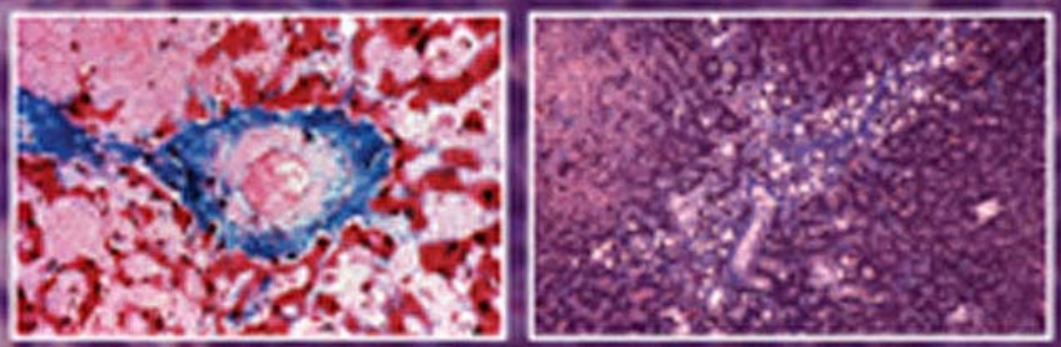
CONTEMPORARY HEMATOLOGY

HEMATOPOIETIC STEM CELL TRANSPLANTATION

SECOND EDITION

Edited by

Robert J. Soiffer, MD



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Hematopoietic Stem Cell Transplantation

CONTEMPORARY HEMATOLOGY

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Hematopoietic Stem Cell Transplantation

Second Edition

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Humana Press

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Cover illustrations: Liver biopsies in an SCT patient (left) and a patient with prior Mylotarg exposure and severe VOD (right). See discussion and complete captions on pp. 468–469.

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Preface

Since the publication of our first edition of *Stem Cell Transplantation for Hematologic Malignancies, Second Edition*, in 2004, the field of transplantation has witnessed continued advances in the understanding of basic transplantation biology and its clinical applications. These advances have created opportunities to address the formidable challenges of transplantation across histocompatibility barriers, stem cell expansion, prevention of complications (e.g., infection, organ damage, graft-versus-host disease) and generation of graft-versus-tumor activity to eradicate residual disease. Transplant physicians, caregivers and patients have more therapeutic options than they did just a decade ago in areas such as donor selection, stem cell source, graft manipulation, GVH prophylaxis, infectious prophylaxis and non transplant treatment options. These require a rigorous ongoing reassessment of the status of the field.

I am delighted that we have been able to expand and update this new edition to reflect the current state of the science. Nine new chapters have been added. Part I introduces the biologic underpinnings of hematopoietic cell transplantation, and includes newly added chapters on basic stem cell biology, immunobiology and histocompatibility. Part II focuses on indications and results of transplantation for specific diseases with the intention of placing the current role of transplantation into perspective as new non transplant therapies are developed. In addition to updating chapters on acute leukemia, chronic myelogenous leukemia, lymphoma, and myeloma, this edition includes new contributions on transplantation for myeloproliferative disorders/myelodsysplastic syndrome, chronic lymphocytic leukemia, bone marrow failure syndromes and hemoglobinopathies. Part III discusses hematopoietic stem cell sources, and includes chapters on unrelated, haploidetical, and umbilical cord blood transplantation, as well as a chapter comparing the use of bone marrow to mobilized peripheral blood. Part IV is devoted to conditioning regimens, and includes a new contribution on ablative regimens, as well as an update on the expanding use of reducing intensity conditioning. Despite improvements in outcome, the morbidity and mortality of transplantation remain significant. Parts V and VI focus on delayed immune reconstitution leading to infectious and lymphoproliferative disorders, lung and hepatic damage, as well as the pathophysiology and clinical aspects of acute and chronic graft-versus-host disease. Part VII is devoted to immune modulation of grafts, including T cell depletion to reduce GVHD, tumor cell purging to prevent disease recurrence, and donor lymphocyte

infusions to promote immune mediated antitumor activity. Part VIII provides a brief glimpse into potential future applications of bone marrow-derived stem cells in the field of cardiac repair.

We hope this edition of *Stem Cell Transplantation for Hematologic Disorders, Second Edition*, will provide physicians, students and other health care professionals with a firm foundation and comprehensive understanding of the current state of transplantation biology, as well as a clear vision into the future. I am grateful to all the authors for their outstanding contributions to this edition.

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Chapter 1

Stem Cell Biology

Elizabeth O. Hexner and Stephen G. Emerson

Stem cells are functionally defined as long-lived cells that can both self-renew and differentiate into multiple cell types. Embryonic stem cells, considered totipotent cells, give rise to all embryonic tissue layers and, consequently, all tissue types. Hematologists/oncologists are perhaps most familiar with hematopoietic stem cells (HSCs): the single pluripotent cell that can give rise to all lymphoid, myeloid and erythroid cell lineages, and repopulate an ablated hematopoietic system [1, 2]. Similar paradigms appear to apply in normal nonhematopoietic tissues as well, including the liver, intestinal epithelium, enodothlium, skeletal muscle and brain. Likewise, using the same framework, cancer biologists have identified cells within a variety of tumors with cancer stem cell properties: rare cells that give rise to tumors, have the capacity to sustain a malignancy and, not surprisingly, share many features in common with tissue specific stem cells [3–5]. These developments create a new sense of urgency to more completely understand the biology of stem cells and their microenvironment: stem cell therapies may become standard outside the subspecialty of hematology, and across disciplines in the emerging field of regenerative medicine, and key molecular differences between normal stem cells and cancer stem cells may emerge as ideal targets for cancer therapy. The goal of this chapter is to provide an overview of current stem cell biology in this context, and its relationship to hematopoietic stem cell transplantation (HSCT).

1. Identifying Stem Cells

Small and rare, HSCs pose an elusive challenge to studies of stem cell biology. HSCs are present in umbilical cord blood with a frequency of just under one in 1 million mononuclear cells (MNCs), and are less frequent in adult bone marrow (one in 3 million MNCs) or mobilized peripheral blood (one in 6 million MNCs) [6]. Given these frequencies, HSCs must undergo at least 20 to 23 divisions on their way to producing mature blood cells, even assuming no cell death along the way. Much effort has been focused on discovering cell surface markers that can identify those cells that possess true functional stem cell properties. Perhaps clinically most familiar is CD34, a glycoprotein present on the cell surface of stem and progenitor cells which is used to enrich stem cells in the

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context of stem cell mobilization and collection for HSCT, but even within the $CD34^+$ population, only a small percentage are HSCs. In experimental transplantation studies in mice, combinations of markers can more highly enrich for HSCs, yielding a population of cells in which 20 percent have the ability to repopulate a recipient mouse [2, 7, 8]. Selection of these cells begins with the depletion cells within the bone marrow bearing any lineage-specific markers, followed by the positive selection of Sca-1^+c-kit^+ cells (Lin^-Sca-1^+c-kit^+ cells or LSK cells), and protocols often integrate an additional selection for Thy-1^{lo} cells [9]. Together, more than 10 monoclonal antibodies are required to select this population of cells, which is feasible in laboratory science using large numbers of cells in suspension, but has clear limitations for clinical applications and *in situ* studies of stem cells. More recently, the signaling lymphocyte activation molecule (SLAM) family of markers were identified by microarray analysis as important discriminators between true HSCs and primitive progenitor cells, and hold promise as a more simple combination of three markers that can as effectively enrich for HSCs as current, more complex combinations [10]. The simplicity of this HSC immunophenotype (CD150+CD244-CD48-) represents an important advance, in that it was also able to be used in tissue sections to provide key geographical insights into where putative HSCs reside within their microenvironment. Moreover, when the SLAM family markers were added to the current complex cocktail of markers, it could more highly enrich for HSCs, with 50 percent of $CD150^+CD48^-$Sca-1^+Lin^-c-kit^+ cells possessing hematopoietic repopulating ability.

Other evidence points to perhaps even more primitive populations of cells isolated based not upon cell surface marker expression, but on their ability to exclude fluorescent DNA binding dyes via cell surface pumps [11, 12]. These side population (SP) cells lack lineage markers and CD34, are Sca-1+ and can repopulate a murine hematopoietic system. Studies of SP HSCs have confirmed that the majority are quiescent cells, but, interestingly, both quiescent and cycling HSCs seem to contribute equally to hematopoiesis, at least in murine experimental systems [12].

Despite these valiant attempts to quantitatively mark and isolate HSCs, to date there is no phenotypic assay that directly identifies and measures HSCs on a cell per cell basis. Rather, the best efforts to measure these rare cells rely on functional assays. The easiest and most popular assays of progenitor cells have been colony-forming cell (CFC) assays, which measure cells that are partially differentiated between HSCs and mature blood cells. These cells, which correlate closely, but inexactly with $CD34^+$ cell numbers, produce easily detectable blood cell colonies in a semisolid medium like methylcellulose after incubation for several weeks (Fig. 1-1). But careful cell fractionation studies have shown that cells of intermediate maturity detected in these assays are not true HSCs, and contribute only very slightly, and temporarily, to hematopoietic recovery after transplantation, since they produce very few long-lived cells following transplantation [13, 14]. So, although $CD34^+$ cell numbers in an unmanipulated graft may correlate with clinical time to engraftment after transplantation, that is likely because the ratio of intermediate progenitor cells to primitive stem cells is fairly constant between clinical samples and, therefore, $CD34^+$ cell numbers only indirectly reflect primitive stem cell numbers transplanted. This assumption, however, may not be true following many *ex vivo* expansion procedures in which progenitors may be greatly expanded while primitive stem cells may not [15]. Better *in vitro* functional assays for

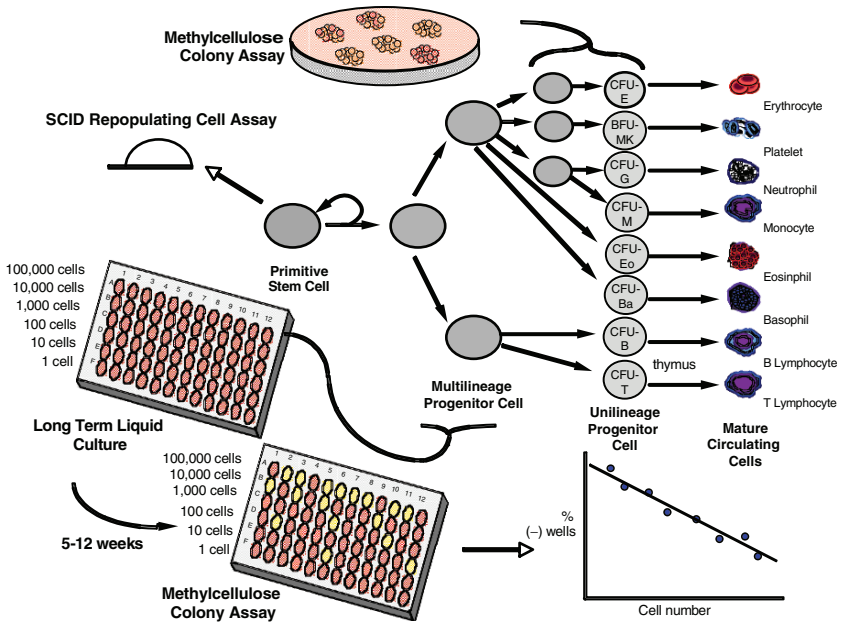

Fig. 1-1. Hierarchy of the hematopoietic system and *in vitro* and *in vivo* functional assays for stem and progenitor cells

primitive stem cells, developed in the late 1980s and 1990s, include the long-term culture initiating cell (LTC-IC) assay and the cobblestone area forming cells (CAFC) assay, where cells are cultured on a stromal cell layer. These assays quantify more primitive, multilineage progenitor cells than do CFC assays. Best of all are the *in vivo* competitive reconstitution assays in mice, or xenograft assays using human cells transplanted into sublethally irradiated immunodeficient NOD/SCID mice. This assay, known as the SCID repopulating cell (SRC) assay, most faithfully reflects true HSCs capable of long-term multilineage repopulation [16]. The ontogeny for mature blood cells has been constructed using observations from these various assays, starting with CFC assays decades ago. This hierarchy, usually schematically depicted in a linear, discrete manner (Fig. 1-1), is probably more accurately described as a developmental pathway on a continuum, heavily influenced by microenvironmental cues. Using the SRC assay and retroviral labeling of cells, it is now apparent that even within the HSC compartment there is considerable heterogeneity: engraftment of all HSCs is not immediate, and HSCs differ in their proliferative potential, with some contributing early and for long periods of time, while others may contribute to hematopoiesis later, for shorter periods of time, or may have relatively less proliferative potential [17–19].

2. The Bone Marrow Microenvironment and the Stem Cell Niche

What determines HSC fate – to enter the cell cycle or not; to differentiate and/or self-renew; to become senescent – is still not clear, although may be largely influenced by cell-extrinsic elements. These microenvironmental cues include

cytokines, contact with other cells within the bone marrow, and probably other factors, including geography within the marrow space and oxygen tension. The construct of a stem cell niche is the specific microenvironment where quiescent stem cells reside, self-renew and where their differentiation is inhibited [20]. HSC niches exist in the bone marrow; whether true HSC niches exist normally outside the marrow in adults is not yet clear. Bone marrow stromal cells include adipocytes, fibroblasts, macrophages, endothelial cells and endosteal osteoblasts; these cells elaborate cytokines and are in close contact with stem cells and progenitors as they differentiate [21]. The relative contribution of each of these cell types to HSC maintenance and differentiation remains obscure, although osteoblasts and endothelial cells are both emerging as critical contributors to the HSC niche. *Ex vivo* experiments using primary osteoblasts and human CD34+ cells in the mid-1990s demonstrated that osteoblasts secrete G-CSF and can be regulated by HSCs to secrete IL-6 and other factors known to be important for HSC survival and differentiation [22–24]. Osteoblasts fail to fully mature in mouse embryos lacking the core binding factor a1 ($Cbfa1^{-/-}$) gene, and the embryos have early blood forming cells, but fail to develop marrow - the deletion lethal in late embryonic life [25]. More recently, transgenic mice without mature osteoblasts were shown to have decreased numbers of HSCs in the marrow, with a compensatory increase in extramedullary hematopoiesis; in this inducible model extramedullary hematopoiesis regresses and bone marrow hematopoiesis was restored when mature osteoblasts were permitted to develop [26]. Two additional recent mouse models point to the central role of osteoblasts in the formation of the HSC niche: one used a conditional knockout of the bone morphogenetic protein receptor 1A (BMPR1A) to reveal an increased number of HSCs which were found to be in contact with an expansion of specialized osteoblasts via N-Cadherin and beta-catenin; another model overexpressed an activated parathyroid hormone receptor in osteoblasts which also resulted in parallel increases in the number of osteoblasts and HSCs, and pointed to the central role of Notch activation in HSCs via increased expression of its ligand, Jagged-1, on osteoblasts [27, 28].

The central role of osteoblasts provides a teleologic explanation of why hematopoiesis might occur primarily within bones. On the other hand, during embryonic life blood cells form in other hematopoietic organs, which continues throughout the lifetime of some animals. Extramedullary hematopoiesis is also observed in humans in certain pathologic states, so clearly other cell types can, at least to some degree, support hematopoiesis. Bone marrow sinusoidal endothelial cells express adhesion molecules and chemokines important for HSC migration and engraftment, and the recent immunoflourescence staining of bone marrow and spleen sections of mice using the SLAM markers demonstrated that while some HSCs were in close apposition to endosteum, most were in contact with sinusoidal endothelium [10, 29, 30]. Some models propose that the osteoblast niche maintains HSC quiescence, while the vascular niche is a secondary niche that supports cycling HSCs, and/or is more permissive for differentiating events, and may be a more dynamic microenvironment that forms during periods of stress such as myeloablative therapy [10, 31, 32].

The cell fate decision to self-renew, differentiate or both is critical to maintenance of normal hematopoiesis over a lifetime, and is profoundly influenced by the HSC niche in particular, and the bone marrow microenvironment in general. A stem cell is defined by its ability to self-renew – that is, divide such that at least one daughter cell maintains its stemness, while the other can either

also remain undifferentiated (symmetric cell division) or differentiate into a multipotent progenitor (MPP; asymmetric cell division). An MPP differs from a HSC in that it too can give rise to all hematopoietic cells, but it has limited self-renewal capacity and cannot maintain hematopoiesis over the long-term. Whether the symmetry of HSC division is determined entirely by the microenvironment or whether cell intrinsic factors play a role remains a matter of debate in vertebrates; in Drosophila it has clearly been observed that asymmetric division can be due to the asymmetric distribution of cellular elements during mitosis [33, 34]. If similar principles apply to mammalian bone marrow, one attractive hypothesis is that bone marrow stromal elements, e.g., osteoblasts or endothelial cells, induce asymmetric distribution of transcription factors promoting self-renewal versus differentiation. Each daughter cell would thereby be directed to divergent cell fates. Alternatively, specific cytokines and/or bioenergetic stimuli could encourage or eschew self-renewal decisions accompanying each HSC division. These signals include Notch, the WNT signaling pathway, angiopoietin-1 and osteopontin (reviewed in [31]). Oxygen concentration has also been implicated in HSC maintenance and differentiation, with the HSC niche being the most hypoxic environment with an increasing oxygen gradient supporting differentiation [35, 36]. *Ex vivo* culture of CD34+CD38- cells under hypoxic conditions increases the frequency of HSCs, as determined by the SRC assay, and may be mechanistically related to stabilization of hypoxia inducible factor-1α (HIF-1α) [35]. Interestingly hypoxia is also inductive of osteoblast differentiation from mesenchymal progenitor cells [37].

3. HSC Homing and Mobilization

Clinicians who care for HSCT patients are conceptually most familiar with the ideas of HSC mobilization and homing, because we often explain it to our patients. The basic biology governing these related processes, however, is still emerging. The interaction between Stromal Derived Factor-1 (SDF-1; also designated CXCL12), and its ligand CXCR4 appears to play a central role in homing and mobilization, and this aspect of basic biology has already found its way into the clinical sphere. As its name implies, SDF-1 is a chemotactic factor expressed by bone marrow stromal cells (osteoblasts and bone marrow endothelium in particular), and circulating HSCs expressing CXCR4 navigate toward the gradient by expressing and secreting metalloproteinases, and via a variety of adhesive interactions, to lodge in the niche [38, 39]. As one might expect, expression of CXCR4 is tightly regulated and can be increased by cytokine stimulation [40]. Both CXCR4 and SDF-1 expression are regulated by HIF-1, and decreasing oxygen tension has been shown to correspond to increased expression of SDF-1 on endothelial cells, again implicating hypoxia as a key microenvironmental regulator of HSCs, through the activity of HIF-1 [41, 42].

Stem cell factor (SCF) - secreted and membrane bound – can increase CXCR4 expression and is another key signal governing HSC homing, as are adhesive interactions between migrating cells and endothelial cells, including those mediated by CD44 and VLA-4/5 (extensively reviewed in [43]). As circulating HSCs migrate into the niche, the high concentrations of SDF-1 are thought to increase HSC survival and promote quiescence [44, 45]. CD26, found to be expressed on CD34+ umbilical cord blood cells, is a cell surface peptidase that cleaves dipeptides from the N-terminus of chemokine receptors,

including SDF-1, and renders it unable to promote migration [46]. Inhibition of CD26 has been shown to increase migration in response to an SDF-1 gradient, and increase homing and stable engraftment of HSCs in experimental mouse models [46, 47]. If CD26 on donor HSCs can be safely inhibited, this observation may have important implications for clinical HSCT, especially in situations where donor cells are limiting, as is often the case in umbilical cord blood transplantation in adults.

At steady state there is a constant, low-level circulation of HSCs in the peripheral blood; whether these are temporarily homeless HSCs, random casualties of the normal bone remodeling process, or whether they circulate to serve a purpose, is not known. In situations of stress, including inflammation or chemotherapy, HSCs are mobilized into the circulation. Clinically, cyclophosphamide and/or granulocyte colony-stimulating factor (G-CSF) are the most common methods for mobilizing HSCs and progenitors into the peripheral blood to be harvested and used in autologous or allogeneic HSCT. Both methods induce the release of proteases from granules in neutrophils in the bone marrow and result in the release of HSCs from the niche; this mechanism of mobilization is probably the same as that induced by physiologic stress [48–52]. Knockout experiments have shown that CD26 is also important in G-CSF mobilization of progenitors/HSCs [53]. Recognizing the importance of the SDF-1/CXCR4 interaction for HSC homing and lodgment within the niche, recent preclinical models using a small molecule antagonist of CXCR4 (AMD-3100) demonstrated that blocking CXCR4 increased the number of circulating mouse and human progenitor cells, and could also increase the number of competitive repopulating cells/SRCs upon transplantation; this increase could be augmented in a synergistic fashion with G-CSF [54].

Pathologic increases in the amount of circulating stem cells have been noted in myeloproliferative disorders, most notably chronic idiopathic myelofibrosis (CIMF), and the number of circulating CD34+ cells correlates with disease severity and extent of extramedullary hematopoiesis [55]. CXCR4 expression has recently been shown to be decreased in circulating CD34+ cells isolated from patients with CIMF [56]. These cells have repopulating ability in SRC assays, and their mobilization also appears to be related to increased protease activity in the disease [57, 58]. Because CXCR4 is also expressed on T cells, it will be important to determine the impact of AMD-3100 on immune reconstitution, in terms of GVHD, antitumor activity and engraftment. AMD-3100 is currently in clinical trials for HSCT mobilization both in the autologous and allogeneic setting.

4. The Molecular Regulation of Stem Cell Proliferation and Self-Renewal

Whatever extrinsic microenvironmental and hormonal cues influence stem cell proliferation and self-renewal, their effects are clearly mediated by cell-intrinsic molecular intermediates which induce differential gene expression. HOXB4, a transcription factor in the homeobox family, was the first such molecule identified and has been extensively studied. Homeobox genes were first identified as critical for body patterning during embryogenesis, and suggested to be involved in hematopoiesis when expression levels were shown to

be highest in the most primitive stem and progenitor cell compartments [59–63]. Overexpression of HOXB4 in murine bone marrow cells led to a 50-fold expansion of HSCs in secondary transplants when compared to retrovirally transduced cells without the HOXB4 gene, without changing peripheral blood parameters [64]. It is worth noting, however, that absolute numbers of HSCs in recipients of HOXB4 overexpressing cells did not exceed normal numbers of HSCs, suggesting that regulatory and/or feedback mechanisms controlling the size of the HSC pool likely supersede the HOXB4 expansion program. There is somewhat less direct evidence that other transcription factors, including Hox family members (HoxA10, HoxC4), LEF (via Wnt signaling) and stem cell leukemia (SCL) protein, are involved in HSC self-renewal (extensively reviewed in [65]).

The Notch receptor Notch-1 and its downstream signaling events play a role in determining HSC cell fate through alteration in cell cycle events. Steir, et al. showed *in vitro* that Notch-1 expression inhibits HSC differentiation (using CFC and LTC-IC assays), and confirmed that the differentiation block was accompanied by an expansion of HSCs using an *in vivo* competitive repopulation assay [66]. Carlesso, et al., also in the Scadden laboratory, had earlier shown (in primary human cord blood cells and cell lines) that the delay in differentiation was associated with an enhanced progression/higher fraction of cells in cycle, suggesting that Notch-1 alters cell cycle kinetics [67]. Recalling that Jagged-1, a Notch ligand, is expressed on osteoblasts, a model for microenvironmental influences of the HSC niche on cell cycling begins to emerge. Cyclin dependent kinase inhibitors such as $P16^{Ink4a}$ and $p19^{Arf}$ (see below), and p21 and p27 have been shown to govern entry into the cell cycle and regulate the size of the HSC and progenitor cell pool [65, 68, 69] (and reviewed in [65]).

Since senescent cells can no longer proliferate, regulated modulation of senescence is, therefore, critical to proper maintenance of the HSC pool. Recently the chromatin modifying cofactor Bmi-1 has been shown to be a critical regulator of HSC senescence [70]. Bmi-1 mRNA levels were first noted to be upregulated in HSCs versus more differentiated hematopoietic cells [71]. Bmi-$1^{-/-}$ mice, in contrast, were viable but had severe neurologic defects, growth retardation and died before two months of life. Peripheral blood findings were significant for lymphopenia and normal numbers of myeloid cells; progenitor cell function by CFC assays were normal. Bone marrows of young mice are hypocellular, and while Bmi-$1^{-/-}$ fetal liver cells do not appear to have an impairment in bone marrow homing, Bmi-$1^{-/-}$ HSCs reconstitute wild type mice only transiently, with bone marrow failure within several weeks. Taken together, these results suggest that absence of Bmi-1 causes a cell-intrinsic defect in self-renewal and a relatively preserved ability to differentiate [70]. Bmi-1 is a member of the *Polycomb* family, proteins that form large complexes and are involved in chromatin remodeling. Bmi-1's role in HSC senescence may be through the *Ink4a* locus that encodes $p16^{Ink4a}$ and $p19^{Arf}$, and is the target of Bmi-1, which normally functions to repress it. $P16^{Ink4a}$ and $p19^{Arf}$ are inhibitors of the Cyclin D and MDM2 pathways, respectively, and their upregulation leads to senescence via a failure of full phosphorylation of Rb, as well as apoptosis via p53 (reviewed in [72]).

The essential function of Bmi-1 does not appear to be limited to the normal HSC compartment: Bmi-$1^{-/-}$ fetal liver cells engineered to be leukemic caused

a phenotypic leukemia in primary recipients, but as in the normal HSC experiments, Bmi-1 deficiency was similarly found to incapacitate the ability to transplant leukemia into secondary recipients, suggesting that Bmi-1 is also essential for leukemic stem cell survival [73]. Bmi-1 is widely expressed, essential for neural stem cell self-renewal and may be involved in other tumor types as well [72, 74].

Senescence is also governed by telomere length. Telomeres are the guanine-rich repeat sequences on the ends of chromosomes that are maintained by telomerase, a ribonucleoprotein. Without telomerase, as the cell divides, DNA polymerase incompletely synthesizes the terminal ends of chromosomes and, therefore, telomeres shorten with each successive generation, as long as the cell lacks the enzyme. HSCs, other tissue specific stem cells and cancer cells express telomerase. Telomerase is not expressed in most mature, somatic tissues, and telomere shortening is thought to contribute to aging and senescence; enforced expression of telomerase extends the replicative ability and lifespan of human cells [75]. Knockout experiments have shown that the phenotype of telomerase deficient mice in initial generations was virtually normal, but with successive generations had more profound effects on the reproductive and hematopoietic systems, not surprising given the highly proliferative nature of these organ systems [76]. Interestingly, while sixth generation telomerase deficient animals had profound differences in progenitor cell numbers as measured by CFC assays, peripheral blood counts remained normal. Normal HSCs exhibit telomere shortening with successive serial transplantations, which may explain why serial transplantation fails after six to eight passages. Competitive repopulating experiments showed an impairment in long-term repopulating ability in telomerase deficient mice, and an increase in genetic instability [77]; it is important to note, however, that the telomerase deficient HSCs could still reconstitute a marrow (even upon secondary transplantation); therefore telomerase is not essential to HSC function in the absolute manner that appears to be the case for Bmi-1.

5. Cancer Stem Cells

In the last decade proof for a cancer stem cell has been increasingly supported by experimental evidence, although the concept, supported by data, has existed for many decades and parallels the timeline for normal stem cell models [78–81]. In the mid-1980s, Sabbath, et al. showed that heterogeneity existed within primary cells from individual subjects with acute myelogenous leukemia (AML) in terms of the cell surface markers expressed, and that clonogenic potential (as determined by the CFC assay) corresponded to the more primitive immunophenotype within each sample [82]. In the late 1990s, using the SRC assay, Bonnet, et al. confirmed this observation and demonstrated that, irrespective of the morphologic differentiation of a particular leukemia sample, the leukemia stem cells (LSCs) were found exclusively within the CD34+CD38- population [4]. Further experiments from the Dick laboratory refined these concepts by using retroviral marking experiments and serial bone marrow transplantation. Like the normal HSC compartment, these studies showed that there was considerable heterogeneity in the LSC population, with some LSCs possessing shorter or longer-term repopulating ability, and some LSC

clones emerging only upon secondary transplantation, indicating that LSCs, like HSCs, can remain quiescent for long periods of time [83]. The frequency of LSCs within the leukemia cell population is between one and 100 per million MNCs, and both their infrequency and shared immunophenotype with normal stem cells may pose further challenges to graft purging efforts [4, 84]. Subsequent to the description of LSCs, cancer stem cells with a distinct immunophenotype have been identified in other tumor types, including breast and those arising in the central nervous system, extending the evidence for cancer stem cells to solid tumors [3, 5].

With the identification of a stem cell homolog within a cancer, we hope to gain more insight into why our therapies too often fail, and how we can specifically target cancer stem cells to improve efficacy and to limit toxicity of cancer therapy in general, and HSCT in particular. Viewed globally, an extremely attractive approach to treating cancer would thus be to identify and pharmacologically target markers or pathways that are aberrantly expressed or dysregulated in the cancer stem cell and not in the normal tissue stem cell counterpart.

Phosphatase and tensin homologue (*Pten*) may be one such target. *Pten* is a tumor suppressor gene, encoding a phosphatase that negatively regulates the phosphatidylinositol-3-OH kinase (PI(3)K)-Akt pathway, a pathway involved in cell proliferation as well as differentiation and migration. It has been shown in mice to be essential for long-term maintenance of normal HSC function; moreover, its deletion appears to drive leukemia [85, 86]. While *Pten* deficient donor cells were able to engraft into irradiated recipients, lodgment of bone marrow cells was found to be impaired in competitive repopulations assays, and long-term normal hematopoiesis could not be sustained [85]. Conditional deletion of *Pten* caused an increase of LSK cells in cycle, and a concomitant depletion of long-term HSCs; the implication of these observations is that *Pten* is critical for transition into cycle, and its loss may favor the activation of HSCs and their forward transition into G1 and/or prevent the reverse transition into a quiescent state. Mice developed a myeloproliferative disorder (MPD), which, in most cases, evolved into acute leukemia - myeloid, lymphoid or biphenotypic diseases - and were associated with additional chromosomal abnormalities [86]. These were true stem cell disorders, in that the leukemias were transplantable and co-purified with the LSK population. That the absence of *Pten* seemed to at once deplete normal HSCs and drive leukemogenesis represented a potential distinction between normal and leukemic stem cells and, therefore, a potential therapeutic target. Yilmaz, et al. tested this hypothesis by treating *Pten* deficient mice (who had not yet developed an MPD) with rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), which is downstream of the PI(3)K-Akt pathway, to determine whether depleted HSCs could be restored and/or leukemia could be prevented. Indeed, rapamycin was effective at restoring HSCs and preventing development of MPD/leukemia, and it could also prolong survival (but not cure disease) in mice with already established leukemia [86]. These studies thus implicate *Pten* as a critical switch in normal HSC function and homeostasis, and a potential therapeutic target because of its distinct effects with respect to normal and leukemic stem cells. Previous studies had already identified PI(3)K itself and mTOR, as well as nuclear factor κB (NFκB), as potential unique therapeutic targets for leukemic stem cells [87–90]. The translation of these findings is currently being tested in the clinic.

Another recently identified unique leukemic stem cell target is CD44, a widely expressed adhesion molecule [91, 92]. Leukemic cells express CD44, and in one study the level of expression of a specific CD44 splice variant on AML cells correlated with poor survival [93]. In a NOD/SCID mouse model of human AML, an activating monoclonal antibody against CD44 had distinct antileukemic activity, apparently via two distinct mechanisms: one involving cell fate (induction of differentiation over self-renewal *in vitro* and *in vivo*), and one that impairs the leukemic stem cell's interaction with the niche [92, 94]. The primary ligand for CD44 is the glycosaminoglycan hyaluronan, abundant in the endosteal region thought to be so critical in formation of the HSC niche, and this latter selective mechanism is particularly interesting in that it provides key evidence that cancer stem cells do indeed maintain niche dependence. Moreover, certain niche stem cell interactions may be selectively vulnerable targets in cancer tissues over normal tissues – engraftment of leukemic stem cells were markedly more sensitive to CD44 antibody treatment than normal HSCs. CD44 interacts with other stromal ligands, and these interactions may prove to be important in other types of cancer, and it is interesting to note that breast cancer stem cells were defined by the $CD44^{+}CD24^{-}Lin^{-}$ immunophenotype [3].

6. Is There a Cancer Stem Cell Niche?

The role of the microenvironment in the biology of cancer stem cells is likely to be as critical as that for normal stem cells. For example, there are rare reports of donor-derived leukemia in recipients of HSCT, and yet no published report has identified disease arising in the donor, suggesting that the host environment is providing key oncogenic signals to susceptible donor cells and allowing them to act as true leukemic stem cells [95]. While reports of donor cell derived leukemia provide intriguing circumstantial evidence that the microenvironment may itself be key to oncogenesis, data from other tumor types provides more direct evidence for such a hypothesis [96, 97]. Genetic profiling studies have, unexpectedly, shown strong correlation between clinical outcome and molecular signatures that correspond to non-tumor cells within a specimen, rather than the clonal cells themselves. Recently, specific alterations in microdissected tumor stroma in squamous cell cancers of the head and neck corresponded both to the size and to the invasiveness of the tumors, suggesting that damage and/or dysregulation by stromal cells are directly related to the aggressiveness of disease [96]. In follicular lymphoma, on the other hand, the molecular signature associated with the best survival corresponded to genes expressed in surrounding T cells, dendritic cells and monocytes within the tumors, and were not expressed in the CD19+ malignant cells when sorting experiments were performed to confirm the observation [97]. Whether this signature means that the immune response primarily determines the more favorable outcome or whether the malignant cells evade or influence the immune response cannot be definitively determined by these data. Nonetheless, these data support the broader notion that tumor behavior is profoundly influenced by surrounding cells, and, more specifically, that immunotherapy – HSCT or otherwise – is a rational therapeutic approach.

In fact, the stem cell model is also being integrated into the immune response itself, in particular long-lived memory responses [98, 99]. In experimental mouse HSCT models of Graft-versus-Host Disease (GVHD) it has

recently been shown that a rare subpopulation of CD8+CD44^{lo}CD62L^{hi} T cells bears all the hallmarks of stem cells: they can sustain themselves as well as generate other memory T cell subsets; they are more proliferative *in vitro* and *in vivo* than are all other T cell subsets; they express high levels of Sca-1 and Bcl-2, and functionally upon secondary adoptive transfer these cells induce GVHD [98]. Molecular support for the notion has come from microarray data comparing memory T and B cell subsets to their naïve and effector counterparts, and demonstrated that the memory cell signatures are distinct and share a self-renewal program with HSCs [99]. These observations should inform our efforts at therapy: for example, it is conceivable that our efforts - many of them frustrated – aimed at preventing and treating GVHD are targeting the wrong cell population; similarly we may be able to augment either endogenous immune responses or graft-versus-tumor effects through augmentation or manipulation of memory cell programs.

7. Conclusions

While the study of the biology of stem cells remains challenging, largely owing to their infrequency, the rewards have been, and will continue to be, fruitful. Within the field of HSCT, a more comprehensive understanding of stem cell self-renewal may finally lead to successful *ex vivo* expansion of HSCs, and allow for efficient manipulation of HSCs so that gene therapy is effective and safe. A more detailed understanding of mobilization, homing and engraftment may broaden the applicability of HSCT and, in particular, may make umbilical cord blood transplantation more safe in adults.

Conventional therapy for hematologic malignancies, as well as all types of cancer, has been largely targeted towards rapidly cycling cells, leading to nonselective normal tissue toxicity and, ironically, possible selective protection of the most malignant cells within a cancer – the quiescent cancer stem cell. A revised approach to cancer therapy should, therefore, have its roots in basic stem cell biology, and holds promise for revolutionizing both the efficacy and tolerability of cancer therapeutics. HSCT, in particular donor HSCT, has in some ways been at the forefront of this revolution, at least in principle: the Graft-versus-Leukemia effect, recognized almost three decades ago, may be an early example of cellular therapy directed against cancer stem cells [100, 101].

Many major questions remain unanswered in the field of stem cell biology, and this review will conclude with two major areas of uncertainty: the microenvironment and cancer stem cell biology. A sketch of the HSC niche is beginning to emerge, but remains obscure. The experimental evidence demonstrating that increased numbers of niche forming cells leads to increased numbers of HSCs suggests that HSC niches are limiting to the engraftment of HSCs, and might be a target for manipulation. This would support the concept in HSCT that conditioning regimens act, in part, to increase the number of functional HSC niches, a concept thus far not supported by evidence. However, indirect evidence to the contrary also exists: experimentation in tolerance induction has shown that, at least following the establishment of microchimerism, long-term engraftment of mismatched HSCs is possible without conditioning in mice, suggesting that HSC niches may not, in fact, limit the size of the HSC pool [102]. More definitive studies are needed to answer this fundamental question. Finally, while the cancer stem cell model is operationally useful, it is still not

known whether cancer stem cells actually arise from the normal stem cell pool or whether committed cell types can be altered and acquire the ability to self-renew. If the latter, defining the stem cell property that is acquired in oncogenesis should have key therapeutic implications.

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Chapter 2

Stem Cell Transplantation for Hematologic Malignancies

Effie W. Petersdorf

1. Introduction

Histocompatibility in transplantation is governed by the products of the human leucocyte antigen (HLA) genes encoded within the major histocompatibility complex (MHC) on chromosome 6p21. HLA antigens define tissue type and are inherited *en bloc* in classical Mendelian fashion. Genotypically identical siblings inherit the same paternal and maternal chromosome 6. Haploidentical relatives share one complete chromosome 6 and the degree of HLA compatibility for the non-shared chromosome 6 is variable. Selection of unrelated donors for hematopoietic cell transplantation (HCT) is based on identity for the classical HLA genes. The precision with which an unrelated donor is "matched" with the recipient is only as rigorous as the typing technology that is used to define the alleles of class I and class II genes. New concepts in immunogenetics in transplantation include the delineation of locus-specific and allele-specific risks associated with donor-recipient disparity, the role of the chromosome 19-encoded natural killer (NK) receptor complex in modulating graft-versus-tumor effects, and the importance of the HLA haplotype as a marker of new transplantation determinants.

2. HLA Genetics

The MHC encodes over 100 loci involved in immune function including the classical and nonclassical HLA genes HLA-A, B, C, DR, DQ and DP [1]. A key feature of HLA genes is their extensive sequence diversity [2] and strong positive linkage disequilibrium (LD) across the HLA-A to HLA-DQ segment [3].

2.1. Polymorphism of HLA Genes

The extensive sequence polymorphism of HLA genes reflects their primary role in the presentation of peptides to antigen-specific T cell receptors [4]. Each HLA allele is unique and given a name by the World Health Organization Nomenclature Committee for Factors of the HLA System [2]. As of April 2006, 469 HLA-A, 794 HLA-B, 244 HLA-C, 525 HLA-DRB, 34 HLA-DQA1, 71 HLA-DQB1, 23 HLA-DPA1 and 124 HLA-DPB1 alleles are currently

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recognized [Steven Marsh, personal communication]. Recently, novel HLA alleles have been discovered through DNA genotyping efforts of ethnic minority populations in volunteer bone marrow registry donors. The frequencies of HLA allele and antigen frequencies vary widely between ethnically diverse populations (*www.allelefrequencies.net*) [5–7]. For this reason, the likelihood of identifying suitable unrelated donors for HCT is highest when the patient and donor are of the same ethnic or racial background [8].

2.2. LD and HLA Haplotypes

LD is defined as the nonrandom association of alleles more often than would be predicted by chance alone. Within the HLA complex there is strong positive LD between the HLA-B and C loci, and between the HLA-DR and DQ loci. There is weak LD between HLA-DP and HLA-A, C,B, DR,DQ. From a clinical perspective, positive LD between HLA alleles and antigens may aid in the identification of well matched unrelated donors. For example, an HLA-A1,B8,DR3-positive recipient will have a good chance of identifying a donor with the same phenotype, because matching for HLA-A1 and B8 will often determine matching for DR3. In turn, HLA-A1, B8, DR3-positive donors and recipients have a high likelihood of being matched for HLA-DRB1*0301 and DQB1*0201 alleles. LD can exist between commonly observed alleles and antigens, as well as between less common ones [9]. When a recipient has inherited a rare combination of alleles and antigens, the probability of identifying a suitable unrelated donor is related to the size and composition of the donor registry.

The physical linkage of HLA genes on the same chromosome is called a haplotype. Haplotypes that encode highly conserved sequences thought to be derived from a common ancestor are known as "ancestral haplotypes" [The Sanger Institute; Human Chromosome 6 Project database *http://www.sanger.ac.uk/HGP/Chr6/*; International HapMap Project database *http://www.hapmap.org/*]. One of the most well-known examples of an ancestral haplotype is HLA-A1,B8,DR3 [10]. Classically, haplotypes are defined by a complete family study of the parents and children. Haploidentical related family members share one chromosome 6 and are variably matched for the non-shared HLA haplotype. A child is haploidentical to each of his/her parents. The genetic relationship between the mother and child can be described as involving "inherited maternal HLA antigens" (IMA), and that between the father and child as involving "inherited paternal HLA antigens" (IPA). HLA antigen and alleles of the non-shared haplotypes are likewise termed "non-inherited maternal antigens" (NIMA) and "non-inherited paternal antigens" (NIPA).

When a family study is not available, haplotype frequencies can be estimated (*www.allelefrequencies.net*) [11]. For example, using a large unrelated donor pool from the NMDP (*www.nmdpresearch.org*), the estimated haplotype frequency of HLA-A1, B8, DR3 in Caucasian, Asian, African and Hispanic Americans is 0.062, 0.003, 0.012 and 0.017, respectively. Estimated haplotype frequencies have been used to determine the ideal size of unrelated donor registries for HCT [12].

2.3. Alleles and Antigens

Alleles define the genotype and antigens define the phenotype [13]. The level of resolution of HLA alleles and antigens is dictated by the specific technique or method used in the laboratory. Serologic methods were historically used to type

HLA antigens; DNA-based methods can also define the equivalent of an antigen (low resolution methods). DNA methods can further define the nucleotide sequence variation that permits the identification of a unique sequence as an allele (high resolution). For this reason, DNA-based techniques have become the gold standard in tissue typing in support of hematopoietic cell transplantation programs for the purposes of donor identification. One serologically defined antigen or phenotype (DR4) may be the product of one or more allele sequences (HLA-DRB1*0401, 0402, etc.) (Table 2-1). In this way, two individuals who share the same HLA

Table 2-1. Concepts in HLA and NK Genetics.

Concept		Definition	Example(s)
HLA Mismatching	Antigen	Donor-recipient difference between two serologically distinct proteins	HLA-A1 *versus* HLA-A2
	Allele	Donor-recipient difference between two unique HLA sequences within a serological specificity	HLA-A*0201 *versus* HLA-A*0205
HLA Vector of Incompatibility	HVG	Donor alleles not present in the recipient	Donor HLA-A1,2 *versus* Recipient HLA-A1,24: recognition of donor A2 by recipient
	GVH	Recipient alleles not present in the donor	Donor HLA-A1,2 *versus* Recipient HLA-A1,24: recognition of recipient A24 by donor
	Unidirectional	Only one vector of incompatibility is present, either HVG or GVH (homozygous recipient with heterozygous donor, or heterozygous recipient with homozygous donor)	HVG: Recipient HLA-A*0201,0201 *versus* Donor HLA-A*0201,0205 GVH: Recipient HLA-A*0201,0205 *versus* Donor HLA-A*0201,0201
	Bidirectional	Both HVG and GVH vectors of incompatibility are present at the same locus (recipient and donor are both heterozygous)	Recipient HLA-A1,2 *versus* Donor HLA-A1, 11; recipient HLA-A*0201, 1101 *versus* Donor HLA-A*0205, 1101
HLA Ligand	One or more amino acid residues of an HLA protein that binds to NK KIR receptor; known ligands include the HLA-Bw4 epitope of certain HLA-B and HLA-A antigens, and residues 77 and 80 of the HLA-C antigen		Bw4 ligand for 3DL1 receptor; C1 ligand for 2DL2, DL3, 2DS2 receptors; C2 ligand for 2DL1 and 2DS1 receptors
KIR Ligand Mismatch	Recipient lacks the HLA epitope present in the donor		Recipient C1, C1 homozygous (eg, HLA-Cw*0701, 0701) *versus* Donor C1, C2 heterozygous (eg, HLA-Cw*0701, 1602)
Missing KIR Ligand	Recipient is missing the HLA epitope for donor inhibitory KIR		Recipient C1, C1 (eg, HLA-Cw*0701, 0701); KIR2DL1 receptor for C2 ligand is not engaged, leading to release of NK inhibition

antigen may be mismatched for two different alleles of that antigen, and explains why unrelated donors who were historically selected based on serological identity for HLA-A, B, DR were later found to encode undetected allele mismatches when modern DNA techniques became available [14, 15]. The immunogenecity of allele mismatches and antigen mismatches is the subject of investigation in unrelated donor HCT [16–21].

2.4. Vector of Incompatibility

The vector of incompatibility refers to the direction of allorecognition between the donor and recipient (Table 2-1). When a donor and recipient are incompatible in the host-versus-graft (HVG) vector, there are donor antigens or alleles not shared by the recipient. HVG vector mismatches correlate closely with graft failure [22]. When a donor and recipient are incompatible in the graft-versus-host (GVH) vector there are recipient antigens or alleles not shared by the donor; GVH vector disparity closely correlates with the risk of acute and chronic GVHD [22]. Unidirectional mismatches refer to disparity in only the HVG or the GVH vector at a given locus. Unidirectional mismatching occurs when the donor is homozygous (donor HLA-A1,1 versus recipient HLA-A1,2 produces a GVH vector mismatch only) or when the recipient is homozygous (donor HLA-A1,2 versus recipient HLA-A2,2 produces an HVG vector mismatch only). Bidirectional mismatching refers to the presence of both HVG and GVH mismatching at the same locus, and occurs when the donor and recipient are each heterozygous for two different alleles or antigens. For example, when a donor is HLA-A1,2 and the recipient is HLA-A1,11, the mismatch between the A2 and the A11 is bidirectional.

3. NK Genetics

The NK family of killer immunoglobulin-like receptor (KIR) genes is encoded within a 150 kb region of chromosome 19q13.4 and segregates independently of HLA. The concept that certain HLA class I gene products may interact with NK cells to distinguish self from non-self has been one of the most important advances in human immunogenetics [23]. The role of NK-mediated alloreactivity provides a unique therapeutic avenue for graft-versus-tumor effects, and the selection of potential donors has recently included consideration of the HLA ligands of NK receptors.

3.1. Ligand-Receptor Diversity

HLA-B, HLA-C and some HLA-A antigens serve as the ligands for inhibitory KIRs [24]. Although the polymorphisms that distinguish HLA-B and C alleles are distributed across three exons, the epitopes that define specificity of KIR receptor binding are governed by residues 77 and 80 of HLA-C and by HLA-Bw4 present on some HLA-B and HLA-A molecules. HLA-C molecules that encode Asn at position 77 and Lys at position 80 define the group 2 (C2) ligands; this polymorphism is present in HLA-Cw2, Cw*0307, Cw*0315, Cw4, Cw5, Cw6, Cw*0707, Cw*0709, Cw*1205, Cw*12041/2, Cw15 (except Cw*1507), Cw*1602, Cw17 and Cw18. Group C2 HLA ligands are recognized by the KIR2DL1 and 2DS1 receptors. HLA-C molecules that encode Ser77 and Asn80 (HLA-Cw1, Cw3 [except Cw*0307, 0310, 0315], Cw7 [except

Cw*0707, 0709], Cw8, Cw12 [except Cw*1205,12041/2], Cw13, Cw14 [except Cw*1404], Cw*1507 and Cw16 [except Cw*1602]) are collectively referred to as the group 1 or C1 ligands; they are recognized by the 2DL2, 2DL3 and 2DS2 KIR receptors. The HLA-Bw4 epitope present in B5, B13, B17, B27, B37, B38, B44, B47, B49, B51, B52,B 53, B58, B59, B63, B77, B*1513, B*1516, B*1517, B*1523 and B*1524 serves as a ligand for the inhibitory KIR3DL1 receptor. The KIR receptor genes display allelic and haplotypic polymorphism [24–31]. The clinical importance of KIR receptor diversity in transplantation is unknown.

3.2. Mechanisms of HLA Ligand- KIR Receptor Recognition in Allogeneic HCT

When HLA-B and/or C ligands are engaged with their inhibitory KIR receptor, the NK cell is inhibited from killing the target cell [32]. Killing of host cells, including residual host leukemia or tumor cells, occurs when the inhibitory KIR of the donor-derived NK cell does not recognize the patient's HLA class I allele, either because the allele is different or because it is not present (Table 2-1). In this situation, eliminating the host leukemic cells leads to lowered post-transplant relapse; the elimination of host antigen-presenting cells leads to lowered GVHD [32, 33]. Among HLA-B and/or C mismatched haploidentical siblings, if the recipient is homozygous for the HLA ligand, or if the recipient is missing an HLA ligand for which the donor NK cells express the inhibitory KIR receptor, then this may lead to release of inhibition of the donor NK cells and result in killing of the target (host) cell. Note that C1, C2 and/or Bw4-homozygous recipient cells may also be the target of donor NK-mediated killing, even when the recipient is HLA matched with his or her donor (Table 2-1).

4. Donor Selection

Donor selection begins with a full evaluation of available family members of the transplant recipient (Fig. 2-1). A family study includes tissue typing of the father, mother, full siblings and additional relatives, where indicated. The family study confirms the recipient's genotype and haplotypes, and provides information on the immediate availability of genotypically matched siblings and haploidentical family members. Further consideration of related donors may include the number of HLA disparities on the mismatched maternal or paternal haplotype, and the presence or absence of disparity and of homozygosity for HLA KIR ligands. For unrelated donors, the number of HLA mismatches and the specific loci mismatched are important elements in the selection of donors (Table 2-2).

4.1. Related Donors

Segregation of chromosome 6 in Mendelian fashion gives rise to a 25 percent probability that two siblings will be genotypically identical, a 50 percent probability that two siblings will be haploidentical (share one paternal chromosome 6 or one maternal chromosome 6) and a 25 percent chance that they inherited different parental chromosome 6s. Clinical outcome after haploidentical transplantation differs depending on whether NIMA or NIPA is involved

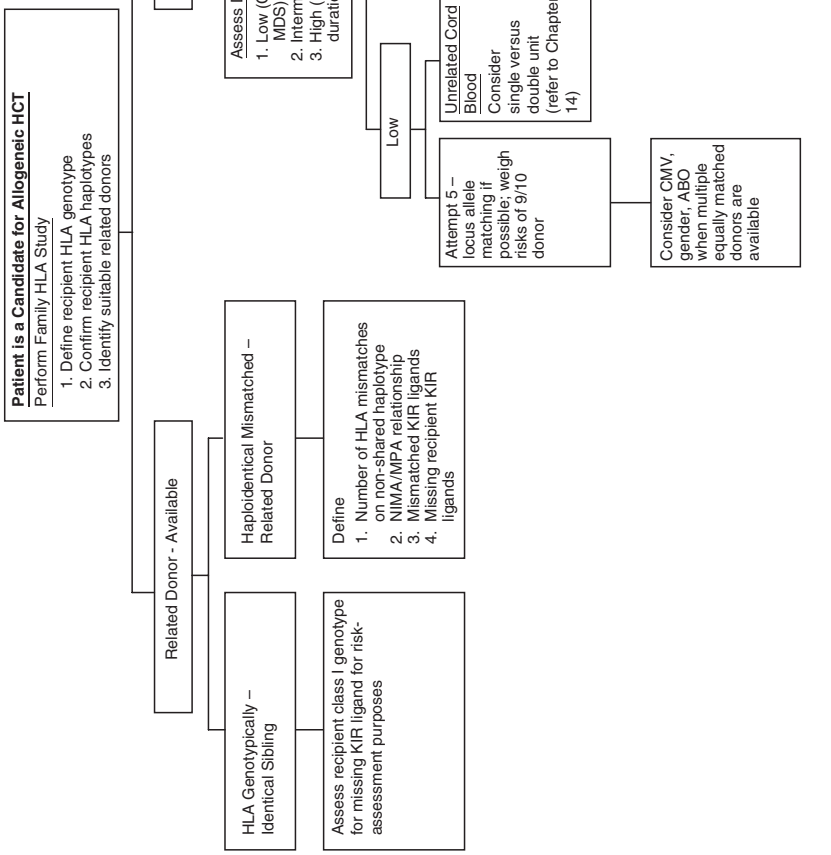

Fig. 2-1. Algorithm for Donor Identification

Table 2-2. Summary of HLA and NK Genetic Matching in Allogeneic HCT.

Number of HLA Disparities	Risks of graft failure and GVHD are associated with degree of donor-recipient HLA mismatching. When a fully matched donor is not available, the donor with the fewest mismatches should be prioritized
HLA Homozygous Recipient	The risk of graft failure is associated with recipient homozygosity at the mismatched locus. When no matched donors are available, avoid donors who are mismatched at the locus for which recipient is homozygous
Allele and Antigen Differences	The risk of graft failure and GVHD are higher with antigen than allele mismatch. When no matched donor is available, donors with allele mismatches should be prioritized over donors with antigen mismatches
Presence of Donor-recipient Disparity for HLA Ligands	Donor-recipient mismatching for HLA KIR ligands is associated with durable engraftment, low risk of GVHD, and low risk of relapse after T cell depleted transplantation from haploidentical related donors for the treatment of AML. Unrelated donor HLA-KIR ligand incompatibility is useful for risk assessment and planning HCT strategies
Recipients Missing HLA Ligands for Donor KIR Receptor	Recipients who are missing Bw4, C1, or C2 HLA ligands have lower relapse after HLA matched and mismatched related and unrelated donor HCT. Pre-transplant genotyping of HLA class I alleles in the patients provides useful information for risk assessment and planning HCT strategies
NIMA Effects	Fetal-maternal chimerism associated with hyporesponsiveness to non-inherited maternal antigens. NIMA mismatched related donors should be considered
Haplotype Effects	Among HLA matched unrelated donors and recipients, patients whose HLA-A,B, DR alleles are in phase with those of the donor have lower risk of GVHD

(described below) and, hence, NIMA and NIPA are used to further select among potential family donors (Table 2-2). Haploidentical siblings may be variably mismatched for the non-shared haplotype if the maternal and paternal HLA haplotypes fortuitously encode the same HLA antigen(s) or allele(s).

In addition to maternal and paternal sharing of HLA antigens, selecting haploidentical family members can also include considering the presence of mismatched HLA KIR ligands encoded by HLA-B, HLA-C and certain HLA-A alleles. For both related and unrelated donors, the availability of high resolution typing of HLA class I alleles of the recipient provides the necessary information to determine whether a patient is Bw4-positive or negative, and whether the recipient is C1/C2 heterozygous, C1/C1 homozygous or C2/C2 homozygous.

4.2. Unrelated Donors

Early in the unrelated HCT clinical experience, matching volunteer donors included consideration of HLA-A, B and DR antigens. As laboratory technology for discriminating HLA alleles became available, a transition from serologically based phenotyping to DNA-based genotyping for DRB1 and DQB1 was made in the early 1990s; application of DNA-based genotyping to class I HLA-A, B and C genes followed soon thereafter.

As robust DNA methods were applied to address research questions on the importance of genetic variation in transplant outcome, new information became available demonstrating the importance of HLA-C [16–18] and HLA-DQB1

[34, 35], and criteria for donor evaluation and selection of volunteer donors became more complex. Today, consideration of 5 loci HLA-A, B, C, DRB1 and DQB1 is considered standard, and gives rise to the 10 possible alleles (two at each loci) for matching ("10/10" allele-matched donor). Although outcome after transplantation from a 10/10 allele-matched donor is outstanding and, in some diseases, approaches the results for HLA identical sibling transplantation, 20 to 80 percent of patients who initiate a donor search are able to find a suitable donor. As described below, a major research impetus has been underway to define specific allele and antigen mismatch combinations that do not increase post-transplant risks. In this way, the use of mismatched donors with "permissible" mismatches may open the possibility that all patients in need of a transplant will have a suitable donor. Overall outcome after unrelated HCT is influenced by the stage of disease at the time of transplantation, and disease activity remains one of the most challenging aspects of searching volunteer donors in a timely manner, particularly for patients with less common genotypes and haplotypes [36]. When more than one equally matched unrelated donor is available, additional criteria include CMV serostatus, donor age, donor gender or ABO [37].

5. Importance of Donor Matchingin Allogeneic HCT

HLA genotypically identical siblings are the preferred donor for allogeneic transplantation because they are genetically identical for the entire chromosome 6, including all non-HLA genetic variation encoded on the MHC haplotype. When a matched sibling is not available, a haploidentical relative, cord blood (CB) unit(s) or a volunteer donor are considered (Fig. 2-1). Choice of these alternative donors is shaped by the urgency of the transplant as well as outcome using specific conditioning regimens and GVHD prophylaxis. When the timing of a transplant is urgent, as in the case of high risk patients whose disease tempo precludes time for a donor search, an available haploidentical donor or CB unit provides a highly attractive strategy. The recent elucidation of the NK KIR receptor complex and its role in donor-antihost alloreactivity after allogeneic transplantation provides a novel approach for optimizing overall results of HCT, especially for patients at high risk for disease recurrence.

5.1. HLA Effects on Transplant Outcome

5.1.1. Haploidentical Mismatched Related Donor Transplantation

A decisive summary of the haploidentical transplant experience is provided elsewhere in this textbook (Chapter 15). The specific risks associated with HLA disparity in this patient population include increased risk of graft failure, GVHD and delayed immune reconstitution [22, 38–43]. The risks of GVHD and graft failure increase as the number of HLA mismatches of the non-shared HLA haplotype [44–47] increase, and are especially high in the presence of mismatching for two or more antigens from a T replete grafting source [44, 46].

Use of alternative donors with limited numbers of HLA mismatches may approach the favorable results observed after HLA-identical related HCT [48–51]. Higher risks of acute GVHD are observed with donors mismatched for one or two antigens, compared to phenotypically identical donors [48]. Additive effects of HLA mismatches are measurable in patients transplanted

from unrelated donors with two or more class I mismatches, or from 6/10 (or lower) haploidentical related donors, compared to matched or single antigen mismatched unrelated donors or 7/10, 8/10 or 9/10 HLA-A, B, C, DR, DQ haploidentical related donors [50]. Compared to matched unrelated donors, use of mismatched unrelated or related donors is associated with higher TRM after transplantation [49], and in patients transplanted from partially matched related donors, increased non-relapse mortality has been a limitation [51].

When multiple haploidentical family members are available to serve as donors, additional HLA criteria may be helpful for prioritizing the optimal donor. Donor-specific suppression of T cell responses against the non-inherited maternal HLA antigens provides a basis for the use of NIMA-mismatched haploidentical donors and may be an effective strategy for patients whose primary risk is TRM [52–54]. Lower risks of acute and chronic GVHD and TRM are observed after T-replete bone marrow transplantation between mother and offspring, compared to father and offspring [52]. These data support an immunological basis for the NIMA effect in which *in utero* exposure to NIMA is tolerizing, whereas exposure to IPA is immunizing. The NIMA effects have been observed after conditioning with myeloablative [53, 54] and reduced intensity [55, 56] regimens, in which overall survival at five years post-transplant is significantly higher and TRM lower among recipients of maternal grafts compared to paternal grafts.

5.1.2. Unrelated Donor Transplantation

There are currently over 10.3 million volunteer donors worldwide (National Marrow Donor Program [NMDP], *www.marrow.org*; Bone Marrow Donors worldwide [BMDW], *www.bmdw.leidenuniv.nl*; World Marrow Donor Association [WMDA], *www.worldmarrow.org*). Good-risk patients who do not have an HLA matched sibling to serve as donor may have superior disease-free survival (DFS) with transplantation from well matched unrelated donors [49, 50, 57–62], and the safety of reduced intensity and non-myeloablative regimens has further broadened the application of unrelated HCT to patients who, due to advanced age or medical infirmities, would not otherwise be considered ideal candidates for traditional myeloablative transplantation procedures [63, 64].

The introduction of polymerase chain reaction (PCR) technology for tissue typing has dramatically changed how unrelated donors are selected. Methods define low (equivalent to serologically defined antigen), intermediate and high (unique allele sequence) resolution of the polymorphic exons that encode HLA molecules. A preliminary search of unrelated donors begins with screening of registry donors typed either at low or high resolution for at least HLA-A, B and DR, and a listing of the HLA types of potentially matched donors. From these potential donors, individual donors who most closely meet the criteria for the planned transplantation procedure are selected for the next phase of "confirmatory typing" (CT) of HLA-A, B, C, DRB1 and DQB1 alleles.

Currently, 20 percent to 80 percent of patients who initiate a search ultimately identify a suitable unrelated donor. The likelihood of identifying a matched unrelated donor depends on the patient's genotype, his or her racial background, and the composition and size of the donor registries [8]. If the HLA criteria for donor selection is too stringent, then few patients will find suitable donors. The need to broaden availability of unrelated HCT for patients who lack a matched donor has provided the rationale to define permissible HLA mismatches.

The optimal set of HLA criteria that allows patients the opportunity for cure while avoiding risks associated with disparity is the subject of ongoing research efforts worldwide.

Retrospective analysis of clinical outcome related to donor HLA match status has played an important role in refining criteria for prospective donor selection. DNA-based methods are now considered the gold standard, as they are reliable, cost-effective and can be applied to samples with low cell concentration and with archived DNA. DNA methods are the chief means through which novel or undetected sequence variation is detected [14, 15, 65] and, therefore, are instrumental in evaluating genotypes of patients and donors representing diverse ethnic and racial backgrounds.

Donors and recipients who share the same serologically defined antigen may differ based on the allele variant of the antigen. HLA class I and class II allele disparities are functional [16–20, 63, 66], and, furthermore, the risks of graft failure and GVHD increase with the increasing degree of donor-recipient incompatibility [16–20, 66–68]. Therefore, comprehensive evaluation of each of the five classical loci at the allele level (.ie., 10 alleles in total) is performed to ascertain not only the match status at each locus, but also the total number of disparities (a single mismatch is referred to as a "9/10" match; two mismatches constitute a "8/10" match, etc).

Current understanding is that some HLA mismatches may be better tolerated than others. Active research questions include whether all six HLA-A, B, C, DR, DQ and DP loci are important for donor matching, whether class I and class II mismatches each contribute to graft failure or GVHD, whether allele mismatches are less detrimental than antigen mismatches and how HLA effects interact with nongenetic factors that affect clinical outcome [16–19, 21, 34, 58, 66, 68–73]. Allele mismatches might be less immunogeneic than antigen mismatches [19–20]; however, the differences may be difficult to measure when disease stage is considered [21].

In a recent analysis by the NMDP and CIBMTR, the risks of HLA disparity and the relative importance of recipient and donor factors in clinical outcome were measured retrospectively in a large study population of patients who received T-replete myeloablative transplantation for leukemia and MDS [74]. The average overall survival rate was 7 to 8 percent lower with each additional HLA mismatch, compared to 10/10 allele matching, and 12 to 15 percent lower if the disease progressed from early to intermediate to advanced stage. Among single mismatches, disparity at the HLA-A locus was associated with a statistically significant risk of mortality and lower DFS. HLA-A, B and C were each associated with increased risk of acute GVHD; there were no HLA associations with chronic GVHD.

Several different strategies have been used to identify permissible HLA mismatches. Analyzing specific class I epitopes involved in T cell recognition has provided one avenue for identifying GVHD risk determinants [75]. Functional assays have been employed to identify tolerable HLA-DP [73] and class I mismatches [76]. Since one underlying mechanism for permissiveness may be related to the specific allele and antigen mismatch combinations, which reflect the ethnic and racial background of the donor and recipient, another approach has been to examine allele mismatches within common antigen groups shared between ethnically diverse transplant populations [77, 78]. These data suggest that HLA and other genetic factors may explain differences in outcomes [79].

5.1.2.1. Non-HLA Factors of Importance in Unrelated HCT

Non-HLA factors that impact transplant outcome include disease stage at the time of transplantation (intermediate versus early; late versus early), CMV-seropositive recipient and recipient age older than 20 years [37, 74]. Younger patients tolerate higher degrees of HLA mismatching [50, 59–61]. Patients transplanted from single allele mismatched donors for low risk CML are at an increased risk of post-transplant complications, compared to patients transplanted from fully matched donors for the same stage of their disease. However, the outcome for patients with higher risk disease following transplantation from mismatched donors is similar to that of matched recipients [21]. These data suggest that the stage of disease is a powerful indicator of transplant outcome and that the detrimental effects of HLA disparity may be obscured by the negative effects of disease stage.

5.1.2.2. HLA Disparity and GVL after Unrelated HCT

Most of the published HLA studies have focused on quantifying risks associated with HLA mismatching and graft failure, GVHD and mortality. One potential benefit of HLA disparity is lower disease recurrence arising from graft-versus-leukemia in patients with clinical GVHD [80]. HLA-DP mismatching has a lower risk of disease recurrence, particularly in patients with lymphoid malignancies [72]. GVL effects may also be contributed by non-HLA loci; lower post-transplant relapse has been observed after non-myeloablative unrelated, compared to related donor HCT, and likely reflect the impact of undetected genetic disparity among unrelated individuals who are HLA matched [64].

5.2. KIR Effects

Demonstrating the powerful effect of HLA KIR ligand on outcome after haploidentical mismatched related HCT is a novel approach for selecting alternative donors and the integrating post-transplant immunotherapy into the treatment plan [32, 33, 81, 82]. Originally described in the setting of megadose *ex vivo* T cell depleted transplantation from NK-alloreactive donors [33], durable engraftment, low risk of GVHD, low post-transplant disease recurrence (particularly in patients with AML) and superior survival have been confirmed in several series [32, 83, 84]. The beneficial effects of donor NK-mediated alloreactivity may not be entirely uniform under different conditioning intensities and GVHD preventive measures, and remains an important research question [85–90].

Since HLA and KIR genes segregate independently, the beneficial effects of donor-mediated GVL may be the result of HLA ligand incompatibility and/or recipient lack of ligand when the donor has a complete repertoire of inhibitory KIR receptors. In the latter scenario, population genetic analysis demonstrates that most individuals encode KIR2DL2, 2DL3, 2DL1 and 3DL1 KIR receptors; however, the distribution of HLA-C and B alleles that define the group C1, group C2 and HLA-Bw4 ligands varies substantially. Even when the donor and recipient are HLA matched, the recipient may lack the ligand for which the donor is the receptor [91].

A test of the potential effect of ligand disparity and missing ligand demonstrates a complex interaction between HLA and KIR-driven alloreactivity [89]. Lowered post-transplant relapse was observed in HLA-mismatched recipients homozygous for HLA-Bw6 and group C1 or C2 (missing KIR ligand) after T-replete marrow, or PBSC after myeloablative conditioning. However, the beneficial effect of lowered relapse was not observed among HLA-matched transplants. These data suggest that KIR ligand absence in the recipient may

be a useful pre-transplant indicator for lowered disease recurrence after T-replete myeloablative conditioning, and could provide clinicians with a strategy to plan specific transplant treatment for patients at highest risk of relapse. Since recipients are now routinely assessed pre-transplant using high resolution HLA typing methods, patients may be readily assessed as to whether they lack C1, C2 or Bw4 ligands to assist in assessing risk.

Research is ongoing to assess the importance of donor KIR receptor polymorphism in donor selection and transplant outcome [90, 92, 93]. Future studies on the clinical impact of ligand/receptor genotype with different conditioning and GVHD preventive regimens are needed to adequately assess the role for pre-transplant receptor genotyping in donor selection.

6. Identifiying Novel Transplantation Determinants

6.1. Microsatellites as a Mapping Tool

Although complete and precise donor-recipient HLA matching can optimize the results of unrelated donor HCT, clinical experience demonstrates that GVHD remains a severe, potentially life-threatening complication. Given the extreme genetic diversity of the MHC, new hypotheses about the potential role of undefined non-HLA MHC-resident genetic variation may be developed. Laboratory approaches to characterizing sequence diversity between the classical HLA loci include direct sequencing and other high throughput platforms for the MHC. Microsatellite (Msat) markers have been used for disease mapping. Although indirect, Msat markers provide information over a great genetic distance of the MHC because Msat alleles are in LD with HLA alleles and haplotypes [94–97]. In this way, Msats have been an effective tool for estimating optimal size and composition of donor registries [98, 99], and for donor selection [100].

Mapping novel transplantation determinants may be developed using either donor-recipient Msat disparity or recipient and/or donor Msat genotype as the marker. Two recent studies have explored the MHC region with such an approach [97, 101]. Although no statistically significant associations between donor-recipient Msat disparity and risks of acute or chronic GVHD, graft failure, relapse or survival were observed in one study, Msat identity for the tumor necrosis factor [TNF] locus was associated with lower survival, compared to matching at this locus among patients developing clinical GVHD [97], suggesting the class III region to be of interest. A second analysis of a large international dataset of unrelated donor transplants uncovered an increased risk of death among patients mismatched for class III and class I Msat markers [101]. Taken together, these studies indicate that undetected genetic variation within the MHC may be functional, and in-depth analysis of the class I, II and III region is warranted.

6.2. Single Nucleotide Polymorphisms (SNPs)

Msats provide an indirect measurement of linked variation. Direct examination of sequence diversity can be attained by directly assaying single nucleotide polymorphisms (SNPs) [1, 102–105]. A catalogue of MHC region SNPs is now available from The MHC Haplotype Project (*http://sanger.ac.uk/HGP/Chr6?MHC*). This work showcases the extreme diversity of the HLA region, and the organization of SNPs into haplotypes. Unique HLA alleles are also defined as part and parcel of these SNP haplotypes [1], and multi-block haplotypes

may define antigens and alleles encoded at HLA-A, B, C and DR loci [106, 107]. These data demonstrate that SNP haplotypes could serve as a surrogate marker for certain HLA alleles, and may provide an alternative strategy for mapping HLA-associated SNPs that may have functional significance.

6.3. Haplotype-Based Approaches for Mapping Genes Important in Transplantation

Donor-recipient matching for blocks of conserved regions within the MHC ("haplotype blocks") has been shown to correlate with improved clinical outcome after transplantation [108, 109]. The HLA haplotype may be an informative approach for understanding the significance of MHC variation. A family study to confirm the haplotypes of an unrelated donor is not feasible, and although statistical methods can be used to infer haplotypes [11, 110], their accuracy in predicting an individual's haplotypes may be limited by the frequency of the donor's HLA alleles [111].

The great genetic distance between HLA loci is a technological challenge. Recently, a novel approach for isolating high quality 2 Mb-long genomic DNA fragments specific for only one of the two HLA haplotypes in heterozygous samples has been developed [112]. The phasing method has been applied to test the hypothesis that haplotype mismatching may occur among HLA identical unrelated donor-recipient pairs, and that haplotype mismatching is a marker for higher risk of GVHD [113]. These data suggest that long-range phasing methods may provide a strategy for mapping MHC-resident variation important in transplantation.

7. Summary and Conclusions

HLA and NK participate in the transplantation barrier. Optimizing transplant outcome includes donor matching for the highly polymorphic HLA loci. When HLA disparity cannot be avoided, judiciously selecting a donor with the fewest HLA mismatches and avoiding certain loci may provide patients with the opportunity for life-saving transplantation. Disease stage remains a strong predictor of overall transplant outcome, and expediency in timing of transplantation for patients with high risk disease is paramount. Haploidentical mismatched related donors and cord blood units provide attractive alternatives for patients whose disease status cannot wait for a prolonged donor search. New research avenues include identifying novel MHC resident genetic variation that may contribute to risks of GVHD and TRM.

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Chapter 3

Immunobiology of Transplantation

Robert S. Negrin

1. Introduction

The immune system is a highly complex and integrated array of cells and molecules capable of providing protection from a hostile environment where pathogens abound. Immune function is critical in the setting of both autologous and allogeneic hematopoietic cell transplantation (HCT). The major cellular populations and mechanisms of action pertinent to the setting of HCT will be discussed. Due to the enormity of the topic great detail cannot be provided, but is available from a number of other sources.

2. Functions of the Immune System

The immune system of vertebrate animals (including humans) is comprised of a complex and overlapping array of cells, secreted proteins and cytokines which allow for survival in a hostile environment. Pathogens are omnipresent as evidenced by the appearance of mold or *aspergillos species*, a deadly pathogen in patients with immune compromise, after just a few days on unpreserved foods. Potentially pathogenic bacteria can be found in the oral cavity, gastrointestinal and vaginal tracts and on the skin. A major role of the immune system is providing protection from infectious microorganisms including viruses, bacteria and fungi. This is especially important at times of tissue injury such as that which occurs following preparation for HCT or with indwelling catheters. In addition, immune-based cells and molecules also participate in wound healing and inflammation. More speculative, yet highly convincing, in the transplant setting is the immune system's role in performing immune surveillance against malignant cells, which is the major mechanism of benefit in HCT [1, 2]. In fact, the impact of immune-based mechanisms compared to relapse rates following identical twin to histocompatible yet not identical sibling transplants is major evidence for such a role of immune function [3]. Immune-based mechanisms are also critically important in the control of organ tolerance and rejection. These diverse functions are maintained in a delicate balance by the coordinated activity of diverse populations of cells that provide rapid, sustained immune function as well as immunological memory. The effector arms of immune function can be divided into humoral and cellular immunity. Cellular

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immunity can be further divided into the broad categories of innate and adaptive immunity.

3. Cellular Basis of Immunity

The diverse functions of the immune system are performed by an array of cells with specific yet overlapping functions. Effective immunity provides both rapid and sustained responses. The regulation of this complex system is of considerable elegance and the subject of ongoing research. All of the cells of the immune system are derived from a common stem cell population termed the hematopoietic stem cell (HSC) [4]. This bone marrow-derived cell is initially found in the yolk sac during embryogeneis with subsequent migration to the fetal liver and finally to the bone marrow. HSCs are capable of two major functions, namely self-replication and multi-lineage differentiation. A stem cell is a cell capable of rescuing lethally irradiated animals which can be re-isolated and rescue a second round of lethally irradiated recipients. This definition has allowed for the isolation of murine HSCs to great purity by cell sorting technology [5, 6]. This assay is obviously not suitable for characterization of human HSCs. Therefore, surrogate assays have been developed for the isolation and characterization of putative human HSCs [6]. Murine HSCs with these properties have been characterized by the expression of the cell surface markers such as stem cell antigen-1 (Sca-1), thy-1, c-kit and the lack of expression of markers typically found on mature myeloid, erythroid and lymphoid cells. In addition, HSCs pump out vital dyes such as rhodamine and are, therefore, rhodamine^{dull}.

4. Stem Cell Model of Hematopoiesis

The basic structure of the hematopoietic system has revealed that all hematolymphoid cells are derived from a common HSC population which has important implications for HCT. HSCs are derived from even more primitive stem cell pools leading back to embryonal stem cells [7]. HSCs are present within the bone marrow at a frequency of approximately 1:10,000 cells [5]. Although the phenotype of HSCs is distinct, morphologically these cells appear similar to small lymphocytes. An important feature of HSCs is that, under certain conditions, they can be mobilized into the circulation and be collected by apheresis [8]. This characteristic of HSCs has considerable practical implications for transplantation procedures since many more HSCs can be collected by apheresis than can be collected during bone marrow harvesting. The reasons why HSCs circulate are not known with certainty, but may be important in tissue repair. A central feature of the stem cell model of hematopoiesis is that all of the blood-derived cells are generated from a common stem cell pool. The stem cells undergo differentiation into progenitor cells, many of which have been isolated. These progenitor cells may have important utility in the transplant setting [9, 10]. Progenitors finally differentiate into mature cells with specific and critical functions. These differentiated cells are released from the bone marrow microenvironment and circulate in the blood where they perform functions often in tissues, lymph nodes or at sites of inflammation or infection. These end-stage cells eventually die after highly variable lengths of time.

Long-lived B and T cell populations are also derived from these common stem cell pools and may persist for many years – even decades – providing critically important immunological memory. Therefore, following a successful HCT the recipient gains the blood type, immune function and allergies of the donor.

5. Organization of the Immune System

The critical functions of the immune system are accomplished through the complex interaction of a number of different cellular components located in different organs of the body. As described above, all of the diverse cells of the immune system are derived from hematopoietic stem cell precursors (Fig. 3-1). Different progeny cell populations migrate to key organs where they undergo activation and, in some instances, selection. Major organs of immune function, in addition to the BM, include the thymus where T cell education occurs, lymph nodes where specific clones of B and T cells are activated and expanded and the spleen where T cell expansion and removal of senescent cells occurs. It is beyond the scope of this chapter to describe the functions of each of these organs in detail, but this may be found in many other sources. The immune system can be broadly divided into two critical and overlapping categories: innate and adaptive immunity.

6. Innate Immunity

Innate immunity is the ability of an array of effector cells to recognize and destroy pathogens without prior exposure. It is likely that during evolution innate immunity developed first and is considered more primitive. A diverse array of cells such as neutrophils, macrophages, eosinophils, basophils and natural killer cells are capable of recognizing a broad array of potential targets

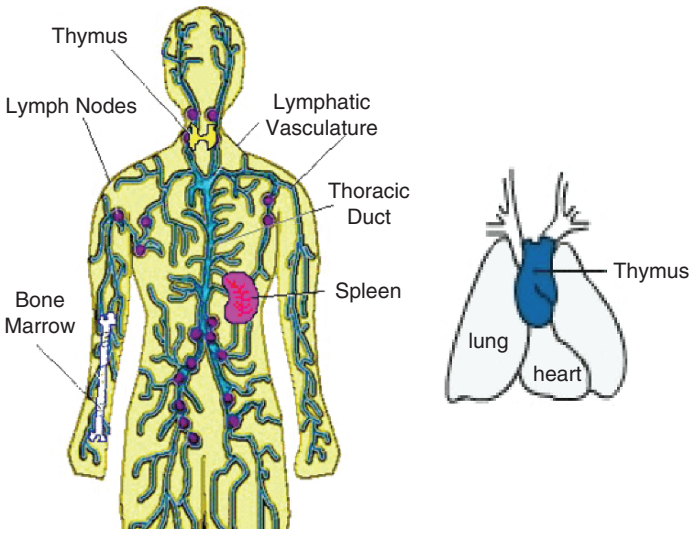

Fig. 3-1. Organization of the immune system

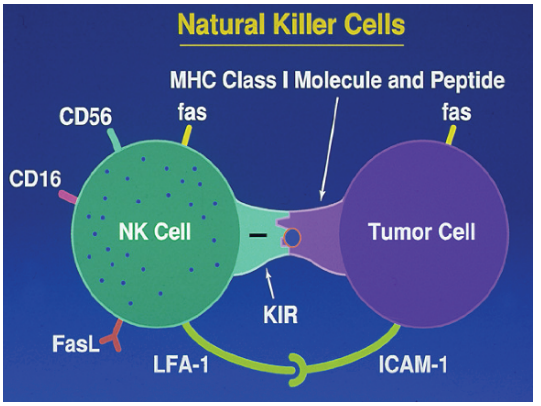

Fig. 3-2. Regulation of function of natural killer cells

including bacteria, fungi, parasites, virally infected cells and likely malignant cells without prior education or priming. These cells provide key first lines of defense and their absence is associated with severe risk of infection. Like all immunological cells these diverse cellular populations are derived from HSCs within the BM and circulate through the bloodstream and lymph system as sentinels ready to respond at a moment's notice. The recognition mechanisms are diverse and vary with each cell type; however, all of these cells contain toxic molecules stored in cytotoxic granules capable of killing their targets such as peforin and granzymes [11]. For example, neutrophils produce toxic molecules such as hydrogen peroxide and oxygen radicals, as well as other molecules which engulf invading bacteria or other pathogens and destroy the invaders in specialized organelles. Protecting the cell from these toxic metabolites and substances is an area of true biological elegance. NK cells have specific cell surface receptors capable of recognizing HLA-class I molecules. Engaging these receptors typically results in inhibition which prevents lysis of normal host cells [12]. The downregulation of class I expression following viral infection or tumor transformation relieves the inhibition of NK cells, resulting in cell lysis [13]. In the mouse these receptors are of the Ly-49 family, and in the human a functionally similar yet structurally distinct family of killer inhibitory receptors (KIRs) performs these functions [14]. This inhibition-based mechanism is summarized in Fig. 3-2. Under certain conditions, such as following haplotype transplantation across MHC class I differences where the appropriate inhibitory receptor is not engaged, raises the possibility of NK cell alloreactivity which is a potent mechanism of antileukemic activity, especially towards myeloid leukemias [15].

More recently, another receptor ligand system has been described which activates upon engagement. In this system expression of NKG2D on NK cells and some T cells react with ligands termed MICA, MICB and other cell surface receptors such as ULB1, 2 and 3 resulting in an active killing mechanism upon engagement [16, 17]. Upregulation of NKG2D ligands occurs following viral infection or tumor transformation in "stressed" cells [18]. The physiological roles of these molecules, outside of their recognition by NKG2D resulting in cell recognition and destruction, are not known. The relative balance of activation and inhibition appears to determine whether an NK cell kills

a given target or not. Interestingly, for reasons that are not yet apparent, NK cells do not appear to cause Graft-versus-Host-Disease (GVHD) following allogeneic HCT [19]. Such conclusions have been reached not only following the adoptive transfer of murine, but also human NK cells [20]. The reason(s) underlying this observation are not yet known; however, in some systems it has been due to the destruction of recipient antigen presenting cells (APCs) responsible for the induction of GVHD in this setting [21].

NK cells also produce an array of cytokines which can recruit other immune-based cells to the site of inflammation, infection or possible tumor growth. These cytokines include interferon-γ, tumor necrosis factor and granulocyte-macrophage colony stimulating factor to name a few. There is now good evidence that the innate and adaptive immune systems are integrally related with cross talk through cytokine production and cell surface receptor engagement. Such cross talk has implicated NK cells as initiators of an immune response where NK destroyed cells are then taken up by resident APCs, and appropriate antigens are presented for adaptive immune cell recognition [22]. NK cells also express receptors for the F_c portion of immunoglobulins and can destroy cells through engagement, a process termed antibody dependent cellular cytotoxicity (ADCC). This mechanism may be extremely important following treatment with monoclonal antibody based immunotherapy. Such an example follows the use of the anti-CD20 MAb rituximab@ where differences in the F_c receptor predicted for outcome following such therapy [23].

Other key members of the innate immune system are monocytes and macrophages which circulate in the blood or are found in tissues, respectively. These cells are capable of antigen presentation upon certain stimulation, both in the body and in the laboratory. Macrophages are found in many organs including the lymph nodes, spleen, GI tract, liver, lungs and brain. Certain infections are killed primarily by macrophages which engulf the offending organism and destroy it. Macrophages also have F_c receptors, produce cytokines and interact with effector cells such as NK and T cells. Cells of the innate system of immunity are critically important to protecting the host from infection, yet they lack precise clonal specificity and memory which are key components of the adaptive immune system. In contrast to adaptive immune function, innate immune function appears to be genetically fixed such that the repertoire of NK cell receptors is maintained and consistent [24, 25].

7. Adaptive Immunity

The evolution of adaptive immunity is one of the truly extraordinary events in biological development. Through this process highly specific cellular components that are capable of massive clonal expansion and persistence (i.e., memory) develop. The mechanisms employed in developing the specificity for such a broad array of potential targets, yet ensuring removal of harmful clones capable of recognizing self, displays biological elegance at its finest. The hallmark of the adaptive immune system is the development of genetic diversity followed by selection of specific subsets of cells which have the desired specificities and reactivities, and deletion or inactivation, of potentially harmful clones. These mechanisms are enormously complex and beyond the scope of this chapter; therefore, this system will be described in relatively simplistic fashion. However, these concepts are important to understand

since they presumably are recapitulated following a successful HCT. Those interested in more detail can find this in many sources including textbooks of immunology.

Key components of the adaptive immune system include B and T cells. B cells ultimately produce antibodies which are molecules capable of recognizing a specific target structure through their variable binding domains and attracting other cell populations to remove the offending protein or cell through their F_c binding domains. Antibodies are produced by B cells when they mature to plasma cells and these generate antibodies with different and unique determinants. Each clonal plasma cell produces a different antibody which is then released into the circulation. The lack of, or diminished, production of antibodies occurs in diseases of B cells, such as chronic lymphocytic leukemia and multiple myeloma, which leaves these patients at significantly increased risk of infection.

T cells function similarly by recognizing major histocompatibility molecules (MHC) on the surface of antigen presenting cells (APCs) through the T cell receptor (TCR). The cell population most efficient at antigen presentation are termed antigen presenting cells (APCs) or dendritic cells (DCs) [26]. Several different types of DCs have been identified based upon morphology, location and cell surface protein expression [26]. DCs have all of the appropriate machinery to initiate an effective immune response which will be described below. Their ability to initiate an immune response has sparked enormous interest in DCs for vaccination and T cell activation purposes [27]. DCs express MHC molecules which are genetically defined and are the major genes which must be matched to limit the risk of GVHD. Due to the outbreeding characteristics of human populations there is enormous diversity in MHC molecules. MHC molecules are subdivided into class I (HLA A, B or C) or class II (HLA D) subgroups. Both classes of HLA molecules present peptides which are short protein fragments. The precise sequence that is effectively bound to HLA class I and II molecules can be defined such that once a protein has been found to be immunogenic, knowing the amino acid sequence allows for the identification of putative peptides that could be recognized which are then further tested in functional assays. Class I MHC molecules are complex with β-2 microglobulin and generally present self or intracellular peptides to CD8 cells [28]. Such peptides are thought to form minor histocompatibility antigens (mHag) which may play a critical role in GVHD and graftversus-tumor (GVT) responses [29, 30]. The identification of mHag is an area of intense research and, thus far, such peptides are derived from housekeeping proteins and appear to be quite complex. Class II MHC molecules generally present peptides derived from extracellular proteins which are engulfed by the APCs, processed and presented to CD4+ T cells [28]. Responding cells recognize MHC determinants through the T cell receptors (TCR) which are clonally expressed and made up of α and β polypeptide chains. The specificity of T cells is generated by the diverse array of different TCRs, each of which is clonally expressed and specifically recognizes a unique MHC-peptide complex. Another population of T cells produces γ and δ TCRs and much less is known about this population of cells due to their relative paucity [31].

This brief description raises three major questions which have important implications in the HCT setting: 1) how is the specificity generated, 2) how is the repertoire shaped, and 3) what is being recognized. The generation of

specificity is a remarkable example of biological efficiency. The nearly limitless array of potential targets makes it nearly impossible for a unique gene to encode every antibody or TCR as this would require too much genetic material. To overcome this limitation an alternative approach has developed over the course of evolution. In this system diversity is generated through the combination of different genetic segments termed common (C), variable (V) and joining (J) regions. These short regions of genetic material are joined together in different ways through gene splicing, ultimately resulting in the translation of different α and β chains which then come together to form the TCR. A similar mechanism is utilized to produce antibodies; the formed antibody is made of heavy and light chains which come together to form the complete antibody molecule which is then secreted as a soluble protein. This strategy of generating diversity through the use of small genetic components uses modest amounts of genetic material to develop enormous diversity. In fact approximately 10 percent of the genome in humans is devoted to immune function. However, this strategy also presents a problem since diversity is generated almost at random by combining different genetic building blocks, and only a fraction of these recombinants will actually be useful and effective. In addition, a number of the recombinants could also be harmful in that they may bind to key elements of the individual and be autoreactive. Therefore, a system of selection is also required to shape the repertoire into functional and useful cellular populations.

Shaping the repertoire is a critical function that is thought to take place within the specialized thymus organ. In the thymus T cells become educated through a process of positive and negative selection. The diverse array of potential T cell reactivities are based upon the random association of TCRs which are selected for those TCR determinants that are effective, have a certain binding affinity yet do not bind too tightly and, therefore, may be autoreactive. Occasionally this process is ineffective and autoreactive T cells develop, the presumed genesis of autoimmune diseases. Peripheral mechanisms are also thought to play a major role in preventing autoimmune reactive clones from resulting in disease (see below). The shaping of the T cell repertoire occurs early in life within the specialized structure of the thymus. The exact nature of positive and negative T cell selection is still an area of ongoing research; however, the efficiency of binding T cells through the TCR is of critical importance such that TCR with the appropriate binding efficiency are allowed to persist, whereas other cells are deleted or anergized. The concept of immunological anergy occurs when cells are inactivated and undergo an active process of quiescence termed anergy. This important concept implies that these T cells are still present, but are incapable of signaling through their respective TCRs. Why such a system is advantageous is not clear; however, this allows for the potential recruitment of anergized T cells at a later date. What biochemical and genetic signals define anergy is an area of active investigation.

T cell selection is a rigorous process where approximately 1 percent or less of the generated T cells are actually selected within the thymus as being functional (Fig. 3-3). Therefore, the requirement for specificity and diversity involves a fair amount of biological waste. As described above, most of the shaping of T cell diversity occurs relatively early in life after which the thymus involutes and loses much of its function. However, the T cell repertoire can still be shaped later in life as evidenced by the development of T cell diversity

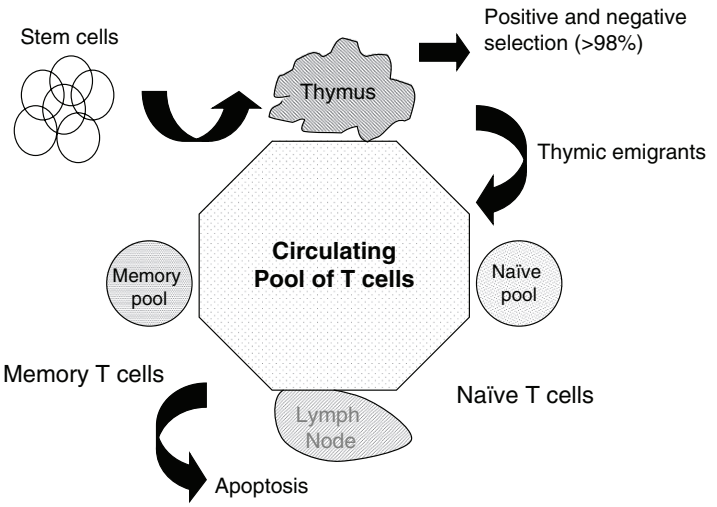

Fig. 3-3. Generation of diversity and T cell selection of the adaptive immune response

following allogeneic HCT in the adult. However, this process appears to take much longer later in life resulting in profound immunodeficiency for six to 12 months following HCT. GVHD can further damage the thymus, causing even more prolonged immunodeficiency and decreased T cell production in such patients [32, 33]. Therefore, understanding the function and mechanisms employed by the thymus, as well as strategies that may improve thymic function later in life, is of critical biological and medical importance since, if one could speed up the process of immunological reconstitution following transplantation, immunodeficiency and associated infections could be significantly reduced. Both keratinocyte growth factor (KGF) and interleukin-7 appear to play a role and investigations using these molecules are under active evaluation in the post-transplant setting [34].

The above sections briefly describe the generation and shaping of the T cell repertoire, yet what do these T cells recognize? The understanding of T cell recognition has developed from a wide array of studies from many investigators. A schematic picture of T cell recognition events is shown in Fig. 3-4. As described above the T cell recognizes antigens through the TCR which is made up of two chains, the α and β polypeptides. The TCR molecules define the specificity of the reaction and recognize MHC molecules on the surface of antigen presenting cells (APCs). The most important and biologically active APCs are DCs which are of major interest for their critical role in initiating an immunological event. APCs are the most effective cells in the body at presenting antigens and, as expected, are found primarily in the lymphoid organs and at sites of entry to the body such as the skin, gut, lungs and liver which may in part be why these organs are major sites of GVHD. A number of different APCs have been described and termed dendritic cells due to their characteristic morphology under the microscope. In the skin APCs are termed Langerhan's cells or dermic dendritic cells depending upon their location with other, at times confusing, terminology in other tissue sites. As discussed above, DCs present antigen in the form of peptides which are eight to 12 amino acid long stretches of particular proteins [35]. These peptides must have particular sequences at

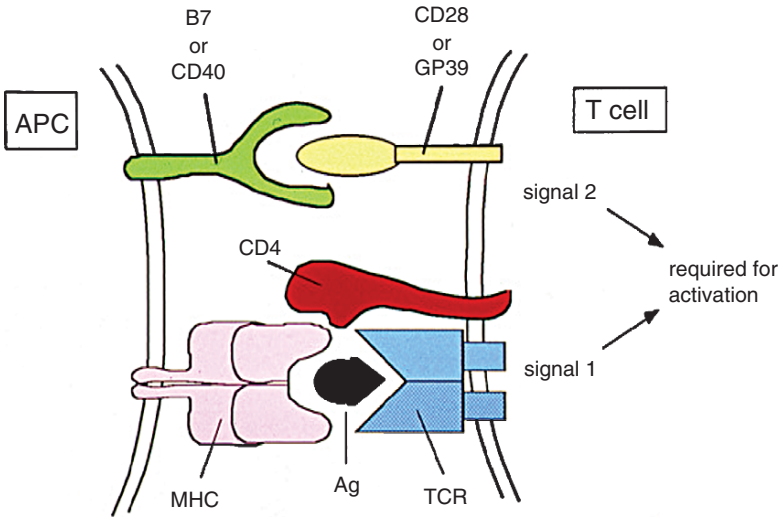

Fig. 3-4. Functional interactions required for a productive T cell interaction

key locations such that they fit into a broad groove on the MHC molecule and are presented to the T cell. Identifying different normal and pathological peptides is an area of intense investigation since loading these peptides onto DCs could direct the immune response to a particular target. DCs have been demonstrated to play a central role in GVHD pathogenesis [36].

In addition to TCR recognition of MHC molecules displaying specific peptides another interaction is critically important for productive T cell activation and expansion. This additional signal is provided by cell surface receptors called costimulatory molecules. The best characterized costimulatory molecule is the CD28/B7 pathway where the CD28 molecule is expressed on T cells and the B7 molecule on APCs. However, more recently a number of other costimulatory receptor ligand interactions have been described. This interaction is critical to the effective activation of T cells and results in clonal expansion of that specific cell population. In the absence of costimulation, but in the presence of specific T cell interactions with MHC molecules, anergy results [37]. Therefore, in the absence of costimulation, not only does an effective T cell response not occur, but those T cells are actually shut down. This observation has helped explain why there may not be a more effective immunological response to tumors since many tumor cells express MHC molecules, but lack expression of costimulatory molecules. Therefore, the resultant outcome is anergy which has been observed in a number of different tumor model systems and patients. These concepts have been translated to the clinic with some evidence of clinical efficacy in controlling graft-versus-Host disease [38]. Further refinements of this approach are in progress and are a rich area of future investigation [39].

CD4 and CD8 T cells have diverse, yet at times overlapping, functions. $CD4^+$ T cells mainly initiate an immunological reaction and amplify the response. $CD4^+$ T cells perform this function through the production of cytokines such as interleukin-2 which is a major growth factor for T cell expansion. Immunosuppressive drugs commonly used in HCT, such as cyclosporine A, have a major impact on the production of cytokines such as IL-2. In addition

$CD4^+$ T cells activate DCs through a molecule called CD40. Activated DCs are much more effective at antigen presentation to $CD4^+$ T cells [40]. The CD4 and CD8 proteins are also part of the recognition event and provide important structural and signaling capabilities. Following antigen presentation and activation $CD8^+$ T cells with the appropriate specificity are activated, resulting in clonal expansion. $CD8^+$ T cells have cytolytic function as described below and are the major effector cells of the immune response. It is apparent that, in some situations, $CD8^+$ T cells can be activated by DCs directly and $CD4^+$ T cells can have cytolytic function depending upon the antigen.

DCs are also activated through another series of cell surface molecules termed toll-like receptors (TLRs) which recognize molecules such as double stranded DNA, lipopolysaccharide and other molecules [41]. These signals can be activated with specific small molecules such as CpG motifs which are being investigated for important clinical functions. DCs which are activated upregulate MHC and costimulatory molecules such that they are much more effective at stimulating an immune response.

If an effective and productive T cell response occurs signals are generated through the TCR resulting in the expansion of that clone of T cells. These events occur within follicles of lymph nodes and the spleen termed germinal centers. The resulting T cells are released from these organs and circulate in the blood to perform their functions of surveillance and cytolysis. These observations, although complex, help explain the major features of the adaptive immune response, including specificity and immunological memory, which are critically important in health and have major relevance to success following HCT. Once a particular clone of T cells has been generated upon subsequent exposure to the same antigen a much more rapid and robust response occurs which is the basis of immunological memory and vaccination.

8. Cytolytic Mechanisms

Once an NK or T cell is engaged both effector cells utilize a similar array of killing mechanisms. These mechanisms include engagement of both cell surface death receptors and the exocytosis of soluble proteins which induce lysis of the target cell. The death receptors include the cell surface expression of a molecule called fas-ligand (FasL) which, upon engagement of the fas receptor, activates programmed cell death (apoptosis). The molecule TRAIL, a member of the tumor necrosis family (TNF), is also capable of directly inducing cell death by activating apoptosis of the target cells. A distinct mechanism of cell lysis involves the exocytosis of molecules called perforin and granzymes which are stored within cytolytic granules of the effector T or NK cells. Upon engagement these cytolytic granules are released within the extracellular space between the killer cell and its target. There perforin induces pores in the target cell membrane which, alone, can result in osmotic lysis and also allows for the introduction of granzymes which activate the apoptotic cascade. Animals rendered genetically deficient in these important effector molecules (knockout animals) have markedly reduced function of both NK and T cells.

Another mechanism of cell death and recruitment of other effector cells to the site of inflammation include the release of cytokines like interferons, tumor necrosis factor and transforming growth factors such as TGF-β. In this

fashion effector cells such as NK and T cells not only directly result in lysis of targets, but also induce the influx and activation of other cytolytic cells inducing a cascade of effector function.

9. Humoral Immunity

An alternative and equally important arm of the immune system utilizes soluble proteins termed antibodies which can recognize infectious agents and target cells. Binding of the antibodies clears the bound molecule through the reticuloendothelial system of the liver, spleen and lymph nodes through F_c receptor engagement. Antibodies are produced by terminally differentiated B cells termed plasma cells, each of which produces a single antibody of defined specificity. While generating antibody specificity is complex and beyond the scope of this chapter, the mechanisms employed are similar to that described for the generation of the T cell repertoire in that defined variable, joining and constant regions are spliced together to produce an antibody molecule. B cell clones which produce useful and non-autoreactive antibodies are further selected. In addition, rearranged genes capable of generating antibodies go through a process of somatic mutation and change resulting in similar antibodies differing in target affinity. The antibody molecule is similar to a T cell receptor in structure except that antibodies are secreted as a soluble protein by the plasma cell which produced it. Each antibody is made up of a heavy and light chain which has variable (V) regions which are unique to that given antibody at one end of the molecule and define reactivity. The antibodies have hinge and constant regions which help define the shape and class of the antibody molecule (e.g., IgA, IgG or IgM). The other end of the antibody molecule is the F_c region which binds to receptors on macrophages and other cells of the RES system and clears the antibody-bound molecule. The remarkable specificity of antibody reactions has been utilized to produce monoclonal antibodies which are tremendously useful diagnostic reagents and therapeutic drugs for the treatment of a variety of conditions including cancer and infectious diseases. Cell processing and hematology laboratories routinely utilize monoclonal antibodies with defined specificity for a wide array of assays including enumeration of stem cells numbers (CD34 analysis), evaluation of T cell content (CD4 and CD 8 analysis) and characterization of malignant disease (immunophenotyping), to name a few. Examples of clinically useful monoclonal antibodies include anti-CD20 (Rituximab™) for the treatment of B cell Non-Hodgkin's Lymphomas and anti-Her-2/neu (Herceptin™) for the treatment of some forms of breast cancer [42].

More recently the role of B cell responses in allogeneic recognition such as GVHD and GVT responses have been observed [43]. These observations have led to the concept of treating chronic GVHD with anti-CD20 monoclonal antibody therapy. Initial results appear promising [44].

10. Regulation of the Immune Response

A central question in immunology is how these various arms of the immune system are regulated to create an effective yet limited immune response capable of protecting the host from infection and possibly tumor growth while avoiding an excessive immune reaction or autoimmunity [45]. The control of the

immune system is complex and incompletely understood, but, in part, related to cell selection and expansion mechanisms described above for shaping of the B and T cell repertoires. Both pro-inflammatory and anti-inflammatory cytokines have been described which induce or dampen an immune response. Clearly, tissue compartments and blood flow are important to contain the immune response to the site of inflammation. In addition, recent discoveries of regulatory T cell populations are likely to be very important in this process. Two relatively well characterized regulatory T cell populations include so called NK-T cells and $CD4^+CD25^+$ T cells. NK-T cells are characterized by a restricted usage of αβ TCR receptors which interact with an MHC-like molecule, CD1 [46, 47]. Upon engagement these T cells produce high levels of IL-4 which help shape and regulate the immune response. NK-T cells are found throughout the immune system, but mainly reside in the bone marrow [48]. In murine models a preparative regimen including total lymphoid irradiation and anti-thymocyte globulin has resulted in reduced GVHD risk by altering the relative balance between conventional and regulatory NK-T cells [49]. Such a strategy has been translated to the clinic yielding early encouraging results and a very low incidence of acute GVHD [50].

$CD4^+CD25^+$ regulatory T (T_{reg}) cells are a recently described population of T cells capable of actively suppressing immune reactions [51]. These regulatory T cells, either isolated fresh or expanded *ex vivo,* have been shown to suppress autoimmune reactions including graft-versus-host reactions [52–54]. This population of T_{reg} express an unique transcription factor FoxP3 which defines this population of cells [55]. Interestingly, inducing expression of the FoxP3 gene in conventional T cells gives s these cells regulatory properties [56]. Adjusting the ratio of conventional and regulatory T cells has resulted in the control of GVHD with preservation of GVT responses [57, 58]. Several studies have also demonstrated that T_{reg} likely plays an important role in human GVHD [59, 40]. Definitive adoptive transfer studies are only beginning to be developed. The role and clinical use of regulatory T cells in specific clinical situations is an area of active and exciting research.

11. Evasion of the Immune Response

Despite the elegant mechanisms of target cell recognition and removal, some harmful pathogens and especially tumors are capable of evading immune recognition [61]. Certain areas of the body are immunologically privileged sites, including the central nervous system, anterior chamber of the eye and testes. In some instances the offending agent may not express unique antigens capable of being recognized, which was previously thought to be a major reason why tumors may remain immunologically silent. However, more recent studies have clearly shown that a number of viral infections and tumors result in downregulation of MHC molecule expression, thereby evading T cell reactivity. This may result in NK cell mediated attack; however, the lack of an adaptive immune response may be inadequate for effective clearance of the offending infectious agent or tumor. Alternatively, tumor cells may express unique antigens yet lack expression of appropriate costimulatory molecules such that the tumor may actually induce anergy to that particular target and inactivate the adaptive arm of the immune system. Efforts in understanding the anergized state and developing

strategies to rescue anergized T cells are in their infancy. Further mechanisms of escape include the observation that many tumor cells express FasL, which may inactivate effector cells by inducing apoptosis through binding of fas on the attacking T cells [62, 63]. Yet other observations have demonstrated that certain tumors express cytokines which inhibit the immune response such as TGF-β and other soluble molecules. Tumor cells that are resistant to the cytolytic mechanisms of T cells such as granzymes, perforin and cytokines have also been described. Understanding the various mechanisms of immune escape enable us to construct strategies to overcome resistance and demonstrate the dynamic interplay inherent in immune mediated events [64].

12. Conclusions

In this chapter it is hoped that the basic concepts of immune function can be better understood. These concepts are central to HCT and a more profound understanding of immunological mechanisms is at the core of improving outcomes following transplantation. Clearly, a system of such complexity and elegance cannot be adequately described in such a short chapter and many simplifications and omissions were required. Ongoing research in immune function and regulation is at the forefront of experimental biology and insights gained are of critical biological and clinical importance. The relevance to these concepts in the field of HCT is clear and advances in our understanding of how to enhance certain immune reactions while controlling others offers the opportunity for significant improvements in patient outcome.

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Chapter 4

Allogeneic and Autologous Hematopoietic Stem Cell Transplantation for Acute Lymphoblastic Leukemia and Acute Myelogenous Leukemia in the Adult

Anthony Stein and Stephen J. Forman

1. Allogeneic Transplantation for Acute Lymphoblastic Leukemia

1.1. Introduction

Acute lymphoblastic leukemia (ALL) is characterized by clonal proliferation, accumulation and tissue infiltration of immature lymphoid cells of the bone marrow. Although ALL accounts for approximately 80 percent of childhood leukemias in the United States, a second peak occurs around age 50, and the number of incidences increase as the population ages. Age greater than 60 years, leukocyte count greater than 30,000, non T cell phenotype, lack of mediastinal adenopathy, poor performance status at diagnosis, Philadelphia chromosome (Ph)+ at cytogenetic analysis, as well as the finding of other chromosomal translocations such as t(4;11), t(1;19) or t(8;14) all predict a poor long-term outcome, even with aggressive chemotherapy. Those patients requiring more than four weeks of induction therapy to achieve remission or who have detectable molecular or immunophenotypic evidence of disease while in remission also have a poorer prognosis [1–4].

1.2. Prognostic Features in the Treatment of ALL in the Adult

Cytogenetic abnormalities found in patients with ALL are important predictors of treatment outcome. In many instances, results of cytogenetic studies can help to direct treatment, highlighting where more aggressive treatment, such as allogeneic transplantation, should be considered. Chromosomal

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Table 4-1. Frequency of Ph+ ALL increases with age.

Age (years)	Ph+ Incidence
15–20	10%
21–30	19%
31–50	24%
>50	44%

(Secker-Walker, et al. **Leukemia**, 1991)

changes are found in 60 to 85 percent of all cases of ALL [5]. Numerical chromosome abnormalities, either alone or in association with structural changes, are found in about half of ALL cases. Although more than 30 distinct nonrandomly occurring rearrangements are presently known in ALL, a few particular cytogenetic anomalies are significantly more common than others and determine the prognosis for the patient. The Third International Workshop on Chromosomes in Leukemia (TIWCL) identified several significant differences between groups of patients, based on results of cytogenetic studies. Translocations t(8;14), t(4;11) and 14q+ correlate with a higher risk of CNS involvement, while t(4;11) and t(9;22) were associated with a higher leukocyte and blast count and risk for relapse.

The most common cytogenetic abnormality in adult ALL is the Philadelphia (Ph) chromosome. Occurring most commonly in chronic myelogenous leukemia (CML), the Ph chromosome brings into juxtaposition the tyrosine kinase *c-abl* on chromosome 9 with the major breakpoint cluster region (m-bcr) on chromosome 22. The Ph+ chromosome appears in about 95 percent of patients with CML, in about 1 to 2 percent of patients with AML, as well as in up to 5 percent of children and 15 to 30 percent of adults with ALL [7], and increases with age (Table 4-1). In contrast to CML, in which patients with the bcr-abl hybrid protein almost always measures 210 kd (the p210 protein), about half of patients with ALL and the Ph+ chromosome have a 190 Kd protein (p195). The development of bcr-abl-specific tyrosine kinase inhibitors has changed the treatment of ALL to include these drugs in the initial induction treatment leading to an improved remission success and potentially improved disease free survival [6–8].

As with any other hematologic malignancy, deciding whether and when to proceed to allogeneic transplant is often dictated by prognostic features identified at diagnosis. Initial treatment of adult patients with ALL has evolved over the past few decades, with an increase in the intensity of treatment and with the addition of consolidation and maintenance arms of treatment. Overall CR rates have risen to as high as 80 to 90 percent of those patients under the age of 60 [9–14]. However, the higher dose regimens do select for disease that is more chemotherapy-resistant when relapses do occur. Second remissions occur with lower frequency than in previous years, and when achieved, tend to be shorter lasting. New treatment protocols incorporating monoclonal antibodies (anti-CD22, anti-CD20, anti-CD52) into the early treatment of patients with ALL are being tested for their impact on improving disease free survival [15].

Currently, the overall disease free survival for adult patients with ALL is 35 percent with those patients with T cell ALL having the better treatment outcomes compared to all other subtypes of ALL in adults [9–15]. Some recent studies suggest that, for young adult patients, a chemotherapy treatment program used for high risk pediatric patients may improve prognosis for this subgroup of patients with adult ALL [16].

1.3. Role of Minimal Residual Disease in ALL

In addition to age and cytogenetic analysis at the time of diagnosis, the most important prognostic factor, and a direct reflection of sensitivity to chemotherapy, is achieving a complete remission. Thus, a slower time to achieve remission is an indicator of relative chemoresistance, similar to what has been observed in pediatric patients. Those patients who take more than one cycle of induction chemotherapy have a poor long-term prognosis and a shorter remission duration [10, 11].

A more quantitative approach to assess the response of an individual to chemotherapy is the measurement of minimal residual disease at various timepoints after therapy. This is emerging as an independent prognostic factor that reflects the resistance of the cells to chemotherapy and allows potential individualized treatment [17, 18]. The assessment identifies potential patients at high risk for relapse despite achieving a morphologic remission, and who may benefit from early transplantation. Studies are being performed to determine the most predictive timepoint for its measurement. It appears that, after consolidation, a high level of MRD at 10^{-4} is associated with a high risk of relapse, with a rising level of MRD on treatment also portending relapse [17, 18]. In some studies now a high level of MRD after induction and consolidation have been identified as high risk features, despite the achievement of a morphologic remission and the absence of high risk cytogenetics [17–19. Conversely, identifying patients who are sensitive to chemotherapy and achieve a low level of MRD (non-detectable) may identify a group of patients who do not need transplantation or can wait until there is clear evidence of relapse [17–20]. In addition, the benefit of transplant in patients who are in first remission, but have evidence of a new factor defining high risk disease, i.e., high MRD, must also be determined. At the present time, the measurement has certain limitations related to the technical procedure which is time consuming, expensive and requires a specialized laboratory to conduct the studies. The testing also involves multiple evaluations with either immunophenotypic flow cytometry analysis or molecular analysis with patient-specific probes for gene rearrangements. Thus, the future of treating adult ALL patients in first remission may be refined to determine those patients who are unlikely to benefit from further chemotherapy and should be considered for transplantation during first remission.

1.4. Allogeneic Transplantation in First Complete Remission (CR1)

Allogeneic transplantation in first CR is generally reserved for those patients who present with poor risk features, such as those described earlier. In several Phase II studies, patients with high risk disease treated with allogeneic transplantation had a disease free survival longer than would have been predicted. Depending on the risk factors present at diagnosis in an individual patient, standard chemotherapy leads to continued remissions ranging from less than 10 percent to more than 50 percent [10]. Studies have indicated that transplant offers some groups of high risk patients long-term disease survival rates of between 40 and 60 percent [21–23]. At the City of Hope and Stanford, two series of patients with high risk features who were transplanted in first CR have been recently updated. Selection criteria included WBC > 25,000; chromosomal translocations t(9;22), t(4;11) and t(8;14); age older than 30; extramedullary disease at the time of diagnosis, and/or requiring more than four weeks to achieve a CR. Two-thirds of the patients had at least one risk

factor and the remaining patients had two or more high risk features at presentation. The majority of these patients underwent HCT in the first four months after achieving a CR. HCT during first remission led to prolonged DFS in this patient population who would otherwise have been expected to fare poorly. At a median follow-up of greater than five years, the probability of event-free survival was 64 percent with a relapse rate of 15 percent [23] (Fig. 4-1).

The French Group on Therapy for Adult ALL conducted a study comparing chemotherapy to autologous SCT and allogeneic BMT [14]. Although the overall results of treatment did not show a treatment advantage for the allogeneic transplant group, subgroup analysis revealed that those patients with high risk disease had a higher five-year survival of 44 percent, as opposed to 20 percent in the other two groups (Fig. 4-2). The recently completed EGOG-MRC trial showed that an allogeneic transplant resulted in better disease control, with benefit seen mostly in younger patients [24].

1.5. Hematopoietic Stem Cell Transplantation for Philadelphia Chromosome Positive ALL

Historically, the dismal outcome with chemotherapy has led to trials focusing on the use of allogeneic transplantation for treatment of adult Ph+ ALL. Most have been single institution studies utilizing a variety of regimens and the cure rate varies from 30 to 65 percent, depending upon age and remission status [25]. Investigators from City of Hope and Stanford have analyzed their experience in 44 patients with Ph+ ALL transplanted from HLA-identical siblings while in first CR between 1984 and 1997, with all patients having 10-year follow-up. All but two patients were conditioned with FTBI (1320 cGy), and high-dose etoposide (60 mg/kg). The three-year probability of disease free survival (DFS) and relapse was 55 percent and 18 percent, respectively. Beyond first remission, stem cell transplantation is curative in a much smaller minority of patients, but remains the treatment of choice.

The development of Imatinib and other tyrosine kinase inhibitors for the treatment of bcr-abl positive hematopoietic malignancy has changed the up-front treatment paradigm and also may affect the outcome after transplantation. Recently, the feasibility of performing allogeneic stem cell transplant after first line Imatinib plus chemotherapy has been reported [26]. In this series, 29 adult patients who completed induction therapy were treated with allogeneic transplantation, and the authors compared their results with 31 patients who had received transplantation in their unit prior to the availability of Imatinib treatment. The data suggests that the risk of relapse was significantly less in the Imatinib group (3.5% versus 47.3%) ($p = 002$), potentially reflecting a lower burden of minimal residual disease at the time of transplant and allowing a higher percentage of patients to come to transplant in a good first remission. The results also indicated a superiority in disease free survival (76% versus 38%) ($p = 001$) without much difference in the transplant-related toxicities (Fig. 4-3). Thus, in the same way that Imatinib may be able to improve the up-front success of induction therapy and potential long-term outcome of patients with Ph+ALL, entering transplant with a lower burden of disease may improve the cure rate for such patients.

Of additional interest is the follow-up of patients with Ph+ ALL, and the impact of detecting MRD after allogeneic transplant. Radich, et al. reviewed

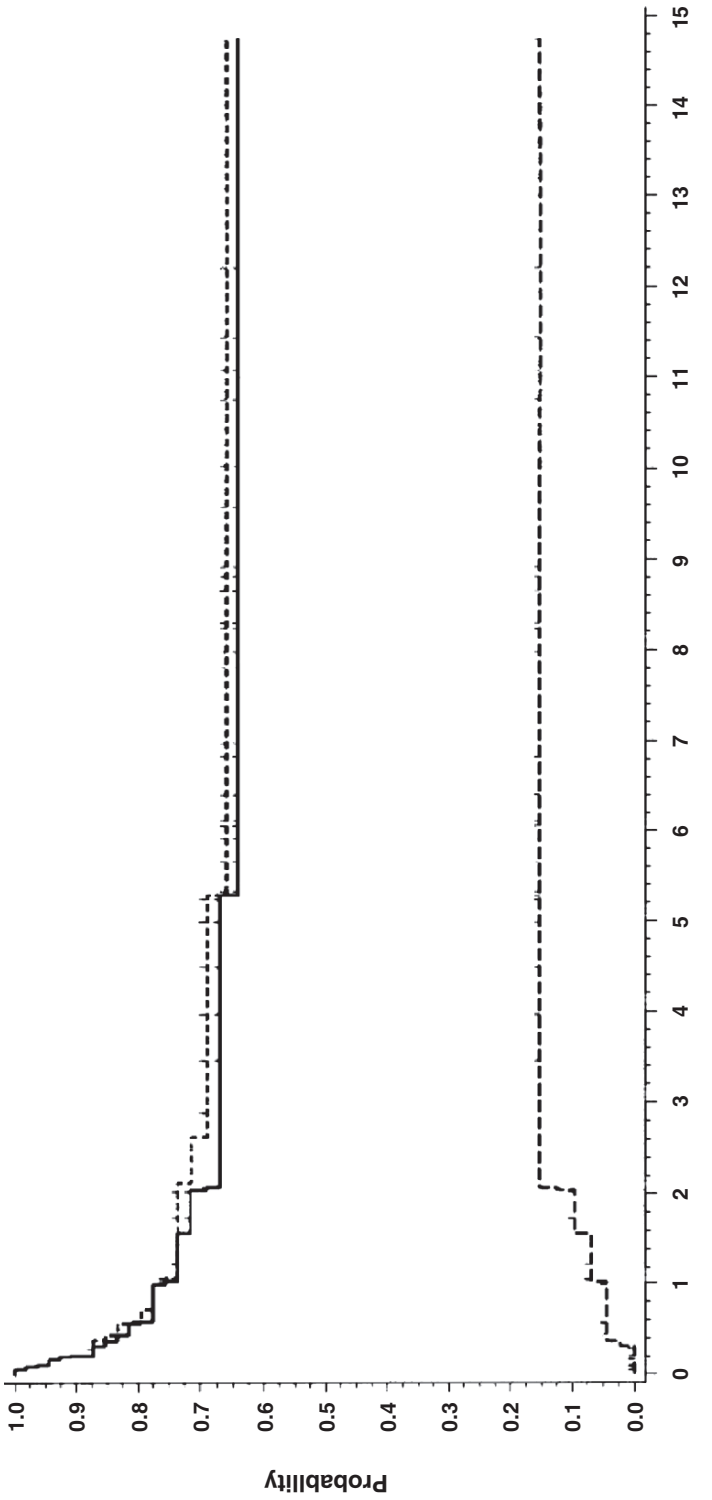

Fig. 4-1. Probability of event-free survival (EFS), overall survival (OS) and relapse for 55 adult patients with high risk acute lymphoblastic leukemia transplanted in first remission. Updated with permission [23]

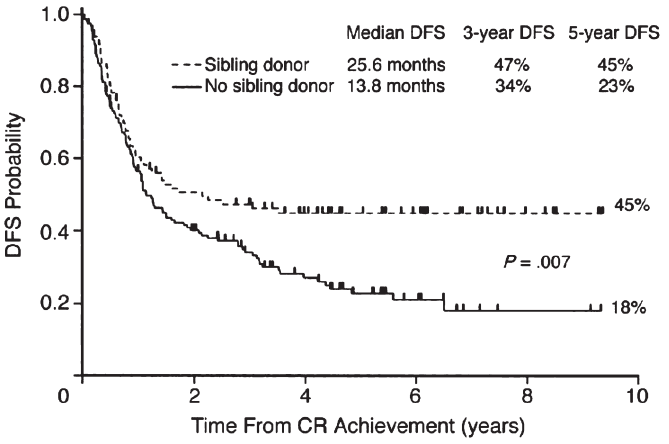

Fig. 4-2. Disease free survival (DFS) according to genetic randomization. The group with a sibling donor comprised 100 patients, whereas that with no sibling donor included 159 patients. CR, complete remission [14]

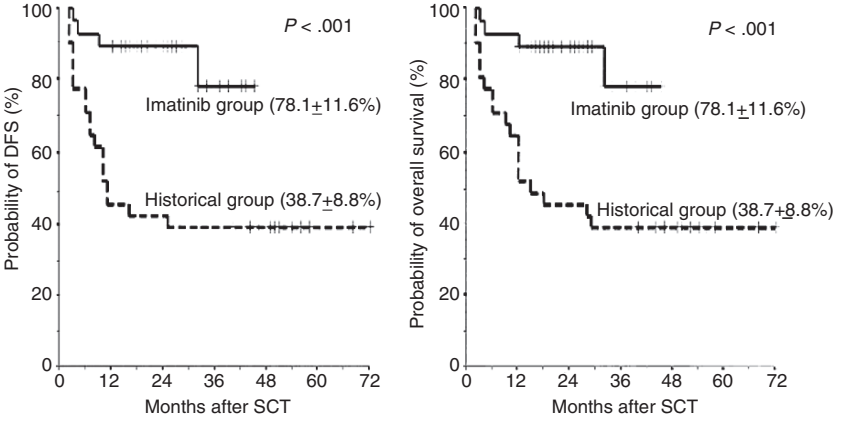

Fig. 4-3. Probabilities of disease free survival and overall survival in the imatinib group versus the historical group. Solid line indicates imatinib group: dotted line, historical group [26]

the transplants of 36 patients with Ph+ ALL [27]. Seventeen were transplanted in relapse, and 19 were transplanted in remission. Twenty-three patients had at least one positive bcr-abl polymerase chain reaction (PCR) assay after transplant either before a relapse or without subsequent relapse. Ten of these 23 relapsed after a positive assay at a median time from first positive PCR assay of 94 days (range, 28 to 416 days). By comparison, only two relapses occurred in the 13 patients with no prior positive PCR assays. The unadjusted relative risk (RR) of relapse associated with a positive PCR assay compared with a negative assay was 5.7. In addition, recent studies have demonstrated the feasibility of giving Imatinib following allogeneic transplant for bcr-abl positive hematologic malignancy, and can be used either preemptively or to treat any MRD detected after transplant prior to relapse instead of using a donor lymphocyte infusion [28].

1.6. Relapsed or Refractory Acute Lymphoblastic Leukemia

ALL is refractory to primary chemotherapy in approximately 10 to 15 percent of patients, and transplant can be successfully used to achieve both a remission and long-term control in approximately 20 percent of patients. Of all those patients who do achieve a first CR to primary therapy, approximately 50 to 70 percent will relapse. Relapsed ALL in an adult is not curable, but remissions are sometimes achieved with re-induction with either a standard vincristine, prednisone and anthracycline, or with a cytarabine-based regimen, particularly high-dose Ara-C (HDAC) combined with an anthracycline or clofarabine [29–32]. Available data from the IBMTR shows that patients transplanted from an HLA-identical sibling for ALL in second CR have approximately a 35 to 40 percent chance of long-term disease free survival, while those transplanted with disease not in remission have a DFS of only 10 to 20 percent. Figure 4-4 shows the overall DFS for patients with ALL, depending on their remission status, who underwent allogeneic transplantation [33].

1.7. Unrelated HCT for ALL

Historically, the outcome after transplantation from unrelated donors has been inferior to that observed after matched sibling transplantation because of increased rates of graft rejection and Graft-versus-Host Disease (GVHD) resulting from increased alloreactivity in this setting. The IBMTR reports a DFS of 44 percent for patients receiving unrelated donor transplantations for ALL in first complete remission (CR1), and 33 percent in second complete remission (CR2). The NMDP reports five-year DFS of 35 percent in CR1 in adults and 46 percent in children, decreasing to 25 percent and 40 percent, respectively, in CR2. Over the past few years, improved results have been

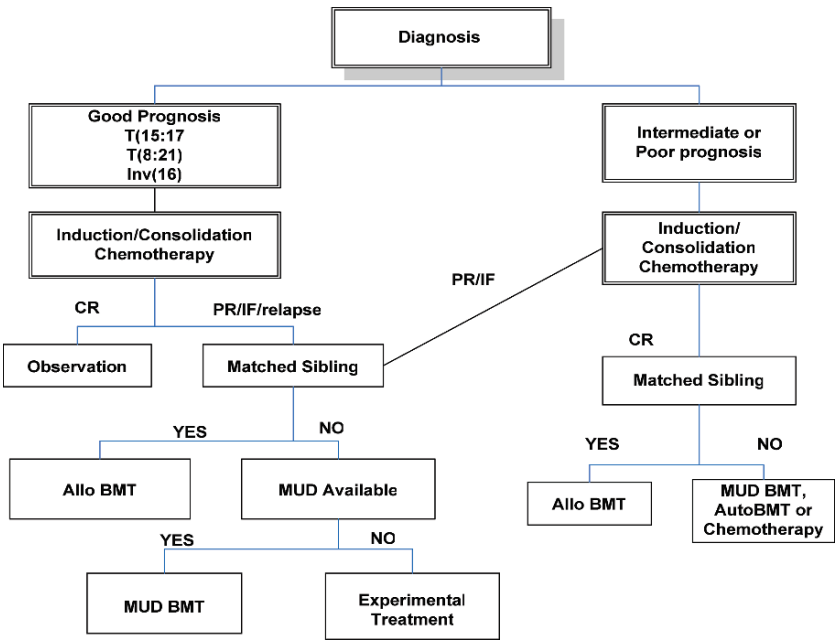

Fig. 4-4. Long-term survival in patients with ALL demonstrating the impact of remission status on the outcome of transplant

reported from several single center studies, particularly in pediatric patients, reflecting improvements in donor/recipient allele level molecular matching in both class I and II histocompatibility genes, GVHD prophylaxis and supportive care [34]. In addition, an NMDP study showed a younger donor and the recipient's age were associated with significantly improved outcome [35]. Recent reports suggest equivalent results for high risk patients from either a related or unrelated donor transplant (43% versus 46%) [36, 37].

1.8. Impact of GVL on Recurrence of ALL

Unlike patients with CML, or even AML, studies of patients with ALL who have relapsed after allogeneic HCT have demonstrated a limited response when immunosuppression is withdrawn or when donor leukocyte infusions are utilized [38]. This has led some to question the existence of a therapeutic GVL effect in ALL. A comparative review of patients who underwent allogeneic HCT for ALL suggests that patients with ALL who have had GVHD have a lower relapse rate than patients who lack the effect (Table 4-2) and provides a rationale for using a reduced intensity transplant in older ALL patients.

1.9. Reduced Intensity Transplant for Treatment of ALL

In contrast to the large number of studies that have evaluated the role of allogeneic reduced intensity transplant in patients with myeloid malignancies, multiple myeloma and low grade lymphoma, fewer studies have been done in ALL. In general, the consensus has been that for patients with ALL, high dose chemoradiotherapy was required for an improved cure rate, but this approach is of limited use in patients over the age of 50. In addition, an evaluation of outcome suggests that the graft-versus-tumor effect is more effective against myeloid malignancy such as AML and CML, and B cell malignancies of mature B cells such as low grade lymphoma and myeloma, but less so with a more undifferentiated B cell like that found in ALL [39]. Nevertheless, a few small studies have suggested that there may be a role for reduced intensity allogeneic transplant even in this disease, particularly in older patients, with 34 percent achieving long-term remission in a report from the EBMT. A recent report of patients undergoing a transplant utilizing either related, unrelated or cord blood donor and a Fludarabine/Melphalan regimen showed an optimistic outcome in a group of patients either at high risk during first remission or who were transplanted after achieving a second and subsequent remission [40]. Since the recent ECOG/MRC study of adult ALL showed surprisingly high

Table 4-2. Relapse after Transplantation for ALL in CR1.

Group	Relapse Probability at 3 Years (%)
Allogeneic, non-T depleted	
No GVHD	44 ± 17
Acute only	17 ± 9
Chronic only	20 ± 19
Both	15 ± 10
Syngeneic	41 ± 32
Allogeneic, T-depleted	
34 ± 13	

toxicity and limited improvement in disease free survival despite better disease control, there is increased interest in exploring this approach in patients with ALL over the age of 35 to 40 who would otherwise be candidates for transplantation based on age, cytogenetics, MRD and response to initial treatment [24].

2. Autologous Hematopoietic Cell Transplantation for Adult ALL

There is much less experience with autologous transplantation for ALL and studies have been focused primarily on those patients in either first or second remission who lacked a sibling or allogeneic donor. Some studies have utilized the same criteria for autologous transplantation as has been utilized for allogeneic transplantation based on the idea that the preparative regimen contributes to the cure of ALL since the allogeneic effect is less potent than in myeloid malignancy. Several groups have reported outcomes for large series of adults with ALL undergoing autologous hematopoietic transplantation in first remission [41–44]. One study from France reported on 233 such patients with long-term, disease free survival at 41 percent [43]. The most important prognostic factor was the interval between achieving a complete remission and proceeding to transplant, with patients being transplanted later having the better disease free survival. This effect may represent the drop out of high risk patients who relapse before transplantation or possibly the effect of consolidation therapy in reducing tumor burden administered prior to hematopoietic cell transplantation. The European Cooperative Group/MRC report on over 1,000 patients indicated a leukemia-free survival of 36 percent while the IBMTR reported a similar plateau at 40 percent [41].

One randomized trial compared the outcome of adults with ALL in first remission treated with chemotherapy versus autologous transplantation. The French LALA 87 trial allocated patients under 40 with HLA-matched siblings to transplantation while the remaining patients received consolidation treatment with modest dose chemotherapy or an autologous transplant [42]. There was a significant drop out rate in the autologous arm due to early relapse, and the long-term follow-up showed no significant difference in overall survival between the two groups, 34 percent for autologous HCT and 29 percent for chemotherapy. This difference applied to both the standard risk and high risk groups. A large trial involving collaboration between the Eastern Cooperative Oncology Group and the MRC was recently reported comparing allogeneic transplant, autologous transplant or chemotherapy in all adult patients with ALL who go into remission [24] and showed equivalent results between autologous transplant and continued chemotherapy. Whether analysis of MRD might be predictive of outcome after autologous transplant is not clear and analysis of a low level of MRD might also predict a good result from chemotherapy.

3. Allogeneic Transplantation for Acute Myelogenous Leukemia

3.1. Introduction

The use of HCT for acute myelogenous leukemia has expanded in the past three decades and has moved from an experimental treatment used only for patients with refractory disease to a first line of treatment for selected patients

with AML in their first remission, depending upon biological characteristics of the leukemic cell and response to initial therapy [44].

Historically, the classification of AML treatment has been based completely on morphologic and clinical observations; however, identifying the molecular events involved in the pathogenesis of human tumors has refined their classification and understanding, including the acute leukemias [45]. In AML, even more than ALL, a large number of leukemia-specific cytogenetic abnormalities have been identified and the involved genes cloned and their clinical significance clarified. These studies have helped elucidate the molecular pathways that may be involved in cellular transformation, provide methods for monitoring patients after chemotherapy and help evaluate the response to therapy correlated with various clinical and phenotypic characteristics [46]. Although the leukemia cells in many patients do not have detectable structural chromosome abnormalities at diagnosis, some may show molecular changes at diagnosis that also predict response to therapy and determine prognosis [47]. Taken together, these observations have led to the concept that AML is a heterogeneous disease with its variants best defined by molecular defects and cytogenetic changes, some of which are more common in different age groups. In previous treatment trials with either standard therapy – allogeneic or autologous transplantation – patients were often treated as a homogeneous group. As described below, recent studies have refined how patients are allocated to various treatments, as well as how data is analyzed, and provides the basis for now making a biologically and response based treatment decision, rather than a global one, for patients with AML [47].

3.2. Cytogenetic Characterization of AML

Cytogenetic risk groups (good, intermediate, poor risk) continue to form the basis for most clinical decision making for post-remission consolidation treatment at the present time [48]. Other disease related factors which influence the risk of relapse after induction chemotherapy include high leukocyte count at diagnosis or extramedullary disease and residual leukemia in marrow exams seven to 10 days after completion of induction therapy and age. The availability of a sibling or unrelated donor also affects the risk assessment for consolidation treatment. HLA typing is now part of the NCCN guideline recommendations for initial evaluation of patients with newly diagnosed AML who do not have comorbid medical conditions which would be a contraindication to transplantation.

3.2.1. Role of Stem Cell Transplantation in the Management of Patients with Acute Promyelocytic Leukemia

The cytogenetic abnormality that predicts the best outcome for patients with AML is the presence of the 15;17 translocation as a sole abnormality. The remission rate and disease free survival is very high with more than 70 percent of patients cured with ATRA-based treatment. Thus, with the advent of increasingly effective ATRA-based therapy and the addition of Arsenic Trioxide and, more recently, Gemtuzumab Ozogamicin, the role of hematopoietic stem cell transplantation as part of the front line therapy of APL has been very limited. The high cure rate from using up-front ATRA and chemotherapy suggests that

there is really no role for hematopoietic stem cell transplantation in patients who are in a first molecular remission if achieved by the end of consolidation therapy [49]. For those patients who have persistent minimal residual disease, therapy with Arsenic Trioxide with or without Gemtuzumab Ozogamicin, followed by hematopoietic stem cell transplant, could be considered. In those patients who remain PCR positive after salvage therapy, an allogeneic approach is recommended, while data suggests that patients who do achieve a molecular remission may be cured by an autologous transplant utilizing a PCR negative hematopoietic stem cell product [49]. Thus, achieving PCR negativity prior to autologous transplant is considered a requirement and is particularly important in those patients who may have limited donor options. For those patients who undergo allogeneic transplantation, there is very little data on the role of reduced intensity transplant and, similar to other patients with AML, it should be utilized in those patients with comorbid conditions or who are older (see below). Although there are some patients who develop therapy-related APL, the prognosis for this group of patients appears to be better than those who develop other forms of therapy-related leukemia. One series confirmed that therapy-related APL generally developed shortly after treatment with Topoisomerase inhibitor drugs such as anthracyclines and Mitoxantrone [50]. Breast cancer is the most common antecedent tumor, followed by lymphoma and Hodgkin's Disease. The same principles that are utilized to treat APL that are not related to prior chemotherapy exposure should be used in this setting as the outcome is very similar with ATRA-based therapy, and transplantation should use the above described principles for *de novo* APL.

3.2.2. Good Risk Cytogenetics

Patients with good risk cytogenetics [t(8;21), inv(16), t(16;16)] may achieve long-term remission with multiple cycles of high dose ARA-C in 40 to 60 percent of patients with relapse as the major cause of treatment failure [51]. Autologous transplant following one or more dose intensive chemotherapy consolidations have shown somewhat better DFS of 60 to 80 percent in some single institution studies [52]. Although molecular probes exist for these translocations, their use in monitoring minimal residual disease in making clinical decisions about transplant is not as clinically useful as the probes for CML or APL [53, 54]. Many patients with t(8;21) in clinical remission remain PCR positive for 10 to 20 years without relapse. Thus, the treatment approach for consolidation therapy of this subgroup would include either (1) multiple cycles of high dose ARA-C with allogeneic transplant reserved for treatment of relapse in patients having a sibling donor, (2) one or two cycles of high dose ARA-C (HDAC) followed by autologous PBSCT in CR, or (3) multiple cycles of HDAC with autologous stem cells collected in remission and reserved for salvage in patients without a sibling donor. For those patients who relapse, depending on the length of the remission, re-induction therapy is often successful and allogeneic transplantation can be successful in eliminating the disease. Recent work in identifying new associated abnormalities in the c-kit gene may provide additional insights into the risk of relapse in this disease and help determine which patients may be better treated with transplant during first remission [55–57].

3.2.3. Intermediate Risk Cytogenetics

The majority of adults with *de novo* AML are in the intermediate risk group. Unfortunately, the disease free survival for this group declines to 30 to 35 percent when HDAC is used alone for consolidation. In this group of patients, both autologous (see below) and allogeneic (sibling) transplant in CR offer an improved DFS of 50 to 60 percent [58, 59].

The largest group of adult patients with AML have no discernible abnormalities utilizing standard cytogenetics [48]. These patients have been the focus of considerable research to define molecular genetic abnormalities that are important in pathogenesis, or that differentiate whether those patients have a good prognosis within this large group [60]. Among the mutations that have been identified are those involving genes encoding NPM1, FLT3 (FLT3-ITD), (FLT3-TKD), CEBPA, MLL and NRAS, and many studies have focused on the prognostic value of single markers identifying subgroups that do not do well [47, 60]. Recent studies have focused on the prognostic value of interactions between these genes to identify groups of patients who do well with chemotherapy or who have a better outcome after transplantation [61]. A recent study from the University of Ulm analyzed 800 patients with normal cytogenetics and found that mutations are identified in NPM1 in 53 percent, FLT3 ITD in 31 percent, FLT3-TKD 11 percent, CEBPA 14 percent, MLL 8 percent and NRAS 13 percent [61]. In this series, patients with a matched related donor went on to transplantation and the impact of these genetic markers on outcome from chemotherapy and transplant were able to identify specific groups. The analysis revealed a marked difference in the outcome, with those patients in the NPM positive FLT3/ITD negative subgroup showing equivalent outcome in relapse-free survival, compared to those who underwent transplantation at approximately 55 percent. In contrast, in the subgroups defined by all other combinations of FLT3-ITD and NPM1 mutations, the benefit of transplantation was significant with marked differences in relapse-free survival of nearly 50 percent for those patients undergoing transplantation and 23 percent for those who do not. Analysis such as this may allow physicians and patients to better understand the significance of a karyotype with normal cytogenetics by focusing on genetic markers such as NPM1 and FLT3 to determine the appropriateness of early transplantation in patients with these markers [61]. Some series have indicated that an allogeneic transplant improves disease control in patients with mutations of FLT3 [62].

3.2.4. Poor Risk Cytogenetics

Patients with loss of chromosomes 5 or 7 or complex karyotypic abnormalities, as well as those patients with antecedent myelodysplasia or therapy-related leukemia, have a very poor outcome when treated with conventional HDAC (10–12% 5 yr DFS) [48]. Autologous transplants have failed to improve on these results in most series. Allogeneic transplants can cure approximately 20 to 40 percent of patients in this group [63]. an unrelated donor search should be initiated early, while the patient is still undergoing induction, for patients with any of these poor risk features who lack a sibling donor.

3.3. Impact of Age on the Outcome of Therapy for Leukemia

Many studies have confirmed the importance of age in the overall prognosis of patients with leukemia [64]. In addition, there are clinical differences between leukemia presenting in older patients and those who are considerably younger, including a different proliferative state, lower white counts and blast percentage, as well as the spectrum of cytogenetic abnormalities with a higher incidence of abnormalities involving chromosomes 5, 7 and 17 and lower incidence of translocations involving favorable treatment outcomes [64]. In addition, expression of P glycoprotein, a marker associated with more resistance, also increases with age [65]. Recent studies have suggested that within each cytogenetic risk group, after accounting for increased incidence of early death from treatment, results tend to deteriorate with increasing age [64]. Thus, for patients with unfavorable cytogenetics, age does not appear to be a major factor in outcome, with all groups of patients having a poor prognosis and disease free survival of under 15 percent. However, for patients with intermediate risk cytogenetics or good risk cytogenetics, age does seem to be a significant factor. Those patients under the age of 55 show a better outcome compared to those patients over the age of 55 and may represent a difference in the molecular genetic differences as described above. This also applies to patients with favorable risk cytogenetics so that those patients over the age of 65, an unusual group of patients, have a prognosis that resembles those with poor risk cytogenetics. This is an important consideration, particularly in considering allogeneic reduced intensity transplant regimens, as both age and cytogenetics should together be factored into the analysis of decisions about prognosis and to determine post-remission therapy.

3.4. Transplant Strategy for Adult Patients with Acute Myelogenous Leukemia

Anthracycline and Cytarabine-based primary induction therapy for newly diagnosed AML will lead to complete remission (CR) in 50 to 80 percent of treated patients [48]. The likelihood of remaining in CR is, however, highly dependent upon prognostic factors found at the time of diagnosis, including cytogenetic analysis, age, molecular analysis, as well as response to treatment. Patients who require more than one cycle of chemotherapy to achieve remission have a poor prognosis regardless of cytogenetic subgroup [66]. Subsequent treatment options for patients who successfully enter first CR after primary induction therapy include (1) repeated courses of intensive consolidation chemotherapy, (2) autologous bone marrow transplantation, or (3) allogeneic bone marrow transplantation.

Currently, when deciding which of the above options to choose the predicted benefit in terms of disease free survival and quality of life, versus risk of morbidity and mortality, should be considered. An important component of this decision depends upon identifing an available matched sibling donor. In nearly all series, allogeneic transplantation results in a lower rate of relapse for patients undergoing HCT for AML in first remission [43], but higher transplant-related morbidity and mortality, and long-term complications and care issues. These results, however, do not always factor in the new information on the biology of AML and the impact of various treatment modalities on the outcome, which can help individualize therapy.

Compared to autologous transplantation or consolidation chemotherapy, allogeneic HCT has a higher potential for complications, with particular difficulty arising from regimen related toxicity, infection and Graft-versus-Host Disease (GVHD), but offers the therapeutic potential of Graft-versus-Leukemia (GVL) Effect. Decision making should also take into account the knowledge that AML treated by allogeneic transplantation at the time of relapse is less likely to induce a lasting remission, compared to transplantation at the time of first remission, because the disease may become treatment-resistant, and develop additional somatic mutations and drug resistance. Patients who relapse and who are then treated with chemotherapy may develop organ dysfunction as a result of chemotherapy, or fungal or bacterial infections, and become less able to withstand subsequent chemotherapy or a bone marrow transplant preparative regimen.

The decision to proceed to allogeneic transplantation thus becomes less controversial as patients move from lesser to greater risk of relapse (and risk of death from leukemia), i.e., beyond first complete remission (CR1), and towards first relapse, second complete remission (CR2), or for primary refractory disease, etc. Much research has, therefore, centered on determining which patients are most likely to benefit from allogeneic bone marrow transplantation early on in their treatment course.

3.4.1. When to Begin Consideration for Bone Marrow Transplantation

Because AML has a high risk of relapse after achieving remission, patients under the age of 50 who have no obvious contraindications for allogeneic HCT should be evaluated regarding the number, health and availability of siblings or other close relations who are potential candidates for stem cell donation. HLA typing can be performed at any time, but should be performed early so that all treatment options can be defined, particularly if the patient does not achieve a remission. This applies particularly to patients with poor risk cytogenetics or other poor prognostic features who are at very high risk for early relapse. This approach provides for minimal delay for transplantation in the possible event of primary refractory disease, early disease relapse after primary therapy or persistent cytogenetic abnormalities in the marrow after CR is attained. In addition, there is currently no evidence that consolidation therapy used before proceeding to full allogeneic transplant has any benefit in reducing relapse after allogeneic transplant [67], but there is evidence that post-remission consolidation improves the outcome of autologous transplant in first remission. Most patients undergoing reduced intensity transplant usually receive at least one cycle of consolidation therapy.

3.5. Outcome after Hematopoietic Cell Transplantation for AML

Studies demonstrate a five-year DFS of 46 to 65 percent for patients treated with allogeneic bone marrow transplantation in CR1 [43]. Cytogenetic analysis also impacts the outcome of transplant. In one series, relapse was 0 percent in patients with good risk cytogenetics and approached 40 percent in those patients with poor risk cytogenetics [68] (Fig. 4-4). A similar result has been observed from the Dana Farber and showed that patient survival after transplant was related to cytogenetic risk group at diagnosis and included patients with AML and MDS [69] (Fig. 4-5).

3.6. Effect of Conditioning Regimen on Survival or Relapse Rate in AML

Several published studies have compared the outcome after different conditioning regimens. Although the use of higher doses of TBI results in a lower rate of relapse, patients suffered a higher incidence of GVHD and transplant-related mortality [70]. Other studies have found no significant differences between conditioning regimens using CY/single dose TBI versus CY/fractionated dose TBI (FTBI) or CT/TBI versus. Melphalan/TBI [71]. There is conflicting data as to whether Busulfan (BU)/CY results in a higher relapse rate than CY/TBI, but recent data suggests that optimal use of Busulfan (intravenous or targeted therapy) may have an impact on both toxicity and relapse [72]. Recent studies utilizing radioimmunotherapy (anti-CD45) designed to target hematopoietic tissue have shown promising results with a low relapse rate and no increase in transplant-related toxicity [73]. Presently, there is no data to determine whether one regimen is more or less effective for each of the cytogenetic subtypes of AML.

3.7. Allogeneic Bone Marrow Transplantation for Acute Myelogenous Leukemia in First Relapse (R1) or second Complete Response (CR2)

For patients in relapse after failure of standard therapy for AML, allogeneic transplantation offers the best chance for cure. For those patients who are able to achieve a second remission, particularly after a long first remission and lack a sibling donor, an autologous transplant is a potentially curative therapy [74, 75]. A common dilemma is the question of whether to proceed directly to allogeneic transplantation at the time of relapse (if a suitable donor has been identified) or whether to proceed to reinduction chemotherapy first in an attempt to reach a second CR (required for autologous HCT). Although no randomized data is available, one study demonstrates statistically nonsignificant survival rate

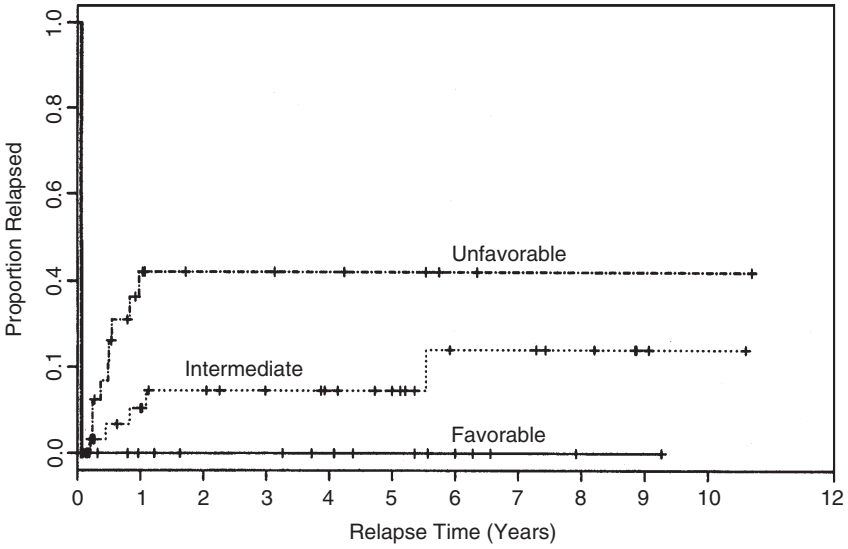

Fig. 4-5. Actuarial relapse rate for patients undergoing allogeneic transplantation for AML in first remission with a regimen of fractionated TBI and VP-16. Based on pre-transplant cytogenetics, those patients with poor risk cytogenetics showed a higher rate of relapse compared to those with more favorable cytogenetic findings [68]

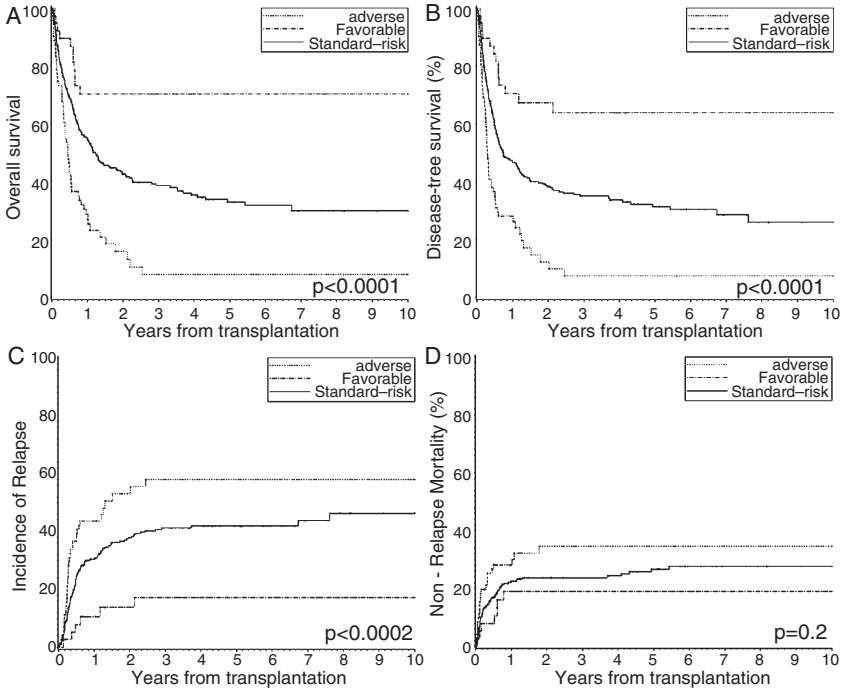

Fig. 4-6. Survival, relapse, and non-relapse mortality for all *de novo* patients, stratified by cytogenetics according to new grouping scheme (**A**) Overall survival; (**B**) Disease free survival; (**C**) Cumulative incidence of relapse; (**D**) Non-relapse mortality [69]

differences of 29 percent in patients transplanted in untreated first relapse versus 22 percent in second remission and in 10 percent with refractory relapse [43, 76]. Another study retrospectively evaluated outcomes in patients transplanted at various stages of disease. Disease free survival was significantly better in patients transplanted in first remission, but no statistical difference was found between the various groups transplanted beyond first CR. Thus, the decision concerning re-induction is often based on the age, condition, duration of first remission and cytogenetic category of the patient with relapsed AML. Generally, patients with remissions lasting greater than one year can be successfully treated. In addition, new epigenetic based therapies may have an impact on reducing disease burden without the infections and organ compromising complications of traditional re-induction programs. Fig. 4-7 shows an approach to the timing and use of HCT based on prognostic features found at diagnosis and response to treatment [39].

3.8. Approach to the Patient with Primary Refractory AML

The survival of patients with AML who do not achieve a remission with primary therapy is very poor and, in general, is independent of all other cellular characteristics. Not achieving remission is the clearest demonstration of the disease's resistance to chemotherapy. Some studies have indicated that the use of allogeneic transplantation in patients who have not achieved a remission may result in long-term disease free survival in approximately 5 to 30 percent

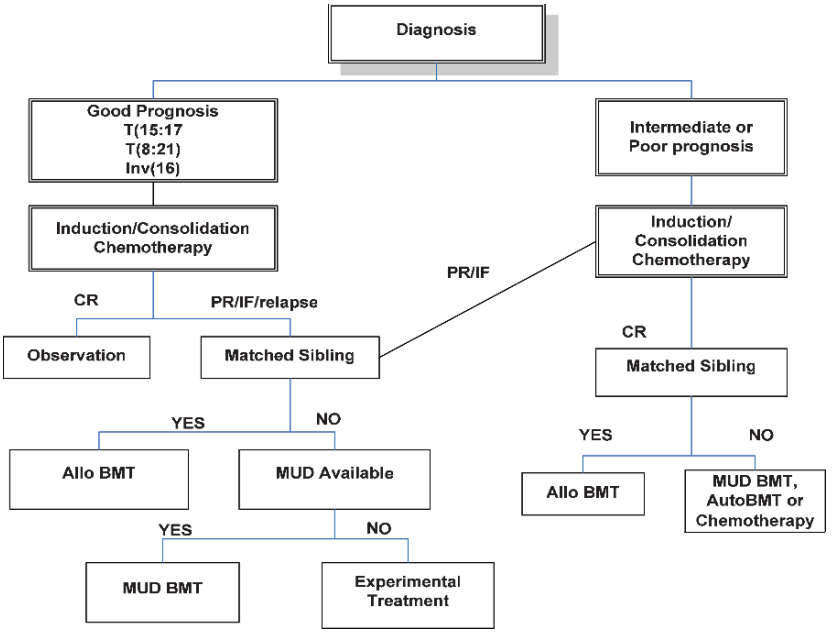

Fig. 4-7. Algorithm for the treatment of patients with acute myeloblastic leukemia. Reprinted with permission from Stockerl-Goldstein, K. and Blume, K.G. in Thomas' Hematopoietic Cell Transplantation, 3rd ed. Blume KG, Forman SJ, Appelbaum FR, eds. [44]

of patients [76–78]. In an analysis of 71 patients with primary refractory AML who underwent a transplant from a sibling donor, an analysis was performed to determine whether there are pre-transplant features of this unique patient population that predict treatment outcome [79]. Although relapse and regimen-related toxicity was high in this high risk patient population, the probability of DFS and relapse at three years was 29 and 54 percent, respectively. Remarkably, cytogenetic analysis before stem cell transplantation was significantly associated with decreased DFS, and a TBI-based regimen appeared to convey a better outcome. The actuarial probability of DFS and relapse at three years was 44 and 38 percent, respectively, for patients with intermediate cytogenetics, and 18 and 68 percent for those patients with unfavorable cytogenetics. Figure 4-7 shows the DFS for a group of patients who failed to achieve a remission and were then treated with an allogeneic HCT from a sibling donor.

The data suggest that allogeneic transplantation can cure some patients with primary refractory AML, and that cytogenetic analysis before stem cell transplantation correlates with transplant outcome as well as relapse. Thus, for patients who do not achieve remission with either one or two cycles of induction therapy, particularly with a high-dose ARA-C based regimen, proceeding to allogeneic transplantation when a sibling donor is identified appears to be the optimal strategy rather than utilizing repeated courses of chemotherapy, which are unlikely to result in remission. Patients who require more than one cycle of chemotherapy to achieve a remission should also be considered at high risk for relapse and should be considered for early transplant [80].

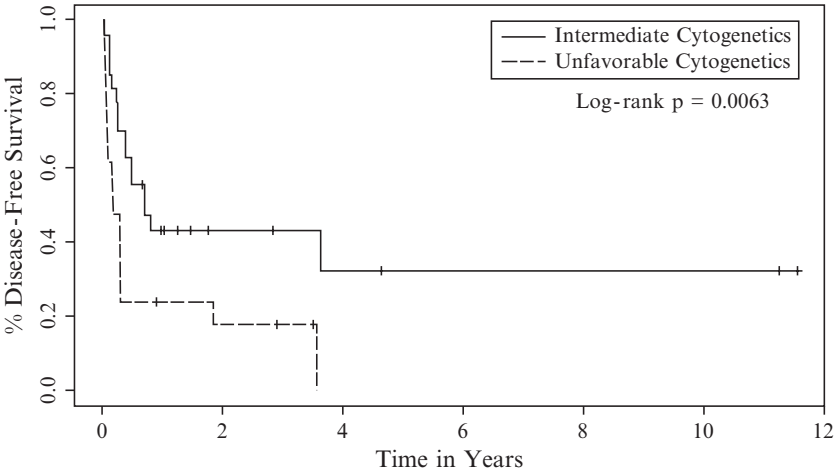

Fig. 4-8. Disease free survival for a group of patients with AML undergoing allogeneic transplantation after having failed to achieve a remission with either ARA-C, idarubicin, or high-dose ARA-C and an anthracycline. Patients with intermediate cytogenetics had a better DFS than those with unfavorable cytogenetics. Overall, the actual probability of DFS at three years was 44 percent for patients with intermediate cytogenetics and 18 percent for those with unfavorable cytogenetics [79]

4. Reduced Intensity Transplantation for AML

It is well known that allogeneic hematopoietic stem cell transplantation offers the best option for cure in patients with myeloid malignancies that lack good prognostic features. It is also recognized that the major limitation is the toxicity of the conditioning regimen and the resulting non-relapse mortality and age. Both of these considerations led to the development of reduced intensity transplant regimens in an attempt to lower the mortality and to offer transplant to patients who otherwise would not be considered candidates because of age or comorbid conditions. The demographics of AML and its increased incidence with increasing age was a major factor in the field to develop regimens that focus more on the graft-versus-tumor effect in mediating an antitumor response and providing potential curative therapy for patients whose prognosis is generally poor because of age. Although there are considerable variations in the reduced intensity regimens, they nearly all rely more on the donor-mediated graft-versus-tumor effect than the transplant regimen dose intensity. To date, it is apparent that the reduced intensity regimens are most effective in patients who are in remission, whereas those patients who have advanced disease require a more intensive regimen to adequately reduce the disease burden and allow the graft-versus-tumor effect to eliminate minimal residual disease over time [81–83]. Figure 4-9 shows the variation in regimens used in the treatment of myeloid malignancy, with full TBI-based regimens being the most intensive, and the low single dose TBI regimen being the least. All of the reduced intensity regimens facilitate full donor engraftment. Fludarabine has, in many programs, replaced Cyclophosphamide because, in addition to being a purine analog that inhibits DNA repair, it is also a potent immune suppressant that allows engraftment of donor stem cells with less conditioning toxicity. Although no direct comparisons have been made for patients with

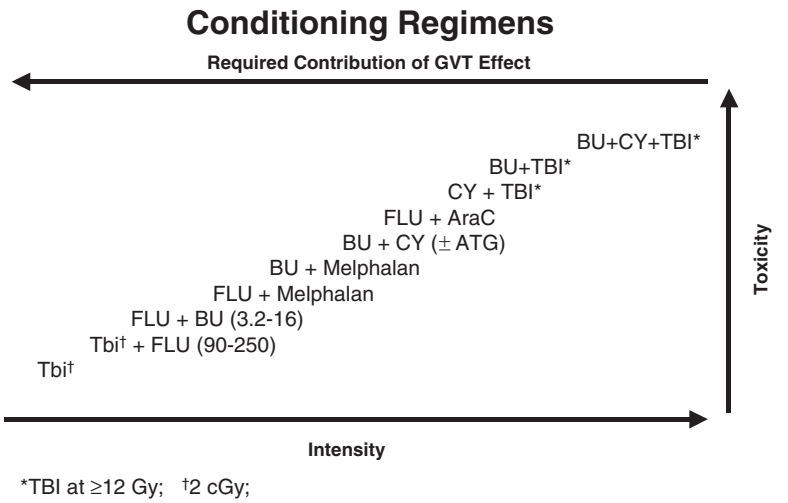

Fig. 4-9. Relationship between intensity of the transplant regimen toxicity for the patient and importance of GVL in the overall outcome. The less intense the regimen, the greater the importance of GVL in controlling disease. (Deeg, et al., Leukemia 20: 1701, 2006)

AML, the data would suggest reduced intensity transplant regimens do allow the treatment of patients with myeloid malignancy who otherwise would not be candidates for a full transplant, but that the risk of relapse may be increased. This has led many investigators to begin to augment the intensity of a regimen that facilitates engraftment and, hence, a graft-versus-tumor effect, with agents that may be less toxic, such as targeted agents utilizing radioimmunotherapy or novel use of other medications. In general, the use of reduced intensity transplant regimens has increased the day 30, day 100 and one-year survival with less toxic deaths from infection during neutropenia, veno-occlusive disease, CMV and interstitial pneumonitis [84, 85].

The European Blood and Marrow Transplantation Group has reported a retrospective analysis of over 700 patients greater than age 50 with AML who underwent conventional myeloablative conditioning with comparable cytogenetics, Fab classification, white blood cell and disease status at the time of transplant [84]. The conditioning regimens analyzed included a variety of agents with varying doses, including Fludarabine and Busulfan, whereas patients conditioned with conventional myeloablative regimens receive either TBI greater than 1,000 rads or Busulfan greater than 8 mg/kg. Their findings included a significant reduction in acute and chronic GVH and non-relapse mortality (18% vs. 32%), with reduced intensity versus conventional myeloablative transplant with a higher relapse rate (41% vs. 24%). Subsequently, the overall two year relapse-free survival did not appear to be different at 47 percent and 44 percent, respectively. Although difficult to determine from these studies, it is clear that there are patients who are undergoing successful allogeneic transplantation with subsequent control of leukemia who otherwise would not have undergone transplant utilizing a more traditional regimen, even after the age of 50. In addition, it is not known from any of the data whether there are subgroups of patients who benefit more from one kind of transplant or the other, and whether the contribution of the preparative regimen versus the GVL may be more important in one

subgroup or another, which subsequent large studies will help determine. Since patients undergoing transplantation for AML do differ in their outcome based on their pre-transplant disease status and cytogenetics, it would be surprising if such information were not important in the reduced intensity setting also. Nevertheless, reduced intensity conditioning is likely preferable in those patients whose age or comorbidity scores preclude consideration for traditional allogeneic transplant to conventional myeloablative conditioning because of reduction of non-relapse mortality. Studies that have been done to date select patients on these considerations and, thus, do not represent true comparisons between patients who could undergo either transplant, and then randomized to one or the other.

5. Autologous Stem Cell Transplantation for AML in First Complete Remission

Many studies have been published utilizing unpurged marrow or purged marrow for treatment of patients with AML in first remission, usually after consolidation therapy [86–89]. Disease free survival for patients in first CR have varied between 34 to 80 percent. Although each trial demonstrates the potential efficacy of the approach, many of these studies have been criticized for including patients who had received widely varying induction therapies, types and numbers of consolidation cycles before autologous hematopoietic cell transplantation, duration of CR before transplant and relatively short follow-up times. In addition, there are differences in the stem cell product manipulation and preparative regimens. In many of these studies, similar to many reports of allogeneic transplant for AML in first remission, a number of patients who otherwise would have been candidates for autologous stem cell transplant suffered a relapse prior to transplant and were not part of the subsequent analysis.

The Medical Research Council Leukemia Working Parties (MRC10) conducted a clinical trial to determine whether adding autologous bone marrow transplantation to intensive consolidation chemotherapy improved relapse-free survival for patients with AML in first remission [87]. After three courses of intensive consolidation therapy, bone marrow was harvested from patients who lacked a donor. These patients were then randomized to receive, after one additional course of chemotherapy, either no further treatment or an autologous bone marrow transplant or preparation with cyclophosphamide and total body irradiation. On an intent to treat analysis, the number of relapses was substantially lower in the group assigned to transplant (37% versus 58%, p = 0007) which resulted in superior disease free survival at seven years (53 versus 40%, p = .04). This benefit for transplant was seen in all cytogenetic risk groups (Fig. 4-10).

In a North American study, patients in first remission with a histocompatible sibling donor were assigned to allogeneic transplantation and the remainder were randomized between autologous hematopoietic cell transplantation utilizing 4-HC purged marrow or one course of HDAC 3 g/m^2 every 12 hours for six days [89]. The preparative regimen for both the allogeneic and autologous transplant was busulfan and cyclophosphamide. The four-year disease free survival for chemotherapy, autologous transplant and allogeneic transplant was 35 percent, 37 percent and 42 percent, respectively; however, as noted above, the

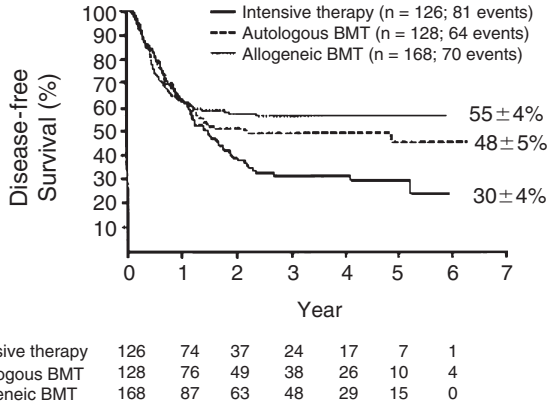

Fig. 4-10. Disease free survival of patients randomized between autologous transplantation, allogeneic transplantation and intensive chemotherapy in the MRC 10 trial. Reprinted with permission [58]

impact of any of these therapies needs to consider the pre-treatment characteristics of the disease in order to assess the efficacy of the post-remission therapy. In the above noted trial, patients were categorized into favorable, intermediate, unfavorable and unknown cytogenetic risk groups based on pretreatment karyotypes which had, as described above, an impact on achieving remission; however, among post-remission patients survival from complete remission varied significantly among the favorable, intermediate and unfavorable groups with significant evidence of interaction between the effects of treatment and cytogenetic risk status on survival [90]. In this trial, patients with favorable cytogenetics did significantly better following autologous transplantation and allogeneic HCT than with chemotherapy alone, whereas patients with unfavorable cytogenetics did better with an allogeneic transplant. This data, combined with that obtained from the CALGB concerning the dose response curve of ara-C in post-remission therapy, again indicates the importance of cytogenetic analysis on the outcome of any particular post-remission induction therapy.

Taken together, these results indicate that autologous transplant in first CR after one or more courses of consolidation therapy can improve disease free survival in selected groups of patients. Questions remain about the number and type of courses of consolidation chemotherapy, the type of regimen used for HCT and the treatment of MRD after transplant. For patients with AML in second CR who lack a matched family or unrelated donor, an autologous transplant can cure up to 30 percent of patients and represents a reasonable option for such patients.

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Chapter 5

Hematopoietic Cell Transplantation for Chronic Myeloid Leukemia

Uday Popat and Sergio Giralt

1. Introduction

No other cancer has been impacted by targeted therapy as much as chronic myeloid leukemia (CML). Likewise, no other disease has seen as much improvement in outcome as CML as the result of a single treatment, in this case the small-molecule tyrosine kinase inhibitor imatinib [1, 2]. Perhaps the biggest benefit from this targeted therapy in patients with CML is that the need for hematopoietic cell transplantation (HCT), which was once the only curative treatment for CML, has declined, though the frequency of use has leveled off recently [3, 4]. There is, however, a continuing role for HCT in the treatment of CML, but this needs to be defined. To help define this role, both now and in the future, we need to better understand the natural history of this disease in the imatinib era and consider both the role of newer tyrosine kinase inhibitors and advances in HCT, including nonablative allogeneic transplantation.

2. Natural History and Conventional Treatment of CML

CML is a clonal hematopoietic stem cell disorder with a characteristic triphasic course consisting of an indolent chronic phase that lasts for several years before turning into a more treatment-resistant, accelerated phase, eventually transforming into an aggressive blast phase akin to acute leukemia that is fatal in most instances. Its annual incidence is one to two per 100,000 per year with a median onset in patients in their 50s and 60s. Patients present with leukocytosis, splenomegaly, basophilia and, to a lesser extent, thrombocytosis. Bone marrow shows hypercellularity with increased numbers of myeloid precursors. Median survival in patients in the absence of treatment is six months [1, 3, 4].

The treatment of CML has evolved as our knowledge of the disease has improved. Busulfan was the first agent developed to control the proliferation of CML cells, prolonging the median survival in patients to five years. Hydroxyurea, which came next, had fewer side effects and produced a better outcome, compared with busulfan [5]. These drugs caused CML to remain in the chronic phase for several years before eventually transforming into the more aggressive accelerated phase and blast crisis, which typically lead to the death of the patient. In randomized trials conducted in the 1980s, alpha

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interferon was found to be superior to hydroxyurea in the treatment of CML [6, 7]. The addition of cytarabine (ara-c) further prolonged survival, but did not effect cure. The only potentially curative therapy at this time was allogeneic transplant, with the standard of care in the 1990s consisting of allogeneic transplant in younger patients who had a matching donor and an interferon-based regimen in older patients or patients who did not have a donor. As noted earlier, the advent of imatinib has now changed the emphasis of treatment.

2.1. Imatinib Treatment

CML is one of the first diseases in which an understanding of the molecular etiology of the disease led to the development of targeted therapy. In fact, discovery of the Philadelphia (Ph) chromosome, (9:22) translocation, unique to the disease launched the entirely new field of cytogenetics. In particular, the understanding that this translocation causes the bcr region on chromosome 22 to be juxtaposed with an abl kinase on chromosome 9, constitutively activating the enzyme and resulting in the uncontrolled proliferation of myeloid cells, led to the development of imatinib [1].

Imatinib has now become the first line therapy for patients with CML, both because of its effectiveness as an orally administered drug and because of its limited side effects. It works by binding to the ATP binding site of constitutively active BCR/ABL kinase [2], thereby suppressing it. It was first studied in phase I trials in 1998, which showed it to be highly effective [8, 9]. It was also studied in comparison with the then standard therapy, interferon plus ara-c, in a multicenter international randomized trial (IRIS) [10]. This trial showed that, in newly diagnosed patients in the chronic phase, imatinib was superior to the interferon plus ara-c combination in terms of all measured endpoints, with 97 percent of patients achieving a complete hematological response. In a recent update to this study, at a median follow up of 60 months, the best complete cytogenetic response in patients receiving imatinib was 69 percent at 12 months and 89 percent at 60 months. Overall survival and progression-free survival rates were 89 percent and 83 percent, respectively. This compares very favorably with the overall survival rate of 68 to 70 percent observed for interferon plus ara-c therapy [11]. This study further showed that 6 percent of patients progressed to the accelerated phase or blast crisis, 3 percent relapsed into the chronic phase and 5 percent showed a loss of the cytogenetic response after a median follow up of 60 months. The estimated treatment failure rate dropped to less than 1 percent per year in the fifth year of the study.

Although patients in a more advanced phase of the disease also respond to imatinib, the results are less impressive and less durable. In particular, at 18 months following the start of therapy in patients in the late chronic phase in whom interferon therapy failed, a complete hematological response was seen in 95 percent, a complete cytogenetic response was seen in 55 percent, the overall survival rate was 95 percent and the progression-free survival rate was 89 percent [12]. At 18 months in patients in the accelerated phase, a complete hematological response was seen in 90 percent, whereas a complete cytogenetic response was seen in only 24 percent [13, 14]. In patients in the blast phase, a hematological response was seen in 52 percent, but a complete cytogenetic response was seen in only 8 percent. The median survival time in these latter patients was six months [15, 16]. Thus, imatinib is only palliative

in patients in the blast phase. In terms of treatment resistance, at 18 months, 80 percent of patients in the blast phase, 50 percent of patients in the accelerated phase and 10 percent of patients in the chronic phase were found to be resistant to imatinib, either initially or at the time of relapse. The effectiveness of imatinib in the long term is limited; that is, despite a good initial response, it is not curative. Most patients who stop treatment have recurrent disease. For this reason, it is currently recommended that patients receive lifelong therapy. Thus, a minority of patients don't respond to it, and some who do respond to it initially become resistant to it. These patients are the ones who need to be identified and considered for early transplantation.

Various clinical and molecular surrogate end points can be used to identify patients with chronic phase CML who are destined to fail imatinib therapy. For example, 97 percent of patients who had a complete cytogenetic response to imatinib therapy at one year did not progress to the advanced phase. Thus, a complete cytogenetic response identifies patients destined to respond well to imatinib. The converse of this, lack of a complete cytogenetic response, identifies patients who should be considered for allogeneic transplant [11]. However, the fact that 93 percent of patients who achieved a partial response at one year, and 81 percent of those who showed a less than partial cytogenetic response at one year remained progression-free in this study indicates that the decision to proceed to allogeneic transplantation should be individualized. Real time quantitative PCR, a more sensitive measure of disease burden, is another tool that could be used to predict eventual disease progression. In one study of this approach, 39 percent of all patients on imatinib showed at least a three-log reduction in BCR-ABL transcripts at one year, and the probability of remaining progression-free was 100 percent among these patients. This was in contrast to a probability of remaining progression-free of 95 percent in those who showed only a cytogenetic complete remission, and 85 percent in those who did not show a complete cytogenetic remission, with both groups showing a less than three-log reduction in BCR-ABL transcripts [17].

2.2. When Should a Transplant Be Done?

Now, with the advances in modern drug therapy for CML, allogeneic transplant cannot be recommended as a first line therapy for patients in the early chronic phase [18], but only at the first sign of an inadequate response. An international expert panel has recommended that allogeneic transplant should be considered at the first sign of drug therapy failure [18]. Based on data from the IRIS trial described above, this has been defined as a lack of hematological response by three months to first line imatinib therapy at a dose of 400 mg daily, no cytogenetic response (Ph+ cells >95 percent) by six months, less than a partial cytogenetic response (Ph+ cells >35 percent) by 12 months, and a complete cytogenetic response not reached until 18 months. In patients failing to achieve the stated response by the said time, the likelihood of eventual response is small and alternative therapy is very reasonable [18]. Allogeneic transplant, with its long track record, should be the appropriate second-line therapy for such patients who have an available donor.

Even more limited data exist regarding other eventualities. For example, patients with blast crisis have a poorer outcome after imatinib and the response is short-lived [9, 15, 16]. In these patients, a transplant should be done as soon

as the second chronic phase occurs, once the best possible response is achieved with imatinib, with or without chemotherapy. In patients with accelerated phase disease, because of the slow tempo of disease compared with blast crisis, a longer trial of imatinib may be permissible. Alternatively, early transplant after a response to imatinib has been obtained is also appropriate. The role of higher doses of imatinib and other tyrosine kinase inhibitors such as desatinib and nirotinib as second line therapy remains unclear. These are certainly effective strategies [19, 20], but the lack of long-term data makes their role uncertain. Thus, they should be used only in patients unable to undergo transplant, or as a short-term bridge to transplant.

2.3. Impact of Pre-Transplant Treatment on Outcome

The use of imatinib prior to transplant does not impair engraftment, increase non–relapse-related mortality, or lead to inferior overall or event-free survival [21]. Even patients with a mutation in the abl tyrosine kinase domain, which confer imatinib resistance, can be successfully salvaged with transplant [21, 22]. Although relatively small retrospective studies found a higher incidence of liver toxicity [23] in patients in which this approach was used, three larger studies did not find an increased incidence of liver toxicity in such patients [21, 24, 25]. Likewise, reports on the incidence and severity of Graft-versus-Host Disease (GVHD) have been mixed, with a higher incidence of GVHD seen in some studies, but not others [21, 24, 25]. In two studies [21, 24], in fact, a lower incidence of chronic GVHD was found in imatinib-treated patients. This is an intriguing finding that can be explained by the fact that imatinib is an immunosuppressive agent, inhibiting T cell [26] and dendritic cell function [27].

Similarly, some studies have indicated poorer overall and disease-free survival in patients treated with imatinib prior to transplant, but most of the larger studies have found no difference in outcome in such patients [21, 24]. In patients with advanced phase CML, imatinib pretreatment before transplant did improve the outcome, particularly in patients who converted to a second chronic phase with < 35 percent Ph+ cells; the three-year overall survival rate in these latter patients was 81 percent [28], but no such improvement was seen in another study [29].

The use of interferon prior to transplant is associated with a poor outcome in some studies, but not in others [30–35]. However, although pretransplant interferon given for a prolonged duration and continued up to the onset of conditioning is associated with a poorer outcome [32], this is not the case if it is used for a short term [31] or if it is stopped at least 90 days prior to conditioning. The five-year overall survival rate was 46 percent for patients who received interferon within 90 days of transplant, and 71 percent for patients who did not. In any case, this is largely a moot topic, as imatinib has now replaced interferon [30–35].

3. Allogeneic Transplant

Allogeneic transplant is a highly efficacious and potentially curative therapy for patients with CML. Its efficacy is due to both the Graft-versus-Leukemia Effect and the effect of conditioning chemotherapy. The therapy had its origins

in the early 1980s, when it was demonstrated that a syngeneic transplant from an identical twin donor cured patients with CML. Since then, stem cells from a variety of sources have been used and the results of transplant have significantly improved [3, 36]. Details on these results, including prognostic factors, are given in the following sections. It is important, however, to bear in mind that these results are from the preimatinib era and, therefore, their place in the imatinib era is unclear.

3.1. Syngeneic Transplant

The first report of a successful identical twin transplant, performed in four patients with chronic phase CML, was published in 1979. Subsequently, the same group reported on another 22 patients who underwent twin transplant. Eight of 12 patients who underwent transplant in the chronic phase were disease free, with a median follow-up of 30 months at the time of the report [37].

The efficacy of a syngeneic transplant clearly illustrates several important principles of the mechanism of leukemia eradication after transplantation. First, high-dose chemoradiotherapy itself can cure some patients with CML, with a three-year leukemia-free survival rate of 59 percent in 34 patients with CML in the first chronic phase [38]. Second, the risk of GVHD is absent and, thus, transplant-related mortality is low at 3 percent, versus 34 percent in patients who receive an allogeneic transplant. Third, the risk of relapse is significantly higher in these patients than in patients who receive an allogeneic transplant (40% versus 7%), suggesting the concept of a Graft-versus-Leukemia immune effect in recipients of allogeneic transplants, a phenomenon equally if not more important than high dose chemotherapy in the eradication of leukemia.

3.2. Matched Sibling Transplant

Since the early report of the efficacy of transplants from identical twin donors, the Seattle team and other transplant teams from around the world have reported on the successful outcomes in patients with CML who receive a transplant from an HLA-identical, related donor. For example, in the first report of its use published in 1982, six of 10 patients were alive and disease free one to three years after transplant [39]. These results were confirmed in a larger series of 198 patients, in which overall survival rates of 49 percent, 15 percent and 14 percent were found in patients in the chronic phase, accelerated phase and blast phase, respectively [40]. The major cause of treatment failure was relapse in patients with advanced disease and interstitial pneumonia in patients in the chronic phase. Several other studies from around the world showed similar results [41–43].

These results have improved over time. In particular, the most recent analysis from the European Blood and Marrow Transplant (EBMT) registry showed that, for all patients, the two-year overall survival rate improved from 53 percent to 61 percent in the most recent years (2000–2003), compared with the years 1980–1990. This improvement was mainly due to a reduction in transplant-related mortality of from 41 percent to 30 percent in all patients, and from 31 percent to 17 percent in patients with low risk disease (EBMT risk score of 0–1). These patients with low risk disease have a two-year overall survival rate of 80 percent [3]. Likewise, a recent study from Seattle has shown an 86 percent three-year survival rate in young patients (median age,

43 years) with CML in the chronic phase receiving transplants from matched siblings with targeted busulfan and cyclophosphamide (Bu/Cy) conditioning [44]. Similarly, a study from Germany showed an improved five-year overall survival rate in the most recent decade – 1995–2005 – compared with the earlier decade –1984–1994 – of 73 percent versus 62 percent, respectively [36].

In the preimatinib era, the outcome after allogeneic transplant was compared with the outcome after interferon-based therapy. In these early studies higher transplant-related mortality negated the benefit of transplant. A retrospective registry study comparing the outcome of patients treated with an HLA-identical sibling transplant and a cohort of patients treated with hydroxyurea or interferon showed a survival advantage for drug therapy in the first four years after transplantation. An advantage for transplant was seen only in the sixth year after diagnosis. This benefit was even delayed for patients with low risk disease [45]. A randomized trial comparing the best available drug therapy (interferon-based) with allogeneic transplant, done to confirm these earlier results, showed significantly superior survival for patients treated with drug therapy after a median follow up of 8.9 years. In particular, the overall survival rates at five years in the transplant group and interferon-based drug therapy group were 62 percent and 73 percent, respectively. This was most pronounced in low risk patients. However, overall survival at 10 years was similar in both groups, at 53 percent and 52 percent, respectively [46]. With advances in transplant technology and, more importantly, with availability of imatinib, these data are largely of historical interest.

The outcome of patients in the accelerated phase and of patients in blast crisis is much worse, with only a 41 percent and 18 percent leukemia free survival rate, compared with 57 percent in patients in the chronic phase, in a registry study of patients who received transplants between 1987 to 1994. This was largely due to a high risk of relapse of 26 percent and 58 percent, respectively [47], in these two groups of patients. Similar results were noted in a study from Seattle, with a 43 percent event-free survival rate in patients with accelerated phase disease [48]. However, the survival was better for patients who had accelerated phase disease solely based on cytogenetics criteria than for patients who had accelerated disease based on other criteria – 66 percent versus 34 percent [48]. Unfortunately, unlike the outcome from transplant in patients with chronic phase CML, the outcome from transplant in patients in the accelerated phase or in blast crisis has changed little in recent years, with most recent EBMT data for patients treated between 2000 and 2003 showing a 47 percent and 16 percent two-year survival rate for patients in the accelerated phase and blast phase, respectively [3].

3.3. Matched, Unrelated Donor Transplant

A matched, unrelated donor (MUD) transplant is an alternative for patients who lack an HLA-identical sibling [49]. Early reports of 196 consecutive patients who received transplants procured by the National Marrow Donor Program (NMDP) confirmed the feasibility and efficacy of this approach: the two-year disease free survival rates were 45 percent, 36 percent, 27 percent and 0 percent in patients who received their transplants in the chronic phase within one year of diagnosis; more than one year after diagnosis, in the accelerated phase, and in the blast phase, respectively [50]. A further update of NMDP

data, in this case for 1,423 patients treated over a period of 8.5 years, showed an improved outcome over earlier findings: the three-year disease free survival rate in patients who received their transplants within one year of diagnosis had risen to 63 percent [51]. Subsequently, better results were reported from single centers. For example, in a series of 198 patients who underwent transplantation between May 1985 and December 1994, the overall survival rate was 57 percent at a median follow-up of five years. Factors found in this study to adversely affect survival were an interval from diagnosis to transplant of one year or more, HLA DRB1 mismatch, and age more than 50 years. The survival rate was 74 percent in those with more favorable characteristics, in particular 50 years and younger, and the interval from diagnosis to transplant within one year of diagnosis [52].

While results for MUD transplants have been somewhat inferior to those for matched sibling transplants [53], some single institution studies have shown similar outcomes in both groups of patients [54, 55]. For example, in a study by Weisdorf, et al., the outcome in patients with CML undergoing transplantation between 1988 and 1999 from a matched sibling donor was compared with that in MUD transplant recipients. Increased risk of graft rejection and GVHD and inferior overall survival and disease free survival were seen in MUD recipients. However, for patients in the chronic phase undergoing MUD transplantation within one year of diagnosis, the outcome was similar or only slightly inferior to that in the matched sibling transplant group [53]. More recently, Gratwohl, et al. reported an improvement in the two-year survival rate between patients treated in the 1980s and those treated after 2000 of 29 percent and 53 percent, respectively [3]. The improved outcome, similar to results obtained for recipients of matched sibling transplants, is likely to be due to reduced treatment-related mortality as a result of better HLA matching at the allele level [56].

3.4. Cord and Haploidentical Related Donor Transplants

For patients who don't have an HLA-identical related or unrelated donor, a transplant from a haploidentical related donor or from cord blood is an alternative. However, these approaches are associated with a higher treatment-related mortality, but some patients do survive long-term leukemia free [57, 59]. Autologous transplant is also an option for some of these patients and has been studied by some groups. The availability of highly effective targeted therapy has made many of these approaches less relevant today, however.

3.5. Source of Stem Cells: Peripheral Blood or Bone Marrow

In randomized trials and a meta-analysis, peripheral blood stem cell grafts were found to improve disease free survival and overall survival compared with bone marrow, though at the expense of a higher incidence of chronic GVHD disease in patients receiving transplants from HLA-identical siblings; therefore, it is a preferred stem cell source in this setting [60, 61]. The benefit is not so clear in patients receiving a transplant from a matched unrelated donor. In particular, use of a peripheral blood stem cell graft from such donors was associated with earlier engraftment, no difference in the incidence of acute GVHD or risk of relapse, a higher incidence of chronic GVHD and a better leukemia free survival rate in patients with advanced CML (33 percent versus 25 percent), but lower leukemia free survival rate in patients in the

first chronic phase (41 percent versus 61 percent), compared with use of bone marrow [62, 63].

3.6. Conditioning Regimen

In the early studies done in the 1980s, the conditioning regimen in the majority of patients consisted of 120 mg/kg cyclophosphamide and 12 Gy of fractionated total body irradiation (Cy/TBI) [40]. Increasing the dose of radiation to 15.75 Gy did not improve overall survival because even though this decreased the relapse rate, the non–relapse-related mortality increased with the higher dose [64]. In the 1980s, busulfan (Bu) and cyclophosphamide (Bu/Cy) were introduced and appeared promising [65, 66]. This was compared with Cy/TBI in four randomized trials. Both regimens were found to be equally effective in early disease, but Bu/Cy was better tolerated and was thought to be an acceptable alternative to Cy/TBI [67, 68].

However, one problem with oral busulfan is that its oral absorption is erratic, which affects patient outcome. Specifically, it was noted that patients with a steady state level of busulfan lower than 917 ng/ml had a higher relapse rate and inferior survival rate, compared with patients with a level that exceeded 917ng/ml [69]. The most recent study from Seattle that examined the Bu/Cy regimen, with a target steady state level of 900–1,200 ng/ml, showed an impressive 86 percent three-year overall survival rate in patients in the chronic phase [44]. In summary, Bu/Cy and Cy/TBI remain the two most commonly used conditioning regimens for patients with CML

3.7. Role of Splenectomy

No clear consensus exists about the impact of splenomegaly and the role of splenectomy prior to transplantation. An increased rate of rejection and delayed engraftment [70] have been observed in patients with a large spleen prior to transplant, and this rate has been found to be reduced in patients who undergo splenectomy in some studies [71], but not in others [72]. Furthermore, an increased risk of leukemia relapse has been reported in patients who undergo splenectomy [73]. Splenectomy [40, 72] and splenic irradiation [74] before transplantation have not been found to improve the overall outcome in patients and, therefore, cannot be recommended as part of the routine pre-transplantation process.

3.8. Nonablative Transplantation

The Graft-versus-Leukemia effect and efficacy of DLI first described in patients with CML make CML a malignancy that is particularly susceptible to the immune effect of transplant and most likely to benefit from nonablative transplantation. Indeed, several studies [75–81] (Table 5-1) have demonstrated the safety and efficacy of this approach, but because these studies have not been randomized comparative trials comparing nonablative transplantation with myeloablative transplantation, the use of nonablative transplantation at this time is limited to patients who cannot tolerate myeloablative transplantation because of age or comorbidity.

Crawley, et al. reported on the outcomes of 186 patients who underwent nonablative transplantation recorded in the EBMT registry. These authors found a two-year non-relapse mortality (NRM) rate of 23 percent, a three-year

Table 5-1. Nonablative hematopoietic cell transplantation for CML.

Study	No. of patients	Conditioning regimen	Median Age	Interval from diagnosis (months)	Disease phase CP1/CP2/ AP/BC	Related/ MUD	Graft rejection/ mixed chimera	GVHD Acute/ acute severe III/ IV/chronic	Median FU (months)	NRM %	DFS %	OS %	Comments
Or, et al. [79]	24	Flu/Bu/ATG	35	9		19/5	0/6	75/29/55	42	13	85	85	Early CSA withdrawal second or third month for mixed chimerism. No relpsess, death due to GVHD
Crawley. et al. [76]	186	Multiple^	50	13.5		133/52	9 %	32/ 9/43	35	23.3	37	58	CP1 69 % 3 year OS
Baron, et al. [75]	21	TBI/Flu	54	26	12//2/5/1	0/21	45 %	82/27/80	867 days	2/21	7/21	14/21	High incidence of rejection
Das, et al. [77]	17	Flu/Bu /ATG or TBI						7/17 – 11/17	30 months		5/17	6/17	
Weisser, et al. [81]	35	Flu/Cy/TBI/ ATG	51		26/9	19/16	0	48/ /23	30	28.5	49 %	63 %	TBI 8 Gy
Kerbauy, et al. [78]	24	TBI/Flu or TBI	58	28	14/4/6/0	24/0	4/24	46/ /29	36	21 %	54 %	54 %	4/8 with TBI alone rejected
Ruiz-Arguelles, et al. [80]	24	Flu/Bu/Cy	41	19	24/0	24/0	0	46/ /30	17	8	92	92	

^largest number (n=95) Flu/Bu.

CP1 = first chronic phase; CP2 = second chronic phase; AP = accelerated phase; BC = blast crisis; MUD = matched unrelated donor; GVHD = Graft-versus-Host Disease; FU – follow-up; NRM = non-relapse mortality; DFS = disease free survival; OS = overall survival; ATG = nti thymocyte globulin; TBI = total-body irradiation

overall survival rate of 58 percent, and a disease free survival rate of 37 percent in this group of patients. However, outcomes varied by the phase of the disease. Specifically, the overall survival rates were 69 percent, 57 percent, 24 percent and 8 percent, and the progression-free survival rates were 44 percent, 30 percent, 10 percent and 0 percent in patients in the first chronic phase, second chronic phase, advanced phase and blast phase, respectively [76]. Similar results were noted in a study of 64 patients treated at The University of Texas M. D. Anderson, which are summarized in Table 5-2 and Fig. 5-1. Similar to the observations of Crawley, et al., a higher EBMT score was significantly associated with poor overall survival rates. Patients with a score of 1–2, 3–4, and > 4 had an overall survival rate of 69 percent, 57 percent and 33 percent, respectively.

GVHD is the major cause of NRM in this group of patients. The overall incidence of GVHD is increased in these patients as a result of the early withdrawal of immunosuppressive treatment and/or the increased use of DLI to promote full donor engraftment and prevent relapse [79]. NRM is higher in patients in the advanced phase of disease than in patients in the chronic phase (20.3 percent versus 11.6 percent) [76]. The incidences of both acute and chronic GVHD are lower in patients who receive antithymocyte globulin(ATG) or alemtuzumab, but this is at the expense of a higher incidence of relapse. The highest risk of relapse, 76 percent at two years, is seen after alemtuzumab use [76].

Although there are no randomized trials comparing different conditioning regimens for nonablative transplantation, the best reported outcome has been for the combination of busulfan, fludarabine and ATG [76]. In contrast, a high incidence of rejection (45 percent) was seen in patients who received a low dose TBI-based regimen, particularly in MUD transplant recipients, likely due to the highly proliferative nature of the disease and the lack of previous aggressive chemotherapy [75]. Hallemeier observed a similarly high graft failure rate (5 of 22 patients) in patients who received Cy/TBI (5.5Gy). However, the graft failure in these patients was followed by recovery of recipient hematopoiesis, unlike the aplasia observed in patients who undergo myeloablative chemotherapy. Patients who rejected the graft also had a low (<40 percent) donor T cell chimerism on day 28 [82]. Marrow grafts in patients who had nonablative transplantation were found to be associated with a higher risk of graft failure than for recipients of peripheral blood stem cell grafts [83]. Sloend, et al. found that the majority of patients who underwent Flu/Cy conditioning failed to achieve remission and required further intervention (DLI, imatinib or a second transplant) for disease eradication [84].

Table 5-2. Nonablative transplant in CML: The University of Texas M. D. Anderson experience in 64 patients.

	AP	BC	CP1	CP2
Median survival (yrs) 95 % CI	0.61 (0.21, 2.94)	0.22 (0.02, 0.41)	4.26 (2.14, –)	3.19 (0.27, –)
Proportion surviving to 2 years	0.38	0	0.77	0.57
Proportion surviving to 5 years	0.23	0	0.46	0.50

AP = acute phase; BC = blast crisis; CP1 = first chronic phase; CP2 = second chronic phase

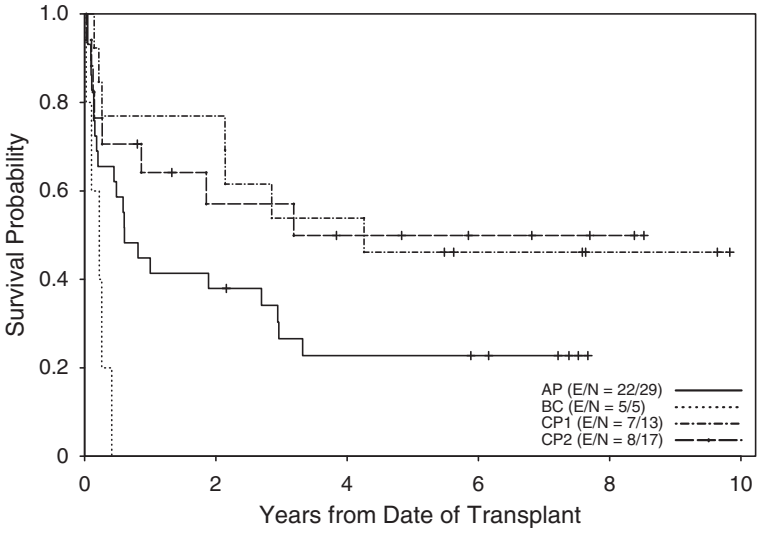

Fig. 5-1. Nonablative transplant in patients with CML: The University of Texas M. D. Anderson experience in 64 patients (AP = accelerated phase; BC = blast crisis; CP1 = first chronic phase; CP2 = second chronic phase)

Molecular remission is achievable, but occurs more slowly or is delayed in patients who receive nonablative, as opposed to conventional, transplants. This usually occurs with or after conversion to full donor T cell chimerism, which is delayed compared with the timing of conversion in recipients of ablative transplants [85], although complete donor chimerism is not necessary for response. A persistently high level of BCR/ABL transcripts 28 to 56 days after transplant predicts relapse in patients who undergo 2Gy TBI-based conditioning [86]. The kinetics of the molecular clearance of disease are similar between patients undergoing nonablative transplantation and those receiving DLI: the maximal response often occurs only after a delay of up to one year after the therapy [87].

4. Prognostic Factors

A transplantation's outcome depends on the disease biology and the health of the recipient. The most important prognostic factor is the phase of the disease; patients in the chronic phase fare better than those with more advanced disease, either in the accelerated phase or blast phase [40]. Even among patients with chronic phase disease, the outcome is better for those who receive transplants early, within one year of diagnosis, as opposed to later [40, 88]. Younger patients also have a better outcome because they are better able to tolerate myeloablative chemotherapy and/or radiotherapy. Recipients of matched sibling transplants were previously expected to have a better outcome than recipients of transplants from a matched unrelated donor, but with better HLA typing and supportive care, the outcome in MUD transplant recipients is improving, matching that of matched sibling transplant recipients in some centers [54, 55].

EBMT took all these factors into account to devise a prognostic index that could be used to predict survival after transplant, help make treatment decisions and counsel patients [89]. This index was subsequently validated [90]. The EBMT score is based on five variables: donor type (HLA-identical sibling donor versus MUD), age (< 20, 20–40, >40 years), disease phase (first chronic, accelerated, and blast), donor–recipient sex combination (female donor-male recipient, other) and interval from diagnosis to transplant (< 1 year versus > 1 year). Risk factors are graded 0 (favorable), 1 or 2, with a global score that ranges from 0–7. Survival rates at five years were shown to be 72 percent, 70 percent, 62 percent, 48 percent, 40 percent, 18 percent and 22 percent for risk scores of 0, 1, 2, 3, 4, 5, and 6 or 7, respectively.

5. Relapse after Allogeneic Transplant

Relapse continues to occur after allogeneic transplant, with an incidence of up to 20 percent in patients who undergo transplantation in the chronic phase, and up to 60 percent in patients who undergo the procedure in the advanced phase of disease [40, 42, 47, 51]. Survival after relapse is limited and is worse for patients in the advanced phase of disease at the time of relapse or at the time of transplant, for patients who undergo transplantation in the late chronic phase more than two years after diagnosis, and for patients who receive transplants from an unrelated donor [91].

Hematological relapse is preceded by molecular relapse, which is indicated by a rise in BCR/ABL transcripts in PCR analyses, and by cytogenetic relapse, which is indicated by the finding of Ph+ cells in cytogenetic studies. However, it is not infrequent to see a transient, intermittent increase in BCR/ABL transcripts, particularly in the first nine to 12 months post-transplant, in patients who subsequently remain in hematological remission. A steadily rising level of BCR/ABL transcripts, however, is eventually followed by hematological relapse. Late relapse is not infrequently seen: the cumulative incidence of relapse at 15 years for patients who are in remission at five years is 17 percent [92].

5.1. Donor Lymphocyte Infusion

Relapse post-transplant can be successfully treated with DLI [93–96], with approximately 70 percent of patients in the chronic phase responding and achieving a prolonged durable remission. The response rate is low (10–20 percent) in patients with advanced disease, but high in patients with molecular or cytogenetic relapse. DLI can sometimes be complicated by fatal GVHD or aplasia. A higher incidence of GVHD and treatment-related mortality has been noted after higher doses of DLI. The first dose of DLI should not exceed 0.2X 10e8 T cells/kg [97] and should, perhaps, be even lower for MUD transplant recipients [98]. To prevent or reduce incidence of GVHD, escalating doses of DLI have been used, administering the least possible cell dose that will result in therapeutic response. Alternatively CD8 depletion of donor lymphocytes has also been successfully used for this purpose [99, 100].

5.2. Imatinib

Relapsed CML after allogeneic transplantation also responds to imatinib [101]. Imatinib treatment in patients who suffer relapse following allogeneic

transplant resulted in a complete hematological response in 100 percent, 83 percent and 43 percent of patients in the chronic phase, accelerated phase, and blast phase, respectively. The main side effects were GVHD and myelosuppression [102, 103]. Interferon alfa is also effective in some patients, but its use has been superseded by more effective drugs such as imatinib [104].

5.3. DLI or Imatinib?

Despite the high rate of response to imatinib, most patients are not cured of their disease and the responses are not as durable as those that have been seen with DLI. For example, in a small study of patients treated with imatinib following allogeneic transplant, nine of 10 patients who suffered relapse after allogeneic transplant responded to imatinib. However, six of these nine patients suffered relapse while being treated with imatinib and the other three suffered relapse when imatinib treatment was stopped [105]. These results are similar to those observed when imatinib was stopped in conventionally treated patients who achieved molecular remission [106, 107]. In contrast, in a study of DLI, 20 of 21 patients treated with DLI (95 percent) achieved a complete molecular remission, with only three (14 percent) of the 21 subsequently suffering relapse. However, 11 of these 21 patients (52 percent) developed acute GVHD, seven developed chronic GVHD and three patients died – two of GVHD and one of recurrent disease [105]. Hence, DLI is associated with a higher treatment-related mortality, lower relapse risk and longer leukemia free survival, compared with imatinib, in patients suffering relapse following allogeneic transplant.

5.4. Monitoring with PCR

Using PCR to monitor for minimal residual disease may allow relapse to be predicted, which will permit early intervention. The finding of three consecutive PCR ABL/ABL ratios of > 0.02 percent or two consecutive values of > 0.05 percent six months after transplantation is considered to signify molecular relapse; the relapse rate in this group is 70 percent. Likewise [108], in another study, a positive PCR finding at six months, but not three months after transplantation, was predictive of relapse, with 42 percent of PCR-positive patients versus 3 percent of PCR-negative patients suffering relapse [109, 110]. However, these results cannot be considered standard, as PCR assays are different at different laboratories. Standardization of these assays among centers will go a long way toward resolving this problem [111].

6. Newer Strategies

Several novel strategies are being studied to improve the outcome from transplantation . For example, modifications of the commonly used Bu/Cy regimen have resulted in the increased safety and efficacy of transplantation. Targeting a steady state busulfan level of at least 900 ng/ml [44], replacing cyclophosphamide with fludarabine [112], and using an intravenous instead of oral preparation of busulfan — all of these approaches have reduced the toxicity of transplant and improved disease free survival [113, 114]. Another approach used by some groups to reduce GVHD and early treatment-related mortality is *ex vivo* or *in vivo* T cell depletion. Early DLI at the time of molecular relapse,

or done prophylactically before relapse occurs, can be paired with T cell depletion to offset the increased risk of relapse associated with the depletion procedure. Encouraging results for this approach have been obtained in some studies [115]. Several clinical trials of the combination of imatinib and allogeneic transplant are ongoing and results of this strategy are keenly awaited.

7. Economic Issues

With the development of targeted therapy (e.g., imatinib), allogeneic transplantation is no longer the most expensive therapy in the treatment of CML; hence, transplantation is a cost-effective treatment option for many patients in middle-income countries [80, 116]. Gratwohl, et al. studied these issues and transplant trends in Europe before and after the introduction of imatinib in the context of both CML and AML. Countries were classified as high-income, middle-income, or low-income based on the gross national product per capita of that country; most of the western European countries were classified as high-income. These authors found that the rate of transplantation increased linearly between 1991 and 1999 from 13 to 38 per 10 million in high-income countries, but from only 0.7 to 15 per 10 million in middle-income countries. After 1999, however, the transplant rate fell in the high-income countries, but remained stable in the middle-income countries, at 15 per 10 million. One would have anticipated a comparable decline in the middle-income countries as well after imatinib, a simple nontoxic therapy, was introduced. The authors hypothesized that a likely reason for this finding was an economic one, in that lifelong drug treatment with a novel agent is very expensive and, therefore, unaffordable for some patients compared with the cost of an early invasive, but curative procedure. This is indicated by the fact that the median cost of imatinib for one year, which is 30,411 euros (range: 28,000–44,000 euros), does not differ between high- and middle-income countries. In contrast, the median cost of allogeneic transplant from an HLA-identical sibling was significantly lower in middle-income countries than in high-income countries, with a median cost of 52,000 euros versus 82,000 euros. This is not surprising, as the cost of transplant is mainly hospital and health care personnel expenses, which are lower in less-developed countries [117]. A similar argument has been made by investigators in a study conducted in Latin America [80], which showed that transplant was favored for economic reasons. This situation may change significantly, however, if a generic or less expensive version of targeted therapy is made available in these countries. A recent patent protection battle taking place in Indian courts may have a significant impact on the availability of imatinib in less-developed nations [118].

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Chapter 6

Stem Cell Transplantation for Hodgkin's and Non-Hodgkin's Lymphomas

John W. Sweetenham

1. Introduction

The role of high dose therapy (HDT) and stem cell transplantation (SCT) in the treatment of Hodgkin's (HL) and Non-Hodgkin's lymphomas (NHLs) continues to evolve. High-dose therapy with autologous stem cell transplantation (ASCT) is now regarded as standard for eligible patients with relapsed and refractory HL or relapsed aggressive B-cell NHL, based on results from prospective, randomized clinical trials. The role of HDT and ASCT as a component of first line therapy for high risk patients with HL and NHL is being clarified as recent randomized studies are reported.

However, there are uncertainties regarding many aspects of SCT for lymphoma. For example, the optimal timing of SCT for most lymphoma subtypes is unclear, as is the best high-dose regimen for each disease, the best stem cell source and the best regimen for cytoreduction prior to SCT. The existence of a Graft-versus-Lymphoma effect has been reported from many animal studies, but at the clinical level, evidence is largely anecdotal. The impact of this effect on outcome for patients undergoing allogeneic SCT for lymphoma is unknown.

The recent improvement in first line therapy for lymphoma raises questions concerning the use of SCT, even in situations which are the current standard of care. For example, using monoclonal antibody therapy in the treatment of B-cell NHL has improved the effectiveness of first line therapy. The effectiveness of HDT strategies to 'salvage' patients relapsing after these therapies is unknown.

The role of SCT as a first line therapy will need to be revisited for certain types of B-cell NHL, for which rituximab/chemotherapy combinations have become a new standard in recent years. Recent studies have suggested that the use of functional imaging during first line treatment may be predictive of subsequent outcome and may, therefore, be a useful modality to identify patients in whom early intensification of therapy with SCT may be beneficial. This requires prospective evaluation.

Despite these uncertainties, new data are beginning to define the impact of SCT in the lymphomas more clearly. Clinical trials have identified patients for whom SCT may produce improved disease free or overall survival compared

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with standard therapy. New studies are exploring novel treatment approaches including the incorporation of radioimmunotherapy into transplant regimens, and manipulation of the immune system in the peri-and post-transplant settings to reduce relapse.

2. Established Indications for High Dose Therapy and SCT

2.1. Relapsed Hodgkin's Lymphoma

Hodgkin's lymphoma (HL) affects approximately 8,000 people each year in the United States. Current conventional chemotherapy and radiotherapy regimens cure 75 percent to 85 percent of patients with early stage disease, and 65 percent to 80 percent of patients with advanced disease. Reported results of conventional dose salvage therapy for patients with HL who relapse after initial chemotherapy have been poor. Early studies conducted in patients relapsing after treatment with MOPP (mechlorethamine, vincristine, procarbazine and prednisone) chemotherapy showed that retreatment with MOPP resulted in second complete remission rates of about 50 percent, with a median remission duration of 21 months [1]. The strongest predictor of outcome in this series was the duration of the initial remission.

Long-term survival was 24 percent for those with an initial remission longer than one year, and 11 percent for those with an initial remission less than one year. Despite an estimated 20-year disease free survival (DFS) of 45 percent, OS was compromised by secondary leukemia and other treatment-related complications. Patients with an initial remission duration of less than 12 months had a significantly worse progression-free and overall survival than those with longer initial remissions. However, even in the 'favorable' group, the long-term outcome was poor. In the NCI series, although 93 percent of patients relapsing after an initial remission of over 12 months obtained a second complete remission, only 17 percent of these patients were alive at 20 years. For patients with an initial remission of less than 12 months duration, only 29 percent achieved a second CR. Similar results were reported from the Milan group, using ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) [2]. In their series, the eight-year OS for relapsed HL patients treated with salvage chemotherapy was 54 percent, 28 percent, and 8 percent for patients with relapse after one year, within one year and with induction failure respectively.

In a randomized study from the Cancer and Leukemia Group B (CALGB), patients who relapsed after initial therapy with ABVD, and retreated with MOPP, had a five-year failure free survival rate of only 31 percent, compared with 15 percent for those receiving MOPP first line and ABVD at relapse [3].

Early single institution and registry-based studies reported superior results for patients treated with HDT and ASCT. Various single institution series reported long-term disease free survival of between 40 percent and 65 percent for patients receiving ASCT at first relapse [4–7]. In a study from the European Group for Blood and Marrow transplantation (EBMT), 45 percent of 139 patients undergoing ASCT at first relapse were in continuing second remission at five years [8].

In a study from Stanford University, the outcome for 60 patients with relapsed or refractory Hodgkin's disease receiving HDT and ASCT was compared

with a matched group of patients receiving conventional dose salvage therapy [5]. Four-year event-free survival (EFS) and freedom from progression (FFP) were higher in the ASCT group (53% versus 27% for EFS; 62% versus 27%, for FFP). Although four-year actuarial overall survival was not significantly different between the two groups (54% for high dose versus 47% for conventional dose),a difference in favor of high-dose therapy was observed for the subset of patients relapsing within 12 months of initial chemotherapy. Two randomized trials have confirmed the superiority of HDT and ASCT over conventional dose therapy for patients with relapsed HL. In the British National Lymphoma Investigation (BNLI) study, 40 patients in first or subsequent relapse were randomized to receive HDT with BEAM (carmustine, etoposide, cytarabine, melphalan) or conventional dose therapy, using the same drugs ('mini BEAM') [9]. A significant difference in event-free survival was observed for patients receiving BEAM (three-year EFS = 53% for BEAM versus 10% for mini-BEAM, p = 0.025), although no overall survival difference was observed. However, since some patients who relapsed after mini BEAM were treated at relapse with BEAM and ASCT, the impact of high-dose therapy on overall survival is difficult to interpret.

In a larger randomized study by the German Hodgkin's Lymphoma Study Group 161 patients with relapsed HD were randomized between two cycles of Dexa-BEAM (dexamethasone, BCNU, etoposide, cytarabine, melphalan) followed either by two further cycles of Dexa-BEAM or HDT and auto-SCT [10]. Patients continued on the protocol only if they had chemosensitive disease (i.e., achieved a PR or CR with the initial two cycles of Dexa-BEAM). Freedom from treatment failure at three years for the chemosensitive patients was significantly improved in the SCT arm (55% versus 34%, p= .019) while OS did not differ significantly between treatment arms (71% versus 65%, p= .331). Again, the failure to demonstrate an overall survival difference is related to the 'crossover' of patients who relapsed on the conventional dose arm and were salvaged by ASCT. These results have recently been updated, with median follow-up now to 83 months [11]. The seven-year FFTF rate was higher in the SCT arm (32% versus 49%). No overall survival difference was observed (56% versus 57% respectively at seven years). The difference in FFTF was seen for patients in early and late first relapse, but no FFTF difference was observed for multiply relapsed patients.

The results of these studies have established HDT and ASCT as the standard of care for patients with relapsed HL after a prior chemotherapy regimen such as MOPP or ABVD, irrespective of the duration of the initial remission. However, with the advent of multidrug dose dense and dose-intensive chemotherapy regimens for initial treatment of advanced HL, the role of HDT and ASCT for relapsing patients is becoming less certain. The Stanford V and escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) regimens both result in high rates of remission and disease free survival, even for poor risk patients with advanced HL [12, 13]. In the phase II study from Stanford, five-year actuarial progression-free and overall survival rates of 89 percent and 96 percent were observed. Of 142 patients treated with this regimen, 16 subsequently relapsed, of whom 11 underwent high-dose therapy and ASCT [12]. The freedom from second relapse in the entire group of 16 patients was 69 percent at five years, suggesting that relapsed patients had a high rate of salvage. The ability to 'salvage' patients who relapse after the BEACOPP regimen is not yet determined.

3. Refractory Hodgkin's Lymphoma

Patients who fail to enter remission with first line chemotherapy have a poor outlook. In a study from the Milan Cancer Institute 29 patients who failed to enter remission after first line therapy with MOPP/ABVD were treated with the CEP (lomustine, etoposide, prednimustine) regimen [2]. Forty-one percent of these patients achieved a complete remission with second line therapy, but the five-year actuarial overall survival was only 12 percent, and all of the surviving patients had evidence of active disease at the time of the report. In a report form the National Cancer Institute, similar results were reported in 51 patients who failed to enter remission after MOPP chemotherapy [1]. Their median overall survival was only 16 months. Comparable results have also been reported from Stanford University, where the four-year overall survival for 29 patients with primary refractory Hodgkin's disease was 38 percent, with a corresponding four-year progression-free survival of only 19 percent [5].

Several studies have addressed the role of HDT and ASCT in this setting. No randomized trials have been performed, and data has, therefore been available only from single institution and registry-based studies. A six-year actuarial progression-free survival rate of 33 percent was reported for 46 patients with primary refractory disease who were treated with BEAM and ASCT at University College Hospital, London, and a similar figure was reported from Memorial Sloan Kettering Cancer Center [7, 14]. At Stanford University, the four-year overall and freedom from progression rates after high-dose therapy were 44 percent and 52 percent respectively. These results were significantly superior to a matched group treated with conventional dose therapy. In a series from the EBMT, patients treated with high-dose therapy and ASCT after failure of induction therapy had a five-year actuarial overall and progression-free survival rate of 36 percent and 32 percent respectively [15]. Very similar results were also reported in a series from the Autologous Blood and Marrow Transplant Registry of North America (ABMTR) [16].

Despite the apparent superiority of high-dose therapy in this setting, these results must be interpreted cautiously. This is partly due to the retrospective nature of all these series, and the potential patient selection, but also because of the definition of refractory disease. Assessment of response in Hodgkin's disease can be problematic, particularly in patients with extensive disease in the mediastinum. The failure to achieve a complete clinical and radiological remission after induction therapy does not necessarily imply that active disease is still present within the residual mass (see above).

Therefore, the group of patients who have stable, or only minimally responsive disease after induction therapy may be distinct from those patients who have obvious disease progression during induction therapy. This issue has only been addressed in one of the retrospective studies to date. In the EBMT series, there was no difference in outcome after high-dose therapy for patients with obvious disease progression on first line therapy, compared with those with stable or only minimally responsive disease [15]. However, the activity of high dose therapy in this setting requires prospective assessment using modern imaging techniques to determine whether an active tumor is present in residual masses.

Functional imaging techniques such as fluorodeoxyglucose positron emission tomography (FDG-PET) may be useful in this context.

The use of early FDG-PET scanning in patients with advanced HL may also lead to a redefinition of refractory disease. Two recent, but preliminary studies have shown that the result of a PET scan performed after two cycles of ABVD in patients with advanced HL is very predictive of subsequent relapse [17, 18]. If confirmed, it is likely that future studies will select patients for intensifed therapy based on the results of an early interim PET scan. The use of HDT and ASCT may be investigated as a potential strategy for intensified therapy in this context.

4. Prognostic Factors after HDT and ASCT for Hodgkin's Lymphoma

Several published series have identified high risk patients with relapsed or refractory HL who may gain the most benefit from SCT [4, 19–22]. In view of the relatively small size of these series, no consistent prognostic factors have emerged. Those identified include performance status, B symptoms at relapse, chemoresponsiveness at time of relapse, disease status at the time of transplant, remission duration, tumor bulk at relapse and extranodal relapse. Disease status at the time of transplant has been the most consistently identified factor across most series. The predictive value of prior remission duration is less clear.

Several groups have published prognostic indices for relapsed and refractory HD patients undergoing SCT (Table 6-1). In a series from Stanford University, disseminated disease, B symptoms, and greater than minimal disease at the time of SCT were identified as high risk factors [4]. Patients with zero, one, two or three risk factors had a three-year FFP of 85 percent, 57 percent, 41 percent and <20 percent, respectively. In another series, more than one extranodal site of relapse, poor performance status and progressive disease at the time of HDT correlated with outcome. Patients with zero, one, or ≥ two factors had a three-year OS of 82 percent, 56 percent, and 19 percent, respectively after HDT and auto-SCT.

A recent study from Vancouver has reported 20-year follow-up for 100 patients with relapsed and refractory HL undergoing HDT and ASCT [23]. For the entire patient population, the only predictive risk factor in multivariate analysis was exposure to at least two prior chemotherapy regimens. For those patients undergoing ASCT in first relapse, this factor retained predictive value, along with extranodal disease at the time of relapse. Recent data suggests that using functional imaging immediately prior to high-dose therapy and ASCT

Table 6-1. Prognostic factors for outcome after high-dose therapy and ASCT for Hodgkin Lymphoma.

Reference	Prognostic factors
4	B symptoms at relapse, pulmonary or bone marrow involvement
6	B symptoms at relapse, extranodal involvement at relapse, initial remission duration <1 year
20	International prognostic score
23	≥2 prior chemotherapy regimens, extranodal disease at relapse

may have predictive value for outcome after transplantation. Moskowitz has reported experience in 152 patients with relapsed or refractory Hodgkin's Lymphoma who responded to second line chemotherapy with ICE (ifosfamide, carboplatin, etoposide) [24]. Functional imaging was performed in all of these patients after completing their salvage chemotherapy regimen, prior to high-dose therapy and ASCT. Patients treated in the mid- to late 1990s were evaluated by gallium scanning, and later patients underwent FDG-PET scanning. In this study, functional imaging performed immediately prior to ASCT was highly predictive of subsequent event-free survival. The five-year EFS for patients with negative and positive functional imaging results were 74 percent and 29 percent, respectively. Since recent data suggest that PET has a higher sensitivity and specificity compared with gallium scanning, it is likely that it will provide higher predictive value in the future. The use of PET may, therefore, identify a group of patients with a very poor outcome after ASCT, in whom experimental approaches may be justified.

The same group has also developed a clinical prognostic model for patients with relapsed HL undergoing ASCT and has used a risk-adapted approach in which patients with poor risk disease receive a more dose-intensive version of the ICE regimen prior to ASCT. They report an apparent improvement in outcome for the poor risk group using this approach, although this represents small single institution series with relatively short median follow-up. Further follow-up and a prospective evaluation of this approach will be required.

5. Relapsed Diffuse Large B-Cell Lymphoma

The use of high dose therapy and ASCT has been regarded as the standard of care for patients with relapsed diffuse large B-cell (DLBCL) and some other types of diffuse aggressive lymphoma for over 10 years. This is based largely on the results of the PARMA randomized trial [25]. This study included 215 patients with relapsed aggressive NHL (mostly DLBCL) who had received adriamycin-based chemotherapy as their initial treatment. These patients were initially treated with two cycles of salvage chemotherapy with DHAP (dexamethasone, high dose cytosine arabinoside, cisplatin) and responding patients were randomized to receive further DHAP chemotherapy, or to proceed to high-dose therapy using BEAC (carmustine, etoposide, cytarabine, cyclophosphamide) and autologous bone marrow transplantation. Patients with bone marrow or central nervous system involvement were excluded from this trial, as were patients over 60 years of age. Significantly superior five-year event-free (46 percent versus 12%, $p=0.0001$) and five-year overall survival (53% versus 32%, $p = 0.038$) rates were observed for the transplant arm compared with the conventional chemotherapy arm. No formal follow-up analysis of the PARMA study has been published. Although this study established ASCT as the standard approach for patients with relapsed chemosensitive DLBCL, the results should be interpreted cautiously. Of the 215 patients entered onto the study, only 109 were randomized, most commonly because the patients did not achieve an adequate response to second line therapy with DHAP (only 56% of patients responded to this chemotherapy). All subsequent survival analyses were restricted to randomized patients only, and no intent to treat analysis was performed. The study was eventually closed due to poor accrual. No statistical

power calculations were provided for the trial and, in view of the poor accrual, it is not clear whether it was adequately powered to detect significant differences in event-free of overall survival.

In addition to these limitations, the relevance of the study in the present context is unclear. Improved supportive care, including the use of peripheral blood progenitor cells, has reduced the morbidity associated with high dose therapy and extended its use to older patient groups, typically up to 70- or 75-years-old. Using PBPCs has also reduced the necessity for an uninvolved marrow at the time of harvesting. The population of patients now receiving ASCT is, therefore, less defined than that in the original PARMA study, raising questions concerning the current relevance of this trial. The development of new effective first line treatments, particularly the addition of rituximab to combination chemotherapy regimens, has improved disease free and overall survival in DLBCL [26, 27]. It is not clear whether patients whose disease relapses after one of these regimens will have the same salvage rates as those treated without monoclonal antibodies as part of their initial treatment. A recent retrospective comparison of outcomes following ASCT for patients receiving first line CHOP or CHOP-rituximab has recently been reported from the University of Nebraska [28]. No difference in event-free or overall survival following ASCT was observed according to initial therapy, although the total study population was only 103 patients. A similar study from our center reported similar findings [29].

High response and survival rates have also been reported for dose intensive and dose dense regimens for DLBCL, including CHOP-14 and the dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab) [27, 30]. It is not clear whether high-dose therapy and ASCT will be effective salvage therapy for patients relapsing after these regimens. A recent report of dose adjusted EPOCH-R as primary therapy in DLBCL has shown a two-year progression-free survival rate of 83 percent with a very similar overall survival, indicating the high activity of this regimen, and the apparent inability to salvage relapsed patients with a standard transplant approach [30].

Although high-dose therapy and ASCT remains the standard of care for patients with relapsed DLBCL which is still sensitive to second line chemotherapy, the true benefit of this approach in the context of modern first line therapies is unclear and requires reevaluation.

6. Prognostic Factors for Relapsed DLBCL

Multiple early single institution and registry studies of ASCT in aggressive NHL demonstrated the importance of disease sensitivity to second line therapy as a predictive factor for outcome after transplantation. Short (less than one year) remission duration, and disease bulk at the time of ASCT were also identified as adverse factors in many studies.

The age adjusted International Prognostic Index (aa-IPI) has predictive value, according to a follow-up report of the PARMA study [31]. The aa-IPI at the time of relapse was available for 204 of the original 216 patients. It proved highly predictive of response to DHAP. Patients with an aa-IPI score of zero had an overall response rate of 77 percent compared to only 42 percent for

those with three adverse factors. The aa-IPI was predictive of overall survival for the entire patient cohort.

When randomized patients were analyzed separately, the aa-IPI was predictive for those receiving DHAP, but not in those undergoing ASCT. In a subset analysis, there was no difference in overall or progression-free survival according to randomized arm for patients with an aa-IPI score of zero, although a significant difference remained for those with scores of one to three.

Hamlin, et al. reported results in 150 patients with relapsed and refractory DLBCL receiving ICE chemotherapy followed by high-dose therapy and ASCT [32]. This study confirmed the predictive value of the aa-IPI in this situation. When analyzed by intent to treat, patients with a score of two or three at the time of relapse had four-year progression-free and overall survival rates of 16 percent and 18 percent, respectively, compared with 70 percent and 74 percent for those with a score of zero.

These subset analyses should be interpreted cautiously, but question whether ASCT offers a survival advantage to low risk patients. In both studies, results are poor in the high risk patients, underlining the need for novel strategies.

7. Other Indications for High Dose Therapy and SCT

7.1. Hodgkin's Lymphoma in First Remission

The use of standard chemotherapy regimens such as ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) for patients with advanced stage Hodgkin's Lymphoma results in long-term disease free survival rates of 60 percent to 70 percent in most large cooperative group studies. Despite these relatively favorable results, various groups have identified poor risk patients with Hodgkin's Lymphoma, with lower projected survival rates using conventional first line chemotherapy regimens. The International Prognostic Factors Project in Advanced Hodgkin's Disease identified seven adverse clinical prognostic factors [33]. The probability of freedom from progression at five years was noted to be predictably lower in relation to the number of adverse factors present. Patients with no adverse factors had a five-year FFP rate of 84 percent compared with only 42 percent for those with four or more risk factors. Several other groups have also produced predictive models, but these have been based on smaller patient numbers, and the International Prognostic System (IPS) above has now become widely accepted. Several studies of high-dose therapy and ASCT for patients with poor risk Hodgkin's Lymphoma have been reported, although the definition of poor risk has varied between these studies, none of which has used the IPS criteria. None of these studies has shown an advantage in progression-free or overall survival for early ASCT. In the largest of these studies 163 patients with poor risk disease (defined according to criteria reported from Memorial Sloan Kettering Cancer Center), achieving complete or partial remission after four cycles of ABVD or similar anthracycline-based chemotherapy, were randomized to high-dose therapy and ASCT (83 patients) or four further cycles of chemotherapy [34]. With a median follow-up of four years, the five-year failure-free survival rates were 75 percent for the transplant arm, compared with 82 percent for the conventional therapy arm ($p = 0.4$). The corresponding overall survival rates were 88 percent versus 88 percent ($p = 0.99$). No benefit was shown for early intensification of therapy.

There is no role for the early use of high-dose therapy and ASCT in advanced Hodgkin's Lymphoma. In addition to the negative results from randomized trials of this approach, the advent of new dose dense and dose intensive regimens in advanced HL has apparently resulted in higher rates of response and tumor control. In studies using the dose escalated BEACOPP regimen, the predictive value of the IPS was lost, suggesting that this intensive regimen had overcome the prognostic significance of the adverse factors identified in the IPS [13].

Future studies may include gene expression profiling or proteomic studies to identify a subgroup of patients with HL with a very poor risk disease at presentation. If so, the potential use of early high-dose therapy may be explored in this group. However, this is likely to represent a very small minority of patients with HL, and the likelihood of conducting a randomized trial of sufficient statistical power to study this issue is small.

8. Diffuse Large B-Cell and Other Aggressive B-Cell Lymphomas in First Remission

Although the use of CHOP and related chemotherapy regimens produces response rates of 70 percent to 80 percent for patients with advanced DLBCL, and long-term DFS rates of 45 percent to 60 percent, a large proportion of patients will either fail to respond to this therapy, or will relapse after achieving a remission. In view of the apparent effectives of high-dose therapy and ASCT as a salvage treatment in aggressive NHL, many groups have explored the potential use of ASCT as a component of first line therapy, particularly for patients identified as having poor risk disease at presentation. Although many trials are now published, inclusion criteria have been variable, as has the definition of poor risk disease, and the type of first line therapy [35–39]. Additionally, some studies have reported retrospective, subset analyses of clinical trials which were not initially stratified according to risk groups. These studies were, therefore, not designed and not powered to detect differences in outcome, so the conclusions must be interpreted cautiously.

One of the largest reported studies from the Groupes D'Etudes Lympomes Aggressif (GELA) in France recruited 916 patients with diffuse aggressive NHL. The patients were all less than 55 years and were defined as high risk according to factors which pre-dated the development of the aa-IPI. Of the original 916 entering the study, 464 achieved CR and were randomized between a conventional dose sequential consolidation protocol, or high-dose therapy and ASCT [40]. For all patients, the three-year DFS was 52 percent for ASCT arm, compared with 59 percent for the conventional dose arm. The corresponding overall survival rates were 71 percent versus 69 percent. A subsequent retrospective subset analysis, restricted to patients with high or high-intermediate risk disease according to the aaIPI, reported an eight-year DFS and OS of 55 percent and 64 percent, respectively, for the ASCT arm compared with 39 percent and 49 percent for the conventional dose arm.

Since this report, multiple prospective studies have been conducted, with eligibility defined according to the aaIPI. Most of these studies have been restricted to high or high-intermediate risk patients according to the aaIPI (Table 6-2). Results have been variable, although most studies have shown equivalent long-term DFS and OS rates for conventional versus high-dose consolidation therapy in this setting.

Table 6-2. Results of HDT and ASCT in first remission for DLBCL and other aggressive NHLs.

Reference	n	Randomization	DFS (conventional chemotherapy vs SCT)	OS (conventional chemotherapy vs SCT)
35	464	Sequential chemotherapy vs HDT and ASCT in patients in CR after induction chemotherapy	52% vs 59% at 3 years (p = 0.46)	71% vs 69% at 3 years (p = 0.6)
36	124	VACOP-B vs VACOP-B plus HDT and ASCT for responding patients	60% vs 80% at 6 years (p = 0.1)	65% vs 65% at 6 years (p = 0.5)
37	194	CHVmP/BV vs CHVmP/BV plus HDT and ASCT for responding patients	56% vs 61% at 5 years (p = 0.712)	77% vs 68% at 5 years (p = 0.336)
38	98	MACOP-B vs high dose sequential therapy including HDT and ASCT	49% vs 76% at 7 years (p = 0.004)	55% vs 81% at 7 years (p = 0.09)
39	370	ACVBP and sequential consolidation vs intensive induction chemotherapy plus HDT and ASCT	76% vs 58% at 5 years (p = 0.004)	60% vs 46% at 5 years (p = 0.007)
41	207	CHOP vs intensive induction chemotherapy plus HDT and ASCT	37% vs 55% at 5 years (p = 0.037)	44% vs 74% at 5 years (p = 0.001)

A recent randomized study from France compared eight cycles of CHOP chemotherapy with two cycles of a more dose intensive first line regimen, followed by high-dose therapy and ASCT for responding patients with advanced diffuse aggressive NHL [41]. Eligible patients were aged 15- to 60-years-old with low, low-intermediate or high-intermediate risk disease according to the aaIPI (a maximum of two adverse risk factors).

Of the 207 patients entered onto this study, 197 were randomized, and 78 percent of patients completed protocol therapy. Survival analyses were conducted by intention to treat. When all randomized patients were analyzed, there was a significantly higher EFS in the high-dose therapy arm (55 percent versus 37%, p = 0.037), although there was no corresponding difference in OS. On subset analysis, a significant difference in OS was seen in the patients with high-intermediate risk disease (p = 0.001). Although interesting, the results of this study

are difficult to interpret in the current context, since the patients did not receive rituximab-based therapy as a component of their first line treatment.

A meta-analysis of HDT and ASCT as a component of first line therapy of diffuse aggressive NHL included 3,079 patients from 15 randomized trials for toxicity data, and 2,018 patients from 13 trials for outcome data [42]. Although a significantly higher CR rate was reported for HDT and ASCT, no differences in EFS or OS were observed. A subgroup analysis according to IPI did not detect a difference in outcome according to risk group, and analyses according to the number of patients with DLBCL, transplant conditioning regimen and response status prior to ASCT also failed to identify a group with superior outcome after HDT.

Based on these results, HDT and ASCT should not be considered a component of first line therapy in patients with diffuse aggressive lymphoma (including DLBCL), irrespective of IPI risk group. Since most of the studies reported above were conducted prior to the introduction of rituximab, or dose dense chemotherapy regimens, their relevance to current management of diffuse aggressive NHL is unclear. The ongoing Southwest Oncology Group 9704 study may be informative in this respect. In this study, patients with diffuse aggressive NHL who are aged 60 or less with high-intermediate or high risk disease according to the aaIPI are randomized to receive eight cycles of CHOP-rituximab therapy alone, or the same therapy followed by HDT and ASCT. This study originally used CHOP chemotherapy alone, but was modified to incorporate rituximab early in its accrual. This is likely to represent a definitive study for the role of first remission ASCT in patients receiving rituximab-based first line therapy.

9. 'Slowly Responding' Diffuse Aggressive NHL

Early studies of combination chemotherapy in aggressive NHL identified slow response to chemotherapy as an adverse factor. Patients who had not achieved a clinical complete response after three or four cycles of chemotherapy had a poor outcome compared with those already in CR. As a result, several studies have examined the use of early high-dose therapy and ASCT for patients not in complete remission early in the course of induction chemotherapy [43–45]. Results of these studies are summarized in Table 6-3. Early intervention with ASCT in slowly responding patients does not seem to improve outcome.

Table 6-3. Results of studies of HDT and ASCT in 'slowly responding' patients with DLBCL and other aggressive NHLs.

Reference	n	Randomization	DFS (conventional chemotherapy vs SCT)	OS (conventional chemotherapy vs SCT)
44	65	CHOP vs HDT and ASCT if in PR after 3 initial cycles of CHOP	85% vs 56% at 4 years (NS)	72% vs 72% at 4 years (NS)
45	49	DHAP vs HDT and ASCT after PR to F-MACHOP or MACOP-B	52% vs 73% at 3 years (NS)	59% vs 73% at 3 years (NS)

With the advent of functional imaging techniques, particularly PET scanning (see above) this issue may need to be re-addressed. Results of PET scanning after one cycle of chemotherapy have been shown to be highly predictive of subsequent relapse-free and overall survival [46–48]. A positive PET scan after one or two cycles of chemotherapy reveals the presence of relatively chemo-resistant tumor cell clones. It is possible that very early intensification of therapy in this situation might overcome chemotherapy resistance.

10. Follicular Lymphoma in First Remission

Several single center and registry-based studies of high-dose therapy and ASCT have been reported in recent years. Results from these studies are summarized in Table 6-4. These results have been variable, but favorable compared with standard initial therapy approaches, although they must be interpreted with caution in view of the potential for referral, selection and reporting bias intrinsic to phase II retrospective studies of this type [49–52].

Three multicenter phase III randomized trials have now been reported. The German Low Grade Lymphoma Study Group (GLSG) conducted a randomized trial comparing HDT and ASCT with α-interferon maintenance in patients with advanced low grade follicular NHL initially treated with CHOP or a CHOP-like induction regimen [53]. Three–hundred-seventy-five eligible patients entered this study, initially receiving two cycles of induction chemotherapy. Patients were randomized after two cycles of therapy between ASCT and α-interferon, but proceeded with two to four more cycles of induction therapy prior to receiving their assigned treatment according to randomization. Patients with disease progression were withdrawn from the study. Of the 375 patients entered, only 279 initiated consolidation therapy, most being excluded from analysis because of ineligible histology. Two-hundred-forty evaluable patients were reported. Consolidation with ASCT resulted in a higher progression-free survival rate than α-interferon (64.7% versus 33.3% at five years, $p = 0.0001$); this effect was largest in patients in PR at the time of consolidation therapy. At the time of this publication, overall survival was not reported since the follow-up time was thought to be too short. Despite the apparently encouraging preliminary results of this study, it is important to note that all survival analyses were performed from the date of completing induction therapy, rather than from the date of study entry. Therefore, no true intent to treat analysis was performed and the study population is potentially biased in favor of patients responding to first line therapy. Additionally, over 90 percent of the patients entered onto this study

Table 6-4. Results of retrospective studies of HDT and ASCT for follicular lymphoma in first remission.

Reference	n	DFS	OS	Follow-up (years)
49	37	86%	97%	10
50	77	66%	89%	3
51	92	67%	84%	4
52	27	55% (RFS)	64%	6

had IPI low risk or low-intermediate risk disease. Comparable PFS rates have been reported for this favorable patient group with other less intensive first line therapies, and more recently with the use of radiolabeled monoclonal antibodies.

The GOELAMS study group in France has assessed the impact of early consolidation with ASCT in a randomized study comparing two distinct treatment approaches [54]. One–hundred-seventy patients were randomized to receive either chemo-immunotherapy with CHVP with interferon versus VCAP and IMVP16 followed by HDT (using cyclophosphamide and TBI). *Ex vivo* manipulation of stem cell products was performed, either by CD34 selection, or negative selection using immunological methods.

Patients in the high-dose arm of this study had a higher response rate (69% versuss 81%, p = 0.045). Five-year EFS rates also favored the transplant arm at 48 percent versus 60 percent (p = 0.05), but this advantage was restricted to patients with high risk disease, according to the FLIPI.

No overall survival difference was observed according to treatment arm, largely because of a high rate of second malignancies in the transplant arm of the study. The GELA group has recently reported results of a randomized trial in which untreated patients with follicular lymphoma were randomized between CHVP plus interferon-α or four cycles of CHOP followed by high-dose therapy with cyclophosphamide, etoposide and TBI followed by ASCT [55]. The study was limited to patients with high tumor burden. Four-hundred-one patients entered the study. Analyses were performed according to intent to treat. The seven-year EFS rates were 45 percent for the ASCT arm compared with 36 percent for the chemotherapy/interferon arm (p = 0.5). A longer overall survival was seen in the transplant arm, which just achieved statistical significance (seven-year OS = 74 percent for chemotherapy arm versus 84 percent for transplant arm, p = 0.05). The apparent discrepancy between the results for OS and EFS in this study is probably explained by the duration of therapy in the conventional chemotherapy arm.

The possibility of inducing excessive rates of second malignancy has been a limitation in the widespread use of early high dose-therapy and ASCT in follicular lymphoma. Results from the three studies above give varying rates of second malignancy, from 0 to 8.5 percent.

These three large randomized studies have shown apparent superiority over early high-dose therapy in terms of EFS, although this has not been reflected in improved overall survival, partly due to excess rates of second malignancy, and partly because patients relapsing after either form of treatment can obtain further remissions from subsequent therapy. The potential use of first remission ASCT must now be assessed in the context of antibody-based therapy as first line treatment in follicular lymphoma. Various rituximab-chemotherapy combinations have now been assessed in first line therapy of follicular lymphoma with long first remissions [56, 57]. Additionally, the use of radiolabeled monoclonal antibodies such as ^{131}I-tositumomab, both alone and in combination with chemotherapy, have been shown to produce high rates of disease free survival [58, 59]. Therefore the use of high-dose therapy and ASCT must be re-evaluated in the setting of these new and highly effective first line regimens, and should still be considered experimental at present.

11. Relapsed Follicular Lymphoma

Several single centers and collaborative groups have reported results of phase II studies that explored the use of high-dose therapy and ASCT in relapsed or refractory follicular lymphoma [60–63]. In general, these studies have included highly selected patient populations, particularly with respect to age and responsiveness to conventional dose chemotherapy given prior to ASCT. Most of the published studies have required patients to be in a state of 'minimal residual disease' (variably defined) prior to undergoing HDT and ASCT. Hence, none of these studies has incorporated an intent to treat analysis since those patients who do not respond adequately to pre-transplant therapy are excluded. The results of some of these studies are summarized in Table 6-5. Although most of these studies have reported encouraging disease free and overall survival rates, they are difficult to interpret one view of the highly selected patient populations.

A single, small randomized trial has been performed in only 89 patients with relapsed follicular lymphoma [64]. Relapsed patients were initially treated with CHOP or a similar regimen and responding patients were randomized between continuing CHOP, ASCT with an un-manipulated stem cell product, or ASCT with purging of the stem cell product. At a median follow-up of 26 months relapse rates significantly improved in patients receiving ASCT compared with conventional chemotherapy, but there was no difference according to manipulation of the stem cell product. Interpretating these results is limited by the small sample size and imbalances in clinical factors between the three arms of the trial. Additionally, since the trial was conducted before the introduction of rituximab, it does not clarify the role of ASCT in the present context. Further studies are required to clarify the role of transplant strategies in relapsed follicular NHL.

12. Mantle Cell Lymphoma

Since patients with advanced stage mantle cell lymphoma (MCL) are known for poor prognosis, high-dose therapy and ASCT, has been investigated by several groups as a component of first line therapy, and for patients with relapsed or refractory disease.

Results of studies in patients with relapsed or refractory disease are summarized in Table 6-6 [65–68]. These series are heterogeneous with respect to extent and nature of previous treatment, type of high-dose regimen, status of disease at the time of transplant and whether or not stem cell products were

Table 6-5. Results of retrospective studies of HDT and ASCT for relapsed and refractory follicular lymphoma.

Reference	n	DFS	OS	Follow-up (years)
60	153	42%	66%	8
61	100	44%	65%	4
62	99	63% (FFR)	69%	5
63	597	31%	55%	5

Table 6-6. Results of retrospective studies of HDT and ASCT for relapsed and refractory mantle cell lymphoma.

Reference	n	EFS	OS	Follow-up
65	150	30%	48%	3
66	28	31%	62%	4
67	80	39%	47%	5
68	20	17%	25%	N/A

manipulated *ex vivo* prior to reinfusion. Additionally, some series did not include routine central histologic review and since diagnostic criteria for MCL have been refined in the last few years, the consistency of diagnosis in some of the earlier studies is doubtful. The significance of these results is difficult to interpret for several reasons. The small patient numbers and heterogeneous populations limit comparisons with non-transplant series. Since there is no clearly defined optimal first line therapy or second line therapy for MCL, the results cannot be compared with a standard approach. As with other subtypes of B-cell NHL, the effectiveness of ASCT may differ in patients previously treated with monoclonal antibodies, particularly rituximab.

Some studies have also investigated the use of high-dose therapy and ASCT as a component of first line therapy in MCL in view of the disease's poor prognosis with conventional chemotherapy. Particularly favorable results have been reported from the MD Anderson Cancer Center for an induction regimen comprising HyperCVAD alternating with high-dose methotrexate and cytarabine, followed by high-dose therapy with autologous or allogeneic SCT for responding patients [68]. Three-year EFS and OS rates of 72 percent and 92 percent, respectively, were reported, which are substantially higher than results reported for conventional dose induction regimens such as CHOP. Whether the superior results of this regimen are related to higher effectiveness of HyperCVAD, first remission SCT or patient selection factors is unclear.

A recent randomized trial from the European MCL Network has investigated the role of ASCT in first remission in this disease [69]. In this study 230 eligible patients with MCL were randomized to receive either six to eight cycles of CHOP-like chemotherapy followed by interferon-α maintenance, or four to six cycles of CHOP-like chemotherapy followed by high-dose therapy (cyclophosphamide and TBI) with ASCT. Only patients in complete or partial remission after four to six cycles of CHOP continued on protocol to receive their assigned therapy. Those with no response to first line therapy were taken off study. Patients randomized to the ASCT had longer PFS (median PFS + 39 months for the ASCT arm compared with 17 months for the conventional dose arm, $p = 0.0108$), but no difference in overall survival was observed. Unfortunately, all survival analyses in this study were calculated from the date of completion of induction therapy. Therefore, no intent to treat analysis was included, making the results difficult to interpret. None of the patients in this study received rituximab as a component of induction therapy, which also makes the results difficult to interpret in the current context. The potential effect of rituximab as part of the induction regimen in MCL is illustrated by experience from MD Anderson Cancer Center in which the HyperCVAD regimen was modified to incorporate rituximab [70]. Preliminary results in 37 patients treated with this regimen showed an overall response rate of 97 percent

with only two patients relapsing after a median follow-up of eight months. In this study, patients in CR after rituximab/HyperCVAD therapy did not proceed to high-dose therapy, but the outcome was comparable to patients in the previous series, all of whom had received allogeneic or autologous SCT. These results raise the possibility that early therapy with rituximab may improve the effectiveness of first line therapy and limit the value of ASCT in first remission. These results are currently being confirmed in a multicenter phase II study of the Southwest Oncology Group.

13. Burkitt's and Atypical Burkitt's Lymphoma

The use of HDT and ASCT has been investigated for Burkitt's and atypical or Burkitt-like lymphoma as a component of first line therapy, and for the treatment of relapsed and refractory disease. Interpreting results of ASCT in this disease is complicated by the varying classification of Burkitt's, and more recently Burkitt-like or atypical Burkitt's lymphoma. The largest series investigating the role of high-dose therapy and ASCT has been reported by the European Group for Blood and Marrow Transplantation (EBMT) [71]. This was a retrospective analysis of 117 cases of Burkitt's and Burkitt-like lymphoma. A three-year overall survival rate of 72 percent was reported for patients undergoing ASCT in first complete remission. Despite this apparently favorable result, recent studies of dose intensive first line chemotherapy regimens for BL have reported comparable and, in some cases, superior results suggesting that these regimens should be used in preference to first remission ASCT. No direct comparative studies of these two approaches have been conducted. First remission ASCT should be considered for patients who do not receive a very intensive first line regimen such as CODOX-M/IVAC, but otherwise has no proven role.

The EBMT series reported a 37 percent three-year overall survival rate for patients with BL transplanted in chemosensitive relapse. No studies comparing this approach to conventional dose salvage have been conducted. However, previous reports from single centers using conventional dose salvage regimens have shown very poor results, suggesting that ASCT should be considered standard of care for patients with chemosensitive relapse of their disease. For those with chemoresistant relapse, the three-year overall survival rate was only 7 percent, suggesting no clear role for ASCT in this situation.

14. Peripheral T Cell Lymphomas

Only limited data have been reported for the use of ASCT in patients with peripheral T cell lymphomas (PTCLs). Interpreting these data is difficult in view of the poor understanding, and marked heterogeneity of PTCL, and the relative rarity of these diseases. Vose, et al. have previously reported outcome for 41 patients with recurrent aggressive NHL, 17 of whom had T cell disease [72]. No difference in two year OS or EFS was observed according to immunophenotype. Single institution series of ASCT for PTCL have reported results generally comparable to those reported for aggressive B-cell lymphomas [73, 74]. In a study from MD Anderson Cancer Center, 36 patients with relapsed or refractory PTCL underwent SCT, seven of whom had allogeneic stem cells,

while the remainder were autologous [75]. They reported a three-year OS and PFS of 36 percent and 28 percent, respectively. Comparable results were recently reported from the Spanish Lymphoma Cooperative Group. Seventy-seven patients with PTCL underwent ASCT either at relapse or in first remission. For the entire group, the five-year actuarial OS and DFS were 49 percent and 44 percent, respectively. For those transplanted in first CR the five-year OS was 80 percent.

Reports of ASCT's use in anaplastic large cell lymphoma have recently been reported [76–78]. For those patients with ALK-1 positive disease, very high long-term DFS rates have been reported for patients transplanted in first remission. However, in view of the favorable prognosis of this disease with conventional dose therapy, there is no clear role for HDT and ASCT as a component of first line therapy. Using ASCT in the salvage setting is recommended, although recent small series have reported poor long-term DFS after ASCT for patients with ALK-1 negative disease, and new strategies, including the use of nonmyeloablative SCT is being investigated for these patients.

15. Precursor T Cell and B-cell Lymphoblastic Leukemia/Lymphoma

Both autologous and allogeneic SCT have been used as consolidative therapy in first remission for patients with these diseases. Interpreting published studies is confounded by the previously variable distinction between lymphoblastic lymphoma (LBL) and acute lymphoblastic leukemia (ALL). Although these two entities are now recognized as identical in the WHO classification, most literature is based on the designation of patients with predominantly nodal disease as LBL, and those with blood- or marrow- based disease as ALL. Retrospective series from registries and single institutions have reported long-term OS rates of 50 percent to 80 percent for patients receiving SCT in first remission [79–82]. These results have generally been comparable to those reported for conventional induction, consolidation and maintenance chemotherapy protocols.

A small randomized trial comparing ASCT with conventional chemotherapy in adult LBL showed no OS advantage for the transplant arm [83]. A trend towards improved RFS was observed in the ASCT arm, although this did not achieve statistical significance.

For patients with relapsed or refractory disease after conventional dose first line therapy, ASCT produces long-term DFS in around 40 percent and 20 percent of patients, respectively.

Although these results are poor, they are superior to results reported for conventional dose salvage regimens, and the use of ASCT in this situation is widely regarded as a standard approach.

16. Allogeneic Stem Cell Transplantation in Lymphomas

16.1. Graft-Versus-Tumor Effects in Lymphoma

There has been increasing interest in the use of allogeneic SCT for patients with malignant lymphomas in recent years, particularly since the advent on nonmyeloablative conditioning regimens.

Allogeneic SCT offers potential advantages over autologous SCT for patients with lymphoma. The risk of contaminating the donor bone marrow with malignant cells is very low, so that there is virtually no potential for infusion of malignancy at the time of transplantation. In addition, the donor marrow will not have been exposed to the damaging effects of previous chemotherapy. The observation of a Graft–versus-Lymphoma effect similar to the Graft-versus-Leukemia effect has, however, been the major stimulus to exploring the role of allogeneic SCT in these diseases.

Evidence for a Graft-versus-Lymphoma effect in lymphoid malignancies includes the apparent effectiveness of modulating immunosuppressive therapy [84], clinical responses to donor lymphocyte infusions [85] and the effectiveness of nonmyeloablative conditioning regimens with allogeneic SCT [86].

Much of the clinical evidence for GvL effects is based on studies comparing autologous and allogeneic SCT in patients with lymphoma. The studies have been mostly registry-based and have used matched patients undergoing allogeneic and autologous SCT. In general, these studies have demonstrated lower relapse rates in patients receiving allogeneic transplants compared with autologous transplants, suggesting that GvL effects may account for this lower relapse rate. However, in general, the lower relapse rate associated with allogeneic transplantation has been offset by higher regimen-related mortality in the patients undergoing allogeneic SCT, so that for most lymphoma subtypes, overall survival has been comparable between the two groups [87].

A recent analysis of patients undergoing syngeneic SCT was conducted by the IBMTR and EBMT [88]. In this study, the outcome for patients undergoing syngeneic transplantation was compared with that for patients receiving allogeneic transplants (both T cell depleted and T cell replete). No differences in relapse, disease free survival or overall survival rates were observed between these groups for any subtype of NHL. Although this study was retrospective and registry-based, no clear evidence for a clinically significant GvL effect was observed.

In summary, although the existence of a GvL effect has been clearly demonstrated in multiple studies, its impact upon outcome for patients with NHL and HL remains uncertain.

Results of studies of allogeneic SCT in lymphoma have the potential for substantial patient selection bias. The high regimen related mortality of this approach, which is closely related to age, has restricted the use of allogeneic SCT primarily to patients below age 50 years. To date, no studies have analyzed outcomes of allogeneic SCT by intent to treat. This is particularly important since donor selection for allogeneic SCT can be lengthy, with a median time of about two to three months in most centers. Patients with very aggressive disease may relapse within this time and never come to transplantation. Therefore, there is a potential for bias in transplant series to include only patients with sufficiently durable remissions to complete the donor selection and evaluation process. Patients with very aggressive disease may be ‘selected out’ by relapsing during that time.

17. Hodgkin Lymphoma

Experience with using allogeneic SCT in HL has been limited, partly due to the increasing effectiveness of modern first line regimens in HL, and partly because of the relatively favorable results with the use of ASCT in relapsed and refractory patients. Early studies using myeloablative regimens, mostly

Table 6-7. Results of studies of allogeneic stem cell transplantation with myeloablative conditioning regimens in patients with relapsed and refractory Hodgkin's Lymphoma.

Reference	n	TRM	DFS
89	100	61% at 3 years	15% at 3 years
90	45	48% at 4 years	15% at 4 years
91	53	49% at 5 years	18% at 5 years
92	53	32% at 10 years	26% at 10 years

with related donor allogeneic transplants, reported very high treatment-related mortality rates of approximately 50 percent to 60 percent (Table 6-7) [89–92]. Despite these high mortality rates, 15 to 20 percent of patients in these series achieved long-term disease free survival. However, comparative analyses of allogeneic versus autologous SCT did not show a survival advantage for allogeneic transplantation.

There has, therefore, been recent interest in using reduced intensity allogeneic transplantation for selected patients with refractory and relapsed HL. In a study from MD Anderson Cancer Center, 40 patients with relapsed or refractory HL, 30 of whom had previously undergone ASCT, were treated with one of two reduced intensity conditioning regimens followed by related (n = 20) or matched unrelated (n = 20) donor stem cell transplantation [93]. At the time of the report, the median follow-up was only 13 months. The 18-month actuarial PFS and OS were 32 percent and 61 percent, respectively.

The significance of these data is difficult to interpret in view of the selected nature of the patient population, and short follow-up.

The use of allogeneic SCT in HL should still be regarded as experimental and should only be performed in a clinical trial context. Studies addressing the potential role of reduced intensity transplantation as consolidation after ASCT for 'bad risk' patients are in progress.

18. Diffuse Large B-Cell and Other Diffuse Aggressive Non-Hodgkin's Lymphomas

There is very limited data to assess the role of allogeneic transplantation in these diseases. Retrospective single center studies are difficult to interpret since most include patients with various histologic subtypes, and many of the patients were included prior to the description of the REAL/WHO classification [94, 95]. A case-matched study from the EBMT compared outcomes for patients with diffuse aggressive NHL undergoing allogeneic and autologous transplantation [87]. Despite the case-matched nature of the study, some imbalances in patient populations were present. In particular there was a higher proportion of patients with chemoresistant disease in the allogeneic population (31% versus 18% for the autologous group). No differences in disease free or overall survival were observed according to stem cell source.

Limited data are available for the use of reduced intensity conditioning regimens and allogeneic transplantation [98–102]. In many cases, patients have been treated with these regimens after relapse or disease progression following autologous SCT. Results of some of these studies are summarized in Table 6–8.

Table 6-8. Results of studies of allogeneic stem cell transplantation with reduced intensity conditioning regimens in diffuse large B-cell lymphoma.

Reference	n	TRM	PFS	OS
96	8	63% at 2 years	13% at 2 years	13% at 2 years
97	20	0% at 100 days	25% (follow up not provided)	N/A
98	9	11% at 100 days	67% at 20 months	78% at 20 months
99	35 (22 DLBCL)	63% at 2 years	34% at 3 years	34% at 3 years

At present, the use of allogeneic transplant strategies in DLBCL and other diffuse aggressive lymphomas should only be conducted in the context of prospective clinical trials. The potential use of reduced intensity conditioning and allogeneic transplantation as consolidation of autologous SCT in high risk patients remains an area of active investigation, but only preliminary data are available.

19. Indolent B-Cell NHL

As with aggressive NHLs, the role of allogeneic SCT in indolent lymphomas is poorly defined.

Early studies of allogeneic transplantation using myeloablative regimens included patients who, based mainly on extensive bone marrow involvement, were not thought to be candidates for autologous SCT. These studies showed remarkably low relapse rates in some cases, but the high treatment-related mortality associated with allogeneic transplantation resulted in overall survival rates comparable with ASCT. A large retrospective study from the IBMTR has reported results for 904 patients with follicular NHL undergoing stem cell transplantation between 1990 and 1999 [63]. One-hundred-seventy-six of these patients underwent allogeneic SCT with myeloablative regimens, and outcomes were compared with patients undergoing purged (n = 131) or unpurged (n = 597) autologous transplants. The five-year relapse rate was significantly lower in patients undergoing allogeneic SCT (21%) compared with ASCT (43% for purged ASCT and 58% for unpurged ASCT). However, the high treatment-related mortality in allogeneic recipients (30% at 5 years) resulted in comparable overall survival in all three groups (51%, 62% and 55%, respectively, at five years).

Few published data exist at present regarding this approach, although the indolent nature of follicular NHL implies that it may be particularly amenable to immune-based therapy. A recently initiated study from the BMT Clinical Trials Network in the United States comparing autologous SCT with reduced intensity allogeneic SCT for patients with relapsed follicular lymphoma will help to clarify its role.

Recent published series of patients treated with myeloablative transplants for chronic lymphocytic leukemia (CLL) are summarized in Table 6-9 [100–102].

Low relapse rates and encouraging outcomes have been reported from many of these series, although they have typically included highly selected, younger CLL patients. Further investigation of this approach is merited, although a recent report of the use of unrelated donor transplantation with myeloblative

Table 6-9. Results of studies of allogeneic stem cell transplantation with myeloablative conditioning regimens in chronic lymphocytic leukemia

Reference	n	TRM	PFS	OS
100	23	17% at 100 days	62% at 5 years	65% at 5 years
101	25	N/A	N/A	32% at 5 years
102	28	11% at 100 days	42% at 5 years	45% at 5 years

regimens in CLL suggests that the treatment-related mortality of this approach outweighs any potential benefit in terms of reduced relapse rates [103].

20. Mantle Cell Lymphoma (MCL)

The advanced median age of patients with MCL has limited experience in the use of allogeneic SCT, particularly using myeloablative regimens. Published experience is largely anecdotal. A study from the EBMT reported 22 patients who were previously treated extensively [65]. The two-year OS and PFS were 62 percent and 50 percent, respectively, although the significance of this observation is difficult to interpret in view of the selected nature of the patient population, particularly with respect to age. Khouri, et al. have reported comparable results in 16 patients, with a median age of 52, undergoing sibling donor transplants for relapsed or refractory MCL or, in five cases, for patients in first CR [104].

The same group has reported initial experience with reduced intensity transplantation in 18 patients with MCL, of whom five had previously undergone ASCT, the remainder have relapsed or refractory disease [105]. Sibling donors were used for 13 of the patients; the remainder receiving matched unrelated transplants. Progression-free survival was 82 percent at three years. Maris, et al. recently reported experience with 33 patients with relapsed or refractory MCL undergoing related (n = 16) or unrelated (n = 17) donor transplants after reduced intensity conditioning. Two-year PFS and OS rates were 60 percent and 64 percent, respectively [106].

21. Other Aggressive NHL Subtypes

Although limited data are available, there are no comparative data which demonstrate a benefit for allogeneic compared with autologous SCT for patients with Burkitt's, Burkitt-like, or lymphoblastic lymphoma [107]. The limited number of comparative studies have shown no survival benefit, and the clinically aggressive nature of these diseases suggests that they are unlikely to be susceptible to graft-versus-tumor effects. Very limited data are available for peripheral T cell lymphomas and cutaneous T cell lymphomas, and the use of allogeneic SCTs for these diseases is still largely anecdotal [108, 109].

22. Technical Aspects of Stem Cell Transplantation

Technical aspects of stem cell transplantation, particularly using autologous stem cells, may influence the outcome of high-dose therapy, but most have not been explored in the context of prospective, randomized trials. The potential effect

of the high-dose regimen and stem cell source (bone marrow versus peripheral blood) and stem cell mobilizing regimen have been investigated in retrospective series, but not in prospective studies, and it is, therefore, not possible to identify optimal high-dose regimens for patients undergoing autologous SCT. Most standard conditioning regimens are regarded as acceptable and equivalent, although there are few data to support this.

The use of *ex vivo* manipulation of autologous stem cell products to reduce the potential contamination from malignant cells is an area of uncertainty and ongoing clinical research.

This has mainly been studied in the context of follicular lymphoma, where most patients have morphologic or molecular evidence of involvement of the bone marrow or peripheral blood. Early studies demonstrated the potential for reinfused lymphoma cells to contribute to relapse, and the potential for reducing the rate of relapse by techniques aimed at *ex vivo* removal of contaminated lymphoma cells ("purging") [110]. The alternative approach of 'positively selecting' CD34-positive hematopoietic progenitor cells using antibody-based methodology has also been investigated by several groups. The impact of *ex vivo* manipulation of progenitor cell products on post-transplant survival is unknown. Conflicting results have been reported by different groups [62, 111]. A small randomized trial has attempted to explore the impact of *ex vivo* manipulation on disease free and overall survival in relapsed follicular lymphoma. In the European CUP trial patients with relapsed follicular lymphoma were randomized to receive either conventional dose salvage chemotherapy (with CHOP or a related regimen), HDT and ASCT with an unpurged stem cell product, or HDT and ASCT with a purged stem cell product [64]. Unfortunately, low patient numbers have limited the interpretation of this study. However, although overall and progression-free survival were longer in patients receiving HDT and ASCT compared with the conventional chemotherapy arm, there was no difference in OS or PFS between the groups receiving purged or unpurged autologous products. In contrast, a retrospective, registry-based study by Bierman, et al. reported a lower relapse rate in patients with indolent lymphoma undergoing ASCT using syngeneic transplants compared with those undergoing purged ASCT which, in turn, had a lower relapse rate than in patients undergoing unpurged ASCT [88]. This was reflected in corresponding improvements in disease free and overall survival, suggesting that contaminating lymphoma cells may be contributing to relapse.

The role of *ex vivo* manipulation is unclear and, in any case, is likely to diminish with the increasing use of monoclonal antibodies such as rituximab. Early phase II studies with rituximab demonstrated that this agent had apparent selective activity in certain body compartments, and an ability to clear malignant B-cells from the bone marrow and peripheral blood even in the absence of a clinical response in lymph nodes. Several groups have subsequently reported on the *in vivo* purging activity of this agent, although to date, no data are available regarding the potential effect of this agent on post-transplant disease progression and survival [112].

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Chapter 7

Transplantation in Chronic Lymphocytic Leukemia

Rifca Le Dieu and John G. Gribben

1. Introduction

Despite the appearance of new agents with activity in CLL, including purine analogs and monoclonal antibodies which have led to improvements in overall and complete response rates and longer duration of remissions, patients invariably relapse and subsequently develop resistance to chemotherapy [1]. Once this occurs, the prognosis is poor with a median survival of less than one year [2].

Although CLL is largely a disease of the elderly with a median age of presentation between 65 and 70 years, 40 percent of patients with CLL are aged under 60 years and 12 percent under 50 years at the time of diagnosis. The disease in these younger patients has no major distinctive features and the prognostic factors are the same as those in older patients, yet more than 90 percent of younger patients die as a result of causes directly related to CLL [3].

Stem cell transplantation (SCT) is not an option for many older patients who would not tolerate the approach, or for those with indolent disease. However, younger patients with poor prognostic disease may have a chance of cure with stem cell transplantation. Currently both the type of transplant and the optimal timing to perform SCT is under debate.

2. Autologous Stem Cell Transplantation (AutoSCT) (See Table 7-1)

This type of transplant offers increased antitumor activity as a result of higher doses of chemotherapeutic agents. The approach is feasible in CLL with a transplant-related mortality of between 2 and 10 percent [4–7]. Among 72 patients reported who had undergone autologous SCT in Finland 1995 and 2005 there were no early treatment-related deaths. The median age of these patients was 57 (range 38 to 69) with a median time from diagnosis of 32 months (range six to 181). The median number of prior therapies was one. The most commonly used conditioning regimen was total body irradiation (TBI) and cyclophosphamide (Cy) (n = 38, 53%). After a median follow-up of 28 months, 37 percent had relapsed or progressed. The projected median progression-free survival (PFS) and overall survival (OS) was 48 months and 95 months, respectively [8].

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Table 7-1. Clinical trials of autologous transplantation for CLL.

No. of patients	Median Follow-up (months)	Continuing complete remission	Treatment related mortality	Reference
137	78	67	5 early 13 MDS/AML 15 other cancer	Gribben, et al. 2005 [4]
77	28	50	0	Jantunen. et al. 2006 [8]
65	36	45	1 early 5 MDS/ AML	Milligan, et al. 2005 [5]
16	37	5	2	Pavletic, et al. 1998 [39]
13	19	12	0	Dreger, et al. 1998 [40]
11	10	2	1	Khouri, et al. 1994 [41]
8 20 enrolled 12 stem cells collected	36	5	0	Sutton, et al. 1998 [42]
5	9	4	0	Itala, et al. 1997 [43]

Detectable molecular disease post-transplant is highly predictive of clinical recurrence, and although molecular remissions can be achieved in more than two-thirds of patients, these are not durable [5, 9–11] and most patients who achieve complete remission (CR) post-autograft will eventually relapse [12].

There has been no prospective study directly comparing outcome after conventional therapy and after autologous transplantation. Two retrospective analyses have been published that show contradictory results [13, 14]. As induction regimens have evolved, it would now be useful to look at the new chemo-immuntherapeutic regimens in direct comparison with autologous transplantation.

Of particular concern are recent reports of myelodysplasia and secondary acute myeloid leukaemia (MDS/AML) post-autograft. In the reported series from the Dana Farber Cancer Institute the actuarial incidence of MDS/AML in CLL patients post-autograft was 12 percent at eight years [4]. This finding has been supported by the MRC CLL Pilot Study. Among 65 newly diagnosed patients treated with fludarabine followed by autologous SCT, eight developed MDS/AML [15]. This equates to a five-year actuarial risk of developing MDS/AML post-autoSCT of 12.4 percent (95% CI 2.5-24%). No potential risk factor analyzed was predictive. The group postulates that potential causative factors may be fludarabine, low cell dose and use of TBI in the conditioning regimen.

2.1. Purging of Autologous Stem Cells

One of the major concerns in autologous transplantation is the reinfusion of tumor cells that theoretically may contribute to the risk of relapse post-autograft. Numerous groups have attempted to improve outcome by “purging”

the graft. The techniques most frequently employed utilize either negative selection using B-cell monoclonal antibodies to deplete tumor cells from the graft, or positive selection of stem cells using CD34 antibodies. Unfortunately these methods remain inefficient at removing CLL cells. In a series from the Dana-Farber, 11 out of 21 patients still had detectable disease in the stem cell harvest after exposure to a cocktail of three monoclonal antibodies [9]. Purging also inevitably results in stem cell loss. If there has been difficulty in obtaining a sufficiently large harvest, purging cannot be performed, as seen in 50 percent of cases in the MRC Pilot Study [16]. This problem could be bypassed by *in vivo* purging using alemtuzumab or rituximab pre-transplant. High-dose alemtuzumab was used for this purpose in the conditioning regimen for autologous transplants in one arm of the CLL3 trial from the German CLL Study Group with unexpected consequences [17]. Sixteen patients were treated in this arm and received a total dose of 43 mg (n = 1), 103 mg (n = 14) and 133 mg (n = 1). A high incidence of initially unexplained skin rashes led to further analysis. Twelve out of 16 patients (87%) developed a skin rash between 43 and 601 days post SCT. In seven patients a diagnosis of Graft-versus-Host Disease (GVHD) could be made, compared with no cases in the TBI/Cy only conditioned patients. Autologous GVHD is an autoimmune syndrome initiated by auto-effector T cells that recognize self MHC Class II antigens. It is usually mild and self-limiting. In this case, however, all cases required immunosuppression and the median duration was 517 days (range 60 to 867). The trial was discontinued due to the high non-relapse mortality. However, the addition of alemtuzumab led to improved disease control at the molecular level. It is interesting that using alemtuzumab in combination with other immunosuppressants prior to allogeneic SCT results in effective prevention of GVHD. In this situation it was postulated that the markedly immunosuppressive regimen depleted regulatory CD4 and CD8 T cells and NK cells, allowing the subsequent development of autoimmune disease. The patients receiving Alemtuzumab/TBI/Cy had a severe CD8 lymphopenia in the first year after SCT. Certainly, future trials investigating *in vivo* purging with alemtuzumab should use a less immunosuppressive conditioning regimen such as BEAM.

The concept of using alemtuzumab for *in vivo* purging should not be discarded. When used at standard doses (10 mg subcutaneously three times per week for six weeks) in 34 patients who had had a clinical response to a fludarabine-based regimen, the CR rate improved from 35 percent to 79.5 percent, with 56 percent achieving minimal residual disease (MRD) negativity. Subsequently, peripheral blood stem cell (PBSC) collection was successfully performed in 92 percent. Eighteen patients underwent autologous transplants with 17 remaining in CR at a median follow-up of 14.5 months post-SCT [18]. Eradication of MRD after alemtuzumab has been demonstrated to result in prolonged survival [19].

3. Myeloablative Allogeneic Stem Cell Transplantation (AlloSCT) (See Table 7-2)

This type of transplant extends the dose intensification effect of autologous transplant by adding cellular immune therapy in the form of the Graft-versus–Leukemia (GVL) effect. This results in better disease control, but at the price

Table 7-2. Clinical trials of myeloablative allogeneic transplantation for CLL.

No.	Median Follow-up (months)	Continuing complete remission	Treatment related mortality	Severe GVHD	Reference
54	27	24	25	18	Michellet, et al. 1996 [21]
25	78	13 8 after DLI	1 early 5 late	5	Gribben, et al. 2005 [4]
25	60	9	7	56	Doney, et al. 2002 [44]
23	24	14	8	47	Pavletic, et al. 2000 [45]
15	35	8	5	26	Khouri, et al. 1997 [46]

of greater toxicity. The morbidity and mortality of alloSCT relate to organ failure caused by the combination of the conditioning regimen, acute and chronic Graft-versus-Host Disease (aGVHD and cGVHD) and infections. As many CLL patients are older these risks are greater.

The feasibility of this treatment in CLL was first demonstrated in 1988 in eight patients, five of whom were alive in CR after a median follow-up of 27 months post-SCT [20]. The high transplant-related mortality (TRM) rate became apparent from registry data with rates of 46 to 50 percent reported [21, 22]. A recent report from Canada examined 30 patients transplanted for CLL between 1989 and 2001. Twenty had related donors and 10 unrelated donors. After a median follow-up of 4.3 years, 47 percent were alive in CR. Actuarial overall and event-free survival at five years was 39 percent. A strong GVL effect was noted and those developing acute or chronic GVHD had nearly complete protection from relapse [23].

A report from the International Bone Marrow Transplant Registry (IBMTR) highlighted that although durable responses were being achieved after allo-SCT, survival was worse than after autoSCT, with the three-year probability of survival reported as 45 percent for allografts and 87 percent for autografts [24]. This lack of benefit over autologous transplantation has been recently illustrated in a series published from the Dana-Farber Cancer Institute [4]. One-hundred-sixty-two patients with high risk CLL underwent transplant procedures between 1989 and 1999. Twenty-five had T cell depleted matched sibling alloSCTs and 137, without matched sibling donors, underwent auto-SCTs. There was a low 100-day mortality of 4 percent for both groups. After a median follow-up of 6.5 years there was no difference in overall survival, cumulative incidence of disease recurrence or deaths without recurrence between the two groups. The progression-free survival, however, was much better following autologous transplant. T cell depletion was used in this study in an attempt to reduce morbidity and mortality-related to alloSCT. However, it is likely that this may have removed the benefits of the GVL effect on pro-gression-free survival and resulted in the late relapses seen in the alloSCT group.

As HLA-matched sibling allogeneic transplants are only possible in a quarter of all potential recipients, the use of unrelated donor stem cells has been investigated. A series of 38 heavily pre-treated patients was recently published [25]. Eleven patients were alive and disease-free at a median of six years. The five-year overall survival rate was 33 percent, TRM 38 percent and

disease-progression rate 32 percent. Of note, 45 percent developed grade 2 to 4 aGVHD and 85 percent had cGVHD. The authors concluded that lasting remissions could be achieved, but that the high TRM illustrated that HLA-mismatched donors should be avoided in future.

4. Reduced Intensity Conditioned Allogeneic Stem Cell Transplantation (RICalloSCT) (See Table 7-3)

A GVL effect in CLL has been demonstrated by the elimination of recurrent disease post-allogeneic transplant after cessation of immunosuppressive therapy, or after the use of donor lymphocyte infusions (DLI) [26–28]. The idea behind the use of RICalloSCT is to capitalize on this GVL effect without the significant morbidity and mortality associated with myeloablative chemoradiotherapy. This approach is particularly applicable to the older age group of patients who develop CLL.

This regimen has been compared to standard allografting in a retrospective analysis of European Bone Marrow Transplant (EBMT) registry data [29]. Seventy-three cases of RICalloSCT were compared to 82 cases of myeloablative procedures performed during the same period. After matching for primary risk factors, conditioning regimen, age, sex, donor type and remission status at time of transplant, the groups were analyzed for effects of type of transplant on TRM, relapse incidence, EFS and OS. As expected, there was a significant reduction in TRM evident in the RICalloSCT population. Owing to an increased relapse incidence in the RICalloSCT group, this did not translate into an improved outcome with EFS and OS showing no difference between the two groups.

The outcome of 64 patients with advanced CLL from the Fred Hutchinson Cancer Research Center has been reported [30]. Forty-four had related donors and 20 had unrelated donors. TRM at day 100 was 11 percent with an incidence of grade 2 to 4 aGVHD of 55 percent. TRM at two years was 22 percent with a rate of cGVHD of 50 percent. After a median follow-up of 24 months, 39 were alive with 25 in CR. The two-year incidence of relapse or progression was 26 percent. The strength of the GVL effect in CLL is illustrated by the fact that unrelated donor SCT resulted in higher CR and lower relapse rates, although the complication rate in this group of patients was higher. A similar high rate of GVHD was seen in a smaller group of patients from an Australian group [31]. Thus, although RICalloSCTs are often termed “mini-transplants,” their cost, particularly in terms of GVHD, is still high. In a series of 46 patients with advanced largely refractory CLL reported from the Dana-Farber Cancer Institute, 67 percent from HLA-matched unrelated donors the two-year OS was 54 percent and PFS 34 percent with a median follow-up of 20 months. The primary cause of treatment failure was relapse, with a two-year cumulative incidence of 48 percent. Predictors for relapse included chemotherapy-refractory disease at transplantation, an increasing number of previous therapies and increasing bone marrow involvement and a low hematopoietic donor chimerism at day +30 [32].

Data of 77 patients were collected from 29 EBMT centers. Median age was 54 (30 to 66) years, and the median number of previous chemotherapy regimens was three (0 to 8). HLA-identical sibling donors were used in 81 percent

Table 7-3. RIC allogeneic SCT for CLL.

n	Age years (range)	Prior regimens (range)	Chemo therapy refractory	Donor (includes mismatch)	GVHD		Treatment related mortality	Survival	Reference
					acute gd 2–4	chronic extensive			
77	54 (30–66)	3 (0–8)	33% 10 prior auto-SCT	81% related	34%	58%	18% 12 m	OS 72% 2 yr PFS 56%	Dreger, et al. 2003 [33]
64	56 (44–69)	4	53%	69% related 31% unrelated	61%	50%	11% at 100 d 22% overall	OS 60% 2 yr PFS 52%	Sorror, et al. 2005 [30]
46	53 (35–67)	5 (1–10)	57% 10 prior auto-SCT	33% related 67% unrelated	34%	43%	17% overall	OS 54% 2 yr PFS 34%	Brown, et al. 2006 [32]
41	54 (37–67)	3 (1–8)	27% 11 prior auto-SCT	58% related 42% unrelated	10% (gd 3–4)	33%* *after DLI	5% at 100 d 26% overall	OS 51 2 yr PFS 45%	Delgado, et al. 2006 [35]
39	57 (34–70)	3 (2–8)	Not stated * 87% "active" disease	90% related 10% unrelated	45%	58%	2% at 100 d	OS 48% 4 yr PFS 44%	Khouri, et al. 2006 [47]
30	50 (12–63)	3 (0–8)	47%	50% related 50% unrelated	56%	21%	13% overall	OS 72% 2 yr PFS 67%	Schetelig, et al. 2003 [48]

of the cases. In 40 percent of the patients, *in vivo* T cell depletion (TCD) with anti-thymocyte globulin or CAMPATH-1H was part of the conditioning regimen. Cumulative TRM was 18 percent (95% CI 9; 27) after 12 months. The two-year probability of relapse was 31 percent with no event occurring later than 12 months post-transplant in the absence of T cell depletion. Only one relapse occurred after onset of chronic Graft-versus-Host Disease. Median follow-up was 18 (1 to 44) months, event-free survival was 56 percent and overall survival 72 percent at 24 months. Donor lymphocyte infusions or secondary transplants were performed in 19 patients with insufficient disease control and/or incomplete donor chimerism post-transplant, leading to a response in seven patients (37%). Multivariate analysis identified less than PR at transplant and alternative donor as significant risk factors for relapse, whereas an increased number of previous regimens, use of TBI and alternative donor were risk factors for survival. The authors concluded that RIC might favorably influence the outcome after allogeneic SCT for CLL by reducing TRM while preserving Graft-versus-Leukemia activity [33].

The incidence of GVHD can be reduced by using alemtuzumab in the conditioning regimen with consequent reduction in TRM. Unfortunately it also delays post-SCT immune reconstitution, increases the risk of infective complications and potentially impairs the GVL effect. This impaired antitumor response may necessitate the early use of DLI post-SCT [34]. A study using alemtuzumab with fludarabine and melphelan as the conditioning regimen has been reported by the British Society of Bone Marrow Transplantation (BSBMT) [35]. Forty-one consecutive patients were treated; 24 had HLA-matched sibling donors and 17 had unrelated volunteer donors (four mismatched). The conditioning regimen had significant antitumor effects, with 100 percent of patients with chemosensitive disease and 86 percent with chemotherapy-refractory disease attaining CR or PR. The TRM rate was 26 percent, overall survival 51 percent and relapse risk 29 percent at two years. GVHD rates were relatively low with aGVHD occurring in 17 (41%) and cGVHD in 13 (33%). The unexpectedly high TRM rate was due to a high incidence of fungal and viral infections. What emerged from this study was the clear adverse prognostic factor of fludarabine refractoriness. This group of patients had a two-year overall survival of only 31 percent.

Patients with unmutated CLL who have undergone autoSCT have a poor outcome [13, 36]. The effect of allogeneic transplantation on outcome in this group has recently been examined [37]. Among 50 patients who underwent SCT, 34 had unmutated immunoglobulin variable heavy chain genes (IgVH) (14 alloSCT and 20 autoSCT) and 16 had mutated IgVH genes (nine alloSCT and seven autoSCT). There was no difference in CR rate between type of transplantation and IgVH mutational status. After a median follow-up of five years, autoSCT resulted in a significantly higher relapse rate than alloSCT in both mutational groups. Thus, the GVL effect of alloSCT may overcome the negative impact of unmutated VH gene status on outcome. Myeloablative conditioning may not be required for this effect as demonstrated in a paper from a Spanish group that looked at RICalloSCT in 30 patients with poor prognosis CLL as defined by mutational status of VH genes and cytogenetic abnormalities (11q-, 17p-) [38]. Overall survival and event-free survival for the poor prognosis group was 90 percent and 92 percent, respectively, and was not significantly different from the good prognosis group.

With the advances in molecular biology it has now become possible to detect minimal residual disease after therapy. The challenge is to find the most reliable method of detection and then to translate results into meaningful data on outcome. These data are now becoming available. It has been established, as previously stated, that alemtuzumab can result in MRD negativity and that this is associated with prolonged survival [19]. MRD post-SCT was the subject of a recent paper. Forty patients undergoing SCT had samples taken for MRD measurement by consensus polymerase chain reaction (PCR), quantitative PCR (qPCR) and flow cytometry. One-hundred-sixty-four of 248 samples were negative for MRD by consensus PCR. Of these 164, MRD was found in 77 (47%) by qPCR and 39 (29%) by flow cytometry. All 84 samples positive by consensus PCR were also positive by qPCR and flow cytometry. Thus, quantitative methods to assess MRD would appear to be more accurate. Fifteen out of 25 patients who underwent autoSCT relapsed. Increasing levels of MRD were detected in all beforehand. In patients undergoing alloSCT (n = 15), MRD detection did not correlate with outcome. Thus, in this circumstance, treatment decisions on the basis of MRD status are not justified [49].

5. Conclusions

Stem cell transplantation should be considered as a possible option in selected patients with CLL. It is not appropriate therapy for those with indolent disease, good prognostic factors (IgVH mutational status, cytogenetics, ZAP-70, CD38) or comorbidities. In fitter patients with aggressive disease or poor prognostic markers, SCT should be considered early on to avoid development of refractory disease which will inevitably occur after several lines of treatment. Autologous stem cell transplantation offers a chance of lasting remission with low TRM rates; however, all will eventually relapse. Furthermore, it is currently unclear if autologous transplantation offers a superior survival advantage over the newer chemoimmunotherapeutic regimens. Autologous transplantation will not overcome the poor prognosis conferred by unmutated VH genes or poor risk cytogenetic abnormalities. In these patients allogeneic stem cell transplantation should be considered. Myeloablative regimens currently offer no survival advantage over autologous procedures and, therefore, a reduced intensity conditioned regimen using fludarabine in combination with melphelan or low dose TBI may be the optimal approach to management. However, the high risk of GVHD needs to be taken into account. The optimal immunosuppressive conditioning regimen that minimizes GVHD while maximizing GVL remains to be defined. Despite encouraging initial results, follow-up of most clinical trials is too short to assess whether SCT can cure CLL. Therefore, continuing to enroll appropriate patients into well designed clinical trials is vital for further progress.

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Chapter 8

Stem Cell Transplantation in Multiple Myeloma

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1. Introduction

Despite advances in both standard dose chemotherapy (SDT) and high-dose myeloablative therapy with autologous stem cell transplantation (HDT), multiple myeloma continues to be characterized by a relapsing and increasingly refractory disease course. Given the increasing availability of novel agents that may complement stem cell transplantation, this chapter re-evaluates the role of autologous and allogeneic stem cell transplantation in the treatment of symptomatic multiple myeloma.

2. Autologous Transplantation

Conventional treatment with melphalan, melphalan and prednisolone, or combination chemotherapy regimens (e.g., cyclophosphamide, melphalan, BCNU, CCNU, adriamycin, vincristine and prednisolone) result in an equivalent median survival of between 24 and 36 months, with approximately 50 percent of patients responding to therapy. However, only a minority (5 to 10%) of patients attained a true complete response (CR), with the disappearance of paraprotein and attainment of a normal marrow [1, 2]. Following the introduction of infusional VAD chemotherapy, the number of patients responding to treatment (70 to 80%) and the level of response achieved increased, with complete response rates of 8 to 28 percent [3, 4]. These responses were often short-lived, and it was with the purpose of improving the duration of response that HDT was introduced [5]. Newer agents like Thalidomide, Lenalidomide and Bortezomib have recently been evaluated as initial therapy, typically in combination with Dexamethasone [6–9]. These studies have shown high response rates (40 to 91%), with complete and near-complete response rates of 20 to 38 percent. However, complete responses thus far have not correlated with a prolonged disease free survival or cure [10, 11], highlighting a continued role for HDT in myeloma management.

2.1. HDT with Single Autologous Transplantation

Initial studies using a single large intravenous dose of melphalan at 140 mg/m^2 (Mel140) gave encouraging results; however, the treatment was associated with a

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prolonged period of myelosuppression which resulted in a significant infection risk and a number of procedure-related deaths. Later this approach was combined with bone marrow (BM) rescue, improving its safety, and a high response rate was noted with up to 50 percent of patients attaining a CR [12]. In the early 1990s, autologous peripheral blood stem cells (PBSC) were reported as an alternative source of stem cell support for the high-dose procedure. There are a number of advantages to such a technique, including ease in collecting stem cells, quicker engraftment times and the possibility of lower transplant-related mortality (TRM). PBSC are currently the preferred source of stem cells for HDT [13].

A number of groups have since shown an improvement in response rates and survival using an HDT approach in both relapsed/refractory and newly diagnosed patients when compared to historical controls [14, 15]. However conflicting evidence also indicated that patients who would be potential candidates for high-dose therapy, but received conventional chemotherapy also had similar survival rates [16]. These nonrandomized studies are difficult to assess because they are more prone to errors of bias and confounding that can lead to inaccurate estimates of effect.

Thus, a number of prospective randomized controlled trials (RCTs) have addressed this question by comparing high-dose therapy with either PBSCT or ABMT support to conventional combination therapy, with variable results (Table 8-1) [17–26]. For instance, three studies have reported an improved overall survival (OS) and progression-free survival (PFS) in patients undergoing high-dose treatment upfront [17, 21, 25]. Two additional studies report an improved PFS, but no OS benefit [18, 19].

A recent systematic review and meta-analysis of all 10 RCTs evaluating upfront HDT versus SDT indicate that HDT with single autologous transplantation offers significant PFS benefit, but not OS benefit, for newly diagnosed myeloma (Figures 8-1and 8-2) [27]. In subgroup and sensitivity analyses, these findings were robust, and did not differ significantly among various clinically relevant subgroups.

2.2. HDT Conditioning Regimens

A number of groups have compared the high-dose conditioning regimen of melphalan 200mg/m^2 (Mel200) versus Mel140, plus total body irradiation (TBI 8Gy). Data from the French Registry showed no improvement in the complete remission (CR) rate, event free survival (EFS) or OS [28]. A randomized trial addressing this question concluded that Mel200 was less toxic than Mel140 plus TBI, with similar response rates and PFS for both conditioning regimens, and indicated improved OS for the Mel200 conditioning [29].

2.3. Purging Autologous Stem Cell Collections

One of the major concerns regarding the reinfusion of autologous progenitor cells following a high-dose procedure was contaminating the harvest with myeloma cells, and whether they have the ability to repopulate the marrow and contribute to relapse of disease.

In the majority of myeloma cases (70%), the contamination as measured by flow cytometry and polymerase chain reaction is < 1 tumor cell in $10^{3–4}$ normal cells. The cases with high tumor contamination tend to be those with persistent disease within the bone marrow at the time of mobilization [30].

Table 8-1. RCTs evaluating upfront HDT with single autotransplant versus conventional chemotherapy (SDT) for newly diagnosed myeloma.

Author	Year started	N	HDT regimen (conditioning regimen)	Stem cells	SDT regimen	CR (SDT)	CR (HDT)	Conclusions
Attal	1990	200	VMCP/BVAP→ (Mel140/TBI-8)	BM	VMCP/BVAP	5%	22%	OS: benefit EFS: benefit
Fermand	1990	185	VAMP→ (Cc/Cy/VP/Mel140/TBI-12)	PBSC	VMCP	5%	19%	OS: no benefit EFS: benefit
Fermand	1991	190	VAMP→ (Bu/Mel140 or Mel200)	PBSC	VMCP	4%	6%	OS: no benefit EFS: benefit
Facon	1992	115	VAD/Mel140→ (Mel140/TBI-12)	BM	VAD/Mel140	18%	32%	OS: no benefit PFS: NR
Child	1993	401	VAMPC→ (Mel200 or Mel140/TBI)	PBSC	ABCM	8%	44%	OS: benefit PFS: benefit
Barlogie	1993	516	VAD→ (Mel140/TBI-12)	PBSC	VAD-> VBMCP	11%	11%	OS: no benefit PFS: no benefit
Blade	1994	164	VBMCP/VBAD→ (Mel200 or Mel140/TBI-12)	PBSC	VBMCP/VBAD	11%	30%	OS: no benefit PFS: no benefit
Segeren	1995	261	VAD-> Mel70x2→ (Cy60x4/TBI-9)	PBSC	VAD-> Mel70x2	13%	29%	OS: no benefit EFS: no benefit
Palumbo	1997	194	VAD→ (Mel100x2)	PBSC	MP	6%	25%	OS: benefit EFS: benefit
Facon	2000	248	VAD→ (Mel100x2)	PBSC	MPT	16%	17%	OS: no benefit PFS: no benefit

The study ID, author, year of initial patient enrollment, study size (number randomized), for HDT and SDT treatment arms are listed. HDT and SDT regimen, source of stem cells: bone marrow (BM) or peripheral blood stem cells (PBSC), and complete response (CR) rates are indicated. Study conclusions regarding overall survival (OS) and progression-free survival (PFS)/event-free survival (EFS) benefit are shown

Abbreviations: ABCM Doxorubicin, Carmustine, Cyclophosphamide, Melphalan; *Bu/Mel* Busulphan, Melphalan; *BVAP* Carmustine, Vincristine, Doxorubicin, Prednisone; *Cc/Cy/VP/Mel140/TBI-12* Lomustine, Cyclophosphamide, Etoposide, Melphalan, XRT; *Cy60* Cyclophosphamide at 60 mg/kg; *Mel70/100/140/200* Melphalan at 70/100/140/200 mg/m^2, *MP* Melphalan, Prednisone; *MPT* Melphalan, Prednisone, Thalidomide; *NR* not reported; *TBI-8/9/12* total body irradiation (XRT) at 8/9/12 Gy; *VAD* Vincristine, Doxorubicin, Dexamethasone; *VAMP* Vincristine, Doxorubicin, Methylprednisolone; *VAMPC* Vincristine, Doxorubicin, Methylprednisolone, Cyclophosphamide; *VBAD* Vincristine, Carmustine, Doxorubicin, Dexamethasone; *VBMCP* Vincristine, Carmustine, Melphalan, Cyclophosphamide, Prednisone; *VMCP* Vincristine, Melphalan, Cyclophosphamide, Prednisone

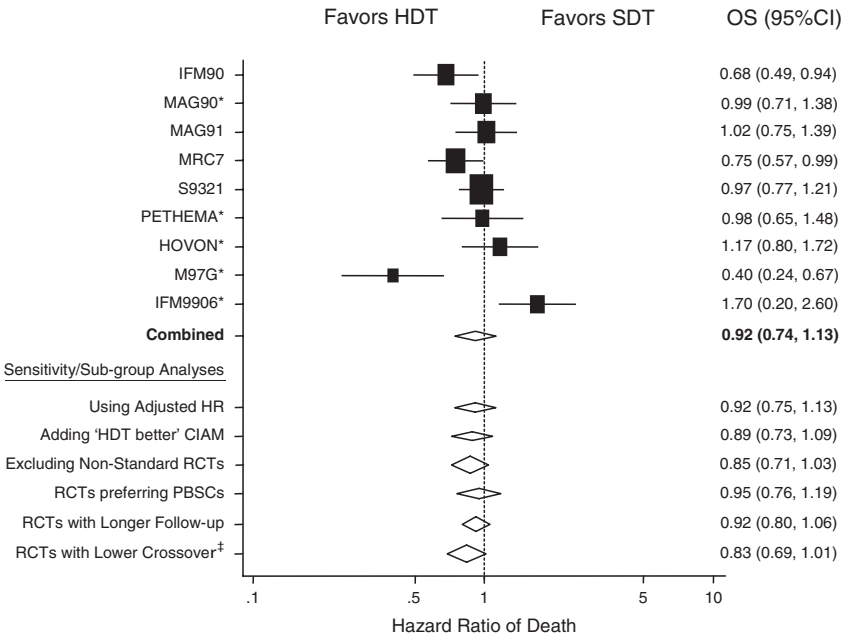

Fig. 8-1. Forrest plot of overall survival (OS) benefit of upfront HDT with single autotransplant in myeloma
The individual RCTs are indicated on the Y-axis. The summary effect estimate (HR) for individual RCTs are indicated by black rectangles (the size of the rectangle is proportional to the study weight), with the lines representing 95 percent CI. The overall summary effect estimate (HR) and 95 percent CI are indicated by the diamond below. Overall estimates after sensitivity and subgroup analyses are shown below. The corresponding values for HR (95% CI) are indicated alongside. (Reproduced with permission)
* non-standard study
‡ 2 negative studies (HOVON, IFM9906) with missing crossover information were omitted from this analysis

Using a more sensitive oligo-specific PCR, which is able to detect one tumor cell in 10^6 normal cells, there is evidence of contamination in almost 100 percent of cases [31]. Whether they are clonogenic is a difficult question to address, but sensitive immunophenotyping suggests that these cells in the apheresis products have a similar phenotype to myelomatous plasma cells from the bone marrow, but express lower levels of syndecan-1 [32]. There is no definitive evidence from mouse studies regarding this matter, but if these cells are reinfused they may contribute to disease relapse.

A number of groups have tried to reduce/eliminate the tumor contamination of harvests by either depleting tumor cells or selecting normal hematopoietic progenitor cells by virtue of CD34 expression from autologous bone marrow or peripheral blood stem cells prior to transplantation [33, 34]. Although these methods may achieve up to a 5-log depletion of tumor cells without affecting engraftment, their clinical benefit is unproven since residual tumor cells are detectable within both the graft and the patient. For purging to be effective the major source of contamination must be considered to be from the graft, with the patient being tumor free, and previous trials of induction chemotherapy suggest that this is unlikely.

A large randomized study assessing the clinical benefits of CD34 selection in myeloma showed that purging had no PFS or OS benefit [35]. Additionally,

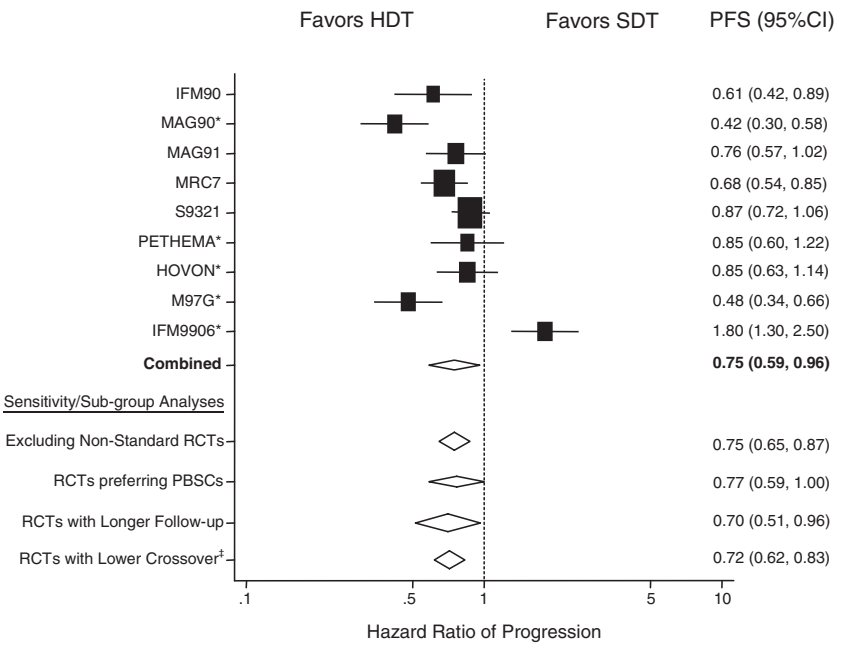

Fig. 8-2. Forrest plot of progression-free survival (PFS) benefit of upfront HDT with single autotransplant in myeloma
The individual RCTs are indicated on the Y-axis. The summary effect estimate (HR) for individual RCTs are indicated by black rectangles (the size of the rectangle is proportional to the study weight), with the lines representing 95 percent CI. The overall summary effect estimate (HR) and 95 percent CI are indicated by the diamond. Overall estimates after additional sensitivity and subgroup analyses are shown below. The corresponding values for HR (95% CI) are indicated alongside. (Reproduced with permission)
* non-standard study
‡ 2 negative studies (HOVON, IFM9906) with missing crossover information were omitted from this analysis

in the meta-analysis of RCTs comparing upfront SDT versus HDT utilizing single autologous transplantation, the presence of differing myeloma burdens in patients (and likely their autologous stem cell collections) at upfront HDT did not impact outcomes. The RCTs limited to patients with chemoresponsive myeloma showed similar outcomes (PFS benefit, but no OS benefit) to those in RCTs that also enrolled patients with chemorefractrory myeloma (and likely higher myeloma burden at transplant [27]). The clinical relevance of myeloma contamination of autografts may be less than initially believed, at least with current treatment regimens.

2.4. HDT for Clinically Relevant Subgroups

The majority of the trials have set an upper age limit of 65 years for considering HDT. A number of reports from single centers have treated older patients that were considered biologically fit (some >75 years) with ablative conditioning (including Mel200), and noted that feasibility, TRM, response and survival rates were similar to younger patients [36, 37]. Two prospective randomized trials of combination chemotherapy versus sequential autotransplants after intermediate dose melphalan at 100mg/m^2 (Mel100) for older patients have

been reported, and are evaluated in the meta-analysis [27]. One study indicates PFS and OS benefit of HDT over chemotherapy with melphalan plus prednisolone [25]. However, in the other study, adding thalidomide to melphalan plus prednisone in the chemotherapy arm, offers significant OS and PFS benefit when compared to upfront HDT with Mel100 × 2 in this population [26].

There are also a number of reports studying the use of HDT (in some cases an attenuated dose of Mel140 was used) in patients with renal failure (some of whom required dialysis). Although in the majority of cases there was no improvement in renal function post-HDT, the procedure was generally well tolerated and the renal disease was not a factor influencing overall survival [38, 39].

Various studies had suggested that having chemo-sensitive disease at the time of transplant is also an important prognostic factor [17, 40]. However, the meta-analysis of randomized studies indicates that response to initial anti-myeloma induction therapy does not impact outcomes differentially. As mentioned previously, in RCTs comparing upfront SDT versus HDT with single autologous transplantation, the studies restricted to patients with chemoresponsive myeloma had similar outcomes – PFS benefit, but no OS benefit – as were noted in studies that also enrolled patients with chemorefractory disease [27].

2.5. Upfront Versus Salvage HDT

The relative merits of HDT, either upfront in the disease course or as salvage therapy for relapse after conventional upfront therapy, have been examined in two RCTs [18, 20]. To date one trial has been published in final form, and showed no difference in OS between patients receiving either early or late (salvage) HDT; however, the Time Without Symptoms and Toxicity (TwisT) favored the early transplant cohort [18]. In the meta-analysis, the subgroup of studies with low SDT crossover (i.e., low proportion of patients on SDT also receiving salvage HDT after disease progression) had similar results to the studies with high crossover in the SDT arm [27]. Both subgroups showed similar findings – PFS benefit, but no OS benefit – with upfront HDT. This indicates that salvage HDT can rescue OS in patients receiving upfront SDT, but by definition will not impact PFS.

Summarizing the data regarding HDT with single autologous transplantation versus SDT, upfront autotransplant may not be mandatory from an OS benefit perspective. However, given the documented PFS benefit, all eligible patients should be considered for HDT, including those with disease refractory to induction therapy. Although the timing of HDT in the disease course remains unclear, it is probably prudent to collect autologous stem cells either at diagnosis or at maximum response to ensure that an adequate harvest is available to support a subsequent high-dose procedure.

2.6. Double Autologous Transplants

In order to improve the response rates and hopefully increase survival, a number of groups have evaluated the use of further courses of intensification therapy following the initial high-dose procedure. Results from the Arkansas group have shown that this approach is effective [40, 41]. To date three RCTs comparing single versus double autologous transplantation have reported interim analyses, and one has been published in final form [42–45]. The approach appears to be feasible, with a low TRM, and with the second HDT

being delivered in a timely fashion in approximately 75 percent of patients. The published trial compared single versus double HDT, each preceded by Mel140+TBI conditioning, and reported a PFS and OS benefit in the double transplant arm at seven years [42]. However, given the superiority of Mel200 conditioning for HDT, the benefit of double HDT in this setting remains to be confirmed. Interim analysis of the other trials show variable results [43–45]. The RCT data available thus far support significant benefit to tandem autologous transplantation, but more mature data from the ongoing tandem transplant RCTs is awaited (Table 8-2). We are not aware of any RCTs comparing SDT versus double autologous transplantation.

2.7. Prognostic Factors

A number of prognostic factors have been identified as important in predicting survival post-HDT. To date nearly all centers have identified β2 microglobulin (β2m) as the single most important prognostic variable, with patients with a high β2m at diagnosis having a shorter survival post-HDT [17, 21, 40]. Patients with hypodiploidy, t(4;14), 11q breakpoints, t(14;16), t(14;20), or deletions of chromosome 13 or of chromosome 17p13 (p53 gene inactivation) also fare worse following HDT [41, 46–50]. When both β2m and chromosome 13 abnormalities are taken into account a group of patients with a particularly poor outlook can be identified [41, 51].

The introduction of HDT has resulted in more patients attaining a CR, and conceptually a CR had been seen as the first step to achieving a cure. Interestingly, however, clinical data equating CR with likelihood of cure has been hard to obtain and, as discussed earlier, the benefit of upfront HDT in trials of patients with chemoresponsive myeloma is not different from trials including patients with chemorefractory myeloma [27]. Post-transplant, it has been suggested that patients who achieve a CR after HDT may have an improved survival, but are still highly likely to relapse. A number of studies have shown an improved PFS and OS in patients who attain a CR with negative immunofixation, although studies using electrophoresis to define CR are less clear-cut [41, 52, 53]. In the context of double autologous transplants with MEL200 conditioning, the benefit of the second transplant is likely restricted to patients who did not achieve a CR or nCR with the initial autologous transplant [42].

Table 8-2. RCTs of single versus double autologous transplantation for newly diagnosed myeloma.

Author	# Transplants	Regimen	CR/nCR[a]	EFS (median)	OS (median)
Attal	Single	Mel140/TBI	42%	25 months	48 months
	Double	Mel140→Mel140/TBI	50%	30 months[b]	58 months[b]
Fermand	Single	MLP140/Cy/TBI-12	39%	31 months	–
	Double	MLP140→MLP140/VP/TBI-12	37%	33 months	–
Cavo	Single	Mel200	35%	21 months	–
	Double	Mel200→Mel120/Bu	48%	31 months[b]	–
Goldschmidt	Single	Mel200	–	23 months	–
	Double	Mel200→Mel200	–	NYR[b]	–

Abbreviations: Cy cyclophosphamide; *Mel* melphalan (dosed in mg/m^2); *MLP* melphalan, carmustine, etoposide; *TBI* total body irradiation (dosed in Gy); *VP* VP16 (etoposide)

[a]nCR > 90% reduction in monoclonal protein

[b]Statistically significant difference

2.8. Minimal Residual Disease

Despite the increase in response rates and improvement in survival following high-dose therapy, several studies have failed to show a plateau of survival, suggesting that all myeloma patients have residual disease after HDT and will eventually relapse [17, 40]. The use of allogeneic and autologous transplantation has increased the CR rate in patients with myeloma, and in order to accurately assess the effects of such treatments more sensitive methods to assess residual disease have been introduced.

The polymerase chain reaction (PCR) can be used to detect rearrangements of the immunoglobulin heavy chain region, although a target is only present in approximately 80 percent of patients. Consensus PCR approaches have sensitivities of up to one malignant cell in 10^4 normal cells. Clonospecific methods are more sensitive (one malignant cell in 10^6 normal cells), but can be labor-intensive and expensive.

Flow cytometry offers a quick and efficient method to detect malignant plasma cells with a sensitivity of greater than one in 10^4 and may offer a clinically useful alternative to PCR. Flow cytometry is an alternative for monitoring minimal residual disease. A recent study suggested that patients who are immunofixation negative and have sustainable levels of plasma cells with a normal phenotype post-transplantation have an improved survival compared to patients who are immunofixation negative with residual plasma cells with a malignant phenotype [54].

It remains unclear whether these technologies provide additional information compared to the simple monitoring of serum or urinary paraprotein levels. A report indicated that cases that were immunofixation negative were also IgH PCR negative, using a fluorescent PCR with a sensitivity of one in 10^4 [53]. ASO-PCR is more sensitive and although the sample size is small, there is a suggestion that PCR-positive patients have a shorter PFS compared to those patients who become PCR negative [55, 56].

These data would suggest that there is little additional benefit for using fluorescent IgH PCR to monitor patients who become immunofixation negative, and that if PCR monitoring is to be clinically relevant the more sensitive ASO-PCR approach should be used. Flow cytometry may be an alternative for these patients.

3. Allogeneic Transplantation

3.1. Ablative Allogeneic Transplantation

Myeloablative allogeneic bone marrow transplantation has not been widely used in myeloma because of the high treatment-related mortality (TRM) and morbidity associated with the procedure in this older patient population (Table 8-3).

Retrospective registry data from the European Group for Bone Marrow Transplantation (EBMT: 1983–1993 and 1994–1998) on 690 patients showed approximately 50 percent of patients achieve a complete response, including some durable responses [57, 58]. Importantly between the two time periods, OS at three years rose from 35 percent to 56 percent and TRM fell from 40 percent to 30 percent. This is presumed to be due to better patient selection, earlier transplantation and less pre-transplant therapy, and improved supportive care. The stage at diagnosis, pre-conditioning remission status, extent of

Table 8-3. Representative studies of myeloablative allogeneic transplantation for myeloma.

Author	*N* (% chemorefractory)	TRM	CR	OS (f/u years)	PFS (f/u years)
Gahrton	162 (46)	41%	44%	28% (7)	55% (5)
Bensinger	80 (71[a])	43%	36%	24% (4.5)	20% (4.5)
Alyea[c]	24 (0[b])	21%	33%[c]	55% (2)	30% (2)
Gahrton	334 (27) BM 1983–1993	46%	60%	35% (3)	40% (3)
	356 (24) BM 1994–1998	30%[d]	60%	55%[d] (3)	55%[f] (3)
	133 (26) PBSC 1994–1998	37%[e]	54%	57%[d,e] (3)	NR

Abbreviations: BM bone marrow for stem cell rescue; *PBSC* peripheral blood for stem cell rescue

[a]<50% reduction in monoclonal protein was considered chemorefractory disease

[b]<40% plasma cells in bone marrow at time of allotransplantation was considered chemosensitive disease

[c]After DLI

[d]Statistically significant difference compared to BM allotransplants in the period 1984–1994

[e]Not statistically different from BM allotransplant outcome in the period 1994–1998

[f]CD6+ T cell depleted transplant plus planned sequential CD4+ DLI

previous treatment and serum β2 microglobulin level were important prognostic factors; males fared less well than females.

In a retrospective case series comparing 189 allogeneic bone marrow recipients to an equal number of autologous stem cell recipients, there was an OS benefit to autotransplantation, at 34 versus 18 months (p=0.0001) [59]. This was primarily due to a markedly lower incidence of TRM with autotransplantation (13%) versus allotransplantation (41%) [60]. Prospective data from the period are limited, but sobering. For instance, in a United States intergroup study initiated in the early 1990s, the upfront allotransplantation arm was closed when an excessive first year TRM of 53 percent was observed after enrollment of 36 eligible patients [22]. Interestingly however, while the seven year-OS was equivalent for the autologous and allogeneic recipients (39%), with follow-up extending to 10 years, the survival curves for the allogeneic group was flat, while there was no plateau of survival in the autologous transplant group.

The use of PBSC rather than BM support has had a likely impact on allogeneic transplantation for myeloma. The more rapid engraftment associated with PBSC has yielded a reduced infection rate, and appears to have made Graft-versus-Host Disease (GVHD) more manageable, despite a greater dose of T cells infused with PB instead of BM [58, 61]. Some studies even suggest that this more rapid engraftment translates to an improved overall survival [61].

In the majority of reports the development of both acute and chronic GVHD has accounted for significant morbidity and mortality, with incidences of up to 50 percent. T cell depletion of grafts offers an approach to reducing the GVHD with a reduction in the associated morbidity and TRM [62]. However, concerns remain regarding a possible increase in infections and a decrease in the therapeutic graft-versus-tumor effect.

Despite the high TRM of conventional allogeneic transplantation in myeloma, the assumption that this mode of treatment is most likely to eradicate the myeloma cells, and the possibility of a significant Graft-versus-Myeloma (GVM) effect has encouraged its further consideration. Data from multiple centers has shown that patients with myeloma relapsed after allogeneic transplantation can achieve marked clinical responses after receiving donor lymphocyte infu-

sion (DLI), due to induction of a GVM effect [63, 64]. In one study there was evidence of GVM response to DLI in 62 percent of cases; however, GVHD occurred in 66 percent of patients and contributed to a procedure-related mortality of 15 percent [63]. Indeed, in a review of patients receiving DLI for relapsed myeloma, GVM effect was noted in 18 of 22 patients who developed GVHD, compared to only two of seven patients who did not develop GVHD [65].

One approach to maintaining the low TRM, but exploiting the GVM effect is to utilize CD8 depleted DLI six to nine months post-CD6 depleted BMT. Alyea, et al. reported the use of this approach in 24 patients with chemoresponsive disease [64]. A significant GVM effect was demonstrated following the DLI for persistent disease in 10 patients (six compete responses and four partial responses); unfortunately this too was associated with 50 percent of patients developing GVHD. Of interest, 10 patients were unable to receive DLI due to transplant-related complications, suggesting that for allogeneic transplantation followed by DLI to be an effective strategy in myeloma a transplantation regimen with less toxicity is needed. As the TRM of ablative allotransplantation is further improved, it may also be reasonable to re-evaluate this modality in the treatment of myeloma.

3.2. Reduced Intensity Conditioning (RIC) Allogeneic Transplantation

A nonmyeloablative or 'mini allogeneic' transplantation approach is currently being evaluated in many centers to reduce the conditioning regimen-related toxicity and attempt to maximize the immunologic Graft-versus-Myeloma effect of allogeneic transplantation. This approach uses immunosuppression rather than cytoreduction to induce donor engraftment with minimal toxicity. It can be used in older individuals or patients who would otherwise not be eligible for conventional high-dose transplantation due to underlying morbidity. This is particularly important in myeloma patients, as less than 10 percent of patients are eligible for a conventional allograft (i.e., aged less than 55 years with a HLA-matched sibling) and the TRM with a myeloablative approach, as mentioned above, is high.

A number of conditioning regimens are being investigated using combinations of low dose radiotherapy, chemotherapy and immunosuppressive agents. Initial reports using low dose total body irradiation (TBI) with mycophenolic acid (MMF) and cyclosporine in end-stage myeloma were disappointing due to poor engraftment and poor response rates. This may be due to the relatively high tumor burden at the time of transplantation [66]. More recent studies have included additional low dose chemotherapy and are more encouraging. Thus far, over a dozen phase II studies involving patients who have received allogeneic stem cell transplantation after RIC regimens have been reported in final or abstract form (Table 8-4) [67–79]. The RIC regimens varied significantly, and include Mel100–140, typically with added fludarabine; TBI (2 Gy), with fludarabine or cyclophosphamide or low dose busulfan. Anti-thymocyte globulin (ATG) or CD52 antibody (alemtuzumab) were included in some regimens to facilitate engraftment and minimize GVHD. There is currently no consensus on which RIC regimen has superior TRM or OS/PFS. Overall, TRM has ranged from 0 percent to 41 percent, with CR rates of 10 percent to 75 percent. Acute (grade 2 to 4) and chronic GVHD rates ranged from 25 percent to 58 percent and 7 percent to 70 percent, respectively.

Recently, EBMT registry data was summarized on 229 patients undergoing RIC allotransplantation at 33 centers [80]. The RIC regimens varied consid-

Table 8-4. Representative studies of RIC allogeneic transplantation in myeloma.

Author	Conditioning regimen	GVHD regimen	N (URD)	Prior Auto[a]	TRM %	CR %	Grade 2–4 acute GVHD%	Chronic GVHD%	OS% (years)
Kroger	Mel100/Flu/ATG	CSA/MTX	17 (8)	17	18	73	38	7	74 (2)
Kroger	Mel100–140/Flu/ATG	CSA/MTX	21 (21)	9	24	40	38	12	74 (2)
Mohty	Bu/Flu/ATG	CSA ± MTX	41 (NR)	0	17	24	36	41	62 (2)
Peggs	TBI/Flu/Alemtuzumab	CSA/MMF	20 (8)	0	15	10	25	NR	71 (2)
Maloney	TBI-2 Gy/Flu	CSA/MMF	54 (0)	54	22	57	45	60	69 (4)
Gerull	TBI-2 Gy/Flu	CSA/MMF	52 (20)	0	17	27	37	70	41 (1.5)
Hoepfner	TBI-2 Gy/Flu	CSA/MMF	19 (6)	0	32	NR	37	NR	50 (2)
Ma	TBI-3 Gy/Flu	CSA/MMF	10 (0)	0	0	30	60	40	100 (1)
Galimberti	TBI-2 Gy/Flu; Flu/Cy	CSA/MMF	20 (0)	20	20	35	25	30	58 (2)
Einsele	TBI-2 Gy/Flu/Cy	CSA/MMF/ATG	22 (15)	0	23	27	38	32	26 (2)
Lee	TBI-2 Gy/Flu/Mel100	CSA	45 (12)	12	38	64	58	13	36 (3)
Giralt	Mel/Flu	FK/MTX	22 (9)	0	41	32	46	27	30 (2)
Perez-Simon	Mel/Flu	CSA/MTX	29 (NR)	10	21	28	41	51	60 (2)

Abbreviations: ATG anti-thymocyte globulin; *Bu* busulfan; *CSA* cyclosporine; *Cy* cyclophosphamide; *Flu* fludarabine; *Mel* melphalan; *MMF* mycophenolate mofetil; *MTX* methotrexate; *TBI* total body irradiation (Gy)

[a]Sequential RIC allotransplant after autotransplant

Table 8-5. Prospective studies of double autologous transplantation versus sequential autologous and RIC allotransplantation in myeloma.

Author	Patients	Conditioning	N	TRM	CR/nCR	Median EFS (months)	Median OS (months)
Garban	Newly diagnosed 'high risk' myeloma (high β2m; del 13); Age < 65 years	Auto Mel200→Auto Mel220	219	5%	33%/18%	25	41
		Auto Mel200->RIC Allo Bu/Flu/ATG	65	11%	33%/29%	30	35
Bruno	Newly diagnosed myeloma; Age < 65 years	Auto Mel200→Auto Mel200 δ	82	2%	26%/NR	29	54
		Auto Mel200→RIC Allo TBI (2 Gy)	80	10%	55%/NR	35*	80[a]

*Statistically significant difference

erably, but most utilized fludarabine with either low dose TBI, melphalan or cyclophosphamide. ATG or alemtuzumab was included in about half the regimens, and most studies used PBSC transplants. Overall, TRM was low at 22 percent, with acute (grade 2 to 4) and chronic (extensive) GVHD occurring in 31 percent and 25 percent of patients, respectively. The three-year OS and PFS were 41 percent and 22 percent, respectively. Interestingly, patients with no chronic GVHD had the worst outcomes, with OS and PFS of 29 percent and 12 percent, respectively. On multivariate analysis, disease status and duration at RIC allotransplantation, and alemtuzumab conditioning were adverse prognostic factors for TRM, PFS and OS.

There are no RCTs comparing allotransplantation with conventional ablative conditioning versus RIC approaches. One nonrandomized study compared 31 patients undergoing RIC allotransplantation with a historical control group of 93 patients undergoing ablative allotransplantation [81]. RIC allotransplantation enjoyed significantly lower TRM (10% versus 29%, p=0.03) and had a trend towards improved OS at one year (71% versus 45%; p=0.09). It is clear that RIC approaches are feasible and appear less toxic by reducing the early transplant-related complications and mortality. They also retain the GVM effect of allotransplantation and are able to induce CRs.

3.3. Sequential Autologous and RIC Allogeneic Transplantation

In order to reduce the myeloma burden before the RIC allotransplantation, a number of groups have combined it with a prior autologous transplant using melphalan conditioning [67, 71]. The procedure is well tolerated with a TRM of 16 to 18 percent, and all patients achieved full donor chimerism. Response rates are good with a high number of patients achieving a CR (up to 73% using stringent criteria), but the incidence of GVHD was high at over 50 percent.

However, when evaluated in a randomized basis, a study comparing 219 high risk patients (high β2m and chromosome 13 deletion) who underwent double autologous transplantation versus 65 patients who underwent autologous transplantation followed by sequential RIC allogeneic transplantation did not find an OS benefit with sequential RIC allotransplantation [82]. Interestingly, the use of high-dose ATG for RIC conditioning in this study likely resulted in a low rate of cGVHD (7%), but also a relatively low CR rate (33%). Whether

RIC regimens without high-dose ATG can overcome the negative prognostic implications of chromosome 13 deletions remains to be determined. Early results from an Italian study comparing the two approaches in standard risk myeloma indicate equivalent TRM, and both OS and PFS benefit to sequential RIC allotransplantation [83]. Additional multi-institution trials comparing the two approaches are ongoing.

RIC allotransplantation regimens now result in reliable engraftment and relatively low TRM compare to ablative regimens. Despite the lower TRM, and preserved immunologic GVM benefit of nonmyeloablative allotransplantation, the occurrence of significant GVHD remains a barrier to using this approach more broadly in myeloma. Additionally, substantial cytoreduction before RIC allotransplantation may be necessary owing to a more limited GVM effect. Longer-term follow-up is required to determine the durability of remissions obtained with RIC approaches.

4. Strategies Complementing Transplantation

Although the results from studies of HDT in myeloma are encouraging, the survival curves show no obvious plateau and suggest that autologous transplantation is not curative. A number of new drugs are being evaluated as part of induction chemotherapy to increase the response rate prior to HDT. In many cases the traditional VAD-like regimens are being substituted by high-dose dexamethasone alone, or with drug combinations including thalidomide, lenalidomide and bortezomib. In order to target residual malignant plasma cells in the bone marrow at the time of transplant, a number of groups are using antibody therapy during the conditioning; examples include Holmium, anti-IL6 and anti-CD138. Unfortunately, in patients with high risk myeloma (high β2m and chromosome 13 deletions) undergoing tandem autologous transplantation, the addition of IL-6 antibody to the second conditioning regimen did not provide survival benefit [84].

One of the major obstacles to curing myeloma is the persistence of minimal residual disease post-HDT. Results with previous maintenance therapy regimens have been disappointing. Trials of maintenance interferon or prednisolone resulted in a small prolongation of survival, at the cost of significant toxicity and impaired quality of life [85, 86]. Recent studies evaluating maintenance thalidomide after tandem autologous transplantation confirmed a PFS benefit, but arrived at variable conclusions regarding OS benefit [87, 88]. A randomized trial of maintenance lenalidomide after HDT with single autologous transplantation is ongoing.

A number of approaches for generating and enhancing anti-myeloma immunity after allogeneic and autologous transplantation are being evaluated. These include antibody directed therapy and immune-based approaches with a variety of vaccination strategies: patient specific idiotype approaches; dendritic cells pulsed with patient specific idiotypic protein or immunization with fusions of myeloma cells with autologous dendritic cells or the infusion of autologous T cells expanded *ex vivo* against patient tumor cells. In the context of immunomodulation, non-cytotoxic approaches utilizing thalidomide or bortezomib, along with DLI for patients with relapsed myeloma after RIC allotransplantation, significantly improved the response rate and resulted in an impressive two-year OS and PFS of 100 percent and 84 percent, respectively, with low rates of GVHD [89, 90].

Further approaches combining myeloma specific agents and immune modulation with autologous and allogeneic transplantation approaches appear promising. As ever, enrollment in clinical trials that evaluate these and other strategies for improving myeloma outcomes are to be encouraged.

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Chapter 9

Allogeneic Hematopoietic Cell Transplantation for Patients with Myelodysplastic Syndrome and Myeloproliferative Disorders

Aravind Ramakrishnan and H. Joachim Deeg

1. Introduction

Myelodysplastic syndromes (MDS) and myeloproliferative disorders (MPD) are clonal diseases of hematopoietic precursors/stem cells. While the course may be protracted, these diseases are progressive in nature and, unless treated effectively, generally prove fatal. MDS represents a complex, heterogeneous group of disorders that are characterized by dysplastic marrow morphology, various cytogenetic abnormalities, peripheral blood cytopenias due to clonally dysregulated hematopoiesis and an increased risk for developing acute myelogenous leukemia (AML) [1]. In many patients with MPD, proliferative features with increased blood cell counts are prominent early in the disease, while cytopenias may develop eventually. Following an initial cellular marrow phase, severe marrow fibrosis, generally associated with splenomegaly, may develop [2]. The recently identified JAK2 (V617F) kinase mutation, which results in constitutive cell activation, may lead to a new classification of the disorders that we currently refer to as Polycythemia vera (PV), essential thrombocythemia (ET) or chronic idiopathic myelofibrosis (CIMF) [3, 4]. Some patients present with clinical and histological features of both MDS and MPD [1, 2]. In fact, chronic myelomonocytic leukemia (CMML), listed under MDS in the original French American British (FAB) classification [5], has been re-classified as a separate (overlap) entity by the World Health Organization (WHO) [6].

MDS and MPD are predominantly, but not exclusively, diseases of the elderly. This is one reason why, until very recently, the standard of care was supportive therapy. Recently, some therapeutic agents, including DNA methyltransferase inhibitors and lenalidomide, have become available. However, as of now, the only treatment modality that has been shown to have curative potential is hematopoietic cell transplantation (HCT) [7].

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2. MDS

MDS is a disease that predominantly affects the elderly (median age at diagnosis 70 years), but younger patients who have received chemotherapy or radiation treatment for other cancers or nonmalignant disorders are also at risk of developing what has been termed secondary or treatment-related MDS. Finally, patients who present with diseases such as aplastic anemia may develop MDS. The underlying pathophysiology of MDS is incompletely understood, although apoptosis does play a central role. The etiology may be multifactorial involving mutations in a hematopoietic stem/precursor cell [1].

Most patients with MDS present with peripheral blood cytopenias, the most common being anemia. Approximately 40 percent to 50 percent of patients with *de novo* MDS also have clonal chromosomal abnormalities that can be detected by karyotype analysis or fluorescent *in situ* hybridization (FISH). Some of these abnormalities, such as isolated del(5q), are associated with a good prognosis, while others, such as abnormalities of chromosome 7, predict a more rapid progression to AML [5–8]. The FAB classification system has been critical for the staging of MDS [5]. Developed in the early 1980s, this system is in the process of being replaced by the WHO modification (see Table 9-1) [6]. Modifications include eliminating the RAEB-T group (all patients with 20% myeloblasts or more are considered as having AML), and separating refractory anemia (RA) into RA (erythroid abnormalities only), refractory cytopenia with multilineage cytopenia (RCMD), the 5q- syndrome and MDS-U. CMML, as indicated, has also been re-classified into a separate MDS/MPD category [6].

These classification systems are used in conjunction with the International Prognostic Scoring System (IPSS) to stratify patients into prognostic groups for the purpose of selecting treatment strategies (Table 9-2). The IPSS incorporates three parameters – the number of blood cytopenias, cytogenetic findings and the myeloblast count in the marrow – to provide a scoring system that predicts prognosis for newly diagnosed patients. Based on those findings, patients are stratified into four categories: low risk, intermediate-1, intermediate-2 and high risk, which carry a median life expectancy of 5.7, 3.5, 1.2 and 0.4 years, respectively [8].

Malcovati, et al. recently proposed a further modified prognostic system based on the WHO classification (WPSS) (Table 9-3) [9]. The WPSS incorporates the WHO classification, karyotype and transfusion requirement to risk-stratify patients into five categories: very low (score 0), low (1), intermediate (2), high (3–4) and very high (5–6), predicting for median survivals of 136, 63, 44, 19 and eight months, respectively. The WPSS categories are also useful for predicting the probability for leukemia progression; patients in the very low group had a 10-year probability of 7 percent while those in the very high group had a probability of 50 percent at eight months [9].

3. MPD

PV, ET and CIMF (also known as agnogenic myeloid metaplasia) are the three main MPDs which will be considered here. Chronic myelogenous leukemia (CML), with the classic t(9;22) translocation leading to the formation of the

Table 9-1. WHO classification and criteria for myelodysplastic syndromes.

Classification	Peripheral Blood	Bone Marrow
Refractory anemia (RA)	Anemia No or rare blasts	Erythroid dysplasia only <5% blasts <15% ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia No blasts	Erythroid dysplasia only <5% blasts >15% ringed sideroblasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Bi- or pan-cytopenia	Dysplasia in ≥10% of cells in two or more myeloid cell lines
	No or rare blasts No Auer rods Monocytes<1000/μL	<5% blasts No Auer rods <15% ringed sideroblasts
Refractory anemia with excess blasts1 (RAEB-1)	Cytopenias	Unilineage or multilineage dysplasia
	<5% blasts No Auer rods Monocytes<1000/μL	5–9% blasts No Auer rods
Refractory anemia with excess blasts 2 (RAEB-2)	Cytopenias	Unilineage or multilineage dysplasia
	5–19% blasts Auer rods± Monocytes<1000/μL	10–19% blasts Auer rods±
MDS-Unclassified (MDS-U)	Cytopenias	Unilineage dysplasia in granulocyte or megakaryocytes
	No or rare blasts No Auer rods	<5% blasts No Auer rods
MDS with del(5q) (5q- syndrome)	Anemia	Normal to increased megakaryocytes with hypolobulated nuclei
	<5% blasts Platelets normal or increased	<5% blasts No Auer rods Isolated del 5q

BCR-ABL fusion gene, is also an MPD, but will not be discussed here. In addition, rare disorders such as chronic eosinophilic leukemia, chronic neutrophilic leukemia and systemic mastocystosis are considered MPD [2].

Similar to MDS, MPD are diseases predominantly affecting the elderly. The hallmark of PV is erythrocytosis with or without leukocytosis or thrombocytosis. ET is associated with highly elevated platelet counts. CIMF is associated with marrow fibrosis, leukoerythroblastosis and extramedullary hematopoiesis, generally associated with massive splenomegaly. In later stages, PV and ET can also progress to myelofibrosis, and all MPDs can evolve into AML [7]. In 2005, several groups simultaneously identified an activating mutation (V617F) in the JAK-2 kinase in the majority of patients with PV and also large proportions of patients with ET and CIMF [3, 4]. While this mutation contributes to the disease, the exact relationship is a matter of debate, and additional mutations are being identified.

Table 9-2. International prognostic scoring system (IPSS) for myelodysplastic syndromes.

Prognostic Value	Score Value				
	0	**0.5**	**1.0**	**1.5**	**2.0**
BM blasts (%)	<5	5–10	—	11–20	21–30
Karyotype[a]	Good	Intermediate	Poor		
Cytopenias	0 or 1	2 or 3			

Scores for risk groups are as follows: low = 0; INT-1 = 0.5–1.0; INT-2 = 1.5–2.0; and high = ≥2.5
[a]Good = normal, -Y, del(5q), del (20q); poor = complex (≥3 abnormalities) or chromosome 7 anomalies; intermediate = other abnormalities

Table 9-3. WHO classification based prognostic scoring system (WPSS) for MDS.

Prognostic Variable	Score Value			
	0	**1**	**2**	**3**
WHO category	RA, RARS, 5q-	RCMD, RCMD-RS	RAEB-1	RAEB-2
Karyotype[a]	Good	Intermediate	Poor	—
Transfusion requirement	No	Regular	—	—

Scores for risk groups as follows: very low = 0, low = 1, intermediate = 2, high = 3–4, very high = 5–6
[a]Good = normal, -Y, del(5q), del (20q); poor = complex (≥3 abnormalities) or chromosome 7 anomalies; intermediate = other abnormalities

Table 9-4. Lille scoring system for myelofibrosis.

Factor Number	Risk Group	Cases (%)	Median Survival (months)
0	Low	47	93
1	Intermediate	45	26
2	High	8	13

Adverse factors: hemoglobin <10 g/dL, white blood cell count <4 or >30 × 10^9/L

While these disorders are chronic in nature, over time patients develop severe secondary complications due to myelofibrosis, including hepatic fibrosis, portal hypertension, heart failure and infectious complications due to cytopenias [2]. Several prognostic scoring systems have been developed for CIMF, the most widely used being the Lille (or Dupriez) scoring system (Table 9-4). This system uses two variables, the hemoglobin level and white blood cell count, to stratify patients into low, intermediate and high risk groups with median survivals of 93, 26, and 13 months, respectively [10].

More recently, the Mayo Clinic group proposed to add thrombocytopenia as another poor risk factor to the variables in the Lille system (Table 9-5). This system predicts median survivals of 155, 69, and 24 months in the presence of 0, 1 or ≥ 2 adverse features respectively (see Table 9-5) [11]. Data from our institution also identified thrombocytopenia as an adverse prognostic factor in patients with CIMF [12].

Furthermore, an Italian study identified the peripheral blood CD 34+ cell count as a prognostic factor associated with leukemic transformation and

Table 9-5. Mayo scoring system for myelofibrosis.

Factor Number	Risk Group	Cases (%)	Median Survival (months)
0	Low	51	155
1	Intermediate	23	69
≥2	High	26	24

Adverse factors: hemoglobin <10 g/dL, white blood cell count <4 or >30 × 10^9/L, Platelet count <100 × 10^9/L

overall survival in 84 patients with CIMF [13]; however, a follow-up Mayo Clinic study failed to support those results [14]. The exact impact of clonal cytogenetic abnormalities remains to be determined [15].

4. CMML

CMML is a disorder with both dysplastic and proliferative features [5, 6]. Peripheral blood monocytosis (>1,000 μL) is the hallmark of CMML. Dependent upon the myeloblast count, it is divided into CMML-1 (blood <5%; marrow <10%), and CMML-2 (blood 5–19%; marrow 10–19%). The WHO has also defined a separate category of CMML with eosinophilia when there is an accompanying peripheral blood eosinophilia. Patients in this category often have a t(5;12)(q31;p12) translocation, which results in an abnormal platelet-derived growth factor receptor (PDGFRβ) fusion gene, and those patients may be responsive to tyrosine kinase inhibitors such as imatinib [6]. Patients may also have other clinical features including splenomegaly, lymphadenopathy and skin rashes. The M.D. Anderson group has developed a scoring system for CMML using four clinical parameters to predict survival: hemoglobin <12 g/L, presence of circulating immature myeloid cells, absolute lymphocyte count >2.5 × 10^9/L and marrow myeloblasts >10 percent (Table 9-6). Based on the presence of one or several of those findings, patients are divided into low, intermediate-1, intermediate-2 and high risk categories, with median survivals of 24,15, eight and five months, respectively [16].

5. Allogeneic Hematopoietic Cell Transplantation (HCT): General Considerations

Allogeneic HCT is currently the only curative therapy for patients with MDS, MPD or CMML. Several important factors should be considered before proceeding with HCT [17]. Patients often have co-existing comorbidities which have a significant impact on the success of HCT. Sorror, et al. recently developed a HCT-specific comorbidity index which provides a useful tool for risk assessment prior to transplantation [18].

The timing of HCT in patients with the chronic myeloid diseases discussed here has remained controversial due to the relatively chronic course without HCT and the treatment-related mortality with HCT due to organ toxicity from conditioning, infectious complications and Graft-versus-Host Disease (GVHD), accounting for 20 to 25 percent mortality [7, 17].

While patients with rapidly progressive disease may have "nothing to lose," patients with indolent (low risk) disease may have a good quality of life for

extended periods of time without any treatment, and may not want to accept the potential risks associated with transplantation [7].

In an attempt to provide guidance, Cutler, et al. carried out a decision analysis in patients with MDS, using a Markov model. This study showed that the IPSS was useful in determining which groups of patients benefited from proceeding to transplantation soon after diagnosis. Patients in the Int-2 and high risk groups (by IPSS) who have HLA-identical siblings should be transplanted early because delaying the procedure resulted in a shortened life expectancy. In contrast, patients in the low risk group had the best life expectancy if HCT was delayed until disease acceleration was confirmed. Some patients in the Int-1 risk group also benefited from delay of HCT, but the benefit was minimal so decisions regarding HCT should be made on an individual basis [19]. For example, some patients in the Int-1 group have isolated neutropenia or transfusion dependent isolated thrombocytopenia and may be at high risk of infectious or bleeding complications, even though their IPSS score was low.

For patients with MPD, few data on the optimal timing of transplantation are available. Most patients with PV and ET are considered for HCT when they develop peripheral blood cytopenias or their disease has progressed to myelofibrosis or transformed to AML [2, 7]. Data from patients with PV, ET and CIMF from our own institution suggest that patient age, Lille score, platelet count and comorbidity score were useful in making the decision to proceed to HCT [20].

G-CSF mobilized peripheral blood progenitor cells (PBPC) are currently the most commonly used source of stem cells. Data from several centers, including our own, suggest decreased rates of graft rejection and relapse in patients with MDS receiving PBPC as compared to marrow. However, the rates of chronic GVHD are higher with PBPC, and overall survival rates may not differ significantly between the two groups [21, 22]. Umbilical cord blood (UCB) is an alternative source of hematopoietic stem cells, and successful transplants have been reported in small groups of patients with MDS [23, 24]. The advantages of UCB include the rapid availability of units from storage banks and greater tolerance for HLA disparity, allowing for the use of mismatched units, thus increasing the potential pool of donors. However, low cell doses in individual cord blood units, an important determinant of engraftment, has limited the use of this transplant modality. Recently, the use of two unit cord blood transplants has garnered more interest; more studies are needed to examine this strategy in patients with MDS and MPD [25, 26].

As discussed elsewhere, major progress has been made in molecular HLA typing. Current techniques allow for selection of unrelated donors based on identity at the DNA sequence level [27]. As a consequence, results with matched unrelated donor transplants are now basically identical to those with HLA genotypically identical sibling donor transplants [22, 27, 28].

Conventional myeloablative conditioning is generally offered only to patients less than 65 or 60 years of age, with HLA-identical siblings and unrelated donors, respectively [7]. Consequently, older patients would not be considered for HCT. In the last decade nonmyeloablative (NMA) or reduced-intensity conditioning (RIC) regimens have allowed for expanding the upper age for HCT, and even patients in their early 70s have been transplanted successfully using these strategies [29, 30]. Treatment-related mortality tends to be lower with these approaches, but this may be at the cost of increased relapse rates. Patients with the lowest disease burden tend to have the best success

rates, an observation that has again raised the question of a potential benefit of pre-transplant debulking chemotherapy [31]. However, the role of cytoreductive chemotherapy before HCT is controversial. A French study showed that patients with secondary MDS who achieved remissions after pre-transplant chemotherapy had a significantly higher probability of relapse-free survival after transplant than patients who did not respond [32]. A recent retrospective analysis conducted at our center also suggested that patients with MDS who received pre-transplant chemotherapy were less likely to relapse after HCT, although there was no significant benefit for relapse-free survival [33]. These data suggest that responses to pre-transplant chemotherapy may select for good risk patients who have chemo-sensitive disease and might have done well even without pre-HCT chemotherapy. Randomized trials that directly address these issues are currently ongoing. Data on the role of debulking chemotherapy in MPD are very limited; studies especially with NMA and RIC regimens may provide more insights.

Thus, the prognosis and pace of disease progression, the number of comorbidities, the stem cell source, the need for pre-transplant chemotherapy and the type of transplant are all important issues to consider before proceeding with HCT.

6. Myeloablative Conditioning and Transplantation

6.1. MDS

6.1.1. Low Risk MDS

The best results with HCT are achieved in patients with low myeloblast counts in the marrow (less <5%) and in patients who lack high risk cytogenetic markers; these are generally patients with low IPSS scores. The European Group for Blood and Marrow Transplantation (EBMT) reported results on 131 MDS patients transplanted from HLA-identical sibling donors. The five-year relapse-free survival (RFS) was 52 percent, and the relapse rate was 13 percent [34]. The same group reported a RFS of 24 percent at two years with a relapse rate of 13 percent for patients transplanted from unrelated HLA-matched donors [35]. The International Bone Marrow Transplant Registry (IBMTR) reported results in 452 patients with MDS transplanted from HLA-matched siblings, showing a three-year RFS of 40 percent, although only 140 patients (31%) had less than 5 percent blasts at HCT. Treatment-related mortality (TRM) was 37 percent, and the relapse rate was 23 percent. High marrow blast count and high IPSS score were significantly correlated with relapse. RFS was 72 percent for patients younger than 18 years [36]. In a cohort of 512 patients with MDS transplanted from unrelated donors, those conditioned with busulfan and cyclophosphamide (BUCY) fared better than patients conditioned with other regimens, in particular regimens containing high-dose total body irradiation (TBI) [37]. We recently reported the FHCRC experience with patients conditioned with a targeted BUCY regimen. The three-year RFS was 68 percent with related donors, and 70 percent with unrelated donors. Among 69 patients with RA/RARS, the non-relapse mortality rate (NRM) was 31 percent, and relapse occurred in 5 percent of patients [38].

6.1.2. High Risk MDS

The success rates of HCT decline as the non-transplant prognosis of MDS worsens, in particular with an increase in the marrow myeloblast count

beyond 5 percent. Intensification of conditioning regimens aimed at reducing the relapse risk has been associated with higher rates of NRM, and no improvement in survival [7]. The IBMTR reported results in 352 patients with high risk MDS transplanted from HLA-matched sibling donors; most were conditioned with TBI-based regimens. The three-year RFS was 63 percent for patients younger than 18 years and 33 percent for older patients [36]. The EBMT reported a five-year RFS for patients transplanted from related donors of 34 percent, 19 percent and 26 percent for RAEB, RAEB-T and tAML, respectively; the relapse rate was about 50 percent for the entire cohort. The two-year RFS for patients transplanted from HLA-matched unrelated donors was 27 percent, 8 percent and 27 percent for RAEB, RAEB-T and tAML, respectively [34, 35]. In a recent study from our center in patients with RAEB transplanted after conditioning with targeted BUCY, three-year RFS was 45 percent with related donors and 40 percent with unrelated donors. The corresponding figures for RAEB-T/tAML were 33 percent and 17 percent, respectively. Our data also indicate that the IPSS correlates strongly with outcome; patients with low IPSS scores had the highest probability of RFS and the lowest rates of relapse [22].

Another trial examined a combination of BU and TBI without high-dose CY. Sixty patients with advanced MDS, tAML or CMML were transplanted from related (n = 20) or unrelated (n = 40) donors with a three-year Kaplan-Meier estimate of survival of 26 percent and a relapse rate of 25 percent [38]. This was comparable to an earlier trial examining the combination of BUCY and TBI [39]. However, NRM was considerable, at 38 percent at 100 days [38]. This observation suggested that further increasing the conditioning intensity (with the aim of reducing relapse frequency) was unlikely to improve overall survival due to high NRM.

In an effort to reduce toxicity, several groups have examined the tolerability and efficacy of fludarabine in combination with targeted oral BU [40, 41]. Bornhauser, et al. prepared 42 patients with high risk hematologic neoplasms (38 with MDS) with a fludarabine plus BU combination for transplantation from related (n = 16) or unrelated (n = 26) donors. The probabilities of overall survival and RFS at a median follow-up of 18 months were 42 percent and 35 percent, respectively. All patients achieved engraftment, and the day 100 mortality was 7 percent [40]. Similarly, the M.D. Anderson group used a regimen of IV BU and fludarabine given consecutively for four days to treat patients with advanced myeloid malignancies (22 patients had MDS). The one-year RFS and TRM were 52 percent and 3 percent, respectively [41]. Encouraging studies such as these have stimulated the interest in further modifying conventional conditioning regimens to reduce toxicity while maintaining efficacy.

6.2. MPD

Initially there was significant concern that patients with myelofibrosis would fail to achieve engraftment of donor cells due to the disturbed marrow architecture. However, several case reports and small series suggested that HCT was feasible and potentially curative therapy [42–45]. These early studies were recently reviewed by van Besien, et al. [46]. It has also been shown both by skeletal MRI and histological examination that myelofibrosis is reversible after successful HCT and eradication of the malignant clone [47].

A retrospective study reported results in 55 patients transplanted at multiple institutions, mostly from matched sibling donors. Thirty-five patients were conditioned with a TBI-based regimen, and 20 were conditioned with BUCY. The five-year overall survival was 47 percent, and NRM at one year was 27 percent. This analysis identified low hemoglobin levels and the presence of osteosclerosis in the marrow as adverse prognostic markers [48].

We recently updated results on 104 patients with PV, ET or CIMF, 18 to 70 years of age, transplanted at our center from allogeneic (56 related; 45 unrelated) or syngeneic (n = 3) donors. The majority of patients received PBPC (n = 61), and engraftment was achieved in 101 of 104 patients. Sixty-three patients are surviving for a seven-year actuarial survival of 61 percent. Eleven patients experienced relapse or had persistent disease, eight of whom have died. Patients conditioned with targeted BUCY had a higher probability of survival than patients conditioned with other regimens. The Lille score, platelet count, patient age and comorbidity score were all significantly associated with survival. These data indicate that HCT is a potentially curative option for patients with MPD [12].

6.3. CMML

Until the recent WHO reclassification, CMML was considered a subcategory of MDS and few studies consider this entity separately [5]. A large series from the EBMT reported results in 50 patients. About half of these patients were conditioned with a TBI-based regimen (n = 26), and the majority of patients (n = 38) received hematopoietic stem cells from HLA-matched siblings. Forty patients received marrow, nine received PBPC and one patient received both. The RFS at two years was 18 percent with a NRM of 55 percent and a relapse incidence of 42 percent. Patients who developed acute GVHD had a lower rate of relapse [49]. The Mayo Clinic group reported results in 17 patients with CMML, the majority receiving TBI-based conditioning; 14 were transplanted from HLA-identical siblings and three from unrelated donors. The TRM was 41 percent, and seven patients (41%) had persistent disease or relapsed; five of the patients who relapsed received donor lymphocyte infusions, and two of these achieved durable remissions. At the time of reporting and with a median follow-up of 34.5 months, three of the 17 patients transplanted were still alive and remained in remission [50].

A total of 43 patients with CMML received HCT from related (n = 21; 18 HLA-identical siblings and three non-HLA-identical family members) or unrelated (n=22; 18 HLA-matched and four HLA-mismatched) donors at our center. The four-year RFS was 41 percent with a relapse incidence of 23 percent. Patients with higher comorbidity scores had an inferior probability of survival. Although the IPSS and M.D. Anderson scores showed no correlation with prognosis, there was a trend for higher relapse incidence among patients with a higher M.D. Anderson score (Table 9-6) [51]. The data suggested that a Graft-versus-CMML effect was provided by HCT [50, 51].

7. Nonmyeloablative/Reduced Intensity Transplants

In an attempt to reduce treatment-related toxicity and to offer the option of HCT to patients who were not considered candidates for conventional conditioning, NMA/RIC regimens have been developed. The basic principle behind

Table 9-6. M.D. Anderson criteria for CMML risk category.

Factor Number	Risk Group	Median Survival (months)
0–1	Low	24
2	Intermediate-1	15
3	Intermediate-2	8
4	High	5

these regimens is to provide sufficient immunosuppression to secure donor cell engraftment and then rely on the graft-versus-tumor effect mediated by donor-derived cells to eradicate the underlying disease. Initial regimens pioneered by Slavin, et al. and by Storb and colleagues consisted of low dose busulfan ± other agents or low dose (200cGy) TBI ± fludarabine [52, 53]. Subsequently, many groups have adopted similar regimens, but have increased the intensity. Conversely, other conventional conditioning regimens have been attenuated, leading to a broad spectrum of regimens aimed at eradicating the patient's disease while minimizing NRM [54]. The general trend has been to develop customized regimens based on disease, patient characteristics and prognosis.

A multicenter study examined the use of a NMA conditioning approach to transplant patients with MDS (n = 77) or MPD (n = 14) from related (n = 49) and unrelated donors (n = 42). The median patient age was 59 (range 6–72) years, and conditioning consisted of fludarabine and 200 cGy of TBI. With a median follow-up of two years, the estimated overall survival for the entire cohort at 18 months was 37 percent with a relapse rate of 43 percent. Patients with high risk MDS had a higher relapse rate than those with low risk disease. Among patients with MPD, 27 percent had evidence of progressive disease after transplantation [53].

Several groups have tested combinations of fludarabine and BU as part of RIC regimens [55, 56]. Kroger, et al. reported on 37 patients with MDS or tAML who were transplanted after conditioning with such a regimen from related (n = 19) or unrelated donors (n = 18). TRM was 27 percent, and with a median follow-up of 20 months, the three-year estimated disease-free survival was 38 percent, with a relapse rate of 32 percent [55]. A Spanish trial reported results on 37 patients with MDS or AML transplanted from HLA-identical siblings following a fludarabine/BU conditioning regimen. The one-year TRM and RFS were 5 percent and 66 percent, respectively [56].

Several studies have incorporated the anti-CD52 antibody alemtuzumab as part of a RIC regimen as a method of *in vivo* T cell depletion [57, 58]. A British study examined the use of a fludarabine/BU/alemtuzumab regimen and reported results on 75 patients with MDS receiving unrelated donor transplants. The three-year actuarial survival was 43 percent, and the cumulative incidence of extensive chronic GVHD was 22 percent. This analysis also indicated that the disease status at transplantation and the comorbidity score were independent risk variables for overall survival; however, patient age and cytogenetic abnormalities did not significantly affect outcomes [57]. A prospective phase II study by van Besien reported results on a RIC conditioning regimen consisting of fludarabine, melphalan and alemtuzumab in 52 patients with AML and MDS. After a median follow-up of 18 months, the relapse rate was 27 percent, TRM was 33 percent and RFS was 38 percent. The cumulative probability of extensive chronic GVHD was 18 percent. High risk disease and performance status were the major adverse factors for outcome [58].

There are few data on these approaches for CMML and MPD. However, several studies have tested NMA and RIC conditioning approaches for myelofibrosis. Devine, et al. first described results in four patients with a median age of 56 (range 48 to 58) years conditioned with a regimen of fludarabine 30 mg/m^2 intravenously daily for five days and melphalan 70 mg/m^2 intravenously daily for two days, followed by PBPCs from HLA-identical siblings. At a median follow-up of 13 months all patients were alive and had full donor chimerism, suggesting that a reduced-intensity approach was feasible in patients with MPD [59]. Investigators at City of Hope Medical Center presented results on nine patients transplanted with a fludarabine-based conditioning regimen. The median patient age was 54 (range, 46 to 68 years). Eight patients received fludarabine 25 mg/m^2/day for five days followed by melphalan 140 mg/m^2 for conditioning, while one patient received fludarabine 30 mg/m^2 for three days followed by 200 cGy of TBI. All patients achieved engraftment, and the probability of one-year survival was 56 percent [60]. Rondelli, et al. reported results in 21 patients with a median age of 54 (range, 27 to 68) years with intermediate or high risk myelofibrosis (based on the Lille score) transplanted at several centers over an extended time interval using a variety of RIC regimens. All but one patient engrafted and, at the time of reporting, 18 patients were alive (17 in remission) with a median follow up of 31 months [61]. Kroger and colleagues also reported results on 21 patients with a median age of 53 (range, 32 to 63) years conditioned with fludarabine, BU and anti-thymocyte globulin followed by transplantation from related (n = 8) or unrelated (n = 13) donors. With a median follow-up of 22 months, the three-year estimated RFS was 84 percent, and the one-year TRM was 16 percent. These reports, albeit in small cohorts of patients, suggest that dose reductions (in comparison to conventional regimens) allow for allogeneic engraftment and sustained remissions in patients with MPD.

8. Nonmyeloablative/Reduced Intensity Versus Myeloablative Conditioning Regimens

While NMA/RIC regimens were developed primarily to reduce toxicity, there has been concern regarding efficacy, raising the question as to the long-term success with either approach. Three recent retrospective analyses sought to address this question in patients with MDS and tAML and all three studies suggested that conditioning intensity did not impact overall survival [62–64].

An analysis of results in 150 patients with AML and MDS transplanted at our center also suggested that conditioning intensity did not significantly impact survival. One–hundred-twelve patients receiving myeloablative conditioning with BUCY were compared to 38 patients conditioned with a NMA regimen of fludarabine 90mg/m^2 with or without 200 cGy of TBI. Patients receiving NMA conditioning were older, had higher risk disease by IPSS, higher comorbidity scores and, importantly, had enjoyed more durable responses to pre-HCT chemotherapy. The three-year RFS and overall survival did not differ significantly between the two groups [62].

Alyea, et al. carried out a retrospective analysis in 136 patients transplanted for MDS and tAML. Outcomes among 97 patients receiving myeloablative conditioning with CY combined with either BU or 1,400 cGy TBI were compared

to those among 39 patients receiving NMA conditioning with fludarabine and low dose BU. This study also showed no statistically significant difference in overall survival and RFS between the two groups. Of note was that patients receiving NMA conditioning had higher rates of relapse, but the impact on survival was counterbalanced by a higher rate of TRM in patients receiving myeloablative conditioning [63].

More recently, the EBMT reported the largest series to date, comparing 836 patients with MDS receiving various myeloablative (n = 621) or RIC (n = 215) regimens. There was no significant difference in survival between the two groups; relapse rates were significantly lower in patients receiving myeloablative conditioning, but as in the other studies, this difference was negated by a higher TRM in patients conditioned with myeloablative regimens [64].

Since patients selected for RIC, as compared to more conventional myeloablative regimens, were not really comparable in regards to risk factors in these retrospective studies, conclusions should be drawn cautiously. Only prospective randomized trials will provide definitive answers. Furthermore, it would be wrong to simply contrast myeloablative to NMA/RIC regimens. As emphasized in several recent reviews [54, 65], a broad spectrum of conditioning regimens using various modalities have been developed. This is illustrated, for example, by the regimens used to condition patients with MPD, as discussed above. It is quite likely that what is "optimal" for one disease category may be "suboptimal" (either too intense or insufficient) for another, and what may yield superior results in patients whose disease is in remission at the time of HCT, may lead to disappointing outcomes in patients with disease burden at HCT.

9. Conclusions

HCT is the only current treatment with curative potential for patients with MDS and MPD. The IPSS is helpful in deciding which MDS patients should be transplanted and when. Patients with high risk MDS by IPSS criteria should undergo early transplantation. Select patients with Int-1 MDS are also likely to benefit from early HCT, particularly if their disease shows multilineage cytopenias requiring heavy transfusion support. Patients with low risk disease by IPSS are likely to have the longest life expectancy if HCT is delayed until there is evidence of disease progression [13]. As new agents for non-transplant therapy become available, the question of timing of transplantation needs to be re-addressed. Although there are fewer data for patients with MPD, patient age, Lille score, platelet count and comorbidity score are all useful parameters in making the decision to proceed with HCT [14].

A broad spectrum of conditioning regimens has been successful in transplanting patients with MDS and MPD. Decreasing conditioning intensity is associated with lower TRM, but often at the cost of increased relapse rates. Future studies are needed to further define the appropriate conditioning that minimizes toxicity while maximizing efficacy.

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Chapter 10

Stem Cell Transplantation for Marrow Failure

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1. Introduction

Aplastic anemia (AA) is a disease characterized by bone marrow hypocellularity and peripheral blood cytopenias, first described by Ehrlich in 1888 [1]. The incidence in Europe and North America is around 2 per million per year, with a biphasic age distribution that peaks at ages 10 to 25, and another at ages over 60 [2]. Beneath the simplicity of its pathological and clinical manifestations, aplastic anemia hides a heterogeneous and fascinating pathophysiology, as well as challenging treatment decisions. Aplasia is actually a manifestation of several distinct diseases, which can be acquired or congenital. In most cases acquired AA is believed to have an immune basis, based on both laboratory and clinical evidence [3]. In a minority of cases, the disease can be traced to exposure to drugs, toxins (most notably benzene), seronegative hepatitis or associated with other conditions (in particular paroxysmal nocturnal hemoglobinuria, pregnancy or eosinophilic fasciitis) [4]. In Asia, where the incidence of AA is higher, there is also epidemiologic evidence for an association with pesticide and animal fertilizer exposure [5]. In most cases, however, acquired AA is an idiopathic disease, where the trigger for the autoimmune attack remains obscure. Even when a trigger is identified, the mechanism leading from the causative agent to aplasia is often unclear, although the end result is either stem cell loss (which is irreversible) or immune-mediated suppression of stem cells (which may respond to immunosuppression or withdrawal of the causative agent). In contrast, congenital AA has a better understood molecular pathophysiology. Tremendous progress has been made in recent years in unraveling the molecular basis for many of the inherited marrow failure syndromes, including Fanconi's anemia, dyskeratosis congenita, Diamond-Blackfan anemia and Schwachman-Diamond syndrome. Their causes have now been traced to DNA damage repair defects, telomerase dysregulation and ribosomal dysfunction [6].

At present, there are two possible approaches to the treatment of acquired AA: immunosuppressive therapy (IST) and allogeneic hematopoietic stem cell transplantation (SCT). The profiles of benefits and toxicities for those

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two approaches are very different, which complicates the treatment decision for a given patient. In this chapter, we will focus on the role of SCT for AA, organizing the discussion around the following questions: why transplant, how to transplant and whom to transplant. The third question is the most relevant one clinically, but its answer depends on the first two and, of course, cannot be addressed without simultaneously discussing the outcomes of IST. There are also several excellent reviews on the topic [3, 7–10]. We have focused on results from Europe and the United States for this review. While the results of both IST and SCT in Asia (in particular Japan and South Korea) are well described, favorable and, in fact, often superior to the results in the West, they may not be directly applicable to patients in Europe or the United States, given the differences in the genetic heterogeneity of the patient populations and the possible differences in the disease itself.

2. The "Why" of Allogeneic Transplantation

Even before the immune basis of acquired AA was recognized, bone marrow transplantation seemed a reasonable strategy to treat patients' empty bone marrows. The first reported attempt was performed by Osgood and his colleagues in Oregon in 1939 on a teenaged school girl suffering from AA [11]. She received an intravenous injection of 18 cc of sternal marrow obtained from her brother, a "compatible donor" (who in fact matched the patient only in ABO group). The transplant was unsuccessful (not surprisingly under the circumstances), and the patient died. An intrasternal sibling marrow transplant performed in 1940 on a 42-year-old man in Brooklyn, NY was reported as successful (although the details of the report, including a platelet recovery in four days, raise questions of whether the diagnosis was truly AA and whether the transplant really contributed to the resolution of the cytopenias) [12]. It was two decades before the first reported successful transplant using a syngeneic donor [13], and another decade before the first successful transplant from a matched related donor (MRD) [14]. In 1976, a randomized trial from Seattle confirmed the survival benefit of MRD SCT over conventional support (at the time consisting of supportive transfusions or androgen treatment) [15]. Of historical note, the early experience in transplantation was one of the important clues to the immune pathophysiology of AA. In 1970, Mathé noted remission of AA in patients for whom antilymphocyte globulin-conditioned transplantation was attempted, but who failed to engraft. He attributed this success to the immunosuppression of the conditioning regimen [16]. In 1980, Appelbaum and colleagues reported on four AA patients treated with syngeneic transplantation without conditioning [17]. Two of the four had recovery of counts, but two did not; the latter patients then received a reinfusion of marrow from their twin donors with an immunosuppressive conditioning regimen, and both engrafted. Moreover, cells from the two patients who did not initially engraft could inhibit donor marrow cell growth in co-culture experiments. These findings, as well as the work of Speck and Kissling on rat models of AA [18], suggested that in some cases AA is caused by immune-mediated suppression of hematopoiesis. Similar observations and conclusions were subsequently published [19].

As more patients have undergone marrow transplantation in the last 30 years, it has become indisputable that this procedure can be curative for many.

Long-term follow-up of young patients transplanted from matched related donors in several studies showed a 10-year survival in excess of 80 percent, with most survivors having a Karnofsky performance status of 100 percent [20–23]. However, SCT entails a number of risks, including immediate complications from the conditioning regimen, graft rejection, acute Graft-versus-Host Disease (aGVHD) and immunosuppression, all contributing to transplant-related morbidity and mortality. There is a substantial incidence of chronic GVHD (cGVHD), which affects 20 to 50 percent of long-term survivors [20, 21]. Transplantation survivors are also at increased risk of growth abnormalities (for children), amenorrhea and infertility, cataracts and hypothyroidism [22]. Finally, there is a small, but definitely increased risk of secondary solid tumors after SCT; in a combined analysis of American and French patients transplanted for AA, the cumulative incidence at 20 years approached 2 percent; the risk was higher in patients who had received radiation as part of the conditioning regimen [24].

These results must be compared to the outcomes after IST. The results of a randomized multicenter German trial demonstrated improved failure-free survival when cyclosporine (CSA) was added to antilymphocyte globulin (ALG) and methylprednisolone [25]. Since that time, the combination of CSA + ALG (or anti-thymocyte globulin, ATG) has become the standard of care for IST [26]. In patients with severe or very severe AA, the four-month response rate was 65 percent; and with median follow-up of over 11 years, this regimen was associated with a 58 percent actuarial survival [27]. These numbers are very similar to those in a non-randomized single-center study of the same regimen at the NIH [28]. However, IST also carries several important risks. First, the rate of relapse is significant, being 45 percent in the above German trial and 35 percent in the NIH trial. Many of the patients who relapse can respond to a second course of IST [29], but this contributes to morbidity and mortality. Moreover, between 15 and 30 percent of patients will need to remain on maintenance CSA indefinitely [26]. More ominously, patients with AA treated with IST have a significant risk of later developing clonal abnormalities, including paroxysmal nocturnal hemoglobinuria (PNH), or worse, myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Those latter two complications are the major cause of death for patients who survive beyond three years after IST. In large series of patients treated with IST, the rate of developing PNH is 15 to 20 percent, while for MDS/AML it is around 10 to 20 percent [3, 30]. Finally, patients treated with IST also seem prone to develop secondary solid tumors, with an incidence around 2 percent, similar to that in transplanted patients [31].

Based on early reports of patients who rejected an allograft after high-dose cyclophosphamide conditioning, but whose AA remitted concomitantly with autologous marrow recovery [32], high-dose cyclophosphamide without grafting has been proposed as an alternative to ATG-based IST. Single-institution results were encouraging [33], but in a randomized trial conducted at NIH this approach showed prohibitive toxicity, leading to early closure of the trial [34]; there was also no evidence of improved remission or decreased risk of clonal progression with longer follow-up [35].

The foregoing discussion highlights the difficulty of comparing IST with SCT. On the one hand, the curative potential of SCT is superior to that of IST. Moreover, SCT essentially eliminates the risk of late relapse and, more

Table 10-1. Comparison of immunosuppressive therapy (IST) and stem cell transplantation (SCT).

	IST	SCT
Patient restrictions	None	Advanced age
		Comorbidities
		Donor unavailability
Curative potential	55–65%	80–90%*
Relapse risk	35–45%	Minimal
Early toxicity	Mild (drug adverse effects)	Graft failure (5–10%)†
		Conditioning toxicity
		Acute GVHD (10–50%)
Late toxicity	Solid tumors (2%)	Solid tumors (2%)
	PNH (15–20%)	Chronic GVHD (20–50%)
	MDS/AML (10–20%)	Other‡

*for young untreated patients with a syngeneic or matched sibling donor
†in modern series
‡including growth retardation, cataracts, hypothyroidism, infertility
Abbreviations: PNH, paroxysmal nocturnal hemoglobinuria; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; GVHD, Graft-versus-Host Disease
See text for references

importantly, the risk of late clonal disorders. On the other hand, SCT carries significant short-term toxicities, and survivors can suffer from long-term debilitating complications. A comparison of the relative merits and dangers of IST and SCT is shown in Table 10-1.

3. The "How" of Allogeneic Transplantation

3.1. The Issue of Graft Failure

SCT for patients with AA presents a unique challenge: to prevent graft failure without incurring undue toxicity. Graft failure (GF) is a particular problem in AA, more so than in other SCT indications, likely because of the anti-hematopoietic immune cells present in the host. These cells can reject the graft by the same mechanism that caused AA in the first place. The rate of GF with MRD transplants was as high as 30 percent in patients transplanted in the 1970s using cyclophosphamide (Cy)-only conditioning, and methotrexate alone for GVHD prophylaxis [36, 37]. Introducing radiation in the conditioning regimen appeared to reduce this risk [38]. A European Bone Marrow Transplant Registry (EBMTR) study confirmed that the rate of GF was lower with radiation conditioning regimens, as well as with use of CSA for GVHD prophylaxis, no treatment prior to SCT and gender matching [37]. There was also a clear time trend, with rates of 32 percent for patients transplanted before 1981, to 8 percent for those transplanted after 1984. In comparable analyses of International Bone Marrow Transplant Registry (IBMTR) data, the use of radiation for conditioning and of CSA for GVHD prophylaxis (versus methotrexate, and even more prominently versus T cell depletion) were confirmed

to be associated with lower risk of GF [39, 40]. Moreover, for patients conditioned with cyclophosphamide (Cy) alone, receiving over 40 transfusions prior to transplant and absence of prior steroid treatment were both associated with a higher risk of GF [39]. In earlier studies from Seattle, a low marrow cell dose was also associated with a higher rate of GF [41, 42]; subsequently, most centers have targeted marrow doses above 2–3 × 10^8 nucleated cells/kg of recipient weight, and (perhaps as a consequence of this) cell dose has not remained associated with GF [39, 40]. For several years, the Seattle group also used donor buffy coat infusions post-transplantation to improve engraftment. While this resulted in a lower incidence of GF, it increased the risk of cGVHD and was, therefore, abandoned [43].

Those findings make physiological sense under the hypothesis that acquired AA is an immune-mediated disease. Pre-transplant transfusions will potentially sensitize the immune system of the recipient to donor cell antigens; more intense conditioning, as provided by the addition of radiation, will better eliminate the recipient immune system, preventing subsequent host-versus-donor attack, and better post-transplant immune suppression (with the use of CSA) will also blunt a possible anti-donor immune attack.

3.2. Choice of Conditioning Regimen

Based on the above, radiation would appear to be of benefit in the conditioning regimen. However, radiation is associated with substantial toxicity. Indeed, in the studies quoted above, there was no survival advantage to total body irradiation (TBI) or total lymphoid irradiation (TLI) regimens; the benefit of improved engraftment was negated by an increase in acute GVHD and pulmonary toxicity [39, 40]. In another retrospective comparison, patients conditioned with Cy + ATG had better survival (~95% versus 55%) than patients conditioned with thoraco-abdominal irradiation (TAI) [44]; acute and chronic GVHD rates were also higher with TAI. Therefore, TBI, TLI or TAI have little role at present in the conditioning of patients receiving a graft from a matched sibling, unless perhaps the patient has been heavily pre-transfused. The situation is different for alternative donor transplantation. Here, the higher rates of GF have made it difficult to eliminate radiation from the conditioning regimen [45]. A recent multicenter study of 87 patients receiving bone marrow from alternative donors (62 matched unrelated donors (MUD) and 25 mismatched unrelated donors (MMUD)) sought to establish the optimal TBI dose (in combination with Cy and ATG) in this setting [46]. GF rates remained tolerable with a TBI dose as low as 200 cGy (1% for MUD and 12% for MMUD), and overall survival (OS) was not compromised (in fact, for MUD transplants OS was highest in the 200 cGy cohort, though this did not achieve statistical significance). Based on those results, a conditioning regimen consisting of Cy 200mg/m2, ATG 90mg/kg, and TBI 200 cGy seems to be a good choice for alternative donor SCT.

Those positive results with low dose TBI even in MUD transplants reflect, in part, the benefit of adding ATG to the conditioning regimen. Cy + ATG was first introduced for patients with graft failure, as part of the conditioning for the third [47], and later for the second, SCTs [48] following graft failure. Based on those encouraging results, ATG was then added to the conditioning regimen for first SCT [49]. In a recent analysis of 81 patients receiving a MRD SCT with Cy + ATG conditioning, very good outcomes were achieved, with

rates of engraftment of 96 percent, aGVHD (grade II-IV) of 24 percent and cGVHD of 26 percent [23]. With long-term (median over nine years) follow-up, 15-year overall survival was 81 percent for patients younger than 18, and 71 percent for patients over 18. Thus, while this has not been demonstrated in a randomized trial, ATG should be part of the conditioning regimen for MRD transplants, where it may advantageously replace radiation [44], as well as for alternative donor transplants, where it may allow safe reduction of the TBI dose.

Newer approaches to conditioning have included using fludarabine and alemtuzumab. An EBMTR study of 38 patients undergoing alternative donor SCT with fludarabine + cyclophosphamide + ATG conditioning (without radiation) showed an engraftment rate of 82 percent (68% in adult patients), and a two-year OS of 73 percent (with short follow-up) [50]. Acute GVHD (grade II-IV) occurred in only 11 percent of patients, and cGVHD in 27 percent. Those early results compare favorably with those using conditioning that contains TBI. Similarly encouraging results were obtained in the MRD setting [51]. Longer follow-up and confirmatory studies will further clarify the role of fludarabine in both the alternative donor and the MRD setting. Another small study described the outcome of seven young patients conditioned for MUD SCT using alemtuzumab with fludarabine and cyclophosphamide [52]. All patients engrafted, with no grade III-IV aGVHD and 17 percent cGVHD. OS in this study was 71 percent. Again, those results are promising and warrant further evaluating this type of combination.

The experience with syngeneic grafts also deserves mention, for the few patients to whom this applies. Hinterberger et al. reported on an IBMTR cohort of 40 patients who received a syngeneic graft between 1964 and 1992 [53]. The salient findings were that, as alluded to above, conditioning greatly improved engraftment rates (64% vs 30%); however, there was no difference in OS between the patients who received conditioning prior to the first transplantation attempt (70%) and those who did not (87%). This reflects the high salvage rates of graft failure using a second transplant from the same donor in patients receiving an unconditioned syngeneic graft (who obviously do not suffer from conditioning regimen toxicity). Nonetheless, since the cure of AA with SCT does not depend on a Graft-versus-Tumor effect, a syngeneic donor, when available, is the best possible choice, with survival approaching 90 percent [7]. In this case, as with matched sibling donors, radiation is no longer favored as part of the conditioning regimen.

3.3. GVHD Prophylaxis

Adding cyclosporine (CSA) to the GVHD prophylaxis regimen has been one of the crucial steps in improving the outcome of SCT for AA. The benefit of CSA was first suggested by a retrospective study of 37 patients receiving Cy-conditioned (mostly) MRD grafts [54]. Twenty-four received CSA, and 14 (historical controls) received Mtx for GVHD prophylaxis. OS was 73 percent in the CSA-treated group, compared to 56 percent in the Mtx group. Engraftment was also superior (92% versus 74%); surprisingly, GVHD was more common in the CSA group. Of note, three patients in this study engrafted, but later lost their graft concomitantly with the withdrawal of CSA. This pattern of late graft failure has been subsequently reported in other patients [55, 56], and associated with mixed chimerism. This suggests that the benefit of CSA in this

context may be not so much in improving GVHD control, but in suppressing the host immune system and thereby preventing rejection of the graft.

The benefit of CSA for engraftment and survival was confirmed in other retrospective studies [39, 57], and in a prospective trial conducted by Storb and colleagues [58]. In this study, 46 patients receiving a MRD graft after Cy-only conditioning were randomized to CSA + Mtx or to Mtx alone for GVHD prophylaxis. Patients receiving combined therapy with CSA + Mtx had a lower risk of aGVHD (18% versus 53%), similar engraftment rates and an improved two-year OS (82% versus 60%), which was significant in multivariate analysis.

There is limited experience with T cell depletion (TCD) in AA transplants. However, most of the data point to an increased risk of GF [39, 59] and, thus, this cannot be recommended outside the context of a clinical trial.

Based on those studies, CSA (or tacrolimus) + Mtx is the standard against which newer regimens should be tested. Whether combination therapy including rapamycin or mycophenolate mofetil (MMF) can improve on this regimen remains to be determined.

3.4. Graft Source

Most studies of SCT for AA have used bone marrow as the stem cell source. Successful use of peripheral blood stem cells (PBSC) has been reported [60, 61]. However, a recent retrospective EBMTR study concluded that using PBSC instead of marrow, despite providing faster engraftment, was associated with an increased incidence of chronic GVHD (similarly to the use of post-transplant buffy coat infusion [43]) and a significantly lower two-year survival (67% vs. 80%) [62]. Therefore, it seems wise at present to use marrow grafts, at least outside of a clinical trial.

Umbilical cord blood (UCB) transplants are also gaining popularity as a way to perform SCT for patients without an HLA-matched sibling. There is little systematic experience with AA, but isolated reports suggest that this is feasible [63, 64].

The issue of related versus unrelated donors (MRD versus MUD) is discussed in the last part of this chapter (sections III.6 and III.7).

3.5. Prognostic Factors for Survival after SCT

Many studies have investigated the prognostic factors for overall survival in patients undergoing SCT, which must be understood prior to comparing SCT with IST. One factor is HLA donor match, with alternative donor transplants yielding consistently lower survivals than MRD transplants [8]. Three other beneficial factors that have consistently emerged from multivariate analyses are younger age (although no strict cut-off exists); use of a CSA-containing regimen for GVHD prophylaxis, and later year of transplantation [7, 36, 65]. Other factors (such as gender matching [65]) also may have prognostic relevance, but the evidence is not as consistent.

4. The "Who" of Transplantation

As discussed in the preceding sections, allogeneic SCT can be curative for many patients with AA. The best situation is a young patient who receives marrow stem cells from a matched sibling, using ATG as part of the conditioning

regimen and CSA as part of the GVHD prophylaxis regimen. However, for older patients or patients without a matched sibling, SCT can still be curative (though with a lower probability). And for all patients there is the alternative option of IST instead of transplantation. This raises the fundamental clinical question in AA: who should receive an upfront transplant and who should receive IST instead? Proposed answers can be found in the literature [7, 10]; below is our own perspective on the question. We do not propose a fixed algorithm, but instead highlight some of the considerations that could be useful to clinicians facing the decision.

4.1. Severity of AA

Severe AA has been defined as marrow cellularity below 25 percent with two of the following: absolute neutrophil count (ANC) < 0.5 $\times 10^9$/L, platelets <20 $\times 10^9$/L or absolute reticulocyte count < 20 $\times 10^9$/L. Very severe AA is the same except that ANC must be below 0.2 $\times 10^9$/L[2]. There seems to be little prognostic difference between severe and very severe AA when treated with SCT [8, 66]; in contrast, for patients treated with IST, very severe AA carries an adverse prognosis in some [66–68], but not all [7] studies. However, it is clear that patients without at least severe AA are best not transplanted, and treated with observation, androgens or IST. Their disease may not progress and may in some cases even remit, and their survival is in excess of 90 percent without SCT [69].

4.2. Inherited Marrow Failure Syndromes

Perhaps the most important issue when evaluating a patient with AA is to consider the possibility of an inherited marrow failure syndrome. Although in most cases the diagnosis is made in childhood, in a minority of patients the manifestations of inherited marrow syndromes may be subtle and the patients may not present with aplasia until early adulthood; furthermore, up to one-third of patients with Fanconi's anemia (FA), the most common cause of congenital AA, do not show any obvious congenital abnormalities [70]. Clues to the diagnosis of Fanconi's anemia include short stature, café-au-lait spots (or other pigmentation anomalies), urogenital abnormalities, hearing defects, radial ray defects, macrocytosis or early presentation of solid malignancies [71]; similarly, nail malformations, a reticular rash, oral leukoplakia, osteoporosis or pulmonary fibrosis may suggest the diagnosis of dyskeratosis congenita (DKC), while exocrine pancreatic insufficiency may be the only clue to the Schwachman-Blackfan-Diamond syndrome [6]. In some cases, the telltale sign may not be present in the patient, but in a family member, underlining the importance of taking a thorough family history. If the diagnosis of FA is suspected, chromosomal breakage testing using mitomycin C or diepoxybutane can be performed. A minority of FA patients display somatic mosaicism, in which case diagnosis may require testing of cultured skin fibroblasts rather than hematopoietic cells [72]. To diagnose the other inherited marrow failure syndromes, sequencing of the relevant genes must be undertaken.

Recognizing an inherited marrow failure syndrome has two critical implications: first, aplasia in those patients will not respond to IST and must, therefore, be treated with SCT; second, patients with DNA repair defects (i.e., patients with FA) are unusually sensitive to certain cytotoxic agents (in particular, alkylating agents), to the point that standard conditioning regimens are lethal. Therefore,

appropriate dose reductions (usually 70 to 90% for cyclophosphamide and 50 to 75% for radiation) are critical. Fludarabine may also be useful in the conditioning regimen to make up for the dose reduction in Cy and TBI [73]. Fortunately, those diseases can still be cured with a judiciously conditioned allogeneic SCT; in fact, for patients with FA, survival following MRD SCT may be close to that of patients transplanted for acquired, although patients with FA may suffer from an increased incidence of GVHD and secondary malignancies [74]. Five-year OS of 60 to 70 percent are typical in retrospective series, including in a large study of patients from the IBMTR [75]. As with acquired AA, survival is worse after alternative donor SCT [76].

4.3. The Relevance of Etiology in Acquired AA

Although most cases of AA are idiopathic, some can be traced to certain triggers such as drugs, hepatitis, pregnancy, etc. Patients with such non-idiopathic AA are usually included in trials and retrospective series and, with rare exceptions of unclear significance [77, 78], their outcome appears to be similar to that of patients with idiopathic AA [7, 21]. Therefore, the etiology of a patient's AA (as long as it is acquired AA) should not, at present, influence treatment decisions.

4.4. Paroxysmal Nocturnal Hemoglobinuria (PNH)

PNH is closely associated with AA, although the basis for this association is still poorly understood [30]. While PNH has a fascinating history and pathophysiology in its own right, a full discussion is outside the scope of this review; nonetheless, several points deserve mention. First, classic PNH (without evidence of AA) has an entirely different pathophysiology than AA, and does not respond to IST. The prognosis of PNH itself is favorable, with few patients requiring treatment beyond intermittent steroids and transfusions [79]. Indeed, a very large French retrospective study of 467 patients with PNH demonstrated an impressive median overall survival of 22 years [80]. Second, many patients with AA have flow cytometry-detectable PNH clones at the time of AA diagnosis, and the presence of such clones may be a favorable prognostic factor for outcome after IST [81, 82]. Third, for patients with PNH (whether isolated or associated with AA) who do require treatment, SCT can be curative, as first demonstrated in the 1970s [83, 84] and subsequently confirmed in many small studies [85–87]. Lastly, the results of recent eculizumab trials hold promise in the treatment of this disease [79], but the role of this drug and its long-term effects have yet to be clarified; furthermore, the drug does not improve the aplasia in patients with AA/PNH.

In summary, for patients with classic PNH (without AA), IST has no role, and SCT should be reserved for patients with debilitating or life-threatening manifestations of the disease. For patients with PNH and AA, the decision is similar to that for patients with AA alone, although the relative benefit of IST may be slightly higher and may tip the balance in borderline cases.

4.5. The Lessons of History

One of the most striking aspects of AA treatment is the dramatic improvement in outcomes over the last few decades. In the case of SCT, improved engraftment and improved survival with time have been documented in multiple large

single-center and registry-based retrospective studies [7, 36, 37, 42, 57, 88]. In those studies, the absolute increase in post-SCT overall survival was 40 to 50 percent over 20 years. Many factors probably account for this improvement (Table 10-2). Some have already been discussed, such as the replacement of TBI by ATG in conditioning, and the addition of CSA to the GVHD prophylaxis regimen. Another important factor is the change in transfusion practices. Receiving multiple transfusions pre-transplantation was recognized early on as an adverse prognostic marker [39, 40, 42], presumably through alloimmunization of the host (who is already immune sensitized to hematopoietic cells). The minimization of pre-transplant transfusions, as well as the use of leukopoor, irradiated blood products and the use of single donor apheresis platelets, has probably contributed significantly to improving engraftment rates and, hence, survival. Finally, concomitant improvements in supportive care (including the use of laminar airflow rooms [89]) have decreased the transplant-related mortality, and have diminished the impact of graft failure (by increasing the probability of survival to a second transplant).

While this history is a ringing tribute to the efforts of clinicians and researchers in this disease, it also carries an important lesson. When comparing the outcome of IST and SCT, long-term retrospective series or older prospective series may not be applicable. The outcomes that matter for this comparison are the ones obtained from studies that use state-of-the-art conditioning, GVHD prophylaxis and supportive care. Importantly, the improvement in outcome has not been the same for SCT and IST [88]. In the latter case, there has not been much recent progress in improving the treatment regimen. The experience with the addition of growth factors or alternative immunosuppressants such as MMF has been disappointing [90, 91], and even the addition of CSA to ATG has not demonstrably improved overall survival [27]. Therefore, what gains have been made in IST are probably related mostly to improvements in supportive care, which do not necessarily affect clinically important outcomes such as relapse and late clonal disease.

Table 10-2. Important advances in transplantation for AA.

Conditioning	Elimination of radiation for MRD and syngeneic grafts [39, 40, 44]
	Decrease in TBI dose for MUD grafts [46]
	Addition of ATG [23, 44]
	Addition of fludarabine to conditioning [50, 51]
GVHD prophylaxis	Combination therapy with cyclosporine and methotrexate [38, 57, 58]
Donor selection	Allele-level matching for unrelated donors [94]
Supportive care	Improved support of cytopenic patients, resulting in lower mortality for graft failure
	Transfusion practices
	Use of leukopoor, irradiated products
	Use of single donor apheresis platelets
	Minimization of pre-transplant transfusions
	Isolation environment [89]

Abbreviations: MRD, matched related donor; TBI, total body irradiation; MUD, matched unrelated donor; ATG, anti-thymocyte globulin; GVHD, Graft-versus-Host Disease

4.6. Patients with an Available HLA-Matched Sibling

The current recommendations for this scenario, proposed by Bacigalupo and colleagues [7], are to transplant patients younger than 40 and treat patients older than 40 with IST (reserving SCT for relapsing or refractory patients). This is based on the very favorable outcomes with MRD SCT in young patients, and the decrease in post-transplantation survival with advancing age. However, it might be reasonable to consider extending the age threshold, based on the following considerations. As discussed in the previous section, the outcomes of MRD SCT have improved dramatically in the last few decades. The relevant benchmark for SCT, therefore, should be a study of patients transplanted after 1990. In a retrospective multicenter EBMTR analysis, the overall survival after SCT for patients over 40 years of age transplanted after 1990 was 54 percent [7]; of note, this study included 14 percent of patients transplanted from alternative donors. The most directly comparable numbers come from another EBMTR analysis of older patients receiving IST [68]. In this study, the five-year OS for patients aged 50 to 59 treated after 1990 was 60 percent. While the two numbers are comparable, SCT carries the additional advantage of avoiding the risk of late relapse and, even more importantly, the risk of late clonal diseases (which was 12 percent at 10 years in the above IST study). Indeed, the survival curves for patients treated with IST in general tend not to plateau as clearly as the corresponding curves for SCT, and there appears to be an up to 10 percent absolute drop in survival between five and 10 years after IST in studies with long-term follow-up [27, 66]. Furthermore, this is the population that is close to the cut-off age for standard transplantation, currently around 55 to 60 years old. Therefore, a patient with AA who is in his/her early fifties and who is treated with an attempt at IST may lose the opportunity of SCT after a few years. Finally, the outcome of SCT appears to be worse for patients who have previously received IST [37, 78].

Taken together, those arguments suggest that it may be worth considering MRD SCT as front line therapy in patients up to the age of 55 or 60. Since, as previously discussed, patients with very severe AA (ANC below 0.2×10^9/l) may do worse than those with severe AA (ANC between 0.2 and 0.5×10^9/l) when treated with IST, but not when transplanted, there may be an even better argument for transplanting patients with a matched sibling donor over the age of 40 when their disease is very severe.

4.7. Patients with an Available Matched Unrelated Donor (MUD)

In this case, the current recommendation is to treat all patients with at least two attempts at IST prior to resorting to transplantation [7]. This reflects the significantly poorer results of MUD compared to MRD SCT. Here again, we would submit an alternative argument. MUD transplantation has benefited from some of the same improvements with time as has MRD transplantation, although the improvement in survival has been less dramatic. In a recent IBMTR retrospective study of patients transplanted between 1988 and 1994, the five-year OS after MUD transplantation was 39 percent, with age over 21 years and poor performance status being adverse risk factors for survival [92]. However, more recent prospective experiences have shown superior results. An EBMTR trial of 38 children and young adults using fludarabine + Cy + ATG conditioning reported a two-year OS of 74 percent, and 84 percent for

patients under the age of 18 [50]. Moreover, in a recent comparison of alternative and matched related donors for pediatric transplants, there was no difference in OS [93]. Finally, in a multicenter study of 87 patients transplanted from alternative donors between 1994 and 2004 examining de-escalating TBI dose, the survival for MUD transplants was 61 percent (66% for those patients conditioned with 200 cGy TBI), and 73 percent for patients under the age of 21 [46]. One plausible explanation for the difference between the EBMTR study of Passweg and colleagues and some of the other studies just mentioned is in the quality of HLA typing. In Passweg, et al.'s study, most patients were typed at low resolution, whereas in other studies, molecular typing was performed. This is very important since the outcome with mismatched unrelated donors is worse than that with matched ones [46, 59]. Therefore, MUD transplantation with molecular typing and modern conditioning regimens can be expected to yield survivals of 60 to 80 percent in young patients. The benefit of allele-level HLA matching has been confirmed in a retrospective French study, in which young patients with HLA-matched donors had a five-year survival of 78 percent [94]. In addition, it must be remembered that, given the current recommendations, patients receiving MUD transplants in clinical trials are patients who have previously failed at least one, and usually more, courses of IST. In Deeg, et al.'s study for example, patients had received a median of three courses of IST [46]. The literature on MRD transplants has repeatedly demonstrated that prior immunosuppression is an adverse risk factor. Therefore, the 60 to 80 percent survival mentioned above may even be an underestimate for patients transplanted upfront.

The foregoing discussion suggests that for young patients (e.g., under 21-years-old, although such cut-offs are, of necessity, arbitrary) with a molecularly typed matched unrelated donor, upfront transplantation may well be a reasonable course of action. For older patients, or for patients without a true MUD, an attempt at IST may still be the better option.

5. Conclusions

Aplastic anemia presents a therapeutic challenge to the clinician. There are two very different possible treatment modalities, which have distinct risks and benefits. Moreover, both approaches are constantly and independently evolving. There are no recent or ongoing randomized trials comparing the two and, even if there were, they would require long follow-up (to account for the late risks of both procedures); by the time the results were mature enough, they would likely be made obsolete by new advances in one or both modalities. Interpretating the results of retrospective registry studies is hampered by the heterogeneity of the techniques used, while small prospective single-center studies are not always generalizable.

Our own interpretation of the available data would lead to the following framework for treatment decisions. We would consider stem cell transplantation for the following patients: (1) patients with an inherited marrow failure syndrome and any available donor; (2) patients under the age of 55 to 60 with acquired AA (regardless of etiology) and a matched sibling donor, and (3) patients with a molecularly matched unrelated donor under the age of 21 or so. For all other patients, an initial attempt at IST is likely the best option, with

transplantation reserved for those who fail a least two courses of IST. For such patients (or for patients with congenital AA), if no MRD or MUD is available, umbilical cord or mismatched donor transplantation should be considered.

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Chapter 11

Stem Cell Transplantation for Hemoglobinopathies

Catherine J. Wu, MD

1. Introduction

In 1982, Thomas, et al. reported the successful marrow transplantation of an infant with thalassemia [1]. Shortly thereafter, in 1984, Johnson, et al. described a child with acute myelogenous leukemia, carrying the concurrent diagnosis of sickle cell disease (SCD), who achieved full donor hematopoiesis with successful replacement of the patient's sickled erythrocytes with donor-derived red blood cells following transplantation of bone marrow derived from her sibling donor who had sickle trait. These early reports raised the tantalizing possibility that these highly morbid genetic nonmalignant diseases could be cured by hematopoietic stem cell transplantation. Today, conventional myeloablative transplantation is considered an established treatment option for thalassemia and SCD. More than 2,400 transplants have been performed worldwide for thalassemia, and over 300 worldwide for SCD (personal communication, Statistical Center of the CIBMTR), with a reported overall (OS) and disease free survival (DFS) rate of 79–93 percent/58–91percent, and 94 percent/84 percent for thalassemia and SCD, respectively [2, 3]. Despite these overall excellent results, debate continues regarding the optimal timing of this intensive therapy for patients with sickle cell disease, given the heterogeneity of its clinical phenotype; and for both diseases, because of the overall poor availability of matched related donors. This chapter reviews the results and controversies surrounding myeloablative allogeneic stem cell transplantation for the hemoglobinopathies. In addition, newer promising developments in the field will be discussed, including the use of lower intensity preparative regimens and alternative sources of donor hematopoietic stem cells that have been generated in the hope of establishing curative therapy for these diseases under conditions of acceptable toxicity.

Chapter Outline

I. Therapeutic challenges in the field of hemoglobinopathies and indications for transplantation
II. Allogeneic marrow and peripheral blood stem cell transplantation for the hemoglobinopathies, using

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a. Myeloablative conditioning
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c. Reduced-intensity conditioning

III. Alternative sources of donor hematopoietic stem cells

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c. Cord blood – haploidentical and unrelated

IV. Autologous stem cell transplantation with gene therapy
V. Summary

2. Therapeutic Challenges in the Field of Hemoglobinopathies and Indications for Transplantation

The hemoglobinopathies, such as sickle cell disease and thalassemia major, constitute a group of genetic diseases associated with significant morbidity and premature death. As extensively reviewed elsewhere, in patients with SCD, a single amino acid substitution leads to a defective β-globin, which causes erythrocytes to sickle in response to deoxygenation. The sequelae of this defect are vaso-occlusive crises, resulting in episodes of bony pain and infarction, acute chest syndrome and strokes [4]. In patients with thalassemia major, the loss of α- or β-globin sub-units results in defective hemoglobin [5] and severe hemolytic anemia. Since red cell transfusions are required as supportive care for both groups of patients, their courses are frequently complicated by alloimmunization and iron overload. In the United States, the prevalence of sickle cell trait is 8 to 10 percent among African-Americans [6], and it is estimated that up to 8 percent of the population of some African countries are afflicted with SCD. While thalassemia major is rare in the United States, its gene frequency in the Mediterranean region, Africa, the Middle East and southeast Asia approaches 3 to 10 percent [5]. Thus, from a global perspective, the hemoglobinopathies present an enormous challenge from the standpoint of cost in human life and in health resource utilization.

Over the years, improvements in the supportive care of the hemoglobinopathies patients have led to longer life expectancies. In the 1970s, the mean survival of patients with sickle cell disease was 14.3 years [7]. With improvements in medical practice, the reported life expectancy in 1994 was 42 years for men and 48 years for women with sickle cell disease [8]. Even with improved supportive care, the median life expectancy of patients wtih thalassemia major has been reported to be less than 35 years even at specialized treatment centers [9–12]. The major improvements in supportive care have been the use of prophylactic antibiotics and blood products. Recently, deferasirox, an oral chelator of iron, has been FDA-approved for clinical use. The availability of this oral agent is anticipated to vastly improve patient compliance for the prevention and treatment of iron overload, which results from frequent and chronic red blood cell transfusion, and contributes to the early mortality of patients with hemoglobinopathy [13–15]. Additional important advances in the treatment for SCD have included the use of agents to stimulate fetal hemoglobin production, which has the potential to ameliorate the clinical symptoms of the disease by prevention of sickling [16].

Despite these advances in supportive care, much room for improvement remains in the clinical treatment of patients with hemoglobinopathy. A number of clinical observations supports efforts to develop more intensive therapies that address the underlying disease. First, chronic anti-sickling agents such as hydroxyurea do not benefit all patients, and there are ongoing concerns that prolonged treatment may increase the risk of secondary leukemia [16–19]. Second, despite the known phenotypic heterogeneity of sickle cell disease, at least three lines of data have suggested that high risk populations can be now identified based on clinical features to determine who could likely benefit from more intensive therapy to correct the underlying disease. One, studies of infants with sickle cell have demonstrated that the observation of a clinical triad of dactylitis, severe anemia and leukocytosis [20], or of early onset of acute chest syndrome [21] portends poor subsequent clinical outcome. Two, in a series of randomized trials, Adams, et al. demonstrated that elevated rates of cerebral blood flow, identified by transcranial Doppler ultrasound, is associated with a high incidence of cerebrovascular accidents, which can be prevented by chronic transfusion therapy. Furthermore, Adams, et al. demonstrated the requirement for indefinite continuation of chronic transfusion, since strokes were observed to return rapidly with cessation of chronic transfusion therapy [22, 23], Three, a separate series of studies have highlighted the impact of chronic intravascular hemolysis, a cardinal clinical feature of the hemoglobinopathies, on endothelial function and end-organ injury, including pulmonary hypertension, in patients with SCD and thalassemia. Recent retrospective and prospective studies have demonstrated that pulmonary hypertension occurs in 20 to 40 percent of adult patients with SCD and is associated with a two-year mortality of 50 percent [24, 25]. Pulmonary hypertension has also been identified to be highly prevalent (>20%) in children and adolescents [26], and is associated with cerebral vasculopathy [27].

From the standpoint of resource utilization, chronic care of this subset of high risk patients is costly. Ho, et al. recently reviewed the costs on greater than 400 patients in Taiwan with β-thalassemics who were transfusion-dependent, and concluded that, given the estimated costs of regular blood transfusions and iron-chelation agents, undergoing successful transplant was at least three-fold more cost effective over a lifetime [28]. Thus, while improved supportive care has succeeded in extending the overall longevity of patients with hemoglobinopathy, one ongoing therapeutic challenge is developing interventions to treat the subset of patients that demonstrate risk factors that predict early mortality and tremendous morbidity. As will be discussed in the next sections, if allogeneic stem cell transplantation can successfully replace the patient's diseased erythrocytes with those from a normal donor under conditions of acceptable toxicity, this therapeutic modality would have an enormous beneficial impact on patients with hemoglobinopathy.

3. Allogeneic Hematopoietic Stem Cell Transplantation

3.1. Myeloablative Transplant

Currently myeloablative allogeneic HSCT is the only potentially curative treatment for severe hemoglobinopathies. Since 1972, >1,500 allogeneic transplants for thalassemia major have been performed worldwide, with the

most extensive experience originating from Pesaro, Italy [29–35]. Review of this extensive myeloablative experience has demonstrated that the efficacy and toxicity of allo-HSCT are closely associated with the extent of end-organ damage prior to transplant. These adverse risk factors include: (1) inadequate iron chelation (2) presence of liver fibrosis, and (3) hepatomegaly (greater than 2 cm). Based on this Pesaro classification, in which patients are defined as Class 1, 2 and 3 based on demonstration of none, one to two or all three risk factors, overall and disease free survival rates at 10-year follow-up of children under 16 years of age have been reported at 93 percent/91 percent, 87 percent/83 percent and 79 percent/58 percent, respectively [36] (Fig. 11-1). Surprisingly, despite the history of chronic transfusion in these patients, overall incidence of graft rejection was low at only 3 to 5 percent, hypothesized to occur as a result of tolerance to RBC antigens.

The overall survival and disease free survival rates of pediatric patients with sickle cell disease undergoing myeloablative marrow transplantation have been quite similar to the thalassemic experience. Using a standard busulfan and cyclophosphamide-based myeloablative preparative regimen for matched related sibling marrow transplant, multiple European and North American consortium experiences have consistently reported an overall survival rate of 94 percent, and a disease free survival rate of 84 percent for children with severely symptomatic disease [37–39]. As shown in Table 11-1, the common indications for undergoing transplant have included stroke, recurrent acute chest syndrome and painful crises. These excellent outcomes are durable. As

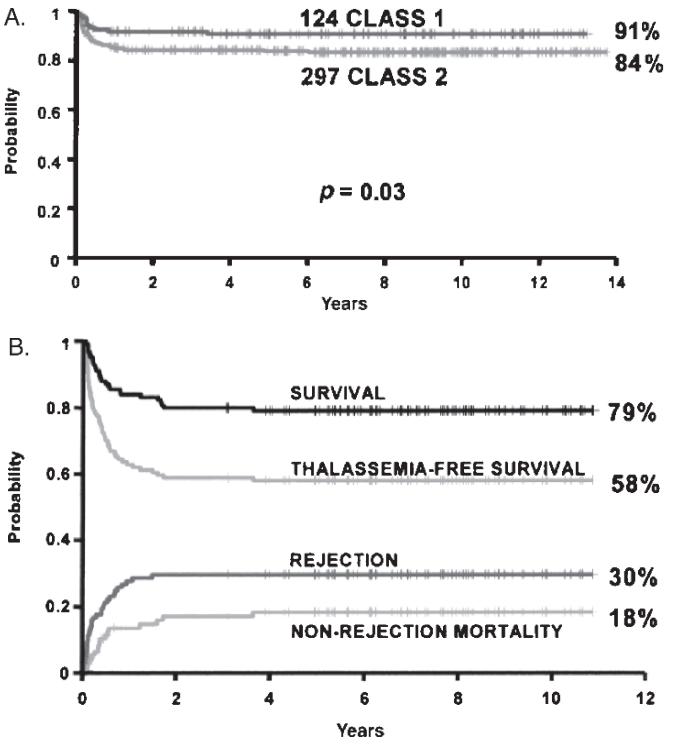

Fig. 11-1. Probability of disease-free survival of pediatric patients (less than 17 years) with Class 1 and 2 (A), and Class 3 (B) thalassemia following myeloablative bone marrow transplantation

Table 11-1. Indications for transplant. (adapted from Walters, et al. [31])

Criteria for inclusion
Sickle cell disease (sickle cell anemia, sickle cell-hemoglobin C disease, or sickle cell– β thalassemia
Age less than 16 years
HLA-identical related donor
One or more of the following:
Stroke or central nervous system event lasting longer than 24 hours
Acute chest syndrome with recurrent hospitalizations or previous exchange transfusions
Recurrent vaso-occulusive pain (>2 episodes per year for several years) or recurrent priapism
Impaired neuropsychological function and abnormal cerebral MRI scan
Stage I or II sickle lung disease
Sickle nephropathy (moderate or severe proteinuria or a glomerular filtration rate 30 to 50% of the predicted normal value)
Bilateral proliferative retinopathy and major visual impairment in a least one eye
Red-cell alloimmunization (>2 antibodies) during long-term transfusion therapy

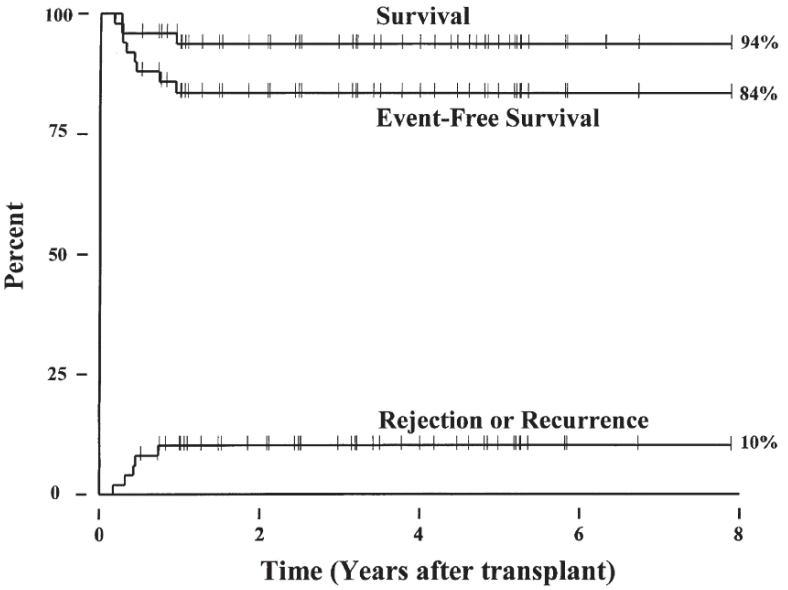

Fig. 11-2. Outcome after transplantation for 50 children with advanced, symptomatic sickle cell disease. Kaplan-Meier estimates for survival and event-free survival and cumulative incidence of graft rejection following marrow transplantation are shown.

shown in Fig. 11-2, Walters, et al. reported up to eight-year follow-up on a group of 50 pediatric patients, and demonstrated that the survival and disease free curves following the first year of transplant are plateaued [37]. In these series, the primary cause of treatment failure was graft failure, occurring at a rate of 10 to 12 percent, which resulted in return of autologous erythropoiesis. This is higher than the <3 percent incidence of graft failure observed following myeloablative HSCT for hematologic malignancies, and this difference has been attributed to the effect of allo-immunization in patients with hemoglobinopathies prior to HSCT.

The use of myeloablative transplantation has not been reported for adults with SCD, as this treatment approach has been considered too toxic for these chronically ill patients. For adults with thalassemia, the experience of using myeloablative allogeneic stem cell transplantation has demonstrated the curative potential of this treatment approach, but the outcomes are worse compared to the pediatric experience. These poorer results have been attributed to a larger proportion of older patients having class III disease. Lucarelli, et al. reported the results of selecting a conditioning regimen based on risk category in 107 thalassemic adults, ages between 17 and 35 years, transplanted with HLA-matched identical siblings. Eighty-seven of the 107 patients had class III disease. Patients with class II disease received 14 mg/kg of busulfan and 200 mg/kg of cyclophosphamide, whereas patients with class III disease received 14 to 16 mg/kg of busulfan with reduced doses of cyclophosphamide (120 to 160 mg/kg). As shown in Fig. 11-3A, using this schema, the OS/DFS was observed at 65 percent/62 percent, with a non-rejection mortality rate of 35 percent, and a 5 percent rate of return of the pre-transplant thalassemic condition [29]. The primary cause of death was due to infectious causes. In this series, in addition to the risk factors considered by the Pesaro classification, chronic hepatitis was identified as an adverse risk factor (Fig. 11-3B).

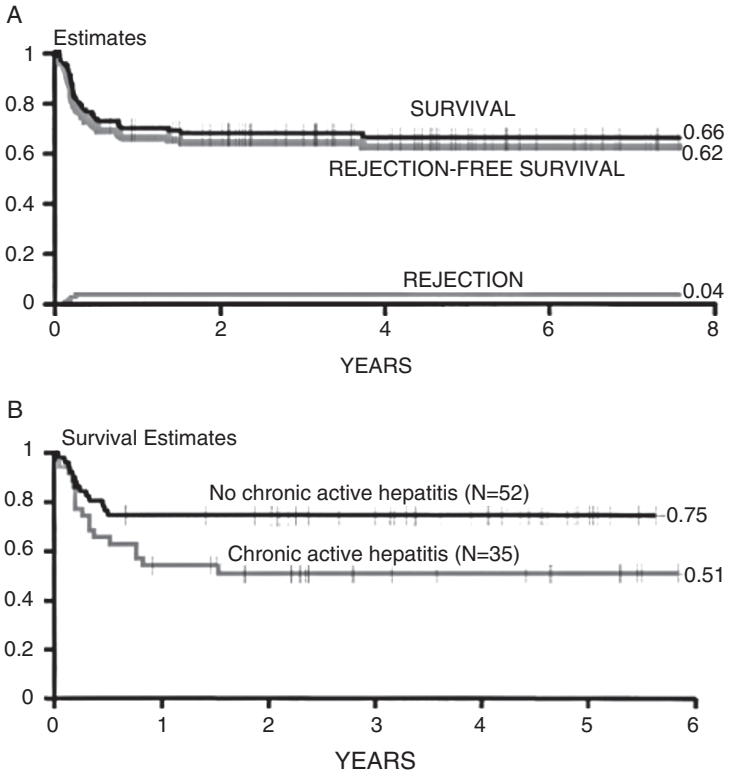

Fig. 11-3. Myeloablative allogeneic hematopoietic stem cell transplantation for adults with thalassemia. **A.** Kaplan-Meier estimates of survival and thalassemia-free survival and cumulative incidence estimates of rejection for 107 adult patients transplanted between November 1988 and September 1996. **B.** Kaplan-Meier estimates of survival for 87 adult patients transplanted between May 1991 and September 1996, categorized on the baiss of presence of chronic active hepatitis at the time of transplantation

Given the overall encouraging results of using myeloablative HSCT for hemoglobinopathies, it might be expected that its use would be more widespread. One factor contributing to the underutilization of this therapeutic modality is the barrier of finding suitable transplant donors, (described in the section "Alternate Sources of Donor Hematopoietic Stem Cells"). Another important factor is that the beneficial effects of transplant — namely freedom from disease manifestations and potential improvement in end-organ function – have to be balanced with the risks of early life threatening toxicity and long-term complications which have been well documented in the literature of allogeneic HSCT for hematologic malignancies. These include infertility and gonadal failure, especially among females, cGVHD and the potential for secondary malignancies [40, 41]. Both beneficial and toxic effects of transplant on growth and organ function have been described in the SCD and thalassemia literature. Piga, et al. monitored the long-term effects of BMT in thalassemia in 33 patients transplanted with matched related donors following Bu/Cy, compared with 155 patients matched for age and treated with conventional therapy. Not only was a significant increase in growth impairment identified, but also a significantly increased risk of fulminant sepsis. However, transplant was associated with improved end-organ function after transplant, including lowered incidence of hypothyroidism, hypogonadism and cardiopathy, compared to conventional therapy. Moreover, undergoing transplant resulted in improved iron chelation, as monthly phlebotomy was instituted, and normalization of iron stores was achieved in 91 percent of cases [12]. Walters, et al. has reported the long-term results of 26 patients with a history of sickle cell disease who achieved stable donor engraftment following myeloablative transplant. In 22 of the 26, complications related to sickle cell disease resolved, and none experienced further episodes of pain, stroke or acute chest syndrome. All 10 engrafted patients with a prior history of stroke had stable or improved cerebral magnetic resonance imaging results. Pulmonary function tests were stable in 22 of the 26 patients, worse in two, and not studied in two. Seven of eight patients transplanted for recurrent acute chest syndrome had stable pulmonary function. Linear growth measured by median height standard deviation score improved from −0.7 before transplantation to −0.2 after transplantation. An adverse effect of busulfan conditioning on ovarian function was demonstrated in five of seven evaluable females [37].

These mixed results have complicated the decision of whether or not to proceed with transplantation for treatment of hemoglobinopathy for both patients and providers [42]. A major deterrant for patients has been the negative impact of myeloablative conditioning regimens on fertility. On the other hand, van Besien, et al. has reported the results of a survey analysis in which symptomatic adults were willing to take on up to 35 percent risk of mortality for the opportunity of cure [43]. Clearly, in order to increase the availability of transplant, improved regimens are needed so that the cost-to-benefit ratio of this treatment approach is sufficiently improved such that it engenders an acceptable level of risk.

3.2. Nonmyeloablative Conditioning

Nonmyeloablative (NMA) conditioning regimens are now routinely used to establish engraftment of donor stem cells in patients with hematologic

malignancies [44–46]. The success of immune suppressive reduced-intensity regimens suggests that immunologic barriers, rather than the creation of "hematopoietic space," are the primary factors that limit engraftment of allogeneic hematopoietic stem cells. These regimens are used primarily in patients who might benefit from allogeneic HSCT, but are ineligible for this intensive treatment because of advanced age or other medical conditions that increase the risk associated with conventional myeloablative therapy. The specific combinations and dose intensities of chemotherapeutic agents used in different nonmyeloablative regimens have varied and some regimens continue to incorporate low doses of total body irradiation. Successful engraftment of donor cells has been reported with the majority of these regimens; thus, a single "standard" NMA conditioning regimen still has not been defined.

Successful engraftment in the hematologic malignancies following conditioning with nonmyeloablative preparative regimens initially generated excitement in the field of hemoglobinopathies, since this could possibly enable donor engraftment under conditions of acceptable toxicity. Although these regimens would not ablate abnormal hematopoiesis, several lines of evidence already suggested that the establishment of even partial stable engraftment of donor stem cells would likely improve disease symptoms in patients with hemoglobinopathies. Following conventional myeloablative marrow transplantation, approximately 10 percent of patients with thalassemia and ~20 percent of patients with sickle cell anemia demonstrate stable mixed chimerism, in which patients' levels of donor engraftment ranged from 10 to 85 percent, that nevertheless is associated with clinical improvement of disease [47, 48]. These results suggested that significant clinical improvement of these hemoglobinopathies can be achieved without complete replacement of recipient hematopoiesis. Murine studies using transgenic sickle cell mice to model the amount of donor engraftment required for clinical improvement have demonstrated that as low as 25 percent engraftment is sufficient for full red cell engraftment, and correction of sickle cell-induced organ damage [49–51]. These studies have been confirmed in patients with severe SCD. Wu, et al. utilized the development of mixed hematopoietic chimerism in SCD patients following nonmyeloablative transplant as a model system to directly compare sickle trait (SA) and sickle cell disease-derived (SS) erythropoiesis [52]. By employing a molecular method to assess erythroid lineage chimerism [53], the extent to which SS or SA erythroblasts developed and matured within the same marrow milieu was directly measured. As shown in Fig. 11-4, these studies demonstrated that, compared to SA erythroblasts, SS erythroblasts are at a competitive disadvantage, and that low levels of donor (SA) erythroid engraftment result in predominantly donor representation in maturing erythroblasts and among mature erythrocytes. Since full replacement of mature erythrocytes can be achieved with relatively low amounts of donor erythroid progenitors, these studies strongly support mixed hematopoietic chimerism as a reasonable endpoint of hematopoietic stem cell transplantation, provided that novel conditioning regimens that can consistently establish stable mixed hematopoietic chimerism can be generated. Moreover, these findings suggest that stem cell-based approaches to treatment of sickle cell disease, including gene therapy, may have a greater impact on the correction of disease than previously expected.

Although achievement of durable donor-derived engraftment following nonmyeloablative conditioning for the hemoglobinopathies has been anecdotally

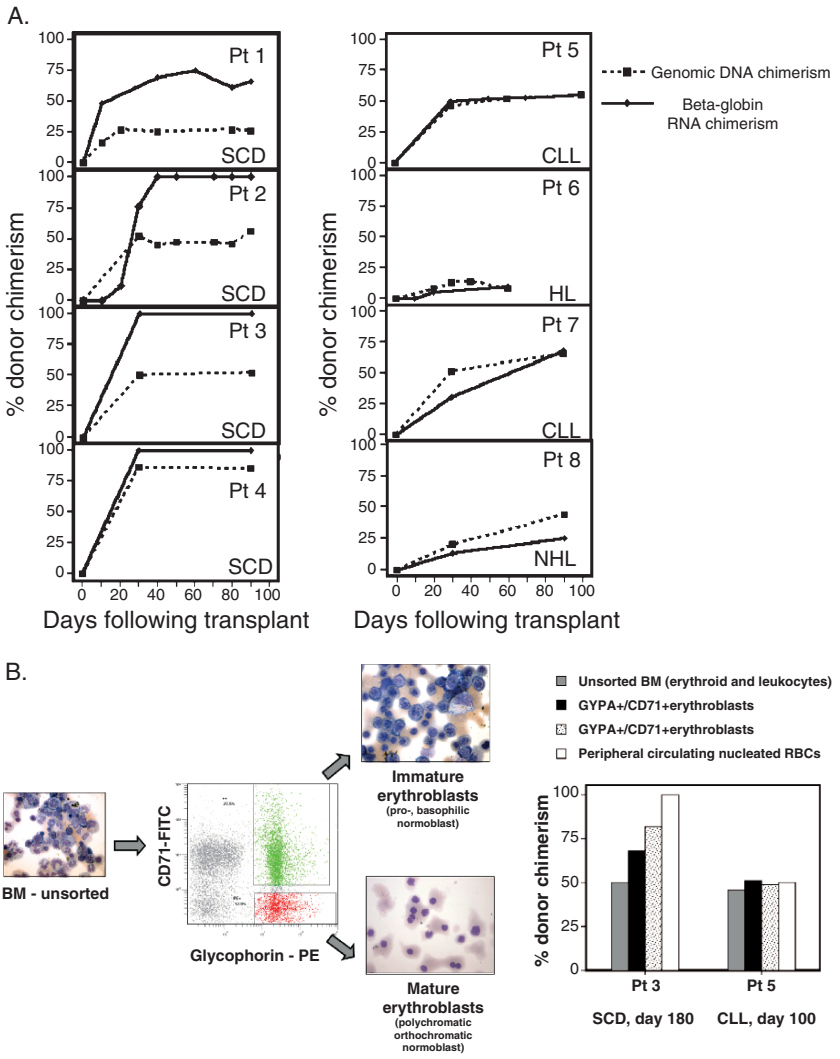

Fig. 11-4. Evidence for ineffective erythropoiesis in patients with sickle cell disease. **A.** Comparison between WBC chimerism (measured in genomic DNA from peripheral blood and RBC chimerism (measured by donor-derived beta-globin RNA) in patients (A) with SCD and (B) without SCD. **B.** Immunophenotyping of marrow mononuclear cells with glycophorin A-PE and CD71-FITC can identify immature from mature erythroblasts, as confirmed by histochemical staining of the sorted populations (inset photographs). Comparison of donor chimerism between unsorted (gray bars), immature (black bars) and mature (hatched bars) marrow erythroblasts isolated from marrow of a SCD compared with a non-SCD patient and comparison with donor chimerism in peripheral circulating nucleated RBC (white bars)

reported (see section on Reduced-intensity Conditioning, below) [54–55], the experience of using truly low intensity regimens for the treatment of hemoglobinopathies has been largely disappointing. In an effort to reduce immediate and long-term treatment-associated toxicities such as a negative impact on fertility and growth, these experiences were designed to test regimens whose intensity represented only 10 to 20 percent of a full myeloablative regimen.

Iannone, et al. described a series of seven children with sickle cell disease and thalassemia who underwent matched related donor marrow transplant following a nonmyeloablative preparative regimen consisting of low dose TBI (200 cGy) and fludarabine (30 mg/m^2/day × 3–5 days), and a combination of mycophenylate mofetil and cyclosporine or tacrolimus for post-transplant GVHD prophylaxis. Six of seven patients achieved initial donor engraftment, ranging from 25 to 85 percent, but all subsequently developed late graft failure with weaning of post-transplant immunosuppression, as shown in Fig. 11-5. In some cases, donor chimerism recovered following resumption of immunosuppressive therapy [56]. Horan, et al. subsequently confirmed that adding the immunosuppressive agent anti-thymocyte globulin to this low intensity regimen of fludarabine and TBI was not consistently successful [57]. Since these patients described in these studies are immunocompetent hosts, with known alloimmunization after frequent RBC transfusion, the precipitous graft loss following withdrawal of immune suppressive medications strongly suggests that immune suppressive therapy administered for GVHD prophylaxis also acted to prevent rejection of donor cells. Taken together, these observations suggest that: (1) the nonmyeloablative conditioning regimen was sufficiently immunosuppressive to permit initial donor engraftment, but (2) not sufficiently immunosuppressive to prevent late rejection of donor stem cells. Although patients and donors were HLA-identical, minor histocompatibility antigen disparities likely became targets for rejection of donor stem cells [58, 59]. Although an alternate mechanism for graft loss would be the failure of donor hematopoietic cells to compete with host hematopoietic cells, which may

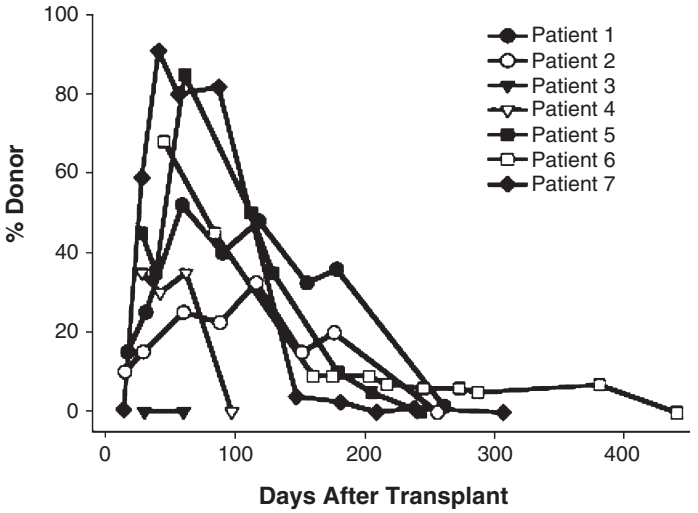

Fig. 11-5. Graft rejection associated with removal of immunosuppression following non-myeloablative bone marrow transplantation of hemoglobinopathy. The percentages of donor engraftment measured in unfractionated blood and bone marrow mononuclear cell preparations are plotted as a function of time in days after transplantation. There was a decrease in donor chimerism 200 days after nonmyeloablative HCT in patients 1 and 2, 60 days after nonmyeloablative HCT in patients 4 and 5, and 120 days after nonmyeloablative HCT in patients 6 and 7. All patients were treated with a conditioning regimen consisting of fludarabine with 200 cGy of total body irradiation +/– horse antithymocyte globulin

persist in much larger numbers than the administered donor cells in the nonmyeloablative setting, the cumulative clinical evidence does not support this as the dominant mechanism of late graft loss in this patient population.

3.3. Reduced-Intensity Conditioning

While the clinical results of HSCT following conditioning with very low intensity regimens have been largely unsuccessful, case reports and small series over the last seven years suggest that, in fact, minimal toxicity and durable stable donor engraftment without the need for chronic immunosuppression can be successfully balanced when using preparative regimens that are moderately intensive and immunosuppressive [60]. Over a decade ago, Lucarelli, et al. described the effects of conditioning regimen dose intensity on overall transplant outcome in HSCT for thalassemia, since the introduction of conditioning regimens containing less than 200 mg/kg of cyclophosphamide resulted in decreased transplantation-related mortality, but a concomittant increase in risk of graft rejection [32]. For example, in pediatric patients with class III disease, the incidence of graft rejection increased from 10 percent to 30 percent following the introduction of a regimen using 120 to 160 mg/kg of cyclophosphosphamide. More recently, Sodani, et al. successfully modified this regimen, resulting in an improved rate of graft rejection from 30 to 8 percent. In addition to busulfan (14 mg/kg) and 160 mg/kg of cyclophosphamide, this regimen uses a combination of intensive hypertransfusion, hydroxyurea and chelation, to reduce erythropoiesis and expansion of thalassemic clones, and addition of fludarabine and azathioprine to increase the level of immunosuppression [61]. Krishnamurti, et al. reported the first successful marrow transplant of a child with sickle cell disease using a reduced-intensity regimen consisting of 8 mg/kg busulfan, 175 mg/m^2 fludarabine, 150 mg/kg of ATG and 500 cGy total lymphoid irradiation. This patient experienced no disease or treatment-related complications and developed neither acute nor chronic GVHD [54]. Using this regimen and similar eligibility criteria as described in Table 11-1, this experience has been recently updated to describe durable donor engraftment in five of five patients who have remained free of SCD-related symptoms for a follow up of one to five years following discontinuation of immunosuppression [62]. Hongeng, et al. has successfully used the same treatment regimen to achieve full donor chimerism in a child with class III β-thalassemia [63].

Other recent studies have demonstrated that it is also possible to utilize G-CSF mobilized peripheral blood stem cells, and alternate means of generating immunosuppressive regimens. Although a number of case reports have described fatal or near-fatal events in patients with SCD following administration of G-CSF [64, 65], Kang, et al. has reported the feasibility of using G-CSF mobilization to collect peripheral blood stem cells from individuals with sickle trait [66]. The successful use of peripheral blood stem cell transplantation with a reduced-intensity regimen was first reported by Gomez-Almaguer, et al. in a 15–year-old patient with β-thalassemia, whose donor was a matched sibling with thalassemia trait, and who was transplanted following conditioning with oral busulfan 4 mg/kg on days −6, and −5, iv cyclophosphamide 350 mg/m^2 on d -4, -3, -2, and iv fludarabine 30 mg/m^2 d-4, -3, -2. Shenoy, et al. also used peripheral blood stem cells mobilized from matched related siblings

to transplant a series of 16 pediatric patients with a variety of nonmalignant diseases, including one infant with SCD and one child with thalassemia major. This study utilized a conditioning regimen consisting of Campath-1H (33–48 mg over days −21 to −19), fludarabine (150 mg/m^2 over days −8 to −4) and melphalan (70–140 mg/m^2 on day −3) [67]. Although severe infectious complications were observed using this CAMPATH-based regimen, this was primarily observed in patients with aplastic anemia. Of note, one patient was reported to successfully conceive and bring to term pregnancy following this transplant. Although anecdotal, the results suggest the possibility that fertility following reduced-intensity conditioning can be preserved.

Since the reduced-intensity conditioning regimens are less intensive than myeloablative regimens, the level of treatment-related toxicity may be acceptable even for adults with significant end-organ damage. A few case reports of this treatment approach have been described in the adult literature [68]. Schleuning, et al. reported the successful transplantation of a 22-year-old man using peripheral blood G-CSF mobilized stem cells from a matched sibling donor, following conditioning with fludarabine (120 mg/m^2) and cyclophosphamide (60 mg/m^2 × 2 days), and using GvHD prophylaxis consisting of MMF and CyA, who developed full donor chimerism. On the other hand, van Besien, et al. observed that highly intensive regimens appear to be too toxic for this chronically ill adult population [69]. He reported two adult patients that were conditioned with 140 mg/m^2 of melphalan, 120 mg/m^2 of fludarabine and 120 mg/kg of ATG, and transplanted with G-CSF–mobilized peripheral blood stem cells. GVHD prophylaxis consisted of tacrolimus and methotrexate. Both patients achieved full donor chimerism by day 30. However, both patients developed acute GVHD and ultimately died of GVHD-related complications. While these reports are anecdotal, murine studies have demonstrated that mixed T cell chimerism is significantly correlated to a decreased risk of acute GVHD after allogeneic stem cell transplantation [70] and, thus, too rapid conversion to donor chimerism in this patient population may have contributed to the adverse outcomes observed.

4. Alternative Sources of Donor Hematopoietic Stem Cells

While allogeneic marrow transplantation is curative therapy for patients with sickle cell anemia, only a small fraction of patients in the United States receive this treatment. Walters, et al. surveyed participants in a multicenter study of marrow transplantation for SCD to determine reasons for not proceeding to transplantation. Among the 4,848 patients less than 16 years of age with sickle cell anemia that were followed in 22 collaborating centers, only 315 (6.5%) patients were reported to meet protocol entry criteria for transplantation, although there was wide variation among the institutions (0.9–36%). For patients reported to meet eligibility criteria, parental refusal and limited financial or psychosocial support were infrequent barriers to transplantation 71]. On the other hand, lack of an HLA-identical donor was found to be a predominant reason for not proceeding with transplant.

4.1. Unrelated Donors

It has been estimated that < 30 percent of eligible individuals with hemoglobinopathies have HLA-identical siblings. Thus, developing regimens

which use HLA-matched unrelated donors (URD) would greatly expand the application of this potentially curative therapy. Based on the current representation in the National Marrow Donor Program (NMDP) registry, ~60 percent SCD and 88 percent thalassemia patients will find at least one potential 6/6 HLA-matched marrow donor or umbilical cord blood (UCB) unit; moreover all patients will find at least one donor or UCB that is a potential 5/6 HLA match [72].

Given the increased immunologic barriers associated with unrelated compared to related donors, we would expect the risk of immune-mediated graft failure to be elevated in the former setting compared to the MRD experience. Nevertheless, using URD transplant to treat thalassemia in Italy and southeast Asia have been encouraging. Contu and La Nasa, et al. reported the first successful unrelated bone marrow transplantation in a patient with β-thalassemia in 1994, who was conditioned with busulfan 14 mg/kg and cyclophosphamide 160 mg/kg [73]. Recently, in a report of the unrelated donor BMT experience in Italy, La Nasa, et al. described an incidence of overall survival and disease free survival with transfusion independence of 79 percent and 66 percent, respectively, in 68 children and adults (median age 15, range 2–37 years), transplanted across six Italian transplant centers. All donors were selected using high resolution molecular typing of both HLA class I and II loci. Under these selection conditions, they observed 13 percent primary or secondary graft failure, and 20 percent death from transplant-related causes. Forty percent of patients experienced grade II to IV aGVHD, and 18 percent had cGVHD. For patients with class III disease, OS and DFS was 65/54 percent. These data demonstrate that when donor selection is based on stringent compatibility criteria, the results of unrelated transplantation in thalassemia patients are comparable to those obtained when the donor is a compatible sibling [74]. Similarly, Hongeng, et al. reported the outcome of myeloablative transplant in Bangkok in 49 children, 21 of which underwent URD marrow transplant, and found no significant differences of thalassemia-free survival nor transplant-related complications, between using MRD or URD [75]. No data are available for the use of URD transplant for the treatment of sickle cell disease.

4.2. Cord Blood: Matched Related

Human umbilical cord blood contains hematopoietic stem cells capable of marrow reconstitution, and represents a potentially good alternative source of allogeneic stem cells [76, 77]. To date, >2,000 cord blood transplants have been performed worldwide [78–81]. Initial studies suggest that cord blood transplantation (CBT) is associated with a reduced incidence of GVHD, even in the setting of incomplete HLA match, attributed to the relative paucity of T cells in cord blood [78, 82, 83]. These characteristics open the possibility of further donor pool expansion and may give patients of ethnic minorities that are poorly represented in bone marrow registries access to HSCT [84].

The first successful related cord blood transplant for the treatment of a child with thalassemia was reported by Issaragrisil, et al. in 1995 [85]. This was followed by a report of CBT in 10 patients based on the Eurocord experience (seven with β-thalassemia, three with SCD) [86]. This series has been recently updated by Locatelli, et al. to include 44 patients (age 1–20) — 33 with thalassemia, and 11 with SCD. Thirty patients received with cyclosporine A alone for GvHD prophylaxis, while 10 patients received a combination of

cyclosporine A and methotrexate. Four patients developed acute GvHD and two patients had cGvHD. The two-year probability of event-free survival was reported at 79 percent and 90 percent for patients with thalassemia and SCD, respectively. In this series, the use of methotrexate was associated with a greater risk of treatment failure [84].

In North America, Walters, et al. has documented the feasibility of collecting – and the relatively high rate of usage of – related cord blood units from families with hemoglobinopathies through the Oakland, California-based Sibling Donor Cord Blood Program. To date, 1,617 units have been collected — 6 percent from families with thalassemia, 28 percent with SCD, 49 percent with malignant disorders and 17 percent with rare hematologic diseases. Thirty-two of 96 donor-recipient pairs were HLA-identical, and 14 have received cord blood transplantation for treatment of thalassemia. Eleven of the 14 transplanted patients survive free of thalassemia after transplantation [87].

4.3. Cord Blood: Haploidentical and Unrelated

Attempts to expand the donor pool by using bone marrow from mismatched related donors as the source of hematopoietic stem cells for myeloablative transplantation of patients with thalassemia have been reported to be associated with high transplant-related mortality, with GvHD as the major contributor of death. Gaziev, et al. reported the results of treating 29 patients with thalassemia with marrow collected from HLA phenotypically identical relatives, HLA nonidentical siblings or parents, and observed a TRM of 38 percent, with greater than 50 percent of deaths attributed to acute or chronic GvHD [88]. The lowered risks of GvHD associated with the use of cord blood as a hematopoietic stem cell source have renewed the possibility of considering the usage of products with greater HLA mismatches, such as a related haploidentical cord blood, in order to further expand the donor pool. Recent studies have begun to explore this approach; a case report described by Hongeng, et al. has demonstrated the feasibility of using mismatched related cord blood in the myeloablative setting for treating a child with thalassemia [89].

To determine the theoretical availability of compatible unrelated umbilical cord blood (UCB) units for treating SCD patients, Adamkiewicz, et al. examined the number registered via Bone Marrow Donors Worldwide for hematopoietic stem cell transplantation (HSCT) of children with sickle cell disease (SCD), matched for DRB1 at high resolution. He found that cord blood units matching at four or more of six HLA loci at acceptable cell doses can be identified for a majority of children with SCD weighing 40 kg or less. Availability of units matched at five or more of six HLA loci was more limited [90]. Adamkiewicz has reported the feasibility of myeloablative CBT using units matched at four of six antigens (A, B and DRB1) for the treatment of SCD. Using a myeloablative regimen consisting of busulfan, cyclophosphamide and equine anti-thymocyte globulin, Adamiekiwicz reported that two of three children with SCD thus treated achieved complete donor hematopoiesis. Three of three transplants were associated with aGVHD (2 × grade 2, one with grade 3), and one with extensive cGVHD [91]. More recently, Jaing, et al. reported rapid and complete donor chimerism at a median follow-up of 303 days after URD mismatched (1–2 of 6 HLA antigens) CBT in five children with β-thalassemia, following a myeloablative preparative regimen (busulfan, cyclophosphamnide and ATG). Five of five

subjects experienced acute GVHD, grade 2–3, but none with extensive cGVHD [92]. Even more recently, Mazur, et al. reported the transplantation of a child with SCD with an unrelated CB unit, after preparation with a reduced-intensity conditioning regimen. This regimen consisted of pretreatment with hydrea, rituximab and alemtuzumab, followed by treatment with thiotepa and low dose TBI before infusion of the URD cord blood unit. Rapid full donor chimerism was observed, associated with improved performance status that was sustained over two years after transplant [93].

5. Autologous with Gene Therapy for Correction of Hemoglobinopathies

Since the hemoglobinopathies are monogenic diseases, an alternative curative approach to allogeneic HSCT, which carries the risk of GvHD, would be to introduce gene therapy to correct the underlying genetic disorder in erythroid progenitor cells, and to engraft the modified cells in the host, by autologous transplantation. The previously described clinical and laboratory studies support the notion that a dosage of approximately 10 to 25 percent RBCs with corrected hemoglobin synthesis would be sufficient for phenotypic correction of these diseases.

There has been long-standing interest in gene therapy for the correction of hemoglobinopathies, but clinical realization of this therapeutic approach has been hampered by major challenges in the control of expression of the globin transgene, which ideally should be erythroid specific, differentiation- and stage-restricted, elevated, position independent and sustained over time [94]. Recenty, May, et al. demonstrated that an optimized combination of proximal and distal transcriptional control elements permits lineage-specific and elevated beta-globin expression, resulting in therapeutic hemoglobin production and correction of anemia in β-thalassemic mice [95]. Using this approach, several groups have demonstrated that it is possible to correct hemoglobinopathies in mouse models [96–98]. Currently this approach is under investigation in phase I/II trials [99].

6. Summary

Clinical studies in the 1980s and 1990s have solidly demonstrated the curative potential of allogeneic stem cell transplantation for the treatment of hemoglobinopathies. Over the past decade, steady progress has been developed towards: (1) identifying clinical features to help identify high risk populations that may benefit from HSCT; (2) developing a mechanistic understanding of how stem cell-based therapies such as HSCT and gene therapy can address treatment of hemoglobinopathies; (3) the development of proof-of–concept studies to test the potential of using regimens that incur lower levels of toxicity compared to myeloablative regimens, and yet are sufficiently immunosuppressive to overcome barriers to lasting donor engraftment, and (4) exploring the use of alternative sources of donor stem cells, including products derived from umbilical cord blood, or unrelated and haploidentical donors. Clearly, broader clinical experience is needed to definitively establish the efficacy and determine the toxicity profiles of these novel regimens. In total, the combination of these

encouraging results suggests that broader availability of this curative treatment modality should be imminently available, and would be anticipated to make a significant impact on this set of devastating diseases.

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Chapter 12

Stem Cell Sources for Allogeneic Transplantation

Corey Cutler

1. Introduction

The discovery that human hematopoietic progenitors are found in the peripheral circulation and can be harvested for use in stem cell transplantation has been both a scientifically enlightening and a clinically useful observation in stem cell transplantation technology. Over the past two decades, our understanding of peripheral blood stem cell (PBSC) properties has increased dramatically and, as a consequence, the use of PBSC in autologous and allogeneic transplantation has increased exponentially. Ease of collection, rapid engraftment and their association with improved outcome make PBSCs the stem cell of choice for all patients undergoing autologous transplantation [1, 2]. In the allogeneic setting, while there have been dramatic increases in the use of PBSC, the conclusive evidence that PBSCs are a superior stem cell source over bone marrow (BM) is not as evident, and in fact some of the putative advantages of PBSCs in the allogeneic setting may have substantial limitations. As a result, some centers have chosen not to adapt PBSC as the main stem cell source for allogeneic transplantation.

Figure 12-1a demonstrates the overwhelming use of PBSC for autologous transplantation and the dramatic increase in PBSC use for allogeneic transplantation in recent years. Greater than 90 percent of autologous transplants and greater than 40 percent of allogeneic transplants reported reported to the CIBMTR were performed with PBSC. Particularly striking is the increased use of PBSCs in the unrelated donor setting in recent years (Fig. 12-1b). The EBMT has noted similar increases in the use of PBSC for allogeneic transplantation [3], and this proportion is expected to increase further in the coming years.

In addition to the short-term (engraftment, acute GVHD) and long-term (chronic GVHD, relapse-free survival) end points considered when choosing stem cells for allogeneic transplantation, other factors such as donor quality of life and economic impact influence decision making. Yet another alternative stem cell source for allogeneic stem cell transplantation now exists, although using umbilical cord blood as a stem cell source in adults is increasing, but remains limited. Cord blood transplantation is compared with adult stem cell

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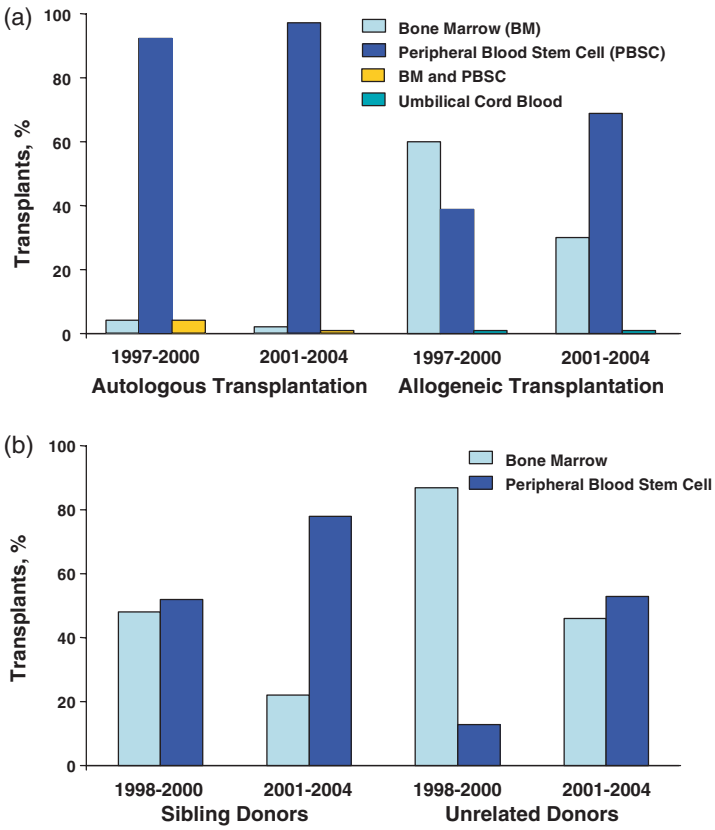

Fig. 12-1. Proportional uses of stem cells in individuals aged 20 or greater, 1997–2004, as reported to the CIBMTR (modified with permission) (a) Use of stem cells in autologous and allogeneic stem cell transplantation (b) Use of peripheral blood and bone marrow among related and unrelated donors

sources in this chapter, but is discussed in greater detail elsewhere in this text (Chapter X). At this time, the decision to employ umbilical cord blood stem cells in adults outside of a clinical trial is based solely on the lack of an available adult donor.

2. Stem Cell Mobilization and Engraftment Kinetics

Circulating peripheral blood stem cells represent less than 0.001 percent of all nucleated cells in circulation [4]. These cells are in continuous recirculation from the marrow to the blood and back to random sites in the marrow cavity. Presumably, this process ensures an even distribution of hematopoiesis throughout the skeletal system. Levels of hematopoietic progenitors rise dramatically during the recovery phase after myelosuppressive chemotherapy and in response to exogenous recombinant human granulocyte colony stimulating factors (rhG-CSF).

Exogenous growth factors have long been the mainstay of stem cell mobilization for both autologous and allogeneic stem cell transplantation. Doses and schedules

of different rhG-CSFs (i.e., G-CSF, GM-CSF) used for mobilization have ranged between two and 24 μg/kg/d for one to five days in reported studies [5–7]. Although different doses, schedules and types of CSFs have been examined in clinical trials prior to autologous transplantation (often in conjunction with myeloablative chemotherapy) [8, 9], trials in healthy PBSC donors have not been reported until recently [10]. Ten μg/kg/d of filgrastim administered for five consecutive days is the regimen recommended by the National Marrow Donor Program for healthy donors. Factors that may affect the cell yield from normal donors include the dose and duration of CSF administration and the timing of the apheresis procedure.

The mechanisms of progenitor cell mobilization have remained unclear for many years. Mobilization is mediated in part through the downregulation and cleavage of adhesion molecules found on stem cell progenitors and marrow stromal endothelium. These adhesion molecules are expressed in high levels in the steady state, and play an important role in the maintenance of localization of the stem cells in the endosteal regions of the marrow space. Once these critical adhesion interactions are disrupted, the progenitor cells migrate through the diaphragmed fenestra of the bone marrow endothelium into the peripheral circulation [11]. Several adhesion partners, including the VLA-4/VCAM-1 complex (targeted by downregulation [12] and neutrophil protease mediated cleavage in response to G-CSF [13]), the selectin molecules, the kit-kit ligand interaction and hyaluronan/CD44 have been thought to be instrumental in maintaining stem cells in the endosteal niche [11]. Most recently, the commercial development of a selective inhibitor of the SDF-1α/CXCR4 interaction has demonstrated that this receptor-ligand pair may in fact be the most critical regulator of stem cell maintenance in the endosteal niche. AMD3100 is a novel bicyclam small molecule reversible inhibitor of SDF-1α binding to CXCR4 [14]. It can be used alone or in combination with G-CSF to mobilize stem cells, and the experience in autologous transplantation suggests that it is effective in poor mobilizers and in mobilizing naïve patients [15]. In the allogeneic setting, where concerns about the differential effects on T cell mobilization have not yet been entirely addressed, one small study suggested that outcomes after AMD3100 and G-CSF combined mobilization are no different than G-CSF mobilization alone [16]. The potential value of AMD3100 lies in the fact that stem cells are rapidly mobilized and ready for collection (within four to 12 hours) after a single dose, without the side effects traditionally associated with prolonged exposure to G-CSF.

As a result of the cytokine and chemokine-induced signaling that occurs upon mobilization, peripheralized hematopoietic progenitors are phenotypically different compared to their marrow stromal-bound precursors. In fact, gene expression profiling studies have demonstrated that cell-cycle promoting genes and genes regulating DNA synthesis and replication are expressed at significantly lower levels in PBSC, while apoptosis-related genes are expressed at significantly higher levels in PBSC [17]. These results corroborate earlier findings demonstrating PBSC to be less metabolically active and less involved in active cell cycling by rhodamine retention studies and S–phase analysis [18]. PBSC have been shown to express higher levels of differentiation markers, such as CD13 and CD33. These committed progenitors may be responsible for the more rapid engraftment noted after PBSCT. In addition, mobilized PBSC have been shown to be composed of higher proportions of $CD34^{+}CD38^{-}$ cells

and $CD34^+Thy\text{-}1^+$ coexpressing cells, which are known to be enriched for long-term culture initiating colonies (LTC-IC) subpopulations. Whether this, in turn, promotes enhanced graft stability and the earlier appearance of more diverse immune reconstitution is not known.

The clinical yields of G-CSF-mobilized PBSC collections are generally superior to bone marrow harvests, when $CD34^+$ progenitor numbers are compared. The differential yield after collecting both bone marrow and PBSC from healthy donors was examined in 40 healthy donors who underwent bone marrow harvesting followed by G-CSF stimulated PBSC collection one week later for their HLA-identical siblings. The recipients were randomized in a blinded fashion to receive either the PBSC or the bone marrow collected from their siblings. Total nucleated cells, $CD34^+$ cell yield and CFU-GM activity were 2.3, 3.7 and 3.7 times higher after PBSC harvesting when compared with bone marrow harvesting. Furthermore, bone marrow harvests were 6.8 times more likely to be insufficient for transplantation (defined as less than 2×10^6 $CD34^+$ cells/kg of recipient weight, $p < 0.001$) [19]. This interpretation may not be valid since, traditionally, a range of $2 - 4 \times 10^8$ total nucleated cells/kg has been used as an arbitrary criterion for a sufficient marrow harvest. In addition, the bone marrow harvest procedure may have artificially enhanced stem cell peripheralization and PBSC yields, making the results difficult to interpret.

A relationship between the dose of $CD34^+$ cells delivered with the transplant and the tempo of hematologic recovery has been demonstrated for both bone marrow [20] and peripheral blood stem cell transplantation [21–24]. Using higher doses of $CD34^+$ cells leads to quicker engraftment, particularly when doses are greatly increased [25, 26]. Platelet recovery appears to be more sensitive to $CD34^+$ doses than neutrophil recovery [26].

A convincing reduction in time to engraftment after both autologous and allogeneic PBSC transplantation has been noted when compared to traditional bone marrow transplantation. This reduction is thought to largely be due to the increased numbers of $CD34^+$ progenitor cells delivered with PBSC grafts, although inherent differences in the stem cells transplanted may be implicated as well. In the allogeneic setting, neutrophil engraftment (to 0.5×10^9 cells/l on three consecutive days) occurred between three and six days earlier with peripheral blood stem cell transplantation when compared with bone marrow transplantation in randomized trials (median time to engraftment: 12 versus 15 days and 15 versus 21 days) [27, 28]. Unsupported platelet counts of 20×10^9/l occurred between five and eight days earlier (median time to platelet engraftment: 15 versus 19 days and 11 versus 18 days) [28, 29]. The results of a large database review are consistent with the results of the randomized trials (median time to neutrophil engraftment 14 versus 19 days, median time to platelet engraftment 18 versus 25 days, $p< 0.001$ for both comparisons) [30]. Finally, a meta-analysis of 11 prospective randomized trials that compared PBSC and bone marrow transplantation from HLA-identical siblings confirmed these findings [31].

The earlier engraftment seen after peripheral blood stem cell transplantation has lead to earlier discharge from the hospital [29, 32, 33], fewer post-transplant transfusions [28, 32, 34, 35] and total lower immediate costs associated with the transplant procedure [27, 36]. The latter is primarily due to fewer dollars spent on hospital room charges, blood products and other supportive measures. However, the costs of stem cell mobilization and collection procedures may be

greater for PBSC than for bone marrow transplantation, primarily due to the use of recombinant human hematopoietic growth factors [36]. Long-term cost issues are more difficult to predict and will be influenced by Graft-versus-Host Disease outcomes after PBSC transplantation (see below).

3. Clinical Transplant Results – Linking Biology to Outcomes Analysis

The ongoing debate over the relative advantages and disadvantages of PBSC when compared to bone marrow in matched, related donor (MRD) allogeneic transplantation has prompted numerous randomized clinical trials. In addition, three meta-analyses have been published to examine the totality of evidence in MRD transplantation [31, 37, 38]. In unrelated donor (URD) transplantation, there have been multiple retrospective analyses and a large, national prospective study is underway to compare PBSC with bone marrow transplantation in a prospective manner.

3.1. Acute GVHD

Phase 2 studies of MRD PBSC transplantation (PBSCT) demonstrated an increased incidence of acute GVHD [39–42], and numerous randomized trials have specifically addressed differences in acute GVHD incidence after PBSCT and BMT [27–29, 32, 34, 35, 43, 44]. The rates of acute GVHD from these trials can be found in Table 12-1 and the summary findings from each of the trials can be found in Table 12-2. In the largest randomized trial involving 350 patients, both grade II–IV and grade III–IV acute GVHD were significantly increased in the PBSCT group (52% versus 39%, $p = 0.014$ and 28% versus 16%, $p = 0.01$, respectively). In this trial, however, an abbreviated three-dose course of methotrexate was used in both arms as GVHD prophylaxis [28]. The Seattle group demonstrated a

Table 12-1. Rates of acute and chronic GVHD from randomized trials.

	Grade II–IV GVHD			Grade III–IV GVHD			Extensive Chronic GVHD		
First Author, Publication Year	**PBSC**	**BM**	**Relative Risk**	**PBSC**	**BM**	**Relative Risk**	**PBSC**	**BM**	**Relative Risk**
Couban 2002	44%	44%	0.99	26%	18%	1.46	40%	30%	1.23***
Bensinger 2001	64%	57%	1.12*	15%	12%	1.23**	46%	35%	1.30
Vigorito 2001	26%	22%	1.20	17%	13%	1.33	67%	30%	2.22°
Schmitz 2001	52%	39%	1.33°	28%	16%	1.75°	25%	11%	2.20°
Blaise 2000	45%	42%	1.06	17%	23%	.74	34%	8%	4.26°
Powles 2000	50%	47%	1.06	NA	NA	NA	NA	NA	NA
Heldal 1999	21%	10%	2.14	NA	NA	NA	15%	7%	2.22
Mahmoud 1999	7%	47%	0.14°	7%	40%	0.17°	NA	NA	NA

° $p \leq 0.05$
* Hazard ratio 1.21 by actuarial methods.
** Hazard ratio 1.27 by actuarial methods.
*** Hazard ration measurement

nonsignificant increase in the hazard ratio for both grade II–IV GVHD (hazard ratio 1.21, 95% CI 0.81– 1.81), and grade III–IV GVHD (hazard ratio 1.27, 95% CI 0.55 – 2.89) [34]. Other trials have reported nonsignificant increases [35, 45] or similar risks in the PBSCT and BMT groups [27, 29, 32] with only one small trial demonstrating a statistically significant increase in acute GVHD in the BMT arm [43]. The IBMTR/EBMT collaborative review of 288 PBSCT and 536 BMT procedures demonstrated a nonsignificant increase in the rate of grades II–IV acute GVHD (RR 1.19, 95% CI 0.9 – 1.56) [30]. Finally, two meta-analyses that examined acute GVHD demonstrated a significant increase in the risk of grade II–IV (RR 1.16, 95% CI 1.04 – 1.28[37]) and grade III–IV acute GVHD (OR 1.39, 95% CI 1.03 to 1.88 [31]). A summary of the important findings from the individual patient data meta-analysis is shown in Fig. 12-2.

There are many possible explanations for the discrepancies noted in the rates of acute GVHD noted in the clinical trials. Factors such as the age of patients and their donors [46], sex mismatch among donor-recipient pairs, the inclusion of higher risk patients in some trials, the use of different conditioning or GVHD prophylaxis regimens and discrepancies in GVHD scoring between transplant centers may influence GVHD occurrence. These differences may be less apparent in the unrelated setting, where rates of GVHD are expected to be higher than in the matched related setting. However, there are no large, prospective trials in URD transplantation upon which to base these hypotheses.

Initial small phase 2 studies of URD PBSCT with retrospective comparisons to BMT did not demonstrate differences in the rates of acute GVHD [47, 48]; however, a large retrospective review of over 1,300 patients from the CIBMTR database has demonstrated that grade II–IV acute GVHD may be more prevalent (56 versus 46%, $p < 0.001$) after URD PBSCT than BMT for leukemia and myelodysplasia [49]. In this analysis, the incidence of grade III–IV acute GVHD was not noted to be different, which contrasts with an NMDP analysis that demonstrated an increased risk of grade III–IV, but not grade II–IV acute GVHD [50].

There are approximately 10-fold more $CD3^+$ T cells delivered with a PBSC graft when compared with a bone marrow graft (279×10^6/kg versus 23.8×10^6/kg

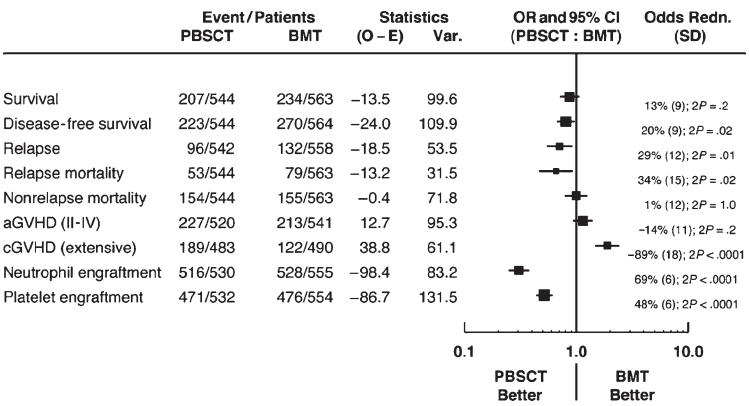

Fig. 12-2. Summary findings from an individual patient meta-analysis of nine randomized trials in matched, related donor transplantation

in one prospective trial [34]), with consistent results among other trials in MRD transplantation [27–29, 44]. This increase in the number of T cells delivered with a PBSC graft is one of the theoretical reasons for the increased rates of GVHD seen after PBSCT and explains, at least in part, the cautious adoption of PBSCT in the allogeneic setting; however, there is very little objective evidence to suggest that there is a relationship between T cell dose and GVHD after PBSCT [51, 52].

In animal studies, G-CSF administration has been shown to polarize mobilized T cells to a type 2 response [53, 54], largely as a result of type 2 dendritic cell stimulation [55, 56]. This has led to diminished rates of GVHD noted in mouse models, but has not been confirmed in all studies. Another explanation for the potential differences in the rates of GVHD that has been explored is that the regulatory T cell subset derived from PBSC and marrow grafts is quantitatively or qualitatively different [57]. It is also possible that in humans, despite a polarization of T cells to the type 2 phenotype, the increased number of T cells is still the driving force behind the increased rates of GVHD noted after PBSCT.

3.2. Chronic GVHD

Chronic GVHD is the most significant contributor to late morbidity and mortality after allogeneic transplantation. There is little doubt that the incidence of chronic GVHD is increased after PBSCT in the MRD setting. The IBMTR/EBMT database review demonstrated a significant increase in chronic extensive GVHD after PBSCT, (65 versus 53% at one year, p= 0.02) [30], subsequently confirmed in a later report (RR 1.65 $p < 0.001$) [58]. Although none of the randomized trials were powered specifically to detect differences in chronic GVHD incidence, every trial has demonstrated at least a trend towards more chronic GVHD, and some have demonstrated statistically significant results (Tables 12-1, 12-2). Relative risks for chronic GVHD have ranged between a 1.29- and a 4.26-fold increase for PBSCT over BMT. The large EBMT randomized trial demonstrated a statistically significant increased risk of chronic GVHD in the PBSCT group (73 versus 55% at 3 years, p=0.003) [28, 59], and the large American and Canadian trials demonstrated trends towards increased chronic GVHD after PBSCT [32, 34]. The individual patient meta-analysis confirmed the statistically significant and clinically meaningful increase in chronic GVHD (47 versus 31 percent incidence of extensive chronic GVHD at three years, $p < 0.001$, Fig. 12-2) [31].

In the URD setting, no differences in the incidence of chronic GVHD was noted in a single published cohort study [47]. However, the lack of a difference in this group may be attributed to the relatively high rates of GVHD noted (61% and 76% for the PBSCT and BMT groups, respectively). Furthermore, the CIBMTR and NMDP analyses both suggested that the risk of chronic GVHD after PBSCT was higher than after BMT (54% versus 40%, $p < 0.001$ and HR 1.3 95%CI 0.8 – 1.9) [49, 50].

The total administered $CD34^+$ cell dose may influence the incidence of chronic GVHD. A retrospective analysis of 181 PBSC transplants performed between 1996 and 1999 demonstrated an increased hazard for chronic GVHD among patients who received more than 8.0×10^6 $CD34^+$ cells/kg (hazard 2.3, $p = 0.001$). This relationship was independent of the number of T cells delivered with the graft [23]. A trend for increased chronic GVHD was noted in the risk

Table 12-2. Clinical of summary of randomized trials comparing PBSC to BM.

First Author, Publication Year	Sample Size	Time to Neutrophil Engraftment	Time to Platelet Engraftment	Acute GVHD	Chronic GVHD	Relapse	Disease Free Survival	Overall Survival
Couban 2002	228	↓ **PBSC**	↓ **PBSC**	ND	↑ PBSC	ND	NA	↑ **PBSC**
Bensinger 2001	172	↓ **PBSC**	↓ **PBSC**	↑ PBSC	↑ PBSC	↑ **BM**	↑ **PBSC**	↑ **PBSC**[*]
Vigorito 2001	56	↓ **PBSC**	↓ **PBSC**	↑ PBSC	↑ **PBSC**	NA	↑ PBSC	↑ PBSC
Schmitz 2001	350	↓ **PBSC**	↓ **PBSC**	↑ **PBSC**	↑ **PBSC**	ND	ND	ND
Blaise 2000	101	↓ **PBSC**	↓ **PBSC**	ND	↓ **PBSC**	↑ BM	ND	ND
Powles 2000	39	↓ **PBSC**	↓ **PBSC**	ND	↑ PBSC	↑ **BM**	NA	↑ PBSC
Heldal 1999	61	↓ **PBSC**	↓ **PBSC**	↑ PBSC	↑ PBSC	↑ BM	↑ PBSC	↑ PBSC
Mahmoud 1999	30	↓ **PBSC**	↓ **PBSC**	↓ **BM**	NA	NA	NA	NA

Thick arrows and bold text represent statistically significant differences ($P \leq 0.05$) Thin arrows represent trends (P = NS)
[*]Patients with advanced malignancy
ND = No difference
NA= Not applicable

factor analysis reported by Przepiorka, et al. [52], but these findings were not confirmed in another similar study [24]. In an analysis of $CD34^+$-selected PBSCT, increased mortality was associated with higher $CD34^+$ cell doses, although the relationship with increased GVHD in this group was not statistically significant [60]. As the dose of $CD34^+$ cells is always higher in PBSC grafts, this may explain some of the clinical differences in chronic GVHD noted.

3.3. Relapse

There is sufficient evidence from nonmyeloablative transplantation, donor lymphocyte infusions (DLI) and immunosuppression withdrawal studies to suggest that a potent Graft-versus-Leukemia (GVL) reaction can occur after stem cell transplantation. With the exception of some experimental rodent studies [61–63], separation of clinical GVL from GVHD has remained elusive. Despite the detrimental effects on survivorship that acute and chronic GVHD can cause, there is evidence to suggest that the presence of acute GVHD, chronic GVHD, or both correlates with disease relapse prevention after bone marrow transplantation [64, 65]. These findings have been confirmed after PBSCT as well, where the presence of chronic GVHD [27, 66] or both acute and chronic GVHD [67] have been demonstrated to protect against relapse, primarily in high risk patients. Brunet, et al., reporting on 136 patients who had undergone allogeneic PBSCT for advanced hematological malignancies, demonstrated a cumulative incidence of relapse of 47 percent in patients without any GVHD, but only 14 percent for patients who experienced both acute and chronic GVHD ($p = 0.002$). This decrease in relapse translated into a long-term survival advantage [67]. Przepiorka, et al. noted a nonsignificant trend for chronic GVHD to be protective from relapse in a retrospective review (Hazard ratio 0.6, $p = 0.2$) [52], and Miklos, et al. demonstrated that the presence of antibodies directed and Y chromosome-encoded minor histocompatibility antigens in the context of chronic GVHD were protective against relapse [68].

Körbling first noted the possible advantage of PBSCT over BMT for refractory leukemia and lymphoma [69]. Elmaagacli, et al. demonstrated a lower incidence of molecular and cytogenetic relapse in a nonrandomized trial comparing matched related PBSCT and BMT for CML [70] and subsequently demonstrated improved survival after PBSCT when compared with BMT in the unrelated donor setting [71]. Similar reductions in disease relapse after PBSC allografting for myeloma have been demonstrated [72].

In the randomized setting, several studies have independently demonstrated a decrease in the rate of disease relapse after PBSCT when compared to BMT (Table 12-2) [27, 29, 43, 73, 74]. The hazard ratio for relapse in the study reported by Bensinger, et al. was 0.49 (95%CI 0.38 – 1.28) among patients transplanted with PBSC and this was associated with an increase in disease free survival for the PBSCT group (65 versus 45%, p=0.03) [34]. The individual patient data meta-analysis also confirmed the reduced relapse associated with PBSCT, and showed that this effect may be more prominent for patients with advanced disease (Fig. 12-2) [31].

In the URD setting, without a randomized trial yet, it is difficult to interpret the results of the retrospective reviews due to the inherent differences in baseline patient characteristics between groups, particularly as they pertain to disease relapse risk. In both the NMDP and CIBMTR retrospective reviews, no differences in the incidence of relapse were noted [49, 50].

3.4. Immune Reconstitution

Immune reconstitution after allogeneic transplantation is critical, since important morbidity and mortality can be ascribed to infectious complications in the post-transplant period. Complete immune reconstitution, or at a minimum, T cell reconstitution may also be important for the development of an effective GVL response as well, although immune reconstitution is often limited in the context of GVHD. Immune reconstitution can be assessed by enumeration of specific immune cell subclasses, by measurement of T cell neogenesis [75], by measurement of immune diversity through V_{β} gene rearrangement spectrotyping [76] and by functional assays of immune activity. These studies can be misleading when performed in the context of active infection, GVHD and immunosuppression.

Despite seemingly normal numbers of mature lymphocytes and granulocytes within weeks to months of allogeneic transplantation, immunosuppressive medications, the occurrence of GVHD and other clinical events alter immune function. Impaired immune function has been noted in a number of studies after allogeneic BMT [77] as well as after PBSCT [78]. In a retrospective study of 115 healthy transplant recipients evaluated at least one year after BMT, risk factors for impaired immune reconstitution (measured by T and B cell numbers, immunoglobulin levels and T cell proliferative responses) included the presence of chronic GVHD, Cytomegalovirus infection, mismatched transplantation, TBI conditioning and advanced recipient age [79]. Earlier studies had noted the correlation between lower T cell counts [80] and lower B cell and monocyte counts [81] with infectious complications after transplantation.

Three of the published randomized studies have addressed issues related to immune reconstitution comparing PBSCT with BMT. The French study

documented higher levels of total lymphocytes, B cells, T cells and T cell subpopulations after PBSCT when compared to BMT, while NK cells and monocyte numbers were not significantly different 30 days after transplantation [82]. Both $CD56^+$ NK cells [83] and $CD19^+$ B cells [19] have been found in greater quantities in PBSC collections than in BM harvests previously. In the Seattle study, transplantation with PBSC was associated with higher $CD4^+$ T cell counts ($CD45RA^{high}$ naïve and $CD45^{low/-}$ mature cells), higher $CD8^+$ T cell counts and higher $CD4^-CD8^-$ T cell counts early after transplantation. The increase in T cell number (without an increase in single T cell function as measured by lymphoproliferation assays) was associated with a lower incidence of confirmed infections (RR 0.59, $p < 0.001$) and confirmed severe infections (RR 0.42, $p = 0.002$) after transplantation [84]. In contrast to an earlier report by the same authors [85], serum immunoglobulin levels were similar to those found in BMT patients, confirming results from smaller studies [86, 87]. Finally, the small Norwegian randomized trial showed similar findings regarding the earlier immune reconstitution after PBSCT over BMT; however, in the context of chronic GVHD, both groups of patients had diminished levels of immunoglobulin, low levels of circulating B cells and increased numbers of $CD8^+$ T cells in the circulation [88].

Owing to reduced thymic function in older individuals, the majority of mature T lymphocytes found in the circulation within the first year after transplantation are felt to have been transferred with the stem cell graft, since evidence of T cell neogenesis does not occur until six months post-transplantation [75]. Tayebi, et al. demonstrated a significant correlation between the infused T cell dose, but not the infused CD34+ stem cell dose, and lymphocyte counts 30 days after transplantation [82]. Since PBSCT is associated with a log-fold increase in delivered T cells, the finding of increased T cell numbers after transplant is not surprising and may explain the differences noted in immune reconstitution studies.

3.5. Survival

While acute GVHD outcomes are important in the short term, long-term survival differences will ultimately drive stem cell choice decision making in allogeneic transplantation. With shorter time to engraftment following PBSCT, it was anticipated that a reduction in treatment-related mortality (TRM) would be noted; however, this has not been universally noted. The Canadian MRD randomized trial demonstrated a reduction in TRM after PBSCT when compared to BMT (2.8 versus 7.6% TRM at 30 days, $p = 0.18$ and 7.4 versus 16.1% TRM at 100 days, $p = 0.07$) [32]. Similarly, a reduction in TRM after PBSCT was noted for individuals with high risk disease in the IBMTR/EBMT review, where TRM was lower for individuals with advanced stage AML and accelerated phase CML, but not different for individuals with AML in first remission or CML in chronic stable phase [30, 58].

Long-term survival after transplantation is affected by early TRM, relapse incidence and mortality resulting from acute and chronic GVHD and infection. Since TRM and relapse may be reduced after PBSCT, GVHD may be more common after PBSCT and immune reconstitution may be more complete after PBSCT, it is unclear how these competing risks will translate into long-term survival differences. In the Canadian trial, an overall survival advantage, largely

attributed to the reduction in early TRM, was noted for the PBSCT group (68 versus 60% survival at 30 months, p = 0.04) [32]. Despite an improvement in disease free survival for individuals transplanted with PBSC (65 versus 45%, p = 0.03), long-term survival differences did not reach statistical significance in the American trial (66 versus 54% 2 year survival, p = 0.06) [34]. However, for individuals with advanced malignancies, there was a significant improvement in overall survival (57 versus 33%, p=0.04). Small differences in survival favoring PBSCT were noted in several other randomized studies (Table 12-2) [35, 45, 73], but in contrast, the EBMT trial has not yet demonstrated a survival advantage for PBSCT [28, 59]. The large meta-analysis suggested a trend for improved survival after MRD PBSCT (Fig. 12-2) [31]. It is also important to note that in contrast to these findings, survival appears to be compromised in children and adolescents transplanted with PBSCs [89].

In unrelated donor transplantation, no advantage to PBSCT was noted in both the CIBMTR retrospective review and the NMDP analysis, although the NMDP analysis demonstrated a reduction in early deaths after transplantation [49, 50]. The results of the prospective randomized trial, therefore, are critical.

The impact of $CD34^+$ cell dose has been explored with respect to overall survival. In a multivariate Cox analysis of predictive factors for TRM and long-term survival after PBSCT, a $CD34^+$ cell dose greater than 3×10^6 cells/kg was associated with a reduction in 180-day TRM (Hazard ratio 0.54, p = 0.03), and a reduction in overall mortality at a median of 3.4 years after transplantation (Hazard ratio 0.55, p= 0.006) [24]. Conversely, in a study of T cell depleted PBSCT, a higher $CD34^+$ cell dose was associated with diminished survival, largely as a result of increased GVHD and infectious complications [60].

4. Implications for Stem Cell Donors and the Economics of Stem Cell Transplantation

The procurement of stem cells from both related and unrelated marrow and peripheral blood stem cell donors must be performed with minimal morbidity to the volunteer donors. Of 1,337 PBSC apheresis procedures reported to the IBMTR/EBMT between 1994 and 1998, complications occurred in 15 donors. One-third of the complications were related to central line venous access (which was required in 20 percent of donors). Complications unrelated to line placement included pericarditis, back pain, hypercalcemia, alterations in blood pressure, nausea, diarrhea and thrombocytopenia [90]. Other commonly reported minor complications include myalgias, headache and fever, all of which responded to mild analgesics in over 80 percent of cases. Rare complications, including splenic rupture and death, have rarely been reported [91, 92]. Complications related to bone marrow harvesting in this retrospective review included prolonged pain at the site of the harvest, infection, anemia requiring red cell transfusion and complications related to either general or spinal anesthesia. There have been two fatalities reported to the IBMTR/EBMT after bone marrow harvesting [90]. Long-term follow-up (up to six years) has confirmed the safety of rhG-CSF administration to healthy donors [92, 93].

Donor preference for either PBSC or BM donation has been examined, as the two procedures differ in the time required for donor preparation (several

days for rhG-CSF administration), in the time needed for the procedure itself (between one and three days for PBSC donation, one day for marrow donation) and in the time to complete recovery (days to weeks for bone marrow donors, nearly immediate for PBSC donors). No differences in self-reported quality of life measures were noted among healthy donors randomized to undergo PBSC or marrow donation in one study [94]; however, an analysis of psychosocial markers of randomized donors in the Canadian randomized trial suggested lower mood, more fatigue and less energy as measured by the Profile of Mood States questionnaire, and persistent negative effects on the quality of life as measured by the SF-36 form in bone marrow donors [95]. These findings were similar to a nonrandomized experience as well [96].

Volunteers randomized to donate autologous PBSC or BM reported higher acceptance of the PBSC donation [97], and seven of eight patients who underwent both procedures also preferred PBSC donation [96]., There are some allogeneic donors who, for personal reasons, prefer either not to receive rhG-CSF or prefer not to undergo general anesthesia, thus dictating the method by which stem cells will be procured. Since there remains conflicting evidence regarding the optimal stem cell source for all patients at this time, the decision should be based primarily on perceived outcomes for individual patients, rather than on donor preference.

Two economic analyses have demonstrated lower costs associated with PBSCT due to a reduced first hospital stay and blood product support; however, both of these analyses did not include long-term costs that could be associated with increased rates of chronic GVHD [36, 98]. Cost comparisons that include cord blood transplantation have not yet been published since the broader acceptance of this stem cell technology [99].

5. Comparisons with Umbilical Cord Blood

The practical aspects of umbilical cord blood procurement and transplantation, as well as umbilical cord blood biology are discussed in Chapter X. Here, the important clinical differences in outcome comparing adult stem cell transplantation and umbilical cord blood transplantation (UCBT) are reviewed.

Two large retrospective reviews have compared the outcomes of unrelated bone marrow transplantation with UCBT [100, 101]. Both reviews have demonstrated consistent findings, with more rapid and complete neutrophil and platelet engraftment in bone marrow recipients. In the American review, there were no differences in the rates of acute GVHD when matched, unrelated bone marrow was compared with UCBT; however, a significantly lower rate of acute GVHD was reported for UCBT recipients in the Eurocord review. Differences in the sample size of the two studies and the inclusion of a higher proportion of single antigen mismatched cord blood donors in the Eurocord study may have influenced these results. Paradoxically, the rate of chronic GVHD was lower in marrow recipients in the American review (albeit with more advanced disease characteristics), although no differences were noted in the Eurocord study. Leukemia-free survival was higher among bone marrow recipients in the American trial, but this difference was not noted in the Eurocord study. The American trial also examined mismatched unrelated bone marrow as compared with UCBT, and in almost all variables examined, mismatched BMT was inferior to UCBT [100].

There are even fewer comparisons between UCBT and PBSCT. A single center analysis that pooled URD, PBSCT and BMT for comparison with UCBT demonstrated similar findings as noted above, with slower engraftment and similar rates of acute GVHD, although this analysis was performed in Japan where the rate of acute GVHD is generally lower than in North America and Europe. Chronic GVHD was more prevalent in the PBSCT/BMT group [102]. With the delay to engraftment noted after UCBT in all comparisons, the risk of infectious mortality after transplantation is thought to be increased. This has been examined in one retrospective review, where the incidence of severe infections was noted to be higher; however, the attributable mortality due to infections was similar in UCBT and BMT groups [103]. Since all comparisons demonstrate, at best, equivalent, but potentially worse outcomes with UCBT when compared with BMT, and until larger comparisons can be made with PBSCT, it would be unwise to offer UCBT when a suitable adult donor can be identified in a timely fashion.

6. Conclusions and Future Directions

Peripheral blood stem cell transplantation is the standard of care in autologous transplantation and has become the *de facto* standard in the allogeneic setting. The major differences in outcomes between PBSCT and BMT include a reduction in the time required for stable neutrophil and platelet engraftment, a higher incidence of acute and chronic GVHD with decreased relapse rates and at least a trend toward improved survival after PBSCT. Whether these findings hold true in the URD setting remain to be seen.

The major limitation to the widespread use of PBSCs is the fear of higher rates of GVHD and a potential negative impact on survival. Introducing novel immunosuppressant regimens may significantly lower the risk of GVHD to allow PBSC to be used more widely. Furthermore, if acute GVHD is adequately controlled, the benefits of reduced disease relapse and improved disease free survival may outweigh the risks of GVHD. Novel immunosuppressive regimens may be particularly helpful in the unrelated setting where GVHD rates are slightly higher, although the benefits of these regimens will need to offset the very high rates of chronic GVHD noted after URD transplantation.

There are several investigational strategies currently being tested that may supplant the use of simple PBSC or bone marrow in allogeneic transplantation. These strategies include the use of combined peripheral blood stem cell and bone marrow transplants [104, 105], the use of rhG-CSF mobilized bone marrow for transplantation [98, 106–109], and the broader use of umbilical cord blood in adult transplantation.

A risk stratification approach may be useful to decide which stem cell source is the most appropriate for individual patients. Considering factors such as patient age, disease stage (early versus advanced) and the evidence for an efficient GVL response, the age of the donor and recipient, recipient comorbidity and future quality of life may influence the stem cell choice. For example, for young individuals with stable phase CML, the slightly prolonged period of neutropenia associated with BMT may be a preferable trade-off for a decreased rate of extensive chronic GVHD, particularly in a disease where relapses are relatively rare and a potent GVL effect with marrow or DLI is

observed. In contrast, patients with relapsed acute leukemia may benefit from the enhanced GVL effects of PBSCT, despite the increased risk of GVHD.

The results of the large BMT Clinical Trials Network randomized trial in unrelated donor stem cell transplantation may sharply reduce the number of PBSC transplants performed if a long-term disadvantage is demonstrated with PBSCs; opposite results could signal the death knell for unstimulated bone marrow transplantation in adults since, in the MRD setting, peripheral blood stem cells do offer some advantages to bone marrow and still remain the stem cell of choice for the majority of patients. As the experience with cord blood transplantation in adults expands and randomized trials are performed we start to predict if cord blood transplantation will ever supplant adult stem cell transplantation.

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Chapter 13

Allogeneic Unrelated Donor Blood and Marrow Transplantation

Daniel Weisdorf

Allogeneic stem cell transplantation can provide curative therapy for patients with hematologic malignancies, marrow failure states, severe immunodeficiencies, hemoglobinopathies and inherited metabolic diseases [1–8]. Unfortunately, only approximately 25 to 30 percent of patients will have a suitable HLA genotypically identical sibling donor available to facilitate transplant therapy. An extensive family search may find a closely matched related donor for only 3 to 5 percent of patients and, thus, in the 1970s HLA-matched unrelated donors (URD) were considered as an alternative [1–3]. In the early 1980s, registries of volunteer marrow donors were established in Europe and North America, but the extreme polymorphism of the HLA system and the immunodominant gene products of the major histocompatibility complex, as well as differential frequencies of HLA phenotypes in different populations, indicated a need for volunteer donor registries of substantial size and great genetic diversity. Over 50 national registries exist in all continents including the National Marrow Donor Program in the United States and the Anthony Nolan Research Centre Registry in the United Kingdom which allow expedited searching for donors among well over 12 million registered volunteer donors.

After 20 years experience, the U.S. National Marrow Donor Program (NMDP) currently lists over 7 million donors with extensive HLA typing (HLA-A, B and DRB1) for nearly 4 million. Donor searching is facilitated by electronic communication, internet access and the multinational linkage of cooperating donor registries including Bone Marrow Donors Worldwide. The extreme diversity of HLA could theoretically yield many millions of possible HLA phenotypes, yet under 500,000 distinct HLA-A, B, DRB1 phenotypes are represented in the several million donors available for searching.

Great efforts have been made to broaden the ethnic diversity of donor registry files. However, the nonrandom linkage disequilibrium associations of certain HLA phenotypes in specific ethnic groups and the somewhat greater diversity of HLA polymorphism in Black and Hispanic individuals compared to northern and western European-derived Whites or other genetically more restricted populations such as Japanese donors, yield differential chances of finding a donor for patients of different racial and ethnic backgrounds.

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1. Donor Selection

1.1. Chances of Finding a Donor

Currently, searching through the National Marrow Donor Program can identify at least one HLA-A, B and DRB1 matched donor for 78 percent of Whites, 38 percent of Blacks, 55 percent of Hispanics and 54 percent of Asian Pacific Islander populations, respectively. Because donor availability and medical suitability for donation further limit these options, the likelihood of a medically suitable available HLA-A, B, DRB1 matched donor are somewhat lower. Presently, through the NMDP suitably matched and available donors are identifiable for approximately 73 percent of Whites, 25 percent of Blacks, 69 percent of Hispanics and 65 percent Asian-Pacific Islanders [9]. Encouragingly, if initial searching identifies more than five potentially compatible donors, the chances of a well matched available donor are much greater, even for the racial and ethnic minorities.

2. Donor Searching

While facilitated electronically and expedited through the experienced and committed network of the NMDP and similar cooperating donor registries worldwide, searching still requires an average of six to eight weeks to identify a donor and a total median time of 10 to 12 weeks to proceed to transplant. Donor contact, counseling, medical evaluation, informed consent, pre-collection donation of autologous red cell units prior to marrow harvest, as well as scheduling, logistics between transplant and donor center, medical complications and preparation of the recipient all contribute to these delays. Ongoing efforts within all cooperating registries are continuing to expedite these logistical and administrative steps. Enhancing the rapid availability of donors for patients in urgent need have gradually shortened, but not eliminated these practical delays. Aggressive expedited procedures at the NMDP have shortened the search time to only three to four weeks when necessary. Recognition of the time required for donor searching is an added element in the choices of alternative therapies and additional treatment options. This time must be considered in order to improve clinical decision making, particularly for patients with acute leukemia or other pressing clinical conditions where remissions, and thus the suitable period for transplantation, may be brief.

In 2006, the Health Resources Services Administration (HRSA) initiated recompetition for a U.S. National Bone Marrow Donor Registry, a Cord Blood Donor Registry and a Single Point of Access to facilitate initiation of donor searches. The NMDP was awarded these three contracts to establish and administer the C.W. (Bill) Young Transplant Donor Program which will provide non-family donors for patients in need.

3. Histocompatibility Matching

The highly polymorphic genes of the major histocompatibility complex defined two major classes which are relevant for donor selection. Class I antigens include HLA-A, -B and -C while Class II includes HLA-DR, -DQ and -DP antigens. Original typing techniques using allo-antisera have been replaced with

DNA-based typing as the gene products and protein structure of HLA alleles have been defined. Certain allelic polymorphisms are recognized by T cells with exquisite specificity and can elicit alloantigenic responses to alleles differing by only a single amino acid at appropriate components of an HLA epitope. HLA antigens inherited from a parent (one-half of the chromosome 6 pair) are referred to as an HLA haplotype. Haplotype structure is preserved across extended haplotypes which show positive linkage disequilibrium. Particularly within families or in racial and minority ethnic populations, linkage frequencies between specific antigens occur considerably more often than chance, thus increasing the likelihood of identifying common haplotypes even in unrelated individuals. While initial definition of a satisfactory donor suggested that serologically defined matching at HLA-A and -B and later allele level matching at HLA-DRB1 was satisfactory, newer information has suggested the importance of matching at additional loci (HLA-C, HLA-DQ and HLA-DP) and at higher resolution (matching at the allele level) [2, 10–25]. While earlier reports suggested that partial matched, unrelated donor transplantation, differing at only a single Class I antigen or a single DRB1 allele could yield satisfactory clinical results, increasing risks of graft failure and GVHD have been noted following these partial matched transplants [11, 27, 28].

Recent analyses confirm the importance of closer matching. A recent analysis from the National Marrow Donor Program confirms the importance of matching at HLA-A, -B, -C and -DRB1 to identify optimal outcome [26]. Mismatching at a single Class I or DRB1 locus led to more frequent graft failure, more frequent GVHD and poorer survival. Multiple mismatches led to even poorer results. Of greatest importance was the recognition that allele level matching at Class I, similar to allele matching at HLA-DRB1, conferred added protection against the major post-transplant complications. Even single allele mismatching may compromise survival [29] though it is important to recognize that near-complete (seven of eight) allele matching at the HLA-A, B, C, DR can yield satisfactory results and, thus, these very closely matched donors are acceptable.

Close though still only partial-matched URD transplants (mismatched at a single Class I allele or at a single DRB1 allele) lead to results nearly as good as fully matched transplants and should not be regarded as imperfect or unsatisfactory matches [19, 20, 26, 29]. Recent work has suggested that single locus mismatch at either the antigen or the allele level leads to similar and promising outcomes [29, 52, 53]. Particularly for racial and ethnic minorities, where fully matched donors may be hard to identify and available, closely matched (but only single antigen or single allele mismatched) donors may yield satisfactory survival. Transplants using serologically defined antigen level matches, particularly for adults, may yield poorer results because of unrecognized multiple allele level mismatches that may exist unless high resolution Class I and DRB1 typing is performed. Even in six antigen matched (antigen class I and allele DRβ1 matched) transplants, 24 percent of patients will have additional allele mismatches present [26].

4. Applications of Allogeneic Unrelated Donor Transplantation

Broad categories of hematologic malignancies, nonmalignant hematologic disorders, metabolic disorders, immunodeficiencies and other malignant diseases have been treated with unrelated donor transplantations (Table 13-1). While

Table 13-1. Diseases treatable with unrelated donor allogeneic hematopoietic stem cell transplantation.

Nonmalignant Diseases
Severe Aplastic Anemia
Hemoglobinopathy and Thalassemia
Immune Deficiencies
Metabolic Storage Diseases
Malignant Diseases
Acute Myeloid Leukemia
Acute Lymphoblastic Leukemia
Chronic Myelogenous Leukemia
Juvenile Myelomonocytic Leukemia
Non-Hodgkin's Lymphoma
Chronic Lymphocytic Leukemia
Multiple Myeloma

experience is greatest in chronic myelogenous leukemia and acute leukemia, other illnesses have been studied. The quality of available data is based on the disease rarity and the international experience evaluating this technique [53].

5. Clinical Results

In general, unrelated donor marrow (URD) transplantation has been performed using similar clinical approaches to HLA-identical sibling transplants including combinations of high-dose chemotherapy and/or total body radiation for pre-transplant conditioning and pharmacologic immunosuppression for Graft-versus-Host Disease (GVHD) prophylaxis after transplantation (Table 13-2). Numerous reports suggest that URD marrow transplantation yields high (90 to 95%) rates of engraftment, but slightly higher risks of graft failure compared to HLA-identical sibling donor transplantation [1, 11, 30–32]. Similarly, GVHD, peri-transplant and post-transplant infectious complications and treatment-related mortality may be substantively higher with URD transplantation compared to sibling donor transplants [11, 30–32]. Initial series reporting results of URD transplantation described disease free survival for patients with well matched donors of 40 to 60 percent for patients with favorable prognosis disease, and 20 to 35 percent for those with more advanced, high risk diseases [1, 3, 4, 7, 10]. Results were better for younger recipients, those with more closely matched URD and those with favorable, pre-transplant performance status. Recent studies suggest comparable outcomes for sibling donor and allele matched URD transplants, particularly in children [33–35].

6. New Graft Sources

Similar to experience with matching sibling donors, most (>70%) URD transplants are now performed using filgrastim-mobilized peripheral blood stem cell (PBSC) grafts [36]. This shift away from bone marrow (BM) grafts has

Table 13-2. Clinical results of unrelated donor transplantation.

Disease Author (ref)	N	Age (years) median (range)	Acute GVHD* Grade III–IV	Non-relapse mortality	Relapse*	Survival**
CML						
Weisdorf [28]	2464	36 (1–62)	matched 35%	N.R.	Chronic phase 5%	Matched 45% 5 year
			partial matched 49%		AP/BP 18%	Partial matched 31% 5 year
CLL						
Pavletic [63]	38	45 (26–57)	45% II–IV	38% 5 yr	32% 5 yr	33% 5 year
						30% DFS 5 year
Brown [72]	46 RIC	53 (35–67)	19%	17% 2 yr	48% 2 yr	54% 2 year
	URD 30					34% DFS 2 year
	Rel 16					
AML						
Sierra [7]	74	20 (1–54)	47%	39%		
	CR1/2+ 21				CR1/2 20–22%	38–58% 5 yr DFS
	Rel 53				Rel 40–70%	15–18% DFS
Hegenbart [62]	122 RIC	58 (17–74)				
	URD		42% II–IV	22%	33%	CR1 63% 2 yr
	Rel		35%	10%	47%	CR1 44% 2 yr
Michel [85]	UCB 95	4.8 (0–15)	35% II–IV	20%	Overall 29%	42% 2 yr DFS
				100 days	CR1 10%	49% 2 yr
					CR2 23%	
					Advanced 61%	
ALL						
Cornelissen [56]	127		31%	CR1 54%	6%	32% 4 yr
	CR1 64	31 (16–54)		CR2/3 75	8	17% 2 yr
	CR2/3 16	27 (17–51)		Rel 64	31	5% 2 yr
	Rel 47	36 (19–51)				
Bunin [54]	363	9 (0–19)	29%	42%	22%	36% 5 yr DFS
Weisdorf [59]	517	14	—	42%	CR1 14%	CR1 51% 3 yr
					CR2 25%	CR2 40% 3 yr
Bishop [88]	159	27 (18–51)	—	43%		
	CR1 76				CR1 15% 5 yr	
	CR2 83				CR2 26%	
Woolfrey [60]	88	9 (0–18)	matched 43%	CR1 20%	10%	CR1 70%
			partial matched 59%	CR2 22%	33%	CR2 46%
				CR3 60%	20%	CR3 20%
				Rel 41%	50%	Rel 9%

(continued)

Table 13-2. (continued)

Disease Author (ref)	N	Age (years) median (range)	Acute GVHD* Grade III–IV	Non-relapse mortality	Relapse*	Survival**
Dahlke [103]	84					
	URD 38	23 (1–60)	15%	~30%	27%	44% 3 yr
	Rel 46	mean				46% 3 yr
MDS						
Castro-Malaspina [86]	510					
		38 (1–62)	47% (II–IV)	54% (2 yr)	14% (2 yr)	29% 2 yr DFS
Arnold [57]	118	24 (0–53)	47% (II–IV)	58%	35%	28% 2 yr DFS
Disease	N	Age (years)	Acute GVHD*	Non-relapse mortality	Relapse*	Survival**
Author (ref)		median (range)	Grade III–IV			
Aplastic Anemia						
Deeg [76]	50	14 (0–46)	61% (II–IV)	—	—	58% 2 yr
Deeg [74]	141		52% (II–IV)	—	—	36% 3 yr
Kojima [75]	154	17 (1–46)	20%	—	—	56% 5 yr
Unrelated donor cord blood versus BM for acute leukemia						
Rocha [97]	UCB 99	6 (2.5–10)	22%	39% 100 day	38%	31% 2 yr DFS
	BM 442	8 (5–12)	30%	17% 100 day	44%	43% 2 yr DFS
Barker [94]***	UCB 31	6 (1–18)	19%	NR	—	53% 2 yr survival
	BM 31	7 (0–17)	8%	NR	—	41% 2 yr survival
Eapen [34]	UCB (85)	9 (0–18) months		31%	CR1 UCB/URD 24%	
	URD BM 81			15%	Sib 47%	54% 3 yr DFS
	Sib BM 81			6%	Advanced CR UCB/URD 45% Sib 65%	49% 3 yr DFS

* Cumulative incidence or Kaplan-Meier incidence
** Survival or DFS = Disease free survival
*** Not all acute leukemia

CP= chronic phase; AP/BP= accelerated phase/blast phase; CR= complete remission; USB= Umbilical cord blood; BM=bone marrow; RIC= reduced intensity conditioning

developed with little or no data supporting the superiority of URD PBSC. Concern about more frequent acute and particularly GVHD may follow PBSC grafts. A recent analysis suggests similar outcomes of URD BM and PBSC grafts [37]. An ongoing prospective randomized trial (Blood and Marrow Transplant Clinical Trials Network 0201) is directly addressing the comparative safety and efficacy of either URD graft source.

7. Umbilical Cord Blood Grafts

Unrelated donor umbilical cord blood (UCB) grafts are increasingly used as a hematopoietic graft source [38]. The greater proliferative potential and immunologic naiveté of UCB cells allow engraftment with a low cell dose (typically 1/5 – 1/10 of a BM or PBSC graft) and tolerable mismatch without overwhelming GVHD. This extends the unrelated donor pool to ethnic and racial minorities with uncommon HLA phenotypes who often fail to find a suitably matched volunteer donor.

UCB grafting is now widely used in children whose smaller body size easily permits a UCB graft >2–3 $\times$ 10^7 cells/kg, a minimum effective cell dose to expect prompt and reliable engraftment. For older children or adults, the limited cell dose provides a major barrier. Newer techniques including two unit grafts have significantly reduced the risks of graft failure (to < 10%) and lessened TRM to < 30 percent at six months [38].

Comparative analysis of UCB versus either matched or partially matched URD transplantation in both children and adults shows that suitably sized and four or five of six HLA locus matched UCB grafts yield engraftment, GVHD and survival rates superior to partially matched URD and similar to matched URD transplants [34]. Clearer data on UCB selection criteria, optimal GVHD prophylaxis and supportive care may improve UCB grafting outcomes even further.

Double cord grafts can substantially improve rates and speed of neutrophil and platelet recovery, especially in adults who may receive only marginally sized single UCB units (100). Intriguingly, data from the University of Minnesota suggests a more potent Graft-versus-Leukemia Effect with significantly reduced risks of relapse observed in early stage leukemia patients receiving two unit UCB grafts. This finding will be prospectively tested in pediatric leukemia patients with an available large UCB graft who will randomly be assigned to receive a second, well matched UCB unit or not (BMT CTN protocol #0501).

8. Complications of Unrelated Donor Transplant

8.1. Graft Failure

Using even well matched URD, primary graft failure of 1 to 7 percent, and secondary graft failure of 3 to 5 percent have been reported [11, 27, 28]. Closer HLA matching, a higher nucleated cell dose and perhaps importantly, a graft containing a higher dose of CD34 positive cells often from PBSC [37] may all be associated with rapid and sustained engraftment following URD transplantation [7, 39]. Acute and chronic GVHD have been more frequent and possibly more therapy resistant in recipients of URD transplantation, compared to those receiving sibling donor transplants [1, 11, 30–32, 39, 40]. Acute GVHD rates of 40 to 90 percent and chronic GVHD rates of 50 to 80 percent have been described. Chronic GVHD may be more frequent and prolonged after PBSC transplantation [37]. Lower risks of GVHD have been observed with closer HLA matching, T-lymphocyte depletion of the donor graft, nonalloimmune (via pregnancy or transfusion) donors, younger recipients and, intriguingly, using younger donors as well [41].

9. Infectious Morbidity

Severe early and late infections have been recognized more commonly after URD transplantation. Only limited formal studies of their immune reconstitution have been reported [42–44]. The greater incidence of acute and chronic GVHD, as well as functionally delayed immune reconstitution following URD transplantation, may necessitate more intensive and extended antibacterial, antiviral and antifungal prophylaxis.

10. Immune Reconstitution

Immune recognition and protective immune response against foreign microbial peptides is most efficiently initiated when presented by self HLA, but not by allogeneic HLA molecules. This HLA-restricted interaction between antigen-presenting cells (APC), naïve T cells and effector immune cells (B lineage and T lineage) may be confounded in recipients of URD transplantation. Donor marrow-derived T cells emanating from the transplanted hematopoietic stem cells must be educated and directed to interact with host APCs, initially by host dendritic cells, and possibly by residual host thymic elements. Disrupting host antigen-presenting function and thymic epithelium by chemotherapy and radiation conditioning regimen may further deplete the capacity for this host and donor T cell interaction, thereby compromising or delaying development of an effective multiparameter immune defense against infection. Further, the development of GVHD and its necessary immunosuppressive therapy prolongs the immunoincompetence of the transplant recipient. Development of antibody diversity, total antibody production, CD4 T cell numbers and effective T cell recognition, proliferation and memory to mount an appropriate immune response may be delayed [42–44]. It takes up to three to six months after transplantation for antibody development and six to 12 months for an effective T cell response. Donor/recipient HLA disparity, clinical GVHD and extended immunosuppressive therapy may extend this recovery interval even further. Recent clinical recognition of late infections, both viral and fungal, even in the non-neutropenic host underscores the importance of this delayed immune reconstitution which is more profound and longer in the URD recipient [45, 46]. Extended infectious disease prophylaxis and ongoing surveillance for opportunistic infection (particularly cytomegalovirus, fungi and *Pneumocystis carinii*) is required.

Recognizing later post-transplant infections may be particularly important in URD recipients. One report from the University of Minnesota identified more frequent late infections, even in unrelated donor recipients without GVHD, compared to recipients of HLA-matched sibling donor transplantation [46]. Comparison of infections after UCB or URD BM or PBSC grafts have noted no major differences in post-transplant infections [47–49]. A pediatric report from Minnesota showed equivalent risks of bacterial, fungal or serious viral infections [47]. A similar report from Barcelona included adult UCB recipients and noted earlier, but not more frequent, CMV infections and overall similar infectious morbidity [49]. EBV infections may increase after UCB transplants, particularly with ATG [50].

11. Protection Against Relapse

The greater donor/host disparity which might augment hazards of GVHD may, in turn, promote a more powerful Graft-versus-Leukemia (GVL) response and better protection against relapse. Clinically demonstrable GVL appears most frequent in those with clinically recognized GVHD, particularly chronic GVHD. Some series have corroborated the comparison of post-transplant relapse rates in sibling versus URD recipients [11, 28, 30–32, 51]. After adjusting GVHD incidence and severity, there was some additionaly protection against relapse after URD transplantation. This may vary in disease settings which express inherently different sensitivity to the immunologically based GVL effect [54–63]. Chronic myelogenous leukemia is most sensitive to GVL and experience to date suggests a particularly low rate of relapse following URD transplantation, at least for transplants performed in the early chronic phase [6, 28, 31, 40, 64]. More advanced CML, ALL and other aggressive malignancies may be less well contained by the URD GVL effect. Notably, however, URD lymphocyte infusions have demonstrable efficacy in inducing durable remissions when infused following relapse after URD BMT [65].

12. Reduced Intensity Conditioning

Similar to broadly developing experience using matched sibling donors, over the last decade increasing numbers of patients have received URD (and some UCB) transplants following nonmyeloablative or reduced intensity conditioning (RIC) [45, 62, 66, 67, 68]. These RIC regimens are modified to be sufficiently immunosuppressive to permit engraftment, but less toxic to lessen multi-organ injury and TRM. RIC regimens were originally used for older patients (> 45 or 50 years), heavily treated (such as a prior autologous transplant) or patients with serious comorbidities such as compromised hepatic or pulmonary function or recent opportunistic infections. They have proven to be modestly safer, but are still accompanied by similar risks of acute and chronic GVHD and variable TRM. They have extended URD and UCB transplantation to an older population most often diagnosed with either AML, lymphomas or plasma cell disorders treatable by allotransplantation [68–73].

Early analyses have demonstrated reasonable TRM and survival in this older and sicker population, but the comparative anti-neoplastic potency of RIC regimens cannot be fairly compared against conventional myeloablative regimens not usually used in this population. Prospective testing in both single and multicenter trials is underway to better define the safety and best applicability of these RIC approaches. An early report of over 200 cases from the NMDP described TRM at six months of 30 percent in patients with a median age of 50 [67]. Three-year survival was 30 to 35 percent in URD RIC recipients with little difference noted with differing diagnoses, regimens or patient subgroups. Another large Minnesota series reported 110 UCB recipients of RIC conditioning with six-month TRM of 25 percent and three-year survival of 40 to 45 percent in similar-aged patients with advanced hematologic malignancies [68]. Further experience with differing regimens and graft types are needed to understand how and when RIC URD or UCB transplants should be done.

13. Survival After Transplantation

13.1. Aplastic Anemia and Nonmalignant Disease

While most patients with aplastic anemia initially receive immunosuppressive therapy, URD transplantation can yield encouraging results and eradication of their aplasia for a sizeable fraction of patients (Table 13-2). In recent reports, 40 to 50 percent of patients survive after URD transplantation, offering encouraging options for those who fail initial immunosuppressive therapy [74, 75]. Modification of conditioning regimens may reduce the peri-transplant toxicity even further and increase the success of such treatment [76]. Severe childhood immunodeficiencies can be well contained after transplantation, particularly classical severe combined immunodeficiency (SCID), while variant immunodeficiency states have been successfully treated as well [5, 44, 77]. Inherited metabolic disorders [78, 79], hemoglobinopathies, thalassemia [80] and Fanconi's Anemia [81] have sometimes been treated with URD transplantation although difficulties with sustained engraftment, hazards of GVHD and peri-transplant morbidity and mortality have limited more broad application of URD transplantation for these disorders.

14. Acute Myeloid Leukemia

Paralleling the successes of sibling donor transplants for acute leukemia, patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) have been successfully treated with URD transplantation [7, 39, 55, 61, 62, 82, 83]. AML patients in second complete remission (CR2) have been regularly treated with well matched URD and 30 to 40 percent of adults enjoy long-term leukemia-free survival (Fig. 13-1). Outcomes in children are more favorable with 40 to 60 percent of patients alive many years post-transplantation. For patients with AML in CR1 with high risk characteristics,

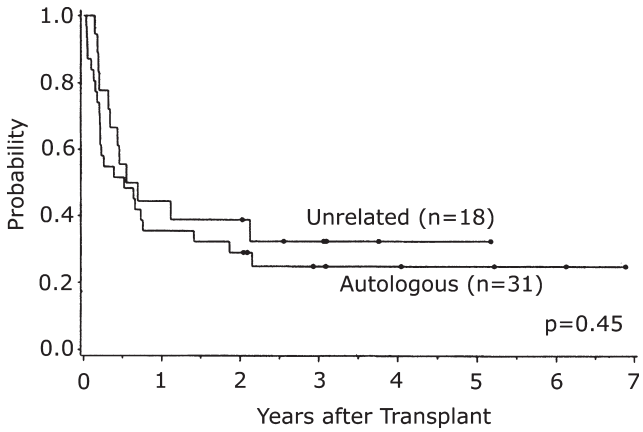

Fig. 13-1. Survival after URD BMT for AML Comparison with autologous transplantation is shown. Adapted from Lazarus, et al. [84]

autologous transplantation or ongoing consolidation and maintenance chemotherapy have proven to be useful. A recent analysis comparing autologous transplantation from the Center for International Blood and Marrow Transplant Research (CIBMTR) to multicenter experience from the NMDP suggests a modest survival advantage for autotransplants both in first and second remission, compared to URD BMT [84]. The excessive morbidity and mortality accompanying GVHD and post-transplant infection overcame the profoundly better protection against relapse accompanying the URD transplants. Newer advances encompassing better donor selection, modifications of GVHD prevention and G-CSF mobilized peripheral blood stem cells may enhance the outcomes of URD transplantation and require revisiting comparisons with autografts in both adults and children [85].

Myelodysplastic syndromes (MDS) have also been treated with URD transplantation [57, 86]. Post-transplant relapse rates are low (14%), but, disappointingly, transplant related mortality is high (54%). Overall 29 percent five-year disease free survival was reported in one large series from the NMDP [86].

15. Acute Lymphoblastic Leukemia

In ALL, similar URD transplant experience has been extensively analyzed [8, 30, 32, 54, 56, 59, 60, 87, 88]. While high risk ALL (e.g., t(4;11), t(9;22)), extreme leukocytosis or mature B cell ALL have inadequate results following conventional, even aggressive chemotherapy, allotransplantation from URD can protect against relapse and yield extended leukemia-free survival for a sizeable fraction of children and a modest number of adults. Several series of Ph^+ALL report 40 to 50 percent extended disease free survival after URD transplantation. For patients lacking a sibling donor this is now recognized as the treatment of choice [56, 87, 88].

Autotransplantation, less widely used for ALL than for AML, has also been contrasted with URD transplantation for patients lacking sibling donors. An earlier report from the University of Minnesota and Dana Farber Cancer Institute compared autografts to URD experience through the NMDP [8]. In that analysis, similar outcomes were observed for most patients. Adjusted multivariate analysis could not define a particular cohort definitively benefiting from the allogeneic approach. A more recent report comparing ABMTR autograft experience versus NMDP URD transplants demonstrated superior disease free survival for standard risk ALL in second or later remission compared to autotransplantation (Fig. 13-2) [59]. Disappointingly, high risk ALL (short initial remission, WBC > 50,000/μl at diagnosis) was not better protected against relapse by the URD allograft. For this group, survival was unsatisfactory after both URD BMT or autotransplantation. The NMDP has reported that children and adults with high risk ALL have satisfactory outcomes after URD transplantation [38, 40, 44]. Thirty-six to 46 percent of children and 20 to 40 percent of adults with high risk features are alive without relapse more than three years following transplantation. As mentioned above, improvements in URD selection and peri-transplant management may yield greater advances in their outcome. A recent similar analysis in adult ALL recipients identified superior survival after URD BMT in CR2, but similar outcomes for patients in CR1 [88].

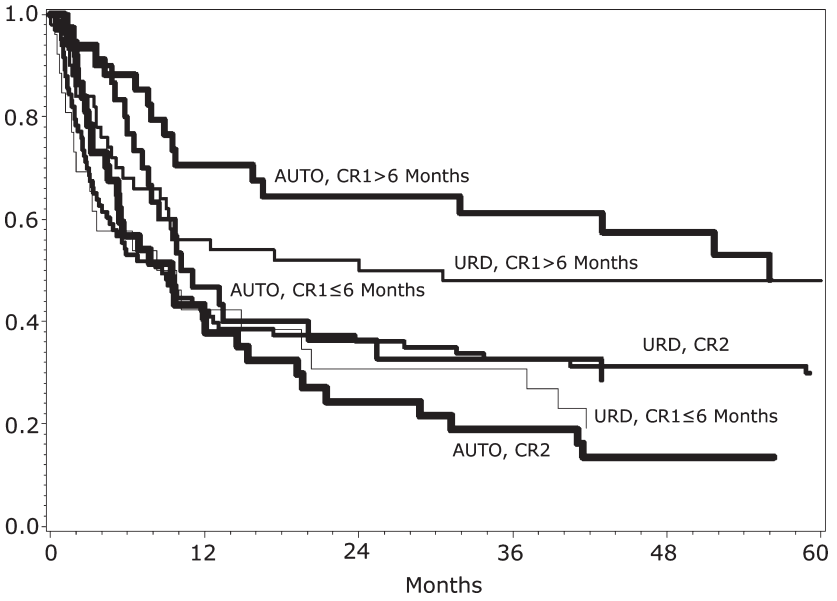

Fig. 13-2. Kaplan-Meier of overall survival based on donor type, remission status and time from achieving first complete remission Adapted from Bishop [88]

16. Chronic Myelogenous Leukemia

Before imatinib (Gleevec) changed the initial management strategies for nearly all patients with CML, URD transplantation was the only curative option for those lacking a matched sibling donor [6, 28, 40]. Decision analyses suggested a survival advantage for URD transplantation compared to extended interferon therapy [89] and some reports recognized the adverse impact of pre-transplant interferon on the outcome of URD BMT [90–92]. Numerous series have documented the efficacy of URD BMT for treatment of CML, particularly in the chronic phase [6, 28, 40, 64], and cost analyses have supported its cost-effectiveness [93]. One report from the NMDP identified 63 percent of young, early chronic phase patients surviving leukemia-free more than three years post-transplantation [6]. Recent comparisons of URD to sibling transplantation suggested slightly, though statistically significant superior survival for recipients of sibling donor BMT, compared to URD BMT [28, 31]. Importantly, the well recognized hazard of delay in time to transplantation exacted a greater reduction in survival after URD transplantation than sibling donor BMT for patients in several age cohorts [28]. As shown (Fig. 13-3), a one to two year delay one to two post-diagnosis had little impact on survival after sibling donor transplantation while 8 percent and 10 percent fewer patients survived with one and two+ years delay for recipients of unrelated donor transplantation. The pathophysiology of this added hazard is uncertain. Young patients, expected to have a high clinical response rate to imatinib therapy, might similarly expect 60 percent five-year leukemia-free survival after

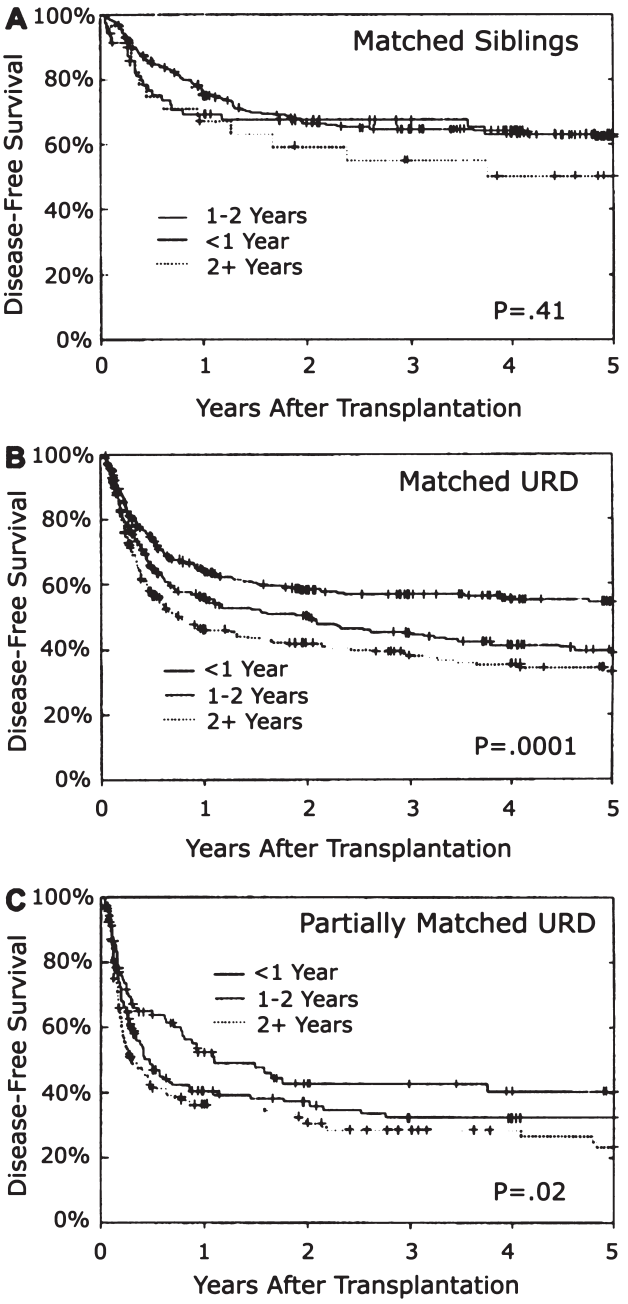

Fig. 13-3. Five-year disease free survival after sibling versus URD BMT for CML in chronic phase: poorer outcome with delayed URD transplantation Adapted from Weisdorf, et al. [28]

URD BMT if a well matched URD donor is identified and the transplant is performed in early chronic phase. Patients aged 30 to 40, or older than 40, can expect slightly poorer outcomes (40 to 50% five-year leukemia-free survival after URD BMT) and may better accept the uncertainties of delay

in contrast to the immediate hazards of an early transplant. Since recent updates document nearly 90 percent durability of response to imatinib at five years, any allogeneic transplants are now restricted to patients who fail to achieve or maintain complete hematologic and cytogenetic responses to imatinib. The clinical impact of pre-transplant imatinib on allotransplants for patients with CML is unknown as relatively few patients have failed therapy and proceeded to URD transplant while still in chronic phase. Cautious and careful analyses of these patients' outcomes is required to guide the decision making for asymptomatic, but imatinib-resistant patients with CML who have no sibling donor.

17. Future Modifications

Overall, peri-transplant and non-relapse mortality is substantially higher following URD transplant compared to sibling donor approaches. For good risk patients with early disease, non-relapse mortality of 20 to 40 percent has been described, but for patients with diagnoses other than CML, many series report non-relapse mortality attributable to the complications of URD transplant up to 40 to 50 percent. Improved donor-recipient matching leads to lesser TRM. Substantive advances in GVHD prophylaxis and management, interventions to facilitate and accelerate immune reconstitution plus more effective and longer duration infectious disease prophylaxis will be required to reduce these hazards and improve the safety of URD transplantation overall. Modified and safer reduced-intensity conditioning regimens may lessen TRM and preserve the potent anti-neoplastic effects of allotransplantation.

Changes in donor selection criteria to identify better matched, and potentially more suitable URD requires more study and more clinical experience. Defining the optimal histocompatibility criteria for matching may only identify a smaller cohort of patients able to enjoy the benefits of a well matched donor while underscoring or actually increasing the minority populations inadequately aided by the donor search and identification process of the worldwide donor registries [29 ,52, 53]. New advances in UCB transplants, which permit safe transplantation across greater histocompatibility barriers and tolerating even two HLA antigen differences, require ongoing experience, particularly for larger children and adult recipients [68, 94–100]. Application of URD transplantation using G-CSF mobilized peripheral blood might realize the same advantages accompanying sibling donor peripheral blood stem cell allotransplantation [37, 101, 102], yet the uncertainty of chronic GVHD risks and the accompanying extended morbidity are inadequately understood and represent a major obstacle to more broad application of URD blood stem cell transplantation. Continued prospective testing of PBSC from URD is essential, especially to quantitatively appreciate the risks of later chronic GVHD-associated morbidity.

Unrelated donor transplantation is a fabulous clinical experiment. It taps the wealth of generosity manifested in the donation of hematopoietic stem cells from unrelated and anonymous distant volunteers and has been lifesaving for many and hope-sustaining for an even greater number still searching for a donor. Advances in the clinical science will broaden these opportunities and enrich the outcomes of many more patients in years to come.

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Chapter 14

Umbilical Cord Hematopoietic Stem Cell Transplantation

Mitchell E. Horwitz and Nelson Chao

1. Introduction

Umbilical cord blood (UCB) is now well accepted as a viable source of hematopoietic stem cells for allogeneic stem cell transplantation [1–3]. Since the first related donor UCB transplant for Fanconi anemia in 1988, and the first successful unrelated donor UCB transplant in 1993, over 8,000 patients have undergone UCB transplantation [4]. Today, this approach is being applied to patients of all ages with a variety of diseases, ranging from nonmalignant hematologic disorders, enzyme deficiencies and congenital metabolic disorders, as well as hematologic malignancies.

Cord blood was first investigated as a potential stem cell source due to the lack of suitably matched related donors for patients who could benefit from allogeneic transplantation. Unfortunately, only 25 to 30 percent of potential recipients have an HLA-identical sibling who can participate as donors. Therefore, the National Marrow Donor Program (NMDP) was established in 1986 to facilitate the search and procurement of stem cells from unrelated donors for patients who lack a matched sibling [5]. Despite an international volunteer donor registry that exceeds 10 million people, less than half of the transplant candidates find a suitably matched unrelated donor. For patients of African, Asian, Native American or Hispanic descent, the success rate is even lower. Since 1993, UCB transplantation from unrelated donors has increased considerably. As of 2004, the Center for International Blood and Marrow Transplant Research found that 34 percent of transplant recipients under age 21 received UCB grafts. In contrast, only 4 percent of adult transplant recipients received stem cells derived from UCB. A number of factors have contributed to this increased popularity. The most important factor, which will be detailed in this chapter, is that patient outcomes continue to improve each year. Furthermore, the cord blood banking infrastructure has improved, significantly increasing the availability of partially matched, high quality UCB grafts. Finally, in comparison to stem cell grafts obtained from unrelated adult donors, UCB stem cells can be procured quicker and with no inconvenience or risk to the donor. Combine these features with the fact that stringent HLA matching with the recipient is not a prerequisite for successful outcome, and it is clear why so

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many investigators have committed themselves to optimizing this approach to allogeneic stem cell transplantation.

2. Cord Blood Banking

2.1. UCB Banks and Registries

Supporting the burgeoning demand for high quality cord blood units is a complex network of collaborative public and private cord blood banks. Transplant centers rely upon these facilities to collect, characterize, cryopreserve and ship the grafts. These banks are linked together by searchable registries that collect data on the grafts and make this data available to the transplant centers. Established in 1997, the NETCORD foundation, headquartered in Leiden, Netherlands, was the first organization to establish standards for cord blood collection and storage for their participating banks. The NETCORD database located in Germany currently lists 115,000 cord blood units from the inventories of 23 cord blood banks in 16 countries. The NMDP, established in 1986 as a registry for volunteer unrelated stem cell donors, has more recently become the dominant registry for cord blood banks in North America. Today, the NMDP's Center for Cord Blood offers a listing of approximately 50,000 units from 16 banks in the United States. In late 2006, NETCORD and the NMDP formed a partnership to help streamline the search process for the transplant centers, allowing for a single portal of access to what is now over 165,000 cord blood units. Furthermore, the partnership establishes more uniform standards to which the individual banks must adhere in order to remain networked with the large registry.

Separate from the NETCORD and the NMDP registries is the National Cord Blood Program of the New York Blood Center. The New York Blood Center's cord blood bank is the largest and oldest public cord blood bank, housing nearly 30,000 cord blood units. At present, transplant centers must undertake a separate search of this bank to gain access to these grafts. Finally, there are numerous smaller cord blood registries scattered throughout the world providing access to independent, for-profit cord blood banks.

In 2006, the Health Resources and Services Administration, a part of the United States Department of Health and Human Services, awarded $12 million to the first group of cord blood banks who will begin collections for the National Cord Blood Inventory (NCBI). The initial goal for the NCBI is to add 150,000 new high quality units to the nation's current registry. Of particular interest is assuring that these units are collected from a diverse population of donors. This award is a component of the larger $50 million C.W. Bill Young Cell Transplantation program aimed at improving the science of stem cell transplantation.

2.2. Practical Issues Surrounding Cord Blood Collection

2.2.1. Ethical Consideration

UCB as an alternative source for allogeneic stem cell transplantation has generated several ethical concerns [6]. In 1997, a working group composed of experts in medicine, blood banking, law, ethics, anthropology and social sciences convened to examine issues such as: 1) questions about ownership of the tissue, 2) the necessity and nature of informed consent from parents who are approached regarding donation of their child's UCB and 3) obligation

to inform the parents of serologic and infectious disease testing results for the donated product [7, 8]. A detailed discussion of these ethical issues goes beyond the scope of this review. However, a detailed description of the collection process, including procurement of informed consent and dissemination of testing information, will be outlined in the subsequent sections.

2.2.2. Informed Consent

Informed consent is routinely a prerequisite for the procedures of UCB banking. Information regarding the risks and benefits of the collection, storage and potential uses of UCB are explained to parents of prospective donors. By convention, the mother must consent by proxy as UCB is technically of fetal origin. Cord blood banks affiliated with the NMDP typically approach the mother before or shortly after collection of the UCB. First and foremost, it is explained that donation of the cord blood is voluntary, confidential and does not result in compensation. Once consent is provided, a history of maternal and family health is obtained. In addition, a behavioral and travel history is obtained to determine the likelihood of exposure to dangerous infectious agents. A sample of maternal blood is obtained for infectious disease testing along with sterility and tissue typing (described below). Parents are informed that they will be notified if the maternal or cord blood testing reveals information that would be important for the well-being of the child.

2.2.3. Collection

Maximizing the yield of UCB stem cells while maintaining sterility are the critical features of the cord blood collection process. This must be done without interfering with the normal activities of the obstetric staff. Blood can be extracted from the placenta after delivery of the baby, but before the placenta has been delivered, or it can be extracted after the placenta has been delivered. In both cases, the blood is aspirated from the umbilical cord vein using a large bore needle. A large, retrospective multicenter analysis of five programs established by the American Red Cross Cord Blood Program concluded that there was no significant difference in nucleated cell recovery using the *in utero* or *ex utero* methods of collection [9]. Factors which have been shown to influence the CD34+ cell content and colony forming units of granulocytes-macrophages (CFU-GM) content include gestational age, length of labor, time of cord blood clamping after delivery, birth weight and birth order. Interestingly, more advanced gestational age increases CD34+ cell content until 37 weeks, after which time the CD34+ content decreases [10, 11].

2.2.4. Processing and Storage

In 1995, the National Heart, Lung and Blood Institute funded three UCB banks at six transplant centers to assist in establishing standard operating procedures (SOP) for the collection, processing and storage of cord blood [12]. While there is some degree of variability among the individual cord blood banks in the SOP, the steps described in this chapter are in keeping with the standards mandated by the National Institutes of Health and the National Marrow Donor Program.

To meet criteria for processing, the unit volume must be at least 40 milliliters, contain greater than 1×10^9 cells and be adequately anticoagulated. The unit must be processed within 48 hours of collection. Most cord blood banks now perform a red cell and plasma depletion step as part of the preparation for cryopreservation. The advantages of this step include less freezer space required

for storage, and less infusional toxicity to particularly small recipients where the red cell dose would be clinically significant. Red cell depletion is facilitated by adding 6 percent hydroxyethyl starch to enhance red cell sedimentation. Following a gentle spin, the red cell depleted graft is isolated. Following a second spin, the nucleated cell fraction is re-suspended in approximately 30 milliliters of autologous plasma containing 10 percent DMSO. The unit is then transferred to a freezer bag in preparation for cryopreservation. The most commonly used freezer bags provide at least two detachable segments of cell product that can be collected without perturbation of the remainder of the unit. These segments are used for confirmatory typing and additional testing. Freezing occurs at a controlled rate. When complete, the unit is transferred to a liquid nitrogen freezer where it may be housed for several years [13, 14].

Preparation of a frozen UCB unit for infusion is similar to the procedure used for peripheral blood stem cell units. The unit is placed in a sterile, sealable plastic bath, submerged, and gently agitated in a 37° C water bath. When thawed, equal volumes of 10 percent dextran and 5 percent albumin are added and, depending on the institution practice, the unit may be washed and re-suspended in the same solution, or directly infused into the patient.

2.2.5. Tissue Typing and Safety Testing

Since both the infant and mother should be considered the donor it is important to obtain a medical history from both. This includes assessing the family for a history of rare genetic disorders, as well as documenting newborn medical screening for disorders such as sickle cell anemia, galactosemia, phenylketonuria (PKU) and other metabolic disorders. Prior to cryopreservation, a battery of testing is performed on all cord blood units and the results are entered into the database (Table 14-1). In an attempt to standardize the procedure for testing of all cord blood units before cryopreservation, the American Association of Blood Banks and the Foundation for Accreditation of Cellular Therapy (FACT-NETCORD) have independently produced documents outlining these guidelines [15, 16]. HLA typing is typically performed from aliquots of cells taken from the granulocyte/red cell enriched pellets from the red cell depletion step of processing. Low resolution class I typing and high resolution, allele level class II typing is performed at a central laboratory and then entered into the database. Infectious disease testing is done from a sample of maternal blood that is collected at the time of delivery.

Infectious organisms can be transferred to recipients of cord blood. The risk of congenital infections depends on the prevalence and incidence in the pregnant women and the variability of the placental barrier to allow the fetus, and thus the cord blood, to pass the infectious agent. The congenital infections that can be passed from mother to infant are vast, and include such agents as human immunodeficiency virus (HIV), cytomegalovirus (CMV), syphilis and rubella. The data regarding transmissibility of these infectious agents vary with subpopulations, but general risks are known. HIV has a prevalence of transmission to the fetus in up to 30 percent of maternal patients not treated with antiretroviral drugs. Primary toxoplasmosis and rubella infections have also been widely known to cross the placental barrier and infect the fetus, causing numerous congenital anomalies. Infections such as hepatitis B, herpes simplex and varicella zoster rarely cross the placenta during gestation, but rather are acquired perinatally. Additionally, during vaginal delivery the umbilical cord and placenta come in contact with vaginal, cervical and perineal skin which

Table 14-1. Common tests performed on UCB.

ABO and Rh Blood groups and types
Antibody Screen
Alanine Aminotransferase
Alanine Aminotransferase in International Units
Cholesterol Level
Cytomegalovirus (CMV) antibody
Hepatitis B Core Antibody
Hemoglobin S (hemoglobin electropheresis)
Hepatitis B Surface Antigen
Hepatitis C Virus Antibody
Human Immunodeficiency Virus ½ combo test
Human Immunodeficiency Virus-1 Antigen p24
Human T-Lymphotropic Virus Type I and Type II
Nucleic Acid Test for HIV-1 and HCV
Serological Test for Syphilis
Bacterial Culture/Contamination

could be sources of contamination with infectious agents such as Candida Albicans, a potentially dangerous pathogen in transplant patients.

A consensus approach to CMV screening of the cord blood graft has yet to be determined. Although the congenital CMV infection rate is low (0.5% to 2% of U.S. births), many cord blood units will test positive for CMV IgG due to transmission of maternal antibodies across the placental barrier [17]. In a retrospective review of 1,221 cord blood transplant recipients, Albano and colleagues found that post-transplant CMV infection was associated with patient pre-transplantation CMV serology, but not cord blood or maternal CMV serologic status [18]. Thus, the focus has been to develop a standardized, polymerase chain reaction (PCR)-based cord blood screening strategy as a means of identifying grafts that come from an infant that has a true congenital CMV infection. Such strategies have yet to be universally instituted.

HIV infection can be transmitted to the fetus in up to one-third of infected mothers not treated with antiretroviral medications. HIV infection can be acquired by the fetus in numerous ways, including perinatally, during fetal life by vertical transmission, or by breast-feeding. Serologic testing could identify most infants at risk, except in mothers with early HIV infection who have not yet developed antibodies. This necessitates testing of maternal blood using PCR for HIV, which can detect infection earlier in the process.

Hepatitis B infections are usually transmitted perinatally to the infant. The risk of infection depends on maternal viremia, particularly at delivery. The Hepatitis B surface antigen does not to cross the placental barrier. Additionally, accurate testing of the fetus for Hepatitis B is currently not readily available, making the testing of the mother necessary. Hepatitis C infection is a known complication of bone marrow transplantation. The route of transmission of the HCV to the infant has not clearly been elucidated. Seroconversion during the first year of life suggests most infections are acquired perinatally or postnatally. This, again, makes the testing of the mother necessary.

2.2.6. Testing Genetic and Metabolic Disorders

Prenatal testing using chorionic villus sampling or amniocentesis is available for prenatal diagnosis of genetic disorders. These include metabolic storage diseases, combined immunodeficiencies, thalassemia, sickle cell, cystic fibrosis and fragile X. However, it is not appropriate to perform such procedures for the sole purpose of cord blood harvest. Other possibilities for obtaining this information include state newborn screening tests, which are routinely done on newborns in all 50 states. However, these screening tests would require consent from the parents to obtain for future use of cord blood. Additionally, not all states screen for the same disorders, making information obtained through these sources incomplete. Testing on the newborn several months after birth may be necessary, yet many parents may be reluctant to have blood testing performed on their baby. Another strategy to obtain this information is to retain DNA from the original cord blood for testing when a match has been found. This manner of screening could minimize costs to the cord bank. The costs of such testing could add substantial cost to the transplantation, which the blood bank may be unwilling to absorb. Also, if any genetic or metabolic testing is to be done it would be necessary to obtain the mother's consent again, maintain records of the mother and infant's identities and notify the parents of any abnormal test results. This could add a great deal of labor to the processing of cord blood.

2.2.7. Cellular Contents of UCB

Flow cytometric analysis of UCB reveals a cellular composition that is quite distinct from bone marrow and peripheral blood stem cell grafts [19, 20]. The absolute number of pluripotent hematopoietic stem cells is significantly reduced in UCB units, compared to bone marrow or peripheral blood grafts. A mean of 0.21×10^6 cells/UCB unit represents approximately 10 percent of the nucleated cellular content of other sources. However, the frequency of primitive hematopoietic progenitor cells is considerably higher (UCB 3.39%, BM 1.47%, PB 1.82%). As would be expected given the low cell dose, the number of lymphocytes in a cord blood graft is considerably smaller than a peripheral blood or bone marrow graft. The frequency of lymphocytes in umbilical cord blood is approximately 18 percent, which is similar to bone marrow, but approximately twice that of a G-CSF-mobilized peripheral blood stem cell graft. Approximately 60 percent of the nucleated cells in cord blood are T cells. This is similar to the T cell representation in peripheral blood and bone marrow stem cell grafts. The major distinguishing factor of the cellular content of umbilical cord blood is the higher frequency of naïve, CD45RA+CD45RO− CD4+ and CD8+ T cells. There is also a higher percentage of NK cells in UCB compared to bone marrow and peripheral blood (24%, 15% and 9%, respectively).

There is evidence that maternal cells contaminate a significant proportion of UCB grafts. Using a highly sensitive PCR assay, Petit and colleagues found evidence of maternal cells in 10 of 47 units tested. In those units that were contaminated, maternal cells were found at a frequency of 1 in 10,000 to 1 in 100,000 cord blood cells [21]. The immunologic implications of maternal blood contamination to the UCB transplant recipients is unclear. However, it does increase the risk of transmitting viral infections carried by the mother to both the child and the transplant recipient.

3. Clinical Use of UCB Stem Cell Grafts for Transplantation

The 1988 report of successful engraftment and outcome of a patient with Fanconi anemia who was transplanted with HLA-identical sibling cord blood generated considerable interest in further development of the transplant approach [3]. From 1988 until 1993, UCB transplants were limited to grafts collected from HLA-identical related donors. This early experience confirmed the pre-clinical observation that contained within the UCB graft were true pleuripotent long-term repopulating cells. What also became apparent from the early experience was that the GVHD causing potential of HLA-matched related cord blood T cells was less than what had been observed from similarly matched bone marrow grafts [22, 23]. The encouraging results in matched related donor cord blood transplantation prompted Kurtzberg and colleagues to perform the first mismatched cord blood transplantation [24]. This series of three patients and the larger series reported by Wagner and colleagues were notable for the engraftment potential and low GVHD potential of these unrelated, cryopreserved grafts [24, 25]. Subsequent to these early reports, UCB banks began to emerge and international registries in Europe and North American began accruing valuable outcome data. The result was a considerable increase in the use of unrelated, partially matched grafts, with relative stability of the use of HLA-identical sibling cord blood grafts for transplantation. Due to the limited number of stem cells contained within the cord blood graft, early experience was limited primarily to children.

3.1. Selecting the Appropriate Candidate for UCB Transplantation; Comparison with Peripheral Blood and Bone Marrow-Derived Stem Cells

All inherited genetic disease types that have proven to be curable with allogeneic stem cells collected from either peripheral blood or bone marrow can be treated with UCB stem cells as well (Table 14-2). There is emerging, albeit controversial preclinical and early clinical evidence that bone marrow or cord blood grafts contain a population of pluripotent stem cells capable of differentation into non-hematopoietic tissue [26–30]. It is hypothesized that UCB contains a higher titer than these primitive cells. If true, UCB transplantation could prove to be an effective treatment for a broad array of congenital and acquired nonmalignant conditions. These studies are ongoing.

Early on it was recognized that cell dose, as measured by nucleated cells per recipient body weight, along with HLA compatibility were the most critical characteristics of the graft that predicted a successful outcome (Fig. 14-1) [31]. Due to the importance of cell dose, the majority of UCB transplants have been, and continue to be, performed on pediatric recipients. This transplant approach has proven to be feasible for adult patients; however, according to the Center for International Blood and Marrow Transplant Research (CIBMTR), the number of adult cord blood transplants was unchanged between 1997 and 2004. For children and adults, a sibling donor who matches completely at the HLA class I, (A, B and C loci) as well as the class II (DRß1 locus) remains the donor of choice for allogeneic stem cell transplantation. Most would agree that a sibling donor who is a single antigen mismatch would also be preferable

Table 14-2. Relevant genetic diseases.

Hemoglobinopathies
Sickle Cell Anemia
Thalassemias
Erythrocyte Enzyme Deficiencies
G6PD deficiency
Adenosine Deaminase (ADA) Deficiency
Dihydrofolate reductase deficiency
Pyruvate Kinase Deficiency
Formamino Transferase Deficiency
Congenital Anemias
Fanconi's Anemia
Dyserythropoietic syndromes
Rh-null Disease
Congenital Immunologic Defects
Severe Combined Immunodeficiency
ADA deficiency
Wiskott-Aldrich Syndrome
X-linked Lymphoproliferative Disorder
Leukocyte adhesion defects
Glycogen Storage Disorders
Hurler Syndrome
Hunter Syndrome
Aplastic anemia
Adrenoleukodystrophies
Metachromatic Leukodystrophy
Infantile Leukodystrophy
Juvenile Leukodystrophy

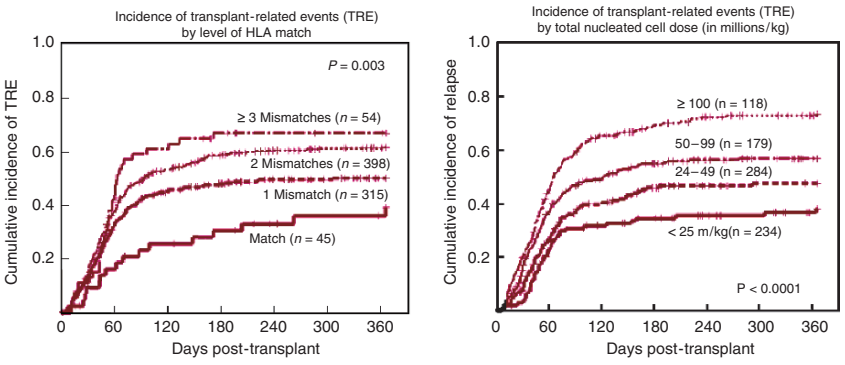

Fig. 14-1. Kaplan-Meier estimates of the cumulative incidence of non-relapse transplant events according to degree of HLA mismatching and by total nucleated cell dose. Transplant-related events include autologous reconstitution, second stem cell transplantation or death. Data from Rubinstein and Stevens [31]

to an alternative, unrelated donor. Once it has been determined that a fully matched or single antigen mismatched family donor is unavailable, the decision to use a cord blood graft depends on age of the recipient, how quickly the donor graft is needed and the ethnic background of the patient. The available data that provide the current algorithm for UCB graft selection will be discussed separately for adults and children below.

3.1.1. Pediatric Patients

Recent outcomes data for children undergoing UCB transplantation are impressive. As a result, there is no uniformly accepted prioritization for selecting a matched unrelated adult or mismatched unrelated cord blood graft. There are no prospective, randomized controlled trials comparing UCB to matched unrelated donor transplantation. The prioritization of UCB graft usage varies among the transplant centers worldwide, depending on the experience and comfort level with either of these approaches. There are three retrospective studies comparing outcomes of UCB to matched unrelated donor transplantation [32–34]. The largest of these retrospective studies was conducted by the Eurocord registry, which compared outcomes of 99 children undergoing mismatched UCB transplantation with 442 children receiving either an un-manipulated (n = 262) or T cell depleted (n = 180) unrelated adult donor graft. The median age of patients in this study was eight years, and all patients had acute leukemia. There were significant differences between the groups with respect to HLA matching, disease characteristics, preparative regimens and GVHD prophylaxis strategies. Non-adjusted estimates of two-year survival and event-free survival rates for patients surviving more than 100 days were 49 percent and 43 percent, respectively, in the unmanipulated unrelated bone marrow transplant group, 41 percent and 37 percent in the T cell depleted unrelated bone marrow transplant group and 35 and 31 percent in the UCB transplant group. After adjustment, differences in outcomes appeared in the first 100 days after transplantation. Compared to the unmanipulated bone marrow transplant group, cord blood transplant recipients had significantly slower hematopoietic recovery ($p < .001$), increased 100 day transplant-related mortality ($p < .01$) and decreased acute GVHD ($p < .001$). The Minneapolis and Montreal groups performed a single center retrospective matched pair analysis of UCB versus matched unrelated bone marrow outcomes. In contrast to the Eurocord registry where overall survival for UCB recipients appeared to be inferior to matched unrelated bone marrow, these centers found overall survival similar among these groups [33]. Dale and colleages report an overall three-year survival of 59 percent for 36 children transplanted with UCB and 57 percent overall survival for 28 children transplanted with matched unrelated donor bone marrow [34]. Barker and colleagues also reported equivalent two-year survival for pediatric recipients of UCB and matched unrelated bone marrow. Equivalence was maintained regardless of whether or not the matched unrelated bone marrow was T cell depleted. Importantly, the incidence of acute GVHD was not higher in the UCB recipients, despite the fact that there was significantly more HLA discordance.

In summary, the available data supports the use of either matched unrelated donor bone marrow or mismatched unrelated UCB as the first choice for donor stem cells in pediatric patients without a matched sibling donor.

3.1.2. Adult Patients

As with children, the first choice for allogeneic donor stem cells is from a matched sibling. There are no randomized controlled trials that compare outcomes using the alternatives such as unrelated or mismatched related donor grafts. Therefore, we must currently rely on registry data and single center retrospective studies to develop a prioritization schema for graft source selection. Complicating the situation is the fact that since around 2000 there has been a progressive transition to allele level matching criteria for both class I and class II loci. The impact on GVHD rates and overall survival rates for recipients of allele level matched grafts has yet to be fully appreciated, but early published data suggest that such patients can expect outcomes similar to those for recipients of matched sibling grafts [35]. In 2004, two observational registry studies using data from Eurocord/European Blood and Marrow Transplant Group (EBMT) and the CIBMTR/New York Cord Blood Center compared outcomes of adult patients with acute leukemia who where transplanted with either bone marrow or UCB from unrelated donors [36, 37]. While not as powerful as a randomized controlled trial, the homogeneity in patient age and disease type enhances the relevance of the observations that are reported. Both studies are restricted to adults under age 60; however, cord blood recipients tended to be slightly younger and had more advanced stages of acute leukemia. Importantly, matching criteria for the unrelated bone marrow and cord blood grafts was based on serologic or low resolution techniques for HLA-A and B and on high resolution molecular typing for HLA-DRß1. Rocha and colleagues, on behalf of Eurocord/EBMT, compared outcomes of 682 adults with acute leukemia who underwent myeloablative allogeneic stem cell transplantation between 1998 and 2002. Of the 682 patients, 98 received mismatched unrelated UCB (96% had 0, 1 or 2 mismatches), and 584 received 6/6 HLA-matched bone marrow. Multivariate analysis showed a lower risk of acute GVHD ($P = 0.01$) and a significant delay in neutrophil recovery ($P < 0.0001$) after UCB transplantation. Although the rate and frequency of sustained donor neutrophil recovery was lower for UCB recipients, the unadjusted incidence of chronic GVHD, transplant-related mortality, relapse rate and leukemia-free and overall survival were not significantly different in the two groups. The multivariate analysis of the data is shown in Table 14-3. Importantly, the authors found no significant difference in relative risk of leukemia-free survival and overall survival between the two groups. As a result, the authors conclude that mismatched cord blood is an appropriate alternative to HLA-matched bone marrow.

The CIBMTR study included 367 recipients of 6/6 HLA-matched bone marrow, 150 recipients of UCB and 83 recipients of 1-antigen mismatched bone marrow [37]. In contrast to the EBMT study, outcomes of UCB recipients were found to be inferior to those who were transplanted with matched bone marrow, but similar to those who received 1-antigen mismatched bone marrow. Among these three groups, the rates of treatment-related mortality, treatment failure and overall mortality were lowest among patients who received matched bone marrow transplants. The CIBMTR/New York Blood Center study differs somewhat from the EBMT study in that the sample included patients with chronic myeloid leukemia and myelodysplastic syndrome. Furthermore, this study includes transplants dating back to 1996, which was less than two years after the first unrelated cord blood transplant was reported. And finally, nearly twice as many cord blood recipients in

Table 14-3. Multivariate analysis comparing outcomes of unrelated cord blood transplantation with unrelated bone marrow transplantation; data from Rocha, et al. [69].

Outcome	Number of Events (UCB/MUD)	Relative Risk* (95%CI)	P (Value)
Neutrophil Recovery	77/528	0.49 (0.41–0.58)	<0.001
Grade II, III, or IV acute GVHD	25/232	0.57 (0.37–0.87)	0.01
Chronic GVHD	18/94	0.64 (0.37–1.1)	0.11
Transplant-related Mortality	43–207	1.13 (0.78–1.64)	0.50
Relapse	24/136	1.02 (0.63–1.65)	0.93
Leukemia-free Survival	67/343	0.95 (0.72–1.25)	0.70
Overall Survival	62/320	0.95 (0.71–1.27)	0.75

*Transplantation with unrelated umbilical cord blood as compared with transplantation with unrelated bone marrow (bone marrow group, relative risk of 1.00)

the CIBMTR sample had two or more antigen mismatches compared to the EBMT sample, (77% versus 41%). Laughlin and colleagues conclude from their data that UCB grafts should be reserved for recipients who do not have an HLA-matched adult donor.

The results of UCB transplantation in Japan are strikingly better than what have been reported from North America and Europe. Takahashi and colleagues compared outcomes of 113 adult patients with hematologic malignancies who were transplanted following myeloablative conditioning with either unrelated bone marrow or unrelated UCB grafts [38]. Owing to the relatively smaller size of Japanese individuals, the median cell dose provided to the UCB recipients was 2.47×10^7/kg recipient body weight. Primary graft failure occurred in 8 percent of cord blood recipients. The incidence grade II-IV GVHD was the same in both groups, but grade III/IV acute GVHD occurred in 4 percent of cord blood recipients and 12 percent of unrelated bone marrow recipients ($p = 0.01$). There was no significant difference in the incidence of chronic GVHD. The Kaplan-Meier estimated probability of two-year disease free survival was 74 percent after UCB transplantation and 44 percent after bone marrow transplantation ($p < 0.01$). A more recent and provocative report from the same group found that outcomes of adult Japanese patients undergoing UCB transplantation were comparable to those that received a peripheral blood or bone marrow graft from a matched or 1-antigen mismatched related donor [39]. One potential explanation for these discordant results is the somewhat restricted HLA diversity found within the Japanese population. If these data can be reproduced by other Japanese centers, it would appear that, at least in Japan, UCB would be the preferred graft for those patients of any age who are without a matched sibling donor.

Perhaps the most significant advance that has occurred in the years that have passed since patients in these largely retrospective studies were transplanted is the efficiency and speed with which one can now perform a search for an HLA-A, B, C and DRß1 allele level matched unrelated donor. For many centers, a 6/6 allele level matched bone marrow or peripheral blood stem cell donor has become a prerequisite for proceeding. Future studies, both prospective and

registry-based, must be performed to describe the impact on both survival and GVHD incidence when these highly matched unrelated donors are utilized.

3.2. Outcomes of Related UCB Donor Transplantation

The clinical experience with UCB transplantation from related donors is limited almost exclusively to children. Wagner and colleagues published the first report on UCB from sibling donors for transplantation [22]. The study analyzed data from 44 sibling donor UCB transplants prior to September 1994. The probability of engraftment for HLA-identical (34 patients) or one HLA locus disparate matches (4 patients) was 85 percent, and was 82 percent for the entire cohort that underwent transplantation. The median time to neutrophil recovery was 22 days (range 12 to 46), and platelet recovery 49 days (range 15 to 117). Graft failure occurred predominately in patients transplanted for nonmalignant disease. The most striking feature of these data was the low risk of acute and chronic GVHD. The probability of Grade II–IV acute GVHD was only 3 percent. The probability of chronic GVHD was 6 percent at one year. The probability of overall survival in this group was 72 percent.

The Eurocord experience was initially published in 1997 and then updated in a review by Rocha in 2006 [2, 40]. The initial review described 78 patients who received related UCB between 1988 and 1996. The donor was HLA-identical in 60 cases. Thirty-two patients had nonmalignant disease, while 46 had malignant disease. One-year survival was 63 percent. Survival at three years was 47 percent in patients with malignancies and 82 percent for patients with bone marrow failure disorders. GVHD occurred in 9 percent of HLA-matched transplants. Engraftment occurred in 85 percent of patients receiving greater than 3.7×10^7 nucleated cells. Younger age, lower weight, HLA identity and negative CMV status were favorable prognostic factors. The incidence of acute and chronic GVHD in related donor UCB transplantation (n = 113) was compared retrospectively to those receiving HLA-identical sibling transplants (n=2052) [23]. The analysis was limited to children under the age of 16 years. The relative risk of acute and chronic GVHD for the children receiving UCB grafts was 0.41 (P = 0.001) and 0.35 (P = 0.02), respectively. Overall survival in both groups was not statistically different.

While these data suggest allogeneic stem cell transplantation using an HLA-identical sibling cord blood graft may provide the best outcome, the rarity of the need for pediatric sibling transplantation and costs associated with cryopreservation of these grafts raises into question the advisability of routine storage of sibling grafts for personal use. However, in situations where there is a family history of hematopoietic malignancies or congenital disorders that are curable with UCB transplantation, collection and storage of family units is recommended.

3.3. Unrelated UCB Transplantation in Children

The published experience from the late 1990s established mismatched, unrelated UCB as a source for hematopoietic stem cells for allogeneic stem cell transplantation [2, 25, 41, 42]. The largest of these studies was updated in 2000 and includes the outcome of 861 patients transplanted through 1999

[31]. Two-thirds of the patients had hematologic malignancies, 25 percent had inherited conditions and 7 percent had acquired diseases. Engraftment was achieved in over 90 percent of recipients. Transplant outcome was influenced by the status of the underlying disease, nucleated cell dose and number of HLA mismatches (Figs. 14-1 and 14-2). The incidence of acute GVHD correlated with the degree of HLA-mismatch. For recipients of 6/6, 5/6 or 3–4/6 HLA-matched grafts, the incidence of grade II–V GVHD was 27 percent, 44 percent and 52 percent, respectively. The event-free survival rates at one year were 30 percent for patients with malignant conditions and 49 percent for those with inherited conditions. As a result of these data, many centers are now using UCB as the first choice for transplant candidates lacking an HLA-identical sibling.

As the experience continues to mount, it is now possible to survey disease-specific outcomes for children undergoing UCB transplantation. Table 14-4 demonstrates the important outcome parameters from a few of the largest studies of specific pediatric disease entities. The Eurocord group recently analyzed the data collected on 323 children undergoing unrelated UCB transplantation for acute lymphoblastic leukemia between 1994 and 2004. All children received myeloablative bone marrow conditioning and 67 percent received cyclosporine and corticosteroids for GVHD prophylaxis. Leukemia-free survival at two years for children transplanted in high risk first complete remission was 42 +/– 6 percent. For children in second complete remission or advanced phase disease, the two-year leukemia-free survival was 41 +/– 4 percent and 24 +/– 4 percent, respectively (Table 14-4) [40].

Michel and colleagues also used Eurocord group data to describe outcomes of UCB transplantation for treatment of childhood acute myelogenous leukemia (AML). Ninety-five children with AML in first complete remission

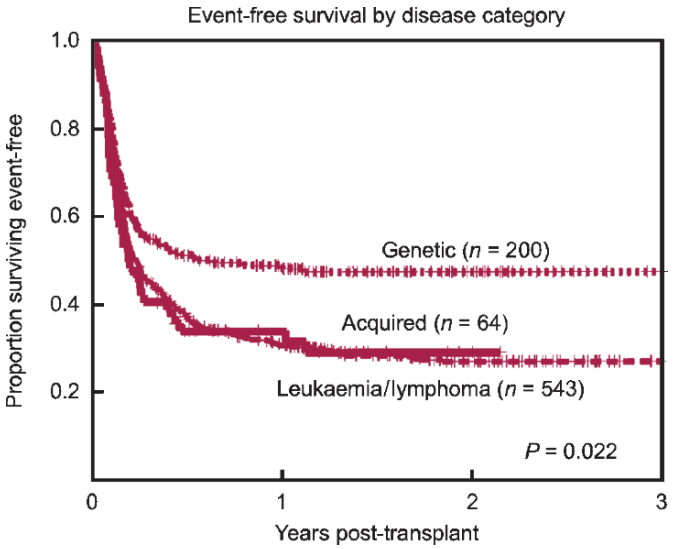

Fig. 14-2. Kaplan-Meier estimate of event-free survival following unrelated UCB transplantation according to disease category Taken from Rubinstein and Stevens [31]

Table 14-4. Disease-specific outcomes of UCB transplantation in children.

	ALL (n = 323)[40] CR1 CR2 Adv.	AML (n = 95)[70] CR1 CR2 Adv.	Infantile Krabbe (n = 25)[46]
Median Cell Dose (10^7 TNC/kg)	5.3 3.7 3.4	..... 4.4	19.1/12.6*
Acute GVHD			
Grade II–IV(%)	46 43 36	..... 35	20
Grade III–IV(%)	NA NA NA	..... 20	8
TRM(%)	22 25 34 (day 100)	..... 20+/−4	16
Relapse (%)	34 37 38 (@2 yrs)	10+/−7 23+/−7 61+/−11	N/A
Survival			
EFS (%)	42 41 24	59+/−11 50+/−8 21+/−9	N/A
OS (%)	NA NA NA	..... 49+/−5	100/40*

*Asymptomatic and symptomatic recipients at time of transplantation, respectively

(n = 20), second complete remission (n = 47) and advanced stage (n = 29) were included in the analysis. The cumulative incidence of neutrophil engraftment was 78 +/– 4 percent, acute GVHD was 35 +/– 5 percent and 100-day treatment-related mortality was 20 +/– 4 percent. The two-year leukemia-free survival was 59 +/– 11 percent when performed in first complete remission, 50 +/– 8 percent in second complete remission and 21 +/– 9 percent for children not in complete remission at the time of transplantation (Table 14-3). A recurrent theme in both of these large registry-based studies is that nucleated cell dose has proven to be an important determinant in a successful outcome.

UCB transplantation activity for nonmalignant conditions is increasing. This includes treatment of patients with inherited immune deficiencies, mucopolysaccharidosis such as Hurlers syndrome, hemoglobinopathies and lysosomal storage diseases such as Infantile Krabbe [43–46].

3.4. Unrelated UCB Transplantation in Adults

The outcomes of adult UCB transplantation are inferior to those seen with children for a number of reasons [47–52]. They include the lower cell dose provided to the larger adult population, which translates into prolonging the vulnerable pancytopenic period and an increased risk of graft failure. Enhanced conditioning-related toxicity is inherent in the adult population regardless of the stem cell source, with comorbid conditions at the time of transplant augmenting this vulnerability. Since adult cord blood transplantation is a relatively new and innovative approach, there is a tendency to select higher risk patients for treatment. Finally, the intrinsic differences in kinetics of immune reconstitution between children and adults is exaggerated after UCB transplantation [53, 54]. The preponderance of naïve T cells in the cord

blood graft and the lack of functional thymus tissue in the adult recipient combine to slow the developing cellular immune system.

The larger single center or registry-based retrospective studies of the outcome of adult UCB transplantation is demonstrated in Table 14-5. Data from the Eurocord group was updated in 2006 and includes 171 patients transplanted in primarily European centers using myeloablative bone marrow conditioning between 1998 and 2003 [49]. These patients had a median age of 29, and were diagnosed with hematologic malignancies. With a cumulative incidence of neutrophil engraftment by day 60 of 73 +/−3 percent, it is clear that graft failure is a major problem with adult cord blood transplantation. Despite this ominous result, 38 +/−7 percent of patients were alive and disease-free at two years if transplantation occurred at an early or intermediate phase of the disease. The multivariate analysis revealed that a nucleated cell dose of $>2.6 \times 10^7$ was the most important factor in predicting successful neutrophil recovery. For patients transplanted in the late phases of disease (defined as acute leukemia in third or subsequent complete remission, refractory acute leukemia, lymphoma in partial remission or resistant relapse), the two-year disease-free survival dropped to 18 +/− 4 percent. The survival rates

Table 14-5. Unrelated UCB transplantation in adult patients.

	Arcese, et al. (Eurocord) (n = 171) [49]	Laughlin, et al. (n = 68) [50]	Long et al. (n = 57) [71]	Cornetta, et al. COBLT (n = 34) [51]	Ooi, et al. (n = 18) [38]
Median Cell Dose; Frozen/infused (10^7 TNC/kg)	2.7/2.1	2.1/1.6	2.1/1.5	2.3/NA	2.51/NA
HLA compatibility (%)*					
6/6	5	3	4	1	0
5/6	46	26	14	29	22
4/6	41	54	77	68	50
3/6	8	17	3	0	22 (1 pt 2/6 matched)
Acute GVHD					
Grade II–IV(%)	32	60	30	34	64
Grade III–IV(%)	16	20	16	NA	6
Chronic GVHD (%) (limited + extensive)**	36 +/− 10	38	32	21	82
Neutrophil Recovery (% estimated probability ANC>500)	72	90	82	66	94 (17 of 18 pts)
TRM(%)	51	47	16	53	5
Survival;					
EFS (%)	27 +/− 4%	26	15	6	77
OS (%)	33 +/− 4	NA	19	17 (@1yr)	

*HLA-A and B matching by serology, HLA-DRß1 matching by molecular, allelic level techniques

**Cumulative incidence

NA- Not Available

were similar in the other studies of UCB transplantation performed in North America or Europe. Outcomes reported by the Japanese group are better. Ooi and colleagues transplanted 18 patients with *de novo* acute myeloid leukemia with unrelated partially matched UCB grafts [38]. The median cryopreserved cell dose was 2.51×10^7/kg. Acute and chronic GVHD occurred at a rate comparable to the other studies; however, the probability of disease-free survival at two years was a remarkable 77 percent. It should be noted that four of the 18 patients were in first complete remission with normal cytogenetics at the time of transplantation, and while the remainder of the patients had more advanced disease (including six with active leukemia at transplant), only two had a classic high risk karyotype (11q23).

The reported data demonstrate the feasibility of adult UCB transplantation; however, significant challenges remain and must be addressed before the approach can be accepted as standard. The importance of cell dose has been recognized and a minimum threshold of $2.5–3 \times 10^7$ nucleated cells per kg recipient body weight has been widely accepted. Techniques designed to expand cord blood stem cells *ex vivo* have been tested clinically, but have yet to show proven efficacy [55]. Using two cord blood grafts to increase the cell dose has shown promise and is currently being studied in a multicenter phase 2 study conducted by the CIBMTR (see below) [56]. HLA matching is also important, and as cord blood banks expand, the ability to find more closely matched grafts of ample size will grow. Finally, the problem of impaired immune recovery and prolonged infection susceptibility is particularly acute in adult patients.

3.4.1. Dual UCB Transplantation

Dual UCB transplantation has recently received considerable attention as a means to increase the cell dose and improve engraftment frequency in adult patients. The idea was initially conceived as a method for safely performing stem cell transplantation of a graft that has been manipulated *ex vivo* by co-transplantation of an un-manipulated graft. Case reports suggested feasibility and safety of this concept [57, 58]. Barker and colleagues conducted a phase I/II clinical trial of dual UCB transplantation using myeloablative bone marrow conditioning consisting of Cyclophosphamide and TBI [56]. Twenty-three patients received dual cord blood grafts and 21 were evaluable for engraftment. All 21 evaluable patients achieved sustained donor neutrophil engraftment. Interestingly, hematopoiesis derived from both cord blood grafts was detected during the first 100 days in five patients. By day 100, all patients had hematopoiesis derived from a single cord blood graft suggesting immunologic clearance by the dominant graft of stem cells from the losing graft (graft versus graft interaction). The incidence of acute and chronic GVHD was in line with other UCB transplant studies. None of the typical graft characteristics, such as nucleated cell dose, CD34+ cell content, HLA matching or T cell content, have been found to be a predictive factor for determining which graft predominates. Future studies comparing single versus dual cord blood transplantation will be needed to confirm efficacy of this approach.

3.4.2. Nonmyeloablative Adult UCB Transplantation

Use of low intensity or nonmyeloablative bone marrow conditioning regimens prior to peripheral blood transplantation has successfully reduced conditioning-related toxicity by approximately 50 percent while still providing the necessary

myelosuppression and immunosuppression needed to facilitate engraftment [59]. It has made allogeneic stem cell transplantation accessible to patients who were otherwise considered too old or sick to withstand the conventional approach. The Minneapolis group employed a reduced intensity regimen consisting of Busulfan or Cyclophosphamide in conjunction with Fludarabine and 200 cGy total body irradiation (TBI) [60]. Dosing of the alkylating agent was 50 percent less than what is given with a conventional myeloablative preparation. Forty-three patients with high risk hematologic malignancies and a median age of 49 were treated. The cumulative incidence of engraftment was 76 percent. For the entire group, overall and disease-free survival was 39 percent and 31 percent, respectively. Treatment-related mortality remained quite high at 48 percent. The authors found that the patients conditioned with Cyclophosphamide containing regimens fared better than those who received Busulfan.

The Duke group further reduced the intensity of the regimen in an attempt to reduce the treatment-related mortality rate in this highly susceptible population of patients [61]. The first 13 patients were conditioned with Fludarabine 120 mg/m^2, Cyclophosphamide 2 g/m^2 and equine Antithymocyte globulin 90 mg/kg. Cyclosporine and Mycophenolate Mofetil were administered for GVHD prophylaxis. Using this approach, only 41 percent of patients achieved sustained donor engraftment. Therefore, 200 cGy total body irradiation was added to the regimen and the minimum nucleated cell dose provided from up to two cord blood units was increased to 3×10^7/kg. Fourteen patients with advanced hematologic malignancies and a median age of 54 were transplanted. Eight patients required dual cord blood grafts to achieve the minimum cell dose. Day 100 treatment-related mortality occurred in four patients (30%) and five patients relapsed (36%). The estimated one-year overall and disease free survival is 25 percent and 17 percent, respectively. The addition of 200 cGy and increasing the cell dose facilitated by dual cord blood transplantation doubled the chance for sustained donor engraftment; however, improved engraftment was accompanied by increased treatment-related mortality, erasing the potential for improved disease free survival [62].

Ballen and colleagues transplanted 21 patients using a reduced intensity regimen consisting of Melphalan 100 mg/m^2, Fludarabine 180 mg/m^2 and rabbit anti-thymocyte globulin [63]. Two partially matched UCB grafts were used, providing a minimum pre-cryopreserved cell dose of 3.7×10^7/kg. All but two of the 21 patients achieved donor engraftment from one or both of the cord blood grafts, and three patients had prolonged donor/recipient mixed chimerism. Grade II–IV acute GVHD was observed in 40 percent of patients. Day 100 treatment-related mortality was 14 percent and one-year disease free survival was 67 percent.

3.5. Immune Reconstitution

Immune reconstitution following UCB transplantation is of concern both on a short-term and long-term basis. Compared to peripheral blood or even bone marrow transplantation, neutrophil recovery is delayed, averaging 13 to 29 days depending on the cell dose. Additionally, platelet recovery is often delayed for up to 100 days to reach a level greater than 50,000.

Of greater concern, particularly in adult UCB transplantation, is the slow pace of lymphoid recovery [53]. In children, median time to both $CD3^+$ and

$CD4^+$ cell reconstitution was 11.7 months, while the median time of $CD8^+$ cell reconstitution was 7.9 months [64]. $CD8^+$ cells recovered faster than $CD4^+$ cells with a characteristic inversion of CD4/CD8 ratio often observed after bone marrow transplantation [65, 66]. Recovery of B cells occurs quicker, taking a median of 5.9 months, and generally as fast as natural killer (NK) cells. Overall, this pattern of recovery in children is similar to what is observed with peripheral blood or bone marrow transplantation [67]. Robust immune recovery has been shown to correlate with a reduction in leukemic relapse and improved survival [68].

Investigators at Duke University analyzed the immunologic reconstitution after myeloablative unrelated UCB transplantation in adults and children to determine the source of the regenerated T cell pool [53]. UCB is phenotypically naïve, but T cell replete, allowing for T cell reconstitution through either central or peripheral mechanisms. Reconstitution of immunity after UCB transplantation occurred over two to three years for both adult and pediatric recipients. Both patient groups eventually demonstrated a normal lymphocyte count with a quantitatively normal distribution of B cells, T cells and NK cells. The sources of T cells, however, are strikingly different. Pediatric patients demonstrated significant numbers of signal joint T cell receptor excision circle (TREC)-containing T cells starting within one year after transplantation, indicating that the T cells were recent thymic emigrants. In contrast, adult recipients did not demonstrate any TREC containing T cells until 1.5 to two years after transplantation, and then only at relatively low levels. These results suggest that adult recipients of cord blood had a very slow recovery of T cell numbers and function. By using a complementary determining region 3 repertoire to measure the changes in T cell receptor diversity that occur when thymic function is restored, skewing of T cell repertoires was demonstrated in adults and children at 12 to 18 months after transplantation. However, this recovered to near normal diversity at two to three years after transplantation. The T cell repertoire seemed more diverse earlier in children than in adults. The analysis provides evidence that early T cell recovery after UCB transplantation occurs primarily through the peripheral expansion of adoptively transferred donor T cells and results in skewing of the T cell repertoire. These data also underscore the importance of preserving the integrity of thymic function, thus allowing rapid recovery of T cells with a complex diversity.

4. Conclusion

Umbilical cord blood is an important source of hematopoietic stem cells for transplantation. The naïve and non-responsive nature of the resident T cells contained within the graft allow for successful outcomes despite HLA discordance. To date the experience with pediatric stem cell transplantation support the use of umbilical cord blood grafts as the first alternative for those patients who do not have an HLA-identical sibling donor. Outcomes in adult patients are not as good due to the low cell dose and due to inherent difficulties in post-transplant immune recovery in patients who are athymic. As cord blood banks expand, there will be improved access to a larger, more diverse pool of high quality grafts that will ultimately translate into improved outcomes for all patient populations.

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Chapter 15

Haploidentical Stem Cell Transplantation

Bimalangshu R. Dey and Thomas R. Spitzer

1. Introduction

The lack of human leukocyte antigen (HLA)-matched related or unrelated donors (or the time necessary to locate and procure stem cells from HLA-matched unrelated donors), and the unavailability of suitable cord blood units are major limitations to the successful application of hematopoietic stem cell transplantation (HSCT). Genetically haploidentical family members represent another potential source of HSC and they are readily available for almost all patients. While initial attempts at haploidentical stem cell transplantation were largely unsuccessful due to an exceedingly high risk of Graft-versus-Host Disease (GVHD) and transplant-related mortality, recent advances in our understanding of the immunologic complications of HLA-mismatched stem cell transplantation, our ability to mobilize and select HSC, and the identification of new immunosuppressive agents and effective prophylactic and preemptive strategies for opportunistic infections, have led to decreased transplant-related mortality and improved survival outcomes. The negative consequences often encountered with haploidentical HSCT, such as increased rates of graft failure, increased probability of disease relapse and delayed immune reconstitution which may lead to fatal infectious complications, are largely due to the rigorous T cell depletion that is necessary to prevent fatal GVHD. Therefore, transferring a functional lymphohematopoietic system from the donor to the recipient in a timely fashion likely determines the ultimate success of haploidentical HSCT, if this can be performed without the risk of severe GVHD. Recently, refinements in transplant strategies, including the transplantation of "mega-dose" peripheral blood stem cells (PBSC) have substantially improved donor engraftment. Furthermore, the add-back of (1) donor lymphocytes that are selectively depleted of host-reactive T cells, (2) regulatory T cells (Tregs and NK/Tregs) or (3) herpes simples virus-tyrosine kinase (HSV-TK) engineered donor lymphocytes are producing encouraging results in terms of earlier immune reconstitution with acceptable rates of GVHD both in preclinical and clinical models. Additionally, novel approaches using the adoptive transfer of *ex vivo* expanded pathogen or tumor antigen-specific effector cells are being evaluated following transplantation with promising early outcomes.

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The obstacles to successful haploidentical HSCT in the clinical setting have been evident over the past two to three decades and will be reviewed in this chapter in detail. The most important immunobiological considerations to successfully transgressing HLA barriers, involving composition of the donor graft and specific genetic disparities between the donor and the recipient, and the cellular environment that exists following haploidentical HSCT will be discussed as a prelude to an overview of the past experiences and future direction of haploidentical HSCT.

2. Haploidentical HSCT: Immunobiological Principles

2.1. Natural Killer Cells and Exploiting KIR Ligand Mismatching in the GVH Direction

Natural killer (NK) cell alloreactivity in the Graft-versus-Host (GVH) or Host-versus-Graft (HVG) directions can be a powerful tool for optimizing the efficacy and safety of haploidentical-HSCT. NK cells are a unique CD56+CD3− cell subset comprising approximately 10 percent of peripheral blood lymphocytes [1, 2] and are involved in innate antiviral and antitumor immune responses [3–5] They constitute a heterogeneous population of different subsets with distinct phenotypic and functional characteristics: the majority (90%) are highly cytotoxic $CD56^{dim}$ cells which likely function as efficient effector cells, whereas a minority (10%) are immunoregulatory $CD56^{bright}$ cells producing abundant cytokines [6, 7]. Following HSCT, including haploidentical-HSCT with selected CD34+ cells, NK cells are the first lymphoid cells to recover, as early as two to three weeks post-transplant by rapid differentiation from engrafted CD34+ cells [8–10].

The "missing self" recognition hypothesis was proposed by Klas Karre two decades ago. He suggested that, unlike T and B-cells, NK cells are activated by the absence of self major histocompatibility complex (MHC) class I molecules on the surface of target cells [11–14]. Expressing appropriate self-MHC molecules on target cells delivers an inhibitory signal to NK cells via inhibitory killer cell immunoglobulin-like receptors (KIRs). In the absence of the inhibitory signal, NK cell alloreactivity, i.e., NK cell-mediated target cell lysis proceeds by default. When NK cell inhibitory receptors are engaged by KIR-specific epitopes, killing is inhibited, whereas killing of target cells occurs when NK cell inhibitory receptors are not engaged, either due to the lack of MHC class I molecules or due to an MHC mismatch. Because virally infected cells and tumor cells downregulate MHC expression to escape adaptive immune surveillance, this ability of NK cells to detect "missing self" is critical in (innate) immune responses against viruses and tumor cells [15, 16]. Virtually all NK cells express at least one inhibitory receptor that is specific for a self MHC class I epitope, thereby preventing autoreactivity [17]. NK cells also possess activating receptors, most notably NKG2D and NKp46, which can trigger NK cell alloreactivity when they are engaged by appropriate antigens on virally infected cells and tumor cells [18–21]. While it is thought that inhibitory signals dominate over activating signals, NK cell activity is regulated by quantitative differences in cumulative inhibitory and activating signals transmitted via KIRs and, therefore, the absence and presence of the respective ligands on recipient cells determines if NK cells will be primed to be alloreactive and kill the targets [2, 22].

The KIRs are classified based on both the number of extracellular Ig domains (either two or three, designated KIR2D or KIR3D) and the length of the intracellular signaling domain (either long or short, designated KIR2DL or KIR2DS). The KIR genes, located on human chromosome 19, and HLA genes, located on human chromosome 6, are inherited independently, but most individuals possess a full complement of KIR genes for inhibitory receptors for the three major class I epitopes: groups 1 and 2 HLA-C alleles, and HLA-Bw4 alleles [23]. NK cell receptors for HLA class I molecules are complex, but the most dominant patterns of alloreactivity are due to two groups of HLA-C epitopes which are determined by the amino acid residue at position 80 of the a-1 helix. "Group 1" HLA-C alleles characterized by an asparagines residue (HLA-C^{Asn80}; -Cw1, -Cw3, -Cw7, -Cw8) are the ligands specific for inhibitory KIR2DL2/3 receptors; "Group 2" HLA-C alleles are characterized by a lysine residue (HLA-C^{Lys80}; -Cw2, -Cw4, -Cw5, -Cw6) and are specific for KIR2DL1; HLA-Bw4 alleles are specific for KIR3DL1 [24]. For Donor-versus-Host NK cell alloreactivity to occur, it is critical that a KIR ligand mismatch exists in the GVH direction, that is, the HLA genotype of the donor includes a KIR epitope that is not a part of the HLA genotype of the host. Donors who express Group 1 HLA-C alleles possess NK cells with KIRs specific for Group 1 HLA-C alleles (KIR2DL2/3) and will be alloreactive against hosts who do not express Group 1 HLA-C alleles, i.e., who are homozygous for Group 2 HLA-C alleles.

NK cell alloreactions following haploidentical HSCT, which are based on the "missing self" hypothesis, were first described in a phenomenon known as hybrid resistance where lethally irradiated heterozygous (parent A × parent B) F1 hybrid mice were found to reject bone marrow transplants from either parent A or parent B, but to accept solid tissue grafts from either parental strain [25–27]. Rejecting solid organs, which is a T cell-mediated process, did not occur since (A × B) F1 recipient mice express all MHC antigens of both parents. Rejection of the parental marrow graft has been proven to be mediated by a subset of host NK cells that lacks the appropriate inhibitory receptors to recognize donor MHC class I ligands. This NK cell-mediated hematopoietic graft rejection has recently been further supported by a series of elegant experiments, including transplants from MHC class I-deficient donors [28–31]. In addition, because of tolerance of the solid organs, this hybrid resistance model indicates that NK cell alloreactivity is restricted to lymphohematopoietic tissues.

By selecting haploidentical stem cell donors whose NK cells are not fully inhibited by recipient MHC class I ligands, i.e., there is a KIR-ligand mismatch in GVH direction, the graft NK cell alloreactivity has recently been utilized for its potent Graft-versus-Tumor (GVT) Effect. Ruggeri, et al. found that an appropriate HLA (KIR Ligand) mismatch in the GVH direction following T cell depleted (TCD) haploidentical-HSCT can be associated with strong NK cell alloreactivity leading to (1) a dramatically reduced relapse rate in patients with acute myelogenous leukemia (AML), (2) a lower rate of graft rejection and (3) a reduction in GVHD. As supported by their animal studies, the reduction in GVHD is due to (1) NK cell-mediated depletion of host antigen presenting cells that are critical in the priming of alloreactive donor T cells and, hence, in the pathogenesis of GVHD, and (2) NK cells that predominantly attack the host hematopoietic cells and spare epithelial GVHD target tissues [32–34].

The lower rates of graft rejection were shown to be due to NK cell-mediated lysis of host residual T cells, thereby preventing them from rejecting the graft. Furthermore, in their murine transplant model they demonstrated that the pre-transplant transfer of NK cells into mice improved engraftment after transplantation of the donor graft, allowing durable full donor chimerism following reduced-intensity conditioning. This NK cell conditioning prevented GVHD efficiently enough to allow safe infusion of otherwise lethal doses of allogeneic T cells for immune reconstitution. While Ruggeri, et al. showed NK cell-mediated antileukemia effects only in AML (and not in acute lymphoblastic leukemia (ALL)), Leung, et al. demonstrated that ALL cells are susceptible to NK cell lysis when the donor NK cells expressed KIRs in the absence of cognate ligand in the recipient [35]. A number of retrospective studies have been undertaken in recipients of unrelated donor transplants to test whether the presence of KIR ligand mismatching in the GVH direction exerted NK-mediated GVT effects [36–39], with many of the studies showing no advantage of transplantation from donors with the potential to exert antihost NK alloreactivity. It is possible that the failure to see clinically meaningful NK alloreactivity in these studies was, at least partly, due to the lack of vigorous T cell depletion since T cell depletion has been suggested as enhancing NK cell recovery and perhaps NK alloreactivity. Recently, improved transplant outcomes were observed in the HLA-identical sibling HSCT setting according to KIR and HLA genotypes, confirming that KIR-driven alloreactivity might be better predicted if the donor KIR genotype is considered in addition to the recipient HLA genotype [40]. In order to favorably impact the outcomes following haploidentical HSCT, adoptive immunotherapy with purified NK cells is being attempted [41]. While efficacy data have not been determined in these small studies, NK cell infusions appear to be feasible with acceptable toxicities.

2.2. Manipulation of Cells to Enhance Immune Reconstitution

Several adverse outcomes are encountered following T cell depleted HSCT including graft failure, an increased probability of relapse and delayed immune reconstitution with resultant risk of infectious complications [42]. Recently, refinements in transplant strategies, including the "megadose" PBSC approach, have substantially improved the restoration of hematopoiesis (engraftment) following haploidentical-HSCT; the reconstitution of a functional immune system, however, remains a substantial challenge. Therefore, strategies are needed to augment immune reconstitution against both residual malignant cells and potentially fatal infectious pathogens without enhancing GVHD.

Recently, new approaches aimed at enhancing engraftment, and reducing GVHD while preserving GVT effects, have been developed with the use of regulatory T cells and mesenchymal stem cells. In mice, two types of regulatory T cells – CD4+CD25+foxp3+ T-cells (Tregs) and natural killer T-cells (NK/Tregs) – can prevent acute GVHD [43–47].

Representing approximately 5 percent to 10 percent of peripheral CD4+ T cells in mice and humans, and identified by their capacity to suppress both CD4+ and CD8+ T cell activation, Tregs (CD4+CD25+Foxp3+ T-cells) promote engraftment, and reduce acute GVHD without the loss of GVT effects [43, 44, 48]. In murine models, Tregs can be successfully expanded *ex vivo*

by *in vitro* stimulation with allogeneic splenocytes plus interleukin 2 (IL-2) and, when added to the donor inoculum containing alloreactive T cells, they efficiently prevent GVHD, favor immune reconstitution and reduce the risk of infection while preserving GVT effects [49]. Preliminary experience addressing the use of expanded Tregs in humans is promising [50], though clinically meaningful expansion of this small effector cell population and its beneficial use outside the prevention of acute GVHD [51] have yet to be determined. These Tregs have been found to enhance immune reconstitution by protecting the thymus and lymphoid tissues from GVHD damage, allowing the generation and expansion of lymphoid cells without impacting the diversity of the T cell repertoire [52].

Constituting only 1 percent to 3 percent of all T cells in the spleen in normal mice, NK/Tregs of either donor or host type have the unique capacity to prevent acute GVHD in mice by secreting IL-4 that prompts donor T cells to acquire an anti-inflammatory, IL-4-secreting Th-2 phenotype [46, 47, 53]. Lowsky, et al. recently evaluated this NK/Tregs-mediated GVHD prevention strategy clinically by using nonmyeloablative conditioning with total lymphoid irradiation (TLI) and anti-thymocyte globulin (ATG). Results included reduced rates of acute GVHD in patients with lymphohematopoietic malignancies that permitted GVT effects [54, 55]. In an MHC-mismatched murine model, this group of investigators recently showed that host NK/Tregs can facilitate the expansion of donor CD4+CD25+Foxp3+ Tregs which play an important role in protecting TLI/ATG treated hosts from lethal GVHD [56].

Mesenchymal stem cells (MSC), which are multipotential nonhematopoetic progenitors and constitute only 0.001 percent of the nucleated cells in human bone marrow [57], have been extensively studied for their regenerative and immunomodulatory properties. Human MSCs have a high *in vitro* proliferative potential and have the capacity to differentiate into several mesenchymal tissues, such as bone, cartilage and fat. They have been found to promote hematopoietic homing and engraftment after both autologous and allogeneic HSCT [57–60]. MSCs have also been shown to inhibit T cell alloreactivity [61–65] and prolong skin allograft survival [62]. In addition, *ex vivo* expanded MSCs have the potential to prevent and treat acute GVHD when used after HLA-matched or mismatched HSCT [60, 66, 67]. Recently, Le Blanc, et al. updated their experience with MSCs. Forty patients with severe grade III–IV GVHD received MSCs; 19 patients achieved complete responses and partial responses were observed in nine patients [68]. While the exact mechanism of immune modulation by MSCs has not been delineated, it appears that they inhibit interferon-gamma (IFN-γ) secretion from Th-1 and NK cells, increase IL-10 secretion from Tregs and increase IL-4 secretion from Th-2 cells, thereby promoting a Th-1 $\rightarrow$Th-2 shift [65].

2.3. Manipulation of Soluble Factors, i.e., Cytokines and Chemokines, to Enhance Immune Reconstitution

A variety of cytokines and chemokines, and their associated monoclonal antibodies, can be applied in a number of ways to affect multiple levels of effector cell development, activation and trafficking, thereby influencing transplant outcomes, including engraftment, immune reconstitution, GVHD and antitumor effects [69–71]. The use of granulocyte-colony stimulating factor (G-CSF)

post-transplant accelerates neutrophil recovery, but recent studies have shown potentially deleterious effects on immune restoration, namely inhibition of NK and T cell function [72–74]. For their ability to enhance the *in vivo* expansion of NK and T cells post-TCD HSCT, IL-2 [75], IL-12 [76], IL-15 [77, 78] and IL-18 [79] are potentially attractive agents for improving immune reconstitution following haploidentical-HSCT. Caution must be exercised, however, since depending upon the timing of administration of these soluble factors, deleterious outcomes such as exacerbation of GVHD may occur [80, 81]. In one murine model, IL-7 improved immune reconstitution by enhancing thymopoiesis in addition to expanding peripheral T cells, NK cells, NK/Tregs, B cells, monocytes and macrophages [82], but in another model acute GVHD was enhanced by this cytokine [83]. Keratinocyte growth factor (KGF) has also been shown experimentally to improve thymic and peripheral T cell recovery after HSCT [84]. Monoclonal antibodies specific for selective cytokines and chemokines are being used to prevent GVHD [85–87] and to enhance GVT effects [70].

2.4. Enhancement of Antigen-Specific Immune Responses

The successful management of opportunistic viral, fungal, bacterial and parasitic infections remains a formidable challenge after haploidentical-HSCT. The failure to successfully treat viral infections, which is largely due to a limited number of non-toxic antiviral drugs, constitutes a major cause of morbidity and mortality following haploidentical-HSCT. Adoptive immunotherapy with *ex vivo* expanded virus-specific cytotoxic T lymphocytes (CTLs) is being currently evaluated in a number of centers. The infusion of donor-derived allogeneic CMV-specific T cell clones into recipients for the prevention or treatment of CMV-related infection has shown considerable promise both for its anti-CMV activity and for the lack of GVHD [88–91]. While much of the research effort has focused on CMV-specific CTLs, these studies have also demonstrated that CMV-specific CD4+ cells are essential for the reconstitution and maintenance of CD8+ CTL responses. Similarly encouraging results have also been achieved when *ex vivo* expanded allogeneic CTL clones specific for EBV were used for the prophylaxis and management of EBV-associated diseases, including post-transplant lymphoproliferative disorder (PTLD) [92–95]. The recent identification of molecules that are specifically expressed by leukemia cells, such as PR1 by chronic myelogenous leukemia (CML) and AML cells and that can be recognized by T cells, has led to the successful *ex vivo* generation of leukemia-specific donor T cells (PR1-CTLs) that promote a GVT effect [96–98]. Notwithstanding the promise of these novel approaches, the large scale therapeutic potential of adoptive transfer of *ex vivo* expanded antigen-specific effector cells has not yet been fully realized because of multiple factors. One of these factors is the limited survival of these effector cells, which is likely due to factors intrinsic to the cells themselves or to the host environment into which the cells are infused.

3. Haploidentical Stem Cell Transplantation: Clinical Applications and Results

While our understanding of the basic immunobiology of HSCT across major MHC barriers has dramatically increased over the past two to three decades, the translating those discoveries into clinical practice has evolved more slowly.

The human immune system is well equipped to resist an antigenically disparate challenge through a variety of well described cellular, humoral and cytokine pathways. With the definition of the HLA complex and the methodology to define at a molecular level the HLA alleles in transplant recipients and their donors, it became apparent early on that the important immunological complications of HSCT – namely, graft rejection and GVHD – were significantly greater obstacles when bone marrow transplants were attempted across major histocompatibility barriers [99–101]. Many of the early failures of haploidentical HSCT were due not to a lack of understanding of the immunologic principles of transplanting across HLA barriers, but to the limited pharmacologic arsenal for preventing graft rejection and GVHD, and for restoring immune competence post-transplant. With new, more powerful and in some cases targeted immunosuppressive medications, and an increased understanding of the immunologic consequences of *ex vivo* and/or *in vivo* depletion of immunocompetent T cells, haploidentical HSCT has become a safer and more effective transplant option. Outcomes of haploidentical HSCT according to the strategy for overcoming the immunological complications of the transplant are summarized in Tables 15-1 and 15-2.

The rationale for considering haploidentical-HSCT has become increasingly clear (Table 15-3). Since there is only a 25 percent chance that a recipient and a potential sibling donor will be HLA-matched, and identifying an HLA-matched unrelated donor within an acceptable time frame for transplantation may meet the overall needs of only approximately one-half of the patients in need of HSCT, consideration is still given to finding related haploidentical donors in selected clinical circumstances. Successfully performing haploidentical HSCT would mean that virtually all patients have a donor available immediately for the transplant and, potentially, for future attempts to modulate the cellular environment by delayed infusions of selected donor cell populations.In preclinical animal models, a stronger GVT effect has been demonstrated when MHC barriers are crossed [102]. Relapse of the underlying malignancy is the primary reason for treatment failure after HSCT for advanced hematologic malignancies, particularly with the use of reduced intensity conditioning [103–105]. One way to at least in part overcome the risk of relapse would be to capture the potentially more potent GVT effect of a haploidentical HSCT. Finally, specific tolerance induction through mixed lymphohemapoietic chimerism will be most applicable if the immunological complications of HLA-mismatched HSCT can be avoided [106, 107].

Haploidentical SCT: Early Lessons

Based on the successful outcomes in children with primary immunodeficiencey syndromes who received HLA-matched sibling donor HSCT [108, 109], attempts were made to perform T cell depleted parental donor haploidentical bone marrow transplantation (BMT). This strategy has subsequently been shown to be successful in a number of immunodeficiency disorders including severe combined immunodeficiency disease and Wiskott-Aldrich syndrome [110, 111]. Despite the early successes of HLA-matched HSCT and the knowledge that only sustained lymphoid chimersim was necessary for functional cure of these illnesses, it was quickly apparent that more aggressive conditioning was required to overcome HLA barriers in the haploidentical-HSCT setting [112, 113].

An early retrospective analysis of transplant outcomes following HLA-1 to 2 antigen mismatched BMT for leukemia by Beatty and colleagues at the Fred Hutchinson Cancer Research Center [100] was very instructive in

Table 15-1. Non-T-cell depleted haploidentical stem cell transplantation.

Center	Disease	Patient #	Conditioning	GVHD prophylaxis	EFS or DFS/OS	Reference
Royal Marsden	AML/ALL	35	TBI/CY or TBI/MEL	CYA+/− MTX	NS/31% at 6 months –3 years	(Powles et al., 1983)
Children's (Boston)	Leukemia, NHL, NMD	12	TB I/CY	Ex vivo T-cell anergization	42%/42% at 4.5–29 months	(Guinan et al., 1999)
China (Multi-center)	Leukemia, MDS	135	BU/CY/ARA-C/MECCNU/ATG	CYA/MTX/MMF	64%/71% at 2 years	(Lu et al., 2006)
Univ. of Tokyo	Leukemia, MDS, NHL	12	TBI/CY+/−VP-16 or BU/FLU + Alemtuzumab	CYA/MTX	42%/58% (not stated)	(Ogawa et al., 2005)
Japan (multicenter)	Leukemia, NHL	35	Myeloablative (*n*=24) Nonmyeloablative (*n*=11)	Microchimeric NIMA-mismatched donor SCT Tacrolimus ± other drugs	40%/43% at 20 months	(Ichinohe et al., 2004)
Johns Hopkins	Leukemia, MDS	13	TBI/CY/FLU post-BMT CY	CYA, MMF	38%/46% at >6 months	(O'Donnell et al., 2004)

AML acute myeloid leukemia; *ALL* acute lymphoblastic leukemia; *HM* hematologic malignancy; *NHL* non-Hodgkin's lymphoma; *MDS* myelodysplastic syndrome; *NMD* nonmalignant disease; *GVHD* graft-versus-host disease; *EFS* event free survival; *DFS* disease free survival; *OS* overall survival; *TBI* total body irradiation; *CY* cyclophosphamide; *CYA* cyclosporine; *MTX* methotrexate; *VP-16* etoposide; *ATG* antithymocyte globulin; *ARA-C* cytarabine; *MECCCU* methyl-CCNU; *TCD* T-cell depletion; *MP* methylprednisone; *MEL* melphalan; *TT* thiotepa; *FLU* fludarabine; *BU* busulfan; *PBSC* peripheral blood stem cells; *NS* not stated; *EFS* event-free survival; *DFS* disease-free survival; *NIMA* noninherited maternal antigen

Table 15-2. T-cell depleted haploidentical stem cell transplantation.

Center	Disease	Patient #	Conditioning	GVHD prophylaxis	EFS or DFS/OS	Reference
U. South Carolina	AML/ALL	201	TBI/VP-16/CY/ARA-C/ATG, CYA	Partial TCD, Steroids, ATG/MP	18%/19% at 5 years	(Mehta et al., 2004)
Basel Univ. Hospital	AML/CML/MDS	10	TBI/VP-16/CY or BU/CY+/−ATG	CYA+/−OKT3	30%/30% at 3–24 months	(Passweg et al., 2000)
Univ. of Perugia	AML/ALL	104	TBI/TT/FLU/ATG	TCD PBSC	48% (AML)[a] 46% (ALL)[a]	Aversa et al., 2005)
Canada (multicenter)	AML	11	MEL/TT/FLU/ATG	TCD PBSC	9%/9% at 9+ months	(Walker et al., 2004)
Emory	HM	28	ATG based	TCD PBSC	NS/7%	(Waller et al., 2004)
Teubingen	HM, NMD	63	TBI or BU + Cy/TT ± FLU	TCD PBSC	48% at 3 years (ALL, NHL in CR)/NS	(Lang et al., 2004)
Catholic Univ. of Korea (Seoul)	AML	11	TBI or MEL + BU/ATG/FLU	TCD PBSC	(Kim et al., 2005)	
Bristol, UK	AML/ALL/CML/MDS	34	TBI/CY+/−ATG	TCD PBSC+/−CYA+/−Alemtuzumab	(Marks et al., 2006)	
Duke	HM, solid tumors	49	CY, FLU + Alemtuzumab	MMF+/−CYA	(Rizzieri et al., 2007)	
MGH	AML, lymphoma	12	CY, Anti-CD2 Mab, Thymic XRT	CYA (≥35 days)+/−ex vivo TCD PBSC	(Spitzer et al., 2004)	

GVHD graft-versus-host disease; *EFS* event free survival; *DFS* disease free survival; *OS* overall survival; *NHL* non-Hodgkin's lymphoma; *AML* acute myeloid leukemia; *ALL* acute lymphoblastic leukemia; *MDS* myelodysplastic syndrome; *CY* cyclophosphamide; *BU* busulfan; *TT* thiotepa; *MEL* melphalan; *ATG* antithymocyte globulin; *Mab* monoclonal antibody; *XRT* irradiation; *TBI* total body irradiation; *BMT* bone marrow transplantation; *CYA* cyclosporine; *TCD* T-cell depleted; *PBSC* peripheral blood stem cells; *MMF* mycophenolate mofetil; *MGH* Massachusetts General Hospital

[a]AML/ALL in remission, EFS only

Table 15-3. Clinical applications of haploidentical stem cell transplantation.

Application	Indication	Methodology
Hematologic malignancy ■ Acute leukemia (in remission) ■ Non-Hodgkin/Hodgkin lymphoma ■ Myelodysplastic syndrome ■ Chronic lymphocytic leukemia ■ Other hematologic neoplasm	Lack of a suitably matched related, unrelated or cord blood stem cell source	Myeloablative vs. nonmyeloablative ex vivo and/or in vivo T-cell depletion ■ T-cell subset depletion ■ Selective allodepletion ■ T-cell anergization
Specific tolerance induction	Avoidance of long-term immunosuppression	Non-myeloablative conditioning, induction of transient mixed lymphohematopoietic chimerism
Cord blood transplantation	Enhancement of engraftment by third party haploidentical stem cells	Co-transplantation of highly purified haploidentical stem cells

describing the range of complications that could occur when HLA barriers were transgressed. Compared with outcomes of HLA genotypically identical BMT, an increased probability of grades II–IV GVHD was seen in the patients who received a nonidentical donor BMT. For patients with acute leukemia in remission, however, overall survival was not significantly different following HLA-matched and single antigen mismatched donor BMT. This outcome was believed to be the result of a stronger GVT effect of HLA-single antigen mismatched BMT balancing the harmful effects of acute GVHD. While the number of patients who received an HLA-2 or 3 antigen mismatched transplant was too small to reach meaningful conclusions, the outcomes of those patients were poor and suggested that the mortality risk of transplants involving donors who were greater than a single antigen mismatch with their recipient was unacceptably high.

The dramatic and frequently catastrophic complications of haploidentical-HSCT using myeloablative conditioning and pharmacologic (cyclosporine with or without methotrexate) GVHD pharmacoprophylaxis were well described by Powles, et al. in 1983 [99]. Thirty-five patients with advanced AML or ALL received an HLA-1 to 3 antigen mismatched related BMT following cyclophosphamide/total body irradiation (TBI) or cyclophosphamide/melphalan conditioning. Twelve patients died from a syndrome consisting of pulmonary edema, seizures, intravascular hemolysis and/or acute renal failure. Ten patients had primary graft failure requiring a second transplant from the same donor. This life threatening syndrome, likely reflecting an inflammatory cytokine cascade at the time of engraftment (or possibly graft rejection), clearly illustrated the immunological consequences of HLA-mismatched BMT.

Subsequently, a large International Bone Marrow Transplant Registry analysis of transplant outcomes following HLA-matched and mismatched related and unrelated donor BMT for AML, ALL and CML was performed. Transplant-related mortality was found to be significantly higher after HLA-1 or 2 antigen mismatched related or matched or single antigen mismatched unrelated donor BMT than after HLA-matched sibling BMT [101]. The large number (n=2055) of patients provided an analysis of outcomes according to the status of the leukemia at the time of transplant. Among patients with early leukemia, all alternative donor transplants were associated with a higher risk of transplant-related mortality than matched sibling donor transplants. The risk of treatment failure was also greater following alternative donor BMT. For patients with more advanced leukemia, treatment failure risk was similar for HLA-matched donor transplants and 1-antigen mismatched related donor transplants, and lower than the treatment failure risk following unrelated donor and HLA-2 antigen mismatched BMT. Although this study demonstrated the importance of the leukemia's status at the time of transplant and the impact of HLA matching on treatment failure risk and survival outcomes, the analysis was limited by the heterogeneous groups of patients (including, for example, both T cell depleted and non-T cell depleted transplants), and the use of exclusively serological methods of HLA typing (thus likely underestimating the HLA disparity that is now identifiable by molecular methods).

Drobyski, et al. at the University of Wisconsin compared the transplant outcomes of 139 patients with hematologic malignancies who received T cell depleted bone marrow transplants from HLA-matched unrelated, single-antigen mismatched unrelated and related haploidentical donors. There were no significant differences in engraftment rates or cumulative incidences of acute or chronic GVHD between the three groups. There was, however, a higher two-year cumulative relapse probability after related haploidentical BMT, and transplant-related mortality risk was higher in both the related haploidentical and single-antigen mismatched unrelated donor groups. Overall survival was superior in the HLA-matched unrelated donor group [114].

4. Recent Advances in Haploidentical HSCT with Myeloablative Conditioning

Ex vivo T Cell Depletion

The early experiences of haploidentical HSCT were notable for a high incidence of moderate to severe GVHD and mortality related to GVHD, and associated immunoincompetence. An abundant preclinical experience [115] and an early randomized clinical trial in patients undergoing HLA-matched BMT [116] showed that GVHD could be effectively prevented, albeit with a higher risk of engraftment failure and a higher risk of relapse of the underlying malignancy, following *ex vivo* T cell depleted BMT. Henslee-Downey and colleagues used TBI-based myeloablative conditioning with partial (1–1.5 log reduction) T cell depletion and post-transplant cyclosporine-based pharmacoprophylaxis [117]. *Ex vivo* T cell depletion was performed with either a T10B9 (anti-alpha beta T cell receptor) monoclonal antibody or OKT3 (anti-CD3) monoclonal antibody. Engraftment was demonstrated in more than

90 percent of their transplants, which was attributable to the intensive conditioning regimen and the incomplete T cell depletion. The incidence of acute GVHD was low in one recent report (with a 13 percent grade >I GVHD risk), and an encouraging survival probability (20%) was observed in patients with advanced hematologic malignancy.

Using high numbers of peripheral blood stem cells to overcome the problem of graft loss, and more vigorous T cell depletion to prevent GVHD, Aversa and colleagues demonstrated a very low incidence of GVHD and impressive event-free and overall survival probabilities following haploidentical-HSCT in patients with acute leukemia [118]. Conditioning therapy consisted of total body radiation, thiotepa, fludarabine and anti-thymocyte globulin. *Ex vivo* T cell depletion was performed using CD34+ cell selection (most recently with a Miltenyi CD34 cell selection device). With "megadose" peripheral blood stem cells containing a median of 13.6 (range 5.1 to 29.7) $\times 10^6$ CD34+ cells/kg, engraftment was reliably achieved with minimal GVHD. In a recently published experience, 104 patients with AML (n = 67) or ALL (n = 37) received a haploidentical T cell-depleted HSCT. Engraftment was ultimately achieved in 100 of 101 evaluable patients. Eight patients had ≥ grade II acute GVHD. Five of 70 patients developed chronic GVHD. A 38 percent non-relapse mortality risk, due primarily to opportunistic infection, occurred. As discussed above, KIR ligand mismatching in the GVH direction was shown to be associated with strikingly less relapse following transplantation for AML [33]. No reduction in relapse probability was observed for ALL, according to KIR ligand compatibility. High numbers of CD34 positive cells and CD8+ T veto cells were shown to facilitate engraftment via a veto effect which may have contributed to the favorable probability of sustained engraftment despite such a vigorously T cell-depleted stem cell graft [119, 120].

Several pediatric HSCT experiences have also shown favorable rates of sustained engraftment, a low incidence of GVHD, and reasonable immune reconstitution following vigorously T cell-depleted haploidentical SCT for nonmalignant hematologic disorders and hematologic malignancy [121–124]. Using positive selection of stem cells with CD34 or CD133-coated magnetic microbeads, or more recently, depletion of T and B-cells using CD3- and CD19-coated microbeads, Lang, et al. demonstrated primary engraftment in > 70 percent of their patients. Of 63 patients transplanted with CD34+ or CD133+ selected cells, 83 percent achieved stable primary engraftment (98% after re-transplant). Grade II – IV acute GVHD occurred in less than 10 percent of patients. Disease free and overall survival probabilities for patients with nonmalignant disease and hematologic malignancy (Non-Hodgkin's Lymphoma or ALL) in complete remission were 60 percent and 48 percent, respectively. A low (< 10%) incidence of fatal viral infections in recently transplanted patients was observed.

Marks and colleagues treated 34 children with acute leukemia and other hematologic malignancies with a TBI/cyclophosphamide and alemtuzumab or ATG conditioning regimen followed by high-dose T cell-depleted (either by CD34+ cell selection or *ex vivo* treatment with) PBSCT [125]. Cyclosporine for GVHD prophylaxis was used only for children who did not receive CD34+ cell selected grafts. Twenty-four patients died due to relapse or infection. Actuarial survival at two years was 26 percent. None of the nine patients with refractory AML survived.

Other centers that have evaluated myeloablative *ex vivo* T cell-depleted (by CD34+ cell selection) high-dose PBSCT for hematologic malignancy have had unfavorable survival outcomes, due to impaired immune reconstitution (resulting in opportunistic infection or relapse). Waller, et al. reported a mortality rate of 93 percent (26 of 28 patients), mostly due to infection or relapse, following ATG-based myeloablative conditioning and high-dose CD34+ cell selected PBSCT [126]. In a Canadian multicenter trial using myeloablative conditioning and CD34+ cell selected peripheral stem cell grafts, 10 of 11 patients died due to infection or leukemic relapse [127]. The reasons for the poor survival outcomes in these trials are unclear, but may reflect patient selection (i.e., the transplantation of patients with more advanced disease) or modifications of the treatment regimen (i.e., the *in vivo* use of ATG resulting in more profound immunodeficiency).

4.1. Induction of Alloantigen Specific Tolerance

Given the poor immune reconstitution following T cell-depleted haploidentical-HSCT, and based on *in vitro* experiments showing the induction of alloantigen-specific anergy by the co-culturing of host and donor bone marrow in the presence of CTLA-4-IG, Guinan and colleagues conducted a trial of *ex vivo* anergized haploidentical BMT in an effort to induce alloantigen specific tolerance [86]. *Ex vivo* anergy was demonstrated by measuring precursor T cell frequencies before and after *ex vivo* treatment of the marrow graft. A multiple log decrease in anti-recipient precursor helper T cell frequency was shown after *ex vivo* anergization. There was, however, no significant change in anti-third party precursor helper T cell frequency. Twelve patients with advanced hematologic malignancy received TBI-based myeloablative conditioning and an *ex vivo* anergized bone marrow graft. Three patients developed gastrointestinal GVHD. Five of 12 patients were alive and disease free from 4.5 to 29 months post-transplant.

4.2. Recent Advances in Haploidentical-HSCT with Nonmyeloablative Conditioning

Nonmyeloablative (reduced-intensity) conditioning for HSCT is associated with significantly less transplant-related toxicity and early mortality than myeloablative conditioning, allowing for the transplantation of older patients and patients with significant comorbidity [103–105, 128]. By various mechanisms including less pro-inflammatory cytokine production and possibly preservation of host "regulatory" hematopoietic elements, nonmyeloablative transplants are likely complicated by less GVHD [129]. A potent GVT effect may be achieved, following either spontaneous or donor lymphocyte infusions (DLI) induced conversion of mixed lymphohematopoietic chimerism to full donor hematopoiesis [104, 130].

4.3. Nonmyeloablative Haploidentical Strategies Using *in vivo* T cell Depletion

Sykes, et al. have shown in murine MHC-mismatched transplant models that mixed lymphohematopoietic chimerism can be reliably induced following nonmyeloablative conditioning, vigorous *in vivo* T cell depletion with anti-CD4

and CD8 monoclonal antibodies and thymic irradiation [130]. Remarkably, these mixed chimeric mice are resistant to the induction of GVHD following delayed DLI, despite a potent lymphohematopoietic GVH response, which converts their mixed chimerism to full donor hematopoiesis. Moreover, a more potent GVT effect has been demonstrated in murine mixed chimeras who convert to full donor chimerism after DLI, compared to full donor chimeras given DLI (with the enhanced antitumor effect shown to result from the preserving host professional antigen presenting cells) [131, 132].

Based on these murine models, a series of haploidentical-HSCT clinical trials have been conducted at the Massachusetts General Hospital (MGH). The initial trials involved cyclophosphamide, equine ATG for *in vivo* T cell depletion and pre-transplant thymic radiation [133]. Cyclosporine was given as GVHD prophylaxis and was tapered by day 35 post-transplant in the absence of GVHD. Delayed DLI were given to patients with mixed chimerism and without GVHD. Due to a high incidence of severe acute GVHD in the initial cohort of patients, MEDI-507 (a monoclonal anti-CD2 antibody) (Med Immune, Inc.; Gaithersburg, MD) was substituted for ATG in subsequent trials [134]. A series of protocol revisions have since been made, including the use of *ex vivo* T cell depleted PBSC (rather than bone marrow), changes in the dose and schedule of MEDI-507, and the addition of fludarabine to address the problems of GVHD and graft rejection. Mixed "split lineage" lymphohematopoietic chimerism has been achieved in all of the patients treated with *ex vivo* T cell-depleted PBSC, usually with an early predominance of donor myeloid chimerism, and a much lower percentage of donor T cell chimerism. In the majority of these patients mixed chimerism in all lineages has converted to full or nearly full donor chimerism, either spontaneously or following DLI. While GVHD has occurred in most of the patients following conversion to full donor chimerism, it has been manageable in most cases. Striking antitumor responses among patients with chemorefractory hematologic malignancies have also been achieved.

An unexpected observation in these clinical trials, as well as HLA-matched nonmyeloablative HSCT trials at the MGH, was durable antitumor responses in some patients with chemorefractory hematologic malignancies occurred despite loss of the hematopoietic graft [135]. Nine of 22 (41%) patients who received an HLA-matched or haploidentical nonmyeloablative SCT achieved a response after loss of their graft. Six patients were alive from 2.5 to 5.5 years post-transplant, four of whom were in a sustained complete remission. Observing ongoing tumor regression after serial DLIs in a patient who lost his graft, accompanied by "spikes" in circulating host CD8 T cells, raised the intriguing possibility that a host-specific antitumor response was induced. In an effort to elucidate the mechanism of these antitumor responses Sykes and colleagues established a murine nonmyeloablative transplant model where recipient lymphocyte infusions (RLI) were administered to cause graft rejection, followed by tumor challenge with host strain-specific malignant cells. A survival benefit was observed in the mice that developed mixed chimerism followed by RLI-induced graft rejection, compared to mice that received conditioning only, conditioning and transplant, or conditioning and RLI alone. The antitumor response has been shown to be mediated by RLI-derived interferon-gamma producing CD8 cells and recipient CD4 cells [136, 137].

Investigators at Duke University Medical Center have used anti-CD52 monoclonal antibody therapy (alemtuzumab) for both *ex vivo* and *in vivo*

T cell depletion in a clinical trial of haploidentical nonmyeloablative HSCT for hematologic malignancies and selected solid tumors. Conditioning consisted of melphalan, fludarabine and alemtuzumab followed by infusion of alemtuzumab treated PBSC. GVHD prophylaxis consisted of MMF for 45 days post-transplant. Of 35 patients, seven developed grade II–IV acute GVHD, and seven chronic GVHD. Three patients experienced primary graft failure. Nine of 12 patients who received DLI developed grade ≥ II GVHD. Complete remission rate and survival at more than three months were 57 percent and 71 percent, respectively (Chao N., personal communication).

4.4. Nonmyeloablative Haploidentical HSCT Involving Depletion of Alloreactive T cells by Post-Transplant Cyclophosphamide

Based on canine experiments in which high-dose post-transplant cyclophosphamide was effective in depleting alloreactive (both in the GVH and HVG direction) T cells, O'Donnell and colleagues performed a phase I trial to determine the minimal conditioning to achieve stable engraftment after an HLA-1–3 antigen mismatched BMT [138]. Thirteen patients with hematologic malignancy received low-dose TBI, fludarabine +/− cyclophosphamide conditioning, and high-dose cyclophosphamide on day + 3 post-transplant. Nine of 13 patients achieved sustained engraftment. Six of 13 patients developed acute GVHD. Six of 13 patients were alive, five in complete remission at a median of 191 days post-transplant.

In an updated report of their experience involving 84 patients treated on this protocol, 16 patients (19%) experienced graft rejection; 14 of those patients recovered autologous hematopoiesis. Interestingly, and consistent with the observations of our group, of the 16 patients who had graft loss, seven achieved an antitumor response, including three of six patients with myelodysplastic syndrome, two of three patients with CML, and both patients with chronic myelomonocytic leukemia [139].

4.5. Selective Allodepletion

Nonselective depletion of T cells from the allograft prior to transplantation effectively prevents severe acute GVHD, but invariably predisposes the recipient to loss of the graft, disease relapse, and an increased incidence of infectious complications. Unmanipulated T cell add-backs are not likely to be effective in preventing these problems without causing GVHD, since the frequency of alloreactive T cells in the peripheral blood are far greater than that of either specific antiviral or antileukemic T cells. One approach to overcome these adverse outcomes is to selectively deplete the graft of the GVHD-causing alloreactive T cells identified by upregulation of activation markers, while conserving cells mediating GVT and antimicrobial immune responses. Several methods of selectively removing alloreactive T cells, which rely on the *ex vivo* stimulation of donor T cells by recipient peripheral blood mononuclear cells (PBMC) in a unidirectional mixed lymphocyte reaction (MLR) culture, have been reported. Host-reactive donor T cells can then be identified by their expression of activation markers (CD25, CD69, CD71), proliferative potential or preferential retention of photoactive dyes, and can be subsequently targeted

for depletion using a variety of methods including an immunotoxin [140, 14], immunomagnetic bead separation [142–145], fluorescence-activated cell sorting [146, 147], photodynamic purging [148, 149] or by Fas-Fas ligand-mediated apoptosis [150]. These selective allodepletion methods have yielded 70 percent to 95 percent reductions in alloreactivity *in vitro* with the retention of immune responses against third party and infectious organisms, with several showing retained alloreactivity against leukemia cells [148, 151–153]. While this promising approach has been found to be feasible in clinical trials [154, 155], several concerns have been raised, including contamination of recipient PBMC by leukemic cells, loss of antileukemic activity and a the induction of clinically significant GVHD. In a recent clinical trial, Amrolia, et al. used different cell doses for allodepleted (via immunotoxin) T cell add-back and demonstrated that patients at a dose level of 10^5 CD3 cells/kg exhibited significantly more rapid recovery of T cells compared with patients receiving 10^4 CD3 cells/kg at three to five months after haploidentical HSCT [156]. The incidence of GVHD was very low and the median time to reach a CD4+ cell count greater than 300/μL was four months in patients at a dose of 10^5 CD3 cells/kg, compared with more than six months in patients at a dose of 10^4 CD3 cells/kg and eight months in the series by Eyrich, et al. without allodepleted T cell add-back. A similar improvement in immune reconstitution without GVHD has been recently demonstrated by another group who used photodynamic therapy (PDT) for selective depletion of alloreactive T cells [157]. However, no matter how small the alloreactive T cell subset of the total T cell population is (<0.1% in the HLA-matched sibling setting; 1%–5% in the HLA-mismatched setting), their complete elimination may be difficult, and doses of unmanipulated T cells as low as 3×10^4/kg can be associated with severe GVHD following haploidentical-HSCT. To address the removal of CD4+CD25+Foxp3+ Tregs along with the depletion of alloreactive T cells, which may negatively impact the development of GVHD, Barrett and colleagues at the NIH demonstrated that selective depletion of alloreactive T cells preserves a CD25−CD4+Foxp3+ fraction of T cells that is capable of undergoing marked expansion post-transplant to Tregs, thereby providing protection against GVHD [158].

5. Non-T Cell-Depleted Blood Stem Cell Transplantation Revisited

Because of the historically high incidence of GVHD and transplant-related mortality following non-T cell-depleted haploidentical-HSCT, most of the recent efforts in haploidentical SCT have focused on *ex vivo* T cell or T cell subset depletion. Recently, however, several groups have reported impressive survival probabilities following non-T cell-depleted PBSCT. Common to these approaches, however, has been the use of polyclonal ATG for *in vivo* T cell depletion.

Lu and colleagues [159] treated 135 patients with a variety of hematologic malignancies with busulfan, cytarabine, cyclophosphamide, rabbit ATG (on days −5 through −2) and non-T cell-depleted bone marrow and/or peripheral blood stem cell transplantation. GVHD prophylaxis consisted

of MMF, cyclosporine and methotrexate. All patients had full donor chimerism at day 30 post-transplant. The cumulative incidence of grades II to IV GVHD was 40 percent and the two-year incidence of transplant-related mortality was 22 percent. The two-year incidence of relapse was 18 percent. Infectious complications included CMV interstitial pneumonitis in 17 percent and hemorrhagic cystitis in 35 percent of patients. Two-year leukemia-free and overall survival probabilities were 64 percent and 71 percent, respectively.

Ogawa, et al. [160] used reduced-intensity conditioning (busulfan, fludarabine, and rabbit ATG) as preparation for HLA-2 to 3 antigen-mismatched non-T cell-depleted PBSCT of 26 patients with high risk hematologic malignancies. GVHD prophylaxis consisted of tacrolimus and corticosteroids. Serum sIL-2R levels were followed and steroid tapering was based in part on the results of these assays. Twenty-five of the 26 patients achieved full donor engraftment. Five patients developed grade II GVHD and five of 20 evaluable patients developed chronic GVHD. Transplant-related mortality was 15 percent (four of 26 patients). CD4+cell recovery was slow, with a median cell count of greater than 100/uL at nine months. Fifteen of the 26 patients were alive and in complete remission at a median of 664 days post-transplant.

The surprisingly low incidence of GVHD in these series compared to historical experiences of non-T cell-depleted HSCT might reflect the use of ATG for *in vivo* T cell depletion, improved GVHD prophylaxis strategies and better prophylaxis and treatment of opportunistic infections. The importance of other factors, such as the use of donors mismatched for non-inherited maternal antigens (vide infra) remains to be determined in future clinical trials of non-T cell-depleted haploidentical HSCT.

6. Haploidentical SCT Based on Feto-Maternal Microchimerism

Several transplant centers have developed haploidentical-HSCT strategies based on the principle of tolerance induction as the result of *in utero* exposure to maternal antigens and the development of long lasting feto-maternal microchimerism [161]. A large International Bone Marrow Transplantation Registry (IBMTR) analysis by van Rood and colleagues showed that the incidence of grade ≥ II acute GVHD following non-T cell-depleted haploidentical-HSCT was related to haplotype inheritance [162]. Transplants from a non-inherited maternal antigen mismatched (NIMA) sibling were associated with significantly less acute GVHD. Transplant-related mortality, moreover, was significantly higher in transplants from a maternal or a paternal donor. Several Japanese transplant centers have performed non-T cell-depleted transplants following either myeloablative or nonmyeloablative conditioning from microchimeric NIMA mismatched donors [163–166]. While the overall incidence of grade ≥ II acute GVHD incidence was high (56 percent of 34 evaluable patients) in one series, a significantly lower risk of acute GVHD was observed in patients who received a transplant from a donor who was NIMA mismatched in the GVH direction.

7. Adoptive Cellular Immunotherapy after Haploidentical-HSCT

Adoptive cellular immunotherapy via delayed DLI has been shown experimentally and clinically to be a potent means of converting mixed chimerism to full donor hematopoiesis and capturing a potent GVT effect after HSCT [167, 168]. In patients with recurrent CML after HLA-matched HSCT, complete clinical and molecular remissions are achieved in the majority of patients following the administration of DLI [169, 170]. A high incidence of GVHD (and marrow aplasia) has tempered the use of this approach, however, and has led to the revision of DLI strategies (e.g., by changing the schedule and/or lowering the dose of infused T cells). The GVHD risk is higher after alternative donor transplantation, and much smaller doses of T cells have usually been used in the haploidentical-HSCT setting. Lewalle, et al. reported that "prophylactic" CD3+ T cell doses of greater than 1×10^4 every three months caused significant GVHD in recipients of B- and T cell-depleted myeloablative PBSC transplants [171]. In our nonmyeloablative haploidentical SCT trial, in which vigorous *ex vivo* and *in vivo* T cell depletion are employed, CD3+ T cell doses of greater than 1.0×10^6/kg have been given in some circumstances with either absent or manageable cutaneous GVHD [134]. Additional studies will be required to establish a safe and effective DLI T cell dose and schedule following haploidentical-HSCT. Identifying specific cellular populations that can be administered as adoptive cellular immunotherapy and that are capable of inducing a strong GVT effect without GVHD is of greater importance.

8. Haploidentical HSCT: New Applications

8.1. Specific Tolerance Induction

Achieving donor-specific tolerance has enormous implications for the field of organ transplantation, which is currently limited by the complications of lifelong immunosuppressive therapy. Multiple preclinical small and large animal models have shown that sustained specific tolerance can be induced following the induction of even transient lymphohematopoietic chimerism [172, 173]. Based on these preclinical observations, we have initiated clinical trials of combined related donor bone marrow and kidney transplantation for patients with end-stage renal disease. In the first of these trials, combined HLA-matched bone marrow and kidney transplantation was offered to patients with multiple myeloma and renal failure. Of six patients who received the combined transplant, three are currently in a complete remission (including one who received a second myeloablative transplant from the same donor) from approximately three to eight years post-transplant. Five of the six patients have normal renal function with two patients completely off of immunosuppressive therapy [107, 174].

In an effort to broaden this application's strategy, we have begun a trial of haploidentical combined bone marrow and kidney transplantation for patients with end-stage renal disease without an underlying malignancy. The preparative therapy was used in earlier trials of haploidenitcal nonmyeloablative HSCT for hematologic malignancies and was notable for the uniform development of transient mixed chimerism and the absence of GVHD. Of the first four patients who received a combined haploidentical transplant, three are off immunosuppressive

therapy with essentially normal renal function from one to four years post-transplant. Evidence of specific tolerance *in vitro* has also been established [173].

Thus, haploidentical combined bone marrow and solid organ transplants are feasible and the proof of principle of specific tolerance induction has been demonstrated clinically. Future trials will likely include the transplantation of other organs, including cadaveric organs.

8.2. Combining Haploidentical PBSC with Umbilical Cord Blood for Transplantation

Umbilical cord blood transplantation, because of the relatively low number of hematopoietic progenitor cells collected and transplanted, has been complicated by slow hematologic recovery and immune reconstitution. In an attempt to hasten hematologic recovery and reduce the complications of poor immune recovery, Fernandez, et al. co-transplanted 11 patients with cord blood cells and CD34+cell selected haploidentical PBSC [175]. Prompt engraftment occurred at a median of 10 (range nine to 17) days. Chimerism studies showed a predominance of the haploidentical genotype in the granulocyte and mononuclear lineages early post-transplant, followed by the progressive replacement by cells of the umbilical cord donor origin. Grade ≥ II GVHD occurred in four patients. Five patients were alive and in complete remission from six to 43 months following HSCT. More recent studies evaluating immune reconstitution showed early recovery of NK 1 cells and B-cells; CD4+ and CD8+-cell recovery was delayed but eventually complete [176].

9. Future Directions

Major strides have been made in our understanding of the immune effectors cells and soluble factors, and the kinetics of their recovery, that affect outcomes following allogeneic stem cell transplantation. Haploidentical-HSCT will hopefully have its therapeutic potential realized, but novel translational approaches that have been carefully evaluated in preclinical models and show considerable potential in improving immune reconstitution and promoting a separation of GVHD and GVT effects, need to be evaluated in clinical trials. First, “megadoses” of TCD hematopoietic progenitors, that can be more efficiently procured in the future with a combination of hematopoietic growth factors and selective chemokine antagonists, along with selective but rigorous allodepletion of the graft will hopefully enable both sustained donor engraftment following nonmyeloablative conditioning and substantial immune recovery while acute GVHD is absent or maximally reduced. *Ex vivo* expanded immunomodulatory cells such as Tregs, NK/Tregs, MSCs and donor-derived NK cells, as well as various soluble factors may be effectively utilized to further enhance engraftment and immune reconstitution of both innate and adaptive immunity, and to favorably modulate alloresponses aimed at capturing GVT effects with acceptable or no GVHD. Unmanipulated DLI can be considered at certain timepoints to convert the mixed chimerism to full donor hematopoiesis in an attempt to optimize its full therapeutic promise, i.e., GVT effect with little or no GVHD. Finally, the adoptive transfer of allogeneic T cells that are specific for viral or tumor antigens may be additional strategies to address the obstacles of infection and disease relapse.

The clinical experience of haploidentical-HSCT has been instrumental in elucidating the challenges of overcoming powerful genetic barriers to sustained engraftment of donor stem cells without GVHD, and the problems of delayed and incomplete immune reconstitution. The future of this field will rely heavily on our sophisticated knowledge of the cellular environment that exists after haploidentical-HSCT, and how to manipulate that environment to optimize clinical outcomes.

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Chapter 16

Ablative Preparative Regimens for Hematopoietic Stem Cell Transplantation

Amin Alousi and Marcos de Lima

The combination of chemo-, radio- and biologic therapies in preparation for hematopoietic stem cell transplantation (HSCT) is referred to as conditioning (or preparative) regimen. As there is no one "standard" regimen, choosing the ideal regimen should take into account numerous factors including: stem cell source (autologous versus allogeneic), diagnosis, disease stage at the time of transplant, prior therapies, and comorbidities. There are few randomized studies comparing regimens and the majority of the data comes from phase 2 or retrospective analysis. Lacking clear-cut evidence supporting the superiority of one conditioning regimen over another, the choice is often based on institutional preference and experience. This chapter will review our current knowledge regarding ablative preparative regimens and some of the controversies that surround the field.

Conditioning Regimens: Goals of Pre-Transplant Therapy

The sole function of the conditioning regimen in the autologous transplant setting is eradication of any residual cancer. As such, dose density is employed to overcome tumor resistance. In the allogeneic setting, the regimen may serve this function, but it must also provide immunosuppression to prevent rejection and create "space" in the marrow microenvironment to allow for engraftment [1]. Traditionally, regimens were designed to provide the highest, tolerable dose of therapy with the hope of more successfully eradicating the malignancy. Agents for which the dose-limiting toxicity is hematologic were chosen as the infusion of hematopoietic cells would circumvent myelotoxicity. Combinations were developed based on non-overlapping side effects and in the allogeneic setting, to allow the inclusion of agents with immunosuppressive properties. Research in this field has, at times, had two competing focuses: reduction of relapse and toxicity. Nonmyeloablative allogeneic transplants have come into design with the goal of impacting the latter. These types of transplants rely, to different degrees, on the therapeutic potential of the donor cells in their Graft-versus-Tumor Effect. They are designed to provide enough immunosuppression to allow for engraftment, but provide less intense anti-malignancy therapy to reduce toxicity. It is accepted that a truly

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nonmyeloablative regimen should allow for autologous marrow recovery in the majority of patients if allogeneic stem cells are not given.

Historical Background

The field of HSCT grew out of studies on the effects of radiation exposure, often for warfare purposes. Researchers in the 1920s established the hematopoietic system as the most radio sensitive normal tissue. This became all too apparent during World War II, when approximately 10 percent of the atomic bomb victims died as a direct result of bone marrow aplasia. In the 1950s, Jacobsen demonstrated that mice could be protected from otherwise lethal doses of irradiation by shielding the spleen [2] or as reported by Lorenz, intravenous infusion of marrow [3]. In 1957, Thomas and colleagues published the outcomes of six patients with advanced malignancies who were given infusion of bone marrow after receiving limited doses of chemotherapy or radiation [4]. They determined the safety of marrow infusion; two of six patients engrafted. In retrospect, this small study was instrumental in demonstrating that, for engraftment to occur, effective conditioning must be administered. The following year, six physicists in Vinca, Yugoslavia were exposed to lethal doses of irradiation in a nuclear reactor accident. Mathe, et al. treated five of the six victims with bone marrow transplantation (BMT). Four of the five survivors had transient engraftment without Graft-versus-Host Disease (GVHD) [5]. They subsequently turned their efforts into therapeutic trials of BMT following (this time purposeful) irradiation doses in patients with leukemia [6]. Throughout the next decade, transplantation continued to be studied, primarily in terminally ill patients with refractory malignancies.

The modern era of transplantation began in Seattle [7, 8]. Based on rodent studies, researchers determined that the most effective conditioning regimen to allow for engraftment would be one that employed total body radiation (TBI), cyclophosphamide (Cy) or a combination of both [9]. Historically, most transplant recipients received TBI-containing regimens, usually in combination with cyclophosphamide, based on these original studies. These early trials in patients with refractory leukemia established that this combination provided the highest leukemic cell kill, compared to either agent given alone, and provided adequate immunosuppression to prevent graft rejection. They also indicated that without successful cytoreduction prior to transplant, the vast majority of patients will relapse. Therefore, they began applying this therapy earlier in the disease process, often after achieving a complete remission. While the focus of this chapter is on conditioning regimens, perhaps the greatest advancements in this field did not come solely through the perfection of regimens, but also via a better understanding of Histoincompatibility issues, prevention and treatment of GVHD and supportive care of patients with bone marrow aplasia [10].

Total Body Irradiation-Based Regimens

Despite years of experience with the use of TBI-containing regimens, there is great debate regarding the fundamental questions of administration dose and fractionation. TBI can be administered as a single-dose (STBI), fractionated

(FTBI) (multiple single-daily doses) or hyperfractionated (HFTBI) (multiple twice-daily or more doses). Fractionating the dose of irradiation is thought to be less toxic to healthy tissue allowing for escalation of the total dose. Thomas, et al. were the first to report superior results of FTBI over STBI. A FTBI regimen of 12 Gy in six fractions was found to improve survival over a STBI regimen administered as 10 Gy in a single fraction in patients with acute myeloid leukemia (AML) in first complete remission (CR) [1]. This was supported by a second trial performed by Deeg, et al., which demonstrated that a similar FTBI regimen resulted in fewer relapses, less toxicity (especially interstitial pneumonia and veno-occlusive disease (VOD) of the liver) and had superior overall survival when compared to STBI delivery [12]. HFTBI was examined as a way of further escalating the dose beyond the maximally tolerated dose achieved by S or FTBI. A prospective randomized study suggested that a HFTBI regimen of 14.85 Gy administered in 11 fractions over five days was equally as safe as a STBI dose of 10 Gy with less hepatic VOD and relapse [13]. Hyperfractionated schedules employing high energy sources combined with etoposide have allowed for dose escalation up to 14.4 Gy administered over five days in nine fractions [14]. Dose escalation studies in patients with advanced lymphoid malignancies of unshielded HFTBI combined with Cy 120 mg/kg have determined the safest dose in adults to be 13.2 Gy administered in thrice daily fractions (children may tolerate a higher total dose of 14.4 Gy) [15]. Lacking standardization across studies with reference to energy source, shielding, schedules and concomitant chemotherapy, it is difficult to draw definitive conclusions regarding the ultimate benefit of fractionation schedules. However, most centers that employ TBI-based therapy use some form of fractionation. Conflicting results remain with respect to the optimum dose for TBI- based regimens as well. The largest randomized studies examining the effect of dose escalation were performed at the Fred Hutchison Cancer Center. These studies in patients with AML in first remission and chronic myeloid leukemia (CML) in chronic phase showed that higher doses of TBI resulted in a decrease in the relapse risk, at the expense of higher treatment-related mortality, GVHD, hepatic VOD, interstitial pneumonia, renal failure and mucositis [16, 17]. The net effect was no improvement in survival for patients who received 15.75 Gy of TBI, compared to those who received 12 Gy. Researchers at the Dana Farber Institute examined whether dose escalation in the setting of T cell-depleted allogeneic transplants would improve overall survival by reducing deaths attributable to GVHD [18]. They examined two dose levels (14.8 and 15.6 Gy) and compared these results to a historical group who received 14 Gy. T cell depletion did not protect against the toxicity of the increased doses of TBI, and recipients of 15.6 Gy had higher acute GVHD and overall treatment-related mortality. Furthermore, the higher doses of TBI did not decrease relapse rates. The best overall survival rates were seen in the patients that received the lowest dose of TBI.

Many of the factors limiting TBI dose escalation are unique to the allogeneic setting (such as GVHD, which may be initiated by radiation-induced inflammation and tissue damage). Furthermore, interpretation of toxicity attribution may be confounded by the co-administration of Cy. A ‘pure’ TBI dose escalation study was performed using partial shielding of the lung and kidneys prior to autologous transplants for refractory lymphoid malignancies. In that setting, TBI dose was escalated to 20 Gy [19]. While high-dose TBI is seldom

given as a single agent, this study determined the non-hematologic toxicity of TBI. Peterson, et al. examined the tolerability of unshielded fractionated TBI in combination with Cy 120 mg/kg as conditioning for autologous or allogeneic transplants, and determined the maximum tolerated dose (MTD) to be 16 Gy [20].

Variables apart from dose and fractionation may also be important and, if nothing else, make direct comparison of TBI regimens problematic. As an example, shielding the lungs may reduce the incidence of pulmonary complications [21]. This benefit is most pronounced in patients who have pre-existing combined ventilation/diffusion capacity impairment. Kidney shielding is also suggested when using TBI regimens that result in biologically effective doses to the kidneys greater than 16 Gy [22]. Some investigators have recommended shielding the eyes to reduce the incidence of TBI-associated cataracts (especially in single-dose regimens) [23]. While many questions remain unsettled regarding dose, fractionation and delivery, Cy 120 mg/kg and TBI (9.2 to 15.75 Gy) continues to be one of the most frequently used preparative regimens. It provides enough immunosuppression for engraftment of hematopoietic stem cells from HLA-identical siblings, haploidentical, matched unrelated bone marrow or cord blood donors. While it is still used for various diseases, it remains a favored regimen in patients with acute lymphocytic leukemia (ALL) (often combined with etoposide) [24].

Non-TBI Containing Regimens

Toxicities such as cataracts, organ damage and impaired growth and development in children prompted the development of non-TBI containing regimens. Chemotherapy-only regimens could be performed at centers lacking radiation capabilities, and for patients that previously received dose limiting radiation therapy. Santos and collaborators sought to replace TBI in the early 1980s [25] by combining Cy to Busulfan (Bu). The somewhat limited immunosuppressive properties of Bu were, to a large extent, compensated by Cy, as shown in a series of preclinical studies [26, 27]. Santos used Bu at a dose of 16 mg/kg divided orally every six hours over four days, combined with Cy 200 mg/kg divided in four daily doses (BuCy4). This regimen was too toxic and was later modified to decrease the dose of Cy to 60 mg/kg for two days (BuCy2) which, together with Cy-TBI, is one of the most used conditioning regimens in HSCT [28].

Randomized Studies of BuCy2 Versus CyTBI

The safety and efficacy of the BuCy2 regimen when compared to Cy-TBI has been evaluated in randomized studies described here. Devergie, et al., on behalf of the French Society of Bone Marrow Graft, performed a multi-institutional study of 120 patients (median age of 36 years) with CML in first chronic phase randomized between BuCy2 or Cy-TBI [29]. The majority of the patients who were randomized to TBI received fractionated TBI with a smaller percentage receiving STBI. With a median follow-up of 42 months, no differences in overall or relapse-free survival, toxicity or acute GVHD were identified between the groups. Similar findings were reported from a

prospective, randomized study performed by Clift, et al. [30]. This study randomized 69 patients with CML between these two regimens. TBI was administered in six fractions of 2Gy. There were no differences in event-free or overall survival between the groups. The BuCy2 regimen appeared to be better tolerated with less acute toxicity including acute GVHD. Ringden, et al. from the Nordic BMT Group performed a randomized, multi-institutional study in 167 patients with leukemia or lymphoma of various types and stages. Radiation was administered per each institution's protocol [31]. BuCy2 produced more treatment-related mortality due to higher incidences of hepatic VOD, hemorrhagic cystitis, fatal acute GVHD and higher rates of chronic GVHD and alopecia. Survival was improved by use of Cy-TBI. Relapse rates were similar. The difference in seven-year transplant-related mortality was most pronounced in those patients with advanced disease (64% versus 22%, $p = 0.004$) [32]. The French Study Group performed a prospective, randomized study comparing these two regimens in 101 patients with AML in CR1 [33, 34]. This study found better treatment-related mortality, relapse-free and overall survival in patients that received the Cy-TBI regimen that elicited the unusually high survival rate of 75 percent and a low treatment-related mortality rate of 8 percent.

Hartman, et al. performed a meta-analysis evaluating the above four randomized trials plus a fifth trial comparing BuCy2 to TBI-etoposide in patients with various leukemias and determined that there was a significantly higher risk of VOD with the BuCy2 regimen, but not for other toxicities or GVHD [35, 36]. Survival and disease-free survival were better with the Cy-TBI regimen, although this did not reach statistical significance. A second combined analysis was performed by Socie, et al. who updated the results of these studies [37]. No difference in overall or disease-free survival was found between the two regimens for CML patients, but a statistically nonsignificant 10 percent survival advantage for Cy-TBI was identified among AML patients. Chronic GVHD rates were similar, but BuCy elicited higher rates of alopecia and TBI caused more cataracts. The major limitation of these trials is the use of oral busulfan. Large variations in Bu levels after oral administration, which can lead to under- and overdosing, respectively exposing patients to graft rejection and relapse, or to toxicity, make the comparison of these regimens intrinsically flawed. The studies have the young age of participants in common and caution is advised when extrapolating those conclusions to older subjects. Nevertheless, despite these otherwise properly conducted randomized studies, it remains unclear whether there is any significant clinical difference between oral Bu versus TBI-based conditioning. It is highly likely that recent modifications to the BuCy regimen (that include pharmacokinetic-guided Bu dosing and the use of an intravenous formulation) negate any potential small advantage of Cy-TBI, at least in the treatment of myeloid leukemias.

Bu Pharmacokinetic-Guided Dosing and Intravenous Formulation

One of the dose limiting Bu side effects is hepatotoxicity. It has been well documented that oral dosing of Bu results in frequent inter- and intra-patient variability in steady state drug concentrations. The area under the concentration

time curve (AUC) can vary 3- to 7-fold in patients given oral Bu based strictly on a weight or body surface area (BSA) calculation [38–42]. High blood levels of Bu have resulted in higher incidences of hepatic VOD [43], while low AUC may be associated with relapse in CML [44] and lower engraftment rates [45–47]. It has been hypothesized that, by effectively reducing this variability, results with Bu containing regimens might be improved. To this end, pharmacokinetic (PK)-guided oral dosing and, lately, an intravenous (IV) formulation of the drug was successfully tested.

The case for Bu PK-guided dosing is even stronger for patients less than four-years-old that have increased clearance of the drug when administered orally due to a higher hepatocyte glutathione conjugation rate [48]. Bolinger, et al. performed one of the earliest trials evaluating targeted dosed Bu in pediatric patients (ages 0.6 years to 18.5 yrs) undergoing allogeneic transplantation [49]. Patients received a test dose of 0.5 mg/kg oral Bu five days prior to admission followed by PK sampling. This information was used to calculate a dose of oral Bu that would achieve a target steady state concentration of 600–900 ng/ml. Non- standard dosing was required in 74 percent of the patients which resulted in 94 percent of the patients successfully achieving the target concentration. This strategy improved the engraftment rate from 74 percent to 94 percent ($p = 0.043$) when compared to a historical control group treated with standard dosed Bu. Bleyzac, et al. documented a lower incidence of hepatic VOD, while others have obtained significant decreases in treatment-related mortality using similar strategies [50–52].

In 1996, a patent was filed for an IV formulation of Bu which was solubilized in a cosolvent mixture of anhydrous dimethylacetamide (DMA), polyethylene glycol 400 (PEG400), and water at a ratio of 1:2:2 (v/v/v) [53]. In 2000, Andersson, et al. at M.D. Anderson Cancer Center determined that a dose of 0.8mg/kg body weight was the IV bioequivalent dose to the oral dose of 1mg/kg [54]. A Phase 2 study suggested that IV Bu-based conditioning resulted in a lower incidence of VOD and a reduction in treatment-related mortality when retrospectively compared to patients treated during the same period of time with the standard oral BuCy2 regimen [55]. The IV formulation resulted in higher inter- and intra-patient consistency with 86 percent of the patients maintaining an AUC between 800 and 1,500 uMol-min [56]. Similar consistency can be obtained with careful dose adjustment of oral Bu, at the expense of multiple PK measurements [57]. Other nonrandomized studies have found similarly low incidences of hepatic VOD and mortality using IV BuCy [58]. Andersson, et al. proposed a therapeutic window for IV Bu when combined to Cy within an AUC of 950–1,520 uMol-min every six hours [59]. It is estimated that 10 percent of the patients may be below the proposed therapeutic window unless adjustments are made, while approximately 10 percent will be above that window with IV Bu, given genetic variations in drug metabolism. These figures are probably twice as high with the oral drug.

Dose Intensification

In an attempt to decrease relapse, additional agents have been added to the Cy-TBI or BuCy regimen. The most common additions have been cytarabine [60], etoposide [61–63], thiotepa [64] or melphalan [65]. Others have given

both Bu and reduced dose TBI [66], often along with Cy [67]. Many of these regimens have been to be too toxic and there is no proven benefit of adding a third agent [68, 69].

The Busulfan-Fludarabine Regimen

Concomitant use of Cy and Bu magnifies the potential for hepatic toxicity due to a variety of factors including competition for the glutathione detoxification mechanism. Fludarabine (Flu) has well known immunosuppressive properties and has been used for many years in allogeneic transplants. It also inhibits DNA repair processes triggered by alkylating agents, resulting in *in vitro* synergy [70]. Investigators have proposed that by replacing Cy with Flu, it would be possible to administer Bu as a single daily dose. This will, in turn, elicit a significantly higher AUC, perhaps benefiting leukemic patients and reducing toxicity.Preliminary experience with the regimen confirms the decrease in toxicity [71–74]. In our institution, the 100-day and one-year regimen-related mortality of patients with AML in CR (median age of 48 years) is 2 percent and 7 percent, respectively. The Bu dose is 130 mg/m^2 IV and Fludarabine 40 mg/m^2 once daily for four days. Among the initial 96 patients so treated, only two developed moderate, reversible hepatic VOD, despite a Bu median daily AUC of 4,891 uMol-min. Many questions remain about giving Bu in this in this daily fashion. It is unknown if the therapeutic window falls in the range of 5,000 uMol-min (as the median AUC would indicate), or if a higher AUC will benefit patients with more advanced disease. Preliminary data at our institution indicates that targeting an AUC of 6,000 uMol-min is both feasible and safe, with mucositis as the most common side effect. This question is currently being addressed in a prospective study at M.D. Anderson Cancer Center.

Combination Therapies in Autologous Stem Cell Transplantation

Malignancy sensitivity (or resistance) to chemo or radiotherapy is not homogeneous, and combination chemotherapy may provide a way to act at different targets within cancer cells. Choice of medications is generally based on the principles of minimizing overlapping toxicity and mechanisms of resistance. Fold dose escalation achievable with the combinations is usually less than with each drug alone. Alkylating agents have a steep dose response curve and myelosuppression as their dose limiting toxicity. Those characteristics made them the most commonly used group of antineoplastic agents in transplantation. Dose escalation is not feasible with agents where extramedullary toxicity occurs at lower doses or at the same dose levels where myelosuppression develops. For instance, mucositis and cardiac toxicity associated with adriamycin cannot be overcome by the use of hematopoietic stem cells, whereas the dose of thiotepa can be escalated MdNY fold with stem cell support [75].

Numerous conditioning regimens have been evaluated for various diseases, and are reviewed in Table 16-1. High-dose chemo-radiation therapy for multiple myeloma and lymphoma, two of the most common indications

Table 16-1. Common conditioning regimens in autologous and allogeneic hematopoietic stem cell transplantation.

Regimen	Agent: Dose (Total)	Indications / Comments	Reference
Busulfan- Based			
BuCy2 (oral)	Bu:16 mg/kg Cy:120 mg/kg	Allogeneic / Autologous, Various indications	[28]
BuCy2 (IV)	Bu:0.8 mg/kg q 6 hrs × 16 doses (IV) Cy:120 mg/kg	Allogeneic / Autologous, Various indications	[55, 56]
BuCyVP-16	Bu:16 mg/kg Cy:120 mg/kg VP-16:30–45 mg/kg	Allogeneic / Autologous, various indications. VP-16 is better tolerated at dose 30 mg/kg with less toxicity and better survival	[61]
BuFlu	Bu:130 mg/m^2 daily ×4 (3.2 mg/kg) Flu:40 mg/m^2 daily × 4 (doses 30–50 mg/m2 reported)	Allogeneic, various indications but predominately MDS and leukemia, PK-guided Bu also reported	[74, 120, 122]
BuMel	Bu:16 mg/kg Mel:140–180 mg/m^2	Autologous or (RIC) allogeneic, multiple myeloma, leukemia, lymphoma, pediatric solid tumor	[123]
BuMelTT (BMT)	Bu:12 mg/kg Mel:100 mg/m^2 TT:500 mg/m^2	Autologous, adult (breast / ovarian) and pediatric (Ewing's sarcoma) solid tumors, lymphoma, myeloma	[124]
TBI-Based			
Cy/ TBI	Cy:120 mg/m^2 FTBI:12 Gy	Allogeneic / Autologous, various indications, FTBI less toxicity than single dose TBI	[11–13] [15–17, 20]
Cy/ TBI/ VP-16	Cy:100 mg/kg FTBI:12 Gy VP-16:60 mg/kg	Allogeneic / Autologous, various indications, leukemia (especially ALL), lymphoma	[125, 126]
TBI/ VP-16	FTBI:13.2 Gy VP16:60 mg/kg	Allogeneic, leukemia, SWOG randomized study comparing it to Bu/Cy in patients with advanced leukemia with equal outcomes	[127, 128]
Melphalan-Based			
Mel	Mel:200 mg / m^2(younger) Mel:140–180 mg/m^2 (older)	Autologous, primarily multiple myeloma, some advocate decreasing dose in older- aged patients	[77–80]
Mel/ TBI	Mel:140 mg/m^2 FTBI:8 Gy	Autologous, primarily multiple myeloma, found to be inferior to Mel 200 mg/m^2 in < 65 yrs age	[77]
Flu/ Mel (FM)	Flu:125 mg/m^2 Mel:140–180 mg/m^2	(RIC) Allogeneic, leukemia or (RIC) allo for solid tumors (renal / breast)	[129, 130]
BCNU-Based			
BEAM / BEAC	BCNU:300–600 mg/m^2 VP16:400–800 mg/m^2 Ara-c:800–1600 mg/m^2 Mel:140 mg/m^2 or Cy:———	300 mg/m^2 800 mg/m^2 800 mg/m^2 ——— 140 mg/kg	Primarily autologous regimen for lymphoma. BEAM has also been used as conditioning for allogeneic HSCT for lymphoma; BEAM < toxic than CBV	[131, 132]
CBV ± Cisplat	Cy:7.2 gm/ m^2 or 100 mg/kg BCNU:600 mg/m^2 VP16:2400 mg/m^2 or 60 mg/kg	Cy:7.2 gm/m^2 BCNU:500 mg/m^2 VP16:2400 mg/m^2 Cisplat:150 mg/m^2	Autologous, primarily lymphoma, may be used as conditioning for allogeneic in lymphoma	[133]

(continued)

Table 16-1. (continued)

Regimen	Agent: Dose (Total)	Indications/ Comments		Reference
STAMP1	BCNU:600 mg/m^2 Cy:5.625 gm/m^2 Cisplat 165 mg/m^2	Autologous; primarily solid tumor (breast)		[134]
Others				
STAMPV	Cy:6 gm/m^2 TT:500 mg/m^2 Carboplat:800 mg/m^2	Cy:5.625 gm/m^2 TT:600 mg/m^2 Cisplat:165 mg/m^2	Autologous; primarily solid tumors (breast, ovarian)	[135, 136]

Ara-c = cytarabine, BCNU = carmustine, Bu = busulfan, Carboplat = carboplatinum, Cisplat = cisplatinum, CNS = central nervous system, Cy = cyclophosphamide, Flu = fludarabine, FTBI = fractionated total body irradiation, IV = intravenous, Mel = melphalan, MDS = myelodysplastic syndrome, PK = pharmacokinetic, RIC = reduced-intensity conditioning, TT = thiotepa, VP16 = etoposide; HSCT = hematopoietic stem cell transplantation

for autologous transplantation, is reviewed below. As new agents are developed it is to be expected that the combinations we use in transplantation will evolve to incorporate newer, hopefully more effective drugs. Good examples are the monoclonal antibodies (MABs) and radioconjugates for B-cell lymphomas and certain leukemias that are discussed separately below. This session does not review peripheral blood stem cell mobilization strategies, which are reviewed elsewhere in this book.

Multiple Myeloma

Balancing efficacy and safety is especially true in the management of patients with multiple myeloma who are often older and suffer comorbidities. This is also one of the few hematologic malignancies for which there is a series of randomized studies comparing regimens for autologous transplantation. It is important, therefore, to review some of these comparisons.

There is a dose-response relationship in the treatment of this disease. High-dose TBI-based therapy with autologous bone marrow support was shown to be superior to standard dose chemotherapy in randomized trials. A French study reported a complete remission and five-year survival rates of 22 percent and 52 percent in the high-dose group versus 5 percent and 12 percent in the standard dose arm [76]. Increasing the dose of Melphalan (Mel) from 100 mg/m^2 to 400 mg/m^2 (200 mg/m^2 for each of two transplants) that is delivered in tandem transplant programs increases the CR rate from 6 percent to 15 to 25 percent in refractory patients, and to 20 to 45 percent in recently diagnosed individuals. The Intergroupe Francophone du Myelome [IFM] 9502 trial was conducted to compare the outcomes following two of the most common regimens used in patients who were under 65 years [77]. Patients were randomized between conditioning with Mel at a dose of 200 mg/m^2 versus Mel 140 mg/m^2 + TBI at a dose of 8 Gy administered in four fractions without lung shielding (n = 282). Mel 200 mg/m^2 was better tolerated and elicited improved 45-month overall survival (66% versus 46%, p = 0.05), with similar EFS. The researchers speculated that the superior survival was a result of the ability to tolerate subsequent therapies. This and other randomized studies have established Mel 200 mg/m^2 as the 'standard' regimen in this setting [78, 79]. The ideal regimen

for older patients with myeloma, however, remains a matter of debate. Badros, et al. determined that Mel-140 mg/m^2 was less toxic and equally as efficacious as Mel-200 mg/m^2 in elderly patients (older than 70 years in this study) [80].

The role of double autologous transplants was investigated in a second protocol of the Intergroupe Francophone du Myelome. Attal, et al. randomized 399 untreated patients younger than 60 years to receive Mel 140 mg/m^2 with TBI 8 Gy (single transplant) or Melphalan 140 mg/m^2 followed by a second transplant using the TBI/Melphalan regimen [81]. Response rate was similar, but overall and EFS were significantly improved in the double transplant arm (seven-year post diagnosis probability of overall and EFS, 42% and 20% versus 21% and 10% in the single transplant arm). The subset of patients that did not achieve at least a partial remission with the first procedure appears to benefit the most with this strategy. This is a rapidly changing scenario, however. All studies that determined the key role of autologous transplantation in this disease were designed and conducted before the development of new 'target' therapies which may radically change the way we treat multiple myeloma in the future.

Lymphoma

High-dose therapy followed by autologous HSCT is considered standard treatment for patients with Non-Hodgkin's Lymphoma and Hodgkin's disease that fail to achieve a remission following initial therapy, or subsequently relapse after an initial remission with chemo-sensitive disease. The basis for dose escalation comes from pre-clinical and clinical studies [82, 83]. Appelbaum, et al. treated 22 patients with refractory lymphoma with high-dose chemotherapy consisting of BCNU, cytarabine, cyclophosphamide and 6-thioguanine in the early 1970s [84]. Twelve of the 22 patients received previously procured, cryopreserved marrow. There was quicker engraftment and less febrile episodes among those patients that received autologous marrow, with a high CR rate. This strategy was later determined to be superior to additional chemotherapy for patients with chemotherapy-sensitive relapsed diffuse large cell lymphoma [85]. However, outcomes for patients with refractory disease have remained poor, illustrating the limitations of dose escalation.

Chemotherapy or radiation-based conditioning regimens have been employed in autologous HSCT for lymphoma. There have been no prospective randomized studies comparing different approaches in this setting. Retrospective studies have arrived at different conclusions with the results of some demonstrating superiority for chemotherapy-only conditioning [86] while others have determined that no major outcome difference exists between TBI-containing versus chemotherapy-only combinations [87, 88].

Monoclonal Antibodies (MABs)

The use of MABs in the management of patients with various malignancies has now become widespread. Rituximab, the most commonly used agent, is a mouse/human chimeric MAB targeting the CD20 antigen that is approved for

use in relapsed low-grade CD20-positive B-cell Non-Hodgkin's Lymphoma. Studies have documented it is beneficial when used with stem cell mobilization regimens as an '*in vivo* purging' tool and post-transplant as a means of treating minimal residual disease [89–93]. Khouri, et al. reported that adding high-dose rituximab before and after autologous HSCT in patients with relapsed, aggressive B-cell lymphoma improved overall and DFS when compared to a historical group of patients with similar disease characteristics who did not receive rituximab [94]. In this study, rituximab was given at a dose of 375 mg/m^2 a day prior to chemo-mobilization followed by 1,000 mg/m^2 seven days later, and on days +1 and +8 after transplantation. Two-year survival was 80 percent versus 53 percent for the historical control group. In a multivariate analysis of 188 patients that received an autologous stem cell transplant for chemo-sensitive, large B-cell lymphoma on a research protocol at M.D. Anderson Cancer Center since 1995, the only predictive factors for progression were FDG-PET/Gallium status pre-transplant and whether higher doses of rituximab were administered, indicating the potential of this agent to improve results of autologous transplants (Fig. 16-1) [95].

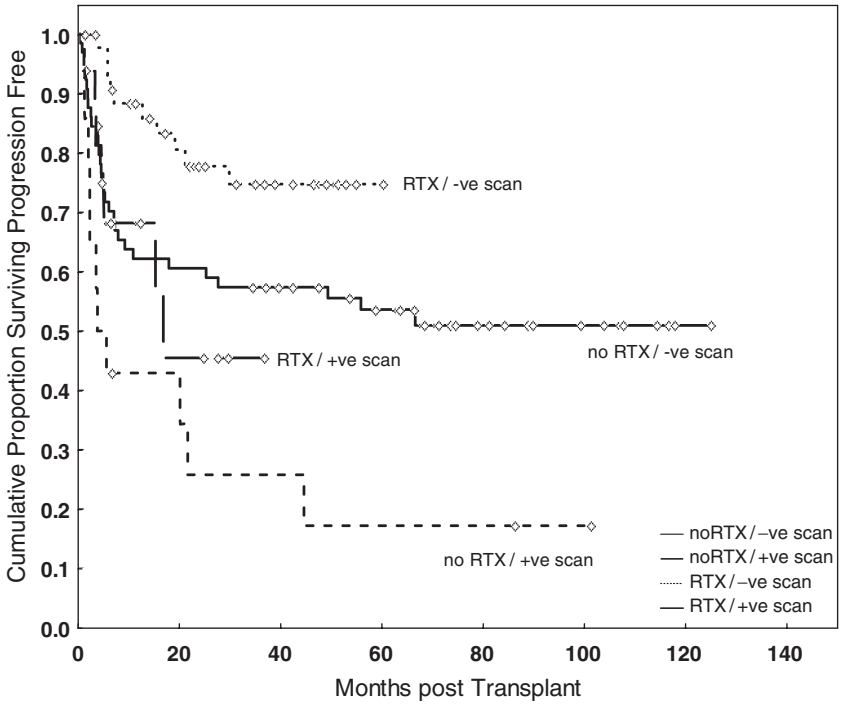

Fig. 16-1. The influence of rituximab use and FDG-PET/ Gallium status in patients with diffuse large B-cell lymphoma undergoing autologous transplant is illustrated here All 188 patients have chemo-sensitive relapsed or primary induction failure disease Treatment consisted of autologous transplants performed under an investigative protocol at the M.D. Anderson Cancer Center between the years 1995 to 2005 Risk factors considered for analysis included age, international prognostic index, number of prior chemotherapies, B2-microglobulin, disease status at transplant, PET/Gallium (PET/G) status and whether high-dose rituximab (HDR) was given with chemo-mobilization and at the time of transplant On multivariate analysis, the only determinants of progression-free survival were pre-transplant PET/Gallium status and whether HDR was given

Radionuclide Therapies

The principle of attaching radioactive particles to MABs is referred to as radioimmunotherapeutics (RIT). It is expected to increase both efficacy and safety by delivering irradiation directly to the target on the tumor cell with relative sparing of the normal tissue. In addition, high energy particles can kill surrounding tumor cells that do not directly bind the antibody through a bystander effect [96]. Currently two radioimmunoconjugates (RICs) are approved by the U.S. Food and Drug Administration for the treatment of CD20+ expressing lymphoma: iodine-131 (I-131) tositumomab and yttrium-90 (Y-90) ibritumomab tiuxetan. Both agents have been tested as a component of high-dose therapy and autologous HSCT.

The first phase 1 study with a RIC administered with stem cell support was conducted by Press, et al. in 1993 with I-131-labled anti-CD20 (B1, 1F5) and anti-CD37 (MB-1) [97]. Diagnoses were refractory or relapsed B-cell lymphoma, and the examined doses were between 10 and 30.75 Gy. Only those patients with favorable biodistribution (increased antibody uptake in tumor sites over critical organs) in dosimetric studies after administration of low doses of the antibody were administered a therapeutic dose. The anti-CD20 antibody was found to be superior to the anti-CD37 antibody in terms of dosing, distribution and toxicity. Patients treated with 23.75 Gy or less to normal organs experienced minimal nonhematologic toxicity. Two patients experienced grade 3 cardiopulmonary complications after receiving doses that resulted in 27.25 and 30.75 Gy to the lung. A total of 16 out of 19 patients treated with the B1 anti-CD20 I-131 antibody achieved a CR. A subsequent phase 2 study by the same investigators confirmed the activity of the regimen [98]. Time-to-treatment failure was 47 months for this heavily pre-treated group and appeared especially encouraging in patients with low-grade lymphomas [99]. Frequency of acute toxicities appeared similar to those observed with standard high-dose conditioning regimens. The only common long-term toxicity was a rise in thyroid stimulating hormone (TSH) in 60 percent of the patients.

Press, et al. sought to determine whether a myeloablative dose of I-131 tositumomab could replace TBI in a traditional combined chemotherapy-TBI preparative regimen. A Phase 1/2 trial of the RIC combined with etoposide 60 mg/m^2 and Cy 100 mg/kg was performed and enrolled 52 patients with relapsed B-cell lymphoma [100]. The MTD was found to be similar to the phase 1, single agent determined dose. Two-year overall and DFS was 83 and 68 percent, respectively. Toxicities were similar to a nonrandomized control group of patients treated during the same period who received a standard TBI/Cy/Etoposide regimen. Similarly encouraging results have been found in patients with refractory mantle cell lymphoma receiving I-131-tositumomab combined with etoposide (30–60 mg/kg) and Cy (60–100 mg/kg) [101]. In addition, I-131-tositumomab administered in a standard total body dose (rather than dosimetric determined) has been safely combined with the BEAM regimen [102]. Similar studies have been conducted employing yttrium-90 ibritumomab. Nadamanee, et al. examined yttrium-90 ibritumomab tiuxetan combined to escalating doses of etoposide (40–60 mg/kg) and Cy 100 mg/kg followed by autologous HSCT. They treated 31 patients with B-cell lymphoma of various histologies with a target dose of 10 Gy to the highest uptaking

normal organ. At a median follow-up of 22 months, overall and DFS were 92 and 78 months, respectively. This study was initially planned for dose escalation of yttrium-90 ibritumomab tiuxetan; however, this was foregone when it became obvious that, at higher doses, the day of stem cell infusion would need to be delayed to allow for clearance of the RIC. Fung, et al. have shown the safety of combining BEAM with the weight-based dose of yttrium-90 ibritumomab tiuxetan of 0.4 mCi/kg prior to autologous transplant. Krishnan, et al. examined the same combination in older adults with a median age of 62 years and found favorable results when retrospectively compared to patients who received BEAM alone, especially for those with relapsed diffuse large cell lymphoma [103]. A dose escalation study of single agent yttrium-90 ibritumomab tiuxetan followed by autologous HSCT has been reported with doses up to 24 Gy to the liver being well tolerated [104]. Trials are underway in several institutions evaluating RIC in the context of allogeneic transplants.

As discussed above, attempts to decrease relapse rates by escalating TBI doses have been hampered by toxicity. However, by increasing the dose of radiation by 3.75 Gy, a corresponding decrease in relapse rates was observed, albeit at the cost of increased toxicity and death. The ability to increase the dose of radiation to the bone marrow and spleen many fold above that to nonhematologic organs, and thus limit extramedullary toxicity, is the basis for incorporating RIT into conditioning for transplant in patients with leukemia. Three different antibodies have been tested – anti-CD33, -CD45 and -CD66 – attached to one of four radionuclides (^{131}I, ^{90}Y ^{188}Re or more recently, ^{213}Bi). Various Phase 1 and 2 studies have evaluated the addition of one of these RICs either to fully ablative (Cy-TBI or BuCy2) or reduced intensity conditioning regimens. The work of investigators at the Fred Hutchinson Cancer Center to develop the radiolabeled antibody- anti-CD45 is presented below. In addition, phase 1 and 2 trials for the anti-CD33 and CD66 RICs have been published and were reviewed by Kotzerke, et al. [105].

The cell surface glycoprotein CD45 is universally expressed on circulating leukocytes and lymphocytes as well as in 70 percent of nucleated cells in the marrow [106], and is present on leukemic cells (85–90% of AML and ALL cases). Furthermore, the antigen is not internalized upon binding of the antibody. In preclinical models, ^{131}I- anti-CD45 antibody had favorable biodistribution [107, 108]. Matthews, et al. reported one of the earliest phase 1 reports with the use of this RIC incorporated into a conditioning regimen for patients with advanced acute leukemia and myelodysplastic syndrome (MDS) [109, 110]. A total of 44 patients were enrolled; 37 had favorable biodistribution after receiving a trace dose of the RIC. Of these 37 patients, a total of 34 went on to receive the ^{131}I- anti-CD45 antibody administered in a dose estimated to deliver an absorbed dose of radiation to the liver (the highest uptaking normal organ in all but one patient) ranging from 3.5 Gy (dose level 1) to 12.25 Gy (level 6). Following administration of the RIC, patients received Cy (120 mg/kg) and TBI (12 Gy divided into six fractions) followed by an autologous or HLA-matched allogeneic transplant. The MTD (mucositis) was determined to be 10.5 Gy. This strategy resulted in supplemental doses of radiation of 24 and 50 Gy to the marrow and spleen. DFS was 29 percent with non-relapse mortality and relapse rate of 18 percent and 50 percent, respectively. There were no cases of severe VOD. Patients in relapse at the time of transplant received higher doses to the bone marrow than those in remission. Based on these

results a phase 1/ 2 trial was conducted in 46 patients with intermediate or poor risk cytogenetics AML in CR1 using the ^{131}I- anti-CD45 antibody combined with targeted oral Bu (AUC of 600–900 ng/mL) and Cy 120 mg/kg, followed by matched related donor transplant [111]. Four patients safely received a dose at level 1 which targeted 3.5 Gy to normal organs with all subsequent patients receiving dose level 2 of 5.25 Gy. This resulted in mean doses of 11.3 and 29.7 Gy to the bone marrow and spleen, respectively. Grade II mucositis occurred in all patients and 71 percent of evaluable patients developed hypothyroidism. The estimated three-year DFS and non-relapse mortality was 61 percent and 21 percent, respectively, with a somewhat high incidence of grade II–IV acute GVHD of 72 percent. These outcomes were compared to BuCy-treated registry patients who were matched for cytogenetics, age and the presence of secondary MDS. This analysis indicated a non-statistically significant survival advantage for RIT-treated patients. In addition, phase 1 studies of reduced-intensity transplantation using RIT combined to low-dose TBI (2 Gy) and fludarabine are being conducted in adults over the age of 50 with advanced AML. RIT offers the prospect of increasing the regimen's intensity without added toxicity. Logistics and costs of this approach, however, are potential obstacles for the widespread use of these promising agents in HSCT.

Side Effects

The attribution of toxicity in patients undergoing transplantation is often a difficult task. Regimen-related toxicity may be arbitrarily defined as adverse reactions occurring within the first 30 days following transplant [112]. However, this definition will miss many delayed toxicities as well as a host of transplant-related long-term complications (Tables 16-2 and 16-3). In addition, factors apart from the chemo- and radiotherapy may influence toxicity. For instance, drugs used to prevent GVHD or infectious complications may magnify side effects. One such example is methotrexate used for GVHD prevention. Using reduced doses of the drug (5 mg/m^2 on days 1,3, 6 and 11 post-transplant) may produce less mucositis post-allogeneic transplant [113]. In certain situations the duration of follow-up after treatment is key to identifying side effects. This is exemplified by the development of secondary cancers, especially MDS and therapy-related AML after autologous transplant employing TBI-based preparative regimens, with a cumulative incidence of 21 percent (10% non-MDS/AML) at 10 years (Table 16-4 [114, 115]. However, frequent bone marrow chromosomal abnormalities discovered prior to transplant illustrate how previous therapies can contribute to hematopoietic stem cell damage [116]. Therefore, a variety of non-regimen-related factors must be considered when analyzing early or late side effects of preparative regimens.

The creation of better supportive medications has minimized many of the acute toxicities. Examples include the incorporation of selective type three 5-hydroxytryptamine (5-HT3) receptor antagonists to decrease the severity of nausea [117], palifermin (recombinant human keratinocyte growth factor) to decrease radiation-induced mucositis [118] and mesna (2-mercaptoethane sodium sulfonate) to decrease the occurrence of chemotherapy-induced cystitis [119]. Unfortunately, progress in reducing long-term toxicity has yet to be realized (Tables 16-3 through 16-5).

Table 16-2. Acute toxicities of conditioning regimens in hematopoietic stem cell transplantation.

Toxicity	Causative Agents	Incidence and Comments	Reference
Oral:			
Mucositis	Melphalan, TBI, Busulfan, Cyclophosphamide, Etoposide, Thiotepa, Methotrexate	Incidence near universal with ablative regimens Most important risk factor is conditioning regimen High-dose melphalan and Cy-TBI / BuCy regimens have the highest incidence.	[137]
		Methotrexate used for GVHD prophylaxis may worsen mucositis.	
CNS:			
Encephalitis	BCNU, ifosfamide (however often not direct chemotherapy toxicity)	Recent series suggest incidence of 16% with the majority occurring in the first 2 months. Allo>auto. Infectious etiology most common followed by metabolic encephalopathy usually as a component of pulmonary or other organ dysfunction.	[138]
Seizures	Busulfan	Incidence approximately 4% with BuCy, prophylaxis minimizes risk.	
Cardiac:			
Acute Cardio-Myopathy / Hemorrhagic Myopericarditis	Cyclophosphamide, TBI	Acute cardiomyopathy, not associated with cumulative dose. Risk factors include older age, prior radiation to chest, pre-existing decreased cardiac function.	[139]
Pulmonary:			
Interstitial Pneumonitis	TBI, Busulfan, Cyclophosphamide, BCNU	Risk factors: prior radiation, allogeneic> autologous, Single-Dose > fractionated TBI	[140]
		Bu/Cy often results in transient declines in lung volumes and a mean drop in DLCO by 15% at 1 yr with usual resolution over time. Acute and chronic forms. Idiopathic pneumonia syndrome classically described as occurring at a median of 50 days post-transplant.	
Diffuse Alveolar Hemorrhage	TBI	May occur with different regimens. Prior radiation to chest is a risk factor.	[141]
Genitourinary:			
Hemorrhagic Cystitis	Cyclophosphamide, TBI, VP-16, Ifosfamide	Higher in cyclophosphamide containing regimens. May lead to severe bladder dysfunction. Acrolein, a metabolite of Cy / ifosfamide, and BK virus may be causative agents. Unrelated > Matched-Related Donor	[142]
Hepatic: Veno-occlusive Disease	Busulfan, Cyclophosphamide, TBI, Carmustine	Criteria for diagnosis includes: ascites, severe weight gain, jaundice, painful hepatomegaly. IV and /or PK-dosed Bu decrease the risk.	[55, 56, 59]
GI:			
Nausea, Diarrhea	Melphalan, TBI, Cyclophosphamide,	Better support medications have decreased the severity, but high incidence remains.	[117]
Renal Failure:	Multiple	Often multi-factorial.	[143]

Table 16-3. Common long-term complications following hematopoietic stem cell transplantation.

Long-Term Complication	Comment	Reference(s)
Cataracts	Greater with TBI; increase risk with steroids (used to treat GVHD)	[23]
Hypothyroidism	Single Fraction (STBI) > FTBI > Chemo-only regimens	[144, 145]
Chronic Pulmonary Disease	Incidence is higher with Bu/Cy and STBI Manifestation on Chronic GVHD (bronchiolitis obliterans)	[32]
Alopecia	More common with BuCy than TBI-based regimens	[32]
Impaired Growth and Development (Pediatrics)	Steroid use may worsen growth delay	[146]
Sterility	High-dose TBI and older age increases the risk of sterility in women. Sperm production is usually severely decreased post-TBI, but less so after Busulfan-based regimens.	[147]
Myelodysplasia & Secondary Cancers	See Tables 16-4 and 16-5	

Table 16-4. Risk factors for development of secondary cancers following autologous HSCT for lymphoma [114, 115, 148].

MDS and Treatment-related AML
• Prior radiation therapy (especially to the pelvis)
• Long duration of exposure to chemotherapy prior to transplant (especially alkylating agents)
• Prolonged interval between initial treatment and transplant
• High-dose TBI regimens
• Slow peripheral blood stem cell mobilizers
Solid Tumors
• Increased age at time of transplant

Table 16-5. Secondary cancers after allogeneic transplant [149].

Risk*	8.3 greater than the general population's
Cumulative Incidence Rate*	2.2% @ 10 years, 6.7% @ 15 years
Types of Cancer with increased risk (risk relative to general population)+	Bone (13.4) Head and Neck Cancers (H/N Ca)) (11.1) Connective Tissue (8.0) Brain / Central Nervous System (7.6) Liver (7.5) Thyroid (6.6) Malignant Melanoma (5.0)
Risk Factors	Higher Doses of TBI Younger Age at the time of Transplant Chronic GVHD and Male Sex (for head and neck cancer only)

* In patients who survived 10 or more years after allogeneic transplant

+ This represents risk relative to general population, not overall frequency

Conclusions

Conditioning regimens are a key part of the HSCT process. Advances in radiation delivery, newer chemotherapy agents, supportive care and Histoincompatibility have contributed to a major reduction in early toxicity after myeloablative autologous and allogeneic transplants. Disease relapse (both autologous and allogeneic setting) and GVHD (allogeneic transplants) remain important obstacles to be overcome. Although a major focus of BMT research is focused on graft engineering and the separation of the Graft-versus-Malignancy effect from GVHD, the biologic revolution is now offering whole new classes of biologic agents that will most certainly help the immunologic component of allogeneic transplants by cytoreduction of the malignancy, or by modulation of immunity during and after hematopoietic transplantation. Development of new regimens will require a new paradigm in clinical research, with incorporation of surrogate molecular endpoints and innovative designs to investigate new drugs is the complex scenario of transplantation where multiple interactions occur simultaneously. Pharmacogenomics will likely play a role here, and hopefully the preparative regimen of the year 2020 will be significantly different from what we use today!

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Chapter 17

Nonmyeloablative Transplantation

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Outline

1. Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) for hematological malignancies was developed in the late 1960s as a way to deliver supra-lethal doses of chemotherapy and/or total body irradiation (TBI) with the aim of eradicating the underlying disease while marrow was infused to restore hematopoiesis. However, confirming observations made in mice 20 years prior [1],

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Weiden, et al. recognized in the late 1970s that the allograft itself conferred immune-mediated antileukemic effects [2, 3]. Indeed, patients who developed acute and/or chronic graft-versus-host disease (GVHD) had lower risks of relapse than those who did not [2, 3]. This antileukemic effect of GVHD was termed the "graft-versus-tumor effect." The existence of graft-versus-tumor effects was then supported by several observations demonstrating higher risk of relapse in patients given syngeneic HSCT, compared to those receiving grafts from allogeneic donors [4] and in those given T cell-depleted grafts [5]. Furthermore, it was found that immune-mediated effects of donor lymphocyte infusions (DLI) were sufficient to eradicate the malignancy in a number of patients who relapsed with chronic or acute myeloid leukemias after allogeneic HSCT [6].

The myeloablative doses of chemotherapy and/or TBI given during the conditioning regimen for conventional allogeneic HSCT can produce significant morbidity and mortality, particularly in older patients, those with medical comorbidities, or those who have failed a myeloablative HSCT [7, 8]. Because of these toxicities, the use of myeloablative allogeneic HSCT has been restricted to younger patients in good medical condition, while median patient age at diagnosis for acute or chronic myeloid leukemia, chronic lymphocytic leukemia, Non-Hodgkin's Lymphoma and multiple myeloma ranges from 65 to 71 years [SEERS (Surveillance, Epidemiology and End Results) data [9]].

2. Nonmyeloablative and Reduced-Intensity Conditioning

Given the increasingly recognized power of Graft-versus-Tumor effects, several groups of investigators explored the feasibility of nonmyeloablative or reduced-intensity conditioning regimens that would allow engraftment of both donor hematopoietic stem cells and donor T cells, and then eradicate the malignancies mainly towards Graft-versus-Tumor effects [10–16] . While Giralt, et al. proposed criteria for reduced-intensity conditioning {1) reversible myelosuppression within 28 days without stem cell support, 2) mixed chimerism (i.e., coexistence of hematopoietic cells of donor and host origin) in a proportion of patients at time of first assessment, and 3) low rates of non-hematologic toxicity} [17], practical definitions for reduced-intensity conditioning regimen varied from one study to another (Table 17-1).

Further, separating what constitutes a nonmyeloablative versus a reduced-intensity conditioning has been somewhat arbitrary (Fig. 17-1). Reduced-intensity conditioning regimens have combined fludarabine (used mainly for its immunosuppressive activity) with consequent (but nonmyeloablative) doses of alkylating agents such as melphalan (140 mg/m^2) [18], thiotepa ($\leq$10 mg/kg) or busulfan (4–8 mg/kg) [19], given to produce significant antitumor effects with the objective of both debulking and controlling the malignancy before the occurrence of Graft-versus-Tumor effects. In contrast, nonmyeloablative conditionings have used potent immunosuppressive regimens to overcome Host-versus-Graft reactions (graft rejection) [15, 16, 20], allowing engraftment of donor hematopoietic and immune cells, and eradication of host-derived hematopoiesis and tumor cells almost exclusively via Graft-versus-Tumor effects. The distinction of what constitutes a nonmyeloablative and what constitutes a reduced-intensity conditioning is clinically relevant

Table 17-1. Practical definitions for reduced-intensity conditioning.

CIBMTR/NMDP [17]
• ≤5 Gy TBI • ≤9 mg/kg total busulfan dose • ≤140 mg/m^2 total melphalan dose • ≤10 mg/kg total thiothepa dose • usually includes a purine analog
EBMT (1) [82]
Fludarabine associated with: • ≤4 Gy TBI • ≤10 mg/kg total busulfan dose • ≤140 mg/m^2 total melphalan dose • ≤10 mg/kg total thiotepa dose
EBMT (2) [46]
Fludarabine associated with: • <3 Gy TBI • ≤8 mg/kg busulfan • or other nonmyeloablative drugs

CIBMTR, Center for International Blood and Marrow Transplant Research; NMDP, National Marrow Donor Program; EBMT, European Group for Blood and Marrow Transplantation; TBI, total body irradiation

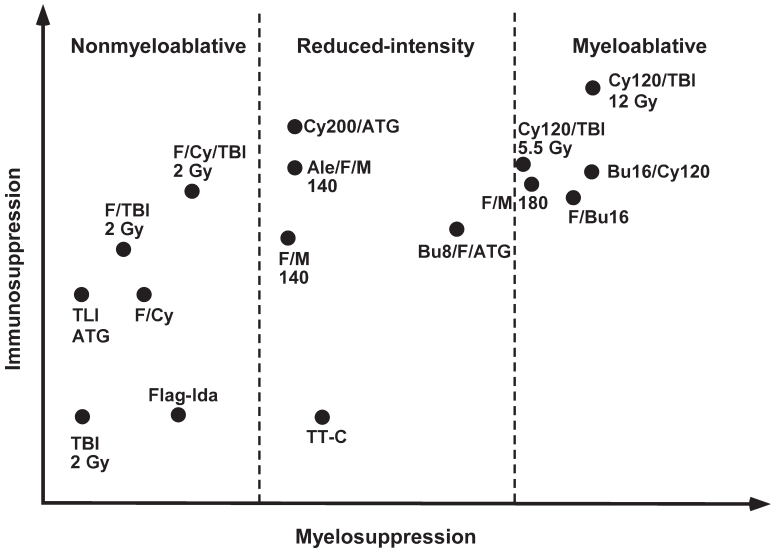

Fig. 17-1. Commonly used conditioning regimens in relation to their immunosuppressive and myelosuppressive properties. Please note that this classification is not based on direct experimentation and is, thus, hypothetical. TBI, total body irradiation; TLI, total lymphoid irradiation; F, fludarabine; Cy, cyclophosphamide; Cy 120, cyclophosphamide 120 mg/kg; Cy 200, cyclophosphamide 200 mg/kg; M, melphalan, M 140; melphalan 140 mg/m^2; M 180; melphalan 180 mg/m^2; Flag-Ida, fludarabine/cytosine arabinoside/idarubicin; TT, thiotepa; ATG, anti-thymocyte globulin; Ale, alemtuzumab; Bu8, busulfan 8 mg/kg; Bu16, busulfan 16 mg/kg. Reprinted from Molecular Therapy, 12:26–41, copyright 2006: F. Baron and R. Storb, "Allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning as treatment for hematologic malignancies and inherited blood disorders (Review)," with permission from Elsevier

since nonmyeloablative conditioning has been associated with a lower degree of donor engraftment, higher risk of graft rejection, decreased risk of non-relapse mortality, and higher risk of relapse compared with reduced-intensity regimens as observed in a study performed at the M.D. Anderson Cancer Center (MDACC) [21].

3. Engraftment Kinetics

By definition, nonmyeloablative and reduced-intensity conditioning regimens usually lead to an initial state of mixed chimerism [22]. Several factors have been associated with kinetics of donor engraftment after nonmyeloablative conditioning. Factors associated with faster donor T cell engraftment included high intensity of the conditioning regimen [21, 22], having had previous myelosuppressive chemotherapy [23, 24], the use of peripheral blood stem cells (PBSC) instead of marrow as a stem cell source [25–27], a high number of $CD34^+$ and T cells in the graft [23, 26, 27], and intense post-grafting immunosuppression [28].

High levels (>50%) of donor T and NK-cell chimerism one month after HSCT have each been associated with a lower risk of graft rejection [15, 24]. When analyzed as a continuous variable, higher levels of donor T cell chimerism one month after HSCT were associated with increased risks of grade II–IV acute GVHD [24] (Fig. 17-2A). Further, achievement of full donor T cell chimerism was associated with a lower risk of relapse (Fig. 17-2B). Finally, in patients with acute myeloid leukemia and myelodysplastic syndromes, the risk of subsequent relapse was substantially higher in patients with <90 percent donor chimerism levels among marrow CD34+ cells on day 28 after HSCT than in those with >90 percent [29].

4. Transplant-Related Toxicities after Nonmyeloablative versus Myeloablative Conditioning

Transplant-related toxicities and infections occurring after myeloablative allogeneic HSCT have been thought to be the consequence of the intense conditioning, of Graft-versus-Host reactions, or of both. A number of retrospective studies compared transplant-related toxicities and infections after HSCT following nonmyeloablative versus myeloablative conditioning to determine the relative contributions of conditioning intensity to these complications.

Not unexpectedly, the hematological changes after nonmyeloablative conditioning were milder than that seen after myeloablative conditioning [30], and patients given nonmyeloablative or reduced-intensity conditioning required less platelet and red blood cell transfusions than those given myeloablative conditioning (reviewed in reference [31]). Similarly, liver, kidney, gastrointestinal, and lung toxicities were significantly reduced with nonmyeloablative conditioning [32–35].

Junghanss, et al. compared the incidence of post-transplant infections in 56 nonmyeloablative recipients to that in 112 matched controls given myeloablative conditioning [36, 37]. The 30- and 100-day incidences of bacteremia were 9 percent and 27 percent in nonmyeloablative recipients versus 27 percent ($P = 0.01$) and 41 percent ($P = 0.07$) in myeloablative recipients, respectively.

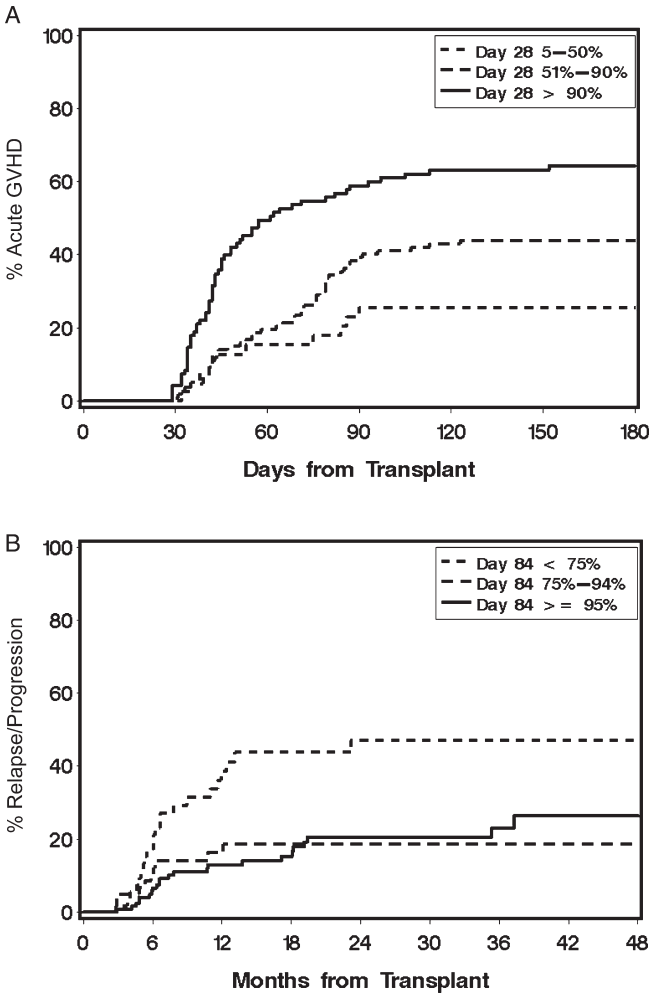

Fig. 17-2 A. Cumulative incidence of grade II–IV acute GVHD (P < 0.0001) according to day 28 donor T cell chimerism levels in 322 patients reported in ref. [54] given grafts after 2 Gy TBI with or without fludarabine **B).** Cumulative incidence of relapse according to day 84 donor T cell chimerism levels in patients reported in ref. [54] given grafts after 2 Gy TBI with or without fludarabine (P = 0.002) Reprinted with permission F. Baron and B.M. Sandmaier, "Chimerism and outcomes after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. Leukemia 2006; 20:1690–1700

In contrast, invasive aspergillosis occurred at a similar rate (15% versus 9% at one year; $P = 0.30$). The onset of CMV disease was significantly delayed among nonmyeloablative compared to myeloablative recipients (medians of 130 versus 52 days; $P = 0.02$) due to the persistence of host-derived CMV immunity early after HSCT in nonmyeloablative recipients [38]. However, the one-year probability of CMV disease for high risk CMV patients was comparable in the two groups.

5. Graft-versus-Host Disease and Graft-versus-Tumor Effects After Nonmyeloablative Conditioning

The biology of reconstitution of donor-derived immunity after nonmyeloablative conditioning differs from what occurs after myeloablative conditioning in several aspects. First, nonmyeloablative conditionings generally lead to an initial state of mixed donor-host chimerism that might favor both Host-versus-Graft and Graft-versus-Host tolerance and, thus, limit GVHD [39]. Secondly, the intensity of the preparative regimens has been shown to contribute to acute GVHD physiopathology, presumably by inducing tissue damage and the release of a "cytokine storm" [40, 41]. In contrast, the number of recipient-derived antigen presenting cells (APC) might be higher after nonmyeloablative than myeloablative conditioning. Since recipient-derived APC are thought to play a major role in the initiation of acute GVHD [42], their persistence in an increased number after nonmyeloablative regimen might favor acute GVHD.

A number of reports have compared incidences of acute and chronic GVHD after nonmyeloablative or reduced-intensity conditioning. Most have shown lower incidences of acute GVHD and similar or lower incidences of chronic GVHD after nonmyeloablative versus myeloablative conditioning [43–47], including one study analyzing age-matched patients treated in a single institution [43]. However, although relatively less frequent, GVHD with or without associated infections has remained the leading cause of non-relapse mortality after nonmyeloablative HSCT.

GVHD incidence could be decreased by the use of anti-thymocyte globulin (ATG) or alemtuzumab, a humanized monoclonal antibody recognizing CD52 that is expressed on lymphocytes and NK cells, but not on hematopoietic stem cells [12, 20, 48]. However, these strategies were associated with increased risk of disease relapse/progression [48, 49].

Another approach aimed at reducing the incidence of acute GVHD has been developed by the Stanford University group. Based on murine experiments [50], the authors investigated a novel nonmyeloablative regimen that favored the presence of a high proportion of regulatory NK-T cells [50]. This regimen consisted of total lymphoid irradiation (TLI, 8 Gy) and ATG (Thymoglobulin, 7.5 mg/kg total dose), and post-grafting immunosuppression with MMF and CSP. First results in 37 patients with various hematological malignancies indicated that this regimen was indeed associated with a low incidence of grade II–IV acute GVHD (one of 37 patients), while Graft-versus-Tumor effects were apparently preserved [20].

As mentioned earlier, GVHD occurrence is strongly associated with Graft-versus-Tumor effects in patients given myeloablative conditioning [3]. Since nonmyeloablative regimens rely nearly exclusively on Graft-versus-Tumor effects for tumor eradication, several groups of investigators looked at the impact of GVHD on HSCT outcomes after nonmyeloablative or reduced-intensity conditioning.

First, Martino, et al. showed that patients with acute myeloid leukemia (n = 17) or myelodysplastic syndrome (n = 20) who experienced acute and/or chronic GVHD had significantly lower risks of relapse than those who did not (P = 0.008) [51]. Kroger, et al. analyzed data from 120 patients with multiple

myeloma who were given allogeneic grafts after reduced-intensity conditioning [52]. While occurrence of acute GVHD was found to have no impact on relapse risks, occurrence of chronic GVHD was associated with significantly lower risk of relapse (P = 0.02) in a time-dependent Cox analysis [52]. Similar observations were made by Crawley, et al. in a cohort of patients given allogeneic grafts after nonmyeloablative or reduced-intensity conditioning at various European Group for Blood and Marrow Transplantation (EBMT)-affiliated centers as treatment for multiple myeloma [49]. More recently, Blaise, et al. analyzed outcomes of 33 patients with acute myeloid leukemia in first complete remission receiving allogeneic HSCT from HLA-identical siblings following reduced-intensity conditioning [53]. In a landmark analysis starting on day 100, occurrence of chronic GVHD was associated with a lower risk of relapse (0% versus 44%, P = 0.007) and better leukemia-free survival (95% versus 53%, P = 0.007).

We analyzed the impact of acute and chronic GVHD on HSCT outcomes in a cohort of 322 patients given nonmyeloablative HSCT as treatment for hematological malignancies [54]. Grades II and III–IV acute GVHD were not significantly associated with lower risks of progression/relapse, but were instead associated with increased non-relapse mortality and lower progression-free survival. In contrast, the occurrence of chronic GVHD correlated with a lower risk of relapse in multivariate time-dependent analyses (HR = 0.4, P = 0.006) and was associated with significantly better progression-free survival (HR = 0.5, P = 0.003) (Fig. 17-3).

Taken together, these observations suggested that new approaches aimed at reducing the incidence of grade II–IV acute GVHD without suppressing chronic GVHD might improve progression-free survival after nonmyeloablative or reduced-intensity conditioning.

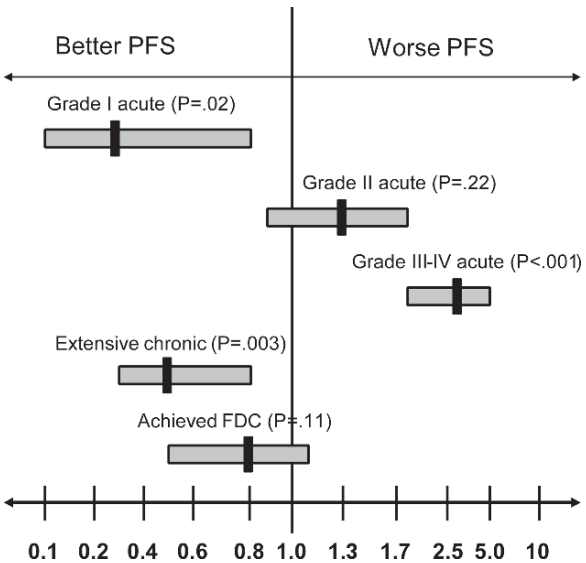

Fig. 17-3. Impact of acute and chronic GVHD and of achievement of full donor T cell chimerism (FDC) on progression-free survival (PFS) 322 patients reported in ref. [54] given grafts after 2 Gy TBI with or without fludarabine

6. Results in Specific Diseases

Tables 17-2 and 17-3 show the results of a number of phase I–II studies assessing post-HSCT outcomes in patients with hematological malignancies who were given nonmyeloablative or reduced-intensity conditioning. Since inclusion criteria varied between the studies, the efficacy of each regimen cannot be compared.

Encouraging results have generally been observed in patients with acute myeloid leukemia in first or second complete remissions (two-year overall survival ranging from 40% to 75%) [46, 53, 55–57] (Fig. 17-4), as well as in patients with myelodysplastic syndrome with < 5 percent blasts at HSCT (two-year overall survival ranging from 33% to 60%) [58, 59], chronic myeloid leukemia (two-year overall survival ≥ 70% for patients in first chronic phase [10, 60, 61]), chronic lymphocytic leukemia (two-year overall survival ranging from 50% to 80%) [62–66] , or indolent or chemotherapy-sensitive aggressive Non-Hodgkin's Lymphoma (two-year overall survival ranging from 50% to 80%) [12, 14, 67–70] (Tables 17-2 and 17-3). Conversely, results in patients with advanced aggressive diseases (such as acute leukemias not in complete remission, chemotherapy-insensitive high-grade Non-Hodgkin's Lymphoma or multiple myeloma, or advanced myelodysplastic syndromes) have been less impressive.

7. Consolidative Allografts Following Planned Autografts

Since Graft-versus-Tumor effects may not be sufficiently fast enough to eradicate large volume disease in patients with aggressive malignancies, an elegant strategy has been to follow a "debulking" autologous HSCT (which can be administered with transplant-related mortality rates of less than 5%) with a nonmyeloablative allogeneic HSCT. This strategy, pioneered by Carella, et al. in patients with refractory lymphoma [71], was evaluated by Maloney, et al. in

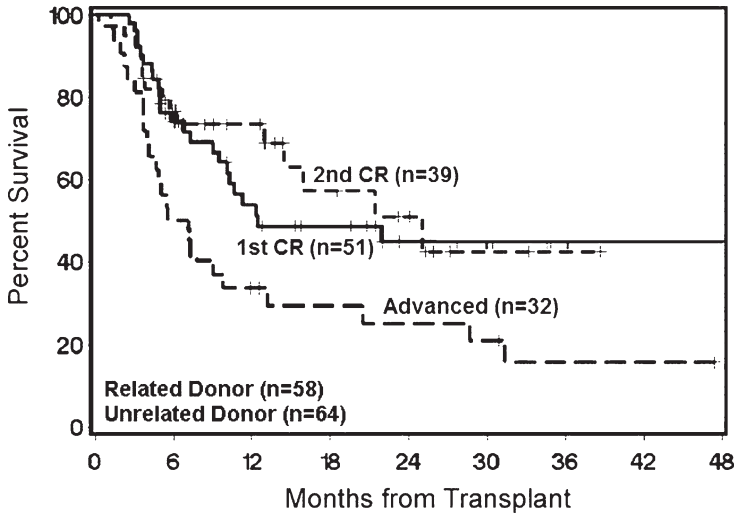

Fig. 17-4. Overall survival in 122 acute myeloid leukemia patients following nonmyeloablative HSCT according to disease status at time of HSCT

Table 17-2. Recent results in patients given HSCT after nonmyeloablative/reduced-intensity conditioning for myeloid malignancies.

						% GVHD		NRM		
Study Group	**Disease**	**Regimen**	**# Pts.**	**Median Pt. Age**	**% Pts. with MRD**	**Grade III–IV Acute**	**Chronic**	**%**	**Follow-up (mos)**	**Survival**
MDACC [21]	AML+MDS	FAI	32	61	81	11	27	29	36	3-yr OS 30% 3-yr PFS 19%
MDACC [21]	AML+MDS	FM	62	54	40	19	39	52	36	3-yr OS 35% 3-yr PFS 32%
CGTP [97]	AML	Various	113	51	44	27	33	53	24	2-yr EFS 29% (all pts) 2-yr EFS 52% (CR1)
King's College London [59]	AML+MDS	FBC	62	53	39	0 (MRD)- 9 (URD)	NR	15	12	1-yr OS 74% 1-yr DFS 62%
Queen Elizabeth Hospital [56]	AML+MDS	FMC	76	52	46	0	11	19	12	3-yr DFS 37% (all patients) 3-yr DFS 42% (CR1, CR2 or CR3)
University of Chicago [98]	AML+MDS	FMC	52	52	44	10	18	33	12	1-yr OS 48% 1-yr PFS 38%
Marseille [53]	AML-CR1	FB+ATG	33	52	100	12	64	5	24	2-yr OS & DFS 76%
HOVON/SAKK/ OSHO [57]	AML-CR1	Flu/TBI	83	62	65	NR	23	22	24	2- yr OS 51% (MRD) / 65% (URD) 2- yr DFS 39% (MRD) / 54% (URD)
Seattle consortium [99]	AML	Flu/TBI	122	58	48	12	36	16	24	2-yr OS 51% (CR1) / 61% (CR2) 2-yr DFS 44% (all pts)
EBMT [46]	AML	Various	315	57	100	8	48	18	24	2-yr OS 53%/60% (CR1/ CR2) 2-yr DFS 40% (all pts)
FHCRC [47]	MDS	Flu/TBI	38	62	68	22	55	41	36	3-yr OS 28% 3-yr PFS 27%
EBMT [82]	MDS	Various	215	56	100	15	45	22	36	3-yr OS 41% 3-yr PFS 33%

(continued)

Table 17-2. (continued)

Study Group	Disease	Regimen	# Pts.	Median Pt. Age	% Pts. with MRD	% GVHD		NRM		Survival
						Grade III–IV Acute	Chronic	%	Follow-up (mos)	
Hadassah-Hebrew University [10]	CML-CP1	FB+ATG	24	35	79	29	55	15	60	5-yr OS & DFS 85%
Seattle consortium [60]	CML	Flu/TBI	24	58	100	12	32	21	36	2-yr OS 70% (CP1) 2-yr OS 56% (>CP1)
EBMT [61]	CML	Various	186	50	61	9	42	19	24	3-yr OS 54% (all pts) 3-yr OS 69% (CP1)

OS, Overall survival; DFS, disease free survival; PFS, progression-free survival; MRD, HLA-matched related donor; URD, unrelated donor; MDACC, MD Anderson Cancer Center; CGTG, Cooperative German Transplant study Group; EBMT, European Group for Blood and Marrow Transplantation; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; CR, complete remission; CP, chronic phase; Flu, fludarabine; TBI, total body irradiation; FAI, Flu 120 mg/m^2 + cytarabine 4 g/m^2 + idarubicin 36 mg/m^2; FM, Flu 100–150 mg/m^2 + melphalan 140–180 mg/m^2; FBC, Flu 150 mg/m^2 + busulfan 8 mg/kg + alemtuzumab 100 mg; FMC, Flu 150 mg/m^2 + melphalan 140 mg/m^2 + alemtuzumab 100 mg; FB + ATG, Flu 180 mg/m^2 + busulfan 8 mg/kg + ATG; Flu/TBI, 2 Gy TBI ± Flu 90 mg/m^2

Table 17-3. Recent results in patients given HSCT after nonmyeloablative/reduced-intensity conditioning for lymphoid malignancies.

						% GVHD		NRM		
Study Group	**Disease**	**Regimen**	**# Pts.**	**Median Pt. Age**	**% Pts. with MRD**	**Grades III–IV Acute**	**Chronic**	**%**	**Follow-up (mos)**	**Survival**
Royal Free and University College London [12]	LG/MCL/HG	FMC	88	48	72	5	7	11 (LG)-38 (HG)	36	3-yr OS 55% (all pts) 3-yr PFS 65% (LG) / 50% (MCL) / 34 (HG)
EBMT [68]	LG/MCL/HG/HL	Various	188	40	89	NR	16	26	12	2-yr OS 50% 2-yr PFS 30%
Seattle consortium [69]	HG	Flu/TBI	42	50	69	19	57	15	12	1-yr OS 63% 1-yr PFS 49%
MDACC [14]	LG	FC ± Rituximab	20	51	100	5	64	16	24	2-yr OS 84% 2-yr DFS 84%
MDACC [70]	MCL	FluCy + Rituximab / FCC	18	57	72	0	36	11	36	3-yr OS 86% 3-yr PFS 82%
Seattle consortium [67]	MCL	Flu/TBI	16	54	49	30	64	24	24	2-yr OS 65% 2-yr DFS 60%
Instituo Nazionale Tumori Milan [100]	TCL	FluCyThio	17	41	82	12	50	6	24	3-yr OS 81% 3-yr PFS 64%
EBMT [101]	HL	Various	311	31	71	NR	20	27	24	2-yr OS 46% 2-yr PFS 26%
Royal Free and University College London [102]	HL	FMC	49	32	63	4	14	16	24	4-yr OS 56% 4-yr PFS 39%
Hospital de la Santa Creu, Barcelona [103]	HL	FM	40	35	93	NR	47	25	12	2-yr OS 48% 2-yr PFS 32%
Berlin [64]	CLL	FB+ATG	30	50	43	20	75	15	24	2-yr OS 72% 2-yr PFS 67%

(continued)

Table 17-3. (continued)

Study Group	Disease	Regimen	# Pts.	Median Pt. Age	% Pts. with MRD	% GVHD		NRM		Survival
						Grades III–IV Acute	Chronic	%	Follow-up (mos)	
MDACC [63]	CLL	FluCy ± Rituximab	17	54	100	12	60	22	24	2-yr OS 80% 2-yr PFS 60%
Seattle consortium [62]	CLL	Flu/TBI	64	56	69	19	50	22	24	2-yr OS 60% 2-yr DFS 52%
Birmingham Heartlands Hospital [66]	CLL	FMC	41	54	58	10	13	26	24	2-yr OS 51% 2-yr PFS 45%
Dana-Farber Cancer Institute [65]	CLL	FB	46	53	33	19	38	17	24	2-yr OS 54% 2-yr PFS 34%
EBMT [49]	MM	Various	229	52	78	NR	50	26	24	3-yr OS 41% 3-yr PFS 21%
Seattle consortium [72]	MM	TBI*	54	52	100	7	46	2	3	2-yr OS 78% 2-yr PFS 55%

OS, Overall survival; DFS, disease free survival; PFS, progression-free survival; MRD, HLA-matched related donor; URD, unrelated donor; MDACC, MD Anderson Cancer Center; EBMT, European Group for Blood and Marrow Transplantation; LG, low-grade non-Hodgkin lymphoma; HG, high-grade Non-Hodgkin's Lymphoma; MCL, mantle cell lymphoma; HL, Hodgkin's Lymphoma; TCL, T-cell lymphoma; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; Flu, fludarabine; TBI, total body irradiarion; FMC, Flu 150 mg/m^2 + melphalan 140 mg/m^2 + alemtuzumab 100 mg; FM, Flu 150 mg/m^2 + melphalan 140 mg/m^2; FB + ATG, Flu 180 mg/m^2 + busulfan 8 mg/kg + ATG 20–40 mg/kg; Flu/TBI, 2 Gy TBI ± Flu 90 mg/m^2; FluCy, Flu 90–125 mg/m^2 + cyclophosphamide 2000–2250 mg/m^2; FCC, Flu 60 mg/m^2 + cisplatine 100 mg/m^2 + cytarabine 2 g/m^2; FluCyThio, Flu 60 mg/m^2 + cyclophosphamide 60 mg/kg + Thiothepa 10 mg/kg; TBI, 2 Gy TBI; FB, Flu 120 mg/m^2 + i.v. busulfan 3.2 mg/kg; * tandem autologous/allogeneic HSCT

54 patients with multiple myeloma. Patients were first given autologous HSCT after a cytoreductive dose of 200 mg/m^2 melphalan; this was followed 1.3–7.6 (median two) months later by allogeneic HSCT from HLA-identical sibling following 2 Gy TBI [72]. The 100-day mortalities after autologous and allogeneic HSCT were 2 percent each. Two-year overall and progression-free survivals were 78 percent and 55 percent, respectively. A large phase III study comparing tandem autologous HSCT with tandem autologous/allogeneic HSCT is currently ongoing in patients with multiple myeloma (BMT-CTN 01–02).

8. Nonmyeloablative HSCT After Failed Autologous HSCT

The outcomes for patients with relapse or secondary myelodysplastic syndromes after autologous HSCT were poor. A second myeloablative HSCT from an allogeneic donor has been a potentially curative option, but this approach has been limited by non-relapse mortality rates of 50 to 80 percent [8]. This prompted several groups of researchers to investigate the feasibility of allogeneic HSCT with nonmyeloablative or reduced-intensity conditioning in patients who had failed autologous HSCT. As shown in Table 17-4, most studies found lower non-relapse mortality, compared to what was seen following myeloablative allogeneic HSCT, and relatively encouraging results in patients with chemo-sensitive disease at HSCT [73–79] .

We recently analyzed data from 147 patients who had treatment failure with myeloablative autologous (n = 135), allogeneic (n = 10) or syngeneic (n = 2) HSCT and underwent HLA-matched related (n = 62) or unrelated (n = 85) HSCT following conditioning with 2 Gy TBI with or without added fludarabine, to determine factors that predict HSCT outcomes [80]. Three-year incidences of non-relapse mortality, relapse and overall survival were 32 percent, 48 percent and 27 percent, respectively, for patients given grafts from related donors, and 28 percent, 44 percent and 44 percent, respectively, for unrelated graft recipients. The best outcomes were seen in patients with Non-Hodgkin's Lymphoma, while patients with Hodgkin's Lymphoma and multiple myeloma had poor outcomes due to high incidences of relapse/progression (Fig. 17-5). Being in partial or complete remission at HSCT (P = 0.002), and developing chronic GVHD (P = 0.03) were associated with lower risks of relapse/progression. Further, being in partial or complete remission at HSCT (P = 0.01), absence of comorbidity at HSCT (P = 0.03) and lack of acute GVHD after HSCT (P = 0.06) were associated with better overall survival.

9. Outcomes with Myeloablative versus Nonmyeloablative Conditioning

Alyea, et al. performed a retrospective analysis of 152 patients (>50-years-old) with hematological malignancies undergoing HSCT after reduced-intensity (n = 71) or myeloablative (n = 81) conditioning [81]. Reduced-intensity conditioning consisted of fludarabine (120 mg/m^2) and intravenous busulfan (3.2 mg/kg), while myeloablative conditioning included mainly cyclophosphamide (3.6 g/m^2) plus TBI (14 Gy). With a median follow-up of 18 months, the cumulative incidences of relapse and non-relapse mortality were 46 percent and 32 percent, respectively, in the reduced-intensity conditioning group, versus 30 percent

Table 17-4. Recent results in patients given HSCT after nonmyeloablative/reduced-intensity conditioning after failed myeloablative HSCT.

						% GVHD		NRM		
Study Group	**Disease**	**Regimen**	**# Pts.**	**Median Pt. Age**	**% Pts. with MRD**	**% Grades III–IV Acute**	**Chronic**	**%**	**Follow-up (mos)**	**Survival**
Massachusetts General Hospital Boston [74]	HM	CyATG-ThyRx	13	38	100	38	40	1 pt.	11	2-yr OS 45% 2-yr DFS 38%
Christie Hospital Manchester [104]	Lymphoproliferative malignancies	FMC	38	44	100	0	15	20	14	14-mo OS 53% 14-mo PFS 50%
MDACC [76]	Chemo-sensitive NHL	FluCy + Rituximab (n = 16) or FCC (n = 4)	20	51	90	0	50	5	36	3-yr OS/PFS 95%
City of Hope Cancer Center, Duarte [77]	HM	FM (n = 24) or Flu/TBI (n = 4)	28	47	50	21	67	21	3	2-yr OS 57% 2-yr DFS 41%
Hospital de la Santa Creu, Barcelona [78]	HM	FM	46	47	100	24	73	24	12	1-yr OS 63% 1-yr PFS 57%
Hadassah-Hebrew University [73]	HM	FB+ATG	12	33	75	17	33	1 pt	3	3-yr OS 56% 3-yr DFS 50%
Seattle consortium [80]	HM	Flu/TBI	147	46	42	19	56	30	36	3-yr PFS 20% (MRD) 3-yr PFS 28% (URD)

OS, Overall survival; DFS, disease free survival; PFS, progression-free survival; MRD, HLA-matched related donor; URD, unrelated donor; MDACC, MD Anderson Cancer Center; NHL, Non-Hodgkin's Lymphoma; HM, Hematological malignancies; CyATG-ThyRx, Cyclophosphamide 150–200 mg/kg + ATG + Thymic irradiation (7 Gy); Flu, fludarabine; TBI, total body irradiation; FMC, Flu 150 mg/m^2 + melphalan 140 mg/m^2 + alemtuzumab 100 mg; FM, Flu 150 mg/m^2 + melphalan 140 mg/m^2; FB + ATG, Flu 180 mg/m^2 + busulfan 8 mg/kg + ATG 20–40 mg/kg; Flu/TBI, 2 Gy TBI ± Flu 90 mg/m^2; FluCy, Flu 90–125 mg/m^2 + cyclophosphamide 2000–2250 mg/m^2; FCC, Flu 60 mg/m^2 + cisplatine 100 mg/m^2 + cytarabine 2 g/m^2

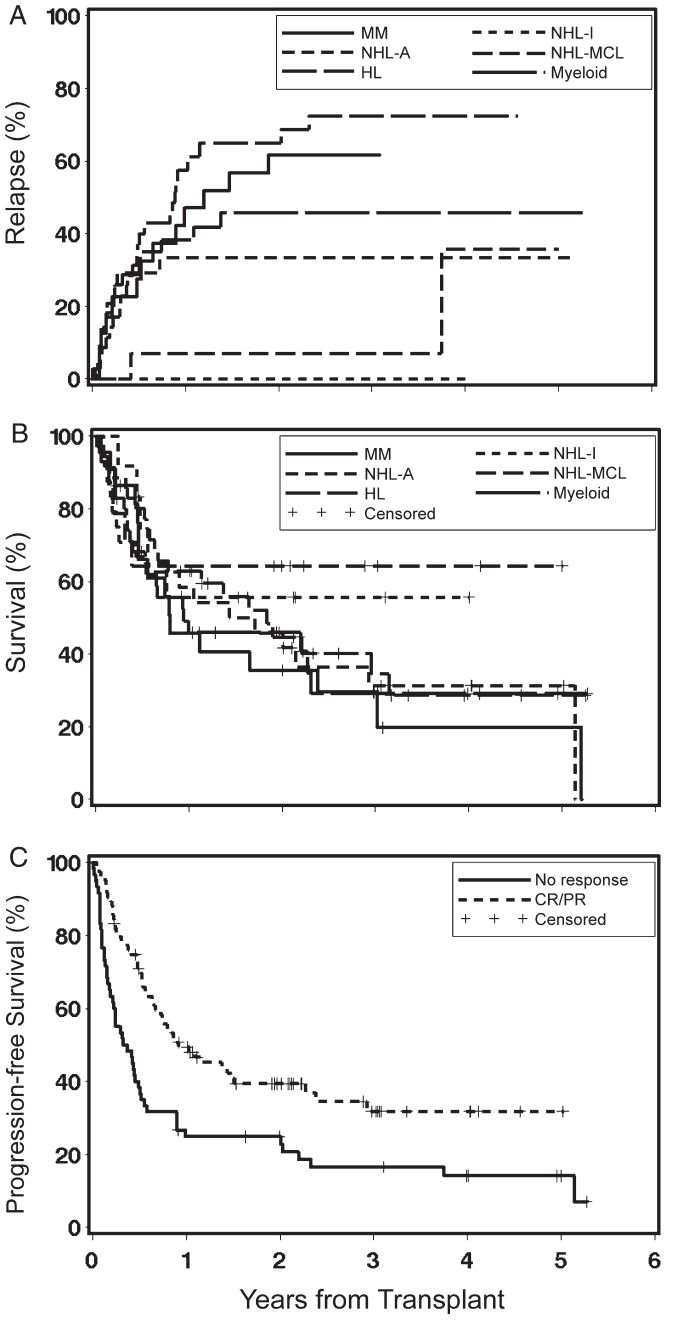

Fig. 17-5. Cumulative incidences of relapse **(A)** and overall survival **(B)** in 147 patients given nonmyeloablative HSCT after failed myeloablative HSCT according to diagnosis category group: HL, Hodgkin's Lymphoma; MM, multiple myeloma; Myeloid, myeloid malignancies including acute myeloid leukemia (n = 16), myelodysplastic syndromes (n = 12), chronic myeloid leukemia (n = 3), and myeloproliferative disorders (n = 2); NHL-A, aggressive Non-Hodgkin's Lymphoma (n = 24); NHL-I, indolent Non-Hodgkin's Lymphoma (n = 12); NHL-MCL, mantle cell lymphoma (n = 14) **(C)** Progression-free survival in 147 patients given nonmyeloablative HSCT after failed myeloablative HSCT according to disease status at HSCT Reprinted from F. Baron, et al., "Factors associated with outcomes in allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning after failed myeloablative hematopoietic cell transplantation." J Clin Oncol 2006; 24:4150–4157. Reprinted with permission from the American Society of Clinical Oncology"

($P = 0.05$) and 50 percent ($P = 0.01$), respectively, in the myeloablative group. Better overall survival was seen in the nonmyeloablative than in the myeloablative group at two years (39% versus 29%; $P = 0.056$).

Scott, et al. compared results of allogeneic HSCT following either nonmyeloablative (2 Gy TBI with or without added fludarabine; $n = 38$) or myeloablative (busulfan 16 mg/kg, targeted to 800–900 ng/mL and cyclophosphamide 120 mg/kg, $n = 112$) conditioning in patients with myelodysplastic syndrome over 40 years of age [47]. In multivariate analyses, three-year progression-free survival (HR = 1.1, P = 0.60), progression incidence (HR = 1.3, P = 0.43) and non-relapse mortality (HR = 1.0, P = 0.94) were comparable between nonmyeloablative and myeloablative patients. Further, in the subgroup of patients with transformed acute myeloid leukemia in morphological complete remission after chemotherapy, progression-free survival (HR = 1, P = 0.93) and progression rate (HR = 0.7, P = 0.64) were similar in patients given nonmyeloablative versus myeloablative conditioning. These observations suggest that Graft-versus-Tumor effects are more important than conditioning intensity in preventing relapse in this group of patients.

Martino, et al. compared HSCT outcomes in 836 patients who received HLA-identical grafts from siblings at various EBMT-affiliated centers after nonmyeloablative (n = 215) or myeloablative (n = 621) conditioning [82]. Nonmyeloablative/reduced-intensity conditioning included fludarabine with intermediate doses of 1–2 alkylating agents (i.e., ≤10 mg/kg p.o. busulfan; ≤140 mg/m^2 i.v. melphalan; or ≤10 mg/kg i.v. thiotepa) or low-dose (2–4 Gy) TBI. Three-year incidences of relapse, non-relapse mortality and progression-free survival were 45 percent, 22 percent and 33 percent, respectively, in nonmyeloablative recipients, versus 27 percent, 32 percent and 41 percent, respectively, in those given myeloablative conditioning. In multivariate analysis, nonmyeloablative recipients had a higher incidence of relapse (HR = 1.64, P = 0.001), but a lower incidence of non-relapse mortality (HR = 0.61, P = 0.015), leading to a similar probability of progression-free survival (P = 0.9).

Aoudjhane, et al. analyzed data from 722 patients with *de novo* acute myeloid leukemia over 50 years of age and given allogeneic HSCT after either reduced-intensity (n = 315) or myeloablative (n = 407) conditioning among EBMT-affiliated centers [46]. Reduced-intensity conditioning regimens were defined as fludarabine combined with low-dose TBI (<3 Gy), or busulfan (total dose ≤ 8 mg/kg) or other nonmyeloablative drugs. Two-year probabilities of leukemia-free survival for patients in first complete remissions at HSCT (n = 416) were 44 percent in patients given reduced-intensity conditioning versus 54 percent (P = 0.26) in patients given myeloablative conditioning. For patients in second complete remissions at HSCT (n = 104), the figures were 55 percent versus 47 percent (P = 0.81), respectively. In multivariate analyses, the use of reduced-intensity versus myeloablative conditioning was associated with a higher risk of relapse (RR 1.8, P = 0.0003), a lower risk of non-relapse mortality (RR 0.48, P<0.0001) and comparable leukemia-free survival (RR 1.15, P = 0.24).

Finally, Dreger, et al. compared data from 155 patients with chronic lymphocytic leukemia who were given allogeneic HSCT after either reduced-intensity (n = 73), or myeloablative conditioning (n = 82) [83]. Two-year rates of relapse, non-relapse mortality and event-free survival were 28 percent, 19 percent and 58 percent, respectively, in nonmyeloablative recipients, versus

11 percent, 26 percent and 62 percent, respectively, in those given myeloablative conditioning. In multivariate analysis, nonmyeloablative recipients had a higher incidence of relapse (HR = 2.46, P = 0.08), but a lower incidence of non-relapse mortality (HR = 0.40, P = 0.03), leading to a similar probability of event-free survival (HR = 0.69, P = 0.22).

Taken together, these studies suggest that nonmyeloablative/reduced-intensity conditioning achieved the goal of reducing early non-relapse mortality, but at the cost of a higher risk of relapse. Prospective studies comparing nonmyeloablative/reduced-intensity versus myeloablative conditioning are needed to define whether there is a role for nonmyeloablative/reduced-intensity conditioning in patients eligible for conventional myeloablative HSCT.

10. Impact of Comorbidities on the Selection of Conditioning Regimens

Since short-term results seem comparable in patients given either nonmyeloablative or myeloablative conditioning, an important question is whether it is possible to determine which patients might benefit from a nonmyeloablative or reduced-intensity conditioning, and which others could safely receive myeloablative regimens. In an effort to answer this question, Sorror, et al. assessed the effect of comorbidities (scored with the Hematopoietic Cell Transplantation-specific comorbidity index (HCT-CI) [84]) on outcomes among patients with acute myeloid leukemia or myelodysplastic syndromes receiving allogeneic grafts after either nonmyeloablative (n = 125) or myeloablative (n = 452) conditioning [85]. Survival for patients with low risk disease (defined as acute myeloid leukemia in first complete remission or myelodysplastic refractory-anemia) and/or no/few comorbidities (HCT-CI scores of 0–Z) was similar among the two groups. However, nonmyeloablative recipients with intermediate or high risk disease and HCT-CI scores of ≥3 had less non-relapse mortality (HR = 0.5, P = 0.05), in multivariate analysis suggesting that nonmyeloablative conditioning could be preferentially used in such patients.

The same group investigated the impact of comorbidities on HSCT outcomes in patients with B-cell malignancies given allogeneic HSCT after either nonmyeloablative or myeloablative conditioning [86]. Among patients without comorbidity at HSCT (HCT-CI = 0), survival was comparable for patients given nonmyeloablative or myeloablative conditioning (P = 0.7). In contrast, among patients with comorbidities (HCT-CI score ≥ 1) at HSCT, the use of nonmyeloablative conditioning was associated with lower non-relapse mortality (HR = 0.5, P = 0.009) and better overall survival (HR = 0.6, P = 0.04).

11. Does Nonmyeloablative HSCT Improve Survival over Chemotherapy in Patients with Hematological Malignancies?

It has been difficult to compare the results of phase I–II studies assessing nonmyeloablative/reduced-intensity conditioning to those obtained in comparable patients given conventional chemotherapy, since one could argue that only fitter patients were referred to transplantation centers and offered HSCT. This

underlines the interest of analyses comparing outcomes in patients who have an HLA-identical sibling donor (and could potentially receive a HSCT) in comparison to those who do not.

11.1. Acute Myeloid Leukemia

Mohty, et al. investigated whether allogeneic HSCT after reduced-intensity conditioning improved progression-free survival in adults with newly diagnosed acute myeloid leukemia who achieved complete remissions after induction chemotherapy, but were ineligible for conventional HSCT because of age or medical comorbidities [87]. Ninety-five consecutive patients {median age 52 (range, 26–65) years old} were retrospectively analyzed. Thirty-five patients had HLA-identical sibling donors (donor group), while 60 did not (no donor group). Twenty-five of 35 patients included in the donor group (71%) received the allogeneic HSCT, while 10 patients with an identified donor did not receive allogeneic HSCT because of patient or donor refusals (n = 6), early relapse (n = 2) or psychiatric disorders (n = 2). The four-year probability of progression-free survival was 54 percent in the donor group, versus 30 percent in the non-donor group (P = 0.01). This was due to a significantly lower risk of relapse in patients who received an allogeneic HSCT (12% at four years), than in those who did not (54% at four years, P<0.001).

The Groupe Ouest Est d'Etude des Leucémies et Autres Maladies du Sang (GOELAMS) recently reported the first results of a phase III study comparing outcomes of patients with acute myeloid leukemia in first complete remission receiving either autologous or allogeneic HSCT [88]. A search to identify an HLA-identical sibling was performed for each patient as they received a first course of consolidation therapy. After a second course of consolidation chemotherapy, patients with an HLA-matched sibling donor were scheduled to undergo an HSCT after either myeloablative (if age ≤50; consisting of 12 Gy TBI and cyclophosphamide 120 mg/kg) or reduced-intensity (if age 51–60; consisting of busulfan 4–8 mg/kg, fludarabine 120 mg/m^2, and ATG) conditioning. Among patients younger that 50 years, disease free survival was significantly better in patients included in the allogeneic arm (n = 111), than in those included in the autologous arm (71% versus 52%, P = 0.007). Among patients aged 50- to 60-years-old, there was better disease free survival in patients given reduced-intensity allogeneic HSCT, than in those given autologous HSCT (62% versus 50%, P = 0.27).

11.2. Multiple Myeloma

The Intergroupe Francophone du Myelome compared autologous HSCT followed by dose-reduced allograft (n = 65) with tandem autologous HSCT (n = 219) in high risk *de novo* multiple myeloma (defined as deletion 13 and/or β2 microglobulin >3 mg/L) [89]. The reduced-intensity conditioning regimen consisted of busulfan (4 mg/kg), fludarabine (125 mg/m^2) and ATG (Imtix; 12.5 mg/kg). Nineteen of the 65 patients with a sibling donor did not receive the allogeneic HSCT because of progressive disease (n = 7), donor/patient refusal (n = 5), ongoing infection (n = 4) or unknown causes (n = 3). On an intent-to-treat basis, survival (P = 0.27) and event-free survival (P = 0.56) did not differ between studies. However, the lack of improved survival in the allogeneic arm might be due to the high-dose ATG used that abrogated Graft-versus-Tumor

effects. Further, the choice of including busulfan instead of melphalan in the conditioning regimen was controversial. Indeed, the use of busulfan in the conditioning regimen was associated with inferior survival (P = 0.01) in the multiple myeloma EBMT study [49]. Results of the ongoing BMT-CTN 01–02 multiple myeloma study will help to better define the role for nonmyeloablative HSCT in patients with multiple myeloma.

12. Conclusions and Perspectives

Reduced-intensity conditioning and nonmyeloablative regimens have allowed older patients, those who had failed a high-dose HSCT, and those with comorbidity to benefit from the potentially curative Graft-versus-Tumor effects. Remarkably, minimally toxic regimens of 2 Gy TBI with or without fludarabine, or TLI plus ATG each followed by post-grafting immunosuppression with MMF and CSP have assured engraftment rates almost similar to those after myeloablative conditioning [15, 20] . Antitumor responses in some disease types require extended periods of time, with some patients achieving complete remissions more than one year after HSCT [15, 54] .

Ongoing efforts are directed at better preventing acute GVHD, at increasing the use of nonmyeloablative regimens in patients given haploidentical grafts [90] or unrelated cord blood [91] and at increasing Graft-versus-Tumor effects by combining nonmyeloablative conditioning with disease-targeted therapy such as imatinib, thalidomide, bortezomib, rituximab or radiolabeled monoclonal antibodies [63, 92–95] . For example, encouraging results have been achieved by combining the anti-CD45 radiolabeled monoclonal antibody with nonmyeloablative conditioning in patients with acute myeloid leukemia not in complete remission at HSCT or with advanced myelodysplastic syndromes [93]. Other groups of investigators are focusing on identifying patients at high risk of relapse early after HSCT and treating them with preemptive DLI or rapid taper of post-grafting immunosuppression [29]. Finally, further progress in adoptive transfer of T cell populations with relative tumor specificity are likely to improve HSCT's effectiveness after reduced-intensity or nonmyeloablative regimens [96].

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Chapter 18

Immune Reconstitution after Allogeneic Transplantation

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Abbreviations Allo: allogeneic; AML: acute myelogenous leukemia; ANC: absolute neutrophil count; ATG: anti-thymocyte globulin; BM: bone marrow; CB: cord blood; CDR3: complementarity-determining region 3; CM: central memory; CMV: cytomegalovirus; DC: dendritic cells; DLI: donor leukocyte infusion; EBV: Epstein Barr Virus; EF: effector; EM: effector memory; Foxp3: forkhead family transcription factor 3; G-CSF: granulocyte colony stimulating factor; GM-CSF: granulocyte macrophage colony stimulating factor; GVHD: Graft-versus-Host Disease; GVT: Graft-versus-Tumor; HHV-6: human herpes virus 6; HLA: human leukocyte antigen; HSCT: hematopoietic stem cell transplant; HSV: herpes simplex virus; IFN: interferon; Ig: immunoglobulin; IL: interleukin; KGF: keratinocyte growth factor; KIR: killer cell Ig-like receptor; LC: Langerhans cells; LLME: L-leucyl-L-leucine methyl ester; NK: natural killer; NST: nonmyeloablative stem cell transplant; PBSC: peripheral blood stem cells; PCR: polymerase chain reaction; PTLD: post-transplant lymphoproliferative disease; PWM: pokeweed mitogen; TCD: T cell-depleted; TCR: T cell receptor; Th1: T helper 1; Th2: T helper 2; TK: thymidine kinase; TNF: tumor necrosis factor; TREC: TCR rearrangement excision circle; Treg: T regulatory cells; TT: tetanus toxoid; VZV: varicella zoster virus

1. Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is an effective and curative treatment for a number of hematological malignancies and immune system or genetic disorders. AlloHSCT allows marrow lethal treatment of the primary disorder ands provides immunotherapy in the form of a Graft-versus-Tumor effect (GVT). Despite more than 35 years of experience with HSCT, the major barriers to this treatment have remained the same. These include Graft-versus-Host Disease (GVHD), the acute and chronic forms, disease progression or relapse, and the rather prolonged period of immune incompetence that occurs as the immune system redevelops. Approaches to reduce

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GVHD often result in exacerbation of immune incompetence, cause problems with engraftment or increase the likelihood of relapse, while attempts to speed engraftment and immune reconstitution have often exacerbated GVHD. Thus, a better understanding of the forces affecting each of these barriers is needed to achieve the right balance to improve HSCT outcome.

HSCT is followed by a period during which the immune system redevelops both by a process of peripheral expansion of transferred mature precursors and via reconstitution from immature progenitors, a process that may take a year or more to complete. Physically the major cellular elements of the immune system recover relatively early (within the first 100 days) and in a fairly predictable pattern in nearly all patients. This is followed by a more prolonged and variable period of functional recovery and maturation of the complex cellular interactions required for full immune competence. The recovery of immune function is further complicated in alloHSCT because of the need for immune suppression to permit engraftment and prevent or treat GVHD. Despite numerous advances in supportive care, morbidity and mortality due to infectious complications secondary to this procedure remain a serious problem. Better antimicrobial drugs, especially antiviral agents, have reduced early transplant mortality, but may have served to delay infections to a later time period in some cases. Late infection (after 100 days) remains one of the leading causes of death following HSCT, especially in recipients of grafts from unrelated donors [1].

Immune reconstitution has been extensively characterized by sequential studies of patient blood sampled at intervals post-HSCT. These studies have identified patient, donor and graft variables that appear to affect the rate of immune reconstitution primarily and, to a lesser extent, the pattern of reconstitution. Such studies are useful in predicting an expected pattern and rate of immune reconstitution for subgroups of transplant patients. Assessments that fall outside of the expected parameters of immune reconstitution for a given time post-HSCT may signal that the patient is at higher risk for infectious complications, thus warranting intervention. In a similar fashion, certain immune phenotypes may be diagnostic of post-HSCT events, such as graft rejection [2] or cytomegalovirus (CMV) reactivation [3]. Knowledge of the kinetics of immune reconstitution and of the patient, donor or graft variables that affect immune reconstitution can provide insights into the function of the immune system, as well as provide useful information relevant to patient care.

This chapter will review the expected pattern of cellular and humoral immune reconstitution following alloHSCT describing both classical and newer methods to evaluate immune function. Some of the more important variables that affect the tempo of immune reconstitution will be identified and approaches that might serve to promote a faster or more complete immune reconstitution in these patients will be described.

2. Patterns of Immune Reconstitution

2.1. Neutrophil Recovery

Neutrophils are essential as a first line of defense against bacterial and fungal infection. Historically, most patients were conditioned for transplant using myeloablative doses of chemotherapy, with or without irradiation, resulting in a period of from nine to 14 days of nearly absolute neutropenia during which there

is a high risk of infection. Without an HSCT rescue, the patient would not be expected to recover hematopoiesis. With myeloablation, the kinetics of neutrophil engraftment are influenced primarily by the graft source, dose of $CD34^+$ progenitor cells, use of hematopoietic growth factors, use of post-HSCT GVHD prophylaxis and, to a lesser extent, by the underlying disease [4]. In general, recipients of mobilized peripheral blood stem cell (PBSC) grafts engraft neutrophils and platelets earlier than recipients of bone marrow (BM) [5, 6], a result of the larger dose of $CD34^+$ progenitors in the PBSC graft as well as to the more differentiated status of peripheral progenitors compared to BM. There is a dose-dependent increase in the rate of neutrophil engraftment up to a threshold number of $CD34^+$ cells (approximately 2.0×10^6/kg) in most studies [7]. Hematopoietic growth factors (primarily granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been widely used to enhance the kinetics of neutrophil engraftment [8], but may be associated with a delay in platelet engraftment [4]. Drugs such as methotrexate that are widely used to prevent acute GVHD are marrow suppressive and may further delay neutrophil engraftment even when growth factors are used [9]. Antiviral agents used to prevent or treat vial infections may likewise be myelosuppressive. The patient's primary disease and the extent of pre-transplant therapy may also affect the rate of neutrophil recovery with patients transplanted for aplasia recovering granulocytes more quickly than those transplanted for acute or chronic leukemia [10]. However, even under the most optimal conditions, one can expect from nine to 16 days during which the absolute neutrophil count (ANC) is below 0.5×10^9/L. Once engraftment occurs, the ANC generally rises rapidly to protective levels above 10^9/L rapidly. With nonmyeloablative stem cell transplant (NST) regimens, the period of neutropenia is considerably shortened or is eliminated, a major factor in the reduced toxicity of these regimens [11].

The ability of the engrafted neutrophils to phagocytize and kill bacteria returns early post-HSCT and neutrophil chemotaxis normalizes by four months unless the patient is experiencing active GVHD or infection [12,14]. Zimmerli, et al. [14] demonstrated that patients who subsequently developed pyogenic infections had poorer neutrophil function than those who did not. In this study, defective skin window migration or combined defects in migration and killing were predictive for late pyogenic infections. Neutrophil migration may be further reduced during infusion of GM-CSF, but normalizes quickly after infusions are stopped [15]. Prophylactic supportive care with antimicrobial agents is of most importance during the engraftment period and for patients with active GVHD.

2.2. Monocyte and Dendritic Cell Reconstitution

Monocytes, like neutrophils, provide a first line of defense by the immune system and this function similarly recovers rapidly post-HSCT. Recipients of BM grafts have shown early recovery of monocyte phagocytic and killing functions, although impairment in tissue-derived macrophages and in monocyte adherence is seen [16, 17]. Monocytes additionally serve as antigen presenting cells (APC) and this function too, is rapidly reconstituted [18]. Monocytes from a minority of patients exhibit suppressor activity in several systems, especially with nonphysiologic ratios of monocytes to T cells or B-cells [18]. Early after engraftment, monocytes appear to be somewhat activated as meas-

ured by increased respiratory burst activity and higher than normal levels of serum neopterin and tumor necrosis factor (TNF) for up to six weeks after alloHSCT. However, these measures of monocyte activation were not associated with GVHD and may represent a nonspecific activation state caused by exposure to infectious agents during this period [19]. Indeed, multivariate analysis has shown that below normal monocyte counts or low B-cell counts at day 80, rather than a low ANC, predicts a higher incidence of infection from day 100 to day 365 in BM-grafted patients [20]. Consistent with this finding, recipients of alloPBSC who recover normal absolute monocytes by one month experience few early or late post-HSCT infections [21].

The essential role of monocytes in immune reconstitution has been strikingly demonstrated in recipients of $CD34^+$ cell-selected haploidentical- HSCT who were given G-CSF to promote neutrophil engraftment [22]. In this study, G-CSF was shown to interfere with the ability of engrafted monocytes to produce interleukin (IL)-12. This defect, in turn, delayed reconstitution of $CD4^+$ T cells with T helper 1 (Th1) activity and skewed T cells to a T helper 2 (Th2) phenotype. The production of IL-4 and IL-10 by Th2 T cells in turn, inhibited monocyte maturation into dendritic cells (DCs) resulting in an overall long-term impairment of immune function beyond that expected. Removal of G-CSF from the post-HSCT regimen delayed, but did not prevent, neutrophil engraftment, but did correct the defect in monocyte IL-12 production resulting in improved recovery of Th1-type $CD4^+$ T cells. Although defects are seen in monocyte function in G-CSF mobilized PBSC donors, these do not seem to translate into clinical immune deficits, likely the result of the shorter duration of the therapy [23]. Monocytes contained within PBSC products have been shown to produce a large amount of IL-10 and to suppress alloreactive T cell responses [24]. This finding may explain why alloPBSC HSCT have not been associated with higher rates of acute GVHD despite the nearly 10-fold larger dose of T cells infused [6, 25].

Early studies looked at the recovery of Langerhans cells (LCs) in skin biopsies at intervals over the first year post-HSCT [26]. Nearly all patients showed very low numbers of LCs through the first four months that subsequently normalized. In most patients, LCs were of host origin through day 49 and in some up to day 120, but by one year LC converted to the donor in most patients [27]. Patients with GVHD had a more prolonged recovery, suggesting that poor antigen-presenting capacity in the skin may contribute to the pathology of this disorder. A lack of skin resident LCs may also explain why patients with chronic GVHD exhibit poor delayed type hypersensitivity reactions to challenge with neo-antigens and recall antigens, unlike patients without chronic GVHD [28]. In contrast, peripheral blood DCs of donor origin reconstitute rapidly following both myeloablative and nonmyeloablative transplant, with approximately 80 percent being donor-derived by day 14 and 95 percent or more by day 56 [29]. While overall DC recovery is rapid there may be a skewing of DC subsets, with a more rapid recovery of the DC1 subset ($CD1c^+CD123^{low}$), than the DC2 subset ($CD11c^-CD123^{bright}$) [30].

Graft–versus-Host Disease may be initiated by the presentation of host alloantigens by host DCs to donor-derived T cells [31]. DCs (and LCs) express the CD52 antigen recognized by the monoclonal antibody, Campath and host type DCs are effectively depleted by preparative treatment with Campath-1G [32]. Host DC depletion during conditioning may contribute to the low rate of acute GVHD in Campath-1G treated patients [33]. Campath-1G conditioning did not affect the tempo of donor-derived DC recovery compared to untreated patients.

DC1 ($CD11c^+$) recovered more rapidly than DC2 ($CD11c^-$) in this group; however, DC numbers were only half those of healthy donors by one year [32].

2.3. Natural Killer Cells

Natural killer (NK) cells derive from a common lymphocyte precursor like T cells and B–cells, but are part of the innate immune system and, as such, are designed for a rapid response to danger (reviewed in ref. [34]). NK cells mediate cytotoxicity both through direct recognition of the infected target (intracellular bacteria, parasites or virus) or transformed target cells, and indirectly via antibody-dependent cellular cytotoxicity. NK cells can also interact with DCs mediating both positive and negative effects on DC function [35]. NK cells bear a complex array of receptors that fall into two main structural types, those with C-type lectin binding domains and those with immunoglobulin (Ig)-like domains. Both types contain activating and inhibitory receptors that regulate NK cell activity. Immature NK cells primarily express the lectin-like receptors, represent approximately 2 percent of the NK cells in healthy adults, and are greatly enriched in fetal and cord blood. The lectin-like receptors consist of a heterodimer of NKG2 and CD69, are of limited polymorphism and recognize the nonclassical human leukocyte antigen (HLA)-E molecule bound with peptides from the leader sequence of classical class I molecules [36]. Immature NK cells bearing CD94/NKG2 receptors have a distinct phenotype, displaying high levels of CD56 ($CD56^{bright}$), low or absent CD16 ($CD16^{dim\ or\ neg}$), high levels of the IL-2 receptor β chain and coexpress CD26. These cells mediate poor resting lytic activity, but respond readily to IL-2 with a broader range of target recognition and increased lytic potential [37]. The killer cell Ig-like receptors (KIR) expressed on more mature NK cells are encoded by a family of genes that vary in number dependent upon the haplotype that is inherited. Two groups of haplotypes are present in the population; group A haplotypes have fewer genes, but carry most of the inhibitory KIR genes with only a single activating KIR gene, while group B haplotypes carry multiple activating genes and fewer inhibitory genes. In addition to a variable number, KIR genes are more polymorphic than CD94/NKG2 receptor genes. The identities of most activating ligands are unknown, but they appear to be primarily expressed by activated or proliferating cells. HLA-C locus antigens provide the strongest inhibitory signals to KIR receptors and differentially bind to specific KIR receptors based on the presence of asparagine (Group 1) or lysine (Group 2) at position 80 of the class I α1 helix. HLA-B locus antigens that fall into the Bw4 group, but not those in the Bw6 group, also provide inhibitory signals to specific KIR receptors. The HLA-A locus antigens, A3 and A11 have been less consistently shown to serve as inhibitory KIR ligands since their ability to inhibit NK cell activity appears to differ based on the peptides that are bound [38]. As they mature, NK cells express lower levels of CD56 ($CD56^{dim}$) and higher levels of CD16 ($CD16^{bright}$), express KIR, but little or no CD94/NKG2, mediate potent resting lytic activity towards NK sensitive targets, and are less well activated by IL-2 than are immature NK cells [37, 39]. NK cells play a role not only in the restoration of post-HSCT immunity, but may also mediate graft rejection, participate in GVHD reactions, as well as serve as GVT effectors (reviewed in [40]).

Similar to neutrophils and monocytes, the number and function of donor-derived NK cells normalize, or even exceed normal levels quite early after

nearly all forms of alloHSCT. During the first three to four months, NK cells may be the dominant lymphocyte population, especially in recipients of grafts that have been depleted of mature T cells [41]. This includes allogeneic recipients of highly purified $CD34^+$ cell-selected grafts [42] and recipients of cord blood grafts [43, 44] where the infused NK cells are mostly immature with poor lytic function [45]. The majority of HSCT patients reconstitute with the more mature $CD56^{dim}CD16^{bright}$ NK subset. However, a subset of patients (35%) receiving autologous or allogeneic T cell-replete grafts have shown an overrepresentation of $CD56^{bright}CD16^-$ cells through four months post-HSCT [46]. As expected these immature NK cells exhibited poor lytic activity when tested fresh, but were highly lytic after culture with IL-2 [39]. In contrast to most other immunological functions, NK activity may be increased in patients with acute GVHD, perhaps secondary to activation from the cytokines produced during this reaction [47, 48]. The pattern of NK cell recovery based on phenotype is illustrated in the upper left panels of Figures 18-1 and 18-2 from our own studies of adult and pediatric recipients of conventional T cell-replete grafts and partially T cell-depleted (TCD)-BM grafts. Here it can be seen that while recipients of TCD grafts recover NK cells the fastest, by 100 days the median absolute NK cell count is within or near the normal range for all patient groups. Long-term NK cell recovery is minimally affected by patient age, extensive chronic GVHD or by T cell depletion.

Fewer studies have looked directly at the recovery of NK cell receptors. Using antibodies reactive with KIR gene products, CD94 and NKG2A, Shilling, et al. [49] examined NK repertoire in 18 recipients of T cell-replete alloHSCT at intervals over the first year post-alloHSCT. The expression of the major KIR receptors and of CD94/NKG2A was compared to NK cells from the donor and the patient obtained prior to transplant. The earliest assessments, prior to day 60, for all patients showed a low frequency of KIR-positive cells associated with increased expression of CD94/NKG2A, as is the pattern in NK cell ontogeny. These data agree with the observation that recipients of T cell-replete grafts have a relatively high percentage of $CD56^{bright}$ $CD16^{low\ or\ neg}$ NK cells during the early period post-engraftment that have poor lytic activity, likely due to the expression of the potent CD94/NKG2A inhibitory receptors [46]. The majority of patients showed a steady recovery of KIR gene expression and loss of NKG2A to a repertoire similar to that of the donor by six to nine months that remained stable even when measured at year three. A second group showed a relative pattern of NK cell receptor expression that was similar to the donor, but with a much lower overall percentage of cells expressing KIR through one year. However, by three years patients who were re-examined did recover to pre-transplant donor levels. The reason for this delayed recovery was not apparent, but could reflect different preparative regimens. The potential clinical relevance of NK cell receptor immune reconstitution is illustrated by the third group of patients in this study who experienced complications post-alloHSCT, including GVHD and relapse, and failed to regenerate NK cells with a repertoire like that of the donor or the patient. A study of pediatric recipients of haploidentical CD34-enriched grafts found abnormal expression of KIR inhibitory receptors at one month, but by three months the patterns were similar to that of the donor [50]. Cooley, et al. [51] used a similar approach to determine the effects of T cell depletion on NK-receptor immune reconstitution. Here only a single time point, day 100, was examined in 77 recipients of unrelated marrow grafts, but even though

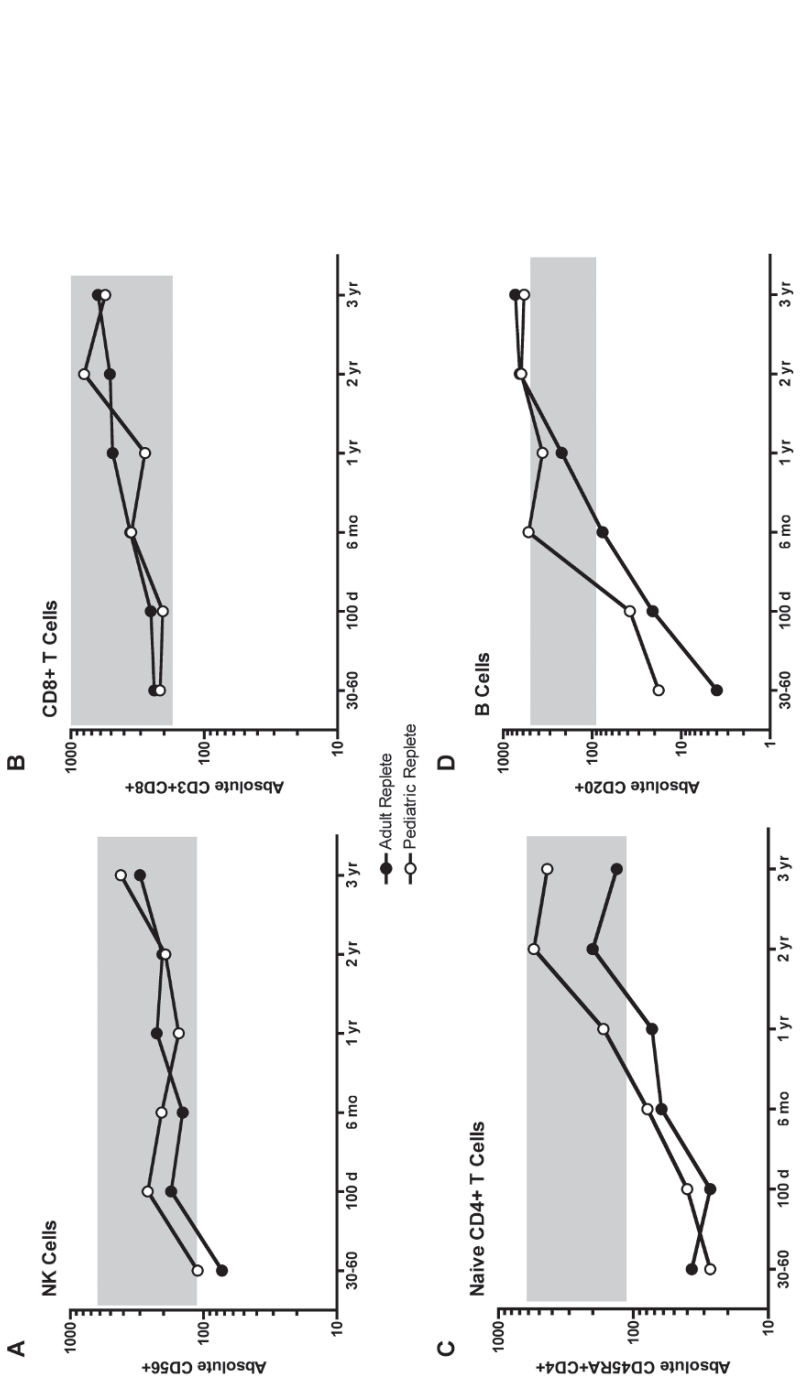

Fig. 18-1. Effect of patient age on lymphocyte subset recovery following T cell-replete HSCT. The median absolute cell counts of $CD56^+$NK cells (panel A), $CD3^+CD8^+$ T cells (panel B), $CD4^+$ $CD45RA^+$ naïve T cells (panel C), and $CD20^+$B cells (panel D) for patients tested at or near the indicated time following HSCT are shown. All patients received T cell-replete grafts and only those patients without extensive chronic GVHD are shown. Adult patients (>18 yr, N = 29) are shown using closed circles and pediatric patients (≤18 y, N = 28) are shown using open circles. Not all patients were tested at each interval, and a given patient was tested only once during an interval. The shaded area represents the 5^{th} to 95^{th} percentile of absolute values from 49 healthy adult donors

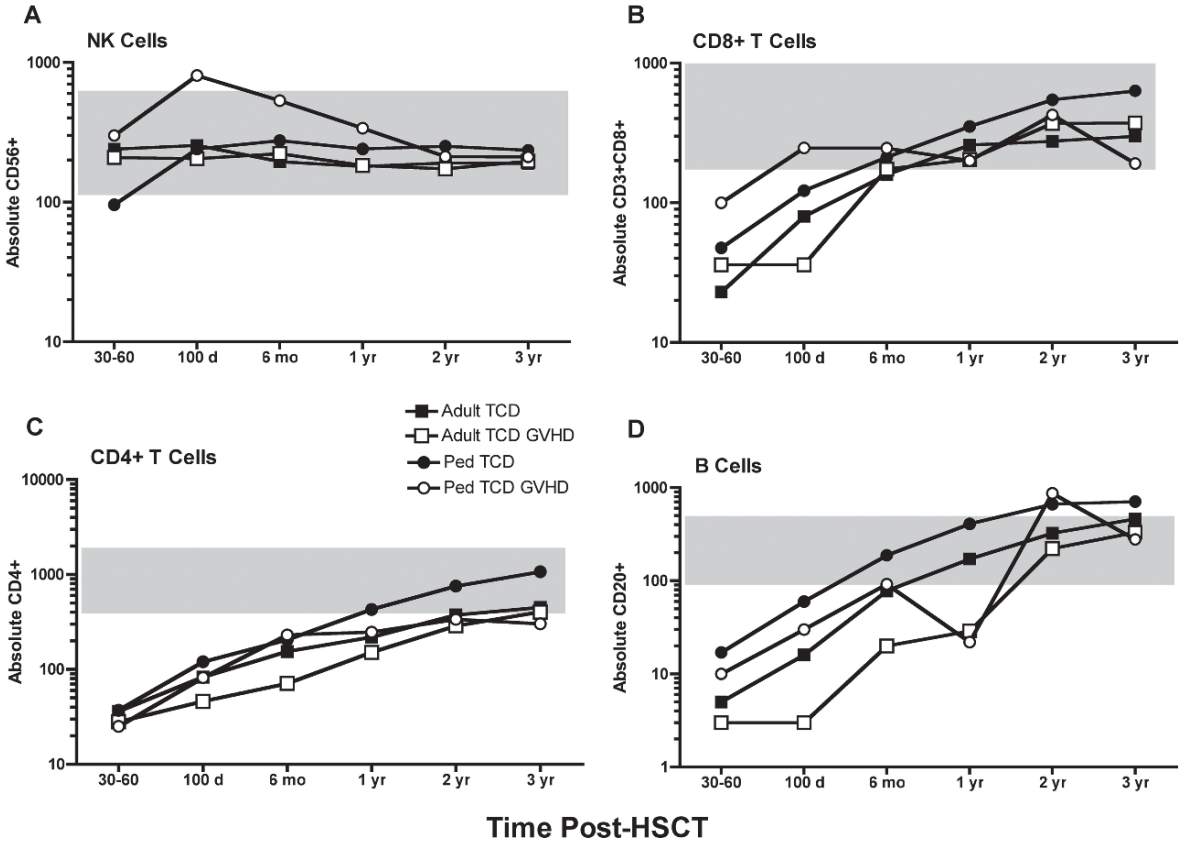

Fig. 18-2. Effect of patient age and GVHD on lymphocyte subset recovery following TCD HSCT. The median absolute cell counts of $CD56^+$NK cells (panel A), $CD3^+CD8^+$ T cells (panel B), $CD4^+$ $CD45RA^+$ naïve T cells (panel C), and $CD20^+$B cells (panel D) for patients tested at or near the indicated time following HSCT are shown. Not all patients were tested at each interval, and a given patient was tested only once during an interval. All patients received grafts purged of T cells by complement-mediated lysis using T10B9-1A1 monoclonal antibody or OKT-3 monoclonal antibody resulting in <2.0 logs TCD requiring post-HSCT cyclosporine as additional GVHD prophylaxis. Adult recipients (>18 yr) without or with only limited chronic GVHD are shown using closed squares (N = 214), adults with extensive chronic GVHD are shown using open squares (N = 50), pediatric recipients (≤18 yr) without or with limited chronic GVHD are shown using closed circles (N = 175) and pediatric recipients with extensive chronic GVHD are shown using open circles (N = 17). The shaded area represents the 5^{th} to 95^{th} percentile of absolute values from 49 healthy adult donors

by this time post-alloHSCT the number of NK cells in recipients of TCD grafts and T cell-replete grafts are similar, differences were seen in NK cell receptor expression. Consistent with the phenotype studies showing higher levels of $CD56^{dim}CD16^{bright}$ in recipients of TCD grafts, NK cells from these patients expressed higher levels of KIR than did NK cells from recipients of T cell-replete grafts. At day 100 neither patient group had recovered an NK cell repertoire similar to their donors and NK cells from both groups over-expressed CD94/NKG2A, as was shown in the Shilling study. As might be expected from their immature status, a higher frequency of NK cells from recipients of T cell-replete grafts produced interferon (IFN)-γ as compared to NK cells from their graft donors or from recipients of TCD grafts. The percentage of NK cells producing IFN-γ in this series was a significant independent variable associated with the incidence of acute GVHD while a higher percentage of NK cells expressing KIR was associated with improved survival [51]. The production of IFN-γ by NK cells has previously been reported to be associated with acute GVHD post-alloHSCT, but whether this is a contributing factor to GVHD, or secondary to GVHD, is unknown [52]. A more limited study of NK-receptor immune reconstitution was performed by Nguyen, et al. [53] on NK cells obtained over the first four months post-alloHSCT from recipients of haploidentical CD34-enriched grafts transplanted for advanced acute myelogenous leukemia (AML). Because all of the patients died from relapse or treatment-related mortality, none fully reconstituted their NK cell receptor repertories. However, like the other studies, there was increased expression of CD94/KNG2A and decreased expression of KIR at both one month and four months post-HSCT. The NK cells were poorly lytic to recipient AML blasts, but lysis could be increased if anti-NKG2A was added to assay to prevent inhibition through this receptor.

Through a stochastic process controlling gene transcription that is not fully understood, individual NK cells may express only a portion of the receptors for which they have genes, a trait that is conferred to daughter cells due to epigenetic methylation of the unexpressed KIR genes. Furthermore, the level of KIR receptor expression may be partially influenced by the individual's HLA phenotype [54]. Most NK cell clones will express minimally one KIR receptor inhibited by a self class I ligand, but rarely two. Those clones that do not express any inhibitory KIR receptor express CD94/NKG2A, while most KIR positive clones lack CD94/NKG2A [55]. Thus, the repertoire of NK-receptor expression differs from individual to individual and is clonally distributed within a given individual. NK cells with low-level or no expression of inhibitory receptors can be triggered to kill targets that do not express class I, express defective class I or lack the specific class I ligand they recognize, provided the target also expresses appropriate activating ligands, along with costimulatory and adhesion molecules (reviewed in ref. [34]). Through this mechanism NK cells recognize tumor targets. Recently, there has been heightened interest in the role of NK cells as GVT effectors after Ruggeri, et al. [56] initially reported high rates of disease-free survival in AML patients transplanted with haploidentical TCD-alloHSCT when the recipient lacked an inhibitory KIR ligand present in the donor. The authors hypothesize that the absence of the inhibitory ligand in the unshared HLA haplotype allowed donor-derived NK cells that would otherwise have been inhibited to expand and eliminate residual leukemia. Indeed, donor-derived NK cell clones isolated

pre-transplant from the donor, as well as those isolated from the patient during the first four months post-alloHSCT, are highly lytic when tested against AML targets that lack HLA ligands for donor KIR receptors [57]. The model of NK cell alloreactivity proposed by Ruggeri assumes that the donor possesses all of the inhibitory KIR ligands, an assumption that is mostly, but not always, correct. A longer-term follow-up of their study continues to show improved survival based on this model, especially in patients transplanted for AML in remission [55], although not all studies have shown a beneficial effect of a missing KIR ligand in the unshared HLA haplotype [53, 58]. Leung, et al. [50] analyzed haploidentical pediatric recipients of TCD alloHSCT and also found a reduced incidence of relapse when donors had KIR ligands missing in the patients, but found a more significant reduction in the risk of relapse when the KIR repertoire of the engrafting NK cells was also considered. Since many individuals will posses KIR receptors for which they lack the corresponding ligand, the hypothesis is that, upon regeneration in the host, those NK cells would be potentially alloreactive before coming under normal regulation [59]. This agrees with the finding by Ruggeri, et al. [57] that alloreactive NK cell clones are only identified early after transplant. In support of this hypothesis, our own studies of recipients of TCD grafts from HLA-identical sibling donors have confirmed a significantly improved disease free survival in patients with AML or myelodysplastic syndrome missing ligands for KIR expressed on donor NK cells [60]. The effect of missing KIR ligands in recipients in the setting of T cell-replete transplant has been more variable, with some studies reporting effects [61, 62] and others not [63]. A recent study of a large number of recipients (N=1770) of T cell-replete unrelated donor transplants showed a lower risk of relapse associated with missing KIR ligands in HLA-mismatched patients for both lymphoid and myeloid leukemia, but in this case no such effect was seen in HLA-matched transplants [64].

The number and type of activating KIR genes on engrafting donor NK cells may also play a role post-alloHSCT. Because of the manner in which KIR genes segregate, one type of haplotype, the group A haplotype, bears the main inhibitory KIR genes and only a single activating gene, while the more common group B haplotype bears multiple activating genes. Activating KIR, while not detected immediately after engraftment, were detected by day 30 post-alloHSCT in one study, and expression of the activating KIR was associated with increased NK lytic activity [65]. Donor KIR haplotype does appear to positively affect transplant outcome by protecting from GVHD [66, 67], relapse [68, 69] or viral infection [70, 71] in some, but not all, studies reported [55]. The combined effect of KIR ligand mismatch and a haplotype with a large number of activating genes, may provide an even stronger survival advantage than either alone [34, 55].

The rapid redevelopment of NK cells post-HSCT is consistent with current knowledge of lymphoid development (reviewed in ref. [72]). T Cells and NK cells likely share a common precursor, but with different sites of differentiation. Whereas T cells normally mature and develop in the thymus, a structure that is mostly atrophied in adult transplant recipients and takes considerable time to recover, NK cells primarily develop in the BM [73]. It might be expected then, that NK cells would re-populate the host before T cells, which must develop in a less optimal site. Although the potential for improving transplant outcome by selecting donors based on their KIR repertoire is

apparent, the optimal requirements for graft T cell content, HLA-disparity and patient disease are less clear.

2.4. T Cells

The recovery of T cells and T cell function post-HSCT has been extensively studied for it is here that we see the most profound and long-lasting deficiency in immune function. Immunophenotype analysis by flow cytometry has been most widely used to characterize T cell immune status post-HSCT, along with other measures, the results of which will be presented in this section. One such method assesses the diversity of T cell receptor (TCR) repertoire using quantitative real-time polymerase chain reaction (PCR)-based methods to expand the TCR complementarity determining region 3 (CDR3) of TCR-Vβ gene families. Due to nonrandom amino acid incorporation in this region, this assay results in a series of from eight to 10 gel bands (or peaks if an amino acid sequencer is used) of varying length that are roughly normally distributed for each Vβ family. The assessment of all Vβ families by this technique has been called CDR3 size spectratyping [74]. CDR3 size spectratypes are complex and complete in healthy individuals, but may be highly skewed when individual T cell clones have expanded or contracted in the setting of disease, or after transplant. Recently an assay has been developed to more precisely measure thymopoiesis than the use of cell surface markers, such as CD45RA, that do not perfectly correlate with thymic selection. This assay uses quantitative PCR to estimate the number of T cells in a sample with excised DNA fragments left over from the TCR rearrangement that occurs during thymus maturation. These fragments, called TCR rearrangement excision circles (TREC), remain in the cytoplasm and are diluted in number as the T cell divides. Therefore, the proportion of T cells with TREC in a population is a measure of recent thymic activity [75].

Assessing the presence (or absence) of T cell subsets can be a good measure of T cell immune reconstitution. However, there is a complex, balanced network of T cell subsets that provide help to B cells and to other T cells, regulate immune response and serve as effectors through soluble mediators and by cell-mediated cytotoxic killing. To fully characterize immune reconstitution post-HSCT assessment of T cell function is also required. In general proliferative responses to mitogens recover to normal levels later than T cell numbers, taking one year or longer even in patients who do not experience GVHD or receive post-HSCT immune suppression [41, 76, 77]. Such early functional defects may be responsible for the increased susceptibility of HSCT recipients to viral infection, particularly reactivation of viruses in the Herpes family, including CMV [78], herpes simplex virus (HSV), human herpes virus-6 (HHV-6) [79] , Epstein Barr Virus (EBV) [80] and varicella zoster virus (VZV) [81] during this period.

Although graft-related, donor-related and patient-related variables affect T cell reconstitution, certain aspects are common to nearly all patients. Much data support the concept that, in contrast to neutrophils, monocytes and NK cells that rapidly reconstitute from progenitors, T cell reconstitution over the first year in adults is largely derived from mature cells contained in the graft expanding in response to the antigenic environment of the host. As a result T cell reconstitution is fastest in recipients of PBSC grafts that contain approximately

10-fold the T cell dose of BM, and is slowest in recipients of grafts that are rigorously depleted of mature T cells. T cell reconstitution is additionally impaired by therapies for the prevention or treatment of GVHD that nearly all target T cells. Furthermore, GVHD itself can directly hinder T cell reconstitution by damaging lymphoid organs, including the thymus, that are needed for T cell redevelopment from stem cell precursors [82, 83].

2.4.1. Effect of Graft Source on Tcell Recovery

The two most commonly used graft sources for HSCT are BM and PBSC. Several comparative studies have shown a faster overall T cell recovery in adult recipients of PBSC grafts as compared to BM graft recipients that is most apparent during the first one to three months [84–86]. Total $CD8^+$ T cells recover faster than $CD4^+$ T cells in both groups, with $CD8^+$ T cells reaching the range of healthy controls by three to six months. Naïve $CD4^+$ T cells ($CD45RA^{bright}$), which are mostly thymic-derived, take longer to recover than memory subsets ($CD45RA^{low/-}$ or $CD45RO^+$). Naïve $CD4^+$ T cells reach the lower limit of normal in recipients of PBSC grafts by one year while BM recipients have still not fully recovered by that time [85, 85]. $CD8^+$ T cells include several distinct subsets that can be distinguished by specific cell surface markers. Like $CD4^+$ T cells, naïve thymic derived $CD8^+$ T cells are $CD45RA^{bright}$ and coexpress CD28, CD62L and lack expression of CD11b. These cells have not previously encountered antigen, produce IL-2 and proliferate strongly in response to antigen, but lack the ability to kill through the granzyme/perforin pathway. Central memory (CM) $CD8^+$ T cells share a similar phenotype to the naïve subset, but are $CD45RA^-$ having previously encountered antigen. Once activated in the lymphoid organs where they reside, CM cells rapidly regain the ability to kill and to produce IFN-γ and TNF-α. In the periphery, effector memory (EM) ($CD45RA^-CD28^-CD62L^-CD11b^+$) and effector (EF) ($CD45RA^+CD28^-CD62L^-CD11b^+$) $CD8^+$ T cells rapidly produce cytokines and mediate cytotoxicity upon antigen encounter (reviewed in ref. [87]). Ferrari, et al. [86] have recently described $CD8^+$ T cell subset recovery during the first 100 days in recipients of PBSC and BM grafts. PBSC grafts contained approximately 10-fold more $CD8^+$ T cells consisting of 20-fold more CM and 4-fold more EM and EF $CD8^+$ T cells than BM grafts. However, despite this larger starting infusion, at both one month and three months these investigators found no significant differences between recipients of BM and PBSC in the absolute number of any of the $CD8^+$ T cell subsets, although by three months EF and EF subsets were 25 percent of normal in the BM group and 50 percent of normal in the PBSC group.

This failure to fully reconstitute the diversity of T cells in the starting produce may be explained by the greatly increased rate of T cell apoptosis seen early post-transplant. $CD4^+$ T cells and most prominently $CD8^+CD45RO^+$ T cells recovered up to one year post-alloHSCT undergo enhanced spontaneous apoptosis during short-term culture [88]. Similar results were seen by Ferrari, et al. [86] who found that T cells obtained shortly after engraftment from recipients of alloPBSC and alloBM grafts displayed significantly higher levels of activation antigens (CD69) and caspase C (a marker of apoptosis) than T cells from recipients of autologous HSCT. The high apoptosis rate decreases in conjunction with the appearance of naïve T cells from the thymus. As might be predicted, CDR3 spectratyping shows that recovery of T cell repertoire

diversity is most rapid in recipients of PBSC HSCT who receive larger T cell infusions [89]. A broader T cell repertoire is largely associated with the presence of $CD4^+CD45RA^+$ T cells as determined by analysis of separated populations [90].

The use of cord blood (CB) as an HSCT source for both pediatric and adult recipients is increasing. Differences in T cell immune reconstitution might be expected based solely on T cell dose, since CB contains approximately 10-fold fewer T cells than marrow and 100-fold fewer T cells than PBSC. However, CB T cells are also nearly completely lacking in $CD4^+$ or $CD8^+$ memory T cell subsets [45, 91]. As might be expected, T cell reconstitution following CB-alloHSCT is delayed compared to BM or PBSC grafts, reflecting the lower number of T cells in the infusion. Unlike the usual fast recovery of the $CD8^+$ T cells seen in PBSC and BM transplants, $CD4^+$ T cells were found to be equal to or greater than $CD8^+$ T cells with a CD4:CD8 ratio above 1.0 by the second month following a CB HSCT that does not normalize to the expected 2:1 ratio until 12 to 24 months [92]. Recipients of CB grafts had higher numbers of $CD4^+CD45RA^+$ cells and markedly fewer $CD8^+CD11b^-$ cells (CM cells) than BM recipients during the first six months after transplant [93], reflecting the immature phenotype of the cells infused. CB T cells exhibited a highly abnormal T cell repertoire the first year following transplant, but attained a higher level of diversity than BM recipients by two years as measured both by TCR CDR3 diversity and by TREC analysis [94]. T cell function as measured by proliferative responses to one or more herpes viruses over the first three yearss post-transplant, but not immunophenotype characteristics of the major lymphocyte subsets, was found to be predictive of disease free survival in recipients of CB grafts [92, 95], indicating the importance of reconstituting immunological memory on transplant outcome.

2.4.2. Effect of Conditioning on T Cell Recovery

The conditioning regimen used for transplant may also affect the tempo of T cell immune reconstitution. Nonmyeloablative stem cell transplant regimens, though varied in their composition, use low or no doses of irradiation that reduces the damage to thymic tissue seen with traditional myeloablative regimens [96]. This, together with the use of PBSC as the graft source in most of these regimens, significantly improves the early immune reconstitution of naïve CD4 and $CD8^+$ T cell subsets in NST recipients [96–98] although later after HSCT differences are less apparent [97, 99]. The result is significantly fewer viral infections during the early post-transplant period in NST recipients [99]. The benefit to immune reconstitution in NST recipients is greater for matched sibling donors, compared to unrelated donors, which is likely secondary to a higher incidence of GVHD in the latter group [100].

Anti-thymocyte globulin (ATG) may be used in transplant conditioning regimens either to eliminate residual host T cells to facilitate engraftment, and/or to provide additional T cell depletion to prevent GVHD [101]. Several studies have shown that using ATG delays T cell immune reconstitution compared to a like group of patients not conditioned with ATG [102, 103]. The detrimental effect of ATG is a function of the dose used and the source, with rabbit-derived ATG having more potent effects than that made in the horse [101]. This may be a direct effect of the ATG on the reconstituting T cells, but can also reflect an effect of ATG on host lymphoid tissue. The effects of ATG

conditioning added to a partial TCD graft are apparent through day 100, as shown in Fig. 18-3, but by six months recovery is on par with that of patients who did not receive ATG.

2.4.3. Effect of T cell Depletion of T Cell Recovery

T cell depletion is an effective way to reduce the incidence and severity of GVHD following alloHSCT. Various methods for T cell depletion have been used, ranging from a partial TCD (1.5–2.0 log depletion) that still requires

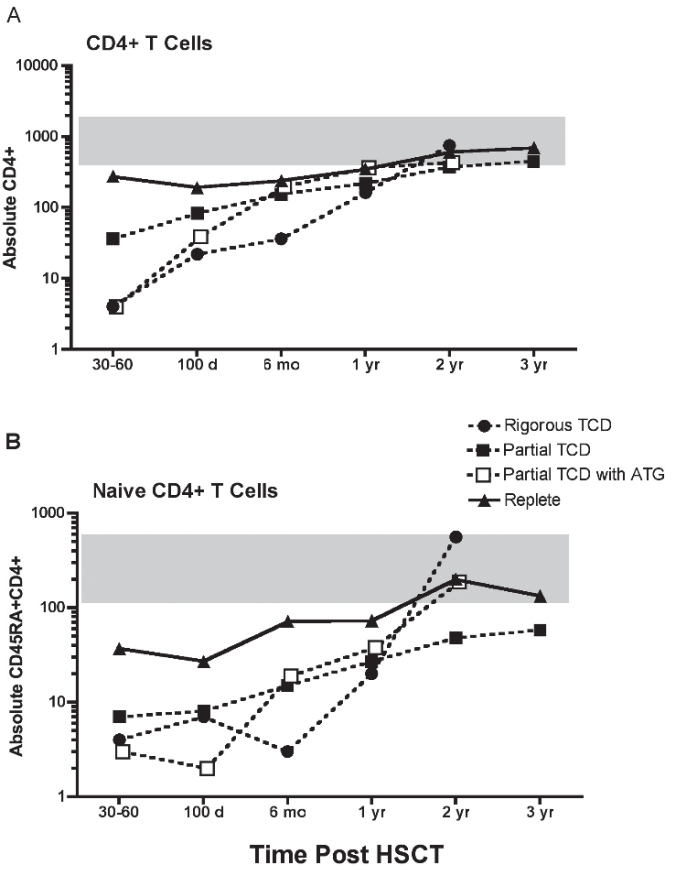

Fig. 18-3. Effect of T cell dose on CD4+ T cell recovery following HSCT. The absolute numbers of $CD4^+$ T cells (panel A) and $CD4^+CD45RA^+$ T cells (panel B) are shown for patients tested at or near the indicated time following HSCT for adult recipients of T cell-replete grafts (closed triangles, N = 29), partially TCD grafts (closed squares, N = 214), partially TCD grafts conditioned with ATG (open squares, N = 15) and rigorous TCD grafts (approximately 4.0 logs) (closed circles, N = 9), without or with only limited chronic GVHD. Partial TCD was by complement-mediated lysis using T10B9-1A1 monoclonal antibody or OKT-3 monoclonal antibody, resulting in <2.0 logs TCD and requiring post-HSCT cyclosporine as additional GVHD prophylaxis. Rigorous TCD was by CD34-enrichment, resulting in 4.0–5.0 log TCD without the need for additional post-transplant immune suppression. Not all patients were tested at each interval, and a given patient was tested only once during an interval. The shaded area represents the 5^{th} to 95^{th} percentile of absolute values from 49 healthy adult donors

the use of post-transplant GVHD prophylaxis, to more rigorous methods (3.0–5.0 log depletion) in which the graft treatment serves as the only GVHD prophylaxis. *In vivo* TCD may also be achieved through the use of monoclonal antibodies before and/or immediately after transplant [33]. All forms of T cell depletion have resulted in a delay in T cell immune reconstitution with the magnitude of the effect directly related to the efficiency of the T cell depletion method. Early studies of T cell depletion in matched sibling recipients of BM grafts at Memorial Sloan Kettering Cancer Center that were approximately 3.0 log depleted of T cells ($<10^5$ CD3+ T cells/kg) showed a fairly modest delay in T cell immune reconstitution, especially in T cell functional assays, compared to recipients of T cell-replete grafts, that was confined to the first three to six months post-transplant [41]. A similar pattern of T cell reconstitution in the absence of post-transplant immune suppression was seen by Soiffer, et al. [77] in recipients of grafts depleted of T cells with a monoclonal antibody to CD6. Here, a significant fraction of the recovering T cells failed to express the CD6 antigen suggesting that reconstitution likely occurred from those few CD6- T cells spared by the T cell depletion procedure. Greater differences are seen between recipients of T cell-replete BM grafts and recipients of grafts that are only partially TCD requiring additional use of post-transplant cyclosporine as immune suppression. This is illustrated by our own experience at the Medical College of Wisconsin using an anti-CD3 antibody plus complement method of T cell depletion, and is especially apparent for $CD4^+$ T cells particularly the naïve subset, as shown in Fig. 18-3. Although total $CD4^+$ T cells had recovered to similar levels in recipients of T cell-replete grafts and partial TCD grafts by six months, $CD4^+CD45RA^+$ T cells remained significantly lower in recipients of TCD grafts through year three. A recent series of haploidentical transplants in pediatric patients using a partial T cell depletion and an NST conditioning regimen has shown improved immune reconstitution compared to fully myeloablative conditioned recipients [104]. Here removing ATG from the conditioning regimen as well as the sparing effect on primary lymphoid organs of no radiation, allowed for a more rapid recovery of naïve thymic-derived T cells, a more rapid and full recovery of TCR repertoire and fewer viral infections in the NST conditioned group. Conversely, adding ATG to T cell depletion protocols further increases the early delay in immune reconstitution, particularly in the recovery of the memory subsets [102, 105] as is also shown for our patients (Fig. 18-3).

The most rigorous methods of T cell depletion employ a highly efficient enrichment of $CD34^+$ progenitor cells from PBSCs, resulting in the infusion of fewer than 5×10^4 T cells/kg (approximately 4.0 log depletion) in grafts that contain significantly larger numbers of $CD34^+$ cells than could be obtained from BM sources [106]. The detrimental effects of such a low number of T cells in the graft on T cell recovery can be profound, especially in adults. This degree of T cell depletion was initially used in the setting of haploidentical-HSCT for children. Handgretinger, et al. [107] achieved engraftment with virtually no GVHD and without the need for post-HSCT immune suppression in the face of full haplotype disparity with this approach. $CD4^+$ T cell recovery in particular was very delayed, but given the pediatric patient population, those who survived eventually did recover T cell numbers and function [108, 109]. Early infectious deaths were increased in this series, and attempts to decrease the relapse rate in this high risk patient group with add-back of donor T cells

resulted in clinically significant GVHD in some patients [107]. Improved immune reconstitution was seen when G-CSF was eliminated from the post-HSCT supportive care regimen [22], although, T cell activation remains subnormal even at one year post-transplant [110]. These results are superior to those seen for adult patients receiving CD34-enriched grafts from HLA-identical siblings who were conditioned for transplant using Campath-1H, an antibody that may have resulted in additional T cell depletion of the infused product. This group had an extremely high early mortality (eight deaths in 11 cases) secondary to infection that was not seen in identically conditioned patients who received a less rigorous TCD graft [111]. The problems with high infection rates and profoundly delayed $CD4^+$ T cell recovery have not been seen in patients receiving allogeneic $CD34^+$ cell-enriched grafts that contain on average 5×10^5 $CD3^+$ cells/kg, showing the important role of T cell dose, rather than the method of T cell depletion on outcome [112]. Care must be taken using approaches that may result in extreme T cell depletion, especially in patients receiving grafts from HLA-disparate donors since there may be insufficient donor T cells to provide protection while new T cells develop from the thymus.

The effect of T cell depletion can also be seen in studies of T cell repertoire recovery. Gorski, et al. [113, 114] performed one of the earliest studies of overall T cell repertoire recovery in recipients of partial TCD grafts at our center. He found a pattern of early limited diversity that persisted in patients with chronic GVHD or active infections, but that ultimately became diverse in longer-term patients without GVHD. Repertoire deficiencies have also been described in recipients of T cell-replete grafts, although to a lesser extent than recipients of TCD grafts [114, 115]. The early limited diversity in T cell response seen in recipients of TCD grafts may be one consequence of T cell repopulation by peripheral expansion of T cells in the graft.

2.4.4. Effect of Age on T Cell Recovery

Overall $CD4^+$ T cells recover faster in children, eventually reaching normal levels in most studies, likely due to the presence of more functional thymic tissue [102, 116, 117]. In contrast to children, residual deficiencies in adults may never fully resolve [118, 119]. The recovery of $TREC^+$ T cells is inversely correlated with age following HSCT and is higher in pediatric recipients of CD34-enriched grafts than in those receiving T cell-replete grafts [75]. Likewise, pediatric recipients of CB grafts achieve normal TREC levels by one year [120] whereas adult recipients may never reach age expected levels of $TREC^+$ T cells [119, 120]. This age effect on T cells and also on B cells is seen in our own series of T cell replete graft recipients, (Fig. 18-1), and partially TCD grafts in the presence or absence of GVHD and with or without T cell depletion (Fig. 18-2).

2.4.5. Effect of GVHD and Donor Relationship on T Cell Recovery

The effect of acute and chronic GVHD on T cell immune reconstitution overshadows the effect of donor source in most reported studies. This is not surprising since GVHD is caused by donor T cells and thus its therapy is directed at reducing T cell numbers and function. However, the GVHD reaction itself targets lymphoid tissue, including the thymus, which may further contribute to the delay in immune reconstitution, even as symptoms come under control. The most profound effects are seen in patients with severe acute

GVHD (grades III–IV) and in those with extensive chronic GVHD. It was recognized early on that patients with extensive chronic GVHD have marked deficiencies in overall T cell reconstitution with a resultant increased risk for viral infections and late bacterial infections [121]. GVHD causes both qualitative and quantitative effects on T cell reconstitution regardless of graft source, graft treatment or patient age, and this agrees with our own data shown in Fig. 18-2. Characterized T cell defects associated with GVHD include: markedly skewed CD4:CD8 T cell ratio [121–123], limited TCR receptor repertoire diversity [74, 124, 125], decreased TREC levels [126], increased numbers of $CD4^+CD27^+$ T cells in patients with acute GVHD [127] and increased numbers of $CD4^+CD29^+$ T cells in patients with extensive chronic GVHD [118]; increased apoptosis in $CD4^+$ T cells associated with grade II or higher acute GVHD [128]; reduced numbers of regulatory T cells in the circulation [129, 130] and in mucosal sites of GVHD [131], and an association of $CD4^+CD134^+$ T cells with therapy-resistant acute GVHD [132].

The effect of donor relationship and T cell immune reconstitution has been more difficult to document separate from the effect of GVHD, its prophylaxis or treatment. One study documented a slower T cell reconstitution in adults, but not pediatric recipients of rigorous TCD grafts (3 to 4 logs) from HLA-matched unrelated donors than their counterparts receiving grafts from sibling donors [133]. One possible speculation involved possible defects in migration to the thymus and subsequent maturation due to undetected HLA disparity in the unrelated group. Significant differences in T cell immune reconstitution between sibling and unrelated or HLA-mismatched related donors has not been seen in our own patient series representing a less rigorous T cell depletion method (average 1.8 logs).

2.4.6. Effect of Virus Infection on T Cell Recovery

Viral infection is primarily controlled by T cells; therefore, it is not unexpected that, given the delays in T cell immune reconstitution, patients are highly susceptible to viral infections during the early period post-transplant. Endogenous herpes family viruses frequently reactivate during this period, and often cause disease. Although $CD8^+$ T cells serve as antiviral effectors, they require viral-specific $CD4^+$ helper T cells to be fully functional [134]. Reactivating CMV in CMV seropositive recipients commonly occurs post-HSCT and has been extensively studied. Differences in T cell immune reconstitution are seen based on the CMV serostatus of patients at the time of transplant and during periods of CMV reactivation or disease. $CD8^+CD57^+$ T cells represent one such T cell subset that is expressed only at low levels (7%±5%) on T cells from healthy donors, but is often seen in abundance (up to 75% of $CD8^+$ T cells) post-HSCT in CMV^+ HSCT recipients, especially in association with viral reactivation [3]. The $CD8^+CD57^+$ T cell subset is largely $CD28^-CD11b^+$ (EF or EM cells), suppresses T cell functions and does not proliferate well to mitogen stimulation, but is capable of mediating cytotoxicity [135, 136]. TCR analysis of the expanded $CD8^+CD57^+$ subset in HSCT patients has shown a limited clonality [137, 138]. However, oligoclonal $CD8^+CD57^+$ T cells are also found in healthy donors and may represent a normal response to the CMV antigen [139]. Indeed, $CD8^+CD57^+$ T cells from individuals that are CMV^+ have been shown to contain a high frequency of CMV-specific cells as measured by cloning or by IFN-γ and TNF-α production in response to

CMV [140]. Although blood from alloHSCT patients with high percentages of $CD8^+CD57^+$ cells proliferated well to autologous CMV-infected fibroblasts, there was only low CMV-specific cytolytic capacity compared to blood from patients with few $CD8^+CD57^+$ T cells [138]. The exact function of this $CD8^+$ T cell subset post-HSCT is unknown. Clearly $CD3^+CD57^+$ T cells expand in response to CMV, but whether this represents an immune response to the virus or a mechanism by which CMV suppresses the immune response to itself is unclear. Our own studies in recipients of TCD grafts support the role of endogenous CMV in the expansion of $CD3^+CD57^+$ T cells post-HSCT. The number of $CD3^+CD57^+$ T cells was normal by day 100 in patients who were CMV^+ at the time of transplant regardless of the serotype of the graft donor, and rapidly rose and remained at above normal levels throughout the post-HSCT course (Fig. 18-4). In contrast, $CD3^+CD57^+$ T cells gradually rose to normal numbers by six months and stayed in the normal range thereafter in CMV^- patients, a finding that was also minimally affected by the serostatus of the graft donor (Fig. 18-4).

Functional studies of T cell immunity are more predictive of viral-specific immune competence than phenotype analysis. The proliferative response to viral antigens is a measure of antigen-specific $CD4^+$ T cells. Proliferation to HSV and VZV is most depressed during the first three months after HSCT when susceptibility to viral infection is highest [141, 142]. Indeed, those patients developing a proliferative response to VZV or HSV following infection were protected from subsequent infection, whereas treatment

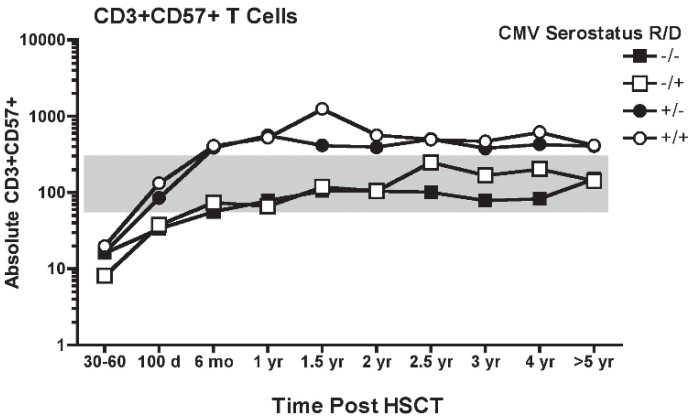

Fig. 18-4. Effect of patient and donor CMV sero-status at the time of transplant on the recovery of $CD3^+CD57^+$ T cells post-HSCT. The median absolute numbers of $CD3^+CD57^+$ T cells are shown for patients tested at or near the indicated time following HSCT for recipient/donor pairs who where both seronegative (–/–, closed squares), recipient negative donor positive (–/+, open squares), patient positive donor negative (+/–, closed circles) or both seropositive (+/+, open circles). Data are from adult and pediatric recipients of TCD and T cell-replete grafts, with or without chronic GVHD. Not all patients were tested at each interval, but a given patient was tested only once during an interval. The shaded area represents the 5^{th} to 95^{th} percentile of absolute values from 49 healthy adult donors of unknown CMV sero-status. Differences between seronegative and seropositive patients were significant ($p < 0.001$) at each interval. Only the difference between +/– and +/+ at 1.5 years reached significance for all other comparisons

with antiviral agents such as acyclovir during the initial infection inhibited the development of viral-specific proliferative responses and increased the likelihood of second infections [143]. The poor antigen-nonspecific T cell proliferative response may involve defects in transmembrane calcium flux, but this has not explained the results in most studies that have been reported [77, 144, 145]. Most likely the poor proliferative response involves T cell subset imbalances together with a decreased ability of post-HSCT T cells to produce IL-2. As for HSV and VZV, the recovery of cytotoxic and proliferative responses to CMV is strongly correlated with protection against infection [78, 146]. Preemptive or prophylactic use of ganciclovir has significantly reduced early mortality due to CMV infection, but with the unintended consequence of delaying the recovery of CMV-specific immunity resulting in a shift to late CMV infection [147]. CMV disease remains a significant problem for patients after alloHSCT. Monitoring CMV-specific T cell immunity post-HSCT may be a useful tool to detect patients in need of intervention to prevent CMV disease (reviewed in ref. [148]). Although PBSC grafts and nonmyeloablative stem cell transplant (NST) are associated with faster T cell reconstitution, the risk of CMV reactivation remains. NST is associated with less CMV antigenemia, viremia and disease in CMV^+ patients with CMV^+ donors prior to the first year, compared with controls receiving myeloablative HSCT. By one year, the overall incidence of CMV disease became similar in both groups, although the onset in NST patients was delayed [99].

Unlike CMV, NK cells may contribute to protection against EBV infection prior to the redevelopment of T cell-specific immunity [149]. However, the critical nature of the T cell response to EBV is indicated by the markedly increased risk of post-transplant lymphoproliferative disease (PTLD) associated with delayed T cell recovery [150], especially if the graft used for HSCT contains B cells [51]. During the first three months there is a poor CTL response to EBV in recipients of TCD grafts that recovers by six months [152]. Patients without EBV-specific CTL precursors were at increased risk for PTLD in this study.

2.4.7. TCRgd+ T Cell Recovery

T cells can express one of two forms of the TCR in association with CD3. Approximately 98 percent of T cells from healthy adult donors use the αβ TCR with nearly all $TCR\alpha\beta^+$ cells expressing either CD4 or CD8. T cells using the γδ TCR range from 1 to 3 percent of lymphocytes in healthy adults and are mostly $CD4^-CD8^-$, although a minority may express CD8αα homodimers. Two major subsets of γδ TCR are seen, those that express Vδ1, representing approximately 25 percent of $\gamma\delta\ TCR^+$ T cells in healthy adults, and those expressing Vδ2, constituting the remaining 75 percent. The Vδ1 subset dominates in fetal life and childhood, suggesting a role of the thymus in their development, and has a more restrictive repertoire than $\gamma\delta TCR^+$ T cells expressing Vδ2. The normal functions of TCR $\gamma\delta^+$ T cells include the recognition of bacterial or vial pathogens, control of immune reactivity by downregulation of activated macrophages and as antitumor effectors (reviewed in ref [153]).

In the setting of HSCT, $TCR\gamma\delta^+$ T cells in the graft may help promote engraftment and do not appear to play a causative role in GVHD [154, 155]. Gratama, et al. [156] were the first to describe $TCR\gamma\delta^+$ T cell recovery post-HSCT and found that, in contrast to the $TCR\alpha\beta^+$ subset, $TCR\gamma\delta^+$ T cells

recovered at nearly equal rates in recipients of TCD and T cell-replete BM grafts. There was no effect of CMV infection on the rate of TCRγδ$^+$ T cell recovery in this study. However, in the setting of solid organ transplants, overexpression of TCRγδ$^+$ T cells has been associated with CMV infection [157]. Consistent with this observation, Cela, et al. [158] described a series of TCD HSCT recipients who showed two patterns of TCRγδ$^+$ T cell reconstitution. One group gradually recovered TCRγδ$^+$ T cells over the first year, although still not reaching control levels, while the second group exhibited unusually high absolute TCRγδ$^+$ T cells on one or more occasions post-HSCT. This second group was distinguished by a high rate of fungal or viral infections during this time period, suggesting that the increase in TCRγδ$^+$ T cells may have been in response to these infections. van der Harst, et al. (159) analyzed TCR-V-region gene usage during TCRγδ$^+$ T cell repopulation and found nearly exclusive use of Vγ9Vδ2 during the first month followed by Vδ1, suggesting that the early appearing TCRγδ$^+$ T cells may have expanded from the cells transferred in the graft. However, Travers, et al. [160] have recently shown that with time post-alloHSCT, the Vδ1 subset is nearly 10-fold more abundant than Vδ2 in recipients older than 30 years, while recovery of a more normal ratio is seen in patients under 30 years, suggesting a long-term impairment in the ability of TCRγδ$^+$ T cells to recognize antigen and exert their normal functions in older adults.

2.4.8. Regulatory T Cell Recovery

Regulatory T cells (Treg) perform a critical role in the immune system by keeping appropriate responses to foreign antigens, and inappropriate responses to self-antigens, in check. There are a number of T cell subsets that can mediate regulatory activity, the most well characterized of which is a subset of CD4$^+$ T cells that constitively coexpress high levels of CD25 (CD25^{bright}), express the forkhead family transcription repressor factor (Foxp3) that is required for their suppressive function, and express several other activation antigens and homing antigens such as CD152, CCR7, CD62L and glucocorticoid-induced tumor necrosis factor receptor (GITR). More recently it has been recognized that CD127, an antigen that is well expressed on most resting T cells, is moderately downregulated on activated T cells, but is expressed weakly, or not at all, on Tregs [161]. Therefore, together with other markers, the lack of CD127 expression may provide a better measure of Tregs than other cell surface markers. At any given time these natural Tregs comprise 5 to 10 percent of circulating CD4$^+$ T cells in healthy adults. However, in humans cytokines such as TGFβ, produced during an active immune response, can induce some CD4$^+$CD25$^-$ cells to temporarily express the phenotype and function of Tregs, including Foxp3. Given that CD4$^+$ T cells are the last T cell subset to recover post-alloHSCT and the fact that activated T cells share many of the same cell surface markers as Tregs, Tregs have been difficult to characterize in HSCT patients. One study by Clark, et al. [162] used the CD4$^+$CD25^{bright} phenotype as a measure of Tregs in patients greater than 100 days post-HSCT and found an increased number of CD4$^+$CD25^{bright} in patients with chronic GVHD. However, studies using intracellular expression of Foxp3 as assessed by quantitative PCR techniques have shown the opposite. Significantly lower levels of Foxp3$^+$ cells are found in the circulation starting shortly before the onset of GVHD and at the tissue sites of GVHD [129–131, 163]. Miura, et al. [163]

found that Foxp3 expression normalized with the resolution of GVHD at the same time TREC$^+$ T cells are detected, indicating that thymic regeneration is necessary for Treg development. In a study of 28 pediatric patients Seidel, et al. [164] found an early reconstitution to donor levels of Foxp3 mRNA with no significant difference in patients with or without GVHD, perhaps due to the superior thymic function of this younger patient group. Preclinical murine models of GVHD have indicated that the transfer of Tregs at the time of transplant can prevent the onset of acute GVHD, but does not prevent the GVT effect of the graft, making this an attractive immunotherapeutic approach for patients at high risk of developing acute GVHD (reviewed in ref [165]). The redevelopment of Tregs is the reason that significantly larger doses of donor-derived T cells are needed to induce GVHD when infused several months after transplant than are needed in the graft at the time of transplant [166].

2.5. B-Cell Reconstitution

The ability of B-cells to produce antibodies is dependent on the help provided by T cells as well as on APC. So it is expected that full B-cell immune reconstitution post-HSCT will parallel that of CD4$^+$ T cells. Although this is partially true, intrinsic B-cell deficiencies are also seen. Like T cells, B-cell immune reconstitution has been characterized primarily using immunophenotype and by assessing Ig gene rearrangement, but also through assessment of nonspecific and antigen-specific production of Ig.

2.5.1. Physical B-Cell Recovery

The absolute number of B-cells is usually low during the first 100 days post-HSCT with or without T cell depletion, although recipients of PBSCs have more B-cells than recipients of BM grafts during this period [85]. Most patients rapidly recover B-cells to normal levels after day 100, whereas the absolute B-cell count in children and recipients of T cell-replete BM grafts may exceed normal levels once recovery is complete [41, 167, 168]. Failure to recover normal B-cell and monocyte numbers in the expected time post-HSCT has been shown to correlate in multivariate analysis to a higher probability for infection after transplant [20]. Prolonged treatment with corticosteroids delays B-cell reconstitution to a greater extent than T cell reconstitution and was found to be associated with a higher infection rate in those patients whose B-cells did not recover by day 100 [169]. Our own data support these findings as shown in our study of T cell-replete and partial TCD transplants in adults and children. As shown in Fig. 18-1D, the rate of B-cell recovery in the absence of extensive chronic GVHD in adult and pediatric recipients of T cell-replete grafts and was similar and rapid, with median absolute numbers in the normal range by six months and above normal by two years. Adult recipients of TCD grafts lagged slightly behind their pediatric counterparts, but were recovered by one year, whereas extensive GVHD delayed recovery of B-cells in both pediatric and adult patients, requiring 18 months to reach the lower range of healthy controls (Fig. 18-2D).

2.5.2. Intrinsic B-Cell Defects

Intrinsic B-cell defects early post-HSCT may be identified by abnormal phenotypes, such as the presence of CD5$^+$B-cells, a phenotype usually seen at birth [167, 170] and the increased number of naïve B-cells expressing surface

IgD [171]. The early reconstituting B-cells may also coexpress fetal-restricted antigens including CD38, CD71, CD1c and CD23 [167, 172]. These immature B-cells may be a source of autoantibodies [173] and display other abnormal functions post-HSCT. Gene rearrangement studies show less diversity in the Ig gene repertoire even at one to two years post-HSCT, with patterns not unlike those seen in fetal ontogeny [174, 175]. Oligoclonal expansions in the IgM and IgG repertoires are revealed by CDR3 spectratyping of Ig V region genes. Skewed IgM repertories normalized by three to four months, whereas the IgG compartment remained restricted for ≥ nine months [176]. Even with repertoire normalization at the gene level, there is less diversity from somatic hypermutation during the first year post-HSCT compared to healthy donors [177]. This factor combined with a defect in class switching and clonal dominance may help explain the defects in mounting a specific humoral immune response during the first year post-HSCT [178].

2.5.3. Recovery of Immunoglobulin Production

Comparative studies of B-cells from recipients of T cell-replete and TCD grafts using a T cell-independent B-cell mitogen showed IgM production by four to six months, and recovery of IgG production to control levels by one year with only quantitative differences in the TCD group [41, 167]. These data suggest that the intrinsic B-cell defects seen during the first year post-HSCT are preventing normal class switching. Other studies of *in vitro* B-cell function have used the T cell-dependent pokeweed mitogen (PWM) Ig production system that reveals defects both in T helper cells and B-cells [179]. PWM-induced Ig production is highly sensitive to inhibition by suppressor T cells of either the $CD4^+$ or $CD8^+$ subsets [180]. $CD3^+CD8^+CD11b^+$ cells are particularly inhibitory to Ig production in these systems [181]. Most helper-T cell defects and increased suppressor activity occur during the first four to six months post-HSCT, but persist in patients with chronic GVHD.

In concurrence with B-cell recovery, Ig isotypes appear at levels like that seen in childhood with an early appearance of serum IgM, IgG1 and IgG3, that is followed later by IgG2, IgG4 and lastly by IgGA. Serum IgM becomes normal by two to six months and IgG1 and IgG3 reach normal levels by nine to 12 months post-HSCT [168]. In contrast, IgG2, IgG4 and both serum and secretory IgA levels are deficient for several years [182, 183] and are not corrected by infusion of intravenous Ig [184]. Chronic GVHD results in more profound defects in secretory and serum IgA [185] and is associated with an increased production of autoantibodies [186]. Children have lower IgA levels at three and six months post-HSCT than adults [187]. The failure to respond normally to encapsulated organisms with an IgG2 or IgA response may contribute to the increase in bacterial infections seen after the first post-HSCT year. These defects may reflect a deficiency in B-cells capable of undergoing isotype switch and are characteristic of B-cells in fetal life and infancy [171]. Serum IgE may be increased during the first 100 days in patients with grade II or higher acute GVHD [188] or active infection [189]. However, patients surviving long-term (20 to 30 years) were found to have completely normal Ig levels, despite some residual defects in $CD4^+$ T cells [119].

2.5.4. Recovery of Specific Humoral Responses

A number of studies show that antigen-specific humoral immunity can be transferred from donor to host. These include the demonstration of antibodies to recall antigens such as tetanus toxoid (TT), diphtheria and measles during the

first 100 days [190, 191]. However, transfer of immunity is best demonstrated in patients who were seronegative pre-transplant to nonlatent viruses such as measles, mumps or rubella to which the donor was immune. Many such patients demonstrate a virus-specific antibody response for up to one year post-HSCT [192]. Transferred immunity is more likely to occur if the donor is immunized prior to harvest and is most likely to occur when both donor and patient are immunized pre transplant [193, 194]. This is likely due to a reservoir of antigen in the recipient. TT-specific helper T cells sharing a predominant donor clonotype are found to be almost exclusively responsible for early TT responses. These, too, are more likely to be present if the donor is immunized prior to transplant [195, 196]. Transferred donor Ig cannot only be detected in the serum, but also in the secretions with peak IgA activity during the first two to three weeks that declines until two months, when levels rise again [197]. Transfer of humoral immunity also occurs in recipients of TCD grafts [198]. Unfortunately, transferred immunity eventually declines, requiring that the patient be vaccinated to maintain protection against these common pathogens [199].

The data support the need to immunize post-HSCT, and a number of studies have examined how this should be best performed. The ability to mount a specific humoral response to a neo-antigen is nearly absent until after day 100 in recipients of T cell-replete BM grafts. The response to neo-antigen recovers to nearly normal levels by day 180 except, of course, in patients with chronic GVHD, who may take much longer or never fully recover [200]. Most clinical studies would support immunization at one year for patients not suffering from extensive chronic GVHD. The best responses are seen to immunizations with protein antigens, with much poorer response to polysaccharide antigens, as might be expected in patients with prolonged IgG2 and IgG4 deficiencies. For patients immunized at two years post-HSCT, fewer than 12 percent responded to a pure polysaccharide pneumococcal vaccine, whereas there was an 80 percent response after two immunizations with a protein-conjugated pneumococcal vaccine [201]. In a similar fashion, children who received the measles, mumps and rubella vaccine responded well to vaccines given two years after matched sibling HSCT [202]. The timing and number of vaccine doses to achieve an optimal response has also been studied. Responses to TT were optimal when vaccination was begun after immune reconstitution was more complete. Patients vaccinated early and repeatedly during the early post-HSCT period had more oligoclonal responses and lower antibody titers than those vaccinated later [203]. Likewise, vaccination to the influenza virus is ineffective at six months with increasingly better responses with time post-HSCT. A single dose of TT vaccine at two years post-HCST was highly effective, even for patients with chronic GVHD [204]. Response to polio vaccine differs somewhat in that patients vaccinated as early as six months received some benefit from two to three sequential doses of inactivated vaccine [199, 205].

3. Approaches to Enhance Post-HSCT Immune Reconstitution

Attempts to significantly enhance immune reconstitution post-HSCT have thus far had only modest success. Many programs have now switched to PBSCs as the primary graft source for transplant, and although this does improve the early rate of T cell reconstitution, the issues of immune suppression

due to GVHD have not been solved. This is especially true because recipients of PBSC grafts are more likely to suffer from extensive chronic GVHD and all the associated immune suppression [206]. The increase in the use of NST has further decreased the period of neutropenia early post-HSCT, but here too there are problems with GVHD, and even with faster T cell immune reconstitution, a period of immune deficiency still exists. In order to transplant patients who lack an HLA-matched or single antigen mismatched donor, centers have turned to approaches that rigorously deplete T cells to allow larger doses of $CD34^+$ cells to facilitate engraftment of haploidentical grafts from family member donors. Here, GVHD has not been a significant problem, but the recovery of T cell immunity, specifically $CD4^+$ T cells, is profoundly delayed, resulting in increased early transplant-related mortality caused by infection. Given the seriousness of the problem, initial approaches to enhance immune reconstitution in this setting have been attempted, and a number of new approaches currently in clinical study or are in the preclinical stages of testing have great promise.

3.1. Growth Factor and Cytokine Therapy

3.1.1. Hematopoietic Growth Factors

Both G-CSF and GM-CSF have been widely used post-alloHSCT to decease the period of neutropenia and, hopefully, reduce the number of early infections. Neutrophil engraftment is enhanced in most studies by two to four days, resulting in a reduced incidence of very early infection [207, 208]. Nonetheless, these effects have not translated to improved outcome and add considerable expense to the transplant procedure if growth factors are used routinely [209]. Our own studies have shown that G-CSF improves neutrophil engraftment at the expense of a delay in platelet engraftment [4]. Furthermore, in the setting of $CD34^+$ cell-selected haploidentical transplants, the use of G-CSF during the engraftment period may actually decrease the rate of $CD4^+$ T cell recovery [22]. Using growth factors to treat neutropenia in association with infection later post-HSCT may be more useful than using them during the engraftment period.

3.1.2. Keratinocyte Growth Factor

Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor family that primarily induces growth and differentiation of epithelial cells, including those in the thymus. KGF is produced by mesenchymal cells in a number of tissues, but can also be produced by mature single positive $TCR\alpha\beta^+$ T cells in the thymus, but not by those in the periphery, and is also produced by $TCR\gamma\delta^+$ T cells in epithelial tissues. Its primary effects are on epithelial cells where it induces both proliferation and differentiation and has been used clinically to reduce the incidence and severity of chemotherapy-induced injury to mucosal tissues (reviewed by [210]). In preclinical mouse models, KGF infused after alloHSCT enhanced thymopoiesis and increased the number of circulating T cells in both young [211] and older animals [212]. Exogenous administration of KGF increases the production of IL-7 in the thymic tissues of treated animals, but also appears to operate through IL-7 independent mechanisms to increase thymopoiesis in the transplant setting [212]. The use of KGF post-HSCT to improve T cell immune reconstitution may be a useful approach.

3.1.3. Interleukin-2

Because IL-2 is a T cell growth factor and IL-2 production early after transplant is severely depressed in nearly all BM recipients [213, 214], administeringIL-2 was among the earliest clinical interventions to improve post-HSCT immunity. The major concern in initiating this treatment was that acute GVHD would be exacerbated. This was not the case; regrettably, neither did IL-2 administration result in enhanceed T cell immunity. Rather, the predominant effect was to further activate NK cells [215–217] and selectively increase the number of circulating Tregs [218]. Continuous infusion of low IL-2 doses are better tolerated than higher dose bolus injections. In spite of this, the enhancing effects of IL-2 on NK activity rapidly declines when infusions are discontinued and this approach offers no real general long-lasting advantage in reconstituting immunity [216, 217].

3.1.4. Interleukin-7

Interleukin-7 is a stromal cell-derived cytokine that has major effects on lymphopoiesis and thymopoiesis (reviewed in ref. [219]). IL-7 is required to maintain the naïve T cell pool and for the peripheral expansion of mature T cells in the lymphopenic host (reviewed in ref, [220]). Preclinical studies using IL-7 in murine models of TCD transplants show effects both on residual mature T cells through upregulation of bcl-2 and on expansion of recent thymic emigrants [221–226]. In the setting of syngeneic transplantation, administration of IL-7 allowed for a protective response to influenza virus infection and could augment responses to subdominant antigens resulting in a broader immune response that was persistent when IL-7 was stopped. Allogeneic transplant models have also shown beneficial effects of IL-7 on T cell immune reconstitution, but have shown mixed results regarding the potential of IL-7 to induce or worsen GVHD reactions [227, 228]. Early results from two phase I studies of IL-7 administration in non-transplanted humans with cancer, showed effects as predicted from the preclinical models [229, 230]. Subcutaneous injections over a two- or three-week period resulted in marked expansion of both $CD4^+$ and $CD8^+$ T cells, predominately in the naïve and memory subsets, with lesser effects on effector T cells. Regulatory T cells, as measured by Foxp3 mRNA copies, declined during IL-7 administration to 50 to 80 percent of the pretreatment levels. Treatment induced a downregulation of IL-7 receptor alpha chain (CD127) detected after the first week of infusion that was associated with a slowing of T cell expansion. CD127 expression recovered to pretreatment levels one week after treatment ended. Significant treatment-related toxicities have not yet been seen, but these studies are still ongoing [230]. Thus far, IL-7 treatment appears to be safe at biologically active doses. However, optimal conditions to achieve the beneficial effects of IL-7 on immune reconstitution without augmentation of GVHD need to be determined. Likely IL-7 will be most beneficial in the setting of rigorous T cell depletion where the risk of GVHD is already low, and immune reconstitution is most delayed [220].

3.1.5. Interleukin 15

Interleukin 15 (IL-15) plays a major role in lymphocyte development and homeostasis, acting as a survival factor that inhibits spontaneous apoptosis in T, B and NK cells by increasing the expression of anti-apoptotic proteins. Given the increased rate of apoptosis seen early post-alloHSCT [86, 88, 128] the use of IL-15 may improve T cell reconstitution by prolonging

T cell survival. Preclinical studies of IL-15 post-alloHSCT have recently been reported indicating that this may be the case [231]. Both young and old IL-15-treated animals showed enhanced recovery of $CD8^+$ T cells that was confined to the memory $CD8^+$ T cell subsets, enhanced NK cell activity and increased numbers of NK- T cells. Neither $CD4^+$ T cells, nor overall thympoiesis was increased in animals receiving IL-15; rather the effects appeared to be due to increased peripheral expansion, and/or reduced apoptosis of memory $CD8^+$ T cells. Recipients of TCD grafts showed no evidence of autoimmunity or GVHD, and when these animals received delayed infusions of T cells, IL-15 appeared to selectively expand non-alloreactive T cells. However, in a T cell-replete transplant model IL-15 did increase the severity of GVHD, suggesting that like IL-7, IL-15 might be most useful in the setting of a TCD transplant.

3.2. T cell Add-Back

3.2.1. Donor Leukocyte Infusion

The first attempts at T cell add-back used increasing doses of peripheral blood buffy coat cells from BM donors following a T cell-replete BM HSCT to promote engraftment in immunized patients transplanted for aplastic anemia. Engraftment was enhanced, but patients at even the lowest doses experienced an unacceptably high rate of extensive chronic GVHD [232]. Data from experimental systems and experience using donor leukocyte infusion (DLI) to re-induce patients into remission who have relapsed post-HSCT, have shown us that donor leukocytes can be tolerated if added back at a later period post-HSCT, and in smaller doses [233]. Animal models indicate that this effect is due to the reconstitution of donor-derived Tregs [166]. Small, et al. [133] described a series of patients who received prophylactic DLI for the prevention of PTLD that resulted in significantly improved immune function, especially in T cell recovery. However, some patients experienced severe GVHD even with the delayed addition of low numbers of intact T cells [234]. Further reduction in the number of cells required to provide antigen-specific immunity might be achieved by immunizing the donor prior to collecting cells for infusion [235]. Such an approach was recently shown to augment CMV-specific immunity in transplant donors vaccinated with a canarypox pp65 vaccine either before transplant, or before DLI [236].

3.2.2. Suicide Gene-Transduced DLI

DLI for immune reconstitution may be safer if the T cells are engineered with a suicide gene such as HSV thymidine kinase (TK) so they can be killed if GVHD occurs. Such infusions done early post-TCD HSCT appear to be safe [237] and promote immune reconstitution [238]. Preliminary results of an ongoing phase II study of HSV-TK engineered DLI infusions between the first and second month post-transplant in recipients of haploidentical-HSCT show improved survival and significantly earlier T cell reconstitution that reached normal levels by one year. These results were significantly superior to patients who did not receive HSV-TK engineered DLI [238]. Attempts to improve methods used for HSV-TK transduction that better preserve the the infused DLI's ability to promote immunity are ongoing [239].

3.2.3. CD8-Depleted Donor PBL

Several centers have demonstrated that $CD8^+$ cell-depleted DLI can be used to provide GVT activity without an undue risk of GVHD by providing missing T cell help [240, 241]. More recently this approach has been used for DLI products intended to boost post-transplant immunity [242, 243]. Meyer, et al. [243] reported on 11 NST recipients where T cell depletion was achieved *in vitro* with the use of Campath 1H (alemtuzumab) in the conditioning regimen. These patients received prophylactic CD8-depleted DLI that was started at the time of CSA taper if there was no evidence of active GVHD. Infusions were started at $10^6/CD4^+$ T cells/kg with escalating doses of 3, 10 and 30×10^6 $CD4^+$ T cells/kg at 60- to 90-day intervals, in the absence of GVHD. Seven of the 11 patients developed acute GVHD following DLI, five with *de novo* grade I skin GVHD that responded well to treatment, and two recipients of HLA-C locus mismatched grafts who developed a more severe GVHD (one grade II and the other grade III) after the first dose level. However, the CD8-depleted DLI was effective in increasing $CD4^+$ T cell counts with a median 2-fold rise within two weeks of infusion that was not seen in a comparable cohort who did not receive DLI. Little or no effect of DLI was seen on $CD8^+$ T cells, NK cells or B-cells, with the exception of the patient experiencing grade III acute GVHD who showed a rise in both $CD4^+$ and $CD8^+$ T cells. Furthermore, in this patient $CD8^+$ T cells specific for the disparate HLA-C locus antigen were detected at the time GVHD developed, but not before DLI, indicating that CD8-depletion alone may not be sufficient to prevent GVHD in HLA disparate transplants. Corradini, et al. [242] recently reported a more rigorous test of the efficiency of CD8-depleted DLI in the face of HLA disparity. In this phase I/II trial doses of CD8-depleted DLI from 10^4 to 10^5/kg were infused at monthly intervals starting at day 45 post-haploidentical- HSCT in recipients of grafts that were rigorously depleted of T cells through CD34-enrichment with additional *in vivo* anti-T cell therapy in the form of alemtuzumab. Here, too, CD8-depletion did not completely eliminate the risk of GVHD, since before DLI there was a 10 percent incidence of grade I-II acute GVHD that increased to 35 percent, all grade II, after DLI. Patients receiving the 10^5/kg dose of DLI were at higher risk of GVHD than those receiving the lower dose. However, $CD4^+$ T cell reconstitution was significantly improved. Thus, while effective in boosting immunity, CD8-depletion of DLI products alone does not eliminate the risk of GVHD in HLA mismatched recipients. Use of this approach may be best avoided in the face of HLA disparity.

3.2.4. LLME-Treated Donor PBL

Haploidentical recipients of rigorously TCD CD34 selected grafts most often have Class II as well as Class I differences with their donors; therefore, for this group, using DLI with the removal of only the $CD8^+$ subset may be even more risky. L-leucyl-L-leucine methyl ester (LLME) is a lysosomotrophic agent that is incorporated into lymphocytes by a dipeptide-facilitated transport mechanism and is converted by the acyl transferase activity of the granule enzyme dipeptidyl peptidase I into hydrophobic polymerization products with membranolytic properties [244–246]. LLME is selective in its action to cells with cytotoxic potential, such as granulocytes, monocytes, NK cells and CTL both of the $CD4^+$ and $CD8^+$ phenotype. LLME induces cell death via apoptosis [246]. In MHC-disparate murine models, LLME-treated progenitor cell

grafts did not mediate GVHD [247]. Disappointingly, this agent could not be used in humans for primary treatment of the stem cell graft, due to toxicity to hematopoietic cell precursors at the concentrations needed to deplete cytotoxic T cells [248, 249]. However, LLME is well suited to selectively deplete cytotoxic $CD8^+$ and $CD4^+$ cells while sparing $CD4^+$ helper T cells in peripheral blood cells to be used for DLI, and has been shown to be effective for this use in murine models [250]. Preliminary data from phase I dose escalation clinical trials to test this hypothesis that are currently ongoing in Philadelphia and at our own center have shown a low risk of GVHD with enhanced recovery of viral immunity and protection from viral infection in recipients of LLME-treated DLI [251].

3.2.5. Allo-Antigen Depleted DLI

Another novel approach to improve the safety of DLI used to enhance immune reconstitution without undue risk of GVHD specificly removes cells with host-specific alloimmunity from the DLI product. Clinical studies in which host-specific alloreactive cells are removed from co-cultures of HLA disparate donor and recipient cells using a CD25-conjugated ricin α-chain immunotoxin [252, 253], CD25-conjugated immunomagnetic beads [254] or through the use of photodynamic therapy that specifically targets alloreactive donor cells [255, 256] are ongoing or planned. Early studies by Andre-Schmutz, et al. [253] demonstrated improved immune reconstitution using CD25-conjugated ricin α-chain immunotoxin allodepleted DLI, but with clinically significant GVHD that was associated with residual recipient-specific alloreactive cells. More recently, Amrolia, et al. [252] achieved a higher degree of alloreactive cell depletion with CD25-conjugated ricin α-chain immunotoxin and treated 16 recipients of haploidentical rigorously TCD grafts. Patients receiving three monthly infusions of 10^4 allodepleted DLI cells/kg showed little or no improvement in immune reconstitution compared to untreated patients, but at a dose of 10^5/kg, significant and rapid improvement was seen in both $CD4^+$ and $CD8^+$ T cells that was almost exclusively confined to the EM subset. Only two of the 16 patients developed GVHD. No significant differences were seen in the recovery of B-cells or NK cells. Viral-specific immunity to CMV and EBV was also detected in most dose-level 2 patients as early as two months post-HSCT, but in only a minority of dose-level 1 patients. However, in this series, relapse remained a problem, suggesting that higher or more frequent DLI doses may be required to achieve the dual effect of improving immunity and decreasing relapse. These data demonstrate that, with a sufficient depletion of alloreactive cells, DLI can be given safely and that high enough doses ($1–3 \times 10^5$/kg) can significantly improve immune reconstitution.

3.2.6. Antigen Specific T Cell Lines or Clones

An effective, but somewhat labor-intensive approach to improve immune reconstitution post-HSCT involves adding back T cell populations that are enriched for cells specific for viral pathogens. The earliest studies were designed to provide CMV immunity for both the prevention and treatment of CMV disease and have included the use of donor-derived CMV-specific T cell clones or lines [257–260]. Such passive immunity can protect against disease without completely suppressing virus, thus allowing for the redevelopment of the patients own immune response to CMV. The studies of Riddell

and Greenberg have also demonstrated the importance of both $CD8^+$ T cell and $CD4^+$ T cell immunity to CMV to protect against disease during the post-HSCT period [134]. $CD4^+$ and $CD8^+$ CMV-specific clones have been successfully used and were shown to be safe, but may not be required for this approach, given the encouraging results using CMV-specific T cell lines for prevention or treatment of disease in patients who have reactivated CMV post-TCD HSCT [261, 262].

As with CMV, approaches to overcome the function immune deficiency to EBV in patients at high risk for PTLD involve the use of donor-derived EBV-specific T cell lines as prophylaxis during the susceptible period post-HSCT [263], or for treatment once PTLD is diagnosed [264]. This approach appears to be safe and effective, even in HLA-mismatched recipients. Several investigators have explored methods to generate lines with specificity for more than one virus in a single culture, such as EBV + CMV or EBV + adenovirus [166, 260, 265]. Results from a recent phase I/II clinical trial indicate that such an approach is feasible and may be effective in providing a protective immunity early after transplant [266].

Neither the use of antigen-specific T cell clones nor T cell lines post-HSCT has been associated with the induction or exacerbation of GVHD. Because healthy donors with previous immunity to CMV or EBV possess relatively high viral-specific T cell precursors, generating highly lytic active and specific T cell lines has been achievable. Antigen-specific therapy for viruses with a lower T cell frequency or for fungal infections, another major cause of post-HSCT morbidity, have been more difficult to develop, although our own studies suggest that this may be possible [267–268]. Although effective and safe, the use of antigen-specific T cell lines to enhance post-HSCT immunity may not be readily exportable given the need for dedicated laboratory facilities of a level beyond those of most transplant centers.

4. Summary and Conclusions

There are many combinations of factors relating to the patient, the donor and the graft that affect immune reconstitution following alloHSCT. Nevertheless, an overall theme emerges that allows us to predict the rate at which immune reconstitution will occur. For granulocytes, monocytes, dendritic cells and NK cells the cellular reconstitution is rapid and occurs with relatively few defects regardless of the combination of factors affecting outcome. For T cell and B-cell reconstitution the story is more complex. During the first year after transplant, T cells primarily derive from those cells that were infused with the graft. The degree of graft T cell depletion is inversely correlated with the speed of T cell reconstitution in this period. Add in immune suppression and there are further delays in T cell recovery. Younger patients with more functional thymus tissue recover faster than older patients, and GVHD impairs recovery both by targeting thymic regeneration and secondary to the immune suppression required to control it. T cell helper function recovers more slowly than cytolytic function and there is a period during which $CD8^+$ T cells with suppressor function may predominate. Recipients of unrelated donor grafts or grafts from HLA disparate related donors are more likely to suffer GVHD and may require more intense

conditioning to allow engraftment, which together may explain the higher rate of post-HSCT infection these patients experience. Because B-cell function is T cell-dependent, B-cell reconstitution suffers from some of the same effects as T cell reconstitution. In addition, there are intrinsic B-cell defects that take time to resolve. Like T cells, much of the early humoral immunity is transferred from the donor and eventually declines until a new immune system develops from the graft progenitor cells. Although we now understand many of the forces that affect immune reconstitution, the challenge to improve it so that more patients may benefit from the potentially curative therapy of HSCT remains. Some exciting new approaches to enhancing post-HSCT immune reconstitution are in preclinical development or are in ongoing phase I/II clinical trials that are showing encouraging results and we are anxiously awaiting their introduction into the clinic.

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Chapter 19

Infection in the Hematopoietic Stem Cell Transplant Recipient

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Introduction

A number of factors interact to determine the risk of infection following HSCT: the underlying disease that led to the need for transplantation; the conditioning regimen employed; the source of the stem cells and preinfusion manipulations such as T cell depletion; the degree of histocompatibility mismatch between donor and recipient; the presence of latent recipient infections; the severity of GVHD that develops and the nature of the immunosuppressive program needed to prevent or treat GVHD; and the environmental exposures to which the recipient has been and will be subjected [1–4].

Environmental exposures of importance include both those experienced in the community and those encountered within the hospital (Table 19-1). Of particular concern are potential hospital exposures to opportunistic molds, *Legionella species,* and resistant gram-negative bacilli. Hospital exposures are further divided into domiciliary and nondomiciliary. Domiciliary exposures are those that occur in the room or on the ward where the patient is housed within the hospital – often there is clustering of cases in time and space [3–5]. Nondomiciliary nosocomial exposures occur when patients are taken to other sites in the hospital environment for procedures and are exposed to contaminated air and/or potable water at those times. Nondomiciliary exposures are more difficult to identify due to the lack of clear-cut clustering of cases, but are actually more common than domiciliary exposures, particularly with the widespread use of HEPA filters on transplant wards. In addition, HSCT recipients are at risk for person-to-person spread of such organisms as methicillin-resistant *Staphylococcus aureus,* vancomycin-resistant enterococci, beta-lactamase producing gram-negative bacilli and azole-resistant yeast on the hands of medical personnel. Finally, as will be discussed subsequently, person-to-person spread of respiratory virus infection (e.g., influenza, respiratory syncytial virus (RSV), parainfluenza, and others) can have a major effect on HSCT patients [6].

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Table 19-1. Infections in HSCT patients due to excessive environmental hazards.

Infections related to excessive nosocomial hazard
Aspergillus species
Legionella species
Pseudomonas aeruginosa and other gram-negative bacilli
Nocardia asteroides
Infections related to particular exposures within the community
Systemic mycotic infections in certain geographic areas
Histoplasma capsulatum
Coccidioides immitis
Blastomyces dermatidis
Strongyloides stercoralis
Community-acquired opportunistic infection resulting from ubiquitous saphrophytes in the environment
Cryptococcus neoformans
Aspergillus species
Nocardia asteroides
Pneumocystis jiroveci (formerly *carinii*)
Respiratory infections circulating in the community
Mycobacterium tuberculosis
Influenza
Adenoviruses
Parainfluenza
Respiratory syncytial virus
Infections acquired by the ingestion of contaminated food/water
Salmonella species
Listeria monocytogenes
Enteric viruses (Rotavirus, Adenovirus, Norovirus, etc.)

Temporal Course of Infection Post-HSCT

There are three key elements of the HSCT procedure that determine the type and timing of the infectious risk profile after transplantation [1, 7]:[1, 7]

a. The duration of neutropenia and mucosal injury which is a function of the conditioning regimen selected (myeloablative or not) and the stem cells' procurement (cord or adult; peripheral or bone marrow acquisition among adult donors).
b. The strategy chosen to prevent GVHD among allogeneic recipients. T cell depletion and other T cell manipulation procedures lead to delayed recovery of lymphocyte function and provide a specific immune deficiency profile.
c. The occurrence and severity of acute and chronic GVHD and its treatment [1, 7].

The temporal course of infection following HSCT can be divided into three time periods (Fig. 19-1) [1, 3, 4, 7]:

1. **Conditioning to Engraftment** The duration of this period has become dynamic and depends on the conditioning regimen itself, the source and

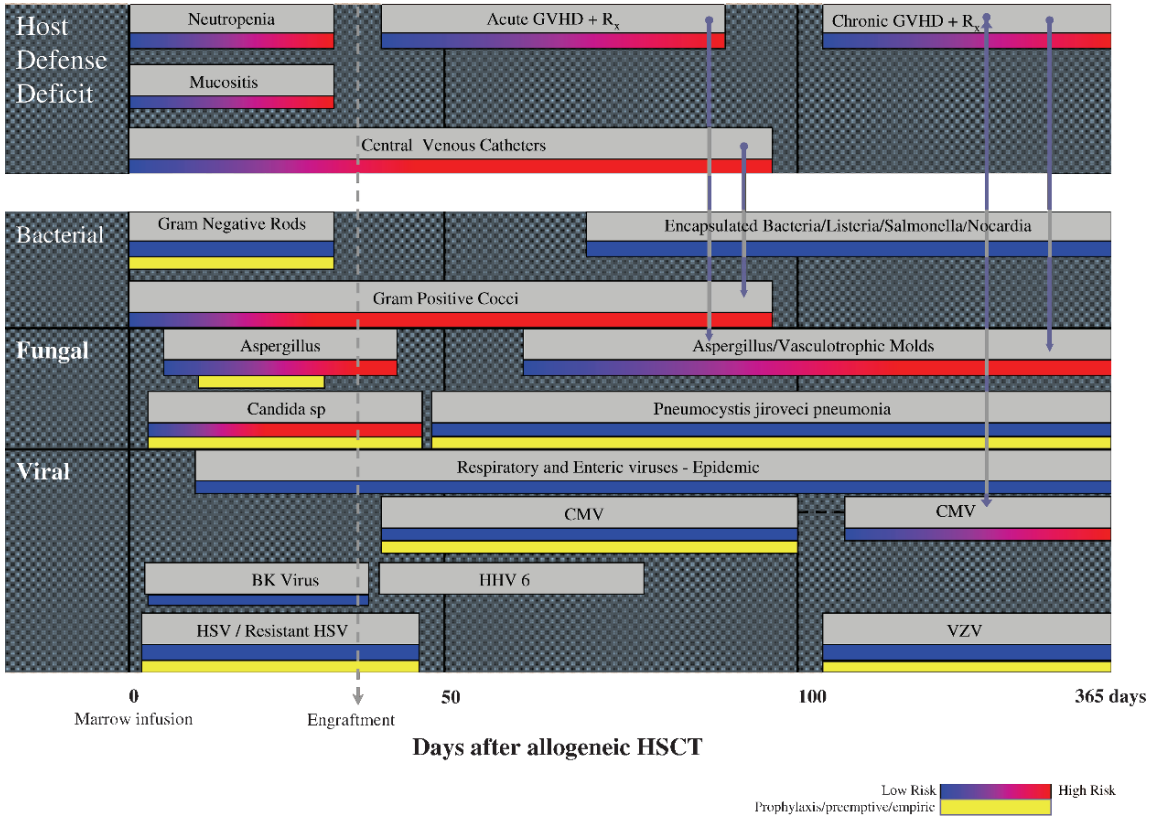

Fig. 19-1. Timetable of infection for allogeneic HSCT patients receiving antimicrobial prophylaxis. This table outlines the time period of the major host deficits and infections which occur during allogeneic HSCT in relation to when targeted pathogen-specific prophylactic, preemptive and empiric therapies are deployed. Risk for infectious complications are temporally dependent and are significantly decreased in the setting of prophylactic, preemptive or empiric therapy The risk of certain infections after transplantation is highly associated with ongoing immunologic manipulation as seen with the therapy for GVHD (linkages noted by vertical arrows).

CVL = central venous line, GVHD = Graft-versus-Host disease, GNR = gram-negative rods, GPC = gram-positive cocci, PCP = *Pneumocystis jiroveci* pneumonia, CMV = cytomegalovirus, HHV-6 = human herpes virus 6, HSV = herpes simplex virus, and VZV = varicella zoster virus.

Standard prophylactic considerations in this context include: GNR= fluoroquinolone or trimethoprim/sulfamethoxazole; Candida sp = fluconazole; PCP = trimethoprim/ sulfamethoxazole, atovaquone, dapsone, or pentamidine; and HSV/VZV = acyclovir. Standard empiric therapeutic considerations in this setting include: GNR = ceftazidime, piperacillin/gentamicin, or imipenem; aspergillus/molds/candida = amphotericin preparations or extended spectrum azoles, and CMV = IV ganciclovir, valganciclovir or preemptive monitoring (by antigenemia or PCR)

dose of stem cells infused and whether growth factors are used. It usually ranges from five (with nonmyeloablative transplants) to 30 days (with bone marrow or umbilical cord blood transplants). The combination of profound granulocytopenia and mucositis with myeloablative conditioning makes the patient particularly vulnerable to bacterial and candidal infections. In addition, infection present in the transplant recipient pre-transplant may be amplified by the granulocytopenic state and deficiencies of T and B-cell numbers and function. Thus, control of pre-transplant infection is needed before initiating the conditioning regimen. Prior to engraftment (both with autologous and allogeneic transplants), approximately 50 percent of patients will have fever of unknown origin, with bloodstream infection in ~12.5 percent and pneumonia in ~10 percent. The risk of an invasive mold infection is related to the duration of neutropenia and the environmental strategy used in a transplant center.

2. **Engraftment to Post-Transplant Day 100** During this time period viral infections, particularly cytomegalovirus (CMV) and the other herpes group viruses, are the major concerns. The occurrence, severity and treatment modalities selected for acute GVHD further modulates and increases the risk of herpesvirus infections, especially CMV and Epstein-Barr virus (EBV), and invasive mold infections [8–11].
3. **More than 100 Days Post-Transplant** In the absence of GVHD, the incidence of infection decreases significantly, with varicella zoster virus (VZV), *Pneumocystis jiroveci* (formerly *carinii)* pneumonia (PCP) and pneumococcal infection being the primary problems of this time period. Routine use of prophylaxis, such as with trimethoprim/sulfamethoxazole and acyclovir, significantly decreases the occurrence of PCP and herpesvirus infections, respectively. In addition, late or relapsing CMV infection may manifest during this time. If GVHD is present, it is typically treated with significant augmentation of immunosuppressive therapy such as with high-dose corticosteroids and monoclonal antibodies. Patients in this last category (GVHD under treatment) are at particular risk for invasive mold infection, CMV reactivation, PCP and other common and opportunistic pathogens.

Principles of Antimicrobial Therapy in the HSCT Recipient

There are four modes in which antimicrobial therapy can be administered to the HSCT patient [4]:

1. A **therapeutic** mode, in which antimicrobial therapy is prescribed for the treatment and eradication of identified microbes causing clinical illness.
2. A **prophylactic** mode, in which antimicrobial therapy is prescribed to an entire population before an event to prevent clinically important infection. For such a strategy to be successful, the infection(s) being targeted must be important enough to justify the intervention, and the antimicrobial therapy prescribed must be nontoxic and inexpensive enough to justify the intervention. By far the most effective antimicrobial prophylactic strategy is low-dose trimethoprim-sulfamethoxazole, which has virtually eliminated the occurrence of *Pneumocystis jiroveci*, *Listeria monocytogenes*, *Nocardia sp,* and

Toxoplasma gondii in patients who adhere to the regimen. Other prophylactic strategies commonly utilized in HSCT patients include acyclovir to prevent herpes simplex virus (HSV) and VZV reactivation, fluoroquinolones [5] to prevent gram-negative sepsis and fluconazole to prevent yeast infection.

3. An **empiric** mode, in which antimicrobial therapy is administered in response to a symptom complex. In this context, empiric antimicrobial therapy is initiated during the period of profound granulocytopenia in response to fever +/− rigors or subtle signs of sepsis (unexplained hypotension, tachypnea, an ongoing volume requirement, or acidosis). In the patient deemed not to be a therapeutic emergency, initial therapy is usually aimed at aerobic gram-negative bacilli (e.g., the Enterobacteriaceae and *Pseudomonas aeruginosa*). A variety of drugs have been utilized for this purpose, depending in part on the nature of particular problem organisms found at a given medical center. Advanced spectrum beta-lactams (e.g., ceftazidime, piperacillin or imipenem), either alone or together with an aminoglycoside or a fluoroquinolone, are the mainstays of this approach. Thus, empiric therapy is based on an algorithm rather than on microbiologic or other studies.
4. A **preemptive** mode, in which antimicrobial therapy is prescribed to a proportion of patients deemed to be at particularly high risk because of clinical/epidemiologic information or the isolation of microbial pathogens. Examples of preemptive therapy in HSCT are the molecular surveillance of CMV linked to deployment of ganciclovir or, more recently, the use of galactomannan monitoring for initiation of anti-*Aspergillus* antifungal treatment [12].

Bacterial Infections

Given the nature, duration and severity of host defense defects present in HSCT patients, it is not surprising that bacterial infection is a regular feature of the post-transplant course. The most common involved sites include blood stream (often catheter-related), lung, gastrointestinal tract and skin/soft tissue. The greatest rate of bacterial infections occur during the period prior to engraftment; this rate is a product of granulocytopenia, mucositis that permits the translocation of bacteria and yeast from the oral cavity and gut into circulation, and the presence of vascular access devices that traverse the skin and serve as direct conduits into the systemic circulation. Thus, the primary mucocutaneous barriers to infection are compromised, and the absence of granulocytes only amplifies the susceptibility of the patient [1, 4, 7].

In an attempt to decrease bacterial infections during the neutropenic period, especially those due to gram-negative bacilli, strategies of prophylactic antimicrobial use have been studied, including the use of trimethoprim-sulfamethoxazole and fluoroquinolones. Some studies, most recently with levofloxacin, have demonstrated benefit in decreasing the occurrence of fever and microbiologically-confirmed bacterial infections [13–15]. However, significant concerns regarding this approach have been raised given that no mortality benefit has been demonstrated, the emergence of resistant organisms, and the impairment this widespread antimicrobial approach has on the use of quinolones in future oral outpatient management. Thus, in many transplant centers, an empiric antibacterial regimen targeting *Pseudomonas*

and other *Enterobacteriaceae* in response to fever or other infectious syndromes remains a preferred approach.

Whereas gram-negative bacteremia was the major cause of blood stream infection 15 to 30 years ago, today gram-positive organisms are the most frequent cause of positive blood cultures. The possible reasons for this shift are many: the widespread use of fluoroquinolones, with their potent activity against gram-negative bacteria, as prophylaxis during this period; the presence of indwelling central venous catheters for prolonged periods; and the widespread use of systemic anti-gram-negative therapy all contribute to the gram-positive predominance. The bacteria isolated during the preengraftment period, then, include staphylococci (especially coagulase-negative *Staphylococcus*), viridans streptococci, enterococci and corynebacteria, with fewer isolates of Enterobacteriaceae or *Pseudomonas aeruginosa* being identified. An increasing problem in the HSCT population is antibiotic resistant organisms, particularly vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, and resistant gram-negative bacilli (such as extended spectrum β-lactamase producing *Klebsiella* and chromosomal inducible β-lactamase producing *Enterobacter species)* [1, 4, 6, 7, 16–20].

The typical approach for the severely granulocytopenic patient at present is the initiation of empiric antibacterial therapy in response to an unexplained fever or other signs of sepsis. What remains controversial is what the regimen should be. Since clinical deterioration can occur rapidly with untreated gram-negative sepsis in the granulocytopenic patient, anti-gram-negative therapy is always employed. The traditional approach of a β-lactam (e.g., piperacillin) plus an aminoglycoside is still favored by some experts, although nephrotoxicity from the aminoglycoside has led to the trial of other approaches, including the substitution of a fluoroquinolone for the aminoglycoside, or the prescription of a single advanced spectrum drug such as ceftazidime, cefepime, imipenem or meropenem. If fluoroquinolone prophylaxis has been utilized, then its use as a therapeutic agent may be diminished. Empiric fluoroquinolone monotherapy is inferior to other regimens, and if pure aerobic gram-negative agents are utilized, (e.g., aztreonam, aminoglycosides) due to confirmed severe beta-lactam hypersensitivity, then the addition of empiric gram-positive coverage that targets aerobic and anaerobic streptococci of the gastrointestinal tract should be considered. Use of extended interval (once-daily dosing) aminoglycoside administration may be safer and as effective.

The second area of controversy is whether empiric gram-positive treatment should be initiated at the same time, given the preponderance of gram-positive infection. As there is typically time to evaluate culture data and deploy targeted gram-positive antimicrobial therapy rather than empiricism, vancomycin should rarely be required empirically. Furthermore, empiric gram-positive coverage is not associated with better outcomes [21]. Indications for the immediate initiation of vancomycin as part of the empiric therapy regimen include the following [6, 16, 18–21]: catheter-related sepsis is likely because of evidence of infection at the insertion site (or within the tunnel), severe illness such as shock and/or respiratory distress are present, the patient is at particular risk for seeding of a prosthetic device (e.g., a prosthetic valve, a hip prosthesis, etc.), or the empiric gram-negative coverage exclusively covers aerobic gram-negative rods – such as the combination of

aztreonam and gentamicin. Vancomycin or other anti-staphylococcal agents should be started if cultures become positive for gram-positive cocci. In our experience, vancomycin can be discontinued safely in patients in whom vancomycin was started empirically, but in whom blood cultures remain negative after 48 to 72 hours and there is no specific syndrome, such as cellulitis, that requires treatment with vancomycin. On the other hand, empirical treatment against gram-negative organisms should be continued until resolution of neutropenia, whether fevers resolve or not [22]. The emergence and persistence of multidrug-resistant organisms should guide local practice in a dynamic fashion.

Indwelling long-term catheters remain a feature of the early post-transplant period to provide chemotherapy, nutritional and blood product support until stable engraftment. Routine anti-gram-positive antimicrobial therapy is not required just because a central catheter is in place for the prevention and management of catheter-related infections [21, 23]. The use of antimicrobial-coated catheters should be studied in this population, especially when non-tunneled catheters need to be used. Nonantimicrobial-based strategies to prevent bacterial infections during the neutropenic period include the systematic use of hand hygiene and the use of mask and gloves by health care personnel and family members. Other nonantimicrobial strategies which may be beneficial in preventing infections, but have not been tested in HSCT, include the use of palifermin to prevent mucositis [24] in patients undergoing myeloablative conditioning.

After engraftment, the risk of bacterial infections depend on the community exposures to common and opportunistic bacteria (e.g., *Nocardia*, *Rhodococcus, Listeria*), the presence of acute and chronic GVHD, the degree of B-cell reconstitution and the use of trimethoprim-sulfamethoxazole prophylaxis. Patients with chronic GVHD are at risk for invasive infection from encapsulated organisms, particularly *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*. It is postulated that the combination of B lymphocyte dysfunction secondary to the conditioning regimen and the effects of GVHD and its treatment have resulted in the loss and failure to develop an opsonizing antibody to these organisms, particularly *Streptococcus pneumoniae*. In addition, for at least one to two years post-transplant, HSCT patients have an inadequate response to pneumococcal vaccine. As IgG levels are often low for some time after HSCT, they should be routinely monitored, with replacement being considered when the IgG level falls below 500 mg/ml [25, 26]. In addition, antimicrobial prophylaxis, such as with low-dose trimethoprim-sulfamethoxazole (one single strength tablet daily for PCP prophylaxis), may afford further protection against this problem [1, 3].

Viral Infections in HSCT Recipients

There are several classes of viral infection of particular importance in the HSCT recipient: those due to herpesviruses (CMV, EBV, HSV, VZV and human herpesvirus-6 [HHV-6]); those due to hepatitis viruses (e.g., hepatitis B [HBV]); those due to respiratory viruses (e.g., influenza, RSV, parainfluenza, adenoviruses, and others), and those due to polyoma viruses.

Herpesvirus

The human herpesviruses share a number of characteristics that make them particularly successful pathogens in HSCT recipients [1, 4, 27]:

1. **Latency** Once infected with a herpesvirus, one is infected for life, with a circulating antibody (seropositivity) in the absence of active viral replication being the classic marker for latent infection. Reactivation from latency may be triggered by tumor necrosis factor (TNF), with the catecholamines epinephrine and norepinephrine and proinflammatory prostaglandins also playing a role. Thus, the virus may be reactivated by such processes as sepsis, GVHD, allogeneic reactions, OKT3 and antilymphocyte globulin. Once a replicating virus is present, medications such as cyclosporine, tacrolimus and prednisone may significantly amplify the viral replication.
2. **Cell Association** These viruses are highly cell-associated, meaning that transmission occurs through intimate person-to-person contact, or transfusion or transplantation of latently or actively replicating cells from a seropositive donor. Humoral immunity is, hence, less important than cell-mediated immunity. Indeed, the key host defense is accomplished by major histocompatibility complex (MHC)-restricted, virus-specific, cytotoxic T cells, just that component of host defense most affected by GVHD and its treatment.
3. **Oncogenesis** Herpesviruses, such as EBV and HHV-8, play a direct role in oncogenesis-causing post-transplant lymphoproliferative disease (PTLD) and Kaposi's sarcoma, respectively. Herpesviruses may also play an indirect role in oncogenesis with symptomatic CMV disease, increasing the incidence of EBV-associated PTLD severalfold.
4. **Indirect Effects** In addition to the direct causation of infectious disease syndromes, human herpesviruses, particularly CMV, have indirect effects that are clinically important. It is believed that cytokines, chemokines and growth factors produced in response to viral replication may be responsible for these effects. They include, in addition to the modulation of oncogenesis, increasing the net state of immunosuppression so that the risk of opportunistic infection is increased. This last point is particularly important, as a variety of experiments have shown that GVHD and infection are closely linked by the production of these mediators. That is, there is a bidirectional trafficking of mediators between these two processes.

Cytomegalovirus

The clinically most important direct effects of CMV in the HSCT recipient are pneumonia and gastrointestinal disease. Before effective antiviral treatment became available, CMV pneumonia occurred in 20 to 30 percent of seropositive recipients and had an associated mortality around 80 percent [28]. CMV commonly causes fever in the absence of preemptive treatment, and end-organ disease (hepatitis, bone marrow dysfunction, retinitis, and encephalitis) may occur. Among allogeneic HSCT recipients, the risk of CMV reactivation (60–80%) and end-organ disease is greatest in the seropositive recipient who receives a graft from a CMV seronegative donor (CMV D−/R+), likely due to the loss of native immunity during the transplant process and immune reconstitution with a CMV naïve allograft [29, 30]. Patients who are CMV

D+/R+ have CMV reactivation (50–60%) and disease risk that is similar to or slightly lower than that of the CMV D−/R+ patient. Patients who are CMV D+/R− have a lower risk of CMV infection (10–30%) and disease, but higher than CMV D−/R− patients (<5%). The risk of CMV infection in this latter group has been greatly decreased by use of leukoreduced blood products or by exclusive use of CMV negative products when available [31]. The risks of CMV reactivation and disease among autologous HSCT recipients is minimal (<1%) [1, 4]. Another major risk factor for the development of CMV reactivation and disease is the occurrence, severity and treatment of acute GVHD [29, 30]. Other potential factors associated with an increased risk of CMV reactivation and disease are reception of T cell-depleted or cord blood allograft, whether the donor is unrelated or mismatched, or donated bone marrow (instead of peripheral stem cells), and whether the conditioning regimen was myeloablative [29, 32].

The most widely used therapy for clinical CMV disease is ganciclovir, which can be administered either intravenously or orally in the form of a prodrug, valganciclovir, with an acceptable bioavailability profile (~50–60%). Typically, the parenteral form is administered until the patient is able to tolerate oral therapy. Gastrointestinal absorption of valganciclovir, even in the setting of mild to moderate GI GVHD, has been demonstrated to be adequate [33, 34]. Duration of treatment depends on the clinical response and the nature of the recovery of native immune function. In the case of serious illness, particularly pneumonia, anti-CMV hyperimmune globulin can be considered as adjunctive therapy. Despite these efforts, the mortality from CMV pneumonia remains high. The major toxicity of ganciclovir is myelosuppression, so that great effort is placed in monitoring these patients closely and adjusting doses appropriately [1, 4]. Occasionally, G-CSF support may be required to preserve an acceptable neutrophil count and to allow adequate therapy of a serious CMV infection. While certain medications, such as ATG and OKT3, are likely to induce CMV reactivation, others like sirolimus may inhibit this [29].

Current strategies are based on preventing CMV disease through prophylaxis or preemption. Prophylaxis with ganciclovir from the time of engraftment until at least day 100 post-transplant has been studied in randomized trials [35, 36]. Although CMV viremia and disease were prevented, there was no overall benefit of this strategy due to secondary bacterial and fungal infections related to ganciclovir-induced neutropenia. Alternatively, a preemptive strategy is employed in which patients are monitored weekly for viremia through either a PCR assay for CMV DNA or an antigenemia assay. Positive results are linked to initiating ganciclovir or other antiviral drugs active against CMV. Typically, these assays turn positive several days to weeks prior to the onset of clinical disease, permitting the use of effective preemptive therapy [1, 4, 29, 33, 37–41]. A preemptive approach significantly decreases the amount of prophylactic medication used, thus minimizing medication-associated toxicity.

In the pre-ganciclovir era, CMV disease typically occurred during the first three months post-transplant. Increasingly, with the widespread use of a prophylactic or preemptive antiviral strategy, breakthrough occurs much later, typically one to three months after the cessation of the antiviral therapy. Risk factors for late CMV disease include chronic GVHD, low CD4-T cell counts, and CMV infection before day 100. Relapse or the emergence of ganciclovir-resistant virus also can occur, particularly in the face of high viral loads and

inadequate courses or dosing of ganciclovir. Foscarnet is the preferred drug in this setting or when further potential myelosuppression with ganciclovir is not advisable. The experience with cidofovir use in the HSCT population is limited. Both foscarnet and cidofovir are potentially nephrotoxic and should be administered with caution [1, 4]. Studies are examining the emerging strategies for the management of CMV infection and the use of CMV vaccines in donors and recipients, adoptive immunotherapy for patients with refractory or relapsing CMV infection and the use of maribavir for prophylaxis.

Epstein-Barr Virus

The major recognizable clinical effect of EBV in the HSCT patient is in the pathogenesis of PTLD. Following the recovery from primary EBV infection (>95% of the adult population), ongoing lytic infection of B-cells occurs in the oropharynx, with latent infection of B-cells in the peripheral blood and lymphoid tissues. These latently infected cells can be transformed and immortalized, resulting in polyclonal proliferation. In the normal seropositive individual, these cells are kept in check by a specific cytotoxic T cell response. In the presence of immunosuppressive therapy, this surveillance system is inhibited in a dose-related fashion, thus permitting continued B-cell proliferation. Such ongoing proliferation results in particular clones being favored and the potential for developing cytogenetic abnormalities, which leads to the development of a truly malignant process- PTLD [1, 4, 27, 42].

The spectrum of clinical disease seen with PTLD is quite broad, ranging from a mononucleosis-like process or a polyclonal proliferation of lymphocytes that usually responds to decreasing immunosuppressive therapy, to a monoclonal, highly malignant B-cell lymphoma. The mononucleosis-like process is seen particularly in children with primary post-transplant EBV infection. The clinical presentation is one of fever, sore throat, cervical adenopathy and tonsillar hypertrophy and inflammation. Unlike B-cell lymphoma in the normal host, in the transplant patient, particularly the adult, the process can be extranodal. Thus, presentations may include central nervous system (CNS) invasion (from involvement of the meninges to focal cerebral lesions), liver, lung and bone marrow diseases. Not uncommonly, involvement of the gut (particularly the small bowel) may lead to recognition of the PTLD, with a clinical presentation of small bowel obstruction, perforation, or occult gastrointestinal bleeding. Disseminated, multi-organ disease is quite common in the HSCT patient [1, 4, 41, 42].

Risk factors for developing PTLD include: primary EBV infection in association with high-dose immunosuppression; interventions such as T cell depletion, umbilical cord blood transplant and the systemic administration of anti-thymocyte globulin increase the risk significantly; and intensive immunosuppression that results in suppression of the key host defense against EBV-transformed cells (MHC-restricted, EBV-specific, cytotoxic T cells) significantly increases the risk of PTLD. In addition to the host characteristics mentioned, high EBV viral loads correlate with an increased risk of PTLD. It has been suggested that EBV viral load surveillance in peripheral blood be carried out in high risk patients (those with primary

EBV infection, anti-T cell antibody therapy for GVHD, HLA-mismatched or T cell-depleted HSCT recipients), with decreased immunosuppression +/– antiviral therapy (acyclovir or ganciclovir) carried out in the setting of high viral loads [1, 4, 41, 42].

Treatment of PTLD remains controversial. All patients with diagnosed PTLD should have a significant decrease in immunosuppressive medications. Many centers also prescribe antiviral therapy. Patients not responding to these measures are usually treated with an anti-B-cell monoclonal antibody (rituximab, an anti-CD20 monoclonal antibody) [43, 44]. After that, therapies have ranged from anti-lymphoma chemotherapy to alpha-interferon and intravenous gamma globulin.

Herpes Simplex Virus

HSV infection prior to the introduction of acyclovir was a major problem in the HSCT recipient. Occurring in the preengraftment period, HSV infection greatly exacerbated the severity of mucositis. Not only were ulcers observed in the oral cavity and anogenital areas, ulcerations of the esophagus, stomach and intestine were also observed. HSV pneumonia was also noted, with rare cases of cutaneous dissemination and encephalitis. The current standard of care is to test all candidates for HSCT for an antibody to HSV, with seropositive individuals then placed on antiviral prophylaxis, beginning prior to HSCT. Effective agents for HSV prophylaxis include acyclovir (intravenous or oral), valacyclovir or famciclovir. Recurrence of HSV may occur later in the course, and should again be treated with an acyclovir regimen, with repeated episodes justifying long-term prophylaxis. Acyclovir resistance is uncommon in this situation, but can occur, and requires treatment with foscarnet [1, 4].

Varicella Zoster Virus

All patients and donors should have serologic testing for VZV prior to transplant. Seronegative individuals post-transplant should avoid exposures to VZV, but if such an exposure occurs, valacyclovir or varicella hyperimmune globulin should be promptly initiated. Before universal prophylaxis with acyclovir became standard, an estimated 40 percent of HSCT patients developed active VZV, with a median time of onset being five months post-transplant. The great majority of these patients had zoster, but approximately 20 percent had a more generalized process resembling primary varicella. A significant concern was visceral involvement in the setting of disseminated disease as well as neurologic complications such as myelitis or encephalitis [1, 45–48]. Prophylaxis with acyclovir in the early period post-transplantation substantially decreases the occurrence of herpesvirus infections, including VZV, and is rarely, if ever, seen during acyclovir prophylaxis. Prophylaxis is typically given for the first year post-allogeniec transplantation. VZV reactivation is often seen three months post-discontinuation of prophylaxis. As the VZV vaccine is a live attenuated viral vaccine, its use is contraindicated for at least two years post-transplantation, and unless a research study or close follow-up is involved, should be omitted.

Human Herpesvirus-6

HHV-6 is a β-herpesvirus (as is CMV) whose role in post-transplant complications is being defined. In the great majority of instances, HHV-6 primary infection occurs by the third year of life, with a seroprevalence rate of 90 percent at one year, and close to 100 percent at three years [49, 50]. The clinical effects associated with primary HHV-6 infection include exanthem subitum (roseola), and a form of encephalitis. In HSCT patients, bone marrow suppression, especially delayed platelet engraftment, and encephalitis have been associated with HHV-6 type B. The encephalitis typically occurs one to two months after transplantation and is associated with profound memory loss, especially short-term memory, and MRI changes in the mesial temporal lobes (limbic encephalitis) [51, 52]. The highest risk patients for this complication are male, umbilical cord blood recipients for whom the attack rate may be as high as 10 to 20 percent. Detecting HHV-6 DNA in the blood of allogeneic HSCT recipients is a common phenomenon occurring transiently in 40 to 60 percent of patients, yet encephalitis is a rather infrequent occurrence (1–2%). As obtaining brain biopsies is not usually feasible early after transplantation, the diagnosis of HHV-6 encephalitis is currently achieved by developing an acute limbic encephalitis syndrome, confirmed with MRI imaging of the brain and by the detection of HHV-6 in the CSF [52]. It remains unclear what the treatment of choice for this virus is. One approach that we currently favor is to use foscarnet. It is possible that anti-CMV preventative strategies with ganciclovir may have a beneficial effect on this virus as well [1, 53].

Respiratory Viruses

HSCT recipients are at significant risk for infection with respiratory viruses circulating in the community. These infections can occur at any time in the post-transplant course, and can be acquired in the community or during hospitalization from infected staff, family and friends. Overall, an estimated 10 to 20 percent of HSCT patients will become infected in the first year post-transplant, with the potential for this figure to rise significantly in the setting of a community-wide outbreak [54]. The dilemma for the clinician is how to prevent these infections, as there is a far higher rate of progression to pneumonia (viral and/or bacterial or fungal superinfection), which carries a far higher morbidity and mortality than what is observed in the general population. In addition, antiviral therapy for these agents is in its infancy. It is important to attempt to make an etiologic diagnosis. Avoiding exposure to infected individuals by systematic infection control measures in both family members and friends, but most importantly in health care workers, is the best preventative strategy available [55–58].

Respiratory Syncytial Virus

Although RSV can be acquired by inhaling an aerosol, direct contact with infected secretions is the usual mode of spread between individuals. In the HSCT patient, both adult and pediatric, RSV is a cause of significant morbidity and mortality. The illness begins with the signs and symptoms of a viral upper

respiratory tract infection (rhinorrhea, sinus congestion, sore throat and/or otitis media), that may progress to pneumonia, especially if the virus is acquired in the preengraftment phase. As with influenza, pneumonic syndromes can be due to RSV itself, but in our experience it is more frequently due to secondary bacterial and fungal infections. The advent of rapid RSV diagnosis by antigen detection in nasopharyngeal swabs has resulted in the recognition that RSV is a significant pathogen for both adults and children, particularly in immunosuppressed patients. Optimal antiviral management, however, remains unclear. There are reports that aerosolized ribavirin +/− anti-RSV polyclonal or monoclonal antibody may have therapeutic benefit, but this remains unproven. There is also interest in prophylaxis with an anti-RSV antibody, although there have been no trials in HSCT patients [55–59].

Influenza

As with RSV, the incidence of influenza infection in HSCT patients reflects the level of influenza activity in the community. The impact of this virus on infected HSCT recipients is demonstrated by the following statistics: ~60 percent of the patients with influenza develop pneumonia and ~25 percent of patients with influenza pneumonia die of progressive respiratory failure. When influenza is identified as a pathogen, use of a neuraminidase inhibitor (oseltamvir or zanamavir) or an amantadate (amantadine or rimanatine) should be considered. The neuraminidase inhibitors are attractive in this setting as they are effective against both influenza A and B and antiviral resistance occurs more slowly compared with amantadine use. Annual influenza vaccination should be considered, but its benefit is attenuated; indeed, it is probably fair to say that maximal benefit from vaccination occurs when the vaccine is administered to health care workers, family, friends and other contacts of the patient. When an infection is diagnosed, early treatment should be considered [58, 60].

Adenovirus

There are more than 50 serotypes of adenovirus and nearly all have been described to cause human disease. Adenovirus disease post-transplantation is likely due to both a newly acquired virus and viral reactivation. The most common adenovirus-associated illness post-transplantation is hemorrhagic cystitis which has been described in a recent report to occur in up to 42 percent of patients in the first year post-transplantation [61]. The overwhelming majority of cases are asymptomatic and require no intervention [62]. Occasionally the severity of hemorrhage or bladder-associated pain is so great that intervention is required. Other important adenovirus-associated syndromes include hepatitis and pneumonitis which may be fatal in the early post-transplant period. In the late post-transplant period adenovirus gastroenteritis may occur which is often a self-limited illness; however, severe disease has been described especially in patients requiring significant levels of immunosuppression for GVHD. Therapeutic options for adenovirus are limited. The role of the antiviral cidofovir is controversial with mixed results having been reported [63]. Decreasing immunusuppression and attempting reconstitution of the native

host immune response is critical. The role for other adjunctive therapies, such as IVIg, is unproven, but can be considered in severe cases. Avoiding exposure to new infection, as with all community-acquired pathogens, is central to optimal care.

Other Respiratory Viruses

Parainfluenza, rhinoviruses, metapneumovirus and coronaviruses are all capable of causing lower respiratory tract infection in HSCT recipients. Of these many viruses, parainfluenza virus type III is especially associated with a high mortality [64, 65]. Again, specific therapy is not available, emphasizing infection control strategies in the hospital setting and avoiding individuals with respiratory tract complaints at home. When upper respiratory tract complaints occur in HSCT patients, a diagnosis should be made, utilizing rapid diagnostic techniques (e.g., antigen detection assays or nucleic acid testing). Preemptive therapy, when available, should be initiated, while immunosuppressive therapy diminished and isolation from other HSCT patients should be accomplished.

Polyomaviruses

BK and JC viruses are the two important species in this family of viruses with a genitourinary and CNS predilection, respectively. Approximately 60 to 80 percent of adults have been infected with one or both of these viruses, typically in childhood. With immunosuppression, reactivation occurs which may lead to disease. BK virus is associated with hemorrhagic cystitis in the early post-transplant period. This virus is commonly found in the urine and rarely requires any therapeutic intervention. JC virus is the etiologic agent of progressive multifocal leukoencephalopathy (PML) which is a rare, but severe post-transplant complication. PML involves the white matter and presents with focal neurologic symptoms associated with the specific area of the CNS where the lesion(s) occur. Diagnosis requires correlating the clinical presentation, radiographic findings (typically by contrast-enhanced MRI imaging) and CSF PCR results for JC virus. Control of JC virus is associated with an intact cell-mediated immune response. Therapy for polyomavirus infection is quite limited with minimizing immunsuppression, when possible, being critical. The role of cidofovir is controversial with mixed results being reported. The use of quinolones for BK viruria is controversial at best and we do not recommend this practice [66]. Although the use of gatifloxacin was advocated by some authors, this drug is no longer available. Leflunomide administration has been used by some for treatment of BK in renal transplant patients, but no randomized trial data exists to support or recommend its use in either kidney or HSCT recipients at this time.

Hepatitis Viruses

Hepatitis B and C viruses may cause chronic infection which often leads to eventual significant liver dysfunction. Given the high global prevalence of these viruses, it is prudent to screen for past or current infection prior to transplantation. When ongoing infection is found, careful assessment of liver function and a pre-transplant liver biopsy should be considered to assess for occult cirrhosis, as this may influence peri-transplant management [67].

HBV infects approximately 350 million people worldwide chronically, and substantially more have had prior resolved infection. The use of the HBV vaccine as a routine childhood immunization will likely decrease the number of chronically infected individuals over the next several decades. The advent of nucleic acid detection technology has allowed a more precise mechanism to detect active HBV replication compared with antigen- (for surface and e) only methods. For patients with evidence of prior HBV exposure (HBV core antibody positive), it is important to consider HBV reactivation in the setting of post-transplant liver dysfunction and to differentiate this from other causes such as hepatic GVHD or medication toxicity, although reactivation initially occurs in the setting of normal liver tests. The best strategy for surveillance post-transplantation remains to be defined. Some recommend routine surveillance for HBV reactivation post-transplantation, whereas others would suggest antiviral prophylaxis. It is important to be aware that old resolved infections, including those with hepatitis core and surface antibody, but without antigen or HBV DNA detected, are at risk for reactivation (seroreversion) post-transplantation, especially in the setting of high levels of immunosuppression [68, 69]. Several therapeutic options have become available over the last several years and include lamivudine [70–72], adefovir [73], entecavir [71, 72] and telbivudine. Other agents such as tenofovir and emtricitabine also have excellent anti-HBV activity. Use of these agents requires careful consideration to minimize the risk for the emergence of resistant virus, which may be as high as 10 percent per year for lamivudine, but is less than 1 percent for adefovir and entecavir.

Epidemiologic studies suggest that more than 170 million people worldwide have been infected with hepatitis C virus (HCV) and the majority (approximately 85%) are chronically infected [67]. Over several decades, chronic HCV infection is associated with progressive hepatic fibrosis, liver failure, and hepatoma. This process is accelerated in certain immunocompromised patients including HSCT recipients [74, 75]. It is important to assess patients for seropositivity to HCV prior to transplantation and in those who are found seropositive, to assess the HCV viral load, genotype and liver pathology. The presence of elevated liver enzymes in the setting of HCV before allogeneic HSCT has been associated with an increased incidence of VOD [76]. A more precise profiling of the HCV-infected patient, including liver biopsy, should be considered to better define the extent of the HCV-induced liver disease, and to optimize the conditioning regimen and frequency of surveillance post-transplantation. Treatment of HCV is limited and typically requires use of an interferon and ribavirin which are likely to be poorly tolerated in the early post-transplant setting. In patients who are infected, it is prudent to counsel them to avoid hepatotoxins, receive the hepatitis A and B vaccines, and minimize the risk of transmission to close contacts.

Fungal Infections in the HSCT Recipient

There are three categories of fungal pathogens that can infect the HSCT patient: a) the classic opportunistic fungi, which cause >90 percent of the invasive fungal infections that occur in the HSCT patient – *Candida, Aspergillus* and *Cryptococcus* being the most important of these infections; b) the geographically restricted systemic mycoses caused by *Blastomyces dermatitidis, Coccidioides immitis* and *Histoplasma capsulatum*; and c) invasive

infection due to the so-called "newly emerging fungi" – *Fusarium,* the zygomycetes and such dematiaceous fungi as *Scedosporium, Scopulariopsis* and *Dactylaria* [4].

Candida is a major cause of fungal bloodstream infection during the preengraftment phase of HSCT. Although there is the possibility that the portal of entry can be vascular access catheters, it is believed that translocation of *Candida species* across gut mucosa damaged by the pre-transplant conditioning regimen is the major route of access to the bloodstream in the granulocytopenic patient [77, 78]. In the past, *C. albicans* and *C. tropicalis* accounted for virtually all of the *Candida* bloodstream infections. The incidence of candidemia was ~11 to 16 percent (with a median time to onset of two weeks post-transplant), resulting in a high rate of tissue invasion and an attributable mortality of nearly 40 percent [79, 80]. With the introduction of empirical antifungal therapy or fluconazole prophylaxis (400 mg/day) during the preengraftment period, the incidence of candidemia has been significantly decreased, hepatosplenic candidiasis has become quite rare, and the attributable mortality has been significantly decreased. Fluconazole-resistant *Candida sp*, *C. krusei* and *C. glabrata*, have emerged as not uncommon causes of candidemia in HSCT patients, as have the other non-*albicans Candida species* [1, 4, 81–87].

It is also important to recognize that other species of yeast (e.g., *Trichosporon sp, Blastoschyzomyces capitatus, Saccharomyces cereviseae* and *Rhodotorula sp*.) can cause clinical syndromes identical to those observed with invasive candidiasis (bloodstream infection, infection metastatic to the skin and subcutaneous tissues, as well as other sites, including hepatosplenic disease identical to that caused by *Candida species*) [88]. In an era of increased use of echinocandins for prophylaxis [89] and empirical antifungal treatment [90], these organisms [88, 91] and echinocandin-resistant *Candida sp*, especially *C. parapsilopsis*, have become emerging causes of fungemia in the HSCT units [1, 4, 82].

Invasive fungal disease has been most commonly caused by *Aspergillus sp*, with *A. fumigatus, A. flavus, A. terreus, A. niger* and *A. nidulans* being the most common causes of invasive aspergillosis. The portal of entry for 90 percent of cases of invasive aspergillosis is the lungs, with the nasal sinuses and the skin accounting for virtually all of the remaining cases. There are two major host defenses that are mobilized in response to inhalation of the *Aspergillus* spores – granulocytes and cell-mediated immunity, specifically cytotoxic T cells. The importance of these mechanisms is demonstrated by the clustering of cases of invasive aspergillosis at two timepoints in the post-transplant course: preengraftment when profound granulocytopenia is present, with the incidence of invasive aspergillosis increasing steadily as the period of granulocytopenia is extended, and after the diagnosis of GVHD and the treatment of this adverse event. Indeed, these late cases of invasive aspergillosis have become more common than the preengraftment cases. Mortality rates have traditionally been high in patients who developed invasive aspergillosis in either time period [1, 4, 92, 93].

The clinical syndromes caused by *Aspergillus* invasion reflect the pathologic consequences of the vasculotropic nature of this mold. The three major consequences of the vascular invasion that characterizes *Aspergillus* invasion include hemorrhage, infarction and metastatic disease. Initial clinical complaints include persistent fever, chest pain, tachypnea, hypoxemia and

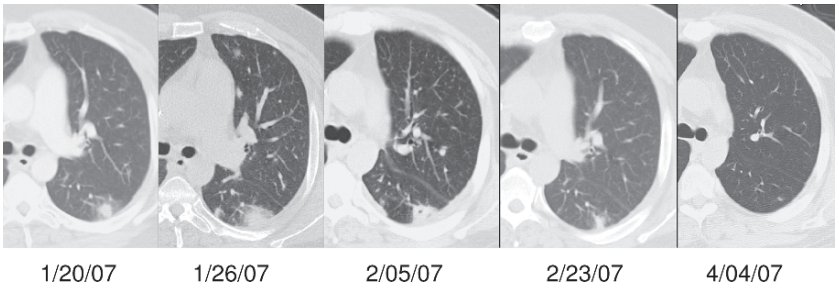

Fig. 19-2. Computerized tomographic scan of the chest in a patient with a "halo sign" due to invasive aspergillosis.
Note that halo signs most commonly occur in granulocytopenic HSCT recipients with invasive aspergillosis. However, it must be emphasized that a halo sign is occasionally seen in patients with *Nocardia*, *Scedosporium*, *Fusarium* and other forms of pneumonia. The patient was treated with voriconazole monotherapy during neutropenia during consolidation chemotherapy for AML and his treatment was continued through allogeneic transplantation.

hemoptysis, as well as symptoms related to metastases. Before the availability of noninvasive fungal markers (galactomannan and β-glucan) and aggressive imaging with spiral chest computerized tomographic (CT) scanning, 50 percent or more of patients experience disseminated infection at the time of first diagnosis, accounting for the high mortality observed in allogeneic HSCT recipients. A particular problem is infection in the CNS, where mortality historically has approached 100 percent. Metastases can present any site, but particularly important is the skin, as innocent appearing skin lesions can lead to early recognition of the disease, and should be aggressively biopsied [4].

Definitive diagnosis of invasive mold infections, including invasive aspergillosis, is usually accomplished by biopsying the site of abnormality. Early diagnosis is the key to effective therapy [94]. Sputum or bronchoscopic samples rarely yield mold on culture. In recent years, considerable effort has been made to find other technology that will lead to an earlier and timely diagnosis. The ones that have been incorporated into practice are the systematic measurement of *Aspergillus* antigens and serial chest CT imaging. Monitoring the serum of HSCT patients for galactomannan or β-glucan is now commercially available and has been incorporated into the current diagnosis guidelines [95, 96]. The detection of circulating fungal DNA in the blood by PCR [97] remains experimental. Findings on chest CT, in particular the halo sign (Fig. 19-2), are associated in the neutropenic patient with invasive aspergillosis (although other pathogens can cause the same radiologic finding: *Fusarium* and other vasculotrophic molds and *Nocardia asteroides* being examples of this). European groups have been advocating protocol serial chest CT scans to find such pathology as a guide to early diagnosis [98]. If prevention fails, then early diagnosis is the key to the patient's survival [4, 92, 93].

Given the limitations of current diagnostic techniques and the significant morbidity associated with invasive fungal infection, two strategies of antimicrobial use are commonly deployed in the HSCT patient. The first is **prophylactic** fluconazole use during the initial transplant period, which has been shown to decrease fungal infections [80] in one study, and overall mortality

[79] in another, when started on day 0 until engraftment [80] or day +75 [79]. It is important to note that a high background rate of *Candida* infections was noted in both of these reports and may not represent the experience of other transplant centers. Echinocandins may be an alternative to fluconazole prophylaxis during this risk period [89]. The second common strategy is **empiric** antifungal therapy in neutropenic patients with persistent fever without a source, despite broad-spectrum antimicrobial therapy for >96 hours [99, 100]. In this setting the primary concern is both *Candida* and invasive mold infection, especially *Aspergillus* [2]. The traditional antifungal therapy utilized as empiric therapy is an amphotericin product [101, 102]. Caspofungin use in this setting has become common because of the favorable side effect profile of this class of agents, but at the expense of a more limited fungal spectrum [90]. Other echinocandins (micafungin, anidulafungin) are likely to be similarly effective, but no randomized comparisons with these latest drugs have been performed. The role of voriconazole in this setting is controversial [102].

When treating invasive aspergillosis several approaches should be considered simultaneously: 1) antifungal therapy, 2) reverse or minimize the host immune defects (decrease corticosteroids, increase neutrophils), 3) control permissive viral infections (e.g., CMV) and 4) consider surgical excision, if possible. Voriconazole has become a cornerstone of therapy for invasive *Aspergillus* infections, though the management of potential side effects is substantial [103–105]. Whether the combination of therapeutic agents (polyenes, azoles and echinocandins) increases the therapeutic benefit has yet to be determined [106]. Increasing experience suggests that voriconazole alone is sufficient in most cases for a successful outcome in invasive aspergillosis and has decreased the morbidity and mortality of this infection [11, 106].

Another significant risk period for invasive fungal infections (IFI) is in the setting of significant GVHD, such as grade III or IV, and its therapy [9]. In this setting, posaconazole (versus fluconazole) prophylaxis has recently demonstrated some benefit in preventing IFI compared to fluconazole (5.3% versus 9.0%, $p = 0.07$) and in preventing probable or proven invasive aspergillosis (2.3% versus 7.0%, $p = 0.006$ – interestingly, these results were largely driven by results from galactomannan assay testing) [107–109]. Posaconazole has activity against the Zygomycetes as well as *Aspergillus* sp [110–112]. When an azole is used in this patient population, careful assessment of drug interactions, both with the initiation and cessation of therapy, is critical.

Therapy for the emerging fungi *Fusarium* and *Scedosporium* should be guided by *in vitro* sensitivity testing done locally or at regional reference laboratories, but voriconazole use should be considered. When therapy for the endemic mycoses is indicated, initial treatment (induction therapy) with an amphotericin preparation should be considered, followed by a prolonged course of consolidative therapy with an oral azole. Cryptococcal disease should be treated initially with an amphotericin preparation, CNS involvement should be excluded by cerebrospinal fluid sampling, and the use of flucytosine should be considered if present.

Pneumocystis jiroveci

Pneumocystis jiroveci, formerly *carinii*, is a ubiquitous environmental organism which is an important cause of pneumonia in patients who are immunosuppressed, such as those who have undergone an HSCT, on chronic prednisone

(typically >20mg per day) or with advanced HIV infection. PCP infection typically presents as an interstitial pneumonitis with marked hypoxemia. Severe infection can be life threatening. Fortunately, universal prophylaxis of high risk patients during the high risk periods with trimethoprim-sulfamethoxazole has markedly decreased this complication. However, intolerance to prophylaxis, use of second line prophylaxis agents (e.g., dapsone, pentamidine, or atovaquone), poor medication compliance or failure to re-institute prophylaxis in the setting of augmented immunosuppression (e.g., treatment of GVHD) are common reasons why cases still occur.

Post-Transplant Management

Several important issues must be addressed after successful HSCT to minimize infectious complications. First, it is important to avoid exposure to pathogens, especially when the immunosuppressive therapy to prevent GVHD is the highest. This includes avoiding gardening and soil exposures, mold exposures such as cleaning out damp basements or smoking marijuana, individuals with active respiratory infections, especially children, and avoiding enteric pathogens. Second, optimal treatment or monitoring for latent infections such as herpesviruses, hepatitis viruses and prior granulomatous diseases (e.g., *Mycobacterium tuberculosis*). Those patients with a positive test for latent tuberculosis should receive secondary prophylaxis, which typically is begun within one month post-transplantation, after the acute regimen toxicities associated with transplantation have subsided, when screening and preventive treatment have not occurred previous to HSCT. The first line therapy for secondary prophylaxis is isoniazid for nine months. However, in patients with significant hepatic dysfunction or peripheral neuropathy alternative regimens need to be considered. Rifamycin-based regimens are difficult given the potential hepatotoxicity, as well as the significant drug interactions, especially with concomitant use of a calcineurin or an azole. A quinolone, such as levofloxacin, with ethambutol may be considered. When a mycobateriologically static regimen is chosen, the duration of therapy often must be extended with some using this combination for 18 months as secondary prophylaxis (Table 19-2).

Third, optimizing vaccinations for routine pathogens such as diphtheria, tetanus, pertussis, influenza and pneumococcus (Table 19-3). This optimal timing of re-vaccination depends on the nature of the transplant, with earlier re-vaccination schedules being considered in the nonmyeloablative setting.

Fourth, prophylaxis for PCP, which is typically continued for approximately one year or until the immunosuppressive medications are tapered off. The optimal medication to use for PCP prophylaxis is trimethoprim-sulfamethoxazole which offers some protection for a variety of other important pathogens including *Pneumococcus*, *Hemophillus influenza*, *Nocardia sp.*, *Toxoplasma*, *Listeria*, *Salmonella sp.*, and other enteric bacterial pathogens. If trimethoprim-sulfamethoxazole is not tolerated due to significant renal dysfunction or bone marrow suppression, then alternative agents for PCP prophylaxis include dapsone, atovaquone or aerosolized pentamidine; however, none of these second line agents afford the broad microbial protection which trimethoprim-sulfamethoxazole affords. And lastly, herpes group viral prevention which should include acyclovir to prevent HSV and VZV and systematic monitoring for CMV, in the allogeneic setting, with early use of a CMV active antiviral if evidence for CMV activation or disease is observed.

Table 19-2. Prevention of infectious complications post-HSCT.

Organism	Primary Prevention or Prophylaxis	Alternative Prevention or Prophylaxis
PCP	Trimethoprim-sulfamethoxazole* either a double strength (DS) 3-times per week or as a single strength (SS) once a day for 1 year	Dapsone 50mg po BID, atovaquone 1,500 mg per day, pentamidine (aerosolized or intravenous)
HSV/VZV	Acyclovir 800 mg BID or 400 mg TID for 1 year	Valacyclovir 500 mg BID, famciclovir 250 mg BID
CMV	Preemption is preferred, where available	Valganciclovir** 900 mg po BID or QD post-engraftment to day 100 Intravenous ganciclovir, foscarnet
HBV***	Monitor for reactivation	
MTb (positive ppd)	Isoniazid 900 mg QD for 9 months**** with pyridoxine	Levofloxacin and/or ethambutol
Encapsulated bacteria	Monitor IgG level and consider replacement with IVIg when < 400–500 mg/dL Trimethoprim-sulfamethoxazole	Amoxicillin 500 mg BID
Candida/Aspergillus	Fluconazole 400 mg QD Posaconazole 200 mg TID*****	

Prophylaxis must be re-assessed in the setting of persistent or augmented immunosuppression, such as in the setting of clinically significant GVHD, regardless of time since HSCT. Medication doses may need to be adjusted for renal dysfunction.

* Trimethoprim-sulfamethoxazole affords modest protection for a broad array of potential environmental and community pathogens including: Nocardia sp, toxoplasmosis, pneumococci, H influenza, listeria, shigella, and slamonella sp.

** Alternatively preemptive monitoring with serial viral antigen or viral load assays can be considered.

*** For those with evidence of prior HBV infection (e.g., Hepatitis B core antibody positive), consider monitoring HBV viral load for evidence of reactivation periodically. If reactivation is detected then consider treatment if persistent HBV viremia detected. Specific HBV antiviral therapy is discussed in the text.

**** Pre-transplant secondary prophylaxis for MTb is preferred.

***** Decision for systemic azole prophylaxis should be based on local epidemiology of invasive fungal infections. Consider posaconazole prophylaxis in the setting of significant GVHD (e.g., Grade 3 or 4) and its therapy. Drug interactions must be carefully managed both with initiation and cessation of azole therapy.

Drug Interactions

An important aspect of antimicrobial therapy in the HSCT patient is the management of drug interactions, especially between antimicrobial agents (e.g., azoles, macrolides) and the immunosuppressive medications (e.g., calcineurin inhibitors, sirolimus) used to prevent and treat GVHD. There are three important categories of interaction to pay particular attention to, two of which are

Table 19-3. Vaccination considerations post-HSCT.

Vaccine	Timing post-HSCT
Tetanus/Diphtheria/Pertussis	12, 14 and 24 months
Pneumococcal	12, 14, 24 months
Haemophilus (HiB)	12, 14 and 24 months
Hepatitis A	12, 18 months
Hepatitis B	12, 14, 18 months
Meningococcal	12 months
Influenza	Annually: as available in the fall
MMR (Live virus vaccine)	At 24 months (if no significant GVHD and minimal immunosuppression)
Inactivated polio	3 doses

related to the major route of drug metabolism for the calcineurin inhibitors, hepatic cytochrome P450 enzymatic metabolism. These interactions are as follows: 1) certain antimicrobial agents (most notably the macrolides [eryth romycin>clarithromycin>azithromycin] and the azoles [ketoconazole>itraco nazole>voriconazole>fluconazole]) will downregulate the metabolism of the calcineurin inhibitors, resulting in elevated blood levels of active drug, and an increased risk of nephrotoxicity, as well as over-immunosuppression and an increased incidence of opportunistic infection; 2) certain antimicrobial agents (such as rifampin and rifabutin) upregulate metabolism of the calcineurin inhibitors, leading to a fall in blood levels and an increased risk of GvHD, and 3) therapeutic blood levels of the calcineurin inhibitors, when combined with such drugs as amphotericin B, aminoglycosides and vancomycin, can cause significant renal toxicity.

Summary and Conclusions

HSCT has become one of the great success stories of modern medicine. It is the therapy of choice for an increasing number of conditions, including a variety of cancers, bone marrow failure states, congenital immunodeficiencies, metabolic disorders and even as a means for introducing new genes. The major hurdle in most of these attempts, however, remains infection. Bacterial and fungal sepsis, as well as herpes group viral infection and community-acquired respiratory virus infection threaten the well-being of these patients. There are two phases of the post-transplant course when the patient is at particular risk: preengraftment with profound granulocytopenia and mucositis, and post-engraftment when GVHD and its therapy render the patient vulnerable to both fungal and viral infection. New preventative strategies are being formulated involving both prophylaxis and preemptive therapy. Similarly, new non-culture diagnostic approaches that rely on antigen detection or PCR detection of microbial DNA are being developed. New therapies, both antiviral and antifungal, have emerged. These should prompt much more effective prevention and therapeutic strategies.

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Chapter 20

EBV Lymphoproliferative Disease after Transplantation

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1.1. Introduction

Epstein-Barr virus (EBV) associated post-transplant lymphoproliferative disease (PTLD) is a serious, life threatening complication post-hematopoietic stem cell transplantation (HSCT) [1–3]. Major risk factors include the use of unrelated or human leukocyte (HLA)-mismatched related donors, T cell depletion of the graft, the use of T cell antibodies for the prophylaxis and therapy of Graft-versus-Host disease (GVHD) and an underlying diagnosis of primary immunodeficiency [4–7]. Over the last decade effective immunotherapies have been developed either reconstituting EBV-specific T cell responses [8, 9] or targeting PTLD with B-cell monoclonal antibodies like rituximab [10–14]. Besides careful clinical monitoring of high risk patients, serial measurement of EBV DNA load in peripheral blood samples by polymerase chain reaction (PCR)-based methods has proven helpful in identifying high risk patients [10, 12, 15–17]. However, the indication for preemptive therapy remains a major challenge since not all HSCT recipients with elevated EBV DNA develop PTLD.

1.2. EBV-Associated Diseases

EBV was the first human virus implicated in oncogenesis and, since its original description, has been linked to a heterogeneous group of nonmalignant and malignant diseases [18, 19]. EBV is a latent herpes virus that infects over 90 percent of the human population worldwide. Primary EBV infection usually occurs through the oropharynx, where mucosal epithelial cells and/or B-cells become primarily infected [20]. The virus produced in these cells may then infect neighboring epithelial cells and B-cells circulating through the mucosa-associated lymphoid tissues. Primary infection results in a self-limiting illness characterized by fever, lymphadenopathy and pharyngitis that is followed by lifelong virus latency in B-cells. In healthy, seropositive individuals, EBV latency is tightly controlled by the cellular immune system. The importance of CD8 positive EBV-specific T cells in the control of primary EBV infection and latency has been well documented [21, 22] and the potential role of CD4 positive EBV-specific T cells has also been highlighted [23–25].

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All EBV-associated malignancies are associated with the virus' latent cycle. Four patterns of EBV latent gene expression have been described, termed type 0, I, II and III, and types I – III are found in malignancies [18, 26]. In all types of latency the EBV-derived viral RNAs, known as EBERs, and the BamHI-A rightward transcripts (BARTS) are expressed, but the pattern of latent viral protein expressing varies. Type 0 latency characterizes EBV latency after primary infection in healthy individuals; the virus persists episomally in resting memory B-cells and, of the almost 100 viral proteins, only EBNA1 is expressed during memory B cell division. In type I latency only EBNA1 is expressed and this pattern is associated with EBV-positive Burkitt's lymphoma and gastric adenocarcinoma. Type II latency, characterized by the expression of EBNA1, LMP1 and LMP2, is found in EBV-positive Hodgkin's Disease and Non-Hodgkin's Lymphomas, and nasopharyngeal carcinoma [27, 28]. While malignancies associated with type I and II latency occur in individuals with minimal or no immune dysfunction, type III latency is associated with malignancies in severely immunocompromised patients. It is characterized by the expression of the entire array of nine EBV latency proteins being EBNAs 1, 2, 3A, 3B, 3C, LP, LMP1 and LMP2 and this pattern of gene expression is found in PTLD after solid organ transplant (SOT) or HSCT, and in EBV-associated lymphomas occurring in patients with congenital immunodeficiency or human immunodeficiency virus (HIV) infections. In addition type III latency is found in lymphoblastoid cell lines (LCL), which 1) can be readily prepared by infecting B-cells *in vitro* with EBV and 2) were instrumental in the generation of EBV-specific CTL for the prophylaxis and therapy of PTLD after HSCT (see section 1.8.2).

1.3. Pathogenesis of PTLD

In EBV-seropositive individuals with compromised T cell function the control of EBV-infected B-cells is impaired leading to an increase in the number of EBV-infected B-cells. PTLD arises from a broad range of EBV-infected B-cells, including naïve, memory or atypical post–germinal center B-cells, illustrating its complex pathogenesis [29–32]. The expansion of latent EBV-infected B-cells occurs without significant reactivation from latency into the lytic cycle, explaining why antiviral agents, like acyclovir, that prevent productive viral replication are of limited therapeutic value [33]. The importance of T cell dysfunction in the pathogenesis of PTLD in HSCT recipients is highlighted by the fact that the majority of PTLD cases occur within the first six months post-transplant when the T cell deficiency is most profound [5]. In addition, as discussed in section 1.4, therapies that selectively deplete T cells or impair their function increase the incidence of PTLD.

Since not all patients with similar T cell dysfunction develop PTLD other contributing risk factors may play a role in the outgrowth of EBV-transformed B-cells, like the local concentrations of cytokines or chemokines, which could potentially promote B-cell proliferation. For example polymorphisms in the promoter regions of cytokines have been implicated in increasing the risk of developing PTLD [34, 35]. Besides EBV and a dysfunctional cellular immune system, genetic alterations in B-cells, including microsatellite instability, DNA hypermethylation, aberrant somatic hypermutation and mutations in specific

genes such as BCL-6, N-ras, p53 and c-myc [36], have also been implicated in the pathogenesis of PTLD, especially in SOT recipients.

1.4. Incidence and Risk Factors

The overall prevalence of PTLD post-allogeneic HSCT is ~1 percent with the majority of cases developing in the first six months after transplantation. However, the incidence is significantly increased by risk factors including 1) the use of HLA-mismatched family members, 2) the use of closely matched unrelated donors, 3) T cell depletion of donor cells, 4) nonmyeloablative conditioning regimens, 5) intensive immunosuppression with T cell antibodies for the prophylaxis and therapy of GVHD and 6) an underlying diagnosis of primary immunodeficiency, (Table 20-1) [5, 37–41]. The incidence is much lower when T and B-cells are depleted simultaneously, indicating that the incidence of PTLD may depend on the balance between EBV-infected B-cells and EBV-specific T cell precursors. In a large review of HSCT recipients treated with the CD52 monoclonal antibody Campath-1, which removes T and B-cells, the incidence of PTLD was less than 2 percent [42]. Other methods of B-cell depletion, like elutriation which removes over 90 percent of B-cells from the donor graft [43], or the addition of monoclonal antibodies for B-cell depletion to the T cell depletion regimen, have also proven to be effective. Using CD19, CD20 or a combination of CD19 and CD20 monoclonal antibodies for the depletion of B-cells in stem cell products prevented the development of PTLD and acute GVHD without reducing the engraftment rate, as compared with historical controls [12, 44].

Only a few cases of PTLD have been described post-autologous HSCT and most have involved patients who had previously received long-standing intensive immunosuppressive therapy or who received a CD34-selected product that may delay immune reconstitution [45]. In addition, the use of rabbit ATG as part of an intense immunosuppressive conditioning regimen followed by autologous CD34-selected HSCT for severe autoimmune disease was associated with an increased incidence of PTLD, compared to equine ATG [46]. The incidence of PTLD after allogeneic umbilical cord blood transplant (UCBT) is low, ranging between 2 to 4.5 percent with myeloabative conditioning regimens [47]. However, a much higher incidence (21%) has recently been reported in patients who received nonmyeloablative condition regimens that

Table 20-1. Risk factors for PTLD after HSCT.

		Relative Risk	
Risk factor	**Bhatia, et al. [114]**	**Curtis, et al. [5]**	**Socie, et al. [6]**
HLA-mismatched Transplant	8.9	3.7	7.5
T cell depletion	11.9	9.1	4.8
ATG as prophylaxis or Therapy for GVHD	5.9	5.5	3.1
Immunodeficiency as primary diagnosis	2.5	NR	NR

NR, not reported

included ATG [48]. Thus, as for allogeneic HSCT, recipient T cell suppressive therapy is an important risk factor for EBV-PTLD after UCBT.

1.5. Clinical Presentation

In HSCT recipients PTLD may present with a diverse spectrum of clinical symptoms and signs, underscoring the need for a high index of suspicion in making the diagnosis. Symptoms and signs include fever, sweats, generalized malaise, enlarged tonsils and cervical lymphadenopathy not unlike that seen in primary EBV infection [20]. EBV-associated B-cell proliferation may involve other organs including lung, liver, spleen, kidneys, small intestine, bone marrow or the central nervous system. Often, diffuse disease is only diagnosed at autopsy in patients thought to have severe GVHD or fulminant sepsis [49].

1.6. Pathology

The pathology ranges from polymorphic B-cell lymphomas to aggressive Non-Hodgkin's Lymphoma [50–53]. The World Health Organization recommends classifying PTLD into three categories: 1) early lesions, 2) polymorphic PTLD and 3) monomorphic, lymphomatous PTLD [54]. The last category mainly consists of diffuse large cell lymphomas and other histologic variants are rarely seen [51]. Differentiating polymorphic from monomorphic PTLD can be difficult, especially since both can be seen in the same biopsy. Despite efforts to standardize the pathological classification of PTLD neither histology nor clonality have proven capable of consistently predicting clinical outcome [55].

1.7. Laboratory Testing

Since the presenting clinical symptoms of PTLD are not specific, there has been a great interest in developing tests which would predict the development of PTLD. The usefulness of PCR-based methods to monitor EBV DNA load has been well documented, and EBV DNA load monitoring is now routinely available in large transplant centers [56]. In addition, functional assays to monitor EBV-specific T cell responses are being developed which may play an important role in the future laboratory assessment of HSCT recipients at risk for PTLD [57, 58].

1.7.1. Monitoring of EBV DNA Load in HSCT Patients

The onset of PTLD in the majority of cases is preceded by a significant increase in EBV load and several investigators have shown that frequent monitoring of EBV DNA load in peripheral blood by PCR is a valuable diagnostic test for early detection of PTLD after both SOT and HSCT [15, 59, 60]. The threshold levels of EBV DNA suggestive of impending PTLD vary according to sample (plasma, serum, PBMC or whole blood) and PCR method of quantifying viral DNA. Different PCR methodologies and their potential shortcomings have been recently reviewed [61]; assay sensitivity ranged from 40 to 100 percent (median 88%), specificity from 61 to 100 percent (median 92%), positive predictive value from 25 to 100 percent (median 62%) and negative predictive

value from 93 to 100 percent (median 100%). We and others currently favor real-time quantitative (RQ) PCR [12, 92] as a detection method since it has several advantages, compared to conventional PCR methods: it is 1) fast and safe requiring minimal specimen handling, 2) flexible allowing the detection of DNA from different specimen material, 3) highly sensitive, 4) reproducible and 5) precise. Initial studies in recipients of T cell-depleted grafts suggested that a high EBV DNA level has a strong prognostic value for developing lymphoma [9, 15, 60]. However, over the past few years it has become clear that while EBV reactivation is a frequent event after both T cell-depleted and un-manipulated transplant, high EBV loads only have a high correlation with development of EBV lymphoma after T cell-depleted transplants [12, 63, 64]. In patients with high EBV DNA load after solid organ transplant, recent studies indicate several distinct patterns of EBV latent gene expression in memory B-cells, with type III latency conferring the highest risk for PLTD development [65]. Thus, an elevated EBV DNA load can lead to early diagnosis of PTLD, although other factors such as clinical symptoms and signs, and results of diagnostic imagining studies must be taken into account before therapy is initiated.

1.7.2. Monitoring EBV-Specific CTL Responses

In contrast to monitoring EBV DNA load in peripheral blood, measuring EBV-specific T cell responses by 1) interferon (IFN)-γ secretion assays using intracellular cytokine staining or Elispot assays, or 2) MHC class I-peptide multimeric complexes for enumerating EBV-specific T cells is not routinely available. Nevertheless, several studies have highlighted the feasibility of this approach [57, 58, 66, 67]. For example, Meij, et al. [67] showed that impaired recovery of EBV-specific, CD8-positive T cells in HSCT recipients with high-level EBV reactivation may identify a subgroup at very high risk for PTLD. Using tetramer analysis and IFN-γ secretion assays in HIV patients van Baarle, et al. [58] demonstrated dysfunctional EBV-specific CD8-positive T cells prior to the development of EBV-associated Non-Hodgkin's Lymphomas (NHL). These studies indicate that functional analysis of EBV-specific T cell responses is feasible and may be useful in assessing the risk of PTLD development in HSCT recipients with increased EBV DNA load.

1.8. Treatment

Treating PTLD has largely focused on strategies to boost the immune response to EBV. In SOT recipients withdrawing immunosuppressive therapy has proven effective, but carries a high risk of graft rejection [68]. Since HSCT patients receive high-dose chemotherapy and/or radiation to completely ablate their immune system, withdrawal of immune suppression post-transplant in the majority cases is ineffective in the HSCT patient with overt PTLD. However, decreasing the immunosuppression in patients with increasing EBV DNA load might accelerate the endogenous recovery of EBV-specific T cell responses and subsequent control of EBV reactivation [69]. For overt PTLD more active immunotherapeutic strategies must be pursued. Therapy with interferon-α and intravenous immune globulin has been used in SOT recipients and in a small number of HSCT patients with some responses [70]. Active immunization is

not feasible because of the patients' severe immunosuppression, and the most successful modalities of therapy have been the adoptive immunotherapy of donor T cells or donor-derived EBV-specific CTL and the infusion of monoclonal antibodies against B-cells.

1.8.1. Treatment with Donor T Cells

Adoptive immunotherapy with donor T cells for PTLD was originally reported by Papadopoulos, et al. [71]. All five patients had therapeutic responses, but three developed GVHD and two died from respiratory insufficiency. In an update of their experience, 17 of 19 patients responded positively to donor T cells (Table 20-2) [72]. Other investigators have also documented success with this approach [60, 73, 74], but emphasize the risk of GVHD and, in some instances, a lower response rate to donor leukocyte infusions than originally reported. In a series of 13 patients post-T cell-depleted allogeneic HSCT, only four (31%) responded to donor leukocyte infusions [60], one of whom died from acute GVHD and another from aspergillosis. Of the two surviving patients who had a complete remission, one also received EBV-specific CTL. Five of the nine patients with disease progression died within 10 days of receiving donor leukocytes, most likely because of advanced PTLD at the time of treatment, prompting the authors to advocate earlier diagnosis and initiation of therapy. In three smaller series none of five patients responded to donor leukocyte infusions [70, 75, 76]. Reasons for the discrepancies in response rates to donor leukocyte infusions (DLI) are unclear, but may reflect different types of disease or better outcome with early diagnosis and treatment. To reduce the risk of GVHD several groups have transduced T cells with 'genetic safety switches.' The most widely used 'safety switch' takes advantage of the herpes simplex virus thymidine kinase (HSV-*tk*) gene which renders transduced cells sensitive to the cytotoxic effects of acyclovir, ganciclovir or valacylovir. In a Phase I clinical trial, donor-derived T cells transduced with

Table 20-2. Treatment and prophylaxis of PTLD with donor T cells or EBV-specific CTL after HSCT.

	Indication for Therapy				
Study	**Prophylaxis**	**Therapeutic**	**Cell Product**	**Response**	**GVHD**
Gustafsson, et al. [9]	+		EBV-specific CTL	1/6 developed LPD	1/6
Rooney, et al. [8]	+		EBV-specific CTL	0/39 developed LPD	1/39
Gross, et al. [70]		+	Donor T cells	0/3	NR
Heslop, et al. [74]		+	Donor T cells	1/1	1/1
Lucas, et al. [60]		+	Donor T cells	4/13*	4/13
Nagafuji, et al. [75]		+	Donor T cells	0/1	0/1
O'Reilly, et al. [72]		+	Donor T cells	17/19	3 acute, 8 chronic
Rooney, et al. [8]		+	EBV-specific CTL	2/3	0/3
Sasahara, et al. [73]		+	Donor T cells EBV-specific CTL	0/1	0/1

* One responding patient received EBV-specific CTL, NR-not reported

the HSV-*tk* gene were infused into HSCT recipients [77]. Six patients developed GVHD and four had a complete resolution of GVHD after ganciclovir treatment. One drawback of this approach is the inherent immunogenicity of HSV-*tk* [78]. Therefore, genetic safety switches using non-immunogenic human components, like CD20, inducible Fas or caspase, have been developed and successfully tested in preclinical animal models [79–81].

1.8.2. Treatment with EBV-Specific Cytotoxic T Lymphocytes

One strategy to reduce the potential risk of GVHD after donor T cell infusions is the administration of *in vitro*-expanded antigen-specific CTL, which was pioneered by Riddell, et al. in Seattle for prophylaxis of CMV disease in HSCT recipients [82, 83]. The CMV-specific CD8-positive CTL clones reconstituted CMV-specific immune responses without adverse effects, and none of the patients developed CMV disease. However, the CTL did not persist long-term except in patients who either endogenously recovered CMV-specific CD4-positive T helper cells or were coinfused with CMV-specific CD4-positive T cell clones, underscoring the need for such cells in the maintenance of CD8-positive CTL populations [84].

In the majority of PTLD cases that occur in HSCT recipients, the transformed B-cells are of donor origin and express all latent cycle virus-associated antigens, providing excellent targets for virus-specific T cells. EBV-transformed LCL also express all latent cycle virus-associated antigens and several costimulatory molecules that facilitate CTL generation. They can be readily prepared from any donor and provide a source of antigen presenting cells that endogenously expresses the appropriate antigens for presentation of HLA class I-restricted epitopes. Most likely, HLA class II-restricted, EBV epitopes are presented through phagocytosis of dead cells [72, 85]. The generation of EBV-specific CTL from seropositive, healthy donors takes eight to 12 weeks, of which four to six weeks are needed to generate sufficient numbers of LCL for CTL stimulation. The resultant EBV-specific CTL are polyclonal and contain both CD4- and CD8- positive EBV-specific T cells which is considered advantageous since the presence of antigen-specific CD4-helper T cells is important for *in vivo* survival of cytotoxic CD8-positive T cell populations [83].

We have infused over 60 recipients of allogeneic T cell-depleted graft products with donor-derived EBV-specific CTL. As prophylaxis, infusions were well tolerated with minimal side effects and no development of acute GVHD. More importantly none of the CTL recipients developed PTLD in comparison to 11.5 percent of untreated historic controls from our institution [8]. The trial was initially designed as a dose escalation study and the first 12 patients received either 4×10^7 cells/m^2 (n = 6) or 1.8×10^7 cells/m^2 (n = 6) over four weeks. At both dose levels efficacy was noted and subsequent dose de-escalation showed that a single dose of 2×107 cells/m^2 was effective with no change in outcome. In nine patients with high EBV DNA load CTL infusion resulted in a 2- to 3-log decline of DNA levels indicating that the infused CTL had antiviral effects and reconstituted cellular immunity to EBV. A subset of patients received EBV-specific CTL, which were gene-marked with the neomycin resistance gene [86]. Gene-marked CTL persisted for up to seven years and in one patient a transient increase in EBV DNA load was mirrored by an increase in gene-marked CTL followed by a subsequent decline

of both values, demonstrating the intricate balance between EBV latency and EBV-specific CTL. Other investigators also showed safety and efficacy of EBV-specific CTL in reducing high EBV DNA load post-HSCT [9]. The infused cell dose was similar to our study (1×10^7 cells/m^2 × 4), however one out of six patients developed fatal PTLD. *In vitro* testing of the infused CTL line of this patient showed only a weak EBV-specific component, which might explain this case of immunotherapy failure.

In our study five patients with overt PTLD received EBV-specific CTL, of whom four had a complete response. In addition, we treated one additional patient with PTLD of the central nervous system with EBV-specific CTL and hydroxyurea [87]. One of the responders experienced a potential complication of CTL therapy: an increase in tumor size due to infiltrating T cells caused airway compromise at a nasopharyngeal tumor site requiring intubation and mucosal ulceration at other tumor sites in the soft palate and intestine [8]. All lesions resolved and the patient is in remission more than nine years post-therapy. The non-responder died 24 days post-CTL therapy and a limited autopsy showed progressive PTLD. Molecular analysis revealed that the patient harbored two genetically distinct viruses prior to CTL infusion, one of which carried a deletion of two immunodominant CTL epitopes [88]. After CTL infusion the epitope deleted virus persisted causing progressive, fatal PTLD. Since CTL mutants have been recently described in other diseases [89, 90], they may present a problem even when polyclonal CTL lines with a fixed epitope repertoire are used for immunotherapy. The incidence of both observed complications of CTL therapy – morbidity secondary to T cell infiltration and occurrence of CTL escape mutants – is most likely to be higher in patients with high tumor burden, supporting the argument to infuse patients with EBV-specific CTL as prophylaxis or with minimal disease.

While therapy with EBV-specific CTLs has proven to be effective the process of generating such lines is labor-intensive and takes 10 to 12 weeks. Koehne, et al. have described methodology for selecting virus-specific cells early in culture by their susceptibility to transduction with a retroviral vector [91] that may allow more rapid CTL generation. In addition, recipients are only protected from one of the many viruses that may cause morbidity and mortality during the period of immunosuppression post-transplant. Several groups have investigated approaches for modifying antigen presenting cells to generate multispecific CTLs. Transduction of LCL with a retroviral vector encoding the CMV protein pp65 has allowed generation of CTL specific for both CMV and EBV [92] while infection of LCL with adenovirus results in generation of CTL specific for both adenovirus and EBV [93]. More recently, our group has generated CTL lines specific for pp65, adenovirus and EBV [94]. Infusion of these multispecific CTL lines was safe and successfully restored virus-specific immunity in HSCT recipients. An alternative strategy to generate broad antiviral immunity for haploidentical SCT recipients is to co-culture donor mononuclear cells or LCL with recipient cells and then deplete populations expressing activation markers such as CD25, which should contain alloreactive cells [95, 96]. The residual allodepleted-T cell product should contain CTL specific for multiple viruses and potentially residual tumor cells. Three phase I/II clinical studies have been published documenting the feasibility of this approach [97–99]. The infusion of allodepleted-T cells was safe and improved T cell recovery after haploidentical SCT without causing severe GVHD.

Other cellular therapies, like the infusion of interleukin (IL)-2 activated killer cells, have been tested in small numbers of SOT recipients with PTLD [100]. However, the initial promising results have not been repeated.

1.8.3. Treatment with Monoclonal Antibodies

In addition to cellular immunotherapy, monoclonal antibodies have been used for to treat PTLD after HSCT (Table 20-3). In a European multicenter study 58 patients were treated with CD21 and CD24 murine monoclonal antibodies and 35 (61%) entered complete remission [101]. However, these antibodies are no longer available and, in addition, murine monoclonal antibodies may cause anaphylaxis and the production of neutralizing human anti-mouse antibodies (HAMA). To reduce the incidence of HAMA, chimeric murine/human monoclonal antibodies have been developed and one of the most successful examples is rituximab (Rituxan; Genentech and IDEC Pharmaceuticals Corporation), a CD20 monoclonal antibody [102–104]. It has been used in the treatment of CD20-positive B-cell NHL as a single agent or in combination with conventional therapy and also is an effective agent for the treatment of PTLD. In a multicenter retrospective analysis of 32 patients with PTLD after SOT or HSCT, rituximab was well tolerated and the overall response rate was 69 percent, with 20 complete responses and two partial responses [105]. To date we have treated 14 patients with rituximab for PTLD after SOT (six liver and one kidney) or HSCT (n = 7) [10, 12, 106] with all patients having a complete response. One patient who had received high-dose steroids and anti-thymocyte immunoglobulin (ATG) for GVHD prior to rituximab therapy, died of aspergillosis. Six of the seven HSCT recipients have now been followed for more than two years after rituximab infusion, with no evidence of PTLD recurrence. Faye, et al. [11] reported 12 patients with PTLD after HSCT treated with rituximab. Eight patients had a complete remission and seven are alive with a median follow-up of almost two years. Of the eight patients two received donor lymphocyte infusion post-rituximab and one patient died of

Table 20-3. Treatment of PTLD after HSCT with rituximab.

	Indication for Therapy		
Study	**Preemptive**	**Therapeutic**	**Response**
Faye, et al. [11]	+		4/4
Faye, et al. [115]		+	1/1
Faye, et al. [11]		+	4/8
Greenfield, et al. [116]	+ (3)	+ (2)	5/5*
Gruhn, et al. [117]	+		3/3
Kuehnle, et al. [10]	+		3/3
Milpied, et al. [105]	+ (4)	+ (2)	4/6**
van Esser, et al. [109]	+		14/15
Wagner, et al. [12]	+		5/5
Weinstock, et al. [118]		+	4/5***

* One responder treated with PTLD also received EBV-specific CTL
** Responders not specified
*** Two responders also received EBV-specific CTL and/or DLI

staphylococcal sepsis. The four non-responders had more extensive disease with mediastinal involvement and a lower CD4 T cell count. The safety and efficacy of rituximab has also been evaluated in a prospective, multicenter Phase II study for SOT patients with biopsy proven PTLD [107]. Forty-three patients were treated with four weekly injections of 375 mg/m^2 rituximab. At day 80, 86 percent of patients were alive and the response rate was 44.2 percent, including 12 complete responses/complete responses unconfirmed. The overall survival rate was 67 percent at one year. While rituximab is clearly effective for the treatment of PTLD the efficacy varied in between studies. As for T cell therapies this most likely reflects better outcome with early diagnosis and treatment.

Rituximab has also been used prophylactically in patients with high EBV viral load. In a study evaluating a humanized CD3 monoclonal antibody in the treatment of GVHD elevated EBV viral load and lymphoproliferative disease developed in two of the first seven patients. Five of the next 10 patients were, therefore, given rituximab when their EBV viral load increased and all had a virological response with none developing lymphoma [108]. Other investigators have also advocated the use of rituximab as preemptive therapy for PTLD [109, 110]. However, because of its known side effects, it should be confined to such clinical scenarios where there is a strong association with developing lymphoma. The profound B-cell depletion induced by rituximab for six to eight months may exacerbate the immunodeficiency in transplant recipients, and a lack of EBV-infected B-cells could potentially delay recovery of EBV-specific immunity, increasing the risk of PTLD late in the post-transplant period. Neither complication was observed in our transplant patients treated with rituximab, but long-term follow-up is necessary to adequately assess these risk factors. It is also possible that monoclonal antibody therapy may result in selection of B-cells negative for the targeted antigen, as reported in some lymphoma patients after anti-CD20 therapy [111] and for a patient with PTLD after lung transplant [112].

Besides B-cell antibodies, IL-6 monoclonal antibodies have been used to treat PTLD [113]. In a multicenter phase I/II clinical trial, 12 patients were treated with IL-6 monoclonal antibodies after SOT with five achieving complete remission and three partial remissions. The treatment was well tolerated and further studies are needed to define the role of IL-6 antibodies in therapy for PTLD.

1.9. Conclusions

Combined B- and T cell depletion has lowered the incidence of PTLD; however, it remains a serious and life threatening complication post-HSCT. Over the last decade effective immunotherapies for PTLD have been developed, including donor-derived EBV-specific CTL and monoclonal antibodies like rituximab. In contrast to EBV-specific CTL, rituximab is readily available and preemptive therapy of imminent PTLD is becoming a clinical reality. Currently, it is advisable to consider preemptive therapy in patients with high EBV DNA load and risk factors such as T cell depletion of the donor graft, infusion of T cell antibodies for GVHD therapy or an underlying diagnosis of primary immunodeficiency. In patients with high EBV DNA levels without risk factors and no clinical symptoms and signs of PTLD, possible side effects of therapy must

be balanced against the risk of developing PTLD. In the future, besides EBV DNA load monitoring by PCR, laboratory tests assessing EBV-specific T cell function may become available to assist in the management of HCST patients who are at high risk of developing PTLD. Since therapy failures have been reported with monoclonal antibodies as well as cellular immunotherapies, one of the major challenges for the future remains how to combine these treatment modalities. Identifying patients at high-risk for PTLD is a prerequisite for such an approach since EBV-specific CTL cannot be generated for every patient as prophylaxis. Patients with imminent PTLD could then receive rituximab alone or in combination with other monoclonal antibodies as preemptive therapy, like anti-IL6, which would allow for the time required to expand EBV-specific CTL. Such an integrative immunotherapeutic approach may ultimately reduce current failure rates and improve long-term outcome of patients with PTLD.

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Chapter 21

Hepatic Veno-Occlusive Disease

Erica Linden and Paul Richardson

Introduction

The clinical syndrome of hepatic veno-occlusive disease (VOD) after hematopoietic stem cell transplantation (SCT) is characterized by liver enlargement and pain, fluid retention, weight gain and jaundice [1–3]. Its onset is typically by day + 30 after SCT, although later onset has been described [4]. Since diagnosis is based on clinical criteria, the incidence reported and severity seen is variable, ranging from 10 to 60 percent, and may be influenced by differences in conditioning regimens and patient characteristics [5, 6]. Prognosis is also variable, ranging in severity from a self-limited disease requiring no specific treatment to critical illness with multi-organ failure and a fatality rate approaching 100 percent [5, 7]. VOD is considered to be part of the spectrum of nonmyeloid organ injury syndromes that can occur after high-dose therapy and SCT, which include idiopathic pneumonitis, diffuse alveolar hemorrhage, thrombotic microangiopathy and capillary-leak syndrome. There is a growing body of evidence indicating that early injury to vascular endothelium, either directly by the conditioning regimen or indirectly through the production of certain cytokines, is a common denominator between these events [8–10]. Supportive care is a mainstay of treatment with close monitoring of fluid status, analgesia, early recognition of infection and prompt initiation of antibiotics. The use of defibrotide (DF) has been studied in prospective multicenter trials and shows consistently promising results in severe VOD with complete response rates of 36 to 42 percent and improved D + 100 survival compared to expected, with a favorable safety profile [11, 12]. Strategies using defibrotide as part of prophlaxis are now underway and when the efficacy of defibrotide is confirmed in this setting, other combination studies incorporating approaches abrogating liver injury will hopefully follow.

Histopathology

Sinusoidal endothelium in the liver is notable for a cobblestone appearance with numerous small pores [13]. These fenestrations create a unique microvascular architecture within the surrounding extracellular matrix and tissues subserved by zone 3 of the hepatic acinus as they drain into the hepatic venules

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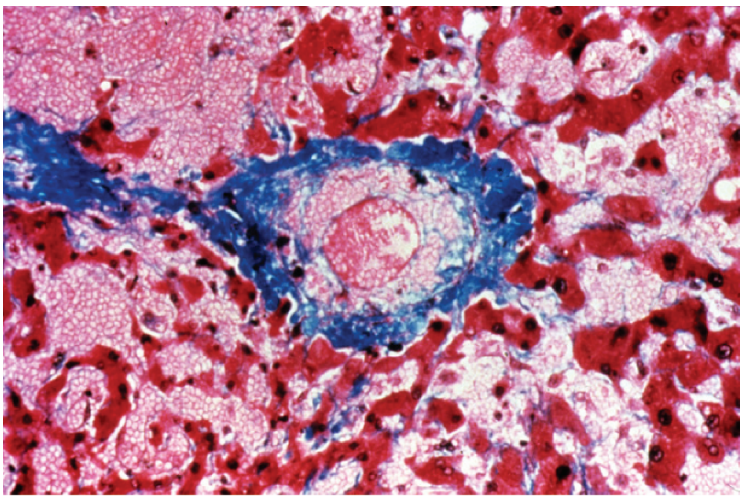
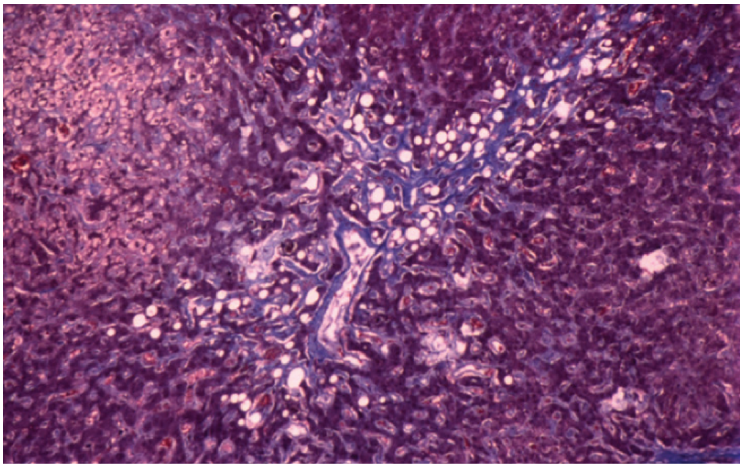
[14, 15]. Hepatic venules manifest the first histologic change in VOD with subendothelial edema and endothelial cell damage with microthromboses, fibrin deposition and the expression of factor VIII/ vWF within venular walls [16]. Dilatation of the sinusoids is also present and hepatocyte necrosis follows with later features including intense collagen deposition in the sinusoids, sclerosis of the venular walls and the development of collagen deposition both within venular lumens and abluminally [15] [See Fig. 21-1a]. This progresses to obliteration of the venule with further hepatocyte necrosis. Advanced veno-occlusion is similar to severe cirrhosis with widespread fibrous tissue replacement of normal liver [15].

Gemtuzumab Ozogamicin (Mylotarg)-related VOD, a recently observed complication of this anti-CD33 monoclonal antibody therapy for acute myelogenous leukemia (AML), is noteworthy for marked sinusoidal obstruction [17–20] and fibrosis [See Fig. 21-1b]. The term "sinusoidal obstruction syndrome" (SOS) has been suggested as an alternate to the established terminology of VOD [17]. While sinusoidal obstruction is clearly apparent in rat models of VOD [21] and is seen in human disease [17], the first recognizable histologic change of liver toxicity in SCT patients is widening of the subendothelial space between the basement membrane and the lumen of central veins [15]. Accompanying venular changes, dilation and engorgement of the sinusoids with extravasation of red cells and frank necrosis of perivenular hepatocytes follow which progress and become more widespread as the extent of venular injury advances [15]. Moreover, correlating histologic findings in a cohort study of 76 consecutive necropsy patients post-SCT found the strongest statistical association between the severity of VOD and the extent of hepatocyte necrosis, sinusoidal fibrosis, thickening of the subendothelium, phlebosclerosis and venular narrowing [15]. Thus, additional prospective



a

Fig. 21-1a Liver biopsy showing characteristic dyes of VOD in an SCT patient with terminal venular fibrosis, fibrin deposition, subendothelial edema and marked zone 3 hepatocellular damage



b

Fig. 21-1b Liver biopsy in a patient with prior Mylotarg exposure and severe VOD: Sinusoidal obstruction is prominent

studies are probably needed and a consensus remains to be reached before the term VOD in SCT patients can be universally changed to SOS; at present, some investigations prefer the term VOD (SOS) which seews a reasonable compromise any pending final agreement [22].

Pathogenesis

Alkylating Agents and Toxic Metabolites

Injury to sinusoidal endothelial cells and hepatocytes in zone 3 of the liver acinus mediated by the conditioning regimen appears to be a key initial event in VOD. Evidence for this include the observation that pyrrolizidine alkaloids cause denigration of hepatic venular endothelium in experimental animals [23]. The ingestion of these compounds in contaminated grains and teas ("bush tea") has been reported to result in VOD in humans independent of stem cell transplantation [24]. Hepatocytes in zone 3 contain both a high concentration of cytochrome P450 enzymes, which metabolize many chemotherapeutic agents used in high-dose regimens, and glutathione S transferase enzymes, which catalyze the reaction of glutathione with electrophilic compounds [25, 26]. Depleting glutathione has been reported to result in hepatocyte necrosis while glutathione mono-8-diester can selectively protect hepatocytes from high-dose alkylator injury [27–29].

A number of trials have shown that higher plasma levels of cytotoxic drugs used in SCT, such as busulphan or the metabolites of cyclophosphamide, are associated with an increase risk of VOD [24–27, 30]. Commensurate with this finding, it has been observed that VOD is more common in patients whose area under the curve of concentration versus time (AUC) of busulphan is elevated [31–33]. Furthermore, when busulphan dosing is adjusted to reduce the AUC in patients whose AUC after first dose is elevated, the incidence of VOD has generally been significantly reduced or even eliminated [31, 34, 35].

In addition, Lee, et al. studied oral versus intravenous busulfan and noted an increase of over two-fold in the incidence of VOD with oral administration, likely due to variability in absorption and first-pass hepatic metabolism [36]. Targeted busulfan dosing has been studied in at least two centers with a corresponding absence of VOD, supporting the belief that individualized dosing of busulfan can reduce the risk of hepatic injury [35, 37].

The importance of busulphan in VOD pathogenesis has been re-evaluated by Slattery, et al. who measured the pharmacokinetics of either busulphan and cyclophosphamide or cyclophosphamide plus total body irradiation in patients prior to SCT [38]. These investigators reported that average plasma steady state concentrations of busulphan directly correlated to the serum levels and, thus, exposure to cyclophosphamide and its metabolites, rather than busulfan. Subsequent studies have confirmed the key importance of cyclophosphamide and its metabolites in sinusoidal endothelial cell and hepatocyte injury [30, 39, 40]. Deleve, et al. demonstrated that the direct exposure of sinusoidal endothelial cells (SEC) to cyclophosphamide did not result in toxicity, but when sinusoidal endothelium was exposed to the metabolites acrolein or 4-hydroxycyclophosphamide, a dose-dependent toxicity was observed [21]. In contrast, when sinusoidal endothelium and hepatocytes were co-cultured in the presence of cyclophosphamide, marked toxicity to sinusoidal endothelium was apparent. This suggested that the increased injury to sinusoidal endothelium, which was greater than that seen to hepatocytes, was due to acrolein generated by the metabolic activation of cyclophosphamide by hepatocytes. In the same study, Deleve demonstrated that the effect was reversed by sustaining levels of hepatocyte glutathione with serine-methionine, and this protective effect was abolished by proparglyglycine, an inhibitor of glutathione synthesis [21]. These studies imply that increased exposure to the toxic metabolites of cyclophosphamide contribute to the development of VOD and that supporting levels of hepatic glutathione might prevent VOD, consistent with earlier experiments done by Teicher, et al. [28]. Additional evidence of the potential importance of glutathione in VOD was demonstrated by the abrogation of the effects of monocrotaline-induced injury in a rat model with the targeted support of sinusoidal endothelial cell glutathione [41], and a clinical report of the successful use of N-acetyl cysteine in the treatment of VOD [42].

Endothelial Cell Injury

Several investigators have reported marked elevations in markers of endothelial injury in patients with VOD. Catani and colleagues measured plasma thrombomodulin (TM) and P-selectin levels prior to and after SCT prospectively in 25 patients, two of whom developed reversible VOD and one who developed fatal VOD. TM and P-selectin levels were normal in all but the one patient with severe VOD, where these endothelial stress products were found to be markedly elevated [43]. Salat, et al. have measured plasma levels of plasminogen activator inhibitor I (PAI-1) in patients undergoing SCT. Levels of PAI-I were increased nearly five-fold in four patients with VOD, compared to 28 patients without VOD [44]. He subsequently reported that levels of PAI-1 were significantly greater in VOD patients than those in other forms of liver injury after SCT, and hypothesized a lipopolysaccharide (LPS)–based mechanism of

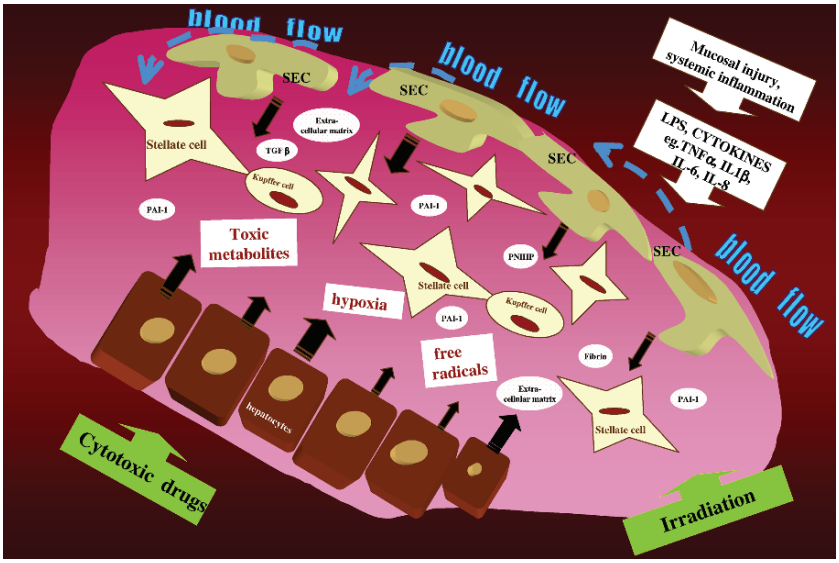


Fig. 21-2. Potential key cellular, biochemical and cytokine events in VOD pathogenesis SEC: Sinusoidal endothelial cell. (with permission)

sinusoidal endothelial injury and Kupffer cell activation illustrated in Fig. 21-2 [45]. Studies by other investigators have subsequently confirmed elevated PAI-1 levels in SCT-associated VOD. In a murine model of VOD, PAI-1 null knockout mice did not develop hepatic dysfunction or venular thromboses. Wild type mice given tiplaxtinin, a PAI-1 inhibitor, were protected from nitric oxide-induced hepatic injury [46]. It is noteworthy that hepatic stellate cells (also known as Ito cells, lipocytes or perisinusoidal cells) produce large amounts of PAI-1 when stressed, with recent evidence pointing to a key role for activated stellate cells in the pathogenesis of VOD through the production of extracellular matrix and the promotion of hepatic fibrosis [47] (see Fig. 21-2). The degree of PAI-1 elevation has been demonstrated to be an independent diagnostic marker of VOD as well as predictive of severity [48]. Other markers of endothelial cell injury have also been found to be elevated in patients with VOD, including tissue factor pathway inhibitor (TFPI), soluble tissue factor (sTF), TM, P and E-selectin [48–53].

The role of cytokines has been an area of interest in the study of VOD pathogenesis [see Fig. 21-2]. Tumor necrosis factor (TNFα) levels in serum are low in established disease, but it has been postulated that high levels of TNFα and interleukin (IL)-1β may contribute to initial endothelial damage [54, 55]. More recent studies of IL-6, IL-8 as well as TNFα and IL-1β levels in patients during SCT have suggested a possible relationship between IL-6 and IL-8 with jaundice, renal dysfunction and pulmonary disease, but in contrast serum TNFα and IL-Iβ concentrations were not predictive of SCT-related complications [56]. Data showing elevated plasma levels of C-reactive protein in allo-SCT patients with severe VOD, as compared to those without, support the possible role of IL-6 in the disease process [57]. Elevation of transforming growth factor β (TGF-β), collagen propeptides and hyaluronic acid have been

observed in VOD [58–61]. Moreover, elevated serum levels of the immunopropeptide of type 3 procollagen (PIIINP) have been reported at the onset of clinically evident VOD in a study by Rio and colleagues [62]. Given that elevated levels of PIIINP have been associated with fibrotic liver disease, it has been speculated that serum levels of PIIINP are surrogates for the intrahepatic accumulation of type 3 collagen in VOD [63, 64].

Plasma levels of certain endothelial stress products increase after high-dose cytoreductive therapy, including von Willebrand factor (vWF) and serum angiotensin converting enzyme [55, 65, 66]. Conversely, levels of anticoagulants fall shortly after high-dose cytoreductive therapy [67–69]. As an extension of these observations and the connection between endothelial damage and VOD, several studies have reported low baseline levels of the naturally occurring anticoagulants in patients who subsequently developed VOD [55, 60, 70], as compared to those who did not develop VOD. The same groups and others have also shown marked fluctuation in both the levels of various procoagulant proteins (including serum proteases and fibrinogen) and fibrinolytic parameters (such as D-dimer) [55, 65, 70, 71]. However, a clear relationship between these levels and the development of VOD remains to be established and these data have been unable to distinguish whether the changes seen in coagulation parameters are directly involved in the pathogenesis of VOD, or are epiphenomena of the disease process.

In patients with established VOD, profound thrombocytopenia and refractoriness to platelet transfusion is common. This may represent splenic sequestration as a result of portal hypertension or consumption continued through endothelial cell injury, and thrombopoietin levels are commensurately high [60, 72]. Factor VII levels are usually low, but it is not known if this is specific to VOD, as a function of increased activation at the endothelial cell surface, or a result of global hepatic dysfunction [55, 65].

Risk Factors

Risk factors for developing VOD can be divided into pre-SCT and SCT-related factors [5, 7, 73]. Pre-SCT factors include elevated liver transaminase levels (specifically AST), older age, poor performance status, female gender, advanced malignancy, prior abdominal radiation, the number of days on broad-spectrum antibiotics pre-SCT, prior exposure to amphotericin B, vancomycin and/or acyclovir therapy, the number of days with fever pre-SCT, the degree of histocompatibility in allogeneic SCT and prior gemtuzumab ozogamicin [2, 5, 18]. Reduced pre-SCT diffusion capacity of the lung may be an independent risk factor for VOD [74]. Norethisterone treatment, previously used in women to minimize menstrual bleeding during the thrombocytopenic period post-SCT, has been incriminated as a risk factor, possibly by causing microthrombotic injury in hepatic venules [75]. Some factors also appear to predict VOD severity; for example, an elevation of AST four-fold above normal and increasing histoincompatibility between donor and recipient have been associated with severe VOD and high fatality [7]. Conversely, in nonrandomized studies, a low incidence of VOD has been found in patients receiving T cell-depleted grafts [76–78].

SCT factors include total body irradiation (TBI) dose, dose rate and dose of busulphan [2, 5]. A randomized study showed a significantly higher incidence

in patients receiving busulphan and cyclophosphamide, compared to cyclophosphamide and TBI conditioning [79]. In a study of 350 patients treated with busulphan 16 mg/kg and cyclophosphamide 120 mg/kg the overall incidence of VOD was 27 percent [80]. In an IBMTR study of 1,717 recipients of HLA-identical sibling SCT for leukemia between 1988 and 1990, variables associated with an increased risk of VOD were conditioning with busulphan, and cyclophosphamide compared to TBI [81]. Conversely, VOD also appears less frequent when peripheral blood progenitor SCT is used compared to bone marrow alone [82]. There may be several reasons for this including more rapid engraftment resulting in less prolonged cytopenia, toxic injury and cytokine disturbance. A more intriguing notion is that this may reflect superior endothelial re-engraftment from stem cells, a hypothesis that has been strengthened by the observation of donor endothelial cell engraftment in coronary vessels post-SCT [83].

An important new risk factor for VOD is the administration of gemtuzumab ozogamicin (Mylotarg or GO), an anti-CD33 monoclonal antibody linked to the potent toxin calicheamycin [18, 84, 85]. Sinusoidal endothelial cells and stellate cells in zone 3 of the hepatic sinus express CD33 and, as a result, significant toxic liver injury has been reported both when this agent is given to AML patients prior to and after SCT, with resultant severe VOD and a high rate of case fatalities [85]. In a study of 62 patients undergoing SCT for AML or MDS, the incidence of VOD was 64 percent in patients with prior gemtuzumab ozogamicin exposure as opposed to 8 percent in patients without prior exposure ($p<0.0001$) [18]. This study also found that the timing of exposure to GO was significant in that nine of 10 patients who received GO within 3.5 months of SCT developed VOD, whereas no patients with exposure after 3.5 months developed VOD. Arceci and colleagues found a 40 percent incidence of VOD in a series of 13 pediatric patients with exposure to GO within 3.5 months of SCT [86]. The Research on Adverse Drug Events and Reports (RADAR) project reported an incidence of VOD ranging from 15 to 40 percent by observational studies in patients with GO exposure within three months of SCT [19]. The conclusions of the RADAR project were to use caution in pursuing SCT in patients with recent exposure to GO, an observation supported by other studies [18].

Diagnosis

The diagnosis of VOD is a clinical diagnosis. Two accepted clinical case definitions used in multiple studies are the Baltimore and Seattle criteria, detailed in Table 21-1 [6, 87]. Although most VOD is apparent within the first three weeks of transplant, there are instances where VOD develops later, which in a limitation of the classic case definitions of VOD. The severity of VOD is defined by the need for treatment and the presence and extent of organ failure: mild disease is defined by no apparent adverse effect from liver dysfunction with complete resolution of symptoms and signs. Moderate disease is characterized by adverse effects of liver dysfunction requiring therapy such as diuresis for fluid retention and analgesia for right upper quadrant pain, but with eventual complete resolution. Severe VOD is defined as VOD with accompanying multi-organ failure, (MOF) typically involving the renal, pul-

monary and/or central nervous systems [4–8]. The majority of patients fall into the mild to moderate category, but a significant fraction of VOD is severe and while occasional patients may recover, most are incurable with a fatality rate ranging from 80 to 100 percent [5, 7], with a recent metanalysis reporting D + 100 mortality of 83% in patients with VOD and MOF (Coppell et al., EBMT 2008). Table 21-2 summarizes some of the clinical features seen in the varying groups by degree of severity, with MOF a hallmark of severe disease [2, 3, 7].

Ultrasound and CT imaging can be useful in identifying hepatomegaly, confirming the presence of ascites and, together with Doppler studies, may be useful in determining whether or not there is attenuation or reversal of venous flow or portal vein thrombosis [88]. Both CT and ultrasound are useful in excluding pericardial effusion, constrictive pericarditis, extrahepatic venous obstruction and mass lesions of the liver [89, 90]. Doppler ultrasound has gained popularity in the assessment of VOD because it is noninvasive and can be performed at the bedside. In a recent study of 244 pediatric patients, reversal of portal blood flow by Doppler ultrasound was associated with

Table 21-1. Clinical criteria for case definition of VOD.

Baltimore criteria [87]	Seattle criteria [7]
By day +21: Hyperbilirubinemia (> 34.2 µM or >2 mg/dL)	Two or more of the following by day +20: Hyperbilirubinemia (> 34.2 µM or >2 mg/dL) Painful hepatomegaly
Plus at least 2 of the following:	Unexplained weight gain (>2% of baseline weight)
Painful hepatomegaly	
Fluid retention or ascites	
Sudden weight gain (>5% of baseline weight)	

Table 21-2. Clinical features of patients with VOD of the liver according to severity of disease [7].

	Mild	Moderate	Severe
Weight gain (% increase)	7.0 (± 3.5%)	10.1(±5.3%)	15.5(±9.2)
Maximum total serum bilirubin before day 20 (mg/dl)	4.73(±2.9)	7.95(±6.6)	26.15(±15.3)
Percent of patients with peripheral edema	23%	70%	85%
Percent of patients with ascites	5%	16%	48%
Platelet transfusion requirements to day 20	53.8(±27.6)	83.6(±5.0)	118.3(±51.8)
Day 100 mortality (all causes) (%)	3	20	98

a transplant-related mortality of 33 percent versus 7 percent in patients with normal flow. However, pulsatile hepatic venous flow can be a relatively nonspecific finding and, as implied from the pediatric experience described above, reversal of portal flow is usually a late feature of VOD. More recently, Doppler measurement of hepatic arterial resistance has been studied prospectively in a limited number of patients with VOD to provide earlier clues for diagnosis and prognosis [91]. MRI has attracted interest, but its role remains to be established, other than as means to exclude other causes of liver dysfunction [92]. Transvenous liver biopsy and wedged hepatic venous pressure gradient measurement (WHVPG) remain gold standards of diagnosis. In this setting, the transfemoral or transjugular method is generally preferred and percutaneous biopsy has little or no place in the evaluation of VOD given the high risk for bleeding [93]. As well as providing tissue, this technique permits measurement of WHVPG, with a gradient of >10 mm of mercury having a 91 percent specificity and 86 percent positive predictive value, but more modest sensitivity at 52 percent [94].

Elevation of PAI-1 may prove to serve as an early marker of VOD. Pihusch, et al. followed PAI-1 antigen levels in 350 stem cell recipients: all patients with a PAI-1 antigen over 120 ng/ml developed VOD, resulting in a sensitivity of 100 percent and specificity of 30.6 percent [53]. Similarly, Lee, et al. followed multiple diagnostic markers in 115 SCT patients: PAI-1 and bilirubin were the markers most predictive of VOD in a multivariable analysis [48].

Prognosis

In attempting to develop an aid to estimate prognosis, a Cox regression analysis was used by Bearman and colleagues to generate risk curves predictive of severe VOD based upon a large cohort of patients from the Seattle Transplant Registry. In these patients, VOD occurrence was defined within the first 16 days post-SCT after preparation with one of three specific regimens: cyclophosphamide and total body irradiation (CyTBI); busulphan and cyclophosphamide (BuCy), or cyclophosphamide, BCNU and VP-16 (CBV) [95]. Severe VOD in turn was associated with a case fatality rate of 98 percent by day + 100 after SCT [See Fig. 21-3]. Calculations were based on total serum bilirubin and percentage weight gain at various timepoints subsequent to SCT, up to day + 16 (see Table 21-2). Similar models have not been proposed for other temporal or therapeutic settings, and models based on possible surrogates, such as cytokines, endothelial stress products or markers of fibrosis have yet to be defined.

Irrespective of time frame and conditioning, the rates of rising bilirubin and weight gain are much higher in patients with severe VOD and the mean maximum bilirubin and percent weight gain are significantly greater in patients with severe VOD as compared to those with milder illness [6] [see Table 21-2]. More recently, both bilirubin and PAI-1 antigen elevation have been shown to be independent predictors of VOD severity [96]. Other clinical features associated with worse outcome include the development of ascites, which occurs in fewer than 20 percent of patients with mild to moderate VOD, compared to 48 percent or more patients with severe disease, and is reflective of increased portal hypertension [6] [See Table 21-2]. Commensurate with this, WHVPG

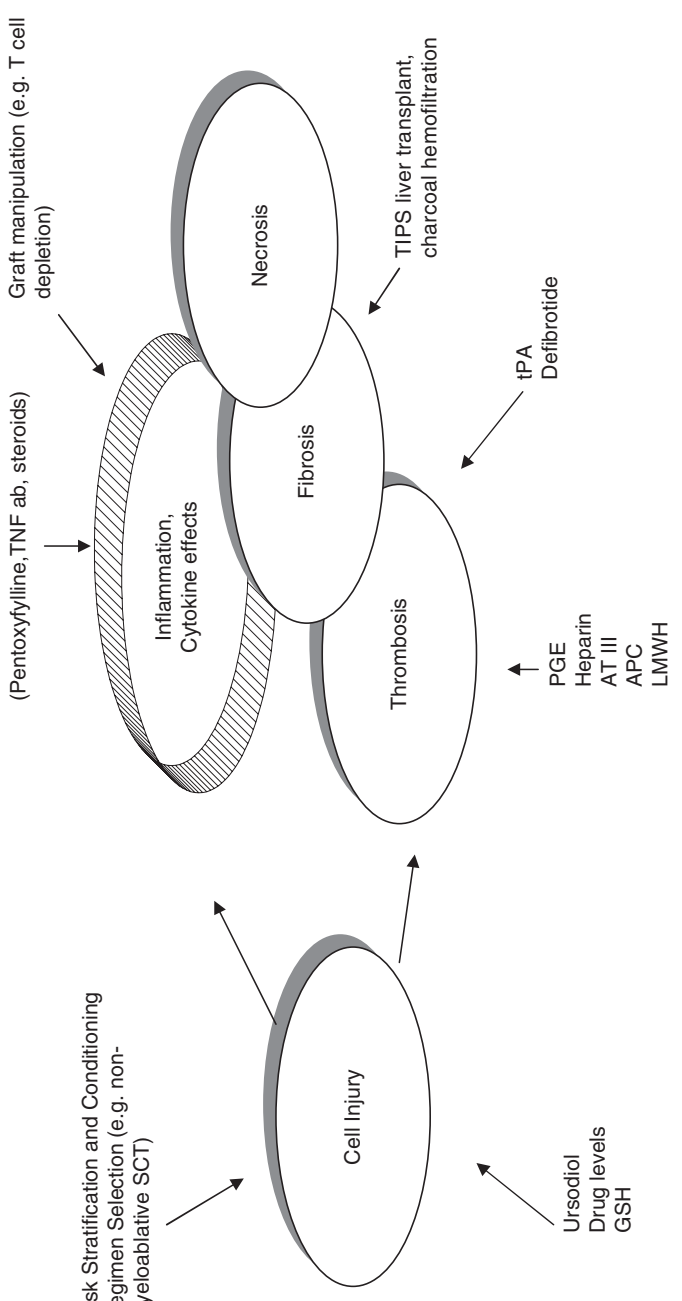


Fig. 21.3 Potential points for intervention in VOD. Whilst cell injury, microthrombosis, fibrosis and necrosis are established as events in VOD pathogenesis, the role of inflammation and cytokine-mediated injury remains to be defined. GSH: Gluthathione; AT III: antithrombin 3; LMWH: low molecular weight heparin; APC: activated protein C; TIPS: transjugular intrahepatic portosystemic shunt; TNF ab: tumor necrosis factor antibody

values in patients with VOD beyond 20 mm of mercury are associated with a particularly poor prognosis [94]. As mentioned previously, a cardinal feature predicting high mortality in VOD is the presence of MOF [2, 3]. In fact, patients with severe VOD usually die of renal, pulmonary and/or cardiac failure, rather than from hepatic insufficiency *per se* [6].

Prevention

Given the lack of approved, effective therapies, preventing severe VOD is a priority. Selection of particular conditioning regimens for patients at high risk is one approach, and this is perhaps best embodied by the emerging field of nonmyeloablative transplant, where the incidence of VOD is low [97]. However, depending on the underlying disease, this may or may not be an optimal therapeutic strategy [97, 98]. Assessing risk by virtue of genetic predisposition to VOD may be one possible avenue in the future. Preliminary studies of genetic polymorphisms in SCT patients have variably suggested a possible association between a mutation of glutathione S transferase synthesis and increased VOD risk [99–102]. Similarly, in a large, prospective study of allelic variants for TNFα in SCT patients, a high incidence of MOF was seen in association with a specific allelic variant (TNF d3), which causes increased TNFα production in response to injury [103]. Therefore, the possibility of risk stratification pre-SCT for both the development and the sequelae of VOD exists, but further, more comprehensive studies are needed to better define such risk. Moreover, the relationship of genetic risk, if able to be defined, to specific regimens and agents will also need to be established.

The most established practice in VOD prevention has been the use of pharmacokinetics to monitor drug levels to minimizie hepatic injury. This approach is currently best illustrated by the monitoring of busulphan levels [23, 31–37]. The observed relationship between elevated busulphan levels and VOD may possibly be due in part to busulphan-mediated depletion of hepatic glutathione, which in turn predisposes hepatocytes to additional injury from ensuing cyclophosphamide exposure. This argument is consistent with data suggesting that increased exposure to the toxic metabolites of cyclophosphamide may contribute to the development of VOD [30, 38, 40]. Moreover, the observation that the ursodeoxycholic acid has important antioxidant properties within hepatocytes may explain why ursodiol has important antioxidant properties within hepatocytes may explain why the observed protective effect ursodeoxycholic acid has been most apparent in patients receiving busulphan-based conditioning [104].

Pharmacologic Prophylaxis

The prophylactic administration of ursodeoxycholic acid (UDCA), a hydrophilic water-soluble bile acid, has been studied in a number of randomized placebo-controlled prospective trials. Several have shown a statistically significant benefit in patients predicted to be at high risk of VOD [104, 105]. A large phase III study by the Nordic Bone Marrow Transplantation group did not demonstrate a statistically significant benefit in reducing the incidence of VOD, though there was a clear decrease in the proportion with marked hyperbilirubinemia (>50 μM), a trend towards decreased acute GVHD and an improved one-year overall survival in patients receiving UDCA of 71 percent versus 55 percent

in those who did not (p=0.02) [106, 107][106,107]. A systemic review of six large studies evaluating the use of UDCA as prophylaxis for VOD showed a statistically significant reduction in both the incidence of VOD (RR: 0.58) [108]. The authors conclude that UDCA should be considered as part of potential prevention strategies in patients undergoing allogeneic SCT [108].

The supplement of hepatic gluthathione has been tested in experimental models [28, 39], but this has been difficult to translate into patients due to concerns regarding tumor protection. The feasibility of restoring hepatic glutathione levels to concentrations that are truly effective in humans is also unclear. However, reports of a significant decline in gluthathione and other antioxidants after chemotherapy in SCT, coupled with a recent report of N-acetyl cysteine supplementation in the successful treatment of VOD, suggest that further evaluation of supportive nutrition, including antioxidants such as vitamin E, is warranted [42, 109, 110].

Other approaches to VOD prevention, such as the role of steroids, have also attracted interest [111]. Given that inflammation does not appear to be a central component to the pathogenesis of VOD, it is difficult to understand why steroids should be of direct benefit. However, it is possible that steroids may abrogate other intercurrent or separate forms of liver injury. Modulating inflammation with pentoxifylline and TNFα neutralization has been unsuccessful to date. Pentoxifylline administration in prospective randomized placebo-controlled trials has been either ineffective or associated with more VOD than placebo [112, 113].

Treatments targeted at preventing vascular injury have been more extensively examined. A small number of randomized trials have studied the effect of low-dose continuous intravenous heparin, but only one randomized study has demonstrated a beneficial effect of heparin prophylaxis [114]. However, this study was conducted mainly in low risk patients and other uncontrolled studies have suggested that heparin was ineffective and/or dangerous, because of increased risk of hemorrhage [5, 75, 115, 116]. In a prospective study the use of ATIII concentrates has been shown to offer no protective value [117]. Low-molecular-weight heparins (LMWHs) seem to be relatively safe and may have some effect in preventing VOD [118, 120]. In a retrospective analysis of 462 patients receiving SCT, patients treated with prophylactic LMWH had significantly less VOD than the other groups (4% versus 11–22%) without an increase in fatal hemorrhage [121]. Prospective, randomized trials of LMWH prophylaxis are needed to confirm these results, but have been hampered by ongoing concerns regarding bleeding risk, effectiveness and cost.

The use of prophylactic fresh frozen plasma (FFP) to replete ATIII and/or protein C has been reported recently [69]. In this retrospective study of 403 patients receiving three different VOD prophylactic strategies of either FFP and heparin, heparin alone, or no VOD prophylaxis, the incidence of VOD was 5.9 percent in the combination group, 20 percent in the heparin alone group and 15.7 percent in the no prophylaxis group ($p<0.01$). A prospective trial has been proposed to further and better evaluate these findings.

Prostaglandin E1 (PGE1) is a vasodilator with cytoprotective effect on endothelium, as well as platelet aggregation inhibitory and prothrombolytic activity [122]. In one trial PGE1 was given in combination with low-dose heparin and the incidence of VOD was 12.2 percent in the PGE1 treated group, compared to an incidence of 25.5 percent in historic controls, suggesting that prophylactic PGE1 might decrease the incidence and severity of VOD [123]. A

randomized trial performed in Buffalo, NY also showed that prophylactic PGE1, heparin and tPA treatment demonstrated an improved D + 100 survival post-SCT, compared to heparin and tPA alone [124]. However, a prospective study by the Seattle group using higher doses of PGE1 in a phase I/II study, without concomitant heparin, could not demonstrate any beneficial effect of this drug, and PGE1 administration was complicated by significant toxicity [95].

Defibrotide has been used primarily to treat severe VOD (discussed below). Given the efficacy seen in established disease and defibrotide's favorable safety profile to date, studies in prevention are an obvious priority. The use of defibrotide as VOD prophylaxis has been reported in 52 patients compared to historical controls: there were no cases of VOD in the study group versus 19 percent in controls. In addition, 100-day event-free survival was better in the defibrotide group as well as a trend towards better overall survival [125]. Several subsequent studies have been performed in prophylaxis, all with promising results, although these trials have to date been limited by the use of historic controls. Future studies to confirm these intriguing findings are warranted and a number of large, multicenter, international trials are either in process or planned.

Treatment

The complex pathophysiology and observed biochemical abnormalities in VOD offer several potential points of intervention and treatment (see Fig. 21-4). Strategies aimed at reducing hepatocyte injury, treating thrombosis, preventing fibrosis, abrogating inflammatory cytokine signals and preventing hepatic failure are, thus, attractive and a number are under investigation.

Based upon the histologic observation of microthrombosis and fibrin deposition, as well as intense factor VIII/ vWF staining in VOD, therapies aimed at promoting fibrinolysis with or without concomitant anticoagulant therapy have been developed [126–128]. Over 100 patients treated to date with thrombolytic therapy, with or without anticoagulation, have been reported in the literature, but only a few series have included more than 10 patients (See Table 21-3). In the largest study published in patients with established VOD, 42 patients received treatment with tissue plasminogen activator (tPA) and concomitant heparin for severe disease. Twelve of 42 (29%) patients responded, with response defined as a reduction in pretreatment bilirubin by at least 50 percent. No patient with MOF (defined as renal insufficiency and/or hypoxemia at the time of treatment) responded and 10 (24%) developed severe secondary bleeding secondary to treatment, with a significant number experiencing fatal hemorrhage. The authors concluded that tPA/heparin should not be given to patients with MOF and treatment should be given early in the disease course or not at all [129].

The administration of ATIII and activated protein C (APC) has been studied with mixed results [130–134]. In one series of 10 patients who received ATIII for the treatment of chemotherapy-induced organ dysfunction following SCT, the probability of dying from multi-organ failure was significantly decreased [134]. In a prospective randomized trial of ATIII in 54 patients with multi-organ failure, there was a trend towards decreased mortality, with decreased severity of illness as well as shorter hospital stays and decreased hospital costs [133]. A literature review of ATIII trials concludes that the evidence of definitive benefit is lacking at this time, although the suggestion of potential benefit still exists [132]. PGE1 infusions for established VOD have been largely unsuccessful [135].

Table 21-3. tPA for the treatment of VOD.

Author	Number of Patients (pt.) (n)	Dose (mg/d)	Duration (days)	Heparin (yes/no)	Number (n) of responses	life threatening or serious hemorrhage
Baglin [166]	1	50	4	no	1	0
Bearman [129]	42	5.4–30	2–4	yes	12	10
LaPorte [167]	1	50	4	no	1	0
Rosti [168]	1	50	4	no	1	0
Ringden [169]	1	50	4	no	0	1
Leahey [127]	9	5–10	2–4	yes	5	0
Feldman [170]	3	15	4	no	3	0
Goldberg [171]	1	20	4	yes	1	0
Higashigawa [172]	1	2–5	4	yes[1]	1	0
Hagglund [173]	10	3–50	3–8	yes[2]	4	4
Lee [118]	3	10–20	7–14	yes	3	0
Yu [174]	8	0.25–0.5[3]	4	no	4	0
Schriber [175]	37	30–40	1–21	yes	10[4]	13
Kulkarni [176]	17	10	1–12	yes	6	0
Litzow [128]	10	10–50	2–4	no	2	0

Notes: [1] pt. also received PGE1

[2] 3 pts. received heparin, 7 pts. did not

[3] dose reported as mg/kg

[4] in pts. who met established criteria for VOD

Defibrotide (DF), a single-stranded polydeoxyribonucleotide with a molecular weight of 15–30 kD [136, has been identified as an agent that might modulate endothelial cell injury without enhancing systemic bleeding and protect host hepatocytes and sinusoidal endothelium without compromising the antitumor effects of cytotoxic therapy [137–139]. DF has specific aptameric binding sites on vascular endothelium, namely adenosine receptors A1 and A2, which are part of the growing family of nucleotide receptors involved in endothelial cell regulation and response to injury [138]. Studies have shown that DF increases prostacyclin (PGI-2), prostaglandin E-2 and thrombomodulin *in vivo*. DF also upregulates TFPI and tPA [140–143]. It decreases thrombin generation and also decreases circulating PAI-1 [144]. Moreover, DF inhibits fibrin deposition and may modulate vitronectin and fibronectin release which, as components of extracellular matrix, are linked to collagen formation and fibrosis [145–147]. Its mechanism of action remains to be defined but its activity as a pharmacologically potent oligonucleotide targetting endothelium has become increasingly clear.

Clinical trials of DF have demonstrated activity in peripheral vascular disease, microvascular thrombotic states, ischemic organ injury and chemotherapy-related hemolytic uremic syndrome (HUS) [141, 146, 148, 149]. Preclinical studies of human-derived, LPS-exposed microvascular and macrovascular endothelium by Falanga, et al. have shown selective and protective effects of DF in LPS–mediated microvascular injury through enhanced fibrinolysis and modulation of sTF and TFPI expression [150, 151]. This differential activity of the drug on microvascular rather than macrovascular endothelium is particularly intriguing in the context of its application to diseases of the microvasculature, such as VOD.

Table 21-4 summarizes the results of reported studies using defibrotide in the treatment of patients with VOD after SCT. The first study was a compassionate

Table 21-4. Defibrotide (DF) for the treatment of Hepatic VOD (from Ho).

Author	N	DF dose (mg/kg/d)	CR rate %	Day+100 Survival %	Patient Characteristics
Richardson, et al. [12]	19	5–60	42	32	High risk patients only: all had MOF
Chopra, et al. [156]	40	10–40	55 (overall) 36 (high risk)	43	28/40 patients considered high risk
Richardson, et al. [11]	88	10–60	36	35	All high risk by Bearman model or MOF
Corbacioglu, et al. [160]	45	10–110	76 (overall) 50 (high risk)	64 (overall) 36 (high risk)	Pediatric 22/45 patients considered high risk
Bulley, et al. [159]	14	11–40	64*	79	Pediatric only Risk group not given
Richardson, et al. ASH Abstr 2006 [158]	102	25 (Arm A) 40 (Arm B)	46	41	All high risk by Bearman model or MOF No CR or OS difference arms b/w 2

*CR rate not reported, 9/14 (64%) discontinued DF due to clinical improvement

use study conducted from 1995 to 1997: only patients with severe VOD were included, defined by a greater than 40 percent risk per the Bearman model or by the presence of MOF [12]. DF treatment was given intravenously, typically every six hours and infused over two hours with a dose range of 10–60 mg/kg/day. In the first cohort of 19 evaluable patients, complete responses (defined as a bilirubin less than 2 mg/dL) were seen in eight patients (42%), most of whom had resolution of MOF and survived to day 100 and beyond. Response was typically evident within the first seven days, and the active dose appeared to be approximately 25 mg/kg/day. None of the non-responders survived past day 100, with a median survival of only 36 days post-SCT (range 15–89) [12].

Additional trials of DF administration to patients with severe VOD and MOF by other groups have produced similar results [152–156]. While the natural history of more moderate VOD is less morbid, the complete response rate in the European experience with DF therapy was higher in patients with moderate, but significant VOD suggesting that earlier intervention may be more effective [156].

Analysis of the expanded United States experience has confirmed the favorable safety profile of DF when used in a multi-institutional setting following specific treatment guidelines [11] where a complete response rate of 36 percent and an overall survival of 35 percent was observed in a total of 88 SCT patients with severe VOD and MOF. Predictors of survival included younger age, autologous SCT and abnormal portal flow, while busulphan-based conditioning and encephalopathy predicted a worse outcome. Decreases in mean creatinine and PAI-1 levels during DF therapy also predicted better survival, suggesting that certain features associated with successful outcome could correlate with DF-related treatment effects, and further evaluation of DF therapy for severe VOD may, therefore, allow better definition of predictors of response or failure [11].

The results of a large multicenter randomized phase II trial attempting to define the optimal dose of DF have been reported; 150 patients who were diagnosed with VOD by Baltimore criteria and had severe VOD, defined as either the presence of MOF and/or a high predicted mortality using the Bearman model [157], were randomized to 25 mg/kg/day [Arm A] or 40 mg/kg/day [Arm B]. The complete response rate was 46 percent and day +100 survival 41 percent [158]. These results were especially noteworthy as almost all of the patients (99%) had MOF, including dialysis-dependence in a third and ventilator dependence in another. No significant difference in outcome was seen between the two dose arms although there was marginally more toxicity with the higher dose arm. Ongoing trials are using the lower dose of DF. Two pediatric trials have established the safety and efficacy of DF in the pediatric population with complete response rates of up to 64 percent reported and impressive D+100 survival [159, 160]. A large, multicenter, historically controlled phase 3 North American and Israeli study is now underway in patients with severe VOD and MOF. Completion is anticipated late in 2008 and, if the results are positive, regulatory approval for DF as treatment for this indication will hopefully follow.

Liver transplant in those able to undergo the procedure has resulted in clinical improvement in about 30 percent as estimated from small cases series, but difficulties with this approach include finding a suitable liver graft, managing the effects of multi-system organ failure and the preventing liver graft rejection [161]. Transjugular intrahepatic porto-systemic shunts (TIPS), which have been used successfully in patients with cirrhosis and bleeding esophageal

varices, ascites and Budd-Chiari syndrome, have also been tested to treat VOD after SCT [162–164]. This procedure involves creating a channel between the hepatic vein and the portal vein using a subcutaneously inserted catheter and maintaining the channel using a metal stent. Although the procedure has appeal because it does not require an open surgical procedure and any bleeding which results is intrahepatic, it probably has no value for patients whose VOD is not characterized by significant fluid retention and ascites. It is also limited in terms of long-term efficacy, as evidenced by the poor overall survival data in the patient series published to date [162–164]. Another modality, charcoal hemofiltration, capable of adsorbing bilirubin and other factors from the circulation, has been reported to be useful [165] and may be helpful as a supportive measure in selected patients.

Conclusion

Hepatic VOD is a manifestation of conditioning regimen-related toxicity in SCT (with a contribution from previous chemotherapy, including newer agents such as Mylotarg) and its incidence is likely increased by allogeneic effects between donor cells, cytokine release and recipient tissues. It is currently a major limiting factor for improving the efficacy of both auto-SCT and allo-SCT, and better methods of prophylaxis and treatment are urgently needed to overcome this much-feared complication. Prevention is clearly a priority, and efforts designed to identify at-risk patients, utilize pharmacokinetics to better individualize chemotherapy administration and prevent vascular and hepatocyte injury are ongoing. The treatment of severe VOD remains inadequate with a very high fatality rate. Current directions in the investigation of VOD therapy target endothelial injury. The use of rh-tPA in conjunction with heparin has been confounded by the risk of serious toxicity. An alternative novel agent, DF, has shown to be active with remarkably little toxicity, as confirmed in several prospective trials with efficacy currently under evaluation in the phase III setting. TIPS, charcoal hemofiltration and liver transplantation are other approaches currently under investigation in severe disease and may have adjunctive roles as better systemic approaches to treatment evolve.

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Chapter 22

Lung Injury after Hematopoietic Stem Cell Transplantation

Kenneth R. Cooke and Gregory Yanik

1. Introduction/Abstract

Allogeneic hematopoietic stem cell transplantation (SCT) is the only curative therapy for a number of malignant and nonmalignant conditions. Unfortunately, this treatment strategy is limited by several side effects including pulmonary toxicity. Despite significant advances in critical care and transplantation medicine, diffuse lung injury remains a significant problem following allogeneic SCT both in the immediate post-transplant period and in the months to years that follow; lung injury occurs in 25 percent to 55 percent of SCT recipients and can account for approximately 50 percent of transplant related mortality [1–6]. Noninfectious lung injury can be either acute (termed idiopathic pneumonia syndrome: IPS) or chronic depending upon onset after SCT and the tempo of disease progression. Historically, approximately one-half of all pneumonias seen after SCT have been secondary to infection, but the judicious use of broad-spectrum antimicrobial prophylaxis has more recently tipped the balance toward noninfectious causes [7]. Chronic lung injury (by definition occurring in patients over 100 days post-transplant) is further subdivided into two types: obstructive and restrictive [8–16]. Although noninfectious lung injury occasionally occurs following autologous transplants, the allogeneic setting significantly exacerbates toxicity in both the acute and chronic time frames; in each case, pulmonary injury is associated with significant morbidity and mortality and responds poorly to standard therapeutic approaches. Clinical and experimental data suggest that the immunologic mechanisms responsible for lung inflammation after allogeneic SCT are similar to those contributing to the development of Graft-versus-Host Disease (GVHD). This chapter will review the definitions, risk factors and pathogeneses of noninfectious lung injury occurring both early and late after allogeneic SCT.

2. Acute Lung Injury: Idiopathic Pneumonia Syndrome (IPS)

2.1. Definition, clinical course, and spectrum of disease

In 1993, IPS was defined as widespread alveolar injury following SCT that occurs in the absence of an active lower respiratory tract infection and

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cardiogenic causes [17]. This form of pulmonary inflammation was considered a clinical *syndrome,* with variable histopathologic correlates and several potential etiologies [17]. As shown in Table 22-1, diagnostic criteria of IPS include signs and symptoms of pneumonia, non-lobar radiographic infiltrates, abnormal pulmonary function and the absence of infectious organisms as determined by broncho-alveolar lavage (BAL) or lung biopsy [2–17]. A variety of histopathologic findings have been associated with IPS including diffuse alveolar damage with hyaline membranes, lymphocytic bronchitis and bronchiolitis obliterans organizing pneumonia (BOOP) [18]. Interstitial pneumonitis, a term historically used interchangeably with IPS, is the pattern reported most frequently [3, 19]. Interstitial pneumonitis is seen in association with diffuse alveolar damage and hemorrhage early after SCT and is accompanied by bronchiolar inflammation and epithelial damage at later timepoints [18].

The incidence of IPS in the first 120 days after allogeneic SCT with myeloablative conditioning ranges between 3 to 15 percent [5–7, 17, 20]. The median time of onset for IPS was initially reported to be six to seven weeks (range 14 to 90 days) after the infusion of donor stem cells [17], and mortality rates ranged from 50 percent to 80 percent overall and greater than 95 percent for patients requiring mechanical ventilation [1, 3, 5–7, 17, 20]. A retrospective study from Seattle showed a lower incidence and earlier onset of IPS than previously reported, but the typical clinical course involving the rapid onset of respiratory failure leading to death remained unchanged [6]. In a recent publication from the University of Michigan, the frequency of IPS after allogeneic SCT ranged from 5 percent to 25 percent depending upon donor source and the degree of antigenic mismatch between donor and recipient [21]. In this series, mortality was 80 percent, and the median time from diagnosis to death was 13 days despite aggressive treatment with high-dose steroids and broad-spectrum antimicrobial therapy.

The clinical spectrum of IPS encompasses several forms of pulmonary toxicity (Table 22-2). In one small subset of patients with IPS, acute pulmonary hemorrhage or hemorrhagic alveolitis occurs. Diffuse alveolar hemorrhage (DAH) generally develops in the immediate post-SCT period and is characterized by

Table 22-1. Definition of Idiopathic pneumonia syndrome.

Evidence of widespread lung injury:
(a) Multi-lobar infiltrates on chest radiograph or computed tomography
(b) Signs and symptoms of pneumonia (cough, dyspnea, rales)
(c) Abnormal pulmonary physiology: increased alveolar to arterial oxygen gradient; or new or increased restrictive lung findings
Absence of lower respiratory tract infection, based upon:
(a) Broncho-alveolar lavage negative for bacterial pathogens and/or lack of improvement with broad spectrum antibiotics
(b) Broncho-alveolar lavage negative for pathogenic non-bacterial micro-organisms
(c) Transbronchial lung biopsy if condition permits
(d) Ideally, second confirmatory negative test for infection two to 14 days following the initial procedure

Table 22-2. The spectrum of non-cardiogenic, pulmonary toxicity defined by IPS.

Interstitial pneumonitis (IP)

- Clinical symptoms: fever, cough, dyspnea, hypoxemia
- Onset: within first 100 days post-transplant
- Etiology: infectious (i.e., CMV, PCP) or noninfectious factors (chemo toxicity: BCNU, bleomycin, busulfan, methotrexate)
- Radiographic findings: bilateral interstitial infiltrates

Bronchiolitis obliterans syndrome (BOS)

- Clinical symptoms: cough, dyspnea, wheezing, lack of fever
- Pulmonary function testing: obstructive findings (diminished $FEV_{1.0}$, or $FEV_{1.0}$ / FVC)
- Onset: three to 24 months post-transplant
- Radiographic findings: hyperinflation; otherwise routinely normal
- Computed tomography (CT): bronchiectasis, centrilobular nodules, septal lines, ground glass appearance
- Histology: lymphocytic bronchitis Bronchiolar inflammation with luminal obliteration

Bronchiolitis obliterans organizing pneumonia (BOOP)

- Clinical symptoms: fever, dry cough, dyspnea
- Onset: two to 12 months post-transplant
- Radiographic findings: Patchy airspace disease, ground glass appearance, nodular opacities
- Histology: peribronchiolar infiltration and fibrosis and the presence of intraluminal granulation tissue

Diffuse alveolar hemorrhage (DAH)

- Clinical symptoms: Progressive dyspnea, cough, rare hemoptysis
- Key finding: progressively bloodier aliquots of lavage fluid
- Onset: early, within first 100 days post-transplant.
- Radiographic findings: diffuse infiltrates, central appearance initially noted
- Histology: Diffuse alveolar damage with alveolar hemorrhage

Peri-engraftment respiratory distress syndrome (PERDS)

- Clinical symptoms: fever, dyspnea, hypoxemia
- Onset: very early, within five to seven days of engraftment, classically after autologous SCT
- Radiographic findings: bilateral interstitial infiltrates

Delayed pulmonary toxicity syndrome (DPTS):

- Clinical symptoms: fever, dry cough, dyspnea
- Onset: late, months to years following autologous SCT for breast cancer
- Responds to corticosteroid therapy

Non-cardiogenic capillary leak syndrome (CLS)

- Clinical symptoms: dyspnea, cough, weight gain, edema
- Onset: early, within first 30 days post-transplant
- Radiographic findings: bilateral perihilar infiltrates, pulmonary edema, pleural effusions

progressive shortness of breath, cough and hypoxemia with or without fever [7, 22–24]. Classically, DAH is defined by progressively bloodier aliquots of BAL fluid, but frank hemoptysis is rare [22]. Mortality from DAH is as high as 75 percent despite aggressive treatment with high-dose (2 mg/kg to 1 gm/m^2) steroids, and death usually occurs within weeks of diagnosis [23]. Sloan and colleagues identified acute hemorrhagic pulmonary edema (the histologic correlate of DAH) postmortem in a subset of SCT recipients, 80 percent of which had received a non-HLA-identical SCT and had previous acute GVHD [25]. Similarly, a separate series reported that death from pulmonary hemorrhage was associated with Grade II or greater GVHD [26].

Peri-engraftment respiratory distress syndrome (PERDS) and delayed pulmonary toxicity syndrome (DPTS) also fall within the definition of IPS [7]. PERDS and DPTS typically occur after autologous SCT [7], and both are characterized by fever, dyspnea and hypoxemia [27–29]. By definition, PERDS occurs within five days of engraftment, whereas the onset of DPTS may be delayed for months and commonly occurs following conditioning regimens that contain cyclophosphamide, cisplatin and bischloroethylinitrosurea (BCNU) as used in SCT for breast cancer [29]. Although PERDS after autologous SCT appears similar to IPS after allogeneic SCT with respect to clinical presentation and time of onset, the two entities differ sharply with respect to overall outcome; injury from PERDS after autologous SCT, even when requiring mechanical support, responds promptly to corticosteroids and is associated with a favorable prognosis [27], whereas IPS occurring in an allogeneic environment responds poorly to standard therapy and results in rapid respiratory failure and death in the majority of patients [6, 21].

2.2. Risk Factors of IPS

As shown in Table 22-3, potential risk factors for IPS include conditioning with total body irradiation (TBI), acute GVHD, older recipient age, initial diagnosis of malignancy other than leukemia and the use of methotrexate (MTX) for GVHD prophylaxis [5, 19, 30, 31]. Moreover, the likelihood of developing IPS increases with the number of identified risk factors [3]. Although recipient age and the use of MTX are not always risk factors, the use of TBI and the development of acute GVHD have been identified as factors in multiple reports [2, 5, 6, 31–33]. Recently, the cumulative incidence of IPS within 120 days of SCT was found to be significantly lower after nonmyeloablative

Table 22-3. Risk factors for IPS.

Graft-versus-Host Disease prophylaxis (methotrexate)
Acute Graft-versus-Host Disease
Increasing recipient
Total body irradiation (≥ 1200 cGy)
Myeloablative conditioning
Decreased pre-transplant performance status
Longer duration from diagnosis to transplant
Transplantation for malignancy other than leukemia
HLA disparity (donor: recipient)

conditioning than was observed following conventional conditioning despite greater patient age and a similar incidence of acute GVHD [34]. Once established, however, pulmonary toxicity was severe in each group and resulted in respiratory failure in the majority of patients. These findings suggest that the intensity of SCT conditioning plays an important role in developing IPS, and they are consistent with data generated from two mouse SCT models showing that the lung is sensitive to the combined effects of radiation and alloreactive T cells [35, 36].

2.3. Etiology of IPS

The potential etiologies for IPS include direct toxic effects of SCT conditioning regimens, occult pulmonary infections and the release of inflammatory cytokines that have been implicated in other forms of pulmonary injury [37–41]. The association between IPS and severe GVHD reported in several large series [2, 3, 5–7, 20] suggest that immunologic factors may also be important. Acute GVHD often precedes IPS, suggesting a possible causal relationship between the two disorders [5, 19, 42, 43]. Although IPS can also occur when signs and symptoms of GVHD are limited or absent [44–47], the consistent association between lung injury and GVHD in experimental models also supports such an etiology [2, 3, 5, 6, 48–52].

Despite the aforementioned clinical association, the lung has not been traditionally recognized as a classic GVHD target organ, and the specific role of alloreactive donor T lymphocytes in the pathogenesis of IPS remains a topic of considerable debate. Epithelial apoptosis is usually attributed to T cell-mediated injury and is considered pathognomonic for acute GVHD. Although epithelial apoptosis has been identified in the lungs of some patients with IPS [18], it has not been consistently observed in allogeneic SCT recipients with pulmonary dysfunction [25, 43, 53, 54]. Lymphocytic bronchitis was, however, reported as a potential histopathologic correlate of GVHD of the lung [43]. This pattern was initially observed in allogeneic SCT recipients with GVHD, but not in patients receiving autologous SCT or in untransplanted controls. However this association was not confirmed in subsequent reports [25, 53, 54]. More recently described is a histologic spectrum of pulmonary GVHD that ranges from diffuse alveolar injury early after SCT to cicatrical bronchiolitis obliterans, a late and irreversible form of lung injury [18]. In this report, bronchitis/bronchiolitis with interstitial pneumonitis (BIP) was the most common finding and included a lymphocytic infiltration around bronchial structures, along with a mononuclear inflammation in the perivascular zones and alveolar septa. The heterogeneity of pulmonary histopathology after allogeneic SCT is complicated further by the nonspecific changes that occur after mechanical ventilation and by the limited quality and quantity of lung biopsy tissue. In spite of the heterogeneity that exists with respect to lung histology, several lines of evidence support the concept that the lung is the target of an immunologic attack after allogeneic SCT.

2.4. Animal Models of Human Disease

The relationship between alloreactivity and IPS has been explored by several laboratories. Rodent SCT models have consistently shown that animals with systemic GVHD develop lung injury, whereas syngeneic, non-GVHD controls

do not [48, 51, 55, 56]. Several patterns of lung injury have been identified including acute hemorrhagic alveolitis, late onset interstitial pneumonitis and lymphocytic bronchiolitis [48]. In several models where the GVHD reaction is induced to 1) minor H antigens, 2) class I or class II MHC antigens only or 3) both major and minor H antigens, two major abnormalities are apparent after allogeneic SCT: a dense mononuclear cell infiltrate around both pulmonary vessels and bronchioles, and an acute pneumonitis involving the interstitium and alveolar spaces [51, 57, 58]. The alveolar infiltrate is composed of macrophages, lymphocytes, epithelial cells and scattered polymorphonuclear cells within a fibrin matrix. Both patterns of inflammation closely resemble those reported in allogeneic SCT recipients [17, 18, 25, 43]. However, evidence for diffuse alveolar injury, including alveolar hemorrhage, edema and hyaline membranes has not been demonstrated in these models.

Lung histopathology is associated with alterations in pulmonary function demonstrating that the observed lung pathology was physiologically relevant [52, 56]. Mice with IPS have significant reductions in both dynamic compliance and airway conductance consistent with changes expected from both the interstitial and peribronchial infiltrates [52]. Furthermore, lung injury correlates with the presence, but not the severity, of GVHD which is consistent with clinical reports of IPS in allogeneic SCT recipients whose signs and symptoms of GVHD were mild or absent [8, 9, 21, 46, 59, 60]. Thus, mouse models of IPS reproduce histologic and functional changes observed during human disease.

2.5. The Pathogenesis of IPS

2.5.1. The Inflammatory Effectors TNFα and LPS

The mixed inflammatory alveolar infiltrates found in mice with IPS are accompanied by significant increases in the total number of lymphocytes, macrophages and neutrophils in the BAL fluid [51]. Cellular inflammation is also associated with increased TNFα levels in both lung tissue and BAL fluid [49–51, 61, 62]. The presence of neutrophils and TNFα in the absence of infection suggests that endogenous endotoxin (LPS) might also contribute to IPS pathophysiology. LPS is a component of the innate immune response and a potent enhancer of inflammatory cytokine release. In non-SCT experimental models, intratracheal administration of LPS elicits a severe, acute inflammatory response in the lungs of animals [63–65]. LPS is also an important effector molecule in the development of acute GVHD; translocation of LPS across a gut mucosa damaged early after allogeneic SCT provides access to the systemic circulation where it stimulates the release of inflammatory mediators that contribute to GVHD target organ damage and dysfunction [66–72]. LPS levels are elevated in the BAL fluid of mice with IPS and the intravenous administration of LPS injection significantly amplifies lung injury in mice with advanced GVHD [51]. The enhanced inflammation is associated with large increases in BAL fluid levels of TNFα and LPS and the development of alveolar hemorrhage [51, 62]. Furthermore, direct antagonism of LPS early in the time course of SCT reduces systemic and BAL fluid levels of TNFα and significantly decreases the severity of IPS compared to control-treated animals [68] (K.R. Cooke, unpublished observation).

Strategies that block TNFα either by neutralizing its effects [61, 62] or by using TNFα–deficient mice as SCT donors [73–74] have established a causal

role for this cytokine in the development of IPS. Administering rhTNFR:Fc (Amgen, Thousand Oaks, CA), a soluble, dimeric, TNF binding protein, at the time of LPS challenge effectively prevents increases in pathology, BAL fluid cellularity and LPS levels, confirming the linkage between LPS and TNFα in this setting [62]. Neutralizing TNFα during the natural development of IPS also reduced the severity of lung inflammation in the absence of LPS challenge [62]. Experiments using genetically altered mice have shown that IPS is dependent upon donor-, rather than host-, derived TNFα and that cytokine production from both donor accessory cells (macrophage/monocytes) and T cells significantly contributes to this toxicity [74]. Moreover, donor-derived TNFα serves as both a facilitator and effector of lung injury. TNFα secreted by donor T cells regulates the chemokine milieu in the lung within the first two weeks after BMT, which directly contributes to the subsequent recruitment of monocytes and macrophages as lung injury progresses [74]. The incomplete protection provided by TNFα neutralization after SCT is consistent with reports from many groups [48, 61, 71, 72, 75–77] and suggests that other inflammatory and cellular mechanisms, such as the Fas-FasL pathway that mediate acute GVHD, may also contribute to the development of IPS [75, 78, 79]. For example, IL-1β, TGFβ and nitrating species including nitric oxide and peroxynitrite have also been implicated in the development of IPS, particularly when cyclophosphamide is included in the conditioning regimen [56, 80, 81].

TNFα likely contributes to the development of IPS through both direct and indirect mechanisms. In addition to being directly cytotoxic, TNFα increases expression of inflammatory chemokines [74] and MHC antigens, modulates leukocyte migration and facilitates cell-mediated cytotoxicity [61, 82]. TNFα may also contribute to lung injury by increasing the severity of GVHD in other target organs such as the gut and liver, thus promoting the release of other inflammatory mediators and their ultimate passage to the pulmonary vascular bed. The ability of TNFα neutralization to reduce BAL fluid LPS levels after the systemic administration of endotoxin strongly suggests that rhTNFR:Fc alters the systemic inflammatory response to LPS "up-stream" from the lung, in addition to directly neutralizing TNFα in the alveolar space [63]. Because damage to the GI tract precedes lung injury in these allogeneic SCT models, we hypothesize that inflammatory mediators (i.e., LPS and TNFα) released in response to GI GVHD later contribute to the development of IPS. From this perspective, the structural and functional integrity of the liver is critical. The liver is pivotally located between the intestinal reservoir of gram-negative bacteria and their toxic by-products, and the rich capillary network in the lung. Kupffer cells in the liver detoxify and subsequently clear endotoxin from the systemic circulation [83] and protect the lung in experimental models of sepsis and ARDS [84, 85]. If the capacity of the liver to clear an endotoxin challenge is exceeded, both inflammatory cytokines and unprocessed LPS can enter into the systemic circulation and damage other organs. Several experimental studies have shown that preexisting injury to the liver decreases its ability to neutralize endotoxins effectively [86–89]. In the setting of acute GVHD, an endotoxin surging into the systemic bloodstream can arise from increased leakage of LPS across damaged intestinal mucosa. In this scenario, underlying damage to the liver could decrease its capacity for LPS uptake and clearance. Animals with mild or no GVHD can effectively detoxify endotoxin and

protect their lungs from further damage, whereas mice with severe GVHD are unable to do so and develop severe extensive lung injury, including alveolar hemorrhage [51].

Collectively, these data demonstrate that the inflammatory mediators TNFα and LPS both contribute to experimental IPS. Moreover, they support the hypothesis of a "gut-liver-lung" axis of inflammation in IPS pathophysiology (**Fig. 22-1**). Any process that ultimately results in excessive amounts of endotoxin and/or TNFα in the pulmonary circulation may contribute to the development of lung injury in this setting. This hypothesis is consistent with the observation that

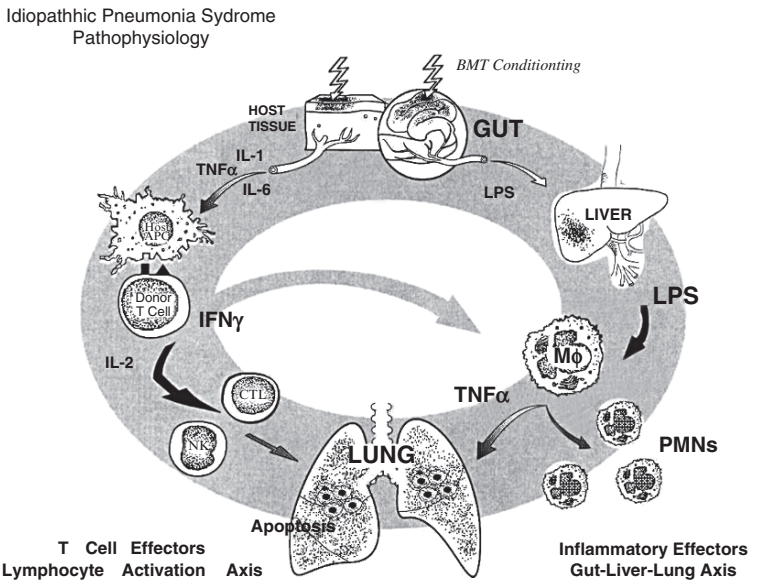

Fig. 22-1. Pathophysiology of IPS after allogeneic SCT
Data generated using murine SCT models have been incorporated into a working hypothesis of IPS physiology. This schema postulates that the lung is susceptible to two distinct, but interrelated pathways of immune mediated injury that occur along a T lymphocyte activation axis and a "gut-liver-lung" axis of inflammation. Chemoradiotherapy of SCT conditioning causes TNFα and IL-1 release that enhance the ability of host APC to present alloantigens to mature donor T cells and upregulate chemokine expression in the lung. Once engaged, donor T cells become activated and secrete IFNγ and IL-2. IFNγ primes donor macrophages (Mϕ) and monocytes, whereas IL-2 facilitates T cell activation and the generation of Th1 lymphocyte effectors that migrate to the lung early after SCT in response to inflammatory chemokine gradients and contribute to pulmonary toxicity via Fas-FasL mediated cell killing. The inflammatory axis focuses on the relationship between the cellular activating effects of LPS and the downstream production of TNFα as it occurs along a gut-liver-lung axis of inflammation. LPS enters the systemic circulation through gaps in the intestinal mucosa. The ability of systemic endotoxin to reach the alveolar space is related to the consequences of GVHD in other target organs, particularly the liver, which is pivotally located immediately downstream (via the splanchnic circulation) of the intestinal reservoir of gram-negative bacteria and their toxic by-products. Underlying liver damage from hepatic GVHD decreases the liver's capacity for LPS uptake and clearance, and allows passage of LPS into the pulmonary circulation. Donor macrophages primed by IFNγ are recruited to the lung where they are triggered by LPS to secrete inflammatory cytokines like TNFα, resulting in enhanced chemokine expression, the recruitment of neutrophils to the lung and increased tissue damage

serum TNFα levels are increased in patients with IPS [90]. A clinical linkage of hepatic dysfunction to lung injury after SCT is also suggested by associations between VOD and IPS, and between hepatic failure and death from IPS [2, 21]. Furthermore, evidence of cytokine activation and LPS amplification observed in the BAL fluid of ARDS patients [91] has been demonstrated in patients with IPS after allogeneic SCT; increased pulmonary vascular permeability and increases in BAL fluid levels of IL-1, IL-12, IL-6, TNFα, LPS binding protein (LBP) and soluble CD14 were also observed in these patients [1].

2.5.2. Cellular Effectors and the Development of IPS

The Role of Donor-Derived T cell Effectors : Although the induction of GVHD fundamentally depends upon interactions between donor T cells and host antigen-presenting cells (APCs) [92], the role of alloreactive donor T cells in the pathogenesis of IPS has been a topic of considerable debate. Pulmonary endothelial and epithelial cells can express MHC Class I, MHC Class II and minor histocompatibility (H) antigens, and the expression of these molecules on vascular endothelium is enhanced by TNFα and IFNγ [93]. It is conceivable, therefore, that pulmonary parenchymal cells can serve as targets for direct cell mediated damage. The recovery of donor-specific, alloreactive T cells in the BAL fluid of heart-lung transplant recipients heralds the onset of acute rejection and provides support for cell mediated lung injury [94]. The importance of lymphocytes to lung injury after experimental SCT has been shown by several groups [49, 56, 95, 96]. Donor T cells are critical to the early proinflammatory events associated with lung injury that develops within the first week of SCT across MHC antigens, whereas in minor H antigen mismatch systems, donor lymphocytes continue to respond to host antigens and contribute to physiologically significant lung injury at later timepoints [52, 56]. Donor T cell clones that recognize CD45 polymorphisms result in a rapidly progressive pulmonary vasculitis within three days of their injection into non-irradiated recipients [49, 96]. The origin and functional capacity of T cells infiltrating the lung have been examined by using differences in the T cell Vβ repertoire between donor and recipient [52]. Flow cytometry demonstrated that the TCRαβ+ T cells found in the lung six weeks after allogeneic SCT were of donor origin. When these donor-derived T cells were re-cultured with irradiated host APCs, they proliferated vigorously and produced significant amounts of IFNγ [52]. Similar results have been obtained using a Parent into F1 (P → F1) mouse model and congenic SCT donors expressing allelic differences of CD45 on all leukocytes. Greater than 95 percent of CD4+ and CD8+ lymphocytes in the broncho-alveolar space were of donor origin by the first week and turnover was complete by week 2 after SCT. A significant proportion of these donor T cells secreted IFNγ and TNFα [74]. Comparable findings were observed for lymphocytes obtained from whole lung digests. These experimental data support the hypothesis that the alveolar lymphocytosis associated with IPS after clinical SCT represents a pulmonary manifestation of GVHD [97].

Both cytotoxic T lymphocytes (CTL) and natural killer (NK) cells may play a role in IPS. Gartner and colleagues showed that pulmonary NK cell activity remained increased over an extended period of time during GVHD, in contrast to the transient and mild increase in NK activity that occurred in the spleen during the same interval [98]. Donor CTL effectors can contribute to lung

injury via two primary cytolytic mechanisms: the perforin-granzyme pathway and the Fas-Fas ligand (FasL) pathway [99–102]. During GVHD, CD4+ T cells primarily use the Fas pathway [103], whereas CD8+ CTL can kill using both perforin and Fas pathways [78, 104]. Each cytolytic pathway can play a role in the development of lung injury in non-SCT models [105–108]. Cytolytic T cells expressing granzyme B are present in the lungs of mice after a fully mismatched allogeneic SCT, and they co-localize with macrophages expressing the costimulatory molecules B7.1 (DC80) and B7.2 (CD86). Pretreating allogeneic SCT recipients with keratinocyte growth factor (KGF) has been shown to decrease B7 and granzyme B expression and hasten repair in the lungs of mice with IPS [109]. Alloantigen-specific killing by donor T cells using both perforin and Fas/FasL pathways has also been identified in the lung after SCT. CTL activity is present as early as week 2 after transplant and persists over time, and in a P → F1 system was primarily mediated by the Fas-FasL pathway [57].

Despite these compelling data supporting a role for alloreactive donor lymphocytes in the development of noninfectious lung injury after SCT, IPS has been reported in patients in whom systemic GVHD is mild or absent, making a causal relationship between the two entities difficult to establish [8, 9, 18, 46, 59, 60]. The relationship between lung injury and GVHD severity has been examined in an SCT model across minor H antigens. T cell depletion (TCD) at the time of allogeneic SCT reduced the number of T cells by greater than 99 percent and eliminated evidence of clinical or histologic GVHD. Nevertheless, significant lung injury was noted after allogeneic TCD SCT and donor lymphocytes reactive to host antigens were present in the BAL fluid, but not the spleens, of these animals [52]. This intriguing result suggested that the lung may be particularly sensitive to the effects of small numbers of host-reactive donor T cells even when systemic tolerance has been established. Consistent with these findings, BAL fluid lymphocytosis has been described after TCD SCT in association with pneumonitis that resulted from a local immune response; pulmonary T cells appeared to be activated despite systemic immune suppression [44]. Furthermore, pulmonary toxicity has been reported after non-conditioned allogeneic SCT for severe combined immunodeficiency (SCID) where donor lymphocytes were noted in the lung during a period of rapid engraftment without evidence of systemic GVHD. Suppression of cellular immunity with high-dose methylprednisolone resulted in complete resolution of lung disease [11].

The Role of Antigen-presenting Presenting Cells: Taken together, these data suggest that donor-derived, IFNγ secreting T cells home to the lungs early after SCT, persistently respond to host antigens and can cause clinically and histologically significant tissue injury, even when systemic GVHD is limited or absent. However, the precise mechanisms by which these cells interact with host antigens and cause injury remain unresolved. This process is likely to be complex and to involve interactions with pulmonary antigen-presenting cells (APCs) [111, 112]. The pulmonary dendritic cell (DC) is significantly more efficient than the alveolar macrophage (AM) in antigen presentation, and functions as the dominant APC in the lung [113–115]. Pulmonary DCs are located in the pulmonary interstitium and in the bronchial epithelium and submucosa where DC tissue density diminishes with decreasing airway diameter [116]. During steady state conditions, pulmonary DCs constitute the sole source

of MHC class II expression within the epithelial lining of the airway [117]. Lung DCs serve as sentinels at the epithelial surface of the airways. DCs can efficiently internalize and process antigens at this location, but paradoxically, their capacity to present antigen and to stimulate T cells is limited. DCs must migrate to regional lymph nodes where they become potent APCs, but at this point their capacity to process antigen diminishes. This maturation process is associated with upregulation of costimulatory adhesion and MHC class II antigens [114]. The antigen-presenting capacity of pulmonary DCs is regulated by AMs which exist in close proximity to DCs in both the airway and lung parenchyma, and this process likely involves the secretion of soluble factors like nitric oxide; adding AMs to *in vitro* DC cultures enhances this regulatory effect, whereas depleting AMs *in vivo* enhances the APC function of freshly isolated DCs [117].

Pulmonary dendritic cells play a critical role in the initiation and regulation of immune responses in the lung, and recent data suggest that they are important to both acute and chronic rejection of lung allografts [115, 118–120]. A $CD8^+$ T cell driven GVHD model emphasized the need for host APCs to generate acute GVHD [92]. These results were recently extended by Teshima and colleagues who showed that alloantigen expression on host APCs alone is both necessary and sufficient to induce a graft-versus-host reaction, and that GVHD target organ damage can be mediated by inflammatory cytokines [121]. Radioresistant host DCs may persist longer in the lung than in other organs and allow for sustained presentation of host antigens in that organ. In experiments using congenic rats, host DC populations in tracheal epithelium were depleted by 80 percent three days after 1,000 cGy of TBI and were completely eliminated 14 days after syngeneic SCT. By contrast, lung parenchymal DCs were only reduced by 50 percent at day 3 and 60 percent by day 6, and declined at a rate that was intermediate between airway and epidermal DC populations [122]. Activated donor T cells might, therefore, remain within the pulmonary microvascular circulation because persistent host DCs function as a continuing site of alloantigen presentation. This scenario could account for the apparent "sanctuary" status of the lung with respect to alloreactive donor T cells, and may have important implications with regard to the evaluation and treatment of IPS after allogeneic SCT, even when clinical GVHD is absent.

It is also possible that donor T cells responsible for IPS initially encounter host APCs residing in more distant secondary lymphoid tissues and are later specifically recruited to the lung. Recent experimental data demonstrate that the subepithelial dome of intestinal Peyer's patches (PPs) is required for the generations of antihost CTL and the resultant induction of acute GVHD [123]. Interrupting donor T cell – host APC interactions at this site by using CCR5 deficient SCT donors, monoclonal antibodies to MAdCAM-1 or PP deficient mice as SCT recipients prevented lethal GVHD resulting from marrow aplasia. However, GVHD target organ histopathology was not examined. These findings suggest PPs are essential for initiating acute GVHD, but whether they are critical for the generation of donor effectors that contribute to damage in target organs, including the lung, must still be evaluated.

The Role of Donor Accessory Cells in the Development of IPS: Experimental data suggest that synergistic interactions between cells from the lymphoid and myeloid lineage are critical to the development of GVHD [67, 68, 72].

Specifically, the production of IFNγ from activated donor T cells primes mononuclear cells and macrophages to secrete cytopathic amounts of TNFα when stimulated with LPS [66]. The contribution of donor accessory cells (monocytes/macrophages) to IPS has been investigated using several models. Kinetic studies of macrophage recruitment to the lung after allogeneic SCT show that the percentage of donor macrophages in the BAL fluid increases from approximately 40 percent at week 1 to > 90 percent by week 4. Additional experiments showed that these donor-derived macrophages are a significant, albeit not the primary, source of TNFα after SCT [74].

The role of accessory cell populations in the pathophysiology of IPS was further examined using SCT donors that differ in their response to LPS [124]. A genetic mutation in the Toll-like receptor 4 (Tlr 4) gene makes C3H/Hej mice resistant to LPS (LPS-r) [124–127]. SCT from LPS-r donors results in a significant decrease in lung injury when compared to SCT using wild type, LPS-sensitive (LPS-s) donors, even though T cell responses to host antigens are identical between the two donor strains [72]. Recipients of LPS-r SCT also develop significantly less GVHD. Furthermore, BAL cells collected from LPS-r recipients produce 30-fold less TNFα when re-stimulated with LPS compared to cells collected after LPS-s SCT, reproducing the phenotype of naïve LPS-r and LPS-s donor cells [73, 128]. Similar findings are observed when cells from SCT donor mice lack CD14 on the cell surface. These results are consistent with the report that monocytes recruited to an inflamed lung upregulate CD14 expression and show enhanced sensitivity to LPS stimulation [129], and with the clinical observation that components of the LPS activating system are elevated in the BAL fluid of SCT patients with IPS [1]. Collectively, these data demonstrate that donor macrophages/monocytes cells are recruited to the lungs of allogeneic SCT recipients, and their secretion of TNFα in response to LPS stimulation directly correlates with IPS severity. Strategies that disrupt the innate immune response by targeting interactions between CD14/Tlr4 and LPS may, therefore, reduce the severity of IPS or prevent its development.

The Role of Neutrophils / Polymorphonuclear (PMN) Cells: Neutrophils are a major component of the inflammatory infiltrates seen in animals with IPS [51]. Neutrophilia is a prominent finding in acute respiratory distress syndrome (ARDS) and in bronchiolitis obliterans syndrome (BOS) which is characteristic of lung allograft rejection [130–135]. PMN products such as elastase, myeloperoxidase, metalloproteinases and oxidants are abundant in the BAL fluid of patients with ARDS and are believed to contribute to the endothelial and epithelial damage that occur in this setting [130, 135]. Increases in PMN activation markers may also be early indicators of BOS after lung transplant [132]. Neutrophils are likely to play a role in lung injury after SCT as well; their appearance in the bloodstream is often temporarily associated with the onset of lung injury [21]. In mouse IPS models, the influx of neutrophils into BAL fluid is prominent between weeks four and six after SCT, and is associated with increases in BAL fluid levels of TNFα and LPS [51, 62]. Neutralizing TNFα with rhTNFR:Fc during this time interval prevents the influx of neutrophils and reduces the progression of lung injury and dysfunction [62]. Administering rhTNFR:Fc following LPS challenge completely abrogates the influx of PMNs into the lungs and prevents further damage (including hemorrhage), underscoring the relationship between neutrophils, TNFα and LPS in this setting.

2.5.3. Mechanisms of Leukocyte Recruitment to the Lung During IPS

Cellular effectors play a significant role in the development of IPS, but the molecular mechanisms by which white blood cells (WBCs) traffic to the lung and cause damage have yet to be fully elucidated. WBC migration to sites of inflammation is a complex process involving interactions between leukocytes and endothelial cells that are mediated by adhesion molecules, chemokines and their receptors [136, 137]. The recruitment of leukocytes from the vascular space and into target tissue can be divided into four steps: 1) weak adhesion of WBCs to the vascular endothelium, 2) firm adhesion of WBCs to endothelial cells, 3) transmigration of leukocytes through the vascular wall and 4) migration of cells through the extracellular matrix along a chemotactic gradient.

Adhesion Molecules and IPS: Selectins and integrins are families of adhesion molecules that are critical to steps one and two, respectively, and the expression of each is enhanced by proinflammatory cytokines like TNFα. Selectins function to slow the passage of WBCs in the main flow of blood. Selectin interactions do not firmly anchor leukocytes, but facilitate their rolling along the endothelial surface [137], whereas firm adhesion of WBCs is dependent on the interaction between intercellular adhesion molecule-1 (ICAM-1) and leukocyte function antigen-1 (LFA-1). The role of adhesion molecules in the development of IPS has been examined using mouse models [58, 138, 139], and mRNA expression of ICAM, VCAM and E-selectin is increased in the lungs of mice with IPS after allogeneic SCT compared to syngeneic controls [139]. Furthermore, the severity of IPS is dramatically reduced when ICAM-deficient (ICAM –/–) mice are used as SCT recipients of either MHC-matched or -mismatched allogeneic donor cells [58, 138]. Surprisingly, in each scenario, ICAM deficiency selectively protects the lung from injury even though ICAM expression is elevated in the liver, colon and spleen of animals with acute GVHD [140].

Chemokines and IPS: Chemokines are a subset of chemoattractant molecules that contribute to steps 2, 3 and 4 of leukocyte migration. Chemokines secreted at the site of tissue injury are retained within the extracellular matrix and on the surface of the overlying endothelial cells [141]. Leukocyte rolling, facilitated by selectin molecules, brings WBCs into contact with chemokines present on the endothelial surface. Chemokine signaling activates leukocyte integrin molecules resulting in arrest and extravasation. Once through the vascular wall, the WBC enters the tissue space where it is exposed to an existing chemokine concentration gradient surrounding the inflammatory stimulus.

Chemokines are classified into four main groups according to the configuration of cysteine residues near the NH2-terminus, and their effects are mediated through a family of seven-transmembrane-spanning, G protein coupled receptors. Chemokines and their receptors can be classified functionally into two broad categories: “inflammatory” and “homeostatic.” Inflammatory chemokines orchestrate the recruitment of leukocytes to sites of inflammation during an immunologic challenge, whereas homeostatic chemokines contribute to the development of secondary lymphoid organs during organogenesis and are responsible for leukocyte migration during routine immune surveillance. Inflammatory chemokines are produced by a variety of hematopoietic and nonhematopoietic cells, and their expression is augmented by molecules such as LPS, IL-1 and TNFα [136]. Receptors for inflammatory chemokines tend

to bind in a more promiscuous or redundant fashion than homeostatic receptors, and are generally expressed on cells with an effector rather than resting phenotype [142].

Chemokines facilitate the recruitment of leukocytes to the lung in a variety of inflammatory states including asthma, ARDS, infectious pneumonia, pulmonary fibrosis and lung allograft rejection [142, 143], and investigators have recently begun to explore their role in IPS. The specific composition of a pulmonary leukocytic infiltrate is determined by the pattern of chemokine expression in the inflamed lung. The mixed pulmonary infiltrate observed after experimental allogeneic SCT, therefore, suggests that those chemokines responsible for the recruitment of lymphocytes, monocytes and neutrophils will be upregulated during the development of IPS.

The pulmonary expression of four inflammatory chemokine receptors of T cell effectors (CCR1 and CXCR3), monocytes and macrophages (CCR2) and neutrophils (CXCR2) and their respective ligands have been analyzed using an irradiated P $\rightarrow$ F1 (C57BL/6 $\rightarrow$ B6D2F1) SCT model wherein IPS develops in response to both minor and major HC antigens. Lung injury in this model is present at week 2 after allogeneic SCT and progresses steadily through week 6. Whole lung mRNA expression was determined at week 1, 2, 4 and 6 after SCT using RNAse protection assays [146]. mRNA expression of each chemokine receptor and ligand is increased in allogeneic SCT recipients, compared to syngeneic controls at every timepoint. Elevations in CCR5 and CXCR3 expression peak early after allogeneic SCT, whereas expression of CCR2 and CXCR2 continue to rise over time. Importantly, the kinetics of chemokine ligand expression correlated with the corresponding receptors; increases in RANTES, MIP-1α (CCR1) and IP-10 (CXCR3) peaked at week 1 and then tapered off, whereas increases in MCP-1 (CCR2) and MIP-2 (CXCR2) peaked at weeks 2 and 4, respectively. These data are consistent with the findings of Panoskaltis-Mortari and co-workers who reported that enhanced expression of monocyte and T cell attracting chemokines in the lungs correlated with lung injury that developed within the first two weeks after SCT [144], and with prominent increases in the expression of IFNγ-inducible chemokines observed in a model of lung injury induced by alloreactive Th-1 cells [145].

Significant increases in chemokine ligand expression in the lung, therefore, precede the development of IPS and herald the influx of leukocyte subsets bearing the corresponding chemokine receptors. Inflammatory chemokine expression is induced by a variety of mediators of the immune response including cytokines and LPS which are also critical to the induction of acute GVHD. We have previously shown that serum levels of IFNγ, TNFα, IL-1 and LPS are significantly increased within the first week after SCT. Thus, upregulation of pulmonary chemokine expression early after SCT occurs in the setting of systemic inflammation. We hypothesize that enhanced chemokine expression and leukocyte infiltration that occurs during the development of IPS can be conceptualized in three distinct steps. These steps describe the kinetics of chemokine receptor expression on activated donor-derived, cellular effectors. In **step 1** of the process, SCT conditioning and the initial allogeneic donor T cell response result in systemic inflammation within seven days of transplant. This proinflammatory environment is characterized by the release of IFNγ, TNFα and LPS, and causes increased chemokine expression in the lung. Increased production of RANTES, MIP-1α and IP-10 recruit donor-derived,

Th1 lymphocyte effectors (CCR1+ / CXCR3+) from day 7 to 14 after SCT. In **step 2**, these T cells proliferate, cause local tissue injury and activate endothelial and epithelial cells to secrete additional chemokines, including MCP-1. Enhanced expression of MCP-1, in turn, recruits CCR2+ donor monocytes, macrophages and additional T cells. In **step 3,** recruited macrophages secrete TNFα in response to LPS stimulation, which results in additional tissue injury, the upregulation of KC and MIP-2 and the recruitment of donor neutrophils. These neutrophils amplify progressive lung injury and dysfunction. This hypothesis stipulates that the migration of each leukocyte subset controls the recruitment of the next wave of effectors. Thus, the recruitment of Th-1 effectors initiates the cascade, and interruption of this first step will have the most profound effect on the development of IPS, whereas prevention of steps 2 or 3 will result in significant, but less complete, amelioration of disease.

Data in support of this hypothesis have recently been generated using CCR2 deficient mice as donors in the B6 → B6D2F1 system described above [146]. CCR2 –/– SCT recipients developed significantly less severe IPS compared to mice receiving allogeneic SCT from wild type donors. Mild lung injury was identified in all animals at three weeks after SCT, consistent with a normal step 1 in both groups. However, the absence of CCR2 on engrafting donor leukocytes completely prevented the increase in lung pathology seen by week 6 in the wild type controls. The reduction in histopathology after CCR2 –/– SCT was associated with decreased macrophages, CD8+ lymphocytes and TNFα levels in the BAL fluid. Similar findings were observed when recipients of wild type SCT were treated with polyclonal antibodies to MCP-1 from day 10 to 28 after transplant. Thus, interruption of CCR2:MCP-1 receptor:ligand interactions significantly impaired the recruitment of cellular effectors to the lung, reduced TNF secretion in the broncho-alveolar space and abrogated the progression pulmonary toxicity after allogeneic SCT. Importantly, experimental data correlated with preliminary clinical findings; patients with IPS have elevated levels of MCP-1 in the BAL fluid at the time of diagnosis [146].

Using the lethally irradiated haploidentical murine model of IPS, investigators also found augmented lung expression of CXCL10 (IP-10) and CXCL11 (I-TAC) that paralleled mononuclear cell recruitment during IPS. Further support of the three-step hypothesis for cellular recruitment is provided by a study using an irradiated murine MHC class I mismatched model wherein IPS and GVHD are mediated by CD8+ T cells. Elevated levels of CXCL9 (MIG) and CXCL10 correlated with the recruitment of CXCR3 expressing $CD8^+$ T cells to the lung as early as day seven after SCT, and *in vivo* neutralization of CXCL9 or CXCL10, or HSCT using $CXCR3^{-/-}$ donor leukocytes resulted in a near complete abrogation of infiltrating $CD8^+$ T cells and IPS severity [147].

2.6. Treatment Strategies for IPS

As shown in Table 22-4, current standard treatment regimens for IPS include supportive care measures in conjunction with broad-spectrum antimicrobial agents with or without intravenous corticosteroids [6, 21]. Although anecdotal reports of responses to standard therapy are available, such responses are limited and the mortality of patients diagnosed with IPS remains unacceptably high [7]. Advances in supportive care, including the early institution of continuous veno-venous hemofiltration, may help to improve survival in some patients, but

Table 22-4. Treatment options for IPS.

Supportive therapy:
• Supplemental oxygen, mechanical ventilation
• Empiric broad-spectrum antimicrobial agents pending culture results
• Management of iatrogenic fluid overload
• Continuous veno-venous hemofiltration (CVVH)
Immunosuppressive therapy:
• Corticosteroids (2 mg/kg/day)
• Investigational: cytokine inhibitors, including anti-TNF agents

prospective studies addressing the treatment of IPS, including the specific use of steroids, are lacking in the literature. Recent reports from the University of Michigan and the Dana Farber Cancer Institute suggest that etanercept (Enbrel, Amgen Corp, Thousand Oaks, CA) may be a useful therapeutic option for IPS [21]. Etanercept was administered in combination with systemic steroids and empiric broad-spectrum antimicrobial therapy to a total of 18 patients with IPS [148]. In all patients, BAL fluid was negative for infection and pulmonary edema from fluid overload or cardiogenic factors was also ruled out before the administration of etanercept. Etanercept was given subcutaneously at a dose of 0.4 mg/kg twice weekly for a maximum of eight doses. Therapy was well tolerated overall. Thirteen of 18 patients were able to completely withdraw from supplemental oxygen support within 28 days of therapy. Survival at day 28 and day 56 (from the first etanercept dose) was 73 percent and 60 percent, respectively. Based upon these encouraging results, larger phase II (pediatric) and phase III (adult) trials are currently open and enrolling patients.

2.7. Summary

IPS remains a frequent and severe complication of allogeneic SCT and, despite significant advances in critical care medicine, mortality rates remain unacceptably high with standard therapy. Extensive preclinical and clinical data suggest that both inflammatory and cellular effectors participate in the development of IPS after allogeneic SCT. TNFα and LPS are significant, albeit not exclusive, contributors to IPS, and cells of both lymphoid and myeloid origin play a direct role in lung injury that occurs in this setting. In particular, the contribution of donor, nonlymphoid, accessory cells may be linked to the cellular activation by LPS and the ultimate secretion of TNFα within a "gut-liver lung" axis of inflammation, whereas donor T cell effectors can home to and damage the lung even when systemic GVHD is mild or absent. These findings have led to the hypothesis that the lung is susceptible to two distinct, but interrelated pathways of injury after SCT involving aspects of both the adaptive and the innate immune response (**Fig. 22-1**). In step 1, interactions between host APC and donor T cells result in the generation of T1 CTL that express CCR1 and CXCR3. This effector pathway is augmented by two related steps wherein the expression of CCR2 and CXCR2 contribute to the sequential recruitment of donor-derived monocytes/macrophages followed by neutrophils, respectively, during the development of IPS. These findings support a shift away from the

current paradigm of acute lung injury after SCT as an idiopathic clinical syndrome and to a process in which the lung is the target of an alloantigen-specific, immune-mediated attack. Mechanistic insights from these experimental models are forming the basis for translational clinical research protocols directed toward treating or preventing IPS.

3. Chronic Pulmonary Dysfunction after SCT: Obstructive and Restrictive Lung Disease

3.1. Definition, Risk Factors and Clinical Course

Two forms of chronic pulmonary dysfunction are common in patients surviving greater than 100 days post-transplant: obstructive lung disease (OLD) and restrictive lung disease (RLD) [8, 9, 14, 60]. The incidence of both patterns of lung toxicity ranges from 20 percent to 50 percent depending upon donor source and the time interval after SCT [8–15, 46]. In each scenario, collagen deposition and the development of fibrosis either in the interstitial (RLD) or peri-bronchiolar space (OLD) are believed to contribute to the patterns of lung dysfunction displayed on pulmonary function testing. While both forms of pulmonary dysfunction exist as late-onset, noninfectious lung complications following allogeneic SCT, RLD and OLD can be distinguished by a number of clinical parameters as described below (Table 22-5).

Restrictive Lung Disease: RLD is defined by reductions in forced vital capacity (FVC), total lung capacity (TLC) and diffusion capacity of the lung for carbon monoxide (DLCO) as measured by standard pulmonary function tests (PFTs). In restrictive disease, the ratio of the forced expiratory volume in one second (FEV_1) to FVC (FEV_1/FVC) is maintained near 100 percent [7, 10, 14, 60]. RLD is common after SCT; significant decreases in FVC or TLC have been reported in as many as 25 percent to 45 percent of allogeneic SCT recipients at day 100 and occur with greater frequency than obstructive abnormalities at this time [10, 14, 15]. An increase in nonrelapse mortality has been associated with a decline in TLC or FVC at 100 days, and one year

Table 22-5. Clinical factors present in OLD versus RLD.

Clinical Factor	Obstructive Lung Disease	Restrictive Lung Disease
Onset	Late (3 to 12 months)	Early (within 3 months)
Symptoms	Dyspnea, non-productive cough	Dyspnea, non-productive cough
Physical exam	Wheezing	Rales
PFTs	Obstructive physiology	Restrictive physiology
FEV1/FVC	Decreased	Normal
TLC	Normal	Decreased
DLco	Decreased	Decreased
Cat Scan findings	Air trapping	patchy consolidation: BOOP
	Bronchial wall thickening	"Ground glass" opacities
	Centrilobular nodules	
Chronic GVHD	Strong association	inconsistent

after SCT compared to pre-transplant values, even if the absolute values for each measurement remained within the normal range [10, 14]. TBI containing conditioning regimens and the presence of acute GVHD have been associated with RLD and higher mortality rates [10, 14, 15, 149–151]. By contrast, the impact of age on the development of RLD is less clear. Early reports suggested that the incidence of RLD is lower in children compared to adults, and that the incidence increases with advancing recipient age [15], but more recent studies have revealed significant RLD in children receiving SCT [152]. In contrast to OLD, RLD after SCT has not been clearly associated with the presence of chronic GVHD.

Histologic features of RLD after SCT are rarely described in the clinical literature, although varying degrees of interstitial and alveolar inflammation and fibrosis, as seen in patients with other forms of interstitial lung disease such as idiopathic pulmonary fibrosis (IPF), would be expected. One exception is bronchiolitis obliterans organizing pneumonia (BOOP). Although reported in less than 2 percent of SCT recipients, BOOP is associated with restrictive (rather than obstructive) changes on PFTs [7]. Presenting symptoms include dry cough, shortness of breath and fever, and radiographic findings show diffuse, peripheral, fluffy infiltrates consistent with airspace consolidation [153]. Diagnosing BOOP requires histologic evidence on lung biopsy of several signature features: patchy fibrosis, granulation tissue within alveolar spaces, alveolar ducts and respiratory bronchioles, and the absence of infectious organisms [153]. The term BOOP should not be used interchangeably with bronchiolitis obliterans (BrOb) or bronchiolitis obliterans syndrome (BOS) to describe a patient with chronic lung dysfunction after SCT, although such usage is unfortunately widespread. The two disorders differ with respect to histopathology, pulmonary function characteristics and, most importantly, response to therapy; BOOP after SCT is quite responsive to corticosteroids and in other settings may resolve spontaneously, whereas as discussed later in this chapter, BrOb and BOS are not [7, 153].

Obstructive Lung Disease: OLD involves enhanced airflow resistance on expiration and reflects conditions in the smaller airways and bronchioles. Obstructive defects are demonstrated by decreases in FEV_1 and in FEV_1/FVC [154]. OLD was first recognized as a complication of allogeneic SCT in the mid 1980s. Of the first 35 patients described, 75 percent had evidence of extensive, chronic GVHD and presented with cough, wheezing or shortness of breath. Despite aggressive therapy with immunosuppressive agents and bronchodilators, only three patients showed improvement in lung function, and the case-fatality rate was 50 percent [154]. OLD is now a well documented cause of morbidity after allogeneic SCT [9, 60, 154–156], and obstructive defects as defined by an FEV1/FVC < 70 percent have been observed in approximately 15 percent to 25 percent of allogeneic SCT recipients by day 100 and can persist for years [10, 46, 60].

OLD results from extensive narrowing and/or destruction of small airways. Lung biopsies from patients with OLD have shown histologic patterns of lymphocytic bronchitis, acute and chronic interstitial pneumonitis and bronchiolar inflammation, including BrOb [8, 46, 155, 157, 158]. This variation in histopathology is complicated further by the methods used to procure lung tissue; transbronchial biopsies rarely include an adequate sampling of distal bronchial structures and, therefore, such specimens can reveal cellular

infiltrates involving larger airways and the interstitium, but may not detect bronchiolar inflammation.

Despite these limitations, BrOb remains the most common form of histopathology associated with OLD and has been used historically to describe "chronic GVHD of the lung" [8, 46, 155, 157, 18]. As the name implies, BrOb depicts small airway inflammation with fibrinous obliteration of the bronchiolar lumen that is classically associated with a fixed obstructive defect on PFT [8, 9, 12, 46, 159]. Airflow obstruction may, however, occasionally exist without BrOb and vice versa [160]. Moreover, in the vast majority of cases, OLD is diagnosed by PFT findings without histopathologic confirmation and, in this context, two phrases that identify affected patients are found in the literature. "Obstructive bronchiolitis" describes functional airflow obstruction in patients with signs and symptoms of bronchial inflammation, and "bronchiolitis obliterans syndrome" (BOS) describes the deterioration of graft function that accompanies chronic lung allograft rejection [161]. BOS is defined as an irreversible decline in FEV1 of at least 20 percent from baseline and is graded using the international heart and lung transplantation criteria: BOS stage 0 = $FEV_1 \geq 80$ percent baseline; stage 1 = FEV_1 from 66 percent to 79 percent; stage 2 = FEV_1 from 51 percent to 65 percent and stage 3 = $FEV_1 \leq 50$ percent of baseline value [161]. This system requires the establishment of a post-transplant baseline value of FEV_1 and is commonly reported in clinical studies of lung allograft rejection.

The wide variation in the reported incidence of OLD after SCT stems from the lack of consistent terminology and the variability in diagnostic criteria for this form of lung injury. Afessa and colleagues found that OLD was reported in 8.3 percent of over 2,000 allogeneic SCT patients in nine studies and was identified in 6 percent to 20 percent of long-term survivors with chronic GVHD [7]. The onset of OLD is later than IPS (ranging from three to 18 months after SCT), and more insidious. A review of 35 allogeneic SCT recipients with OLD from a single institution demonstrated that approximately 40 percent of cases developed between 50 and 150 days after transplantation, whereas the remainder occurred between 150 and 500 days [154]. Respiratory symptoms include cough, dyspnea and wheezing, but many patients remain asymptomatic despite signs of moderate to severe airway obstruction on PFTs [8, 60]. Chest radiographs are most often normal except for signs of hyperinflation, but patchy, diffuse infiltrates can be present [8, 46, 59]. Likewise, chest CT findings range from normal early in the course of disease to extensive peribronchial inflammation and bronchiectasis with significant air trapping and diffuse parenchymal hypoattenuation at later timepoints [9, 162, 163].

The clinical course of OLD also varies from mild to severe with necrotizing bronchiolitis, a rapid decline in FEV1 and a mortality rate of 25 to 50 percent and, unfortunately, clear predictors of outcome have not been identified [8, 9, 11–13, 59, 154]. Since airflow obstruction tends to be fixed rather than reversible, response to bronchodilator therapy is usually marginal. Similarly, responses to immunosuppressive therapy (including various combinations of steroids, calcineurin inhibitors and azathioprine) are limited and are typically associated with preservation of remaining lung function rather than in significant improvement. Since enhanced immunosuppression significantly increases the risk of infection, the utility of such therapy is questionable when a clinical response is not seen within the first several months of treatment or when pulmonary dysfunction is long-standing. The

partial response to immunsuppressive therapy suggests that early detection of disease may be important [8, 9, 13, 154]. In this light, two reports have suggested that a decrease of maximum mid-expiratory flow rates (MMFR) may be an earlier indicator of OLD than changes in FEV1 [13, 59]. Additional studies are required to determine whether such a parameter can serve as an early diagnostic tool and improve efforts to prevent the development of significant, irreversible dysfunction.

There are several risk factors for OLD including the effects of pre-transplant conditioning regimens, concomitant infections, chronic aspiration and GVHD. OLD has been reported in association with older donor age, the use of methotrexate for GVHD prophylaxis, the presence of esophageal dysfunction (with aspiration), the use of mismatched donors and the use of busulfan (rather than TBI) in SCT conditioning regimens [8, 9, 13, 60, 154, 164]. From an infectious disease perspective, the CMV status of neither donor nor recipient prior to SCT correlates with the development of OLD. However, previous RSV and adenoviral infections are possible risk factors for the higher incidence of OLD in the pediatric population [9]. Developing OLD is strongly associated with chronic GVHD, particularly in patients with low serum IgG levels [8, 154] and with chronic hepatic GVHD [9]. Furthermore, recipients of HLA-mismatched related donor or matched unrelated donor grafts have a much higher incidence of OLD than patients receiving 6/6 HLA-matched related donor transplants (40% versus 13%) [9]. Thus, OLD and chronic GVHD may share immunopathogenic mechanisms.

3.2. Pathogenesis of Chronic Pulmonary Toxicity

The pathophysiology of chronic lung injury after SCT is less well defined than for IPS, particularly because of the paucity of correlative clinical data and the lack of suitable animal models for either OLD or RLD. The development of chronic pulmonary toxicity likely involves an initial insult to lung parenchyma followed by an ongoing inflammatory process involving the interplay between recruited immune effector cells and the resident cells of the pulmonary vascular endothelium and interstitium. Most of what is known about the pathogenesis of OLD is based upon observations made in lung allograft recipients and from data generated in murine heterotopic tracheal transplant models. The absence of an initial inflammatory response from SCT conditioning regimens and the presence of a "Host-versus-Graft" rather than a "Graft-versus-Host" reaction are just two of the issues that limit the extrapolation of such data to OLD after hematopoietic SCT. Similarly, our understanding of the mechanisms responsible for RLD is inferred from patients with various forms of interstitial fibrosis and from animal models of these diseases. Despite these limitations, lung allograft rejection and pulmonary fibrosis are characterized by epithelial cell injury in the terminal bronchioles or alveoli, respectively, and by a profound defect in reepithelialization and normal repair. Each response may also involves T cell activation, leukocyte recruitment and enhanced expression of inflammatory mediators, all of which are likely operative in chronic lung injury after SCT.

3.2.1. The Pathogenesis of OLD

As noted, mechanisms of OLD have been extrapolated primarily from clinical and experimental models of lung allograft rejection, which is classified as acute or chronic histologically. Acute rejection is driven primarily by T helper

lymphocytes that recognize donor MHC epitopes, secrete cytokines and facilitate the generation of CTL effectors. The factors responsible for the progression from acute to chronic rejection, which is characterized by fibrous obliteration of epithelialized bronchial structures, remain to be determined. The direct correlation between the intensity and duration of acute rejection episodes and the subsequent development of chronic rejection suggests, however, that the obstructive lesions characteristic of chronic rejection and BOS progress through a series of events involving persistent and antigen-dependent injury of endothelium and epithelium followed by the induction of repair mechanisms. Although direct cytotoxicity by cellular or cytokine effectors may be responsible for damage and loss of airway epithelium in the transplanted lung, the inability to regenerate and heal injured epithelium is believed to be an equally important factor to the development of BOS [165]. In this context, interactions between activated epithelial cells and lung fibroblasts, rather than inflammatory cells, may be critical [166, 167].

Experimental and clinical data support the hypothesis that developing OLD during chronic lung allograft rejection involves the secretion of inflammatory cytokines and chemokines, and interactions between APCs and activated lymphocytes. Analysis of BAL fluid and biopsy specimens obtained from lung allograft recipients with BOS has revealed elevations in IL-1ra, TGFβ, IL-8 and MCP-1, all of which have been implicated in other fibro-proliferative processes [132, 168–170]. Alveolar macrophages from patients with BOS also produce significant amounts of TNFα and RANTES after stimulation with IFNγ [171]. Since IL-8 is a potent chemoattractant for neutrophils, BAL fluid elevations of this chemokine in patients with BOS are consistent with the observed influx of neutrophils into the bronchial walls and broncho-alveolar space [21, 135, 172].

Murine models of lung allograft rejection have confirmed many of the clinical associations and have begun to elucidate the mechanisms that contribute to leukocyte migration and rejection of allograft epithelium. Boehler and colleagues found that rat heterotopic lung allografts undergoing rejection showed a strong Th1 immune response even after fibrosis and airway obliteration was complete [173]. This response was characterized by enhanced production of IL-2 and IFNγ, and was associated with upregulation of MCP-1 and RANTES. Work by this same group demonstrated that administering IL-10 either in the recombinant form or by gene transfer using an adenoviral vector inhibited the development of fibrous airway obstruction [174]. Subsequent studies by Belperio and others showed enhanced expression of TNFα and TGFβ during allograft rejection and ultimately revealed critical roles for both RANTES and MCP-1 in the development of experimental BOS [168, 175, 176].

Donor-specific, alloreactive lymphocytes have also been identified in the BAL fluid and lung biopsy specimens from patients with acute and chronic rejection [165, 179, 180]. In particular, the clonal expansion of T cells responding to a limited number of immunodominant epitopes has been identified in the peripheral blood and BAL fluid of patients with BOS, and the oligoclonal expansion of circulating CD4+ T cells recognizing alloantigen determinants had 100 percent specificity and 80 percent specificity for the presence or imminent development of allograft rejection [180]. Dendritic cells expressing the costimulatory molecules CD80 and CD86 have also been identified in the lungs of patients with BOS [181, 182], and experimental studies have shown

that CD28-B7 interactions contribute to the alloreactive response since the use of CTLA4Ig abrogates the development of OLD [183]. Furthermore, rodent heterotopic tracheal allograft models have demonstrated that developing BOS requires APCs from the transplanted trachea and can be initiated against either MHC class I or class II antigens on donor tissue. These findings suggest that direct allorecognition by either CD8+ or CD4+ cells is critical to the development of this form of airway injury [184].

3.2.2. The Pathogenesis of RLD

The mechanisms responsible for RLD after SCT are also poorly characterized. As with OLD, factors contributing to the development of disease likely include underlying injury to lung epithelial cells, immune cell activation and the secretion of inflammatory chemokines and cytokines [185]. The prevailing hypothesis holds that most forms of interstitial fibrosis are initiated by an acute inflammatory event that injures the lung and subsequently modulates pulmonary fibrogenesis [186]. The etiology of the initial cellular insult may involve direct toxicity from drugs, toxins or ionizing irradiation. Alternatively, the insult may represent a dysregulated immune response to an environmental antigen, (e.g., hypersensitivity pneumonitis) or to an aberrantly expressed self-antigen, as occurs when interstitial fibrosis accompanies autoimmune disorders [187].

The role of T cells in the development of interstitial fibrosis is variable and depends upon the inciting stimulus. T cells significantly contribute to hypersensitivity pneumonitis and pulmonary fibrosis that complicates collagen vascular diseases, whereas they are less critical for interstitial fibrosis secondary to radiation and other toxins [187–189]. Significant increases in eosinophils and lymphocytes accompany the airway remodeling and peribronchial fibrosis that characterize the chronic, allergic, airway disease that results from the inhalation of *Aspergillus fumigatus* [190]. Furthermore, macrophages and T lymphocytes contribute to pulmonary fibrosis induced by the instillation of bleomycin or silica, but fibrosis caused by the intratracheal administration of FITC can occur in the absence of T cells [189].

By contrast, the etiology of the inciting injurious event and the potential role for antigen-specific T cell responses in patients with idiopathic pulmonary fibrosis (IPF) are matters of considerable debate [186, 191, 192]. The lungs of patients with IPF show increases in lymphocytes, macrophages and neutrophils in interstitial and intra-alveolar spaces. Some believe that T cell responses occur early in the time course of IPF and may contribute to direct epithelial injury and the modulation of disease progression [187]. Activated lymphocytes and alveolar macrophages can secrete inflammatory mediators that propagate epithelial cell injury and destroy the basal lamina and the deposition of fibrin-rich exudates in the intra-alveolar space. If these exudates are not successfully cleared, fibroblasts migrate to the inflamed area and release matrix proteins that contribute to scar formation [187]. Recent data suggest, however, that acute inflammation may not be a critical pathogenic event in IPF; epithelial injury without ongoing inflammation may be sufficient to stimulate the development of fibrosis [186, 193]. In this alternative hypothesis, injured alveolar epithelium stimulate parenchymal fibroblasts (rather than leukocytes) to produce an exuberant reparative response characterized by proliferation, differentiation to myofibroblasts and excess matrix deposition

[186, 193]. The function of lung fibroblasts has traditionally been limited to normal structural maintenance and the latter stages of tissue repair. However, recent data demonstrate that fibroblasts can both respond to and secrete a variety of inflammatory mediators, including CC and CXC chemokines, which allow these cells to migrate to sites of ongoing inflammation, subsequently recruiting activated immune cells [194, 195]. In this context, pulmonary fibroblasts may represent an important link in the development of both RLD and OLD after allogeneic SCT.

Several proteins that are known to have pro-fibrotic activity including TNFα, IL-1, IL-6, TGFβ, IL-8, MCP-1 and MIP-1α have been identified in the BAL fluid of patients with IPF and in animal models of interstitial fibrosis. A growing body of scientific evidence suggests that the cytokine profile present during an evolving immune response determines its pathologic outcome. In this context, a Th1/Th2 paradigm has emerged to help predict whether a specific pulmonary response will ultimately resolve or progress toward end-stage fibrosis. Much of the scientific evidence supporting this concept is derived from studies showing that the interferons, and specifically IFNγ, have profound regulatory activity on collagen synthesis and possess potent anti-fibrotic effects, whereas Th2 cytokines like IL-4 and IL-13 activate fibroblasts and stimulate the production of extracellular matrix proteins. Recent data also demonstrate that a variety of cell types contribute to pulmonary fibrosis, including epithelial cells, macrophages, eosinophils and fibroblasts. Since these cells can synthesize type-2 cytokines, it may be more appropriate to consider certain diseases in terms of their predominant cytokine profile rather than by the T helper cell subsets involved [185]. The Th2 dominant paradigm appears to be particularly useful for pulmonary inflammation engendered by antigens to either the parasite *S. mansoni* or the fungus *A. fumigatus* [190, 196], whereas its relevance in other models of fibrosis is less clear [197–200].

The role of TNFα in interstitial fibrosis has also been underscored in several experimental studies and clinical reports. TNFα may stimulate fibroblast proliferation and collagen gene upregulation through a TGFβ or platelet-derived growth factor pathway [201]. Human alveolar macrophages and type II epithelial cells from patients with IPF express increased amounts of TNFα [202, 203], and recent data suggest an association between polymorphisms in the promoter region of the TNFα gene, and an increased risk of developing IPF [204]. Furthermore, TNFα gene expression increases after administering agents that cause pulmonary fibrosis [205–208]. Abrogation of TNFα signaling, either by antibody neutralization or by using mutant mice deficient in both TNF receptors (p55 –/– and p75 –/– mice), significantly reduces lung fibrosis in murine models [205, 206, 209]. When TNFα is specifically overexpressed in the lungs of rodents, animals develop a lymphocytic infiltration followed by progressive alveolar and interstitial fibrosis that can be accompanied by the enhanced expression of TGFβ [210–212]. TNFα is also upregulated in models where Th2 cytokines like IL-4 and IL-13 are operative, suggesting that it may have a significant role in fibrogenesis even in a Th2 skewed cytokine environment [208].

3.2.3. The Evolution of Chronic Lung Injury After SCT

A tri-phasic model of chronic, noninfectious lung injury after SCT is proposed wherein alloantigen recognition represents the inciting stimulus of the immune

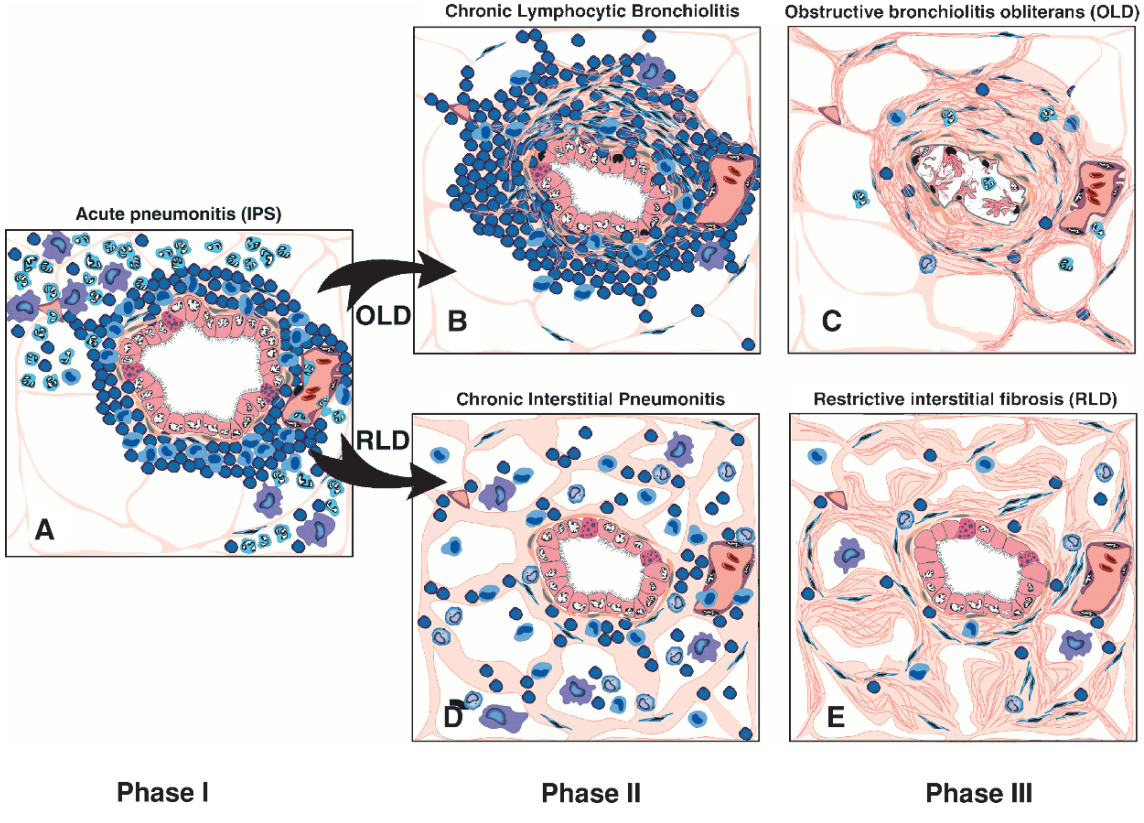

Fig. 22-2. A triphasic model of both OLD and RLD following allogeneic SCT
Immune mediated injury to the lung after allogeneic SCT can be conceptualized in three phases. In phase I, acute lung injury develops as a consequence of an allogeneic immune response and results in the sequential influx of lymphocytes, macrophages and neutrophils into an inflamed pulmonary parenchyma (A). Persistence of an inflammatory signal in the setting of dysregulated repair mechanisms promotes the transition from acute to chronic injury in phase II (B and D). As chronic inflammation proceeds to phase III, lung fibroblasts increase dramatically in number and contribute to the enhanced deposition of collagen and granulation tissue in and around bronchial structures, ultimately resulting in complete obliteration of small airways and fixed OLD (C). If, by contrast, the principal target of early damage is the alveolar epithelium, fibroblast proliferation and intra-septal collagen deposition ultimately results in interstitial fibrosis and RLD (E).

response (**Fig. 22-2**). Phase I of disease is characterized by the development of a mixed leukocytic infiltrate and acute interstitial and peribronchial inflammation similar to that observed in IPS (Fig. 22-2A). This injury is initiated early after allogeneic SCT by a systemic proinflammatory environment that leads to chemokine upregulation, the promotion of leukocyte recruitment and the secretion of inflammatory cytokines in the lung (see section 2.5). Donor T cells recruited to the lungs within the first two weeks following allogeneic SCT are followed by the influx of donor monocytes, macrophages and neutrophils all of which contribute to the secretion of TNFα and the propagation of tissue damage. **(see Fig. 22-1).**

In phase II, the persistent expression of MHC antigens on pulmonary epithelial and vascular endothelial cells, coupled with the sequestration of alloreactive T cell effectors in the pulmonary microcirculation, result in ongoing epithelial activation and injury. A persistence inflammatory signal results in a dysregulated reparative response in the lung and promotes the transition from acute to chronic lung injury. This transition is accompanied by a change in the character of the leukocytic infiltrate (to one that is predominantly lymphocytic in nature) along with a shift to a pro-fibrotic environment and a proactive role of the lung fibroblast. The histologic changes that occur during phase II are dependent upon the primary site of lung injury. When inflammation to the bronchiolar epithelium predominates, phase II is associated with a progressive, concentric lymphocytic infiltration, collagen deposition, and early fibrosis in the peribronchial areas resulting in a chronic bronchiolitis (Fig. 22-2B). Activated lymphocytes then migrate through the basement membrane of the respiratory epithelium and into the airway mucosa and result in epithelial cell apoptosis and necrosis [18]. Continued epithelial injury leads to areas of denudation and ulceration. As chronic inflammation proceeds into phase III, lung fibroblasts increase dramatically in number and contribute to 1) the proliferation of endothelial cells, 2) enhanced collagen deposition and 3) the development of intraluminal granulation tissue and dense, concentric, periluminal fibrous bands (Fig. 22-2C) [18, 166]. Ultimately, this process results in complete obliteration of small airways (cicatricial bronchiolitis obliterans) and significant OLD.

By contrast, if epithelial cells in the alveolar septae are the principal targets of injury, persistent antigenic stimulation results in recruitment of lymphocytes and monocytes into the interstitial space, eventually resulting in RLD. Alveolar architecture is preserved in the early stage of phase II, but the alveolar walls are expanded by edema and infiltration of leukocytes (Fig. 22-2D). The resultant inflammation is associated with apoptosis and loss of septal epithelial cells, exudation of proteinecious material, the recruitment and proliferation of fibroblasts and the deposition of intra-septal granulation tissue [18, 166, 213]. If this extracellular matrix is not resorbed, chronic inflammation progresses to phase III wherein collagen is deposited within the alveolar septae and the chronic leukocytic infiltrates are less evident. This results in interstitial thickening, septal fibrosis and loss of alveolar architecture leading to dilated cystic air spaces or "honeycombing" (Fig. 22-2E). Such end-stage histopathology is associated with significant volume reduction and severely impaired gas exchange that is characteristic of severe RLD.

Precisely what determines the anatomic specificity (peribronchiolar versus interstitial) of chronic lung injury remains unclear, and developing either

pattern of chronic lung injury (OLD or RLD) does not necessarily exclude the other; each pattern may develop simultaneously in an individual patient as suggested in a recent report from Trisolini and colleagues [213]. Furthermore, the extremely poor survival seen in patients with IPS has precluded our ability to follow the natural history of lung injury after allogeneic SCT. Recently, PFTs completed on patients with IPS who were treated with etanercept have revealed persistent restrictive changes months after the initial episode of acute lung injury (K.R. Cooke, unpublished observation). It should be noted, however, that the role of acute inflammation and specifically of alloreactive effector cells in the initial damage to the alveolar or bronchiolar epithelium and the subsequent progression to chronic pulmonary injury are not well established. Some have suggested that an early, robust inflammatory phase may not be a prerequisite for subsequent fibrosis; persistent epithelial damage and subsequent "cross talk" between epithelial cells and fibroblasts may be sufficient to develop fibrotic lung disease [214]. This mechanism could explain why some patients with chronic lung dysfunction do not have a clear antecedent history of acute lung inflammation; in the setting of imbalanced immune regulation, a subclinical injury, such as an allogeneic response to lung epithelial cells, could initiate a dysregulated reparative response resulting in scarring of either the terminal airways (OLD) or the interstitial space (RLD).

As noted above, clinical and experimental data suggest that the progression to a chronic, pro-fibrotic and more irreversible form of pulmonary toxicity involves the secretion of inflammatory proteins that can stimulate the proliferation of myofibroblasts, promote collagen synthesis and enhance leukocyte recruitment to inflamed tissue [36, 166, 210]. In this context, TNFα may be a central factor in the tri-phasic model proposed above. As noted, TNFα is an important mediator of both acute and chronic lung injury after SCT [1, 21, 50, 51, 62] and directly contributes to the development of RLD and interstitial fibrosis in several non-SCT models [166, 210–212]. Strong evidence for a role of TNFα in the transition from acute to chronic lung injury comes from a study using transgenic mice with targeted overexpression of TNFα in the lungs [211]. Early lung histopathology includes a lymphocytic infiltrate similar to that seen in experimental IPS models [48, 51], whereas the histologic changes associated with more prolonged exposure to TNFα closely resemble those seen at later timepoints after SCT [18, 36]. Similar observations were made in other studies wherein TNFα was overexpressed in normal pulmonary tissue of adult rats wherein an acute inflammatory response is followed weeks later by the upregulation of TGFβ, the activation of fibroblasts and the development of interstitial fibrosis [210]. Finally, TNFα producing cells have been associated with both acute and chronic rejection of rat lung allografts and were specifically identified in close proximity to MHC class II positive epithelial cells at later timepoints of the immune response [215].

The linkage between TNFα and OLD after SCT is more indirect, but increased levels of TNFα are also known to increase the expression of several proteins that may contribute to this process. For example, neutralizing TNFα secreted in a human MLR downregulates the production of MCP-1 that occurs during this response [216]. Increased levels of TNFα are also associated with enhanced MCP-1 expression in a murine lung allograft rejection model and correlate with upregulation of CCR2 and the recruitment of mononuclear phagocytes into the allograft [168]. Interruption of MCP-1/CCR2 signaling

using CCR2 deficient recipients results in a reduction of macrophage infiltration and less rejection [168]. Signaling via CCR2 is also critical to the induction of profibrotic cytokine cascades following the intratracheal administration of FITC and bleomycin [208]. Finally, clinical studies have shown that enhanced MCP-1 is associated with the progression from acute to chronic allograft rejection and with the evolution of IPF [168, 217–219]. Studies from our laboratory have demonstrated significant roles for both TNFα and CCR2/MCP-1 in the development of IPS [62, 74, 146], and experiments are ongoing to determine whether these interactions are operative in the transition from acute to chronic lung injury after SCT.

3.3. Treatment of Chronic Lung Injury After SCT

The clear association between OLD and chronic GVHD has resulted in a general consensus that this form of lung damage is immunologically mediated. Thus, "standard" therapy of OLD combines enhanced immunosuppression in conjunction with supportive care including supplemental oxygen therapy and broad-spectrum antimicrobial prophylaxis. Unfortunately, the response to multiple agents including steroids, cyclosporine, tacrolimus and azathioprine is limited and tends to occur only early in the course of treatment [8, 13, 46, 59, 154]. Patients with more severe disease at the start of treatment have poor prognoses and high mortality rates, suggesting that early recognition of OLD may be important [13, 59, 154]. The poor response to standard therapy and the unacceptable morbidity and mortality associated with chronic lung injury after SCT are underscored by the need for lung transplant in some SCT recipients with severe OLD [18, 220].

The published literature contains a paucity of therapeutic trials for chronic lung injury after SCT, and most retrospective reports focus on patients treated for OLD rather than RLD. Although no agent or combination of agents has proven efficacy with respect to treating OLD, a study by Payne and colleagues showed that using cyclosporine and methotrexate as GVHD prophylaxis prevented the development of OLD when compared to historical controls receiving prednisone and methotrexate [221]. Unfortunately, results of prospective, randomized trials studying the impact of GVHD prophylaxis regimens on the incidence and severity of OLD have not been reported. A recent clinical trial using inhaled steroids, in addition to standard systemic immunosuppression, to prevent BOS after lung allografting was recently completed, but no benefit was observed compared to placebo controls [222].

The potential role for TNFα in the pathogenesis of both OLD and RLD suggests that agents which neutralize this protein may have promise as novel therapeutic agents for these disorders. Etanercept was recently evaluated in an open label pilot study in nine patients with IPF [223]. All patients had worsening disease that was refractory to conventional therapy. Five of nine patients reported had objective improvement in oxygen requirement, DLCO and A-a gradient after an average follow-up of 11 months. Etanercept has also been used to treat steroid refractory chronic GVHD in a series of 10 patients, and three of four patients with lung disease were reported to have objective response (increased DLCO) as well as subjective improvement [224]. A phase I-II clinical trial using etanercept specifically for patients with either OLD or RLD has recently been completed at the University of Michigan. Preliminary

results demonstrate that etanercept can be safely administered in this patient population and six of 15 patients showed at least a 10 percent improvement from baseline in either FEV1, FVC or DLCO within the first two months of finishing therapy [225], thus prompting the development of a larger phase II trial that is currently accruing patients. Additional prospective trials are needed to enhance our understanding of the immunologic mechanisms responsible for this complication, to identify diagnostic and prognostic predictive factors and to test new agents or treatment strategies in this clinical setting.

3.4. Conclusion

Noninfectious lung injury remains a significant problem following allogeneic SCT both in the immediate post-transplant period and in the months to years that follow. Although such lung injury occasionally occurs following autologous transplants, the allogeneic setting significantly exacerbates toxicity in both the acute and chronic setting. Historically, much of this injury was assumed to be due to occult and unidentifiable infections, but animal models have clearly shown immunologic mechanisms to be operative. Is the lung a target of GVHD? The weight of conceptual and experimental evidence seems to favor rather than disfavor this possibility.

As detailed above, a large preponderance of experimental data now demonstrates that IPS has a major immunologic component. Like the gut and skin, the lung is a critical immunologic interface between the sterile body sanctuary and the outside environment. As such, the lung is a rich source of histocompatibility antigens and professional APCs and is the site of complex immunologic networks that involve cytokine production and lymphocyte activation. Inflammatory mediators such as TNFα and LPS, along with donor-derived Th1 effector cells which are known to play a role in acute GVHD, are associated with acute lung injury in the experimental and clinical setting. From a clinical perspective, however, evidence supporting the concept that the lung is a target organ of acute GVHD is limited, and the major obstacle has been the lack of apoptotic epithelial injury. However, other GVHD target organs such as the thymus do not express this particular form of injury, and recent experimental data demonstrate that direct recognition of alloantigen on host epithelium by cytotoxic effectors is not required for GVHD induction or target organ injury. The unique aspects of epithelial anatomy in the lung may also be important factors in this discrepancy. Since there is no stratification or layering of pulmonary epithelial cells as in the skin or intestine, the histopathologic repertoire of pulmonary damage is very limited making a potential diagnosis of acute GVHD in the lung by histologic criteria difficult.

In the chronic setting, the lung is widely accepted as a target of GVHD. The striking similarities between the histopathologic features of bronchiolitis obliterans seen in association with OLD after allogeneic SCT and those observed during lung allograft rejection, along with reports of improved lung function with immunosuppressive agents, strongly support this concept. The case for immunologic mechanisms contributing to RLD is more tenuous and the data from experimental allogeneic models is still scant. Nonetheless, TNFα may be viewed as a common thread between acute IPS and chronic lung disease of either the obstructive or restrictive type. It is hoped that as animal models of lung injury post-SCT yield further insights, our understanding of these

disease processes will improve and ultimately lead to new therapeutic strategies to diagnose, treat and prevent pulmonary toxicity in our allogeneic SCT recipients.

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Chapter 23

Quality of Life Issues Post-Transplantation

Stephanie J. Lee

1. Overview

Quality of life (QOL) refers to every dimension of life except its length, and includes physical abilities, symptoms, social well-being, psychoemotional status and spiritual/existential qualities. It reflects how well people feel, what they can accomplish, how satisfied they are with their lives and whether their lives have meaning and purpose. Within this broad concept, health-related quality of life (HRQOL) refers to aspects of QOL that are attributable to health, disease or medical treatment (in this chapter, the abbreviation QOL will be used for simplicity). Following hematopoietic stem cell transplantation (HCT), QOL can range from perfect, with no physical, emotional or social sequelae and a greater appreciation for life, to severely compromised with physical disability, pain and psychological despair. Of course, most patients who have undergone HCT fall within this spectrum. The goal of this chapter is to provide an overview of concepts and published work rather than review individual studies exhaustively. Additional sections address how to evaluate QOL studies and use QOL data in the care of individual patients.

2. Definitions

Figure 23-1 shows the general taxonomy of QOL. Overall or global quality of life is at the top of the pyramid and is the short answer to the question, "How are you doing today?" Conceptually, global QOL is based on a composite of domains representing physical, emotional, social, functional and spiritual/existential considerations. These domains are best assessed by familiar queries: "Are you bothered by any symptoms? How are you holding up?" These domains reflect specific issues e.g., "Do you have any mouth sores? Can you walk up a flight of stairs? Have you gone back to work yet? Are you sleeping alright? How are things at home?" The exact segregation of some QOL components varies according to the author. For example, sexual functioning has been grouped variably under the physical, functional and social domains.

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By definition QOL is multidimensional, individual and subjective [1]. Studies of concurrent measures of patient- and surrogate-reported quality of life show that physicians, nurses, spouses and parents often think differently about patients' QOL than they do [2]. For example, nurses thought some specific problems would have a greater impact on QOL than patients reported [3]. These findings suggest that QOL is best assessed by patients, and that surrogate assessments may be misleading.

Several qualitative studies have probed the meaning of QOL for HCT patients. Ferrell analyzed survey responses to six open-ended questions about QOL from 119 HCT survivors (63% response rate, transplanted 1976 to 1990). Content analysis suggested six themes about the meaning of QOL. These may be summarized into two overarching concepts: appreciating and cherishing what one has (family, relationships, life) and desire to regain pre-transplant functioning (independence, health, return to work, normalcy). The impact of HCT on QOL emphasized several losses (side effects, decreased strength and stamina, limited work and activities) and some positive effects (second chance, opportunity to improve QOL, increased spirituality and meaning, increased appreciation for life). When asked what physicians or nurses could do to improve QOL, patients responded with many practical suggestions (be accessible, provide support groups, provide education and coping strategies, increase patient participation in decision making) [4, 5,]. Haberman studied 125 adult HCT survivors (64% response rate, transplanted prior to 1983, 87% allogeneic recipients) more than six years post-transplantation using an open-ended questionnaire. Many patients reported using cognitive and attitudinal strategies (acceptance, looking on the positive side) and behavior and action-oriented strategies (staying active, setting goals, taking care of oneself) to cope with problems. Patients who believe HCT improved their QOL focused on the lessons learned through the experience and improved health. Patients who reported poorer QOL after HCT focused on physical and social limitations, and increased stress in their lives from finances and job [6]. Baker interviewed 84 survivors after their departure from the Johns Hopkins Oncology Center, and again at six months and one year (80% to 84% response rate, 42% allogeneic recipients). In addition to physical and psychological problems, respondents noted problems with resuming roles put on hold because of illness. Specifically, they reported issues with returning home, work and social situations such as stigmatization, problems with family and children, financial and job concerns [7].

3. Specific Issues Post-Transplantation

Survivors generally report high global quality of life following HCT, but many specific symptoms [1, 8–13] and limitations on their daily activities [14]. However, despite many problematic long-term complications, almost all patients indicate they would undergo the procedure again given similar circumstances [12, 15–17]. For some problems such as fatigue, sleep and sexual functioning, the incidence of problems must be placed in the context of the high dissatisfaction seen in the general population and chemotherapy-treated patients, too [10, 18]. The following section briefly highlights the major themes reported in the literature.

3.1. Physical Functioning and Physical Symptoms

The physical domain encompasses strength, stamina and symptoms. HCT patients, particularly those suffering from chronic Graft-versus-Host disease (cGVHD), report lower physical functioning and poorer overall health than the general population [10, 13, 18–20]. HCT can be associated with a variety of irreversible physical sequelae including cataracts, premature menopause, infertility and avascular necrosis of bone. Treatment-related pulmonary, cardiovascular and renal complications occur [21, 22]. Many of the medications prescribed after HCT have bothersome side effects.

Patients report many specific symptoms, particularly those involving skin changes, fatigue, weakness, pain, stiff joints, headache, poor appetite, mouth sores, dry eyes and mouth and frequent colds. Fatigue and lack of stamina may be overwhelming [23] (Table 23-1).

3.2. Psychoemotional

The psychoemotional domain encompasses emotions (e.g., anxiety, depression and fear) and cognition. Rates of true psychiatric diagnoses are high including a 20 to 30 percent incidence of depression [24, 25] and a 5 percent incidence of post-traumatic stress disorder [24–27]. Surprisingly, most studies find a

Table 23-1. The percentage of autologous and allogeneic recipients agreeing or strongly agreeing with qualitative statements about recovery at six, 12 and 24 months following transplantation Reprinted, with permission, Lee, et al. (2001).

	Autologous			Allogeneic		
Variable	**6 mos**	**12 mos**	**24 mos**	**6 mos**	**12 mos**	**24 mos**
N	93	69	35	112	79	45
Life has returned to normal	53%***	61%	63%	31%***	58%	68%
I feel back to my old self	42%	62%	60%	31%	56%	47%
I have been able to enjoy my normal activities since BMT	42%**	62%	60%	21%**	48%	62%
I have been able to enjoy socializing with family and friends since BMT	75%***	87%	83%	52%***	77%	84%
I have been able to put my illness and BMT behind me and get on with life	55%***	68%	66%	33%***	59%	67%
I am satisfied with my physical appearance	76%***	72%	77%*	43%***	58%	56%*
I have recovered from my transplant	55%*	65%	65%	41%*	66%	71%
In general, my health is very good or excellent	37%	46%	46%	33%	39%	36%

Asterisks refer to comparison of autologous and allogeneic patients at identical time points: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

similar rate of psychoemotional problems between autologous and allogeneic recipients [28, 29], or even better functioning in allogeneic recipients [30, 31]. Patients continue to worry about relapse and whether they will recover to their pre-transplant level of functioning [7, 19, 32].

Post-HCT neurobehavioral outcomes are strongly predicted by baseline cognitive and social functioning [33]. Mild cognitive deficits are seen in 20 percent of patients even before HCT, and an early decline in cognitive function is observed during the hospitalization [34]. One of the most common patient concerns is loss of memory and ability to concentrate and, indeed, short-term memory problems do increase with time after HCT [33]. A cross sectional study of 66 HCT patients a mean of 34 months post-procedure showed that 37 percent of allogeneic and 17 percent of autologous patients had abnormal neuropsychological exams with detectable deficits in orientation, memory and reasoning. Concurrent MRI exams showed abnormalities in most of these patients [35]. In children, intelligence quotients are lower one year after HCT, although no further decline was seen at three years [36].

Harder and colleagues studied 101 HCT patients and 82 untransplanted patients with hematologic malignancies and reported similar rates of cognitive impairment, even though the HCT patients reported greater subjective difficulties. They suggest that cognitive performance is impaired prior to HCT and that there may be slight improvement with time [37, 38].

3.3. Social

Social functioning encompasses relationships, roles and leisure activities. Social relationships are generally preserved or even enhanced post–transplant [12]. However, one study reported that social support decreases with greater time since HCT [31]. Dissatisfaction with appearance is common. Sexual problems are also very common, with women reporting more sexual difficulties than men. Estrogen and testosterone levels, whether endogenous or due to replacement, seem to correlate with sexual satisfaction [11, 39–45].

3.4. Functional

Approximately 60 to 90 percent of HCT survivors eventually return to work, with higher rates noted in office workers compared to people with physically demanding jobs [20, 31, 46–53]. Return to work is significantly associated with better QOL, but both may be attributable to physical and mental health [47, 54]. Failure to return to work or school is common even in untransplanted patients with hematologic malignancies (26%) and, thus, may not be attributable to HCT itself [52, 55]. Concerns over finances [44] and obtaining health insurance are common after HCT [46], as they are for other cancer survivors. Sleep difficulties are also noted [56, 57].

3.5. Spiritual/Existential

The spiritual/existential domain refers to religion, spirituality, hope and the meaning of life. Many patients experience a greater appreciation for life compared to patients not treated with HCT [4, 9, 10, 23, 53, 58–60]. A sense that life has purpose may facilitate adaptation [61] as can the ability to find meaning in illness [62]. Viewing survival as a second chance for a different, perhaps more appreciated life can accentuate QOL, while struggling to regain

a lifestyle and outlook similar to pre-HCT may lead to dissatisfaction. Indeed, reordering priorities is a common theme for survivors [31], and while gains in the spiritual/existential domain may greatly improve overall QOL, at least one author has suggested that full recovery is not complete until patients are no longer acutely aware and appreciative of being alive [63].

4. Phases of Transplantation

HCT is a procedure with substantial risks of treatment-related morbidity and mortality. Beyond the early risks, survivors are faced with increased susceptibility to infections and restrictions on a normal lifestyle for months to years after the procedure. Patients are generally advised to wear protective masks and gloves and avoid crowded public areas until they have physically recovered. They are not allowed to return to work until six to 12 months following HCT so, for many patients, their roles as a worker, parent and caregiver are simultaneously threatened [47, 54]. In addition, the persistent risk of relapse, potential need for re-hospitalization and late complications can interfere with complete recovery.

It is helpful to conceptualize the HCT process into three broad phases that have particular implications for QOL [1, 64, 65]. The first phase begins when the decision is made to pursue HCT and ends with commencement of the procedure. Researchers have focused on the psychoemotional distress associated with this period including high levels of anxiety and depression as patients await transplantation [66–70]. The second phase encompasses the period of hospitalization for HCT through early recovery. Quality of life during this time is compromised by physical discomfort, fatigue, social isolation, depression and fears of imminent death [71]. However, most patients view this phase as temporary and not representative of long-term QOL. In fact, Zittoun has reported that overall quality of life is most influenced by depression and fatigue, and not physical symptoms during this period [72].

Whereas physical concerns and survival predominate early after HCT, over time concerns shift towards integrating survivorship and moving on [7, 17]. The third phase entails long-term readaptation and return to some form of normalcy [10, 16], although the transplant experience may inexorably alter an individual's perceptions of "normal" [65]. Reports on the trajectory of recovery vary. Continued improvement over time has been observed [31, 50, 73–75], while others report a plateau after one to two years [14, 44, 76] (Table 23-2). Yet another study suggests that problems vary over time with physical recovery dominating early after transplantation and then ebbing, and issues of reintegration, discrimination and the ability to attain long-range goals becoming increasingly important as time from transplantation increases [17]. Patients struggle to regain or redefine their roles as spouses, parents, caregivers, students or workers within the context of their HCT experience and any sequelae from the procedure.

5. Summary of Large HCT Survivor Studies

In 1999, it was estimated that there were over 20,000 patients surviving more than five years post–HCT [77]. Most studies report very good health and adaptation [51] although up to 31 percent of survivors report serious functional limitations or poor QOL [43, 78, 79]. The following section briefly

Table 23-2. Bothersome symptoms reported by autologous and allogeneic recipients reported at six, 12 and 24 months following transplantation Patients reported being bothered a lot or extremely bothered. Reprinted, with permission, Lee, et al. (2001).

	Autologous			Allogeneic		
Variable	**6 mos**	**12 mos**	**24 mos**	**6 mos**	**12 mos**	**24 mos**
N	93	69	35	112	79	45
Fatigue	42%	30%	35%	44%	35%	33%
Anxiety	15%	7%	11%	13%	10%	18%
Depression	9%	9%	11%	8%	4%	16%
Pain	11%	7%	14%	12%	5%	11%
Difficulty concentrating	14%	13%	17%	9%	14%	13%
Feeling isolated	8%*	3%	3%	20%*	5%	11%
Mouth sores	2%	1%**	0%	2%	15%**	9%
Painful joints	17%	12%	11%	12%	14%	20%
Skin changes	9%	4%***	11%	18%	24%***	16%
Memory loss	13%	9%	17%	9%	13%	16%
Finances	23%	17%	11%	25%	27%	22%
Sexual difficulties	24%	29%	37%	21%	29%	36%

Asterisks refer to comparison of autologous and allogeneic patients at identical time points: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

summarizes eight cohorts in which long-term or longitudinal QOL has been evaluated after HCT.

Andrykowski assembled a large cross sectional cohort of over 200 patients from five transplant centers. Approximately half had an allogeneic transplant procedure, and the average time since HCT was 3.5 years. Patients were surveyed by mail using several validated instruments. A subset (N = 172) were interviewed by phone at the time of enrollment and 137 returned a follow-up survey 18 months later. The authors concluded that less than half of survivors report normal functional status in most domains. Fatigue and sleep disturbances were common for both autologous and allogeneic patients. Many specific physical symptoms and limitations persisted, particularly for allogeneic recipients. Interestingly, discordance between pre-HCT expectations for returning to normal and current health status was associated with the greatest psychological distress [16, 50, 56].

Sutherland mailed a self-administered questionnaire to 251 allogeneic recipients and achieved a response rate of 93 percent (N = 231). Patients completed the Short Form 36, Satisfaction with Life Domains Scale and a symptom scale at a median of 40 months post-HCT. The major finding was that patients within three years from HCT had significantly worse scores compared to the general population on all SF36 subscales except the mental health scale. In contrast, patients more than three years post-HCT displayed better functioning

on the social, mental health and vitality subscales, and equivalent functioning on the other SF36 scales compared to the general population. However, Kiss reported 28 subjects from this same population who had survived at least 10 years following allogeneic transplantation for CML. In these very late survivors they found poorer physical functioning, role physical functioning and general health than an age-adjusted normative United States population. Several late medical complications adversely affected quality of life including chronic GVHD, relapse, osteoporosis and need for medications [12, 13].

Bush has measured QOL in autologous and allogeneic survivors within four years of transplantation (N = 415) or greater than six years post-HCT (N = 125) via self-administered surveys with the QLQC30, a symptom scale, demands of BMT recovery, Profile of Mood States, health perceptions survey and an open-ended module. They found that overall QOL was good for most survivors and improved over the first four years post-transplant, but many patients had residual deficits. Approximately 5 percent of long-term survivors rated their health as poor [11,31].

Syrjala and colleagues prospectively studied 405 adults, including 137 long-term survivors, from before HCT up to 10 years post-transplant. They also assembled a cohort of age, sex and race-matched controls. They report that QOL continued to improve up to three to five years post-transplant. By 10 years post-transplant, survivors were similar to controls in many respects, but had more medical issues and were more likely to be denied life and health insurance [80, 81].

The Hopkins group reported a cohort of 135 patients (86% response rate, 71% allogeneic recipients) who were at least six months post-transplant. Participants completed a battery of instruments measuring functional status (SF36), occupational information, self-esteem, coping, optimism, social ties, satisfaction, mood and positive and negative affect. Approximately 70 percent reported good to excellent health, felt that social and physical functioning was normal or only slightly limited, and had returned to work. Many positive changes were seen in relationships and existential/psychological domains, and 75 to 90 percent of patients were able to maintain their family, friend and homemaker roles. However, 23 percent reported job discrimination, 39 percent had insurance problems and 22 percent were dissatisfied with their sexual functioning [9, 39, 46, 54, 58].

Broers reported a prospective, longitudinal study of 125 patients (approximately 50 percent allogeneic). Subjects completed surveys measuring QOL, functional limitations, psychological distress, anxiety and depression, self-esteem and health locus of control prior to HCT and one month, six months, one year and three years afterwards. Follow-up of survivors was 63 to 80 percent at each timepoint. The authors report that 25 percent of patients still had severe functional limitations at three years, although 90 percent reported overall good to excellent health [79].

Lee studied 320 patients (70% response rate, 63% allogeneic) by self-administered survey prior to transplantation, and at six, 12 and 24 months post-HCT. Instruments included the SF36, two utility measurements, a symptom scale and qualitative questions. Results showed that although autologous patients had fewer physical symptoms and better perceptions of recovery at six months, by 12 months autologous and allogeneic patients were indistinguishable and little further gains were made by two years [44].

Andrykowski and colleagues reported results from a large, cross sectional, multicenter study of HCT survivors (N=662) a mean of seven years post-HCT. Results were compared with a group of peer-nominated controls showing that the HCT survivors experienced poorer health status than controls in all domains except psychological and interpersonal growth, where they experienced better health [82].

6. Predictors of Better Quality of Life Post-Transplantation

Better post-HCT adaptation and QOL are predicted by younger age, male sex, higher educational level, better quality of life and social support at the time of HCT, longer time since HCT and absence of late complications including chronic GVHD [11, 20, 32, 43, 50, 73, 83–88]. Surprisingly, the type of HCT procedure does not seem to be influential as autologous and allogeneic recipients have remarkably similar QOL given the differences in their risks for treatment-related mortality and late complications (Fig. 23-2) [44, 52, 60, 73, 89]. In autologous HCT, use of PBSC is associated with fewer physical symptoms within the first three months than marrow, although overall QOL was similar in a randomized study of 91 patients (62 received peripheral blood, 29 received marrow) [90]. In allogeneic HCT, QOL comparisons have evaluated the method of GVHD prophylaxis, graft source and conditioning regimen. No differences were seen in an observational study of 146 recipients of unrelated donor marrow whether T cell depletion (TCD) or a methotrexate-based regimen was used as prophylaxis [91]. A randomized study of 410 recipients of unrelated donor bone marrow comparing TCD with methotrexate-based prophylaxis showed similar QOL at one year [92]. Bevans and colleagues reported similar QOL two years following reduced-intensity conditioning or myeloablative conditioning in 76 patients [93]. De Souza and colleagues assessed QOL at least one year after participation in a small randomized study of peripheral blood versus bone marrow and reported that bone marrow recipients had less difficulty with pain and discomfort, mobility and the activities of daily living [94]. Thus, with the exception of graft source for autologous patients and use of bone marrow instead of peripheral blood for allogeneic patients, very few modifiable factors have been associated with post-HCT QOL.

In solid organ transplantation where donors are a limited resource, the concept that psychosocial profiles predict survival and success of the procedure is widely accepted and is part of the patient selection process. In HCT, studies of QOL and survival have focused more on depression and coping styles with contradictory results [95–99]. Several psychological assessment scales to

Fig. 23-1. Components of quality of life

identify HCT patients at high risk for poor quality of life and shortened survival post-transplant have been proposed, but they have either not been shown to predict outcome or are not widely applied [8, 18, 19, 100–106].

7. Quality of Life Following Transplantation Compared to Other Medical Procedures

Although HCT patients appear to have poorer QOL relative to normal, healthy people, they appear remarkably similar to other patient populations. Comparing HCT patients to those with hematologic malignancies treated with chemotherapy alone [8, 18, 19, 102, 104, 107] or recipients of solid organ transplants [25, 83] suggests more similarities than differences. When differences exist, there are usually greater physical problems in HCT patients, but better psychoemotional functioning than comparison groups [52, 90]. However, most of these comparative studies included fewer than 100 patients, and differences may have been missed. Molassiotis used a cross sectional design to study 91 long-term HCT survivors and 73 matched controls treated with chemotherapy alone. Measures included psychosocial adjustment to illness, anxiety and depression, symptoms, social support and psychosexual functioning. They concluded that transplant patients were doing as well or better than chemotherapy patients [52, 90]. Zittoun reported a cross sectional comparison of 98 patients participating in a multicenter study comparing allogeneic HCT (assigned to this arm if an HLA-matched sibling was available, N=35), autologous HCT (N=29) or intensive chemotherapy (N=34) (randomized between these latter two options). Patients were surveyed a median of four years after attaining complete remission using a multidimensional QOL instrument, an HCT-specific symptom scale and a measure of perceived changes in several domains. The authors concluded that allogeneic HCT survivors had poorer overall QOL and more physical problems than the other groups, but were similar in cognitive, emotional and social outcomes [18]. Hjermstad longitudinally assessed 177 (41 allogeneic recipients, 51 autologous patients, 85 chemotherapy recipients) with the EORTC QLQ C30, an anxiety and depression scale, and a symptom scale. They found that at one year, all groups had lower physical, role and social scores than a population sample which did not appreciably improve at three years post-HCT. However, in the two transplanted groups, the rate of anxiety and depression, and quality of life was similar [19, 89, 107, 108].

Watson and colleagues measured QOL in 481 patients treated on the UK MRC AML 10 trial, which genetically assigned patients with AML to allogeneic HCT (if they had an HLA-matched sibling). Patients without a matched sibling were randomized to an autologous transplant or continued chemotherapy. Patients completed self-administered surveys more than one year after completing therapy. The authors concluded that allogeneic recipients had the worst QOL, followed by autologous recipients, then chemotherapy patients [109].

8. Clinical Uses for Quality of Life Data

8.1. Patient Counseling

Despite acknowledgement that QOL considerations may be paramount for some patients, studies suggest that, for most patients and physicians, potential

length of life is more important when choosing treatment options [110–113]. This is supported by observations that many patients seem to have already made up their minds to pursue HCT even before they are aware of what the procedure entails [10, 64, 96, 114, 115]. For many HCT candidates it may seem there is "no choice" since transplantation offers the only realistic hope of cure [116]. Indeed, many physicians have observed that patients wish to avoid discussion of the specific risks and QOL issues surrounding HCT once they have made the decision to proceed [9, 10, 67, 117].

However, even if QOL considerations do not influence treatment choice [113], understanding probable QOL outcomes may help patients and their families prepare for challenges they will face during and after the procedure [44]. Some authors hypothesize that realistic expectations facilitate better recovery, while overly optimistic expectations impede adaptation. However, given the wide range of possible outcomes and the inability to predict individual problems accurately, it is difficult to know what patients' expectations should be. Also, even on a population basis, concrete estimates of symptom prevalence after transplantation and reasonable expectations for recovery are rarely presented in the literature.

8.2. Designing Interventions to Improve Quality of Life

While observational data are descriptively useful and to help identify vulnerable patients at risk for poor QOL post-HCT, for most researchers the ultimate goal is to improve the QOL of present or future patients. However, most interventions either have not been tested in clinical trials or have failed to show significant improvements over standard practice.

As noted above, one proposed approach to improving post-HCT QOL is to align expectations with experiences. In theory, fostering realistic expectations may help adjustment following HCT and actually improve perceptions of QOL [16, 24, 67]. However, operationalizing and scientifically testing this concept is difficult.

Another psychological strategy to improve QOL is to help patients retain as much personal control as possible during HCT [28]. However, a fact sheet designed to empower patients that listed common concerns and suggested sources for additional information actually increased feelings of helplessness and hopelessness, although patients were less anxious, better prepared and reported fewer problems [118].

A third approach advocates a systems approach to psychosocial complications and quality of life issues. In theory, this would help establish a therapeutic relationship before it's needed, normalize the experience of distress to make it more acceptable, integrate psychosocial providers into the treatment team, and allow routine screening for distress [7, 65, 108]. However, a randomized trial of psychosocial screening and disclosure to treating physicians failed to demonstrate a benefit of intervention during the acute hospitalization (N = 178) [72].

A final approach to improving quality of life is to institute procedures to counteract specific problems. For example, several studies show that monitored or self-administered aerobic exercise programs improve fatigue, physical performance and psychological distress in HCT patients [119–124]. A randomized controlled trial of a comprehensive coping program involving

preparatory information, cognitive restructuring and relaxation with guided imagery administered to patients undergoing autologous transplantation for breast cancer (N = 110) demonstrated less nausea and anxiety in the intervention group within seven days post-transplantation, but did not affect pain or psychological distress [125]. Syrjala performed a randomized controlled trial (N = 94) with four different groups: standard care, therapist support, relaxation and imagery, and a package of cognitive-behavioral techniques that included relaxation and imagery. The two groups that were taught relaxation and imagery had significantly less pain. However, the addition of cognitive-behavioral techniques to one group did not improve pain control, and no intervention improved nausea [126]. Thus, while aerobic exercise and relaxation imagery have shown benefits in small studies, they have not been embraced by the HCT community as part of routine care.

9. Quality of Life Issues for Special Populations

9.1. Pediatrics

Studies of pediatric QOL in HCT have been limited, but the bulk of evidence suggests better recovery in children than adults. Badell interviewed 98 disease free survivors more than three years after pediatric HCT (74% allogeneic recipients) and compared results with 58 healthy control subjects. Transplant survivors reported higher global QOL than the controls. They reported better sleep, family and friend relationships and leisure possibilities, but perceived more problems with physical appearance, school and work possibilities than their peers. The authors concluded that pediatric survivors seem to value both their lives and their free time more highly [60]. Schmidt interviewed 212 survivors (90% participation rate) at least one year following allogeneic transplantation using the City of Hope/Stanford survivor index. Fifty subjects were pediatric survivors. Adults suffered from more chronic GVHD, frequent colds and skin changes than pediatric survivors. Thirty-six percent of adults and 50 percent of children rated their overall QOL as 10 out of a possible 10. The authors conclude that younger patients recover more fully from HCT than adults [48]. Matthes-Martin assessed 73 pediatric allogeneic survivors at least one year post-transplantation. They reported that 75 percent of patients (or surrogates for patients less than 12–years-old) had excellent quality of life, and few had detectable organ impairments, although 27 percent had chronic GVHD [127].

Phipps and colleagues studied 153 children longitudinally during HCT at St. Jude's. They describe a high level of psychological and physical distress before HCT that peaks at approximately one week, substantially improves by one month and continues to ebb over the first six months post-transplant. Older age, allogeneic procedure and lower socioeconomic status predicted greater levels of distress [128, 129]. This study, as well as others, compared child ratings with parental ratings. While there was some correlation, collecting the quality of life data directly from children is recommended [128, 130–132].

9.2. Family Members

HCT is a process that families go through together and every family member's quality of life is probably impacted by the demands of the procedure. Dermatis

and Lesko studied 61 parents prior to transplantation of their children and found very high levels of psychological distress, with mothers displaying more difficulties than fathers [133]. Manne studied 111 mothers of children surviving at least 18 months after HCT and reported that approximately 20 percent had clinically significant distress reactions [134].

Surprisingly little is reported about HCT's impact on adult patients' families. Qualitative studies hint at the family distress instigated by HCT [5, 7]. Keogh studied 28 patients and 25 relatives (not the donor) prior to transplant, and at three, six and 12 months after HCT. The study design specified that if a patient died, the relative was no longer contacted. The authors concluded that relatives were quite stressed prior to transplant and at three months, but most were no longer distressed by 12 months. However, qualitative statements suggested that the support of others (not the patient) "kept them going" (68%) and that most expected life to get back to normal by three to six months. When this did not occur, they were surprised by the "reluctance" of patients to do so and by the patient's unhappiness and irritability. Forty-five percent reported tension and conflict around this issue and 64 percent expressed feelings of resentment and frustration at the continued dependence of the patient [135]. Results from a large, cross sectional, multicenter study of HCT survivors, significant others and controls suggest that spouses suffer considerably from social isolation (John Wingard, personal communication, Feb 2002).

Although many patients have dependent children, little is written in the HCT literature about effects on children and ways to minimize adverse impacts on their development and parent-child relationships [136]. Similarly, given that HCT is a semi-elective procedure associated with extremely high mortality rates, little is written about approaches to manage family stress or family bereavement issues.

9.3. Donors

For every patient undergoing allogeneic transplantation, there is a donor somewhere whose quality of life may be affected by the procedure. Although the absolute risk of severe or life threatening complications is quite low [37], donors undergo painful procedures to provide stem cells. Although almost all recover and return to normal function, this may take a prolonged period of time and may not be complete for some patients. Studies suggest that unrelated donors, who provide anonymous stem cells for purely altruistic reasons, report less discomfort than related donors [138]. Certain techniques, such as use of long-acting local anesthetics after marrow donation, may further decrease the pain [139]. Even when marrow aspiration is avoided, as with peripheral cell donation, donors receive injections of growth factors and undergo leukapheresis to remove stem cells. Using growth factors in normal donors is commonly associated with a need for narcotic pain relief, particularly for severe back pain. There are rare reports of major complications (splenic rupture). Nevertheless, several studies suggest that recovery is faster with peripheral blood donation than bone marrow [140, 141]. The QOL of peripheral blood related donors was better than bone marrow donors up to one month after donation in a randomized Canadian study [141].

Two studies of the first 493 unrelated donors who participated in the National Marrow Donor Program suggest that the risk of acute complications

is low (6%), and that most donors experience positive psychological benefits from marrow donation. At one year, 87 percent felt donation was "very worthwhile" and 91 percent would donate again in the future. However, some donors did experience stress and inconvenience as a result of the donation, and longer collection times predicted poorer outcomes. At one week post-harvest, more than half still reported fatigue, pain at marrow collection sites and low back pain. Mean recovery time was 15.8 days, but 10 percent took longer than 30 days to recover fully [142, 143]. Qualitative interviews with 52 unrelated donors participating in the early years of the National Marrow Donor Program suggest that donors have high self-esteem and believe that marrow donation is consistent with their values and personal concepts. Interestingly, score on the self-esteem scale one year after donation correlated with whether the patient was still alive [144]. Another study of 565 unrelated Japanese marrow donors showed that physical functioning, role functioning and pain were significantly affected one week after harvest, but functioning returned to baseline after three months. Prior to harvest, donors scored above the national average in all areas of functioning [145].

The Blood and Marrow Transplant Clinical Trials Network is conducting a randomized trial of unrelated peripheral blood versus marrow transplantation that includes assessment of donor quality of life and symptoms.

10. Research issues

10.1. Topics

QOL research seems poised to move to the next level, and evolve from observation to intervention and influence. This will depend on the field's ability to: (1) translate the observations of the past 20 years into successful interventions that maintain or improve QOL for future patients [65]; (2) incorporate QOL endpoints alongside clinical trials to help present a balanced picture of outcomes [10],and (3) translate findings of QOL studies into meaningful data that is accessible and influential to clinicians, patients and policy makers [146].

10.2. Study Design Challenges

Because by definition quality of life is individual, patients are the only reliable source of data. In addition, quality of life data must be collected in real time, as patients do not accurately recall their past quality of life [147], and data cannot be collected retrospectively from chart review. Missing data pose serious analytic problems if they are missing informatively (because death, poor health and even good health cause missing assessments). Counterbalancing these potential problems is the fact that QOL studies in HCT survivors routinely enjoy very high response rates (70–90%) [116]. Methods to address the missing data problem include rigorous attempts to collect complete data from surviving patients, depiction of results as absolute proportions (that take into account patients who die or fail to provide quality of life data) [44] and statistical models that combine quality of life and survival data (Fig. 23-2) [148, 149].

The Internet offers a powerful, efficient and convenient way to study large numbers of patients. However, this research tool has its own limitations

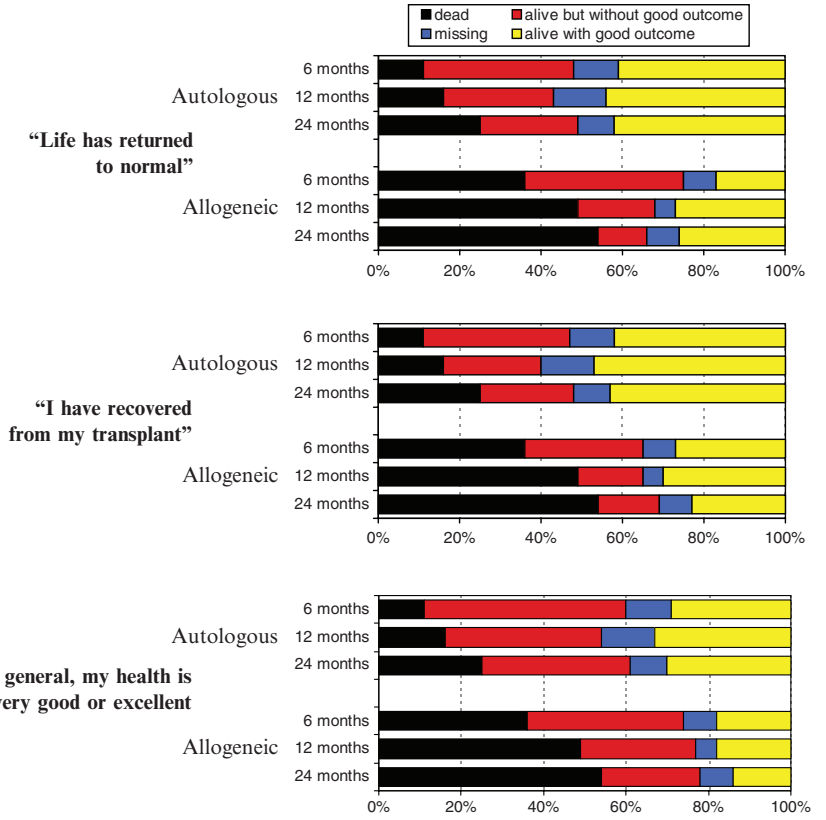

Fig. 23-2. Absolute proportions of patients at six, 12 and 24 months somewhat or strongly agreeing with the listed statements of indicating very good or excellent health Reprinted, with permission, Lee, et al. (2001)

including lack of universal Internet access, computer familiarity and concern about security issues. Bush and colleagues have piloted brief, daily assessments by the Internet and more detailed monthly assessments. While subject satisfaction with the assessment method was high, attrition due to disease relapse, death and illness and initial refusal to participate meant that fewer than half of patients who were approached for enrollment completed the study [150].

Choosing QOL instruments will vary depending on the study population and the research question. Complications during the early hospital phase may be effectively measured by certain instruments [151], whereas other instruments are designed to capture the full spectrum of QOL issues from acute hospitalization to long-term follow-up [152, 153]. Late complications such as chronic GVHD may best be captured by specific instruments [154].

Readers evaluating the quality of QOL studies should consider how QOL was measured, how representative of the universe of patients the study population is, and whether appropriate analytic methods were used. When available, a pre-transplant baseline and appropriate repeated measures techniques should be used since many deficits already exist prior to HCT [155, 156] and means can be deceptive as some subjects improve while others worsen

[157]. Comparison or control groups should be included whenever possible to acknowledge the variation in QOL and help place the results in context. Specific broad domains, as well as symptoms, should be measured because HCT patients tend to report very high global QOL while suffering from multiple problems. Finally, readers should consider whether the reported outcomes are clinically meaningful or not, and whether the authors have helped readers translate results into communicable concepts for patients.

11. Future Directions

While most patients and health care providers acknowledge the importance of QOL, several factors in HCT have conspired to limit the extent to which this concern is translated into practice and decision making. First, QOL after HCT is variable, to a large extent unpredictable and, thus far, unmodifiable. It is difficult to factor QOL into treatment decisions when outcome varies so much. Second, most patients have diseases for which there is no other reasonable chance of cure. Thus, QOL concerns may be relegated to a more informational role. Third, most reports do not translate results of QOL studies back into language and concepts that are accessible to patients and practicing physicians. Finally, several authors have suggested the field needs to move beyond small cross sectional studies. Larger, longitudinal studies evaluating potential interventions to improve QOL or that seek QOL differences between modifiable transplant practices should be the new standard. Only by moving to the next level of research studies will we be able to actually improve QOL and return the investment of so many patients who faithfully fill out our surveys.

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Chapter 24

Pathophysiology of Acute Graft-versus-Host Disease

Pavan Reddy and James L. M. Ferrara

I. Introduction

Allogeneic hematopoietic cell transplantation (HCT) represents an important therapy for many hematological and some epithelial malignancies, and for a spectrum of nonmalignant diseases [1]. The development of novel strategies such as donor leukocyte infusions (DLI), nonmyeloablative HCT and cord blood transplantation (CBT) have helped expand the indications for allogeneic HCT over the last several years, especially among older patients [2]. However, the major toxicity of allogeneic HCT, Graft-versus-Host disease (GVHD), remains a lethal complication that limits its wider application [3]. Depending on when it occurs after HCT, GVHD can be either acute or chronic [4–7]. Acute GVHD is responsible for 15 percent to 40 percent of mortality and is the major cause of morbidity after allogeneic HCT, while chronic GVHD occurs in up to 50 percent of patients who survive three months after HCT [1, 7]. Research efforts over the years have provided increasing insight into the biology of this complex disease process.

The GVHD reaction was first noted when irradiated mice were infused with allogeneic marrow and spleen cells [8]. Although mice recovered from radiation injury and marrow aplasia, they subsequently died with "secondary disease [8]," a syndrome that causes diarrhea, weight loss, skin changes and liver abnormalities. This phenomenon was subsequently recognized as GVHD disease (GVHD). Three requirements for the developing GVHD were formulated by Billingham [9]. First, the graft must contain immunologically competent cells, now recognized as mature T cells. In both experimental and clinical allogeneic BMT, the severity of GVHD correlates with the number of transfused donor T cells [10, 11]. The precise nature of these cells and the mechanisms they use are now understood in greater detail (discussed below). Second, the recipient must be incapable of rejecting the transplanted cells (i.e., immunocompromised). A patient with a normal immune system will usually reject cells from a foreign donor. In allogeneic BMT, the recipients are usually immunosuppressed with chemotherapy and/or radiation before stem cell infusion [2]. Third, the recipient must express tissue antigens that are not present in the transplant donor. This area has been the focus of intense research that

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has led to the discovery of the major histocompatibility complex (MHC) [12]. Human leukocyte antigens (HLA) are proteins that are the gene products of the MHC and that are expressed on the cell surfaces of all nucleated cells in the human body. HLA proteins are essential to the activation of allogeneic T cells [12, 13] (discussed below).

This chapter on the pathophysiology of acute GVHD will place the genetic basis and the immuno-biological mechanisms of Billingham's postulates in perspective.

II. Genetic Basis of GVHD

Billingham's third postulate stipulates that GVHD reaction occurs when donor immune cells recognize disparate host antigens [9]. These differences are governed by the genetic polymorphisms of the HLA system and the non-HLA systems such as the killer immunoglobulin receptors (KIR) family of NK receptors, nucleotide binding oligomerization domain (NOD) 2 and cytokine gene polymorphisms [2, 13].

A. HLA matching

The HLA antigens are encoded by the MHC gene complex on the short arm of chromosome 6 and can be categorized as Class I, II and III. Class I antigens (HLA A, B and C) are expressed on almost all cells of the body at varying densities [12]. Class II antigens include DR, DQ and DP antigens and are primarily expressed on hematopoietic cells (B cells, DCs, monocytes), although their expression can also be induced on many other cell types following inflammation or injury [12]. The incidence of acute GVHD is directly related to the degree of MHC mismatch [14–17]. The role of HLA mismatching in cord blood transplant (CBT) is more difficult to analyze than in unrelated HSCT, because allele level typing of CB units for HLA-A, B, C, DRB1 and DQB1 is not routinely performed [18]. Nonetheless, the total number of HLA disparities between the recipient and the CB unit has been shown to correlate with risk of acute GVHD, and the frequency of severe acute GVHD was lower in patients transplanted with matched (6/6) CB units [18–20].

B. Minor Histocompatibility Antigens (MiHAs)

Despite HLA identity between a patient and donor, substantial numbers (40%) of patients receiving HLA-identical grafts and optimal post-grafting immune suppression, develop acute GVHD due to differences in MiHAs that lie outside the HLA loci [21, 22]. Recent efforts have identified some MiHAs such as, HA-1 and HA-2, that are primarily found on hematopoietic cells [23]. These proteins may, therefore, induce GVHD. By contrast, other MiHAs, such as H-Y and HA-3, are expressed ubiquitously (see Table 24-1) [22]. The role of specific and immunodominant MiHAs that are relevant in GVHD has not been systematically evaluated in large groups of patients.

C. Other Non-HLA genes

Genetic polymorphisms in several non-HLA genes such as in KIRs, cytokines and NOD2 genes have recently been shown to modulate the severity and incidence of GVHD.

Table 24-1.

Minor Histocompatibility Antigens	Tissue Distribution
HA-1	Hematopoietic
HA-2	Hematopoietic
HB-1	Hematopoietic
BCL2A1	Hematopoietic
HA-3	Ubiquitous
HA-8	Ubiquitous
UGT2B17	Ubiquitous
HY (A, B, DR, DQ) antigens	Ubiquitous

1. KIR Polymorphisms

KIR receptors on NK cells that bind to the HLA class I gene products are encoded on chromosome 19. Polymorphisms in the transmembrane and cytoplasmic domains of KIR receptors governs whether the receptor has inhibitory potential (such as KIR2DL1, -2DL2, -2DL3 and 3DL1 and their HLA class I ligands HLA-C and HLA-Bw4) or activating potential; at this time, there is limited information on the clinical significance of activating KIR genes. Two competing models have been proposed for HLA-KIR allorecognition by donor NK cells following HSCT: "mismatched ligand" and the "missing ligand" models [24–28]. The former posits that NK alloreactivity occurs when donor NK cells recognize recipient target cells that lack the class I allele of the donor (HLA mismatching between the donor and recipient in the GVHD direction); the latter hypothesizes that donor NK alloreactivity occurs when the host lacks the correct HLA class I ligand(s) to provide the inhibitory signal for donor KIR [27, 28]. Both models are supported by some clinical observations, albeit in patients receiving very different transplant and immunosuppressive regimens [25, 29–31]. Further validation is warranted and it is likely that the immuno-biology of the interface between HLA and KIR genetics will be an area of intense future investigation.

2. Cytokine Gene Polymorphisms

Proinflammatory cytokines involved in the classical "cytokine storm" of GVHD (discussed below), cause pathological damage of target organs such as skin, gut and liver [32]. Several cytokine gene polymorphisms, both in hosts and donors, have been implicated. Specifically, TNF polymorphisms (TNFd3/d3 in the recipient, TNF-863 and -857 in donors and/or recipients, and TNFd4, TNF-α-1031C and TNFRII-196R- in the donors) have been associated with an increased risk of acute GVHD and transplant-related mortality [33, 34]. The three common haplotypes of the IL-10 gene promoter region in recipients representing high, intermediate and low production of IL-10 have been associated with the severity of acute GVHD following allo-BMT after HLA-matched sibling donors [35]. By contrast, smaller studies have found neither IL-10 nor TNF-α polymorphisms to be associated with GVHD after HLA-mismatched cord blood transplants [34, 36]. IFN-γ polymorphisms of the 2/2 genotype (high IFN-γ production) and 3/3 genotype (low IFN-γ) have been associated with decreased or increased acute GVHD, respectively [34, 37]. A small study

in pediatric recipients of unrelated HSCT suggested that the presence of the IL-1α-889 allele in either donor or recipient decreased transplant-related mortality, but did not decrease GVHD [34]. NOD2/CARD15 gene polymorphisms in both the donors and recipients were recently shown to have a striking association with GI GVHD and overall mortality after both related and unrelated allogeneic HSCT [38]. It is likely that non-HLA gene polymorphisms might play differing roles depending on the donor source (related versus unrelated), HLA disparity (matched versus mismatched), source of the graft (CB versus PBSC versus BM), and the intensity of the conditioning.

III. Immunobiology

It is helpful to remember two important principles when considering the pathophysiology of acute GVHD. First, acute GVHD reflects exaggerated, but normal inflammatory mechanisms that occur in a setting where they are undesirable. The donor lymphocytes that have been infused into the recipient function appropriately, given the foreign environment they encounter. Second, donor lymphocytes encounter tissues in the recipient that have often been profoundly damaged. The effects of the underlying disease, prior infections and the intensity of conditioning regimen all result in substantial changes not only in the immune cells, but also in the endothelial and epithelial cells. Thus, the allogeneic donor cells rapidly encounter not only a foreign environment, but one that has been altered to promote the activation and proliferation of inflammatory cells. Thus, the pathophysiology of acute GVHD may be considered a distortion of the normal inflammatory cellular responses [39]. The development and evolution of acute GVHD can be conceptualized in three sequential phases (Fig. 24-1) to provide a unified perspective on the complex cellular interactions and inflammatory cascades that lead to acute GVHD: (1) activation of the antigen-presenting cells (APCs) (2) donor T cell activation, differentiation and migration and (3) effector phase [39].

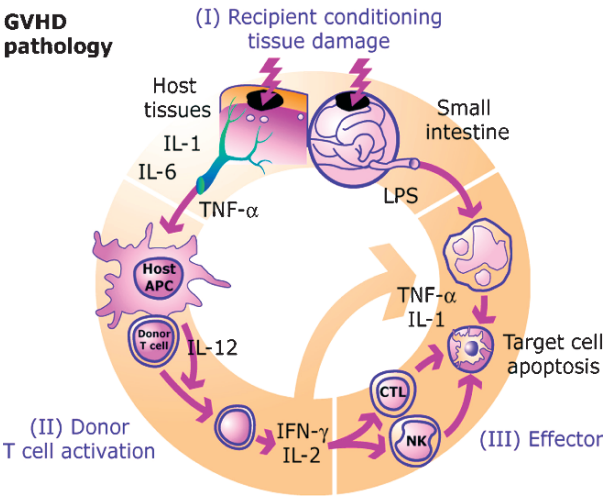

Fig. 24-1 Three phases of GVHD immuno-biology

A. Phase 1: Activation of Antigen Presenting Cells (APCs)

The earliest phase of acute GVHD is set into motion by the profound damage caused by the underlying disease and infections, and further exacerbated by the BMT conditioning regimens (which include total body irradiation (TBI) and/or chemotherapy) that are administered even before the infusion of donor cells [40–44]. This first step results in activating the APCs. Specifically, damaged host tissues respond with multiple changes, including the secretion of proinflammatory cytokines, such as TNF-α and IL-1, described as the "cytokine storm" [42, 43, 45]. Such changes increase expression of adhesion molecules, costimulatory molecules, MHC antigens and chemokines gradients that alert the residual host and the infused donor immune cells [43]. These "danger signals" activate host APCs [46, 47]. Damage to the gastrointestinal (GI) tract from the conditioning is particularly important in this process because it allows for systemic translocation of immuno-stimulatory microbial products such as lipopolysaccaride (LPS) that further enhance the activation of host APCs, and the secondary lymphoid tissue in the GI tract is likely the initial site of interaction between activated APCs and donor T cells [43, 48, 49]. This scenario accords with the observation that an increased risk of GVHD is associated with intensive conditioning regimens that cause extensive injury to epithelial and endothelial surfaces with a subsequent release of inflammatory cytokines, and increases the expression of cell surface adhesion molecules [43, 44]. The relationship among conditioning intensity, inflammatory cytokine and GVHD severity has been supported by elegant murine studies [45]. Furthermore, the observations from these experimental studies have led to two recent clinical innovations to reduce clinical acute GVHD: (a) reduced-intensity conditioning to decrease the damage to host tissues and, thus, limit activation of host APC and (b) KIR mismatches between donor and recipients to eliminate the host APCs by the alloreactive NK cells [26, 50].

Host type APCs that are present and have been primed by conditioning are critical for the induction of this phase; recent evidence suggests that donor type APCs exacerbate GVHD, but in certain experimental models donor type APC chimeras also induce GVHD [47, 51–53]. In clinical situations, if donor type APCs are present in sufficient quantity and have been appropriately primed, they too might play a role in the initiation and exacerbation of GVHD [54–56]. Amongst the cells with antigen-presenting capability, DCs are the most potent and play an important role in the induction of GVHD [57]. Experimental data suggest that GVHD can be regulated by qualitatively or quantitatively modulating distinct DC subsets [58–63]. In one clinical study persistence of host DC after day 100 correlated with the severity of acute GVHD while elimination of host DCs was associated with reduced severity of acute GVHD [55]. The allo-stimulatory capacity of mature monocyte derived DCs (mDCs) after reduced-intensity transplants was lower for up to six months compared to the mDCs from myeloablative transplant recipients, thus suggesting a role for host DCs and the reduction in "danger signals" secondary to less intense conditioning in acute GVHD [64]. Nonetheless this concept of enhanced host APC activation explains a number of clinical observations, such as increased risks of acute GVHD associated with advanced stage malignancy, conditioning intensity and histories of viral infections.

Other professional APCs such as monocytes/ macrophages or semi-professional APCs might also play a role in this phase. For example, recent data suggests

that host type B-cells might play a regulatory role under certain contexts [65]. Also host or donor type nonhematopoietic stem cells, such as mesenchymal stem cells or stromal cells when acting as APCs have been shown to reduce T cell allogeneic responses, although the mechanism for such inhibition remains unclear. The relative contributions of various APCs, professional or otherwise, remain to be elucidated.

B. Phase 2: Donor-T-Cell Activation, Differentiation and Migration

The infused donor T cells interact with the primed APCs and initiate the second phase of acute GVHD. This phase includes antigen presentation by primed APCs, the subsequent activation, proliferation, differentiation and migration of alloreactive donor T cells.

After allogeneic HSC transplants, both host- and donor-derived APCs are present in secondary lymphoid organs [66, 67]. The T cell receptor (TCR) of the donor T cells can recognize alloantigens either on host APCs (direct presentation) or donor APCs (indirect presentation) [68, 69]. In direct presentation, donor T cells recognize either the peptide bound to allogeneic MHC molecules or allogeneic MHC molecules without peptide [69, 70]. During indirect presentation, T cells respond to the peptide generated by degradation of the allogeneic MHC molecules presented on self-MHC [70]. An experimental study demonstrated that APCs derived from the host, rather than from the donor, are critical in inducing GVHD across MiHA mismatch [68] Recent data suggest that presenting distinct target antigens by the host and donor type APCs might play a differential role in mediating target organ damage [71, 72]. In humans, most cases of acute GVHD developed when both host DCs and donor dendritic cells (DCs) were present in peripheral blood after BMT [55].

1. Costimulation

The interaction of donor lymphocyte TCR with the host allo-peptide presented on the MHC of APCs alone is insufficient to induce T cell activation [73]. Both TCR ligation and costimulation via a "second" signal through interaction between the T cell costimulatory molecules and their ligands on APCs are required to achieve T proliferation, differentiation and survival [74]. The danger signals generated in phase 1 augment these interactions and significant progress has been made on the nature and impact of these "second" signals [75, 76]. Costimulatory pathways are now known to deliver both positive and negative signals and molecules from two major families; the B7 family and the TNF receptor (TNFR) family play pivotal roles in GVHD [77]. Interrupting the second signal by blockade of various positive costimulatory molecules (CD28, ICOS, CD40, CD30, 4-1BB and OX40) reduces acute GVHD in several murine models while antagonism of the inhibitory signals (PD-1 and CTLA-4) exacerbates the severity of acute GVHD [2, 78–83]. The various T cell and APC costimulatory molecules and the impact on acute GVHD are summarized in Table 24-2. The specific context and the hierarchy in which each of these signals play a dominant role in the modulation of GVHD remain to be determined.

2. T cell subsets

T cells consist of several subsets whose responses differ based on antigenic stimuli, activation thresholds and effector functions. The alloantigen composition of the host determines which donor T cell subsets proliferate and differentiate.

Table 24-2

T Cell costimulation	T cell	APC
Adhesion	ICAMs	LEA-I
	LEA-1	ICAM~
	CD2 (LEA-2)	LFA-3
Recognition	TCR/CD4	NIIIC hi
	TCR/CD8	Mi-Icc I
Costimulation	CD28	CD80/86
	CD152 (CTLA-4	CD8O/86
	ICOS	B7H/B7RP-1
	PD-1	PD-L1, PD-L2
	Unknown	B7-H3
	CD 154 (CD4OL)	CD4O
	CD134 (0X40)	CD134L (OX4OL)
	CD137 (4-IBB)	CDI37L (4-1IBBL)
	HVEM	LIGHT

Abbreviations: HVEM HSV glycoprotein D for herpesvirus entry mediator; LIGHT, homologous to lymphotoxins, shows inducible expression and competes with herpes simplex virus glycoprotein D for herpes virus entry mediator (HVEM), a receptor expressed by T lymphocytes

CD4+ and CD8+ cells

CD4and CD8 proteins are coreceptors for constant portions of MHC class II and class I molecules, respectively [84]. Therefore, MHC class I (HLA-A, -B, -C) differences stimulate CD8+T cells and MHC class II (HLA-DR -DP, -DQ) differences stimulate CD4+T cells [84–87]. But clinical trials of $CD4^+$ or $CD8^+$ depletion have been inconclusive [88]. This may not be surprising because GVHD is induced by MiHAs in the majority of HLA-identical BMT, which are peptides derived from polymorphic cellular proteins that are presented by MHC molecules [89]. Because the manner of protein processing depends on genes of the MHC, two siblings will have many different peptides in the MHC groove [89]. Thus, in the majority of HLA-identical BMT, acute GVHD may be induced by either or both $CD4^+$ and $CD8^+$ subsets in response to minor histocompatibility antigens [88]. The peptide repertoire for class I or class II MHC remains unknown and likely to be distinct between one individual to the next [90]. But it is plausible that only a few of these many peptides might behave as immunodominant "major minor" antigens that can potentially induce GVHD. In any event, such antigens remain to be identified and validated in large patient populations.

Central deletion by establishment of stable mixed hematopoietic chimeric state is an effective way to eliminate continued thymic production of both $CD4^+$ and $CD8^+$ alloreactive T cells and thus reduce GVHD [91–93]. In contrast peripheral mechanisms to induce tolerance of $CD4^+$ and $CD8^+$ T cells appears to be distinct [94, 95]. The T cell apoptosis pathways by which peripheral deletion occurs can be broadly categorized into activation-induced cell death (AICD) and passive cell death (PCD) [96]. Experimental data suggests that

deletional tolerance by AICD is operative via the Fas (for CD4+) or TNFR (CD8+) pathways in Th1 cells and when the frequency of alloreactive T cells is at much greater [97–102]. PCD or "death by neglect" is due to rapid downregulation of Bcl-2 and appears to be critical in non-irradiated, but not after irradiated BMT [103]. Thus, distinct mechanisms of tolerance induced by apoptosis have a dominant role depending on the T cell subsets, the conditioning regimens and the histocompatibility differences. Nonetheless strategies aimed at selective elimination of donor T cells *in vivo* after HCT, either by targeting a suicide gene to the allo-T cells or by photodynamic cell purging, appears to be promising in reducing experimental acute GVHD [104–110].

Naïve and Memory Subsets: Several independent groups have intriguingly found that, unlike memory ($CD62L^-$) T cells, the naïve ($CD62L^+$) T cells were alloreactive and caused acute GVHD across different donor/recipient strain combinations [111–114]. Furthermore, expression of the naïve T cell marker CD62L was also found to be critical to regulate GVHD by donor natural regulatory T cells [115, 116]. By contrast, another recent study demonstrated that alloreactive memory T cells and their precursor cells (memory stem cells) caused robust GVHD [117, 118].

Regulatory T cells: Recent advances indicate that distinct subsets of regulatory $CD4^+CD25^+$, $CD4^+CD25^-IL10^+$ Tr cells, γδT cells, DN^- T cells, NK T cells and regulatory DCs control immune responses by inducing anergy or active suppression of alloreactive T cells [59, 60, 119–127]. Several studies have demonstrated a critical role for the natural donor $CD4^+CD25^+$ Foxp3+ regulatory T (Treg) cells, obtained from naïve animals or generated *ex vivo*, in the outcome of acute GVHD. Donor $CD4^+CD25^+$ T cells suppressed the early expansion of alloreactive donor T cells and their capacity to induce acute GVHD without abrogating GVL effector function against these tumors [128, 129]. $CD4^+CD25^+$ T cells induced/generated by immature or regulatory host type DCs and by regulatory donor type myeloid APCs were also able to suppress acute GVHD [59]. One of the clinical studies that evaluated the relationship between donor $CD4^+CD25^+$ cells and acute GVHD in humans after matched sibling donor grafts found that, in contrast to the murine studies, donor grafts containing larger numbers of $CD4^+$ $CD25^+$T cells developed more severe acute GVHD [130]. These data suggest that coexpression of $CD4^+$ and $CD25^+$ is insufficient because an increase in $CD25^+$ T cells in donor grafts is associated with greater risks of acute GVHD after clinical HCT. Another recent study found that Foxp3 mRNA expression (considered a specific marker for naturally occurring $CD4^+CD25^+$Tregs) was significantly decreased in peripheral blood mononuclear cells from patients with acute GVHD [131, 132]. But Foxp3 expression in humans, unlike mice, may not be specific for T cells with a regulatory phenotype [133]. It is likely that the precise role of regulatory T cells in clinical acute GVHD will, therefore, not only depend upon identifying specific molecular markers in addition to Foxp3, but also on the ability for *ex vivo* expansion of these cells in sufficient numbers. Several clinical trials are underway in the United States and Europe to substantially expand these cells *ex vivo* and use for prevention of GVHD.

Host $NK1.1^+$ T cells are another T cell subset with suppressive functions that have also been shown to suppress acute GVHD in an IL-4 dependent manner [126, 127, 134]. By contrast, donor NKT cells were found to reduce

GVHD and enhance perforin mediated GVL in an IFN-γ dependent manner [135, 136]. Recent clinical data suggests that enhancing recipient NKT cells by repeated TLI conditioning promoted Th2 polarization and dramatically reduced GVHD [127]. Experimental data also show that activated donor NK cells can reduce GVHD through the elimination of host APCs or by secretion of transforming growth factor-β (TGF-β) [136]. A murine BMT study using mice lacking SH2-containing inositol phosphatase (SHIP), in which the NK compartment is dominated by cells that express two inhibitory receptors capable of binding either self or allogeneic MHC ligands, suggests that host NK cells may play a role in the initiation of GVHD [137].

3. Cytokines and T Cell Differentiation

APC and T cell activation result in rapid intracellular biochemical cascades that induce transcription of many genes including cytokines and their receptors. The Th1 cytokines (IFN-γ, IL-2 and TNF-α) have been implicated in the pathophysiology of acute GVHD [138–140]. IL-2 production by donor T cells remains the main target of many current clinical therapeutic and prophylactic approaches, such as cyclosporine, tacrolimus and monoclonal antibodies (mAbs) against the IL-2 and its receptor to control acute GVHD [141, 142]. But emerging data indicate an important role for IL-2 in the generation and maintenance of $CD4^+CD25^+$ Foxp3+ T regs, suggesting that prolonged interference with IL-2 may have the unintended consequence of preventing the development of long-term tolerance after allogeneic HCT [143–146].

Similarly the role of other Th1 cytokines IFN-γ or their inducers as regulators or inducers of GVHD severity depends on the degree of allo-mismatch, the intensity of conditioning and the T cell subsets that are involved after BMT [147–149]. Thus, although the "cytokine storm" initiated in phase 1 and amplified by the Th1 cytokines correlates with the development of acute GVHD, early Th1 polarization of donor T cells to HCT recipients can attenuate acute GVHD suggesting that physiological and adequate amounts of Th1 cytokines are critical for GVHD induction, while inadequate production (extremely low or high) could modulate acute GVHD through a breakdown of negative feedback mechanisms for activated donor T cells [140, 149–152]. Several different cytokines that polarize donor T cells to Th2 such as IL-4, G-CSF, IL-18, IL-11, rapamycin and the secretion of IL-4 by $NK1.1^+$ T cells can reduce acute GVHD [153–160]. But Th1 and Th2 subsets cause injury of distinct acute GVHD target tissues, and some studies failed to show a beneficial effect of Th2 polarization on acute GVHD [161]. Thus, the Th1/Th2 paradigm of donor T cells in the immuno-pathogenesis of acute GVHD has evolved over the last few years and its causal role in acute GVHD is complex and incompletely understood.

IL-10 plays a key role in suppressing immune responses and its role in regulating experimental acute GVHD is unclear [162]. Recent clinical data demonstrate an unequivocal association between IL-10 polymorphisms and the severity of acute GVHD [35]. TGF-β, another suppressive cytokine, was shown to suppress acute GVHD, but to exacerbate chronic GVHD [163]. The roles of some other cytokines, such as IL-7 (that promotes immune reconstitution) and IL-13, remain unclear [164–167]. The role for Th17 cells, a recently described novel T cell differentiation in many immunological processes, is not

yet known [168]. In any case, all of the experimental data so far collectively suggest that the timing of administration, the production of any given cytokine, the intensity of the conditioning regimen and the donor-recipient combination may all be critical to the eventual outcome of acute GVHD.

4. Leukocyte Migration

Donor T cells migrate to lymphoid tissues, recognize alloantigens on either host or donor APCs and become activated. They then exit the lymphoid tissues and traffic to the target organs, causing tissue damage [169]. The molecular interactions necessary for T cell migration and the role of lymphoid organs during acute GVHD have recently become the focus of a growing body of research. Chemokines play a critical role in the migration of immune cells to secondary lymphoid organs and target tissues [170]. T-lymphocyte production of macrophage inflammatory protein-1alpha (MIP-1α) is critical to the recruitment of $CD8^+$, but not $CD4^+$ T cells to the liver, lung and spleen during acute GVHD [171]. Several chemokines such as CCL2-5, CXCL2, CXCL9-11, CCL17 and CCL27 are overexpressed and might play a critical role in the migration of leukocyte subsets to target organs liver, spleen, skin and lungs during acute GVHD [169–172]. CXCR3+ T and CCR5+ T cells cause acute GVHD in the liver and intestine [169, 173–175]. CCR5 expression has also been found to be critical for Treg migration in GVHD [176]. In addition to chemokines and their receptors, expression of selectins and integrins and their ligands also regulate the migration of inflammatory cells to target organs [170]. For example, interaction between $\alpha4\beta7$ integrin and its ligand MadCAM-1 are important for homing of donor T cells to Peyer's patches and in the initiation of intestinal GVHD [48, 177]. $\alpha L\beta2$ / ICAM1, 2, 3 and $\alpha4\beta1$ / VCAM-2 interactions are important for homing to the lung and liver after experimental HCT [169]. Expressing CD62L on donor Tregs is critical for their regulation of acute GVHD, suggesting that their migration in secondary tissues is critical for their regulatory effects [67]. The migratory requirement of donor T cells to specific lymph nodes (e.g., Peyer's patches) for the induction of GVHD might depend on other factors such as the conditioning regimen, inflammatory milieu, etc. [48, 178]. Furthermore, FTY720, a pharmacologic sphingosine-1-phosphate receptor agonist, inhibited GVHD in murine, but not in canine models of HCT [179, 180]. Thus, there might also be significant species differences in the ability of these molecules to regulate GVHD.

C. Phase 3: Effector Phase

The effector phase that leads to the GVHD target organ damage is a complex cascade of multiple cellular and inflammatory effectors that further modulate each others' responses either simultaneously or successively. Effector mechanisms of acute GVHD can be grouped into cellular effectors (e.g., CTLs) and inflammatory effectors such as cytokines. Inflammatory chemokines expressed in inflamed tissues upon stimulation by proinflammatory effectors such as cytokines are specialized for the recruitment of effector cells, such as CTLs [181]. Furthermore the spatio-temporal expression of the cyto-chemokine gradients might determine not only the severity, but also the unusual cluster of GVHD target organs (skin, gut and liver) [169–182].

1. Cellular Effectors

Cytotoxic T cells (CTLs) are the major cellular effectors of GVHD [183, 184]. The Fas-Fas ligand (FasL), the perforin-grazyme (or granule exocytosis) and TNFR-like death receptors (DR), such as TNF-related apoptosis-inducing ligand (TRAIL: DR4, 5 ligand) and TNF-like weak inducers of apoptosis (TWEAK: DR3 ligand), are the principle CTL effector pathways that have been evaluated after allogeneic BMT [184–189]. The involvement of each of these molecules in GVHD has been tested by utilizing donor cells that are unable to mediate each pathway. Perforin is stored in cytotoxic granules of CTLs and NK cells, together with granzymes and other proteins. Although the exact mechanisms remain unclear, following the recognition of a target cell through the TCR-MHC interaction, perforin is secreted and inserted into the cell membrane, forming "perforin pores" that allow granzymes to enter the target cells and induce apoptosis through various downstream effector pathways such as caspases [190]. Ligation of Fas results in a death-inducing signaling complex (DISC) and also activates caspases [191, 192].

Transplantation of perforin deficient T cells results in a marked delay in the onset of GVHD in transplants across MiHA disparities only, both MHC and MiHA disparities (126), and across isolated MHC class I or MHC Class II disparities [184, 193–197]. However, mortality and clinical and histological signs of GVHD were still induced even in the absence of perforin-dependent killing in these studies, demonstrating that the perforin-granzyme pathways play little role in target organ damage. A role for the perforin-granzyme pathway for GVHD induction is also evident in studies employing donor-T cell subsets. Perforin- or granzyme B-deficient CD8+ T cells caused less mortality than wild type T cells in experimental transplants across a single MHC class I mismatch. This pathway, however, seems to be less important compared to the Fas/FasL pathway in CD4-mediated GVHD [196–198]. Thus, it seems that CD4+ CTLs preferentially use the Fas-FasL pathway, whereas CD8+CTLs primarily use the perforin-granzyme pathway.

Fas, a TNF-receptor family member, is expressed by many tissues, including GVHD target organs [199]. Its expression can be upregulated by inflammatory cytokines such as IFN-γ and TNF-α during GVHD, and the expression of FasL is also increased on donor T cells, indicating that FasL-mediated cytotoxicity may be a particularly important effector pathway in GVHD [184, 200]. FasL-defective T cells cause less GVHD in the liver, skin and lymphoid organs [195, 198, 200]. The Fas-FasL pathway is particularly important in hepatic GVHD, consistent with the keen sensitivity of hepatocytes to Fas-mediated cytotoxicity in experimental models of murine hepatitis [184]. Fas-deficient recipients are protected from hepatic GVHD, but not from other organ GVHD, and administration of anti-FasL (but not anti-TNF) MAbs significantly blocked hepatic GVHD damage occurring in murine models [184, 201, 202]. Although the use of FasL-deficient donor T cells or the administration of neutralizing FasL MAbs had no effect on the development of intestinal GVHD in several studies, the Fas-FasL pathway may play a role in this target organ, because intestinal epithelial lymphocytes exhibit increased FasL-mediated killing potential [203]. Elevated serum levels of soluble FasL and Fas have also been observed in at least some patients with acute GVHD [204, 205].

Using a perforin-granzyme and FasL cytotoxic double-deficient (*cdd*) mouse provides an opportunity to address whether other effector pathways are

capable of inducing GVHD target organ pathology. An initial study demonstrated that *cdd* T cells were unable to induce lethal GVHD across MHC class I and class II disparities after sublethal irradiation [194]. However, subsequent studies demonstrated that cytotoxic effector mechanisms of donor T cells are critical in preventing host resistance to GVHD [188, 206]. Thus, when recipients were conditioned with a lethal dose of irradiation, *cdd* CD4+ T cells produced similar mortality to wild type CD4+ T cells [188]. These results were confirmed by a recent study demonstrating that GVHD target damage can occur in mice that lack alloantigen expression on the epithelium, preventing direct interaction between CTLs and target cells [189].

The participation of another death ligand receptor signaling pathway, TNF/TNFRs, has also been evaluated. Experimental data suggests that this pathway is crucial for GI GVHD (discussed more below). Recently, several additional TNF family apoptosis-inducing receptors/ligands have been identified, including TWEAK, TRAIL and LTβ/LIGHT, and are all assumed to play a role in GVHD and GVL responses 2, 207–213]. However, whether these distinct pathways play a more specific role for GVHD mediated by distinct T cell subsets in certain situations remains unknown. Intriguingly, recent data suggest that none of these pathways might be critical for mediating the rejection of donor grafts [207, 214]. Thus, it is likely that their role in GVHD might be modulated by the intensity of conditioning and by the recipient T cell subsets. Existing experimental data suggest that perforin and TRAIL cytotoxic pathways are associated with CD8+ T cell–mediated GVL [184]. The available experimental data are strongly skewed toward CD8+ T cell–mediated GVL based on the dominant role of this effector population in most murine GVT models; however, CD4+ T cells can mediate GVL and might be crucial in clinical BMT depending on the type of malignancy and the expression of immunodominant antigens.

Taken together, although experimental data suggest there might be some distinction between the use of different lytic pathways for the specific GVHD target organs and GVL, the clinical applicability of these observations is as yet largely unknown

2. Inflammatory Effectors

Inflammatory cytokines synergize with CTLs resulting in the amplification of local tissue injury and further promotion of an inflammation, which ultimately leads to the observed target tissue destruction in the transplant recipient [32]. Macrophages, which had been primed with IFN-γ during step 2, produce inflammatory cytokines TNF-α and IL-1 when stimulated by a secondary triggering signal [215]. This stimulus may be provided through Toll-like receptors (TLRs) by microbial products such as LPS and other microbial particles, which can leak through the intestinal mucosa damaged by the conditioning regimen and gut GVHD [216, 217]. It is now apparent that immune recognition through both TLR and non-TLRs (such as NOD) by the innate immune system also controls activation of adaptive immune responses [216, 218]. Recent clinical studies of GVHD suggested the possible association between TLR/NOD polymorphisms and the severity of GVHD [38, 219, 220]. LPS and other innate stimuli may stimulate gut-associated lymphocytes, keratinocytes, dermal fibroblasts and macrophages to produce proinflammatory effectors that play a direct role in causing target organ damage. Indeed experimental data

with MHC-mismatched BMT suggest that, under certain circumstances, these inflammatory mediators are sufficient in causing GVHD damage even in the absence of direct CTL-induced damage [51]. The severity of GVHD appears to be directly related to the level of innate and adaptive immune cell priming and release of proinflammatory cytokines such as TNF- α, IL-1 and nitric oxide (NO) [51, 217, 221–223].

The cytokines TNF- α and IL-1 are produced by an abundance of cell types during processes of both innate and adaptive immunity; they often have synergistic, pleiotrophic and redundant effects on both activation and effector phases of GVHD [140]. A critical role for TNF- α in the pathophysiology of acute GVHD was first suggested over 20 years ago because mice transplanted with mixtures of allogeneic BM and T cells developed severe skin, gut and lung lesions that were associated with high levels of TNF- α mRNA in these tissues [224]. Target organ damage could be inhibited by an infusion of anti-TNF- α MAbs, and mortality could be reduced from 100 percent to 50 percent by the administration of soluble TNF- α receptor (sTNFR), an antagonist of TNF- α [42, 45, 222]. Accumulating experimental data further suggest that TNF -α is involved in a multi-step process of GVHD pathophysiology. TNF-α can (1) cause cachexia, a characteristic feature of GVHD, (2) induce maturation of DCs, thus enhancing alloantigen presentation, (3) recruit effector T cells, neutrophilis and monocytes into target organs through the induction of inflammatory chemokines and (4) cause direct tissue damage by inducing apoptosis and necrosis. TNF-α also involves donor-T cell activation directly through its signaling via TNFR1 and TNFR2 on T cells. TNF-TNF1 interactions on donor T cells promote alloreactive T cell responses, and TNF-TNFR2 interactions are critical for intestinal GVHD [210, 225]. TNF-α also seems to be an important effector molecule in GVHD in skin and lymphoid tissue [224, 226]. Additionally, TNF-α might also be involved in hepatic GVHD, probably by enhancing effector cell migration to the liver via the induction of inflammatory chemokines [227]. An important role for TNF-α in clinical acute GVHD has been suggested by studies demonstrating elevated serum levels or TNF- α or elevated TNF- α mRNA expression in peripheral blood mononuclear cells in patients with acute GVHD and other endothelial complications, such as hepatic veno-occlusive disease (VOD) [227–230]. Phase I-II trials using TNF-α antagonists reduced the severity of GVHD suggesting that it is a relevant effector in causing target organ damage [231, 232].

The second major proinflammatory cytokine that appears to play an important role in the effector phase of acute GVHD is IL-1 [233]. Secretion of IL-1 appears to occur predominantly during the effector phase of GVHD of the spleen and skin, two major GVHD target organs [234]. A similar increase in mononuclear cell IL-1 mRNA has been shown during clinical acute GVHD. Indirect evidence of a role for IL-1 in GVHD was obtained by administering this cytokine to recipients in an allogeneic murine BMT model. Mice receiving IL-1 displayed a wasting syndrome and increased mortality that appeared to be an accelerated form of disease. By contrast, intraperitoneal administration of IL-1ra starting on day 10 post-transplant reversed the development of GVHD in the majority of animals, giving treated animals a significant survival advantage [235]. However, the attempt to use IL-1ra to prevent acute GVHD in a randomized trial was not successful [236].

As a result of activation during GVHD, macrophages also produce NO, which contributes to the deleterious effects on GVHD target tissues, particularly immunosuppression [223, 237]. NO also inhibits the repair mechanisms of target tissue destruction by inhibiting proliferation of epithelial stem cells in the gut and skin [238]. In humans and rats, the development of GVHD is preceded by an increase in serum levels of NO oxidation products [239–242].

Existing data demonstrate the important role of various inflammatory effectors in GVHD. The relevance of currently studied, or as yet unknown specific effectors, might, however, be determined by other factors, including the intensity of preparatory regimens, the type of allograft, the T cell subsets and the duration of BMT. In any event, both experimental and clinical data suggest an important role for both the cellular and inflammatory mediators in GVHD-induced target organ damage.

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Chapter 25

Acute Graft-versus-Host Disease

Martin Benesch and H. Joachim Deeg

1. Introduction

Graft-versus-Host Disease (GVHD) has remained the most frequent complication after allogeneic hematopoietic cell transplantation (HCT). GVHD was first described as "secondary disease" in mice that had their marrow function ablated by total body irradiation (TBI) [1] and were then given viable splenocytes from histoincompatible donors. The syndrome was soon found to be caused by immuno-competent donor cells, specifically T lymphocytes [2-4]. Arguably, the first case of GVHD in humans was observed after marrow cells were used to treat survivors of a nuclear accident in former Yugoslavia [5]. With the establishment of the scientific basis for human HCT, GVHD emerged as a challenging problem even with transplantation of cells from sibling donors who were identical with the patient for the antigens of the major histocompatibility complex (MHC), termed HLA (human leukocyte antigen) in humans.

An early acute form of GVHD and a delayed chronic form were originally described [6]. However, a clear distinction between acute and chronic GVHD is no longer tenable. Observations in patients transplanted with reduced-intensity conditioning (RIC) regimens or in patients receiving donor lymphocyte infusions (DLI) at various time intervals after HCT indicate that patients may have acute GVHD several months after transplantation [7]. Conversely, GVHD with characteristics of the "chronic" form can occur as early as two months after transplantation [7, 8]. The recent NIH Consensus Conference suggested two categories of GVHD: 1) acute GVHD (absence of features consistent with chronic GVHD), comprising a) classic acute GVHD (before day 100) and b) persistent, recurrent or late acute GVHD (after day 100, often upon withdrawal of immunosuppression), and 2) chronic GVHD, comprising a) classic chronic GVHD (no signs of acute GVHD) and b) an overlap syndrome, in which features of both acute and chronic GVHD are present [9].

GVHD can also occur after blood transfusions (transfusion-associated GVHD) or in patients receiving autologous or syngeneic transplants (autologous or syngeneic GVHD) [10].

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2. Pathophysiology

The basic requirements for GVHD were summarized by Billingham [4]: 1) immuno-competent cells in the graft; 2) expression of tissue antigens in the patient that are not present in (and therefore novel to) the donor; 3) inability of the recipient to reject or destroy the donor cells; research over the past 40 years has generated a large amount of data and led to additional insights, e.g. that inappropriate recognition of self antigens (after reinfusion of autologous cells) can also lead to a GVHD-like disease (autologous GVHD) [10]; however, the basic Billingham criteria are still valid.

The current understanding of acute GVHD is best reflected by a three-step model (reviewed in [11]). In this model, TBI or other cytotoxic therapy results in the release of inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α, causing apoptosis in endothelial cells; in the intestinal tract, this process may lead to lipopolysaccharides (LPS) and other breakdown products entering the circulation. This process further enhances cytokine release and finally results in activation and expansion of donor cells, including T lymphocytes. Murine experiments have shown that host antigen-presenting cells, in particular dendritic cells, are essential, and that the cytokines released by tissue damage upregulate MHC gene products on those cells. CD4+ T cells interact primarily with MHC class II molecules (and associated peptides), and CD8+ cells with MHC class I. Minor histocompatibility antigens (miHA) are also presented by MHC molecules. Numerous cytokines are released from activated T cells, including interferon (IFN)-γ, IL-2 and TNF-α. IL-2 plays a central role in T cell expansion; however, the overall response depends upon the polarization to a Th1 (IL-2, TNF-α etc.) versus a Th2 (IL-10, IL-4, etc.) pattern. The interactions are complex and described in detail in recent reviews and monographs [11]. These events are followed by the generation of cytotoxic effectors including (more) inflammatory cytokines, cytotoxic effector cells (using Fas- and perforin-mediated mechanisms), large granular lymphocytes (LGL) and nitric oxide. There is good evidence for interactions of innate (LGL/NK cells) and adaptive (alloreactive T lymphocytes) immune responses that lead to end-organ damage. The recent description of natural killer T (NKT) cells and regulatory T cells (Treg) (for a detailed review see [11]) revealed the pathophysiology of GVHD to be even more complex.

3. Risk Factors for and Incidence of Acute GVHD

Several risk factors for GVHD were identified for cohorts of patients, but not for individuals. *In vitro* tests were developed, aimed at determining whether a given pre-HCT patient would develop GVHD if transplanted from a specific donor [12]. One test used patient skin biopsies in co-cultures with lymphocytes from the prospective donor. The pattern of infiltration of skin tissue by lymphocytes and the resulting histologic changes were used to predict the development of GVHD. In the initial study, a correlation of *in vitro* findings with the occurrence of GVHD of the skin, but not in other organs, was described [13, 14]. However, as with other tests, results have been difficult to reproduce [15, 16], and they have not been widely adopted. Others have attempted to assign GVHD risk based on the donor and patient polymorphisms

of CD31, IL-10 or TNFα, but the predictive value of those tests has remained controversial [17–21].

3.1. Histocompatibility

Histoincompatibility and the transfer of immuno-competent donor cells, specifically T lymphocytes that recognize "foreign" antigens in the recipient, are the two major risk factors for GVHD.

Histoincompatibility has the most profound effect when MHC (i.e., HLA barriers) are involved. Differences for class II antigens (HLA-DR,-DP,-DQ) are more relevant for GVHD, whereas differences for class I antigens (HLA-A,-B and especially -C) may interfere with engraftment [22]. Certain "mismatches," for example isolated differences for HLA-DQ, are tolerated and do not negatively impact transplant outcome [23].

Developing GVHD in HLA-identical recipients, particularly with sibling donors who had inherited the same HLA haplotypes on the two parental chromosomes "en bloc," is presumably due to differences in miHA outside MHC [24]. More insights into the role of miHA may come from currently ongoing studies on haplotype effects in recipients of unrelated donor transplants. Male individuals express unique H-Y antigens encoded on the Y chromosome, which are recognized by female donor cells in the context of HLA compatibility and trigger GVHD, particularly if the female donor is allosensitized [25-27]. Other miHA include HA-1 to -5, and probably antigens that have not yet been characterized [26, 28].

3.2. Alloreactive Donor Cells

In murine models, both CD4+ and CD8+ T cells are involved in triggering GVHD, somewhat dependent upon the histocompatibility barrier that is crossed [29]. The distinction in humans is less clear. Selective CD4 and CD8 depletion studies have not allowed for definitive conclusions [30-32]. Depletion of CD3+ T lymphocytes by two or three logs is effective in preventing or reducing the incidence of acute GVHD, although this approach may be associated with an increased risk of graft failure and disease recurrence [33, 34]. T cell reduction (rather than depletion) or graded add-back of T lymphocytes may circumvent these problems [30, 32]. The value of limiting dilution analyses of antihost-specific donor T helper and cytotoxic cells as predictors for the development of GVHD has remained controversial [35-38].

Recent data show that a high degree of donor T cell chimerism early (< day 28) after HCT is associated with an increased probability of acute GVHD, particularly in patients prepared with reduced-intensity conditioning (RIC) regimens, although it is not clear whether the high degree of chimerism is a risk factor for or a reflection of GVHD [39].

3.3. Other Risk Factors

Older age of the recipient as well as the donor is associated with an increased incidence of GVHD [27, 40]. Donor/patient sex mismatch increases the risk of GVHD; with a female donor into a male recipient the risk is increased about two-fold compared to same sex transplants [41]. Allosensitization of the donor (see above), disease stage and intensity of the conditioning regimen are further

risk factors [7, 8, 27, 40, 42-47]. Recent reports suggest that acute GVHD, especially severe GVHD, is less frequent in patients conditioned with RIC regimens [7, 48]. More controversial is the impact of certain HLA alleles [45, 49], prior splenectomy [42, 50], exposure to herpes viruses [51], ABO incompatibility [52] and CD34 cell dose [43]. Interestingly, the infusion of "mega-doses" of CD34+ cells, especially in children given haploidentical transplants, seems to be associated with an acceptable incidence of acute GVHD [53].

3.4. Incidence

Most studies only report the incidence of Grades II–IV (not Grade I) acute GVHD. Among patients transplanted from HLA-identical siblings with T cell-replete marrow, following conditioning with a conventional myeloablative regimen (e.g., TBI plus cyclophosphamide or busulfan plus cyclophosphamide) and given prophylaxis with cyclosporine (CSP) or tacrolimus (FK506; TAC) plus methotrexate (MTX) or mycophenolate mofetil (MMF), about 40 to 60 percent develop acute GVHD that requires therapy. Adding steroids to such a combination in some studies has reduced the incidence to 10 to 20 percent. Among patients transplanted for nonmalignant diseases who are generally conditioned with less intensive regimens, the incidence has ranged from 20 to 40 percent [54]. With the use of T cell-depleted marrow and CSP prophylaxis the incidence of severe Grades III/IV acute GVHD can be reduced to 15 to 20 percent compared with T cell-replete marrow and standard GVHD prophylaxis with CSP and methotrexate (MTX) (37%) [55]. The use of a gnotobiotic environment has been effective in reducing GVHD incidence at some centers (see below).

In patients transplanted from unrelated donors, acute GVHD incidence rates as high as 60 to 80 percent have been reported. Among patients prepared with RIC regimens and transplanted from HLA-identical siblings, the incidence may be 40 percent [7, 48].

4. Clinical Presentation and Diagnosis

4.1. Diagnosis of GVHD

Acute GVHD is a clinical syndrome primarily involving skin, liver and gastrointestinal tract. The skin is most frequently involved and, in most cases, accompanies manifestations in other organs. However, the diagnosis of GVHD may be difficult. For instance, an elevated direct serum bilirubin is the principal parameter for hepatic GVHD. Short of a liver biopsy, hyperbilirubinemia may be ascribed to GVHD when it is actually related to infection or transplant-related toxicities. Diarrhea may be caused by infections, conditioning or other drug toxicity, or GVHD. Similarly, GVHD skin rashes may be difficult to distinguish from drug eruptions, even on histology. Although GVHD is primarily a clinical diagnosis, histopathological confirmation should be attempted and has to fit the clinical picture.

Inter-observer variability in assessing GVHD severity can be considerable [56]. In a multicenter phase III trial, the incidence (and severity) of GVHD as determined by an independent committee was substantially lower than reported by the investigators [57]. Nevertheless, an experienced clinician will generally be able to establish a working diagnosis of GVHD that is satisfactory for a meaningful prognostic assessment [58].

4.2. Time of GVHD Onset

The onset of GVHD depends upon a patient's individual risk factors and the regimen used for GVHD prophylaxis. A severe and potentially fatal form of GVHD with fever, erythroderma and fluid retention may occur within a week of HCT in patients with major HLA mismatches and in patients who receive T cell-replete transplants without or with inadequate *in vivo* GVHD prophylaxis [59]. In patients receiving conventional GVHD prophylaxis, e.g., a combination of CSP or TAC and MTX, the median onset of GVHD is 15 to 30 days after HCT; with *in vitro* T cell-depleted transplants the onset may be delayed [60]. Thus, a rash and diarrhea by one week after HCT would likely represent GVHD, if minimal or ineffective prophylaxis were given. The same kinetics would be unlikely with CSP or TAC or *in vitro* T cell depletion of the stem cell inoculum.

A similar, but less ominous syndrome in the first one to two weeks after donor cell infusion has been termed "engraftment syndrome," which may be seen with either allogeneic or autologous HCT [61]. It is thought to be caused by a wave of cytokine production as the donor graft starts to recover, distinct from the "cytokine storm" of acute GVHD [62]. In most patients this syndrome responds promptly to steroids [63]. However, in allogeneic transplant recipients this syndrome must be distinguished from hyper-acute onset GVHD.

4.3. Skin and Mucosa

The most common manifestation of GVHD is a maculopapular, occasionally morbilliform, sometimes confluent, erythematous exanthema often involving palms and soles – a useful clinical hint, since drug eruptions are less likely on the palms. However, a painful, blistering acral erythema can also be related to conditioning therapy (e.g., busulfan) [64, 65]. Skin manifestations of GVHD may be asymptomatic, pruritic and painful. Erythema typically starts in sun-exposed areas like the shoulders, face and arms. A skin biopsy may be helpful in substantiating the diagnosis, but by itself will not prove the presence of GVHD. In its mildest form, GVHD may involve less than 25 percent of the body surface, but it can progress to whole body erythema, desquamation, bullae formation and sloughing of the skin (Table 25-1). In its severe forms GVHD can be difficult to distinguish from Stevens-Johnson syndrome or toxic epidermal necrolysis. It is noteworthy, however, that the conjunctivae are infrequently affected by acute GVHD.

The loss of integrity of the integument leads to an increased risk of infection with normal skin flora. In severe GVHD of the skin, the management resembles that of patients with severe burns, and consultation with a burn specialist is, therefore, advisable.

Mucositis was not part of the classic description of acute GVHD. However, if infection is excluded, mucosal lesions that fail to heal with hematologic recovery may signify mucosal (oral, conjunctival and vaginal) GVHD.

4.4. Liver

Hepatic GVHD is graded on the basis of total serum bilirubin levels (Table 25-2), but alkaline phosphatase and transaminase levels may also be elevated. Acute GVHD after DLI, i.e., with some delay after HCT, may

Table 25-1. Grading of acute GVHD.

A) Consensus Grading, Modified[a]

	Organ / Extent of Involvement		
Grade	**Skin (rash % BSA)**	**Liver (bilirubin; mg/dL)**	**Intestinal Tract**
0	None	None	None
I	< 50%	None	None
Iia	<50% not progressing rapidly	< 3mg/dL	Diarrhea <1,000 mL/d (<20 mL/kg/d) No blood, no cramping
Iib	≥ 50% *or* rapidly progressing	≥ 3 mg/dL[b]	Diarrhea ≥ 1,000 mL/d ±blood, cramping
III	—	≥ 6 and ≤ 15 mg/dL	*or* Diarrhea ≥ 1,500 mL/d Abdominal pain ± ileus
IV	Generalized erythema and bullae formation	*or* >15mg/Dl	—

B) IBMTR Severity Index[c]

	Organ / Extent of Involvement					
					Gastrointestinal Tract	
Index	**Skin (rash % BSA)**		**Liver (bilirubin; mg/dL)**		**Diarrhea (mL/day)**	**UGI**
A	<25	<3.4	<500	—		
B	25–50	*or*	3.5–7.9	*or*	500–1,500	Nausea, vomiting or epigastric pain
C	>50	*or*	8.0–15.0	*or*	>1,500	Positive biopsy
D	Bullae	*or*	>15.0	*or*	Severe pain, ileus	—

[a] Adapted from the Consensus Report by Przepiroka, et al. [90] with incorporation of a split of grade II into two subgroups
[b] ≥ 6 mg/dL if other hepatic complication are present
[c] Index is assigned on the basis of maximum involvement in any organ system (modified from [58]
Abbreviations: BSA = body surface area; UGI = upper gastrointestinal tract

take the form of "hepatitic" GVHD, including prominent transaminase elevations [66]. Bilirubin may be elevated only three- to four-fold or may reach levels of 10–20 mg/dL or higher; in extreme cases there may be a loss of liver synthetic function. As specific tests are lacking, diagnosing hepatic GVHD on clinical grounds may be tenuous. Drug toxicity (e.g., CSP), parenteral nutrition, hepatic sinusoidal obstruction syndrome, infection (e.g., gram-negative bacteria, CMV), cholangitis lenta, cholelithiasis, acalculous cholecystitis and other conditions may coexist or be confused with hepatic GVHD. In contrast to sinusoidal obstruction syndrome, hepatic GVHD rarely leads to weight gain, capsular pain or ascites.

Thus, the diagnosis of acute GVHD of the liver is difficult. Transvenous biopsies are associated with a low morbidity and, if results are unequivocal, may lead to changes in the clinical diagnosis (and management) in as many as 50 percent of patients [67, 68]; however, the small biopsy sample may still leave diagnostic uncertainty.

Table 25-2. Randomized trials of combination immunosuppression for prevention of acute GVHD.

Center (reference)	Diagnoses included	Patients (*n*)	Regimens compared	Median age (year)	GVHD (%)
HLA-identical Sibling Donors					
Minneapolis [134]	Nonmalignant and malignant diseases	32	MTX+ATG+PSE vs.	16	21 (p=0.01)
		35	MTX	16	48
Seattle [192]	AML in CR1 and CML in CP	43 50	MTX+CSP vs. CSP	30 30	33 (p=0.01) 54
City of Hope [193]	Acute leukemia and CML	53	MTX+PSE vs.	26	47 (p=0.05)
		54	CSP+PSE	26	28
Baltimore [194]	Nonmalignant and malignant diseases	42 40	CSP+MP vs. CY+MP	23 24	32 (p=0.05) 68
Seattle [195]	Nonmalignant and malignant diseases	59	MTX+CSP+PSE vs.	32	46 (p=0.02)
		63	MTX+CP	28	25
Stanford [196]	Malignant diseases	74 75	CSP+PSE vs. MTX+CSP+PSE	32 28	23 (p=0.02) 9
Multicenter [197]	Hematologic malignancies	164	MTX+CSP vs.	40	44 (p=0.01)
		165	MTX+TAC	40	32
Stanford [198]	Hematologic malignancies	96 90	MTX+CSP vs. MTX+CSP+PSE	34 34	20 (n.s) 18
Helsinki [199]	Hematologic malignancies	55	MTX+CSP vs.	41	56 (p=0.001)
	Severe aplastic anemia	53	MTX+CSP+PSE	42	19
Cleveland [127]	Hematologic malignancies	21 19	MMF+CSP MTX[a]+CSP	NA NA	48 (p=0.40) 37
HLA-nonidentical Donors					
Multicenter [57]	Nonmalignant and malignant diseases	90 90	MTX+CSP vs. MTX+TAC	35 34	74 (p=0.001) 56

[a] MTX at 5 mg/m^2 on days 1, 3, 6, and 11

Abbreviations: AML=acute myeloid leukemia; ATG=antithymocyte globulin; BC=donor buffy coat cells; CML=chronic myeloid leukemia; CP=chronic phase; CR=complete remission; CSP=cyclosporine; CY=cyclophosphamide; GVHD=graft-vs.-host disease; MP=methylprednisolone; MTX=methotrexate; n.s.=not significant; NA=not applicable, PSE=prednisone, TAC=tacrolimus

4.5. The Intestinal Tract

Involvement of the gut by GVHD may cause nausea, anorexia, pain and watery, secretory diarrhea. In severe cases, mucosal damage may result in protein-losing enteropathy, hypoalbuminemia, hemorrhage, or ileus. These manifestations, along with fluid losses through damaged skin and decreased hepatic synthetic function, render management difficult. Frequent monitoring of electrolytes and careful intake and output measurements are mandatory. Infection with *C. difficile* or other organisms, conditioning-related toxicity, lactose intolerance and nonspecific mucosal damage may mimic or enhance manifestations of gut GVHD. In contrast to skin histopathology, the histology of rectal and gastric or duodenal mucosal biopsies is more distinct or even pathognomonic [69-71].

Isolated GVHD of the upper gastrointestinal tract is not uncommon [72] and is commonly the cause of otherwise unexplained nausea and vomiting. It must be distinguished from herpesvirus infection, candidiasis and nonspecific gastritis [69, 73, 74]. Endoscopy and mucosal biopsies will usually lead to the correct diagnosis. GVHD of the upper intestinal tract tends to be sensitive to corticosteroid therapy, given systemically or by mouth in the form of beclomethasone [75].

Radiographic findings of intestinal GVHD are nonspecific and include increased bowel wall thickness (edema) and vascularity, as well as fluid-filled bowel loops. Transit time tends to be rapid, and Doppler studies may show increased arterial flow to the inflamed organ. A magnetic resonance imaging (MRI) study may show generalized bowel wall contrast enhancement [76, 77].

4.6. Other Organs

The involvement of other organs by acute GVHD has remained controversial. The most likely candidate is the lung [78]. Manifestations of lung toxicity, including interstitial pneumonitis and alveolar hemorrhage, occur in 20 to 60 percent of allogeneic, but in few autologous transplant recipients. Causes of pulmonary damage other than GVHD include "engraftment syndrome," infection, radiation pneumonitis and chemotherapy-related toxicity (e.g., MTX, busulfan). At least one retrospective analysis failed to link severe pulmonary complications to clinical acute GVHD per se [79]. Mortality due to pneumonia increases with the severity of GVHD. Nevertheless, the lungs are likely targets of GVHD because of their extensive reticuloendothelial system and direct exposure to the environment, which is strongly supported by studies in murine transplant models [80]. In addition, other chronic pulmonary syndromes, especially bronchiolitis obliterans, may be manifestations of chronic GVHD.

Renal and urinary tract symptoms are common, but are generally attributed to the conditioning regimen, immunosuppressive agents, or infection. There is no convincing evidence for a role of acute GVHD. Similarly, neurologic complications are common after transplantation, but most can be attributed to drug toxicity, infection, or vascular insults. Nevertheless, data on vasculitis of the CNS, possibly associated with GVHD and peripheral neuropathy, have been presented [81-83].

5. Histopathology

The histopathology of GVHD has been described elsewhere [71]. Programmed cell death (apoptosis) in the tissue layer responsible for proliferation and regeneration is a typical histologic feature of GVHD. In the skin, the dermoepidermal junction is most severely affected. Histopathologic findings include epidermal and basal cell vacuolar degeneration, disorganization of epidermal cell maturation, eosinophilic body formation and melanocyte incontinence [71, 84-86]. However, histopathologic features of acute GVHD are mimicked by the effects of chemo-radiotherapy and drug reactions [87]. Hepatic small bile ducts may show segmental disruption, injury to the periductular epithelium, bile duct atypia and cellular degeneration. Cholestasis may be present [88, 89]. Mucosal ulcerations and crypt destruction are present in the intestinal

tract, with the crypt bases being most severely affected. The colon is more frequently involved than the ileum. Often mononuclear cell infiltrates are mild. The involvement of the "stem cell" layer of the organ, i.e., the base of the crypts in the gut and the dermo-epidermal junction in the skin, results in a reduced ability to repopulate the damaged epithelium, with the consequence of blister formation, loss of villi and associated dysfunction.

6. Staging and Grading of Acute GVHD

The original grading system was proposed by Glucksberg, et al. [6]; it was subsequently modified into a "consensus" system (Table 25-2) [90] and complemented by the International Bone Marrow Transplant Registry (IBMTR) GVHD Severity Index (Table 25-2) [58]. An important modification in the IBMTR index is the inclusion of upper gastrointestinal findings (nausea, vomiting, pain, positive biopsy). This IBMTR system has been validated in a retrospective analysis, and severity as determined by this system was reflected in the incidence of transplant-related mortality [58]. IBMTR levels A, B, C, and D correspond approximately to Glucksberg Grades I, II, III and IV, respectively [56]. A recent prospective trial indicates that both systems are comparable in their ability to assess GVHD severity and prognosis [91]. Another modification, originally proposed by Vogelsang, et al. [92], involves a dividing Grade II into IIa (with a better prognosis) and IIb, dependent upon extent of the disease. If further clinical studies provide compelling evidence for manifestations of acute GVHD in other organs [78], and an impact on prognosis, these features will need to be considered in future grading systems.

Mild to moderate GVHD (Grades I or II [level A or B by IBMTR index]) is associated with limited morbidity, but is a significant risk factor for the development of chronic GVHD [93]. Grades III and IV (levels C and D by IBMTR index) GVHD carry a grave prognosis. In patients with Grade IV GVHD (Severity index D), mortality reaches 90 to 100 percent.

All these grading systems assess the maximum severity of acute GHVD, but do not consider the disease course over time. To overcome these limitations, Leisenring, et al. presented an "acute GVHD Activity Index" in which they assessed GVHD at 10-day intervals (Fig. 25-1). Skin rash, bilirubin, diarrhea, caloric intake, body temperature, medications administered and performance level were considered. Bilirubin values, uncontrolled anorexia (nausea, vomiting, caloric intake <40% of requirements), use of prednisone (or more intensive immunosuppressive therapy) and poor performance status were associated with an increased risk of non-relapse mortality by day 200 [94]. This activity index is awaiting validation in other disease cohorts.

7. Syngeneic and Autologous GVHD

Some 10 to 15 percent of autologous or syngeneic transplant recipients have been reported to develop a clinical picture that resembles GVHD, primarily of the skin, that usually responds promptly to corticosteroid therapy. However, the liver may also be involved [95], and a recently reported study showed that 13 percent of patients developed gastrointestinal GVHD following autologous transplants [96].

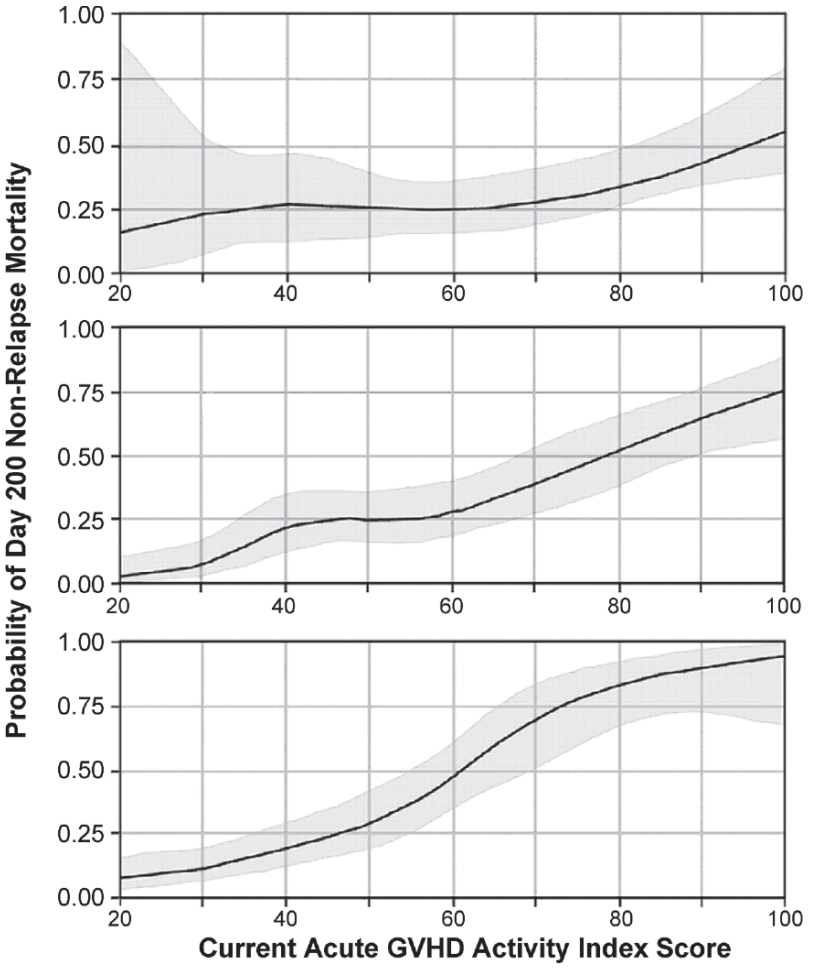

Fig. 25-1. Predicted non-relapse mortality by day 200 as a function of current acute GVHD activity. Shown are index scores at three different time intervals after transplantation and their impact on mortality at day 200. Within each panel, the solid line represents the predicted median activity index scores, and the shaded areas represent 95 percent confidence intervals (This research was originally published in Blood: Leisenring WM, Martin PJ, Petersdorf EW, Regan AE, Aboulhosn N, Stern JM, Aker SN, Salazar RC, McDonald GB. An acute graft-versus-host disease activity index to predict survival after hematopoietic cell transplantation with myeloablative conditioning regimens. Blood 2006; 108: 749-755. © The American Society of Hematology)

Hess and colleagues have proposed that infused T cells recognize MHC class II antigens in association with a peptide from the invariant chain (CLIP) (public determinants) [97, 98]. Another hypothesis was proposed by Nelson, who showed that in some individuals maternal cells transmitted during fetal development remain present throughout life [99]. This suggests the possibility that small numbers of HLA-incompatible cells (derived from the donor's mother) may be transmitted with "HLA-identical transplants." Transplacentally transferred maternal cells may also play a role in the development of neonatal GVHD [100].

8. Transfusion-Associated GVHD

Blood products administered to immunocompromised patients are irradiated or leukocyte-depleted to avoid the transfusion of viable alloreactive T cells [101]. Typically, MHC incompatibility between donor and recipient results in rapid clearance of transfused T cells by the recipient's immune system. However, transfusions from donors homozygous for one of the recipient's MHC haplotypes may not be recognized as foreign by the recipient [102]. These cells can engraft and mount an immunologic attack against the unshared haplotype in the patient, resulting in transfusion-induced GVHD [101, 103]. Transfusion-associated GVHD differs from GVHD after HCT in regards to its kinetics and manifestations insofar as the recipient marrow is a major target, as also observed with DLI after HCT. Since the number of stem cells in the offending blood product is inadequate, there is no hematopoietic recovery from donor cells. This syndrome is generally fatal due to refractory pancytopenia.

9. Prevention of GVHD

Prevention of GVHD involves the elimination or control of recognized risk factors.

9.1. Donor Selection

The preferred donor is a young HLA-identical sibling who has not had CMV exposure (CMV-negative), is not allosensitized, and is of the same sex as the patient, although it is noteworthy that stronger Graft-versus-Leukemia effects are observed with HLA-nonidentical or unrelated donors. In fact, some recent reports, particularly with RIC, suggest superior transplant outcome with unrelated donors in some disease categories [104].

While HLA-nonidentical related donors may be acceptable, dependent upon the degree of mismatch, the underlying disease, and the willingness of the patient to be enrolled in investigational protocols, generally a search for an unrelated donor is initiated if an HLA-identical sibling is not available. With the increasing number of potential donors registered (presently about 10 million individuals) and the progress in molecular HLA typing, many patients can find an unrelated donor identical for HLA at the DNA level.

Another potential source of stem cells are cord blood cells, which in virtually all cases will not be HLA-identical with the patient, but due to their immaturity as well as cell composition and function, are associated with a lower incidence of GVHD than expected on the basis of HLA disparity [105, 106].

Selecting donors on the basis of certain cytokine (e.g., TNF-α; IL-10) polymorphisms is currently not a realistic option.

9.2. Host Factors

Murine studies showed that transplantation in a gnotobiotic (i.e., sterile) environment prevented GVHD [3]. Studies in human patients who were placed in isolation (laminar air flow or other isolation rooms) and underwent both gut and skin decontamination have shown partial efficacy, particularly in patients with nonmalignant diseases [107, 108] and, at some centers, in all patients

so treated [109]. It is noteworthy that two recent reports describe a lower frequency of GVHD in patients given prophylactic metronidazole (combined with ciprofloxacin) and fluconazole, respectively [110, 111]. In animal models the use of LPS (or TNF-α) neutralizing agents may decrease intestinal GVHD [112]. IL-11 and keratinocyte growth factor have been tested, but there is no evidence that either one provides potent anti-GVHD protection [113, 114].

9.3. Conditioning Regimen

As described under **Pathophysiology**, intensive conditioning, especially with high-dose TBI, leads to endothelial injury and associated tissue damage. In agreement with preclinical observations, clinical results suggest that less toxic conditioning regimens (e.g., cyclophosphamide alone or various RIC regimens) are associated with a lower incidence and less severe acute GVHD [7, 48, 107, 115]. However, a recent report suggests that the intensity of the pre-transplant conditioning regimen (myeloablative versus nonmyeloablative) might have no major impact on the incidence of serious aGVHD (i.e., death, lengthy hospitalization, major disability, recurrent major infections related to GVHD) [116].

9.4. *In Vivo* Prophylaxis

9.4.1. Pharmacologic Agents

Early studies used single-agent MTX (based on the seminal work by Uphoff, using α-aminopterin [117] or cyclophosphamide for GVHD prophylaxis. Pilot trials omitting prophylaxis showed an incidence of acute GVHD close to 100 percent [59], whereas patients given MTX (15 mg/m^2 on day 1, and 10 mg/m^2 on days 3, 6, 11 and weekly until day 102) experienced GVHD Grades II–IV in 30 to 60 percent of cases [118]; abbreviated MTX regimens were less effective [119].

The introduction of calcineurin inhibitors, CSP [120] and TAC [121], offered new approaches to prevent GVHD. CSP is administered starting one to several days *before* transplantation, at doses of 1.5 to 2.5 mg/kg i.v. every 12 hours (or as a continuous infusion) with a switch to oral drug, 6 mg/kg every 12 hours, when tolerated. Dependent upon the regimen and the method used to measure CSP, blood levels of 150–400 ng/mL should be maintained. Tapering typically starts on day 50 (earlier if necessitated by toxicity) and continues over six to 12 months.

TAC is often given as continuous infusion, 0.03–0.04 mg/kg/day, and switched to an oral preparation when tolerated (0.15 mg/kg/day). Blood levels are targeted at or below 15 ng/mL. In a randomized Japanese trial, TAC was superior to CSP with an incidence of acute GVHD of 13 to 21 percent (HLA-identical/nonidentical) versus 41 to 54 percent in patients given CSP [122].

Based on preclinical studies [123] clinical trials tested single agents against drug combinations or combinations against each other (Table 25-2). With MTX plus CSP combinations, the incidence rates of acute GVHD Grades II–IV were 20 to 56 percent with HLA-identical sibling transplants. With CSP combined with methylprednisolone (MP) at doses of 0.5–1.0 mg/kg for various intervals after HCT, the GVHD incidence ranged from 23 to 32 percent. With a triple combination including MTX, CSP, and MP, the incidence varied from 9 to 46 percent. The spread of these results reflects not only differences in the dose schedules, but also patient selection, patient age, the conditioning regimen used and variations in the clinical grading of GVHD [124]. Importantly, even

though drug combinations may have resulted in a lower incidence of GVHD, in many trials this was not reflected in improved survival (due to increased rates of infections and relapse), particularly with the incorporation of MP as a third drug. Nevertheless, overall drug combinations are superior to single agents, and the use of TAC appears to have an advantage over CSP [125].

The replacement of MTX by MMF has reduced the frequency and severity of mucositis; however, outcome, both in regards to GVHD and survival, was not superior to that observed with MTX [126, 127].

Many investigators have reduced the amount of MTX administered [128, 129], and Przepiorka has reported a "mini-MTX" regimen [130]. Such a strategy may reduce problems with mucositis and allow for better compliance with concurrently given CSP or TAC. Thus, many current regimens use MTX at doses of 7.5 (rather than 15 or 10) mg/m^2 or even lower, and often give it only on days 1, 3, and 6. There is evidence, however, that omitting the day 11 dose is associated with an increase in the frequency of GVHD [44]4=. On the other hand, a recent meta-analysis suggests that giving the fourth dose of MTX resulted in an increased relapse risk in patients transplanted with marrow [131].

Cutler, et al. replaced MTX by sirolimus (rapamycin) in a phase II trial in combination with TAC, and observed an incidence of acute GVHD of 10 percent, and a day 100 relapse-free survival of 93 percent with HLA-identical sibling transplants [132]. The same investigators then used a triple combination of TAC, sirolimus and low-dose MTX with unrelated donor transplants and observed an incidence of Grades II–IV acute GVHD of 26 percent [133]. However, so far these results have not been reproduced by other investigators.

9.4.2. In Vivo Antibody Prophylaxis

Several recent trials have used the anti-thymocyte globulin (ATG) preparation, Thymoglobulin (rabbit), as part of the conditioning and GVHD regimen, resuming a strategy proposed by Ramsay, et al. in 1982 [134]. These reports show that ATG given during the pre- and peri-transplant period reduces the incidence of acute (and chronic) GVHD and may be associated with lower late morbidity [135-139]. With this approach, Russell, et al. reported an incidence of acute GVHD of 8 percent with related, and 19 percent with unrelated donors [135]; incidence figures reported by others have been higher [138, 140]. A very low incidence of acute GVHD (0% and 9% in patients receiving grafts from matched related or unrelated donors, respectively) was recently reported following nonmyeloablative conditioning with ATG and total lymphoid irradiation [141]. Very high doses of Thymoglobulin, however, are associated with more infections and abrogate its positive impact on GVHD [140]. A dose in the range of 6–8 mg/kg may be appropriate.

The impact of large doses of intravenous immunoglobulins on GVHD, despite initial promising results, is controversial. One undesired effect is interference with immune recovery [142].

The most widely used monoclonal antibody given *in vivo* for GVHD prophylaxis is probably Campath 1H (anti-CD52), which has been found to reduce the incidence of GVHD and non-relapse mortality in related and unrelated transplant recipients [143]; the antibody has also "graft facilitating" activity. Antibodies to the IL-2 receptor (CD25) have shown some benefit [144]. The use of the ricin-conjugated anti-CD5 antibody did not result in a significant advantage [60, 154, 146].

9.4.3. In Vitro T Cell Depletion

Theoretically, removing the host-reactive donor T cells should solve the problem of GVHD, and, in fact, all T cell depletion studies have shown a reduction in acute (albeit not in chronic) GVHD. However, sustained engraftment and disease relapse occurred in a larger proportion of patients than observed with T cell-replete transplants. Nevertheless, trials using various monoclonal antibodies with or without a nonhuman source of complement, E-rosetting/lectin methods, elutriation techniques, or functional inactivation via phototherapy, have been used [143, 146, 147]. In an attempt to circumvent problems associated with global T cell removal, strategies of selective or partial removal of CD4+ or CD8+ cells or specific T cell subsets have been pursued [30]. Another strategy in a small study of HLA-nonidentical HCT has been the removal of activated T cells by incubation with CTLA4-Ig (in the presence of donor antigen-presenting cells) [148].

Recently Wagner, et al. presented the results of a randomized, multicenter trial comparing the use of MTX plus CSP (n=204) to T cell depletion plus CSP (n=201) as GVHD prophylaxis. T cell depletion used either elutriation or treatment of donor cells with the monoclonal antibody T10B9 (targeting the T cell receptor αβ), plus rabbit complement [55]. The incidence of GVHD Grades III–IV was 19 percent with T cell depletion and 29 percent with MTX plus CSP (p=0.017). However, there was no significant difference in long-term survival, and the incidence of relapse among patients with CML was 20 percent versus 7 percent in patients given MTX plus CSP (p=0.009); there was also a higher incidence of CMV infection with T cell depletion (p=0.023). Others have used T cell depletion and, by design, administered additional DLI following HCT with the intent of obtaining the benefit of T depletion without risking relapse [147].

T cell depletion has also been applied to PBPC [149] since PBPC carry a higher risk of GVHD, in particular chronic GVHD, than marrow. One consideration here is the fact that following administration of G-CSF to the donor there is a shift in favor of type 2 dendritic cells (DC2) [150]. DC2 cells activate Th2 cytokines IL-4 and IL-10 and are unable to initiate proliferation of naïve T cells. Thus, the concept is that partial removal of CD3+ T cells, while maintaining the remainder of cells, will preserve the favorable DC2 action and reduce the risk of GVHD [150, 151].

9.4.4. Other Methods

Ruutu, et al. reported on the use of ursodeoxycholic acid (UDCA), originally with the aim of reducing hepatotoxicity after related and unrelated donor transplants [152]. Patients given UDCA, 12 mg/kg/day, from before conditioning until day 90 showed a non-relapse mortality of 19 percent (versus 34% in patients not given UDCA; p= 0.01) and a survival of 71 percent (versus 55%). In addition, acute GVHD of Grades III–IV was less frequent (p=0.01).

10. Treatment of GVHD

Despite sufficient prophylaxis, some 10 to 90 percent of patients develop acute GVHD that requires therapy. The probability of survival depends upon the response to therapy [153-155]. Some treatment trials are summarized in Table 25-3. A treatment algorithm is given in Fig. 25-2.

Table 25-3. Treatment of acute GVHD.

Center (reference)	Patients (*n*)	Regimens compared	Median Age (year)	Response (flare) (%)	Overall Survival (%)
Seattle [200]	20	MP vs.	24	65	40 (n.s.)
	17	ATG	24	35	24
Seattle [201]	39	MP vs.	27	41 (p=0.039)	28 (n.s.)
	38	CSP	26	61	24
Minneapolis [202]	16	MP long taper vs.	33	(13) (n.s.)	81 (n.s.)
	14	MP short taper	29	(29)	66
France [203]	34	MP+CSP+placebo vs.	29	63 (n.s)	59 (n.s)
	35	MS+CSP+CD25 mAB	25	70	66
Seattle [169]	114	MP+placebo vs.	30	25 (p=0.019)	(45) (n.s.)
	129	MP+CD5-IT	29	40	49
Italy [156]	47	MP 2 mg/kg vs.	26	68 (n.s.)	63 (n.s.)
	48	MP 10 mg/kg	28	71	62
Seattle [158] (Enteric GVHD)	29	MP+placebo vs.	39	41 (p=0.02)	NA
	31	MP+Beclo	34	71	NA
Minneapolis [170]	46	MP vs.	28	55 (p=0.02)	50 (n.s.)
	50	MP+ATG	23	27	40
Multicenter [204]	53	MP + Daclizumab vs.	45	29 (p=0.002)	25[a] (p=0.005)
	49	MP+placebo	42	60	56

[a]Disease free survival at one year

Abbreviations: ATG = antithymocyte globulin; Beclo = beclomethasone; CSP = cyclosporine; GVHD = Graft-versus-Host Disease; IT = immunotoxin; MAB = monoclonal antibody; MP = methylprednisolone; NA = not available; n.s. = not significant; PSE = prednisone

10.1. Primary Therapy

Corticosteroids (e.g., prednisolone [PRED] or MP, 2 mg/kg/day for 14 days or longer) remain the mainstay of acute GVHD therapy. Lysis of lymphocytes during interphase and anti-inflammatory effects may lead to prompt improvement. Complete responses occur in 20 to 25 percent, and partial responses in 40 to 50 percent of patients with Grades II to IV acute GVHD. A prospective, randomized study comparing 2 mg/kg/day of MP to 10 mg/kg/day failed to show an advantage of the higher dose [156]. The same investigators subsequently presented results in 211 patients with Grades I–IV GVHD treated with MP, 2 mg/kg/day [157]. In 150 patients (71%) the dose could be tapered beginning after five days of therapy, whereas 61 patients (29%) required continuation of therapy. The non-responders were randomized to receive MP at 5 mg/kg/day for 10 days, alone or combined with Thymoglobulin (6.25 mg over 10 days); 26 percent of patients had complete responses. Non-relapse mortality for the day 5 responders and non-responders was 27 percent and 49 percent (p=0.009), and five-year survival 53 percent and 35 percent, respectively (p=0.007). There was no significant difference between the two secondary therapies.

A randomized trial in 60 patients with intestinal GVHD compared oral MP, at 1 mg/kg/day, plus placebo to MP plus beclomethasone. The durable

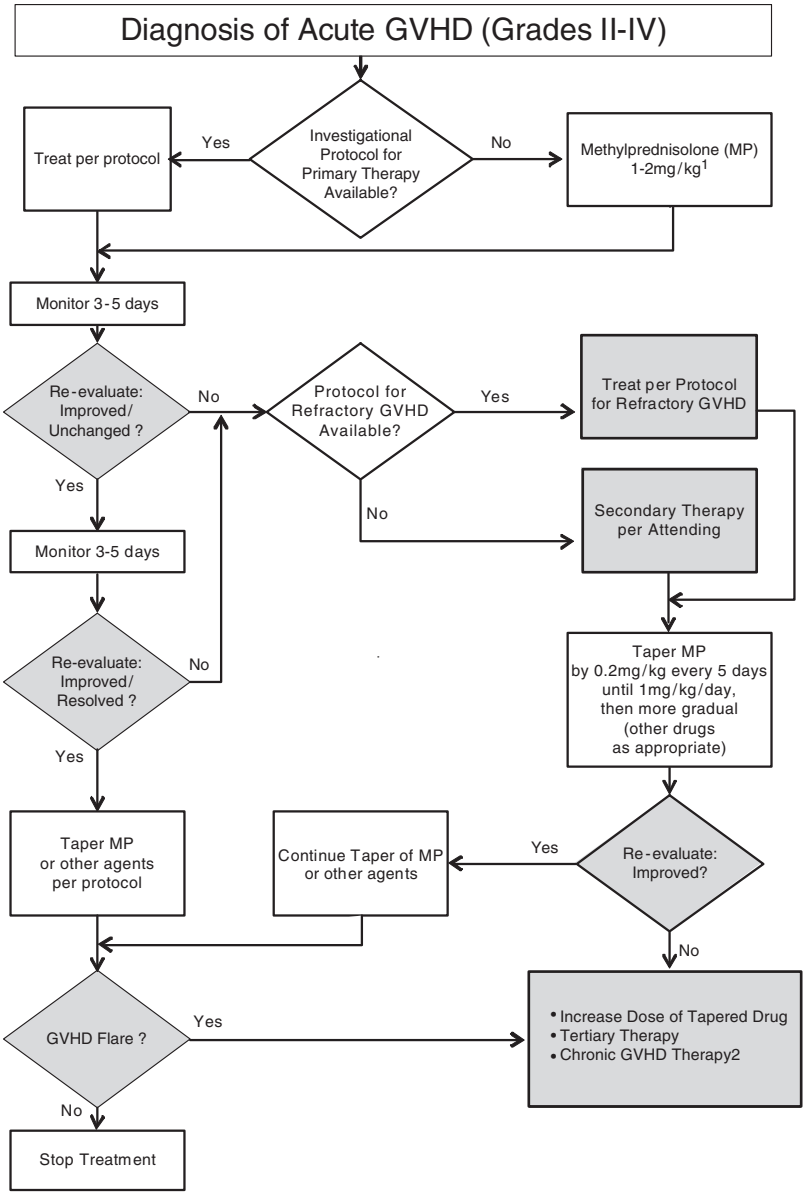

Fig. 25-2. Treatment algorithm for acute GVHD. Protocol treatment is preferred. Days indicated at the left margin are intended to serve as a guide for the timing of therapeutic decisions (This research was originally published in Blood Online. Deeg, HJ. How I treat refractory acute GVHD. Blood. Prepublished January 18, 2007; DOI 10.1182/blood-2006-12-041889)

responses by day 30 were 41 percent and 71 percent for the two groups, respectively (p=0.02) (158,159). A follow-up study by McDonald, et al. (unpublished observations) shows a day 200 survival of 92 percent for patients on beclomethasone and 76 percent for placebo-treated patients (p=0.01). In a multivariate analysis, only the use of beclomethasone was significant (p=0.05)

for day 200 survival. Patients with recurrent GVHD had greater steroid exposure and a higher risk of death.

TAC and CSP have been used with some success therapeutically in patients who had not received these agents as part of their prophylaxis [160]. In our experience, a switch from CSP to TAC was beneficial only in patients who were switched because of CNS toxicity [161].

Equine (ATGAM) or rabbit ATG (Thymoglobulin) are potent anti-T cell agents that achieve responses in 20 to 30 percent of patients even after steroid failure [153, 155, 162, 163]. However, infections and thrombocytopenia are common complications and, in some trials, patient survival was as low as 10 percent [164]. A combination of TAC and ATG has yielded promising results in one study [165].

MMF and rapamycin (sirolimus) have also been used for the treatment of acute GVHD [166]. MMF may be effective in some patients in combination with CSP and prednisolone [167]. Toxin-conjugated monoclonal antibodies have shown encouraging results [168]. However, a randomized trial comparing MP with MP plus a ricin A-conjugated anti-CD5 antibody, while showing a higher response rate (40% versus 25%; p=0.019), revealed no significant difference in the incidence of chronic GVHD or survival [169]. The same was true for a comparison of MP and MP plus ATG [170].

In recent studies, the addition of *ex vivo*-cultured adult human mesenchymal stem cells to corticosteroids for primary treatment of acute GVHD has resulted in encouraging response rates [171, 172].

10.2. Secondary Therapy (Steroid–Nonresponsive GVHD)

Various strategies have been used to treat steroid non responsive GVHD (Table 25-4). CSP, TAC, ATG, and MMF have all been used to treat steroid-unresponsive acute GVHD. Recent trials with rapamycin showed a response rate of about 50 percent, and suggested improved survival compared to historic controls [166, 173].

Table 25-4. Treatment options for steroid-nonresponsive acute GVHD.

AXgent/Strategy	Selected References
PUVA	[205, 206]
ECP	[185, 207]
Anti-TNFα agents	[208–210]
Human anti-CD3	[178, 179]
Denileukin diftitox	[211]
Anti-IL-2 receptor antibody	[210, 212]
Sirolimus	[166, 213]
Tacrolimus	[214]
ATG	[153, 214]
Pentostatin	[215, 216]
ATG versus ABX-CTL	[177]

Abbreviations: ATG = antithymocyte globulin; ECP = extracorporeal photopheresis; PUVA = psoralen sensitization and exposure to ultraviolet A light; TNF = tumor necrosis factor

A broad array of monoclonal antibodies in murine or humanized form with pan T or T-subset reactivity has been used in patients with GVHD not responding to corticosteroids. Responses, sometimes sustained, are observed with anti-CD2, anti-CD3, anti-CD5, and other antibodies [34, 174, 175]. In a trial using the anti-CD147 antibody, ABX-CBL, more than half of the patients with steroid-refractory acute GVHD responded, and survival was superior to that observed in a historical comparison group treated with horse ATG [176]. However, a subsequent phase III trial comparing ABX-CBL with ATG (ATGAM) failed to show a significant difference [177]. Remarkable results have been obtained with a humanized antibody, HuM291 (visilizumab, directed at the T cell receptor zeta chain), by Carpenter, et al. [178]. Among 15 patients with steroid-refractory GVHD, seven achieved complete and eight partial responses. Notably, sustained responses were achieved with a single dose of antibody. A follow-up multicenter study enrolled 44 patients, 86 percent of whom had Grades III–IV acute GVHD. The overall response rate at 42 days was 32 percent, with 14 percent complete responses. Survival at six months was 32 percent [179]. In both trials, 40 to 50 percent of patients experienced a rise in plasma titers of EBV DNA, which was controlled by administration of the anti-CD 20 antibody, rituximab.

Another treatment strategy for steroid-refractory acute GVHD is cytokine blockade. A monoclonal antibody to the IL-2R (B-B10) was found to be experimentally and clinically effective [180]. Pilot trials using genetically engineered human monoclonal antibody specific for the alpha subunit of the IL-2R (anti-Tac, daclizumab) in patients who had failed to respond to corticosteroids showed responses in about 40 percent of patients [181, 182]. Although experimental data suggest that blocking other receptors (e.g., IL-1) may also be beneficial, a recent report showed a lack of efficacy in patients [114]. One clinical report suggested efficacy of an anti-TNF-α monoclonal antibody (infliximab) in steroid-refractory acute GVHD [183]. This approach may require special caution as some cases of tuberculosis reactivation have been reported [184].

PUVA treatment (photosensitization with 8-methoxypsoralen and UVA irradiation) is effective in the treatment of acute and chronic GVHD of the skin in some patients. Extracorporeal exposure of the recipient's peripheral blood mononuclear cells to the photosensitizing effect of 8-methoxypsoralen and UV light and their subsequent reinfusion has shown efficacy in treating acute (and chronic) GVHD refractory to conventional treatment [185-187].

11. Summary and Perspective

Acute GVHD is a complex clinical syndrome. There are no simple laboratory tests to confirm the diagnosis. However, it is important to recognize the disease, since the survival of patients may depend on the accuracy of the clinical diagnosis and prompt institution of therapy. None of the prophylactic strategies currently available have been completely successful, and the most effective ones have been associated with other complications such as an increased incidence of disease recurrence. There is evidence that acute GVHD may be less frequent and less severe in patients conditioned with

RIC regimens; however, the incidence and severity of chronic GVHD has not been affected.

Qualitatively new approaches such as risk-adapted first line treatment or preemptive therapy might have an impact on outcome. A better definition of predictive parameters is needed. Endothelial cells have only recently been identified as important targets of GVHD [188], and agents modulating endothelial activation have not been tested. Finally, the increasingly recognized role for regulatory T cells (CD4+CD25+ T cells or T regulatory type 1 cells) [189-191] suggests that strategies aimed at modulating subpopulations of cells or the therapeutic use of regulatory cells may be useful for the prevention and therapy of GVHD.

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Chapter 26

Chronic Graft-versus-Host Disease

Javier Bolaños-Meade and Georgia B. Vogelsang

1. Introduction

Chronic Graft-versus-Host Disease is the most common and significant late morbidity of allogeneic transplantation. It may effect up to 60 percent of the patients undergoing HLA-identical allografts who survive beyond 100 days [1]. It is the leading cause of death in patients surviving longer than two years after transplant [2]. Chronic Graft-versus-Host Disease has repeatedly been found to be the main adverse factor affecting health status and quality of life post-transplant [3–6]. The increasing use of alternative donors, older patient age, use of donor lymphocyte infusions, the use of peripheral blood as a stem cell source, and improvements in supportive care resulting in more patients surviving acute Graft-versus-Host Disease are all likely to contribute to the high frequency of this complication. The increasing importance of this problem was recently highlighted by the National Institutes of Health-sponsored Chronic Graft-versus-Host Disease Consensus Conference [7–12].

2. Definition and Diagnosis of Chronic Graft-versus-Host Disease

Historically, Graft-versus-Host Disease was defined by time post-transplant. Any Graft-versus-Host Disease before day 100 was called acute, and any thereafter was called chronic. This distinction persisted because of the simplicity of the definition, even when it was very clear that this distinction had no biologic or clinical meaning. Graft-versus-Host Disease is now defined by the clinical manifestations of the disease. It is a multisystem alloimmune and autoimmune disorder characterized by immune dysregulation, immunodeficiency, impaired organ function, and decreased survival seen in the setting of cellular therapy. Patients with chronic Graft-versus-Host Disease have clinical features suggestive of spontaneously occurring autoimmune disorders. Most have skin changes, but almost any organ can be involved (see below). The most important abnormality, however, is the immune dysfunction seen with the disease, which is compounded by the immunosuppressive therapy given to treat the disorder.

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One of the major accomplishments of the National Institutes of Health Consensus Conference was to formulate diagnostic criteria for chronic Graft-versus-Host Disease to use in clinical trials [7, 13]. However, this is also intended as a very functional definition to be used in clinical practice. The minimal criteria for a diagnosis of chronic Graft-versus-Host Disease require the presence of at least one diagnostic clinical sign of chronic Graft-versus-Host Disease without biopsy confirmation, or the presence of at least one distinctive sign confirmed by biopsy or other laboratory or imaging test in the same or another organ. Biopsy of involved tissue is always encouraged and often valuable to confirm the presence of chronic Graft-versus-Host Disease but it is not always feasible and is not mandatory if the patient has clinical manifestations diagnostic of chronic Graft-versus-Host Disease. A biopsy "consistent with" or "unequivocal to" Graft-versus-Host Disease will be considered sufficient to support the diagnosis of chronic Graft-versus-Host Disease if accompanied by at least one distinctive clinical manifestation. An in-depth discussion of chronic Graft-versus-Host Disease histopathology and the National Institutes of Health Working Group's recommendations can be found in the article by Shulman, et al. [8]. Diagnostic signs and symptoms of chronic Graft-versus-Host Disease refer to those manifestations that establish the presence of chronic Graft-versus-Host Disease without further testing or evidence of other organ involvement (examples include poikiloderma, sclerotic skin features, oral lichen-type changes, fasciitis, or bronchiolitis obliterans diagnosed with lung biopsy). Distinctive signs and symptoms refer to those manifestations that are not ordinarily found in acute Graft-versus-Host Disease but are not considered sufficient to establish an unequivocal diagnosis of chronic Graft-versus-Host Disease without further testing or additional organ involvement (for example, nail dystrophy, vitiligo-like depigmentation, bronchiolitis obliterans diagnoses based only on pulmonary function tests and computerized tomography findings). The recommendations also address other features of chronic Graft-versus-Host Disease that are rare, controversial or nonspecific features that cannot be used to establish the diagnosis, but can be acknowledged as part of chronic Graft-versus-Host Disease symptomatology if the diagnosis is confirmed (examples include hypo and hyper pigmentation, keratosis pilaris, exocrine pancreas insufficiency, thrombocytopenia, eosinophilia, autoantibodes, nephrotic syndrome, and others). The final group of manifestations can be encountered in chronic Graft-versus-Host Disease, but are common signs and symptoms that can be found in both chronic and acute Graft-versus-Host Disease (erythema, maculopapular rash, nausea, vomiting or diarrhea, or elevated liver function tests).

Distinguishing between chronic and acute Graft-versus-Host Disease is a major issue. The working group recognizes two main categories of Graft-versus-Host Disease (acute and chronic), each with two subcategories (classic acute; persistent, recurrent or late onset acute; classic chronic; overlap syndrome). Classic acute Graft-versus-Host Disease (maculopapular erythematous rash, gastrointestinal symptoms, or cholestatic hepatitis) occurs within 100 days post-transplant or donor lymphocyte infusion. The second category is persistent, recurrent or late acute Graft-versus-Host Disease presenting beyond 100 days of transplantation or donor lymphocyte infusions. The arbitrary day 100 distinction is retained to facilitate standardized reporting in clinical trials. Acute Graft-versus-Host Disease should occur without diagnostic or

distinctive chronic Graft-versus-Host Disease manifestations. Classic chronic Graft-versus-Host Disease consists of manifestations that can only be ascribed to chronic Graft-versus-Host Disease. Acute and chronic overlap syndrome have features of both acute and chronic Graft-versus-Host Disease occurring together. A very important point is that in the absence of histological or clinical signs or symptoms of chronic Graft-versus-Host Disease, skin, gut or liver abnormalities should be classified as acute Graft-versus-Host Disease regardless of the time after transplantation. The exclusion of other possible diagnoses (infection, drug effect, etc.) is very important as many patients believed to have chronic Graft-versus-Host Disease may not be suffering from this complication [14].

3. Pathogenesis

Less is understood about the pathogenesis of chronic than acute Graft-versus-Host Disease. Although the reasons for this are many, one important factor has been the lack of an animal model which duplicates the clinical manifestations of the disorder. In murine models, for example, manipulating the age of the donors and/or recipients, strain combinations, number and type of donor cells, preparative regimen (or the lack thereof in the case of parent into F1 hybrid models), degree and type of matching, etc. alter the clinical manifestations produced. Moreover, the animals do not receive Graft-versus-Host Disease prophylaxis which significantly alters the recovery of immune cells. The most striking and consistent findings are a wasting syndrome with lymphoid atrophy, but both lupus-like skin lesions and fibrosis in the skin and other organs can be produced. The parent into F1 hybrid model, for example, also produces a severe renal injury and splenomegaly, findings rarely seen in humans. Despite these limitations, these models do indicate some common threads. The disease appears to be a T cell-mediated disorder in which immune tolerance to self-antigens is broken [15]. The generation of these autoreactive cells is influenced by the Th2 cytokines (IL-4, IL-6, IL-10). Other cytokines (IL-12, IL-18), costimulatory blockade (4-1BB, cytotoxic T-lymphocyte antigen 4 [CTLA-4], inducible costimulator ([ICOS], CD28) and chemokine antagonists (CC chemokine receptor 7 [CCR7]) can prevent the development of clinically evident chronic Graft-versus-Host Disease. However, the exact timing of administration of these immune mediators very much influences their effect. The data that is emerging strongly suggest that there is no simple, one-size-fits-all explanation for chronic Graft-versus-Host Disease. It is a complex interplay of age, integrity of the immune system, timing and mixture of immune mediators present, degree and type of match, and source of stem cells. The injury may not be the immunologic explosion seen in acute Graft-versus-Host Disease, but a smolder where precise correction would stop the immunologic injury fire before it really gets burning. Clearly, more work is needed to understand how to do this sort of targeted therapy for chronic Graft-versus-Host Disease.

Human data on chronic Graft-versus-Host Disease is relatively sparse. One area of recent interest and controversy is the role of Regulatory T cells (CD4+/CD25+ T cells or Treg cells) in chronic Graft-versus-Host Disease [16]. In this study, flow cytometry was used to measure the size of the Treg pool in 40 patients who survived more than 100 days after allogeneic transplant. Patients with

chronic Graft-versus-Host Disease had significant increased Treg cells, expressed both as a percentage of CD4+ T cells or as absolute counts, compared to patients without chronic Graft-versus-Host Disease. Further, the investigators showed that purified Treg cells from patients with chronic Graft-versus-Host Disease showed suppressive capabilities *in vitro*, which may help explain the significant immune unresponsiveness seen in patients with chronic Graft-versus-Host Disease. Two other groups have examined the role of Treg cells, with contradictory results [17, 18]. The differences need further study, but may represent more differences in the assays used, rather than true biologic differences *in vitro* functional assay to demonstrate that these cells display suppressive capabilities.

There are data on B-cell activation with the presence of autoantibodies [19–22]. In patients followed to study immune reconstitution, 83 percent developed chronic Graft-versus-Host Disease, and 71 percent had autoantibodies [22]. The fact that some patients with chronic Graft-versus-Host Disease respond to therapy with rituximab further supports the role of B-cells in the pathobiology of the disease [23–26]. However, the exact role of B-cells in the pathogenesisi of chronic Graft-versus-Host Disease remains a mystery.

4. Risk Factors for the Development of Chronic Graft-versus-Host Disease

There are several factors associated with the development of chronic Graft-versus-Host Disease. Some of the most relevant risk factors will be discussed here.

4.1. Stem Cell Source

There has been a great deal of discussion on the importance of stem cell source on the risk of chronic Graft-versus-Host Disease [27–30]. Several studies have addressed this issue in the related setting. Of the eight randomized trials published [31–38] only one reported a statistically significant increase in Grades II–IV acute Graft-versus-Host Disease with the use of peripheral blood stem cells, compared to bone marrow (52 versus 39%) [37]. Regarding chronic Graft-versus-Host Disease, the results are as follows: three studies have shown an increase of chronic Graft-versus-Host Disease with peripheral blood stem cells as opposed to bone marrow [33, 37, 39]. One study showed a trend towards an increase in chronic Graft-versus-Host Disease with the use of peripheral blood stem cells [34]. A meta-analysis by Cutler, et al. confirmed that both acute and chronic Graft-versus-Host Disease are more common after peripheral blood stem cells than bone marrow [28]. Registry data from pediatric patients showed that chronic Graft-versus-Host Disease was more frequent (and had higher mortality) after peripheral blood stem cells than after bone marrow [29]. In adults, chronic Graft-versus-Host Disease is also more prevalent [40]. Umbilical cord stem cells have also been a source of grafts in children and young adults. As children tolerate mismatches better than adults, interpreting risk in this group is difficult, but it seems that the rate of chronic Graft-versus-Host Disease is low for this stem cell source, especially considering that almost all grafts are one to three antigen mismatches [41, 42]. Long-term follow-up of these patients will be needed to evaluate the long-term immune competence of these grafts.

4.2. HLA typing

HLA matching is an important risk factor for the development of Graft-versus-Host Disease. Its impact in survival was recently reviewed [43]. The incidence of Graft-versus-Host Disease increases with the level of HLA mismatch [44–46]. Utilizing DNA testing, it is known that a mismatch in HLA-A, B, -C, or -DRB1 increases the risk of Graft-versus-Host Disease and has a negative effect on survival [47–51], and HLA-DRB1 has been associated with an increase in severe acute Graft-versus-Host Disease [52]. Minor histocompatibility antigens are major histocompatibility complex-associated peptides, which trigger T cell responses that mediate Graft-versus-Host Disease [53].

4.3. T Cell Depletion

T cell depletion from marrow grafts decreases the risk of acute Graft-versus-Host Disease [54], but its effects on chronic Graft-versus-Host Disease are less clear. In HLA-matched sibling marrow transplants T cell depletion is associated with less chronic Graft-versus-Host Disease [55]. *In vivo* T cell depletion with alemtuzumab produced very low rates of chronic Graft-versus-Host Disease [56, 57]. *In vitro* T cell depletion has also given encouraging results with low rates of chronic Graft-versus-Host Disease [58]. Montero, et al. reported on 148 patients that received T cell-depleted allogeneic peripheral blood stem cell grafts [59]. The goal of this study was to determine the outcome of T cell-depleted peripheral blood stem cell grafts with T cells given after recovery from the transplant. The results showed a strong Graft-versus-Leukemia effect and no mortality due to chronic Graft-versus-Host Disease. However, the incidence of chronic Graft-versus-Host Disease was similar to that published with conventional bone marrow grafts. Pavletic, et al. reported a very large randomized multicenter trial with over 400 patients receiving unrelated grafts comparing T cell depletion plus cyclosporine to pharmacologic prophylaxis (methotrexate and cyclosporine). Despite a reduction in acute Graft-versus-Host Disease, there was no difference in the incidence of chronic Graft-versus-Host Disease or survival [60].

4.4. Nonmyeloablative Transplants

Busca, et al. compared 29 patients who underwent nonmyeloablative transplants to an age-matched cohort of 29 patients who received myeloablative transplants. They did not find major differences between patients receiving ablative or nonmyeloablative transplants with respect to timing, clinical characteristics and incidence of chronic Graft-versus-Host Disease. By multivariate analysis, nonmyeloablative transplantation was the only independent, prognostic factor for the development of refractory chronic Graft-versus-Host Disease [61]. Mielcarek, et al. retrospectively analyzed the incidence of Graft-versus-Host Disease between 44 patients who underwent nonmyeloablative and 52 who underwent myeloablative transplants and found no differences in the cumulative incidence of chronic Graft-versus-Host Disease requiring therapy [62].

4.5. Ethnicity

It would be predicted that isolated ethnic populations (such as Japanese and certain Scandinavian cohorts) would have lower rates of acute and, consequently, chronic Graft-versus-Host Disease than more diverse populations,

especially when examining matched unrelated donors. Oh, et al. compared the results of HLA-identical sibling transplantation for acute leukemias and chronic myeloid leukemia, performed between 1990 and 1999 [63]. They included different ethnic groups, 562 Japanese, 829 White Americans, 71 Black Americans, 195 Scandinavians and 95 Irish. Multivariate analyses of adult patients showed that White Americans, Black Americans and Irish cohorts were at significantly higher risk for acute Graft-versus-Host Disease than Japanese or Scandinavian cohorts. No differences in the risk for chronic Graft-versus-Host Disease, relapse, and overall survival were noted. In the pediatric cohort (limited to Japanese and White Americans), WhiteAmericans were at significantly higher risk for acute Graft-versus-Host Disease. No differences in other clinical outcomes were noted.

4.6. Donor Lymphocyte Infusions

Both, acute and chronic Graft-versus-Host Disease are common complications after donor lymphocyte infusions [64]. The incidence of chronic Graft-versus-Host Disease after donor lymphocyte infusion has been between 33 to 61 percent [64–66]. Unfortunately, some patients that receive donor lymphocyte infusions do not survive long enough to develop chronic Graft-versus-Host Disease. At the same time, the development of Graft-versus-Host Disease correlates well with a Graft-versus-Tumor effect [64–66].

4.7. Acute Graft-versus-Host Disease

Atkinson, et al. analyzed risk factors for chronic Graft-versus-Host Disease in 2,534 recipients of HLA-identical sibling transplants surviving at least 90 days after transplantation [67]. The most important risk factor for chronic Graft-versus-Host Disease was acute Graft-versus-Host Disease. The three-year probabilities of chronic Graft-versus-Host Disease were 28 percent, 49 percent, 59 percent, 80 percent and 85 percent for patients with Grades 0, I, II, III and IV acute Graft-versus-Host Disease, respectively. The probability of developing this complication was 46 percent. Among patients with Grade II through IV acute Graft-versus-Host Disease, no other risk factor predicted chronic Graft-versus-Host Disease.

4.8. Other Factors

Age of the recipient is also very important; the older the patient, the higher the risk of developing chronic Graft-versus-Host Disease [67–69]. Donor parity and sex mismatch (female donor to male recipient), and cytomegalovirus positivity have also been associated with chronic Graft-versus-Host Disease [70–74]. Transplantation for chronic myeloid leukemia or aplastic anemia [67, 75, 76] and low levels of vascular endothelial growth factors have been associated with an increased risk of Graft-versus-Host Disease and non-relapse mortality [77]. Early complete chimerism has been associated with the development of extensive chronic Graft-versus-Host Disease [78]. In patients with aplastic anemia, the use of cyclophosphamide alone (compared with cyclophosphamide with limited field radiation, and cyclophosphamide plus total body radiation) resulted in less chronic Graft-versus-Host Disease than the other conditioning regimens [79]. High numbers of CD34+ cells in the graft correlate with the risk of chronic Graft-versus-Host Disease [80].

Recipient IL-10 ATA and Fas -670 genotype were independent risk factors for chronic Graft-versus-Host Disease.

5. Chronic Graft-versus-Host Disease and Graft-versus-Tumor Effect

Chronic Graft-versus-Host Disease has been associated with a Graft-versus-Tumor effect. It is difficult to describe a specific Graft-versus-Tumor effect to chronic Graft-versus-Host Disease that is different from acute Graft-versus-Host Disease. Most patients with chronic Graft-versus-Host Disease have had prior acute Graft-versus-Host Disease, so the sample size of those chronic only patients (previously referred to as *de novo*) is modest. Moreover, many of these patients actually had signs and symptoms that now would be labeled acute Graft-versus-Host Disease, but were called chronic simply because of the timing of presentation. Thus, historic data sets using time dependent designations for Graft-versus-Host Disease are not ideal to address the question of the antitumor effects of chronic Graft-versus-Host Disease. Despite these concerns, the available data suggests that there is an antitumor effect associated with chronic Graft-versus-Host Disease. For example, Sullivan, et al. studied the impact of Graft-versus-Host Disease in 1,202 patients with acute myeloid leukemia, acute lymphoblastic leukemia and chronic myeloid leukemia after HLA-identical sibling transplants done in Seattle [81]. A significant association of Graft-versus-Host Disease with a decreased relative risk of relapse in patients transplanted in advanced disease was found. Among patients developing either acute or chronic Graft-versus-Host Disease, mortality or relapse were decreased in patients with acute lymphoblastic leukemia transplanted in relapse, and chronic myelogenous leukemia in blast crisis. Among patients with acute leukemia transplanted in relapse, the probability of relapse after day 150 was 74 percent without Graft-versus-Host Disease, 45 percent with acute and chronic Graft-versus-Host Disease, 35 percent with only acute Graft-versus-Host Disease, and 34 percent with only chronic Graft-versus-Host Disease. Among patients with chronic leukemia in acceleration or blast crisis, the probability of relapse after day 150 was 65 percent without Graft-versus-Host Disease and 36 percent with Graft-versus-Host Disease.

6. Chronic Graft-versus-Host Disease Prophylaxis

Based on the success of using pharmacologic prophylaxis for acute Graft-versus-Host Disease, several groups have examined using prophylaxis for chronic Graft-versus-Host Disease. Multiple different regimens have been used in an attempt to prevent the development of chronic Graft-versus-Host Disease in high risk patients. At this point, no approach has really shown efficacy [82]. Generically, approaches such as T cell depletion, which substantially lower or eliminate acute Graft-versus-Host Disease, also reduces or eliminates chronic Graft-versus-Host Disease, but often at the risk of increased graft failures and higher relapse rates [60]. Using anti-thymocyte globulin may reduce the rates of extensive chronic Graft-versus-Host Disease and lung dysfunction in the unrelated transplant setting [83]. Bacigalupo, et al. recently updated their randomized trial on the use of cyclosporine/methotrexate with or without anti-thymocyte globulin for

Graft-versus-Host Disease prophylaxis [84]. The median follow-up for surviving patients was 5.7 years. At last follow-up, chronic Graft-versus-Host Disease was scored in 60 percent of non-anti-thymocyte globulin and in 37 percent of anti-thymocyte globulin patients, and extensive chronic Graft-versus-Host Disease was present in 41 percent and 15 percent, respectively. Chronic lung dysfunction was diagnosed in 51 percent versus 19 percent of patients. The proportion of patients with Karnofsky scores higher than 90 at four years were 57 percent versus 89 percent in non-anti-thymocyte globulin, versus anti-thymocyte globulin patients. For patients who survived one year, transplant-related mortality was 25 percent versus 3 percent. Although this data is suggestive, the study was not designed to look at chronic Graft-versus-Host Disease, all inferences must be made with due caution. Basara, et al. studied 155 patients with leukemia receiving unrelated grafts [85]. Chronic Graft-versus-Host Disease developed significantly more frequently in patients receiving no anti-thymocyte globulin group as part of their Graft-versus-Host Disease prophylaxis.

The length of immunosuppressive therapy was recently studied to see if it translated into a decrease in chronic Graft-versus-Host Disease. Burroughs, et al. retrospectively analyzed outcomes among 185 patients with hematologic malignancies who were given grafts from HLA-matched related donors following conditioning with total body irradiation alone or in combination with fludarabine [86]. Post-grafting immunosuppression consisted of mycophenolate mofetil (days 0–27) in combination with three different cyclosporine regimens: taper from (A) days 35 to 56, (B) days 56 to 77, and (C) days 56 to 180. The overall incidences of Grades II–IV and III–IV acute Graft-versus-Host Disease, and extensive chronic Graft-versus-Host Disease were 52 percent, 13 percent, and 56 percent, respectively to A, B and C. The duration of cyclosporine prophylaxis did not significantly influence the overall rate of acute Graft-versus-Host Disease (Grade II–IV), extensive chronic Graft-versus-Host Disease, or non-relapse mortality. In another study, patients who had received allogeneic transplants and had acute Graft-versus-Host Disease or histologic evidence of subclinical Graft-versus-Host Disease were randomly assigned to receive either six or 24 months of oral cyclosporine [87]. Clinical extensive chronic Graft-versus-Host Disease developed in 39 and 51 percent of those in the six- and 24-month treatment groups, respectively ($p = 0.25$). Montero, et al. also studied different cyclosporine schedules (in T cell-depleted peripheral blood stem cell grafts) and found no difference between the different schemes [59]. A recent meta-analysis concluded that day 11 methotrexate had no effect on the rates of chronic Graft-versus-Host Disease compared to patients who did not receive the drug [88].

Thalidomide was examined in a trial where 59 patients were randomly assigned to treatment with thalidomide (200 mg twice a day) or placebo [89]. The study was terminated after an interim analysis showed a higher mortality and incidence of chronic Graft-versus-Host Disease among the group receiving thalidomide.

After the observation that thymic atrophy was present in patients with chronic Graft-versus-Host Disease, thymus tissue implants, thymic endothelial cells or thymic hormones (thymosin fraction five and thymopentin) were given to HLA-matched sibling transplants [90]. No difference in the incidence of chronic Graft-versus-Host Disease was found in patients receiving thymic tissue or thymic hormones compared to controls.

7. Classification and Scoring

There are several ways to classify chronic Graft-versus-Host Disease: according to the type of onset, need for systemic immunosuppressive therapy, or mortality risk. The disease may evolve directly from acute Graft-versus-Host Disease ("progressive") or not ("interrupted") Graft-versus-Host Disease. Patients may also develop *de novo* chronic Graft-versus-Host Disease. Data from the International Bone Marrow Transplant Registry shows the distribution of chronic Graft-versus-Host Disease onset for HLA-matched siblings is 20 to 30 percent progressive, 30 to 40 percent interrupted and 35 percent *de novo*. Data from the National Marrow Donor Program for unrelated donor recipients, where the incidence of acute Graft-versus-Host Disease is higher, shows the spectrum of onset as 19 percent progressive, 69 percent interrupted and 12 percent *de novo* onset [1]. As discussed above, this distribution is likely to change now that the Graft-versus-Host Disease designation is based on clinical symptoms, not time post-transplant.

In 1980 the Seattle group proposed "limited/extensive" staging based on a retrospective clinical and pathological review of 20 patients with chronic Graft-versus-Host Disease after an HLA-identical sibling donor transplant [91]. In that report, three patients had limited chronic Graft-versus-Host Disease (characterized by localized skin involvement and/or hepatic disease without chronic aggressive histology) with relatively favorable prognosis. Most patients, however, had extensive disease with a progressive course. In these patients, severity correlated best with Karnofsky performance status and recurrent bacterial infections (more common in those with extensive disease) [91]. However, the classification of "limited/extensive" has significant limitations.

The National Institutes of Health Chronic Graft-versus-Host Disease Consensus Project Working Group on Diagnosis and Staging proposed a new severity scoring system [7]. Elements included in the proposed scoring system include both the number of organs or sites involved and the severity within each affected organ. Mild chronic Graft-versus-Host Disease involves only one or two organs or sites (except the lung), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites). Moderate chronic Graft-versus-Host Disease involves (1) at least one organ or site with clinically significant, but no major disability (maximum score of 2 in any affected organ or site) or (2) three or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). A lung score of 1 will also be considered moderate chronic Graft-versus-Host Disease. Severe chronic Graft-versus-Host Disease indicates major disability caused by chronic Graft-versus-Host Disease (score of 3 in any organ or site). A lung score of 2 or greater will also be considered severe chronic Graft-versus-Host Disease [7]. This new scoring has not been validated yet.

8. Prognosis

Several investigators have reported prognostic grading scales based on large numbers of observed patients with survival as the primary end point. Several studies have shown that thrombocytopenia (platelet count $<100 \times 10^9$/L), progressive onset, skin involvement, poor performance status and gastrointestinal

involvement are associated with a poor prognosis [1, 91–93]. The Johns Hopkins model stratified patients into risk categories according to the presence or not of extensive skin involvement, thrombocytopenia and progressive-type onset [93]. This model was validated using data from a total of 1,108 patients from the International Bone Marrow Transplant Registry, Fred Hutchinson Cancer Center, University of Nebraska and the University of Minnesota [94]. The International Bone Marrow Transplant Registry has also reported a prognostic model, using data from 1,827 HLA-matched sibling marrow recipients reported to the registry [1]. Karnofsky performance score, diarrhea, weight loss and cutaneous and oral involvement were found to be independent prognostic variables, from which a grading scheme was generated. This scheme, the limited/extensive classification system, and a classification based on clinical impression of overall chronic Graft-versus-Host Disease severity (mild, moderate, or severe) was assessed in a parallel analyses of 1,092 HLA-matched sibling transplant recipients from the International Bone Marrow Transplant Registry and 553 recipients of unrelated donor marrow from the National Marrow Donor Program. The presence of chronic Graft-versus-Host Disease was associated with fewer relapses, but increased treatment-related mortality. All schemes predicted treatment-related mortality. The survival of the most favorable chronic Graft-versus-Host Disease group in each scheme was similar or better than those of patients without chronic Graft-versus-Host Disease [1].

9. Clinical Manifestations

The clinical manifestations of chronic Graft-versus-Host Disease can be present before day +100 post-bone marrow transplant, but rarely after day +500; the median day of diagnosis is day +201 when an HLA-identical sibling donor is used, day +159 when an HLA-non-identical related donor is used, and day +133 after an unrelated donor bone marrow transplant [95]. The chronic Graft-versus-Host Disease Consensus Project stated that "the syndrome has features resembling autoimmune and other immunologic disorders such as scleroderma, Sjögren syndrome, primary biliary cirrhosis, wasting syndrome, bronchiolitis obliterans, immune cytopenias and chronic immunodeficiency," as such and, given the systemic nature of the problem, the constellation of signs and symptoms is enormous [7]. A biopsy is recommended to confirm the diagnosis. It is not uncommon to blame chronic Graft-versus-Host Disease for any problem that the patient develops after bone marrow transplant. In a study of 123 patients referred to Johns Hopkins for management of chronic Graft-versus-Host Disease, nine patients had no evidence of ever having this problem, and 26 had no evidence of active disease [14]. Therefore, it is important that patients are evaluated by a multidisciplinary team familiar with this problem. The requirements for obtaining a biopsy, and the criteria for interpretation, were recently published by the National Institutes of Health Chronic Graft-versus-Host Disease Consensus Project [8]. However, making the diagnosis remains one of the most important steps in the management of these patients; that way patients will not be unnecessarily exposed to immunosuppression or will not be denied therapy when needed [96]. As a baseline for all patients with chronic Graft-versus-Host Disease, we usually perform a complete physical examination, good dental examination, pulmonary function tests, complete blood counts, liver function tests, ophthalmologic examination

with Schirmer test and range-of-motion evaluation by a physical therapist, as well as a consultation with a social worker. The National Institutes of Health Consensus Conference has proposed a comprehensive examination that may limit its applicability outside of the clinical trial setting [97]. Depending on the clinical manifestations of the disease, the evaluation may need to focus on specific organs or systems [11].

Flowers, et al. analyzed the results of a study comparing bone marrow versus peripheral blood as the source of stem cells, to determine whether the clinical characteristics of chronic Graft-versus-Host Disease were different [98]. No significant differences were found in the incidence, time and type of onset of clinical extensive chronic Graft-versus-Host Disease or in the frequency of complications associated with severe morbidity. Involvement of skin and female genital tract was more frequent in peripheral blood stem cell recipients than in bone marrow recipients. The cumulative incidence of chronic Graft-versus-Host Disease at three years was similar in the two groups, but the number of successive treatments – and duration of steroid therapy needed to control it – were higher after peripheral blood than after bone marrow grafts, suggesting that disease in patients receiving peripheral blood stem cells may be (not just more frequent as discussed above, but) more difficult to treat.

In pediatric patients, chronic Graft-versus-Host Disease and/or its therapy can effect growth. The long-term effects of chronic Graft-versus-Host Disease and its therapy are more profound in children. One of the goals of cooperative pediatric oncology groups has been to address the late sequelae of therapy. This same goal is now being applied to chronic Graft-versus-Host Disease.

A detailed description of clinical manifestations present in chronic Graft-versus-Host Disease follows.

9.1. Skin and Dermal Appendages

Chronic Graft-versus-Host Disease often presents with a lichenoid eruption, an erythematous, papular rash that resembles lichen planus, and has no typical distribution pattern. Sclerodermatous Graft-versus-Host Disease may involve the dermis and/or the muscular fascia and clinically resembles systemic sclerosis. The skin is thickened, tight and fragile with very poor wound healing capacity. Hypo or hyperpigmentation may occur. There may be poikiloderma. Deep fibrosis can cause immovable skin that cannot be pinched. In advanced disease, nonhealing ulcers can be present. Hair loss and destruction of the sweat glands are common. Fingernails and toenails may be affected. Nails develop vertical ridges and cracking and are very fragile. Nail problems may persist even after skin changes have resolved. Hair loss in areas of affected skin may also persist after treatment, although recovery of hair is frequently a sign of recovery. Brittle hair often precedes alopecia. Premature graying of hair, eyebrows and eyelashes may be seen. An illustrated review on skin changes present in Graft-versus-Host Disease has recently been published [99].

9.2. Musculoskeletal System

Muscle cramps are a common complaint. Myositis, with tender muscles and elevated muscle enzymes, may start as a proximal myopathy [100]. As with other manifestations of the disease, it must be distinguished from the side

effects of therapy [101]. Fascial involvement in sclerodermatous Graft-versus-Host Disease is usually associated with skin changes, but may develop with normal, but fixed overlying skin making the diagnosis difficult to the inexperienced physician. Fasciitis often affects forearms and legs causing significant limitations in range of motion and joint contractures.

9.3. Mouth

Oral Graft-versus-Host Disease presents with xerostomia and/or food sensitivity. Physical exam reveals erythema with white plaques, and more extensive disease has lichenoid or hyperkeratotic changes. Pseudomembrane, nonhealing ulcers may be found anywhere in the mouth including the tongue and palate, but are often along the bite lines. Salivary dysfunction occurs and that translates into dental decay. Mucoceles are frequently seen. Patients complain of pain or discomfort with spicy food, carbonated beverages, or toothpaste. Local infections may cause changes in symptoms without changes in physical findings. Secondary infections with viruses and yeasts are very common. Fibrosis causing decreased oral range of motion is a very late manifestation [102].

9.4. Respiratory

Chronic Graft-versus-Host Disease of the lung can cause dyspnea, wheezing, cough, air trapping, bronchiectasis, pneumothorax, pneumomediastinum, subcutaneous emphysema, microbial colonization or infection and obstructive or restrictive changes on pulmonary function testing. Gastroesphageal reflux and silent pulmonary aspiration can be present. Chronic and acute sinusitis are frequently seen. Bronchiolitis obliterans and cryptogenic organizing pneumonia (also known as bronchiolitis obliterans with organizing pneumonia) are the pulmonary syndromes most likely to occur [103, 104].

9.5. Gut and Liver

Gastrointestinal symptoms are quite common in patients with chronic Graft-versus-Host Disease. Dysphagia, odynophagia, poor gut motility, heartburn, anorexia, nausea, vomiting, abdominal pain, cramping, diarrhea, pancreatic insufficiency, weight loss and malnutrition may occur. Esophageal webs are common. It is usually unclear whether these symptoms are directly related to chronic Graft-versus-Host Disease, stigmata of acute Graft-versus-Host Disease, or if they have a different etiology. Therefore, confirming the diagnosis is very important (14, 105]. It is also important to remember that patients with extensive chronic Graft-versus-Host Disease show an increase in resting energy expenditure and alterations in fat and carbohydrate oxidation rates [106]. Therefore, weight loss may not reflect gut Graft-versus-Host Disease exclusively.

Liver chronic Graft-versus-Host Disease can present as cholestasis (elevated alkaline phosphatase and/or serum bilirubin) or hepatitis (elevated transaminases which is more commonly, although not exclusively, seen after donor lymphocyte infusions) [107] which can symptomatically present as fatigue, jaundice, or pruritus. Abnormalities should be systematically evaluated and patients with rapid rises in liver enzymes should be strongly considered for liver biopsy, if there is no clear cause of the abnormality (such as drugs). Liver biopsy is particularly important in patients with isolated rising liver function tests and in those without tissue diagnosis [11]. Many patients

may have low platelets so it is important to correct any coagulation deficit before proceeding with the biopsy. Hemochromatosis must be excluded (serum ferritin, liver magnetic resonance imaging, biopsy, etc.) in patients who are long-term survivors from a transplant as therapy needs to be started before the damage is irreversible, and this complication can be confused with Graft-versus-Host Disease.

9.6. Eyes

Ocular Graft-versus-Host Disease often presents with irritation, burning, dry eyes, or photophobia from irreversible destruction of the lacrimal glands. It affects up to 80 percent of patients with Graft-versus-Host Disease. Keratoconjunctivitis sicca, cicatricial conjunctivitis, corneal ulcerations and scleritis are common features [108]. Excessive tearing may precede dry eyes.

9.7. Blood

Cytopenias are seen post-autologous [109] and allogeneic stem cell transplantation [110]. They can have different etiologies including Graft-versus-Host Disease [110–112]. Chronic Graft-versus-Host Disease can induce a state of anemia of chronic disease. Thrombocytopenia at the time that chronic Graft-versus-Host Disease is diagnosed is associated with poor prognosis [93, 94], but may also be due to idiopathic thrombocytopenia and, thus, responsive to steroid or rituximab treatment. Eosinophilia has been seen in both acute Graft-versus-Host Disease and chronic Graft-versus-Host Disease [113, 114]. In chronic Graft-versus-Host Disease, it has been associated with elevated levels of IL-5(115) and can herald or represent disease activity [116].

9.8. Reproductive Organs Tract Graft-versus-Host Disease

The incidence of genital Graft-versus-Host Disease has been reported at 35 percent at one year and 49 percent at two years [117]. Symptoms and signs include vulvar irritation, discharge, vulvar Lichenoid changes, vaginal stenosis, dryness, dyspareunia and pain. Topical therapy is effective in most cases, and self-maintenance of vaginal capacity by dilator or intercourse is quite useful. In males, some patients will be infertile, and this may be more common on patients with Graft-versus-Host Disease [118].

9.9. Immunodeficiency

Chronic Graft-versus-Host Disease causes profound immune dysfunction and autoimmunity, and most chronic Graft-versus-Host Disease deaths are attributable to infection. Functional asplenia with an increased susceptibility to encapsulated bacteria, particularly pneumococcus, is common. Patients are also at high risk for invasive fungal infections and *P. jiroveci* pneumonia [11]. A detailed description about antibiotic prophylaxis can be found elsewhere in this manuscript, as well as in the consensus paper by Couriel, et al. [11].

9.10. Other Organs

Heart involvement has been described as bradycardia [119]. Renal and central nervous system manifestations have been described, but these associations are not universally accepted [120–123]. Peripheral neuropathy can also be present

[124]. Virtually any autoimmune disease can be associated with chronic Graft-versus-Host Disease [96].

10. Therapy

10.1. Initial Therapy

The most commonly employed first line therapy for treating chronic Graft-versus-Host Disease is cyclosporine and prednisone. In patients classified as high risk on the basis of thrombocytopenia ($<100 \times 10^9$/l), treatment with prednisone alone only yielded 26 percent five-year survival. When a similar group of patients was treated with alternating day cyclosporine and prednisone, the five-year survival exceeded 50 percent [125]. Based on these findings, Koc, et al. conducted a trial comparing prednisone as a single agent to prednisone plus cyclosporine in patients with extensive chronic Graft-versus-Host Disease without thrombocytopenia [126]. In this trial with 287 patients, the cumulative incidence of transplant-related mortality, survival, relapse, need for salvage therapy, and discontinuation of immunosuppression were not different between the two arms. Tumor free survival and avascular necrosis of the hip were higher in the prednisone-only arm ($p = 0.03$). Because of the increased toxicity in the steroid-only arm, most transplant groups favor combining an immunophilin with a steroid for initial treatment.

Will the addition of other agents increase the response rate and/or decrease the length of therapy needed? This question is being examined in an ongoing trial looking at the addition of mycophenolate mofetil. A trial reported by Arora, et al. randomly assigned 54 individuals to receive cyclosporine and prednisone alone, or in combination with thalidomide, as initial therapy for chronic Graft-versus-Host Disease [127]. Response rates were similar in the two arms and complications were greater in the thalidomide arm.

10.2. Salvage Therapy

The most commonly used agent for steroid-refractory chronic Graft-versus-Host Disease is mycophenolate mofetil. The City of Hope group reported a retrospective review of their use of mycophenolate mofetil for *de novo* or refractory chronic Graft-versus-Host Disease [128]. As primary therapy, 90 percent of patients who received mycophenolate mofetil with prednisone or a calcineurin inhibitor responded. An impressive 75 percent of patients receiving mycophenolate mofetil for refractory chronic Graft-versus-Host Disease had a response. There have also been multiple smaller trials reported, all showing response rates above 45 percent in refractory patients [129–133]. The concerns raised in these trials included higher than expected infection rates, gastrointestinal toxicity and difficulty obtaining adequate blood levels. Despite these limitations, mycophenolate mofetil is a promising agent and the results of ongoing trials are eagerly awaited.

Pentostatin, a nucleoside analog with potent immunosuppressive properties, has been studied in acute and chronic Graft-versus-Host Disease [134–136]. It is an interesting agent with modest myeloid toxicity that kills lymphocytes by inhibiting adenosine deaminase, blocking the metabolism of 2'-deoxyadenosine [137]. In heavily pre-treated patients with chronic Graft-versus-Host

Disease, the response rate observed was over 50 percent, with infections as the main side effect of therapy. Renal function most be monitored in patients receiving this drug [135].

Sirolimus, which blocks mTOR, has been reported to have a 63 percent response rate when combined with tacrolimus, and corticosteroids in patients with steroid-refractory chronic Graft-versus-Host Disease [138]. Renal toxicity and a high rate of thrombotic microangiopathy were reported. Johnston, et al. reported that 15 of 16 evaluable patients with steroid-refractory chronic Graft-versus-Host Disease had a response to sirolimus, but again with a high rate of renal toxicity [139].

Directing a monoclonal antibody against a B-cell antigen may at first seem like an unlikely agent to use in Graft-versus-Host Disease. Rituximab is an anti-CD20 antibody which mediates B-cell lysis by complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and induction of apoptosis. It was initially developed for the treatment of B-cell malignancies, but has also been used to treat a number of autoimmune diseases. Although chronic Graft-versus-Host Disease is considered a T cell disorder, it is also characterized by autoimmunity. Many patients with chronic Graft-versus-Host Disease have evidence of B-cell activation with the presence of autoantibodies [19]. The correlation of the development of autoantibodies to the minor histocompatibility antigens encoded on the Y chromosome in sex mismatch transplants [21]. Given the low toxicity of rituximab compared to other agents, it is also an attractive potential therapy. There have been three small case series and a larger trial of 21 patients reported [23–26]. All of the reported patients received the standard regimen of 375 mg/m2/week for four weeks, with responders often receiving a second course. In the largest trial, 21 patients were treated although the response rate was high (70%); unfortunately, responses were limited to skin and musculoskeletal manifestations of chronic Graft-versus-Host Disease. Interestingly, antibody titers against Y chromosome–encoded minor HLA antigens fell in responders.

Extracorporeal photochemotherapy (or photopheresis) has been reported to give encouraging results. Couriel, et al. retrospectively evaluated 71 patients with severe chronic Graft-versus-Host Disease treated with this modality [140]. The response rate was 61 percent with 14 patients achieving a complete response. The best responses were observed in skin, liver, oral mucosa and eye. Thrombocytopenia was associated with a lower response rate, and there was a trend toward a higher response rate in *de novo* disease. Some of their responses were sustained. The cumulative incidence of steroid discontinuation at one year was 22 percent. The overall survival since initiation of therapy was 53 percent at one year. Foss, et al. reported on 25 patients receiving extracorporeal photochemotherapy with chronic Graft-versus-Host Disease [141]. Skin and visceral improvement was observed in 71 percent and 61 percent. One interesting finding (despite the small sample) was that there was no difference between those patients treated weekly and those treated every other week. Apisarnthanarax reported on 32 patients heavily pre-treated that received extracorporeal photochemotherapy [142]. Cutaneous involvement was extensive in 28 patients (88%) and was accompanied by visceral (hepatic, gastrointestinal) disease in 23 patients (72%). Complete responses were achieved in 22 percent and partial in 34 percent of patients. Greinix, et al. reported 15 patients treated with extracorporeal photochemotherapy [143]. Patients had extensive disease with

involvement of skin (n = 15), liver (n = 10), oral mucosa (n = 11), ocular glands (n = 6) and thrombocytopenia (n = 3). Cutaneous disease completely resolved in 12 patients. Contractures of knees and elbows due to scleroderma resolved only partially. Oral mucosal ulcerations resolved in all patients. Seven patients with liver involvement had complete responses. Table 26-1 lists the results of some of these approaches. It should be emphasized that all patients should be enrolled in clinical trials whenever possible.

10.3. Supportive Therapy

Infection is the leading cause of death in patients with chronic Graft-versus-Host Disease and a major cause of morbidity. The Ancillary Therapy and Supportive Care Working Group Report of the National Institutes of Health Consensus Project have suggested guidelines for preventing opportunistic infections among hematopoeitic stem cell transplant recipients [11]. Briefly, prophylaxis against *P. jiroveci* should be administered to all patients undergoing treatment of chronic Graft-versus-Host Disease. Lifelong splenic dysfunction occurs with chronic Graft-versus-Host Disease, and prophylaxis against encapsulated bacteria is recommended. At our center we recommend antibiotic prophylaxis before dental procedures, but there was no consensus about this issue [96]. Although no studies have evaluated the degree of protection provided by pneumococcal, Hib conjugate, or influenza vaccination in patients with chronic Graft-versus-Host Disease, most experts advocate their use. No live viruses, including the live attenuated influenza vaccine and Measles Mumps and Rubella, should be given. Household contacts should not be given oral polio vaccine. Intravenous immunoglobulins may be considered for patients > 90 days after bone marrow transplant who have recurrent sinopulmonary infections and serum IgG levels < 400 mg/dl. Some experts recommend monitoring IgG levels and administering intravenous immunoglobulin routinely in these patients, but there are no data to support this. Antifungal prophylaxis is an area of uncertainty. Current evidence does not support routine administration of antiviral prophylaxis for *Herpes simplex* virus in patients with chronic Graft-versus-Host Disease. Even when no definitive recommendations were given for *Cytomegalovirus* monitoring or prophylaxis, at our center we routinely obtain a *Cytomegalovirus* antigen on patients receiving therapy [11, 96]. Aggressive supportive care is of paramount importance to have a successful outcome for these patients. For further details about supportive care please refer to the document by Couriel, et al. [11].

11. Conclusions

The recent National Institutes of Health-sponsored Chronic Graft-versus-Host Disease Consensus Conference has given new life to the treatment of this disease [7–12]. Its implications are profound as it reached an agreement on areas of diagnosis, staging, strategies to validate and develop clinical trials and response criteria, supportive care, etc. [7–13]. This is very important since, even though many of these proposals need to be validated, they establish uniform criteria in this very complex disease. Also, it is expected that the future will bring a better understanding about the biology of this problem. Once the biology of chronic Graft-versus-Host Disease is better understood, it is possible

Table 26-1. Salvage therapies used in chronic Graft-versus-Host Disease (Modified from Lee SJ, et al. [144]).

Agent	Success	Hypothesized Mechanism of Action	Side Effects	References
High-dose corticosteroids	48% major response rate	Lympholytic at these doses	Infection, glucose intolerance, osteoporosis, avascular necrosis, cataracts, psychological effects including psychosis, insomnia	[45]
Pentostatin	Over 50% response rate	Inhibits adenosine deaminase	Nausea, vomiting, mild myelosuppression, renal toxicity	[135, 136]
Tacrolimus	35% response rate	Binds to FKBP-12 and inhibits T lymphocyte activation, concentrates in liver	Renal dysfunction, neurotoxicity, hypertension	[129, 146, 147]
Mycophenolate mofetil	46% objective response	Prodrug of mycophenolic acid that is a non-competitive reversible inhibitor of inosine monophosphate dehydrogenase. Cytostatic for T and B lymphocytes since they lack salvage pathways	Nausea, vomiting, diarrhea, neutropenia	[129–133, 148]
Sirolimus	63–90% response rate	Binds to FKBP-12 and mTOR to inhibit cytokine-driven T cell proliferation	Hyperlipidemia, hypertension, rash, nephrotoxicity	[138, 139]
Extracorporeal photopheresis	33%–80% response rates	Induces apoptosis in alloreactive T cells, normalization of CD4/CD8 ratios by decreasing CD8 cells, increases natural killer cells, decreases dendritic cells	Gastrointestinal upset, potential need for central IV access	[143, 149–151]
Rituximab	70% response rate	Chimeric anti-CD20 monoclonal antibody	Allergic reactions	[23–26]
Psoralen and ultraviolet A	40% complete and, 38% partial responses	Interferes with antigen presentation and inflammatory cytokine production by Langerhan's cells, increases IL-10 production by keratinocytes	Increase in skin cancer, phototoxicity, nausea, hepatotoxicity	[152–156]
Ultraviolet B radiation	Case series	Treats epidermis only, induces IL-10 in human epidermal cells	Increase in skin cancer, phototoxicity	[157]
Thalidomide	9–42% complete response rate	Anti-inflammatory and immunosuppressive properties	Neuropathy, somnolence, constipation, neutropenia	[158–160]
Etretinate (no longer available), acitretin	74% improvement	Synthetic vitamin A derivative, may affect production of cytokines	Skin scaling, breakdown, nail cracking, xerosis, cheilitis, pruritus, rare pseudotumor cerebri	[161]

(continued)

Table 26-1. (continued)

Agent	Success	Hypothesized Mechanism of Action	Side Effects	References
Azathioprine	Unknown	Cleaved to mercaptopurine	Gastrointestinal symptoms, neutropenia, thrombocytopenia	
Hydroxychloroquine	9% complete and 44% partial responses	Interferes with antigen processing and presentation, proliferation, tumor necrosis factor alpha production and cytotoxicity, synergistic with cyclosporine and tacrolimus *in vitro*	Gastrointestinal symptoms, rare retinal toxicity, myopathy	[101, 162]
Ursodeoxycholic acid	33% decreased in bilirubin.	Replaces native human bile acids, reduces class I HLA expression on hepatocytes	Diarrhea, abdominal pain, headache	[163]
Clofazimine	55% partial response	Atypical immunomodulatory effects	Abdominal cramping, hyperpigmentation	[164]
Anti-thymocyte globulin	Unknown	*In vivo* T cell depletion	Anaphylaxis, serum sickness	
Etanercept	Case series	Tumor necrosis factor inhibitor	Hypersensitivity reactions, infections	[165]
Daclizumab	Case series	Humanized anti-IL2 receptor antibody	Infections	[166, 167]
Infliximab	Case reports	Chimeric IgG monoclonal antibody, binds to tumor necrosis factor alpha and prevents binding with receptors	Hypersensitivity reactions, infections	[166]
Total lymphoid radiation	Case series		Leukopenia	[168, 169]
Topical azathioprine	Case report	Purine analog metabolized to 6MP	Rash, fever, pancreatitis, arthralgias, malaise, nausea, diarrhea, pancytopenia, hepatitis, infections, malignancy	[170]
Topical tacrolimus	Case series	0.1% ointment	Localized skin burning, pruritus, irritation, toxic absorption	[171, 172]
Ophthalmic cyclosporine	Case series	1% solution	None	[173]
Intravenous lidocaine	Case report	Vascular and anti-inflammatory properties	Seizures, drowsiness, tremors, hypotension	[174]

that future therapies can be targeted to treat specific defects and, therefore, decrease toxicities, both acute and long-term.

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Chapter 27

Graft Manipulation:
T Cell Depletion and Tumor Cell Purging

Vincent T. Ho

1. Introduction

When clinical hematopoeitic stem cell transplantation (HSCT) was first performed several decades ago, it was believed that high-dose chemotherapy or total body radiation would be sufficient to eradicate tumor cells, and the stem cell graft was recognized only as a means for rescuing hematopoiesis. Advances in transplantation immunology have created a paradigm shift, and demonstrated that perhaps even more so than the conditioning regimen, the components of the stem cell graft itself are crucial to transplant outcomes, both in terms of relapse and Graft-versus-Host Disease (GVHD). With technology to separate different cellular components of the stem cell graft, investigators can now dissect and "engineer" grafts aimed towards improving transplantation outcomes.

In autologous transplantation (autoSCT), disease relapse may occur because the high-dose conditioning regimen is insufficient to eradicate the cancer. Alternatively, relapse may occur because the autologous graft is contaminated with tumor cells, and the cancer is, in effect, reinfused into the patient after the chemotherapy. To enhance outcomes after autoSCT, graft manipulation techniques have been applied to purge malignant cells from the autologous stem cell product. In the allogeneic transplant setting (alloSCT), tumor contamination is not an issue since the graft is taken from a healthy donor. However, donor and recipient HLA differences render GVHD the most frequent and significant cause of morbidity and mortality after transplantation. In alloSCT, graft manipulation is almost exclusively used in the context of T cell depletion to reduce the incidence of GVHD. This chapter will review our current knowledge of graft manipulation as a means of tumor cell purging in autoSCT, and as donor T cell depletion in alloSCT.

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2. Graft Manipulation in AlloSCT: T Cell Depletion

Despite recent advances in HLA typing and improved supportive care, acute and chronic GVHD remain formidable complications after allogeneic blood stem cell transplantation (SCT). Consistent with the notion that GVHD is induced by donor T cells co-infused with the graft, T cell depletion (TCD) is one of the most effective methods available for preventing GVHD. However, T cell depletion has not gained widespread application because TCD is commonly associated with impaired immune reconstitution, graft failure, increased risk of post-transplant EBV-associated lymphoproliferative disorders, and loss of Graft-versus-Tumor activity. Most studies using TCD, including a recently published multicenter randomized phase II–III trial, [1, 2] have not demonstrated a survival benefit for T cell depletion over conventional pharmacologic GVHD prophylaxis. Despite these limitations, there remains interest for developing and improving technology to separate components of the donor grafts. These techniques offer hope that with better understanding of cellular subsets and their function, we will one day be able to engineer a graft that is competent in establishing engraftment, Graft-versus-Tumor activity, and effective immune reconstitution without GVHD.

3. Methods of TCD

T cell depletion strategies include the *ex vivo* removal of T cells or their subsets from the donor stem cell product, or through the *in vivo* reduction of donor and host T cell numbers by anti-T cell antibody administration. *Ex vivo* depletion of T cells from the stem cell product can be achieved by negative selection of specific T cells/subsets, or by the positive selection of CD34+ stem cells, thus allowing the lymphocyte components of the product to be elutriated.

Two of the more successful physical separation methods are lectin agglutination followed by rosetting with sheep red blood cells [3],or counterflow centrifugal elutriation, which separates cells based on sedimentation velocity due to differences in cell size and density between lymphocytes and progenitor cells [4-6]. Immunologic methods of T cell depletion rely mainly on monoclonal antibodies that target T cell antigens with restricted expression [7-9]. A common and versatile monoclonal antibody method for T cell depletion includes a set of antibodies known as CAMPATH, which targets CD52, an antigen expressed on lymphocytes and many antigen-presenting cells. Because CAMPATH antibodies can lyse cells in human serum, they are effective in both *ex vivo* or *in vivo* applications [10].

In vivo negative selection techniques commonly employed in HSCT include equine or rabbit-derived anti-thymocyte globulin (ATG) and alemtuzumab, the fully humanized version of CAMPATH. The benefit of *in vivo* TCD is that it simultaneously inhibits both recipient T cells that could mediate graft rejection, and donor T cells that might induce GVHD [11-13]. ATG and alemtuzumab have been particularly useful in nonmyeloablative transplantation or haploidentical transplantation, where both GVHD and graft rejection are common concerns. The availability of humanized alemtuzumab or ATG preparations has allowed transplant centers to offer TCD allogeneic transplants without a cell processing facility.

In the 1990s, several T cell depletion methods were adapted for the processing of peripheral blood stem cells (PBSC). However, T cell depletion studies

in PBSC transplantation have been limited to certain extents due to factors inherent to the PBSC product. As the mobilized PBSC product typically contains an average of 1 log excess in T cells relative to marrow grafts, older T cell depletion techniques, such as lectin agglutination or monoclonal antibody with complement, cannot be readily applied to PBSC due to cost, timing and efficiency considerations. While *in vivo* T cell depletion with alemtuzumab has been used with success in allogeneic PBSCT, *ex vivo* T cell depletion of PBSC has required the development of simple, automated and efficient medical devices that provide rapid and reliable separation of the cellular components of the PBSC graft. The most common physical method for TCD of PBSC is the CD34+ positive selection column. These systems enrich CD34+ based on the principle of immunoadsoption via biotin-avidin columns (Cellpro Ceprate SC), or by indirect or direct immuno-magnetic bead separation (Baxter Isolex 300i; Miltenyi CliniMACS, etc.) This process effectively reduces the lymphocyte content up to 5 logs in the eluted adherent fraction of CD34 cells. A critical issue with using a CD34-selected PBSC product is that this graft is, in effect, much more than a T cell-depleted graft. It is also devoid of B-cells, NK cells and other cellular components that could potentially be important for supporting engraftment, immune reconstitution, and antitumor immunity. Bypassing this issue, newer separation devices using immuno-magnetic beads coated with anti-CD8 or anti-CD3 monoclonal antibodies are now available, and should allow for direct anatomic depletion of T cell subsets from PBSC products.

4. T Cell Dose and GVHD

The extent of T cell depletion needed to prevent GVHD varies and depends not only on T cell dose, but also other factors including donor source, stem cell source and degree of major and minor HLA disparity, and other potential polymorphisms between donor and recipient pairs [14]. An average patient transplanted with unmodified bone marrow generally receives a T cell dose on the order of $1–3 \times 10^7$ cells/kg recipient body weight. Limiting dilution assays suggest that a 2-log depletion of T cells to $(1–3) \times 10^5$ cells/kg is necessary to prevent GVHD in the HLA-identical related donor marrow transplant setting without exogenous immune suppression [6, 15, 16].

G-CSF mobilized blood stem cell products contain approximately 10-fold more of T cells than unstimulated bone marrow grafts. CD34+ selection of PBSC with a 3 to 4 log reduction in CD3+ count to about 1×10^5 cells/kg is associated with lower rates of GVHD, but this benefit is counterbalanced by a higher incidence of graft failure, infection and disease relapse [17-19]. Although there appears to be a threshold effect at about 1×10^5 CD3+ cells/kg for PBSC transplantation, this T cell number must be considered in the context of the stem cell dose as well, since CD34+ count is also an important determinant of engraftment, GVHD and survival after PBSCT [20-23].

5. Specificity of TCD

Although TCD is regarded as a single concept, there is great variability in outcomes even among TCD studies, likely reflecting differences in separation techniques and depletion specificities. For example, some broad T cell depletion

strategies may also eliminate natural killer (NK) cells, immature thymocytes, B-cells and antigen-presenting cells along with the T cells. These other cellular elements may indeed play an important role in immune surveillance, engraftment and elimination of residual disease post-transplantation. Furthermore, even within the T cell compartment, it remains unclear how distinct subsets of T cells contribute to the development of GVHD, and whether depletion of specific subsets of T cells can improve outcomes. An important study of alternative donor transplantation from the IBMTR suggests that it is valid to segregate TCD techniques based on whether they have a "narrow" versus "broad" spectrum of reactivity. Examples of "narrow" specificity techniques include antibodies targeting mature T cells only, such as anti-TCR, anti-CD6, or anti-CD5, or T cells with specific functional roles (e.g., anti-CD8). Broad specificity techniques include antibodies such as CAMPATH, multiple antibody combinations and physical separation techniques such as lectin agglutination. In the IBMTR analysis, patients who received T cell depletion using narrow specificity antibodies had a superior five-year leukemia-free survival compared to those receiving broad specificity T cell depletion, primarily due to increased relapse and transplant-related mortality in the broad specificity TCD cohort [24].

6. TCD and Acute GVHD

6.1. Matched Related Donor Transplantation

In the early 1980s, investigators first demonstrated that acute GVHD could be reduced in human matched sibling BMT by treating the marrow with ATG [25]. Later studies employing various anti-T cell monoclonal antibody plus complement methods, as well as CAMPATH antibodies *in vivo* or *ex vivo*, confirmed that the Grade II–IV acute GVHD incidence can be reduced to less than 20 percent in the matched sibling marrow setting, sometimes even in the absence of additional immune suppression [26-37].

In the 1990s, several devices were developed for CD34 selection that would prove useful for the processing of mobilized peripheral blood stem cell (PBSC) products in matched sibling transplantation. A study comparing three products (CEPRATE, Isolex 300i, CliniMACS) found no apparent differences in the allograft composition or clinical outcomes, although ease of processing did differ among the three devices [38]. Initial studies using biotin-avidin CD34 selection columns showed that a 2 to 3 log reduction in T cells from matched related donor PBSC was insufficient to control GVHD despite the use of cyclosporine ± methrotrexate as post-transplant immune suppression [39, 40]. However, a subsequent study from the Spanish group found that when the T cell content in the sibling PBSC graft was reduced by 3.0 to 3.5 logs using the CEPRATE or Isolex 300i systems, Grade II–IV acute and extensive chronic GVHD outcomes were markedly improved, at 10 percent and 12 percent, respectively [41]. This difference in GVHD incidence in the Spanish trial suggests a possible threshold effect for the development of GVHD in matched related donor PBSCT when the CD3+ T cell dose exceeded 0.5×10^6 cells/kg.

6.2. Alternative Donor Transplantation

Because an HLA-identical sibling is available in only 25 to 30 percent of patients requiring transplant, most transplant candidates will need an alternative

graft source, such as an unrelated donor or a partially HLA-mismatched family member. Transplantation from these "alternative" donors is generally associated with a higher risk of GVHD, although, in recent years, this gap appears to be narrowing in unrelated donor transplantation due to improved HLA typing techniques.

6.2.1. Matched Unrelated Donor (MUD)

Retrospective data from the national marrow donor program (NMDP) and other sources have shown that the incidence of Grade II–IV GVHD after unrelated donor TCD BMT ranges from 20 to 50 percent, which is lower than the 40 to 75 percent incidence reported in a series of URD BMT where TCD was not employed [42-45]. In the initial report from the NMDP, the use of TCD was the most significant factor predicting freedom from severe (Grade III–IV) acute GVHD [42]. A subsequent analysis from the IBMTR involving 1,868 leukemia patients receiving marrow transplants from donors other than HLA-identical siblings revealed that the Grade II–IV acute GVHD incidence were 34 percent and 38 percent for the TCD groups, compared to 57 percent for the non-TCD cohort (P <0.0001) [24].

6.2.2. Randomized Trials of TCD versus Non-TCD in MUD BMT

In a recent prospective multicenter randomized phase II-III trial comparing TCD versus conventional GVHD prophylaxis in unrelated donor marrow transplantation, 410 patients with hematologic malignancies were randomized to receive either T cell-depleted bone marrow (by the monoclonal antibody $T_{10}B_9$ or counterflow centrifugal ellutriation) with post-transplant cyclosporine (CsA) alone, versus T-replete bone marrow with post-transplant CsA and methotrexate (MTX) as GVHD prophylaxis [1]. Conditioning consisted of cyclosphosphamide (1,200 mg/kg over two days) and total body irradiation (1,320-1,375 cGy over four days). Participants in the TCD arm received additional treatment to promote engraftment. Anti-thymocyte globulin (60 mg/kg over two days) was added in recipients of TCD by elutriation, and cytarabine (9 gm/m2 over three days) was added to Cy/TBI in the recipients of TCD by T10B9 antibody. Mean T cell dose infused for the TCD and non-TCD arms were 2.8 vs. 30.1×10^6 CD3 cells/kg, respectively (p <0.0001). There was no difference in incidence of primary graft failure in the two groups. The incidence of acute GVHD was significantly lower in the TCD arm compared to the non-TCD arm. Cumulative incidence estimates of acute GVHD Grades II–IV were 39 percent and 63 percent for those receiving TCD versus CsA/MTX (P<0.0001), and the cumulative incidence of Grades III–IV acute GVHD were 18 percent versus 37 percent, respectively (p< 0.0001). TCD patients also had significant reduction in regimen-related toxicity within the first 28 days of transplant, with less stomatitis (P<0.0001), hepatic (P=0.0003), pulmonary (p=0.012) and CNS complications (p=0.024) compared to the CsA/MTX arm. However, these reductions in acute GVHD and regimen-related toxicity did not improve transplant-related mortality because of a higher incidence of severe infections in the TCD group, especially with cytomegalovirus. Another important finding in this trial was that TCD in the unrelated donor setting did not adversely affect relapse, with the exception of patients transplanted for chronic myelogenous leukemia in first chronic phase. Overall disease free survival at three years was 30 percent, and there was no difference between the TCD and non-TCD arms (27% versus 34%, respectively p=0.16). Important

long-term results regarding chronic GVHD from these randomized trials was published in a separate report by Pavletic and colleagues [2]. Although there was a reduction in acute GVHD with TCD, the incidence of chronic GVHD at two years was similar between the TCD and non-TCD arms, 29% versus 34% (P=0.27), respectively. Overall survival at three years from diagnosis of cGVHD was also similar. In summary, this important randomized study showed that partial (1 log) *ex vivo* depletion of T cells from unrelated donor marrow reduced acute GVHD and early regimen-related toxicity. However, TCD was also associated with a greater risk of severe infections, and failed to reduce the incidence of cGVHD, such that in the end, no net benefit in disease free or overall survival is achieved.

Investigators from the Gruppo Italiano Trapianti Midollo Osseo (GITMO) have also published the results of two randomized trials testing different schedules of ATG (7.5 mg/kg vs. 15 mg/kg) with cyclosporine versus cyclosporine/methotrexate after MUD BMT. They found that the only higher dose ATG (3.75 mg/kg IV × 4 days from day –5 through day-2) was associated with a significant reduction in Grade III–IV acute GVHD compared to the CsA/MTX control arm (11% versus 50%, p=0.001). However, this reduction in severe acute GVHD did not modify transplant-related mortality because 30 percent of the patients on the high-dose ATG arm died of severe infections, and the final overall survival was not affected by the use of either low-dose or high-dose ATG [46].

6.2.3. Partially Mismatched Related Donor Transplantation (PMRD)

Overcoming major HLA barriers to enable transplantation of mismatched grafts remains a central goal for the use of T cell depletion. In the PMRD setting, reported Grade II–IV GVHD incidences have ranged from 18 percent to 40 percent after TCD with monoclonal antibodies plus complement [47, 48]. A more recent series of 201 patients receiving T cell-depleted myeloablative BMT from partially HLA-mismatched family donors reported Grade II–IV acute GVHD of 13 percent when partial TCD was achieved with OKT3 or T10B9 antibodies [49]. However, a majority of these patients also received steroids, ATG and cyclosporine as additional prophylaxis, and survival was reduced by a high incidence of infectious complications and leukemia recurrence.

6.2.4. Haploidentical Donor Transplantation

Early studies of TCD in full haplotype mismatched BMT reported low incidence of acute GVHD, but were complicated by high rates of graft failure and morbidity from infection [50]. Better engraftment results have been achieved in recent years by using high doses of CD34+ selected mobilized peripheral blood cells as the graft source [51, 52]. Successful engraftment of haploidentical family donor PBSC using a nonmyeloablative conditioning consisting of fludarabine, cyclophosphamide and alemtuzumab was also recently published [53]. This FC+ alemtuzumab conditioning regimen, together with mycophenolate mofetil +/– cyclosporine post-transplant, yielded a 16 percent incidence of Grade II–IV acute GVHD (8% Grade III–IV).

7. TCD and NK Cell Reactivity

Haploidentical transplantation may be employed in conjunction with extensive TCD as a platform for establishing natural killer (NK) cell activity to reduce leukemia relapse without GVHD after transplant. Donor recipient disparity

in HLA class I alleles, particularly at HLA-C, could translate to mismatches in killer immunoglobulin receptors (KIRs) which form the basis for NK cell allorecogniton and activation. The biology of KIR mismatch and NK cell reactivity is discussed in detail in other chapters of this book. The concept of NK cell reactivity in clinical transplantation was first reported by Ruggeri and colleagues from Perugia Italy [54]. In this study, acute leukemia patients underwent haploidentical transplantation with an intensive myeloablative conditioning regimen coupled with extensive T cell depletion using sheep RBC rosetting and CD34 selection of the PBSC product. No post-transplant immune suppression was given. Among 60 patients transplanted, only four cases of GVHD were observed. Notably, patients in this group whose donor-recipient HLA disparity predicted for killer immunoglobulin receptor (KIR) mismatch and NK cell reactivity in the Graft-versus-Host direction experienced no graft failure, acute GVHD, or myeloid leukemia relapse. To prove that this phenomenon was a result of donor NK cell activity, investigators were able to detect high frequencies of alloreactive donor NK cells in the recipient post-transplant that specifically lysed "missing KIR ligand" leukemia targets, and not control target cells that expressed the "self" KIR ligand [54].

These results generated speculation that HLA class I mismatching in the unrelated donor setting would also lead to leukemia relapse if the mismatch predicts for a KIR mismatch in the GvH direction. However, as of today, only one retrospective study in mismatched unrelated donor transplantation, in which cyclosporine, methotrexate and pre-transplant ATG was used as GVHD prophylaxis, has demonstrated that KIR ligand mismatch in the GVH direction improved TRM, disease free and overall survival [55]. A majority of other similar studies in conventional non-TCD mismatched URD transplantation, including a recent analysis from the combined CIBMTR, EBMT and Dutch registry, have failed to demonstrate any benefit in outcomes for recipients with KIR mismatch [56-59]. It is possible that in the non-TCD setting, the beneficial effects of NK cells were offset by the deleterious effects of alloreactive T cells in the absence of TCD. Recent studies indeed suggest that vigorous TCD is necessary and that TCD may enhance NK cell recovery and NK cell reactivity after transplantation [60]. Large multicenter prospective clinical trials of HLA-mismatched or haploidentical SCT with extensive TCD will be necessary to assess the true contribution of NK cell reactivity to transplant outcomes.

8. TCD and Chronic GVHD

TCD's effect on the development of chronic GVHD after SCT remains unclear. While a retrospective analysis from the NMDP had previously demonstrated that TCD reduced the risk of both acute and chronic GVHD [42], a recent prospective multicenter randomized trial of unrelated donor marrow transplantation failed to demonstrate any difference in the incidence and time to development of cGVHD (29% versus 32%, P=0.27) between patients receiving TCD versus standard cyclosporine/methrotrexate GVHD prophylaxis, although overall incidence reported in this trial was low and may not represent true results [2]. It has been suggested that perhaps the 1 log TCD achieved by elutriation or T10B9 monoclonal antibody in this prospective study was insufficient to protect against chronic GVHD. However, the lack of protection

against cGVHD despite lower risks of acute GVHD may also reflect intrinsic differences in the pathogenesis in these two conditions. A retrospective study from the IBMTR examinig the effect of broad versus narrow specificity TCD also demonstrated no protection for TCD against chronic GVHD despite a reduction in acute GVHD [24]. In the multivariate analysis, the use of narrow specificity TCD methods was, in fact, associated with a 1.5-fold increased risk of chronic GVHD ($p=0.0003$) compared to pharmacologic GVHD prophylaxis. This finding suggests that accessory cells not removed in the narrow specificity T cell depletion process may play a role in promoting cGVHD.

9. Organ Dysfunction after TCD BMT

T cell depletion may be associated with less organ toxicity early post-transplant compared to conventional BMT. In single institution series, TCD has been associated with a lower incidence of hepatic, renal and pulmonary complications [61-63]. In an analysis of 199 allogeneic transplants, the incidence of severe pulmonary complications was 8 percent among those who received CD6 TCD as the sole form of GVHD prophylaxis, but 33 percent among those who received cyclosporine and methotrexate ($P<0.0001$) [64]. The protective effect of TCD against pulmonary complications was independent of the diagnosis of acute GVHD. Reductions in organ toxicity were also reported in a randomized trial of TCD plus cyclosporine versus cyclosporine plus methotrexate [1]. Regimen-related toxicity as assessed by the Bearman toxicity scale was significantly lower in the TCD cohort. It is possible that the reduction in organ dysfunction after TCD is related to absence or reduced use of immune suppressive drugs such as methotrexate. Alternatively, TCD may result in lower levels of inflammatory cytokines or chemokines that are damaging to the endothelium and/or epithelium in vital organs such as the liver, lungs and kidneys.

10. TCD and Graft Failure

Graft failure is an uncommon occurrence (<1%) after conventional bone marrow transplantation, and is most often restricted to patients with aplastic anemia who had been previously allo-immunized though blood product transfusions [65-67]. However, graft failure can be a significant problem after T cell-depleted transplants. This is not surprising since donor T cells co-infused with the graft also play an important role in promoting engraftment. BMT series using various methods of TCD in the 1980s and early 1990s consistently reported incidences of graft failure ranging from 5 percent to 50 percent [6, 10, 27, 28, 30, 66, 68-81]. In an analysis from the IBMTR, T cell depletion was associated with a significantly increased risk for graft failure compared to unmanipulated marrow transplantation (relative risk 9.29, $P<0.0001$) [82].

Graft failure after TCD marrow transplantation may occur in two patterns: (1) failure of initial engraftment or graft rejection within two weeks of BMT, and (2) delayed graft failure which can occur months post-transplant [68]. Early graft failure after TCD transplantation likely represents primary immunologic rejection of donor hematopoietic elements by host lymphoid cells that have survived the conditioning regimen. Host T lymphocytes with donor-specific

activity have been isolated from the blood of patients at the time of graft rejection [71, 83-92]. It is likely that TCD or marrow manipulation removes cells that are important for promoting engraftment. Some murine models have shown NK cells to be critical for establishing engraftment, while others have implicated CD8+ T cells. In a clinical trial of HLA-mismatched URD BMT where patients were given marrow grafts engineered to contain graded doses of CD4 cells and CD8 cells, investigators found that it was the CD8+ cell dose, but not CD4+, that was associated with graft rejection [93].

The mechanism behind late graft failure remains to be elucidated. Unlike primary rejection, donor-specific host T lymphocytes have not been isolated from the blood of recipients at the time of late graft failure. Since mixed hematopoietic and lymphoid chimerism is common after TCD transplantation, late graft rejection may result when this state of immune tolerance is broken by some event. Viral infections with cytomegalovirus (CMV) or human herpes virus 6 (HHV-6) may also contribute to late graft failure after BMT [94-98]. Although TCD patients have a higher incidence of CMV reactivation [99-101], there is no direct evidence linking these viruses to graft rejection.

Clinical evidence supporting the role of immunologic rejection in graft failure include observations that HLA disparity between donor and recipient is a primary risk factor, and that by increasing host immune suppression, either via intensified conditioning regimen or additional immune suppressive agents post-transplant, the risk of graft failure can be mitigated [68, 78, 81, 82, 102-104]. Aside from intensifying the conditioning regimen, other attempts to reduce graft failure after TCD transplantation have involved narrowing the breadth of TCD, use of *in vivo* TCD techniques such as ATG or CAMPATH that result in concurrent host immune suppression post-transplant, and infusion of increasing doses of CD34+ cells in the graft.

Graft failure can also be a major complication in allogeneic PBSC transplantation when extensive TCD is employed. In this setting, the risk of graft failure is inversely correlated with the CD3+ content in the PBSC graft. In one analysis of 257 patients undergoing matched sibling PBSC transplantation with CD34 selection, the incidence of graft failure was 18 percent among recipients whose grafts contained $\leq 2.0 \times 10^5$ CD3+ cells/kg, compared to 1 percent among those who received $> 2 \times 10^5$ CD3+ cells/kg (P=0.001), and low CD3+ dose was a significant predictor of graft failure in the multivariate analysis [19]. Similarly, an NIH/NHLBI study demonstrated that a CD3+ dose $< 0.83 \times 10^5$ cells/kg was an independent factor associated with poor survival, primarily from graft failure and disease relapse [105].

11. Delayed Immune Reconstitution after TCD BMT

Delayed immune reconstitution has been well documented with various methods of T cell depletion [106-113]. In phenotypic analyses, total lymphocyte numbers are usually higher early after BMT in recipients of conventional marrow transplants than those who receive TCD grafts. Further, the reconstituted T cell compartment after TCD BMT is often deficient in CD4+ cells relative to CD8+ cells. This can result in an inverted CD4+ to CD8+ ratio that can last for up to two years [110]. CD4+ cell numbers normalize at seven to nine months after conventional BMT, but this process is delayed after TCD BMT [106].

Functional recovery of T cells is also impaired after TCD BMT. In recipients of TCD BMT using lectin agglutination, the proliferative response of peripheral blood mononuclear cells to exogenous IL-2 stimulation remains abnormal for up to six months, compared to only one month for recipients of conventional BMT [109]. Proliferative response of T cells to mitogenic stimuli are impaired for up to 18 months after TCD BMT [110]. T lymphocytes from recipients of TCD BMT also have restricted variability in their T cell receptor repertoires [111, 113].

12. EBV Reactivation and PTLD after TCD

Delayed immune reconstitution and loss of TCR repertoire diversity may place TCD BMT recipients at increased risk for opportunistic infections and post-transplant EBV-associated lymphoproliferative disease (PTLD). A number of studies have demonstrated a higher probability of Epstein-Barr virus (EBV) reactivation in recipients of TCD grafts [31, 114, 115]. Reported incidence of PTLD after TCD BMT ranges from 5 to 30 percent [31, 114, 115]. Recipients of TCD transplants using HLA-mismatched or unrelated donor marrow appear to be at particularly high risk [114, 116, 117], as are patients with severe GVHD and those treated with certain anti-T cell monoclonal antibodies [31, 114, 118]. Recipients of cord blood transplantation are also at high risk for EBV-PTLD, especially if they are conditioned with a nonmyeloablative regimen that includes anti-thymocyte globulin (ATG) [119]. The pathogenesis and biology of EBV-LPD is discussed in another chapter dedicated to this topic.

13. Leukemia Relapse after TCD BMT

The association between use of T cell depletion and increased risk of disease relapse is well recognized, especially in patients transplanted for chronic myelogenous leukemia (CML) [69, 82, 120-126]. The increased incidence of relapse after TCD BMT has been linked, at least in part, to the reduction in GVHD and concomitant loss of Graft-versus-Leukemia (GVL) activity. It is well established that allogeneic SCT is superior to autologous or syngeneic transplants because donor T cells can mediate allo-immune responses, and patients who develop clinically significant GVHD have a better leukemia-free survival after BMT [127-129]. Direct evidence of the importance of donor T cells in the GVL effect have been derived from DLI studies of patients with CML who have relapsed after allogeneic BMT, where complete remission rates of 70 to 80 percent are achieved [130, 131].

In patients receiving matched sibling BMT for first chronic phase CML, the risk of relapse with TCD has been estimated to be 5- to 6-folds higher than conventional BMT [82, 121]. This relative risk is as high as 18 for patients transplanted in the accelerated phase [123]. Interestingly, the incremental risk of relapse is less apparent after TCD transplantation using matched unrelated marrow [129, 132-134]. In one series, the three-year probability of relapse for CML chronic phase was only 8 percent for recipients of unrelated TCD allografts, compared to 47 percent for those who received TCD marrow from HLA-identical siblings [134]. A study from the EBMT demonstrated that in patients with CML who received URD BMT, TCD was not associated

with a higher incidence of relapse in the multivariate analysis [135]. In the recent multicenter randomized trial of TCD using T10B9 or elutriation versus cyclosporine/methotrexate as GVHD prophylaxis, the three-year relapse incidence was higher in the TCD patients transplanted for CML (20% vs. 7%, $p = 0.017$), but not different in patients transplanted for acute leukemia [1].

In contrast to CML, TCD appears to have minimal effect on the relapse rates of patients transplanted for acute leukemia. Reported rates of relapse after matched sibling TCD BMT for acute myelogenous leukemia (AML) in first remission range from 3 percent to 31 percent [36, 136, 137, 227]. Retrospective data from the IBMTR showed that TCD is associated with a 1.7- to 2.0-fold increased risk for recurrence in patients with acute lymphoblastic leukemia (ALL) in any phase, and in patients with AML who are transplanted in relapse or in first complete remission (CR) [82]. In this same analysis, AML patients transplanted in CR2 actually had a lower risk of relapse with TCD. In two separate randomized trials comparing TCD with methotrexate and cyclosporine as GVHD prophylaxis for patients undergoing HLA-matched related or unrelated BMT, a higher relapse rate was observed after TCD BMT only in patients with CML, not in patients with acute leukemia [138].

The risk of leukemia relapse may also vary depending on the extent and specificity of TCD employed. A retrospective analysis from the IBMTR reported that leukemia-free survival for recipients of grafts T-depleted with "narrow specificity"antibodies (e.g., anti-CD5, anti-CD6, anti-CD8, anti-TCR$\alpha\beta$, etc.) were significantly higher compared to "broad specificity" TCD methods (anti-CD2, CAMPATH, elutriation, lectin agglutination, etc.). The five-year probability of leukemia relapse was 28 percent for recipients of narrow specificity TCD BMT, versus 51 percent for recipients T-depleted by other techniques ($P<0.001$) [24]. The five-year relapse rate for recipients of narrow specificity TCD were similar to that observed in patients who received unmanipulated BMT. These results suggest that, at least in the unrelated or mismatched marrow transplant setting, TCD using narrow specificity antibodies can reduce GVHD without significant loss of GVL activity.

14. TCD in Nonmyeloablative Transplantation

Over the past decade, allogeneic transplantation employing reduced-intensity or nonmyeloablative conditioning regimens is increasingly used. The low incidence of regimen-related toxicity associated with these nonmyeloablative transplants (NST) has rendered transplantation feasible for patients who are otherwise precluded from HSCT due to old age or comorbid medical conditions. Conditioning regimens for NST often include low doses of chemotherapy and/or TBI at doses sufficiently immune suppressive to permit donor cell engraftment, but likely not intense enough to kill significant numbers of tumor cells. As such, disease eradication and long-term "cure" after NST relies primarily upon the development of a GVL effect from the donor graft. Thus, there would appear to be little rationale for TCD in NST since TCD could blunt the GVL effect, and defeat the purpose of the transplant itself. Furthermore, the nonablative preparative regimens in NST might not be sufficiently immune suppressive to support and sustain the engraftment of

T cell-depleted stem cells or marrow. ATG and alemtuzumab have been used to achieve simultaneous depletion of donor and host T cells to ensure engraftment and reduce GVHD in NST, especially in the URD and mismatched setting [139-141]. CD34 selection is another TCD method employed in NST [142-143]. Unfortunately, these TCD methods are associated with poor immune reconstitution and a high incidence of treatment failure from disease relapse and infections, especially from CMV and adenovirus [13, 144-149]. Many studies of TCD NST thus incorporate planned donor lymphocyte infusion or T cell add-back post-transplant to improve immune function, facilitate full donor chimerism, and restore the GVL effect [150-152]. In a comparative analysis of two prospective studies using alemtuzumab/cyclosporine versus cyclosporine/methotrexate as GVHD prophylaxis after fludarabine/melphalan (FLU/MEL) reduced-intensity conditioning, there was a significantly lower incidence of acute and chronic GVHD in the alemtuzumab NST patients, but a higher incidence of CMV reactivation and disease relapse/progression [13]. However, long-term disease and overall survival status were similar between the two cohorts when responses were considered after DLI in the patients on the alemtuzumab arm. These data suggest that TCD can be used to induce a state of mixed chimerism with minimal GVHD after NST, and provide a platform for additional adoptive immunotherapy interventions such as DLI or vaccinations to bolster immune function and GVL.

15. Strategies to Reduce Relapse After TCD

Efforts for reducing leukemia relapse after TCD have primarily focused on immune-based strategies to enhance GVL activity without comprising GVHD prevention. These approaches have included: (1) selective purging of T lymphocyte subsets, such as CD8+ cells, which may preferentially mediate GVHD over GVL; (2) efforts to induce NK cell mediated antitumor activity; (3) prophylactic donor lymphocyte infusion (DLI), and potentially (4) strategies utilizing cancer vaccinations after transplant to fortify the GVL effect.

15.1. CD8+ T Cell Depletion

Selective CD8 T cell depletion has attracted particular interest because preclinical data suggest CD8 T cells preferentially mediate GVHD[153]. Clinical trials in HLA-identical sibling BMT in the early 1990s, including a prospective randomized study, have demonstrated that CD8+ depletion with post-transplant cyclosporine alone could reduce the incidence and severity of GVHD without compromising GVL [33, 35]. However, data on CD8 T cell depletion in the unrelated donor setting have been disappointing. In a matched unrelated donor BMT series using ablative busulfan-based preparative regimens, a 2-log depletion of CD8+ cells was associated with a 67 percent incidence of Grade II–IV aGVHD (19% grade III–IV), and 15 percent graft failure [154]. Two-log CD8+ depletion also appears insufficient to abrogate GVHD in PBSC transplantation. In two phase II trials of patients receiving MRD and MUD PBSC after cytoxan/TBI conditioning, 2-log depletion of CD8+ cells to median cell doses between 2 –8 $\times$ 10^5 CD8+/kg was associated with 44 percent and 88 percent incidence of Grade II–IV acute GVHD, respectively [155]. Although acute GVHD incidence was high, most cases of GVHD in these two trials

presented as a steroid responsive skin rash at the time of neutrophil recovery, with little long-term sequelae. Importantly, Graft-versus-Leukemia was preserved, as only three of 41 patients transplanted have relapsed despite extended follow-up, and the three-year relapse-free survival was 56 percent.

15.2. Induction of NK Cell Activity

Recent evidence suggests that NK cells could also be important mediators of antileukemic responses after HSCT. Preclinical models have shown that adoptive transfer of CD8+ NK/T cells expanded in culture exhibit potent cytotoxic activity against allogeneic tumor targets without causing GVHD [156]. NK cell antileukemic alloreactivity has also been demonstrated in human transplantation. In haploidentical transplantation with KIR mismatches in the GVH direction, donor-derived alloreactive NK cell clones capable of lysing recipient target leukemia cells have been isolated at a high frequency early post-transplant in the absence of clinical GVHD [54]. These results suggest that, at least under certain circumstances, HLA-mismatched transplantation combined with intensive conditioning and extensive T cell depletion could provide a platform for the induction of NK cell-mediated antitumor activity without GVHD. Whether such NK cell-mediated antileukemic activity exists in mismatched transplantation in the absence of extensive TCD remains to be elucidated.

Low doses of IL-2 administration may also enhance GVL activity through NK cell stimulation without inducing GVHD. Prolonged infusion of low-dose recombinant IL-2 following autologous or TCD allogeneic BMT is well tolerated and results in a marked increase in NK cell numbers [157]. In a pilot clinical trial, infusion of IL-2 of at least four weeks after TCD BMT appeared to lower the incidence of disease relapse relative to historical controls [158]. Further studies are needed to confirm the potential beneficial impact of low-dose IL-2 therapy.

15.3. Prophylactic Donor Lymphocyte Infusion (DLI)

Donor lymphocyte infusion or T cell add-back strategies are commonly used in conjunction with TCD as a strategy to restore GVL activity and prevent disease relapse. Prophylactic DLI strategies may be particularly useful after TCD SCT using CD34 selection, ATG or CAMPATH, where the extensive depletion leads to profound and long lasting impairment of immunity [13, 144, 146, 149]. Unfortunately, GVHD is common after these DLI because the T cell infusion is often administered early after transplant following rapid withdrawal of immune suppression. While the incidence of chronic GVHD after prophylactic DLI is similar to conventional transplantation, a recent report suggests that this cGVHD after T cell add-back is associated with less mortality, but retains its protective effect on relapse [159].

15.4. Cancer Vaccination after TCD HSCT

As new cancer vaccines are being developed, they may also be applied in the allogeneic SCT setting to stimulate tumor-specific activity without GVHD. Tumor vaccine response may be potentiated early after TCD because of the homeostatic T cell expansion and activation that occurs

early after lymphodepletion [160], possibly through the elimination of CD4+CD25+ regulatory T (T-reg) cells [161], or removal of cellular elements that act as sinks for cytokines needed to augment tumor-specific T cell responses [162]. Consistent with this, administration of irradiated tumor cells engineered to secrete GM-CSF in mice six weeks after TCD BMT elicited lasting antitumor immunity without GVHD [163]. However, this vaccine effect was restricted to animals of TCD BMT, and was not seen in non-TCD BMT animals that developed GVHD. Rather than immunizing recipients, it may also be possible to vaccinate donors prior to stem cell harvest, thereby transferring tumor-specific effector cells to the host. Still to be determined are the precise antigens and adjuvants needed for optimal immunization, and whether TCD will eliminate transfer of protective effector cell populations.

16. Investigative Strategies for Graft Manipulation

16.1. Alloreactive T Cell Depletion

To avoid the potential deleterious effects of nonspecific T cell depletion, some investigators are turning to TCD techniques whereby alloreactive T cells are selectively removed from the graft. Methods for removing alloreactive donor T cells include photoinactivation [164], or immunologic agents that target T cell activation markers, such as CD25 or CD69 [165-168]. It is postulated that these methods would preferentially purge alloreactive lymphocytes responsible for causing GVHD, but retain "nonreactive" T cells which may improve post-transplant immune reconstitution, enhance engraftment and the GVL effect. In a pilot clinical trial of elderly patients receiving matched related donor nonmyeloablative transplantation, *ex vivo* selective depletion of alloreactive T cells using an anti-CD25 immunotoxin did not hamper engraftment, immune recovery, or GVL. Acute GVHD incidence was reasonably low (48% Grade II–IV; 12% Grade III–IV), especially considering that cyclosporine was given as sole post-transplant immune suppression, and was discontinued by day +100 [167]. A concern with the approach of targeting CD25+ alloreactive T cells is the issue of concurrent depletion CD4+CD25+ regulatory T cells from the graft. Whether this loss of regulatory T cells will result in an increase in autoimmune disease and/or cGVHD after transplantation remains under investigation.

16.2. Induction of T Cell Anergy

An alternative prophylactic approach has focused on the induction of host-directed antigen-specific anergy in donor T cells through the blockade of costimulatory pathways (e.g., CD28/B7, LFA-1/ICAM, CD40-CD40L). In a small pilot study of patients transplanted with HLA-mismatched marrow treated *in vitro* with CTLA4-Ig as a method of T anergy induction, a 27 percent incidence of acute GVHD was reported [169]. These results suggest that induction of anergy may be a feasible method for the prevention and treatment of GVHD in the future. If distinct targets for GVHD and GVL can be identified, it may ultimately be possible to induce GVHD-specific anergy, while preserving the T cell response to tumor antigens for a full GVL effect.

16.3. HSV-TK Suicide Gene Insertion

Herpes simplex thymidine kinase (HSV-TK) gene insertion into donor T cells represents another novel approach for controlling GVHD after allogeneic transplantation or DLI. In a pilot study, infusion of HSV-TK+ T lymphocytes with TCD marrow graft did not hamper engraftment, and resulted in stable establishment of circulating TK+ cells in the recipient post–transplant [170]. A case of chronic cutaneous GVHD responsive to ganciclovir has been reported in a patient who had received T cells bearing the HSV-TK gene at the time of BMT [171]. HSV-TK gene insertion has also been studied in the DLI setting [172, 173]. In a recently published report, 23 patients with relapsed hematologic malignancies after BMT were given HSV-TK transduced DLI (TK-DLI), with 29 percent CR and 35 percent PR, similar to that expected for unmodified DLI [174]. The authors further demonstrated a direct correlation between antitumor response with peak levels of TK+ cells in circulation, and that circulating TK+ cells could be disrupted by the administration of ganciclovir. Although the development of an immune response against the TK protein was also associated with reduced circulating TK+ cells, a recent report found that development of HSV-TK-specific CD8+ T cells did not interfere with the TK-DLI response [175].

16.4. Adoptive Therapy with Regulatory T cells (Tregs)

In recent years, CD4+CD25+ regulatory T cells (Tregs) have been identified as important mediators of self tolerance, auto/alloimmunity and immune reconstitution after HSCT. Murine transplant models have consistently demonstrated that Tregs are potent suppressors of alloimmune responses, and can protect against GVHD without abrogating Graft-versus-Tumor activity [176, 177]. Correlative studies in human HSCT have also demonstrated that lower levels of Tregs are associated with the development of acute and cGVHD [178, 179]. These findings have set the stage for the potential therapeutic application of Tregs in human transplantation. Clinical trials involving adoptive transfer of Tregs as treatment or prevention of GVHD are underway, and could one day unlock the secret to the separation of GVHD and GVL.

17. Summary: T Cell Depletion

In the two plus decades since the first clinical trials of TCD were performed, we have greatly extended our understanding of the immunologic basis of engraftment, GVHD and GVL. While there is no debate that TCD reduces the risk and severity of acute GVHD and, in most cases, transplant-related organ toxicity, randomized trials to date have failed to prove that TCD improves overall survival after transplantation. With improved understanding of the cellular effectors involved, we may yet be able to engineer a graft that will minimize GVHD while preserving immunologic integrity to fight infection and eradicate residual disease. As new immunologic targets of GVL are discovered, opportunities for cancer vaccination strategies with TCD will emerge that may further improve transplant outcomes without GVHD.

18. Graft Manipulation in AutoSCT: Tumor Cell Purging

As success in autologous stem cell transplantation depends primarily on the cytotoxic conditioning regimen and not Graft-versus-Tumor activity after transplant, conventional wisdom suggests disease relapse would be more likely if contaminating tumor cells are reinfused into the patient with their autologous graft. Detractors from this theory would argue that outcomes of most phase II studies of purged versus unpurged autoSCT are similar, and that patients often relapse post-autoSCT in spite of previous disease, suggesting that the treatment failure resulted from incomplete tumor eradication by the conditioning regimen, and not reinfused contaminating tumor cells. Nonetheless, there is compelling data showing that, at least in some cases, relapse after autoSCT is caused by tumor cells reinfused with the graft. For example, in studies of autoSCT where reinfused contaminating tumor cells were marked by a neomycin resistance gene, the same marker was detected in the resurgent cancer cells in all patients at the time of relapse [180, 181]. Studies in follicular NHL showing disease relapse is reduced in recipients of PCR negative BM grafts provide further evidence that tumor contamination contributes to disease recurrence after transplantation, and that effective purging of the graft could improve autoSCT outcome [182].

19. Tumor Cell Contamination in AutoSCT

The risk of tumor contamination in autologous grafts depends on a number of variables, including the underlying malignancy, disease status at harvest and stem cell source. For example, marrow contamination is extremely uncommon in patients with germ cell tumors, but far more common in those who have NHL or leukemia. Likewise, patients with a higher tumor burden at time of collection are more likely to have marrow contamination compared to those who are in complete remission. In addition, the ability to detect tumor contamination relies on the sensitivity of the assay used. Evidence of tumor contamination in autologous grafts have come from studies whereby occult tumor cells can be detected in morphologically normal marrow by immunocytochemistry, clonogenic tumor assays and polymerase chain reaction (PCR) techniques [183-185]. Tumor contamination is also well documented in PBSC transplantation [186, 187]. In a study of unmanipulated autologous PBSCT for multiple myeloma, contaminating tumor cells were detected from the leukopheresis products in eight of 14 patients [188]. In light of these facts and gene marking studies proving contaminating tumors cells contribute to disease recurrence, it appears prudent to explore tumor cell purging of the marrow or PBSC graft as a mechanism to reduce relapse and improve clinical outcomes after autoSCT.

20. Methods of Purging

Similar to TCD in the allogeneic transplant setting, early studies of tumor cell purging of bone marrow involved the use of monoclonal antibodies with complement. These techniques were often limited by variability of complement lots that resulted in inconsistent cytolysis. The use of complements could

be bypassed with the use of immunotoxins that have direct cytotoxic activity, immunomagnetic bead depletion, or monoclonal antibody-coated microparticle sedimentation [183, 189, 190]. Effective *ex vivo* purging of leukemia cells has also been achieved with cytotoxic drugs such as 4-hydroperoxycyclophosphamide (4-HC) [191] or its related compound mafosfamide [192]. With mobilized PBSC becoming the preferred source of stem cells in autologous transplantation, CD34+ selection has become the most widely used strategy for *ex vivo* removal of contaminating tumor cells from PBSC product. In one study, CD34 selection achieved a 2.7- to 4.5-log reduction in myeloma cells from the stem cell infusate [188]. A disadvantage of CD34 selection of PBSC is that the concurrent loss of immune cells results in slower reconstitution of the immune system post-transplant, leading to a higher rate of infectious complications [193], especially from cytomegalovirus(CMV) and adenovirus infections [194, 195].

In recent years, the availability of humanized antibodies has made it possible to administer these agents to the patient just before their stem cell harvest as a method of so-called *in vivo* purging. *In vivo* purging is advantageous in that it is easy to perform, does not require a cell manipulation laboratory and treats not only the stem cell product, but the entire patient as a whole. The antibody that is currently most widely used in this setting is rituximab in patients receiving autologous SCT for B-cell Non-Hodgkin's Lymphoma [196]. In a recent randomized trial comparing *ex vivo* CD34 selection with *in vivo* purging using rituximab for patients receiving autologous transplantation for NHL, investigators found that both were similarly efficient in eliminating tumor B-cells in the infusate, but Rituxan purging was associated with less CD34+ cell loss and faster time to myeloid engraftment [197].

21. Clinical Efficacy of Tumor Cell Purging

Tumor purging in autologous marrow transplantation was first performed in Non-Hodgkin's Lymphoma, and has been most widely studied in this disease [183, 198, 199]. Additional studies have been performed in multiple myeloma [200-202], ALL [203-205] and AML [191, 206-209]. Although a number of studies suggest that *ex vivo* purging of autologous grafts reduces relapse, this benefit has generally not been demonstrated in larger multicenter studies, including randomized trials [202, 210]. Whether this failure to demonstrate an advantage for purging is related to the relative deficiency of the purging techniques involved, the inability to eradicate the putative clonogenic tumor stem cell, or the resistance of the cancer to the high-dose chemotherapy, remains unclear.

21.1. Purging in Non-Hodgkin's Lymphoma

In a large single center a study, 114 patients with follicular NHL received high-dose cytoxan with TBI followed by infusion of autologous marrow purged with anti-B-cell moABs plus complement [183]. Residual disease in the processed marrow was assessed by PCR amplification of the IgH-BCL2 translocation locus. Recipients of marrows negative for lymphoma by PCR had a significantly lower incidence of relapse, compared to those who received marrow grafts that were still PCR-positive after monoclonal antibody purging

[182]. Furthermore, PCR positivity after purging remained the strongest predictive factor of lymphoma relapse in multiple regression analysis, independent of lymphoma histology, degree of marrow involvement at harvest and disease status at transplant. Clinical benefit for the same purging technique with autologus BMT was not observed in patients who were transplanted for mantle cell lymphoma [199]. The lack of efficacy for purging in mantle cell NHL may reflect intrinsic resistance of this histology to *ex vivo* purging [211]. Results demonstrating no clinical benefit for purging have been reported in other autologous transplant series involving patients with progressive or aggressive NHL [212, 213].

The effect of purging in autologous transplantation for NHL has also been assessed in large BMT registry analyses. The European Blood and Marrow Transplant Lymphoma Registry compared the results of 270 patients whose autologous marrow grafts had been purged compared to 270 case-matched unpurged control patients. No difference in progression-free survival was observed with the purging, although there was improved overall survival in the subset of patients transplanted for low-grade NHL [210]. In a more recent study reported from the lymphoma working committee of the IBMTR and EBMT, patients with low-grade NHL who received unpurged autografts had significantly lower disease free survival compared to recipients of syngeneic transplants (RR 2.28; $p = 0.04$) or purged autologous grafts (RR 1.59; $p = 0.003$), suggesting that an uncontaminated graft minimizes the risk of relapse [214]. However, this benefit of purging was restricted to patients with low-grade NHL, and was not seen in patients transplanted for intermediate or high-grade NHL. In a prospective randomized trial from the EBMT (CUP trial), 140 patients with chemotherapy sensitive relapsed follicular lymphoma were randomized to receive conventional chemotherapy, autologous transplant, or autologous transplant with purging using an antibody cocktail against B-cell differentiating antigens [215]. Although this trial detected a significant survival benefit for patients who underwent autologous transplant compared to chemotherapy, there was no definite improvement in four-year overall survival (77% versus 71%, $p = 0.079$) for purging versus no-purging in the autologous transplant patients. Taken together, these studies suggest that purging may offer a benefit in subsets of patients with low-grade NHL, particularly follicular lymphoma, although definitive conclusions will require larger randomized clinical trials.

In recent years, small series using CD34 selection or anti-B-cell nickel bead sedimentation have demonstrated efficient purging of follicular lymphoma cells from PBSC products, with 2 to 3 log depletion [216, 217]. Despite processing, there was frequent residual lymphoma contamination of the product, and assessment of the impact on relapse is limited by small sample size. Studies assessing *in vivo* purging using rituximab are ongoing, with some encouraging preliminary results [218].

21.2. Purging in Multiple Myeloma

Although plasma cell contamination of the PBSC graft is well documented [188] and associated with disease progression post–autoSCT [219], clinical trials using *ex vivo* purging in multiple myeloma have generally been disappointing. Smaller series using immunologic negative selection techniques

have demonstrated purging does not impede engraftment, and is associated with reasonable rates of disease progression [220-222]. In contrast to negative depletion, CD34 selection of PBSC has been more widely studied as a purging method in autologous transplantation for multiple myeloma. In a prospective phase III randomized trial of patients undergoing CD34+ selected (n=93) versus unselected (n=97) autologous PBSCT for myeloma, no difference in progression-free survival or overall survival was observed [202]. This result is particularly notable considering that the plasma cell burden in the stem cell product was reduced by a median of 3.1 logs by the CD34+ selection process. Other studies comparing CD34 selected versus unselected autologous PBSCT for multiple myeloma in prospective, nonrandomized trials, have likewise demonstrated no difference in survival [223, 224].

21.3. Purging in Acute Myeloid Leukemia

High-dose chemotherapy with autologous peripheral stem cell transplantation is a potential consolidation strategy for patients with AML in first complete remission [225]. To reduce the risk of AML contamination in the marrow infusate, investigators have employed a number of different purging techniques, including 4-hydroperoxy-cyclophosphamide (4-HC) [191, 225] and the anti-CD33 monoclonal antibody anti-MY9 [206]. Although a small study using 4-HC purged marrow in patients with AML CR1 demonstrated a three-year disease free survival of 54 percent [191], a large randomized trial led by ECOG demonstrated that autologous transplantation with 4-HC purging did not provide any advantage compared to standard consolidation with high-dose cytarabine [226]. In a more recent reported series from Germany, 98 patients with high risk AML underwent mafosfamide purged ABMT in first or second or higher complete remission with cytoxan/TBI conditioning. Twenty-seven (30%) remained in continuous CR with a median follow-up of 11 years. However, long-term toxicity was significant with this treatment, as 44.4 percent of survivors developed cataracts, and 4.4 percent developed secondary myelodysplasia [209].

22. Conclusions

Despite improvements in graft engineering techniques that allow for *ex vivo* physical separation of contaminating tumor cells in the marrow or stem cell product, it remains to be proven that *ex vivo* purging improves relapse or survival outcomes after autologous stem cell transplantation. To date, randomized clinical trials in multiple myeloma, AML and Non-Hodgkin's Lymphoma have been disappointing. It is possible that *ex vivo* purging as a concept is flawed because many cases of relapse after autologous SCT result not from tumor contamination of the graft, but from failure of the chemo-radiotherapy to eradicate residual disease in the host. To improve disease outcomes and survival, future studies may thus need to combine *ex vivo* purging with better conditioning regimens, or novel agents that will achieve further purging *in vivo* during and after the transplantation process.

Despite the negative results of prospective randomized trials for tumor cell purging and T cell depletion, there remains great enthusiasm for studies of graft engineering in stem cell transplantation. There is no question that the

composition of the graft matters and affects transplant outcomes. The observations that T cell depletion abolishes GVHD after alloSCT and genetically marked contaminating tumor cells directly cause relapse after autoSCT are testaments to this fact. The challenge ahead remains whether we will be able to "fine tune" these cellular components in such a way that will optimize transplant effectiveness. The future of transplantation may well be one where the composition of the graft is prescribed on an individual basis, adjusted based on the patient's unique profile, disease status and, in the allogeneic setting, the donor characteristics and degree of donor-recipient HLA disparity.

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Chapter 28

Donor Lymphocyte Infusions

Clinical Applications and the Graft–versus-Leukemia Effect

Edwin P. Alyea

The success of donor lymphocyte infusion (DLI) in inducing long lasting remissions in patients' chronic myelogenous leukemia provided the first direct evidence of the existence of a Graft-versus-Leukemia (GVL) effect. In the decade since the first reports of DLI's use by Kolb and Slavin, the diseases which response to DLI have been identified and efforts to further enhance the GVL response have been explored [1, 2]. Graft-versus-Host-Disease (GVHD) is the major complication of DLI and strategies aimed at limiting this toxicity have been investigated. Demonstrating the profound antitumor effect mediated by the donor graft has led to the development of nonmyeloablative, or mini-transplants, which depend upon the GVL response for success.

Extensive laboratory efforts to identify the effector mechanism of response and potential targets of the GVL reaction have been undertaken. Interestingly, despite the dramatic clinical responses that have been observed, the targets and effector mechanisms of the GVL effect remain unclear. Many potential targets have been proposed including alloantigens, such as minor histocompatibility antigens, as well as tumor-specific antigens. Responses may be mediated either by cellular mediated direct cytotoxicity, or indirectly via inflammatory cytokines. The role of humoral immunity is also now being explored, and may also play a role in the response to DLI. This chapter will review the clinical applications and outcomes of DLI. We will also discuss future strategies to enhance the GVL response mediated by DLI, with focus on potential targets of the GVL effect.

1. GVL: Experimental Models

Early preclinical studies of transplantation suggested the presence of a GVL effect. In 1956, Barnes and colleagues observed that radiation alone was not sufficient to eliminate 100 percent of leukemic cells in murine transplant models and proposed the existence of the GVL effect mediated by the allogeneic splenocyte graft [3, 4]. This initial observation was confirmed by numerous investigators using a variety of murine models demonstrating that adoptively transferred lymphocytes, given either prior to or following transplant, are able

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to eliminate residual leukemia cells [5]. The effector cell population and the target of the GVL reaction in these models is dependent both on the HLA relationship between donor and host cells and the antigens expressed by the malignant cells.

Indirect evidence suggested the existence of a GVL effect in human transplantation. This indirect evidence included the observation of a higher relapse rate in recipients of syngeneic transplants compared with allogeneic transplants from sibling donors [6, 7]. In addition, numerous studies demonstrated a higher relapse rate in recipients receiving T cell-depleted transplantation compared with patients receiving T cell-replete transplantation [7-10]. The differences in relapse rates between T cell-depleted transplantation and T cell-replete transplantation were most pronounced in patients with CML, the disease now recognized as most sensitive to the GVL effect. In addition, there appears to be a tight link between the GVL effect and the development of GVHD. A reduced risk of relapse was also observed in patients who developed GVHD after BMT [11, 12]. Further observations supporting the GVL effect and its link to GVHD includes the withdrawal of immune suppression in relapsed transplant patients resulting in remission, often in the presence of GVHD [13-15]. Direct evidence of the existence of a GVL effect was obtained when donor lymphocyte infusions (DLI) were successfully used to treat patients with CML who had relapsed after BMT.

2. Clinical Results

2.1. Donor Lymphocyte Infusion for Chronic Myelogenous Leukemia

Since the initial reports by Kolb and Slavin, numerous series have confirmed the high remission rates of DLI in patients with CML who have relapsed after allogeneic BMT [16-21]. Registry reports about CML patients from Europe and North America who relapsed after HLA-matched sibling donor transplants demonstrate a complete cytogenetic response rate of >70 percent when they were treated in either cytogenetic or hematologic relapse (Table 28-1) [2, 22]. These studies also consistently demonstrate that patients with CML in more advanced stages of relapse, accelerated phase or blast crisis, have a much lower response rate following DLI [17, 22]. An analysis of 593 donor lymphocyte infusions demonstrated responses for patients with CML in molecular relapse, cytogenetic relapse, chronic phase and accelerated/blastic phase

Table 28-1. Results of chronic myelogenous leukemia treated with donor lymphocyte infusion.

Stage of Disease	North American [22]	%	EBMTR [140]	%
Early Relapse	27/38	71%	126/164	78%
Cytogenetic	3/3	100%	40/50	80%
Hematologic	24/35	74%	88/114	77%
Advanced Phase	5/18	28%	13/36	36%
Accelerated,	4/12	33%		
Blast Phase	1/6	17%		

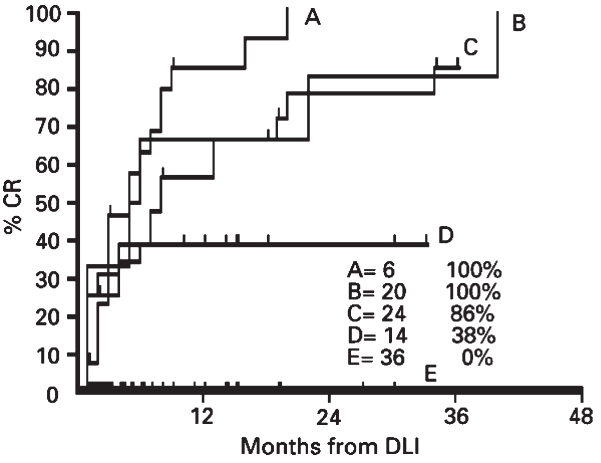

Fig. 28-1. Response to DLI in patients with CML

relapse were 100 percent, 90 percent, 75 percent and 35 percent, respectively (Fig. 28-1) [23].

Responses observed in patients with CML after DLI appear to be durable. Two studies have reported long-term follow-up of patients who achieved a complete remission following DLI. Five (13%) of 39 patients who achieved complete cytogenetic remission after DLI relapsed with extended follow-up[24]. The three-year overall survival for these 39 patients was 70 percent. The EBMTR has reported on 44 patients with CML who achieved a molecular remission after treatment DLI [25]. Four (9%) of 44 patients developed evidence of recurrent disease by PCR with extended follow-up. The three-year overall survival for this group of patients was 95 percent. While these results are encouraging, longer follow-up will be needed to fully assess the impact of this treatment. When relapse does occur, isolated extramedullary involvement without evidence of systemic disease can be observed. The mechanism of "immune escape" for cells in these myeloblastomas is not clear.

DLI is also effective in treating patients with CML relapsing after unrelated donor transplant [26]. Eleven (46%) of 24 patients treated with DLI from unrelated donors achieved a complete response with seven (58%) of 12 patients treated in the early phase of relapse obtaining remission. Similar to the results of DLI from matched siblings, the response to DLI in advanced stage CML remains poor with only four (31%) of 13 patients achieving remission. It is interesting to note that the results of unrelated and related donor DLI are similar despite greater antigenic differences in unrelated donor-recipient pairs.

Studies have attempted to identify characteristics which may be predictive of a response to DLI in patients with CML. Analyzed factors included the degree of donor chimerism at the time of DLI, stage of disease at the time of DLI and dose of cells infused. The degree of donor chimerism at the time of DLI is not predictive of response in patients with CML. While a high degree of donor chimerism was associated with a more rapid response, patients with <10 percent donor chimerism at the time of DLI had a similar complete remission rate as patients with high degrees of donor chimerism at the time

of DLI [27]. Therefore, low degrees of donor chimerism is not an indicator for not proceeding with DLI.

Confirming prior observations, stage of disease at the time of transplant is the most significant predictor of response in patients with CML. Studies consistently demonstrate that patients receiving DLI in cytogenetic or hematologic relapse have a much higher response rate than patients treated in more advanced phases of the disease. T cell dose also appears to impact both response rate and risk of developing GHVD. When the impact of cell dose on response was assessed in the large registry studies, no clear correlation between cell dose infused and response was noted [2, 22]. However, the doses of cells infused in the majority of these patients may have been so high that the beneficial effects of low-dose cell infusion were not apparent. In a large retrospective analysis of 344 patient with CML treated at 51 centers, an initial cell dose of less than 2×10^7 mononuclear cells was associated with less GVHD, less myelosuppression, similar response rate, improved survival, improved progression-free survival and reduced DLI-related mortality compared with higher doses of DLI [28].

Two prospective trials of un-manipulated DLI have analyzed T cell number and the impact on response and GVHD. MacKinnon, et al. have reported a high response rate and low incidence of GVHD in patients receiving 1×10^7 CD3+ cells/kg [29]. Among the eight patients receiving this dose only one developed GVHD. Lower doses of cells appear less effective with no responses seen in patients receiving less than 1×10^7 CD3+ cells/kg. A subsequent trial compared a single bulk dose regimen with an infusion of escalating doses of T cells [30]. Patients receiving a single bulk infusion received a median infusion of 1.5×10^8 T cells/kg while patients receiving the escalating regimen received 1×10^7, 5×10^7 and 1×10^8 T CD3+ cells/kg if no response or toxicity was observed after each infusion. The incidence of GVHD was significantly lower with the escalating dose regimen (10%) compared with the single bulk infusion (44%) ($p=0.011$). There was no difference in the remission rate. Supporting these observations, a retrospective analysis of 30 patients with relapsed CML treated with DLI, patients who received $<1 \times 10^8$ CD3+ cells/kg and $<8 \times 10^7$ CD4+ cells/kg had a better survival and similar response rate [31]. In a small trial, the addition of alpha-interferon allowed much lower doses of donor cells to be infused and was associated with response rates similar to those seen with higher cell doses [32]. In this study 12 of 13 patients in cytogenetic or molecular relapse who received 2 to 6×10^6 CD3+ T cells/kg combined with low dose alpha-interferon obtained a complete cytogenetic response. In DLI from unrelated donors, no correlation between cell dose and response rate or incidence of acute GVHD has been noted.

The time to complete cytogenetic response in patients with CML after DLI is prolonged, often eight to 16 weeks following the initial infusion of donor cells [33]. The time to complete molecular response, defined by elimination of the BCR-ABL transcript as detected by PCR, can be six months or greater after cell infusion (Fig. 28-2). Several studies have demonstrated that alpha-interferon is not required to achieve a response in patients with CML treated with DLI. Both the number of cells infused and the time after transplant when cells are infused appear to be important factors in limiting GVHD.

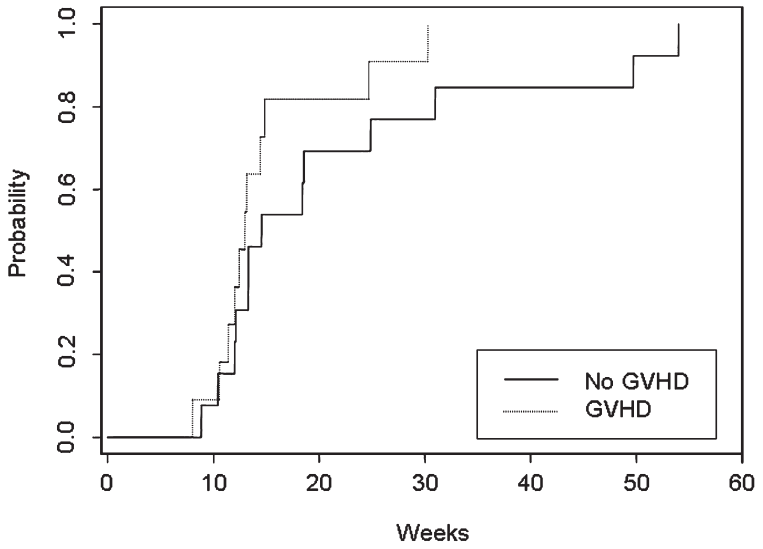

Fig. 28-2. Time to complete cytogenetic and molecular response in patients with CML

Table 28-2. Results of multiple myeloma treated with DLI.

	N	Prior Chemotherapy	CR (%)	PR (%)	Overall RR (%)
Salama, et al. [141]	25	3	7 (33%)	2 (8%)	9 (36%)
Lokhorst, et al. [37]	27	13	6 (22%)	8 (29%)	14 (52%)
DFCI	21	0	9 (43%)	6 (29%)	15/21 (71%)

3. Multiple Myeloma

Several studies of allogeneic transplantation in patients with myeloma had suggested the presence of a Graft-versus-Myeloma (GVM) effect [34-36]. DLI studies have provided direct evidence of the GVM effect with DLI inducing significant responses in patients with multiple myeloma who have relapsed after transplantation (Table 28-2). The overall response rate to DLI in patients with myeloma approaches 45 percent, with complete responses noted in about 25 percent of patients. Durable complete responses are noted in half of the patients who obtain a complete remission, with follow-up over seven years in some patients.

Both the dose of infused cells and the timing of DLI after transplantation for myeloma may influence response rates. Lokhorst and colleagues reported that patients receiving $> 1 \times 10^8$ CD3+ cells/kg had an improved response; however, responses have been noted in patients with dose infusions as low as 1×10^7 CD3+ cells/kg [37]. Early administration of DLI after allogeneic transplantation may improve response rates and improve GVM after transplantation.

Researchers have explored the use of T cell depletion to reduce GVHD and transplant toxicity early after allogeneic HSCT, followed by DLI to restore the

GVM effect. In that trial prophylactic DLI was given to 14 myeloma patients six to nine months after T cell-depleted myeloablative allogeneic transplantation [38]. Of the 14 patients receiving DLI, 11 patients had evidence of disease at the time of DLI. Ten of the 11 patients with evidence of disease demonstrated significant GVM responses, with six patients obtaining a complete remission. While a significant GVM effect could be induced by the addition of prophylactic DLI after allogeneic transplant, only 58 percent of myeloma patients were able to receive DLI after transplantation. Additional patients could not receive DLI because they had developed complications, such as GVHD limiting the utility of this approach.

Another study reported on 16 patients who received DLI for relapsed or persistent myeloma one year after T cell-depleted myeloablative BMT [39]. Ten (63%) developed GVHD after DLI, and eight of 16 (50%) had disease response (6 CR; 2PR). Investigators from Europe have also administered DLI to patients with residual or progressive myeloma after *in vivo* T cell-depleted allogeneic HSCT using reduced-intensity conditioning [40]. Fourteen of 20 patients received escalating dosesm of DLI for residual/progressive disease >six months post-transplant. Seven (50%) patients had a clinical response. Demonstrating the close link between the Graft-versus-Myeloma effect and GVHD, five of the seven patients who developed GVHD after DLI developed either PR or a CR. Unlike CML, these responses were less durable, and most patients in this study had progression of disease within one year despite persistent full donor chimerism.

Studies have attempted to identify characteristics which may be predictive of a response to DLI in patients with relapsed multiple myeloma. In an analysis of 63 myeloma patients treated with DLI, 19 percent received a complete response and 19 percent a partial response [41]. Seven patients (11%) died from DLI-related complications. Significant factors associated with response included the development of acute and chronic GVHD which developed in 38 percent and 43 percent of patients, respectively. A report by Lokhorst had demonstrated an overall incidence of acute and chronic GVHD in 66 percent and 56 percent, respectively, in patients with myeloma receiving DLI [37]. In the same study, acute and chronic GVHD developed in 87 percent and 85 percent of patients responding, respectively, demonstrating the close association of GVHD and GVM.

There is optimisim that combining DLI with other immune modulator agents may improve the response to DLI. Low-dose thalidomide in combination with DLI was used in 18 patients with relapsed myeloma with an overall response rate of 67 percent and a complete response rate of 22 percent [42]. Limited toxicity was observed with only 11 percent of patients developing evidence of chronic GVHD. The safety and efficacy of combining bortezimib or lenolinomide with donor lymphocyte infusion will need to be explored in future clinical trials.

4. Myelodysplastic Syndrome and Acute Leukemia

The results of DLI in patients with acute leukemia and myelodyslplastic syndrome (MDS) have not been as encouraging as in patients with CML and multiple myeloma. Overall the response rate has been lower and the

Table 28-3. Results of MDS and acute leukemia treated with DLI alone.

Disease	North American Experience [22]	European Experience [140]
Myelodysplasia	2/5 (40%)	3/9 (33%)
Acute Myeloid Leukemia	6/39 (15%)	12/42 (29%)
Acute Lymphocytic Leukemia	2/15 (13%)	1/22 (5%)

duration of response is limited. The results of patients with MDS and acute leukemia who have been treated with DLI are outlined in Table 28-3. In the North American registry report, complete responses were noted in two of five patients with MDS treated with DLI while in the European experience three of nine patients with MDS achieved a remission [22, 43]. Response rates in acute myelogenous leukemia (AML) and acute lymphocytic leukemia (ALL) are low and are similar to the response rates noted in patients with advanced stage CML. The complete response rate to DLI in patients with AML is 15 to 29 percent and 5 to18 percent in ALL. In a study assessing the long-term outcome of patients treated with DLI, 36 percent of patients with acute leukemia who achieved remission after DLI relapsed, including four of 15 with AML and three of four with ALL [24]. The median time to relapse was 10 months (range, one to 37 months).

DLI from unrelated donors in patients with acute leukemia is associated with a higher response rate than that seen with DLI from related donors with eight of 19 patients (42%) achieving a complete response after unrelated DLI [26]. Of patients achieving a complete response after DLI, 30 percent died of treatment-related complications and 30 percent relapsed. The median survival of patients receiving unrelated DLI was only 11 weeks.

Patients with relapsed acute leukemia after allogeneic transplantation have also been treated with chemotherapy followed by DLI. In many cases chemotherapy was administered because of rapidly progressive disease or in an attempt to de-bulk patients prior to DLI. While the overall response rate to chemotherapy plus DLI is higher than DLI alone, long-term outcome does not appear significantly improved. A clinical trial which combined chemotherapy and DLI demonstrated an overall complete response rate of 47 percent [44]. Unfortunately, the toxicity associated with this approach was high with a treatment related mortality of 23 percent and a disappointing two-year overall survival for all patients of 19 percent. A second study in 12 patients with relapsed acute leukemia using high-dose ara-C followed by dose escalated donor lymphocyte infusions also demonstrated a low overall complete response rate (33%) and the majority of these were not durable [45].

5. Chronic Lymphocytic Leukemia and Lymphoma

DLI experience in patients with chronic lymphocytic lymphoma (CLL) and low-grade lymphoma is limited. Patients with CLL have obtained a complete response following DLI. The time to complete response may be prolonged; one patient was followed for 12 months after a single infusion of donor lymphocytes before obtaining a remission [46]. There are case reports of patients with follicular lymphoma responding to DLI [47]. In one report six of seven

patients with indolent lymphoma responded to DLI [48]. Responses have also been noted in Hodgkin's Disease [49].

6. EBV-Associated Lymphoproliferative Disorders after BMT

DLI is a highly effective treatment for post-transplant EBV-associated lymphoproliferative disorders (EBV-LPD). Papadapoulos, et al. treated five patients with post-BMT EBV-LPD using infusions of un-irradiated donor leukocytes at a dose of 1.0×10^6 CD3+ T cells per kilogram body weight ($1/10^{th}$ the usual CD3+ cell dose used for treating relapsed disease), and observed a 100 percent pathologic and clinical response without significant GVHD [50]. The therapeutic effect was attributed to the high concentration of EBV-specific CTLs normally present in the circulating lymphocyte pool. As an extension of this strategy, researchers have now demonstrated that administering *in vitro* cultivated EBV-specific CTLs alone is sufficient to eradicate EBV-LPD [51-54]. PCR tests are now available which can quantify EBV DNA and offer a method of diagnosing patients prior to the onset of clinically evident EBV-LPD [52]. With this tool for early detection, prophylactic administration of EBV-specific CTLs can now be used as preemptive therapy against EBV-LPD after BMT [53, 54]. DLI has also been used to treat other viral illness such as human herpesvirus-6 encephalitis [55].

7. DLI after Nonmyeloablative Transplantation

The role of DLI after nonmyeloablative or reduced-intensity conditioning (RIC) stem cell transplantation remains to be defined. DLI has been used after RIC in two ways: treatment of relapse or as a method to convert patients from a mixed chimeric state to full donor chimerism. Use of DLI to treat relapse after NST has been limited both by the high incidence of chronic GVHD seen after NST, as well as the development of GVHD as immune suppression is tapered when recurrent disease is identified. When DLI has been used, the response rates are low. In a study of 446 patients receiving NST, 53 patients received DLI for treatment of relapsed disease. The overall response rate was 25 percent with only 13 percent of patients receiving a CR [56].

When anti-T cell agents such as CAMPATH or ATG are used as part of the RIC regimen, the incidence of recurrent disease after transplantation is increased and many patients demonstrate mixed chimerism after transplantation. A strategy of using dose escalated DLI in this setting has been associated with a low incidence of GVHD while inducing Graft-versus-Malignancy effects in a variety of diseases in this setting [40, 57].

Prophylactic DLI has also been explored in the RIC setting. Seventy-five patients with high risk AML or MDS received low-dose conditioning with fludarabine, cytarabine and TBI. GVHD prophylaxis included cyclosporine and ATG [58]. Patients were to receive prophylactic DLI after day 120 if there was no evidence of GVHD and were off immune suppression medications. Of the 75 patients enrolled, only 12 patients were able to receive prophylactic DLI demonstrating the limitation of this approach. Another study used prophylactic CD8-depleted DLI after NST [59]. In that study 11 of 23 patients were able to receive prophylactic DLI. Patients receiving CD8-depleted DLI had accelerated immune reconstitution and minimal GVHD.

8. Graft-versus-Host Disease Following DLI

Graft-versus-Host Disease is the principle complication of DLI. GVHD occurs in 45 to 100 percent of patients with CML who achieve a complete cytogenetic response [2, 22, 60]. GVHD, which develops after DLI, often has characteristics of chronic GVHD involving the liver and skin; however, GVHD with characteristics of acute GVHD has also been noted. A variant of hepatic GVHD manifesting primarily as elevated serum transaminases and lobular hepatitis histologically has been described in patients after DLI [61]. Complications related to its treatment are the primary reason for the 10 to 20 percent treatment-related mortality associated with the early DLI experience. The association between response to DLI and the development of GVHD suggests that GVL and GVHD may be closely related. However, clinical responses are noted in some patients without the development of GVHD, suggesting that these processes are distinct (Fig. 28-3). Efforts to separate GVL and GVHD both experimentally and in the clinic is an area of active investigation (Table 28-4). These efforts include infusion of low doses of cells, defining the proper timing of cell infusion after BMT and selective T cell infusions.

As previously discussed, infusing low doses of T cells results in high response rates in CML patients with minimal GVHD. This has led to a strategy of using escalating doses of lymphocytes with infusion of higher doses of cells being reserved for patients who do not respond to the initial DLI [30]. To minimize toxicity associated with the infusion of larger numbers of cells, patients must be followed for prolonged periods since response to DLI may be delayed. Responses have been noted up to nine months after a single course of DLI. The relationship between cell dose, response and toxicity is not well established in other diseases. No relationship between cell dose and response or the development of GVHD was noted in patients receiving DLI from unrelated donors; however, the number of patients available for evaluation was limited [26].

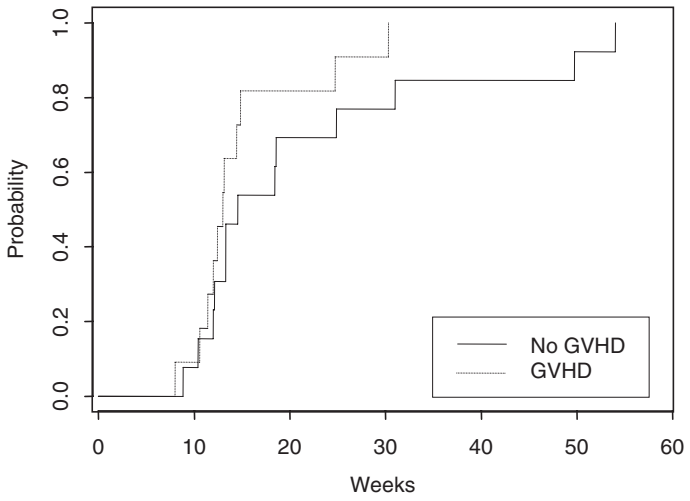

Fig. 28-3. Impact of GVHD on response in patients with CML

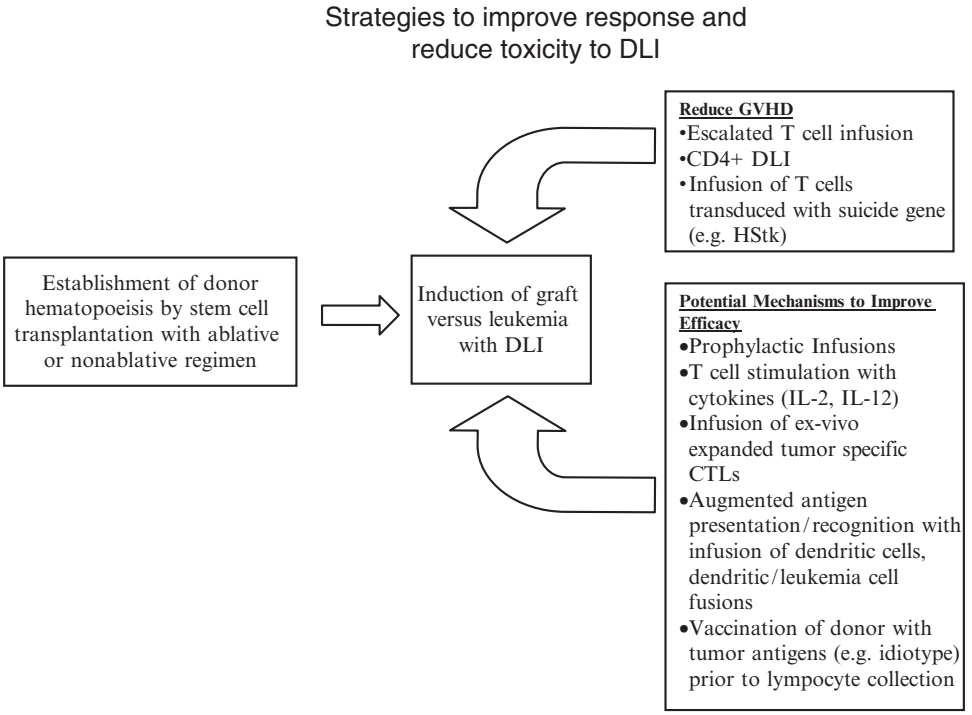

Fig. 28-4. Strategies to improve response to DLI

Administering DLI very early after transplantation is associated with significant GVHD. A very high incidence of acute GVHD was noted when DLI was given within the first weeks after BMT [62]. Barrett, et al. noted an increased risk of GVHD associated with T cell infusion at day 30 after BMT compared with infusions at day 45 after T cell-depleted allogeneic BMT [63]. Larger registry studies of DLI did not demonstrate an increased risk of GVHD when DLI was administered either within the first year or beyond one year after transplantation [2, 22].

Two strategies using selective T cell infusion have been explored to limit GVHD while preserving GVL: DLI depleted of CD8+ cells or DLI in which a suicide gene has been transduced into the infused cells. In clinical transplantation, evidence suggests that CD8+ cells play a role in the development of GVHD in humans. This evidence includes the observation that patients with a higher number of circulating CD8+ T cells during the period of early lymphoid reconstitution have an increased risk of developing GVHD [64]. In a clinical transplant model, selective T cell depletion of donor marrow with an anti-CD8 monoclonal antibody was capable of reducing the incidence of GVHD without leading to an increased risk of relapse [65].

Two trials of CD8+ cell depletion prior to DLI have been performed [33, 66]. The incidence of GVHD noted in these trials was low when compared with trials using un-manipulated donor cell infusions. In one study, approximately 50 percent of patients with CML who achieved a complete cytogenetic response did not develop evidence of clinical GVHD (Fig. 28-2). In addition, no patient receiving CD8-depleted donor lymphocytes developed GVHD in

the absence of a response. GVHD has been noted to occur in some patients who have not achieved a response when treated with un-manipulated DLI. These two studies suggest that CD4+ DLI are capable of inducing a GVL effect while reducing the risk of GVHD, and the responses to CD4+ DLI also appear durable [67]. A direct comparison of CD4+ DLI with un-manipulated DLI administered six months after T cell-depleted DLI has been performed and a significantly lower incidence of GVHD was noted in patients receiving CD4+ DLI. Larger comparative trials will be needed to confirm this finding.

As an alternative strategy to separate GVHD from GVL, investigators have designed donor T cells transduced with a suicide gene, the herpes simplex virus thymidine kinase (HStk), which could be activated if the patient develops GVHD after DLI [68-70]. These transduced cells appear to remain fully functional [71]; however, these cells may be killed by the administration of ganciclovir [72]. This strategy may allow for the induction of a GVL response which is able to be terminated when GVHD begins to develop by the administration of ganciclovir. Several trials using this technology have been reported.

9. Methods to Enhance the GVL Response Mediated by DLI

Strategies to enhance the GVL effect mediated by DLI have included activation of the infused cells as well as methods to improve potential target antigen presentation. In an attempt to activate and enhance the immune response, Slavin, et al. has administered IL-2 to patients following DLI. In addition, some patients received allogeneic cells which had been activated *ex vivo* by IL-2 [1].

Another approach has been to prime donor T cells by immunizing donors with immunoglobulin idiotype as in multiple myeloma. This approach has been used in patients with myeloma undergoing conventional transplantation [73]. Similarly other investigators have attempted to prime the transplanted immune system at the time of relapse. In a small pilot study, donor dendritic cells combined with soluble Id conjugated to KLH were been administered to patients with myeloma who have relapsed after transplantation. This approach appears safe, but data on its efficacy in a large number of patients is not available [74].

A third approach has been to generate *in vitro* and infuse T cell clones which have antileukemic acitivity. Falkenburg, el al. have reported the successful treatment of a patient with accelerated phase CML using this approach [75]. A feasibility study demonstrated that large scale production of cytotoxic T cell clones is possible; however, given the complex and time consuming techniques required, the applicability of the approach is limited [76]. Slavin has reported the use of *in vitro* primed donor lymphocytes [77] where cells from the donor are incubated with irradiated lymphocytes obtained from the recipient in an attempt to "immunize" the donor cells. The efficacy and practicality of this approach has not been assessed in a large trial.

10. Mediators of the GVL Effect

The majority of evidence suggests that donor T cells mediate the GVL effect in animal models. In murine models the relative contribution of either CD8+ or CD4+ T cell subsets in mediating the GVL effect depends on the HLA and minor antigen relationship between donor and host, as well as the target anti-

gens expressed by the malignant cell. CD8+ cells appear to mediate the GVL effect in the majority of models through direct cytotoxicity of the target cell. Demonstrating the importance of CD8+ cells, mice receiving bone marrow depleted of CD8+ cells had an increased risk of leukemia relapse compared with mice receiving marrow depleted of CD4+ cells [78]. In contrast, infusion of CD8-depleted marrow with the addition of CD4+ T cells leads to a low incidence of GVHD while preserving GVL in other models [79]. The mechanism by which CD4+ cells mediate a GVL response is not clear. Host antigen-presenting cells also appear to play an important role in murine models and their presence after transplantation is important in the development of both GVL and GVHD post-DLI [80].

Indirect evidence suggests that T cells mediate GVL in humans. Clinical trials have demonstrated that T cell-depleted BMT results in the significant loss of GVL. This loss of GVL is responsible for the increased relapse rate seen in CML patients after T cell-depleted BMT which approaches 40 to 60 percent as compared with only 10 to 20 percent after non-T cell-depleted BMT. *In vitro* both CD4+ and CD8+ T cell subsets which demonstrate antileukemic activity have been generated [81-84]. CD4+ T cells with selective cytotoxicity of Ph+ clones have been identified *in vivo,* but appears to wane with prolonged culture specificity [82].

Serial phenotypic analysis has not revealed the *in vivo* expansion of either a population of CD8+ or CD4+ T cells in patients responding to DLI. T cell repertoire analysis has also been employed as a more sensitive method to assess changes in the T cell compartment following DLI. In some patients with CML and myeloma who respond to DLI, selective T cell clonal expansion has been noted at the time of response [85, 86]. In some cases the expanded CD8+ donor cells demonstrate cytolytic activity against recipient hematopoietic cells suggesting that the target is not tumor-specific [87]. In patients with myeloma, clonal expansion of CD3+CD8+ which recognizes patient myeloma cells in an HLA class I restricted manner have been identified [88]. Interestingly, these cells can be identified in patients prior to receiving DLI, but are expanded after the patient receives and responds to DLI. In one patient with relapsed myeloma who responded to DLI, donor T cells recognized an activation-induced minor histocompatibility antigen encoded by the ATP dependent interferon responsive gene [89]. In the majority of patients responding to DLI, the specific target of expanded T cells remains elusive.

NK cells have also been identified as potential mediators of GVL. NK cells appear during hematopoietic recovery after allogeneic BMT and are able to recognize differences in the target MHC class I [90, 91] and class II molecules [92]. Activated NK cells mediate cytotoxicity through MHC unrestricted killing. A correlation between the high number of circulating NK cells and remission status has been noted in patients after BMT [93]. Murine models do not support the role of NK cells in the GVL reaction mediated by DLI [94].

Three pathways by which the GVL effect may eliminate tumor cells have been suggested [95-97]. Killing leukemia cells directly through perforin and granzyme attack, mediated by CD4+ or CD8+ cytotoxic lymphocytes or NK cells, has been postulated. T cells may also mediate killing via cytokines such as TNF-α and INF-γ which have been shown to inhibit hematopoiesis [98]. The involvement of Fas/Fas ligand interactions and the induction of apoptosis has also been implicated in the GVL effect. Fas-Fas ligand appears to be an

important pathway for T cells to mediate antigen-specific killing. Both chronic and acute leukemias have been shown to express the Fas antigen [99]. A more thorough understanding of the GVL reaction's mechanisms will lead to targeted strategies which enhance the GVL effect and limit toxicity (Fig. 28-3).

11. Potential Targets of the GVL Effect

Potential targets include both tumor-specific antigens and allo-specific antigens. It is likely that the target of the GVL effect may vary by disease (Table 28-5). Non-disease-specific targets of the GVL response after HLA-matched allogeneic transplantation include minor histocompatibility antigens (mHag) [100] and gender-specific H-Y proteins in sex mismatched donor-recipient pairs [101-105]. Disease-specific antigens include proteins that are restricted to the malignant cells. For example, in chronic myelogenous leukemia, the protein product of the BCR/ABL gene fusion is a potential GVL target [106], as are other leukemia-specific proteins, including the proteinase 3-derived peptide PR-1 [107, 108], CML28 [109, 110], CML66 [110, 111] and the anti-apoptosis protein surviving [112, 113]. Other tumor-specific antigens include immunogloblin idiotype as in patients with lymphoma or multiple myeloma. Recognition of targets is anticipated to vary by HLA types due to varied antigen presentation by HLA subgroups.

12. Minor Histocompatibilty Antigens as Targets of GVL

Over the last several years that has been an increased understanding of mHLA antigens and their role in allogeneic transplantation. GVH and GVL may arise when alloreactive donor T cells respond to mismatched minor histocompatibility antigens (mHAgs) expressed on recipient cells [114, 115]. Many of these antigens are ubiquitous, including HA-3, HA-4, HA-6 and H7, while other minor antigens are specific for tissues of hematopoietic origin, including HA-1 and HA-2. Leukemia cells express a large number of mHAgs on their surface, and mHAg-specific CTLs have been demonstrated to recognized and lyse leukemia cells *in vitro* [116, 117]. Ubiquitously expressed mHAgs such as HY, HA3, HA-4, HA-6, HA-7 and HA-8 may be targets for both GVH and GVL reactions. In contrast, lineage-specific mHAgs, such as the HLA-A_2 restricted HA-1 and HA-2, which are expressed only on hematopoietic-derived tissue, could theoretically result in GVL activity without clinical GVHD [118]. This specificity would also explain the frequent conversion from mixed chimerism to complete donor hematopoiesis after response to DLI [119]. Demonstrating the tight link between GVHD and GVL, HA-1 was the first mHAg demonstrated to be associated with acute GVHD after HSCT [120, 121]. The discordance between hematopoietic-restricted expression of HA-1 and the occurrence of GVHD in nonhematopoietic tissue that do not express HA-1 could potentially be explained by a "cross priming" phenomenon. In this model, recipient HA-1 positive antigen presentation cells (e.g., Langerhans cells and dendritic cells in skin or gut) are lysed by the allo-specific donor T cell clones, and their destruction leads to release of inflammatory cytokines and tissue antigens, which stimulates the influx of T cells with other specificities to the area and triggers GVHD.

Several clinical examples illustrate this "cross priming" concept. Seven CD8+ alloreactive T cell clones were generated from a patient with relapsed BCR/ABL positive ALL after SCT who achieved complete remission and concomitantly developed extensive chronic GVHD two months post-DLI. The clones were generated by stimulating the post-DLI remission PBMCs with a patient's pre-transplant mature dendritic cells, demonstrating that the target of the alloreactive cells was restricted to HA-1As [122]. In another report, three HA-1 and/or HA-2 positive patients with relapsed disease (two CML, one MM) after using alloSCT responded to DLI from their HA-1 and/or HA-2 negative donors [123]. They were able to demonstrate the emergence of HA-1 and HA-2-specific CD8+ T cells in the blood of the recipients five to seven weeks after DLI, and that emergence of these cells was immediately followed by complete remission of disease, as well as conversion to full donor chimerism. All patients also developed mild GVHD in association with their disease response. These investigators were able to demonstrate *in vitro* that HA-1/HA-2-specific CTL clones isolated during clinical response strongly inhibited, in a dose-dependent manner, growth of HA-1 and HA-2 expressing malignant recipient cells, but not normal donor hematopoietic progenitor cells. These results suggest the direct involvement of HA-1 and HA-2-specific CTLs in the GVL response, and imply that *in vitro* generated HA-1 or HA-2-specific CTLs could be used as adoptive immunotherapy to treat relapsed malignancies after HSCT [123].

Gender mismatched transplants provide an opportunity to identify male-specific minor antigens which may be important targets for GVHD and possibly GVL responses. CTL clones specific for the H-Y antigen *SMCY* have been identified in male patients with acute and chronic GVHD after sex-mismatched BMT [124, 125]. DBY, which is HLA-DQ5 (class II) restricted, has also been shown to be the target of a CD4+ CTL clone isolated from a male patient with acute GVHD after transplantation of stem cells from a female donor [101]. Recent evidence suggests that B-cell responses against DBY may be important in the pathogenesis of chronic GVHD as well. In 60 male patients who received stem cell grafts from female donors, 29 (48%) were found to have the anti-DBY antibody at four to eight months post-transplant, as compared to 2/39 (5%) in male patients with male donors [103]. The antibody response appears to correlate with decreased relapse rates. A larger analysis involving 3,238 sibling transplants supported this observation with female donor-male recipient pairs being found to have a significantly lower hazard ratio for leukemia relapse, but higher odds for GVHD, compared to all other gender combinations [126].

13. Tumor-Specific Targets

13.1. CML

Junctional p210 peptides derived from the BCR/ABL fusion gene have been generated and are immunogenic *in vitro*, and perhaps may serve as leukemia-specific targets in patients with CML. T cells clones have been generated *in vitro* that recognize proteins created by the BCR/ABL fusion product [127-130]. Four peptides specific for the b3a2 fusion have been identified as having high or intermediate binding efficiency to HLA A3, A11 and B8 [127]. Supporting evidence that the fusion protein is a relevant target, a p210 specific CD4+CD3+

T cell clone of donor origin has also been generated *in vitro* by stimulating PBMCs obtained post-DLI with BCR/ABL junctional peptides [131]. *In vivo* BCR/ABL peptide-specific, T cell proliferative responses and DTH responses have also been observed after vaccinating chronic phase CML patients with b3a2 fusion peptides [132]. Despite these reports, it is unclear if *in vivo* clinical response in CML patients are directed against these target antigens.

Other potential targets of GLV responses in CML have also been identified by looking at humoral responses. Using SEREX, 13 leukemia-associated target antigens have been identified in patients with CML one year after response to DLI [133] and two represented novel genes, which have since been identified as CML66 and CML 28 [109, 111]. CML66 is a broadly expressed tumor antigen in hematopoietic malignancies, as well as in solid tumors such lung cancer, prostate cancer and melanoma [111]. CML28 is a tumor antigen that is widely expressed in highly proliferative malignancies, including AML and CML blast crisis. Elevated titers of IgG specific for CML 28 have also been detected in 10 to 33 percent of patients with melanoma, lung or prostate cancer [109]. Despite the detection of these antibody responses *in vivo,* it remains unclear if these are targets of the GVL response.

14. Myeloid Malignancies

PR1 is a potential target of the GVL response in myeloid malignancies. PR1 is a peptide from the primary granule enzyme proteinase 3 and is aberrantly overexpressed in myeloid leukemias. PR1-specific CTL clones have been generated from healthy donors and these CTLs can lyse leukemia blasts [134] and CFU-GM from HLA-A2.1 patients with CML [108]. PR1-specific CTLs have been found in 11 of 12 CML patients who responded to interferon therapy, compared to zero of the seven who did not respond. In patients receiving allogeneic transplantation, six of eight CML patients who responded developed PR1-specific CTLs in their blood [135]. PR1-specific CTLs exist in both high and low TCR avidity forms, and CML target cell killing correlates with TCR avidity. High avidity PR1-specific CTL have been identified in patients in cytogenetic remission after interferon therapy [136]. Clinical trials with PR1 peptide and adoptive cellular therapy using PR1-specific CTLs may provide direct evidence that this is a valid target of the GVL response.

15. Myeloma

Potential targets of the Graft-versus-Myeloma response include minor histocompatibility antigens as well as myeloma-specific targets. The minor histocompatibility ATP dependent interferon responsive gene has been identified as a potential target of donor T cell reactivity in patients with myeloma [89]. Using SERAX to evaluate patients who had responded to DLI , antibodies were identified which were directed towards several highly expressed myeloma-associated antigens [137, 138]. One of the B-cell antigens identified in myeloma patients was B-cell maturation antigen (BCMA), a transmembrane receptor of the tumor necrosis factor (TNF) superfamily that is selectively expressed by mature B–cells [139]. Further characterization of this antigen as a potential target of the GVM response is being performed.

16. Future Directions

While responses to DLI demonstrate the presence of a Graft-versus-Malignancy effect, defining a clear and effective role of DLI in allogeneic transplantation remains elusive. The success of DLI for treating relapse after transplantation is limited and efforts are needed to make DLI more effective in this setting. An improved understanding of both the effector cells and targets of the GVL response may allow for more selected therapies to be developed in the future. Perhaps the most appropriate platform for DLI in the future is combining it with nonmyeloablative transplantation. Future efforts should focus on prophylactic DLI being used in patients at high risk of relapse after nonmyeloablative transplantation as a method to reduce relapse.

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Chapter 29

Regenerative Medicine:
Application in Cardiovascular Diseases

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1. Introduction

Stem cells are self-replicating cells that can generate, sustain and replace terminally differentiated cells. Embryonic stem cells, which are derived from the cell mass of the blastocyst, are pluripotent, meaning that they can generate any terminally differentiated cell derived from any one of the three embryonic germ layers. Adult stem cells—the focus of this chapter—are intrinsic to specific tissues of the postnatal organism and are multipotent, meaning that they are committed to differentiate into cell lineages from only those tissues.

Adult bone marrow–derived stem cells are presently the cell types most widely used in cardiac stem cell therapy. A heterogeneous subset thereof, termed autologous bone marrow–derived mononuclear cells (ABMMNCs), comprises the following types of stem cells that have potential therapeutic uses:

- Adult mesenchymal (stromal) stem cells (MSCs) are found in various niches of adult tissue, including bone marrow and adipose tissue. They can be expanded in culture and differentiated into several specific mesenchymal cell lineages. MSCs are rare in bone marrow (<0.01% of nucleated cells, by some estimates) and 10 times less abundant than hematopoietic progenitor cells.
- Hematopoietic stem cells are found mostly in the bone marrow. They give rise to all types of blood cells, including the myeloid (monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets and some dendritic cells) and lymphoid (T cells, B cells, natural killer cells and some dendritic cells) lineages.
- Endothelial progenitor cells can be isolated from the mononuclear fraction of the bone marrow or peripheral blood, as well as from fetal liver or umbilical cord blood.

Lastly, although not "true" stem cells, skeletal myoblasts can also be harvested and expanded for use in cardiovascular therapy. Skeletal myoblasts are tissue-specific stem cells located between the basal lamina and the sarcolemma on

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the periphery of mature skeletal muscle fibers. These cells have the availability and capability to form myotubules, which could help replenish muscle cells in the failing heart.

2. Delivery of Therapeutic Stem Cells

Stem cells can be delivered by several methods, including direct surgical injection, intracoronary infusion, retrograde venous infusion and transendocardial injection. The intracoronary route is the simplest approach to administer cells. Unfortunately, Vulliet, et al. [1] observed acute myocardial infarction (AMI) and subacute myocardial microinfarctions after intracoronary arterial injection of bone marrow stem cells (BMSCs) in dogs. Even more worrisome were observations of such complications in healthy dogs with normal epicardial coronaries. The microinfarctions are believed to have been caused by microcirculatory "plugging." The findings of Vulliet, et al. agree with our own findings concerning intracoronary infusion of BMSCs in a canine model of acute ischemia (unpublished data). The clinical significance of these preclinical data has yet to be determined, but further preclinical work is warranted to assess the safety of intracoronary delivery.

In contrast, transendocardial delivery of stem cells appears to be safer. This technique uses the NOGA electromechanical mapping system to guide a catheter with attached syringe directly to areas targeted for injection (Fig. 29-1). Although this technique has not been tested in humans after AMI, our work at the Texas Heart Institute in a canine model of acute ischemia has demonstrated an excellent safety profile (unpublished data). Moreover, transendocardial delivery has been proven safe in extensive preclinical and clinical testing in the setting of chronic myocardial ischemia [2–4]. The transendocardial route has also been used to deliver skeletal myoblasts. Smits, et al. [5] documented improvement in global left ventricular ejection fraction (LVEF) after transendocardial delivery of skeletal myoblasts in five patients with ischemic heart failure.

Transendocardial delivery of stem cells offers two important advantages over intracoronary infusion in the setting of chronic ischemia. First, the

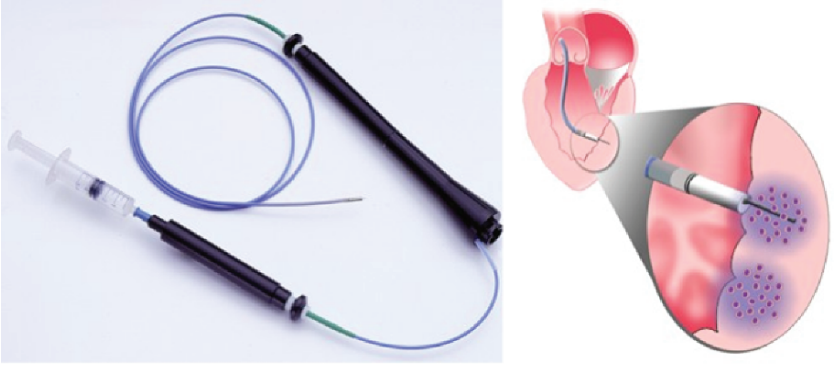

Fig. 29-1. Left, Myostar catheter with attached syringe. Right, catheter traversing aortic valve. Inset shows transendocardial extension of the needle with cell delivery. Reprinted with permission from [3]

transendocardial route provides a means by which tissues with different degrees of viability and ischemia can be mapped in detail, allowing therapy to be precisely targeted (e.g., at the border zone of an infarct). Targeted cell therapy is gaining importance as the ineffectiveness of injecting BMSCs into scar tissue becomes increasingly evident. This has been confirmed by Agbulut, et al. [6], who found that injecting bone marrow-derived $CD133^+$ cells into scarred myocardium was associated with less engraftment (and therefore less efficacy) than injection of skeletal myoblasts. Targeted therapy may also be important in treating chronic disease, in which homing of stem cells to disease areas may be less intense. Conversely, skeletal myoblasts can survive even in a severely oxygen-deprived milieu and have been shown to engraft in scar tissue [7]. The second advantage of transendocardial delivery in patients with chronic ischemia is that injection is not limited to areas supplied by patent coronary arteries. This is particularly important in patients with end-stage ischemic heart failure, in whom most of the epicardial beds are chronically occluded. Thus, based on the evidence available at this time, transendocardial delivery is likely to be the first choice for BMSC delivery in treating chronic myocardial ischemia.

3. Safety of Stem Cell Therapy

Cardiac stem cell therapy is very well tolerated with respect to left ventricular function. With respect to potential toxicity and side effects, however, it is important to distinguish BMSCs from skeletal myoblasts. Skeletal myoblasts do not establish structural connections with host cardiomyocytes and can, therefore, trigger ventricular arrhythmias [8, 9]. Indeed, initial clinical experience has confirmed the potential for lethal ventricular arrhythmias after skeletal myoblast transplantation [9]. This high incidence of ventricular arrhythmias has led to protocol changes involving systematic implantation of cardiac defibrillators in phase I and II trials of skeletal myoblast therapy. In contrast, proarrhythmic effects of BMSCs have not been demonstrated to date, so no limits have been imposed on this type of cell transplantation.

Nevertheless, BMSCs can cause other deleterious effects. There were early concerns about abnormal transdifferentiation and tumorigenesis, although these have subsided somewhat. However, the potential for accelerated atherogenesis—the development of arterial plaques—remains an active issue, given the limited clinical experience and the small number of treated patients. Another potential deleterious effect of BMSCs is myocardial calcification. Yoon, et al. [10] reported that direct transplantation of unselected BMSCs into acutely infarcted myocardium may induce significant intramyocardial calcification. Notably, however, mononuclear BMSCs (which are widely used in clinical trials) in the same study did not elicit the same effect.

4. Clinical Trials in Ischemic Heart Disease

4.1. Refractory Angina

Tse, et al. [4] conducted one of the first studies evaluating the use of ABMMNCs to treat patients with refractory angina. In this study, ABMMNCs were transendocardially injected into eight patients with severe ischemic heart

disease and preserved left ventricular function, as indicated by the LVEF. After three months of follow-up, the researchers observed an improvement in symptomatology and myocardial perfusion. Cardiac MRI revealed improved perfusion and contractility in the ischemic region.

Around the same time, Fuchs, et al. [11] conducted a clinical feasibility study of transendocardial delivery of filtered, unfractionated autologous bone marrow-derived (not mononuclear) cells in 10 patients with severe chronic, symptomatic myocardial ischemia not amenable to conventional revascularization. Twelve targeted injections (0.2 ml each) were administered into ischemic, non-infarcted myocardium that was pre-identified with single photon emission computed tomography (SPECT) perfusion imaging. The patients had no serious adverse effects, including arrhythmia, infection, myocardial inflammation or increased scar formation. Treadmill exercise duration (data available for nine patients) did not change significantly (391 ± 155 seconds before treatment versus 485 ± 198 seconds after treatment; P = 0.1), but there was improvement in Canadian Cardiovascular Society angina scores (3.1 ± 0.3 vs. 2.0 ± 0.94; P = 0.001) and in stress scores of segments within the injected regions (2.1 ± 0.8 vs. 1.6 ± 0.8; P < 0.001).

5. Heart Failure

Perin, et al. [3] conducted the first clinical trial of transendocardially injected ABMMNCs in patients with end-stage ischemic heart failure with severe systolic dysfunction. This trial provided the first objective evidence of perfusional and functional improvement in patients with severe ischemic heart failure treated solely with cell therapy. The first 14 patients formed the treatment group, and the last seven formed the control group. All patients were subjected to baseline laboratory tests, exercise stress (ramp treadmill) studies, two-dimensional Doppler echocardiography, SPECT perfusion scanning (Fig. 29-2A) and 24-hour Holter monitoring. Bone marrow-derived mononuclear cells were then harvested from each patient and processed for injection by a NOGA catheter. A total of 15 injections (0.2 cc each, totaling ~30×10^6 cells per patient) were administered into areas of viable myocardium (areas with an electromechanical mapping unipolar voltage greater than 6.9 mV). The treatment area was further correlated with baseline SPECT findings, and only points with preserved viability were injected. All patients underwent noninvasive follow-up tests at two months, with the treatment group undergoing additional noninvasive testing and invasive electromechanical mapping at four months.

Demographics and exercise test variables did not differ significantly between the treatment and control groups. No procedural complications occurred, and no periprocedural arrhythmias were identified. At two months, quantitative SPECT analysis revealed a significant reduction in the total reversible defect in the treatment group compared to the control group (P = 0.02) (Fig. 29-2A). At four months, the LVEF had improved from a baseline of 20 to 29 percent (P = 0.003) in the treated patients, and the end-systolic volume was also reduced (P = 0.03). Electromechanical mapping revealed significant mechanical improvement of the injected segments (P < 0.0005) (Fig. 29-2B). More importantly, at six and 12 months, the total reversible defect measured by SPECT perfusion scanning was significantly reduced in the treatment group compared

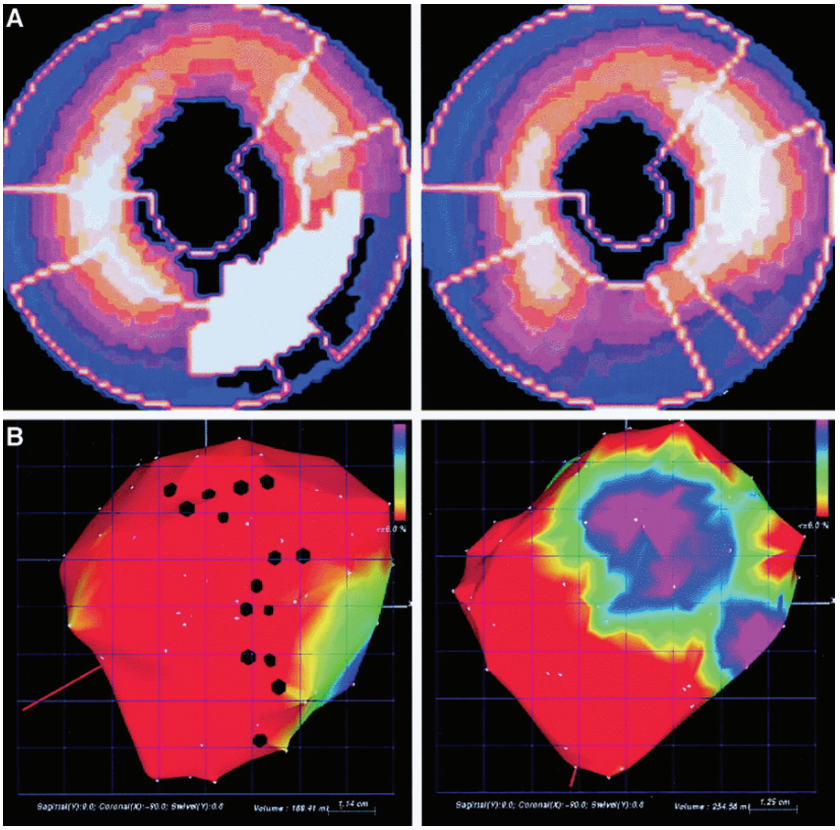

Fig. 29-2. (**A**) Left, SPECT polar map at baseline, showing an area of inferolateral, reversible ischemia in white and nonreversible stress defect in black. Right, follow-up SPECT at two months, showing complete resolution of the ischemic and basilar nonreversible defects and a decrease in the nonreversible apical defect. (**B**) Electromechanical maps from the same patient viewed from the inferior position. Left, mechanical map at the time of the injection procedure shows the 15 injection sites (black dots) distributed along the inferior wall. Right, follow-up mechanical map at four months shows marked improvement in contractile function in the injected area. Reprinted with permission from [3]

with the control group. Exercise capacity was also significantly improved in the treatment group at 12 months (Table 29-1) [12].

Overall, transendocardial injection of ABMMNCs has been safe and effective in preliminary clinical testing. Table 29-2 summarizes the current experience with stem cell therapy in chronic ischemic heart disease.

6. Skeletal Myoblasts

Clinical trials of skeletal myoblasts have focused on the treatment of patients with ischemic cardiomyopathy and systolic dysfunction. Overall, these trials have resulted in improved segmental contractility and global LVEF (Table 29-3). The preferred delivery route has been surgical intramyocardial injection, although one feasibility trial of transendocardial injection has been reported in the literature thus far.

Table 29-1. Baseline and follow-up clinical data.

	Baseline		2 Months		6 Months		
	Treatment	Control	Treatment	Control	Treatment	Control	P^*
SPECT							
Total reversible defect, %	15.15 ± 15	10.71 ± 16.6	4.53 ± 16.6	32.23 ± 37.5	8.8 ± 9	32.7 ± 37	0.04
Total fixed defect (50%), %	41 ± 11	36 ± 10	39 ± 8.8	36.4 ± 12	38 ± 6.7	36.4 ± 12	0.3
Ramp treadmill							
VO_2 max, ml/kg/min	18 ± 9	17.75 ± 6.8	23.3 ± 7	18 ± 6.2	24.15 ± 7	17.3 ± 6	0.02
METS	5.09 ± 2.5	5.07 ± 1.96	6.68 ± 2.35	5.16 ± 2.45	7.19 ± 2.4	4.92 ± 1.7	0.01
PVC, n	2507 ± 6243	672 ± 1085	901 ± 1236	2034 ± 4528	3902 ± 8267	1041 ± 1971	0.4
dQRS, ms	136 ± 15	145 ± 61	145.9 ± 25	130 ± 27	144.8 ± 25	140 ± 61	0.62
LAS 40, ms	50 ± 24	70 ± 76	54 ± 33	48 ± 20	25 ± 25	66 ± 79	0.47
RMS 40, μV	22.2 ± 22	23.3 ± 23	23.3 ± 19	24.6 ± 28	25 ± 25	30 ± 27	0.70

SPECT, single-photon emission computed tomography; METS, metabolic equivalents; PVC, premature ventricular contraction; LAS 40, duration of terminal low-amplitude signal less than 40 mV; RMS 40, root mean square voltage in the terminal 40 ms of the QRS complex; dQRS, filtered QRS duration

* *P* for comparisons between treatment and control groups by ANOVA

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Table 29-2. Selected clinical studies of bone marrow–derived stem cell transplantation in chronic ischemic heart disease.

Study	Design (n)	Cell Type	Delivery Method	Results	Adverse Events
Perin, et al. [3]	Series (14) + control (7)	BMMC	Transendocardial	Improved EF and regional perfusion and symptoms	None reported
Fuchs, et al. [11]	Series (10)	BMMC	Transendocardial	Improved symptoms and regional perfusion	None reported
Tse, et al. [4]	Series (8)	BMMC	Transendocardial	Improved symptoms and regional function	None reported
Stamm, et al. [22]	Series (12)	AC133+	Epicardial + CABG	Improved EF and regional perfusion	None reported
Erbs, et al. [23]	RCT, double blind (26)	CPC	Intracoronary post-CTO recanalization	Decreased infract size, increased EF, viability and coronary flow reserve	None reported
Hendrikx, et al. [24]	RCT (20)	BMMC	Epicardial + CABG	Improved regional function	None reported
TOPCARE -CHD [13]	3 phases; randomized controlled crossover (75)	BMMC vs. CPC	Intracoronary	Improved EF and regional contractility in patients treated with BMMC	3 Coronary dissections after infusion procedure; 1 VF during infusion
FOCUS	RCT, single blind (30)	BMMC	Transendocardial	Ongoing	–

BMMC, bone marrow-derived mononuclear cells; EF, left ventricular ejection fraction; CABG, coronary artery bypass graft; RCT, randomized clinical trial; CPC, circulating progenitor cell; CTO, chronic total occlusion; VF, ventricular fibrillation

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Table 29-3. Selected clinical studies of skeletal myoblast transplantation in cardiomyopathy.

Study	Design (n)	Cell Type	Delivery Method	Results	Adverse Events
Hagege, et al. [26]	Series (9)	SKM	Epicardial + CABG	Improved NYHA, EF, regional contractility	4 cases sustained VT
Herrerros, et al. [27]	Series (12) + historical control	SKM	Epicardial + CABG	Improved EF, regional contractility, viability	None reported
Siminiak, et al. [28]	Series (10)	SKM	Epicardial + CABG	Improved NYHA	4 cases sustained VT; 1 early death (nonrelated)
POZNAN [29]	Series (10)	SKM	Transcoronary sinus	Improved NYHA, EF	Injection impossible in 1 patient
Dib, et al. [30]	Series (30)	SKM	Epicardial + CABG/ LVAD	Improved EF, viability Long-term survival of SKM	3 episodes of NSVT
MAGIC [31]	RCT, double blind (97) + Dose testing	SKM	Epicardial + CABG	No effect on EF, positive remodeling in high-dose group	No increase in ventricular arrhythmia

SKM, skeletal myoblast; CABG, coronary artery bypass graft; NYHA, New York Heart Association functional class; EF, Left ventricular ejection fraction; VT, ventricular tachycardia; LVAD, left ventricular assist device; NSVT, nonsustained ventricular tachycardia; RCT, randomized clinical trial
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7. Acute Myocardial Infarction

Most of the clinical experience gained with stem cells has involved therapy for AMI, particularly intracoronary infusion of bone marrow cells. Table 29-4 summarizes the experience to date. In all of these trials, revascularization was performed promptly after the initial myocardial infarction, and left ventricular systolic compromise was minor (although in the Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration [BOOST] trial, the baseline LVEF was 50%).

The Transplantation of Progenitor Cells and Regeneration Enhancement in AMI (TOPCARE-AMI) trial [13] was one of the earliest trials. Patients were randomly assigned to receive either bone marrow–derived mononuclear cells or endothelial progenitor cells by intracoronary infusion. Compared with nonrandomized, matched reference patients and regardless of the cell type used, treated patients had a significantly improved global LVEF as assessed by left ventricular angiography. In a subgroup of this study population, LVEF was significantly increased, as assessed by cardiac MRI, and infarct size was reduced, as assessed by late-enhancement MRI. Interestingly, the infused cells' ability to migrate was the most important predictor of infarct remodeling. Coronary flow reserve also increased, suggesting that neovascularization had occurred. The one-year results of TOPCARE-AMI [14] reinforced the notion that stem cells protect against ventricular remodeling. Despite the limited number of patients, contrast-enhanced MRI revealed a significantly increased LVEF ($P<0.001$), significantly reduced infarct size ($P<0.001$) and no reactive hypertrophy, suggesting that the infarcted ventricles had been functionally regenerated. Scientific criticism of this trial has focused on the method of cell delivery, which included

Table 29-4. Selected clinical studies of cell therapy in acute myocardial infarction.

Study	Design (n)	Cell Type	Delivery Method	Results	Adverse Events
Strauer, et al. [32]	Series (10)	BMMC	Intracoronary	Feasible	None reported
TOPCARE [13, 14]	Randomized (59)	BMMC vs. circulating EPC	Intracoronary	Improved EF and perfusion; reduced infarct size	None reported at 1 year
Chen, et al. [16]	RCT, open label (69)	MSC	Intracoronary	Improved EF and viability	None reported
BOOST [17, 18]	RCT, open label (60)	BMMC	Intracoronary	Transient EF improvement; improved diastolic dysfunction	None reported at 18 months
Fernandez-Aviles, et al. [33]	Series (20)	BMMC	Intracoronary	Improved EF	None reported
REVIVAL-2 [34]	RCT, double blind (114)	G-CSF-mobilized PBSC	–	No effect on EF or infarct size	Muscle discomfort
MAGIC Cell-3-DES [35]	RCT (96)	G-CSF-mobilized PBSC	Intracoronary	Improved EF	DES prevented restenosis
Janssens, et al. [19]	RCT, double blind (67)	BMMC	Intracoronary	Reduced infarct size and improvement in regional recovery	None reported
ASTAMI [20]	RCT, double blind (100)	BMMC	Intracoronary	No benefit of BMMC	None reported
REPAIR-AMI [21]	RCT, double blind (204)	BMMC	Intracoronary	Improved EF	None reported

BMMC, bone marrow mononuclear cell; EPC, endothelial progenitor cell; EF, left ventricular ejection fraction; RCT, randomized clinical trial; MSC, mesenchymal stem cell; G-CSF, granulocyte colony-stimulating factor; PBSC, peripheral bone marrow stem cell; DES, drug eluting stent
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transient coronary occlusion and flow cessation, and its potential for ischemic preconditioning. Such preconditioning has been shown to improve outcomes during AMI and may have contributed to the functional improvement noted in this trial. Moreover, safety concerns were raised when one patient experienced in-stent thrombosis three days after receiving cell therapy.

Bartunek, et al. [15] further investigated this concern of restenosis after intracoronary infusion. In their study, 35 patients with AMI were treated with intracoronary infusion of $AC133^{+}$ bone marrow cells (mean dose of 12.6 million cells) an average of 11.4 days after the index event. At four-month follow-up, the treated patients showed improvement in mean LVEF, but had higher rates of stent restenosis, stent reocclusion and *de novo* coronary artery lesions compared to the control subjects. Thus, this study confirmed concerns that intracoronary infusion of enriched BMSCs could result in disequilibrium between pro-atherogenic and anti-atherogenic factors.

Despite the concerns with BMSCs, the intracoronary route has been used to safely deliver autologous MSCs. Chen, et al. [16] reported the first randomized clinical trial of autologous MSCs in 69 patients who underwent a primary percutaneous coronary intervention within 12 hours after an AMI. Either MSCs or saline was injected into the target coronary artery. At three-month follow-up, left ventricular perfusion and the LVEF had significantly improved in the treatment group. Thus, now that intracoronary infusion of either ABMMNCs or MSCs (in this study) had been safely used with some improvement in LVEF, this set the stage for large randomized trials to exam the safety and efficacy of intracoronary infusion.

The BOOST trial [17] was the first randomized study of stem cell therapy in AMI. In this trial, 60 patients received either ABMMNCs or optimal medical treatment after percutaneous coronary intervention. Stem cell therapy resulted in an increased LVEF and a reduced end-systolic volume, as assessed by MRI. This improvement was attributed principally to increased contractility around the infarct zones. Unlike the earlier nonrandomized trials, however, the BOOST trial did not show a significant reduction in infarct size. The recently published 18-month follow-up of patients in the BOOST trial [18] revealed that the initial improvement in LVEF in the stem cell-treated group was not sustained compared to the control group. However, the speed at which LVEF recovered over the 18-month period was significantly higher in the stem cell-treated group.

A study by Janssens, et al. [19] provided further insight into the prevention of left ventricular remodeling after AMI with intracoronary delivery of ABMMNCs. ABMMNCs were infused into 67 patients 24 hours after mechanical reperfusion. The patients were monitored for adverse events during the first seven days after infusion and were then discharged. Follow-up acetate PET scanning, echocardiography with tissue Doppler and cineMRI were performed at four months. The primary end point—mean global LVEF—was similar between patients in the treatment and placebo groups at four months (P=0.36). Myocardial perfusion and metabolism, as measured by acetate PET scanning, were similarly increased in both groups. However, compared with the placebo infusion, ABMMNC infusion was associated with a significant reduction in myocardial infarct size (ABMMNC treatment effect of 28%, P=0.036) and a better recovery of systolic function. Although the primary end point was not met, this additional reduction in infarct size in patients who received prompt revascularization and ABMMNC injection suggests that the stem cell treatment has a potentially beneficial effect on left ventricular remodeling.

The discrepancy in LVEF results in the two aforementioned trials led Lunde, et al. [20] to investigate the effects of intracoronary injection of ABMMNCs into 100 patients with AMI. Patients were randomized to receive either placebo or ABMMNC injection four to eight days after AMI. LVEF, infarct size and left ventricular volumes were assessed by echocardiography, SPECT and MRI at two to three weeks after AMI and then again six months after injection. In total, 47 patients underwent ABMMNC infusion with a mean of 87 million cells using the stop-flow technique. Compared to the placebo, ABMMNC infusion did not result in any difference in left ventricular function, infarct size, left ventricular end-diastolic volume or adverse events. Although the injections used in this trial did not contain as many cells as those used in the BOOST and Janssens trials, this trial did add to the growing support for ABMMNC treatment of AMI.

The most recent and methodologically sound study of AMI stem cell therapy to date is the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial [21]. In this study, 204 patients with AMI were randomly assigned to receive an intracoronary infusion of bone marrow-derived progenitor cells or placebo medium into the infarct artery three to seven days after successful reperfusion therapy. There was a significantly greater absolute improvement in global LVEF in the stem cell-treated patients than in the placebo-treated patients (5.5 ± 7.3% vs. 3.0 ± 6.5%; P = 0.01). Patients with a baseline LVEF at or below the median value of 48.9 percent derived the most benefit. Most importantly, at one year, stem cell therapy resulted in a reduction in the prespecified combined clinical end point of death, recurrence of myocardial infarction and any revascularization procedure (P = 0.01).

Taken together, the phase I intracoronary delivery trials have taught us that the improvement seen after intracoronary infusion of stem cells may result from prevention of remodeling. The timing of cell delivery relative to the index event is important, as was evident in the Janssens study [19]. Early infusion of stem cells may result in even lower engraftment rates or higher rates of cell death, given the adverse environment into which the cells are delivered. The modest therapeutic benefit seen in some of the trials may have resulted from patient selection, as most of the intracoronary studies were performed in patients with small areas of infarction and a preserved LVEF. The REPAIR-AMI data corroborate that theory and set the stage for future clinical trials, possibly in patients with lower post-MI LVEF.

8. Conclusions

Despite many unresolved issues related to treatment dose, timing and delivery, the clinical potential of stem cell therapy for cardiovascular disease is enormous, and the field is advancing rapidly. However, to achieve the full potential of stem cell therapy will require continued cooperation and close collaboration between basic and clinical scientists. Translational preclinical large-animal research is likely to have a key role in the advancement of the field. The future of cardiac stem cell therapy is particularly bright with respect to its potential to prevent post-MI left ventricular remodeling and the potential to set a new standard for the treatment of AMI, but clinicians in every specialty will need to pay close attention to the field of regenerative medicine.

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