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Acute Leukemias

Prognostic Factors and Treatment Strategies

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Preface

The progress in the treatment of childhood and adult acute leukemias within the last 10–15 years was pioneered by the work of single institutions incorporating modern leukemia cell research and pharmacology into the design of chemotherapeutic regimens. These new approaches, however, had to be validated by representative prospective studies in order to clearly demonstrate the efficacy of special concepts and to identify prognostic determinants and differences between patient subgroups.

It was therefore the aim of the First International Symposium "Acute Leukemias: Prognostic Factors and Treatment Strategies" held in Münster in 1986 to provide a broad overview over the present status of chemotherapy and bone marrow transplantation in childhood and adult leukemias with special emphasis on the results of multicenter trials recruiting large numbers of patients in Europe and oversea countries. In addition, new aspects of cytogenetics, leukemia cell biology and modern diagnostic techniques as well as improvements in supportive treatment were covered.

In an attempt to summarize the major results presented during this meeting, which are given in detail in the present book, the following conclusions may be drawn: In adults with acute myeloid leukemia complete remissions are achieved in 60 to 70% of all patients and translate into long-term remissions in 15 to 25% of responders. Monthly maintenance chemotherapy was found to prolong remission duration significantly. In addition, late intensification, early consolidation or, as a new approach. double induction therapy seem to improve remission duration whereas intensive immunotherapy with neuraminidase-treated blasts failed to influence the outcome. Treatment of childhood acute myeloid leukemia by extended multidrug chemotherapy or intermittent consolidation resulted in 40–60% long-term remissions as reported by three different studies. In children with acute lymphoblastic leukemia the overall cure rate approaches 70–75% based on risk-adapted treatment stratifications. Similar concepts for the therapy of acute lymphoblastic leukemia in adults have produced long-term remissions in approximately 40–50% of responders and may be further improved by intensive consolidation. Additional progress in first line therapy of acute leukemias can be expected from new second line regimens producing high response rates in refractory leukemias. Encouraging results were also reported, both in children and adults, after allogeneic bone marrow transplantation in first remission and in more advanced disease. preliminary data of autologous bone marrow transplantation with or without in vitro purging seem also promising.

The results of these trials obviously open new ways of effective anti-leukemic therapy and certainly stimulate the evaluation of other therapeutic approaches. The hope for further increasing cure rates in acute leukemias seems therefore justified and will result from the combined efforts of clinical and basic research.

Münster, Spring 1987

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Biology, Cytogenetics and Morphology in Acute Leukemias

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Cell Kinetics in Leukemia and Preleukemia*

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A cell lineage affected by acute leukemia is distinguished from the corresponding normal lineage by a prevalence of immature blast cells and a deficit of mature cells. Currently there are two different concepts of antileukemic therapy that might profit from a clearer understanding of the causes of the excess of blast cells. These concepts are either to sweep out the leukemic cells by cytotoxic treatment, or to induce them to differentiate in a fashion like normal cells. We are interested to know if cell kinetics can add to this understanding.

In general, neoplastic lesions are associated with an increased number of proliferating cells. However, in the full-blown neoplastic state the proliferative activity of the individual cells is not necessarily increased. In fact, it may even be decreased. The primary cause of the increased number of proliferating cells is as yet unresolved. It might be a defect in the regulation of either proliferation or differentiation. The absolute number of proliferating cells in a tumor results from the ratio of the rates of relative cell production and cell loss. Cell loss means either death or disappearance of cells from a compartment by differentiation. While there are tools for studying the relative cell production rate in humans by dividing the labeling index by the DNA synthesis time in a compartment [2, 3], there are no such means for determining the rate of cell loss as far as steady state-like conditions are concerned. Many of our studies, therefore, deal with only one side of the coin, and it is only infer-

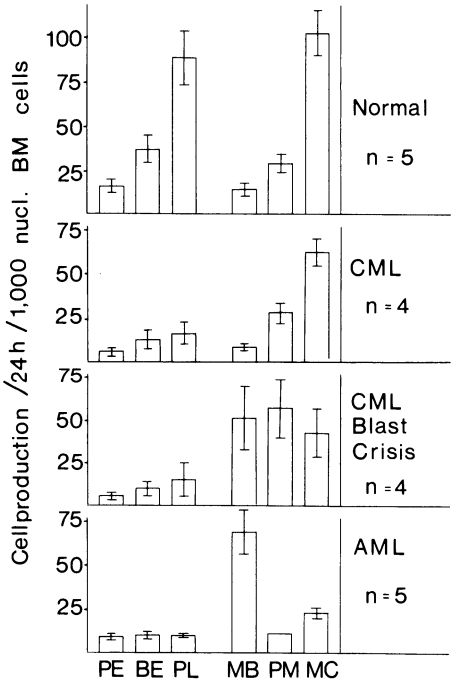

Fig. 1. Relative rates of cell production in the various bone marrow cell compartments of healthy individuals and patients with myeloproliferative disorders. *Vertical bars* indicate ± SEM. *BM*, bone marrow; *PE*, proerythroblasts; *BE*, basophilic erythroblasts; *PL*, polychromatic erythroblasts; *MB*, myeloblasts; *PM*, promyelocytes; *MC*, myelocytes. (From [5])

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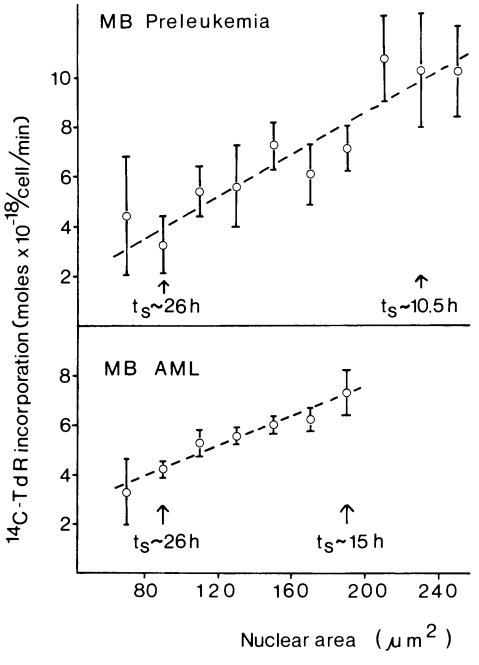

Fig. 2. DNA synthesis rates of myeloblasts *(MB)* of the same patient in the stage of preleukemia, and 2 years later in overt leukemia. Myeloblasts are grouped according to nuclear size. *Vertical bars* indicate ± SEM. For the extreme values of DNA synthesis rate the corresponding figures of DNA synthesis time (t_s) have been calculated

red that the other side is roughly equivalent, which may be permissible as long as there is no gross change in the total cell number during the observation period.

When the relative rates of cell production (Fig. 1) in normal hemopoiesis and in chronic myeloid leukemia (CML) are considered, a comparable increase from myeloblasts to promyelocytes, and from promyelocytes to myelocytes is seen. On the other hand, erythropoiesis in CML shows reduction in the relative production rate of polychromatic erythroblasts consistent with the notion of premature cell death in the compartment of polychromatic erythroblasts [5, 17]. All cases of CML and acute myeloid leukemia (AML) as well as two out of four cases of blast crisis were studied prior to antileukemic treatment. In AML a high relative cell production rate is only seen in the myeloblast compartment. Promyelocytes and myelocytes exhibit the so-called maturation arrest. Although erythropoiesis in AML shows a reduced relative cell production rate present as early as at the proerythroblast stage, the absolute proerythroblast production rate may be still normal when allowance is made for the increased total number of cells. On the other hand, in agreement with morphological findings [16], a marked amount of premature cell death can be deduced from the relative production rates of basophilic and polychromatic erythroblasts, since there is no increase over the respective preceding compartment. Blast crisis in CML shows a kinetic pattern intermediate between AML and CML.

In summary, these data suggest a primarily proliferative lesion in CML without alteration of the differentiation into granulocytes. Maturation arrest becomes obvious at the stage of transition to blast crisis, which thus might be a secondary process. Concerning erythropoiesis, a defect in differentiation is also apparent, however, without changes in proliferation in the earliest recognizable compartment. This may suggest that the deregulation of proliferation and differentiation is caused by different molecular mechanisms. In AML cell kinetics provide no definite answer as to whether the primary lesion concerns proliferation or differentiation.

The cell kinetic properties of individual leukemic blast cells may be quite similar to those of corresponding normal cells. Gavosto et al. [8] showed some 20 years ago that the labeling index of the blast cells decreases with nuclear size. On the other hand, we observed a long time ago [4] that the DNA synthesis time of blasts in leukemia as well as preleukemia also depends on nuclear size (Fig. 2). These findings have been essentially confirmed in a human leukemic cell line by Yen et al. using the labeled mitoses technique [20]. It is therefore plausible that, comparable to normal hemopoiesis, there is also some proliferative hierarchy in leukemic blasts, such that large cells have short cycle times and divide into smaller cells with longer cycle times. These, in turn, transit into small nonproliferating cells that eventually die. Concerning the mode of self-renewal of AML blasts, it is still speculative whether a lineage-like model with a small self-maintaining precursor compartment or a recruitment model with the same probability for any blast to serve as a stem cell applies

Table 1. DNA synthesis time and labeling index in euploid cases of AML

Case no.	t_s (h)	Li (%)
1	10.8	17.0
2	12.0	5.2
3	12.6	3.9
4	15.7	9.6
5	16.3	4.9
6	18.8	19.5
7	21.6	6.8
8	25.6	8.9

t_s, DNA synthesis time; Li, labeling index.

[14, 15]. Cell surface investigations favor the former model by demonstrating that the precursor compartment has properties of more immature cells than do the cells grown to colonies [11, 13, 19]. This field is definitely complicated by findings of the Fialkow group indicating that in some cases of acute nonlymphocytic leukemia (ANLL), only the granulocytic lineage is involved in the leukemic process, while in other cases the stem cell is at least bipotential [7].

In untreated acute myeloid leukemias selected for euploidy in terms of DNA content, a broad distribution of the mean blast DNA synthesis time is encountered (Table 1). The mean value for normal myeloblasts is roughly 13 h [1]. It is obvious, as has been shown by many others before, that the labeling index is markedly lower than in normal myeloblasts. There is no correlation between DNA synthesis time and labeling index in these cases. However, a close correlation between the labeling index and the fractional birth rate of blasts is encountered (Fig. 3). The labeling index determined by either autoradiography or an adequate flow cytometric method can thus be taken as a measure of the relative cell production rate of these blasts.

Comparable observations were made in childhood acute lymphoblastic leukemia (ALL) [6]. Here we also find a close correlation of the labeling index with the relative cell production rate of blasts. We further observed (Fig. 4) that the DNA synthesis time and the labeling index of hyperdiploid cases were increased by the same factor over diploid cases. This means that the mean DNA synthesis rate is equal in both groups. Look et al. [12] were the first to report a relationship between aneuploidy and the S-phase fraction in childhood ALL and suspected that hyperdiploidy is associated with a prolonged DNA synthesis time. Based on observations of a better prognosis in hyperdiploid than in diploid cases, they have inferred that a higher probability exists for the individual cell of hyperdiploid cases to be hit by an antileukemic drug while staying in the S-phase. Our data support their suspicion. However, we would hesitate to subscribe to their interpretation of the better prognosis, since hyperdiploidy may equally indicate higher genetic instability and thus a higher vulnerability towards antileukemic treatment.

An interesting correlation exists (Fig. 5) between the DNA synthesis rate of leukemic blasts in bone marrow and peripheral blood of the same patient [18]. This includes ALL as well as ANLL cases. In all cases the rate of DNA synthesis in peripheral blood is higher by roughly the same factor than in bone marrow. We are unable to define the rules of this relationship. However, it seems permissible to state that leukemic blasts show some adaptative behavior towards their environment. The so-called autono-

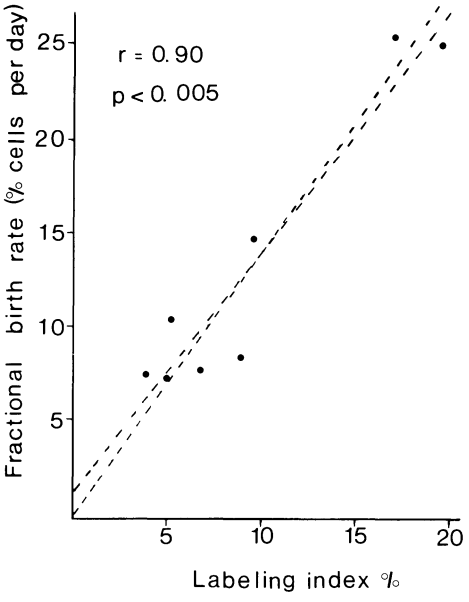

Fig. 3. Relative cell production rates of blasts in eight cases of euploid AML plotted against the autoradiographically determined labeling indices

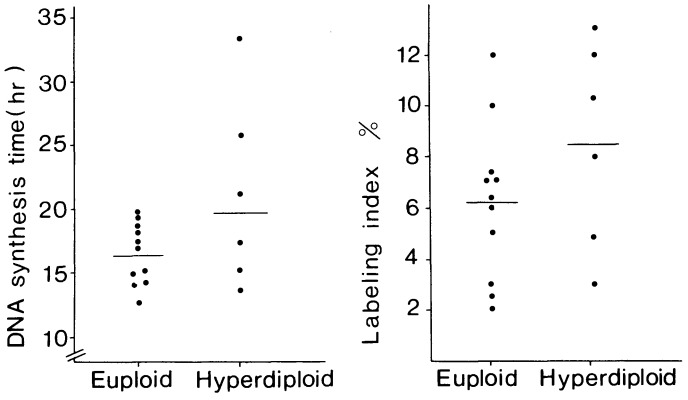

Fig. 4. DNA synthesis time and labeling index of ALL blasts subdivided into 2 groups with either euploid or hyperdiploid stem line. Arithmetic means per group are indicated by horizontal bars

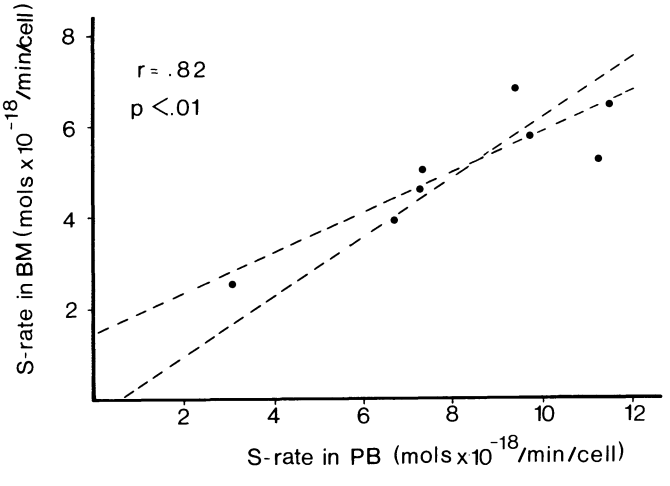

Fig. 5. DNA synthesis rates of ALL and ANLL cases determined in both bone marow *(BM)* and peripheral blood *(PB)*. PB values are higher than BM values by a factor of 1.6

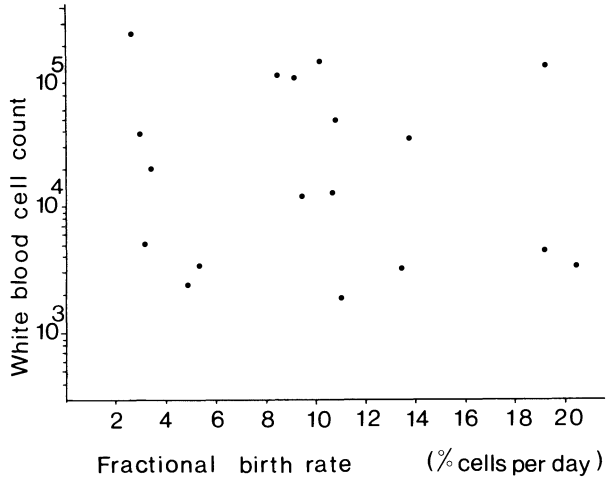

Fig. 6. White blood cell counts (cells/μl) in 19 cases of childhood ALL plotted against the fractional birth rates of ALL blasts in the bone marrow. (From [6])

mous growth of leukemic blasts thus does not preclude their capability for some adaptation. On the other hand, the most significant type of adaptation has been lost: to adapt the production rate of cells to the actual cell mass in a compartment. Figure 6 shows that in childhood ALL there is no correlation between the blast cell count in peripheral blood, used here as an indicator of total leukemic cell mass, and the cell produc-

Table 2. Effect of cytostatic treatment in CML (chronic phase + blatic crisis) on duration of DNA synthesis. Statistical evaluation (t-test) of untreated vs. treated group

	Normal ($n=5$)	CML untreated ($n=4$)	CML treated ($n=4$)	P
Proerythroblasts	9.4±1.9[a]	9.7±2.0[a]	14.2±2.8[a]	0.05
Basophilic erythr.	11.0±2.3	12.0±3.7	18.6±3.0	0.05
Polychrom. erythr.	17.0±3.4	19.5±5.7	25.4±5.3	n.s.[b]
Myeloblasts	13.2±1.8	13.3±2.2	17.9±2.3	0.05
Promyelocytes	13.4±0.9	13.2±2.0	18.5±1.5	0.05
Myelocytes	14.1±1.1	16.9±2.5	19.9±2.7	n.s.

[a] Means±SD.
[b] n.s., not significant.

tion rate. While this suggests an adaptative defect, it also indicates that total cell mass is not a consequence of proliferative activity. Apparently, the balance between cell production and cell loss rate determining the total tumor mass varies from one case to another.

All these data show few ways in which antileukemic therapy could profit from specific cell-kinetic properties of cells in acute leukemia. On the other hand, it cannot be excluded that antileukemic therapy has some influence on the kinetics in leukemias, for example, by way of a clonal selection process or by a general alteration of the replicative capability. We have compared the DNA synthesis time of four CML patients prior to busulfan treatment (Table 2), which was normal, with that of four other cases after busulfan treatment. In this latter group a treatment-free interval of at least 4 weeks preceded the cell-kinetic study. A prolongation of DNA synthesis time was present in all cell compartments and in most cases was statistically significant. However, before stating that busulfan prolongs the DNA synthesis time it has to be confirmed that this is not due to the natural history of CML, since in the second group the disease had lasted for a longer period. In view of an increasing genetic instability with time of the CML cells the latter possibility needs clarification.

Preliminary data from a recently finished prospective study on the kinetics in preleukemia will be briefly described. We found a close correlation (Fig. 7) between the degree of ineffective granulocytopoiesis and the colony-forming unit-culture (CFU-C) numbers in bone marrow. This indicates

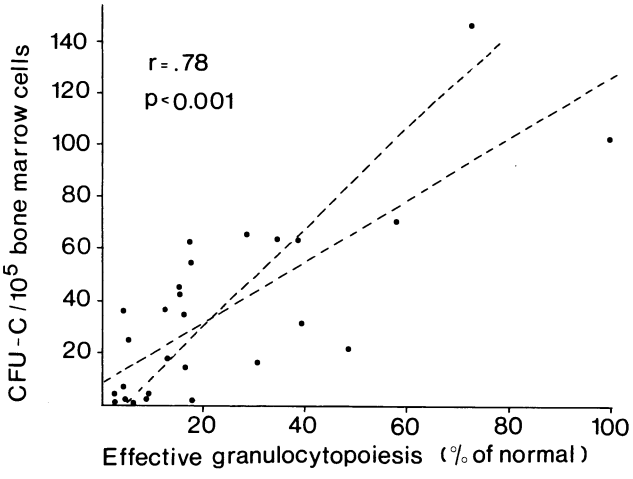

Fig. 7. Number of CFU-C colonies in patients with myelodysplastic syndrome plotted against the degree of effective granulocytopoiesis

that in disorders associated with ineffective granulocytopoiesis, CFU-C numbers do not necessarily tell much about the size of the precursor compartments. The same was true for erythropoiesis. Thus, no reliable information on the size of the precursor compartments in AML is derived from conventional precursor studies. It was further investigated whether the transition from preleukemia to overt leukemia is a continuous or a discontinuous process. Several cases of refractory anemia with excess of blasts (RAEB) showed that both processes may be found. In some patients there was a continuous increase in the blast count and number of blasts in S-phase in the bone marrow associated with a corresponding decrease of effective granulocytopoiesis. Other cases of RAEB, however, did not show significant kinetic changes over a long period. Then a sudden change with an acute increase in the number of blasts in S-phase and a decrease of effective granulocytopoiesis was observed. This suggests the evolution of a new clone with different kinetic properties.

In clonclusion, cell kinetics as a method of dynamic cell population statistics do show basic alterations of leukemic cells, and in some instances point to the type of defect in the underlying molecular mechanisms. In other instances the underlying mechanisms remain obscure. Therapy can profit only little from differences between leukemic and normal cell kinetics. Cell death kinetics in vivo as pioneered by Hiddemann et al. [9, 10] could be of higher significance for treatment procedures than cell production kinetics, but routine methods for this purpose are not yet available. Cell production kinetics, on the other hand, are easily accessible by determination of the labeling index.

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Acute Nonlymphocytic Leukemia in Adults: Pathophysiology, Status of Current Therapy, and New Approaches

R. H. Mertelsmann and F. Herrmann[1]

New Pathophysiologic Aspects of Leukemogenesis and Leukemic Growth

Recent information concerning the cell biology of leukemias has provided new insights into the pathophysiology and pathogenesis of acute leukemia, involving the detection of leukemia viruses, oncogenes and their products, and the discovery of factors supporting clonal leukemic growth. Murine, avian, and cat leukemia viruses are well characterized. To date, only HTLV I appears to be a likely candidate as a human leukemia virus. For both avian and murine viruses, there is a fundamental classification distinction between long-latency viruses (LLV) and acute transforming viruses (ATV). The ATV are replication defective and must be propagated with a helper virus. They have within their genome an identifiable oncogene. The LLV do not contain such an oncogene and presumably act by "promotor insertion", e.g., a retroviral long terminal repeat (LTR) inserted 5′ to the cellular oncogene. Acutely appearing neoplasms are probably not clonal, but reflect infection of multiple target cells. Long-latency neoplasms, however, are clonal and probably reflect expansion of a random oncogenic event. There are usually differences between in vitro and in vivo target cell specificities for these viruses. Furthermore, pathogenicity in the animal is greatly affected by animal age, inoculum route, and the genetics of the recipients. Target cell specificities will hopefully be classified by new in vitro culture techniques for hematopoietic cells. Three avian ATV are interesting because of their apparent target cell specificity:

AEV (erythroblastosis virus), AMV (myeloblastosis virus), and MC 29 (myelocytomatosis virus). Relative numbers of target cells appear to differ for these viruses: 50 for AEV, 700 for AMV, and 3500 for MC 29. AEV (V-erb B) affects erythroid precursors (presumably BFU-E), AMV (V-myb), early myeloid precursors, and MC 29 (V-myc), macrophage-like cells. In vitro AEV and MC 29, but not AMV, transform fibroblasts.

These viruses illustrate general issues concerning acute transforming oncogenic viruses: (a) is the defect the result of a block at a specific point in a hematopoietic lineage? (b) Is the block affected by constitutive or high-level production of crucial cellular genes particular to that stage of differentation? (c) If so, can analogous cellular gene products be found in these cell types?

Pragmatically, ATV are capable of expanding a specific compartment of hematopoietic cells, which makes possible their molecular characterization (e.g., Friend erythroleukemic virus).

An example of "promotor insertion" appears to occur in the murine WEHI-3B cell line, where LLV insertion near the interleukin-3 gene results in its constitutive synthesis and subsequent autocrine stimulation of cell growth [1]. Similarly, in human leukemia, the "leukemogenic event" may result from virus infection with expression of the viral oncogene or possibly from the juxtaposition of cellular proto-oncogenes with genes

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undergoing rearrangement during normal hematopoietic development. In this context, C-fms is of interest.

C-fms, the cellular homologue to V-fms, the oncogene of a feline sarcoma virus, was found to be related to the receptor for CSF-1, a lineage-specific growth factor for the proliferation of monocytoid precursor cells [2]. Overexpression of CSF-1 receptors may result in abnormal proliferation of the respective cell type. Since C-fms is located on chromosome 5(q), an absence of the CSF-1 receptor, as seen in $5q^-$ syndrome, may have complex effects resulting in acute nonlymphocytic leukemia (ANLL). Also, autocrine stimulation of leukemic growth may take place in the pathogenesis of human ANLL. It is difficult to determine primary oncogenic events, since proto-oncogene amplification or constitutive expression may only reflect an additional selective growth advantage acquired after the initial deregulation of growth control. However, analogously to the events causing myeloid leukemia in animal models, there may be corresponding mechanisms of leukemogenesis for humans. It might be hypothesized that a leukemic event such as transformation by a human leukemia virus or activation of one or more proto-oncogenes by other mechanisms, take place in a normal myeloid progenitor cell, which then expands as a clonal population of cells with progenitor cell properties, unrestricted growth, and limited differentiative capacity.

A small subset of leukemic cells ($< 1\%$ of the total leukemic population) from many patients with ANLL has been shown to form clones in vitro in semisolid medium [3]. These clonogenic cells, leukemic colony-forming cells (L-CFC), have several properties that are not shared by the majority of leukemic cells: A high fraction of L-CFC is in S-phase, the cells have the ability to divide rapidly in vitro and have self-renewal capability [4, 5]. L-CFC share these properties with normal myeloid progenitor cells. It has been proposed that L-CFC act as progenitor cells in vivo and are responsible for the maintenance of the leukemic blast cell population. Growth of L-CFC ist dependent on growth factors that are only poorly defined. The possibility that some autocrine growth factors are secreted and utilized has also been considered, but not investigated extensively. However, recent studies with recombinant granulocyte/macrophage colony-stimulating factor (GM-CSF) have suggested that L-CFC proliferation in a large proportion of ANLL patients can be effectively induced by that factor [6]. Moreover, in some patients, it was possible to demonstrate the constitutive secretion of a growth factor with GM-CSF biological activity by leukemic cells. The autonomous growth of L-CFC by these patients was further substantiated by Northern blot analysis of patients' mRNA with a specific cDNA probe for GM-CSF and immunologic identification with anti-GM-CSF monoclonal antibody [7]. Further investigations are currently in progress to establish whether all leukemic cells secrete GM-CSF or other CSF species or whether only a subset is responsible (proliferating or nonproliferating cells). It is possible, for example, that only the latter differentiated and nonproliferating cells secrete CSF. If so, this would presumably be in a constitutive, nonregulated manner which could contribute to the proliferative thrust of the tumor, since normal differentiated myelomonocytic cells do not secrete CSF without inducing factors [8].

Basic research on the molecular basis of leukemogenesis, the regulation of expression of relevant genes, and their modulation by agents with therapeutic potential should eventually lead to more rational and effective models of therapy.

Status of Current Therapy

Acute nonlymphoblastic leukemia can now be cured in some adult patients. At present, 10%–30% of all patients started on treatment survive more than 5–10 years. Attempts to recognize prognostic factors identifying patients with good or poor prognosis at diagnosis have led to several interesting observations which eventually should allow stratification to more or less intensive treatment regimens based on prognostic parameters.

The standard induction treatments for ANLL employ a combination of an anthracycline and cytosine arabinoside (ara-C) at

intermediate, and more recently, at high doses, giving remission rates of between 50% and 90%, depending on patient age, risk factors, and other prognostic parameters. Induction treatment is followed by one or more consolidation cycles with either the same regimen used for induction or with a sequence of potentially non-cross-resistant combinations. In children and young adults, very intensive consolidation treatment cycles are well tolerated and appear to be associated with longer remissions in these patients [9, 10]. Older patients do not tolerate repeated cycles of nadir-inducing regimens very well, and deaths in complete remission can be a major problem when using an aggressive consolidation approach. Studies reporting advantages for intensive consolidation over no consolidation have not been randomized, and patient selection has to be considered as a factor responsible for the observed differences between patients with and without "intensification" [11, 12]. There is very little evidence to suggest that maintenance treatment is of benefit in ANLL. Similarly, the role of early and late intensification has not been clearly identified. The single-arm studies presented to date have not taken into account the fact that patients who are in continuous complete remission at 1 year (the usual time point selected for late intensification) have a 10-year survival of approximately 50% even without "late intensification".

Ongoing studies of ANLL and related disorders include both clinical studies and laboratory projects to elucidate mechanisms of leukemogenesis and leukemia pathophysiology in an effort to design new therapeutic strategies. The clinical studies focus on (a) further phase I, II, and III trials of new chemotherapeutic agents; (b) allogeneic bone marrow transplantation; (c) autologous marrow transplantation with and without in vitro elimination of leukemic cells ("purging"); (d) prognostic factor analysis with stratification to different therapeutic approaches for prognostic subgroups; and (e) phase I, II, and III trials of biological response modifiers (BRM). Preclinical in vitro and in vivo evaluation of new agents include BRM and attempts at purging bone marrows of leukemic cells for later autologous transplantation.

Prognostic Factor Analysis

The ongoing analysis with respect to prognostic factors of all patients with ANLL treated on recent protocols has documented advanced age, an absence of Auer rods, and elevated terminal deoxynucleotidyl transferase activity (Tdt) as unequivocally associated with a poor prognosis. ANLL developing after a preceding myelodysplastic syndrome (MDS) or secondary ANLL developing after chemotherapy and/or radiotherapy also respond poorly to conventional ara-C/anthracycline combinations, but might be more responsive to high-dose ara-C/anthracycline combinations or to low-dose ara-C. Upon completion of the statistical analysis of prognostic factors, stratification is planned in order to explore new treatment strategies, using BRM, for example, for patients expected to do poorly on convential regimens.

Recently, a subgroup of myelomonocytic leukemia with eosinophilia (M4Eo) and a specific chromosome inversion (16, p13q22) has been recognized that is associated with a high rate of complete remission (>90%), long remissions (median, 18 months), and long survival (median 34 months). This group was further characterized by a high incidence (35%) of CNS disease. It is anticipated that more precise classification of leukemias by cytogenetic and recombinant DNA techniques will lead to further definition of prognostic subgroups with therapeutic implications, as evidenced by the M4Eo subcategory.

New Approaches

New Drugs and Schedules

The introduction of new anthracyclines and related agents has increased the spectrum of drugs that can be used successfully for remission induction in ANLL. So far, however, only decreased toxicity or a different spectrum of toxicities, rather than increased therapeutic efficacy have been achieved and might lead eventually to the replacement of daunorubicin by other agents as the anthracycline of choice. The combination of AMSA (Z. Arlin, personal communication) or daunorubicin [13] together with high-

dose ara-C might prove to be the most effective induction/consolidation regimen available today. While the search for more effective and less toxic drugs will have to continue, alternative forms of therapy will have to be pursued more actively in view of the consistently poor overall results, especially for older patients with ANLL [14]. The pharmacologic, biological, and clinical effects of low-dose ara-C are under active investigation here and elsewhere. Although not very efficacious, it appears to offer a less toxic therapeutic alternative for poor-risk patients with ANLL or MDS (for review, see [15]).

Autologous Marrow Transplantation

The indications for allogeneic marrow transplantation in young patients with ANLL in complete remission and with a compatible sibling donor appear to have been established. Autologous marrow transplantation without [16, 17] or with in vitro elimination of residual leukemic cells, in particular L-CFC, by immunologic [18] or chemotherapeutic [19, 20] techniques for older adult patients or those without a suitable donor is an area of intensive research pursued at several centers [21].

Biological Response Modifiers

With the rapidly expanding knowledge of the molecular basis of leukemogenesis [22], as well as of the control of growth and differentiation [23], more rational approaches to leukemia control can be designed. Differentiation-inducing agents such as 13-cis retinoic acid and 1,25-dihydroxy vitamin D3 have been very active in various in vitro systems, but their clinical use has so far been rather disappointing. New agents under study include hexamethylenebisacetamide (HMBA) and, possibly, physiologic differentiation-inducing peptides produced by hematopoietic cells. The observation that alpha-interferon is a uniquely effective treatment for hairy cell leukemia would suggest that other lymphokines and cytokines might also be of therapeutic benefit in specific hematopoietic neoplasias. Cytokines in current or planned clinical trials at our department include interleukin-2, gamma-interferon, tumor necrosis factor, and colony-stimulating factors. These agents should also prove useful in the in vitro manipulation of bone marrows in preparation for autologous transplantation.

Outlook

Although complete remission rates for ANLL, especially in younger patients, have increased dramatically in recent years, remission durations have been far less encouraging. It is hoped that further understanding of the pathogenetic mechanism underlying leukemogenesis and the remission status will lead to a more rational approach to the use of chemotherapeutic and BRM agents, as well as of autologous and allogeneic bone marrow transplantation. More efficacious treatments and approaches associated with less morbidity have to be developed for this disease, which is still fatal for the majority of patients.

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Prognostic Significance of Chromosome Changes in Acute Leukemia

A. A. Sandberg[1]

The recognition that chromosome changes constitute an independent variable bearing upon the prognostic aspects of acute leukemia has now been well established [1–3]. This applies equally to acute nonlymphocytic leukemia (ANLL) and acute lymphoblastic leukemia (ALL). The present paper will deal with a succinct evaluation and clarification of the relative importance of established cytogenetic changes in the prognostic aspects of acute leukemia. The chromosomal (karyotypic, cytogenetic) abnormalities concerned with the prognostic aspects of acute leukemia can be classified as follows, in the order of their relative importance:

1. Specific (primary) karyotypic change
2. Secondary chromosome changes
3. The presence or absence of cytogenetically normal cells in the marrow
4. The presence of double minute chromosomes (DMS) or homogeneously staining regions (HSR)
5. Numerical chromosome changes (without morphologic abnormalities)

Specific (Primary) Karyotypic Change

It is now generally accepted that the primary (specific) chromosome change in acute leukemia is probably related to, if not necessary for, the process of malignant transformation and closely associated with those molecular events which may, in fact, be responsible for this transformation, if not for the causation of the disease [4–6]. A major feature of the specific chromosome changes is the recognition that they may characterize well-defined subgroups within an existing acute leukemia entity (Tables 1 and 2) [7]. For example, the FAB M2 type of acute myeloblastic leukemia (AML) has been shown cytogenetically to be a rather heterogeneous disease in that subgroups within this entity can now be defined karyotypically and include cases with translocations between chromosomes 8 and 21, t(8;21)(q22;q22), other cases with t(6;9)(p23;q34), and still others with a Philadelphia (Ph) chromosome due to t(9;22)(q34;q11). Furthermore, the AML cases with t(8;21) are usually associated with Auer bodies in the leukemic cells, a ready response to therapy with long complete remissions, and a relatively long survival among the ANLL cases. On the other hand, AML with a Ph chromosome appears to have a rather poor prognosis with short-lived complete remissions, when these can be attained, and a rather short survival. A similar situation applies to M4 (acute myelomonocytic) type of ANLL, in which several subcategories, including that with an 11q−, another with inversion of chromosome 16, and still others with a Ph chromosome; appear to exist within this entity. The important point to stress is that the primary chromosomal event appears to determine the basis biology of the disease and thus the response to therapy, survival, as well as other clinical aspects of the acute leukemia.

Although a few conditions among the acute leukemias have been studied [8–11], what remains to be determined are the basic molecular events, such as involvement and

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Table 1a. Specific (primary) karyotypic changes in ANLL and related disorders

Type of acute ANLL	Chromosome change
M1, M2	inv(3) (q21q25–27)
M2, M4	+4
M1, M2, M4, M5, M6	−5 or 5q− (q13q31)
M1, M2	t(6; 9) (p22.2; q34)
M1, M2, M4, M5, M6	−7 or 7q− (q31.2q36)
M1, M2, M4, M5, M6	+8
M2	t(8; 21) (q22.1; q22.3)
M2, M4, M5a	t(9; 11) (p22; q23)
M1, M2	t(9; 22) (q34.1; q11.2)
M3	t(15; 17) (q22; q11.2)
M2, M4, M5b	inv(16) (p13.1q22.1) or 16q− (q22)
M2	t(3; 5) (q26; q22)

Table 1b. Specific karyotypic changes in myelodysplastic or myeloproliferative disorders and preleukemia, conditions which may precede or change into ANLL

t(1; 3) (p36; q21)
t(1; 7) (p11; p11)
t(2; 11) (p11; q23)
del(9) (q13q22)
del(20) (q12q13)
t(3; 17) (q26; q22)
t(11; 21) (q22; q21)
21q−
del(13) (q14)
+8

expression of particular oncogenes and other genes in each cytogenetically defined subgroup of acute leukemia and the nature of their products, particularly proteins, which may play a direct role in the causation and biology of the leukemia. Thus, the establishment of the primary chromosome change can serve as an important guide to molecular biologists in recognizing the genes involved in the process of malignant transformation and affected by the karyotypic changes [12]. Since, as mentioned above, in each category of the FAB classification of ANLL and ALL a number of subentities are apparently defined cytogenetically in terms

Table 2. Specific (primary) karyotypic changes in ALL

Type of ALL	Type of cell involved	Specific chromosome change
L1 (L2)	Pre-B cell	t(1; 19) (q21−q23; p13.3)
L3	B-cell	t(2; 8) (p11–13; q24.1)
(L1), L2	Early pre-B precursor	t(4; 11) (q21; q23)
L2	Common B-cell	del(6) (q21q25)
L3	B-cell	t(8; 14) (q24.1; q32.3)
L3	B-cell	t(8; 22) (q24.1; q11)
L1	Common B-cell or T-cell	9p−
L1, (L2)	Early pre-B cell	t(9; 22) (q34.1; q11.2)
(L1), L2	T-cell	t(11; 14) (p13–14; q11.2–13)
L1, (L2)	Common B-cell	t(11; 14) (q13; q32)
(L1), L2	Common-B-cell	12p− (p12)
L2	Common B-cell	+21
L1, L2	T-cell	14q+(q32) or 14q−(q11)
L1, (L2)	B-cell	Near haploid

Table 3. Relation of primary karyotypic change to prognosis in ANLL

Type of ANLL	Primary karyotypic change	Relative prognosis
M2	t(8; 21) (q22; q22)	Good
M1 or M2 or M4	+8	Intermediate
M2 or M4	t(6; 9) (p23; q34)	Intermediate
M4	Inv(16) (p13q22) or 16q− (q22)	Good
M1 or M2	t(9; 22) (q34; q11)	Poor
Secondary	−5, 5q−, −7, 7q−	Poor
M5	t(9; 11) (p21; q23)	Poor

Table 4. Relation of primary karyotypic change to prognosis in ALL

Type of ALL	Primary karyotypic change	Relative prognosis
L1 or L2	Numerical, e.g., +21	Good
L1 or L2	6q−	Intermediate
L1 or L2	t(1; 19) (q23; p13)	Good
L3	t(8; 14) (q24; q32)	Poor
L2	t(4; 11) (q21; q23)	Poor

of the primary chromosomal event, with the latter serving as a key prognostic index, it is important to continue to define further various subentities in ANLL and ALL, cytogenetically and/or molecularly.

Tables 3 and 4 show the relative roles played by the primary chromosomal changes in some ANLL and ALL entities in the prognosis of acute leukemias.

Secondary Chromosome Changes

In all probability, each form of acute leukemia is at its lowest level of malignancy when the leukemic cells contain the primary karyotypic change only. The biology of the disease is almost invariably worsened by the appearance of secondary chromosome changes, regardless of the nature of the primary karyotypic event. Thus, in cases with t(8;21) the disease has a rather "benign" course until secondary changes, usually consisting of loss of a sex chromosome, +8 or other abnormalities, appear in the leukemic cells. Once such a secondary change occurs, the disease assumes a more aggressive course with resistance to chemotherapy and difficulty in obtaining complete or long remissions. In some acute leukemias, the course of the disease appears to change for the worse without the appearance of secondary chromosome changes, and in these circumstances it is possible that the secondary events take place at the molecular level, e.g., either abnormal activation of oncogenes (or other genes) with overproduction of normal products or the production of abnormal products. That leukemic cells in which only a primary chromosomal event is present are capable of expressing a number of oncogenes has been established [10, 11, 13], though the order in which such oncogenes are activated and the nature of the activation (transient or permanent, normal or abnormal) has yet to be established.

Whether there is a nonrandom pattern to the appearance of secondary chromosome changes in acute leukemia has not been established with certainty. For example, trisomy 8 is a common event in some of the leukemias, but whether there is a specificity to this secondary change has not been ascertained.

In addition to the qualitative secondary chromosome changes in acute leukemia, the prognostic aspects appear also to be related to quantitative anomalies [14]. Thus, the presence of major karyotypic changes (MAKA) appears to reflect a much poorer prognosis than the presence of minor karyotypic abnormalities (MIKA). Thus, one ob-

tains the impression that the appearance of more and more karyotypic changes worsens the prognosis of a particular disease, those associated with MAKA having a much shorter survival than those with MIKA.

Presence or Absence of Cytogenetically Normal Cells

Within each category of acute leukemia associated with an established primary karyotypic change, a factor affecting prognosis is related to the presence or absence of cytogenetically normal cells in the bone marrow, a factor first pointed out by us more than 10 years ago [15]. Thus, patients whose marrow contains only abnormal cells (AA cases) appear to have a much poorer prognosis than those who have some cytogenetically normal cells (AN cases) admixed with the abnormal ones or only normal cells in the marrow (NN cases). It must be unterstood that this classification is basically a quantitative one, since there ist little doubt that if an inordinately large number of cells were to be examined in either AA or NN cases, normal or abnormal cells would be encountered, respectively. What these findings do indicate is that the presence of a substantial number of normal cells in the marrow of a patient treated with chemotherapy possibly allows repopulation of the marrow by the normal cells once the leukemic cells have been eradicated by such therapy. In the absence of cytogenetically normal cells, the marrow is not readily repopulated with the normal ones, and the patient is then subject to complications of marrow deficiency, e.g., anemia, bleeding due to thrombocytopenia, and, most importantly, overwhelming infections due to the shortage of proper leukocytes. The value of the AA, AN, and NN classification appears to have been established more firmly for ANLL than for ALL, though there are reports in which correlations between the presence or absence of cytogenetically normal cells in ALL appears to bear definitely upon the prognosis of the disease [16, 17].

Establishing a meaningful and reliable AA, AN, or NN classification of a marrow requires examination of a relatively large number of cells (e.g., more than 50), though considerable information may be obtained from a lesser number of cells. The examination does not require unusual expertise, since the recognition of even a single normal metaphase in a marrow allows it to be classified as AN, thus affording the clinician important information regarding the therapeutic approaches and ultimately the prognostic aspects of the disease.

Double Minute Chromosomes and Homogenously Staining Regions

The presence of DMS in leukemic cells, particularly those of ANLL, has been thought to carry with it a rather poor prognosis [1]. Though it is possible that DMS may be associated with gene amplification and thus possibly lead to drug resistance [18], recent evaluation has led us to believe that the primary chromosomal event plays a much more profound role in the prognostic aspects of acute leukemia than the presence of DMS. For example, some cases of ANLL with DMS in otherwise karyotypically normal cells have been described [19] and the response of these patients to therapy and, hence, their survival appear to have been related more to the basic karyotype than the presence of DMS. Nevertheless, future evaluations of the role of DMS vis a vis the primary chromosomal change, as far as prognosis of acute leukemia is concerned, will have to be undertaken in a larger series of patients, with the type of the acute leukemia and particularly the primary karyotypic event taken into careful consideration.

HSR which are thought to be related to DMS [18] are very seldom seen in cells of acute leukemia; in all probability what has been said about DMS applies to HSR as far as the prognostic aspects of this phenomenon in acute leukemia are concerned.

Numerical Changes in Acute Leukemia

Some acute leukemias, particularly ALL, may be associated solely with numerical chromosomal changes. Hyperdiploid ALL cases, ANLL cases with trisomy 8, and secondary leukemias with either a -5 and/or -7 are examples of such numerical changes.

When such numerical changes represent the primary karyotypic event, they probably have a role, as far as the prognosis of the acute leukemia is concerned, similar to that of other primary events, such as translocations, deletions, insertions, or inversions. Nevertheless, in ALL it appears that the presence of only numerical karyotyic changes, particularly those leading to hyperdiploidy, is associated with a much better prognosis than with karyotypic changes of the morphologic type [3, 17]. The same may be true of ANLL, though here the situation is more complicated since the number of cases of ANLL with numerical changes only is rather small as compared with ALL. In the case of secondary leukemia monosomy 5 or 7 invariably indicates a very poor prognosis with very rare complete remissions, and when such are achieved they are of relatively short duration with the result that survival in the particular acute leukemia (almos always ANLL) is very short [2].

Absence of Metaphases in Marrow

In some cases of acute leukemia, and this applies more to ALL than ANLL, no metaphases can be found in the bone marrow preparation, and the question arises as to the significance of this finding. Obviously, repeat cytogenetic examinations should be performed just in case a particular specimen does not contain metaphases, possibly due to a number of technical and other reasons. However, the consistent absence of metaphases in the bone marrow of patients with acute leukemia tends to indicate a rather poor prognosis, as based on general experience [3, 20]. Why that is so is not clear, but the possibility exists that since dividing cells are more susceptible to the effects of chemotherapy, the absence of a sufficient number of such cells may render the therapy much less efficacious than when metaphases are encountered in the marrow.

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Morphological and Cytochemical Classification of Adult Acute Leukemias in Two Multicenter Studies in the Federal Republic of Germany*

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Introduction

In 1978, two pilot studies evaluating the efficacy of new treatment strategies for morphologically defined acute lymphoblastic leukemia (ALL) or acute undifferentiated leukemia (AUL) and for acute myelogenous leukemia (AML) were initiated in the Federal Republic of Germany. These studies were followed by controlled randomized trials in 1981 and 1982 respectively. While the main objective of these studies was to improve remission quality and duration, the trials further aimed at establishing prognostic factors. It was therefore decided to concentrate the morphological and cytochemical characterization and the immunologic surface marker studies at single institutions for all patients enrolled in the ALL/AUL trial. For patients with AML, a central review panel judged all equivocal cases after a primary diagnosis had been made by the local hematologist. The results obtained from the investigation of patients participating in either of these studies are presented in this report.

Material and Methods

ALL/AUL Group. Samples of 471 patients were examined independently by two different central review institutions (Kiel and Munich), one being responsible for morphological and cytochemical investigation and the other for immunologic studies. Patients were recruited by the German Multicenter Therapy Trial [1] between January, 1981, and April, 1985.

The morphological appearance of blasts on smears of bone marrow and peripheral blood stained with May-Grünwald-Giemsa was classified according to the FAB criteria [2]. Cytochemical examination of bone marrow and blood smears included the following reactions according to standard procedures: (a) myeloperoxidase, (b) periodic acid Schiff reaction (PAS), (c) acid naphthyl acetate esterase (ANAE), (d) acid phosphatase (AcP), (e) dipeptidylaminopeptidase IV (DAP IV) [3]. Terminal deoxynucleotidyl transferase (TDT) was qualitatively determined by immunofluorescence [4] using commercially available antisera (Bethesda Research Labs, Eggestein, Federal Republic of Germany; P-L Biochemical, St. Goar, Federal Republic of Germany).

Immunologic phenotypes of leukemic blasts were characterized by immunofluorescence techniques detailed elsewhere (see Thiel et al., this volume).

ALL and AUL were defined by morphological and cytochemical examination, irrespective of the results of immune phenotyping, thus allowing independent evaluation of both diagnostic methods. Blasts lacking myeloperoxidase and monocyte-type esterase were regarded as ALL if a block or coarse granular PAS reaction and/or a focal AcP reaction (or both patterns of PAS and AcP) were present. Cases negative for mye-

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loperoxidase, PAS, and AcP, were defined as AUL, irrespective of ANAE, DAP IV, and TDT reactions. ANAE reaction was considered positive if dot-like staining was present. Presence of DAP IV was regarded positive independently of the localization of the staining pattern. Immune phenotypes were classified as follows: (a) CALLA/pre-B/pre-pre-B type (C/pB), (b) T type (T), (c) pre-T type (pT), (d) B type (B), (e) O type (O), and (f) mixed lymphomyeloid type mixed).

All cases were examined for myeloperoxidase and PAS, 96.2% of all cases for AcP, 95.1% for ANAE, 67.5% for DAP IV, 77.3% for TDT, and 70.9% of all patients were examined by immunologic phenotyping.

Statistical analysis was done by χ^2 test.

AML Group. The diagnosis of AML and its subtypes was based on May-Grünwald-Giemsa–stained marrow aspirates and blood smears of 407 patients. Patients were recruited by the German AML Cooperative Group [5]. In this study, the primary diagnosis was made by the local hospital, but the smears were reviewed during regular slide conferences by members of the diagnostic comittee. In equivocal cases, discriminating stains including peroxidase and esterase were obligatory.

Results

Diagnostic Groups and Subgroups

ALL was diagnosed in 392 (83.2%) of 471 patients, while AUL was present in 79 (16.8%) patients. Morphological classification revealed a predominance of L2 in 67.6% of all patients, while the L1 type occurred in 27.5% and L3 in only 4.9% (Fig. 1A). Immunologic investigation revealed C/pB type in 44.8% of the patients, O type and T type in about equal proportions (23.6% and 22.7% respectively), pT type in 5.7%, and B type in 3.0% of cases. The blasts of one patient showed a pattern of mixed type immunologically (Fig. 1B). While cases of AUL had to be negative for PAS and AcP by definition, typical dot-like ANAE positivity was detected in 6.4% of AUL vesus 12.4% of ALL (not significant). Positivity for DAP IV was exclusively restricted to ALL. TDT was present in 85.9% of ALL versus 50.9% of AUL ($p<0.001$).

Characterization of Morphological Subtypes (FAB)

Blasts of L3 type were present to a significantly higher degree in AUL (10.1%) as compared with ALL (3.8%, $p<0.05$). Although L1 morphology was more frequent in ALL (29.3%) as opposed to AUL (17.7%) and L2 distribution was slightly more common in AUL (72.2%) as opposed to 66.8% for ALL these differences were not statistically significant.

Cytochemical reactions within FAB subgroups (Fig. 2) were significantly different for PAS in L1 as opposed to L2 and L3 ($p<0.01$), for AcP in L1 as opposed to L2 and L3 ($p<0.01$), for DAP IV in all subgroups ($p<0.05$), and for TDT in L3 as opposed to L1 and L2 ($p<0.001$).

Distribution of immunologic types within each of the three FAB groups revealed a significantly higher percentage of T type in the L1 subgroup ($p<0.05$) and of B type in the L3 subgroup ($p<0.001$), while the C/pB type was far less common the in L3 subgroup ($p<0.001$). One case of mixed type occurred in the L1 group (1%).

Correlations Between Cytochemical Markers

Significant correlations could be observed between (a) AcP and ANAE ($p<0.001$), (b) AcP and DAP IV ($p<0.001$), and (c) ANAE and DAP IV ($p<0.05$).

Characterization of Immunologic Subgroups

As has already been pointed out, distribution of the different immunologic phenotypes in the FAB subgroups was not at random. Thus, each of the immunologic subgroups was composed of different proportions of morphological types (Table 1). B-type blasts were mainly of L3 and, to a lesser, degree, of L2 morphology, while L1

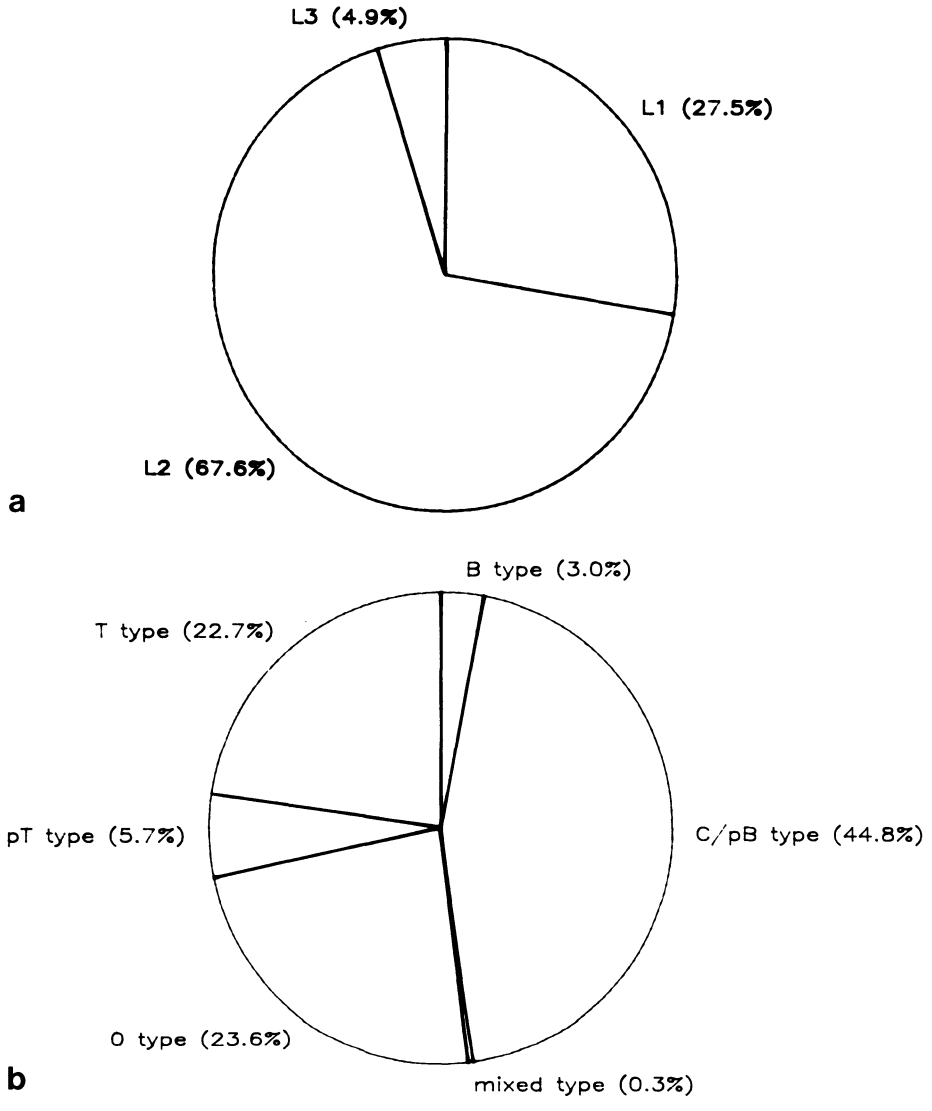

Fig. 1a, b. Proportions (in percent) of morphological subgroups of **a** 471 patients with ALL ($n=392$) or AUL ($n=79$) (FAB classification) and **b** 334 patients with ALL ($n=288$) or AUL ($n=46$)

Table 1. Distribution of morphological subtypes (L1, L2, L3) in different immune phenotypical groups

	C/pB		T		pT		O		B
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)
L1	45	30.0	31	40.8	5	25.0	18	23.1	0
L2	104	69.3	42	55.2	14	70.0	55	70.5	3
L3	1	0.7	3	4.0	1	5.0	5	6.4	7
n	150	100	76	100	20	100	78	100	10

The significance of the distribution of the various immunologic groups is as follows: B/L3 vs. L1, L2: $p<0.001$; C/pB/L1, L2 vs. L3: $p<0.001$; T/L1 vs. L2, L3: $p<0.05$).

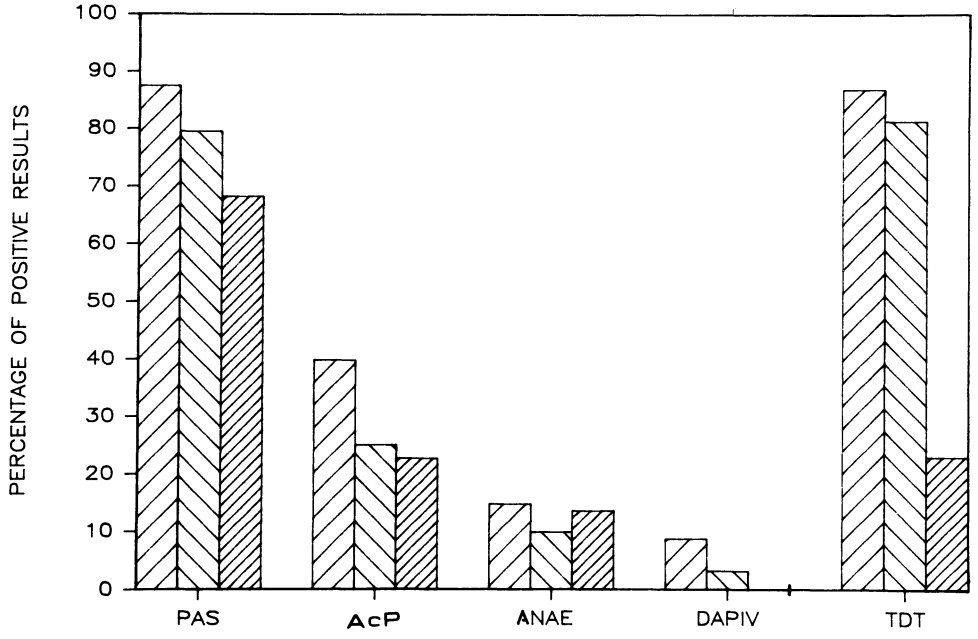

Fig. 2. Cytochemical patterns (percentage of positive results) of morphological subgroups (FAB classification) of patients with ALL or AUL. For abbreviations, see text. L1, ▨; L2, ▧; L3, ▨. *PAS:* L1 vs. L2 vs. L3, $p<0.05$; *AcP:* L1 vs L2, L3, $p<0.01$; *DAP IV:* L1 vs. L2 vs. L3, $p<0.05$; *TDT:* L3 vs. L1, L2, $p<0.001$

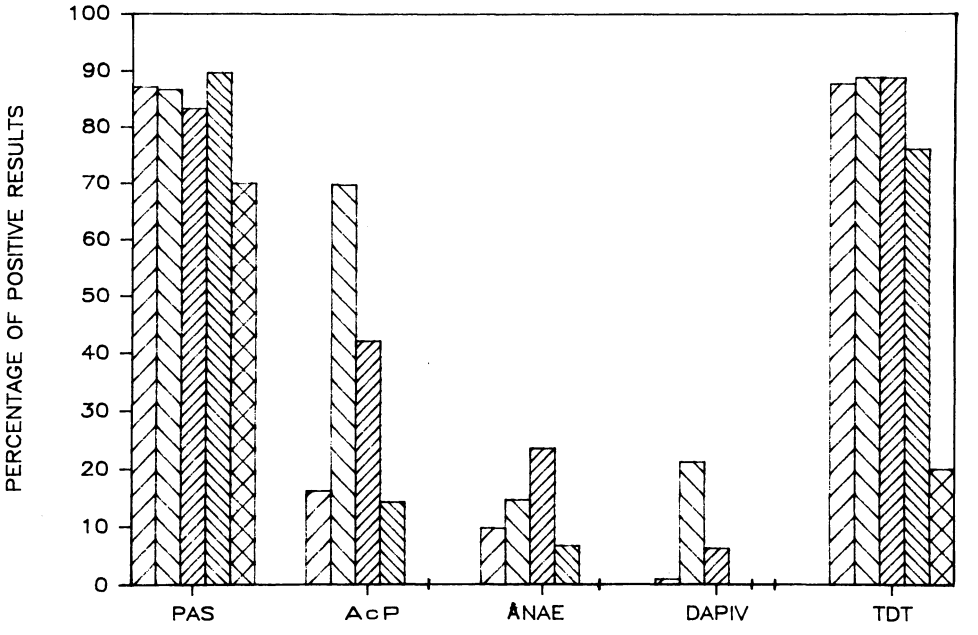

Fig. 3. Cytochemical patterns (percentage of positive results) of immunologic subgroups of patients with ALL or AUL. For abbreviations, see text. C/pB type, ▨; T type, ▧; pT type, ▨; O type, ▧; B type, ▨. *AcP:* T, pT vs. C/pB, O,B, $p<0.001$; *DAPIV:* T, pT vs. C/pB, O, B, $p<0.001$; *TDT:* C/pB, T, pT vs. O, $p<0.05$; *TDT:* O vs. B, $p<0.05$

morphology was not found within this category. The proportion of L3 morphology in the other immunologic subgroups ranged from 0.7% (C/pB type) to 6.3% (O type). The frequency of L1 morphology was highest in T types, which revealed a lower percentage of L2 morphology.

Cytochemical reactions of the different immunologic subgroups are indicated in Fig. 3. The main differences consisted of significantly higher percentages of AcP and DAP IV in T type and pT type as opposed to other immunologic subtypes ($p < 0.001$). In addition, the percentage of AcP positivity in T as compared with pT type was also significant ($p < 0.05$). While the AcP reaction was not completely restricted to ALL of T lineage, DAP IV positivity only occurred in T and pT type, with the exception of one patient whose leukemia had been classified as C/pB type immunologically. The higher percentage of ANAE positivity in the T and pT types as opposed to the C/pB and O types did not reach significance ($\chi^2 = 3.37$), nor did the lower percentage of PAS in the B type as compared with the remaining immunologic subgroups. One of five patients of B type exhibited TDT-positive blasts; immunologically, these blasts revealed rather immature B characteristics (surface immunoglobulin positive, cytoplasmic immunoglobulin negative, B antigen positive, CALLA positive).

In relation to the diagnostic subgroups of ALL and AUL, the former comprised more C/pB and T types, while the O and B types were represented to a considerably higher degree in the latter group.

AML Subgroups

In our study, the original FAB categories were used. FAB M4 Eo and M7 were not included at that time.

Frequenc distributions of the FAB subtypes were as follows: 106 patients, M1; 140 patients, M2; 14 patients, M3; 92 patients, M4; 44 patients, M5; and 11 patients, M6. While there was no difference in the percentage of relapse-free survival between the various subtypes, there is some indication of a worse prognosis for patients with the M5 subtype in the maintenance therapy arm; at the moment, however, the figures are too small for meaningful statistical evaluation.

Relapse-free survival of patients with the M2 subtype is significantly better if these patients are treated after obtaining complete remission, as compared with patients randomized to the observation arm.

Discussion

The objective of our study was to investigate the correlation of morphology (according to FAB criteria), cytochemistry, and immunologic classification using a battery of routinely available methods. The results of morphological characterization, which were supplemented by the evaluation of TdT activity in 476 adults with ALL or AUL being treated in the cooperative trial in the Federal Republic of Germany, were achieved independently of immunologic analysis.

Among the morphological classifications which have been proposed to define ALL, the FAB classification is now widely used. We applied the criteria originally described by Bennett et al. [2]. Regardless of the immunologic characterization, AUL was defined as an acute leukemia lacking any marker thought to identify AML or ALL positively: Thus, the PAS and focal AcP reactions had to be negative.

One of the interesting features is the correlation between L3 and the B phenotype. In our series B-ALL blasts were mostly, but not exclusively, of L3 morphology, since three out of ten cases with immunologically proven B-ALL belonged to the L2 category. Regarding all cases of L3 morphology, there was a considerable and statistically highly significant predominance of the B type, but other immune phenotypes were also present in this group: one C-All, three T-ALL, one pre-T-ALL, and five O-ALL. In L1 and L2 groups, C-ALL is the most frequent, and T-ALL is significantly correlated with L1, but this is not of clinical relevance.

Comparing FAB categories with cytochemical phenotypes, there is a significant correlation between AcP and L1 and between DAP IV and L1, which in both cases can be related to immunophenotypes with more statistical power and clinical relevance. In addition, we compared the cytochemical

patterns described earlier with the immunophenotypes determined in our study (see Thiel et al, this volume). There is a highly significant correlation between focal AcP and the T and, to a lesser degree, the pre-T phenotypes, compared with only a small proportion of C/pB and O-ALL disclosing this pattern of AcP reaction. The most specific, but least sensitive technique is the DAP IV method: With only one exception, it was exclusively positive in the T and pre-T-ALL cases. Since the first description of ALL with a focal AcP pattern and the clinical features of T-ALL [6] and the correlation to E-rosette–positive ALL [7] this relationship has been confirmed in several publications. It is correlated to earlier stages of T-cell development than the E-receptor–positive T-ALL.

DAP IV has been shown to be expressed by a proportion of T-lymphocytes at different stages of development belonging in most cases to the Tμ subpopulation [3]. It has been demonstrated in some cases of T-lymphoblastic lymphoma, as well as in T-ALL. Our investigations confirmed the value of this method, which is the most specific cytochemical technique for a subset of T-ALL. Preliminary statistical analysis of our data aiming at correlating morphological and cytochemical analysis to clinical data showed a significantly higher complete remission rate and a longer remission duration in cytochemically defined ALL compared with AUL (85% vs. 61%; median not reached vs. 17 months), independently of immune phenotype.

In the AML cooperative trial, the frequency distribution of the subtypes was in the range of other comparable studies, M2 being the most frequent subtype (140 of 407 patients).

At the moment, the only statistically significant result is related to the M2 subtype: Relapse-free survival of patients with this subtype is significantly better if they are treated after complete remission as compared with patients randomized to the observation arm.

Recently, several groups revealed M4 and M5 morphology as a negative prognostic factor in children and adults with AML [8, 9]. Others showed a higher relapse rate after allogeneic bone marrow transplantation or chemotherapy in these subtypes [10–12]. So far, it has not been able confirm this in our study, although there is a tendency for a worse prognosis for M5 leukemia with patients given maintenance therapy.

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Acute Myeloid Leukemia in Adults

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Therapeutic Strategies in Acute Myelocytic Leukemia: A Status Report of the Experience of CALGB*

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Introduction

This is a summary report on the progress made by Cancer and Leukemia Group B (CALGB) in the treatment of acute myelocytic leukemia (AML) in the adult during the past 10 years. CALGB Study 7421 [1] was initiated in April 1974 to determine through a large, randomized multiinstitutional trial whether intensive chemotherapy delivered with the aim of rapidly ablating the marrow will result in an increase in the incidence of complete remission (CR), as shown in a small single-institutional pilot study by Yates et al. [2]. The importance of achieving CR is shown in Fig. 1, which depicts the survival of 352 patients studied in CALGB 7421 [1]. Virtually all 162 Patients who failed to achieve a response died, 75% of them within 2 months and most others within a year. Those achieving a partial response fared only slightly better than the nonresponders. In contrast, the survival curve of 152 patients who achieved CR is significantly better than that of the two groups.

The objectives of our efforts during the past decade were the following:

1. To find methods of treatment which would increase the incidence of CR in AML
2. To find methods of treatment which would keep the patients in CR once CR was achieved

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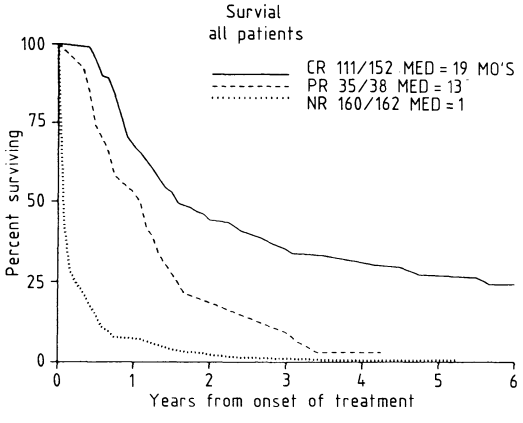

Fig. 1. Duration of survival according to the outcome of remission induction therapy in 352 patients with AML. CR, Complete remission; PR, partial remission; NR, no response. Number of patients at risk for each 1-year interval is as follows:

Year	0	1	2	3	4	5	6
CR	152	105	67	53	44	36	11
PR	38	21	7	5	1	0	0
NR	162	12	4	2	1	1	0

Remission Induction

The three successive trials, CALGB 7421 [1], 7521 [3], and 7721 [4], covering the time periods 1974–1975, 1975–1977, and 1977–1979 respectively, demonstrate the achievement of a relatively unchanging, yet moderately high incidence of CR during this 5-year time span. The subsequent studies, CALGB 7921 [5] and 8221 [6] have been analyzed only during the past few months, and these reports, now being finalized by Harvey Preisler and Robert Mayer respectively, demonstrate a substantial increase in the CR rate in the second 5-year time span.

In CALGB 7421 [1], we demonstrated that intensive chemotherapy consisting of continuous IV infusion of cytosine arabinoside (100 mg/m^2 per 24 h from days 1 to 7 and IV injection of daunorubicin (45 mg/m^2 per day) on days 1, 2, and 3 (this therapy is now well known as the standard "7-and-3 regimen") induced a CR in 56% of patients (59% in the group less than 60 years and 45% in the group aged 60 and over). This was a significantly higher incidence than the less aggressive 5-and-2 regimens. Thus, the 7-and-3 regimens contributed to the improvement in the mean rate of CR among all the 376 patients treated on study 7421. In studies 7521 [3] and 7721 [4], this approach of attempting a fast ablation of marrow with intensive induction therapy was refined to the extent that the results for a large number of patients in each of the three major age groups studied became predictably reproducible. These resuls are shown in Table 1.

These results on 1774, patients demonstrate an overall CR rate of 43%, which is almost twice as high as the CR rate we were able to achieve just a decade earlier [7]. The CR incidence initially remained at 57% for the age group under 40 and increased to 65% in study 7721. For the other age groups, those aged 40–59 and 60 and over, these rates remained very stable at about 43% and 25% respectively.

Although there have been several reports suggesting some benefit from the prophylactic use of cotrimoxazole in patients with acute leukemia by reducing the incidence of severe life-threatening septic complications during induction therapy, none of these was definitive [8]. Therefore, because the exact role of cotrimoxazole during initial chemotherapy in AML was unclear, it was decided to investigate this on large numbers of patients in a controlled clinical trial in 1979. In CALGB 7921 [5] we randomized patients prior to the initiation of induction chemotherapy either to receive cotrimoxazole or not to receive it. Each of these two arms included more than 320 patients. We found no difference in either the incidence of severe or life-threatening sepsis or in the CR incidence between the groups receiving or not receiving cotrimoxazole. The three chemotherapy arms (by random allocation) for remission induction phase of this study were the 7-and-3 regimen, TAD (thioguanine, cytosine arabinoside, daunorubicin), and the 10-and-3 regimen (10 days of cytosine arabinoside by continuous infusion). More than 210 patients were included in each of thse three regimens, and the overall CR incidence was almost constant for all groups at about 56%.

Finally, in the recently concluded study 8221 [6] in which the dose of cytosine arabinoside was increased to 200 mg/m^2 per day in the 7-and-3 regimen, 68% of 205 patients achieved a CR, including 85% of those aged under 40 years ($n=73$), 63% of those aged 40–60 years ($n=71$), and 52% of those aged 60 and over ($n=61$).

These results of the CALGB studies of AML (for all adults over the age of 18) clearly demonstrate that the overall incidence of CR has increased from 43%

Table 1. CALGB data on CR 1974–1979

Age group	<40 years		40–59 years		>60 years		Total all ages	
Study	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
7421	84/147	57	44/110	40	27/119	23	155/376	41
7521	142/249	57	91/208	44	63/229	28	296/686	43
7721	145/224	65	102/237	43	62/251	25	309/712	43

(achieved up to 1979) to the currently achieved levels of 68%. Although very high incidences of CR were reported [9, 10] several years ago by investigators involved in clinical trials at single institutions, the results of CALGB reported here represent a major increase in CR rates in large multiinstitutional, cooperative, randomized trials.

Duration of Remission

In studies 7421 [1], 7521 [3], and 7721 [4], we treated patients in the postremission induction phase with monthly courses of cytosine arabinoside given for 5 days along with the addition of another chemotherapeutic agent on a rotational schedule. This second drug was one of the following: 6-thioguanine, cyclophosphamide, daunorubicin, 1-(-2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) on a 4-month rotation in 7421, or 6-thioguanine, daunorubicin, or a combination of vincristine and prednisone in the other studies. The median durations of remission and of survival of 760 patients [11] observed over an 8-to-10-year period were 1.1 years and 1.6 years respectively. The largest proportion of relapses (and deaths) occurred during the first 2 years, approximately 41% in the 1st year and 30% in the 2nd year. However, relapses were seen continuously throughout 9 years of follow-up, although the percentage of relapses dropped to less than 10% during the 3rd and 4th year of remission in our study, in which the minimum follow-up period was 6 years. In study 7921, the maintenance therapy was randomly chosen either to be discontinued after 8 months of treatment or to be continued until relapse [5]. There were about 75 patients in each of these two arms neither of which show any significant difference in the duration of remission or of survival between these two regimens (about 1.4 years and about 2 years respectively).

The latest study, CALGB 8221 [6], which was a pilot to our currently active group-wide study 8525, showed that postremission induction phase consolidation therapy can be safely recommended with single-agent cytosine arabinoside in high doses, given for 5 days at monthly intervals. The maximum tolerated dose on intermittent dosage is 3 g/m^2 IV by infusion over a 3-hour period; a 12 h given on days 1, 3, and 5 (6 doses in 5 days); or 400 mg/m^2 per 24 h for 5 days by continuous infusion. Only 24 and 29 patients respectively following these two regimens are evaluable at this time. These early observations indicate that the median durations of remission and of survival have so far not been reached in either of these groups with a maximum follow-up of about 1 year.

In summary, prior to CALGB study 7421, the median duration of remission in CALGB studies was 1.5 years [1] with approximately 24% of patients in continuous CR at 5 years. With 760 patients in CR on long-term follow-up (minimum period of 6 years) in studies 7421, 7521, and 7721, the median duration of remission was 1.1 years with 22% in CR at 5 years [11]. The currently active studies of CALGB are still ongoing, but the initial evaluations at this time strongly suggest that early consolidation therapy in the immediate postremission induction period seems to prevent early relapses.

The progress witnessed by CALGB both in remission induction and in prolongation of remission in AML in adults reflects that achieved by other institutions around the world as well. This progress offers a significantly better outlook for these patients in 1986 than was the case in 1976.

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The Ninth British Medical Research Council Trial for the Treatment of Acute Myeloid Leukaemia

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The Ninth British Medical Research Council trial for the treatment of acute myeloid leukaemia (AML) opened in February 1984 to all patients with primary or secondary forms of the disease; there was no age limit. Patients were randomised to receive *either* a 1+5 DAT combination (daunorubicin 50 mg/m^2 i.v. on day 1, cytosine arabinoside 100 mg/m^2 i.v. every 12 h on days 1–5, and 6-thioguanine 100 mg/m^2 every 12 h on days 1–5) *or* a 3+10 DAT regime (daunorubicin at the same dose on days 1, 3, and 5 and cytosine arabinoside and 6-thioguanine again at the same doses as in the 1+5 combination but on days 1–10).

Following remission, patients were randomised for a second time to

either	2		C		2		C
	+	►	O	►	+	►	O
	7		A		7		A
	DAT		P		DAT		P
or	2		M		2		M
	+	►	A	►	+	►	A
	7		Z		7		Z
	DAT		E		DAT		E

COAP consists of cyclophosphamide 600 mg/m^2 i.v. on day 1, Oncovin 1.5 m^2 i.v. on day 1, Ara-C 100 mg/m^2 i.v./s.c. on days 1–5, and prednisolone 60 mg/m^2 orally on days 1–5.

MAZE consists of m-AMSA 100 mg/m^2 i.v. on days 1–5, 5-azacytidine 100 mg/m^2 i.v. on days 1–5 and etoposide (VP-16) 100 mg/m^2 i.v. on days 1–5.

The interval between consolidation courses in the COAP arm is 21–28 days. In the MAZE arm the intervals are longer – generally between 28 and 40 days.

Patients who are to receive either an allogeneic or autologous bone marrow transplant (BMT) are not randomised for consolidation therapy.

When consolidation therapy is complete, patients are randomised a final time between maintenance therapy (eight courses of cytosine arabinoside and 6-thioguanine every 12 h for 5 days each month followed by four courses of COAP) and stopping all treatment.

In the 2 years up to the end of January 1986, 441 patients were accepted into the trial; 14 additional patients were deemed ineligible because of an incorrect diagnosis. The median age is 54 years. Although paediatric cases are accepted into the study, very few have been entered (seven cases) because a separate study has been devised for the management of paediatric AML in Britain with which the majority of centres are collaborating.

Table 1. Remission rates by age group

Age group	All cases		De novo cases[a]	
	(*n*)	(%)	(*n*)	(%)
0–39	79/ 98	81	78/ 94	83
40–59	76/119	64	73/113	65
60+	59/133	44	52/114	46

[a] Excluding patients with secondary leukemias.

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Table 2. Remission rates age group/therapy

Age group	Treatment A		Treatment B		All cases	
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
0–39	40/46	87	33/46	72	73/ 92	79
40–59	34/58	59	40/59	68	74/117	63
60+	28/64	44	29/67	43	57/131	44

Table 3. MRC AML 9: Supportive care to remission or death

	No. evaluable patients	Mean	Range	Median
Units blood	190	16.2	0–107	14
Units platelets	188	66	0–347	48
No. days i.v. antibiotics	190	22	0/ 79	19

Table 4. MRC AML 9: Supportive care to remission or death

	1+5	3+10	
Units blood	17.3	13.9	$p=0.05$
Units platelets	71	59	$p=0.17$
No. days IV antibiotics	24.8	18.4	$p=0.005$

This report gives the preliminary results on the first 350 patients in the study for whom there has been an adequate period of follow-up. Among this group are 29 patients with secondary leukaemia. The overall remission rate is 61% (214/350). The remission rate for the cases of AML arising de novo is 63% (203/321), and 11/29 (38%) of the patients with secondary leukaemias achieved complete remission. The remission rates by age group are shown in Table 1.

There is no significant difference between the remission rates among the patients receiving 1+5 DAT compared with the 3+10 DAT as shown in Table 2, which excludes ten patients who were not randomised at the beginning of the trial but were given 3+10 DAT – all entered complete remission.

The average number of courses of the 1+5 regime required to achieve complete remission was 2.5 after an average of 46 days in hospital. For those patients receiving the 3+10 combination, the average number required was 1.3 after an average period of 38 days in hospital.

The amount of supportive care required during induction therapy has been calculated for the first 190 patients and a comparison made between patients receiving the different forms of induction (Tables 3 and 4).

The reasons for failure to enter remission were classified according to the following categories:

A. Inadequate trial. Patient dies during or less than seven days after completing the first course of therapy.

Table 5. Failure type by age/treatment group

Age group	1+5							3+10						
	A	B	C	D	E	F	Total	A	B	C	D	E	F	Total
0–39		2		6	3	2	13	2			2	1		5
40–59	2	2	3	2	6	3	18	6	1	6	7	1	3	24
60+	6	1	10	4	11	3	35	12	1	10	1	6	5	35
							66							64

B. Marrow hypocellularity attained but regenerating population consists predominantly of blast cells.

C. Marrow hypocellularity with no peripheral blood blasts attained but patient dies during the hypoplastic period from haemorrhage or infection.

D. Decrease in bone marrow blast cell population to 10%–15% (partial remission).

E. Failure of therapy to achieve any or significant effect on the marrow blast cell poplation.

F. Any other course of events not covered by A–E.

Table 5 shows that the number of patients classified as having refractory disease is, as one might expect, lower among those reveiving the more intensive treatment. Consolidation therapy was designed to be intensive, but unfortunately this has produced irreversible myelosuppression in 21 patients who have died in remission of haemorrhage or infection; the majority were over the age of 60.

The period of follow-up is at the moment too short – the longest is 2 years – for any conclusions to be reached on the relative values of the two forms of induction and consolidation therapy in maintaining long-term remissions and survival.

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Long-Term Results of Two Swiss AML Studies*

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Summary

The Swiss group for clinical cancer research (SAKK) completed two first line protocols for the treatment of acute myelogenous leukemia (AML). In the first protocol (SAKK AML 74, from August 1974 to April 1977; 107 patients) the effectiveness of immunization with viral oncolysate during maintenance treatment was tested. After succesful induction treatment, the patients were randomized in two groups: Group A: monthly maintenance chemotherapy for 2 years and group A+IT, which received the same maintenance chemotherapy regimen plus, on day 15, injection of viral oncolysate. Of the 107 patients, 57 (53%) achieved complete remission, 29 then being randomized to group A, 28 to group A+IT. As of August 1984, there is no statistical difference between the survival times of the two groups ($p=0.288$). Ten years after the start of the study there is still no clear plateau of the survival curve. Nine percent of all patients (18% of the remission patients) are alive today. With the treatment of the mid-seventies, therefore, the cure of a patient suffering from AML was a rare event.

The second protocol (SAKK AML 77, from April 1977 to April 1982; 162 patients) was designed to evaluate the usefulness of a prolonged maintenance treatment after early consolidation. The 74 patients who were still in remission after early consolidation treatment were assigned to either maintenance chemotherapy or to observation only. At 3.5 years after the last patient's entry there was no difference between the groups in duration of survival ($p=0.332$). Patients above 40 years of age survived longer after early consolidation (median 4 years) than did patients aged 40 and below (median 1.75 years, $p=0.0001$).

At 4.5 years the survival curves of both protocols meet. Both studies show a similar proportion of long-term survivors. The conclusions drawn a few years ago hold up: Viral oncolysate does not prolong survival; maintenance chemotherapy after early consolidation treatment does not prolong survival either.

Introduction

Results of treatments of patients suffering from acute myelogenous leukemia (AML) are mostly published after a medium observation time of a few years. The usual Kaplan-Meier presentation [1] of survival curves then shows plateaus at different levels depending on the observation time. Of course these plateaus do not represent the percentage of cured AML patients, since every late relapse lowers the plateau. We therefore thought it important to analyze our AML studies 10 and 8.3 years after their implementation to better clarify the curability of AML with modern cytotoxic chemotherapy. At the same time we would be able to judge long-term effects of our immunologi-

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cal manipulation with viral oncolysate and of early consolidation with or without maintenance treatment.

Patients and Methods

First Protocol (SAKK AML 74)

From August 1974 to April 1977, previously untreated patients suffering from AML were admitted to this study irrespective of age provided they had never received any of the drugs prescribed in this study. One-hundred seven patients were adequately documented according to the protocol. Sixteen patients who were announced for the protocol but then were either not treated according to the treatment plan or insufficiently documented could not be evaluated. The characteristics of the 57 patients entering the randomized maintenance study described below were the following: Median age 41 (2–76) years, 27 women, 30 men. The cut off for study analysis was August 1984. Induction treatment consisted of cytosine arabinoside (Ara-C) continuously administered intravenously for 7 days at 100 mg/m^2 and daunorubicine at a dose of 45 mg/m^2 by direct intravenous injection on days 1, 2, and 3. If complete remission as defined by Ellison et al. [2] was achieved, the patients were randomized in one of the two maintenance regimens (A or A+IT) as described later. If complete remission was not achieved, a second course of induction treatment reduced to 5 days of Ara-C and 2 days of daunorubicine was given. Those patients achieving complete remission now entered the maintenance protocol as well.

Maintenance treatment (group A) consisted of 5-day courses every 4 weeks of Ara-C 100 mg/m^2 i.v. every 12 h. In each course one of the following drugs was added: 6-thioguanine (200 mg/m^2 per os per day for 5 days), cyclophosphamide (1000 mg/m^2 i.v. on day 1), CCNU (75 mg/m^2 per os on day 1), daunorubicine (45 mg/m^2 i.v. on days 1 and 2). This 4-month cycle was repeated five times or until relapse. The patients randomized to group A+IT received the same chemotherapy. In addition, on day 15 of each 4-week cycle, 1.0 ml viral oncolysate was injected as described elsewhere in detail [3].

Second Protocol (SAAK AML 77)

During the period of this study (April 1977–April 1982) previously untreated patients with AML were admitted to the study if their AML met the criteria of the French-American-British (FAB) classification [4] and if they were aged under 65 years. Of the 195 patients admitted, 33 were not evaluable because of protocol deviations. The 162 evaluable patients consisted of 96 (59%) women and 66 (41%) men. Their median age was 43 (range 7–65 years). The last study analysis was done in August 1985. A summary of induction and early consolidation treatment is shown in Table 1. Patients who successfully completed their last consolidation course were randomized to maintenance treatment (for details see [5]) or observation only.

Statistical Evaluation

The duration of survival was measured from time of diagnosis of AML. The probability of staying alive was calculated according to the Kaplan-Meier method [1]. Survival curves were compared by the use of the log-rank test [6]. To test the influence of factors

Table 1. Induction and early consolidation treatment of protocol SAKK AML 77

Drug	Dosage mg/m^2/day	Route	Day course 1, 3, 4	Day course 2
Cytosine arabinoside	100	Continuous i.v. infusion	1–7	1–5
Daunorubicine	45	i.v.	1, 2, 3	1,2
Vincristine	0.8	i.v.	10	8

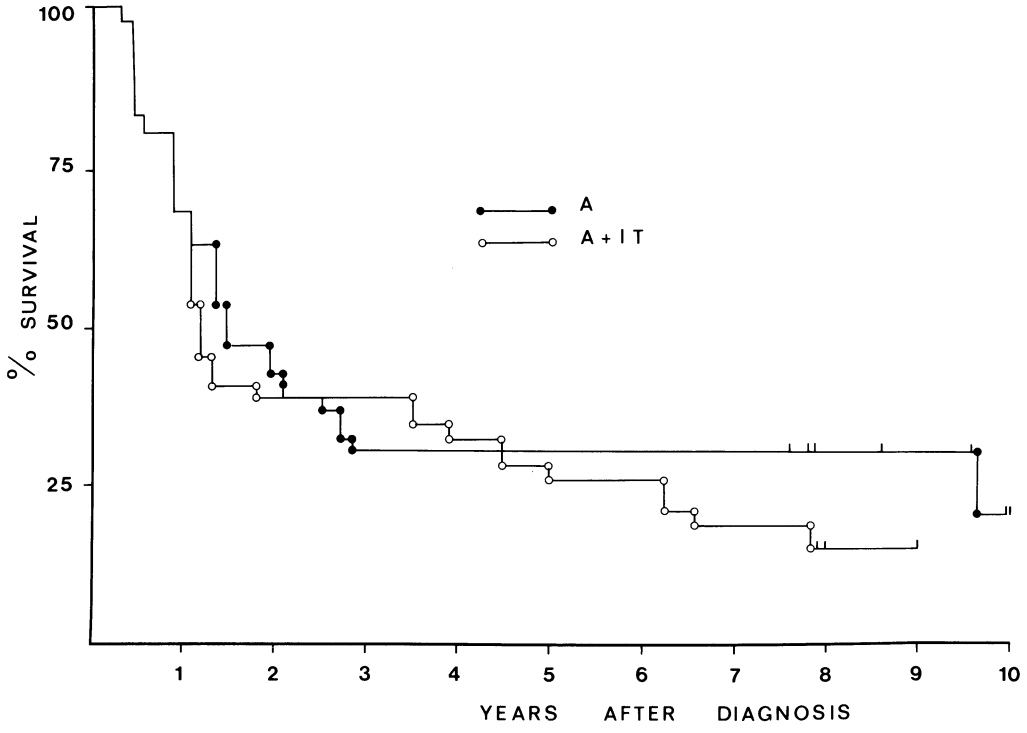

Fig. 1. Survival of remission patients from SAKK AML 74. *A*, patients with maintenance chemotherapy; *A+IT*, patients with maintenance chemotherapy plus viral oncolysate

Table 2. Results of the induction treatment of protocol SAKK AML 77

Patients entered	195	
Patients evaluable	162	
Bone marrow remission	117 (72%)	
– Early relapse		25 (15%)
– Off protocoll in remission before randomization to maintenance or observation (bone marrow transplantation, protocol deviation)		18 (11%)
– Reaching randomization for maintenance or observation		74 (46%)

such as sex and age on survival, these variables were used as covariants in a proportional hazard model. Calculations were done with the BMDP2L program.

Results

Protocol SAKK AML 74

The results of the induction treatment were as follows: Of the 107 evaluable patients, 57 (53%) achieved a complete remission (CR). All patients not achieving a CR died within 1 year of diagnosis. The survival of the 57 CR patients is shown below in Fig. 5. Ten patients (18% of the CR patients or 9% of all evaluable patients) are alive as of August 1, 1984. The last death due to AML relapse occurred 9.5 years after diagnosis. Of the 57 patients in CR, 29 were randomized to group A maintenance treatment, 28 to group A+IT. The survival of the two groups is shown in Fig. 1. There is no statistical difference between the two survival curves ($p=0.288$).

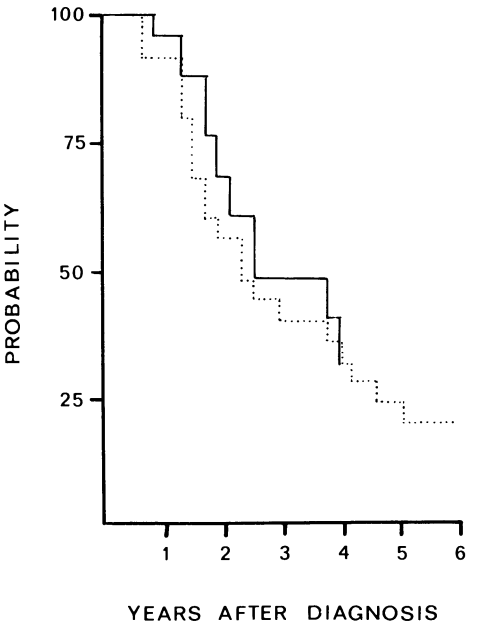

Fig. 2. Survival of remission patients from SAKK AML 77. *Continuous line* indicates observation (26 patients), and *dotted line* indicates maintenance chemotherapy (48 patients)

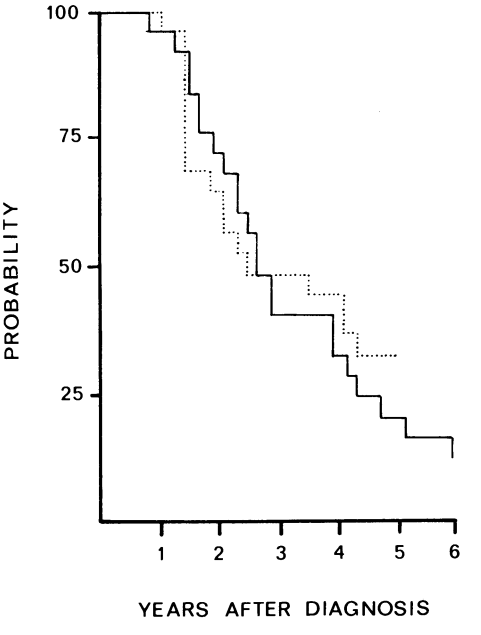

Fig. 3. Survival of remission patients from SAKK AML 77: Number of inductions to achieve remission. *Continuous line* indicates bone marrow remission only after more than one induction cycle, and *dotted line* indicates bone marrow remission after first induction cycle

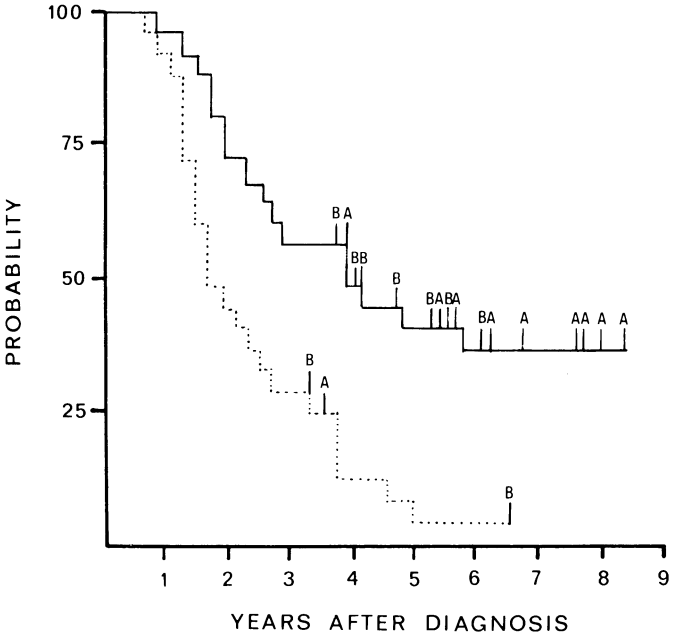

Fig. 4. Survival of remission patients from SAKK AML 77. *Continuous line* indicates patients aged 40–65 years (41 patients), and *dotted line* indicates patients aged less than 40 years (33 patients). *A*, maintenance; *B*, observation

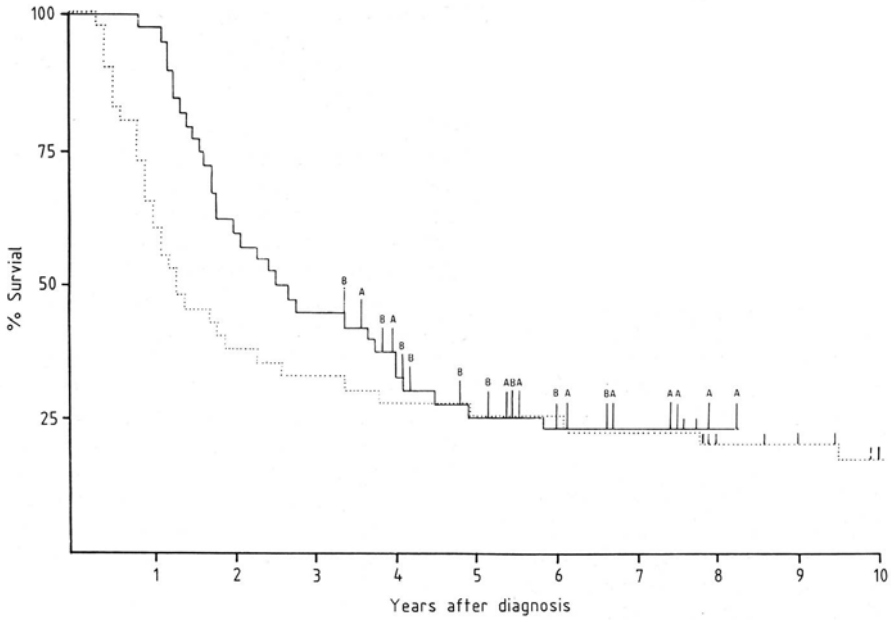

Fig. 5. Comparison of survival of remission patients of SAKK AML 74 *(dotted line)* and SAKK AML 77 *(continuous line)*. *A*, maintenance; *B*, observation

Protocol SAKK AML 77

The results of the induction treatment are shown in Table 2. Eighty-three percent of the patients under 40 and 65% of the patients above 40 ($p=0.011$) achieved bone marrow remission. All patients not achieving CR or relapsing during consolidation died within 1.5 years of diagnosis. The survival data are presented in Figs. 2–5. For both groups (maintenance and observation) the median survival time was 2.5 years. There was no difference with respect to long-term survival ($p=0.332$; Figs. 2 and 5). Covariance analysis of the influence of sex ($p=0.543$), number of induction treatment courses to achieve remission ($p=0.385$; Fig. 3), and age (<40 years vs. >40) showed that only age significantly affected prognosis ($p=0.0001$; Fig. 4).

Discussion

Cytosine arabinoside (Ara-C) and daunorubicine (DNR), both introduced in the 1960s, remain the main drugs used for the treatment of AML. The mode of application (Ara-C 100 mg/m^2 continuous infusion over 7 days and DNR 45 mg/m^2 i.v. on days 1, 2, and 3) has remained the standard induction treatment for 10 years now. The strategy, however, has changed today. Early consolidation with the same drugs is more the rule [7], while prolonged maintenance treatment is not routinely applied any more [5, 8]. Comparing the survival curve of the CR patients in the study SAKK AML 74 with the one of the successive Swiss AML study (SAKK AML 77) where early consolidation was applied, the main difference was during the first 4.5 years: Median survival was little more than 1 year in the study of 1974 vs. 2.5 years in the successive one. The 5-year survival, however, according to a Kaplan-Meier estimate [1], is about the same with 25% survivors (Fig. 5). The chances of AML patients achieving a long-term remission (except perhaps for the rare patient who gets a bone marrow transplantation) might therefore in the mid-eighties be the same as they were in the mid-seventies.

Figure 5 shows that even at 9 years there is no plateau. Relapses between 4 and 7 years are well known [9]. Therefore, we hesitate to state that the 9% survivors of our study of 1974 are cured. We prefer to call them, for the time being, long-term re-

mission patients. Compared to the Swiss results of the early 1970s, when only 0.7% survivors were observed at the end of 6 years [10], the treatment of the mid-seventies definitely improved survival of AML patients in Switzerland.

To improve long-term survival many "immunotherapy" trials were implemented in the 1970s [11]. Our study of 1974, which for the first time took advantage of the immunopotentiating effect of myxoviruses [12] in a randomized trial, did not show any beneficial effect of the immunization by viral oncolysates. As with all immunotherapeutic trials, two main questions have to be asked: 1. Were the patients immunocompetent? 2. Were tumor-associated antigens (TAA) present in the immunizing material? The answer to the first question is certainly yes, since delayed skin reactions [3] and anti-FPV antibodies [13] could be observed. The immunization with respect to viral antigens was successful. There was an inverse correlation between the production rate of anti-FPV antibodies and prognosis [14]. The answer to the second question could well be no. In animal experiments, antiviral antibodies parallel tumor immunity [15], which apparently was not the case in the present study. This could mean that there were no TAA in the allogeneic immunizing material. TAA might therefore be specific – if present at all – for each individual AML patient. Studies using autologous viral oncolysate would be interesting, but technically difficult to realize.

The analysis of August 1985 of the study SAKK AML 77 produced similar results as in February 1983 [5]. The median remission times of the maintained and unmaintained groups are both around 18 months, a favorable result compared to the recently published German study. Here the median remission time of the maintained group is 13 months versus 8 months for the observation group [16]. Two years of maintenance treatment did not affect survival as shown in Fig. 2.

The conclusions of these two studies are:

1. Immunization with viral oncolysate does not prolong survival. The rate of antiviral antibody production is a prognostic indicator.
2. Early consolidation increased the median survival time of remission patients from 1.3 years (SAKK AML 74) to 2.5 years (SAKK AML 77).
3. After the first induction cycle the evaluation of the success is too early. About one-half of the prospective remission patients reach a normal bone marrow only after the second induction cycle [5].
4. After early consolidation AML patients do not benefit from conventional maintenance treatment.
5. Patients up to 65 years should be treated as aggressively as younger patients. Survival of CR patients above 40 is significantly longer then below 40.
6. The long-term results of these two studies with about 10% survivors call certainly for new treatment strategies in order to improve the prospects for the mid-nineties.

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Prediction of Induction and Duration of Complete Remission in Acute Myelogenous Leukemia: Value of Clonogenic Cell Properties

R. Zittoun, J.P. Marie, D. Brilhante, and A. Delmer[1]

Introduction

The in vitro clonogenic assay in acute myelogenous leukemia (AML) has brought the possibility to study the proliferative and differentiating capacity of the clonogenic cells as well as their in vitro sensitivity to antileukemic drugs. Although these clonogenic cells do not represent the true leukemic stem cells, their properties of colony formation and in vitro self-renewal have been considered to reflect the characteristics of the leukemic proliferation and be correlated with the outcome of the disease [8].

The in vitro evaluation of anticancer drugs using the human tumor clonogenic assay has been widely applied over the past 15 years [15] and is proposed for screening of new drugs, in vitro phase II studies, as well as drug selection in individual patients. However, such selection is hardly feasible in most untreated acute leukemic patients, due to the necessity of starting chemotherapy in less time than that required for the in vitro assay. Therefore, the clonogenic assay must at the moment be considered mainly in AML for its prognostic value.

Several studies have shown that the in vitro sensitivity of clonogenic leukemic cells correlates with the outcome of the remission induction treatment [1, 3, 6, 9, 11]. However the practical value of such a correlation is still questioned [13] and only McCulloch et al. [8] have attempted to integrate the clonogenic assay among the various clinical and biological parameters which have been shown to be of prognostic importance in AML [4, 5, 12, 16]. Moreover, the recent introduction of the clonogenic assay precluded the attempt to assess its prognostic value for remission duration. We had confirmed previously the prognostic value of the clonogenic assay in AML for remission induction [7]. In the present work we have attempted to study the place of the clonogenic assay among other clinical and biologic variables for remission induction through a multivariate analysis, and to look for a possible role of clonogenic cell properties for the prediction of remission duration.

Patients and Methods

Ninety-one AML patients (77 not previously treated and 14 in relapse) have been studied. Clonogenic assay was performed as previously described [7]. Briefly, bone marrow blast cells were separated using density 1077 MSL (Eurobio). T cell depletion was performed by rosetting with sheep erythrocyte. 3×10^6 cells were incubated in alpha medium containing 10% FCS for 30 min with 10^{-7}, 10^{-6}, and 10^{-5} M daunorubicin (DNR), pelleted, washed twice in alpha medium, and plated. For cytosine arabinoside (Ara-C), continuous exposure to 10^{-7}, 10^{-6}, and 10^{-5} M was realized by including the drug to the culture medium. Cells incubated with both DNR (10^{-6} M/30 min) and Ara-C (10^{-7} M continuous exposure) were also plated. For measurement of the suicide index, cells were incubated for 20 min with 3H-thymidine (0.1 mCi, 25 Ci/mmol, Amersham Lab), washed with cold thy-

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midine, and plated. A total of 2×10^4 T-depleted cells in 1 ml alpha medium were plated with methylcellulose (0.8%), 20% FCS, and 10% PHA-leucocyte conditioned medium, in 1 ml microwells (Titertek Lab), with a minimum of 4 microwells for each drug concentration or control, and incubated in a moist atmosphere with 6% carbon dioxide. Aggregates greater than 20 cells were counted in the control microwells after 7 days (plating efficiency 1, or PE1), and colonies were pooled for replating under the same conditions to study the self-renewal (plating efficiency 2, or PE2). Drug inhibition was assessed at day 7 and expressed as a percentage of control.

The following biological studies have been also performed: measurement of the S-phase fraction using a cytofluorograph equipment, in vitro inhibition by DNR (10^{-6} and 10^{-7} M) and Ara-C (10^{-5} to 10^{-10} M) of 3H-thymidine uptake in all blast cells, in vitro growth on agar medium using placenta conditioned medium ("CFU-GM"). Cytogenetic analysis was not considered for this analysis for practical reasons.

The first eight patients were treated according to the EORTC AML 5 protocol with adriamycine 50 mg/m^2 on day 1, vincristine 1 mg/m^2 on day 2, and Ara-C 80 mg/m^2 q 12 h on days 3–9, with five patients achieving a CR.

Most patients (62) were treated according to the EORTC AML 6 protocol with one or two cycles of DNR: 45 mg/m^2 on days 1–3, vincristine: 1 mg/m^2 on day 2, and Ara-C: 200 mg/m^2 on days 1–7 (half by continuous i.v. infusion and half by i.v. push every 12 h 33 patients achieved a CR. Four patients aged more than 65 years received only 30 mg/m^2 DNR days 1–3 (AML 7 protocol) with two achieving a CR.

Eight patients were treated according to the AML 8 pilot study with DRN 45 mg/m^2 on days 1–3 and Ara-C 200 mg/m^2 i.v. continuous infusion on days 1–7, with five achieving a CR. Finally eight relapsing patients received an induction treatment with high dose Ara-C 2 g/m^2 q 12 h on days 1–6 and AMSA 120 mg/m^2 on days 5–7, with six patients achieving a CR.

The correlation between single parameters and induction treatment outcome was obtained through chi-square method or comparison of means using the t test. Multivariate analysis was performed using the Cox's stepwise logistic regression model (BMDP program). Comparison of remission duration curves was made using Kaplan-Meier's method.

Results

The main clinical and biological single variables which appeared to have a significant prognostic value at a minimum of $p < 0.1$ are listed in Tables 1 and 2. One can see that the main clinical parameters are age and secondary leukemia, and the main cytological parameters are the number of circulating blast cells – or the WBC count as well – and the presence of Auer rods.

The in vitro growth using a CFU-GM assay with placenta conditioned medium had a borderline value. The in vitro inhibition of leukemic clonogenic cells correlated with clinical sensitivity to antileukemic combination (CR versus resistance) at the $p < 0.01$ for Ara-C 10^{-5} M, at the $p < 0.1$ for DNR 10^{-6} M, and at $p < 0.01$ for DNR 10^{-6} M + Ara-C 10^{-7} M.

All other single variables were not significant for induction treatment outcome: sex, hemoglobin, FAB subtype, LDH, fibrinogen, S-phase fraction, in vitro inhibition of 3H-thymidine uptake by DNR and Ara-C, PE1, PE2, suicide index, and in vitro inhibition of leukemic clonogenic cells by other concentrations of DNR and Ara-C.

The prognostic value of the main clinical and biological factors was studied using a stepwise logistic regression. The value of the index proposed by Keating et al. [5] was confirmed ($p = 0.011$); Auer rods and the number of circulating blast cells did not add to this value, but the fibrinogen level appeared as a supplementary prognostic factor at a second rank ($p = 0.024$). The in vitro sensitivity to Ara-C 10^{-5} M had more value ($p = 0.005$) than the sensitivity to DNR ($p = 0.026$). By combining the index of Keating et al. (KI) and the clonogenic assay, both appeared as important and independent prognostic variable with the KI sorting first when looking at distinction between CR and resistance + death (KI = 0.001, sensitivity to Ara-C + DNR = 0.016), while the vitro sensitivity

Table 1. Prognostic value of main significant single clinical or biological factors and of the Keating's index for the remission induction outcome

	n	CR	Resistant disease	Death during induction	*p*
Age	91	44±16	51±19	63± 9	<0.001
Auer rods	80 < +	29	15	2	<0.01
	80 < −	12	10	12	
Number of circulating blast cells ($\times 10^3/\mu l$)	86	27±49	42±48	67±75	<0.05
Secondary Leukemia	91 < yes	4	9	3	<0.1
	91 < no	41	21	13	
CFU-GM	84 < nl or 0	21	17	4	<0.1
	84 < micro or macro Cl.	19	11	12	
Keating's index (%)	74	60±22	47±23	37±22	<0.01

Table 2. Prognostic value of clonogenic assay for CR induction

	n	CR	Resistant disease	*p*
CFU-L inhibition by				
Ara-C 10^{-5} M	71	62.7±31	43±29	<0.01
DNR 10^{-6} M	59	74.7±27	62±22	<0.1
Ara-C 10^{-7} M + DNR 10^{-6} M	79	79 ±25	62±19	<0.01

sorted first when looking at difference between CR and resistant disease. The model which fitted best for probability of CR versus resistance corresponded to the logistic regression equation:

$$\log e \frac{p}{1-p} = (0.053 \times CA) + (0.045 \times KI) - 6, \quad (1)$$

where CA is the in vitro simultaneous sensitivity to both DNR and Ara-C on clonogenic assay and KI the index defined by Keating et al.

The prognostic value of the clinical and biological factors studied for the remission duration is shown in Table 3. Although the number of patients is relatively low in this series, one can see that the clonogenic cell properties were the only ones which correlated with CR duration, when comparing patients who relapsed within 8 months, i.e., the median duration of the whole series, with patients with CR longer than 8 months: early relapses were characterized by a higher suicide index of the clonogenic cells, a paradoxical higher in vitro sensitivity to Ara-C 10^{-7} M, a lower in vitro sensitivity to DNR, and a higher in vitro self-renewal (PE^2). A significant correlation was observed between suicide index and in vitro sensitivity to Ara-C 10^{-7} M ($r=0.65$, $p<0.02$). All other single parameters had no significant prog-

Table 3. Prognosis of CR duration

	n	CR > 8 months	CR < 8 months	*p*
PE^2	14	9±11	37±32	<0.05
Suicide index	19	21±17	39±23	=0.05
CFU-L inhibition by Ara-C 10^{-7} M	35	27±23	48±25	<0.05
CFU-L inhibition by DNR 10^{-6} M[a]	20	81±26	58±25	<0.05

[a] In patients treated with 3 days' DNR + 7 days' Ara-C.

nostic value for remission duration age, hemoglobin, number of circulating blast cells, LDH, fibrinogen, Auer rods, S-phase ratio, number of courses to CR, and in vitro inhibition of clonogenic leukemic cells by other concentrations of DNR and Ara-C.

Discussion

Our study confirms the prognostic value of the in vitro sensitivity of the leukemic clonogenic cells to the antileukemic drugs currently used for remission induction. As emphasized in a previous study, the multivariate analysis indicates a best fit when both anthracyclines and Ara-C are combined in the in vitro test system, with short exposure to anthracyclines and continuous exposure to Ara-C [6, 11].

This sensitivity appears to be independent of the other variables with known prognostic value for CR induction. Our data show that, contrarily to recent statements [14], clonogenic assay can be actually considered as of major prognostic importance for both remission induction and duration. The relative importance of other biological determinants, such as drug uptake or cytogenetics, must await further studies.

The practical interest of the in vitro sensitivity for remission induction remains, however, open to discussion in AML: in most patients, treatment must be started before the results of the clonogenic assay are available. The possibilities which could be examined in the future are related to the simultaneous study of the in vitro response to other antileukemic agents (i.e., AMSA, mitoxantrone, VP-16 213, etc.). One could hope, when looking by day 7 both at the results of the clonogenic assay and the bone marrow cellularity at that moment [14], to identify the patients more likely to resist the first conventional combination and the drugs which could be combined for an immediate second step induction treatment.

Our study also shows that the properties of the leukemic clonogenic cells could be of paramount importance for the prognosis of remission duration. The in vitro self-renewal capacity, as indicated by the second plating efficiency, has been related to the proliferative capacity of leukemic stem cells [10]. A high suicide index of the CFU-L, as well as a high sensitivity to Ara-C 10^{-7} M – two data which have been correlated in our study – indicates that a high percentage of clonogenic cells are in S phase and that most of these cells are actively dividing. Such conditions have been frequently correlated with a high rate of early relapses, perhaps through an increased occurrence of mutations and acquired drug resistance.

On the other hand, a high in vitro sensitivity of CFU-L to DNR correlated with longer duration of CR. In another multivariate analysis of factors associated with outcome of treatment in AML, the remission duration was correlated with the total dose of DNR administered during remission induction [16], highlighting the contribution of anthracyclines in leukemic cell kill during induction and consolidation.

Our results differ from those reported by McCulloch et al. [8]. While our methods of clonogenic assay were identical – and differed from those utilized by other authors [3, 6, 11, 12] – the in vitro drug sensitivity correlated significantly in our study with remission induction or clinical resistance; on the other hand the self-renewal correlated for McCulloch both with remission induction and overall survival, whereas its prognostic value was limited in our study to remission duration.

Finally, it is interesting to point out that contrarily to recent statements, some in vitro assays which had been shown in the past as significantly correlated with the outcome of AML, i.e., the S-phase ratio for remission induction and duration, and the inhibition of 3H-thymidine uptake for CR induction [2, 16], had no prognostic value in our present study. This shows that the prognostic value of most clinical and biological factors can vary with time, depending especially on the efficacy of the treatment protocols. Moreover, it constitutes a major argument in favor of considering the clonogenic leukemic cells as a subset of major biological importance and as the key target, rather than the bulk of leukemic blast cells.

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Remission Induction and Maintenance Modalities in Acute Myeloid Leukemia: A Multicenter Randomized Study

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Introduction

While both remission rates and duration have improved in acute myeloid leukemia (AML), the overall prognosis remains poor. Large multicenter studies using DAT or DAT-derived remission induction regimens have failed to reproduce the complete remission (CR) rates (75%–85%) obtained in small single-institution studies [1–3]. Maintenance therapy has been demonstrated to improve CR duration [4, 5]; yet ultimately, less than 20% of all treated patients will achieve long-term disease-free survival (DFS) [6–8]. Furthermore, modalities of maintenance regimens remain debated: while both early intensification and semi-continuous maintenance therapy have improved DFS in large studies [4, 8, 9], they carry drug-related lethal toxicity in 5%–10% of patients [8–11]. Prognostic factors that could affect both indications and modalities of therapy are still poorly understood in AML, thus preventing the design of more specific treatment.

In previous studies we failed to show any benefit of three- and four-drug regimens over Ara-C-zorubicin combination therapy [12]. Thus, the Leukemia Group of the French Society of Hematology undertook in 1981 a study in order to:

1. Improve CR rates in AML using high-dose zorubicin and Ara-C combination chemotherapy, since previous studies and experimental data [13] strongly suggested a dose-effect relationship in tumor burden reduction achieved with anthracyclins. Zorubicin was chosen because of its improved therapeutic index over daunorubicin (DNR) [13]. The possible benefit of the addition of cyclophosphamide (CPA) over this two-drug regimen – suggested by results of allogeneic bone marrow transplantation – was also studied.
2. Define better maintenance regimens.
3. Define some prognostic indicators in AML.

Preliminary results of this study involving 17 departments of hematology are reported.

Patients and Methods

Patients

From September 1981 to December 1985, 444 patients were enrolled in the 01AM81 protocol. To be eligible, patients had to be between 01 and 65 years old; diagnosis of de novo AML according to FAB classification had to be confirmed by the Cytology Group of the French Society of Hematology: Patients with M3 leukemia were treated according to specific modalities and thus not included in the 01AM81 protocol. Patient characteristics are shown in Table 1.

Remission Induction Modalities

Patients were randomized to receive Ara-C 200 mg/m^2/day for 7 days given as continuous infusion and RBZ 200 mg/m^2/day for 4 days given as 1 h infusion (regimen A) or

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Table 1. Characteristics of 444 patients aged 1–65 ($m=46$) at presentation. *FUO*, fever of unknown origin; *major infection:* septicemia and/or lung, liver, gastro-intestinal tract infection; *DIC*, diffuse intravascular clotting

		n	%
FAB	M1	125	28
	M2	165	37
	M4	66	15
	M5	57	13
	Other	31	7
WBC	> 50 g/l	103	23
	<100 g/l	43	10
Platelets	<50 g/l	103	23
	<10 g/l	19	4
Infection	FUO	41	9
	Minor	105	24
	Major	50	11
Complications	DIC	35	8
	Renal failure	9	2
	Leukostasis	4	1

regimen B where cyclophosphamide (1500 mg/m^2 day 5) was added to regimen A. Bone marrow was studied at day 15: in case of persisting leukemic cells, patients received either Ara-C (200 mg/m^2/d for 3 days) and RBZ (200 mg/m^2/day for 2 days) or Ara-C and AMSA (200 mg/m^2/day for 2 days). In case of persisting leukemic cells, patients were classified and reported as failures even though CR could be achieved using other regimens or investigational agents. Depending on the institution's facilities, patients were cared for either in a highly protected environment with oral nonabsorbable antibiotics or in a conventional reverse isolation room. Prophylactic red blood cell and platelet transfusions were used in all patients. CR was defined as disappearance of all clinical and hematological evidence of leukemic infiltration with normal hematopoiesis recovery lasting more than 30 days.

Maintenance Modalities

When CR was achieved, patients were randomized to one of three maintenance arms.

Arm 1. Sequential courses of chemotherapy with Ara-C 100 mg/m^2/12 h subcutaneously for 5 days and alternatively prednisone (40 mg/m^2/d for 5 days) and vincristine (1.4 mg/m^2 day 1); methylglyoxal (400 mg/m^2 day 1 and 4); CPA (600 mg/m^2 day 1); RBZ (120 mg/m^2 day 1–2) every 6 weeks; RBZ was stopped after 12 months of continuing CR. All therapy was stopped after 36 months of continuing CR.

Arm 2: Similar regimen where 6-mercaptopurine (200 mg/m^2/day for 5 days) replaced Ara-C.

Arm 3. The first four courses associated Ara-C and m-AMSA (120 mg/m^2 d1); thereafter therapy was identical to arm 1.

Patients with acute monoblastic leukemia (M5) and/or hyperleukocytosis at presentation received CNS prophylaxis (intrathecal methotrexate (12 mg/m^2) for 6 weeks and skull irradiation delivering 24 Gy) and maintenance regimen identical to arm 1.

Patients less than 30 years with an HLA identical sibling could receive allogeneic bone marrow transplantation; such patients were not evaluated for CR duration.

Statistical Evaluation

CR was defined as above; failure as described; deaths were classified as toxic deaths (without persisting leukemic cells in the marrow) or failure deaths. Comparison between induction arms and analysis of prognostic factors related to CR were done using chi-square analysis.

Survival was calculated from the start of treatment to death; DFS was calculated from day of CR to either day of relapse or death. Life table analyses were done according to Kaplan and Meier [14]. Comparison between induction and maintenance arms and prognostic factors related to DFS were done by the log rank test [15].

Results

Remission Induction Regimen

CR was obtained in 358/444 patients (81%); five patients (1.4%) died before completion of the first 5 days of induction regimen; in 40 patients CR was not achieved by planned

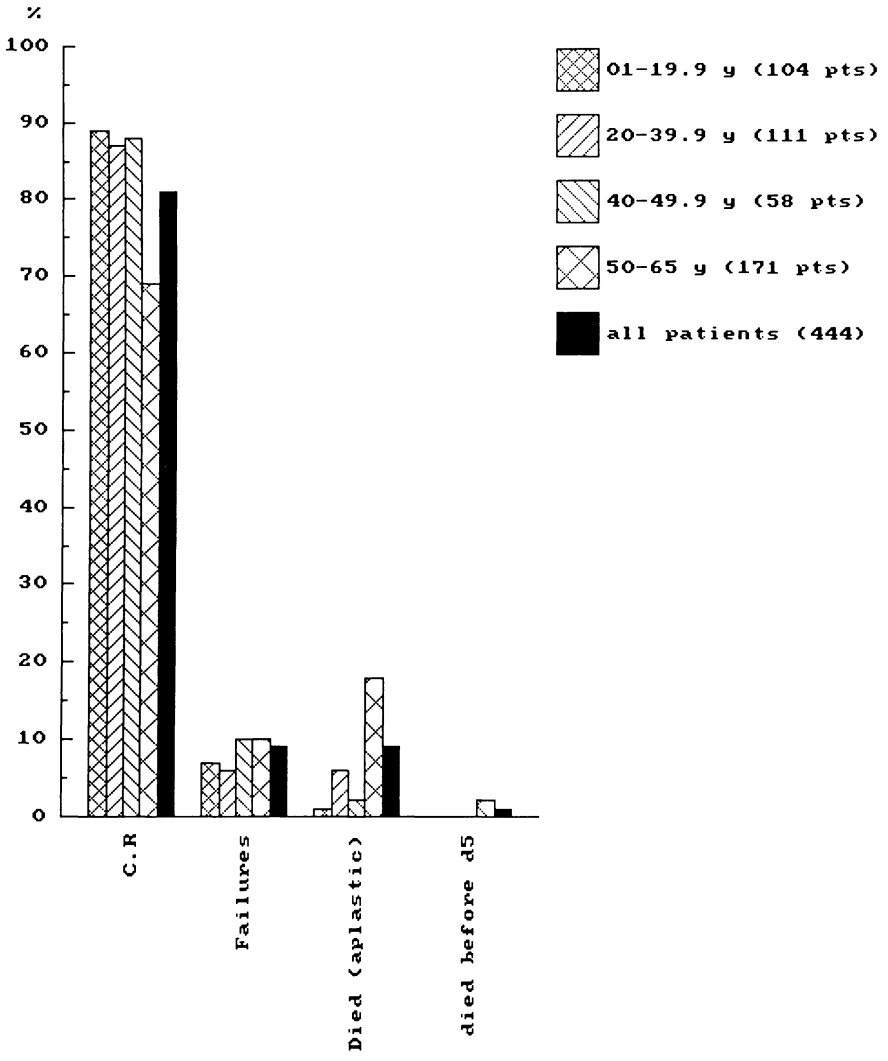

Fig. 1. Results of remission induction therapy

chemotherapy (9%) although a further regimen achieved CR in seven of them; finally, 41 patients (9%) died after bone marrow aplasia was obtained (Fig. 1).

After the first course of combination chemotherapy, 326 CR were achieved (91% of CR); 32 (9%) after a second course. Median duration of pancytopenia (WBC <0.5 g/l and/or platelets <30 g/l) has been 21 days (14–44 days).

CR rate was strongly dependant upon age: 231/260 patients younger than 50 years (88%) achieved CR as compared to 127/184 patients (69%) over 50 years ($p=0.000002$).

Irrespective of age, significantly higher CR rates were obtained in patients with myelomonocytic leukemia (CR = 90%; ($p=0.02$) and in patients with platelets below 50 g/l at presentation (84%; ($p=0.004$). On the other hand, neither tumor masses, leukemic complication (DIC, leukostasis, renal failure), infection at presentation, nor abnormal cytogenetics influenced CR rate. Finally, addition of CPA to Ara-C and RBZ did not improve remission induction results (79% vs. 82% CR).

Remission Duration, Survival, Disease-Free Survival

Overal remission duration and survival are shown in Fig. 2. As previously stated, they

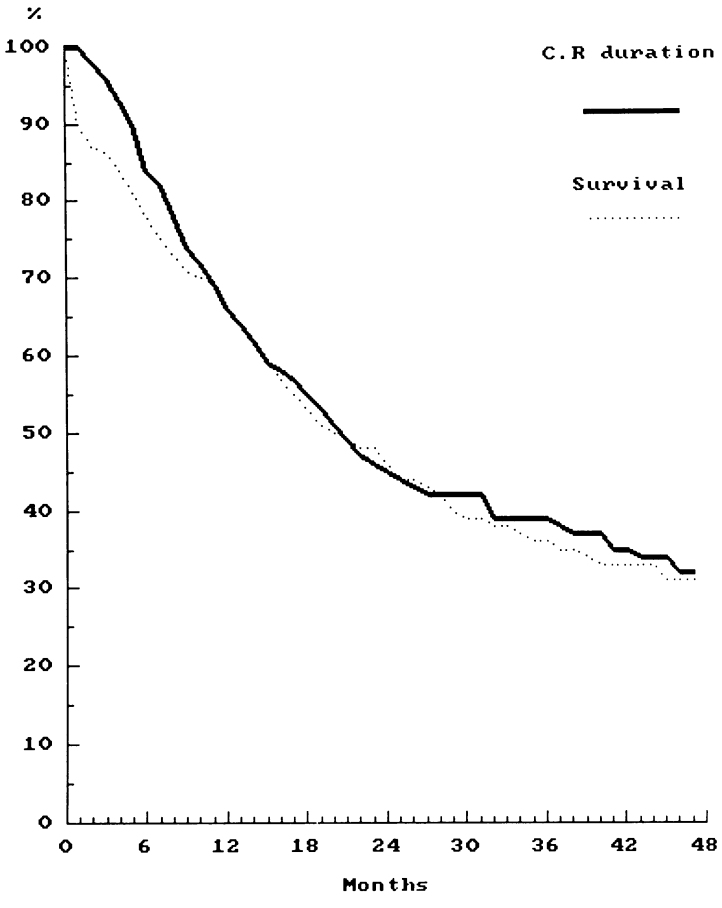

Fig. 2. Remission duration and overall survival (444 patients)

have been computed only for those 422 patients who did not receive allogeneic bone marrow transplantation: median duration of CR was 19 months with a projected CR rate of 29% at 48 months; overall median survival was 20 months with 32% of patients projected to survive 48 months. A plateau has not been reached as yet, and five relapses have already occurred in 43 patients after the end of therapy.

As the number of patients at risk after 36 months is still limited within each subgroup, comparisons have been carried only up to 36 months.

CR duration was strongly dependent upon age at diagnosis: median CR duration was 13 months in 144 patients less than 30 years with 30% of them in continuing CR at 36 months as compared with 22 months and 40% in 278 patients over 30 years ($p <= 0.005$).

Actuarial CR duration according to maintenance arm is shown in Fig. 3. Thirty-two patients could not be analyzed because of early deaths (10 patients) or major protocol violation (22 patients). Median CR duration and percentage of patients in continuous complete CR were 20 months and 37% in 81 patients treated in maintenance arm 1; 14 months and 21% in 72 patients in arm 2; 22 months and 37% in 77 patients in arm 3; 25 months and 47% in arm 4.

Thus, maintenance arm 2 carried a significantly worse prognosis than other maintenance arms. On the other hand, no significant difference was found between other maintenance modalities. Only three meningeal relapses have occurred; one in a patient with M5 type after CNS prophylaxis; two in patients who did not receive CNS prophylaxis.

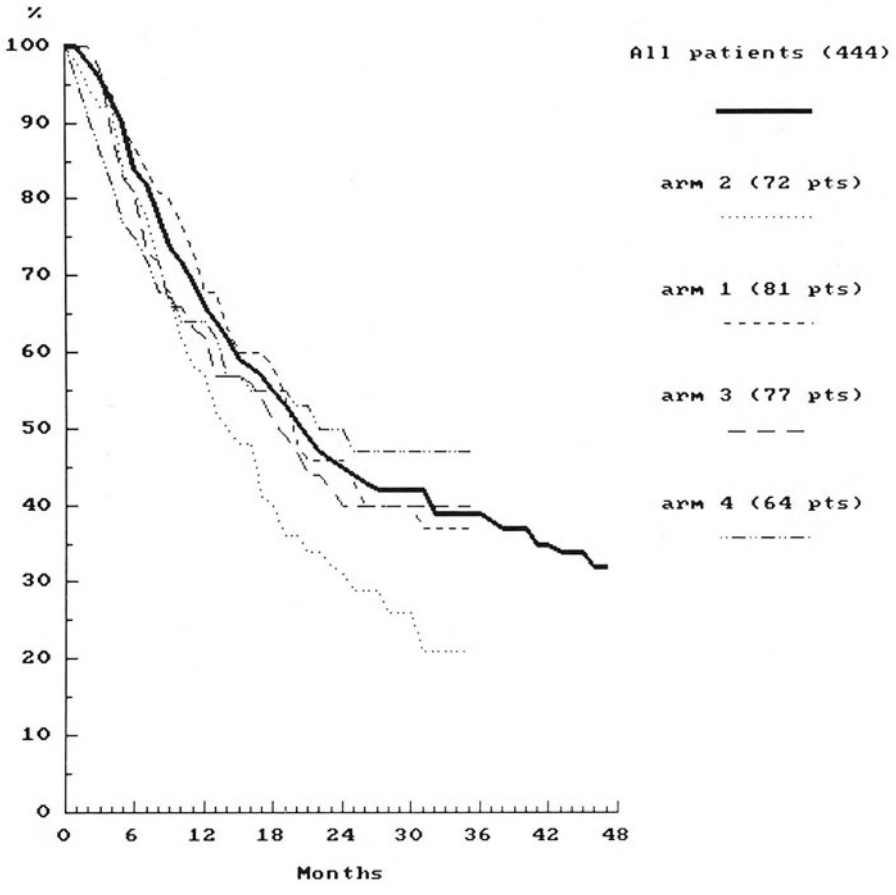

Fig. 3. Actuarial remission duration according to maintenance modalities

CR duration did not appear to be influenced by FAB type, WBC, platelet count at presentation, tumor masses, abnormal cytogenetics. Patients who needed two courses to achieve CR had shorter remission duration (15 months) (NS).

Finally the addition of CPA to Ara-C/RBZ induction regimen failed to improve CR duration.

Discussion

In this multicenter study of 444 patients with AML, Ara-C-zorubicin combination achieved 81% CR, the highest CR rate reported to date in a multicenter study. Presentation and age of patients do not appear to differ from those described in large multicenter trials [1, 3, 6–9]. The fact that M3 leukemia was not included in this trial cannot account for such results since M3 leukemia accounts for less than 5% of AML [16] and we have achieved 82% CR rate using same induction regimen in M3 leukemia (unpublished data). Ara-C-RBZ combination could therefore provide optimal remission induction regimen for ANLL. Addition of cyclophosphamide failed to improve those results. We previously obtained similar results when adding vincristine-prednisone or 6-mercaptopurine-methylglyoxal to Ara-C-RBZ combination [12]. A possible benefit of three- or four-drug combinations remains, therefore, questionable. That higher CR rates are achieved in younger patients has indeed been found in most studies: this relates mostly to better tolerance. Although we do confirm in this study our prior finding of increased chemosensitivity in M4 [12, 13], similar results have not been reported by others.

CR and DFS duration thus remains the major unsolved therapeutic problem in ANLL. The value of some (any) type of maintenance therapy is now widely accepted [4]. However, one should keep in mind that – as in experimental models – CR duration is strongly dependent upon initial tumor burden reduction. Although these data arise from sequential studies [12, 13], median CR duration has increased from 9 to 20 months using similar intermittent maintenance therapy as doses of Ara-C and zorubicin used in remission induction therapy were increased. The benefit of early consolidation therapy has been suggested in large studies [4, 6, 8, 11]; however, median duration of CR did not exceed 20 months in most studies: in 01AM81 protocol, similar values were achieved without early intensification while Ara-C-AMSA early intensification significantly improved these values. Rees at al. have shown that late intensification using COAP will increase 4 years RFS from 23% to 40%; this could also lead to the conclusion that sustained intensive monthly courses up to at least 12 months are useful. That therapy should not be stopped after 1 year is strongly suggested in our study by a linear monthly relapse rate up to at least 36 months. That patients with M5 and/or initial hyperleucocytosis do not carry a worse prognosis was somewhat unexpected: it strengthens previous findings that CNS prophylaxis will prevent high CNS relapse rate in these patients [17]; furthermore, less than 3% CNS relapses were observed leading to the conclusion that other forms would not benefit of CNS prophylaxis.

As others [9], we have found that median duration of CR is shorter in younger patients. Opposite results have generally been reported [4]. No imbalance between characteristics at presentation was found in younger and older patients, and the mechanisms underlying this difference remain unknown.

Preliminary conclusions from this study are:

1. Ara-C-RBZ appears to be an optimal remission induction regimen in AML.
2. Prolonged DFS or cure should be achieved in 20%–25% of patients, thus leading to a significantly increased cure rate in AML.
3. While maintenance therapy appears mandatory, optimal modalities are still questionable and will need further studies.

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Postinduction and Preremission Chemotherapy Alternatives for Adult AML: Three Multicenter Studies of the AML Cooperative Group*

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Various intensive induction regimens for adult AML involving a total of 3380 patients produced a median of 60% complete remissions (CR) ranging from 47% to 72% in multicenter [2, 4, 8, 10–12, 14] and major monocenter [5, 6, 9, 13] studies. The probability of continuous CR in these studies ranges from 8% to 45% (median, 24%) at 3 years. Corresponding data projected to 5 years yield a continuous CR rate of 10%–28% (median, 21%). No clear correlation has been found between CR duration and the type of induction or postinduction treatment. In particular, the role of consolidation and long-term maintenance therapy has remained controversial.

Initiated in 1978, the AML Cooperative Group in Germany has been conducting three multicenter trials on the role of major postinduction treatment variables (1978 pilot study and 1981 randomized study) and a new concept of double induction treatment (1985 pilot study).

Patients and Therapy

The design of the studies is shown in Figs. 1 and 2. So far, 877 adult patients with AML according to FAB criteria M1–M6 [1] have been treated in the three studies. The courses administered in the different phases of treatment have been as follows:

Induction and Consolidation. TAD9 [2] induction courses contained thioguanine (TG) 200 mg/m^2 daily p.o. on days 3–9, ARA-C 100 mg/m^2 daily by continuous i.v. infusion on days 1 and 2 and by 30-min i.v. infusion q 12 h on days 3–8, and daunorubicin (DNR) 60 mg/m^2 daily by i.v. injection on days 3–5.

In the 1978 pilot and 1981 randomized study, TAD9 induction was repeated once if the bone marrow on day 16 contained 5% or more leukemic blasts. TAD9 was administered in the same manner for consolidation.

In the 1985 pilot study, a second induction course was given to each patient. This course was applied prior to achievement of CR, if possible, and patients were randomized to receive TAD-9 or high-dose ARA-C plus mitoxantron (HAM) as second course. HAM comprised ARA-C 3 g/m^2 q 12 h by 3-h i.v. infusion on days 1–4 and mitoxantrone 10 mg/m^2 daily by 30-min. i.v. infusion on days 2–5. In a reduced version (HAM mod), ARA-C was given on days 1–3 and mitoxantrone on days 3–5 only. Consolidation and maintenance, as for the pilot phase of this new trial, has been uniform for all patients and identical to the two previous studies. In the 1985 pilot study, patients 60 years of age and older were treated differently from the younger patients, receiving a second induction course only if required and

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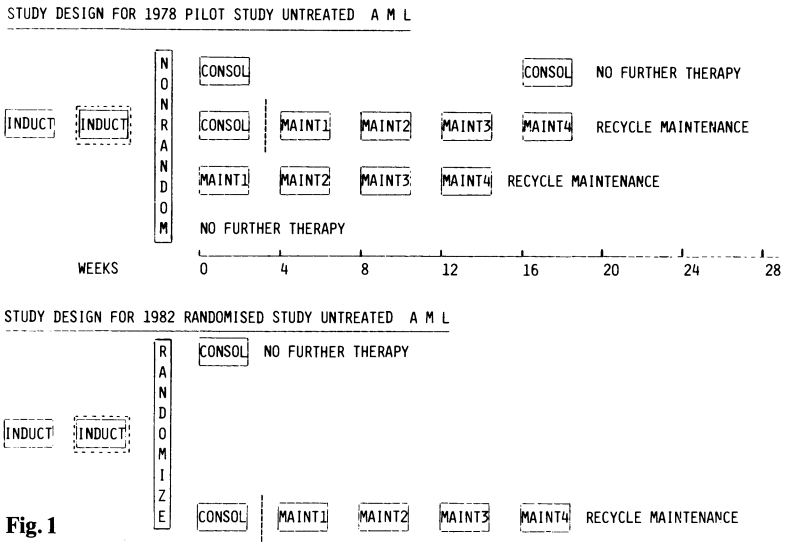

Fig. 1

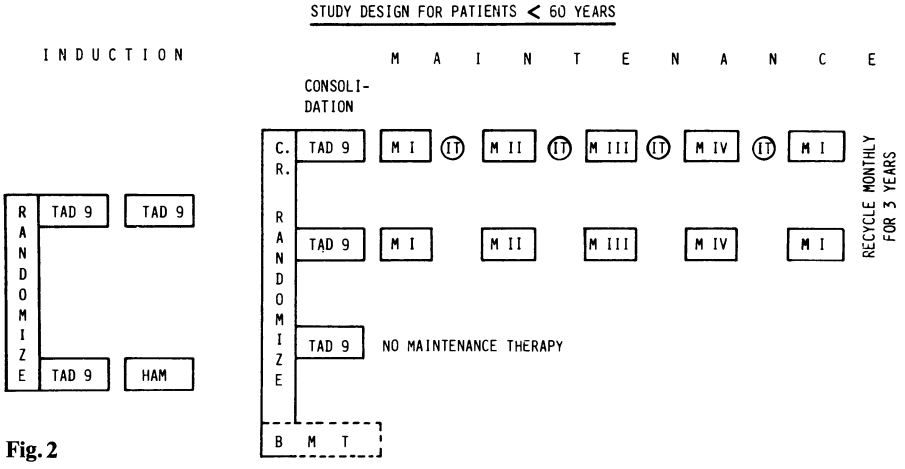

Fig. 2

being randomized to receive TAD9 induction courses either by original or 50% dosage for DNR. In addition, DNR in consolidation is administered at 50% dosage uniformly in the higher age group, and maintenance is the same as for younger patients.

Maintenance. Patients receive monthly 5-day courses of ARA-C 100 mg/m^2 q 12 h alternatingly combined with DNR 45 mg/m^2 i.v. injections on days 3 and 4 (Maint 1), TG 200 mg/m^2 p.o. on days 1–5 (Maint 2 and 4), or cyclophosphamide 1 g/m^2 i.v. injections on days 3 (Maint 3). Maintenance courses are recycled until relapse or for 3 years.

In the 1978 pilot study, patients in CR at the different centers nonrandomly received either one to two courses of consolidation, consolidation and maintenance, or maintenance alone. A fourth group did not receive any treatment in CR either by protocol or as a result of early relapses.

In the 1981 randomized study, patients in CR randomly received consolidation with our without maintenance. Patients in CR at three centers entered a special subtrial on immunotherapy presented separately in this volume (see Urbanitz et al.).

Results

In the 1978 pilot study, 242 patients at a median age of 50 (range, 15–78) years were

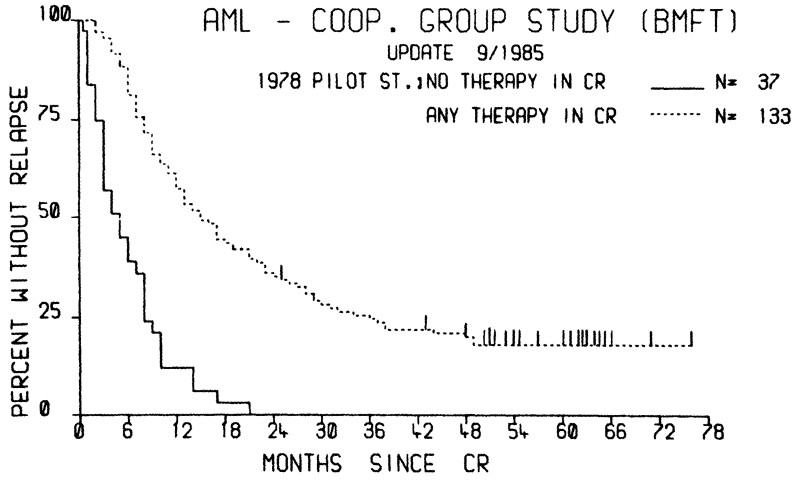

Fig. 3

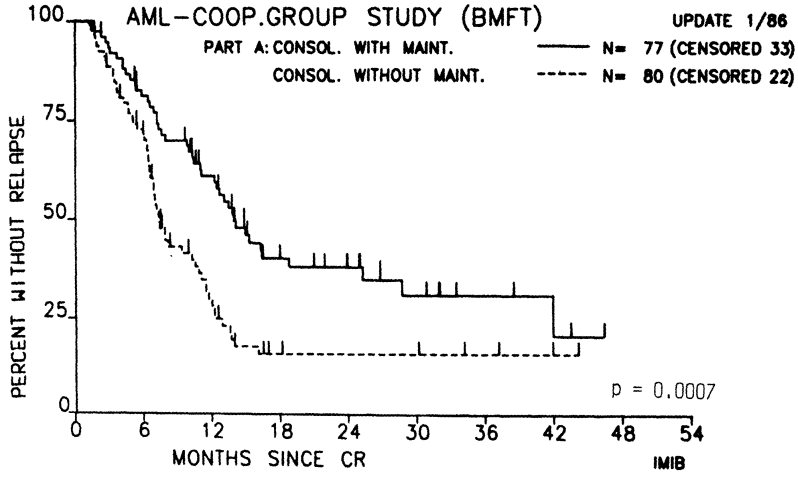

Fig. 4

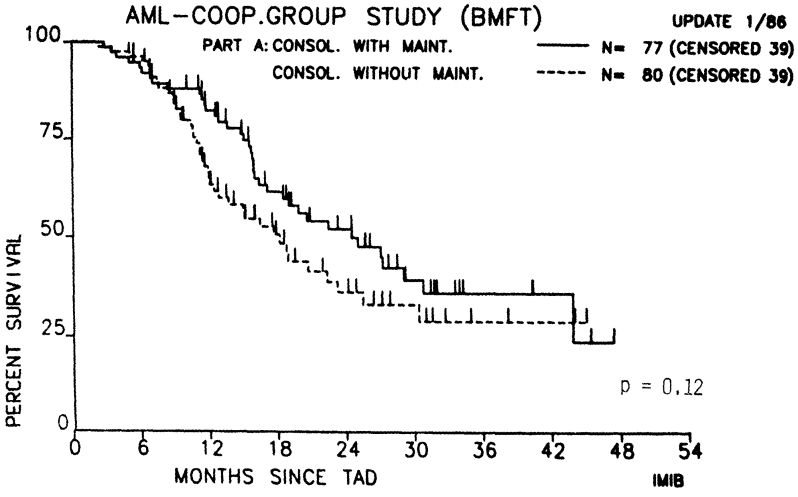

Fig. 5

Table 1. AML Cooperative Group randomized studies. Response data in the 1981 randomized study and the 1985 pilot study for patients of all ages and for patients in the older and younger age groups

Age	16–59 years		60–80 years		Total	
Study	1981	1985	1981	1985	1981	1985
Patients (*n*)	454	46	148	19	602	65
CR (%)	64	67	42	53	59	63
PR (%)	6	7	7	5	6	6
NR (%)	15	11	17	21	15	14
ED (%)	15	15	34	21	20	17

CR, complete remission; PR, partial remission; NR, nonresponse; ED, early death within 6 weeks from treatment start.

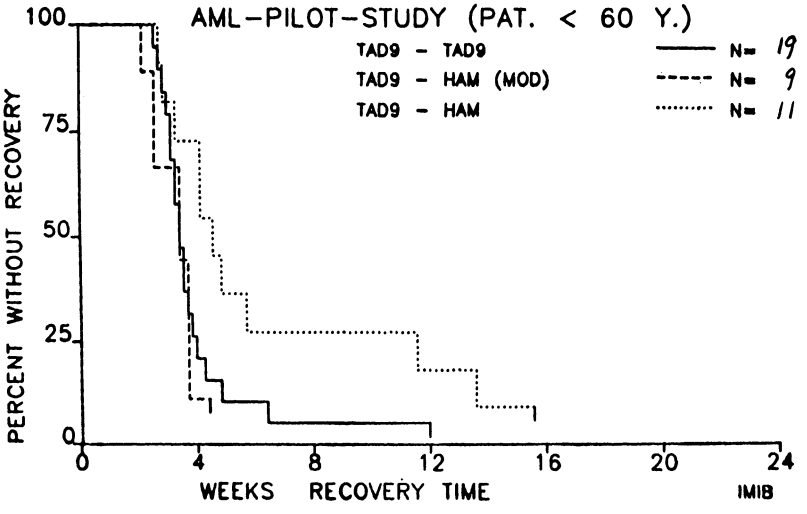

Fig. 6

Table 2. AML cooperative group 1985 pilot study: data on intestinal toxicity during and after the second induction course for the three regimens administered

Second course	TAD9	HAM	HAM mod
Patients (*n*)	20	12	8
Diarrhea (%)	30	8	25
Ileus: reversible (%)		8	
Ileus: irreversible (%)		12	

evaluable, and 70% achieved a CR. Of this group, 65% achieved a CR after only one TAD9 course. Remission duration did not differ significantly for the different treatment types in CR in this nonrandomized trial. Remission duration was significantly shorter, however, in patients without treatment in CR, and none of the patients in this group achieved a long-term CR (Fig. 3).

For the 1981 randomized study, response data are given in Table 1. The median age is 48 (range, 16–80) years. In this study, 70% of CRs were achieved with one TAD9 course, only. Remission duration and survival for the two treatment arms in CR, excluding patients entering the immunotherapy subtrial (see Urbanitz et al.) are shown in Figs. 4 and 5.

Response data from the 1985 pilot study are given in Table 1 and compared with those of the 1981 randomized study. In the younger age group, two induction courses

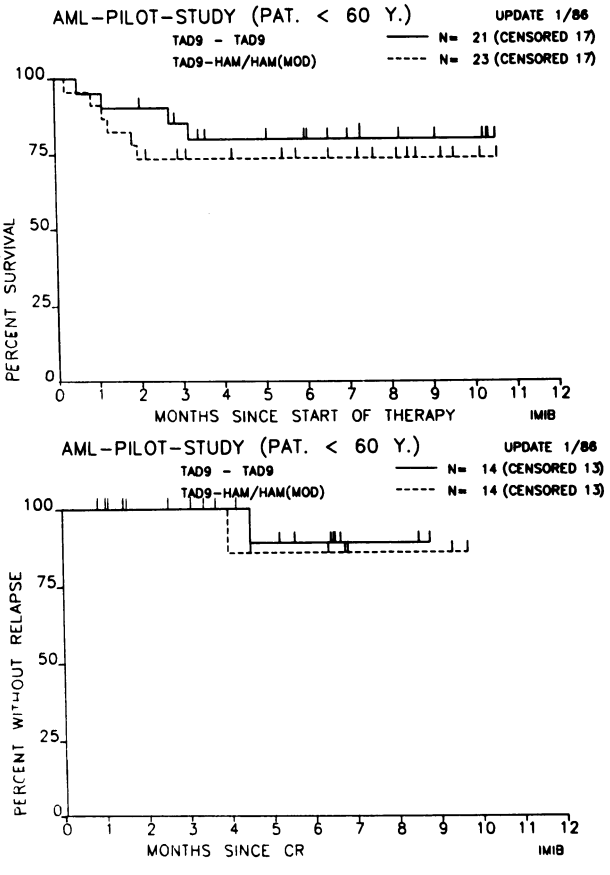

Fig. 7

have been given to 40 patients so far. Starting with HAM at the original dosage, unacceptable gastrointestinal toxicity (Table 2) and prolonged recovery time for granulocytes and platelets (Fig. 6) were observed. The subsequently reduced version HAM mod was found to be identical to TAD-9 in its toxicity (Table 2) and recovery time (Fig. 6). Preliminary survival of all treated patients entered and CR duration of those receiving two courses of induction are given in Fig. 7.

Discussion

As shown by our 1978 pilot and 1981 randomized studies [2, 3] the TAD9 intensified induction regimen was found to be highly effective, combining high remission rates with rapid response in multicenter trials. Even the 59% CR rate in the 1981 trial is in the upper range of comparable studies and is exceeded only by studies using bone marrow criteria [11]; excluding very early deaths [12], patients over 50 [13] or patients over 69 years [4]; or including children [11]. The rapidity of response may be higher by TAD9 than by the original TAD [7], as the median time to CR was 33 days for TAD9 as opposed to 45 days for TAD [5].

Myelosuppressive postremission chemotherapy was found to be a prerequisite of long-term remissions, as already shown by the 1978 pilot study [2]. There was not a single long-term remission in the nontreatment group, although two-thirds of the patients remained untreated for reasons other than early relapse [3].

The importance of myelosuppressive chemotherapy of a certain duration is underlined by the 1981 randomized study showing significantly superior remission duration in the monthly maintenance arm. In addition,

the 30% continuous CR rate at 3 years may represent a therapeutic improvement, as this has not been reached by any other multicenter study and has been markedly exceeded by only one monocenter result in patients up to 50 years [13]. Thus, it was possible to establish the validity of monthly maintenance after intensive induction and consolidation in our study.

It is a common finding in all comparable AML studies that the highest rate of relapses occurs during the first year of CR. The most recent concept of multiple intensive consolidations aims at preventing this early steep decline in the remission curve (see Rai et al., this volume). As an alternative approach, the AML Cooperative Group has been designing a new study introducing a concept of intensive two-course preremission therapy. In addition, we are randomly comparing a recycling to an alternating version of induction. Through a pilot phase of this trial and its first update, we can demonstrate the practicability of this new approach, which does not result in increased early lethality and shows a promising CR rate, total survival, and remission duration. We have also been able to contribute data on the toxicity and the optimum dosage of the new combination HAM as a second induction course, a regimen proving highly effective in refractory AML, as seen in a multicenter study of our group presented in this volume (see Hiddemann et al.).

In the next 2–3 years, we should see whether the long-term results in AML can be improved by either postinduction or preremission intensification of chemotherapy.

Summary

Major chemotherapeutic alternatives for AML have been implemented and compared in three multicenter studies, including a total of 877 adult patients of all ages. The results strongly suggest that myelosuppressive postinduction treatment is a prerequisite for the achievement of long-term remissions. In addition, it was possible to establish an important antileukemic effect of monthly maintenance chemotherapy. Initial results from an intensive two-course preremission therapy concept revealed good practicability and acceptable toxicity, as well as promising response and remission durations by this new approach.

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Neuraminidase-Treated Allogeneic Blasts for Maintenance In Acute Myelogenous Leukemia: Results of a Prospective Randomized Trial

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Summary

Between July 1, 1981, and July 1, 1985, 167 patients with acute myelogeneous leukemia (AML) were treated with one or, if necessary, two courses of a modified TAD regimen (TAD9) for induction. 96 patients (58%) achieved a complete remission (CR); 62 achieved CR after one and 34 patients after two courses of TAD9. 29 patients (17%) were considered early deaths, and 42 patients (25%) nonresponders. For CR maintenance 64 patients were eligible according to protocol criteria; 33 patients were randomized to chemotherapy, only, (CT) by monthly courses of cytosine arabinoside (ARA-C) alternatingly combined with daunorubicin or thioguanine or cyclophosphamide, while 31 patients were randomized to receive immunotherapy in addition to chemotherapy (CIT) by intradermal injections of 10^{10} neuraminidase-treated viable allogeneic blasts per immunization interspersed between the CT courses. Maintenance therapy was given for up to 3 years. The median survival in CT patients is 23 months, while in CIT patients the median has not been reached after 54 months; corresponding median relapse-free survival is 15 months for the CT patients as opposed to 40 months for the CIT group. The differences are not significant.

Comparing CT with CIT, the survival data show a persistent trend in favor of CIT; under the conditions of the study, however, a substantial benefit of immunotherapy may be restricted to a certain subset of patients with low risk for early relapse.

Introduction:

Since AML in CR represents a minimal residual disease with a greatly reduced tumor load [1] one important condition for a potentially successful immunotherapeutic approach is given. Out of several different immunotherapy trials related to AML in remission, the most encouraging results have been reported by Bekesi et al. [2, 3]. By using neuraminidase-treated allogeneic blasts in a high dosage, they have obtained a highly significant prolongation of both CR and survival. Until now, however, studies confirming these results have not been published.

Therefore, in 1981 we decided to initiate a randomized study using the same maintenance chemotherapy and immunotherapy as Bekesi and his group.

Patients and Methods

Induction Therapy

Patients with AML according to FAB criteria [4] admitted to the hospitals between July 1981 and July 1985 were treated with a modified TAD regimen, the TAD9 protocol [5] for induction: Ara-C 100 mg/m^2 per day by continuous infusion on days 1 and 2 followed by a 30-min infusion of Ara-C 100 mg/m^2 every 12 h from day 3 to day 8, thioguanine 200 mg/m^2 per day per os

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Table 1. Results of induction therapy

Patients admitted between 6/81 and 6/85 (n):	195
Excluded from TAD9 (n):	28
Evaluable patients (n):	167
Mean age (years):	48.6 (range, 17–76)
CR (n):	96 (58%)
NR (n):	42 (25%)
ED (n):	29 (17%)

from day 3 to day 9, and daunorubicin 60 mg/m^2 per day i.v. on days 3, 4, and 5. Patients expiring within 6 weeks after onset of induction therapy were considered early deaths (ED), those not achieving CR after two cycles of TAD were designated nonresponders (NR).

Maintenance Therapy

Patients achieving CR after one or two cycles of TAD9 were randomly allocated to one of two maintenance regimens: chemotherapy only (CT) or chemotherapy and immunotherapy (CIT). For CT, monthly courses of Ara-C alternatingly combined with daunorubicin, thioguanine, or cyclophosphamide were given according the CALGB protocol [6]. A permanent dose reduction of 50% for all drugs was necessary if two severe episodes of cytopenia had occurred (granulocytes $<500/mm^3$ and/or platelets $<20\,000/mm^3$ with a further reduction to 25% of the original dose after another two severe cytopenic episodes.

CIT included for the same protocol and in addition immunotherapy with doses of 10^{10} viable allogeneic neuraminidase-treated blasts injected intracutaneously at 50 different sites [3]. The effectiveness of the neuraminidase treatment was proven by estimation of the amount of neuraminic acid split off in every case. The original dose of 10^{10} viable blasts per immunization was maintained by taking into account only viable blasts in the final blast suspension. Immunotherapy was given on day 14 after the onset of each preceding CT cycle if platelet levels had recovered to $>50\,000/mm^3$ and leukocyte levels to $>1500/mm^3$; otherwise, immunotherapy was delayed for 1 week. Analogous delays were provided in the chemotherapy group. CT was administered 2 weeks after each immunotherapy course.

Table 2. Results of maintenance therapy

Patients randomized (n):	64
For CT(n):	33 (mean age, 42.9 years)
For CIT(n):	31 (mean age, 43.5 years)
Median observation time	
For survival:	31.5 months
For CR duration:	28.0 months
Median survival	
CT:	23 months
CIT:	54+ months
Median relapse-free survival	
CT:	13 months
CIT:	17 months

Maintenance therapy was continued for up to 3 years.

Statistics

Life table analyses were performed according to Kaplan and Meier; significances were determined by the logrank test.

Results

The data concerning induction therapy are depicted in Table 1. The reasons for exclusion from TAD9 included medical contraindications ($n=16$), protocol violation ($n=10$), and refusal ($n=2$).

For the results of maintenance, see Table 2.

The reasons for excluding 32 patients from randomization according to protocol criteria involved refusal ($n=11$), medical contraindications ($n=8$), no blasts for immunization available ($n=7$), social reasons ($n=3$), protocol violation ($n=2$), and death before randomization ($n=1$).

The life table analyses for survival and relapse-free survival (Figs. 1 and 2) disclose nearly identical patterns for CT and CIT patients for the first 18 months of remission; for longer-lasting remissions, a trend in favor of CIT is observed. The differences are not significant. After the exclusion of cases involving death before or during the first CT course ($n=2$), relapse before the first course ($n=1$), meningeosis after the first course of IT ($n=1$), and randomization despite persistent extramedullary leukemia ($n=1$) or severe diabetes mellitus ($n=1$), survival and re-

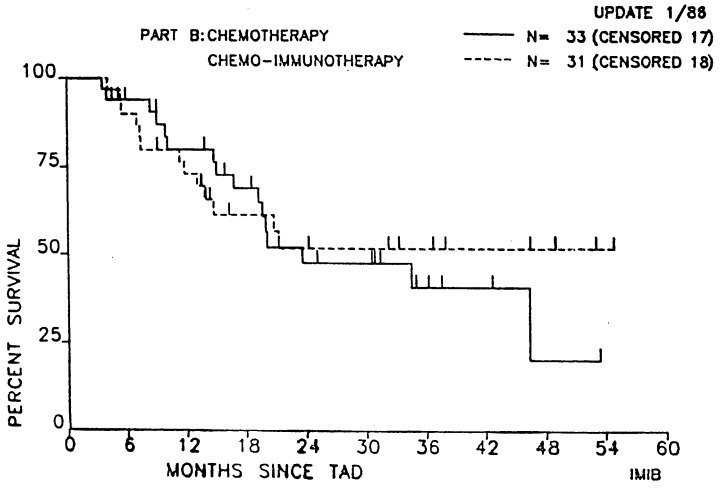

Fig. 1. Survival by maintenance arm

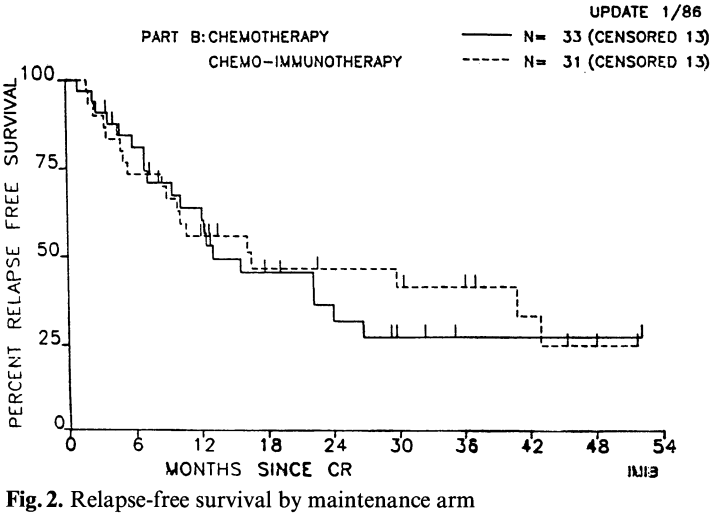

Fig. 2. Relapse-free survival by maintenance arm

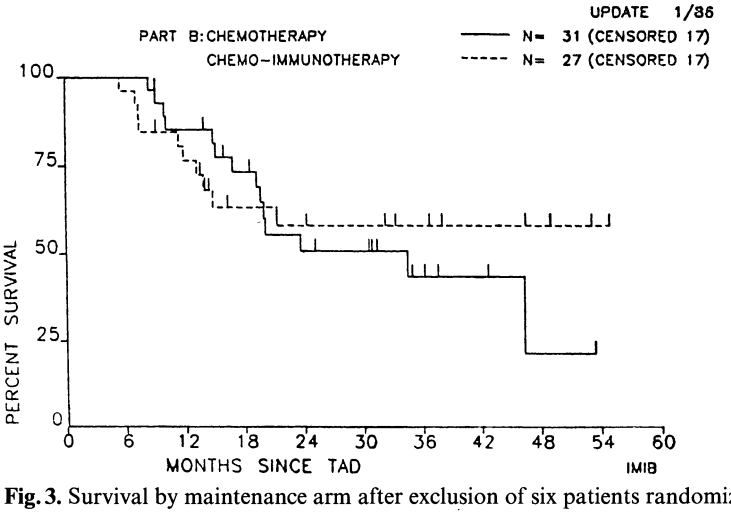

Fig. 3. Survival by maintenance arm after exclusion of six patients randomized

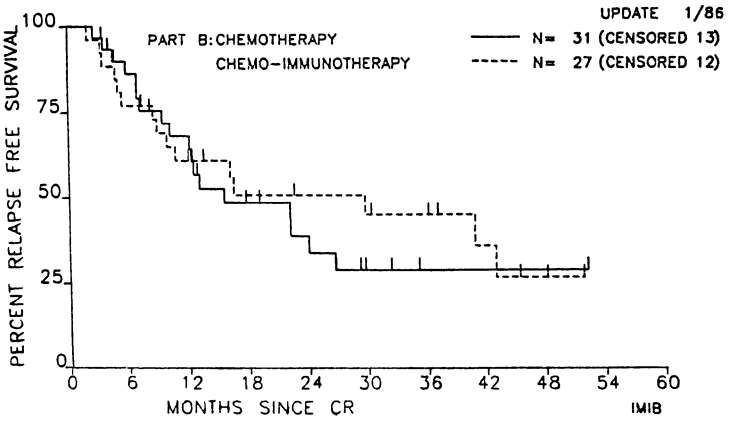

Fig. 4. Relapse-free survival by maintenance arm after exclusion of six patients randomized

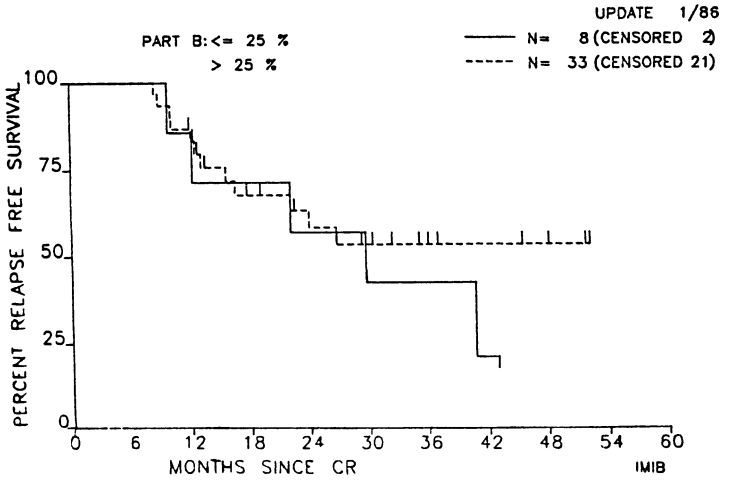

Fig. 5. Relapse-free survival by dose reduction of maintenance chemotherapy

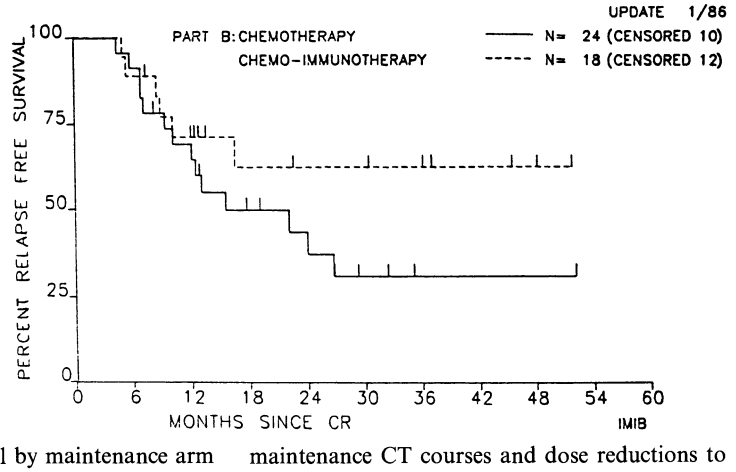

Fig. 6. Relapse-free survival by maintenance arm after exclusion of patients with less than three maintenance CT courses and dose reductions to 25% or less of original dose

lapse-free survival (Figs. 3 and 4) show a slightly enhanced tendency in favor of CIT.

Focusing on the three late relapses in the CIT group (28, 39, and 43 month's CR duration respectively), a common feature of these patients is a dose reduction for maintenance chemotherapy to $\leqq 25\%$. Only eight of the 64 patients randomized required dose reductions to 25% or less. These patients seem to have a poor prognosis since no long-term remission has been observed among these patients compared with those tolerating 50% or more of the original dose (Fig. 5).

After exclusion of patients with less than three cycles of maintenance chemotherapy and dose reduction to 25% or less (regardless of being randomized for CIT or CT), a marked tendency in favor of CIT is evident (Fig. 6), but again the difference is not significant ($p = 0.1$).

Discussion

The survival data show nearly identical values over the first 2 years of CR for the two treatment groups, confirming the data of Bekesi et al. who obtained an advantage for the CIT arm only after CR of more than 2 years duration. A possible explanation of this phenomenon could be that the blasts used for immunization are only weak immunogens and have to be administered several times in order to elicit a clinically relevant biological response. After more than 2 years of CR, a consistent trend in favour of immunotherapy is observed which increases if patients with dose reductions of cytostatics to $\leqq 25\%$ are excluded. A dose reduction to $\leqq 25\%$ was not equivalent to a relapse, since bone marrow monitoring did not reveal blast regrowth and the time period from dose reduction until relapse lasted for 2–3.5 years in the three CIT patients with late relapse.

The inability of patients to tolerate reasonable doses of CT in remission may be a reflection of impaired stem cell function and/or a reduced number of stem cells, which itself may be due to a high residual tumor load.

Taking these considerations into account, patients with a longer-lasting CR (permitting a certain number of immunotherapy courses) and with a sufficient CT dose may be candidates for an effective immunotherapeutic approach of this kind.

Under the conditions of the study, we were not able to confirm the results of Bekesi et al. who achieved a significant advantage for the entire group of CIT patients. Results of other authors using identical maintenance therapy for CT/CIT have not been published.

Immunologic data as presented by Pielken et al. (see this volume) point to an immunosuppressed state in CR patients, regardless of whether they receive CT or CIT. Whith increasing remission duration the immune response recovers partially.

A more intensive induction of early consolidation regimen as proposed by the German AML Cooperative Group (see Büchner et al., this volume) potentially leading to more long-term remissions may improve conditions for an immunotherapeutic approach toward AML in remission.

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Acute Myeloid Leukemia in Children

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The Childhood AML Studies BFM-78 and -83: Treatment Results and Risk Factor Analysis*

U. Creutzig, J. Ritter, H. Riehm, M. Budde, and G. Schellong[1]

Within the last 10 years the prognosis of childhood AML has been improved by more intensive combination chemotherapy and better supportive care, but not in the same degree as in childhood ALL [1]. The German AML studies, BFM-78 and -83, demonstrate increasing rates of long-lasting remissions. The large number of patients have allowed us to define prognostic factors in childhood AML.

Patients and Methods

One-hundred fifty-one children under the age of 17 years entered the AML study BFM-78 between December 1978 and October 1982. One-hundred forty-three patients were registered on BFM-83 between December 1982 and January 1986. Patient characteristics for both studies are given in Table 1.

Diagnostic criteria and treatment of children in the study BFM-78 have been described [2]. In the study BFM-83, an 8-day intensive induction precedes the induction-consolidation and maintenance therapy of the BFM-78 study: Cytosine arabinoside (100 mg/m^2 per day) 24-h infusion for 2 days followed by 2 × (100 mg/m^2 per day) 30 min infusion for 6 days; daunorubicin (60 mg/m^2 per day) days 3–5, and VP-16 (150 mg/m^2 per day) 60-min infusion days 6–8.

Response to induction therapy is evaluated on day 15. If the bone marrow (BM) contains < 5% blasts, the induction-consolidation therapy of the BFM-78 study with minimal modifications begins after hematological recovery of the peripheral blood. In case of more than 5% blasts the same induction-consolidation therapy should be given without delay if the patient's condition is judged stable enough to proceed. Complete remission (CR) is documented when the bone marrow contains < 5% blasts and there is no evidence of disease at other sites. In the study BFM-83, the CALGB criteria of hematological recovery [3] were followed, which was not possible in the BFM-78

Table 1. Patient characteristics

		AML-BFM 78	AML-BFM 83
Number of patients		151	143
Age, median (years; months)		9; 11	9; 3
Sex (% m:f)		54:46	52:48
WBC (median × 10^3/μl)		24.0	28.5
CNS involvement (%)		9	7
Extramedullary organ involvement (%)		18	32
FAB[a]	M 1 (%)	24	22
	M 2 (%)	23	20
	M 3 (%)	4	2
	M 4 (%)	26	22
	M 5 (%)	21	28
	M 6 (%)	2	3

[a] In 2 children of the study BFM-83, the FAB type is unclassifiable.

* Supported by the *Bundesminister für Forschung und Technologie*, FRG.

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study, because the continuous induction-consolidation did not allow full recovery.

Definitions and assumptions of Kaplan-Meier life table analyses [4] were:

1. Event-free survival (EFS): in the total group of patients, all events leading to remission failure or termination of survival in first CR were evaluated.
2. Event-free interval (EFI): in patients achieving CR, the time of first relapse or death in remission was evaluated.
3. Relapse-free interval (RFI): only the first relapse was counted as failure; death in remission was censored.
4. Withdrawals from the study were always censored at that time point.

Statistical comparisons between life table curves were performed with the log rank test. The importance of prognostic factors has been examined by the analysis of contingency tables (Yule's association analysis, X^2-test, stratified analysis) [5]. The following initial features were evaluated: age, sex, white blood count (WBC), platelet count, hemoglobin, liver and spleen enlargement, extramedullary organ involvement (liver and spleen excluded), and CNS involvement. Extramedullary organ involvement was mainly seen in the skin, tonsils, submandibular and lacrimal glands, testes, lung, and pericardium. In the BFM-83 study, involvement of the orbits, kidneys, and bones were also documented. It should be mentioned that multivariate analysis by Cox's regression or logistic regression gave no further insight into the data [5, 6].

Results

The overall results are presented in Table 2. Eleven children (4%) died prior to therapy of hemorrhage and/or leukostasis. Of the treated children, 119/149 (80%) in BFM-78 and 104/134 (78%) in BFM-83 achieved complete remission. In both studies 19/283 (7%) patients had early deaths from hemorrhage and/or leukostasis, and 7/283 (2%) patients died due to other complications within the first 6 weeks of treatment. Thirty-four of the 283 (12%) children were partial responders or nonresponders. In the BFM-78 study, 54 relapses (8 with CNS involvement) occurred after a median follow-up time of 5.3 years (range 3.3–7.0 years). The life table estimations for the EFS and EFI after 7.0 years are 38% (SD 4%) and 47% (SD 5%) respectively (Fig. 1).

Table 2. Results of the AML studies BFM-78 and-83, January 1986

	BFM-78	BFM-83
Patients	151	143
Death before onset of therapy	2	9
Death during induction		
hemorrhage/leukostasis	12	7
other complications	5	2
Partial/nonresponder	13	21
Complete remission achieved	119	104
Death in remission	6	4
Withdrawals[a] (BMT)	5 (2)	6(6)
Relapses	54	25
In CCR	54	69
Alive	66	88

BMT, bone marrow transplantation.
[a] Three children were withdrawn because of alteration or refusal of chemotherapy or loss to follow-up.

In the BFM-83 study, 25 relapses occurred (four with CNS involvement) after a median follow-up of 1.8 years (range 0.2–3.1 years). The life table estimations are EFS 48% (SD 5%) and EFI 62% (SD 6%) (Fig. 1). By comparison, the corresponding values after 3.1 years of the BFM-78 study are EFS 41% (SD 4%) and EFI 51% (SD 5%).

Results by morphological subtypes are given in Table 3. The rate of early death from hemorrhage and/or leukostasis was highest in the M5 type, while nonresponders were almost equally distributed among the four subtypes with a greater number of patients. In the BFM-78 study, relapse rates were high in the subtypes M2 and M4, which in contrast holds till now for the M5 type in the BFM-83 study. Life table estimates for EFS show that patients with the M1 type have the best prognosis. The difference in EFS between the M1 and M4 subtypes in patients in both studies is significant ($p < 0.05$, log rank test). Four of nine patients with M3 type had early deaths, whereas no relapses have occurred but two children have died in

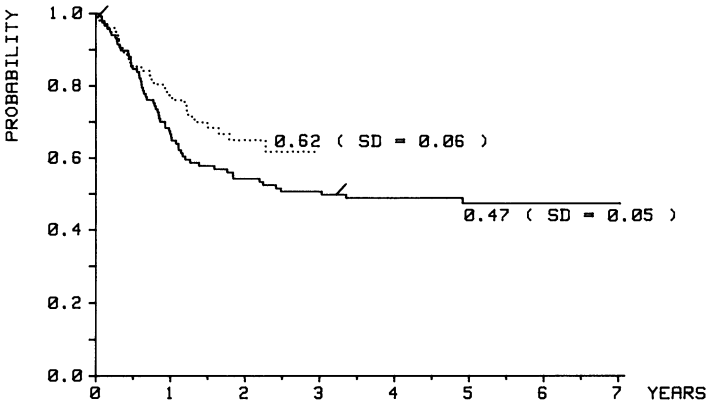

Fig. 1. Probability of event-free interval in the AML studies BFM-78 and -83. *Diagonal* indicates last patient entered the group. *Solid line*, AML-BFM 78 ($n=119$, 54 in CCR); *dotted line*, AML-BFM 83 ($n=104$, 69 in CCR)

CR from infection. All seven children with M6 type achieved CR, of whom three had early relapses and one received a bone marrow transplantation in first remission.

For the analysis of the risk factors for early death and nonresponse, the patients in both studies were evaluated together, but for the risk of relapse only the children of the BFM-78 study were analyzed because of their longer follow-up time.

The risk for early fatal hemorrhage and/or leukostasis is significantly increased in children with M5 type, with hyperleukocytosis ($\leqq 100 \times 10^3/\mu l$), and with extramedullary organ involvement. The combination of these features is extremely unfavorable.

The risk of nonresponse to treatment is also increased in patients with hyperleukocytosis; 12/34 nonresponding patients had hyperleukocytosis in contrast to only 31/223 responders ($p<0.05$, χ^2 test). The prognostic value of the percentage of blasts in the BM on day 15 is seen in the BFM-83 study: 9/14 nonresponders had $\geqq 5\%$ blasts in the BM in contrast to only 25/95 responders ($p<0.05$, χ^2 test).

The risk of relapse also depends on the size of the WBC (Fig. 2): In patients with a

Table 3. Results by FAB subtypes[a]

	AML-BFM 78				AML-BFM 83			
	M1	M2	M4	M5	M1	M2	M4	M5
Total (*n*)	36	34	40	32	32	29	32	40
Death before onset of therapy	–	–	–	2	–	–	1	8
Death during induction	3	–	4	7	1	3	2	2
(hemorrhage/leukostasis)	(3)	–	(2)	(6)	(1)	(2)	(1)	(2)
Nonresponder	4	1	5	3	4	4	7	5
Complete remission achived	29	33	31	20	27	22	22	26
Death in remission	1	–	3	1	–	1	1	1
Withdrawals	3	–	1	1	–	–	4	1
Relapses	10	19	16	6	5	5	5	9
In CCR	15	14	11	12	22	16	12	14
EFS[b] (%)	48	41	26	42	66	47	41	36
EFI[b] (%)	59	42	33	63	78	62	58	46

[a] For results of FAB M3 and M6 see text.
[b] Kaplan-Meier estimation (standard deviations according to patient numbers 8%–14%).

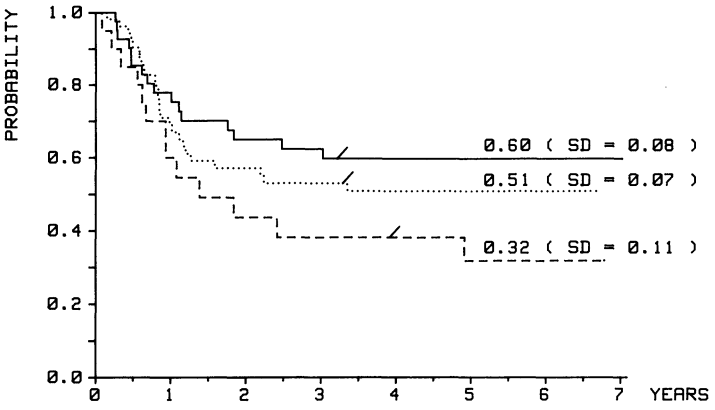

Fig. 2. Probability of relapse-free interval related to WBC in AML study BFM-78. *Diagonal* indicates last patient entered the group. *Solid line*, WBC $< 10 \times 10^3/\mu l$ ($n = 42$, 16 relapses); *dotted line*, $10 \times 10^3/\mu l <$ WBC $< 100 \times 10^3/\mu l$ ($n = 57$, 25 relapses); *dashed line*, WBC $\geqq 100 \times 10^3/\mu l$ ($n = 20$, 13 relapses)

low WBC ($< 10 \times 10^3/\mu l$) the RFI is 60% (SD 8%) and in those with a WBC $\geqq 100 \times 10^3/\mu l$ it is 32% (SD 11%) after 7.0 years ($p = 0.06$, log rank test).

Initial CNS involvement is also a risk factor for relapse. RFI estimations for the patients of both studies are CNS positive: 32% (SD 14%), 9 relapses in 16 patients; CNS negative: 57% (SD 4%), 69 relapses in 201 patients after 4 years ($p = 0.05$, log rank test).

Analysis of toxicity in the BFM-83 study is preliminary. In the BFM-78 study six children died from sepsis or pneumonia in the pause after the first 4-week phase or during the second phase of induction-consolidation therapy, while in the BFM-83 study only one child has died of infection in association with the second phase of therapy. Although the frequency of sepsis was nearly 30% during the first 2 weeks of BFM-83 induction, no child has died from these complications. The most important cause of early death was fatal hemorrhage or leukostasis, which occurred in a well-defined risk group of children (see above) in both studies.

Discussion and Conclusions

The present data of the two consecutive German AML studies in childhood show that

Risk \ FAB		M1	M2	M3	M4	M5	M6
Early death Hemorrh./ Leukostasis	Incidence	+	+	++	+	+++	
	WBC ↑			?			?
	Organ +						
Non-responder	Incidence	+	+		++	+	
	WBC ↑						
	Organ +						
Relapse	Incidence	+	+		+	+	+
	WBC ↑						
	Organ +						

Fig. 3. Pattern of risk factors in childhood AML

nearly 80% of patients achieve complete remission and that the probabilities of continuous complete remission (EFI) are over 50% after 3 years. Special analyses show that the intensification of induction therapy in the BFM-83 study has improved the treatment results in the FAB types Ml to M4 but not in the M5 type.

Risk factor analysis is difficult because of the heterogeneity of AML (Fig. 3). Hyperleukocytosis is the main risk factor for early death from hemorrhage or leukostasis, for nonresponse, and for relapse. We have seen this in the morphological subtypes with great enough numbers of patients (M1, M2, M4, M5) and cannot comment on how they relate to the M3 and M6 types, because of small numbers of patients and generally low WBC. In the myeloblastic subtypes M1, M2, and M4, the risk of fatal events increases with the degree of hyperleukocytosis ($\geqq 100 \times 10^3/\mu l$). In the M5 type, the risk, especially of early fatal hemorrhage and leukostasis, begins with an even lower WBC ($< 100 \times 10^3/\mu l$). In the monocytic subtypes, M4 and especially M5, the extramedullary burden of leukemic blasts increases the risk of fatal events not only early after diagnosis, but also after achieving remission. This was not seen in the M1 and M2 types with extramedullary organ involvement.

We conclude that intensification of therapy in childhood AML may improve the long-term prognosis without increasing the incidence of early deaths due to toxicity of therapy. New initial treatment strategies are necessary for children with high risk of early death from hemorrhage and/or leukostasis, i.e., especially with M5 type and hyperleukocytosis or extramedullary organ involvement. In addition, a modification or intensification of chemotherapy is warranted in the M5 type because of the high relapse rate.

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Improved Treatment Results in Childhood Acute Nonlymphoblastic Leukemia with the BFM-AML Protocol 78 in a Multicenter Study in the GDR

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Introduction

In contrast to recent advances in the treatment of acute lymphoblastic leukemia (ALL) where cure rates of nearly 70% have been obtained [1, 2], the outcome of the treatment for acute nonlymphoblastic leukemia (ANLL, AML) is less successful. In general, remission rates of about 70% have been obtained [3–9]. But the relapse rate is high, and so CCR rates of 50% after 2–3 years are exceptions [3, 4, 10].

In this paper we will report the most important results of the AML study I/82 of the GDR multicenter study group Pediatric Hematology and Oncology. The patients were treated as laid down in the BFM-AML protocol 78 [11].

Patients and Methods

Patients

A total of 87 patients were treated in one of the 14 centers of Pediatric Oncology of the GDR. Sixteen children entered a pilot study between June 1979 and December 1981, and 71 consecutive patients from January 1982 to February 1986. All patients were under 16 years of age. A pretreatment for ALL of up to 14 days was permitted.

Diagnosis

The diagnosis of the ANLL subtypes was made in the respective treatment center and the study center (Jena) simultaneously. The subtypes of ANLL were determined according to the FAB classification [12] on Pappenheim stained bone marrow and blood smears and special stains, including PAS, POX, Sudan black, and alpha-naphthylacetate esterase with and without NaF inhibition. In some cases an immunologic examination with monoclonal antibodies (VIM 2, 12, 13, and D 5) was performed.

Treatment

Patients with initial leukocyte counts of more than 20 Gpt/l and/or hepatosplenomegaly received a pretreatment with low doses of 6-thioguanine (6-TG), 30 mg/m^2 p.o. and cytosine arabinoside (Ara-C), 40 mg/m^2 i.v. daily for up to 10 days.

The initial treatment consisted of two phases of 4 weeks each (Fig. 1). During phase I (induction) patients received prednisolone, vincristine, adriamycin, Ara-C, and 6-TG. After a treatment-free interval of 1–2 weeks cyclophosphamide,, Ara-C, 6-TG, and adriamycin were given in phase II (consolidation). At the same time the patients received CNS prophylaxis consisting of four intrathecal injections of methotrexate and cranial irradiation. Therapy-free intervals of several days were allowed during phase II in patients with severe bone marrow depression. The bone marrow status was performed after phase I and after phase II in cases of partial response or nonresponse.

Maintenance therapy was started 2 weeks after the end of phase II with 6-TG (40 mg/m^2 p.o.), Ara-C (40 mg/m^2 s.c.) daily for 4

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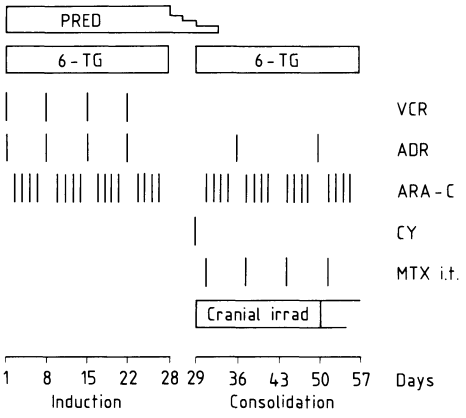

Fig. 1. Induction/consolidation therapy of AML study I/82 (BFM-AML 78).*PRED,* prednisolone, 60 mg/m^2 p.o. daily for 28 days; *6-TG,* 6-thioguanine, 60 mg/m^2 p.o. daily for 28 days × 2; *VCR,* vincristine, 1.5 mg/m^2 i.v. × 4; maximal single dose 2 mg; *ADR,* adriamycin, 25 mg/m^2 i.v. × 6; *Ara-C,* cytosine arabinoside, 75 mg/m^2 i.v. × 32; *CY,* cyclophosphamide, 500 mg/m^2 p.inf. × 2; *MTX,* methotrexate, 12.5 mg/m^2 i.t. × 4; *Cranial irradiation,* 12 Gy in 1st year of life, 15 Gy in 2nd year of life, 18 Gy after 3rd year of life

days every 4 weeks and adriamycin (25 mg/m^2 i.v.) every 8 weeks for the 1st year. Maintenance therapy was stopped after 2 years.

Colistin or neomycin, nystatin and cotrimoxazole as well as i.v. gammaglobuline and appropriate care of mucous membranes were given to all patients.

Statistical Methods

Life table analyses were performed according to the method of Kaplan and Meier [13]. They were based on the following definitions:

Survival. The time from diagnosis to death was evaluated for the total group of protocol patients.

Event-Free Survival (Disease-Free Survival). All events in the total group leading to remission failures (early death, nonresponse) or to termination of survival in remission (relapse, death in remission) were evaluated.

Event-Free Interval (Disease-Free Interval). The analysis was based on patients of the remission group. The time of first relapse or death in first remission was evaluated. Transplanted patients were censored at time of transplantation.

Relapse-Free Interval. The analysis was based on patients of the remission group. The time of first relapse was evaluated. Transplanted patients were censored at time of transplantation.

Results

Total Group

Eighty-seven patients had entered the study by 15 February 1986 (Table 1). Four patients had not yet achieved remission at the time of evaluation. Seventeen patients (20%) died before attaining remission eight of whom (10%) died of hemorrhages. Eight patients failed to respond (10%). Thus, the remission rate was 70%. One patient (M 4) died of septicemia 1 year after diagnosis in remission. Six patients received a bone marrow transplantation. Two of these patients died (one of encephalopathy and one of fatal GVHD). Twenty-three children (28% of the total group) relapsed. The life table analysis revealed a probability for event-free survival of 39% and an event-free interval of 51% after 68 months (Figs. 2 and 3).

Results Obtained with ANLL Subtypes M 1 to M 5 (Table 1 and Fig. 4)

Acute Myeloblastic Leukemia without Maturation (M 1). Fifteen patients belonged to this group. The remission rate was 87%. Three patients relapsed. Of the children in the remission group, 74% are in CCR.

Acute Myeloblastic Leukemia with Maturation (M 2). Of the 18 patients in this group 75% attained remission. The relapse rate was 25% (5 patients). Fifty-four percent of the children in the remission group remained in CCR.

Acute Promyelocytic Leukemia (M 3). The one girl of this group attained remission but relapsed after 30 months and died.

Table 1. Distribution of ANLL subtypes and results of therapy (0–68 months) – 15 February 1986

Results of therapy	Total		M1		M2		M4		M5		M3	M6
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(*n*)
Study patients	83	100	15	100	20	100	24	100	22	100	1	1
+not yet in CCR	+4	–	+2	–	–	–	–	–	+2	–	–	–
Early deaths (4 w.)	17	20	2	13	4	20	5	21	6	27	–	–
(Bleeding)	(8)	(10)	(1)	(7)	(2)	(10)	(2)	(8)	(2)	(9)	(–)	(–)
Nonresponder	8	10	–	–	1	5	5	21	3	14	–	–
Remission	58	70	13	87	15	75	14	58	14	64	1	1
Deaths in first CCR	1	1	–	–	–	–	1	4	–	–	–	–
BMT	6	7	2	13	2	10	2	8	–	–	–	–
Patients with relapses	23	28	3	20	5	25	5	21	9	41	1	–
Event-free survival	0.362		0.654		0.410		0.340		0.171		–	–
SD	0.056		0.125		0.12		0.102		0.10		–	–
Event-free interval	0.508		0.741		0.546		0.584		0.256		0.0	1.0
SD	0.072		0.12		0.15		0.14		0.14			

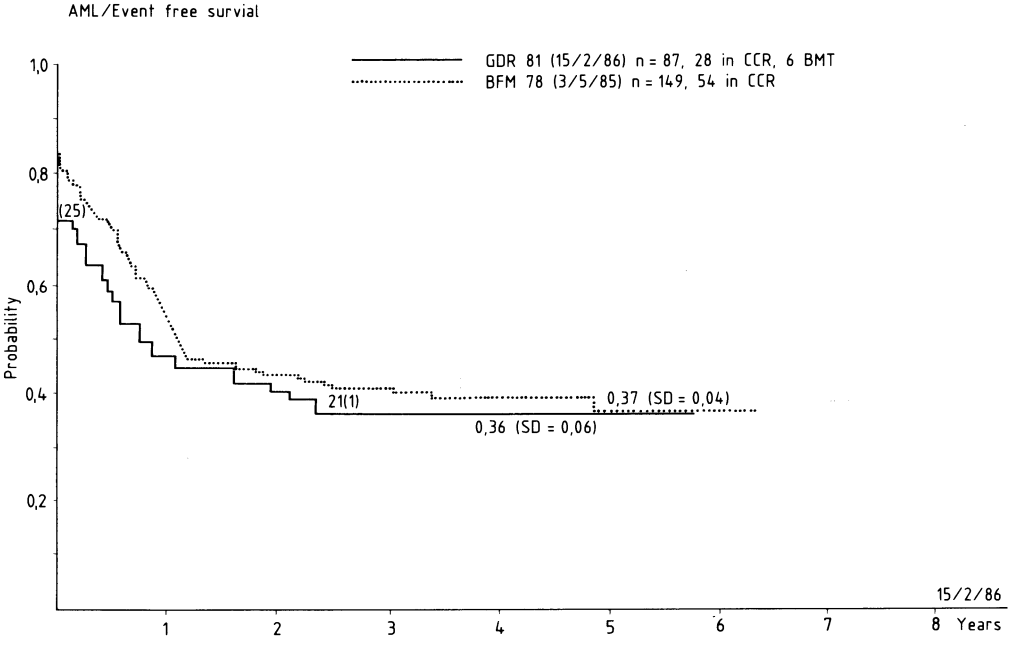

Fig. 2. Probability of event-free survival. *Solid line* denotes AML I/82 GDR, *dotted line* denotes BFM 78

Acute Myelomonocytic Leukemia (M 4). Only 14 of 24 children in this group achieved remission (58%). Ten children died before attaining remission or were nonresponders. Five patients (21%) relapsed. Thirty-four percent of the total group and 58% of the remission group are in CCR after 64 months. One patient of this group died in remission.

Acute Monocytic Leukemia (M 5). Twenty-four children belonged to this group. There were 6 cases of early death. Two of them

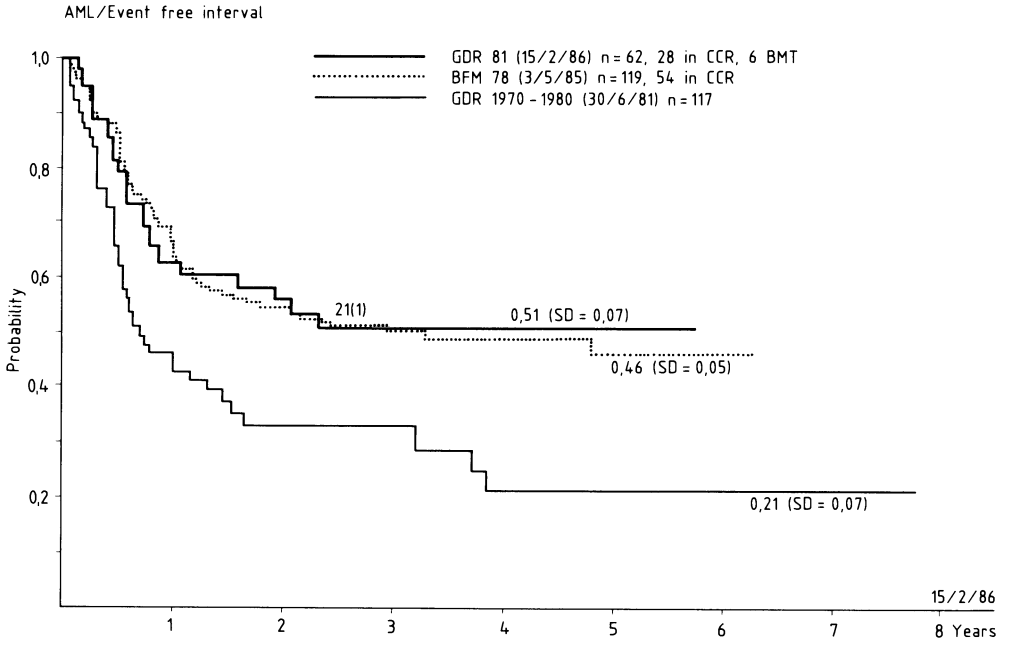

Fig. 3. Probability of event-free interval. *Solid line (thick)* denotes AML I/82 GDR, *dotted line* denotes BFM 78, *solid line (thin)* indicates retrospective study GDR 1970–1980

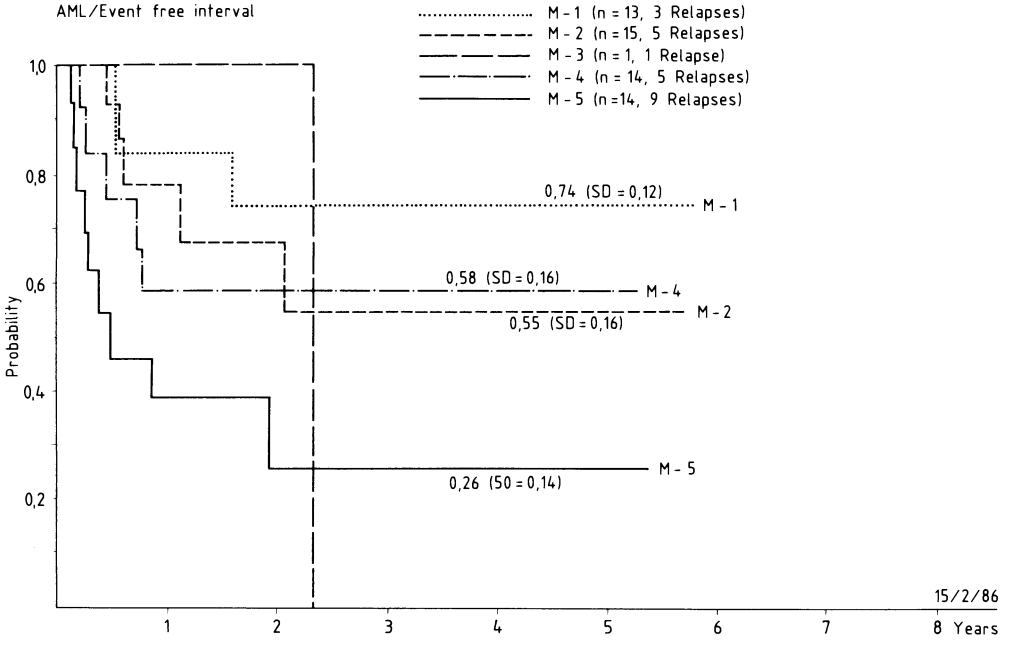

Fig. 4. Probability of relapse-free interval for ANLL subtypes

Table 2. Frequency and sites of relapses in ANLL study I/82 (0–68 months) – 15 February 1986

Relapses	Total		M1		M2		M4		M5		M3	M6
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(*n*)
Patients in remission	58	100	13	100	15	100	14	100	14	100	1	1
Relapses	23	40	3	23	5	33	5	36	9	64	1	–
BM	15	26	3	23	4	27	3	21	5	36	–	–
BM + Skin	1	2	–	–	–	–	–	–	1	7	–	–
BM + CNS	1	2	–	–	–	–	1[a]	7	–	–	–	–
Skin	1	2	–	–	–	–	–	–	1	7	–	–
CNS	1	2	–	–	1	7	–	–	–	–	–	–
Eye	2	3	–	–	–	–	1	7	1	7	–	–
Testes	1	2	–	–	–	–	–	–	1	7	–	–
Abdomen	1	2	–	–	–	–	–	–	–	–	1	–

[a] After primary CNS involvement.

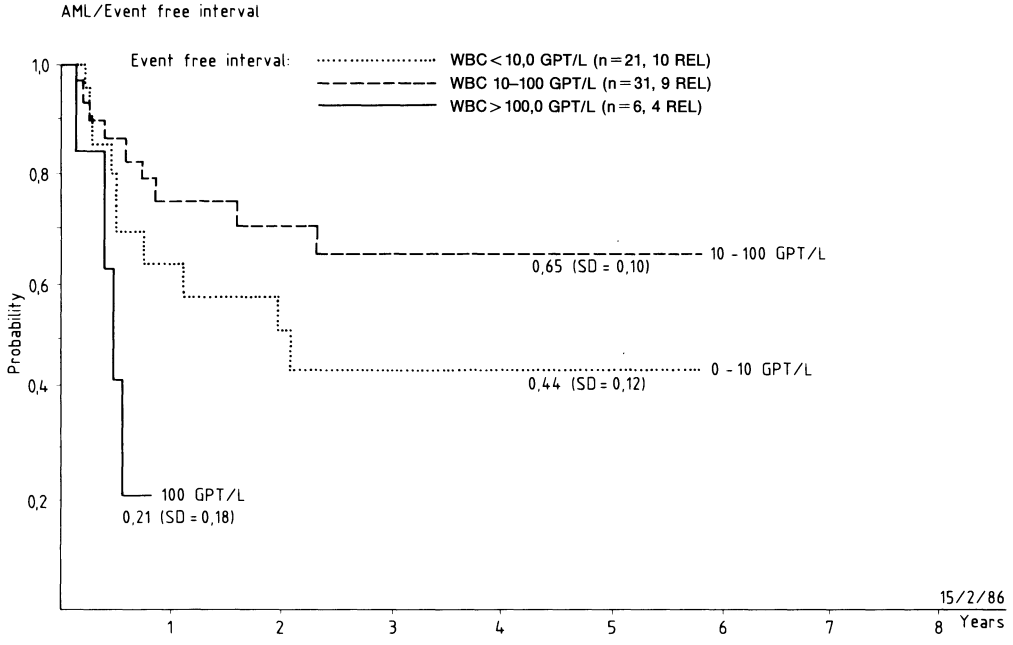

Fig. 5. Probability of relapse-free interval according to initial leukocyte counts

died of hemorrhages and 3 were non responders. Of the patients, 64% attained complete remission. The relapse rate of 41% was very high; 17% of the total group and 26% of the remission group remain in CCR. Of the 9 relapses, 7 occured during the first 6 months of remission.

Relapses

Twenty-three (40%) of the 58 patients in remission relapsed. Table 2 summarizes the localization of relapses. Specific organ involvement (skin, eye, testes) was found exclusively in myelomonocytic and monocytic

leukemia. Two CNS relapses were registered, one after initial CNS involvement (M 4, combined CNS and bone marrow relapse). As shown in Fig. 5, the highest risk factor for getting a relapse seems to be an initial leukocyte count of more than 100 Gpt/l.

Discussion

In contrast to adults, there is relatively little information about ANLL therapy studies in childhood [3, 4, 6–8, 10, 14]. As a rule, there are remission rates of more than 60% in these studies. Most of them have a high relapse rate, and so the remission level is mostly below 30% after 2–3 years. We had similarly discouraging results in our research group from 1970 to 1980 (Fig. 3). Only few studies achieved a higher rate of continuous complete remissions [3–5, 10].

The therapy concept presented here was developed from the BFM-ALL protocols by Riehm and Schellong. Therapy was intensified, cytostatics (6-TG, Ara-C) particularly effective in ANLL were increasingly applied and asparaginase, which has little effect in AML, was omitted.

The total results of our study AML I/82 exactly correspond with those of the BFM-AML study 1978 (Figs. 2 and 3). But if the results of the subtypes (Schellong, personal information) are compared, there are considerable discrepancies which can be constantly demonstrated from the very beginning.

This is particularly evident in relapse-free interval (RFI). While in the BFM group M 1, M 2, and M 4 have remarkably worse results, these are significantly better in M 5 (RFI 0.63 BFM vs. 0.27 GDR after 5 years).

The toxicity of the protocol is acceptable. Whereas the induction therapy can mostly be carried out without notable interruption, intervals are frequently necessary in the consolidation therapy due to bone marrow depression. the maintenance therapy is easily controllable. Only one patient (the first of the pilot study) died of septicemia in bone marrow depression 1 year after the beginning of the therapy.

Bone marrow transplantation has brought about an essential improvement of ANLL prognosis: 50%–70% of patients can be cured with it [10, 15–17]. However, this therapy is limited to patients with histocompatible bone marrow donors. The main risk factors are toxicity of therapy, fatal infections, GVHD, and recurrent leukemia. Two of six children died of encephalopathy after total body irradiation and of fatal GVHD.

Conflicting recommendations have been published for treatment of pediatric patients with ANLL in first remission. In Seattle and Minnesota, results are sufficiently superior with marrow grafting to justify transplantation of all children with matched donors. Other authors, however, believe that the superiority of either chemotherapy or transplantation has not been established and that studies comparing the best chemotherapy regimens with marrow grafting need to be completed. For a child with newly diagnosed ANLL, the modality recommended will depend on the institution's expertise [10, 18]. Because of the good relapse-free interval in patients with M 1, M 2, and M 4 (74%, 54%, and 58%) in our study, we can only recommend BMT in first remission for monocytic leukemia.

Summary

Eighty-seven children with acute nonlymphoblastic leukemia were treated with the AML protocol BFM 78 between June 1979 and February 1986 in a multicenter study in the GDR. Seventeen children (20%) died from early complications, eight did not respond to therapy. Fifty-eight patients (70%) achieved a complete remission. Twenty-three patients relapsed. The life table analysis revealed after 5 years a probability for event-free survival of 36% (SD = 6%) and an event-free interval of 51% (SD = 8%). Six patients were transplanted in first remission. Two of them died; one (M 1) on day + 19 from encephalopathy and one (M 4) on day + 60 from acute GVHD. The overall results are in good correlation with the original BFM study, but there are differences in the subtypes. Results are superior to other AML protocols in our group.

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A Comparison of Cytokinetically Based Versus Intensive Chemotherapy for Childhood Acute Myelogenous Leukemia*

G. V. Dahl, D. K. Kalwinsky, J. Mirro, and A. T. Look [1]

Introduction

In 1976 we designed a treatment protocol for children with acute myelogenous leukemia (AML) that featured a cytokinetically based induction regimen and 30 months of maintenance chemotherapy employing vincristine-doxorubicin-cyclophosphamide, cytarabine, and 6-mercaptopurine [1]. The intent was to determine if relatively nontoxic chemotherapy would cure a significant number of patients, with the benefit of fewer adverse side effects. About one-third of the patients were randomized to a splenectomy group in an effort to determine if the spleen is a clinically important sanctuary for leukemic cells. In 1980 we drastically changed our therapeutic strategy to an intensive induction followed by either sequential intensive chemotherapy or bone marrow transplantation [2].

These two protocols represent very different approaches to the treatment of childhood AML. In this article, we compare the antileukemic effectiveness and toxicity of the two chemotherapeutic strategies. Our analysis indicates that intensive chemotherapy, as described here, is more effective than cytokinetically based treatment for early control of leukemia, but does not yield a significantly higher proportion of long-term survivors. Although not included in the formal comparison, bone marrow transplantation in the AML-80 protocol has produced excellent short-term results and appears to offer the greatest likelihood of securing permanent remissions.

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Materials and Methods

One hundred eighty-two consecutive untreated patients with AML were admitted to two successive clinical trials at St. Jude Children's Research Hospital. From January 1976 to February 1980, 95 children were admitted to the AML-76 study, and from March 1980 to October 1983, 87 were admitted to the AML-80 study. None of the patients had preleukemic syndromes or second malignancies. AML was diagnosed by standard morphologic and cytochemical criteria, and cases were classified by the French-American-British (FAB) system [3].

All investigations were approved by the institution's clinical trials committee; informed consent was obtained for all patients.

Treatment

In the AML-76 study [1], remission induction therapy consisted of weekly daunorubicin (Dauno, 25 mg/m^2 i.v. day 1), vincristine (1.5 mg/m^2 i.v. day 1), 6-azuridine (15 mg/m^2 i.v. daily $\times$ 3), and cytarabine (Ara-C, 150 mg/m^2 i.v. daily $\times$ 4) administered according to a cell kinetic rationale for up to 6 weeks. Children who achieved complete remission were randomized to undergo or not

undergo splenectomy. Subsequently, all patients received monthly vincristine, doxorubicin, cyclophosphamide, and weekly Ara-C and 6-mercaptopurine for 30 months. All patients in continuous complete remission received late intensive therapy (prednisone, vincristine, methotrexate and 6-mercaptopurine) on months 31 and 32 before cessation of chemotherapy.

In the AML-80 study [2], remission induction therapy consisted of Dauno [45 mg/m^2 i.v. daily × 3) and Ara-C (100 mg/m^2 by continuous infusion daily × 7) with an additional course of Dauno (daily × 2) and Ara-C (continuous infusion daily × 5) administered if hypoplasia was not induced or when hematopoietic recovery occurred (consolidation phase). Those failing to achieve remission with two courses of Dauno/Ara-C were treated with etoposide (VP16, 250 mg/m^2 i.v. daily × 6) and 5-azacytidine (5-Az, 300 mg/m^2 i.v. daily × 4). Remissions were maintained with intensive chemotherapy consisting of sequenced drug pairs – doxorubicin/Ara-C, VP16/5-Az, 6-thioguanine/Ara-C – administered monthly over 12 months. Patients in complete remission who had HLA-matched siblings were eligible for transplantation within 16 weeks of the date of remission.

Statistical Analysis

The Kaplan-Meier procedure was used to estimate the proportion of patients in complete remission; the resulting curves were compared by the Cox-Mantel test. Follow-up observations extended through February 1986. Deaths in remission were considered relapses, while withdrawals were evaluated up to the time the patients left the study.

Results

Initial Clinical Features

The presenting clinical features of the patients in these studies were essentially identical (Table 1). For AML-76, the 95 patients ranged in age from 3 months to 19 years 11 months (median, 8.8 years), and had a median leukocyte count of 26.0×10^9/L. In AML-80, the 87 patients ranged in age from 2 months to 19 years 9 months (median 11.4 years), with a median leukocyte count of 24.6×10^9/L. No child had Down's syndrome, and only one had the Philadelphia chromosome.

Remission Induction

Sixty-eight (72%) of the 95 patients treated in AML-76 attained a complete remission. Of the 27 failures, eight died within 14 days after admission, four died before marrow recovery, three recovered with leukemic cells, and 12 failed to achieve marrow hypoplasia despite 3–5 weeks of therapy. Promyelocytic leukemia accounted for one-third of the cases of drug resistance.

Similarly, in AML-80, 65 (75%) of 87 patients entered complete remission. Six of the 22 who failed died within 14 days of the initiation of therapy; ten died during the anticipated period of marrow aplasia induced by Dauno/Ara-C; and six failed to achieve hypoplasia despite receiving VP16 and 5-Az. Four of the six early failures had promyelocytic leukemia, emphasizing the high likelihood of failure associated with this subtype of ANLL.

Remission Duration

Postinduction responses are compared in Fig. 1. The 68 patients achieving a complete remission in the AML-76 study have been followed for a median of 7.7 years. There have been 50 relapses (74%) but no deaths during remission. A similar proportion of patients have relapsed in the chemotherapy arm of AML-80, 33 of 50 (66%), with a median follow-up of 3.7 years. Complete remission durations in the two groups are not significantly different ($p = 0.13$), and the estimated probabilities of 3-year relapse-free survival are similar ($29 \pm 11\%$ versus $35 \pm 20\%$). Patients undergoing splenectomy in the AML-76 study did not fare better than the group without splenectomy ($p = 0.25$).

Although no ultimate therapeutic benefit could be demonstrated for the AML-80 re-

Table 1. Comparison of presenting features: AML-76 versus AML-80

Feature	Category	AML-76	AML-80
Sex	M	42	52
	FF	53	35
Age (yr)	< 3	31	16
	3–11	31	30
	12–17	25	31
≧ 18	8	10	
Race	W	81	76
	B	14	11
Auer rods	Yes	50	41
	No	45	46
Leukocyte count (10^9/L)	< 20	43	39
	≧ 20	52	45
FAB type	M1	11	27
	M2	38	20
	M3	6	6
	M4	24	18
	M5	14	16
	M6	2	0
CNS at diagnosis	Yes	14	26
	No	81	58
Liver size (cm)[A]	< 5	74	72
	≧ 5	21	15
Spleen size (cm)[a]	< 5	79	70
	≧ 5	16	17
Platelet count (10^9/L)	<100	72	60
	≧100	23	27
Hemoglobin (g/dL)	< 9	59	45
	≧ 9	36	42
Coagulation abnormalities	Yes	20	16
	No	75	71

[a] Edge palpable below the costal margin.

gimen, it should be noted that the patterns of failure in the two studies were quite different. Whereas the majority (64%) of patients in AML-76 relapsed before 1 year from the date of complete remission, failures in the more aggressively treated group tended to occur later (Fig. 1). The median duration of complete remission was 20 months for the AML-80 group versus <12 months for patients treated in AML-76. Incidences of extramedullary relapse in these studies were 10% and 12%.

Of the 64 children who achieved complete remission in AML-80, 21 had HLA/MLC matched siblings for marrow donors. Thus, 15 patients underwent bone marrow transplantation, with six excluded because of parental refusal (2), clostridial osteomyelitis (1), systemic aspergillosis (1), severe neuropathy (1), and young age (1).

The median time from remission induction to transplantation was 72 days (range, 41–102). Seven patients have failed (three from transplant-related complications and four from recurrent marrow disease), while eight (53%) remain in continuous complete remission for 27+ to 56+ months (median, 34+ months). Three patients have moderate-to-severe chronic graft-versus-host disease (GVHD).

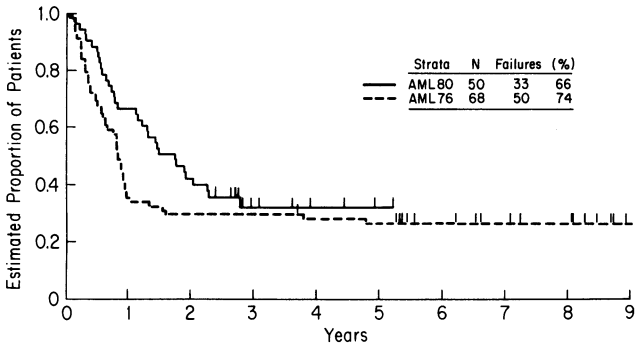

Fig. 1. Comparison of complete remission durations among patients treated in AML-76 and AML-80 (chemotherapy group only). The Kaplan-Meier plots are not significantly different ($p=0.13$)

Toxicity

Chemotherapy in AML-76 was generally administered in the outpatient clinic and was well tolerated. Although 83 of 95 patients required hospitalization during induction therapy to receive antibiotics for fever and neutropenia, the median number of hospital days was only 14 (range, 1–70). Complications during continuation therapy were rare, with an occasional patient admitted for treatment of infection associated with neutropenia. There were no deaths in complete remission.

In AML-80, by contrast, induction chemotherapy could not be given in an ambulatory setting. All 87 patients required hospitalization to receive chemotherapy and for management of hypoplasia-related complications. The median number of hospital days during induction therapy was 38 (range 1–148). During the intensive sequential maintenance chemotherapy, 50 patients were hospitalized for a median of 63 days for treatment of infections associated with neutropenia. Two children treated with chemotherapy died of bacterial sepsis, and three who received a marrow transplant died of hyperkalemia [1], interstitial pneumonitis [1], and chronic GVHD [1].

Discussion

The outcome of the AML-76 study suggested that cytokinetically based induction chemotherapy, followed by relatively nontoxic continuation treatment, will induce remissions in a respectable proportion of patients. An alternative approach, intensive induction therapy with sequentially administered drug pairs or bone marrow transplantation for remission maintenance, was tried in the subsequent protocol, affording the opportunity to compare end results obtained in a single institution.

The remission induction rate for AML-80 was not different from that in AML-76, nor was there a difference overall in complete remission durations. This suggests that chemotherapy failed to adequately reduce the initial leukemic cell burden or to adequately control residual leukemia in patients who attained complete remission. Intensification of chemotherapy to improve prognosis in AML is a relatively recent concept that remains controversial. Vaughan et al. [4] have reported a 38% 4-year relapse-free survival rate in adult patients treated with timed-sequential chemotherapy that included two cycles of daunorubicin and moderate-dose Ara-C, one for induction and for consolidation. Weinstein et al. [5] using very intensive postinduction chemotherapy, have projected a 4-year continuous complete remission rate of 50%. More recently, investigators in West Germany reported that 52% of children treated in their BFM-78 study (intensified induction and consolidation therapy) will remain in complete remission for over 4 years [6].

Current results in AML-80 show little improvement over those obtained with less aggressive chemotherapy. Moreover, there was a marked increase in hospitalization of patients and a greater interruption of normal activities. Two patients died in complete remission as a result of treatment-related aplasia. Reasons for this lack of efficacy are not clear, but may be related to inadvertent selection of a favorable-prognosis group for marrow transplantation or to the sequenc-

ing of drug pairs during remission. If the timing for introduction of each pair was not optimal or if some drugs used in combination were cross-resistant, one could expect the development and overgrowth of drug-resistant leukemic cells.

The acceptance of marrow transplantation as the treatment of choice for AML in children in first complete remission has been limited by the necessity for HLA-matched donors. While transplantation has generally been restricted to younger patients with an HLA-matched sibling, it is acknowledged to yield long-term relapse-free survival in 40% –70% of patients [7–10]. Results in our AML-80 study indicate a 3-year complete remission rate of 53%, for bone marrow transplantation, although GVHD persists in one-third of this group and there was appreciable early mortality even when suboptimal candidates were excluded.

The fact that the majority of patients with AML still relapse within 2 years indicates the inability of chemotherapy, as described here, to eradicate small numbers of residual leukemic cells. Our comparison of two strategies of chemotherapy for AML suggests that disease control in AML is more likely to be improved in the future by experimental therapies, such as bone marrow transplantation. Increased understanding of normal and leukemic cell growth may suggest an innovative approach to therapy, including use of lymphokines to modify cell growth or agents such as retinoic acid to induce cell maturation.

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Postremission Induction Intensive Sequential Chemotherapy for Children with AML – Treatment Results and Prognostic Factors

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Introduction

Advances in chemotherapy and supportive care have been associated with an increase in the complete remission rate of patients with AML and the percentage of patients, especially children, in long-term continuous remission (>than 5 years) [1–3]. Although these results have been gratifying, approximately 25% of children still fail to enter complete remission and 50%–70% relapse after treatment with chemotherapy. Previous analyses of many adult and pediatric ANNL studies have failed to identify consistent prognostic factors.

Between 1976 and 1984, we treated children with AML with two successive protocols using adriamycin or daunorubicin and cytosine arabinoside for remission induction followed by 12–14 months of post remission intensive sequential chemotherapy. We have reviewed these two protocols to update the results of the treatment programs and to identify prognostic factors that were associated with either failure to induce complete remission or leukemic relapse.

Material and Methods

Sixty-one consecutive, previously untreated patients less than 18 years of age were evaluated and entered into the VAPA protocol between February 1976 and May 1980. The next 64 patients were entered into a successor protocol (80-035) from May 1980 through March 1984. The diagnosis for AML was based on morphologic examination of bone marrow aspirate and on histochemical staining. The morphologic subgroups were classified according to the French-American-British classification. The clinical and laboratory features of the patients treated on both protocols were similar and are therefore presented as a combined group in Table 1.

Table 1. Patient characteristics

Feature	Patients	
	(*n*)	(%)
Patients entered	125	
Sex		
Male	53	42
Female	72	58
Age (years)		
<2	35	42
2–18	90	72
Initial WBC		
<100000	103	82
>100000	22	18
FAB type		
M1 and 2	48	38
M3	8	6
M4	42	34
M5	17	14
M6	5	4
UNCL	5	4
No. with CNS leukemia at diagnosis (80035)	11	17

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Table 2. Induction of remission

Drug	Dosage	Route	Course 1 day	Course 2 day
Daunorubicin	45 mg/m^2	IV	1, 2, 3	1, 2
Cytosine arabinoside	200 mg/m^2	Continuous IV infusion	1–7	1–5
Cytosine arabinoside	40 mg/m^2	Intrathecal	1	1

Table 3. Intensive sequential therapy

Sequence I (Courses 1–4)	Sequence II (Courses 5–7)	Sequence III (Courses 8–11)
Drugs	Drugs	Drugs
Daunorubicin 45 mg/m^2 Day 1	Daunorubicin 30 mg/m^2 day 1	Cytosine arabinoside 200 mg/m$_2$ day 1–5 (continuous sq infusion)
Cytosine arabinoside 200 mg/m^2 Day 1–5 (constinuous sq infusion)	Azacytidine 150 mg/m^2 day 1–5 (continuous IV infusion	Thioguanine 200 mg/m^2 day 1–5 orally
Thioguanine 200 mg/m^2 Day 1–5 orally		
(Given 4 times at 3–4 week intervals)	(Given 4 times at 4 week intervals)	(Given 4 times at 3–4 week intervals)

Intrathecal Ara-C 40 mg/m^2 – Courses 1, 2 (day 1,5), courses 5, 7 (day 1), and courses 8, 11 (day 1).

Treatment

Details of the VAPA protocol have been published [4]. The 80-035 protocol is detailed in Tables 2 and 3. As in the VAPA protocol, patients were removed from the study and induction therapy was considered a failure if a complete remission (CR) was not reached after two courses. Patients who entered CR were treated with 12 months of intensive sequential chemotherapy.

The major differences between the VAPA and 80-035 protocols included (a) the substitution of daunorubicin for adriamycin, (b) doubling of the cytosine arabinoside dose during remission induction, (c) the periodic administration of intrathecal cytosine arabinoside, and (d) the addition of thioguanine to early and late intensification.

Statistical Analysis

Duration of remission extended from the time bone marrow remission was confirmed and the duration of survical was measured from the time of initial therapy. Kaplan-Meier analyses were performed for survival and disease-free survival estimates. Statistical tests of significance were made with the log-rank test or Cox model when appropriate. Remission deaths were evaluated as relapses.

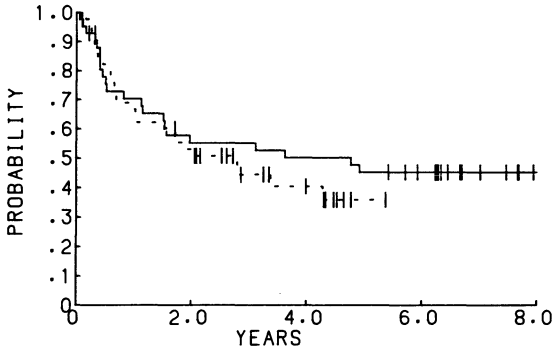
Results

Induction of Remission

Rates of complete remission were 74% (45/61) and 70% (45/64) for VAPA and 80-035, respectively. They were not statistically significantly different by protocol, age, sex, initial white cell count, or FAB subtype of AML.

Duration of Remission

The median (range) follow-up for patients in continuous complete remission is 6.7 years (5.5–9.4) and 3.4 years (1.8–5.4) for the



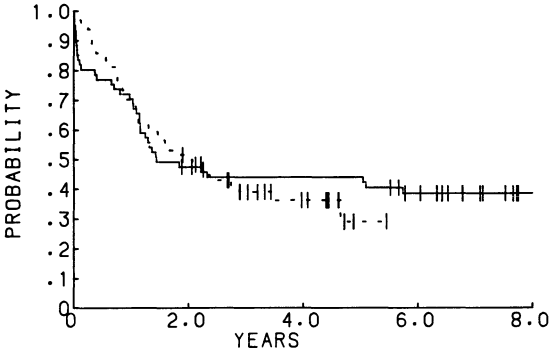
TREATMENT	CCR	FAIL	TOTAL	MEDIAN
—— VAPA	23	22	45	4.8
- - - 80-035	19	26	45	2.8

Fig. 1. Kaplan Meier plots probability of disease-free survival by protocol treatment for the complete responders

Table 4. Causes of failure during complete remission

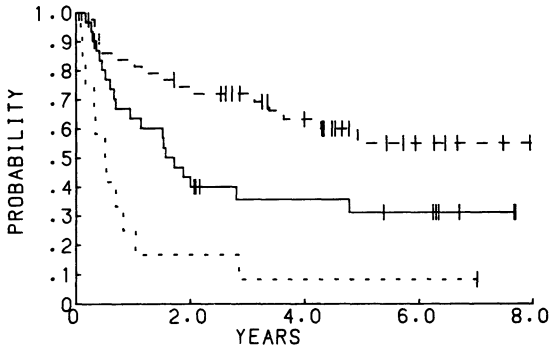
	VAPA	80-035
No. in complete remission	45	45
Deaths during remission	0	6
Relapses (total no.)	22	20
CNS	8	3
Bone Marrow	13	17
Myeloblastoma	1	0

VAPA and 80-035 protocols, respectively. Figure 1 shows Kaplan-Meier plots for the probability of remaining disease-free for the VAPA and 80-035 protocol treated patients. For patients treated with VAPA, the 4-year actuarial disease-free survival is 50%. For patients treated with 80-035, the 4-year acturarial disease-free survival is 40%. The curves in Fig. 1 are not statistically significantly different ($p=0.4$). Tabel 4 lists the causes of failure during complete remission. Eight of 22 relapses in VAPA occurred initially in the central nervous system (CNS). In contrast, only three of the 20 relapses in 80-035 occurred in the CNS. Six other failures in 80-035 were secondary to deaths in complete remission (1 from hemorrhage, 2 from infections, 1 from broncholitis obliterans 22 months in remission), and 1 from pneumocystis pneumonia in a patient who was withdrawn from the study for a marrow transplant. There were no remission deaths in VAPA. Figure 2 shows Kaplan-Meier plots of the probability of survival for all patients. The 4-year survival probability esti-



TREATMENT	ALIVE	DEAD	TOTAL	MEDIAN
—— VAPA	24	37	61	1.4
- - - 80-035	24	40	64	2.0

Fig. 2. Kaplan Meier plots of probability of survival by protocol treatment for all patients



	FAB GROUP	CCR	FAIL	TOTAL	MEDIAN
——	M4	11	20	31	1.7
- - -	M5	1	11	12	0.5
– –	OTHER	30	17	47	UNDEF

Fig. 3. Kaplan-Meier plots of probability of disease-free survival by FAB type for the combined protocols

mates are 44% for patients treated with VAPA and 36% for patients treated with 80-035.

Prognostic Factors for Remission Duration

Factors that may have influenced the duration of remission were analyzed for each study separately and with the protocols combined. Clinical and laboratory parameters tested included white blood count at the time of diagnosis, age, sex, FAB subtype of AML, and the number of courses of chemotherapy needed to achieve a complete response. The M5 FAB subtype ($p=0.000$), age less than 2 years at diagnosis ($p=0.006$), and WBC greater than 100 000 ($p=0.01$) all predicted for short durations of remission in a univariate analysis of the combined protocols. The other factors analyzed show no statistically significant difference. A step-up Cox model was used to weigh the relative importance of these factors for duration of complete remission. Monocytic subtype had the most significant influence on disease-free survival.

Toxicity

There was a 50%–75% likelihood of a patient developing fever with or without a documented infection during the granulocyte nadir of each intensification course with cytosine arabinoside. Two patients treated with 80-035 died of infection during complete remission at a time of chemotherapy-induced neutropenia. A third patient in 80-035 died of hemorrhage during a platelet nadir after a course of intensification. Cardiotoxicity, manifested as congestive heart failure, was observed in two children in the VAPA study and in five patients in the 80-035 study. The original cumulative dose of daunorubicin in the 80-035 study was 665 mg/m^2, and this was subsequently reduced to 495 mg/m^2 after the early experience with cardiotoxicity. After this modification, there was only one episode of cardiotoxicity which developed at a cumulative daunorubicin dose of 465 mg/m^2.

Following the azacytidine courses (sequence 2), there was often a long interval before the granulocyte and platelet nadirs and slow hematopoietic recovery. The interval between courses averaged 4–5 weeks (range 3–9 weeks).

Discussion

The 125 patients described here were treated with intensive chemotherapy and had a 70%–74% complete remission rate with 40%–50% projected leukemia-free survival for complete responders. The overall data from both studies continues to be very encouraging.

Our most remarkable finding is the marked influence of M5 (monocytic leu-

kemia) on disease-free survival in both studies. Four of 12 patients with M5 AML who achieved CR had primary CNS relapses. In other reported studies patients with M4 and M5 leukemia also had an increased risk for CNS relapse [5]. In 80-035, intrathecal cytosine arabinoside did not statistically significantly reduce ($p=0.09$) the incidence of primary CNS relapse when compared to the VAPA study. In the German AML study, BFM-78, cranial irradiation and intrathecal methotrexate prevented primary CNS relapse, and FAB type had no impact upon remission duration [2]. Perhaps, more effective CNS prophylaxis in our studies would have resulted in better disease-free survival for the M5 subgroup. Odom et al. have reported that infants with M5 AML have a particularly favorable outcome when treated with VP 16 or VM 26 [6]. These observations are provocative but require confirmation in a larger study. Data from several bone marrow transplant studies, interestingly, have also indentified M5 leukemia as an unfavorable prognostic variable [7].

Data from other childhood AML studies have demonstrated that white blood count or platelet count at diagnosis, spleen size, and age predicted for short durations of remission [3, 8]. We have found that age less than 2 years and white blood count greater than 100 000 at diagnosis were statistically significantly associated with shorter durations of remission. Because age and FAB subtype were confounding variables (12 of 17 patients with M5 leukemia in both protocols were less than 2 years of age), a multivariate analysis was performed which showed that M5 type was the most significant prognostic variable.

Analyses investigating the prognostic significance of various clinical and laboratory features must be interpreted within the context of the treatments. Investigators must also be cautious about concluding that a variable is of no prognostic significance. Studies like ours involving small numbers of patients have large type 2 errors (i.e., high chance of missing a clinically significant prognostic factor). In addition, our analyses were based on a retrospective review designed to identify prognostic variables rather than to verify predetermined hypotheses.

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Acute Lymphoblastic Leukemia in Adults

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Clinical Relevance of Blast Cell Phenotype as Determined with Monoclonal Antibodies in Acute Lymphoblastic Leukemia of Adults*

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Introduction

Over the past 10 years, leukemias and lymphomas have been analyzed extensively with antibodies and for enzyme activity [1, 2]. More recently, the introduction of monoclonal antibodies (MoAbs) has improved dramatically the precision, practicability, and standardization of immunophenotyping. Meanwhile, immunologic analysis of leukemia cells has become routine clinical practice, and evidence that cell typing with antibodies contributes to final diagnosis, differential diagnosis, and classification of leukemias is compelling [3].

Less clear is the question of whether the immunophenotype of leukemia cells is of prognostic relevance. Earlier studies indicated for instance a worse prognosis for T- and B-ALL compared with the large non-T group, but the meaning of those retrospective studies is strongly hampered by small patient numbers, mixing children with adults, and a lack of homogeneous treatment. Some later studies questioned any prognostic correlation when clinical factors of prognosis were analyzed in parallel [4]. Additional confusion arises from differences in typing methods and classification schemes, and the profusion and often obscure nomenclature of MoAbs has not contributed to clarification so far.

In a recent report, however, the clinical usefulness of MoAb phenotyping has been exemplified in a prospective study of childhood acute lymphoblastic leukemia (ALL) [5]. In the following, we summarize our experience with immunophenotyping in the central laboratory of a prospective large multicenter trial of ALL and acute undifferentiated leukemia (AUL) in adults which has been continuing since 1978. The typing procedures were started with well-defined, extensively absorbed polyclonal heterologous antisera together with rosette assays, as described [6], and were then extended to MoAb testing beginning in 1980. Since 1981–1982 all cell typing for this trial has been performed with selected lineage-specific or maturation-linked monoclonal reagents. In spite of these technical variations resulting from the refinement of methods, it has been possible to preserve the rough classification scheme consisting of the four categories T-/pre-T ALL, B-ALL, common ALL, and null-ALL throughout the study. This in turn has made feasible a multivariate analysis of the prognostic implication of the immunophenotype in relation to clinical prognostic factors. The results indicate that immunologic blast cell phenotype is an independent prognostic factor in ALL/AUL of adults treated by a current, intensive multidrug regimen.

Materials and Methods

Pretreatment specimens (heparinized marrow and/or blood) were obtained from 586

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Table 1. Prospective multicenter therapy trial of ALL and AUL in adults in the Federal Republic of Germany

Description of trial

Pilot study since 1978, main phase 1980–1983, since July 1983 risk-adapted trial

Evaluation date: April 30, 1985	
Patients in trial	$n=706$
Central immunologic marker analysis of pretreatment specimens	$n=586$ (83%)
Inadequate specimens (insufficient cells, aleukemic blood)	$n=$ 64 (10.9%)

Immunologic subclasses[a]

Total	Common ALL	Null-ALL	T-ALL	Pre-T ALL	B-ALL	Mixed leukemias
$n=522$ (100%)	$n=266$ (50.9%)	$n=120$ (23%)	$n=98$ (18.8%)	$n=20$ (3.8%)	$n=13$ (2.5%)	$n=5$ (0.9%)
Pilot study ($n=88$)	61.4%	23.8%	11.3%			
Main phase ($n=200$)	48.5%	22.5%	23.5%			
Risk-adapted phase ($n=234$)	51.7%	23.1%	17.5%			

[a] The immunologic subclasses are defined in Table 3.

patients of the BMFT-ALL/AUL trial of adults from 1978 to April 30, 1985. Specimens were inadequate for phenotype analysis in 64 cases (10.9%), mainly owing to insufficient cells, low blast count, or aleukemic blood samples. In 522 cases, complete phenotyping was performable (see Table 1).

Blast cells for phenotype determination were isolated by standard Ficoll-Isopaque density gradient centrifugation. Rosette assays with untreated and AET-treated sheep erythrocytes and with 7s-coated ox erythrocytes and immunofluorescence stainings with polyclonal anti-T, anti-CALLA, anti-myeloid, and anti-Ig antisera were performed as described [6]. The binding of murine MoAbs and of an ascites control was assessed with fluorochrome-labeled, affinity-purified IgGF(ab')$_2$ fragments of goat anti-mouse Ig by means of fluorescence microscopy and in part by flow cytometry, as described [7]. The following MoAbs according to WHO nomenclature [8] were used, with mixtures of MoAbs against important antigens composed in order to avoid false-negative results owing to the lack of a given epitope: VIL-A1 (CD10) against CALLA (kindly provided by W. Knapp); OKI1 against Ia-(HLA-DR)-antigens; OKT6 and NA134 (CD1) against cortical thymocyte antigen HTA-1; Lyt 3 and OKT11 (CD2) against E-receptor-associated antigen; UCHT1 (kindly provided by P. Beverly) and OKT3 (CD3) against mature T-cell-receptor–associated antigen; Leu1 and Lyt2 (CD5) against a pan-T and B-subset/CLL antigen; WT1 (kindly provided by W. Tax) against a pan-T antigen (CD7); BA-1 against a pan-B and mature granulocyte antigen (CD24); B1 (CD20) against a B-/pre-B antigen; 63D3 and VIM13 against monocytic antigens; and VIM D5 and VIM2 against myeloid antigens (kindly provided by W. Knapp). The sources and references have been given elsewhere [2]. Comparisons of MoAbs with polyclonal anti-T and anti-CALLA antisera in phenotyping of frozen cell samples revealed a 95% concordance of VIL-A1 and rabbit anti-CALLA, whereas a battery of MoAbs against T cells (CD1, CD2, CD3, CD5) were needed to substitute T-cell-specific absorbed rabbit antithymocyte sera [6]. The WT1 MoAb (CD7), however, turned out to be a nearly complete substitute for rabbit anti-T, but was introduced at a later occasion (since 1983). Since many samples have been kept frozen and have meanwhile been retyped by MoAbs, only 52 of the 522 cases have been phenotyped without the help of MoAbs.

In addition, retyping with another battery of MoAbs has been performed by one of us (B.D.) in 120 cases using the APAAP immunocytochemical method [9]. Besides being confirmatory in most cases, further subdivisions became evident by the use of an extended anti-B MoAb battery, as will be published elsewhere (Dörken et al., in preparation). Some of the results with HD37 (CD19) [10] are considered here.

Results and Discussion

Hematologic Diagnosis of ALL or AUL and Immunophenotypes

The diagnostic value of immunophenotyping becomes most evident when hematologic diagnoses based on morphology and cytochemistry are compared with immunologic diagnosis made with the same specimens. Besides routine morphological and cytochemical analysis performed at the institution of origin, in more than 80% of the cases, an additional central evaluation of morphology and cytochemistry (peroxidase, PAS, acid phosphatase, unspecific esterases, DAB IV) has been performed by one of us (H.L.). AUL was defined as an absence of morphological or cytochemical (POX, NAS) criteria for myeloid differentiation and negativity for PAS staining and acid phosphatase. Thus, subdivision into ALL or AUL was performed primarily on hematologic grounds in this study. An AUL diagnosis was made for one-third of the patients. When we analyzed 100 consecutive AUL cases for immunophenotype, the following allocation to immunologic subclasses resulted: in 49%, common ALL (C-ALL); in 18% T/pre-T ALL ; in 27%, null-ALL. In 9% of AUL cases, negativity for CALLA and B and T antigens, but strong positivity for myeloid and Ia antigens indicated a myeloid differentiation on immunologic grounds. The latter phenotype correlated with that of more than 200 additional samples received with a provisional diagnosis of acute leukemia which turned out to be nonlymphocytic by hematologic reevaluation. With a few exceptions, immunophenotyping was confirmatory in these cases (in around 5%–10% of apparently myeloid leukemias, neither myeloid nor monocytic antigens are demonstrable in our experience). We can conclude that most of the so-called AUL cases can be classified, since the majority of those cases had blast cells expressing B- or T-cell antigens.

When a hematologic diagnosis of ALL was made, the immunophenotype was not only confirmatory in the majority of cases, but also helped to classify them in subgroups. It should be noted, however, that in a small minority of 5%–10% of hematologic ALL diagnosis based on morphology and strong PAS positivity, a myeloid immunophenotype (CALLA and T- or B-antigen negative, HLA-DR and myeloid/monocytic antigen positive) was recorded. A comparison of hematologic and immunologic categories are given in Table 2. Thus, at least five main immunologic categories (C-ALL T/pre-T-ALL, B-ALL, null-ALL, myeloid immunophenotype) can be linked to both an ALL and an AUL diagnosis. This means that hematologic heterogeneity exists between immunologic subgroups, e.g., PAS-positive (ALL-type) C-ALL or PAS-negative (AUL-type) C-ALL.

Table 2. Comparison of hematologic and immunologic classification categories in peroxidase- and esterase-negative ALL and AUL

Hematologic diagnosis	Immunologic diagnosis
1. ALL (L1, L2, or L3; PAS and/or acid phosphatase positive)	ALL (C-, pre-T/T-, B-, or null-ALL) or myeloid immunophenotype[a]
2. AUL (mostly L2-like; PAS and acid phosphatase	ALL (C-, pre-T/T-, B-, or null-ALL) or myeloid immunophenotype[a]

[a] Despite the myeloid immunophenotype, a diagnosis of myeloid null-AL(L) was made when a diagnosis of acute non-lymphocytic leukemia was excluded by morphology and cytochemistry.

Immunologic Classification of ALL

As a consequence of the definition of an increasing number of immunologic cell markers, the nomenclature of immunologic leukemia classifications has changed during the last decade [1, 2, 4]. Starting from the hematologic subdivision of acute leukemias in to ALL, AUL, and ANLL, the application of the first two immunologic markers in 1972, namely the E-receptor and surface Ig, allowed a classification as T-, B-, or the widely prevalent non-T non-B ALL (see Fig. 1). The introduction of specific heteroantisera disclosed a small group of E-R negative but T-antigen-positive blasts (called pre-T ALL) and a large C-ALL group with the so-called CALL-antigen (CALLA). Thus, five subtypes were defined by 1975, namely B-ALL, T-ALL, pre-T ALL, C-ALL, and unclassified or null-ALL. The latter was defined as T- and CALL-antigen–negative, non-T non-B ALL. The demonstration of cycloplasmic Ig in around a third of C-ALL cases indicated a B-cell differentiation of this subtype called pre-B ALL. The demonstration of B-cell or T-cell antigens by MoAbs in nearly all ALL cases indicated that ALL consists of precursor-B or precursor-T cell types, except for some rare and still poorly defined myeloid-like subtypes. The view that ALL cells correlate to early lymphatic precursor cells mostly of the B lineage was supported by the demonstration of rearranged Ig heavy chains and sometimes also of light-chain gene reorganizations [11]. It appears, however, insufficient on clinical and biological grounds to allocate leukemias only to T or B lineage. It remains of the utmost importance to distinguish subclasses according to T or B maturation degree. This is best exemplified when we consider that C-ALL with its pre-pre-B phenotype and B-ALL with its activated B-blast phenotype are both B leukemias, but differ markedly in the biology of the respective disease and in their response to treatment.

Table 3 shows the classification we are using based on the reaction pattern of selected MoAbs. We still distinguish four large subgroups, namely C-ALL with the pre-B subset, T-ALL with three subsets according to T11 or HTA-1 expression, B-ALL, and null-ALL with a B-differentiated major subset and a subset of B- and T-negative myeloid-like phenotype without enzymes such as peroxidase in light microscopy. The frequencies of cases for the respective subgroups are listed for the 522 consecutive patients typed in the prospective BMFT-study of adults (see Table 1). Five cases were exceptional in that, besides having a majority of lymphoid blast cells with CALLA and B-antigen expression, an additional blast population with peroxidase was registered. These leukemias with overt myeloblastic and lymphoblastic differentiation were grouped in the category "mixed leukemias". They were

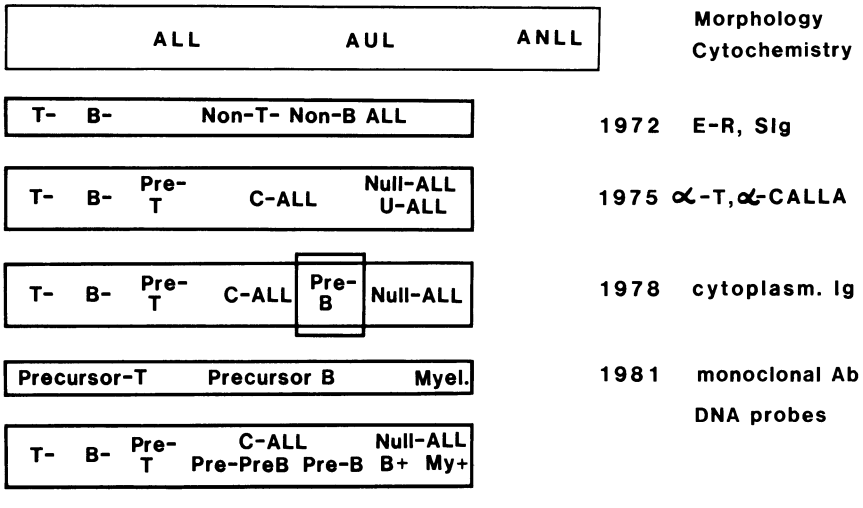

Fig. 1. Development of immunologic ALL classification during the last decade

Table 3. Antigenic profiles of ALL phenotypes

Marker (clusters; clones)	ALL								AML
	Precursor T				Precursor B				
	T-ALL			B-ALL	C-ALL		Null-ALL		
	Pre-T	Thy	T		Pre-pre B	Pre-B	B-lymphoid	myeloid	
CALLA (VIL-A1, J5; CD10)	−	+	−	±	+	+	−	−	−
B-AG (B4, HD37; CD19)	−	−	−	+	+	+	+	−	−
HLA-DR (OKIa)	−	−	−	+	+	+	+	+	+
Cytoplasmic Ig	−	−	−	−	−	+	−	−	−
Surface Ig	−	−	−	+	−	−	−	−	−
Pan-T (WT1, 3A1; CD7)	+	+	+	−	−	−	−	−	−
HTA-1 (NA134, OKT6; CD1)	−	+	−	−	−	−	−	−	−
E-R (Lyt3, OKT11; CD2)	−	+	+	−	−	−	−	−	−
MyA (VIMD5, VIM2, My9)	−	−	−	−	−	−	−	+	+
TdT	+	+	+	−	+	+	+	±	−

rather rare and should not be confused with a part of leukemias of the null-ALL group with simultaneous occurrence of immature B-differentiated (only B antigen without CALLA or Ig) and myeloid-like myeloperoxidase-negative blast cells.

Complexity of the Null-ALL Subgroup

As pointed out before, some leukemias of apparently myeloid-like immunophenotype which were diagnosed hematologically as AUL or even as PAS-positive ALL, were included in a heterogeneous group which also contained very immature ALL immunophenotypes with B antigens (CD19 and/or CD24) and TdT, but without CALLA, B1, or Ig. Besides these B-type and myeloid-like subtypes, additional subsets of still lineage-undefined immunophenotype or of mixed or hybrid phenotype (e.g., myeloid antigen-positive blasts and B-antigen-positive blasts or, rarely, blasts with simultaneous expression of myeloid and lymphatic antigens) became evident (see Table 4). In a re-evaluation of 30 cases of the null-ALL category with My7–My9 and with HD37 (CD19), only three cases (around 10%) remained in the unclassified subset. This amounts to 2% –3% of the whole ALL/AUL collective, of which null-ALL is diagnosed in 23% of cases (see Table 1). Myeloid-associated antigens have recently been recorded in several studies of ALL in children and adults [12–14]. It is presently unclear whether these myeloid antigen-positive cases represent variant forms of ALL or true cases of acute immature myelogenous leukemia (AML)

Table 4. Subsets of the null-ALL subgroups[a] in 58 patients

	HLA-DR (OKI 1)	B antigen (BA-1)	Myeloid antigens (VIMD5, VIM2)	Frequency % of null-ALL
1. B	+[b]	+	−	38
2. Myeloid	+	−	+	25
3. Mixed, Hybrid	+	+	+	9
4. U (unclassified)	±	−	−	28[c]

[a] CALLA-negative, Ig-negative, pan-T (WT1)-negative ALL or AUL.
[b] In some cases, faint CALLA-expression is demonstrable on some blast cells by sensitive techniques.
[c] The percentage of this group drops to 10% when additional MoAbs (HD37 and My 7, 9) are used.

with unusual morphological and cytochemical (myeloperoxidase-negative, PAS-positive) characteristics. In four of 14 cases of null-ALL, one of us (D. H). identified with ultrstructural and refined cytochemical studies characteristics of myeloid cells, namely, granules, ultrastructural peroxidase, peroxidase of eosinophiles, and naphthol AS-D chloroacetate esterase. Furthermore, in an earlier ultrastructural study of undifferentiated leukemias by light microscopy, a substantial number of cases disclosed myeloid differentiation [15]. In differentiation induction experiments with AUL, cell markers of early myeloid cells (MCS-2, My8, My9) were induced in some cases, together with nonspecific esterase and sometimes chloroacetate esterase, whereas TdT activity decreased [16, 17]. We must conclude, therefore, that a portion of the cases typed as null-"ALL" probably show immature myeloid differentiation. Apparently, a primitive bipotential or pluripotential stem cell stage of differentiation closely corresponding to the bifurcation of the lymphocyte/myeloid pathway can be assumed for many cases of this subgroup. Good examples illustrating this hypothesis are cases with mixtures of blasts with lymphatic or myeloid antigens, cases with hybrid expression of markers of both lineages, and cases with sequential expression of lymphoid and myeloid phenotypes during the course of the disease [12–14, 18].

A substantial number of cases in the null-ALL subgroup, however, appear to show "true" lymphatic differentiation: In addition to HLA-DR antigens, the blast cells express B antigens of the CD24 (BA-1) and the CD19 (HD37, B4) clusters. Since later B antigens (B1, B2) are not expressed and since the blasts are CALLA negative but TdT positive, an immature B differentiation status just before the phenotype of the C-ALL group has to be assumed for this subset. Accordingly, this subset had been included with the C-ALL group as B-lineage ALL in a recent report [14]. Besides CALLA expression, C-ALL differs markedly from B-positive null-ALL in 5'nucleotidase activity [19]. We therefore continue to distinguish the CALLA-postive ALL cases as intermediary to the ALL cases of precursor B origin.

Prognostic Relevance of Immunophenotypes

In contrast to the abundance of reports on phenotype characteristics of leukemias, there is still a paucity of controlled prospective studies on clinical and prognostic implications of immunophenotypes. In adults, we are aware of only one report that relates observations of remission time to blast cell immunophenotype [20], and in the following discussion, we will extend these data of the ongoing BMFT-trial with more patients and longer observation times. In another recent publication on adult ALL, only remission rates are reported [14]. Interestingly enough, only two of six myeloid antigen ALL cases responded with complete remission in this report, whereas nine of nine CALLA-negative B-lineage ALL achieved complete remission [14]. The number of patients in this study, however, is too small to draw definite conclusions. In another study of children, all 18 patients with ALL and myeloid markers entered complete remission [12]. In a large prospective ALL study (the German BMFT-ALL/AUL trial of adults), no substantial difference in remission rates was recorded for the null-ALL group [20]. However, remission duration was significantly influenced by blast cell immunophenotype, as shown in Fig. 2. The design of the trial and the therapy protocol have been published elsewhere [20]. The curves of the first consecutive phenotyped patients of the pilot and main phase are given (see Fig. 2). All patients were treated identically. There was no difference in remission duration for typed and untyped patients (data not shown). Surprisingly, it turned out that the T-ALL subgroup was the best, followed by C-ALL, whereas patients of the null-ALL category revealed a substantially worse remission curve (see Fig. 2). Re-evaluations of significance and relation to clinical prognostic factors by multivariate analyses revealed again that the null phenotype was an independent prognostic factor, together with leukocytes, age, and time to remission. Thus, the data already published for smaller numbers of patients and shorter observation times [20] have been confirmed. This is summarized in Table 5 and compared with data from an analysis of the prognostic relevance of the

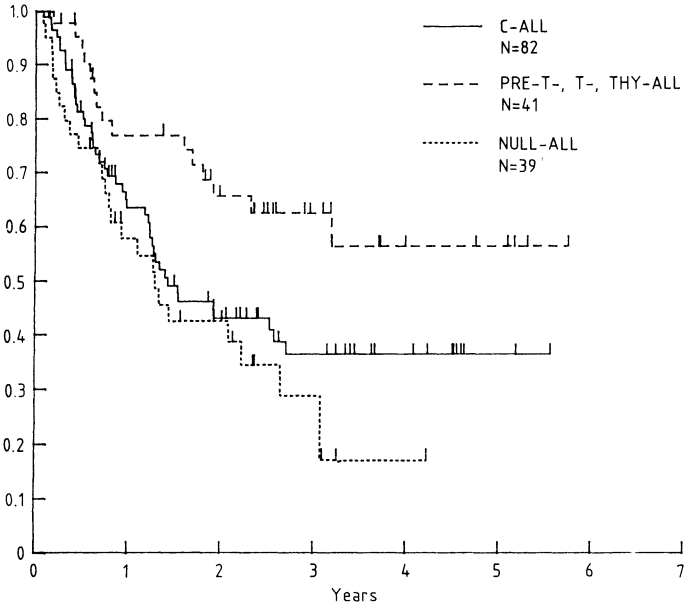

Fig. 2. Remission duration of 162 patients in the BMFT-ALL/AUL trial for adults according to immunologic subgroup

Table 5. Prognostic relevance of ALL immunophenotypes in the context of particular therapeutic regimens

I. Adults		– Immunophenotype (null-ALL T-ALL or C-ALL) is an independent prognostic factor together with – Age (>35 years) – WBC (>30000) – Response time to induction therapy
	Sequence:	T-ALL > C-ALL > null-ALL > B-ALL
II. Children[a]		– Immunophenotype only of borderline significance (significant within clinical risk groups) – WBC – Age (<2 years, >10 years) – Sex – CNS, splenomegaly, thrombocytopenia (<50000)
	Sequence:	C-ALL > T-ALL > null-ALL > B-ALL

[a] Data in [2].

ALL phenotype in 320 children, as published elsewhere [2].

In summary, we can conclude that immunophenotyping of acute leukemias is not only of importance for diagnosis, differential diagnosis, and classification, but also of prognostic relevance in relation to current therapy protocols. The heterogenous group of null-ALL, which contains leukemias with bipotential or multipotential stem cell characteristics, appears to be compromised by its early and high relapse rate. The heterogenous composition of those leukemias may be the cause of early re-emergence of blast cell variants which are resistant to ALL-type chemotherapy. This subgroup is rather rare in children, but occurs with substantial frequency in adults (see Table 1). In this connection, it is of interest that a recent analysis of 90 infants under 18 months of age identified a 51% occurrence of non-(T, B, pre-B) ALL with CALLA-negative blasts, as compared with only 7% in children aged 18 months–10 years of age [21]. The well-

known poor prognosis of infants was convincingly related to clinical factors and to the unfavorable null-ALL phenotype in this study [21]. ALL thus appears to be a convincing example of the prognostic relevance of biological tumor cell features such as immunophenotype, as described here, and chromosomal abnormalities [22].

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Risk Groups in Adult Acute Lymphoblastic Leukemia*

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Introduction

When the German multicenter trial for the treatment of adult acute lymphoblastic leukemia (ALL) and acute undifferentiated leukemia (AUL) was started in 1978, one of the main objectives was to establish prognostic factors for the definition of groups with increased risk of relapse. At that time, on the basis of large cooperative studies of childhood ALL, children could already be classified as being of standard risk or high risk [1]. It was also of interest whether the prognostic factors might differ when a comparable treatment strategy was applied to adults.

In the German multicenter therapy trial, prognostic factors for disease-free survival were extracted retrospectively in a pilot study on 162 patients entered between October 1978 and June 1981 [2]. These factors were then analyzed prospectively in an ongoing study with the same treatment protocol until Juni 1983 for a total of 384 patients [3]. Based on these prognostic factors and the risk groups of that study they served to define a new risk-adapted protocol with intensified therapy for high-risk patients which was started in July 1983.

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Design of Clinical Trials

In both trials, a central diagnosis was obligatory for all study patients, including morphological and cytochemical studies of blood and bone marrow smears (H. Löffler, Kiel) and surface marker analysis of fresh blood or bone marrow samples (E. Thiel, Munich). Details of the procedures and criteria of classification are given elsewhere [2, 4].

Treatment Regimen: Trial 01/81

The treatment protocol was based on a therapy regimen successful in treating childhood ALL [5]. The underlying principle involved intensive induction and consolidation therapy with conventional (6-mercaptopurine/methotrexate) maintenance therapy without further reinforcement cycles (Fig. 1). The 8-week induction regimen consisted of two phases. In the first 4 weeks, the following was administered: prednisone 60 mg/m^2 p.o. daily; vincristine 1.5 mg/m^2 i.v. weekly; daunorubicin 25 mg/m^2 i.v. weekly; and L-asparaginase 5000 U/m^2 i.v. on days 1–14. The second 4-week phase comprised: three doses of cyclophosphamide 650 mg/m^2 i.v.; four courses of cytosine arabinoside 75 mg/m^2 i.v. for 4 days each course; and 6-mercaptopurine 60 mg/m^2 p.o. daily. CNS prophylaxis consisted of methotrexate 10 mg/m^2 intrathecally (i.t.) weekly and CNS irradiation with 24 Gy. After 3 months, a 6-week consolidation course followed that also consisted

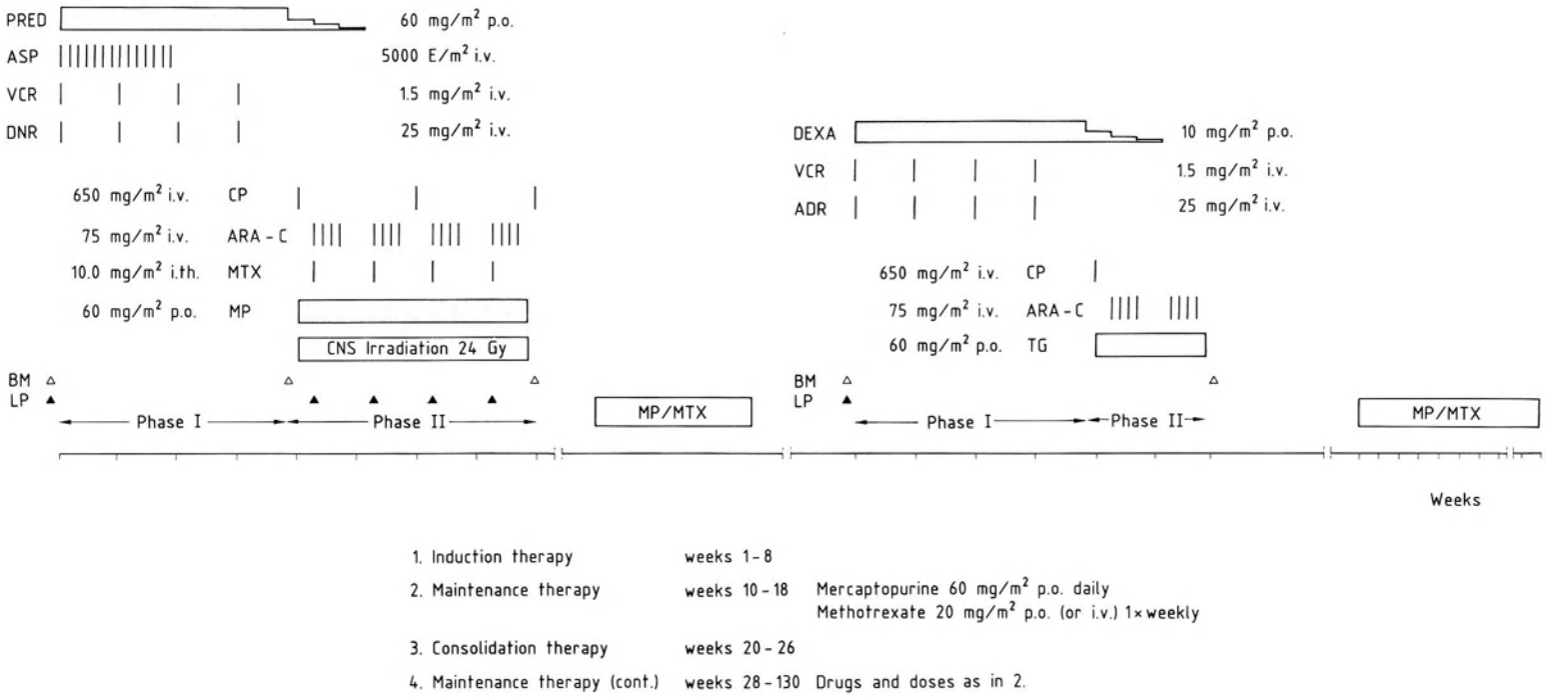
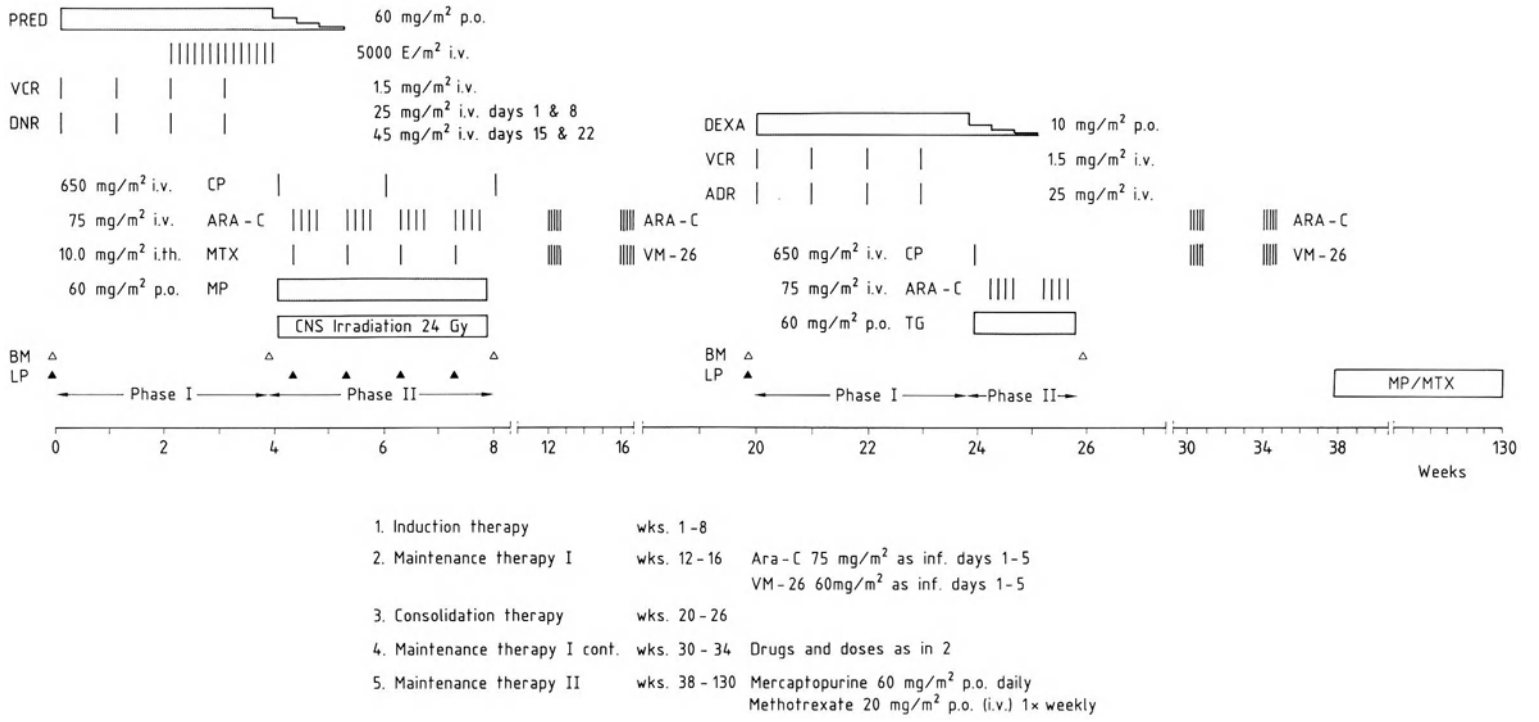


Fig. 1. Therapy regimen in the German multicentre therapy trial for adult ALL/AUL, 01/82. *PRED*, prednisone; *ASP*, L-asparaginase; *VCR*, vincristine; *DNR*, daunorubicin; *CP*, cyclophosphamide; *ARA-C*, cytosine arabinoside; *MTX*, methotrexate; *MP*, 6-mercaptopurine; *DEXA*, dexamethasone; *TG*, thioguanine, *BM*, bone marrow aspiration; *LP*, lumbar puncture



1. Induction therapy	wks. 1-8	
2. Maintenance therapy I	wks. 12-16	Ara-C 75 mg/m² as inf. days 1-5 VM-26 60mg/m² as inf. days 1-5
3. Consolidation therapy	wks. 20-26	
4. Maintenance therapy I cont.	wks. 30-34	Drugs and doses as in 2
5. Maintenance therapy II	wks. 38-130	Mercaptopurine 60 mg/m² p.o. daily Methotrexate 20 mg/m² p.o. (i.v.) 1× weekly

Fig. 2. Therapy regimen for high-risk patients in the German multicenter risk-adapted therapy trial for adult ALL/AUL, 02/84. For definitions of abbreviations, see Fig. 1

of two phases. The first 4-week phase included: dexamethasone 10 mg/m^2 p.o. daily; vincristine 1.5 mg/m^2 i.v. weekly; and adriamycin 25 mg/m^2 i.v. weekly. In the 5th and 6th weeks, the following drugs were given: cyclophosphamide 650 mg/m^2 i.v. once; two courses of cytosine arabinoside 75 mg/m^2 i.v. for 4 days each course; and thioguanine 60 mg/m^2 p.o. daily. Maintenance therapy comprising 6-mercaptopurine 60 mg/m^2 p.o. daily and methotrexate 20 mg/m^2 p.o. i.v. weekly was given between induction and consolidation therapy and was continued for 2 years.

Treatment Regimen
Risk-Adapted Trial 02/84

Several alterations were made to the standard protocol described above in order to intensify the treatment for high-risk patients (Fig. 2). These were:

1. Increasing the daunorubicin dose from 25 mg/m^2 to 45 mg/m^2 in weeks 3 and 4 to increase the complete remission (CR) rate within the first 4 weeks
2. Postponing the L-asparaginase from weeks 1 and 2 to weeks 3 and 4, since L-asparaginase had often been given for 8–10 days only instead of 14 days because of bleeding tendencies
3. Disregarding the dose limit (maximum single dose 1000 mg) for the three doses of cyclophosphamide in phase 2, since patients with a large surface area, especially male patients, might not have received an optimal dose
4. Adding four consolidation cycles consisting of cytosine arabinoside and teniposide (VM-26). The sequence of the four cycles at 1 and 2 months after both induction therapy and consolidation therapy was chosen because of the high relapse rate within the first 6 months. It was also an attempt to improve the poor outcome for the null-ALL patients in the first trial.

The other parts of the thearpy schedule remained unchanged.

Overall Results

In trial 01/81, a total of 384 patients from 33 institutions were entered during the recruitment period from October 1978 to June 1983 (Table 1). Of 368 evaluable patients, 272 achieved a complete remission (73.9%), 17 (4.6%) a partial remission, and 79 (21.5%) were failures, which also included deaths occurring during the induction period.

In the risk-adapted trial 02/84, 413 patients from 43 institutions were recruited between July 1983 and the last evaluation date, November 1985. At that time, 293 patients had completed the induction therapy; of these, 229 (78.2%) achieved CR, 12 (4.1%) partial remission, and 52 (17.7%) counted as failures, which included patient deaths during induction therapy.

Table 1. German multicenter therapy trials for adult ALL

	Trial 01/81 (*n*)	Trial 02/84 (risk-adapted) (*n*)
Recruitment period	10/78–6/83	7/83–11/85
Institutions	33	43
Total patients entered	384	413
Patients evaluable	368	293[a]
Results of induction therapy		
Complete remission	272 (73.9%)	229 (78.2%)
Partial remission	17 (4.6%)	12 (4.1%)
Failure	79 (21.5%)	52 (17.7%)

[a] Induction therapy completed.

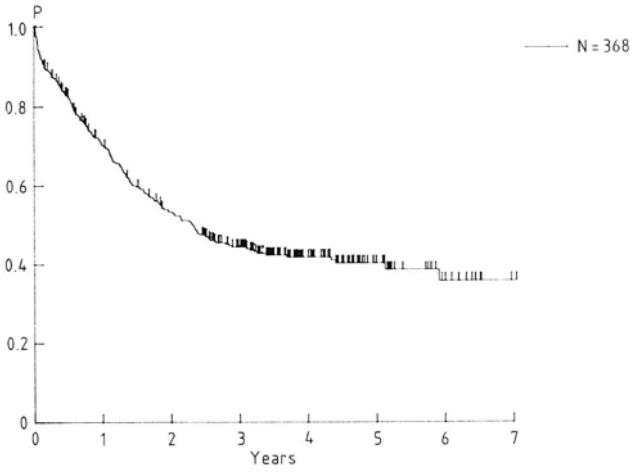


Fig. 3. Actuarial survival of patients in therapy trial 01/81

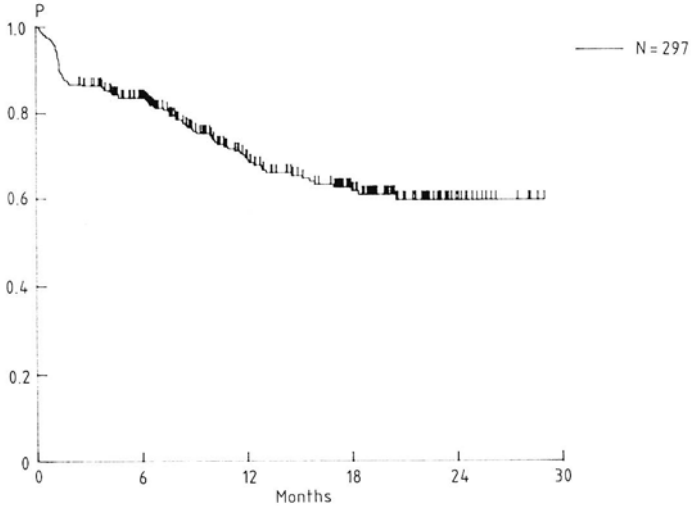


Fig. 4. Actuarial survival of patients in therapy trial 02/84

Survival

In trial 01/81, the median survival time for all 368 patients is 28.1 months. The probability of being alive at 2 years is 0.53, at 5 years 0.40 (Fig. 3), and at 7 years (last observation), 0.36. The last relapse occurred at 61.3 months.

In trial 02/84, for the 293 patients so far evaluable, the median survival has not yet been reached; the probability of being alive at 2 years is 0.59 (Fig. 4). The median observation time is, however, only 8.7 months.

Prognostic Factors

Despite improved results with chemotherapy in adult ALL, 50%–60% [2, 3, 6, 7] of adult patients are expected to die as a result of relapsing leukemia within 5 years after achieving complete remission. Thus, evaluation of prognostic factors and the definition of risk groups is urgently needed to optimize chemotherapy and to select patients for alternative therapies such as allogeneic or autologous bone marrow transplantation in first remission.

Table 2. Median remission duration and *p*-values for the four prognostic factors in trial 01/81 at varying observation periods

Prognostic factor	Patients in CR	Evaluation 30. November 1982 CR patients ($n=126$)		Evaluation 30. November 1984 CR patients ($n=268$)	
	(n)	MRD (months)	p value	MRD (months)	p value
Time to CR					
<4 weeks	95	23.3	0.0021	31.2	0.0002
>4 weeks	31	9.8		11.8	
Age					
<35 years	98	23.4	0.0055	32.1	0.0096
>35 years	28	12.7		15.7	
Leucocytes					
<30000/µl	85	28.0	0.0108	33.2	0.0032
>30000/µl	41	14.8		15.6	
Immunologic subtype					
c-ALL	31	23.4		23.6	
T-ALL	15	Median not reached	0.0427		0.0166
null-ALL	12	12.7		15.4	

The first 162 patients (Table 2) treated according to protocol 01/81 were analyzed retrospectively (evaluation date, 30 November 1982), resulting in four factors which could predict the length of disease-free survival. These factors were, in order of significance, the time required to achieve complete remission, (i.e., whether less or more than 4 weeks after start of therapy, age under or over 35 years, initial white blood cell count below or above 30000/µl, and immunologic subtype. When the patients who were entered subsequently into the study were analyzed (evaluation date 30 November 1984), these four prognostic factors were confirmed and proved to be independent by multivariate analysis (Table 2).

On the basis of these prognostic factors, patients could be classified into a high-risk or low-risk group. Those patients having none of the adverse factors were defined as low-risk patients; those having any one or more of the following criteria at diagnosis were defined as high risk: (a) time to achieve CR of >4 weeks; (b) age >35 years; (c) leukocyte count of >30 000/µl; (d) immunological subtype null-ALL (non-T, non-B, CALLA-negative).

At the evaluation date of November 1984, the median for remission duration (MRD) had not been reached in the low-risk group, compared with a MRD of 15.7 months for high-risk patients.

The risk-adapted trial 02/84 was activated in July 1983 with stratification of the patients into low-risk or high-risk groups. For the low-risk patients, the treatment schedule remained nearly unchanged; for high-risk patients, it was intensified even more (Fig. 2). A preliminary evaluation suggests that the intensification of the protocol might have benefited patients over the age of 35. However, the observation time for the risk-adapted trial has not yet been sufficiently long to assess the success of this new strategy for all high-risk patients.

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Prognostic Factors in Acute Lymphoblastic Leukemia in Adults: The Memorial Hospital Experience *

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Introduction

Acute lymphoblastic leukemia (ALL) has become a curable disease through the development of effective treatment strategies based on polychemotherapy with non-cross-resistant drugs, effective prophylaxis of central nervous system leukemia, and prolonged maintenance chemotherapy [1–4]. Lymhoblastic leukemia in children, in particular, has been treated successfully, with the majority of children surviving 5 years [5]. It was recognized during the early studies that patients had very different responses to standard therapy, and a number of models were developed to identify patient groups with different prognoses. As a consequence, children with ALL were treated differently, based on their classification as "low,"- "standard,"- or "high"-risk patients. Risk group assignments were mainly based on age, white blood cell count (WBC), and involvement of lymph nodes, hepatomegaly or splenomegaly in some series [5].

When adult patients were treated with similar protocols, for example, with the LSA_2/L-2 protocol at Memorial Hospital, they were all considered high risk because of their more advanced age, as compared with pediatric patients. The results of these early studies, which started in 1968 at Memorial Hospital, produced a significant proportion of patients with long-term remissions. Subsequent protocols, which used modifications of the initial regimen, did not lead to significantly improved results [3]. We have therefore combined our experience with the L-2, L-10, L-10M, L-17, and L-17M protocols and conducted a thorough analysis of prognostic factors. We have examined conventional and more recently developed laboratory parameters in conjunction with a large variety of clinical variables. As a result, we are now able to discriminate three groups of patients with probabilities of 77%, 47%, and 18% for maintaining remissions of 4 years or longer. As a novel prognostic variable, the ribonucleic acid (RNA) content of leukemic cells at the time of diagnosis was found to be of prognostic importance.

Patients and Methods

This study included 149 newly diagnosed, untreated, consecutive patients with ALL, who were diagnosed between 1969 and 1982. Patient follow-up is updated through April 1, 1984. The patients were treated according to the L-2, L-10, L-10M, L-17, and L-17M protocols [1–3]. Twelve patients with known Philadelphia chromosome were excluded. Since karyotype analysis has been performed on only 46% (68 of 149) of the patients in recent years, this does not exclude all patients who might have been Philadelphia positive.

A large number of clinical variables were investigated, including age, sex, weight loss, liver or spleen enlargement, absence or presence of peripheral lymphadenopathy, and mediastinal involvement.

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Laboratory variables included WBC, hemoglobin levels, platelet count, differential blood cell count including the percentage of blasts, bone marrow blast cell count, morphological characterization by FAB criteria [6], and serum glutamic oxaloacetic transaminase (SGOT), albumin, lactic dehydrogenase (LDH), calcium, protein, and alkaline phosphatase levels.

Special Studies

Cytogenetic analysis was performed on 68 of 149 patients, of whom 19 had no analyzable metaphases [7].

Surface marker analysis was done in 68% of patients (101 of 149) and used to classify ALL as T-cell, B-cell, or "null" cell type. Common-ALL antigen was studied in an insufficient number of samples to be considered for uni- or multivariate analysis.

Flow cytometric determination of the deoxyribonucleic acid (DNA) index, proliferation and the RNA index was performed with 56 patients, starting in 1977. The method has been described previously [8]. In short, bone marrow aspirate material was separated on a Ficoll-Hypaque gradient. Staining for cellular DNA and RNA content was done using the acridine orange (AO) two-step technique. Under certain conditions, this technique allows simultaneous staining of cellular RNA, which stains metachromatically red, and of native double-stranded DNA, which stains orthochromatically green. Simultaneous measurements of green (DNA), red (RNA), and green pulse width (GPW) were performed. The GPW measurements allow discrimination of single cells and cell aggregates, which were excluded from further analysis. Measurements were done in a Cytofluorograph, Model FC-200 (Ortho Instruments, Westwood, MA), and the data for 5000 cells per sample were stored in a Nova 1220 minicomputer. The DNA index was determined as per the "Convention on Nomenclature of DNA" [9], cell cycle analysis was performed using our "Peak Symmetry Model" [10], and the RNA index was determined as the ratio of ten times the mean RNA content of the $G_{0/1}$ cells of the sample to the median RNA content of normal lymphocytes [8, 10, 11].

Statistical Methods

A stepwise logistic regression analysis of prognostic factors for remission incidence (the probability of achieving complete remission) and a stepwise Cox regression analysis [12] of prognostic factors for remission duration (the rate of relapse) were performed using the BMDP statistical package [13]. For each of the two outcome variables, a final model containing a most important set of prognostic factors was determined.

The likelihood ratio test criterion was used in the logistic regression analysis, and the score test criterion was used in the Cox regression analysis. Both criteria are comparable [14, 15]. Univariate tests of association between a categorical variable and the outcome variable were performed using the Pearson (uncorrected) chi-squared and Mantel-Haenszel logrank tests [16, 17]. Pearson chi-squared tests of association between two categorical variables, *t*-tests of the difference between two means, and *t*-tests for linear correlation were also performed. Log transformed values were used here if the distribution was highly skewed.

Using the regression coefficients in the final Cox multiplicative model for remission duration, patients were separated by their log hazard ratios (a negative correlate of prognosis) into prognostic groups. The log hazard ratio is the sum of each variable multiplied by its model coefficient. Kaplan-Meier [18] remission duration curves were then used to display the differences among these groups. Kaplan-Meier curves were also performed separately for each prognostic variable.

Patients with missing covariate information were usually excluded from the fitting of a particular regression model with multiple covariates. However, too many missing values existed for two variables that were prognostically important as single factors: RNA $G_{0/1}$ index for remission incidence and remission duration, and cell surface markers for remission duration. In order to consider these variables for entry into the final logistic and Cox models, missing values were replaced by the observed mean of the variable. For a zero-one covariate, the observed mean is simply the proportion of patients with the value "one." This procedure for handling

missing data in a multiple regression model is known as the zero-order estimator [19–21]. The assumption that patients with a missing value represent a random sample from the overall group was reasonable here; therefore, the procedure was applied.

Results

Clinical Results

In this study, 149 newly diagnosed, previously untreated patients were started on induction therapy. Of these, 16 patients (10.7%) died during induction therapy, and ten patients (6.7%) failed induction therapy; i.e., they did not achieve complete remission after induction therapy or after the first course of consolidation therapy. Another 123 patients (82.6%) achieved complete remission, characterized by normal bone marrow with $\leqq 5\%$ blasts (M1), normal peripheral blood counts, and no abnormal physical findings. Of these 123 patients, 61 are continuing in on-going complete remission (as of April 1, 1984) (Figs. 1 and 2a). Another 57 patients relapsed, and five patients died in complete remission owing to bleeding or infection within 6 months after complete remission was achieved. The observed medians for remission duration and disease-free survival (deaths in complete remission are included as failures) were 44 and 39 months, respectively. These data have been reported previously [3]. Information regarding patient response to reinduction therapy was available for 47 of the relapsed patients. The percentage of patients who achieved a second remission was 55% (26 of 47), and six patients (13%) died during the reinduction chemotherapy.

Clinical and Laboratory Pretreatment Data

The median age was 25 years, the median WBC was 9600/mm^3. The median bone marrow blast count was 79%, and 57% of the patients were male (85 of 149). By FAB criteria, the majority of patients (81% or 121 of 149) had L-1 morphology. L-2 morphology was observed in 11 patients, L-3 morphology in six patients, and unclassified morphology in 11 patients.

Hepatomegaly was found in 25% (37 of 148) of patients, and splenomegaly in 40% (59 of 149). Mediastinal involvement was observed in 11% (16 of 148) and lymphadenopathy in 50% (74 of 149) of patients. Approximately 28% (41 of 149) of our patients suffered more than 5% loss of body weight prior to therapy. The median LDH level was 465 (U/liter), and the mean albumin level was 4.1. A total of 19% (25 of 129) of patients had albumin $\leqq 3.5$ (g/dl). Cytogenetic abnormalities were observed in

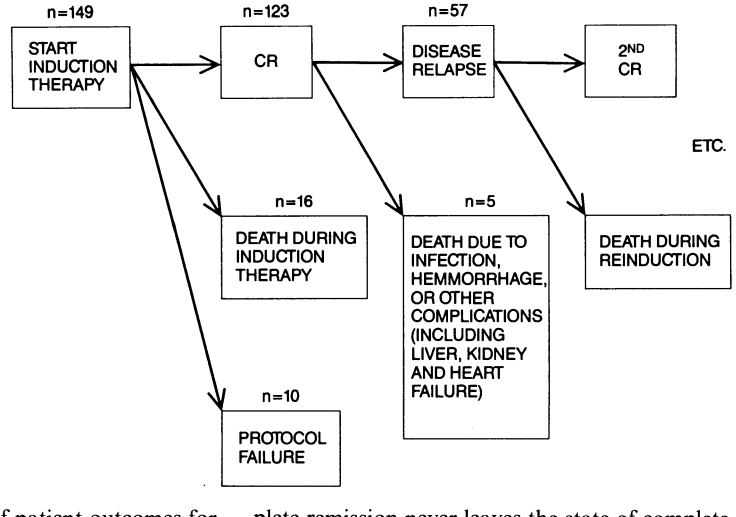
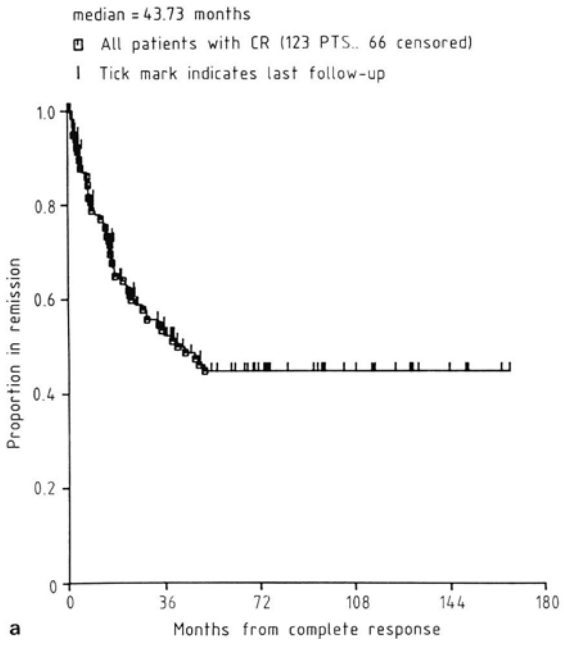


Fig. 1. Stochastic process of patient outcomes for adults with ALL. A patient in continuous complete remission never leaves the state of complete remission



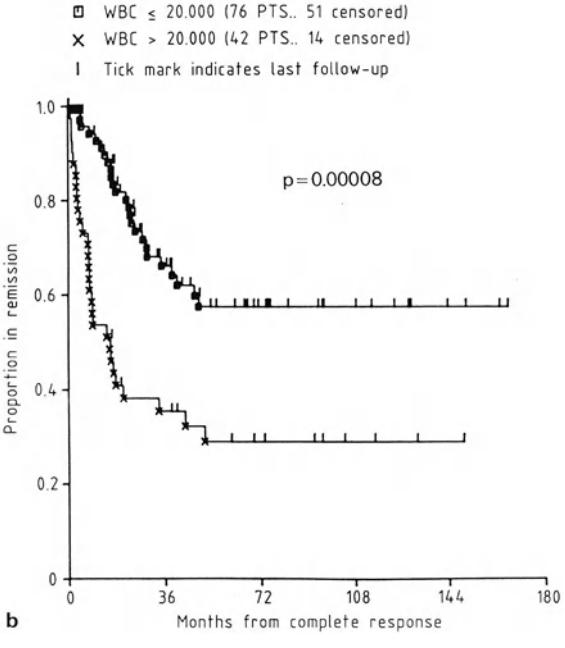


Fig. 2. a–g. Kaplan-Meier curves for remission duration in adult patients with ALL in the Memorial Hospital study. **a** Remission duration for the entire group. **b** Remission duration for patients with a WBC > or ≦20000. **c** Remission duration for patients with > or ≦80% circulating blasts in peripheral blood. **d** Remission duration for patients with a WBC ≦ or >20000 and a percent age of circulating blasts ≦ or >80%. **e** Remission duration curves for patients with T-ALL as compared with B- or null-ALL. **f** Remission duration curves for patients with RNA index > or ≦14. **g** Remission duration for patients who achieved M-1 marrow (≦5% blasts) in ≦ or >28 days

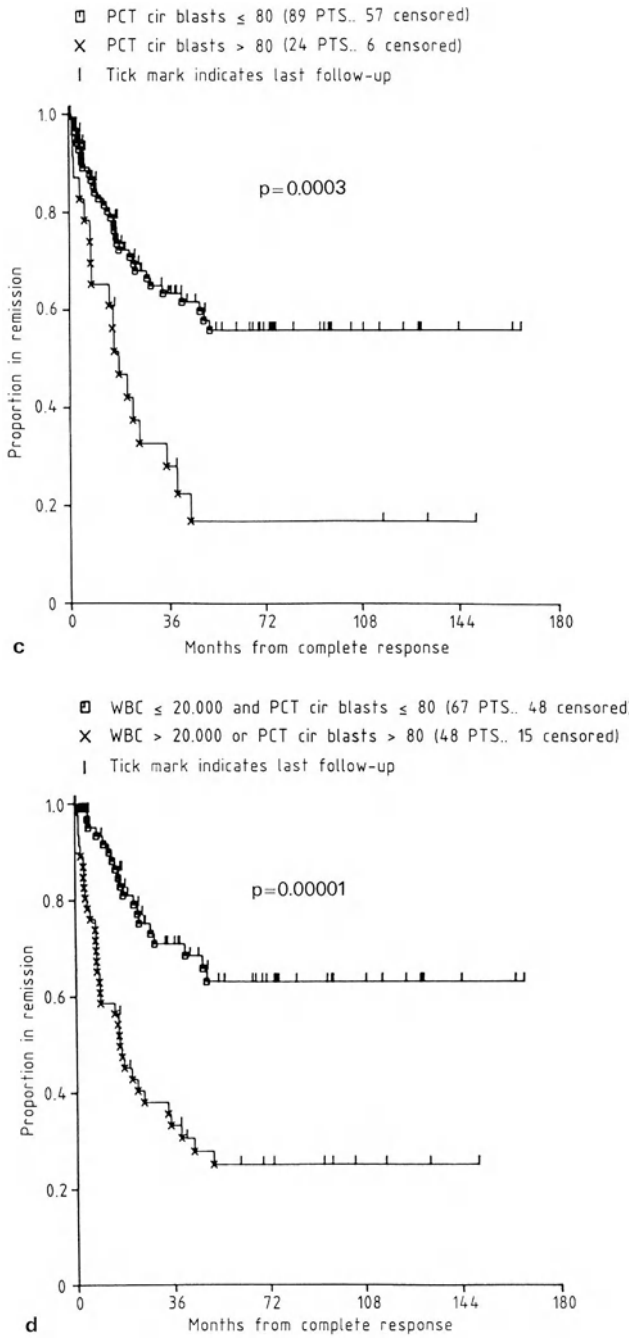
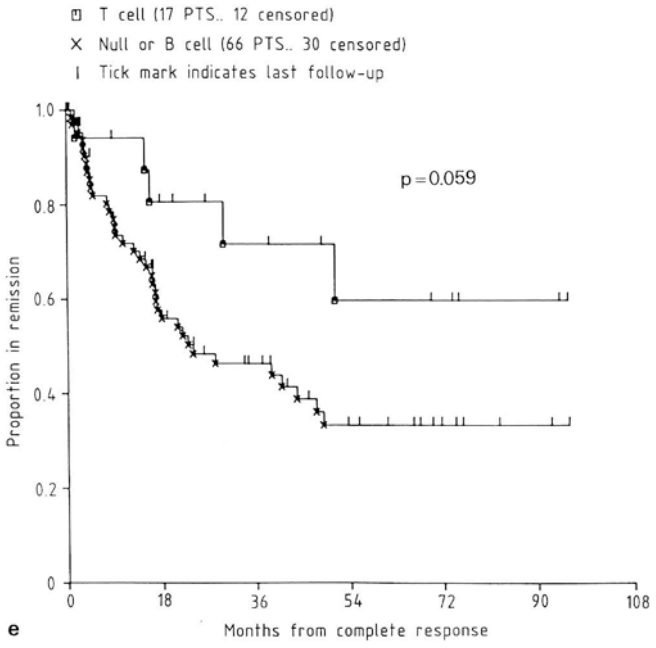


Fig. 2. c, d (continued on pp. 116–117)

28% (19 of 68) of patients studied. The karyotype was not analyzable in 19 patients, 30 patients had a normal karyotype, seven were hyperdiploid, five were hypodiploid, and seven were pseudodiploid. Cell surface marker analysis showed null or B-cell phenotype for 80% (81 of 101) of patients studied.

Flow cytometric determination revealed mean S-phase of 7.2%. The DNA index was aneuploid in 30% (17 of 56); one patient was hypodiploid, and 16 patients were hyper-



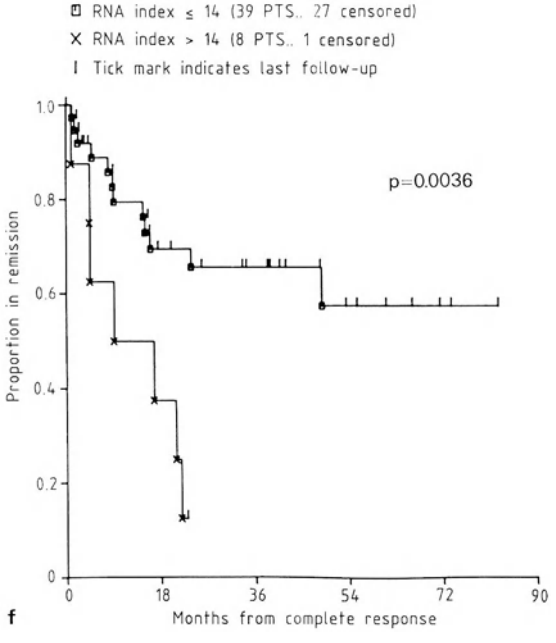


Fig. 2. e, f (continued from p. 115)

diploid. The median RNA index of bone marrow cells was 12.1.

Of 123 patients, 43 had a cytoreduction to less than 5% marrow blasts after 28 days of chemotherapy; the remaining 80 patients required 28 days or less to achieve this level.

Significant Associations Pretreatment Variables

A number of variables were associated with other variables. Table 1 summarizes significant associations. Low albumin was associ-

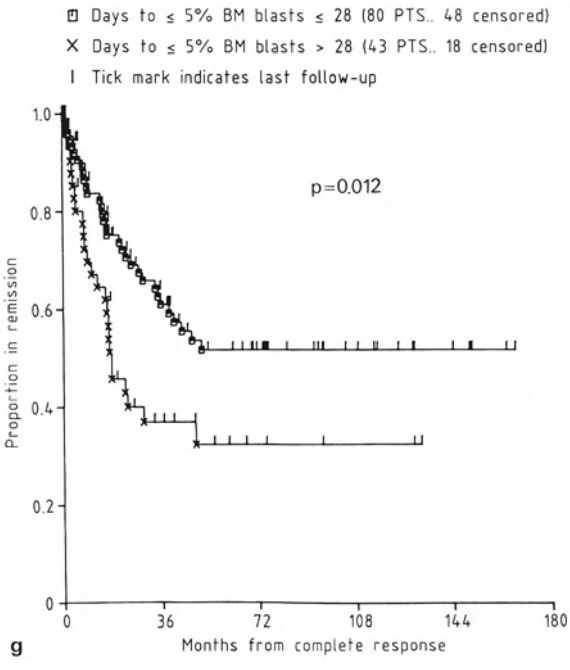


Fig. 2. g (continued)

Table 1. Significant associations between pretreatment variables in ALL

		p value
Entire group of patients		
Low albumin	Age > 50 years	0.0
	WBC $\leqq 2000/mm^3$	0.0002
	High percentage of weight loss	0.002
	Liver enlargement	0.025
	L3 or AUL morphology	0.001
L3 or AUL	Age > 50 years	0.001
	Weight loss > 5%	0.013
	Liver enlargement	0.026
L2, L3 or AUL	Male sex	0.003
High log RNA $G_{0/1}$ index	L3 or AUL	0.07
High log WBC	High percentage of circulating blasts	<0.00002
Patients achieving complete remission		
High log WBC	High log LDH	0.0005
Age >60 years	Number of days to achieve ≦5% blasts in the marrow >28	0.037
Percentage of circulating blasts >80%	Percentage of circulating monocytes ≦1%	<0.0004
High log WBC	High log absolute number of circulating blasts	<0.00002
High percentage of circulating blasts	High log absolute number of circulating blasts	<0.00002
High log WBC	High percentage of circulating blasts	<0.00002

ated with age over 50, WBC of $\leqq 2000$, high percent age loss of body weight, liver enlargement, and L-3 or AUL morphology. L-3 or AUL morphology was also associated with age over 50, loss of body weight of over 5%, and hepatomegaly. L-2, L-3, or AUL morphology were positively associated with male sex. L-3 or AUL morphology showed borderline association with a high RNA index ($P=0.07$). Not surprisingly, a high WBC was correlated with a high percentage of circulating blasts.

Other associations include those between high WBC and high LDH levels, age over 60 years and age over 60 years and more than 28 days to achieve $\leqq 5\%$ bone marrow blasts, a percentage of circulating blasts of over 80% and a percentage of circulating monocytes of $\leqq 1\%$.

Analysis of Remission Incidence

Table 2 summarizes the results of remission incidence analysis. Results are given for the univariate and logistic regression analyses. Unfavorable characteristics were L-3 or AUL morphology. a WBC of over 10 000/mm^3, more than 5% loss of body weight, a low RNA index, and older age at diagnosis. These five factors were selected into the final logistic model (their likelihood ratio test p values are listed in Table 2). Additional unfavorable prognostic factors for remission incidence were low albumin levels, and to a lesser degree, SGOT levels of $\leqq 20$ U/dl, liver enlargement, and male sex.

Analysis of Remission Duration

Figure 2a shows a Kaplan-Meier plot of remission duration. It is apparent that relapses occur for the first 48 months after achievement of complete remission. Subsequently, the curve flattens out, indicating a much lower rate of relapse. If remission duration is analyzed by WBC, patients with a WBC of $\leqq 20\,000/\text{mm}^3$ have a significantly longer remission than patients with a higher WBC ($P = 0.00008$) (Fig. 2b).

Patients with $\leqq 80\%$ of circulating blasts in their peripheral blood at the time of diagnosis have a significantly longer remission duration than patients with $>80\%$ blasts ($P=0.0003$) (Fig. 2c).

If patients are grouped by WBC and percentage of circulating blasts, two groups can

Table 2. Results of the remission incidence (yes/no) analysis listing unfavorable characteristics

Characteristic[a]	Patients with a measured value (n)	Univariate test P value	Selection into the final logistic model[b] and likelihood ratio test P value	Model coefficient and standard error
L3 or AUL morphology	149	0.00001	0.0008	(−2.65, 0.85)
WBC $>10000/\text{mm}^3$	144	0.013	0.0009	(−1.89, 0.63)
Weight loss $>5\%$	149	0.0047	0.010	(−1.50, 0.58)
Low log (RNA $G_{0/1}$ index)	56	0.027	0.011	(4.55, 2.29)
Older age at diagnosis	149	0.003	0.012	(−0.040, 0.016)
Low albumin level	129	0.005		
SGOT $\leqq 20$ (U/liter)	130	0.023		
Liver enlargement	148	0.025		
Male sex	149	0.069		

[a] This table includes zero-one covariates (1 if unfavorable, 0 otherwise) for morphology, WBC, percentage of weight loss, liver enlargement, sex, and SGOT levels. Log (RNA $G_{0/1}$ index), age at diagnosis, and albumin levels are represented as continuous covariates.

[b] The observed mean of log (RNA $G_{0/1}$ index) was substituted for a missing value in this variable's consideration for entry into the final logistic model. The final model is based on 144 patients; five patients with missing WBC information were excluded.

Table 3. Results of the remission duration analysis listing unfavorable characteristics (covariate = 1 if unfavorable, 0 otherwise)

Characteristic	Proportion of patients in complete remission with characteristic (*n*)	Univariate logrank test *P* value	Selection into the final Cox multiplicative model and score test *P* value[a]	(Model coefficient and standard error)
WBC >20000 or percentage of circulating blasts >80%	48/115	0.00001	0.0	(1.46, 0.31)
Null or B-cell ALL	66/83	0.059	0.014	(1.51, 0.65)
RNA $G_{0/1}$ index >14	8/47	0.0036	0.017	(1.19, 0.51)
Age at diagnosis >60 years	7/123	0.002	0.049	(1.24, 0.65)
Time to achieve ≦5% blasts in the marrow >28 days	43/123	0.012	0.066	(0.56, 0.31)
WBC >20000/mm^3	42/118	0.00008		
Percentage of circulating blasts >80%	24/113	0.0003		
Absolute number of circulating blasts >10000	31/113	0.001		
Percentage of circulating monocytes ≦1%	68/114	0.014		
Time to achieve complete remission >28 days	67/123	0.027		
LDH >300 (U/liter)	82/110	0.034		

[a] For the zero-one covariates defining the unfavorable effects of null- or B-cell ALL and RNA $G_{0/1}$ index >14, the observed proportions of patients with these unfavorable characteristics (i.e., the observed covariate means), 0.795 (66/83) and 0.170 (8/47) respectively, were substituted for missing values in their consideration for entry into final Cox model. The final Cox multiplicative model is based on 115 patients; eight patients with missing peripheral blood information were excluded.

be discriminated (Fig. 2d): Those with WBC ≦20 000/mm^3 and a percent age of circulating blasts of ≦80% have better than a 60% likelihood of continuing complete remission, while the remaining patients have a likelihood of less than 30% ($P = 0.00001$).

Table 3 shows uni- and multivariate analyses of factors affecting remission duration. Patients with null or B-cell surface markers had a borderline shorter remission duration than patients with T-cell characteristics in univariate analysis ($p = 0.059$) (Fig. 2e), and this comparison was significant in the multivariate analysis ($P = 0.014$). Patients with a high RNA index (>14) had a significantly shorter remission duration in both the univariate ($P = 0.0036$) and multivariate analyses ($P = 0.017$) /Fig. 2f). Patients over 60 years of age at the time of diagnosis had a significantly shorter remission duration ($P = 0.002$), and patients who required more than 28 days to achieve ≦5% bone marrow blasts had a shorter remission duration ($P = 0.012$) (Fig. 2g).

Other characteristics associated with a higher rate of relapse (i.e., shorter remission duration) were an absolute number of circulating blasts >10 000/mm^3, a percent age of circulating monocytes <1%, a time to achieving complete remission of >28 days, and serum LDH levels of >300 U/dl. However, these variables did not qualify for the final model because of their significant associations with the other variables.

Final Cox Model for Remission Duration

Table 3 lists the model coefficients for the five variables selected into the model: WBC and percent age of circulating blasts, age, RNA index, days to M-1 marrow (≦5% blasts), and cell surface markers. The model

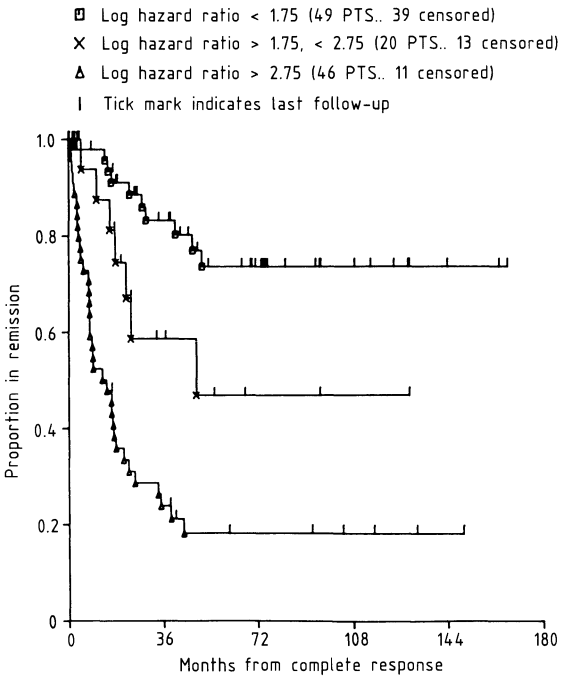


Fig. 3. Remission duration based on the final Cox model. Three prognostic groups are distinguished with good (□), intermediate (X), and short (▲) remission duration

is based on 115 patients; eight patients with missing peripheral blood information were excluded.

Based on this model, patients were separated into three prognostic groups (Fig. 3). The most favorable had a log hazard ratio of $\leqq 1.75$. It included 49 patients, of whom only ten had relapsed. Their Kaplan-Meier remission duration probability is 77% at 4 years. The intermediate group (log hazard ratios of >1.75 and $\leqq 2.75$) included 20 patients, with 13 patients showing no evidence of disease. Their remission duration curve is 47% at 4 years. The high-risk group included 46 patients, of whom only 11 show no evidence of disease. Their log hazard ratio is greater than 2.75, and they have less than a 20% likelihood of long-term remission duration (18% at 4 years). If the patients with no information on cell surface markers are excluded (36 of 115), the separation in the curves for the three prognostic groups remains essentially unchanged (figure not shown).

The most favorable group is comprised of patients with no more than one of the five negative characteristics in the final model (see Table 3). The five patients with a WBC of $>20\,000$ in this group have T-cell ALL. The model coefficient for the effect of the number of days required to achieve $\leqq 5\%$ blasts in the marrow is less than one-half the magnitude of the other variables' coefficients. Thus, the intermediate group includes patients requiring >28 days to achieve an M1 marrow with only one additional negative characteristic. The poor prognostic group is comprised of patients with null or B-cell ALL and one of the following factors: WBC $>20\,000/mm^3$, percentage of circulating blasts of $>80\%$, RNA $G_{0/1}$ index of >14, or age >60 years (time to achieve $\leqq 5\%$ blasts in the marrow of >28 days is not required).

Discussion

The results of remission incidence and remission duration analyses in adult ALL confirm previous data, published by us and others [1–4]. They confirm the importance of

WBC and age as major prognostic factors for remission incidence. Burkitt-like or undifferentiated/unclassified morphology was also confirmed to be a poor prognostic sign. As new clinical variables, weight loss of >5% body weight at the time of diagnosis and low albumin levels were indentified as unfavorable for achievement of complete remission. Weight loss is known to be a sign of poor prognosis in patients with non-Hodgkin's lymphomas (as part of the definition of "B" symptoms), but had not yet been identified for patients with acute leukemia. Both low albumin levels and weight loss may indicate poor nutritional and/or metabolic status. Results of a cause-specific hazard rate analysis to delineate how these variables are associated with remission incidence will appear in a separate paper [22].

The importance of tumor mass, as measured by WBC, confirms the results of other studies. Cell surface marker analysis (T vs null) did not contribute to the analysis of remission incidence. Patients with a low RNA index, however, had a significantly lower likelihood of achieving complete remission. Low RNA index has been associated with cell kinetic quiescence in many cell systems [23], and this result is consistent with the idea of an increased resistence to chemotherapy of quiescent cells. The results in adult ALL confirm the results by Redner et al. in pediatric ALL [24]. In their analysis of ALL in children, patients with a low RNA index had a low likelihood of achieving complete remission in 14 days, and patients who did not achieve complete remission within 14 days of treatment had a higher likelihood of subsequent relapse. In vitro investigations of lymphoblasts indicate that it may be possible to increase RNA index by the addition of interleukin-2 (IL-2) [25]. Though lymphoblasts did not have a high level of IL-2 receptors, the exogenous addition of IL-2 to these cells in tissue culture resulted in an increased level of IL-2 receptors on these cells. A similar phenomenon was observed in normal lymphocytes, where it was possible to induce IL-2 receptors by exogenous IL-2 [26]. This phenomenon may be exploited by pretreating patients with IL-2 prior to initiation of chemotherapy.

S-phase, as measured by flow cytometry, did not prove to be of prognostic importance for remission duration. This is at variance with some reports [27–29], but confirms data obtained in some series of pediatric ALL [24, 30].

Remission duration was, at least in part, affected by similar factors that were important for achievement of complete remission. A high WBC and age over 60 were unfavorable for both remission incidence and remission duration. LDH levels were significant in the univariate analysis of remission duration, but were not independent of the effect of WBC. As in other series [4, 24], slow response to chemotherapy was unfavorable for a long remission duration: Patients who did not achieve an M-1 marrow in 28 days of chemotherapy were prone to have shorter remission durations.

The unfavorable effect of a high WBC on remission duration, along with the effect of a slow response to the chemotherapy, is shown in a separate paper to diminish over time [22]. That is to say, their effects on the rate of relapse do not follow a proportional hazards model. Patients with a WBC of >20 000, as shown in Fig. 2 b, have a significantly higher probability of relapsing early; however, their prognosis is no longer unfavorable once a continuous complete remission of 1–2 years has been achieved.

Patients with a high RNA index (>14) had a significantly shorter remission duration than patients with a low RNA index. RNA index was independent of other variables and was therefore selected into the final Cox model. Patients whose leukemic cells had a high RNA index at diagnosis showed a higher likelihood of response probably because they were in the G_1 phase of the cell cycle. This feature, however, was unfavorable for long-lasting remission.

A final Cox model was developed that permits us to describe remission duration for adult patients with ALL. Three prognostic groups can clearly be discriminated based on this model, and therapeutic consequences have been drawn. Patients with the highest risk of short remission duration will be randomized to (a) chemotherapy or (b) autologous bone marrow transplantation after achieving complete remission. If a compatible donor is available, the patient will receive an allogeneic bone marrow transplant. This is a marked change, since previously only

ALL patients in second remission were candidates for bone marrow transplantation. This important change in strategy has been incorporated in the design of the present protocol for adult ALL at Memorial Hospital (L-20). One hopes that this approach will lead to improved long-term survival for patients who are presently at high risk of early relapse.

Other groups have recently obtained similar results in adult patients with ALL. The German cooperative ALL group achieved 77.8% complete remissions in 162 previously untreated adults, with a median remission duration of 20 months [4]. This group also found the T-cell phenotype to be a favorable prognostic feature for remission duration. The Italian group has achieved 79.2% complete remissions in 293 previously untreated adults, with a median disease-free survival of 16 months [31]. Finally, the SWOG group, using the L-10M protocol, has achieved 65.8% complete remissions in 158 previously untreated adults, with medians for remission duration and disease-free survival of 39.6 and 23.7 months respectively (Hussein KK, Waddell CC, Head DR, 1985, Treatment of Acute Lymphoblastic Leukemia in Adults Using Intensive Induction, Consolidation and Maintenance Chemotherapy: A Southwest Oncology Group Study, unpublished manuscript). The treatment outcomes reported from these groups are not much different from our own results.

It is therefore evident that a high number of remissions and significant remission durations can be achieved in adult patients with ALL.

We hope that selection of a high-risk group (based on a prognostic model) to receive a new treatment modality such as bone marrow transplantation will improve the remission duration and survival of these patients. Using this strategy, patients with an intermediate-to-favorable prognosis on standard therapy would not be exposed to the unproven regimen.

Conclusion

A group of 149 newly diagnosed, previously untreated adults with ALL was treated with Memorial Hospital Protocols. Variables associated with remission incidence and remission duration were identified, and stepwise logistic and Cox regression analyses were performed. Complete remission was achieved in 82.6% of patients, and of these, 44% are expected to remain in complete remission after 4 years. Variables associated with a low incidence of complete remission were: L3 or undifferentiated morphology, WBC $>10\,000$, weight loss $>5\%$, low RNA content of bone marrow cells, and greater age. Unfavorable variables for long remission duration were: WBC $>20\,000$ or $>80\%$ circulating blast cells, null or B-cell phenotype, a high RNA content (RNA index >14), age >60 years, and slow response to chemotherapy, as measured by cytoreduction to $\leqq 5\%$ marrow blasts in >28 days. A final Cox model was developed that identifies three groups of patients with probabilities for remissions at 4 years of 77%, 47%, and 18% respectively. This model includes cellular RNA content, as measured by AO flow cytometry, as a novel variable. Patients expected to have high risk for early relapse are now being randomized to alternative treatment modalities.

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Treatment of Adult Acute Lymphoblastic Leukemia. Preliminary Results of a Trial from the French Group*

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Introduction

Treatment of acute lymphoblastic leukemia (ALL) in children with effective regimens including induction with vincristine, cytoxan, asparaginase, and prednisone followed by prolonged maintenance with various drug combinations results in complete remission (CR) rates of approximately 90%, with more than 50% of the patients being possibly cured [1]. Use of similar regimens in patients over 15 has, however, been far less successful [2]. During the last decade, considerable improvement has been achieved in the treatment of adult ALL using more intensive regimens, and several protocols including more aggressive induction with anthracyclines followed by intensive consolidation and multidrug maintenance over several years have yielded therapeutic results similar to those obtained in children [3–6] and possibly similar to those achieved by allogeneic bone marrow transplantation [7] with approximately 50% long-term survivors. These regimens, however, are very toxic and may not be suitable for all patients.

The aims of the present trial were (a) to study the usefulness of aggressive CR induction and intensive CR consolidation in the treatment of adult ALL; (b) to study the value of allogeneic bone marrow transplantation performed in early first CR.

We present here preliminary results of this trial after 244 patients have been included with a median follow-up of 24 months.

Material and Methods

Patient Recruitment

Between January 1983 and March 1985, 244 patients were included in this study by the 33 institutions participating in the French Group for the Treatment of Adult ALL (FGTAALL). Criteria for eligibility were age between 15 and 60 and diagnosis of ALL of FAB L1 or L2 type based on morphological examination and cytochemistry of bone marrow smears obtained prior to therapy. Seventeen patients were subsequently declared ineligible and excluded from the study for the following reasons: six patients were over 60, six had acute nonlymphoblastic leukemia, four had non-Hodgkin lymphoma, and one had FAB L3 ALL. The results presented here concern the 227 remaining eligible patients who had a median age of 33 years.

Treatment Protocol

Induction therapy was randomized with one-third of patients receiving VCP regimen, including vincristine 1.5 mg/m^2 i.v. and cytoxan 400 mg/m^2 i.v. on days 1, 8, 15, and 22, and prednisone 60 mg/m^2 po on days 1–15, and two-thirds of patients receiving the more aggressive VRAP regimen including vincristine 1.2 mg/m^2 i.v. on days 1 and 5,

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rubidazone 450 mg/m^2 i.v. on day 1, cytosine arabinoside (araC) 100 mg/m^2/day i.v. in continuous infusion on days 1–5, and prednisone 80 mg/m^2/day po on days 1–5.

Consolidation therapy, initiated 60 days after the beginning of induction, was realized through AAA regimen, including adriamycin 40 mg/m^2 i.v. on day 1, araC 60 mg/m^2/day s.c. on days 3–7, and asparaginase 1000 μ/kg/day i.m. on days 8–12 administered in 3 monthly courses to all patients who initially received VCP induction and randomly to one-half of the patients who received VRAP induction.

Maintenance chemotherapy, administered to all patients, was initiated immediately after achievement of CR in VRAP induced patients who did not receive AAA and 1 month after the third AAA course in all other patients and comported 4-week cycles of 6-mercaptopurine 90 mg/m^2/day po and methotrexate 15 mg/m^2/week i.m. over a 2-year period with a 2-week interval between cycles. Reinduction courses were administered during the interval between maintenance cycles using alternatively R1 regimen, including vincristine 1.5 mg/m^2 i.v. and cytoxan 600 mg/m^2 i.v. on day 1 and prednisone 60 mg/m^2/day po on days 1–8, and R2 regimen, including adriamycin 40 mg/m^2 i.v. on day 1 and araC 60 mg/m^2/day s.c. on days 1–5.

Central nervous system prophylaxis was realized through IT methotrexate, 10 mg/m^2/week during the first 6 weeks of treatment, and cranial radiation (24 Gy) administered between day 45 and day 60 after initiation of induction chemotherapy.

Patients in CR having a suitable donor for HLA-identical allogeneic BMT were excluded from the postremission chemotherapy protocol and were scheduled to receive BMT within 3 months of achievement of CR. They did not receive cranial radiation and were administered R1 reinduction regimen every 3 weeks until transplantation.

Evaluation of Response

Complete remission was defined as less than 5% blasts in a normocellular bone marrow on days 28–35 after the beginning of induction therapy with normal blood counts. Patients who did not achieve CR after a first course of induction received a salvage therapy using VRAP regimen whatever the induction arm in which they were initially randomized.

Results

Remission Induction

Seven eligible patients died early before initiation of induction therapy and two patients were not evaluable for CR induction because of major protocol violations. The results for 218 evaluable patients after one course of induction chemotherapy are reported in Table 1. Of the 72 patients who received VCP regimen, 65% achieved CR and 35% had resistant disease while no death occurred during the first course of induction. Of the 146 patients who received the more aggressive VRAP regimen, 66% entered CR, 21% had resistant disease, and 13% died. Patients not achieving CR after one course of induction chemotherapy received a

Table 1. Results of the first course of induction chemotherapy in our patients

Induction regimen	Patients	CR		Resistant disease		Death	
	n	*n*	%	*n*	%	*n*	%
VCP	72	47	65	25	35	0	
VRAP	146	97	66	31	21	18	13
Total	218	144	66	56	26	18	8

CR, complete remission; *VCP*, vincristine, cytoxan, prednisone; *VRAP*, vincristine, rubidazone, araC, prednisone.

Table 2. Results of salvage therapy with VRAP after failure of initial induction by VCP or VRAP

Initial induction	Patients	CR		Resistant disease		Death	
	n	*n*	%	*n*	%	*n*	%
VCP	24	16	66	4	17	4	17
VRAP	28	9	32	11	39	8	29
Total	52	25	48	15	29	12	23

CR, complete remission; *VCP,* vincristine, cytoxan, prednisone; *VRAP,* vincristine, rubidazone, araC, prednisone.

Table 3. Overall results of remission induction according to initial induction therapy

Initial induction	Patients	CR		Resistant disease		Death	
	n	*n*	%	*n*	%	n	%
VCP	72	63	87[a]	5	7	4	6
VRAP	146	106	73[a]	14	10	26	17
Total	218	169	77	19	9	30	14

CR, complete remission; *VCP,* vincristine, cytoxan, prednisone; *VRAP,* vincristine, rubidazone, araC, prednisone.
[a] $p=0.01$.

salvage therapy using VRAP regimen; the results are indicated in Table 2, which shows considerably higher efficacy and lower toxicity of VRAP regimen when administered after failure of VCP regimen (66% CR and 17% deaths) than when administered after failure of a first course of VRAP (32% CR and 29% deaths). Overall results of CR induction for patients initially randomized in both arms are indicated in Table 3, which shows a significantly higher CR rate in patients randomized to receive VCP induction when compared with patients randomized to receive VRAP (87% vs. 73% CR, $p=0.01$). Ovrall CR rate for the whole group of patients was 77%.

Postremission Chemotherapy

A total 169 patients achieved CR. Forty-six patients with an HLA-identical sibling were excluded from the postremission chemotherapy protocol in order to receive allogeneic bone marrow transplantation (BMT) and 25 patients had major protocol violations and are unevaluable for CR duration, including eight patients who had dangerous infections following induction and five patients who were scheduled to receive intensive chemotherapy followed by autologous BMT. Table 4 shows the results of postremission chemotherapy in the 99 evaluable patients with a median follow-up of 24 months: interestingly, disease-free survival (DFS) was significantly longer in patients receiving AAA consolidation than in patients receiving only maintenance chemotherapy ($p<0.005$).

Allogeneic Bone Marrow Transplantation in First CR

Of the 46 patients who were excluded from the postremission chemotherapy protocol for allografting, six relapsed before BMT and 38 patients were actually grafted. Five patients relapsed after BMT and eight died

Table 4. Disease free survival according to induction and post remission chemotherapy

Treatment received	Patients	Median DFS (months)	% DFS	
	n		1 year	2 years
VCP+CS+M	38	29	83	53
VRAP+CS+M	32	NR[a]	77	64
VRAP+M	29	15[a]	71	23
VCP/VRAP+BMT	38	NR	78	72

DFS, disease-free survival; *VCP*, vincristine, cytoxan, prednisone; *VRAP*, vincristine, rubidazone, araC, prednisone; *CS*, consolidation; *M*, maintenance; *BMT*, allogeneic bone marrow transplantation; *NR*, not reached.
[a] $p < 0.005$.

of other complications. Overall results of BMT are indicated in Table 4. At present there is no significant difference in DFS between patients aged under 40 treated in the two chemotherapy arms with AAA consolidation and patients receiving BMT.

Prognostic Factors

Detailed analysis of prognostic factors is not yet available in our patient population. Preliminary analysis shows that none of the initial clinical or biological characteristics of our patients are predictive for achievement of CR, while WBC $> 50 \times 10^9/1$ and platelet count $< 100 \times 10^9/1$ are significantly associated with a shorter CR duration ($p < 0.005$). Age, sex, and immunological phenotype of the blasts were of no predictive value for CR achievement or for CR duration. Although numbers of patients are too low to reach statistical significance, patients having initially high WBC or low platelet count seem to have a shorter DFS after grafting when compared with other patients. Patients grafted beyond 3 months of achieving CR also tend to have a shorter DFS when compared with patients grafted earlier.

Discussion

In our group, intensification of induction chemotherapy did not significantly increase CR rate, and this was due to an increase in toxic deaths when intensive induction was performed as the first therapy and to a remarkable efficacy of the intensive induction program when performed as a salvage therapy in the 35% of patients who failed to achieve CR with a first attempt of less intensive chemotherapy in VCP arm. This does not confirm recently published studies reporting CR rates of up to 85% with aggressive induction regimens [4–6]. Those studies however did not include a control arm with less aggressive chemotherapy, and only one of them was reported from a cooperative group [5]. Increased toxicity in our aggressive induction arm by comparison with the above mentioned studies may be due to the administration of high dosages of chemotherapy over a short period of only 5 days in VRAP regimen.

Our trial establishes the value of intensive consolidation to prolong CR, and this is in accordance with the results of several studies using intensive consolidation on a nonrandomized basis and reporting continuing CR rates of 40%–50% at 5 years [4–6]. Patients allografted in first CR appear to have a slightly longer disease-free survival than patients treated in the best chemotherapy arm. However, at the time of analysis and taking into account the still short median follow-up in our series, this difference is not statistically significant. The high relapse rate in patients waiting for allograft in our series is striking, demonstrating that when allograft is considered, it should be performed as early as possible in first CR or after a course of intensive consolidation.

Summary

We present here the results of a cooperative trial in 244 adult patients with acute lymphoblastic leukemia. Induction therapy with

vincristine, cytoxan, and prednisone (VCP) gave the same complete remission rate after one course as more aggressive induction with vincristine, rubidazone, araC, and prednisone (VRAP) due to increased toxic death in the aggressive arm. Because of high efficacy of salvage therapy with VRAP regimen in patients failing to achieve CR with VCP regimen, patients initialy randomized to receive VCP had a significantly higher CR rate than patients initialy receiving VRAP (87% vs. 73%, $p=0.01$). Patients randomized to receive postremission consolidation using adriamycin, araC, and asparaginase (AAA) prior to maintenance had a significantly longer remission than patients not receiving consolidation ($p<0.005$). At the time of analysis allogeneic bone marrow transplantation does not significantly increase disease-free survival when compared with intensive consolidation chemotherapy.

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Therapy for Adolescent and Adult Acute Lymphoblastic Leukemia: Randomization of Induction and Consolidation Therapies (Preliminary Results of EORTC Study 58791)

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Introduction

The results of treatment of acute lymphoblastic leukemia (ALL) in adolescents and adults were poor 10 years ago [1] and have improved considerably since then. A recent review [2] of the subject has indicated that, indeed, around three-quarters of the patients can achieve complete remission (CR) and that median durations of CR lasting over 2 years have now been reached.

The present study was designed to investigate whether these results could be improved by adding cytosine-arabinoside (Ara-C) in the induction treatment and whether the toxicity of the efficient protocol (L-10) designed by the Memorial Sloan-Kettering Institute [3] could be decreased by omitting a 3-month consolidation phase combining methotrexate (MTX), Ara-C, and 6-thioguanine (6TG).

Concerning the first question, Ara-C was considered for the following reasons: (a) this drug was known to be effective in treating ALL [4]; (b) given as a bolus injection even at very high doses, it had been shown to have no hematologic toxicity in hematologically normal individuals [5]; (c) in acute myeloblastic leukemia (AML) at least, it had been shown that a bolus injection produced a long-lasting decrease in protein synthesis in all the leukemic cells, irrespective of their position in the cell cycle [6, 7]; and (d) it was known that a reasonable concentration of Ara-C can be found in the cerebrospinal fluid (CSF) and that, owing to a low level of deaminase, this concentration is maintained longer than in the blood.

The second question related to the consolidation phase appeared to provide an important conceptual link to the issues of (a) the efficiency of antimetabolites in the consolidation therapy of adult ALL, (b) the toxicity of a 3-month consolidation phase, and thus (c) the therapeutic index of such a consolidation therapy.

Material and Methods

The study started in 1980 and is still open to patient entry. So far, 124 patients comprising adolescents and adults (16–65 years old) treated in eight different institutions have entered the study. Four patients are ineligible, four are inevaluable, 16 are too recent, and 100 are evaluable. All patients with the diagnosis of ALL were eligible, this diagnosis including common ALL (c-ALL), T-ALL, B-ALL, and undifferentiated acute leukemia. The patients with Philadelphia chromosome (Ph^1 c)-positive ALL, an enlarged mediastinum, or meningeal infiltration at diagnosis were not excluded. Patients with overt liver or renal failure, those who had already received chemotherapy, and those with another malignancy (except epithelial skin tumor) were excluded from the study.

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The patients eligible were randomized to receive induction treatment with or without one pulse injection of high-dose Ara-C. The patients were stratified for randomization according to institution. The patients who reached CR by day 42 were randomized a second time for consolidation therapy (long or short consolidation). They were stratified according to the first randomization branch.

The induction therapy comprised adriamycin 30 mg/m^2 of body area intravenously (IV) as a bolus injection on days 8, 29, and 43; prednisone 60 mg/m^2 given as three daily doses from day 1–day 28 and then tapered off over 2 weeks (days 29–43); and vincristine 1.4 mg/m^2 IV as a bolus injection on days 1, 8, 15, 29, and 43. Ara-C was given only to the patients allocated to this therapy by randomization and was administered as a bolus injection of 2500 mg/m^2 IV on day 1 (or 2 or 3 in case of high tumor mass and fear of tumor lysis syndrome).

Central nervous system (CNS) presymptomatic treatment was administered between day 1 and day 57. This consisted of preservative-free MTX administered intrathecally (i.t.) by lumbar puncture at a total dose of 12 mg (in a volume of 10 ml CSF) on days 1 (or 2 or 3 if there was severe thrombocytopenia or a high WBC) 15, 29, 36, 43, and 57. Radiotherapy to the brain was given to the skull, the base of the skull, and the first two cervical vertebrae at a dose of 1800 rads midplane delivered in ten sessions over 12–14 days.

Consolidation therapy was started on day 58, i.e., after completion of CNS presymptomatic treatment. The patients were randomized to receive either a long (4 months) or a short (1 month) consolidation.

The long consolidation, started at week 8, comprised a phase of methotrexate given at 15 mg/m^2/day (maximum daily dose of 25 mg) from day 1 to 3 for the first course and, if well tolerated, from day 1 to 4 or 5 for the following courses. This was followed at week 10 by a phase comprising 6-TG 120 mg/m^2 p.o. every 12 h from day 1 to 5 and Ara-C 150 mg/m^2 IV every 12 h, also from day 1 to 5. At week 13, MTX was repeated as at week 8, but the duration (4 or 5 days) was adapted according to the patient's tolerance during the first cycle. At week 15, 6-TG and Ara-C were given for 5, 6, or 7 days depending on the tolerance of the previous cycle, and one dose of MTX i.t. was also administered. At week 18, MTX was given again IV as at week 13. At week 20, the treatment of week 15 was repeated. At week 21, asparaginase 1000 units/kg of body weight was given IV six times over 2 weeks. At week 23, cyclophosphamide 1200 mg/m^2, or only 1000 mg/m^2 to patients over 60 years, was administered IV.

The short consolidation comprised only asparaginase (week 8) six times over 12 days and cyclophosphamide (on week 10) once at the end of the L-asparaginase cycle exactly as given in the long consolidation therapy.

Maintenance therapy was given for 3 years and consisted of two successive phases: high maintenance (approximately 60 weeks) and low maintenance (approximately 96 weeks). The high maintenance phase consisted of six courses of 70 days. Each course started with prednisone 180 mg/m^2/day p.o. in three divided doses from day 1 to 7 and vincristine 2 mg/m^2/week on days 1 and 8. On day 15, the treatment conisted of either adriamycin (on courses 1, 3, and 5) at doses of 20 mg/m^2/day IV on days 15, 16, and 17 or bis-chloroethyl-nitrosourea (BCNU) and cyclophosphamide IV on day 1 (on courses 2, 4, and 6) at respective doses of 80 and 800 mg/m^2/day. Other drugs were started on day 29, comprising: 6-mercaptopurine (6MP) (90 mg/m^2/day p.o.) (from day 29 to 56), MTX 20 mg/m^2/day p.o. (on days 36, 41, 46, 51, and 56), and MTX (12 mg total daily dose i.t.). The course was terminated by one injection of actinomycin D 1 mg/m^2/day IV on day 64. Another course was started on day 70. After 6 courses of consolidation of this type, the low maintenance was started and given until completion of 3 years of maintenance therapy. It comprised daily 6MP 90 mg/m^2 p.o., weekly MTX 20 mg/m^2 p.o., and, every 3 months, discontinuation of 6MP and MTX and administration of vincristine 1.5 mg/m^2 on days 1, 8, and 15 and prednisone 40 mg/m^2 p.o. on days 1–15. In most patients, these doses had to be decreased, primarily according to hematologic tolerance.

CR was defined as the disappearance of clinical and hematologic signs of leukemia. CR blood showed no lymphoblasts and

Table 1. Patient and disease characteristics at diagnosis

Median age	26.7 years
Range	16–65 years
WBC > 50000 µl	22%
Initial liver and spleen enlarged	29%
Initial CNS infiltration	6%
CALLA +	55%
T-All	12%
Ph[1] chromosome	12%

Table 2. Overall preliminary results of EORTC 58791

Death during induction	8%
CR	74%
Median duration of CR	32 months
CR at 3 years	52%
CR at 5 years	38%
Median survival of CR	33 months
Median survival of ALL	25 months
Treatment-related death during CR	0/69
Isolated extra BM relapses/ all relapses	2/30
Isolated CNS relapses/All CR	1/69
Latest relapse	46 months

more than 500 granulocytes and >75 000 platelets/µl. CR in the bone marrow (BM) was defined as normal cellularity; normal representation of erythroid, myeloid, and megakaryocytic elements; and a percentage of blasts equal to or less than 5%.

The tests performed for immunophenotyping have been modified during the course of the study. It is presently possible to define five groups: (a) T-ALL, as defined by E-rosetting in the beginning or later by a T monoclonal antibody (more than 20% T-type lymphoblasts); (b) pre-pre B or c-ALL, as defined by an anti-CALLA antibody (more than 20% CALLA-positive blasts); (c) B-ALL, as defined by more than 20% blasts bearing a monoclonal surface membrane immunoglobulin (SmIg); (d) undiffer-

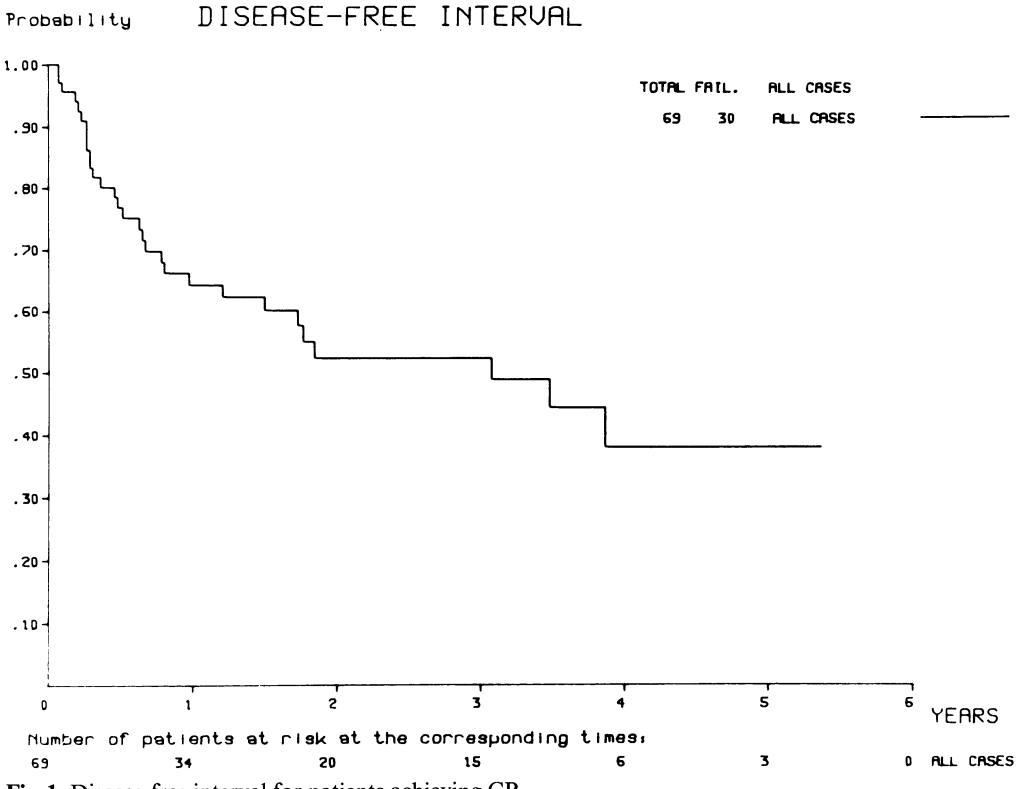

Fig. 1. Disease-free interval for patients achieving CR

entiated ALL with <20% T blasts, <20% SmIg-positive blasts, and <20% CALLA-positive blasts; (e) unclassifiable ALL, for which insufficient tests were performed to allocate it to one of the above-mentioned groups.

Successful cytogenetic analysis was obtained in only 47 of the 100 evaluable patients. This analysis showed a cytogenetic abnormality in 20 patients, of whom six showed a Ph^1c.

All data were collected, processed, and analyzed at the EORTC Data Center. The criteria of evaluation were the percentage of patients achieving CR, disease-free interval (DFI), and survial. To determine the correlation between a categorical variable and the percentage of CR, the usual chi-square test has been used. Actuarial curves were calculated according to the Kaplan-Meier method, and the prognostic value of different varibles related to the duration of survival and DFI have been tested using the log rank test.

Results

Table 1 shows the patients' characteristics, while Table 2 summarizes the overall results. In Fig. 1, the DFI of the patients who reached CR is represented. It can be seen that the majority of relapses occured within 1 year of CR, but that some BM relapses occurred between the 3rd and the 4th year of CR. Only two patients showed isolated extramedullary relapse, one in the mediastinum and one in the CNS. This CNS relapse occurred in the only patient not receiving CNS irradiation but rather, MTX i.t. through an intraventricular Ommaya reservoir because of an anatomic malformation making intralumbar MTX administration impossible.

Figure 2 shows that the WBC at diagnosis (< or > than 50 000/μl) has significant prognostic value for DFI.

Figure 3 A illustrates the prognostic value for DFI of the simultaneous presence of a palpable liver and spleen at diagnosis. This

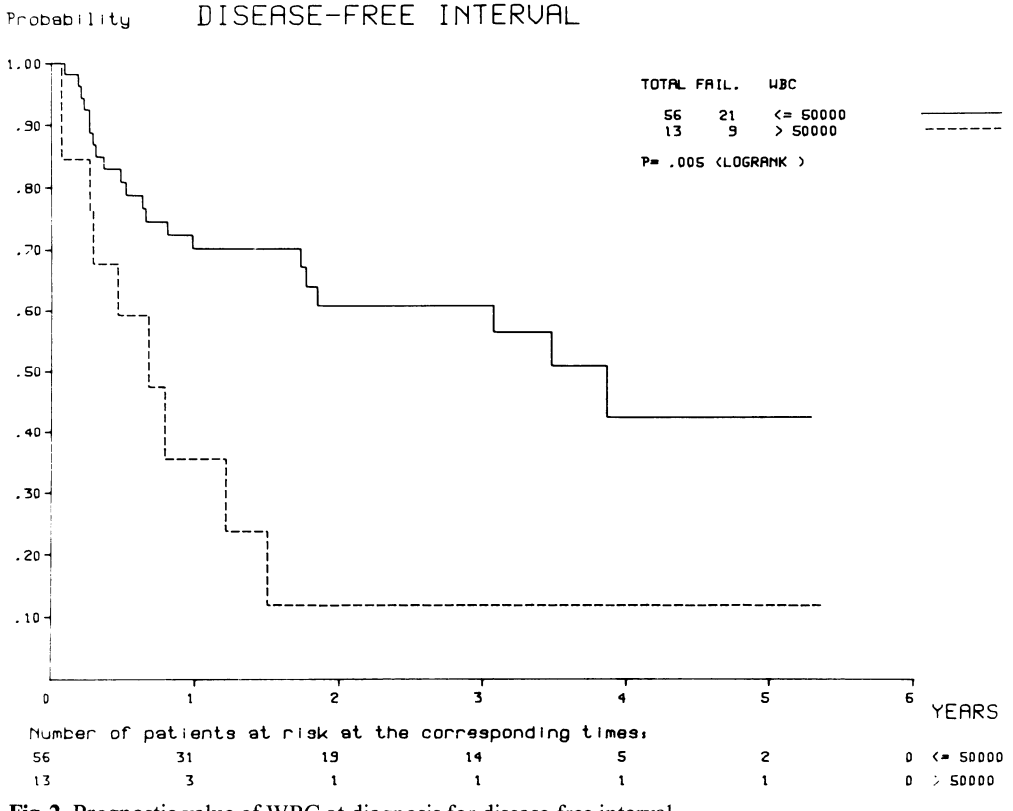

Fig. 2. Prognostic value of WBC at diagnosis for disease-free interval

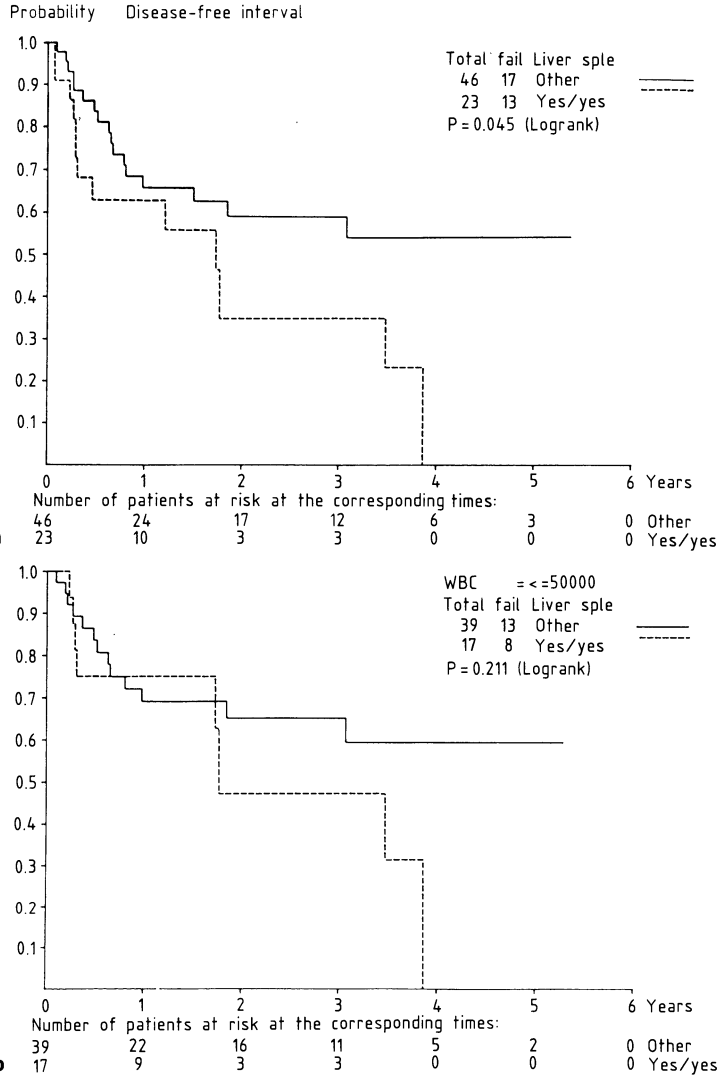

Fig. 3 a, b. Prognostic value for disease-free interval of **a** hepatosplenomegaly at diagnosis and **b** hepatosplenomegaly at diagnosis as related to a WBC of $< 50\,000/\mu l$. ---, patients with palpable liver and spleen at diagnosis; ——, all other patients (including those with palpable spleen or liver or with impalpable spleen and liver)

anomaly was correlated with the WBC, but was prognostically independent. In other words, it was related to a shorter DFI among the patients with fewer than 50 000 WBC/μl (Fig. 3 B), as well as among those with over 50 000 WBC/μl (not shown).

Table 3 summarizes the prognostic value of some patient, disease, and treatment characteristics in terms of the percentage of CR, DFI, survial of remitters, and overall survival. It is apparent that performance status at diagnosis seems to influence survival and that age does not influence either the incidence of CR or survival. Most important is the fact that neither the type of induction (pulse Ara-C vs. no Ara-C) nor the type of consolidation (long vs. short) seems to influence the prognosis. The time required to reach CR (more or less than 4 weeks) did not influence the prognosis either. In the present study, the percentage of blasts remaining in the BM on day 7 was the only prognostic

Table 3. Prognostic significance of patient, disease, and treatment characteristics related to the percentage of CR, duration of CR (t CR), and duration of survival (tS) for patients in CR and for all patients (remitters and nonremitters)

Features	% CR	t CR	tS (CR)	tS (all)
Age (< or >30 years)	NS	NS	NS	NS
Sex	NS	NS	NS	NS
PS (< or >60%)	NS	0.02	0.007	0.002
WBC (< or >50000/μl)	NS	0.005	0.02	0.04
Spleen (palpable or not)	NS	NS	NS	NS
Liver (palpable or not)	NS	NS	0.02	NS
Liver and spleen (palpable or not)	NS	0.045	0.006	NS
Induction	NS	NS	NS	NS
Consolidation		NS	NS	NS
% BM blasts at day 7	0.01	NS	NS	0.03
% BM blasts at day 28		NS	NS	

The induction treatment was with or without ARA-C, and the consolidation phase was long or short. The response to treatment was estimated by examination of the BM either at day 7 (expressed as more or less than 50% blasts) or at day 28 (expressed as more or less than 5% blasts).
PS, Karnofsky performance status; NS, not significant.

predictor of achievement of CR at day 42:88.6% achieved CR if there were <50% blasts on day 7, compared with 59.3% if there were >50% blasts.

Discussion

The present results are very similar to those of other studies, as summarized in a recent review by Clarkson [2], and particularly to those of the German Multicenter Trial for Adult ALL presented at this meeting and showing a DFI rate at 5 years of 38%. Such results represent progress compared with the situation 10 years ago, but also serve to emphasize the need for other therapies. BM transplantation most probably plays an important role in the therapy of adult ALL; however, the various lethal complications associated with its use make it crucial to exclude from this therapeutic procedure the ALL patients with very good prognosis and thus to develop a means of identifying these patients.

The prognostic value of a palpable liver and spleen at diagnosis, seen in the present study, is reminiscent of the value attributed to the size of spleen and liver in the study of Riehm et al. in childhood ALL [8]. These authors have hypothesized that the size of these two organs, in addition to the number of peripheral blood blasts, is a good reflection of the overall tumor mass. Moreover, they have shown that the size of the tumor mass estimated with these parameters is the most important prognostic factor in childhood ALL. The present study of adults and adolescents is thus apparently in agreement with this concept. Indeed, Fig. 3 B makes clear that, by combining hepatosplenic size and WBC, it is possible to define a subgroup of patients (no hepatosplenomegaly and a WBC below 50 000/μl) with a DFI rate at 5 years of 60%. These results are similar to those presented by the German BMFT ALL/AUL Study Group at the present meeting and earlier [9]: 60% continuous CR at 5 years for 1/3 of patients who presented none of the four high-risk factors which this study group identified (i.e., time to CR <4 weeks, leukocyte count <30 000/μl, age <35 years, and the T-ALL or c-ALL subtypes.

We could not confirm in the present study the good prognosis in terms of DFI for the patients reaching CR in 4 weeks, for those under 35 years, or for those with T-ALL or c-ALl. It should be admitted, however, that the number of patients adequately immunophenotyped was probably too low (approximately 50% of all the patients) to draw defi-

nitive conclusions concerning the value of immunophenotypes in the present study. As for the WBC at diagnosis, it showed a highly prognostic value when the limit was put a high as 50 000/μl, but not at 30 000/μl, as in the German study.

It should be emphasized that none of the patient or disease characteristics at diagnosis were prognostic in terms of achievement of CR. The only parameter which was helpful in this regard was an early consequence of treatment, namely, the percentage of blasts remaining in the BM at day 7 of induction (Table 3). Indeed, if this value was less than 50%, the chances of achieving CR by day 42 were significantly higher than if more than 50% blasts remained. This parameter thus appears to be an important piece of information for early adaptation of therapy during the course of treatment.

Up to now, the two consolidation therapies which have been compared show no difference as to their therapeutic effect. The data concerning their relative toxicities has not yet been analyzed completely, but it is interesting to note that so far neither consolidation caused lethal toxicity in CR patients.

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Acute Lymphoblastic Leukemia in Children

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Therapy Results in Five ALL-BFM Studies Since 1970: Implications of Risk Factors for Prognosis

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for the BFM Study Group

Introduction

Prognosis in childhood acute lymphoblastic leukemia (ALL) after risk-adapted therapy is first of all dependent on the quality of therapy. Conventional risk factors such as WBC, age, sex, organ involvement, and other features certainly have lost their prognostic significance in varying degrees during the evolution of risk-adapted and necessarily intensive therapy. Still, the tumor burden and other ill-defined or unknown factors are responsible for therapy failure. Obviously, the patient group with therapy failure must be the target of future efforts.

Of the initial 119 patients in the nonstratified study ALL-BFM 70 (BFM, Berlin-Frankfurt-Münster) 63 patients remain in the first complete continuous remission (CCR) 10–16 years after diagnosis [1] which indicates a 54% event-free survival according Kaplan-Meier life table analysis (Fig. 1). In all of the consecutive studies ALL-BFM 76–83 [2–4], the introduction of an intensive reinduction therapy element (protocol II) early in remission [5] for approximately one-third of patients at increased risk for relapse (discriminated by a risk score) further improved long-term survival by approximately 20%. Of 1641 patients enrolled in these studies, 1258 (76.6%) are currently in complete uninterrupted remission. Probability for long-term event-free survival according life table analysis is 67% (SD 4%). These results imply that the major improvement of treatment occurred in the initial two studies, since the following trials did not further increase the over-all cure rates (Fig. 1) in spite of minor modifications in respect to drug dosage (daunorubicin), timing (L-asparaginase), and introduction of a new compound (intermediate dose methotrexate). It must be kept in mind, however, that "positive" modifications may have been neutralized by "negative" ones. Minor changes from one study to the next which have been considered at that time to be appropiate have not been carried out in a one-step fashion.

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Patients at Risk for Relapse

Detailed analysis of the ALL-BFM 70 study with regard to prognosis showed a significant correlation to the initial number of peripheral blood blasts, and the sizes of the liver and spleen. Assuming the total tumor load at diagnosis to be the decisive prognostic predictor, a risk factor (RF) has been calculated according to the equation:

$$RF = 0.2 \log (\text{Blasts} + 1) + 0.06 \times \text{Liver} + 0.04 \times \text{Spleen}$$

(organ size in cm below costal margin).

The RFs of all patients in studies ALL-BFM 76 and ALL-BFM 79 have been calculated retrospectively. Despite risk-adapted therapy in these studies, the RF nevertheless discriminated on different levels for the various risk groups quite appropiately, children with a RF of more than 1.7 still being at a clear disadvantage [6, 7]. The nomogram for

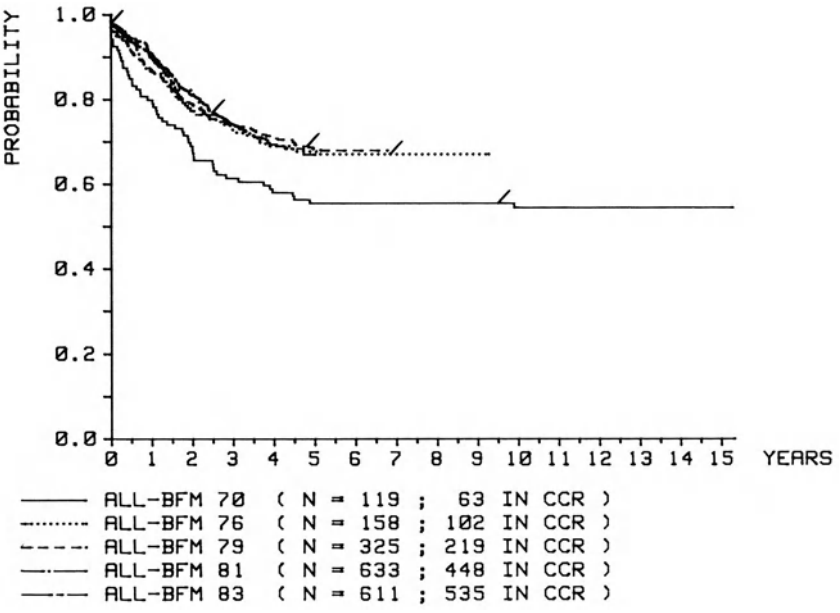

Fig. 1. Probability of event-free survival in five consecutive studies ALL-BFM 70–83. Data for probability of CCR (p-CCR) according to life table analysis (Kaplan-Meier): ALL-BFM 70, 0.54 (SD = 0,05); ALL-BFM 76, 0.67 (SD = 0,04); ALL-BFM 79, 0.68 (SD = 0,03); ALL-BFM 81, 0.67 (SD = 0,02); ALL-BFM 83, 0.76 (SD = 0,03). $p < 0.03$ for ALL-BFM 70 vs. BFM 76. Slashes indicate last patient on individual studies, as in all other figures

its calculation is given in Fig. 2. In studies ALL-BFM 81 and ALL-BFM 83 this RF has been utilized to classify patients to receive risk-adapted therapy (except B-ALL). In study ALL-BFM 81 three therapy branches have been considered to cope with the relapse problem by applying adequate risk-adapted therapy [3, 5]. Standard risk (SR) patients were defined by a RF below 1.2, medium risk (MR) patients by a RF 1.2–1.7, and all patients with a RF of more than 1.7 as high risk (HR) patients. In study ALL-BFM 83 the standard risk group has been further subdivided into patients with standard risk low (RF <0.8, SR-L) and those with standard risk high (RF 0.8– <1.2, SR-H). Randomizations for preventive CNS irradiation in SR patients, duration of therapy, and the introduction of a more intensive therapy in HR patients have been the rationals and questions in study ALL-BFM 81. The recognition that B-ALL is a separate biological entity led consequentely to a more specific therapy protocol [8] followed by remarkably improved results. Unfortunately, none of the individual risk parameters such as WBC, liver and spleen size, and CNS disease at diagnosis [6, 7] have disappeared completely in these studies, obviously because other biologically more fundamental principles have still not been taken in account or cannot be influenced properly (Tables 1–4). Certainly age (e.g., infants less than 2 years of age as well as teenagers) and probably the thrombocyte count and hemoglobin level also have some impact on prognosis.

Therapy Results in Studies ALL-BFM 81 and ALL-BFM 83

Therapy results to the two most recent ALL-BFM studies are presented in Tables 1 and 2. The analyses were performed in May 1986 with a comparable number of patients in both studies (ALL-BFM 81, $n = 633$; ALL-BFM 83, $n = 611$). Induction therapy according to protocol I [1] led to a rate of complete remissions (CR) of more than 98%. Only patients with a very high tumor burden (HR patients) and patients with B-ALL had

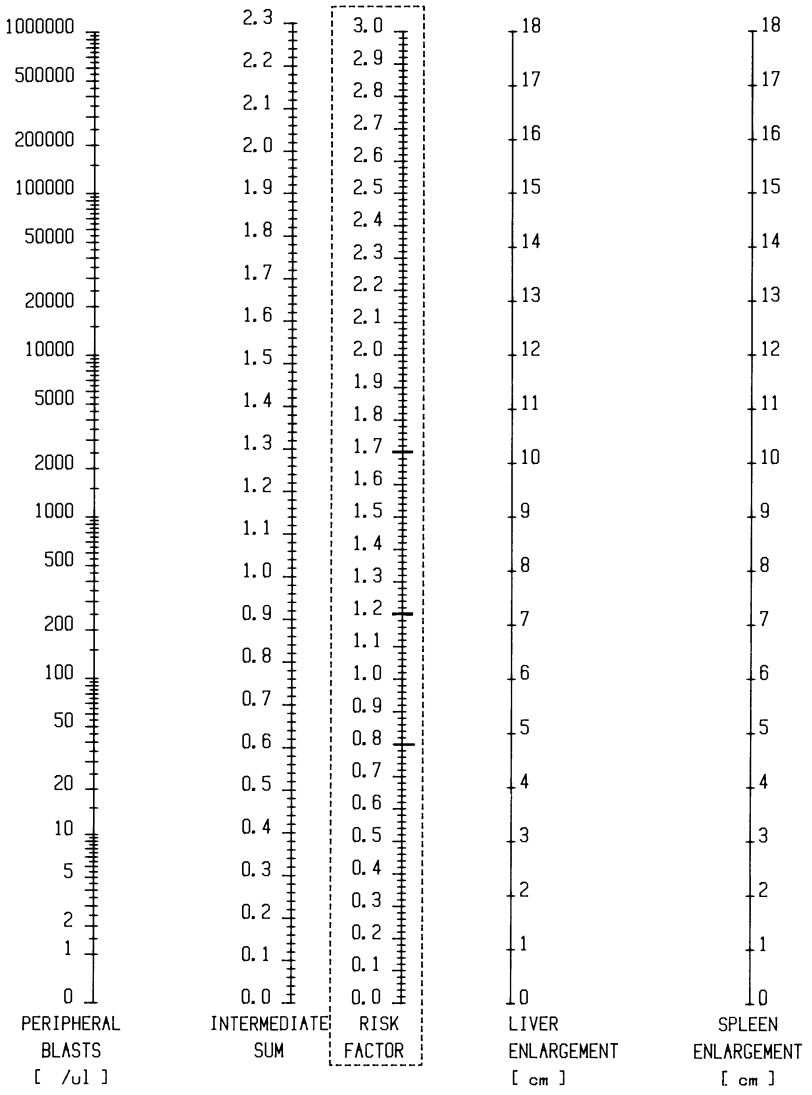

Fig. 2. Diagram for determination of risk factor according to $RF = 0.2 \times \log(\text{Blasts} + 1) + 0.06 \times \text{Liver} + 0.04 \times \text{Spleen}$. The absolute number of leukemic blast cells in the peripheral blood is marked in the first scale of the diagram and a line drawn to the mark of liver size (centimeters below costal margin in medioclavicular line, patient lying supine). A line is then drawn from the point of intersection with the intermediate sum to the mark of spleen size (centimeters below costal margin in longitudinal axis). This will cross the number of the risk factor (RF) in the middle scale

a CR rate in the range of 90%. In both studies only a small minority of patients did not achieve remission (nonresponders), with the highest proportion being among HR patients. None of these patients survived (Tables 1 and 2). In late responders (i.e., no remission after phase 1 of protocol I but remission after phase 2 of protocol I), prognosis has been grave despite specific efforts such as bone marrow transplantation in some patients and highly experimental chemotherapy in others. In study ALL-BFM 81 five out of eight late responders have died in the meantime, whereas at the present four out of five late responders in ALL-BFM 83 are alive.

Table 1. Results of therapy in ALL-BFM 81

	Therapy strategy					
	SR-A	SR-B	MR	HR	B-ALL	Total
Number of patients	184	178	204	45	22	633
Complete remission (%)	180 (97.8)	177 (99.4)	200 (98.0)	41 (91.1)	21 (95.5)	619 (97.8)
Nonresponders[a]				2	1	3
Late responders[b]	2	1	5			8
Death after nonresponse (%)				2	1	3 (0.5)
Death from ALL and/or therapy before CR (%)	3	1	4	2		10 (1.6)
Death from therapy after CR (%)	3	1	8	1	1	14 (2.2)
Relapses (%)	30 (16.3)	46 (25.8)	52 (25.5)	17 (37.8)	10 (45.5)	155 (24.5)
Patients in CCR (%)	146 (79.3)	129 (72.5)	140 (68.6)	23 (51.1)	10 (45.5)	448 (70.8)
Probability of CCR	0.75	0.69	0.65	0.46	0.45	0.67

SR-A/SR-B, Risk factor (RF) < 1.2 (SR-B with no CNS irradiation, but CNS prevention with MTX); MR, RF $1.2 - < 1.7$; HR, RF $> = 1.7$.

[a] Nonresponders: Patients not in remission after protocol I.

[b] Late responders: Patients not in remission after phase 1 of protocol I but in remission after phase 2 of protocol I.

Table 2. Results of therapy in ALL-BFM 83

	Therapy strategy					
	SR-L	SR-H	MR	HR	B-ALL	Total
Number of patients	176	181	192	44	18	611
Complete remission (%)	175 (99.4)	178 (98.3)	190 (99.9)	40 (90.9)	16 (88.9)	599 (98.0)
Nonresponders[a]		2	1	2	1	6
Late responders[b]		1	1	3		5
Death after nonresponse (%)		2	1	2	1	6 (0.9)
Death from ALL and/or therapy before CR (%)	1	2	1	2	1	7 (1.1)
Death from therapy after CR (%)	1		2	2		5 (0.8)
Relapses (%)	4 (2.3)	15 (8.3)	25 (13.0)	8 (18.2)	6 (33.3)	58 (9.5)
Patients in CCR (%)	170 (96.6)	162 (89.5)	163 (84.9)	30 (68.2)	10 (55.6)	535 (87.6)
Probability of CCR	0.91	0.80	0.69	0.60	0.50	0.77

SR-L, Risk factor (RF) < 0.8; SR-H, RF $0.8 - < 1.2$; MR, RF $1.2 - < 1.7$; HR, RF $> = 1.7$.

[a] Nonresponders: Patients not in remission after protocol I.

[b] Late responders: Patients not in remission after phase 1 of protocol I but in remission after phase 2 of protocol I.

Table 3. Incidence and localization of isolated and combined relapses in patients treated according to ALL-BFM 81

	Therapy strategy					Total	
	SR-A	SR-B	MR	HR	B-ALL	*n*	%
Number of patients	184	178	204	45	22	633	
Relapses	30	46	51	17	10	154	24.5
%	16.3	25.8	25.0	37.8	45.5		
Localization of relapse							
BM (isolated)	19	22	20	7	2	70	11.1
CNS (isolated)	4	11	11	3	5	34	5.4
Testes (isolated)	5	2	7	1		15	2.4
BM/CNS (combined)	1	7	7	2		17	2.7
BM/testes (combined)		1	3	1		5	0.8
Others	1	3	3	3	3	13	2.0
Patients in CCR	146	129	140	23	10	448	70.8
%	79.3	72.5	68.6	51.1	45.5		

Table 4. Incidence and localization of isolated and combined relapses in patients treated according to ALL-BFM 83

	Therapy strategy					Total	
	SR-L	SR-H	MR	HR	B-ALL	*n*	%
Number of patients	176	181	192	44	18	611	
Relapses	4	15	25	8	6	58	9.5
%	2.3	8.3	13.0	18.2	33.3		
Localization of relapse							
BM (isolated)	3	10	14	2	5	34	5.6
CNS (isolated)	1	4	4	2		11	1.8
Testes (isolated)			2			2	0.3
BM/CNS (combined)		1	5	4	1	11	1.8
Patients in CCR	170	162	163	30	10	535	87.6
%	96.6	89.5	84.9	68.2	55.6		

Despite expansion of the participating hospitals in both studies to a maximum of 45, the mortality rate before CR has been as low as 1.6% in the ALL-BFM 81 study, and 1.1% in the ALL-BFM 83 study, both of which are smaller than in previous studies. Therapy-related morbidity after CR has been a minor problem, and the death toll has been low (ALL-BFM 81 2.2%, ALL-BFM 83 less than 1%).

The major challenge for the future is therapy failure due to relapse despite risk-adapted therapy, as shown by data in Tables 1–4. The relapse incidence after a median duration of 48 months in the ALL-BFM 81 study is 24.5%. Despite major therapeutic efforts the relative majority of relapses occured in HR patients (37.8%), compared to 16.3% in SR-A patients (Table 3), which is prognostically the most favorable category. The dynamic of relapses in ALL-BFM 83 seems to be identical to that of the previous study (Fig. 1 and Table 4). Life table analyses (Figs. 3 and 4) demonstrate that the probability of event-free survival (EFS) is still obviously related to the RF. Probability

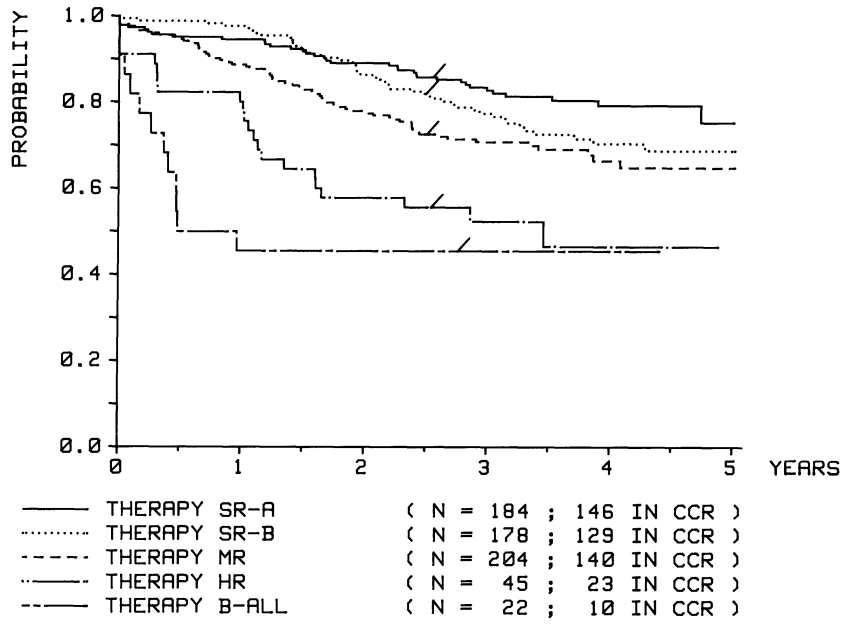

Fig. 3. Probability of event-free survival for all therapy subgroups in ALL-BFM 81 as defined in the text. Data for p-CCR: SR-A, 0.75 (SD = 0.05); SR-B, 0.69 (SD = 0.04); MR, 0.65 (SD = 0,04); HR, 0.46 (SD = 0.09); B-ALL, 0.45 (SD = 0.11). *p*-values: SR-A vs. SR-B, 0.10; SR-A vs. MR, 0.003; SR-A vs. HR, 0.000001; SR-B vs. HR, 0.0001; MR vs. HR, 0.0067

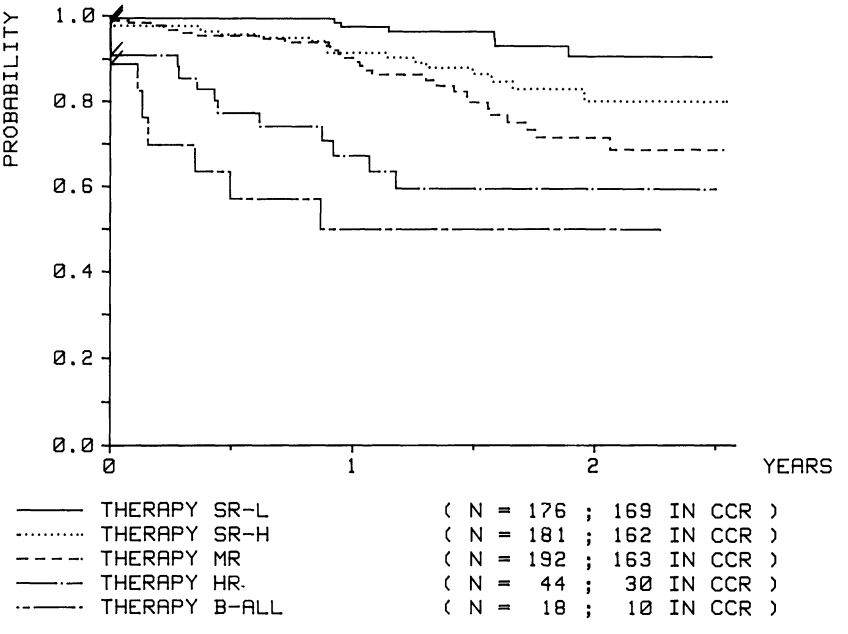

Fig. 4. Probability of event-free survival for all therapy subgroups of ALL-BFM 83 as defined in the text. Data for p-CCR: SR-L, 0.91 (SD = 0.04); SR-H, 0.80 (SD = 0,05); MR, 0.69 (SD = 0.06); HR, 0.60 (SD = 0.09); B-ALL, 0.50 (SD = 0.13). *p*-values: SR-L vs. SR-H, 0.002; SR-L vs. MR, 0.00002; SR-L vs. HR: 0.000; SR-H vs. MR, 0.22; SR-H vs. HR: 0.001; MR vs. HR: 0.01

for EFS (ALL-BFM 81) is slightly higher than 70% for SR patients (representing 60% of all patients), 65% for MR patients (30% of all patients), and 46% for HR and B-ALL patients (10% of all patients). P-CCR is more favorable in SR patients if irradiation with 18 Gy is given as a preventive measure against CNS relapse (EFS 75% for patients receiving therapy SR-A compared to a EFS of 69% for those receiving therapy SR-B with no preventive CNS irradiation, Fig. 3). Patients classed for SR-L in ALL-BFM 83 are doing even better with a p-CCR currently at 91% (Fig. 4), the median duration of this study being 15 months. Preliminary results of ALL-BFM 83 in respect to the randomized therapy strategy SR-L (reinduction vs. no reinduction with protocol III, a reinforced therapy element applied early in remission), and strategy SR-H (preventive CNS irradiation with 12 Gy vs. 18 Gy) have so far not revealed any differences. This means that in strategy group SR-H preventive CNS irradiation with a dose as low as 12 Gy may offer an adequate protection for eventual CNS disease; this dose can be considered to be noncritical in respect to late sequelae, e.g., intellectual dysfunction and the emergence of brain tumors.

Therapy Response as a New Prognostic Factor

In ALL-BFM 83 induction therapy starts with a 7-day exposure to corticosteroids [9], since they are considered to be the most effective antileukemic substance. Furthermore, they can be applied safely and can be easily adjusted to each individual patient. We are not afraid that the emergence of a drug-resistant clone during a short steroid exposure jeopardizes the prognosis. The extent of leukemic blast cell reduction in the peripheral blood at day 8 has been correlated with the incidence of failures. In fact, the analysis of 476 patients revealed an impressive correlation between the corticosteroid response and outcome, at least considering early failures. At present, an absolute number of less than 1000/mm^3 leukemic cells at day 8 defines a group of patients with a p-CCR of 83%, irrespective of risk factor. In contrast, patients with more than 1000/mm^3 blasts at day 8 are doing markedly worse, the p-CCR being 38% (Fig. 5). Nonresponders to corticosteroids, as defined before, were only recruited from group SR-H, MR, and HR, however. Furthermore, if this therapy response is utilized in the analysis of

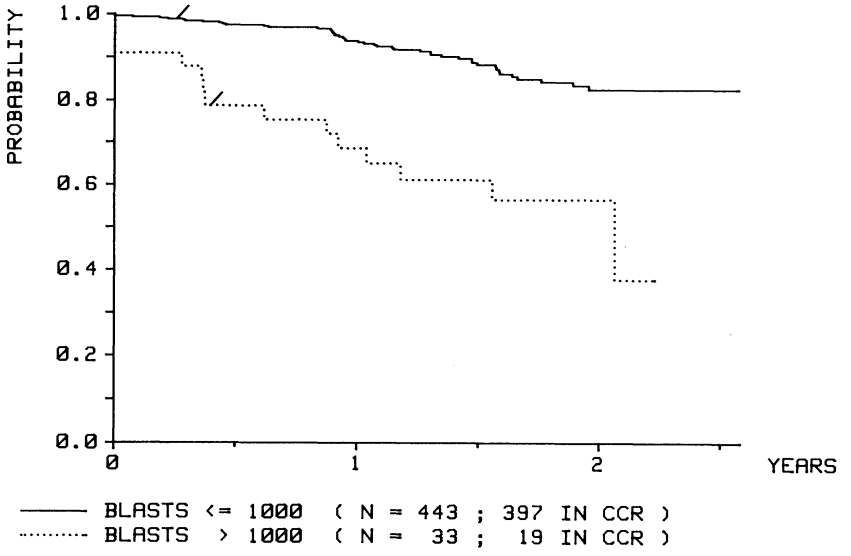

Fig. 5. Probability of event-free survival in ALL-BFM 83 as related to adequate or inadequate initial corticosteroid response (absolute blast number in the peripheral blood at day 8 of therapy below or above 1000/mm^3); data for p-CCR, 0.83 and 0.38, respectively. $p < 0.0001$

ALL-BFM 83 by excluding those patients who exhibit poor response to corticosteroids from the strategy arms, the probability for EFS increased by approximately 3% in SR-H, 5% in MR, and 13% in HR patients.

It seems reasonable to use the in vivo response to corticosteroids for trials in the near future as a supplement to other risk factors such as tumor burden and other phenotypes in individual patients and their leukemic cells.

In spite of these efforts it is unlikely that combinative chemotherapy and its counterpart – supportive care – will be the ultimate solution for cure in patients with leukemia. The problem of minimal residual tumor load in biologically protected locations (extra-compartments) make therapeutic approaches necessary which are more specifically directed to these targets.

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The BFM Relapse Studies in Childhood ALL: Concepts of Two Multicenter Trials and Results after 2½ Years*

G. Henze, S. Buchmann, R. Fengler, and R. Hartmann [1]

Introduction

Different mechanisms may be responsible for the occurrence of relapse of acute lymphoblastic leukemia (ALL). As a consequence, treatment schedules for relapse should take into account the possible or assumed mechanism with the aim of attempting to develop adequate treatment strategies.

Figure 1 shows in a simplified schema the interrelationships of underlying mechanism and the resulting type of relapse. One reason for treatment failure may be the existence of an anatomic barrier which prevents antineoplastic drugs from reaching their target at therapeutically effective concentrations. Central nervous system (CNS) relapse is the main representative of this group. Because of possible reseeding of cells to other sites, treatment has to be local and systemic as well.

Another reason for relapse is drug resistance, either primary or secondary, resulting in nonresponse to treatment or early sys-

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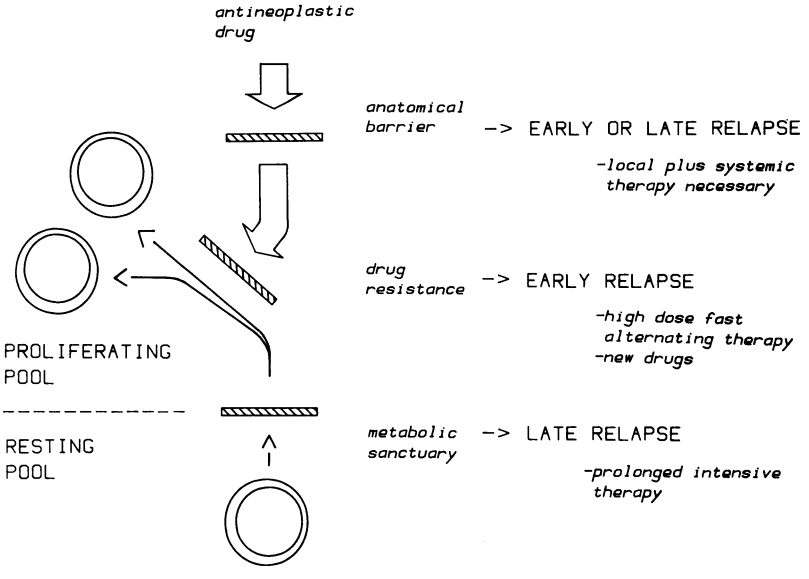

Fig. 1. Interrelationships of mechanisms of relapse and resulting types of relapse and implications for treatment

temic relapse. At the present time, the possibilities of chemotherapy in this condition are very limited. Drug resistance may be overcome by the introduction of high-dose combination chemotherapy delivered in fast alternating cycles or by using not previously applied new drugs.

The third possible mechanism of relapse might be that cells are hidden in "metabolic" sanctuaries, i.e., prevented from being recruited into the cell cycle and thus not being accessible to chemotherapy. The remaining of leukemic cells in the resting pool may be an individual property of the cell or could also be due to altered metabolic environmental conditions as exist in the CNS or in the testicles, resulting in a much lower proliferative activity. It becomes clear that there is probably an overlapping of anatomical and metabolic sanctuaries. Implications for systemic treatment of these relapses are that therapy should be prolonged and intensive and, if necessary, complemented by local measures.

First results of two BFM ALL relapse studies will be presented.

Description of Trials

Study ALL-REZ BFM 83

The first multicenter and prospective study for treatment of relapsed ALL in the Federal Republic of Germany was started in 1983. Aims of study were:

1. Improvement of prognosis of children with relapsed ALL by a newly designed chemotherapy regimen and adaptation of treatment to time and site of recurrence. In patients with an HLA-identical sibling bone marrow transplant was to be done soon after remission was achieved.
2. Carrying out a trial with largely uniform treatment of different sites of relapse in order to create a baseline for future approaches, as there are only few reliable data available from large scale trials.
3. Looking for as yet unknown prognostic factors by extensive diagnostic procedures in case of relapse, which would possibly have to be taken into account at the initial manifestation of ALL in future patients.

The treatment design is shown in Fig. 2. Patients were subdivided into three categories, groups A, B, and C, depending on time and site of recurrence. In children with early bone marrow relapse (occurrence during or until 6 months after the end of initial treatment, group A) remission was to be achieved by applying most aggressive induction therapy (protocol E, Fig. 3). This was followed by alternating administration of the main constituents of the treatment schedule, blocks R-1 and R-2 (Fig. 4). After eight treatment courses had been completed, maintenance therapy was to be started with daily oral 6-thioguanine (50 mg/m^2) and i.v. methotrexate (MTX) every 2 weeks (50 mg/m^2) for 2 years.

For patients with late bone marrow relapse (occurrence beyond 6 months after the end of initial treatment, group B), blocks R-1 and R-2 were used for remission induction and consolidation. After eight R-blocks, maintenance therapy was given in the same way as in patients of group A.

Group C patients, i.e., those with early or late extramedullary relapse, were to receive four R-blocks followed by only 1 year of maintenance therapy as in groups A and B.

Local measures included orchiectomy in boys with testicular relapse and cranial radiotherapy (craniospinal was also admitted) in patients with CNS relapse. In the latter group, treatment was supplemented by triple intrathecal chemotherapy with prednisone, cytarabine (Ara-C), and MTX on days 1 and 5 of each R-block and every 6 weeks thereafter up to 1 year. Radiotherapy to sites other than the CNS was only to be given if there was incomplete response to chemotherapy.

Study ALL-REZ BFM 85

In 1985, the treatment schedule was modified as it was evident that the aggressive protocol E was not capable of producing satisfactory results in group A patients as far as remission rates and long-term disease-free survival were concerned. Hence, high-dose therapy with the drugs MTX and Ara-C was introduced, resulting in the design of protocol F as outlined in Fig. 5.

Patients of all groups were randomized to receive MTX either at a dose of 1 g/m^2

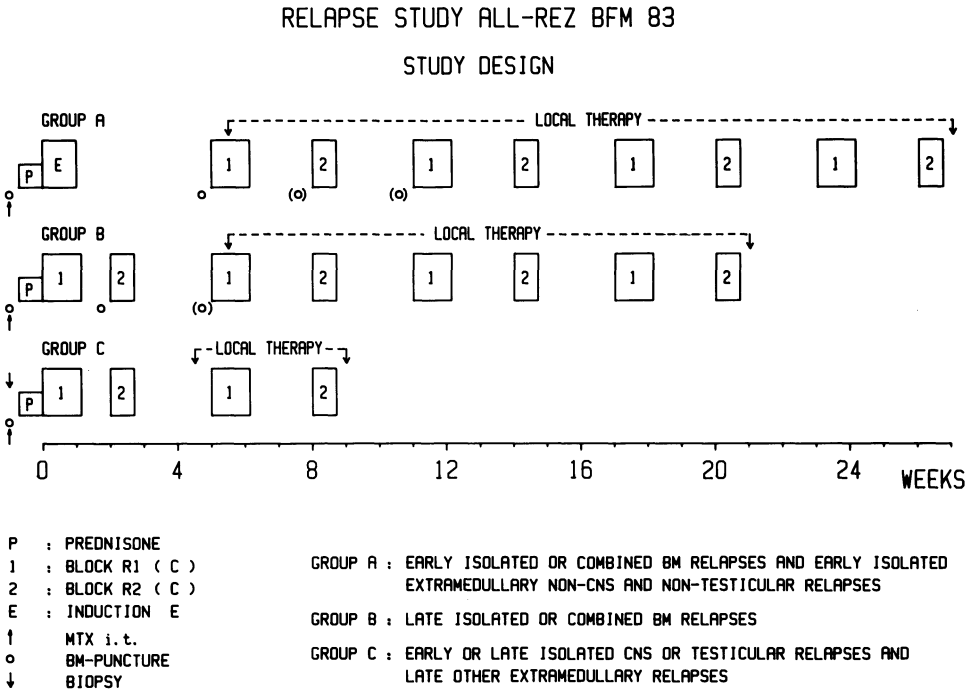

Fig. 2. Treatment design of relapse study ALL-REZ BFM 83. In the subsequent study ALL-REZ BFM 85 induction protocol "E" was replaced by "F" and the number of R-blocks in group C increased from four to six; furthermore, in this study patients were initially randomized to receive methotrexate at a high or intermediate dose (see text for further explanation)

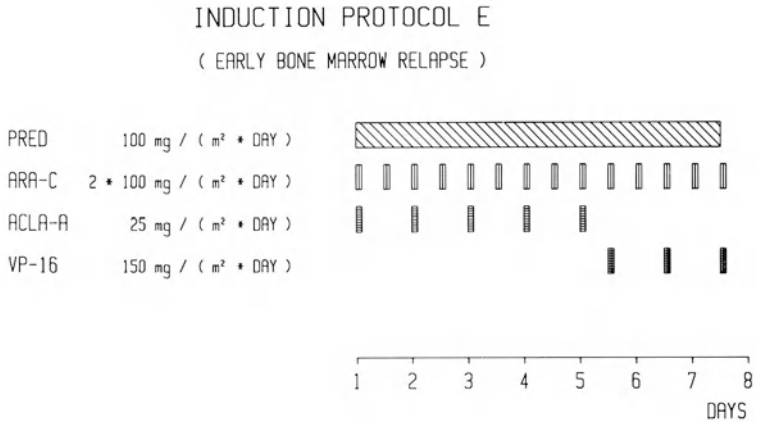

Fig. 3. Induction protocol E for children with early medullary relapse in study ALL-REZ BFM 83

(limb M) delivered over a 36 h infusion period followed by conventional leucovorin rescue with 2 doses of 15 mg/m^2 each, or at a dose fo 12 g/m^2 given within 4 h and rescued by eight doses of leucovorin. This randomization was done for all therapy elements, i.e., protocol F and blocks R-1 and R-2 (see footnotes in Fig. 4 and 5).

For patients of group C, the number of R-blocks was increased from four to six, be-

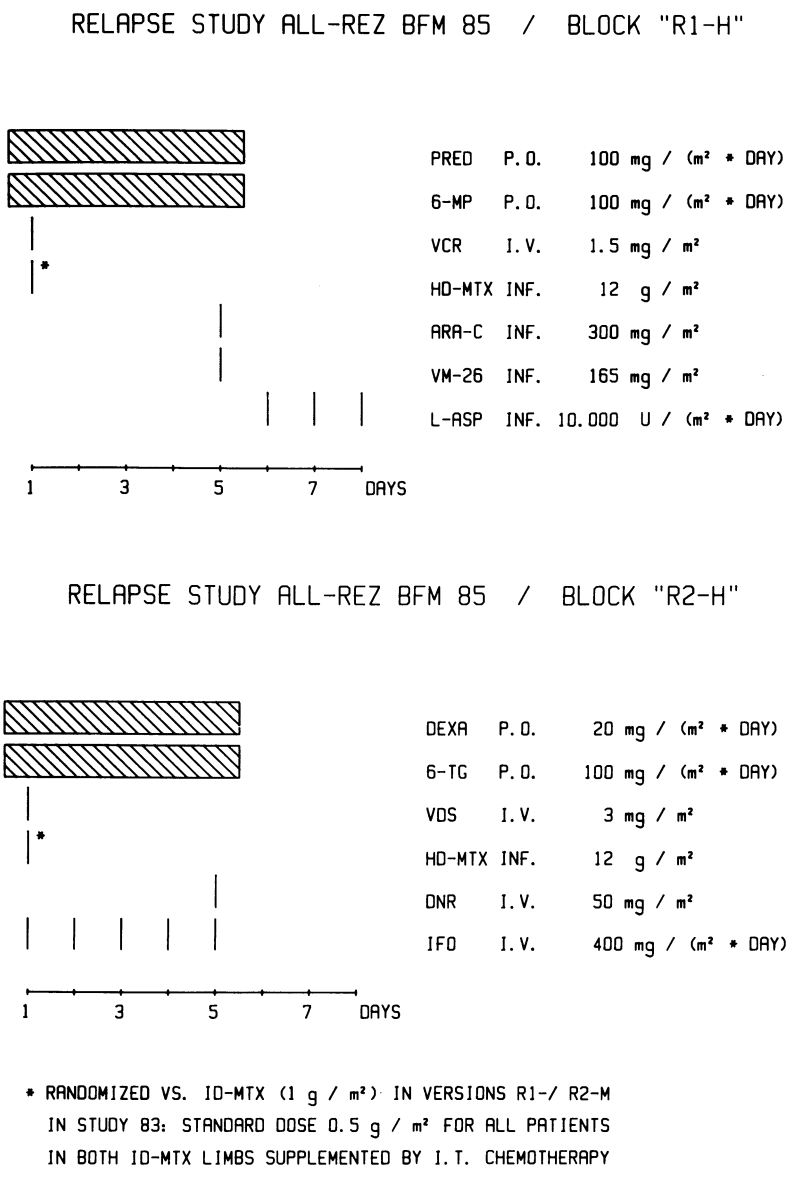

Fig. 4. Polychemotherapy blocks R1 and R2 with high dose methotrexate (version "H") as used in study ALL-REZ BFM 85. The same combination was given in study ALL-REZ BFM 83 with the one exception that the methotrexate dose was uniformly 0.5 g/m^2 for all patients (see also text)

cause at that time there was a small but relevant incidence of systemic relapses following initial isolated CNS relapse. Otherwise the protocol was unchanged compared with study ALL-REZ BFM 83. The design is outlined in Fig. 2 (see legend for explanation of differences between studies 83 and 85).

Patients and Diagnostic Criteria

From June 1983 to August 1985, 95 children and adolescents with their first relapse of ALL were enrolled in study ALL-REZ BFM 83. Four patients had to be removed because of protocol violations, resulting in a

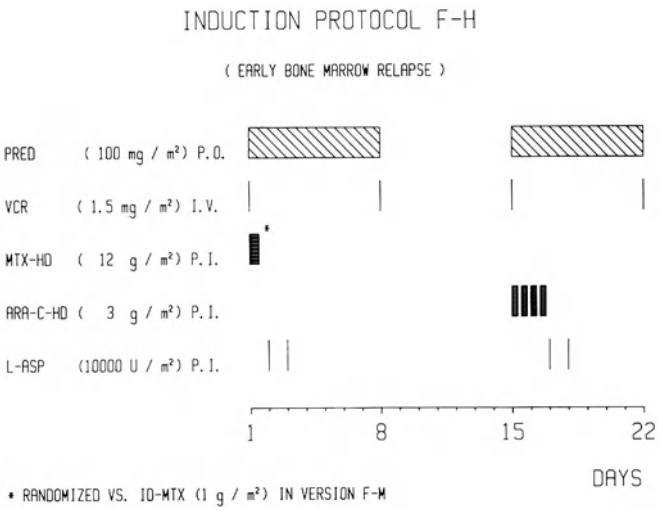

Fig. 5. Induction protocol F with high-dose methotrexate (version H) as used for children with early medullary relapse in study ALL-REZ BFM 85

Table 1. Distribution of age and sex in study ALL-REZ BFM 83

	Patients		Age (median) (years)
	n	%	
Boys	59	64.8	$9^{10}/_{12}$
Girls	32	35.2	$7^{8}/_{12}$
Total	91	100.0	$8^{3}/_{12}$

Table 2. Time and site of recurrence in patients at entry to study ALL-REZ BFM 83

Relapses	Early	Late	Total
Isolated BM	17	23	40
Isolated CNS	19	2	21
Isolated testes	2	5	7
Combined BM	11	7	18
Other sites	4	1	5
All sites	53	38	91

Table 3. Distribution of age and sex in study ALL-REZ BFM 85

	Patients		Age (median) (years)
	n	%	
Boys	54	79.4	$7^{3}/_{12}$
Girls	14	20.6	$10^{5}/_{12}$
Total	68	100.0	$8^{4}/_{12}$

Table 4. Time and site of recurrence in patients at entry to study ALL-REZ BFM 85

Relapses	Early	Late	Total
Isolated BM	27	16	43
Isolated CNS	4	2	6
Isolated testes	6	–	6
Combined BM	6	7	13
Other sites	–	–	–
All sites	43	25	68

total number of 91 evaluable patients. Patient characteristics for age and sex as well as for time and sites of recurrence at study entry are given in Tables 1 and 2. Up to now 68 children have been registered for study ALL-REZ BFM 85 (Tables 3 and 4). Several differences in the patient distribution of the two studies have to be noted. In study 85 there was an even more pronounced preponderance of males than in study 83. Age of boys compared to girls is inversely distributed in both studies. The ratio of early to late relapses compares favourably in both studies. A much higher percentage of early bone marrow relapse has to be noted in study 85, whereas in study 83 the CNS constituted the most frequent site in the group of isolated early relapses. For the present the observed

imbalances between patient characteristics of both studies cannot sufficiently be explained.

The diagnosis of medullary relapse was made by the detection of at least 25% blast cells in Wright stained bone marrow smears. Cytocentrifuge preparations of CSF with the presence of lymphoblasts were essential for the diagnosis of CNS relapse. Testicular relapse had to be proven by histology positive for lymphoblasts of the removed testis or biopsies. The diagnosis of "isolated" extramedullary relapse was compatible with a bone marrow infiltration of less than 5% blasts. If there were blast cells in excess of or at the 5% level, patients were by definition diagnosed as having combined relapses.

If possible, extensive diagnostic studies were to be done, including immunological phenotyping of blasts, chromosome studies, and measurements of the cellular DNA content by means of flow cytophotometry. First results of cytogenetic and flow cytophotometric investigations are described elsewhere in this volume (Harbott et al., Gromball et al.).

Criteria for a second complete remission were an M1 marrow (< 5% blast cells) after a maximum of two blocks of chemotherapy in patients with bone marrow relapse. In children with CNS relapse, the CSF had to be cleared from leukemic cells, and in patients with other extramedullary relapse complete regression of a preexisting leukemic infiltrate had to be documented.

Results

Tables 5 and 6 show the results of both studies with respect to treatment response and follow-up.

In study 83, second complete remissions (CR) were obtained in 77 out of 91 patients.

Table 5. Remission rates and follow up data of patients from study ALL-REZ BFM 83

	Patients	No 2nd CR		2nd CR		Death in CR	Relapse	In CCR
	n	ED	NR	*n*	%			
BM involved								
Early	28	4	6	18	64	1[a]	14	3
Late	30	–	2	28	93	4[a]	6	18
Other sites								
Early	25	2	–	23	92	1	10	12
Late	8	–	–	8	100	–	1	7
All sites	91	6	8	77	85	6	31	40

[a] After BMT in 2nd CR.

Table 6. Remission rates and follow up data of patients from study ALL-REZ BFM 85

	Patients	No 2nd CR		2nd CR		Death in CR	Relapse	In CCR
	n	ED	NR	*n*	%			
BM involved								
Early	33	1	1	31	94	1	5	25
Late	23	2	–	21	91	–	–	21
Other sites								
Early	10	–	–	10	100	–	1	9
Late	2	–	–	2	100	–	–	2
All sites	68	3	1	64	94	1	6	57

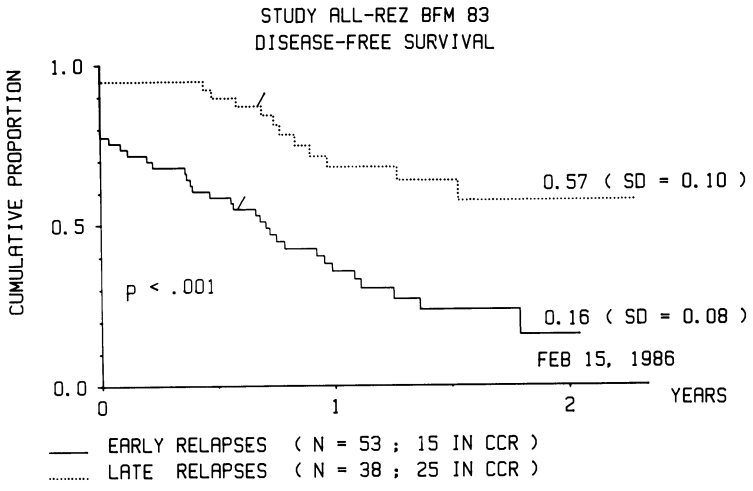

Fig. 6. Probability of second continuous complete remission: Comparison between early and late relapsed patients in study ALL-REZ BFM 83. *Diagonal* indicates last follow-up

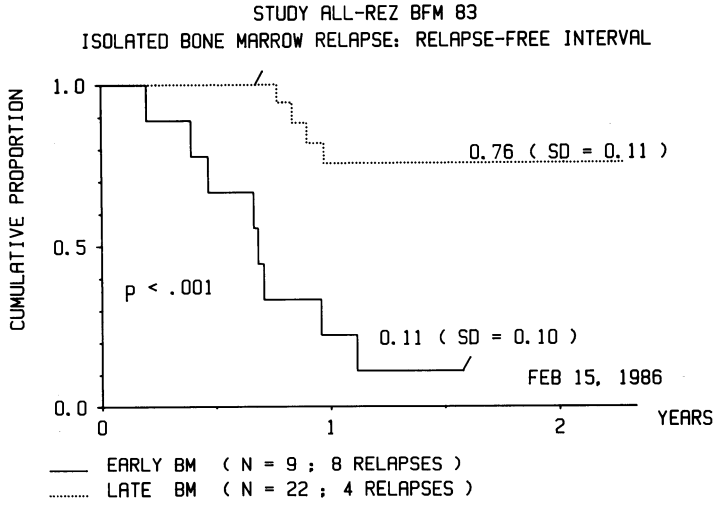

Fig. 7. Comparison of relapse-free intervals between patients with early and late isolated bone marrow relapse in study ALL-REZ BFM 83. For this kind of analysis only patients who achieved second CR were considered and adverse events in second CR other than relapses censored at date of occurrence. *Diagonal* indicates last follow-up

The remission rate was distinctly lower in children with early relapses, which was in part due to obvious nonresponse and to an equal extent a consequence of lethal complications following the application of protocol E. Toxic deaths occurred in six children and were caused by bleedings or infections. Hence, a striking difference between remission rates for early (64%) and late (93%) bone marrow relapses could be observed ($p < 0.01$).

Much more favorable results were found in study 85 when protocol F was used for remission induction in group A patients. The remission rate was well above 90% because nonresponse and early death were much less frequent causes of treatment failure. Thus, in study 85 remission rates of children with early and late bone marrow relapses compare favorably. The difference between remission rates of group A patients in studies 83 and 85 is significant at $p < 0.01$.

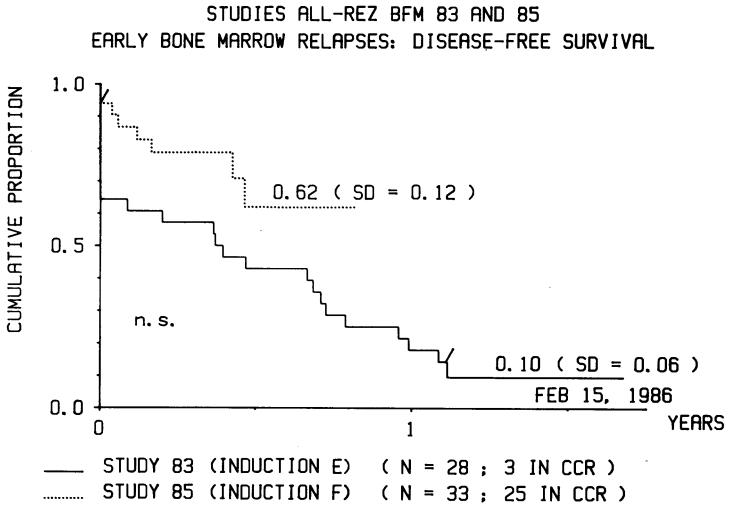

Fig. 8. Comparison of the probabilities of second continous complete remission between patients with early medullary relapse in studies ALL-REZ BFM 83 and ALL-REZ BFM 85, having received different treatment for remission induction. *Diagonal* indicates last follow-up

In study 83, six children died in second CR. In five of them death was related to bone marrow transplant (BMT). In fact, none of the 11 transplanted patients of this study have suffered a second relapse thus far, whereas one out of four transplanted patients of study 85 had a second bone marrow relapse 3 months after BMT.

As expected, second relapse was the major cause for treatment failure, especially in children with early medullary relapse at study entry. Only three out of 28 patients of study 83 continue in second CR. Calculated by life table analysis the probability of continuous complete remission after 28 months is 0.16 for patients with all kinds of early relapse and 0.57 for patients who had late relapse ($p < 0.001$, Fig. 6). When looking at patients who achieved a second CR and censoring all adverse events other than relapses, the probability of being in remission after just 2.5 years yields 0.22 and 0.71 for early and late relapsed children, respectively. These results differ at $p < 0.001$.

So far, the probability of a relapse-free interval of nearly 2½ years after late isolated marrow relapse is 0.76 in a group of 22 patients (three in CCR after BMT). This is in contrast to 0.11 in a group of nine children with early isolated marrow relapse, the only one who was transplanted still being in CCR (Fig. 7).

A distinct, though not yet significant, difference can be observed between life table curves of group A patients of both studies in favor of study 85. This gives rise to cautious optimism for further outcome of these poor prognosis patients (Fig. 8).

There is no significant difference between randomisation limbs H and M in study 85, neither for remission rates nor for subsequent relapses.

Discussion

Although there are a number of well-organized trials for treatment of firstly diagnosed childhood ALL, reports on systematic multicenter studies in relapsed patients are lacking. As relapse of leukemia is usually considered a condition with an extremely poor prognosis, it is often difficult to decide what to do with these patients. Should treatment be restricted to palliation or would it be justified to attempt a second cure? If the latter would be accepted and, as in the majority of patients, there is no compatible donor for BMT, what treatment can offer a real perspective? There is no doubt that the results of treatment for relapse depend on several factors, such as time and site of recurrence, but most probably on the intensity of preceding treatment as well. Therefore, re-

ported results with a certain treatment schedule in case of relapse may not be true for differently pretreated patients.

For these reasons, it seemed to be desirable to start a nation-wide study on relapsed ALL in Germany in order to help physicians deciding how to treat their patients and to gain information on the effectiveness of a newly designed treatment strategy. A subsequent step would be to alter the schedule on the basis of careful analysis of data.

After 2½ years of the first trial, results are still preliminary. Keeping in mind that all patients in Germany had intensive initial treatment, remission rates in study 83 are acceptable, though not satisfying in children with early bone marrow relapse when compared with other trials [1–4]. As to be expected, long-term results proved to be poor. Therefore, a completely different induction regimen was designed for this patient group in study 85. Not only was it possible to reduce toxicity and the frequency of early death but also significantly to enhance the remission rate. At the present time, no conclusions can be made concerning long-term results. Nevertheless, the most important finding is that increasing toxicity is not necessarily followed by better results.

Remission rates obtained in children with late bone marrow relapses can be judged satisfactory, i.e., over 90% in both studies. The percentage of patients with long-lasting second remissions has been reported to vary [5–9]. Note, however, that most studies included only a few patients and pretreatment was distinctly less intensive than in the present series. The probability of about 0.70 of being in CCR 2½ years after relapse is in a similar range, if not better, than reported for BMT [10, 11]. Undoubtedly, this will not be the final result but on the other hand it has to be kept in mind that late second relapses may occur also after BMT.

Patients with early extramedullary relapses, most of which were localized in the CNS, fared relatively poorly. At present, it cannot be judged whether or not prognosis will be improved by the modifications introduced in study 85.

In conclusion, the treatment results obtained so far are encouraging and give rise to the hope that it may be possible to achieve acceptable second remission durations also after intensive initial treatment in a proportion of children with relapsed ALL.

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Limiting Toxicities During Intensified Remission Induction Chemotherapy for Childhood Acute Lymphocytic Leukemia*

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The leukemias of childhood have provided an excellent model with which to test novel therapeutic strategies for human neoplasias [1]. In 1984, we devised an intensive multidrug regimen for treatment of newly diagnosed patients with acute lymphocytic leukemia (ALL). This therapy is being evaluated in Study XI of the Total Therapy series at St. Jude Children's Research Hospital, and features drug combinations and scheduling that differ radically from those tested in previous studies. Our rationale follows predictions of the somatic mutation theory of Goldie and Coldman [2] that early and repeated use of nonspecific but intensive combination chemotherapy should decrease the likelihood of drug resistance and, therefore, improve the end results of treatment. However, we encountered unexpectedly severe toxicity from early intensification of therapy that led to amendment of the protocol [3]. In this article, we review the toxic effects of the original treatment regimen and its subsequent modifications, emphasizing the potential hazards of intensive combination chemotherapy for ALL.

Original Study

All patients entered in the study, irrespective of risk status, received a 6-week multidrug induction treatment, as outlined in Fig. 1. The regimen featured rapid (every 3 or 4 days) rotation of six antileukemic agents administered orally, intramuscularly, intravenously, and intrathecally. A conventional combination of prednisone, vincristine, and L-asparaginase was given at standard dosages to all patients. VM26 dosage was 150 mg/m^2 i.v. on days 4, 15, 25, 29; cytarabine (Ara-C) 300 mg/m^2 i.v., was administered on days 4, and 25; and high-dose MTX, 2 mg/m^2 i.v., was given on days 8 and 36. In addition, four injections of triple IT MTX, hydrocortisone, and Ara-C therapy were given on days 4, 8, 36, and 43 to all patients. Intrathecal therapy was also followed by leucovorin rescue 24 h later. The first course of high-dose MTX was administered on day 8–4 days after the first doses of VM26, Ara-C, and intrathecal therapy.

In January and February of 1984, we treated 13 consecutive patients, eight of whom developed unexpected severe gastrointestinal toxicity. Five patients in this group had symptoms of life-threatening toxicity, and one died. The remainder had similar but less severe toxicity. The side effects were characterized by diffuse mucosal involvement with acute bleeding during the first 3 weeks. This syndrome was associated with significant body weight losses of 15%–20% and complicating infections, mostly fungal. The time to recovery of bone marrow function was prolonged. The median duration of neutropenia (< 500 phagocytes/cu mm) was 26 days (range, 12–40 days). Five patients developed systemic candidiasis and required total parenteral nutrition for acute weight loss, antifungal therapy, and other support-

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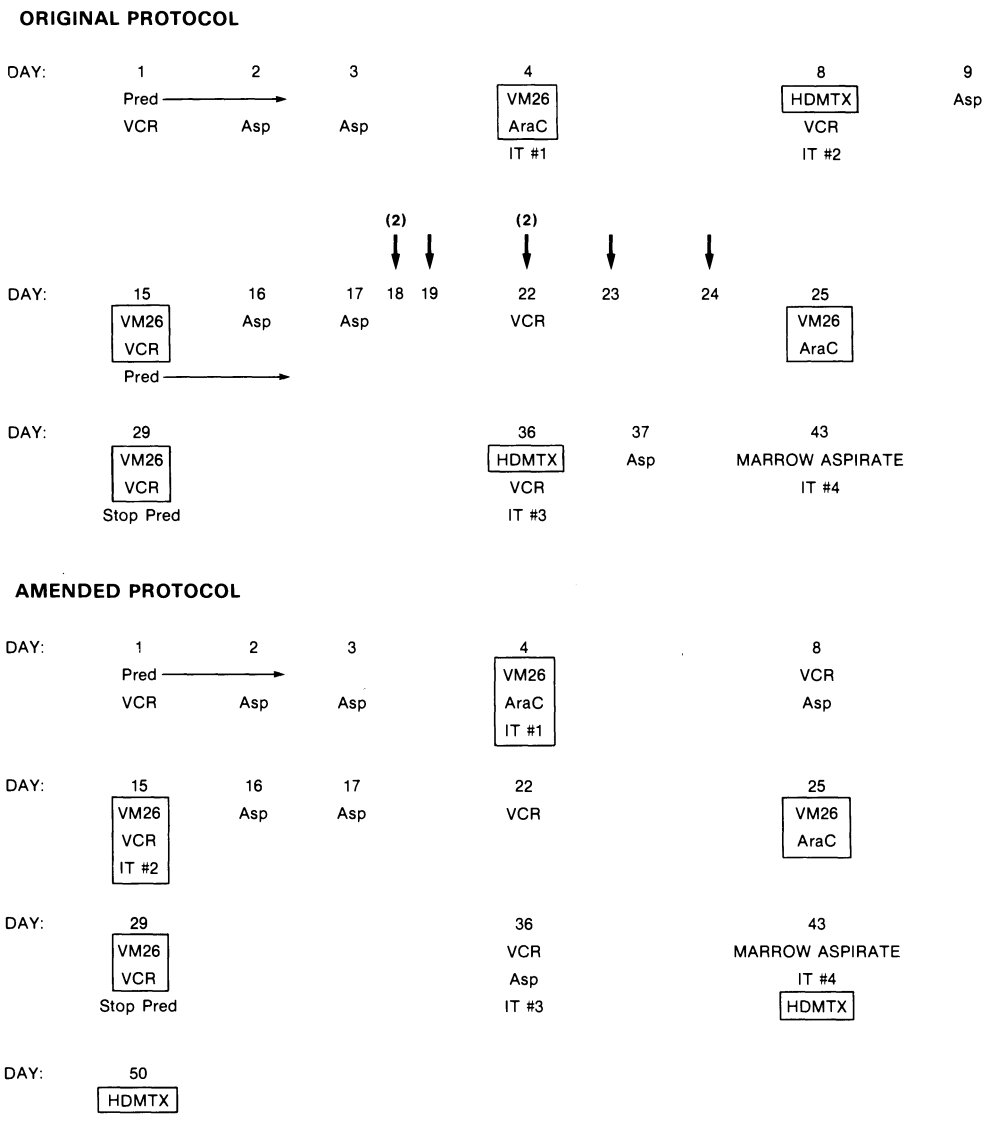

Fig. 1. Schema of early therapy in original and amended protocols. *Vertical arrows* indicate onset of acute gastrointestinal toxicity in eight patients. *Pred,* prednisone; *VCR,* vincristine; *Asp,* asparaginase; *VM-26,* teniposide; *Ara-C,* cytosine arabinoside; *HDMTX,* high-dose methotrexate; *IT,* intrathecal MTX + hydrocortisone + Ara-C therapy. In the amended protocol, HDMTX was omitted on days 8 and 36

ive measures. The median duration of hospitalization was 70 days (45–130). With the exception of the child who died, all patients attained complete remission.

Because of the excessive toxicity, only one-half to two-thirds of the planned induction chemotherapy could be administered to the patients; consequently, there was a median delay of 35 days before continuation treatment could begin. It was not possible to identify the causative agents with certainty, as all patients had received multidrug therapy. However, circumstantial evidence indicated that high-dose MTX, acting alone or with other agents, may have been a chief contributor to the observed toxic effects.

Additionally, rotation of the drug combinations may have been too rapid, so that host tolerance was overwhelmed, and the original therapeutic intent defeated.

First Amendment

We modified the original protocol in March 1984 by omitting the two courses of high-dose MTX on days 8 and 36 from the induction regimen and including them as consolidation treatment on days 43 and 50, after patients had attained an initial remission. It was reasoned that delaying MTX therapy for at least 1 month would permit mucosal recovery and restoration of normal hematopoiesis. The total number of doses and agents remained the same but were delivered over 8 instead of 6 weeks.

Of the 121 patients who were entered in the amended study through June 1985, 120 were evaluable. None developed the previously described syndrome of gastrointestinal toxicity; however, 14 demonstrated neurotoxicity in the form of seizures (Table 1). The time of seizure onset ranged from day 8 to 59 (median, day 24); 11 patients had seizures during the initial 6 weeks of induction and three during the consolidation phase with high-dose MTX (weeks 7–8 of early therapy). No clear temporal relationship between a particular treatment and the occurrence of seizures could be established. For example, some children developed seizures after receiving only two of the planned six doses of asparaginase, while others had received five doses.

Ochs et al. have demonstrated an increased seizure rate among patients whose early therapy included IT injections of MTX, and noted an apparent relationship of about 1 week between IT injections and seizure activity [4].

Our amended protocol specified IT MTX on days 4, 15, 36, and 43, but we did not find any clear association between the timing of IT chemotherapy and seizure development. Furthermore, most children continued to receive their scheduled IT therapy thereafter without developing subsequent seizure episodes. Analysis of presenting clinical fea-

Table 1. Characteristics of patients who developed seizures during early therapy

Patient no.	Diagnostic features			Day of onset	Cause
	Age (yr)	WBC ($\times 10^9$/L)	CNS		
Induction therapy ($n=11$)					
1[a]	8	479	+	8	CVA
2[a]	17	12	+	10	Unknown
3	9	2.2	−	12	CVA
4	10	1.4	−	16	VCR
5[a]	8	230	+	18	Unknown[b]
6	12	6.4	+	24	Unknown[b]
7	9	109	−	24	Unknown
8	4	24	−	29	Unknown[c]
9	4	5.7	−	36	Unknown
10[a]	12	174	−	39	CVA
11	12	194	+	47	Encephalopathy[d]
Consolidation therapy ($n=3$)					
12	14	4.0	−	53	Unknown
13[a]	17	4.0	−	55	Unknown
14	12	2.4	−	59	Unknown

[a] T cell ALL.
[b] *CVA*, cerebrovascular accident.
[c] Toxic death, no postmortem examination.
[d] Toxic death without CNS lesions at postmortem examination.

tures that might be expected to influence seizure activity likewise failed to suggest any significant relationships. For instance, of the 21 patients with CNS leukemia at diagnosis, only five had seizures. Similarly, seizures were not related to hyperleukocytosis (five episodes among 23 patients).

Laboratory findings included thrombocytopenia (< 100 000 platelets/cu mm) in four patients, hypofibrinogenemia (< 100 mg/dL) in five, and hyponetremia [< 135 mEQ/L) in two. Despite sequential computed tomography scans of the head and repeated neurologic and cerebrospinal fluid examinations, etiologic factors for seizure development could not be identified for most patients. Cerebrovascular accidents could be documented in only three of the 14 patients, and in each instance, they were likely associated with *L*-asparaginase therapy. There was one case of vincristine-induced neurotoxicity and another of diffuse encephalopathy of undetermined etiology.

Ten of the patients had uneventful recoveries and have not experienced recurrent seizures while receiving prolonged anticonvulsant therapy. Three patients died of systemic infections and had no evidence of leukemia or CNS lesions at postmortem examination. An additional patient died but autopsy permission was not granted.

In an effort to identify presenting features that might predispose a child to seizures while receiving intensive induction therapy, we compared the characteristics of patients with and without seizure episodes. Only two variables proved significant. By *t*-test analysis, the mean age of patients who had seizures was greater than that of the alternative group (10.6 vs. 6.2, $p = 0.0002$). Similarly, a T cell immunophenotype conferred a higher risk of seizure development than did other immunologic species of ALL ($p = 0.02$ by contingency table analysis). Sex, race, hemoglobin level, leukocyte and platelet counts, CNS leukemia at diagnosis, FAB classification, and blast cell karyotypes were not significant predictors of neurotoxicity.

In the context of previous institutional experience [5] and reports of others on acute toxicity during induction therapy for childhood ALL [6–7] a seizure frequency of > 10% is unacceptable. Thus, the 12% incidence of seizure episodes we encountered warranted further modification of the protocol to reduce neurotoxicity.

The median time for recovery of hematopoiesis remained prolonged (day 25), despite the switch of high-dose MTX from days 8 and 36 to days 43 and 50. Of the 120 patients who received the amended therapy, 11 (9%) were considered induction failures, five because of toxic events and four because of residual leukemia.

The early death rate in both the original and the amended protocols was 4% (5 of 133 patients), which is not significantly different from rates in other trials for childhood ALL. Nonetheless, we concluded that additional modifications of the protocol were needed to decrease morbidity rates.

Summary and Conclusions

Intensification chemotherapy offers a rational approach to curative treatment of acute lymphoblastic leukemia (ALL). Factors potentially responsible for treatment failure in ALL include inadequate reduction of the initial leukemia cell burden, the development of drug resistance by residual cells, and the presence of "sanctuaries" from chemotherapy, such as the central nervous system (CNS). Therapists have chosen several different methods of treatment intensification to overcome these problems: use of (a) additional drugs, often in high doses, during remission induction or continuation therapy [8]; (b) "pulses" of chemotherapy during the continuation phase with agents the patient has not previously received [9]; and (c) an intensive phase of treatment shortly after remission induction [10]. In Study XI, we elected to intensify therapy by rapid rotation of non-cross-resistant drug pairs throughout the different phases of treatment. Despite its theroretical appeal, this strategy resulted in prohibitive toxicity in about 15% of patients who were receiving early therapy. Not only were patients subjected to severe side effects, but in many instances continuation treatment had to be delayed, increasing the risk for relapse. In complex, multidrug regimens such as the type described here, untoward drug interactions may comprise the ability of patients to maintain effective immune response or to recover normal leukocyte

counts within an acceptable time. We recommend critical evaluation of all drug scheduling in intensified regimens for childhood ALL.

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Stratification by Prognostic Factors in the Design and Analysis of Clinical Trials for Acute Lymphoblastic Leukemia*

G. D. Hammond, H. Sather, W. A. Bleyer, and P. Coccia[1]

Children with acute lymphoblastic leukemia (ALL) were treated until a decade ago as a homogeneous group of patients with the same diagnosis. Experience has illustrated the extraordinary clinical and biologic heterogeneity of patients with this diagnosis. Now numerous characteristics of the disease which can be evaluated at the time of diagnosis are known to be associated with either favorable or poor outcome to current treatments and, therefore, are important indicators of prognosis. With the identification of an increasing number of such prognostic factors, it has become possible to identify subsets of patients with ALL which can be expected to have a very favorable outcome to current treatments and other subsets that are at high risk of early relapse and death. It has become essential for the design and analysis of clinical trials to use important prognostic characteristics to stratify patients with ALL into relatively homogeneous subgroups and to design clinical trials which pose therapeutic questions appropriate for each stratum. Apart from clinical trials, the knowledge and judicious use of the most important prognostic factors are necessary for the selection of the most appropriate treatment for each patient group.

I wish to report the experience of the Childrens Cancer Study Group (CCSG) in the use of prognostic factors for the design and analysis of a series of eight successive studies of ALL which were initiated between 1972 and 1981 and into which over 5000 children were entered. The study designs stratified patients into subgroups of differing prognosis, and the clinical trials posed different questions for up to five different subsets of patients. This appears to have accomplished the purpose of optimizing their treatment and also accomplishing clear, analyzable study designs.

The most recent series of studies which have been followed long enough to illustrate long-term outcome is the CCG-160 series. These studies entered 2887 children with newly diagnosed ALL from 1978 to 1983. Patients were stratified by age and white blood cell count at diagnosis into three subgroups, each eligible for admission into a different study: CCG-161 for patients expected to be at low risk of relapse, CCG-162 for patients at average risk, and CCG-163 for patients expected to be at high risk of relapse and early death. Those with initial WBC 50 000 or greater (approximately 22% of all patients) were considered to be at high risk of relapse regardless of age. They were entered into CCG-163. Patients aged 3–6 years and with WBC less then 10 000 (approximately 27%) were entered into the low-risk study, CCG-161. All others (51%) were entered into the study for intermediate risk, CCG-162.

Following initial induction of remission and consolidation during a 2-month period, maintenance therapy was given to the completion of 2 or 3 years. The overall survival of patients entered on the 160 series is ap-

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proximately 70% at 7 years and the event-free survival (EFS) is 57%. EFS analysis considered all adverse events including induction failure, death, bone marrow relapse, and testicular or CNS leukemia. It also considered occult testicular relapse found on routine biopsy to be an adverse event. Testicular biopsy was required after 3 months of maintenance therapy for males with high-risk ALL and also at the end of 2 years of maintenance therapy for all males. Thus, the EFS analysis followed the most stringent criteria.

Between 5 and 7 years following diagnosis, which was 2–5 years after therapy had been discontinued, the EFS declined less than 1% per year. Thus, the 5-year EFS is relatively stable and predicts long-term EFS as determined by life table methods. The survival curve is approximately 15% higher than the EFS curve from 3.5 to 5.5 years following diagnosis and declines to a 12% difference between 6 and 7 years.

When the 5-year EFS of all risk groups was analyzed, it became apparent that infants less than 1 year of age had the worst rates regardless of the initial WBC. Children between 10 and 16 had the next poorest outcome. In this group, 5-year EFS was directly related to the WBC. Those with WBC less then 20 000 had a 5-year EFS of slightly over 50%, while those with WBC over 50 000 had less than 30% five-year EFS. The most favorable responders were those between the ages of 1 and 10 years with initial WBC below 20 000. In this age group, those with WBC over 50 000 at diagnosis fared much less well.

The obvious advantage of having a large population of patients for study is that it can be stratified into multiple subgroups according to several important factors. If a population of children with ALL is stratified into only two groups, by whatever prognostic factor, considerable information will be obscured in the analysis. It is recommended strongly to select, by reliable criteria, those at lowest risk of relapse and those at highest risk of relapse. In between these clearly definable extremes is the largest group of patients, those at intermediate risk. If only two strata are used, the analysis will be made more difficult by the contamination of either or both the low-risk and high-risk groups by admixtures of patients that do not fit logically into either category.

Alternatively, if a large study population is available, a detailed analysis of subsets stratified by multiple prognostic factors and analyzed by multivariate techniques can disclose very important information. For example, the most favorable outcome of the CCG-160 series was in patients between the ages of 1 and 10 years with initial WBC of 10 000 or below. Of this group, 80% achieved 5-year survival with no adverse events of any kind, and a high rate of cure certainly is expected. These became the eligibility criteria for our successor study for low-risk ALL, CCG-104.

Since the heterogeneity among patients with ALL, as disclosed by important prognostic factors, is often greater than the detectable therapeutic difference between regimens, prognostic factors may have a more powerful effect on the outcome of a clinical trial than the therapies under study. We have studied a large number of potential prognostic factors, the effects of which can be seen only among subsets of patients which have been stratified initially by important prognostic factors already known. In such subgroups of relatively homogeneous patients, the association of additional factors on outcome to treatment can be seen.

The presence at diagnosis of an abnormal mediastinal mass has frequently been cited as a bad prognostic factor. Our data on over 4700 patients shows this to be true when the presence or absence of a mediastinal mass is analyzed as a single, independent variable. However, its significance in the CCG-160 series patients was confined to those with an initial WBC between 10 000 and 50 000. Mediastinal mass was not a significant predictor of poor outcome among patients having initial WBC below 10 000 or above 50 000. This is an example of a prognostic factor of considerable importance as an independent variable, but which upon multivariate analysis is found to be so closely linked to the WBC that, when patients are stratified according to high WBC, one has at the same time selected the majority of patients that have mediastinal mass.

We evaluated the morphology of marrow lymphoblasts of over 3500 patients according to the French-American-British (FAB)

morphology criteria. We found that patients with 10% or more blasts of L2 morphology had outcomes much less favorable than those with predominantly L1 blast morphology. The effect of this variable is continuous, so the outcome is worse as the percentage of L2 blasts increases above 10%. In multivariate analysis, it is seen that blast morphology is an independent variable, not linked to the initial WBC level. It is an important predictor of outcome at all levels of initial WBC. It provides important new prognostic information in addition to the prognostic implications of the initial WBC.

With present remission induction chemotherapy regimens in wide use, up to 98% of large groups of patients with ALL on clinical trials achieve complete bone marrow remission; therefore, attainment of complete remission does not distinguish among patients with varying risk of relapse. Examination of the bone marrow after only the first 2 weeks of chemotherapy reveals that if malignant blasts have disappeared from the marrow the long-term prognosis is very favorable. If 25% or more malignant lymphoblasts remain at the 14th day of therapy, the long-term prognosis is poor, even for patients who achieve a blast-free marrow by 28 days. Thus the rapidity of the response to initial therapy is another important predictor of long-term outcome.

There are two other interesting and important observations about the CCG marrow response data. In our experience, only 90% of infants less than 1 year of age at diagnosis achieved a complete remission. Infants who achieve a marrow free of malignant blasts by the 14th day of therapy do not have a favorable long-term outcome even if they have a low initial WBC. Since infants as a group have poor outcome to current therapies, even though they may have a constellation of favorable prognostic factors, we have developed a different protocol specifically for infants less than 12 months of age (CCG-107).

These few examples illustrate the importance of prognostic factors and that prognostic variables must be examined in detail to detect their interaction. We have examined the rank order of importance of 17 variables, all of which were studied in a group of over 1500 patients. When these are examined as independent variables, their relative statistical significance can be determined by ranking them according to standardized chi square values, or by their *P* values, or by the relative risk of relapse they confer (Table 1). The relative order of importance as independent prognostic factors of significance was as follows: Initial WBC, mediastinal mass, sex, splenomegaly, age at diagnosis, day-14 marrow response, hepatomegaly, platelet count, lymph node enlargement, blast morphology, IgM level, E rosette reactivity, race, hemoglobin level at diagnosis, and CNS leukemia at diagnosis.

Since each of these factors may be associated with other factors, the same data were subjected to multivariate analysis to permit ranking the variables according to their prognostic importance in relation to all others. The analysis showed that the rank order among the factors was not changed very greatly (Table 2). However, only eight factors retained statistical significance in a multivariate ranking. Moreover, the degree of statistical significance as measured by standardized chi square values, and to some extent by *P* values, was substantially reduced. In this analysis the factors of greatest multivariate importance ranked by chi square significance were: initial WBC, sex, mediastinal mass, day-14 marrow response, age, platelet count, hepatomegaly, and blast morphology.

Since many of the variables have an association with the WBC level, an additional analysis was done to determine the importance of such linkages. Examination of the chi square values for each factor, both before and after adjustment for the prognostic importance of the WBC, disclosed the strength of the association between each factor and the WBC (Tables 3 and 4). Many had a marked reduction in their prognostic significance, as measured by chi square values, after adjustment for WBC. The prognostic significance of E-rosette reactivity and CNS leukemia at diagnosis was eliminated by adjustment for WBC. Their chi square values were reduced over 90%. The significance of mediastinal mass, splenomegaly, hepatomegaly, node enlargement, and race was reduced 40%–60% by adjustment for WBC. The prognostic significance of age at diagnosis, day-14 marrow response, plate-

Table 1. Univariate analysis to determine relative importance of factors predicting event-free survival (considered individually). 1490 patients with all variables[a]

Order of importance	"Standardized"[b] χ^2 value	p value	Relative risk	
1. White blood count	110.4	<0.0001	>20000:<20000	=2.2
2. Mediastinal mass	61.5	<0.0001	Yes:No	=2.8
3. Sex	44.2	<0.0001	M:F	=1.8
4. Splenomegaly	40.8	<0.0001	Mark:N+Mod	=1.9
5. Age	36.9	<0.0001	< 1:1–9	=4.1
			≧10:1–9	=2.0
6. Day-14 marrow	27.4	<0.0001	M3:M1	=2.7
			M2:M1	=1.7
7. Hepatomegaly	20.2	<0.0001	Mark:N+Mod	=1.6
8. Platelet count	15.5	0.0001	<50000:>50000	=1.4
9. Node enlargement	14.9	0.0001	Mark:N+Mod	=1.8
10. Blast morphology (FAB)	9.6	0.0001	L2:L1	=1.9
			L1/L2:L1	=1.5
11. IgM level	9.7	0.002	Depr:N	=1.4
12. E-rosette reactivity	8.5	0.004	Pos:Neg	=1.5
13. Race	4.4	0.04	Non-Wh:White	=1.3
14. Hemoglobin level	3.4	0.06	>8 g:<8 g	=1.2
15. CNS at diagnosis	3.0	0.09	Yes:No	=1.5
16. IgA level	0.0	0.89	Depr:N	=1.0
17. IgG level	0.0	0.99	Depr:N	=1.0

[a] CCSG ALL Studies 1978–1983.
[b] χ^2 value divided by degrees of freedom.

Table 2. Multivariate analysis to determine relative importance of factors predicting event-free survival (considered simultaneously). 1490 patients with all variables[a]

Order of importance	"Standardized"[b] χ^2 value	p value	Relative risk	
1. White blood count	39.3	<0.0001	>20000:<20000	=1.7
2. Sex	37.4	<0.0001	M:F	=1.8
3. Mediastinal mass	21.6	<0.0001	Yes:No	=1.9
4. Day-14 marrow	18.3	<0.0001	M3:M1	=2.4
			M2:M1	=1.6
5. Age	14.2	<0.0001	10+:1–9	=1.7
			<1:1–9	=1.5
6. Platelet count	13.1	0.0003	<50000:>50000	=1.3
7. Hepatomegaly	11.7	0.0006	Mark:N+Mod	=1.5
8. Blast morphology (FAB)	7.8	0.0004	L2:L1	=2.1
			L1/L2:L1	=1.4
9. IgM level	2.8	0.09	Depr:N	=1.2
10. Splenomegaly	1.7	0.19	Mark:N+Mod	=1.2
11. IgA level	1.0	0.31	Depr:N	=0.9
12. Race	0.9	0.34	Non-Wh:White	=1.1
13. IgG level	0.7	0.42	Depr:N	=0.9
14. E-rosette reactivity	0.4	0.53	Pos:Neg	=0.9
15. Hemoglobin level	0.3	0.58	>8 g:<8 g	=1.0
16. Node enlargement	0.3	0.59	Mark:N+Mod	=1.1
17. CNS at diagnosis	0.1	0.72	Yes:No	=1.1

[a] CCSG ALL studies 1978–1983.
[b] χ^2 value divided by degrees of freedom.

Table 3. Significance of prognostic factors before and after adjustment for white blood count

Factor	Order of importance		Standardized χ^2		Change	p value
	Before	After	Before	After	%	after
WBC	1	1	–	–	–	–
Sex	3	2	44.2	44.3	–	<0.0001
Mediastinal mass	2	3	61.5	36.0	41	<0.0001
Age	5	4	36.9	26.5	28	<0.0001
Day-14 marrow	6	5	20.7	17.4	16	<0.0001
Splenomegaly	4	6	40.8	16.4	60	<0.0001
Hepatomegaly	7	7	20.2	12.5	38	<0.0004
Platelet count	8	8	15.5	11.7	24	<0.0006
FAB morphology	10	9	11.0	9.5	–	0.003
IgM level	11	10	9.7	8.3	13	0.004
Nodal enlargement	9	11	14.9	7.2	48	0.007
Hemoglobin level	14	12	3.4	3.4	–	0.06
Race	13	13	4.4	2.4	45	0.12
E-rosette	12	14	8.5	0.4	96	0.56
CNS at diagnosis	15	15	3.0	0.2	93	0.66

let count, and IgM level was reduced 13%–28%. The prognostic significance of sex, marrow blast morphology, and hemoglobin level was not affected by adjustment for WBC. This identifies them as independent factors which provide prognostic information of value in addition to the WBC.

Additional prognostic factors continue to be reported, some of which may give new information of considerable value independently of other known factors. We are not reporting here the cytogenetic studies of lymhoblasts which have been performed in recent CCG studies and which are now known to have important prognostic implications independent of other factors. Similarly, the detection of pre-B phenotype has been found to identify patients with poor prognosis who would otherwise be considered as having favorable prognostic features. Thus, this procedure will be important in identifying high-risk patients who currently are identified incorrectly as patients with minimal risk of relapse.

In summary, multiple clinical and laboratory observations, which can customarily be made at the time of diagnosis of ALL, are known to have important predictive value in determining long-term outcome with currently available therapies. Prognostic factors of importance can be used to stratify patient populations into subgroups that will predictably have different outcomes. The outcome differences predicted by such prognostic factors are freqently as great as, or greater than, a therapeutic trial can detect. Therefore, it is mandatory that groups of patients with the same diagnosis be stratified according to important prognostic variables, both to enable the clinician to select the best available therapy for the particular patient and also to be able to design clinical trials to obtain answers to questions which are appropriate only for specific subgroups.

Table 4. Prognostic factors in acute lymphoblastic leukemia: association with white blood count

Very strong	Relatively independent
E-rosette reactivity	Age at diagnosis
CNS at diagnosis	Platelet count
Spenomegaly	Day-14 marrow
	IgM level
Strong	**Independent**
Node enlargement	Sex
Race	Blast morphology
Mediastinal mass	Hemoglobin
Hepatomegaly	

Unless patient populations under study in clinical trials are sufficiently large to permit

outcome analysis of subgroups that have been stratified according to multiple prognostic factors, the true results may be obscured. It is no longer appropriate to treat children with ALL as if all patients with the diagnosis deserve the same therapy. Subgroups of patients can be identified which have minimal risk of relapse and which will respond very favorably to currently available therapies, and even to less intensive therapies, with a high probability of cure. Such patients should not be subjected to toxic therapies with potentially deleterious late adverse effects. Conversely, patients who can be predicted to have high risk of early relapse and death certainly deserve a trial of therapy carefully designed to provide more effective treatment for their disease than the standard therapies which are known to be ineffective.

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Strategies for the Treatment of Children with Acute Lymphoblastic Leukemia and Unfavorable Presenting Features *

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Background

The Childrens Cancer Study Group (CCSG) is an association of over 100 institutions whose members are responsible for the care of the majority of children with neoplastic disease in the United States and Canada. Over the last 30 years, clinical trials have been conducted for children with acute lymphoblastic or undifferentiated leukemia (ALL). The voluminous data collected have permitted the identification of presenting features that are strongly predictive of outcome and maintain this power in multivariate analyses [1, 2]. These include age, lymphoma syndrome, white blood count (wbc), and percent marrow blasts FAB L2 [3, 4].

The diagnosis of lymphoma with leukemic transformation is by definition limited to patients with lymphoma and $<25\%$ marrow lymphoblasts. Lymphoma syndrome patients, on the other hand, have a lymphomatous mass (massive splenomegaly, massive lymphadenopathy, and/or large mediatinal mass) and $\geqq 25\%$ marrow lymphoblasts (FAB L1 or L2). One laboratory finding is also required (hemoglobin $\geqq 10$ g/dl wbc $\geqq 50\,000/mm^3$, and/or E-rosettes $\geqq 25\%$ positive). When patients with lymphoma syndrome are compared with those with lymphoblastic lymphoma, one finds similar blast cell morphology and histochemistry, similar distribution of blast cell phenotypes, and propensity for early marrow and/or extramedullary relapse [5].

Early CCSG attempts to improve the outcome of high-risk patients met with only limited success. On CCG-141, neither the addition of cyclophosphamide (cpm) to a vincristine (vcr), prednisone (prd), and L-asparaginase (L-asp) induction, nor employment of alternating courses of POMP [prd, vcr, 6-mercaptopurine (6-mp), and methotrexate (mtx)] and POCA (prd, vcr, cytosine arabinoside [c-a], and adriamycin [adr]) in maintenance, improved outcome in children with wbc $\geqq 20\,000/mm^3$ [2]. On CCG-163, the high-risk population was defined by wbc $\geqq 50\,000/mm^3$ and/or marrow blasts $>25\%$ FAB L2. Additive continuous maintenance added q 4 week courses of cpm, c-a, or adr to standard maintenance consisting of q 12 week intrathecal (i.t.) mtx, q 4 week vcr/prd, q week mtx, and q day 6-mp. Cyclic maintenance consisted of q 12 week i.t. mtx and cyclic pulses of (a) POMP; (b) vcr, prd, cpm; (c) POCA; or (d) mtx by 42-h infusion with citrovorum rescue each 3 weeks. Additive continuous maintenance was superior to cyclic maintenance, but preliminary analyses demonstrate no general improvement over historical controls [6].

Following experience on CCG-143 [7], however, the dose of prophylactic whole brain X-ray therapy was reduced from 2400 to 1800 rads and an age-based dosage schedule was adapted for i.t. medication. Children aged <1 year, $\geqq 1$ and <2 years, $\geqq 2$ and <3 years, and $\geqq 3$ years receive 6 mg, 8 mg, 10 mg, and 12 mg i.t. mtx, respectively. I.t. therapy was begun early in in-

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duction and continued in maintenance. The cumulative incidence of central nervous system (CNS) relapse in high-risk patients fell from about 25% to 6% at 3 years [8]. This has become the standard approach to CNS prophylaxis on current CCSG studies for high-risk patients, age $\geqq 1$ year.

The estimated 3-year event-free survival on CCSG-163 was about 40%.

Encouraging results from BFM 76/79 [9] suggested that better therapy might be available. Rather than embark on a randomized trial immediately, however, a group wide pilot was undertaken to enable participating investigators to gain personal experience so that any therapeutic benefit in the eventual trial would not be masked by errors in compliance and supportive care. Work at Memorial Sloan Kettering also suggested that a modification of LSA2L2 [10] – the New York regimen – might also be efficacious [5]. In May of 1981 two pilot studies were opened for group wide participation, CCG-192P and CCG-193P. Both employ the CNS prophylaxis described above.

New York Regimen (Continuously Intensive Therapy, CCG-192P)[5]

LSA2L2 has proven efficacy in the treatment of lymphoblastic lymphoma. Review of reported trials shows inclusion of a number of patients with >25% marrow lymphoblasts who might alternatively have been classified as ALL. Outcome for these patients and those with <25% marrow blasts are not appreciably different [11].

New York therapy is an attempt to improve the LSA2L2 protocol (Table 1). Patients receive daunomycin (dnm) on day 1 and 2 of induction in order to achieve more rapid cytoreduction and to allow earlier recovery of counts. L-asp is administered in induction and consolidation, and a vcr/prd course is administered in maintenance at times when counts are suppressed precluding more myelosuppressive therapy. BCNU and hydroxyurea, seemingly less active agents, are omitted. Intravenous methotrexate is added to consolidation. In maintenance, the dose of mtx is escalated to tolerance as is suggested by recent data regarding individual variations in mtx clearance and a possible relation to outcome [12].

Morbidity was substantial but managable. Estimated event-free survival is 68% at 5 years with 100 "high risk" patients on study.

Table 1. New York regimen

Induction (29 days)			
cpm	1200	mg/m^2	d 0
prd	60	mg/m^2	d 1–28 and taper
vcr	1.5	mg/m^2	d 1, 8, 15, 22
dnm	60	mg/m^2	d 2, 3
1-asp	6000	μ/m^2	d 15, 17, 19, 22, 24, 26
c-a i.t. by age			d 0
mtx i.t. by age			d 15
X-ray therapy to extra-abdominal bulk disease			
Consolidation (31 days)			
vcr	1.5	mg/m^2	d 0
c-a	150	mg/m^2	d 0–7
6-tg	75	mg/m^2	d 0–7
1-asp	6000	μ/m^2	d 8–19
mtx	10	mg/m^2	d 20–24
mtx i.t. by age			d 0, 7, 14, 21, 31
X-ray therapy to the whole brain			
Maintenance (56 days/course)			
I)			
mtx i.t. by age			d 0
6-tg	300	mg/m^2	d 0–3
cpm	1200	mg/m^2	d 4
	(600 mg/m^2 1st cycle)		
II)			
vcr	1.5	mg/m^2	d 11, 18
prd	180	mg/m^2	d 11–17
III)			
vcr	1.5	mg/m^2	d 25
mtx	150	mg/m^2	d 25
escalate by 50 mg/m^2/course to toxicity			
IV)			
adr	15	mg/m^2	d 39, 40
c-a	40	mg/m^2	d 41–43 q12h
6-tg	35	mg/m^2	d 41–43 q12h

BFM (Delayed Intensification Therapy, CCG-193P) [13]

BFM 76/79 [10] served as the basis for CCSG version (Table 2). Differences in-

Table 2. BFM therapy (CCSG "100 series" version)

Induction (35 days)			
vcr	1.5	mg/m^2	d 0, 7, 14, 21
prd	60	mg/m^2	d 0–27 and taper
dnm	25	mg/m^2	d 0, 7, 14, 21
1-asp	6000	u/m^2	d 3, 5, 7, 10, 12, 14, 17, 19, 21
Consolidation (35 days)			
cpm	1000	mg/m^2	d 0, 14
6-mp	60	mg/m^2	d 0–27
c-a	75	mg/m^2	d 1–4, 8–11, 15–18, 22–25
mtx i.t. by age			d 1, 8, 15, 22
Interim maintenance (56 days)			
6-mp	60	mg/m^2	d 0–41
mtx	15	mg/m^2	d 0, 7, 14, 21, 28, 35
Delayed intensification (49 days)			
			Reinduction (28 days)
vcr	1.5	mg/m^2	d 0, 7, 14
dxm	10	mg/m^2	d 0–20 and taper (dexamethasone)
adr	25	mg/m^2	d 0, 7, 14
1-asp	6000	u/m^2	d 3, 5, 7, 10, 12, 14
			Reconsolidation (21 days)
cpm	1000	mg/m^2	d 0
6-tg	60	mg/m^2	d 0–13
c-a	75	mg/m^2	d 1–4, 8–11
mtx i.t. by age			d 1, 8
Maintenance (84 days/course)			
vcr	1.5	mg/m^2	d 0, 28, 56
6-mp	75	mg/m^2	d 0–83
mtx	20	mg/m^2	d 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77
mtx i.t. by age			d 0

clude (a) CNS prophylaxis as described above; (b) L-asp administered t.i.w. × 9 in induction and in reinduction; (c) an additional week of rest in induction and reinduction in order to assure recovery of counts. Therapy could be interrupted on days 0 and 14 of consolidation and day 0 of reconsolidation until peripheral counts recovered (absolute neutrophil count $>500/mm^3$ and platelets $>100\,000/mm^3$). Otherwise, in induction, consolidation, reinduction and reconsolidation, therapy was not to be interrupted for uncomplicated myelosuppression.

Two hundred-nine patients were entered with wbc $\geq 50\,000/mm^3$ or lymphoma syndrome. Event-free survival was 63% at 3 years. This result is encouraging in this population and includes a 6% remission death rate in the delayed intensification phase. The BFM regimen was then further modified for the open randomized trial.

Current "100 Series" ALL Trial

The current "100 series" ALl trial divides children with unfavorable presenting features into three subsets. Extensive cell phenotype data are obtained for all patients by means of regional "resource laboratories" for use in future analyses. All children aged <1 year are eligible for CCG-107. All children aged ≥ 1 and <22 years aged with lymphoma syndrome are eligible for CCG-123. All other children aged ≥ 1 and <22 years with:

(a) wbc $\geq 50\,000/mm^3$ or
(b) marrow blasts $\geq 10\%$ FAB L2 and
    (1) age ≥ 1 and <2 years or ≥ 10 years or
    (2) age ≥ 2 and <10 years and wbc $\geq 10\,000/mm^3$

are eligible for CCG-106. Patients with marrow blasts $\geq 25\%$ FAB L3 are excluded. The studies opened in May of 1983.

Infants (CCG-107)

Infants <1 year old make up 3% of the study population (see Table 3) on past CCSG studies. They are more likely to have marked leukocytosis, hepatosplenomegaly, and CNS leukemia at diagnosis. They are less likely to achieve remission by day 14, but 90% achieve remission by day 28. The median duration of remission is 8 months. The 5-year event-free survial is 21% with leukemic relapse rather than treatment toxicity being the principal problem. The appearance of CNS relapse seems unrelated to whether or not the patient received prophylactic cranial X-ray therapy. One-half of the few long-term survivors manifest severe neuropsychological sequelae [14].

Infants were included in the New York pilot with provision for delay of cranial X-ray

Table 3. Expected outcome based on past[a] CCSG studies

	CCG-107	CCG-123	CCG-106
Percent of population	3%	14%	21%
Event-free survival			
3 years	24%	42%	47%
5 years	21%	39%	40%
Isol. CNS relapse[b]			
3 years	3%	10%	8%
Isol. test relapse[b]			
3 years	0%	20%	12%
Survival			
3 years	37%	56%	61%
5 years	27%	44%	46%

Isol, isolated; CNS, central nervous system; test, overt testicular.

[a] Based on CCG 141, 141A, 161, 162, 163 as of July, 1982.

[b] Based on CCG 161, 162, 163 as of July, 1982.

therapy until the child had passed his first birthday. Twenty-seven patients were entered. Twenty-five achieved remission (93%). The median duration of remission is 17 months. Six patients have had marrow relapse, three isolated central nervous system relapse, one isolated testicular relapse, and two died in remission (1 g negative sepsis, 1 pneumocystis pneumonia). Estimated 2-year event-free survival is 49%. All three central nervous system relapses occurred in patients who had received X-ray therapy [15].

Poplack and others have shown that high-dose mtx (6.0 g/m^2 over 1 h followed by 1.2 g/m/h $\times$ 23 h with citrovorum rescue) can produce cerebrospinal fluid levels similar to i.t. mtx and prevent CNS relapse in standard risk patients with ALL [16, 17].

CCG-107 is a single arm trial. Patients receive a four-drug induction followed by a consolidation phase requiring 3 infusions of very-high-dose mtx as above. After a 4-week interim maintenance phase, patients receive delayed intensification together with an additional mtx infusion. Maintenance is q 12 week i.t. mtx, q 4 week vcr/prd, weekly mtx (oral), and daily 6-mp. No patient receives cranial X-ray therapy. The study is in progress.

Lymphoma Syndrome (CCG-123)

Fourteen percent of patients have lymphoma syndrome. Past CCSG data (see Table 1) suggests a 39% 5-year event-free survival with a cumulative incidence of isolated CNS relapse of 10% and testicular relapse of 20% (in boys) at 3 years. CCG-123 compares the CCSG version of BFM (regimen A), LSA2L2 with cranial X-ray therapy (regimen B), and LSA2L2 without cranial X-ray therapy (regimen C). The outcome of patients with lymphoma syndrome treated on LSA2L2 will be compared with the outcome of patients with lymphoblastic lymphoma and lesser marrow involvement similarly treated on CCSG-502. The outcome of patients treated with BFM can be compared with the outcome of patients treated with BFM on the standard risk protocol (CCG-105) and on the other high-risk protocol (CCG-106). Future plans include is testing the New York regimen in this population.

Other High-Risk Patients (CCG-106)

Twenty-one percent of patients are eligible for CCG-106. Past CCSG experience suggests (see Table 1) a 40% 5-year event-free survial with a cumulative incidence of isolated cns relapse of 8% and testicular relapse of 12% (in boys) at 3 years. The current study compares the CCSG version of BFM, New York, and standard therapy. All patients receive cranial X-ray therapy. In November of 1984, patients on standard therapy were shown to have a poorer event-free survival. This disadvantage persisted in comparisons stratified by age, wbc, sex, and FAB. Assignment to standard therapy was halted. The study continues as a two armed trial. Twenty-four month event-free survival stands at about 85% on both experimental regimens [18].

The Future

Prospects seem brighter for children with ALL and unfavorable presenting features. Greater success with the high-risk population forces reexamination of current strate-

gies for the standard risk population. Pre-B phenotype may predict poor outcome in an otherwise standard risk patient [19]. Preliminary analyses suggest that slow early response to therapy may similarly portend a poorer outcome [20]. Culling these patients should leave the stndard risk group more homogeneous and with yet better outcome on nontoxic therapy. More intensive treatment of the identified subset may improve its outcome.

Successful strategies have involved increasing the intensity of therapy – the amount of drug administered per unit time. Future strategies should be directed at identifying subsets of patients who have FAB M1 AnLL, Philadelphia chromosome positive ALL, acute megakaryocytic leukemia, etc. and are not likely to benefit from modifications of ALL therapy, increasing the intensity of therapy without major increases in its morbidity, and reinvestigating the role of immunotherapy in patients with minimal disease burden. The impact of delays and dose reductions on outcome requires thorough study.

Past successes will delay the identification of future therapeutic advantage. Attention will shift from the 2- or 3-year event-free survival to the 5-or 6-year event-free survival. Careful monitoring of morbidity and late effects will become yet more critical as a greater percentage of patients can expect to survive their disease.

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Supportive Care in Acute Leukemia

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Infection Prevention and Immediate Antibiotic Therapy in the Neutropenic Patient

F. Wendt and G. Maschmeyer[1]

Severe infections are the major cause of death in patients with acute leukemias [6, 10, 11] (Table 1). Whereas lethal hemorrhagic complications have been reduced by improvement of platelet transfusion therapy, the incidence of lethal severe infection is still high. In particular, it contributes significantly to the early death rate during remission-induction therapy, particularly in older age groups of patients.

Neutropenia is the most important factor in the pathomechanism of severe infection (Fig. 1), as could be shown in an early study by Bodey et al. 1966 [2]. The more intensive and the longer lasting the neutropenia, the higher the risk that a particular patient will acquire a fatal infection. Pizzo [16] in Fig. 2 summarized the factors influencing the risk of acquiring infection in granulocytopenic patients and discussed the many open questions regarding prophylaxis and therapy of this type of infection.

Severe bronchopulmonary infections and bacteremias are the most important infections in these patients (Table 2). Organisms responsible vary remarkably. Whereas in the 1950s gram-positive cocci, especially *Staphylococcus aureus,* were most frequently found, in the 1960s and 1970s gram-negative organisms, mostly Enterobacteriaceae and *Pseudomonas* species dominated [3, 4]. With the introduction of more effective prophylactic measures and early antibiotic therapy with modern β-lactam and aminoglycoside antibiotics, the proportion of severe gram-negative infections reclined with concomitant increase of gram-positive infections and systemic mycoses.

It has been established that most severe infections in the neutropenic patient origi-

Table 1. Causes of death in acute leukemia

Ref.	*n*	Infection	Infection + Hemorrhage
Hersh et al. [9]	170	44%	23%
Schimpff et al. [14]	48	70%	ND
Inagaki et al. [10]	816	47%	ND
Chang et al. [6]	315	66%	9%
Brown [5]	109	54%	14%

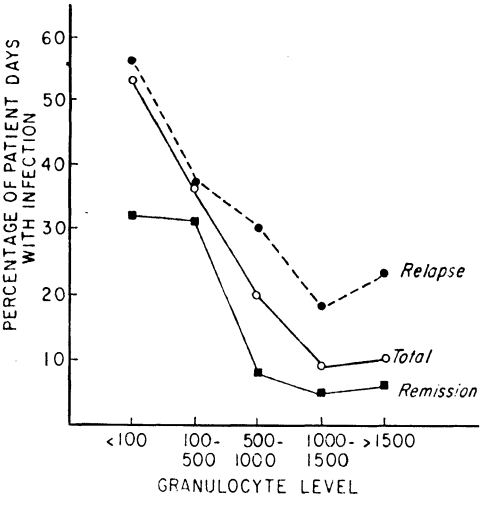

Fig. 1. The effect of granulocyte level on the presence of identified infection (from Bodey et al. 1966)

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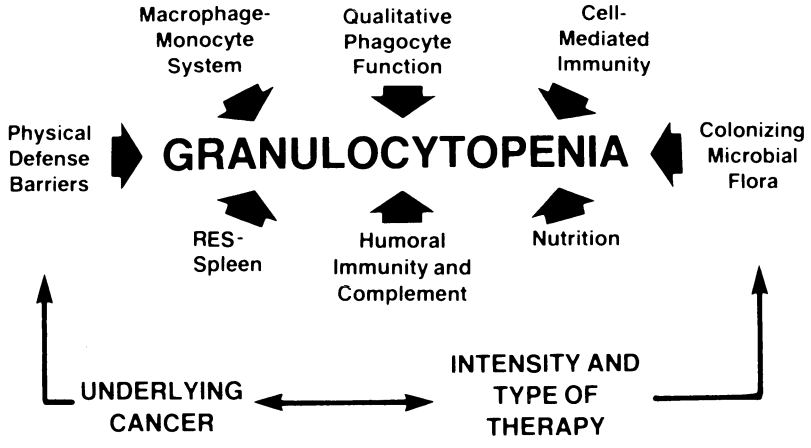

Fig. 2. Host-related factors influencing the risk of infection in granulocytopenic patients (from Pizzo 1984)

Table 2. Sites and organisms causing infection in leukemic patients (from Schimpff et al. 1974)

	Gram-positive	*St. aureus*	*Streptococcus*	*Pneumococcus*	Corynebacterium	Gram-Negative Bacilli	*E. coli*	Klebsiella	*P. aeruginosa*	Serratia	Enterobacter	Proteus	Salmonella	Other
Disseminated	25	16	6	0	3	294	98	73	70	17	26	3	2	5
Pneumonia	5	3	0	2	0	131	21	65	25	4	8	4	0	4
Cellulitis	17	16	1	0	0	68	10	9	32	9	4	4	0	0
Urinary tract	1	0	0	0	1	75	29	12	13	6	4	11	0	0
Gastrointestinal	0	0	0	0	0	14	0	9	1	0	0	0	3	1
Upper respiratory	3	2	1	0	0	5	1	1	1	1	0	1	0	0
Anorectal	0	0	0	0	0	18	10	0	3	0	1	4	0	0
Pelvic inflammatory	0	0	0	0	0	2	0	2	0	0	0	0	0	0
Hepatic*	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Meningitis'	1	0	0	1	0	1	0	0	1	0	0	0	0	0
Peritonitis	0	0	0	0	0	3	2	0	0	0	0	0	0	1
Bone and joint	0	0	0	0	0	4	1	2	0	1	0	0	0	0
Other[1]	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Total	52	37	8	3	4	617	172	174	146	38	43	27	5	12[§]

nate from the endogenous microbial flora [16, 17] (Table 3). Of the microorganisms found in severe infections, 50%–80% could previously be detected in surveillance cultures taken from fecal material, urine, oral washings, or swabs. This range was reproducible in our group.

From these data, concepts were developed to reduce the endogenous flora responsible for severe infections. The most rigorous strategy of strict protective reverse isolation and complete decontamination of the patient's endogenous microflora, the "germ-free patient", proved only partially effective [1, 3]. Institutional and cooperative multicenter studies showed that elimination of infections could not be realized and that resistant microorganisms increasingly caused

Table 3. Previous detection of microorganisms causing severe infection in acute leukemia patients 1981–1983

Infection	Germ(s)	Previous detection
Septicemia	*S. faecalis*	Oral washing
	Pseudomonas sp.	Oral washing
Septicemia	*St. epidermidis*	∅
Septicemia	Klebsiella/Enterobacter	Feces
Septicemia from pyelonephritis	*Proteus mirabilis*	Urine
Septicemia from pleuropneumonia	*E. coli*	Oral washing
	C. albicans	Feces
Septicemia	*St. aureus*	∅
Pneumonia	*Proteus vulgaris*(?)	Feces, oral washing
Pneumonia	Klebsiella/Enterobacter	∅
Pneumonia	Klebsiella/Enterobacter	∅
Pneumonia	Klebsiella/Enterobacter	∅
	C. albicans	Feces
Pneumonia	*St. aureus*	∅
	Pseudomonas sp.	∅

Table 4. Mechanisms responsible for colonization resistance of the gastrointestinal tract (from van der Waaij 1979)

Mechanical clearance
Mucin secretion
Cell desquamation
IgA secretion
Anaerobic bacteria
Lowering of pH and redox potential
Competition for essential nutrients
Production of bacteriocins
Production of volatile fatty acids
Deconjungation of bile salts/acids
"Wall paper" adherence on mucosal surface

Table 5. Antibiotics not affecting colonization resistance (from van der Waaij 1979)

Nonabsorbable	
Neomycin	Low dosages
Paromomycin	
Tobramycin	
Gentamicin	
Aztreonam	
Polymyxin B + E	
Cephaloridin	
Absorbable	
Pipemidic acid	
Nalidixic acid	
Quinolones	
Cephradine	Low dosages
Cefaclor	
Cephalexine	
Pivmecillinam	
Doxycycline	
Co-trimoxazole	

severe complications [7, 14, 20]. The colonization of resistant microorganisms was favored by selection pressure and by complete elimination of the physiologic anaerobic flora resulting in decreased colonization resistance of the gastrointestinal tract [15] (Table 4).

So, methods of prophylactic antimicrobial medication were studied which lead to selective elimination of potentially pathogenic microorganisms leaving the anaerobic gut flora intact, thus preserving colonization resistance. A certain number of nonabsorbable and absorbable antimicrobial drugs, shown in Table 5, possess this property under certain defined conditions [18, 19].

This concept of selective decontamination was studied in several institutions and several cooperative multicenter trials with differing results and more or less success [8, 12]. It could be established that regimens with constituents which exhibit some systemic effects are slightly superior to those regimens consisting only of nonabsorbable drugs. A particular trial of the E.O.R.T.C. Gnotobiotic Project Group showed the combination of co-trimoxazole + polymixin + amphotericin B suspension effective in prolonging the time to onset of first infection during the course of long-lasting severe neutropenia

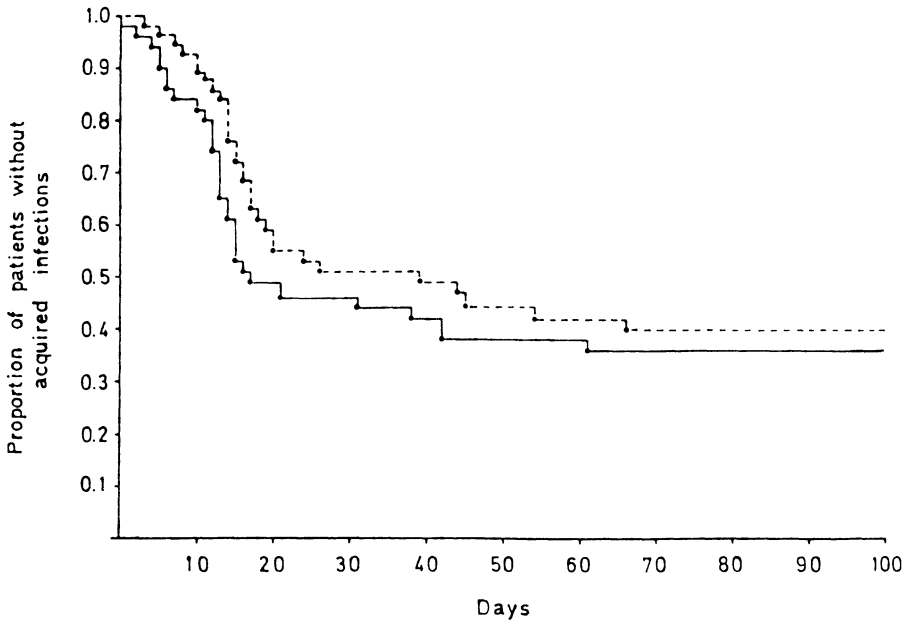

Fig. 3. Proportion of patients without acquired infections in the two different regimen with cotrimoxazole-polymyxin-amphotericin B (*dotted line*) against neomycin-polymyxin-amphotericin B (*full line*) (from Kurrle et al. 1986)

Table 6. Summarizing results, E.O.R.T.C. Gnotobiotic Project Group Study 1981–1983. (From Kurrle et al. 1986)

1. Advantages of group B (TMP−SMZ+polymyxin B)
Lower incidence of acquired septicemias ($p=0.015$) and of febrile days ($p<0.01$)
Significant reduction of days on systemic anbiotic therapy
Lower number of gram-positive infections
2. No difference in
Onset of the first acquired infection
Incidence of FUO
Incidence of local infections
Insidence of gram-negative infections
Patients' compliance
Outcome of antileukemic therapy
Duration of hemocytopenia

[13] (Fig. 3). The incidence of infection compared with the control group (Table 6) could be reduced. The efficiency of this method is comparable to complicated reverse isolation procedures despite the fact that this prophylaxis is pursued under normal hospital care conditions. Whether 4-quinolone derivatives will further improve infection prevention by prophylactic application is still under study in several institutions including the E.O.R.T.C. Gnotobiotic Project Group.

In the case of an infection occurring in a neutropenic patient despite prophylactic measures, immediate antibiotic therapy is necessary. Only bactericidal drugs can be expected to be effective. The regimen of application should serve the strategy that optimal drug concentrations in blood and tissues should rapidly be obtained. The choice of drugs depends upon the microorganisms expected to be responsible for the particular infection. Some institutional and big multicenter trials, in particular the E.O.R.T.C. International Antimicrobial Therapy Cooperative Group [9], have shown that initially a combination of modern β-lactam antibiotics, penicillins, or cephalosporins, with aminoglycoside antibiotics is most effective. A double β-lactam combination (ureidopenicillin + third generation cephalosporin) is claimed to be comparatively effective, and a few groups claim ceftazidime alone to be comparably effective in the order of 70%–80% cure rate of an infection in a neutropenic patient. In judging these controversial

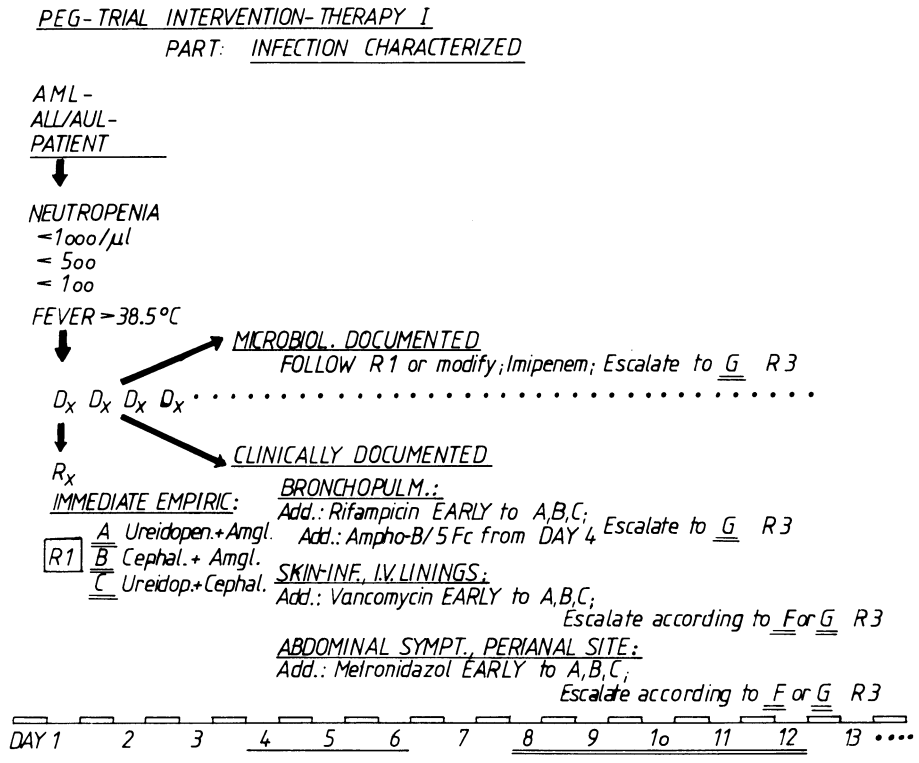

Fig. 4. Principal treatment strategy in the case of "infection characterized"

results some considerations should be made:

Those studies which differentiated the outcome depending upon the degree and the time course of neutropenia found that the success rate is higher and easier to obtain the less severe and the less long-lasting neutropenia is, calculated from the start of the severe infection. This effect is more pronounced than the effect of certain microbiological findings of particularly virulent microorganisms as, for instance, *S. aureus* or *Pseudomonas* species or Enterobacteriaceae.

Furthermore it became clear that, after day 4 of the particular infection, the phenomenon of either selection of a different potential pathogen or the rise of another acquired microorganism resistant to the drug regimen being given might decisively influence the course of infection.

This leads to a sophisticated intervention therapy regimen which contains several steps. These should be taken into account if, despite antimicrobial prophylaxis, an infection occurs in a neutropenic patient. A model of such an intervention therapy regimen is the trial design of the P.E.G. multicenter study [15] (Figs. 4 and 5).

Immediately following the initial diagnostic measures with clinical, radiological, and microbiological techniques to characterize infection in a febrile episode in a neutropenic patient, a combination of two drugs of the three different classes ureidopenicillins, third generation cephalosporins, and aminoglycosides is given in a high dose. During the following 4–6 days this infection episode might be specified as "microbiologically documented" as bacteriemia, urinary tract infection, or other, and antibiotic therapy might be adjusted to the microbiological findings.

In the case of negative microbiologic findings despite repeated diagnostic measures, infection might be specified as related to the bronchopulmonary system by X-ray findings, or as related to the gastrointestinal system or the perianal region depending upon certain specific symptoms. In this case, the antibiotic regimen should be adjusted by the

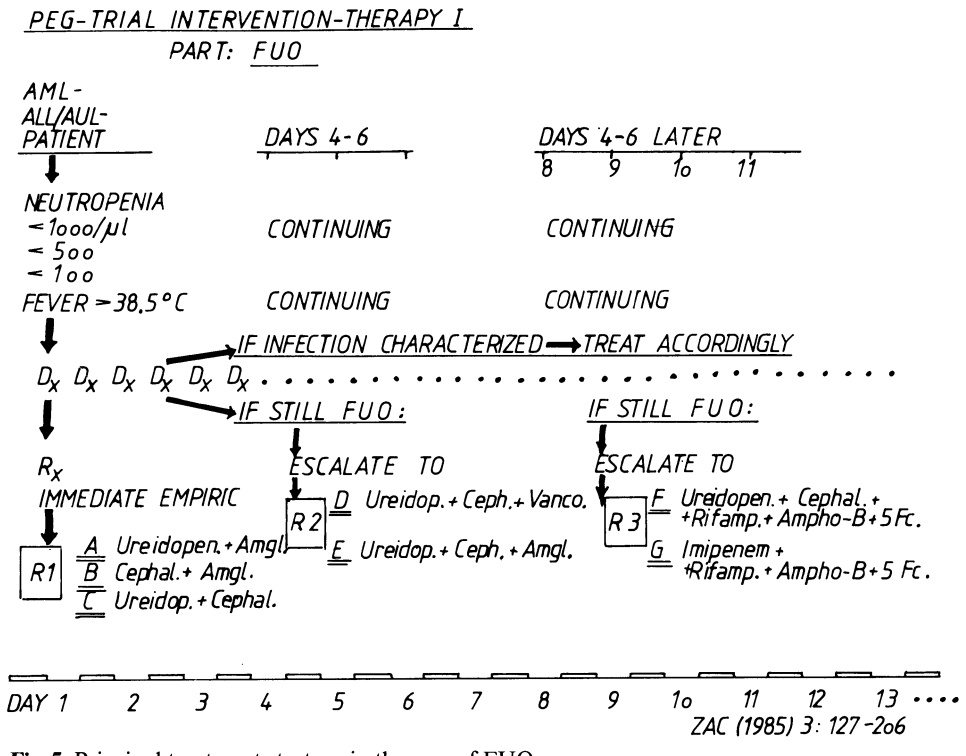

Fig. 5. Principal treatment strategy in the case of FUO

addition of drugs which aim at particular microorganisms frequently found in connection with this particular type of infection. In the case of skin infection, the skin flora and staphylococci have to be taken into consideration at the beginning by adding specifically active antibiotics to the initial double drug combination.

It should be mentioned that the preceding prophylactic antibiotic regimen has to be continuously applied during the whole period of systemic antibiotic application in order to prevent further selection of intestinal potentially pathogenic microorganisms and acquisition and colonization of new organisms with oral intake.

In the case of the infection episode remaining undetermined by repeated microbiological testing and repeated clinical follow-up investigations, the situation is regarded as FUO. FUO constitutes a certain percentage of infection episodes in neutropenic patients worldwide, and is concomitant with the increasing risk of secondary infection by opportunistic outgrowth of fungi in particular, but of other microorganisms as well. This leads to the strategy of escalating antimicrobial therapy early during the course of the infection episode by drugs active against gram-positive cocci, such as vancomycin and rifampicin, and against fungi, such as combination of amphotericin B and 5-fluocytosin. If even these combinations remain ineffective, the patient remains neutropenic and febrile, newer drugs, such as imipenem, might be added. It should again be emphasized that the complete course of an infection episode should be followed up with repeated clinical investigations and with microbiological surveillance investigations in order to detect clinical signs which might lead to change in strategy or causative microorganisms showing up.

The physician responsible for an acute leukemia patient in neutropenia who has acquired an infectious episode must be aware of the complications that may occur during the course of the treatment. The recovery of the patient usually comes along with the rise in peripheral neutrophils. The prognosis of

an individual infection episode in a neutropenic patient, therefore, will be better when infection appears late during the course of neutropenia. In order to get more patients reaching this prognostically better situation, infection prophylaxis as well as immediate infection therapy have to be improved.

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Special Aspects of Supportive Therapy in Childhood Acute Leukemias

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Leukemias and solid tumors in childhood have become potentially curable diseases as a result of increasingly more aggressive chemo-radiotherapy protocols. The limiting factor to both chemo- and radiotherapy is the tolerance of physiological tissues. Further advances in treatment results will depend largely on the development of new concepts and techniques of supportive care.

In this paper we present data on two special topics of supportive care in childhood malignancies: a) results of a randomized hypertransfusion study in children with acute lymphoblastic leukemia (ALL); and b) prophylaxis and treatment of varicella zoster virus infections during immunosuppressive therapy.

Hypertransfusion in Childhood Acute Lymphoblastic Leukemia During Remission Induction Therapy

Granulocytopenia and thrombocytopenia often complicate the treatment of malignancies, predisposing the patient to infections and bleeding with considerable morbidity and even mortality. Furthermore, chemotherapy has to be interrupted in these situations, and the ultimate chance of cure may be diminished. Animal studies have provided evidence that accelerated differentiation of pluripotent hematopoetic stem cells into either the erythropoetic or ganulopoetic pathway may be accompanied by diminished differentiation into the other pathway (stem cell competition [10, 17]). Toogood et al. found a significantly more rapid rise in neutrophils, a lower incidence of infection, and less interruption of chemotherapy in hypertransfused children with ALL during a two-drug remission induction therapy [18]. However, these results could not be reproduced by others [11, 19].

In 1979 we initiated a prospective randomized trial in children with ALL who were treated according to the BFM protocol 79/81 [12] to test the hypothesis whether hypertransfusion during remission induction therapy may shorten the period of granulocytopenia and/or thrombocytopenia and reduce the infection rate in these patients.

Patients, Treatment Protocol, and Methods

Sixty-one children with newly diagnosed ALL entered the study during a 15-month period from 1979 to 1980. Thirty children were randomized in the hypertransfusion group T_1, and 31 children were randomized into the control group T_0. The male:female ratio, the median age group, the ratio of T-ALL, the mean risk score BFM 79/81 [12], and the proportion of high-risk patients with a risk score >2 were comparable in the patient groups (Table 1).

Patients in group T_1 were initially hypertransfused with packed red blood cells to a hemoglobin content >15 g/dl. During the first 28 days of the remission induction protocol I, the Hb was maintained >12 g/dl in these patients. Patients in group T_0 were

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Table 1. Patient characteristics

	Hyper-transfused (T_1; $n=30$)	Not hyper-transfused (T_0; $n=31$)
Male:female	19:11	17:14
Median age (range)	6.2 (0.5–15) years	6.4 (1.5–15) years
T-ALL	6	5
Risk score (mean ± SD)	2.4 ± 2.1	2.6 ± 2.2
Therapy B (risk score > 2)	11	11

only transfused if the hemoglobin fell below 9 g/dl. Patients in group T_1 with a Hb < 12 g/dl ($n=4$) and in group T_0 with a Hb > 12 g/dl ($n=4$) during the first 28 days of remission induction were excluded from the study and are not included in Table 1.

Blood counts were taken every day to every other day in all patients. Hb, WBC, and platelet counts were measured by a Coulter counter. Blood smears were reviewed by an experienced technician. A control bone marrow aspiration was done on day 28 in all patients to document the remission state. Infectious complications, days with fever, and delay of chemotherapy were prospectively documented in all patients. Differences between the groups were evaluated by the χ^2 test, the Mann-Whitney U-test, or the student's *t*-test.

Results

Figure 1 shows the absolute neutrophil counts (ANC) during the first 28 days of remission induction therapy in patients who were or were not hypertransfused during this period. There were no statistically signifi-

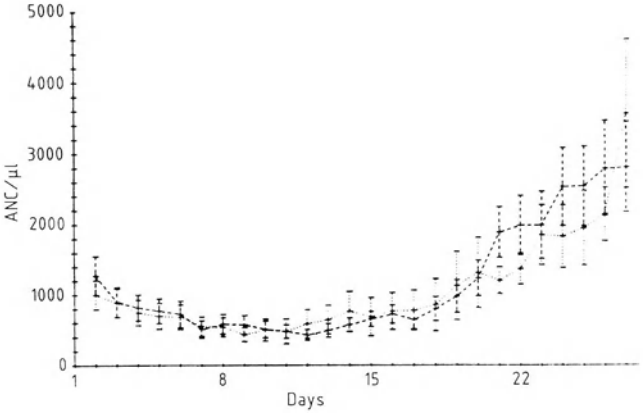

Fig. 1. Absolute neutrophil count (mean ± SEM) in children with ALL with and without hypertransfusion during remission induction therapy. --- Hypertransfusion group T_1, ... control group T_0

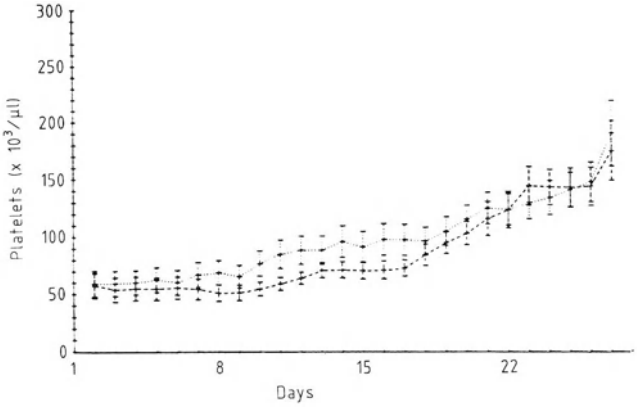

Fig. 2. Platelet count (mean ± SEM) in children with or without hypertransfusion during remission induction therapy. --- Hypertransfusion group T_1, ... control group T_0

Table 2. Myeloid:erythroid precursor ratio in the bone marrow on day 28

	Hyper-transfused (T_1; $n=30$)	Not hyper-transfused (T_0; $n=31$)
M:E ratio		
median	1.9:1	0.65:1
range	0.1–99:1	0.06–9.4:1

$p<0.05$ (Mann-Whitney's U-test).

cant differences between the two groups during the whole period.

Unexpectedly, the platelet counts were slightly higher in the control group during days 8–18, as shown in Fig. 2, but again these differences were not statistically significant. The myeloid:erythroid (M:E) precursor ratio in the control bone marrow on day 28 was significantly higher in the hypertransfused patients (Table 2).

In Table 3, the complications during remission induction therapy in both groups are compared. Significantly fewer patients in the hypertransfused groups T_1 developed severe infections during this period. Furthermore, in more patients of the control group T_0 chemotherapy had to be delayed due to infectious complications. The remission rate was equal in both groups (Table 3).

The late results of the study after more than 6 years are shown in Table 4. There is no statistically significant difference in the number of patients in complete continuous remission after 4–6 years in the two groups. The same is shown in the life table analysis:

Table 4. Late results of the ALL hypertransfusion study BFM 79/81 (Feb. 21, 1986)

	Hyper-transfused (T_1)	Not hyper-transfused (T_0)
Patients	30	31
Early death/ nonresponder	1	1
Complete remission	29	30
Death in remission	2	0
Relapses	8	11
CCR (after 4–6 yrs)	19	19
pCCR (life table method)	63%; SD 9%	61%; SD 9%

pCCR in group T_1: 63%, SD = 9%; pCCR in group T_0: 61%, SD = 9% (Table 4).

Discussion

In the present study, no effect of hypertransfusion on the time to recovery of neutrophils and platelets could be demonstrated in children with ALL during intensive four-drug remission induction therapy with protocol BFM 79/81. Failure of hypertransfusion was also recorded by Helson et al. [11] and Weiss et al. [19]. The beneficial effect of hypertransfusion in the study of Toogood et al. [18] may be due to either less intensive remission induction therapy – two-drug regimen rather than the four-drug regimen in the present study – or due to the slightly different transfusion schedule used by these

Table 3. Complications during remission induction therapy

	Hypertransfused (T_1; $n=30$)	Not hypertransfused (T_0; $n=31$)
Local infections	3	7
Severe infections (septicemia, pneumonia)	3[a]	12[a]
Fever of unknown origin	6	5
Delay of therapy	2	5
Severe hemorrhage	0	1 (fibrinogen ↓)
Death during remission induction	0	1 (toxic pancreatitis)
Nonresponse	1	0
CR	29	30

[a] $p<0.05$ (χ^2 test).

authors – initial transfusion to a hemoglobin to 16–18 g/dl rather than 15 g/dl in the present study.

Although erythropoetin levels were not available in the present study, a clear hypertransfusion effect was demonstrated in the control bone marrow on day 28 in our study. Furthermore, the relative and absolute numbers of reticulocytes were significantly lower in our hypertransfused patients as compared with the controls.

Although the ANC was comparable in both groups, the number of severe infections – pneumonia, septicemia – was significantly lower in hypertransfusion patients as compared with the controls. This effect may be explained by better tissue perfusion in hypertransfused children, but these results need confirmation by further studies. However, these studies must keep in mind that at least in children with hyperleukocytosis rapid transfusion of packed red blood cells may be disastrous because of the increased bleeding tendency especially within the central nervous system [1]. Thus, hypertransfusion cannot be recommended for all children with acute leukemia during remission induction therapy.

Prophylaxis and Treatment of Varicella Zoster Virus Infections in Children During Immunosuppressive Therapy

Varicella zoster virus (VZV) can be a serious threat to immunocompromised patients. Primary varicella (chickenpox) and varicella zoster (shingles) infection can complicate the course of cancer chemotherapy or organ transplantation in children and adults.

Prior to effective antiviral treatment, a mortality rate of 7%–14% was reported for overt VZV disease [6, 8]. Several reports have demonstrated that early application of zoster immunoglobulin (ZIG) after incubation with VZV can prevent or mitigate overt VZV infections in immunocompromised patients [3, 7, 13].

Overt VZV disease can be effectively treated with different antiviral drugs as interferon [2], Vidarabine [20], or acyclovir [4, 15]. In the following, we will present data on the prophylaxis and treatment of VZV infections in immunocompromised children with malignancies at our institution.

Patients and Methods

Case reports of 317 children with malignancies at the University Children's Hospital Münster were retrospectively evaluated for susceptibility to the varicella zoster virus. Seronegative children with a negative history for varicella were defined as susceptible to VZV. All patients were treated with intensive chemotherapy and/or radiotherapy according to the actual BFM or GPO protocols. After known exposure to varicella zoster virus, all children received intravenous standard immunglobulin (Intraglobin) or intramuscular zoster immunglobulin (Immunglobulin Anti-Varicelle SRK; Gammaprotect-Varicelle; Varicellon). Since 1981 all immunocompromised children with overt VZV disease (chickenpox and shingles) received intravenously acyclovir (3×10 mg/kg as 1-h infusion) for 5–7 days.

The incidence of VZV infections in the different patient groups and morbidity and mortality rates were evaluated. Differences between two patient groups were evaluated with the χ^2 test.

Results

As shown in Table 5, the incidence of manifest varicella after exposure to chickenpox (6.2%) or shingles (5.6%) in susceptible immunocompromised children was in the same range.

The incidence of varicella was significantly ($p < 0.001$) higher after household exposures (37.5%) as compared with all other exposures (3.5%) (Table 6).

Table 5. Incidence of manifest varicella after exposure to chickenpox or shingles in susceptible children with malignancies

	Exposures	Manifest varicella
Exposure to chickenpox	161	19 (6.2%)
Exposure to shingles	54	3 (5.6%)

p = n.s. (χ^2 test).

Table 6. Incidence of manifest varicella after exposure to VZV in susceptible children with malignancies

	Exposures	Manifest varicella
Household exposure	16	6 (37.5%)
Others (school, kindergarten, Dept. of Oncology)	199	7 (3.5%)

$p < 0.001$ (χ^2 test)

Table 7. Effect of ZIG prophylaxis on the course of manifest VZV disease

	Course of chickenpox	
	With ZIG	Without ZIG
"Normal"	10	4
Severe, life-threatening	1	6
Death due to VZV disease	0	2

$p < 0.05$ (χ^2 test).

Table 8. Incidence of manifest varicella after ZIG in susceptible children with malignancies

	Interval between exposure and ZIG application	
	< 24 h	> 24 h
Exposed children	60	94
Manifest chickenpox	2	9

$p = 0.08$ (χ^2 test).

Table 9. Treatment of VZV disease with acyclovir in 31 children with malignancies (Univ. Children's Hospital Münster; 10. 81–2. 86)

	Varicella	Herpes zoster
ALL	11	8
HD	0	5
Sarcomas	3	4
Other diseases (Dermatomyositis; AHA; kidney transplantation)	3	3

Table 10. Death due to VZV in children with malignancies

	Without ACV treatment	With ACV treatment
VZV disease	63	31
Severe course	6	2[a]
Deaths	2	0

[a] Treatment was started on day 3 after manifest disease, no prior ZIG prophylaxis.

Application of ZIG could significantly ($p < 0.05$) mitigate the later course of varicella (Table 7). The only two deaths due to varicella in immunocompromised children in our department occurred in patients whose parents did not inform us about the possible exposure to the virus and who therefore did not receive ZIG.

We found a clear trend ($p = 0.08$) that application of ZIG within 24 h of known exposure to the virus is more effective than later application (Table 8).

Since October 1981, 31 children with malignancies and six immunocompromised children with other diseases have been treated with intravenous acyclovir at our hospital (Table 9).

Since the introduction of acyclovir, no death due to varicella has occurred in our institution (Table 10). The only two severe courses occurred in two patients in which acyclovir treatment was started late after manifestation of chickenpox (day 3) and who did not receive ZIG prophylaxis after exposure (Table 10).

Discussion

Our data have shown effective prevention or mitigation of overt VZV infection by ZIG in immunocompromised patients. These data are in accordance with recent data by others [3, 7, 13]. ZIG should be given as early as possible after incubation of susceptible children with VZV, but application later than 72 after incubation still may have some effect. The incidence of manifest VZV disease is significantly higher after household exposure than after all other exposures. Unexpectedly, our data demonstrate that expo-

sure of susceptible children to both chickenpox and shingles can be followed by overt varicella.

Overt VZV infection can effectively be treated with intravenous acyclovir. In a recent randomized study, Shepp et al. clearly demonstrated the superiority of acyclovir as compared to Vidarabine in the treatment of VZV infections in bone marrow transplantations [16]. The morbidity of VZV infections in immuncompromised children can be significantly reduced, and the mortality of overt VZV infections is zero in our study and other similar studies [4, 5, 14, 15] as compared with a significant mortality reported by others [6, 8] and ourselves in the pre-acyclovir era. Thus, at the moment there seems to be no urgent need for active immunisation [9] against varicella zoster virus in immunocompromised children.

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Prevention of Infection in Patients with Acute Nonlymphocytic Leukemia by Several Drug Treatment Regimens

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Introduction

Infections are a frequent consequence of the severe granulocytopenia in patients with acute leukemia. Patients with acute nonlymphocytic leukemia are especially susceptible to bacterial infections during remission induction treatment. They usually become infected by aerobic gram-negative rods from the alimentary tract [1–3]. The most common infections are pneumonia, oesophagitis, pharyngitis, skin infections, and perianal and perirectal lesions.

Various methods to prevent infections in these patients have been studied. The results of using protective isolation or prophylactic nonabsorbable antibiotics to prevent infections are not always convincing and comparable [4–6]. Combinations of oral nonabsorbable antibiotics used for alimentary tract decontamination are often poorly tolerated, expensive, and do not always protect against acquisition of resistant microorganisms. Another approach has been suggested by van der Waay et al.: the selective suppression of the microbial flora in the gut [7, 8]. These workers have tried only to eliminate the aerobic flora of the alimentary tract. With the anaerobic flora left intact, the resistance to colonization by aerobic gram-negative rods is considered to be maintained and subsequent infection prevented. Antimicrobial agents such as trimethoprim-sulfamethoxazole, nalidixic acid, and colistin suppress only the aerobic flora. Therefore, these agents can be used for selective decontamination of the alimentary tract. The results of infection prevention by selective decontamination that have been evaluated are encouraging [9–11]. Wade et al. [12] showed the effect of the combination of trimethoprim-sulfamethoxazole and nystatin. In another study [13], an additional benefit of using trimethoprim-sulfamethoxazole was reported in patients already receiving the combination of framycetin, colistin, and nystatin. However, one of the potential problems of using trimethoprim-sulfamethoxazole as a prophylactic agent is the emergence of resistant microbes and the possible side effects, such as skin reactions [14]. Therefore, we investigated the effect of several prophylactic regimens such as trimethoprim-sulfamethoxazole (TMP-SMX), TMP-SMX together with colistin, neomycin and colistin, and one of the new quinolone derivates, ciprofloxacin.

Patients and Methods

All adult patients with acute nonlymphocytic leukemia, first diagnosed or in relapse, who were admitted to our hospital between 1 December 1978 and 1 November 1980 were randomized to a control group or to a group receiving trimethoprim-sulfamethoxazole prophylactically; from 30 November 1980 and 1 December 1981 to a regime of trimethoprim-sulfamethoxazole together with colistin; from 1 December 1981 to 1 December 1983 randomized to a group receiving colistin plus trimethoprim-sulfa-

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methoxazole or to a group receiving neomycin and colistin, and after 1 December 1983 to either a group receiving ciprofloxacin or trimethoprim-sulfamethoxazole plus colistin.

Trimethoprim-sulfamethoxazole (240 mg + 1200 mg) was given orally twice a day; colistin sulfate tablets (150 mg) and neomycin (250 mg) was administered every 6 h; ciprofloxacin (500 mg) was given orally twice daily. All patients received amphotericin B (suspension and tablets, each 200 mg) four times a day.

No other prophylactic measures were taken. Patients were cared for in single rooms or in an open ward. The groups were similarly treated in every other respect. The antileukemic treatment consisted of a combination of doxorubicin, cytarabine, and vincristine; sometimes thioguanine was added. The prophylactic treatment was started shortly before cytotoxic treatment was initiated and ended when the granulocyte count was above 500/μL and no further cytotoxic treatment was indicated.

Clinical Investigations

Patients were examined daily for mucositis, cellulitis, or perianal lesions. Fever was defined as an oral temperature above 38 °C lasting more than 12 h. Clinically documented infections were defined as the presence of signs and symptoms of an infection not followed by a positive culture result. Bacteriologically documented infections were clinically documented infections in which pathogenic microorganism(s) could be isolated from the site of the infection or from the blood. All granulocytopenic patients with suspected infections, fever, or both were treated intravenously with gentamicin and cephalothin or cefuroxim while the prophylactic treatment was continued.

Microbiological Investigations

Surveillance cultures were obtained from the throat, nose, urine, and feces of each patient at time of admission and thereafter once or twice weekly. Fecal samples were placed in transport medium containing cysteine hydrochloride (Fluka AG, Buchs, Switzerland), peptone (Oxoid Ltd., Basingstoke, England), and yeast extract (Merck AG, Darmstadt, West Germany). After homogenization, tenfold serial dilutions were made. For the isolation and enumeration of aerobic bacteria the dilutions were subcultured on MacConkey's agar; blood agar (Blood Agar Base, with 7% defibrinated sheep blood, Oxoid Ltd.) for staphylococci and streptococci; and Sabouraud's dextrose agar (Difco Laboratories, Detroit, Michigan), selective for yeasts. For the isolation and enumeration of anaerobic microorganisms, serial dilutions were plated on blood agar containing Iso-sensitest agar (Oxoid Ltd.), 7% defibrinated sheep blood, 5% saponin (Merck), and kanamycin (125 μg/mL). For the first 20 patients, quantitative anaerobic samplings of stool were done. *Enterobacteriaceae* were identified with API 20E (API System; S. A. La Balme, Les Grottes, France), anaerobic microorganisms with API 20A, and *Staphylococcus aureus* by deoxyribonuclease and coagulase assays. Antimicrobial susceptibility was tested by agar diffusion method on Iso-sensitest agar (Oxoid Ltd.) with Neo-sensitabs (Rosco; Taastrup, Denmark).

Results

A total 146 patients participated in the different studies. the results of the different studies are summarized in Tables 1–4. The conclusion of the first study was that a significant decrease in the number of acquired infections was found in the trimethoprim-sulfamethoxazole group. There were 16 acquired infections compared with 31 in the control group. Five patients with trimethoprim-sulfamethoxazole had no fever during the whole granulocytopenic period, while all control patients receiving only amphotericin B experienced at least one fabrile episode. However, one disturbing factor of this study was that patients receiving trimethoprim-sulfamethoxazole became colonized and infected with multiresistant organisms (Table 4). This led us to add colistin to prevent the emergence of multiresistant organisms. As can be seen also the regimen trimethoprim-sulfamethoxazole plus co-

Table 1. Clinical data and acquired infections

	Control	T/S	T/S+C	Colistin+ Neomycin	CF
Patients (*n*)	26	26	30	15	24
Acute nonlymphocytic leukemia (*n*)	26	26	18	12	20
Granulocytopenic episodes (*n*)	40	39	38	20	39
Granulocyte count 101–499/μl[a]	10.4	10.5	11.3	9.6	10.2
Granulocyte count <100/μl[a]	17.9	27.8	26.7	31.1	27.3
Granulocytopenic days with fever (%)	32	22	15	35	19
Acquired infections	31	16	14	14	20
Bacteriologically documented	20	9	6	9	5
Major infections (bacteremia)	13 (7)	6 (4)	2 (2)	6 (6)	4 (3)
Minor infections	7	3	4	3	1
Clinically documented	11	7	8	5	15

[a] (mean) days.
T/S, trimethoprim/sulfamethoxazole; C, colistin; CF, ciprofloxacin.

Table 2. Sites of bacteriologically documented infections

	Control[a]	T/S[a]	T/S+C	Colistin+ Neomycin	CF
Patients (*n*)	26	26	30	15	24
Bacteriologically documented infections	20	9	6	9	5
Pneumonia	3	2	0	0	1[b]
Upper respiratory tract	0	1	0	1	1
Anorectal	4	1	0	0	0
Urinary tract	3	0	1	0	0
Skin and soft tissue	6	2	3	2	1
Not site found (septicemia)	4	3	2	6	2

[a] Yeast infections are not included.
[b] Candida infections.
T/S, trimethoxazole/sulfamethoxazole; C, colistin; CF, ciprofloxacin.

listin led to a reduction of infection (Tables 1 and 2). But only two patients became colonized with resistant microbes (two enterobacteriacease strains), compared to 20 in the trimethoprim-sulfamethoxazole group (Table 4).

We concluded from these results that trimethoprim-sulfamethoxazole prevented infections in deep granulocytopenic patients. However, one could argue that the effect of trimethoprim-sulfamethoxazole was not an effect on the gutflora per se, but merely trimethoprim-sulfamethoxazole prevented infection by its capacity to reach relatively high tissue levels thereby preventing invasion of the tissues by bacteria. Therefore, we studied the effect of two nonabsorbable drugs neomycin and colistin and compared their efficacy with that of trimethoprim-sulfamethoxazole plus colistin. This small study (only 15 patients participated) shows a clear tendency towards a better effectiveness of trimethoprim-sulfamethoxazole plus colistin. Ten patients in the latter group had no infections compared with only three in the colistin-neomycin group (Table 1).

One major problem that arose during the study was the development of allergic reactions against trimethoprim-sulfamethoxazole. About 20% of the patients receiving trimethoprim-sulfamethoxazole developed cutaneous manifestations, most often against the sulfa component of the combina-

Table 3. Microbiological documentation of acquired infections

	Control	T/S	T/S+C	Colistin+ Neomycin	CF
Patients (*n*)	26	26	30	15	24
Acquired infections	31	16	14	9 (6)	20
Bacteriologically documented	13 (7)[a]	9 (4)	6 (2)	9 (6)	5 (3)
Aerobic gram-negative bacteria	6 (2)	1	1	6 (3)	3 (2)
S. aureus	4	1	–	3	–
S. epidermidis	–	–	–	1 (1)	–
Streptococci (β-hemolytic)	2 (2)	–	–	2 (2)	2 (2)
S. epidermidis + streptococci	–	–	1	–	–
Corynebacterium spp. and other gram-positive bacilli.	1 (1)	–	–	–	1
Aerobic gram-negative bacteria	13 (4)	7 (3)	4 (1)	2 (2)	0
Enterobacterieceae	9 (3)	5 (3)	1	–	–
Pseudomonas spp.	1	2	3 (1)	1 (1)	–
Enterobacterieceae + *Pseudomonas*	3 (1)	–	–	–	–
Haemophilus influenzae	–	–	–	1 (1)	–
Aerobic gram-positive and gram-negative bacteria	–	–	–	–	–
Anaerobic bacteria	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Yeast or fungi	3	2	1	1	1

[a] ( ), cases with bacteremia.
T/S, trimethoprim/sulfamethoxazole; C, colistin; CF, ciprofloxacin.

Table 4. Acquisition of, and infection with, resistant[a] gram-negative bacilli and yeast

	Control	T/S	T/S+C	Colistin+ Neomycin	CF
Patients (*n*)	26	26	30	15	24
Transient[b] gram-negative bacilli	12	29	10 (1)	9	6
Enterobacterieceae			5 (1)	2	–
Pseudomonas spp.			5	2	3
Acinetobacter spp.			–	2	3
Colonizing[c] gram-negative bacilli	6 (2)	20 (6)	2	5	0
Enterobacterieceae	6 (2)	19 (5)	2	5	–
Pseudomonas spp.	–	1 (1)	–	–	–
Colonizing yeast	9	10	8 (2)	5	7

[a] Resistant to CF or T/S.
[b] Microorganisms appearing in one single culture.
[c] Microorganisms appearing in consecutive cultures.
[d] Indicate number of patients with infections.
T/S, trimethoprim/sulfamethoxazole; C, colistin; CF, ciprofloxacin.

tion. Therefore, a new drug, ciprofloxacin, was introduced. Again, 49 patients entered the study, 25 receiving trimethoprim-sulfamethoxazole plus colistin and 24 ciprofloxacin. As can be seen, ciprofloxacin was extremely effective in preventing infection due to gram-negative microbes, only infections due to gram-positive bacteria were observed *(S. epidermidis, S. viridans)*. Data on the second arm of this study (25 patients receiving trimethoprim-sulfamethoxazole plus colistin are not shown); these were comparable with the data of our first trimethoprim-sulfamethoxazole plus colistin group.

Discussion

All agents used in the several prophylactic studies were able to reduce the infection rate in granulocytopenic patients with acute nonlymphocytic leukemia.

In the first study we observed a significant reduction in the number of acquired infections in patients with acute nonlymphocytic leukemia who received trimethoprim-sulfamethoxazole, However, infections seen in these patients were caused by resistant bacteria. To prevent the emergence of these resistant strains, we therefore decided to treat patients with acute nonlymphocytic leukemia with a prophylactic regimen of trimethoprim-sulfamethoxazole plus colistin during remission induction treatment. With the exception of *Proteus* spp., most aerobic gram-negative microorganisms are sensitive to colistin, which ist not absorbed from the alimentary tract. The combination of trimethoprim-sulfamethoxazole plus colistin was well tolerated in our patients, and its efficacy in preventing infections was at least as good as trimethoprim-sulfamethoxazole alone. The addition of colistin significantly reduced the acquisition rate and the number of infectionsly resistant Enterobacteriaceae [15].

In the third study two regimens were used for infection prevention in patients with acute leukemia: one with only nonabsorbable drugs (colistin plus neomycin). The results show that both regimens are similarly effective in preventing infections caused by aerobic gram-negative microorganisms. Colonization of the alimentary tract with potential pathogens occurred only in a few cases. The appearance of *Proteus* spp. in patients receiving colistin with neomycin is not surprising because these microorganisms are not sensitive to colistin. This is not necessarily a disadvantage of this regimen because colonization with *Proteus* is rarely followed by a subsequent infection. The low incidence of colonization of the oropharynx in both groups support the idea that the gut flora and the oropharyngeal flora are closely related, and that the intestines are the most likely reservoir for these microorganisms. Selective decontamination of the microbial flora of the gut seems to be the most important way to prevent colonization of the oropharynx. For this purpose a systemic antibiotic effect, e.g., trimethoprim-sulfamethoxazole, is not needed. A favorable effect of selective decontamination on the oropharyngeal flora may result in a decrease of bacterial pneumonia. However, one should be aware of a relative increase in pneumonia caused by *Aspergillus* spp. Of the total 115 patients who received selective decontamination, we have observed five cases of acquired pneumonias caused by *Aspergillus* spp.

The addition of systemic antibiotics such as trimethoprim-sulfamethoxazole reduced the number of infections caused by gram-positive bacteria, confirming our previous findings. There was a lower incidence of septicemias in the group of patients treated with colistin plus trimethoprim-sulfamethoxazole: no episodes of septicemias were seen compared with six in the colistin-neomycin group ($p = 0.05$). We concluded that the systemic effect of trimethoprim-sulfamethoxazole contributed to the lower infection rate.

The introduction of new 4-quinolones, antimicrobial agents with a broad activity against potentially pathogenic aerobic gram-negative rods and with virtually no effect on the anaerobic part of the normal bacterial flora, allows us to study the ability of one of these drugs, ciprofloxacin, to decontaminate selectively the alimentary tract, and therefore assess its possible value for prevention of infection. Ciprofloxacin is well absorbed, and only two tablets (500 mg each) were taken daily. In none of our 24 patients who were treated for at least 25 days were serious side effects (such as nausea, vomiting, diarrhea, dizziness, headache, and skin rash) observed. In all patients the use of ciprofloxacin led to a rapid and persisting reduction in number of Enterobacteriaceae in fecal samples. No effect was seen on anaerobes, such as *Bacteroides* and *Clostridium* species, although some effect was seen on anaerobic gram-positive bacteria, such as nonsporeforming rods and cocci [16]. During the study period few ciprofloxacin-resistant aerobic gram-negative rods were cultured, and always in a low number. Such findings never led to colonization or infection. Seven patients became colonized with *S. epidermidis*.

In 24 patients receiving ciprofloxacin for a mean duration of 25 days, no infections caused by gram-negative rods occurred. Most of the acquired infections were caused by gram-positive cocci, especially *S. epidermidis,* in patients with i.v. catheters and by β-hemolytic streptococci from the oropharynx. This study shows that ciprofloxacin is effective for selective decontamination of the alimentary tract in leukemic patients during remission induction treatment. It is possible that the use of this quinolone derivative may lead to a change in the spectrum of infections seen in these patients: from causative organisms such as Enterobacteriaceae or *Pseudomonas* to *S. epidermidis* and other gram-positive cocci. Therefore, studies with new quinolones most be accompanied by surveillance cultures. Another possible disadvantge which needs careful follow-up is the possibility that when patients with prophylactic agents develop fever, cultures may remain negative because of the clinical drug given. The percentage of clinically documented but not bacteriologically proven cases will then increase, with possible difficulties for selecting appropriate therapeutic agents as a consequence.

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Bone Marrow Transplantation in Acute Leukemias

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The Role of Bone Marrow Transplantation in Acute Myelogenous Leukemia

R. P Gale[1]

Over the last 15 years bone marrow transplantation has emerged as an important therapeutic modality in acute myelogenous leukemia (AML). It is useful in selected patients with AML in precisely defined clinical circumstances. Generally three types of transplants have been investigated: transplantation from a HLA-identical sibling, transplantation from partially or fully HLA-matched related or unrelated donors, and autologous transplantation using the patient's bone marrow cryopreserved during remission. Results of bone marrow transplantation will be discussed in this context.

HLA-Identical Sibling Transplants

The basic principles of bone marrow transplantation in patients with leukemia have been reviewed recently [1]. Patients receive high-dose chemotherapy and total-body radiation followed by "rescue" with donor bone marrow cells. Engraftment occurs over a period of 3–4 weeks, and hematologic values usually return to normal within 2 or 3 months. Several centers have reported 15%–20% 3–5-year disease-free survival in selected patients with resistant AML receiving transplants from HLA-identical siblings (reviewed in [2, 3]). Relapse rates were high, 60%–100% in most studies. Survival rates may be as high as 30% when an identical-twin donor is available [2, 4, 5].

The high incidence of relapse following transplants in resistant leukemia led to trials of transplantation in remission. The concept behind this approach is that the antileukemic effect of bone marrow transplantation might be greater when transplants were performed before the development of resistant disease, when the leukemia cell burden is low, and when the patient is in relatively good clinical condition.

Data from the International Bone Marrow Transplant Registry and several transplant centers indicate that 20%–35% of patients with AML transplanted in second to fourth complete remission can achieve >3-year leukemia-free survival [1–6]. These results are clearly superior to those achieved with conventional and investigational chemotherapy, and transplantation is the optimal therapy for these individuals.

Almost 1000 transplants have been reported in patients with AML in first remission [1, 2, 5–14]. In most studies, high doses of cyclosphosphamide [120 mg/kg) or cytarabine (24–36 g/m^2) followed by single-dose or fractionated total-body radiation (7.5–12 Gy) are used to prepare patients for transplantation. Most studies indicate 3-year leukemia relapse rates of 25% (range 0–45%).

Actuarial survival at 3 years is 45% (range 35%–70%). Because these results suggest a substantial potential cure rate for transplantation, it is important to consider the relative efficacy of chemotherapy versus bone marrow transplantation for patients with AML in first remission. Data from a review of the literature are indicated in Fig. 1. It is clear from these data that the likelihood

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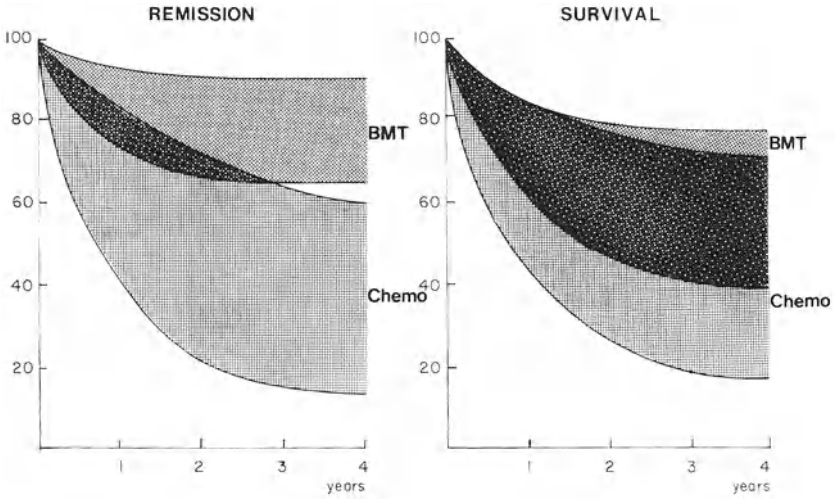

Fig. 1

of leukemia relapse is significantly decreased by bone marrow transplantation. Results of survival analyses are less certain with considerable overlap of data. This situation arises because, although bone marrow transplantation is more effective in eradicating leukemia, it introduces new problems such as graft-versus-host disease (GvHD) and interstitial pneumonia.

Prospective randomized trials are required to determine the relative efficacy of chemotherapy versus bone marrow transplantation in first remission. Results of three trials have been reported [7, 13, 14]. The UCLA and Seattle studies were prospective, controlled trials; the Marsden study was not. Both the UCLA and Marsden trials confirm a decreased risk of recurrent leukemia following bone marrow transplantation; survival data are more difficult to interpret. The only full-length report of the Marsden trial failed to indicate improved survival; a subsequent letter suggested a significant survival advantage but study details were not provided. The Seattle trial showed only a borderline improvement in overall survival; there was, however, a significant relapse-free survival advantage after 6 months for the transplant group. Although there was a favorable trend towards improved survival in the transplant group in the UCLA study, this difference was not significant. The reasons for these disparate results from different centers is complex. Trial designs varied, as did details of chemotherapy and bone marrow transplantation. Some trials included genetically identical twins or partially HLA-matched donors whereas others included only HLA-identical siblings. One important factor was the mechanism by which patients declining treatment assignment were analyzed; in one trial they were censored, in others, included. Finally, there is a high probability that a clinically important benefit of transplantation might not achieve statistical significance because of the relatively small sample size (or type 2 error).

Based on these studies it is likely that transplantation is more effective in preventing leukemia relapse than chemotherapy but neither treatment can be definitively recommended as producing superior survival. Since there is a trend towards improved survival with transplantation and no evidence of an adverse affect, it is reasonable to recommend transplantation for individuals <45 years with an HLA-identical sibling donor. Transplantation in older patients as well as transplants from HLA-nonidentical related or unrelated donors are less likely to produce favorable results, and this approach should be regarded as investigational in patients with AML in first remission. For patients in second or subsequent remission, there are no or only few cures with chemotherapy alone. Transplantation from an HLA-identical sibling results in approximately 30% long-term survival and is the preferred therapy. Since patients in second or later remission have such a poor prognosis, it is reasonable to consider investigational approaches as transplants from par-

tially or fully HLA-matched related or unrelated donors. It should be noted that some recent trials of reinduction chemotherapy in AML, such as with high-dose cytarabine, have reported 10%–15% long second remissions. If these data are confirmed, it may be necessary to reassess the role of bone marrow transplantation in this setting.

One controversial area is whether a patient relapses after an initial remission and is a transplant candidate, should be transplanted immediately, or should receive additional chemotherapy in an attempt to achieve a second remission followed by a transplant if remission is achieved. Randomized trials addressing this question have not been reported, and there are considerable, contradictory data. One recent large study found no difference in transplant outcome. The optimal approach probably depends on several factors including the likelihood of achieving a second remission, the leukemia cell mass, and clinical condition at the time of relapse. Patients with a high leukemia cell mass and with a reasonable probability of achieving a second remission should probably receive chemotherapy first. In an attempt to increase remission, patients with low numbers of leukemia cells and those with a poor probability of response may be candidates for immediate transplantation. This decision is best individualized until results of randomized trials are available.

Another question which arises from these studies is whether there are specific subsets of patiens with AML who are most likely to benefit from transplantation. Transplant results are superior in younger individuals in most but not all studies. Patients <16–24 years have 2–3 year survival at 50%–70%. Although it seems reasonable to conclude that transplantation is the preferred therapy in these individuals, it is important to consider that 2–3 year continuous disease-free survival of 50%–70% also been reported with chemotherapy in this age group in some but not all studies [15–17]. Again, a definitive conclusion is not possible presently; a CCSG trial which should answer this question is nearing completion.

A second group of individuals who might benefit from transplantation are those predicted to have an inferior outcome with chemotherapy. As indicated, these individuals are difficult to accurately identify but might include patients with acute monoblastic leukemia (M5a), extramedullary or central nervous system leukemia, leukemic cell counts >50–100×10^9/l, or with selected chromosome abnormalities including t (4; 11) and t (9; 22). Although this approach seems reasonable, it presupposes that these prognostic factors will not have a similar adverse influence on the outcome of transplantation. This is unknown; three studies reported adverse effects of several of these factors on survival following transplantation [12, 18, 19]; one study found no effect [7].

The mechanism by which bone marrow transplantation is effective in eradicating leukemia is controversial. Although most investigators believe it is related to the antileukemic effects of high-dose chemotherapy and radiation, this concept has recently been questioned. One recent study reviewed data in patients with AML in first remission who received a transplant from an HLA-identical sibling or a genetically identical twin. The relapse rate in twins (60%) was threefold higher than that observed in patients receiving transplants from HLA-identical siblings (20%) [20]. Similar data have been reported by others. An antileukemic effect of allogeneic bone marrow transplantation is well documented in animals (for review see [21]) and in patients with acute or chronic GvHD who receive transplants during relapse [22–24]. This is generally referred to as a graft-versus-leukemia effect. In animals, graft-versus-leukemia is clearly separable from graft-versus-host disease; it is not known whether a similar conclusion applies to man. Furthermore, a graft-versus-leukemia effect has been reported in experimental systems in which graft-versus-host disease does not occur, such as gnotobiotic mice [25]. Based on these data, it appears that the mechanism by which bone marrow transplantation is effective in AML is complex and may involve at least two different effects; direct antileukemic activities of drug and radiation and graft-versus-leukemia. The latter may be identical or clinically indistinguishable from graft-versus-host disease in man. Therefore, although GvHD is an important problem in transplant patients, it also may have an important beneficial antileukemia effect. This is supported by data from a recent trial in

which GvHD was presented by T-cell depletion using a monoclonal anti-T antibody. The incidence of leukemia relapse was significantly increased [26]. This has led some investigators to try to induce limited GvHD intentionally in young transplant recipients with acute lymphoblastic leukemia [27]. Data from this trial are too preliminary for critical analyses.

These data on the mechanism of leukemia and radication have important implications for the use of bone marrow *autotransplants* in AML in first remission implying a minimum 60% relapse rate in this setting; the relapse rate could be higher if the cryopreserved bone marrow contained leukemia cells. There are two caveats regarding the potential anti leukemic effect of allogeneic transplantation. First, it may be important in some but not all stages of AML or may be difficult to detect when the relapse rate is low. In three recent analyses in patients transplanted in first remission, an association between GvHD and leukemia relapse was found in two studies. The second caveat is that the demonstration of an allogeneic antileukemic effect may depend upon the efficacy of the pretransplant conditioning regimen. Some regimens are associated with few if any relapses and it is therefore difficult to demonstrate any graft-versus-leukemia effect. Interestingly, however, twins receiving these regimens have a relapse rate similar to twins receiving regimens thought to be less effective in the context of allogeneic transplantation.

Bone marrow transplantation during remission, even if potentially useful, is limited by patient age and availability of an HLA-identical sibling donor. Most investigators agree that optimal candidates for bone marrow transplantation are those <30 years and usually <20 years. Thus, bone marrow transplantation, even if superior to chemotherpy, is applicable to only a small proportion (approximately 5%) of patients with AML and alternative approaches are required.

Partially of Fully HLA-Identical Related or Unrelated Donors

An increasing number of transplants have been performed using donors other than an HLA-identical sibling, including partially or fully HLA-matched related or unrelated donors [28–31]. Studies from several centers have reported >150 transplants between related donors sharing one HLA-haploidentical. In some instances these individuals were matched for one or more HLA-antigens of the second HLA-haplotype. Most of these transplants have been performed in individuals in $\geqq$ second remission or relapse. Recent analyses of these data indicate that when two additional antigens are shared on the second HLA-haplotype, survival results are not significantly different from those of recipients of HLA-identical sibling transplants if the patient is in remission. Results of transplants in patients in relapse or using related donors sharing only a single HLA-haplotype are less satisfactory; survival is possible in young individuals (<16 years) but poor in older patients [30]. Graft-rejection may be a problem, particularly when T cells are removed from the graft with antibodies and complement or immunotoxins to modify GvHD. This problem may be less when physical separation techniques are used or if more intensive post-transplant immune suppression is given. Pulmonary toxicity and early infections are also common amongst recipients of mismatched transplants. Although the incidence and rate of onset of acute GvHD were increased in some studies using partially HLA-matched donors, this did not always result in decreased survival.

Recently, several centers have reported transplants using partially or fully HLA-matched unrelated donors, primarily in patients with advanced AML [31–33]. Preliminary data indicate acceptable results, but very few completely HLA-matched transplants have been reported and follow-up is brief. Presently transplants from donors other than an HLA-identical sibling should be reserved for patients unresponsive to other approaches.

Autologous Bone Marrow Transplantation

Autologous bone marrow transplantation is an area of considerable recent interest (reviewed in [2, 5, 34, 35]). Typically, bone marrow is cryopreserved while the patient is in remission. When relapse occurs, the patient

receives high-dose chemotherapy and/or radiation followed by "rescue" with the cryopreserved autologous bone marrow. This approach is attractive since it can be used in patients without an HLA-identical donor and avoids the immunologic problems of graft rejection and graft-versus-host disease; the risk of interstitial pneumonia is also decreased. One reservation regarding this approach is the high probability that the cryopreserved remission bone marrow contains residual leukemia cells. It is possible that these cells can be eliminated by physical, immunologic, or pharmacologic techniques. This approach has been primarily evaluated in patients with acute lymphoblastic leukemia (ALL) in ≧ second remission in whom the cryopreserved bone marrow was treated in vitro with antisera or monoclonal antibodies reactive with ALL cells or was fractioned on a density gradient [34–39]. Recovery of hematopoiesis has been reported and approximately 20% of patients survive >2 years. It is not certain whether these results are a consequence of the autotransplant or the in vitro treatment; additional data are required before definitive conclusions can be reached regarding the efficacy of this approach.

Several investigators have reported antisera or monoclonal antibodies reactive with AML cells [40–44]. Most antibodies react with only a proportion of cases of AML. Even when the antibodies are reactive, they are rarely cytotoxic to 100% of the leukemia cells. Furthermore, it is uncertain whether these antibodies react against leukemia progenitor cells [45, 46]. In most instances antibodies in AML cells are also reactive with normal hematopoietic stem cells. Some in vitro data suggest that antibodies to HLA-DR might be effective since they react with AML cells but apparently not with some human-pluripotent hematopoiectic stem cells as detected by long-term bone marrow cultures. Again these antibodies may react with only a proportion of leukemia cells; proliferating cells may display decreased levels of HLA-DR [11]. Some patients with advanced AML have been treated with monoclonal antibodies; results have not been encouraging [47].

As indicated, bone marrow autotransplants in first remission are likely to be complicated by a substantial risk of leukemia relapse. This may occur from persistence of leukemia in the patient, in the cryopreserved bone marrow, or both. Recently there have been reports of beneficial results of patients with AML receiving autotransplants in first remission [48, 49]. These studies are difficult to evaluate critically since they involve highly selected patients who have been in remission for several months to years. Presently there are no convincing data that the continued leukemia-free survival in these individuals is a consequence of the bone marrow autotransplant. To address this question, controlled trials in which patients are randomized to postremission chemotherapy versus transplantation are needed. Until these data are available, autotransplants in first remission should be regarded as investigational.

Autotransplants in second or greater remission are less difficult to critically evaluate since there is no long-term suvival in patients receiving chemotherapy. Results of autotransplants in AML in ≧ second remission and relapse have been disappointing with actuarial relapse rates >80% and survival <10% at 1–2 years [50]. Recently, two studies reported results in patients in second remission in whom the cryopreserved bone marrow cells were treated with 4-hydroperoxycyclophosphamide to remove residual leukemia cells [51, 52]. These data are encouraging with actuarial survival of >30% and relapse rates of >60% but must be considered preliminary since in vitro studies have failed to indicate a selective toxicity of this drug for leukemic versus normal stem cells [53]. Other approaches using monoclonal antibodies or purification of normal stem cells have not been critically evaluated in man. Clearly, additional data are required before any of these approaches can be regarded as effective.

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Allogeneic Marrow Transplantation for Treatment of Leukemia: Results of the Munich Cooperative Group

H. J. Kolb, C. Bender-Götze, R. J. Haas, S. Thierfelder, and W. Wilmanns [1, 2]

Allogeneic bone marrow transplantation has improved the survival of a group of patients with otherwise poor prognosis provided the patient is of younger age and an HLA-identical marrow donor is available [1]. In Munich, a cooperative group for bone marrow transplantation was founded following the first successful transplants in 1975 [2]. Ninety-seven patients with leukemia were transplanted in the Departments of Internal Medicine and Pediatrics of the University of Munich, the Community Hospital München-Schwabing, and the Technical University according to common protocols. Six patients were grafted with autologous marrow four with marrow from their monozygous twin, and nine with marrow from other allogeneic donors than HLA-identical siblings. This report summarizes the results of 78 patients who were grafted with marrow from HLA-identical siblings.

Acute Lymphoblastic Leukemia (ALL) and Acute Undifferentiated Leukemia (AUL)

Thirty-nine patients were grafted for treatment of ALL and AUL (Fig. 1). At 2 years the probability of survival for patients grafted in third or later remission or in refractory relapse is 22% and for patients grafted in second remission 55%. Patients grafted in first remission were adults with a high risk of recurrence according to the ALL/AUL study in adults [3]. In one patient leukemia recurred 4 months after grafting. A second remission was induced and maintained since more than 11 months with chemotherapy. The relapse of leukemia was of host type, marrow and blood cells during remission are again of donor karyotype. One patient grafted in first remission died of sepsis. Twenty-two of 34 patients grafted in an advance stage of leukemia died with recurrence of leukemia (12), infection (2), interstitial pneumonia (4), graft-versus-host disease (GVHD) and interstitial pneumonia (1), GVHD and infection (1), or graft failure (2). Four patients grafted for treatment of advanced leukemia have survived more than 5 years in ummaintained remission.

Acute Myelogenous Leukemia (AML)

Fifteen patients were grafted in an advanced stage of AML (Fig. 2). Ten of these patients have died. The cause of death was toxicity in three cases, infection in two, interstitial pneumonia in two, and recurrence of leukemia in three. Two patients grafted in an advanced stage have survived more than 2 years in unmaintained remission. Two of nine patients grafted in first remission died of GVHD followed by interstitial pneumonia or infection. The probability of survival and remission for patients grafted in first remission at 1 year is 75%.

Chronic Myelogenous Leukemia (CML)

Eleven patients were grafted in chronic phase and four patients in advanced phase.

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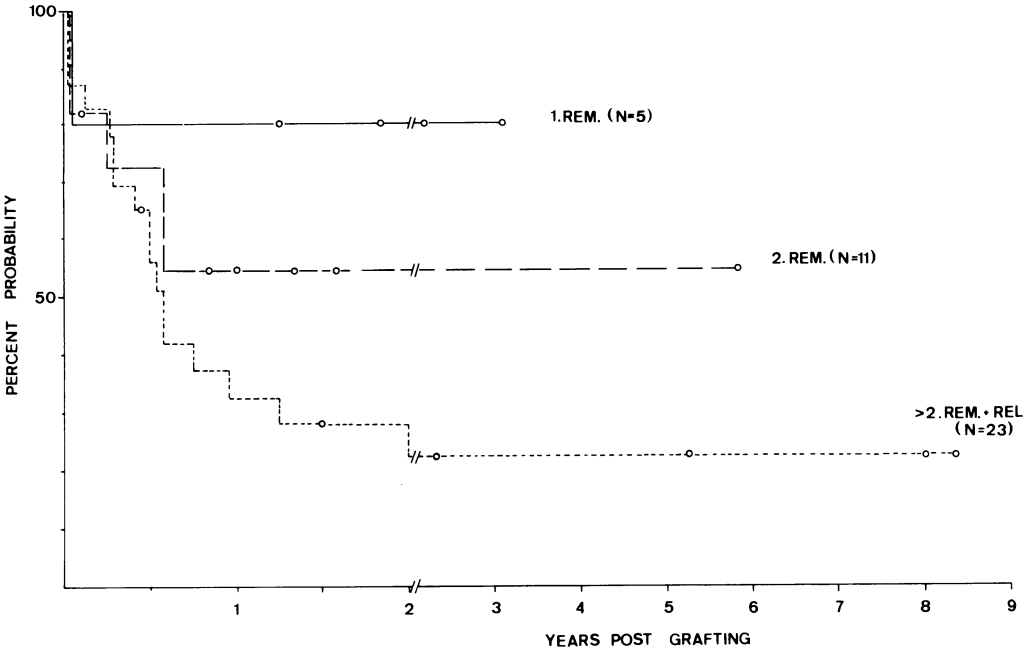

Fig. 1. Survival of patients with acute lymphoblastic and acute undifferentiated leukemia following HLA-identical marrow transplantation. Results per February 1986. *1. Rem.*, patients grafted in first remission; *2. Rem. + Rel.*, patients grafted in third or later remission or in relapse; *numbers in parenthesis* indicate number of patients grafted; *open circles* indicate living patients

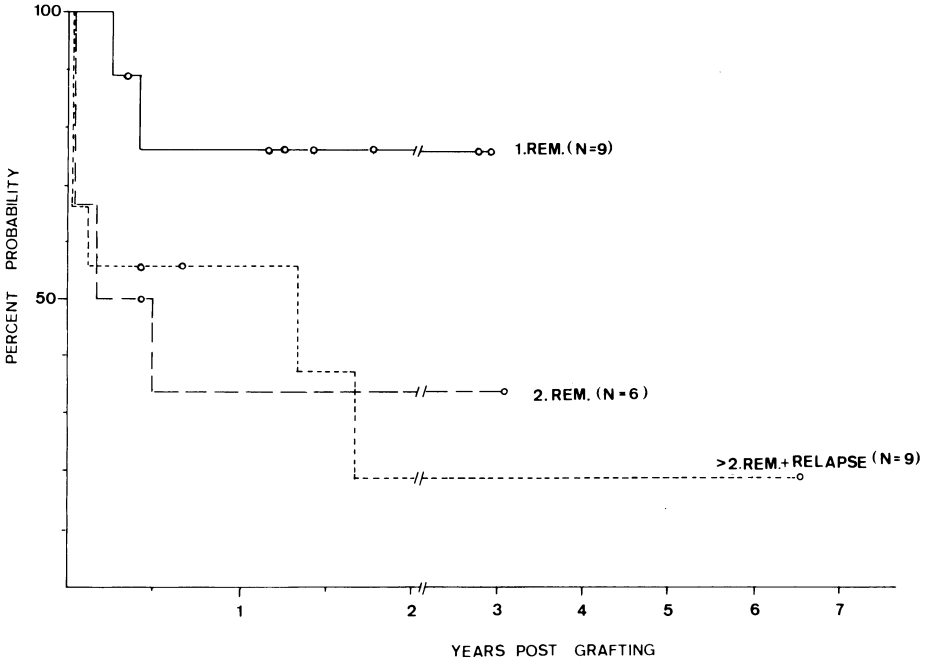

Fig. 2. Survival of patients with acute myelogenous leukemia following HLA-identical marrow transplantation. See legend to Fig. 1

Advanced phase comprised second blast crisis in one, accelerated phase in one, and second chronic phase in two patients. Infection, interstitial pneumonia, GVHD and interstitial pneumonia, and recurrence of blast crisis were causes of failure in patients with advanced CML. We lost three patients grafted in chronic phase due to toxicity (one case with veno-occlusive disease, one case with Lyell syndrome) and to graft rejection. The probability of survival at 1 year is 72%. Repeated cytogenetic analyses failed to show karyotypes with the Philadelphia chromosome after transplantation.

Conditioning Regimens

Three regimens of antileukemic and immunosuppressive conditioning were used consecutively. Only patients grafted in an advanced stage of leukemia, i.e., second or later remission or refractory relapse were evaluated, since recurrence of leukemia has been rare in our patients who were grafted in an early phase (1 of 25). So far we have not lost any patients due to leukemia during the first 3 months after transplantation. Therefore, the probability of remission was evaluated only in patients who survived more than 3 months.

The first regimen (BAC-TBI) included intensive chemotherapy with BCNU (bischloroethylnitrosourea, 200 mg/m^2 on day 12 and 11 before grafting) and cytosine arabinoside (200 mg/m^2 daily as continuous infusion from day 10 through 6 before grafting) in addition to the Seattle protocol of cyclophosphamide (60 mg/kg on day 5 and 4 before grafting) and total body irradiation (TBI) in a single dose of 9.3 Gy on the day before grafting. TBI was administered from two opposing cobalt sources at a dose rate of 4.5 cGy/min in body midline. This regimen has been less well tolerated by adult patients than by children. Therefore, BCNU was reduced to one dose, and cytosine arabinoside was given as continuous infusion of only 100 mg/m^2 daily and additional injections of 50 mg/m^2 in the morning and the evening. Twenty-one of 25 patients were in third or later remission or relapse. Five of 25 patients have been more than 5 years in continuous unmaintained remission (Fig. 4). Fifteen patients survived more than 3 months, the probability of remission is 33% (Fig. 5).

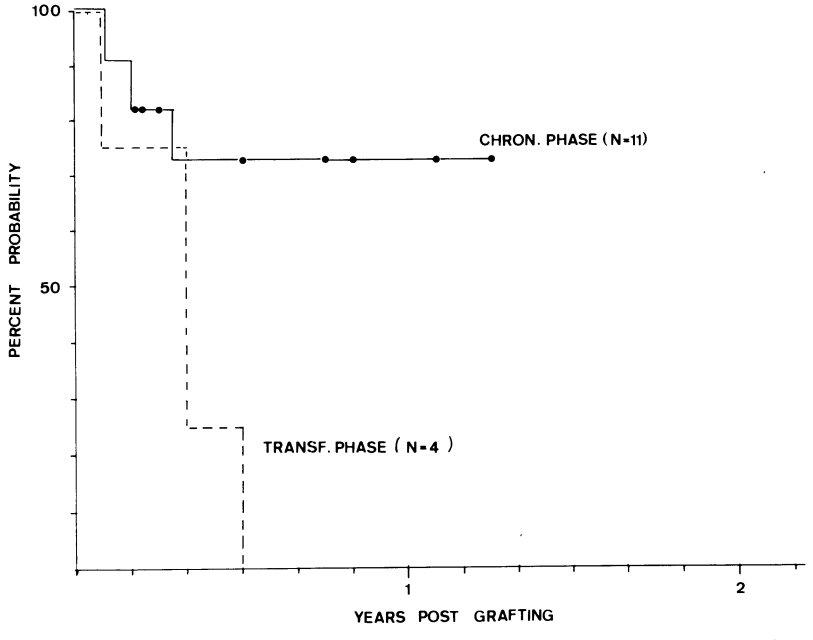

Fig. 3. Survival of patients with chronic myelogenous leukemia following HLA-identical marrow transplantation, chron., chronic phase; transf., transformed phase including one patient in second blast crisis, two patients in second chronic phase and one patient in accelerated phase

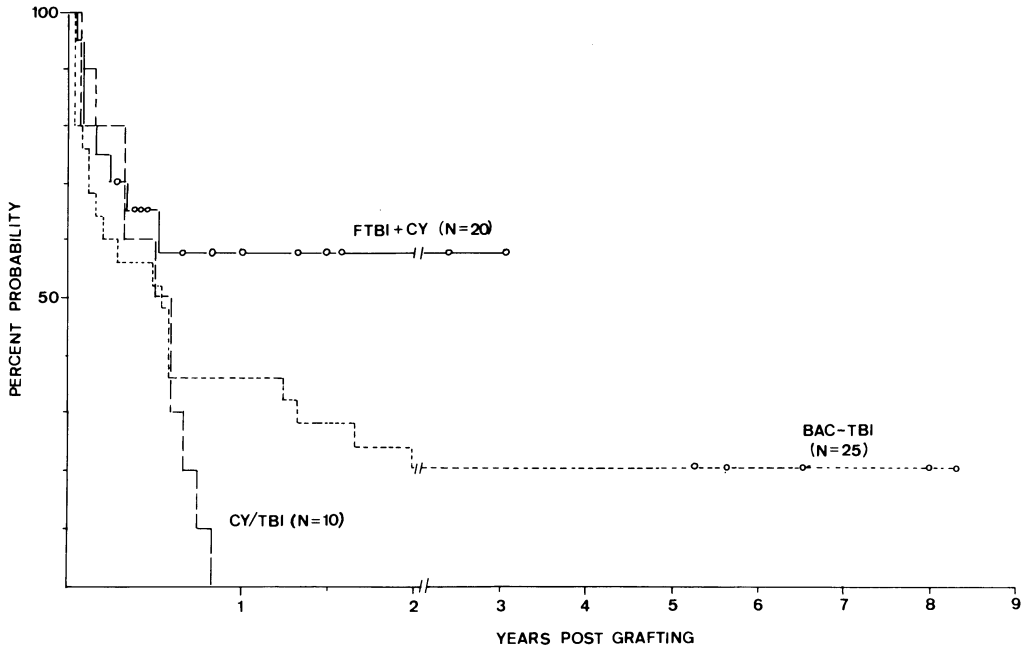

Fig. 4. Survival of patients with advanced leukemia following HLA-identical marrow transplantation. *FTBI,* fractionated total body irradiation; *CY,* cyclophosphamide; *TBI,* total body irradiation; *BAC,* BCNU, cytosine arabinoside, cyclophosphamide; *open circles* indicate living patients

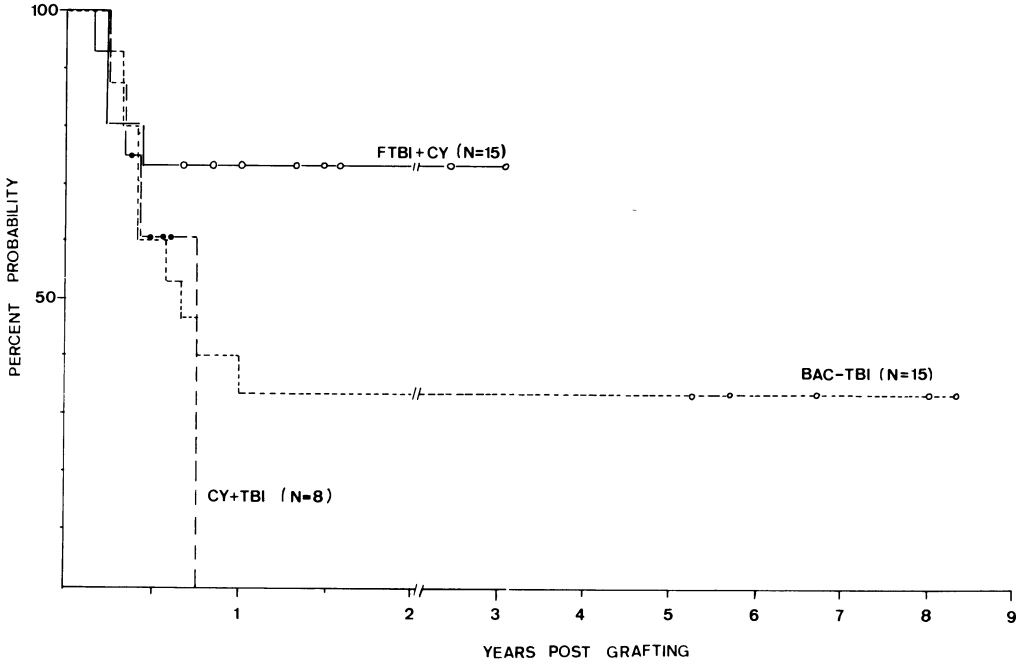

Fig. 5. Probability of remission in patients with advanced leukemia following HLA-identical marrow transplantation. See legend to Fig. 4. *Solid circles* indicate censored patients who died of other causes than leukemia

Ten patients were treated according to the Seattle protocol with only cyclophosphamide and TBI [1]. Unfortunately, all the patients died. One heavily pretreated patient died of toxicity, three patients of interstitial pneumonia, two of GvHD with subsequent infection, and four of recurrent leukemia.

The presently used conditioning regimen consists of fractionated TBI with 4 Gy on each of three days (day 8, 7, and 6 before grafting) followed by 50 mg/kg cyclophosphamide on each of four days (day 5, 4, 3 and 2 before grafting) in order to improve immunosuppression. At the time of transplantation, 10 of 20 patients were in third or later remission or in refractory relapse. Twelve patients are alive, 11 in continuous remission. One year after grafting, the probability of survival is 57%, the probability of remission 73% (Fig. 4 and 5). However, the median observation time is only 5 months.

Graft-versus-Host Disease and Methods for Prophylaxis

Prevention of GvHD was attempted by prophylactic treatment with either methotrexate (MTX) according to the Seattle protocol or cyclosporin A (CSA) as continuous infusion with 10 mg/kg day −1, 5 mg/kg day 0 through 4, 3 mg/kg 5 through 28, thereafter orally between 3 and 6 mg/kg twice a day providing serum levels of between 50 and 200 ng/ml or the combination of CSA and only three doses of MTX (Table 1). Similar to earlier experiments in animals, we have tried to prevent GVHD by in vitro treatment of the marrow graft with absorbed rabbit-anti-thymocyte globulin (ATCG) which was added to the marrow in a concentration of 1:200 for 30 min at 4 °C before infusion [4–6]. Another group of patients received marrow that was treated with a monoclonal rat-anti-human lymphocyte antibody (Campath 1) produced by Hale and Waldman [7]. This antibody activates human complement in vitro [7]. It was added in a concentration of 1 mg per 100 ml marrow for 10 min at room temperature and than mixed with 20 vol% donor serum as a source of complement for 40 min at 37 °C.

Although patients were not randomly assigned to the various methods of prophylaxis of GVHD, patients given untreated marrow were comparable to those given T cell depleted marrow with regard to disease, stage of the disease, and age. Only patients with engraftment as indicated by a rise in white blood count and reticulocyte count were evaluable for GVHD. The incidence of GVHD of clinical grade II–IV was lower in the groups of patients given antibody-treated grafts (Table 1). In a Kaplan-Meier plot, the probability of remaining in remission appeared higher in patients with GVHD (Fig. 6). Moreover, relapses of leukemia occurred later than in patients without GVHD. However, survival was not better in patients with GVHD (Fig. 7). Antibody treatment of the graft for depletion of T cells did not improve the survival (Fig. 8) despite its effect on GVHD. This may be due to the higher probability of relapse seen in

Table 1. Graft-versus-host disease, recurrence of leukemia, and survival of patients with advanced leukemia following HLA-identical marrow transplantation (AG-KMT Munich, Jan. 1986)

GVHD prophylaxis	No. of patients			
	Transplanted	With GVHD[a] per evaluable	With rec. leukemia per surviving 3 mo.	Surviving per transplanted
MTX	14	6/10	3/7	4/14
MTX+ATCG	18	3/16	7/12	4/18
CSA	6	3/6	1/6	4/6
CSA+Camp. 1	5	1/4	4/4	0/5
MTX+CSA	9	4/7	2/6	5/9

[a] GVHD of grade II or more; incidence of GVHD lower in groups with t cell depletion ($p < 0.05$). GVHD, graft-versus-host disease; MTX, methotrexate; ATCG, absorbed rabbit-anti-human-thymocyte globulin; CSA, cyclosporin A; CAMP 1, Campath 1, monoclonal rat-anti-human-lymphocyte antibody.

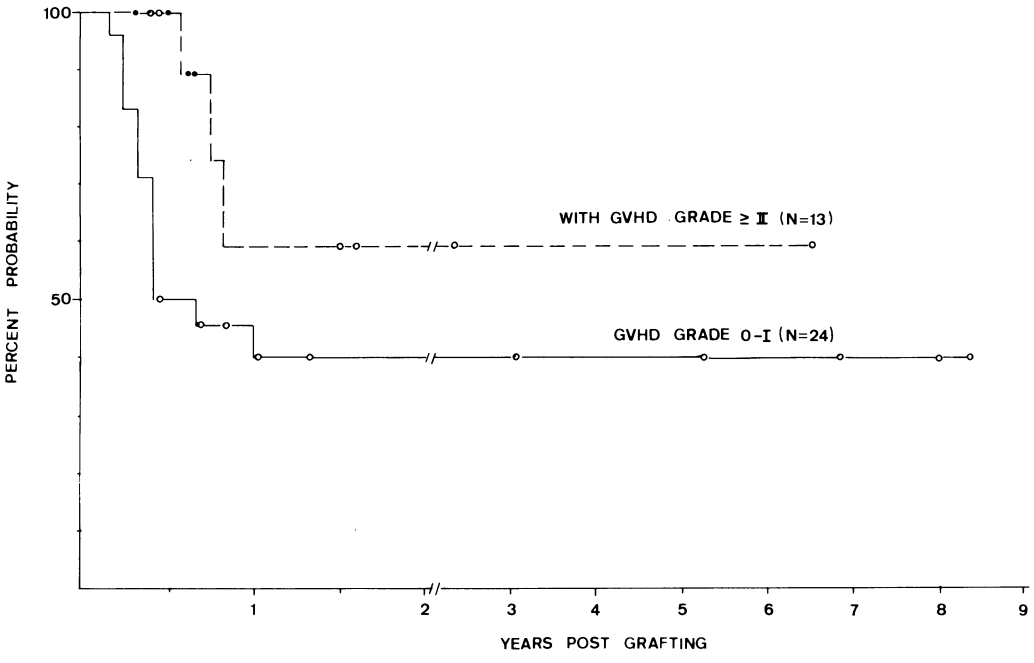

Fig. 6. Probability of remission following HLA-identical bone marrow transplantation for advanced leukemia. See legend to Fig. 4. GVHD, graft-versus host disease

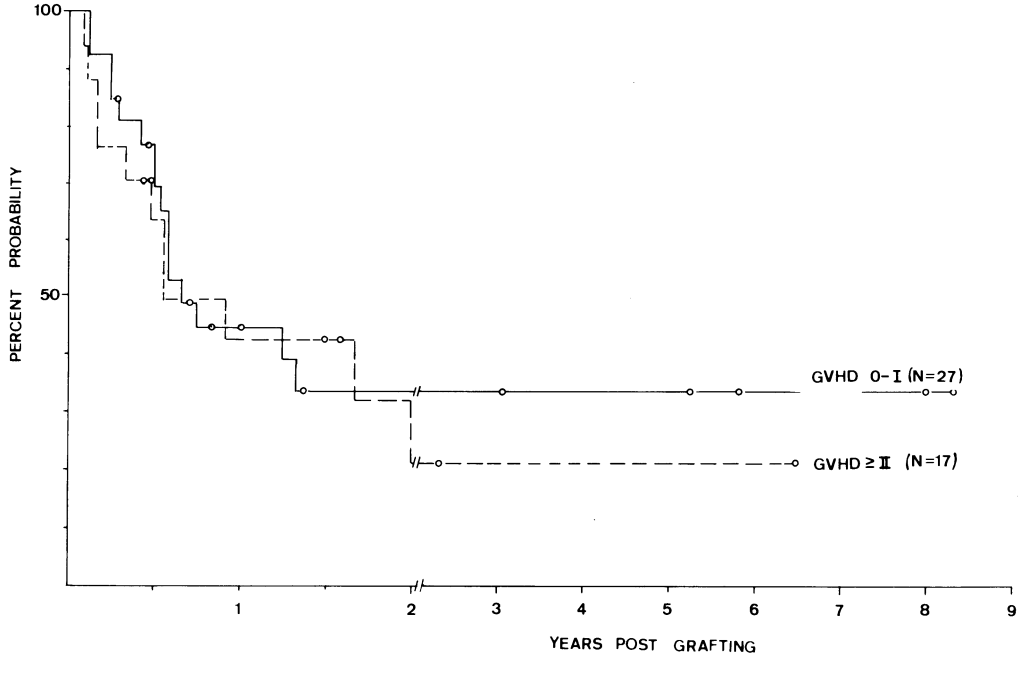

Fig. 7. Survival of patients with advanced leukemia following HLA-identical marrow transplantation

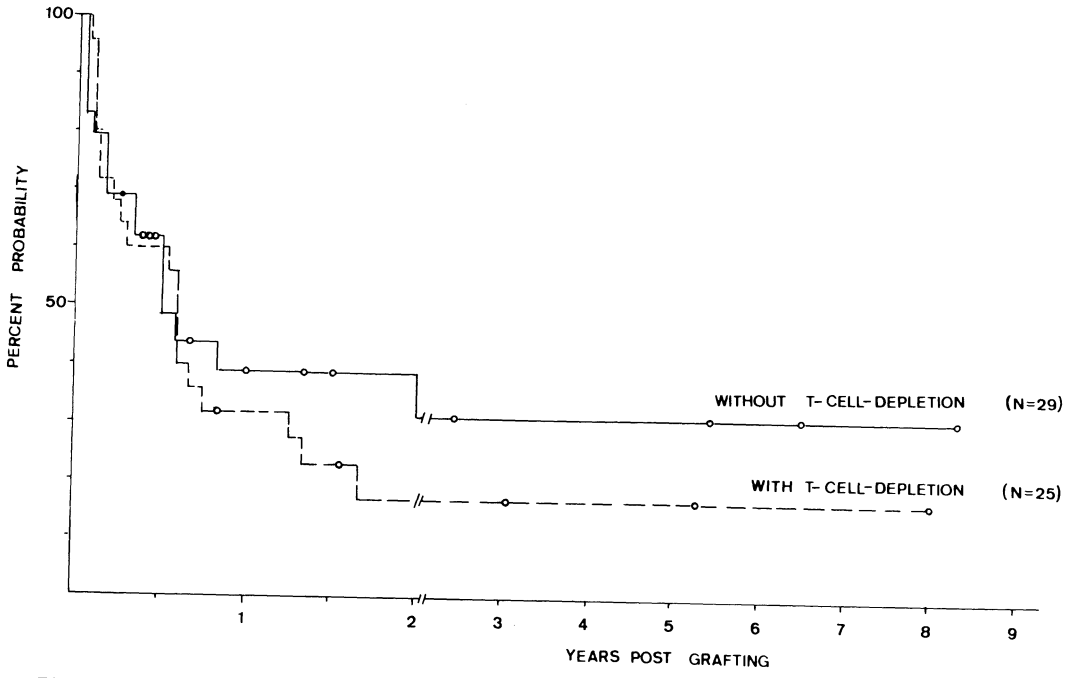

Fig. 8. Survival of patients with advanced leukemia following HLA-identical marrow transplantation. *T cell depletion,* in vitro treatment of marrow with ATCG or Camp. 1 prior to infusion

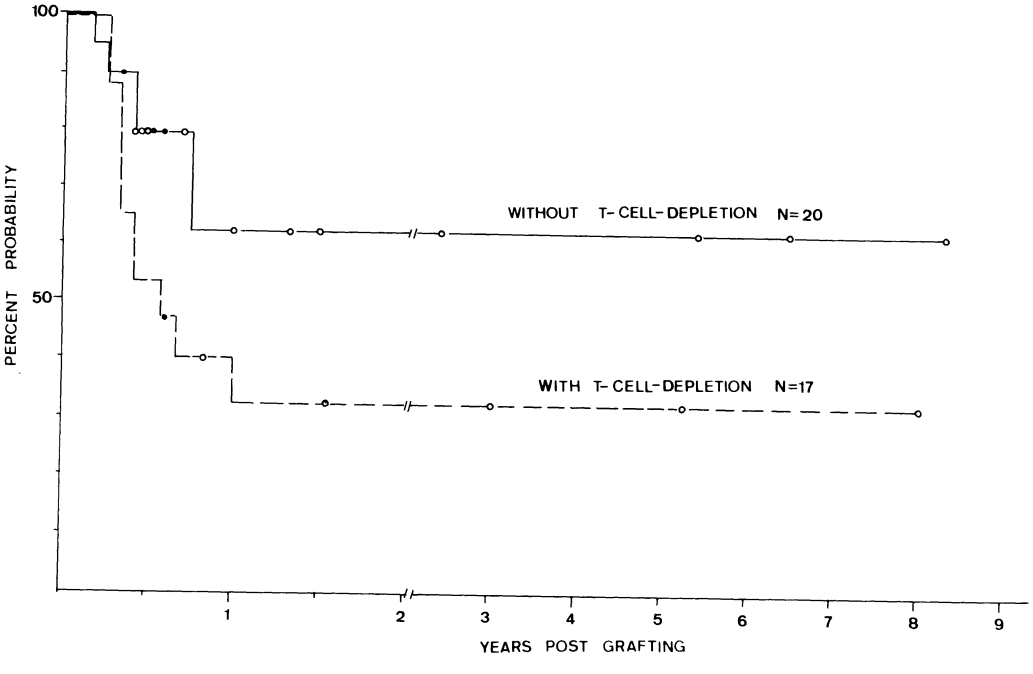

Fig. 9. Probability of remission following HLA-identical marrow transplantation for advanced leukemia. See legend to Fig. 8

the group given antibody treated marrow (Fig. 9). Two patients rejected the antibody-treated marrow and died of aplasia. Nevertheless, the proportions of early deaths were comparable in both groups.

Discussion

During the past 11 years we have been engaged in experimental and clinical bone marrow transplantation. The clinical facilities have been modest until recently, when the number of transplant beds was increased to four in the Department of Internal Medicine and two each in two Pediatric Departments. Therefore, the number of patients transplanted per year has been small and only increased recently. Time for collecting data on treatment regimens has been long, but the Munich cooperative group has adhered to common protocols. Nonetheless, we were able to confirm the results of large centers [8–11] in the treatment of advanced leukemia as well as of earlier stages. Five of our patients with relapsed leukemia have been in continuous remission for more than 5 years and may be cured. More than one-half of our patients grafted in an earlier stage are alive and free of leukemia. We have tried to compare the conditioning regimens and the methods of GvHD prophylaxis retrospectively with regard to survival and recurrence of leukemia. Twenty percent of our patients with advanced leukemia, most of them refractory to conventional chemotherapy, have become long-term survivors following treatment with BAC-TBI for conditioning. Other centers had abandonned intensive chemotherapy in addition to cyclophosphamide and TBI because the results were not improved [12, 13]. However, none of the intensive regimens had been compared in a larger number of patients with the standard cyclophosphamide-TBI regimen. Only recently have some groups started to investigate intensive chemotherapy in addition to TBI [14, 15]. We have left the BAC-TBI regimen because it was too toxic in full dosage for adult patients. Instead we use fractionated TBI and full doses of cyclophosphamide in order to improve immunosupression of the host. The rationale of this regimen is that improved immmunosuppression of the host would allow a better function of the graft in any possible way against residual leukemic cells. It is too early for any firm conclusion. So far the results of survival and remission duration have been encouraging.

Based on our experiments in the mouse and the dog, we started in 1978 to treat human donor marrow with absorbed antithymocyte globulin for prevention of GvHD [4–6]. Although we saw less GVHD in patients given ATCG-treated marrow, we could not improve the overall results in patients with advanced leukemia. A similar impression was gained after the use of Campath 1. The only possible explanation for this discrepancy, which is supported by this retrospective comparison, is a higher incidence of recurrent leukemia in patients given antibody treated marrow. Similar observations have been reported by Prentice et al. [16]. The antileukemic effect of GvHD is well known from animal experiments [17] and has been described in human patients by Weiden et al. [18]. Even in our small number of patients, we have seen a small difference in probability of remission. However, the difference between patients given untreated marrow and those given treated marrow was larger than that between patients with and without GVHD. These variant probabilities of recurrence of leukemia ar compatible with the assumption that T cells of the allogeneic graft have an influence on recurrence of leukemia without requiring clinically severe GVHD.

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Haematology and Blood Transfusion Vol. 30
Acute Leukemias
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Bone Marrow Transplantation in Acute Leukemia*

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Introduction

Aggressive chemotherapy of acute lymphoblastic leukemia (ALL) in childhood has led to long-term survival and potential cure in more than 50% of the patients. Despite recent advances in the treatment of adult acute leukemia, the majority of these patients die due to recurrence of the disease within 2 years of diagnosis.

In the last decade, bone marrow transplantation (BMT) has become an established therapeutic modality in acute and chronic myeloid leukemia. The concept underlying BMT is the replacement of the malignant hemopoietic system by normal donor hemopoietic stem cells. This is preceded by conditioning the recipient with a high dose of cytoreductive therapy.

In the early 1970s, following the establishment of HLA typing to identify histocompatible sibling donors, increasing numbers of successful allogeneic transplants were carried out [1–3]. At our center, the first clinical BMT was performed in December 1975. By February 1986, the total number of transplantations had reached 140. The following report deals with our experience in BMT for acute leukemia.

Patients, Material, and Methods

In the period between December 1975 and February 1986, 74 patients suffering from acute leukemia received a marrow graft. Seventy transplantations were allogeneic, two isologous, and three autologous. The bone marrow donors were HLA compatible and MLC-test mutually unresponsive family members. In one case of acute myeloid leukemia (AML), an unrelated compatible donor was chosen. In two cases of AML, cryopreserved autologous marrow harvested during remission was grafted in relapse. One patient with ALL received preserved autologous marrow during second remission.

For the AML patients the remission induction therapy consisted of daunomycin, cytosine arbinoside, ± thioguanin. This was followed by either a cyclic two-drug maintenance therapy or two courses of the COAP regimen (cyclophosphamide, oncovin, Ara-C, prednisone) for consolidation. In ALL, most of the patients were treated according the German BMFT protocol [4].

The age of the patients was between 9 and 46 years. In patients grafted in remission, the median duration between the entrance of remission and BMT was 4 months (1–18).

Conditioning Regimen

The conditioning regimen consisted of total body irradiation (TBI) and cyclophosphamide (60 mg/kg/day), on two consecutive days. A few patients received an alternative conditioning program with high dose

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busulfan (4 mg/kg/day × 4 days) and cyclophosphamide (60 mg/kg/day × 2 days or 50 mg/kg/day × 4 days). The details of the irradiation procedure are given in Table 1.

Either a linear accelerator or a cobalt 60 source were used. In 48 patients, the irradiation dose (8.60 Gy) was applied in one session, while 22 patients received a fractionated TBI (4×2.50 Gy) with pulmonary shielding (total lung dose 8.00 Gy).

The marrow was collected from the donor by multiple punctures of the iliac bones in general or spinal anesthesia. It was infused through a central venous catheter, and contained $2–4 \times 10^8$ nucleated marrow cells per kg body weight of the recipient.

Supportive Care

In the aplastic phase prior to engraftment, the patients received platelet concentrates every other day, and red cell concentrates if indicated. In exceptional cases, granulocytes have been transfused. All blood products were irradiated before being transfused (15 Gy). The occurrence of stomatitis in such patients makes a 30-day parenteral feeding program necessary. Intravenous methotrexate was given for prevention of graft-versus-host disease (GvHD) in the first 100 days.

Gnotobiotic Care

The first 26 patients were admitted to laminar flow isolators (LAF), while the following patients were treated in single rooms under strict barrier nursing conditions (BN). All material introduced into the LAF isolators or the BN rooms, including food, was sterilised. Oral washings, fecal and midstream urine samples from all patients were cultured twice weekly for anaerobes, aerobes, and fungi. In cases of fever above 39 °C, blood cultures were done. Total decontamination was attempted using nonabsorbable antimycotics and antibiotics.

All patients received trimethoprim-sulfamethoxazole tablets prophylactically against infections with *Pneumocystis carinii*. The last 60 patients were treated prophylactically with intravenous hyperimmune globulin against cytomegalovirus. In addition, only CMV negative blood transfusions were administered.

Table 1. Total body irradiation techniques

I	Linear accelerator	
	Energy	5.7 MeV
	Beam direction	fixed, horizontal, bilateral
	Patient position	supine, extended
	Dose	1×8.6 Gy or 4×2.50 Gy
	Dose rate	0.11 Gy/min
	Lung dose	without shielding 10 Gy ± 20%
		with shielding 8 Gy ± 15%
II	Cobalt-60	
	Beam direction	vertical, pa/ap patient translation
	Patient position	prone/supine, extended
	Dose	1×8.6 Gy or 4×2.50 Gy
	Dose rate	(overall) 0.025 Gy/min
	Dose rate	(instantaneous) 0.16 Gy/min
	Lung dose	without shielding 9.2 Gy ± 10%
		with shielding 8.0 Gy ± 10%

Results

Take. In all patients who survived for more than 4 weeks, engraftment could be proven. Apart from rising peripheral white blood cell and reticulocyte counts, other markers were sometimes available to prove chimerism, e.g., ABO blood groups and sex chromosome difference. The earliest signs of engraftment could be observed after 12–14 days.

Table 2. Disease-free survival

	n	Survivors (%)	Disease-free survival of survivors (median/range)
ALL-AML-relapse	23	0	(up to 6 years)
AML 1 CR	29	16 (55)	41 (2–70) months
AML 2 CR	6	5 (83)	7 (4–12) months
AML 3 CR	1	0	
AML 4 CR	1	0	
ALL 1 CR	6	2 (33)	5 (1–9) months
ALL 2 CR	7	4 (57)	10 (3–34) months
ALL 4 CR	1	1	17 months

Table 3. Incidence of graft-versus-host disease in 120 patients

GVH grade	Patients at risk (survival >14 days, allogeneic BMT)			
	SAA (%)	AML (%)	ALL (%)	CML (%)
Acute I–II	0	6	9	20
Acute III–IV	14	8	0	23!
De novo-chronic	14	12	0	20
Total incidence	28	26	9	63!

Table 4. Incidence of lethal interstitial pneumonia

	Radiation technique			
	1 × 8.60 Gy 5.7 MeV	4 × 2.50 Gy 5.7 MeV lung shielding	1 × 8.60 Gy 60 Co	4 × 2.50 Gy 60 Co lung shielding
Acute leukemia	10/41 (24%)	3/14 (21%)	6/7 (86%)	1/8 (12%)
CML	5/19 (26%)	4/7 (57%)	6/8 (75%)	0/6

Survival. The survival dates are shown in Table 2.

GvHD. The incidence of graft-versus-host disease (GvHD) is shown in Table 3.

Leukemic Relapse. Relapses of acute leukemia have been observed in six allogeneic, two isologous, and one autologous cases. All but three relapses occurred in patients grafted during relapse. With exception of a patient who relapsed after 6 years, all other relapses were in the period between 3 and 18 months after BMT. All relapses were of recipient type.

Interstitial Pneumonia (IP). The incidence of lethal IP in relation to the applied irradiation technique is given in Table 4. Using 8.60 Gy from a cobalt 60 source as a single exposure without pulmonary shielding, the incidence of IP was very high. This was the reason for stopping further irradiation according to this technique in our center.

Discussion

In accordance with the experience of other centers our results clearly demonstrate that marrow grafting in remission of the disease will markedly improve long-term survival. In our center, the survival of AML patients who received a marrow graft in first remission is 55%. The median observation time is 41 months. Early BMT markedly decreases the incidence of leukemic relapse.

To date, only one patient out of 35 acute leukemic cases transplanted in first remission had a leukemic relapse. In the group of 23 patients grafted in relapse, six patients had recurrence of the disease following BMT. In one patient the relapse even occurred 6 years post transplantation [5].

The incidence of acute GvHD in our patients is relatively low as compared with patients in other centers. This is presumed to be related to strict gnotobiotic care [2, 6]. Animal experiments and experience in some other centers support this assumption [7–9].

In SAA patients, the incidence of GvHD is comparable with its incidence in acute leukemia patients. However, the same does not apply to patients with CML: the incidence of GvHD is significantly higher. The reason for this difference is still unsettled.

The main cause of death in our patients is interstitial pneumonia (IP). In most of the cases pneumonia was idiopathic, since no causative agent could be demonstrated. A major factor in the pathogenesis of IP was

single exposure TBI, with a high instantaneous dose rate and without pulmonary shielding. The incidence of IP markedly decreased following fractionation of TBI and pulmonary shielding (total lung dose 8 Gy). In 10% of IP cases, CMV was demonstrated to be the causative agent. It is not yet settled whether this low incidence of CMV-associated IP is related to routine prophylaxis with CMV hyperimmune globulin.

Summary

In Essen 142 bone marrow transplantations were carried out between December 1975 and February 1985. In 74 cases the indication was acute leukemia in relapse ($n = 23$) or in first or consecutive remission ($n = 51$). The conditioning regimen consisted of cyclophosphamide or the combination of cyclophosphamide and total body irradiation. All patients were treated under strict gnotobiotic care. To mitigate the risk of CMV infections, intravenous CMV-hyperimmune globulin and CMV-negative blood products have been applied routinely for 2 years. MTX was used as prophylaxis against GvHD.

In the prognostically unfavorable group of acute leukemia in relapse, only one patient showed long-term survival. In this patient, leukemic relapse occurred 6 years after transplantation. The survival rate of AML patients grafted during the first remission is 55% (16/29) with a median observation time of 41 months. For patients grafted in first or consecutive remission of acute lymphoblastic leukemia, the survival rate is 50% (7/14) with a maximal observation time of 34 months.

The overall incidence of GvHD in patients at risk was 28% in aplastic anemia, 26% in AML, 9% in ALL, and 63% in CML. In aplastic anemia, no patient developed an interstitial pneumonia. In leukemia, the risk of fatal interstitial pneumonia was 34%.

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Bone Marrow Transplantation in Childhood Leukemia in West Germany *

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Introduction

About 10 years ago allogeneic bone marrow transplantation (BMT) became established in West Germany as a therapeutic method for hematological malignancies. Between 1975 and April 1984, more than 300 patients were transplanted for various forms of leukemia [1]. Between 1975 and the end of 1985, 108 children up to the age of 16 years with leukemia were transplanted in five different centers. They now present a combined report for the first time. Sixty percent of the children were transplanted in the last 3 years whereas the remaining 40% were treated in the last 7 years. The total number of patients is rather small compared with other countries, and it is only recently that transplantations have been performed frequently in children in this country. The main reason for this slow development are the good results of conventional chemotherapy of acute lymphocytic leukemia (ALL) [2] and acute nonlymphocytic leukemia (ANLL) [3]. Not a single child with ALL, and only nine children with ANLL and two with acute undifferentiated leukemia (AUL) have been transplanted during first complete remission. In contrast, 39 children (36%) were not in remission at the time of transplantation. This point and the fact that the relapses of acute leukemia occurred after the very intensive chemotherapy used in this country has to be considered when the combined results are reviewed in the following.

General Considerations

In all German centers, BMT is part of the general therapeutic program of the departments. In two of the hospitals, transplants in children are performed by a special team, whereas in the other centers children and adults are treated by the same team. In the first years some patients were isolated only in single rooms, whereas now all centers isolate the patients in reversed isolation, such as laminar flow rooms or plastic isolators. Selective oral decontamination with nonabsorbable antibiotics has been used, and cotrimoxazole has been applied prophylactically. Intravenous gammaglobulin preparations were given to most patients following BMT. During the last 3 years, a special immunoglobulin preparation with a high titer against cytomegalovirus (CMV) has been used successfully in the majority of centers with almost complete prevention of CMV-induced interstitial pneumonias [4].

In the first years, antileukemic therapy generally comprised single dose total body irradiation (10 Gy) and cyclophosphamide (2×60 mg/kg body weight). In some cases, BCNU and cytosine arabinoside has been added. Since 1982 fractionated irradiation with a total dose of 12 or 12.5 Gy has been used in most centers (lung shielding after 8 or 10 Gy, and in some centers a boost to the ribs and underlying soft tissue to a total dose of 10 or 12 Gy). Methotrexate has been used

* With the Support of the Deutsche Forschungsgemeinschaft and Deutsche Krebshilfe.
Bone Marrow Transplantation Teams at the Universities of Tübingen[1], München[2], Kiel[3], Ulm[4] and Essen[5], Federal Republic of Germany.

Table 1. Diagnosis and number of patients transplanted in the different centres and the year of the first transplant of a child with leukemia

Centre	Year of first transplant	*n*	ALL/AUL						ANNL				CML		
			First CR	Second CR	Third CR	Fourth CR	Rel./ Nonresponse	Total	First CR	Second CR	Rel./ Nonresponse	Total	First CP	Second CP	Total
Munich	1975	41	2	6	2	–	18	28	3	2	7	12	1	–	1
Tübingen	1979	35	–	12	3	2	4	21	2	3	1	6	7	1	8
Ulm	1982	12	–	5	2	–	–	7	2	1	–	3	1	1	2
Essen	1982	7	–	1	–	–	1	2	2	–	–	2	3	–	3
Kiel	1983	13	–	2	2	–	1	5	–	1	7	8	–	–	–
Total	1975–1985	108	2	26	9	2	24	63	9	7	15	31	12	2	14

CR, complete remission; Rel./Nonresponse, relapses or nonresponding to chemotherapy; CP, chronic phase.

for the prophylaxis of graft-versus-host disease according to the Seattle protocol [5]. This has been replaced by cylosporin A in most of the centers in the last three years – at least in children above the age of 10 years. In some centers, the transplanted marrow was incubated with an anti-human T-cell globulin if more than 2×10^8 cells/kg body weight of the recipient were available [6, 7]. In the majority of cases, the donor was an HLA-identical sibling. Ten children with acute leukemia received the marrow from an identical twin, and in nine cases the donor was either a phenotypically identical parent or a nonidentical family member.

A monoclonal anti-T-cell antibody or other means of removal of T cells were used in a few cases. In summary, there are some variations in the prophylactic and therapeutic procedures used in the patients reported in this combined report. The results will be demonstrated according to the type of leukemia leading to transplantation. The data reflect the situation of bone marrow transplantation in childhood leukemia during the last 10 years, in which the vast majority of the grafted cases of acute leukemia (either ALL or ANLL) were bad risk cases. Survival curves were calculated by nonparametric estimation for incomplete observations [8]. Survival was assessed as of January 1, 1986. Table 1 summarizes the patients with regard to diagnosis, stage of disease, and center where they have been treated.

Results

Acute Lymphocytic Leukemia

A total of 63 children with ALL and AUL have been transplanted. The probability of survial for the 60 children with ALL is 39% (Fig. 1). None of the children with ALL was transplantated in first complete remission (CR). For the 26 children transplanted in second CR, the probability of survival is 50% (Fig. 2). For the 11 patients transplanted in third or fourth CR, this values is 34% and 28% for those being treated during relapse or while nonresponding to chemotherapy ($n = 23$). In the latter group, most children died in the 1st year following transplantation either of complications or relapse

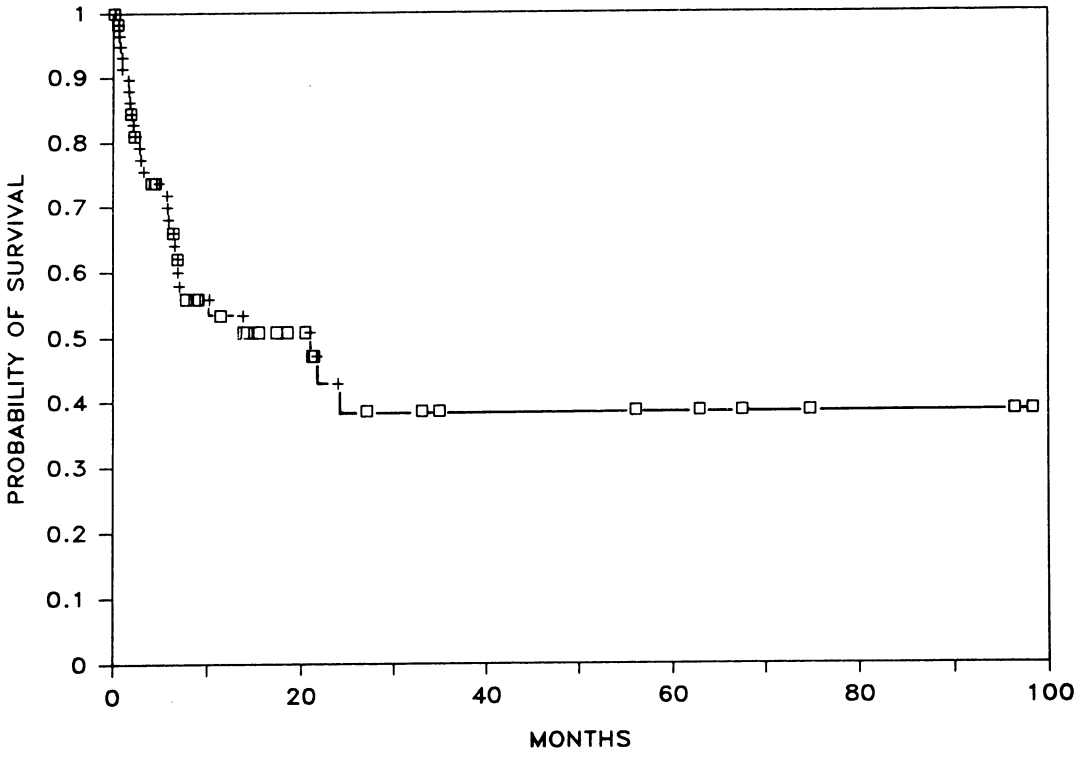

Fig. 1. Probability of survival of all transplanted 60 children with ALL (three children with AUL excluded). □, alive; +, dead

Table 2. Relapses of leukemia following bone marrow transplantation. $n=29/108$

Diagnosis Disease at time of transplant	*n*/total	%	Day of death or relapse, + still alive
ALL/AUL			
First CR	1/ 2	21	120
Second CR	5/26		100, 107+, 348, 421, 662
Third CR	2/ 9	22	180, 639
Fourth CR	0/ 2		
Rel./Nonresp.	10/24	42	72, 90, 120, 176, 193, 210, 210, 215, 733+, 1241+
	18/63	29	
ANLL			
First CR	0/ 9	–	
Second CR	4/ 7	57	141, 150, 441, 934
Rel./Nonresp.	4/15	27	21, 28, 122, 467
	8/31	26	
CML			
First chronic phase	1/12		485+
Second chronic phase	1/ 2		190
	2/14	14	

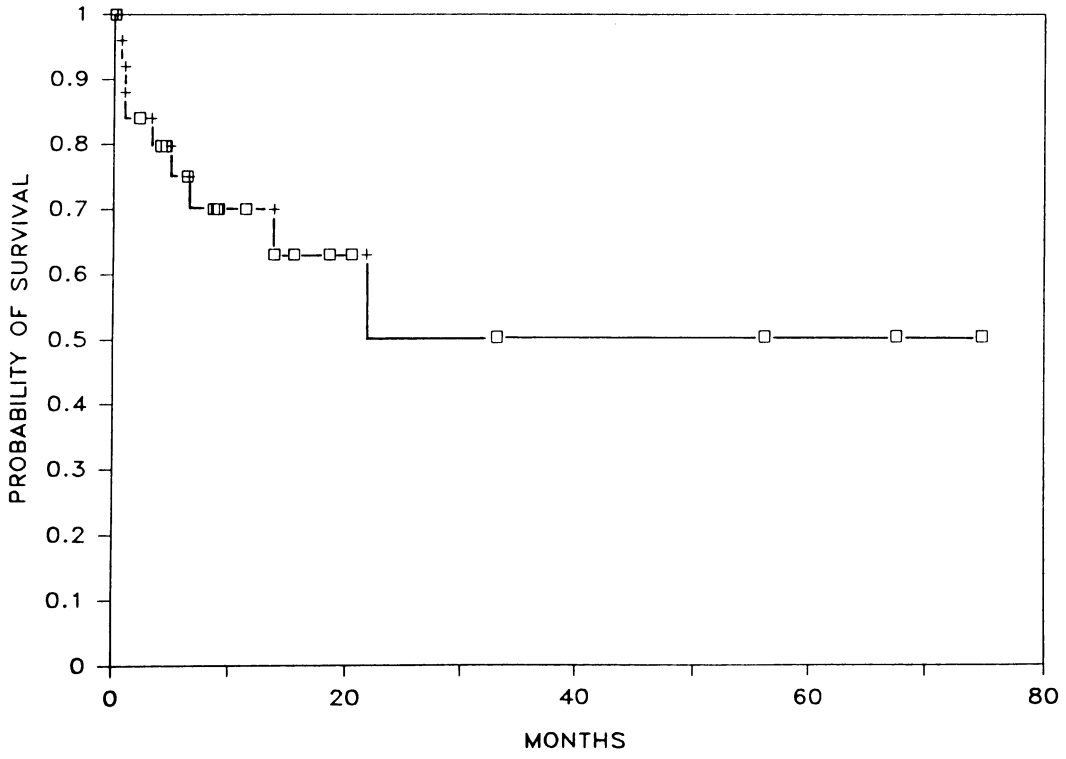

Fig. 2. Probability of survival of 26 children with ALL transplanted in second CR (AUL excluded). □, alive; +, dead

($n=10$) (Fig. 3 and Table 2). Four of the seven patients with an identical twin as donor survived between 75 and 6 months, including one patient transplanted during second relapse (22 months).

One child died on day 151 post transplant from typical hemolytic uremic syndrome at the age of 4 years, being just at the end of the typical age range for this disease.

Acute Nonlymphocytic Leukemia

Only 31 children with acute nonlymphocytic leukemia have been transplanted at various stages of their disease. The overall probability of survivl is only 29% (Fig. 4). These rather disappointing results, however, can be easily explained after closer scrutiny. Only nine children were transplanted during first CR with a probability of survival of 55% (Fig. 5). An additional seven children were treated during second CR (probability of survival 20% [Fig. 5]). The overall figure for children transplanted in first and second CR was only 34%, whereas that for the 13 children transplanted during relapse or while not responding to chemotherapy was 22%. The data include five patients with a non-HLA-identical sibling. Only one of these children has survived – now more than 200 days after transplantation in first relapse using the mother as donor. Three antigens were mismatched in this situation and the T cells were removed by E-rosetting. The other five patients died early from complications. None of the children transplanted during the first CR had a relapse, while this was a major cause of death in those transplanted during second CR (Table 2).

Chronic Myeloid Leukemia

BMT in CML was first performed in this country at the beginning of 1982 after the first positive results were reported in identical twins transplanted in chronic phase [9,

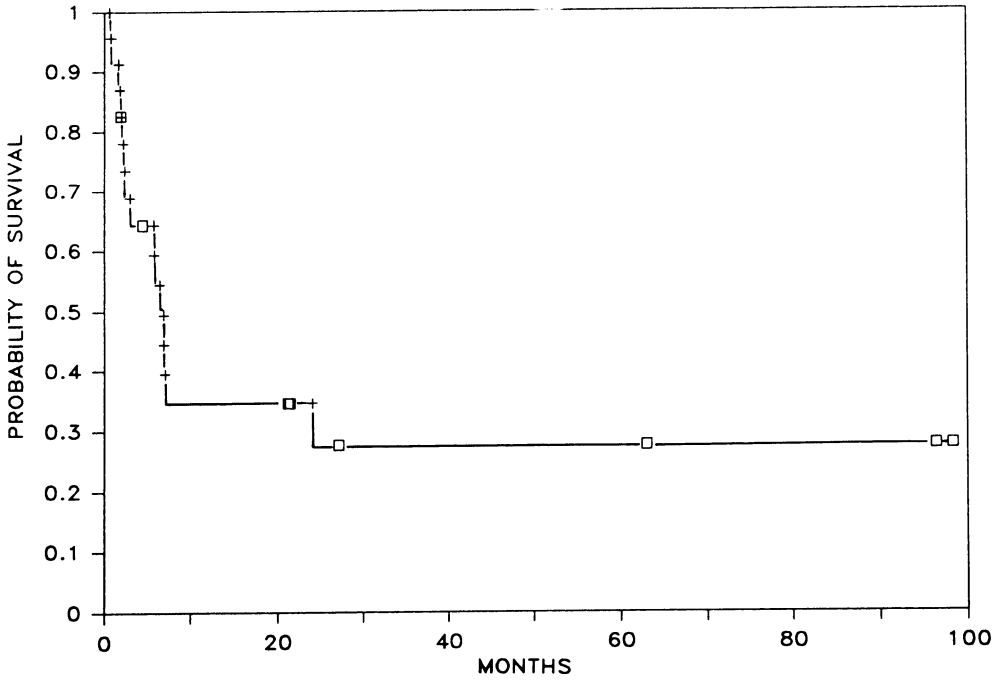

Fig. 3. Probability of survial of 23 children with ALL transplanted during relapse or after nonresponding to chemotherapy (AUL excluded). □, alive; +, dead

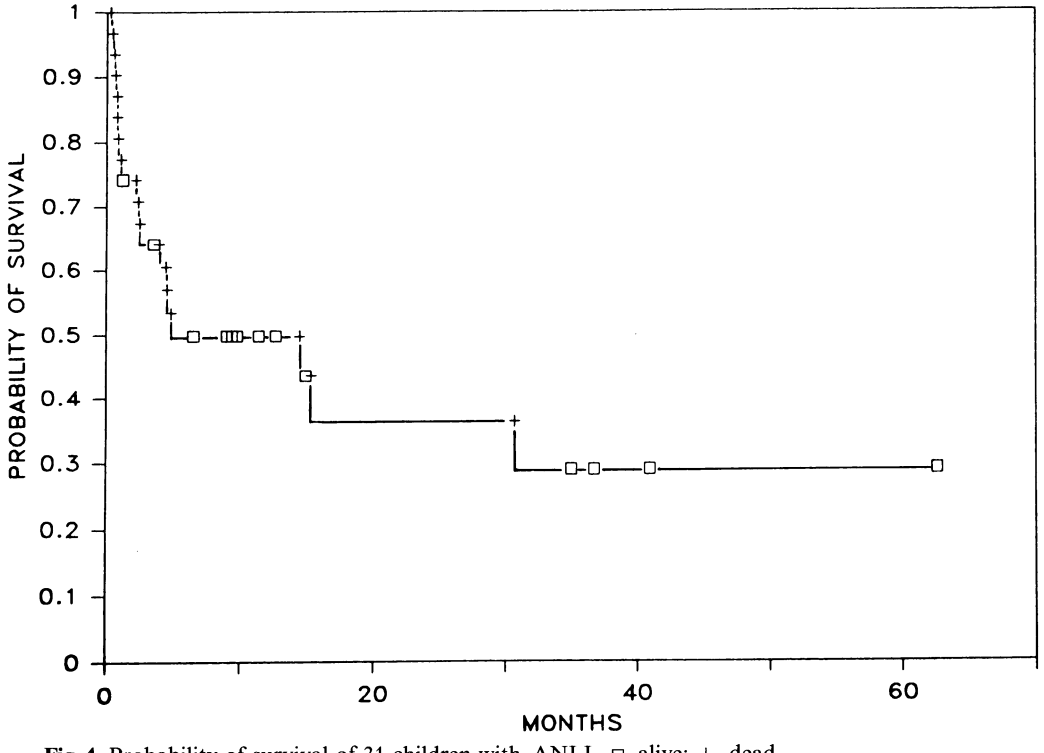

Fig. 4. Probability of survival of 31 children with ANLL. □, alive; +, dead

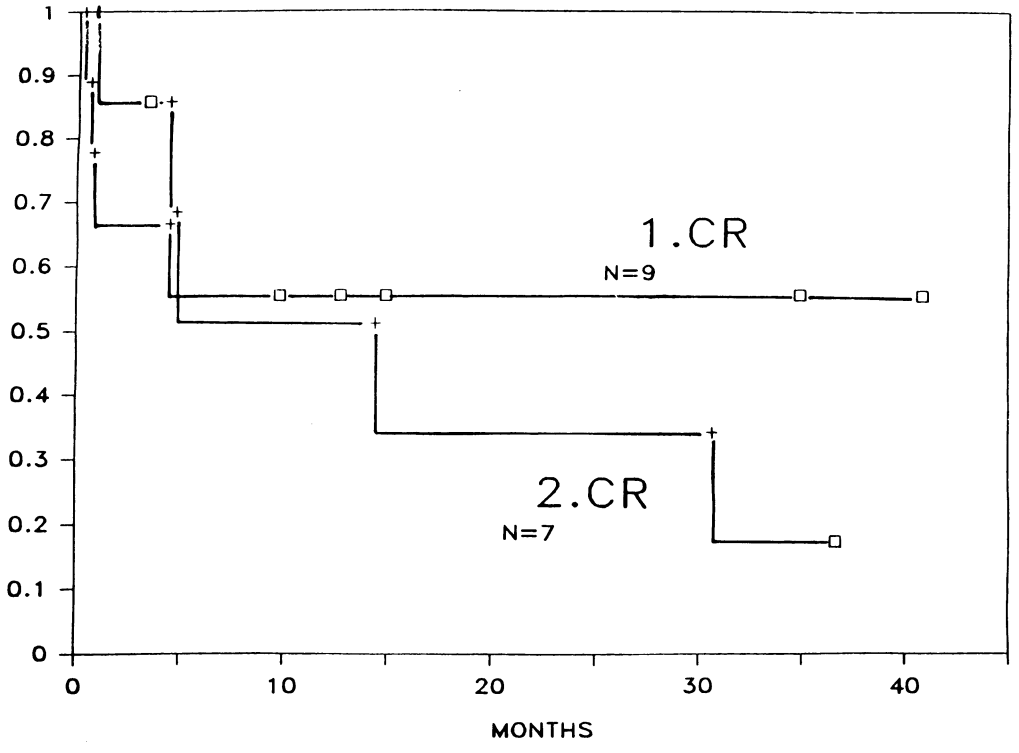

Fig. 5. Probability of survival of nine children with ANNL transplanted in first CR and seven children transplanted in second CR. □, alive; +, dead

10]. Fourteen children have been transplanted during chronic phase. Two of them were in second or third chronic phase of the disease. In one child, the CML was diagnosed following induction therapy of ALL. A second blast crisis (again C-ALL-antigen positive ALL) occurred a few days before BMT was scheduled. It was finally transplanted in third chronic phase. Thirteen of the children were Ph chromosome positive. One child got the graft from a nonidentical sibling. The results are shown in Fig. 6. One child died from severe chronic graft-versus-host disease and three from infectious complications. One patient died 103 days after BMT from secondary aplasia probably due to the incubation of the marrow with a monoclonal antibody (CAMPATH). The child with the mismatched graft died from the consequences of graft rejection at day 130. Two children relapsed at day 190 and 2 years post grafting. The second child is still in chronic phase 6 months later. The child with the Ph^1 negative CML is still free of disease 27 months after BMT.

Causes of Death

Graft-versus-host disease has not been a major problem in the patients with an identical sibling donor. But as the data came from eight different hospitals this statement has to be treated with caution. The primary causes of death were infectious complications and recurrences of leukemia. Twenty-eight children had a relapse of their disease (Table 2). Only 3 occurred more than 2 years after BMT. One child with ALL had a relapse 3.4 years after grafting. The girl had been transplanted during the fifth relapse following various kinds of intensive chemotherapy protocols. Altogether, the numbers are too small to allow a clear evaluation, but the pattern is similar to that reported in the literature. An important point is that none of

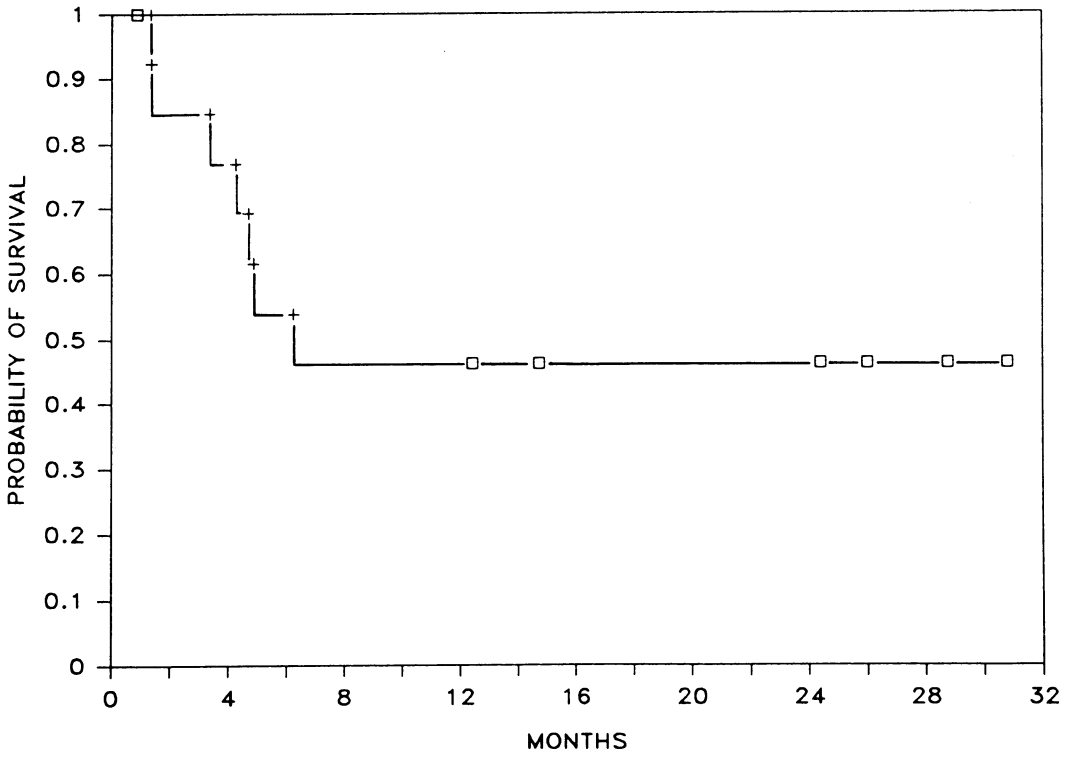

Fig. 6. Probability of survival of 14 children with CML transplanted in chronic phase

the children with ANLL transplanted in first CR have yet relapsed.

Discussion

In this report the results of BMT in 108 children who were transplanted for acute or chronic leukemia in five different German centers between 1975 and 1985 are summarized. Due to the various subgroups (types of leukemia, transplantation in relapse or remission, number of remissions prior to transplantation, donor-host combination) any statistical analysis has to be regarded with great caution. Different procedures of antileukemic treatment or prevention of graft-versus-host disease render the situation even more complicated. The report also includes nine patients with an histoincompatible donor, of whom seven died and one is too early to evaluate. Nevertheless, there are findings which have to be discussed. Up to 1981 only single cases were transplanted for acute leukemia in spite of the fact that the first children with aplastic anemia were grafted in 1975. The main reason for this were the good results of combination chemotherapy in ALL [2] and ANLL [3] in this country. Fourteen of the first 15 patients transplanted in Munich between 1975 and 1980 for acute leukemia were grafted during relapse, most of them resistant to chemotherapy. Three of the latter and the patient transplanted in remission became long-term survivors (4/15). Combining all 39 patients with acute leukemias (ALL, AUL and ANLL) transplanted during relapse or as an nonresponder to initial chemotherapy, the probability of survival was about 25% (28% for 23 patients with ALL), which is at least as good as the data reported from Seattle [11]. In recent years, the vast majority of the patients were transplanted in remisson.

The results of BMT in acute lymphocytic leukemia in remission are encouraging. The probability of survival is 50% in second CR

and 34% in third and fourth CR, comparable to the data reported from Seattle [12] in children with ALL. There cannot be much doubt that BMT is superior to conventional chemotherapy in children when a relapse has occurred. The better results in second CR are compatible with the idea that the procedure of BMT has only limited value for overcoming resistance developed during chemotherapy. Nevertheless, the observation time is still not long enough for most cases. So presently it cannot be judged whether the long-term results in ALL transplanted in second CR will be worse in Germany than in other countries due to the intensive primary chemotherapy generally administered to children with ALL in this country.

For the nine children transplanted in first CR of acute nonlymphocytic leukemia, the probability of survival is 55%. This is again comparable to the results of 70% reported for children in the literature [13]. The results in second CR are not as good, which is also in agreement with the literature [14]. Nevertheless, in spite of the intensive ANLL protocol in Germany, patients seem to have a second chance of survival by BMT. In the future, BMT in first CR should be aimed at for the results are better than those of the best chemotherapy [3].

The combined results of BMT in children with chronic myeloid leukemia (46% in chronic phase) are not as good as the 70% reported in the literature [15]. The rather bad results are most likely due to the small sample. One center transplanted more than half of the patients, and seven out of eight are alive and in good health after 1–4 years, one with a relapse of the disease. The six other patients transplanted in three other centers all died from complications. None of the patients was transplanted during blast crisis or accelerated phase. One child transplanted in the third chronic phase has been disease free for 2 years, whereas another child transplanted in second chronic phase relapsed 180 days post grafting. In a new report the chance of relapse after transplantation in chronic phase is 10% and in more advanced disease 42% [16]. Ther can be no question that BMT in CML should be performed in the first chronic phase whenever possible.

This combined report represents the activities in BMT during the last 10 years in West Germany. In future, the number of patients will increase more rapidly. At least two or three more centers will start BMT very soon. Oncologists in this country have now accepted the value of this procedure, and so BMT in ALL in second CR, in ANLL in first or second CR, and in CML in first chronic phase might become available for almost all patients with a donor. In addition, it is now easier to define risk groups in ALL and ANLL in which BMT could be of value in first CR. Since almost all children enter protocols for acute leukemias, BMT will in future be part of the primary treatment (ANLL) or the relapse protocol (ALL). This should lead to a better comparison of the results of conventional chemotherapy and BMT under defined conditions. In addition, attempts to cross the HLA barrier in children without an identical sibling will increase in the next future. During an international meeting on this subject a few months ago, it became evident that the time of this restriction due to genetic reasons might be over [17]. It is now conceivable that BMT might soon be available for all those children with leukemia who have no chance of survival with conventional treatment.

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Allogeneic, Syngeneic, and Autologous Bone Marrow Transplantation in the Acute Leukemias – Baltimore Experience *

G. W. Santos, A. M. Yeager, and R. Saral[1]

The rationale, history, and results of the therapeutic application of allogeneic and syngeneic marrow transplantation in acute leukemias have been discussed in a number of reports and reviews [1, 2]. The humble beginnings with transplantation of patients with end-stage disease in the late 1950s to the early 1970s have rapidly improved to the application of marrow transplantation in the treatment of patients earlier in the course of their disease and in a situation of minimal residual disease. Most problems of leukemia relapse, graft-versus-host disease (GvHD) and viral infections have been identified, and solutions to these problems are being actively pursued in many centers around the world.

We will report our experience with allogeneic, syngeneic, and autologous bone marrow transplantation (BMT) in the acute leukemias.

Allogeneic Transplantation for Acute Lymphoblastic Leukemia (ALL)

From January 1980 to July 1985 we performed 56 marrow transplants using genotypic HLA-identical allogeneic family member donors (1 father, 55 siblings) in patients with ALL who were in complete remission (CR) 1 (15) with median age of 24, CR2 (24) with a median age of 13.5, or CR3 (17) with a median age of 10. All patients were prepared with cyclophosphamide (CY) (50 mg/kg daily × 4 days) followed by total body irradiation (TBI) (300 rad/day × 4 days with lung shielding for the third dose). Following transplantation, prophylaxis for GvHD included CY, CY and prednisone, cyclosporine and prednisone, or cyclosporine alone depending on what our prophylaxis study was current at the time of transplant. The actuarial disease-free survival (DFS) curves are shown in Fig. 1 [3].

The actuarial DFS for patients in CR1 and CR2 was similar at 45% but different for patients in CR3 where the actuarial disease-free survival was 30%. Seven patients in CR1 survive disease-free for 15.7–38 months (median, 24.1 months), twelve patients in CR2 survive disease-free for 5.5–70 months (median, 33.0 months) and five patients in CR3 survive in continuous remission for 22–67 months (median 42.0). One patient transplanted in CR1 relapsed at 108 days and died at 214 days after a second transplant. One patient in second remission relapsed at 14.2 months and subsequently died of leukemia. Six patients transplanted in their third remission relapsed 2–15.6 months following transplant. Five of these patients subsequently died. The sixth patient who relapsed at 15.6 months achieved a fourth remisson with chemotherapy and then received a second transplant from the same donor following preparation with busulfan (BU) and CY as outlined below for patients with acute nonlymphoblastic leukemia (ANLL). This patient survives free of

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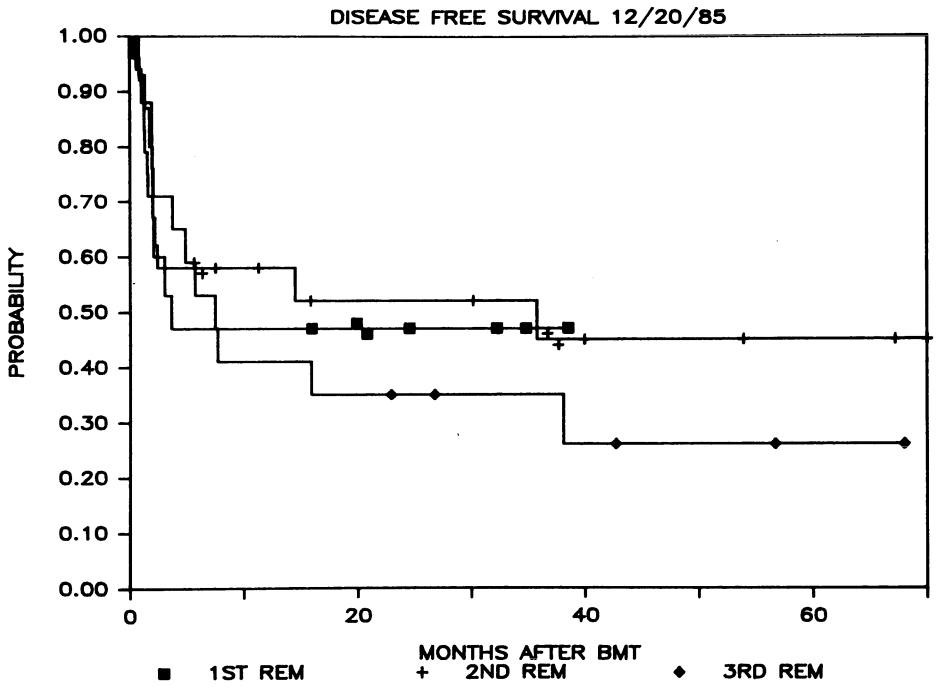

Fig. 1. Survival analysis of 56 patients undergoing allogeneic bone marrow transplantation for acute lymphoblastic leukemia in first (15 patients), second (24 patients), and third remission (17 patients). Disease-free survival is shown. Individual living patients are shown by *symbols*

disease 19.2 months after the second transplant and 3.6 years after the first transplant. The actuarial relapse rate in CR3 was 50% (data not shown).

Allogeneic Transplantation for ANLL

We have performed 68 transplants using HLA-identical sibling donors in patients with ANLL who were in CR1 (35) with a median age of 23 years (4–41 years), CR2 (19) with a median age of 23 years (6–39 years). Transplants were performed between April 1979 and July 1985. All patients were given BU (1 mg/kg orally four times daily for 4 consecutive days – total dose 16 mg/kg). This was immediately followed by Cy given i.v. at a dose of 50 mg/kg daily for four consecutive doses. Post-transplantant prophylaxis for GVHD was the same as outlined above for ALL.

The actuarial DFS for the 35 patients transplanted in first remission was 47%. The actuarial DFS for 33 patients transplanted in subsequent courses of their disease was 36%. This difference did not reach significance. Twenty-one of the patients transplanted were 20 years or younger and 47 were age 21–40. The actuarial DFS for those 20 years or younger was 67% and 30% for those older (Fig. 2). Only two relapses were seen, one in a 36-year-old man transplanted in CR3 who relapsed at 349 days and another in a 19-year-old woman transplanted in CR2 who relapsed at 1859 days (5.1 years). The median time of follow-up for the patients surviving continuously free of disease is 33.1 months (5.8–81.0 months).

Causes of Death after Allogeneic Transplantation

The causes of mortality in a given patient are often very difficult to assign since one entity may lead to another, and eventually multiorgan failure due to many secondary causes may ensue. Nevertheless, we have examined what we believe to be the major contributing

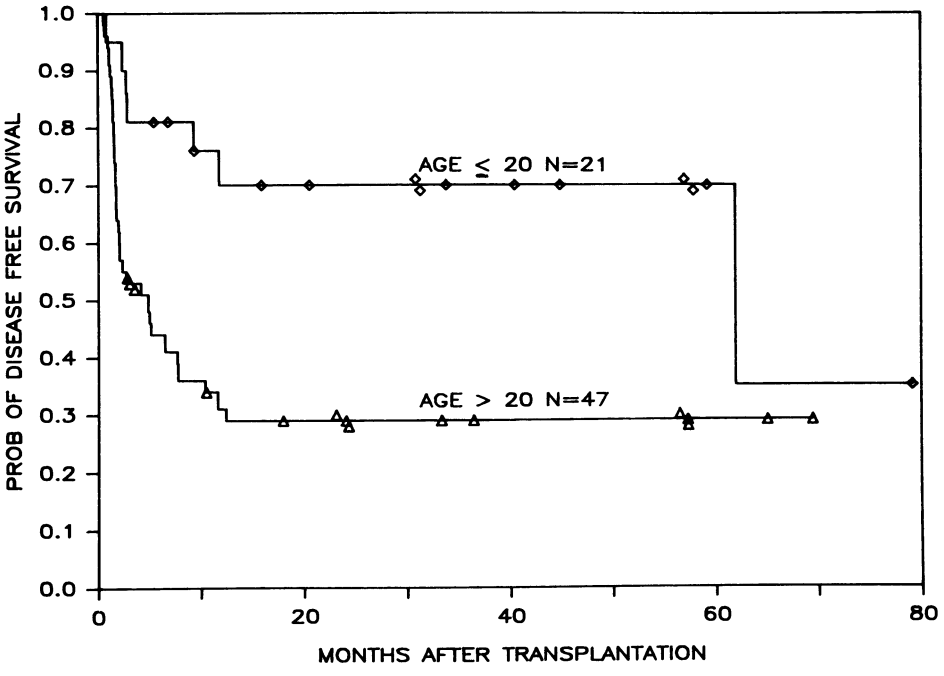

Fig. 2. Kaplan-Meier product limit estimates of disease-free survival in all patients with ANLL transplanted – the effect of age. *Symbols* represent patients living in continuous remission

causes of death in our allogeneic patients. In the present group of patients, two-thirds of deaths were related to GVHD and viral infections. There are, however, promising approaches currently being pursued in a number of centers toward solutions to these problems.

The other major contributing causes of death, each with about a 3%–5% incidence, include sepsis, veno-occlusive disease of the liver, adult respiratory distress syndrome, renal failure, chronic pulmonary failure, and other causes.

Syngeneic Marrow Transplantation in Acute Leukemia

Marrow transplantation employing monozygotic twins as donors (syngeneic) affords the rather rare opportunity to study the therapeutic as well as the toxic effects of marrow transplantation without GVHD. Elsewhere, we have suggested that it represents a standard or bench mark for autologous marrow transplantation in the acute leukemias [4].

We have performed syngeneic transplants in 13 patients with acute leukemia. Four patients with ANLL in CR1, three patients with ANLL in full relapse, two patients with ALL in CR1, one in CR3, one in early relapse, and two in full relapse were transplanted with the same preparative regimens used for our allogeneic transplants. All patients transplanted in full relapse achieved remission, but all subsequently relapsed 73–262 days following transplantation and eventually died of their leukemia. Five of eight patients transplanted in complete remission and early relapse survive in continuous disease-free status for 2.5–6.5 years (median of 4.0 years). The actuarial DFS (not shown) is 62%.

Autologous Marrow Transplantation in Acute Leukemia

The requirements for successful autologous marrow transplantation as well as its rationale and laboratory data have been published. The use of 4-hydroperoxycyclo-

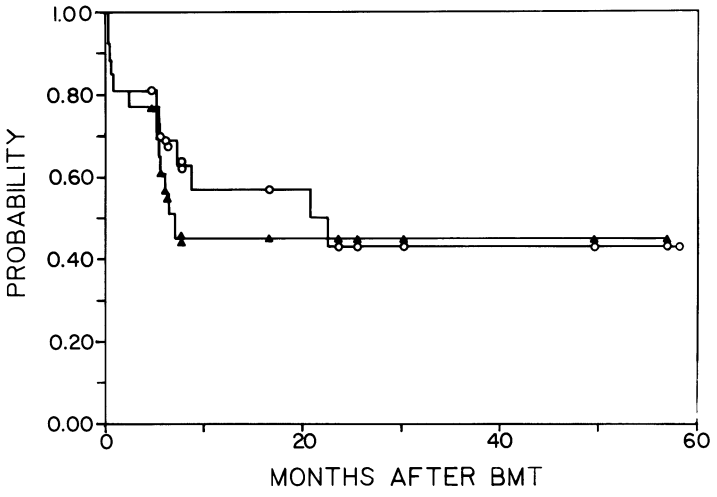

Fig. 3. Kaplan-Meier product limit estimates of survival (0) and disease-free survival (0) in 24 patients with ANLL (19 in second remission and 5 in third remission) given autologous marrow transplants

phosphamide (4HC) as a purging agent has also been discussed [4]. Our experience with the use of 4HC in ALL and ANLL in phase 1 clinical studies formed the basis of our phase 2 studies [5]. The preparative regimens were the same as applied for the allogeneic and syngeneic transplants.

Fifteen patients with ALL in second complete hematologic remission received autologous marrow transplantation with 4HC treated marrow (100 μg/ml). The median age of these patients was 21 years (range, 3–39 years) and the median duration of first remission was 14 months (range, 3–63 months). There were no deaths in the immediate post-BMT period from sepsis or from toxicity of the preparative regimen. One patient, a 36-year-old man, died with necrotizing *Pseudomonas* pneumonitis while in unmaintained CR2 5.5 months after BMT; there was no evidence of persistent or recurrent leukemia at the time of death. One patient, a 5-year-old-boy, had an isolated central nervous system relapse 9.5 months after transplantation. He received both systemic and intrathecal induction chemotherapy and is in maintained remission 40 months after transplantation. Twelve of the remaining 13 patients have relapsed at a median of 4 months (range, 2–15 months) after transplantation. Only one patient remains in continuous complete remission 19.5 months after transplantation, but his duration of CR1 was 32 months.

Twenty-four patients with ANLL aged 4–53 years (median 31 years) were transplanted with autologous bone marrow incubated ex vivo with 4HC. Nineteen patients were in second remission and five in third remission. All patients were transplanted within 3 months of obtaining the remission (second or third). Four patients died with bacterial or fungal sepsis in the 1st month after transplantation before evaluation of persistent engraftment was possible. A fifth patient had persistent marrow hypoplasia and died of sepsis 155 days after transplantation. Seven patients (an actuarial relapse rate of 37.5%) relapsed at 73–213 days (median, 165 days). Twelve patients (eight in second remission and four in third remission) remain continuously free of disease at a median of 277 days (range, 185–1531 days) following transplantation. The Kaplan-Meier product-limit estimate curves [3] for survival and DFS for these patients is shown in Fig. 3.

Discussion

Allogeneic and Syngeneic Transplants

Until recently, the majority of bone marrow transplantation centers have employed the

identical or slightly modified Seattle preparative regimen of Cy (60 mg/kg) given on 2 successive days followed by a single dose of TBI (800–1000 rad) or a daily fractionated dose up to 1200 rad. This has often been referred to as the "standard", and relatively high relapse rates have been seen in those patients transplanted in their second remission. These data have been reviewed in a recent publication [6].

Two groups have reported a very low relapse rate in patients with ALL transplanted in CR2. The Memorial Sloan-Kettering group employs a preparative regimen of hyperfractionated TBI for a total dose of 1320 rad delivered in 4 days followed by Cy (60 mg/kg) given on each of 2 consecutive days. Of those transplanted in second remission, 15 of 22 survived, 14 in complete remission with a median follow-up of 24 months. The projected 2-year actuarial disease-free survival was 62%. There were two observed relapses in this group at 9 and 15 months. In third remission patients, six of 15 survived, five in complete remission for 15–20 months (median 16 months). The projected 2-year disease-free survival was 27%. Six of 15 patients were observed to relapse 1–9 months after transplantation [7].

Coccia et al. [8] reported a series of 14 patients with ALL in second remission who were transplanted following preparation with cytosine arabinoside (3000 mg/m^2 q 12 h for 12 doses) followed by TBI (200 rad q 12 h for six doses). Three patients died 1–2.5 months following transplantation of infection (two patients) or GvHD and sepsis (one patient). One patient relapsed at 17 months and was alive in remission at the time of the report 30 months following transplantation. Ten of the remaining patients enjoyed a disease-free survival of 1–42 months (median 26.5 months).

Our data in allogeneic transplants for ALL reviewed above suggests a third preparative treatment regimen that holds considerable promise in regard to its antileukemic properties. The data presented with syngeneic transplants supports this notion. The relapse rate in patients transplanted in their third remission is universally too high. We have begun to approach this problem using Bu and Cy in such patients. Clearly other approaches are also needed. At the moment, all one can say is that the three preparative regimens noted above appear to offer more of a therapeutic advantage than others.

Although most centers have reported excellent results following transplantation of patients with ANLL in first remission, a review of the results [6] has indicated that a high relapse rate is seen in patients transplanted in second or subsequent remission. The Seattle group has projected an actuarial relapse rate for these patients of 45% with an actuarial survival of 25% [9], a result not better than their experience in transplanting patients in first relapse [10].

Two transplant regimens appear to offer a greater antileukemic effect. The Memorial Sloan-Kettering group employs the same regimen for ANLL that they have employed in ALL noted above. Thirty patients were transplanted in first remission with an actuarial disease-free survivl of 55%. Only three patients relapsed. Eleven patients transplanted in second remission showed a projected disease-free survival of 64% which was not different from patients transplanted in first remission. There were no relapses in this group [11]. The other very effective regimen for ANLL is Bu and Cy as we have reported above. These two regimens at the moment appear to be the most attractive.

Unfortunately, increasing age of patients is a negative factor for the outcome of allogeneic bone marrow transplantation. It is hoped that effective prevention or management of GVHD and viral infections may extend the benefits of the procedure to the older patients. Although there is interest and stated need in employing unrelated matched marrow donors for those who do not have a related donor, the use of autologous transplantation already shows results that may in itself supplant this need [12]. It is also evident at the moment that the older patient with autologous transplantation may do better than they can expect with allogeneic transplantation.

Autologous Transplants

Autologous BMT in ALL has resulted in a DFS of 30%–35% using marrow purged with monoclonal antibody and complement [13, 14]. Our results reported herein are,

therefore, quite disappointing. Currently we are evaluating the effect of a combination of 4HC and methylprednisone for marrow purging and will in the next year evaluate Merocyanine 540 as a purging agent in this disease [15].

No significant leukemia-free survival has been reported when autologous bone marrow transplants are carried out with untreated remission marrow with ANLL in relapse [16] or in second or subsequent remission [17]. The observed disease-free survival of 12/24 or 50% after autologous transplant with 4HC-treated marrow in the present series of patients is encouraging and is atleast comparable to the results reported for allogeneic or syngeneic marrow transplantation [6]. Of interest is the overall DFS of 58% (7/12) in patients over age 30 years who receive autologous transplants, since the mortality rate is high after allogeneic marrow transplantation in this age group [18, 19]. These findings suggest that autologous marrow transplantation may present fewer risks of transplant-related complications to older patients and may be associated with a definite likelihood of cure of ANLL.

Summary and Conclusions

In presentation of our data in context of other clinical series, we have identified a few bone marrow transplant preparative regimens that appear to have a superior advantage over others as far as their antileukemic effects are concerned.

So far, the delayed and late effects of the treatment appear to be quite acceptable considering the ultimate fatal outcome in the acute leukemias, in particular those in their second remission.

GvHD and viral infections account for about two-thirds of the mortalities in series where leukemic relapse is very low. It is hoped that solutions or at least partial solutions will not only improve the overall therapeutic results, but will allow us to extend the procedure to older patients.

It is suggested that the need for unrelated HLA "matched" donors may be supplanted by the use of autologous bone marrow transplants, particularly in the older patients.

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Treatment of Patients with Acute Myeloid Leukemia in First Remission with Marrow Ablative Therapy and Autologous Bone Marrow Transplantation

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The use of high-dose chemo- and radiotherapy followed by allogeneic bone marrow transplantation has proved to be an effective treatment for patients with acute myeloid leukemia. In particular when applied during first remission, a significant proportion of patients (approximately 50%) will remain in continuous remission. The major limitations of the treatment relate to the complications of interstitial pneumonia, infections and graft-versus-host disease (GvHD), and as a consequence its use is currently restricted to patients aged below 45 years and only to those patients who have a genotypically HLA-identical donor [1–4].

In order to circumvent these limitations, current research programs deal with the development of new therapeutic strategies. One of these modalities is the use of autologous bone marrow, taken from the patient himself at the time of full remission [8, 9]. It is assumed that in this stage of the disease, the bone marrow is contaminated with only minimal numbers of tumor cells and that the reinfusion of small numbers of tumor cells will not necessarily lead to relapse following i.v. infusion. In fact, animal studies have shown that the establishment of tumors in a host following i.v. transfer requires a cell dose above a critical threshold [7]. This threshold is thought to be determined by the amount of clonogenic cells in the intravenous inoculate and by the seeding efficiency of the cells to appropriate tissues in the recipient. On the other hand, while autologous bone marrow transplantation has the advantages of not being associated with graft-versus-host disease and interstitial pneumonia, these advantages should be weighed against the price of a higher probability of relapse of leukemia. In addition, in the setting of autologous transplantation, the age restrictions are far less strict.

Here we report our current experience of transplantation of autologous bone marrow in 12 patients with acute myeloid leukemia in first remission.

Methods

Bone marrow was collected from the iliac crest and pelvic spine in 2–4 ml aspirates and collected in bottles containing heparinized Hanks Balanced Salt Solution. The buffy coat (prepared by centrifugation at 2000 g) was filtered through a nylon gauze and then through a glass filter. Nucleated cells were counted in Türck solution. Samples from the graft were sent for bacteriological and GM-CFU cultures. Cells were frozen in 10% DMSO and 20% calf serum using a controlled rate freezer (Cryoson, Beemster, Holland) at 1 °C/min and stored at −196 °C in liquid nitrogen [5]. Cyclophosphamide (60 mg/kg) was administered in saline during a 1-h infusion on 2 subsequent days under conditions of forced diuresis (>125 ml/h). Mesna was given in divided portions at −10 min, +4 h, +8 h, and +12 h following the start of the cyclophosphamide infusion up to a total dose of 48 mg/kg on each of 2 days. Total body irradiation (25 MV photon beam; average treat-

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ment distance, 420 cm) was administered on day −1 with two horizontal beams (AP and PA; with patient on either side) and delivered in one session at an average dose rate of 15.0 cGy/min (patients no. 1 and no. 2) or 5.5 cGy/min (patients no. 3–12). The total dose was 8.0 Gy to the midline of the body with partial lung shielding resulting in a dose of 7.0 Gy to the lungs.

The marrow graft was thawed on day 0 and stepwise diluted with HBSS according to previously described methods [5] and then reinfused within 30–45 min. GM-CFU cultures were done by a double agar layer technique with a leukocyte feeder as previously described [6].

Blood products for transfusion were irradiated [15 Gy); leukocyte-poor cotton-wool filtrated red cells were given; platelet and granulocyte transfusions were always prepared from single donors using an Aminco continuous flow cell separator.

All patients were nursed in reverse isolation in a room (patients no. 1, 4–12) or a laminar air flow unit (patients no. 2 and no. 3) from about day −10 until the granulocyte count had reached a value of 0.5×10^9/l. No autologous BMT in patients with AML performed in our institution were excluded from the analysis. Clinical data were evaluated as of February 1, 1986.

Results

Table 1 contains a summary of the selected clincal characteristics of the 12 patients subjected to autologous bone marrow transplantation. The median duration of complete remission at the time of translantation was 7 months. The bone marrow transplants had been harvested and cryopreserved and were used without any attempt to remove residual leukemia. The numbers of nucleated cells as well as myeloid precursors (CFU-GM) per kg b.w. are indicated in the table as well.

At the time of analysis, five of these 12 patients had died (Table 2). Four patients died from AML relapse as the primary cause of death. One patient showed insufficient hemopoietic recovery and died finally from septicemia (pattient no. 7). The other seven patients survive disease-free at the time of this report. Three of these patients are now beyond 2 years after transplantation. Common complications in the early post-transplant period were infections, usually of bacterial etiology. Granulocyte recovery to a level of 0.5×10^9/l was reached within 22–63 days (median 28 days). The day of platelet recovery to 50×10^9/l showed a significantly broader range, i.e., 39–179 days. These data indicate that a subgroup of patients showed

Table 1. Clinical characteristics and graft size in twelve patients with AML

		ABMT in AML – first remission			
Patient	Sex/age	FAB	Duration of CR until BMT (months)	Nucleated cells $\times 10^8$/kg	CFU-GM $\times 10^4$/kg
1	F, 30	M4	7	2.5	3.1
2	F, 48	M4	7	1.5	2.1
3	M, 15	M3	10	1.9	1.0
4	F, 35	M2	4	3.8	8.2
5	F, 44	M1	5	2.8	6.2
6	F, 45	M4	4	4.0	2.3
7	F, 45	M4	12	2.6	2.9
8	M, 47	M5	7	1.7	5.4
9	M, 42	M1	11	3.7	3.7
10	M, 37	M2	6	2.3	2.3
11	F, 57	M4	5	2.6	8.0
12	F, 39	M2	3	2.8	5.6
Mean			7	2.7	4.2
Median			7	1.6	3.1

Table 2. Hematopoietic recovery and clinical outcome following autologous BMT in 12 patients with first remission AML

Patients	Day granulocytes above $0.5 \times 10/1$	Day platelets above $50 \times 10^9/l$	Complications	Survival status (months)
1	36	67	Pneumonia	72^+
2	23	39	–	14 dead; relapse
3	27	39	Hidradenitis/herpes simplex/ *Staphyl. epid.* septicaemia	46^+
4	24	40	–	9 dead; relapse
5	24	48	Septicaemia *Staphyl. epid.*	24^+
6	28	68	–	12 dead; relapse
7	63	NR	Prolonged thrombopenia	5 dead; insufficient hematological recovery
8	20	47	–	10^+
9	30	46	*Streptococcus* septicaemia	9^+
10	40	53	*Streptococcus septicaemia*/ encephalopathy	3 dead; relapse
11	38	179	Perinal infection, *Aspergillus sepsis*	9^+
12	60	154	*Enterobacter septicaemia*	7^+
Mean	34	71		
Median	28	48		

NR, not reached.

a slow recovery and that prolonged granulocytopenias and thrombocytopenias were noted in approximately 25% of these patients.

Conclusion

Our intitial experiences of the use of nonmodified whole autologous bone marrow in patients with AML in first full remission indicate that continuing lasting remissions can be obtained without purging and without additional maintenance chemotherapy. The major cause of death is relapse, as would be expected from extrapolations from data on allogeneic bone marrow transplantation. The fact that analogous bone marrow with subclinical leukemia is used as well as the absence of graft-versus-host disease facilitate relapse of AML following transplantation when compared with allografting. Indeed, four of our 12 patients have so far shown a relapse. On the basis of these results, it is impossible to make definite conclusions on the value of the therapeutic approach of autologous bone marrow transplantation. As the feasibility study performed in these patients indicates that, in general, adequate hemopoietic recoveries and stable remissions can be obtained, the time has been set for a prospective study. Such a study should aim at evaluating autologous bone marrow transplantation in nonselected patients, i.e., those who enter the study at the time of diagnosis. Such a study has recently been initiated in The Netherlands with four participating centers.

It is apparent from our results that the rate of hemopoietic recovery may vary quite significantly. For instance, platelets may remain depressed below $50 \times 10^9/l$ up to 6 months. The reasons for this delay in hemopoietic recovery are not certain but are probably attributed to the use of bone marrow which has been preexposed to intensive chemotherapy (during remission induction treatment) or the fact that the marrow has been cryopreserved. Another possible explanation could be that the autologous marrow derived from patients with AML have intrinsically inferior repopulation capacities, a

feature which might be disease related. Though we cannot definitely explain the basis of the lasting marrow depression after autologous bone marrow transplantation in a subgroup of patients, it is important to realize that adequate supportive care is necessary for performing this type of treatment.

At this time it is impossible to conclude how the advantages and disadvantages of autologous bone marrow transplantation compare with those of allogeneic bone marrow transplantation. These factors can be appreciated on theoretical grounds, but prospective studies should definitely resolve the value of this treatment modality.

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Poster Session

Basic Research

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Modification of tRNA and Its Applicability for the Assessment of Prognosis, State of Differentiation, and Clonality in Human Leukemias and Lymphomas*

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Introduction

The central agents in protein synthesis are tRNA molecules to which amino acids are attached prior to their polymerisation into polypeptides. Besides its role in protein synthesis, tRNA also plays an important role in the regulation of cell metabolism. These regulatory functions involve alterations of tRNA modifications. The modified nucleosides occur at well-defined positions in specific tRNAs (Fig. 1 a).

The tRNAs specific for aspartic acid, asparagine, histidine, and tyrosine of most organisms contain an unmodified guanosine (G) or the hypermodified nucleoside queuosine (Q) [7-(4,5 *cis*, dihydroxy-1-cyclopentene-1yl-3-aminomethyl)-7-deazaguanosine] in position 34 of the anticodon (wobble base) [1]. These tRNAs of the so-called Q-familiy read the codons NAU and NAC where N is A, U, C, or G, respectively. The extent of this modification varies with the physiologic and developmental state of the organism. Accumulation of tRNA having G in place of Q, i.e., (Q-)tRNA, was particularly found in unmatured and tumor cells [2, 3]. Several experimental findings indicate that free queuine might control the transcription of specific genes, encoding enzymes involved in redox reactions, whereas alterations in the Q content of tRNA cause changes in gene expression at the translation level by affecting the choice of the codon [4, 5]. Queuosine is the only modified nucleoside so far discovered which is derived from deazaguanine (Fig. 1 b). Queuine is inserted into the tRNA by a specific tRNA transglycosylase [2]. This enzyme has been isolated from a wide variety of sources. The bases that can be incorporated into tRNA differ depending upon the source of the enzyme and the tRNA used as acceptor. The *Escherichia coli*-tRNA transglycosylase (E.C.2.4.2.) catalyzes incorporation of queuine precursors as well as guanine into (Q-)tRNAs and cannot catalyze an exchange of guanine with any Q-containing tRNAs, i.e., (Q+)tRNA. Therefore, the *E. coli* enzyme can be used for determining the amount of (Q-)tRNA in total tRNA probes. The mammalian tRNA transglycosylase, however, incorporates queuine as well as its queuine precursors only into mammalian (Q-)tRNA. These enzymes were found to be subject to inhibition by pteridines in a competitive manner [6]. Interestingly, anticancer agents such as the synthetic guanine analogues 8-azaguanine and 6-thioguanine are efficiently incorporated into (Q-)tRNA by the rat liver enzyme [2].

In order to investigate the biologic and conceivable clinical significance of the tRNA modification in human hematopoietic malignancies, we studied the extent of Q modification and the number of tRNA molecules involved in various human leukemias and lymphomas.

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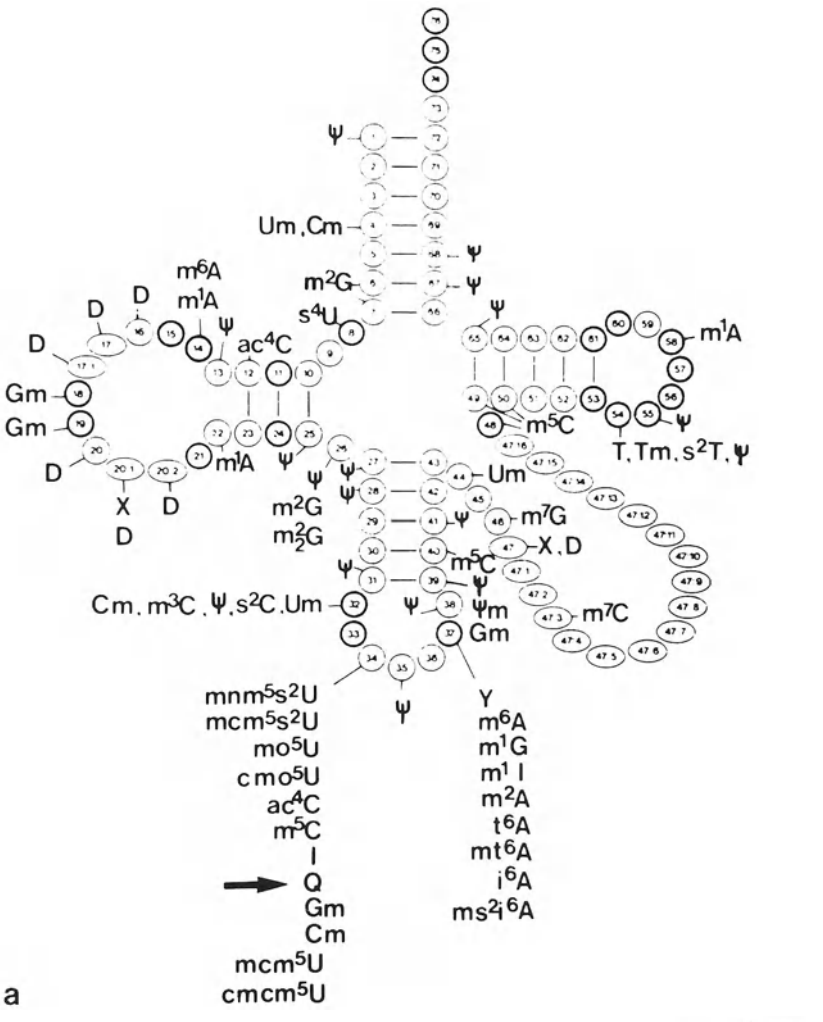

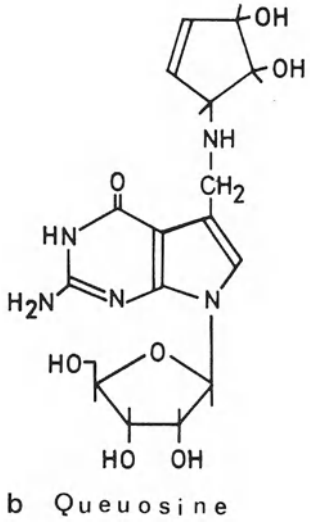

Fig. 1. a, b. General cloverleaf structure of tRNA and positions of modified nucleosides. *Arrow*, position of queuosine *(Q)*. **b** structure of the modified nucleoside queuosine

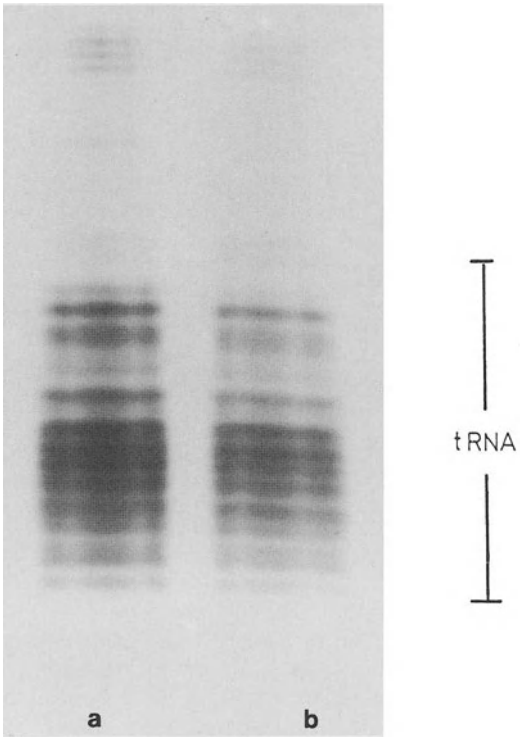

Fig. 2 a, b. Electrophoretic pattern of total tRNA preparation from lymphoma biopsies. **a** lymph node of an immunoblastic lymphoma. **b** spleen of a hairy cell leukemia. Total tRNA was subject to a 10% PAGE in 4 *M* urea and stained by ethidium bromide

Materials and Methods

Cells. Heparinized blood and lymph node biopsies were collected from untreated patients. Leukemic blast cells or lymphoma cells were separated on Ficoll-Isopaque and characterized as previously described [7].

Preparation of Total tRNA. Leukemic cells or lymphoma specimens of a minimum wet weight of 0.5 g were rapidly frozen in liquid nitrogen and stored at −20 °C. The frozen material was minced in liquid nitrogen and extracted with buffer. After the treatment with phenol, tRNA was purified on a DEAE-cellulose column. The tRNA fraction was desalted by Sephadex G-25 column chromatography and further purified on a Sephadex G-150 column. Elution was performed by a buffer containing 10 m*M* Tris-HCl (pH 7.5), 200 m*M* NaCl, 10 m*M* $MgCl_2$, and 7 m*M* NaN_3; tRNA was subsequently desalted on a Sephadex G-25 column. The quality of total tRNA preparation was checked by one-dimensional 10% polyacrylamide gel electrophoresis (PAGE)/4 *M* urea (Fig. 2).

Determination of the Amount of (Q-)tRNA. The amount of tRNA with guanosine at position 34 in place of queuosine was tested by the transglycosylase reaction:

E. coli-tRNA guanine transglycosylase

$$^3\text{H-Gua} + (\text{Q-})\text{tRNA} \rightleftharpoons {}^3\text{H-Gua-tRNA} + \text{Gua} \quad (1)$$

The assay contained in 100 µl: 0.5 A units of tRNA and 100 µg of *E. coli*-tRNA guanine transglycosylase prepared according to [8], 1 nmol (H^3) guanine sulfate (7.7 Ci/mmol) purchased from Radiochemical Centre, Amersham, England, 20 m*M* $MgCl_2$, and 70 m*M* Tris-HCl (pH 7.5).

Electrophoretic Analysis of (Q-)tRNAs. After guanylation, tRNAs were separated on 10% polyacrylamide gels in 4 *M* urea and tRNA species containing (^{3}H)guanine were visualized by fluorography as described previously [7].

Statistical Methods. The nonparametric test, Mann-Whitney U test, was applied to assess

the statistical significance of differences in the mean values for the amount of (Q-) tRNA in the various groups.

Results and Discussion

Undermodification in Chronic Lymphocytic Leukemia Lymphocytes

The relation of incomplete Q modification in tRNA to the prognostic stage of the disease is clearly demonstrated by the findings in chronic lymphocytic leukemia (CLL) (Fig. 3). In all patients immunologic surface marker analyses have proved a unique early B cell marker profile with restriction to one immunoglobulin light chain class, the expression of the GP 67 antigen detected by the Leu 1 or Lyt 2 monoclonal antibody, early B cell antigens detected by BA-1 monoclonal antibodies and Ia antigens detected by OKT Ia or L 243 monoclonal antibodies as well as rosetting with mouse erythrocytes in a variable degree. Hence, lymphocytes of these CLL patients represent the same stage of B cell differentiation. But the amount of the (Q-)tRNA increases significantly ($P<0{,}01$) from favorable prognostic stage A (mean $\pm$SD, 4.72 ± 2.58) to the worst prognostic stage C (20.36 ± 10.08) of the Binet classification. The difference in the mean values of group B (7.85 ± 2.69) and group C is significant at a level of $P<0.05$. The pathophysiologic mechanisms causing the variation in disease progression of CLL is not clearly understood but there are several findings supporting the view that the change from the favorable to the infavorable prognostic stage is linked with an increase of the growth fraction of the malignant B cell clone [10, 11]. Increasing undermodification in CLL lymphocytes may therefore be due to an increasing cell proliferation rate. The high amount of Q-lacking tRNA found in regenerating rat liver indicates that the lack of Q in tRNA is correlated with cell proliferation in nonmalignant cells too [12]. This interpretation is further supported by the clinical course of our patients, in whom rapid lymph node enlargement or rapid increment of blood lymphocytes was accompanied by a high content of (Q-)tRNA.

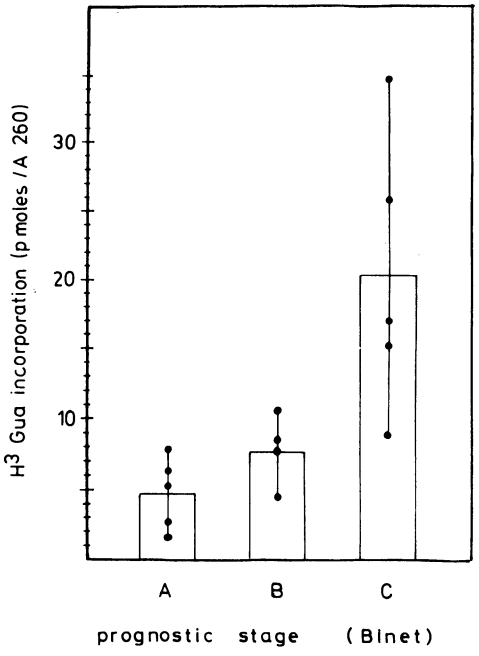

Fig. 3. Amount of (Q-)tRNA in CLL lymphocytes from patients in different prognostic stages according to the Binet classification [9]

Undermodification in Low-Grade and High-Grade Lymphomas

The extent of undermodification was measured in total tRNA preparations from biopsies of six high-grade and nine low-grade lymphomas, and seven non-neoplastic lymphatic tissues (Fig. 4). The amounts of (Q-)tRNA in the low-grade malignant lymphomas, which were mostly germinal center cell lymphomas, are very low (4.67 ± 3.58). They are not significantly different from those found in non-neoplastic lymphatic tissue (4.25 ± 3.17) such as tonsils, lymph nodes or spleens. Considerably higher amounts of (Q-)tRNA are found in high-grade lymphomas [37.18 ± 20.71 ($P<0.01$)]. In these heterogenous diseases, tRNA from a lymphoblastic lymphoma of T cell type and that from a Hodgkin's sarcoma were extremely undermodified. The other probes were a histiocytic high-grade lymphoma and three high-grade lymphomas of immunoblastic type. These findings show that the

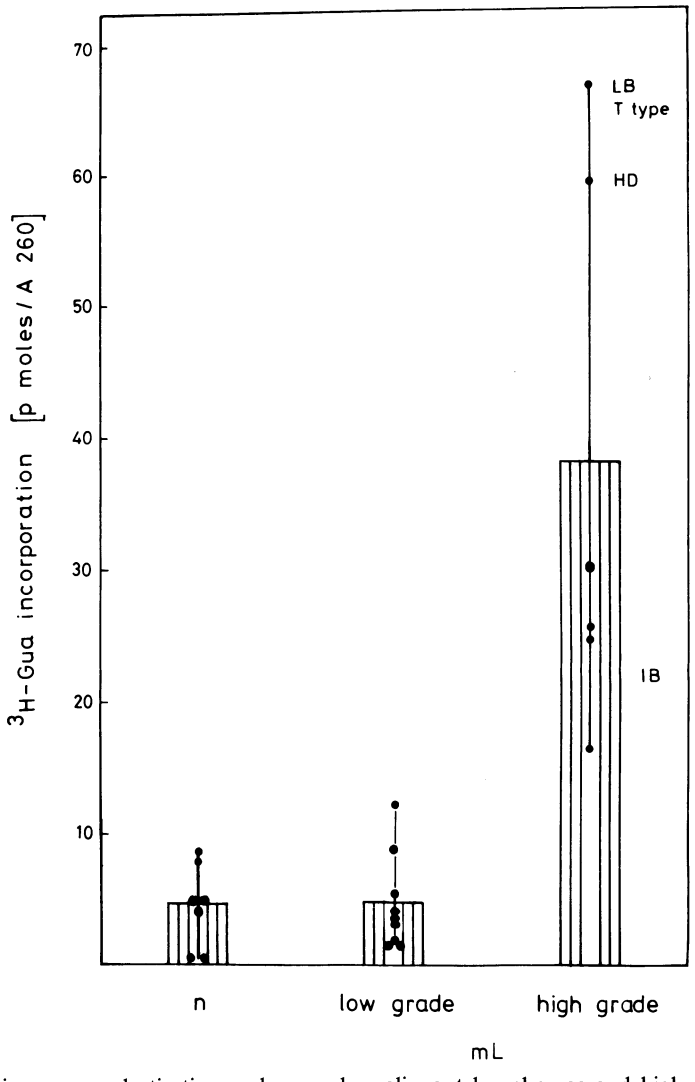

Fig. 4. Amount of (Q-)tRNA in non-neoplastic tissues, low-grade malignant lymphomas and high-grade malignant lymphomas

extent of undermodification of tRNA with respect of Q is well-correlated to the grade of malignancy, defined by histopathologic grading. Since in high-grade lymphomas the percentage of cells in S phase is found to be significantly higher than in low-grade lymphomas [13], it is conceivable that the amount of (Q-)tRNA found in the biopsies may also reflect the different proliferative capacities of these lymphomas.

However, comparison of probes from patients without clinical signs of rapid tumor growth but with lymphomas representing early, intermediate and late B cell differentiation stages, i.e., CLL stage A, hairy cell leukemias, lymphoplasmacytoid immunocytomas with IgM paraprotein secretion, and a solitary plasmacytoma also revealed an increase of (Q-)tRNA in matured B cell lymphomas (see Fig. 5). This may be an indication that the extent of tRNA undermodification in human lymphomas is not generally a consequence of cell proliferation but may be influenced by other factors such as the state of cell maturation. Provided that this interpretation is correct, the alteration of Q

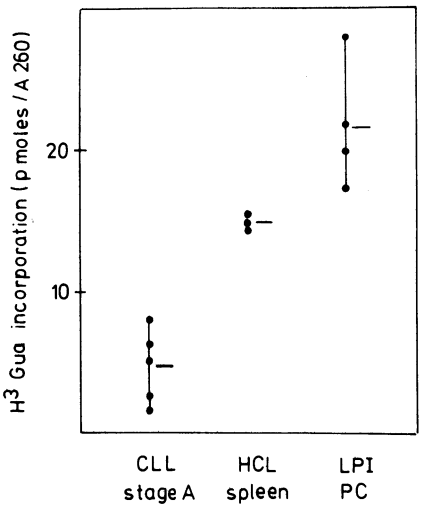

Fig. 5. Amont of (Q-)tRNA in lymphomas representing early, intermediate and late B cell differentiation stages. *CLL,* chronic lymphatic leukemia; *HCL,* hairy cell leukemia; *LPI,* lymphoplasmacytoid immunocytoma; *PC* = solitary plasmacytoma

modification in B lymphocyte maturation would be the reverse of that observed in murine erythroleukemia cells, where the amount of (Q-)tRNA decreases from unmatured to mature cells [14].

Extent of Undermodification in Leukemic Blasts

The amounts of (Q-)tRNA measured in total tRNA preparations from leukemic cells of patients with acute myeloblastic leukemia (AML), acute lymphoblastic and acute undifferentiated leukemia (ALL/AUL) and chronic myeloid leukemia-blast crisis (CML-BC) are outlined in Table 1. Only in 5 of 18 patients were the values for (Q-)tRNA higher than those found in non-neoplastic tissues. Elevated values were found in the following patients: AML (M_4) developed from a myelodysplastic syndrome (MDS) (no. 8), common acute lymphoblastic leukemia (no. 10), AUL (no. 12), CML-BC with myeloid differentiation (no. 15) and

Table 1. Amount of (Q-)tRNA in leukemic blast cells from patients with acute leukemias

Patient no.	Age	Sex	Diagnosis	Differentiation of blasts	Blast counts per µl	Response to induction chemotherapy (survival in months)	Duration of 1st remission (months)	H^3 Gua incorporation (pmol/A 260)
1	56	m	AML	M_2	19475	CR	17+	6.6
2	38	f	AML	M_2	64584	CR	6	3.5
3	55	f	AML	M_3	149400	Early death		5.1
4	44	m	AML	M_4	26226	CR	5	3.4
5	53	f	AML	M_4	44145	Early death		9.0
6	16	m	AML	M_4	120900	NR		4.3
7	63	f	AML	M_5	72485	NR		4.8
8	78	f	AML	M_5	281520	Early death		4.6
9	35	m	MDS-AML	M_4	58029	Early death		11.7
10	61	m	MDS-AML	M_4	62730	Early death		1.5
11	26	f	cALL	L_2 [a]	59760	NR		17.0
12	38	f	T-ALL	L_2 [b]	45000	CR	7+	1.7
13	32	f	AUL		13352	CR	16	15.3
14	78	m	CML-BC	POX+	38420	Survival 1.0		2.2
15	39	m	CML-BC	POX+	6000	Survival 12.0		6.4
16	61	m	CML-BC	POX+	140000	Survival 0.5		24.7
17	55	m	CML-BC	TdT+	80000	Survival 4.0		31.9
18	67	f	CML-BC	PAS+	8000	Survival 1.0		9.1

[a] Common acute lymphoblastic antigen (cALLA)+.
[b] OKT 3, 4, 6, 8+; Leu 1+; E-R+; cALLA+.
CR, complete remission; NR, no response.

Table 2. Electrophoretic pattern of guanylated tRNAs

Patient no.	Diagnosis	H^3 Gua incorporation (pmol/A 260)	No. of bands								
			1	2	3	4	5	6	7	8	9
1	CML-BC (my)	3.7			+++						
2	CML-BC (my)	6.4			+++						
3	CML-BC (my)	24.7			+++						
4	CML-BC (ly)	31.9			+++						
5	CML-BC (ly)	9.1			+++						
6	AML (M_2)	6.6	+	+	++	++	+	+			
7	AML (M_4)	9.0		+	++	+					
8	AML (M_4)	11.3			++	+					
9	AML ($M_{4/5}$)	4.8			++						
10	AML (M_5)	4.6			++	+					
11	CLL/A	3.4	+	+	++	++		+	+	+	+
12	CLL/A	4.4	++	++	++	++	+	+	+		
13	CLL/A	8.0	+	+	++	+	+	+			
14	CLL/B	8.5	+	++	++	+	+				
15	CLL/C	8.9	+	++	++	++	+	+	+	+	
16	CLL/C	15.3	+	+	++	++	+	++	++		
17	CLL/C	17.0	+++	++	+++	+++	+	+	+	+	
18	Centrocytic malignant lymphoma	12.1			+						
19	Centroblastic/centrocytic malignant lymphoma	4.0	+	+	+						
20	HCL	14.8	+		++	+++					
21	HCL	14.4	++		++	++					
22	Lymphoplasmacytoid immunocytoma	17.0	++	++	++	++		+	+	+	+
23	Lymphoplasmacytoid immunocytoma	21.6	+++	++	+++	+++	+	+	+	+	
24	Solitary plasmacytoma	27.9	+		++						
25	Hodgkin's sarcoma	59.5	+	+++	+++	+++					
26	Histiocytic sarcoma	29.9		+	+++	+++	+++				
27	Immunoblastic malignant lymphoma	25.0	+	+++	+++	+++					
28	Ln (non-neoplastic)	8.6		+	+						
29	Spleen (non-neoplastic)	3.9			+						

CML-BC with lymphoid differentiation (no. 16). The extent of undermodification seems to be independent of clinical characteristics such as differentiation of the blast defined by the FAB classification, surface markers, initial blast counts, or response to chemotherapy.

Analysis of (Q-)tRNA by PAGE

The Q-lacking tRNA species can be separated after labeling with (^{3}H) guanine by PAGE and are thus specifically detectable by fluorography among the broad spectrum of tRNA bands on the gel demonstrated in

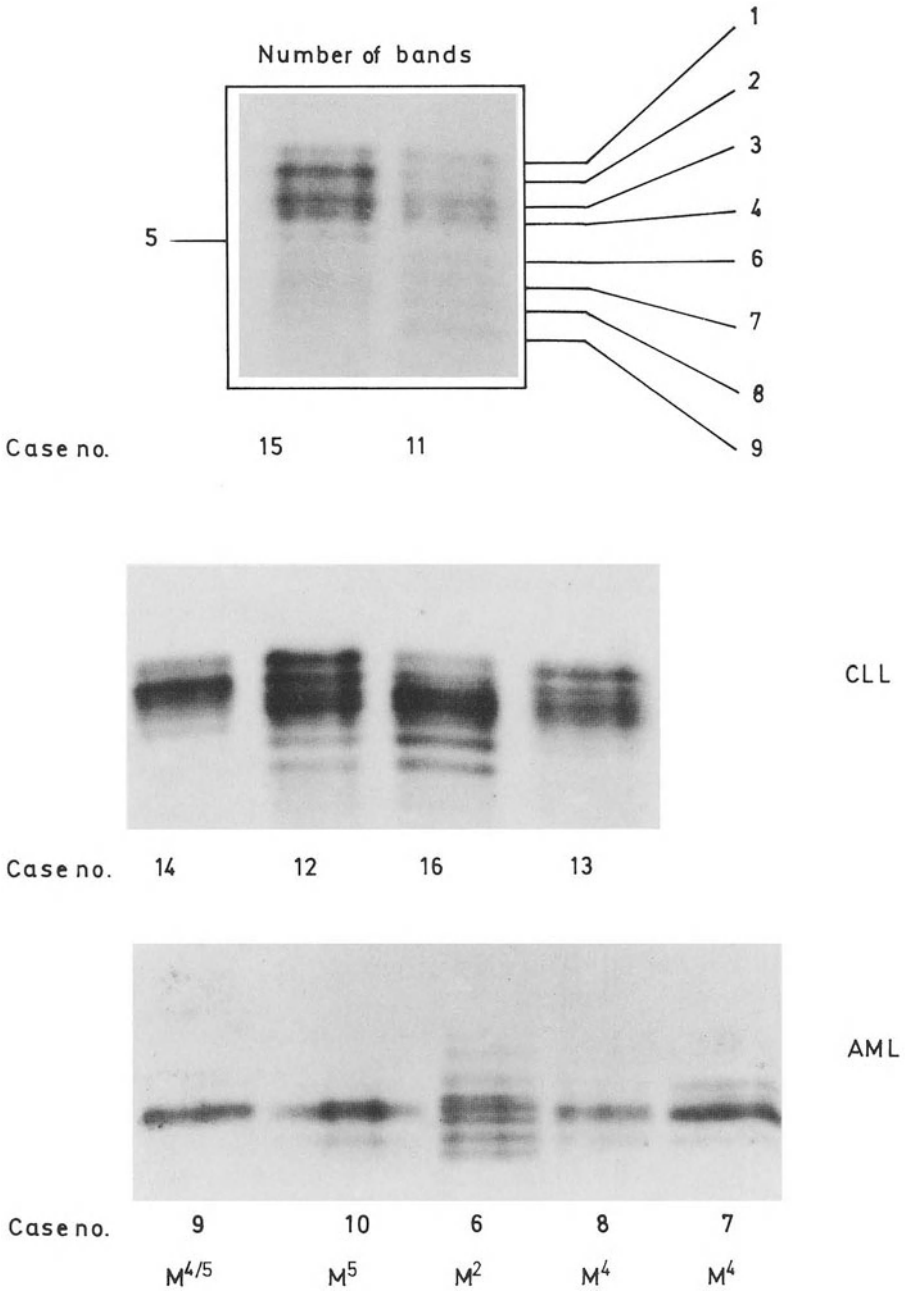

Fig. 6. Fluorographs of separated undermodified tRNA species of the Q family

Fig. 2. During differentiation of lower eukaryotic cells the pattern of guanylatable tRNAs is characteristically changed [4]. We therefore asked whether the pattern of guanylation of the tRNA species of the Q family may vary in normal and neoplastic cells and according to the type of the neoplastic cell. This phenotypic analysis was done in cell or tissue probes of 29 patients (Table 2). Nine well-separated radioactive bands were ob-

served on the autoradiographs. Thus, nine (Q-)tRNA species with different electrophoretic mobilities can be distinguished (Fig. 6). In non-neoplastic lymphatic tissues only one or two bands were detectable, whereas in CLL lymphocytes and leukemic cells of immunocytomas up to eight bands could be found. Interestingly, tRNA preparation of blasts from patients with CML-BC and the probe from a centrocytic malignant lymphoma exhibit only one band. The restriction to one band was independent of the extent of undermodification and the presence of myeloid or lymphoid markers. In AML blasts with M_4 or M_5 differentiation a restriction to band 3 with low radioactivity in position 2 and/or 4 could also be observed. The patterns found in the seven CLL lymphocytes are heterogenous and not correlatable to the amount of (^{3}H) guanine incorporated into total tRNA nor to the prognostic stage. The pattern found in the three high-grade lymphomas is very similar with pronounced bands in positions 2, 3, and 4. Since specific (Q+) and (Q−)tRNA of humans are not yet available, the bands cannot be correlated to $tRNA^{Asn}$, $tRNA^{His}$, $tRNA^{Tyr}$, or $tRNA^{Asp}$. The fact that more than four labeled bands are detectable indicates, however, that isoaccepting tRNA molecules for some of these species of the Q family must be expressed. In experimental models we are beginning to understand the physiologic role of the different Q-lacking tRNA molecules in the regulation of gene expression on transcription and translation levels [4, 5]. The wide variation in the expression of different Q-lacking tRNA molecules in human hematopoietic malignancies underlines the complex role of these regulator molecules in cellular transformation and differentiation of human cells.

Attempts to determine monoclonality of an individual neoplasm have classically used restriction to one immunoglobulin light chain; specific cytogenetic abnormalities, the expression of only a single glucose-6-phosphate dehydrogenase allele within the tumor of heterozygous female; and recently, the rearrangement of genes [15, 16]. Each of these approaches has limitations. From this aspect the observed specific electrophoretic variants of (Q-)tRNA seem to be of clinical interest both for diagnosis and for predicting clonality. However, further studies are necessary before this aspect can be judged definitively, as the origin of polymorphism in (Q-)tRNA patterns seems to be complex. On the one hand, it is dependent on the expression of tRNA genes for histidine-, tyrosine-, asparagine- and aspartic acid-specific tRNA molecules. On the other hand, the modification, i.e., the exchange of G by Q, is a post-transcriptional process which is probably also regulated by genetically independent mechanisms; for instance, the inhibition of the tRNA transglycosylase by pteridine or other metabolic or exogenous factors [17].

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Immunoglobulin and T Cell Receptor Gene Rearrangements in Acute Leukemias*

A. Raghavachar[1], C. R. Bartram[2], E. Kleihauer[2], and B. Kubanek[1]

Introduction

Acute leukemias are commonly classified on the basis of morphologic, cytochemical and immunologic criteria. Immunologic techniques, particularly surface antigen analysis using monoclonal antibodies, have related leukemic cells to normal cell counterparts [1]. In general, lineage fidelity is maintained in leukemic blast cells. However, there are exceptions [2, 3]. Recently, molecular analysis using specific Ig and T cell receptor gene probes has become a valuable addition to morphologic, cytochemical and surface marker studies in determining lineage, clonality, and differentiation of leukemic cell populations [4, 5]. We therefore investigated the diagnostic potential of molecular analyses (a) to develop an understanding of the cellular origin of leukemias which cannot be allocated to one of the defined myeloid or lymphoid lineages by cellular phenotypes, and (b) to reveal the clonal development of phenotypic conversion in acute leukemia.

Patients and Methods

Leukemia Cells

Ficoll-Hypaque-enriched blasts from a total of 148 consecutively diagnosed patients with acute leukemia (Depts. of Hematology/Oncology and Pediatrics II, University of Ulm) were investigated for morphologic and immunologic markers. From these, patients who were lacking morphologic and immunologic features characteristic of a particular cell lineage were selected for molecular analyses. In addition, we included two patients with acute undifferentiated leukemia (AUL) showing phenotypic conversion during the course of disease, 18 acute nonlymphoid leukemia (ANLL) patients and nine patients with hairy cell leukemia (HCL) since considerable controversy has surrounded the cellular origin and stage of differentiation of HCL [6, 7].

Multiple Marker Analysis

The analysis included standard staining techniques (Wright-Giemsa, PAS, MPO, the latter at the ultrastructural level), karyotyping, immunofluorescence assays for TdT, B lineage, T lineage, common acute lymphocytic leukemia (cALL) and granulocytic-monocytic lineage-associated antigens as described in detail elsewhere [8].

DNA Analysis

DNAs were extracted from mononuclear cells after Ficoll-Hypaque gradient centrifugation by standard techniques. DNA 15 µg was codigested with *Bam*HI and *Hind*III, electrophoresed on a 0.7% agarose gel, blotted and hybridized to a J_H probe (Oncor, Gaithersbury, USA) that detects a 5.6-kb heavy chain Ig gene germline fragment. Fol-

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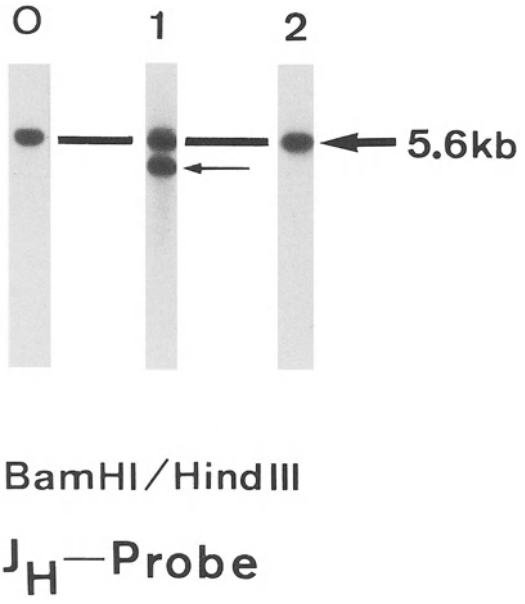

Fig. 1. Southern blot analyses of two AUL patients. One allele is rearranged in case *1*, both alleles are in germline in case *2*. *0*, placenta control DNA

lowing *Eco*RI or *Bam*HI digestion DNAs were hybridized to a $C_T\beta$ probe (Oncor, Gaithersbury, USA) that detects 12-kb and 4.2-kb or 23-kb germline bands of the T cell receptor β chain genes. After hybridization the filters were washed under stringent conditions and exposed to XAR-5 film (Kodak) using Dupont Lightning-Plus intensifying screens for 12–60 h at −70 °C.

Results and Discussion

Despite the lack of MPO at the ultrastructural level, expression of some myeloid-specific antibodies was detected in five cases. Southern blot analysis revealed a rearrangement of Ig heavy chain sequences in eight cases, the majority following a monoclonal pattern (Fig. 1, lane 1). In three cases, including one case with t (4; 11), more than two rearranged fragments were detected indicating oligoclonality. Two patients exhibited no rearrangements within the Ig heavy chain sequences covered by the J_H probe and a Cμ probe (kindly provided by Philip Leder) (Fig. 1, lane 2). T cell receptor β chain sequences were in germline in all ten leukemic cell DNAs. We conclude from these results that most common acute lymphoblastic antigen (cALLA)-negative non-T non-B ALLs are diseases of early B cells at the bifurcation of the lymphoid-myeloid dif-

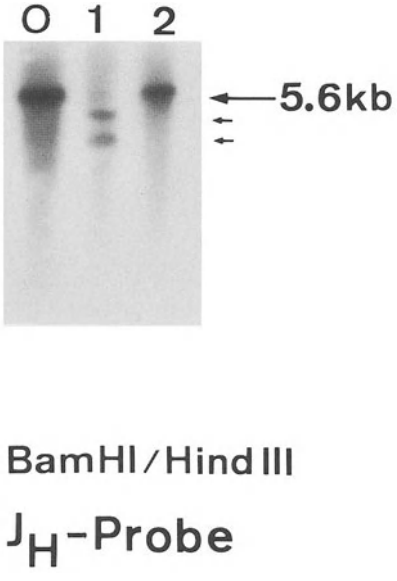

Fig. 2. Southern blot analyses of two AML patients. Patient *1* exhibits a biallelic Ig heavy chain rearrangement, patient *2* has both Ig heavy chain alleles in germline. *0*, placenta control DNA

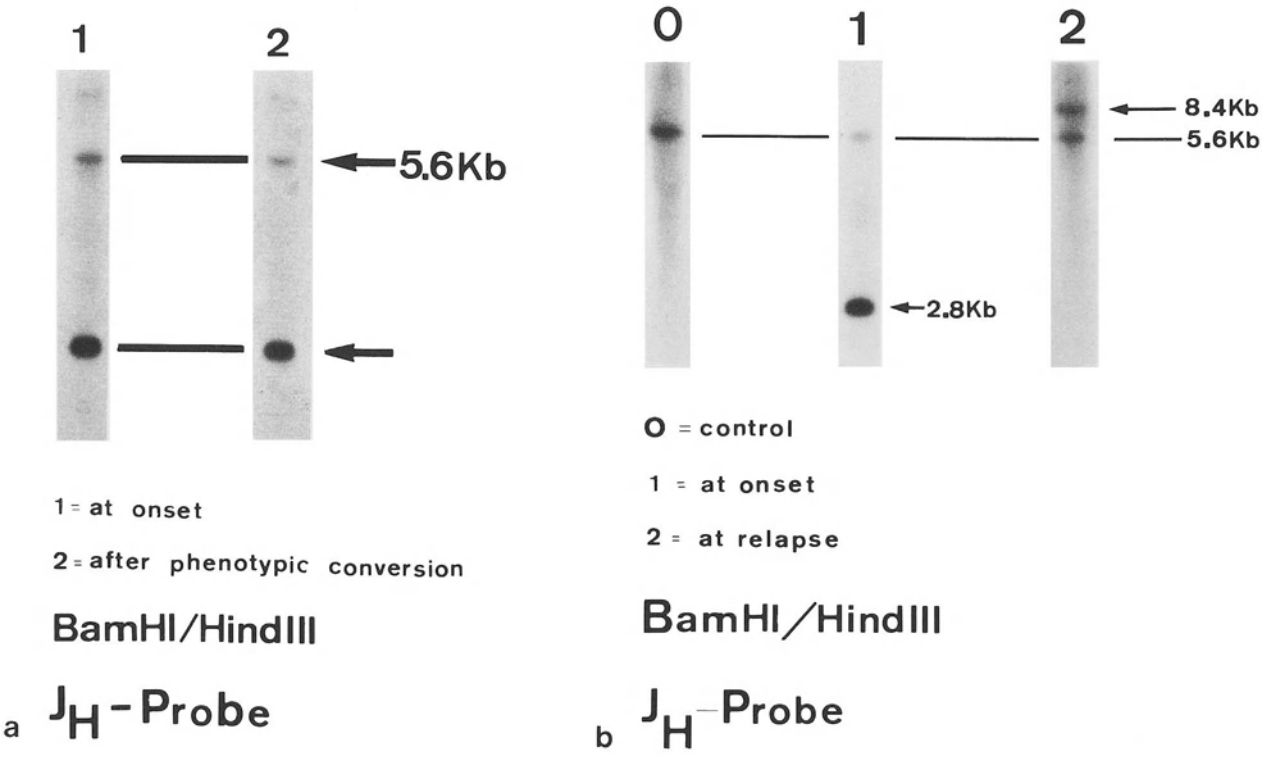

Fig. 3a, b. Southern blot analyses of two AUL patients with phenotypic conversion. **a** Patient with identical patterns of JH gene rearrangement; **b** patient in whom different rearranged fragments were detected

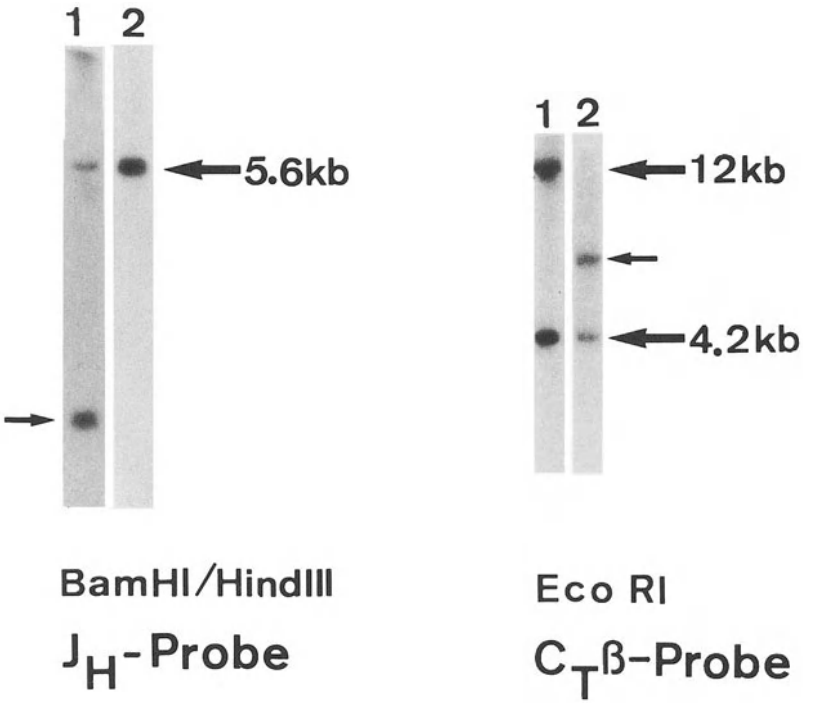

Fig. 4. Southern blot analyses of tow HCL patients. In patient *1* one Ig heavy chain allele is rearranged, the other deleted. T-β genes are in germline. In patient *2* Ig heavy chain genes are in germline, the normal 23-kb Tcβ_1 fragment is deleted on one allele and shows a rearrangement on the other allele

ferentiation pathways. However, the detection of Ig heavy chain gene rearrangements per se does not necessarily imply a B cell lineage commitment as this type of recombination may also occur in about 10% of phenotypically defined ANLL (Fig. 2) [10].

In two cases of AUL with phenotypic conversion the diagnostic potential of molecular analysis was demonstrated (Fig. 3). In one patient (Fig. 3a) who progressed from AUL to acute myelomonocytic leukemia (AMML) within 3 weeks of starting treatment, the detection of identical rearranged Ig heavy chain fragments in respective leukemic cell samples supports the concept of the capability of AUL to differentiate into myeloid lineage [11]. However, in the other of these patients with AUL (Fig. 3b), who relapsed after allogeneic bone marrow transplantation (BMT) with cALL, different autoradiographic bands were demonstrated on Southern blots in AUL and cALL phases and this implies the development of a new leukemic cell clone after BMT.

In HCL either an Ig (eight cases) or a T cell receptor gene rearrangement (one case) could be demonstrated (Fig. 4), supporting the view that HCL represents a clinicopathologic entity comprising heterogeneous leukemic subtypes.

In summary, our studies illustrate that a combination of both immunologic and molecular approaches appears to be essential in order to help our understanding as to the lineage commitment in human leukemias, and this combination will influence our therapeutic approach in future.

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Abnormal Production and Release of Ferritin by Immature Myeloid Cells in Leukemia

E. Aulbert[1] and H. Fromm

Introduction

Serum ferritin concentration has been shown to reflect total body iron stores in iron deficiency and iron overload [1]. Elevated serum ferritin levels have also been described in several malignant diseases such as leukemia, malignant lymphomas, and different types of solid tumors [2–6]. However, data reported within the last few years have been contradictory and show highly differing results. In this paper we present results indicating that increased serum ferritin concentrations in immature myeloid leukemia are due to an increased production and release of ferritin by immature myeloid blasts.

Methods

Serum ferritin concentrations were measured with respect to various hematologic parameters before therapy was started and during the course of the disease in 176 patients with leukemia. There were 45 cases of acute myeloblastic leukemia (AML), 60 cases of chronic myeloid leukemia (CML), 40 cases of acute myelomonocytic leukemia (AMML), and 31 cases of acute lymphoblastic leukemia (ALL). All patients were adults. Ferritin was determined by means of a standardized solid-phase enzyme immunoassay. A serum ferritin level of 150 ng/ml was valued as the upper limit of normal range.

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Intracellular ferritin content of the leukemic cells was measured after ultrasonic disruption of the isolated leukemic cells in a hypotonic medium. The intracellular ferritin concentration was expressed in terms of femtograms (fg) per cell.

Results and Discussion

Serum ferritin concentrations of all patients with leukemia at presentation were significantly elevated compared with values of normal subjects. Patients were divided into six subgroups according to the FAB classification of acute myeloid leukemias (M_1-M_6) [7], one subgroup of ALL and three subgroups of CML (chronic phase, blast crisis, and complete remission after bone marrow transplantation). Extremely high serum ferritin concentrations were found in patients with immature AML (M_1) and AML (M_2) before treatment. The mean ferritin concentrations were 2235 ± 870 ng/ml and 1355 ± 532 ng/ml, which is a 22-fold and 13-fold increase compared with normal values (96 ng/ml). In contrast, serum ferritin concentrations in patients with AMML and acute monocytic leukemia (M_4, M_5) were found to be significantly lower, i.e., 450 ± 252 ng/ml and 463 ± 254 ng/ml (Fig. 1). Even lower levels were found in ALL (362 ± 151 ng/ml). There was no correlation between serum ferritin levels and bone marrow storage iron, serum iron concentration, or transferrin saturation. We could not confirm earlier reports of a correlation between serum ferritin levels and peripheral white blood cell count [8].

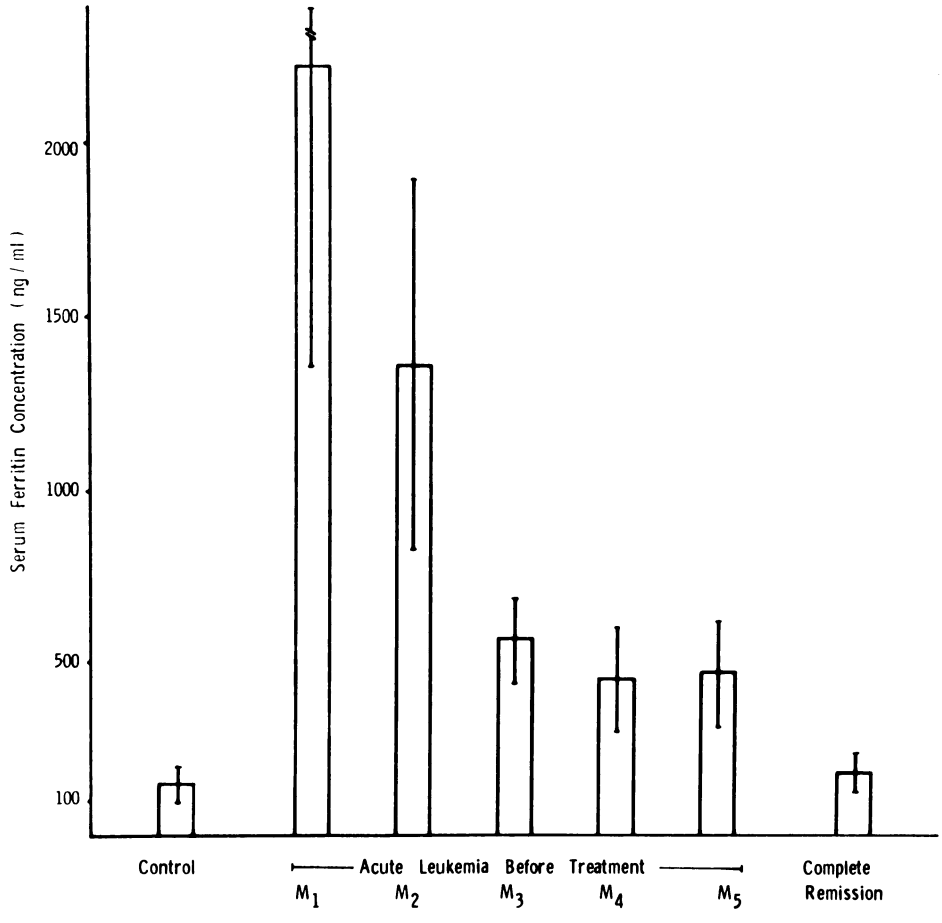

Fig. 1. Serum ferritin concentrations in patients with AML, before treatment and in complete remission

Investigation of the intracellular ferritin concentration revealed a direct correlation between serum ferritin concentration and the amount of ferritin within the leukemic blasts: the intracellular ferritin content was 72 ± 16 fg/cell in the immature myeloid leukemic blasts, 9.5 ± 4 fg/cell within the myelomonocytic leukemic cells and 3.6 ± 2 fg/cell within the lymphatic leukemic blasts compared with 5.5 ± 2 fg/cell within the normal granulocyte (Table 1). These data suggest the possibility that the high serum ferritin concentrations in patients with immature myeloblastic leukemia may be derived from the leukemic cells themselves and that it may be due to an increased synthesis and release of ferritin. Our data are consistent with previous observations that leukemic cells synthesize ferritin at a much higher rate than do normal leukocytes [9]. The more mature leukemic cells obviously synthesize less ferritin than do immature leukemic cells. Accordingly, reports of increased incorporation of ^{14}C-leucine into ferritin of less mature leukemic cells reflect a greater ferritin synthesis in these cells [10].

In addition, our investigations of patients with CML confirm this concept. During the chronic phase of CML we found normal serum ferritin concentrations (84 ± 51 ng/ml), whereas myeloid blast crisis was associated with a 21-fold increase in serum ferritin levels (2180 ± 867 ng/ml). In cases of myelomonocytic type of blast crisis and in one case of lymphatic blast crisis a significantly lower increase of serum ferritin concentrations was

Table 1. Serum ferritin concentration and intracellular ferritin content within leukemic cells

	FAB classification	Serum ferritin concentration	Intracellular ferritin concentration
AML	M_1	2235 ± 870 ng/ml	72 ± 16 fg/cell
	M_2	1355 ± 532 ng/ml	
Acute Promyelocytic L.	M_3	565 ± 135 ng/ml	
AMML	M_4	450 ± 252 ng/ml	9 ± 4 fg/cell
Acute Monocytic L.	M_5	463 ± 254 ng/ml	
Acute Erythroleukemia	M_6	598 ± 145 ng/ml	
ALL		362 ± 151 ng/ml	4 ± 2 fg/cell
CML	Chronic phase	84 ± 51 ng/ml	3 ± 2 fg/cell
	Blast crisis	2180 ± 867 ng/ml	52 ± 12 fg/cell
Normal		96 ± 42 ng/ml	5 ± 2 fg/granulocyte

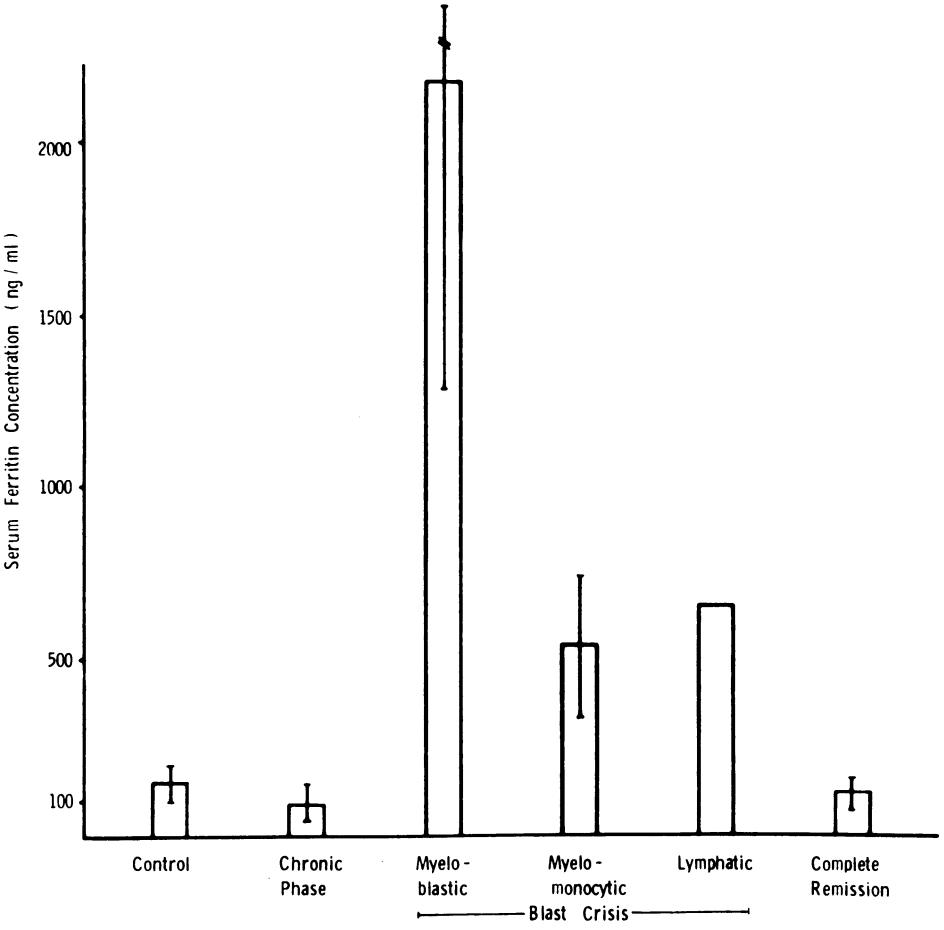

Fig. 2. Serum ferritin concentrations in patients with CML, in chronic phase, in blast crisis, and in complete remission (after bone marrow transplantation)

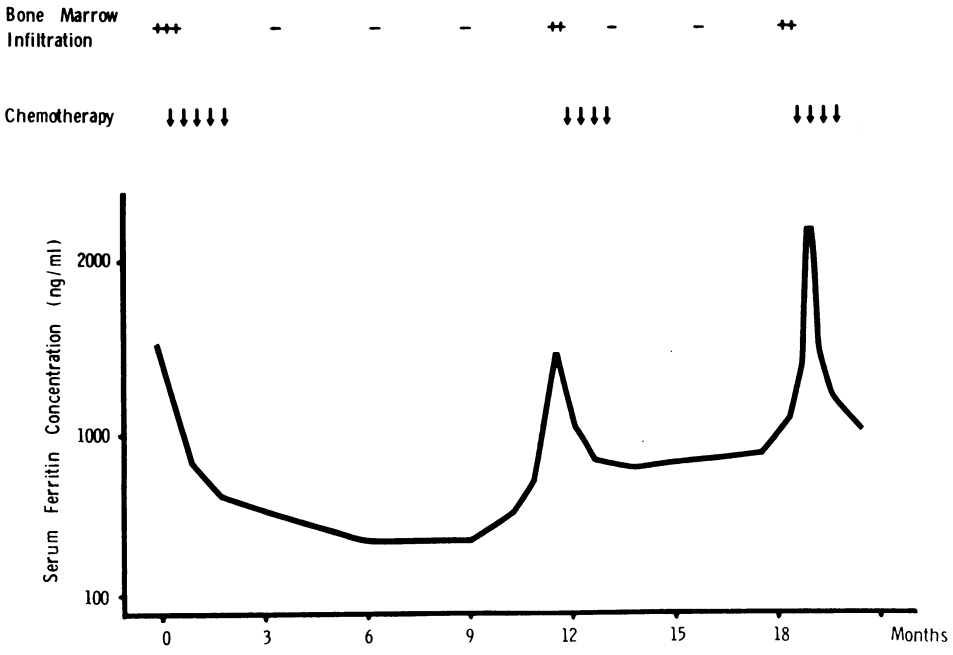

Fig. 3. Serum ferritin concentration during the course of a representative case of acute leukemia

recorded (537 ± 199 ng/ml and 648 ng/ml, respectively) (Fig. 2). There was also a direct correlation between serum ferritin levels and intracellular ferritin content of myeloid cells in the chronic phase (3.2 ± 2 fg/cell) and in myeloid blast crisis (52 ± 12 fg/cell) (Table 1).

In longitudinal investigations of patients with AML we found that serum ferritin reflected the clinical course of the disease: high pretreatment serum ferritin levels decreased during tumor regression, reached normal values in complete remission and increased again in relapse (Fig. 3). Similar results were obtained in the patients with CML: patients who entered blast crisis showed highly increased serum ferritin levels, whereas during tumor regression under chemotherapy serum ferritin levels decreased again. In patients treated by bone marrow transplantation and reaching a complete remission serum ferritin concentrations decreased to normal values (Fig. 2).

Nevertheless, the precise mechanism for the increase of serum ferritin levels in leukemia is not yet clear and may be influenced by other factors: elevation of serum ferritin concentration can, for instance, also occur by cell damage after treatment. In patients who were continuously observed during the induction of remission by chemotherapy, a further increase of serum ferritin concentration during cytotoxic chemotherapy was found. It was evident that this increase of serum ferritin concentration under chemotherapy was conspicuous in those forms of leukemia where high intracellular ferritin concentrations were found within the leukemic cells. This phenomenon indicates that increased serum ferritin levels may also be due to the release of intracellular ferritin from damaged leukemic cells. Furthermore, during the therapeutically induced myelosuppression, red blood cell transfusions are frequently necessary and these produce a transitory iron overload with increasing ferritin levels [1]. Thus, in the first few months after the start of induction chemotherapy serum ferritin levels are of limited value as indicators of disease activity. Despite this restriction, during the later course of the disease serum ferritin can be used as a helpful and simple parameter in monitoring the activity of the disease. This could be particularly useful for controlling tumor regression under a successful antineoplastic therapy. In

particular, serial determinations of serum ferritin concentration are relevant in the management of patients in complete remission in order to allow an early assessment of potential relapse.

Conclusion

We conclude from our results that immature leukemic blasts produce and release ferritin at a very high rate resulting in a highly raised serum ferritin concentration. Thus, serum ferritin concentrations in AML and in CML must be valued as a clinically useful tumor-associated marker. There is no correlation between the highly elevated serum ferritin concentrations and the total body iron stores in these diseases. A direct correlation between serum ferritin concentration and the intracellular ferritin content within the leukemic blasts indicates that increased serum ferritin levels may be due to increased synthesis and release of ferritin by the leukemic cells themselves. In patients who were continuously observed during the course of the disease, serum ferritin could be shown to be a helpful and simple parameter in monitoring the disease activity.

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Induction of Early Myeloperoxidase in Acute Unclassified Leukemia*

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Introduction

In a small percentage of acute leukemias the lineage specificity of blasts remains undetermined after morphologic, cytochemical, and immunologic analyses. Some of these cases may coexpress markers of different cell lineages, suggesting their origin from a multipotent precursor cell or an aberrant gene expression [1–3].

For further analysis of their lineage affinity, two diagnostic tools have provided additional information: (a) detection of myeloperoxidase by transmission electron microscopy (TEM) as strong evidence for the commitment to the myelomonocytic lineage [4, 5]; and (b) differentiation induction in vitro of these undifferentiated blasts by various chemical inducers [6, 7].

The aim of the present study was to trace, by in vitro culture with 12-O-tetradecanoylphorbol-13-acetate (TPA) as a differentiation-inducing agent, the lineage affiliation of leukemic blasts in two patients with unclassified leukemias presenting with surface markers characteristic for several distinct lineages.

Patients and Methods

The leukemic blasts of two patients suffering from acute unclassified leukemia (Table 1) were isolated from the peripheral blood by separation on a Ficoll-Isopaque gradient (1.077 g/ml) and cryopreserved prior to the present studies. The diagnosis of acute undifferentiated leukemia (AUL) was defined by the absence of both morphologic and cytochemical lineage specific markers [8]. The immunologic diagnosis 0-AL was made if there were no lineage specific markers or a coexpression of both myeloid and lymphoid lineage specificities [9].

Suspension Culture. The blasts were cultured in vitro for 4 days at 1×10^6 cells/ml in RPMI 1640, as previously described [10]. TPA (Sigma) at a final concentration of 5×10^{-9} *M* was used as an inducing agent [7].

TEM Cytochemistry. Endogenous peroxidase acitivity was studied by ultrastructural analysis using the method of Roels et al. [11]. The cells were incubated unfixed in a medium of 20 mg diaminobenzidine (DAB, Sigma), dissolved in 10 ml of 0.05 *M* Ringer-Tris buffer, pH 7.4 and H_2O_2 (0.003%) for 1 h at room temperature. The cells were then processed and embedded as for TEM mor-

Table 1. Data of the patients

No.	1	2
Morphologic diagnosis	AUL	ALL
Immunologic diagnosis	0-AL	0-AL
WBC/mm	130000	367000
Blast cells (%)	95	95
Genotype	ND	Diploid

ND, not done.

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Table 2. Immunologic analysis of the blast cells prior to and after culturee

Patient no.	Duration of culture days	Inducing agents	Cells/ml ($\times 10^{-5}$)	% positive cells								
				Myelomonocytic antigens				cALLA	B antigens		T antigens		
				MY-7	VIM-2	B4.3	OKM-1	J5	B4	pan-B	WT-1	OKT6	OKT3
1	0	–	10.0	12	21	–	5	–	–	–	66	–	–
	4	TPA, 5n*M*	4.8	70	64	–	6	–	–	–	45	–	5
2	0	–	10.0	–	59	26	–	8	98	20	4	–	ND
	4	TPA, 5n*M*	17.8	1	60	45	–	21	29	25	2	–	ND

ND, not done.

phology except for the block staining with uranyl acetate.

Phenotypic Analysis with Antibodies. Phenotypic analysis was performed using the immunoalkaline phosphatase method as described before [10]. Each test consisted of an examination of 200 cells.

Double-immunofluorescence staining. Double-fluorescence staining was accomplished by using combinations of monoclonal antibodies of different immunoglobulin classes [12].

Results

According to morphology and cytochemistry the clinical diagnosis of the patients was acute undifferentiated leukemia (AUL) and acute lymphocytic leukemia (ALL) (Table 1).

The immunologic phenotype of the blasts in both cases in the study was characterized by the presence of both myeloid and lymphoid surface antigens on the same cells (Table 2).

After 4 days in vitro there was a significant increase of the reactivity with the myeloid specific antibodies in all patients. Double-immunofluorescence staining of these cells again revealed the coexpression of both myeloid and lymphoid markers (Table 2).

TEM Cytochemistry. The ultrastructural analysis of the blasts prior to culture (Fig. 1 a) revealed the morpholgy of undifferentiated blasts. No specific peroxidase activity was found. After exposure to TPA (Fig. 1 b) a strong expression of peroxidase activity was observed in the perinuclear membrane, the endoplasmatic reticulum, and Golgi apparatus in the vast majority of the blasts in all cases studied.

Discussion

The lineage affiliation of two cases of null-AL was studied by differentiation induction in vitro using TPA as an inducing agent. The differentiation capacity was monitored by the ultrastructural demonstration of myelo-

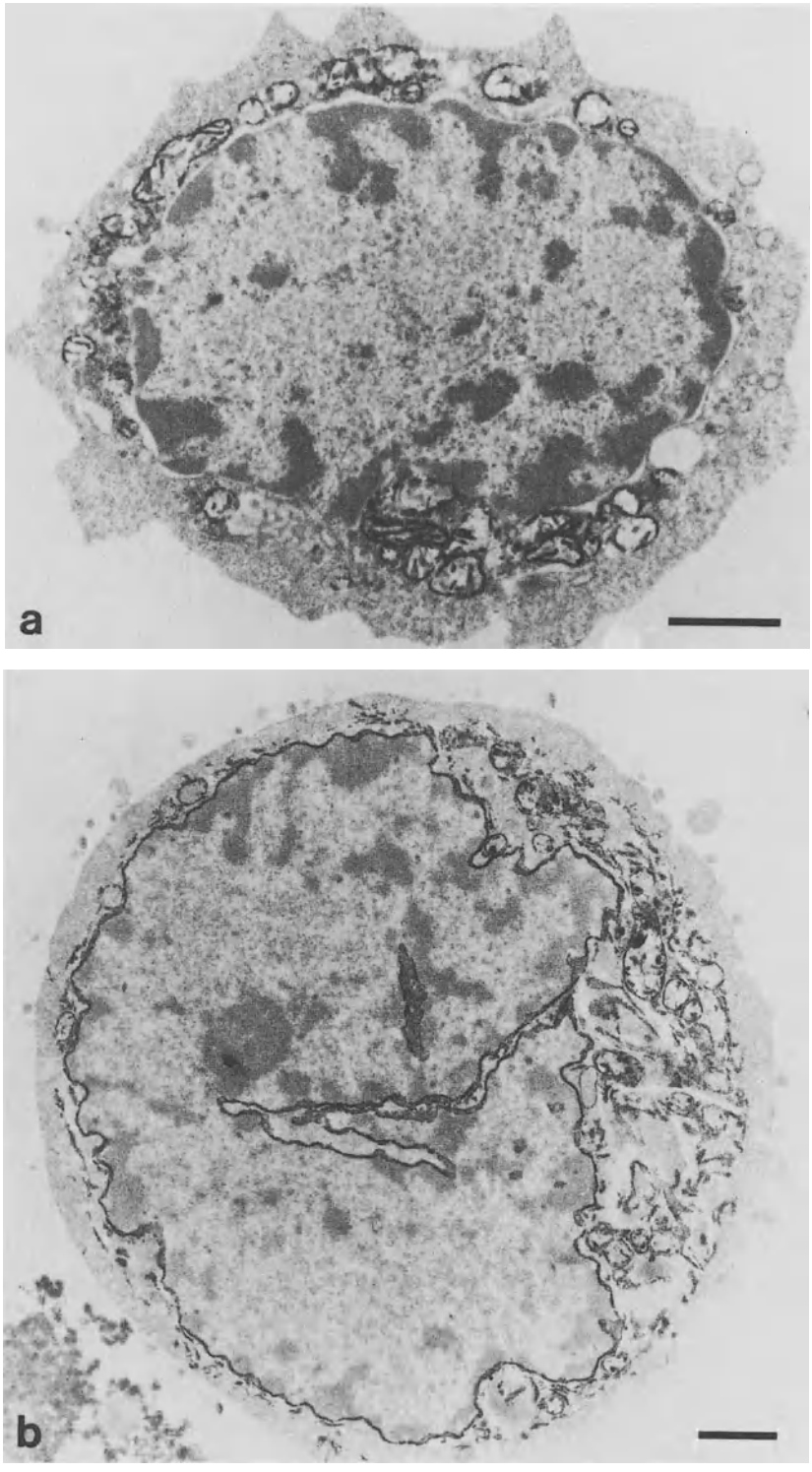

Fig. 1 a, b. Ultrastructural cytochemistry of blasts prior to culture. **a** reveals no specific activity of myeloperoxidase but a nonspecific staining of the mitochondria due to oxidative phosphorilation enzymes. **b** after the culture, peroxidase activity is found in the perinuclear membranes, the endoplasmatic reticulum, and Golgi apparatus. No granular reaction is visible. The sections are stained with lead citrate (**a**) or unstained (**b**). (*Bars* = 1 μm)

peroxidase, the only lineage specific marker available at present [13, 14].

Prior to culture the blasts coexpressed both myeloid and lymphoid markers indicating true cases of lineage infidelity. The ultrastructural analysis of myeloperoxidase was negative, allowing no further classification of the blasts [3].

After 4 days in vitro the coexpression of myeloid and lymphoid markers was unchanged, the slight increase of the myeloid marker frequency suggested a preference for the myeloid pathway. These cells now revealed a strong activity of myeloperoxidase, which clearly indicates a differentiation induction along the myelomonocytic pathway in all cases in the study [4, 15].

Since TPA is an effective inducer of both myeloid and lymphoid differentiation our data provide indirect evidence for a myelomonocytic commitment of these cells [7, 10, 16].

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Abnormal Marker Expression in Acute Leukemia (AL) Characterized by Monoclonal Antibodies and Flow Cytometry*

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Introduction

The application of refined immunologic and enzymatic markers to conventional morphologic and cytochemical techniques has revealed an unexpected heterogeneitiy in acute leukemia (AL). Since the development of monoclonal antibodies (MoAbs) to lineage specific differentiation markers, there have been several reports of AL patients whose blast cells represent relatively homogeneous populations with phenotypic features of more than one cell line [1–5] or are characterized by the coexistence of separate cell populations each demonstrating either lymphoid or myeloid features [6–10].

Different terms, e.g., hybrid acute leukemia, acute mixed-lineage leukemia (AMLL), biphenotypic leukemia, and intra-/interlineage infidelity have been proposed to describe these leukemias (reviewed in [11]).

We report ten cases with biphenotypic AL (according to the definition proposed in [12]) and two cases with biclonal AL diagnosed by applying a panel of lineage-associated MoAbs for phenotype determinations in 401 consecutive children with acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML). Analysis of cellular DNA content by flow cytometry (FCM) was performed in 11 out of 12 patients with biphenotypic/biclonal AL to establish leukemia cell ploidy.

Materials and Methods

The 12 patients described in this study were immunophenotyped in a 2-year period during which samples from 81 children with AML and 320 children with ALL were referred for marker analysis as part of the AML and ALL BFM trials 83 in childhood.

In ten patients bone marrow and/or peripheral blood were taken for immunologic and DNA analysis prior to the administration of induction chemotherapy. Two patients were studied at relapse.

Details of methods for immunologic and DNA analyses are described elsewhere [13]. Briefly, leukemic blasts were isolated by standard Ficoll-Hypaque density gradient centrifugation. For phenotype determinations, blasts were first incubated in heat-inactivated pooled AB serum to avoid nonspecific binding to Fc receptors, and then washed three times in phosphate-buffered saline. The binding of MoAbs was assessed by indirect immunofluorescence with fluoresceinated goat $F(ab')_2$ antimouse IgG plus IgM. Fluorescence of cells was evaluated with an epilluminated fluorescence Zeiss microscope or with a fluorescence-activated cell sorter (Epics V, Coulter Electronics). Background fluorescence, determined by using nonreactive MoAbs of the same isotype as the test MoAbs, was subtracted. In selected cases with adequate cell

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Table 1. Panel of MoAbs utilized in this study

Group	MoAbs	CD[a]	Reactivity	Reference/source
Granulomonocytic lineage	VIM-D5	CD15	Granulocytes, monoblasts	Majdic et al. [15]/ Dr. Knapp[b]
	My9		Granulo-, monocytes	Griffin et al. [16]/Coulter
	AML-2-23		Granulo-, monocytes	Ball et al. [17]/Hybritech
	Leu-M3	CDw14	Monocyte/macrophage	Dimitriu-Bona et al. [18]/ Becton Dickinson
B cell lineage	B4	CD19	Pan-B-specific	Nadler et al. [19]/Coulter
	VIB-C5	CD24	Pan-B and granulocytes	Majdic et al. [20]/ Dr. Knapp
T cell lineage	Leu-9	CD7	Pan-T	Link et al. [21]/ Becton Dickinson
	OKT11	CD2	Pan-T (E rosette receptor)	Verbi et al. [22]/Ortho
CALLA	J5	CD10	Common ALL antigen	Ritz et al. [23]/Coulter
HLA-DR	OKla1		Ia-like antigen	Nadler et al. [24]/Ortho

[a] Cluster designation.

[b] The authors wish to thank Dr. Knapp for providing the VIM-D5 and VIB-C5 MoAbs.

numbers, double-fluorescence staining was performed as described elsewhere [14].

The panel of MoAbs selected for this study is depicted in Table 1.

For intranuclear terminal deoxynucleotidyltransferase (TdT) staining, cytospin cell preparations were fixed in cold methanol, and TdT was detected by an indirect immunofluorescence assay as described elsewhere [25].

For analysis of cellular DNA content, cells were stained with ethidium bromide and mithramycin in combination, and DNA measurements were carried out by flow cytometry (ICP 11, Bartec, Basle/Switzerland) hooked up to a 1.024-channel multichannel analyzer. The degree of DNA aneuploidy was quantified on the basis of the DNA index, which was calculated by dividing the $G_{0/1}$ DNA content of the sample by that of reference (diploid) lymphocytes.

Morphologic diagnosis was determined by standard techniques using Pappenheim-stained smears and cytochemical staining (peroxidase, nonspecific esterase, periodic acid-Schiff, acid phosphatase).

Results

Table 2 summarizes the patient characteristics investigated in this study. Seven patients were morphologically considered lymphoid and three patients myeloid on the basis of FAB criteria. In one patient, the morphologic diagnosis was uncertain (M_1/L_2 form with inconclusive cytochemistry), and one other patient was observed to develop ALL and AML consecutively within 1 week. Table 3 shows the results of cytochemical staining, phenotype determinations and DNA measurements. Myeloid cell-associated antigens (VIM-D5 or My9) were found in five patients of otherwise typical ALL (nos. 1–5). T-cell-associated antigen (T11) was expressed in two patients with common ALL (nos. 6 and 7). Myeloid antigen (My9) was observed in two patients with T-All (nos. 8 and 9), and blast cells from patient no. 10 with morphologically determined AML expressed both myeloid and B-cell-restricted antigens.

The overlap in percentages of blasts reactive with myeloid- or T-cell-associated antigens and B-cell-restricted antigens clearly indicates the biphenotypic features of these cases. In addition, double-fluorescence staining performed in patient nos. 2, 3, 11 confirmed the expression of myeloid antigens on TdT-positive blast cells and the simultaneous expression of myeloid and B cell antigens on individual leukemic cells. Interestingly, patient no. 3 demonstrated a lineage switch from AML to ALL, and cytoge-

Table 2. Patient characteristics

Patient no.	Study time[a]	Age (years)	Sex	Morphologic diagnosis	Immunologic diagnosis	Response to antileukemia therapy[e]
1	RP	8	M	ALL	Common ALL	NR
2	DG	1	F	ALL	Null-ALL	NR
3	RP	4	M	AMOL/ALL[b]	Null-ALL	CR
4	DG	15	M	ALL	Common ALL	NR
5	DG	14	M	Uncertain[c]	Common ALL	CR
6	DG	7	M	ALL	Common ALL	CR
7	DG	16	F	ALL	Common ALL	CR
8	DG	8	M	ALL	T-ALL	CR
9	DG	15	M	ALL	T-ALL	CR
10	DG	1	M	AML	AML	CR
11	DG	13	F	AMOL	Biclonal AL	CR
12	DG	0.5	F	ALL/AML[d]	Null-ALL/AML	CNS relapse

[a] RP, relapse; DG, diagnosis.
[b] At diagnosis AMOL (M5a); at relapse ALL with t (4;11).
[c] M_1/L_2 according to FAB classification.
[d] At diagnosis ALL; developing AML 1 week later during induction chemotherapy for ALL.
[e] NR, no response; CR, complete remission.

netic analysis of blast cells at relapse disclosed a t (4; 11) consistent with recent findings that t (4; 11) has a pluripotent target cell [26].

In patient no. 11, double-fluorescence staining revealed a heterogeneous population with blast cells expressing both myeloid and lymphoid antigens (My9 and B4), as well as separate cells with myeloid (My9+, TdT−) and lymphoid (B4+, TdT+) features.

At presentation, blast cells of patient no. 12 showed the typical phenotype of 0-ALL with only a low proportion of cells expressing myeloid antigens. One week later, after administration of induction chemotherapy for ALL, large blasts with monocytoid features appeared in the peripheral blood, and surface marker studies confirmed the AML phenotype. Similar cases have recently been published [8, 10, 27].

Analysis of cellular DNA content by FCM showed a diploid DNA index in ten cases and an aneuploid DNA content in one patient (Table 3). Different DNA stem lines could not be detected in these patients.

Discussion

The immunologic analysis of AL by MoAbs reacting with lineage-associated or -restricted cell surface antigens has revealed a spectrum of unexpressed marker profiles, including AL with a coexistence of blast cell populations showing either myeloid or lymphoid features, defined as biclonal AL [12], and a single population of leukemic cells with markers of both myeloid and lymphoid lineage, termed biphenotypic AL [12].

Different mechanisms have been proposed to explain the origin of biclonal/biphenotypic AL, i.e., leukemic transformation of a pluripotential stem cell, aberrant gene expression, existence of a rare hematopoietic progenitor cell with expression of different lineage-specific characteristics, or the probably unusual evolution of two distinct malignant transformations at the level of both myeloid- and lymphoid-committed precursor cells [1, 2, 5, 11].

The cases in this study can be divided into two types: patient nos. 1–10 (Table 3) expressed both lymphoid and myeloid cell-associated antigens or B- and T-lineage-associated antigens. The simultaneous or sequential occurrence of two distinct populations with lymhoid and myeloid antigens could be demonstrated in patient nos. 11 and 12. Although AL with two stem lines has been recently described [28, 29], the measurement of DNA content by FCM did not reveal different stem lines in any of our patients, thus emphasizing the monoclonal origin of AL

Table 3. Comparison of cytochemistry, immunophenotypic features and DNA index

Patient no.	Cytochemistry[b] (% pos. cells)				Immunologic phenotype (% pos. cells)											DI[e]
	POX	NSE	PAS	AP	VIM-D5	My9	AML-2-23	Leu-M3	B4	VIB-C5	J5	Leu-9	OKT11	OKla1	TdT	
1	ND[c]	ND	ND	ND	40	0	ND	ND	65	75	75	10	ND	85	90	ND
2	0	0	55	0	75	5	ND	ND	60	10	2	2	ND	90	95	1.0
3	0	40	<10	0	30	10	ND	1	60	2	3	5	ND	84	55	1.0
4	0	0	50	17	63	0	ND	ND	50	36	80	5	ND	90	85	1.15
5	1	< 5	26	< 5	10	66	ND	2	56	ND	45	15	ND	55	70	1.0
6	0	0	0	0	0	0	ND	ND	60	55	40	8	50	85	50	1.0
7	0	0	0	0	2	2	ND	ND	55	55	85	6	45	85	90	1.0
8	0	21	54	80	4	70	ND	ND	0	0	65	85	80	3	85	1.0
9	0	0	76	51	5	55	ND	ND	4	0	50	90	70	2	90	1.0
10	0	0	65	0	5	2	50	3	55	30	0	4	ND	22	5	1.0
11	(+)[d]	+	++	ND	18	45	ND	4	40	10	0	0	ND	50	50	1.0
12	0	0	0	0	10	0	2	5	35	15	0	2	ND	50	45	1.0
12[a]	0	0	0	0	40	45	77	40	12	ND	0	9	ND	45	0	ND

[a] 1 week later (see text).
[b] POX, peroxidase; NSE, nonspecific esterase; PAS, periodic acid Schiff; AP, acid phosphatase.
[c] ND, not done.
[d] POX: faintly pos. (monoblasts); NSE: diffuse pos.; PAS: diffuse pos.
[e] DI, DNA index.

evaluated in this study. The implications of biphenotypic/biclonal AL with respect to remission induction and prognosis are not yet clear [2, 5]. Nine out of ten patients studied at diagnosis achieved complete remission with standard induction therapy for ALL or AML, one patient did not respond and one other patient had a CNS relapse with TdT-positive blasts 4 months after achieving complete remission (Table 2). However, longer follow-up evaluation of these patients is necessary to determine the prognostic significance of this type of AL. Our data support recent results showing that biphenotypic/biclonal AL may be an underdiagnosed group [5] that requires better characterization by a combination of different techniques including double-fluorescence staining, cytogenetic analysis, FCM DNA measurements, Ig or T cell receptor gene rearrangement studies and in vitro induction of differentiation.

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Urinary GP41 Excretion in Patients with Acute Leukemias Treated with Intensive Induction Polychemotherapy*

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Introduction

Alterations of cell membrane glycoproteins are involved in several crucial biologic processes, e.g., contact inhibition of growth, influence on cellular adhesion, cellular differentiation, malignant transformation and development of metastases [1–3]. Cells in acute leukemias and lymphomas express typical glycoproteins depending on their stage of differentiation [4]. These glycoproteins are frequently shed or secreted from the cells and can be detected in serum and urine [5, 6]. Previous studies in a patient with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) showed that the leukemic blasts contain a glycoprotein with a molecular weight of 41 000 (GP41) and a strong affinity to Concanavalin A. It could be demonstrated that GP41 was secreted in the urine in response to leukemic cell reduction [7]. The object of this study was to investigate the following questions in a larger number of patients with acute leukemias: Do all leukemic blasts express GP41? Are the kinetics of excretion correlated to the clinical response and can GP41 excretion be used as an early response parameter in acute leukemias? Early response parameters are thought to be of prognostic relevance and may be of importance for individualizing the mode, intensity and duration of chemotherapy [8].

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Material and Methods

Patients

Glycoproteins from leukemic cells and from urine collected over a period of 24 h were investigated in 7 patients with ALL, 18 patients with AML and two patients with high-grade non-Hodgkin's lymphomas. All the patients were undergoing intensive induction chemotherapy. The clinical characteristics of these patients are listed in Table 1.

Preparations of Leukemic Cells and Urine

Leukemic cells were only investigated in patients with 70% or more leukemic blasts in peripheral blood. The mononuclear cells were separated on Ficoll-Isopaque and washed three times with phosphate-buffered saline (PBS). Glycoproteins were prepared by direct extraction with Triton-X 100 (1%) as follows: 0.2 ml 1% Triton-X 100 in 10 m*M* Tris HCl (pH 7.5) was added to 200 mg (wet weight) of cells, mixed and centrifuged at 1000 g. The supernatant containing the membrane glycoproteins was boiled in an equal volume of solubilizing buffer containing 2% sodium dodecyl sulfate (SDS), 10% glycerine, 0.02% bromphenolblue, 0.1 *M* dithioerythritol and 10 m*M* Tris HCl (pH 8.0). The samples were frozen and stored at −20 °C. Urine taken over a 24-h period was collected into 5 ml NaN_3 (1%). The whole volume was concentrated by ultrafiltration to a constant volume of 3.5 ml containing all proteins with a molecular

Table 1. GP41 excretion in urine of patients with acute leukemias, high-grade lymphomas and controls

No.	Age	Sex	Diagnosis	Type (FAB class.)	Stage	Treatment	Clinical response	Urinary GP41
1	15	f	cALL	L_1	1st manifestation	V, P, D, ASP,CYT, A, MP	CR	+++
2	14	m	cALL	L_1	1st manifestation	V, P, D, ASP,CYT, A, MP	CR	+++
3	10	m	ALL		1st manifestation	V, P, D, ASP,CYT, A, MP	CR	+++
4	10	f	cALL	L_1	1st manifestation	V, P, D, ASP,CYT, A, MP	CR	+++
5	18	m	O-ALL	L_1	1st manifestation	V, P, D, ASP,CYT, A, MP	CR	+++
6	19	m	O-ALL		1st relapse	HD A	F	+
7	38	f	T-ALL	L_2	1st manifestation	V, P, D, ASP,CYT, A, MP	CR	−
8	29	m	AML	M_1	1st manifestation	TAD 9	CR	+++
9	53	m	AML	M_1	1st manifestation	TAD 9	PR	++
10	61	m	AML	M_1	1st manifestation	TAD 9	CR	+++
11	73	m	AML	M_1	1st manifestation	TAD 9	F	+
12	55	m	AML	M_1	1st manifestation	TAD 9	CR	+++
13	57	m	AML	M_1	1st manifestation	TAD 9	CR	+++
14	56	m	AML	M_2	1st manifestation	TAD 9	CR	+++
15	31	m	AML	M_2	1st manifestation	TAD 9	F	+
16	39	f	AML	M_2	1st manifestation	TAD 9	CR	++
17	33	m	AML	M_2	1st manifestation	TAD 9	CR	+++
18	60	m	AML	M_4	1st relapse	HD A, Mitox	CR	++
19	41	m	AML	M_4	1st manifestation	TAD 9	CR	+++
20	35	m	AML	M_4	1st manifestation	TAD 9	PR	++
21	18	m	AML	M_4	1st manifestation	TAD 9	PR	++
22	24	m	AML	M_5	1st manifestation	TAD 9	PR	++
23	58	m	AML	M_5	1st relapse	HD A	PR	++
24	47	f	AML	M_5	2nd relapse	VP 16, A	F	(+)
25	55	m	AML	M_6	1st manifestation	TAD 9	PR	++
26	20	f	LB ml		1st manifestation	COPBLAM	CR	+++
27	63	f	IB ml		1st manifestation	COPBLAM	CR	+++
21 controls (8 SCLC responding to polychemotherapy, 3 patients with CLL under COP chemotherapy, 4 patients with non-neoplastic diseases (SLE and nephrotic syndrome) and 6 healthy subjects)								Negative

V, vincristine; P, prednisone; D, daunorubicin; ASP, asparaginase; M, methotrexate; MP, mercaptopurine; A, cytosine arabinoside; HD, high dose; CYT, cyclophosphamide, COPBLAM, cyclophosphamide, vincristine, prednisone, bleomycin, adriamycin, procarbazine; Mitox, mitoxantrone; CR, complete response; PR, partial response; F, failure.

weight of 10000 and more; 0.1 ml of the ultrafiltrate was mixed with 0.1 ml of solubilizing buffer, boiled, frozen and stored at −20 °C.

Electrophoresis and Lectin Affinity Labeling of Glycoproteins

Both urine and cell samples were subjected to SDS polyacrylamide gel electrophoresis (PAGE) with an exponential gradient from 5% to 15%. The gels were stained with coomassie blue and then labeled with (J^{125}) Concanavalin A in a modification of the method described by Bramwell and Harris [9]. Concanavalin A (10 mg) was suspended in 200 µl PBS containing 20 m*M* glucose and 25 µl lactoperoxidase (10 mg/2 ml); 10 µl glucoseoxidase (1 mg/ml) was added and the mixture incubated with 1 mCi natrium (J^{125}) iodide for 30 min. The reaction was stopped by addition of 1.5 ml ice-cold saturated ammonium sulfate solution and the labeled complex was then centrifuged for 15 min at 2000 *g*. After additional washing, the pellet was solubilized in 2 ml PBS, counted and then diluted to a final volume of 500 ml PBS. The gel was washed four times in PBS followed by incubation with the (J^{125}) Concanavalin A solution for 16–

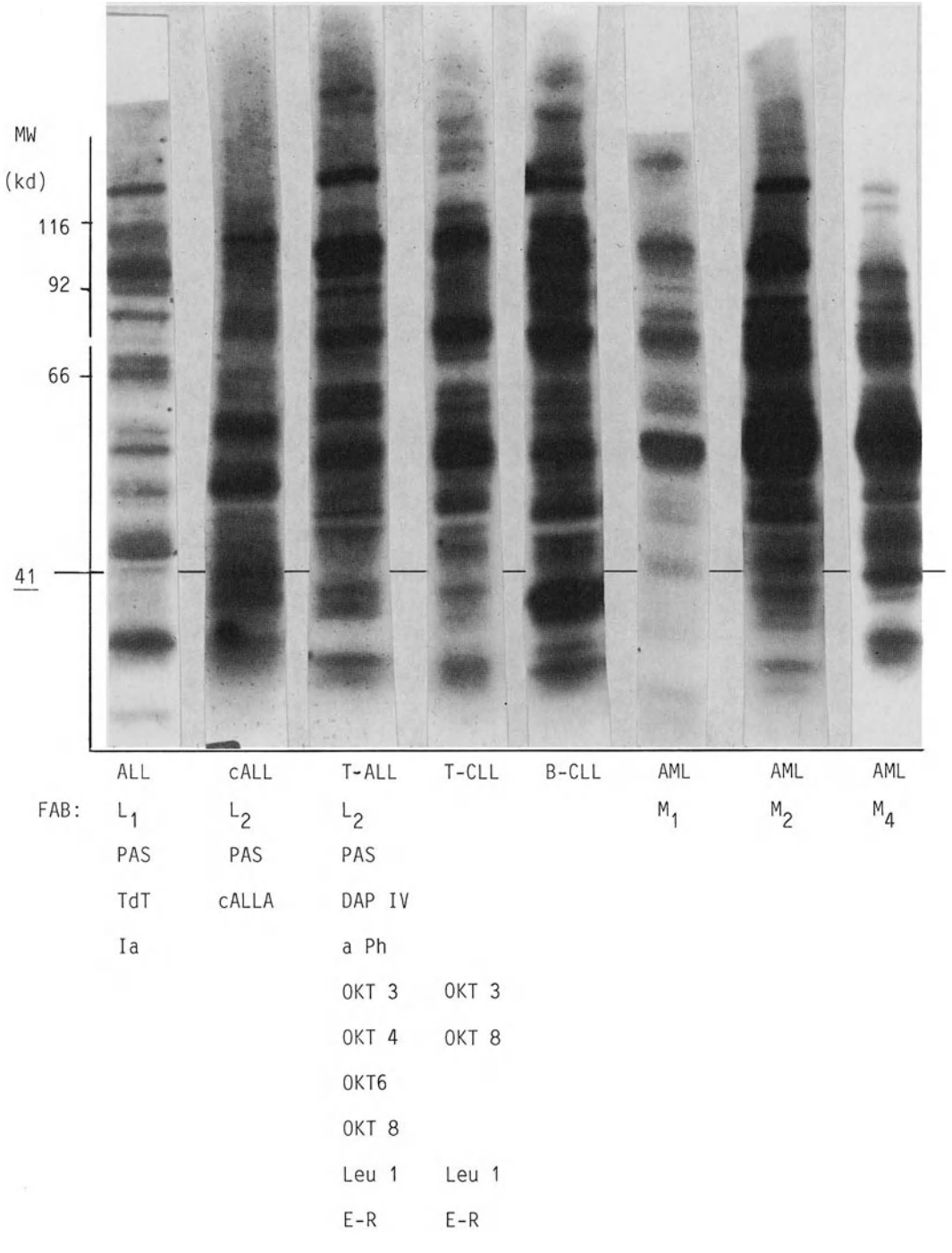

Fig. 1. Cellular membrane glycoprotein pattern and the morphologic, cytochemical and immunologic characteristics of leukemic blasts from patients with different acute and chronic leukemias. *ALL,* acute lymphoblastic leukemia; *AML,* acute myeloid leukemia; *FAB,* French, American, British classification of acute leukemias; *PAS,* periodic acid-Schiff; *TdT,* terminal deoxyribonucleotidyl transferase; *cALLA,* common acute lymphoblastic antigen; *DAP IV,* diaminopeptidase IV, *a Ph,* acid phosphatase; E-R, sheep erythrocyte rosettes. *kd,* kilodalton

24 h. After washing and drying the gels were exposed to Kodak-x-omat films for about 3 days. The relative amount of glycoprotein excretion was determined according to a score system (+ = slight excretion; + + = medium excretion; + + + = strong excretion) by two independent examiners, and in some cases semiquantitatively by computer-assisted laser densitometry of the autoradiographs.

Results and Discussion

Cellular Membrane Glycoprotein Patterns of Leukemic Blasts

The cellular membrane glycoprotein patterns of leukemic cells separated from heparinized blood samples from patients with different leukemias are shown in Fig. 1. It illustrates that blasts from patients with ALL and AML, as well as chronic lymphatic leukemias (CLL) each express characteristic glycoproteins indicated by a different number and intensity of bands. Similar results were obtained previously by Andersson and coworkers using a different labeling method [10, 11]. GP41 was found in all leukemic blasts from patients with AML and ALL with the exception of one case with T-ALL (see lane 3, Fig. 1). Furthermore, GP41 was not observed in T-CLL lymphocytes (see lane 4) and in any of 15 B-CLL lymphocyte preparations (see lane 5).

Urinary Glycoproteins in Patients with Acute Leukemia

The excretion of glycoproteins in the urine of a patient with AML (M_1 of the FAB classification) undergoing TAD-9 induction therapy [12] is shown in Fig. 2. In contrast to healthy subjects, patients with acute leukemias excrete several glycoproteins in the urine, which generally have molecular weights ranging from 14 to 116 kilodalton. Of these urinary glycoproteins only GP41 fulfilled the criteria for a tumor related glycoprotein, i.e., it only appeared in the urine in response to tumor cell reduction and there was a corresponding cellular glycoprotein.

Specificity of GP41 Excretion

The specificity of GP41 excretion was tested in 25 patients with acute leukemias, two patients with high-grade non-Hodgkin's lymphomas, three patients with CLL, eight patients with small cell lung carcinomas (SCLC) treated with adriamycin, cyclophosphamide and vincristine and four patients with nonmalignant kidney disease [systemic lupus erythematodes (SLE) and nephrotic syndrome], and six healthy persons. The results are shown in Table 1. GP41 was observed in the urine of all patients with acute leukemias apart from one with T-ALL. GP41 was also found in the two patients with high-grade non-Hodgkin's lympho-

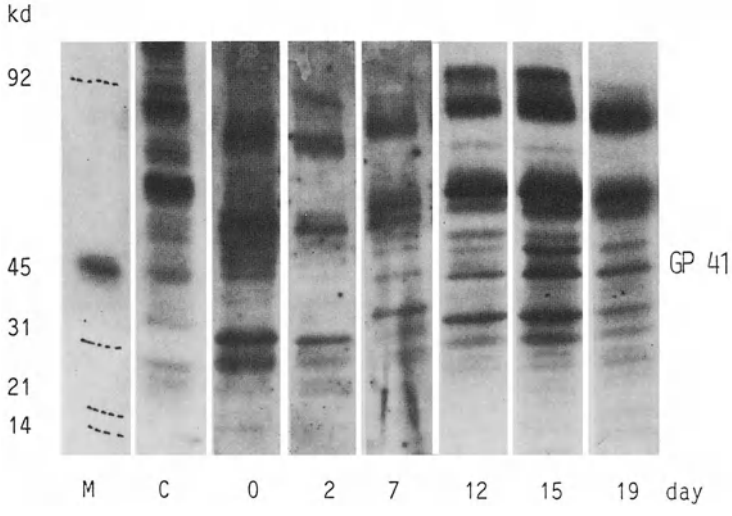

Fig. 2. Urinary glycoproteins excreted in a patient with AML before and under treatment with the TAD-9 induction regime. *M*, molecular weight markers; *kd*, kilodalton; *C*, cellular membrane glycoproteins; *0, 2, 7, etc.*, urinary glycoproteins from day 0, 2, 7, etc. of the treatment regimen

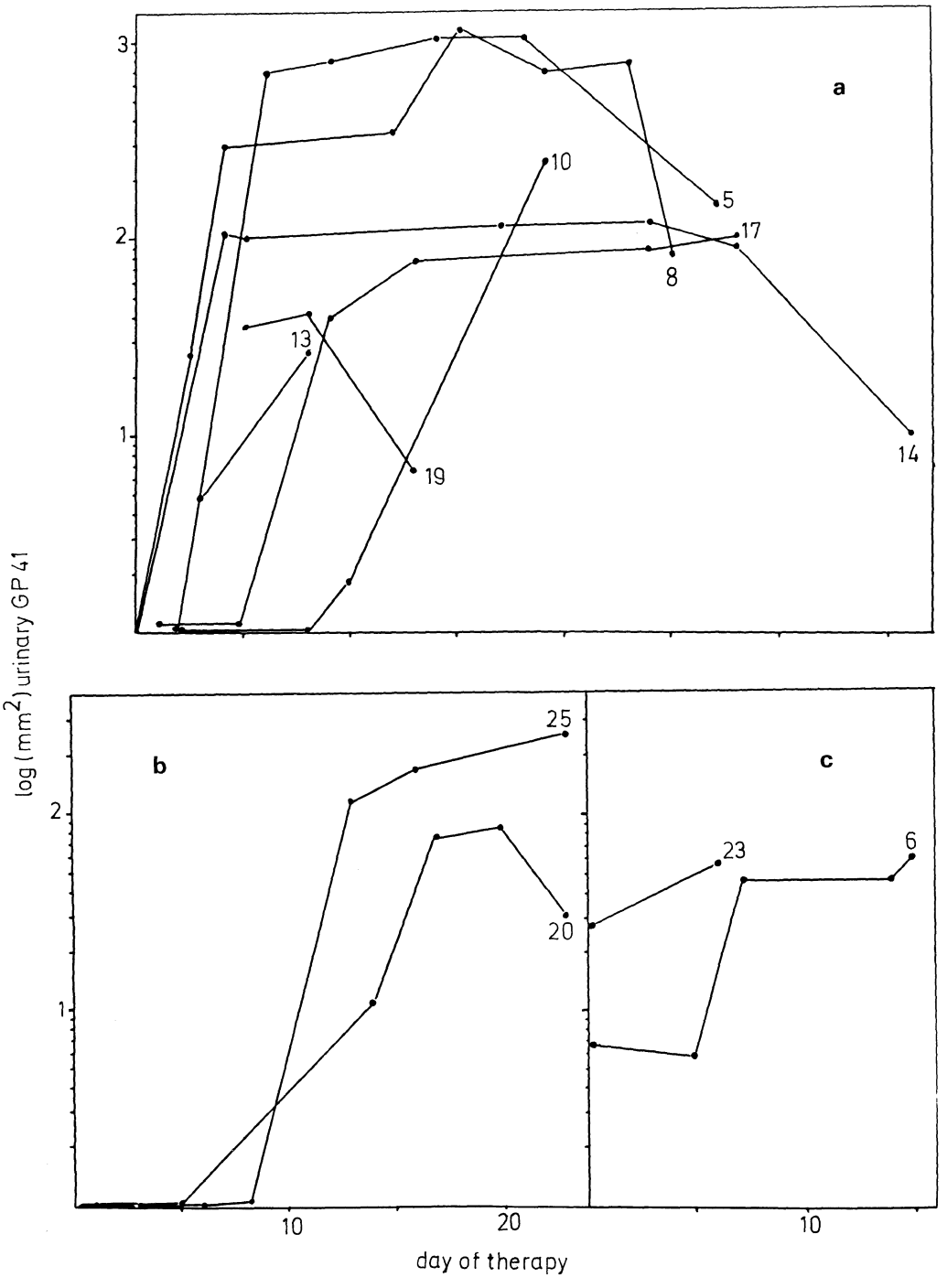

Fig. 3a–c. Kinetics of GP41 excretion determined in patients with acute leukemias by computer-assisted laser densitometry. **a** Patients with first manifestation achieving complete remission; **b** patients with first manifestation receiving partial response; **c** patients with relapsed acute leukemias achieving only a partial response *(no. 23)* or no response *(no. 6)*. For clinical characteristic of the individual patients see Table 1. The number of the patient is indicated *at the end of the kinetic curve*

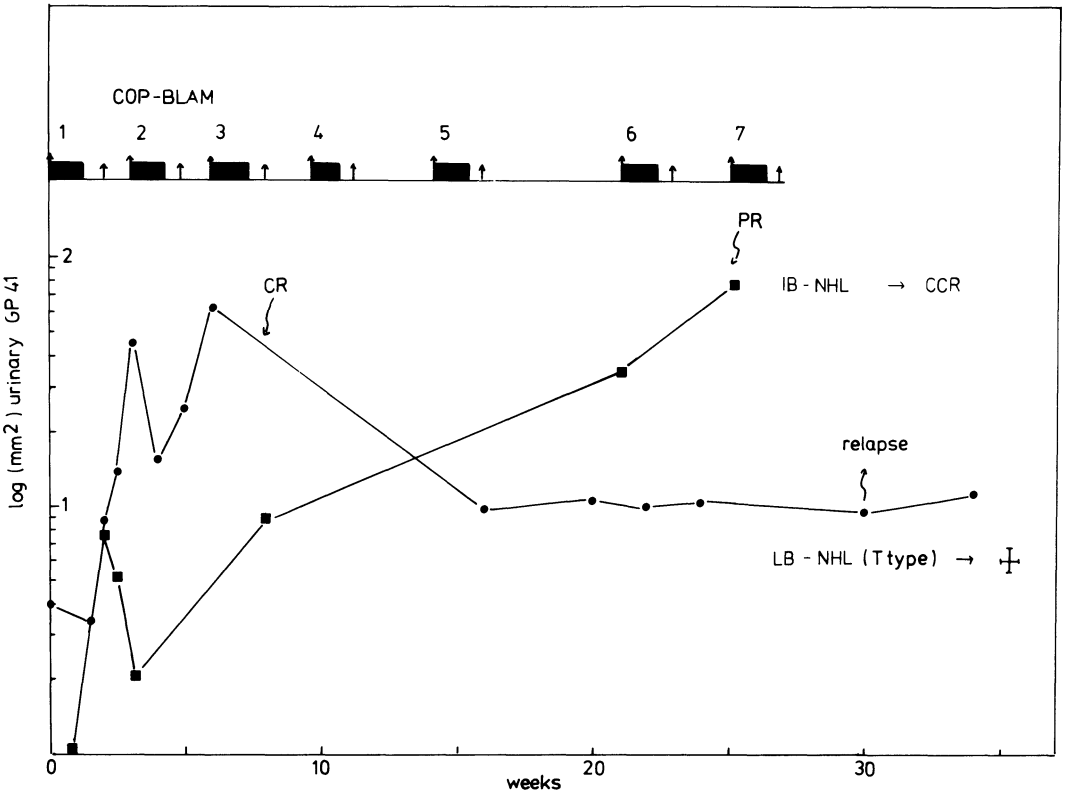

Fig. 4. Urinary GP 41 excretion in high-grade non-Hodgkin's lymphomas under long-term, multidrug polychemotherapy. GP 41 excretion was determined as described in this paper by computer-assisted laser densitometry. *IB-NHL*, immunoblastic lymphoma; *LB-NHL*, lymphoblastic lymphoma; *CCR*, continuous complete remission; *PR*, partial response

mas, but not in the urine of any of the control groups.

Relationship of GP41 Excretion to the Clinical Response

In a previous study we showed that the excretion of GP41 in the urine correlated with the reduction of the peripheral leukemic cells [7]. In the study described here it was found that the relative amount of GP41 excretion, evaluated by two independent examiners according to the score system, also correlates with the clinical response. Slight excretion (+) was found in patients who did not respond to chemotherapy, whereas excretion in responding patients was always medium (++) to strong (+++). This finding was further supported by the more objective measurement of the kinetics of GP41 excretion by computer-assisted laser densitometry. This was done in 11 patients with AL with different response and/or stages of the disease. As shown in Fig. 3, GP41 appeared to be excreted faster and to a greater extent in patients who achieved a complete remission on initial treatment (group A) than in patients who showed only a partial response (group B) or who had relapsed leukemias (group C). The excretion curves of most patients in group A (complete remission) showed a steep initial slope with a high plateau, whereas patients in groups B and C had a lower plateau and in some cases a delayed and less steep initial slope. Detectable GP41 excretion before start of treatment was only found in patients with relapsed leukemia, and might reflect the high tumor burden in these patients.

Furthermore, urine of two patients with high-grade non-Hodgkin's lymphomas was

analyzed under long-term polychemotherapy consisting of cyclophosphamide, vincristine, prednisone, bleomycin, doxorubicin, and procarbacine (COP-BLAM regime). In both patients, GP41 excretion also reflects the kinetic of tumor cell reduction (see Fig. 4). In the case of the lymphoblastic lymphoma of T-cell type, GP41 excretion increased rapidly in the first 6 weeks of treatment, and the patient was clinically in a complete remission after three cycles of COP-BLAM. Later on, however, GP41 excretion dropped to a low level, and the patient relapsed even though chemotherapy was continued. In the other case of an immunoblastic lymphoma, complete remission with a duration of 24+ months was achieved only after 10 cycles of COP-BLAM, and here GP41 excretion also follows the slow but continuous tumor cell reduction.

Conclusion

The results indicate that the urinary glycoprotein GP41 is derived from leukemic cells and is excreted in response to leukemic cell reduction. The extent of excretion appears to be related to the response to treatment. Thus, GP41 seems to be suitable for the assessment of early response in AL. At present, these results are semiquantitative and based on a small group of patients, since the method is relatively time consuming. A more sensitive assay based on monoclonal antibodies directed to GP41 and suitable for more wide-scale application is under development and will be used to study the prognostic value of GP41 excretion in more detail.

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Toxicity and Mutagenicity of 6 Anti-Cancer Drugs in Chinese Hamster Cells Co-Cultured with Rat Hepatocytes*

M. J. Phillips, M. Dickins, K. Wright, and N. K. Todd[1]

Aims

Most studies of the genetic effects of anti-cancer drugs have been concerned with their ability to cause cytogenetic damage. Gene mutations have been screened in bacteria [1–4], *Drosophila* [5, 6], and cultured mammalian cells [7, 8].

This report gives data on toxic and mutagenic effects of six anti-cancer drugs: cyclophosphamide (cyclo), adriamycin (ADR), methotrexate (MTX), cytosine arabinoside (Ara-C), 6-mercaptopurine (6MP), and vincristine (VCR) using Chinese hamster V79 cells as the target system. It has previously been shown that the use of these cells in isolation severely limits the detection of mutagens as they lack key drug-metabolising enzymes [8]. However, the co-culture of V79 cells with hepatocytes overcomes this limitation and also substantially improves the correlation of mutagenicity with carcinogenicity [9].

Methods

The V79 cells were cultured in complete medium. To ensure a low spontaneous mutation frequency (less than 1 mutant/10^5 viable cells) cells were initially cultured at a density of 1000 cells/25 cm^2 T-flask for 10 days. Cells were then trypsinised and spread in complete medium containing 6-thioguanine (TG) at a concentration of 3 μg/ml to determine the spontaneous mutation frequency, i.e. mutation recognised as cells resistant to TG. Isolated rat hepatocytes were prepared on the day of each experiment by the method described by Dickins and Peterson [10], and modified by Dickins et al. [11]. Hepatocyte viability (typically 85%–95%) was assessed by trypan blue exclusion.

The drugs under test were dissolved in Williams' E serum-free medium immediately before use, with the exception of MTX and 6MP which were pre-dissolved in 0.05 *M* NaOH. They were then added to the culture flasks and incubated at 37 °C for 24 h. Following this period of exposure, cell cultures were washed, trypsinised and resuspended in complete medium. A portion of the cell suspension was diluted to contain 250 cells and seeded on 3 cm × 6 cm plates to determine the surviving fraction immediately after treatment. The remaining cells were transferred to new flasks and grown for 6–8 days with subculturing to maintain exponential growth. At least 1.5×10^6 viable cells were transferred at each subculture. Cells were then dissociated and plated at 250 cells/6 cm dish × 3 for estimation of surviving fraction and at 10^5 cells/10 cm plate × 10 in complete medium containing TG for estimation of the mutant fraction. Plates were incubated for 7 and 10 days respectively and scored after treatment with methanol and Gurr's NRG stain. Full details of methodology are given by Dickins et al. [11].

Preliminary experiments to establish the optimal number of hepatocytes to be used

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were carried out using a known mutagen, dimethylnitrosamine (DMN), which requires metabolism prior to exertion of its biological effects. The toxic and mutagenic effects of DMN were assayed as described above and the compound was used as a positive control for the hepato-mediated system in subsequent experiments.

Results

The DMN experiments revealed that the optimal density of hepatocytes was 10^6 hepatocytes/flask for a range of DMN concentrations tested and this density was used in all experiments. Fig. 1 shows the effects of expression time on DMN induction of TG-resistant mutants in V79 cells in the presence of rat hepatocytes.

The toxicity and mutagenicity of the six drugs to V79 cells both with and without hepatocytes are shown in Fig. 2.

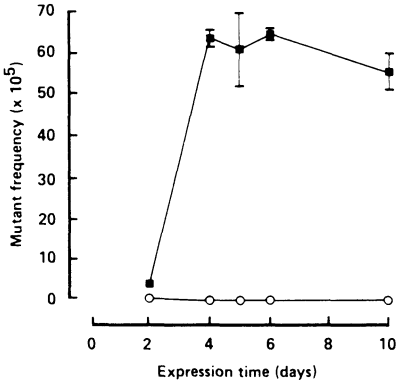

Fig. 1. Effects of expression time on DMN induction of TG-resistant mutants in V79 cells in the presence of rat hepatocytes. *Square symbols,* DMN-treated cells, *round symbols,* control cells

Cyclophosphamide (Fig. 2a)

Non-toxic and non-mutagenic in the absence of hepatocytes but there was an expo-

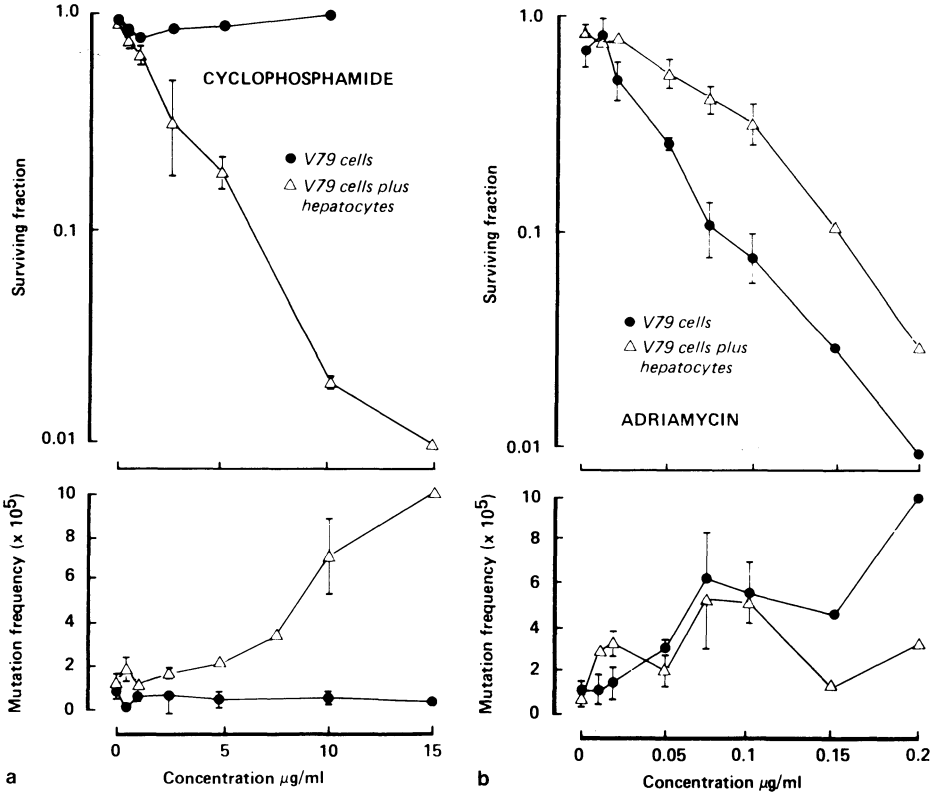

Fig. 2 a–f. Toxicity and frequency of TG-resistant mutants in V79 cells by **a** cyclo, **b** ADR (continued on p. 280)

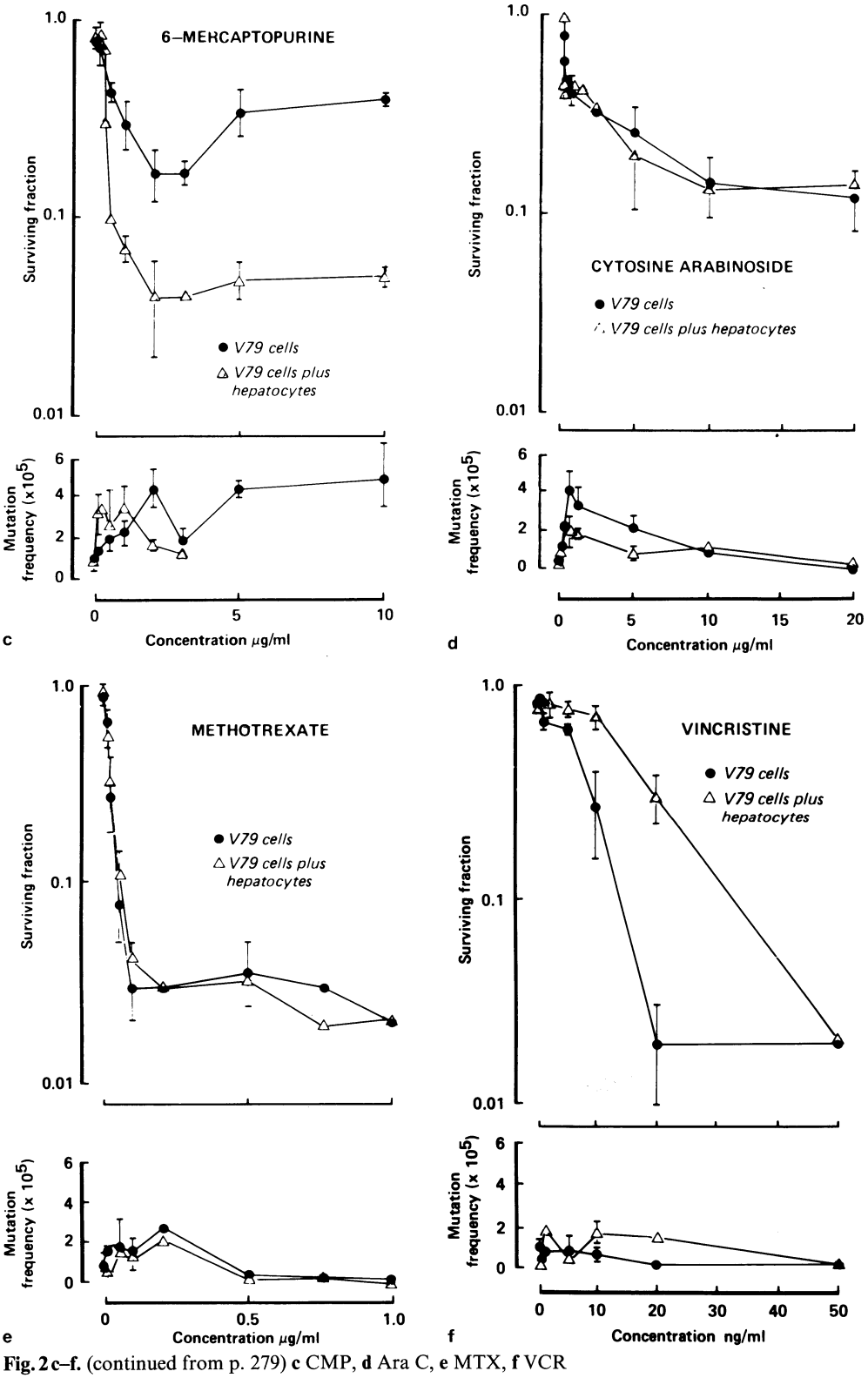

Fig. 2 c–f. (continued from p. 279) **c** CMP, **d** Ara C, **e** MTX, **f** VCR

nential toxic response in their presence. There was a clear dose-related mutagenic effect in the presence of hepatocytes.

Adriamycin (Fig. 2 b)

Toxic both in the absence and presence of hepatocytes although toxicity was significantly enhanced when liver cells were absent. A mutagenic effect was found under both experimental conditions, with a dose response more apparent in the absence of hepatocytes.

6-Mercaptopurine (Fig. 2 c)

Toxic under both experimental conditions, more pronounced in the presence of hepatocytes. An increased mutagenic response above spontaneous was observed with V79 cells alone but in the presence of hepatocytes only lower concentrations showed an increase in mutation frequency.

Cytosine arabinoside (Fig. 2 d)

Toxic with or without the presence of hepatocytes and to the same degree. In both cases the toxicity curve plateaued at higher concentrations. In the absence of hepatocytes a weak mutagenic response was present up to a concentration of 1 μg/ml but with hepatocytes no effect was apparent.

Methotrexate (Fig. 2 e)

Toxic and here also the presence of hepatocytes made no difference to the response. No mutagenic effects were found.

Vincristine (Fig. 2 f)

Less toxic in the presence of hepatocytes and no mutagenicity was shown.

Conclusions

The results indicate that this is a viable and sensitive test of the mutagenic potential of anti-cancer drugs. The co-culture of mammalian cells with hepatocytes plainly allows both the necessary metabolism of certain drugs to produce their cytotoxic metabolites, e.g. cyclo, or the detoxification of others, e.g. ADR. The toxicity studies may provide valuable indications of optimal dose selection of the drugs tested.

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Pharmacokinetics of Daunorubicin as a Determinant of Response in Acute Myeloid Leukemia*

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Abstract

Twenty-one adult patients with acute myeloid leukemia (AML) were treated with the EORTC LAM-6 remission induction protocol [daunorubicin (DNR) (45 mg/m^2, days 1–3), cytarabine (200 mg/m^2, days 1–7) and vincristine (1 mg/m^2, day 2)]. Pharmacokinetics of DNR were studied at day 1. The concentration of DNR and daunorubicinol were determined in plasma, in white blood cells and in bone marrow. A large variability was observed with respect to (1) the plasma area under the curve (AUC) 0–24 h (range: 0.06–0.37 nmol × h/ml); (2) the white cell AUC 0–24 h (range: 0–441 nmol × h/10^9 cells); and (3) the 1 h bone marrow concentration (range: 0–27 nmol/10^9 cells). In eight patients treated twice, a small intraindividual variability of these parameters was observed. Concentrations in plasma did not correlate with cellular concentrations. All pharmacokinetic parameters in plasma and white cells did not correlate with response to therapy. In patients reaching complete remission (CR), however, the tumor load, as expressed by the number of blast cells present in the untreated bone marrow, was significantly lower than the number of blast cells in patients not reaching CR.

* This work was supported by a grant from The Netherlands Cancer Foundation "Koningin Wilhelmina Fonds".
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Introduction

The anthracyclines daunorubicin (DNR) and doxorubicine (DOX) are effective drugs in the treatment of acute myelocytic leukemia (AML). DNR is administered for remission induction (RI) as well as for consolidation therapy. The clinical response of AML patients treated with DNR-containing chemotherapy shows considerable variation. In spite of a high complete remission (CR) rate (60%–70%), a significant proportion of cases remains primarily refractory [1]. Among the patients who attain CR the relapse rate is high due to incomplete eradication of the leukemic cells. The duration of the remission may vary from 2 months to several years. Efforts to elucidate the role of the pharmacokinetics of DNR and DOX in establishing their therapeutic effect have been unsuccessful. Monitoring of DNR and DOX plasma levels in AML patients during RI yielded little information to add to our understanding of the reasons for interindividual variation of the initial response and eventual relapse [2]. However, it is most likely that DNR exerts its major effect through intercalation into DNA [3] and the drug is tightly bound in tissues for hours or even days. Therefore, the determination of DNR concentrations in leukemic cells rather than in plasma might be a better parameter when it comes to establishing a relationship between drug pharmacokinetics and the clinical response to therapy. The main goal of the study presented here was to determine the in vivo uptake and retention of DNR in leukemic cells from blood and bone marrow of AML patients during RI treatment, and

to assess whether these are related to the plasma distribution kinetics of this drug. In addition, we investigated whether the in vivo uptake and retention of DNR in leukemic cells had a predictive value regarding the clinical outcome of therapy.

Materials and Methods

Twenty-one patients with previously untreated AML participated in this study. The criteria of the French-American-British working party (FAB classification) were used to diagnose AML [4]. The patients were treated according to the EORTC LAM-6 protocol. RI chemotherapy contained DNR (45 mg/m^2 body surface on days 1–3 by i.v. bolus), cytosine arabinoside (Ara-C) (200 mg/m^2 i.v. on days 1–7) and vincristine (1 mg/m^2 i.v. on day 2). Concentrations of DNR attained in vivo in plasma and circulating nucleated cells were determined up to 24 h after the first course of DNR therapy. Bone marrow aspirates were obtained at 1 h and 24 h after the start of DNR therapy. The nucleated cells from the samples were isolated as described previously [5]. The DNR content in plasma and in cells was determined using high-performance liquid chromatography [6]. The criteria for evaluating response were those established by the Cancer and Acute Leukemia Group B [7]; failures of the induction treatment were characterized according to Preisler et al. [8]. The distribution and elimination kinetics of DNR were analyzed by fitting a two-compartment model to the observed plasma and intracellular (blood nucleated cells) DNR concentrations [5, 9]. The DNR area under the concentration-time curves (AUC) in plasma and blood nucleated cells, the volume of the central (plasma) compartment (V_1), the apparent distribution volume (Vd area), and the total body clearance were calculated from this model.

Results

Twenty-one previously untreated evaluable patients with AML (sex: 18 m and 3 f; age:

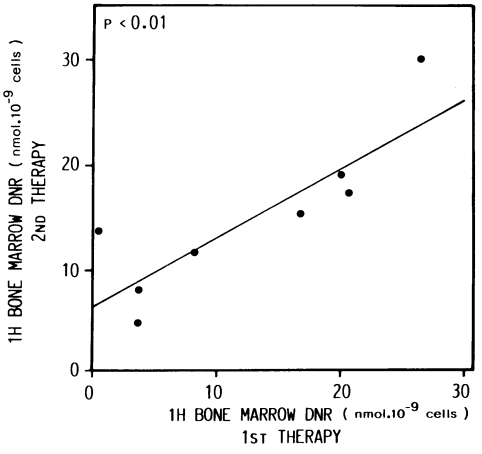

Fig. 1. Relationship between the 1 h bone marrow daunorubicin levels during two successive courses of daunorubicin-containing chemotherapy

Table 1. Plasma pharmacokinetic parameters of daunorubicin in 21 AML patients after i.v. bolus injection at $t=0$ h

		Mean value	Range
Dose	(mg)	81.43	(60 – 100)
$t_{1/2\alpha}$	(h)	0.09	(0.01– 0.30)
$t_{1/2\beta}$	(h)	1.48	(0.11–11.94)
V_1[a]	(liters)	190	(10 – 600)
V_d area[b]	(liters)	570	(0.20– 1640)
Clearance	(liters/h)	590	(90 – 2770)
Plasma AUC[c] 0–4 h	(nmol · h/ml)	0.38	(0.06– 0.78)
Plasma AUC[c] 0–24 h	(nmol · h/ml)	0.43	(0.06– 1.08)
Plasma (DNR) 4 h	(nmol/ml)	0.03	(0 – 0.08)

[a] Volume of the central (plasma) compartment.
[b] Apparent distribution volume.
[c] Area under the concentration-time curve.

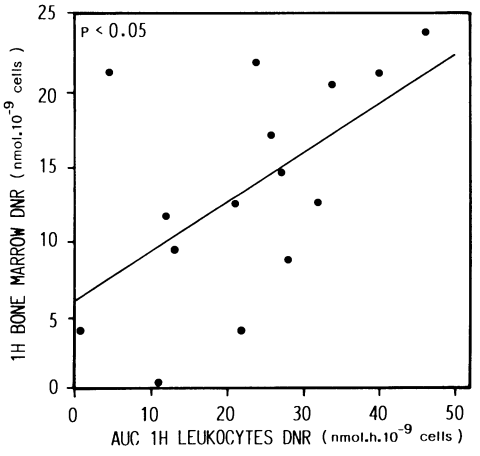

Fig. 2. Relationship between the daunorubicin content of peripheral leukocytes and bone marrow nucleated cells in acute myelocytic leukemia

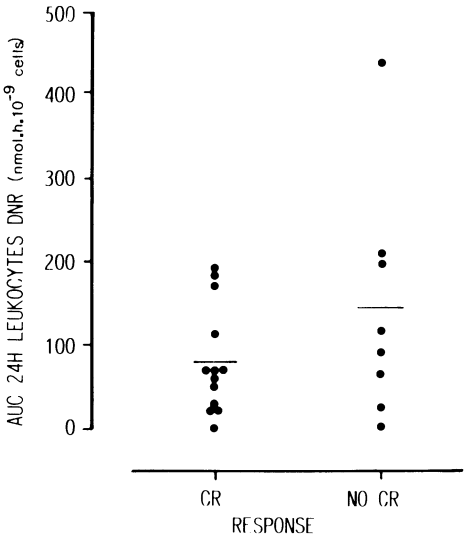

Fig. 3. Relelvance of the daunorubicin levels (AUC 0–24 h) in peripheral leukocytes for the outcome of remission induction therapy

mean 48 years, range 17–74 years; FAB classification: 5 M_1, 6 M_2, 8 M_4, 2 M_5) were studied during the first RI therapy and eight of these patients were studied during two successive courses of RI therapy. The outcome of therapy was determined after one or two courses of RI therapy: 13/21 (62%) complete remission (CR) and 8/21 (38%) no CR [six (29%) partial remission (PR); two (9%) failures type 2].

Table 1 shows the calculated plasma pharmacokinetic parameters of DNR of the patients. A wide range can be observed with respect to all plasma parameters studied. In addition, a large variability was observed between patients with respect to: (1) the amount of DNR present in nucleated blood cells (AUC 0–24 h range: 0–441 nmol × h/10^9 cells); and (2) the bone marrow concentration of DNR at 1 h and at 24 h after DNR administration (range: 0–24 nmol/10^9 nucleated bone marrow cells and 0–14 nmol/10^9 nucleated bone marrow cells, respectively). In contrast to the high interindividual variability of the DNR pharmacokinetic parameters, the intraindividual variability of these parameters was small in

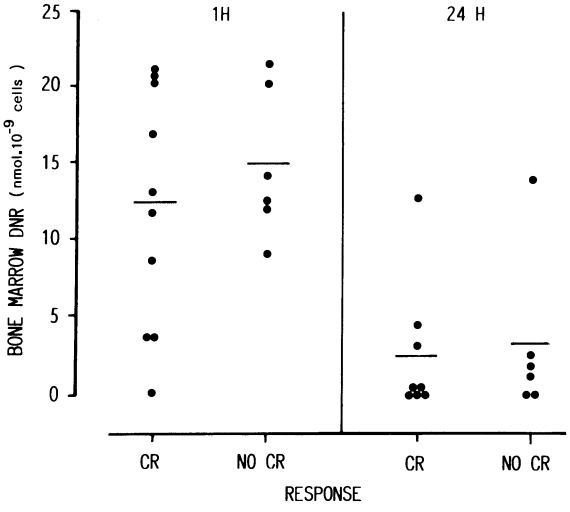

Fig. 4. Relevance of the daunorubicin levels (1 h and 24 h) in bone marrow nucleated cells for the outcome of remission induction therapy

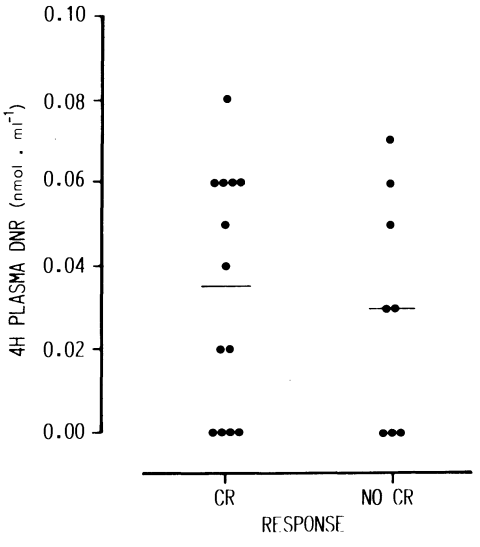

Fig. 5. Relevance of the 4-h plasma daunorubicin levels on day 1 of therapy for the outcome of remission induction therapy

those eight patients who were studied twice. Also, the content of DNR in bone marrow nucleated cells was comparable during two successive courses of DNR-containing chemotherapy (Fig. 1).

DNR plasma concentrations did not correlate with intracellular DNR concentrations of nucleated cells in blood and bone marrow (data not shown), suggesting that no rapid equilibrium exists between plasma and cells. Therefore, these cells should be regarded as separate compartments. The uptake of DNR in peripheral nucleated cells showed a significant positive relation with the 1-h DNR concentration in bone marrow nucleated cells ($P<0.05$) (Fig. 2). However, neither the amount of DNR present in peripheral nucleated cells nor in bone marrow nucleated cells was predictive for the clinical response to therapy (Fig. 3 and 4). In addition, none of the plasma pharmacokinetic parameters studied correlated with treatment outcome as is shown, for example, by the lack of relevance of the 4-h plasma DNR levels on day 1 of therapy for the outcome of RI therapy (Fig. 5).

In patients reaching CR, however, the tumor load, as expressed by the number of blast cells present in the untreated bone marrow, was significantly lower ($P<0.05$) (Wilcoxon test) than the number of blast cells in patients not reaching CR. The number of nucleated cells present at the start of therapy was not different (Fig. 6).

Discussion

To gain a better understanding of the large variation in individual response to DNR-containing chemotherapy in AML patients, individual monitoring of DNR levels of leukemic cells is of interest. In the group of 21

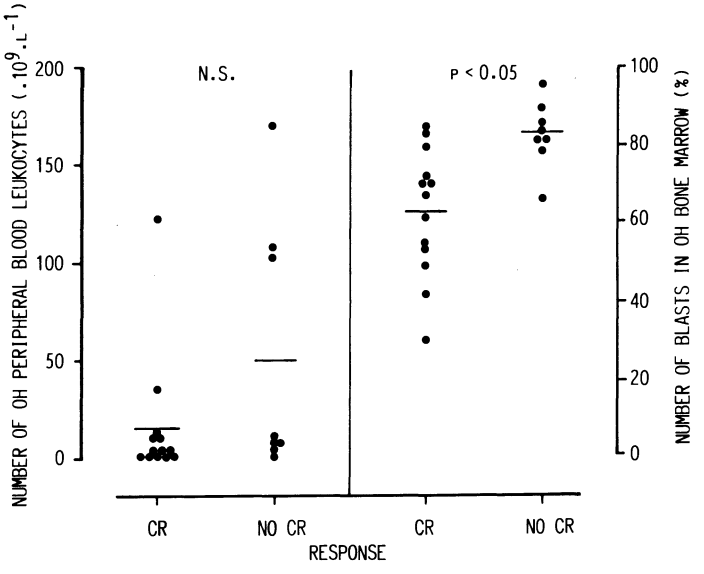

Fig. 6. Relevance of tumor load before therapy [expressed as the number of peripheral leukocytes ($\times 10^9$/liter) or the number of blasts in the bone marrow (%)] for the outcome of remission induction therapy

evaluable AML patients investigated during their first RI course of therapy, it was found that all the pharmacokinetic parameters of DNR studied showed a large interindividual variability; in the eight patients studied twice during two successive courses of therapy, only small intraindividual variability of these parameters was observed.

No correlation could be found between the plasma DNR levels and the intracellular DNR levels of nucleated cells in blood and bone marrow, suggesting that these cells should be regarded as separate compartments. These findings are in agreement with the results of DeGregorio et al. [10], who indicated that plasma concentrations of DNR and Daunomycinol (DOL) are not useful in estimating the (in vitro) inhibition of DNA synthesis. The lack of relevance of the 4-h plasma DNR levels at day 1 of therapy for the outcome of RI therapy is consistent with conclusions of Preisler et al. [2], who showed that the range of values of the 3-h plasma DOX and doxorubicinol levels at day 1 of therapy were similar for CR and resistant disease patients. However, Preisler also found that high 3-h plasma DOX levels were associated with both death during RI therapy and long remissions for those who survived induction therapy and entered remission. Prediction of therapeutic effects on the basis of plasma pharmacokinetics presupposes that changes in plasma drug levels reflect changes within target tissues. However, the neoplastic process is heterogeneous with respect to, for example, differentiation, metabolic activity, clonogenic potential, and degree of vascularity. This heterogeneity may complicate the correlation of plasma drug concentration with the therapeutic effect. It may well be that plasma pharmacokinetic analysis will be useful in predicting toxicity, since the response of normal tissue to given drug concentrations shows less variability. The intracellular DNR concentrations of blood nucleated cells and bone marrow nucleated cells are closely correlated. It is possible that these cells represent rapid exchangeable populations and hence exhibit similar uptake and retention of DNR.

Poor treatment results (no CR) are not associated with low concentrations of DNR in nucleated cells in blood or in bone marrow, indicating that no simple relation could be found between the DNR uptake of nucleated cells in vivo and the clinical response to therapy. The pretherapy bone marrows of patients who would enter CR contained less leukemic blast cells than patients who would fail to enter remission because of persistant leukemia. It remains to be investigated whether the DNR uptake specifically in leukemic bone marrow blast cells will offer better information about the predictive value of drug concentrations with respect to the clinical outcome of therapy.

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Pharmacokinetic Study of Cytosine Arabinoside in Patients with Acute Myelogeneous Leukemia*

P. Preusser[1], H. J. Pielken[1], and H.-J. Bauch[2]

Introduction

The aim of the study was to evaluate the pharmacokinetics of cytosine arabinoside (Ara-C) and its extracellular metabolite uracil arabinoside Ara-U in patients with acute myelogeneous leukemia (AML) in correlation to response in therapy. There are few data [4] that indicate that high and long-lasting Ara-U plasma levels increase the incorportion of Ara-C into DNA and decrease the catabolism of Ara-C, and might also play a role in getting a response in therapy. We therefore investigated the pharmacokinetics of Ara-C and Ara-U in plasma of 17 patients with AML.

Methods and Patients

We developed a rapid, specific and sensitive high-pressure liquid chromatography (HPLC) method for measuring Ara-C and Ara-U in patients' plasma because the published methods [2, 3, 6, 7] indicated low sensitivity and/or a short life span of the analytical column.

Conditions

Reversed-phase C-18 column (3 µm, 125 mm × 4.6 mm), 0.05 *M* phosphate buffer pH 7/MeOH 98/2 (v/v), flow rate 1 ml/min, UV detection 254 nm, 0.01 AUFS, external quantification.

Specifications

Detection limit 20 ng/ml for Ara-C and Ara-U, recovery >95%, linearity from 20 ng/ml to 40000 ng/ml, variation coefficient <5%, analysis number 700–1000/column, analysis speed including sample preparation 30 min.

Figure 1 shows standard and patients' samples. Blood samples (2 ml) were collected at defined time intervals in tubes containing tetrahydrouridine (blood deaminase inhibitor). After centrifugation 10 µl of the ultrafiltrate was analysed by HPLC.

Ten patients with AML in first-line therapy with TAD (Ara-C infusion 100 mg/m^2 for 48 h, thioguanine, daunorubicin) and seven patients in second-line therapy with high-dose Ara-C, mitoxantrone (HAM) (Ara-C 3 g/m^2 every 12 h for 4 days as 3 h infusion, mitoxantrone) were investigated. The characteristics of patients receiving TAD are listed in Table 1 and of those receiving HAM, in Table 2.

Results

The areas under curve (AUC) for TAD patients are listed in Table 3 and for HAM patients in Table 4.

TAD Patients

Three out of four responders show lower Ara-C plasma levels on day 2 than on day 1 of therapy, four out of five nonresponders

* Supported by the *Deutsche Forschungsgemeinschaft* (PR 220/1-1).
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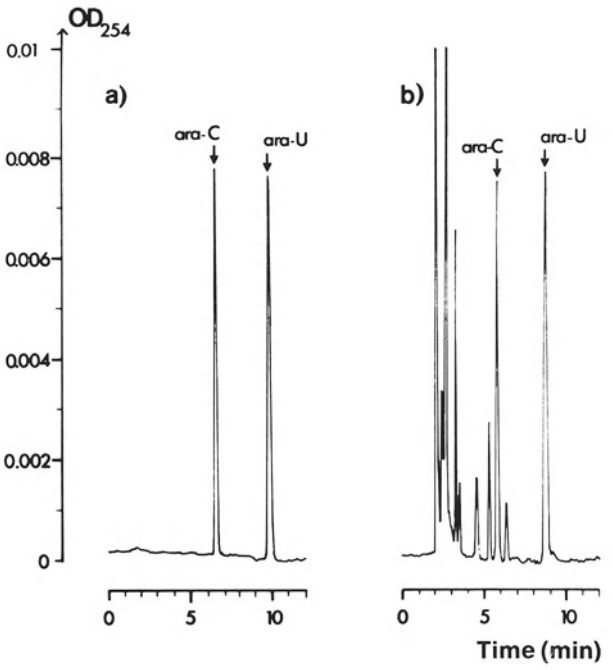

Fig. 1. a Standard, **b** patients' sample before therapy

Table 1. Characteristics of patients in TAD therapy

Patient no.	Sex	Age (years)	Leukocytes (per mm^3)	Bone marrow blasts (%)	Peripheral blasts (%)	Response
1	m	30	37800	90	29	CR
2	m	44	4300	70	66	CR
3	m	46	1400	70	0	CR
4	f	50	17600	98	93	CR
5	f	20	6500	95	85	NR
6	f	33	25600	85	85	NR
7	f	46	1400	70	21	NR
8	f	57	116000	98	97	NR
9	f	61	61000	80	40	NR
10	m	69	45000	80	60	NR

FAB classification: 4 patients M_1, 3 patients M_5, 2 patients M_2, 1 patient M_4.
Response criteria: complete response/no response (CR/NR).

Table 2. Characteristics of patients in HAM therapy

Patient no.	Sex	Age (years)	Leukocytes (per mm^3)	Bone marrow blasts (%)	Peripheral blasts (%)	Response
1	m	26	3800	18	0	CR
2	f	30	2500	70	6	CR
3	m	41	20000	90	70	CR
4	m	43	133600	98	84	CR
5	m	45	2400	80	40	CR
6	f	20	255000	70	94	NR
7	f	39	8300	20	0	NR

FAB classification: 3 patients M_4, 2 patients M_5, 1 patient M_1, 1 patient M_3.
Response criteria: complete response/no response (CR/NR).

Table 3. AUC (μg/ml × h) for TAD patients

Patient no.	Arc-C		Arc-U		Response
	day 1	day 2	day 1	day 2	
1	7.18	6.58	9.09	15.03	CR
2	15.61	13.77	4.56	8.59	CR
3	7.51	12.66	3.42	4.23	CR
4	3.93	2.89	4.42	9.64	CR
5	10.32	11.52	3.95	3.73	NR
6	–	–	4.42	9.65	NR
7	6.9	3.3	2.99	8.39	NR
8	10.35	6.24	6.24	7.32	NR
9	7.2	13.04	5.08	11.02	NR
10	2.95	9.67	6.61	14.88	NR

Response criteria: complete response/no response (CR/NR).

Table 4. AUC (μg/ml × h) for HAM patients

Patient no.	Arc-C		Arc-U		Response
	day 1	day 2	day 1	day 2	
1	110.25	105.1	311.5	737.25	CR
2	2186.75	1336.55	762.0	773.05	CR
3	330.75	1942.45	472.55	641.55	CR
4	1242.3	785.3	502.15	648.7	CR
5	1192.6	2166.1	533.05	693.74	CR
6	5783.98	3867.8	1033.00	782.15	NR
7	1113.85	2220.55	806.1	732.55	NR

Response criteria: complete response/no response (CR/NR).

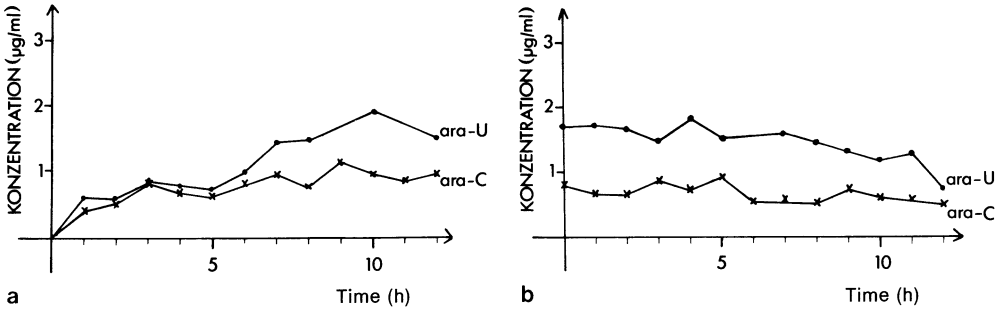

Fig. 2 a, b. Kinetic curves of responders to TAD therapy, **a** day 1, **b** day 2

show higher Ara-C plasma levels on day 2 than on day 1 of therapy.

HAM Patients

Both nonresponders show higher Ara-C plasma levels than all five responders on day 2 of therapy. All five responders show an increase in Ara-U plasma levels from day 1 to day 2 of therapy and a higher plateau, both nonresponders show a decrease in Ara-U.

Kinetic curves for TAD responders are shown in Fig. 2 and for TAD nonresponders in Fig. 3. Kinetic curves for HAM re-

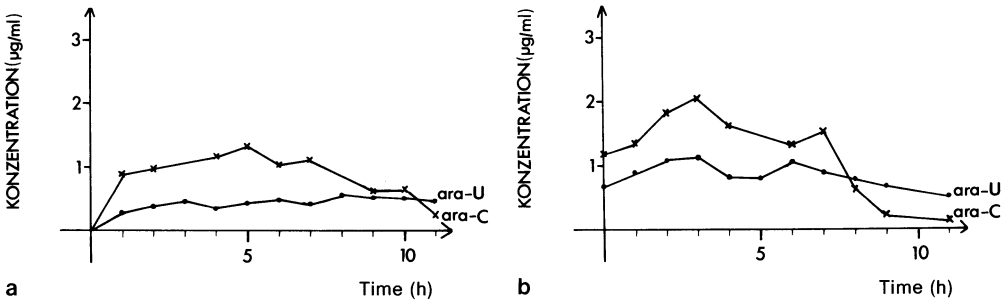

Fig. 3 a, b. Kinetic curves of nonresponders to TAD therapy, **a** day 1, **b** day 2

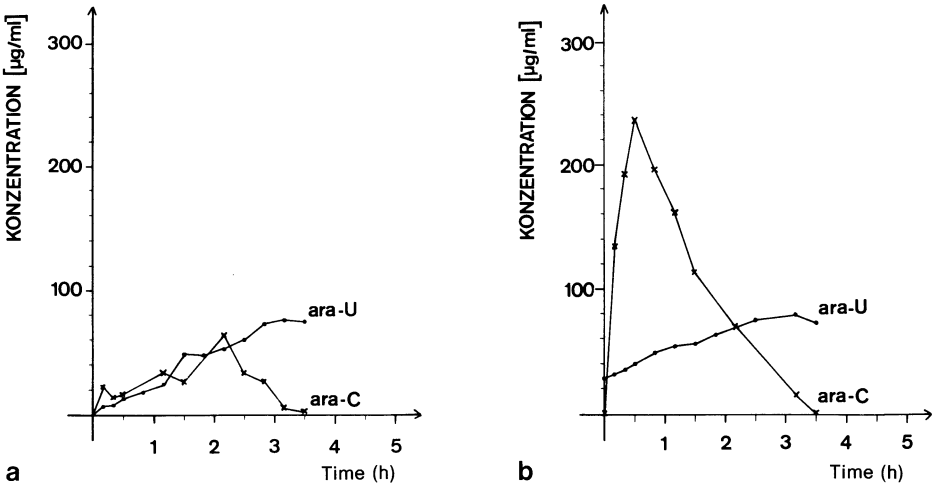

Fig. 4 a, b. Kinetic curves of responders to HAM therapy, **a** day 1, **b** day 2

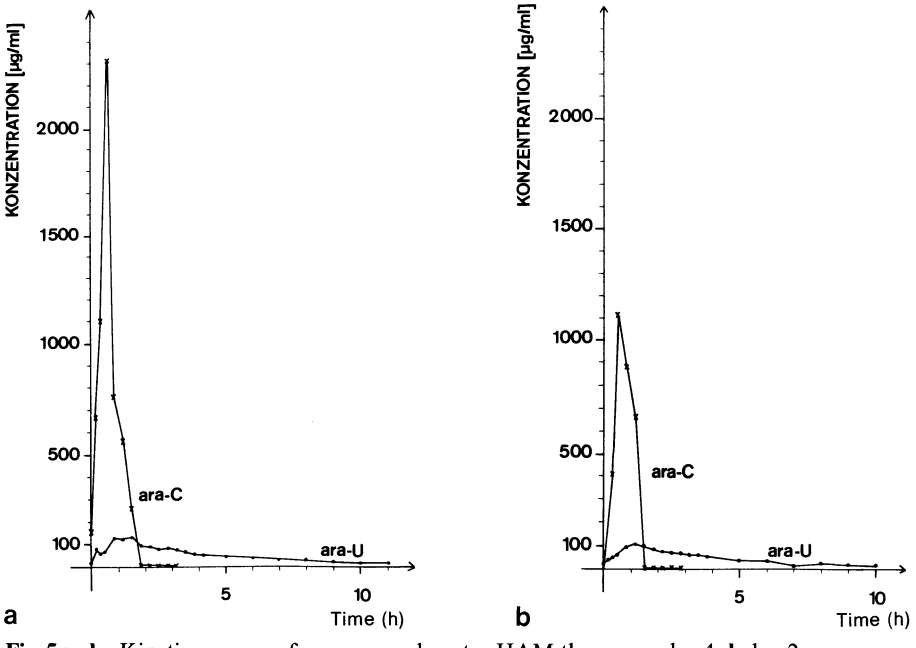

Fig. 5 a, b. Kinetic curves of nonresponders to HAM therapy. **a** day 1, **b** day 2

sponders are shown in Fig. 4 and for HAM nonresponders in Fig. 5.

Discussion

Three out of four TAD responders and all five HAM responders show lower Ara-C plasma levels on day 2 of therapy in contrast to four out of five TAD nonresponders and both HAM nonresponders.

All five HAM responders show an increase in Ara-U plasma levels from day 1 to day 2 of therapy and a higher plateau in contrast to both HAM nonresponders.

Ara-CTP is incorporated into DNA [1, 3, 8, 10] and Ara-CTP formation and retention in blasts is correlated with reponse [5, 9]. Because of the lower Ara-C plasma levels of TAD and HAM responders on day 2 of therapy, the increase in Ara-U plasma levels in HAM responders from day 1 to day 2 of therapy, and the higher plateau of Ara-U, it might be postulated that there is a higher influx of Ara-C into blasts of responders and a higher rate of Ara-CTP formation. Therefore, the study is being continued with a greater number of patients and with the aim of doing statistical evaluations to see if the above results are significant for the group of patients suffering from AML in our hospital. This continued study will also evaluate Ara-CTP pharmacokinetics in peripheral and bone marrow blasts to determine a possible correlation with response in therapy.

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Pharmacokinetics of Oral Methotrexate in Bone Marrow During Maintenance Treatment of Childhood Acute Lymphocytic Leukemia

P. Sonneveld[1], K. Nooter, F. Schultz, and K. Hählen[2]

Introduction

The efficacy of methotrexate (MTX) in maintenance therapy for childhood acute lymphocytic leukemia (ALL) is well established. Unpredictable serum concentrations of MTX have been observed in several studies [1–6].

Following intermediate-dose MTX added to conventional therapy, its rapid systemic clearance is associated with a higher probability of relapse [1]. It is conceivable that hematologic relapse may be the result of fast systemic clearance and low bone marrow concentrations of MTX. Because of the variability in clearance, single measurements of MTX may not be used to estimate the probability of hematologic relapse.

Therefore, we studied the plasma pharmacokinetics together with the bone marrow concentrations of MTX during successive courses of maintenance therapy.

Patients and Methods

Eighteen patients (ten male, eight female) with ALL in complete remission (CR) were included in the study. Their ages were 3–15 years. They were treated according to the protocol of the Dutch Childhood Leukemia Study Group. In this regimen, MTX (30 mg/m^2) is given p.o. weekly for 120 weeks. No additional treatment was given for at least 7 days preceding pharmacokinetic studies, with the exception of daily trimethoprim in ten patients. The pharmacokinetic studies were performed under clinical observation at weeks 9, 30, 58, 86, and 114 after start of treatment. All children received MTX (30 mg/m^2) as 2.5 mg tablets after an overnight fast. Blood samples were obtained at −5, 30, 60, 90, 120, 150, and 180 min and at 4, 5, 6, and 24 h after MTX administration. A bone marrow aspirate was obtained by crista puncture at 24 h and stored at −20 °C. Urine was collected during 24 h following p.o. MTX.

All blood, bone marrow, and urine samples were analyzed with high-pressure liquid chromatography. This assay is sensitive to MTX concentrations of 2×10^{-8} *M* and separates MTX from its major metabolites 7-hydroxymethotrexate and 4-amino 4-deoxy N_{10} methyl pteroid acid (DAMPA). Also, the assay is capable of separating and detecting the MTX polyglutamates MTX-PG_1, MTX-PG_2, and MTX-PG_3 [7].

The MTX absorption curve was calculated using a two-compartment model described by

$$C(t) = A \cdot E^{-\alpha t} + B \cdot e^{\beta t} + F \cdot E^{-k\alpha t}, \quad (1)$$

where $C(t)$ is the plasma concentration at time t; $k\alpha$ is the elimination rate constant and F is the fraction of the dose absorbed. The plasma area under the curve was also calculated with this model.

Results

With the standard dosage of 30 mg/m^2 the peak concentration of MTX in plasma

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showed a considerable variation between patients. A mean peak level of 1.51 ± 0.07 μM (mean $\pm$ SE of 56 curves) was observed with a range of 0.41 μM to 2.77 μM (Fig. 1). The time of maximum MTX concentration in plasma varied from 60 to 180 min with a mean of 98 min. No double peaks were observed (Fig. 1). The area under the plasma MTX curve (AUC) during 24 following p.o. administration varied from 65.7 to 565.0 $\mu M \cdot min^{-1}$ (mean $\pm$ SE: 330.1 ± 56.4 $\mu M \cdot min^{-1}$) (Fig. 1).

The AUC of MTX in plasma during the initial 6 following administration varied from 63.7 $\mu M \cdot min^{-1}$ to 481.8 $\mu M \cdot min^{-1}$ (mean $\pm$ SE: 268.2 ± 12.3 $\mu M \cdot min^{-1}$). Fast absorbers, who had a peak concentration of MTX within 90 min or less, did not reach higher peak concentrations than did slow absorbers (mean $\pm$ SE: 148.1 ± 9.7 vs. 156.8 ± 10.1). Also the plasma AUC of MTX did not differ between fast and slow absorbers. However, the plasma AUC of MTX clearly showed a correlation with the plasma peak concentration (Fig. 2).

At 24 h after p.o. MTX, concentrations in plasma were below 10^{-8} M (7/56) or undetectable (49/56). Concentrations of 7-hydroxymethotrexate exceeded those of MTX at time points beyond 2 h in all patients.

Concentrations of DAMPA were negligible. The postabsorbance plasma levels of MTX were best fitted to a first order decay curve. The goodness of fit (r^2) was greater than 0.95 in all cases. The plasma clearance of MTX varied from 33 ml/min to 592 ml/min with a mean of 193 ± 14.1 ml/min (mean $\pm$ SE). Urinary elimination of MTX was determined following 37/56 p.o. administrations. During 24 h following p.o. MTX, $45.7\% \pm 4.2\%$ of dose (mean $\pm$ SE) was excreted. The renal elimination of MTX was proportional to the plasma AUC.

A large interindividual and intraindividual heterogeneity of bone marrow levels of MTX was observed in this group of patients.

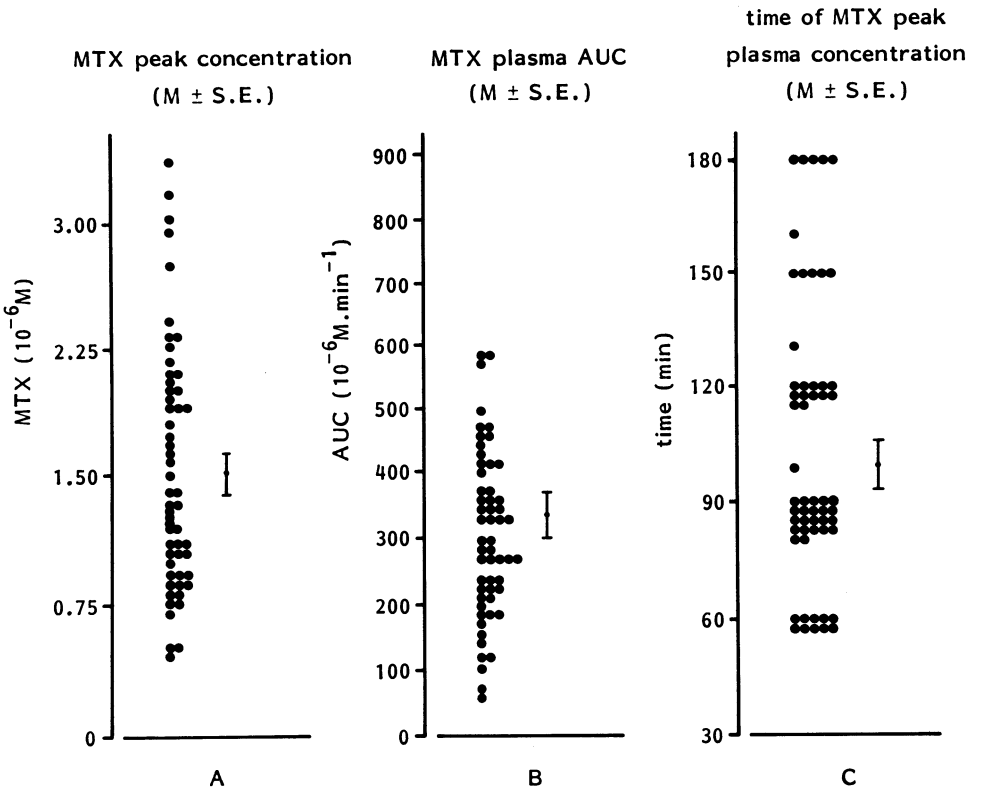

Fig. 1. Plasma peak concentration (*A*), plasma area under the curve (*B*) and time of plasma peak concentration (*C*) of methotrexate following p.o. administration of 30 mg/m^2 (mean $\pm$ SEM)

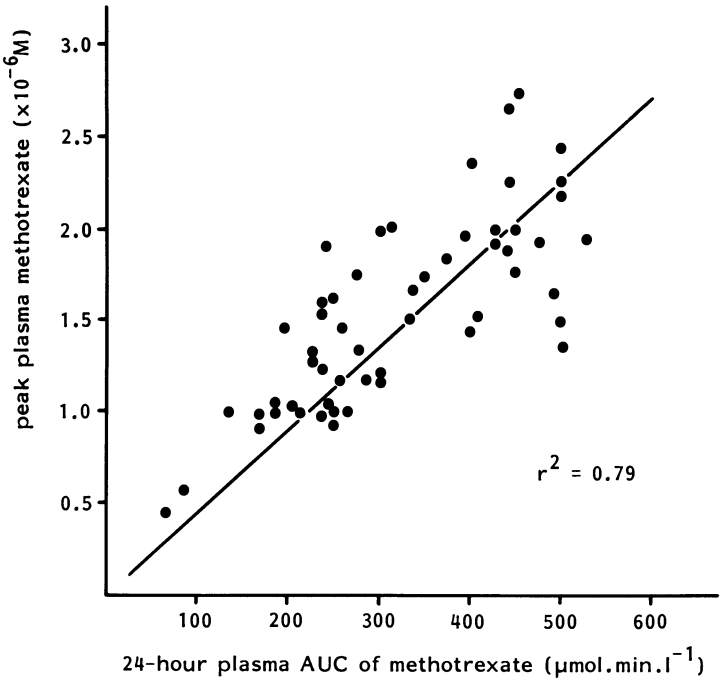

Fig. 2. Scatter diagram of 24-h plasma AUC. There is a significant correlation with the peak plasma concentration of MTX ($r^2 = 0.79$)

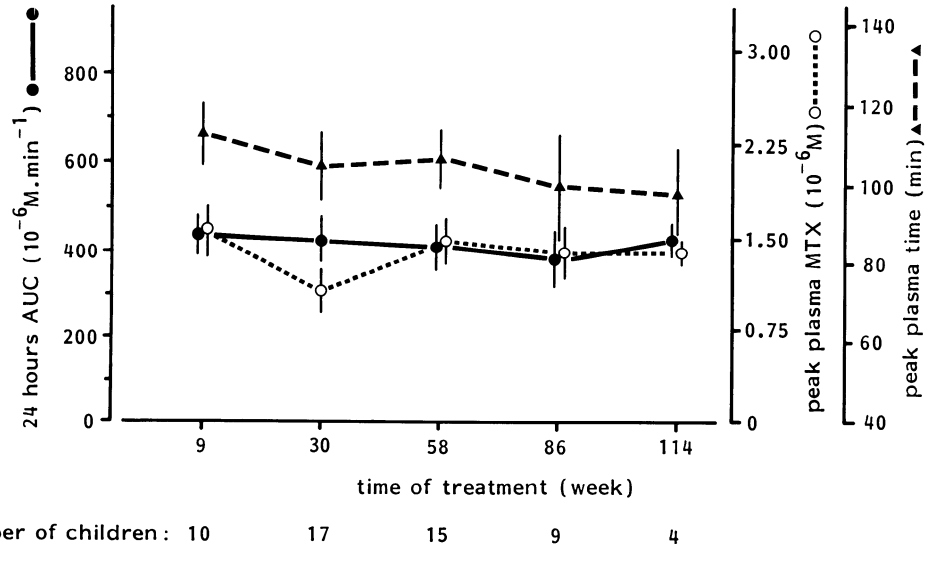

Fig. 3. Plasma AUC, plasma peak concentration and time of plasma peak concentration of MTX during prolonged weekly p.o. treatment (mean ± SEM)

At 24 h the drug concentration in bone marrow varied from 5-706.10^{-9} *M* (mean ± SE: 226.0 ± 31.1 × 10^{-9} *M*). A large fraction of the drug in bone marrow consisted of 7-hydroxymethotrexate (mean ± SE: 87% ± 13%), while a small fraction was MTX (mean ± SE: 13% ± 6%). Polyglutamates of MTX (PG_1, PG_2, PG_3) could not be identi-

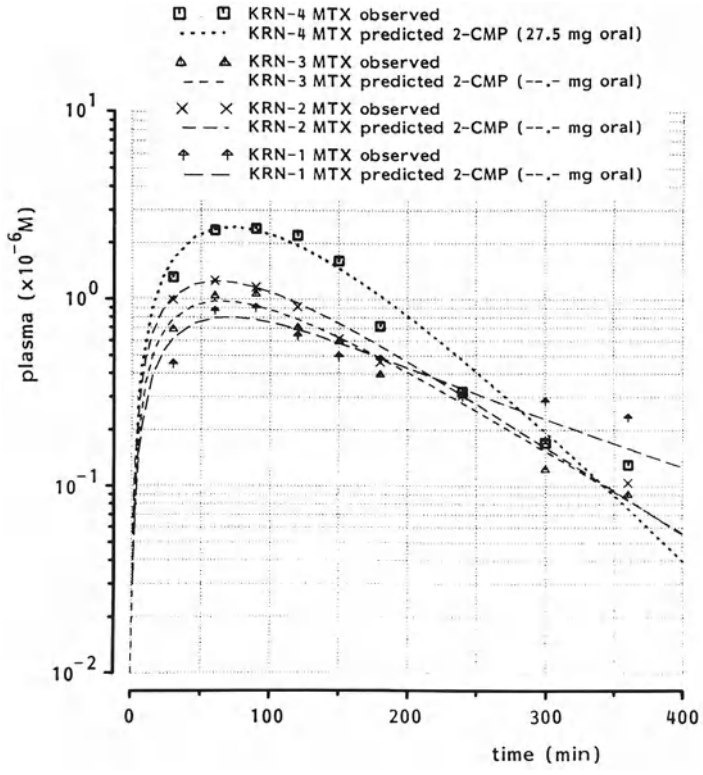

Fig. 4. Plasma time concentration curves of a patient, showing measured and predicted disappearance. The concentrations were determined following separate p.o. administrations of MTX at weeks 9, 30, 58, and 86 of maintenance treatment

fied. The bone marrow concentrations of MTX and 7-hydroxymethotrexate were not correlated with the plasma AUC, nor with the absorption pattern of MTX, when defined as the time of its peak plasma concentration. In this group of patients, plasma pharmacokinetics and 24 h bone marrow concentrations of MTX did not show significant fluctuations when studied at several periods of maintenance treatment (Fig. 3 and 4). There were no indications of reduced absorbance of MTX over a 114-week maintenance period.

Discussion

Several studies have demonstrated considerable interindividual variability in MTX absorption in children with ALL [2–5, 8, 10, 11] although the absorption of MTX following doses below 30 mg/m^2 is essentially complete [9].

As in previous studies, our results demonstrate a wide interindividual variation in plasma concentrations of MTX following a uniform oral dose. Both the peak concentration and the interval between the oral dose and the maximum plasma level were not related to the absolute dose. In some patients (6/17) an intraindividual variation in peak plasma concentration of more than 50% of the initial value was also detected. This variation could not be attributed to food intake, nor to gastrointestinal disease, nor to improper sampling procedures.

A wide variation of the plasma AUC was observed in these patients, which can be explained by the variation in the peak concentrations of MTX. Under standard conditions, no significant effect of the rapidity of the MTX absorption on the plasma avail-

ability could be demonstrated. It remains to be determined why there was such a variable absorption of MTX. Using HPLC we found that 7-hydroxymethotrexate was present in greater quantities than MTX itself at times beyond 2 h. Thus, the plasma peak concentration and the plasma AUC of MTX alone do not reflect the total absorbance from the gastrointestinal tract.

The variability in plasma MTX levels following p.o. administration led us to determine the actual concentrations in a target organ, i.e., bone marrow, which is a site of possible hematologic relapse in ALL.

Surprisingly, 7-hydroxymethotrexate accounted for 87% of the total drug content at 24 h following p.o. administration. At that time, drug levels in plasma had fallen to zero. No other metabolites could be detected in bone marrow. The large proportion of 7-hydroxymethotrexate in bone marrow may result from the high plasma levels of this metabolite, which is less soluble than the parent drug and is hardly eliminated through glomerular filtration [10]. MTX and 7-hydroxymethotrexate are competitive substrates for the carrier-mediated active transport system into the cell, which has an affinity constant of 1–10 μM [12]. Thus, high concentrations of 7-hydroxymethotrexate may interfere with the uptake of MTX into the cell, and thereby prohibit sufficient antifolate activity.

The accumulated MTX and 7-hydroxymethotrexate in bone marrow at 24 h did not show any correlation with the plasma AUC of the drug. Thus, monitoring of plasma levels does not reflect their concentrations in this target organ. Finally, the plasma pharmacokinetic parameters and bone marrow concentrations of MTX were repeatedly determined during the maintenance treatment. No significant reduction of MTX absorption was observed. Thus, there are no reasons to alter the route of administration or the formulation of the drug. It is obvious, however, that the pharmacokinetic parameters of MTX in plasma are not sufficient to assess the presence of adequate drug levels in bone marrow. In view of the frequent occurrence of hematologic relapse of ALL, further evaluation of the in vivo pharmacodynamics of MTX in bone marrow may be warranted.

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Determination of the Cellular Uptake of Daunorubicin in Human Leukemia in vivo: Method of Examination and First Results*

M. E. Scheulen[1], K. Lennartz[2], T. Heidrich[1], G. Host[1], and B. Kramer[1]

Introduction

Daunorubicin (DNM) and other anthracycline antibiotics are among the most active agents in the cytostatic chemotherapy of acute leukemias and are widely used in clinical oncology. Their clinical use is limited by toxic side effects such as cardiotoxicity and by the development of resistance, respectively. Clinical pharmacology is one of the tools to determine and possibly predict antitumor efficacy as well as toxicity of antineoplastic drugs in man. As the cellular pharmacology of cytostatics in tumor cells and organ tissues may better contribute to the understanding of the antineoplastic effect and the toxic side effects of cytostatic chemotherapy and thus may better predict drug resistance individually, attempts are necessary to develop appropriate methods in man in vivo. The repeated aquirement of tumor cells to establish kinetics of intracellular drug content is ethically justified and can be easily performed only in patients with leukocytic leukemias.

Thus, we have established two methods for the determination of the cellular uptake of DNM in leukoblasts in vivo: one method by high-performance liquid chromatography (HPLC), the other method by use of flow cytometry with a fluorescence-activated cell sorter (FACS II), respectively.

Material and Methods

Preparation of leukoblasts. All preparation steps except the incubation were performed at 4 °C. Immediately after venipuncture heparinized blood was diluted by phosphate buffered saline (PBS) (10/6, v/v), layered on top of lymphoprep (Nyegaard and Co., Oslo, Norway) and centrifuged at 400 g for 17 min. The leukoblast layer was washed in PBS and centrifuged at 400 g for 10 min. Leukoblast pellet was incubated in 0.83% NH_4 Cl/300 m*M* Tris-KCl-buffer (9/1, v/v) for 10 min at 37 °C to lyse decontaminating erythrocytes. Leukoblasts were centrifuged at 400 g for 10 min and washed in PBS twice as before. Final leukoblast pellets contained 10^7 nucleated cells.

Extraction of DNM. Leukoblast pellets were resuspended in 200 µl 3N HCl/ethanol (1/1, v/v). For internal standardization 50 µl 1 µ*M* doxorubicinol was added. After vigorous shaking and sonification with a Branson sonifier B12 cell debris were centrifuged.

Separation and Identification of DNM by HPLC. 50 µl supernatant was injected into an HPLC system with a Bondapak phenyl column (3.9 mm × 300 mm) in 20 m*M* H_3PO_4/acetonitril (67.5/32.5, v/v) at a flow rate of 3 ml/min. DNM and metabolites were identified with a Kratos FS 970 spectrofluorometer with a GM 970 monochromator (excitation 254 nm, emission 580 nm)

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and quantified by internal standardization with a Hewlett Packard 3390A integrator.

Measurement of the DNM Content of Leukoblasts by flow Cytometry with a FACS II. Alternatively, the DNM content was individually measured in a modified FACS II (Becton and Dickinson, Mountain View, Ca., USA) according to the method described by Speth et al. [1]. The argon ion laser was tuned at 488 nm (0.5 W) for excitation. The scatter light was blocked by a 530 nm long pass filter. The intensity of the fluorescence, after correction for the background fluorescence, is proportional to the DNM content of the leukoblasts. 20000 nucleated cells were analyzed per run.

Results

According to the determinations of the intracellular DNM content of human leukoblasts in vivo by HPLC, maximal concentrations varied between 2 and 12 ng/10^6 cells during the first 4 h after i.v. bolus injection of up to 60 mg/m^2 (Table 1).

Altogether, 13 determinations of the intracellular kinetics of DNM have been performed up to now. Two typical kinetics are shown in Fig. 1 and 2. There is a large variation in maximal intracellular DNM concentrations as well as in the areas under the curve (AUC) during the time between 5 min and 2 h post injection (Table 1), which can be demonstrated interindividually and in-

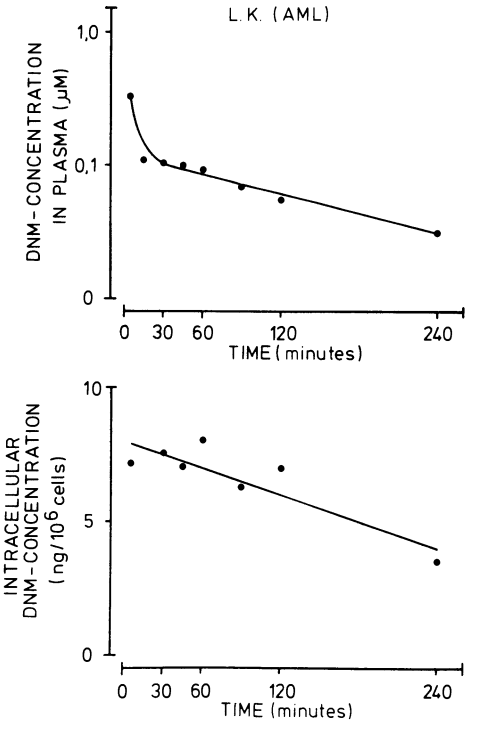

Fig. 1. Plasma kinetics and cellular uptake of DNM in vivo in patient L. K. with AML after administration of 19 mg/m^2 DNM i.v.

Table 1. Cellular uptake and plasma kinetics of daunorubicin in patients with acute leukemias

Patient	Diagnosis	DNM-Dose (mg/m^2)	Max. conc. plasma (µmol/liter)	Max. conc. leukoblasts (ng/10^6 cells)	AUC[a] plasma (µmol · h/liter)	AUC[a] leukoblasts (ng · h/10^6 cells)
E. P.	AML_{sens}	60	0.81	10.4	0.55	15.8
J. S.	AML_{sens}	57	0.82	2.3	0.49	3.5
H. S.	AML_{sens}	28	0.82	8.4	0.83	14.4
H. V.	ALL_{sens}	25	0.17	6.4	0.19	10.8
U. W. (I)	AML_{sens}	19	0.75	12.1	0.36	19.1
U. W. (II)	AML_{res}	19	0.36	5.9	0.21	10.6
U. W. (III)	AML_{res}	19	0.41	5.7	0.18	8.4
L. K.	AML_{sens}	19	0.33	8.0	0.21	14.0
L. G. (I)	AML_{sens}	19	0.38	4.3	0.45	7.2
L. G. (II)	AML_{sens}	19	0.25	4.2	0.31	6.9
E. K.	AML_{sens}	18	0.44	5.7	0.40	9.9
C. J. (I)	AML_{res}	16	0.43	6.7	0.19	8.1
C. J. (II)	AML_{res}	16	0.41	8.0	0.27	9.1

[a] Area under the curve (5–120 min post injection).

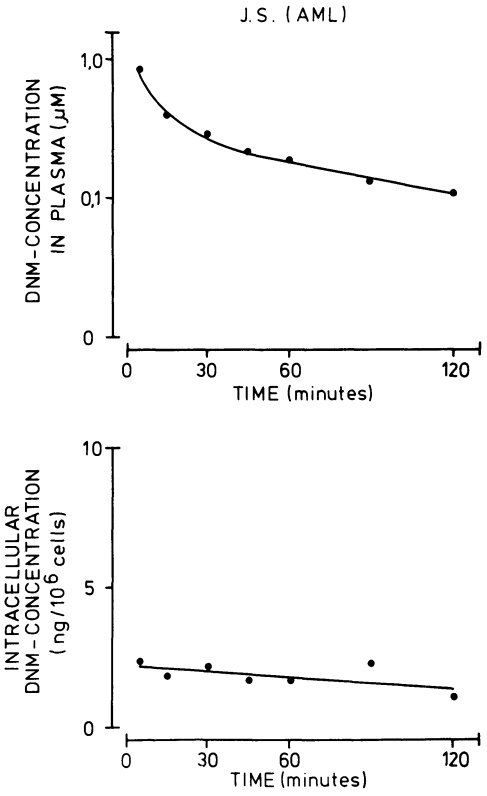

Fig. 2. Plasma kinetics and cellular uptake of DNM in vivo in patient J.S. with AML after administration of 57 mg/m^2 DNM i.v.

traindividually when more than one therapy is analyzed.

The plasma kinetics of DNM have always been determined simultaneously (Fig. 1 and 2). According to the comparison of plasma kinetics and intracellular kinetics of DNM, our results do not show a significant correlation between plasma levels and intracellular levels of DNM. Predictions of the antileukemic efficacy of DNM per se by comparing the maximal concentrations or by the AUC are hampered by the fact that individual patients have been treated by combination chemotherapy including DNM.

The determination of the relative fluorescence intensity by flow cytometry with the FACS II well reflects the cellular uptake of DNM (Fig. 3) and is in agreement with the values measured by HPLC (Fig. 4).

Discussion

Up to now, flow cytometry studies on intracellular anthracyclines in leukemic cells have only been performed in vitro [2–6], and correlated with HPLC methods in one report [1].

We have used both methods for the determination of the cellular uptake of DNM in leukoblasts in man in vivo. The HPLC method has the advantage of discriminating intracellular metabolites of DNM. On the other hand, the FACS method allows the measurement of the overall content of DNM and its metabolites in individual cells and thus may discriminate different cell populations.

Investigations in other tumor cell systems clearly demonstrated that one important factor for DNM resistance is a reduction of the intracellular DNM steady state concentration [7] caused by an active outward transport of the drug in resistant cells [8]. Up to now, our results are too preliminary to draw any conclusion on the antileukemic ac-

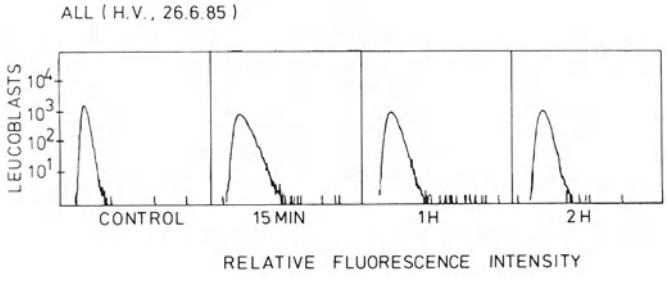

Fig. 3. Time-dependent analysis of the DNM content of leukoblasts in patient H.V. with ALL in vivo by determinants of the relative fluorescence intensity by flow cytometry of 20 000 cells with the FACS II

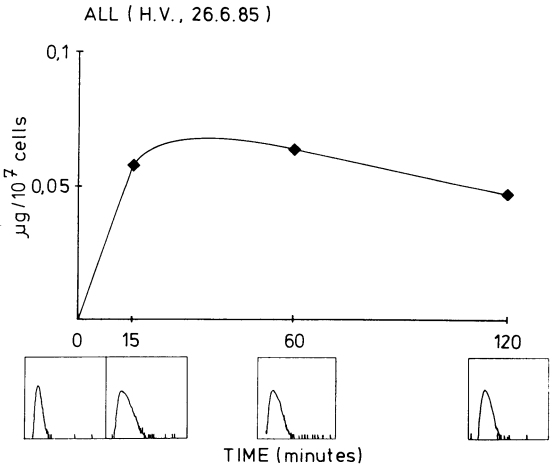

Fig. 4. Correlation between the determinations of the DNM content of leukoblasts in patient H. V. with ALL in vivo by HPLC and flow cytometry with the FACS II, respectively

tivity of DNM individually. Interindividual differences in maximal concentrations or in the AUC for DNM cannot be correlated with the clinical response to cytostatic chemotherapy as individual patients have not been treated by DNM alone. Nevertheless, the significant differences in drug uptake between patient L. K., who received only 19 mg/m^2 DNM and had a maximal intracellular concentration of 8.0 ng/10^6 cells (Fig. 1), and patient J. S., who received 57 mg/m^2 DNM and had a maximal intracellular concentration of only 2.3 ng/10^6 cells (Fig. 2) is notable. There are intraindividual differences in daunomycin uptake on repeated investigations as well. In patient U. W. (Table 1), who was treated three times with DNM alone, the reduction in intracellular uptake of the drug may be the reason for the concomitant development of resistance.

In conclusion, the measurement of the cellular uptake of DNM and other anthracycline antibiotics is feasible in patients with acute leukemias in vivo. Thus, the determination of the cellular pharmacology of anthracyclines in patients with acute leukemias may be important for (1) the prediction of prognosis; (2) the early recognition of the development of resistance; (3) the development of new anthracyclines; (4) the evaluation of the efficacy of chemosensitizers concomitantly administered.

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Cytoskeletal Organization in Acute Leukemias*

A. Schmitt-Gräff[1], M. E. Scheulen[2], and G. Gabbiani

Introduction

The cytoplasm of eukaryotic cells contains three fibrous systems: microfilaments, intermediate filaments and microtubules. Microfilaments are polymers of actin, microtubules are made up of tubulin subunits and intermediate filaments are composed of at least five different classes of proteins according to tissue origin. It is now widely recognized that cytoskeletal and their associated proteins play an important role in cell motility, cell shape, endo- and exocytotic processes, organelle movement, and control of surface receptor functions[1].

In nonmuscle cells, actin exists as a globular monomer (G-actin) which assembles reversibly to form microfilaments (F-actin). F-actin is assumed to be the functional form of actin and is also an important structural comoponent of nonmuscle cells. Actin-dependant nonmuscle cell motility is thought to play a role in the spreading of neoplastic cells through the body and during tumor invasion [2]. In leukocytes, actin is one of the most abundant proteins and is responsible together with associated proteins for the locomotory activity of leukocytes [3]. The degree of actin assembly seems to reflect the motile and functional behavior of neutrophils [4].

Intermediate filament proteins provide information on cell origin and can be used as markers to identify the origin of tumor cells [1]. The intermediate filaments of lymphocytes and polymorphonuclear leukocytes (PMNs) have been shown to be composed of vimentin in normal and neoplastic conditions [5].

We have focused our study on actin and vimentin in circulating lymphocytes and PMNs from normal donors and in blasts from patients with common acute lymphoblastic leukemia (cALL) and acute myeloid leukemia (FAB classification M_1 and M_2).

Materials and Methods

Samples of heparinized blood were obtained from normal subjects and from patients with ALL and AML. Mononuclear cells were separated by centrifugation on a Ficoll-Hypaque gradient. PMNs were collected from sediments after hypotonic lysis of red blood cells. Myeloblasts and PMNs were pretreated with 0.002 *M* diisopropylfluorphosphate to prevent proteolysis. Cell suspensions were lysed by addition of a lysis buffer containing 1% triton X-100 [6]. Proteins of the lysate were precipitated by acetone for the determination of total actin and vimentin content. Total cell extracts were centrifuged for 60 min at 144 800 × g to pellet F-actin. Total extracts and pellets were dissolved in 2% Sodium dodecyl sulfate (SDS)

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sample buffer, sonicated and boiled. Proteins were electrophoresed on 10% SDS-PAGE (polycrylamide gel electrophoresis), stained with coomassie blue and destained. Quantification of total actin, vimentin and F-actin as a percentage of total cellular protein was done by densitometric scanning of SDS-PAGE [7].

Samples of fresh blood cells were glutaraldehyde-fixed and processed for transmission electron microscopy using standard techniques. Immunofluorescence staining on isolated cells was done with monospecific antibodies and rhodamine phalloidin for actin and with monoclonal antibodies for vimentin.

Results

Our biochemical studies demonstrated that ALL blasts lacked evidence of significant changes in total actin and vimentin content when compared to normal lymphocytes (Tables 1 and 2). In contrast, F-actin decreased by about 50% when normal lym-

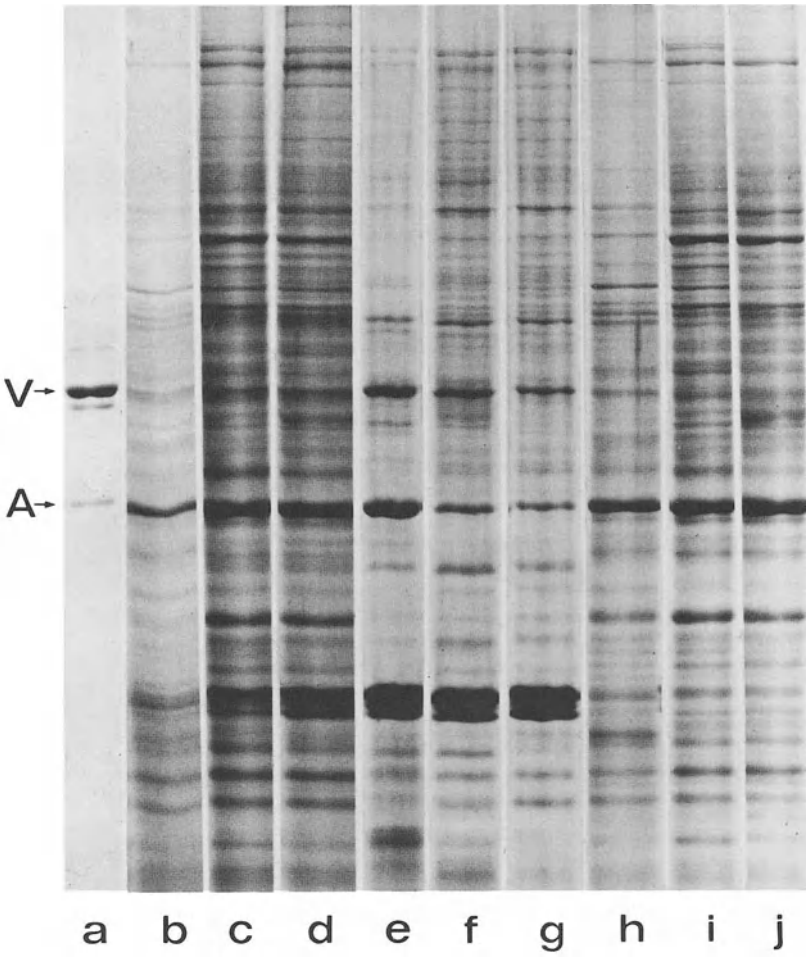

Fig. 1. SDS-PAGE of control lymphocytes (lanes *b, e, h*) and of lymphoblasts from patients with ALL *(c, d, f, g, i, j)*. Lanes *b, c, d* are loaded with total extracts of cell lysates (smaller charge of protein in lane *b*); lanes *e, f, g* with ultracentrifugation pellets; lanes *h, i, j* with ultracentrifugation supernatants; lane *a* with cultured baby hamster kidney cells as a marker for actin *(A)* and vimentin *(V)*. Lane *e* (pellet of control) shows a band of actin larger and more intensely stained than lane *f* and *g* (pellets of lymphoblasts)

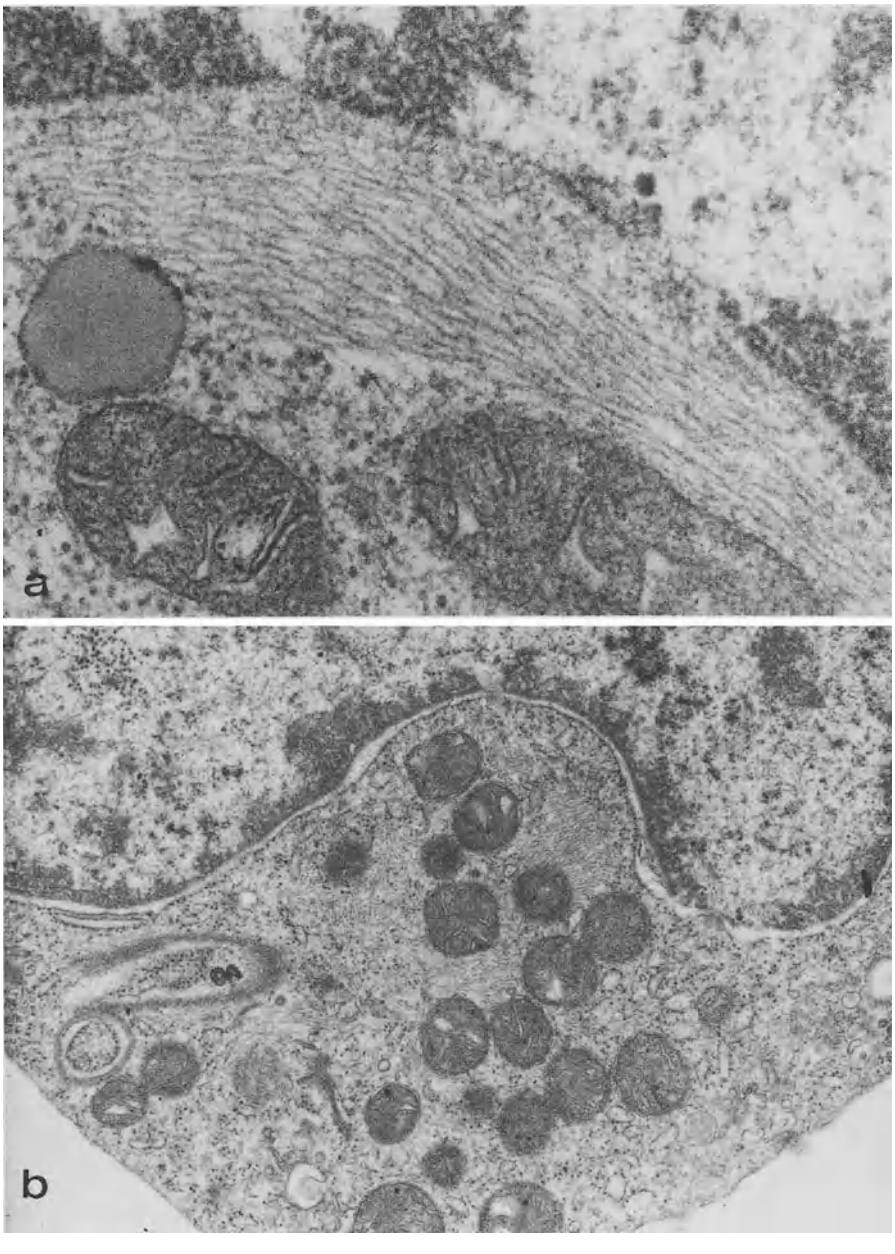

Fig. 2 a, b. Electron micrographs of an ALL blast (**a**) and an AML blast (**b**) yielding large bundles of intermediate filaments in close association with the nucleus and mitochondria. **a** ×55 500, **b** ×32 400

phocytes were compared with lymphocytes from patients with ALL (Table 1, Fig. 1).

The total and F-actin contents were found to be significantly lower in the poorly differentiated myeloid blasts from patients from AML, M_1, than in the more differentiated myeloid cells from patients with AML, M_2, and in mature PMNs (Table 1). The amount of vimentin was very similar in both leukemic cell populations. However, we ob-

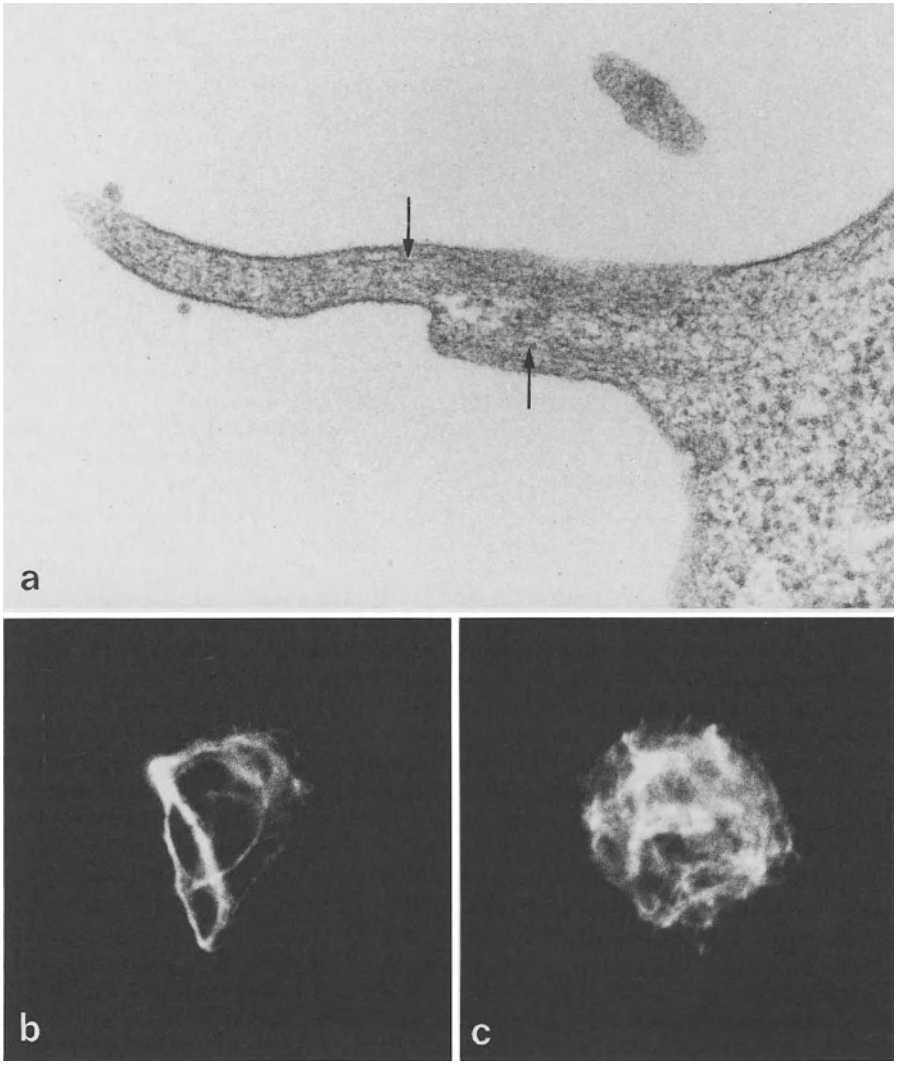

Fig. 3. a Surface ruffles of a myeloid leukemia cell from a patient with AML, M_2, are filled with a microfilamentous meshwork *(arrows)* when viewed by transmission electron microscopy. ×57000 **b,c** Immunofluorescence staining with antivimentin antibodies discloses a well-developed network of intermediate filaments in peripheral blood lymphoblasts from a patient with ALL. ×1000

Table 1. Actin content of circulating lymphocytes and polymorphonuclear leukocytes (PMNs) from healthy donors and of blasts from patients with ALL and AML (FAB M1 and M2)

Fraction	Percentage of actin in total protein				
	Lymphocytes ($n=6$)	ALL ($n=6$)	PMNs ($n=4$)	AML, M1 ($n=9$)	AML, M2 ($n=7$)
Total extract	14.74±0.79	13.77±0.83	15.77±0.50	9.45±0.77	11.09±0.78
144800×g Pellet	9.11±0.55	5.19±0.70	12.50±0.16	6.75±0.65	11.35±0.75

Table 2. Vimentin content in peripheral blood lymphocytes and polymorphonuclear leukocytes (PMNs) from control persons and in blasts from patients with ALL and AML (FAB M1 and M2)

Fraction	Percentage of vimentin in total protein			
	Lymphocytes ($n=6$)	ALL ($n=6$)	PMNs ($n=4$)	AML (M1 and M2) ($n=16$)
Total extract	3.35 ± 0.11	3.29 ± 0.29	1.97 ± 0.77	3.09 ± 29

served a significant increase in the vimentin content when myeloid blasts were compared to normal PMNs (Table 2).

On electron microscopic examination, the cytoplasm of leukemia cells from ALL and AML patients exhibited large arrays of intermediate filaments often in close association with the nucleus and mitochondria (Fig. 2a and b). All blasts and blasts from patients with AML, M_1, showed a poorly developed microfilament network. Surface ruffles of myeloid cells from AML with maturation (M_2), however, were filled with a well-developed microfilamentous meshwork (Fig. 3a). Staining for actin with antiactin antibodies and rhodamine phalloidin yielded a greater intensity of fluorescence in control granulocytes and in M_2 leukemia cells than in M_1 blasts. Similar results were obtained when staining of normal lymphocytes was compared to that of ALL blasts. When stained with antivimentin antibodies, leukemic cells of ALL and AML disclosed a well-developed filament network extending throughout their cytoplasm (Fig. 3a and b). In contrast, PMNs showed only few filaments, usually located near the nucleus.

Discussion

There is a lot of evidence indicating that cytoskeletal organization may reflect differentiation phenomena in cells.

Up to now, changes in cytoskeletal organization have not been documented in acute leukemias when immature variants are compared to more differentiated subgroups and to mature lymphocytes and PMNs. An increase in actin content has only been described during induced maturation of myeloid leukemia cell lines in culture [8, 9]. In our study, analysis of SDS-PAGE demonstrated a marked increase in total and F-actin content in the more mature myeloid leukemias (M_2) when compared to leukemias without maturation (M_1).

F-actin decreases by about 50% in the immature ALL blasts compared to normal peripheral lymphocytes. Thus, our observations suggest that actin cytoskeletal organization may correlate with cellular differentiation phenomena and reflect biochemical and functional events occurring during the maturation pathway of hematopoietic cells.

A rich intermediate filament network in leukemic blasts was revealed by immunofluorescence and electron microscopy. These findings were confirmed by the high vimentin content of ALL and AML cells as determined by densitometric scanning of SDS-PAGE. In contrast, control PMNs contain only a small amount of vimentin. It is worth noting that immunofluorescence studies have indicated an alteration of vimentin expression during differentiation of normal human hemopoietic cells [10]. In previous studies, evidence was obtained that an increased vimentin content is typical of cells having a high replication rate, such as vascular smooth muscle cells in vivo [7] and in vitro [11]. Thus, the high vimentin content of leukemic cells may be related to blast growth potential and cellular proliferative activity.

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Pitfalls in the Evaluation of Prognostic Factors*

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Introduction

Any treatment can be regarded as an experiment with an unknown outcome. Physicians and patients, however, would like to know – prior to the beginning of treatment – what the outcome will be, or more exactly, what the likelihood of different possible outcomes will be. To fulfil this demand prognostic factors are called for. Prognostic factors can be defined as factors, measured before the beginning of therapy, which allow for the prediction of outcome. There is plenty of literature about the statistical methods used to extract prognostic factors from clinical data [1, 2], but much less about inherent problems and pitfalls. These are, however, obvious, especially when the evidence for prognostic factors is compared in various studies. The purpose of our contribution is to analyze possible reasons for contradictory results and to give recommendations which can improve the assessability of reported prognostic factors.

Material and Methods

We analyzed 12 major contributions, published in relevant journals since 1979 regarding prognostic factors in adolescent or adult acute lymphocytic leukemia (ALL) or acute undifferentiated leukemia (AUL). Publications comprising mixed samples of children and adults were excluded. In this context we considered the outcome criterion "remission duration". A description of important design features of the 12 studies and the results of the analyses concerning prognostic factors are shown in Table 1. All the potential prognostic factors, which were examined in at least one study, are listed.

Our descriptive analysis shows a marked variability in alleged prognostic factors: RNA-index was examined only once [3], whereas age was examined in 10 out of 12 publications. Comparing the results of these examinations we have to acknowledge that they sometimes correspond and sometimes contradict each other. Concentrating on the latter, we shall present examples of possible reasons which might change the values of specific prognostic factors from one study to the other.

Among the more important reasons are: (1) heterogeneous patient samples and different diagnostic techniques; (2) different therapeutic regimens; (3) different statistical methods, e.g., the use of univariate or multivariate analysis, as well as different sample sizes or study designs.

Heterogeneous Patient Samples and Different Diagnostic Techniques

To show the effect of heterogeneous patient samples age distribution is considered, and this is frequently reported as a prognostic factor in literature.

Patients' age distributions vary between the cohorts, starting with an age of 11 [4] or

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12 [5, 6], and ending with an age of between 57 [5] and 82 years [7]. The cut points of age which were used for stratification of remission duration differed in most papers: 20 years in [8] and [9], 25 years in [3], 30 years in [10] and [4], 35 years in [11]. It is not quite certain why these particular cutoff points were chosen. One reason for finding positive and negative correlations could be the different ranges of age distribution and different definitions of strata. In two papers [7, 8] a significant influence of age on the probability of obtaining a complete remission was shown, so the remaining sample, evaluating remission duration, was most probably highly selected regarding age. This might be a reason why these papers did not show age as an influence on remission duration.

The methods of diagnosing immunologic subtypes have changed considerably during recent years. Lazzarino [6] classifies patients into T-, B-, or non-T-, non-B-ALL, whereas Clarkson [3] uses T-, B-, and null-ALL, as well as a "not studied" subgroup, Burns [12] distinguishes only between T- and null-ALL. Therefore a comparison of the subtypes in the respective samples is not possible, and thus equal prognostic factors cannot be expected.

Different Therapeutic Regimens

Generally, prognostic factors are only valid under specific conditions, i.e., the therapeutic regimens under which they were evaluated. Because none of the mentioned authors used exactly the same regimen, it is hardly possible to extract the interaction between prognostic factor and treatment. Shuster [13] found that in a randomized trial of the Pediatric Oncology Group a patient characteristic had a different prognostic significance within two therapies.

Different Statistical Methods

An inadequate statistical method is one possible reason why Brun [7] did not find a correlation between age and remission duration whereas Hoelzer [11] did. The Mann-Whitney test is only to be applied to uncensored data, i.e., each patient has to be observed until relapse. If the patient is still in complete remission at the date of evaluation, his/her remission duration is considered to be a censored observation. In this case Life-Table methods are the correct statistical instrument.

Univariate and multivariate analyses may result in a different pattern of prognostic factors. When prognostic factors are cited they are mostly supposed to be independent of each other. Most authors did not check multicollinearities, i.e., correlations between prognostic factors. Hoelzer [11] found blast cell count to be highly correlated with WBC count and of no further prognostic value in the risk to patients.

Sample Size and Study Design

One reason for the nonsignificance of a possible prognostic factor might be a small sample size. Omura [8] and Gingrich [9] failed to show a correlation between remission duration and age whereas Baccarani [4] and Hoelzer [11] succeeded (Table 2).

Suppose there are 40 patients in each of the two age groups. To detect a significant influence of age on remission duration the median remission duration of one group has to be increased by a factor of approximately 2 in comparison to the median of the other group ($\alpha = 0.05$, $\beta = 0.2$) [14].

In general, observational studies and randomized trials present less heterogenous samples than selective groups with regard to diagnostic techniques, treatment and follow up. Homogeneous samples will reveal clear structures more easily than heterogeneous samples, i.e., they will detect true correlations and avoid sham correlations.

Conclusions

The given examples show that results regarding the evaluation of prognostic factors on remission duration are hardly comparable between the publications and rarely transferable to further studies.

It is not possible to pool the results because of the heterogeneous samples which were diagnosed and treated in different ways.

Table 1. Review of papers concerning prognostic factors for remission duration in adult ALL/AUL

Author/ year	Patients entered. CR achieved	Age range $\tilde{X}$: median	Exclusion criterion	Study design	Treatment regimen	FAB	Sub-type	TDT	Cyto-genetic	RNA in-dex	Time to re-mission
Ruggero [5] 1979	32 25	12–57 $\tilde{X}$: 20	Pretreatment	Retrolective cohort	Three different regimens						
Henderson [10] 1979	149 107	≧20 $\tilde{X}$: 37	>1 week pretreatment V, P, 6-MP, MTX	Randomized	V, P, DNR, L-Asp; MTX, 6-MP, V, P						
Omura [8] 1980	99 79	15–70 $\tilde{X}$: 23	Pretreatment	Randomized	V, P, MTX; MTX, 6-MP, CVP; ARA-C, 6-TG, V, P, L-Asp	–					
Brun [7] 1980	92 65	14–82 $\tilde{X}$: 39	Pretreatment	Retrolective cohort	Three different regimens						
Burns [12] 1981	23 17	16–60 $\tilde{X}$: 30	Pretreatment	Retrolective cohort	V, P, ADR, L-Asp; V, P, ADR, 6-MP, MTX, BCNU, C, D	+					
Baccarani [4] 1982	293 232	≧11	Pretreatment	Retrolective cohort	Several different regimens	$(+)^{L}$					
Lazzarino [6] 1982	62 45	12–59	Pretreatment	Observational study	V, DNR, P; MTX; 6-MP, MTX, DNR	*	*				
Garay [15] 1982	241 ?	>15	Pretreatment	Observational study	V, P, DAU; MTX vs none; C, ARA-C vs none						
Lister [16] 1983	112 74	15–68 $\tilde{X}$: 27	Pretreatment	Observational study	ADR, V, P, L-Asp/C; MTX, ARA-C; 6-MP, C, MTX	*					
Gingrich [9] 1985	48 39	15–72 $\tilde{X}$: 23	Pretreatment	Observational study	V, P, L-Asp, A; MTX; V, P, A, 6-MP, D, B	–	*				
Clarkson [3] 1985	149 123	≧15 $\tilde{X}$: 25	Philadelphia chromosome positive, pretreatment	Observational study	L2-L17M protocols	*	*	–	*	*	*
Hoelzer [11] 1984	162 126	15–65 $\tilde{X}$: 25	>2 weeks V, P,	Observational study	V, P, L-Asp, A; MTX; V, P, A, 6-MP, D, B		*	–	–		*

*, significant correlation; +, $0.05 \leqq p < 0.1$, trend; –, not significant; $(+)^{L}$, multicollinearity with WBC count; V, vincristine; P, prednisone; L-Asp, L-asperaginase; A, doxorubicine; MTX, methotrexate; D, dactinomycine; B, carmustine; C, cyclophosphamide; DNR, daunorubicine; 6-MP, 6-mercaptopurine; ARA-Co, arabinocide; 6-TG, 6-thioguanine; ADR, adriamycine; DAU, daunomycine; DXM, dexamethasone.

From a biometric point of view, results concerning prognostic factors should be described with regard to the following points:

- definition of outcome criterion (here: remission duration)
- description of the diagnostic techniques used
- the sample in complete remission (CR) should be described regarding the distribution of all patient characteristics which are to be evaluated in respect to their prognostic value
- the reason why continuous variables (e.g., age, WBC count) are classified with certain cut points, should be explained
- the statistical procedure regarding the use of uni- and multivariate analysis should be described (e.g., whether multicolinearities were checked)
- patient characteristics which were not found to be of prognostic value should be

Table 1 (continued)

Sex	Age	Fever, infection	WBC count	Blast cell count	Platelets	Hemoglobin	Lactic dehydrogenate	CNS infiltration	Mediastinal tumor	Liver enlargement	Spleen enlargement	Lymphoadenopathy	Statistical method	Remark
	*			*	–								Log rank test	Case reports
	*												Log rank test	Outcome variable is survival time
–	–		–		–	–			–	–	–	–	Log rank test Cox model	
–	–			*					–				Mann-Whitney test	
													Log rank test	$\tilde{X}$:
+	(+)		*	–	–	–		(+)[L]	–	–	–	–	Log rank test	
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	*		*	–									Log rank test	Abstract
				*									Long rank test	Abstract
	–			*									Log rank test multiple regression	
+	*	–	*	+					–				Log rank test	
–	*	–	*	(+)[L]	–			–	–	–	–	–	Log rank test Cox model	

Table 2. Coherence between sample size and significant result

Author	Sample size of		Correlation
	Young patients	Elderly patients	
Omura	33	46	Not significant
Gingrich	12	27	Not significant
Baccarani	146	79	Significant
Hoelzer	98	28	Significant

mentioned; failure to achieve significance does not prove the absence of a prognostic value. Attention should be paid to the β-error in small sample sizes, i.e., the probability of not detecting a dependence although one exists

- patient characteristics at time of achieving CR, as well as the calculated remission du-

ration, should be reported for each patient, especially when the sample size is below 100, e.g., in [5]
- an interpretation from a medical point of view should sum up the statistical findings.

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AML in Adults

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Low-Dose Ara-C in Myelodysplastic Syndromes and Acute Nonlymphoid Leukemia. Experience with Seven Patients*

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Introduction

Treatment by low-dose cytosine arabinoside (LDAC) is effective in several cases of acute nonlymphoid leukemia (ANLL) and myelodysplastic syndromes (MDS), but the mechanism of remission induction is still being debated. The results of some clinical investigations are consistent with induction of leukemic cell differentiation [1–4] while others favor a cytotoxic effect of LDAC [5, 6]. Ishikura et al. [7] and Degos et al. [8] found in a large number of patients that the mechanism(s) of LDAC in inducing remission may involve a cytotoxic effect as well as a differentiation-inducing effect. We treated seven nonconsecutive patients with LDAC: four with ANLL (M_1, M_2 according to the FAB classification [9], and two MDS [refractory anemia (RA) and refractory anemia with excess of blasts in transformation (RAEB-t), according to the FAB classification [9]]. This report summarizes the results obtained in a preliminary trial. The results seem to confirm that remission by LDAC can be achieved either by leukemic cell differentiation or by cytostatic effect.

Patients and Methods

Seven male patients were studied. The age range was 31–79 years, mean age 61. Five patients had ANLL and two had MDS (RA and RAEB-t) at the time of treatment with LDAC. FAB subtypes are given in Table 1. One patient developed overt acute leukemia (FAB classification M_1) after an RA and only one patient had de novo ANLL (FAB classification M_2). The other three cases included secondary leukemia to multiple myeloma, leukemia resistent to conventional therapy, and leukemia in relapse. Cytosine arabinoside (Ara-C) treatment was given by s.c. injections at a dose of 10–11 mg/m^2 every 12 h, and only on two occasions by continuous infusion (c.i.) (Table 1).

Patient 1. DFG, 58 years old. RA with thrombocytopenia and neutropenia was diagnosed in October 1981 (hemoglobin (Hb) 7.2 g/dl, neutrophils 1.1×10^9/liter, platelets 11×10^9/liter, bone marrow blasts 3%–5% in several aspirations). The patient received regular blood transfusions, and was then treated with LDAC (10 mg/m^2 every 12 h s.c. for 14 days, daily dose 40 mg). A significant improvement was observed in anemia, neutropenia and thrombocytopenia (Hb 12.4 g/dl, reticulocytes 4.4%, neutrophils 2.1×10^9/liter, platelets 52×10^9/liter). The improvement was temporary and the patient became pancytopenic once again. A second and a third course (8 days) of LDAC (10 mg/m^2 every 12 h s.c., daily dose 40 mg) were given but the patient did not recover from severe pancytopenia and worsened after LDAC courses. Blood and platelet transfusion requirements increased and the patient died of high uncontrolled fever (septicemia?).

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Table 1. Patients treated with low-dose Ara-C and results of treatment

Case	Age (years)	Sex	Diagnosis (FAB)	Dose of Ara-C mg/m^2	Administration	Route	Duration (days)	Result
1 DFG	58	M	RA	10	q 12 h	s.c.	14, 8, 8	NR
2 TV	79	M	RAEB-t	10	q 12 h	s.c.	11	NR
3 PG	58	M	ANLL-M2 sec	10	q 12 h	s.c.	14	NR
4 MF	43	M	ANLL-M2 res	11	q 12 h	s.c.	7, 20	NR
5 DVF	69	M	ANLL-M1 (RA)	10	q 12 h	s.c.	7	NR
6 SV	31	M	ANLL-M2 rel	11	q 12 h	s.c.	25	CR
					Continuous infusion	i.v.	24, 26	
7 BG	72	M	ANLL-M2	10	Continuous infusion	i.v.	21	PR
					q 12 h	s.c.	23, 20	

Sec, secondary; res, resistent; rel, relapse; NR, nonresponder; CR, complete remission; PR, partial remission.

Patient 2. TV, 79 years old. RAEB-t was diagnosed in November 1982 (Hb 7.2 g/dl, neutrophils 0.930×10^9/liter, platelets 14×10^9/liter, bone marrow blasts 29%). The patient received regular blood transfusions. Then he was treated with LDAC (10 mg/m^2 every 12 h, s.c. for 11 days, daily dose 36 mg). Limited, temporary hematologic improvement in Hb, platelet, and neutrophil values was observed but the percentage of bone marrow blast cells did not change significantly. The patient again became dependent on transfusions. He died of ictus.

Patient 3. PG, 58 years old. An ANLL (FAB classification M_2) secondary to multiple myeloma was diagnosed in November 1981, after two years of melphalan and prednisone therapy. The patient was unsuccessfully treated with four courses of daunorubicin, Ara-C, thioguanine and then he was started in LDAC (10 mg/m^2 every 12 h s.c. for 14 days, daily dose 32 mg). Two weeks after the therapy with LDAC the peripheral blood showed the same percentage of blasts, no improvement in Hb, neutrophil, and platelet values and the same percentage of blasts was observed in bone marrow (80%–85%). The patient died of leukemia 2 weeks after the LDAC course.

Patient 4. MF, 42 years old. ANLL (FAB classification M_2) was treated with daunorubicin and Ara-C (two courses) and a complete remission was obtained. The remission was of short duration since after a consolidation therapy (daunorubicin, Ara-C, thioguanine) the blast cells reappeared in the peripheral blood (4%) while the bone marrow contained 12.5% blasts. The patient was treated with LDAC (11 mg/m^2 every 12 h s.c., daily dose 40 mg, two courses: the first of 7 days, the second of 20 days). No response was obtained. The clinical and hematologic conditions deteriorated and the patient died of disease-related complications (high fever, sepsis?).

Patient 5. DVF, 69 years old. RA (according to the FAB classification) with moderate neutropenia and severe thrombocytopenia was diagnosed in 1977 (Hb 5.8 g/dl, neutrophils 1.2×10^9/liter, platelets 38×10^9/liter). Since then the patient had been regularly transfused to keep his hemoglobin level between 9–11 g/dl while neutropenia and thrombocytopenia persisted. Several bone marrow examinations showed evidence of impaired erythropoiesis without blasts until October 1984 when bone marrow aspiration showed 16% blasts and peripheral blood values were: Hb 5.6 g/dl, neutrophils 0.34×10^9/liter, platelets 19×10^9/liter. The diagnosis of RAEB was made. Three months later – January 1985 – bone marrow blasts were 38.2% with 6% (0.09×10^9/liter) of blasts in peripheral blood. A diagnosis of

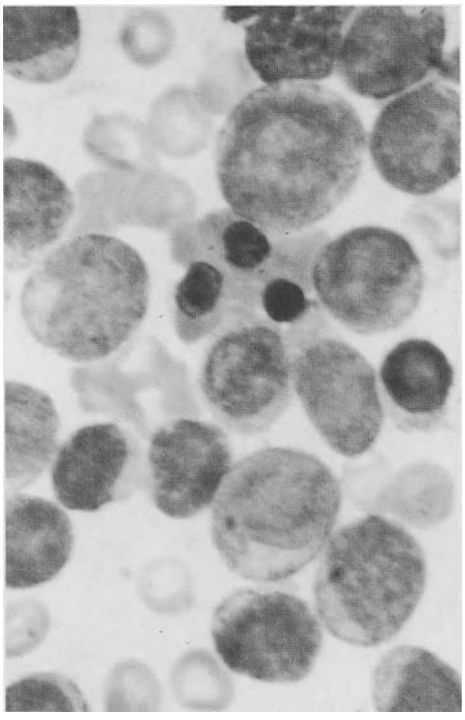

Fig. 1. Bone marrow at diagnosis, patient no. 6. ×1000

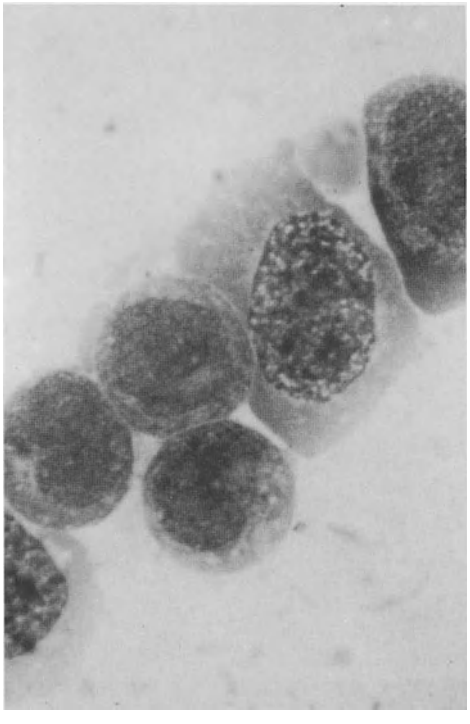

Fig. 2. Bone marrow with megaloblasts and leukemic cells, patient no. 6. ×1000

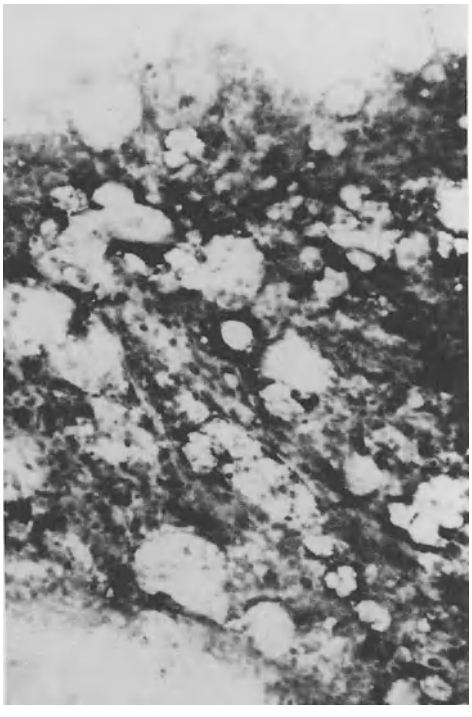

Fig. 3. Aplastic phase, patient no. 6. × 1000

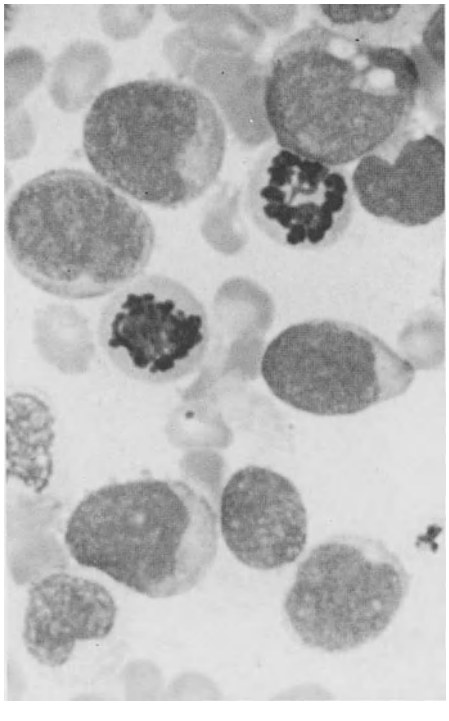

Fig. 4. Bone marrow morphology before LDAC treatment. Patient no. 7. × 1000

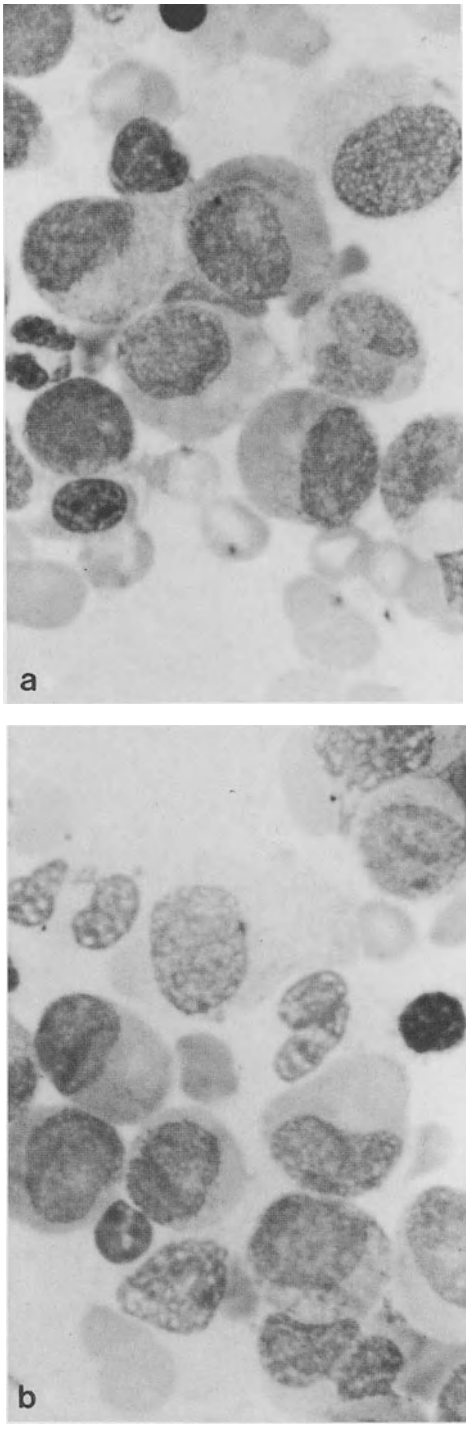

Fig. 5 a, b. Shift to a more differentiated cell population (morphologically promyelocyte- and myelocyte-like) after 21 days of LDAC therapy. $\times 1000$

ANLL (FAB classification M_1 supported by cytochemical reactions) was made. The patient received LDAC (10 mg/m^2 every 12 h s.c.; daily dose 30 mg) for 7 days. The clinical and hematologic conditions deteriorated and the patient died of pulmonary edema with uncontrolled fever. Peripheral blood values were: Hb 7.2 g/dl, neutrophils 0.02×10^9/liter; platelets 4×10^9/liter, blasts 52%, bone marrow blasts 54%.

Patient 6. SV, 31 years old, FAB classification M_2 (Fig. 1). The patient, in second relapse after two complete remissions with DAT and Bloomfield-Peterson protocols, achieved three complete consecutive remissions with LDAC given as s.c. injections, in the first course (11 mg/m^2 every 12 h s.c. for 25 days, daily dose 44 mg), but as continuous infusions in the second and third course (22 mg/m^2 per day, daily dose 44 mg for 24–26 days). Before each complete remission the patient experienced bone marrow hypoplasia with megaloblastic changes (Fig. 2), then bone marrow aplasia (Fig. 3). In this patient the complete remissions (maintained with a 6-day course every month of LDAC, 10 mg/m^2 per day, daily dose 20 mg) were of short duration (6–8 months). After the last complete remission (28. 3. 1985), a consolidation protocol (4 courses of daunorubicin, thioguanine, Ara-C) was given and the patient is now off therapy in complete remission since September 1985.

Patient 7. BG, 72 years old, FAB classification M_2 (Fig. 4). In this patient the diagnosis of ANLL was made in September 1985 (Hb 8.1 g/dl, neutrophils 3.3×10^9/liter, blasts 66%, platelets 36×10^9/liter, granular bone marrow blasts 84%) and LDAC was given as first-choice therapy (10 mg/m^2 every 12 h, c.i., daily dose 32 mg, 21 days). A complete clearance of blast cells from peripheral blood was observed after one course of therapy and pancytopenia was present. Serial bone marrow aspirations during the LDAC course showed a progressive decrease in blast cells but not a total clearance (blasts 8% at the end of treatment). Neither hypoplasia nor aplasia was observed but bone marrow examination revealed the presence of normal promyelocytes and maturing myeloid islets coincidently with a persistence of myeloblasts (Fig. 5 a and b). A second and a third course of LDAC were given (23 and 20 days respectively). The patient now has pancytopenia with 2% blasts (Hb 8.9 g/dl, neutrophils 0.9×10^9/liter, platelets 76×10^9/liter) and moderate bone marrow hypoplasia with 14.4% blasts.

Results

The results obtained with LDAC therapy are summarized in Table 1. No significant favorable long-lasting effect was observed in five patients. These patients died either of leukemia or of disease-related complications. Two patients with ANLL achieved complete (patient no. 6). and partial (patient no. 7) remission, the first patient after bone marrow aplasia, the second without aplasia but with morphologic evidence of granulocytic differentiation.

Discussion

Considering the limited number of patients it is not possible to evaluate the effectiveness of LDAC therapy in MDS and ANLL, but one aspect may be emphasized: the remission induction mechanism. It appears even from our limited results that with the regimen used more than one remission induction mechanism must be attributed to LDAC in responsive patients. One of our patients achieved three complete remissions after bone marrow aplasia preceded by megaloblastic changes. This sequence proves that the cytotoxic mechanism of Ara-C, even in low doses, depends on the inhibition of DNA synthesis and cell replication, accumulation in S phase, and cell death. It is our impression that this patient, as well as others who respond to LDAC therapy after an aplastic phase, has a malignant clone which is extremely sensitive to Ara-C.

A second patient achieved partial remission without aplasia but with morphologic evidence of granulocytic differentiation of the leukemic cells. In agreement with this theory of the mechanism of remission induction are experimental findings demonstrating that Ara-C at low doses may

induce maturation of murine and human leukemic cells in vitro [10–12].

Chromosomal findings of Hossfeld et al. [13] suggest a cytostatic as well as a differentiating effect of LDAC. These two mechanisms are not mutually incompatible: cytotoxicity should not in fact exclude the hypothesis of differentiation and maturation since the inhibition of DNA synthesis might restrain the proliferative capacity of leukemic blast cells and permit their maturation.

Summary

Seven patients were treated with low dose Ara-C (LDAC). Five patients had acute nonlymphoid leukemia (ANLL), two patients had myelodisplastic syndrome (MDS): refractory anemia (RA) and refractory anemia with excess of blasts in transformation (RAEB-t). Ara-C treatment was given by s.c. injections at a dose of 10–11 mg/m^2 every 12 h and only on two occasions by continuous infusion.

No improvement, or limited improvement, was observed in five patients and they died of leukemia or of disease-related complications. Two patients with ANLL achieved remission: the first patient after bone marrow aplasia, the second without aplasia but with morphologic evidence of granulocytic differentiation of leukemic cells.

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Low-Dose Cytosine Arabinoside in Patients with Acute Myeloblastic Leukemia and Myelodysplastic Syndrome

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Introduction

In previous studies, treatment with low-dose cytosine arabinoside (LDAC) has been reported as inducing high rates of complete remission (CR) in patients with acute myeloblastic leukemia (AML) and myelodysplastic syndrome (MDS) [2, 3, 5, 9]. More recent investigations have led to conflicting results and have even stressed drug-induced bone marrow hypoplasia. From these studies it was concluded that the mechanism of remission induction by LDAC involves a cytotoxic effect rather than induction of blast cell differentiation [4, 6, 8].

The present unicentric study was designed: (a) to evaluate the benefit of LDAC treatment in a large group of patients; (b) to identify subgroups with a good therapy response; and (c) to study the influence of different routes of administration.

Material and Methods

Patients

At the University of Düsseldorf 31 patients were treated with LDAC. These patients could be divided into five subgroups with the following diagnoses (Table 1): (1) de novo AML; (2) AML secondary to MDS; (3) AML in relapse; (4) myelodysplasia; (5) chronic myeloid leukemia (CML) in acceleration.

In group 2, transformation of MDS to full-blown AML was demonstrated by serial bone marrow aspirations. Group 3 comprised three patients with AML in relapse refractory to aggressive reinduction regimens (e.g., TAD, high-dose cytosine arabinoside). Morphologic diagnoses of MDS patients are detailed in Table 1. Two patients presented with CML in acceleration refractory to conventional chemotherapy (e.g., busulfan, hydroxyurea).

Ara-C Treatment

Twenty-five patients were treated by s.c. bolus injections at a dosage of 10 mg/m^2 every 12 h. Ara-C was given to 6 patients by continuous i.v. infusion at a dosage of 20 mg/m^2 every 24 h. The duration of the induction course was designed to be 21 days in both groups and after achieving remission, maintenance therapy was given in the same dosage for 7 days at monthly intervals. Often induction therapy had to be shortended because of severe thrombocytopenia (Table 3), so that duration of induction treatment was in the range of 7–22 days.

Results

Remission Rates

Responders were divided into three categories: (1) CR; and (2) partial remission (PR) according to the CALGB group; (3) "responders" (R) were patients showing a decrease in peripheral blast count of more

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Table 1. Patients treated with low-dose Ara-C, total number: 31

Diagnosis	Age (years)	Sex
De Novo AML: 11 – M1: 6 – M2: 3 – M4: 1 – M5: 1	Range 40–80 Median 67	M 5 F 6
AML secondary to MDS: 10 – M1: 2 – M2: 5 – M4: 2 – M5: 1	Range 41–76 Median 62	M 2 F 8
AML in relapse (after TAD): 3 – M2: 3	Range 55–67 Median 66	M 1 F 2
Myelodysplasia: 5 – RAEB: 2 – RAEB-t: 2 – CMML: 1	Range 19–72 Median 49	M 2 F 3
CML in acceleration: 2	Range 30–64	M 1 F 1

Table 2. Results of treatment with low-dose Ara-C

Total number of CR: 5 (=17%)

Duration of CR: 4, 4, 9, 5+, 5+ months

De Novo AML ($n=11$): CR: 3 (=27%) PR: 1 R[a]: 4 NR: 3	AML secondary to MDS ($n=10$): CR: 1 (=10%) PR: 0 R[a]: 4 NR: 5
Myelodysplasia ($n=5$): CR: 1 PR: 0 R[a]: 1 NR: 3	AML in relapse ($n=3$): R[a]: 1 NR: 2 CML in acceleration ($n=2$): fall in leukocyte count ≧50%: 1 NR: 1

[a] Fall in peripheral blast count ≧50%.

than 50%. All other patients were classified as nonresponders (NR) (Table 2). In 31 patients treated with LDAC CR was obtained in five cases and PR in one. Another ten patients could be classified as R. Duration of CR was 4; 4, 9, 5+ and 5+ months, respectively. Among the patients who did not respond there were two cases of early death. The patients died of leukemic progression during the first week of therapy. All other AML and MDS patients with initial leukocytosis (>10 000/ μl) showed a significant decrease in leukocyte count (>50%).

The highest CR rate was obtained in patients with de novo AML ($n=11$): 3 CRs, 1 PR, 4 Rs, and 3 NRs. CR rate in patients with AML secondary to MDS ($n=10$) was significantly lower: 1 CR, 4 Rs and 5 NRs. Among the MDS patients, one patient with refractory anemia with excess of blasts in transformation (RAEB-t) achieved CR. Due to the small number of MDS patients

Table 3. Side effects of treatment with low-dose Ara-C

	Bolus s.c. injection	Continuous i.v. infusion
No. of patients	25	6
No. of courses	73	9
No. of courses interrupted because of severe fall in thrombocyte count	17	1
No. of patients with decrease in leukocyte count below 1000/μl	8	0
Decrease in BM cellularity	12	1

treated by LDAC a definite CR rate cannot be given. In one patient with CML in acceleration (peripheral blast 16%, bone marrow blast 10%) refractory to other forms of therapy (vincristine, vinblastine, prednisone, hydroxyurea), LDAC led to a significant decrease in leukocyte count and halted the progression of splenic enlargement. A significant reduction of relative peripheral blast count could not be observed. One patient with chronic myelomonocytic leukemia (CMML) showed a similar response.

Side Effects

LDAC therapy was accompanied in many patients by marked bone marrow hypoplasia. In 18 cases induction therapy had to be interrupted because of a severe decrease in thrombocyte count ($<20000/\mu l$). One patient with RAEB-t, whose initial thrombocyte counts were already less than $10000/\mu l$, died of cerebral bleeding. Eight patients developed leukocytopenia with a leukocyte count below $1000/\mu l$. In 13 patients a decrease in bone marrow cellularity after therapy could be proved by serial bone marrow aspiration. All patients who achieved remission showed bone marrow hypoplasia. Hepatotoxicity due to LDAC administration was not observed.

There is a striking difference in the rate of bone marrow hypoplasia depending on the method of administration, with the continuous i.v. infusion having the advantage (Table 3). However the number of patients treated this way is not large enough to permit a reliable assessment as to whether the method of administration influences the remission rate. Five CRs were obtained in the group of 25 patients treated by s.c. bolus injections and one of the six patients treated with continuous i.v. infusion showed PR of de novo AML.

Discussion

The present study shows that of the patients treated with LDAC, those presenting with de novo AML achieved the highest remission rate (27%), while the prognosis of patients with AML secondary to MDS is worse (CR rate 10%). A similar difference in prognosis is also known for patients treated with aggressive chemotherapy (e.g., TAD). Remission rate for AML patients treated with LDAC (CR rate 27%) is significantly lower as compared with AML patients treated according to the TAD regimen (CR rate 65%) [1]. This is probably not due to the high number of elderly patients in our LDAC group (range 40–80 years), because in the group of elderly patients treated with TAD (range 65–78 years), CR rate is still twice as high (51%). There is further evidence that TAD therapy is much more effective. We treated three patients (two de novo AML, one AML secondary to MDS), refractory to LDAC or in relapse after LDAC-induced CR, according to the TAD regimen. All patients achieved CR. On the other hand, three patients presenting with AML in relapse and refractory to further aggressive reinduction regimens were treated with LDAC (s.c. bolus injections). Only one of these patients responded by a transient decrease in peripheral blast count and all three patients died of progressive AML. Duration of CR in AML patients treated with LDAC (median 5 months) seems to be much shorter compared to AML patients treated with TAD (median 12 months).

Table 4. Possible indications for low-dose Ara-C therapy

AML
– for patients in poor condition (e.g., elderly patients)
– if TAD is contraindicated (e.g., infection)
MDS
– RAEB, RAEB-t, CMML
CML in acceleration
– refractory to conventional therapy

On the other hand, LDAC induces a high rate of bone marrow hypoplasia with patients being threatened by thrombocytopenia and agranulocytosis.

Therefore, LDAC should only be used for patients in whom TAD is contraindicated (e.g., infection) or patients in poor condition (e.g., elderly patients). Patients with severe acute infection can be treated with LDAC to halt further leukemic progression while the appropriate anti-infectional therapy is given. After overcoming the infection, aggressive chemotherapy (e.g., TAD) may be possible. LDAC can also be used for MDS patients because CR can be achieved. Due to the small number of MDS patients treated with LDAC we are not yet able to determine the CR rate. There might also be some benefit of LDAC therapy for patients presenting with CML in acceleration refractory to other drugs (Table 4).

Regarding the side effects, it is obvious that a number of patients, including all patients achieving CR, showed bone marrow hypoplasia with a severe decrease in thrombocyte and leukocyte count. Perhaps the rate of bone marrow hypoplasia can be reduced by continuous i.v. administration. This might be due to pharmakokinetic differences in Ara-C plasma levels demonstrated by Spriggs et al. [7]. These considerations led us to change the administration to continuous i.v. infusion. It remains to be seen whether the remission rate is the same as in patients treated with s.c. bolus injections.

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Low-Dose Ara-C Treatment in Elderly Patients with Acute Myeloblastic Leukemia*

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Aim of the Study

Established induction treatment regimens have so far failed to achieve long remissions in patients with myelodysplastic syndromes (MDS) in transformation to acute leukemia, chronic myeloid leukemia (CML) in transformation to blast crisis, and in elderly patients suffering from acute leukemia. In particular, commonly used high-dose chemotherapy, including anthracyclines or high-dose cytosine-arabinoside (Ara-C), is very often too toxic for these patients.

Based on the in vitro experiments by Lotem [1] and Sachs [2] indicating the capacity of Ara-C to induce differentiation of leukemic cells, many authors introduced low-dose (LD) Ara-C into the treatment of patients with acute myeloblastic leukemia (AML) and MDS and observed complete and partial remissions (CR and PR) [3–20].

It is not quite certain if LD Ara-C can induce differentiation of leukemic cells in vivo too, or if the cytotoxic effect of this drug is capable of achieving the clinical improvement. Probably both effects exist, and some publications point to this possibility [14, 21–25]. All the published results suggest that in general LD Ara-C is well-tolerated. Therefore this form of application appeared to offer a useful alternative to intensive induction chemotherapy in those patients in whom such treatment is usually poorly tolerated, and it also had the advantage that it could be given to outpatients.

Material and Methods

LD Ara-C was given to (a) 22 AML patients over 60 years; (b) eight patients with CML in blast crisis (cML-BC); and (c) 11 patients with MDS in transformation to acute leukemia.

The malignant transformation of MDS and CML was defined as more than 20% blast cells in bone marrow and/or more than 5% blasts in peripheral blood. No prior chemotherapy was accepted, except busulphan or dibrommannitol for CML in the chronic phase.

Exclusion criteria were a decreased renal function (creatinine over 175 µmol/liter or 2.0 mg-%), or an important extramedullary involvement with severe thrombocytopenia (below 40 GPT/liter or 40000/mm^3). The treatment schedule was 10–15 mg/m^2 (about 20 mg) Ara-C s.c. (or as a short infusion), every 12 h daily for at least 14 days (up to 4 weeks). If necessary, the cycle was repeated after 2–4 weeks.

In all patients cytomorphology and cytochemistry of bone marrow was examined before treatment and 2 weeks after treatment or after the aplastic phase. Cytochemistry included PAS, POX and ANAE staining.

Criteria for complete remission were blast cells below 5% and normal cellularity in bone marrow, granulocytes over 1.5 GPT/liter, platetets over 100 GPT/liter and hemoglobin over 6.2 mmol/liter or 10 g/100 ml in peripheral blood.

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Results

The data are summarized in Tables 1–4 and Fig. 1. In the cases with AML, seven CRs and eight PRs were obtained in 22 patients receiving LD Ara-C. The duration of the CRs has so far been a mean 15.5 months, four patients are still in therapy. It seems that FAB classification types M_2 and M_4 are particularly suited to this form of treatment.

Table 1. Acute nonlymphatic lrukemias

Number of patients	FAB	Age/sex	Number of cycles	CR	PR	F
3	M_1	66±3 2 f, 1 m	3	–	1	2
6	M_2	71±5 3 f, 3 m	2.2	3	3	–
2	M_3	61.5±0.5 2 m	3	–	–	2
8	M_4	69±6 5 f, 3 m	2.4	4	3	1
2	M_5A	63.5±0.5 1 f, 1 m	3	–	–	2
1	M_5B	66 1 f	3	–	1	–
Total 22	–	∅ 67.8 12 f, 10 m	∅ 2.5	7	8	7

Table 2. CML in blast crisis

Number of patients	Age/sex	Number of cycles	Response	Failure
8	64±4 5 f, 3 m	2.4	5	3

Table 3. MDS in transformation to acute leukemia

Number of patients	Age/sex	Number of cycles	Response	Failure
11	67±6 7 f, 4 m	2.2	6	5

Table 4. Tentative results of response duration

Diagnosis	Number of patients	Average values (in days)	Lowest and peak values	Patients still in therapy[a]
AML CR	7	466	246–544	4
AML PR	8	254	57–387	3
CML in blast crisis	5	104	31–212	1
MDS in transformation	6	189	75–291	3

[a] Reinduction between 4 to 10 weeks ober 5 days.

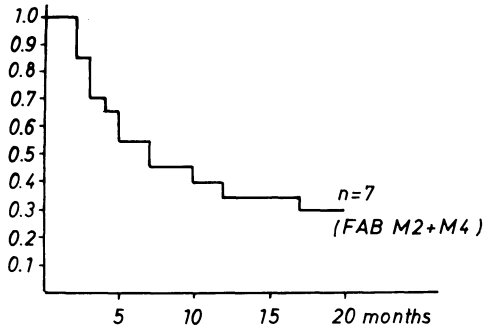

Fig. 1. Survival of all AML patients observed in the study ($n=22$)

In CML-BC in elderly patients five responses of only 3.5 months median duration were registered; however, there is one patient still in response after 212 days. In the cases of MDS in transformation six responses to treatment were seen. The median duration of response is 6.3 months, three patients are still in therapy after more than 9 months.

The mean survival duration in nonresponders to LD Ara-C was 3.6 months (range 1–6 months) in the 15 patients in all diagnostic groups. Eleven of these patients have received further antileukemic therapy with other agents. During treatment, peripheral pancytopenia developed or worsened in all patients, necessitating red cell and platelet transfusions. In most responders a rapid rise in platelets and leukocytes was noticed about ten days after the end of the cycle. Bone marrow cytology revealed hypoplasia in 18 of the 26 responders. In seven patients clear signs of maturation of blast cells could be seen. The treatment was subjectively well-tolerated without nausea, vomiting, hair loss or serious infectious complications. There were no treatment-related deaths. However, the toxic effect upon the bone marrow was considerable.

Conclusion

LD Ara-C treatment seems to be a possible and sparing therapy, especially for AML in old age. The results, for instance in the FAB classification types M_2 and M_4 are no worse than in high-dose chemotherapy. On the other hand, LD Ara-C shows only few side effects and is well-tolerated. Therefore, the given treatment schedule can be recommended for those cases where intensive induction chemotherapy is usually poorly tolerated. Powell et al. [26] showed that in patients aged 60 years or more the higher CR rate with high-dose Ara-C treatment is associated with a high induction death rate.

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Acute Leukaemia in the Elderly, Remission Induction Versus Palliative Therapy

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Most patients with acute myeloid leukaemia (AML) are over 60 years old [1], remission rates are lower and survival poorer for this group in both AML [2] and for older patients with acute lymphocytic leukaemia (ALL) [3]. Current intensive therapy protocols have been proposed as the treatment of choice for these patients although published studies in AML advocating this approach contain a lower percentage of patients over 60 than would otherwise be expected [4, 5]. Such studies therefore do not appear to reflect the whole problem of acute leukaemia in the elderly.

We have reviewed the outcome in 25 consecutive patients over 60 with all forms of acute leukaemia (except chronic myeloid leukaemia [CML] blast crisis). These patients were seen over a 3-year period and accounted for 60% leukaemic referrals during this period. Characteristics are detailed in Table 1, median age of the series was 73 years. A documented myelodysplasic syndrome (MDS) was present in six patients before diagnosis of acute leukaemia; four patients had refractory anaemia with excess of blasts (RAEB) and two had primary acquired sideroblastic anaemia (PASA). Three patients had a previously treated malignancy: breast carcinoma treated by irradiation in two patients and bronchial carcinoma treated by chemotherapy in one.

Remission induction (RI) with standard regimes was attempted in 12 patients (10 AML, 1 acute undifferentiated leukaemia AUL, 1 ALL); complete remissions (CR) were achieved in six (50%) (5 AML, 1 ALL); no response occurred in two patients and three patients died before treatment could be evaluated, the remaining patient achieved a

Table 1. Acute leukemia in over-60s – all patients

Number of patients		25
Age range (years)		60–87
Median age (years)		73
Mean age (years)		72.5
Type of leukemia:	AML	22
	ALL	3
	AUL	1
Preceding myelodysplasia[a]		6
Previous radiotherapy of chemotherapy		3
Survival range (weeks)[b]		0.5–90+
Median survival (weeks)		7.5

[a] See text.
[b] As at 31/12/85.

Table 2. Remission induction patients

Number of patients		12
Age range (years)		60–75
Median age (years)		68.5
Mean age (years)		66.9
Type of leukemia:	AML	10
	ALL	1
	AUL	1
Preceding myelodysplasia[a]		2
Previous radiotherapy of chemotherapy[b]		1
Survival range (weeks)[c]		1.4–90+
Median survival (weeks)		9

[a] See text; 1 RAEB, 1 PASA.
[b] Irradiation of breast carcinoma.
[c] As at 31/12/85.

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Table 3. Remission induction patients – outcome

Number of patients	12
Achieved	
complete remission (CR)	6 (5 AML, 1 ALL)
partial remission	1
no response	2
Supportive deaths	2
Early death	1
(Total	12)
Remission duration (weeks)[a]	29–75+
Early remission deaths	2 (1 AML, 1 ALL)

[a] As at 31/12/85.

Table 4. Palliatively treated patients

Number of patients	13
Age range (years)	61–87
Median age (years)	73
Mean age (years)	77.7
Type of leukemia: AML	11
ALL	2
Preceding myelodysplasia[a]	4
Previous radiotherapy or chemotherapy[b]	2
Survival range (weeks)	0.4–23.3
Median survival (weeks)	7

[a] See text: 3 RAEB, 1 PASA.
[b] 1 irradiation of breast carcinoma, 1 chemotherapy of bronchial carcinoma.

Table 5. Contraindications to remission induction

Co-existent serious medical problems	7
Adverse psychological or social factors	4
Judged by physician not be able to withstand treatment	9
(Total: 13 patients in group)	

partial remission. Details are given in Tables 2 and 3; the two deaths early in remission were due to pneumonia (1 ALL) and haemetemesis (1 AML).

Supportive therapy was given to 13 patients, additional palliating single-agent treatment with prednisolone, hydroxyurea or cytosine was used in six patients in this group. Although no long-term survivors occurred in the group, eight of nine patients who survived more than 4 weeks spent less than 26% of their survival time in hospital. Details and outcome of this group are given in Table 4.

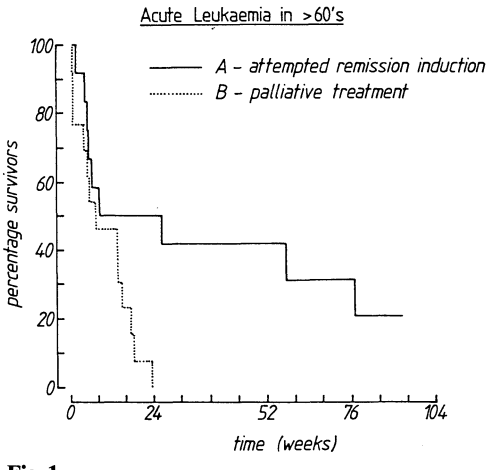

Fig. 1.

Examination of age and presenting Karnofsky status showed only age to be significant in separating the two groups, untreated patients were significantly older ($P < 0.001$). Decisions to offer (RI) therapy were clinical; reasons not to attempt (RI) came under three headings and are listed in Table 5. Individual patients in this group had at least one contra-indication present.

Survival graphs for each group are shown in Fig. 1. Median survivals for those intensively treated and those managed palliatively were similar but survival beyond 26 weeks only occurred in the treated group. One year survival rate for all patients with acute leukaemia was 20% (5 of 25 patients). Of the remitters one patient remains in CR beyond 75 weeks, the others relapsed at 29 weeks, 40.5 weeks, and 52.4 weeks.

From this study it appears that at least 50% of patients over 60 with acute leukaemia are unsuitable for presently used intensive chemotherapy. Studies on the treatment of elderly leukaemic populations must take such patients into account [6, 7]; a small number of elderly patients do benefit from intensive treatment, even so most elderly leukaemics still die within 26 weeks. Further studies on optimum management of acute leukaemia in the over 60s are needed.

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The Use of Amsacrine plus Intermediate-Dose Cytosine Arabinoside in Relapsed and Refractory Acute Nonlymphocytic Leukemia

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Introduction

In a previous study [1] we found that cytosine arabinoside (Ara-C) in an intermediate dose of 500 mg/m^2 every 12 h for 6 days was an effective therapy with only a mild degree of toxicity in not heavily pretreated patients with acute nonlymphocytic leukemia (ANLL). This dose was calculated to be sufficient for saturation of the phosphorylating enzymes. Amsacrine (AMSA) monotherapy appears to be active in refractory or relapsed ANLL [2]. In this study we investigated the efficacy and toxicity of a combined regimen of Ara-C with AMSA in 27 patients with relapsed or refractory ANLL or myeloid blastic crisis of chronic myeloid leukemia (CML). The remission induction therapy consisted of the optimal dose schedule for AMSA, 150 mg/m^2 every 24 h [2], and Ara-C, 500 mg/m^2 every 12 h for 5 days each [1]. The same combination was used as consolidation treatment but only for 3 days. Patients were evaluated for response using the standard criteria of the Cancer and Acute leukemia Group B (CALGB). Toxicity was evaluated according to the WHO criteria [3].

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Results

A total of 27 patients were treated, 22 with ANLL in relapse or refractory to conventional therapy [4] and five with untreated myeloid blastic crisis of CML. The median age was 47 years ranging from 17 to 71 years. The result of treatment with AMSA and intermediate-dose Ara-C was evaluated after one course of treatment (Table 1). Of the 27 patients, 11 (41%) achieved complete remission (CR), the median duration of CR was 6 months. Out of 12 patients with drug resistance four died of infection and one of pulmonary toxicity. The gastrointestinal side effects were substantial (Table 2). The degree of nausea and vomiting was moder-

Table 1. Response to AMSA plus intermediate-dose Ara-C in patients with acute nonlymphocytic leukemia

	No. of patients	CR (%)	PR	Failure (%)	Death during aplasia
Refractory ANLL	10	3 (30)	–	7 (70)	–
First relapse ANLL	8	5 (63)	–	2 (25)	1
Second relapse ANLL	4	2 (50)	–	–	2
BC-CML	5	1 (20)	1	3 (60)	–
Total	27	11 (41)	1	12 (44)	3 (11)

Median duration of complete remission: 6 months, range 4–14 months.

Table 2. Side effects of *remission induction* with AMSA plus intermediate dose Ara-C in 27 patients

	No. of patients			
	Grade 1	Grade 2	Grade 3	Grade 4[b]
Gastrointestinal				
Nausea/vomiting	0	9	17	1
Diarrhea	4	4	5	3
Stomatitis/oseophagitis	3	7	5	1
Hepatic				
Hyperbilirubinemia	13	0	0	1
Elevated transaminases	7	4	0	0
Conjunctival[a]	1	0	0	0
Cutaneous	6	0	0	0
Neurologic	0	0	0	0
Cardiac	0	0	0	0
Pulmonary	0	0	0	1

[a] Prophylactic glucocorticoid eye drops were used.
[b] WHO grades of toxicity.

Table 3. Side effects of *consolidation* with AMSA plus intermediate-dose Ara-C in 11 patients

	No. of patients			
	Grade 1	Grade 2	Grade 3	Grade 4[b]
Gastrointestinal				
Nausea/vomiting	3	7	1	0
Diarrhea	0	1	0	0
Stomatitis/oesophagitis	3	1	0	0
Hepatic				
Hyperbilirubinemia	3	0	0	0
Elevated transaminases	3	0	1	0
Conjunctival	0	0	0	0
Cutaneous	2	0	0	0

No neurologic, cardiac or pulmonary toxicity.
[a] WHO grades of toxicity.

ate to severe in 67% of the patients and severe diarrhea occurred in 30%. About 50% of the patients had stomatitis. The toxicity, however, in patients receiving the consolidation therapy was mild (Table 3). Hematologic recovery occurred with a median of 21 days (range, 11–37) after therapy. Although remission induction therapy was given for 5 days and consolidation therapy for 3 days, the same degree of myelosuppression was observed. There were 23 infections during 38 courses of cytotoxic therapy, 8 of 12 bacteremias were caused by α-hemolytic streptococci.

Discussion

The combination of AMSA with intermediate-dose (ID) Ara-C appears to be an effective therapy in relapsed ANLL (CR 58%) and also has a moderate effect in refractory leukemia (CR 30%). These results are comparable to regimens using high-dose (HD) Ara-C with or without anthracyclines or AMSA [5–9]. The toxicity of HD Ara-C is well-known [10] and of a higher degree than caused by ID Ara-C [1]. In our present regimen gastrointestinal side effects and mucositis were substantial. It is likely that the

high total dose of AMSA used in this regimen caused the observed mucosal toxicity [2]. AMSA and ID Ara-C used for 3 days each, as consolidation treatment, was associated with only a mild degree of mucositis but with the same intensive myelosuppression as observed after the 5-day regimen. These results suggest that a 3-day therapy with AMSA in this regimen could be as effective as a 5 days with AMSA in remission induction therapy with lower toxicity, and this deserves further evaluation.

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Phase I/II Trial of High-Dose Cytosine Arabinoside and Mitoxantrone in Adult Refractory Acute Myeloid Leukemia

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Introduction

In spite of a high initial response rate of 60% –80% the vast majority of adult patients with acute myeloid leukemia (AML) still cannot be cured of the disease and ultimately die from recurrent and refractory leukemia. The development of new therapeutic approaches and of more effective drugs therefore seems warranted.

Besides amsacrine (mAMSA), etoposide and 5-azacytidine, high-dose cytosine arabinoside (HD Ara-C) and the newly developed anthracendion and anthracycline derivatives mitoxantrone and aclacinomycin-A have proved to be effective in relapsed or refractory AML within the last few years [2, 7, 8, 10, 15, 16, 18–20, 22, 24]. On the basis of a standardized first-line treatment in the multicenter trial of the German AML Cooperative Group [3, 4] the combination of HD Ara-C and mitoxantrone was applied to patients whose AML was considered refractory against conventional regimens in order to assess the toxicity and antileukemic activity of the two-drug combination.

Patients, Treatment Protocol, and Methods

The study comprised 33 patients from 7 centers in the Federal Republic of Germany. Three patients had an antecedent myelodysplastic syndrome (MDS) which turned into an overt AML, one patient had been treated with radiation therapy for breast cancer 2 years before the onset of leukemia.

All patients were recruited from the German Cooperative Group AML trial and had received cytosine arabinoside at conventional doses and daunorubicin at doses between 260 and 990 mg/m^2.

Patients were eligible for HD Ara-C/mitroxantrone if they met the following criteria defining refractory disease [12, 13]:

1. Primary resistance against 2 TAD-9 induction courses ($n=8$)
2. Early relapse within the first 6 months of achieving complete remission (CR) ($n=10$).
3. Later occurring relapse with nonresponse to one additional induction-type TAD-9 cycle ($n=6$).
4. Second and subsequent relapses ($n=9$).

The treatment regimen consisted of HD Ara-C 3 g/m^2 every 12 h by a 3-h infusion on days 1–4. Mitoxantrone was started at 12 mg/m^2 per day on days 3, 4 and 5 and was escalated in subsequent cases to 4 and 5 doses of 10 mg/m^2 per day on days 2–5 and 2–6, respectively.

Toxicity was assessed according to WHO criteria, while the antileukemic efficacy was judged according to CALGB criteria [17, 23, 25].

Results

Thirty of the 33 patients were treated with one course of HD Ara-C/mitoxantrone while a second cycle was given in three cases,

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accounting for a total of 36 treatment courses.

CR was achieved in 17 patients after one ($n=15$) or two ($n=2$) courses of treatment. One additional patient achieved a partial remission (PR) after one cycle. Twelve patients succumbed to infectious complications within the first 5 weeks after the start of therapy, one patient died because of an acute cardiomyopathy after the first dose of mitoxantrone on the 2nd day of treatment. Persistant or recurrent AML was observed in three cases.

Except for the one case of sudden cardiac death, toxicity was mild to moderate consisting mainly of nausea and vomiting, mucositis and diarrhea. Central nervous system (CNS) toxicity of grades 1 to 3 was encountered in five cases.

Two of the 17 CR patients underwent a bone marrow transplantation after 1+ and 2+ months while 13 cases remained untreated. Two patients were started on a monthly maintenance regimen consisting of two doses of HD Ara-C 3 g/m^2, and one dose of mitoxantrone 10 mg/m^2. Ten patients have relapsed after 1–5 months while four cases are still in CR at 1+, 3+, 4+, and 6+ months.

Discussion

The response rate of 52% CR achieved by HD Ara-C/mitoxantrone in heavily pretreated AML patients whose disease was considered refractory to conventional regimens clearly demonstrates the high antileukemic efficacy of the two-drug combination. These data compare favorably with other HD Ara-C combinations and underline preclinical evaluations indicating a possible synergistic effect of the two drugs [1, 5, 6, 9, 11, 14, 21, 26].

Although the high incidence of early deaths seems disturbing one must take into account that new aggressive approaches in advanced stages of AML carry a high risk of complications during the treatment-induced phase of bone marrow aplasia. It must also be considered that patients with antecedent hematologic disorders and breast cancer were included in the present study, and that their chances of responding to an antileukemic therapy are already substantially diminished from the onset of their leukemic phase.

Hence, the present combination of HD Ara-C/mitoxantrone may be considered as one of the most effective antileukemic regimens and deserves incorporation during earlier stages of AML treatment.

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Mitoxantrone and VP-16 in Refractory Acute Myelogenous Leukemia

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Introduction

Despite improvement in the complete remission (CR) rate of adult acute myelogenous leukemia (AML), the majority of patients will relapse within 2 years of attaining CR [1–4]. The most effective agents for remission induction are daunorobucin, cytarabine and 6-thioguanine. Of the patients who relapsed or who became refractory, few achieved a second CR upon further treatment with the initial induction regimen.

Recently, some studies have reported on the effectiveness of mitoxantrone in relapsed and refractory acute leukemia [5–8]. In a previous multicenter cooperative study, we treated 24 patients who had refractory or relapsed acute leukemia with mitoxantrone as a single agent. Four of the 13 patients with AML achieved a CR, the longest duration of which is now 20+ months [9]. Etoposid (VP-16) as a single agent has also been shown to be effective in the treatment of AML [10–11]. This multicenter phase I/II study was undertaken to evaluate the efficacy of the combination of mitoxantrone and VP-16 in refractory AML.

Materials and Methods

Patient Eligibility

Adult patients (>15 years) with refractory AML were eligible for the study if they had normal liver and renal functions and did not have active infections or signs of cardiac insufficiency. Diagnosis and classification of the AML were made according to the FAB criteria [12]. Patients with the followings are considered to be refractory: (1) failure to achieve a CR after two induction courses of conventional TAD, which consists of 6-thioguanine 100 mg/m^2 every 12 h for 7 days, cytarabine 100 mg/m^2 every 12 h for 9 days and daunorubicin 45 mg/m^2 per day for 3 days; (2) relapse within the first 6 months after achieving the first CR; (3) failure to achieve a second CR by one other induction course in first relapse, occurring more than 6 months after CR; (4) first relapse under continuous maintenance therapy, which consists of monthly administration of cytarabine 100 mg/m^2 every 12 h s.c. for 5 days, combined alternately with daunorubicin, 6-thioguanine, and cyclophosphamide [13]; (5) second or subsequent relapses.

Treatment Protocol

Mitoxantrone was given at a dosage of 10 mg/m^2 per day, for days 1 to 5 as rapid i.v. infusion (15 min). The initial schedule of VP-16 was 100 mg/m^2 per day for days 1 to 3. In subsequent groups of at least five patients each, escalation of VP-16 was achieved by increasing the number of days of therapy to a maximum of 5. The cardiac ejection fraction was assessed by means of echocardiography or by multigated isotope ventriculography (MUGA) before and after treatment. If no CR was achieved with the first course and there was a blast reduction of >50%, judged when marrow cellularity

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was >2+, a second course was to be administered. Patients who achieved a CR were to be given one course of consolidation therapy, which consisted of mitoxantrone 8 mg/m^2 per day, days 1–5; VP-16 75 mg/m^2 per day, days 1–5 and cytarabine 75 mg/m^2 every 12 h for days 1–5. The study protocol was reviewed by each institution's protocol review committee and informed consent was obtained before therapy.

CR was defined as less than 5% blasts in a normocellular bone marrow with a granulocyte count of >1000/μl and a platelet count of >100000/μl in peripheral blood and with normal physical findings. Partial remission (PR) was defined as either less than 5% blasts in marrow but incomplete recovery of blood counts or 5%–25% blasts in bone marrow but normalization of platelet and granulocyte counts in peripheral blood. Any status less than CR or PR was categorized under no response (NR). Toxicity was assessed by WHO criteria [14]. Duration of response was counted from the day of diagnosis of CR to the day of bone marrow relapse. Patient entry began on 1st December, 1984.

Results

As of 15th February, 1986, 32 patients were evaluable for response and toxicity. 22 patients were male and 10 female. Their median age was 46.0 years, ranging from 20 to 69 years. Other clinical data of these patients are summarized in Table 1.

Twenty-five patients received one induction course and seven patients received two induction courses. Higher response rates seemed to be achieved at higher dosages of VP-16 (Table 2). Two of the ten patients who received 3×100 mg/m^2 per day attained a CR, whereas CR was achieved in four of ten patients (40%) who received 4×100 mg/m^2 per day and eight of twelve patients (66.7%) who received 5×100 mg/m^2 per day VP-16.

Table 2. Treatment results in relationship to escalation levels of VP-16

	Escalation levels of VP-16		
	3× 100 mg/m^2 per day	4× 100 mg/m^2 per day	5× 100 mg/m^2 per day
Total	10	10	12
CR	2	4	8
PR	1	0	1
Median time to CR (days)	37	43	48.7

The median interval between initiation of therapy to diagnosis of CR was 41.0 days with a range of 25–82 days. One patient underwent autologous bone marrow transplantation 56 days after diagnosis of CR and is still in continuous CR after 393+ days. The median duration of CR was 52.0 days with a range from 7+ to 243 days, excluding the patient who received autologous bone marrow transplantation.

The toxic effects of this regimen are summarized in Table 3. This combination was subjectively well-tolerated by most patients. The most common nonhematologic compli-

Table 1. Clinical data of the patients in relationship to treatment results

	Diagnosis									
	Morphology					Status				
	M_1	M_2	M_3	M_4	M_5	Res	ER	R+Res	R+M	>2nd R
CR	1	4	0	5	4	4	4	0	3	3
PR	0	0	1	1	0	1	1	0	0	0
NR	4	3	1	7	1	8	3	3	1	1
Total	5	7	2	13	5	13	8	3	4	4

Res, primary resistance to TAD; ER, early relapse; R+Res, relapse and refractory; R+M, relapse under maintenance therapy; >2nd R, Second or subsequent relapse.

Table 3. Nonhematologic and hematologic toxicities of the regimen. The number of courses in which the corresponding side effects were observed are given. Total number of treatment courses was 39. Hematologic toxicity was expressed by the average number of transfusions and by the duration of parenteral antibiotic treatment required during one course

WHO grade	1	2	3	4
Nausea/vomiting	9	9	9	0
Stomatitis	7	11	4	0
Diarrhea	1	11	1	0
Obstipation	3	1	0	0
Hepatotoxicity	13	2	1	0
Infection/fever of undetermined origin	0	0	27	2
Cardiotoxicity	2	0	0	0
Alopecia[a]	0	0	12	0

[a] Present in 20 patients as a result of previous therapy.

Average number of transfusions per treatment course: erythrocytes: 8.5 ± 5.3 (mean + SD) units thrombocytes: 5.7 ± 5.9 units. Average duration of parenteral antibiotic treatment: 15.6 ± 14.1 days.

cations were mild nausea and vomiting, mild to moderate stomatitis, and mild hepatotoxicity as shown by elevations of either SGOT, SGPT, or bilirubin. Fever of unknown origin occurred in 14 patients and infections in 15 patients. The infections included 11 cases of pneumonia, two of septicemia and one case each of periorbital phlegmone and perianal abscess. Hematologic toxicity is expressed by the number of transfusions of erythrocytes (8.5 ± 5.3 units, mean $\pm$ SD) and of platelets (5.7 ± 5.9 units) required per treatment course and by the duration of parenteral antimicrobial treatment, on average 15.6 ± 14.1 days. There was only one report of cardiac toxicity (tachycardia of >120/min) persisting for 2 days and only one case of early death due to severe infection within the first 5 weeks of treatment.

Discussion

In this study we have demonstrated that the combination of mitoxantrone and VP-16 is active in the treatment of refractory leukemia. A CR rate of 43.8% was achieved.

As the toxic side effects associated with this combination might be unpredictable, patient safety was the main concern and a careful dosage escalation of VP-16 was done. We were aware that the initial schedule with 3×100 mg/m^2 per day of VP-16 might be inadequate, but was justified in order to reduce the incidence of toxicity. The present evaluation shows that higher remission rates are observed in patients receiving 4×100 mg/m^2 per day and 5×100 mg/m^2 per day, respectively. As the last escalation schedule has an acceptable toxicity risk and a considerable CR rate was achieved, we would suggest that a total dose of 100 mg/m^2 per day $\times 5$ is the optimal dose.

Other than nausea and stomatitis, the nonhematologic toxicities were mild. However, severe aplasia was observed in all patients and was usually of long duration. In those patients who responded, the mean time to recovery of granulocytes ($>1000/\mu l$) and of platelets ($>100\,000/\mu l$) was 37 days from initiation of therapy. The profound hematologic toxicity was reflected by the units of transfusions required per treatment course: on average 8.5 units of packed erythrocytes and 5.7 units of platelets. The degree of granulocytopenia was expressed by the duration of parenteral antimicrobial treatment which was 15.6 days (mean).

In conclusion, the present study shows that mitoxantrone combined with VP-16 is effective in refractory AML with an acceptable toxicity risk. 43.8% of the patients with resistant AML entered CR. It is possible that if this regimen is used in primary treatment, either for induction or for consolidation, an improved remission rate or prolonged remission duration would result.

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4-Demethoxydaunorubicin (Idarubicin) in Relapsed and Refractory Acute Myeloid Leukemia

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Introduction

Despite the progress in the treatment of adult acute myeloid leukemia (AML), most of the patients achieving complete remission (CR) with induction chemotherapy will relapse within 2 years. Therefore, there is an urgent need for new developments in the treatment of adult AML. The application of new chemotherapeutic drugs may be one way to achieve success. Because anthracyclines are highly effective in the treatment of AML, it may be possible that anthracycline analogues will increase the therapeutic results.

Idarubicin is the 4-demethoxy derivative of daunorubicin [1]. In preclinical studies it proved to be five times more potent than daunorubicin in L 1210 and P 388 leukemia [2]. The cardiotoxicity of idarubicin compared to adriamycin and daunorubicin was lower, resulting in a higher therapeutic index than the parent drugs have [3]. Idarubicin may be administered both i.v. and orally [4]. When administered i.v., the terminal half-life of idarubicin is 18.4 h. Idarubicin has a major and at least one minor metabolite. The plasma half-life of the major metabolite, 13-hydroxy-idarubicin, is 50–55 h [5]. Only 6.5% of the drug will be eliminated in the urine within 24 h [5]. Cytosine arabinoside (Ara-C) does not significantly alter the pharmakokinetics of idarubicin [6].

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Patients and Methods

The efficacy of idarubcin was tested in adult AML patients who fulfilled the following criteria: all patients had to be pretreated with the TAD-9 protocol [7], and (1) were primarily resistant to two cycles of TAD-9; (2) had a first relapse of AML within 6 months of achieving CR; or (3) had second or further relapse of AML.

We treated ten consecutive AML patients who fulfilled these study criteria. The seven male and three female patients had a median age of 42 years. The youngest patient was 30, the oldest 70 years of age.

According to the FAB classification three had M_1, four had M_2, two had M_4, and one had M_5 types of AML. Four patients had previously had two courses, five patients three courses, and one patient even four courses of the TAD-9 regimen. Four patients had additionally received 350–1050 mg aclacinomycin-A.

One of the ten patients was primarily resistant to TAD-9 therapy. Four patients had early relapse after 2½, 4, 5, and 6 months of CR, respectively. Five patients had a second or subsequent relapse.

All patients received idarubicin in combination with other cytostatic drugs. At the beginning of the study patients received idarubicin in sequential combination with Ara-C according to a proposal of Polli et al. [8] (regimen I). The next group of patients was treated with a simultaneous combination of idarubicin, Ara-C and etoposide according to a protocol of Carella et al. [9] (regimen II). In the last group we started to combine idarubicin with high-dose Ara-C

Table 1. Treatment regimens for refractory and relapsed AML patients

Regimen			Dosage	Administration	Days
I[a]		Idarubicin	10 mg/m^2 per day	i.v.	1–3
	+	Ara-C	2 × 120 mg/m^2 per day	i.v.	4–8
II[b]		Idarubicin	8 mg/m^2 per day	i.v.	1–3
	+	VP-16	150 mg/m^2 per day	i.v.	1–3
	+	Ara-C	150 mg/m^2 per day	24-h infusion	1–5
III		Idarubicin	8 mg/m^2 per day	i.v.	1–4
	+	High-dose Ara-C	2 × 3 g/m^2 per day	3-h infusion	1–4

[a] Modified from Polli et al. [8].
[b] Protocol of Carella et al. [9].

(regimen III). The dosages and schedules are listed in Table 1.

The first five patients were treated with one course of regimen I. The next four patients received two cycles each of regimen II. Additionally, one patient not responding to regimen I was treated with regimen II. One patient with early relapse was primarily treated with one course of regimen III and two patients received regimen III as secondary therapy after treatment with regimen II. Response to treatment was judged according to the CALGB criteria.

Results

The results of the idarubicin combination treatment in the ten refractory or relapsed AML patients are summarized in Table 2.

Table 2. Results of idarubicin-based salvage treatment in refractory and relapsed AML patients

	n	CR	PR	NR	ED
Primary resistance	1	–	–	1	–
Early relapse	4	–	2	2	–
2nd or further relapse	5	2	–	2	1
Total	10	2	2	5	1

The patient primarily resistant to two cycles of TAD-9 achieved no remission (NR) after treatment with two courses of regimen II and died half a month after regimen III therapy.

Two out of four patiens with early relapse came into partial remission (PR). One PR was obtained after one cycle of regimen II, but could not be completed by a further one. The other PR was achieved by one course of regimen III. Two patients with early relapse did not respond to one course of regimen I or two courses of regimen II, respectively.

Two out of five patients with a second relapse came into CR, both after one course of regimen I or II, respectively. The patient responding to regimen I had been heavily pretreated with anthracyclines. He had previously received 1080 mg daunorubicin and 1050 mg aclacinomycin-A. Two patients with a second or fourth relapse, respectively, showed a marked decrease in the initially high peripheral white blood cell counts, but had no significant blast cell reduction in the bone marrow. Therefore, they had to be classified as treatment failures. The fifth patient with a second relapse died from septicemia four weeks after beginning regimen I therapy [early death (ED)]. He had bone marrow aplasia without blast cells. This patient had been pretreated with four courses of TAD-9 with a cumulative daunorubicin dose of 1420 mg. A subsequent therapy with a total dose of 840 mg aclacinomycin-A in combination with Ara-C had not altered the leukemic infiltration in the bone marrow.

All the patients have died since the study. Their median survival from start of idarubicin-combination salvage therapy was 4 months ranging from 1 to 10 months. The duration of the two CRs was 2 and 4 months, respectively. The two PRs lasted 1 and 3.5 months, respectively.

The treatment was relatively well-tolerated. However, all patients needed platelet transfusions and antibiotic therapy. Some patients suffered from diarrhea. Two patients had a short and reversible liver function abnormality. No patient showed clinical signs of congestive heart failure or cardiac arrhythmias.

Discussion

The outlook for refractory or relapsed patients with AML is still poor though the published results of the salvage treatments vary widely. This may be related to the different kinds and the intensity of pretreatment patients had received.

In the literature 14 CRs and 79 previously treated acute leukemia patients were achieved by single-agent treatment with idarubicin [8, 10, 11]. These patients had AML as well as acute lymphocytic leukemia (ALL). The pretreatment of the patients was different and in most instances not as aggressive as the TAD-9 regimen. The kind of relapse was not stated exactly. The same holds true for the published results with idarubicin and Ara-C combination chemotherapy. Here, five CRs were obtained in 28 pretreated patients [8, 12]. Our results with idarubicin-based combination tratment confirm a CR rate of 20% and an overall remission rate of 40% in heavily pretreated patients who fulfilled strict criteria for prospective evaluation.

In conclusion, idarubicin in combination with Ara-C ± etoposide is effective in relapsed AML, even in intensively pretreated patients. The combination of idarubicin and Ara-C ± etoposide does not show a complete cross resistance to other regimens containing anthracycline, e.g., the TAD-9 protocol. The duration of remission and survival in heavily pretreated patients is short. In our opinion idarubicin is a promising drug and deserves further evaluation in the treatment of AML.

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Intensive Induction and Consolidation Chemotherapy for Adults and Children with Acute Myeloid Leukaemia (AML) Joint AML Trial 1982–1985

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Introduction

Complete remission (CR) can now be obtained in approximately 75% of patients presenting with AML [1–4]. It is not certain, however, whether subsequent intensive therapy ("consolidation") will prolong remission duration in patients achieving CR [5]. Furthermore, if consolidation therapy is important, the optimum number of courses remains to be established. The role of allogeneic bone marrow transplantation (BMT) in such patients is not clear. Although it seems that relapse rates after BMT are lower than those observed after chemotherapy, overall survival in the former group may not be superior due to increased deaths following transplant-related complications [6, 7].

In this study of intensive induction and consolidation chemotherapy for adults and children with AML we have attempted to address the following questions. First, we wished to confirm the high CR rates obtained by others. Secondly, we compared a consolidation protocol consisting of five courses of non-cross-reacting agents with a protocol containing three courses, including high-dose melphalan as the final course. Finally, we compared the survival of patients under the age of 40 years who received an allogeneic BMT with those who received further chemotherapy.

Materials and Methods

Patients

Patients with AML were eligible for inclusion in the trial if they were less than 60 years of age at diagnosis, had no evidence of preexisting myelodysplasia and had received no prior chemotherapy.

The diagnosis of AML was made on Romanowsky-stained films of blood and bone marrow, further defined by the use of cytochemical stains (Sudan black and α-naphthyl acetate esterase) and more recently by specific myeloid monoclonal antibodies. The subtype of leukaemia was classified according to the criteria of the FAB group.

Treatment Protocols

All patients had induction chemotherapy with daunorubicin, 50 mg/m^2 given by rapid i.v. infusion on days 1, 3 and 5, and 10 days each of cytosine arabinoside (Ara-C) (100 mg/m^2 by 24 h continuous infusion) and 6-thioguanine (75 mg/m^2 b.d. by mouth). Patients who entered CR then had a further identical course after peripheral blood and bone marrow recovery. Patients not in CR after one course were given the second course at the discretion of the clinician. Patients who had no response to one or two courses were withdrawn from the

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trial and second-line chemotherapy was given. All patients in CR then received further chemotherapy with the combination of amsacrine, 5'azacytidine and etoposide all at a dose of 100 mg/m^2 for 5 days (MAZE).

Patients under 40 years, in CR after recovery from this course, were offered allogeneic BMT if they had an HL-A-matched sibling, other patients were randomised to receive one further course of chemotherapy consisting of a single dose of melphalan 50–80 mg/m^2 according to age, or to three further courses. These consisted of: (1) daunorubicin, 50 mg/m^2 for 2 days, with Ara-C, 200 mg/m^2, and 6-thioguanine, 150 mg/m^2 for 5 days; (2) MAZE; (3) Ara-C, 1 g/m^2 by 2-h infusion b.d. for 3 days, with 6-thioguanine, 150 mg/m^2 for 5 days, and a single dose of daunorubicin, 50 mg/m^2.

All patients had a Hickman line inserted at diagnosis. They received selective gut prophylaxis which varied from centre to centre. All received empirical antibiotics for febrile neutropenic episodes.

All patients received lumbar punctures and an instillation of methotrexate, 10 mg/m^2, on five occasions after achievement of CR.

Results

Since 1982 we have recruited 151 patients: 85 were adults (>16 years) of whom 47 were men and 38 women; their mean age was 37 years (16–60). There were 66 children, 30 boys and 36 girls whose mean age was 6 years.

Complete Remission Rates. CR was achieved in 127 patients (84%). CR rate was 91% in children and 79% in adults (P=NS). In 111 patients CR was achieved after one course, 16 further patients required a further course of therapy to enter CR, 24 patients did not achieve CR, 10 patients were resistant to chemotherapy (Table 1) and 14 patients died of other causes, usually infection, before response to treatment could be assessed (Table 2). The difference in treatment failure rate between adults and children (21% and 8%, respectively) was not significant.

Overall Survival. Median overall survival for adults is 26 months, and has not been reached in children. Patients with M_5-AML had the worst survival compared with patients in the other FAB categories (Fig. 1).

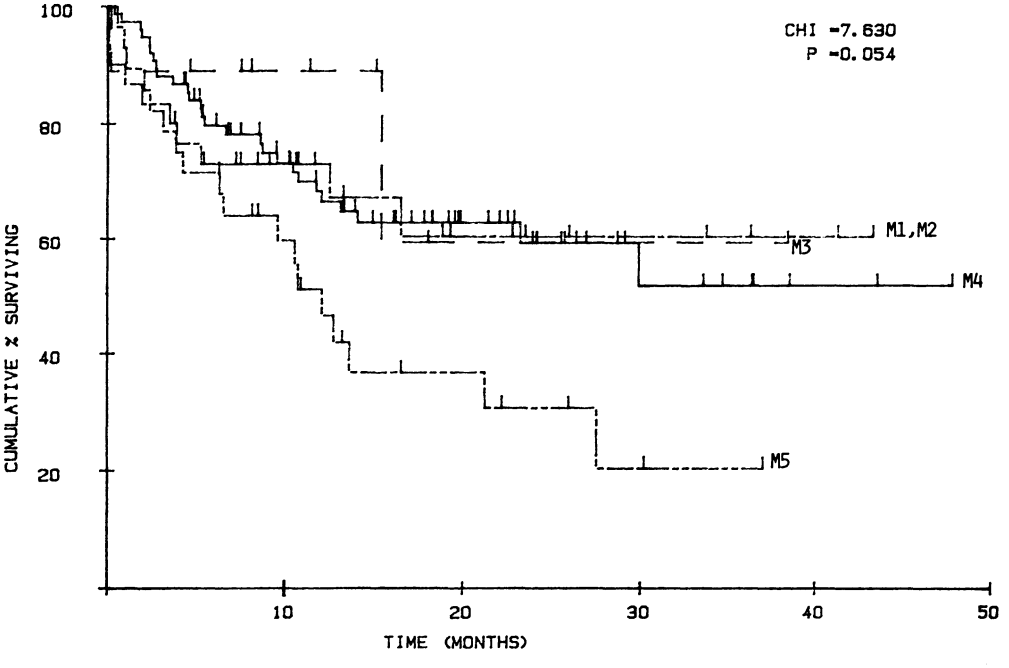

Fig. 1. Overall survival by FAB group

Table 1. Early deaths – patients dying before response to treatment assessed

	Adults	Children	Total
Bacterial sepsis	8	2	10
Fungal sepsis	1	–	1
DIC	1	–	1
Tumour lysis	1	–	1
Bleeding	–	1	1
Total	11 (13%)	3 (4%)	14 (9%)

DIC, disseminated intravascular coagulopathy.

Table 2. Leukemia resistance

	Adults	Children	Total
No response to "3+10"	2	2	4
Rapid recrudescence	2	1	3
Partial remission after three courses	3	–	3
Total	7 (8%)	3 (4%)	10 (7%)

Table 3. Deaths in complete remission. 22 patients have died in CR (15 adults and 7 children)

	No. of patients Total (Children)		Cause
After course			
1 (3+10)	1		Aspergillus
2 (3+10)	9		
		6 (3)	Gram-negative sepsis
		1	Aspergillus
		1 (1)	Prolonged aplasia
3 (MAZE)	2		Gram-negative sepsis
After randomisation			
Melphalan	1		Gram-negative sepsis
Courses 4,5,6	–		
BMT	9 (3)		7 GVHD/cytomegalovirus pneumonia 2 aspergillus

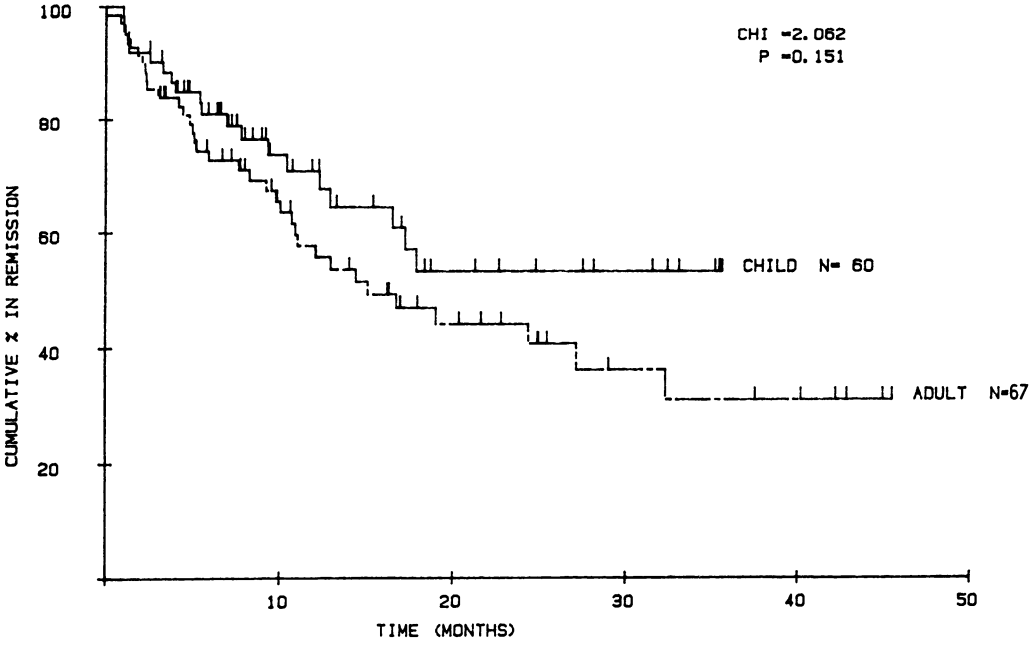

Fig. 2. Disease-free survival (remitters only)

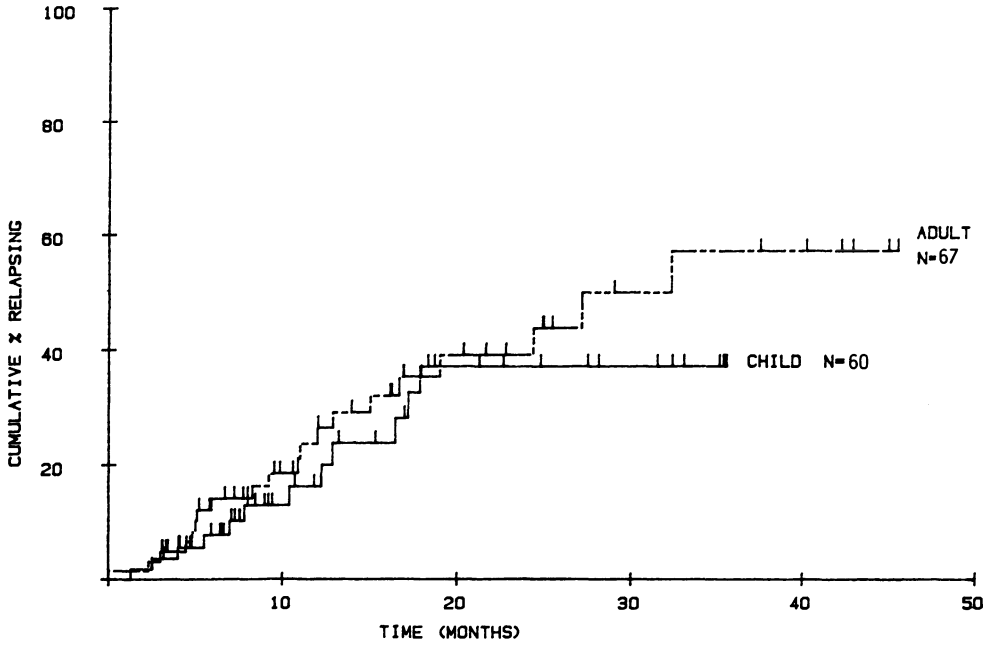

Fig. 3. Actuarial risk of relapse after entering CR

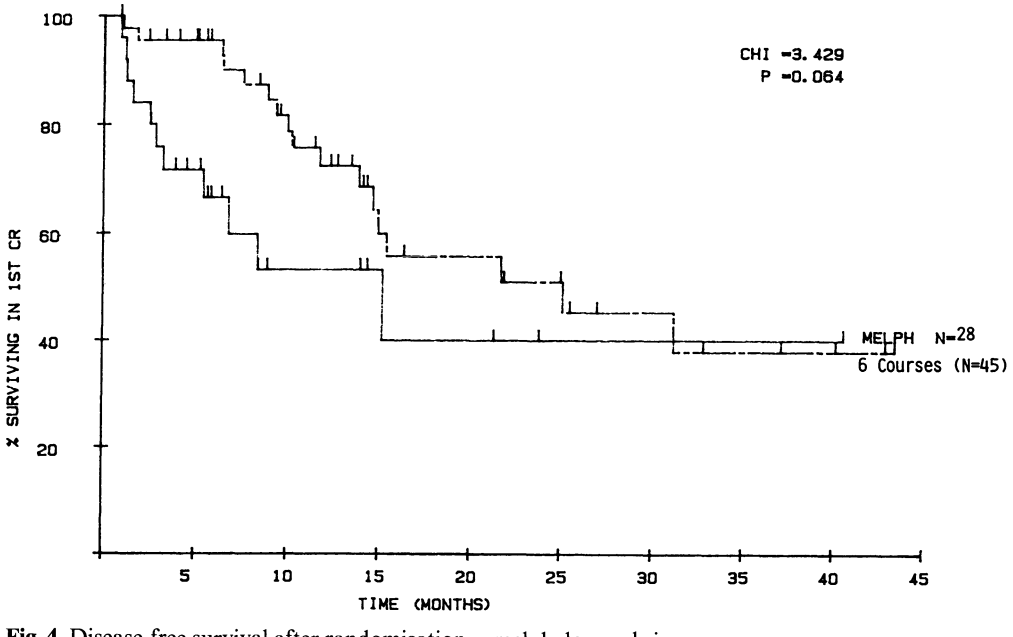

Fig. 4. Disease-free survival after randomisation – melphalon and six courses

Death in CR. The consolidation protocol was associated with 22 deaths in CR (Table 3). Nine of these died after the second course of DAT 3+10, most of gram negative sepsis. Thirteen deaths (9% of all remitters) occurred in those receiving chemotherapy, and nine in recipients of BMT.

Remission Duration. Median remission duration is 14 months in adults and has not

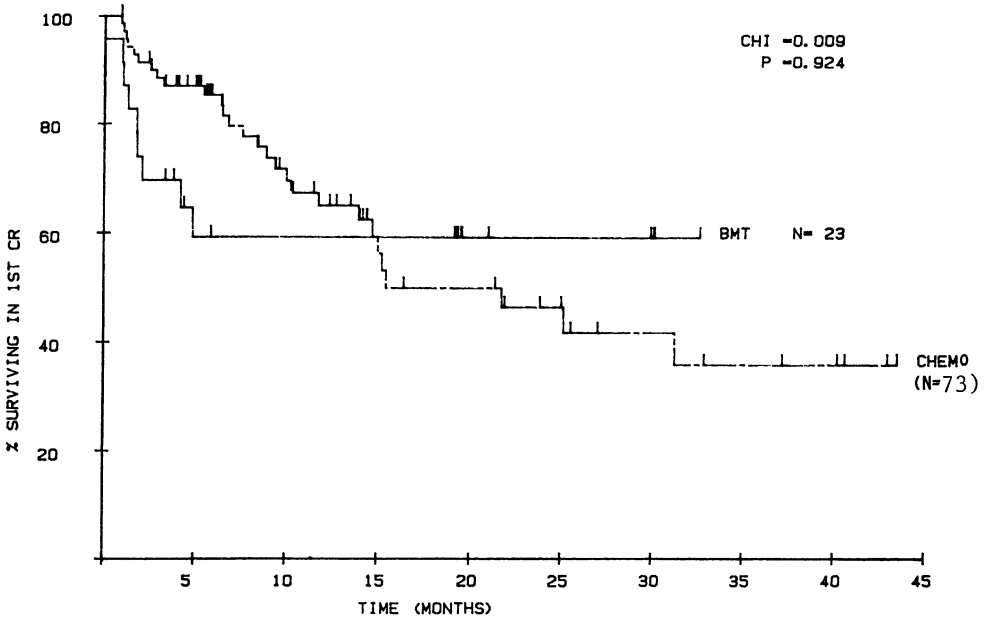

Fig. 5. Disease-free survival after randomisation

been reached in children (Fig. 2). 42% of children in CR are in continuous CR compared to 29% of adults. This difference has not reached statistical significance. Actuarial risk of relapse is 59% for adults and 37% for children (Fig. 3).

Duration of remission after randomisation. A total of 96 patients were alive in first CR after three courses. Of these 23 had an HL-A-matched sibling and underwent allogeneic BMT. Of the remaining patients 25 received melphalan and 48 received three further courses of chemotherapy. Patients who received melphalan relapsed earlier than those who received the six-course protocol but at 3 years, 37% of patients in both groups are in continuous CR (Fig. 4). 59% of patients receiving BMT remain in continuous CR (Fig. 5). Nine of these 23 have died, eight from transplant-related complications and one in relapse.

Fifteen children underwent allogeneic BMT; 70% remain in continuous CR at 30 months compared to 35% receiving chemotherapy. This is in contrast to adults of whom only 36% of those receiving BMT remain alive in first CR compared with 38% of those receiving chemotherapy.

Discussion

Preliminary results from this trial confirm that high CR rates may be obtained with intensive induction protocols. Overall survival and median remission rates are comparable to other recent reports with worst survival in adult patients and those suffering from acute monocytic leukaemia (FAB classification-M_5). We observed significant mortality in CR after course 2; the majority of these patients died of sepsis. When organisms have been isolated in blood cultures they are the same as those identified in surveillance cultures of faeces or rectal swabs. This suggests that a second course of Ara-C, known to be toxic to the gastro-intestinal tract, given within 4–6 weeks of the first may exert a cumulative toxic effect on the lining of the gut, permitting organisms to enter the bloodstream. We have now reduced the number of days of Ara-C to 8 in course 2 in the light of this experience.

Patients receiving melphalan relapse earlier than those who receive a total of six courses; however, the proportion of patients in continuous CR in both arms was identical at 3 years. This suggests that the six-course protocol delays the onset of relapse but may

not increase the cure rate. Longer follow-up is necessary to reach a firmer conclusion.

In this study BMT in adults has not proved superior to chemotherapy due to a high incidence of fatal transplant complications. This contrasts with results in children where the mortality of the procedure is lower, and a larger percentage receiving BMT remain in continuous CR compared with those who receive chemotherapy.

This early analysis of our data suggests that short courses of highly intensive chemotherapy may produce optimal results, but that such intensive therapy may be associated with significant mortality in CR. Allogeneic BMT seems to be superior in children but its place in adults in first remission AML is still not defined.

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Remission Induction with Cytarabine and Daunorubicin With or Without 6-Thioguanine in Adult Patients With Acute Myelocytic Leukemia

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Introduction

Cytosine arabinoside (Ara-C) and daunorubicin (DNR) are the two most active agents in the treatment of acute myelocytic leukemia (AML) with complete remission (CR) rates of 20%–30% and 33%–55%, respectively [1]. Combinations of the two drugs given in their optimal schedules have shown CR rates of 55%–66% in patients with AML [2, 3]. In contrast to Ara-C and DNR, 6-thioguanine (6-TG) is known to be only marginally active against AML when given as a single agent [1]. On the other hand, CR rates of up to 76% have been achieved using combinations of Ara-C and DNR together with 6-TG [4]. However, there are only a few direct comparative studies concerning the effect of 6-TG on the efficacy of Ara-C and DNR in AML [5, 6]. In these studies, the drugs were given in suboptimal schedules resulting in relatively low CR rates in regimens with or without 6-TG. The present report deals with the clinical results in 116 adult patients with AML who were treated for remission induction (RI) either with a combination of Ara-C and DNR or an intensified combination of these two drugs together with 6-TG. The patients were treated in a single institution under comparable routine supportive care.

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Patients and Treatment

In a total of 116 consecutive patients with de novo AML, the results were analyzed retrospectively with regard to the regimen used for RI. Sixty-five of the 116 patients were treated between 1974 and 1979 for RI with one or two courses of Ara-C (100 mg/m^2, 24 h infusion, days 1–7) together with DNR (45 mg/m^2 i.v. days 1–3) (AD regimen). In the remaining 51 patients treated between 1980 and 1984, RI consisted of Ara-C (100 mg/m^2, 24 h infusion, days 1–2, followed by 30 min infusion q 12 h days 3–8), DNR (60 mg/m^2 i.v. days 3–5) and 6-TG (200 mg/m^2 p.o. days 3–9) (TAD 9 regimen). In both treatment groups supportive care included broad-spectrum antibiotics for fever in the presence of granulocytopenia, and transfusions of erythrocytes and platelets when required.

All patients had bone marrow and blood smears stained by May-Grünwald-Giemsa, peroxidase, periodic acid-Schiff, and nonspecific esterase with and without sodium fluoride. Criteria for the diagnosis of AML were: hypercellular marrow with greater than 50% infiltration by blast cells. Leukemic subtypes included acute myeloblastic, acute promyelocytic, acute myelomonocytic, acute monocytic, and acute erythrocytic leukemia. Criteria for CR were the absence of disease-related symptoms, normal cellular marrow with less than 5% blasts and peripheral blood granulocyte and platelet counts greater than 1.5×10^9/liter and 100×10^9/liter, respectively.

Statistical Analysis

Patients who did not achieve CR after at least two courses of therapy or who died during RI were considered as induction failures. Time to CR was counted from the start of treatment. The χ^2 test was used to determine the differences in response rates or frequency of occurrence.

Results

Table 1 presents the characteristics of the patient population treated on each of the two RI regimens. The two patient groups were comparable with regard to the distribution of sex and age as well as the subtypes of AML and the initial total leukocyte count in the peripheral blood. Of the 116 patients treated, 70 (60%) achieved CR. The CR rate was 55% (36/65) in patients treated with the AD combination and 67% (34/51) in those treated with the TAD-9 combination ($P<0.15$) (Table 2). The mean number of chemotherapy courses to achieve CR was 1.5 in both groups, and the median number of days to achieve CR was 36 in the first and 34 in the second group. Fifty percent of the complete responders to the AD regimen ob-

Table 1. Characteristics of patients treated with AD or TAD-9 regimen

	AD regimen	TAD-regimen
No. of patients	65	51
Sex:		
male	31 (48%)	26 (51%)
female	34 (52%)	25 (49%)
Age (years):		
range	15–71	17–70
median	43	43
WBC (count/mm^3):		
range	800–467000	1000–213000
median	13400	20600
Morphologic variants of leukemia (FAB):		
AML (M_1, M_2)	48 (73%)	33 (65%)
APL (M_3)	3 (5%)	1 (2%)
AMM, AMoL (M_4, M_5)	14 (22%)	16 (31%)
AEL (M_6)	–	1 (2%)

AML, acute myeloblastic leukemia; APL, acute promyelocytic leukemia; AMML, acute myelomonocytic leukemia; AMoL, acute monocytic leukemia; AEL, acute erythroleukemia.

Table 2. Results of remission induction therapy in patients with AML treated with AD or TAD-9 regimen

	AD	TAD	Both groups
Patients treated	65	51	116
Patients achieving CR	36 (55%)	34 (67%)	70 (60%)
Patients died in aplasia[a]	12 (18%)	8 (16%)	20 (17%)
Nonresponders	17 (27%)	9 (17%)	26 (23%)
Median no. of courses to CR	1.5	1.5	
Median no. of days to CR	36	34	
% of CRs after only 1 course	50	53	

[a] Early death within the first 30 days.

Table 3. Influence of age on the outcome of remission induction in patients with AML treated with AD or TAD-9 regimen

	AD			TAD		
		< 50 years	≧ 50 years		< 50 years	≧ 50 years
Total	65	45	20	51	33	18
CR	36	27 (60%)	9 (45%)	34	25 (76%)	9 (50%)
ED[a]	12	4 (9%)	8 (40%)	8	3 (9%)	5 (28%)
No response	17	14 (31%)	3 (15%)	9	5 (15%)	4 (22%)

[a] Early death within the first 30 days.

tained CR after only one course, while 54% of the responders to the TAD-9 regimen did so. In both groups, the CR rate and the frequency of early death within the first 30 days appeared to be related to the age of patients (Table 3). Sixty percent of the patients under age 50 and 44% of patients 50 years of age and older obtained CR when treated with the AD combination. The TAD-9 combination induced 76% and 50% CRs, respectively. The rate of early death was 18% in patients treated with the AD regimen and 16% in those treated with the TAD-9 regimen. In patients under age 50, there was an early death rate of 9% in both treatment groups. In older patients, however, early death occurred in 40% of patients with the AD regimen and in 28% of those with the TAD-9 regimen.

Discussion

The CR rates of 55% obtained with the AD regimen and of 67% obtained with the TAD-9 regimen are favorably comparable to the results of other studies using similar induction regimens [2, 4, 7]. The data reported in this paper demonstrate that intensified drug administration and the addition of 6-TG to Ara-C and DNR are superior to the two-drug combination in inducing CR in patients with AML. With both induction regimens, however, the treatment results are less satisfactory in patients 50 years of age and older than in younger patients. This observation is consistent with that in a series of studies, indicating a significant relationship between age, response to RI therapy and mortality [2, 8–10]. In our analysis, however, the risk of early death did not appear to increase with the intensification of therapy in patients under age 50 or in those over age 50. Furthermore, TAD-9 and AD regimens were found to be comparable with respect to the number of courses and the time required to achieve CR or the probability of CR after each course.

In conclusion, the data demonstrate a clear trend favoring the TAD-9 combination over the AD combination, particularly in patients younger than 50 years. In addition, the use of the three-drug regimen does not seem to be associated with an increase in fatal complications when compared to the two-drug regimen.

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Contribution of Clonogenic Leukemic Cell Characteristics to Therapy Outcome in Patients With Acute Myeloblastic Leukemia

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Introduction

Unlike acute lymphoblastic leukemia, prognostic factors predicting the response to chemotherapy in patients with acute myeloblastic leukemia (AML) have yet to be identified. Several clinical and hematologic features of the disease, previously thought to contribute to therapy outcome, have lost significance with changes in chemotherapy [1]. Recently, culture techniques have been described for the growth of blast cell colonies in patients with AML [2, 3]. These colonies are derived from clonogenic leukemic cells (CFU-L) which possess a substantial proliferative capacity and are capable of self-renewal [4]. It has been suggested that the growth characteristics of CFU-L reflect the biologic behavior of individual AML clones and may, therefore, be related to treatment outcome [5].

In this study, we examined the contribution of several CFU-L properties to outcome variation in 15 consecutive patients with de novo AML. In contrast to other reported series, all patients were previously untreated and received uniform remission induction (RI) therapy (TAD-9).

Materials and Methods

Patients

A total of 15 consecutive patients with de novo AML at initial presentation were studied. Clinical and hematologic data are given in Table 1. All patients were uniformly treated using the TAD-9 protocol of the German AML Cooperative Group [6]. Six patients achieved complete remission (CR) after one or two courses of chemotherapy. Failures of induction therapy were classified according to Preisler [7]. Type I and II failures characterize patients with resistant disease (RD).

Colony Assay

Clonogenic leukemic cells were assayed using the method by Minden et al. [4]. Briefly, mononuclear cells from peripheral blood were depleted of T lymphocytes and plated at concentrations of 2×10^5 cells/ml in Iscove's modification of Dulbecco's minimum essential medium (IMDM) with 30% fetal calf serum (FCS), 5% phytohemagglutinin-lymphocyte-conditioned medium (PHA-LCM), and 0.9% methylcellulose. Cultures were routinely grown in 0.1 ml volumes in flat-bottom microwells. In each experiment, 20 microwells were plated. After 5–7 days of incubation, blast colonies containing a minimum of 20 cells were counted (first plating efficiency, PE1).

Cytosine Arabinoside Suiciding

The fraction of CFU-L in S phase of the cell cycle was determined using the cytosine arabinoside (Ara-C) suicide technique [8]. T-depleted cells (1.5×10^6/ml) were exposed to Ara-C (1 μM) for 1 h at 37 °C, washed twice

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Table 1. Clinical data and results of colony formation (CFU-L)

Patient	Age (years)	Sex	FAB subtype	WBC (/μl)	Blasts (%)		PE1	Suicide (%)	PE2	Therapy	Outcome[a]	Survival (months)
					PB	BM						
M.E.	56	M	M_2	16800	27	87	16	63	0	TAD-9	CR	10+
W.P.	44	M	M_3	27700	87	90	23	67	0	TAD-9	FV	0
H.W.	58	M	M_2	30600	85	98	41	62	1	TAD-9	CR	14+
G.O.	62	M	M_2	6600	50	89	8	53	1	TAD-9	FV	0
V.K.	33	F	M_2	5900	24	90	9	98	2	TAD-9	CR	15+
K.K.	68	F	M_1	2300	30	80	3	67	2	TAD-9	CR	7+
M.B.	64	M	M_5	51000	20	75	9	42	3	TAD-9	CR	10+
K.S.	68	F	M_2	5400	26	54	26	93	10	TAD-9	FIII	2
A.A.	63	M	M_5	216000	62	85	19	93	11	TAD-9	CR	7+
E.O.	41	F	M_2	12800	76	50	16	73	57	TAD-9	FII	7
J.L.	69	M	M_2	25600	80	97	14	53	80	TAD-9	FI	1
A.K.	63	F	M_1	9200	40	96	21	67	102	TAD-9	FI	1
R.R.	52	F	M_2	84600	93	95	67	55	108	TAD-9	FI	2
E.S.	36	M	M_1	15000	73	80	80	62	146	TAD-9	FI	1
G.W.	66	M	M_1	28600	89	97	27	79	ND	TAD-9	FII	7

[a] Failures classified according to preisler [7].
PE1(2), first (second) plating efficiency (colonies/2×10^4 cells).

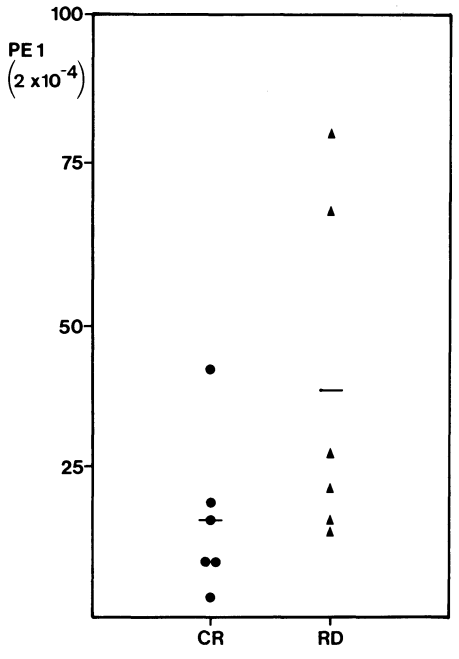

Fig. 1. First plating efficiency (PE1) of CFU-L in patients with sensitive (●) and resistant (▲) disease

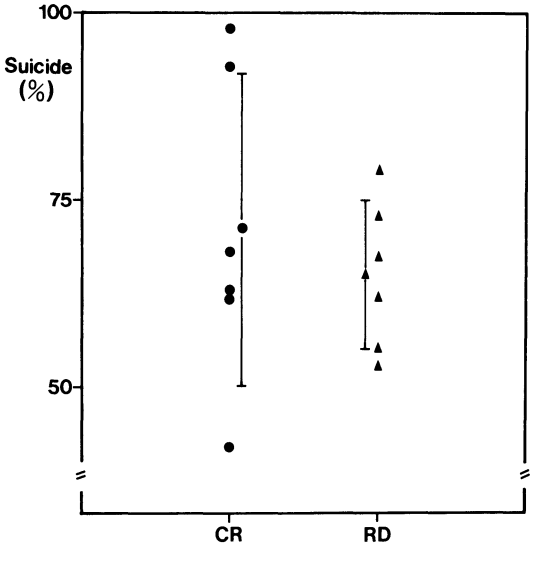

Fig. 2. Ara-C suicide rates of CFU-L in patients with sensitive (●) and resistant (▲) disease

with an excess of IMDM and plated as described above. Sucide was expressed as percentage loss of colony formation compared to unexposed samples which were identically processed.

Measurement of Self-Renewal

Pooled primary colonies were harvested, redispersed, washed twice in IMDM and then replated. In all experiments, cell yield was sufficient to set up at least 5 microwells. At day 5–7 of incubation, secondary colonies were counted (second plating efficiency, PE2).

Results

In all patients with AML, blast cell colonies could be grown from T-depleted blood cells. In contrast, colony formation was not observed in three patients with acute lymphoblastic leukemia and two normal volunteers. Colonies usually contained 20–200 cells with blast-like morphology, as shown by Pappenheim staining. In two patients, Auer rods could be demonstrated in cultured cells. Contamination with T cell colonies was excluded by the inability of cells to form erythrocyte (E) rosettes.

PE1 of blast cells varied between 1.5×10^{-4} and 4×10^{-3} (25 ± 22 colonies/2×10^4 cells). A significant correlation was found between peripheral blast cell concentration and in vitro colony formation ($r = 0.635$; $P < 0.01$). Although PE1 was lower in responders than in patients with RD (Fig. 1), the difference did not reach significance ($P > 0.1$).

Following short-time exposure of cells to Ara-C (1 μM), a substantial reduction of colony formation was observed in all cases. Suicide levels were higher than in previously published studies using hydroxyurea or ^{3}H-thymidine as suicide agents [9, 10]. Conditions of Ara-C incubation were equivalent to those used by Dresch et al. for in vitro suicide of CFU-C [8]. Comparing the suicide levels of CFU-L in patients with CR ($71 \pm 21\%$), and RD ($65 \pm 10\%$), no statistical difference was found (Fig. 2).

In contrast, self-renewal of CFU-L as determined by replating experiments was found to be significantly correlated with therapy outcome ($P < 0.001$). Secondary colonies could be grown in 13 cases. Five of seven patients presenting with low numbers of PE2 entered CR, whereas all patients with a high capacity for self-renewal were resistant to chemotherapy (Fig. 3).

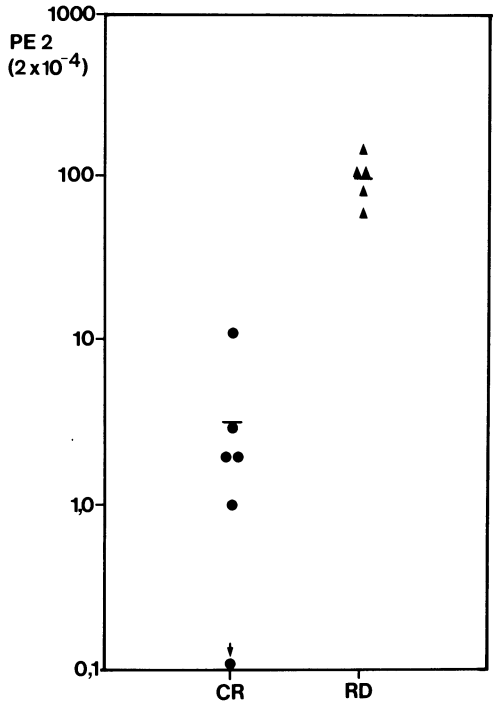

Fig. 3. Second plating efficiency (PE2) of CFU-L in patients with sensitive (●) and resistant (▲) disease

Discussion

The results presented here show that leukemic colony-forming cells can be readily detected in the peripheral blood of patients with AML. Using the culture technique by Minden et al. [3], we were able to obtain leukemic colonies in all patients examined. Similarly, other authors have reported on the successful growth of clonogenic leukemic cells from blood and marrow samples [10]. As shown in previous studies, the removal of T lymphocytes prior to plating improves cloning efficiency and allows colony formation even in preleukemic patients [11].

Recently, the specificity of the blast cell assay has been questioned by Kubota et al. [12], who studied the growth of clonogenic cells from patients with acute leukemia under different culture conditions and concluded that "blast cell colonies" were derived from defective CFU-C. In our study, the leukemic origin of colony-forming cells was suggested by (1) the morphology of cultured cells found to contain Auer rods in two cases; (2) kinetics of colony growth; (3) significant correlation between peripheral blast cell concentration and the number of colonies formed; and (4) capacity of CFU-L for self-renewal.

This study was carried out to evaluate the relationship between in vitro characteristics of CFU-L and in vivo response to chemotherapy in patients with de novo AML. In contrast to other reported series [13], all patients were previously untreated and received uniform RI therapy (TAD-9). Patients who died early after the start of treatment or due to severe bone marrow hypoplasia were excluded from the analysis.

The PE1 of blast cells and Ara-C suicide rates were not found to be different in patients with sensitive and resistant disease. In contrast, we could demonstrate a significant correlation between self-renewal of CFU-L and the outcome of chemotherapy. A considerable patient-to-patient variation in the frequency of secondary colonies was observed. Low values of PE2 characterized patients with high incidence of CR, while extensive capacity for self-renewal was associated with RD. These results confirm earlier data by McCulloch et al. [5]. In a study of 53 patients, these authors showed that the self-renewal of CFU-L represents an important prognostic factor, predictive both of CR and survival. A similar relationship was not observed by Marie et al., but their studies were done on bone marrow CFU-L [10].

In summary, the results presented here indicate that studies of CFU-L self-renewal are of value in predicting therapy outcome in AML. They provide further support for the relevance of cell culture data to clinical studies.

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Expression of CD-15 Antigen on Leukemic Cells – A New Prognostic Factor for Ability to Achieve Complete Remission and for Survival in ANLL

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Introduction

One of the key problems in acute leukemia is the optimal treatment based on adjusted management while at the same time taking into consideration the risk factors [1]. The monoclonal antibodies (MoAbs) directed against cell differentiation antigens have contributed greatly to the immunotyping of leukemia and several investigators have proved their usefulness in the identification of subsets of patients with acute lymphoblastic leukemia who have a better or worse prognosis than on average [2]. In this publication, based on the analysis of a large group of patients, we present evidence that the CD-15 antigen detected by VIM-D5 MoAbs can be regarded as the immunologic marker in acute nonlymphocytic leukemia (ANLL) which predicts both the ability to achieve complete remission (CR) and survival.

Materials and Methods

Our analysis is based on the observation of 247 untreated patients (117 men and 130 women, median age 48 years) with ANLL.

The mononuclear cells isolated from the peripheral blood and/or bone morrow were submitted to immunologic typing by means of a panel of 15 MoAbs of VI series [3, 4], donated by Prof. W. Knapp of the University of Vienna. Only samples with more than 70% blasts in differential count were analyzed. In the paper, special attention will be paid to the results obtained with VIM-D5 MoAbs detecting the CD-15 antigen [3] and to the VIM-2 MoAbs [3, 4]. Reactivity of cells was tested in indirect immunofluorescence using FITC-labeled goat $F(ab)_2$ antibodies against mouse IgG + IgM (Grubb) as secondary reagent. The criterion for CD-15 or VIM-2 positivity was expression of the marker by at least 15% of the blast cell population. After immunotyping, patients were submitted to remission induction (RI) treatment using one of the following protocols: (1) daunorubicin + cytosine arabinoside (Ara-C), 3+7 days; (2) TAD. In separate randomized studies it had been proved that the results in patients treated according to these schemas were similar [5, 6]. Survival curves were constructed according to the Kaplan-Meier product limit procedure and the results were compared according to the Wilcoxon test and the log-rank test. Linear regression analysis was used to estimate the correlation coefficient of CD-15 antigen expression and the survival of 93 patients.

Results

The proportion of leukemic blasts which expressed the CD-15 antigen ranged from 0% to 95% with a median of 28%. Out of 247 patients 167 displayed CD-15 positivity and 80 were identified as negative.

In the CD-15 positive group, 54% of patients achieved CR, whereas in the CD-15

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Table 1. Relation of CR rates to CD-15 positivity of blasts and other risk factors

Proportion of VIM-D5 positive blasts	No. of cases	% CR	Age years	WBC	Blasts
				10^6/l	
0–14%	80	35	46	43	41
		$p<0.01$	$p<0.9$	$p<0.9$	$p<0.9$
≧15%	167	54	48	48	42
0–49%	168	42	48	47	43
		$p<0.02$	$p<0.9$	$p<0.9$	$p<0.9$
≧50%	79	61	44	47	38

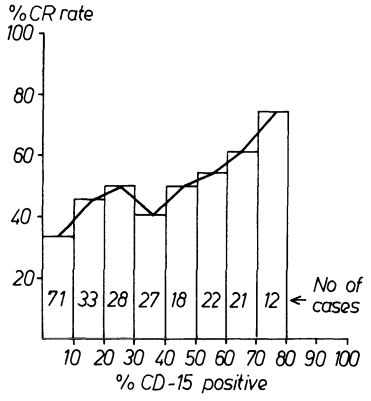

Fig. 1. The relationship between expression of CD-15 antigen on leukemic cells and CR rate in 232 ANLL cases

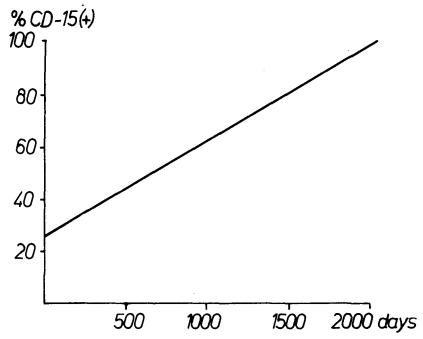

Fig. 3. Significant positive correlation between CD-15 antigen expression and survival of patients who died within the observation time. The correlation index, $r = +0.44$, $p<0.01$, linear regression analysis

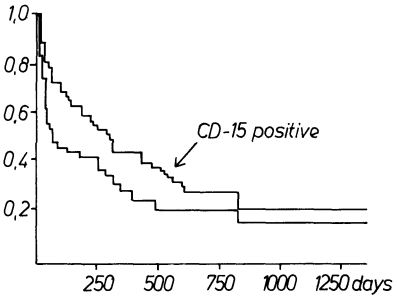

Fig. 2. Comparison of Kaplan-Meier survival curves. Patients with CD-15 expression had a significantly better outcome ($p<0.02$, Wilcoxon test)

negative group only 35% of patients did so ($p<0.01$, χ^2 test, Table 1). A significant difference in the CR rate was also proved between the subgroups of patients, with the proportion of CD-15 positive blasts above 50% compared to those with lower values. The CR rates in these groups equaled 61% and 42%, respectively ($p<0.01$, Table 1).

An attempt was made to exclude the influence of other risk factors on the CR rate in the foregoing analysis. We found no statistically significant differences in age, WBC and blastosis between the subgroups (Table 1). This confirms our preliminary suggestion that the proportion of patients achieving CR is related to the CD-15 positivity of ANLL blast cells.

Figure 1 shows this positive relationship between expression of CD-15 antigen on leukemic cells and CR rate in subgroups of patients arranged according to the percentage of CD-15 positive blasts.

No significant interrelationship could be found between expression of VIM-2 antigen and CR rate in the 86 patients analyzed hitherto. Comparison of the Kaplan-Meier survival curves (Fig. 2) suggests that the CD-15 positive group of ANLL patients had a sig-

nificantly better outcome when compared to the CD-15 negative group ($p<0.02$, Wilcoxon test). This difference in the probability of survival was particularly marked within the first 3 months following diagnosis ($p<0.01$ Wilcoxon test and log-rank test).

Regression analysis (Fig. 3) proved the significant positive correlation between CD-15 antigen expression and survival (coefficient $=0.44$, $p<0.001$). A similar correlation was found in a subgroup of patients who achieved CR (coefficient 0.32, $p<0.05$).

Analogous analyses demonstrated that the VIM-2 expression does not correlate to survival (coefficient 0.08) in spite of significant correlation of the expression of CD-15 antigen and VIM-2 antigen on leukemic cells (coefficient 0.48, $p<0.001$).

Discussion

For about 15 years, patients with various subtypes of myeloid leukemia have been treated with very similar chemotherapeutic programs, regardless of their exact pretreatment characteristics. Today, when a number of significant therapeutic options are available, an attempt should be made to individualize therapy and use potentially more aggressive or new therapies only in prognostically poor groups of patients. The number of prognostic factors which enable the identification of patients with different prognosis is limited and new ones have been sought as the progress of science has continued. New possibilities were opened up with the introduction of immunotyping of leukemia but until now it has been proved mainly only in ALL [2]. In ANLL several investigators [1, 7] have identified at least 40 objective quantitative parameters influencing response to therapy but none of them are of an immunologic nature.

Our observation raises the problem of the prognostic value of immunotyping in ANLL and suggests the CD-15 positivity of ANLL blasts to be a new favorable prognostic factor in the ability to achieve CR and for survival. The curve expressing the interrelationship between the degree of CD-15 expression (Fig. 1) and CR rate shows that the proportion of patients achieving CR is related to the CD-15 positivity of leukemic blasts. It has also been proved by statistical comparison of the CR rate in subgroups of patients with low and high expression of CD-15 antigen (Table 1). Therefore we conclude that examination of CD-15 expression contributes to the prediction of the probability of achieving CR. Comparison of the survival curves (Fig. 2) suggests that the subgroup of patients displaying CD-15 positivity also had a significantly better outcome then the CD-15 negative ones.

Additional analysis proved that the difference in probability of survival is most obvious within the first few months of the follow up. The prognostic significance of CD-15 expression for survival has also been confirmed by regression analysis in patients who died within the follow-up time (Fig. 3). This analysis revealed a significant positive correlation both in the entire group and in the subgroup of patients with CR. Despite these suggestive results, they should be interpreted with caution until the independent predictive value of CD-15 expression is confirmed by multivariate analysis.

Explanations for the differences in CR rate and probability of survival between the CD-15 positive and negative groups of patients are purely speculative. CD-15 antigen can be found on normal granulocytic cells from blasts through promyelocytes to granulocytes [3, 4]. It appears to be more specific for the granulocytic line when compared to VIM-2 antigen [3], which in our experience has no prognostic significance in ANLL.

Assuming that patients of marker expression by normal hematopoietic cells are conserved by their malignant counterparts, it appears that CD-15 positivity reflects, at least to some degree, the maturity of leukemic cells. From this point of view our findings are in agreement with the observations indicating better prognosis in more mature subtypes of AMl [1, 8]. On the contrary, an immature phenotype appears to be a major biologic feature associated with a high proliferative tendency and drug resistance.

From a practical standpoint, CD-15 estimation is routinely available for all newly diagnosed leukemic patients with the use of VIM-D5, VIM-C6, or BMA 0200 MoAbs [4] and is relatively inexpensive to perform.

Thus, if further studies confirm the present findings, examination of CD-15 expression could be a useful addition to existing systems of risk assignment in ANLL and additionally, it could contribute to an improvement in therapy.

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Prognostic Significance of Morphologic and Cytogenetic Findings for Progression in Myelodysplastic Syndromes *

G. Kerndrup, B. Pedersen, J. Ellegaard, and P. Hokland[1]

Introduction

Within the group of myelodysplastic syndromes (MDS) two subgroups are defined [refractory anemia (RA) and RA with ringed sideroblasts (RA-S)] where the percentages of peripheral blood and bone marrow blast cells, by definition, do not exceed 1% and 5%, respectively [1]. Therefore, the applicability of these parameters would be expected to be of limited value with regard to the prediction of progression to RA with an excess of blasts (RAEB) and/or acute myeloid leukemia (AML). The present investigation was undertaken to evaluate the predictive value of peripheral blood cytopenia and to investigate whether a causal relationship between peripheral blood cytopenia, bone marrow morphology, and cytogenetic abnormalities could be envisaged.

Material and Methods

A total of 27 patients were found to fulfill the diagnostic criteria proposed by the FAB group [2] (Table 1). To simplify correlations, the patients were grouped into two cytopenia groups (Table 1). Bone marrow blast cell percentages were the mean of two independent observations where at least 200 nucleated bone marrow cells were counted.

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Table 1. Clinical data of patients

		Initial diagnosis		Clonal abnormalities[c]	Progression to RAEB or AML	$\bar{x}$ bone marrow blast cell percentage[e]
		RA-S (n=10)	RA (n=17)	(n=15)	(n=12)	
Cytopenia[a]	Group A	6	3	2	1	1.05
	Group B	4	14	13	11	1.75
	P value	0.04[b]		< 1.05[d]	< 0.05[d]	=0.05[e]

[a] One point was assigned to each of the following cytopenia values: granulocyte count $<2\times10^9$/liter; hemoglobin $\leqq$6 mmol/liter; thrombocyte count $\leqq100\times10^9$/liter. Group A: 0–1 point; group B: 2–3 points.
[b] Fisher's exact test. An initial diagnosis of RA was significantly correlated to severe cytopenia (group B).
[c] The International System for Human Cytogenetic Nomenclature was used.
[d] χ^2-test.
[e] The percentages for group A and group B patients were ranked and compared by means of Mann-Whitney's test.

The bone marrow cellularity was estimated semiquantitatively on sections of bone marrow biopsies or the bone marrow clot [3]. The bone marrow cells obtained for cytogenetic analysis were cultured according to the high-resolution technique described by Yunis [4]. Banding and photography were carried out as earlier described [5].

Results

From Table 1 it appears that severe peripheral blood cytopenia was correlated to an initial diagnosis of RA ($P=0.04$), the initial occurrence of clonal cytogenetic abnormalities ($P<0.05$), progression to RAEB or AML ($P<0.05$) and to bone marrow blast

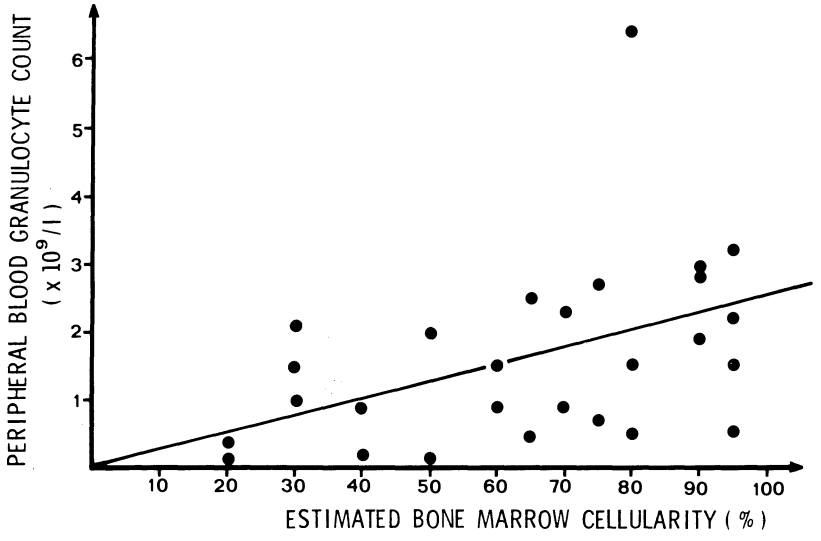

Fig. 1. Relationship between peripheral blood granulocyte count and estimated bone marrow cellularity. Correlation coefficient parameters: $Y=0.17+0.02x$; $r=0.4173$; $P(r)<0.05$

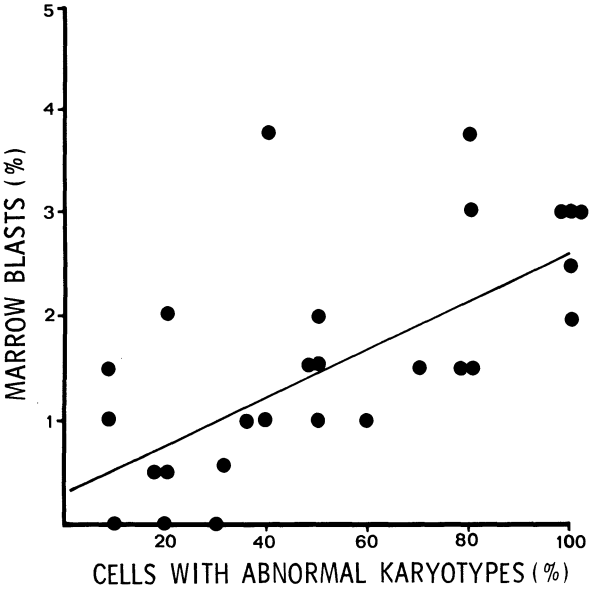

Fig. 2. Relationship between frequencies of abnormal metaphases (clonal and nonclonal) and bone marrow cellularity. Correlation coefficient parameters: $Y=85.83-0.4x$; $r=-0.55$; $P(r)<0.01$

Table 2. Correlation coefficients (r) and p values of correlation coefficients [p(r)] for the relationship between frequencies of abnormal karyotypes, bone marrow cellularity, and bone marrow blast cells

		Clonal	Nonclonal	Clonal + nonclonal
Bone marrow cellularity	r	−0.42	−0.07	−0.55
	p	<0.05	>0.05	<0.01
Bone marrow blast cells	r	+0.73	−0.06	+0.71
	p	<0.001	>0.05	<0.001

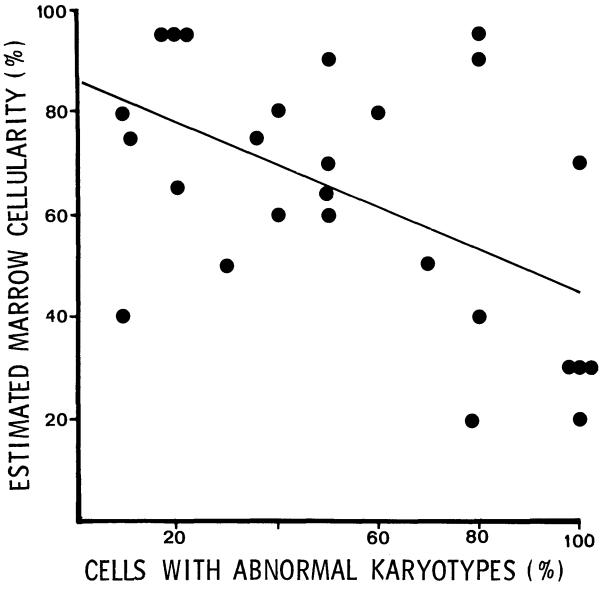

Fig. 3. Relationship between frequencies of abnormal metaphases (clonal and nonclonal) and bone marrow blast cell percentage. Correlation coefficient parameters: Y = 0.36 + 0.02x; r = 0.71; P(r) < 0.001

cell percentage ($P = 0.05$). Peripheral blood granulocytopenia was correlated to bone marrow cellularity (Fig. 1). Moreover, a positive relationship between cytogenetic abnormalities and bone marrow blast cell percentage (Fig. 2 and Table 2), and a negative relationship between cytogenetic abnormalities and bone marrow cellularity (Fig. 3 and Table 2) was found.

Discussion

From the results presented in Table 1 it appears that severe cytopenia is centrally positioned with regard to progression within the FAB subtypes or to AML. This agrees well with the findings of Mufti et al. [6].

Furthermore, the relations depicted in Figs. 1–3 and Table 2 may explain the effects of cytogenetic abnormalities in MDS: the genetic damage accompanying chromosome abnormalities can, alone or together with other factors, interfere with the processes governing proliferation and maturation of primitive precursor cells. This is morphologically evidenced by a decline in cellularity in a former hypercellular bone marrow (Fig. 3). This leads to a decrease in the number of granulocytes in the blood (Fig. 1). Because of interference with maturation, blast cells will gradually accumulate (Fig. 2) and morphologic examination will reveal a hyperplastic bone marrow with an excess of blasts or, as the processes evolve, dominated by immature blast cells diagnostic of AML. That such an evolutionary pattern exists is supported by the observation of similar relationships in another stem cell disease, chronic myeloid leukemia [7, 8], and the

finding of an increased probability for transformation to AML in preleukemic patients with prolonged bone marrow cell generation times [9].

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Analysis of Prognostic Factors in Acute Leukemias in Adults

E. Krykowski, E. Polkowska-Kulesza, T. Robak, W. Matusewicz, H. Urbańska-Rys, and A. Hołub[1]

Introduction

Many factors have been associated with the possibility of achieving remission and the duration of survival. In this study we have analyzed the clinical and laboratory characteristics of patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) treated during the last 10 years.

Patients and methods

Of a total number of 239 patients analyzed there were 197 patients with AML (109 M and 88 F) and 42 with ALL (23 M and 19 F). The ages of patients ranged from 14 to 77 years (mean 42; for AML 45.6 and for ALL 26 years).

Most patients had been treated with combined chemotherapy regimens, in AML consisting of cytarabine and daunomycin or doxorubicin; in ALL mainly of vincristine, doxorubicin, cytarabine, and prednisone. The results of three kinds of treatment, i.e., monotherapy, polychemotherapy alone, or improved by prophylactic care, were compared.

The role of pretreatment risk factors (Table 1) potentially influencing response to therapy and survival time was analyzed separately in AML and ALL. Coexisting diseases which made the treatment more difficult were included (e.g., stomach ulcer; diabetes; tuberculosis; heart, kidney, and liver failure). Negative prognostic features (NPF) present were considered below 50 points according to Karnovsky's criteria, with the addition of sweating, loss of weight and anorexia. Critical levels were assumed as follows: erythrocytes 3.0×10^{12}/liter, leukocytes 30.0×10^{9}/liter, thrombocytes 30.0×10^{9}/liter.

The patients were divided into three groups according to survival time (A: less than 3 months, B: 3–12 months, and C: more than 12 months) and according to remission (A: nonresponders, B: less than 6 months, and C: more than 6 months).

Results

The results of our investigation regarding survival time are presented in Table 1. The age of patients had an essential prognostic significance in AML ($P<0.01$) but not in ALL. AML patients younger than 50 survive considerably longer than older ones ($P<0.001$). The distribution of sex was similar in all three groups. The coexisting diseases in AML patients influenced their survival time (A vs. C groups $P<0.05$). NPF presence had an important significance in both types of leukemia (AML: $P<0.001$, ALL: $P<0.01$). Infections occurred at a similar rate in all groups. The presence of hemorrhage, which did not always correlate with platelet count, only influenced survival time in AML patients ($P<0.01$). Platelet count differed significantly in extreme groups of AML ($P<0.05$) and ALL ($P<0.01$) patients. The differences in the

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Table 1. Statistical analysis of prognostic factors in AML and ALL patients divided into three groups

Factor	Groups compared	Results of statistical analysis	
		AML	ALL
Age in five groups	ABC	$P<0.01$	NS
<50 vs. ≥ 50	ABC	$P<0.001$	NS
Sex	ABC	NS	NS
Coexisting diseases	ABC	NS	Not investigated
	AC	$P<0.05$	
NPF	ABC	$P<0.001$	$P<0.01$
Infection	ABC	NS	NS
Hemorrhage	ABC	$P<0.01$	NS
	AC	$P<0.008$	NS
Morphology (FAB classification) M_1+M_2	ABC	NS	Not investigated
vs. M_3	ABC	NS	
vs. M_4+M_5	ABC	NS	
Erythrocytes $<3.0\times10^{12}$/liter	ABC	NS	$P<0.05$
Leukocytes $>30.0\times10^{9}$/liter	ABC	NS	NS
Platelets $<30.0\times10^{9}$/liter	ABC	$P<0.08$	NS
	AC	$P<0.05$	$P<0.01$
Percentage of blasts in blood	ABC	$P<0.05$	NS
	AC	$P<0.01$	NS
Percentage of blasts in marrow	ABC	NS	Not investigated
Spleen enlargement	ABC	NS	NS
Treatment strategy	ABC	$P<0.001$	NS

A, survived <3 months; B, survived 3–12 months; C, survived >12 months.

Table 2. Comparison of prognostic factors in patients with AML according to achievement and duration of remission

Factor	A′ (no remission) $n=25$	B′ (remission <6 months) $n=12$	C′ (remission ≥ 6 months) $n=25$	Statistical analysis
Age (mean)	46.1	31.4	34	$P<0.01$
<50 years[a]	52	100	81	
Sex M/F[a]	60/40	25/75	50/50	NS
NPF present[a]	80	50	46	NS
Infection present[a]	60	58	38	NS
Hemorrhage present[a]	56	42	38	NS
Morphology (FAB)	Predominant M_2		Predominant M_1, M_2	–
Erythrocytes $\leq 3.0\times10^{12}$/liter[a]	76	75	73	NS
Leukocytes $>30.0\times10^{9}$/liter[a]	36	33	15	NS
Platelets $<30.0\times10^{9}$/liter[a]	52	67	38	NS
Duration of remission in months	–	3.9	14.8	
Survival in months	1.8	11.5	22.5	

[a] In percentage of patients.

frequency of severe anemia were observed only in ALL groups ($P<0.05$). The analysis of the influence of morphologic subtypes in AML on survival did not show significant dependence, even when leukemic patients were grouped into two similar types (M_1+M_2 and M_4+M_5) excluding M_3. The greater percentage of blasts in peripheral

Table 3. Comparison of prognostic factors in patients with ALL according to achievement and duration of remission

Factor	A′ (no remission) $n=13$	B′ (remission <6 months) $n=5$	C′ (remission $\geqq 6$ months) $n=16$	Statistical analysis
Age (mean)	36	23.4	26	NS
<50 years[a]	61	100	94	NS
Sex M/F[a]	61/39	80/20	62/38	NS
NPF present[a]	80	40	44	NS
Infection present[a]	46	0	37	NS
Hemorrhage present[a]	38	20	12	NS
Erythrocytes $\leqq 3.0 \times 10^{12}$/liter	69	60	43	NS
Leukocytes $>30.0 \times 10^{9}$/liter	38	60	18	NS
Platelets $<30.0 \times 10^{9}$/liter	69	40	25	NS
Duration of remission in months (mean)	–	4.0	12.0	
Survival in months	2.1	10.8	17.7	

[a] In percentage of patients.

blood, but not in bone marrow, in AML correlated with shorter survival time ($P<0.01$) while in ALL patients such a relationship was not observed.

The treatment strategy in AML patients, especially the supportive therapy, played a significant role in lengthening survival time in AML ($P<0.001$) but not in ALL patients.

Analyzing the influence of various prognostic factors on the chance of obtaining remission and its duration time, we obtained only one statistically significant difference, namely in age groups of AML ($P<0.01$) (Tables 2 and 3). Survival time of patients correlated clearly with the duration time of the first remission.

Discussion

The importance of several risk factors has been confirmed in predicting survival time and remission achievement in acute leukemias. Leukocyte count, previously reported to be associated with response or survival [1], was found not to be significant as a prognostic factor. Some authors claim, that the normal hemoglobin (Hb) level has prognostic significance. Our observation proved this claim but only in ALL patients. Passe et al. [2] did not find any blood or marrow values, besides Auer rods, to be significantly related to the rate of remission. We found a relationship between the percentage of blasts in peripheral blood and survival in AML patients.

Amaki et al. [3] in analyzing the cytology of blasts found no difference between M_1 and M_2 and a significant difference between M_4 and M_5 in the response to treatment: the longest remission in M_3 and the shortest in M_5 and M_6. In all the subtypes, we did not find any statistical correlations between the cytologic type of AML and survival but we did have very few M_5 and M_6 cases.

NPF, according to Bernard et al. [4], set the collection of several clinical and hematologic factors responsible for bad prognosis. We decided to use this definition to describe the patients' general clinical condition. It seems to us that these "factors", which are to some extent subjective, play an important role along with the other prognostic characteristics. Age is an important prognostic factor both in AML and ALL [1, 4]. Most studies support the notion that increasing age is associated with shorter survival. Our study, together with others, found no such correlation in adult ALL. Bernard et al. and other authors have forced the opinion that for those patients over 60 prognosis is much poorer [4–6]. Contrary to others [4, 7], we did not find a sex difference in response rate and survival. The influence of infection on survival has been discussed [7]. Surprisingly,

the patients with an infection were found to have longer remission than did those without an infection [2]. We made the same observation in several patients after lobar pneumonia. Hepatic and renal lesions and other coexisting diseases however, made the leukemia worse.

Keating et al. [7] found that a history of antecedent hematologic disorders and insufficient metaphases had a major significance in prognosis. We did not investigate these factors here, but we have made some observations confirming this opinion. The same applies to leukemia occurring in patients who have had treatment with an alkylating agent. The achievement of complete remission is the most important factor determining survival [8, 9]. Our AML patients with remission lasting less than half a year (median 3.9 months) survived an average 11.5 months, and those with longer remission (median 14.8 months) had a median survival time of 22.5 months. The treatment strategy and supportive care have an important influence on the remission rate.

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Acute Myelocytic Leukemia in Adults: A Long-Term Analysis

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Introduction

Cytosine arabinoside (Ara-C) and daunorubicin (DNR) have been shown to be the most active single agents in the treatment of acute myelocytic leukemia (AML) leading to 20%–30% and 35%–55% complete remissions (CRs), respectively [1]. In 1973, Yates et al. [2] reported on a CR rate of 63% in adult patients with AML treated with a 7-day continous infusion of Ara-C together with three daily doses of DNR. The present report deals with the long-term results of such remission induction (RI) therapy in 65 adult patients with AML as well as with cyclic maintenance therapy in those patients who achieved CR. Maintenance chemotherapy was applied as an attempt to prolong the remission duration and possibly to eradicate persisting leukemic cells during remission. Furthermore, this report deals with a number of pretherapeutic and therapeutic variables tested for their predictive value for RI and survival in the patients treated.

Patients and Therapy

A total of 65 patients with previously untreated AML (34 female, 31 male), ranging in age from 15 to 71 (median 43) years were treated between 1974 and 1979. Follow up ranged up to 10 years. RI consisted of 7-day courses of Ara-C (100 mg/m^2 24-h infusion) together with DNR (45 mg/m^2 per day i.v.) on days 1, 2, and 3. Supportive care consisted of broad spectrum antibiotics for fever in the presence of granulocytopenia, and transfusions of erythrocytes and platelets when required. Criteria for CR were the absence of disease-related symptoms, normal marrow cellularity with less than 5% blasts and peripheral blood granulocyte and platelet counts greater than 1.5×10^9/liter and 100×10^9/liter, respectively. For remission maintenance, combinations of Ara-C (i.v. 100 mg/m^2 q 12 h $\times$ 10) with each of four drugs, 6-thioguanine (p.o. 100 mg/m^2 q 12 h $\times$ 10), cyclophosphamide (i.v. 800 mg/m^2 on day 1), chloroethyl cyclohexyl nitrosourea (CCNU) (p.o. 100 mg on day 1) or DNR (i.v. 45 mg/m^2 on days 1 and 2) were given in rotational sequence every 4 weeks during the first year and every 6 weeks during the second year of remission.

Statistical Analysis

Patients who did not achieve CR after at least two or three courses of therapy or who died during RI were considered as induction failures. Time to CR was counted from the start of treatment. The χ^2 test was used to determine differences in rates or frequency of occurrence. Survival was calculated from start of treatment to death, remission duration from achievement of CR to relapse, and relapse-free survival from achievement of CR to relapse or death. Remission duration, relapse-free survival and overall survival were calculated using the Kaplan-Meier method. Differences between the survival curves were tested using the generalized Wil-

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coxon and generalized Savage methods. Prognostic factors related to survival were assessed using Cox's proportional hazards model.

Results

Of the 65 patients 36 (55%) attained CR. Of the 29 patients who failed to achieve CR, 17 (27%) had definitely resistant leukemia and 12 (18%) died during RI of infection or hemorrhage. The mean number of courses and the median number of days to achieve CR were 1.5 and 36, respectively. Sex and leukemic type seemed to have no effect on the remission rate. Age, in contrast, appeared to correlate with CR rate inversely and to predispose to fatal complications, since the frequency of early death increased significantly ($p=0.02$) from 9% (4/45) in patients aged 49 or under to 40% (8/20) in those aged 50 or over. The median remission duration for the 36 responders was 11 months. The median survival time for all patients was 31 weeks, for those responding to therapy 74 weeks and those not responding 8 weeks. The probability of a 10-year survival for all patients was 10%, and for those achieving CR 20%. Comparisons of Kaplan-Meier plots of groups with different pretherapeutic and therapeutic characteristics (sex, age, subtype of leukemia, initial WBC count, number of courses to achieve CR) revealed a significant ($p=0.03$) difference in disease-free survival and overall survival only between patients with an initial WBC count higher than 30000/mm^3 and those with a WBC count below this level (Fig. 1). In addition, age over 50 years appeared to be of negative prognostic significance ($p=0.02$) for survival, particularly during the initial phase of treatment (Fig. 2). Furthermore, there was a suggestive, but statistically not significant, inverse correlation between survival time and the number of induction courses to

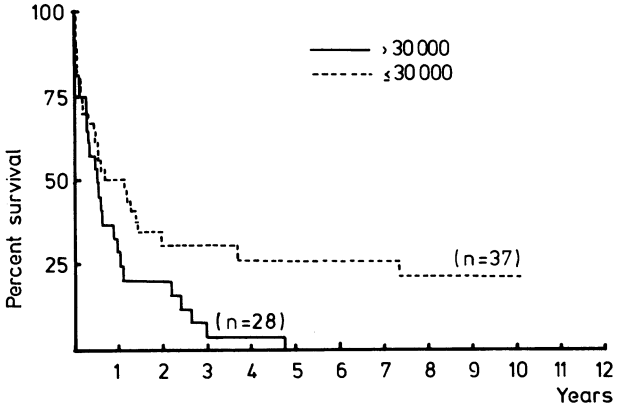

Fig. 1. Survival according to initial WBC count/mm^3; $p=0.19$ (generalized Wilcoxon's test); $p=0.03$ (generalized Savage's test)

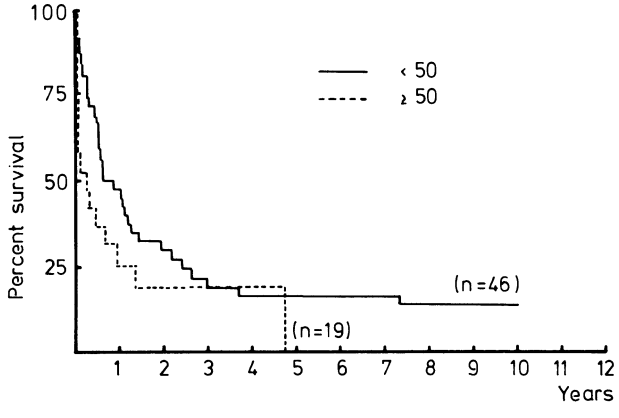

Fig. 2. Survival according to age (years); $p=0.02$ (generalized Wilcoxon's test); $p=0.07$ (generalized Savage's test)

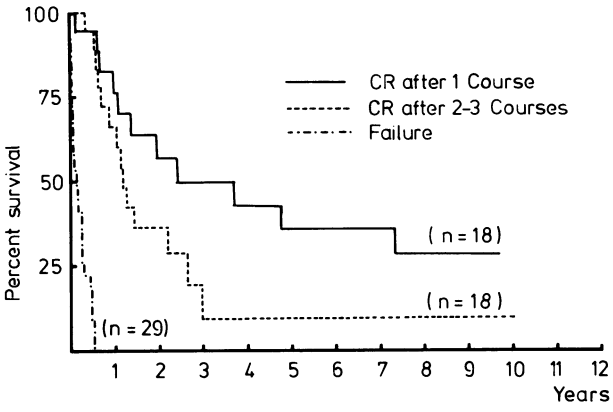

Fig. 3. Survival by response to induction therapy and by number of courses required to achieve CR; $p = 0.14$ (generalized Wilcoxon's test); $p = 0.09$ (generalized Savage's test)

achieve CR (Fig. 3). Predictive values of sex, age, type of leukemia, initial WBC count, lymph node enlargement, hepatosplenomegaly, bleeding, and infection for survival were also evaluated using the Cox model. Of these variables, only initial WBC count was found to be of prognostic significance ($p = 0.04$).

Discussion

The therapeutic results obtained in this study compare favorably with those reported by Rai et al. [3] who used the same RI and maintenance therapy. These authors reported on a CR rate of 55% and a median remission duration of 10 months in adult patients with AML. In patients who responded, the probability of remaining in CR was approximately 25% at 5 years. Among the different variables tested in our study, age appeared to be the only major determinant for the outcome of RI therapy. This observation is consistent with the results of a series of studies indicating an adverse effect of age over 50–60 years on CR rate [3–7]. The relatively low CR rate in this patient group is mainly due to a high mortality rate during RI. For long-term survival, however, an initial WBC count greater than 30000/mm^3 was found to be the only factor of negative prognostic significance. The number of courses required to achieve CR showed a suggestive, but statistically not significant, inverse correlation to survival. The risk factors reported in this and other studies, however, have to be considered valid only in the context of the therapeutic regimens used. Differences in the therapeutic protocols might result in different prognostic factors, as reported in some other studies [8, 9].

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Prognostic Factors in Acute Myelogenous Leukemia

L. Čevreska[1], and R. P. Gale[2]

Introduction

Recently, there has been considerable progress in the therapy of acute myelogenous leukemia (AML). Much of this progress results from the use of intensive remission induction (RI) regimens typically consisting of cytarabine and daunorubicin with or without thioguanine. In recent studies, individuals who achieve a remission receive intensive post-remission chemotherapy, referred to as consolidation or intensification. Maintenance chemotherapy is also given in some studies.

The use of high-dose chemotherapy, although effective, is associated with considerable morbidity and mortality. Because of this, it is important to identify accurately individuals likely to benefit from treatment. In this study we analyzed data from 152 reports of prognostic factors in more than 5000 patients with AML. Factors were divided into clinical, laboratory, or special investigations, and considered as either dichotomous or continuous variables in univariate and multivariate analyses. Based on a critical analysis of positive and negative studies we were able to rank variables for their likelihood of predicting treatment outcome.

Methods

A Medline search of reported prognostic factors in AML was performed using a 1975–1985 database. Relevant citations were checked for cross-references. We identified 152 reports in this period which analyzed one or more prognostic factors for effect on treatment outcome. The 42 variables contained in these reports are outlined in Table 1. Variables could be divided into three groups: clinical features, laboratory studies, and special investigations. Three potential treatment outcomes were identified: remission rate, remission duration, and survival. Factors were considered either as dichotomous or continuous variables dependent on the method of reporting. In each report we consider several aspects, including significant positive and negative correlations as well as factors determined not to significantly influence outcome. Typically significant correlations were supported by a $P\alpha \leqq 0.05$. When possible, we calculated the statistical power of analyses that failed to find significant correlations.

In the second stage of this analysis we ranked each of 31 variables, for which there were sufficient data, for likelihood of predicting outcome using a tertiary scale of highly predictive, probably predictive, and possibly predictive. Eleven variables were reported in single studies and could not be critically evaluated for likelihood of predicting outcome.

Results

Results of the analysis of 152 studies involving >5000 individuals with AML are summarized in Table 1. Ten clinical factors were analyzed (Table 2). Those highly correlated

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Table 1. Prognostic factors in AML[a]

Variable	Favorable	Remission	
		Rate	Duration
Age	Young	+	+
Sex	Female	+	−
Performance status	High	+	−
Liver	Normal	+	−
Spleen	Normal	+	−
Blasts	$<50 \times 10^9$/liter	+	+
Platelets	$>70 \times 10^9$/liter	+	−
LDH	<400 IU/dl	−	+
Fibrinogen	>250 mg/dl	−	+
Creatinine (BUN)	<2.0 mg/dl (<18 mg)	+	−
Chromosomes	Normal, t (8;21)	+	−
Autoimmune hemolytic disease	No	+	+
Hepatitis	Yes	−	+
Labeling index	Low	−	+
Growth pattern in vitro	Cluster	+	+
FAB subtype	$M_{1,2,3}$	+ ($M_{1,2}$)	+ (M_3)
Auer rods	Present	+	+
CNS leukemia	No	+	+
Extramedullary leukemia	No	+	+
Drug sensitivity in vitro	Sensitive	+	+
Ara-CTP incorporation	High	+	+
Rate decrease blasts	Rapid	−	+
Time to CR	Rapid	−	+
Cycles to CR	Few	−	+

[a] +, indicates an influence of the variable on outcome; −, no influence. These conclusions are based on a consensus of reports.

Table 2. Clinical features of patients and laboratory and special investigations carried out

Clinical features	Laboratory tests	Special investigations
Age	WBC	FAB classification
Antecedent hematologic disorder	Level circulating myeloblasts	Cytogenetics
		Auer rods
Therapy-linked AML	Platelets	Interval or cycles to remission
Sex	Hemoglobin	Reduction in marrow myeloblasts/cellularity
CNS involvement	LDH	Ara-CTP retention/incorpation
Performance status	Alkaline phosphatase	Labeling index
Infection	Fibrinogen	Growth pattern in vitro
Bleeding	Creatinine	Self-renewal
Associated pathology	Lysozyme	Premature chromosome condensation
Hepatosplenomegaly	Bilirubin	Drug sensitivity in vitro
	Hepatitis	TdT
		Reverse transcriptase
		Leukemia-associated antigen
		Immune complexes
		Antiviral antibody
		Glucocorticoid receptors
		Abnormal PMNs
		Leukapheresis

Table 3. Scoring of prognostic Factors

Highly correlated	Probably correlated	Possibly correlated
Age	Sex	Infection
Antecedent hematologic disorder	CNS involvement	Bleeding
Therapy-linked AML	Performance status	Associated pathology
WBC	Auer rods	Platelets
Level circulating myeloblasts	Interval cycles to remission	Hemoglobin
FAB classification	Reduction bone marrow myeloblasts	Alkaline phosphatase
Cytogenetics		Fibrinogen
		Creatinine
		Lysozyme
		Bilirubin
		Hepatitis
		Ara-CTP retention/incorporation
		Labeling index
		Cell growth in vitro
		Self-renewal in vitro
		Reduction in myeloblasts/cellularity
		Premature chromosome condensation
		Drug sensitivity in vitro

with outcome included age, presence of therapy-linked AML, antecedent hematologic disorders, and central nervous system (CNS) involvement at diagnosis. Less impressive correlations were determined for hepatomegaly, splenomegaly, and sex. Performance status, associated pathology, and infection or bleeding at diagnosis were inconsistently predictive of outcome. The influence of some factors such as age appeared restricted to one outcome, such as remission rate, but not duration. In other instances, such as antecedent hematologic disorder or therapy-linked AML, both remission rate and duration were adversely affected.

Eleven routine laboratory tests were evaluated (Table 2). The WBC or absolute level of circulating myeloblasts was reproducibly correlated with outcome, including remission rate and/or duration. An elevated level of hepatic enzymes following induction chemotherapy was correlated with longer remissions in most, but not all, studies. Moderately reproducible correlations were also found for hemoglobin, platelets, fibrinogen, creatinine, lysozyme, lactic dehydrogenase (LDH), alkaline phosphatase, and bilirubin.

Nineteen more specialized investigations were reported (Table 2). Highly correlated factors included French-American-British (FAB) classification, cytogenetics, Auer rods, and percentage of bone marrow myeloblasts and/or cellularity. Probable correlations were found for interval or numbers of cycles of chemotherapy required to achieve remission, in vitro drug sensitivity, in vitro cell growth pattern in semisolid matrices, pretreatment labeling index, and terminal deoxynucleotidyl transferase (TdT) positivity. Possible correlations included self-renewal capacity and premature chromosome condensation. Variable correlations were reported for leukapheresis, reverse transcriptase levels, level of leukemia-associated antigens, circulating immune complexes, antiviral antibody responses, glucocorticoid receptors on leukemia cells, and abnormal morphologic features of polymorphonuclear neutrophils. Eleven factors were analyzed in single studies and could not be critically evaluated.

We next ranked the likelihood of each variable predicting outcome. This ranking was based on reproducibility between studies as well as the statistical power of these studies; these data are summarized in Table 3.

Seven factors were determined to be highly correlated with treatment outcome, including age, antecedent hematologic disorder, therapy-linked AML, WBC, level of myeloblasts in the blood, FAB classification, and chromosome abnormalities. Each

of these correlations was complex. For example, some chromosome abnormalities such as t(9;22), t(4;11) or −5, −7, or −11 were correlated with an adverse outcome with regard to both remission rate and duration. Other abnormalities such as t(8;21) t or inv (3;3), or inv or del (16) were associated with favorable outcomes.

Six variables were considered probably predictive of outcome including sex, CNS leukemia at diagnosis, performance stutus, Auer rods, time or numbers of induction courses to remission, and extent or rate of reduction of bone marrow myeloblasts.

Eighteen variables were considered possibly predictive of outcome; and eleven were noted in single reports and are indicated in Table 3.

Discussion

In this study we evaluated 42 factors reported to predict treatment outcome in individuals with AML. Some factors were highly correlated with either remission rate, remission duration, or survival in most, if not all, studies. These correlations were typically highly significant; studies which failed to find a correlation usually had a low statistical power.

The effect of these factors was complex. For example, increasing age resulted in lower remission rates in most studies. In contrast, there are few data indicating an effect of age on remission duration or subsequent survival. The impact of FAB classification was also complex; acute progranulocyte leukemia (M_3), had an adverse effect on remission rate but was associated with longer remissions.

Other variables were usually but not invariably correlated with outcome; we term these probably predictive of outcome. In this instance, most, but not all, studies reported a correlation including some with considerable power of analysis. In addition, the level of significance was lower than noted with factors highly predictive of outcome. These factors were found to be less complex with two exceptions. Several studies have reported a correlation between time to remission and subsequent remission duration. Since time to remission is correlated with the number of cycles of induction chemotherapy, these variables are confounded; it is not presently possible to distinguish between these alternatives. Analysis of bone marrow myeloblasts is also complex. Some studies suggest that the extent of reduction is critical whereas others suggest a primary role for the rate of reduction independent of extent. Again, these factors are correlated and the analysis, therefore, confounded. It is likely that both factors play a role and that some dynamic transgenerated variable might be most highly correlated.

Eighteen variables were inconsistently correlated with treatment outcome and 11 were reported in single studies. The importance of these factors is less certain and should probably not to be used for clinical decisions until confirmed in prospective studies.

By combining and weighting the prognostic implications of the variable considered in this study, it is possible to construct a scoring system to predict treatment outcome. These data should prove useful in comparing data from different studies and may be useful to explain diverse results obtained with identical treatment protocols. They should also prove useful in the design of new therapeutic strategies. They may also provide an insight into the biology of AML.

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Serum Zinc and Copper as Prognostic Factors in Acute Nonlymphocytic Leukemia

Y. Beguin, J. Bury, J. M. Delbrouck, G. Fillet, G. Robaye, I. Roelandts, and G. Weber[1]

Abstract

A total of 44 patients were treated with intensive induction chemotherapy for acute nonlymphocytic leukemia (ANLL). A complete remission (CR) was obtained in 29/44 (66%) patients. Serum zinc (Zn) and copper (Cu) were studied as possible prognostic factors in the determination of the chance of a patient attaining remission. Pretreatment Zn was higher in patients attaining a remission (0.99 ± 0.05 μg/ml) than in patients failing to attain a CR (0.78 ± 0.06 μg/ml) ($P = 0.0216$). There was no further difference between the two groups during aplasia. However, when response to treatment was evaluated about day 28, the difference reappeared: 1.06 ± 0.05 μg/ml for CR patients vs 0.77 ± 0.07 μg/ml for failures ($p = 0.0012$). Pretreatment Cu was higher in responding (1.44 ± 0.07 μg/ml) than in nonresponding (1.06 ± 0.05 μg/ml) patients ($p = 0.0002$). The difference between the two groups remained highly significant at days 7, 14, 21, and 28. At the time of response evaluation, the values were 1.46 ± 0.05 μg/ml for CR patients vs 1.19 ± 0.08 μg/ml for non-CR patients ($P = 0.0070$). We conclude that the measurement of serum Zn and Cu may be helpful in the prediction of response to chemotherapy in patients treated for ANLL.

Introduction

Significant progress has been achieved in the treatment of acute nonlymphocytic leukemia (ANLL) since the use of anthracyclines and cytosine arabinoside (Ara-C) [1, 2]. However, a substantial fraction of the patients (20%–40%) will not respond to chemotherapy [1, 2]. Therefore, attention has been focused on prognostic factors possibly determining the chance of a patient attaining remission [3]. Serum trace elements are a subject of increasing interest in human health and diseases. Many reports have dealt with the significance of copper in lymphomas and Hodgkin's disease [4] but few observations are available on trace elements in acute leukemia [5–13]. We have undertaken a prospective study of serum trace elements in ANLL, with special reference to their prognostic significance in patients receiving induction chemotherapy.

Patients and Methods

A total of 44 patients with ANLL were studied. There were 28 men and 16 women. Age ranged from 11 to 85 years (mean 48.6 years). Thirty patients were seen at first presentation (new patients) and 14 patients at relapse (relapsing patients).

New patients received an association (DOA) of daunorubicin (45 mg/m^2, days 1, 2, 3), vincristine (1 mg/m^2, day 2), and cytarabine (CAR, 200 mg/m^2, from day 1 to day 7). Other therapeutic regimens (AMSA + CAR, AMSA + VP16, CAR + VP16, high-dose CAR, high-dose melphalan) were used in patients failing to respond to the DOA protocol and in relapsing patients.

Serum trace elements (STE) were measured by PIXE (proton-induced X-ray emis-

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sion), allowing for a simultaneous determination of chlorine, potassium, iron, calcium (Ca), copper (Cu), zinc (Zn), selenium (Se), and bromine (Br) [14]. Samples were obtained before chemotherapy (day 0) and then twice weekly for 3–20 weeks.

Results

Overall Results

CR was obtained in 29/44 (66%) patients. The CR rate was higher in new (23/30, 77%) than in relapsing (6/14, 43%) patients. Pretreatment STE values are presented in Table 1. Ca and Br were not significantly different in patients and in controls. Cu was higher in patients (1.31 ± 0.06 μg/ml) than in controls (1.10 ± 0.02 μg/ml) ($P < 0.002$). Zn was lower in patients (0.92 ± 0.04 μg/ml) than in controls (1.10 ± 0.02 μg/ml) ($P < 0.001$). Se was also reduced in patients (0.078 ± 0.005 μg/ml) as compared with controls (0.097 ± 0.004 μg/ml) ($P < 0.01$).

Comparison of CR and non-CR patients

STE were compared in CR and non-CR patients (Table 2). Se, Ca, and Br were not different in the two groups. Pretreatment Zn was significantly higher in Cr (0.99 ± 0.05 μg/ml) than in non-CR (0.78 ± 0.06 μg/ml) patients ($P = 0.0216$). Pretreatment Cu was also more elevated in responding (1.44 ± 0.07 μg/ml) than in nonresponding (1.06 ± 0.05 μg/ml) patients ($P = 0.0002$). A multivariate Hotelling T2 test on pretreatment Ca, Cu, Zn, Se, and Br gave a P value of 0.0040.

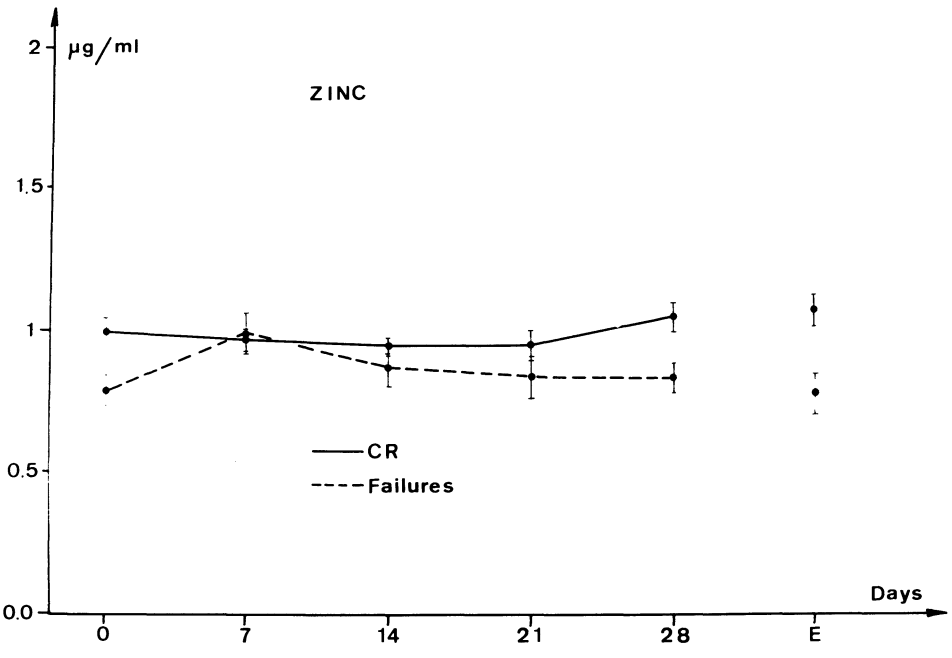
Various combinations of pretreatment STE and serum proteins were tested in discriminant analysis between CR and non-CR patients. The best combination was provided by Cu, Zn, and serum proteins (entered in this order), allowing for a correct classification of 87% of the patients. The patients incorrectly classified were: one case of death 3 weeks after CR, one case of second CR of very short duration, one case of infection before chemotherapy (thus depressing serum Zn level), and one unexplained case. The patient incorrectely classified as CR was a patient who died during aplasia. For Zn (Fig. 1), the difference among CR and non-CR patients was not significant during aplasia, but reappeared at the time of response evaluation: 1.06 ± 0.05 μg/ml in CR vs 0.77 ± 0.07 μg/ml in non-CR patients ($P = 0.0012$). For Cu (Fig. 2), the difference

Table 1. Serum trace elements (mean ± SEM, μg/ml) in 44 ANLL patients and 100 normal controls

	Controls ($n = 100$)	Patients ($n = 44$)	p value
Calcium	102.5 ±0.7	102.2 ±2.2	NS
Copper	1.10 ±0.02	1.31 ±0.06	$p < 0.002$
Zinc	1.10 ±0.02	0.92 ±0.04	$p < 0.001$
Selenium	0.097±0.004	0.078±0.005	$p < 0.01$
Bromine	5.36 ±0.19	5.04 ±0.81	NS

Table 2. Pretreatment serum trace elements (mean ± SEM, μg/ml) in CR patients and in failures

	CR	Failures	p value
Calcium	104.1 ±2.8	98.4 ±3.4	NS
Copper	1.44 ±0.07	1.06 ±0.05	$p = 0.0002$
Zinc	0.99 ±0.05	0.78 ±0.06	$p = 0.0216$
Selenium	0.080±0.0006	0.073±0.009	NS
Bromine	4.88 ±0.62	5.36 ±2.17	NS



CR	0.99 ± 0.05	0.96 ± 0.04	0.93 ± 0.03	0.93 ± 0.05	1.03 ± 0.05	1.06 ± 0.05
Failures	0.78 ± 0.06	0.98 ± 0.07	0.85 ± 0.06	0.82 ± 0.07	0.82 ± 0.05	0.77 ± 0.07
P Value	P = 0.0216	NS	NS	NS	NS	P = 0.0012

Fig. 1. Mean ± SEM serum zinc in CR patients and in failures, from day 0 to the day of response evaluation *(E)*

remained highly significant on days 7, 14, 21, and 28. At the time of response evaluation, the values were 1.46 ± 0.05 µg/ml for CR patients vs $1.19 \pm 0{,}08$ µg/ml for failures ($P = 0.0070$).

Comparison of New and Relapsing Patients

There was no difference in STE levels between new and relapsing patients, except for Cu which was more elevated in new than in relapsing patients, although the difference became only significant by day 7. The two groups were analyzed separately but the results must be interpreted cautiously as complete pretreatment data are available for few non-CR new patients and CR relapsing patients. However, both in new and in relapsing patients Cu and Zn were significantly higher in CR than in non-CR patients.

Discussion

Few authors have reported on Cu and Zn in acute leukemia. Cu was generally increased in active disease [5–7, 9, 10, 12], returned to normal values in patients attaining a CR [6, 7, 9, 10, 12] but remained high in non responsive patients [8]. So Cu was considered to be valuable in monitoring response to chemotherapy [6, 7, 12] but long-term studies ruled out Cu as a useful index of disease activity after CR has been obtained [13]. Pretreatment Zn was found to be somewhat lower in leukemic patients than in controls [7]. However, these studies were carried out in acute lymphocytic leukemia (ALL) or in inhomogeneous groups of ALL and ANLL patients, and generally in children. None examined Cu or Zn as possible prognostic factors.

We found moderately but significantly decreased Zn and increased Cu levels in ANLL patients. Cu and Zn did not return to

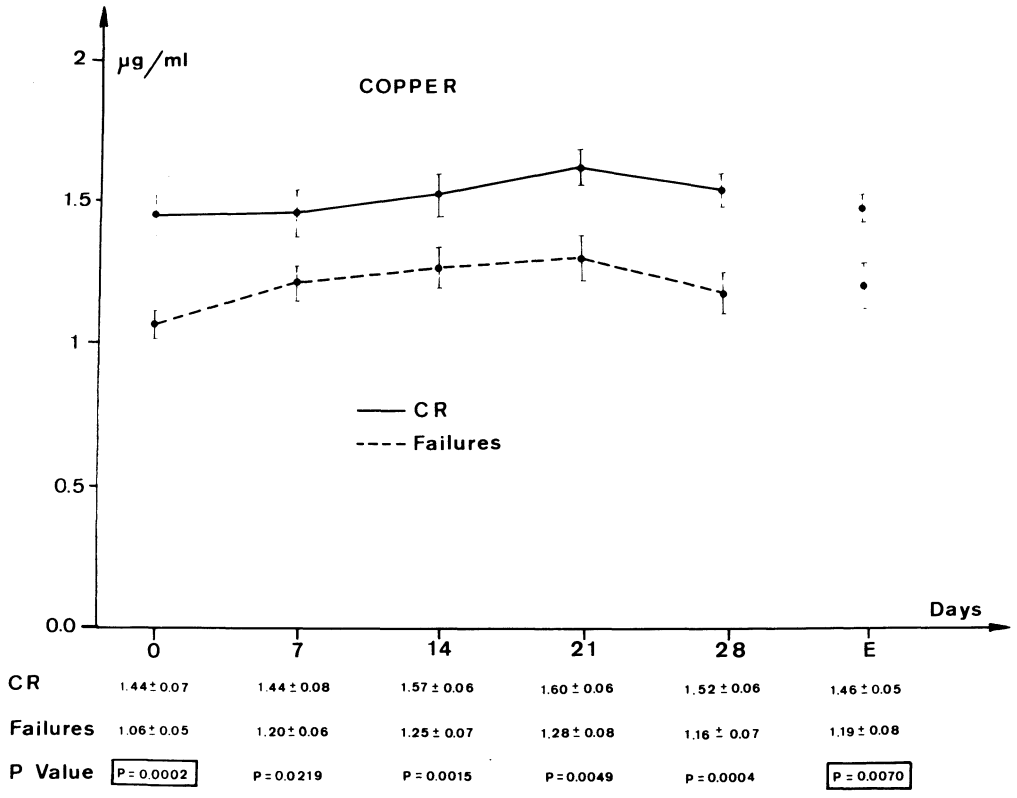


Fig. 2. Mean ± SEM serum copper in CR patients and in failures, from day 0 to the day of response evaluation *(E)*

normal by day 28. However, as most of our patients experienced infectious episodes during aplasia, this might have prevented correction of their Cu and Zn levels.

Pretreatment Cu and Zn were found to be significant prognostic factors of response to induction chemotherapy, both being higher in CR than in non-CR patients. Relapsing patients had a lower Cu than new patients but, after correction for difference in the stage of the disease, the prognostic significance of Cu and Zn was still observed. Furthermore, 87% of the patients were correctly classified into CR and failures by discriminant analysis based on their pretreatment Cu, Zn, and serum proteins. Reasonable explanations could account for most of the incorrectly classified cases.

Our results suggest that pretreatment serum Zn and Cu might be useful prognostic factors in ANLL patients. The use of STE together with other clinical and biologic parameters could be of considerable interest in developing predictive models for response to chemotherapy [3]. Further studies are needed to evaluate the potential role of STE in monitoring patients during CR and in detecting early relapse or extramedullary disease [10].

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Immunological Monitoring in Remission Acute Myeloid Leukemia During Maintenance Therapy

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Introduction

In a study beginning in 1981 43 adult patients with acute myeloid leukemia (AML) in remission were treated with cytosine arabinoside (Ara-C) alternately combined with daunorubicin, thioguanine, or cyclophosphamide in maintenance therapy every 4 weeks. 22 of the 43 patients were randomized for chemotherapy (CT) only and 21 for chemo-immunotherapy (CIT). CIT patients received immunotherapy with additional neuraminidase-treated allogeneic viable blasts given between the chemotherapy courses. During maintenance therapy immunologic monitoring was carried out.

Skin Testing

Skin tests with recall antigens were performed at the start of the maintenance therapy and during ongoing remission.

All patients and controls received intradermal injections of 10 U SK- 2.5 U/SD /0.1 ml (Varidase, Lederle), 0.1 ml mumps skin test antigen (Lilly) undiluted, 100 PNU/ 0.1 ml candida allergen-extract (Hollister Stier) and 5 TU/0.1 ml PPD (Tuberculin, purified protein derivate, Parke-Davis) into the forearm. The delayed hypersensitivity reaction was measured 48 h after injection.

If tests were carried out before the start of maintenance therapy patients showed a significantly impaired reactivity to SK/SD ($p \leqq 0.0001$), mumps ($p \leqq 0.001$) and candida-antigen ($p \leqq 0.02$) compared to normal subjects. Only in the skin test with PPD ($P \geqq 0.1$) was there no difference. During ongoing remission an improvement in skin test reactivity could be observed.

Lymphocyte Stimulations

Between chemotherapy courses lymphocyte stimulations were performed every 4 weeks, provided that the count of leukocytes was $\geqq 1500$ and the thrombocytes $\geqq 50\,000/\text{mm}^3$.

Lymphocytes were purified using the Ficoll-Hypaque sedimentation technique, incubated with phytohemagglutinin (PHA) or pokeweed mitogen (PWM) for 48 h, thereafter ^{3}H-thymidine was added; 24 h later all cells were harvested.

In stimulations using PHA before the start of maintenance therapy a dose-related increase of thymidine uptake was found for a mitogen concentration of 0.156 y/ml, 0.625 y/ml, 2.5 y/ml and 10 y/ml; followed by a decrease in thymidine uptake for a concentration of 40 y/ml. The same results were shown in stimulations using PWM. Compared to normal subjects, patients' lymphocytes reached a significant lower thymidine uptake in stimulations with both mitogens (PHA: 0.156 y/ml, $p = 0.177$; 0.625 y/ml, $p = 0.129$; 2.5 y/ml, $p = 0.0001$; 10 y/ml, $p = 0.0001$; 40 y/ml, $p = 0.0001$; PWM: 0.025 y/ml, $p = 0.065$; 0.25 y/ml, $p = 0.005$; 2.5 y/ml, $p = 0.0001$ y/ml; 25 y/ml, $p = 0.001$; 250 y/ml, $p = 0.017$).

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The mitogen responsiveness of the patients' lymphocytes was reduced before the eighth therapy course, but before the 12th course there was no difference between the thymidine uptake of patients and that of the normal subjects.

No difference in mitogen responsiveness was found over all the tests between the two therapy groups.

Lymphocyte Subpopulations

Simultaneously, lymphocyte subpopulations were analyzed with the monoclonal antibodies OKIa1, OKT3, OKT4 and OKT8, and were examined at the same time as the lymphocyte stimulations.

The mononuclear cells were separated using the same technique as above, and then cytospin preparations were performed. After incubation with the monoclonal antibodies the reaction was visualized by peroxidase-conjugated anti-mouse immunoglobulin.

Compared to normal subjects the count of B cells, T cells, and helper-cells in patients was significantly reduced ($p \leqq 0.01$), whereas the absolute number of suppressor cells was identical.

At the same time patients showed a significantly ($p \leqq 0.01$) reduced absolute peripheral blood lymphocyte count while the absolute granulocyte count was comparable.

Discussion

Lymphocyte stimulations and skin tests indicate that the immune system of patients in remission AML is suppressed. In addition, the T4/T8 ratio is significantly reduced in patients compared to controls ($T4/T8 = 1.36$, $p \leqq 0.01$). During ongoing remission an improvement of mitogen responsiveness and skin test reactivity was observed.

No differences were seen between the patients who received only chemotherapy or additional immunotherapy; but to compare both therapy groups one must consider that the number of patients in longer remission in both therapy groups (over 2 years) is too small to give an answer to this question at the moment.

Further investigations are needed to confirm whether the depression of the immune system is related to chemotherapy or to an underlying disease.

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AML in Children

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Treatment of Childhood Acute Nonlymphocytic Leukemia with Individually Scheduled High Doses of Cytarabine: Preliminary Results of Study ANLL-82 of the Dutch Childhood Leukemia Study Group (DCLSG)*

K. Hählen[1], A van der Does-van den Berg, L. P. Colly, L. A. Smets, J. A. J. M. Taminiau, and J. M. Vossen

Introduction

Experimental studies in animals have shown that high doses of cytosine arabinoside (HDARA-C) induce synchronization and possible recruitment of leukemic cells. Administration of a second HDARA-C injection at the moment of maximal accumulation of cells in S-phase resulted in the most effective reduction (one log) of leukemic cells [1]. In a pilot study (ANLL-80) of 24 children with acute nonlymphocytic leukemia (ANLL), 12 injections of HDARA-C were administered at 24-h intervals, followed by two injections of adriamycin, which resulted in a remission rate of 71%. Without further treatment, however, early relapses occurred in the majority of these children. Cell kinetic studies in children with relapsed ANLL have shown a considerable range in the proliferative status of their disease, measured by flow cytometry. The percentage of cells in S-phase in the bone marrow (BM) at the time of relapse varied between 3.5% and 14%. An inverse relationship between the percentage of cells in S-phase at relapse and the time interval up to maximal accumulation of cells in S-phase after one injection of HDARA-C (1 g/m^2) was established. The correlation between these two parameters was laid down in a "calibration curve" [2]. The current protocol, ANLL-82, with an individually scheduled induction course consisting of 12 injections of HDARA-C and one injection of adriamycin, is based on these findings. In children in remission this was followed by allogeneic bone marrow transplantation (BMT) or maintenance treatment according to the VAPA-10 protocol [3].

Patients and Methods

Between January 1983 and May 1985, 29 children with newly diagnosed ANLL were admitted to this study. The diagnosis of ANLL and subtyping according to the FAB classification were made by institutional examination of BM smears and independently confirmed by the DCLSG laboratory. All follow-up BM smears were also sent there. A heparinized sample of the diagnostic BM aspirate was sent to the laboratory of the Antoni van Leeuwenhoekhuis in Amsterdam. The percentage of leukemic cells in S-phase was determined by flow cytometry as previously described [2]. From the calibration curve, the time to maximal accumulation of cells in S-phase was estimated. This time, minus 4 h, was taken as the "interval time" between the HDARA-C injections. Just prior to the second HDARA-C injection, a BM sample was taken to determine the effect of the first injection on the distribution of cells in the cell cycle. On that basis, the interval time was further maintained or, if necessary, adjusted. The result of induction treatment was evaluated after recovery of BM aplasia or, at the latest, on the 28th day after administration of adriamycin.

* This report is also on behalf of G. E. van Zanen, J. de Koning, J. A. Rammeloo, W. A. Kamps, E. F. van Leeuwen, F. A. E. Nabben, E. J. M. Sjamsoedin-Visser, G. A. M. de Vaan, F. C. de Waal, R. S. Weening, and E. R. van Wering.

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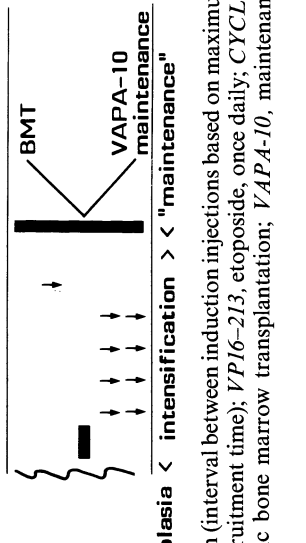

Fig. 1. Treatment scheme of DCLSG study ANLL-82. *ARA-C*, cytosine arabinoside; *push*, within 1 min (interval between induction injections based on maximum recruitment time); *ADRIA*, adriamycin (interval between induction injections based on maximum recruitment time); *VP16–213*, etoposide, once daily; *CYCLO*, cyclophosphamide, once daily (not to be delivered to patients undergoing BMT); *BMT*, allogeneic bone marrow transplantation; *VAPA-10*, maintenance treatment according to VAPA-10

An outline of the treatment protocol is given in Fig. 1.

Results

Eighteen girls and 11 boys entered the study. The ages ranged from 0 to 14 years (median 6 years). Initial white blood cell counts (WBC) ranged from 1.3 to 381.0×10^9/liter (median 25.8×10^9/liter). Eleven children had WBC $> 50 \times 10^9$/liter, six of these $> 100 \times 10^9$/liter. According to the FAB classification, three children had M1, five M2, two M3, nine M4, seven M5, one M6, and two acute undifferentiated leukemia. The interval times for administration of the induction injections are given in Table 1. Complete remission (CR) was achieved in 18 (69%) of 26 evaluable children; 2 of them reached CR only after additional chemotherapy. Eight children died: for one child treatment was refused, six died of early hemorrhages, and one died of refractory leukemia. In three children the results of induction treatment could not be evaluated because of treatment alterations or missing data; all three, however, achieved CR later.

Of the 21 children with CR, seven had a compatible donor and underwent allogeneic BMT; all are alive and in continuous CR for 6^+–33^+ months. Fourteen children were treated with intensive maintenance chemotherapy according to the VAPA-10 protocol. Five of these are still in continuous CR after 8^+–25^+ months, four being off treatment; one died in CR, presumably of toxicity of high-dose 6-mercaptopurine;

Table 1. Definitive interval times between drug administrations during induction in study ANLL-82

Interval (h)	Number of patients
< 11	2
12–17	6
18–23	7
24–29	7
> 30	6
Not done (death before treatment)	1
Total	29

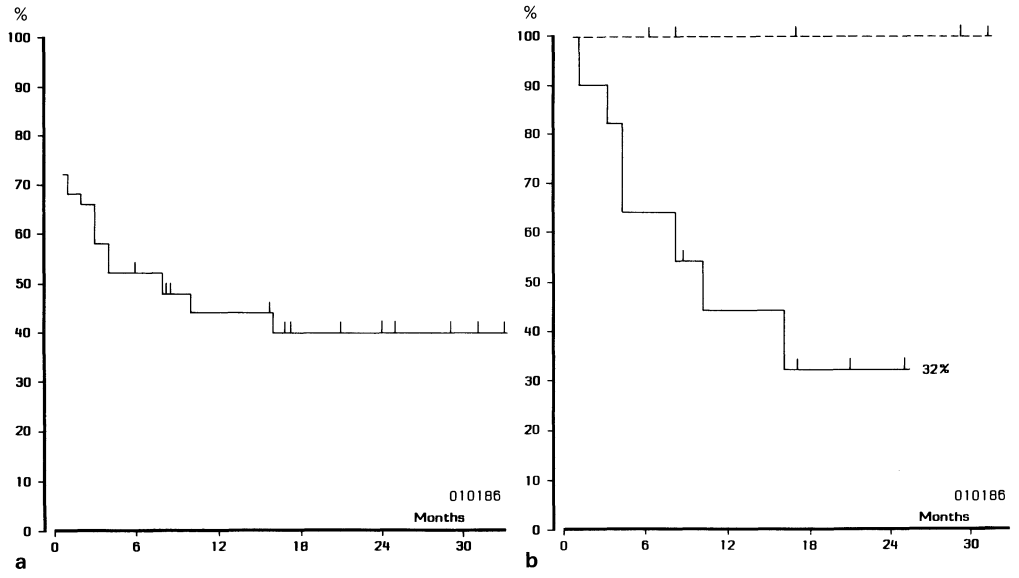

Fig. 2a, b. Kaplan-Meier life-table analysis for disease-free survival. **a** All patients registered in study ANLL-82; **b** patients achieving CR on ANLL-82 induction treatment *only*, followed by VAPA-10 chemotherapy (—) or allogeneic BMT (- - -)

eight children relapsed hematologically after 1–16 months. The Kaplan-Meier analysis for disease-free survival, updated as of 1 January 1986, is shown in Fig. 2.

Discussion

The remission rate (69% of 26 evaluable children) achieved in this study is within the range (60%–80%) of other regimens [4, 5]. Sixteen children (62%) achieved CR with the individually scheduled induction scheme alone. This result is encouraging. However, a high rate of relapses occurred during maintenance treatment according to the VAPA-10 protocol. Reasons for these results may be: (a) In contrast to other studies, only one course of induction treatment was given, which may have resulted in insufficient initial leukemic cell reduction. (b) HDARA-C was administered at intervals, on the basis of the cell kinetic properties of the majority of the leukemic cells. A subpopulation with different cell kinetic properties may have largely escaped the effect of HDARA-C. (c) One injection of adriamycin after HDARA-C may have been insufficient to kill cells primarily resistant to HDARA-C. (d) The delay in treatment after the induction course may have been too long and, therefore, may have allowed regrowth of leukemic cells. (e) The distribution of initial characteristics in this small patient population seems to be skewed with regard to possible prognostic factors (age, WBC, FAB type) [5, 6]. In conclusion, individually scheduled injections of HDARA-C are effective as induction treatment. However, earlier intensification seems to be indicated in order to improve the treatment results. The results of allogeneic BMT are excellent thus far. At the moment, for children with ANLL in first CR and with a compatible donor, allogeneic BMT is the treatment of choice.

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Aclacinomycin-A in the Induction Treatment of Childhood Acute Myelogenous Leukemia

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Introduction

The treatment of childhood acute myelogenous leukemia (AML) remains a serious problem. Improved remission rates of about 70% have been achieved by several groups [1, 2]. The regimens, however, cause considerable toxicity. Anthracyclines (doxorubicin, daunorubicin [DNR]) are of great value in the induction therapy for AML. They are herefore used in high cumulative doses which may cause severe myocardial toxicity.

Aclacinomycin-A (ACLA-A) is a relatively new anthracycline antibiotic. In animal studies its cardiotoxicity was significantly lower than that of doxorubicin or DNR [3–5]. In recent phase I and II studies ACLA-A showed significant antileukemic activity, particularly in acute myelogenous leukemia in adults [6–8].

In 1984 a cooperative multicentric study (AML-IGCI-84) was initiated to evaluate ACLA-A combined with cytosine arabinoside (Ara-C) and VP-16/213 in the induction therapy for childhood AML.

Materials and Methods

Patients and Diagnosis

Children with newly diagnosed untreated AML were eligible for the study. They were treated at several pediatric clinics in Austria and Hungary. Leukemias were classified according to the cytologic and cytochemical criteria of the FAB classification [9]. In addition, in most of the cases, immunologic classification of cell surface markers, determination of terminal deoxynucleotidyl transferase (TdT), and chromosome analysis were performed.

Treatment

The study design is presented in Fig. 1. The basic structure of the protocol is the current AML-BFM-83 protocol. In contrast to the BFM-83 protocol, all patients received the induction therapy course I1 (Ara-C, ACLA-A, VP-16/213; Fig. 2). If complete remission (CR) (< 5% blast cells in bone marrow aspirate) was achieved on day 21, consolidation therapy (BFM-83; Fig. 3) and maintenance therapy (BFM-83) followed. If only partial remission (PR) or nonresponse (NR) was found on day 21, the original BFM-83 induction therapy course I2 (ARA-C, DNR, VP-16/213; Fig. 2) followed immediately before consolidation and maintenance. Maintenance was started 2 weeks after the end of the consolidation therapy with daily 6-thioguanine (40 mg/m^2 p.o.), Ara-C (40 mg/m^2 s.c.) for 4 days every 4 weeks, and adriamycin (ADR) (25 mg/m^2 60-min i.v. infusion) every 8 weeks 4 times only (during the first year). Maintenance was stopped 2 years after diagnosis. Patients with high initial white blood cell counts (WBC) (> 50.0 G/l) and/or extensive organomegaly received a cytoreductive pretreatment with 6-thioguanine and Ara-C.

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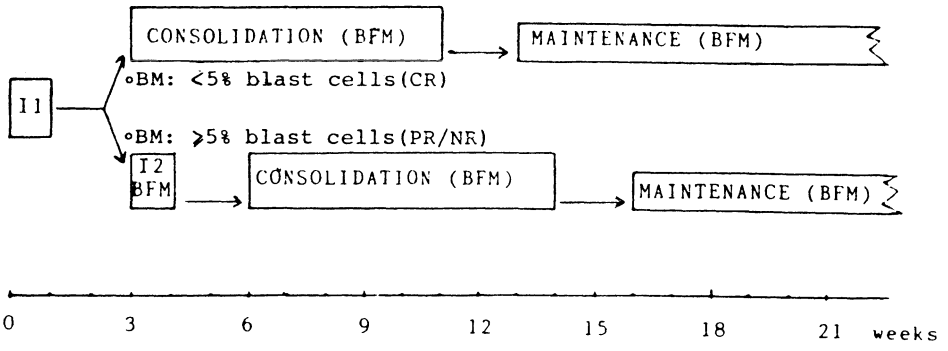

Fig. 1. AML-IGCI-84 study design

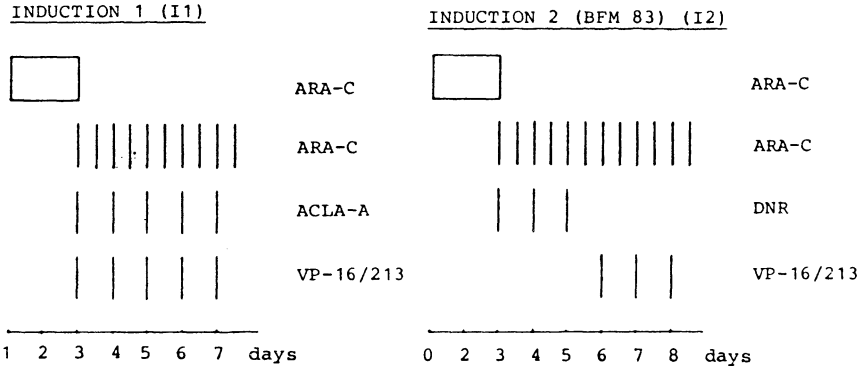

Fig. 2. Therapy courses I1 and I2 for induction of therapy study AML-IGCI-84. I1: Ara-C (100 mg/m^2 day 24-h i.v. infusion on days 1 and 2, 100 mg/m^2/12-h 30-min i.v. infusion on days 3–7); ACLA-A (25 mg/m^2/day 60-min i.v. infusion on days 3–7, VP-16/213 100 mg/m^2 day 60-min i.v. infusion on days 3–7). I2 (BFM-83): Ara-C (100 mg/m^2/day 24-h i.v. infusion on days 1 and 2, 100 mg/m^2/12-h 30-min i.v. infusion on days 3–8); DNR (60 mg/m^2/day 60-min i.v. infusion on days 3–5); VP-16/213 (150 mg/m^2/day 60-min i.v. infusion on days 6–8)

Results

From January 1984 to January 1986, 38 patients were admitted to the study: 18 boys and 20 girls, with a median age of 6 4/12 years (range 2/12–15 2/12 years). The median initial WBC was 25.0 G/l (range 1.8–1350.0 G/l). The results are summarized in Table 1. FAB M1 morphology was diagnosed ten times, M2 eight times, M3 once, M4 ten times, and M5 eight times. In one case the blast cells were positive for immunologic markers of megakaryopoietic precursors.

Seven early deaths (deaths before day 28 of treatment) occurred, three of which were due to hemorrhage and four to septic complications. One patient already had documented CR. Thus, 32 patients were evaluable for treatment response. CR was achieved in 29/38 (76.3%) patients of the whole group and 29/32 (90.6%) of the evaluable patients (patients were evaluable if a control bone marrow aspiration scheduled for day 21 was done after I1). CR was achieved for 23/32 (71.9%) after one induction therapy course (I1), 5/32 (15.6%) after a second course (I2), and 1/32 (3.1%) after completion of consolidation therapy; 3/32 (9.3%) patients were nonresponders.

By the date of evaluation (16 January 1986) 7/29 (24.1%) relapses had occurred and three patients had died in CR. Thus, 19/29 (67.8%) of the patients who reached CR and 19/38 (50.0%) of the whole group remained in first CR. The median follow-up period was 7 months (range 0–22 months).

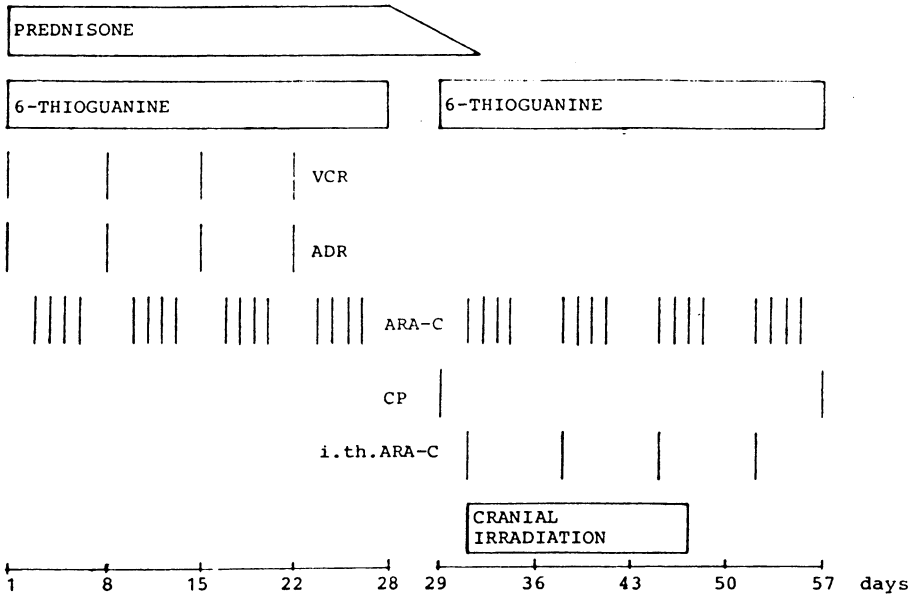

Fig. 3. Consolidation regimen (BFM-83) of therapy study AML-IGCI-84. Prednisone (40 mg/m^2/day p.o.), tapering in 3 3-day stages at one-half, one-fourth, and one-eighth of the starting dose; 6-thioguanine (60 mg/m^2/day p.o.); VCR (vincristine) (1.5 mg/m^2 i.v.); ADR (25 mg/m^2 60-min i.v. infusion); Ara-C (75 mg/m^2 i.v.); CP (cyclophosphamide) (500 mg/m^2 i.v.); i. th. Ara-C age dependent (<1 year 20 mg, 1–2 years 26 mg, 2–3 years 34 mg, >3 years 40 mg); cranial irradiation, whole brain irradiation with age-dependent focal dose (<1 year 12 Gy, 1–2 years 15 Gy, >2 years 18 Gy)

Table 1. Results of therapy study AML-IGCI-84 and analysis according to FAB classification. Median follow-up 7 months (0–22 months)

	n	%	FAB Morphology					Megakaryocytic
			M1	M2	M3	M4	M5	
Patients	38	100.0	10	8	1	10	8	1
Early deaths (before day 28)	7[a]/38	18.4	1	0	0	2	3	1
Due to hemorrhage	3/38	7.8	0	0	0	1	2	0
Patients evaluable for treatment response	32/38	84.2	9	8	1	8	6	0
CR achieved	29/38	76.3	8	8	1	8	4	0
	29/32	90.6						
By 11	23/32	71.9	7	5	0	7	4	0
By 12	5/32	15.6	0	3	1	1	0	0
By consolidation	1/32	3.1	1	0	0	0	0	0
PR/NR	3/32	9.3	1	0	0	0	2	0
Relapses	7/29	24.1	1	2	0	2	2	0
Deaths in CR	3/29	10.7	2	0	0	0	1	0
In 1 CCR	19/38	50.0	5	6	1	6	1	0
	19/29	67.8						

[a] 1 already in CR.

Table 2. Influence of age on results

Patient age	*n*	Early deaths	CR	Relapses	FAB M5
<1 year	6	4	1	0	3
1–7 years	16	3	12	5	5
>7 years	16	0	16	2	0
All patients	38	7	29	7	8

Table 3. Influence of initial WBC on results

Initial white blood cells (WBC)	*n*	Early deaths	CR	NR	Relapses
< 10 G/l	12	2	9	1	4
11–19 G/l	18	3	15	1	2
>100 G/l	8	2	5	1	1
All patients	38	7	29	3	7

Table 4. Therapy-related deaths

	n	Early deaths	Deaths in CR
Hemorrhages[a]	3	3	1
Septic complications[b]	6	4 (1 in CR)	2
Together	9	7 (1 in CR)	3

[a] Cerebral 3×, intestinal 1×.
[b] Aspergillosis 1×, histoplasmosis 1×, bacterial septicemia 4×.

Kaplan-Meier life table analysis predicts a probability of survival of 55.0% at 23 months for the whole group ($n=38$) and a probability of relapse-free survival of 55.8% at 22 months (deaths in CR counted as failures; $n=29$).

Patient age had an influence on prognosis (Table 2). Six patients were treated in the first year of life. There were four early deaths (including the only CR patient in this age group), and the remaining two patients were nonresponders. Of the 16 patients from 1 to 7 years old, 12 achieved CR but five relapsed. All the 16 patients older than 7 years reached CR and only two relapsed. FAB M5 morphology was unequally distributed: every second patient under 1 year of age and every third patient from 1 to 7 years showed M5, but none of the older patients did so.

In eight patients FAB M5 subtype was diagnosed. Three early deaths occurred, two of them due to cerebral hemorrhage, and two patients did not respond to therapy. The third nonresponder had an initial hyperleukocytosis of 1350.0 G/l and M1 morphology.

In our study a prognostic influence of the initial WBC was not demonstrable (Table 3). Early deaths and nonresponders were equally distributed among patients with low, intermediate, and high WBC. Surprisingly, the highest incidence of relapses occurred in patients with low WBC (4/12).

Nine deaths were related to therapy (Table 4). Seven patients died before day 28 of treatment, one of them already in CR. Three of these early deaths were due to cerebral hemorrhage and four to septic complications (one aspergillosis, one histoplasmosis, and two bacterial septicemias). The two patients who died later in CR succumbed to bacterial septicemia and intestinal hemorrhage, respectively.

Table 5. Comparison of different induction regimens for AML

	Patients studied	CR achieved		CR after 1 course	Ref.
		n	%	%	
I1 (IGCI)	38	29	76.3	60.5	Present study
BFM 83	95	73	76.8	63.2	BFM study group, personal communication 1985 [10]
VAPA	83	58	69.9	48.2	Weinstein et al. 1980 [2]

Discussion

This prospective cooperative multicenter study, AML-IGCI-84, is based on the concept of the study AML-BFM-83 currently used by the BFM group. With the aim of reducing cardiotoxicity, we introduced a different induction therapy course including ACLA-A instead of DNR. There is no other difference between the two treatment protocols. Therefore, it is reasonable to compare the preliminary results (10) 2 years after commencement of the study.

The rate of CR (76.3% – 29/38) is the same as in the current BFM study (76.8% – 73/95; Table 5). The results are comparable to other studies with relatively high CR rates, such as the VAPA protocol (69.9% – 58/83) (2). The chance of achieving CR already with the first induction therapy course (I1) (60.5% – 23/38) is again nearly the same as in the BFM study (63.2% – 60/95), but improved in comparison to the VAPA protocol (48.2% – 40/83). By the date of evaluation the relapse rate (24.1% – 7/29) was similar to that in BFM-83 (21.9% – 16/73) after a similar short follow-up.

Thus ACLA-A and DNR seem to be equally effective when combined with Ara-C and VP-16/213 in the induction therapy for childhood AML. Cardiotoxicity of AML induction regimens may be reduced by partly substituting ACLA-A for DNR.

Patient age at diagnosis was an important prognostic factor. Infants younger than 1 year are at highest risk for early death and nonresponse. Children older than 7 years have the best chances of achieving CR; the risk of early death is low. To some extent the reason may be the unequal distribution of FAB M5 subtype, which is far more common in the younger age groups.

Patients with M5 morphology did badly. They had a high incidence of early fatal hemorrhage and nonresponse. Although one of the three nonresponders had an extreme hyperleukocytosis, we were not able to demonstrate a prognostic influence of the initial WBC.

The incidence of therapy-related deaths was high. Five of the nine events were due to septic complications, four of the five fatal septic complications occurring in the bone marrow aplasia after the first induction therapy course. This experience points to the importance of optimal supportive care, which can be supplied only by experienced teams.

Conclusions

When combined with Ara-C and VP-16/213, ACLA-A and DNR seem to be equally effective in induction chemotherapy regimens for childhood AML. The cardiotoxicity of such regimens may be reduced by partly substituting ACLA-A for DNR. Since the incidence of therapy-related deaths is high, optimal supportive care by an experienced team must be supplied at all times during the period of intensive chemotherapy.

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High-Dose Cytosine Arabinoside and Retinol in the Treatment of Acute Myelogenous Leukemia in Childhood*

S. O. Lie and S. H. Slørdahl[1]

Acute myelogenous leukemia (AML) in children is considerably more resistant to chemotherapy than acute lymhoblastic leukemia, where progress is well known and well documented [1]. In recent years, however, more aggressive chemotherpy has resulted in an increasing proportion of children who achieve complete remission. With intensive consolidation therapy, the proportion of children remaining in complete remission is also increasing, and long-term survival figures of more than 40% have been reported [2–4].

In a single institution study we have obtained a high proportion of long-term relapse-free survivors among children, using conventional induction therapy, but with high-dose cytosine arabinoside (ARA-C) as consolidation [5, 6]. In addition, we have used high-dose retinoids during maintenance. This paper describes our observations both on the effect and toxicity of the chemotherapeutic program and on the toxicities of high-dose retinyl palmitate given to children with AML in first remission.

Materials and Methods

Twenty-five children with AML diagnosed during 1973–1980 were treated with a moderate protocol containing as induction ARA-C (100 mg/m^2 × 2 on days 1, 2, and 3) and adriamycin 60 mg/m^2 given as the DNA complex [7, 8] on day 4, repeated three or four times with 14- to 16-day intervals. Maintenance therapy did not follow any strict protocol. Most patients received the same course as during induction therapy, once monthly for the 1st year and every 6th week during the 2nd year.

From 1981 through 1984, 12 children were treated with an intensified induction regimen including 6-thioguanine and with high-dose ARA-C as consolidation (see Table 1). The dose of ARA-C in consolidation was 2 g/m^2 diluted in 100 ml of isotonic saline, given in a 2-h infusion every 12 h for six doses. Some children also received maintenance chemotherapy of moderate intensity (Table 1). After remission was obtained, the children were given retinyl palmitate in a dose of 50000 IU per m^2 orally daily. They were followed closely with respect to clinical signs of vitamin A toxicity. Plasma retinol, retinol-binding protein, and retinyl ester were measured every 2nd month 48 h after the previous intake of vitamin A.

Results

We have used historical controls in order to evaluate the effectiveness of high-dose ARA-C and retinol in the maintenance of remission in children with AML.

Figure 1 shows the relapse-free survival of children with AML diagnosed from 1973 to 1980 and from 1981 to 1984. In the first period, 22 out of 25 children achieved complete remission. However, the duration of remission was far from satisfactory, with

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Table 1. Details of children with AML treated with high-dose ARA-C as consolidation therapy

Patient	Age at diagnosis (years)	WBC at diagnosis ($\times 10^9$ µl)	FAB morphology	No. of induction courses[a]	No. of maintenance courses[b]	No. of high-dose ARA-C courses[c]	Months of vitamin A medication[d]	Months of relapse-free survival
1	13	200.0	M1-M2	4	9	2	34	37
2	9	29.4	M4	5	8	2	35	51+
3	$1^{8}/_{12}$	13.0	M1	3	9	2	36	48+
4	2	13.0	M1	1	10	1	33	37+
5	$^{3}/_{12}$	88.0	M1 (M6?)	4	2	2	31	32+
6	$^{3}/_{12}$	158.0	M6	4	2	2	30	31+
7	2	18.0	M2	2	3	2	30	31+
8	12	98.0	M4	3	0	4	29	30+
9	$^{10}/_{12}$	203.0	M4	2	0	4	24	25+
10	$1^{1}/_{12}$	10.4	M1	3	0	4	12	16+
11	$10^{2}/_{12}$	10.0	M6	3	0	4	12	15+
12	$1^{6}/_{12}$	6.6	M6	3	0	4	12	16+

[a] ARA-C and 6T6 100 mg/m^2 × 2 on day, 1, 2, 3, 4
Adria-DNA 75 mg/m^2 on day 5.
[b] ARA-C 80 mg/m^2 × 2 and
6TG 100 mg/m^2 on days 1, 2, 3, 5 monthly.
[c] ARA-C 2 g/m^2 × 2 every 12 h × 6 (except no. 5, who received 1 g/m^2 per dose).
[d] Retinyl palmitate – 50 000 IU/m^2 daily.

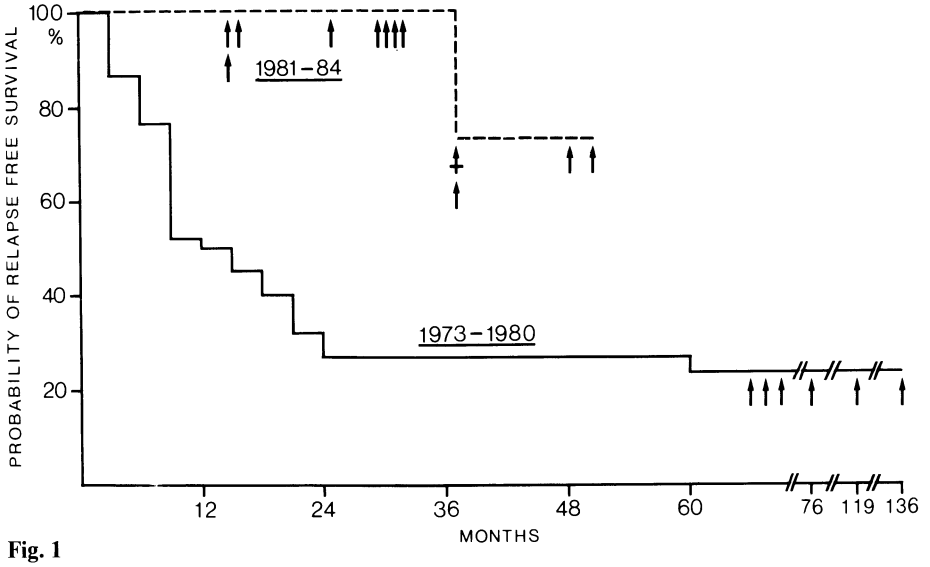

Fig. 1

only six long-term survivors. Nevertheless, we must believe that these six are cured of their disease.

Table 1 details the characteristics of the children treated in the second period, and Fig. 1 their relapse-free survival curve. The results are dramatically improved. We have observed only one relapse at 37 months. The girl – 13 years old at diagnosis – was easily induced into a 2nd remission and treated

Table 2. High-dose ARA-C in children

Dose	Durations of leukopenia			Complications	
	Day of nadir (range)	$<1 \times 10^9 \times$ liter		Septicemia	CNS symptoms
		No.	Mean no. of days (range)		
1 g/m^2 × 6 (6 courses)	12 (6–16)	4/ 6	2 (1– 5)	1/ 6	1/ 6
2 g/m^2 × 6 (3–5 courses)	13 (6–23)	16/35	4 (1–11)	4/36	1/43
3 g/m^2 × 8 (10 courses)	13 (9–18)	8/10	7 (1–13)	2/20	0/10

again with high-dose ARA-C as consolidation for 4 courses followed by Maze [9] three times. She is now off therapy for the second time.

Clinical toxicities of the induction regimen were acceptable, with no therapy-related deaths in this phase. High-dose ARA-C was well tolerated. Table 2 gives the details of the duration of leukopenia and the complications observed. The problems are manageable and not more than one has to accept in the therapy of such an aggressive disease.

Retinyl palmitate has been given for periods of up to 36 months. We have not seen any significant clinical or biochemical complications. Skin biopsies will be performed on the children to see whether or not there is an increased accumulation of retinol in this tissue.

Discussion

ARA-C has been labeled the "mainstay of current treatment for acute myeloblastic leukemia" [10]. Although this drug was introduced more than 15 years ago [11], we still do not know its correct dosage and administration. Doses from 10 mg/m^2 up to 6 g/m^2 have been advocated [6]. It is quite clear that the drug can act both as an inducer of differentiation in low doses as well as a cytostatic agent in higher doses. High-dose ARA-C certainly attacks the leukemic cell in a different way than the conventional dose, since resistance to the latter can be turned in to sensitivity by increasing the dose.

We wanted to take advantage of both methods of action and have therefore induced remission with the conventional dose and consolidated with high-dose ARA-C. This should also give protection to sanctuaries, including the central nervous system. The side effects of consolidation have been acceptable and less than those reported in the literature. This is what could be expected when this phase I agent is administered to a normal marrow.

Our observations on vitamin A in high doses are essentially a phase I study. What we have shown is that retinyl palmitate can safely be given in doses of 50 000 IU per m^2 orally for many years. Long-term side effects cannot yet be ruled out, however.

The results of our protocol are encouraging indeed but should be considered as preliminary. The terror of small numbers may still be the explanation. However, we do believe that optimal AML therapy today should include an intensive induction regimen followed by a different but intensive consolidation. Whether or not an inducer of differentiation, such as retinol [12, 13], has any role in our results can only be clarified through further observation and clinical trials.

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CHOP Treatment of Childhood Acute Myelogenous Leukemia with Monocytic Differentiation: A Report on Five Cases

T. Urasiński, and W. Podraza[1]

Summary

Five children with M4 or M5 acute myelogenous leukemia (AML) not responding to previous treatment or in relapse were treated with a four-drug protocol consisting of cyclophosphamide, adriamycin, vincristine, prednisone, and CNS prophylaxis. There were two treatment failures; the remaining three patients have achieved complete remission, lasting 18+, 13+, and 12+ months respectively. Further follow-up is to be performed.

Introduction

In contrast to the great progress in the treatment of acute lymphoblastic leukemia during the last two decades, results achieved in children with acute nonlymphocytic leukemia are still not satisfactory. Even though the remission rate is relatively high, ranging from 51% to 79%, remission duration has remained disappointingly short [1, 2]. For those who have not achieved remission or have relapsed the prognosis is very poor [2].

It has been proved that malignant histiocytosis – malignant proliferation of cells of monocytic origin – can be cured with a four-drug protocol consisting of cyclophosphamide, VCR, adriamycin and prednisone [3, 4]. Taking this fact into consideration, we decided to introduce the CHOP protocol into the treatment of children diagnosed as having acute myelogenous leukemia (AML) with monocytic differentiation who had not responded to previous chemotherapy or who had responded and subsequently relapsed.

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Patients and Methods

The study covered five consecutive children admitted to our Department between July 1983 and July 1984. Criteria for the diagnosis of AML with monocytic differentiation were as follows: M4 or M5 morphology according to the FAB classification [5] and positive reaction for nonspecific esterase [6]. Four of our patients had previously been treated according to the DAT protocol. One of them did not respond to therapy (case no. 1), whereas the rest achieved remission but subsequently relapsed within 3–9 months. One patient, who received AML-BFM 83 protocol, achieved remission but relapsed 8 months later. They were all systemic relapses without CNS or testicular involvement. The clinical characteristics of the patients are summarized in Table 1.

All patients were treated according to the CHOP protocol. Treatment consisted of cyclophosphamide 1000 mg/m^2 on day 1, adriamycin 50 mg/m^2 on day 1, VCR 1.5 mg/m^2 on day 1, prednisone 100 mg/m^2 days 1–5, intrathecal methotrexate, and cranial irradiation (18 Gy) in patient no. 1, who had not previously been irradiated. Courses were repeated every 3 weeks. Adriamycin was administered to the total cumulative dose of 450 mg/m^2 and was then omitted. Details of the treatment are presented in Table 2.

Table 1. Clinical characteristics

Patient	Age (years)	Sex	Diagnosis	Non-specific esterase	Previous therapy	Previous remission (months)
1	$6^{1}/_{12}$	F	M5	+	DAT	0
2	$4^{11}/_{12}$	M	M4	+	DAT	3½
3	$3^{11}/_{12}$	F	M4	+	DAT	4
4	$2^{1}/_{12}$	F	M4	+	DAT	3
5	$10^{5}/_{12}$	M	M4	+	AML-BFM 83	9

Table 2. Details of CHOP protocol

Cyclophosphamide	1000 mg/m²	!
Adriamycin	50 mg/m²	!
VCR	1.5 mg/m²	!
Prednisone	100 mg/m²	┌ - ┐
		├ - ┤
		1 2 3 4 5 day

Results

One patient did not respond to the treatment. Bone marrow examination performed after two courses of CHOP revealed an increased percentage of blastic cells. Partial response (disappearance of clinical symptoms and reduction of blast percentage in bone marrow) was observed in patient no. 4, but leukemia recurred after the third course of CHOP. The girl died of the disease one month later. The remaining three patients achieved a second complete clinical and hematologic remission, lasting 12+, 13+, and 18+ months. All these patients are still in treatment. The survival of our patients is 8, 15+, 19+, 20+, and 20+ months, respectively.

Moderate myelosuppression was the most common side effect in all five patients. The white blood count never dropped below 1000/μl and a platelet count below 50000/μl was never seen. All children experienced nausea and vomiting while in treatment. ECG changes suspected of being caused by anthracyclines were noticed in patient no. 5.

Table 3. Results of CHOP treatment

Patient	Diagnosis	Response to therapy	Remission duration (in months)	Survival (in months)	Follow up and side effects
1	M5	Remission	18+	20+	Still on treatment, recurrent urinary tract infections
2	M4	Remission	12+	19+	Still on treatment, tissue necrosis due to drug extravasation
3	M4	Remission	13+	20+	Still on treatment, recurrent respiratory tract infection
4	M4	Partial response	0	8	Died of disease 1 month later
5	M4	No response	0	15+	In relapse refractory to treatment, ECG changes

Tissue necrosis due to drug extravasation occurred in patient no. 2. Patient no. 1 developed a recurrent urinary tract infection, and recurrent respiratory tract infections were observed in patient no. 3. The results of the treatment are presented in Table 3.

Discussion

Childhood AML is relatively rare, representing approximately 15%–20% of leukemias in children [1, 2, 7]. Of these, cases with monocytic features occur at a frequency of 43%–47% [1, 7]. Ritter et al. [8] point out that the number of children achieving complete remission in the monocytic subtypes M4 and M5 is relatively low and remission duration is short; this fact is in concordance with our own observations. Thus, we think that clinical trials introducing new forms of treatment of relapsed AML with monocytic features are justified especially in cases in which bone marrow transplantation is not available.

We do not know of any reports describing the results of treatment of childhood refractory or relapsed leukemia with M4 or M5 morphology. A small group of adult patients with refractory ANLL with monocytic features treated with VP-16-213 was presented by Hurd et al. [9]; their preliminary results were very promising.

The small number of patients, the short period of observation, and the fact that our patients are still in treatment limit the possibility of forming any conclusions; further follow-up needs to be done. We would only like to draw attention to the fact that some patients with M4 or M5 leukemia may respond to the CHOP protocol. We also think that our results support the hypothesis that M4 and M5 leukemia may represent a disseminated form of malignant histiocytosis [10].

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Effective Remission Induction in Children with Recurrent Acute Myeloid Leukemia by mAMSA, Ara-C, and VP 16

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Summary

Five children treated for acute myeloid leukemia according to the BFM protocol AML 83 experienced first bone marrow relapse after 7, 10, 14, 18, and 30 months and were retreated for second remission induction. The chemotherapy consisted of mAMSA (100 mg/m^2 per day i.v., days 1–3), ARA-C (100 mg/m^2, twice daily, days 1–6), and VP 16 (150 mg/m^2 per day, days 4–6). Four of the children achieved a complete second remission after one course of chemotherapy, and the fifth child died of pneumonia during bone marrow aplasia. All surviving children received an identical second course within 4–5 weeks, followed by maintenance chemotherapy. Remission duration was 0, 3, 4, 5, and 5 months.

Toxicity was confined to heavy bone marrow depression with thrombocytopenia (nadir 2–7000, days 7–13) and leukocytopenia (nadir 0–400, days 8–14). Bleeding episodes could be prevented by substitution with platelets. Four patients experienced infections (pneumonia, septicemia). We conclude that combination chemotherapy using mAMSA, ARA-C, and VP 16 is effective in inducing a second remission in patients with early bone marrow relapse. The main side effect was considerable bone marrow toxicity.

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Introduction

Highly intensive chemotherapy using cytarabine, daunorubicin, and etoposide (VP 16) is now able to achieve 80%–85% complete remissions in children with acute myeloid leukemia (AML) [1]. Unfortunately, as many as 40% of the responders experience bone marrow relapse within 3 years [1]. The success rate in inducing a second remission is thought to depend on the preceding regimen, with better results in less aggressively treated patients and poorer results in more aggressively treated ones. Here we report a high response rate in a small series of heavily pretreated children with AML, obtained at a single institution.

Patients

Five consecutive children with first bone marrow relapse of AML entered the trial. All of them were uniformly pretreated according to the BFM protocol AML 83 consisting of intensive chemotherapy for remission induction (cytarabine 100 mg/m^2 per day, 24-h infusion for 2 days followed by 2 × 100 mg/m^2 per day, 30-min infusion, for 6 days; daunorubicin 60 mg/m^2 per day, days 3–5; VP 16 150 mg/m^2 per day, days 6–8) and achieved complete remission. After a consolidation course (prednisone, thioguanine, vincristine, adriamycin, cytarabine, cyclophosphamide, skull irradiation) maintenance therapy (thioguanine 40 mg/m^2 per day, cytarabine 40 mg/m^2 per day, days 1–4 every 4 weeks, adriamycin 25 mg/m^2 per day every 8 weeks × 4) was scheduled for up to

Table 1. Chracteristics of patients with recurrent AML at diagnosis and relapse

Name, sex	Blasts in bone marrow at diagnosis (%)	Blast count in peripheral blood at diagnosis (/nl)	FAB morphology	Duration of first remission (months)	Blasts in bone marrow at relapse (%)	Blast count in peripheral blood at relapse (/nl)
N. M., f	82	45.6	M2	14	60	0.1
T. R., m	97	28.8	M2	10	68	1.6
M. W., m	70	15.4	M1	7	96	1.0
H. P., m	56	0.2	M4	30	42	0
D. W., m	98	28.8	M5	18	81	12.0

Table 2. Treatment schedule for children with first relapse of AML

Agent	Dosage	Route	Schedule
mAMSA	100 mg/m^2 per day	30-min infusion	Days 1–3
Cytarabine	2 × 100 mg/m^2 per day	1-h infusion at 12-h intervals	Days 1–6
Etoposide	150 mg/m^2 per day	1-h infusion	Days 4–6

2 years from diagnosis. Four out of five patients relapsed during maintenance therapy and one boy 6 months after the end of chemotherapy. The characteristics of the patients are listed in Table 1. All but one were hyperleukocytotic (15–46 blasts/nl at the beginning). After relapse two children received low-dose cytosine arabinoside (ARA-C) for 14 days without any effect on their remission status. Anthracycline dosis (daunorubicin, adriamycin) prior to entering the study was 375–400 mg/m^2. The therapeutic regimen is set put in Table 2. Prophylactic oral nystatin and trimethoprim-sulfamethoxazole were begun on day 1 of therapy and were continued throughout the trial. The four patients surviving the two induction blocks (Table 2) received modified maintenance therapy (6-mercaptopurine, mitoxanthrone, cytarabine) until recurrence of the disease or death.

Results

Remission Induction and Survival

Four out of five patients achieved a complete marrow and hematologic remission

Table 3. Response of children with recurrent AML to mAMSA, cytarabine, and etoposide

Name	Number of courses	Remission status after first course	Duration of second remission (months)	Follow-up
N. M.	2	Complete remission	3	Died of staphylococcal septicemia
T. R.	2	Complete remission	5	Died of progressive disease 6 months later
M. W.	1	Bone marrow aplasia	0	Died of pneumonia during bone marrow aplasia
H. P.	2	Complete remission	5	Died of Coli septicemia
D. W.	2	Complete remission	4	Died of progressive disease 5 months later

Table 4. Side effects and complications related to treatment with mAMSA, cytarabine, and etoposide

Name	Leukocytopenia (/nl)		Thrombocytopenia (/nl)		Complications
	Nadir	Day[a]	Nadir	Day[a]	
N. M.	0.4	10	3.0	13	Septicemia, mucositis
T. R.	0.1	8	4.0	8	Pneumonia
M. W.	0	8	2.0	7	Pneumonia, empyema
H. P.	0.1	14	7.0	13	Septicemia, mucositis
D. W.	0.2	13	5.0	12	–

[a] Counted from day 1 of chemotherapy.

21–35 days after beginning induction (Table 2 and 3). Complete remission was defined by less than 5% blasts in normocellular or moderately hypocellular marrow *and* recovery of peripheral leukocyte count to >1.5/nl without blasts and platelets to >100/nl. The fifth child died of staphylococcal pneumonia on day 17 during the aplastic phase of bone marrow which was cleared of blasts. The remaining four patients received a second course for consolidation. The median duration of second remission was 4 months. All patients subsequently died of progressive disease or septic complication during maintenance therapy.

Toxicity

The main side effects and complications are listed in Table 4. Severe myelosuppression occurred in all patients requiring antibiotics and transfusions of packed red blood cells. Vigorous platelet substitution prevented major bleeding episodes. Septic complications occurred in four cases (staphylococcal pneumonia, Coli septicemia, pneumonia, fever of unknown origin) during the first course. Though there was considerable bone marrow depression during the second block with mAMSA–ARA-C–VP 16, septic complications were not seen. Severe mucositis was found in two patients. Nausea was treated with levomepromazine. Phlebitis, cardiac toxicity (electrocardiographic changes, cardiomyopathy), impairments of liver (transaminases, bilirubin) and renal function (uric acid, creatinine, urea), and neurologic side effects were not observed. Hyperuricemic nephropathy was prevented by allopurinol, intravenous hydration, and urinary alkalization.

Discussion

The acridine derivative mAMSA has been demonstrated to be effective as a single agent in refractory or recurrent AML in 9% –33% [2–4]. Addition of VP 16 did not seem to yield better results [5]. In combination with high-dose ARA-C, the response rate improved to 58% [6] and 70% [7]. We report here a small, uniformly pretreated series of five children, all of whom responded to the combination mAMSA–ARA-C–P 16. The only difference between induction therapy at diagnosis and induction therapy at relapse consisted in the replacement of daunorubicin by mAMSA, supporting the idea of non-cross-resistance between anthracyclines and mAMSA. Remarkably, these excellent response rates were obtained from heavily pretreated children with early bone marrow relapse. In two patients second relapse occurred 4 and 5 months later; the other three patients died of septic complications, the latter being related in one case to the induction therapy. Bone marrow toxicity was considerable in all patients and required vigorous supportive care. Although the study population is limited, this drug combination appears to be useful in children with recurrent AML and warrants further trial in that disease.

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Alteration of Blast Phenotype after Low-Dose Cytarabine in Children with Acute Myeloid Leukemia

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Summary

Two children with acute myeloid leukemia (FAB M1 and M2) experienced bone marrow relapse during maintenance chemotherapy 7 and 10 months after diagnosis. Low-dose ARA-C monotherapy (2×10 mg/m^2 per day s.c. for 14 days) was then initiated, as suggested by others reporting induction of differentiation and achievement of remission without toxic side effects. In contrast to these reports, remission induction was not observed in the two children after low-dose ARA-C but was achieved by subsequent high-dose chemotherapy. However, blast cell characteristics revealed some alterations.

Blast count and chromosome pattern remained unchanged. Cytochemistry revealed the appearence of esterase- (0→11%), 0→21%) and PAS- (0→74%, 0→45%) positive cells in the patients and a remarkable increase (patient 1: 0→71%) and decrease (patient 2: 90→12%) in acid phosphatase positivity. Expression of myeloid marker VIM D5 decreased distinctly (70→4%, 77→11%). However, the biologic relevance of these alterations remains in question. The failure to respond clinically to low-dose ARA-C in both children is discouraging.

Introduction

Cytarabine (ARA-C) is an S-phase specific antileukemic agent acting primarily as a competitive inhibitor of DNA polymerase and is considered a major cytostatic drug in the treatment of acute myeloid leukemia (AML). In vitro data suggest a second possible mechanism demonstrating that low-dose concentrations of ARA-C may induce differentiation in mouse leukemic cells [1] as well as in human myeloblastic (ML-1) [2], promyelocytic (HL 60) [3], and histiocytic (U-937) [4] cell lines. The differentiation-inducing effect was attributed to hypermethylation of DNA resulting in changes of gene expression [5]. This idea has been supported by several clinical observations. In patients with preleukemic syndromes [6, 7] and in some patients with AML [7–13] administration of low-dose ARA-C was able to induce remission. The toxicity of the regimen was low, with rare vomiting and no hepatic or neurologic side effects. Some patients were reported to achieve complete remission without a significant bone marrow reduction phase [8]. We used low-dose ARA-C as an attractive alternative approach to intensive chemotherapy in two children with first relapse of AML. Here we report changes in blast phenotype expression as judged by cytology, cytochemistry, immunotyping, and cytogenetics without achievement of clinical remission.

Patients and Studies

Patient 1 first presented AML (FAB M1) at the age of 4½. He was treated according to the AML protocol BFM 83, using highly intensive chemotherapy for remission induction ARA-C, daunorubicin, etoposide) and for consolidation (thioguanine, cytarabine,

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Table 1. Marker expression in myeloid blasts before and after low-dose ARA-C treatment

	Patient 1		Patient 2	
	Before	After	Before	After
Blast count in bone marrow	92%	96%	70%	68%
Cytochemistry				
Peroxidase-positive cells	60%	50%	51%	35%
Exterase-positive cells	0%	11%	0%	21%
Acid phosphatase-positive cells	0%	71%	90%	12%
PAS-positive cells	0%	74%	0%	45%
Immune marker				
VIM D5	70%	4%	77%	11%
MY 7	27%	50%	56%	38%
HLA-DR	40%	65%	49%	41%
Chromosome pattern	No change before/after: hyperdiploidy 47 XY trisomy 8 complex translocation involving chromosome 2, 10, 11 duplication 17q marker chromosome		No change before/after: hyperdiploidy 45 XY t (7;12) t (7;17) del 1q-	

vincristine, adriamycine, cyclophosphamide, prednisone, skull irradiation). The maintenance chemotherapy consisted of thioguanine, adriamycine and cytarabine. Complete remission was achieved after the first course of chemotherapy; however, bone marrow relapse occurred during maintenance therapy 7 months from diagnosis. Low-dose ARA-C monotherapy (2×10 mg/m^2 per days s.c.) was then administered for 14 days in order to induce maturation of the blast cells without toxic side effects. In contrast to reports in adults (7–13), remission induction was not observed, but clearing of bone marrow from blast cells was able to be achieved by subsequent high-dose chemotherapy (ARA-C, mAMSA, etoposide). The boy died of septicemia during extreme bone marrow hypoplasia.

Table 1 presents characteristics of bone marrow blasts before and after low-dose ARA-C treatment. Blast count and chromosome pattern remained unchanged. The cytologic appearance was very similar, though minor changes may have occurred (increasing cell size and granulation). Cytochemistry revealed esterase-, PAS-, and acid phosphatase-positive cells after low-dose ARA-C. Expression of the myeloid marker VIM D5 decreased distinctly, while other markers showed no alteration.

The AML of *patient 2* was diagnosed at the age of 3½ and with primary CNS involvement (FAB M2). He was treated according to the AML protocol BFM 83 as was patient 1, achieved complete remission and experienced 10 months later bone marrow and CNS relapse during maintenance therapy. Treatment of relapse with low-dose ARA-C (2×10 mg/m^2 per day s.c., days 1–14) showed no efficacy in remission induction, which was achieved later by high-dose chemotherapy (ARA-C, mAMSA, etoposide). However, a second bone marrow relapse occurred 5 months later and the boy died of progredient disease. As in patient 1, blast count in bone marrow and chromosome was identical before and after low-dose ARA-C. Blast cell morphology may have shown some increase in cell size, cytoplasmic granulation, and nucleus indentation, while more distinct changes were seen by cytochemistry (presence of esterase-positive blasts, decrease of acid phosphatase positivity) and surface marker analysis (decrease of VIM D5).

Discussion

Successful remission induction by low-dose ARA-C has been reported primarily in pre-

leukemic syndromes [6, 7, 10] and elderly patients with AML [8, 12]. Efficacy achieving good response rates seemed to be correlated with low bone marrow cellularity [12] or with a subacute type of leukemia with a limited number of blast cells and some signs of maturation in the bone marrow [9]. Moreover, the quality of remissions achieved by low-dose ARA-C was not uniform, since both persistence of clonal abnormality [13] and disappearance of chromosomal abnormality [11] during remission have been reported. One may conclude that efficacy of low-dose ARA-C is dependent on slowly growing malignant clones (preleukemic syndromes, elderly patients) and that persistence of chromosomal abnormality during remission is an indication of the inferior quality of remission achieved rather than evidence of the maturation of malignant into normal cells. The two patients reported here were children with rapidly proliferating recurrent leukemic clones replacing normal hematopoiesis. Subcutaneous administration of low-dose ARA-C for 2 weeks did not have any measurable effect on remission status (general condition, blood count, ratio of malignant/nonmalignant cells in bone marrow). To our knowledge, those children are the first reported pediatric patients, which means that the number for drawing more general conclusions is much too small; however, the results are certainly not encouraging.

The identical chromosome pattern of blasts before and after low-dose ARA-C suggests genotype conservation, whatever the expression of the altered chromosomal structure might be. Alterations of blast phenotype included the appearance of esterase- and PAS-positive cells in both patients, a remarkable increase (patient 1) and decrease (patient 2) in acid phosphatase positivity, and a distinct decrease in VIM D5 expression. However, the biologic significance of these alterations remains in question.

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Biphenotypic Leukemia in Childhood: Presentation of Five Cases

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Introduction

Biphenotypic leukemias are characterized by both a lymphoid and a myeloid differentiation. They represent a heterogeneous subgroup of leukemias and include the following types:

1. Sequential manifestation: Leukemias with a conversion of the leukemic phenotype on relapse ("lineage switch") [1–4]
2. Simultaneous manifestation:
   a) Leukemias with lymphoid and myeloid determinants present on the same cell ("lineage infidelity") [5, 6]
   b) Leukemias with different cell lines present at the same time [7–10]

Since 1980, five children with biphenotypic leukemias have been treated in the Department of Pediatric Hematology and Oncology at Düsseldorf University. On average, 25 children with leukemia are admitted to this hospital every year. Two of the five children were referred with relapse, the first diagnosis of leukemia in these children (cases 1 and 2) having been made elsewhere prior to 1980 (1973 and 1979, respectively).

Case Reports

1. A 2-year-old girl presented with a 1-week history of fever, tonsillitis, pallor, and hematomas. The blood count showed an extreme anemia with a hemoglobin (Hgb) of 3.6 g/dl and a thrombocytopenia of 20.0×10^9/l; the white blood cell count (WBC) was 3.8×10^9/l. The bone marrow was hypercellular and lymphoblasts were predominant. A complete remission was achieved following treatment with the standard therapy for acute lymphocytic leukemia (ALL) at that time ("total therapy VII") [11]. Two years after cessation of therapy the child again fell ill with fever and pallor. The peripheral blood count showed anemia (Hgb 7.7 g/dl) and thrombocytopenia (79.0×10^9/l; the WBC was normal (5.2×10^9/l). Bone marrow smears revealed an ALL relapse, and immunologic marker analysis provided the diagnosis of c/T-ALL. Remission was obtained with a modified BFM-ALL protocol therapy [12]. During maintenance chemotherapy, however, a second leukemic relapse, again with anemia, thrombocytopenia, and normal WBC in the peripheral blood occurred. The morphology of the blasts in the bone marrow smear showed a change from lymphoblasts to myeloblasts, and peroxidase was positive in 48% of the cells.

Consequently, treatment was started according to the BFM-AML protocol [13]. One week later myeloid blasts (peroxidase-positive) were still found in the bone marrow (12%). At 5½ months after the start of AML therapy, a relapse of ALL was again diagnosed: hypocellular bone marrow showed 79% lymphoblasts; myeloblasts were not present. Six weeks later, following this third leukemic relapse, the girl died under palliative chemotherapy at the age of 7.

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2. A 5-month-old girl presented with a 1-month history of febrile lymphadenitis. The blood count showed an elevated leukocyte count (45.0×10^9/l), accompanied by anemia (Hgb 6.0 g/dl) and thrombocytopenia (32.0×10^9/l). Bone marrow smears showed a predominance of uniformly shaped lymphoblasts. Complete remission was obtained with chemotherapy, using the 6801 ALGB protocol for ALL [14]. A year after cessation of therapy, the girl presented with cerebral symptoms, including headache, morning nausea and vomiting, and fever. The peripheral blood count showed a leukopenia of 0.8×10^9/l, the Hgb level was 9.8 g/dl), and the platelet count 273.0×10^9/l. Bone marrow smears revealed an ALL relapse, and examination of the CSF showed an involvement of the CNS. Complete remission was obtained with treatment according to the Lapoca scheme [15].

A second leukemic relapse occurred 5½ years later, with a change of morphology from lymphoblasts to monoblasts. The blasts were peroxidase-negative but esterase-positive. The WBC at this time was 2.7×10^9/l, the Hgb level 11.8 g/dl, and the platelet count 128.0×10^9/l. The girl responded to treatment according to the BFM-AML protocol [13] with a complete remission, which has now lasted 3½ years. However, it is complicated by cirrhosis of the liver, which is probably drug induced.

3. An 11-year-old boy presented with a 1-week history of cervical lymph node swelling. The WBC was 91.9×10^9/l, the Hgb level 13 g/dl, and the platelet count 155.0×10^9/l. Bone marrow smears were hypercellular with a predominance of lymphoblasts and a simultaneous presence of about 20% myeloid cells. Immunologic analysis showed the presence of c-ALL antigen; in addition, a weak positive reaction with a monoclonal antibody against AML blasts was demonstrated. Cytogenetical analysis of the blasts was normal; impulse flow cytophotometry (ICP), however, showed a hyperdiploid DNA aneuploidy. Owing to the predominance of lymhoblasts, induction therapy was started according to the COALL 82 study [16]. After 1 month, therapy was changed to the BFM-AML protocol [13] because of blast cell persistence in the marrow (38%). At this time blasts were found in the CSF. Under the BFM-AML regimen [13] complete remission was obtained but lasted only 13 weeks. Cervical lymph nodes, which had diminished rapidly under treatment, swelled again. The peripheral blood count at this time showed a Hgb of 13.0 g/dl, a WBC of 2.5×10^9/l, and a platelet count of 125.0×10^9/l. Biopsies of the cervical lymph nodes and of the bone marrow showed a leukemic relapse with a predominance of myeloid cells. Unfortunately, ICP was not done at that time; cytogenetics remained normal. In spite of intensive chemotherapy with methotrexate, cyclophosphamide, asparaginase, VM-26, and cytosine arabinoside, large cervical lymph node swellings appeared again 7 weeks later. The peripheral blood count showed a Hgb of 13.8 g/dl, a WBC of 3.1×10^9/l, and a platelet count of 55.0×10^9/l. The histology of a new lymph node biopsy revealed a second leukemic relapse: this time, the lymphoid lineage seemed to have relapsed. After an unsuccessful attempt at treatment with mitoxantrone, the boy received palliative chemotherapy and local radiation until death, 10 months after the initial diagnosis.

4. A 10-month-old boy presented with a brief history of weariness and slightly raised temperature. On examination, the kidneys were found to be enlarged. The peripheral blood count showed a hyperleukosis of 222.0×10^9/l, a Hgb of 10.5 g/dl, and a platelet count of 195.0×10^9/l. Bone marrow smears contained 94% blasts, which were difficult to classify. An L_1 morphology (FAB) and cytochemistry suggested that most of the blasts were of lymphoid origin; immunologically, these blasts were classified as pre-pre-B and pre-B blasts. About 10% were of myelomonocytic morphology and esterase-positive. Treatment according to COALL 82 protocol [16] was begun and after 1 month the boy entered complete remission. Cytogenetic analysis of bone marrow cells in remission revealed 95% normal cells but 5% aberrant cores with marker chromosomes. Five months after diagnosis, routine blood count controls showed a continuous rise in the leukocyte count and a fall in the platelet count. Peripheral blood counts recorded a Hgb of 10.5 g/dl, a WBC of 36.8×10^9/l, and a platelet count of 30.0×10^9/l. Bone marrow smears were hy-

percellular with blasts of myelomonocytic morphology dominating, 50% were peroxidase-positive, and 100% esterase-positive. Cytogenetic analysis now showed abnormalities in nearly all cells. In view of the histologic and cytochemical diagnosis, the treatment was changed to the BFM protocol for acute myeloid leukemias [13], and complete remission was again obtained. Sixteen months later, a successful bone marrow transplantation using marrow from the boy's identical twin was performed, following total body radiation and administration of cyclophosphamide. Three months later, there was again a rise in WBC (106.0×10^9/l) and a fall in platelets (22.0×10^9/l), with a Hgb of 11.6 g-%. Examination of the bone marrow provided the diagnosis of c-ALL. The boy died 4 months later under palliative chemotherapy, $1^5/_{12}$ years after initial diagnosis.

5. An 8-year-old girl presented with a facial nerve palsy. The WBC was 350.0×10^9/l, the Hgb 10.5 g/dl and the platelet count $195. \times 10^9$/l. Bone marrow smears showed 87% blasts, predominantly lymphoblasts (FAB: L_2), but a myeloid lineage with the presence of Auer bodies in some of the cells was also identified; 10%–15% of the blasts were peroxidase-positive. C-ALL antigen was found in some blast cells, while others had a myeloid surface marker. Cytogenetic analysis of the blasts showed a Philadelphia-chromosome translocation, indicating chronic myeloid leukemia (CML). CSF examinations showed the presence of blast cells. Owing to the predominance of lymphoid blasts, treatment according to the COALL 82 protocol [16] was started, and after 1 week the lymphoblast-to-myeloblast ratio in the peripheral blood had changed in favour of peroxidase-positive myeloblasts. Treatment was changed to the BFM protocol for AML [13]. The facial nerve palsy rapidly improved under chemotherapy, but the bone marrow showed only a partial remission with the persistence of the Philadelphia chromosome. Bone marrow transplantation (BMT) following total body radiation and administration of cyclophosphamide, was carried out 9 months after initial treatment.

Hematologic reconstitution was good and the Philadelphia chromosome was no longer demonstrable. Six months later, there was a sudden rise in WBC to 160.0×10^9/l, the Hgb level was 14.3 g-% and the platelet count 98.0×10^9/l. Bone marrow smears showed a pathologic infiltration of lymphoid blasts which reacted immunologically to c-ALL specific monoclonal antibodies. Treatment according to the BFM-ALL relapse protocol [17] was started, but the girl did not respond and died $1^4/_{12}$ years after initial diagnosis.

Discussion

According to the classification mentioned, the five patients can be divided into two groups: patients 1 and 2, who had a sequential manifestation of biphenotypic leukemia, and patients 3, 4, and 5, who had a simultaneous manifestation.

Patients 1 and 2 presented ALL on initial diagnosis and on first relapse, and AML on second relapse, with myeloblastic and monoblastic cells respectively. The interval until lineage switch lasted $4^8/_{12}$ years in patient 1 and $9^5/_{12}$ years in patient 2. The choice of treatment corresponded to the phenotype. In this way, long periods of survival were attained (5 years and 13+ years respectively), despite several relapses. Stass et al. [1] and Perentesis et al. [2] have also described the possibility of continuous complete remissions, despite conversion of the leukemic cell type when treatment was tailored to morphology. On the other hand, two children described by Stass et al. [1] died after lineage switch from ALL to AML, as conversion of the leukemic phenotype was not recognized in time and the children did not respond to ALL reinduction. For this reason, the importance of prompt recognition of lineage switch and selection of an appropriate treatment regimen cannot be emphasized enough.

Specific causative factors for lineage switch in acute leukemias have not yet been identified, although at least two general mechanisms seem possible. In the first one, the existence of a pluripotent stem cell capable of differentiating into both lymphoid and myeloid clones is postulated [2, 18]; chemotherapy which eradicates the dominant clone present at diagnosis may allow for ex-

pansion of the secondary clone with a different phenotype [1]. In the second mechanism, lineage switch may be related to chemotherapy-induced mechanisms; either drug-induced changes of the original clone may cause phenotypic shift [1] or the secondary leukemic cell line represents a therapy-induced second malignoma.

The second group of biphenotypic leukemias was formed by those patients with simultaneous manifestations of lympho- and myeloblastic phenotypes at initial presentation. CML, as seen in patient 5, is the best known example of mixed leukemia [19, 20]; less commonly, a mixed lymphoid and myeloid leukemic population is described in Philadelphia-chromosome-negative acute leukemias [8, 10, 21]. The cases of mixed leukemias support the theory of a pluripotent stem cell capable of differentiating into both lymphoid and myeloid clones. Presumably, the malignant transformation has already occurred in such a stem cell, otherwise the appearance of different malignant cell clones at the same time would be hard to explain [18].

A large leukemic cell mass at diagnosis was present in all three children with leukocyte counts above 90×10^9/l and an extramedullary tissue involvement. Cytogenetic abnormalities and an abnormal DNA content of the leukemic blast cell, respectively, were characteristic findings: hyperdiploid DNA aneuploidy in patient 3; Philadelphia-chromosome translocation in patient 5, indicating a combined blast crisis of CML; in patient 4, cytogenetic analysis demonstrated the selection of the therapy-resistant clone on relapse, as the number of aberrant cores had increased from 5% in remission to nearly 100% in relapse. In our patients, remissions were obtained with an individualized chemotherapeutic schedule combining elements from both ALL and AML induction regimens, but unfortunately remissions were not long lasting. Despite intensive chemotherapy and allogeneic BMT in two cases, treatment results were poor, as all children died within 1½ years from diagnosis. It should be mentioned, nevertheless, that circumstances for bone marrow transplantations in our cases were unfavourable; the donor in case 4 was the patient's identical twin, and patient 5 had never entered complete remission before BMT.

The poor prognosis of mixed lymphoid and myeloid leukemias is described in the literature [7, 10, 18]. However, Pui et al. [21] reported remissions in two children with mixed lymphoid and myeloid phenotypes (Ph^1-negative), with standard therapy for ALL. Mertelsmann et al. [8] achieved a complete remission in a young woman with combined ALL and AML treatment. However, their observation time was rather short (4 and 5 months respectively [21] and 16 months [8] of continuous remission).

Conclusions

Biphenotypic leukemias represent a poor prognostic group. Compared to the patients with simultaneous expression of both lymphoid and myeloid determinants, patients with a conversion of the leukemic phenotype on relapse seem to have a better prognosis. Prompt recognition of the lineage switch is of great importance for the selection of an appropriate treatment. As for those patients with simultaneous manifestations of biphenotypic leukemia, different regimens need to be evaluated for remission induction, maintenance, and pre-BMT conditioning.

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Surface Marker Analysis by Monoclonal Antibodies: A Valuable Technique in Childhood Acute Myeloid Leukemia*

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A considerable number of monoclonal antibodies (MoAbs) with myeloid activity have been described during the last few years (summarized in [1]). These MoAbs have been applied to the study of normal myeloid differentiation, as well as to the surface marker analysis of acute myeloid leukemia (AML) [2–6]. Although there is a strong tendency for morphological differentiation to correspond to surface antigen differentiation of malignant myeloid cells [2, 3], a recent report has failed to correlate the FAB classification system with immunologic categories of AML [6].

More importantly, MoAbs recognizing myeloid progenitor cells have proven useful in distinguishing undifferentiated forms of AML and ALL [7, 8]. In addition to their potential diagnostic utility in AML, MoAbs have been used to identify prognostically important subgroups in AML [9].

The purpose of our study was to analyze prospectively the immunologic phenotype of a large number of children with AML, using a panel of well-characterized MoAbs. The results of immunologic typing were compared and contrasted with those obtained by conventional morphological and cytochemical techniques. Furthermore, the value of surface antigen analysis should be determined in cases of acute leukemia with inconclusive morphology/cytochemistry.

Materials and Methods

Marker analyses were performed in 81 children with AML. All the children were under 17 years of age and had been referred for phenotype determinations as part of the German cooperative study BFM 83.

The patients were classified as M1–M6 AML according to FAB criteria by standard methods using both morphology and cytochemical stains, as described elsewhere [10]. Pretreatment specimens of heparinized bone marrow and/or peripheral blood were separated by Ficoll-Hypaque density gradient centrifugation. For phenotype determinations, blasts were first incubated in heat-inactivated pooled AB serum to avoid nonspecific binding to Fc receptors and then washed three times in phosphate-buffered saline. The binding of MoAbs was assessed by indirect immunofluorescence with fluoresceinated goat $(Fab')_2$ antimouse IgG plus IgM. Fluorescence of cells was evaluated with an epilluminated fluorescence Zeiss microscope or with a fluorescence-activated cell sorter (Epics V, Coulter Electronics). Background fluorescence, determined by using nonreactive MoAbs of the same isotype as the test MoAbs, was subtracted.

The characteristics of the MoAbs used in the evaluation of AML are shown in Table 1. The criterion for immunologic marker positivity was expression of the marker by at least 20% of the blast cell population.

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Table 1. MoAbs selected for childhood AML phenotype determination

Group	MoAbs	CD	Reactivity	Reference/Source
Granulomonocytic lineage	VIM-D5	CD15	Granulocytes, monoblasts	Majdic et al. [11]/Dr. Knapp[a]
	My7	CDw13	Granulocytes, monocytes	Griffin et al. [12]/Coulter
	My9		Granulocytes, monocytes	Griffin et al. [13]/Coulter
	Leu-M3	CDw14	Monocyte/macrophage	Dimitriu-Bona et al. [14]/ Becton Dickinson
	VIM13	CDw14	Monocyte/macrophage	Knapp et al. [15]/Dr. Knapp[a]
	OKM1	CD11	Granulocytes, monocytes, null cells	Breard et al. [16]/Ortho
Megakaryocytic lineage	J15		Platelet gp IIb/IIIa	Vainchenker et al. [17]/ Dr. Mc Michael[a]
Erythroid lineage	VIE-G4		Glycophorin A	Liszka et al. [18]/Dr. Knapp[a]
T cell lineage	Leu-9	CD7	Pan-T	Link et al. [19]/Becton Dickinson
B cell lineage	B4	CD19	Pan-B specific	Nadler et al. [20]/Coulter
CALLA	J5	CD10	Common ALL antigen	Ritz et al. [21]/Coulter
HLA-DR	OKla1		Ia-like antigen	Nadler et al. [22]/Ortho

CD, cluster designation.

[a] The authors wish to thank Dr. Mc Michael and Dr. Knapp for providing the MoAbs J15, VIM-D5, VIM13 and VIE-G4.

For intranuclear terminal deoxynucleotidyl transferase (TdT) staining, cytospin cell preparations were fixed in cold methanol, incubated with rabbit anti-calf TdT for 30 min at room temperature, and then incubated with FITC-conjugated goat antirabbit IgG.

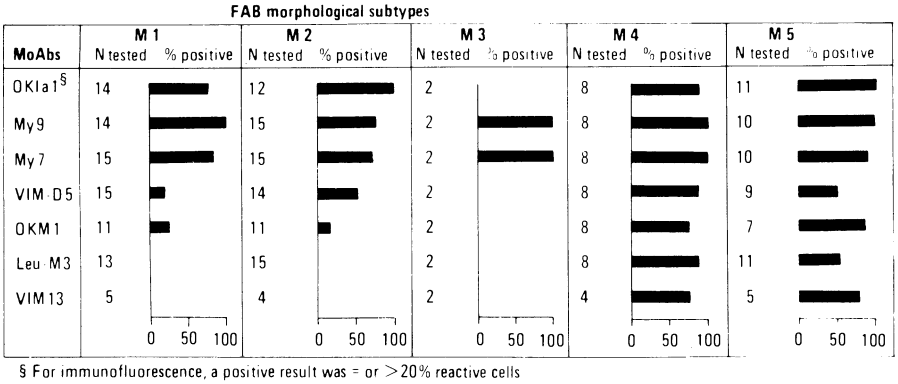
Complete marker profiles were not possible in nine patients because of insufficient numbers or decreased viability of cells.

Results

The results of the classification according to FAB criteria in 79 children with AML are shown in Table 2. Figure 1 illustrates the reactivity of MoAbs in 52 cases of childhood AML in which the immunologic phenotype closely corresponded with the morphologic classification. As expected from recent re-

Table 2. Classification of 79 patients with AML according to FAB criteria

	FAB					
	M1	M2	M3	M4	M5	M6
Patients (*n*)	23	15	2	15	23	1



§ For immunofluorescence, a positive result was = or >20% reactive cells

Fig. 1. Surface marker profile in childhood AML

Table 3. Composite antigenic phenotype not correlating with FAB morphological subtype

Patient no.	Age	Sex	MoAbs (% cells positive)							Cytochemistry		Morphology
			OKla1	My9	My7	VIM-D5	OKM1	Leu-M3	VIM13	POX	NSE	
1	6yr	F	40	23	55	11	21	27	ND	90	(+)	M1
2	7yr	M	45	50	42	27	34	4	10	20	(+)	M4
3	9yr	M	3	76	38	35	37	6	ND	50	(+)	M4
4	2yr	F	4	75	62	45	18	ND	12	100	(+)	M4
5	14yr	F	70	30	60	5	ND	2	5	38	(+)	M4
6	3wk	M	20	35	56	50	34	5	10	80	(+)	M4
7	15yr	M	31	49	70	38	24	12	ND	40	(+)	M4
8	2yr	F	30	80	85	65	30	8	ND	18	(+)	M4
9	6yr	F	41	58	18	66	ND	ND	7	0	++	M5
10	11yr	F	65	70	ND	65	ND	ND	3	0	++	M5
11	13yr	M	65	30	75	60	ND	5	10	3	++	M5
12	13yr	M	10	89	90	89	ND	7	ND	90	++	M5
13	14yr	F	2	0	0	48	0	0	0	1	++	M5
14	1yr	M	52	39	61	50	27	10	ND	1	++	M5
15	2yr	M	72	70	50	15	18	4	10	1	(+)	M5a
16	3yr	F	80	70	40	66	5	2	0	0	(+)	M5
17	1yr	M	56	33	0	48	5	0	ND	1	++	M5
18	NK	NK	80	80	16	33	2	2	2	NK	NK	M5a

POX, peroxidase; (% cells positive); NSE, nonspecific esterase; (+), faintly positive; ++, strongly positive; ND, not done; NK, not known.

ports [3, 5, 23], My9 and My7 reacted with the majority of cases of both monocytic and nonmonocytic AML, whereas the percentage of VIM-D5 positive cells was low in the immature subgroup of AML (M1 versus M2/M4), and OKM1 stained predominantly cells from patients with M4 or M5 AML. None of these MoAbs clearly distinguished granulocytic from monocytic forms of AML. Although the monocytic MoAbs VIM13 and Leu-M3 reacted exclusively with M4/5 subtypes, a substantial proportion of morphologically/cytochemically diagnosed M4/M5 AML was VIM13/Leu-M3 negative or weakly positive (Table 3).

In two patients with undifferentiated forms of acute leukemia, blast cells could be affiliated with erythroid (VIE-G4+, My7−, My9−) or megakaryocytic (J15+, My7, and My9 only weakly+) lineage. Leukemic cells from one patient with acute undifferentiated leukemia (POX−) expressed myeloid antigens (My7+, My9+) and could be classified as immature AML. In one patient initially diagnosed as having null ALL, AML developed within 1 week after administration of induction chemotherapy for ALL, indicating hybrid acute leukemia. Details of surface antigen expression in this patient are described elsewhere [Ludwig et al., this volume]. Nine patients with AML without morphologic/immunologic lymphoid features exhibited an elevated (>10%) level of TdT-positive cells.

Discussion

The clinical utility of MoAbs in the evaluation of AML has been shown in several studies [2, 3, 23], and a highly reproducible classification system based on an antigenic phenotype of malignant myeloid cells has been proposed [3]. In our study of 79 children with AML classified according to FAB criteria, it was possible clearly to differentiate in 52 cases the AML subtypes M1/2, M3, and M4/5 on the basis of the surface antigens detected by the panel of MoAbs applied (Fig. 1). However, immunologic results were inconsistent with routine morphology/cytochemistry in 18 cases predominantly classified as monocytic variants (M4/5) of AML according to FAB criteria (Table 3). The fact that routine morphology may be misleading in some of these cases is demonstrated by case no. 5; though this was morphologically diagnosed as M4 AML, leukemic cells were negative with MoAbs reacting against monocytic antigens, and cytogenetical analysis revealed a *t* (8; 21) typical for FAB type M2 [24]. On the other hand, blast cells from two cases (nos. 15 and 18) with morphologically unequivocal M5a AML lacked detectable monocytic antigens. These results confirm that monocytic involvement in AML is more reliably assessed by morphological studies used in combination with immunologic and cytogenetical findings.

The potential value of MoAbs reacting with myeloid cells in acute undifferentiated leukemia could be demonstrated in three cases identified as M1, M6, and M7 AML by immunologic analysis (data not shown).

Information about the progostic significance of surface antigen expression in AML cannot as yet be derived from our data because of the short follow-up of patients included in this analysis. It is noteworthy, however, that out of nine TdT-positive AML patients, only four are still in complete remission; three relapsed within a year, one achieved only a partial remission, and another did not respond. These results may support earlier reports that TdT-positive AML patients have a poor prognosis [25].

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Infant Leukemia: A Single Pattern of Nonlymphocytic Leukemia?

E. R. van Wering and W. A. Kamps[1]

At present, the majority of children with acute lymphoblastic leukemia can expect a favorable outcome. However, leukemia in infants (0–1 year) still runs a dismal course. The first year of life also has a unique pattern of hematopoietic activity. A possible association between these two findings was investigated by us.

We examined the characteristics of 51 infant leukemia patients diagnosed from 1975 to 1984; 48 of the 51 had evaluable data. Infants had a high frequency (39.6%) of hyperleukocytosis ($>100 \times 10^9$/L), early central nervous system involvement (27.1%), and cutaneous infiltration (18.8%). The respective numbers in patients >1 year were 13.9%, 4.8%, and 1.0%. Acute nonlymphocytic leukemia was diagnosed in 18 of 48 infants (37.5%), compared with 128 out of 892 (14.3%) in older patients. Infants had only acute leukemia of FAB class M5 (10/19) or acute leukemia with unclassifiable but monocytoid blasts (8/19).

This study confirms the presence of poor prognostic features in infant leukemia. The almost exclusive occurrence of monoblastic or monocytoid acute leukemia parallels the high proliferation rate of monocytes in this age group, and the leukemic cells may thus well reflect frozen stages of monocytic differentiation.

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ALL in Children

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Growth of Children with Acute Lymphocytic Leukemia: Preliminary Results

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In 1975, Onoyama et al. [1] reported growth disturbances in 50% of the children irradiated with 30.0 Gy in the hypothalamic pituitary region. At the same time, Shalet and Beardwell [2] demonstrated a decreased response to growth hormone in a comparable group of ten patients. Following CNS prophylactic irradiation of the skull (24 Gy and a daily dose of 2 Gy) in patients with acute lymphocytic leukemia (ALL), a growth hormone deficiency occurred as well [3]. However, this group of patients showed a diminished growth hormone response to insulin but not to arginine [4]. It is suggested that the decreased response of growth hormone may be temporary [5] and would be restored after 6–12 months. However, no other studies have confirmed this suggestion. After cessation of the treatment, height growth shows a slight increase – catch-up growth. The extent of this catch-up growth is unknown.

In this retrospective study, we present preliminary results of a statistical evaluation of height growth in children with ALL treated in our department.

Methods

Sixty-six children (25 boys, 41 girls) with ALL were treated according to standard treatment protocols that include cranial or craniospinal irradiation. The treatment schedules consisted of daily prednisone (40–80 mg/m^2) for 6 weeks, weekly intravenous injections of vincristine (2 mg/m^2), and from week 4 to week 6 daily asparaginase (200 U/kg i.v. or i.m.). Children with high-risk ALL also received cyclophosphamide (1200 mg/m^2) on day 1 and adriamycin (50 mg/m^2) on days 21 and 22. After bone marrow remission in patients with standard-risk ALL had been achieved, the central nervous system was treated prophylactically by cranial irradiation (25 Gy in daily fractions of 2 Gy for 2½ weeks), together with five intrathecal injections of methotrexate (12 mg/m^2) and prednisolone (12 mg/m^2). In children with high-risk ALL, the complete craniospinal axis was irradiated with 18 Gy over the skull and 12 Gy over the spine. In relapse patients who had previously had standard-risk ALL, reinduction was performed by the high-risk scheme, resulting in a total radiation dose of 43 Gy over the cranium and 12 Gy over the spine. In all patients, remission was maintained by daily 6-mercaptopurin (50 mg/m^2) and weekly methotrexate (30 mg/m^2) orally, alternated after 5 weeks by a consolidation treatment consisting of 14-day prednisone (40 mg/m^2) together with vincristine (2 mg/m^2) on days 1 and 8. The maintenance and consolidation treatments were continued for a period of 2 years, after which all cytotoxic agents and corticosteroids were stopped. All 66 children received cytotoxic agents and corticosteroids: 45 (=68%) children received cranial irradiation; 8 (=12%) craniospinal irradiation; 1 child was not irradiated at all.

At every consultation, the height of the children was measured. For easy assimilation of the data, four values a year, at regular intervals, were selected and compared

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Table 1. Irradiation regimens for children with standard-risk, high-risk, and relapse ALL

Method	(*n*)	%	Maximum follow-up period (years)
Cranial irradiation (25 Gy)	45	68	10
Craniospinal irradiation (18 Gy cranial; 12 Gy spinal)	8	12	6
Relapse treatment (total radiation dose: 43 Gy cranial; 12 Gy spinal)	12	18	9
No irradiation	1	2	10

with values for the normal population of that age group. The standard deviation (SD) of this mean height was determined. The values of the normal population were obtained from a recent study of the height of normal Dutch children [6]. To make the height of two ages in one child comparable and in order to compare two different children in this height growth; we calculated the standard deviation score (Z score):

$$\text{Z score} = \frac{\text{measured height} - \text{height of the standard population}}{\text{SD of the standard population}}.$$

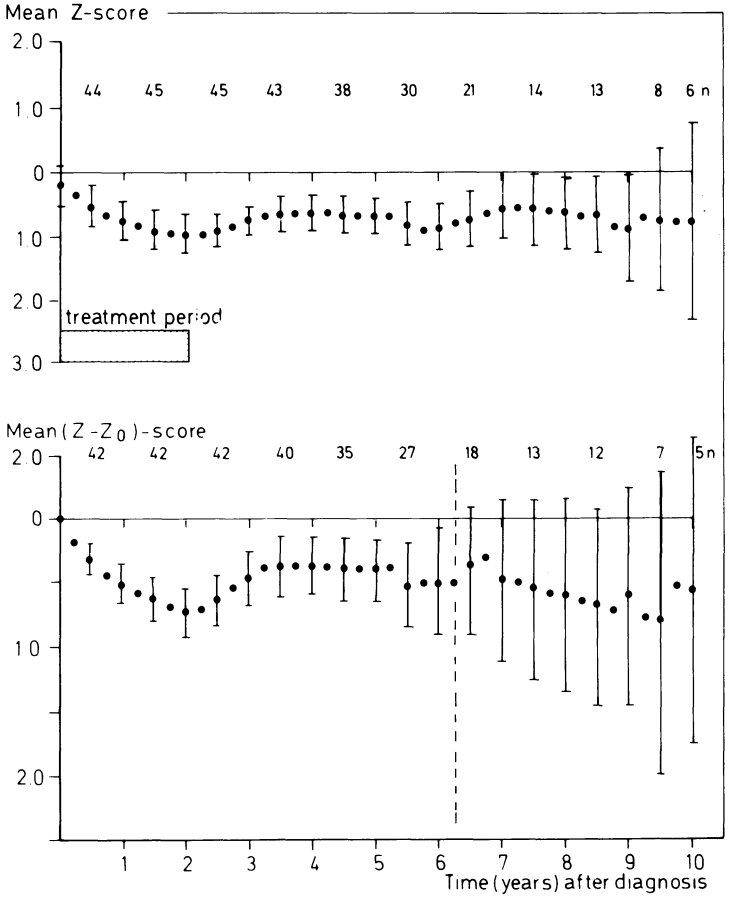

Fig. 1. Course of standard deviation scores and the relative standard deviation scores for height growth in 45 children with normal-risk ALL; cranial irradiation with 25 Gy, daily fraction dose. 2.0 Gy

The relative standard deviation score (Z_r score) is the mean of changes of the individual Z scores in relation to the age at diagnosis (Z_r score at diagnosis = zero), simplifying the comparison of the changes in height growth from the time of diagnosis.

In 12 (18%) children with standard-risk ALL, a relapse occurred, and they were retreated according to the high-risk protocol. The differences in radiation treatment are summarized in Table 1.

Results

1. Children with Standard-Risk ALL Receiving Cranial Irradiation (Fig. 1).

Because the range of the Z_r score can never be more than zero, the useful follow-up period was 6½ years. During this period, the standard deviation score of the height growth decreases during the treatment period up to a Z score of -1. After the cessation of the treatment a catch-up growth of $+0.3 \times$ SD occurred, resulting in an ultimate loss-of-height score of -0.7. This signifies a mean loss of prognostic height of about 5 cm.

2. Children with High-Risk ALL Receiving Craniospinal Irradiation (Fig. 2).

The group of children is rather small (eight patients), but some peculiarities can be found. The height at diagnosis is $-0.6 \times$ SD of the normal population. Because the height of the parents did not differ from that of the normal Dutch population, this fact cannot easily be explained. In these children, the Z score of the height also decreased during the treatment period with a loss of up to -1.4. A catch-up growth of $+0.4 \times$ SD resulted in an ultimate loss of Z score of -1.0. This signifies a mean loss of prognostic height of about 7 cm.

3. Children with a Relapse of a Standard-Risk ALL (Fig. 3).

In these children, no catch-up growth is observed, probably because the relapse occurred between the 1st and the 3rd year of

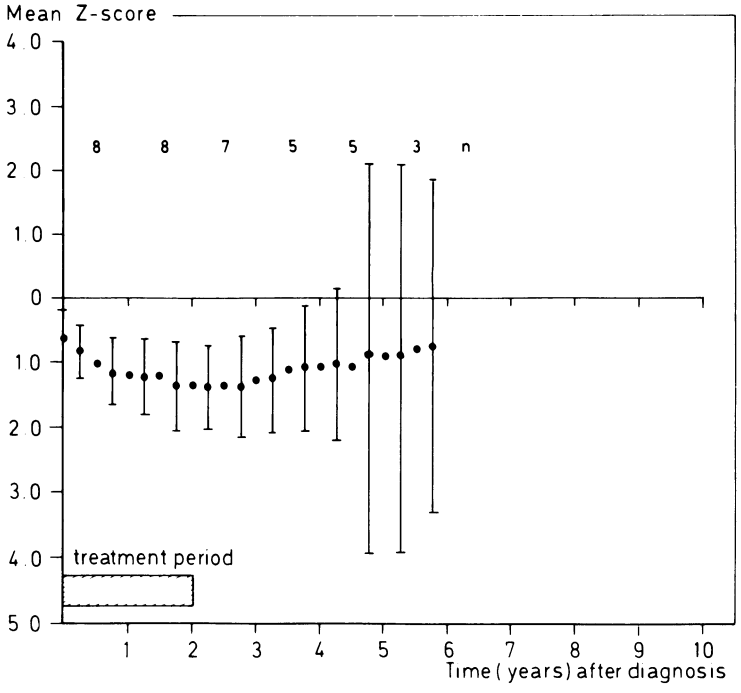

Fig. 2. Course of standard deviation scores for height growth in eight children with high-risk ALL; craniospinal irradiation: cranial 18 Gy, spinal 12 Gy, daily fraction dose 2.0 Gy

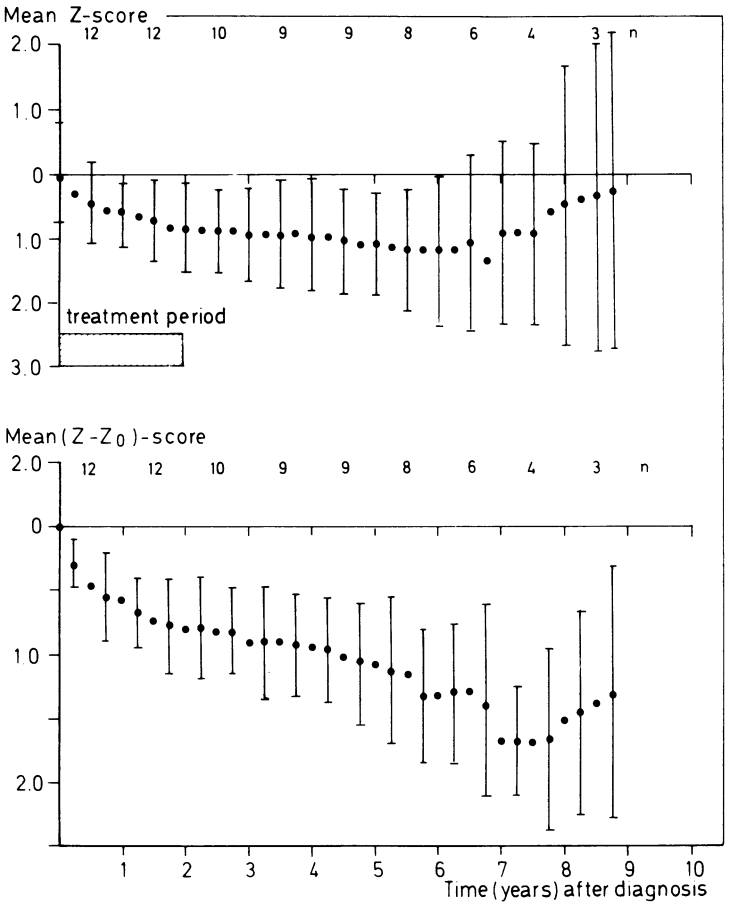

Fig. 3. Course of standard deviation scores and relative standard deviation scores for height growth in 12 children treated for relapse ALL

the follow-up, including the period of catch-up growth in the first two groups. These children show a loss of prognostic ultimate height of at least 10 cm.

Discussion and Conclusions

The treatment of children with ALL results in a reduction in height growth. The most likely cause of this phenomenon is irradiation over the hypothalamic pituitary region. Further, the influence of the regular use of corticosteroids should not be underestimated. However, in those patients where catch-up growth is possible, the retardation in height growth will be mild. On the other hand, in the children treated for a second time because of a relapse, catch-up growth does not occur, and a progressive retardation in height growth will result in relatively short stature. The cause of catch-up growth is not yet clear. It may be influenced by the cessation of regular corticosteroid administration. However, this phenomenon requires supplementary investigation. The comparison of bone age development with the patterns of height growth may give some indication as to the influences of corticosteroids.

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Two Unexpected Courses in Four Children with Lymphoblastic Leukemia of B-Cell Type (B-ALL)

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Introduction

There is now good evidence that ALL of L3 morphology (FAB) is mainly of B-cell origin [4, 7]. B-ALL is one of the fastest growing human malignancies [6, 11]. Prognosis has always been rather poor, particularly with conventional ALL treatment that is highly effective in non-B-ALL [3, 8, 13, 15]. With the advance of new B-ALL treatment protocols prognosis has been somewhat improved [10, 12, 14]. Thus, the probability for disease-free survival is now about 50% for patients treated with the B-ALL protocol of the German BFM study group [9]. However, 50% of patients will either not respond to treatment or relapse early and die of their disease.

In this presentation, we report the clinical course of four patients with B-ALL. Two patients had the typical rapidly fatal course with bone marrow (BM) or CNS relapse during or shortly after treatment. Surprisingly, another patient has been in continuous complete remission (CCR) for 3 years and 2 months after only a very short course of chemotherapy. Another patient had a late relapse 11 months after achieving complete remission (CR).

In retrospect, we cannot find any clinical, hematologic, or immunologic parameter that might enable us to predict one of the different clinical courses.

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Materials and Methods

Cytology. Diagnosis of B-ALL was made by detecting more than 25% blasts of L3 morphology (FAB classification) in BM aspirates. In all cases, diagnosis was confirmed by Prof. Dr. H.J. Riehm (Kinderklinik der Medizinischen Hochschule, D-3000 Hannover, FRG). Surface markers were analyzed by Dr. W.-D. Ludwig (Medizinische Klinik, Klinikum Steglitz der Freien Univ., D-1000 Berlin, FRG) and in our own laboratory. Histochemical stains for PAS, alkaline phosphatase, POX, esterase, and TdT were negative.

Cytogenetic studies were performed by Dr. J. Harbott (Kinderklinik der Universität, D-6300 Gießen, FRG). Flow cytophotometry was done by Dr. W. Hiddemann (Medizinische Klinik der Univ., 4400 Münster, FRG).

Treatment Protocol. The patients were treated according to the BFM study group protocol with two slightly different, alternating chemotherapy blocks [9].

Block 1 consisted of cyclophosphamide (CPM) 200 mg/m^2 on days 1–5; methotrexate (MTX) 500 mg/m^2 on day 1 with leucovorin rescue; teniposid (VM 26) 165 mg/m^2 on day 5; cytosine arabinoside (ARA-C) 300 mg/m^2 on day 5; MTX intrathecally (ith) on day 1 or via Rickham reservoir MTX 3 mg on days 1–4, and ARA-C 40 mg on day 5. Since 1983, dexamethasone 10 mg/m^2 has been given on days 1–5. Block 2 consisted of Adriamycin (ADR) 50 mg/m^2 given on day 5 instead of ARA-C/VM 26.

Finally, chemotherapy is followed by cranial irradiation.

Case Reports

Patient I. R. On admission to hospital, the 11-year-old boy had pain in both knees and fever; liver and spleen were 2 cm below the costal margins. He had stomatitis, generalized lymphadenopathy, ataxia, and left-sided abducens paralysis. A full blood count and a BM aspirate were normal. Twelve days later, he developed renal failure. Ultrasound examination revealed very large kidneys, and a kidney biopsy showed diffuse infiltrations by lymphoblasts. At that time, there was complete BM metaplasia with blasts of L3 morphology.

Renal function improved rapidly after small doses of prednisone. During the first round of chemotherapy (Block 1), generalized herpes zoster, bacterial septicemia, and generalized seizures occurred. He was treated with acyclovir, antibiotics, and granulocyte transfusions and made a complete recovery. A repeat BM aspirate taken 5 weeks after initiation of chemotherapy showed CR. A second B Block (2) was then started but had to be stopped after 4 days because of generalized seizures, somnolence, blindness, hearing loss, and severe trigeminal neuralgia. Eight weeks later, all neurologic symptoms had disappeared with the exception of convulsions occurring about every three weeks BM examination revealed 5% blasts of L3-like morphology. Because of serious toxic side effects, the parents refused any further treatment. Three years and 2 months later, the boy is in good health without any signs or symptoms of relapse.

Patient R. H. The $3^{9}/_{12}$-year-old boy was admitted to surgery because appendicitis was suspected. Laparotomy revealed a tumor mass 16 cm in diameter at the site of the ileocecal region. A biopsy revealed Burkitt-like non-Hodgkin lymphoma (NHL).

CR of BM and CSF was obtained after the first Block 1. After four chemotherapy blocks, a residual lymphoma 2 cm in diameter was removed during a second-look laparotomy. At that time, BM examination revealed 5% L3 blasts. BM infiltration increased to 50% during the fifth B Block. High-dose CPM and high-dose MTX were without effect. The boy died from drug-resistant B-ALL 4 months after initiation of chemotherapy.

Patient R. N. The $13^{9}/_{12}$-year-old boy presented petechial hemorrhages. Cytoreductive therapy was started with prednisone and low-dose CPM but had to be stopped 5 days later because of transient renal failure. A BM aspirate taken 7 days later showed marked hypoplasia with only 2% L3 blasts. The boy went into CR after the first Block 1. He then received a full course of further five B Blocks followed by cranial irradiation with 24 Gy.

Eleven months after achieving the first CR, he had cutaneous lymphoblastic infiltrations on his scalp. At that time, CSF analysis revealed two blasts of L3 morphology per µl, and his BM showed 2% L3 blasts. He achieved CR again after one Block 1. He was then treated for 10 months altogether according to a French B-ALL/NHL protocol [12]. A second BM relapse was diagnosed 3½ months after cessation of treatment and 11 months after the second CR. The boy again went into CR after one Block 1. He was further treated with two cycles of COPAD-M and one cycle of CAM according to the French protocol. He was then referred to Ulm University for autologous bone marrow transplantation (ABMT). There, he died from "venous occlusive disease" 12 days after ABMT and 2½ years after diagnosis of B-ALL.

Patient M. W. The 8-year-old boy was admitted to hospital because of severe pain in his jaw, loosening of teeth, and swelling of gingiva. He also went into CR after one Block 1. A Rickham reservoir for ith. chemotherapy was then implanted. He was treated with five additional B Blocks followed by cranial irradiation with 30 Gy. Leptomeningeal relapse with 400 blasts per µl CSF occurred 4 weeks after irradiation. A BM aspirate showed normal hematopoiesis but revealed about 5% L3 blasts. A second CR of CSF and BM was obtained after one COPAD-M cycle according to the French protocol. He is still under treatment. ABMT will be considered.

Results and Discussion

The clinical, hematologic, and immunologic characteristics of the patients are summa-

Table 1. Clinical, hematologic, and immunologic characteristics of patients with B-ALL at the time of diagnosis

	J. R.	R. H.	R. N.	M. W.
Age (years)	11	$3^{9}/_{12}$	$13^{9}/_{12}$	8
Duration of history (weeks)	6	1	2	4
Peripheral blood				
WBC/μl	6400	8700	8300	3000
L3 blasts (%)	1	15	16	7
Hb (g/dl)	6.9	9.9	10.6	8.5
Platelets × 10^3/μl	90	28	61	37
LDH (U/liter)	2394	2813	3443	3340
Uric acid (mg/dl)	6.6	9.4	15.4	13.2
EBV serology	Negative	Negative	Negative	Negative
Immunoglobulins	Low	Normal	Normal	Low
Bone marrow blasts				
Morphology (FAB)	L3	L3	L3	L3
Percentage	93	88	92	95
Surface markers				
Ig	nd	+	+	+
IgM	+	+	nd	nd
Y29/55	+	+	+	nd
B1	nd	+	nd	+
HLA-DR	+	+	+	+
Impulse cytophotometry	nd	Euploid	Euploid	nd
S phase: G2M	nd	18,2:6,0	26,4:5,6	nd
Chromosomal analysis	nd	nd	Normal	t (8;14)
Involvement of				
CNS	+	+	∅	+
Liver	+	∅	∅	∅
Spleen	∅	+	∅	+
Lymph nodes cervical	+	∅	∅	+
abdominal	+	+++	∅	+
Kidney	+++	∅	∅	∅
Bone	+++	∅	∅	∅

nd, not done.

rized in Table 1. On admission to hospital, all patients exept one (R. N.) were anemic, and all were thrombocytopenic. Peripheral white blood counts (WBC) were normal. Serum lactate dehydrogenase (LDH) and uric acid were consistently elevated, thus indicating a very high proliferative turnover of leukemic blasts. All patients had BM metaplasia with more than 50% blasts of L3 morphology with the typical surface markers of B cells.

Although all patients rapidly went into CR as far as BM and CSF are concerned after only one BFM-B Block 1, they differed as to the final outcome. Patients R. H. and M. W. had early relapses either during or shortly after treatment. These early relapses might be due to the emergence of drug-resistant clones within the blast population, as demonstrated for patient R. H.

The clinical courses of the other two patients are unusual. Patient I. R. has been in CCR for the last 3 years and 2 months after receiving only a short course of chemotherapy. A similar observation was made by Pees et al. [11]. Their patient with B-ALL had also been treated according to the BFM protocol. He has now been in CCR for about 19 months, although treatment had to be stopped after three BFM Blocks because of life-threatening complications. It might be speculated that, in these patients, all leukemic

blasts were in such a vulnerable phase of the generation cycle that they could be totally destroyed by a short course of chemotherapy.

Finally, there is the clinical course of patient R. N. Such a late relpase, which is very rare in African Burkitt's lymphoma [1], had never before been reported in B-ALL. This patient even relapsed twice but promptly achieved CR again with the same B Block. It could well be that some malignant blasts remained in GO phase and reentered the cell cycle when chemotherapy was stopped altogether. If this is true, the overall duration of treatment was too short in this case.

From our clinical observations, we would like to suggest that B-ALL is not a uniform disease entity. It seems that under B-specific treatment it can take one of at least four different clinical courses: (a) CCR after a full course of chemotherapy, with or without cranial irradiation; (b) non-response or early relapse during or shortly after treatment; (c) late relapse after a full course of treatment; (d) CCR after a very short course of chemotherapy.

At present, the benefit-risk ratio of current B-ALL treatment protocols seems satisfactory for patients belonging to group a. However, it could well be that some patients in group a actually belong to group d. These patients might receive too intensive and prolonged treatment that could eventually lead to life-threatening complications. On the other hand, treatment protocols are inefficient for patients in groups b and c at present.

To prescribe adequate treatment, it would be necessary to detect clinical, hematologic, or immunologic characteristics that would enable us to predict one of the different clinical courses for the individual patient. However, in our group of patients, there were no correlations with WBC, levels of LDH, serum immunoglobulins, Epstein-Barr virus serology, DNA content, chromosomal aberrations, or even CNS involvement. Obviously, it is not the origin of B-ALL that matters. In two of our patients with bulky abdominal disease, the malignancy arose most probably from an extramedullary site. One might assume that bulky NHL with secondary ALL would need more aggressive chemotherapy than ALL without bulky disease [2, 5]. This is, however, not confirmed in the case of patient I. R. Philip et al. [12] have already suggested that patients with bulky B-NHL, regardless of the extent of BM involvement, would have far better prognosis than patients with B-ALL without bulky disease.

It will be of special interest to see whether modern molecular techniques will provide us with additional parameters to predict B-ALL subsets of good and poor prognosis [6].

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Progress in Treatment of Children with non-Hodgkin Lymphoma: A Report of the Polish Leukemia and Lymphoma Study Group *

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Introduction

During the past decade an evident increase in the curability of children with non-Hodgkin lymphoma (NHL) has been achieved. Intensive multidrug therapy regimens involving local radiotherapy and prophylaxis of the central nervous system have been used with notable success [1–3]. The increasing efficiency of modern therapy of childhood NHL has stimulated further studies. It has been demonstrated that the remarkable heterogeneity of NHL in the relapse risk is related to the primary location of the tumor and its dissemination, histology, and immunology (4–9). During its 6-year observation of children with non-Hodgkin lymphoma treated according to the modified LSA_2L_2 protocol, the Polish Children's Leukemia and Lymphoma Study Group has accumulated experience which, together with other studies, could be the background for further refinment of the treatment. This paper summarizes the long-term results in the treatment of NHL with the modified LSA_2L_2 protocol. The evaluation of the early effects of therapy of disseminated NHL with two other regimens, COAMP [5] and Murphy-Bowmann [4], also is reported.

Materials and Methods

Patients

From January 1979 to January 1986, a total of 243 previously untreated children aged 1–17 years with non-Hodgkin lymphoma were entered in the study, representing all cases of NHL admitted to the six Polish Children's Hematology Centers. The male-to-female ratio was 4:1. In all cases, a histopathologic diagnosis was established prior to starting the therapy. A Kiel histologic classification scheme was used [10].

Evaluation and Staging

Pretreatment evaluation included physical examination, complete blood count, renal and hepatic function test, and percutaneous bone marrow aspiration. Spinal fluid cell count and cell morphology examination were carried out for all children. Wherever possible, fresh tumor cell suspensions were studied for immunologic markers by standard methods in order to confirm the B-cell origin through the presence of surface immunoglobulins. Imaging studies, including chest and bone radiographs, intravenous urography, and lower-extremity lymphangiography, were performed if required by clinical judgment. More recently, ultrasonography and computed tomography (CT) were used to evaluate intra-abdominal disease. In selected cases, CT scans of the head and chest were done.

Treatment

Three treatment protocols were applied to the children evaluated in this study. In 1979–1982, all eligible patients with NHL were treated according to the modified LSA_2L_2 regimen (Fig. 1). From 1983 to 1985, all the

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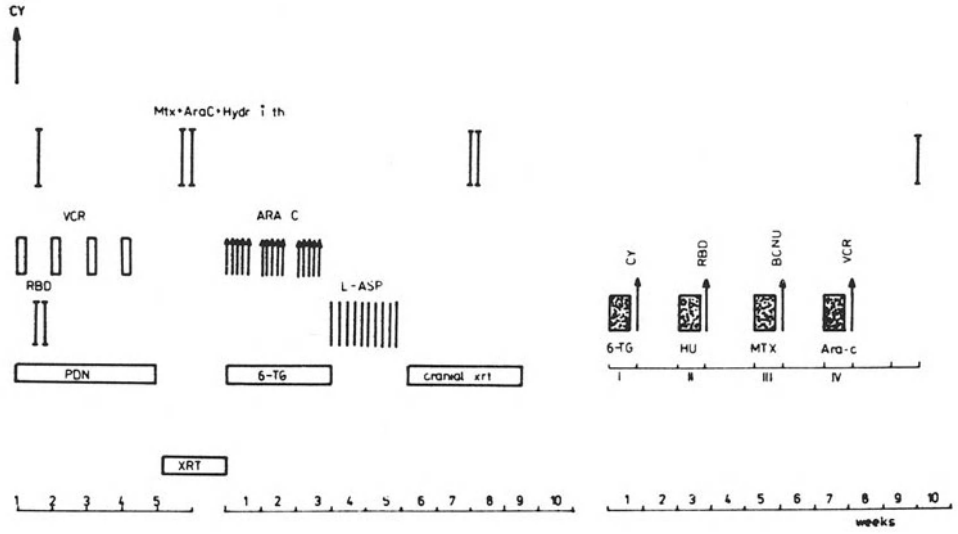

Fig. 1. Modified LSA_2L_2 protocol. CY, cyclophosphamide; mtx, methorexate; VCR, vincristine; XRT, radiotherapy; PDN, prednisone; RBD, rubidomycine

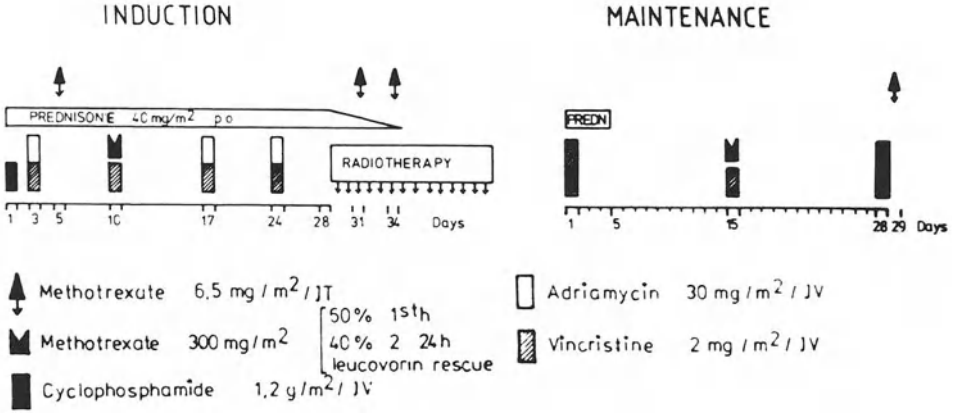

Fig. 2. COAMP protocol. PREDN, prednisone

registered children with stages III and IV were assigned to one of the two treatment programs: COAMP (Fig. 2) or the Murphy-Bowmann regimen (Fig. 3). There were 194 patients treated with LSA_2L_2, 25 with COAMP, and 24 with the Murphy-Bowman protocol. Treatment was carried out for 24 months with the LSA_2L_2, 18 months with the COAMP and 6 months with the Murphy-Bowmann protocol. Involved field radiotherapy was given to 59 children treated with LSA_2L_2 after completion of the induction phase. The doses delivered to our patients were in the range of 1400–3500 rad. The children in stages II, III, and IV on the LSA_2L_2 protocol and the patients in stages III and IV on the COAMP regimen received cranial radiation after achieving remission.

Analysis of Data

The survival curves showing estimated distribution of disease-free survival were calculated using the product-limit method of Kaplan and Meier [11].

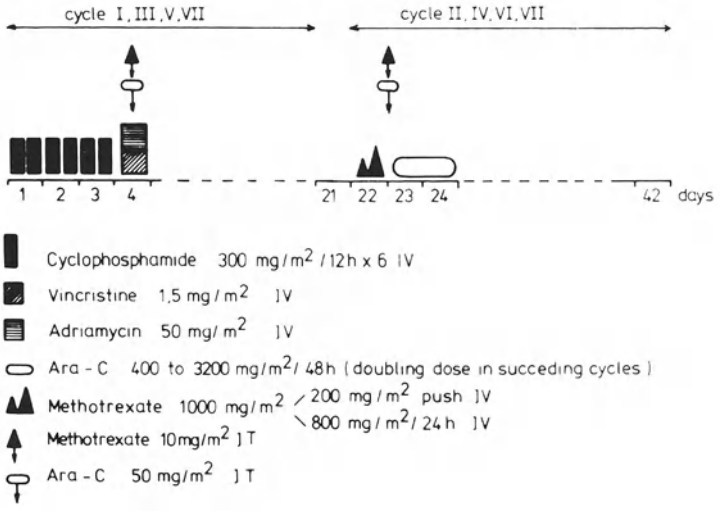

Fig. 3. Murphy-Bowmann protocol

Results

At the end of the induction phase, 70% of patients treated with the LSA_2L_2 protocol were surviving in complete remission. The response rate in children with localized disease was 90% and in children with nonlocalized disease, 64% (Tables 1 and 2). Four patients with localized NHL who did not respond to initial LSA_2L_2 terapy included three with primary tonsil involvement and one with primary cervical involvement. Of 136 responders, 45 relapsed during the maintenance therapy. There were 33 relapses in 56 responders in stage III and 21 relapses in 46 responders in stage IV. a total of 73 patients were off treatment at the time of analysis. The actuarial estimated disease-free survival rate in localized disease was 95% at 85 months (Fig. 4). In contrast, failure-free survival in stages III and IV was 66% and 50% respectively. It indicated clearly that the LSA_2L_2 regimen, which produced good

Table 1. Overall results achieved for 196 children with NHL treated with LSA_2L_2 protocol

	(*n*)
Total number of patients	194
Complete remission	136 (70%)
No response	58 (30%)
Relapse	45
Still in complete remission	91 (67%)
Off therapy	73 (54%)
Median time of follow-up (months)	29

Table 2. Response rate related to stage in NHL treated with LSA_2L_2

	Stage				
	I	II	III	IV	Total[a]
	(*n*)				
Complete remission	11 (84%)	23 (92%)	56 (63%)	46 (69%)	136
No complete remission	2	2	33	21	58
Relapse	2	3	19	21	45
Still in complete remission	9	20	37	25	91

[a] Primary tonsil involvement: 3 cases; cervical lymphnode involvement: 1 case.

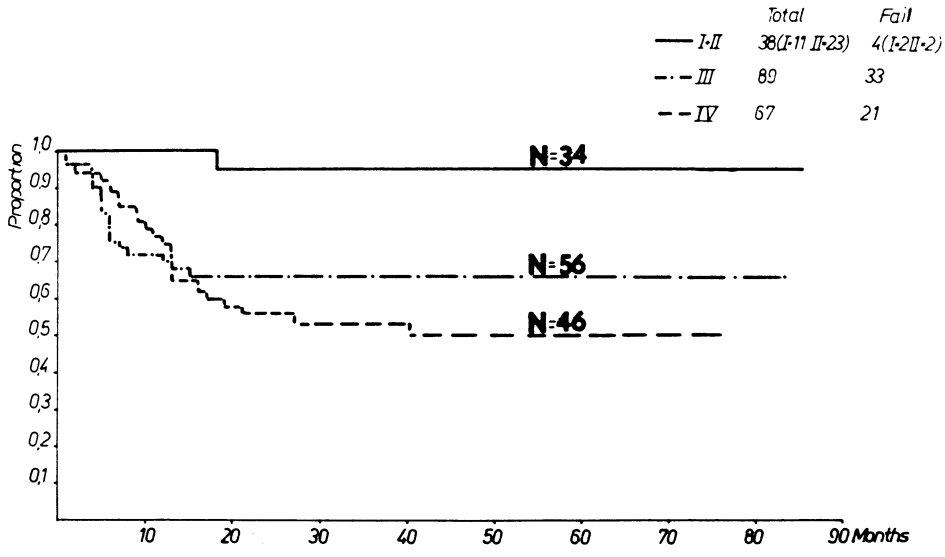

Fig. 4. Probability of disease-free survival of children with NHL treated with LSA_2L_2 protocol as related to stage

Table 3. Overall results of treatment of nonlocalized NHL with three different protocols

	LSA_2L_2	COAMP	Murphy-Bowmann
	(*n*)		
Total number of patients	156	25	24
Complete remission	102 (65%)	22 (88%)	19 (79%)
No complete remission	54	3	5
Relapse	40	5	9
Still in complete remission	62 (61%)	17 (77%)	10 (53%)
Off therapy	50	4	7
Median time of follow-up (months)	29	15	12

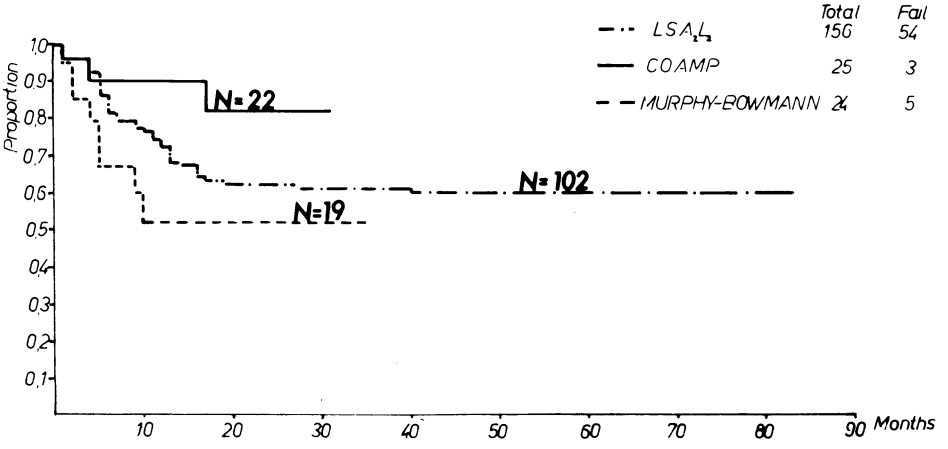

Fig. 5. Probability of disease-free survival of children with nonlocalized NHL (stages III and IV) treated with three different protocols

Table 4. Response rate related to main clinical presentation in children with NHL treated according to three different protocols

	Primary anatomic site of tumor involvement															
	Intrathoracic			Abdomen			Head-neck			Peripheral nodes			Others			
	LSA_2L_2	COAMP	Murphy-Bowmann	LSA_2L_2	COAMP	Murphy-Bowmann	LSA_2L_2	COAMP	Murphy-Bowmann	LSA_2L_2	COAMP	Murphy-Bowmann	LSA_2L_2	COAMP	Murphy-Bowmann
	(*n*)			(*n*)			(*n*)			(*n*)			(*n*)		
Number of patients	59	1	1	55	18	19	27			7	6	3	8		1
Complete remission	49	1		21 (36%)	16 (86%)	15 (79%)	21			6	5	3	5		1
No complete remission	10		1	34	2	4	6			1	1		3		
Relapse	17	1	1	9	3	5	11			5	1	2	3		1

results in localized childhood NHL, was not curative for the majority of patients with advanced, nonlocalized disease. Therefore, in 1983–1986, two other regimens-COAMP and Murphy-Bowmann – were applied to cases of nonlocalized disease with a prevalent primary abdominal location. The comparison of the results achieved in nonlocalized disease with the three therapy regimens is presented in Table 3. Of the children with nonlocalized NHL, 83% treated with COAMP and 79% of those treated with the Murphy-Bowmann protocol successfully entered a complete remission; these results compared favourably with 64% of patients treated according to the LSA_2L_2 protocol. In children with disseminated NHL, the response rate to initial therapy improved markedly with the COAMP and the Murphy-Bowmann protocol. Most of the 92 children studied with stage III and IV disease showed, at admission, abdominal tumors (Table 4). Within this group, there were only 36% responders on the LSA_2L_2 protocol, as compared with 89% on the COAMP and 79% on the Murphy-Bowmann regimens. At the time of this report, 40 of 102 responders on the LSA_2L_2 protocol, 5 of 22 responders on COAMP, and 9 of 19 on the Murphy-Bowmann protocol had relapsed.

The disease-free survival rate for nonlocalized disease is 82% for children treated with COAMP, 52% for those treated with the Murphy-Bowmann and 60% for those treated with the LSA_2L_2 protocol (Fig. 5). As shown in Fig. 6, the actuarial estimate of the proportion of disease-free surviving children with disseminated B-NHL was distinctly higher when they were treated with COAMP, as compared with the Murphy-Bowmann protocol.

Discussion

In the long-term follow-up, the excellent prognosis in localized stages of childhood NHL treated with the modified LSA_2L_2 protocol has been shown. However, the proportion of children who failed to achieve remission was higher in our series than in that reported originally by Wollner [1]. As we have shown, the poor response to the initial LSA_2L_2 therapy was observed in children

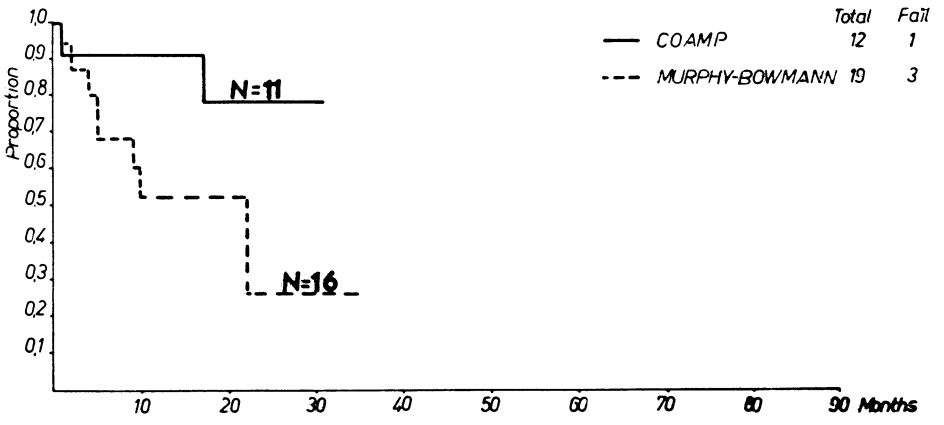

Fig. 6. Probability of disease-free survival of children with nonlocalized B-NHL treated with COAMP and Murphy-Bowmann protocols

with massive tumor in the abdominal cavity and B-cell histology. In contrast to other studies in which LSA_2L_2 protocol was used, the high rate of initial failures and relapses has also been observed in children with intrathoracic tumor. The reason for our worse results may have been the ommission of radiation of the mediastinal mass, which was combined with chemotherapy in other studies [6, 9]. Comparison of the effects of therapy achieved in nonlocalized disease with the three different regimens would indicate that the most promising results are obtained with the COAMP program, especially in respect to NHL with B-cell histology. These results seem to be as good as the best ones reported by the BFM group [2] and Lemerle [7]. The high incidence of failure observed in our patients with disseminated disease treated according to Murphy is in striking contradiction with that previously reported by her [4], despite comparable selection of patients. A larger group of patients and a longer follow-up are needed before firm conclusions can be drawn.

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Addition of Rubidomycin to Induction Treatment with Vincristine, Prednisone, and L-Asparaginase in Standard-Risk Childhood Acute Lymphocytic Leukemia (Study ALL V): A Report on Behalf of the Dutch Childhood Leukemia Study Group

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Introduction

In childhood acute lymhocytic leukemia (ALL), the initial reduction of the leukemic cell mass by intensive combination chemotherapy is important both for inducing a complete remission and for long-term control and cure of the disease [1]. Intensification of induction treatment, consisting of vincristine (VCR) and prednisone (Pred), by addition of a third drug, L-asparaginase (L-Asp), has increased the remission rate and

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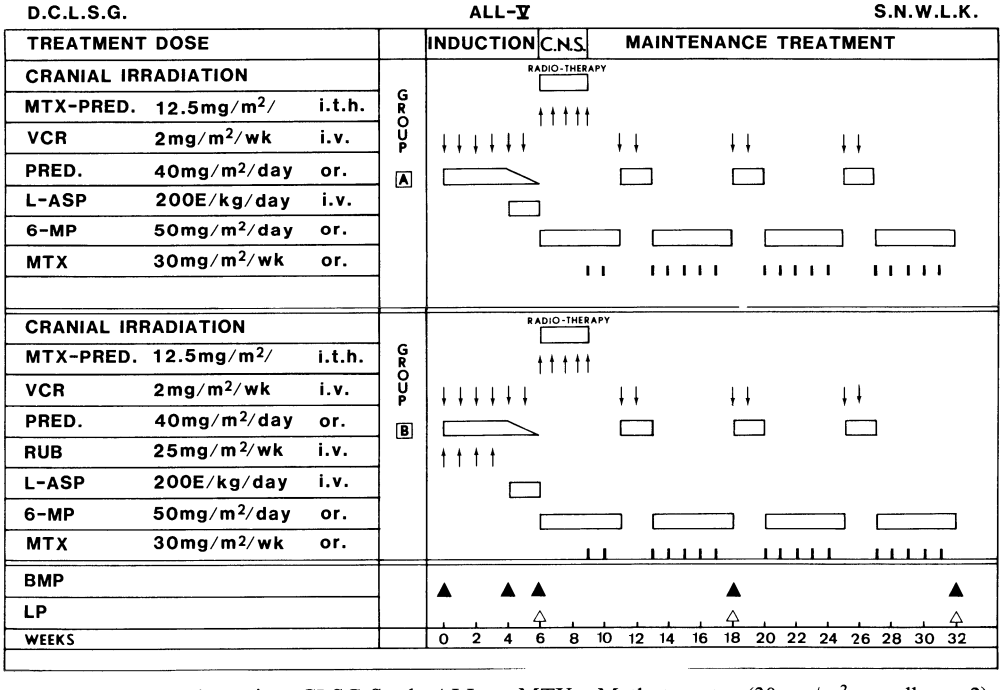

Fig. 1. Treatment scheme in DCLSG Study ALL V. VCR, Vincristine (2.0 mg/m^2, i.v. $\times 6$); Pred, Prednisone (40 mg/m^2, orally, daily, for 28 days tapering off to 42 days); L-Asp, L-Asparaginase (200 E/kg, i.v., for 14 days, days 28–42); Rub, Rubidomycin (25 mg/m^2, i.v. $\times 4$); 6-MP, 6-Mercaptopurine (50 mg/m^2, orally, daily, for 5 weeks); MTX, Methotrexate (30 mg/m^2, orally $\times 2$); MTX-Pred, Methotrexate and Diadreson-F (12.5 mg/m^2, i.th. $\times 5$); cranial irradiation, <1 year: 1500 cGy; 1–2 years: 2040 cGy; >2 years: 2500 cGy; BMP, bone marrow puncture (aspiration); LP, lumbar puncture

the duration of the remission [2]. The current study, ALL V, was undertaken to evaluate the effectiveness of further intensification of the induction treatment with a fourth drug, rubidomycin (Rub), in children with standard-risk ALL. Criteria for standard risk, based on a previous study ALL II [3] by the Dutch Childhood Leukemia Study Group (DCLSG) included, age of 0–15 years, initial leukocyte count of $< 50 \times 10^9$/liter, absence of mediastinal mass, and absence of cerebromeningeal involvement by the end of induction treatment.

Patients and Methods

Patients. In the period of accrual from May 1979 to December 1982, 351 consecutive children with ALL were diagnosed. Of this group, 252 patients (71.8%) fulfilled the criteria for standard risk and 240 entered study ALL V. Eight centers for pediatric oncology and 51 pediatric departments in general hospitals participated in the study.

Diagnosis. The diagnosis of ALL was made by institutional examination of bone marrow (BM) smears, followed by confirmation and subtyping according to the criteria of the FAB classification by the DCLSG laboratory. Immunophenotyping was done by the Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam (M. B. van 't Veer, MD, PhD).

Treatment. An outline of the protocol is shown in Fig. 1. At diagnosis, the patients were randomized into two groups. *Group A* received VCR and Pred for 6 weeks and L-Asp during the last 2 weeks. *Group B* received Rub (25 mg/m^2) weekly during the first 4 weeks in addition to VCR, Pred and L-Asp as administered in group A.

All children achieving complete remission (CR) 6 weeks after the start of induction treatment received the same central nervous system (CNS) prophylaxis and maintenance and consolidation treatment. The duration of the treatment was 24 months after CR.

Definitions. CR was defined as $< 5\%$ blast cells in the BM and no evidence of disease at

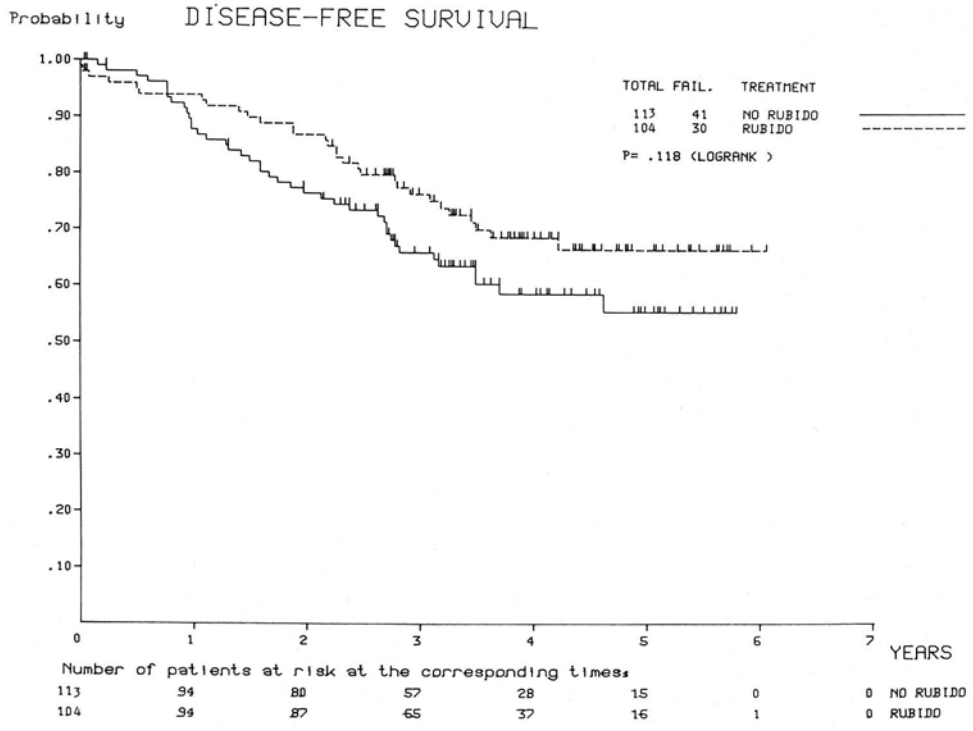

Fig. 2. Disease-free survival in DCLSG Study ALL V

any other site. The criterion for partial remission (PR) was 5%–20% blast cells in the BM. Relapse was defined as >20% blast cells in the BM or evidence of disease at any other site. CNS leukemia was defined as the presence of blast cells in the cerebrospinal fluid or clinical evidence of cerebral leukemic infiltrations.

Statistical Methods. The Kaplan-Meier life-table analysis was based on the following definitions: The analyis of disease-free survival was based on patients in CR at week 6. The time of first relapse or death in CR was evaluated. Withdrawals from the study were censored at the time of withdrawal ("off study"). The reasons for withdrawal were: major protocol violation, noncompliance of the patients, treatment refusal, and second tumors.

Duration of Survival. The analysis was based on all the patients entered in the study, and duration was calculated from diagnosis to time of death. The statistical comparison of life-table curves was performed using the log-rank test. All results were updated as of 15 July 1985 (see Fig. 2).

Results

Patient Characteristics. A total of 122 children were randomized into group A and 118 children into group B. The patients were well balanced in the two treatment groups for age, sex, initial leukocyte and platelet counts, FAB type, and immunophenotype.

Results of Treatment. The overall results of treatment are shown in Table 1.

Induction Treatment. CR was achieved in 113 of 117 (96.6%) evaluable patients in group A and in 104 of 108 (96.3%) evaluable patients in group B. In group A, no patients suffered from severe toxicity. In group B, three patients died by the end of or shortly after induction treatment, and four patients suffered from severe toxicity, resulting in renal failure (one patient), CNS injury (two patients), and bone marrow aplasia (one patient). These patients did achieve CR within 6 weeks after the start of treatment but were not considered in the analysis.

Table 1. Results of treatment in DCLSG Study ALL V

	Group A (*n*)		Group B (*n*)	
Total patients		122		118
Early death (<10 days after start of therapy)		2		–
Partial remission/ no remission		2		4
Withdrawn ("off study")		5		10
Violation	5		6	
Toxicity			4	
Complete remission		113		104
Withdrawn ("off study")		9		5
Treatment refused	2		–	
Noncompliance	1		1	
Protocol violation	4		3	
Second tumor	2		1	
Relapses		39		26
Bone marrow	15		10	
CNS	18		10	
Bone marrow/CNS	4		3	
Testis	1		2	
Bone marrow/testis	–		–	
Bone marrow/med.	–		1	
Gastrointestinal tract	1		–	
Death in CR		2		4[a]
Alive, in CCR off therapy for 6–48 months		63		69

[a] 3 patients died shortly after achieving CR.

Maintenance Treatment and Consolidation Treatment. Thus far, 39 patients in group A and 26 patients in group B have relapsed (Table 1). In both groups, the CNS relapse rate was high, accounting for 43% of the relapses. Isolated testicular relapses and combined bone marrow and CNS relapses were rare. Nine patients in group A and five patients in group B were withdrawn from the study. The reasons are shown in Table 1. Second tumors occurred in three patients and were, respectively, a malignant fibrohistiocytoma of the right tragus, a T-cell malignant lymphoma (stage IV), and an oligodendroglioblastoma.

The probability of remaining alive and in CR 4 years after first remission is 58.8% (±10.04%) for patients in group A and

68.9% (±9.8%) for patients in group B (log-rank: $p=0.118$) (Fig. 2). The probability of being alive 4 years after start of induction is respectively 75.5% (±8.4%) for patients in group A and 83.7% (±7.2%) for patients in group B (log-rank: $p=0.124$).

Conclusion

The addition of Rub to induction treatment with VCR, Pred, and L-Asp has neither improved the remission rate nor significantly increased disease-free survival in standard-risk ALL patients. Nevertheless, the long-term benefit of Rub, currently ±10% at 4 years, might increase in the future [4]. Treatment intensification with Rub did increase toxicity during induction, causing death in three patients and severe complications in four patients. Thus far, second tumors are rare in childhood ALL. Prolonged follow-up is necessary to determine the exact risk of second malignancies in children with standard-risk ALL.

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Medical Research Council Childhood Leukaemia Trial VIII Compared with Trials II–VII: Lessons for Future Management *

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Introduction

Over the last 15 years, the Working Party on Childhood Leukaemia of the Medical Research Council (MRC) has conducted a series of therapeutic trials on acute lymphoblastic leukaemia (ALL). The broad principles, including early CNS prophylaxis and prolonged maintenance chemotherapy, had been established by 1972, and in the series of trials II–VII, various aspects of the basic protocol were tested. In general, the results were disappointing, with less than 50% of the 1470 patients entered into the studies between 1972 and 1979 remaining in first remission at 4 years. UKALL VII (1979–1980) gave somewhat better results for a small group of good-prognosis patients but not as impressive as the results emerging from certain American trials, and especially from the BFM West German group [1, 2]. Therefore, in 1980, the MRC sought permission to adopt a protocol developed by the United States Children's Cancer Study Group (CCG) for average-risk patents and used this protocol for all children with lymphoblastic leukaemia from ages 0–14, no matter what their prognostic features. After the first year, a single randomised variable was introduced for patients to receive or not a dose of daunorubicin, 45 mg/m^2 intravenously on days 1 and 2. Subsequently, in line with the CCG 160 series, a second randomised variable was introduced to decide duration of maintenance of between 2 and 3 years.

Patients and Methods

In 1980, all children aged 0–14 years inclusive were entered into a single-arm study (Fig. 1) identical to the CCG 162 Arm 1A protocol. After the first year, a single variable of two doses of Daunorubicin on days 1 and 2 was introduced. Subsequently, a late randomisation of between 2 and 3 years' maintenance was also introduced. This trial was closed to entry in December 1984. The major differences between this and previous UKALL trials were:

1. A prolonged course of nine intramuscular injections of asparaginase was commenced on day 4 and given i.m. 3 times a week for 3 weeks.
2. Daily mercaptopurine in full dosages was continued throughout the CNS prophylaxis phase.
3. No gaps in therapy during induction and between induction and maintenance were permitted.
4. Mercaptopurine and methotrexate were given in maintenance with commencement at full dosage and only reduced by specific aliquots in response to significant and sustained depression of neutrophil or platelet counts.
5. In maintenance, vincristine and prednisolone were always given irrespective of peripheral blood counts.

* Research Grant support is afforded to M.P. Shaw and S. Richards by the Medical Research Council of the United Kingdom.
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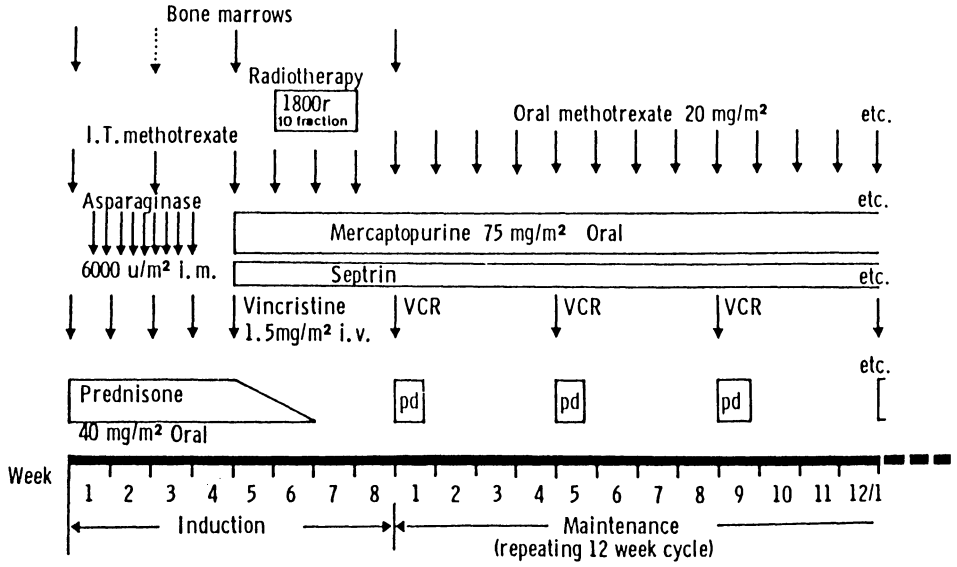

Fig. 1. UKALL VIII Study Protocol. Treatment was similar in the following UKALL VIII Trial except for the randomised addition of two doses of daunorubicin on days 1 and 2

6. Cotrimoxazole was introduced to combat the high initial incidence of interstitial pneumonitis recorded in both the CCG studies and the first 6 months of UKALL VIII.
7. The emphasis was upon strict doctor compliance with the protocols, with limits on the brand forms of drugs used and restrictions on any deviations.

Previous Trials

All analyses are restricted to patients who were randomised in the previous trials, UKALL II–VII inclusive, at notification. These previous regimens were divided into two groups, standard and non-standard, for the purpose of comparison with UKALL VIII. All trials except UKALL II modified and UKALL III intensive included a standard treatment arm with at least 3 weeks of conventional remission/induction with daily steroids and weekly vincristine, short courses of asparaginase, CNS prophylaxis, and continuous or nearly continuous maintenance in which chemotherapy was not interrupted for more than a week and cyclophosphamide was not given. Non-standard regimens are defined as those in which initial induction or maintenance was intermittent or cyclophosphamide was given during maintenance. This division was based on the following considerations:

1. A reduction in the duration of disease-free survival was observed in UKALL II in patients allocated to receive i.v. cyclophosphamide during maintenance ($p = 0.06$ [3]), and in UKALL V in patients allocated to intermittent high-dose maintenance ($p = < 0.05$).
2. In UKALL IV, both intermittent high-dose maintenance which included high-dose cyclophosphamide and the addition of cyclophosphamide and cytosine arabinoside during induction gave inferior results, and disease-free survival of patients who received either or both was significantly worse than those who did not ($p < 0.01$).

For the purpose of this analysis, treatments have therefore been classified as non-standard if they included any of these features. Certain aspects at variance with the standard regime which appear to make no difference in outcome included the effects of cytosine arabinoside and L-asparaginase given in maintenance [4] and some changes in chemotherapy following the induction of remission between weeks 4 and 10. The bias that such post hoc selection may have introduced could exaggerate the overall differences between standard and non-standard

Table 1. MRC trials ALL (1972–84)

Trial	Period	Patients eligible	No.	Standard	Non-Standard
I Modified	1972	All	205	No cyclo phosphamide	Cyclo phosphamide
II Modified	1973	All	89	–	All had cyclo phosphamide
III Ordinary	1973–74	WBC,0–20 × 10^9/litre <14 years	136	–	–
III Modified	1975	WBC,0–20 × 10^9/litre <14 years	110	–	–
III Intensive	1973–74	WBC < 20 × 10^9/litre (all ages)	70	–	Intensive multiple drug induction
IV	1975–78	WBC > 20 × 10^9/litre (all ages)	167	No added drug	+drugs in induction +intensive maintenance
V	1976–79	WBC < 20 × 10^9/litre <14 years	524	Continuous plus gaps maintenance	Intermittent intensive maintenance
VI	1978–80	WBC > 20 × 10^9/litre (all ages)	169	–	–
VII	1979–80	WBC < 20 × 10^9/litre <14 years	83	–	–
VIII Study+ Trial	1980–84	All	829	–	–

WBC, white blood cells.

treatment, but as the results of non-standard treatment were in every trial either similar to, or in most instances worse than, the standard treatment, the bias would always intend to improve the overall results of standard treatment.

The main purpose of this review was to determine whether the results of UKALL VIII were in fact superior to any of the best previous results, and therefore all analyses have been with the standard treatment of earlier trials.

Actuarial survival curves and significant levels were calculated in the usual way [5] and randomised treatment comparisons within trials are based on allocated treatment. All relevant randomisations were allocated at entry, although in several trials the randomisation did not affect treatment until the beginning of maintenance treatment (approximately 12 weeks after entry) and in UKALL II cyclophosphamide was not given until week 22. In UKALL VIII, the late randomisation has not been taken into consideration for this analysis, since any analysis of 2 vs. 3 years' maintenance is premature. The principal details of previous trials and their distinguishing features are shown in Table 1.

Results

Mantel-Haenszel risk ratios and associated significance levels comparing patients allocated to non-standard regimes against patients allocated to standard treatment on the same trial are shown in Table 2. Figure 2 indicates that the only significant difference in trials II–VII was an improvement for patients with an initial white count $< 20 \times 10^9$/litre compared with previous trials ($p < 0.05$).

The induction phase of UKALL II was similar to UKALL VII and the early results on the two trials were comparable, but there were several late relapses even amongst low white count patients where the prognosis was best in UKALL II. We are unable to define at the present time the specific features of UKALL VII which gave the improved survival for low white count patients [6].

Table 2. Disease-free survival of patients (*n*) aged 1–13 years in UKALL II to UKALL VII

Trial	Allocated treatment						Significance levels
	Standard			Non-Standard			
	N	O	E	N	O	E	
UKALL II ordinary	105	59	69.5	100	71	60.5	$P=0.06$
UKALL II[a] modified				89	60		
UKALL III ordinary	136	78					
UKALL III modified	110	63					
UKALL III[a] intensive				70	61		
UKALL IV	38	23	36.8	129	108	94.2	$P<0.01$
UKALL V	348	185	205.0	176	118	98.0	$P<0.05$
UKALL VI	169	112					
UKALL VII	83	32					

Numbers of patients (N) and Mantel-Haenszel observed (O) and expected (E) numbers of first events (relapse or death) are given comparing standard and non-standard treatment.
[a] All patients were allocated to a single regimen in UKALL II modified and UKALL III intensive.

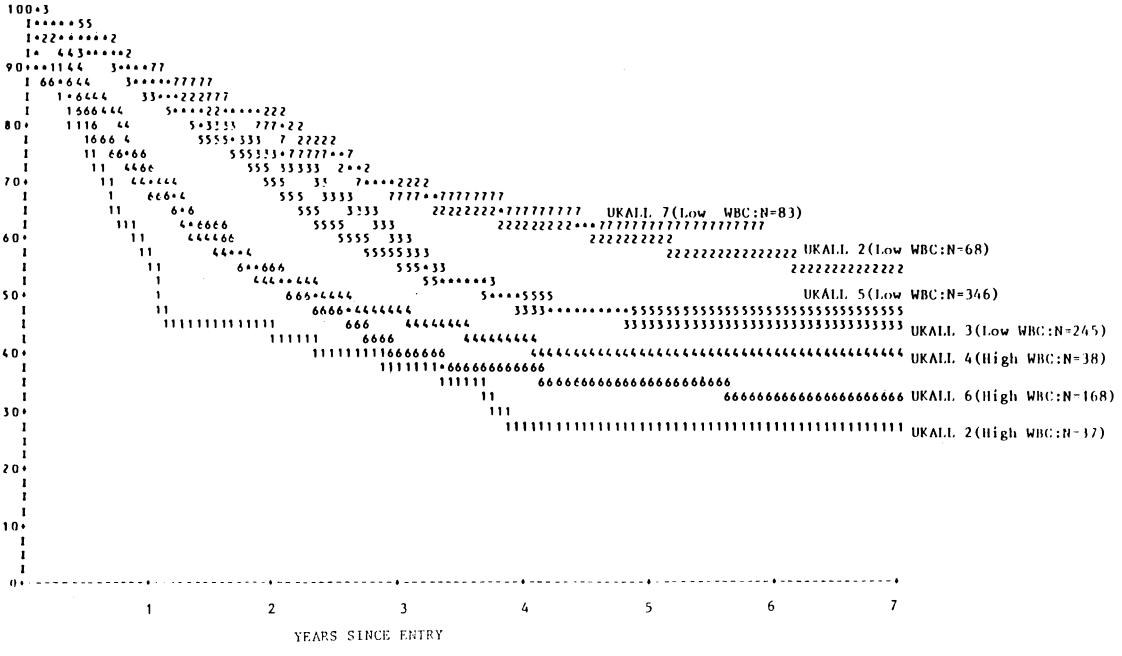

Fig. 2. Disease-free survival in patients aged 1–13 allocated standard treatment by initial WBC (up to 20×10^9/litre or over 20×10^9/litre

In Figs. 3–5, the results in previous trials are compared with those in UKALL VIII for, respectively, those with initial white counts of <20, 20–50 and $>50\times10^9$/litre. The relapse rate has been consistently lower in UKALL VIII during the first 4 years than in any previous trials and the disease-free survival at 4 years is 15%–20% higher in each of these white cell count ranges, compared with the best previous standard treatments. Even in UKALL VII, which achieved better results than any previous trial, disease-free survival was lower than in UKALL VIII. Clearly, longer follow-up will be re-

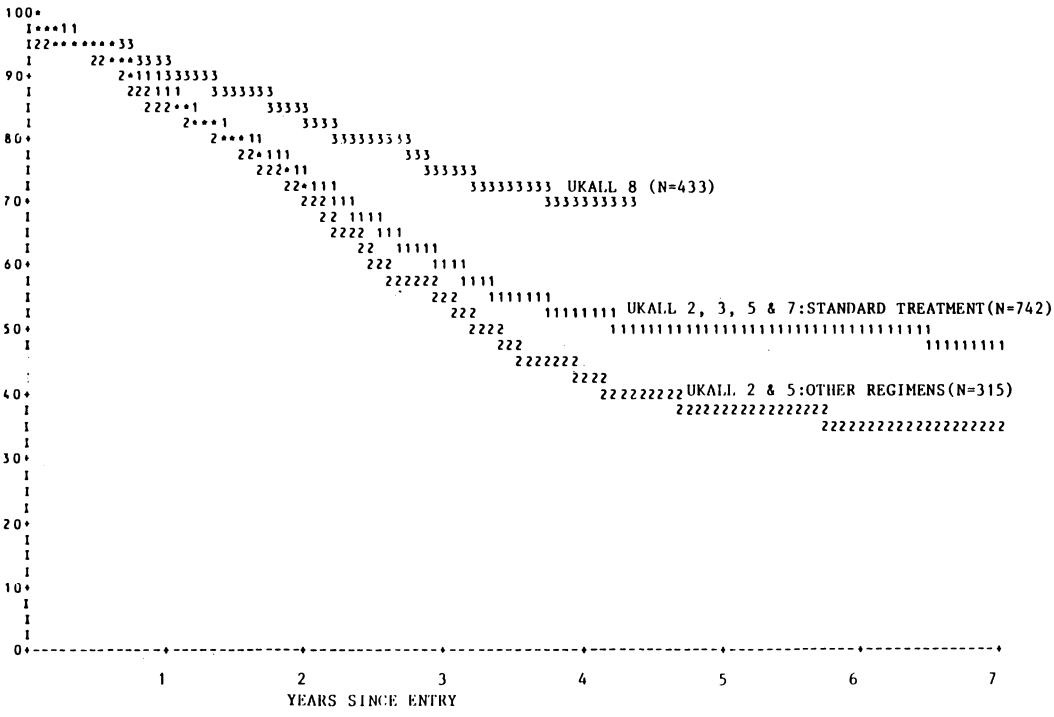

Fig. 3. Disease-free survival in UKALL II–VII compared with patients in UKALL VIII combined for WBC of up to 20×10^9/litre

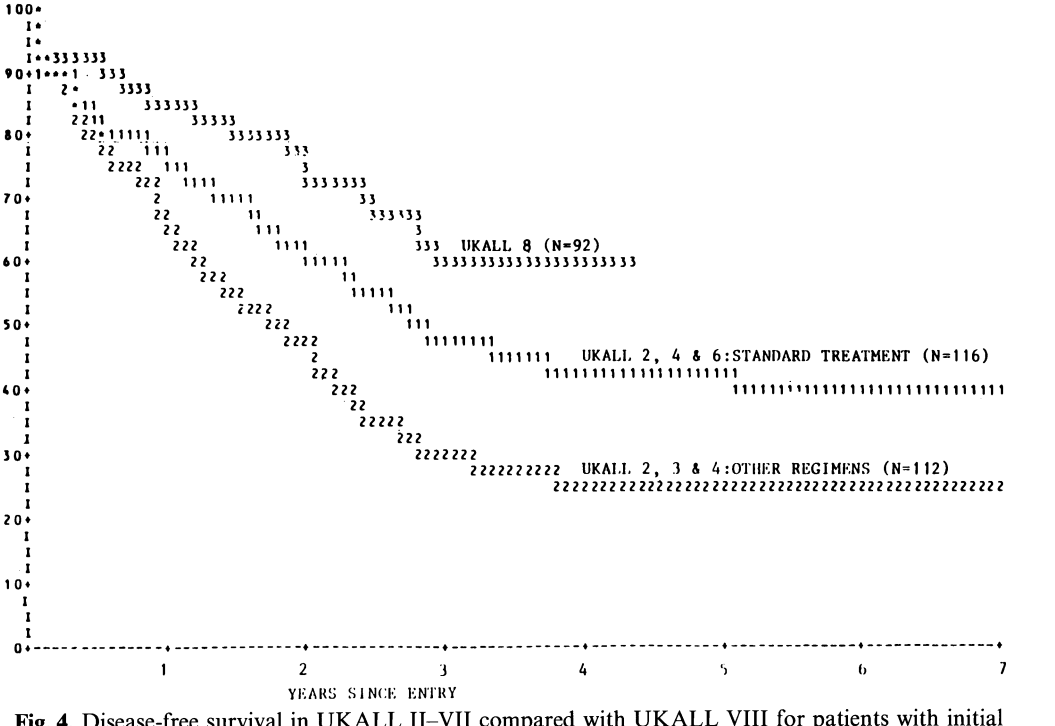

Fig. 4. Disease-free survival in UKALL II–VII compared with UKALL VIII for patients with initial WBC of between 20×10^9 and 50×10^9/litre

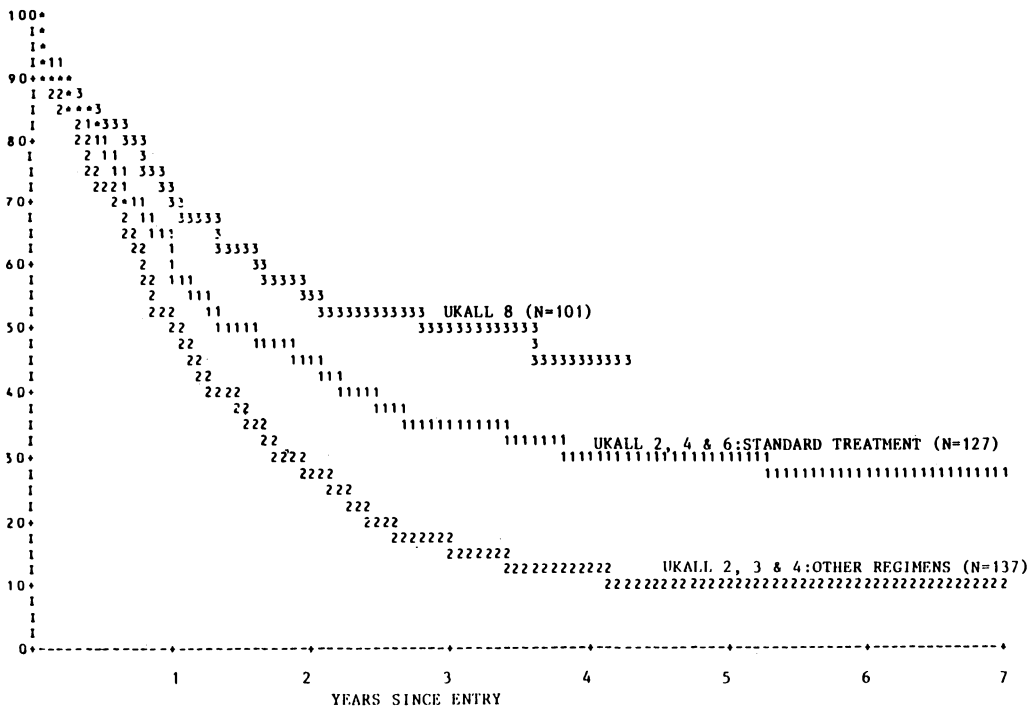

Fig. 5. Comparison of disease-free survival in UKALL II–VII with UKALL VIII for patients with initial WBC of greater than 50×10^9/litre

quired before long-term disease-free survival rates can be predicted accurately, but if as before the relapse rate after 4 years is as low as in previous trials, it would appear that, even for those patients with an initial white count exceeding 50×10^9/litre, long-term remission may approximate to 50% and be at or around 65% for those with a white count of $< 20 \times 10^9$/litre.

Morbidity

The more sustained nature of the induction programme with no interruptions was associated with initial increased morbidity and mortality. Certain initial problems were defined:

Induction Deaths. In the UKALL study, there were ten induction deaths, three from gastro-intestinal haemorrhage, two from septicaemia complicated by haemorrhage, one from convulsions with intracranial haemorrhage, one from septicaemia with liver dysfunction, one from measles pneumonitis and two from fungaemia. These ten deaths all occurred in the first 6 months of the study, and in the second 6 months, owing to increased vigilance, more rapid detection of infection and intervention led to no deaths during the period. There was also an increasing conversion to the use of *Erwinia* asparaginase (see below).

In the UKALL VIII trial arm A, the induction mortality had been reduced from 5% to 1.6%, but in arm B, which included the 2 days of daunomycin, the rate was 6.5%, the risk being principally one of a much increased septicaemic incidence (in arm A, 13.4% of entrants had septicaemia during the first 4 weeks of induction, compared with 31.7% of arm B). The sepsis in arm B was more frequently associated with haemorrhage as a result of more protracted myelosuppression. This detrimental effect of daunomycin was balanced by the reported relapse rate of 15.3% in arm A to date, compared with only 9.8% in arm B. Awareness of the very high risk of sepsis and aggressive treatment thereof would clearly reduce this morbidity and mortality. Much of the hae-

Table 3. Comparison of toxicity possibly associated with Asparaginase *Erwinia* v. *Escherichia coli*

Toxicity	*Escherichia coli* ($n=275$)		*Erwinia* ($n=483$)	
	(%)	(*n*)	(%)	(*n*)
Neurological (coma ± seizures)	4.4	(12)	2.1	(10)
Bleeding	5.5	(15)	3.3	(16)
Pancreatitis	1.8	(5)	0	
Diabetes	1.5	(4)	0.2	(1)
Hepatomegaly + +	1.1	(3)	0.6	(3)
Malabsorption (>20% weight loss)	1.5	(4)	1.4	(7)
Anaphylaxis	0.4	(1)	0.2	(1)
Overall incidence of severe toxicity	16	(44)	7.9	(38)
Deaths from above	3.6	(10)	1.9	(9)

morrhagic problem will be discussed further.

Pneumonitis. In the UKALL VIII study, 35 out of the 199 entrants (17.6%) developed interstitial pneumonitis within the first 44 weeks of treatment (median onset 10 weeks), and 20% died. High-dose trimethoprimsulphamathoxazole was successful in treating 26 out of 33 cases (78%). An improvement in survival was noted in our series in those children given steroids. The introduction of prophylactic cotrimoxazole has removed this early problem, but cases have occurred later on when cotrimoxazole has been stopped. Many centres now advocate continuation of this drug in low dosage throughout the period of maintenance. One death occurred 7 months after cessation of all chemotherapy, including cotrimoxazole, from pneumocystis pneumonitis. International collaboration with CCG co-ordinators and increasing national co-operation in communication of individual centre problems enabled quicker identification and response to this specific problem.

Acute Encephalopathy During Induction. Of the 829 patients analysed, 21 suffered acute neurological crises during induction, the majority occurring between weeks 3 and 4 of induction. Eighteen of the patients had convulsions (12 multiple convulsions), and nine went into coma for periods of from a few days up to 6 weeks, with one patient never regaining consciousness. Two patients died, and two subsequently developed hydrocephalus, but the remaining patients all recovered. The evidence is difficult to decipher fully, but a major causative role for asparaginase seems likely in view of the timing of the problems and the full recovery occurring in the majority. Clearly, asparaginase has a number of effects upon the CNS, including depletion of L-asparagine and L-glutamine, which is undetectable in the CNS even after a single intramuscular injection of asparaginase for 5–7 days. Intracranial haemorrhage, with or without thrombosis, and associated with complex involvement of the coagulation and fibrinolytic systems resulting in prolonged partial thromboplastin and prothrombin times, decreased fibrinogen and antithrombin III levels, and elevated ammonia levels. The incidence of CNS complications was lower with *Erwinia* than with *Escherichia coli* asparaginase (see below) in terms of overall CNS problems. Despite even prolonged coma, if supported and if the patient was continued on ALL therapy (except for the asparaginase), recovery was complete in the majority of cases, and the overall leukaemic prognosis was good.

Major Toxicity Possibly Related to Asparaginase. Escherichia coli asparaginase was initially used for the trial in line with the CCG 162 protocol, but as supplies to the United Kingdom ceased, a non-randomised sequential conversion to *Erwinia* asparaginase became necessary. In this study, a consistently lower incidence of toxicity was encountered when *Erwinia* rather than *Escherichia coli* asparaginase was used, with in particular fewer neurological problems and no pancreatitis. In Table 3, the comparison of the two forms of asparaginase is outlined. In all instances, the incidence of toxicity relatable to asparaginase is lower with the *Erwinia* product. The most marked feature is the very low incidence of anaphylaxis with intramuscular administration. It has been known for some time that it is more difficult to produce a pure product with *Escherichia coli* than it is with *Erwinia* because of the nature of the enzymes and their method of separation. For much of the toxicity, it is thought

that the common denominator is upon the liver, the hepato-toxicity resulting in the disturbance of the coagulation and fibrinolytic factors. It has been suggested that glutaminase-free asparaginase may reduce this hepato-toxicity, and research is continuing with regard to this. All patients given prolonged courses of asparaginase may indeed have disturbance of their coagulation system, but this is only a major problem if they first have significant and prolonged thrombocytopenia and septicaemia. Careful observation and awareness of this problem may reduce its effect on morbidity and mortality.

Summary

This improvement in medium disease-free survival is probably a result of sustained early cell kill, and UKALL VIII has enabled us to define risk categories requiring even further continuous intensification, as now introduced in MRC UKALL X.

Thanks to the greater availability of blood products, for example, the rational use of antibiotics and the development of expertise amongst nurses and doctors, such sustained therapy can now be delivered on a multi-centre basis, but only in experienced centres. The monitoring and removal of morbidity are essential if the advantages of this more sustained chemotherapy are to be realised. All elements of therapy require controlling and patients, parents and, above all, doctors must comply with protocol requirements in order to build further upon these initial promising results.

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Early Intensification Therapy in High-Risk Childhood Acute Lymphocytic Leukemia: Lack of Benefit from High-Dose Methotrexate *

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The prognosis for acute lymphoblastic leukemia (ALL) in childhood has improved considerably in the last two decades. Intensive chemotherapy soon after diagnosis has made a major contribution to this success [3, 12, 14], especially in patients with adverse prognostic factors [6]. In COALL-80, a cooperative West German treatment study for ALL, high-risk patients received intensive combination chemotherapy after a modification of the West Berlin protocol BFM 79/81 [7]. In the subsequent study, COALL-82, high-dose methotrexate was added to the intensive phase regimen. The results of both studies, which included 95 high-risk patients, are reported in this paper.

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Patients and Methods

High risk was defined as an initial leukocyte count >25/nl. In COALL-82, patients with T-cell leukemia and acute undifferentiated leukemia with leukocytes below 25/nl were also entered into the high-risk protocol but were excluded from the present analysis.

COALL-80. The therapy regimen is shown in Figs. 1 and 2. Reinduction therapy was given 4 weeks after the intensive phase. Maintenance treatment consisted of daily 6-mercaptopurine per os and weekly methotrexate

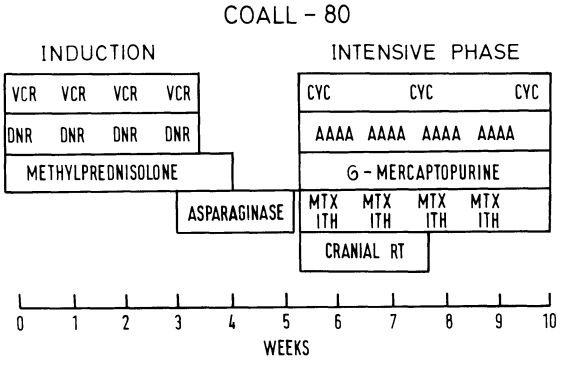

Fig. 1. Treatment protocol COALL-80. Drug doses: Methylprednisolone 2.0 mg/kg p.o.; *VCR* (vincristine) 0.06 mg/kg i.v.; *DNR* (daunorubicin) 1.0 mg/kg i.v.; asparaginase 200 U/kg i.v. on 14 successive days in induction and 400 U/kg i.v. twice weekly ×4 in reinduction; *CYC* (cyclophosphamide) 40 mg/kg i.v.; *A* (cytarabine) 3 mg/kg i.v.; 6-mercaptopurine 2.4 mg/kg p.o.; *MTX ITH* (methotrexate intrathecally) in age-adjusted dose [1]; cranial RT (cranial radiotherapy) with 24 Gy; ADR (adriamycin) 1.0 mg/kg i.v.; 6-thioguanine 2.4 mg/kg p.o.

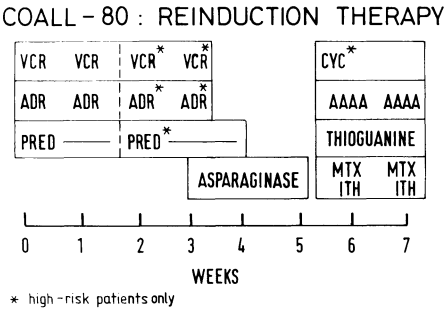

Fig. 2. COALL-80 reinduction therapy. For treatment protocol, see Fig. 1

per os. Patients were randomized for reinforcement therapy ("pulses") with vincristine or medium-dose methotrexate during the 1st year of maintenance treatment [9]. Therapy was electively discontinued after 24 months. From November 1978 to November 1982, 39 high-risk patients were entered into the study. The initial characteristics of the patients are shown in Table 1.

COALL-82. The therapy regimen is shown in Figs. 3 and 4. Reinduction therapy was started after an interval of 3 months, during which the patients received 6-mercaptopurine and methotrexate per os. Cranial irradiation (24 Gy) was given at the beginning of this interval. Maintenance treatment and total duration of therapy were the same as in COALL-80. Randomization for vincristine or methotrexate reinforcement therapy was stopped in November 1983 (eight patients still received two to three pulses) because it was considered to be ineffective on the basis of the analysis of COALL-80 data [9]. From January 1982 to April 1985, 56 high-risk patients were entered into the study. The initial characteristics of the patients are shown in Table 1.

Table 1. Patient characteristics at diagnosis in the reduced groups (total number of patients less remission failures and deaths in remission)

	COALL-80		COALL-82	
Patients (*n*)	36		52	
	(*n*)	(%)	(*n*)	(%)
Median age (years)	4.3		4.8	
age <2 or >10	10	(28)	19	(36)
Male sex	26	72)	33	(63)
Leukocytes > 50/nl	24	(66)	31	(60)
>100/nl	12	(33)	15	(29)
Mediastinal mass	2	(6)	7	(13)
CNS involvement	3	(8)	3	(6)
Liver/spleen > 5 cm	10	(28)	13	(25)
Immunological subtype				
Common ALL	12	(57)[a]	29	(69)[b]
T-ALL	6	(43)[a]	12	(31)[b]
AUL	3		1	

[a] Percentage of 21 patients tested.
[b] Percentage of 42 patients tested.
AUL, acute undifferentiated leukemia.

The comparison of prognostic factors within the two study groups shows no appre-

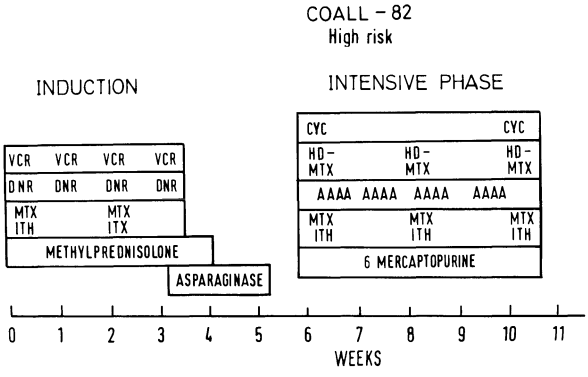

Fig. 3. Treatment protocol COALL-82. Drug doses are the same as in COALL-80 except: *DNR* 1.2 mg/kg i.v.; asparaginase 3000 U/kg i.v. twice weekly ×4; *CYC* 30 mg/kg i.v.; *HD-MTX* (high-dose methotraxate) 100 mg/kg as 4-h infusion, followed 24 h later by leucovorin 0.05 mg/kg p.o. q 6 h × 10; *ADR* 1.2 mg/kg i.v.; *VM* (VM 26, teniposide) 5.5 mg/kg i.v.; *A* 3 mg/kg i.v. in intensive phase, 10 mg/kg i.v. in reinduction

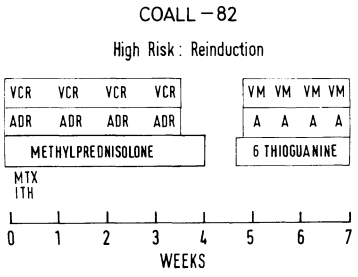

Fig. 4. COALL-82 high-risk reinduction therapy. For treatment protocol, see Fig. 3

ciable differences in sex, age, hepatosplenomegaly, CNS involvement, and range of leukoyte counts (Table 1). Unfavorable immunologic subtypes (T-ALL, AUL) were slightly more frequent in COALL-80, whereas a higher proportion of the patients had a mediastinal mass in COALL-82.

Results

As of September 1985, 8 of 36 high-risk patients in COALL-80 and 18 of 52 high-risk patients in COALL-82 had suffered a relapse (Table 2). The probability of continuous complete remission is 73% after 6 years for COALL-80 (median observation time, 47 months) and 44% after 3.5 years for COALL-82 (median observation time, 23 months) (Fig. 5). This difference is statistically significant ($p < 0.05$). The poorer results in the COALL-82 study were mostly due to an increased relapse rate in the bone marrow (Table 2). There were also more

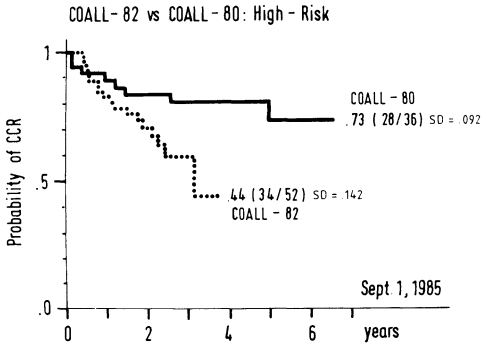

Fig. 5. Probability of continuous complete remission (CCR) in the reduced groups by the life-table method

Table 2. Clinical results

	COALL-80	COALL-82
Patients (*n*)	39	56
Remission failure (*n*)	1	1
Death in remission (*n*)	2	3
Total relapses	8	18
Bone marrow	5[a]	11[b]
CNS	2	6
Testes	1	1
Common ALL	4	12
T-ALL	2	5
Leukocytes > 50/nl	5	14
In continuous complete remission	28/39	34/56

[a] 2 combined relapses.
[b] 1 combined relapses.

CNS relapses (17% VS 7%), but this difference is not statistically significant.

Of the eight patients who still received reinforcement therapy during the 1st year of maintenance therapy, three suffered a relapse.

High-dose methotrexate infusions were well tolerated by all patients except two: one developed renal failure requiring peritoneal dialysis and one showed hepatotoxicity with a peak plasma concentration for bilirubin of 15 mg% and for serum glutamic pyruvic transaminase of 1345 U/liter. Both patients recovered and did not receive further high-dose methotrexate. Methotrexate peak plasma levels, measured in several patients, reached values of between 100 and 300 µmol/liter, but fell below 1 µmol/liter within 12–24 after the end of the infusion.

Discussion

Intensive chemotherapy soon after diagnosis, as given in the BFM studies, resulted in a high relapse-free survival rate for patients with risk factors that was not inferior to that of low-risk patients (6, 7). Accordingly, in COALL-80, which was modified after BFM 79/81, patients with an initial white blood count of > 25/nl had the same good prognosis as low-risk patients [10]. However, in most ALL treatment studies, patients with adverse prognostic factors have a higher re-

lapse rate, even with more aggressive chemotherapy [3, 4, 11, 15]. The definition of risk factors varies from study to study, but a high white blood count at diagnosis is universally accepted as a poor prognostic parameter. The excellent results of study COALL-80 were thus regarded with reservation, and it was decided to provide further intensification of therapy in the subsequent study, COALL-82. As a major modification, three infusions of methotrexate at a dose of 100 mg/kg over 4 h were given additionally in the intensive phase. Methotrexate peak plasma levels of above 100 μmol/liter were reached. At this concentration, methotrexate enters the cell not only by active transport but also by passive diffusion [16], and enzymes other than dihydrofolate reductase are inhibited [2]. A 20% increase in the dose of anthracyclines and the replacement of cyclophosphamide/cytarabine by VM 26/cytarabine, a combination highly effective in ALL [13], were also considered to be more effective chemotherapy.

Why, then were the results of COALL-82 significantly worse than those of COALL-80? Since no appreciable differences in the initial prognostic factors were evident within the two studies (Table 1), a detailed analysis of the actual cumulative drug doses and treatment times was made. Patients with and without relapses showed no significant differences (Student's t test); thus, only the figures for the total group are given (Table 3). Apparently, the dose of the anthracyclines and antimetabolites cannot have been responsible for the poorer results of COALL-82. Furthermore, the time to complete treatment phases was not much different in both studies. The interval between the intensive phase and reinduction was longer in COALL-82, but the results of BFM 79/81 show that a delay of 1 as opposed to 3 months does not influence the prognosis [7]. Moreover, six relapses occurred shortly after the intensive phase, a fact which points to ineffective initial chemotherapy.

Only two doses of cyclophosphamide were given in COALL-82 because 21 of 36 patients in COALL-80 had not been able to tolerate three doses, owing to severe bone marrow toxicity. The difference between the mean cumulative doses of cyclophosphamide and also between the other drug doses in both studies (Table 3) is not statistically significant (Student's t test). This result has to be interpreted with caution, however, since the individual drugs are not independent of each other. Nevertheless, cyclophosphamide is not considered to be a drug of first choice in ALL and it seems doubtful that a reduction in its dose has an influence on a higher relapse rate. In COALL-82, asparaginase was omitted from reinduction therapy and was given in higher doses and at longer intervals in induction therapy. Asparaginase plays an important role in the treatment of ALL and has been shown to improve relapse-free survival rates when given during maintenance treatment [15]. It is as effective in a daily as in a twice-weekly dose [8]. Five patients in COALL-82 had received asparaginase in reinduction according to the COALL-80 protocol, and two of them relapsed. Its omission from the regimens of the other patients may still have been a factor in the higher relapse rate.

Table 3. Cumulative drug doses and treatment times

	COALL-80	COALL-82
	(n)	
Mean cumulative drug dose (mg)		
Anthracyclines	7.9	9.4
6-Mercaptopurine	52.0	59.4
6-Thioguanine	23.0	34.4
Cytarabine	61.4	74.7
Cyclophosphamide	115.2	57.9
Asparaginase		
Induction	200 U/kg × 14 days	3000 U/kg twice weekly × 4
Reinduction	400 U/kg twice weekly × 4	
High-dose methotrexate		3 doses
VM-26		4 doses
Mean treatment time (days)		
Induction phase	43.0	42.6
Intensive phase	39.2	43.1
Reinduction phase	56.5	64.3

Could high-dose methotrexate/leucovorin therapy have been a disadvantage in COALL-82? The 4-h methotrexate infusion was followed by prolonged leucovorin rescue 24 h later according to the treatment used in osteogenic sarcoma. In lymphoblastoid cells, which in contrast to osteogenic sarcoma cells have an active transport system for methotrexate and hence also folates [16], this treatment could have resulted in neutralization of the antileukemic effect of methotrexate. Furthermore, it has to be discussed whether the ample doses of folate, which is a growth factor for leukemic cells [5], may even have had a detrimental influence on the prognosis of our high-risk patients. Finally, differences in the distribution of chromosomal abnormalities, an important prognostic factor not examined in our patients, have to be considered.

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Intermediate-Risk Childhood Acute Lymphoblastic Leukemias: Amsacrine + Cytosine Arabinoside Versus Intermediate-Dose Methotrexate for Consolidation, and 6-Mercaptopurine + Methotrexate + Vincristine Versus Monthly Pulses for Maintenance

G. Schaison, G. Leverger, A. Bancillon, M. Marty, D. Olive, G. Cornu, C. Griscelli, S. Lemerle, J.L. Harousseau, M. Bonnet, F. Freycon, D. Dufillot, M. Demeocq, F. Bauters, J.P. Lamagnere, and O. Taboureau[1]

The prognosis for childhood acute lymphoblastic leukemia improved dramatically in the late 1970s, with 50%–70% of long-term survivors being reported following multiagent chemotherapy regimens. Unfortunately, the survival rate is still poor for relapsing patients.

It has been shown that in leukemias with a good prognosis, it is possible to decrease the treatment and avoid skull irradiation [1]. The usefulness of early consolidation with intermediate-dose methotrexate (ID MTX) has been demonstrated by Moe [2] and, with a multiagent regimen, by Riehm [3]. In relapsing patients previously heavily treated combination of amsacrine (AMSA) and cytosine arabinoside (Ara-C) [4] had been reported as resulting in 60% complete remission; we therefore tried this combination as a powerful consolidation. We have previously reported the results of the very increased risk childhood acute lymphoblastic leukemia (VIRCALL) protocol [5] for very high-risk patients [mediastinal mass and/or white blood count (WBC) over 100000/mm^3]: With an aggressive induction including five drugs and high-dose daunorubicine (DNR), complete remission (CR) is achieved in 90% of cases within a 21-day aplastic phase. After CR, there was no permanent maintenance but heavy monthly pulses with asparaginase (Aase), cytosine arabinoside (Ara-C), Cytoxan (CTX) and/or vincristine (VCR) or DNR. Permanent maintenance was started after 6 months. By Kaplan-Meier analysis, the disease-free survival curve is in a plateau at 69%. The plateau is reached at 26 months. On the other hand, good results have been demonstrated in average-risk patients with continuous chemotherapy.

According to these preliminary results, a new regimen was designed in a French multicenter cooperative study in 1983. Intermediate-risk patients were treated with a five-drug induction and then randomized for consolidation and maintenance. The aims were to compare two types of consolidation – ID MTX versus AMSA and ARA-C – and two types of maintenance – permanent maintenance with 6-mercaptopurine (6-MP) and MTX with VCR-prednisone (P) pulses, as for low-risk patients, versus aggressive monthly pulses, as for high-risk patients. The therapeutic challenge in intermediate-risk patients was to increase survival by using a protocol known to give good results in high-risk patients.

Materials and Methods

Three groups of patients were defined: in group I (good-risk patients) the criteria are: age, 24–120 months; WBC of fewer than 15000/mm^3; Hb of less than 10 g/dl; no large involvement of spleen or lymph nodes;

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no thymic mass; and no CNS or testis involvement. All these criteria must be met simultaneously. In group III (very high-risk patients), only one of the following criteria must be met: WBC of over 100000/mm^3, mediastinal mass, or initial CNS involvement. Group II (intermediate-risk patients) comprises all the patients not included in groups I or III, children younger than 24 months or over 10 years of age, and failures at 15 or 29 days in group I. Cytogenetics and immune markers were not included in this multicentric study.

Protocol. Induction therapy used one course of P (100 mg/m^2, days 1–5), DNR (80 mg/m^2, days 2–4), VCR (2 mg/m^2, day 1), CTX (600 mg/m^2, day 2), and Aase (1000 U/kg, days 7–16). Urate oxydase, intravenous hydratation, and alkalinization were instituted prior to beginning chemotherapy in an attempt to prevent the development of metabolic imbalance secondary to rapid tumor lysis. Patients not in CR at day 16 received supplementary treatment. Children who failed to achieve CR following the completion of induction chemotherapy were considered to be treatment failures.

Consolidation was started at day 25 and randomized in two arms. Arm A comprised two courses of AMSA+Ara-C: AMSA 75 mg/m^2 (day 1), Ara-C 1 g/m^2 push followed by 50 mg/m^2 subcutaneously every 12 h × 9. The second course was scheduled after 20 days. Arm B comprised four courses of ID MTX 500 mg/m^2 administered as one-third push and two-thirds in a 4-h infusion followed by folinic acid rescue every 10 days.

Maintenance was then randomized in two arms. Arm alpha consisted 6-MP and MTX for 3 years, with monthly pulses of VCR+P the first year and every 3 months the second year; arm beta called for alternative monthly pulses always including Ara-C 50 mg/m^2 twice a day for 5 days (1–5), Aase 1000 U/kg on days 6–10, and/or VCR+CTX, or VM 26, or DNR+CTX. DNR was omitted after 6 months.

All patients received CNS prophylaxis with skull irradiation (1800 rads) and 12 doses intrathecal MTX (six during the first 2

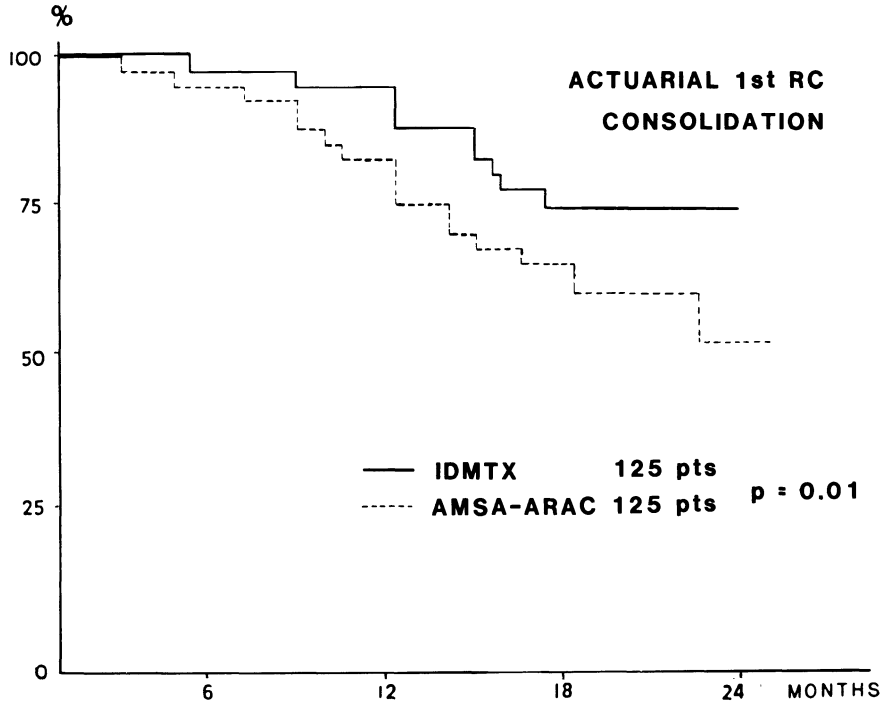

Fig. 1. Actuarial first remission curve. Consolidation: ID MTX (——) versus AMSA+Ara-C (– – –)

months and six during the first year of maintenance).

Results

Out of 271 patients, 250 (92%) achieved CR within 24 days. Another 21 patients were nonresponders or died in the aplastic phase. Causes of death were infections or bleedings. Consolidation B with ID MTX was well tolerated, but the incidence of hematologic toxicity was high in consolidation A with AMSA + Ara-C, and in 67% of cases the second course was postponed or reduced. There are 125 patients in each arm of consolidation. Actuarial first CR at 30 months is 52% in arm A and 75% in arm B ($p = 0.01$) (Fig. 1). A total of 216 patients were randomized for remission. Actuarial first at 30 months is 73% in arm alpha and 52% in arm beta ($p = 0.001$) (Fig. 2). Within the consolidation group, remission duration was studied according to maintenance. With AMSA + Ara-C consolidation, actuarial first CR is at 65% with maintenance alpha and 37% with maintenance beta ($p = 0.001$) (Fig. 3). With ID MTX consolidation (arm B), actuarial first CR is at 87% with maintenance alpha and 65% with maintenance beta (not significant) (Fig. 4). There were no cases of CNS or testis isolated relapse.

Discussion

We have reported here the preliminary results, at 2.5 years, of a multicenter trial in intermediate-risk children with acute lymphoblastic leukemia. The data on initial response with intensive induction regimen compare favorably with the results of less intensive induction from other multicenter studies. The rate of responders achieving complete remission was 92%. When considering the two types of consolidation, it appears that ID MTX is less toxic and more effective than the combination of AMSA and ARA-C. These daxa indirectly confirm

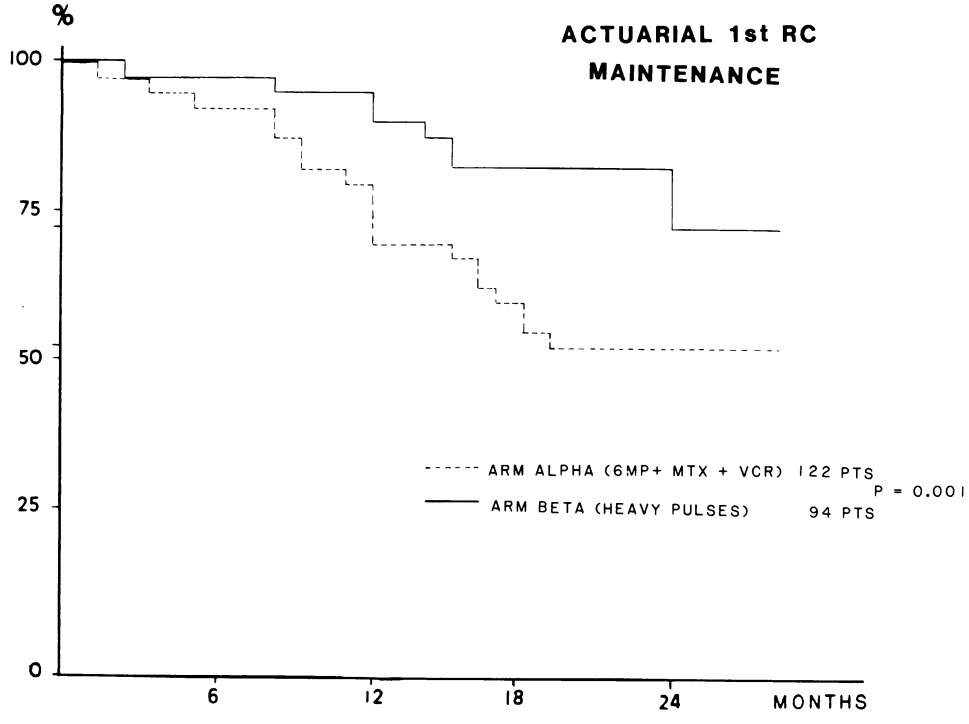

Fig. 2. Actuarial first remission curve. Maintenance: 6-MP + MTX and VCR pulses (– – –) versus heavy pulses alone (——)

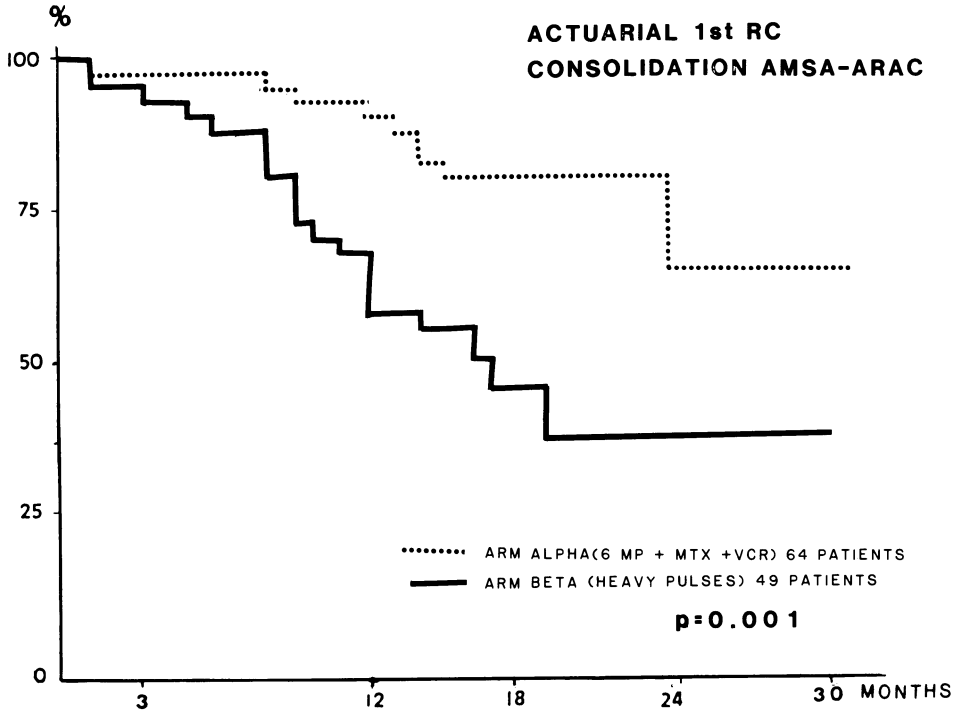

Fig. 3. Actuarial first remission curve. Consolidation: AMSA + Ara-C. Comparison of maintenance: 6-MP + MTX and VCR pulses (...) versus heavy pulses alone (—)

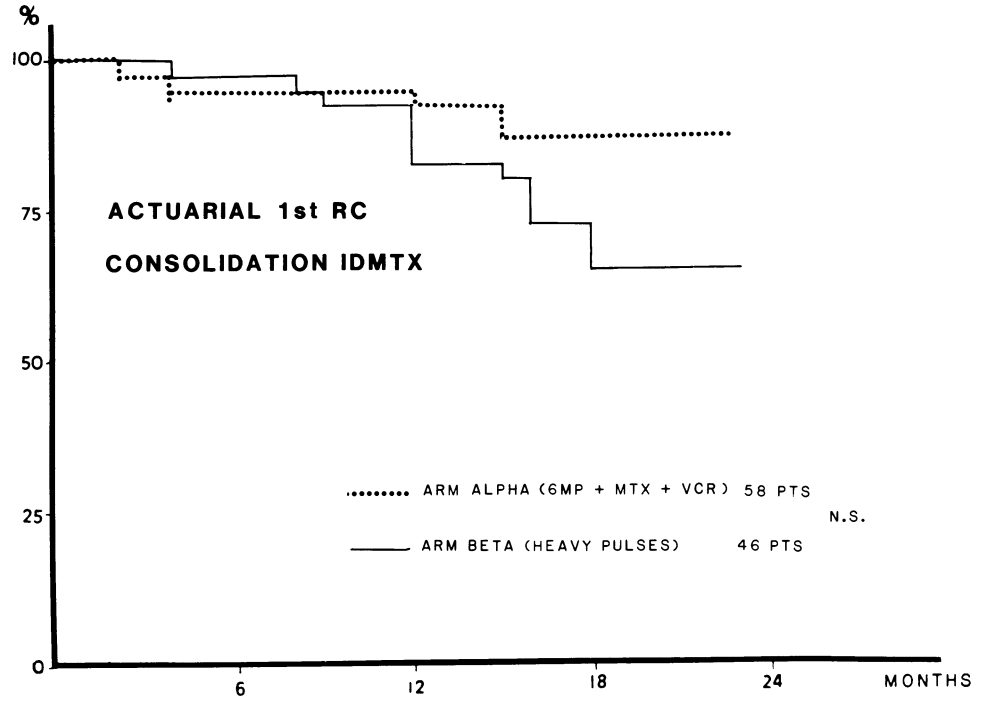

Fig. 4. Actuarial first remission curve. Consolidation: IDMTX. Comparison of maintenance: 6-MP + MTX and VCR (...) pulses versus heavy pulses alone (—)

the usefulness of consolidation in average-risk patients. When considering the two types of maintenance, it is clear that continuous maintenance chemotherapy is superior to intermittent treatment; intermittent monthly pulses of good efficacy in high-risk patients are less effective in intermediate-risk patients. The results of the best maintenance, i.e., permanent treatment, are enhanced by the best consolidation and lessened by the worst.

A five-drug induction with high-dose DNR, followed by ID MTX and 6-MP, and MTX maintenance with monthly VCR pulses seems to be one the best treatments for intermediate-risk patients, with 87% of patients in CR at 2.5 years. These results are higher than those of Moe et al. [2] with similar consolidation and maintenance. The better results of the present protocol can be explained by the more aggressive induction chemotherapy.

These preliminary results have to be confirmed by a longer follow-up and explained by a better knowledge of leukemic cells' kinetics.

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Treatment of Acute Lymphoblastic Leukemia in Children with the BFM Protocol: A Cooperative Study and Analysis of Prognostic Factors

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In 1981, 13 French and Belgian institutions started a clinical study using the German BFM protocol for the treatment of children with acute lymphoblastic leukemia (ALL). The objectives of the study were (a) to evaluate the feasibility and toxicity of this aggressive regimen, (b) to reevaluate the weight of the prognostic factors (number of circulating blasts, hepatomegaly, and splenomegaly) used for the classification of patients into different risk categories, and (c) to identify other possible prognostic features.

Materials and Methods

Patients under 16 years of age with newly diagnosed ALL were treated according to the 1981–1983 version of the BFM protocol (Riehm, personal communication) with some adaptations. They were classified according to the risk factor (RF) as defined previously [1]: $RF = 0.2 \times \log_{10}(1 + \text{blasts}) + 0.06$ hepatomegaly $+ 0.04$ splenomegaly (splenomegaly and hepatomegaly expressed in cm under the rib border). Patients with

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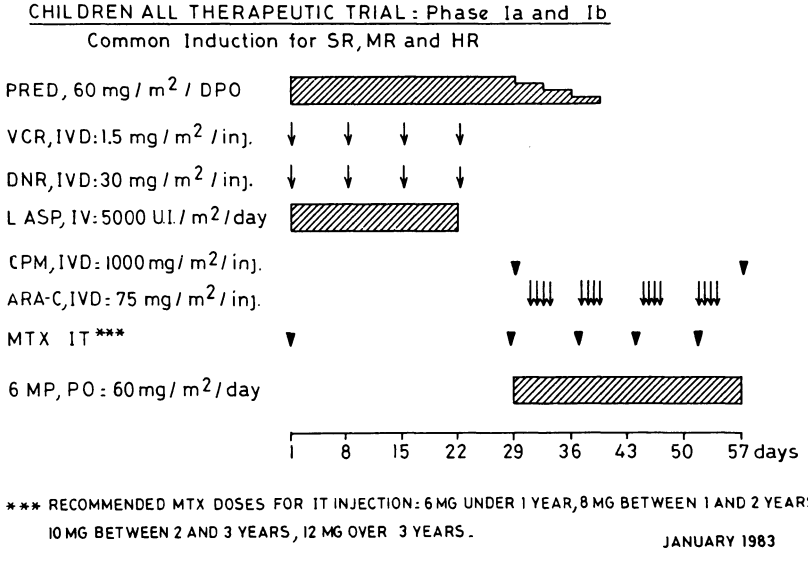

Fig. 1. Induction course for all patients. *PRED*, prednisone; *VCR*, vincristin; *DNR*, daunorubicin

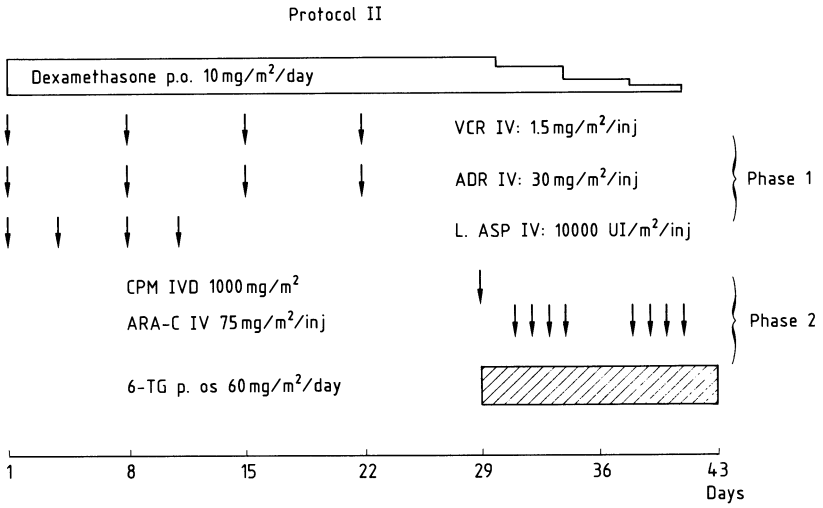

Fig. 2. Reinduction course for MR + HR patients. *ADR*, adriamycin

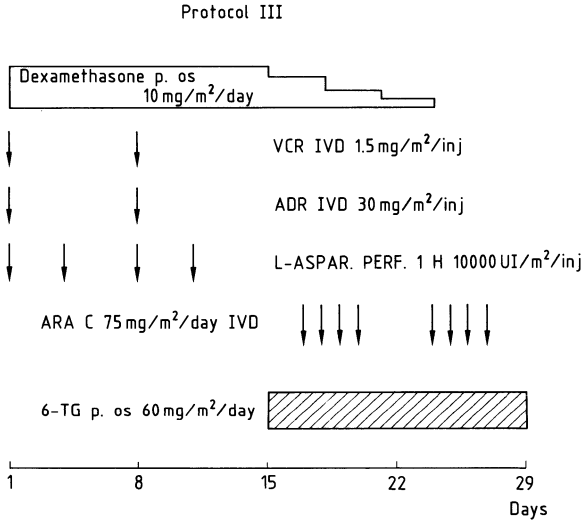

Fig. 3. Reinduction course for SR patients

RF < 1.2 were considered as standard-risk (SR), those with RF ≧ 1.2 and < 1.7 as medium-risk (MR), and those with RF ≧ 1.7 as high-risk patients (HR), respectively. MR and HR patients received identical treatment. Induction of 8 weeks' duration was the same for all patients (Fig. 1). It was followed by a maintenance type of treatment [6-mercaptopurine (6-MP) and methotrexate (MTX)] during 8 weeks therapy and afterward by reinduction of 4 weeks' duration for SR patients (Fig. 3) or of 6 weeks' duration for MR and HR patients (Fig. 2). Subsequent maintenance therapy with 6-MP (50 mg/m² daily) and MTX (20 mg/m² weekly) was stopped 2 years after diagnosis. Fourteen SR patients received i.v. MTX (500 mg/m²) simultaneously with i.t. MTX during the interval phase and no radiation to the brain. For all the others, radiotherapy to the cranium (18 Gy in SR patients and 24 Gy in MR and HR patients) and i.t. MTX were given for prophylaxis of relapses in the central nervous system (CNS).

Results

From January 1981 until July 1983, 141 children entered the study. Complete remission (CR) was induced in 133 patients (94%). Three patients failed to achieve CR, and five died during induction. Presently, 93 patients remain in first CR. Based on the population of patients who achieved CR, actuarial disease-free survival is 67% at 4 years, with a median follow-up of 3 years (Fig. 4). It is 70% in SR and 65% in MR + HR patients. Twenty-seven patients relapsed: 11 of these involved the bone marrow (8%), and four the CNS (3%), four the gonads (3%), while eight cases involved two or more sites simultaneously (6%). Thirteen patients died in CR. The incidence of death in CR markedly decreased during the course of the study (Table 1). Of the 20 patients registered in 1981, five (25%) died in CR, as opposed to one of 45 registered in 1983 (2%). Factors

Table 1. Deaths in induction phase and in CR according to year of registration

Year of registration	1981	1982	1983
	(*n*)		
Total patients	20	76	45
Deaths in induction	0	4	1
Patients achieving CR	20	71	42
Deaths in CR	5 (25%)	7 (10%)	1 (2%)
Total deaths in induction and in CR	5 (25%)	11 (14%)	2 (4%)

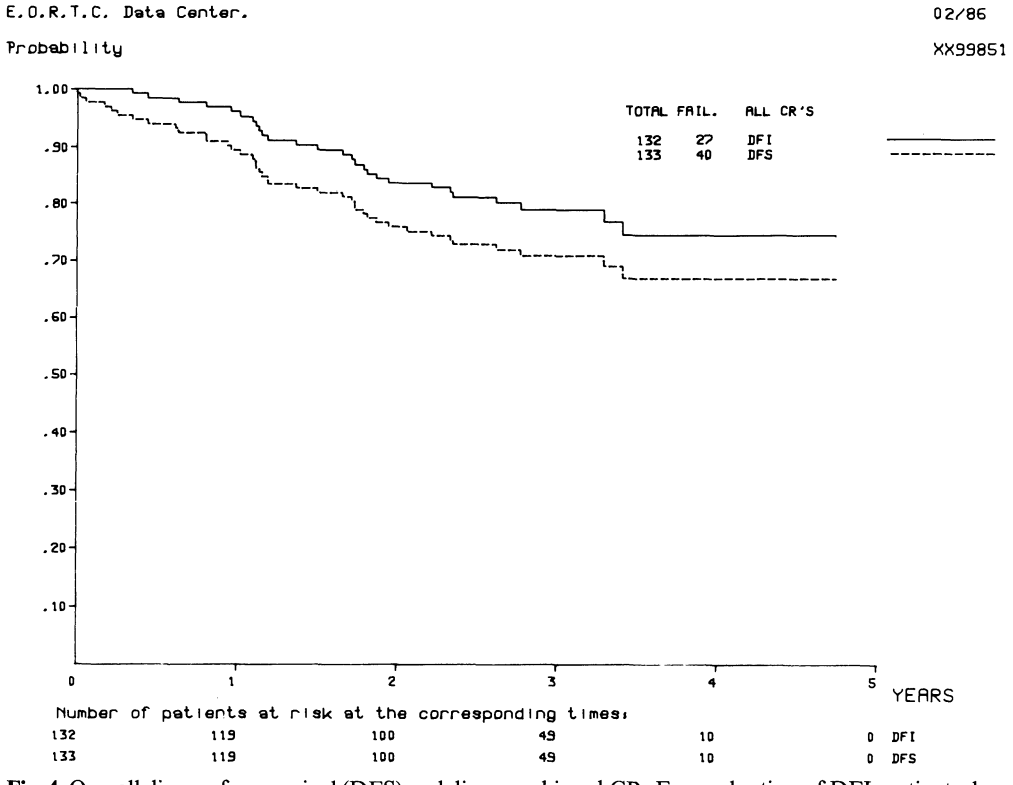

Fig. 4. Overall disease-free survival (DFS) and disease-free interval (DFI) of the 133 patients who achieved CR. For evaluation of DFI, patients dying in CR were censored

Table 2. Factors predictive of longer disease-free interval

Not significant	*p* value	Significant	*p* value
Gender	0.55		
Age	0.09	Splenomegaly $\leqq 5$ cm	0.022
Hepatomegaly $\leqq 5$ cm	0.93		(0.12)[a]
Absence of enlarged mediastinum	0.77	Granulocytes $< 10^9$/liter	0.03
			(0.07)[a]
Non-T versus T-cell type	0.28	Hb $\leqq 8$ g/dl	0.078
Leukocyte count $< 25 \times 10^9$/liter	0.10		(0.04)[a]
Blast count $< 10 \times 10/^9$liter	0.13		
RF < 1.2	0.13	Hb $\leqq 8$ g/dl in MR + HR patients	0.005

[a] Adjusted by RF.

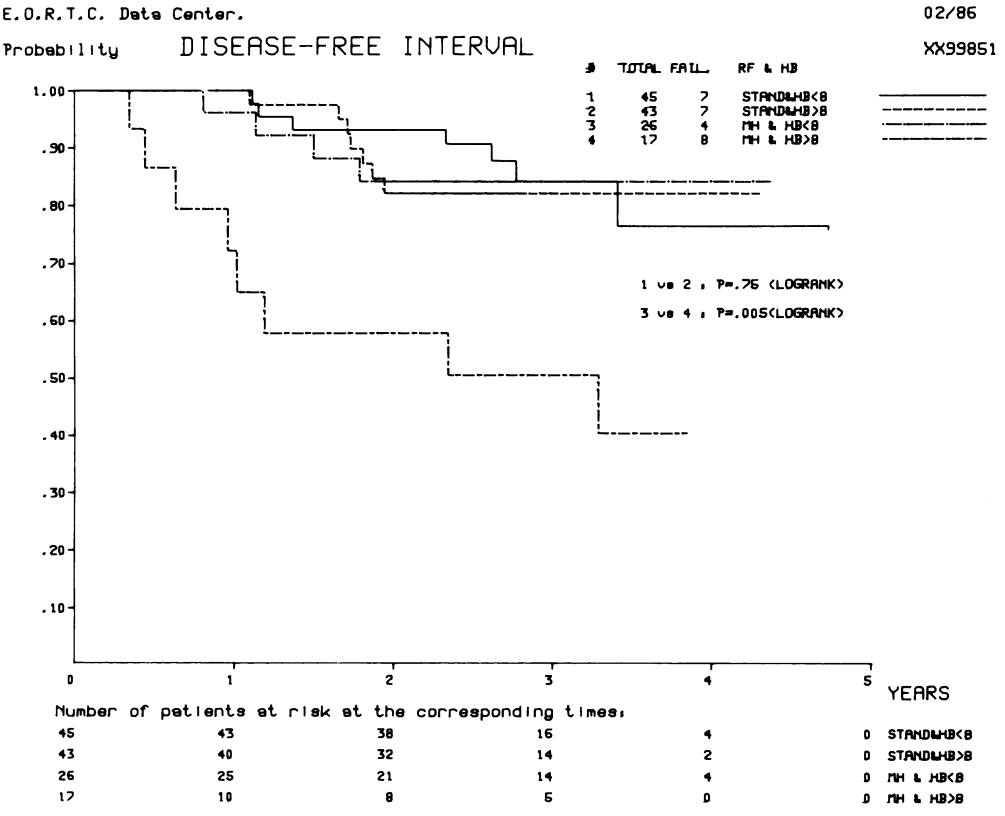

Fig. 5. Disease-free interval (DFI) according to RF and level of Hb ($\leqq 8$ g/dl or > 8 g/dl). DFI is significantly lower for MR and HR patients with Hb > 8 g/dl

found to correlate with a better outcome were absence of bulky splenomegaly (less than 5 cm under the rib border), a granulocyte count of fewer than 10^9/liter and hemoglobin (Hb) $\leqq 8$ g/dl (Table 2). The latter factor was of greatest prognostic significance within the MH+HR subset of patients (Fig. 5).

Discussion

The results of this study confirm the overall effectiveness of the BFM treatment regimen [2]. Lethal toxicity was unusually high at the beginning of the study, as shown by the 25% death rate in CR among the first 20 patients who entered the study, but it decreased

markedly thereafter as the participants became progressively better acquainted with the protocol. The patients were allocated to either of two protocols of different aggressiveness according to their risk factor [1]. As patients with RF $\geqq$ 1.2 were given more intensive reinduction, it should come as no surprise that neither hepatomegaly nor blast count remained predictive of outcome any more. However, in spite of this therapeutic adjustment, splenomegaly was still a significant prognostic factor (Table 2). This observation suggests that splenomegaly should perhaps be given greater weight in the calculation of RF. Within the subgroup of MR + HR patients, high Hb (>8 g/dl) was significantly correlated with bad prognosis (Fig. 5). This is in accordance with the results of some other studies [3–5]. In future, the combination of high RF and high hemoglobin level might be used to define a subset of patients for whom new therapeutic approaches should be investigated.

Although the results achieved with the BFM protocol are very encouraging, one may wonder whether this intensive regimen does not represent unnecessary overtreatment for those patients with the best prognostic features. This issue is currently being addressed in a controlled trial for SR patients, who are randomized to receive either the full BFM induction course or the same course without cyclophosphamide. Simultaneously, medium- and high-risk patients have been entered into a randomized trial designed to evaluate the usefulness of brain irradiation when given after four intravenous courses of high-dose methotrexate (2.5 g/m^2).

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Results of Acute Lymphoblastic Leukemia Therapy in Childhood with a Modified BFM Protocol in a Multicenter Study in the German Democratic Republic

F. Zintl, W. Plenert, and H. Malke[1]

Considerable progress in the treatment of acute lymphoblastic leukemia (ALL) has been made in the last 30 years, particularly childhood ALL. The basis of this progress has been modern multidisciplinary management and controlled clinical trials. The most important problem in the therapy of ALL in childhood is the relapse of leukemia. From 1978 to 1981, we did not succeed in raising the relapse-free survival of children with high-risk factors above 30% [1] with the LSA_2L_2 protocol [2]. In a study carried out from 1970 to 1976 [3], it was shown that the intensification and prolongation of the remission-induction phase resulted in a higher percentage of relapse-free long-term survival. We adopted the principles of the BFM protocol in 1981 and started the controlled and randomized multicentric ALL study VII/81.

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Material and Methods

Patients. A total of 382 children, without prior treatment, suffering from ALL were entered into a multicentric controlled randomized study between 1 September 1981 and 31 December 1985.

Table 1. Summary of results

Results of therapy (0–51 months)	Total		SR (64%)		MR (30%)		HR (6%)	
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
Patients	374	100	240	100	112	100	22	100
Not yet in remission	8		4		3		1	
Early deaths	15	4	8	3	5	4	2	9
Death in initial stage	6	1.6	4	1.6	2	1.8		
Complete remission	353	94	228	95	105	94	20	91
Death in remission	15	4	10	4	4	4	1	5
Lost to follow-up	1	0.3	1	0.4				
Relapses, total	61	16	30	13	21	19	10	45
Bone marrow (BM)	33	9	16	7	11	10	6	27
CNS	10	3	6	3	1	0.9	3	14
CNS + BM	11	3	6	3	4	4	1	5
Testes	1	0.3			1	0.9		
Testes and BM	4	1	1	0.4	3	3		
Mediastinum	2	0.5	1	0.4	1	0.9		
In first remission	277	74	188	78	80	71	9	41
Proportion CCR	0.618 ± 0.0036		0.659 ± 0.046		0.590 ± 0.063		0.295 ± 0.116	

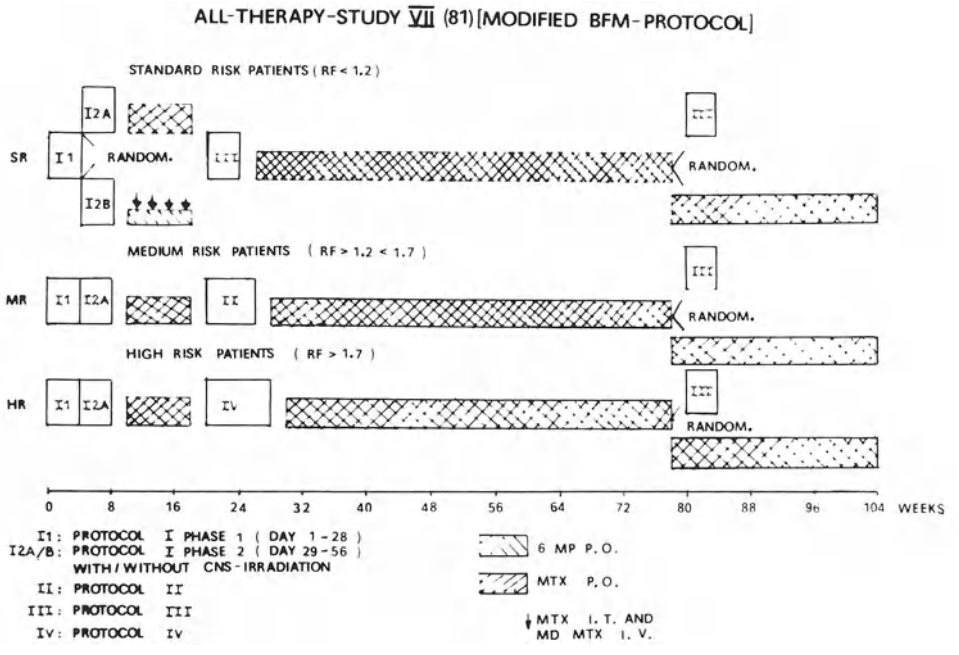

Fig. 1. Protocol VII/81 with classification and randomization of different risk groups of children with ALL

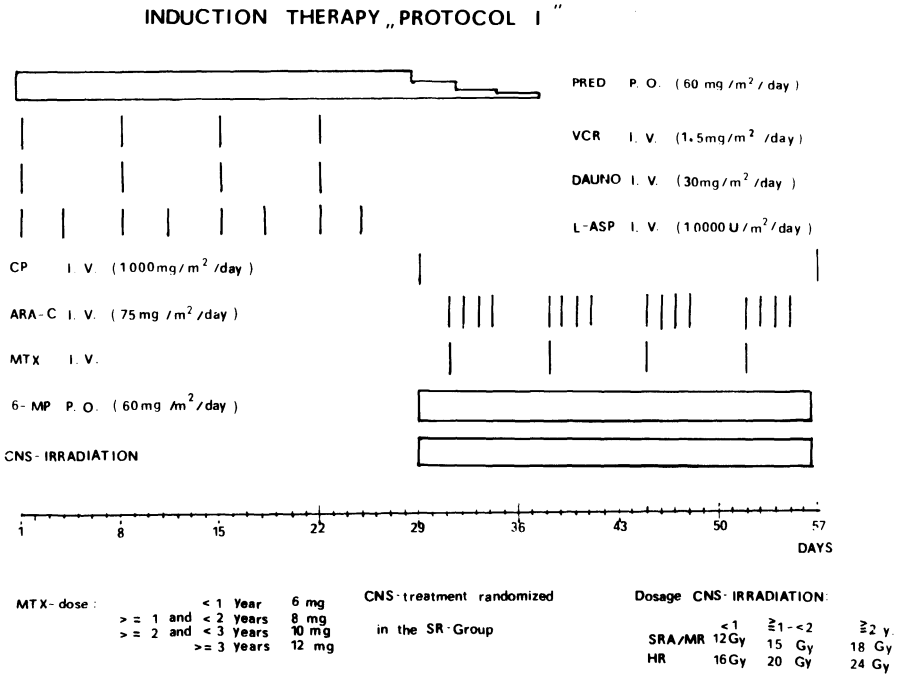

Fig. 2. Induction therapy (protocol I), which is identical for all risk groups, consists of phases I and II

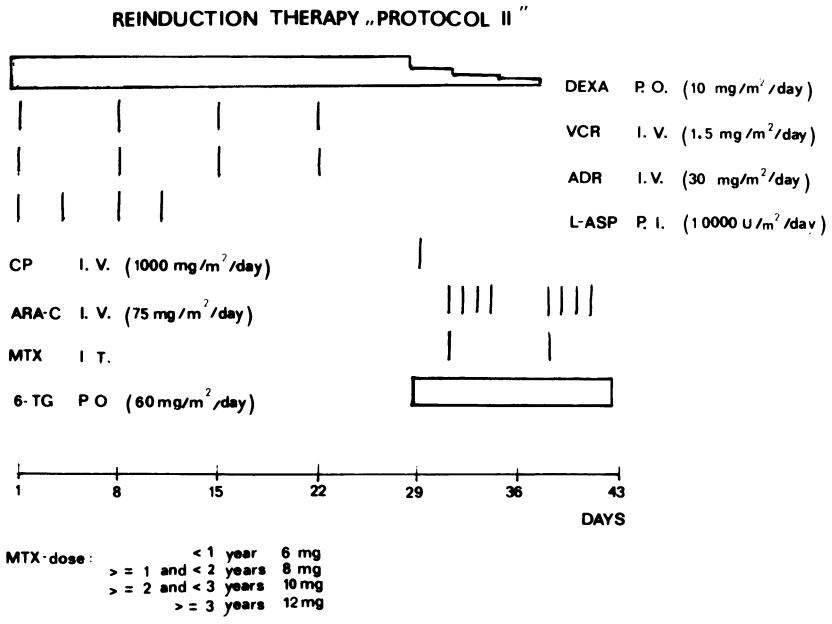

Fig. 3. Reinduction protocol II for SR patients

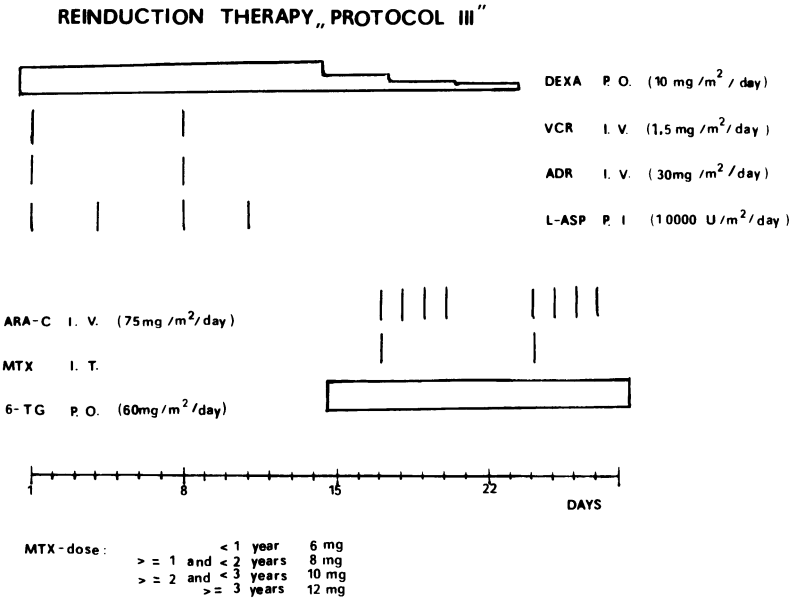

Fig. 4. Reinduction protocol III for MR patients

Patients' characteristics are given in Table 1. In all, 14 pediatric hospitals took part in the study. Diagnosis was made on the basis of morphological criteria. Furthermore, peroxidase, PAS, unspecific esterase, and acid phosphatase were evaluated for all patients. Patients with less than 25% lymphoblasts in bone marrow were diagnosed as non-Hodgkin lymphoma (NHL). NHL and B-ALL are not included in this study.

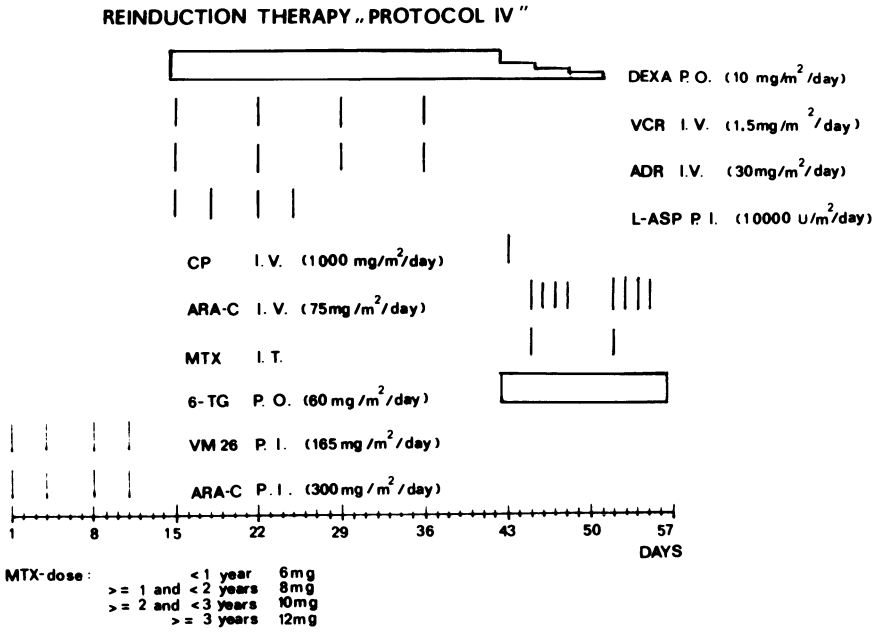

Fig. 5. Reinduction protocol IV for HR patients

Treatment. Patients were divided into three risk groups (Fig. 1) by calculating a risk factor (RF) on the basis of the number of initial leukemic cells and liver and spleen enlargement: standard-(SR), medium-(MR), and high-risk (HR) groups [4].

The treatment comprises induction therapy with CNS prophylaxis, reinduction therapy, and maintenance therapy. Protocol I (Fig. 2) consisted of two phases and was identical for all risk groups. After an interval therapy of 12 weeks, reinduction protocols followed: these had a different intensity for each risk groups: protocol III (Fig. 3) for SR patients, protocol II (Fig. 4) for MR patients, and protocol IV (Fig. 5) for HR patients. For prophylactic CNS therapy in the SR group, patients were randomized to receive cranial irradiation and intrathecal methotrexate (MTX) or an intermediate dose of MTX (500 mg/m^2) and intrathecal MTX (Fig. 1). For the duration of maintenance therapy, patients were randomized after 78 weeks to receive MTX and 6-mercaptopurine (6-MP) for another 6 months or protocol III. The induction therapy of the BFM protocol was modified by reducing L-asparaginase in dose and duration (10000 IU/m^2/day, twice a week). The second modification of the BFM protocol was the reinduction with protocol III before stopping therapy.

Definition and Statistical Analysis

Complete remission (CR) was defined as less than 5% leukemic cells in bone marrow and no evidence of extramedullary leukemia. Kaplan-Meier analyses [5] were performed for event-free survival. Failure is defined as induction failure, initial relapse at any site, death during remission, or death during initial CR.

Results

The results are summarized in Table 2. Out of 382 patients, there were 244 (64%) with standard risk, 115 (30%) with medium risk, and 23 (6%) with highest risk. It was found that 94% of patients attained CR after 4 weeks of therapy. Twenty-one children (5.6%) died within the first 28 days of therapy without having achieved remission. Causes of death were infections [6] and cerebral bleeding [5]. Ten patients did not re-

spond to therapy, and 15 died in the phase of first CR, mostly from infections.

The probability of event-free survival for 374 patients amounts to 0.62 ± 0.04 (Fig. 6). Therapy results for patients with different risks are shown in Fig. 7. Continuous complete remission (CCR) probability amounts to 0.62 ± 0.05 for the SR group, 0.59 ± 0.06 for MR patients, and 0.29 ± 0.12 for HR patients.

Of 353 patients who had achieved CR, 61 relapsed (Table 2). In the SR group, 13% relapsed; in the MR group, 19%; and in the HR group, 45%. Twenty-one CNS relapses (6%) have occurred up to now, with bone marrow being simultaneously affected in 11 cases.

Both groups with different CNS prophylaxis – SRA (irradiation plus intrathecal MTX) and SRB (intermediate-dose MTX plus intrathecal MTX) – have not yet shown any statistically significant difference in the probability of the event-free interval (0.71 ± 0.05 versus $0.78 \pm 0{,}09$) at the moment. However, with regard to CNS relapses only (isolated and combined), the irradiation group reveals a much better survival rate than the MTX group (0.96 ± 0.02 versus 0.80 ± 0.09; Fig. 8). On the other hand, bone marrow relapses amount to 9.3% in the irradiation group and 1.3% in the MTX group. The importance of different treatment duration (18 months plus protocol III versus 24 months) is indicated in Fig. 9. The probability of event-free survival for the 18-month

Table 2. Characteristics of patients in study VII/81

Patients' characteristics	(*n*)	(%)
Boys	210	55
Girls	172	45
Median age (years)	$4^{10}/_{12}$	
< 2	36	9
>10	69	18
Leukocytes		
> 25000	123	32
> 50000	80	21
>100000	44	12
CNS involvement	19	5
Mediastinal tumor	40	11
Liver 5 cm	119	31
Spleen 5 cm	99	23
Total patients	382	

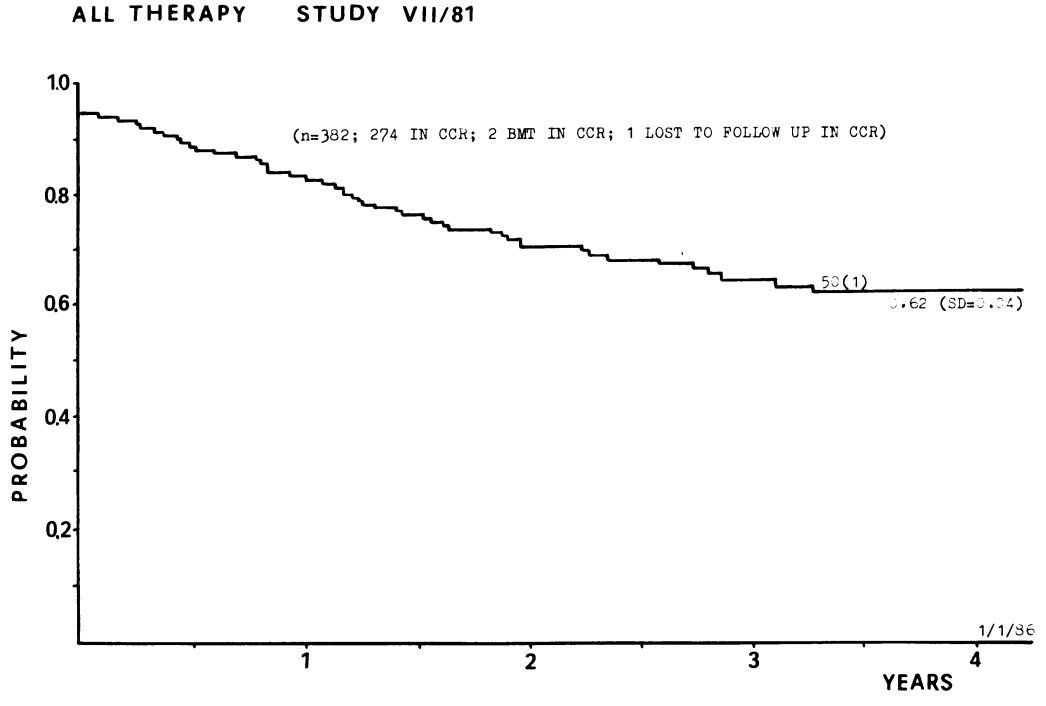

Fig. 6. Life-table curve for the total number of patients (after Kaplan-Meier)

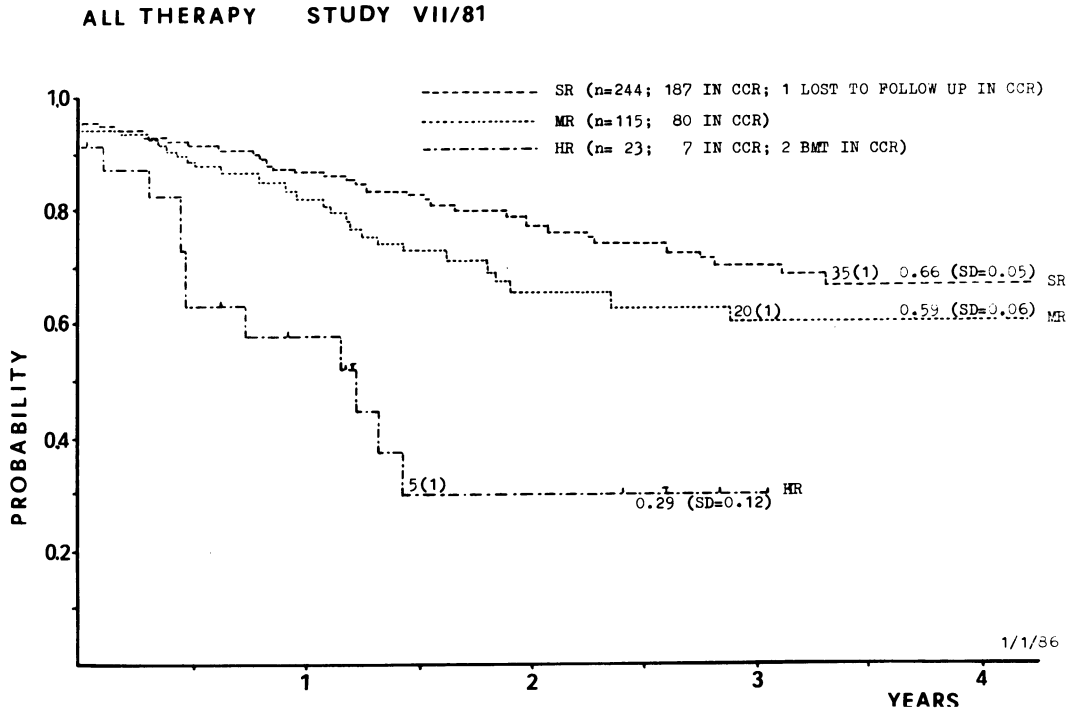

Fig. 7. Life-table curves for different risk groups

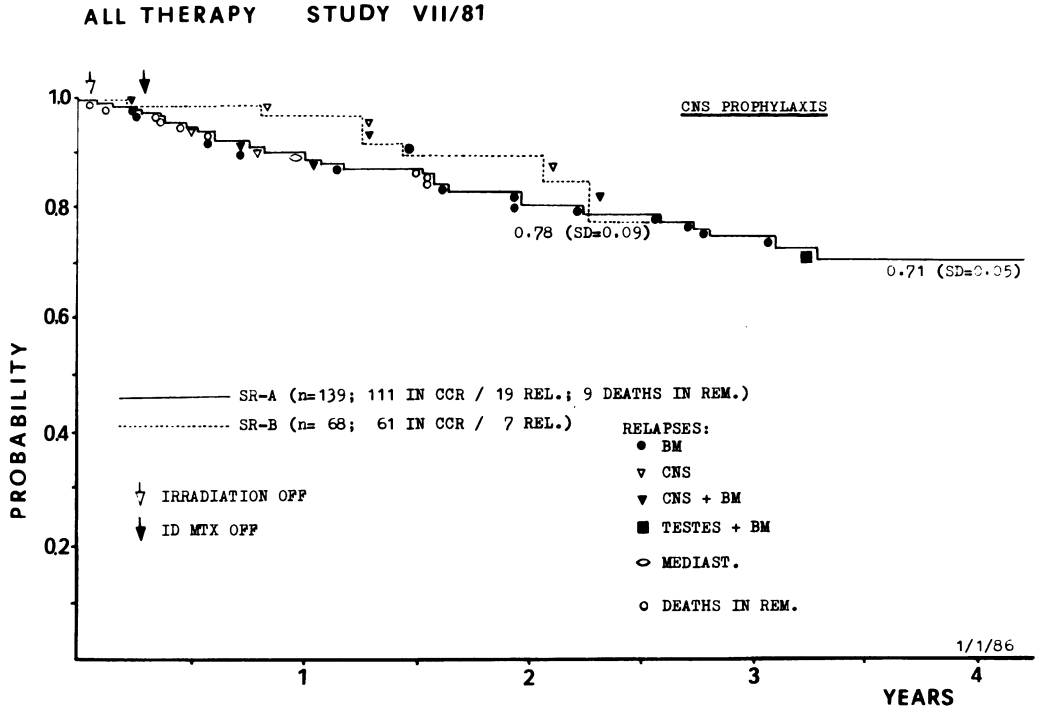

Fig. 8. Influence of different CNS preventive treatment protocols on SR patients

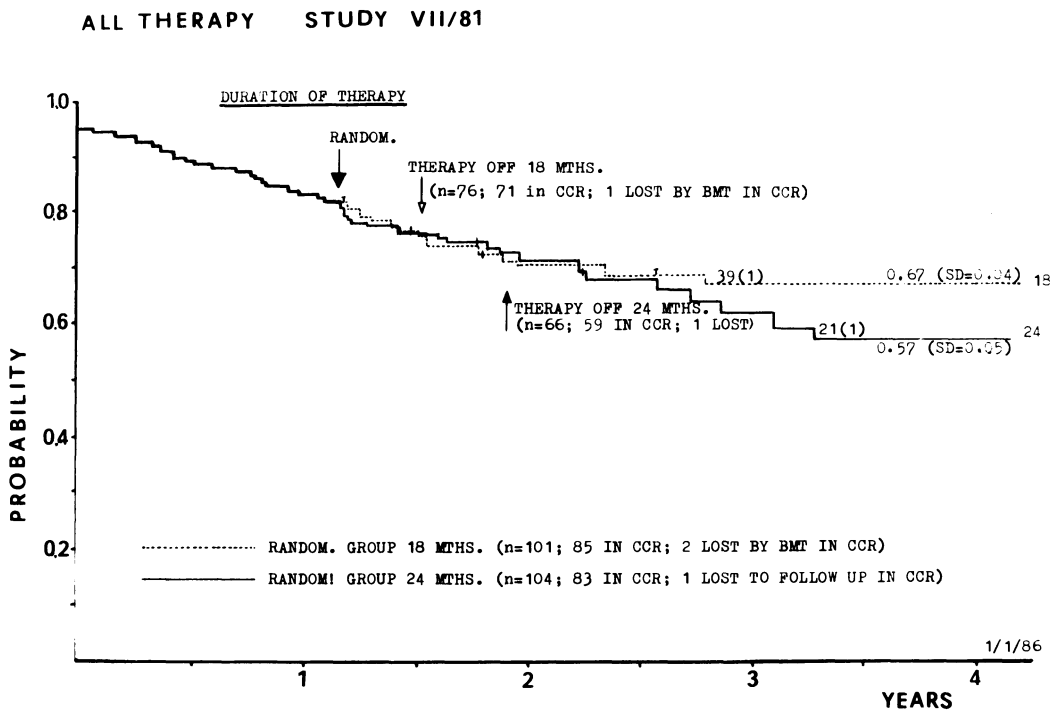

Fig. 9. Influence of therapy duration of 18 and 24 months respectively on the event-free survival rate

group amounts to 0.67 ± 0.04 and to $0.57 \pm 0{,}05$ for the 24-month group.

Discussion

In the last 10 years, the therapy of childhood ALL has been characterized by the efforts to replace the uniform therapy for all patients by a more sophisticated therapy for certain risk groups of patients. The reason for that is the knowledge that ALL is a heterogeneous group [4, 6, 7], Furthermore, Riehm et al. (1986) [3] found that a more intensive and longer incubation phase leads to a higher percentage of long-term disease-free survivors. The BFM group could show that the prognosis of ALL patients was determined by three essential indicators, i.e., the mass of leukemic cells, the initial number of lymphoblasts per µl, and the size of liver and spleen [4]. This finding was the basis for the stratification of risk groups in the BFM study 1981. In our group, study VII/81 (modified BFM protocol) represented considerable progress, compared with previous protocols [1, 9], though our expectations were not fully met. This is firstly true for the relatively bad outcome of HR patients. A probability of event-free survival of only 0.29 ± 0.12 was achieved in the HR group in spite of intensified reinduction with protocol IV with the application of the podophyllotoxin drug VM-26. The bad outcome of HR patients, whose group is relatively small with only 23 patients (6% of all patients), is due both to more frequent complications in the early phase of therapy (9%) and the evidently higher relapse rate (45%). The corresponding values of the relapse rate for SR and MR patients (13% and 19% respectively) are clearly below this frequency. Since, for the time being, none of the protocols used brings real progress, it is in our opinion absolutely necessary to enter these patients in the bone marrow transplantation program already during first remission (90% have achieved this stage).

The most effective and least toxic prophylactic CNS therapy has to be defined accord-

ing to risk groups. The data of this study, which compared cranial radiotherapy with 1800 rad with intermediate-dose MTX, both combined with intrathecal MTX for SR patients, revealed a higher rate of CNS relapses but a lower rate of bone marrow relapses in the intermediate-dose MTX group.

The same results obtained in the BFM group were the reason for further randomized trials to determine whether lower doses of radiation for a certain group of standard patients will adequately protect them from CNS relapses (H. Riehm 1984, personal communication). The good event-free survival rate of patients who were only treated for 18 months, in comparison with the 24-month group, corresponds with our opinion that the fate of patients is decided at a very early phase of therapy. The fact that the 18-month group with a slightly better remission rate might profit from late reinduction cannot be proved with statistical relevance at present.

The results obtained in this study will stimulate us to follow this treatment strategy in the future, with some important modifications such as intermediate-dose MTX for all patients, cranial irradiation for a certain risk group of standard patients, or more intensive treatment for patients with poor prognostic biologic features.

Summary

Between 1 September 1981 and 31 December 1985, 382 previously untreated children with ALL were entered into study VII/81, a multicentric and randomized study with a modified BFM protocol. Patients were divided into three risk groups according to the initial lymphoblast count and liver and spleen enlargement: standard- (SR), medium- (MR), and high-risk (HR) groups. Of all patients, 94% attained complete remission. The actuarial probability of event-free survival is 0.62 ± 0.04 (SR group, $0.66 \pm 0{,}06$; HR group, 0.29 ± 0.12). Sixty-one patients relapsed, 10 had isolated CNS relapses, and 11 CNS relapses were combined with bone marrow relapses. Concerning the duration of maintenance therapy, patients were randomized into two groups of 18 and 24 months respectively. Up to now, there has been a slight advantage for the 18-month group. Two different methods of CNS preventive therapy for SR patients (irradiation plus intrathecal methotrexate and intermediate-dose methotrexate (IDMTX) plus intrathecal methotrexate) were used and revealed a higher rate of CNS relapses but a lower rate of bone marrow relapses in the intermediate-dose MTX group.

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Intensive Therapy in Childhood Acute Lymphoblastic Leukemia: A Report from the Polish Children's Leukemia and Lymphoma Study Group After 11 Years

D. Michalewska, U. Radwańska, M. Kaczmarek, J. Armata, J. Bogusławska-Jaworska, R. Cyklis, D. Derulska, T. Newecka-Samól, M. Ochocka, B. Rodziewicz, R. Rokicka-Milewska, D. Sońta-Jakimczyk, and M. Sroczyńska

A total of 1160 patients with acute lymphoblastic leukemia (ALL) were treated from 1973 to 1984, all of them according to three intensified therapeutic programs (Table 1).

The classification of prognostic groups was based on the criteria summarized in Table 2. In the low-risk category were included patients with a score of lower than 3 and in the high-risk category, those with a score of 3 and over.

It may be seen from Table 3 that the groups of children treated according to all three programs were comparable in sex, age, initial mediastinal mass, and white blood count (WBC). The only remarkable difference was a greater number of children with initial central nervous system (CNS) involvement in the first group treated according to program I.

The continuous remission probability according to Cutler and Ederer [1] was evaluated only for those children who had entered remission, that is, for 1056 children (see Table 4). The results were evaluated up to 31 May 1985.

As may be seen from Fig. 1 and Table 4, there has been a considerable increase in continuous 3-year remission probability – from 0.41 (program I) to 0.57 (program II), and 0.82 (program III).

A 6-year follow-up of the patients provided a 0.31 probability of continuous complete remission (CCR) for children in program I and a 0.47 probability for those in program II (Table 4).

Departments of Hematology – Medical Academies of Poznań, Krakow, Wroclaw, Warszawa and Zabrze, Poland.

Table 1. Therapeutic programs

Years	Programs	*n*	
1973–1979	I. Memphis VI, VII [3, 6]		426
1979–1981	II. Low-risk group Memphis + consolidation[a]	240	
	High-risk group LSA_2L_2 [7]	106	346
1981–1984 [2, 4, 5]	III. BFM 79/81 adapted to risk groups		388

[a] Our own modification: L-ASP, ARA-C, 6-TG.

Table 2. Determination of risk index at the time of diagnosis

Findings at diagnosis	Score
Initial WBC $\geq 50 \cdot 10^9$/liter/ $\geq 25 \times 10^9$/liter[a]	3
CNS involvement	2
Mediastinal mass and/or other significant extranodular mass	2
E-rosette and/or AcP positivity	2
Age <2 or >10 years	1
Risk index	Score sum

[a] Since 1983.

Therapy effects relative to low- and high-risk patients treated according to programs II and III are presented in Fig. 2. It may be seen that in the group treated according to program II, the probability of a 3-year CCR

Table 3. Characteristics of patients in study

	Therapeutic program						Total	
	I		II		III			
	n	%	*n*	%	*n*	%	*n*	%
Total patients in study	426		346		388		1160	
Sex Girls	192	45.1	145	41.9	160	41.2	497	42.8
Boys	234	54.9	201	58.1	228	58.8	663	57.2
Age (years) < 2	56	13.2	36	10.4	51	13.1	143	12.3
2–0	300	70.4	255	73.7	284	73.2	839	72.3
>10	70	16.4	55	15.9	53	13.7	178	15.4
Thymic involvement	33	7.7	24	6.9	27	7.0	84	7.2
CNS involvement	24	5.6	7	2.0	7	1.8	38	3.3
WBC $<50\times10^9$/liter	358	84.0	286	82.7	332	85.6	976	84.1
$\geqq 50\times10^9$/liter	68	16.0	60	17.3	56	14.4	184	15.9

Table 4. CCR probability

Program	Children who have entered remission	CCR	
		After 36 months	After 72 months
I	384	0.41	0.31
II	322	0.57	0.47
III	350	0.82	unknown

amounts to 0.61 for low-risk patients and only 0.46 for high-risk patients.

Parallel values for children treated according to program III reach, respectively, 0.84 and 0.77.

Conclusions

1. Intensification of ALL-therapy made it possible to increase the percentage of children with a 3-year CCR from 41%

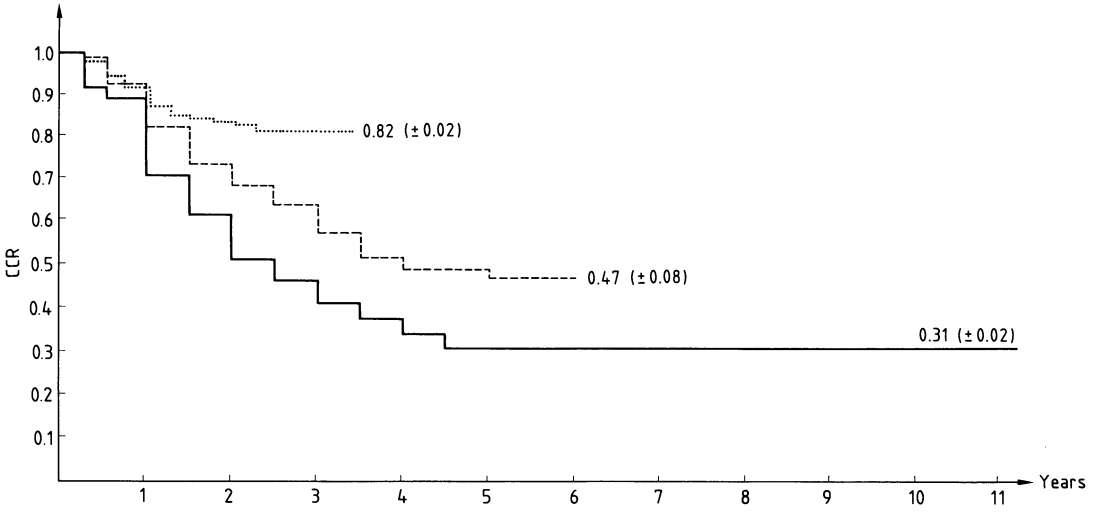

Fig. 1. CCR probability: —— Program I; – – – Program II; ... Program III

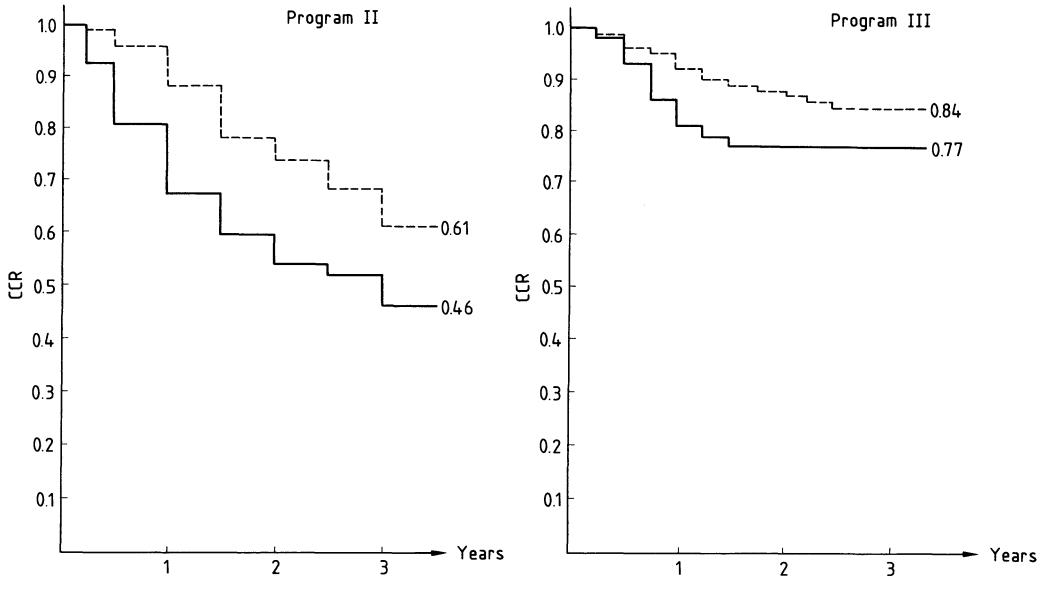

Fig. 2. CCR probability in low- and high-risk patients: —— High risk; --- Low risk

(program I) to 57% (program II) and 82% (program III).

2. Intensification of therapy for high-risk patients in program III (BFM) equaled the chances for a 3-year CR in low- and high-risk children. At present, 84% of low-risk patients and 77% of high-risk patients remain in 3-year remission. The respective values for the earlier program II reached 61% and 46%.

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Treatment of Standard- and High-Risk Childhood Acute Lymphoblastic Leukaemia with Two CNS Prophylaxis Regimens

J. J. Ortega, G. Javier, and T. Olive[1]

By the mid-1970s the common therapy for acute lymphoblastic leukaemia (ALL) in children consisted of an induction treatment with three drugs (prednisone, vincristine, and asparaginase) followed by CNS prophylaxis, usually cranial irradiation combined with intrathecal methotrexate, and a 3-year maintenance therapy with mercaptopurine and methotrexate, with or without reinforcement treatments. With this strategy, about 95% of the patients achieved remission and a 5-year disease-free survival rate could be obtained in 50% [1–5]. Analysis of results indicated that a classification into different risk groups could be established, based on initial clinical and haematological characteristics [6, 7]. It could be shown that a group of patients with some prognostic factors had an increased risk of developing relapses and their disease-free survival rate was statistically lower than the one attained by the other patients. At the same time, some studies, such as BFM 70/76, seemed to indicate that intensification of induction therapy could result in a decrease in the number of relapses [8]. Consequently, it was thought that the use of different protocols for patients with standard and increased risk could benefit the overall cure rate [9].

CNS prophylaxis with cranial irradiation (24 Gy) and intrathecal methotrexate had reduced the incidence of CNS disease to less than 10%. However, increasing evidence of the toxicity of this combined treatment, including neurotoxic effects and intellectual impairment [10–15], called for alternative treatments. Also, since 1975, there had been increasing concern about the testes as a not uncommon site of first relapse and particularly of late relapse occuring after therapy was discontinued [16, 17]. Some authors began to report that testes biopsies in asymptomatic patients, before the suppression of cytostatics, showed occult leukemic infiltrates in some [18, 19].

In this context, the aims of the Pethema [2] 7/78 study conceived at the end of 1977 were (a) to reduce the proportion of relapses in increased risk patients by intensification of the induction treatment, (b) to compare an irradiation-free CNS prophylaxis with conventional treatment, seeking an effective and less toxic therapy; and (c) to detect the presence of occult leukaemic infiltrates in boys after 2 years in remission by routine bilateral testicular biopsies, in order to identify a subgroup of patients with increased risk of late relapse and apply treatment before an open relapse appeared. This study was carried out in the *Hospital Infantil Vall d'Hebrón,* which was the pilot centre for parallel multicentric trial involving 11 hospitals.

Patients and Methods

Between April 1978 and December 1983, 87 patients with ALL under 15 years of age were entered in the study. Patients' charac-

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[2] Co-operative Group of the Spanish Society of Haematology for Treatment of Malignant Haemopathies.

teristics are shown in Table 1. An initial classification of the patients into one of two risk groups, i.e. high-risk (HR) or standard-risk (SR), was established according to a risk index (RI). This index was obtained from the sum of the scores of a series of clinical and haematological factors (Table 2). The RI was based on analysis of the results of two previous studies in the pilot centre (data not shown). When the RI was $\geqq 3$ the patient was included in the HR group; patients with a RI < 3 were enrolled in the SR group.

The treatment programme is outlined in Table 3. Induction therapy consisted of prednisolone, vincristine and asparaginase for the SR group and the same *plus* daunorubicin for the HR group. Patients achieving remission were randomly allocated, on a stratified basis according to the risk group, to one of two regimens of CNS prophylaxis. Regimen *A* consisted of cranial irradiation (24 Gy in 12 fractions) and methotrexate intrathecally (6 doses at 5-day intervals). Regimen *B* consisted of methotrexate *plus* cytosine arabinoside intrathecally, six times during this phase followed by four doses at 1-month intervals during the maintenance phase. Mercaptopurine (40 mg/m^2/day^1) was also administered during the period of CNS prophylaxis in both regimens. All patients received maintenance therapy with oral mercaptopurine (60 mg/m^2/day^1) *plus* intramuscular methotrexate (15 mg/m^2 week^1). Patients in the HR group also received 2-week reinforcement courses of prednisolone, vincristine (two doses) and daunorubicin (one dose) every 12 weeks over 3 years. During maintenance, the doses of mercaptopurine and methotrexate were modified if the white blood cell (WBC) count was below 2×10^9/liter or the platelet count below 100×10^9/liter.

A bilateral testes biopsy was performed in male patients in continuous remission (CR) at 24 months. Patients in whom leukaemic infiltrates were found received complementary treatment with local irradiation (25 Gy to both testes) and a further 4-week induction treatment with prednisolone and vincristine.

After three years of CR, female patients were taken off chemotherapy. The duration of maintenance therapy in male patients was prolonged for 2 more years in order to ascertain whether, with this prolongation, the higher relapse rate observed in boys in previous studies could be reduced.

Table 1. Pethema study ALL 7/78. Initial characteristics of patients

		$n=87$	(%)
1. Sex:	Male	47	(54)
	Female	40	(46)
2. Age:	0– 1	4	(4.6)
	1– 9	78	(90)
	10–14	5	(5.4)
3. Spleen (cm, bcm)	<5 cm	70	(80)
	$\geqq 5$ cm	17	(20)
4. Liver (cm, bcm)	<5 cm	75	(86)
	$\geqq 5$ cm	12	(14)
5. Adenomegalies	>2 cm	10	(11.5)
6. Mediastinal mass		2	(2.3)
7. CNS infiltrates		1	(1.1)
8. Other tumoral infiltrates		4	(4.6)
9. Leukocytes ($\times 10^9$/liter)			
	< 20	41	(47)
	20–99	35	(40.2)
	$\geqq 100$	11	(12.8)
10. Platelets ($\times 10^9$/liter	<50	60	(69)
	$\geqq 50$	27	(31)
11. Hb:	<9 g/dl	75	(86.2)
	$\geqq 9$ g/dj	12	(13.8)
12. Blast cell morphology			
(FAB classification):	L_1	62	(71.3)
	L_2	25	(28.7)
	L_3	0	
13. Cell markers:			
T markers		12/80	(15)
B markers		0	
Non-T, non-B markers		68/80	(85)
SR: Risk index	<3	65	(75)
HR: Risk index	$\geqq 3$	22	(25)

Survival and relapse-free survival were calculated as of January 1986. Minimum follow-up was 25 months and the median 62 months. Deaths of patients in CR were considered as relapses in the statistical evaluations. Life-table analysis was performed by the Kaplan-Meier method and differences in patterns were studied with the log-rank test. For other comparisons the chi-square test for contingency table was used.

Table 2. Determination of risk index according to findings at diagnosis and classification of patients into two risk groups

Findings at diagnosis	Score	
	Protocol 7/78	Protocol 12/48
1. Age less than 1 year	2	3
10–14	1	2
2. Leukocyte count ($\times 10^9$/liter)		
20–99	1	
20–49		1
50–99		2
$\geqq 100$	2	3
3. CNS involvement	3	3
4. Tumoral burden		
Splenomegaly $\geqq 5$ cm bcm	1	1
Hepatomegaly $\geqq 5$ cm bcm	1	1
Adenomegalies >2 cm	1	1
Mediastinal mass	2	2
Other tumoral infiltrates	1	1
5. T-cell markers	2	2
B-cell markers	3	Excluded from protocol

Risk index (RI) = score sum.
Standard risk (SR) RI <3; High risk (HR) RI $\geqq 3$.

Table 3. Pethema ALL 7/78. Protocol treatment programme

1. Induction	2. CNS prophylaxis	3. Maintenance phase
4 weeks	*4 weeks*	*Girls, 3 years; boys, 5 years*
Standard risk SR: PRED, VCR, ASPAR	SR *A*, HR *A*: cranial irradiation, i.t. MTX, 6 doses	SR *A*: MP orally, daily; MTX i.m. weekly
	All patients: MP, oraly	SR *B*: Same as SR *A* plus i.t. MTX+ARA-C, monthly $\times 4$ (3rd-6th month)
High risk HR: PRED, VCR, ASPAR, DAUNO	SR *B*, HR *B*: i.t. MTX+ARA-C, 6 doses	HR *A*: Same as SR *A* +reinforcements
		HR *B*: Same as SR *B* +reinforcements
		Reinforcements: only group HR
		Every 3 months: PRED, VCR, DAUNO $\times$ 2 weeks[a]

Dosages: PRED (prednisolone) 40 mg m^{-2} day $\times 28$; VCR (vincristine) 1.5 mg m^{-2} week $\times 4$; ASPAR (asparaginase) 10000 U/$m^{-2} \times 6$ doses (weeks 3–4); DAUNO (daunorubicin) 30 mg m^{-2} week $\times 2$ (weeks 1–2); i.t. MTX (methotrexate) 10 mg m^{-2} (max. dose 10 mg); i.t. MTX+ARA-C (cytosine arabinoside) 30 mg m^{-2} (max. dose 30 mg); MP (mercaptopurine) 60 mg m^{-2} day; MTX i.m. (methotrexate) 15 mg m^{-2} $week^{-1}$.
[a] VCR, 2 doses; DAUNO, 1 dose.

Results

Risk groups and remission induction. Twenty-two patients (25%) had a RI of 3 or more and, consequently, were included in the HR group; the other 65 (75%) with a RI of less than 3 constituted the SR group. All 87 patients attained initial remission and no deaths were registered during this phase. One patient in the HR group died of cranial traumatism after 18 months in remission and was excluded from further evaluation.

CNS treatment regimen assignment. The characteristics of patients in the two regimen groups were very similar. Ten of the 44 patients randomly assigned to regimen *A* and 11 of the 42 assigned to regimen *B* were HR patients.

Overall results. The course after remission induction, with relapse frequency and deaths in CR, is shown in Table 4. Up to the date of evaluation, 25 patients (29%) had relapsed and 56 patients (65.1%) were in continuous complete remission (CCR). Most relapses were haematological and only 3 CNS relapses (3.5%) were registered. Three patients died of infectious complications during remission: two interstitial pneumonias and one necrotizing enterocolitis. The probability of disease-free survival for all the patients entered in the study is 65% (SD 6%), as shown in Fig. 1. Life-table curves show a 67% probability of CCR for SR and 58% for HR Fig. 2).

Results According to CNS Prophylaxis Regimen. Patients treated with regimen *A* (in-

Table 4. Pethema ALL 7/78. Protocol status of patients at January 1986 evaluation (median follow-up: 62 months)

Patients	Total		Standard-Risk		High-Risk	
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
First CR	87	(100)	65	(100)	22	(100)
Evaluable	86		65		21	(1 died in CCR, accidentally)
Relapses	25	(29)	18	(27.7)	7	(33.3)
BM relapse (±others)	19	(22)	15	(23)	4	(19)
CNS relapse	3	(3.5)	1	(1.5)	2	(9.5)
Testes relapse	2	(4.3 males)	1	(2.8 males)	1	(8.3 males)
Deaths in CR	3	(3.5)	2	(3.1)	1	(4.7)
In CCR	56	(65.1)	43	(66.0)	13	(62)
Survival	58	(67.4)	45	(69.2)	13	(62)

Table 5. Remission status and CNS prophylaxis regimen

Patients	Regimen *A* With cranial irradiation		Regimen *B* No cranial irradiation	
	n	%	*n*	%
Evaluable and randomized	44		42	
SR/HR	34/10		31/11	
Relapses	16	(36.3)	10	(23.8)
CNS relapses	2	(4.5)	1	(2.4)
Deaths in CR	1	(2.3)	2	(4.8)
In CR	27	(61.4)	30	(71.4)
Survival	28	(63.6)	31	(73.8)

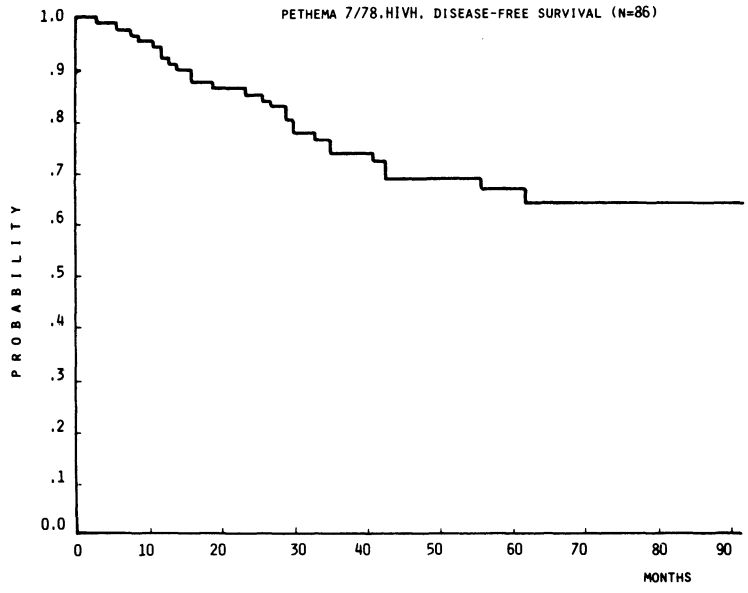

Fig. 1. Pethema ALL 7/78 protocol. Disease-free survival probability for all patients. Median follow-up, 62 months

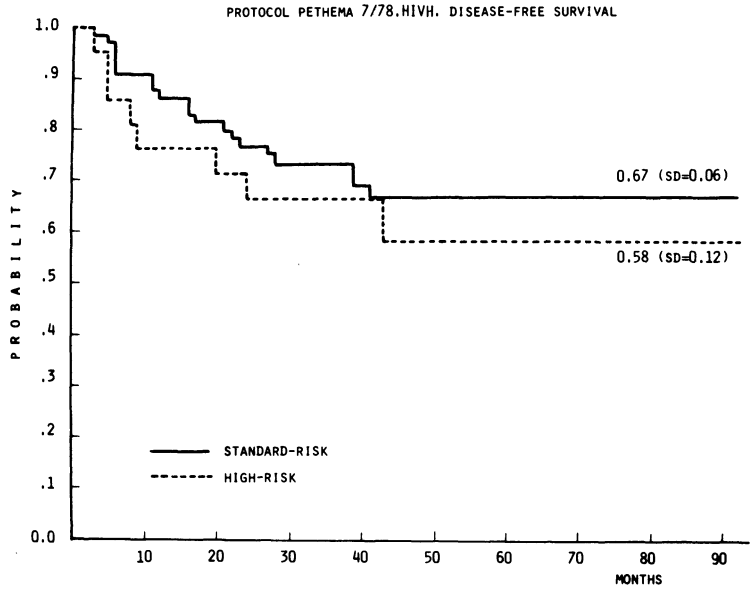

Fig. 2. Pethema ALL 7/78 protocol. Duration of complete remission in standard-risk (75%) and high-risk (25%) patients

cluding cranial irradiation) suffered more relapses than those given regimen *B*, as shown in Table 5 and Fig. 3. Nevertheless, the differences were not statistically significant. The proportion of CNS relapses in both regimens was very low: 4.5% in patients treated with regimen *A* and only 2.4% in those not receiving cranial irradiation (regime B). Two patients given regimen *A* presented encephalopathy at 14 and 26 months, respectively, after CNS therapy: one was left with severe intellectual sequelae and in the

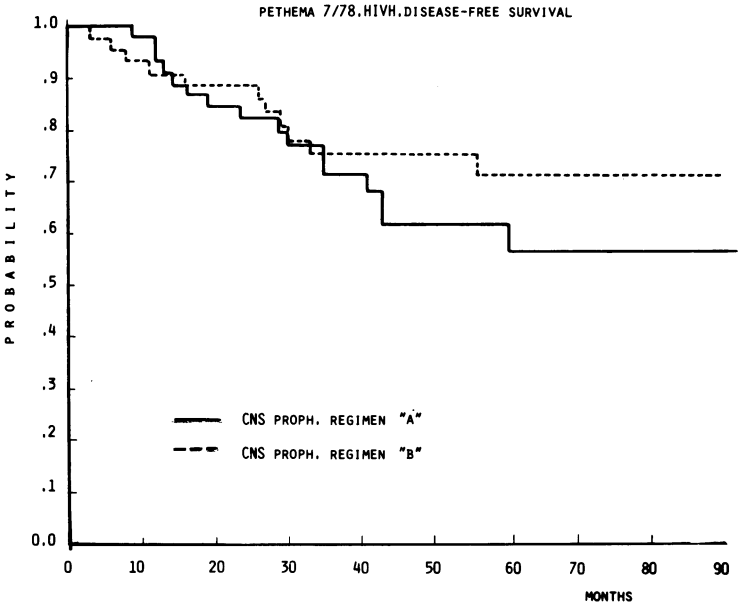

Fig. 3. Pethema ALL 7/78 protocol. Disease-free survival probability according to CNS prophylaxis regimen. Regimen *A* was cranial irradiation *plus* i.t. methotrexate; regimen *B* was ten doses of i.t. therapy with methotrexate and cytosine arabinoside over a 6-month period

other verbal impairment persists; a third patient presented with akinetic seizures. On the other hand, none of the patients treated with regimen *B* had encephalopathy or seizures, but one presented with a transient paraparesis after the sixth intrathecal dose and two others have cerebral calcifications with no neurological symptoms. Comparative psychomotor evaluation showed a slightly lower mean full-scale IQ in the irradiated group.

Testes Involvement. Only 2 of the 47 male patients presented with isolated clinical testicular relapse. One was an early relapse, at 3 months after entering remission, in a 6-month-old infant with an initial WBC count of 1000×10^9/liter. The other was a late relapse in a 9-year-old boy after 39 months CR. Another patient, a 9-month-old infant, suffered simultaneous relapse in bone marrow and testes. Routine biopsies performed in 36 boys after 24 months in remission showed interstitial leukaemic infiltrates in 10. These ten patients received the aforementioned complementary therapy and none of them had presented relapses up to the date of evaluation. On the other hand, 3 of the 26 with negative biopsies presented relapses: two in bone marrow and one in testes (previous false-negative biopsy).

Multicentric Trial. A first evaluation of this trial was reported in 1983 [20] and update results will be published elsewhere. In summary, 243 out of the 256 evaluable patients (95%) attained remission after induction treatment. A total of 108 relapses were registered, 19 of which (7.8%) were CNS relapses. The estimated long-term survival rate is 46% (SD 3%). A total of 114 patients (86 SR and 28 HR) were assigned to receive CNS prophylaxis with regimen *A* and the other 129 (97 SR and 32 HR) were treated with regimen *B*. No significant differences in relapse rates according to the CNS prophylaxis regimen were found. However, between HR subgroups a slight tendency, not statistically significant, favourable to regimen *A* was registered.

Discussion

The estimated long-term disease-free survival rate for all patients entered in this

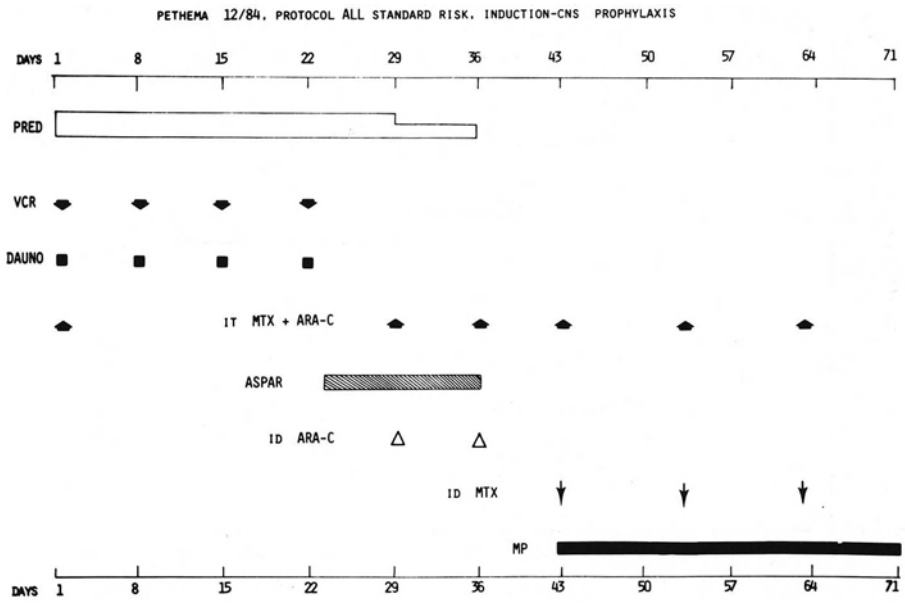

Fig. 4. Schema of induction-consolidation phase in Pethema ALL 12/84 protocol for standard-risk patients. *PRED* prednisolone) 40 mg m^{-2} day^{-1}, orally; *VCR* (vincristine) 1.5 mg m^{-2} $week^{-1}$; *DAUNO* (daunorubicin) 30 mg m^{-2} $week^{-1}$, *IT MTX + Ara-C*, intrathecal methotrexate 10 mg/m^2 (max. dose 10 mg) *plus* cytosine arabinoside 30 mg/m^2 (max. dose 30 mg); *ASPAR* (asparaginase) 5000 U m^{-2} day^{-1} × 14 i.m.; *ID Ara-C* (cytosine arabinoside) 200 mg/m^2 in 3-h infusion; *ID MTX* (methotrexate) 500 mg/m^2 in 6-h infusion *plus* leucovorin 15 mg/m^2 24 h later; *MP* (mercaptopurine) 60 mg m^{-2} day^{-1}, orally

study is 65% (SD 6%). This rate is comparable to those obtained in other contemporary studies [2, 21–23]. The lower proportion obtained in the multicentric trial may be explained by the heterogeneous characteristics of the 11 participating centres. The use of a RI based on our own data gathered from previous studies [5, 24] resulted in a high-risk group that included 25% of all patients – a lower rate than that obtained in most studies, which generally ranged from 35% to 53% [22, 23, 25, 26]. In spite of the more restrictive criteria used to select the increased-risk group, the 58% rate of long-term CR compares favourably with the rates reported in other studies [22, 23] but is lower than the rates in protocols BFM 76/79 and 79/81 [25, 26]. In these two studies induction and consolidation therapies in both risk groups were much more intensive, particularly in high-risk patients. In our protocol ALL 12/84 emphasis has also been placed on reinforcement of the initial treatment. In the standard-risk group a total of seven drugs are given over a 10-week period, and nine drugs over a 16-week period are administered to increased-risk patients (Fig. 4 and 5). In both groups, maintenance treatment is given over 24 months. The protocols have been in progress since the end of 1983 and to date 50 patients have been included in our centre. Preliminary results show a disease-free survival rate of 96.5% at 12 months.

The second aim of the present study was to compare, through a prospective randomized trial, an alternative treatment based on intrathecal chemotherapy alone with the common CNS prophylaxis of cranial irradiation and intrathecal methrotrexate. As has been shown, ten doses (six at 1-week intervals followed by four at 1-month intervals) of intrathecal therapy with methotrexate plus cytosine arabinoside were as effective in terms of CNS prophylaxis as the combined treatment; with both regimens the CNS relapse rates were very low (4.5% in the regimen with cranial irradiation and 2.4% in the alternative). More interestingly, the dis-

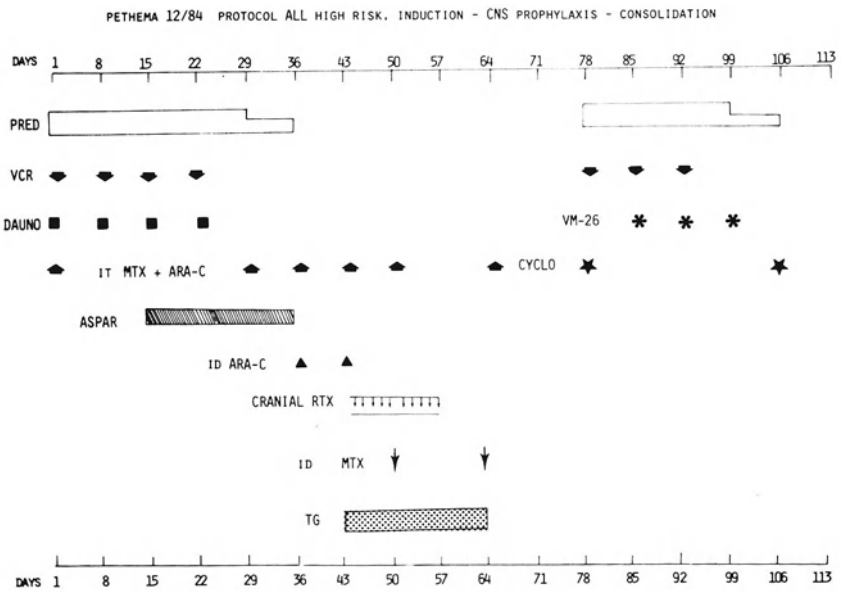

Fig. 5. Schema of induction-consolidation phase of Pethema ALL 12/84 protocol for high-risk patients. *PRED* (prednisolone) 60 mg m^{-2} day^{-1}, orally. *VCR* (vincristine) 1.5 mg m^{-2} $week^{-1}$, *DAUNO* (daunorubicin) 30 mg m^{-2} $week^{-1}$; *IT MTX + Ara-C,* intrathecal methotrexate 10 mg/m^2 (max. dose 10 mg) *plus* cytosine arabinoside 30 mg/m^2 (max. dose 30 mg); *ASPAR* (asparaginase) 10000 U/m^{-2}/day^{-1} × 21 i.m.; *ID Ara-C* (cytosine arabinoside) 200 mg/m^2 in 3-h infusion; *CRANIAL RTX* (holocranial irradiation) 18 Gy in 2 weeks (15 Gy in children 1–2 years of age); *ID MTX* (methotrexate) 500 mg/m^2 in 6-h infusion *plus* leucovorin 15 mg/m^2 24 h later; *TG* (thioguanine) 60 mg m^{-2} day^{-1}, orally; *VM-26* (or teniposide) 150 mg/m^2 i.v.; *CYCLO* (cyclophosphamide) 1000 mg/m^2 in 1-h infusion

ease-free survival rate was higher in the non-irradiated group (71.4% vs. 56.4%), although the differences were not statistically significant. If only increased-risk subgroups are compared, the results are no different from those obtained for all patients. The parallel multicentre trial with 256 patients, using the same protocol, has confirmed the efficacy of the ten-dose intrathecal chemotherapy; only by comparing high-risk subgroups did we find a small, not statistically significant difference in favour of irradiation. The evaluation of intellectual abilities showed a lower average IQ score in patients given cranial irradiation. Two cases of encephalopathy with motor and intellectual sequelae were observed in irradiated patients but none among non-irradiated patients.

The incidence of open testicular relapse was only 4.2% (2 cases among 47 males), clearly lower than the rate observed in a previous study in which 8 of 36 male patients (22.2%) presented open testicular relapse (27). Bilateral testicular biopsies performed in the 36 males at 24 months in CR showed the presence of leukaemic infiltrates in 10 (27.7%). These ten patients were given local irradiation (25 Gy) and induction therapy, and all have continued in remission. Consequently, we believe that through biopsy a subgroup of patients with increased risk of relapse may be identified and that, with the complementary therapy given, open relapses in some of these patients may be avoided.

The mortality rate due to infection in patients in remission was a low 3.5%, which we consider to be partly due to the continuous use of anti-infectious prophylaxis with cotrimoxazol.

In summary, from the analysis of results of study 7/78 and the preliminary results of study 12/84 it may be concluded that (a) intensification of chemotherapy given in the initial phase is an effective method for reducing the early relapse rate in all risk groups, and (b) ten doses of intrathecal chemother-

apy with methotrexate *plus* cytosine arabinoside administered over the first 6 months of treatment are an effective alternative CNS prophylaxis therapy. Cranial irradiation is therefore unnecessary for the majority of children with ALL.

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Aggressive Combination Chemotherapy of Bone Marrow Relapse in Childhood Acute Lymphoblastic Leukemia Containing Aclacinomycin-A: A Multicentric Trial *

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Summary

An intensive 7-day combination chemotherapy protocol was designed to reinduce children with early bone marrow relapse of acute lymphoblastic leukemia (less than 6 months after the end of or during preceding treatment). This aggressive approach seemed to be justified for a group of patients who were at the highest risk for ultimate treatment failure. In all, 38 children were enrolled for study. The ratio of male (median age, 10 years) to female (median age, 13 years) subjects was 27:11. Thirty patients were treated for their first relapse and eight for their second or subsequent relapse. Isolated bone marrow involvement was present in 24 cases. All patients had received heavy pretreatment including anthracyclines with cumulative doses of between 120 and 240 mg/m^2.

22 of these patients, achieved complete remission, ten did not respond to therapy, and six died from the toxicity of the protocol. Cardiac failure was the cause of death in one child (after additional radiotherapy for a mediastinal mass). No further clinical manifestation of cardiomyopathy could be observed. The other five patients died from hemorrhages or infectious complications. The main side effects were fever, gastrointestinal problems, stomatitis, and severe bone marrow aplasia lasting for about 2 weeks with nadirs of platelets and white blood count around days 10–14. The remission rate of 60% was acceptable, though not satisfactory. Only four children survived disease-free for 13+, 14+, 20+, and 22+ months after diagnosis of relapse.

Introduction

While today's therapy for childhood ALL at initial diagnosis is successful in almost 70% of patients, resulting in long-term disease-free survival, treatment for relapse has been a frustrating endeavor in the overwhelming majority of cases. This was confirmed by the retrospective analysis of patients treated for relapse in three BFM ALL studies following the same chemotherapy protocols that had already been used in the primary approach. Similar disappointing results were reported by other study groups [1–9]. Acceptable long-term results were published for boys with testicular relapse only [10]. Prognosis turned out to be poorest for children with bone marrow relapse occurring while they were still on initial treatment [1, 4, 9] and for patients with multiple relapse [7]. The chances of achieving a prolonged second remission were slightly less unfavorable for patients who relapsed after cessation of therapy [2, 3].

Treatment

Bearing in mind the extremely poor prognosis for children with early relapse, the impact of most aggressive chemotherapy seemed to be justified. Thus, in relapse study ALL-REZ BFM 83, a pilot protocol comprised of

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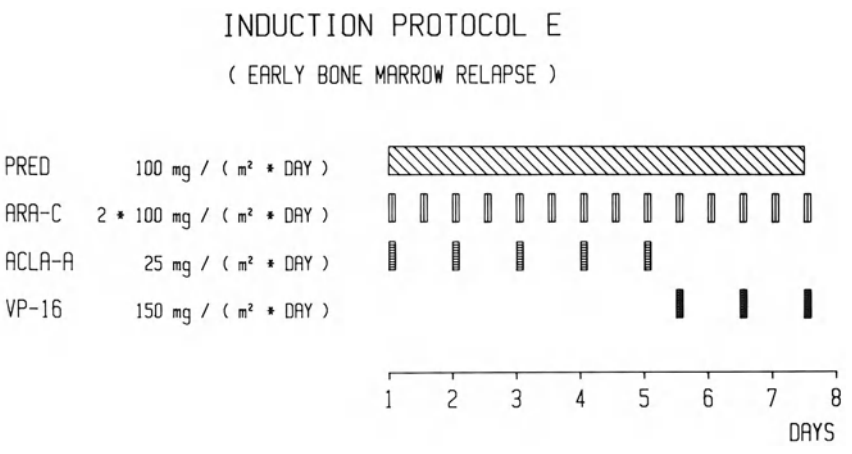

Fig. 1. Reinduction protocol E for children with early bone marrow relapse

prednisone, intermediate-dose cytarabine, etoposide, and aclacynomycin-A (Fig. 1) was introduced for administration to patients with bone marrow relapse during, or less than 6 months after the end of, the preceding treatment. The anthracycline aclacinomycin-A was selected with the intention of reducing the risk of cardiotoxicity in those children, who had just previously been intensively treated.

Patients' Characteristics

The final analysis included 38 children, 30 of whom were enrolled for their first relapse, seven for their second, and one for his third. Bone marrow was the single site of relapse in 24 children. In 12 patients, blast cells were additionally found in other compartments (Table 1). Two boys had an anterior mediastinal mass, one of them in combination with extensive prostate involvement. These conditions were considered to be of equally unfavorable prognosis as marrow relapse, and the patients were therefore included in the study. The male-to-female ratio of 27:11 was distinctly higher than in primarily diagnosed ALL patients. The median age for girls was 13 years and 10 years for boys.

All children had been heavily pretreated according to the West German BFM-ALL or COALL protocols, including anthracyclines at doses of 120–240 mg/m^2. Approximately one-third of patients had been classified as standard risk at initial manifestation of ALL. The remainder belonged originally to groups with increased or high risk of relapse. The results of immunologic marker analysis are given in Table 2. The proportion of children with T-cell markers on their leukemic cells is clearly higher when com-

Table 1. Number and site of early relapses

Site of relapse $n=38$	Relapses (n)	
	First	Multiple
BM	16	8
BM + CNS	5	–
BM + testes	2	–
BM + others	5	–
Other sites	2	–
Total	30	8

Table 2. Immunologic subtypes at entry into study

Immunologic subtype	(n)
C-ALL	21
PRE-B-ALL	2
T-ALL	9
PRE-T-ALL	3
C/T-ALL	1
O-ALL	1
ND	1
Total	38

pared with the characteristics of primarily diagnosed patient series.

Results

Of the 38 patients, 16 did not achieve complete remission after protocol E (Table 3). The remission rate was slightly higher in children with first relapse than in those with second or subsequent relapse. Six patients died from side effects related to the toxicity of the protocol. Death was due to hemorrhage in three patients, and the other three children died from infectious complications, two of which included interstitial pneumonia. Frequently observed, but not life-threatening, side effects were mucositis, diarrhea, and vomiting (Table 4).

Severe bone marrow depression was seen in all patients and required extensive supportive treatment, with a repeated need for red cell transfusions in 35 of the 38 children. Substitution of platelets and of irradiated granulocytes was necessary in 34 and five of the 38 patients respectively.

Nadir values of white blood count and platelets were seen around days 10–14, with slow recovery thereafter. Clinically relevant cardiotoxicity occurred in only one patient. This 6-year-old boy with T-ALL received radiotherapy (24 Gy) to a bulky mediastinal mass. While still on intensive chemotherapy, he suffered a second relapse and died of cardiomyopathy after repeated unsuccessful antineoplastic therapy with high-dose cytarabine. It was not completely clear whether cardiac failure was related only to the toxicity of chemotherapy or to additional underlying infection and leukemia, or, as seems probable, to a combination of several factors.

Results are also disappointing when current remission status is evaluated [11]. Only four patients have survived disease-free until now (Table 5).

Table 3. Response to protocol E

	Total	First relapse	Multiple
CR	22 (57.9)	19 (63.3)	3 (37.5)
NR	10 (26.3)	6 (20.0)	4 (56.0)
ED	6 (15.8)	5 (16.7)	1 (12.5)
Total	38	30	8

NR, no remission; ED, early death.

Table 4. Side effects of protocol E

	(*n*)	WHO grading				
		0	1	2	3	4
Nausea/emesis	38	3	10	12	12	1
Stomatitis	38	14	20	3	1	
Diarrhea	38	17	16	4	1	
Fever	37		1	24	12	
Major bleeding	29	—— Not done ——				
Infection	23	—— Not done ——				

Table 5. Current status of 22 patients who achieved remission after protocol E (as of 15 February 1986)

CR	Total ($n=22$)	First relapse ($n=19$)	Multiple relapse ($n=3$)
	(*n*) (%)	(*n*) (%)	(*n*) (%)
Relapse	17 (77.3)	14 (73.7)	3[a] (100)
Death in second CR	1 (4.5)	1 (5.3)	
In second CR	4 (18.2)	4 (21.0)	

[a] Including one patient again in CCR after bone marrow transplantation.

Discussion

Though highly toxic, the proposed protocol has achieved the goal of remission induction in only 60% of our patients with early bone marrow relapse. However, this result had to be expected in the light of the experience of other study groups [1, 4, 9] and taking into account the fact that only patients with the poorest prognostic features had been included. Furthermore, the quality of remission measured in terms of relapse-free interval remained highly insufficient. No significant improvement of prognosis could be obtained in our series, in contrast to results reported in the literature.

The aim of avoiding lethal cardiotoxicity without canceling out anthracyclines failed

in one patient. However, taking into account the extensive exposure to other antineoplastic drugs and additionally applied intensive local radiotherapy, this fatal outcome cannot necessarily be ascribed to cumulative anthracycline toxicity.

Three major conclusions can be drawn from the experience with this induction regimen:

1. Aclacinomycin-A within an intensive reinduction protocol as used in this study is not likely to carry inappropriate risks.
2. Long-term results and the number of therapy-related deaths in our group of patients strongly support the thesis that increasing toxicity is not inevitably followed by better results, but possibly by ineffective treatment.
3. With regard to the course of disease in early relapsed ALL patients, the question of adequate treatment still remains a major challenge in pediatric oncology.

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Prognostic Meaning of Chromosome Aberrations in Acute Lymphocytic Leukemia and Acute Nonlymphocytic Leukemia Patients of the BFM Study Group *

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Introduction

Consistent numerical and structural aberrations of chromosomes are well known as an important additional diagnostic factor in all kinds of leukemia. The prognostic meaning of these abnormalities, however, is still being discussed. To show the influence of chromosomal aberrations on the course of the disease, all cytogenetically investigated patients have to be treated in the same way, for therapy is one of the most important factors of prognosis. The following cytogenetical data were obtained from the analysis of bone marrow blast cells of children treated according to protocols ALL-83 and AML-83 of the BFM study group.

Materials and Methods

Bone marrow samples, mostly received by mail (80%–90%), were washed twice in RPMI 1640 and then either prepared directly or incubated in RPMI 1640 + 20% FCS for a 24-h culture with MTX synchronization for 17 h. The cell suspension was then brought to hypotonic solution (KCl, 15 min) and fixed in a solution of methanol and acetic acid (3:1). After being washed six to eight times, the cells were then dropped on a cold wet slide to spread the metaphases. G-banding was done with Giemsa stain after a trypsine pretreatment 3–5 days later.

Results

Cytogenetic analysis of bone marrow samples of 100 children with acute lymhocytic leukemia (ALL) at diagnosis was performed successfully. Chromosomal abnormalities could be detected in nearly 60%. The most common finding (22%) was a hyperdiploid karyotype with 50 or more chromosomes, whereas pseudodiploidy and hyperdiploidy with 47–49 chromosomes appeared less often (17% and 15% respectively). Hypodiploidy was only seen in three cases (Fig. 1).

Numerical changes were mostly found in common ALL (c-ALL), an immunologic subtype defined by the presence of common ALL antigen (CALLA) and HLA-DR on leukemic blasts. The other immunophenotypes of ALL, however, were found to have a close relationship with pseudodiploidy (Table 1). The consistent structural aberrations are listed in Table 2 and demonstrated in Fig. 2.

In contrast to the results at diagnosis, striking differences became visible regarding ploidy groups at relapse ($n = 31$) (Fig. 1). Samples with a normal karyotype were found in nearly the same percentages as at diagnosis. The number of pseudodiploid cells, however, increased to 25.8%, and hyperdiploidy with 47–49 chromosomes appeared more often (19.3%). On the other

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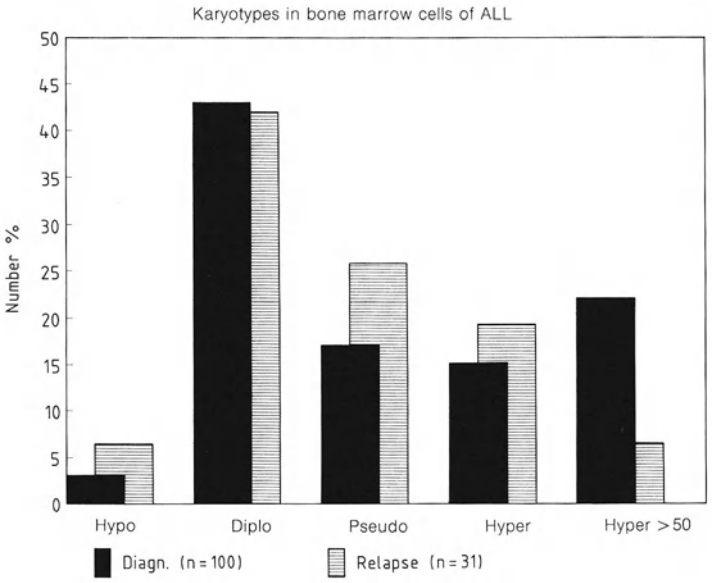

Fig. 1. Cytogenetic findings of bone marrow cells of children with ALL at diagnosis and relapse

Table 1. All patients with different immunophenotypes in the ploidy groups

	Immunophenotype						
	c-ALL		B-ALL	T-ALL	O-ALL	Total of B-, T-, O-ALL	
	(*n*)	(%)	(*n*)			(*n*)	(%)
Hypodiploid	1	1.7	1			1	3.3
Diploid	24	40.7	1	12	1	14	46.7
Pseudodiploid	3	5.1	3	6	3	12	40.0
Hyperdiploid 47–49	10	16.9	1	1	1	3	10.0
Hyperdiploid >49	21	35.6					0.0

hand, the percentage of samples showing a hyperdiploid karyotype of 50 or more chromosomes decreased to 6.5%.

To find out more about the relationship between karyotype and prognosis, the clinical outcome of children in whom the chromosomes were initially investigated was studied. Table 3 includes only those patients who lived without relapse for at least 1 year or who had a relapse. Whereas five out of nine children (55.6%) with pseudodiploid karyotype relapsed and two died, only one patient in each of the other abnormal groups experienced ALL relapse. It might be noted that marker chromosomes were detected in their metaphase plates.

Cytogenetic analysis of bone marrow of children with acute nonlymphocytic leukemia (ANLL) was successfully performed in 37 cases. Most of the samples were pseudodiploid. However, there were no striking differences between them and those with normal or hyperdiploid karyotype, as chromosome numbers of more than 50 were very rare and only one group was seen in this range (Fig. 3).

Regarding structural aberrations, consistent translocations were found which were

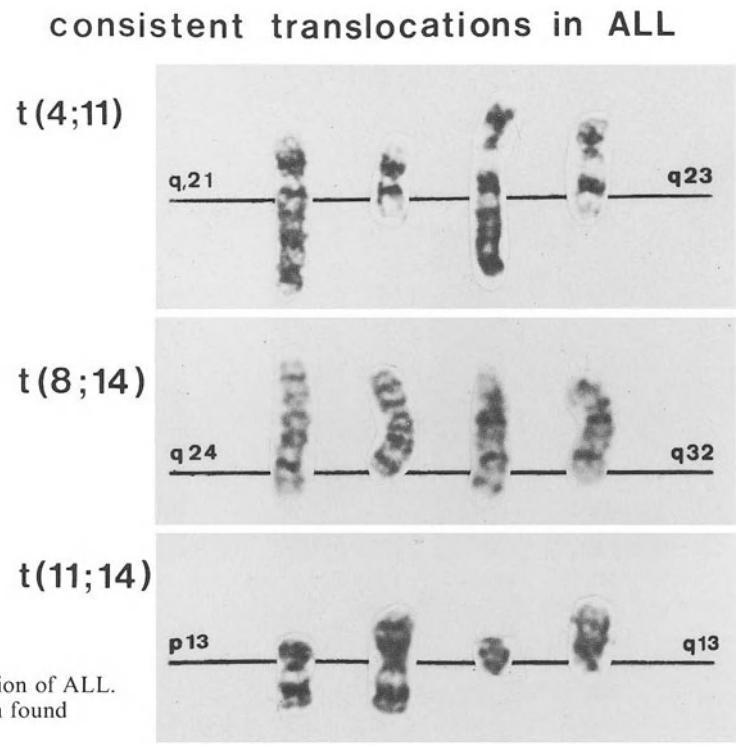

Fig. 2. Consistent aberration of ALL. The t (1; 19) had not been found hitherto

Table 2. Number of cases with consistent aberrations in ALL with different immunophenotype

Aberration	Immunophenotype			
	c	B	T	O
t (4;11)				4
t (11;14)			3	
t (8;14)		5		
t (9; 22)	2			

specific for some of the FAB groups (Table 4, Fig. 4). Whereas t(8; 21) was only found in M1 and M2, t(15; 17) was only found in M3, and t(9; 11) only in M5. Whereas trisomy 8 appeared in M2, M4, and M5, a derivation of the long arm of chromosome 11 [der (11q)] was detected in M4 and M5. Besides these specific abnormalities, several other random aberrations were found, but none of them appeared more than once.

Table 3. Outcome of ALL patients with different ploidy groups (time without relapse, at least 1 year)

	Total	Relapsed		Died	
	(*n*)	(*n*)	(%)	(*n*)	(%)
Hypodiploid			0.0		0.0
Diploid	18	6	33.3	1	5.6[a]
Pseudodiploid	9	5	55.6	2	22.2
Hyperdiploid 47–49	8	1	12.5[b]		0.0
Hyperdiploid >49	11	1	9.0[b]		0.0

[a] Marker chromosome at relapse.
[b] +Marker chromosome.

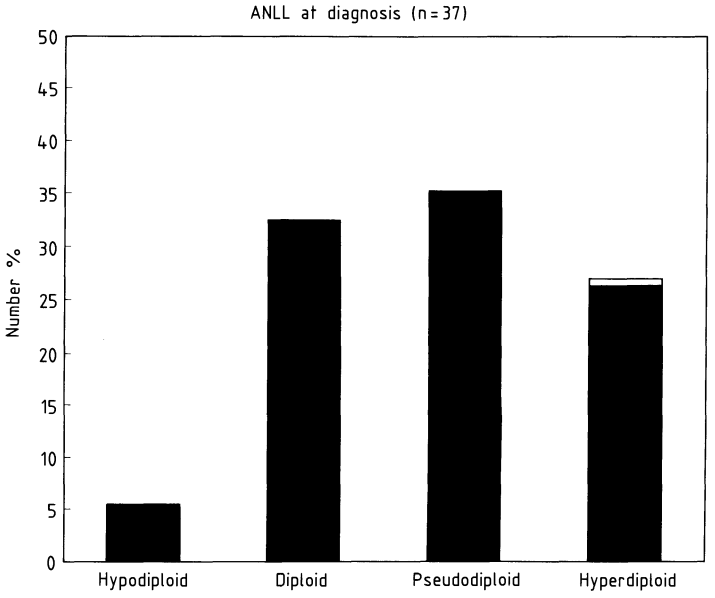

Fig. 3. Cytogenetic findings of bone marrow cells of children with ANLL at diagnosis

Table 4. Consistent and random chromosomal aberrations in FAB groups of ANLL

	Aberrations in ANLL in the different FAB groups: AML study group (BFM 83)					
	M1	M2	M3	M4	M5	M6
t (9;22)	–	–	–	1[a]	–	–
t (8;21)	2	4	–	–	–	–
t (9;11)	–	–	–	–	3	–
t (15; 17)	–	–	1	–	–	–
inv (16)	–	–	–	–	–	–
+8	–	1	–	1	2	–
der (11q23)	–	–	–	1	2	–
t (8;10)	–	1	–	–	–	–
t (9;10)	–	–	–	–	1[a]	–
t (2;12)	–	–	–	–	–	1
der (8)	–	–	–	1	1	–
inv (21q)	–	1	–	–	–	–
17p+	–	–	–	–	–	1
Other number	–	1	–	1	–	–

[a] Biclonal leukemia.

Comparing the initial results of chromosome analysis with the outcome of the patient, there is no evidence for a relationship between consistent or random aberrations and relapse or death either within the FAB groups or in total (Table 5).

Discussion

To find out more about the relationship between the karyotype of blast cells and the prognosis of acute leukemias, cytogenetic analysis was performed on bone marrow

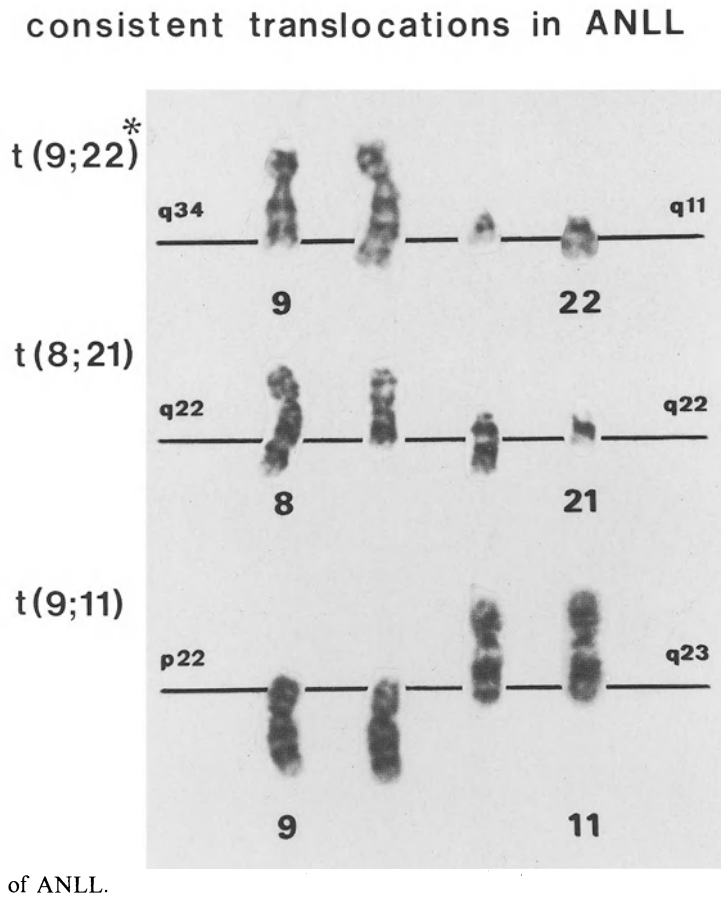

Fig. 4. Consistent aberration of ANLL. The t (15; 17) and inv (16) had not been found hitherto

cells of 100 children with ALL at diagnosis and 31 at relapse. The percentage of samples within the ploidy groups is very similar to those described in other groups [1–3]. Between relapse and diagnosis, however, there is a striking difference within the ploidy groups.

Whereas 22% of all investigated samples had a number of more than 50 chromosomes, only 6.5% were found at relapse. On the other hand, the number of pseudodiploid cells increased in relapses (+8.8%), as did that of hyperdiploids with 47–48 chromosomes (+4.3%). As children with relapse were mostly not investigated cytogenetically at diagnosis, it is not clear whether chromosomal aberrations found in relapse cells were identical to those at diagnosis. Therefore, the outcome of children in whom the karyotype was analyzed at diagnosis was studied. Although there were only a few patients ($n=46$) with a relapse-free survival of at least 1 year, it appears that children with structural aberrations have a worse prognosis: Five out of nine patients with a pseudodiploid karyotype had a relapse (55.6%) and two of them died, whereas there was only one relapse in each group of hyperdiploidy. It might be noted that marker chromosomes were found in the cells of those patients.

The meaning of cells with normal karyotype remains unclear. It is supposed, however, that subtle chromosome changes become visible by means of new banding tech-

Table 5. Aberrations and outcome of patients with ANLL cytogenetically investigated at diagnosis

Chromosomal aberrations in ANLL study group AML-BFM-83						
	No.	Study No.	Date of remission	Relapse	Death	Aberration
M1	04	KI 1	08/08/83	−	−	n
	06	HS 2	09/22/83	−	−	n
	07	GI 5	09/05/83	+[a]	+	n
	15	T 2	12/28/83	−	−	n
	19	N 4	04/01/84	−	−	n
	21	N 5		pr	+	n
	26	KI 3	11/23/84	−	−	n
	34	A 1	02/22/85	−	−	ca t (8;21)
	44	B 9	07/15/85	+	−	ca t (8;21)
M2	10	E 1	01/23/84	−	−	ca t (8;21)
	12	GI 6	02/21/84	+	+	ra
	17	E 2		pr	+	ra
	22	D 5	07/19/84	−	−	ca t (8;21)
	28	GI 9	12/09/84	−	−	ca t (8;21)
	32	MS 6	02/18/85	−	−	ra
	42	T 5	*	pr	−	ra
	47	B 10	*			ca t (8;21)
M3	30	HS 2	02/22/85	−	−	ca t (15;17)
M4	14	MS 5	12/27/83	−	−	n
	24	GI 8	08/26/84	−	−	n
	31	M 16		pr	+	ra
	33	MR 2	03/01/85	−	−	n
	40	M 17	05/01/85	+	+	ra
	43	M 18	06/23/85	−	+	ra
	45	GI 10	*			ca +8
M5	03	GI 3	06/20/83	+	+	ca t (9;11)
	09	KI 2	09/21/83	+	+	n
	11	M 11	10/28/83	−	−	ra
	18	S 1		nr	+	n
	23	E 4	08/28/84	+	+	n
	25	B 7	10/16/84	+	+	n
	29	FB 6	03/10/85	pr	−	ra
	36	U 3	*			ra
	37	E 5	*			n
	39	FB 7	*			ra
	46	GI 11	08/08/85	−	+	ca t (9;11)

*, date of diagnosis after 01/31/85; n, normal karyotype; ca, consistent aberration; ra, random aberration; nr, nonresponder; pr, partial remission.
[a] Chromosome aberration in relapse.

niquès and may show that no normal cells exist in childhood acute leukemia [2].

Karyotyping of leukemic blast cells of children with ANLL was performed on 37 bone marrow samples. The specificity of chromosomal aberrations in the FAB groups became clear, but there is no evidence at the moment for a relationship between karyotype and outcome of patients. Further cytogenetic investigations have to be done in ANLL at diagnosis and especially at relapse.

To find out whether chromosomal aberrations are an independent prognostic fac-

tor, we plan to investigate the outcome of patients with different karyotypes within the same immunologic subgroup of ALL or FAB group in ANLL. Further karyotyping will elucidate the role of random aberrations in childhood acute leukemia.

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Karyotype, Immunophenotype, and Clinical Outcome: Correlations in Childhood Acute Lymphoblastic Leukemia

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The correlation between classical subgroups of childhood acute lymphoblastic leukemia (ALL) (clinical presentation, morphologic and cytochemical characteristics) and immunologic and cytogenetic subgroups has yet to be defined. The present retrospective study was done to establish the prognostic significance of and correlations between clinical, immunologic, and cytogenetic anomalies and outcome.

Materials and Methods

Patients. From May 1984 through September 1985, 30 children with ALL (60% of our patient population) were studied with regard to lymphoblast morphology (FAB), immunophenotyping, and chromosomal analysis of bone marrow blasts. Four patients were excluded because of unsuccessful chromosomal analysis. Low-risk patients were treated according to the French National Protocol: Fralle I (age 24–120 m, white cell count (WBC) less than 15×10^9/liter, Hb less than 10 g/dl, absence of extramedullary leukemia). High-risk patients were treated according to Fralle III (age less than 24 m, WBC greater than 100×10^9/liter or greater than 50×10^9/liter with Hb more than 10 g/dl, mediastinal mass and/or central nervous system or tesicular leukemia at diagnosis). Patients not eligible for Fralle I or III were treated with the Fralle II protocol (intermediate risk).

Immunophenotyping. Surface blast immunophenotypes were determined using conventional antisera and monoclonal antibodies (positive if 40% or more blasts show surface fluorescence). B-cell lineage was defined by the presence of sIg, cIg, and/or presence of antigens CD19, CD21, and CD24. T-cell markers found were CD2, CD5, and/or CD7. Other markers analyzed were CD9, CD10 (CALLA), DR, and TdT. Lymphoblasts with no demonstrable antigens were called undifferentiated; the immunophenotype was called "undetermined" when complete typing was not performed. Cells were considered double lineage when both B and T antigens were present.

Chromosomal Analysis. Cytogenetical studies were performed on bone marrow lymphoblasts after short-term cultures (24 and 48 h) and methotrexate cell synchronization [1]. When material was sufficient, direct technique was also performed. At first, slides were prepared by conventional Giemsa staining and mitosis was localized. Then Q-banding analyses [2] were carried out on the same mitosis. Using this technique, 26/30 (87%) analyses were successful.

Twenty-one patients were studied at the time of initial diagnosis and five patients were studied at the time of bone marrow relapse. A mean of 20 mitoses were analyzed for each patient.

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Table 1. Clinical and biologic data

Name	Age	Sex	Fralle score	FAB	WBC (10^9/liter)	Immunophenotype	Karyotype	Time of *CCR (months)
Patients alive in CCR*								
B CE	6y 12mo	F	II	L1	45.6	DR+ CD 10− CD 7+	46, XX	13
G AS	6y	F	II	L2	6.8	DR− CD 10− CD 2+ CD 5+	46, XX	7
L C	13y 4mo	F	III	L1	12.7	DR− CD 10− CD 7+	46, XX	8
V L	3y 4mo	F	II	L1	2	DR+ CD 10+ CD 19+ CD 20+	46, XX	16
R F	10y 11mo	M	III	L1	120	DR+ CD 10+ CD 19+ CD 20+ CD 21+ CD 24+	46, XY	4
L V	4y 5mo	F	II	L2	451	DR+ CD 10− CD 19+	46, XX	6
E Mo	1mo	M	III	L1	13.9	DR+ CD 10? CD 19+	46, XY	7 (BMT)
L D	3y 7mo	M	II	L1	4.3	DR+ CD 10+	46, XY	20
G M	6y 3mo	F	I	L1	2.1	DR− CD 10− CD 19+ CD 2+	46, XX/47, XX, +8	9
B J	3y 6mo	M	II	L1	14.9	DR+ CD 10− CD 19+	46, XY/55, XY	9
E Ma	3y 10mo	M	II	L1	13.9	DR− CD 10+ CD 19+ CD 24−	46, XY/56, XXY	7
S M	3y 5mo	M	II	L1	3	DR+ CD 10− CD 2+ CD 7+	46, XY/46, XY, 7p−, −9, −20, +3 mar	17
R C	15y 4mo	F	II	L2	9.7	DR− CD 10+ CD 19+	46, XY/46, X, −3, −8, +3 mar	16
Patients alive after relapse								
D S	12y 11mo	M	II	L2	4.4	DR− CD 10+ CD 19+	46, XY, −21, +mar	0
P A	3y	M	II		20.5	DR+ CD 10+ CD 19+ CD 24+	56, XY, +mar/46, XY	0
M S	5y	M	II	L1	49.8	DR+ CD 10− CD 19+ CD 24+	46, XY/46, XY, t(6;12) (q23;p12)	0
P F	6y 6mo	M	II	L1	7.6	DR+ CD 10+ CD 2+ CD 7+	46, XY	0
H E	13y 6mo	M	II		12.7	DR− CD 10− CD 19+	[a]46, XY/65, XXY, 1p+, 2q+, 3q+	12
C J	1y	M	III	L1	120	DR− CD 10+ CD 19−	[a]46, XY/45, XY, t(7;12) (q11;p12), −7, 8q+, 10q−	20
Patients deceased								
B C	9y	M	II		7.5	DR− CD 10+ CD 19+	[a]46, XY	77
P S	11y	M	II	L2	9.4	DR+ CD 10+	[a]46, XY/54, XY	24
B V	7y	M	III	L1	450	DR+ CD 10+ CD 2+ CD 7+	46, XY/46, XY, t(6;7) (q23, q33)	4
S N	1mo	F	III	L1	2000	DR− CD 10− CD 19+	46, XX/47, XX, iso 11q, +mar	3
A MA	5y 6mo	F	I	L1	7.1	DR− CD 10− CD 19+	[a]45, XX, t(9;22), −4, −7, −8, −17, +22, +2 mar	12
N E	13y 3mo	M	III	L1	340	CD 10−	46, XY/47, XY, t(6;16) (q21;q24), +mar	16
R A	9mo	F	III	L1	7.5	DR+ CD 10−	46, XX, t(1;11) (p32;q23)	0

[a] Studied at relapse.
BMT, bone marrow transplantation.
* Complete continuous remission.

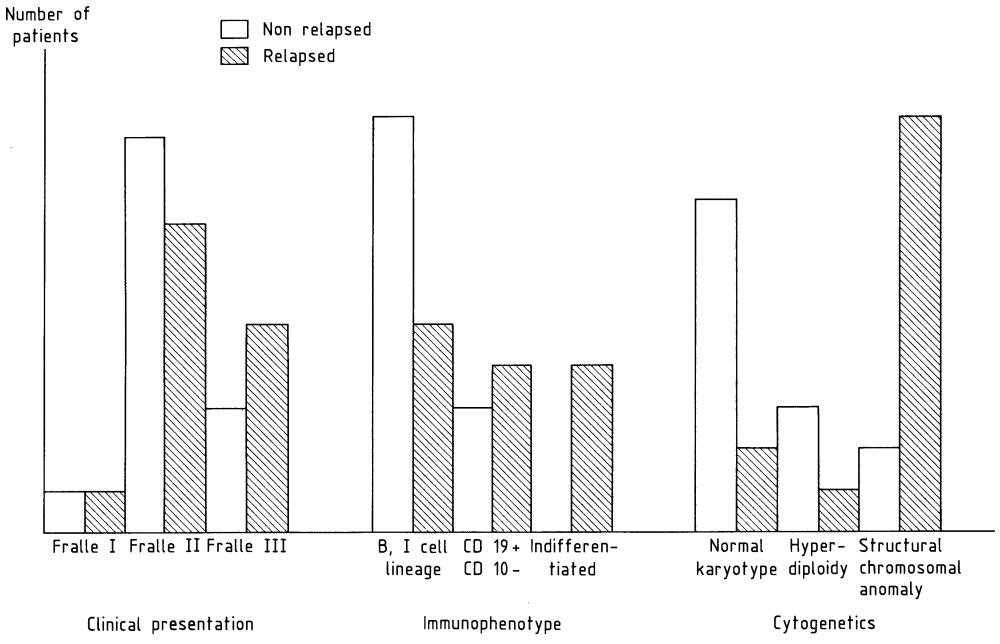

Fig. 1. Distribution of relapsed and nonrelapsed patients according to clinical immunophenotype and cytogenetic subgroups

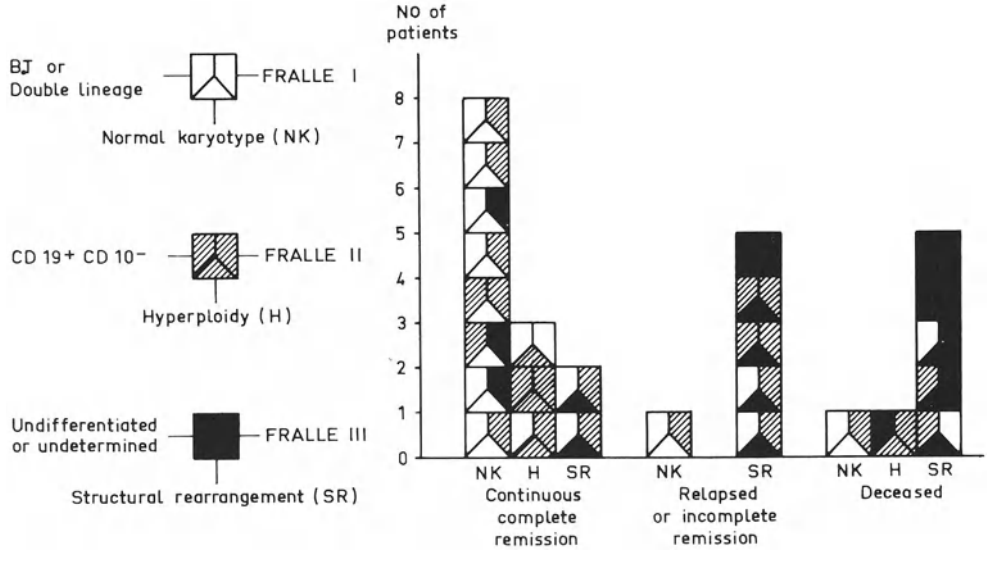

Fig. 2. Correlation between clinical presentation, immunophenotype, karyotype, and outcome

Results

The clinical, immunologic, and cytogenetic data are presented in Table 1.

Twenty-one patients were studied at the time of initial diagnosis: 12 males, 9 females; mean age 6 years 1 month (range 1 month–15 years 4 months). FAB criteria were L1 for

14 patients, L2 for 6 patients, and nonclassified for 1 patient. Mean follow-up was 10 months. Five patients were studied at the time of bone marrow relapse: 4 males, 1 female; mean age 8 years 6 months (range 3 years–13 years 5 months). Two patients were low risk; 16 patients, intermediate risk; and 8 patients high risk. B-cell lineage was found in 14 patients, T-cell markers in 6 patients, and double lineage in 2 patients. Four patients had either undifferentiated or undetermined immunophenotypes. Patients were subdivided into three groups: 15 patients with phenotypic features typical of either B and/or T lymphoblasts cells; 7 patients with B-cell precursors (CD19+ CD10−); and 4 patients with undetermined or undifferentiated lymphoblasts. Ten patients had normal karyotypes (38.5%). Twelve patients had structural chromosomal anomalies of which three were translocations involving the long arms of chromosome 6 (MS, NE, BV) and two, the short arms of chromosome 12 (MS, CJ); one patient had a Philadelphia chromosome (AMA). Four patients had hyperdiploid karyotypes without structural anomaly. Figures 1 and 2 show the correlation between clinical presentation, immunophenotype, karyotype, and outcome.

Discussion

In Burkitt's lymphoma, a clear-cut relationship between clinical, morphologic, immunologic, and cytogenetic anomalies has been found, leading to better understanding of leukemogenesis and to the design of specific chemotherapy protocols. In our sample group, there is no correlation between clinical presentation and immunophenotype, but patients with B- or T-cell lineage generally had normal karyotypes; only two of ten patients had structural chromosomal anomalies, and both relapsed. Five out of seven patients with a CD19+ CD10− immunophenotype had abnormal karyotypes; four relapsed or had incomplete bone marrow (BM) remission. The four patients with undifferentiated immunophenotypes had structural chromosomal anomalies and all have relapsed.

Current patient classification based only on clinical presentation is unsatisfactory and only partially correlates with outcome. Although our sample size is small, there does not seem to be a direct correlation between clinical presentation and chromosomal anomalies since ½ low-risk patients, $^{6}/_{16}$ standard-risk patients, and $^{5}/_{8}$ high-risk patients have structural rearrangements.

In the intermediate-risk group, five out of seven patients with a normal karyotype correlate with continuous complete remission (CCR), whereas seven out of nine patients with an abnormal karyotype have either relapsed or had incomplete BM remission. Only two of the six patients with structural chromosomal anomalies are in CCR. Of our eight patients with high-risk ALL, five have relapsed, all of whom had cytogenetic anomalies, whereas the three patients who remain in CCR all had a normal karyotype at the time of diagnosis. Of patients with ALL 50%–70% have either numerical or structural chromosomal anomalies [3–5]. Cytogenetic anomalies are an independent prognostic factor and are closely correlated with outcome [6–8].

Although long-term follow-up is lacking for our patient population, there is an obvious relationship between structural chromosomal anomalies and poor outcome: only 2 out of 12 patients with structural chromosomal anomalies are in CCR, whereas 8 out of 10 patients with normal karyotypes had a favorable outcome.

It is conceivable that as cancer cytogenetic analysis improves, all or most patients will be found to have cells with abnormal karyotypes, thereby raising the question as to the significance of chromosome change. However, one might hypothesize that this would lead to better subclassification of ALL and subsequently to more specific therapeutic approaches. It is of interest that using standard patient classification, patient AMA was considered low risk. She had, however, a philadelphia chromosome – an extremely poor prognostic feature [9] – and relapsed a year later, never achieving a second remission.

In conclusion, on the basis of our findings, we propose that a better patient classification taking into account chromosomal analysis is a prerequisite for both clarifying the mechanism of oncogenesis and designing more rational chemotherapy protocols.

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DNA Aneuploidy in Children with Relapsed Acute Lymhoblastic Leukemia as Measured by Flow Cytometry

J. D. Beck[1], J. Gromball[1], T. Klingenbiel[2], J. Ritter[3], G. Henze[4], H. Riehm[5], and W. Hiddemann[3]

Summary

An aneuploid DNA stem line has been detected by flow cytometric measurements in 17 (29%) out of 59 children entered in the BFM 83 pilot study for ALL relapse. Of 17 DNA aneuploidies, 15 were hyperdiploid. Euploidy was observed in 28 of 34 patients with an early relapse, whereas 11 of 25 children suffering a late recurrence of disease showed aneuploid DNA stem lines. In contradistinction to ALL relapse, a significantly higher frequency (38%) of pretreatment DNA aneuploidy was measured in 376 newly diagnosed patients of the BFM trials. Our findings may reflect the impact of therapy on leukemic cell clones and their relapse pattern.

Introduction

Conflicting results are reported on the prognostic significance of pretreatment DNA aneuploidy in blast cells of acute lymhoblastic leukemia (ALL). Look et al. [9] found a better treatment response in standard-risk children whose DNA index [1] exceeded 1.16 Hiddemann et al. [7] did not observe differences in remission rates in three BFM ALL studies [79/81, 81/83, and 83/86) among patients with and without DNA aneuploidy. In all three ALL studies, a tendency toward a lower frequency of DNA aneuploidies in children with non-T/non-B–ALL and a high-risk index [7] was detected. For remission duration, however, a significant advantage in long-term remissions was revealed only in the BFM 79/81 study for patients with DNA aneuploidy [7]. These inconsistent findings on the prognostic significance of DNA aneuploidy seem to reflect the impact of different treatment strategies.

Hitherto, one investigation has been published on DNA stem lines in children with relapsed ALL: Suarez et al. [10] detected the same aneuploidy frequency in children with recurrent disease as in newly diagnosed cases. We report here on the aneuploidy pattern of relapsed patients who were entered in the BFM 83 pilot study for recurrent disease.

Methods

Lymphoblasts from heparinized bone marrow aspirates were isolated by a Ficoll-Hypaque gradient (density 1.078 g/ml). After being washed three times in RPMI medium, the cells were fixed in 96% ice-cold ethanol and stored at 4 °C. For DNA measurements the cells were centrifuged for 5 min at 1000 g, resuspended with 1–2 ml 0.5% pepsin HCl solution, and stained with DAPJ (Serva, Heidelberg, West Germany) in a final concentration of 5 μg/ml or ethidium bromide and mithramycin in combination [2].

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For the determination of the DNA stem line [6] and the identification of DNA aneuploidies, every sample was mixed with diploid mononuclear reference cells from normal blood donors in two concentrations. After 15–30 min of staining, the DNA measurements were carried out on a pulse-cytophotometer (ICP 11 or a Partec model) connected to a multichannel analyzer. The appearance of a second G 0/1 peak and its variation according to the number of admixed reference cells indicated a DNA aneuploidy [1].

The BFM 83 pilot study for ALL relapse defines early relapse as occurring within 6 months after cessation of the preceding chemotherapy. Late relapses occur after this deadline.

Table 1. BFM 83 pilot study for ALL relapse

Patients (*n*)			Sex	Site of relapse	
			Male: 102 Female: 56	Isolated bone marrow	76
			Age (years) Range: 3–22 Medium: 9	Isolated CNS	23
				Isolated testis	13
91	44	23		Isolated other organs	11
First relapse	Repeated relapses	No protocol patients		Combined bone marrow	36

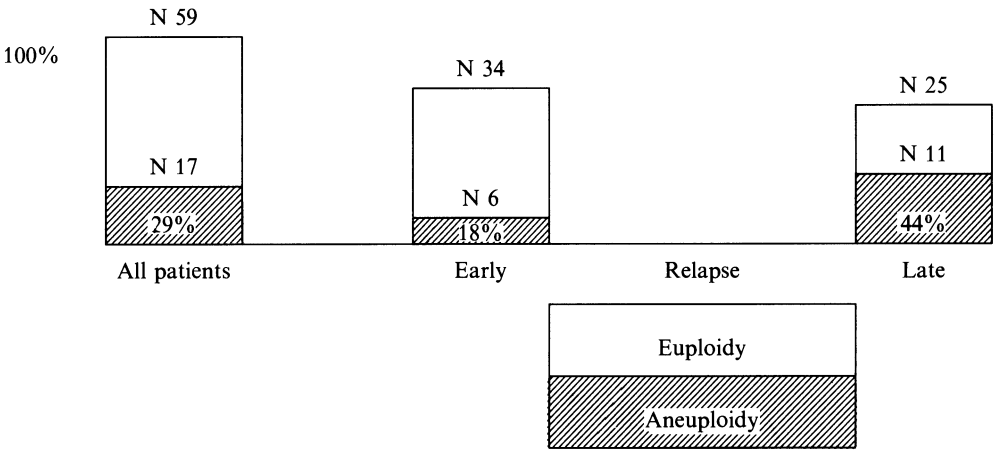
Deadline for admission of new patients was August 1985.

Table 2. Bone marrow blasts of 59 relapsed patients, analyzed by flow cytometric measurements

Patients (*n*)		Sex	Site of relapse	
		Male: 38 Female: 21	Isolated bone marrow	37
		Age (years) Range: 3–20 Medium: 10	Isolated CNS	3
34	25		Isolated testis	0
Early relapse	Late relapse		Other organs	4
No protocol patient were excluded from our analysis			Combined bone marrow	15

An *early relapse* occurs within 6 months after cessation of the preceding chemotherapy.
A *late relapse* is defined as a recurrence after this deadline.

Table 3. DNA stem line in 59 relapsed patients



Results

A total of 158 patients were entered into the BFM 83 pilot study for ALL relapse (Table 1). Bone marrow blasts of 59 patients were analyzed by flow cytometric measurements (Table 2). Lymphoblasts of 17 (29%) children revealed an aneuploid DNA stem line (Table 3). There was no difference between boys and girls. Hyperdiploidy was found in 15 of 17 aneuploid cases. Euploidy was observed in the majority (28 of 34) of early relapses (Table 3), in contradistinction to 11 of 25 aneuploid stem lines in late recurrences.

Discussion

A different frequency of DNA aneuploidy has been observed among children with newly diagnosed ALL (38%) and relapsed patients (29%) in the BFM studies [7]. The latter group, however, is composed of only 59 children, whereas pretreatment results were obtained from 376 patients [7]. There was no difference in DNA stem lines between boys and girls in the relapsed group. Hyperdiploidy was measured in 15 of 17 aneuploid cases. Late recurrences ($n=25$) showed the greatest number of ($n=11$) of the aneuploid stem lines in contradistinction to 28 of 34 euploid measurements in early relapses.

Different results obtained by other investigators comparing DNA stem lines in both ALL groups may reflect the impact of various therapy modalities on leukemic cell clones and their relapse pattern. Suarez et al. [10] conducted the only investigation outside the BFM trials in 34 relapsed children and found the same aneuploidy frequency (40%) in newly diagnosed cases as in patients with recurrent disease. Our own findings and the results of Suarez et al. [10], however, are preliminary because of the small number of relapsed patients who were analyzed. Clinical features [8] and biologic characteristics of blast cells in relapsed children will be compared at the time of initial diagnosis [3–5] and of recurrence in a further study of the BFM trials and may help to identify a specific risk group.

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Hyperdiploid Childhood Acute Lymphocytic Leukemia: Cellular Properties and Prognostic Implications

L. A. Smets[1], H. Behrendt[2], G. de Vaan[3], K. Hählen[4], and F. C. de Waal[5]

Introduction

Alterations in modal DNA per cell content or numerical increases in chromosome number are frequently observed in childhood acute lymphocytic leukemia (ALL). In most instances, these karyotypic changes involve a 20% increase in modal DNA content accompanied by a chromosome number of $n > 50$. This ALL subtype is usually referred to as hyperdiploid ALL (HD-ALL). Whereas in most malignant conditions aneuploidy of either DNA content or chromosome number is a sign of poor prognosis, several studies have assigned a favorable prognosis to HD-ALL [4, 6, 8, 9]. However, negative [3] or even contrary conclusions [5] as to the existence of such a correlation have also been reported.

Our working party has addressed two questions regarding hyperdiploid ALL in children. In its first study, alterations in modal DNA content as detected by flow cytometry were compared with other karyotypic changes and with cell biologic properties. This study [8] has resulted in a more refined characterization of the hyperdiploid category of ALL. Subsequently, the possible prognostic importance of hyperdiploidy was retrospectively evaluated in a multicenter study with 114 standard-risk patients treated according to a standard protocol, with a median observation period of 4.5 years.

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Results

DNA Flow Cytometry. Mononuclear leukocytes from aspirates of bone marrow and peripheral blood were analyzed by flow cytometry of cellular DNA content. A fairly large proportion (>30%) of patients presented with an increased modal DNA content of the leukemic cells. The DNA index (DI), representing the ratio of the DNA values in leukemic versus normal cells, averaged 1.20. The narrow distribution of DI values in HD-ALL patients contrasted with the broad range found in acute myeloid leukemia, lymphoma, or relapsed ALL (Fig. 1). Comparison with clinical data revealed that hyperdiploidy was associated with low leukocyte count and the expression of the CALLA antigen but was unrelated to age, sex, or FAB classification. Hyperdiploidy was rare in high-risk patients (2/45), as defined by a white blood cell count (WBC) $> 50 \times 10^9$/liter.

Karyotypic Changes. Chromosome analysis of HD-ALL revealed a typical and consistent pattern of numerical changes. Tetra- and trisomy of chromosome 21 was observed in all patients of this category, as were increases in a few other chromosomes [8]. This characteristic pattern was confirmed in a subsequent extension of the study (Slater et al., this volume). Thus, a constant increase in modal DNA content is associated with nonrandom increases in specific chromosomes.

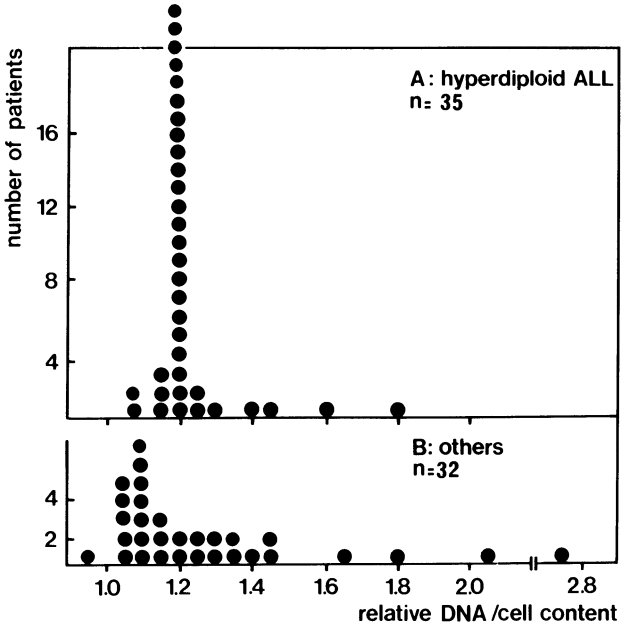

Fig. 1. Distribution of DNA values in untreated children with hyperdiploid ALL *(A)* compared to the DNA values encountered in other hematologic malignancies *(B)*. The value 1.0 represents the DNA/cell content of normal diploid cells. (From [8]. Reproduced by permission of the Br J Haematol)

Oncogenes. Genomic DNA was extracted from leukemic cells and investigated by Southern blot analysis for amplifications or rearrangements of various known oncogenes. In a pilot study with 13 diploid and 7 hyperdiploid patients, no specific changes differentiated the two types of disease. However, in two patients with high-risk features whose disease had a fatal course, activation of the N-*ras* oncogene by a point mutation in codon 12 was detected. These preliminary observations indicate that diploid and hyperdiploid ALL are not discernible by overt molecular biologic changes but that more refined risk classifications, based on oncogenic changes, may well be possible in the future.

Maturation Properties. A striking feature encountered in the peripheral blood leukocytes of HD-ALL patients was a discrepancy between the number of lymphoblasts and cells with hyperdiploid DNA content. On average, the number of hyperdiploids exceeded twofold the number of peripheral blasts, suggesting that many cells of leukemic origin displayed a normal morphology. Velocity sedimentation at unit gravity was applied to separate peripheral blood leukocytes according to size. By this method hyperdiploid cells of lymphocyte morphology, expressing the CALLA antigen, could be separated from hyperdiploid blasts on the one hand and from normal diploid lymphocytes on the other hand (Fig. 2). Apparently, HD-ALL cells are capable of partial differentiation into cells of normal lymphocytic morphology that persistently express the CALLA antigen.

This maturation capacity was expressed by a maturation index, calculated as the fraction "hyperdiploid lymphocytes / all hyperdiploid cells" and represented as a function of peripheral leukocyte count (Fig. 3). From this analysis, two populations were apparent. One subgroup consisted of patients with a relatively low leukocyte count and a high degree of leukemic cell maturation. This category contained seven clinically aleukemic patients with a varying fraction of hyperdiploid lymphocytes in the peripheral blood (maturation index $=1.0$). The other population contained patients with elevated WBC values and a low degree of maturation of peripheral leukemic cells. These data suggest a cause-and-effect relationship between maturation capacity and low peripheral spread in HD-ALL.

Prognosis. In a previous study [8] the prognosis of HD-ALL was more favorable than that of diploid disease. However, the difference was of borderline significance ($p=$

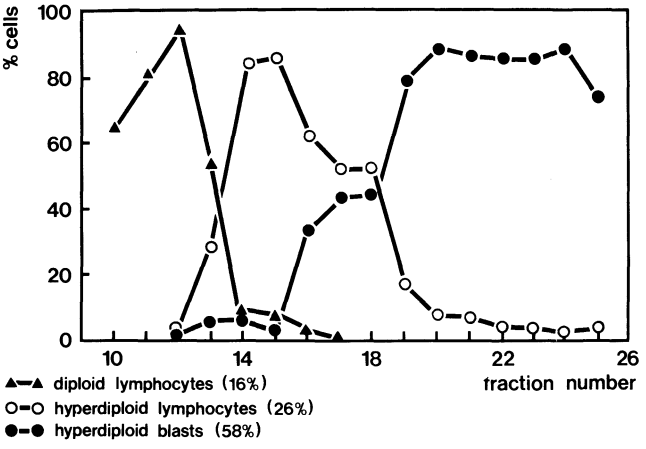

Fig. 2. Separation of peripheral blood leukocytes from a patient with HD-ALL by velocity sedimentation at unit gravity. With increasing fraction number (=cell size), normal diploid lymphocytes, hyperdiploid lymphocytes, and hyperdiploid blasts were successively isolated

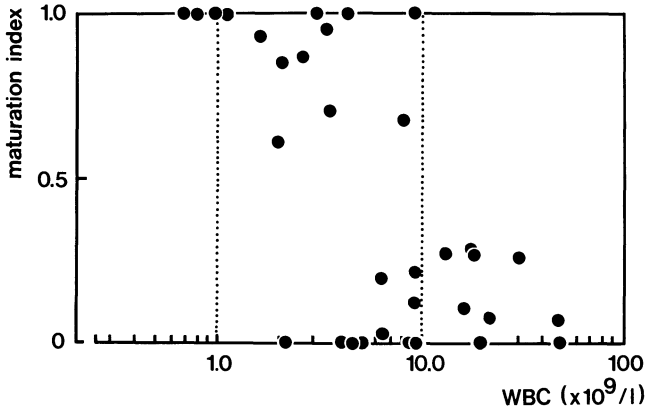

Fig. 3. The maturation indices of peripheral leukemic cells from patients with HD-ALL distributed according to WBC at presentation

Table 1. Frequency of hyperdiploid disease (DNA index 1.15–1.30) and relapse rates in 114 children with ALL. All patients were untreated at diagnosis and belonged to the standard-risk category of WBC (50×10^9/liter). Data were from four Dutch centers collected from January 1979 to April 1984, and follow-up as of February 1986 (median 4.5 years)

	No. of patients (frequency)	No. of relapses (rate)
All patients	114 (100%)	34 (30%)
Diploids	71 (62%)	28 (39%)
Hyperdiploids	43 (38%)	6 (14%)

0.058) and the median observation time was short (24 months). The prognostic impact of HD-ALL was therefore reinvestigated in a multicenter study. Cytophotometric and clinical data of 114 standard-risk patients, collected in four Dutch centers, were combined in this study. All patients had been treated according to protocol ALL-V of the Dutch Childhood Leukemia Study Group. Data were collected from January 1979 to April 1984, and follow-up as of 1 February 1986. The preliminary results of this analysis are summarized in Table 1. The frequency of HD-ALL was high (38%), and the relapse rate in this subcategory (14%) was lower than in diploid disease. In fact, the data indicated a twofold lower probability of relapse for hyperdiploid versus diploid patients. The number of relapses in patients on maintenance therapy and in those off therapy was the same i.e., 17 in each category, and not different between diploid and hyperdiploid disease.

Discussion

Childhood HD-ALL, defined by a DNA/cell content between 1.15 and 1.30 times the

value of normal diploid cells or by chromosome numbers of $n > 50$, is evidently a discrete diagnostic entity associated with specific karyotypic and cell biologic properties. Most larger studies agree that patients in this category enjoy a better prognosis in terms of lower probability of relapse. The absence of prognostic importance of hyperdiploidy in the BMF-ALL 81/83 study [3] may well be explained by the superior overall treatment results in this study. On the basis of their experience with a large patient sample, Look et al. [4] have proposed that HD-ALL patients be treated with less intensive therapy. This proposal is the more interesting as the diagnosis of hyperdiploidy is conveniently and reliably made in ethanol-fixed cell samples which can be submitted for centralized analysis.

However, there remains the crucial question of whether hyperdiploidy is indeed an independent prognostic factor or whether it clusters with other factors known to be associated with low risk. The latter was the case in study BMF-ALL 79/81 [3], and also in our study [8] HD-ALL was associated with low WBC and the expression of the CALLA antigen. In contrast, the study at St Jude's [4] revealed that hyperdiploidy conferred low risk only on patients with a WBC $< 25 \times 10^9$/liter, and was thus independent of the prognostic impact of low leukocyte count alone. This would mean that other factors than low WBC associated with HD-ALL are responsible for better treatment responses, for instance, the capacity of terminal maturation as described in this report.

Basically, a low risk of relapse signifies either a better response to induction treatment or less likelihood of the emergence of (drug resistant) variants. Although both properties are not unrelated [1], their relative contribution to the outcome of treatment of HD-ALL should be known in order to find out at what stage reduction of treatment might be considered. In model studies [7] we have observed that the cytolytic action of glucocorticoids is restricted to noncycling leukemic cells. Sensitivity to this drug is therefore correlated with the capacity of cell cycle exit. This would link the observed maturtion capacity of HD-ALL with increased sensitivity to glucocorticoid hormones during remission induction. Rapid ablative responses are of known positive prognostic value in ALL [2]. In conclusion, our data confirm that hyperdiploid disease is a fairly large and characteristic subcategory of childhood ALL with a lower probability of relapse. However, the mechanism of protection in this diagnosis and the relationship with other prognostic factors are still unclear and require further investigation.

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Supportive Care

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Intensive Care Therapy for Patients with Hematological Diseases

B. Anger[1], T. Schmeiser[1], H. Sigel[2], and H. Heimpel[1]

Introduction

Treatment of patients with hematological diseases can be complicated by life-threatening problems such as shock, pneumonia, sepsis, or major bleeding. Some of these problems may result from direct effects of a malignant disease or from the cytopenias associated with some of the nonmalignant hematological diseases. But the side effects of bone marrow transplantation, radiation, chemotherapy, and surgery may also harm body defenses and vital organ system. The decision to admit a patient to an ICU should be guided by the patient's prognosis. Patients with a hematological malignancy have very low survival rates once admission to an ICU becomes necessary [1–8]. This report concerns mortality and discharge rates of 84 admission of 77 patients with hematological diseases to the ICU of our hospital.

Patients and Methods

The medial ICU of Ulm University Hospital (8 beds) is a modern, fully equipped facility for the management of dysfunction of vital organ systems. The patients are cared for by house staff of the medical service and are supervised by members of the Division of Cardiology.

Admission records for the period 1 January 1976 to 30 Juni 1984 were reviewed. During this time, there were 4561 admissions, of which 84 were for 77 patients with hematological diseases. Every admission was used for the statistical analysis, even if the patient was admitted twice (5 patients) or three times (1 patient). The following information was searched for: Age, sex, hematological diagnosis, reason for ICU admission, length of stay on the ICU, survival after admission, laboratory parameters at the time of admission or shortly thereafter (pH, pO_2, thrombocyte and granulocyte counts, shock index, 24-h urine volume), clinical syndromes at the time of admission (pneumonia, sepsis, shock, heart insufficiency, renal failure, respiratory insufficiency, major bleeding, coma, graft-versus-host disease, residual malignant disease), and the need for intubation and respirator therapy. For statistical analysis the chi-square test and the log-rank test were used.

The risk-score was constructed by simply adding one point for each of the following risk-factors: pneumonia, sepsis, shock, respirator therapy, and active residual disease in a patient with malignancy.

Results

In all 4561 patients, among them 77 patients with hematological diseases, were admitted to the ICU: 10 patients had a bone marrow transplantation (4 with acute myelocytic leukemia, 3 with acute lymphatic leukema and 3 with aplastic anemia). A further 47 patients had malignant diseases (including 24 with acute leukemia and 7 with myeloproliferative syndromes), and 20 patients had nonmalignant diseases (including 7 with

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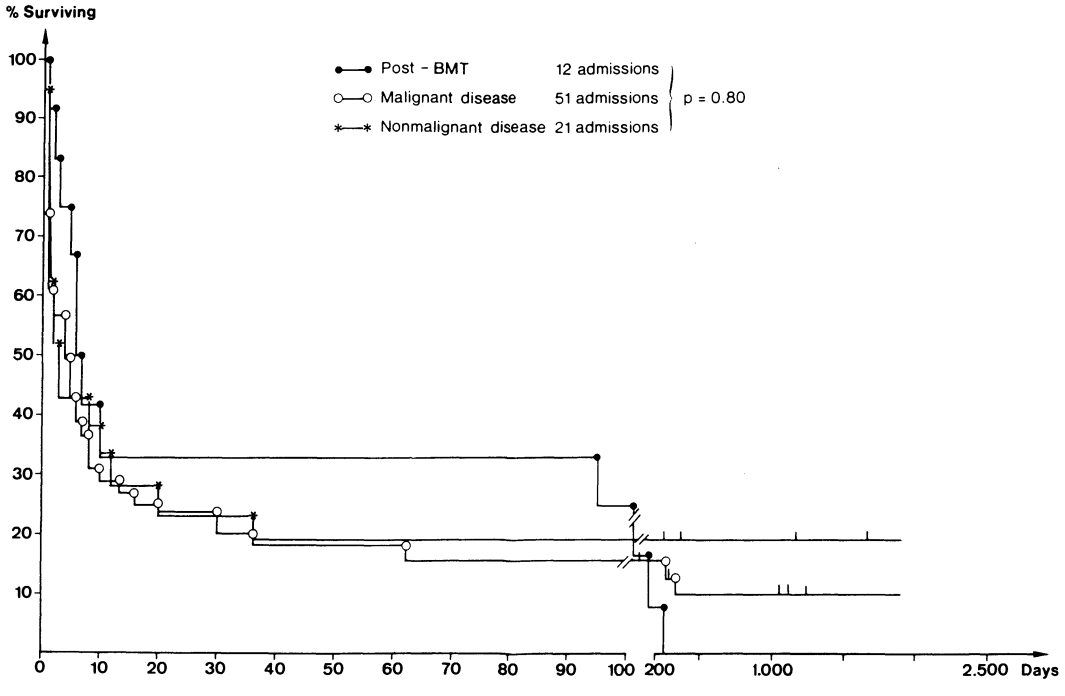

Fig. 1. Survival after admission to the ICU. ●———●, after bone marrow transplantation; o———o, malignant disease; *———*, nonmalignant disease

agranulocytosis and five with immunothrombocytopenia).

Common reasons for admission to the ICU were pneumonia (40%), respiratory insufficiency (40%), shock (38%), sepsis (31%), major bleeding (30%), coma (17%), heart insufficiency (18%), GvH (8%). Granulocyte counts were below 1×10^9/liter in 39% of the patients admitted and below 0.1×10^9/liter in 25%. Thrombocyte counts were below 50×10^9/liter in 52% and below 20×10^9/liter in 25%.

The mortality of the patients with hematological diseases (59 of 77 = 70%) was sig-

Table 1. Influence of clinical syndromes on survival in patients with hematological diseases admitted to the ICU

Factor	I	II	III	Total	*p*
Shock	44% (26/59)	9% (1/11)	29% (4/14)	37% (31/84)	0.025
Pneumonia	47% (28/59)	27% (3/11)	21% (3/14)	40% (34/84)	0.025
Septicemia	39% (23/59)	9% (1/11)	14% (2/14)	31% (26/84)	0.025
Bleeding	29% (17/59)	18% (2/11)	36% (5/14)	29% (24/84)	n.s.
Coma	17% (10/59)	9% (1/11)	21% (3/14)	17% (14/84)	n.s.
Heart insufficiency	20% (12/59)	27% (3/11)	0% (0/14)	18% (15/84)	n.s.
Leucopenia	39% (23/59)	36% (4/11)	43% (6/14)	39% (33/84)	n.s.
Reversal of granulocytopenia during stay on ICU	4% (1/23)	25% (1/4)	83% (5/6)	21% (7/33)	Numbers too small
Respirator therapy	70% (41/59)	36% (4/11)	36% (5/14)	60% (50/84)	0.025
CR/PR in group B patients	17% (6/36)	0% (0/7)	50% (4/8)	20% (10/51)	0.050

Note: I: Death in ICU; II: Death in hospital; III: Left hospital alive.

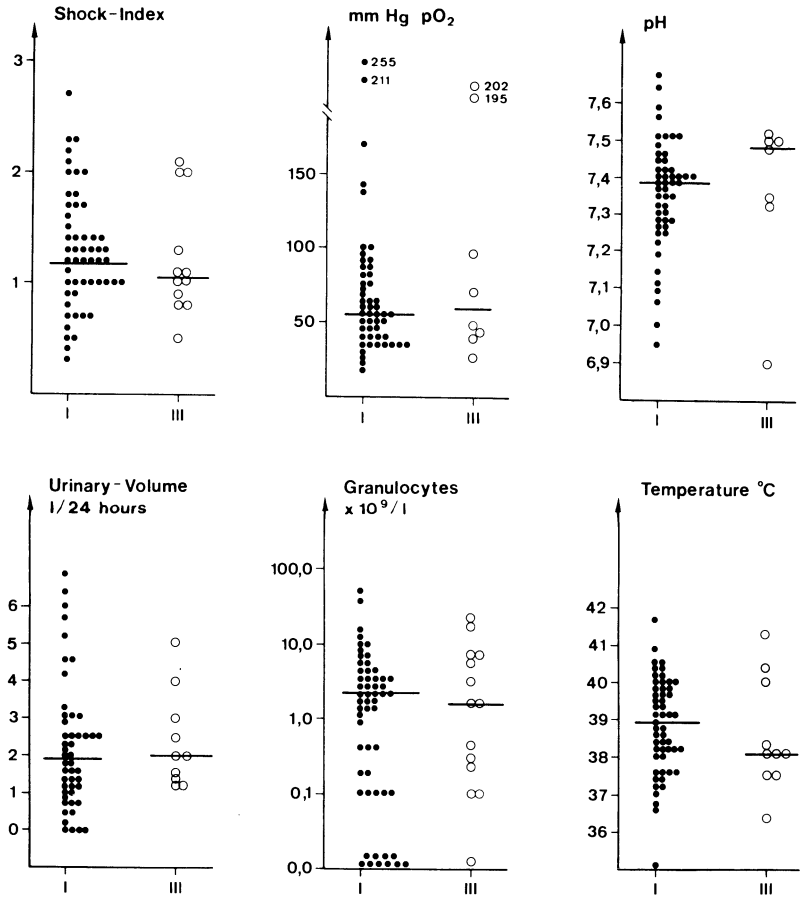

Fig. 2. Laboratory parameters on admission to the ICU. ●, patient died in the ICU; ○, patient left the hospital alive

nificantly higher than the mortality of the patients with other diseases (1205 of 4484 = 27%; $p = 0.0001$). Once admission to the ICU was necessary there was no significant difference in mortality in the ICU for patients with malignant disease (70% died), with nonmalignant disease (66% died), or after bone marrow transplantation (71% died). Long-term survival was also not statistically different (see Fig. 1), but three of the six long-term survivors had nonmalignant hematological diseases.

No significant difference in the values of the admission laboratory parameters was found between patients that died in the ICU and patients that left the hospital alive (see Fig. 2).

Pneumonia, sepsis, shock, residual malignant disease, and the necessity for respirator therapy had a significantly negative influence on survival (see Table 1). The reversal of granulocytopenia during the stay in the ICU may have had a positive influence on survival (statistically not significant because the numbers were too small).

None of the 31 patients admitted with a risk score of 3, 4, or 5 left the ICU alive. Of the 53 admitted with a risk score of 0, 1, or 2, 14 (26%) left the hospital alive (see Figs. 3 and 4). Six patients (11%) in this last risk group were alive more than 1 year after discharge from the ICU.

Discussion

Patients with hematological diseases and patients who have received bone marrow trans-

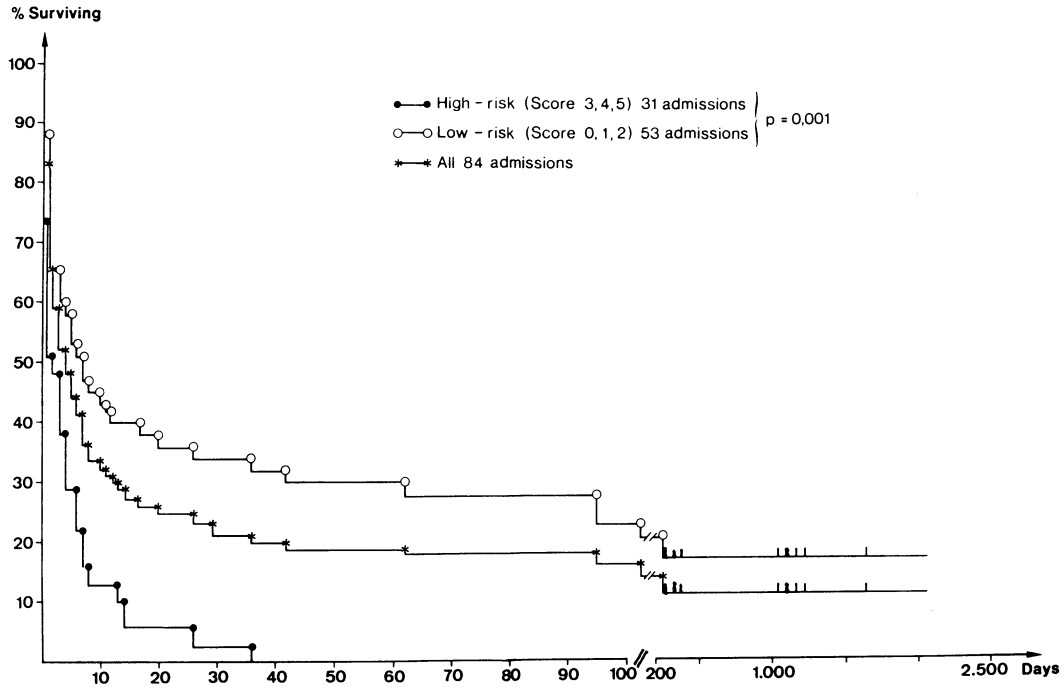

Fig. 3. Survival after admission to the ICU. o———o, low-risk score (0,1,2); ●———●, high-risk score (3,4,5); *———*, all 84 admissions

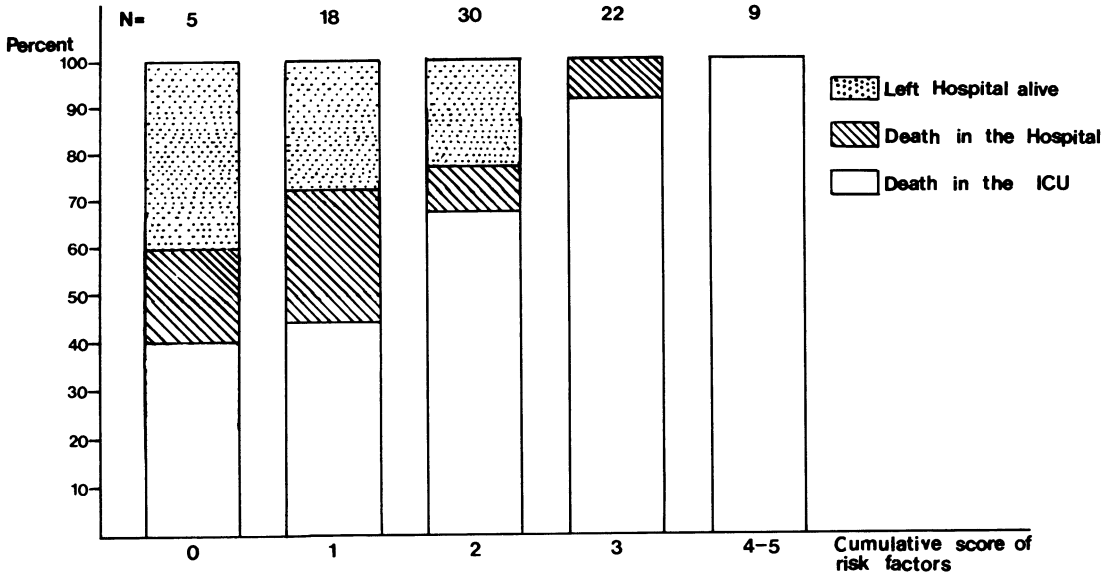

Fig. 4. Survival in relation to the number of risk factor

plantations are prone to life-threatening complications. There can be no doubt that for some of the patients medical intensive care increases the likelihood of survival. The majority of these patients, however, die in the ICU. In our unit the mortality of patients with hematological diseases was 70%, which was much higher than that of patients with other diagnoses (27%). Other groups found mortality rates ranging between 55% and 74% for patients with cancer who required admission to an ICU [1–8]. In a study by Schuster et al., 46 of 77 patients with hematological malignancies (60%) died in the ICU, only 15 (21%) left the hospital alive. These figures are almost identical to what we found [4]. To our surprise, the prognosis of patients with nonmalignant disease was not different from that of patients with malignant disease or from that of patients after bone marrow transplantation.

Laboratory parameters measured at the time of admission to the ICU were not statistically different for survivors and those who died. We identified five clinically detectable risk factors that were associated with a poor prognosis: pneumonia, sepsis, shock, respirator therapy, and residual malignant disease. Survival was not only related to these risk factors individually but also to their cumulative number.

The risk score constructed from these risk factors may allow clinical identification of those patients who are hopelessly ill and will probably not benefit from medical intensive care. Should life support and maximal therapeutic efforts be continued for such patients? This is a serious medical, emotional, legal, and economic question. Although relatively few such patients are encountered in an ICU (1.6% of all admissions to our ICU), their presence generates medical and moral problems out of proportion to their number [9–13]. In our view continued maximal efforts including admission to an ICU constitute a reasonable attempt for patients with malignancy who have not more than two risk factors accumulated and for patients with nonmalignant disease, regardless of their risk score, because most long-term survivors fell in these two categories.

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The Problem of Early Death in Childhood AML *

U. Creutzig[1], K. Stahnke, H. Pollmann, A. Sutor, J. Ritter, M. Budde, and G. Schellong

Introduction

In the last 10 years the treatment results of childhood AML have been improved by intensification of chemotherapy and better supportive care. However, approximately 10%–15% of children could not receive adequate chemotherapy because of early death (ED) prior to or during the first 6 weeks of therapy.

Retrospective analysis of the 37 ED patients from the two German AML studies BFM-78 and BFM-83 indicate that certain prognostic factors are associated with a high risk of early death. Therapeutic strategies which might alleviate these complications are proposed and discussed.

Patients and Methods

A total of 294 children with AML under the age of 17 years entered the AML studies BFM-78 ($n=151$) and BFM-83 ($n=143$) from December 1978 to January 1986.

All children who died prior to receiving protocol therapy and children who died during the first 6 weeks after starting treatment are defined as ED patients.

Hyperleukocytosis is defined by white blood counts (WBC) $\geqq 100 \times 10^3/\mu l$. Extramedullary organ involvement indicates leukemic infiltrations excluding liver, spleen and CNS. Leukostasis was defined as an accumulation of blasts in the vessels [1, 2] and can be proven only by autopsy. Pulmonary signs of leukostasis are: tachypnea, dyspnea with hypoxia, and diffuse interstitial infiltrations revealed by chest radiography [3]. Clinical signs of hyperleukocytosis with or without hemorrhage in the CNS are stupor, delirium, dizziness, tinnitis, ataxia, visual blurring, papilledema, and retinal vein distension. In addition priapism and vascular insufficiency have been reported [4].

Results

Of the 294 patients entered in these studies, 71 did not achieve complete remission, 37 (13%) because of ED and 34 (12%) due to lack of response to the planned treatment. Among the 37 patients with ED, 30 died of hemorrhage and leukostasis within the first 12 days and prior to receiving chemotherapy, while 7 (2%) died of other complications.

Initial hyperleukocytosis and extramedullary organ involvement, liver enlargement, female sex, and age $\leqq 2$ years and $\geqq 10$ years were found more often in ED patients with hemorrhage and/or leukostasis than in patients surviving the first 12 days (for p-values see Fig. 1). The portion of patients with initial CNS involvement, spleen enlargement, low hemoglobin, and thrombopenia was the same in both groups of patients.

Association analysis shows that hepatomegaly is not independent of hyperleukocytosis and that it is also correlated with the factor female sex. Extramedullary organ involvement is correlated with hyperleukocytosis and with age less than 2. Age of more than 10 years may be an independent nega-

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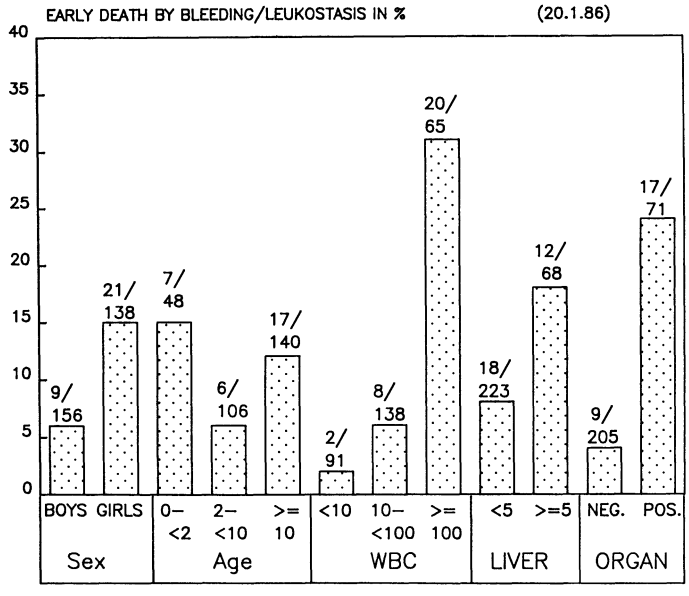

Fig. 1. Dependence of early death on prognostic factors in the AML-BFM studies; P-values (χ^2-test): sex $=0.008$; age <2 years (vs. 2–10 years) $=0.065$; age $\geqq 10$ years (vs. 2–10 years) $=0.084$; WBC $\geqq 100 \times 10^3/mm^3$ (vs. $<100 \times 10^3/mm^3$) <0.001; liver enlargement $=0.023$; organ involvement <0.001

Table 1. ED from hemorrhage and/or leukostasis: Association with FAB types, hyperleukocytosis, and extramedullary organ involvement

FAB	ED/total group		WBC $\geqq 100 \times 10^3/\mu l$		Organ involvement	
	n	(%)	*n*	(%)	*n*	(%)
$M_{1/2}$	6/131	(5)	4/25	(16)	1/20	(5)
M_3	2/9	(22)	–	–	–	–
M_4	4/72	(6)	3/22	(14)	3/20	(15)
M_5	18/72[a]	(25)	13/18[a]	(72)	13/30[a]	(43)
Others	–/10	–	–	–	–/1	–
Total	30/294	(10)	20/65[a]	(31)	17/71[a]	(24)

[a] χ^2-test $p \leqq 0.001$.

tive factor. Additionally, the morphological subtype M_5 is a negative factor for ED from hemorrhage and/or leukostasis (Table 1).

Of 30 EDs due to fatal hemorrhage and/or leukostasis, 18 (60%) occurred in children with M_5 type. For patients with this subtype and simultaneous hyperleukocytosis, the risk of ED from hemorrhage and/or leukostasis increased to 72%, while 43% of those with M_5 type and simultaneous organ involvement developed this complication.

Risk factors for ED Patients

Besides the initial features already mentioned, the clinical course monitored by decrease or increase of blasts and the coagulation abnormalities, including the reduction of platelets, are important. Children with ED were subdivided into four groups.

Group A: ED before onset of therapy ($n=11$)

Group B1: ED from hemorrhage and/or leukostasis with hyperleukocytosis (in general WBC $\geqq 100 \times 10^3/\mu l$) ($n=12$)

Group B2: ED from hemorrhage with WBC $<20 \times 10^3/\mu l$ ($n=7$)

Group C: ED from other complications ($n=7$) (see Table 2).

Table 2. Clinical data of the ED patients

	n	Age years median (range)	Sex m:f	WBC	Platelets	FAB		Extramed. organ involvement	Survival days median range	Cause of death			
				$\times 10^3/\mu l$ median (range)		$M_{1/2}$ M_3	M_4 M_5			H	LS	H/LS	Others
A	11	12;6 (0;7–16;6)	4:7	220 (10–350)	79 (16–126)	–	1 10	6	1 (0–6)	6	3	2	–
B1	12	12;2 (0;6–15;10)	5:7	176 (42–370)	38 (10–160)	4 –	2 6	8	3 (2–9)	5	–	7	–
B2	7	2;0 (0;4–13;8)	0:7	11 (2–18)	20 (3–118)	2 2	1 2	3	10 (4–12)	7	–	–	(2)
C	7	8;5 (1;10–15;2)	6:1	43 (2–245)	23 (8–220)	1 2	3 1	1	37 (17–41)	(2)	–	–	7

H, hemorrhage; *LS*, leukostasis; *H/LS*, hemorrhage and leukostasis; *others*, see text.

Group A. There were 11 children who died before treatment. The median initial WBC was $220 \times 10^3/\mu l$, while the median platelet count was $79 \times 10^3/\mu l$. Of these 11 patients, 10 had monocytic leukemia (FAB M_5). An initial diagnosis of ALL had been recorded in 3. Signs of bleeding were noted on admission in 7 of these 11 children, and 2 had symptomatic CNS hemorrhage. Cerebral, hemorrhage was the ultimate cause of death in 6 patients, in 2 of these in association with CNS leukemic infiltrates. One child died of pulmonary hemorrhage. In 2 children, leukostasis or leukemic infiltrations of all organs caused respiratory and myocardial insufficiency. One child with leukemic blasts in the cerebrospinal fluid died after stupor, probably with central inhibition of respiration. There was incomplete information for one child, who developed sudden hypotension and a drop in hemoglobin, indicating hemorrhage as the likely etiology. The three children with leukostasis did not show severe signs of bleeding.

Group B: This group consisted of 19 children who died of hemorrhage and/or leukostasis during the first 12 days of treatment. In *group B1,* among the 12 patients the median WBC was $176 \times 10^3/\mu l$ and 10 of 12 had a WBC $\geqq 100 \times 10^3/\mu l$. In one child with an admission WBC of $42 \times 10^3/\mu l$ the WBC increased to $125 \times 10^3/\mu l$ during the first 2 days of treatment. Another child had WBC of $87 \times 10^3/\mu l$. Leukostasis and leukemic infiltrates together with hemorrhage were the cause of death in seven patients; four had WBC $> 240 \times 10^3/\mu l$. In the remaining five patients the fatal hemorrhage occurred at the time of rapid cytoreduction after daunorubicin (3 patients), cytosine arabinoside infusion (1 patient), and leukapheresis (1 patient). Coagulation abnormalities were seen in all patients; four children had thrombopenias ($< 20 \times 10^3/\mu l$).

For the seven children in *group B2* the causes of death were heterogeneous: Two children had the coagulation abnormalities of acute promyelocytic leukemia (APL, FAB M_3), while in two patients bleeding occurrd together with sepsis and pneumonia. Two children did not respond to induction therapy and died early on day 12 of thrombopenic bleeding; autopsy showed leukemic

Table 3. Treatment of 13 patients with hyperleukocytosis and severe hemorrhage and/or leukostasis surviving the first 12 days

FAB	WBC $\geqq 100 \times 10^3/\mu l$	Hemorrhage		Leukostasis	Effective therapy in preventing fatal hemorrhage
		Mild	Severe		
$M_{1/2}$	21	2	4	1	2 × P, H, Ex, HU, PT, Ind
M_4	19	5	6	1	2 × P, H, 4 × Ex, FFP, PT
M_5	5	–	1	–	HU, FFP
Total	45	7	11	2	

P, platelets; *H*, heparin; *Ex*, exchange transfusion; *FFP*, fresh frozen plasma; *PT*, pretreatment (Ara-C/TG); *HU*, hydroxyurea; *Ind*, induction protocol.

infiltrations of all organs. The seventh patient already had cerebral bleeding at admission, probably due to thrombocytopenia.

Coagulation abnormalities were completely examined only in 12 patients at the Children's Hospital in Munster. A low plasminogen level (<60 mg-%) initially or during the first days after admission or after treatment indicated a high risk of fatal hemorrhage, but thrombocytopenia and the parameters prothrombin time, aPTT, fibrinogen, AT III, and fibrinogen degradation products (FDP) did not.

Group C. These seven children died of other complications. Deaths occurred after the first course of therapy on days 17–41, and none occurred before this time. In three children sepsis and aplasia were the cause of death. One child with M_4 type and WBC $245 \times 10^3/\mu l$ received three exchange transfusions for blast reduction and died of uremia and sepsis in aplasia 17 days after admission to the study and after the 8-day induction therapy. Another two children died of thrombopenic bleeding in aplasia. One child probably had a cardiomyopathy after anthracyclines. Two children of this ED group belonged to the subtype APL.

Therapy for Preventing Early Death from Hemorrhage and/or Leukostasis in Children in Groups A and B1

According to the protocols, children with a high WBC were initially given cytosine arabinoside (30 mg/m^2 i.v.) and 6-thioguanine (30 mg/m^2 p.o.) daily to provide slow cytoreduction. Three children did not receive this initial cytoreduction and died of hemorrhage after rapid blast reduction following daunorubicin and cytosine arabinoside infusion, while the WBC increased in four children who were given this pretreatment, and after initiation of the protocol therapy fatal hemorrhage occurred in association with the sudden blast reduction by daunorubicin in three patients and after leukapheresis in one child.

Other prophylactic and therapeutic steps taken were the use of different coagulant agents or anticoagulants or blood components. Blood transfusion caused an increase of the cytocrit (=hematocrit + leukocrit) and subsequent leukostasis and hemorrhage in one child. The bleeding was transiently stopped in one patient by neurosurgical intervention. Leukopheresis and exchange transfusion were done in one child but only after the onset of cerebral hemorrhage.

There were 45 patients with hyperleukocytosis who *survived the initial phase* (Table 3). Eleven had severe but controllable bleeding, three with signs of cerebral hemorrhage. Another two children had symptoms of pulmonary and cerebral leukostasis. Exchange transfusion used effectively in five patients prevented early hemorrhage and/or leukostasis. In four patients thrombopenic bleeding was stopped by platelet transfusion.

Discussion

In contrast to AML in adults, where most of the ED are due to infection [5, 6], early fatal hemorrhages and/or leukostasis are the ma-

jor initial problems in childhood AML. Through our analysis we have identified a well-defined high-risk group for early fatal hemorrhage and/or leukostasis (group A and B1). A second group of patients has different but distinct initial features and heterogeneous problems during induction (group B2 and C).

High risk is associated particularly with children who have M_5 morphology and simultaneous hyperleukocytosis or organ involvement. The prognostic significance of hyperleukocytosis as a risk factor for early death is known mainly in adults with AML [7–9]. The association of monocytic subtypes with extreme leukocytosis and extramedullary organ involvement has been described and the danger of leukostasis emphasized [3, 10]. A correlation with coagulation abnormalities was seen in patients with monocytic leukemia regardless of the age group [11, 12].

The proportion of children with AML of M_5 morphology is higher than that of adults, and more than 50% of children with this subtype are under 2 years of age [13]. Especially in this age group we have seen the combination of M_5 type with extramedullary organ involvement.

Our analysis of treatment in this high-risk group indicates that exchange transfusion is the most effective initial therapy. Parallel use of other prophylactic and therapeutic steps is necessary: intensive care, cautious hydration with exact balance, alkalinization of the urine, and allupurinol to prevent nephropathy. Platelet concentrates are necessary in thrombopenia and fresh-frozen plasma for coagulation abnormalities. Blood transfusions should be postponed, if possible, until the blast reduction is below $100 \times 10^3/\mu l$. This is particularly important in patients with high WBCs, in whom the cytocrit increases after blood transfusion and leukostasis may occur. A gradual reduction in the blast count is likewise important; hydroxyurea or low-dose cytosine arabinoside is recommended.

The second group (B2) of patients with initial fatal hemorrhage and low WBC is heterogeneous, as is the group (C) of patients with ED resulting from other complications. Fatal hemorrhage was seen in children with APL (M_3 type), who are well known to be at risk of bleeding during induction therapy. Most commonly this is due to disseminated intravascular coagulation [14, 15]. Nonresponse to treatment is accompanied by severe long-lasting thrombopenia; these patients die in aplasia after several weeks of treatment (group C) and only exceptionally early on day 12 (two patients in group B2).

Table 4. Definitions of AML children with a high risk for ED from hemorrhage and/or leukostasis

FAB	In association with
M_5	WBC $\geqq 100 \times 10^3/\mu l$, extramedullary organ involvement with even lower WBC
M_1, M_2, M_4	WBC $\geqq 150 \times 10^3/\mu l$ ($\geqq 100 \times 10^3/\mu l$?)
M_3	–
All types	Plasminogen level $<60\%$ initially or during cytoreduction

In other patients hemorrhages occurred together with sepsis or fulminant pneumonia, probably in association with disseminated intravascular coagulation (as seen in one patient of group B2). Other patients died of sepsis in aplasia. Another particular problem in children with a high WBC is the nephrotoxicity due to metabolic disturbances after rapid cytolysis. One child of group C probably died of the metabolic consequences of renal failure.

In conclusion, to prevent ED it is necessary to recognize the risk groups (Table 4) and to start prophylactic treatment at once, because a large proportion of patients are at high risk of early death during the first 3 days after admission.

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Passive and Active Anti-Hepatitis B Immunization of Children with Hematological Malignancies

J. Bogusławska-Jaworska [1], E. Gorczyńska, H. Seyfried, A. Gładysz, and M. Zalewska

Introduction

The risk of hepatitis B virus (HBV) infection is evidently higher in children with hematological malignancies than in the general population [1–4]. Unlike healthy children, a high proportion of those receiving chemotherapy for leukemia and lymphoma who are infected with HBV become chronic carriers [4]. Most of them remain highly contagious throughout the cytostatic therapy. Their infectivity is associated with a frequent occurrence of HBV antigen in their tissues and body fluids and its excretion in saliva and urine [5]. Controlling the HBV infection chain is a difficult problem. The passive and active specific immunization may provide the solution. In this paper we report results of the study on the efficacy of the two different immunization formulae which we used during the epidemic in 1982–1985.

Patients and Methods

Design of Trial

Children admitted to the hospital with acute lymphatic leukemia (ALL), acute myeloblastic leukemia (AML), and non-Hodgkin's lymphoma (NHL) and eligible for this trial had no evidence of present or past hepatitis B infection according to past medical history and screening for HBV antigens HBsAg) and anti-HBsAg. HBsAg-negative subjects received their first injection of anti-HBV immunoglobulin (HBIg) i.m. Sera of the immunized patients were collected monthly for 12 months after starting the trial and examined for the presence of HBsAg, anti-HBsAg, and alanine aminotransferase (ALAT) level.

Forty-two patients were specifically immunized according to the two different schedules (Fig. 1). In years 1983–1984 a total number of 15 children received Hepatect and HB-Vax according to schedule I (group II). These patients received their first dose of Hepatect between weeks 1 and 6 after admission. All the children in group III (28 patients) immunized according to schedule II received the first dose of anit-HB immunoglobulin on the day of admission. Control group I was composed of 33 nonimmunized children admitted to the ward in 1982 (Table 1). All the patients with ALL and AML were treated according to BFM protocols [4, 5]. The children with NHL were treated according to LSA_2L_2 [6]. Repetitive doses of HBIg were given throughout the induction and consolidation therapy, whereas vaccination was performed during the maintenance treatment.

Laboratory Methods

HBsAg screening of children in groups I and II was done by the immuno-osmo precipitation method. In group III, all tests (HBsAg, HBeAg, HBcAg, anti-HBsAg, anti-HBeAg, anti-HBcAg) were perfomed by immunoenzymatic assay (Abbot Laboratories).

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Studied group	Immunisation schedule										
	day 0	1 week	1 mo	2 mo	3 mo	4 mo	5 mo	6 mo	7 mo	8 mo	12 mo
I		HBIg[1]		HBIg		V[1]		V	V		V
II	HBIg[2]		HBIg	HBIg	HBIg	HBIg	HBIg	HBIg	V[2]	V	V
III /control/	-	-	-	-	-	-	-	-	-	-	-

HBIg[1] Hepatect /Biotest/, 10 IU/kg
HBIg[2] Ig anty HBV/Inst. Hematol. Warszawa/, 20 IU/kg
V[1] HB-Vax/Inst. Boehring/age 10 ys: 0.5 ml/kg, age 10 ys: 1 ml/kg
V[2] HB-Vax/Inst. Boehring/age 10 ys: 1 ml/kg, age 10 ys: 2 ml/kg

Fig. 1. Immunization schedule

Table 1. Characteristics of children studied

Primary diagnosis	Treatment group			
	I	II	III	Total
ALL	20	12	22	54
AML	4	1	4	9
NHL	9	2	2	13
Total	33	15	28	76

ALAT levels were determined by the spectrophotometric method and expressed in IU/liter, the upper limit of normal being 25.

Definition of Hepatitis Events

HBV infection (HBV viral event) was diagnosed when two or more sequential blood samples were positive for HBsAg. Hepatitis B infection was divided into the following categories, according to the course of infection: (a) Acute hepatitis B evidenced by elevation of ALAT above 100 IU/liter in at least two consecutive samples. (b) Chronic active B hepatitis was diagnosed by histologic and immunologic exmination of liver specimens taken by oligobiopsy in chronic HBsAg carriers. (c) Fulminant hepatitis was an acute liver failure developing during hepatitis B, associated with coma hepaticum. (d) Category HBsAg included only the cases with normal or sporadically elevated ALAT that did not exceed 100 IU/liter. (e) Category anti-HBsAg comprised the seroconverted patients without any elevation of ALAT.

Sera and Vaccine

Two different preparations of anti-HBV immunoglobulins were used in our studies. In children immunized according to schedule I, Hepatect (Biotest) was administered. In patients immunized according to schedule II the anti-HBV immunoglobulin produced in the Department of Serology, Institute of Hematology in Warsaw (H. Seyfried) was used. In both groups the same vaccine HB-Vax (Inst. Boehring) was given.

Results

Serum Levels of Anti-HBsAg Antibodies

The aim of the study was to find a way of controlling the persistence of anti-HBsAg antibodies which were passively transmitted and the level of these antibodies formed in response to the vaccine. These studies were perfomed only in the group treated according to schedule II. The antibody titers were determined in weeks 1, 2, 3, 4, and 6 after

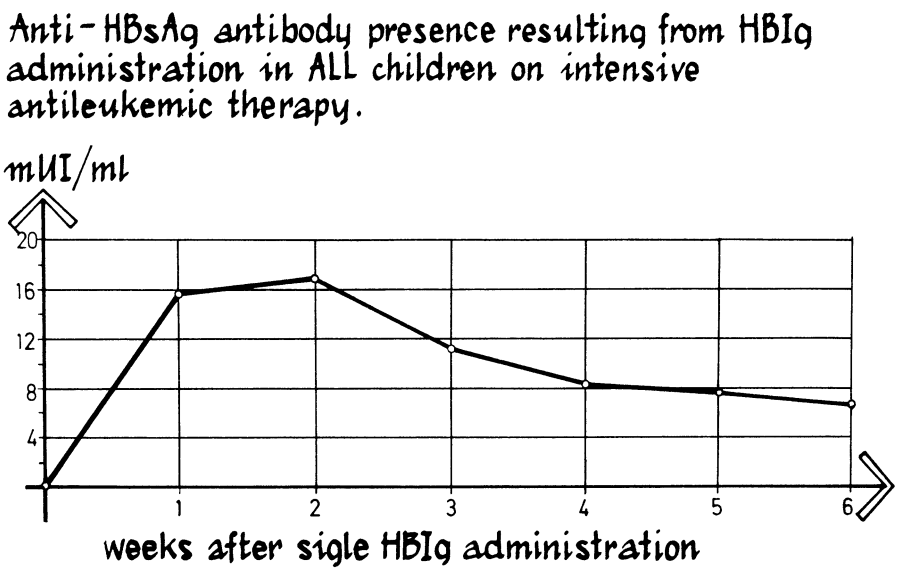

Fig. 2. Anti-HBsAg antibody presence resulting from HBIg administration in ALL children on intensive antileukemic therapy

Table 2. Immune response in children vaccinated during maintenance therapy

ALL $n=7$	
Negative	Positive
5	2 (123 mUI/ml) (466 mUI/ml)

HBIg injection (Fig. 2). The weak antibody titer was present up to the 6th week after injection of immunoglobulin.

In seven children with acute leukemia vaccinated during the remission maintenance therapy the antibody titer was determined for 1.5–3 months after vaccination. Only two patients developed antibodies, one of them after first and the second after third vaccination (Table 2).

HBV Infection

As shown in Table 3 and Fig. 3 in children treated according to schedule I immunization did not influence the HBV infection rate. In this group, as in the control group, 86% of children were infected, with HBV events peaking 4–7 months after admission. However, the advantage of this treatment was that there was only one acute hepatitis event in this group in one patient: The chronic HBsAg carrier developed fatal fulminant hepatitis during the immunologic re-

Table 3. Efficacy of passive and active immunization in clinical observations

Diagnosis	Group					
	I		II		III	
	Total	HBV-infected	Total	HBV-infected	Total	HBV-infected
ALL	20	16	12	11	22	7
AML	4	3	1	0	4	0
NHL	9	9	2	1	2	1
Total	33	28 (84%)	15	13 (80%)	28	8 (27%)

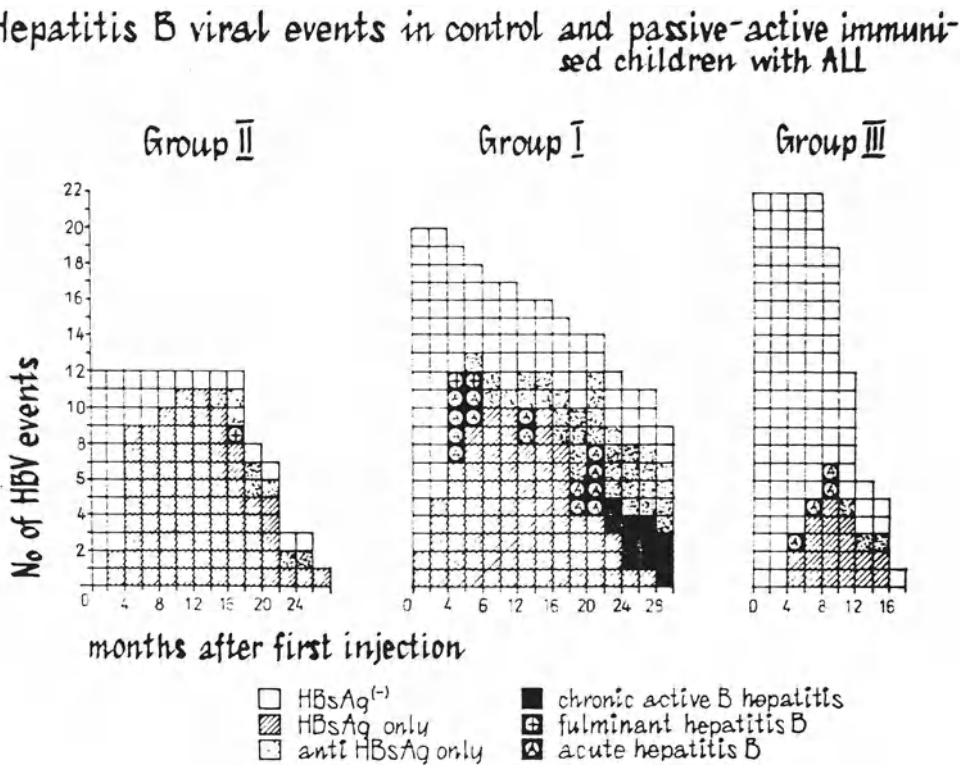

Fig. 3. Hepatitis B viral events in control and passive-active immunized children with ALL

bound 3 weeks after withdrawal of the cytostatic therapy. Much better results were obtained in patients treated according to schedule II, who received five doses of HBIg given at 4-week intervals, beginning from the day of admission. The protection rate in this group was 72%. It was also shown that in the remaining 28% the peak HBV acquisition was shifted to 8 months after diagnosis of ALL (Fig. 3).

Discussion

The aim of our study was to find whether the hepatitis B infection chain in the hematologic unit could be controlled. This seems to have been achieved, with a protection rate of about 70%, by repeated administration of HBIg throughout intensive anticancer therapy. Our results indicate that it is important to start the immunization on the day of admission to the ward. Our formula immunization was not sufficient to eliminate all HBV infection. Acquisition of HBV infection between the 8th and 10th months was observed in about 30% of children who had earlier been efficiently protected by monthly repeated administration of HBIg. It suggests the need for prolongation of the passive immunization period at least in children exposed to contact with contagious HBV carriers.

The results of our vaccination trial are disappointing. The frequency of antibody response was low in our patients despite extension of the administration of vaccine beyond the period of initial intensive anticancer therapy. The low response rate may be attributable not only to the special population of immunocompromised patients but perhaps also to inefficient antigenic potency of the vaccine. We must now consider using another vaccine preparation which can induce the immune response. Low rates of anti-HBs responses to the HB-Vax Institut Boehring vaccine have been reported [9]. In contrast, 88% of dialyzed patients treated

with Netherlands Red Cross Blood Transfusion vaccine produced anti-HBs antibodies [9]. The timing and number of vaccine doses also seems to be important for induction of the immune response, since better results have been reported with four doses of vaccine given at monthly intervals [2, 9].

All these data should stimulate further studies directed at the development of more efficacious vaccine or vaccination schedules for immunocompromised patients.

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Increased Awareness of Aspergillosis in Acute Leukemia Patients

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Introduction

Patients with acute leukemia are highly susceptible to severe infections due to neutropenia, ulcerations and, in addition, the immunosuppressive effects of cytostatic chemotherapy [1]. In the present study, we examined the significance of flexible bronchoscopy combined with bronchial lavage and bronchial brush in the assessment of pulmonary infiltrates in patients with acute leukemia. In addition, we determined the therapeutic implications of these procedures and the outcome, and correlated the clinical data with autopsy findings.

Patients and Methods

Between August 1984 and December 1985, all patients in the Department of Hematology with a rectal temperature above 38.5 °C and newly developed pulmonary infiltrates were entered on study. Fiberoptic bronchoscopy (FOB) was exclusively performed by one examiner (G.H.). Depending on the clinical condition of the patient, initially bronchial secretions were obtained by means of either an unprotected cytologic brush or a protected telescoping brush catheter in eight patients. Subsequently, all patients underwent bronchoalveolar lavage (BAL) of the effected subsegment of the lung with the endoscope in a wedge position.

In all specimens, the following stains were used: Gram H&E, Grocott, auramine, Giemsa. Culture for bacteria, fungi, mycobacteria, viruses; immunofluorescence for *Legionella* spp. (CDC-Atlanta).

The study population comprised 12 men and 4 women, with a mean age of 59 years (28–84 years) (Table 1). All patients had re-

Table 1. Demographic data of patients undergoing fiberoptic bronchoscopy

Patients		16
Age (mean, range in years)		59 (28–84)
Sex ratio (f/m)		4/12
Underlying disease (number)		
Acute lymphocytic leukemia (ALL)		2
Acute nonlymphocytic leukemia (ANLL)		12
FAB classification		
M_1	3	
M_2	2	
M_4	5	
M_5	1	
M_6	1	
Acute undifferentiated leukemia		1
Acute double leukemia (ANLL M_4 – ALL)		1
Cytotoxic chemotherapy[a]		
TAD		8
TAD + aclacynomycine/Vepeside		2
ALL – induction chemotherapy		4

[a] *TAD,* cytosine arabinoside 100 mg/m² i.v. infusion days 1–3, 100 mg/m² b.i.d. days 3–8; Daunoblastin 60 mg/m² i.v. days 3–5; thioguanine 100 mg/m² b.i.d. orally days 3–9.
ALL-induction therapy, prednisolone 60 mg/m² orally days 1–28; vincristine 1.5 mg/m² i.v. days 1, 8, 15, 22; Daunoblastin 24 mg/m² i.v. days 1, 8, 15, 22: L-aspariginase 5000 units/m² i.v. days 1–14.

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Table 2. Microbiological findings established by fiberoptic bronchoscopy, postbronchoscopic sputa, and additional tests

	Source
Monobacterial origin (6 patients)	
Staphylococci, Coagulase negative	BAL, BC
Staph. aureus	BAL, B
E. coli	BAL, BC
Ps. aeruginosa	BAL
Ps. maltophila	BAL
Lactobacilli	BC
Polybacterial origin (1 patient)	
E. coli + *Enterococci*	BAL, BC
Monomycotic origin (4 patients)	
Aspergillus fumigatus (2)	BAL, B/PBS
Aspergillus flavus	BAL
Absidia	PBS
Polymycotic origin (1 patient)	
Aspergillus fumigatus + *C. krusci*	BAL, BC
Polymicrobial origin (3 patients)	
Aspergillus fumigatus + *Pneumocystis carinii*	BAL, BC
C. albicans + *Legionella pneumophila*	BAL, serology
Aspergillus fumigatus + *Legionella pneumophila* (4)	BAL

BAL, bronchoalveolar lavage; *PBS*, postbronchoscopic sputum; *B*, brush; *BC*, blood culture; serology, in one patient candida HA and candida antigen.

ceived cytostatic chemotherapy, immunosuppressive therapy, or steroids with neutrophil counts in the peripheral blood below 1000/μl (mean count 90/μl, range 0–888/μl). All patients were judged to be severely ill, on the basis of fever, tachypnea (above 30/min), cyanosis, and hypoxemia (below 55 mmHg in seven patients tested). The predominant radiological types of pulmonary infiltrates were localized consolidations in 13 patients; only in 3 patients were the infiltrates defined as diffuse and interstitial.

Table 2 summarizes the microbiological diagnoses established by fiberoptic bronchoscopy. In only six patients (38%) pneumonia of monobacterial origin was found. In eight patients (50%), fungi were the significant etiologic agents for pneumonia. Monomycotic pneumonia was identified in four cases, three caused by *Aspergillus spp.* and one by *Absidia*. In one patient, two different fungi were isolated, *A. fumigatus* in BAL and *Candida krusei* in BAL and blood cultures. In three patients, a polymicrobial cause for the infiltrates was found. One patient was suffering from *Candida albicans* and *Legionella pneumophila* pneumonia and one from *A. fumigatus* and *Legionella pneumophila* pneumonia; one patient had an infection with *Pneumocystis carinii* and *A. fumigatus*.

To evaluate risk factors which might make fungal infections more likely in acute leukemia patients, some clinical parameters were calculated separately for the fungal and nonfungal subgroup. No significant differences could be seen between the subgroups in exposure to penicillins, cephalosporins, or aminoglycosides. Patients with fungal disease experienced a higher total number of days with antibiotic therapy and prophylaxis (36 days vs. 31 days). A statistically significant difference could be calculated for total number of days with parenterally administered antibiotic therapy (21 days vs. 15 days: $p = 0.1$). Similarly, the total duration of neutropenia during the entire hospitalization period was longer in the fungal group than in the nonfungal patients. Patients with mycotic disease had a mean of 33 neutropenic days, compared with 24 days in the controls ($p = 0.1$).

Similarly, the only factor which was highly predictive for improvement in the whole study group was the recovery of bone marrow. In six of the seven surviving patients, rising neutrophil counts could be noted during the period of clinical improvement and defervescence. Only one patient improved, despite falling PNM counts in the peripheral blood. In contrast, in eight out of nine deceased patients, persistent profound neutropenia with PNM counts below 100/μl was present. Thus, aplasia was the major risk factor for death in patients with bacterial pneumonia.

Nine of the sixteen patients (56%) died, two of seven (29%) with pneumonia of bacterial origin and six of nine (67%) with fungal pneumonia. In six of nine patients (67%) the infection was the only cause of death due to profound respiratory failure. In five of the nine deceased patients (56%) autopsy was

performed. Fungal pneumonia was the predominant finding in four patients (three *Aspergilli,* one *Absidia*). In one of these patients, bronchoscopic and postbronchoscopic specimens had been negative for *Aspergilli* (false-negative FOB). The lungs were the only organ in which *Aspergilli* could be demonstrated at autopsy. No systemic dissemination could be noted.

Comment

In 16 neutropenic patients with acute leukemia, fiberoptic bronchoscopy, blood cultures, postbronchoscopic sputum, and serologic examinations were performed for evaluation of newly developed pulmonary infiltrates. In 15 out of 16 patients, the pathogenic microorganisms could be evaluated (95%). In ten patients, only one pathogen could be isolated; in five patients, the infiltrates were of polymicrobial origin. *Aspergillus* spp. (*A. fugimatus* 6, *A. flavus* 1) were present in seven patients (43%). This corresponds to the results of McCabe et al. and other authors [2–4]. In three additional patients, *Candida albicans, Candida krusei,* and *Absidia corymbifera* were found. *Pneumocystis carinii* was present in only one case. Ten patients had bacterial pneumonia (63%), and in two patients *L. pneumophila, Pseudomonas* spp. and *E. coli* could be detected. No major complications due to the invasive procedure were noted. The outcome in these patients was not correlated with a specific diagnosis. The only predictive factor for survival was the recovery of bone marrow. Antifungal therapy did not increase the survival rate. Thus, these data underline the significance of fungal pneumonia in acute leukemia patients and support the need for an effective antimycotic prophylaxis in these patients.

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Cytomegalovirus Hyperimmunoglobulin and Substitution with Blood Products from Antibody-Negative Donors. A Pilot Study in Bone Marrow Transplant Recipients

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Introduction

A major cause of death after allogeneic marrow transplantation is interstitial pneumonitis (IP), frequently associated with cytomegalovirus (CMV) infection [1]. It is presumed that most CMV infections are either acquired by transfusion of blood products or represent reactivation of a latent endogenous virus. Because the prospects for treatment of CMV are relatively remote, attention has centered on prevention. Recently, controlled trials of passive immunization for the prevention of CMV disease in bone marrow allograft recipients have been done [2–4].

The results showed that recipients of CMV immune globulin who had no antibodies of their own to CMV (seronegative) and were not given prophylactic granulocyte transfusions had significantly fewer CMV infections after bone marrow transplantation (BMT) than did control patients.

As one of the main sources of CMV infection after BMT is transfusion with blood products from seropositive donors, we tried in this study to eliminate a potential exposure by using blood products from seronegative donors in addition to passive immunization with a hyperimmune globulin. This report deals with the preliminary results of the trial in 19 transplant recipients.

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Materials and Methods

Nineteen patients with acute or chronic leukemia who received allogeneic or syngeneic marrow grafts were included in this study. The conditioning regimen consisted of 120 mg/kg cyclophosphamide and total body irradiation (TBI). The irradiation was applied either as 8.6 Gy as a single exposure or in fractions of 2.5 Gy each on 4 consecutive days.

Methotrexate was used for graft-versus-host disease (GVHD) prophylaxis. Manifest GVHD was treated with cyclosporin A, antithymocyte globulin, methylprednisolone, or a combination of these. Only CMV seronegative donors were selected for blood component substitution. None of the patients received granulocyte transfusions. CMV antibody titres were determined prior to BMT and every 2 weeks thereafter using a complement fixation test and an ELISA technique.

In the case of a patient's death the lungs, liver, and intestine were histologically examined for CMV. If IP was the cause of death cultures to isolate CMV were set up from the lung tissue. The serological diagnosis of CMV infection was based upon a quadruple rise of the antibody titres in the complement fixation test or the appearance of IgG or IgM antibodies detected by the ELISA technique in a previously seronegative patient.

For prophylaxis of CMV a hyperimmune globulin, CMV-Polyglobin, was used in a dose of 2 ml/kg on days -7 and $+13$, $+33$, $+53$, $+73$, $+93$, the day of BMT being counted as day 0.

Table 1. Patient characteristics and CMV serology data prior to BMT

	Sero-negative	Sero-positive
Number of patients	16 (84%)	3 (16%)
Median age (range) (years)	25 (15–41)	33 (17–42)
Sex		
Male	8 (42%)	1 (5%)
Female	8 (42%)	2 11%)
Primary disease		
AML	5 (26%)	1 (5%)
ALL	5 (26%)	1 (5%)
CML	6 (32%)	1 (5%)
CMV serology of donor		
Negative	12 (63%)	2 (11%)
Positive	4 (21%)	1 (5%)
Transfusions (number)		
Red blood cells	6 (2–29)	22 (7–33)
Platelets	10 (2–49)	24 (6–37)

Results

The results of CMV serology before and after BMT are given in Table 1. Of 16 patients who were primarily seronegative, 13 remained so following BMT. Five patients in this group developed IP and in four of them this complication was the main cause of death. In one patient CMV could be cultured from lung tissue at autopsy. Three patients were seroconverted following BMT, and one of them died due to idiopathic IP.

Two of three patients in the primarily seropositive group developed lethal IP. In neither of the two cases could CMV be isolated in culture at autopsy or demonstrated in histological sections.

The incidence of CMV-associated IP was 1/19 (5%), and the overall incidence of IP was 8/19 (42%).

With the exception of one case all other interstitial pneumonias were idiopathic. In four patients IP developed following acute GVHD. The incidence of IP was not significantly different in the single exposure TBI group (40%) as compared with the fractionated TBI group (44%).

Table 2 gives the incidence of IP in relation to CMV serology data after BMT, the occurrence of acute GVHD, and the TBI modality used.

Discussion

CMV immune globulin is effective in the prevention of CMV infection when given to seronegative patients with marrow transplants who have not received granulocyte transfusions. The effect was greatest in patients whose marrow donors where also seronegative [3].

Other studies have demonstrated that anti-CMV IgG, when given prophylactically during the period of highest risk, significantly reduce CMV-related IP in seronegative and positive recipients of allogeneic bone marrow [2, 4, 5].

Asymptomatic CMV infections must be distinguished from symptomatic ones. The true test of prophylactic efficacy should be the prevention of symptomatic CMV infections. This distinction was made by Winston et al. [6], who found that CMV immune plasma prophylaxis did not prevent asymptomatic CMV infection after BMT but did significantly reduce the incidence of symptomatic CMV disease. Our findings are in

Table 2. The incidence of idiopathic and CMV-associated IP in relation to CMV serology, occurrence of acute GVHD and TBI modality

	Idiopathic IP	CMV-associated IP	IP (total)
Negative serology	4/13 (31%)	1/13 (7%)	5/13 (38%)
Seroconversion	1/3 (33%)	0/3 (0%)	1/3 (33%)
Positive serology	2/3 (66%)	0/3 (0%)	2/3 (66%)
Acute GVHD	3/9 (33%)	1/9 (11%)	4/9 (44%)
No GVHD	4/10 (40%)	0/10 (0%)	4/10 (40%)
Fractionated TBI (4×2.5 Gy)	4/9 (44%)	0/9 (0%)	4/9 (44%)
Single exposure TBI (1×8.6 Gy)	3/10 (30%)	1/10 (10%)	4/10 (40%)

accordance with the previous study in which the rate of asymptomatic infection was 19%. Only one case (5%) has developed CMV associated IP.

The serologic tests are not sufficient for the diagnosis of CMV-associated IP. In such cases cultures from buffy coat cells or urine should be performed. Another promising method is the detection of early structural proteins of CMV in culture by monoclonal antibodies. In our opinion, three questions will have to be answered in future studies: How far will seropositive patients benefit from a CMV hyperimmune globulin prophylaxis? What is the optimal dose and duration of the prophylaxis following BMT? What is the value of a combined CMV prophylaxis (as done in this study) compared with passive immunization only?

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Prophylactic Application of an Anti-Cytomegalovirus Hyperimmunoglobulin in Allogeneic Bone Marrow Transplant Recipients

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Introduction

Interstitial pneumonia (IP) is still a life-threatening complication after allogeneic bone marrow transplantation (BMT). It is reported to occur in about 40% of all patients [1]. Almost half the cases of IP are associated with cytomegalovirus (CMV), whereas one-third of IP remains idiopathic. CMV-associated IP has a mortality of up to 90% [1].

The prevention of CMV pneumonia by antiviral chemotherapy has proven to be ineffective to date [2]. Several prospective randomized trials using passively administered immunoglobulins against CMV have suggested a positive effect on the incidence and course of CMV infections and CMV-associated IP [3–7]. Nevertheless, no survival benefit has been established, and passive immunization failed to prevent CMV pneumonia in some patients. This might be attributed to an inadequate mode of administration.

In order to assess the prophylactic value of a hyperimmunoglobulin against CMV, we started an open prospective trial including all patients undergoing allogeneic BMT for hematologic malignancies and severe aplastic anemia.

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Patients and Methods

Between 1 January 1984 and 31 December 1985, a total of 44 patients were admitted to our hospital for allogeneic BMT. Two patients were excluded from the study: one for nonmedical reasons, while the second died of septicemia on day 5 after BMT. The remaining 42 patients had a median age of 26.5 years (range 5–44); 22 were women and 20 men. The underlying diseases were ALL in 13 cases, AML in 13, CML in 13, and SAA in 3. Forty-one patients received bone marrow from HLA-A, -B, -C, and -DR-identical, MLC-negative siblings. One patient had a DR-locus mismatch.

For conditioning 30 leukemic patients had cyclophosphamide 60 mg/kg per day on 2 successive days followed by total body irradiation with 10 Gy in a single dose (7.5 cGy/min). The last nine patients had fractionated total body irradiation with 6×200 cGy (25 cGy/min). Patients with severe aplastic anemia (SAA) were conditioned with cyclophosphamide 50 mg/kg per day on 4 successive days. All patients were kept in laminar flow units with skin and mucosal decontamination. No granulocyte transfusions were used. Frozen deglycerolized red cell concentrates were given to keep the hemoglobin above 10 g/dl. Platelet count was maintained above 20×10^9/liter by platelet concentrates mainly from the marrow donor. For prevention of graft-versus-host-disease (GvHD) cyclosporine-A was used.

The CMV hyperimmunoglobulin (Cytotect®, Biotest Inc., Frankfurt, FRG) contains 110 mg/ml protein with 95% mono-

meric IgG. The CMV IgG titer is 50 PEIE/ml. 1 ml/kg was administered i.v. before BMT on day −7 and after BMT on day +13, +33, +53, +73, +100, +120, +150 and +180.

CMV monitoring was performed serologically and by viral cultures. Serum of donors and marrow recipients was tested before BMT either by an ELISA [8] (for patients 1–18) or by an IF assay (Virgo® Reagents, ENL Inc., Columbia, USA) (for patients 19–42). Serological testing was repeated prior to each application of the hyperimmunoglobulin. Samples of urine and buffy coat from the donor were cultivated pre BMT. Samples of urine, buffy coat, feces and saliva of the recipient were cultivated pre BMT and at least every 2 weeks after BMT by conventional fetal fibroblast cultures. In a subgroup of patients kinetics of the hyperimmunoglobulin were studied before and after the onset of GvHD by an ELISA technique [9].

A fourfold increase in CMV antibody titers and/or positive viral cultures after BMT was considered indicative of a diagnosis of CMV infection. An IP was considered to be CMV associated when CMV was cultivated from lung specimen or bronchial lavage or when there was histological evidence for CMV.

Results

Of 42 patients, 26 (62%) are alive a median of 398 days (range 56–763 days) after BMT. Sixteen patients died: four of IP (one CMV associated, three idiopathic), seven of GvHD (four with idiopathic IP at autopsy), one of veno-occlusive disease (VOD), one of bone marrow aplasia, two of leukemic relapse, and one of intracerebral hemorrhage.

CMV infection was documented in 21 patients (50%). Symptomatic infection was seen in eleven patients: two had CMV-IP, one a biopsy proven CMV hepatitis, eight a clinical syndrome. Sixteen episodes of IP occurred in fifteen patients (38.1%). Median onset was on day 60 (range 19–129). Twelve IP were idiopathic (28.6%) and four CMV associated (9.5%). Two of four CMV-IP were documented at autopsy in the patients who died of VOD and intracerebral hemorrhage. The only patient who died of CMV-IP already had active CMV infection before BMT and developed pneumonia on day 41 after BMT [10]. The fourth patient with CMV-IP had interstitial pneumonia on day 19 after BMT and recovered after a therapeutic course of CMV hyperimmunoglobulin [11]. Nevertheless, he died on day 70 of a second episode of IP. Autopsy gave no cultural or histological evidence of CMV in lung, liver, gut, or kidney.

CMV infections were classified according to Table 1. In 29% of all patients there was still no evidence of CMV infection. They amounted to 60% of seronegative patients before BMT. Latent infection was present in 21% of all patients. Primary infection was found in 19%. One of the eight patients of this group had CMV-IP. He had received seropositive platelet transfusions. Reactivation or reinfection was seen in 31%. Three CMV-IP (two with seronegative donors) were included in this group of 13 patients.

The highest incidence of CMV infection (64%) was seen in seropositive recipients of marrow from seropositive donors. The lowest incidence (33%) occurred when both recipient and donor were seronegative. The incidence was 59% in patients with seropositive donors and 40% when the donor was seronegative.

Under 9 years of age one of four patients had CMV-IP, between 10 and 39 years 3/34, above 40 years none. Almost half the patients between 10 and 39 years of age, however, had CMV infection, and all four patients above 40 years.

CMV infection was present in 6/13 patients with ALL, in 8/13 with AML, in 6/13 with CML, and in 1/3 with SAA. CMV-IP was present in 3 ALL and 1 CML patients.

CMV infection occurred in 4/11 patients (36%) with GvHD grade 0–I. None of these patients had CMV-IP, but 1 had idiopathic IP. Of the 31 patients with GvHD II–IV, 17 (55%) had CMV infection, 4 CMV-IP (13%), and 11 idiopathic IP (36%); 81% of CMV infections occurred in this group.

Results of hyperimmunoglobulin kinetics are available for one patient with severe GvHD. Measurements of serum samples before and after the first administration before BMT on day −7 yielded a half-life of 11.5 days. The same measurements on day 39

Table 1. Type of CMV infection according to CMV serology and viral cultures

	CMV serology		Viral cultures after BMT	Number of pts.	Percentage of all pts.	Percentage of pts.	
	Before BMT	After BMT				seroneg. before BMT	seropos. before BMT
No CMV infection	−	−	−	3	29	60	
	−	*	−	9			
Primary infection	−	−	+	2			
	−	*	+	4	19	40	
	−	↑	+/−	2			
Reactivation/reinfection	+	*	+	12			
	+	↑	+/−	1	31		59
Latent infection	+	*	−	9	21		41

Asterisks, passive transfer of CMV IgG antibodies with the CMV hyperimmunoglobulin and/or stable CMV antibody titers; *arrows,* at least four-fold increase in CMV antibody titers after BMT.

after BMT, when severe GvHD of the gut with hemorrhagic diarrhea was present, showed a half-life of 6.8 days.

Discussion

In this prospective open prophylaxis study with an anti-CMV hyperimmunoglobulin, CMV infection occurred in 50% of cases and was symptomatic in 26%. The overall incidence of CMV infection was similar to that in other reports [3, 5, 12]. However, symptomatic infections were less frequent than in control patients in published randomized controlled studies, in whom an incidence of 41%–44% has been reported. In the same studies passive immunization with different anti-CMV globulin preparations lowered the occurrence of symptomatic infections significantly to 6%–21% [3, 4, 7].

Interstitial pneumonia had an incidence of 38.1% and was CMV associated in 9.5%. Meyers et al. [1] found a 16% incidence of CMV-IP in 525 allogeneic BMT patients. Controlled trials with anti-CMV immunoglobulin prophylaxis revealed a significant reduction in the incidence of CMV-IP compared with control patients [3, 5, 6]. Our incidence is in the range of these reports.

Of the 16 patients who died, four had CMV-IP. Two were detected at autopsy in patients who died of other causes. The only patient who died of CMV-IP had an active CMV infection before BMT [10]. A second patient with florid pretransplant CMV infection had a clinical syndrome with proven CMV hepatitis after BMT. Therapeutic use of the hyperimmunoglobulin [11] might have simultaneously prevented the onset of CMV-IP.

Forty percent of the seronegative and 59% of the seropositive patients had CMV infection after BMT. According to Meyers [12] a positive serology before transplant is a major risk factor for CMV infection. The donor serostatus also seemed to have an influence in seronegative recipients. Fifty percent with seropositive and 33% with seronegative donors developed CMV infection. In one case with seronegative donor and recipient, CMV-associated IP occurred. Seropositive platelet transfusions might have contributed to the onset of CMV infection in this patient. Eighty-one percent of CMV infections occurred in patients with severe acute GvHD. With one exception all IP occurred in this group. A multivariate analysis of 545 patients showed a significant increase in the incidence of CMV infections when acute GvHD developed; however, patients with CMV infection did not have an increased risk of acute GvHD [12].

The preliminary results of kinetic studies in a patient with severe GvHD in the gastrointestinal tract revealed a significant

shortening of the half-life of the hyperimmunoglobulin. This could be explained by a substantial enteral loss of the hyperimmunoglobulin or other causes, such as the catabolic state of the patient. Further investigations are needed to evaluate whether another mode of administration of the hyperimmunoglobulin (higher doses, shorter intervals) is required to prevent CMV infections in patients with severe GvHD.

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Incidence and Treatment of Fungal Infections in Neutropenic Patients

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The incidence of bacterial and/or fungal infections in neutropenic patients is high [1–3]. While bacterial infections can be successfully treated with newer cephalosporins or other broad-spectrum antibiotics, fungal infections remain a diagnostic and therapeutic problem [4, 5, 7]. To find out the value of prophylactic treatment with ketoconazole in preventing fungal infections, we investigated the clinical course of neutropenic patients treated prophylactically with either ketoconazole or amphotericin B orally.

Patients and Methods

We investigated the clinical course of 60 patients in 107 neutropenic periods. The underlying diseases were acute myeloid leukemia (39 patients) acute lymphoblastic leukemia (13 patients), and acute megakaryocytic leukemia (two patients). In all 29 males and 31 females were entered on the study. The median age was 49.7 years (15–81 years).

We studied a total of 107 neutropenic periods, 98 of whom were evaluable. The median duration of neutropenia was 26 days (range 5–159).

Administration of Drugs

Amphotericin B was administered pro during 45 neutropenic periods. Ketoconazole was given at a daily dosage of 400 mg during 38 neutropenic periods and at a daily dosage of 600 mg during 15 neutropenic periods.

Results

A total of 20 fungal infections occurred during the neutropenic periods. The predominant sites of fungal infections were lung (9), perityphlic abscess (1), stomatitis with ulcerative esophagitis (1), and fungal sepsis (4); fever due to nonlocalized fungal infection was observed in four patients.

Of 20 fungal infections, 12 occurred 2–4 days after successful treatment of a bacterial infection. The rest were either primary infections or occurred more than 3 days after treatment of bacterial infection.

Six of the fungal infections were observed in the treatment arm with 400 mg ketoconazole daily, i.e., an incidence of 15% for the whole of the treatment period at this dosage. Three fungal infections occurred in the treatment arm with 600 mg ketoconazole daily, the incidence being 15% of the treatment weeks so far.

On the other hand we observed 11 fungal infections in patients treated with amphotericin Borally, which means a 24% incidence in the weeks of treatment.

Fungal infection lead to death in 7 of the 20 patients affected. The causative organisms were *Mucor* (1), *Aspergillus* (1), *Trichosporon* (1), and *Candida* sp. (4).

Thirteen patients were successfully treated with amphothericin and 5-fluorcytosine.

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Discussion

Fungal infection remains a serious problem in the supportive treatment of neutropenic patients [8, 9]. Amphotericin B, the most effective agent in the treatment of fungal infections, causes problems because of its toxicity. Thus, the decision to treat must be based on findings suggesting the presence of a fungal infection. Sufficiently early acquisition of data remains the main problems. Preliminary clinical results published so far seem to show that ketoconazole is effective in preventing fungal infections [2, 3]. Our data suggest that there is no statistical difference in the incidence of fungal infections in the different treatment arms, even with a dosage of 600 mg ketoconazole daily.

The majority of fungal infections occurred after successful treatment of bacterial infections with antibiotics. We therefore conclude that signs of infection after successful antibacterial treatments are highly suggestive for fungal infection. This may be sufficient reason to start treatment with amphotericin B, despite its toxicity.

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First Experiences with a Permanent Catheter System in Acute Leukemia

H. A. Vaupel[1], J. H. Hengstmann[2], K. Straif[3], and M. Westerhausen[1]

Introduction

To reduce complications due to central venous catheterization in the treatment of acute leukemia, we began to implant permanent catheter systems in adult patients suffering from various hematological diseases, on the basis of our positive experience with permanent catheters in the treatment of solid tumors.

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Materials and Methods

From August 1982 to January 1986 20 patients suffering from acute leukemia received a permanent catheter system (Port-A-Cath,

Table 1. Patient characteristics

Patient no.	Initials	Sex	Age	Diagnosis	Implantation	Explantation	Remarks	Duration
1	K.G.	m	18	ALL	7/85		Died in early relapse	5
2	S.E.	f	20	AML rel.	1/83	2/83	Local infection	0
3	T.I.	f	25	AML	2/84		Died in second relapse	14
4	K.T.	m	25	ALL	5/84		Died after BMT	6
5	G.G.	m	26	ALL	6/84		Died in first relapse	9
6	M.K.	f	28	ALL	8/82		Lost to follow-up 8/84	24
7	M.O.	m	31	ALL	1/85			13
8	A..B.	m	31	ALL	5/85	11/85	Prior to BMT	6
9	P.S.	m	36	AML	1/86			1
10	G.P.	f	40	AML	7/85			7
11	M.S.	f	44	AML	3/85	5/85	Local infection	2
12	M.G.	m	45	ALL rel.	4/85			8
13	G.H.	f	45	AML	11/85			3
14	K.S.	m	46	AML	8/85			6
15	M.P.	f	48	AML	12/85			2
16	U.F.	f	58	AML	9/85			5
17	J.M.	m	60	AML	11/85			3
18	H.N.	f	62	ALL	3/85		Died 2/86 from CNS relapse	11
19	W.W.	m	64	AML	11/85			3
20	W.N.	m	64	AML rel.	1/86			1
								129 months

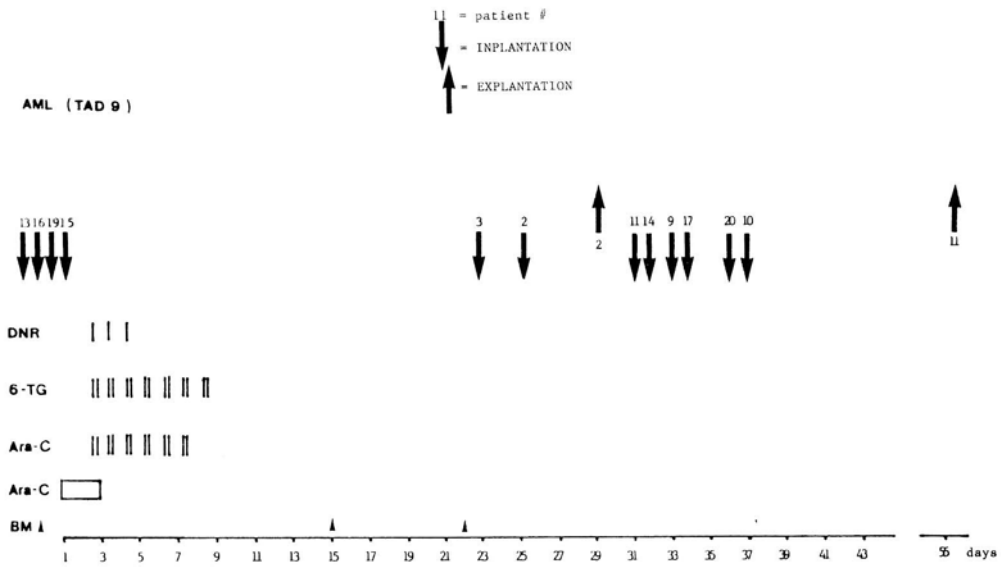

Fig. 1. Dates of implantation (explantation) in relation to ANLL induction therapy

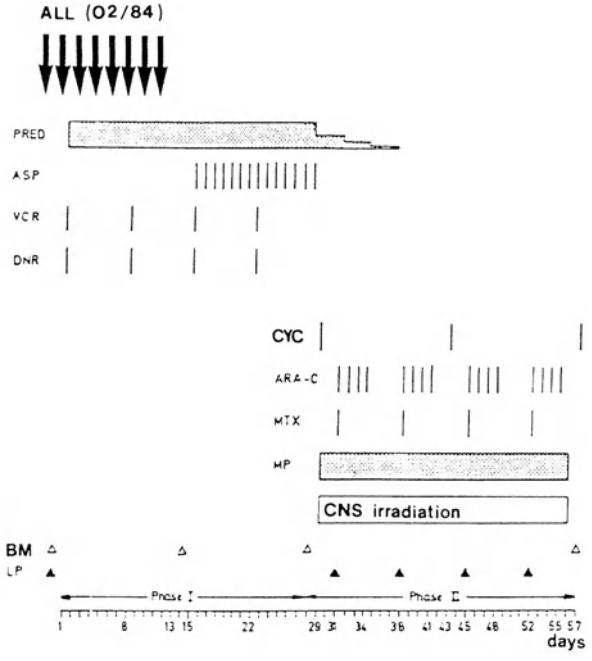

Fig. 2. Dates of implantation in relation to ALL induction therapy

Deutsche Pharmacia, 7800 Freiburg) before, during, or shortly after induction therapy of leukemic disease.

Table 1 shows the patient characteristics and data concerning the time of use of each catheter.

We treated 12 patients with ANLL according to the BMFT-TAD 9 study initiated by Büchner et al. (six female patients aged 20, 25, 40, 45, 48, and 58, mean 39 years; six male patients aged 36, 44, 46, 60, 64, and 64, mean 52 years). The dates of implantation in

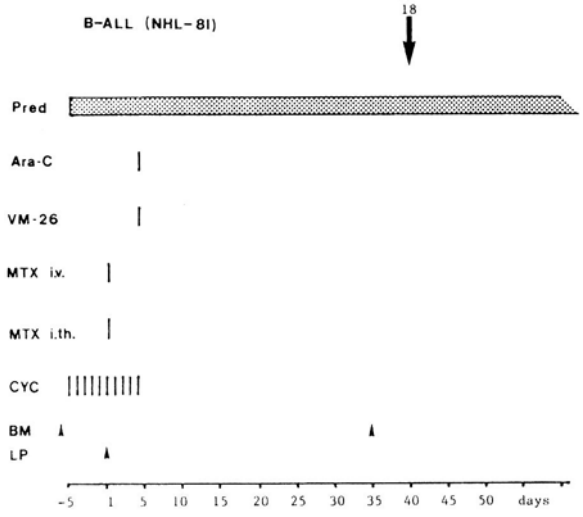

Fig. 3. Date of implantation in relation to B-ALL induction therapy

relation to the treatment of ANLL are shown in Fig. 1. The first implantations (patients 6 and 2) were performed after achievement of a complete or partial remission after initial treatment, confirmed by bone marrow aspiration cytology before an identical course of induction or consolidation chemotherapy.

Seven patients suffering from ALL were treated according to the protocol BMFT 01/81 and the risk-adapted trial BMFT 02/84 designed by Hoelzer et al. In general, the mean age was lower (29 years) than in patients with ANLL, with a high dominance of male patients of younger age (six men aged 18, 25, 26, 31, 45, and one woman aged 28). The implantation was performed at a very early stage of treatment before the beginning of L-asparaginase medication to avoid L-asparaginase-induced bleeding complications (Fig. 2). Therefore, all patients received the catheter system within the first 10 days of treatment.

An elderly woman of 62 years suffering from B-ALL was treated according to the protocol BMFT NHL-81 first utilized for childhood B-ALL by Riehm et al. This patient received the catheter 45 days after the onset of treatment after a long phase of severe pancytopenia (Fig. 3). For implantation we only accepted patients without bleeding or coagulation disorders, who were free of fever and had a granulocyte count above $500/mm^3$ and thrombocyte count above $50\,000/mm^3$.

Results

In 20 cases of acute leukemia (17 previously untreated patients, three patients with first relapses of ANLL) we used a permanent catheter system with an overall period of use of 129 months ranging from 1 to 24 months (mean 6 months).

Complications were seen in two cases and led to explantation of the system. The first catheter (patient 2) had to be explanted after only 4 days. On the day of implantation the patient developed septicemia, which led to local infection at the implantation site. The second system (patient 11) had to be removed after 26 days due to local infection of the implantation site during the first complete remission after consolidation chemotherapy of ANLL. Another catheter was removed in preparation for bone marrow transplantation and replaced by a Hickman catheter without any complications.

During the observation period of 129 months, no cases of thrombosis, perforation, penetration, or leakage occurred. Temporary occlusion was seen in two cases. In

two more cases bacterial contamination without fever was observed.

Discussion

Permanent catheter systems such as Port-A-Cath have been in use since the early 1980s, particularly in patients with solid tumors and those in whom it seems unlikely that venipuncture can be safely accomplished for administration of cytotoxic drugs. Such systems offer the chance of long-term administration of drugs that are harmful to the veins or blood components and also of blood sampling with a reduced risk of the complications seen in catheterization of large veins. We therefore tried them in cases of acute leukemia. On the whole, our results were encouraging and led to the implantation of catheter systems in a very early stage of disease even in ANLL of elderly patients. Our conclusions relating to the use of such systems in acute leukemia are as follows:

1. Catheter implantation should only be performed in patients who are free of fever and of bleeding or coagulation disturbances and have a granulocyte count above 500/mm^3 and a thrombocyte count of more than 50 000/mm^3.
2. The puncture procedure should be performed only by skilled personnel under aseptic conditions.
3. The needle used should remain in the port no longer than 3 days and must then be exchanged.
4. Other fields of application of permanent catheter systems in hematologic disorders are hemolytic disorders, hemophilia, severe aplastic anemia, and myelodysplastic syndromes.

Bone Marrow Transplantation

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On the Fate of Leukemic Cells Infused with the Autologous Marrow Graft *

A. Hagenbeek[1] and A. C. M. Martens[2]

Introduction

Autologous bone marrow transplantation (ABMT) is presently being performed in patients with acute leukemia who lack a suitable bone marrow donor [1–4]. The graft is usually harvested in first complete remission, because at that time (a) the number of leukemic cells is low, i.e., below the detection level, and (b) pluripotent hematopoietic stem cells (HSC) have been subjected "only" to two or three courses of chemotherapy. After freezing in liquid nitrogen, subsequent thawing and reinfusion, HSC are generally capable of fully repopulating the lethally treated host.

As with conventional chemotherapy, after ABMT leukemia relapse remains the major obstacle. A recent survey of results with ABMT in first remission of acute myelocytic leukemia (AML) in Europe showed a 50% relapse rate (European Bone Marrow Transplantation Group; N. C. Gorin, personal communication, 1985). This percentage is likely to increase further as the followup of the majority of patients is still rather short (median follow-up: 1.5 years). However, so far it appears that ABMT is not inferior to chemotherapy as regards survival in the first 1–2 years after treatment.

The origin of a leukemia relapse after ABMT is uncertain. It might be due to residual leukemia in the host, surviving high-dose chemoradiotherapy, or to reinfusion of leukemic cells with the graft, or to a combination of the two. That relapse from residual disease might play an important role is indicated indirectly by the 59% relapse rate observed in AML patients receiving marrow from their identical twins [5].

Finally, at present several groups are attempting to eliminate leukemic cells from autologous grafts in vitro by using either in vitro chemotherapy [2, 6, 7] or immunological methods [8]. To date, results from these studies are unevaluable as there are no data to show that these "purging" procedures are at all effective.

From the above it appears that there are valid arguments for turning to relevant preclinical models to further analyse current clinical problems. In fact, the BN rat acute myelocytic leukemia (BNML) has served for some years now in a number of centers as a model for human AML [9, 10]. As a basis for

Table 1. Questions related to relapse from leukemic cells present in the autologous marrow graft

1. The number of clonogenic leukemic cells surviving cryopreservation
2. The number of clonogenic leukemic cells infused with the graft
3. The fate of reinfused clonogenic leukemic cells (ED_{50}):
   a) Lodging at favorable/unfavorable sites
   b) Regrowth kinetics in microenvironment previously subjected to high-dose chemoradiotherapy
   c) Immunological reactivity of the host

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clinical trials, data have been published on various methods to separate clonogenic leukemic cells from HSC [7].

The present paper tries to answer some of the questions related to a relapse from leukemic cells present in the autologous marrow graft (Table 1).

Experimental Designs and Results

Determination of the ED_{50} for BNML cells

The ED_{50}, i.e., the number of leukemic cells which – after i.v. injection – cause death from leukemia in 50% of the recipient rats, was determined by injecting graded low numbers of BNML cells in groups of normal BN rats (ten rats per group). With the in vitro serial dilution procedure, siliconized glassware was used. As derived from probit analysis, 24.7 BNML cells are needed to cause overt leukemia in 50% of the rats. If 100 or more leukemic cells are injected all rats die from subsequent leukemia.

Influence of Previous High-Dose Chemoradiotherapy on Regrowth of Subsequently Infused Leukemic Cells

Normal rats ($n=8$) were subjected to high-dose cyclophosphamide (100 mg/kg i.p. at day −25; ASTA Werke, Bielefeld, FRG), followed by total-body irradiation (TBI 850 cGy X-rays at day −24; see [11]). To prevent the animals from dying from aplasia, 10^8 isologous normal BN bone marrow cells were injected i.v. directly after TBI. Finally, at day 0, 10^3 BNML cells were injected i.v. Non-pretreated rats ($n=8$) injected with BNML cells on the same day served as controls. As a measure of the growth rate of leukemia the survival times were recorded.

As shown in Table 2, it is clear that heavy previous chemoradiotherapy results in significantly longer survival after a subsequent challenge with leukemic cells. In this respect it should be remembered that "one log less leukemic cells" corresponds to an increase in life span of 4 days.

To exclude the possibility that the lodging of i.v. infused leukemic cells would have changed due to pretreatment, the distribution pattern of ^{51}Cr-labeled BNML cells was studied in the same experimental setting. Figure 1 shows quite similar organ uptake of leukemic cells in cyclophosphamide/TBI pretreated rats and in nontreated controls.

Table 2. Influence of supralethal chemo-radiotherapy in normal rats on growth of subsequently injected leukemic cells (BN acute myelocytic leukemia)

	MST (days)	$\bar{x}$ST (days) ±SE
I. Day −25: 100 mg Cy · kg^{-1} i.p. Day −24: 850 cGy TBI $+10^8$ nBM i.v. Day 0: 10^3 BNML i.v.	37 (35–39)	36.8±0.8
II. Day 0: 10^3 BNML i.v.	31 (29–36)	31.6±0.9

Cy, cyclophosphamide; *MST*, median survival time; *TBI*, total body irradiation; $\bar{x}ST$, mean survival time; *nBM*, normal isologous bone marrow; Student's-*t*-test: I–II: $p<0.001$.
Eight rats per group.

Discussion

The ED_{50} value for human acute leukemia cells is unknown. However, from a variety of experimental rodent leukemias it appears that the minimum number of leukemic cells required to induce leukemia after i.v. transfer ranges from 1 to 10^5 cells [12–14]. For the BNML an ED_{50} of 24.7 cells was found. This significant difference between models might be due to (a) variations in the fraction of in vivo clonogenic leukemic cells; (b) different seeding patterns upon i.v. injection, with different proportions of cells lodging in sites unfavorable for the regrowth of leukemic cells; or (c) immunological reactivity of the host against the injected tumor cells. As far as human leukemias are concerned little is known on these items. From in vitro culture studies with human AML estimations have been made on the fraction of clonogenic leukemic cells, i.e., ranging from 0.1% to 1.0% [15]. No data are available on distribution and lodging of human AML

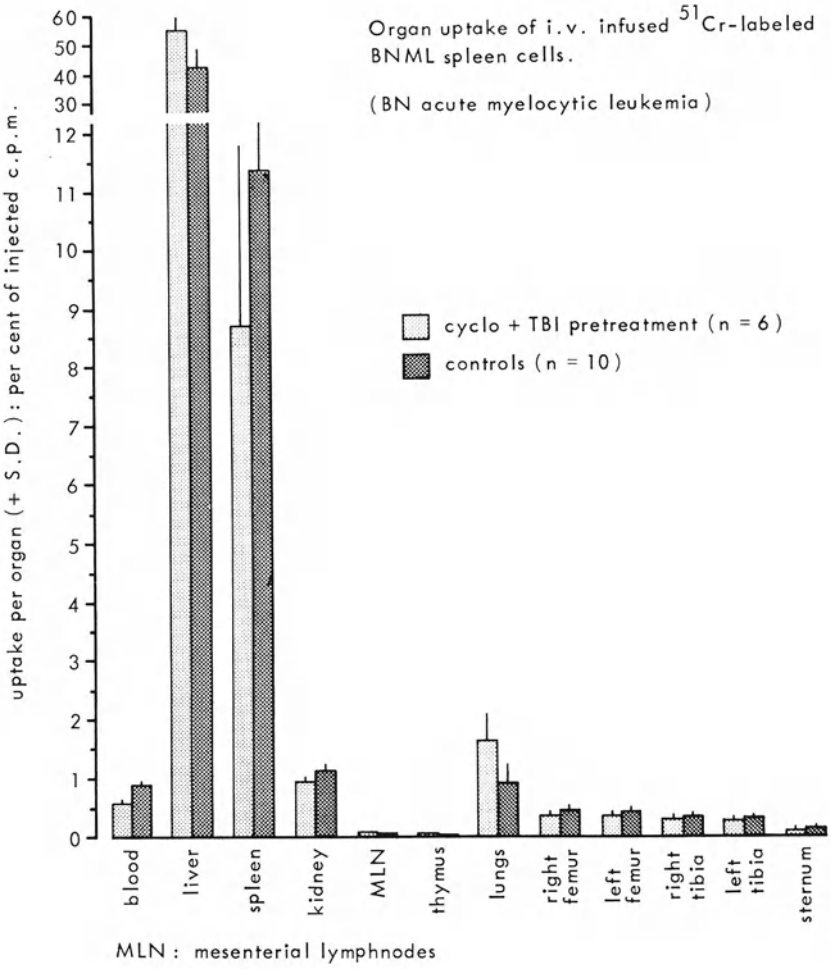

Fig. 1. Pattern of lodging of ^{51}chromium-labeled leukemic cells injected after high-dose chemoradiotherapy in normal rats. The measurements were performed 24 h after injection. Cyclo, cyclophosphamide; TBI, total body irradiation

cells upon i.v. transfer. Finally, as human AML cells, like BNML cells [16], lack specific leukemia-associated antigens, immunological rejection of the grafted leukemic cells by the heavily immunosuppressed host seems most unlikely.

In the rat model presented (BNML) more information is available. First of all, based on concentration experiments combined with assays for clonogenic leukemic cells, using a modified spleen colony assay (LCFU-S), it appeared that all leukemic cells have clonogenic potential [10]. Secondly, the seeding pattern of injected leukemic cells has been well established [17].

From the data presented it appears that previous supralethal high-dose chemoradiotherapy significantly influences the regrowth of subsequently injected low numbers of leukemic cells (Table 2). From Fig. 1 it appears that the lodging pattern is similar in both groups. Thus, three possible explanations remain for the observed differences in survival time. Firstly, in the pretreated group a significant number of leukemic cells might have died in the microenvironment that has been altered by high-dose cyclophosphamide and TBI. This initial kill of infused leukemic cells would then be in the order of 90%–99%, given the linear relationship be-

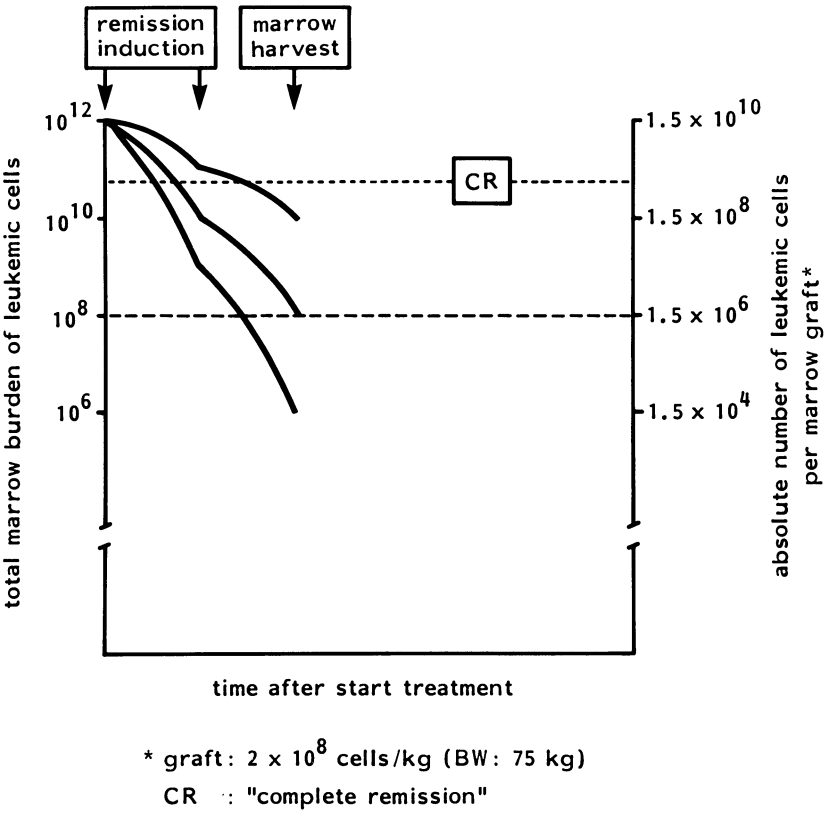

Fig. 2. Relationship between the log leukemic cell kill induced by remission-induction chemotherapy and the AML cell content of the autologous marrow graft

tween the number of injected BNML cells and the survival time, from which it can be deduced that every 4-day increase in life span corresponds with a 1-log decrease in the number of leukemic cells [9]. Secondly, treatment-induced damage to the microenvironment in the organs critical for leukemia growth (the bone marrow, the spleen, the liver) might be the underlying responsible factor for the hampered regrowth of leukemia. This letter hypothesis is supported by (a) an hampered recovery of pluripotent hematopoietic stem cells in the bone marrow of mice pretreated with either cyclophosphamide or ionizing irradiation and by (b) changes in the growth rate of experimental solid tumors implanted at previously pretreated sites.

Thirdly, there is the possibility that 3–4 weeks after chemoradiotherapy and bone marrow transplantation select subpopulations of lymphoid cells may have recovered. These might to some extent influence the outgrowth of infused leukemic cells.

Whether one of these mechanisms is operational after autologous bone marrow transplantation for acute leukemia in man remains to be established. If it were, it would clearly be advantageous.

From experiments to be published elsewhere it has become clear that cryopreservation results in much better survival of normal stem cells (CFU-S) compared with in vivo clonogenic leukemic cells (LCFU-S): about a one-log difference.

The presently available data on human isologous and autologous bone marrow transplantation in first complete remission AML indicate a relapse rate in both groups of 50%. However, given the relatively short follow-up of the ABMT group (1.5 years median), this percentage is expected to increase further. If it is assumed that (a) the distribution of individual tumor loads is

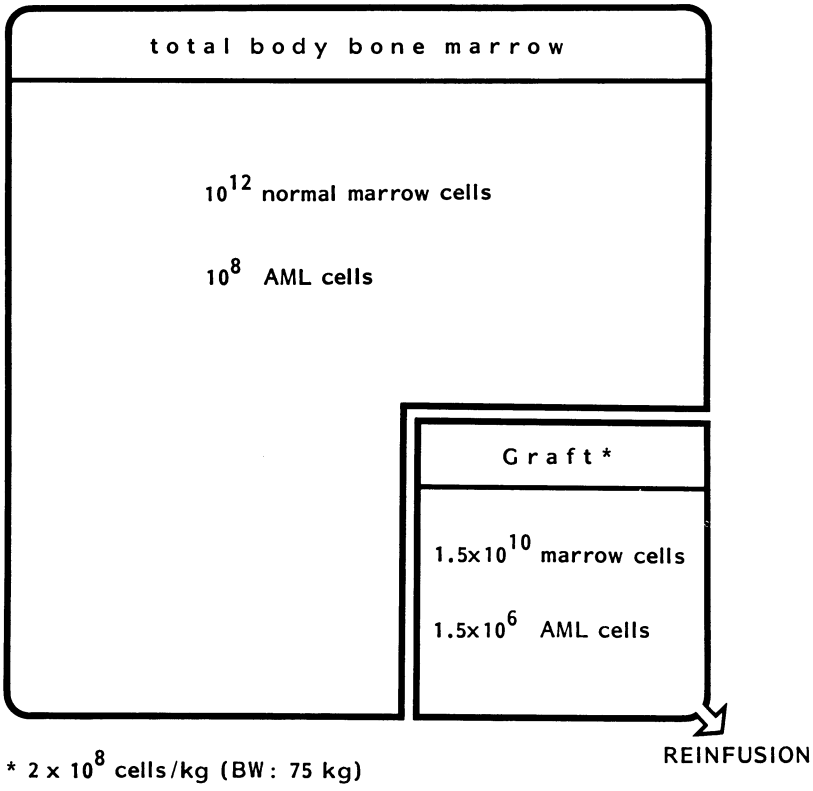

Fig. 3. The composition of the human autologous marrow graft after a 4-log leukemic cell kill by remission-induction chemotherapy

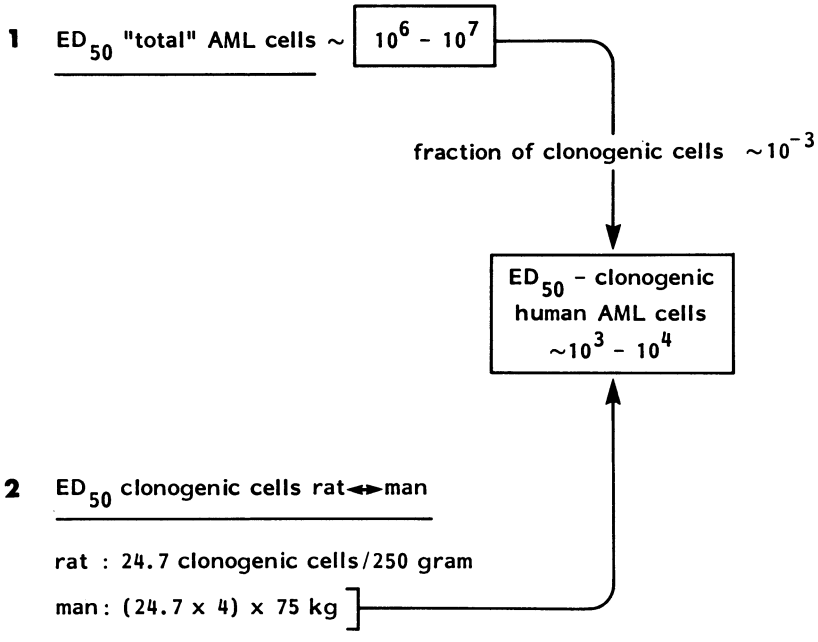

Fig. 4. The number of human in vivo clonogenic leukemic cells which reinduces a relapse in 50% of the recipients (ED_{50}) after autologous bone marrow transplantation: the hypothesis

similar in both groups and (b) the cytoreductive effect of the conditioning regimen is similar too, it should be concluded that the eventual excess relapse that might be observed in the ABMT group is due to reinfusion of leukemic cells with the graft.

Figure 2 shows the calculated maximum number of leukemic cells in the human autologous marrow graft as related to the total body tumor burden at the time of marrow harvesting. Obviously, the number of leukemic cells in the marrow is directly dependent on the efficacy of the preceding courses of remission-induction chemotherapy. From studies in the BNML it appeared that high-dose cyclophosphamide followed by supralethal TBI yields an 8-log leukemic cell kill [11]. If this can be extrapolated to the human situation, patients with a tumor load of 10^8 or fewer leukemic cells should be cured by the conditioning regimen. This would mean that, given a total human marrow compartment of 10^{12} cells, the graft would contain at the most 1 leukemic cell per 10^4 normal marrow cells. Thus, a graft containing 1.5×10^{10} cells (2×10^8 cells per kg body weight; body weight: 75 kg), would hide about 1.5×10^6 leukemic cells, as indicated in Fig. 3. This then would imply that the ED_{50} for human AML cells injected i.v. would be between 10^6 and 10^7 cells at the most. How this number relates to the number of *in vivo clonogenic* AML cells is unknown for man. If only 1 cell per 1000 human AML cells would be clonogenic in vivo [15], the ED_{50} for human clonogenic AML cells would be in the order of 10^3 to 10^4 cells (Fig. 4). This value is significantly higher than the ED_{50} found for the BNML, i.e., 24.7 cells in a rat weighing 250 g. If there is a direct correlation between the ED_{50} value and body weight, a 75-kg man would indeed require $24.7 \times 4 \times 75 = 10^4$ clonogenic leukemic cells to reintroduce the leukemia. Which of the proposed extrapolations is acceptable?

To solve this dilemma, it is crucial that the detection level of residual leukemic cells in remission marrow be lowered. In this way it should be possible to define prognostic factors at the time of harvesting of the graft, conditioning and ABMT related to the tumor load per individual patient. In the rat model, it is now possible to detect 1 leukemic cell per 10000 normal marrow cells using monoclonal antibodies and fluorescence-activated cell sorting (FACS; [18]). If this becomes feasible in human AML, the hypothesis presented in Fig. 4 can be tested.

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Hematological Reconstitution After Autologous Peripheral Blood Transplantation *

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Introduction

The rebound increase in circulating granulocyte-monocyte progenitors (PB CFU-GM) levels during bone marrow recovery from induction therapy in acute leukemia is a known phenomenon [1]. Collection of mononuclear cells by cytapheresis at this time could provide a high number of autologous stem cells for hematological reconstitution after intensive treatment. We have demonstrated that the PB CFU-GM peak after induction treatment coincides in time with that of immature myeloid cells and monocytes, and is preceded by the platelet rise above 100×10^9/liter [2]. These simple hematologic features could indicate the best moment when cytaphereses should be performed.

We describe our first attempt at autologous peripheral blood transplantation in a patient with acute lymphoblastic leukemia (ALL) in complete remission.

Peripheral Blood Cell Harvest

Peripheral blood cells were harvested on days 22 and 23 of the induction course, during the PB CFU-GM peak, using a Fenwall CS 3000 cell separator. Mononuclear cells were separated on Fycoll-Hypaque gradient and washed using a IBM 2991 cell separator. Cells were then suspended in RPMI medium and frozen in 20% DMSO and 4% human serum albumin.

CFU-GM were assayed in a methylcellulose semi-solid culture system with human placental conditioned medium as the source of colony-stimulating factors as previously described [2]. Results of the two cytaphereses are given in Table 1.

Table 1. Results of the two cytaphereses performed on days 22 and 23 of the induction course

	Day 22	Day 23
Patient values		
Leukocytes ($\times 10^9$/liter)	12.6	26.9
Immature cells ($\times 10^9$/liter)	3.5	9.7
Monocytes ($\times 10^9$/liter)	1.7	2.9
CFU-GM/ml	7.4×10^3	10.1×10^3
Cytapheresis final product		
Leucocytes ($\times 10^9$/liter)	46	99
CFU-GM/ml	73.5×10^3	77.9×10^3
Volume (ml)	327	380
Total number of CFU-GM	24×10^6	30×10^6
CFU-GM/kg	3.5×10^5	4.2×10^5

Case Report

A 35-year-old woman was diagnosed as having an ALL (DR+, CALLA+, B1+, B4+). Complete remission was obtained after a single course of chemotherapy (daunorubicin: 100 mg/m^2 day 1 to day 3, vincristine: 1.4 mg/m^2 day 1 and day 8, cyclophosphamide: 400 mg/m^2 day 1 and day 8, prednisone: 40 mg day 1 to day 15). The pa-

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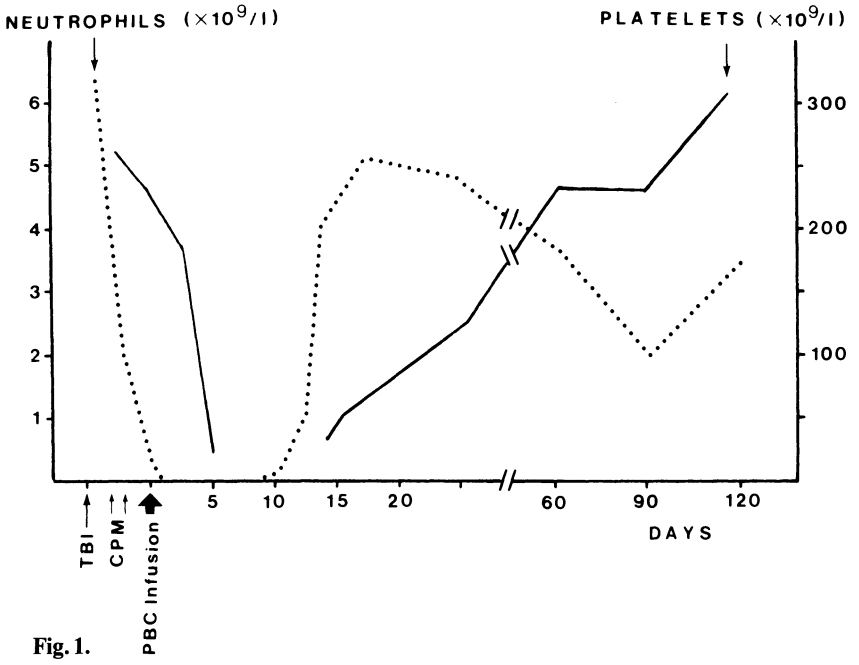

Fig. 1.

tient received three additional monthly courses of consolidation chemotherapy with adriamycin, cytarabine, and L-asparaginase. Bone marrow rescue was harvested after the second consolidation course. The conditioning regimen before transplantation consisted of total-body irradiation (10 Gy) on day -5 and Cyclophosphamide 60 mg/kg on days -3 and -2. Peripheral blood cells were thawed and infused on day 0.

Hematological reconstitution was prompt with neutrophils above 0.5×10^9/liter at day 11 and platelets above 50×10^9/liter at day 15 (Fig. 1). At day 30, neutrophils were 4.4×10^9/liter, platelets 130×10^9/liter, and reticulocytes 105×10^9/liter; bone marrow examination confirmed the persistence of complete remission features, and the patient was discharged from hospital. The patient received six erythrocyte and four platelet transfusions. No further transfusions were needed after day 14.

At day 120, hemoglobin was 11 g/dl, neutrophils 3.2×10^9/liter, and platelets 300×10^9/liter.

Discussion

There are serious theoretical arguments to support the view that peripheral blood cells collected during bone marrow recovery after induction treatment of acute leukemia may have less leukemic contamination than bone marrow cells harvested during stable remission [1]. The possibility of hematopoietic engraftment of circulating stem cells after supralethal therapy has been considered on several occasions [3, 4], but such attemps in humans remain few and inconclusive [5, 6], except in the case of chronic granulocytic leukemia [7]. Recently, Juttner et al. reported on incomplete hematopoietic reconstitution after circulating autologous stem cell transplantation in two patients with relapsed acute nonlymphoblastic leukemia [8]. The complete engraftment, and especially the thrombopoietic reconstitution, in our patient may be explained by the uncommonly high number of PB CFU-GM at the time of cytapheresis, which certainly influences the number of pluripotent stem cells.

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Depletion of T Cells from Bone Marrow Grafts with Soybean Agglutinin and Sheep Red Blood Cells for Prevention of Graft-Versus-Host Disease

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Introduction

Acute (and chronic) graft-versus-host disease (GVHD) is a principal cause of morbidity and mortality in allogeneic bone marrow transplantation (BMT) despite HLA matching of donor and recipient and post-transplant immunosuppressive regimens. Previous studies in animals have demonstrated that mature T cells in the marrow graft are primary mediators of GVHD [1, 2]. Recently, various methods have been developed in man to deplete the marrow graft from mature T cells as attempts to prevent GVHD [3–6].

We report our results in 14 recipients of allogeneic BMT who received T cell depleted marrow grafts from HLA-identical (12 patients) or HLA-nonidentical (two patients) donors for treatment of hematologic malignancies or severe aplastic anemia.

Materials and Methods

Patients. Fourteen patients (median age of 32 years) with acute lymphoblastic or non-lymphoblastic leukemia in CR, chronic myeloid leukemia in chronic or accelerated phase, or severe aplastic anemia were treated with cyclophosphamide (120 mg/kg) and total body irradiation (800 rad; 16 rad/min, 8 MEV linear accelerator) followed by T cell-depleted marrow transplantation (Table 1). Marrow was obtained from 12 HLA-identical and 2 HLA-nonidentical donors. Cyclosporin A, for additional GVHD prevention, was given in eight patients, and six patients received no immunosuppressive prophylaxis after transplantation. The patients were treated in single rooms with reversed isolation, received selective decontamination of the gastrointestinal tract with colistin, trimethoprim-sulfamethoxazole, amphotericin B, and nystatin suspension, and were given semi-sterile food until the granulocyte counts were above 0.5×10^9/liter. In addition, all patients received parenteral hyperalimentation. All blood products were irradiated (3000 rad).

GVHD was defined as acute if beginning < 80 days after BMT, and severity was defined according to the Seattle criteria [7].

T Cell Depletion. T cell depletion was carried out using an immunomechanical method [3]. In brief, marrow suspension was filtered and centrifuged (Haemonetics, blood cell separator) to obtain the buffy coat. Isolation of mononuclear cells was done by centrifugation over Ficoll-Isopaque (1077). Enrichment of stem cells was made by agglutination with soybean agglutination (SBA) and differential sedimentation over bovine serum albumin (5%) gradient. Removal of T cells from the unagglutinated (SBA−) cells was done by incubation of SBA− cells with AET-treated sheep red blood cells (SRBC) and removal of SRBC-rosette-forming T cells by centrifugation over Ficoll-Isopaque.

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Table 1. Clinical data of 14 patients

Patient	Sex/ age (year)	Diagnosis	Donor		aGVHD prophylaxis	aGVHD (grade)	Engraftment	Rejection
			Sex/ age	MHC match				
1	M/37	C-ALL (CR2)	M/38	+	+	−	+	−
2	F/31	O-ALL (CR1)	F/35	+	+	−	+	−
3	M/43	B-ALL (CR1)	M/38	+	−	+ (I)	+	−
4	M/33	C-ALL (CR2)	M/42	+	−	−	+	−
5	F/33	ANLL, M_4 (CR1)	M/31	+	−	NE	−	NE
6	F/32	ANLL, M_4 (CR1)	M/21	+	−	+ (II)	+	−
7	M/32	ANLL, M_1 (CR1)	F/40	+	+	−	+	−
8	F/20	CML (CP1)	F/20	+	−	−	+	−
9	M/37	CML (AP)	M/28	−	−	+ (IV)	+	−
10	F/31	CML (CP1)	M/30	+	+	+ (II)	+	−
11	M/30	CML (CP1)	F/26	+	+	−	+	−
12	M/36	CML (CP3)	M/24	+	+	−	+	−
13	F/22	SAA	F/20	−	+	NE	−	NE
14	F/26	SAA	F/30	+	+	+ (II)	+	−

C-ALL, common acute lymphoblastic leukemia; *O-ALL*, non B, non T acute lymphoblastic leukemia; *B-ALL*, B cell acute lymphoblastic leukemia; *ANLL*, acute nonlymphoblastic leukemia (FAB classification); *CML*, chronic myeloid leukemia; *SAA*, severe aplastic anemia; *CR1,2*, first or second complete remission; *CP1,3*, first or third chronic phase; *AP*, accelerated phase; *MHC match*, HLA-A,B,C and D identical; *aGVHD prophylaxis*, cyclosporin A (3 mg/kg per day, i.v.); *NE*, not evaluable.

Table 2. T cell depletion and engraftment data

Patient no.	Harvested bone marrow		T cells $\times 10^5$/kg	Infused bone marrow		T cells $\times 10^5$/kg	Hematopoietic recovery: days to achieve counts of granulocytes-thrombocytes	
	Nucleated cells $\times 10^8$/kg	CFU-GM $\times 10^4$/kg		Nucleated cells $\times 10^8$/kg	CFU-GM $\times 10^4$/kg		$\geqq 500/\mu l$	$\geqq 50000/\mu l$
1	3.1	6.0	51	0.04	2.4	0.4	25	30
2	3.3	9.5	123	0.07	1.6	0.2	15	15
3	3.3	7.8	167	0.08	4.9	1.0	19	36
4	3.1	5.8	256	0.09	2.4	0.8	20	28
5	2.6	5.5	120	0.04	3.5	0.4	–	–
6	4.3	8.2	134	0.09	7.0	0.4	19	20
7	3.6	4.1	259	0.08	1.8	1.0	25	26
8	3.5	8.0	153	0.09	5.3	0.9	20	24
9	3.5	13.0	212	0.09	5.1	1.0	36	58
10	4.4	10.9	236	0.26	6.3	1.1	17	35
11	2.5	3.7	127	0.07	2.5	1.0	28	56
12	3.1	7.6	145	0.10	5.2	1.0	21	20
13	3.1	8.9	84	0.05	3.6	4.7	–	–
14	3.4	7.2	152	0.13	5.3	1.0	23	16
Mean	3.3	7.6	159	0.09 (3%)	4.1 (54%)	1.1 (0.7%)	22	30

(% of harvested).

Results

aGVHD Incidence. All evaluable patients had a follow-up of >3 months (Table 1). Seven of twelve evaluable patients did not develop acute GVHD. One patient had grade I acute GVHD, three patients had grade II acute GVHD, and one patient had grade IV acute GVHD. The patient with grade IV acute GVHD (no. 9) had CML in accelerated phase and received HLA-nonidentical marrow graft. Acute GVHD yielded to steroids in all, except patient no. 9. Two of seven patients receiving CyA prophylaxis had acute GVHD, and three of five patients not receiving CyA and acute GVHD. Up to now, we have observed one patient who developed chronic GVHD (patient no. 4).

T Cell Depletion. The mean number of nucleated cells harvested was 3.3×10^8/kg, which included a mean number of 7.6×10^4 CFU-GM/kg and 159×10^5 T cells/kg (Table 2). After in vitro marrow treatment, patients received a mean number of 0.09×10^8 nucleated cells/kg (3%), which included a mean number of 4.1×10^4 CFU-GM/kg (54%) and 1.1×10^5 T cells (0.7%). Excluding patient no. 13, who intentionally received less T cell depletion, T cell depletion was always >99.2% (mean 99.5%).

Engraftment. In two patients engraftment failed. Patient no. 5 received several thrombocyte concentrates in the preceding remission-induction phase from his donor because of HLA antibodies; a second, untreated, marrow graft from the same donor again failed to engraft. Patient no. 13, who had previously not responded to ATG, received marrow from a haploidentical sibling but engraftment failed, as happened with subsequent untreated marrow graft from her mother. Cyclosporin A was not initially given to patient no. 5 but was given to patient no. 13 and with the second transplantation to patient no. 5.

Engraftment was prompt in other patients. The time to recovery to $\geqq 500/\mu l$ granulocytes was 15–36 days (mean 22) and that to $\geqq 50\,000/\mu l$ thrombocytes was 15–58 days (mean 30). So far no late rejection has occurred.

Discussion

Our study confirms the efficacy of depleting >99% of T cells from marrow graft by means of soybean agglutinin and sheep red blood cells. This procedure is completed in about 7 h and does not damage marrow function. Impressive results of abrogation of GVHD by this technique have been reported [8]. Our results are favorable, though not as impressive as others reported. The much higher age of our patients may account for this difference. Consequently, all our patients will receive CyA from now on.

Nonengraftment (and rejection) remains a problem in T cell-depleted marrow grafting for patients with HLA-nonidentical marrow donors, and probably also for patients sensitized to marrow donors before marrow transplantation. In these circumstances, less T cell depletion and/or more intensive immunosuppressive regimens will be necessary.

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Bone Marrow Transplantation for Chronic Granulocytic Leukemia: Results of the French Cooperative Group (GEGMO)

A. Devergie[1], J. P. Vernant[2], D. Guyotat[3], D. Maraninchi[4], M. Michallet[5], J. Pico[6], and E. Gluckman[1]

Introduction

Chronic granulocytic leukemia (CGL), characterized by the Philadelphia chromosomal abnormality [1], is a clonal [2] myeloproliferative disorder with poor prognosis [3]. The main cause of death is blast crisis, which is often preceded by an accelerated phase. Blast crisis is generally refractory to chemotherapy and fatal within a few months. Conventional chemotherapy can improve the quality of life during the chronic phase but cannot delay the onset of blast crisis. Aggressive chemotherapy during the chronic phase gives poor results because cytogenetic conversions are uncommon, incomplete, and usually transient. Expectations that autografting would prolong survival for patients in blast crisis have not been confirmed. Because of these disappointing results, several investigators began to evaluate supralethal chemoradiotherapy followed by bone marrow transplantation (BMT) as a potential therapeutic modality [4–8]. We report here the results of a retrospective analysis of 160 patients who received transplants between 1981 and 1985 in 18 French centers.

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Methods

Questionnaires were collected by the French Cooperative Group for BMT (GEGMO) from all members for their consecutive transplants in patients with CGL. The 160 patients included 79 males and 81 females aged 7–46 years (median 31). The median duration of disease before BMT was 26 months (range 3–160 months).

Table 1. Clinical features of 160 patients with chronic myeloid leukemia

Disease classification	*n*	Sex m/f	Age, range and median (years)	Time to transplant (months)	Splenectomy
First chronic phase	100	54/46	7 → 46 31	5 → 143 27	78
Accelerated phase	40	20/20	13 → 44 30	3 → 160 40	31
Blastic phase or second chronic phase	20	5/15	13 → 43 30	9 → 61 30	9
Total	160	79/81	–	–	118

The different phases of CGL were defined by the criteria of the International Bone Marrow Transplant Registry: 100 patients were in the first chronic phase. 40 patients were in the accelerated phase, and 20 patients were in blast crisis or second chronic phase (Table 1). All patients received bone marrow from an HLA A-B-D/DR-identical sibling donor. Twins were excluded from this study. The patients received high-dose chemoradiotherapy before transplantation: cyclophosphamide (60 mg/kg body weight per day on days −5 and −4) followed by total-body irradiation with lung shielding. The radiation technique varied from center to center: 113 patients were given single-dose total-body irradiation with 8–10 Gy (median 10 Gy) with a dose rate of 2.2–5 cGy/min (median 4 cGy/min) and a total applied lung dose of 4–8 Gy (median 8 Gy). Forty-seven patients were treated with fractionated irradiation with 10–13 Gy (median 11 Gy) with a total applied lung dose of 6–10 Gy (median 7 Gy). One-hundred eighteen patients were splenectomized before transplantation, and 42 were not. All patients received prophylaxis for graft-versus-host disease (GVHD). This consisted of cyclosporin in 72 patients, methotrexate in 62, and a combination of cyclosporin plus methotrexate in 23. Forty patients received bone marrow that was treated ex vivo with monoclonal antibodies to deplete the donor's T cells, three of them without other GVHD prophylaxis.

Results

Of the total 160 patients, 96 are alive 2–57 months after transplant. The median duration of followup among survivors is 13 months. The actuarial survival is shown in Fig. 1 (60% in patients grafted in first chronic phase versus 36% in patients grafted in accelerated or blastic phase of the disease).

Of the 160 patients, 59 (37%) had acute GVHD of grade II or worse. It was fatal in 17 patients (10%). The incidence of GVHD was strongly correlated with prophylactic

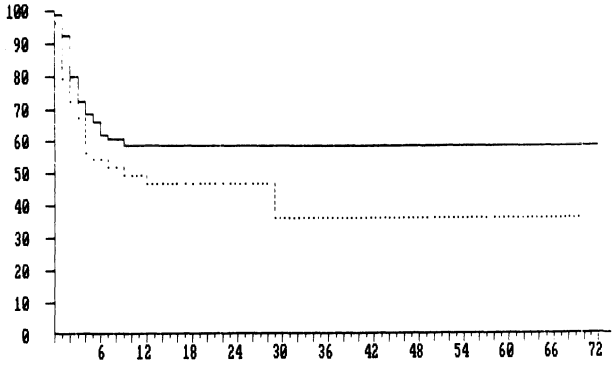

Fig. 1

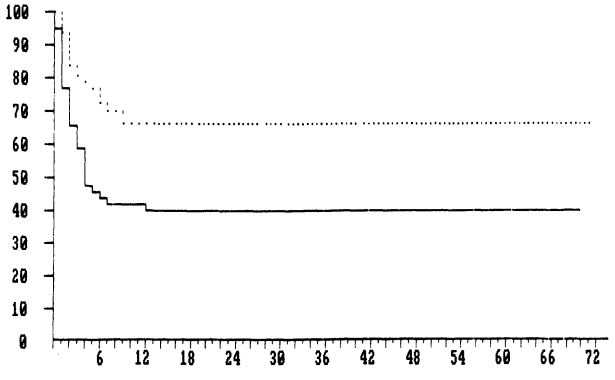

Fig. 2

Table 2. Incidence of GVHD according to type of prophylaxis

	Metho-trexate	Cyclos-porin	MTX + CYA	T-depleted marrow alone	Total
Acute GVHD, Grade 0–I	28	55	16	2	101
Acute GVH, Grade II–III	34 (55%)	17 (23%) $p < 0.001$	7 (30%)	1	59 (37%)
Total	62	72	23	3	160
Chronic GVHD patients at risk	13/29	16/41	5/11	0/2	34/83 (40%)

treatment with cyclosporin A or methotrexate (Table 2). It was lower in patients treated with cyclosporin (23%) than in patients treated with methotrexate (55%), and actuarial survival was significantly better in the first group (Fig. 2) ($p < 0.001$). The incidence of severe acute GVHD was also lower in patients who received marrow depleted of T cells (18%) than in those who received untreated marrow (43%).

Of the 40 patients who received T cell-depleted marrow. 27 were in the first chronic phase at time of BMT: they received either cyclosporin A (21 patients), or methotrexate (three patients) or no further GVHD prophylaxis (three patients). In this small group of patients, the actuarial survival was excellent (82%): only three patients died, one with acute GVHD, one with fungal infection, and one with secondary lymphoma.

Of the 83 patients who survived until day 150, 34 (40%) had some degree of chronic GVHD, which resolved completely in most cases. Only five patients still have severe chronic GVHD.

Interstitial pneumonitis occurred in 43 patients (27%) and was the primary cause of death in 24; it was associated with acute fatal GVHD in 8; only in 11 cases did interstitial pneumonitis resolve. The main cause was cytomegalovirus (17 cases); 13 cases of interstitial pneumonitis were idiopathic.

Ten patients had relapses with cytogenetic or hematologic evidence of leukemia. Six had died by the time of this report. Only one of these patients had received a transplant in the chronic phase of his disease, and he is still alive in the chronic phase. Of the 48 patients to whom bone marrow was grafted in the accelerated phase or in the second chronic phase. 4 (8.3%) had a relapse. But 5 of 12 patients who received grafts in blastic crisis had a relapse (41%), and 4 have died.

Discussion

The preliminary results of treating patients with CGL by allogeneic BMT were published from a number of centers [5–13]. Actuarial survival is about 60%–70% at 1 year for patients grafted in chronic phase of their disease with a relatively low risk of relapse. The probability of surviving transplantation is lower and the risk of relapse higher if transplantation is performed in a more advanced phase. Our results confirm this finding. There was no correlation between age at

Table 3. Cause of death

	Acute GVHD	Interstitial pneumonitis	Relapse	Other	Total
First chronic phase	8	17	0	9	34/100
Accelerated phase	8	4	2	4	18/40
Second chronic phase	1	1	0	2	4/8
Blastic crisis	0	2	4	2	8/12
Total	17	24	6	17	64/160

time of BMT and survival; in patients who received grafts in a chronic phase, actuarial survival was 65% among the 38 aged 7–29 years, and 55% among the 62 older patients aged 30 years or more ($p=0.5$ non significant).

The benefit of splenectomy is controversial [14–16] and long-term followup is required before any conclusion can be drawn about its possible value in preventing relapses. In this series, no difference in survival could be detected between the splenectomized patients and those with intact spleens. The incidence of acute GVHD, interstitial pneumonitis, and relapse was the same in the two groups. The main cause of death was interstitial pneumonitis; there was no difference between patients grafted in a chronic phase and patients grafted in a more advanced phase of their disease. The inferior survival of patients grafted in accelerated phase or in blast crisis was due to the combined effects of the increased risk of dying of transplant-related complications, especially acute GVHD (15% versus 8%) and the increased risk of dying of leukemic relapse (10% versus 0%) (Table 3). The phase of the disease when BMT was performed and the prophylactic treatment of GVHD were the two main prognostic factors.

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An HLA Lost Mutation May Lead to Leukemic Relapse of Recipient Type Six Years After Bone Marrow Transplantation *

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Introduction

Products of the major histocompatibility complex (MHC) serve to direct interactions amongst cells of the immune system. Cytolytic T cells recognize their targets by means of an antigen-specific receptor, but this recognition also requires a match of MHC antigens between effector and target cells (MHC restriction) [1]. Cytolytic T cells have been implicated in the elimination of malignantly transformed cells [2]. One mechanism by which tumor cells might escape immune destruction would be a reduction of the expression of MHC antigens, i.e., introduction of restriction elements. This situation occurs in cells transformed with an oncogenic strain of adenovirus which have switched off the expression of class I MHC antigens [3].

We report here on a case of AML in a 20-year-old female patient who was treated by total-body irradiation and bone marrow transplantation from her HLA haploidentical but MLC (=HLA-D)-compatible brother, resulting in a full hematological and immunological reconstitution. A relapse occurred 6 years later with outgrowth of AML of recipient type [4].

Materials and Methods

HLA blood group typing of the patient and her relatives (Fig. 1) was done in the NIH microcytotoxicity assay, using more than 120 specific cytotoxic antisera for HLA-A, B, C antigens and more than 80 antisera for HLA-DR antigens. The MLC tests were done in a microculture system with 50000 responding cells and X-irradiated stimulating cells.

For biochemical analysis of class-I MHC antigens cryopreserved AML blasts from the patient before transplantation as well as from the relapse were labeled with ^{35}S-methionine. HLA-A, B antigens were isolated from detergent extracts with the monoclonal antibody W6/32 [5] and analysed by one-dimentional isoelectric focusing [6] using two different concentrations of the detergent extracts [1 : 1 and 1 : 2; see Fig. 2).

Discussion

After total-body irradiation this 20-year-old patient with acute myelogenous leukemia (AML), received marrow from her HLA haploidentical but MLC (=HLA-D)-compatible brother. Six years later she had a leukemic relapse of recipient type, as disclosed by karyotyping and blood group markers. Serological HLA typing of the relapse cells revealed a loss of expression of the mismatched HLA-A, B, C haplotype between donor and recipient. Biochemical analysis of the class I antigens by immunoprecipitation and one-dimensional isoelectric focusing confirmed these findings.

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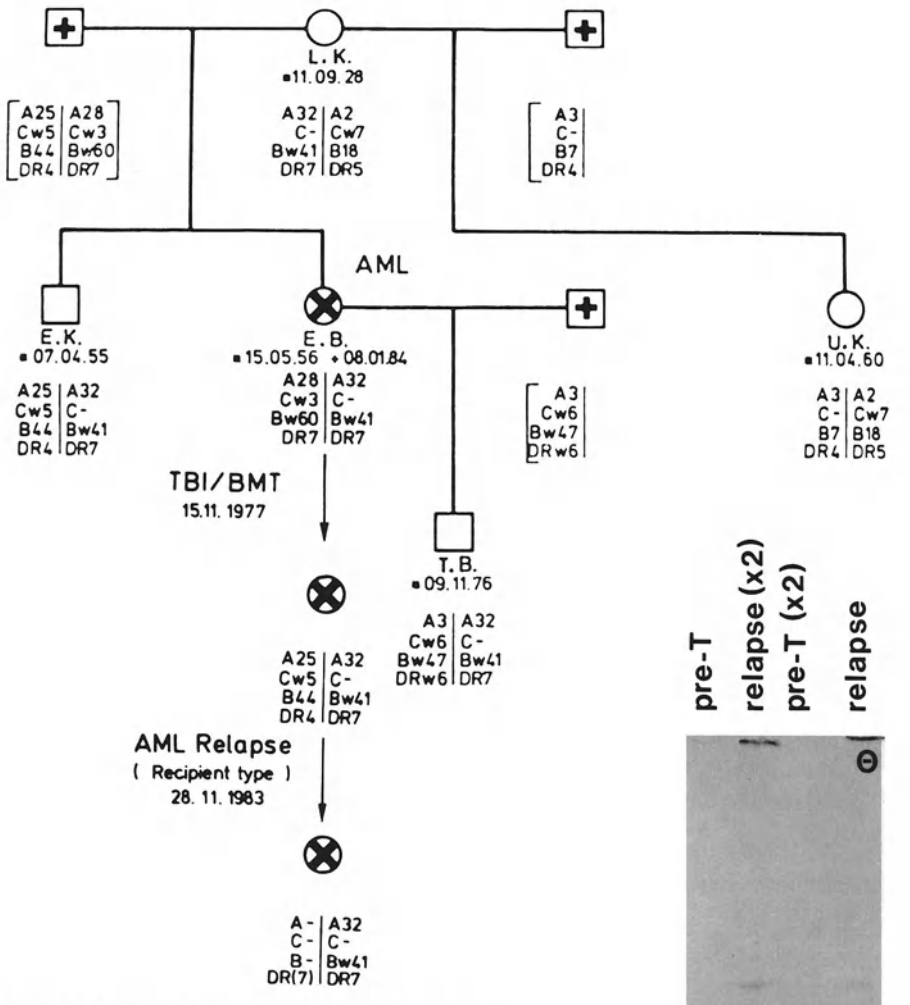

Fig. 1 Pedigree and HLA haplotypes of AML patient E. B.

A plausible scheme for explanation of this phenomenon is that one or more hematopoietic progenitor cells escaped surveillance of the donor immune system due to deletion of the mismatched HLA haplotype. The reappearance of recipient leukemia as a result of transformation of residual host cells damaged by radiation and/or chemotherapy does not argue against MHC-restricted im-

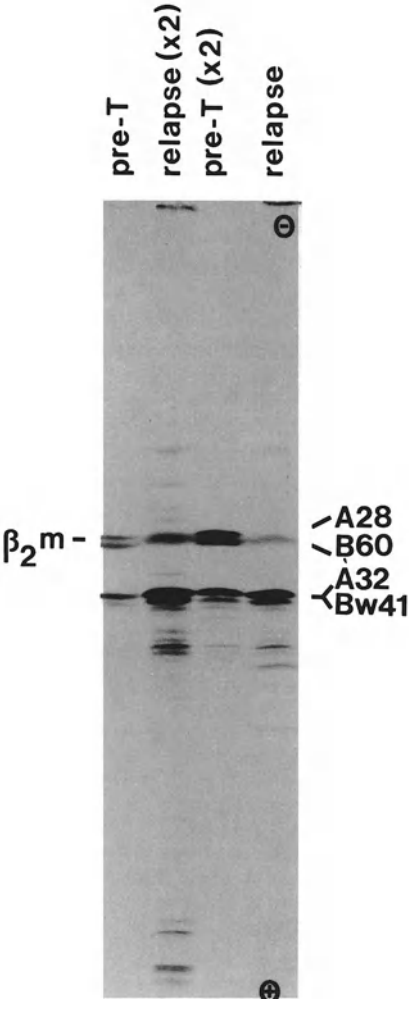

Fig. 2. 1D-IEF pattern of detergent extracts from AML blasts before transplantation *(pre-T)* and after late relapse *(relapse)*. $\beta_2 m$, beta-2-microglobulin

mune surveillance for elimination of malignantly transformed cells.

So far as we are aware, this is the first description of an in vivo MHC (HLA) lost mutation in man. The circumstances under which this lost mutation was observed point to the immunological relevance of HLA antigens in tumor cell recognition and destruction.

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Toxoplasmosis After Bone Marrow Transplantation *

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Introduction

Bone marrow transplantation (BMT) is being used with increasing success as part of treatment programs for acute leukemia, chronic granulocytic leukemia, severe aplastic anemia, and congenital immunodeficiency syndromes [1].

Patients submitted to BMT become severely immunodeficient for prolonged periods (6–12 months) and are at risk of developing a variety of opportunistic infections [2, 3].

In the early post-transplantation phase bacteria, fungi, viruses, and protozoan parasites may lead to life-threatening infectious complications [2]. Concerning the protozoal infections, attention was formerly focused on *Pneumocystis carinii*. Following the introduction of trimethoprim-sulfomethoxazole prophylaxis for *Pneumocystis carinii*, infections caused by this organism have become rare [4]. There are few published data on toxoplasma infections in marrow transplant recipients [5, 6]. This report deals with four cases of toxoplasmosis among 138 patients who received a marrow transplant at our center.

Patients and Methods

The patient characteristics are given in Table 1. The conditioning regimen for all four cases consisted of 60 mg/kg body weight cyclophosphamide on days −6 and −5, followed by 8.6 Gy total-body irradiation (midline dose) given on day −1 before BMT. Patients were isolated in single rooms under barrier nursing conditions and received autoclaved food. Total decontamination of the gastrointestinal tract was performed with nonabsorbable antibiotics and antimycotics.

Two weeks prior to BMT a *Pneumocystis carinii* prophylaxis with trimethoprim-sulfo-

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Table 1. Recipient and donor characteristics of the four patients who developed manifest toxoplasmosis after bone marrow transplantation

UPN	Age (years)	Sex	Underlying disease	Donor	Age (years)
54	33	F	AML − 1st relapse	HLA-genotypically identical sister	42
66	33	F	Ph^+CGL (chronic)	HLA-genotypically identical brother	36
79	21	M	AML − 1st relapse	HLA-genotypically identical sister	16
84	32	F	Ph^-CGL (chronic)	HLA-genotypically identical brother	30

AML, acute myeloid leukemia; *CGL*, chronic granulocytic leukemia.

methoxazole was started. For prevention of graft-versus-host disease (GvHD) methotrexate was used according to the Seattle protocol [7]. Diagnosis of acute GvHD was based on clinical findings and on skin or liver biopsies according to the criteria published by Glucksberg et al. [8]. Immunosuppressive treatment of manifest acute GvHD was done with high-dose methylprednisolone. For blood product substitution washed red blood cells and cell separator platelet concentrates were given. Granulocyte transfusions were not given in any of these patients.

Results

In none of the four patients could the diagnosis of toxoplasmosis be established before postmortem examination. The clinical features and the main causes of death are given in Table 2. Three patients already had acute GvHD when toxoplasmosis first became clinically manifest. The following signs must in retrospect be interpreted to be consequences of toxoplasmosis:

1. Generalized seizures (UPN 54)
2. Necrotizing retinochorioiditis (UPN 84)
3. Spastic hemiparesis, disturbances of consciousness (UPN 84)
4. Ventricular extrasystoles and ST depressions in ECG traces (UPN 54)
5. Cardiomegaly and congestive heart failure (UPN 66, UPN 79)

Histopathological sections demonstrated a varying degree of diffuse encephalitis. (Fig. 1 and 2) and involvement of the heart muscle in the form of myocarditis (Fig. 3). The inflammatory reaction was characterized by ruptured pseudocystes, free trophozoites in the tissue, a surrounding mononuclear cell invasion, and zones of necrosis. In one patient (UPN 84) a necrotizing inflammation of the retina could be demonstrated on ophthalmoscopy (Fig. 4).

Discussion

Toxoplasma gondii is ubiquitous in nature – achieving prevalence rates in excess of 50% in many population groups – and is capable of lying latent in multiple tissues for the life

Table 2. Clinical features of manifest toxoplasmosis, primary cause of death and contributory cause of death of the four patients who developed manifest toxoplasmosis after bone marrow transplantation

UPN	GvHD	Immunosuppressive treatment of GvHD (started days after BMT)	Clinical signs of toxoplasmosis (day first observed after BMT)	Cause of death (day after BMT)	Contributory cause of death
54	Acute grade I	High-dose MP (33)	Generalized seizures (55), ventricular extrasystoles (46), ST depression in ECG tracings	Toxoplasma myocarditis (71)	Toxoplasma diffuse necrotizing encephalitis
66	–	–	Congestive heart failure (30)	Toxoplasma myocarditis (39)	Aspergillosis of the lungs
79	Acute grade II	High-dose MP (49)	Left ventricular failure (67)	Idiopathic interstitial pneumonitis (73)	Toxoplasma myocarditis
84	Acute grade II	High-dose MP (40)	Progressive lethargy (148), diminution of vision (161), spastic hemiparesis (210)	Idiopathic interstitial pneumonitis (245)	Toxoplasma diffuse necrotizing encephalitis

GvHD, graft-versus-host disease; *MP*, methylprednisolone.

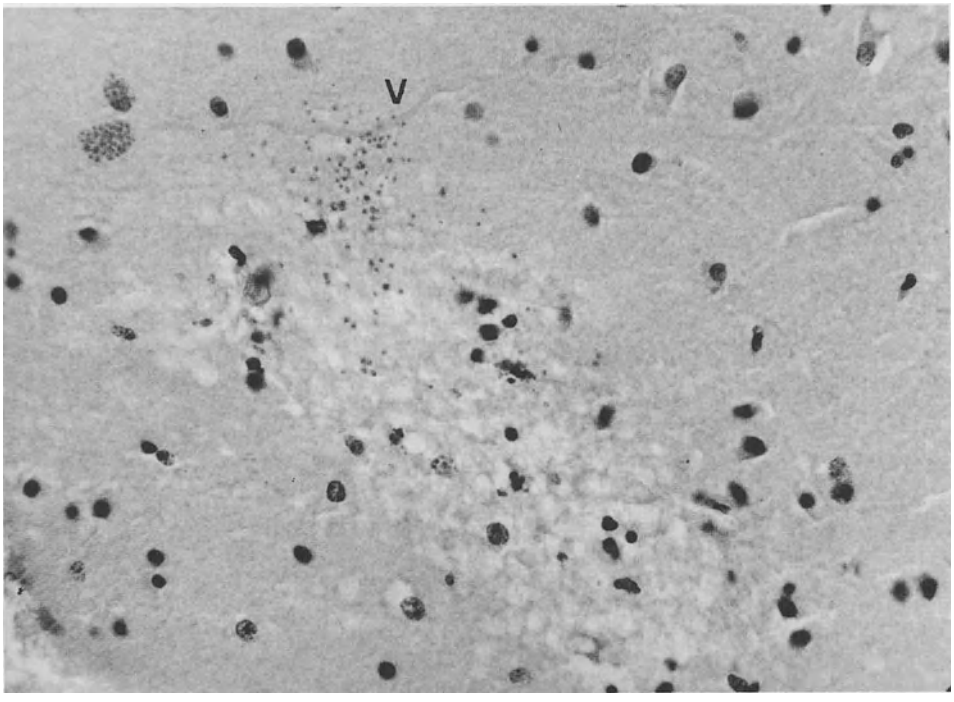

Fig. 1. Manifest toxoplasmosis of the cerebellum: The reaction is characterized by necrosis, edema, and free trophozoites (V). In the *upper left quadrant*, two intact pseudocystes are seen. H&E, ×350

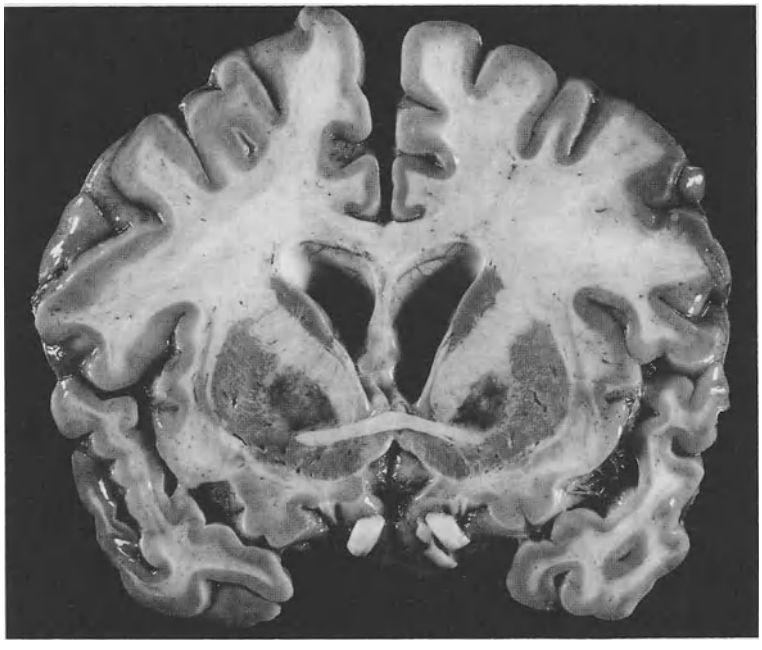

Fig. 2. Coronal section through the brain. Yellow cystic necrotic foci are seen in the pallidum

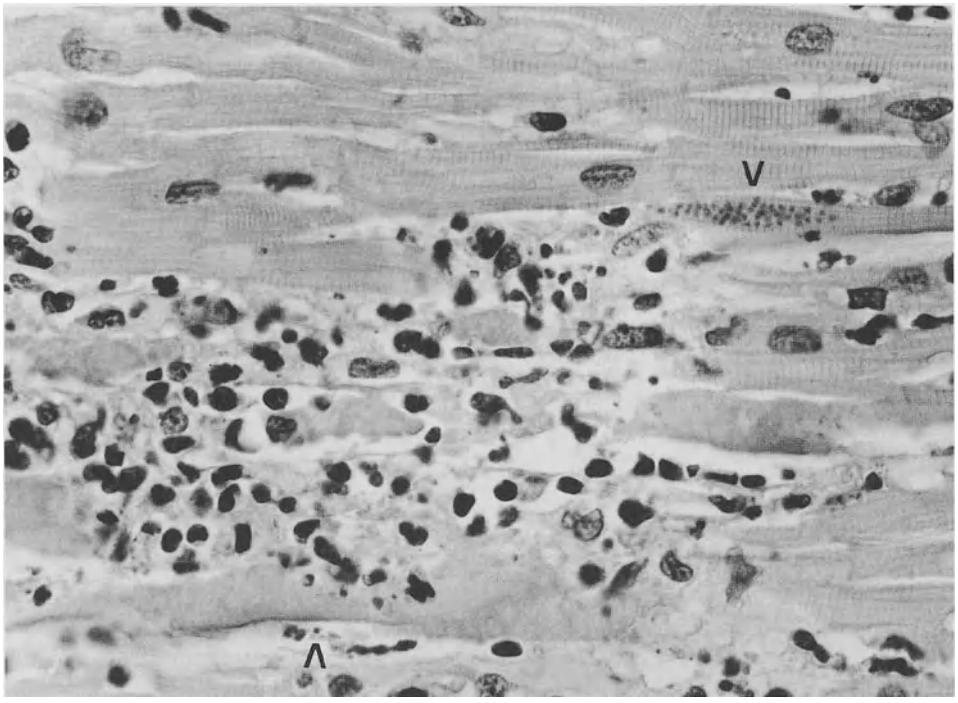

Fig. 3. Manifest toxoplasmosis of the heart muscle: The reaction is characterized by necrosis of muscle fibers and mononuclear cell invasion. In the *upper right quadrant* (V) intact pseudocystes are seen. Free trophozoites are seen among the necrotic tissue (Λ). H&E, × 350

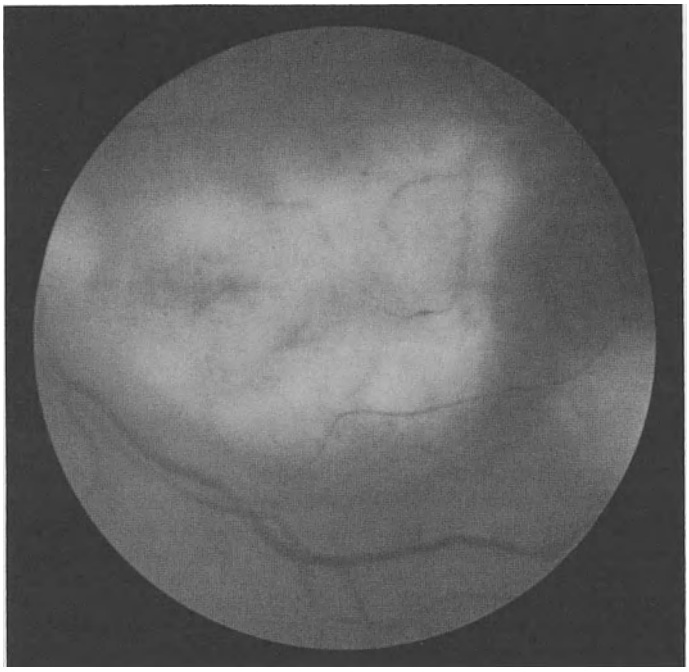

Fig. 4. Toxoplasma: retinochorioiditis. The macular region of the left eye is demonstrated to show necrotic foci and a very slight inflammatory reaction of the choroid, retina, and vitreous humor

of its host [9]. In most immunocompromised patients, disseminated toxoplasmosis results from reactivation of remotely acquired latent infection. However, exogenous acquisition of the organism might well produce an acute, equally devastating illness in immunologically compromised individuals and yet cause no symptoms at all in normal subjects [9].

Following BMT patients are particularly at risk of acquiring such an infection, as cellular and humoral defense mechanisms are still impaired. This possibility is even higher if GvHD occurs or immunosuppressive treatment becomes necessary [2]. In most cases, including our four, the central nervous system and the myocardium are affected [9]. The predominant clinical picture is that of diffuse encephalitis or myocarditis complicated by congestive heart failure.

Pre- and post-BMT serologic diagnosis of toxoplasmosis was not performed for our cases, and therefore it is not known whether infection was newly acquired or represented activation of a latent one. It is extremly unlikely that our patients could acquire an exogenous infection through the autoclaved food. However, another route of infection, e.g., that of contaminated blood products, cannot be ruled out.

One theoretical objection to serologic diagnosis of toxoplasmosis following BMT is the plausible argument that such persons may fail to mount a measurably significant circulating antibody response to the parasite. In one of the published cases, a rising titer after BMT could be demonstrated to occur during active illness, while in the other the serodiagnosis failed [5, 6].

In practice, antemortem diagnosis was only achieved in two reported cases after BMT, one of them through animal inoculation of brain biopsy material and lymph-node tissue.

Inoculation of blood or cerebrospinal fluid from the same patient failed to demonstrate the parasite [6].

It is essential to stress that serologic confirmation of acute toxoplasma infection, especially in adults, is hampered by the widespread prevalence of antitoxoplasma antibodies in the normal population. For the same reason it is impossible to exclude patients with positive titers from BMT programs. In addition, the clinical picture of the various infections to which patients are subjected in the early post-transplantation phase makes it difficult to establish the diagnosis exclusively on clinical grounds, as some of them mimic the manifestations of toxoplasmosis. So the disease must be presumed and diagnosed by exclusion. In case of doubt, patients developing cerebral or cardiac symptoms of unknown origin should receive treatment with pyrimethamine and sulfadiazine in combination with folinic acid to allay the adverse effect of pyrimethamine on the bone marrow.

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