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Osteosarcoma in Adolescents and Young Adults: New Developments and Controversies

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SPRINGER SCIENCE+BUSINESS MEDIA, LLC

Cancer Treatment and Research is indexed in the National Library of Medicine MEDLARS system.

Library of Congress Cataloging-in-Publication Data

Osteosarcoma in adolescents and young adults : new developments and controversies / edited by G. Bennett Humphrey . . . [et al.]; editorial assistants, Anneke Johnson-Hoekzema, Lodewijk Martijn.
p. cm.—(Cancer treatment and research; v. 62)
Includes bibliographical references and index.
ISBN 978-1-4613-6561-7 ISBN 978-1-4615-3518-8 (eBook)
DOI 10.1007/978-1-4615-3518-8
1. Osteosarcoma in children. 2. Teenagers—Diseases. 3. Young adults—Diseases. I. Humphrey, G. Bennett (George Bennett), 1934– II. Series.
[DNLM: 1. Osteosarcoma—in adolescence. 2. Osteosarcoma—in adulthood. 3. Osteosarcoma—therapy. W1 CA693 v. 62 / WE 258 0845]
RC280.B6O85 1993
618.92'99471—dc20
DNLM/DLC
for Library of Congress 92–49668
CIP

Originally published by Kluwer Academic Publishers in 1993
Softcover reprint of the hardcover 1st edition 1993

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Preface

OSTEOSARCOMA: New Developments, Controversies and Current Practice is the fifth volume in the series Cancer Treatment and Research devoted to pediatric oncology. Like its immediate predecessor, Volume 4: Hodgkin's Disease: Current Practice and Controversies, this volume will also deal with the current state of affairs, where do researchers differ in opinions or firmly held beliefs, what does the future hold for research or for the patients who have benefitted from research and are now cured, etc.

The first part deals with preclinical and clinical issues that relate to the disease and the second part deals with the results of recent trials and in some cases ongoing trials. It could be argued that section B Pharmacology, section D Radio-therapy and section E Surgery of part one belong to part two, however in these sections we have tried to focus on one discipline and in part two, we have tried to focus on the results of multidisciplinary trials. A third part includes a few critiques and editorial comments.

This volume was prepared and planned for both basic and clinical oncologists. It is hoped that the basic scientists will gain some insight into the problems of their clinical colleagues and that the clinicians will be willing to read to the more basic articles. It is not a text for students (or for that matter physicians) who want all things to be laid out in "black and white". But it is a text for those students who want to know how vibrant this field of research is and are willing to stand in awe of how much research needs to be done.

Almost all of the invited participants for this volume provided us with a manuscript. This resulted in a large volume of submitted material and not enough space to publish each manuscript in its entirety. Therefore, all manuscripts were edited by us and in most cases the contents reduced. A few authors were kind enough to edit their own text. Thus the reader will note that the introductory comments are very brief, data are presented in either the text or a figure/table but not both and references have been limited in many cases. Some data were eliminated where there was duplication (e.g., discussions of surgery in institutional reports were deleted because of the invited reviews of controversies in surgery that make up section D). If any of the articles seem to be fragmented or lacking in depth, solidity or

comprehensiveness the fault lies with the editors and not the authors who delivered painstakingly written manuscripts.

The editors wish to express their appreciation to all for the willingness not only to review the current status of research in osteosarcoma, but also to speculate on future direction for research.

Osteosarcoma in Adolescents and Young Adults: New Developments and Controversies

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Osteosarcoma in Adolescents and Young Adults: New Developments and Controversies

page 245, last complete sentence, should read

The dosage of CCDP (Platinol 0.5 mg/ml, Bristol Myers SAE, Spain) used for the perfusion was 30 mg/l extremity volume [4].

1. Osteosarcoma at the end of the 20th century

G. Bennett Humphrey, W.M. Molenaar, H. Schraffordt Koops, and A. Postma

Introduction

As we approach the end of the 20th century, there may be some value in looking at what has been accomplished, what detours were taken from an orderly progression of science, and what has not been achieved in research in pediatric oncology. We should also try to tabulate what controversies have been solved and which ones remain active. In this introduction to a book on osteosarcoma, we will tackle some of these issues in relation to this tumor type.

Advances

There clearly have been some advances. There has been a dramatic improvement in the disease-free and overall survival, as well as improvements in the quality of life (e.g. limb-salvage procedures) for patients with nonmetastatic osteosarcoma. These advances can be attributed to better surgical and chemotherapeutic approaches to patient management. Osteosarcoma has been the proving ground for neoadjuvant chemotherapy, that is, chemotherapy given after a definitive biopsy but before definitive surgery. For some patients there has been some progress in the management of isolated relapses in the lungs or at the primary site.

The advances have not been limited to clinical research. During the past 20 years a great deal has been learned about the DNA content of osteosarcoma and its importance in diagnosis, about growth factors, and about genes and tumor markers. These represent valuable methods for the pathologist to classify bone tumors and to better define which tumors of the bone are malignant and which are benign.

In the field of surgical oncology, there have been significant achievements in limb salvage. The ambitions and imagination of surgical oncologists to save limbs have been a driving force in the management of patients with nonmetastatic osteosarcoma of the extremities. The availability of such techniques have required chemotherapists to develop not only adjuvant but also

G. Bennett Humphrey (ed.), OSTEOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS.
 ISBN 0-7923-1905-2

neoadjuvant chemotherapeutic regimens. Limb-salvage techniques are not only available for the fully grown adolescent and young adult, but expandable prostheses have been developed for very young patients. At present these require a minor invasive procedure for expansion; however, a modular endoprosthetic system that can be expanded by an external magnetic field is under development.

At least one new drug, ifosfamide, has recently been added to the list of known active agents for osteosarcoma and other pediatric malignancies. There are two new derivatives of known active agents that have activity in osteosarcoma and that may be effective alternatives to the more toxic parent compounds. They are 4′ epidoxorubicin, which is less cardiotoxic than doxorubicin, and carboplatin, which is less nephrotoxic and ototoxic than cisplatin.

Detours

Retrospectively, there have been some detours. Whether these could have been avoided is a matter for an ongoing debate. It might be better to simply reflect on these issues and try to profit from experience.

There were a number of immunotherapy trials in osteosarcoma, as there were in the acute leukemias and a few other pediatric malignancies. Transfer factor and BCG have been completely dropped from clinical protocols, and interferon, while still being used in a few centers, is no longer in the mainstream of experimental therapy. Does immunotherapy represent a detour or just a reasonable idea that needed to be proven and accepted or abandoned, based on clinical experience? It could be argued that we do not know enough about tumor immunology and/or the mechanism controlling tumor growth or rejection, to rationally design immunotherapeutic trials, and therefore any immune stimulation or passive transfer of immune competent or activated cells is premature. This is probably too harse a judgment. Drug development is, in general, a matter of trial and error so why should not immunotherapy also be allowed to be judged on the same basis.

A more interesting issue that may have truly been a detour from the orderly progress of clinical science was the suggestion that there was a change in the natural history of osteosarcoma. It was argued that the adjuvant therapy being used at a large number of centers and by cooperative groups could not account for an increase in survival. This is because in the 1970s one American and one European center noted an increased survival after surgery alone. This issue resulted in two trials in patients with nonmetastatic osteosarcoma of an extremity. Patients were randomized to either adjuvant therapy immediately after definitive surgery or no therapy until there was evidence of disease (local reoccurence or metastatsis). Both trials proved conclusively that (1) adjuvant therapy was required for a cure rate of 50% or greater, (2) there was no change in the natural history of osteosarcoma, and (3) definitive

surgery alone resulted in cure rates of only 20%. This 20% survival is the same poor outcome that was reported for decades all over the world prior to the advent of adjuvant chemotherapy.

Dr. James Holland has argued that these trials were not necessary [1]. Dr. Joseph Bertino has argued the opposite, as have others [2]. Whether or not this is a detour is probably not the issue. Each clinical scientist must decide whether or not to enter patients into a randomized trial just to prove that something does not work when there is no compelling scientific basis for the trial. On the one hand, there was no reason to believe that there had been a change in the exposure to a possible etiological agent for osteosarcoma (as none are known to exist for humans).

In Europe and North America there was no significant change in nutrition, sanitation, or the standard of living in the period in question; nor was there any precedence that the natural history of any spontaneous sarcoma of childhood could change in such a short time. (The natural history of childhood leukemias/lymphomas can change with the industrialization of a country, but this takes many decades—a far different time frame than that suggested for osteosarcoma.) On the other hand, no pediatric oncologist wants to give unnecessary chemotherapy to any child. This latter point probably warranted the "detour" taken to definitively prove the value of adjuvant therapy.

What has not been achieved

While the definition of osteosarcoma is clear, the distinction between benign and malignant cells may be difficult. We have been unable to apply some of the advances in cellular and molecular biology to better delineate osteosarcoma. Also, we are as yet unable to recognize subsets of osteosarcoma that are clinically relevant. DNA ploidy could go a long way toward improving diagnosis, and routine DNA characterization would be useful for cooperative groups to make comparisons between protocols more meaningful. The same might be true for the use of monoclonal antibodies that identify cells that are in cycle; however, the experience with this methodology is not as extensive as that with DNA analysis. Finally, chemotherapy in the individual patient is still a matter of trial and error, and we cannot predict which tumor will respond to which drug. Multiple drug-resistance gene studies might help to resolve this issue to some extent.

At present we do not have effective protocols for the following patients:

1. Those who present with metastasis at diagnosis
2. Those who present with central lesions of the pelvis, spine, etc.
3. Those who relapse with multiple pulmonary metastasis or metastases to the bone

These problems are not unique to osteosarcoma but are also problems that are seen with most solid tumors of childhood. (Hodgkin's disease is an exception to this rule.)

Controversies

Probably all pediatric malignancies contain controversies, but those within the field of osteosarcoma, we think, are especially challenging and important in patient management. Some of them are resolvable, others are irresolvable, and at least one has been generated more from rumors and not from lack of data or lack of properly controlled trials.

In his 1988 article, "*Controversies in the treatment of osteosarcoma*" [3] Dr. James B. Nachman lists three major controversies that concern patient management:

"1) Do neoadjuvant chemotherapy programs improve long-term disease-free survival when compared with standard adjuvant programs?;
2) Within neoadjuvant programs, will increasing the percentage of patients who show a good histological response in the primary tumor produce an improved over-all long-term disease-free survival?;
3) and opposed to standard amputation procedures, does limb-sparing surgery have an adverse effect on long-term disease-free survival?" [3].

This article goes on to summarize and tabulate these and additional controversies. The results of research available at that time (up to and through 1987) have been nicely summarized elsewhere [3, p. 409].

Additional controversies can be added as follows:

General

1. In the initial evaluation and follow-up of patients, is nuclear magnetic resonance spectroscopy a valuable addition for the assessment of the osteosarcoma tumor burden, as has been suggested by a few articles in the recent literature?
2. What price are patients paying in late effects from the aggressive and toxic chemotherapeutic regimes that are currently being used to increase the percent of long-term survivors?
3. Should all patients of all ages be managed on the same protocol, or should there be one protocol for children and young adolescents, and another for older adolescents and adults?

Pharmacology

4. Is it possible to itemize chemotherapeutic agents in a list that prioritizes which drug is the most active in osteosarcoma, which is second most active, etc.?
5. Is any form of immunotherapy worth pursuing in the treatment of patients with osteosarcoma?

Radiotherapy

6. Is there a rational role for radiation therapy in the treatment of osteosarcoma?

Surgery

7. From the surgical point of view, should all patients undergo a limb-salvage procedure rather than an amputation or rotationplasty for osteosarcoma of the lower extremity?

8. Is there a therapeutic or surgical management advantage for the use of intraarterial chemotherapy as opposed to systemic administration of the same dose? In the same vein (no pun intended), what is the therapeutic advantage for isolated limb perfusion with cisplatin or for intraoperative radiotherapy?

Institutional reports

9. Are the rumors that have circulated for the last few years true, that longer follow-up of patients receiving therapy of the T10 type, as originally described by Memorial Sloan-Kettering, results in a significantly lower disease-free survival rate than originally reported by Dr. Rosen?

Aim of this volume

The contents of this volume are addressed at individuals who are interested in osteosarcoma. It is intended for those individuals involved in any kind of related research or the care of patients with osteosarcoma. We will, therefore, not include an introductory chapter on incidence, etiology, diagnostic evaluation of patients with bone lesions, etc. These subjects are adequately covered in many excellent textbooks.

What we do hope to accomplish is the presentation of material that will shed some light on recent research and will give some insight into the current practice at those institutions that are caring for patients but do not have sufficient patients or resources to mount a research program. We hope the material in this book will resolve some of the controversies in the field, or at least help us to focus more clearly on some issues that cannot be resolved at present.

References

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2. Retroviruses and oncogenes associated with osteosarcomas

L. Michiels and J. Merregaert

Introduction

The first demonstration that tumors can be induced by viruses dates from the early 1900s, when Ellerman and Bang extracted a "leukemia inducing factor" from blood of leukemic chickens. In 1911, Rous isolated a sarcoma-inducing agent in a filtered cell free extract from a fibrosarcoma, which was identified as the Rous sarcoma virus. Viral cancer induction was highly disputed over several decades, mainly due to irreproducible experimental evidence; nevertheless, viral oncology was born. Over the last three decades many types of DNA viruses and one class of RNA viruses, capable of inducing neoplastic transformation in different species, have been identified [1,2]. Recent experiments revealed that both DNA and RNA tumor viruses induce neoplastic transformation through host cellular genes, which are important for cellular growth regulation, such as processes leading to cell renewal, terminal differentiation, growth arrest, senescence, or even programmed cell death. Those genes have also been shown to be potential oncogenic themselves [3,4]. In this chapter we will focus on RNA-containing type-C retroviruses associated with osteosarcomas, and more specifically on two members of this group, which induce exclusively osteosarcomas in mice. Finally, we will review oncogenes reported to be activated in *H*uman (H-) and *M*urine (M-) *OS*teosarcomas (OS). Due to limitations of space, reviews or recent publications containing overviews have been used as references instead of the original publications.

Retroviruses in OS

Type-C retroviruses are divided into two different groups. (1) The *slow-acting viruses* [also called chronic, replication competent or leukemia viruses, (*LV*)] induce predominantly leukemias after a latency period of months or years, but they cannot transform cells in vitro. (2) The *acute transforming viruses* [transducing viruses, sarcoma viruses (*SV*)] are capable of transforming cells in culture and of inducing tumors in vivo after a few weeks. The

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latter type of virus replaces viral genetic material for an oncogene, resulting in a replication-deficient virus, which therefore needs a helper virus to complete its life cycle [2]. The lifestyle of these viruses and how they manage to transform cells will be discussed later.

Retroviruses have been identified in or isolated from the majority of radiation-induced and spontaneous OS in animals, and from both tumor-bearing animals and cell lines derived therefrom. These isolates consist of activated endogenous or recombinant viruses, mostly of the slow-acting type, which induce lymphomas, osteopetrosis, and osteomas upon infection of newborn mice. These data suggest that these viruses have distinct effects on bone tissue [5,6]. Endogenous, ecotropic retroviruses have been found to be amplified in radiation-induced osteosarcomas of Balb/c mice, and newly somatically acquired proviruses are frequently found in radiation-induced osteosarcomas [6]. The viruses derived from molecularly cloned, somatically acquired proviruses from two radiation-induced osteosarcomas of the Balb/c mouse also induce malignant lymphomas and osteopetrosis (J. Schmidt, personal communication). The broad range of diseases induced by these viruses and the mouse-strain-related differences in the presence of virus particles in osteosarcomas strongly suggest that they contribute indirectly to osteosarcomagenesis by a yet unidentified mechanism [7].

OS-inducing acute transforming retroviruses

Two different isolates of murine sarcoma viruses that induce bone tumors after infection of susceptible mice have been characterized. The *F*inkel-*B*iskis-*J*inkins-*Mu*rine virus complex (FBJ-MuSV/LV) was isolated from a spontaneous bone tumor of a CF1 mouse, and the *F*inkel-*B*iskis-*R*eilly-*Mu*rine virus complex (FBR-MuSV/LV) was isolated from a radiation-induced osteosarcoma of an X/Gf mouse [5]. Both virus isolates appear to have a different host range, a property entirely related to the helper virus: an N-tropic FBJ-MuLV [8] and a B-tropic FBR-MuLV [9]. The most striking feature of the FBJ and FBR transforming viruses is their exclusive association with osteogenic sarcoma.

Retroviruses are characterized by their ability to reverse transcribe their RNA genome into a DNA intermediate, which in turn is integrated within the host chromosomal DNA and becomes a provirus. The typical life cycle of a type-C retrovirus has been reviewed by Varmus [2]. During the stay of a provirus in host DNA, rearrangements may occur in which a cellular gene becomes entrapped within the virus, leading to the formation of an acute transforming virus. The osteosarcoma-inducing virus isolates FBJ- and FBR-MuSV/MuLV exhibit a comparable pathology, so the question has been raised whether these viruses transduced the same cellular gene. Indeed, both transforming viruses contain sequences derived from the *c-proto-fos* gene of the mouse, but differences in oncogenic potential were observed. FBR-MuSV

transduced, in addition, *fos*-unrelated sequences, which, at the time were called *fox* [10], but which recently have been identified as invertedly transduced mouse *fau* sequences [11]. Figure 2-1 resumes the structural organization of the mouse *c-proto-fos* gene and the *fau* cDNA and how they are transduced in the respective osteosarcoma viruses.

Fos

The *fos* gene has numerous upstream regulatory sequences (e.g., response elements for Ca^{2+}; cAMP; serum factors; PDGF-, estrogen-, and TNF-inducible factors), influencing both the basal and inducible expression of *c-fos* mRNA. Morgan and Curran [12] have reviewed the promotor region of the *c-fos* gene. Once transcribed, *c-fos* mRNA is rapidly degraded due to two destabilizing elements in the 3′ untranslated region and within 200 bp of the 3′ end coding sequences, respectively [13]. Finally, the Fos protein itself is multipotential. Fos is, as a nuclear phosphoprotein, a member of the Fos-Jun family, which contains active transcription activators upon dimerization. Other such families exist, such as C/EBP and ATF/CREB, and all are modular in structure. Each of these modules is responsible for specific tasks. The leucine zipper is necessary for protein dimerization, resulting in a specific set of basic DNA-binding domains from both participating proteins defining the specificity of the bound DNA sequence (e.g., the AP-1 site sequence TGACATCA is recognized by the Fos-Jun complex). Competition in dimerization between members of the same family leads to different *trans*-activation or -repression activities (e.g., Jun-FosB or Jun-Jun dimers will exhibit different *trans*-activating activities from Jun-Fos itself). The possibilities of such regulation systems are reviewed in Lamb and McKnight [14]. Also, inhibitory complexes are formed with IP-1, a nuclear protein that binds to both Fos and Jun proteins, leading to "activator sequestering" [15].

The complexity of transcription repression and activation in *trans* makes families of transcription factors such as Fos, Jun, Ets, CREB, and C/EBP master switches that turn short external signals to long-term genetic events [16].

Fau

The fox sequences transduced in FBR-MuSV (see Figure 2-1) were of unknown origin until we realized that reverse transcriptional transduction of a new cellular gene, *fau* (*F*BR-MuSV *A*ssociated *U*biquitously expressed gene), is involved. Therefore, the fox sequences in FBR-MuSV represent antisense sequences to the mouse *fau* gene. This was the first demonstration of antisense sequences present in mammalian retroviruses in vivo. Moreover, the presence of these antisense *fau* sequences doubled the transformation potential of FBR-MuSV in vitro [11]. Both mouse and human *fau* cDNAs encode a protein of 133 amino acids (98% identity), whereas *fau*-specific DNA

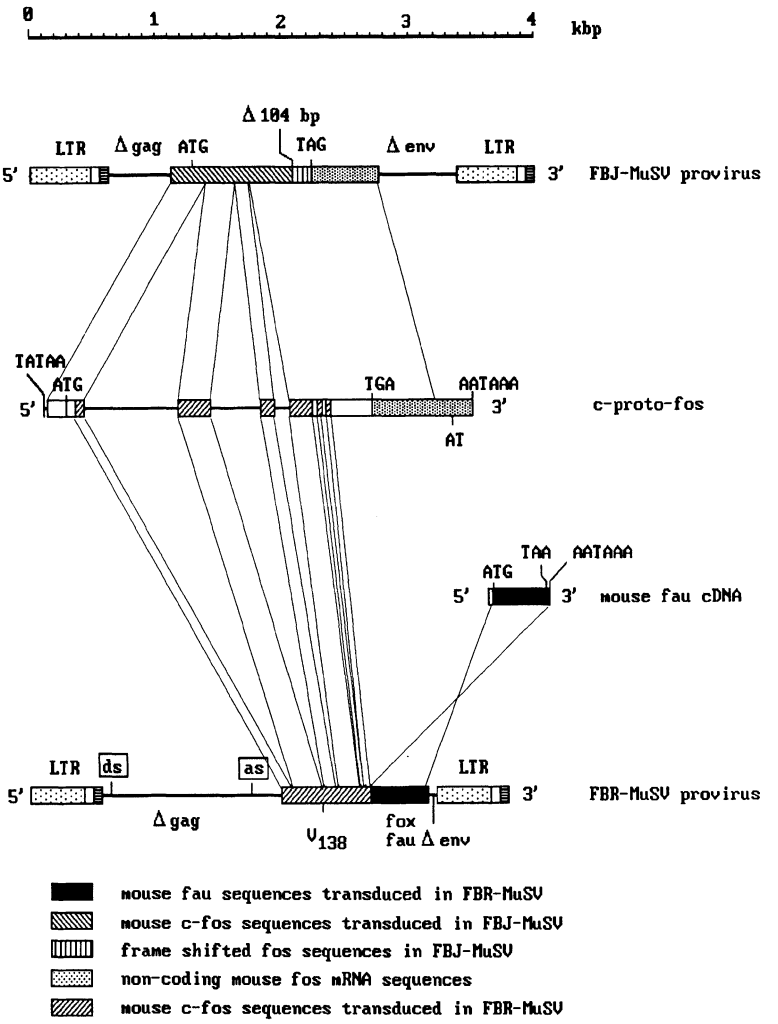

Figure 2-1. Schematic representation of the FBJ-MuSV and FBR-MuSV proviral genomes. The mouse *fos* genomic DNA [21] shows the four exons (boxes), of which the hatched parts are transduced into FBR-MuSV and the stippled area corresponds to the untranslated mRNA region [10]. The black boxes in mouse fau cDNA and in the provirus represent the sequence transduced invertedly into FBR-MuSV [11], whereas V_{138} indicates a mutation in FBR-MuSV that, affects its immortalizing capacity [20]. The vertically hatched box in FBJ-MuSV represents frame-shifted translated *v-fos* sequences due to an internal deletion of 104 bp [21]. AT refers to the AT-rich region destabilizing the fos mRNA [13].

hybridization probes revealed conserved sequences in the genomic DNA of several species. Uniform expression in different tissues tested did not only led to its name but suggested that *fau* may be a housekeeping gene. Mouse and human genomic DNA revealed at least four loci in which *fau* sequences reside. Moreover, these loci themselves seem to accommodate different numbers of *fau* genes.

In situ immunodetection showed that the Fau protein is located in the nucleus. The Fau protein resembles the multifunctional ubiquitin in its form fused to the so-called *C*arboxy terminal *E*xtension *P*roteins (CEP) and contains a nuclear localization signal. The first half of Fau is 55% homologous to Ubiquitin (including amino acid residues important for protein degradation), whereas the second half of Fau physically resembles the CEPs, which happen to be ribosomal proteins (for a review on Ubiquitin and CEPs, see Monia et al. [17]). Analogous to Ubiquitin-CEPs, Fau is transported to the nucleus, where the C-half of the protein may participate in ribosome biogenesis and the N-half may function as a signal for oncoprotein degradation or may participate in transcriptional processes [18]. Such a mechanism may provide basic cell growth regulation.

Activation of Fos in FBJ and FBR M-OS inducing viruses

As already mentioned, Fos is a nuclear phosphoprotein associated with members of the Jun Family in the AP-1 transcription complex. If, however, distortions in regulatory areas of the *c-proto-fos* gene, in mRNA stability, in translation, in protein stability, or in protein interactions are introduced, the c-Fos protein becomes an active oncogene [19,20]. c-Fos is unable to transform primary cultures of fibroblasts in vitro; this contrasts with murine retrovirus v-Fos, FBJ- and FBR-MuSV, which induce osteogenic tumors in mice. The elimination of the carboxy-terminal part of the coding sequence and of a short 3′ noncoding AU-rich domain of the *c-fos* gene converts it to an oncogene in vitro [13]. However, the activation of c-Fos is achieved in different ways in FBJ- and FBR-MuSV (see Figure 2-1). An out-of-frame deletion in v-Fos^{FBJ} results in a completely different Fos protein C terminus [21]. FBR-MuSV has accumulated several point mutations and its protein (v-Fos^{FBR} = p75$^{gag\text{-}fos\text{-}fox}$) ends in the unrelated *fox* sequences [10]. These changes markedly enhance both the immortalizing properties and the transforming capacity of FBR-MuSV, in contrast to FBJ-MuSV [11,20]. A point mutation ($Glu_{138} \rightarrow Val_{138}$) is responsible for the immortalizing properties of FBR-MuSV [20]. The substitution of the C-terminus of c-Fos by eight *fox*-derived amino acids activates the c-Fos protooncogene. The *fox* sequence represents a *fau* sequence that has been transduced in the inverted transcriptional orientation. Therefore, FBR-MuSV gained at the same time antisense sequences, resulting in a higher transformation efficiency compared with FBJ-MuSV [11]. This implies that the *v-fau/fox* effect can be split: first eliminating the negative regulatory sequences in *c-fos*, stabilizing the mRNA,

together with the properly ending of the $p75^{gag\text{-}fos\text{-}fox}$. Secondly, the effect of antisense *fau* sequences support a cooperative effect of *fau* inhibition in v-Fos^{FBR}-induced transformation in vitro.

Revertants of both FBJ-MuSV [22] and FBR-MuSV [23] nonproductively transformed rat fibroblasts have been isolated. These revertants seem to contain functional viruses, expressing v-Fos, but fail to retransform even upon superinfection with the original virus, or eventually after the introduction of several oncogenes. Thus, one or more cellular mutations (dominant and/or recessive) are important for the suppression of v-Fos transformation. These observations are in agreement with the biological activity of various FBR-MuSV constructs, which are recombinant in *fau* sequences, showing different transformation efficiencies without changes in v-Fos^{FBR} protein levels. Therefore, *fau* may be a candidate cellular gene affected in these revertants [11].

Fos activation in radiation-induced and spontaneous OS

In addition to the effect of v-Fos in bone tumor inducing retroviruses, deregulated c-Fos expression also affects bone growth and differentiation. Moreover, the overexpression of Fos in transgenic mice clearly interferes with bone development, such as hyperplasia found with excessive new bone formation. Fifteen per cent of these bone lesions gave rise to osteosarcomas after 9.5 months [24]. Furthermore, Wang and coworkers demonstrated that chondrogenic cells and earlier progenitors are specifically transformed by Fos/Jun, and therefore mesenchymal cells represent a target cell for transformation through c-Fos overexpression [25]. The same type of results have emerged in vitro using a tissue culture model for osteogenic differentiation, such as mandibular condyles from neonatal mice. They contain distinct layers of phenotypically different cells. Mesenchymal-like stem cells differentiate upon cultivation in vitro into osteogenic cells and form chondroid bone [26]. Closs et al. [27] showed that Fos expression precedes this osteogenic differentiation process. Infection of this organ culture with the FBR-MuSV/LV virus complex leads to the transformation of the mesenchymal progenitor cells, resulting in a transplantable osteosarcoma-like lesion [28].

Wu and coworkers [29] reported that in 61% of human osteosarcomas c-Fos expression is elevated. Further, one murine osteosarcoma derived from a spontaneous etiology has been shown to be the consequence of an interchromosomal translocation between the *fos* gene and sequences from mouse chromosome 1, resulting in constitutive Fos expression. The altered allele has been designated *non-fos*. There is some evidence that the *non-fos* sequences (or perhaps the loss of the 3′ *fos* sequences) have contributed to the conversion of *c-fos* into an oncogene, since the gene isolated from the tumor has the capacity to induce morphological transformation in vitro and

is tumorigenic in vivo. However, it is not yet known whether there is any additional positive or negative role for the appended *non-fos* sequences (N. Teich, personal communication). We have also reported an aberrant fos mRNA in a cell line derived from a radiation-induced M-OS [30].

On the other hand, 5 of 8 DNA's of ^{90}Sr-induced bone tumors from CF1 mice revealed a rearranged *fau* gene [11]. Fos-Jun also plays an important role in stimulus-transcription coupling in the nervous system [12]. Therefore the question arises as to whether the identification of a primary intracerebral osteosarcoma [31] can be due to the deregulation of the Fos-Jun genomic response.

Other oncogenes and growth suppressor genes affected in OS

Other genes have also been reported to affect bone tumor formation. First, it has been known for a long time that bone tumors arise frequently as secondary tumors in children cured from retinoblastoma. The Rb gene, the inactivation of which is at the basis of this eye tumor [4,32], is rearranged in several osteosarcomas (Table 2-1). Moreover, the reintroduction of an intact Rb gene in cell lines derived from bone tumors strongly reduces tumor growth [33]. Secondly, several research groups have reported changes in another tumor suppressor or growth suppressor gene, namely *p53*. Again, over-expression of a wild-type *p53* in an osteosarcoma-derived cell line (SAOS-2) blocks the growth of tumor cells [4,34].

These two types of tumor suppressor or growth suppressor genes have severe effects on bone tumor growth but do not, nevertheless, give a definite predisposition to osteosarcoma [35]. Numerous reports have linked dominant oncogenes to osteosarcomas, and a list of observations is summarized in Table 2-1.

Conclusions and new perspectives

The study of OS and cancer, in general, relies on the study of primary tumors, naturally occurring in animals and humans, or experimentally induced with bone-seeking radionuclides or acute transforming retroviruses. Otherwise, cell lines can be generated from these primary tumors or OS can be induced in vitro using mandibular condyle cultures. However, when studying these cancer cells in vivo, and surely in vitro, we deal with "changed" cells. These are not the result of an ordered array of molecular genetic events but rather chaos. Indeed, cancer cells frequently show amplification of genome segments, proving their genetic instability [36,37]. Thus, when comparing tumor cells to their normal counterparts, numerous changes are noticed, but the question of whether it is cause or effect remains unresolved. Recent achievements in

Table 2-1. Oncogenes described in osteosarcomas

Gene	Source
Oncogenes	
fos	FBJ-and FBR-MuSV M-OS[a] [10,20,41] Overexpression or altered transcription in spontaneous[b] and radiation-induced [30] M-OS overexpression in H-OS[a] [29]
myc	Amplification or overexpression in viral or radiation-induced M-OS [6,30,42–44] Amplification in mouse SEWA tumors from polyoma virus-induced M-OS [45] Amplification or overexpression in H-OS [46–48] PDGF-induced expression in HOS cell line [49]
met	Activated by MNNG-induced translocation in HOS cells [50]
sis	Multiple transcripts in human U_2-OS cells [51] Amplification in spontaneous canine OS [52] Overexpression in radiation OS [30]
Ki-Ras	Mutated, amplified and overexpressed in OHA OS [53] Ki-ras transformation of HOS revertants [54]
Ha-Ras	Ha-MuSV induced OS in rats and hamsters [55] Highly variable allele methylation pattern in the VTR region of MNNG-treated HOS cells [56] Multiple transcripts and overexpression in radiation- and FBR-MuSV-induced M-OS [30]
raf-1	Amplification in H-OS [48]
mos	Mo-MuSV-induced OS in rats and hamsters [55]
fps	Implicated in in vitro transformation of osteoblasts [57]
abl	Overexpression in FBR- and radiation-induced M-OS [30]
Tumour/growth suppressor genes	
rb	Gene rearrangements, inactivation in OS [58,59]
p53	Gene rearrangements, inactivation in OS [60,61]
Other	
fau	Gene rearrangement in radiation OS [11]

[a] M-OS: murine osteosarcoma; H-OS: human osteosarcoma.
[b] N. Teich, personal communication.

overall molecular oncology have been reviewed recently by several authors in a special issue of *Cell* (Volume 64, January 1991).

As for radiation-induced OS, endogenous retroviruses frequently are activated or even recombined to form new types of viruses. The possible causal effect of these viruses in OS formation is still obscure. However, insertional mutagenesis of these viruses has been shown to influence the expression of oncoproteins in radiation-induced lymphomas [6]. Therefore, virus integration may have a direct effect on carcinogenesis or may merely provide a continuous

intracellular mitogenic signal. Otherwise, viruses can be generated from the "chaotic" genetic situation of an irradiated cell, such that it escapes repression activity.

The *fos*-bearing M-OS-inducing viruses (FBJ-MuSV and FBR-MuSV) revealed a definite link between viruses and bone tumors. However, the dominant position of Fos in bone formation, differentiation, and transformation has been supported by numerous reports, as, summarized in this chapter (viruses, transgenic mice, mandibular condyles, overexpression in human osteosarcomas); the *fos* gene has also been transduced in a chicken retrovirus (NK-24), which induces nephroblastomas [38], while c-Fos is implicated in several cellular functions [12,19].

In addition to the numerous reports about *fos* gene changes in OS, we reported the rearrangement of *fau* loci in several radiation-induced OS, while *fau* antisense sequences cooperate with v-Fos^{FBR} in FBR-MuSV transformation (see Section 2.3). Taken together, our data support the hypothesis that Fau may act as a nuclear tumor suppressor gene on v-Fos^{FBR} transformation and, eventually, on c-Fos-induced bone tumors. However, more study is needed to verify whether this tumor-suppressing activity is tumor independent or is restricted to tumors associated with nuclear transcription factors (e.g., Fos, Jun, and Ets) and how this is related to the cellular function of Fau.

In comparison to Ubiquitin-CEP, Fau may be associated with transcription of DNA through its effect on the chromatin structure. Dynamic Ubi- and de-Ubiquitination is required for active DNA transcription [39]. Otherwise Ubiquitin is needed for the turnover of short-lived oncoproteins in the nucleus [40]. In this regard, *fau* may be a member of a superfamily of genes [18], related to ubiquitin, which play a pivotal role in several basic cellular functions (transcription, protein degradation, ribosome biogenesis, receptor binding, . . .) [17].

Numerous other oncogenes are implicated in OS. Some show different oncoproteins activated in one tumor; other cases are reported to have one oncogene affected up to now (Table 2-1). Also growth suppressor genes (or antioncogenes) are inactivated in several cases of OS (Table 2-1), while reintroduction results in growth arrest, implicating an antitumoral effect. However, unlimited growth is only one aspect of cancer cells, which is different from the transformed status. Therefore, the predisposition to OS resulting from heterozygous inactivation of Rb or p53 remains to be proven.

It is widely accepted that the accumulation of changes result in a cancer cell with unlimited growth, probably through the genetic instability of an initial triggered cell. Therefore, the identification of genes and proteins affected in cancer cells remains important in order to study their normal counterpart. Indeed, the study of oncoprotein ancestors and how they manage, interactively, both the cell growth and differentiation pathways will reveal basic break points (trigger points) leading to the chaotic cellular state from which cancer cells eventually develop.

Acknowledgments

The work at the authors' laboratory was supported by the "Kankerfonds" from the "Algemene Spaar en Lijfrentekas" and the Belgian "Fonds voor Geneeskundig Wetenschappelijk Onderzoek".

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3. In vivo ^{31}P nuclear magnetic resonance spectroscopy of osteosarcoma

E.L. Mooyaart, R.L. Kamman, and W.J. Boeve

Characterization of tumor processes and studying their metabolism is one of the main challenges in diagnostic clinical oncology. Magnetic resonance spectroscopy (MRS) provides a noninvasive method to monitor tissue metabolism. Since the early work of Griffiths [1], an increasing number of papers have been published on in vivo measurement of tumor metabolism using MRS. Detailed accounts of the methods of NMR spectroscopy and NMR imaging have been published [2,3] and are beyond the scope of this review.

In vivo MRS requires a homogeneous high magnetic field created by an NMR magnet. Currently the highest field-strength magnet approved for human applications is 2 Tesla (T). The strength of the earth magnetic field is approximately 0.5 Gauss, and 10.000 Gauss is equal to 1 T. MRS can provide information about a number of biologically important nuclei, such as ^{1}H, ^{13}C, ^{23}Na, and ^{31}P. However, since all MRS studies of in vivo spectroscopy of osteosarcoma have used ^{31}P, this chapter is limited to ^{31}P-MRS.

^{31}P-MRS can detect phosphorylated compounds when they are present at greater than roughly 100 μmol concentration [4]. These phosphorylated compounds include metabolites such as adenosine triphosphates (ATP), phosphocreatine (PCr), inorganic phosphate (Pi), and phosphomonoester (PME) and phosphodiester (PDE) compounds. The adenosine triphosphate peaks are predominantly the three phosphorous nuclei of ATP. The PME peaks give information about membrane synthesis, and the PDE peak provides information about membrane degradation. The PME peaks have been attributed to sugar phospates, AMP, or phosphocholine (PC) plus phosphoetanolamine (PE) [5,6]. The PDE peaks derive from the membrane breakdown products glycerolphosphorylcholine and glycerophosphorylethanolamine [6]. Since no method for reliable absolute quantitation of the concentrations of these metabolites is available, relative peak heights or peak areas (peak ratios) are used to describe differences between normal and tumorous tissue, and to monitor changes in the spectrum during therapy. The Pi peak shifts position with pH, which allows the measurement of both intracellular and extracellular pH; however, at a magnetic field strength of 1.5–2T the Pi is broad and is not resolved into its intracellular and extracellular components, making the pH an average with a large standard error [7]. In normal muscular tissue, PCr is

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 ISBN 0-7923-1905-2

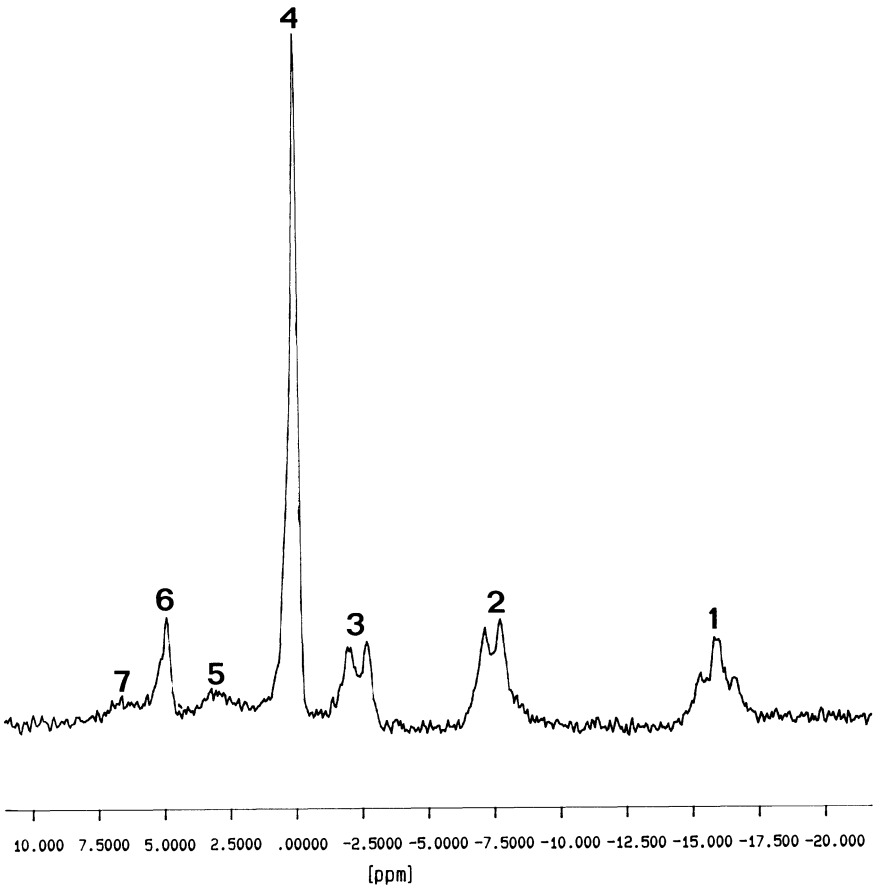


Figure 3-1. ^{31}P spectrum of the gastrocnemius muscle. 1 = βATP, 2 = αATP, 3 = γATP, 4 = phosphocreatine (PCr), 5 = PME, 6 = Pi, 7 = PDE. The PME peaks give information about membrane synthesis, and the PDE peaks about membrane degradation. The PME peaks have been attributed to sugar phosphates, AMP, or phosphocholine (PC) plus phosphoethanolamine (PE). The PDE peaks derive from the membrane breakdown products glycerolphosphoryl-choline and glycerophosphorylethanolamine. In normal muscle tissue PME and PDE are hardly discernable.

the dominant peak in the phosphor spectrum, whereas PDE and PDE peaks are hardly discernable (Figure 3-1).

During the last 5 years results of ^{31}P-MRS in osteosarcoma in a limited number of patients have been described [8–15]; moreover, tumor response to therapy has been monitored with ^{31}P-spectroscopy in a number of patients [7,8,9,11]. In most studies osteosarcoma was studied within a large group of other bone and soft tissue tumors. In these studies no characteristic spectra for different histologic types of tumors could be identified [7,8,10–12]. Most publications report an elevation of the PME, PDE, and Pi peaks compared

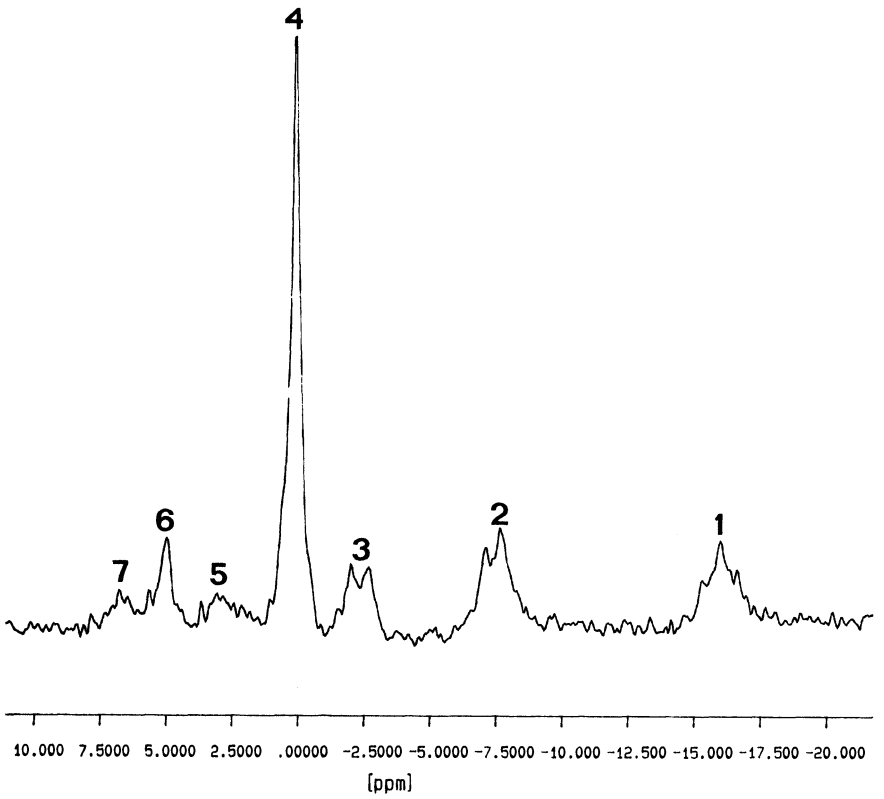
to normal muscular tissue [7–15]. Ross et al. also described an elevation of ATP [8]. The pH of osteosarcoma did not differ from normal tissue in this study. Redmond et al. [9] found a significantly high PME/PCr ratio, whereas the Pi/PME ratio was significantly decreased. The pH of the tumors in this study was within normal limits. Semmler et al. [7] used the least squares fit method of analyzing the spectra and found increased PCr/Pi and PCr/βATP ratios. Our results with localized ^{31}P MRS in osteosarcoma show an elevation of the PME and PDE peaks as well [14,15].

When tumor metabolism during therapy is monitored, usually a decrease in the PME and PDE peaks is observed [8,9,11]. Some authors have described an increase in inorganic phosphate after therapy. This elevation is attributed to cell death; however, no correlation was found between changes of PME/ATP or Pi/PME and necrosis [9].

All MR spectroscopists face the major problem of contamination of the tumor spectrum by surrounding tissue. This contamination jeopardizes the reliability of the tumor spectra and sheds doubt on the results. When the sensitivity profile of the surface coil is used as the only method of localization [7–13], a significant contamination of surrounding tissue occurs. The degree of contamination from adjacent tissue relative to the intended volume of interest depends on the tumor size and the sensitive volume of the coil used. The smaller the tumor size with respect to the coil dimensions, the larger the contribution from signals outside the tumor. When one-dimensional chemical-shift localization techniques are used, spectra more characteristic of tumor can be obtained. Ross et al. [8] described a marked improvement in results when a one-dimensional localization technique was applied, compared to the results of whole-volume spectroscopy. Phosphocreatine was consistantly lower in localized tumor spectra compared to the whole-volume spectra. A high PCr peak in a tumor spectrum, therefore, may indicate a significant contribution of surrounding tissue to the tumor spectrum.

Zlatkin et al. [10] also used whole-volume spectroscopy and found 10 cases of high-degree muscle contamination in a series of 23 patients with bone and soft tissue tumors. They observed that the spectra of superficially located tumors with a relative larger tumor volume had less contamination. When one-dimensional chemical shift localization was used, the spectra became characteristic of tumors with higher levels of PME, PDE, and Pi, and decreased levels of PCr in slices deeper in the tumor. Also, our spectra of osteosarcoma show an significant muscle contamination when whole-volume spectroscopy is used (Figure 3-2). The PCr peak originating from muscular tissue is significantly higher in the whole-volume, compared to the localized, spectrum.

When tumor metabolism is monitored during therapy, tumor shrinkage is likely to occur and muscle contamination will increase when no adequate localization techniques and applied. The whole-volume spectra of an osteosarcoma during chemotherapy and radiotherapy published by Semmler et al. [7] show a high PCr peak, indicating significant muscle contamination.

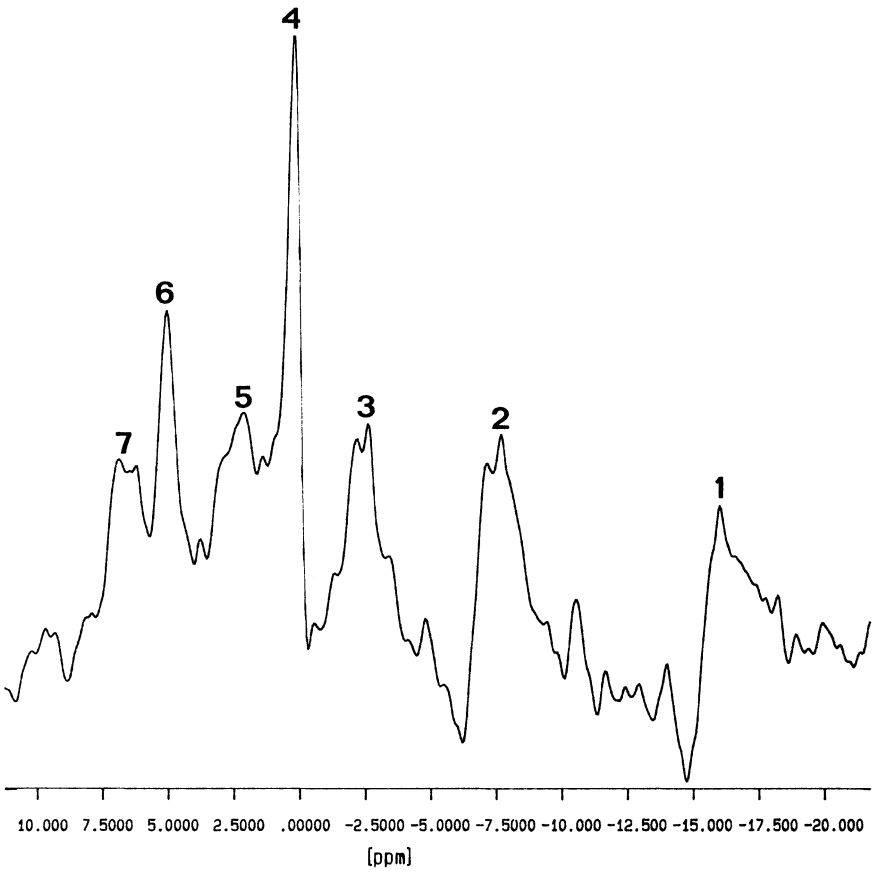


A

Therefore many authors state the necessity of volume-selective spectroscopy to measure real tumor spectra [8,10,12]. This agrees with our findings with localized MRS of osteosarcoma: The use of adequate localization techniques is a prerequisite for obtaining reliable tumor spectra [15].

Technical shortcomings of MRS put severe restrictions on the clinical applicability of ^{31}P MRS in osteosarcoma. First, the low sensitivity of ^{31}P-MRS limits the measurement of localized spectra to larger volumes. When spectra of smaller tumors have to be obtained, unacceptable acquisition times are necessary. Second, because the sensitive volume of a surface coil is limited to a hemisphere with a radius of approximately half the coil diameter, only superficially located tumors can be measured.

Although the initial results of ^{31}P-MRS in bone tumors are tempting, this technique is not recommended as a routine procedure in the clinical management of patients with osteosarcoma. Until technical improvements in localization techniques and surface coil sensitivity are available, ^{31}P-MRS of osteosarcoma should be limited to research institutes and should be used as a promising noninvasive technique to monitor tumor metabolism.



B

Figure 3-2. **A:** whole-volume spectrum of an osteosarcoma. PDE (5) is slightly elevated. PCr (4) is high, indicating contamination by surrounding tissue. **B:** Specially localized spectrum of the same osteosarcoma. PME (5), Pi (6), and PDE (7) contributions are more prominent with respect to PCr (4).

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4. Perioperative blood transfusions and survival in osteosarcoma

Rina Chesi, Battista Borghi, and Stefano Lari

Introduction

Various authors have observed and reported an association between perioperative blood transfusions and disease-free survival time in patients with malignant tumors. Interest in this relationship began from the observation that perioperative transfusions improved graft survival in patients undergoing kidney transplantation [1]. Based on these clinical data, in 1981 Gantt stated his belief that blood transfusions in patients with malignant tumors would give the tumor "a better chance to survive" [2]. Since that time clinical investigations have been carried out to confirm the relationship between perioperative blood transfusions and a worse prognosis. Most of the data concern colerectal cancer [3–7], lung cancer [8, 9], breast cancer [7,10–12], soft tissue sarcomas [13], and head and neck cancer [14].

Our experience [15] is based on a study of 155 patients with nonmetastatic osteosarcoma of the long bones who were all treated with amputation and adjuvant chemotherapy. The following variables were analyzed: (1) age upon referral (mostly between 5 and 20) (2) sex, (3) site of the tumor, (4) time lapse between onset of symptoms and diagnosis, (5) radiologic features of the tumor, (6) tumor size in relation to the total length of the bone segment, (7) alkaline phosphatase value, (8) type of biopsy, (9) number of perioperative transfusions, (10) interval between surgery and appearance of metastases, and (11) survival period. Variables (10) and (11) were monitored for 8 years after the surgery; for this reason, the data collected are considered definitive.

The patients were divided into two groups, transfused and nontransfused; then an accurate statistical elaboration was performed showed similar characteristics for almost all variables except perioperative blood transfusions, which revealed a significant difference in disease-free and overall survival after surgery (Figures 4-1 and 4-2).

It can be assumed that massive transfusions could be the symptom of a more severe pathology or larger tumor size, but we must keep in mind that in our series, in order to avoid the prognostic variance due to surgical stress, we included only amputated patients.

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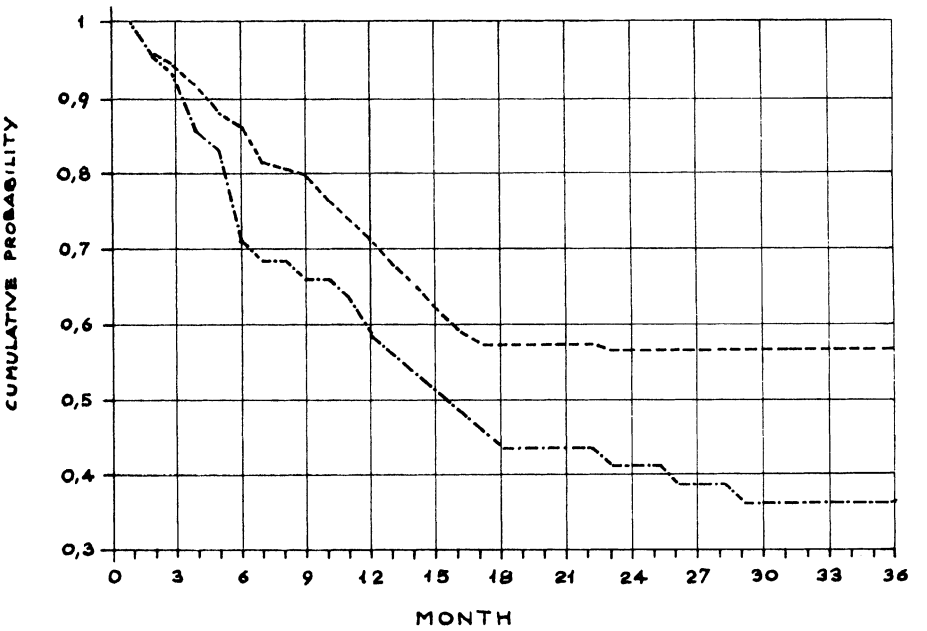

Figure 4-1. Disease-free survival. Comparison between transfused and nontransfused patients. – – – nontransfused patients · – · – · transfused patients

The immunodepressive effect of hemotransfusion has now been widely proven by both clinical and experimental studies. Waymack et al. [16,23] tested the effect of blood transfusions on tumor growth in a rat sarcoma model, confirming a significant increase of tumor growth when syngeneic transfusions were used. The same author observed an impairment in macrophage function [17] and increased synthesis of the immunosuppressive metabolite prostaglandin E [18,19]. These studies reported a decreased cell-mediated immune response, as measured by hapten sensibilization to dinitrofluorobenzene, alteration in macrophage/monocyte function, and diminished ability to phagocytose and kill bacteria [17].

Extensive data on human studies confirm the immunosuppressive effect of hemotransfusions. Fisher et al. analyzed the effect of transfusions on washed, packed RBCs in kidney graft recipients and saw that transfusion of one to three units suppressed cellular immunity, as determined by the mixed lymphocyte response to both mitogenic and antigenic stimulation [1]. Moreover, Smith et al. found evidence of increased T lymphocyte suppressor cell activity in patients transfused with two units of packed RBCs [20]. Kessler et al. found that patients receiving blood transfusions or even factor VIII concentrate alone for hematologic disorders compared with nontransfused patients with the same disease presented a low natural killer cell activity and decreased T4/T8 ratios due to an increase in T8 cells [21].

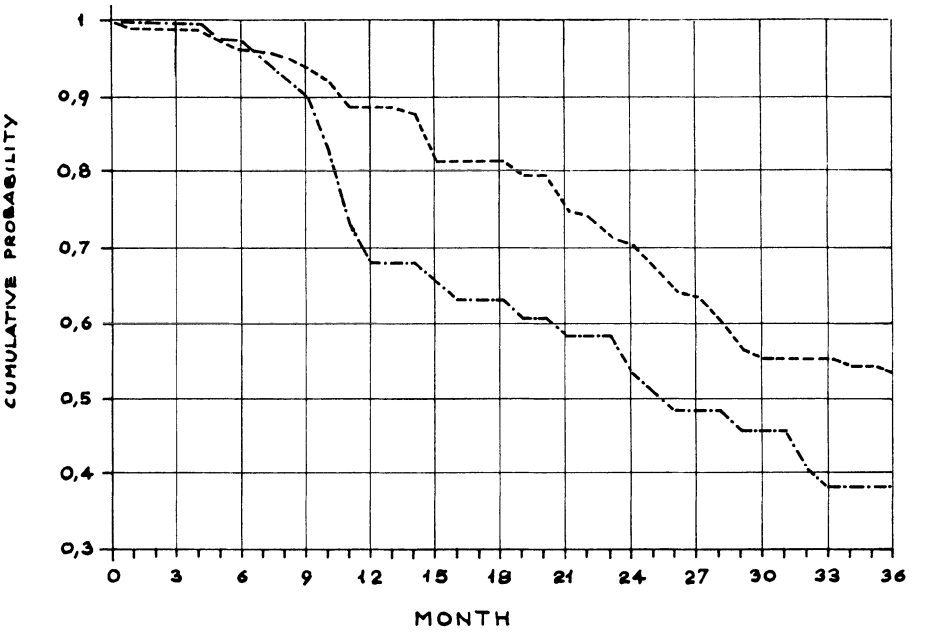

Figure 4-2. Overall survival. Comparison between transfused and nontransfused patients. ·–·–· transfused patients – – – nontransfused patients

The scenario described by these data indicates evidence of immunodepression after hemotransfusion. As a therapyeutic practice, the need for reducing perioperative syngeneic transfusions as much as possible has been demonstrated to be mandatory. As an effective alternative, the extensive use of autologous transfusions, hemodiluition, and correct antianemic therapies may also be programmed.

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5. Adjuvant interferon treatment in human osteosarcoma

Hans Strander, Henric C.F. Bauer, Otte Brosjö, Andris Kreicbergs, Johan Lindholm, Ulf Nilsonne, Claes Silfverswärd, and Alfred Szamosi

Introduction

The protocols of Rosen and collaborators have been used for several years in Scandinavia [1], with the exception of our institution, where adjuvant IFN has been utilized for 20 years [2]. The present report is an update of our IFN-treated osteosarcoma series conducted over the period 1971–1984; control patients representing a high-dose chemotherapy group and a nonadjuvant group are also presented.

Patients and methods

During the years 1971 and 1984, 77 osteosarcoma patients were seen at Karolinska Hospital. Nine patients had metastases at presentation, leaving 68 patients for the IFN trial; these 68 patients constitute the IFN-treated group. Two control groups have been elected. One is a nonadjuvant group of 32 patients treated elsewhere in Sweden between 1971 and 1976. The other is a chemotherapy group of 20 patients treated elsewhere in Sweden from 1977 to 1980. The IFN and both control groups comprise all osteosarcoma patients without metastases at presentation in Sweden during the years studied. Hence, these groups together constitute a nonselected series of patients followed for a minimum of 5 years.

IFN treatment

The IFN preparations used in the trial consisted of natural leukocyte IFN processed according to two methods, both yielding semipurified solutions [2]. The IFN was administered (i.m.) daily during the first month after diagnostic open biopsy at a dose of 3×10^6]U and then three times per week for another 17 months.

The side effects of this type of treatment have been described previously in detail [3]; they are typical of those reported for natural IFN alpha therapy. No patients had to stop IFN treatment because of side effects.

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Chemotherapy

Chemotherapy was given with either high-dose methotrexate or high-dose Adriamycin according to local protocols.

Clinicopathologic features

Different clinicopathologic features considered to be of prognostic significance were assessed during the course of treatment, as described previously [2]. The surgical margins were not equally distributed among the three groups. There were more radical and wide margins in the two control groups. In fact, an inadequate margin, i.e., a marginal or intralesional, was recorded in as many as 21 of 68 patients in the IFN group, while inadequate margins were recorded in only two patients in the chemotherapy group and in one patient in the nonadjuvant group.

Results

The local recurrence rate was much higher in the IFN group than in the other two groups, i.e., 30% compared to 0% in the chemotherapy group and 4% in the nonadjuvant group. The 5-year metastasis-free survival was 0.38 in the IFN group, 0.50 in the chemotherapy group, and 0.32 in the nonadjuvant group. The overall survival was 49% in the IFN group, 54% in the chemotherapy group, and 35% in the nonadjuvant group (Figure 5-1).

Discussion

The rationale for using IFN in the adjuvant setting of osteosarcoma is multifold. Experimentally, exposure of osteosarcoma cells to IFN alpha has been shown to cause partial reversion of the malignant phenotype, inhibition of proliferation, loss of cell-overlapping capability in confluent cultures, and marked reduction of tumorigenicity [4]. Kirstein and Baglioni [5] reported that human tumor necrosis factor stimulated the proliferation of human osteosarcoma cells and that mitogenic activity could be abolished by IFN. The monocytes probably play a role in host defense against osteosarcoma cells in vivo. The monocyte function of patients with osteosarcoma is normal [6] and can be stimulated by IFN.

Human leukocyte IFN is capable of inhibiting the growth of human osteosarcoma cells in tissue culture [7]. Glasgow and Kern demonstrated an inhibitory effect of IFN on osteosarcoma in rodents in one system, but not in another [8,9]. Transplanted human osteosarcomas growing in nude mice are

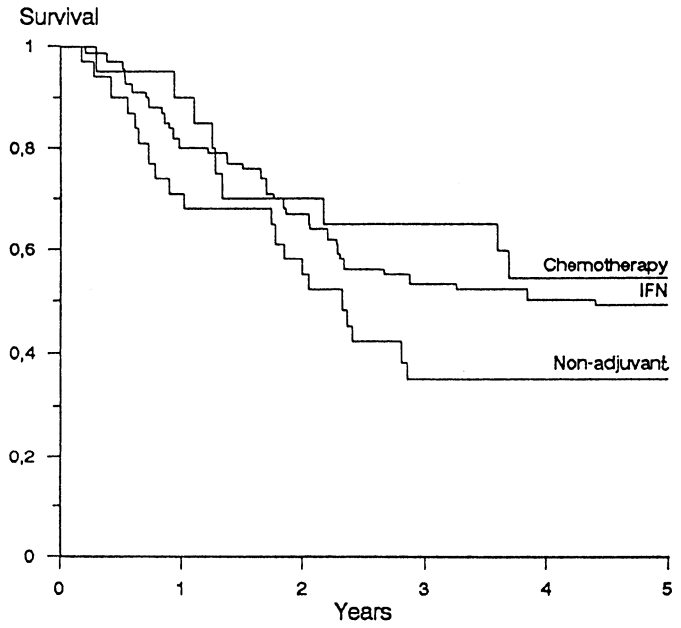

Figure 5-1. Overall survival of the three groups.

inhibited by human IFN alpha [10–13]. The agent should be given frequently in a high dose for a long period. It is likely that the regimen of the German/Austrian COSS trial on IFN beta used doses that were too low over an insufficient period of time [14].

In metastatic osteosarcoma, Rosen and coworkers could not see any effects of IFN [15]. However, partial regression of osteosarcomas has been reported by others using human alpha IFN [16]. A Japanese trial showed that 2 out of 4 osteosarcoma patients with pulmonary metastatic disease responded to treatment with natural IFN alpha [17]. Nonetheless, it must be emphasized that lung resection should always be considered in the primary salvage treatment of patients with a single or few pulmonary metastases [18].

Summary

An update of the adjuvant trial on osteosarcoma in Sweden comparing patients receiving natural interferon (IFN) alpha with a high-dose chemotherapy group and a nonadjuvant group is presented. The overall survival for the IFN group is 49%, for the chemotherapy group 54%, and for the nonadjuvant group 35%. Trial evaluation was complicated by group differences with respect to various clinicopathologic features of prognostic significance. The role of IFN in the treatment of osteosarcoma can still not be established.

Acknowledgments

The study was supported by grants from the Cancer Society of Stockholm and the Karolinska Institute Funds.

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6. Difference between osteosarcoma in southwest England and the Kanto area of Japan in relation to age, sex, and localization

Rikuo Machinami and Tudor Wickremaratchi

Introduction

Osteosarcoma usually affects the long bones during the second decade of life. Although there are several reports on clinicopathological aspects of many osteosarcomas in the European, American, and Japanese literatures [1–5], no comparison has been made between European or American cases and Japanese cases. Therefore, an attempt was made to compare the age, sex, and localization of histologically confirmed osteosarcomas in southwest England with those in the Kanto area of Japan. Several reports on the age and sex distribution and localization of osteosarcomas have already been published from the Bristol Bone Tumour Registry in England [2,6–8], and we have reported previously [1] on 62 cases of histologically confirmed osteosarcomas examined mainly at the University of the Tokyo Hospital. The data from both institutes have been updated in the current study, and Japanese cases of osteosarcoma from several hospitals in the Kanto area of Japan have been included.

Surgical specimens of 270 English and 163 Japanese histologically confirmed osteosarcomas—excluding parosteal, periosteal, or extraskeletal osteosarcomas—were used in this study. The English cases were examined over 37 years, i.e., between 1946 and 1983, at the Bristol Bone Tumour Registry; while the Japanese cases were examined over 20 years, i.e., between 1966 and 1986, including 95 cases at the University of Tokyo Hospital, 44 cases at the Cancer Institute Hospital, 20 cases at the Gunma University Hospital, and four cases at Toranomon Hospital. Osteosarcomas were divided into "typical" and "atypical" cases according to their localization and the age of the patients. Those cases occurring between the first and third decades of life that were localized in the long tubular bones were grouped as "typical" osteosarcomas. Osteosarcomas in the short and flat bones, and the cases localized in the long tubular bones in patients over 30 years of age, were grouped as "atypical" osteosarcomas.

Although a similar male preponderance was found in English and Japanese cases, the percentage of male patients was slightly higher in the Japanese

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cases (64.4%) than in the English cases (58.5%). A high peak in incidence was found in the second decade of life in both the English and Japanese cases (Figures 6-1 and 6-2). The percentage of cases in patients over 30 years was twice as high in Bristol (36.3%) as in the Kanto area of Japan (15.3%). The percentage of atypical osteosarcomas was twice as high in English cases (43.3%) as in Japanese cases (20.9%) (Figures 6-1 and 6-3). The most striking difference between English and Japanese osteosarcomas was that more than half (52.6%) of the osteosarcomas of Bristol in those patients over 40 years occurred in patients with Paget's disease and no osteosarcomas with Paget's disease were observed in Japanese cases (Figures 6-4 to 6-6). A low peak incidence in the sixth decade cases seems to correspond to the cases with Paget's disease. It has been reported from the Bristol Bone Tumour Registry that 30% of osteosarcomas are associated with Paget's disease of bone. This has been reported to be 60% in patients with osteosarcoma aged over 50 years [6]. This is in contrast to a report from the United States that only 20 (3.3%) of 600 osteosarcomas arose in bone affected by Paget's disease [3]. The high incidence of Paget's osteosarcoma seems to correspond to the higher prevalence of Paget's disease in Britain as compared to other European countries and North America. Age- and sex-standardized prevalences of Paget's disease have been reported to be 4.6% in Britain, 0.4–2.7% in other European countries, and 0.9–3.9% in the United States [9]. Paget's disease of bone is very rare in Japan, and therefore no Paget's osteosarcoma has been reported in the Kanto area. The incidence of sarcoma occurring in Paget's disease was reported from Latin America to be 6.28%. The most common tumor type was osteosarcoma (62.9%), followed by fibrosarcoma (24.2%), chondrosarcoma, malignant fibrous histiocytoma, and reticulum cell sarcoma [11]. A similar incidence of Paget's sarcoma has been reported in the United States; the percentages of osteosarcoma, fibrosarcoma, chondrosarcoma, and giant cell tumor are 93.9%, 3.7%, 1.2%, and 1.3%, respectively [12]. The incidence of Paget's osteosarcoma in the Bristol Bone Tumor Registry was 48.5% for osteosarcoma, 19.7% for fibrosarcoma, 3.0% for malignant lymphoma, and 7.6% for undifferentiated sarcoma [9].

The frequency of localization was similar between the two countries: distal femur proximal tibia proximal humerus. Atypical osteosarcomas of the long tubular bones were higher (54.5%) than those of the short and flat bones in cases from Bristol, while short and flat bone cases were higher (55.9%) in Japanese cases. Almost no male preponderance was found in all atypical osteosarcomas of Bristol, while in the Japanese cases of the short and flat bones there was a 63.2% male predominance.

The distal femur and pelvis were the most frequently involved sites for atypical osteosarcoma of the long tubular bones and the short and flat bones, respectively, both for the English and Japanese cases. No peaks in age incidence were found in atypical osteosarcomas of the short and flat bones among the English cases, while a low peak in age incidence was found in the third decade of life in those from Japan.

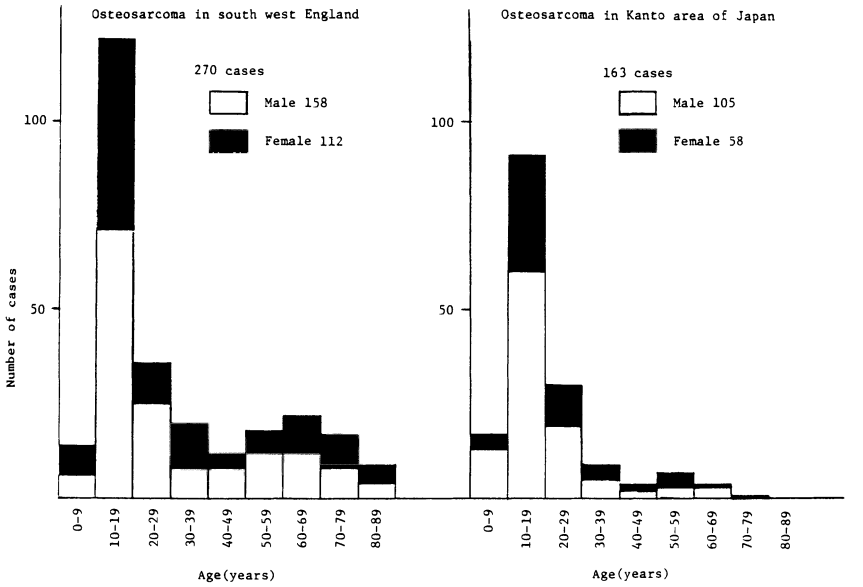

Figure 6-1. Age and sex distribution of osteosarcoma in southwest England and Kanto area of Japan.

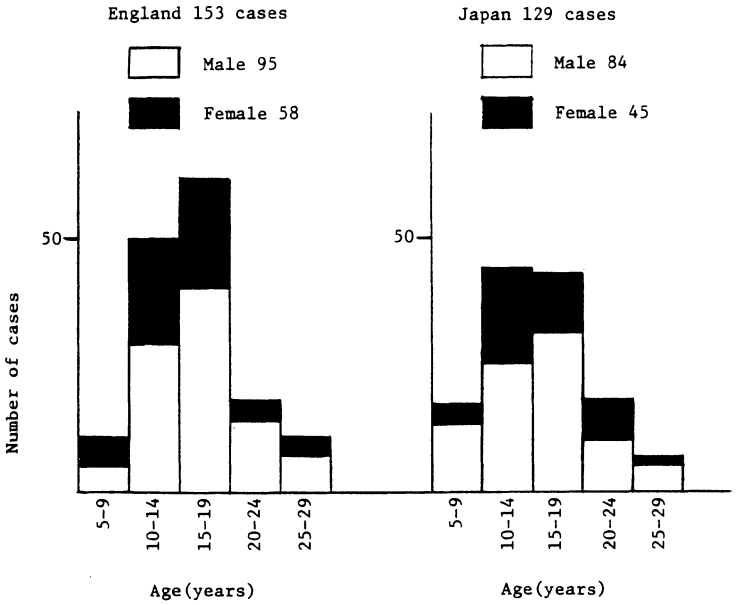

Figure 6-2. Age and sex distribution of typical osteosarcoma in southwest England and Kanto area of Japan.

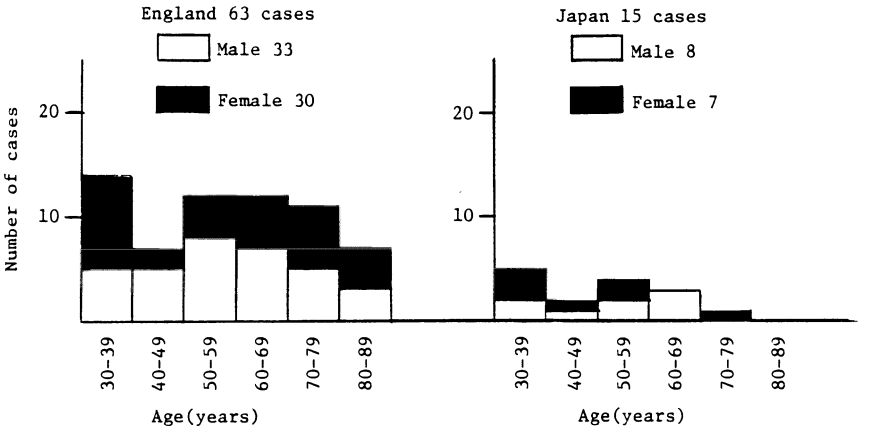

Figure 6-3. Age and sex distribution of atypical osteosarcoma of the long tubular bones in southwest England and Kanto area of Japan.

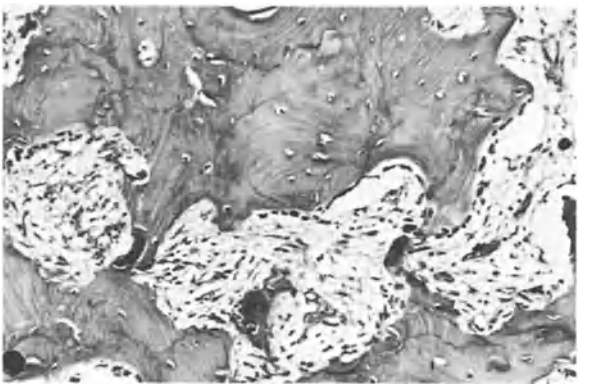

Figure 6-4. Histology of Paget's disease of the left femur in a 58-year-old female with osteosarcoma of the same bone. The lamellar bone trabeculae with irregular cement lines and reactions of osteoblasts and osteoclasts are seen (H&E, ×100).

Acknowledgments

Dr. N.G. Snerkin supported us in the examination of cases from the Bristol Bone Tumor Registry, while one of the authors (R.M.) stayed in the registry from October 1 to November 23, 1984 as a visiting pathologist supported by the Ministry of Education, Science and Culture of the Japanese Government. We would like to thank the staff of the Pathology Departments of the Bristol Bone Tumor Registry, University of Tokyo, Gunma University, Cancer Institute (Tokyo), and Toranomon Hospital.

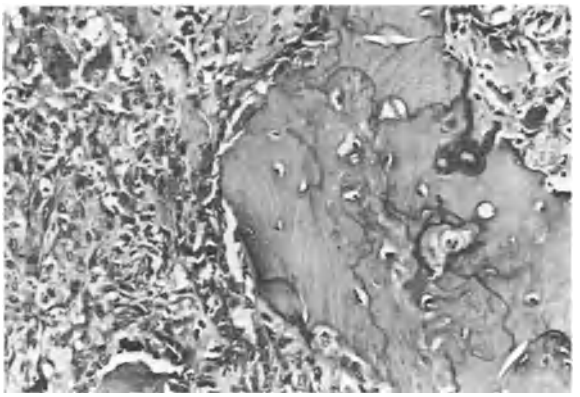

Figure 6-5. Histology of Paget's osteosarcoma of the same patient as in Figure 6-4. Osteoblastic osteosarcoma tissue and the bone trabeculae with irregular cement lines are seen (H&E, ×100).

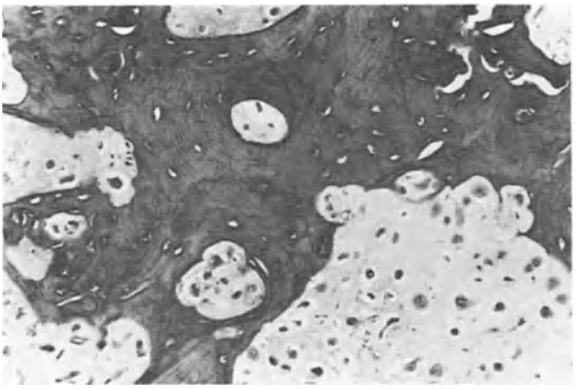

Figure 6-6. Histology of Paget's osteosarcoma of chondroblastic type in a 73-year-old female patient (H&E, ×200).

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7. Psychological effects of amputation in osteosarcoma

Cameron K. Tebbi

Introduction

Amputations have been performed for centuries to treat a variety of disorders including cancer. Historically amputees often are geriatric patients with diabetes and peripheral vascular disease, or those receiving injuries as a result of an accident or war [1–3]. In fact, in 1866 after the Civil War in the United States, one-fifth of the entire revenue of the State of Mississippi was spent on artificial limbs for amputees [4]. Nevertheless, the full impact of amputation, or the more modern version of it; i.e., limb-salvage operation, has not been fully explored.

In the United States in 1991 it is estimated that nearly 2000 persons will be diagnosed with bone cancer and 1050 will die from it [5]. Osteosarcoma and Ewings sarcoma account for the majority of these patients. With the propensity of osteosarcoma for long bones, amputation of extremities or removal and replacement of the bones with grafts is commonly performed. The loss of limb adds to the burden of cancer and can become a constant reminder of the disease and its consequences.

At the present time, especially in the treatment of cancer in childhood, therapy is given with curative rather than palliative intents. As the rate of survival from cancer increases, the importance of psychological and long-term effects of therapy becomes more evident. In osteosarcoma it is now documented that chemotherapy substantially increases the survival of patients approximately 20% beyond that expected with amputation [6–9]. The increased numbers of survivors pose a new problem, as these individuals need to return to a society that is at times less than optimistic about their cure and still has significant misgivings about and resistance to accept cancer patients [10].

Amputation vs. limb salvage

While some distinction in the course of the psychological outcome of patients undergoing limb salvage is possible, overall the outcome in both groups appears

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 ISBN 0–7923–1905–2

to be similar [11–13]. In recent years, whenever possible, attempts have been made to salvage a patient's limb and to replace the bone with an allograft or a metallic implant. Some of the prerequisites for this procedure make the population undergoing this procedure somewhat different from that of amputees. Limb sparing is most applicable to patients with relatively small tumors that do not significantly involve vascular and nerve structures. In addition, proper candidates must have bone growth near the stage of epiphyseal closure. Thus, this group of patients usually are older adolescents or young adults. The functional result is variable and may or may not be superior to amputation. An argument often made in favor of limb-sparing procedures over amputation is the psychological outcome. While data comparing the two procedures are limited, available information has so far failed to demonstrate any significant psychological advantage for limb-salvage surgery [12–14].

Some studies have found that a greater length of denial and a longer period of time is needed for adaptation for patients undergoing limb salvage as compared to amputation [14]. It appears that the former group focuses their emotional efforts on saving their limb, whereas amputees, after a relatively short period of mourning the loss of their limb, go on to deal with their disease and its treatment. This may be in part due to the need for a longer period of surgical care and hospitalization after a limb-salvage operation.

The emotional rehabilitation of patients undergoing limb salvage was also found to be more difficult than amputees [14]. In another study 26 young and middle-aged adults with extremity sarcoma who had undergone amputation were compared to a similar group who had been treated with limb-salvage therapy [13]. A battery of tests for measurement of the impact of the cancer, such as adjustment, level of function, mobility, daily living activities, sexual relationships, pain, treatment effects on the patient, etc., were used. Based on these data, the quality of life in salvage therapy did not appear to be superior to that of amputees [13]. A comparative longitudinal study of 14 amputees and 19 patients with salvaged limbs is also available [12]. This study has measured cognitive functioning, global psychological adjustment to illness and surgery, and lifetime prevalence of psychiatric disorders before and after surgery. The amputees and limb-salvage groups were similar in terms of age, time lapsed between surgery and interview, and sociodemographic parameters. The study finds no statistically significant differences in cognitive capacity, anxiety, and depressive symptomatology between the two groups [12]. It appears that to date a psychological outcome advantage for limb-salvage surgery compared to amputation has not been demonstrated.

Vocational and economical effects of amputation

With increased survival, the importance of rehabilitation of former cancer patients has significantly increased [15–18]. Nevertheless, unfortunately at

the present time the cure of individuals with cancer is viewed by most societies with a great degree of skepticism. At times former cancer patients are discriminated against in jobs and face restrictions and rejections when applying for employment and insurance. The addition of a visible handicap; e.g., loss of a limb, adds to their difficulties [10,18]. In turn, vocational status is closely related to the sensitive adolescent and young adult issues of autonomy and independence [19–21]. It is now widely accepted that individuals with invisible handicaps such as cancer are victims of insidious social forces, including stigma, negative attitudes, and career ostracism.

Despite fear of discrimination and the difficulties employees have in recognizing the potential for recovery and function of cancer patients, many cancer survivors, including amputees, return to work and perform well. [17,18,22,23,24–27]. A study of adjustment of adolescent cancer amputees in the United States found that in 27 patients who had survived their disease, only one had failed to gain employment or go back to school [10]. The occupations covered a wide range and included many physically demanding positions [10]. In patients who had survived beyond 5 years, 85% were employed at least on a part-time basis. Nevertheless, nearly half had faced discrimination in job hiring or promotions [10]. The same study repeated in Brazil found no evidence of job discrimination. However, while 50% of the students had returned to school after amputation, 67% could not keep up with school work. Of 13 patients who were employed prior to amputation, only five had returned to their positions. In this study, patients who had lost or had to change their jobs perceived amputation as the cause [23]. A relatively low unemployment rate is reported in other types of cancer in adults [24–27]. Among 93 survivors of childhood cancer in the United States, only 14% were unemployed [10,18]. In one study of adolescent/young adult cancer, the mean income of cancer patients as a group far exceeded the matched control [18]. This, at least in part, may be due to high levels of motivation and attention to training of these individuals. A survey of the vocational achievements of young adults who had undergone amputation as children found that those who received extensive rehabilitation in childhood had skills for jobs with high employment potentials [28].

Quality of life

The past two decades have seen significant improvement in the therapy and survival of cancer patients. With increased survival, attention has been focused on the quality of life. The definition of quality of life is difficult, and even illusive, since it embodies numerous items and considerations. The meaning of a "good" life appears to be very subjective and cannot be generalized and, despite various methods devised, is difficult to measure [29]. Lifestyles of amputees often need to change to accommodate the patient's physical limitations and decreased mobility [23]. The degree of change not only depends

on the type and level of amputation and prosthesis, but the nature and degree of rehabilitation and the patient's will and desires. Most amputees, if adequately rehabilitated, are capable of independent living in and out of their home [10]. In one study 70% of patients reported no or minor changes in the kinds of activities they performed after surgery, and only 30% had a great deal of alteration in their lifestyle [30]. Well-motivated amputees are capable of performing well. Nearly 60% of cancer amputees reported no change or increased change in their physical activities. The monumental and well-publicized attempt at running across Canada by Terry Fox, a cancer amputee, is an extreme example. Others have mastered sports such as skiing, tubing, snowmobiling, swimming, etc. [31]. In contrast to expectations, sedentary activities decreased or remained unchanged in 64% of cancer amputees [10]. Despite the popular assumption, depression is not common in adolescent and young adults with cancer [32]. With regard to social activities, these patients appear to adjust well in their social interactions. In the above survey nearly 67% of participants reported an increase in their level of social activities. While direct data regarding cancer amputees are not available, based on a study of patients with cancer most find security in their religion, but the level of their practice and beliefs does not appear to differ from that of controls. The degree of religiosity does not appear to change significantly during the course of cancer [33,34].

If the quality of life can be partially operationalized in terms of happiness, satisfaction, achievements, and overall sense of well-being, cancer amputees appear to do well, considering their limitations. In one study, based on the results of an exhaustive questionnaire examining the ability to function successfully, socially, educationally, vocationally, etc., the majority of cancer amputees were judged to be "well adjusted" [10]. This and other information obtained from cancer amputees indicates that, given appropriate support, training, rehabilitation, and encouragement, the majority of cancer amputees will adjust well and have an accepted quality of life. The knowledge that cancer amputees can live normal active and productive lives [10] should provide additional incentive for maximizing the efforts to reduce psychological trauma and to provide optimum rehabilitation for this group of patients. In the long run, such an effort not only results in a favorable outcome in human terms, but it pays dividends in economic terms to society as well.

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8. Late effects of therapy in survivors of childhood and adolescent osteosarcoma

H. Stacy Nicholson and John J. Mulvihill

Introduction

Survival rates for children and adolescents with osteosarcoma have increased significantly: The Survival, Epidemiology, and End Results (SEER) program of the National Cancer Institute (USA) has shown the 5-year survival rate for whites diagnosed with bone cancer before age 15 between 1981 and 1986 to be 54% [1]; two decades earlier, the rate was 20% [2].

Although little is known about late effects of therapy in osteosarcoma survivors, studies of adults who have survived various childhood or adolescent malignancies and studies of late effects following specific treatments similar to ones used in osteosarcoma are germane to osteosarcoma survivors.

Late effects of specific therapies

Most ongoing protocols for osteosarcoma utilize multiple agents, including one or more alkylating agents. Alkylators have been associated with second cancers and impaired fertility when used alone or in combination with radiotherapy [3,4].

Doxorubicin, a very effective agent against osteosarcoma, is potentially very toxic. Survivors treated with doxorubicin have a risk of delayed cardiac damage, including congestive heart failure; those with cumulative doses over 500 mg/m^2 are at greatest risk [5]. Although recovery from heart failure may be complete, heart transplantation has also been required for end-stage anthracycline cardiotoxicity [6]. Still other survivors may be asymptomatic until a cardiovascular stress occurs, such as pregnancy [7]. Finally, cardiac abnormalities following doxorubicin therapy may occur at lower doses than previously thought, as recently reported in survivors of acute leukemia [8].

Although osteosarcoma is not a radiosensitive tumor, some treatment protocols have included prophylactic lung irradiation for the micrometastatic disease likely to be present at diagnosis [9]. All patients so treated have transient, restrictive pulmonary function shortly after radiation; thus, these survivors may have an increased risk of pulmonary fibrosis and chronic lung

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 ISBN 0-7923-1905-2

disease, and should be followed by pulmonary function testing. Furthermore, radiation therapy that included the heart in the field has been associated with late cardiac effects, including ischemic heart disease [10].

Potential late effects following osteosarcoma

Late relapses and premature mortality

Although relapses beyond 5 years following diagnosis are rare [11], osteosarcoma has recurred as many as 13 years after diagnosis [12]. In our study of 82 adult survivors of osteosarcoma who were diagnosed prior to age 21 and followed to an average age of 33 years, three deaths occurred, one each from progressive osteosarcoma, multiple trauma, and a central nervous system hemorrhage during resection of a brain mass [13]. Overall, 3.3 deaths occurred per 1000 person-years of follow-up in this cohort of osteosarcoma survivors.

A large population-based study of long-term survival in children with cancer included 196 three-year survivors of osteosarcoma [14]; of these, 77% were still alive 10 years after diagnosis. Although eight excess deaths occurred per 100 person-years in the third and fourth years after diagnosis, by 10 years, only one excess death per 200 person-years occurred.

Second cancers

Second malignancies following osteosarcoma include fibrosarcoma, cervical carcinoma, breast carcinoma, second primary osteosarcoma, malignant fibrous histiocytoma, cutaneous malignant melanoma, acute myelogenous leukemia, and esophageal cancer [13,15–17]. Second cancers occur less frequently after osteosarcoma than after many other childhood cancers [18].

Other general health issues

The osteosarcoma survivors in our study reported no excess of hypertension, heart disease, arthritis, renal disease, diabetes, pituitary dysfunction, adrenal dysfunction, thyroid dysfunction, problems with sight or hearing, or emotional problems compared to their siblings [13]. However, when asked to classify their health as excellent, good, fair, or poor, the survivors were more that twice as likely as their siblings to perceive their health as fair or poor. This effect was increased for females but not significant for males.

Musculoskeletal

Sequelae of amputations include local pressure sores from ill-fitting prostheses, phantom sensations, and phantom limb; in those with amputations

performed prior to cessation of growth, overgrowth of bone may occur [19]. Other late consequences of amputations may become apparent in osteosarcoma survivors as they age; for example, an increased frequency of abdominal aortic aneurysms were seen in elderly World War II veterans who had undergone above-knee amputations [20]. Potential late effects of limb-sparing procedures include deep infections, neurological complications, mechanical problems such as fracture or loosening of the prosthesis, and limb-length discrepancy. Some of these late complications may require revision of the prosthesis or amputation. Osteosarcoma patients treated with amputation considered their spared limb to be poor in approximately one third of cases. As time elapsed from diagnosis increased, there was less satisfaction with the spared limb. Psychosocial effects of amputation are addressed in Chapter 7 of this volume.

Fertility

Among 32 male osteosarcoma patients treated with cisplatin, doxorubicin, and dacarbazine, most had resumed sperm production 2 years after diagnosis despite having had azoospermia during treatment. However, only 43% of those who received more than 600 mg/m^2 of cisplatin recovered, compared to 95% of those who had received lower doses; no dose-response effect was demonstrated for other agents [21]. Transient testicular dysfunction has also been documented in osteosarcoma patients following combination therapy with high-dose methotrexate and vincristine [22].

Pregnancy outcome and offspring

When 2308 offspring of adults survivors of cancer diagnosed prior to age 20 were compared with 4719 of their cousins, no significant excess risks of cancer or birth defects were noted in the offspring [23,24]. In this cohort, none of the 101 offspring of the 82 osteosarcoma survivors had been diagnosed with cancer.

Summary

Adult survivors of childhood or adolescent osteosarcoma require ongoing medical follow-up in order to monitor for potentially life-threatening consequences of therapy, including second cancers and anthracycline cardiotoxicity. In the future, additional knowledge of tumor biology will likely change staging methods and allow intensive therapy to be given only to those most likely to benefit from it [25]; those with less risk of relapse may require less toxic therapy and still achieve acceptable levels of survival.

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9. An opinion supporting the role of high-dose methotrexate in the treatment of osteosarcoma

Gerald Rosen

Introduction

To properly assess the role of any drug for a specific disease, it would be prudent to examine the benefit to risk ratio of each drug. In this chapter these factors will be examined for not only high-dose methotrexate with leucovorin rescue, but also for the other currently useful drugs in the treatment of osteogenic sarcoma, namely, high-dose ifosfamide with mesna and cisplatinum combined with doxorubicin. Comments will be made about other proposed treatments that, at the current time, have no proven place in the treatment of patients with newly diagnosed primary osteosarcoma.

High-dose methotrexate with leucovorin rescue

High-dose methotrexate with leucovorin rescue was first described for the treatment of metastatic osteosarcoma in the early 1970s by Djerassi and Jaffe [1]. During the mid 1970s a great deal of work was done on defining the dose to which patients with osteosarcoma would respond to high-dose methotrexate. For the pediatric age group the dose settled upon is now almost universally accepted as 12 g/m^2 [2,3].

Pharmacokinetic studies [4,5] demonstrated that high-dose methotrexate can be safely and effectively given while limiting 24-hour urine output for the first 24 hours after the administration of the drug to approximately 1400 cc/m^2. These same studies also demonstrated the safe way to administer leucovorin, to prevent lethal toxicity and, in general, to prevent almost all toxicity from the treatment. It was found that leucovorin rescue need only be started approximately 24 hours after the beginning of the high-dose methotrexate infusion and need only be given at doses as little as 10 mg total dose, p.o., every 6 hours for approximately 12 doses, or until the serum methotrexate level is below 0.1 μM. In addition, guidelines were developed to prevent toxicity in patients who abnormally metabolize high-dose methotrexate or experience transient renal failure following drug administration. Depending on the type of abnormal excretion pattern noted, the protracted

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 ISBN 0-7923-1905-2

administration of leucovorin or the possibility of a higher leucovorin dose could still prevent toxicity. These studies were published in 1982 and established safe and effective guidelines for the administration of this drug [4].

In the early days of the T7 chemotherapy protocol at the Memorial Sloan-Kettering Cancer Center (1976–1978) and of the T10 chemotherapy protocol (1978–1981) [6], approximately 50% of the patients had documented responses to high-dose methotrexate. During the period of the T10 protocol (from 1978 until the present time) there has been no lethal toxicity associated with high-dose methotrexate and leucovorin rescue in our experience with literally thousands of drug administrations.

When properly administered, the advantages of high-dose methotrexate in the treatment of osteosarcoma include the following: (1) responses are often complete and indeed dramatic; (2) one can resume treatment with chemotherapy after only 1 week from the administration of high-dose methotrexate; (3) when properly administered there is virtually no toxicity, including no bone marrow suppression, no stomatitis, no alopecia, and no nephrotoxicity or neurotoxicity. Neurotoxicity is extremely rare, except in patients with brain tumors following maximally tolerable brain irradiation. The more common transient neurologic syndrome, which we described following high-dose methotrexate, occurred in less than 2% of patients treated with high-dose methotrexate, and very rarely did this complication have any sequela [7].

The major disadvantage of high-dose methotrexate in the treatment of osteosarcoma is that approximately 50% of patients will not respond to this treatment. Nonresponders appear to be completely drug resistant and tend to have progressive disease while on this treatment. Another disadvantage arises in smaller centers physicians and nurses probably do not have experience with carefully monitoring the patients. Overhydration, to "ensure safety," may only ensure an ineffective treatment.

Until approximately 2 years ago, high-dose methotrexate with leucovorin rescue appeared to have the highest response rate of all single agents used in the treatment of osteosarcoma. For methotrexate-resistant patients, tumor can progress while wasting time with an ineffective treatment. This latter phenomenon may have led to the high local recurrence rate that was observed in the T12 protocol [8].

In a recent study we reported the use of thallium-201 to rapidly identifying the tumor response [9]. Serial thallium-201 scans done prior to the start of preoperative chemotherapy have been able to identify chemotherapy-responsive patients. In a study of 27 patients with primary osteosarcoma (24 patients) and malignant fibrous histiocytoma of bone (3 patients), serial thallium-201 scans accurately detected responses to preoperative chemotherapy confirmed by histologic analysis of the resected tumor specimen [10]. Thus a significant decrease in thallium uptake following preoperative chemotherapy correlated with a complete histologic response (>95% tumor necrosis). These changes can be detected as early as 2 weeks following two weekly high-dose

Table 9-1. Chemotherapy for osteosarcoma

Active single agents	Dose	Resp. rate
Mitomycin C	0.5–1.0 mg/kg	20%
Adriamycin	90 mg/m	30%
High-dose MTX	8–12 g/m	50%
Cisplatinum	120 mg/m	20%
Ifosfamide	6–14 g/m	20–67%

methotrexate treatments, with 100% accuracy in 33% of the patients, and have high statistical significance ($p < 0.0005$) [10].

Ifosfamide

This author considers high-dose ifosfamide to be the most universally effective treatment in osteosarcoma. As noted in Table 9-1, a dose of 14 g/m^2 produces a response rate of 67% [11]. Lower doses, in the range of 6–10 g/m^2, have produced response rates varying between 20% and 33%. This doubling or tripling of the response rate by raising the dose to a level at which the myelosuppression is approximately that obtained from cisplatinum combined with doxorubicin has led to much more effective treatment. There is a sharp dose-response curve with ifosfamide in the treatment of osteosarcoma. Because of the lack of myelosuppressive dose-limiting toxicity at lower doses of ifosfamide, several authors have included the drug VP-16 in combination with ifosfamide. In this author's experience, VP-16 has no activity at all in the treatment of metastatic osteosarcoma. Indeed, VP-16 added to cisplatinum in patients who had exhausted doxorubicin treatment showed no increase in response rate over that of cisplatinum alone given at the same dose. Thus the addition of VP-16 to lower doses of ifosfamide is not very prudent.

High-dose ifosfamide chemotherapy has some renal toxicity associated with it, namely, renal tubular acidosis. This can be managed by the proper metabolic replacement of bicarbonate and potassium in most patients. Cisplatinum alone or combined with doxorubicin may produce permanent renal injury, making subsequent treatment with methotrexate and ifosfamide even more difficult. Therefore, we prefer to use cisplatinum and doxorubicin after high-dose ifosfamide in the chemotherapy sequence (Figure 9-1). Most of the serious toxicity associated with ifosfamide chemotherapy is in the form of myelosuppression, which can be greatly abrogated through the use of granulocyte colony stimulating factors (GCSF or GMCSF). The reported dose-limiting toxicity of neurotoxicity associated with high doses of ifosfamide has not been our experience when one uses continuous infusion of the drug and vigorous replacement of bicarbonate to prevent patients from becoming

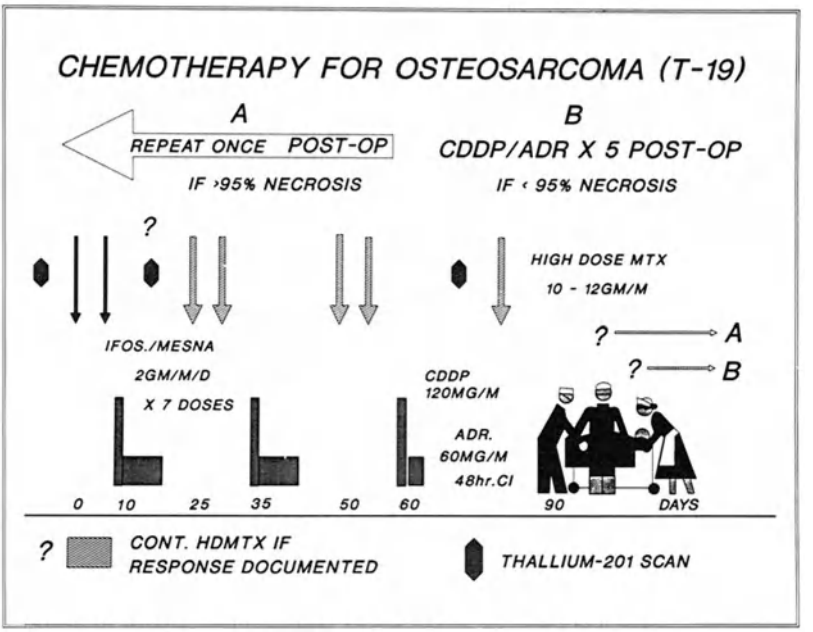

Figure 9-1. The current treatment regimen used by this author for osteosarcoma of bone. High-dose methotrexate is given in two weekly doses and then the patient is reevaluated with a repeat thallium-201 scan. If an obvious clinical response is not readily documented, serial thallium-201 scans are used to determine whether to continue high-dose methotrexate. If there is a significant decrease in thallium uptake and it is determined that high-dose methotrexate with leucovorin rescue is an effective treatment in a given patient, treatment is continued, including high-dose methotrexate. If there is no obvious clinical regression of tumor and the repeat thallium-201 scan does not show a significant decrease in the uptake in the lesional area, high-dose methotrexate with leucovorin rescue is not given to the patient in subsequent chemotherapy. Patients who do not have a complete histologic response in the primary tumor (nominally defined as >95% necrosis) to preoperative chemotherapy receive only cisplatinum combined with doxorubicin postoperatively.

acidotic. It is our policy to determine the serum bicarbonate on a daily basis in patients undergoing high-dose ifosfamide treatment and to replace 100–200 mEq sodium bicarbonate per day as an additional i.v. supplement. Under these circumstances high-dose ifosfamide with mesna produces a very high response rate in both metastatic and primary osteosarcoma.

Cisplatinum combined with doxorubicin

Cisplatinum at a dose of 120 mg/m^2 combined with doxorubicin at a dose of 60 mg/m^2 produces a partial and complete response rate of approximately 40–65% in patients with osteosarcoma. Most patients who do not have a

major response to this combination have stable disease, and seldom do we see progressive disease in nonresponding patients. This treatment combination appears to be more reliably effective in metastatic osteosarcoma than the older combination of bleomycin, cyclophosphamide, and dactinomycin (BCD). The addition of the more active high-dose ifosfamide to high-dose methotrexate and cisplatinum combined with doxorubicin has allowed us to delete BCD chemotherapy from our treatment regimen for primary osteosarcoma.

The major drawback of cisplatinum is its extreme toxicity, including probable permanent renal and ototoxicity, as well as severe nausea and vomiting, which is associated with a great deal of morbidity. Nevertheless, cisplatinum combined with doxorubicin is the standard, most reliable treatment for osteosarcoma based on prior studies both by this author and others [3,6,8,12]. Our current treatment regimen limits this combination to only two doses in those patients who have a complete histologic response to preoperative chemotherapy (Figure 9-1). However, in patients who do not respond completely to high-dose methotrexate, high-dose ifosfamide, and one cisplatinum and doxorubicin treatment given preoperatively, we feel the only reliable therapy that can be given in the face of this presumed resistance is five more postoperative cisplatinum and doxorubicin combination treatments. Rarely can more than six courses of this treatment be given due to renal toxicity and patient tolerance.

Conclusions

High-dose methotrexate with leucovorin rescue remains an important and central treatment in the overall management of the patient with osteosarcoma. Its main advantages are rapid onset of response, lack of significant toxicity, and the ability to proceed with other forms of treatment within 1 week of high-dose methotrexate treatment. Its major disadvantages include the fact that 50% of the patients who are so treated may experience progressive disease during the time they are being administered treatment. Through the use of careful evaluation techniques, including serial thallium-201 scans, a rapid decision about the efficacy of high-dose methotrexate in each individual patient can be made, allowing the clinical investigator to eliminate this treatment from the patient's treatment course if necessary. The overall effect of investigating the role of high-dose methotrexate in each individual patient will, hopefully, further increase the cure rate in osteosarcoma, as well as decrease the morbidity of treatment.

Two of the first six patients treated on the T-19 protocol (Figure 9-1) who did not initially respond to high-dose methotrexate, but had a complete response to preoperative chemotherapy, relapsed early with pulmonary metastases after the completion of the abbreviated chemotherapy as depicted in Figure 9-1. This prompted a review of over 300 patients treated with preoperative

chemotherapy. This experience has caused us to conclude that, regardless of apparent response of the primary tumor to high-dose methotrexate, there was definite survival benefit associated with 18 or more high-dose methotrexate treatments. Therefore, we have amended our protocol to reflect this experience, and all patients are currently receiving 20 high-dose methotrexate treatments (similar to that given in the T-7 protocol)[2], with the addition of ifosfamide and cisplatinum combined with adriamycin as described in this text.

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10. Doxorubicin: Role in the treatment of osteosarcoma

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Introduction

The objective of this chapter is to review the role that doxorubicin (DOX), one of the most active single agents for the treatment of osteosarcoma, has played in the neoadjuvant and adjuvant treatment of osteosarcoma during the past several decades. A brief review of the mechanism of action, pharmacokinetics, and pharmacodynamics of DOX will be presented, since an understanding of these parameters is essential to the optimal use of this agent. Results from recent clinical trials that have incorporated DOX (either alone or in combination chemotherapy regimens) will then be reviewed, and potential strategies to maximize the antitumor potential of DOX in future combination regimens will be discussed. The comprehensive review published in 1988 by Grem et al. [1] will be updated, and reference citation will be limited to the recent literature (1988 to the present).

Clinical pharmacology of doxorubicin

Mechanism of action

Although anthracyclines have classically been thought of as intercalating agents that interfere with DNA replication, transcription, and repair after insertion into the DNA helix [2], the mechanism of action of anthracyclines remains controversial. Postulated mechanisms of antitumor activity include (1) intercalation between DNA bases, (2) free radical formation, and (3) topoisomerase-II mediated strand breaks [3,4]. However, no single mechanism has been consistently identified as the primary determinant of cell cytotoxicity, and the importance of different intracellular targets may vary among cell lines [3,5].

Intercalation of DOX between DNA base pairs can result in inhibition of RNA and DNA synthesis through inhibition of RNA and DNA polymerases [15]. However, since DOX inhibition of DNA and RNA polymerases via intercalation occurs only at anthracycline concentrations far in excess of

G. Bennett Humphrey (ed.), OSTEOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS.
 ISBN 0-7923-1905-2

those achievable in vivo [5], this mechanism is unlikely to be of clinical significance.

Chemical reduction of anthracyclines by intracellular flavin-dependent enzyme systems can result in free radical formation and cell toxicity by either one- or two-electron reduction mechanisms [5]. One-electron reduction of DOX results in semiquinone free radical intermediates that can react with molecular oxygen, leading to the formation of highly reactive superoxide, hydrogen peroxide, and hydroxyl radicals, which cause oxidative damage to cellular macromolecules. Two-electron reduction of DOX can lead to the formation of a quinone methide, a potential monofunctional alkylating agent. The latter mechanism of DOX reduction is most likely a pathway for drug inactivation, since the reactive intermediates are quickly converted to relatively noncytotoxic deoxyaglycones. The former mechanism has been implicated as a major cause of the cardiac toxicity of these compounds and may also play a role in the cytotoxic activity observed in some cell lines. The significant cytotoxic activity of the anthrapyrazoles (e.g., piroxantrone), which generate much lower concentrations of reactive oxygen species than DOX, suggests that free radical production is not a prerequisite for anthracycline cytotoxic activity [6]. Additional evidence for the importance of non-free radical mechanisms for DOX cytotoxicity is the maintenance of the cytotoxic effects of DOX when it is combined with iron chelators that decrease free radical formation. The controversy regarding the role of oxygen radical formation and cytotoxicity following exposure to DOX has been discussed in detail in several recent reviews [3–5].

In many cell types, DNA cleavage, mediated by the DNA repair enzyme topoisomerase II, occurs following exposure to DOX [14]. DOX-mediated topoisomerase II inhibition correlates well with cytotoxicity in murine leukemia cell lines and occurs at DOX concentrations achievable in vivo. This mechanism of action is supported by studies of cell lines selected for resistance to topoisomerase II inhibitors (e.g., VM-26). These cell lines show altered topoisomerase-II activity, as well as resistance to DOX and other anthracyclines [3,7–9]. However, the reversal of DOX resistance in some tumor cell lines by agents that reduce glutathione concentration is better explained by free radical-mediated mechanisms of cytotoxicity than by topoisomerase II-mediated mechanisms and suggests that multiple mechanisms for DOX cytotoxicity may be operative [10,11].

Mechanisms of resistance

DOX, a natural-product cytotoxic agent, belongs to that class of compounds for which pleiotropic drug resistance has been described. Just as the mechanism of action for DOX cytotoxicity is probably multifactorial, the mechanisms of both de novo and acquired multidrug resistance (MDR) to this agent also appear to be multifactorial. Potential mechanisms of resistance to DOX include (1) increased expression of the P-170 glycoprotein, which mediates

efflux of DOX as well as of other antineoplastic agents; (2) non-P-170 glycoprotein mediated MDR, associated with decreased accumulation of DOX [12,13]; (3) alterations in topoisomerase II, associated with decreased DOX-induced DNA strand breaks and cytotoxicity [8,9,14]; and (4) alterations in the glutathione-S-transferases, enzymes involved in the detoxification of electrophilic substances [15].

Clinical trials using the calcium-channel blocker verapamil to circumvent P-170 glycoprotein mediated MDR have been performed in patients with multiple myeloma and non-Hodgkin's lymphoma (NHL) [16], refractory ovarian cancer [17], refractory NHL [18], and in pediatric and adult patients with a variety of other tumors [19]. The calmodulin inhibitor trifluoperazine has also been combined with DOX to attempt reversal of multidrug resistance (MDR) [20]. While results from some studies are particularly promising [18], confounding variables (e.g., changes in dose and schedule of administration of cytotoxic agents) prevent simple interpretations of these trials [21]. Other potential means of reversing P-170 glycoprotein mediated resistance include therapy with agents such as quinidine, amiodarone, cyclosporine (and related analogies), and tamoxifen, as well as the use of monoclonal antibodies directed against this glycoprotein [5]. The identification and clinical development of anthracyclines that are not transported by the P-170 glycoprotein is another possible option for circumventing MDR [22].

Elucidation of the mechanism(s) of clinical resistance of osteosarcoma tumor cells will help determine and prioritize the therapeutic strategies most likely to be successful in circumventing the clinical problems associated with drug resistance.

Pharmacokinetics/pharmacodynamics

The instability of DOX in an acidic environment precludes its oral administration, and its severe vessicant properties prevent both subcutaneous and intramuscular administration. Therefore, DOX must be administered intravenously (i.v.) or intraarterially (i.a.), either as a bolus or as an infusion. Following i.v. bolus administration, DOX plasma concentrations fall rapidly as a result of avid tissue binding of the drug. The initial half-life is only 10 minutes, followed by a prolonged terminal phase with a half-life of greater than 24 hours [?]. This prolonged terminal phase accounts for more than 70% of the total drug exposure. Plasma concentrations remain above levels known to be cytotoxic in vitro for almost a week following a single dose of 60 mg/m^2 [5]. DOX is eliminated primarily by hepatic biotransformation and by biliary excretion; less than 10% of the drug is excreted in the urine. Doxorubicinol, the primary metabolite of DOX, is less cytotoxic than the parent compound and plasma exposure is approximately one-half that of DOX.

The antitumor activity of DOX is not schedule dependent and correlates better with total drug exposure as measured by the area under the plasma concentration-time curve (AUC) than peak drug levels [5]. This observation

is of importance in determining dosing schedules for clinical trials, since the cardiac toxicity of DOX appear to correlate more with peak drug levels than with AUC [23]. Administration of DOX by continuous infusion can result in reductions in peak DOX concentrations by several orders of magnitude without compromising the total AUC [23]. Further discussion of the significance of dosing schedule for DOX administration will be presented in the discussion.

Adjuvant therapy

Single agent activity

DOX is one of the few antineoplastic agents with documented single-agent activity in patients with osteosarcoma. Clinical trials with DOX have been previously reviewed [1].

Combination chemotherapy regimens including doxorubicin

The reader is referred to the comprehensive review by Grem et al. of the multiple neoadjuvant and adjuvant osteosarcoma trials, many of which incorporated DOX [52]. A brief review of the highlights of those regimens and an update of trials since that review appeared will be presented here (Table 10-1).

DOX was initially used in combination therapy with methotrexate (MTX), the first antineoplastic agent that was shown to have significant activity against osteosarcoma.

Cisplatin (CDDP) has also shown significant single-agent activity against osteosarcoma, with response rates ranging from 18% to 50%.

Ifosfamide (IFOS) has also been recently identified as another antineoplastic agent with good single-agent activity in patients with osteosarcoma [24,25]. Preliminary results of a trial for previously untreated patients with osteosarcoma that included IFOS as the initial therapy followed by high-dose methotrexate (HDMTX)/CDDP/DOX have been reported by Meyer et al. The clinical plus radiographic response rate to initial therapy with IFOS in this trial was 44% [25]. Chawla et al. have reported a 62.5% response rate to high-dose IFOS therapy as a single agent in patients with recurrent, refractory osteosarcoma. IFOS has therefore been increasingly incorporated into adjuvant combination chemotherapy regimens for the treatment of patients with osteosarcoma.

In the German COSS-82 trial [26] patients were randomized to receive neoadjuvant chemotherapy with HDMTX/bleomycin, cyclophosphamide, dactinomycin (BCD) or DOX/CDDP/HDMTX. Patients with a good response (≥90% tumor necrosis as determined by pathology review following primary surgery) to preoperative chemotherapy received the same therapy postoperatively. Patients with a poor response (<90% tumor necrosis) to

HDMTX/BCD received DOX/CDDP/HDMTX postoperatively, while those with a poor response to preoperative DOX/CDDP/HDMTX received BCD plus CDDP/IFOS postoperatively. Only 26% of patients on the MTX/BCD neoadjuvant arm had a good response vs. 60% on the DOX/CDDP/MTX neoadjuvant arm ($p < .001$). At 5 years, the actuarial disease-free survival (DFS) rate for the MTX/BCD neoadjuvant arm was 45% vs. 68% for the DOX-CDDP-MTX neoadjuvant arm ($p < .05$) [26]. Thus, the attempt to spare patients from the toxicities of DOX and CDDP was unsuccessful in this trial. Patients receiving neoadjuvant therapy with DOX-CDDP had a significantly better histologic response and overall survival.

In the COSS-86 trial (Table 10-1), high-risk patients received neoadjuvant chemotherapy with DOX/HDMTX/IFOS, and either i.v. or i.a. CDDP. The percentage of good responders to preoperative therapy was 68% after i.a. vs. 69% after i.v. CDDP, suggesting no benefit for i.a. administration of CDDP compared to i.v. administration for this particular combination chemotherapy regimen. Postoperatively, high-risk patients received therapy with the same agents for 40 weeks. It is too early to determine the impact of IFOS on the cure rate or the overall disease-free survival rates for this study [27].

In a nonrandomized neoadjuvant study at the M.D. Anderson Cancer Center (Table 10-1) [28], 71% of patients had a good response to preoperative therapy with DOX (90 mg/m^2 as a 96-hour continuous i.v. infusion) immediately followed by i.a. CDDP (160 mg/m^2/24 hr). Postoperatively, good responders received three additional cycles of the same chemotherapy administered intravenously; while poor responders received alternating cycles of HDMTX/DOX/(DTIC)/BCD for 9–12 months. The continuous disease-free survival at 4 years was 81% following a good response vs. 51% after a poor response. Of note, patients with a good response to preoperative DOX/CDDP never received therapy with HDMTX/BCD.

In a Rizzoli Institute trial (1983–1986) (Table 10-1) patients were randomized to receive neoadjuvant chemotherapy with i.v. CDDP and either intermediate-dose methotrexate (IDMTX; 750 mg/m^2) or HDMTX (8g/m^2). The postoperative chemotherapy regimen was determined by the histologic response to the preoperative therapy. The postoperative regimen was initially designed such that good responders (≥90% tumor necrosis) received two cycles of the preoperative chemotherapy regimen, fair responders (60–80% tumor necrosis) 24 weeks of therapy with DOM/MTX/CDDP, and poor responders (<60% tumor necrosis) five cycles of DOX/BCD. However, due to the occurrence of four early relapses in the first 15 patients with a good response, the therapy for this group of patients was subsequently intensified so that it was identical to the therapy of the fair responders. This subsequent modification of therapy, which included the addition of DOX as well as a prolongation of the total postoperative therapy time, ultimately resulted in a better cumulative disease-free probability at 5 years for the good responders vs. the fair or the poor responders (67% vs. 42% vs. <10%) [29]. This study suggested that there may be an advantage to high-dose vs. intermediate-dose

Table 10-1. Recent trials of neoadjuvant and adjuvant chemotherapy in osteosarcoma[a]

Investigator	Dose and schedule	Disease characteristics	No. of evaluable patients	Response	Comment
Bacci et al. [67]	Preoperative MTX, 750 mg/m^2 iv, days 1, 21 CF CDDP, 120–150 mg/m^2/72 hr ia, days 7, 27 *OR* MTX, 7500 mg/m^2 iv, days 1, 21 CF CDDP, 120–150 mg/m^2/72 hr ia, days 7, 27 Surgery, day 49 Postoperative GR 2 cycles preop chemo FR ADM, 45 mg mg/m^2 iv days 1, 2 MTX, week 4 (same dose as preop) CF CDDP, week 5 (234 weeks total)	Primary tumor	127	Preoperative GR—52% FR—36% PR—12% Postoperative 66 (51%) CDFS at median of 47 months Actuarial 5 yr CDFS HDMTX—58% IDMTX—42%	GRs received postoperative chemotherapy as FRs after first 15 patients secondary to 4 early relapses; GRs have a better response than fr if postoperative chemo sufficiently prolonged and included DOX; HD-MTX may be better than IDM; No improvement in prognosis for PRs with different postoperative chemo

	PR ADM, day 1 BCD, week 4 Bleo, 15 U/m²/d × 2 Ctx, 600 mg/m²/d × 2 Dact, 0.6 mg/m²/d × 2 (5 cycles)				
Bacci et al. [68]	Preoperative MTX, 8 g/m², iv, day 1 CF CDDP, 120 mg/m²/72 hr, ia, day 6 DOX, 60 mg/m²/8 hr, iv, day 9 Repeat cycle × 1 at wk 3 Postoperative GR 3 cycles of above, iv, 30 wks total therapy PR Preoperative therapy, iv, plus IFOS, 2 g/m² × 5 days VP-16, 120 mg/m² × 3 days 40 wks total therapy	Primary tumor	125	Preoperative GR—75% PR—25% Postoperative GR—87% CDFS at median 26 months (7–37) PR—58% CDFS	Role of drug dose intensity (DDI) on CDFS: DDI > 80% = 87% vs. DDI < 80% = 65% (p = 0.01)

Table 10-1 (continued)

Investigator	Dose and schedule	Disease characteristics	No. of evaluable patients	Response	Comment
Benjamin et al. [4]	Preoperative DOX, 90 mg/m²/96 hr, iv CDDP, 160 mg/m²/24 hr, ia following DOX (Continue cycles until maximum tumor response by subtraction angiography) Postoperative GR 3 cycles of preop chemo, iv; Replace CDDP if neurotoxic with DTIC, 750 mg/m² infused simultaneously with DOX PR HDMTX; DOX/DTIC; BCD; Alternate cycles for 9–12 months postoperative	Primary tumor	50	Preoperative GR—71% PR—29% Postoperative 72% CDFS at 4 years (81% for GRs and 51% for PRs)	Good histologic responders have good CDFS without having received MTX therapy

Winkler et al. [9]	COSS-86 Preoperative DOX, 45 mg/m^2/d, iv days 1, 2 HDMTX, 12 g/m^2 iv, days 15, 22 CF IFOS, 3 g/m^2/d iv × 2 days 29, 30, 50, 51 CDDP, 150 (120) mg/m^2 iv, days 31, 52 *OR* Same as above except ia CDDP Surgery, week 11 Postoperative DOX, wks 12, 20, 29, 38 HDMTX, wks 13, 14, 18, 19, 22, 23, 27, 28, 31, 32, 36, 37, 40, 41 IFOS, wks 15, 24, 33 CDDP, wks 15, 24, 33	High risk[b]	109 50—ia CDDP 59—iv CDDP	Preoperative GR—34/50 (68%) after ia vs. 41/59 (69%) after iv CDDP	Patients not strictly randomized to ia vs. iv CDDP Overall rate of histologic GR not different after preoperative ia vs. iv CDDP Dose of CDDP decreased for 150 to 120 mg/m^2 and infusion time increase from 60 min to 5 hr in both arms secondary to ototoxicity Too early to tell impact of IFOS on CDFS

Table 10-1 (continued)

Investigator	Dose and schedule	Disease characteristics	No. of evaluable patients	Response	Comment
Meyers et al. [69]	Regimen I Preoperative HDMTX BCD DOX/CDDP Postoperative Repeat cycles preoperative therapy *OR* Regimen II Preoperative HDMTX BCD Postoperative GR HDMTX BCD DOX PR HDMTX BCD DOX/CDDP		31	Preoperative Regimen I GR (Huvos grade III/IV)—8/15 (53%) vs. Regimen II GR (Huvos grade III/IV)—3/16 (19%)	CDFS too early to tell; Better preoperative histologic response in preoperative regimen containing DOX/CDDP

Stine et al. [95]	Preoperative DOX, 90 mg/m²/96 hr, iv CDDP, 150 mg/m², ia, day 5 (4 cycles total) Surgery, week 15 Postoperative BCD, wks 17, 20, 26 Bleo, 15 U/m²/d × 2 Ctx, 600 mg/m²/d × 2 Dact, 0.6 mg/m²/d × 2 DOX, 50 mg/m²/d, iv, wk 23, and q 3 wks until total dose 460 mg/m² CDDP, 100 mg/m², iv, wk 23 and q 3 wks VP—16, 200 mg/m², iv, on day 2 following CDDP; starting after total DOX received	Primary tumor	8	Preoperative 6/8 good local control—limb salvage all with good histologic response Postoperative 5/8 (63%) CDFS at median 18 months (range, 12–21 months)	Small pediatric series demonstrating safety and efficacy of neoadjuvant therapy

[a] MTX = methotrexate; HDMTX = high-dose methotrexate; IDM = intermediate-dose methotrexate; CF = leucovorin; CDDP = cisplatin; DOX = doxorubicin; Bleo = bleomycin; Ctx = cytoxan; Dact = dactinomycin; IFOS = ifosfamide; VP-16 = etoposide; ia = intraarterial; iv = intravenous; GR = good response (≥90% tumor necrosis); FR = fair response (60–80% tumor necrosis); PR = poor response (<60% tumor necrosis); CDFS = continuous disease-free survival; DDI = drug dose intensity.

[b] High risk = one or more of the following signs or findings present: (1) tumor size greater one third of the involved bone or truncal lesion, (2) greater than 20% chondroid substance formation in the biopsy specimen and (3) less than 20% reduction in early and late phase bone scan activity after 4 weeks of preoperative chemotherapy.

MTX in that the 5-year actuarial continuous DFS (CDFS) was 58% for those who received HDMTX vs. 42% for those who received IDM.

In the more recent Rizzoli Institute nonrandomized osteosarcoma trial (1986–1988) (Table 10-1), patients received neoadjuvant chemotherapy with HDMTX/CDDP/DOX. Overall, 75% of patients had a good response to the preoperative therapy. Postoperatively, good responders received three cycles of the preoperative chemotherapy, while poor responders received the agents administered preoperatively plus IFOS and etoposide (VP-16) for a total duration of 40 weeks. Continuous disease-free survival at a median follow-up time of 26 months is 87% for the good responders vs. 58% for the poor responders [30]. A retrospective analysis of the drug dose intensity (DI) that patients actually received was performed for this trial and will be discussed further.

In a single-institution trial at Memorial Sloan-Kettering Cancer Center (Table 10-1), patients were randomized to receive preoperative chemotherapy with either HDMTX/BCD or HDMTX/BCD/DOX/CDDP. Although it is too early to determine survival rates, the histologic response to therapy was significantly better in the regimen containing DOX/CDDP compared to the control arm (53% vs. 19%, respectively) [31].

Miser et al. have piloted an intensive regimen employing DOX and IFOS with HDMTX in the neoadjuvant setting. The high percentage of patients with >90% tumor necrosis following 15 weeks of chemotherapy (79%) of patients) is an impetus for further study of this drug combination [32].

Discussion

Significant improvements in the treatment of osteosarcoma have occurred in the past two decades with the utilization of neoadjuvant and adjuvant multidrug chemotherapy regimens. Two definitive trials have established that adjuvant chemotherapy with an intensive multiagent regimen that includes HDMTX/DOX/BCD ± CDDP results in a superior DFS and overall survival compared to no adjuvant therapy for patients with extremity osteosarcoma. A recent update by Link et al. [33] of the Multi-Institutional Osteosarcoma Study (MIOS) that compared postoperative chemotherapy with BCD/HDMTX/DOX/CDDP to no adjuvant therapy showed a projected 6-year CDFS of 63% for the 165 patients who received adjuvant chemotherapy vs. only 15% for the 36 patients who did not receive postoperative chemotherapy ($p < 0.001$). Additionally, a significant survival advantage was noted for those patients receiving adjuvant chemotherapy compared to the control arm, 74% vs. 49% ($p = 0.011$). However, the contribution of individual agents to the efficacy of the combination regimens of this and other past trials is difficult to ascertain, since these studies were not designed to answer such questions. The relatively bad prognosis for patients with a poor histologic response to neoadjuvant chemotherapy indicates the urgent need to identify new agents

and/or alternative salvage strategies. Another rational approach to the treatment of patients with refractory or metastatic osteosarcoma is to optimize the delivery of currently available antineoplastic agents in an attempt to improve clinical activity. The discussion below attempts to dissect the impact of DOX dose intensity in treatment regimens for patients with osteosarcoma and then examines potential strategies to increase DOX dose intensity.

Dose intensity refers to the amount of drug delivered per unit time (i.e., mg/m^2/week) regardless of schedule of administration. In a retrospective analysis of dose intensity in the adjuvant treatment of osteosarcoma, a significant positive correlation was reported between the actual dose intensity received by the patient (calculated as) a percentage of the planned dose intensity of the protocol) and the continuous disease-free survival of good histologic responders [30]. The overall continuous disease-free survival was 87% in patients who received 80% or more of the projected dose intensity vs. 65% for those who received less than 80% of the scheduled dose intensity ($p < 0.01$) with an average follow-up time of 26 months. Furthermore, as already described, Cortes et al. demonstrated by retrospective analysis that significant reductions from the planned DOX dose substantially decreased the cure rate and/or relapse-free survival rate in patients receiving DOX as a single agent in the adjuvant setting [1]. Although there are problems inherent in retrospective analyses of this type, the data are provocative and suggest that systematic review of dose intensity should be incorporated into subsequent trials of adjuvant therapy in osteosarcoma to confirm whether a relationship between dose intensity and survival can be established.

Further evidence supporting the importance of DOX dose intensity in the successful treatment of osteosarcoma is obtained by correlating the DOX neoadjuvant dose intensity with the extent of tumor necrosis in trials in which this parameter was measured. The strong relationship between survival and the extent of tumor necrosis following presurgical chemotherapy supports the validity of using the percentage of patients with "good" tumor necrosis as a measure of the effectiveness of chemotherapy regimens [26,29,30,34–36]. The DOX dose intensity and percentage of patients with "good" tumor necrosis (>90%) for 11 chemotherapy regimens are shown in Table 10-2. In each of these trials, DOX was combined with other cytotoxic agents. In spite of the variation among trials in the cytotoxic agents combined with DOX, there is a strong correlation between DOX dose intensity and the percentage of patients with >90% tumor necrosis following neoadjuvant DOX (Figure 10-1). Preliminary analysis of the dose intensity of the other cytotoxic agents used in these 11 regimens shows that only the DOX dose intensity strongly correlated with histologic response (R. Simon and M. Smith, unpublished observations).

The correlation between dose intensity and clinical response for a variety of tumors has been an impetus for the clinical investigation of methods to optimize the dose intensity of currently utilized antineoplastic agents. For DOX, these investigations have predominantly focused on ways to reduce the risk of cardiac toxicity and to overcome myelotoxicity while maintaining

Table 10-2. Neoadjuvant doxorubicin dose intensity and percentage of patients with >90% tumor necrosis

Trial	# Patients	DOX DI[a]	% Patients with GR[b]
Provisor, 1987	192	0	30
Winkler, 1988	57	0	26
Bacci, 1990	127	0	52
Hudson, 1990	80	0	43
Rosen, 1982	57	5.6	39
Winkler, 1988	31	10	52
Winkler, 1988	37	10	56
Winkler, 1988	109	9	69
Winkler, 1988	58	12	60
Miser, 1990	19	15	79
Prasad, 1990	125	15	75

[a] DOX dose intensity in mg/m^2/week prior to surgical excision.
[b] Percentage of patients with ≥90% tumor necrosis at surgical excision of primary tumor.

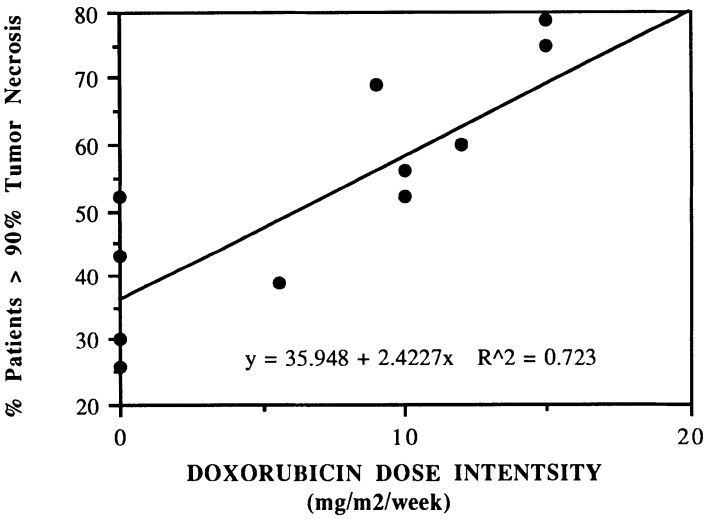

Figure 10-1. The percentage of patients with >90% tumor necrosis as a function of neoadjuvant doxorubicin dose intensity (in mg/m^2/wk) for the 12 regimens cited in Table 10-2, with best fit by linear regression analysis.

dose intensity. Such therapeutic strategies that have been or are currently undergoing investigation include the following: (1) DOX administration using alternate dosing schedules (e.g., continuous infusion); (2) delivery of DOX in liposome-encapsulated formulations; (3) administration of DOX in conjunction with the cardioprotective agent ICRF-187; and (4) administration of DOX with cytokines [e.g., granulocyte-macrophage stimulating factor (GM-CSF) and granulocyte stimulating factor (G-CSF)].

DOX administration by continuous infusion may provide a more favorable pharmacologic profile by maintaining the total systemic drug exposure while avoiding high peak plasma levels. Several recent clinical trials utilizing a continuous infusion schedule have demonstrated decreased cardiac toxicity (without compromising antitumor activity) when compared to DOX given on a bolus schedule [37]. This has allowed higher total cumulative doses of DOX to be administered without increased cardiac toxicity [43]. The myelotoxicity associated with DOX has essentially been the same after either bolus or continuous infusion dosing schedules. However, mucositis may be more severe with increasing durations of infusion and can become dose limiting [23]. Other potential disadvantages of DOX administration by continuous infusion include the need for placement of a central venous catheter to minimize the risk of severe tissue injury associated with local extravasation of DOX, and the potential increased costs and inconvenience of drug administration via the continuous infusion schedule secondary to the need for hospitalization and/or a portable infusion pump. Despite these potential disadvantages, administration of DOX by continuous infusion has been shown to be quite feasible in both adult and pediatric patient populations [37–39].

The administration of DOX following pretreatment with the cardioprotective agent ICRF-187, an investigational bispiperazinedione iron-chelating agent that prevents DOX free-radical formation in the myocardium [40], has been shown to decrease the cardiac toxicity of DOX in a wide variety of animal models [41]. In a recent randomized controlled trial by Speyer et al. patients with advanced breast cancer received either 5-fluorouracil, DOX, and cyclophosphamide or the same combination plus ICRF-187. Both arms had equivalent response rates and duration of time to disease progression. However, the ICRF-187 treated patients had significant cardioprotection as determined by clinical examination, changes in left ventricular ejection fraction measured by multigated nuclear scans, and histologic changes on cardiac biopsy [42]. Eleven patients on the ICRF-187 arm tolerated DOX doses above 600 mg/m^2 vs. only one patient on the control arm. Although the patients receiving ICRF-187 had slightly greater myelosuppression than the control arm, the incidences of significant adverse effects were similar on both arms. In a subsequent clinical trial, DOX, ICRF-187 and granulocyte-macrophage colony-stimulating factor (GM-CSF) were combined in an attempt to increase dose intensity while minimizing myelotoxicity [43]. Although significant hematologic toxicity was observed in this trial, it is likely that different doses and schedules of administration and/or other cytokines may allow significant increases in dose intensity with acceptable cardiac and hematologic toxicity.

In animal models liposome-encapsulated forms of DOX have also been shown to reduce the cardiac toxicity of DOX [44]. Several phase I studies in humans have demonstrated the feasibility of administration of liposome-encapsulated DOX [45,46]. In a recently reported phase II trial of liposome-encapsulated DOX in patients with advanced breast cancer, 12 patients received total cumulative DOX doses of greater than 400 mg/m^2 administered

intravenously as 60–75 mg/m^2 doses every 3 weeks. Objective responses occurred in 9 of the 20 patients (45%) enrolled in this trial. Patients were evaluated for cardiac toxicity by radionuclide ventriculograms, and in some patients endomyocardial biopsies were also performed. Little to no cardiac toxicity was observed, while the noncardiac toxicities (primarily myelosuppression and gastrointestinal) appeared to be milder than anticipated for patients receiving equivalent doses of free doxorubicin [47]. The results of this trial suggest that the cardiac toxicity in humans may be reduced by liposome encapsulation of DOX, while antitumor activity is not compromised.

Granulocyte colony-stimulating factor is another agent that has been used to increase the intensity of treatment with DOX in women with advanced breast and ovarian cancer [94]. Administration of G-CSF beginning 1 day after bolus DOX allowed escalation of the DOX dosage from 75 mg/m^2 to 150 mg/m^2 and resulted in a dose-limiting toxicity of mucositis at the latter dosage. Cycles of therapy were given every 2 weeks, providing DOX dose intensities of over 60 mg/m^2/wk. In comparison, the highest DOX dose intensity investigated to date in patients with osteosarcoma is 15 mg/m^2/wk. Thus, significant increases in DOX dose intensity appear feasible when cytokine support is provided.

Future directions

Although it is difficult to make conclusions about the superiority of particular combination chemotherapy regimens for osteosarcoma because of the tremendous interstudy heterogeneity with regard to drug dosages, schedule of administration, and timing of administration, the data from recent clinical trials as reviewed above support the significant contribution of DOX-containing chemotherapy regimens to the successful treatment of patients with osteosarcoma.

Many critical issues specifically pertaining to the use of DOX in the treatment of osteosarcoma remain unresolved. Dose response and dose intensity issues are among the most important issues to be addressed in current and future studies. Alternative dosing schedules for DOX administration, DOX administration in conjunction with ICRF-187, the use of liposome-encapsulated DOX, or utilization of cytokines to ameliorate myelotoxicity may allow significant increases in DOX dose intensity and duration of therapy while minimizing both the acute and cumulative toxicities associated with DOX administration. Further research into the mechanism of cytotoxicity of DOX in osteosarcoma, the mechanisms of DOX drug resistance in patients with osteosarcoma, and the optimization of DOX cytotoxicity by combination with other cytotoxic agents may ultimately enhance our ability to use DOX efficaciously in patients who present with metastatic disease or who relapse after receiving adjuvant therapy.

In conclusion, it is evident that neoadjuvant/adjuvant chemotherapy should

be considered for all patients with osteosarcoma and that DOX at intensive dosages should be included in these regimens. Carefully designed prospective randomized clinical trials designed to address the issues discussed above will be required to determine the optimal neoadjuvant/adjuvant regimens for patients with osteosarcoma.

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11. Pediatric osteosarcoma: Treatment of the primary tumor with intraarterial cis-diamminedichloroplatinum-II (CDP)—Advantages, disadvantages, and controversial issues

Norman Jaffe

Introduction

This communication will review therapeutic and toxic effects of intraarterial cis-diamminedichloroplatinum-II (CDP) in the treatment of the primary tumor and will discuss controversial issues [1,2,3].

Intraarterial cis-diamminedichloroplatinum-II (CDP) as a single agent

The first formal investigation of the use of intraarterial (i.a.) CDP for treatment of the primary tumor in pediatric osteosarcoma was published by the M.D. Anderson Cancer Center in 1983 [4]. It comprised a phase I–II study to determine the optimum dosage, safety, tolerance, and efficacy. The regimen is outlined in Figure 11-1. Responses were assessed by clinical, radiographic, angiographic, and pathologic criteria [4–7]. In the initial studies four patients were entered and achieved complete or partial responses. Subsequently intraarterial CDP was utilized as definitive preoperative treatment of the primary tumor in seven osteosarcoma patients and in one with malignant fibrous histiocytoma of bone. The overall response was 50% with two patients exhibiting total tumor destruction.

Concurrent investigations also revealed an augmented regional CDP concentration. This was demonstrated by consistently elevated concentrations in the local vein as opposed to the peripheral vein (Figure 11-2). The differences in the two circulations during the first 2 hours were highly significant ($p < 0.025$).

The highest single concentrations in the local vein were achieved at 60 and 90 minutes, and a steady state was approached in the systemic circulation in 2–3 hours at a serum level of 4 μg/ml [4].

Tissue concentrations in the tumor and the adjacent bone demonstrated that a dose of approximately 17–40 μg/g CDP was associated with tumor destruction of 60–100% [4]. This contrasted sharply with concentrations of 12 μg/g or less producing tumor destruction under 60%. Using a one-tail t-test,

G. Bennett Humphrey (ed.), OSTEOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS.
 ISBN 0-7923-1905-2

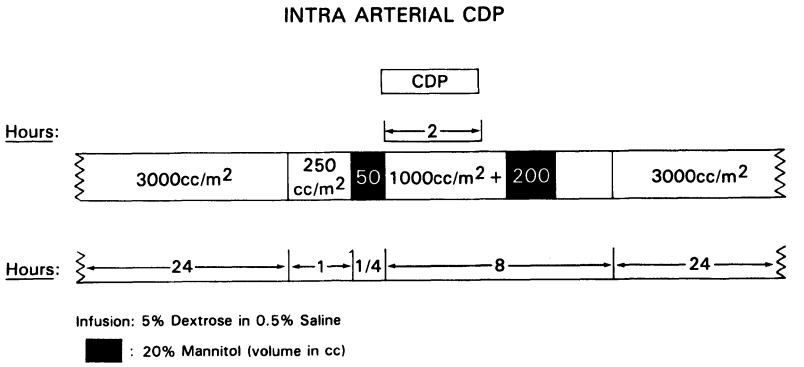

Figure 11-1. Schedule for cis-diamminedichloroplatinum-II (CDP) treatment. Patients received a maintenance i.v. infusion of 3000 ml/m^2 of 5% dextrose in 0.5% saline solution. This infusion was interrupted for $9\frac{1}{4}$ hours to permit administration of the following: 250 ml/m^2 5% dextrose in 0.5% saline solution (1 hour); 50 ml of 20% mannitol ($\frac{1}{4}$ hour), and 200 ml of 50% mannitol dissolved in 1000 ml/m^2 of 5% dextrose in 0.5% saline solution (8 hours). Intraarterial CDP was administered over 2 hours concurrent with the initiation of the latter infusion. The maintenance infusion was then reinstated. CDP was dissolved in 300 ml of normal saline to which 3000 IU of heparin were added. The depicted volumes of 20% mannitol (50 ml, 10 g, and 200 ml, 40 g) were utilized for a surface area of 1–1.5 m^2. Appropriate adjustments were made for children with smaller surface areas.

this was significant at a level of 0.025. CDP tumor concentrations were also related to the number of infusions: Three or four infusions were associated with increased tumor destruction, as opposed to two infusions. This also correlated with CDP uptake: More extensive destruction was associated with an increased CDP concentration. CDP uptake also varied with the tumor subtype: Smaller concentrations were detected in telangiectatic osteosarcoma and malignant fibrous histiocytoma as opposed to the chondroblastic variety.

As a result of this experience, a therapeutic program was devised to deliver a minimum of four courses, and optimally seven courses, for treatment of the primary tumor [5]. After 42 patients had been treated, the therapeutic effects of cumulative courses of intraarterial CDP, each administered at a standardized dose of 150 mg/m^2, were evaluated. Responses correlated with the number of CDP courses: 1–3 vs. 4–7 and tumor subtype. A significant therapeutic effect (over 60% destruction) was observed with four or more courses in one of nine tumors (one to three courses) vs. 26 of 33 tumors (four to seven courses) ($p = 0.01$). These data were consistent with the initial observations that a minimum of four courses were required to achieve optimum effects. It also appeared that osteoblastic osteosarcoma was a highly responsive tumor.

Clinical and radiographic variables also confirmed that responses were more impressive in patients who received four or more courses. These responses included more substantial evidence of bone healing and calcification, and reduction in soft tissue mass and remodeling of tumor-bearing bone [6,7].

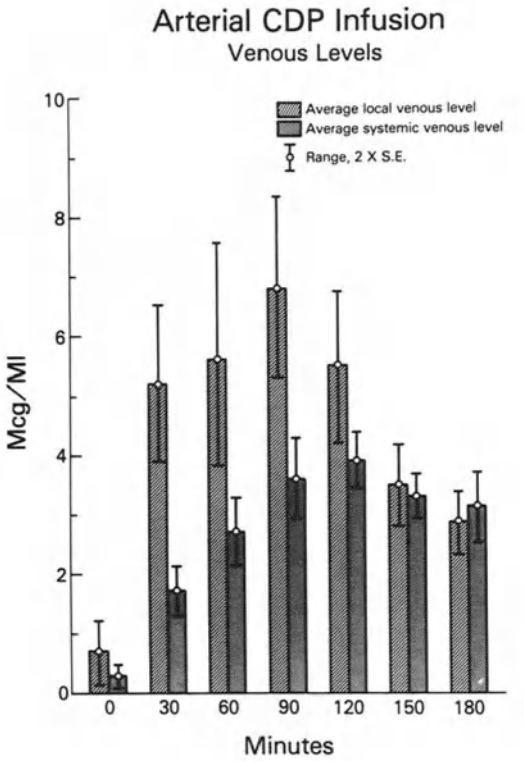

Figure 11-2. Venous concentrations of cis-diamminedichloroplatinum-II (CDP) (μg/ml) in the local draining vein (local venous level) and peripheral circulation (systemic venous level). The levels reflect the average concentration in five patients during intraarterial CDP infusion.

This was also associated with the complete disappearance of tumor neovascularity and stain and resumption of normal range of motion. Whether this was in reality a function of treatment or time or whether it would also have been seen later in other patients who were subjected to earlier surgery is unknown.

Treatment with intraarterial CDP also achieved healing of bone in patients with pathologic fractures, improved the opportunity for limb salvage in questionable candidates, and enhanced the safety of the surgical approach [9]. In a later analysis of 50 primary tumors utilizing pathologic criteria, a 76% response rate was achieved in patients treated with a minimum of four courses [10]. Responses achieved with preoperative therapy were utilized for the selection of postoperative adjuvant treatment yielding a disease-free survival of 60% [1,10] (Figure 11-3). A majority of patients were also afforded the opportunity of limb salvage for tumor extirpation [10]. Treatment strategies revealed that only extent of tumor necrosis and tumor size were significant prognostic factors.

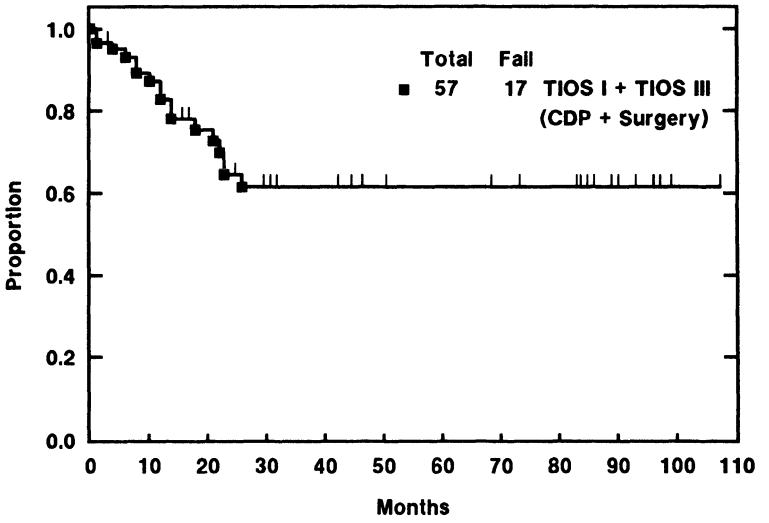

Figure 11-3. Disease-free survival in patients treated with preoperative intraarterial cis-diamminedichloroplatinum-II. Total patients 57; failed 17. Reproduced with permission from Hudson et al. J Clin Oncol 8:1988–1997, 1990.

In the initial studies, one to three courses of intraarterial CDP failed to induce a response in patients with pulmonary metastases despite an effect on the primary tumor [4]. Subsequently, with accumulated experience, 7 of 14 patients with pulmonary metastases achieved responses (6 CR and 1 PR) and one stable disease with four to seven courses (unpublished data). Concurrently, tumor escape or failure to respond (in the primary tumor and pulmonary metastases) was noted in four additional patients treated with one to three courses.

Systemic toxicity

Systemic toxicity characteristic of intravenous CDP administration was also encountered with the intraarterial route. This comprised excessive nausea and vomiting during the infusion. The symptomatology occasionally persisted for 2–3 weeks and eventually abated. Conventional forms of antiemetics were only mildly effective in controlling the side effects. Approximately 10–20% of patients experienced transient forms of hypertension, usually after the second or third course [11]. In some instances hypertension persisted for approximately 6 months.

All patients demonstrated a reduction in the corrected creatinine clearance to the extent that by the seventh course clearance was reduced by 25–50% of its baseline value [12]. Patients also demonstrated auditory deficits [13]. Many also complained of a metallic taste during treatment. Finally,

Table 11-1. Intravenous cis-diamminedichloroplatinum-II in metastatic osteosarcoma

Investigator	Number	Response	Dosage	Frequency
Rosen [15]	20	4	60 mg/m^2 × 2 120 mg/m^2	q 3 wk q 3 wk
Pratt [16]	13	7	30 mg/m^2 120 mg/m^2	q wk q 3 wk
Sarna [17]	7	0	20 mg/m^2/d × 4	q 3 wk
Ochs [18]	8	4	20 mg/m^2/d × 4 120 mg/m^2/d	(4 courses) q 3–4 wks
Freeman [19]	13	7	100 mg/m^2	q 3 wk
Baum [20]	18	3	3–4–5 mg/g	q 3 wk
Nitchke [21]	3	2	15 or 20 mg/m^2 d × 5 1 mg/kg/d	q 3 wk q wk
Gasparini [22]	37	7	100 mg/m^2 24°	q 3 wk
Total	119	34 (29%)		

approximately 30% of patients developed areas of hyperpigmentation. This was particularly evident around the base of the neck and around the nail beds of the fingers and toes.

Local toxicity

Pain in the musculature surrounding the tumor occasionally occurred and was attributed to localized deposition of drug, leading to muscle spasm or minor necrosis. Similar depositions in the skin and subcutaneous tissue occurred at the tip of the catheter in approximately 90% of patients. It manifested as induration, erythema, and pain, and was attributed to drug deposition by laminar flow. The symptomatology was generally relieved with aspirin and the application of localized heat (warm soaks or a heating pad). With the use of a pulsatile pump that induced turbulence and mixing of CDP, a slight reduction in the incidence of the complication was achieved [14]; however, it was not entirely eliminated.

Intravenous cis-diamminedichloroplatinum as a single agent

CDP administered by the intravenous route was reported to produce responses varying from 0% to 53% [15–22] (Table 11-1). The studies were conducted in patients with metastases located principally within the thorax, although isolated cases with bone metastases were also treated. In all reported cases,

Table 11-2. Intravenous or intraarterial cis-diamminedichloroplatinum-II and multiple-agent chemotherapy

Investigator	Chemotherapeutic Agent				Duration (wk)	Resp (%)
	MTX-CF (g/m^2)	ADR (mg/m^2)	CDP (mg/m^2)	Ifos (g/m^2)		
Winkler						
Coss—80 [24]	12 × 4	90 × 1	120 × 1		10	55
Coss—82 [25]	12 × 4	60 × 2	90–120 × 2		10	60
Coss—86 [26]	12 × 2	45 × 2	120 or 150 × 1 (i.v.[a] 1 or 5 hr)	3 × 2	8	69
Coss—86 [26]	12 × 2	45 × 2	120 or 150 × 1 (i.a.[b] tourniquet infusion 1 hr)		8	68
Weiner [27]	8–12 × 4		75 × 2		8(–16)	84
Benjamin [28]		90	120 (i.a.)[c]		12–28	57
Picci [29]	7.5 v 0.75		120–150 (i.a.)		6	52
Stine [30]		90 × 4	150 (i.a.) × 4		12	100
Graham-Pole [31]	7.5 × 4	40	100 (i.a. and i.v.)		8–10	54% (i.v.) 57% (i.a.)

[a] Intravenous route.
[b] Intraarterial route.
[c] Treatment is administered at 4-weekly intervals until the angiogram is "dry."

the dosage was under 120 mg/m^2 and the schedule was less intense than that utilized with the intraarterial regimen.

We are unaware of any human investigations to determine the effects of intravenous CDP administered as a single agent for treatment of the primary tumor. However, studies in dogs have demonstrated that intravenous CDP (70 mg/m^2 q 3 wk × 2) produced results that were inferior to CDP administered by the intraarterial route. An increase in the percent tumor necrosis was strongly predictive for local tumor control [23].

Intraarterial cis-diamminedichloroplatinum-II in combination chemotherapy

Several investigators reported substantial responses following intraarterial CDP in combination with other agents for the treatment of the primary tumor. In the majority of cases the responses varied from 80% to 100% [24–31] (Table 11-2). The combinations of agents comprised high-dose methotrexate with citrovorum factor (leucovorin) rescue and Adriamycin (ADR). The rationale for combination chemotherapy was derived from biochemical synergism utilizing agents with different mechanisms of action and nonoverlapping dose-limiting toxicity. Included in these studies is an attempt to determine the efficacy of intravenous and intraarterial CDP after pretreatment with MTX-CF, ADR, and ifosfamide; and MTX and ADR [26]. These investigations failed to discern any differences in the therapeutic effects of CDP administered by either route.

Discussion

The rationale underlying the delivery of a chemotherapeutic agent by the intraarterial route is based upon an attempt to deliver a high local (regional) concentration and thereby to achieve a greater biological effect in a limited anatomic site. With this approach, it is assumed that an increased extraction of the chemotherapeutic agent occurs with the initial treatment ("first-pass" effect). The effect is also related to the steep dose-response characteristic of certain cytotoxic agents, i.e., the higher the concentration, the greater the antitumor effect [and concomitantly, also, the greater the potential for (local) side effects]. This differential dosage is usually operative during the infusion and is retained until the tumor repositories are saturated.

Higher local drug concentrations may be advantageous if tumor resistance is a consequence of inadequate exposure to, or penetration by, the drug. This is related to primary resistance of some or all tumor cells and defects in membrane transport. Duration of exposure (concentration x time) may also have an impact on the result. Local therapeutic effects (and toxicity) may also be related to tumor vascularity, mechanism of action, and environment (pH, pO_2, and pCO_2). The regional advantage (Ra) for intraarterial treatment is also related to total body clearance [C1 TB (ml/min)] and inversely related to regional plasma flow [Q (ml/min)] of the artery utilized for the infusion [32]. This may be calculated from the formula Ra = 1 + C1 TB/Q. Based on an assumed flow of 1000 and 100 ml/min, the calculated Ra for CDP is 1.4 and 5 respectively.

Systemic effects and toxicity of a drug are influenced by transportation, distribution, diffusion, metabolism, and excretion. These factors may also be operative after the "first-pass" effect with intraarterial treatment and are dependent upon the extent of local drug uptake by tumor and tissues, and the concentration escaping into the systemic circulation. Systemic concentrations entering via normal venous drainage could consequently be equal to, or less than, that achieved by the intravenous route. Whatever the circumstances, systemic tumoricidal concentrations, and toxic effects with intraarterial CDP are certainly attained. This is clearly evident from the experiences reported herein and from published reports [4,33–36].

A major criticism in the use of intraarterial CDP in humans resides in the inability to demonstrate differences in the response between intravenous and intraarterial treatment of the primary tumor. These investigations were conducted with CDP administered with combination chemotherapy [26,30,31]. In one study, an inability to achieve a regional pharmacokinetic advantage with CDP and failure to correlate CDP tissue content with tumor necrosis was cited in support of this claim [26]. However, CDP concentrations may be misleading, since tissue content may decrease with time [26].

An alternative explanation resides in the possibility that a regional advantage was achieved but was not translated into an improved (overall) response. This explanation may be tenable since in the cited studies a limited number

of reduced doses (<120 mg/m^2), at more prolonged intervals (in association with multiple agent chemotherapy), were utilized. As a consequence, an improved local effect could have occurred but was too small to evaluate. In these circumstances, combination chemotherapy prior to CDP could have produced a finite degree of necrosis, with CDP contributing to the end result [26,30,31].

Responses induced by an agent are related not only to the route of administration but also to the dose and schedule of treatment. The intraarterial approach advocated treatment with CDP every 2 weeks to a maximum cumulative dose of 1050 mg/m^2 over 3 months. This is an intensive regimen when compared to singled and multiple-agent CDP-based protocols. It was also contemplated that more intensive treatment would increase the cumulative uptake and enhance the potential for response. This concept was substantiated by the results: Necrosis in the primary tumor (local effect) and elimination of pulmonary metastases (systemic effect) could be correlated significantly with the number of courses administered [5]. A corollary to these findings was the incidence of renal and auditory toxicity [12,13].

Responses with intravenous CDP comparable to intraarterial CDP in the treatment of the primary tumor have been attained only with combination regimens (Table 11-2). Dose intensity, therefore, was judged to be a critical factor in attaining local control and optimum survival, and no attempt was made to incorporate or interpose other agents that could compromise or undermine the schedule of initial treatment. The strategy is not new [37,38]; it was utilized successfully in MTX-CF studies and represents a departure from the conventional administration of cyclic combination regimens.

Comparable intravenous studies, utilizing CDP in a dose intensity equivalent to the intraarterial protocols, are not available; however, it appears that with an identical regimen similar responses could possibly be attained. Investigations along these lines, with emphasis on an augmented dose intensity, appear to be an urgent consideration.

Contrary to earlier claims [2], responses achieved with primary intraarterial CDP were reliable in terms of selecting postoperative adjuvant treatment [1]: Disease-free survival was similar to that reported in studies utilizing primary intravenous multiagent chemotherapy [24–31,39,40]. Prognostic determinants comprised percentage of tumor necrosis induced by intraarterial CDP and tumor size (burden). The response in the primary tumor also enhanced the safety of the surgical procedure, particularly limb salvage.

Summary

Cumulative courses of intraarterial CDP are highly effective for treatment of the primary tumor in osteosarcoma. The response permitted limb-salvage procedures to be performed in a majority of patients. Responses induced in the primary tumor with multiagent chemotherapy, including reduced doses of

CDP, were similar to those obtained with cumulative courses of intraarterial CDP. It is suggested that identical results may be attained with single-agent intravenous CDP by augmenting the dose intensity.

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12. Intraarterial chemotherapy for osteosarcoma: Does the result really justify the effort?

Stefan S. Bielack, Patricia Bieling, Rudolf Erttmann, and Kurt Winkler

Abstract

The use of multiagent chemotherapy has led to improved survival for osteosarcoma patients. Infusion of cisplatin by the intraarterial route in order to devitalize the primary tumor more effectively has been advocated in this context. While systemic drug levels do not seem to be compromised by this regional approach, the envisioned increase in platinum content of osteosarcoma tissue has not been substantiated.

As far as clinical results are concerned, neither a comparison of various protocols including cisplatin by either intraarterial or intravenous application, nor the only controlled study dealing with the subject (COSS 86), were able to demonstrate a correlation between the route of cisplatin administration and tumor response to chemotherapy.

Therefore, the use of the intraarterial route for cisplatin treatment of osteosarcoma cannot be considered standard therapy but is still an investigational approach without proven benefits. In addition, the risk of side effects and the increased cost of this procedure should be considered before deciding to use intraarterial treatment for osteosarcoma.

Why use intraarterial therapy for osteosarcoma?

Intraarterial (i.a.) therapy of osteosarcoma has been advocated by a multitude of authors [1–11]. What could the theoretical advantage of intraarterial treatment be? In the ideal case, local treatment would be intensified: Higher local drug concentrations would lead to better local tumor cell kill. However, local relapse rates have been traditionally low in osteosarcoma, even when amputations were increasingly replaced by less mutilating but more complicated limb-salvage procedures, so that only very few patients would be saved from local relapse by improved local chemotherapy.

Could a significant systemic advantage due to an improved local effect of intraarterial treatment be achieved? Hardly! Occult pulmonary metastases must be presumed to be present even before the initiation of therapy. Their

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 ISBN 0-7923-1905-2

growth will not be influenced by treatment modifications made in some other, distant part of the body. Therefore, the possible benefit of intraarterial therapy for an osteosarcoma patient, even if this form of treatment really should prove to be more effective against the primary tumor, is limited.

On the other hand, intraarterial therapy has some definite risks and discomforts. For example, Winkler et al. observed considerable or even intractable pain due to the procedure in more than half of their i.a. infusions performed without general anesthesia when a tourniquet was used to obstruct the blood flow distal to the tumor [12]. Local complications, such as necrosis, thrombosis, or infection, can prohibit limb-salvage procedures in otherwise eligible candidates. Also, disabling neuropathies [13,14], and even compartment syndromes [15], have been reported with i.a. cisplatin.

In addition to these "local" problems, trapping of active drug during the first pass through the tumor could lead to decreased systemic drug availability, with loss of efficacy against metastatic disease. Finally, before deciding to use intraarterial therapy one has to remember that it is a time- and money-consuming procedure, requiring space and medical staff, all of which might be used more meaningfully.

Choice of drug for intraarterial therapy

The number of agents with proven activity against osteosarcoma is small. If the possible, limited benefits of intraarterial therapy for osteosarcoma discussed above are considered worth the additional effort, which drug would be the most promising to use? From a pharmacokinetic point of view, the theoretical regional advantage of intraarterial drug application depends on no more than two factors: It is positively correlated with the total body clearance of the drug used and inversely correlated with plasma flow through the tumor [16].

As osteosarcoma is usually a well-vascularized tumor with high regional blood flow, appreciable benefits from intraarterial application could only be expected from agents with a very rapid clearance. Due to its low systemic clearance, the potential benefit of giving methotrexate intraarterially is negligible. Ifosfamide has to be activated by the liver before becoming effective [17] and is therefore not a candidate for regional infusion at all. Doxorubicin has been given intraarterially to osteosarcoma patients [9,18,19]. However, due to its aggressive nature, the rate of local complication is rather high [19].

Cisplatin is the substance most commonly used in the intraarterial treatment of osteosarcoma. For this drug, a regional pharmacokinetic advantage for intraarterial over systemic infusion of 5 has been calculated for tumors nourished by arteries with low plasma flow (100 ml/min), while an advantage of only 1.4 was predicted for arteries with high flow (1000 ml/min) [16].

However, even this rather low theoretical benefit could only be utilized if

there was an appreciable dose/response effect for cisplatin in osteosarcoma. So far, no controlled studies dealing with this subject have been reported, so that it remains open to discussion. No impact on osteosarcoma response rates was noted when cisplatin doses were lowered from 120 to 90 mg/m^2 in the trial COSS 82 [20].

Pharmacokinetic studies

Pharmacokinetic studies of i.a. vs. i.v. cisplatin, mostly using flameless atomic absorption spectroscopy for the determination of free or protein-bound platinum, have been performed in various animal models as well as in several human tumor types. The first question requiring an answer is: Are systemic drug levels (essential for control of metastatic disease) compromised by intraarterial infusion?

A significant decrease of systemic platinum exposure was seen by Hecquet et al. with bilateral hypogastric artery infusion for advanced tumors of the uterine cervix [21] and by Ratto et al., who compared intrapulmonary artery and i.v. infusion in pigs [22]. While et al., however, saw such differences only after isolated limb perfusion or i.a. infusion with outflow occlusion, but not after simple i.a. infusion or i.a. infusion with distal stop of flow in rabbits [23]. No differences of total platinum exposure or platinum peaks attributable to the cisplatin application route were seen by Mangues in the peripheral compartment of patients with brain gliomas, patients with extremity sarcomas, or in a dog model [24]. When biodistribution of radiolabeled cisplatin was measured by Shani et al. using scintigraphic dynamic imaging, blood levels of either free cisplatin or proteinated species showed no differences between i.a. and i.v. application [25]. In osteosarcoma patients, no decrease of systemic drug availability was found after i.a. compared to i.v. cisplatin given at 150 mg/m^2 over 1 hour according to the COSS-86 protocol of the cooperative German/Austrian osteosarcoma study group. Peak levels of total platinum in plasma (5464 ± 1186 i.a. vs. 5820 ± 2559 i.v. ng Pt/ml) and free platinum in plasma ultrafiltrate (3115 ± 1160 i.a. vs. 3107 ± 812 i.v. ng Pt/ml), as well as the proportion of platinum excreted in the urine, were basically identical after both modes of application [26].

Does the route of cisplatin administration influence platinum concentrations in the tumor region? Stewart et al. measured increased platinum levels after i.a. compared to i.v. treatment in human tumor autopsy samples. However, the time from last cisplatin treatment and cumulative dose of cisplatinum received were more closely related to the observed platinum levels [27]. Mangues compared peripheral and local tumor draining veins during i.a. cisplatin in various settings, including extremity sarcoma, and found more ultrafiltrable platinum in the latter [24]. Jakowatz et al. saw higher tissue platinum levels after i.a. as compared to i.v. cisplatin only after 48-hour

infusions in a rat limb tumor model, while no differences were seen with 30-minute infusions [28]. A similar observation was made by Kishimoto et al. in rat uterus tissue [29].

No difference of tumor platinum content after i.a. vs. i.v. cisplatin has been seen in human osteosarcoma. In osteosarcomas resected 3 weeks after the second of two cisplatin infusions given over 1 hour at 150 mg/m^2 according to the aforementioned COSS-86 trial, tumor platinum was not influenced by the mode of cisplatin application. Also, there was no correlation between platinum level and the degree of tumor response. A large intertumor and even intratumor variability was seen, with levels ranging from <50 to several hundred picograms of platinum per milligram of tumor [26].

Clinical results

What about the clinical efficacy of intraarterial cisplatin in osteosarcoma? No advantage over i.v. application has been demonstrated thus far! A comparison of neoadjuvant osteosarcoma protocols including either intravenous or intraarterial cisplatin does not show an advantage of any one mode (Table 12-1) [2–6,12,20,30–33]. Histological response rates, usually defined as >90% tumor cell necrosis, are in the same range after preoperative chemotherapy including either i.a. or i.v. cisplatin. However, the possible effects of regional cisplatin application could have been obscured by other variables of the treatment protocols, such as different concomitant medications, varying length of preoperative treatment, or unequal distribution of patient characteristics. Therefore, the prospective, controlled study COSS-86 of the cooperative German/Austrian osteosarcoma study group, the only controlled trial of intraarterial vs. intravenous cisplatin in osteosarcoma reported to date, was designed to specifically address this question [12].

Preoperative chemotherapy included two doses of either i.a. or i.v. cisplatin as well as one course of doxorubicin and two doses each of high-dose methotrexate and ifosfamide. Equal distribution of risk factors between the two treatment arms was achieved by central stratification and was ensured by multivariate analysis. Again, the envisioned advantage of i.a. treatment was not seen: Response rates (percent of tumors with >90% necrosis) were practically identical at 68% (34 of 50 tumors) after i.a. vs. 69% (41 of 59 tumors) after i.v. cisplatin.

A possible criticism of COSS-86 might be that the use of three other active drugs could have obscured differences that might only become apparent during single-agent cisplatin therapy. Also, Jaffe et al. have argued that more than three cycles of i.a. cisplatin should be given preoperatively in order to achieve optimal results [3], while the COSS-86 trial included no more than two courses. However, as interesting as these points might be academically, they seem to be of little practical value in the clinical setting: Prolonged preoperative monotherapy with intraarterial cisplatin as a single agent does not seem to be

Table 12-1. Cisplatin-based preoperative chemotherapy for osteosarcoma

Reference	Cisplatin		Other drugs	Duration	Pts	RR%
	mg/m²	Cycles		(weeks)		
Intravenous cisplatin						
Winkler [20]	120	1	DOX × 1; MTX × 4	10	27	55%
Winkler [30]	90–120	2	DOX × 2; MTX × 4	10	58	60%
Winkler [12][a]	120–150	2	DOX × 1; MTX × 2 IFO × 2	10	59	69%
Weiner [32]	75	2	DOX × 2; MTX × 4	8–16	25	84%
Intraarterial cisplatin						
Jaffe [3]	150	1–7	none	var.	42	43%
Malawer [6]	120	2	DOX × 2	n.s.	22[b]	45%[c]
Picci [31]	120–150	2	MTX × 2	6–7	85	52%
Sierrasesumaga [4]	120	3	DOX × 3	18	18	67%
Winkler [12][a]	120–150	2	DOX × 1; MTX × 2 IFO × 2	10	50	68%
Benjamin [2]	160	Var.[d]	DOX × var.	Var.	50	71%
Ruggieri [33]	120	2	DOX × 2; MTX × 2	8	116	76%
Weiss [5]	120	3	DOX × 1–2; FUDR × 1–2	4–8	50	90%[e]

Published data reporting histological response rates after preoperative therapy including either intraarterial or intravenous cisplatin for osteosarcoma.
pts = patients evaluable for tumor response; RR = response rate: percent of tumors with >90% necrosis after preoperative treatment; var. = variable; n.s. = not specified; DOX = doxorubicin; MTX = methotrexate; IFO = ifosfamide.
[a] Randomized trial of i.a. vs. iv. cisplatin.
[b] 18 osteosarcoma, 3 MFH, 1 other.
[c] >95% necrosis.
[d] Until maximum response.
[e] ≥85% necrosis.

the most promising choice for osteosarcoma patients. Based on a mathematical model, Goldie and Coldman have shown that as many effective agents as possible have to be used at maximum dose intensity from the very beginning of treatment in order to avoid the emergence of drug resistance [34]. In accordance with this theory, salvage therapy for poor responders has failed in osteosarcoma [35–38], even when inadequate preoperative treatment was replaced by highly active therapy postoperatively [30].

Also, Green et al. saw relapses in 5 of 6 cases treated by single-agent intraarterial therapy preoperatively, while 8 of 13 patients without such treatment remained disease free [39]. Consequently, any possible benefit of using the intraarterial route for cisplatin therapy of osteosarcoma would have to be demonstrated within an effective multiagent setting in order to be of any clinical relevance, and this approach has failed. Interestingly, a randomized comparison of intraarterial versus intravenous therapy in soft tissue sarcoma,

studying the effect of infusion mode of doxorubicin, has also failed to show any benefit of regional application [40].

Considering the fact that it has not even been possible to demonstrate an increased local efficacy of intraarterial cisplatin, it should not come as a surprise that its impact on disease-free survival is even less well defined. No controlled study addressing this question has been published so far.

Conclusions

In osteosarcoma, the potential clinical benefit of giving cisplatin using the intraarterial route is limited. Pharmacokinetic studies in various models have not led to uniform results as far as systemic availability and possible regional differences are concerned. No superiority of i.a. over i.v. cisplatin has been proven in the clinical setting. Application of CDDP by the intraarterial route for osteosarcoma should be considered an experimental approach without proven benefit but with definite risks for the patient.

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13. Thermal chemosensitization of cDDP-resistant cells

A.W.T. Konings, J.V.E. Hettinga, and H.H. Kampinga

Introduction

The use of selected anticancer drugs combined with local hyperthermia may improve the ability of oncologists to control locally advanced tumors. This article addresses this possibility and especially discusses recent developments in the area of hyperthermic chemosensitization of cis-diamminedichloroplatinum (cDDP) resistant cells. Only in vitro data will be discussed.

Drug resistance

General introduction

The first contact of drugs with cells is at the level of the plasma membrane. The plasma membrane may play a role in drug resistance, resulting in a decreased accumulation of the drug in the cell. It is mostly drug efflux over the membrane and not drug uptake that seems responsible for diminished accumulation. In a number of cases a membrane-spanning protein, P-glycoprotein, is thought to actively pump drugs out of cells. Resistance to a wide range of drugs may be due to alteration or overexpression of the gene coding for this pump [1]. Once the drug has entered the cytoplasm, it may be exposed to detoxification mechanisms, potentially rendering the drug inactive and the cell resistant. When the drug has reached its ultimate target (e.g., DNA), cells may be resistant because of their greater capacity to repair the damage. The main evidence that DNA repair capacity is an important determinant of response to chemotherapy comes from the inherited human diseases that are repair deficient. Amongst the many examples, xeroderma pigmentosum cells are hypersensitive to UV and cDDP, Fanconi's anemia cells to nitrogen mustard and cDDP, and ataxia telangiectasia cells to VP-16 and ionizing radiation. Thus, very often cells that have a reduced capacity to repair DNA are hypersensitive to a number of anticancer agents. Biochemical mechanisms of drug resistance usually have a genetic origin and can arise either by mutation [2] or by adaptation by switching on genes [3].

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 ISBN 0-7923-1905-2

One of the frequently used approaches to study mechanisms of drug resistance is the in vitro development of drug-resistant cell lines. This is often done by exposing the cells to a low concentration of the drug for a long time or/ and to short treatments with a high dose. There are indications [4] that the method of selection or induction is of importance for the type of resistance obtained.

cDDP resistance

For cDDP resistance several factors may play a role, such as
1. Decreased accumulation capacity of the cells
2. Enhanced detoxification in the cells
3. Structural protection of potential targets, e.g., by altered chromatin
4. Enhanced DNA repair capacity

Decreased cDDP accumulation. Although in a number of resistant cell lines a decreased accumulation capacity has been found, this was not always the case. cDDP-resistant cell lines do not overexpress P-170 glycoprotein, as is the case in the Multi-Drug-Resistance (MDR) system. MDR cell lines are not cross resistant to cDDP. Recently, enhanced expression of a 200-kDa membrane glycoprotein was identified in a cDDP-resistant lymphoma cell line [5]. This may be a cDDP-specific system with some analogy to the MDR phenotype.

Enhanced detoxification. Several compounds and enzymes in the cell may be responsible for detoxification reactions. Reduced glutathione (GSH) is one. Hydrolysis of cDDP in the cell yields electrophylic molecules that easily react with thiol groups. When GSH is involved, the reaction of cDDP with cellular DNA is diminished. GSH may also react with Pt-DNA monoadducts. As a result the production of interstrand and intrastrand crosslinks is (partly) prevented. In a number of cases cDDP-resistant cell lines show a higher GSH content. Also, enhanced levels of the enzyme glutathione-S-transferase (GST) have been found in a number of a cDDP-resistant cell lines. In our laboratory Ehrlich Ascites Tumor (EAT) cells have been made resistant to cDDP by continuous exposure to increasing (just not completely toxic) doses of the drug. Just prior to cloning of the cells, they were exposed to a high dose of cDDP over a short period of time. Although these cells contained 10–40% more GSH than the (parent) normal cells, depletion of GSH by BSO did not have any effect on the cDDP sensitivity of these cells. It is possible that only GSH bound to the nucleus is effective in detoxification, and these molecules are probably not removed by routine depletion procedures. These findings indicate that the level of GSH, as normally assayed, cannot be the only cause of cDDP resistance. Moreover, recently Freeman et al. [6] chronically enhanced the GSH level in CHO cells; cDDP sensitivity did not change! The thiol groups of metallothioneine proteins (MTs) form an

important source of intracellular protein-SH. These molecules bind to heavy metals and afford cellular resistance to these poisons. Bakka et al. [7] found cross-resistance to cDDP in a Cd-resistant cell line with enhanced levels of MTs. After cDDP treatment of these cells, about 70% of Pt was bound to the MTs while in control cells only 5% was bound. There are indications that in a number of cases of cDDP-resistant cell lines enhanced levels of MT are present [8,9]. To induce resistance in these cells high concentrations of cDDP were used, yielding very resistant cells!

Structural changes in chromatin. Because of altered chromatin in cDDP-resistant cell lines, the Pt compounds may be less harmful. Milan et al. [10] showed a protective effect of polyamine depletion on cDDP toxicity. This phenomenon indicates a role of DNA conformational changes, because polyamines bind to DNA. Sometimes "enhanced tolerance" is claimed in cDDP resistance [11]. The observed effects may, however, be explained by enhanced repair. This field of research is rather undeveloped as yet.

Enhanced DNA repair. As mentioned before, xeroderma pigmentosum (XP) cells, having a defect in excision repair of DNA damage, appear to be hypersensitive to cDDP [12,13]. Furthermore, enhanced unscheduled DNA synthesis (UDS) is found in cDDP-resistant cell lines [14–16] as well as increased levels of mRNA of DNA polymerase [α] and [β]; also the levels of these enzymes were increased [17]. Enhanced removal of Pt-DNA adducts has been found in a number of cDDP-resistant cell lines [11,18,19]. Inhibitors of DNA repair (e.g., aphidicolin, an inhibitor of polymerase α and β) can enhance cDDP sensitivity [14,16]. Two research groups [12,20,21] have identified a DNA binding protein that recognizes a DNA fragment damaged by cDDP (or UV radiation). This protein is deficient in XP(E) cells and is enhanced in cDDP-resistant HeLa cells. The protein may play a role in recognizing specific types of DNA damage. As a result, more efficient repair can take place in resistant cells.

Hyperthermic treatment of cDDP-resistant cells

Three articles have been published on the interaction of hyperthermia and cDDP resistance. The reported results are not straightforward. Wallner et al. [22] and Mansouri et al. [23] mentioned a reversion of cDDP resistance by hyperthermia. In the studies of Herman et al. [24], no decreased resistance was reported. A closer look at the published data reveal a different and more detailed picture. When thermal enhancement ratios (TERs) are calculated from the data given in the publications and when these are compared for the different cell lines and different temperatures, the effect on the resistance factor (RF) can be made visible. Furthermore, the cDDP-resistant cells may have a different heat sensitivity when compared to the cells from which they

Table 13-1. Interference of hyperthermia with cDDP resistance; thermal enhancement ratios (TER[a]) and resistance factors (RF[b]) after cDDP treatments at various temperatures

Temperature (1 h at T indicated)	Parent cells	Resistant cells		Cell line	Reference
	TER	TER	RF		
37°C	(1.0)	(1.0)	1.5	CHO	28
39°C	1.5	1.3	1.8		
41°C	1.9	2.1	1.3		
43°C	4.3	3.8	1.7		
37°C	(1.0)	(1.0)	2.5	CHO	28
39°C	1.5	1.3	3.1		
41°C	1.9	1.9	2.6		
43°C	4.3	5.9	1.8		
37°C	(1.0)	(1.0)	3.5	RIF	32
43°C	2.7	5.6	1.7		
37°C	(1.0)	(1.0)	~30	Human	21
42°C	5.0	4.0	~40	carcinoma	
43°C	7.5	20	~15		
37°C	(1.0)	(1.0)	1.7	EAT	This
42°C	2.74	2.25	2.1		report
43°C	5.44	5.65	1.6		

[a] $\text{TER} = \dfrac{\text{Conc. cDDP killing 90\% of the cells at 37°C}}{\text{Conc. cDDP killing 90\% of the cells at the HT temperature}}$.

[b] $\text{RF} = \dfrac{\text{Conc. cDDP killing 90\% of the resistant cells}}{\text{Conc. cDDP killing 90\% of the parent cells}}$.

are derived. The cells studied by Wallner et al. [22] and by Mansouri et al. [23] were more sensitive to heat alone. The cells of Herman et al. [24] were equally heat sensitive. This is, of course, of advantage during tumor therapy with a combination of hyperthermia and chemotherapy, when the drug-resistant cells are more heat sensitive. In Table 13-1 a summary of calculated data (from the literature) is given. The data have been corrected for the effect of heat alone and they have been obtained from 1-hour heat exposures. The latter limits a general interpretation of the results because it is possible that different TERs are found at shorter or longer heat treatments.

In our laboratory three EAT cell lines have been compared for cDDP sensitivity and its interaction with hyperthermia. Cell survival data of the three cell types after exposure to different concentrations of cDDP over 90 minutes at 37°C are given in Figure 13-1. The parent line (EAT-N) has a cDDP sensitivity intermediate between the sensitive EAT-S and the resistant EAT-R lines. The resistance factors for cDDP sensitivity at the level of 10% survival are 0.4 and 1.7, respectively.

All three cell types have been exposed to hyperthermia at 42°, 43°, and

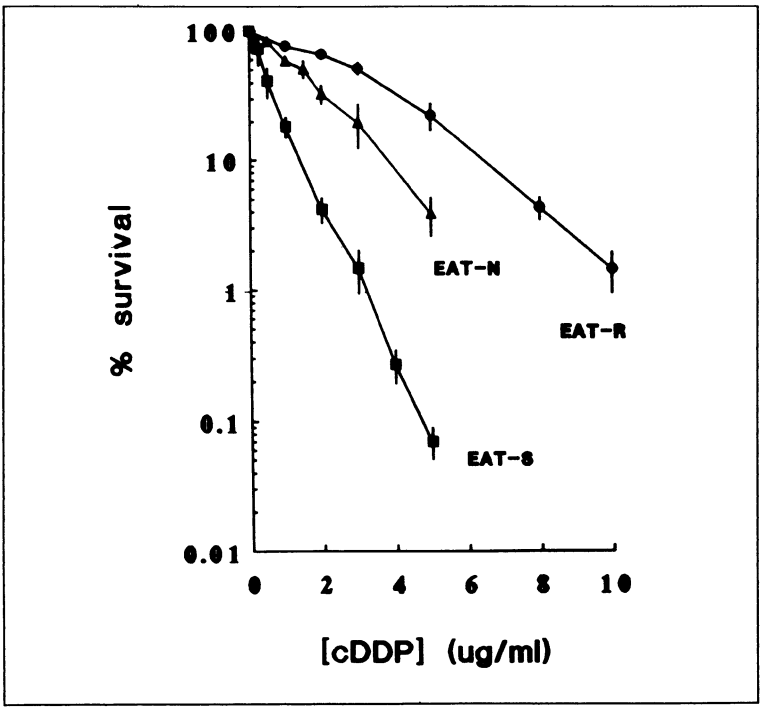

Figure 13-1. Effect of cDDP on the clonogenic ability (survival) of three Ehrlich ascites tumor (EAT) cell lines (EAT-R, EAT-N, EAT-S). Cells were exposed to cDDP for 90 minutes at 37°C and clonogenicity was determined on soft agar.

44°C for different times. The EAT-R cells were more resistant to heat alone than EAT-N. In all cases thermal chemosensitization was observed and expressed as TERs, as can be seen in Figure 13-2. The TERs for the three cell lines after different times at 42°C is graphically presented in Figure 13-2A. No major differences can be observed. The resistant cell line seems to have the lowest TERs over the entire time scale. For 43°C heating, comparable results have been obtained until a heating time of 45 minutes (see Figure 13-2B). In this case the resistant cells tend to have somewhat higher TERs, while heating at 44°C gave more or less identical results for all three cell types.

From a part of the work of Wallner et al. [22], as well as from the experiments performed by Mansouri et al. [23] and Herman et al. [24], it seems that higher temperatures are more suited to partly overcome cDDP resistance by hyperthermia. This, however, is not true for the EAT cells discussed above nor for one of Wallner's CHO lines.

An interesting situation emerges when for the 43°C treatment the TERs for the three EAT cell lines are compared with the killing capacity of heat alone. This is illustrated in Figure 13-2D. Down to about 98% cell killing by heat alone, the TER of the resistant line is significantly higher than the TER

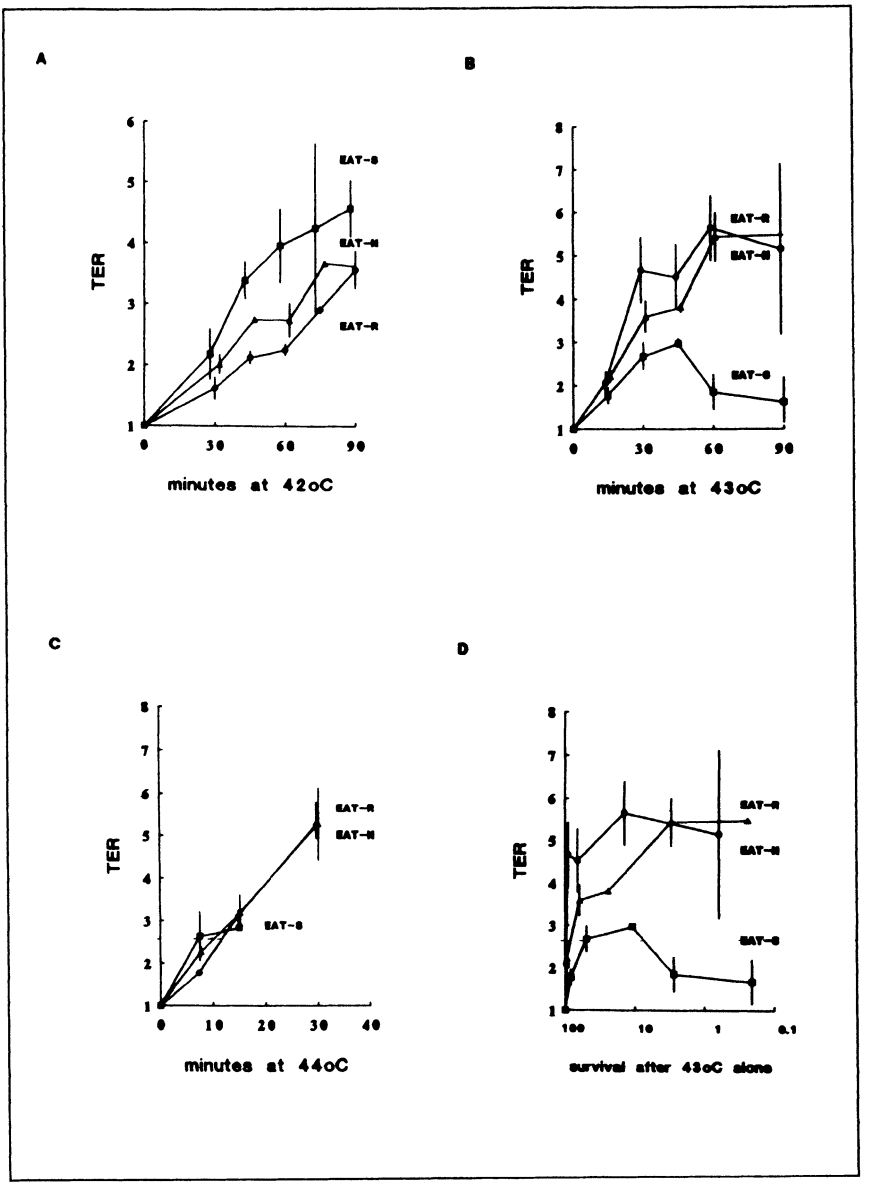

Figure 13-2. Thermal chemosensitization is expressed as TER and is plotted as a function of time at 42°C (A), 43°C (B), and 44°C (C), or as a function of the extent of heat killing at 43°C by heat alone (D).

$$\text{TER} = \frac{\text{Conc. cDDP killing 90\% of the cells at 37°C}}{\text{Conc. cDDP killing 90\% of the cells at the HT temperature}}.$$

of the parent line and is much higher than the TER of the sensitive line. When it is assumed that the mechanism of cell killing by heat alone in the three cell lines is comparable, then Figure 13-2D indicates that the mechanism of thermal chemosensitization is not directly coupled to the molecular reactions leading to killing by heat alone. This conclusion is in accordance with the observations of Neilan et al. [25] in which the state of thermotolerance of the cells did not interfere with cDDP sensitivity.

Although it is somewhat disappointing that a decrease in the cDDP-resistant factor, as a result of hyperthermia, is not generally found in the different studies, it should also be noted that in almost all cases tested, hyperthermia still sensitizes cDDP-resistant cells. Because an enhanced repair capacity for cDDP-induced DNA lesions seems to be one of the causes of cDDP resistance, it is important for future developments to concentrate on efforts to inhibit this type of DNA repair. Masuda et al. [16] showed that aphidicolin could substantially decrease the cDDP RF of the resistant cell line at a concentration that did not affect the sensitivity of the parent line. Whether DNA repair inhibitors can be used clinically for these purposes remains to be seen. Inhibition of repair of cDDP-induced DNA damage solely by hyperthermia might be an option when heat treatment is given after cDDP treatment. More research is needed to obtain insight into these possibilities.

Acknowledgments

The authors thank Prof. Dr. G.B. Humphrey for his support and SKOG, Foundation of Pediatric Oncology Groningen for financing part of the experiments reported.

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14. Liposome-encapsulated muramyl tripeptide: A new biologic response modifier for the treatment of osteosarcoma

Eugenie S. Kleinerman, Miho Maeda, and Norman Jaffe

Introduction

The majority of children who present with osteosarcoma have pulmonary metastases at the time of diagnosis. Despite surgical resection of the primary tumor and aggressive adjuvant chemotherapy, the 2-year metastasis-free survival (MFS) is approximately 66%. The implication of this observation is that these patients harbor "drug-resistant" tumor cells. Therapy failure can be explained by an inherent drug resistance of some metastatic cells in the lung, by an inability to deliver sufficient quantities of drug to the metastatic cells because of side effects, or by the metastatic cells being located in an area where chemotherapy cannot reach them. New forms of therapy are therefore clearly needed if we are to make a further impact in the treatment of osteosarcoma.

In an attempt to do so, we propose to incorporate the biologic response modifier liposome-encapsulated muramyl tripeptide phosphatidylethanolamine (MTP-PE) into traditional chemotherapy regimens to eradicate the residual micrometastases not eliminated by adjuvant chemotherapy. MTP-PE is a synthetic lipophilic analogue of muramyl dipeptide (MDP), the minimal structural unit of mycobacteria with immune potentiating activity [1]. MTP-PE is encapsulated into multilamellar liposomes, which allow selective delivery of the agent directly to pulmonary macrophages and circulating monocytes, thus reducing undesirable side effects.

Human monocytes/macrophages efficiently phagocytose liposomes containing MTP-PE and subsequently kill tumor, but not normal, cells [2]. Macrophages activated by liposomal MTP-PE kill phenotypically diverse tumor cells, including cells resistant to anticancer drugs such as Adriamycin (ADR) [3]. When liposomes containing MTP-PE were injected intravenously (i.v.) into mice bearing established metastases in the lung and lymph nodes, 8–10% localized in the pulmonary microvasculature. Pulmonary macrophages became tumoricidal without local or systemic toxic effects [3,4]. Furthermore, repeated i.v. injections of liposomal MTP-PE completely eradicated metastases at these sites in 70% of mice [4]. Similarly, repeated systemic administration of liposome-encapsulated MTP-PE to dogs with spontaneous osteosarcoma produced a 40% long-term survival rate [5].

G. Bennett Humphrey (ed.), OSTEOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS.
 ISBN 0–7923–1905–2

It is unlikely, however, that liposomal MTP-PE alone can successfully treat metastatic disease. If the ratio of macrophages to tumor cells required for optimal macrophage-mediated tumoricidal activity in vivo is similar to that in vitro, a tumor burden exceeding 10^9 cells would be too large for the number of macrophages in the body to have a significant effect. Indeed, animal studies have verified that liposomal MTP-PE is not an effective therapy for bulk disease and can only eradicate small numbers of tumor cells in specific areas of the body where the agent can be delivered [3].

Such a therapy is precisely what we need for osteosarcoma. The lung is the most frequent site for metastases and is an organ in which macrophages are plentiful and drug delivery can be achieved. The major tumor burden is eliminated by surgery. If combination chemotherapy can kill 90% or 98% of the metastatic tumor cells in the lung, activated macrophages could perhaps destroy those drug-resistant cells left behind.

Since the majority of patients with osteosarcoma who relapse will do so during their adjuvant chemotherapy protocol, we envision combining liposomal MTP-PE with chemotherapy early in the treatment course, rather than waiting until after all chemotherapy has been given. Preclinical and clinical studies (summarized below) have demonstrated that this is indeed a reasonable treatment goal.

Mechanism of monocyte activation by liposomal MTP-PE

Monocyte tumoricidal function has been linked to both interleukin 1 (IL-1) and tumor necrosis factor (TNF) [6,7]. We have recently demonstrated that liposomal MTP-PE upregulated IL-1α, IL-1β, and TNF mRNA and stimulated the production of these proteins [8]. TNF secretion peaked at 8 hours and was sustained for up to 72 hours. Intracellular IL-1 levels also peaked at 8 hours and decreased by 24 hours. Antibody studies proved that this intracellular IL-1 activity was due to the presence of both IL-1α and IL-1β. These data indicate that liposomal MTP-PE activates monocyte tumoricidal function through upregulation of the TNF and IL-1 genes, and the subsequent secretion of those monokines.

Cytotoxic function of monocytes from osteosarcoma patients and the influence of chemotherapy on activation by liposomal MTP-PE

The tumoricidal properties of monocytes from patients with osteosarcoma could be activated by liposomal MTP-PE to levels equal to or greater than those expressed by normal control monocytes [9]. No intrinsic monocyte defect was demonstrated. Single-agent chemotherapy consisting of cisplatin (CDP),

high-dose methotrexate (MTX), cytoxan (CTX), or ADR did not interfere with this activation process [9,10]. This was determined by collecting blood monocytes before and 24 hours after chemotherapy administration and assaying liposomal MTP-PE's ability to activate the tumoricidal function of these cells in vitro. Cytotoxicity values obtained after therapy were compared to those obtained before therapy. A suggestion of enhanced activation potential followed the administration of ADR [9, 10]. Monocyte cytotoxic function remained unchanged after the other agents were administered. We therefore concluded that liposomal MTP-PE can be combined with ADR, CDP, MTX, or CTX, but that ADR plus liposomal MTP-PE is the most effective combination.

Phase I trial of liposomal MTP-PE

A stable reproducible preparation of liposomal MTP-PE is produced by Ciba-Geigy, Ltd. (Basel, Switzerland) for clinical use. In a phase I trial, liposomal MTP-PE (CGP 19835A) infused over 1 hour twice weekly for 9 weeks in 24 patients showed moderate toxic effects (≤grade II), consisting of fever, chills, malaise, fatigue, and myalgias [11]. The maximum tolerated dose was 6 mg/m^2. In 4 of 4 patients evaluated, ^{99m}Tc-labeled liposomes containing MTP-PE were taken up by the liver, spleen, lung, nasopharynx, and thyroid; similar results had been observed in the mouse studies. In two of these patients, uptake in pulmonary metastases was also observed. Imaging of the lung metastases was presumably due to tumor-associated macrophages laden with liposomes.

Significant ($p < 0.05$) increases in white blood cell count (WBC), absolute granulocyte count, and the serum levels of the acute-phase reactants ceruloplasmin, β_2-microglobulin, and C-reactive protein occurred in those patients receiving ≥2 mg/m^2 liposomal MTP-PE. These patients also had decreases in serum cholesterol. IL-1β activity was demonstrated in the sera of 6 of 10 patients 1 hour after liposomal MTP-PE infusion. Five of these six patients had received ≥2 mg/m^2 liposomal MTP-PE. No IL-1β was detected in serum samples from 10 control patients [11].

Patients receiving 0.55–2.0 mg/m^2 liposomal MTP-PE had significant elevations in their monocyte tumoricidal activity at 24, 72, and 96 hours after infusion ($p < 0.005$). Those patients receiving higher doses (>2 mg/m^2) had elevations after 24 hours *only*, and no significant elevation in cytotoxic function could be demonstrated in patients receiving <0.5 mg/m^2 of the agent. Since the other biologic parameters measured (serum IL-1β, acute-phase reactants, increased WBC and granulocyte counts, decreased cholesterol) showed significant change following infusion of 2 mg/m^2 liposomal MTP-PE, we have defined this to be the optimal biologic dose of the agent, well below the maximum tolerated dose.

No change in lymphocyte surface antigens (T_3, T_4, T_8, T_{10}, T_{11}, Ia), surface immunoglobulin-positive B cells, skin test responses to recall antigens, ANA, rheumatoid factor, or serum IFN-α levels was demonstrated. Also, no objective tumor response was observed.

Phase II trial of liposomal MTP-PE in osteosarcoma

We recently began a phase II trial of liposomal MTP-PE therapy in patients with osteosarcoma who developed pulmonary metastases during adjuvant chemotherapy or who presented with pulmonary metastases that persisted despite chemotherapy. Eligible patients were rendered free of visible disease by surgery. Liposomal MTP-PE (CGP 19835A at 2 mg/m^2) was infused twice weekly for 3 months. The rationale for using liposomal MTP-PE in this group of patients was that (1) these patients are known to have a poor prognosis, with a disease-free interval of less than years; (2) salvage chemotherapy has been largely unsuccessful in this group of patients, emphasizing the need for new forms of therapy; (3) these patients presumably have a subpopulation of cancer cells that is either resistant to chemotherapy or situated where adequate drug levels cannot be obtained; and (4) these patients can be rendered free of visible disease by surgery, making the remaining tumor burden favorable for eradication by activated macrophages.

Because this study is still in progress, no conclusion on the clinical efficacy of this agent can be reached. However, several salient findings, particularly some striking pathologic observations, have emerged.

In 5 of 10 patients with an age range of 13–44 years, a single tumor nodule recurred within 6 weeks after completion of MTP-PE therapy. These lesions were resected and submitted for pathologic examination. Tissue specimens obtained after therapy were compared with those obtained before therapy. All patients showed a change in the histologic characteristics of the pulmonary tumors. In three patients, peripheral fibrosis surrounded the tumor and inflammatory cell infiltration and neovascularization were present. Lesions resected following chemotherapy, however, had central necrosis with viable peripheral tumor cells and no inflammatory response. In a fourth case, evidence of early fibrotic changes was found. The fourth and fifth cases also showed a change in malignant characteristics, from high grade before liposomal therapy to low grade after therapy. The tumor was infiltrated with chronic inflammatory cells after liposomal therapy in all five cases. Immunohistochemistry studies using the calcium-binding proteins MRP-14 and MRP-8 specific for inflammatory macrophages and using the antimacrophage antibody CD68 confirmed these cells to be histiocytes-macrophages.

Patient 6 developed chest wall, pleural, pulmonary recurrences at 3 months and subsequently received chemotherapy; thus no histologic comparisons could be made. Patient 7 had no evidence of disease (NED) for 12 months, and patients 8 and 9 have been NED for 19 and 23 months. The 10th patient

Table 14-1. Summary of peak cytokine levels measured following liposomal MTP-PE infusion[a]

Cytokine	Time of peak increase
TNF	1–2 hr
IL-6	2–3 hr
CRP[b]	24 hr
Neopterin	24 hr
IL-1α	neg[c]
IL-1β	neg
IFNγ	neg

[a] Plasma samples were collected from 10 patients immediately before and 0.5, 1, 2, 3, 4, 24, 48, and 72 hours after infusion of liposomal MTP-PE.
[b] CRP = C-reactive protein.
[c] neg = not detected.

developed a relapse in the bone at 8 months with no evidence of lung metastases. His lungs remain free of disease at 15 months.

Immunologic stimulation in this phase II trial was demonstrated by the elevations in plasma C-reactive protein, neopterin, TNF, and IL-6 following the infusion of liposomal MTP-PE. The times of peak elevations are summarized in Table 14-1. In addition, as observed in the phase I study, the tumoricidal activity of blood monocytes was also elevated following the administration of liposomal MTP-PE. Taken together, the pathologic findings, the elevations in plasma cytokines, and the increased monocyte activity following administration provide evidence for a biological effect of liposomal MTP-PE.

Summary and conclusions

We have demonstrated that monocytes from osteosarcoma patients can be rendered tumor cytotoxic by both in vitro incubation with liposomal MTP-PE and i.v. administration of this agent. Chemotherapy did not interfere with this activation process. We have further demonstrated in phase I and phase II trials that liposomal MTP-PE can be given safely i.v. to both adults and children with minimal side effects.

The findings of peripheral fibrosis with neovascularization and infiltration of the tumor with chronic inflammatory cells after liposomal MTP-PE therapy are unlike any observed following chemotherapy or surgery. Subsequent to chemotherapy, osteosarcoma lung metastases usually exhibit a zone of central necrosis, with viable tumor cells growing at the periphery of the lesion. However, in our patients following liposomal MTP-PE viable tumor cells were observed in the center of the lesion, with necrosis and fibrosis at the periphery. These changes were thus interpreted as a specific response to liposomal MTP-PE.

The peripheral fibrosis observed in these tumors is reminiscent of the appearance of pulmonary tuberculosis lesions. Initially, the lesion is walled off and slow necrosis proceeds from the outside so that the lesion is replaced by fibrous tissue. Eradication of tuberculosis by chronic inflammation is a slow process. Viable bacilli can persist for months. Thus, our choice of a 3-month treatment course may have been insufficient. We have now extended our protocol to allow 6 months of therapy.

Osteosarcoma appears to be an ideal disease in which to employ liposomal MTP-PE as an additional adjuvant to present chemotherapy regimens. The lung is the most frequent site for metastases, and pulmonary micrometastases are considered to be present in the majority of patients at diagnosis. Approximately 40% of children with osteosarcoma develop pulmonary metastases despite the administration of adjuvant chemotherapy [12–15]. Based upon the above data, we believe that liposomal MTP-PE may be effectively combined with chemotherapy in the adjuvant setting. Liposomal MTP-PE could activate pulmonary macrophages to destroy the residual tumor cells that are not eliminated by chemotherapy. If in the pulmonary tumor lesions the central necrosis associated with chemotherapy and the peripheral necrosis associated with liposomal MTP-PE are additive, then the entire lung lesion could be eradicated. Furthermore, new vessel formation stimulated in and around the tumor by liposomal MTP-PE may enhance the delivery of chemotherapy to these lesions.

Liposomal MTP-PE was shown to be effective as a single agent in spontaneous canine osteosarcoma [5]. MacEwen et al. have also reported preliminary data indicating that the combination of cisplatin plus liposomal MTP-PE is more effective than cisplatin alone [16], once again supporting the use of combination therapy early in the treatment course.

Acknowledgments

This work was supported by National Institutes of Health grant CA 42992, CIBA-GEIGY, Ltd., Basel, Switzerland, and a rehabilitation award for patient travel from the American Cancer Society.

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15. Pathological diagnosis of osteosarcoma: The validity of the subclassification and some new diagnostic approaches using immunohistochemistry

Yoshimichi Ueda, Albert Roessner, and Ekkehard Grundmann

Introduction

Osteosarcoma of bone is a disease with considerable histologic and anatomic heterogeneity affecting the biological behavior of the tumor. On the basis of clinical features, anatomic location, histologic subtype, cytologic grading, and biological behavior, Dahlin and Unni [1] subclassified osteosarcoma into a "conventional" type and 11 important recognizable varieties, a system that has been generally utilized over the last 15 years. These days, however, our knowledge of osteosarcoma has greatly increased, compelling us to modify the original implications of those entities and to add a few novel varieties. Moreover, estimation of biological behavior is another important problem that should be settled by pathologists. Thus far, we have found no effective indicator other than cytological grading.

In the first part of this article the subclassification of osteosarcoma will be critically reviewed based on the most recent knowledge on osteosarcoma as well as on our experience in the Bone Tumor Registry of Westfalia. In the second part, newly developed cytomorphological methods, such as electron microscopy, immunohistochemistry, and enzyme histochemistry, and their practical applications in the diagnosis of osteosarcoma will be addressed [2]. Special emphasis will be placed on immunohistochemistry, particularly with regard to extracellular matrix components of bone, proliferation-associated nuclear proteins, and tumor associated antigens.

Critical review of the classification of osteosarcoma

Osteosarcoma of bone is now generally classified into the 14 subclasses listed in Table 15-1 [3].

Conventional osteosarcoma

Conventional osteosarcoma is a common, high-grade malignant mesenchymal tumor producing osteoid or bone directly, even if only in small foci (Figure

G. Bennett Humphrey (ed.), OSTEOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS.
 ISBN 0-7923-1905-2

Table 15-1. Subclassification of osteosarcoma

Central
Primary
Conventional
Telangiectatic
Small cell
Malignant fibrous histiocytoma-subtype
Low-grade intraosseous
Multicentric
Gnathic
Secondary
Paget's disease
Radiation induced
Associated with other benign preexisting condition (e.g., fibrous dysplasia)
Juxtacortical
Parosteal
(Dedifferentiated parosteal)
Periosteal
High-grade surface

Modified from Klein et al. [3], with permission.

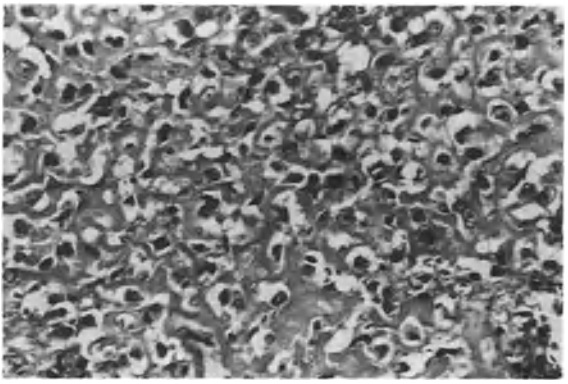

Figure 15-1. Conventional osteosarcoma showing typical irregular-shaped, lacelike tumor osteoid produced directly by highly anaplastic cells (H&E, ×330).

15-1). It generally involves the metaphysis of a long bone, especially around the knee, of adolescents and young adults. It was further subclassified into osteoblastic, chondroblastic, and fibroblastic osteosarcoma, depending on the predominant matrix pattern. However, the histologic multipotentiality of the tumor and the lack of significant statistical differences in prognosis between the subtypes make the subdivision artificial [4].

Osteosarcoma sometimes shows a histological appearance closely resembling benign lesions, such as osteoblastoma, chondroblastoma, or giant cell tumor, or even metastatic carcinoma [5]. Raymond and his colleagues presented a unique case of high-grade osteosarcoma that was located primarily in the epiphysis of the distal femur and comprised mostly tumor components simulating clear-cell chondrosarcoma, in addition to a small but distinct area showing the histology of typical anaplastic osteosarcoma; they called the tumor "chondroblastic osteosarcoma, clear cell variant" [6]. In making a pathological diagnosis of bone tumors, we should always bear in mind that osteosarcoma may arise in any part of any bone and may show histological appearances corresponding to any benign or malignant bone tumor.

Telangiectatic osteosarcoma

Telangiectatic osteosarcoma is characterized by a purely lytic radiographic appearance, and a macroscopic and microscopic resemblance to aneurysmal bone cyst. The prognosis of this variant was thought to be extremely poor since the Mayo Clinic Group had reported a series of 25 cases [7]. This was countered by the Memorial Hospital Group [8]. Later, it is demonstrated that telangiectatic osteosarcoma was more sensitive to chemotherapy than conventional-type osteosarcoma and was potentially curable [9]. Dahlin and Unni agreed that no significant prognostic difference between telangiectatic and conventional osteosarcomas had been observed in the cases diagnosed and treated after 1976 [4]. The cause of this change of prognosis is not quite clear. The establishment and understanding of this entity, which made early diagnosis and referral to therapy possible, seems to be one of the factors involved.

Small cell osteosarcoma

Small cell osteosarcoma was first reported by Sim et al. [10] and has subsequently been studied clinicopathologically by others [11,12] (Chapter 17). Roessner et al. [13] described a sclerosing osteosarcoma with small cell foci resembling Ewing's sarcoma, and recently we saw another case. These tumors may represent a low-grade variant of small cell osteosarcoma.

Malignant fibrous histiocytoma subtype

This subtype comprised, in the original material of Dahlin and Unni [1], 18 tumors that could be separated from osteosarcomas because of their histologic similarity to malignant fibrous histiocytoma [MFH] of soft tissue. However, according to more detailed understanding of such lesions [14–16], they were finally classified as a genuine "MFH of bone" because of the absence

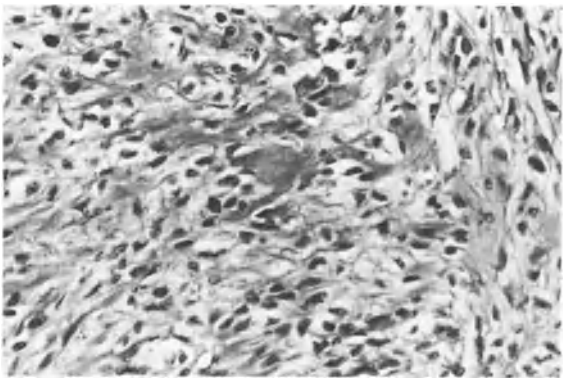

Figure 15-2. Malignant fibrous histiocytoma of bone. Osteoidlike material in otherwise typical histology of malignant fibrous histiocytoma. Interpretations of such material are different among pathologists (H&E, ×330).

of definite osteoid or chondroid matrix [4]. Therefore, the MFH subtype of osteosarcoma is currently defined as a tumor with predominant areas histologically simulating MFH, in addition to small but distinct components producing osteoid or cartilaginous matrix. This lesion has been described by various terms, such as *MFH-like osteosarcoma* [7], *fibrohistiocytic type of osteosarcoma* [18,19], and *MFH subtype of osteosarcoma* [20], but we feel unable to agree that it is a distinct entity. Conventional osteosarcoma may also contain a large number of macrophages or histiocytes, which have been considered characteristic of the MFH subtype [21]. Moreover, no clinical, radiographic, or prognostic differences were revealed between conventional osteosarcoma and this subtype, except for its tendency to occur more often in older patients than conventional osteosarcoma [19,20]. The significance of this subtype merely remains histomorphological. Differentiation between this subtype and true MFH is sometimes difficult and must remain arbitrary, because accurate histological definition of osteoid is difficult with conventional histological methods (Figure 15-2). Adequate sampling combined with radiographical findings is indispensable for correct diagnosis of this lesion.

Low-grade intraosseous osteosarcoma

Low-grade intraosseous osteosarcoma is rare and comprises less than 2% of all osteosarcomas [22]. Histologically, it is a low-grade fibro-osseous lesion, ranging from parosteal osteosarcoma patterns, to fibrous dysplasia patterns, to desmoid patterns (Figure 15-3). For differential diagnosis from fibrous dysplasia, with which it is often confused, the recent study of 80 cases of low-grade intraosseous osteosarcomas stressed certain atypical roentogenographic features, such as cortical destruction, erosion, and poor margination, and the

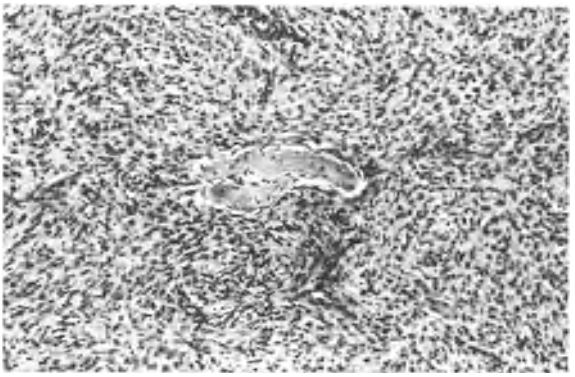

Figure 15-3. Low-grade intraosseous osteosarcoma showing a histology reminiscent of fibrous dsyplasia. Nuclei have a regular shape with few atypical cells and occasional mitotoic figures (H&E, ×316).

histologic feature of a permeative pattern [23]. The lesion can be successfully treated by wide excision. Local excision is frequently followed by recurrence, 15% of which is associated with high-grade osteosarcoma [22,23].

Osteosarcoma arising on the surface of long bones

Osteosarcoma that arise from the surfaces of long bones are far less common than those within bone. There had been conflicting terminologies regarding those tumors, but Schajowicz et al. [24] have now summarized the literature and have subdivided osteosarcomas into three subgroups using histological and biological criteria: parosteal (juxtacortical), periosteal, and high-grade surface osteosarcoma [24,25].

Parosteal osteosarcoma. Parosteal osteosarcoma, the most frequent surface osteosarcoma, is usually seen in patients of the third and fourth decades. The vast majority of these tumors arise from the metaphyseal region of a long tubular bone, especially the posterior distal femur. Radiographically they present as a heavy ossified mass with a broad base attached to the underlying cortex. Histologically the lesion is characterized as a low-grade malignant (Broders' grade 1 or 2 [26]) spindle-cell tumor with well-formed, parallel, trabecular bone (Figure 15-4). High-grade (grade 3) lesions included in the series of Ahuja et al. [27] and Campanacci et al. [28] have to be reclassified as high-grade surface osteosarcomas. The prognosis of these patients is good, and wide excision is successful in a vast majority [24,29]. Wold et al. [30], however, reported that about 20% of parosteal osteosarcomas developed into high-grade osteosarcomas as a local recurrence after curative surgery. The prognosis for the patients with dedifferentiation was as poor as for those

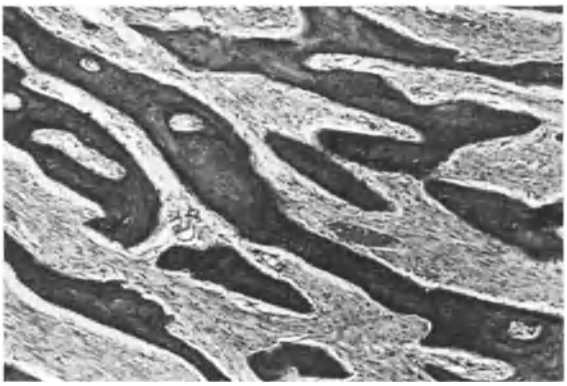

Figure 15-4. Parosteal osteosarcoma. Atypia of spindle-shaped cells between bone trabeculae is minimal (H&E, ×100).

with conventional high-grade osteosarcoma. Bertoni et al. [3] revealed 33% of dedifferentiation at the time of presentation in a series of 36 conventional parosteal osteosarcomas; they clearly demonstrated a combination of deep radiolucent areas with dedifferentiated areas when whole-organ histologic sections were examined. Ayala et al. [32] showed that such radiolucent areas related to dedifferentiation were angiographically hypervascular and that selective biopsies of those hypervascular radiolucent areas permitted the preoperative diagnosis of dedifferentiated parosteal osteosarcoma. The prognosis for patients with these lesions may be further improved by intensive preoperative chemotherapy, whereas diagnosis is ensured preoperatively by proper imaging.

Periosteal osteosarcoma. Periosteal osteosarcoma is a rare subtype of surface osteosarcoma involving the diaphysis of long bones in adolescence and with moderately well-differentiated (grade 2 or 3) chondroblastic histology [33]. The prognosis in this lesion is better than that for high-grade surface osteosarcoma. However, it is less than satisfactory [24], although Unni and his associates insisted that it was about as good as with parosteal osteosarcoma [33]. Criteria chosen for diagnosis of periosteal osteosarcoma caused some confusion with juxtacortical chondrosarcoma, as described by Schajowicz [34], but Bertoni et al. [35] later clearly demonstrated that these were two distinct entities with marked clinical, radiographic, histologic, and prognostic differences. The problem of the acceptance of medullary involvement proposed by Hall et al. [36] has to be answered by further investigations.

High-grade surface osteosarcoma. This is a very unusual, highly malignant tumor that sometimes invades the medullary cavity. Its histology and prognosis are the same as those of conventional osteosarcoma [37].

Recent development in the immunohistochemical approach

Extracellular matrix components

One of the important characteristics of osteosarcoma cells is the production of extracellular matrix. Osteosarcoma is diagnosed by the presence of tumor osteoid, which is usually identified on the basis of its characteristic histological features. However, it is often difficult to differentiate osteoid from chondroid or hyalinized collagenous tissue, particularly if there is no mineralization. Some histochemical methods have been employed to distinguish osteoid from other matrices [38,39], but there is no absolute staining method for the identification of osteoid at present. However, recent advances in biochemical research on extracellular matrix components of skeletal tissue have brought a new approach to the immunohistochemical diagnosis of osteosarcoma [40–43]. Organic components of the extracellular matrix of osteosarcoma are composed of collagenous and noncollagenous bone proteins.

Collagenous protein. Collagen, the major structural component of the extracellular matrix, is now recognized as a family of proteins of at least 13 different molecular species [44–46]. Normal bone matrix is made up predominantly of type I and a small amount of type V collagen [47]. Immunohistochemical studies demonstrated that in normal and reactive skeletal tissues, type I collagen is the main component of bone and osteoid, and type V is confined to osteoid. Type III and type VI collagens, which are usually codistributed with type I in ordinary fibrous connective tissue, as well as type II and type IV collagen, are absent in bone and osteoid matrix [48–55]. In tumor osteoid, the composition of collagen subtypes is basically similar to that of non-neoplastic osteoid. However, particularly in the case of immature tumor osteoid, a small amount of type III collagen is sometimes mixed with type I collagen, as indicated by Remberger and Gay [49] and Roessner et al. [56], and type V collagen is occasionally lacking (Table 15-2) [55]. The characteristic composition of collagen types in tumor osteoid found by immunohistochemistry has been supported by quantitative analysis of collagen types performed on short-time cultured tumor cells. Tumor cells from osteosarcoma produced almost exclusively type I collagen and constantly small amounts of type V collagen. The synthetic activity of type III collagen, which comprised about 10% of type I collagen in dermal fibroblasts, was extremely low in osteosarcoma cells, about 1% of type I collagen [55].

Although no single type of collagen is specific to tumor osteoid, the combination of immunoreactivities to type I–VI collagen antibodies seems to be helpful in defining tumor osteoid in osteosarcoma. In addition, in the differential diagnosis between chondroblastic osteosarcoma and chondrosarcoma, the absence of immunoreactions to type II and VI collagen indicates that the matrix is not of chondroid but of osteoid nature, since both collagen types are almost constantly present in the matrix of chondrosarcoma [57]. Moreover,

Table 15-2. Summary of tissue localization of collagen types in different areas of osteosarcomas

Area of tissue	Immunoreactivity with type-specific anticollagen					
	I	II	III	IV	V	VI
Conventional osteosarcoma						
Tumor osteoid	+ +[a]	−	− ~ ±	−	± ~ +[a]	−
Fibroblastic area	+[a]	−	+	− ~ ±	±	+
Chondroblastic area	+ +	+ ~ + +	±	−	± ~ +[a]	+
Anaplastic area	±	−	±	−	−	±
Telangiectatic osteosarcoma	+ +[a]	−	− ~ ±	± ~ +	− ~ ±	± ~ +
Intramedullary well-differentiated osteosarcoma						
Tumor osteoid	+[a]	−	− ~ ±	−	+	−
Fibrous stroma	+[a]	−	+ +	±	−	+

+ +, constantly and strongly positive; +, constantly positive; ±, variably and weakly positive; −, negative.

[a] Intracellularly positive.

an immunohistochemical approach using antibodies to different collagen types is helpful in understanding the biological properties of osteosarcoma cells with diverse differentiation, as suggested by electron microscopic evidence, such as angioblastic in telangiectatic osteosarcoma [58,59].

Noncollagenous bone proteins. Noncollagenous bone proteins comprise about 10% of the organic component of bone matrix. Since type I collagen, the principal constituent of bone matrix, is itself not specific to bone tissue, the unique quality of bone matrix is attributed to noncollagenous proteins; albumin and IgG account for a large share, but several unique proteins, such as osteonectin [60], osteocalcin [61], bone sialoprotein I, II [62], proteoglycan I, II [63], and bone morphogenetic protein [64], have also been isolated.

Osteonectin is a phosphorylated glycoprotein, with a molecular weight of 32000 Dal, that binds selectively to both hydroxyapatite and type I collagen [60]. Bone tissue contains 500 to 1000 higher concentrations than soft tissue [65], and immunohistochemical studies using a polyclonal antibody to osteonectin have also shown the exclusive localization of osteonectin in osteoblastic cells of normal and reactive bones, indicating that osteonectin is a marker of osteoblastic differentiation [66]. However, the specificity of osteonectin for osteoblastic differentiation is now questionable. Several osteonectin-homologue proteins that show a wide variety of anatomical distribution, such as SPARC [67], BM40 protein [68], 43 kDμ protein [69], and platelet osteonectin [70], have been discovered, and osteonectin is now considered as a multifunctional protein [71]. The value of osteonectin for the diagnosis of osteosarcoma as proposed by Schulz et al. [72] and Jundt et al.

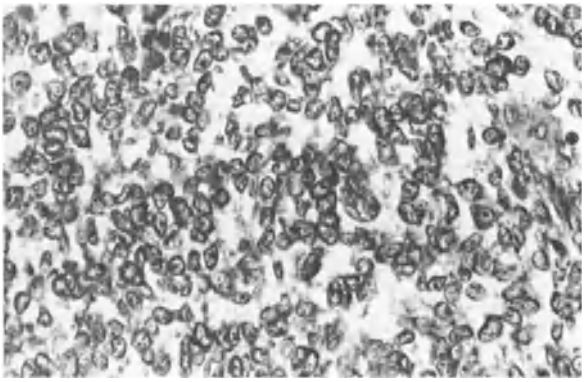

Figure 15-5. Ewing's sarcoma stained with antiosteonectin antibody. Many tumor cells show positive reactions in the small cytoplasmic rims (anti-osteonectin, ×550).

[73] is also questionable. Immunohistological studies at the Münster Institute using the same polyclonal antibody against osteonectin have disclosed positive reactions, not only in osteosarcoma, but also in various other malignant bone tumors, including malignant fibrous histiocytoma, chondrosarcoma, and Ewing's sarcoma (Figure 15-5) [74]. In the future, however, immunohistochemical studies using monoclonal antibodies against osteonectin [75] and a quantitative approach of osteonectin content in tumor tissues may provide helpful information on osteonectin as a marker for osteoblastic differentiation.

Osteocalcin (bone Gla-protein) is another major noncollagenous bone protein, with a molecular weight of 5200–5800 Dal, characterized by the presence of two or three gamma carboxy glutamic acids in the molecule. The most obvious properties of osteocalcin are the specific Gla-dependent binding of Ca^{2+} and the high affinity for hydroxyapatite crystals [61]. Immunohistochemically osteocalcin was exclusively demonstrated in osteoblasts, osteocytes, and bone matrix of normal and reactive bone [76,77]. In the study of bone tumors, Ushigome et al. [78] (Chapter 16) showed that the differential diagnosis between osteosarcoma and malignant fibrous histiocytoma could be accomplished by immunohistochemistry using an antibody to osteocalcin. However, in a recent study osteocalcin was detected not only in tumor cells of osteosarcoma, but also in those of chondrosarcoma and malignant fibrous histiocytoma (G. Jundt, personal communication). Further studies are required to evaluate the value of osteocalcin in the immunohistochemical diagnosis of osteosarcoma.

Bone morphogenetic protein is a minor component of noncollagenous bone proteins, which induces differentiation of host mesenchymal cells to form bone in heterotopic sites [64]. Immunohistochemical studies demonstrated bone morphogenetic proteins in osteoblasts, osteocytes, and stromal

mesenchymal cells, as well as bone matrix in periosteum, developing fetal bone, and the tissue of healing fractures [79]. In studies of bone tumors, bone morphogenetic protein was demonstrated in the tumor cells of chondrosarcoma as well as in those of osteosarcoma [79, 80]. The bone morphogenetic activity of the primary osteosarcoma tissues, which was bioassayed as ectopic new bone formation on implantation of freeze-dried fractions of tumor tissue in athymic nude mice, was shown to correlate with a high incidence of metastases to lungs and bones and a poorer prognosis of patients [81]. Such a correlation should be also confirmed immunohistochemically with a significant number of cases of osteosarcoma.

Monoclonal antibodies to osteosarcoma-associated antigen(s)

Several reports were published on the production of monoclonal antibodies against osteosarcoma-associated antigens [82–88]. Embleton et al. [82] developed an antibody (791T/36) by immunization of an established osteosarcoma cell line. This monoclonal antibody reacted not only with several human osteosarcoma cell lines but also with several carcinoma cells. Monoclonal antibodies (OST6,7,15) to osteosarcoma-associated antigens were established by immunization of fresh osteosarcoma tissue [83]. Those antibodies, however, cross-reacted with chondrosarcoma, and a reactivity with serum alkaline phosphatase was also later demonstrated [89]. Monoclonal antibodies TP-1,3 [84] and B-OS12 [86] recognized no osteosarcoma-specific, but largely sarcoma-related antigens distributed in various sarcoma types. Since specificity is the most desirable factor of monoclonal antibodies, a broad reactivity of such monoclonal antibodies must hamper their practical application in the diagnosis of osteosarcoma. However, recently established antibodies, such as OSA-1,2 [85], 2H1 and 2D3 [87], appear specific to human osteosarcoma-associated antigens, at least according to the data presented so far, although further screening of cross-reactivity with a wide variety of normal and neoplastic tissues, in particular tumors and tumorlike lesions of bone, are required to verify their practical application.

It should be realized at this stage that, as yet, no monoclonal antibody has been adequately assessed as an aid for the morphological distinction of osteosarcoma from other primary bone tumors and tumorlike lesions. It might be difficult, though not impossible, to establish monoclonal antibodies absolutely specific for osteosarcoma. However, further development of several monoclonal antibodies to tumor-associated and differentiation-related antigens of osteosarcoma will facilitate the histopathological diagnosis of osteosarcoma. Tsai et al. [88] generated a unique monoclonal antibody (TMMR-2) by immunization of an established osteosarcoma cell line. The antibody recognizes an osteoblast/osteocyte surface antigen (MW 26000) distributed in normal, reactive, and neoplastic bone. The inhibitory effect of TMMR-2 on DNA synthesis in cultured osteosarcoma cells indicates an important growth-inhibiting role of this surface antigen. Further investigations using monoclonal antibodies

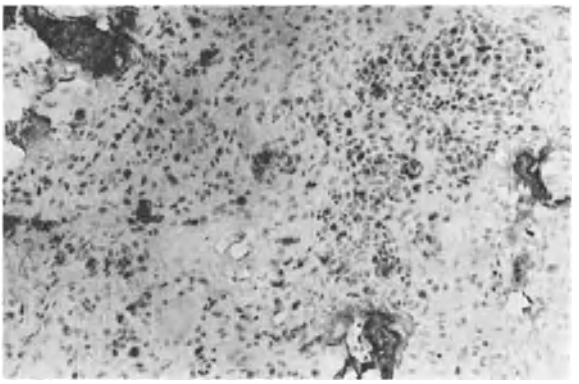

Figure 15-6. Highly malignant osteosarcoma stained with anti-Ki-67 antibody. Many tumor cells are in the active phases of the cell cycle (anti-Ki-67, ×160).

against osteosarcoma-associated antigens will advance our understanding of antigens, present mostly on the cell membrane, and closely related to cell activation and proliferation.

Proliferation-associated nuclear proteins

While most immunohistochemical studies on osteosarcoma have focused on the histogenetic origin and grade of differentiation of tumor cells, markers for the prognostic evaluation of a tumor's biological behavior are very rare. The rate of proliferation is a meaningful criterion for the tumor's biological activity. Ki-67, a monoclonal antibody against a nuclear antigen that exists in all active phases of the cell cycle (G1, S, G2, M), makes it possible to determine on unfixed frozen sections which cell population in a given neoplasm is actually proliferating [90,91]. The immunohistochemical study of 97 bone tumors, including 20 osteosarcomas documented in the Bone Tumor Registry of Westfalia has demonstrated a good correlation between Ki-67 expression and proliferative behavior (Figure 15-6) [92] and suggests that Ki-67 is helpful in verifying and supplementing conventional histologic grading. Particularly in osteosarcoma, the cell kinetic data obtained by immunohistochemistry may contribute to the differentiation of anaplastic giant-cell containing osteosarcoma from giant cell tumor, telangiectatic osteosarcoma from aneurysmal bone cyst, and also high-grade osteosarcoma from its low-grade counterpart [92]. The limitations of Ki-67, which can be used only on fresh frozen sections, will be conquered by the introduction of new monoclonal antibodies against proliferating cell nuclear antigen (a 36 kDμ, S-phase-associated nuclear protein) such as PCNA/cyclin [93] and PCNA/PC-10 [94], which are applicable to paraffin-embedded tissues fixed in alcohol and even in formalin in the case of the latter antibody.

Conclusions and perspectives

The present review of the current subclassification of osteosarcoma has assured its clinicopathologic significance, calling attention to altered implications and problems still to be resolved in some subclasses. Among the newly developed approaches—such as immunohistochemistry for extracellular matrix components, proliferation-associated nuclear proteins, and osteosarcoma-associated antigens, and DNA cytometry (Chapter 18)—none appears in itself to be authentic enough to distinguish osteosarcoma from other bone tumors and to estimate plausibly the biological aggressiveness of osteosarcomas. However, a combination of findings obtained with new sophisticated methods will eventually improve the accuracy and quality of the pathological diagnosis. The new methods will also contribute to our understanding of the biological features of osteosarcoma. In summary, we would currently recommend, for a complete pathologic workup of a new case with a possible diagnosis of osteosarcoma, immunohistochemistry for collagens I, II, III, V, and VI, and for noncollagenous proteins such as osteocalcin and osteonectin, in order to clarify the osteoblastic nature of the tumor, and DNA cytometry and immunohistochemistry for proliferation-associated nuclear proteins in order to comprehend the biological aggressiveness of the tumor.

Recently, cytogenetic studies have disclosed the involvement of mutation of tumor suppressor genes, such as retinoblastoma susceptibility (Rb-1) gene and p53 gene, in the development of human osteosarcomas [95,96]. Both DNA analysis and immunohistochemistry using monoclonal antibodies specific to mutant type p53 [97] will enable us to utilize data on cytogenetic abnormalities in order to distinguish osteosarcoma from benign and reactive lesions. The most serious problem in osteosarcoma is pulmonary metastasis, and it is still not possible to predict the metastatic potential of osteosarcoma in individual cases. An immunohistochemical approach using antibodies against such elements as type IV collagenase (gelatinase) [98–100] and integrin receptors [101], which play important roles in the process of metastasis, may enable us to solve that perplexing problem.

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16. Histologic subclassification of osteosarcoma: Differential diagnostic problems and immunohistochemical aspects

Shinichiro Ushigome, Kazuhito Nakamori, Takashi Nikaido, and Masayuki Takagi

Histologic subclassification of osteosarcoma and diagnostic problems

Histologic subclassification of osteosarcoma, with the exception of parosteal osteosarcoma [1–3], has not been regarded to be useful as a prognostic parameter. Nevertheless, it is now applied in routine surgical pathology. A generally accepted subclassification is the one by Dahlin and Unni of the Mayo Clinic [4,5] (Table16-1). Our experience is shown in Table 16-2. MFH-mimicking osteosarcoma, giant-cell-rich osteosarcoma, and dedifferentiation of intraosseous well-differentiated osteosarcoma are added.

Unni stated that the advent of intensive chemotherapy makes subclassification of osteosarcoma more important [5]. It is true that some osteosarcomas may be treated by surgery only and others may be very sensitive to chemotherapy. However, one does encounter osteosarcomas that are difficult to differentiate from other tumors or tumorlike conditions, such as malignant fibrous histiocytoma, giant cell tumor, osteoblastoma, Ewing's sarcoma, malignant lymphoma, aneurysmal bone cyst, fibrous dysplasia, etc. This applies especially to biopsy interpretation [6,7]. The recent advent of immunohistochemistry may be expected to give reliable objective findings on final histologic evaluation.

We first discuss the subtypes that are often seen in younger subjects and their problems in differential diagnosis or their significance as an entity. We then describe the usefulness of immunohistochemical application in tissue diagnosis of some varieties of osteosarcoma.

Intraosseous well-differentiated osteosarcoma

This was defined as a subtype by Unni et al. in 1977 [8]. The age of the patients ranges from 10 to 65 years [8], but most are in the third decade. Biopsy material may be easily misinterpreted as fibrous dysplasia or osteoblastoma. Minimal cytologic atypia of stromal cells, at least in some areas, is seen on careful observation (Figure 16-1). Complete resection of the involved portion is generally recommended because there is usually no development of local recurrence or metastasis with such therapy. Therefore,

G. Bennett Humphrey (ed.), OSTEOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS.
 ISBN 0-7923-1905-2

Table 16-1. Classification of osteosarcoma

Conventional osteosarcomas
Osteoblastic osteosarcoma
Chondroblastic osteosarcoma
Fibroblastic osteosarcoma
Osteosarcoma with epithelioid cells
Osteosarcoma with small cells
Osteosarcoma with giant cells
Osteosarcoma resembling osteoblastoma
Osteosarcoma of jaw bone
Osteosarcomas secondary to precursor lesions
Osteosarcoma as a portion of dedifferentiated chondrosarcoma
Telangiectatic osteosarcoma
Low-grade central osteosarcoma
Osteosarcomas predominantly involving the surface of bone
Parosteal osteosarcoma
Periosteal osteosarcoma
High-grade surface osteosarcoma

From Unni [5], with permission.

Table 16-2. Histologic subtypes of osteosarcomas and their distributions in subjects younger and older than age 20 in our series (1976–1990)

Subtypes	Age <20	Age >21
Conventional osteosarcomas		
Osteoblastic	32	9
Chondroblastic	5	4
Fibroblastic	2	1
Osteosarcoma of jaw	2	3
Telangiectatic osteosarcoma	2	3
Intraosseous, well differentiated	0	2
(dedifferentiation)	0	(1)
MFH mimicking osteosarcoma	4	1
Giant-cell-rich osteosarcoma	0	2
Osteoblastoma-like osteosarcoma	1	0
Postradiation osteosarcoma	0	3
Periosteal osteosarcoma	1	1
Parosteal osteosarcoma	0	5
High-grade surface osteosarcoma	0	1
Miscellaneous	0	1
Total	49	36

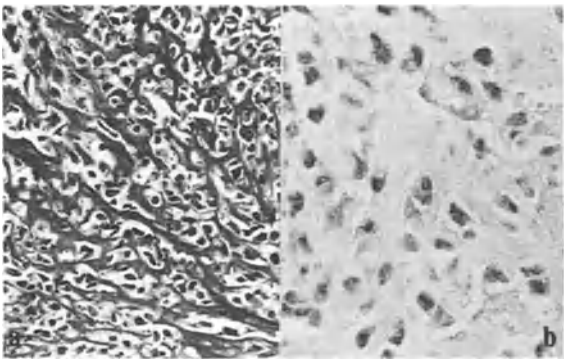

Figure 16-1. Histology of intraosseous well-differentiated osteosarcoma (28-year-old male, femur). In higher magnification tumor cells with atypical nuclei are seen (b, H&E. stain; a, ×200; b, ×800).

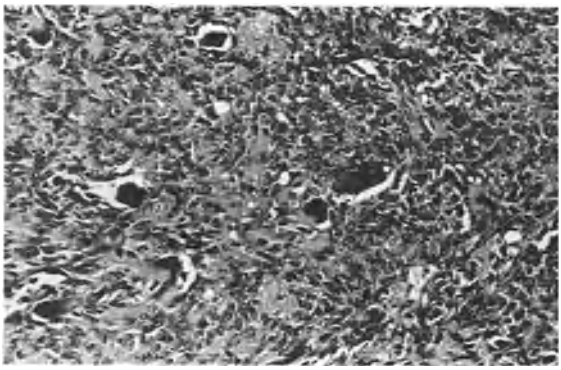

Figure 16-2. Dedifferentiation in well-differentiated osteosarcoma. Same case as Figure 16-1. See more cellular osteosarcoma with many multinucleated giant cells (H&E stain, ×200).

pathologists, radiologists, pediatric oncologists, and orthopedic surgeons should recognize this entity. However, we have seen an intraosseous well-differentiated osteosarcoma with well-circumscribed foci of dedifferentiation in a 28-year-old male (Figure 16-2) [9] who soon developed multiple bone-to-bone metastases. Dedifferentiation has not been reported before in this subtype, although it has been reported in parosteal osteosarcoma [10]. The presence or absence of dedifferentiation in intraosseous well-differentiated osteosarcomas should be considered in the selection of therapy. Recent progress in imaging techniques, particularly MRI, has resulted in the ability to recognize any dedifferentiated focus in a given case of well-differentiated osteosarcoma, either intraosseous or parosteal, prior to surgery.

Immunohistochemistry does not appear to be useful for the differential diagnosis of this tumor. However, it might be useful for the dedifferentiated portion in primary or metastatic foci.

Telangiectatic osteosarcoma

This subtype has become popular since first proposed by Matsuno et al. in 1976 [11], but there are still problems regarding the diagnostic criteria and the differential diagnosis. Microscopic foci of hemorrhagic and cystic changes may be seen in conventional osteosarcoma, and these changes in themselves are not diagnostic for the telangiectatic subtype. Radiological, gross, and microscopic features should be evaluated together [12,13]. The layered architecture characteristic of aneurysmal bone cyst [14] may also be seen in telangiectatic osteosarcoma. The presence or absence of atypia of stromal cells is important for discrimination.

The evaluation of the biologic behavior in this subtype is also under discussion. A worse prognosis was emphasized in the first report [11], but a recent study from the Mayo Clinic suggests essentially no difference from that of conventional osteosarcoma, because of a relatively high sensitivity to chemotherapy [15]. At present, the usefulness of immunohistochemistry for differential diagnosis has not been reported.

Osteosarcoma histologically simulating malignant fibrous histiocytoma (MFH)

Ballance et al. [16] reported six cases of osteosarcoma with predominant features simulating MFH and proposed this as a subtype of osteosarcoma in 1989. Mirra also recognized a subtype of MFH-like osteosarcoma [17], but it has not been generally recognized. We speculate that osteosarcoma with MFH-like features may be categorized as telangiectatic osteosarcoma or simply MFH.

We also emphasized careful differential diagnosis with true MFH using immunohistochemistry [18,19] (see below). True MFH of bone is now generally regarded as a clinicopathologic entity [20], usually occurring in adults in the fourth decade [3]. It may occur in teenagers or even in infants, but in those cases MFH has always to be distinguished from osteosarcoma or fibrosarcoma. If no definite tumor osteoid is seen in the biopsy specimen, the differential diagnosis between MFH and osteosarcoma must be carefully made. In adults, metastatic carcinoma with sarcomatoid changes or anaplastic pleomorphism, for example renal cell and lung carcinomas, may simulate MFH. Huvos et al. [20] pointed out that the lesion should be considered an osteosarcoma when osteoid or primitive bone, even one microscopic focus, is directly formed by the tumor cells. We support this concept, since in most of our cases minute foci of tumor osteoid were eventually found (Figure 16-3).

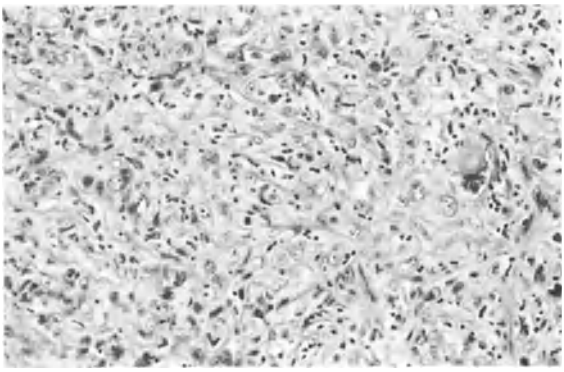

Figure 16-3. MFH-mimicking osteosarcoma (6-year-old male, femur). The tumor is predominantly composed of pleomorphic tumor cells simulating the features of MFH. Minute osteoid was seen in limited areas. (H&E stain, ×200).

When MFH-like features are predominant and only minute foci of tumor osteoid are seen in the resected specimen, the tumor is classified as MFH-mimicking osteosarcoma. In such a case lung metastases may show unequivocal osteoid production by tumor cells. The significance of a MFH-like component in osteosarcoma remains to be solved, although it may simply represent a sort of dedifferentiation.

Giant-cell-rich osteosarcoma

The designation of this entity as a subtype is still not settled. Osteoclastlike giant cells are often seen in osteosarcomas [5,20], but some of them show many and need to be differentiated from conventional or malignant giant cell tumors [21]. Unni classifies them simply as osteosarcomas with giant cells [5] and Mirra used the terms *giant cell tumor-like osteosarcoma* or *benign giant cell rich osteosarcoma* [17]. Unni mentioned a metastatic osteosarcoma with many giant cells simulating a benign giant cell tumor [5]. If definite foci of tumor osteoid are recognized in a tumor with giant cells, it should be categorized as an osteosarcoma [21,22].

Bathurst et al. [23] reported nine cases of osteoclast-rich osteosarcoma in young patients (average 16.5 years, younger than 20 in seven cases), presenting with lytic lesions involving the shaft of a long bone. In contrast, our four cases ranged in age from 28 to 46 (average 40.3 years) and were distributed in the epiphyseometaphyseal region of long bones (Figure 16-5).

When unequivocal tumor osteoid is not seen in a biopsy of a giant cell tumor with malignant stromal cells, the discrimination between malignant giant cell tumor or giant-cell-rich osteosarcoma becomes ambiguous. In such cases immunohistochemistry may be useful for the differential diagnosis (Figure 16-6).

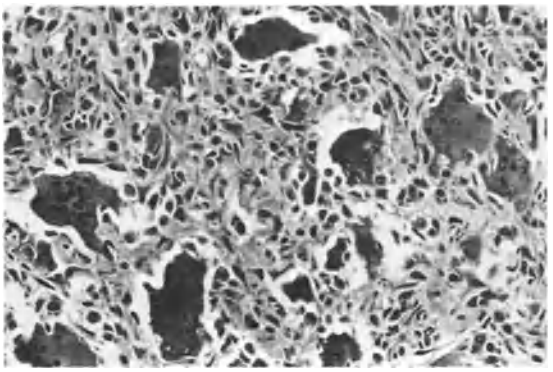

Figure 16-5. Giant-cell-rich osteosarcoma (distal femur). See fine tumor osteoid between atypical stromal cells and multinucleated giant cells (H&E stain, ×200).

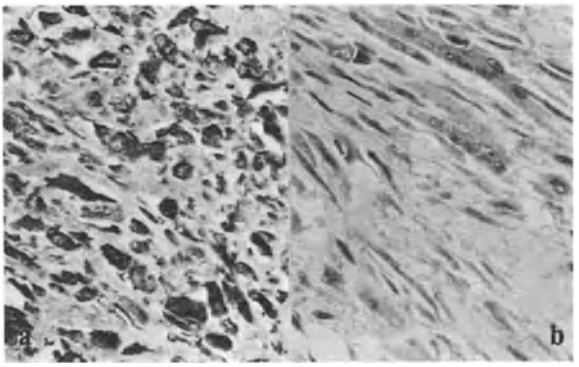

Figure 16-6. Immunoreactivity of giant-cell-rich osteosarcoma in the same case as in Figure 16-5. **a**: reactivity for BGP in stromal cells. **b**: reactivity for ALPase in stromal cells. (a,b ×400).

Small cell osteosarcoma

This is a unique subtype of osteosarcoma and was first defined by Sim et al. [24] and was further discussed by Ayala et al. [25] (Chapter 17).

Osteosarcoma with epithelioid features

Reports of osteosarcoma with epithelioid features simulating undifferentiated carcinoma are rare [5,7,26], and we have seen only one case (Figure 16-7). When tumor osteoid is scanty, the tumor might be misinterpreted as metastatic carcinoma and immunostaining with osteoblast and epithelial markers is useful.

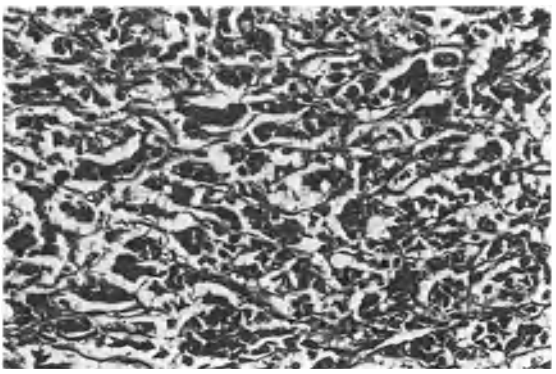

Figure 16-7. Osteosarcoma with epithelioid features (26-year-old male, vertebra). Alveolar structures of tumor cells are mimicking carcinoma. Definite tumor osteoid was seen in other portions (H&E stain, ×200).

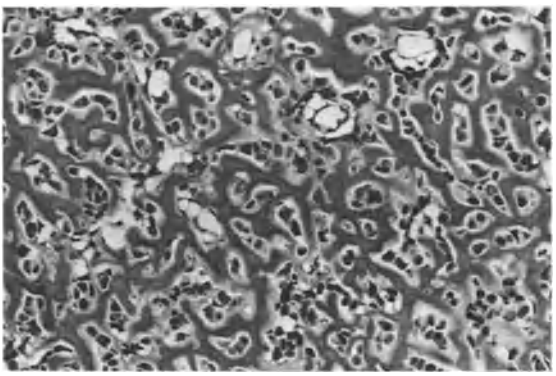

Figure 16-8. Osteoblastoma-like osteosarcoma (12-year-old female, proximal femur).

Osteoblastoma-like osteosarcoma

One may encounter osteosarcomas that histologically resemble osteoblastoma, especially in the spine [5]. Seventeen patients with osteosarcoma of this type have been reported by Bertoni et al. [27] who ranged in age from 11 to 58, and half of them were younger than 20. Permeation of surrounding tissue and lack of "maturation" toward the edge were emphasized in distinguishing osteoblastoma-like osteosarcoma from osteoblastoma [27]. Anaplasia of tumor cells is naturally important for the discrimination (Figure 16-8). However, the diagnosis is always difficult in a biopsy. No useful immunohistochemical study for the tumor has been reported.

Table 16-3. Histologic discrimination of tumor osteoid from other fibrous matrix

	Malignant osteoid	Reactive osteoid	Collagenous matrix
Shape	Irregular, Lacy Diffuse	Strap shaped Sausage shaped	Fiberlike, linear Bandlike
Border	Generally distinct	Distinct	Generally indistinct, sometimes distinct
Stainability			
H&E	Relatively dense	Relatively dense	Less dense
Masson	Deep blue	Deep blue	Less deep blue
Structure	Woven	Woven	Fibrillary, often wavy
Distribution	Poorly oriented	Well oriented, at periphery	Poorly oriented
Tumor cells	Closely related	Not related	Related, along between
Rim	Rimmed by atypical cells	Rimmed by non-neoplastic osteoblasts	No rimming
Lacunae	May be seen	Often seen	None
Cartilage	May be associated	May be associated	Never associated
Calcification	May be seen	May be seen	Usually none

Surface osteosarcoma

Three types of surface osteosarcoma, proposed by the Mayo Clinic, are now generally accepted, i.e. parosteal, periosteal, and high-grade surface osteosarcoma [4–6,28,29]. In addition, dedifferentiation of parosteal osteosarcoma has been reported [10]. Periosteal [30] and high-grade surface osteosarcoma [31] may be seen in children and adolescents, but both subtypes are rare. Radiological as well as histological features are important for a conclusive diagnosis. Immunohistochemical studies cannot be expected to be useful for the differential diagnosis.

Histologic differential diagnosis between tumor osteoid and simple hyalinized fibrous matrix

Evaluation of tumor osteoid (malignant osteoid) and stromal cells is essential for a conclusive diagnosis of osteosarcoma. Recognition of even minute foci of tumor osteoid is significant to make the diagnosis of osteosarcoma. On the other hand, MFH frequently shows a prominent fibrous matrix with hyalinization, either extensively or focally. Since true MFH of bone is rather uncommon in children, the histological distinction between osteosarcoma with prominent MFH-like features and MFH is particularly important but may be difficult.

Table 16-3 summarizes histologic differential points between malignant osteoid, reactive osteoid, and simple collagenous matrix. In cases when tumor osteoid is not definite in open or needle biopsy specimens but osteoblast

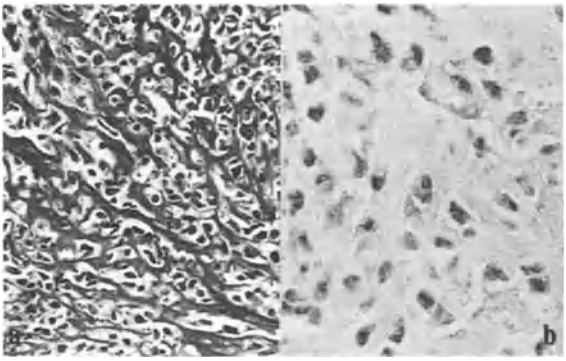

Figure 16-9. Osteoblastic osteosarcoma (20-year-old female, femur) BGP is positively demonstrated in tumor cells (b) (a, H&E stain ×200; b, BGP ×400).

markers mentioned below are positively demonstrated in tumor cells, the possibility of osteosarcoma is high, and even minute tumor osteoid should be found. In our experience Masson trichrome stain is more useful than H&E. stain for the detection of fine tumor osteoid.

Application of immunohistochemistry for the differential diagnosis

We reported that osteocalcin (bone Gla protein, BGP) [19], one of the non-collagenous proteins, and alkaline phosphatase (ALPase) are reliable markers for either neoplastic or non-neoplastic osteoblasts, using immunohistochemistry in paraffin section [18]. Adequate fixation and decalcification are important in order to obtain good immunoreactivity. In our study good results were obtained in 10% buffered formalin fixation and decalcification in 4°C using Plank-Rychlo's solution. Using this technique, BGP and ALPase were often demonstrated in osteoblastic (Figure 16-9) and fibroblastic (Figure 16-10) osteosarcoma, but not in MFH and fibrosarcoma (Table 16-4). Such so-called histiocytic markers as lysozyme and alpha-1-antichymotrypsin were positively demonstrated not only in MFH but also in osteosarcoma, while they were not demonstrated in fibrosarcoma (Table 16-4).

Osteosarcoma vs. MFH

In our study, osteosarcomas with predominant fibroblastic components or MFH-like features, BGP, and ALPase were positively demonstrated in tumor cells (Figure 16-4), while MFH or fibrosarcoma cells were negative. Ballance et al. [16] emphasized positive reactivity for alpha-1-antichymotrypsin and lysozyme in tumor cells of osteosarcoma of the MFH subtype, but no reactivity for these histiocyte markers in conventional osteosarcoma. In contrast, our

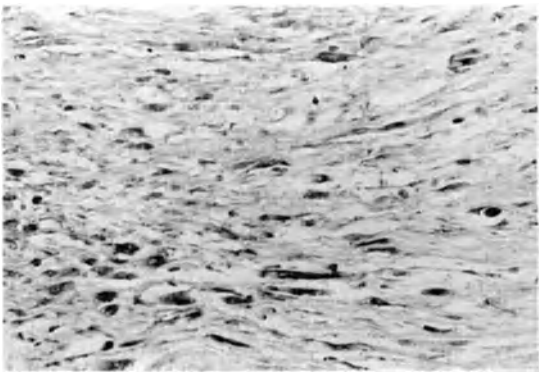

Figure 16-10. Fibroblastic osteosarcoma (16-year-old male, tibia) BGP is positively seen in spindle tumor cells (×200).

Table 16-4. Immunoreactivity of osteosarcoma, MFH, and fibrosarcoma cases

Subtypes	BGP	ALPase	Alpha-1-ACT	Lysozyme
Osteoblastic O-S	+20/21	+9/10	+10/13	−10/13
Fibroblastic O-S	+5/5	+3/3	ND	ND
MFH-mimicking O-S	+5/7	+6/7	−2/3	+2/3
Giant cell rich O-S	+4/4	+3/3	+3/3	−3/3
Fibrosarcoma	−3/3	−2/2	−2/2	−2/2
MFH	−16/16	−11/16	+11/16	−13/16

O-S = osteosarcoma; MFH = malignant fibrous histiocytoma; BGP = osteocalcin; ACT = antichymotrypsin; ND = not done; − = negative reactivity; + = positive reactivity.

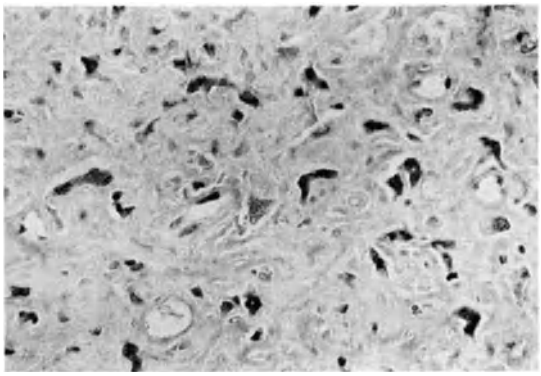

Figure 16-4. BGP in tumor cells of same case as Figure 16-5. Tumor cells revealed a positive immunoreactivity for BGP (×400).

study disclosed positive immunoreactivity for such markers, even in cases of conventional osteosarcoma [18] (Table 16-4) and carcinoma cells with sarcomatoid changes.

Fibroblastic osteosarcoma vs. fibrosarcoma

In cases in which tumor osteoid is obscure in biopsy material, an immunohistochemical approach with osteoblast markers may be very useful. BGP and ALPase were demonstrated in spindle cells of most fibroblastic osteosarcomas (Table 16-4) but not in those of fibrosarcomas [18]. In two cases with fibroblastic malignancies that necessitated the differential diagnosis of fibrosarcoma, MFH, and fibroblastic osteosarcoma, the tumor cells disclosed immunoreactivity for BGP (Figure 16-10), and fine lace-like tumor osteoid was found on careful observation.

Giant-cell-rich osteosarcoma vs. (malignant) giant cell tumor

BGP was demonstrated in four and ALPase in 3 of 4 cases of giant-cell-rich osteosarcoma (Figure 16-6; Table 16-4). These markers are generally not demonstrated in giant cell tumors. Alpha-1-antichymotrypsin, KP-1 (=CD68, a macrophage/myeloid marker) and Factor XIIIa were not useful for the differential diagnosis [18].

Small cell osteosarcoma vs. Ewing's sarcoma or malignant lymphoma

We do not have any experience of immunohistochemistry of BGP and ALPase in small cell osteosarcoma. Theoretically, leucocyte common antigen and other lymphoma cell markers (L26, UCHL-1, etc.) are not expected in small cell osteosarcoma. Neural markers such as neuron-specific enolase, neurofilament proteins, and Leu-7, often demonstrated in Ewing's sarcoma and related tumors [32–34], are also supposedly negative in small cell osteosarcoma.

Osteosarcoma with predominant epithelioid features

In our case of osteosarcoma with epithelial arrangement of tumor cells, BGP was positively demonstrated in tumor cells, but keratin and EMA were negative. The case described by Yoshida appeared positive for ALPase [26]. Therefore, immunohistochemistry may be useful for the differential diagnosis.

Conclusions

Although osteosarcomas are generally of high-grade malignancy and may show histologic diversity, some subtypes are known to have a better prognosis.

The selection of treatment and the evaluation of the outcome should be based on correct tissue diagnosis in biopsy material, because tissue diagnosis in resected specimen may be made impossible by recent intensive therapy.

The usefulness of immunohistochemistry with osteoblast markers was emphasized for the differential diagnosis of some subtypes of osteosarcoma.

Acknowledgments

This study was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan, and the Vehicle Racing Commemorative Foundation, Tokyo, Japan.

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17. Small cell osteosarcoma

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A. Kevin Raymond, and Jack Edeiken

Introduction

Small cell osteosarcoma (SCO) is a rare but distinct variant of osteosarcoma. Although Hutter et al. [1] in 1966 and Jacobson in 1977 [2] described small cell tumors of bone capable of differentiating into bone and cartilage, Sim and collaborators [3], reporting in 1979 on 24 patients at the Mayo Clinic, were the first to delineate the clinicopathological features of this entity. Further clarification was given by other investigators [4–7], but few large series of patients with these lesions have been studied [3–7], and there have been only several case reports [8–10] of these lesions.

In this chapter we have compiled the major aspects of 73 SCO cases accrued from the major series including our experience [3–7] and a few case reports [8–10].

Incidence

In several large series, the incidence of SCO has been reported to vary from 1.1% to 4.0% [3,6,7]. The incidence of SCO has been reported to be the lowest among the variants of osteosarcoma [11].

Age

Although the age at presentation ranges from 6 to 83 years, most patients experience this disease in the second decade of life [3–10], which is not different from that of conventional osteosarcoma [11]. The age distribution of the 73 cases reported in the literature is presented in Figure 17-1.

Gender

Among the 73 patients reported, 40 were female and 33 male [3–10], the slight shift in distribution to the female gender contrasting with the higher incidence of conventional osteosarcoma among males [11].

G. Bennett Humphrey (ed.), OSTEOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS.
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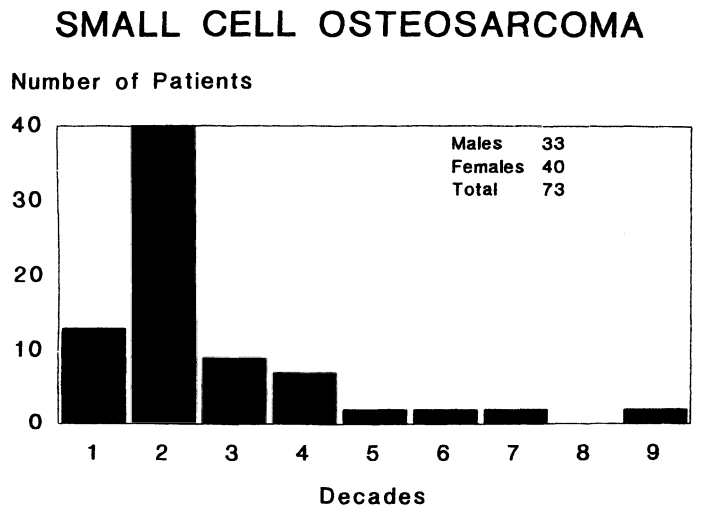


Figure 17-1. Age in decades of 73 patients.

Race

The SCO patients' race was reported in only one of the series [6], in which the patients were predominantly white (19 of 27), with five blacks and three Hispanic patients. Race becomes an important clinical factor in the differential diagnosis of small cell osteosarcoma from Ewing's sarcoma because the latter tumor is almost never seen in black persons [12].

Skeletal distribution

The lesions' skeletal distribution is shown in Figure 17-2. By far the most common location was the metaphysis of the long bones. In 24 of 73 patients the distal end of the femur was affected followed by nine patients whose lesions were in the proximal tibia [3–10]. In three patients, the mandible was the site of occurrence; this is a relatively high incidence when the total number of SCO patients is considered. Although one patient in the Sim et al. [3] series had two simultaneously occurring lesions, SCO is a unicentric disease.

Symptoms and signs

Pain and swelling of the affected areas were the most common symptoms. In the Mayo Clinic series [3], all patients presented with pain but only eight developed swelling. In many patients pain seemed to be the first manifestation [7]; the duration of symptoms was relatively short, usually from a few weeks to several months, and most patients had symptoms for less than 1 year [3–7]; however, Sim et al. [3] reported three patients whose symptomatology lasted

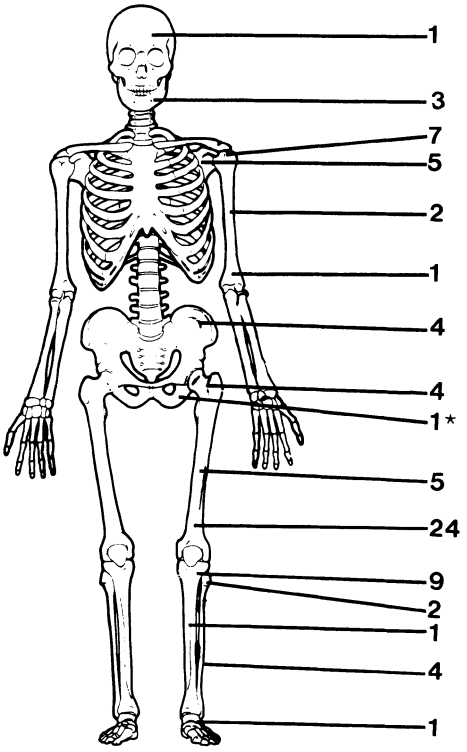


Figure 17-2. Skeletal distribution of 74 lesions. * Note that one patient in the Sim et al. [3] series had two lesions, one in the femur and one in the ischium.

3 years and one with 4 years of symptoms. One patient reported by Roessner et al. [9] also experienced nearly 4 years of symptomatology.

Pathologic features

In gross appearance the tumors resembled conventional osteosarcomas. They ranged in size from 4 to 20 cm at their greatest diameter [3,7] and were described as masses involving both the medullary cavity and cortex, often with an extraosseous component. Histologically, small cell osteosarcomas contain undifferentiated small cells and osteoid [3,7]; small cells range in size from three to five times that of a mature lymphocyte, with nuclear characteristics described as having three different appearances [6]: Ewing's-like, large cell lymphoma-like, and small, short spindle cells. The Ewing's-like type, the most common, is characterized by relatively monotonous round-to-oval nuclei (three to four times larger than a small lymphocyte) that have a fine nuclear chromatin pattern with a few small nucleoli. The amount of cytoplasm is

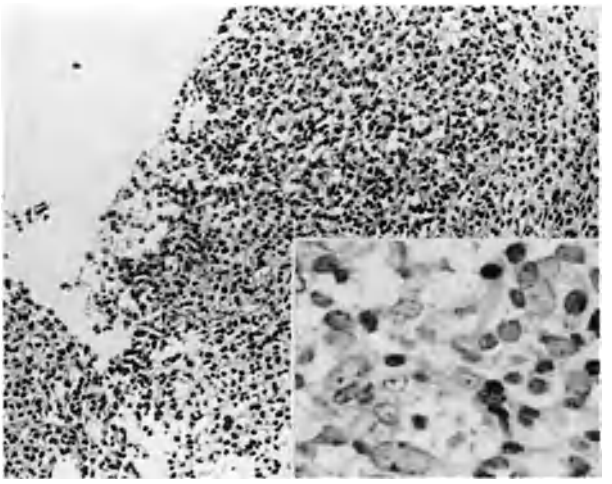


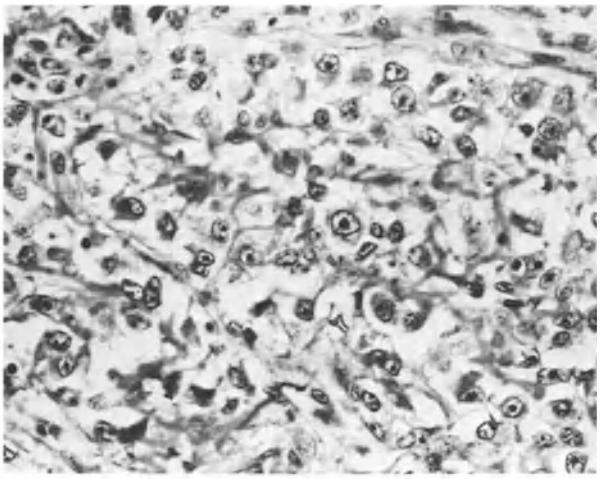
Figure 17-3. Needle biopsy demonstrates diffuse proliferation of small round cells mimicking Ewing's sarcoma (H&E, ×100). Insert displays round-to-oval nuclei with a fine chromatin pattern and small nucleoli (H&E, ×300).

relatively small and cell membranes are generally obscured by cell overlapping; mitoses as well as areas of necrosis may commonly be found (Figure 17-3). The lymphoma-like type shows larger nuclei, about four to five times the size of a lymphocyte and, in addition to a fine nuclear chromatin pattern, the nucleoli are large and prominent (Figure 17-4). The short spindle-cell type generally has tumor cells with a denser chromatin pattern and indistinct and scant cytoplasm (Figure 17-5). These cell types may be associated with a hemangiopericytic type of arrangement [3]. Interestingly, the lymphoma-like appearance, though not mentioned by Martin et al. [4], was depicted in Figure 5 of their review.

Osteoid production, a *sine qua non* condition for diagnosing SCO (Figure 17-6), is usually lacelike and may be difficult to find, especially in tissue from small or needle biopsiès. It is not unusual for an SCO to have large areas totally made up of pure small cells without osteoid formation. Under higher magnification, however, osteoid is invariably present—without it a diagnosis of small cell osteosarcoma cannot be made. In our group's experience [6], the osteoid in lymphoma-like SCO is much easier to find than in the Ewing's-like SCO pattern. Broad bands of calcified osteoid may be seen in some of the tumors. In fact, some of the tumors have large areas with broad bands of bone or osteoid associated with small-cell proliferation.

Glycogen has been found in SCO by several research groups [3,4,6,7,9] using the periodic acid Schiff technique with and without diastase treatment in more than half of the cases.

A recent article on ultrastructure of SCO by Dickersin and Rosenberg [13]



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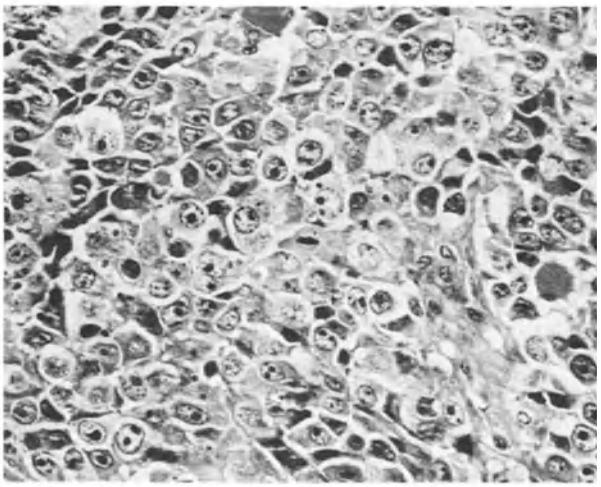

B

Figure 17-4. Large cell lymphoma-like pattern. **A**: Cells shows nuclei with large prominent nucleoli; interlacing bands of osteoid are shown in background (H&E, ×300). **B**: Solid area shows similar cells but without osteoid (H&E, ×300).

has shown that the most commonly found features of this tumor include small cells with a high nuclear cytoplasmic ratio, numerous cytoplasmic ribosomes and mitochondria, small junctions, and envelopment of individual and groups of cells by matrix. Dilated rough endoplasmic reticulum, many polyribosomes, and lysosomes may be found, as well as intercellular spaces containing collagen fibrils and amorphous dense deposits that have been

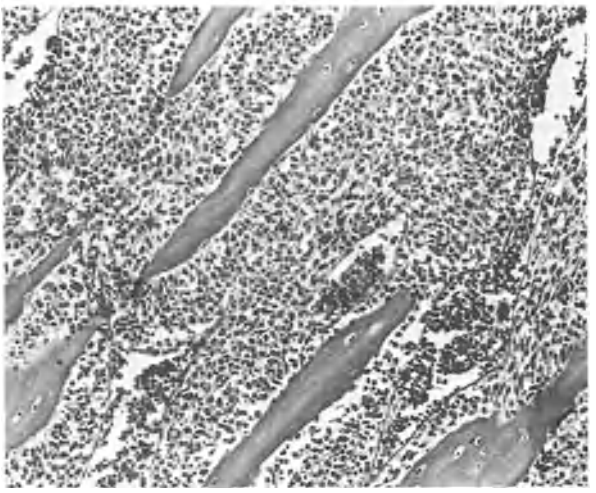

Figure 17-5. Small spindle and round cell situated between trabeculae of mature bone (H&E, ×160).

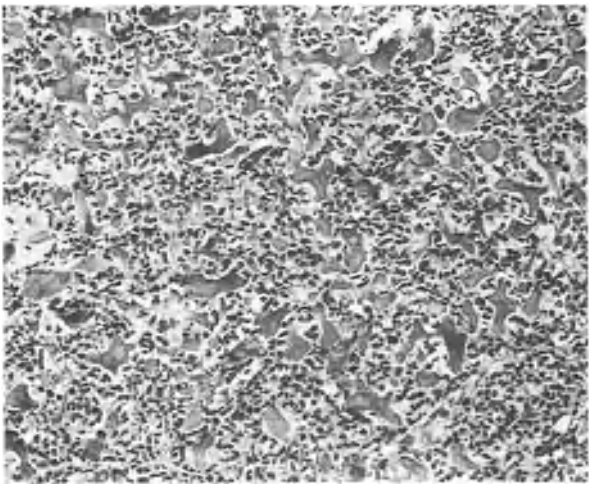

Figure 17-6. Abundant osteoid production by small cells (H&E, ×160).

described as nonpolymerized collagen [14,15]. When Ringus et al. [15] compared 10 cases of Ewing's sarcoma with their case of SCO, they found the SCO cells slightly larger and having a greater amount of rough endoplasmic reticulum than those of Ewing's sarcoma; intercellular amorphous material was present only in the SCO.

Although there are no pathognomonic ultrastructural findings in SCO,

Dickersin and Rosenberg [13] believe that most small-cell neoplasms can be ruled out based on the electron microscopic findings.

Recently, Noguera et al. [10] reported a chromosomal translocation involving chromosomes 11 and 22 in a patient with SCO. This 11; 22 translocation is known to occur in neuroectodermal tumors, such as Ewing's sarcoma/peripheral neuropithelioma, Askin tumor, esthesioneuroblastoma, and neuroendocrine carcinoma of the small intestine [10]. Since this chromosomal abnormality is identical in Ewing's sarcoma and SCO, Noguera and collaborators believe that these two bone tumors may be related [10]. This is an interesting observation that, deserves further confirmatory studies.

Radiologic features

Edeiken et al. [16] described the radiological features on 13 patients at the M.D. Anderson Cancer Center, and these cases were included in our subsequent report [6]. Radiologically, SCO is not different from conventional osteosarcoma; it may be a totally permeative lesion (Figure 17-7), but the majority of cases show a mixed lytic and blastic pattern (Figure 17-8). These facts should be remembered because when a small biopsy shows only small cells without osteoid, the radiologic features should alert the examining pathologist to the possibility of an SCO. The lesions are generally large, involve the metaphysis, and may extend into the epiphysis; most lesions break through the cortex with a soft tissue component, which may be totally lytic or may contain matrix formation.

Differential diagnosis

SCO must be differentiated from other small cell tumors, including Ewing's sarcoma, large cell lymphoma, and mesenchymal chondrosarcoma. Since the differential diagnosis of SCO may be difficult, clinical radiological and pathological findings need to be put together to arrive at its diagnosis.

Ewing's sarcoma may be extremely difficult to differentiate from SCO because, radiologically, both tumors may show a permeative pattern and both may have a blastic component in the medullary cavity. Production of matrix outside the bone, however, is seen only in osteosarcoma. Although a predominantly permeative pattern without matrix production may occur in SCO, its occurrence is rare. The lesions may be predominantly lytic, but in most series the predominant radiographic presentation was of a mixed lytic-blastic type [4,6,7].

From the histologic point of view, SCO with the Ewing's-like pattern may not be differentiated from Ewing's sarcoma unless one finds osteoid [6]. If osteoid is not present, the examining pathologist should always look for radiographic parameters of osteosarcoma. Glycogen stain does not help to

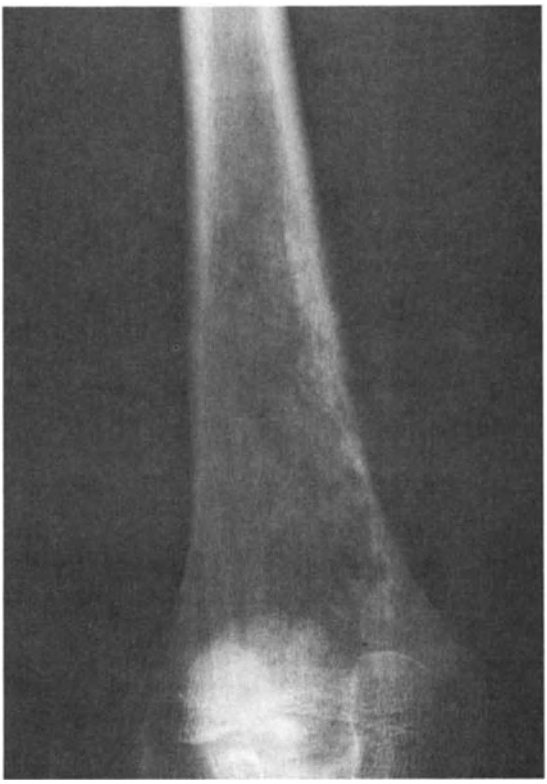

Figure 17-7. Metaphysis of distal femur shows a diffuse permeative lesion involving medullary cavity, breaking through cortex and extending into soft tissue.

differentiate SCO from Ewing's sarcoma because both tumors may contain it. Electron microscopic findings may be helpful but not conclusive. If a small cell sarcoma shows peripheral neuroectodermal differentiation including cytoplasmic processes and neurosecretory granules, the tumor should be ruled out from the SCO category [17]. Although neuron-specific enolase has been reported to be positive in the cells of Ewing's sarcoma/peripheral neuroectodermal tumor and such a finding may point away from SCO, we are not aware of any studies of neuron-specific enolase in SCO.

Large cell lymphoma of bone (LCLB) shares radiologic characteristics with SCO and Ewing's sarcoma, and may be difficult to differentiate from SCO. LCLB may be difficult to differentiate from SCO. LCLB may be seen in patients during all decades of life but is more common in adulthood at a mean age of 46.1 years [18,19], in contrast to SCO, which has a peak incidence in the first and second decades of life. A good histologic preparation may be sufficient to differentiate SCO from LCLB because osteoid is not difficult to find in the former. In difficult cases, however, an immunohistochemical study

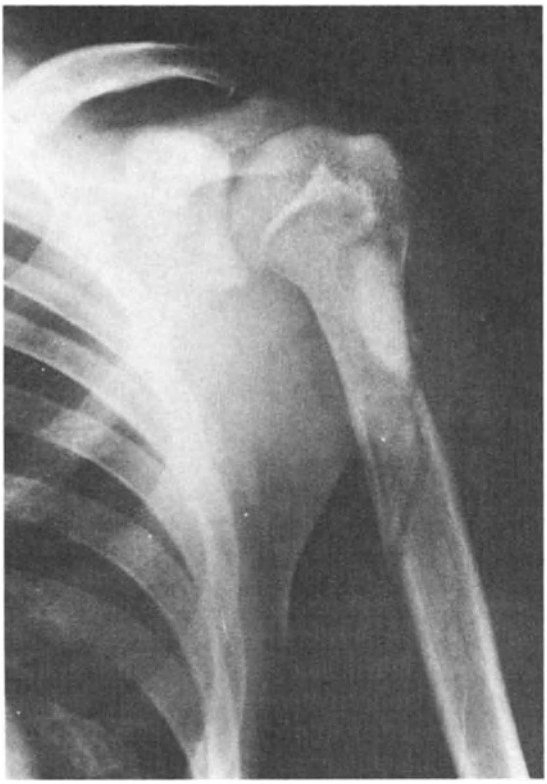

Figure 17-8. Radiograph of humerus shows blastic and lytic components. The pathologic fracture courses through the permeative component.

with leukocytic common antigen and Ki-1 should differentiate the two tumors, as LCLB is usually positive for one or both stains [20,21].

Mesenchymal chondrosarcoma (MC) may pose another differential-diagnostic problem. MC is predominantly seen in patients during the second and third decades of life, and, although it may affect nearly any bone, the distal femur location is rare and was found in only four cases in a series of 11 patients [22]. Radiologically, MC resembles ordinary chondrosarcoma of bone, with osteolytic and destructive features showing stippled calcification [22]. Histologically, MC may be confused with SCO when the biopsied tumor tissue is scanty. MC is characterized by sheets of undifferentiated small cells alternating with zones of differentiated cartilaginous tissue. The small cells may have oval to spindle or round nuclei, and they often are arranged in a hemangiopericytic pattern. The cartilaginous component forms islands of mature tissue or may have a low-grade malignant appearance. Osteoid may be seen as part of the chondroid matrix maturation, but the lacelike osteoid typical of osteosarcoma is not seen [22].

Management

Management of patients with SCO has varied from institution to institution [3–10]. Since these studies dealt with the use of different modalities of treatment, the impact of therapy is difficult to evaluate. The primary lesion seems to be sensitive to chemotherapy [6] or radiation therapy [5], but metastatic disease is difficult to control [6]. It seems, therefore, that despite aggressive chemotherapy for SCO, the results are at best the same or slightly worse than those obtained in treating ordinary osteosarcoma [22].

Currently at the M.D. Anderson Cancer Center, the basic therapy for SCO in adult patients includes i.a. CDDP and systemic ADR [23]. Recently, we began strongly to consider the addition of ifosfamide with or without HDM if the tumor's response to the above therapy is not optimal or if the lesion is located in a flat bone. Children, after completing several courses of i.a. CDDP and surgery, are given systemic chemotherapy with Adriamycin, vincristine, ACT-D, and CTX (A-VAC) [24].

In summary, SCO is a rare variant of osteosarcoma that affects the methaphysis of the long bones, especially the distal femur of children and young adults, and it is slightly more common in females than males. Because of the relatively small number of cases reported and the diversity of treatment regimes, additional prospective studies will have to be done to learn more about this disease. We believe, however, that chemotherapy is the primary treatment for this tumor.

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18. Current status of DNA cytometry in osteosarcoma

Henrik C.F. Bauer

Introduction

Modern treatment of osteosarcoma, based on surgery and adjuvant chemotherapy, has improved the survival rate from less than 20% to more than 50%. Ideally, the treatment should be based upon the individual patient's risk of metastasis and the tumor cells' sensitivity to chemotherapy. However, clinical and histopathologic criteria are of limited prognostic value [1]. Osteosarcoma commonly exhibits histologic features indicative of high-grade malignancy [2,3], but there is a great morphologic variability among the tumors, sometimes even within an individual lesion. This heterogeneous morphology of osteosarcomas may cause diagnostic problems, including confusion with benign bone tumors, such as osteoblastoma and aneurysmal bone cyst [4–8]. Hence, there is a need for better characterization of this tumor entity.

Cytometric DNA studies have shown that for several tumor types there is a relationship between cellular DNA content and clinical course [9–11]. In general, a diploid DNA content is associated with a more favorable prognosis than an abnormal (nondiploid) DNA content. In chondrosarcomas and soft tissue sarcomas, the proportion of nondiploid lesions increases with increasing histologic malignancy, but DNA content has been the stronger prognostic factor [12–16]. The purpose of this chapter is to review the background, methodology, and clinical application of DNA analysis in osteosarcoma.

Background

The normal human nonproliferating cell is characterized by an invariable DNA content, corresponding to 46 chromosomes. It is referred to as euploid or *diploid.* Cytogenetic studies have shown that neoplastic cells may be characterized by structural and/or numerical chromosome changes. When the numerical abnormalities are sufficiently pronounced, they can be detected by quantitative DNA cytometry. With this technique, cell populations can be detected that have a DNA content that is at least 10% lower or higher than normal cell populations. Such abnormal cell populations are called *nondiploid.*

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 ISBN 0-7923-1905-2

Although nondiploidy, in general, signifies high-grade malignancy, all diploid cell populations are not necessarily normal. There are certain tumor entities that are diploid but of high-grade malignancy. For instance, the majority of Ewing's and synovial sarcomas exhibit specific chromosomal aberrations that do not lead to an increased DNA content [17–19]. Hence, these lesions are diploid but highly malignant.

Another common feature in neoplasia is increased growth rate, as reflected by a high fraction of cells in the S and G_2+M phases of the cell cycle. The fraction of proliferating cells in a normal growing cell population may at the extreme reach 15%. Higher values can be regarded as reflecting neoplastic abnormality. Cell populations that have a high growth fraction are commonly sensitive to chemotherapy and radiotherapy.

Methodological aspects

Cellular DNA analysis is based on either absorbence or fluorescence of dyes specifically bound to DNA, providing a direct relation with the DNA content of the measured cell. However, the method gives only the relative DNA content, not the absolute amount of DNA. Therefore, the DNA content of the measured tumor cells has to be related to a normal control cell population, such as lymphocytes or fibroblasts. The DNA content of a cell population is given in relative values, where DNA Index (DI) 1.0 denotes diploidy [20].

Basically, there are two means, both optical, for determining cellular DNA content: microspectrophotometry and flow cytometry. Notably, both techniques can be applied to cells regardless of whether DNA is present in chromosomal or dispersed form. For a detailed discussion of methodological problems associated with DNA cytometry of mesenchymal tumors, see Kreicbergs review [21].

Microspectrophotometry (MSP)

MSP is based on static measurement of DNA absorbence of cell nuclei stained with pararosaniline, the Feulgen stain [22]. MSP can also be applied to fluorescein-stained nuclei, a technique that may become more popular with the recent development of rapid slide cytofluorometers.

MSP is applied to cells on slides under visual control. Routinely, 10–50 normal cells are measured to obtain the diploid reference value. Subsequently, 100–200 tumor cells are analyzed and their DNA content is related to that of the control cell population. MSP can be applied to both imprint preparations and tissue sections. The advantage of the former is that whole cell nuclei are measured. With tissue sections an artifact of measuring partly sectioned nuclei is introduced. Due to this artifact, the exact DI cannot be determined with MSP of tissue sections [23]. However, the major advantage is that it offers DNA analysis of tissue with preserved architecture. Thus, cells from different

tissue areas of interest can be selected for measurement. This may be especially important in osteosarcoma, which commonly exhibits histologic heterogeneity.

Flow cytometry (FCM)

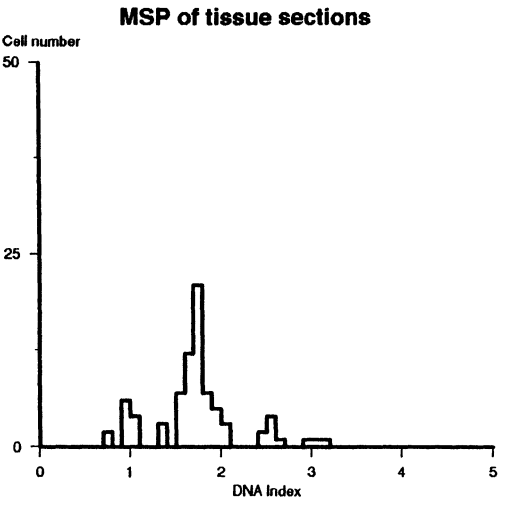
FCM is based on dynamic measurement of a suspension of fluorescein-stained cells [24]. The cell suspensions are prepared from solid tissue by mechanical and chemical disaggregation. Hence the tissue architecture is destroyed by the preparation technique. The cells in suspension are then stained with a fluorescent DNA dye, commonly ethidium bromide or propidium iodide. Before DNA measurement, the preparation must be treated with RNAase, since these dyes also stain RNA. The cell suspension is measured in a flow cytometer equipped with a mercury lamp or laser. As the cells, one by one, pass the light beam the fluorescence is excited and the emitted light is recorded by a photomultiplier. The fluorescence intensity is directly related to the DNA content of each cell. Trout or chicken red blood cells and human lymphocytes are measured simultaneously for control of the normal DNA content.

In studies of sarcoma, requiring long follow-up periods to be conclusive, retrospective studies using archival paraffin-embedded material are often preferable to prospective studies. Previously, only MSP of tissue sections could be applied to archival specimens, which have been shown to retain adequate Feulgen DNA stainability [25,26]. However, techniques for the preparation of cell suspensions from paraffin-embedded specimens by dewaxing and disaggregation have now been described [27].

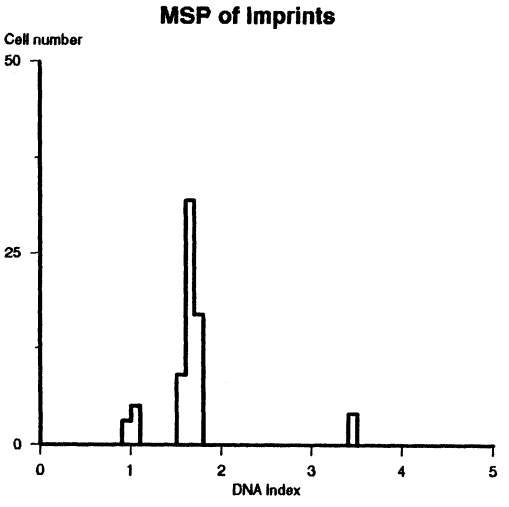
Ploidy classification

Both MSP and FCM provide histograms of the relative DNA content of the cells in the measured sample. Tumors that exhibit cells with the same DNA content as the normal controls are called *diploid* (DI 1.0). Tumors exhibiting cell populations with a DNA content different from the normal control are called *nondiploid.* Nondiploid tumors can be either *tetraploid* or *aneuploid.* Tetraploid lesions have a DNA content exactly double the normal DNA content, i.e., DI 2.0. Aneuploid lesions are those exhibiting a DNA content that is not a multiple of the diploid DNA content. Exact ploidy determination is feasible from DNA histograms obtained by FCM or by MSP of imprint preparations. In MSP of tissue sections, the methodological error of measuring sectioned nuclei makes exact determination of the DI unreliable. However, the method permits conclusive discrimination between diploid and nondiploid bone tumors, based on the proportion of cells with an abnormal DNA content [23] (Figure 18-1).

For most practical purposes it is sufficient to distinguish between diploid and nondiploid lesions. However, it has been reported in genitourinary nondiploid lesions that tetraploid variants are associated with a better prognosis than aneuploid ones [11].


MSP of tissue sections
Cell number
50
25
0
0
1
2
3
4
5
DNA Index

A


MSP of Imprints
Cell number
50
25
0
0
1
2
3
4
5
DNA Index

B

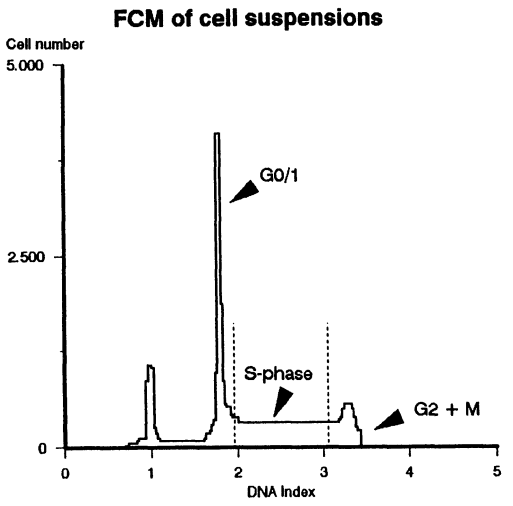


Figure 18-1. **A–C**: Schematic DNA histograms of a nondiploid osteosarcoma analyzed by MSP (A and B) and FCM (C) showing DNA index 1.7. The cell-cycle fractions of the nondiploid cell line are shown in the histogram obtained by FCM.

Table 18-1. Methodological differences between MSP and FCM

	MSP	FCM
Number of cells	100–200	>10,000
Visual control	Yes	No
Selection of different histologic areas for measurement	Yes	No
Speed	30 min	5 min
Archival tissue	Yes	Yes
Exact determination of DNA index	No	Yes
Determination of growth fraction	No	Yes
Multiparameter	?	Yes

The choice of relying on MSP or FCM is difficult. Comparative studies based on osteosarcoma have shown that there is a good correlation with regards to the discrimination between diploid and nondiploid lesions [23,28]. Hence either MSP or FCM should suffice for routine DNA analysis for clinical purposes, but there are several reasons to apply both techniques (Table 18-1). The advantage of FCM is the speed of the analysis and the exact determination of the DI of aneuploid peaks. FCM also permits assessment of growth fractions. However, the analysis is made without visual control of the measured specimen. A diploid DNA histogram may be the result of measurement of an unrepresentative tumor specimen. Hence, diploid tumors should

Table 18-2. Comparative DNA analysis of MSP and FCM of bone and soft tissue tumors

	Microspectrophotometry				
Flow cytometry	Diploid	Tetraploid	Aneuploid	Inconclusive	Total
Diploid	58	3	8	8	
Teraploid	—	2	—	1	
Aneuploid	—	—	41	2	
Inconclusive	9	1	9	18	37
Total				29	160

always be reassessed by MSP to ascertain that the analysis is based on representative tumor cells.

During the last year we have applied both MSP and FCM to bone and soft tissue tumors. The proportion of inconclusive DNA measurements is considerably reduced when both techniques are applied (Table 18-2). Based on a series of 160 tumors, MSP was inconclusive in 18% of cases, FCM in 23% but only 11% were inconclusive with both techniques. Furthermore, all lesions that were diploid according to MSP were also diploid by FCM. Eleven of 69 lesions that were diploid by FCM were nondiploid according to MSP. These findings emphasize the importance of using both techniques for routine DNA analysis. Especially diploid lesions, as assessed by FCM, should be reevaluated with MSP.

Clinical application

Diagnosis

In early studies of osteosarcoma analyzed by FCM and MSP, all high-grade osteosarcomas were found to be nondiploid [29,30]. Parosteal low-grade variants were diploid. Collective data of subsequent studies (Table 18-3) showed that a small fraction of high-grade osteosarcomas is diploid [31–35]. On the other hand, all parosteal osteosarcomas and all benign bone tumors are diploid. Hence, among bone-forming tumors, nondiploidy is only seen in high-grade osteosarcoma.

In our study of osteosarcoma, there were 17 cases that had caused diagnostic difficulties as to being bone tumors or osteosarcomas [36]. DNA analysis of these 17 cases showed that seven were diploid and 10 nondiploid. None of the seven patients with diploid lesions had local or distant metastases. Among the 10 with nondiploid lesions, eight developed local recurrences, three had died of tumor, and one was alive with lung metastases. Thus in this group of patients, where differential diagnostic problems as to being benign

Table 18-3. Collective results of DNA measurements of benign and malignant bone-forming tumors

	Benign tumors		Parosteal osteosarcoma		Osteosarcoma	
Study	Diploid	Nondiploid	Diploid	Nondiploid	Diploid	Nondiploid
Heliö et al. 1985	24	0	—	—	2	13
Mankin et al. 1985	49	0	8	0	9	34
Hiddeman et al. 1987	—	—	3	0	3	18
Xiang et al. 1987	—	—	3	0	0	16
Bauer et al. 1987	43	0	4	0	5	97
Bauer unpublished	50	0	1	0	2	15
Total	116	0	19	0	21	193

or malignant prevailed, recurrence or death was consistently associated with nondiploidy.

The combined findings suggest that whenever doubts arise about the diagnosis of osteosarcoma vs. a benign lesion, DNA cytometry may offer decisive information. Thus, the finding of nondiploidy precludes benignity and, instead, suggests the presence of a highly malignant lesion. Diploidy does not rule out high-grade osteosarcoma, but the diagnosis should not be accepted without careful reevaluation.

Recently, van Oven and coworkers [37] reported a case of parosteal osteosarcoma with a superficial high-grade osteosarcoma component in an otherwise typical, sclerotic low-grade osteosarcoma. FCM of the high-grade component revealed aneuploidy, supporting the correlation between aneuploidy and high-grade malignancy. Unfortunately, FCM of the low-grade part was unsuccessful.

One major concern when applying DNA analysis as a diagnostic tool is the representativity of the analyzed specimen. This is especially important in osteosarcoma, often exhibiting histologic heterogeneity. To determine whether the DNA content varies within a lesion, the DNA content of areas with chondroblastic differentiation were compared to those with osteo-/fibroblastic differentiation within 12 osteosarcomas [38]. The study, based on MSP in tissue sections, showed that the DNA content was the same in different parts of the lesions. Most important, all tumor areas analyzed were proven nondiploid. It should be emphasized that histologic evaluation of the representativeness of the analyzed specimen is a prerequisite for correct interpretation of the DNA analysis, especially in studies based on FCM.

At the Karolinska Hospital, DNA analysis has been adopted as part of the routine diagnostic evaluation of bone tumors. In the vast majority of cases this provides complementary information, i.e., benign lesions are diploid and osteosarcomas nondiploid. Occasionally cases are encountered that pose major differential diagnostic challenges. We have found that DNA analysis often provides conclusive and decisive information in these difficult cases.

Prognosis

The prognostic information of DNA analysis has so far been limited [39]. As already mentioned, in most DNA studies of solid tumors the dichotomization between diploidy and nondiploidy has been prognostic. In osteosarcoma, this gives very little information since there are so few diploid variants and it is not known whether the exceptional diploid variants are associated with a better prognosis.

In a further attempt to extract prognostic information from DNA analysis of osteosarcoma, we related the clinical course to the DI of 35 osteosarcomas analyzed by FCM [35]. Disappointingly, neither the peak DNA value nor the presence of multiple nondiploid stem lines were of prognostic value. However, cell-cycle analysis, feasible in 28 cases, seemed to provide prognostic information. Thus, for the seven patients with tumors containing <15% S-phase cells, the 3-year metastasis-free survival rate was 0.71 compared with 0.27 for the 21 with >15% S-phase cells. Interestingly, it has recently been shown that the S-phase percentage in canine osteosarcoma is correlated with the DI, diploid lesions exhibiting the lowest values [40].

In another prognostic FCM study of osteosarcoma by Look et al., near-diploid variants responded more favorably to adjuvant chemotherapy than other nondiploid lesions [41]. Hence, among nondiploid osteosarcoma it may still prove possible to identify subgroups with a more favorable prognosis.

Experimental studies of DNA ploidy of primary tumors compared to recurrences and metastases [42–45] imply that the DNA value of the aneuploid stem cell line is relatively stable in osteosarcoma. This has also been conformed in a clinical study of primary osteosarcomas and their local recurrences and metastases [38]. In 15 cases studied, 24 recurrences and the corresponding primary lesions, MSP showed that the nondiploid characteristic was retained in all. Furthermore, in six cases studied by FCM, five metastatic cases exhibited the same DI as their primary lesion. In one patient treated with interferon and lung irradiation prior to thoracotomy, the primary tumor was aneuploid and the lung metastasis diploid.

Experimentally, interferon-induced growth inhibition of osteosarcomas leads to a depletion of aneuploid tumor cells [46]. However, the aneuploid cell lines regain pretreatment proportions when growth resumes after interferon is stopped. The sensitivity to irradiation may vary among different aneuploid cell lines in multiclonal osteosarcomas, so that radiation treatment leads to a selection of cells with a higher DNA content, less sensitive to radiation than near-diploid cell populations [47]. These findings, although mainly based on animal models, indicate that DNA analysis can be an important tool for monitoring the effects of adjuvant treatment.

In conclusion, there is now overwhelming evidence that the vast majority of osteosarcomas are nondiploid. Although of limited prognostic value, DNA analysis appears to be of differential diagnostic value. Routine DNA characterization would make comparison of treatment results from different centers

more meaningful. In the future, prognostic information may be gained from DNA measurements by more accurate assessment of the growth fraction, e.g., by bromodeoxyuridine uptake. Multiparameter FCM with simultaneous measurement of DNA content and other tumor features, such as nuclear size, RNA, and total protein, may also give more prognostic information. More sophisticated quantitative cytologic characterization of osteosarcomas promises to make DNA cytometry an important tool in predicting sensitivity to adjuvant treatment and in monitoring treatment effects.

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19. Osteosarcomatosis and metastatic osteosarcoma

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Richard P. Moser, Jr.

Definition and classification of osteosarcomatosis

Multiple secondary osseous foci that appear within 5 months after the diagnosis of symptomatic, radiographically dominant tumor are classified as synchronously appearing multifocal osteosarcoma, so-called osteosarcomatosis (Figure 19-1). Because of its rapid onset and generally symmetric distribution, several theories have been proposed as to the origin of osteosarcomatosis. Several authors (1–6) have theorized that these multiple skeletal lesions represent multiple primary osteosarcomas of varying sizes and occur from the "multipotent preosseous tissue of the periosteum" [1]. Associated primitive preosseous rests [7] and humoral and cell-mediated immunity induced by specific neoplastic antigens [8,9] have also been proposed as possible etiologies. However, other authors [9–13] have suggested that these multiple synchronously appearing bone lesions actually represent rapidly appearing metastases.

Three separate systems have been proposed to classify osteosarcomatosis [2,9,14]. These systems are compared in Table 19-1. The classification system proposed by Amstutz [14] covers all aspects of multifocal osteosarcoma and appears to be the most commonly used in the medical literature. Therefore, we chose this system to evaluate the secondary osseous foci found among 690 cases of osteosarcoma in the radiographic files of the Armed Forces Institute of Pathology (AFIP) in Washington, D.C. This system divided osteosarcomatosis into types I and II (synchronously appearing less than 5 months after diagnosis of a symptomatic, radiographically dominant primary) and metastatic osteosarcoma types IIIa and IIIb (metachronously appearing over 5 months after the diagnosis of a symptomatic, radiographically dominant primary). The major difference between Amstutz type I and type II osteosarcomatosis is the age of the patient at the time of initial diagnosis. Amstutz type I osteosarcomatosis includes those patients 18 years and under, and type II includes all patients over 18 years of age. Amstutz type IIIa (early metachronous metastatic osteosarcoma) includes any patient (regardless of age) with secondary skeletal lesions occurring more than 5 months and up to 24 months after diagnosis of a primary osteosarcoma. Amstutz type IIIb (late metachronous metastatic osteosarcoma) includes any patient (regardless of

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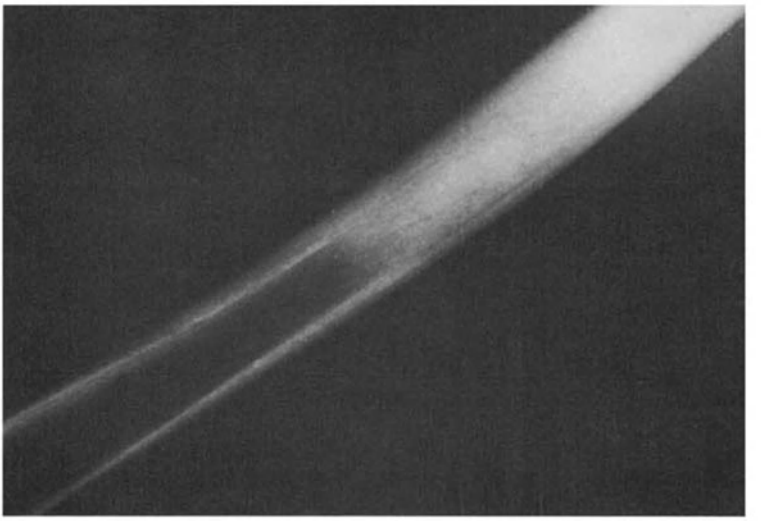
 ISBN 0-7923-1905-2



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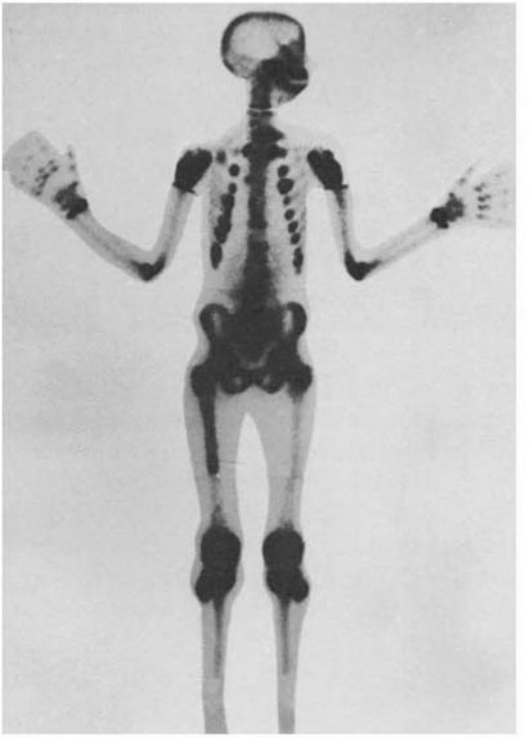

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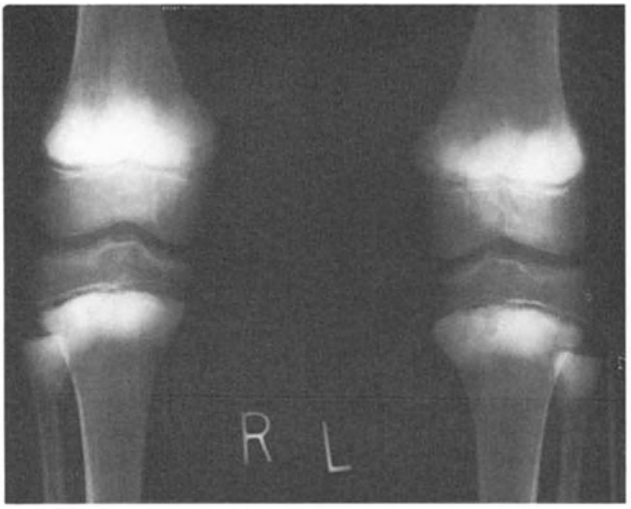

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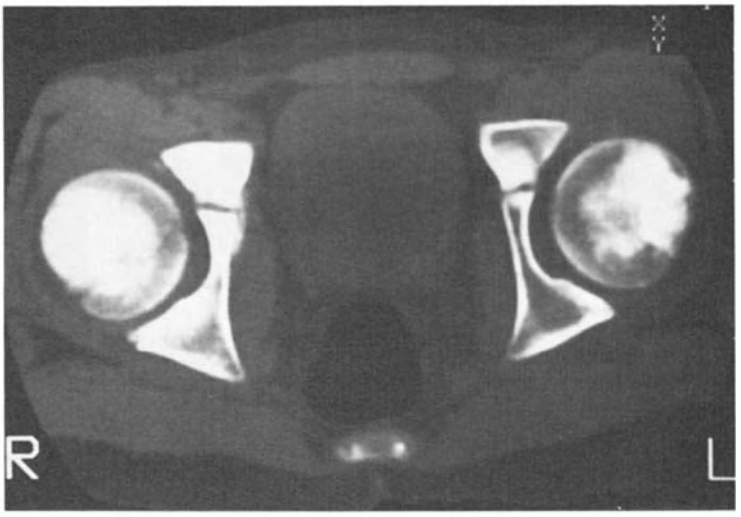

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Figure 19-1. Images of 14-year-old boy with a 3-month history of right thigh pain. **A**: Radiography shows a long sclerotic diaphyseal osteosarcoma in the right femur. **B**: Bone scan demonstrates numerous secondary bone lesions, including symmetric metaphyseal lesions in most long tubular bones. **C**: Radiography shows metaphyseal lesions in knee. **D**: CT scan shows lesions in femoral heads and acetabula. Reprinted with permission of Hopper et al. [10].

Table 19-1. Classification of osteosarcomatosis

Type		Description	Age range	Time sequence of other bone lesions
Amstutz [14] Mahoney [2] Lowbeer [9]	Type I Type A Type IIA	Child-adolescent, multiple, almost simultaneous, symmetric long bone metaphysis; sclerotic, anaplastic lesions	≤18 years	≤5 months
Amstutz [14] Mahoney [2] Lowbeer [9]	Type II Type B Type IIB	Adult low-grade multiple malignancies; generally symmetric long bone metaphysis; generally sclerotic lesions	≤18 years	≤5 months
Amstutz [14] Mahoney [2] Lowbeer [9]	Type IIIa Type c Type I	Early metachronous; metastatic predominantly spine, pelvis; metastatic lesion usually lytic; anaplastic primary	All ages	≤5 months but ≤24 months
Amstutz [14] Mahoney [2]	Type IIIb Type D	Late metachronous; metastatic from anaplastic primary; lytic to sclerotic lesions to usually spine or flat bones; questionable new primary	All ages	>24 months

age) with secondary skeletal lesions appearing more than 24 months after diagnosis of a primary osteosarcoma. Amstutz does not attempt to further subdivide these types on the basis of the numbers of skeletal lesions or on the presence of pulmonary metastases.

The AFIP experience: metastatic osteosarcoma

Of the 690 cases of osteosarcoma reviewed from the files of the AFIP, 10% showed multiple sites of skeletal involvement. These included 15 patients with secondary foci, either in the same bone or transarticularly on the opposite side of an adjacent joint of a bone having primary, radiographically dominant osteosarcoma. As these tumors likely represent manifestations of the primary osteosarcoma ("skip" lesions), they are not considered further in this discussion of metastatic osteosarcoma. Fifteen and 14 patients were found to have types I and II osteosarcoma, respectively. Eighteen patients had type IIIa metastatic osteosarcoma, and nine were assigned a type IIIb classification.

The demographics of patients with types I, II, IIIa, and IIIb metastatic osteosarcoma are outlined in Table 19-2. Of these 56 patients, 55 had a radiographically dominant primary tumor that was clearly larger in size and demonstrated an aggressive associated periosteal reaction and the usually expected tumor extension in adjacent soft tissues, as would be expected in any patient with primary osteosarcoma. One patient with type II osteosarcomatosis did not have a definite radiographically dominant primary osteosarcoma. All primary osteosarcomas visualized were in bone, with the exception of one patient having type II osteosarcomatosis. The secondary foci, their number, and symmetry, varied significantly between types I, II, IIIa, and IIIb metastatic osteosarcoma (Figure 19-2, Table 19-3). The number of sites also varied dramatically, with type I averaging eight secondary foci per patients vs. three, two, and one secondary foci in individuals with type II, IIIa, and IIIb. The majority of all secondary foci, regardless of classification type, sclerotic. The majority (50–73%) of all four types of metastatic osteosarcoma developed pulmonary metastases simultaneously with or prior to the appearance of this secondary skeletal foci. In patients with type I and type II osteosarcomatosis, their pulmonary metastases developed an average of 0.6 to 1.9 months after the diagnosis of a radiographically dominant primary osteosarcoma.

Treatment and the changing face of metastatic osteosarcoma

The dramatic advances that have occurred in the multidisciplinary treatment of osteosarcoma over the past two decades have dramatically improved the prognosis of patients with osteosarcoma. Because of the incidence on limb time of these patients, as well as a greater emphasis on limb-salvage treatments,

Table 19-2. General data for osteosarcoma patients

Category	Type I (n = 5)	Type II (n = 14)	Type IIIa (n = 18)	Type IIIb (n = 9)	All osteosarcomas (n = 690)
Age (y)	11.6 ± 4.3	30.3 ± 13.0	26.1 ± 17.4	28.1 ± 21.5	24.3 ± 16.5
Range	4–18	19–63	8–76	10–78	6 mo–99
Race (%)					
White	81.8	87.5	92.3	100	82.9
Black	9.1	12.5	7.7	0	11.0
Asian	9.1	0	0	0	4.4
Sex (%)					
Male	53.3	71.4	88.9	62.5	68.5
Female	46.7	28.6	11.1	37.5	31.5
Frequency (%)	2.2	2.0	2.6	1.3	—

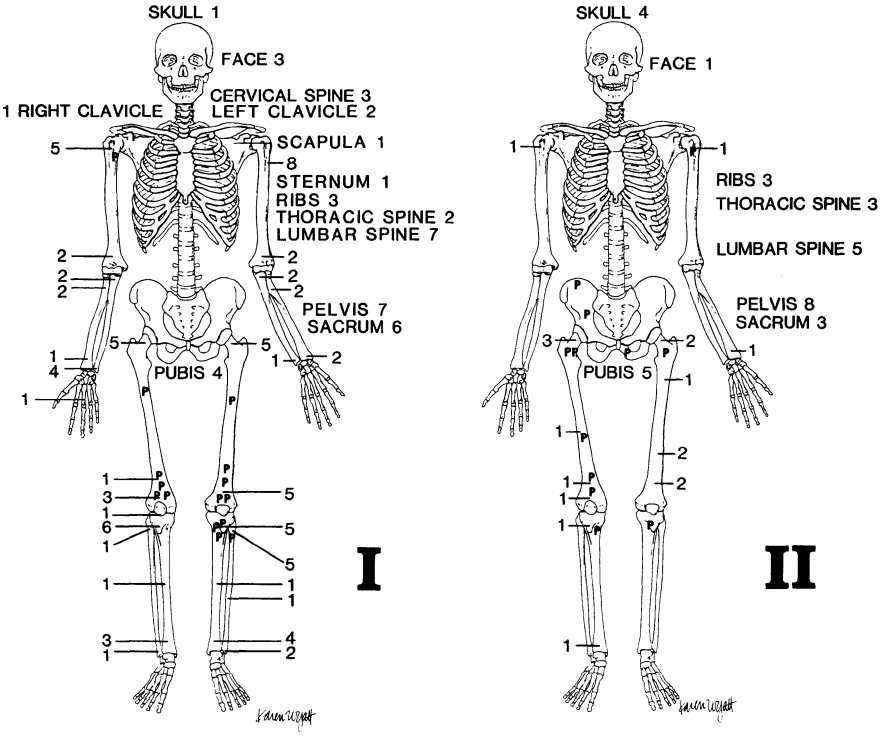

Figure 19-2. The skeletal distribution of the secondary foci are depicted for type I (A), type II (B) osteosarcomatosis. The epicenter of each radiographically dominant tumor is indicated (p = primary). Only 12 primary lesions for type II are shown because one patient had no radiographically dominant lesion and another had a soft-tissue retroperitoneal primary osteosarcoma.

including chemotherapy, the incidence of multifocal osteosarcoma can be expected to increase.

Conclusions

Our data demonstrate that types I and II osteosarcomatosis actually represent forms of metastatic osteosarcoma. A radiographically dominant tumor was found in nearly all of these patients that was indistinguishable in appearance and presentation from other patients with primary osteosarcoma. This conclusion is further supported by the large proportion of both patients with type I and type II osteosarcomatosis who developed pulmonary metastases, usually before the manifestation of the skeletal secondary foci. The differentiation of osteosarcoma patients with osseous metastases into types I, II, IIIa, and IIIb is probably arbitrary, as all represent a metastatic process. The use of the term osteosarcomatosis for type I and type II multifocal

Table 19-3. Secondary osteosarcoma lesions with and without lung metastasis

Category	Type I (n = 15)	Type II (n = 14)	Type IIIa (n = 18)	Type IIIb (n = 9)
Secondary bone lesions				
Patients with no dominant primary lesion	0%	6.7%	0%	0%
Patients with secondary bone lesion present at time of initial diagnosis	73.3%	64.3%	0%	0%
Patients with secondary bone lesions present with 1 month of initial diagnosis	93.3%	71.4%	0%	0%
Range of occurrence (mo)	0–4	0–5	6–23	24–60
Mean ± 1 standard deviation	0.5 ± 1.1	1.1 ± 1.7	9.0 ± 4.3	38.0 ± 12.6
Range of no. of sites involved	1–33	1–33	1–6	1–2
Mean ± 1 standard deviation	8.1 ± 8.6	3.2 ± 3.3	2.3 ± 1.8	1.1 ± .4
Symmetry	80.0%	21.4%	20.0%	0%
Secondary bone lesions and lung metastases				
Patients with lung metastases	73.3%	50.0%	61.1%	55.6%
Patients with lung metastases with or before secondary bone lesions	100%	100%	90.9%	100%
Range of occurrence of lung metastases (mo)	0–4	0–5	5–16	24–36
Mean ± 1 standard deviation	0.6 ± 1.2	1.9 ± 2.0	9.5 ± 3.4	28.4 ± 4.9
Range of no. of sites of secondary bone lesions in patients with lung metastases	1–20	1–13	1–6	1–2
Mean ± standard deviation	7.6 ± 5.4	4.7 ± 4.5	2.1 ± 1.7	1.3 ± .5

osteosarcoma will probably continue, although it would perhaps be more correct to label them type I and type II metastatic osteosarcoma. Lastly, increasing survival, changing treatment, and the utilization of routine periodic scintigraphic surveillance in patients with osteosarcoma will probably lead to an increased incidence and/or detection of metastatic osteosarcoma.

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20. Experience of the EORTC Radiotherapy/ Chemotherapy Group in osteosarcoma trials

J.M.V. Burgers

One of the earliest cooperative groups of the GECA, the forerunner of the EORTC, was the Radiotherapy Chemotherapy Group, comprising mainly French, Belgian, and Dutch centers. Next to work on lymphomas, this group initiated randomized trials on bonesarcoma in children and young adults, respectively for Ewing's sarcoma in 1969 and for osteosarcoma of the limbs in 1970. At that time chemotherapy for osteosarcoma was not yet available, and the radiosensitivity of the tumor is limited. Observations and calculations of Profs. Breur [1] and Abbatucci [2] had shown that the radiosensitivity of osteosarcoma is sufficient to eradicate tumor nodules of 10^4 to 10^5 cells with a dose of 20 Gy over 10 days, that is, the maximum dose that is tolerable for both lungs. The chance that subclinical lung metastases at the time of diagnosis are of this size or smaller is about 20%. Therefore prophylactic bilateral pulmonary irradiation directly after amputation or after irradiation of the primary tumor should increase the survival level by this proportion. This first study of the Radiotherapy Chemotherapy Group, 02, collected 86 patients. The late Prof. Breur was study coordinator, together with Dr. Schweisguth [3]. The 3-year disease-free survival rose from 28% to 43% ($p = 0.056$). The study could not collect sufficient patients because the first reports on the beneficial effects of adjuvant Adriamycin or methotrexate appeared around 1974 and thereafter patient accrual diminished.

The same collaborative group started a second trial on adjuvant treatment for osteosarcoma of the limbs after some years of deliberation in 1978. "Controlled trial on adjuvant therapy in the treatment of osteosarcomas" protocol nr. 20781, protocol committee K. Breur, P.A. Voute, O. Schweisguth, P. Cohen, D. Machine, later M van Glabbeke, secretary: E van der Schueren, later J.M.V. Burgers. In the first arm adjuvant chemotherapy was given, alternating Adriamycin and vincristine-methotrexate every 2 weeks over the first 12 weeks, followed by a consolidation phase in which these schedules were alternated with cyclophosphamide every 4 weeks over 6 months. The total adjuvant treatment time lasted 41 weeks (Figure 20-1). The second treatment arm was identical to trial 02, with prophylactic bilateral pulmonary irradiation at a dose of 20 Gy (after air correction). In the third treatment arm the induction chemotherapy of arm 1 was followed after 12 weeks by bilateral pulmonary

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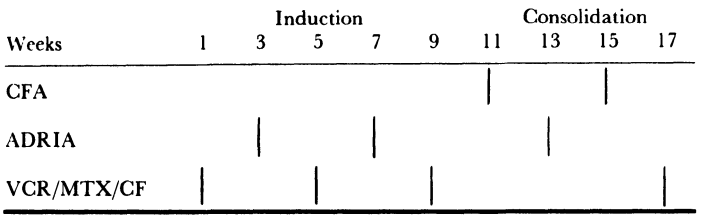
 ISBN 0-7923-1905-2



Cycles of consolidation chemotherapy to be given for a total of 4 times, up to week 41.

Methotrexate	6000 mg/m^2/6 hour infusion	MTX
Citrovorum factor	15 mg/6 hour/x 12	CF
Vincristine	1.5 mg/m^2/i.v. (max. 2 mg)	VCR
Adriamycin	70 mg/m^2/i.v.	ADRIA
Cyclophophamide	1200 mg/m^2/i.v.	CFA

Figure 20-1. Schedule of adjuvant treatment for arm 1. Cycles of consolidation chemotherapy to be given for a total of four times, up to week 41. In arm 3 consolidation treatment was dropped and instead elective bilateral lung irradiation given. MTX = methotrexate (6000 mg/m^2/6 hr infusion; CF = citrovorum factor (15 mg/6 hr/x12); VCR = vincristine (1.5 mg/m^2/i.v. (max. 2 mg); ADRIA = adriamycin (doxorubicin) (70 mg/m^2/i.v.; CFA = cyclophosphamide (1200 mg/m^2/i.v.).

irradiation. This trial collected 205 patients over 5 years. Preliminary results favored the chemotherapy arm, but this was not substantiated after further follow-up [4], as 5-year survival was identical at 43% and the disease-free survival at 5 years was 24%. In this trial 19% of the patients had undergone radiotherapy as treatment for the primary tumor, while 52% had amputation and 29% had exarticulation. The local recurrence rate in the patients with primary radiotherapy was 43%, while the functional result was moderate. In the amputated patients the local recurrence rate was 10%. In all treatment arms the first distant metastases were in the lungs in 62% of patients and in bone in 24%. However there was some difference in the location and number of metastases in the patients who had undergone radiotherapy. In an unpublished analysis by Busson [5] it was shown that the localization of pulmonary metastases was mainly behind the dome of the diaphragm and behind the heart and mediastinum in those patients who were irradiated. These areas had received a smaller dose, as the irradiation passes partly through nonaerated tissue. The toxicity of the lung irradiation was minimal during the observation period. In the chemotherapy arms three toxic deaths occurred; WHO grade 3–4 toxicity was observed in 20% in arm 1 and in 7% in arm 3. As for late toxicity, a decrease in lung function was seen in 5% of patients in the chemotherapy only arm and in 14% of the patients who did not develop lung metastases in the radiotherapy arms. Late cardiac toxicity and late hematologic toxicity were also reported in the chemotherapy and mixed treatment arms.

Since the introduction of neoadjuvant chemotherapy with more active drugs, many patients can have limb-sparing surgery. Still the risk of lung metastases is substantial in patients who have an insufficient histological response to this

chemotherapy [6]. For these patients bilateral pulmonary irradiation as adjuvant therapy directly after surgery might help to reduce this risk. A slightly higher dose of radiotherapy seems feasible, with a booster dose to the areas behind the dome of the diaphragm and the mediastinum. To evaluate the toxicity of such treatment given after earlier extensive chemotherapy, the EOI has now designed a pilot study for which patients are selected who have already undergone one thoracotomy for a first pulmonary metastasis.

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21. Intraoperative radiation therapy for osteosarcoma

Takao Yamamuro and Yoshihiko Kotoura

Introduction

In 1964, Abe et al. [1] developed intraoperative radiotherapy (IOR) for advanced abdominal cancers with significantly better local control than other methods. Since 1978 we have used IOR in combination with chemotherapy for treating primary and metastatic malignant bone tumors in an attempt to preserve the affected limb [2]. In 1983 we demonstrated by serial histological examination of the removed tumors receiving IOR that direct irradiation of a large dose (50–60 Gy) of electron beams was extremely effective in controlling local lesions in the extremities, although IOR was not considered to have any influence on the survival rate of the patient [3].

This chapter describes the procedure of IOR for malignant bone tumors in the extremities and the results of combined treatment for osteosarcoma using chemotherapy, IOR, and surgery.

Indications for IOR

As a rule, IOR is not indicated for the tumors arising in the spine, shoulder girdle, and pelvic girdle. The proximal part of the humerus and femur are indicated for IOR only when their heads can be dislocated from the joint without exposing the tumor directly. When a bone tumor has not expanded into the soft tissues according to such imaging techniques as angiography, bone scintigraphy, computed tomography (CT), and magnetic resonance imaging (MRI), IOR is indicated for most parts of the four extremities.

IOR Procedure

The irradiation area is exposed in the operating room. An extensive skin incision is made over the tumor. The skin, muscles, vessels, and nerves are retracted away from the irradiation area as much as possible so as to avoid damage by IOR. If the tissue is suspected of being invaded by the tumor, or if the safety margin is not clear, the tissue is included in the irradiation area. In many instances ligaments around the joint are often included into the

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 ISBN 0-7923-1905-2

Figure 21-1. Exposed irradiation area for an osteosarcoma that developed in the distal femoral diaphysis. Small air bags and polyethylene catheters are used to retract the skin, muscles, vessels, and nerves away from the irradiation area. The tumor is covered with a layer of normal soft tissues. A treatment cone covered with a sterile drape is attached to the betatron for the mediolateral irradiation.

irradiation area, but their damage is not so severe as to result in marked contracture of the joint. When the tumor is thus sufficiently exposed, the wound is closed by wrapping sterile drapes over the affected limb and the patient is transferred to the betatron room for irradiation.

As a rule, irradiation is performed with 12- to 26-MeV electron beams from a betatron at a dose of 50–60 Gy. In cases of juxtacortical osteosarcoma, which is more radioresistant than other osteosarcomas, 100 Gy is used. The dose distribution of electron beams in the focus is studied preoperatively by the CT number with a computer so that the focus is subjected to at least 80% of the irradiation dose. We found that the multifocal bilateral irradiation method is best for minimizing complications of the soft tissues and increasing the dose distribution of electron beams in the focus. A treatment cone (the maximum size is 8×10 cm) covered with a sterile drape is attached to the betatron (Figure 21-1). When the size of the treatment cone is not large enough to cover the entire area of irradiation, the cone is moved proximally or distally, overlapping by 1 cm with the adjacent area to compensate for dose reduction in the margins. For example, three irradiation fields with a 10×8 cm treatment cone with 1 cm overlap at the margins of each irradiation field cover an area of 10×22 cm. After irradiation on the lateral side, the treatment cone is rotated 180° and the corresponding medial side is similarly irradiated. Formerly, when irradiation was only unilateral, skin and nerve damage and muscle contracture were observed on the contralateral side. These side effects of radiation therapy have been almost completely eliminated since we began using the bilateral irradiation method in 1981.

The irradiation usually takes about 30–40 Min. During irradiation, the patient under general anesthesia is observed through a TV camera, and the electrocardiogram, pulse, and blood pressure are monitored in the control room. After the irradiation, the wound is carefully washed and closed, and a drain is installed.

Post IOR management

The drain is removed on the third postoperative day in most cases. Passive and active joint exercise is started 7–10 days after IOR. In osteosarcoma of the osteolytic type arising in the lower extremities, full weight bearing is not permitted until prosthetic replacement is performed in order to avoid pathological fracture. With the osteoblastic type, joint exercise and full weight bearing can be started a few days after the skin wound has healed.

Patients and site of the lesion

Thirty-two patients with osteosarcoma were treated by IOR between December 1978 and December 1990. These patients consisted of 25 males and seven females and were aged 6–51 years at the time of IOR. The site of the lesion was the distal femur in 18 cases, middle femur in one, proximal tibia in 11, proximal humerus in one, and iliac bone in one. Of these 32 patients, 24 received IOR in combination with chemotherapy and the others underwent prosthetic replacement about 3 months after IOR.

Results

Skin at the site of IOR

In the majority of cases, the surgical wound heals primarily with no particular problems after IOR, and almost no irradiation injuries of the skin are noted when the bilateral irradiation method is employed. Among 32 patients who underwent IOR, two with tibial osteosarcoma developed extensive skin necrosis, probably due to the unilateral irradiation method, and they eventually underwent amputation. Two other cases of tibial osteosarcoma and one case of femoral osteosarcoma developed moderate skin necrosis due to extensive skin dissection during IOR, and they underwent rotational skin plasty, which covered the site of skin necrosis successfully. Thus, particularly in the case of tibial osteosarcoma, extensive skin dissection for IOR sometimes results in ischemic skin necrosis, even if the bilateral irradiation method is used, since the microvessels in the underlying tissues soon become necrotic after IOR. To prevent such ischemic skin necrosis, a long skin incision on the medial surface of the tibia and unnecessary dissection between the skin and underlying muscles should be avoided.

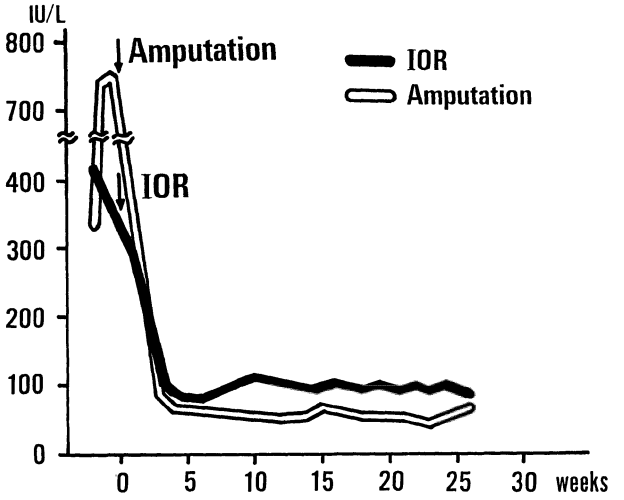

Figure 21-2. Serum alkaline phosphatase levels expressed as average values of 20 cases who underwent IOR and chemotherapy, in comparison with those who underwent amputation. Note the rapid decrease in the levels in all patients 2–3 weeks after IOR. This decrease is similar to that observed in patients who underwent amputation without IOR.

Serum alkaline phosphatase level

The serum alkaline phosphatase level was examined at regular intervals in 20 patients. The level, which was more or less elevated before IOR, returned rapidly to normal 2–3 weeks after IOR in all patients (Figure 21-2). This reduction was comparable with that observed in patients who underwent limb amputation without IOR. If the level remains high even after IOR, metastasis to other bones or the lung is suspected.

Histological findings

Serial histological examination of the resected primary lesions was carried out in 10 patients who underwent limb amputation or prosthetic replacement 2–10 months (average 3 months) after IOR. In two patients who had received unilateral irradiation, a few scattered tumor cells appeared viable in the superficial layers in spite of the presence of marked cellular changes and extensive destruction of the tumor. In the other eight patients who had received multifocal bilateral irradiation, complete necrosis of the tumor cells was observed throughout the specimen, except for a few scattered, markedly altered, presumably nonviable tumor cells in small clusters (Figure 21-3) [3].

Local recurrence of tumor

Local recurrence of tumor was found in 3 patients, excluding the two who underwent limb amputation. Two patients showed recurrence in the

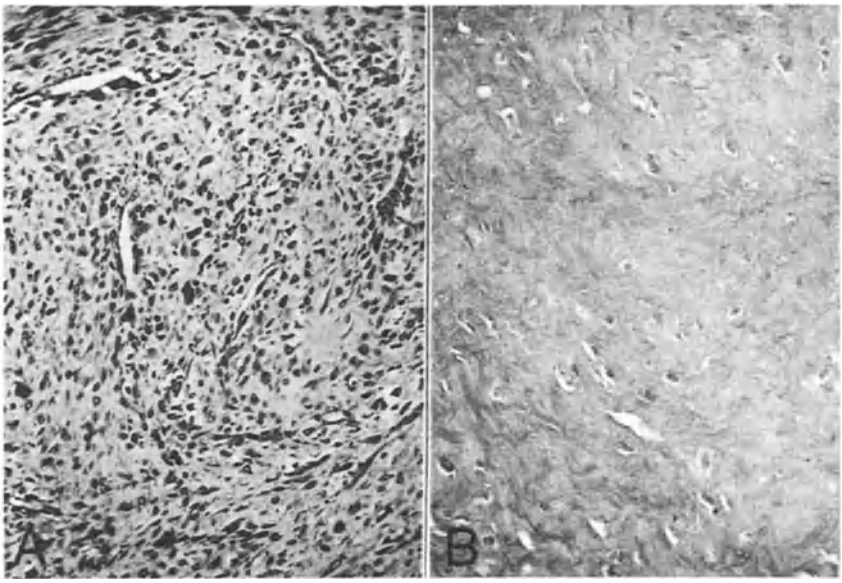

Figure 21-3. Histological findings of osteosarcoma developed in the left proximal tibia in a 21-year-old male. **A**: Microphotograph of the tumor tissue taken by biopsy before treatment shows a typical findings of osteosarcoma (×20). **B**: Microphotograph of the tumor removed 3 months after IOR shows complete necrosis of the tissue except for a few scattered, markedly altered, presumably nonviable cells.

non-irradiated area, one due to tumor implantation in the muscle during the biopsy procedure and the other due to insufficient selection of the irradiation area. Therefore, particular caution is necessary during the biopsy technique and the irradiation method. No recurrence of the tumor was observed in the irradiated area, except for one case of juxtacortical osteosarcoma, which showed bone scintigraphic signs of recurrence 2 years and 7 months after IOR. As juxtacortical osteosarcoma is less malignant and more radioresistant than conventional osteosarcoma, this case underwent IOR again at a dose of 100 Gy. Thus, sufficient local control of osteosarcoma can be achieved by IOR at a dose of 50–60 Gy. However, it is considered that a larger dose, such as 70–100 Gy, is required for juxtacortical osteosarcoma or chondrosarcoma to obtain complete cytocidal effect using IOR.

Function of the joint after IOR

Since most osteosarcomas arises in the metaphysis of long bones, the irradiation area of IOR inevitably includes the adjacent joint. Surprisingly, however, functions of this joint are very well preserved after IOR in most instances, presumably because the capsular ligaments and joint cartilage are much more

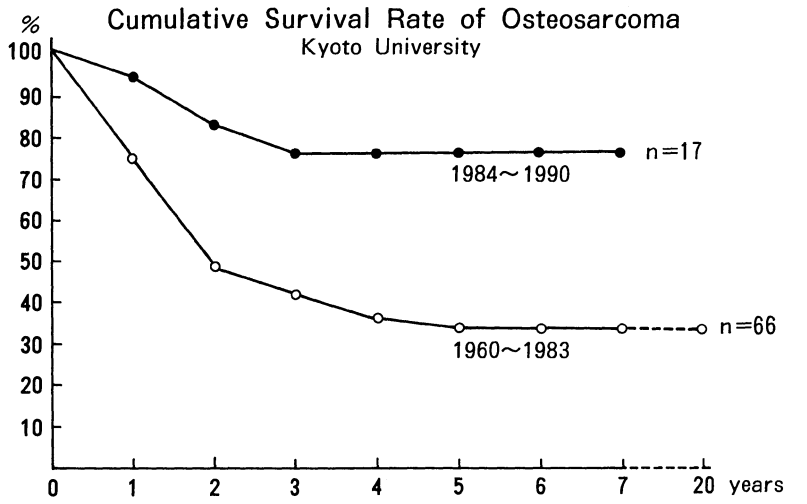

Figure 21-4. Cumulative survival rate of osteosarcoma treated in Kyoto University Hospital between 1960 and 1990. Note the significant increase in the survival rate after 1984, when cisplatinum was introduced into the chemotherapy protocol.

radioresistant than the skin and muscles. When active and passive exercise of the joint is started 7–10 days after IOR, usually full extension and 70–90% flexion of the joint are preserved. This is one of the great advantages of IOR in the treatment of osteosarcoma.

Pathological fracture after IOR

Among the patients who underwent neither limb amputation nor prosthetic replacement and survived longer than 1 year after IOR, about 58% sustained pathological fracture through the lesions. All attempts of osteosynthesis failed to fuse the fracture site. The fracture occurred much more frequently in the osteolytic tumors than in the osteoblastic ones. Therefore, in cases of osteolytic tumors prosthetic replacement is indicated 3 months after IOR when all the irradiated tissues become completely necrotic [4]. The reason we dare to perform prosthetic replacement at least 3 months after IOR are first that marginal or even intralesional resection of the tumor is not risky, and second that the bone segment undergoing prosthetic replacement can be made considerably smaller than that without IOR, as irradiated but nonosteolytic parts of the bone do not have to be removed.

In cases of osteoblastic or less osteolytic tumors, intramedullarly nailing with special rods after IOR seems to be the best method to prevent pathological fracture as well as to preserve good joint functions of the affected limb [5].

Cumulative survival rate

The overall cumulative survival rate in 66 cases of osteosarcoma treated in our clinic between 1960 and 1983 was 32%. These patients received chemotherapy with cytoxan, mitomycin C, methotrexate, and Adriamycin, but none of these treatments was significantly effective when compared with cisplatinum, which was introduced into our chemotherapy protocol in 1984. Before 1984, 15 patients underwent IOR, and their cumulative survival rate was almost similar to the rate of those who did not undergo IOR. After 1984, 17 patients received chemotherapy with cisplatinum and Adriamycin in combination with IOR, and their cumulative survival rate was 78% (Figure 21-4). Thus IOR has little advantage in terms of the survival rate, although it is extremely effective in the local control of osteosarcoma. IOR must, therefore, be performed in combination with adequate chemotherapy.

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22. Surgery of Osteosarcoma of the extremities: Indications and complications in the recent Experience at the Istituto Ortopedico Rizzoli

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Introduction

In the surgical treatment of localized osteosarcoma of the extremities, limb-salvage procedures are currently used whenever wide surgical margins can be achieved [1–3]. The increased indications for conservative surgery in osteosarcoma rely mainly on accurate preoperative staging through diagnostic imaging tools and the use of adjuvant or neoadjuvant chemotherapy [1,2].

In most reported studies limb salvage has not shown any adverse impact on the survival of patients with osteosarcoma when chemotherapy is used and wide surgical margins are achieved [1–5]. However, the increased use of limb-salvage procedures and better long-term survival of osteosarcoma patients imply a higher rate of immediate and delayed complications than ablative surgery [1,6].

Materials and methods

The present study enrolled 144 patients with localized osteosarcoma of the extremities treated at the Istituto Ortopedico Rizzoli between September 1986 and December 1989. There were 75 males and 69 females, ranging in age from 3 to 41 years (65 patients under 14 years). All patients had high-grade classic osteosarcoma of the extremities, which was extracompartmental and without metastases at presentation.

Histological diagnosis of osteosarcoma was obtained by needle biopsy in 91 cases and by incisional biopsy in 53 cases. Histology revealed the following types of osteosarcoma: osteoblastic in 93 cases (65%), chondroblastic in 21 (15%), teleangiectatic in 14 (10%), fibroblastic in 10 cases (6%), and other subtypes in 6 cases (4%).

There were 11 osteosarcomas grade 3 and 133 osteosarcomas grade 4. All patients received preoperative chemotherapy. Surgery was performed 3 weeks after the second preoperative cycle, and it was always preceded by a complete restaging of the lesion. The aim of the surgical procedure was to achieve wide margins; therefore, ablative surgery was restricted to those cases in

G. Bennett Humphrey (ed.), OSTEOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS.
 ISBN 0–7923–1905–2

Table 22-1. Surgical procedures according to tumor site

Site		n	Resections	Amputations	Rotationplasties
Humerus	Proximal	18	17 (94%)	1 (6%)	—
	Diaphysis	1	1 (100%)	—	—
	Distal	1	1 (100%)	—	—
Femur	Proximal	7	6 (86%)	1 (14%)	—
	Diaphysis	5	5 (100%)	—	—
	Distal metadiaph.	1	1 (100%)	—	—
	Distal	59	47 (80%)	3 (5%)	9 (15%)
Tibia	Proximal	34	30 (88%)	4 (12%)	—
	Prox. metadiaph.	2	2 (100%)	—	—
	Diaphysis	2	2 (100%)	—	—
	Distal	4	3 (75%)	1 (25%)	
Fibula		9	6 (67%)	3 (33%)	—
Distal Radius		1	1 (100%)	—	—

which limb-salvage procedures could not obtain oncologically adequate margins or for the preservation of a limb that could at least be partially functional after reconstruction.

Following surgery the specimen was studied and surgical margins were assessed both macroscopically and microscopically according to Enneking's classification [7]. The rate of tumor necrosis was histologically evaluated according to the previously reported method [8]. The response to preoperative chemotherapy was considered "good" if tumor necrosis was ≥90% or "poor" if it was <90%. Postoperative chemotherapy was given as reported by Bacci and coworkers in this book.

Surgery consisted of amputation in 13 cases, rotationplasty in nine cases, and limb-salvage procedures in 122 cases. The type of surgery performed and the different reconstructive techniques used, as related to the location of the tumor in bone, are summarized in Tables 22-1 and 22-2. Of the four patients who underwent hemiresection and grafts, in three a hemiarticular allograft was used and in one (proximal femur) the hemiresection was reconstructed with fibular autografts. Of the six patients who had reconstruction with vascularized fibula, the vascularized fibula was used without other grafts in one case (diaphysis of humerus), while in the other five cases it was associated with allografts.

The prosthesis used for the proximal humerus was a modular cemented prosthesis made at the Istituto Rizzoli (modular resection shoulder prosthesis, MRS) (Figure 22-1). In 10 cases of proximal humerus involvement an intra-articular resection of the proximal humerus was done and reconstruction was obtained with a cemented MRS prosthesis in nine cases and with a composite allograft in one case in which the length of the resection required did not

Table 22-2A. Surgical techniques

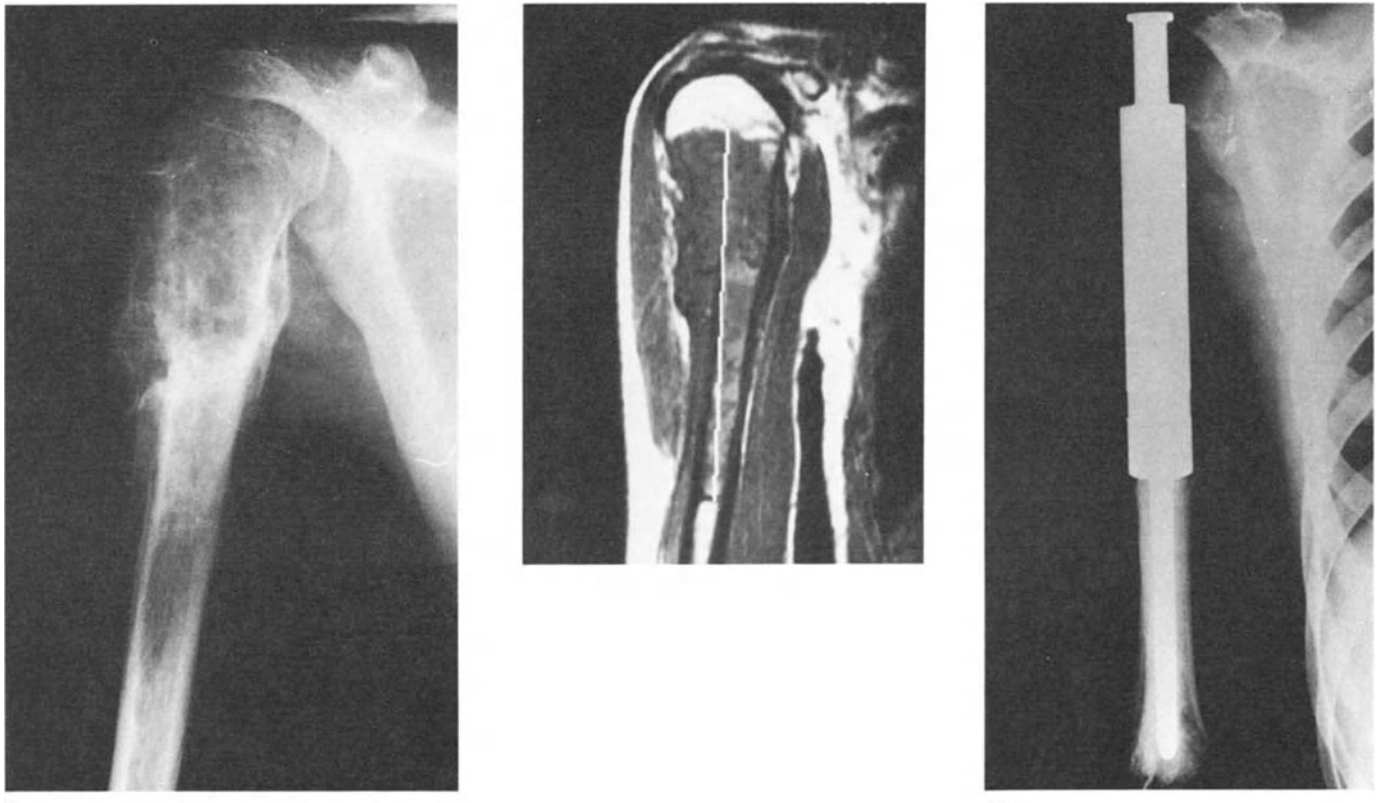
Site	No reconstruction	Prosthesis	Composit allograft	Hemiresection + graft	Osteoarticular allograft	Allograft + K. rod	Allograft + G. K. rod
Proximal humerus		16	1				
Diaphysis of humerus							
Distal humerus					1		
Distal radius							
Proximal femur		1		1			
Diaphysis of femur							3
Metadiaphysis distal femur							
Distal femur		33		1	1	6•	2•
Proximal tibia		21		3		1•	2•
Metadiaphysis proximal tibia							
Diaphysis of tibia							
Distal tibia							2•
Fibula	6						

• = Arthrodesis; K. = Kuntscher; G. K. = Grosse-Kempf.

Table 22-2B. Surgical techniques

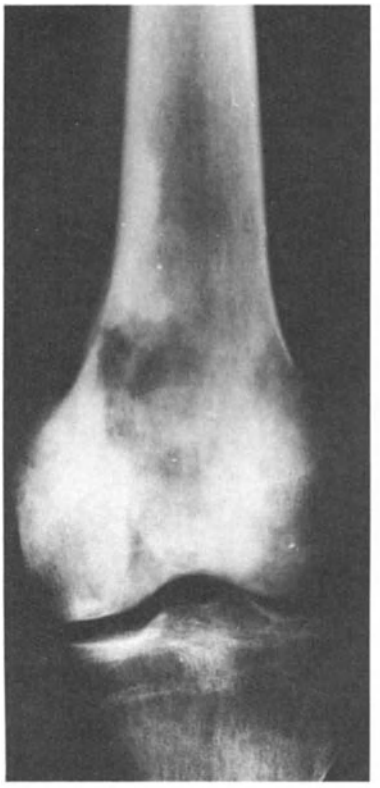
Site	Allograft + plate	K. + cement	Plate + cement	Vascularized fibula	Fibula pro-radius	Rotation plasty	Amputation
Proximal humerus							1
Diaphysis of humerus				1			
Distal humerus							
Distal radius					1		
Proximal femur	4•						1
Diaphysis of femur			2				
Metadiaphysis distal femur				1			
Distal femur		4•				9	3
Proximal tibia	2•			1•			4
Metadiaphysis proximal tibia				2			
Diaphysis of tibia	1		1				
Distal tibia				1•			1
Fibula							3

• = Arthrodesis; K. = Kuntscher; G. K. = Grosse-Kempf.



A C

Figure 22-1. Teleangiectatic osteosarcoma in a 15-year-old male. **A**: Standard x-ray. **B**: MRI showing the intramedullary extension. **C**: x-ray control after resection and reconstruction with a cemented prosthesis.



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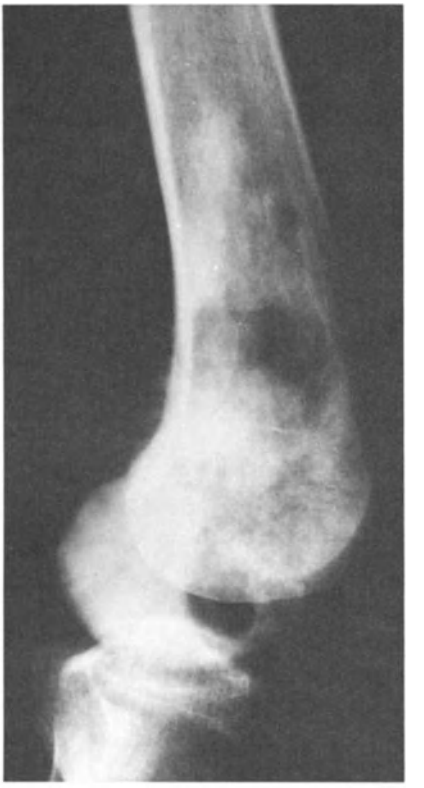

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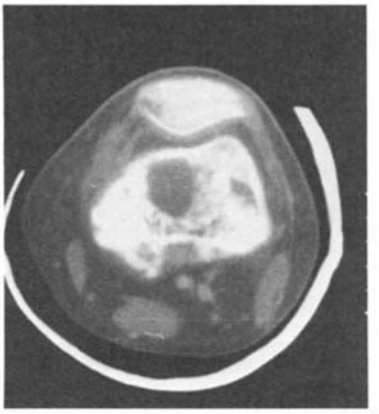

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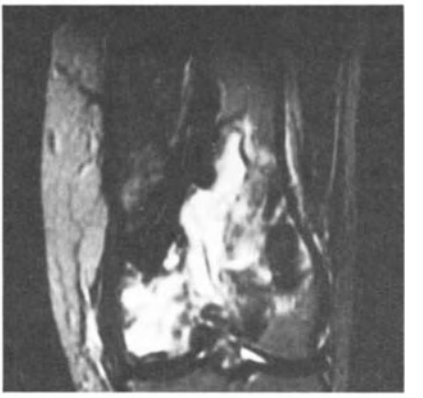

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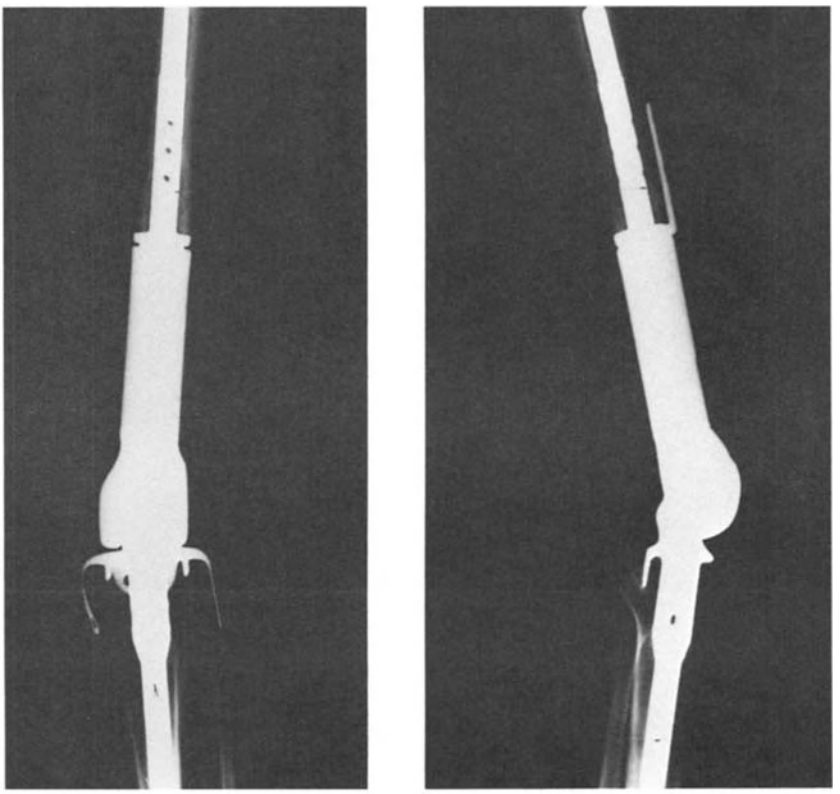

E F

Figure 22-2. Osteoblastic osteosarcoma in a 24-year-old male. **A, B**: Standard x-rays of the distal femur. **C**: CT scan of the lesion. **D**: MRI on a coronal view, showing the longitudinal extension of the tumor; **E, F**: Wide resection and reconstruction with K.M.F.T.R. prosthesis.

allow implant of the prosthetic stem in the short portion of the spared distal humerus. In seven cases of proximal humerus involvement the resection was extraarticular: in two cases a classical Tikhoff-Linberg resection and in five cases a modified Tikhoff-Linberg resection, sparing the scapular body were performed. The lower limb prosthesis used was always the modular uncemented K.M.F.T.R. System prosthesis [9] (Figure 22-2). For the proximal femur the resection was intraarticular in one case and extraarticular in five cases. For the distal femur the resections performed were intraarticular in 43 cases and extraarticular in four cases (Figure 22-3). For the proximal tibia all the 30 resections performed were intraarticular. Also the three resections of the distal tibia were intraarticular. In the reconstruction following intraarticular resection of the distal radius, the proximal fibula of the same patient was used as a nonvascularized graft.

Complications observed were classified in minor and major and were graded

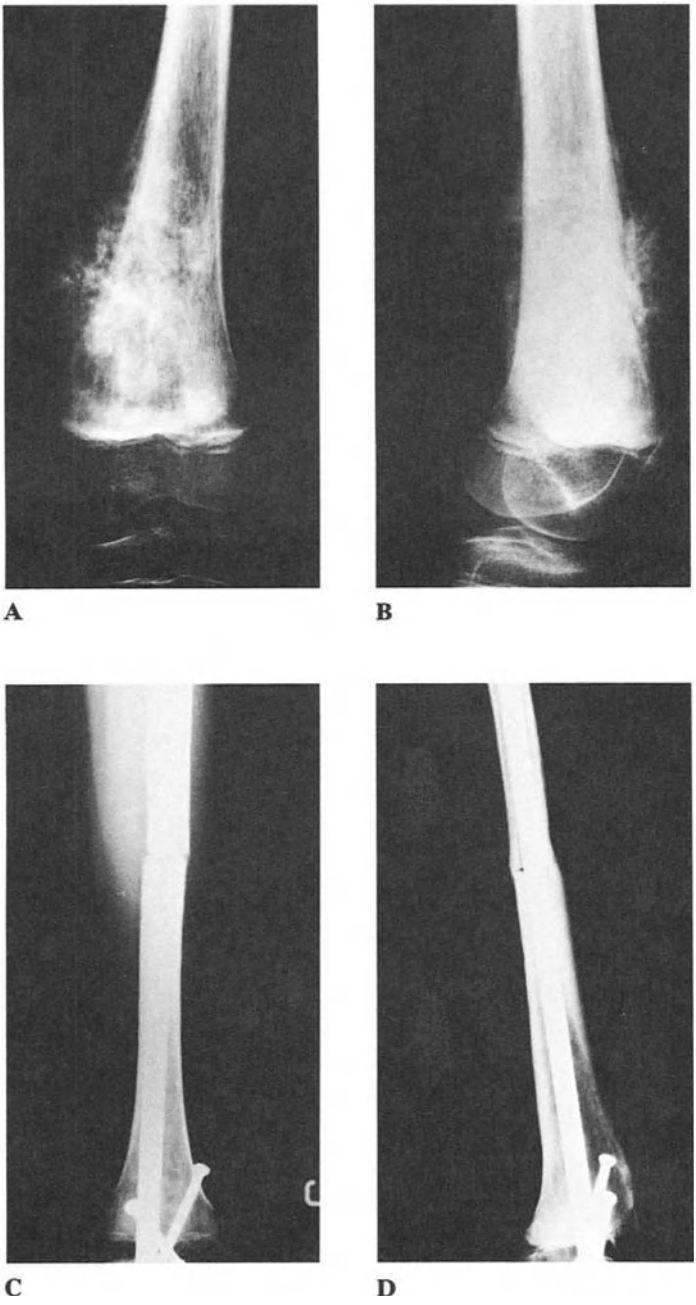

Figure 22-3. Osteoblastic osteosarcoma in a 10-year-old female. **A, B**: Standard x-rays. **C, D**: Wide resection and reconstruction with an intercalary allograft arthrodesis: x-ray control at 9 months showing a good fusion of the graft.

Table 22-3. Grading of complications

Minor complications (not requiring surgery)	Grade 1:	Prolonged time of recovery, no adverse effect on the functional result
	Grade 2:	Prolonged time of recovery, adverse effect on the functional result
Major complications (requiring surgery)	Grade 3:	Surgery required does not imply complete removal or substitution of the primary reconstructive device
	Grade 4:	Surgery required implies complete removal and substitution of the primary reconstructive device
	Grade 5:	Surgery required is (1) removal of the reconstructive device without new reconstruction or (2) amputation

from 1 to 5, as reported in Table 22-3. Complications were considered minor when they did not require any surgical treatment, whereas major complications required surgery. Minor complications grade 1 may include hematoma, compound fractures, and transient nerve palsies. Minor complications grade 2 include subluxation of the head of humeral prosthesis. Major complications grade 3 may include substitution of polyethylene bushes in K.M.F.T.R. prostheses, additional bone grafting in massive allografts, surgical debridement in infected wounds, and plastic surgery in wound sloughs.

Major complications grade 4 or 5 may include deep infections, allograft rejections, and prosthesis loosening. Complications grade 4 and 5 represented complete failures of the primary reconstruction and therefore were the worst complications observed.

Functional results were evaluated according to the newly modified functional evaluation system proposed by Enneking [10] at the M.S.T.S. for a clinical trial. This new system evaluates the following criteria in the lower limb: pain, function, emotional acceptance, supports, walking ability, and gait. For the upper limb the criteria considered include: pain, function, emotional acceptance, hand positioning, dexterity, and lifting ability. Each of these criteria is divided into six grades, from 0 to 5, so that a total score can be obtained for the limb and rated in a percentage referred to the total score that a normal limb can reach (30 points). All patients were followed after surgery with clinical examination, x-rays of the operated limb, and chest x-rays every 2 months for 2 years, every 3 months in the third year, and subsequently every 6 months.

Results

Surgical margins, according to Enneking's Classification [7], were radical in eight cases (6%), wide in 110 cases (76%), marginal in 12 (8%), intralesional

Table 22-4. Disease-free survival (DFS) and relapses in 144 patients, related to surgery and grade of necrosis

		Resection	Amputation	Rotationplasty
Patients DFS	GR	86	4	7
	PR	22	6	1
Metastases	GR	13	1	1
	PR	1	2	—
Local recurrences	GR	1[a]	—	—
	PR	—	—	—

GR = good responders; PR = poor responders.
[a] This patient also had lung metastases.

in seven (5%), and wide but contaminated in seven cases (5%). When related to surgery performed, surgical margins in amputations were radical in eight cases and wide in five; in rotationplasties they were wide in eight cases and intralesional in one; in limb-salvage procedures they were wide in 97 cases, marginal in 12 cases, intralesional in six and wide but contaminated in seven cases.

Tumor necrosis induced by preoperative chemotherapy was ≥90% in 112 patients and <90% in 32 patients. Therefore, 112 patients were judged good responders (78%) and 32 poor responders (22%) (Table 22-4).

Oncological results were evaluated in December 1990, at an average follow-up of 30 months (min. 12 to max. 51): 126 patients remained continuously disease free, 18 had lung or bone metastases, and one of these 18 patients developed a local recurrence. The local recurrence was observed at 21 months from the operation (hemiresection and bone allograft in a small osteosarcoma of the distal femur involving the lateral condyle) in a patient who had a good necrosis and a wide but contaminated surgical margin. The actuarial disease-free survival curve is reported in this volume by Bacci et al. [11].

In the 18 patients who had metastases, the time to relapse ranged from 3 to 36 months (average 17.7 months), and the first metastasis was in the lungs in 15 cases and in bone in three. Of these 18 patients, 10 are presently alive and disease free (at 3–21 months from the treatment of metastasis), four are alive with uncontrolled disease, and four have died.

In Table 22-4 the data concerning type of relapse related to the grade of necrosis and surgery performed are summarized. Related to the rate of tumor necrosis, the continuously disease-free survival rate was 86.6% (97/112) for patients who were good responders and 90.6% (29/32) for poor responders. This difference is not statistically significant. There were no differences in the continuously disease-free survival related to surgery: 88.5% in limb-salvage procedures, 76.9% in amputations, and 88.8% in rotationplasties.

Surgical complications observed are reported in Table 22-5, related to the surgery performed and reconstructive techniques used. Complications observed

Table 22-5A. Postoperative complications

	No recontruction	Amputation	Rotationplasty	Prothesis		Osteoarticular allograft
				Humerus	Lower limb	
Nerve palsy				1	10	1
Graft fracture						
Prosthesis loosening					2	
Subluxation of prosthesis				1		
Prosthesis failure				3	2	
Osteosynthesis devices failure			1			
Fracture					1	
Delayed union[a]						
Postoperative thrombosis			1			
Extrusion of cement				1		
Patellar tendon detachment					1	
Wear of polyethylene bushes					24	
Hematoma					2	
Wound slough			1	1	3	
Infection				2	3	

[a] Requiring bone grafting or vascularized fibula.

Table 22-5B. Postoperative complications

	Intercalary Allograft	Allograft arthrodesis (rod or plate)	Rod or plate + cement	hemiresection + graft	vascularized fibula
Nerve palsy		4			1
Graft fracture	2			1	6
Prosthesis loosening					
Subluxation of prosthesis					
Prosthesis failure					
Osteosynthesis devices failure	1	2	5	1	1
Fracture					
Delayed union[a]	1	4			
Postoperative thrombosis					
Extrusion of cement					
Patellar tendon detachment					
Wear of polyethylene bushes					
Hematoma					1
Wound slough		2			
Infection			1		

[a] Requiring bone grafting or vascularized fibula.

Table 22-6. Grade of complications in surgical procedures

Compl.[a]	Amputation (13)	Rotationplasty (9)	Resection (122)
Grade 1			23
Grade 2			1
Grade 3		3	48
Grade 4			18
Grade 5			1
Total	0	3	91

[a] Complication.

were also graded according to the above-mentioned classification (see Table 22-3), and the results of this grading are reported in Table 22-6. In 16 cases a nerve palsy was observed postoperatively: in 15 patients the nerve involved was the peroneal nerve and in one it was the radial nerve. All but one of the peroneal palsy and the radial nerve palsy were transient; therefore, there were 15 grade 1 complications and one grade 3 complication. The permanent palsy of the peroneal nerve was considered grade 3, since it required a muscular transposition. Ten fractures of the grafts were observed in eight patients (two patients had two stress fractures, each one in vascularized fibulae). Six stress fractures in vascularized fibula reconstructions did not require any surgical treatment (six grade 1 complications). In one osteoarticular allograft of the elbow, the fracture required substitution of the allograft (grade 4 complication). In two cases of intercalary allograft, a fracture required surgery: in one of these patients it consisted in new apposition of autografts from the iliac crest and a new osteosynthesis (grade 3); in the second case at the reconstruction a vascularized fibula was added (grade 3). In another patient who had had a hemiresection of the tibial plateau and allograft, an articular fracture of the graft was observed that healed spontaneously (grade 1). Two cases of loosening of prostheses were observed (both were in the lower limb): one had a beginning loosening of the stem at 34 months and did not require surgery because of "autosetting" of the stem anchorage (grade 1) and consequent clinical and radiographical improvement; the second had loosening of the acetabular cup at 24 months and required substitution of this component (grade 4). In one case of humeral prosthesis there was a subluxation of the polyethylene head of the prosthesis at 25 months: surgery was not required and only a minor adverse effect on the functional result was observed (grade 2).

Prosthetic failure was observed in five cases: three were cemented humeral prosthesis that had the stem broken in two cases and bent in one and required substitution with a new M.R.S. prosthesis (three grade 4 complications); two were uncemented lower limb prosthesis (K.M.F.T.R.) that had breakage of the stem and required a new prosthetic implant (grade 4). Failure of the osteosynthesis devices was observed in 11 patients: these were breakage of rods or plates, or loosening of plates, and required substitution

of the reconstruction in seven cases (four of which had secondarily a vascularized fibula) and a new osteosynthesis in the remaining four (seven grade 4 complications and four grade 3).

In five other cases a delayed union or non-union was surgically treated before causing failure of osteosynthesis devices: one of these patients had a diaphyseal intercalary allograft and was treated at 11 months with apposition of new bone grafts from the iliac crest (grade 3); four patients had arthrodeses with allografts requiring new bone grafts in three cases (grade 3) and a vascularized fibula in one case (grade 4). In one patient who had had a resection of the distal femur and reconstruction with a K.M.F.T.R. prosthesis, a fracture of the neck of the femur (proximally to the prosthetic stem) was observed at 9 months: this complication was treated with a new reconstruction with a total femur K.M.F.T.R. prosthesis (grade 4). In one patient who had a rotationplasty 1 day postoperatively, a vascular thrombosis (of both the artery and vein) was observed, requiring immediate surgical revision and repair of the vascular anastomosis (grade 3). In a patient who had had a proximal humerus resection and a cemented humeral prosthesis, a revision operation was required 3 months later to remove the cement extrused into the olecranon fossa (grade 3). In one case of K.M.F.T.R. prosthesis of the proximal tibia, there was detachment of the patellar tendon that required a surgical reinsertion of the tendon to recover active extension of the knee (grade 3).

In 24 K.M.F.T.R. prostheses, there was wear of the polyethylene bushes, requiring substitution: in 10 patients the bushes have already been substituted, whereas 14 are waiting for treatment (all considered grade 3). In three patients a hematoma developed postoperatively, requiring surgical drainage (grade 3). In seven patients there was a wound slough requiring revision of the wound and/or a new suture (grade 3). Six cases of deep infections were observed: two in humeral prostheses, three in K.M.F.T.R. prostheses, and one in an arthrodesis with Kuntscher rod and cement. Three of these patients healed after repeated surgical debridements and local antibiotic therapy (grade 3), two required also removal of the prosthesis and later a reconstruction with a new prosthesis (grade 4); in one patient with infection of a K.M.F.T.R. prosthesis an amputation was necessary (grade 5). In the whole series of 144 patients, 94 complications were observed in 78 patients, thus resulting in 54% (78/144) of the patients being affected by surgical complications. Each of the patients with complications had an average of 1.2 complications.

No complications were observed in amputations and there were three grade 3 complications in nine rotationplasties (33.3%). Ninety-one of the 94 complications were registered in limb-salvage procedures (91/122, 75%), and most of these depended on the reconstruction technique used and not on the surgical procedure of resection.

Minor complications observed in limb-salvage procedures were 24/122 (20%). Major complications were 67 over 122 resections (55%). Only one

complication required amputation (and was therefore graded 5) over 122 resections (0.8%).

Functional results assessed according to the above-mentioned Enneking's new system [10] were divided into three groups, depending on the percentage of score referred to a normal limb having 100% of function:

1. Patients with a percentage above 75%
2. Patients between 50% and 75%
3. Patients with less than 50%

Of the 144 patients, 10 were not evaluable for different reasons, whereas 134 patients were evaluated (93%), including seven rotationplasties, 11 amputations, and 116 resections. All the seven rotationplasties had a score between 50% and 75%. Of the 11 amputated patients, four (36%) had a score between 50% and 75%, and seven (64%) had a score of <50%. Of the 116 evaluated patients who had limb-salvage procedures, seven (6%) had a score of 75%, 81 (70%) were between 50% and 75%, and 28 (24%) had <50%. A 100% score was obtained only in one patient who had a diaphyseal resection of the tibia and reconstruction with plate and cement.

Discussion

Complications

In the present series a high incidence of postoperative complications were registered, with 78 of the 144 patients (54%) having at least one complication. This incidence of complication is not different from the 55.4% already reported with neoadjuvant chemotherapy in a previous paper of the Istituto Rizzoli [6]. In the present study no complications were observed in amputated patients and only three were observed in rotationplasties; consequently the rate of complications is higher in limb-salvage procedures (74.5%).

It is already well accepted in the literature that limb-salvage procedures result in a higher rate of complications than do amputations [1,6] and that neoadjuvant chemotherapy influences the incidence and severity of complications [1,6]. Most complications require additional surgery and may lead to loss of the involved limb. Moreover, major complications may cause deviations and/or delay of chemotherapy treatment and therefore may influence the scheduled dose/intensity of chemotherapy regimens that seem to be crucial for the effectiveness of the treatment. The real impact of these deviations or delay of chemotherapy on survival is not known or not easily evaluable, but may be important [1]. Minor complications were observed in 20% of patients who had limb-salvage in the present series, whereas major complications were seen in 55%. It must be stressed that major grade 3 complications included 24 cases of substitution of polyethylene bushes in K.M.F.T.R. prostheses that required minor surgery.

Complications requiring surgery in all but one case could be successfully managed without causing loss of the involved limb or removal of the reconstructive device without a new reconstruction. Only one complication (a deep infection) required an amputation (and was therefore graded 5).

Functional results

The evaluation of the functional results after surgery for osteosarcoma is a critical task, since—whatever the system used—it is extremely difficult to compare different types of surgery, and particularly different types of reconstructions after limb-salvage. It must be also stressed that different reconstructive techniques may require a different recovery time and offer a different durability. In the present study the functional results achieved with limb-salvage were considered above 50% of a normal limb function in most patients (76%), while amputations in more than half of the patients had a score of less than 50% and the rotationplasties did not register any functional result of less than 50% of the score. However, these data should be regarded cautiously in consideration of the difficulties in trying to make an objective functional evaluation. In the literature data have been reported for the lower limbs on gait analysis of patients with limb salvage for osteosarcoma [1,12]. Also with gait analysis it was difficult to measure and interpret the data since patients with limb-salvage have different methods of gait compensation, even with the same type of reconstruction. Analysis of oxygen consumption has been used in evaluating the energy expended during gait, and therefore the function of the lower limb. The studies reported indicated that mobile replacement of the knee requires less energy (in terms of oxygen consumption), followed by rotationplasties and arthrodeses, which again require less energy than above-the-knee amputations [1,12].

Surgical indications

Limb-salvage surgery has been widely demonstrated to be successful in the treatment of osteosarcoma when associated with chemotherapy [1,2,4–6,11]. In the present study the very low incidence of local recurrence (1/144 patients) and the functional results obtained seem to further encourage the use of limb salvage in osteosarcoma. Certainly neoadjuvant chemotherapy allows more conservative surgery, but it does not justify inadequate surgical margins [3]. Therefore we feel that in defining surgical indications, first priority should be given to the oncological adequacy of surgery and wide removal of all of the tumor without contamination is required. Thus, complete staging at diagnosis with modern diagnostic tools and also "restaging" after preoperative chemotherapy are mandatory. When the staging indicates that it is not possible to achieve adequate margins with limb-salvage, then ablative surgery should be favored. If wide margins can be achieved with limb-salvage, then a resection is preferable.

In "expendable" bones (scapula, ribs, clavicle, proximal fibula) no reconstruction is required. In all other sites one must choose the type of reconstruction. At the Bone Tumor Center of the Rizzoli Institute presently, with regard to articular reconstruction, after resection in the treatment of classic osteosarcoma prostheses are mostly used. In the proximal humerus a cemented prosthesis is used, since the non-weight-bearing condition does not cause problems of loosening. In lower limb reconstructions with K.M.F.T.R. uncemented modular prosthesis is used. Exceptions to the use of a prosthesis in our indications are in the knee: (1) those cases of resection of the distal femur in which the muscle excision required involves more than three muscular heads of the quadriceps, since the functional result of the prosthesis and the prospective of durability would be worse [13]; (2) resection of the proximal tibia requiring sacrifice of the extensor apparatus or extraarticular resection. In these situations we prefer to offer the patient an arthrodesis, usually performed with a massive bone allograft. Other situations for which we give first priority to the use of bone allografts are diaphyseal locations of the tumor or rare cases of a metadiaphyseal location in which the epiphysis can be preserved: in these cases intercalary allografts are used in the reconstruction.

The indications for the use of osteoarticular allografts (or hemiarticular allografts) in the treatment of osteosarcoma in our experience are not frequent, since a wide removal of the capsule and ligament insertions are often required in the resection, and an adequate soft tissue coverage to the allograft can rarely be given. Consequently we use osteoarticular allografts in the reconstructions only in some rare osteosarcomas that are intracompartmental or have a little extraosseous component.

Special problems are raised for the surgeon in limb salvage in children [14], mainly in the lower limbs. Our indications favor rotationplasty [9,15] (Figure 22-4) in patients usually under 11 years of age when a limb length discrepancy above 10 cm at the end of growth would be expected. In patients aged more than 11 years and/or with an expected final limb length discrepancy between 5 and 10 cm, we prefer reconstruction with an arthrodesis with bone allograft and delayed lengthening in the following years, which can be obtained with different techniques (i.e., the Ilizarov technique). If the expected final limb length discrepancy is less than 5 cm, then an arthrodesis can be performed along with a contralateral epiphysiodesis. Finally in very rare selected cases with a metadiaphyseal location not involving the epiphysis a careful intraepiphyseal resection can be done along with reconstruction with a vascularized fibula in conjunction with a bone allograft (a "combined" graft).

Acknowledgment

This work was supported in part by the Rizzoli Research Fund.

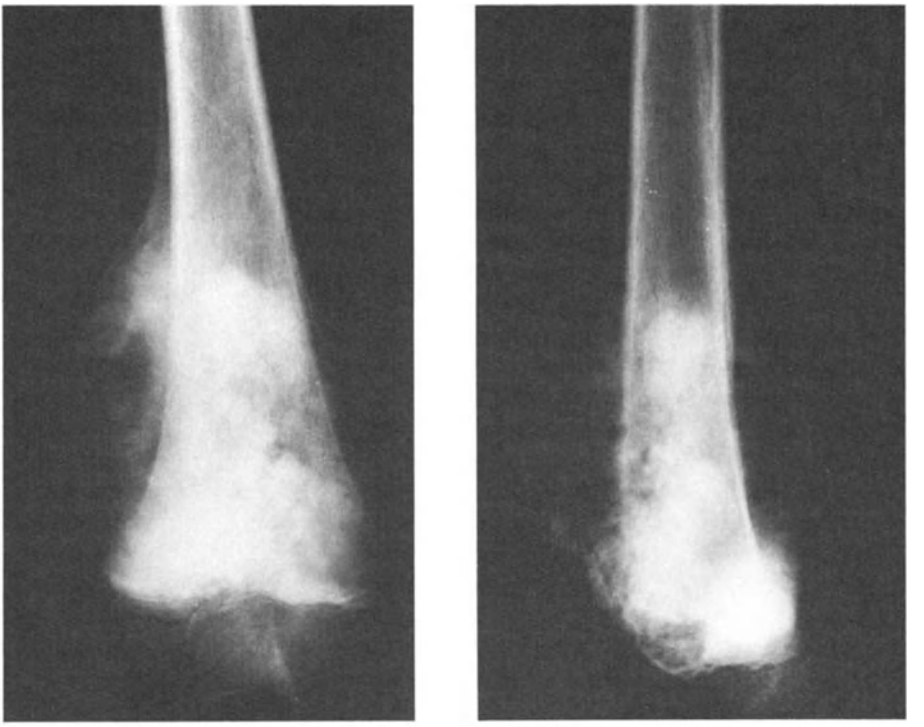

Figure 22-4. Osteoblastic osteosarcoma in a 7-year-old female. **A, B**: Standard x-rays of the distal femur showing the involvement of the growth plate.

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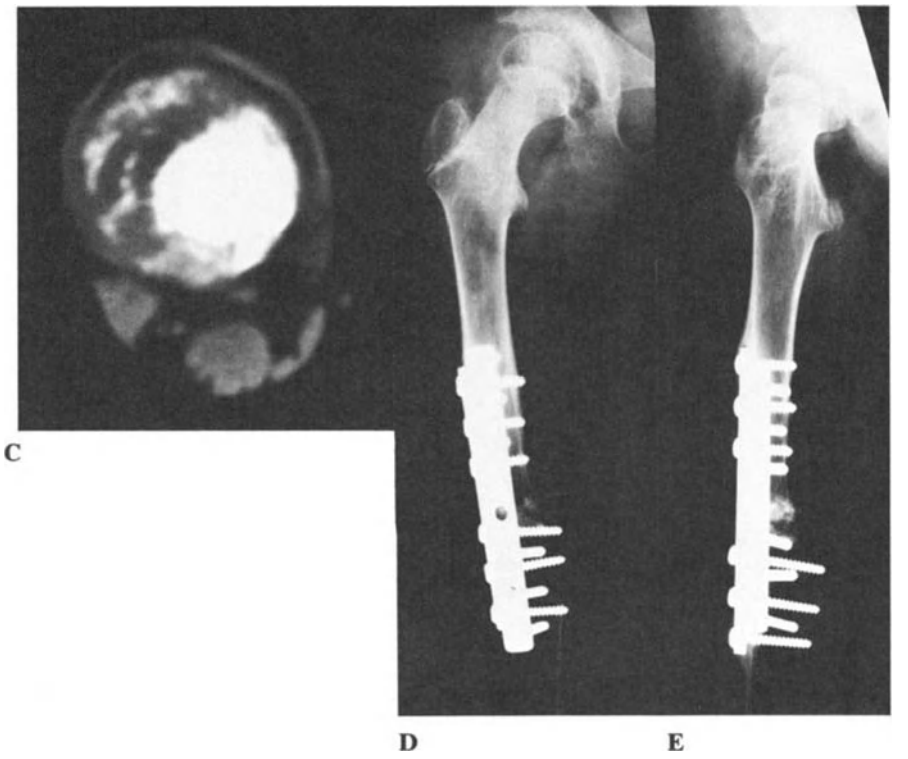

Figure 22-4. (cont.) **C**: CT scan shows the extension of the tumor in the adjacent soft tissue. **D, E**: Wide resection of the tumor and rotationplasty.

induced by chemotherapy—regional mapping of viable and nonviable tumor. Cancer 56:1515–1521, 1985.
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23. Limb sparing for skeletally immature patients with osteosarcoma: The expandable prosthesis

Samuel Kenan, Daniel P. DeSimone, and Michael M. Lewis

In the last decade there have been dramatic improvements in chemotherapeutic protocols for osteosarcoma and, as such, there has been a tremendous increase in the disease-free survival period [1,10,13]. Therefore many more children will survive to adulthood after successful treatment of their tumors. Limb-sparing surgery has become an acceptable alternative to amputation in adults with primary bone tumors [5]. Reconstruction of a large segmental defect with the loss of one or more growth plates in a growing child continues to pose a difficult challenge. Recent advances in the field of orthopedic bioengineering have led to the development of prostheses that are durable enough for children and can be periodically expanded to compensate for limb length discrepancy [11,16–18].

Local recurrence of malignancy is a devastating complication of limb-sparing surgery at any age. It has been reported as 5–7% in limb-salvage surgery compared to a 2% recurrence rate following the same level of amputation [2,20,21]. How this difference affects the long-term survival is a very important question. A worldwide multiinstitutional study on a large group of patients with osteosarcoma clearly indicates that survival after resection for limb sparing was similar to that after the appropriate level of amputation. More precise surgical planning prior to resection may help to reduce local recurrence of the disease [5, 7–9,15,21].

In this review the authors wish to present their experience with the use of the Lewis Expandable Adjustable Prosthesis (L.E.A.P.) and the problems that may arise during the growing years. Between 1983 and 1989, thirty-three children with high-grade osteosarcoma underwent limb-sparing surgery with the placement of a L.E.A.P. Two patients had stage III lung metastases at the time of initial presentation. All patients received preoperative chemotherapy. There were 18 females and 15 males. The ages ranged from 3 to 16 years. There were 10 children less than 8 years; 6 children between 9 and 12 years, and 17 children between 13 and 16 years. The anatomic locations included the distal femur in 15 children, the proximal femur in one child, the proximal tibia in 10 children, the entire femur in four children, and the proximal humerus in three children. Periodic limb lengthening was necessary in all patients younger than 13 years. The purpose of using the expandable prosthesis in

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 ISBN 0–7923–1905–2

children above 13 years was to be able to intraoperatively adjust the length of resection and to overlengthen the limb to compensate for possible limb length discrepancy.

Functional assessment was performed using the criteria and rating scale recommended by the American Musculoskeletal Tumor Society (AMTS) [6].

Local control of malignancy was achieved in all patients except two who presented with osteosarcoma at the proximal tibia with stage III lung metastases. The total amount of lengthening required varied from 2 to 7 cm. One patient with a distal femoral replacement reached the maximal lengthening of 7 cm, and her prosthesis was revised with a new expandable prosthesis, which was lengthened an additional 3 cm, allowing her to reach skeletal maturity with limb length equality. Newer prosthetic designs with improvement of the expansion mechanism have allowed for reliable lengthening.

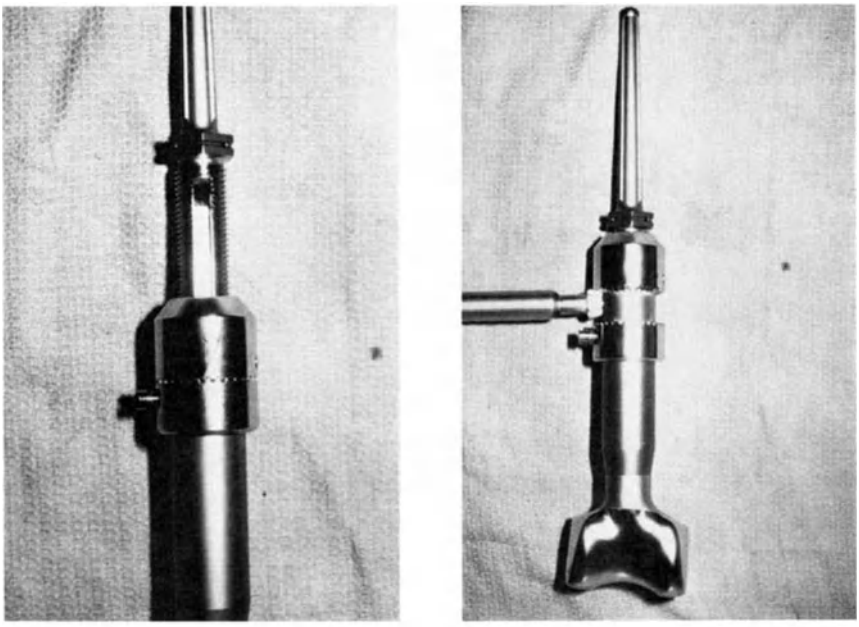
Our functional results with distal femoral replacement were excellent in three, good in two, fair in four, and poor in two patients. In patients younger than 12 years, the migration of the prosthetic stem within the femoral shaft and subsequent remodelling of the bone in relation to the new position of the stem was a major problem. At the femoral level the prosthesis is subjected to tremendous shearing forces created by the loss of the normal muscle envelope, which was sacrificed as part of the surgical margin. This resulted in stem loosening in nine patients, which eventually required surgical revision. All revisions were successful, with good functional results.

Proximal tibial replacement is frequently associated with a large surgical defect, creating a problem with soft tissue coverage of the prosthesis and wound closure. Our functional results with 10 children who underwent proximal tibial replacement included three with good results, four with fair results, and three with poor results. There were two early and one late infections. Two were revised successfully and one required amputation.

Proximal tibial replacement required partial or total sacrifice of the patellar tendon. This results in the inability to overcome gravity in full extension. All patients, however, had full passive extension and active flexion. The lack of active extension did not significantly interfere with the ability to walk.

A solution to the problem of limb length inequality in growing children after wide resection for osteosarcoma has come from the development of telescopic implants that can be serially lengthened to match bone growth of the contralateral limb [12,16,19].

The L.E.A.P. that we have been using is made of a hollow titanium alloy tube assembled over a threaded shaft and fitted with a Jacobs chuck adjustable ring (Figure 23-1A and 23-B). Expansion is achieved by a simple but invasive procedure, requiring overnight hospitalization. The expansion amount varies from 1 to 2 cm at a time and could be repeated up to a total length of 7 cm (Figure 23-2A to 23-2C). Other prototypes of expandable prostheses with different expansion mechanisms are also available, including one designed by Scales that also requires an invasive procedure for lengthening [19]. For several other prototypes now undergoing experimental testing use an external force



A B

Figure 23-1. New model of expandable distal femoral prosthesis with ring locking device, hexagonal collar, and fluted stem. **A**: Closed. **B**: Opened.

that is converted into hydraulic energy, and another uses an electromotor with an inductive power supply. A new and promising design utilizes a rotating magnetic field [14].

We have had no complications related to the expansion procedure and are very satisfied with our results. We will continue to use this method of expansion until a better noninvasive mechanism is designed that will overcome the forces imposed on the expansion component by the surrounding fibrous membrane.

The vast majority of our complications were related to loosening and subsequent migration of the femoral stem of the prosthesis. Thus, the major problem facing us is limb-salvage surgery is the longevity of the prosthesis fixation [4]. The problems of fixation and stress distribution are more intensified in growing children. In these patients there is an increased bone turnover, and the medullary cavity diameter and its shape show continual remodelling, which may affect long-term fixation. Other factors that may affect the durability of the fixation are the level of resection and the amount of skeletal muscle that is resected. In order to maintain axial loading without abnormal stress forces, one must have intact muscle. In the proximal femur, where the normal loading forces are approximately 0.25–4 times the patient body weight, one must have intact muscle, such as the quadriceps, adductors, hamstrings, and

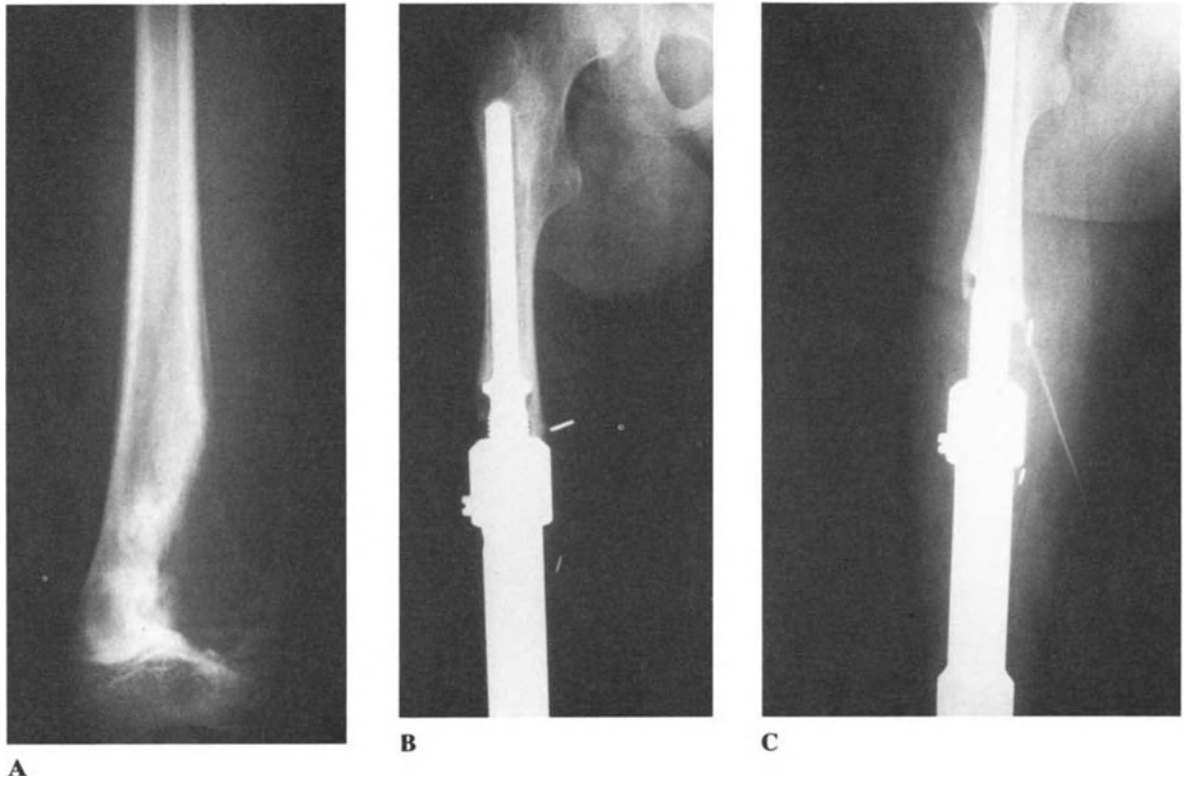


Figure 23-2. **A**: Plain roentgenogram of an 8-year-old male with osteosarcoma of the right distal femur. **B**: Following wide resection of the distal femur and reconstruction with an expandable prosthesis. **C**: The patient is fully ambulatory after 3 years and two lengthening procedures.

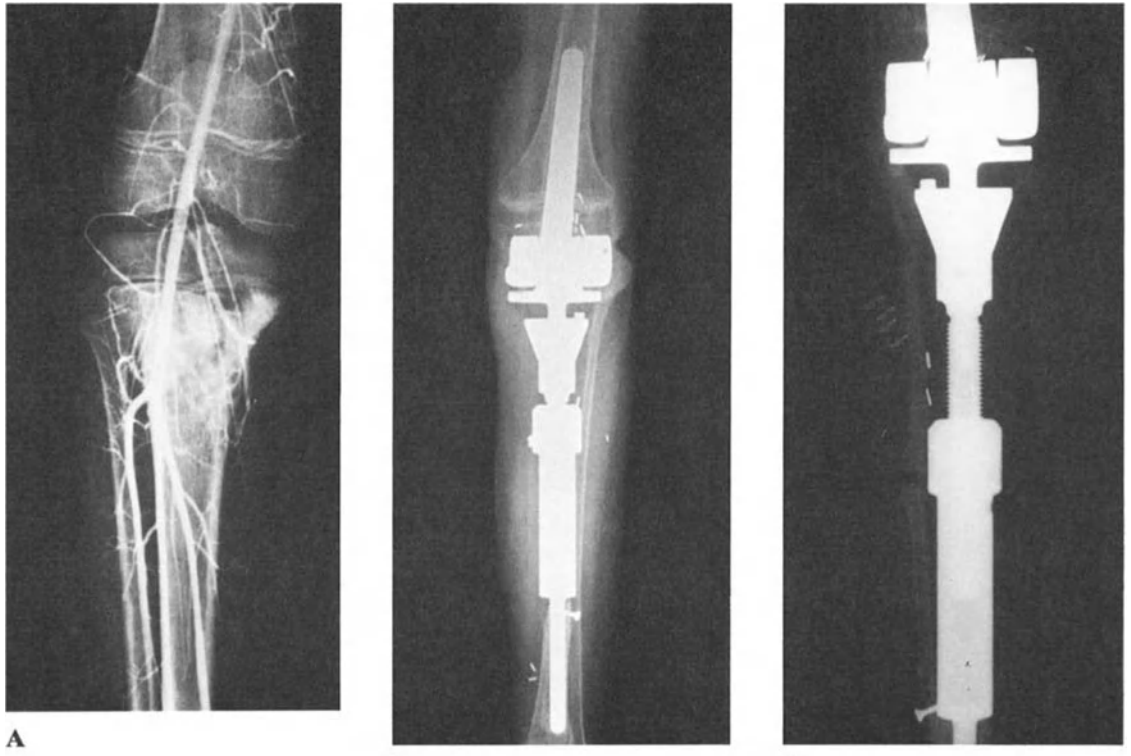


Figure 23-3. **A**: Arteriogram of an 8-year-old male with osteosarcoma of the proximal tibia. **B**: Following wide resection of the proximal tibia and reconstruction with an expandable prosthesis. **C**: Follow-up 3 years later, after the third lengthening.

iliotibial band. Once this muscle balance is compromised, it causes a shift in loading forces and consequently the creation of shear forces that eventually will lead to loosening and subsequent stem migration. In cemented prostheses the bone, prosthesis, and cement are subjected to cyclic oscillating forces of 4 million times per year. Cemented prostheses have the known complication of cement granulomatous disease, bone resorption, with the loss of critical bone stock and stress shielding. Taking these factors into consideration, the preferred method of fixation in growing children is a cementless press-fit prosthesis [3].

Limb-sparing surgery in growing children has proven to be very effective from an oncologic standpoint. When performed by experienced surgeons, limb sparing neither compromises the survival nor significantly increases the rate of local recurrence, and the disease-free survival period has been shown to be equivalent to that of amputation. The expandable prosthesis addresses the problem, of limb length inequality (Figure 23-3A to 23-3C). Several designs for expansion are currently in clinical or experimental use. The main problem with the noninvasive prosthesis is overcoming the extreme forces across the expansion component created by a sleeve of dense fibrous connective tissue. Now that chemotherapy has significantly expanded the lifespan of these children, we are confronted with the problem of prosthesis fixation longevity. New methods of prosthesis fixation combining biological fixation with newer stem design will increase the longevity of fixation. Once growth ceases, the expandable prosthesis can be replaced by an adult prosthesis or a biologic substitute.

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24. A modular endoprosthetic system, noninvasively extendable, for young patients with osteosarcoma

Bart Verkerke, Heimen Schraffordt Koops, René P.H. Veth, Liedeke Postma, and Henk J. Grootenboer

Introduction

Patients having a malignant bone tumor (usually at the distal femoral metaphysis) can be treated with limb-saving surgery. Resection of the affected region can be reconstructed with the aid of an endoprosthetic system [1–3]. A modular system has many advantages [4,5]. With a limited number of modules many different compositions can be created. Another advantage is that a modular endoprosthesis allows replacement of one component for another, in case of failure or merely to adapt the endoprosthesis to altered needs of the patient. Existing fixations to bone can remain unimpaired.

When an endoprosthetic system is used to replace resected tumor involved bone in a growing patient, the endoprosthetic system should include an extendable element to prevent leg-length discrepancy, since resection of the affected region implies the loss of the distal femoral and proximal tibial epiphyses. Several lengthening systems have been developed, all of which must be adjusted invasively [1–3]. A lengthening element that can be adjusted *non*invasively has many advantages because operations for adjustment can be avoided. Consequently the risk of infection is diminished.

The endoprosthetic system, that we have developed [6] is of the modular type and includes an element that can be adjusted noninvasively to match the growth of the other leg.

Description of the modular endoprosthetic system

Besides three lengthening elements with lengthening capacities of 40, 60, and 80 mm, the endoprosthetic system (Figure 24-1) contains:

- Two semiconstrained knee prostheses, in both the left and right configurations; one type will be used in the case of a femoral tumor, the other in the case of a tibial tumor
- A hip prosthesis in the left and right configurations to be provided with various heads of different materials and sizes. The anteversion angle is adjustable. For acetabular replacement each system with appropriate cup diameters can be used

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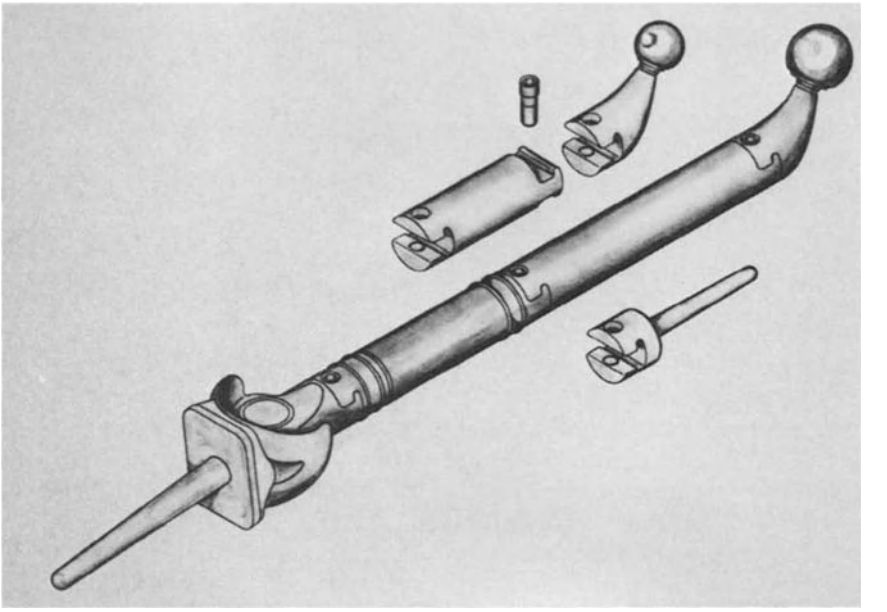

Figure 24-1. Extendable Modular Endoprosthetic System.

- Connectors—20, 40, 60, 80, and 100 mm long—to connect the knee component and the lengthening element with the hip component. The connectors are composed of a hollow shaft with two lids. The connectors are available in several lengths to create an endoprosthesis of an appropriate length.
- Fixation elements to couple the elements to the remaining bone.

All elements can be linked together with a specially developed universal connection to assemble prostheses of any composition for each patient. The endoprosthetic system is suitable for girls from 8 years old and boys from 10 years old with a tumor in the femur, the knee, or the proximal tibia. The composition of the endoprosthesis depends on the length of the resected bone, on the kind of resected bone (femur or tibia), and on the expected growth of the normal leg compared with the involved leg.

Lengthening element

The lengthening element [7] (Figure 24-2) consists of two tubes and is adjusted noninvasively. This is achieved by using an external rotating magnetic field (Figure 24-3), which causes rotation of a small permanent magnet in the inner tube. The magnet drives a motion screw via a gearbox. This screw rotates in the outer tube and forces the two telescopic tubes apart. The polygonal shape of the inner tube prevents rotational movement between the

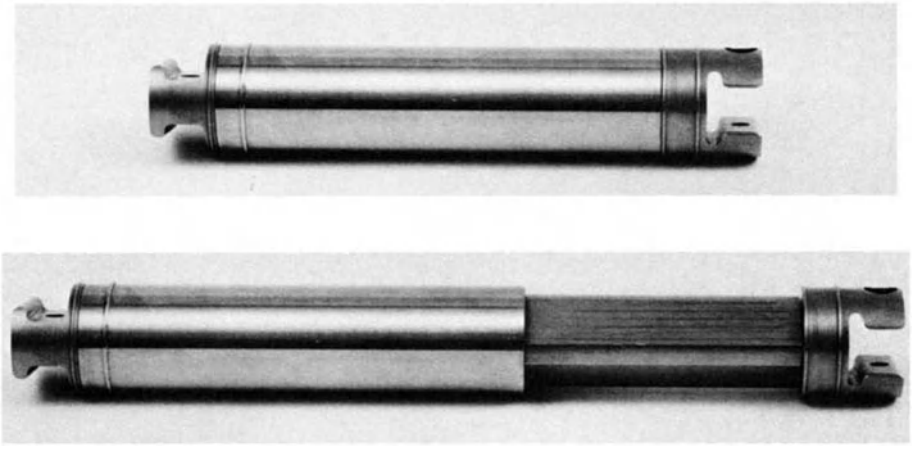

Figure 24-2. Lengthening element without bellows before (above) and after (below) full extension.

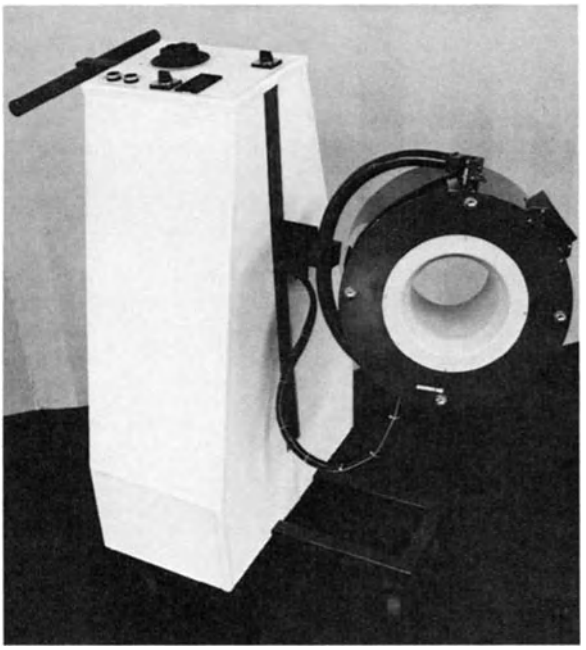

Figure 24-3. Electromagnet on trolley.

tubes. To shield the lengthening element from moisture, a bellows made of silicone rubber is glued to the lengthening element.

Universal connection

To link the different modules of the endoprosthetic system, a universal connection was created [9]. After assembly possible play is eliminated by using a bolt that pulls the two parts together. Advantages are low assembly and disassembly forces, and miminal (≤15 mm) implant elongation during manipulation so that the surrounding tissues are protected.

Fixation elements

The endoprosthesis, composed from the Modular Endoprosthetic System, is fixed to the remaining part of the femur with a fixation element, having custom-made, press-fit stem on which the contours of the bone are transposed. Extracortical side plates with unicortical screws provide for the primary rotation stability. The fixation to the remaining part of the tibia is performed by a stem cemented into the medullary canal. At the Groningen University Hospital these fixations have been applied for the fixation of prostheses [8]. After an observation period of 2–8 years no failure has been observed.

In-vitro tests of components

Passive mechanical strength of the lengthening element, universal connection, and connectors were successfully tested by subjecting several prototypes to maximum and fatigue torsional and bending loading. To check the effectiveness of the power supply of the lengthening element, a prototype was load with 450-N, 30-mm eccentric acting and was lengthened by a magnetic field of 0.02 T. Extension appeared to be possible, even after 5 days of standing still. While lengthening, forces of at most 200 N are expected [10]; this test verified the proper function of the lengthening element. The bellows and glue bonding proved to be watertight: after 4 days of immersion in Ringer's solution no moisture infiltrated the prototype.

In-vivo tests of components

Materials

The lengthening element and the universal connection were tested in vivo. Two identical endoprostheses were used. They consisted of two parts, the lengthening element with the distal stem and the proximal stem. Both parts were fitted with the universal connection. The maximal extension of the endoprosthesis was 28 mm. In total six animal experiments were performed.

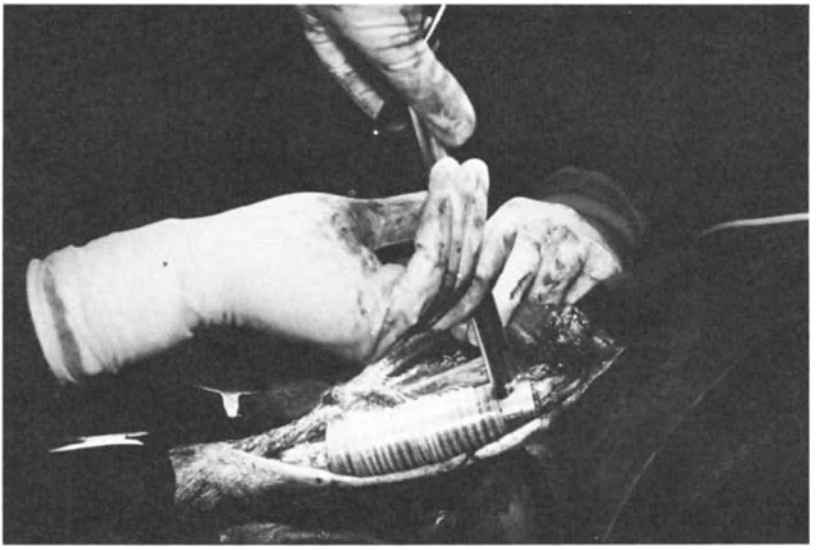

Figure 24-4. Endoprosthesis implanted in a goat.

Operative technique

The prostheses were implanted in the tibia of adult goats. After resection of the diaphysis of the tibia the two parts of the prosthesis were cemented into the medullary cavities, slid together, and fixed with a bolt (Figure 24-4). A plaster cast provided external support to the operated leg in order to prevent fracture of the bone. All animals tolerated the operation very well.

Extensions

Two weeks postoperatively lengthening was started. The goats were anesthetized to reduce the risk for fractures. Before and after each 4-mm extension an x-ray was taken (Figure 24-5) to check the actual elongation. This procedure was repeated every week until eight extensions were performed. In the last two animal experiments the final extension was postponed to 6 months and 1 year postoperatively to obtain information about the longevity of the prosthesis. The prosthesis was removed immediately after the last extension. Representative biopsies were taken for histological examination. Also, a bacteriological culture was prepared. The bellows was examined for signs of leakage, and the lengthening element was dismantled to check for signs of mechanical damage.

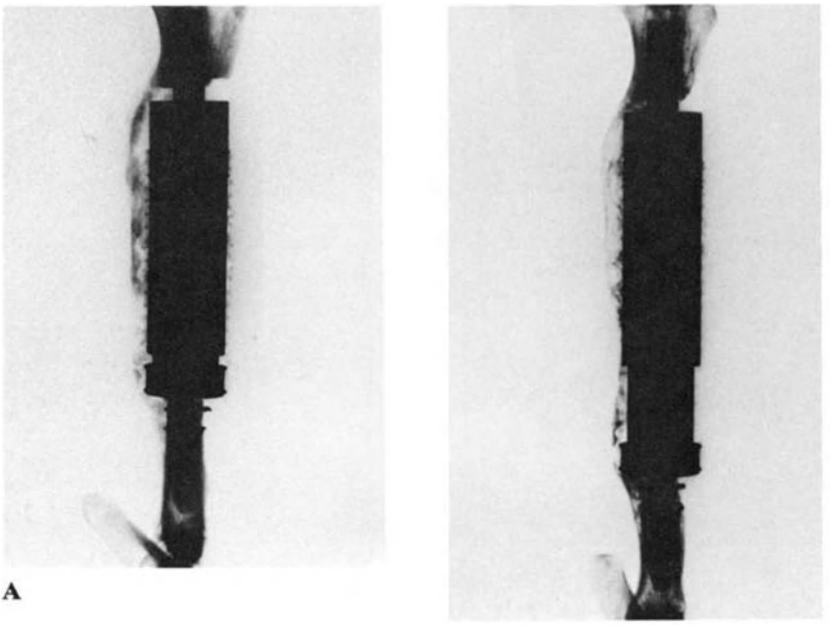

Figure 24-5. X-rays of the prosthesis. To the left of the prosthesis the bony bridge is visible. **A**: Before the first lengthening. **B**: After the last lengthening.

Results

All elongations but one were performed successfully, in spite of ectopic bone formation, which was about 5 mm thick and had bridged the prosthesis completely (Figure 24-5). The cause for the unsuccessful (first) elongation was use of a lower magnetic field. The bone bridge demanded a larger field then was calculated. Once the field was adjusted, no problems occurred. Analysis of the prostheses after sacrificing the animals revealed no mechanical problems. The histologic examination of the tissue around the bellows showed fat and connective tissue with some lymphoplasmacellular infiltrates, reflecting a nonspecific tissue reaction.

Patient safety

The only two materials that are brought in contact with body tissues are Ti6A14V (titanium-aluminium-vanadium alloy) and silicone rubber. Both materials have proven their biocompatibility and durability in many other clinical applications. In addition, the lifetime of the lengthening element of

approximately 7 years should suffice to cover the period between implantation and the end of growth. Then the expandable element can be replaced by an element with a fixed length. The strength of the applied magnetic field, at most 0.052 T, is far less than the magnetic field used in MRI, which is considered safe.

Conclusions

With the Extendable Modular Endoprosthetic System a correctly sized endoprosthesis can be designed for boys from age 10 on and girls from age 8 on, whose tibia or femur has been resected as treatment for a malignant bone tumor.

Future developments

The clinical research studies to finalize the development of the modular endoprosthetic system will start soon at the Groningen University Hospital. These studies will obtain scientific information on the patient/prosthesis interaction. If the assessment of the feasibility of the endoprosthetis system is positive, clinical trials will be started and will involve multiple centers. After successful clinical trials, the Extendable Modular Endoprosthetic System will be introduced to the market by a well-known company that already distributes endoprosthesis.

Acknowledgments

This research was supported by the Technology Foundation (STW), the Dutch Cancer Society (Koningin Wilhelmina Fonds), and the Groningen Pediatric Oncology Foundation (Stichting Kinderoncologie Groningen).

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25. A critique of techniques for reconstruction after internal hemipelvectomy for osteosarcoma

R.P.H. Veth, H. Schraffordt Koops, H.K.L. Nielsen, J. Oldhoff, G.J. Verkerke, and A. Postma

Introduction

Osteosarcoma may affect any bone but is mainly encountered in long bones like the femur and is rarely observed in flat bones [1]. According to Schajowicz [2] high-grade osteosarcoma occurs in the pelvic area in approximately 3.5% of cases. Involvement of the pelvis by paracortical osteosarcoma is even rarer (2%) [2].

A hemipelvectomy is a mutilating procedure in both somatic and psychical ways [1,4]. Artificial limbs do not fit properly in most patients [4]. From this point of view, a limb-saving procedure such as an internal hemipelvectomy seems to be justified [1,5]. However, due to the usually large size of these tumors, large resections of bone, joint, and soft tissue are often necessary in order to obtain adequate surgical margins. This is probably the main reason why reconstructions in this area most frequently do not yield an acceptable function. Either the bone is absent, which permits adequate fixation of the graft or implant, or the muscles and even nerves are absent, which are indispensable to activation of the reconstructed bony area and the affected limb. So the aim of each type of reconstruction is a compromise, and these reconstructions will never result in normal limb function.

Staging studies, as in all malignant tumors, are of the utmost importance. Magnetic resonance imaging and computed tomography provide information on the local extent of the tumor [1,7]. The critical structures are the ischial and femoral nerve, the iliac and femoral vessels, and the periacetabular region [1].

Critical review

In Enneking stage IIA and IIB tumors [12], like osteosarcoma, at least wide or radical margins should be obtained. In the pelvis one may distinguish three major types of resection, according to Enneking [1] (Figure 25-1):

I: Iliac; IA: iliac, including the gluteal muscles
II: Periacetabular; IIA: periacetabular including the hip joint;
III: Ischiopubic

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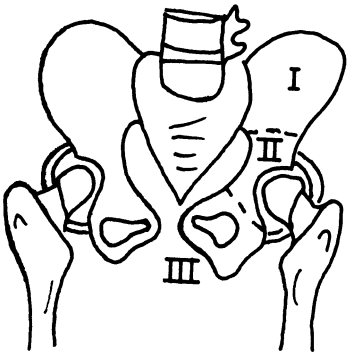

Figure 25-1. Pelvic resection types according to Enneking. I: iliac; II: periacetabular; III: ischiopubic.

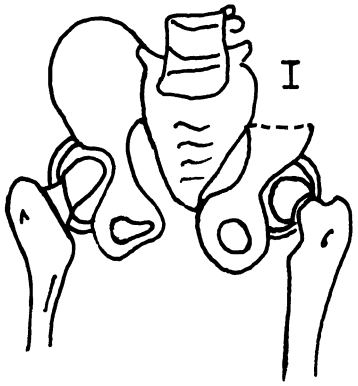

Figure 25-2. Type I resection.

To this classification one may add resection of the sacrum. These types of resection serve as the guidelines for the following review.

Type I and IA resection

In type I and IA resections (Figure 25-2): the hip joint is preserved and restoration of the pelvic ring can be accomplished by bone grafting between the sacrum and the remaining ileum [8,9,13]. Preferably this goal is met by interposition of a vascularized fibular graft. Allografts and prostheses are unnecessary in this area. The Winkelmann solution [17], in which a fusion is performed between the acetabulum and the sacrum, seems to be exaggerated. According to Enneking [1] the functional results of a IA resection,

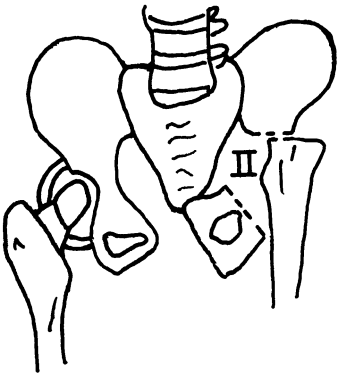

Figure 25-3. Type II resection and iliofemoral fusion.

which include both the gluteal muscles and the ischial nerve, are superior to those after hemipelvectomy.

Type II and IIA reconstruction

In the hypothetical assumption that only one third of the acetabulum has to be resected because of osteosarcoma, reconstruction is unnecessary [1]. In most cases of periacetabular osteosarcoma, however, the entire acetabulum has to be resected with the femoral part of the hip joint (Figure 25-1). This situation is a challenge for reconstructions. As all solutions have their drawbacks, we certainly do not pretend to know the best.

For instance an attempt at an iliofemoral arthrodesis (Figure 25-3) often ends up in a pseudarthrosis [1,6,10], and this iliofemoral pseudarthrosis should be compared functionally to a flail hip [1]. An ischiofemoral arthrodesis (Figure 25-4) often turns into a pseudarthrosis [3,6], and most patients who have undergone an ilio- or ischio-femoral fusion or pseudarthrosis end up with a leg length discrepancy [1,10], use one or two crutches or canes, and lack stability of the hip [14].

A successful fusion, either iliofemoral or ischiofemoral, can only be accomplished in 42% of attempted cases [10], despite the use of wire fixation at the site of the fusion and despite immobilization in a plastercast for a period of 6 months. Alternatives are the use of an allograft [14], prosthesis [4,15], or a combination of both methods.

The results of allograft reconstruction are conflicting. Several authors have mentioned good results, but the follow-up has been rather short; others stress that the risk of non-union, especially after chemotherapy, hampers the process of revitalization [7,14] and conclude that the use of an allograft is an experimental method of reconstruction [6,8].

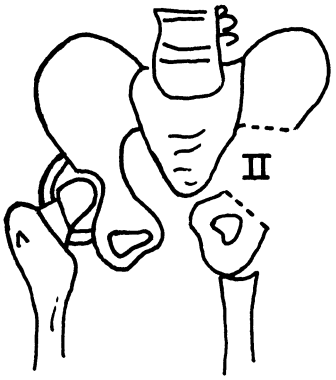

Figure 25-4. Type II resection and ischiofemoral fusion.

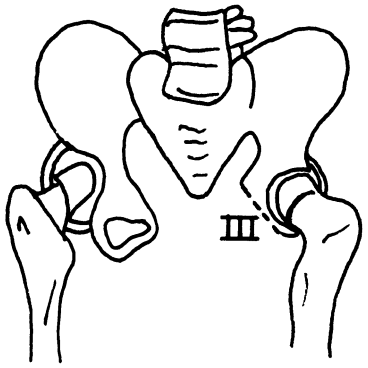

Figure 25-5. Type III resection.

The occurrence of a Charcot joint (trophic disturbance) and late fractures are important complications related to the use of an allograft [6,16]. Additionally, in cases in which the entire hip joint has been transplanted, early degenerative changes are to be expected [1]. Another example of periacetabular reconstruction is the use of an endoprosthesis. The options are a pelvic prosthesis [4,15,17] (Figure 25-5), including the hip joint or a saddle prosthesis [18]. Although the goal of the reconstruction, the restoration of the pelvic ring, seems to be met by the first option, the long-term results of this type of reconstruction are generally poor [6]; the main complications are loosening, dislocation, and infection. This also applies to the combination of an allografted acetabulum and a prosthetic hip replacement [16].

Implantation of a saddle prosthesis seems to result in fewer complications than in pelvic prosthetic replacement [18] (Figure 25-6). In all types of periacetabular reconstruction, general complications are to be expected, including infection, skin necrosis, nerve and vascular damage, and large hematomas [1,3,6,9,11,16]. The more complex the method of reconstruction,

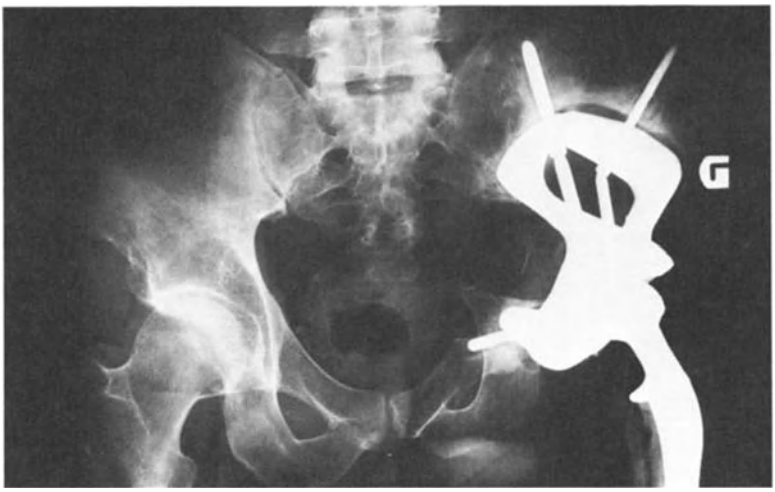

Figure 25-6. Pelvic prosthesis after type II resection.

the greater the risk of infection. The frequencies of these complications vary from 20% to 65%.

Type III ischiopubic resection and combined I, II, III resection

The type III resection (Figure 25-7) does not require complicated methods of reconstruction. The pelvic ring may be interrupted, but this does not impose extraordinary stress on the ipsilateral hip or sacroiliac joint [10]. A more complex situation occurs, however, when a combination of a type III resection with type II or type II and I has to be performed.

Essentially the situation after combined type II (IIA) and III resection is not different from a II or IIA pelvic resection. Similar methods can be used for reconstruction, such as an iliofemoral arthrodesis, pseudarthrosis, allograft, pelvic prosthesis, or saddle prosthesis [1,3,19]. Even a flail hip can be accepted. The most complex situation is created by a combined type I (IA), II (IIA), and III resection. This is the area where surgeons and engineers need utmost ingenuity. One possibility is a sacrofemoral fusion, with the aid of a (vascularized) fibular graft [16], but this procedure requires long-term immobilization and there probably will be a leg length discrepancy. In addition there is a major risk of fracture of the graft. Another possibility is the insertion of a hemipelvic allograft or endoprosthesis [20]. The complications of this procedure have been mentioned earlier in this paper. Winkelmann [21] has adapted the lower leg rotationplasty to the pelvic area and uses the rotated knee joint as a hip and the ankle joint as a knee. These patients

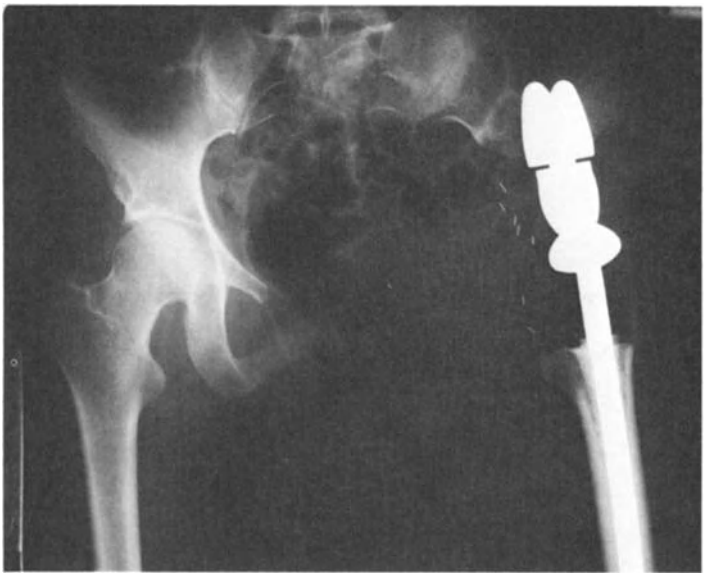

Figure 25-7. Saddle prosthesis after combined II and III resection.

obviously need an external prosthesis. Additionally the cosmetic appearance is still open for discussion.

In a combined I, II, or IIA resection, an acceptable reconstruction can be performed by a partial transposition of the ipsilateral femur, which fuses to the sacrum and the pubic/ischial bone. A proximal femur-hip megaprosthesis, with a special cup fixation (to the distal part of the transposed femur) could provide for mobility and restoration of the junction between the remaining ipsilateral femur and the reconstructed pelvis (Figure 25-8). The drawbacks of this method are the time that is required for the autograft to consolidate with the sacrum and ischial bone and the fact that the muscles that are used for this type of reconstruction are often insufficient or even absent due to the resection. One advantage of this method can be that in case of failure of the cup one can still try a saddle component, which "articulates" to the junction between the ischial bone and the transposed femur. A premise for this construction is the use of a modular system, which enables the conversion of the hip into a saddle component.

Sacrum resection and reconstruction

Resections of the sacrum are almost inevitably associated with neurological deficit and loss of stability [13,22]. In IIA or IIB [12] osteosarcoma, a complete excision of the sacrum usually has to be performed. Wide margins [12]

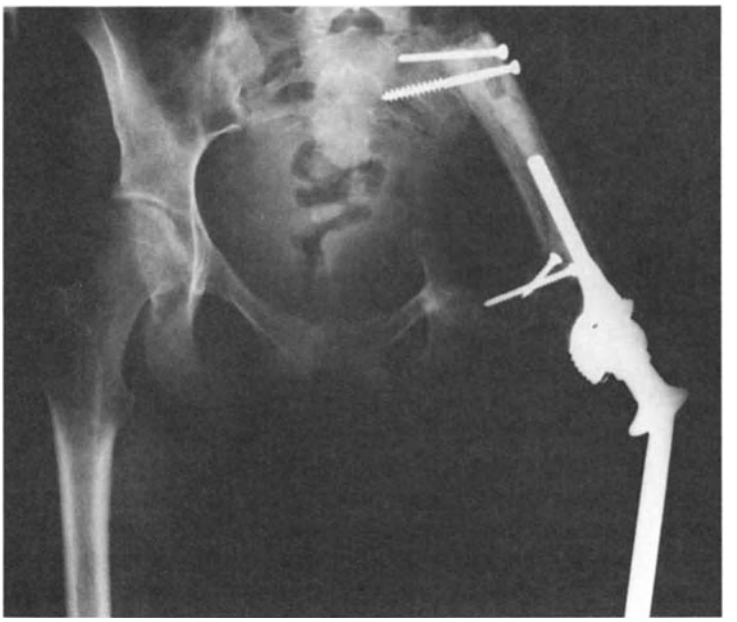

Figure 25-8. Reconstruction after combined I and II resection.

may be obtained by this procedure, but it is often very difficult to obtain radical margins. In fact, radical margins may be obtained only by hemicorporectomy. Resection of the sacrum is often associated with incontinence, paraplegia, and impotence [13,22,23]. Reconstructions in these cases, which are highly demanding, should be regarded as experimental procedures with uncertain functional results. They can be performed in different ways. Their main purpose is refixation of the iliac bones to the vertebral column [20,23].

Future directions

It is obviously impossible to give simple answers to the questions that arise with respect to reconstruction after pelvic resection. One has to search for the simplest method of reconstruction that guarantees maximal stability. Lack of stability and complex reconstructive procedures are connected with a high risk of complications.

The high frequency of local recurrence [3,6,9] after reconstruction—up to 50% in high-grade malignant tumors—is an omen. This information reflects the fact that adequate oncological margins in pelvic resections often do not permit reconstruction. In an ultimate attempt to restore the integrity of the pelvis and leg, the medical and surgical team search for methods to increase

the number of reconstructions. Perhaps under these circumstances they rely more on the efficiency of adjuvant therapy than they should. Although the leg is preserved by this attitude, the health and life of the patient are brought into danger.

Although the early results of allografts appear to be promising, though experimental, several authors question its durability [7,8]. They may act like semibiological spacers that are subjected to resorption and immunological processes. A flail hip appears to be a bad solution, and many authors agree with this statement with respect to ischiofemoral fusion and pseudarthrosis. The reputation of the sacrofemoral fusion and iliofemoral pseudarthrosis are poor as well, and the same applies to the pelvic prosthesis. Perhaps in the future the best solution will be the use of as many autografts as possible; if mobility is the goal of the procedure, then a combination of an autograft and the most simple endoprosthesis should be pursued. Long-term success, however, is not guaranteed. Revision surgery of the prosthetic replacement will be inevitable. Nevertheless, in pelvic disease adequate local control, obtained by wide or preferably radical margins, remains the primary goal of surgical treatment, especially in high-grade malignant tumors such as osteosarcoma.

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26. Diffusion of methotrexate from surgical acrylic cement

P. Hernigou, B. Brun, A. Astier, D. Goutallier, and J.P. le Bourgeois

Introduction

There are two causes of failure in the surgical treatment of bone tumors. One is local recurrence of the tumor, which is not always avoidable, even after wide surgical excision and systemic chemotherapy. The other is difficulty in controlling metastases after surgical treatment. For these reasons, chemotherapy treatment is now recommended preoperatively, and it is continued as soon as possible after definitive surgery but not, in theory, before skin healing has taken place. To limit the postoperative period with chemotherapy, we thought that it would be interesting to apply perioperative and immediate postoperative chemotherapy by adding an antimitotic agent to the acrylic cement used to fill the loss of bone substance or to fix reconstructive prostheses, as is already done with many antibiotics, which would diffuse into the surrounding tissues.

We have already described the experiments used to demonstrate that the surgical cement may be used as a supporting vehicle for the diffusion of drugs for local chemotherapy and the experiments done in animals to test the general and local tolerance of the antimitotic-loaded cement [1–4]. Here we report our preliminary clinical investigations with pharmacological data from patients.

Clinical investigations

Indications

Thirty patients with bone tumors (primary or metastatic) were selected for local chemotherapy diffusing from acrylic cement, since general chemotherapy was inappropriate because of the age of the patient. In primary bone tumors the indication for selection was the age of the patient or because the excision of the tumor was too marginal (pelvis, spine, sacrum).

Local chemotherapy was also used in 32 metastatic bone lesions because it was necessary to add methylmethacrylate to the internal fixation to produce adequate stability in pathologic fractures of the femur or in spine metastases

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 ISBN 0-7923-1905-2

(anterior stabilization). Local irradiation is an essential adjuvant for metastases, but in some patients this irradiation has been done before the fracture or the surgical treatment and cannot be used again because the maximal dose has been delivered. Thus we thought that local chemotherapy might be a useful adjuvant for these patients.

Surgical technique

The lytic lesions of the metastases were first removed; for femoral lesions, as for spinal metastases, the first operation consisted of internal fixation; after internal fixation the resected space was filled with antimitotic-loaded cement. The defect was filled with freshly made methotrexate-containing cement (100 mg of methotrexate) and the acrylic cement hardened in situ.

Pharmacological data

Pharmacological data confirmed the high local concentration of MTX (10,000 times the blood concentration in vacuum drainage), the general chemotherapeutic effect during the first days, and the urinary excretion of MTX up to at least the third week. The release and diffusion of MTX from cement was about the same at all sites and shapes of cement. This local chemotherapy was well tolerated. No patient had general MTX toxicity; there was no MTX-related anemia, depressed platelet count, or leucopenia, and there was no change in creatinine clearance.

Clinical results

A delay in wound healing occurred in two patients with tumors of the pelvis and required ablation of the cement block in one patient. No neurologic complication occurred, and there was no clinical evidence of an adverse effect due to methotrexate diffusing from the cement on the dura or neural elements during spinal metastases. During the follow-up of this technique (4 years), there was only one recurrence (kidney metastasis from the pelvis).

Discussion

Clinical application of antimitotic-impregnated acrylic cement constitutes the use of an approved drug for unapproved indications. The value of such local chemotherapy in preventing local recurrence or as an adjuvant therapy may be argued. However, since our initial report in 1986 [1,2], other authors [5,6] have tested this method both experimentally and clinically.

It is possible to use "orthopedic materials" (acrylic surgical cement) as supporting vehicles for the diffusion of drugs for local chemotherapy. Until now perioperative regional chemotherapy has been limited to an anatomical region using one of the following methods: intraarterial infusion, infusion via tourniquet, and regional isolated perfusion with extracorporeal circulation. Although these methods have shown encouraging results, they produce complications that may require amputation. If further studies confirm the initial results obtained by our experiment [1–4], this method of local chemotherapy could serve as another complementary therapeutic measure in the treatment of bone tumors.

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27. Hyperthermic isolated perfusion using cisplatin for treatment of the extremities

Shinsei Takeyama, Akio Tateishi, Shozoh Higaki, and Masayuki Yamanashi

Introduction

Since 1960 we have performed isolation perfusion chemotherapy as a preoperative procedure combined with surgery in 146 patients with osteosarcoma of the extremities [1–3]. The technique of regional isolated perfusion has remained essentially the same since it was introduced in 1958 by Creech et al. [4]. In combination with perfusion, hyperthermia seems to increase the cytotoxicity of anticancer agents. In this report we present the results of hyperthermic isolation perfusion of the lower extremity with the use of cisplatin in the treatment of osteosarcoma.

Materials and methods

Materials

Between May 1986 and July 1989, 12 patients with osteosarcoma of the lower extremity were treated by hyperthermic isolation perfusion using CDDP. Eleven of the 12 patients had localized disease, and one had evidence of pulmonary metastases at the time of treatment. Of these 12 patients, 11 had conventional osteosarcoma and one had high-grade surface osteosarcoma of the distal femur. Their ages ranged from 8 to 29 years, with an average of 16 years. Eight patients were males and four were females. The primary site was the distal femur in seven patients, the proximal tibia in three, the proximal fibula in one, and the distal fibula in one.

Treatment protocol

Our protocol for the treatment of osteosarcoma is shown in Figure 27-1. After open biopsy, preoperative chemotherapy consisted of two cycles of intraarterial CDDP (3 mg/kg), hyperthermic isolation perfusion (CDDP 60 mg), and two cycles of HD-MTX. Ten weeks later, the evaluation of each cases was performed to determine whether a limb-salvage operation was feasible. Depending on the age of the patient, the tumor location, the degree

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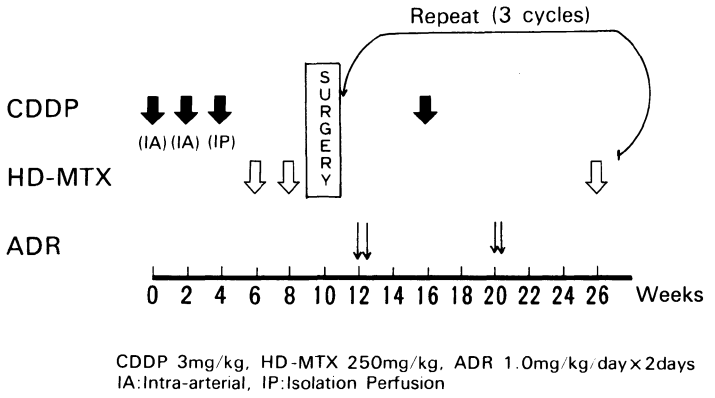

Figure 27-1. Our protocol for the treatment of osteosarcoma.

of involvement of the surrounding soft tissues, and the effect of preoperative chemotherapy, the patients underwent limb-salvage operations or amputation. Postoperative chemotherapy consisted of three alternating cycles of ADR, CDDP, ADR, and HK-MTX continued for about 1 year.

Technique of hyperthermic perfusion

Our hyperthermic perfusion circuit consisted of a roller pump, a pediatric membrane-type oxygenator, and a heat exchanger. For lower extremity isolation, the pneumatic tourniquet was applied at the proximal thigh, and then the femoral artery and vein were canulated (Figure 27-2). The temperature of the perfusate was maintained at 42–43.5°C, and the flow rate was set at about 300 ml/min. The temperature during perfusion was monitored continuously by thermocouples at six or seven points in the perfused limb. The dosage of CDDP was 100 μg/ml perfusate. Hyperthermic isolation perfusion was performed for 60 minutes. At the end of treatment, the circuit was washed out with low molecular weight dextran, which was then replaced with whole blood, and the canulated vessels were then repaired.

Results

Prognosis

The follow-up time for patients was 1.6–4.7 years, with a median duration of 3.1 years. Of the 12 patients, 11 were evaluated, the exception being with pulmonary metastases at the time of hyperthermic isolation perfusion. Of the 11 patients, seven remained continuously disease free, three had no evidence of disease, and one died of disease. The cumulative 5-year survival rate of the

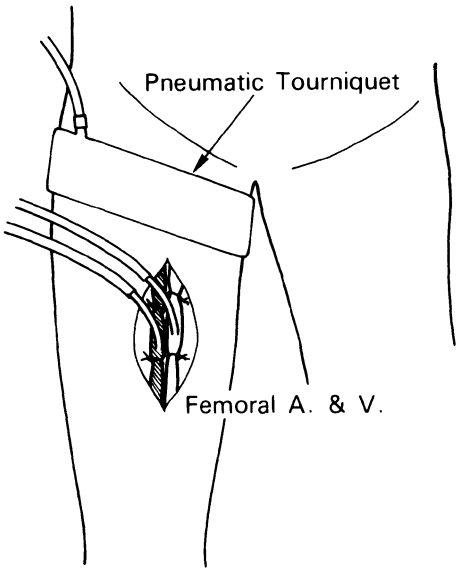

Figure 27-2. Technique of isolation perfusion in the lower extremity.

11 patients was 91%. Of the 12 patients, eight had limb-salvage operations and four underwent amputation.

Tissue temperature during perfusion

In the first six patients, the temperature within the tumor ranged from 40.0 to 41.9°C, while in the latter six patients the temperature ranged from 42.0 to 43.9°C. Within the tumors, the highest temperature during perfusion ranged from 40.8 to 44.4°C, while in the surrounding muscles it ranged from 40.2 to 45.4°C. Of the 12 patients, only one had a temperature of more than 44°C within the tumor tissue.

As shown in Figure 27-3, the temperature of the tumor tissue increased gradually, and cisplatin was administered when the tumor tissue temperature reached 40°C. After 15 minutes, a stable temperature level (42.0–43.9°C) was obtained and was maintained for more than 60 minutes. The temperature within the tumor tissue was directly proportional to that of the adjacent muscle tissue. However, the skin temperature was 2–4°C less than the temperature within the tumor tissue.

CDDP concentration within the perfusate and tissue concentration of CDDP

The CDDP concentration within the perfusate was assessed at 1, 5, 10, 15, 20, 25, 30, 30, 50, and 60 minutes after CDDP administration. The total Pt

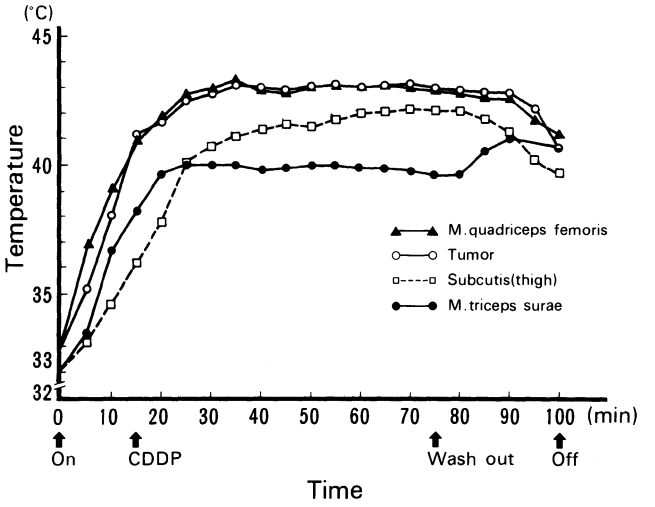

Figure 27-3. Temperature curves for hyperthermic isolation perfusion: 8-year-old girl with osteosarcoma of the distal femur.

concentration decreased gradually during perfusion, and the average level was 24.1 μg/ml. Similarly, the free Pt concentration decreased gradually during perfusion, with the average level being 11.8 μg/ml (Figure 27-4).

Tissue concentrations of CDDP after preoperative chemotherapy are shown in Figure 27-5. The tumor tissue showed the highest CDDP level (8.2 μg/g), followed by the level in nerve tissue (3.5 μg/g), skin (1.3 μg/g), muscle (1.2 μg/g), and bone marrow (0.5 μg/g) in that order. The CDDP tumor tissue level was significantly higher than that in skin or muscle.

Ratio of necrotic to viable tumor cell after preoperative chemotherapy

The tumors were examined microscopically after preoperative chemotherapy, and the ratio of necrotic to viable tumor cells was calculated. The degree of tumor necrosis was classified as excellent (≥95%), good (80–94%), fair (50–79%), and poor (<50%). Of seven patients, the outcome for three was excellent, for two was good, and for two was fair.

Complications

In all 12 patients there were no systemic complications, and only one patient had femoral arterial thrombosis and compartmental syndrome of the leg. Because of muscle damage and skin necrosis of the lower extremity, he required amputation after wide resection and total knee replacement as initial surgery.

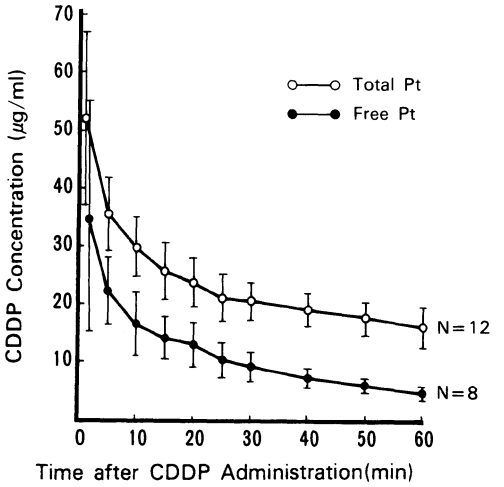

Figure 27-4. Cisplatin (CDDP) concentration curves for the perfusate during isolation perfusion.

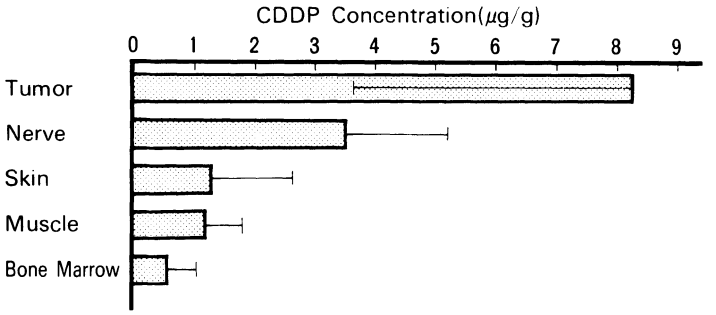

Figure 27-5. Cisplatin (CDDP) concentrations in tissues after preoperative chemotherapy. Data from four samples of tumor, nerve, muscle, and bone marrow, and from two nerve samples.

Discussion

Administration of regional chemotherapy is an attempt to augment the efficacy of a chemotherapeutic agent by achieving high drug levels in the target region while at the same time reducing systemic toxicity. In 1978, the drug cisplatin (CDDP) was first shown to have activity in the treatment of osteosarcoma. Although the response rate was only approximately 20%, most of the responses were complete or good partial responses.

Since 1983 we have used cisplatin as the agent for isolation perfusion chemotherapy in over 30 patients with bone and soft tissue sarcomas of the extremities [3]. In 1987 Cavaliere et al. reported on the role of hyperthermic perfusion therapy in the treatment of limb osteosarcomas [5]. The 5-year

survival rate for hyperthermic perfusion followed by amputation was 50.9%, while the 5-year survival rate for hyperthermic chemotherapy perfusion followed by amputation was 71.4%. Moreover, the 5-year survival rate for hyperthermic chemotherapy perfusion followed by en bloc resection and bone reconstruction was 63.5%. These results seemed to indicate that hyperthermic chemotherapy perfusion allowed conservative rather than ablative surgery to be used in the treatment of limb osteosarcoma. Our results are similar to those of Cavaliere.

It is known that above-normal temperatures (42–42.5°C) cause selective damage to neoplastic cells. Generally speaking, hyperthermia is safe and effective if the temperature within the tumor tissue during isolation perfusion ranges from 42.0 to 43.9°C. Our data showed that the temperature within tumor tissue was directly proportional to that of the neighboring muscle. It is thus sufficient for the temperature of the adjacent muscle around the tumor to be monitored continuously during perfusion. It is important that a muscle temperature of 44°C is never exceeded.

Out of our seven patients, excellent necrosis of more than 95% was seen in three tumors (42%), and two tumors (28%) showed good necrosis (80–94%). It is thus suggested that tumor necrosis after hyperthermic isolation perfusion is more complete than after intravenous and intraarterial infusion. Cavaliere et al. divided complications into five grades as follows: grade 1, no subjective or objective evidence of any reaction; grade 2, slight erythema and/or edema; grade 3, considerable erythema and/or edema with some blistering, slight impairment of motility permissible; grade 4, extensive epidermolysis and/or obvious damage to the deep tissues causing definite functional disturbances, and threatening or manifest compartmental syndrome; and grade 5, reactions that could require amputation [5]. Of the 12 patients, 11 were grade 1 or 2 and only one was grade 5. This was probably caused by the excessive hyperthermia (more than 44°C).

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28. Hyperthermic isolation limb perfusion (HILP) in the management of extremity melanoma and sarcoma with particular reference to the dosage, pharmacokinetics, and toxicity of cisplatin

William S. Fletcher, Eugene A. Woltering, H. Stephens Moseley, Gary Bos, Luis Lebredo, David Brown, and Karen Small

Introduction

Hyperthermic Isolation Limb Perfusion (HILP) is a technique of extremity chemotherapy developed by Dr. Oscar Creech et al. At Tulane University in the late 1950s and early 1960s [1]. The technique was adopted by relatively few institutions around the world, essentially all of whom claim superior results in comparison to conventional treatment, i.e., surgery alone. There are, however, no convincing randomized trials that precisely document the value of HILP; thus, the technique remains controversial. While the technique and drugs used are continuing to evolve, there is little question that HILP has a role in the management of advanced and high-risk extremity melanomas and sarcomas.

Between 1960 and December 1, 1990, we utilized HILP 531 times in the management of patients with melanoma or sarcoma of the extremities. Published results with stage I, stage II, and acral lentiginous melanoma indicate a substantial increase in disease-free survival over published reports utilizing surgery alone [2–4]. Similarly, HILP in the management of soft tissue sarcomas of extremities results in a marked decrease in local recurrence over that found in the literature [5,6]. Our initial experience, like that of most others, was in using 1-phenylalanine mustard (1-Pam) for melanomas and a combination of 1-Pam and actinomycin-D for sarcomas (1-Pam 1.0 mg/kg for upper extremities, 1-Pam 1.5 mg/kg for lower extremities, and actinomycin-D 20–25 μg/kg). Following this we utilized HILP in an escalating dose with dimethyl triazine imidazole carboxamide (DTIC), with substantially inferior results than previously experienced with 1-Pam. Despite published reports that DTIC is a useful agent when activated by light during perfusion [7], our experience suggests that the drug requires activation by the liver and is probably not an optimum choice for HILP [7].

In an attempt to define the maximum tolerated dose of cisplatin by HILP, we have steadily escalated the dose while keeping all other parameters of the perfusion constant [8,9]. We have studied the pharmacokinetics of the drug in seven patients at low doses, and subsequently in 10 patients at high doses. The dose initially was 0.75 mg/kg, based on either the actual weight or the

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 ISBN 0-7923-1905-2

ideal weight. This dose was selected on the basis of a rabbit perfusion model that indicated a substantial muscle toxicity at higher doses [10]. Between 1983 and December 1, 1990, 131 patients with melanoma or sarcoma of the extremity were perfused. The dosage was steadily escalated at 1–3 mg/kg until one melanoma patient, treated at 1.7 mg/kg, developed a compartment syndrome in all of the compartments of the lower extremity. This complication required extensive fasciotomy, prolonged hospitalization, and rehabilitation. Exhaustive review of the procedure revealed no aberrations of dosage, temperature, or other alterations in the technique. Thereafter, 10 patients treated at 1.6 mg/kg suffered no toxicity. Prior to the case treated at 1.7 mg/kg, no attempt had been made to control the pH of the perfusate, and subsequently the pH has been kept near normal by the use of carbogen (95% O_2, 5% CO_2) in the oxygenator. Other conditions of the perfusion included priming of the oxygenator with one unit of fresh whole or packed red blood cells, supplemented with saline to provide an adequate level in the reservoir. Flow rates were maintained at the level necessary to maintain a stable level in the oxygenator reservoir. In sarcoma patients, external hyperthermia was utilized, generally attaining an intratumor and muscle temperature in the range of 38–39°C; muscle temperatures above 40° were avoided. Cisplatin was added in two increments, at 0 and 5 minutes, into the reservoir, not into the arterial line.

Toxicity

Under the conditions just described, HILP with cisplatin was well tolerated at dosages below 250 mg/m^2. Patients treated with doses in excess of 250 mg/m^2 may develop rhabdomyolysis with myoglobinuria, massive edema, erythema, local muscle wasting, hyponatremia starting on the fourth day, local nerve conduction abnormalities, paresthesias, and hearing loss. All of these adverse effects are completely reversed, with the possible exception hearing loss. We conclude that 250 mg/m^2 under the conditions of this trial is the maximum tolerated dose of cisplatin for hindquarter HILP [11].

Pharmacokinetics

The primary advantage of isolated hyperthermic limb perfusion may rest in the high regional levels of drug that can be achieved while minimizing systemic drug levels and thus systemic toxicity. Our original observations on the pharmacokinetics of cisplatin involved 22 patients with melanoma treated with doses of 0.75–2 mg/kg. In these 22 patients, the mean perfusate cisplatin concentration at 5 minutes was 35.175 μg/ml, and the corresponding systemic cisplatin concentration was 0.499 μg/ml. This yields a 70:1 therapeutic advantage. Fifteen minutes after the beginning of cisplatin perfusion, the mean

cisplatin perfusate concentration was 8.5 μg/ml and the corresponding systemic cisplatin concentration was 0.746 μg/ml. This represents a therapeutic advantage of 11:1. The mean systemic cisplatin level of these patients 60 minutes after tourniquet release was 0.98 μg/ml, and the systemic cisplatin concentration 24 hours after tourniquet release was 0.25 μg/ml. These patients then underwent approximately a 3- to 4-week wait before their tumor was excised. At that time, the mean tissue concentration of cisplatin was 0.89 μg/g in the peripheral tumor and 0.421 μg/g in the central tumor. Subsequently, we have studied additional patients at the maximum tolerated drug dose [4–6 mg/kg (190–200 mg/m^2)]. In these patients the therapeutic advantage was similar; however, 1 hour after tourniquet release the peripheral plasma cisplatin level was 5.4 μg/ml, and at 24 hours after the release of the tourniquet the peripheral plasma level was 3.0 μg/ml. This later experience with high-dose cisplatin in isolated hyperthermic limb perfusion would indicate that we have achieved extremely high perfusate levels of cisplatin and, in addition, have provided adequate systemic platinum levels similar to those achieved with adjuvant systemic platinum therapy. Currently we are studying the red cell uptake of blood used in the isolated hyperthermic perfusate circuit. It may be feasible to wash and reinfuse these red cells, thus limiting the patient's exposure to additional autologous blood.

Discussion

While publishing the detailed results of treatment of melanomas and sarcomas is premature, several observations are note worthy. First, of the 71 sarcomas treated with HILP only three have recurred locally, and these were recurrent at the time of the initial perfusion. Second, no patient has lost an extremity or been disabled as a result of the perfusion. Third, the incidence of distant metastases is on the order of 22%, but essentially all of the patients dying of metastases retain a functional extremity. Only one patient with osteosarcoma of the femur has been treated by this technique and subsequently underwent a limb-salvage procedure with a metallic prosthesis, followed by further adjuvant therapy with high-dose methotrexate. He remains disease free at 1 year. This case brings to light the possibility that HILP may be a more effective and less toxic method of utilizing cisplatin for the treatment of osteosarcoma compared with the repeated infusional technique described by Jaffe [12].

Conclusions

The technique of HILP in the management of extremity melanoma and sarcoma is still evolving. Although we believe that we have defined the maximum tolerated dose of cisplatin under the conditions utilized, it is not certain

that we have achieved the optimum dose. Quite possibly, lower cisplatin doses and longer perfusion times may optimize drug delivery. A myriad of other questions need to be evaluated. These include the possibility of late washout of the drug from edematous or high tumor burden extremities, the role of cisplatin HILP in the management of osteosarcoma, and the role of cisplatin HILP in combination with other agents. It is hoped that these data will be helpful to others wishing to pursue further investigations.

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29. Effect of isolated limb perfusion with cisplatin (CDDP) on canine osteosarcoma

H.J. Hoekstra, F.J. Meutstege, J.W. Oosterhuis, J. De Vries, and H. Schraffordt Koops

Introduction

Cisplatin (CDDP) is one of the most effective chemotherapeutic agents in the treatment of osteosarcoma used as neoadjuvant and adjuvant treatment. The potential effectiveness of CDDP is limited due to nephrotoxicity and ototoxicity. With isolated limb perfusion (ILP), followed by an exchange transfusion of the perfusate and whole blood, it is possible to attain very high local drug concentrations in a limb with minimal systemic toxicity. The biological behavior of canine osteosarcoma is similar to human osteosarcoma. The tumor occurs predominantly in the long bones, with early hematogenous metastases to the lungs [1]. The efficacy of ILP with CDDP on osteosarcoma was studied in spontaneous canine osteosarcomas to provide guidelines for its clinical use.

Material and methods

Dogs

Twenty-eight dogs with spontaneous, histologically proven, previously untreated primary osteosarcoma of the extremity, without radiographic evidence of distant metastases, underwent an ILP with CDDP. Preoperatively all dogs were thoroughly clinically evaluated and underwent a complete blood count, serum chemistry profile, and radiographs of the primary tumor and thorax. The characteristics of the dogs are summarized in Table 29-1.

Perfusion treatment

All isolated limb perfusions were pressure regulated and performed under physiological optimal conditions [2]. The dosage of the cytostatic agent was based on the volume of the affect limb [3]. The dosage of CDDP (Platinol 0.5 mg/ml, Bristol Myers SAE, Spain) used for the perfusion was 30 mg/ml extremity volume [4]. The limb was perfused with the aid of a pump oxygenator;

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 ISBN 0-7923-1905-2

Table 29-1. Dogs characteristics and Huvos reaction after isolated limb perfusion with CDDP

Dogbreed	Sex	Age (yrs)	Weight (kg)	Tumor site	Extremity vol.	Huvos 2 wk	6 wk
Group I							
1 Rotweiler	M	8	48	Tibia	1.9	—	—
2 Rotweiler	M	1	35	Tibia	1.8	IV	III
3 Bouvier	F	2	49	Radius	2.2	IV	—
4 St. Bernard	M	6	60	Radius	2.0	III	II
5 Irish wolf	F	6	47	Femur	2.0	—	—
6 Doberman	M	9	52	Radius	1.5	III	II
7 Bouvier	F	10	37	Radius	1.6	III	III
8 Bouvier	F	9	42	Radius	1.8	II	II
9 G. Shephard	M	9	42	Femur	1.8	I	I
10 B. Shephard	M	5	40	Ulna	1.3	III	III
11 Mongrel	M	8	30	Radius	1.2	II	II
12 Boxer	M	9	31	Radius	1.6	III	II
13 Labrador	M	9	43	Metatarsal	1.6	III	III
14 New Foundland	M	2	43	Tibia	1.8	III	III
Group II							
15 Bouvier	M	5	34	Radius	1.2	II	II
16 St. Bernard	M	7	67	Radius	3	II	II
17 B. Shephard	M	7	33	Radius	1	II	*
18 Rotweiler	M	6	47	Ulna	1.4	I	II
19 Boxer	M	7	32	Tibia	1.4	I	I
20 St. Bernard	F	9	58	Radius	2	II	III
21 Great Dane	F	6	54	Radius	1.8	IV	*
22 Bouvier	M	8	44	Radius	1.4	I	II
23 Rotweiler	M	9	47	Radius	1.6	II	*
24 Great Dane	M	6	61	Radius	2.5	IV	II
25 Bouvier	M	3	38	Radius	1.6	II	II
26 Leeuwenberger	M	6	50	Radius	1.6	—	—
27 Hovawart	F	6	33	Radius	1	IV	I
28 Great Dane	F	6	60	Radius	1.6	I	II

* Unable to classify.

the perfusate consisted of 350 ml 5% dextran 40 in glucose 5% (Isodex, Pharmacia AB, Sweden), 500 ml whole blood, 30 ml 8.4% $NaHCo_3$, and 0.5 ml 5000 IU/ml heparin (Thromboliquine, Organon BV, Oss, the Netherlands). CDDP was added to the circulated perfusate over 10 minutes. ILP was performed for 1 hour at an extremity temperature for Group I (14 dogs) of 39–40°C, and for Group II (14 dogs) of 40–41°C. All dogs were followed for local and systemic side effects of CDDP perfusion.

Histology

Three drill bone biopsies from the tumor were taken at 2 and 6 weeks post perfusion treatment. The effect of CDDP on the tumor was histologically

Table 29-2. CDDP effect on tumor tissues according to Huvos

	Group I		Group II		Total	
Huvos histology	2 wks N = 12	6 wks N = 11	2 wks N = 13	6 wks N = 10	2 wks N = 25	6 wks N = 21
I—no reaction	1 (8%)	1 (10%)	4 (30%)	2 (20%)	5 (20%)	3 (14%)
II—moderate	2 (17%)	5 (45%)	6 (46%)	7 (70%)	8 (32%)	12 (57%)
III—good	7 (58%)	5 (45%)	—	1 (10%)	7 (28%)	6 (29%)
IV—necrosis	2 (17%)	—	3 (23%)	—	5 (20%)	—
Mean	2.83	2.36	2.15	1.90	2.48	1.90

scored according to the criteria described by Huvos: no reaction, I; moderate effect, II; good effect, III; and total necrosis, IV [5].

Results

Three dogs (1, 5, 26) died within 24 hours of perfusion due to anesthesia. A fourth dog [3] died 1 week postperfusion from a large myocardial infarction. Postmortem examination showed a complete necrotic tumor. Twenty-five dogs could be analyzed. All perfused extremities showed an initial slight edema, which reached a maximum on the third postoperative day and disappeared within the first week.

The effect of CDDP on the tumor was classified according to Huvos [5]. After 2 weeks biopsy: no reaction, Huvos I in five dogs (20%); moderate effect, Huvos II in eight dogs (32%); good effect, Huvos III in seven dogs (28%); total necrosis, Huvos IV in five dogs (20%). Six weeks biopsy: no reaction, Huvos I in three dogs (14%); moderate effect, Huvos II in 12 dogs (57%); good effect, Huvos III in six dogs (29%); total necrosis, Huvos IV in none of the dogs.

The mean histological score for the total group at 2 weeks was 2.48, and at 6 weeks 1.90; for Group I at 2 weeks 2.83 and at 6 weeks 2.36; and for Group II at 2 weeks 2.15 and at 6 weeks 1.90 (Table 29-2).

Discussion

Cisplatin is one of the most effective cytostatic agents in the treatment of osteosarcoma. CDDP is cell-cycle independent, it is used in short-term, high-dose treatments, but the total delivered CDDP dosage is limited due to its nephrotoxicity and ototoxicity. Bielack found no difference in the effectiveness of intravenous or intraarterially delivered CDDP in the treatment of human osteosarcoma [6]. In contrast, Powers demonstrated the superiority of the intraarterial to the intravenous route of CDDP infusion in canine

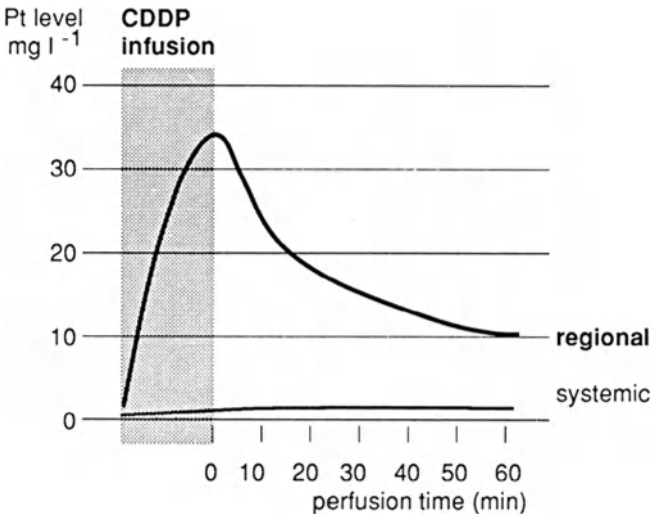

Figure 29-1. Regional and systemic platinum levels in serum during the perfusion. *CDDP*-cisdiamminedichloroplatinum.

osteosarcoma [7]. The superiority of CDDP perfusion to the intraarterial or intravenous route was demonstrated by Kar in an experimental pharmacokinetic study [8]. With CDDP perfusion a substantial drug extraction occurs, with minimal leakage to the systemic circulation (Figure 29-1) [4]. The fraction-free (active) platina increased by a factor of 4–20 in comparison with the normal intravenous route of CDDP administration due to the low protein content of the perfusate [9].

This study demonstrated that ILP with CDDP is feasible and beneficial. The tumor response achieved with CDDP perfusion in this canine osteosarcoma model is better than the clinical results with high-dose methotrexate (HDMTX) in human osteosarcomas [10]. There was no significant improvement in the Huvos score between 2 and 6 weeks. This might indicate tumor regrowth and may have implications for the further clinical use of ILP with CDDP.

Hyperthermia enhanced CDDP toxicity [11]. Tumor blood flow may be enhanced by perfusion. When hyperthermia is added to the perfusion there may be enhanced blood flow due to vasodilatation [12], with enhanced cellular drug uptake [13], tissue extraction [14], and DNA crosslinking [15], and decreased DNA repair [16]. No therapeutic effect, according to the Huvos score, could be demonstrated by increasing the limb temperature 1°C.

The results of ILP with CDDP in spontaneous canine osteosarcomas justify study of the clinical applicability of ILP with CDDP for human osteosarcomas in a Phase I-II study. With ILP considerable higher fractions of free platinum can be achieved without systemic toxicity. ILP offers the opportunity to modify the perfusate composition such that it influences CDDP kinetics favorably [9].

Acknowledgments

This work was partly supported by the Groningen Pediatric Oncology Foundation (SKOG) and Department of Surgery, Groningen University Hospital.

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30. Salvage surgery for childhood osteosarcoma

Ugo Pastorino, Marco Gasparini, Alberto Azzarelli, Luca Tavecchio, and Gianni Ravasi

Rationale

The concept of salvage surgery in osteosarcoma is based on solid knowledge of the natural history of this disease. Unlike many other solid tumors, sarcomas manifest the peculiar characteristic of organ-restricted spread [1,2]. In most cases, lung metastases are the only site of distant relapse, occurring in 50–80% of osteosarcomas [1,3]. Although, if the cure rates for childhood osteosarcoma have dramatically improved over the last 20 years, lung metastases still remain a major cause of failure, occurring in 40–50% of patients [4] and being refractory to salvage chemotherapy [5–7]. A summary of the reported results of salvage surgery in osteosarcoma is given in Table 30-1 [8–15].

The results of recent trials in osteosarcoma have suggested that optimal adjuvant chemotherapy might improve the chances of salvage surgery at the pulmonary level by selecting the chemoresistant component of the disease [5,6,16].

Diagnosis and staging

In osteosarcoma the median disease-free interval after primary surgery is less than 12 months (Figure 30-1), and nearly 90% of pulmonary metastases are detected within 2 years, although a few relapses may still occur after 3 years [18].

A prominent concept of salvage surgery in osteosarcoma is that optimal results require intensive follow-up after primary treatment [17]. Active follow-up for salvage surgery should include chest x-rays at least every 2 months, and CT scan or linear tomograms of the chest at least every 6 months for the first 2 years after primary treatment.

Presurgical evaluation at the time of occurrence of lung metastases requires CT scan or MRI of the chest, bone scans, and ventilatory function. Other examinations, such as brain CT or liver ultrasound, may also be applied. Bronchoscopy is only exceptionally required for centrally located lesions.

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 ISBN 0-7923-1905-2

Table 30-1. Results of salvage surgery for lung metastases

		N	Survival %		Ref.
Martini	1971	22	32	(5 yr)	20
Rosen	1978	14	71	(3 yr)	21
Telander	1978	28	57	(4 yr)	22
Waine Flye	1984	26	44	(4 yr)	23
Goorin	1984	11	82	(3 yr)	24
Gundry	1984	24	50	(4 yr)	25
Mountain	1984	56	51	(5 yr)	26
Beattie	1984	22	27	(10 yr)	19
Eilber	1987	21	60	(2 yr)	13
Pastorino	1987	27	47	(3 yr)	27

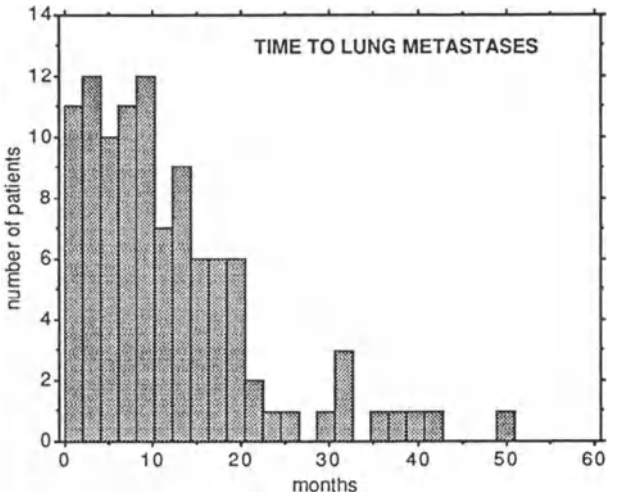

Figure 30-1. Distribution of time of lung metastases in a consecutive series of 174 primary childhood osteosarcomas, curatively resected in the years 1970–1988.

Sternotomy vs. Thoracotomy

Salvage surgery in metastatic sarcomas should be aimed at achieving the highest chance of complete resection with the minimum functional damage. One should keep in mind from the start that two or three subsequent thoracotomies are often necessary to control pulmonary disease. Preservation of pulmonary function not only requires lung-sparing surgical procedures, but also a conservative approach to the thoracic muscles. Wedge or segmental resections are the procedure of choice, and mechanical staplers now available offer a wide spectrum of technical facilities to the thoracic surgeon.

Although the majority of surgeons still prefer the traditional posterolateral thoracotomy, recent experiences suggest that median sternotomy is a more effective approach [19–22]. In fact, median sternotomy allows for bilateral surgical staging and one-stage resection. In our experience, half of patients present with bilateral pulmonary spread, while occult contralateral disease occurs in about one third of monolateral clinical metastases [18]. In the absence of a randomized control it is impossible to assess the ultimate benefit of such early resection, but at least unnecessary rethoracotomy may be avoided in these patients.

Moreover, midsternal split prevents surgical damage of the thoracic muscles and nerves, and disruption of the parietal pleura. In the hands of an experienced surgeon, virtually any pulmonary resection can be performed through the median approach, although left lower lobe segmentectomy is more difficult than through the lateral approach and may trigger transient arrhythmias. Post-operative complications are usually limited to transient air leakage and accumulated bronchial secretion, which seldom require repeated bronchial suction. Postoperative ventilatory failure and intercostal pain are substantially reduced by median sternotomy, as well as intrathoracic bleeding and the subsequent occurrence of pleural adhesions. Reoperation may be performed through the median or lateral approaches, depending on the site of pulmonary relapse. Many thoracic surgeons are still reluctant to perform re-do sternotomy due to the high reported complication rate of this procedure in cardiac surgery. In pulmonary surgery, however, the intact pericardium prevents troublesome adhesions, and we have performed re-do sternotomy in several cases without any problem [22].

Prognostic factors

As in primary tumors, where surgery plays a curative role, in salvage surgery for metastatic osteosarcoma the tumor burden is associated with the prognosis. Nevertheless, we still lack clear-cut criteria for exclusion from surgery based on prognostic factors [23]. In 1985 the NCI group reported a multivariate analysis of the long-term results of metastasectomy for sarcomas, stating that patients with more than four metastases at first thoracotomy had a 6-month median survival and did not benefit from resection [24]. However, the same authors in a later analysis demonstrated that the number of nodules resected at thoracotomy did not have an impact on long-term survival, provided that the lung disease could be completely resected [25].

Our recent data on 44 patients who underwent metastasectomy for osteosarcoma in the years 1984–1990, confirm that the survival of patients with favorable prognostic factors is different from that of patients with untoward prognostic factors (Figure 30-2). Kaplan-Mayer estimated survival of patients with a single pathological lesion and a free interval >12 months (good prognosis) at 62% at 5 years, compared to 47% for patients with multiple lesions

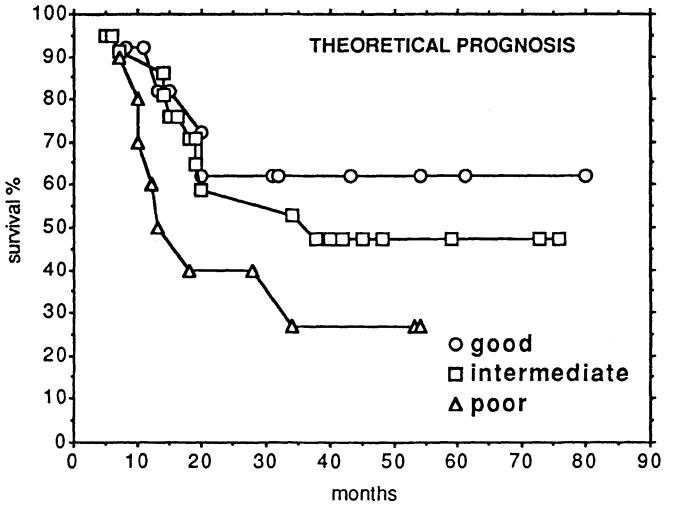

Figure 30-2. Overall survival after complete resection of pulmonary metastases according to theoretical prognosis: single pathological lesion and free interval >12 months (12 patients, good prognosis), multiple lesions or free interval ≤12 months (22 patients, intermediate prognosis), and both multiple lesions and short intervals (10 patients, poor prognosis).

or a free interval ≤12 months (intermediate prognosis), and 27% for patients with both multiple lesions and a short interval (poor prognosis). Taking into account the number of lesions only, patients with a single pathological lesion had a 60% survival rate at 5 years, compared to 51% for patients with two or three lesions, and 24% for patients with four or more lesions (Figure 30-3). In the latter group, 4 out of 14 patients were alive at 36 months after the first operation.

Although the chance of surviving after lung resection may be influenced by the extent of disease or the tumor growth rate, for the time being the choice of candidates for salvage surgery should be based only on complete resectability. Given the heterogeneity of the patient population and the small numbers of patients available at single institutions, a cooperative research effort is needed, possibly by pooling data in a meta-analysis, in order to establish the optimal selection criteria as well as the real prognostic factors.

Synchronous metastases

The issue of osteosarcoma that is metastatic at presentation is very controversial. Thoracic surgeons are more frequently asked to cope with this problem in an attempt to rule out false-positive lesions, to avoid unnecessary amputation in the case of disseminated disease, and possibly to improve the chances of permanent eradication of the disease. In the majority of surgical series, the rare synchronous resected metastases are often combined with

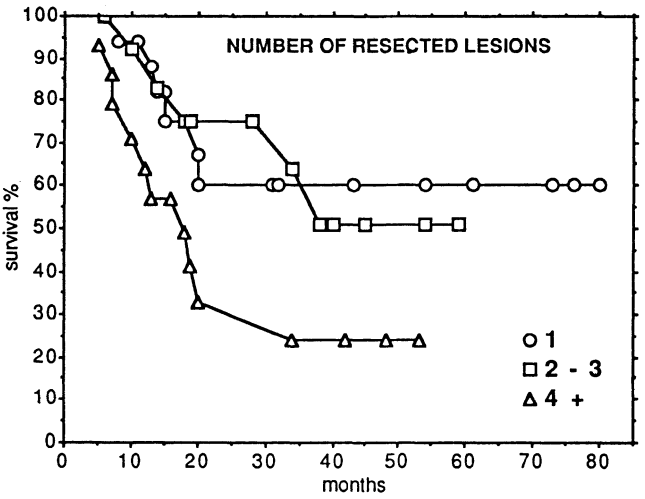

Figure 30-3. Overall survival after complete resection of pulmonary metastases according to the number of pathological lesions: single lesion (17 patients) two or three lesions (13 patients), four or more lesions (14 patients).

those occurring during the first months of follow-up, giving rise to a considerable amount of confusion. Theoretically, early lung metastases (with a free interval <6 months) may be the expression of chemoresistance, thereby manifesting a poorer prognosis than synchronous metastases.

Two recent studies have reported conflicting results for salvage surgery of synchronous lung metastases. The first paper from the Rizzoli Institute includes 10 patients, only two of whom (20%) were alive and disease free at 12 and 30 months [26]. The other study, from the German-Austrian Cooperative Osteosarcoma Study Group (COSS-80 and COSS-82), includes 31 patients and 15 long-term survivors, with a median survival of 57 months and an estimated 6-year survival of 45% [16]. Such encouraging results need to be verified through a longer follow-up.

Combined treatments

Both chemotherapy and radiotherapy have been applied as salvage treatment for metastatic osteosarcoma with limited success. Patients with unresected lung metastases have a median survival of 6–8 months, and nearly all patients die within 2 years [5–7]. Adjuvant irradiation of both lungs represents another option in patients with multiple resected lesions, but the benefits of this procedure remain to be established. Biological therapy with IL-2, alone or in combination with activated cells (LAK, TIL), is a promising new tool for childhood osteosarcoma whose potential applications are to be tested in the near future.

Table 30-2. Average number of thoracotomies and resected pulmonary metastases in long-term survivors

		Survivors	Thoracotomies[a]	Metastases[a]	Ref.
Beattie	1971	6	3	8	20
Rosen	1978	10	2.2		21
Telander	1978	16	2.2	3	22
Waine	1984	12	1.5	9	23
Goorin	1984	9	2	4	24
Gundry	1984	11	1.9	5	25

[a] Average number.

Follow-up and surgical rescue

A relevant concept of salvage surgery is that the first operation is not always able to achieve permanent control of the disease, and the same criteria chosen for the first lung resection should also be applied to pulmonary relapsed. In fact, looking at the surgical management of long-term survivors reported in the literature (Table 30-2), the average number of thoracotomies applied to each patient ranges between two and three, with the number of resected metastases ranging from three to nine.

The long-term follow-up of the pilot study on 22 children with multiple pulmonary metastases from osteosarcoma published by Beattie in 1971 [8] and 1984 [27] has been recently updated with a surprising outcome [28]. Four of the six 10-year survivors survived more than 19 years after as many as nine thoracotomies, and 3 out of 6 (50%) developed second primary cancers during the second decade of follow-up.

Clinical follow-up after pulmonary resection should be tailored according to the expected time of relapse. In our experience (Figure 30-4), the median disease-free interval after the first lung resection is 6 months (means 8.5, range 1–40). Optimal outpatient monitoring after salvage surgery should include chest x-rays and an alkaline phosphatase test every month, and a chest CT scan every 3 months up to 1 year, followed by chest x-rays every 2–3 months from the second year on.

Overall contribution of metastasectomy

The overall contribution of systematic salvage surgery to the management of childhood osteosarcoma is difficult to define. Randomized comparisons are inapplicable today, given the expected benefit of surgery. On the other hand, retrospective evaluation is affected by the changes that have occurred in staging procedures and in the selection of candidates for salvage surgery. We have tried to assess the real benefit of salvage surgery in a consecutive series

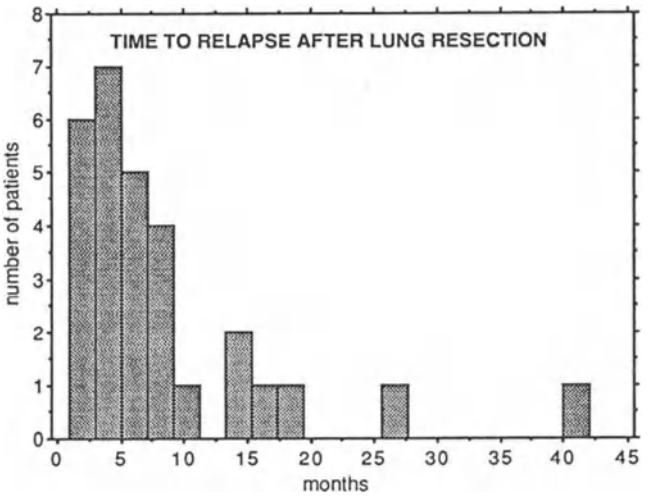

Figure 30-4. Distribution of time to pulmonary relapse after the first resection of lung metastases.

of 174 primary childhood osteosarcomas, that were curatively resected at our institute in the years 1970–1988:[18]. Two historical periods were compared, corresponding to different modalities in the treatment of the primary tumor as well as in the prevention or control of pulmonary relapse: 72 children were treated over the years 1970–1981 and 102 children over the years 1982–1988. In the latter period, adjuvant chemotherapy was replaced by neoadjuvant programs and new criteria were adopted for the management of lung metastases, consisting of early bilateral surgical staging and lung resection through median sternotomy for all patients with purely intrathoracic relapse. The follow-up was updated in December 1989. During the last period, the overall 5-year survival improved significantly from 35% to 58% ($p < 0.001$). The disease-free survival rose from 38% to 45% at 5 years, with median values of 15 vs. 33 months, while the frequency of isolated lung metastases dropped from 58% to the actuarial 48%. The proportion of patients who underwent complete resections of their pulmonary metastases rose from 17% (7/42) to 55% (27/49), without operative mortality.

Due to such a high proportion of patients eligible for salvage surgery, the overall survival from detection of lung metastases (Figure 30-5) improved from 0 to 28% at 5 years ($p < 0.001$). Five patients underwent subsequent lung resections. The improvement in survival for metastatic patients in the years 1982–1988 appeared to be related to the systematic application of salvage surgery (Figure 30-6). In fact, the survival of patients who achieved complete surgical resection of lung metastases was 47% at 5 years ($p < 0.001$), with a median survival of 3 years and six patients still alive at 4 years. Unresected lung metastases showed the same dismal prognosis observed in the prior series, with a median survival of 8 months and no survivors beyond 42 months.

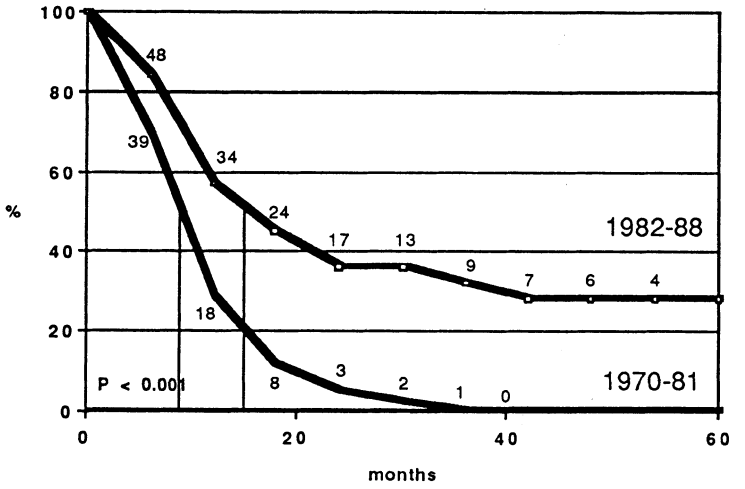

Figure 30-5. Overall survival of patients developing lung metastases, calculated from the time of detection, for the periods 1970–1981 and 1982–1988.

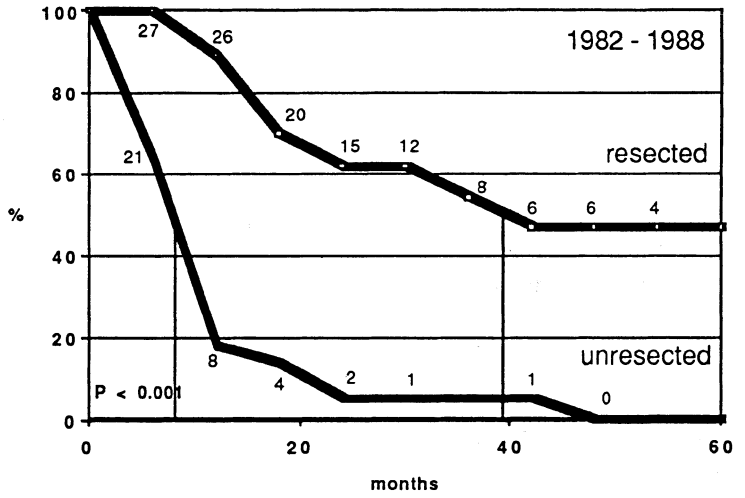

Figure 30-6. Overall survival from detection lung metastases, according to salvage surgery, in the years 1982–1988.

These data indicate that systematic bilateral pulmonary resection, in combination with optimal neoadjuvant chemotherapy, has improved the final cure rate in childhood osteosarcoma. Salvage surgery should be considered the gold standard for treatment of limited metastatic disease and should be used as a stratification parameter in the analysis of adjuvant trials.

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31. The multi-institutional osteosarcoma study: An update

Michael P. Link

Introduction

The Multi-institutional Osteosarcoma Study (MIOS) was initiated in June 1982 and was designed to test the role of adjuvant chemotherapy in the treatment of osteosarcoma of the extremity. At the time of initiation of this trial, the role of adjuvant chemotherapy in the treatment of osteosarcoma was controversial, because the outcome of patients treated with only surgery and without adjuvant chemotherapy appeared to be improving over time at certain institutions [1,2]. Preliminary [3] and updated [4] results of this trial have been published and have confirmed the favorable impact of adjuvant chemotherapy on event-free survival for patients with osteosarcoma of the extremity; the outcome for patients treated with immediate adjuvant chemotherapy was significantly better than for those treated with only surgery.

Methods

Patients

Patients were entered into the MIOS from the majority of the institutions of the Pediatric Oncology Group (POG), the Dana-Farber Cancer Institute, the Pediatric and Surgical Oncology Branches of the National Cancer Institute, Children's Hospital of Philadelphia, and the London Solid Tumor Group. Eligibility requirements have been previously reported [3,4]. While the study was open for randomization between June 1982 and August 1984, patients were randomly assigned to a group receiving immediate intensive adjuvant chemotherapy or to a control group treated with observation alone without adjuvant therapy after surgery. Eligible patients who declined randomization but who accepted treatment according to one of the two study arms were also entered and were followed in an identical fashion as patients who accepted randomization. After the randomization was closed in August 1984 (see above), eligible patients were all treated with adjuvant chemotherapy as prescribed in the protocol.

G. Bennett Humphrey (ed.), OSTEOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS.
 ISBN 0-7923-1905-2

Chemotherapy was instituted 2 weeks after surgery of the primary tumor for patients assigned to this treatment. The chemotherapy utilized in this study is shown in Figure 31-1 and featured high-dose methotrexate with leucovorin rescue, Adriamycin, the combination of Adriamycin and cisplatin, and the combination of bleomycin, cyclophosphamide, and actinomycin D. Chemotherapy was continued for approximately 45 weeks. Follow-up of patients included monthly chest radiographs, thoracic CT scanning every 4 months, and radionuclide bone scanning every 6 months for 2 years after surgery.

Statistical considerations

The statistical considerations of this study have been discussed in detail elsewhere [3].

Results

Between June 1982 and August 1984, while the randomization was open, 113 eligible patients were entered into the study. Thirty-six accepted randomization: 18 were assigned to immediate adjuvant chemotherapy and 18 to observation alone. An additional 77 eligible patients declined randomization but were followed according to one of the treatment groups of this study; 59 of these patients elected immediate adjuvant chemotherapy and 18 elected observation alone. These 113 patients are included in the updated life tables presented in Figure 31-2, which address the randomized question of the MIOS. In August 1984, when a preliminary analysis of this study indicated a significant event-free survival advantage for patients treated with immediate adjuvant chemotherapy, the randomization was closed and immediate adjuvant chemotherapy was recommended for all subsequent patients after definitive surgery of the primary tumor. An additional 88 eligible patients were entered into the study between August 1984 and October 1986, all of whom received adjuvant chemotherapy according to the treatment regimen of this protocol. All 165 patients treated with adjuvant chemotherapy are included in the analysis of the efficacy of the chemotherapy regimen and the analysis of prognostic factors.

The outcome of treatment for the 113 patients entered into the MIOS while the randomization was open between June 1982 and August 1984 is shown in Figure 31-2. Of the 36 patients treated with observation alone in the control group, 31 have relapsed (including 16 of 18 randomized patients and 15 of 18 nonrandomized patients). Among the 77 patients assigned to immediate adjuvant chemotherapy by randomization or by choice, 28 have relapsed and two patients have died without evidence of recurrent tumor. Whether randomized or nonrandomized patients are considered, the projected 8-year event-free survival for the control group is 11% compared to 61% for

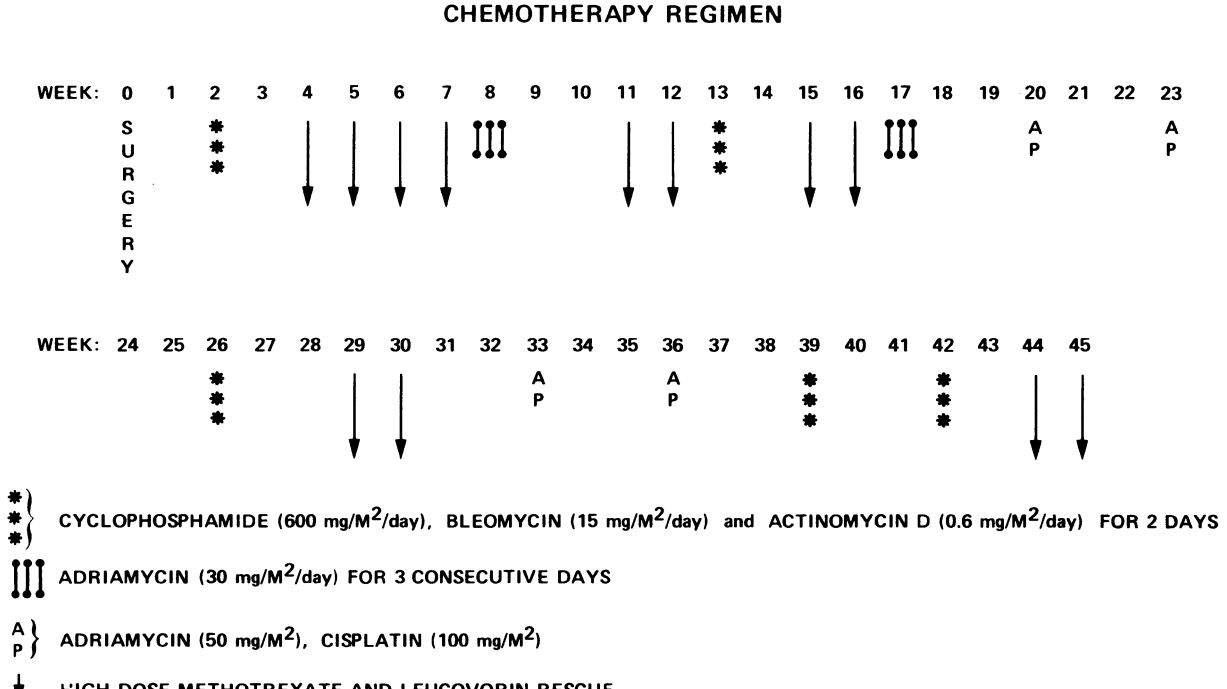
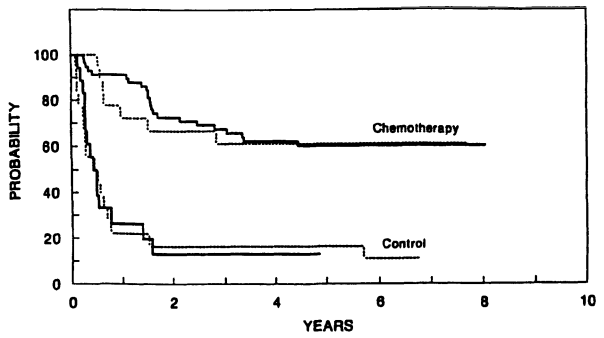


Figure 31-1. Chemotherapy regimen of the Multi-Institutional Osteosarcoma Study.



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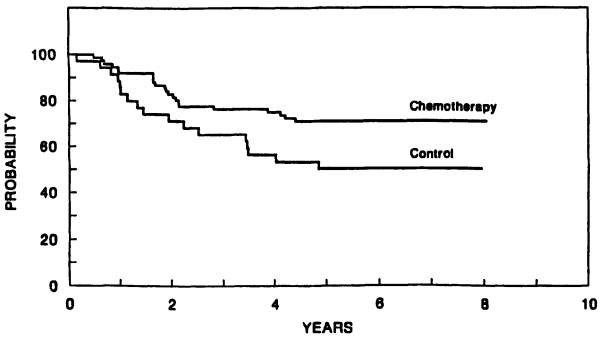


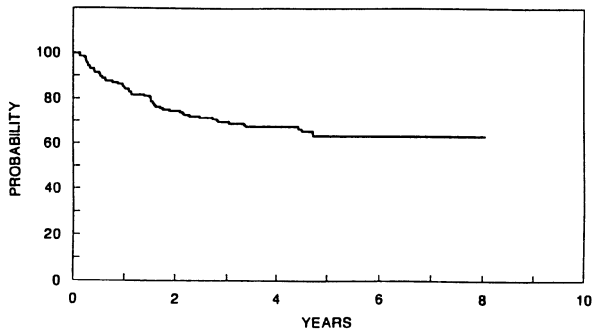
B

Figure 31-2. Outcome of patients entered on theMIOS while the randomization was open. **A**: Life tables of event-free survival for patients accepting and declining randomization according to assigned treatment. Dashed lines indicate randomized patients; solid lines indicate nonrandomized patients. **B**: Life tables of overall survival for randomized and nonrandomized patients pooled according to treatment.

the group treated with immediate adjuvant chemotherapy (Figure 31-2A). The life tables demonstrate that the overall outcomes of randomized and nonrandomized patients were similar and that the difference in outcome between chemotherapy and the control groups (a difference that is highly statistically significant) is equally dramatic whether randomized or nonrandomized patients are considered.

Life table analysis of overall survival for these 113 patients is shown in Figure 31-2B. Although no difference in overall survival is demonstrable among randomized patients (because of small numbers; data not shown), when randomized and nonrandomized patients are pooled according to their assigned treatment the difference in overall survival is apparent. At 8 years the projected overall survival for patients treated with immediate adjuvant chemotherapy is 71% compared to 50% for patients treated initially only with surgery and salvaged after relapse with thoracotomy and chemotherapy.

The remaining analyses of this report include the 165 patients treated with



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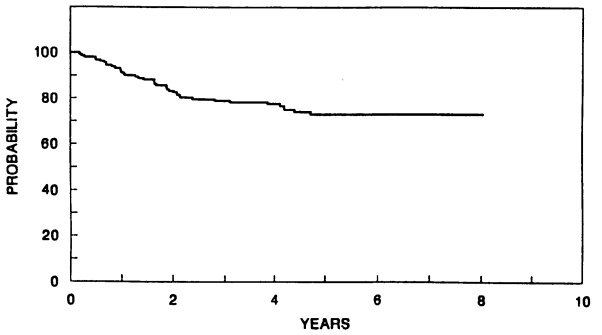

B

Figure 31-3. **A**: Life table of event-free survival for all 165 patients treated with adjuvant chemotherapy on the MIOS. **B**: Life table of overall survival for all 165 patients treated with adjuvant chemotherapy on the MIOS.

immediate adjuvant chemotherapy on the MIOS and do not include patients treated initially on the observation arm of the study. Fifty-seven of these 165 patients have suffered adverse events and 108 survive free of recurrent disease. Life table analysis of event-free survival for all 165 patients treated with adjuvant chemotherapy on the MIOS is shown in Figure 31-3A. With a minimum follow-up of 4 years, the projected event-free survival at 2 years is 75%, and at 8 years it is 63%. The overall survival is indicated in Figure 31-3B. Seventy-three percent of the patients are projected to remain alive at 8 years.

When all of the patients treated with immediate adjuvant chemotherapy were included in an analysis of prognostic factors, neither age, sex, nor level of serum alkaline phosphatase proved to be prognostic for disease outcome, nor did patients undergoing limb-sparing resection appear to have a significantly increased risk of developing recurrent disease as compared to those undergoing amputation (8-year event-free survival for patients undergoing limb sparing resection = 56.5% compared to 8-year event-free survival of 65% for patients undergoing amputation). Elevation of the serum lactic

dehydrogenase (LDH) at diagnosis proved to be the factor most predictive of an adverse outcome among patients treated with adjuvant chemotherapy. Thirty-nine (31%) of the patients were found to have LDH levels that were abnormally elevated. At 8 years the projected event-free survival for patients with elevated LDH is 40% compared to 74% for the remaining patients who had normal LDH at diagnosis ($p < 0.001$). Similarly, the site of the primary tumor proved to be prognostically important. Patients with primaries in the tibia (with a projected 8-year event-free survival of 79%) fared significantly better than patients with tumors at other sites (8-year event-free survival of 56%; $p = 0.005$). In a univariate analysis, size of the primary tumor proved to be a significant prognostic factor. Patients with a tumor diameter larger than 5.0 cm fared significantly worse with an 8-year event-free survival of 49% compared to 72% for patients with tumor diameters smaller than 5.0 cm ($p = 0.002$). However, after correcting for LDH and primary site, tumor size was no longer of independent prognostic value. It is notable that the prognostic value of elevated LDH remains significant after correcting for tibia vs. nontibia as the primary site.

Discussion

From the results of the MIOS, the favorable impact of adjuvant chemotherapy on event-free survival in patients with osteosarcoma of the extremity appears incontrovertible. Our results have been confirmed in another study from UCLA [5]. Further, when randomized and nonrandomized patients from the MIOS are analyzed together, the favorable impact of adjuvant chemotherapy on overall survival also emerges. These results have now been widely accepted, and it is clear that adjuvant chemotherapy should be a component of treatment for all patients with high-grade osteosarcoma.

The chemotherapy regimen utilized in this study has proven to be highly effective, since 63% of the patients treated with immediate adjuvant chemotherapy are projected to remain free of recurrent disease and almost three quarters of the patients are projected to be alive 8 years from diagnosis. These results compare favorably with results reported from other recent multiinstitutional trials utilizing a variety of chemotherapy regimens [6–8].

Examination of prognostic variables in this large group of patients with osteosarcoma treated on a single chemotherapy regimen allows the identification of several factors predictive of adverse outcome. In this study, elevation of the serum LDH at diagnosis proved to be the single factor most predictive of adverse outcome. While elevation of serum LDH has been reported to be an adverse prognostic factor in children with Burkitt's lymphoma and in those with Ewing's sarcoma, it has not been previously demonstrated to be an adverse prognostic factor in children with osteosarcoma. Children with primary osteosarcomas in the tibia have a more favorable outcome, although this was demonstrated in the prechemotherapy era.

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32. Treatment of osteosarcoma: Experience of the Cooperative Osteosarcoma Study Group (COSS)

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Adjuvant chemotherapy

The cooperative osteosarcoma study (COSS) group of the German Society of Pediatric Oncology (GPO) has been conducting adjuvant chemotherapy trials in children and adults with Osteosarcoma (OS) of the extremities since 1977. An outline of chemotherapies used for the different trials is given in Figure 32-1, and the metastases-free survival (MFS) probabilities in Figure 32-2.

Figure 32-2 shows a significant increase in the MFS rate from the COSS-77 study to the COSS-80 study, which may be attributed mainly a doubling of the high-dose methotrexate with leucovorin rescue (HDMTX) dose from 6 to 12 g/m^2 [1]. Thereafter only following the introduction of the ifosfamide (IFO)/cisplatinum (CPL) combination in the COSS-86 study could an additional improvement in MFS be achieved. In addition, important findings influencing our actual policy and future plans have been made.

Primary (neoadjuvant) chemotherapy and delayed surgery

An important step was the switch from adjuvant chemotherapy in the COSS-77 study to neoadjuvant (primary) chemotherapy and delayed surgery in subsequent studies. The expectations of that strategy were

1. Improved systemic tumor control due to immediate and stringent chemotherapy undisturbed from wound healing problems after major surgery.
2. Improved local tumor control and facilitation of limb-salvage procedures.
3. Gain in prognostic information from in vivo observation of tumor response and thus an opportunity for individual modulation of treatment.

Improved systemic tumor control

No data from controlled studies comparing adjuvant vs. neoadjuvant chemotherapy are available at present. The MFS rate of patients from the COSS-80 study or the COSS-82 study, who underwent amputation following

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 ISBN 0-7923-1905-2

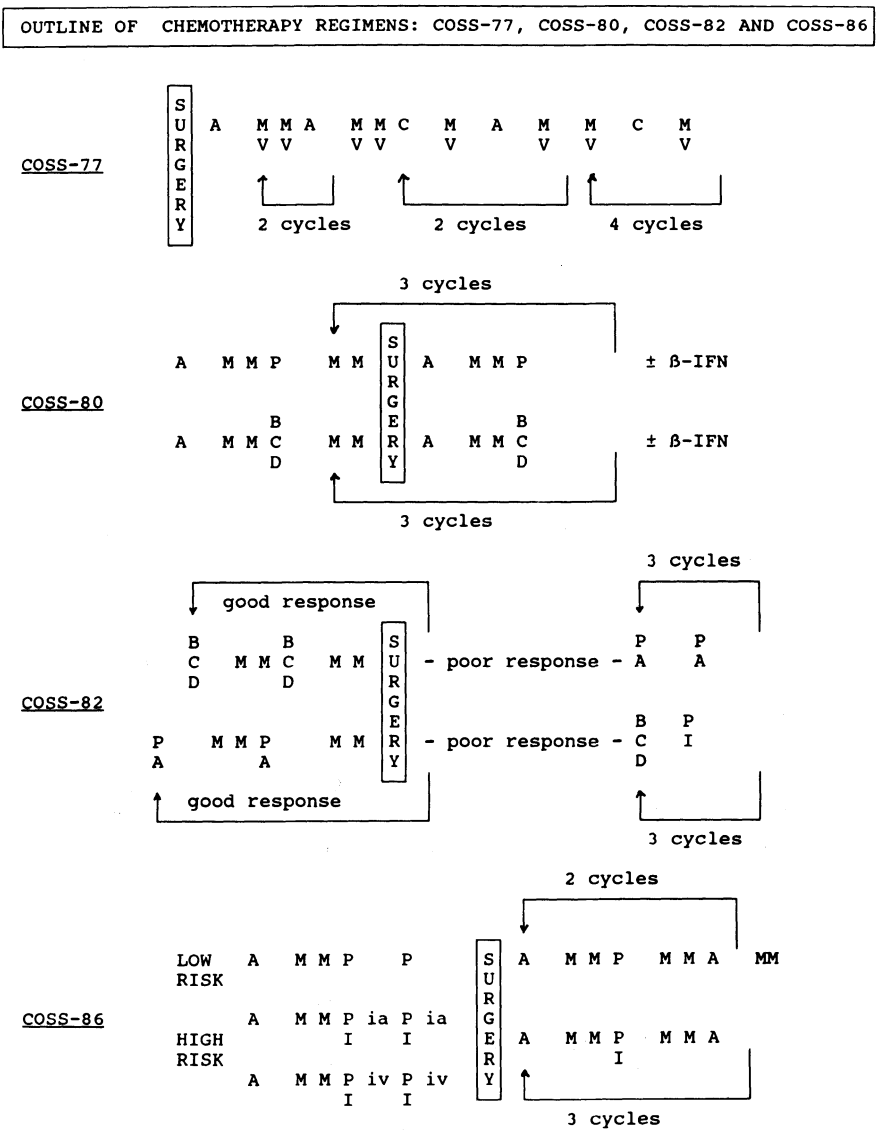

Figure 32-1. Outline of chemotherapy regimen of the cooperative osteosarcoma studies COSS-77, COSS-80, COSS-82, and COSS-86. A = doxorubicin; M = high-dose methotrexate with leucovorin rescue; V = vincristine; c = cyclophosphamide BCD = bleomycin + C + dactinomycin; P = cisplatinum; I = ifosfamide; β-IFN = interferon β; ia = intraarterial; iv = intravenous. There was no difference in the COSS-80 study between the cisplatinum, and BCD arms nor between patients receiving β-interferon after completion of chemotherapy or not. All the results were thus combined and reported as the COSS-80 results.

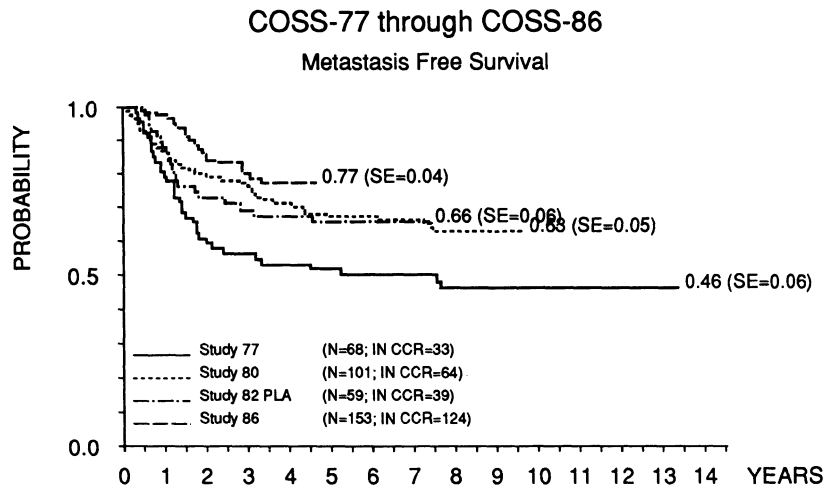

Figure 32-2. Eastimated metastasis-free survival (MFS) rates of 379 patients with classic osteosarcoma of the extremities after adjuvant (neoadjuvant) chemotherapy according to the consecutive cooperative osteosarcoma studies COSS-77 through COSS-86. Only the aggressive control arm (PLA) of the COSS-82 study is shown in this figure.

neoadjuvant chemotherapy compares favorably with that from patients receiving the same chemotherapy following primary amputation that was performed elsewhere prior to referral of the patients to one of the COSS institutions (Figure 32-3), [2]. Even though the patient characteristics of the two groups were comparable, the results of this nonprospectively controlled investigation has to be regarded cautiously. However, it seems, appropriate to state that delaying surgery for 10–12 weeks in order to give preoperative chemotherapy has no untoward effect on survival.

Local control and surgical procedure

Orthopedic surgeons point out that there is an advantage in operating on tumor that has shrunken after chemotherapy and has become demarcated by a firm pseudocapsule. The latter allows the definition of resections as "wide" at most narrow margins conceivable. Also the value of the gain of time for preparing limb-salvage procedures need not be explained. This time may be used for thoroughly establishing the indication and preparing for the planned type of definite surgery, for asking for consultation from experienced surgeons, and for obtaining the material required for reconstruction.

The policy of the COSS group towards limb-salvage procedures in the past was quite restrictive. In the majority of cases ablative procedures or rotation-plasties were performed. Correspondingly, the overall local control in the COSS-77 through COSS-86 studies is excellent (445/467, 95.3%). The local failure rate, however, is 11.1% after resection, which is five times higher than

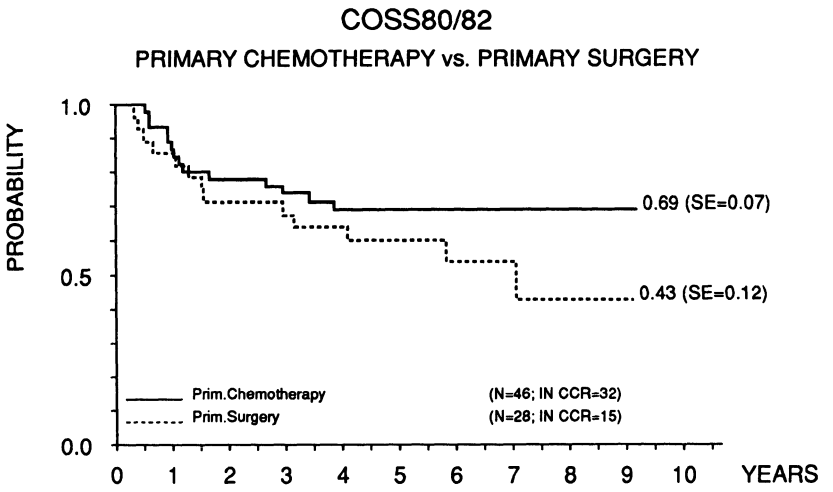

Figure 32-3. Estimated metastasis-free survival rate after primary chemotherapy and delayed amputation vs. primary amputation elsewhere, followed by chemotherapy according to the cooperative osteosarcoma studies COSS-80 and COSS-82. In the latter study, primarily operated patients were allocated to the aggressive PLA control arm without postsurgical salvage treatment. (Difference not significant.)

after ablative procedures (2.2%, $p < 0.05$). Therefore it is apparent that limb-salvage procedures, though relatively safe, definitely reduce on the chance of cure. In an earlier analysis we found that this fact was also reflected by a diminished MFS rate after limb-salvage procedures [3]; others, however, have not been able to confirm this observation [4,5]. An increased local failure rate following resective surgery, especially in tumors not responding to primary chemotherapy, was also reported from the Rizzoli Institute in Bologna [6].

Despite limb-salvage procedures in 87% of cases after primary chemotherapy, the Bologna group had only one local failure in 116 patients with osteosarcoma [7]. The striking difference compared to our own experience may reflect the extraordinary expertise of surgeons from a single institution as compared to a cooperative study with more than 70 participating institutions. However, it also has to be kept in mind that in our analysis not only were local failures included as first events (as usual), but also cases with simultaneous metastases and local failures following the manifestation of metastases. By evaluating local failures as first events only, our local control rate would be 459/467 (98%).

With the aim of making limb-salvage procedures safer and more feasible for more patients, in the COSS-86 study we tried to improve the effect of preoperative CT on the primary tumor. Cisplatinum was administered as an intra-arterial infusion, in addition to systemic high-dose methotrexate, doxorubicin, and ifosfamide [8]. No regional advantage (improved local response rate)

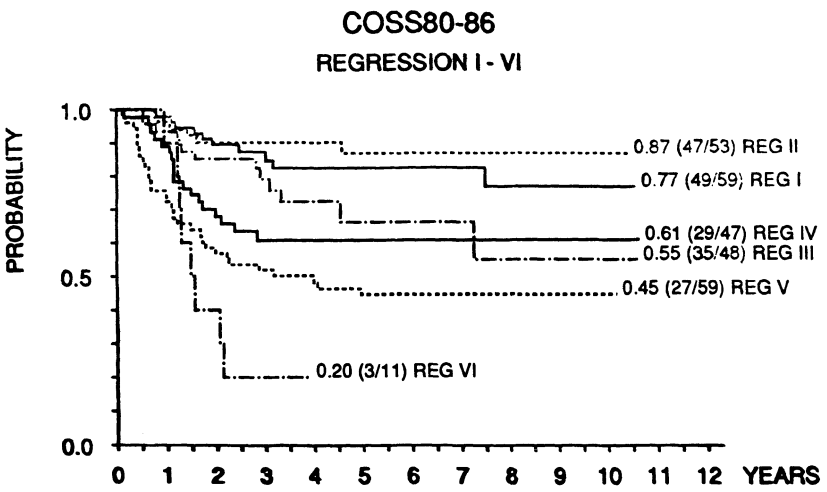

Figure 32-4. Estimated metastasis-free survival rate of 266 patients from the cooperative osteosarcoma studies COSS-80, COSS-82, and COSS-86, according to Salzer-Kuntschik histologic grades of tumor response (REG). REG I = no vital tumor cells; II = single vital tumor cells or single vital focus <0.5 cm diameter; III = less than 10% vital tumor; IV = 10–50% vital tumor; V = more than 50% vital tumor; VI = no effect of chemotherapy. The higher the extent of tumor necrosis, the better the prognosis, but no cutoff point for a definition of good vs. poor responders is visible.

could be obtained within this setting. For further information see the article by Bielack et al. in this issue.

Prognostic significance and modulation of treatment according to tumor response

The tumor response, defined as >90% necrosis histologically [9], was found to be highly predicative for MFS. Hence it is clear that even patients with a poor response achieved a 6-year MFS rate of 44%, which is far higher than historical controls without chemotherapy [10] or for the zero control group in Link's study [11].

No cutoff point separating good from poor responders could be defined when analyzing the 3 year MFS-rates using the Salzer-Kuntschik grades of tumor response in almost 300 patients on whom adequate information was available (Figure 32-4).

Our attempt to improve the prognosis of poor responders by offering them an alternative "salvage" chemotherapy after surgery failed [12]. In that study (COSS-82) it was intended to spare some patients from the dreadful late effects of DOX and CPL by using for induction treatment a relatively nontoxic combination consisting of HDMTX and BCD (bleomycin + cyclophosphamide + dactinomycin). In good responders this treatment was continued postoperatively, while poor responders were switched to DOX and

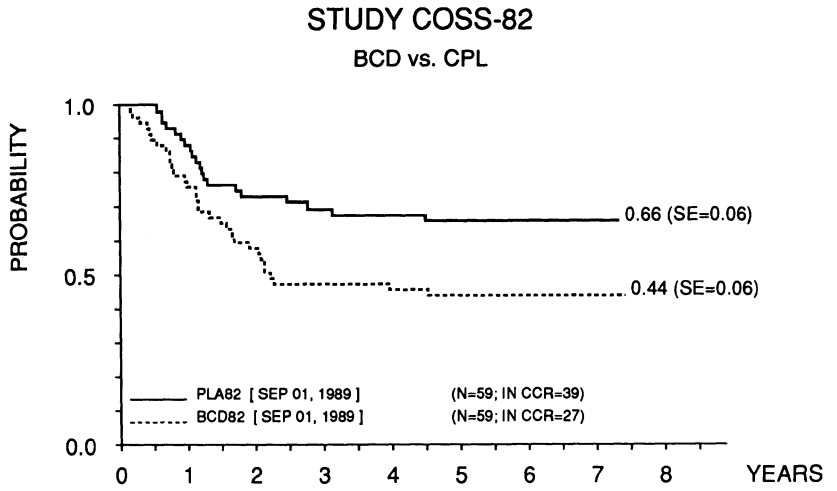

Figure 32-5. Estimated metastasis-free survival (MFS) rates of the low-toxicity study arm (BCD) vs. the initially aggressive control arm (PLA) and selection of postsurgical chemotherapy according to tumor response ($p < 0.05$). The low response rate after BCD treatment could not be compensated for by postsurgical use of PLA, and the MFS rate after PLA induction could not be improved by switching to an alternative chemotherapy in poor responders.

CPL. In a control arm DOX and CPL were used with HDMTX from the beginning. Patients from that arm were salvaged with BCD and CPL/IFO.

As expected, the rate of good responders in the study arm was significantly lower than in the control arm (26% vs. 60%, $p < 0.001$). Postsurgical salvage chemotherapy, however, was not able to improve the prognosis for poor responders as compared to previous experience without salvage strategy. Not even in the study arm were quite a number of potential good responders left behind for salvage after weak induction chemotherapy [12]. Consequently the 5-year MFS rate of the study arm was significantly lower than that of the initially aggressive control arm (Figure 32-5). The conclusion from that study is that DOX is mandatory at present for the treatment of osteosarcoma and that it has to be used early, since resistance emerges quite rapidly. The dependence of treatment success in OS on doxorubicin has also been supported by other groups [13,14].

These results confirm that intensive initial therapy is the crucial element in treatment. Therefore, in the COSS-86 study early intensification has been introduced for high-risk patients (large tumor and/or abundant chondroid matrix [15] and/or an unsatisfying scintigraphic response at week 5 [16]. Intensification consisted of CPL not as a single drug, as for low-risk patients, but in combination with ifosfamide. The response rate in this study is >70% and is higher than in our previous studies. This improvement, according to a preliminary analysis, also seems to result in an improved MFS rate (see Figure 32-2).

Conclusions

Further improvement in survival may be difficult to accomplish until more effective drugs are available. With the given drugs and the aim of simultaneously reducing the rate of late sequelae, there are only two treatment options. One is to increase the dose rate but at the same time decrease the total dose, and hence the treatment duration. The other is to look for more effective and less toxic modes of drug administration.

With regard to doxorubicin, prolonging the infusion time in order to decrease toxicity might compromise its efficacy [17]. Prolongation of the infusion time of cisplatinum or split-course treatment can be expected to reduce toxicity [18] but, unlike DOX, continuous infusion also might increase efficacy in osteosarcoma. This is suggested from a very high response rate of more than 80% after only two courses of HDMTX followed 1 week later by doxorubicin and a 72-hour intraarterial infusion of cisplatinum [19]. Using a very similar approach but a 5-hour intraveneous cisplatinum infusion, we achieved only a 60% response rate [12], and intraarterial as compared to intraveneous infusion (1–5 hours) of cisplatinum did not improve the response rate [8]. Ifosfamide nephrotoxicity however, might even be higher after a continuous infusion [20,21]. Moreover in soft tissue sarcomas, bolus injections have been found to be more effective than a continuous infusion [22].

Thus our new protocol, which just now is being piloted, uses the three mutual two-drug combinations of doxorubicin, ifosfamide, and cisplatinum (DOX/IFO, DOX/CPL, CPL/IFO) only once each before and once after surgery. In addition, HDMTX—as two weekly infusions—is given once prior to surgery following DOX/IFO and three times after surgery following each of the three mentioned drug combinations. The cumulative dosages of the respective drugs are very moderate; 240mg/m^2 for DOX, 24 g/m^2 for IFO, and 480 mg/m^2 for CPL. The scheduled treatment duration is short (24 weeks) but the dose rate is high.

Summary

Using high-dose methotrexate, doxorubicin, and cisplatinum (or BCD) for adjuvant chemotherapy in osteosarcoma of the extremities, we achieved 8-year metastasis-free survival rates of 60–70%. No relapse has been observed after that time. A dose of 12 g/m^2 of high-dose methotrexate seems superior to 6 g; doxorubicin was found to be indispensable for efficient therapy and administering ifosfamide in addition seemed to be beneficial. Primary chemotherapy appeared to be safe and to facilitate surgery. The response on chemotherapy provided valuable prognostic information. Salvage of poor responders by alternative postsurgical chemotherapy was unsuccessful. Intraarterial, as opposed to intraveneous, use of cisplatinum, in addition to systemic three-drug chemotherapy, did not improve the local tumor response rate.

The local failure rate was low (4.7%); it was higher, however, after limb-salvage procedures than after amputation and rotationplasty (11.1% vs. 2.2%, $p < 0.05$). The outcome after local failure was almost universally fatal.

The most intriguing late sequelae of chemotherapy were cardiomyopathy due to doxorubicin and hearing loss due to cisplatinum. Given the limited number of effective drugs, it might be difficult to further improve the cure rate and also to diminish late toxicity. Exploration of the most effective but least toxic mode of drug administration might be one possibility. Another might be reduction of the cumulative doses and therapy duration, while simultaneously increasing the dose rate.

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33. The European Osteosarcoma Intergroup (E.O.I.) studies 1980–1991

A.W. Craft and J.M.V. Burgers

In 1980 there was considerable confusion surrounding the management of osteosarcoma. Those involved in the management of this disease were faced with a series of conflicts. The early promise of chemotherapy as initially reported by Jaffe appeared to have been logically extended by Rosen in a series of studies culminating in the startling results of T10 wherein over 90% of patients were disease free at 2 years. However, the use of chemotherapy in Europe was producing surprisingly little improvement in survival over that achieved by amputation alone. Both the E.O.R.T.C. Studies and that undertaken by the Medical Research Council (M.R.C.) in the United Kingdom were disappointing, although in retrospect it is clear that the intensity of chemotherapy was inadequate [1,2]. At the same time the Mayo Clinic was reporting a survival of 50% with surgery alone and no chemotherapy, one of their explanations being that the natural history of the disease had changed and that chemotherapy was no longer necessary [3].

It was against this background that a number of European cancer study groups met together and formed the European Osteosarcoma Intergroup (E.O.I.). These groups were the M.R.C., United Kingdom Children's Cancer Study Group (U.K.C.C.S.G.), the Bone and Soft Tissue Sarcoma Group of the E.O.R.T.C., and the International Paediatric Oncology Society (S.I.O.P.). They were later joined by the Canadian Sarcoma Group (C.S.G.). Initially the Institute Gustav Roussy (I.G.R.) was involved in joint discussions but later decided not to participate in the E.O.I. studies and to pursue a Rosen T10 approach. Results of the I.G.R. studies are also given in this volume by Kalifa. The "no chemotherapy" question was soon settled by the MIOS and U.C.L.A. studies [4,5] so that the E.O.I. was able to concentrate on determining the optimum chemotherapy.

The first E.O.I. study (80831) began as a randomized phase II trial in which two short intensive chemotherapy regimes were to be compared for toxicity and for response with a view to comparing one of these with a multidrug Rosen-type regime in a subsequent study. The three most active drugs were used in 80831, i.e., cisplatinum (DDP), doxorubicin (DOX) + methotrexate (MTX). The study scheme is shown in Figure 33-1. All patients under the age of 40 years with biopsy-proven osteosarcoma were eligible for

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 ISBN 0-7923-1905-2

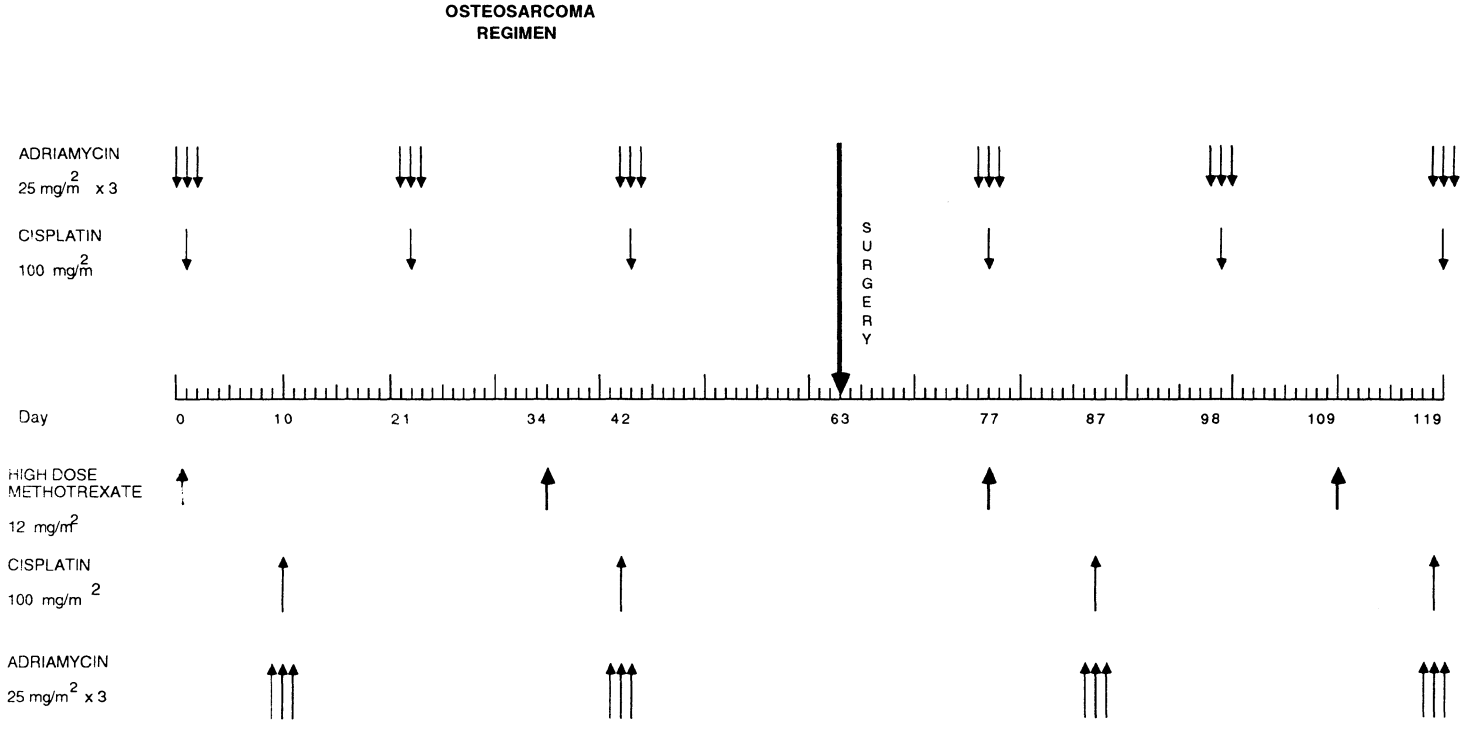

Figure 33-1. The study scheme for E.O.I. 80831.

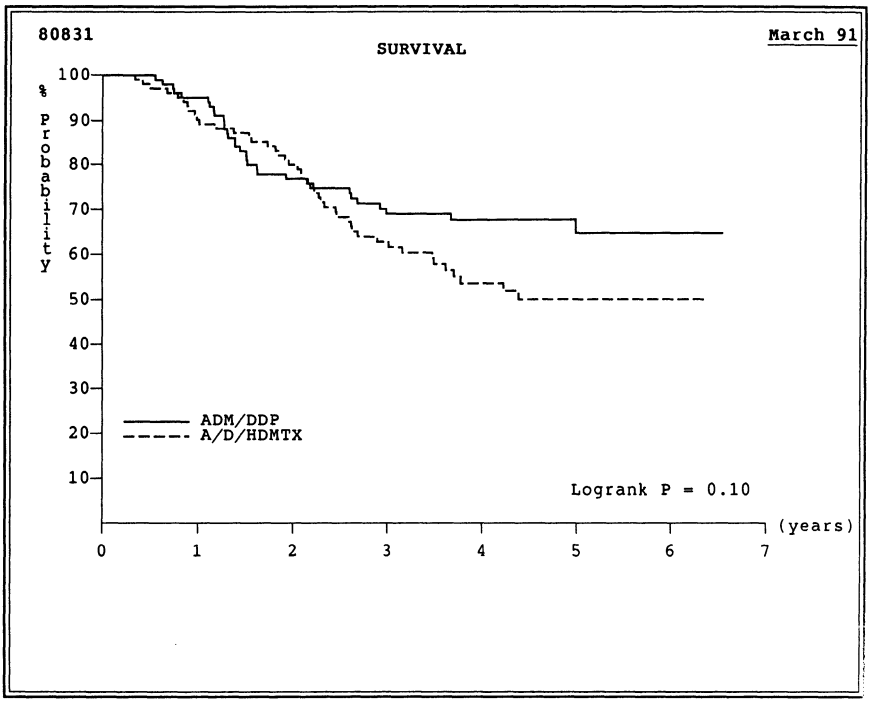

Figure 33-2. The survival according to treatment arm. The median follow-up is 40 months.

the study. Patients with axial skeleton primary tumors and those with metastases at diagnosis were eligible for study but this report will concentrate on only those nonmetastatic limb primary cases, some of whom had amputation prior to chemotherapy (adjuvant group) and some who had surgery midway through the chemotherapy regime. Although 80831 was set up as a toxicity and response study, it accrued patients very rapidly so that the target of 30 patients in each arm was soon passed, and the trial was therefore expanded into a formal phase III study with survival and disease-free survival as additional end points.

Between 1983 and 1986, 307 patients were registered, 207 of whom had limb primaries, no metastases, and were evaluable for the trial. A total of 102 received DOX/DDP and 105 the DOX/DDP/MTX arm; 163 (79%) successfully completed the allotted chemotherapy, 44 (21%) terminated chemotherapy early because of toxicity—19 (9%), early relapse—10 (5%), or patient refusal—7 (3%). There were eight (4%) major protocol violations.

The outcome according to treatment arm is shown in Figure 33-2 for survival (S) and Figure 33-3 for disease-free survival (DFS). The median follow-up at the time of production of these survival curves is 40 months. DFS is significantly better for the two-drug than the three-drug arm ($p < 0.02$), and although

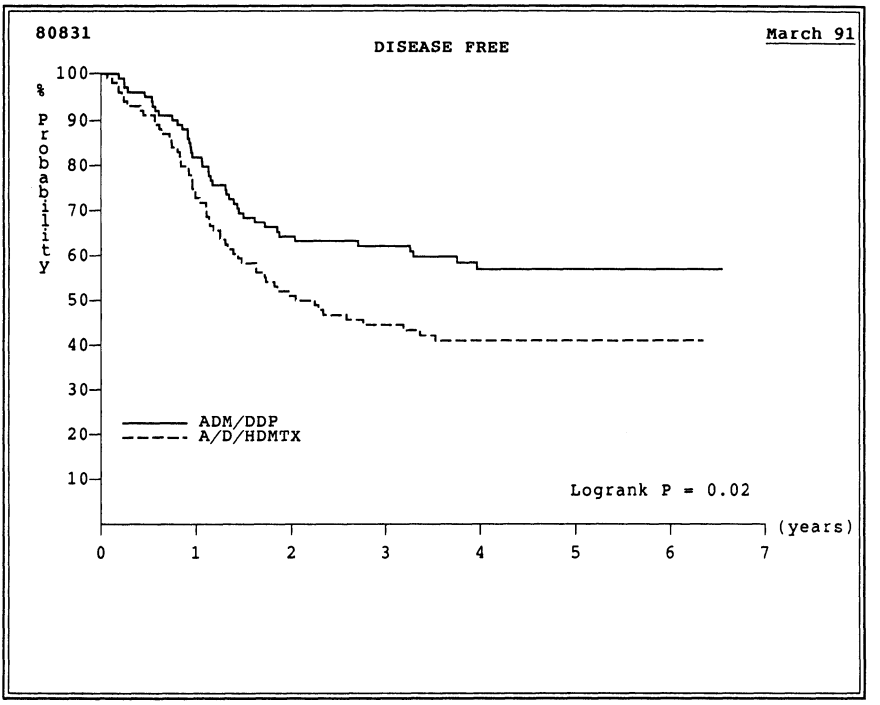

Figure 33-3. The disease-free survival according to treatment arm. The median follow-up is 40 months.

survival is better it does not reach statistical significance ($p < 0.15$). The only differences in toxicity between the two arms were an excess of hepatic toxicity in the MTX-containing arm and a slight excess of neurotoxity in the two-drug arm. There was one toxic death due to infection in the two-drug arm.

A total of 186 patients had surgery during chemotherapy and 21 had amputation before entry. Prior to entry into the study the registering doctor was asked to record the surgical plan for that patient, either amputation or conservation. Of 58 patients who initially planned to have amputation, conservative surgery was undertaken in 18, and of the 128 who were planned for conservation 27 had an amputation. DFS was better for those patients planned to have conservation than amputation ($p = 0.07$).

Both treatment arms were tolerable, with no serious differences in toxicity. DFS was better for the two-drug arm without MTX. However, the dose intensity of DDP/DOX was different in the two-arms, as was the total dose of these two drugs given (Figure 33-1). Patients in the three-drug arm were to receive only 66% of the total DDP/DOX dose that those in the two-drug arm were given, and the DDP/DOX "pulses" were 31 rather than 21 days apart. It is concluded, therefore, that the major finding of the 80831 protocol

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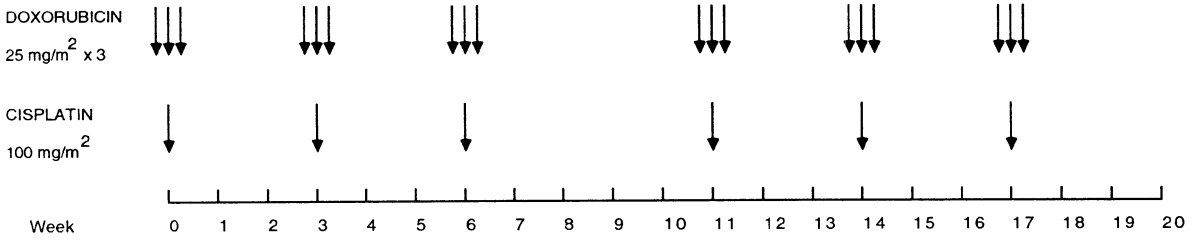

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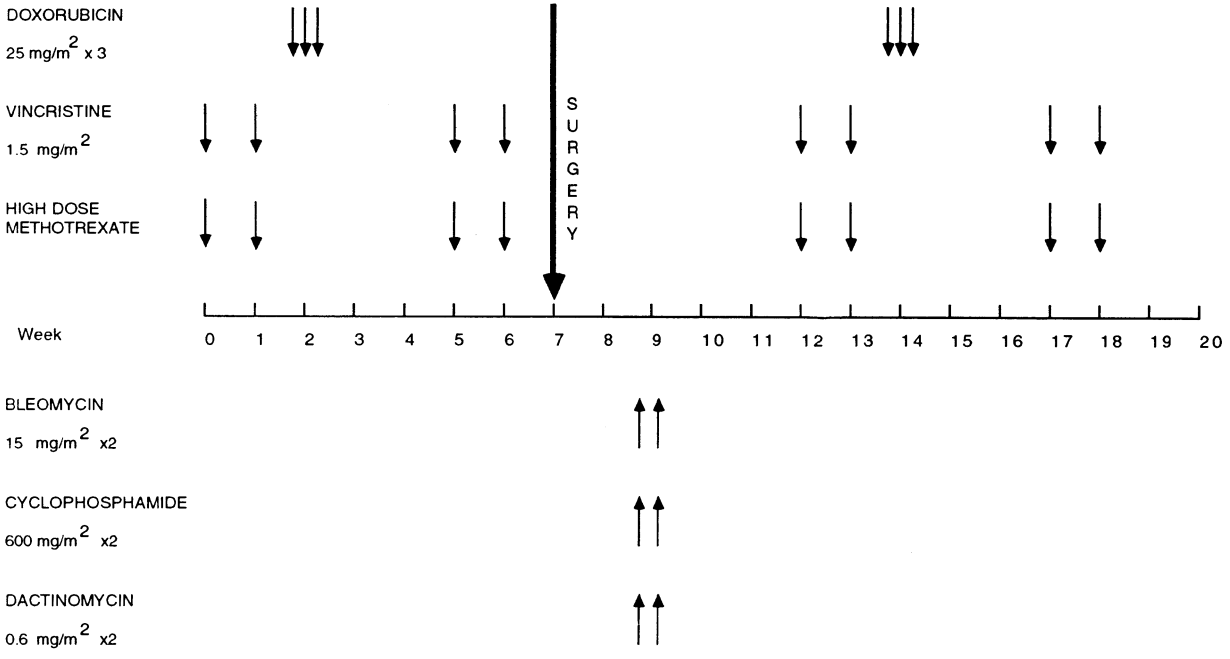

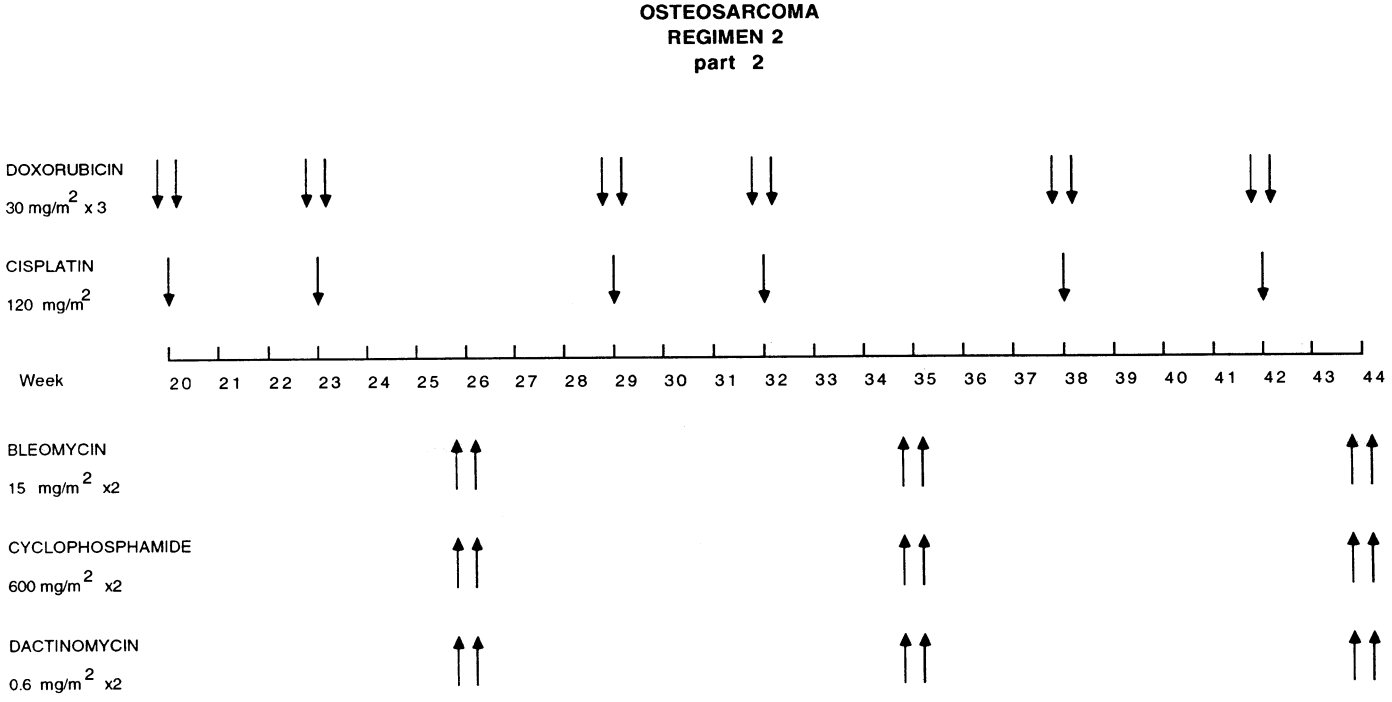

Figure 33-4. The scheme for 80861. The chemotherapy regimen is the two-drug arm from the 80831 study (A), and the multidrug regimen 2 (B) is similar to Rosen's T10 regime.

is that total dose and dose intensity are important predictors of outcome. It was decided to carry forward the DDP/DOX arm to the next study.

The second E.O.I. study (80861) commenced recruitment in 1986 and had accrued 310 patients by March 1, 1991. It is a randomized trial of two chemotherapy regimes in the treatment of operable, nonmetastatic osteosarcoma. The chemotherapy regimes are the two-drug arm from the 80831 study and a multidrug regime designed to be similar to the Rosen T10 regime. In the T10 scheme there is a switch of chemotherapy after surgery, depending on the histological response to the presurgery treatment. In the 80861 multidrug arm, there is a: "fixed switch" to DDP/DOX after surgery, which is independent of the postchemotherapy histology. The primary aim of the study is to determine whether a short, intensive chemotherapy lasting 18 weeks differs in terms of survival and DFS from a more prolonged regime lasting 42 weeks. The target number of patients to be entered is 400, and it is expected that this will be achieved in 1992. The study scheme is shown in Figure 33-4A and 33-B.

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34. The Childrens Cancer Group (CCG) Studies

James S. Miser and Mark Krailo

Introduction

The Childrens Cancer Group has undertaken studies using chemotherapy for osteosarcoma since 1976. These trials were based on the premise that the progression-free survival of patients with high-grade osteosarcoma was 25% or less when adjuvant chemotherapy was not used. Thus, randomized trials assessing the overall role of adjuvant chemotherapy were not undertaken: instead, the thrust of the CCG studies was an attempt to improve chemotherapy by evaluating new ways to administer the therapy or by evaluating new strategies to treat the disease.

Study I (CCG-741)

Objectives

The main objective of this trial was to compare high-dose methotrexate and moderate-dose methotrexate in the context of a multiagent regimen including Adriamycin and vincristine in treating high-grade osteosarcoma.

Methods

(Treatment protocol (Figure 34-1).) Patients were randomized to receive either high-dose methotrexate or moderate-dose methotrexate given with Adriamycin and vincristine. The high-dose methotrexate was given in a dose of 7500 mg/m^2 administered over 6 hours followed by folinic acid. The moderate-dose methotrexate was given in the dose of 690 mg/m^2 over the first hour, followed by 15 mg/m^2/h for 41 hr, followed by folinic acid. Methotrexate therapy for both treatment arms was started 3 weeks after the tumor was resected and was repeated every 6 weeks for a total of 12 courses. Vincristine (2 mg/m^2) was given 1 hour after starting methotrexate on both treatment arms. Adriamycin was given at 30 mg/m^2 on the day of surgical resection and at 90 mg/m^2 (30 mg/m^2 day $\times$ 3 days) every 6 weeks $\times$ 6 for a total of 570 mg/m^2.

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 ISBN 0–7923–1905–2

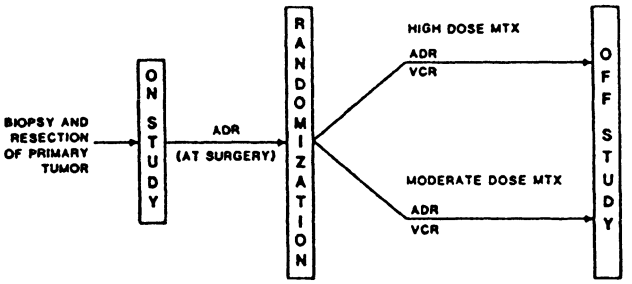

Figure 34-1. Schema for CCG-741.

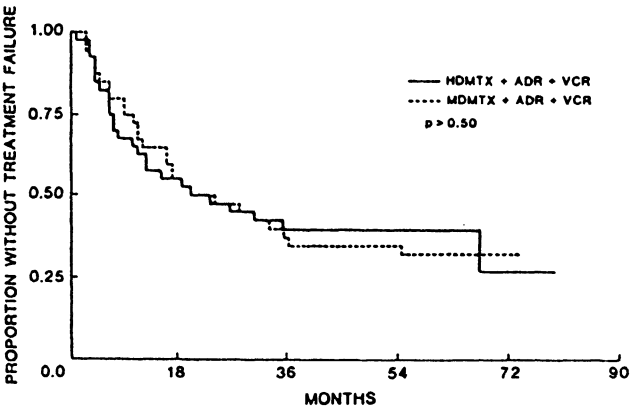

Figure 34-2. Disease-free survival related to treatment with high-dose methotrexate (——) compared to moderate-dose methotrexate (------).

Results

Two hundred thirty-four patients were registered in this study from 1976 to 1981. Sixty-eight were excluded from the analysis primarily because they did not fit the eligibility requirements. One hundred sixty-six patients who had extremity lesions that were completely resected and who were randomized are included in this analysis.

Thirty-eight percent of the patients (standard error = 4.0%) remain disease free after 48 months in the study. There was no statistically significant difference in disease-free survival when the two regimens were compared ($p > .50$). The occurrence of grade III or IV toxicities was not different between the two arms (Figure 34-2). Only the presence of spontaneous tumor necrosis at diagnosis was found to be independently prognostic ($p = 0.03$); decreased disease-free survival was associated with the presence of necrosis.

Conclusions

Moderate-dose methotrexate given as postoperative adjuvant therapy is of equivalent efficacy to high-dose methotrexate when used in the modest doses and schedules employed by this protocol and given with Adriamycin and vincristine. The presence of spontaneous necrosis was associated with relatively poor disease-free survival.

Study II (CCG-782)

Objectives

- To improve the disease-free survival in patients with newly diagnosed untreated nonmetastatic osteosarcoma of the extremities
- To use the histologic response of the primary tumor to preoperative chemotherapy to determine the optimal postoperative chemotherapy
- To evaluate a uniform histologic grading system for tumor response to therapy
- To examine patient characteristics that might influence event-free survival

Methods

Treatment protocol. Following diagnostic biopsy, all patients received two weekly courses of high-dose methotrexate (8 g/m^2 in older patients and 12 g/m^2 in younger patients) with vincristine (1.5 mg/m^2; maximum dose 2.0 mg) followed by leucovorin. At the third week a course of bleomycin (10 U/m^2), cytoxan (600 mg/m^2/day × 2 days), and dactinomycin (600 μg/m^2/day × 2 days)—BCD—was given; two additional courses of vincristine, high-dose methotrexate, and vincristine were then given to complete the induction phase.

Surgery was then performed. Following surgery all patients without local tumor progression in induction received a consolidation with one course of BCD, four courses of vincristine and high-dose methotrexate with leucovorin rescue, and one course of doxorubicin (30 mg/m^2/day × 3 days). Following completion of this consolidation, patients were assigned to maintenance therapy based on the histologic response of the primary tumor to preoperative chemotherapy. If there was less than 95% necrosis, then the patient received six cycles of cisplatin (3 mg/kg or 90 mg/m^2, whichever was less) in combination with doxorubicin (30 mg/m^2/day × 2 days) and three cycles of BCD. If there was 95% or more necrosis, the patient received three courses of BCD, four doses of high-dose methotrexate with vincristine and leucovorin, and three doses of doxorubicin (30 mg/m^2/day × 3 days).

Histopathologic grading system. The histopathologic response of the tumors to chemotherapy was graded in the following manner:
Grade I: No identifiable chemotherapy effect
Grade II: Less than 95% necrosis
Grade III: Scattered foci of viable tumor seen with 95% or more necrosis
Grade IV: No viable tumor seen
This histopathologic grading of each tumor was reviewed centrally.

Results

Two hundred and sixty-eight patients were registered on the study between August 1983 and October 1986. Thirty-six patients were not evaluable for the primary study questions. Of 232 patients, 209 were evaluable for histopathologic response. Fifty-five (27%) of the patients had a good histopathologic response to the induction chemotherapy; 117 had a poor histopathologic response without local disease progression, 32 (16%) developed local disease progression in the induction phase of the protocol.

The actuarial 4-year event-free survival (EFS) and overall survival of the 232 eligible patients were 58% and 70%, respectively. The 3-year EFS and survival were 88% and 94% respectively for the good histopathologic responders compared with 57% and 73% for the poor histopathologic responders. Patients with progressive disease in induction fared more poorly: only 48% remained event free at 3 years. Tumors of the proximal humerus and proximal femur were associated with an increase in risk for adverse event and death. Similarly, an elevated alkaline phosphatase level at diagnosis was also associated with a poor prognosis.

There have been 88 adverse events thus far. Eighty patients developed distant metastases: 60 in lung only, 10 in bone, six in multiple sites, one in brain, and three in unknown sites. Four patients had a local recurrence. Two patients developed a second malignancy: One died of a pulmonary embolus and one died of doxorubicin cardiotoxicity. Slightly less than one-half of the patients underwent limb-salvage procedures, with most of these having marginal to wide resections. Neither amputation nor limb-salvage surgery was statistically related to an increased risk of adverse events or death. Of the four local recurrences, two occurred in patients who had marginal resections.

Conclusions

- Chemotherapy improves event-free survival for the entire group of patients with nonmetastatic osteosarcoma of the extremity.
- Patients with a good histopathologic response to preoperative chemotherapy have an excellent event-free survival and overall survival.

- Patients with tumors arising in the proximal humerus and proximal femur have a poorer prognosis than those with tumors arising in other extremity sites.
- Limb-salvage surgery is not associated with an increased risk of an adverse event.

Future studies

The main thrust of future studies of the treatment of osteosarcoma in CCSG will be to evaluate in randomized trials the role of specific agents in the context of multiagent chemotherapy regimens. The first study will evaluate the role of ifosfamide, the second study will evaluate the role of high-dose methotrexate, and the third study will focus on the platinum analogs. An additional focus of these investigations will be to evaluate the effect of immunotherapy, specifically muramyl tripeptide phosphatidylethanolamine, when given in the adjuvant setting.

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35. An update of Scandinavian studies of osteosarcoma

Inkeri Elomaa

Introduction

The total population of all Scandinavian countries is about 22 million. These countries have a similar social structure, a modern medical service covering all inhabitants, and an effective registration system for all cancer patients. Based on these similarities, the Scandinavian Sarcoma Group (SSG) was founded in 1979 with the intention of improving the prognosis for patients with sarcoma. In the first study period 1982–1989, a nonrandomized trial based on Rosen's T10 protocol [1] for patients with operable osteosarcoma was used. Preliminary results have been published [2], and the complete data are currently under analysis. The second study, started in April 1990 and is based on the results and experience obtained from the preceding protocol and recent reports from other centres. This article gives an update of both trials. In addition, the Finnish 10-year results of osteosarcoma treatment are reviewed [3].

Finnish study

The main objective of these treatments was to decrease mortality. The series includes 26 children and 30 young adults (mean age 15 years, range 6–32) [3]. All patients were treated between 1976 and 1987 at the University Central Hospital of Helsinki. The patients had high-grade osteogenic sarcoma, located in an extremity (55 patients) and in the spine (one patient). The tumor penetrated the periost, growing into surrounding soft tissues in 52 patients.

Surgery

The operation most often used was wide excision, including, when necessary, (1) resection and osteosynthesis (two patients) (2) rotationplasty (three patients), and (3) endoprosthetic replacement (one patient). All 56 patients were treated with combination chemotherapy: 20 patients according to either Rosen's T4 or T7 protocols during 1976–1979 [4] and 32 patients after 1980 according to Rosen's T10 protocol [1].

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 ISBN 0-7923-1905-2

Thirteen of 56 patients developed metastases about 11 months after diagnosis. The actuarial disease-free survival was 80% in the first and 73% in the second to eighth years. There was no statistically significant difference in survival between the chemotherapy protocols used (T10 vs. T4 & T7; $p < 0.27$ Mantel-Cox) or between good or poor responders ($p < 0.38$ Mantel Cox). Two children died due to toxic side effects: one because of septicemia (T4 protocol) and the other because of doxorubicin (total dose <420 mg/m^2) induced heart failure 7 years after discontinuation of chemotherapy (T4 protocol). An irreversible failure of spermatogenesis has been noted in patients who received cisplatin (T10 protocol) [5].

Conclusions

The Finnish study confirms that various protocols of multiagent chemotherapy, as described by Rosen, decrease mortality from osteosarcoma. However, on the basis of small number of the patients it remains uncertain whether preoperative chemotherapy is crucial and whether the use of additional active agents improves the overall disease-free survival of drug-resistant tumors.

SSG study (closed)

The aim of this study was to increase the disease-free survival rate for patients with operable osteosarcoma.

Study design

Ninety-seven patients (median age 16 years, range 6–36) with high-grade nonmetastatic osteosarcoma of an extremity were treated according to Rosen's T10 protocol [1,2]. Ninety percent of the tumors infiltrated surrounding soft tissues.

Treatment

Eighty-eight patients received four weekly courses of HDMTX alone (8 g/m^2 for patients >8 years and 12 g/m^2 for patients <8 years) as preoperative treatment. Surgery was carried out after 4 weeks and consisted of amputation in 75% of cases. Limb-saving surgery was possible only in a limited number of patients.

Results

The histological response to chemotherapy on the primary tumor was as follows: GI in 18 patients (21%), GII in 53 patients (60%), and GIII/IV in 17 patients (19%). The serum concentration of HDMTX after 24 and 48 hours was significantly lower in nonresponders than in responders [6].

The acturial 5-year overall survival was 63% and the disease-free survival 55% [6]. In the group of patients with an osteosarcoma in the lower leg, the overall survival was 87%, and the disease-free survival after 4 years was 70% [2]. GI responders had a significantly poorer overall survival than GIII/IV responders (49% vs. 89%, $p = 0.01$) [5]. No life-threatening side effects due to chemotherapy occurred.

Conclusions

Although the main objective of this study has been achieved, the results are inferior to those originally reported by Rosen et al. [1]. Less effects were observed on the primary tumor by preoperative chemotherapy, and only 19% of our patients had complete or near-complete tumor necrosis vs. 40% in Rosen's series. One explanation for this difference may be that our patients were treated exclusively with four weekly HDMTX infusions, whereas many of Rosen's patients received a combination of drugs for a longer period of time (16 weeks).

SSG study (active)

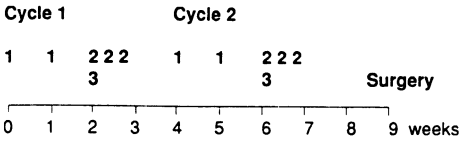
Objective

In order to obtain a further statistically significant increase in survival in the magnitude of 15%, about 300 patients would have to be recruited for a randomized study. Since this is impossible within a reasonable period of time, another one-armed study was initiated based on our experience in the previous study.

The results of the first SSG study showed that single-drug HDMTX was inferior in terms of its effect on the primary tumor. The most effective drugs (methotrexate, doxorubicin, and cisplatin) are being used in both preoperative and postoperative treatment in several ongoing investigations [7,8]. The drug sequence and the times of application vary between the studies. Ifosfamide is also an active drug, although less extensively studied [9]. On the other hand, the value of bleomycin, cyclophosphamide, and actinomycin D has recently been questioned [7]. In addition, the drugs contribute to the toxicity of the treatment and may adversely affect the dosage of more effective agents. Weiner and colleaques have concentrated the preoperative treatment, including methotrexate, doxorubicin, and cisplatin, over a relative short period of time. In this study 90% of patients showed total or nearly total necrosis of the primary tumor. The disease-free survival at two years was 77% [8]. Based on our own results and the encouraging results of this study with a limited number of patients, we decided to apply a similar regimen for our new study (Figure 35-1). Ifosfamide and etoposide (VP 16) were reserved for patients with a poor response (GI) [10].

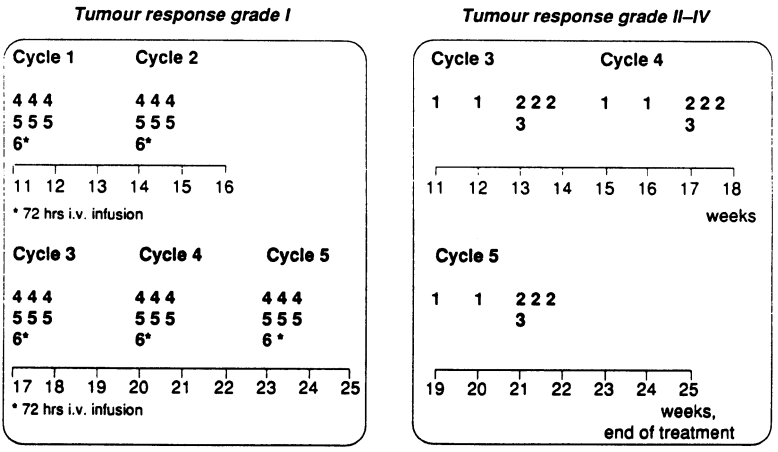
SSG VIII
COMBINATION CHEMOTHERAPY IN PRIMARY OSTEOSARCOMA

PREOPERATIVE TREATMENT



A responding tumor may increase in size clinically and by CT or MRI. Alkaline phosphatase may be the most reliable parameter at this stage. If progression is obvious, immediate surgery should be considered.

POSTOPERATIVE TREATMENT



		Dose reduction scheme		
		Leukocytes L x 10^9/l	Trombocytes T x 10^9/l	Dose reduction
1 Methotrexate	<12 years: 12 g/m² >12 years: 8 g/m² No dose reduction	<2	< 100	Delay start of CHT
2 Adriamycin	25 mg/m² daily (for 3 days)	<3	<100	Delay start of CHT
3 Cisplatin	90 mg/m²	<3	<100	Delay start of CHT
4 Ifosfamide	1.5 g/m² (for 3 days)			
5 Mitexan	600 mg x 3 i.v. injection hours 0, 4, 8 after Ifosfamide day 1–3			
6 VP–16	600 mg/m² (as 72 hrs i.v. infusion)			
2–6	Reduce 20 % if nadir value is	<1.0	<50	

Figure 35-1. Humphrey

During the previous study, extremity-conserving operations have been developed as an alternative to ablative surgery for many osteosarcoma patients. By potentiating the preoperative treatment, such techniques could probably be used for more patients with less risk of local recurrence.

In the first osteosarcoma study, doses of cytotoxic drugs often had to be reduced or delayed due to toxicity. Some patients treated by cisplatin developed permanent hearing defects, hypomagnesemia, and azoospermia. If the cumulative dose of cisplatin was 720 mg/m^2, given 120 mg/m^2 as i.v. infusions over 4 hours, which was the guideline in the first protocol, recovery of spermatogenesis did not occur [5]. However, Meistrich and colleagues have reported normal gonadal function in 78% of osteosarcoma patients 2 years after therapy. However, the cumulative dose of cisplatin in this study was less, with a median of 540 mg/m^2, and the drug was administered directly into the tumor by the intraarterial route [11]. In our new protocol the total dose of cisplatin is 450 mg/m^2. In the light of these facts and previous experience that 10–30% of patients will cure by surgical treatment alone, we feel that the overall treatment time should be shortened to limit long-term toxicity and the dose intensity should be increased to improve survival rates. Hence, the treatment period is now 25 weeks, instead of the previous 43 or 46 weeks.

Thus, the objectives of the present study are (1) an increase in disease-free survival, (2) an improvement in the effects of preoperative treatment on the tumor, and (3) a reduction in long-term toxicity. Future research will be aimed at investigating the nature of the tumors using chromosomal and ploidy studies [12–15].

Acknowledgment

The author would like to thank the Finnish Cancer Foundation for their support of this work.

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36. Neoadjuvant chemotherapy for nonmetastatic osteosarcoma of the extremities: The recent experience at the Rizzoli Institute

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Introduction

In the late 1970s Rosen et al. was the first to report the advantages of primary chemotherapy and delayed surgery (neoadjuvant chemotherapy) over adjuvant chemotherapy. The excellent results reported by these authors induced us to introduce neoadjuvant chemotherapy in 1983. In our first neoadjuvant study (OS/NEO/IOR-1°) preoperative chemotherapy consisted of two cycles of MTX and cisplatinum (CDP) (Figure 36-1). The rationale for the combined use of these two drugs has been described in a previous paper [1].

Postoperative chemotherapy differed depending on the grade of necrosis. Patients with poor necrosis (<60% tumor necrosis) were classified as *poor responders* and received a 45-week treatment with Adriamycin (ADM) and bleomycin, cyclophosphamyde, and dactinomycin (BCD). Patients with necrosis between 60% and 89% were classified as *fair responders* and received a 24 week of ADM, MTX and CDP. MTX was given at the same doses as preoperatively. Patients with necrosis >90%, considered *good responders*, received postoperatively only two cycles of MTX and CDP at the beginning of the study.

Because of four early relapses among the first 15 good responders postoperatively treated with this regimen, after December 1983 this arm of the study was closed and the good responders were postoperatively treated with the same regimen scheduled for fair responders (i.e., 24 weeks of MTX, CDP, and ADM). Of the 127 patients treated with this protocol between March 1983 and August 1986, 63 (49.6%) remained continuously disease free. This percentage was not significantly different from the percentage obtained with adjuvant chemotherapy. However, in this first neoadjuvant study only 26% of patients were amputated, compared to 82% in our previous adjuvant studies.

The results of this first neoadjuvant study have been reported in detail elsewhere and from these data we concluded that:

1. With neoadjuvant chemotherapy limb salvage was possible in about 70% of patients with osteosarcoma.
2. Patients who histologically have a good necrosis also have a good prognosis.

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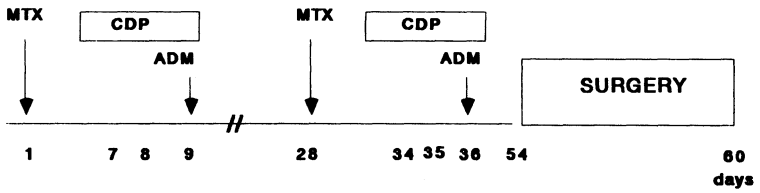
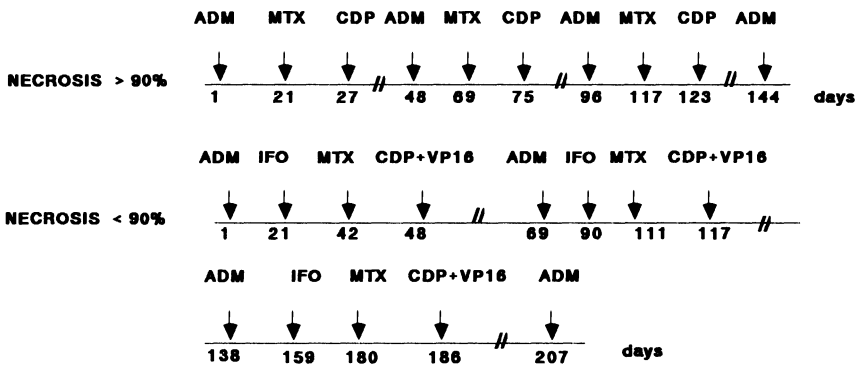
 ISBN 0-7923-1905-2

PREOPERATIVE CHEMOTHERAPY



MTX - Methotrexate 8 g/m2 i.v in 6 hrs followed by Citrovorum Factor rescue
CDP - Cisplatinum 120 mg/m2 for intrarterial continuous infusion of 72 hrs
ADM - Adriamycin 60 mg/m2 for i.v infusion of 8 h starting 48 hrs after the beginning of CDP

POSTOPERATIVE CHEMOTHERAPY



ADM - Adriamycin 45 mg/m2/day i.v for 2 consecutive days in 4 hours infusion
IFO - Ifosfamide 2g/m2/day i.v for 5 consecutive days in 90 min. plus MESNA
CDP - Cisplatinum the same doses used preoperatively, i.v
VP16 - Etoposide 120 mg/m2/day in 1 hour infusion for 3 days

Figure 36-1. Chemotherapy performed in the second neoadjuvant study.

3. The break point between good prognosis and poor prognosis is 90% of tumor necrosis.
4. Also in good responders, postoperative chemotherapy must be sufficiently protracted and must include ADM. Our attempt to reduce the postoperative treatment and to avoid the use of ADM in good responders failed.
5. In poor responders the prognosis remained poor, though the drugs in the postoperative treatment were changed.
6. In our multidrug regimen, high doses of MTX are more effective than moderate doses.

In both arms of this study toxicity was moderate and there were no deaths related to chemotherapy. In addition, in this study we were not able to demonstrate any factor that could predict the grade of histological response before treatment.

Therefore when in 1986 we started our second neoadjuvant study for osteosarcoma (OS/NEO/IOR-2°), the following points were considered:

1. The existence of a strict correlation between the necrosis induced by preoperative chemotherapy and the prognosis
2. Our inability to identify poor responders before treatment
3. The lack of efficacy of a different postoperative chemotherapy in poor responders
4. The efficacy of high doses (HD) of MTX over moderate doses

For these reasons the aim of this second study was to increase the percentage of good responders, and we tried to achieve this goal by adding ADM to HDMTX and CDP in the preoperative treatment. The purpose of this paper is to report the results obtained in 144 patients treated according to this second protocol.

Materials and methods

The study was started in September 1986 and closed in December 1989.

Patient selection

Patients were considered eligible for the study if they fulfilled the following criteria: (1) typical radiographic and histologic features of osteosarcoma (primary, central, and high grade); (2) tumor located in the extremities; (3) no prior history of cancer and no prior treatment elsewhere; (4) age <50 years; (5) no evidence of metastases. Among the 319 cases of osteosarcoma observed at the Rizzoli Institute between September 1986 and December 1989, 187 were eligible for the study. The reasons for exclusion of 132 cases are reported in Table 36-1.

Of the 164 patients who entered the study, 20 were not evaluable for the following reasons: two patients, complication of preoperative chemotherapy; four refused surgery; six patients refused further chemotherapy; and 8

Table 36-1. Patients who entered the second neoadjuvant study, patients excluded, and causes of exclusion

Total cases observed		319
Ineligible patients		132
Varietes of osteosarcoma	67	
Primary metastases	33	
Osteos not arising in the extremities	24	
Age >50 years	7	
Previous therapy	1	
Eligible patients		187
Patients who refused to enter the trial	23	
Patients who entered the trial	164	
Evaluable		144
Not evaluable		20

patients had major deviations in chemotherapy treatment. The remaining 144 patients are the subjects studied in this paper. The data concerning these patients are reported in Table 36-2.

Preoperative chemotherapy

The outline of preoperative chemotherapy is given schematically in Figure 36-1. Hydration of the patients over the first 24 hours after the administration of drug was performed according to the guidelines suggested by Rosen et al. [2].

Surgery

Surgery was scheduled 3 weeks after the end of preoperative chemotherapy, i.e., 7 weeks after the beginning of treatment and is discussed in Chapter 22 in this volume by Ruggieri et al.

Pathological examination

Surgical margins were classified as radical, wide, marginal, or intralesional [3]. The percentage of tumor necrosis induced by preoperative chemotherapy was evaluated by histological examination of the entire coronal slice of the tumor [4]. The response to preoperative chemotherapy was rated *good* (>90% tumor necrosis) or *poor* (<90% tumor necrosis).

Postoperative chemotherapy

As illustrated in Figure 36-1, patients with good necrosis (good responders) were treated for 21 weeks with cycles of ADM, MTX, and CDP, while patients

Table 36-2. Continuously disease-free survival of the 144 evaluated patients according to several variables

Sex		
Male	63/75	84%
Female	63/69	91%
Age		
<14 years	57/65	87.6%
>14 years	69/79	87.3%
Surgery		
Amputation	10/13	76.9%
Rotationplasty	8/9	88.8%
Resection	108/122	88.5%
Site		
Femur	61/72	84%
Tibia	34/42	90%
Humerus	17/20	85%
Fibula	9/9	100%
Radius	1/1	100%
Size		
<1/3 of the involved bone length	93/106	87.7%
>1/3 of the involved bone length	33/38	86.8%
Histology		
Osteoblastic	82/93	88.1%
Chondroblastic	16/21	76.1%
Telangiectatic	13/14	92.8%
Fibroblastic	9/10	90.0%
Other subtypes	6/6	100%
Grade		
3°	11/11	100%
4°	115/133	86.4%

with poor necrosis (poor responders) received a longer chemotherapy (30 weeks), which also included ifosfamide and VP-16.

Results

Clinical and radiological response to preoperative chemotherapy

The correlation between the clinicoradiographic response and the percentage of tumor necrosis was generally good but not always constant. In the five patients who had a poor clinical and radiologic response, necrosis was poor, while in the 120 patients with a good clinicoradiographic response, all rates of necrosis were observed. Among the criteria used to assess tumor response to

Table 36-3. Correlation between many variables and histological response

	good responses/ No. of cases	% good responses
Sex		
Male	57/75	76%
Female	55/69	80%
Age		
<14 years	53/65	82%
>14 years	59/79	79%
Surgery		
Amputation	5/13	38%
Rotationplasty	8/9	88.8%
Resection	99/122	81%
Site		
Femur	61/72	85%
Tibia	30/42	71%
Humerus	15/20	75%
Fibula	6/9	66%
Radius	0/1	
Size		
<1/3 of the involved bone length	87/106	82%
>1/3 of the involved bone length	25/38	65%
Histology		
Osteoblastic	73/93	78%
Chondroblastic	10/21	47%
Telangiectatic	14/14	100%
Fibroblastic	9/10	90%
Other subtypes	6/6	100%
Grade		
3°	8/11	72%
4°	104/133	78%

chemotherapy, reduced vascularity on angiograms was, as previously reported [5], the most predictive of histological response.

Histological response to preoperative chemotherapy

The response to chemotherapy according to necrosis was good in 112 patients (77.7%) and poor in 32 (22.3%) (Table 36-2). This difference is statistically significant ($p < 0.01$).

The rate of "good necrosis" was not correlated with tumor grade, or the site, age, or sex of the patient (Table 36-3). On the contrary, in contrast to our first neoadjuvant study [1], the histological subtype and size of the tumor seemed to influence the histological response. In the present study chondroblastic tumors showed a significant lower percentage of good responders than

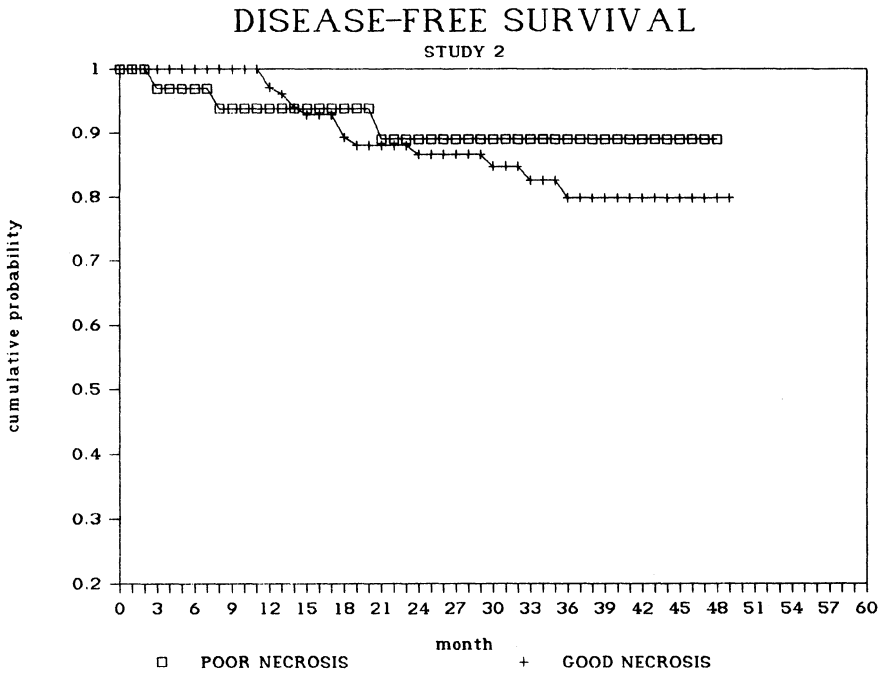


Figure 36-2. Continuously disease-free survival: comparison between good and poor necrosis in the second neoadjuvant study.

other subtypes (10/21 to 47.6% vs. 102/123 to 82.9%: $p < 0.001$), and the smaller tumors also had a higher percentage of good responses in comparison with the larger ones.

Continuously disease-free survival (CDFS)

Until December 1990, with an average follow-up of 30 months [12–51], 126 of the 144 (87.5%) patients remained continuously disease-free (CDF) and 18 relapsed with metastases. One of these patients had local recurrence before pulmonary metastases. The 2-year actuarial continuously disease-free survival rate was 0.84.

Considering that only the 82 patients who entered the study before January 1988 had a minimum follow-up of 24 months, the CDFS is 88.3% (71/82). In osteosarcoma treated with adjuvant chemotherapy, the probability of subsequent relapse after 2 years is, in our experience, only 9% [6], therefore, with this new protocol we think we will be able to achieve a cure rate of >75%.

The percentage of CDFS was 86.6% (97/112) for the good responders and 90.6% (29/32) for the poor responders (Figure 36-2). This difference is not statistically significant. The rate of CDFS was not related to the sex or age

of the patient; to site, size, histological subtype, nor grade of the tumor; nor to the roentgenological feature or extension, the type of surgery performed, nor the surgical margins.

In the 17 patients who relapsed with metastatic disease, the first metastasis was the lung in 14 cases and in bones in three. In all but one case metastases appeared after the completion of postoperative chemotherapy. The average time to metastases was 17.7 months (3–36) and appeared shorter for poor responders (10 months: range 3–21) than for good responders (20 months: range 12–36).

Of the 18 patients who relapsed with metastases, 10 are alive and disease free (3–21 months after metastasectomy), four are alive with uncontrolled disease, and four have died.

Chemotherapy toxicity

Chemotherapy was fairly well tolerated. However, in terms of dose intensity only 60 patients (41.6%) received 90% or more of the scheduled treatment, 46 (31.9%) a dose intensity between 80% and 89%, and the remaining 38 (26.3%) had a dose-intensity treatment between 52% and 79%. Reductions in dose intensity were mainly due to delays in treatment commonly caused by persistent leuko/thrombopenia (about 90% of cases) or by surgical complications. Four patients had clinical cardiotoxicity due to Adriamycin and two died. The other major systemic complications of chemotherapy were: two patients who developed lung tuberculosis, one patient who developed a transient ascites after the last MTX, and 12 patients had severe bone marrow depression requiring hospitalization. Other than the two Adriamycin cardiotoxic deaths, all patients recovered after appropriate medical management.

Discussion

In neoadjuvant chemotherapy for osteosarcoma, the combinations of MTX-BCD, MTX-ADM-BCD, ADM-CDP, and ADM-MTX have been widely used in preoperative treatment. However, the combination MTX-CDP has been scantily investigated, in spite of the fact that this combination has proven to be very effective in the treatment of metastatic osteosarcoma [7,8].

In our previous neoadjuvant study of localized osteosarcoma of the extremities [1], the preoperative combination of MTX (i.v.) followed by CDP (i.a.) was very effective, with a large percentage of good response (tumor necrosis >90%) in more than 50% of cases. Rosen [2], as have others [10,11,12], demonstrated that patients who had a good histological response to preoperative chemotherapy have a much better prognosis. This predictive value of necrosis is also true when preoperative chemotherapy is intraarterially delivered, as previously reported by our group [1] and by others [12,13].

In contrast to Rosen's results [14], several authors [9,10,11] have reported that "salvage" chemotherapy after surgery in poor responders did not improve prognosis. In our first neoadjuvant study our findings also demonstrated that in poor responders salvage chemotherapy with BCD after surgery was ineffective. Since it is not possible to identify, before treatment, the patients who will have a poor response to preoperative chemotherapy, one possible method of improving the overall cure rate is to give all patients more aggressive preoperative chemotherapy.

The preliminary results of our second neoadjuvant study seem to confirm the efficacy of this method. With more aggressive chemotherapy, adding Adriamycin to high-dose methotrexate and cisplatinum without prolonging preoperative treatment, we were able to increase the percentage of good responders to about 25%, and consequently to increase the percentage of disease-free survival at 2 years. In addition to the increase in CDFS, in this study, the salvage chemotherapy performed in poor responders by adding HDMTX, CDSP and ADM, ifosfamide, and VP-16 also contributed. These data seem to demonstrate that in neoadjuvant treatment of osteosarcoma, salvage chemotherapy, when performed with drugs that are effective in osteosarcoma, such as ifosfamide and VP-16, works very well.

This increase in CDFS was, however, associated with higher cardiotoxicity from ADM, since the cumulative dose in the second study was 480 mg/m^2, instead of the 360 mg/m^2 used in the first study. In neoadjuvant treatment of osteosarcoma, the problem of ADM cardiotoxicity when the drug is used at dosage >400 mg/m^2 has recently been stressed by the German COSS-86 study as well (K. Winkler, personal communication). These data suggest that a cumulative dose of 400 mg/m^2 of ADM should not be exceeded.

In our study more aggressive preoperative chemotherapy was also associated with a higher percentage of limb salvage. It is not possible to establish whether this increase in conservative surgery resulted only from more effective preoperative chemotherapy or from the simultaneous improvement in reconstruction techniques and, perhaps, also from the surgeon's different indications as well.

In conclusion, for the treatment of osteosarcoma of the extremities the reported protocol of neoadjuvant chemotherapy was effective in about 75% of cases, and it will probably be difficult to increase this percentage by only modifying chemotherapy regimens. Therefore, our aim in the future will be to select prognostic factors that allow us to identify before treatment those patients who will relapse and to use in these patients a new, more aggressive therapeutic approach.

Acknowledgment

This work was supported by Rizzoli Research Funds.

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37. Osteosarcoma of the extremities: Chemotherapy experience at Memorial Sloan-Kettering

Paul A. Meyers, Glenn Heller, and Vaia Vlamis

Introduction

At the Memorial Sloan-Kettering Cancer Center (MSKCC) multiagent chemotherapy for the treatment of osteosarcoma (OS) was introduced by Dr. Gerald Rosen in 1973[1]. Rosen and Marcove developed the concept of an initial period of chemotherapy prior to definitive surgery of the primary tumor (neoadjuvant chemotherapy). The initial impetus for this strategy came from the need to create custom prostheses for limb-sparing surgery. Rosen and Huvos examined the tumors removed at definitive surgery for the degree of necrosis present following neoadjuvant chemotherapy [2]. They demonstrated a strong correlation between the histologically evaluated response and subsequent disease-free survival (DFS).

By 1975 the treatment strategy for OS at MSKCC was accepted to include aggressive multiagent chemotherapy and wide en-bloc resection or amputation of the primary tumor. Whenever possible, chemotherapy was administered prior to definitive surgery. From 1975 to 1984, Rosen and colleagues performed a series of trials for the treatment of OS [1–4]. During this interval all patients were treated with high-dose methotrexate with leucovorin factor rescue (HDMTX), doxorubicin, and the three-drug combination of bleomycin, cyclophosphamide, and actinomycin-D (BCD). When it became clear that patients with a poor histological response to neoadjuvant chemotherapy had an inferior prognosis, an attempt was made to modify that prognosis by the addition of cisplatin to the postoperative chemotherapy regimen [2].

We have recently completed a retrospective review of all of the patients seen at MSKCC during the years 1975–1984. During this interval we saw 255 newly diagnosed patients with fully malignant, high-grade OS of the extremity who presented without clinically detectable metastatic disease. We excluded from the analysis patients who had received more than 3 weeks of treatment prior to referral to MSKCC or who had developed OS following Paget's disease or prior radiation.

G. Bennett Humphrey (ed.), OSTEOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS.
 ISBN 0-7923-1905-2

Treatment

A series of protocols was utilized to treat these patients: T5, T7, T10, and T12 [1–4]. Slightly more than half of the patients had this accomplished with limb-sparing surgery. Treatment for OS underwent frequent modification to incorporate the experience acquired with the preceding patients. Patient treatment was individualized for response to therapy using a number of indicators. When patients received neoadjuvant chemotherapy, the response to therapy was assessed by changes in symptoms, tumor size, alkaline phosphatase, radionuclide imaging, and the histologic response of the tumor at the time of definitive surgery. In some cases chemotherapy during the neoadjuvant period was modified because of a clinical impression of a poor response. Most patients received cisplatin after a poor response to neoadjuvant chemotherapy documented by a poor histologic response. Four patients received cisplatin in the neoadjuvant period because of a poor clinical response prior to surgery.

Results

For all of the factors analyzed the correlation with DFS and survival were equivalent. All results are reported only in terms of DFS.

Surgery

The treatment strategy called for all patients to receive neoadjuvant chemotherapy prior to definitive surgery. Some patients referred to MSKCC had already undergone definitive surgery prior to referral. Others were determined to require primary definitive surgery because of large tumor size, a pathological fracture, or intractable pain. Fifty-five patients had primary surgery and did not receive neoadjuvant chemotherapy. Univariate analysis showed no difference between patients who underwent primary surgery followed by adjuvant chemotherapy and patients who received neoadjuvant chemotherapy followed by definitive surgery and subsequent adjuvant chemotherapy ($p = 0.36$). The treatment strategy called for patients to have limb-sparing surgery whenever possible. There was no difference between patients who underwent limb-sparing surgery and patients who had an amputation ($p = 0.45$).

Age

Patients in the age range of 13–21 years at diagnosis had a higher probability of DFS than patients ≤12 years. The latter group, in turn, fared better than patient ≥22-years old at diagnosis ($p = 0.41$, Figure 37-1).

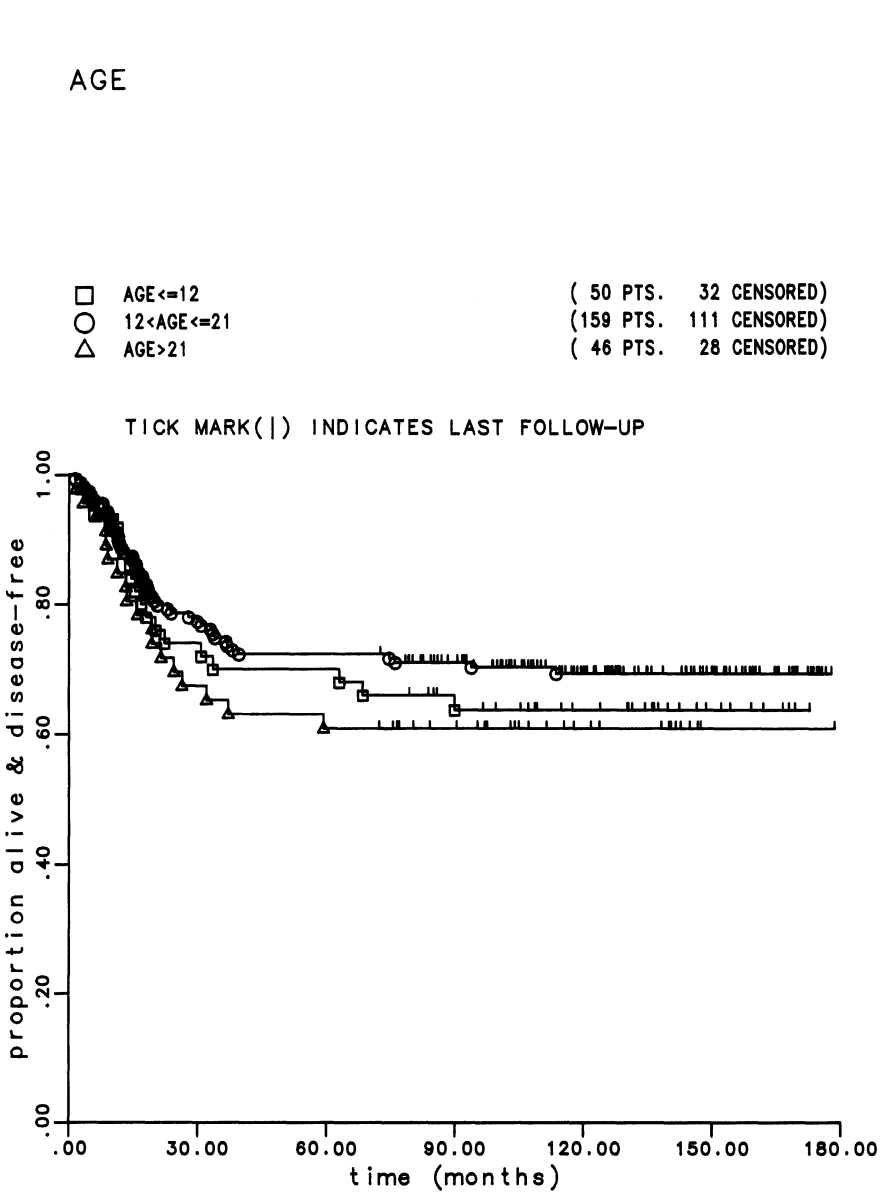

Figure 37-1. DFS as a function of age at diagnosis.

Sex

There was no correlation between patient sex and DFS.

Race

Patients were classified as white, black, Hispanic, and other. White patients had the highest probability of DFS; Hispanic patients were very similar. Black patients had an inferior probability of DFS ($p = 0.04$, Figure 37-2).

Primary site

The site of the primary tumor predicted DFS ($p = 0.04$, Figure 37-3). The proximal tibia and the proximal humerus were the most favorable sites, with a probability of DFS of 80% and 74%, respectively. Sixty-one percent of patients with a distal femur primary are continuously free of disease. All other extremity sites had a probability of DFS of 60%.

Serum alkaline phosphatase and LDH

The initial serum alkaline phosphatase and LDH both correlated strongly with DFS ($p = 0.05$ and $p = 0.05$, respectively; Figures 37-4 and 37-5). There was a steady increase in the risk of relapse both with increasing alkaline phosphatase and with LDH.

Histological response

Huvos and Rosen introduced the concept of histological evaluation of tumor response. Tumors removed from patients at the time of definitive surgery were examined for the degree of necrosis observed following neoadjuvant chemotherapy. Tumors were overlaid with a grid of 20–30 areas. Representative samples were submitted for histological analysis from each area. Tumors in which no viable tumor was observed in any of the sections were classified as a grade IV response. Tumors in which no more necrosis was observed than could be seen spontaneously in a large tumor were classified as a grade I response. Tumors with no more than a few foci of scattered viable tumor cells were classified as a grade III response. All others were classified as a grade II. Grade III and IV histological responses were considered complete responses to neoadjuvant chemotherapy.

There was a strong correlation between histological response grade and DFS ($p = 0.01$; Figure 37-6). DFS at 10 years ranged from 90% for patients with a grade IV histological response to 47% for patients with a grade I histological response. Fifty-five patients were not assessed for histologic response, either because they underwent primary surgery or because the analysis was not performed in all patients in the early years of this series. These

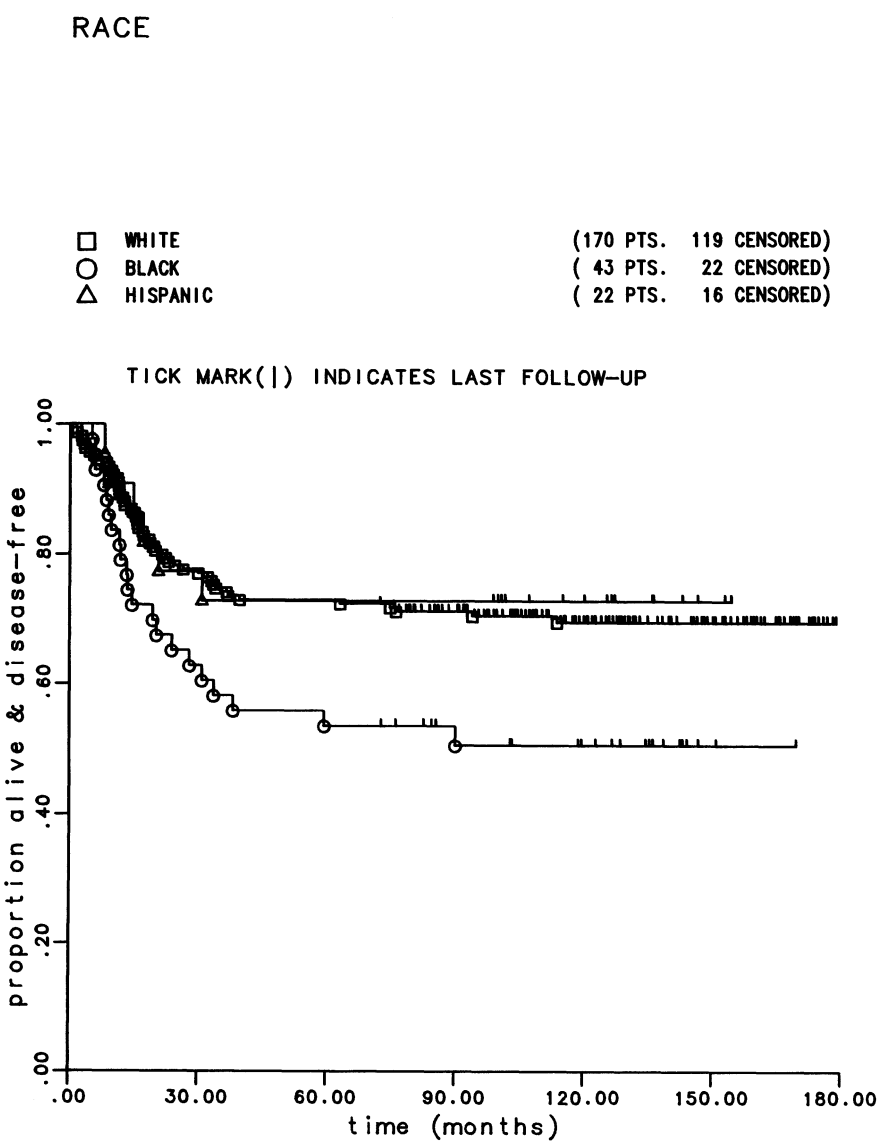

Figure 37-2. DFS as a function of race.

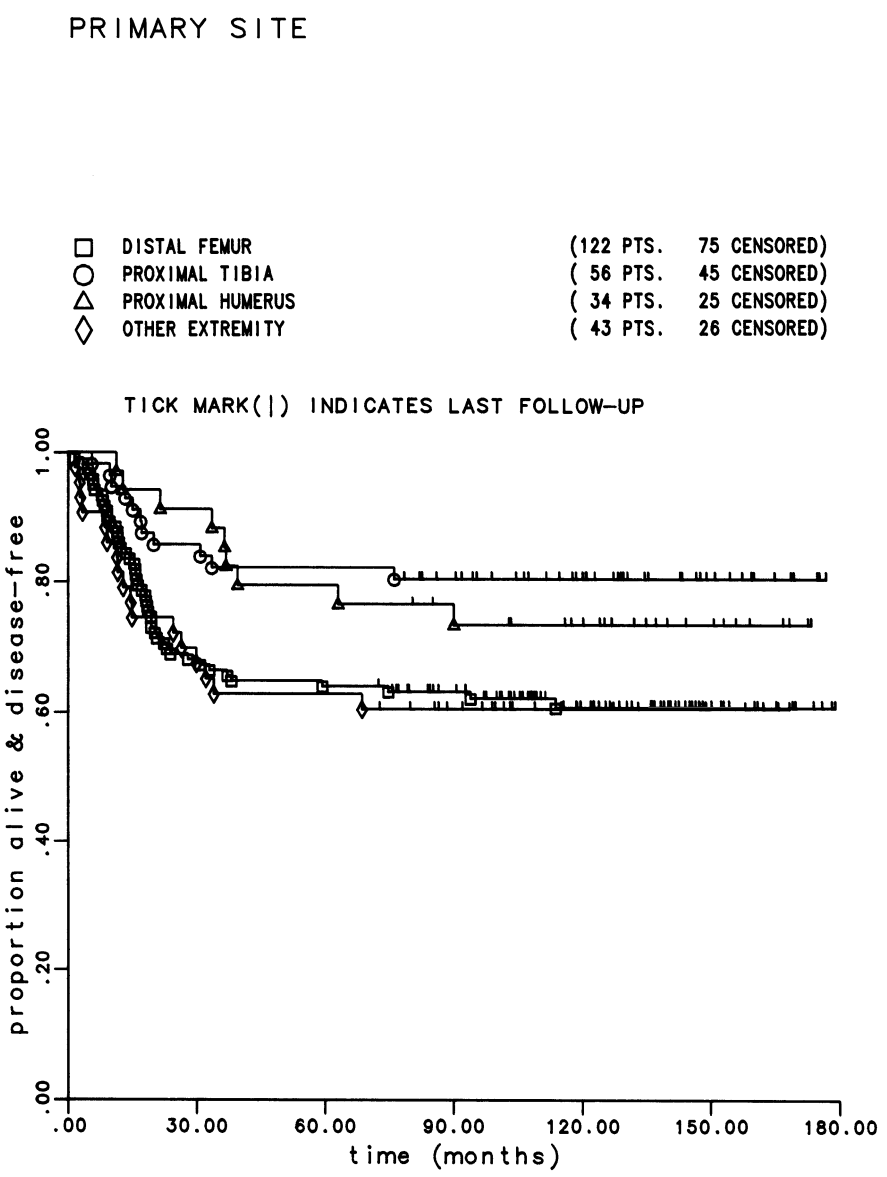

Figure 37-3. DFS as a function of primary site.

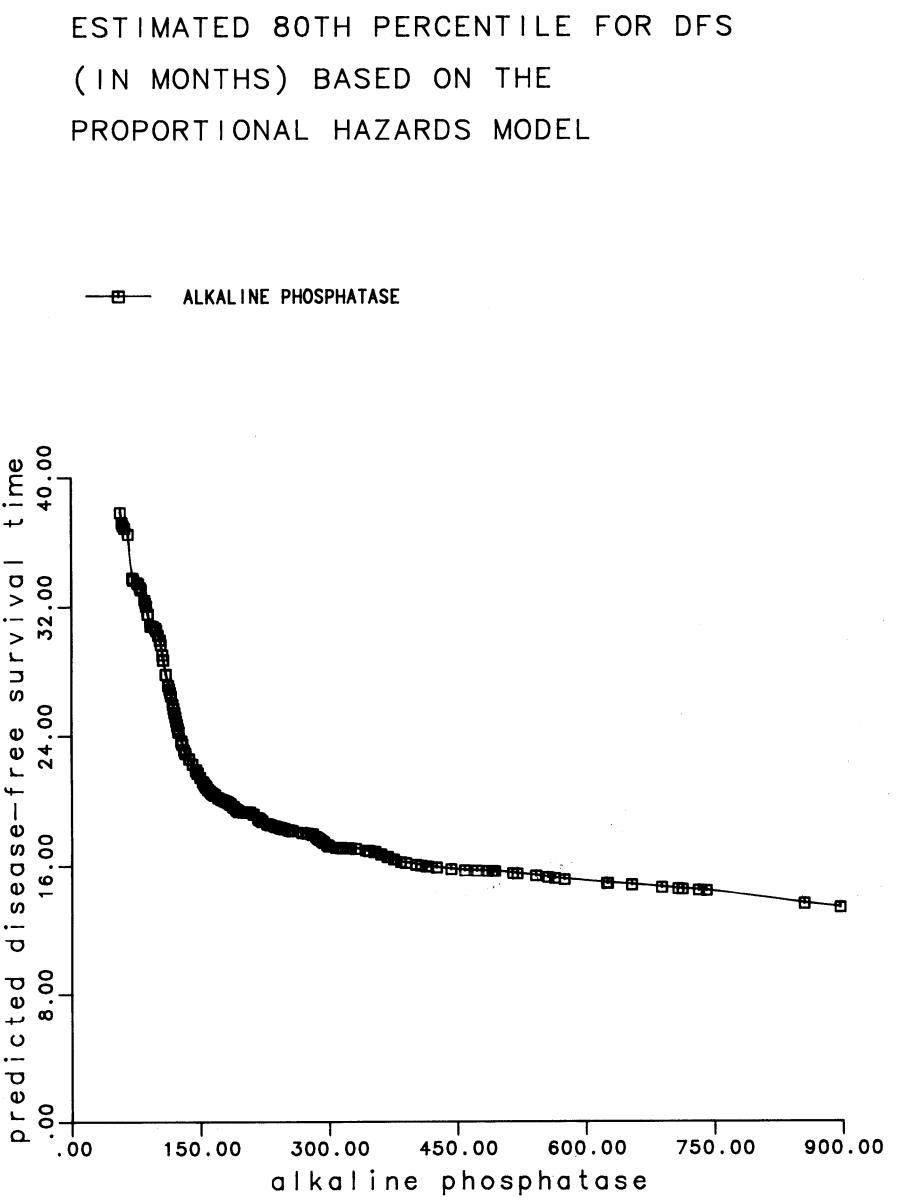

Figure 37-4. Predicted 80th percentile for DFS as a function of baseline alkaline phosphatase based on the proportional hazards model.

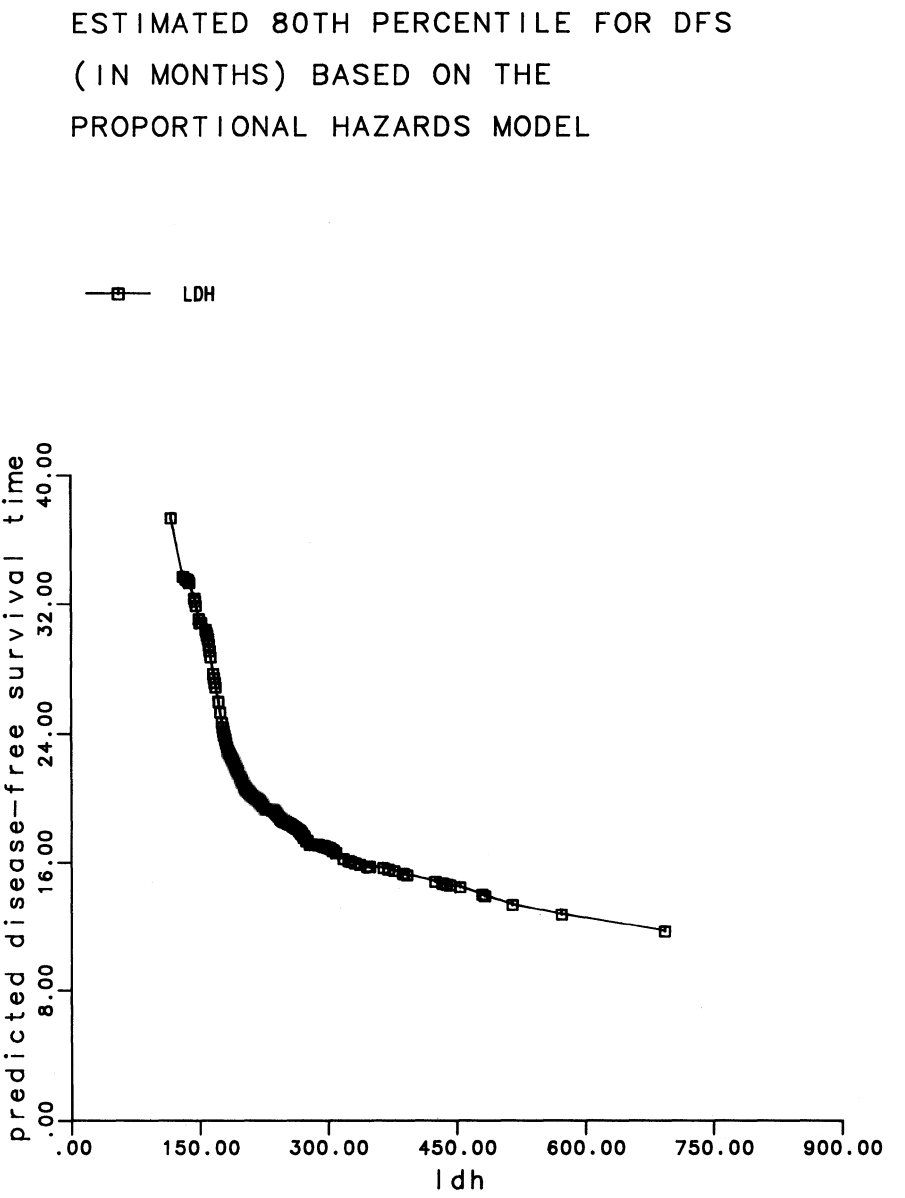

Figure 37-5. Predicted 80th percentile for DFS as a function of baseline LDH based on the proportional hazards model.

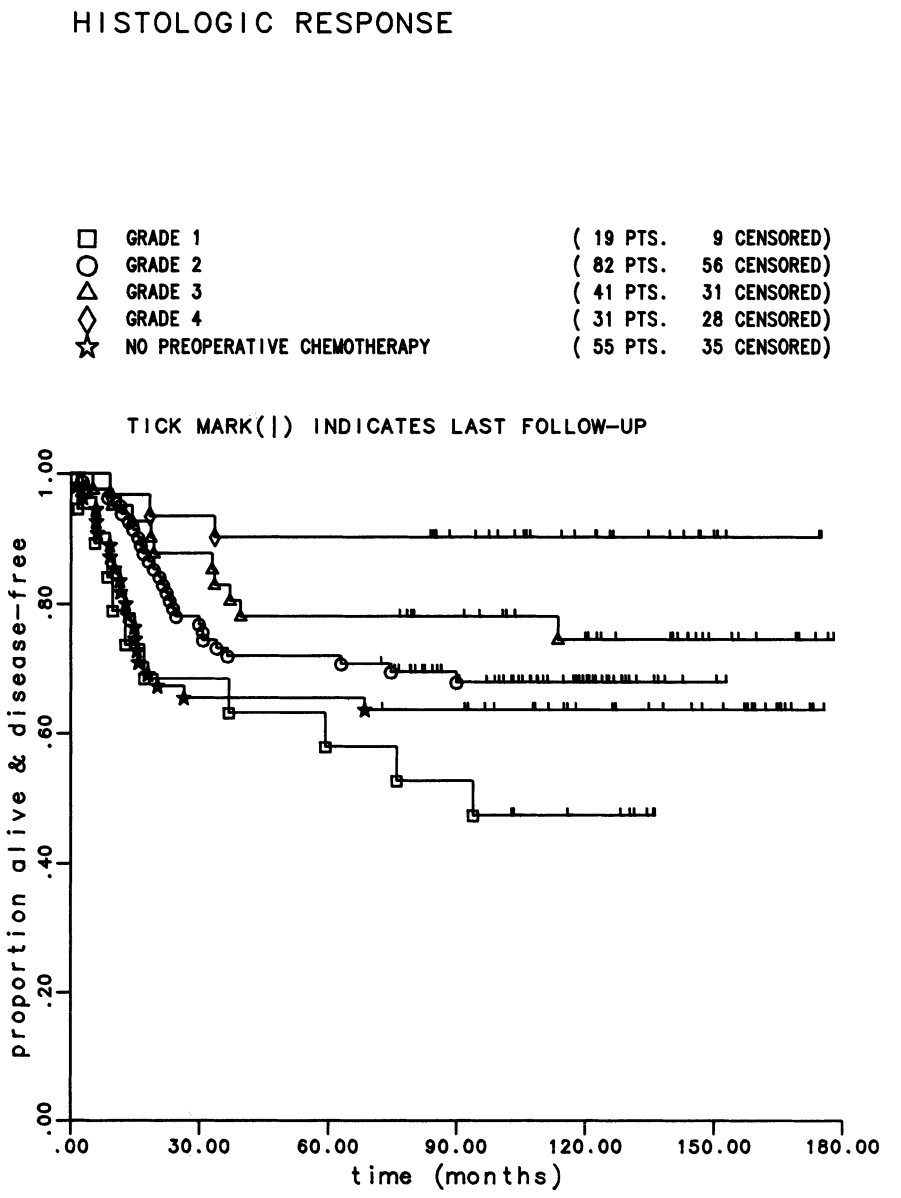

Figure 37-6. DFS as a function of histological response at the time of definitive surgery.

Table 37-1. Proportional hazards model for disease-free survival (n = 212)[a]

Covariate	Relative risk	P value
Histologic response		0.018
Grade I	6.59	
Grade II	3.48	
Grade III	2.70	
Grade IV	1.00	
No pre-op chemo	4.96	
ln LDH	exp[(ln LDH − 5.42)*0.54]	0.047
ln alk phos	exp[(ln AP − 5.28)*0.34]	0.096

[a] Significant covariates obtained from the multivariate analysis using Cox's proportional hazards model.
LDH = lactic dehydrogenase; Alk phos, AP = alkaline phosphatase; ln = natural logarithm.

patients as a group did not have a probability of DFS that was inferior to all the patients who were assessed for histological response, but they had a DFS that was inferior to patients with a grade III or IV response.

Chemotherapy

We were not able to demonstrate a correlation between dose or dose intensity and DFS for any of the chemotherapeutic agents used in this series. Not all patients with a poor (grade I or II) histological response to neoadjuvant chemotherapy received subsequent adjuvant cisplatin. The addition of cisplatin to the regimen for patients with a poor histological response did not improve their probability of DFS (p = 0.31).

While no patients were treated exclusively with HDMTX, 58 patients received HDMTX only in the neoadjuvant phase of treatment. Of these patients, 17% had a grade III or IV histological response, which is effectively a complete response to neoadjuvant chemotherapy. This compares with a complete response rate of 54% for patients who received multiagent neoadjuvant chemotherapy.

Multivariate analysis

Multivariate analysis of the variables identified in univariate analysis was performed, including, race, primary site, LDH and alkaline phosphatase, and histological response to neoadjuvant chemotherapy. Race appears to correlate with histological response and is no longer a significant factor in multivariate analysis. The probability of DFS correlates with lower LDH and alkaline phosphatase and a favorable histological response (Table 37-1). Primary site did not remain significant under multivariate analysis.

Discussion

Prognostic factors are influenced both by the intrinsic biology of a tumor and the treatment. Factors that predict outcome for one treatment regimen may not be valid for alternative forms of treatment. Using a series of protocols that depend heavily on HDMTX and doxorubicin, we have identified several factors that predict DFS for patients with OS. Baseline serum alkaline phosphatase and LDH have a strong and independent ability to predict outcome. Higher levels predict a greater likelihood of relapse. Tumor primary site correlates well with DFS. The proximal tibia and humerus are favorable sites; the distal femur and other extremity primary sites have an inferior prognosis.

Since a poor histological response to neoadjuvant chemotherapy identifies patients with a poor prognosis, it is appropriate to try to modify that prognosis by the addition of other active agents after definitive surgery. In an earlier publication with a relatively short follow-up, Dr. Rosen suggested that the addition of cisplatin following a grade I or II response to neoadjuvant therapy resulted in improved DFS [2]. With a larger number of patients and longer follow-up, we were not able to demonstrate any improvement in DFS with the introduction of cisplatin. This may be due in part to the fact that cisplatin began only after at least 20 weeks of neoadjuvant and adjuvant therapy with other agents. In the German experience, however, earlier introduction of cisplatin for the poor responders was not able to improve a very poor prognosis [5]. In that study patients with a poor response to neoadjuvant HDMTX and BCD received only cisplatin and doxorubicin following definitive surgery. A poor histological response to neoadjuvant chemotherapy does not imply that the agents have no value in the treatment of an individual patient's OS. In our series, patients with no apparent effect of chemotherapy (grade I response) had a DFS of 47%, even when no change in therapy was made. The lack of apparent activity in the primary tumor does not preclude activity against micrometastases.

The histological response to neoadjuvant chemotherapy correlates strongly with DFS. The greater the degree of necrosis at the time of definitive surgery, the greater the probability of DFS. This result, first reported from MSKCC, has been confirmed in other settings, notably the CCSG and the German cooperative OS trials [5,6].

An interesting strategy to improve DFS for patients with OS would be to utilize more agents in the neoadjuvant period to increase the degree of necrosis in the primary tumor prior to definitive surgery. This is the basis of the current randomized prospective trial for OS at MSKCC. All patients receive neoadjuvant chemotherapy; they are randomly assigned to receive either HDMTX and BCD prior to definitive surgery or a more intensive neoadjuvant regimen with HDMTX, BCD, doxorubicin, and cisplatin. The more intensive regimen results in a significantly higher rate of good histological response; there is not yet a significant difference in DFS between the two regimens [7].

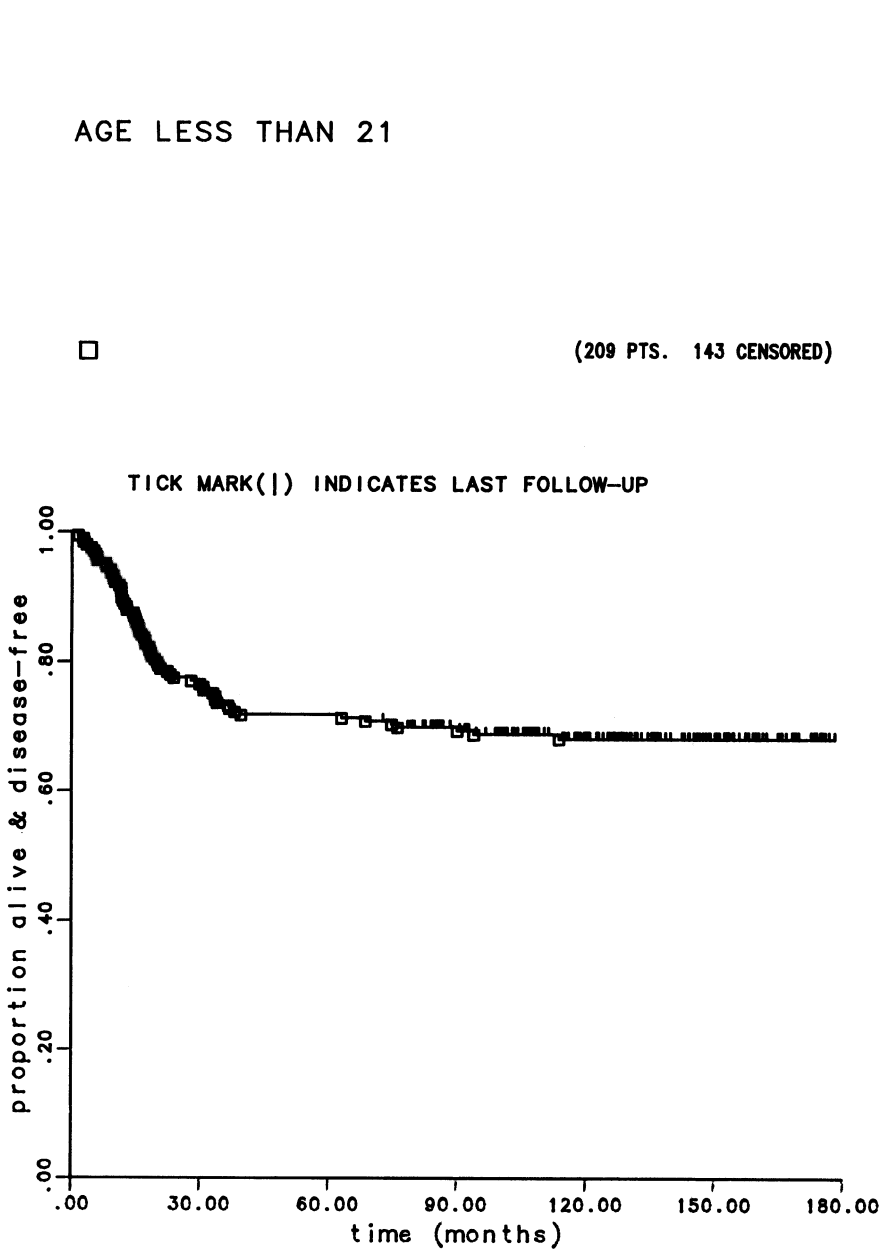

Figure 37-7. DFS for patients who were ≤21 years old at diagnosis.

HDMTX is widely used for the treatment of OS. Questions have been raised regarding the relative merits of high-dose and moderate-dose methotrexate. Our experience with HDMTX as a single agent in the neoadjuvant phase provides evidence of a significant ability to produce a substantial rate of complete responses. In addition, HDMTX can be administered with minimal toxicity, allowing repeated courses in a short interval or the addition of other agents with minimal delay. The CCSG has reported a trial comparing HDMTX and moderate-dose methotrexate [8]. There was no difference in DFS, but both treatment arms had a DFS distinctly inferior to the subsequent CCSG study, which in turn had a DFS inferior to the MSKCC series. HDMTX differs from other forms of chemotherapy in being subject to a far greater number of variables of administration. These include dose; rate and volume of administration; alkalinization; and dose, timing, and route of administration of leucovorin. For example, the CCSG 782 study called for leucovorin 15 mg every 6 hours; at MSKCC we have always used 10 mg. This may account for some of the differences between our series.

The three-drug combination chemotherapy BCD was introduced for the treatment of OS at MSKCC after demonstrating limited activity in relapsed patients with OS [9]. More recently a Phase II trial of BCD in patients with relapsed metastatic OS at St. Jude's failed to demonstrate any activity in eight patients [10]. There is widespread skepticism about the role of BCD in the treatment of OS. However, our results in the treatment of OS are among the best reported. For newly diagnosed patients with OS of the extremity without metastatic disease diagnosed at age 21 or younger, we have obtained a DFS of 68% with long follow-up (Figure 37-7). There does not appear to be a deleterious effect on outcome from the use of BCD.

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38. Osteosarcoma studies at St. Jude Children's Research Hospital from 1968 through 1990

C.B. Pratt, W.H. Meyer, B.N. Rao, D.M. Parham, and I.D. Fleming

Introduction

St. Jude Children's Research Hospital opened its doors in 1962. By 1968, stage-related treatment protocols for osteosarcoma were established. This paper will be limited to our investigations of the relatively new agent ifosfamide, adjuvant chemotherapy protocols, and the treatment of metastatic disease revealed at diagnosis.

Ifosfamide

Clinical studies with ifosfamide, an isomer of cyclophosphamide, began in 1983 [1,2]. Five of 22 patients with osteosarcoma in a phase II trial (1983–86) had complete or partial responses. Ancillary studies identified the relationship between the neurotoxicity, hematotoxicity, and nephrotoxicity associated with ifosfamide and prior therapy with cisplatin [3–5]. Increased urinary concentrations of the renal tubular enzyme, N-acetyl-β-D-glucoseaminidase, suggested renal tubular damage [6–8]. Neurotoxicity involved the central and peripheral nervous systems, and the amount of a dechloroethylated metabolite of ifosfamide, chloroacetaldehyde, correlated with clinical measures of neurotoxicity [9].

In 1987, we instituted a Phase I study administration of daily doses of ifosfamide with mesna (2-mercaptoethane sulfonate sodium) for 3 days every 3 weeks and noticed the difference in neurotoxicity between patients who did or did not have prior cisplatin treatment [10]. For patients with brain tumors, ifosfamide/mesna 3 g/m^2 every other day × 3 was well tolerated, irrespective of prior cisplatin treatment. For patients with other solid tumors 2133 mg/m^2 daily × 3 was well tolerated by those who had received prior cisplatin, yet 2560 mg/m^2 daily × 3 was associated with both neurotoxicity and myelosuppression. For patients who had no previous cisplatin, a dosage of 2560 mg/m^2 daily × 3 was well tolerated without neurotoxicity, and there was acceptable myelosuppression.

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 ISBN 0–7923–1905–2

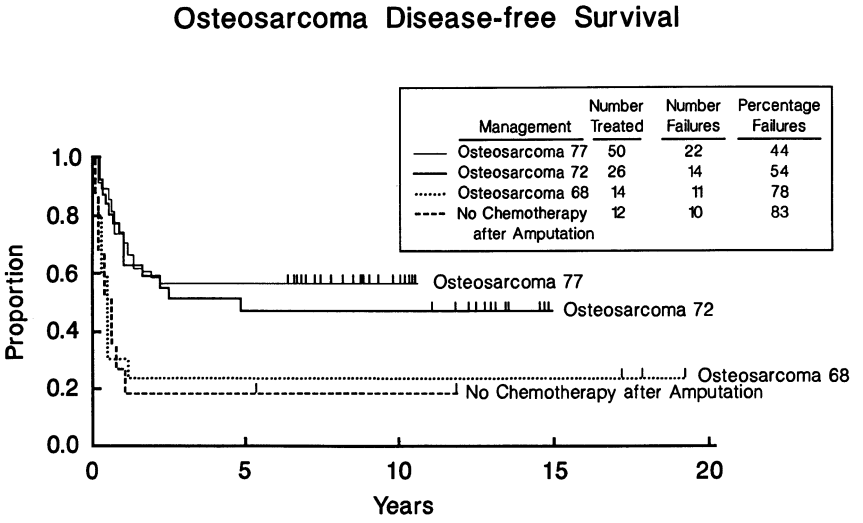

Figure 38-1. Disease-free survival for patients treated with three adjuvant chemotherapy protocols (Osteosarcoma 68, Osteosarcoma 72, and Osteosarcoma 77) at St. Jude Children's Research Hospital. The results are compared with those of patients who received no adjuvant chemotherapy following amputation.

Assessment of chemotherapy protocols

Surgery and chemotherapy are the principal treatments for primary and metastatic osteosarcoma. Classic, high-grade osteosarcoma of the extremity was treated by two protocols initiated in 1972 and 1977, respectively [11,12]. Both protocols used high-dose methotrexate, leucovorin, cyclophosphamide, and doxorubicin. All 76 patients, after appropriate amputations, had chemotherapy. In the 1977 protocol, drug dosage was increased. The rates of long-term, disease-free survival were 46% in the 1972 protocol and 56% in the 1977 protocol (Figure 38-1). An improved disease-free survival outcome was obtained for the group of patients who received the more intensive chemotherapy.

The results of the 1972 and 1977 protocols were compared with the results for patients who received adjuvant vincristine and cyclophosphamide between 1968 and 1972 (OS68 trial). The combination of vincristine and cyclophosphamide was ineffective and was no better than amputation alone (Figure 38-1). Results of the protocols begun in 1972 and 1977 have not changed since they were recently reported, except that the survivors have added another year to their lives [12,13]. The analysis of the patients who failed these adjuvant chemotherapy regimens has been reported elsewhere [14].

Between 1981 and 1986, more than 40 patients from our center were added to the Multi-Institutional Study for the Treatment of Osteosarcoma [15]. Because ifosfamide has been shown to be effective in treating osteosarcoma,

it was added to a multimodal scheme as the initial therapy for previously untreated patients [16,17]. Each course consisted of 1.6 g/m^2 of ifosfamide administered daily for 5 days at 3-week intervals. The preliminary results of this trial, presented in 1990, indicated that approximately 67% of previously untreated patients with osteosarcoma had demonstrable responses after receiving two or more courses of ifosfamide before receiving high-dose methotrexate with leucovorin rescue, doxorubicin, cisplatin, and definitive surgery.

Surgery for osteosarcoma

The treatment of metastatic osteosarcoma may require thoracic surgery. Aggressive resection of pulmonary nodules with adjuvant chemotherapy is usually recommended for the management of patients with pulmonary relapse. Since 1968, 67% of our 40 patients who developed pulmonary metastases after adjuvant chemotherapy have had thoracotomies [18–20]. In 1987, an analysis of this practice indicated that 9 of these 40 patients who had thoracotomies were alive with no evidence of disease [20]. Factors that correlated by univariate analysis were sex, number of nodules detected radiographically and resected, completeness of resection, and bilateral or unilateral disease. By Cox regression analysis, only sex and the number of nodules detected radiographically or during surgery, and resected, significantly correlated with survival. Males had a 44% survival compared to females, who had an 8% survival. Patients with fewer than three nodules detected radiographically had a 42% survival compared to patients with greater than three nodules, for whom there were no survivors. Likewise, for patients with fewer than six nodules that were resected, survival was 42% compared to lack of survival for patients with six or more nodules. Patients with bilateral disease determined radiographically had a shorter median survival time.

Conclusions

Our current institutional protocol for adjuvant chemotherapy is being used for patients who have unresectable or metastatic disease at diagnosis [16,17]. The final evaluation of whether the combination of ifosfamide with high-dose methotrexate, doxorubicin, and cisplatin will significantly increase the overall survival of patients with osteosarcoma requires time for accrual of patients and analysis of the results.

Acknowledgments

The authors are grateful to Ann Morris for editorial review and to Ann Shinall for typing the manuscript.

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39. A monocentric therapy study: An approach to optimize the results of the treatment of osteosarcoma by protocols based upon HDMTX, associated with systematic conservative surgery

N. Delepine, G. Delepine, and J.C. Desbois

Introduction

In order to understand why Rosen's protocol [1] has not been reproduced by most groups [2–5] that used the same scheme, we started preliminary study in 1984 using T10 to analyze methotrexate (MTX) pharmacokinetics and its correlations with clinical and histological results. From 1985 to 1989 a second pilot study was conducted to optimize the results of Rosen while conducting a pharmacokinetic analysis to understand the reasons for failures in attempts to reproduce T10 and to emphasize the fundamental points for curing osteosarcomas.

Study 1 (T10C protocol)

Objectives

- To prove the reproductibility of the T10 protocol in patients treated at our institution
- To define a biologic index for measuring therapeutic intensity that is simpler than the area under the curve
- To correlate this therapeutic aggressiveness index with the histologic response in order to determinate the optimal level of the biologic index needed to obtain a good response and low toxicity
- To use systematic conservative surgery and to improve reconstructive procedures
- To decrease the role of local radiotherapy in the treatment of osteosarcoma

Methods

Treatment protocol. Patients were treated using the T10 protocol [6]. The first part of treatment started soon after biopsy (the same day in 90% of our

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 ISBN 0-7923-1905-2

patients when immediate histologic examination permitted the diagnosis). *The length of preoperative chemotherapy was always less than to 1 month*, according to our preliminary demonstration of the major importance of a short duration of preoperative chemotherapy [7]. Patients >15 year, received 8 g/m^2 MTX, and those aged <15 years received 12 g/m^2 MTX. Conservative surgery was performed by the same surgeon after three to four courses of HDMTX and was followed by perioperative day 1 (D1) and day 2 (D2) BCD (bleomycine 15 mg /m^2 J1 J2 i.v. bolus, cyclophosphamide 600 mg/m^2 J1 J2 2 hours infusion, dactinomycin 600 μg/m^2 J1 J2 i.v. bolus). Following histologic grading, poor responders received T10 A (two cycles of 2 ADR-CDDP + BCD) and good responders T10 B (three cycles of 2 HD MTX + BCD + 2 HD MTX + ADR [6]. HDMTX and folinic acid rescue was performed following the exact recommendations of Rosen, in particular avoiding intravenous overhyperhydratation. No local radiotherapy was performed for good responders. Poor responders received a prophylactic irradiation of 35–49 Gy to eradicate permeation nodules around the tumor bed.

Follow-up protocol. Patients were followed by repeated clinical examination; standard X-ray; thoracic, cerebral, and local MRI and/or CT scan. Bone scan was performed at the beginning of treatment, after neoadjuvant chemotherapy (1 month), and every 3 months for 2 years and every 6 months over the next 2 years.

Biologic studies. MTX infused with a pump over 6 hours. The serum level of MTX was measured at the end of the infusion (6 hours) and at least three times during the first day after the beginning of MTX infusion (at 8, 12, and 18 hours) and then at 24, 48, and 72 hours to manage folinate rescue, as previously described.

Statistical analysis was performed by one of us on computer. The MTX serum level distribution was studied by the Kolmogorov-Smirnov test before calculating the mean value and variance. The research for a relationship between quantitative characters was performed by multivariant analysis, the partial correlation coefficient, and when positive, by the linear regression curve.

Materials

Ten patients aged 5–35 years (average, 17) with non-metastatic, untreated, primary high-grade limb osteosarcoma were included in pilot study 1 from October 1984 to December 1985. During the same period, 41 other patients with trunk location, metastatic or radioinduced osteosarcoma, or high-grade chondrosarcoma, aged from 8–63 years (average, 32), received high-dose MTX following the same preoperative scheme and completed the tests for MTX pharmacokinetic analysis.

Results

Oncologic results. Analysis of the 10 patients enrolled in study 1 showed that 6 out of 10 patients were poor responders (following Huvos classification) and 5 out of these 6 poor responders presented a CMax <1000 μmol/1, in contrast to 4 out of 4 good responders who had a value that exceeded this level, indicating the value of this CMax limit for common osteosarcoma.

In this group (without adjustment for MTX dosage), we observed three relapses (one bone relapse, two pulmonary relapse at 12 and 18 months), and two of these patients died. One patient with pulmonary metastatis underwent an operation and is in long-term (42 months) second remission. The overall survival is 80% at 66 months. The actuarial disease-free survival is 80% at 66 months. The event-free survival is 70% at 66 months. This study reproduces globally Rosen's T10 results, with initially conservative surgery in all patients (but two late amputations were performed in this group for infection).

Tolerance and toxicity. In pilot study 1 the tolerance was good and toxicity was minimal in the 10 patients. There were no lethal deaths and no life-threatening side effects.

Pharmacokinetic results. After infusion the decreasing curve of serum MTX concentration was exponential over the first 12–15 hours. This phase responds to a single compartmental model. Therefore the maximal concentration obtained at the end of the infusion represents a good index of the half-life and of the area under the curve (AUC) in each patient and probably a good correlation with treatment intensity. We chose the serum level at 6 hours of infusion as an index of individual therapeutic intensity (or aggressiveness). The statistical computations showed that the serum level at 6 hours depends on the dose per m^2 ($p < 0.001$). For a fixed dosage, it depends on age ($p < 0.01$). For a fixed dosage and age, there is large interindividual variability (the standard deviation is 45% of the mean value), which does not depend on sex. There is no correlation between the MTX levels at 6 and 24 hours; the 24-hour level depends mainly on the second pharmacokinetic compartment, whose influence becomes evident during the 16th to 24th hour after beginning the infusion. These results have been previously published [8].

Study 2 (DD1 protocol)

Objectives

- To rescue all patients (even poor responders)
- To confirm the correlations between the therapeutic aggressiveness index and therapeutic efficiency

- To optimize the dose-effect response in the preoperative phase of treatment by using individual pharmacokinetics to adapt HDMTX dosages in order to obtain the highest response rate
- To verify if the dose-effect time-intensity concept is of value in osteosarcoma

Methods

Treatment protocol. Patients were treated following the DD1 protocol, which is a modified T10. The first phase of treatment began immediately after biopsy and immediate histologic diagnosis. The first course of HDMTX was given at dosages adapted to the age of the patient (12 g/m^2 under 18 years, 8 g/m^2 over 18 years). The dosages of MTX in other courses were adapted to individual pharmacokinetics in order to obtain 1000 μmol at the end of the sixth hour. The dosage was increased from 2 to 4 g/m^2 as a function of the result of CMax at the first course.

En-bloc extratumoral resection was performed in all cases by the same surgeon immediately after the fourth course of HDMTX (between the fourth and seventh day after this course). Perioperative chemotherapy (BCD) was given the day following surgery (D1 or D2). Postoperative chemotherapy was adapted to the response and graded according to our previously published score [9].

Good responders received postoperative chemotherapy according to T10 B, with dosages of HDMTX determined by the fourth preoperative course; poor responders received six cycles of two HDMTX; BCD and ifosfamide (IPA). Local radiotherapy (35 Gy) was only applied to poor responders, along with marginal resection.

Results

Tolerance and toxicity. Tolerance was good and toxicity acceptable, with no lethal death. Globally tolerance of HDMTX was excellent and toxicity was limited to headaches during the 12th course in a 9-year-old girl and moderate elevation of transaminases during the last courses of HDMTX. Hematologic toxicity of IPA was always important with fever and in some cases was life-threatening due to septicemic episodes. Cure was obtained in all cases with antibiotics and antifungal treatments.

Oncologic results. In pilot study 2 with individual adaptations of HDMTX in the preoperative phase of treatment, we observed 13 out of 21 good histologic responders and two relapses (one pulmonary at 12 months and one bone metastasis at 24 months). At a median follow-up of 45 months, the actuarial overall survival and the actuarial disease-free survival are 100% at 60 months. The actuarial event-free survival is 88% at 60 months.

Pharmacokinetic correlations. Analysis of the pharmacokinetic data from these patients and from patients coming from other centres after failure of the "T10" protocol indicates the following:

- The common osteosarcoma response to preoperative chemotherapy is significantly correlated with the dose intensity (DI = $g/m^2/wk$) and the serum level of MTX (SI = mean value of H6/wk).
- Obtaining on effective serum level of MTX requires individual adaptation of the MTX dosage.
- Poor responders can be cured like good responders if the length of preoperative chemotherapy is not too long (in our protocol DD1, all poor responders are disease free).
- Failure to reproduce Rosen's results is due to preoperative chemotherapy that is too long, drug and serum levels that are too low, and stopping MTX too early in poor responders. Our new protocol, DD11 (activated in 1990), provides the same postoperative chemotherapy to all patients.

Conclusions

With the individualized multidisciplinary approach described here, primary limb-localized osteosarcoma becomes a curable disease not requiring amputation [10]. Randomized studies using other protocols whose results not as good [11,12] are now useless, dangerous, and may be unethical.

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40. The Mayo Clinic studies

James S. Miser, Douglas J. Pritchard, Michael G. Rock, Thomas C. Shives, Gerald S. Gilchrist, William A. Smithson, Carola A.S. Arndt, John H. Edmonson, and Daniel J. Schaid

Introduction

The Mayo Clinic has undertaken studies of chemotherapy for osteosarcoma since 1976. The first study, which opened in 1979 and closed in 1980, evaluated the role of high-dose methotrexate in combination with vincristine. From 1983 through 1986 patients were entered into a Childrens Cancer Group trial evaluating multiagent chemotherapy in the treatment of osteosar-coma. In 1988, a pilot protocol (OGS PILOT-1) was opened in collaboration with the University of Minnesota, the University of Wisconsin, the University of Chicago, the University of Michigan, and the Children's Hospital of Los Angeles. This pilot protocol evaluated the histopathologic response to the combination of ifosfamide, Adriamycin, and high-dose methotrexate. This protocol was closed to patient entry in January 1991, and a second pilot protocol (OGS PILOT-2) was opened with the same collaborators. This second pilot protocol is evaluating the feasibility of a four-drug induction of cisplatin, ifosfamide, Adriamycin, and high-dose methotrexate and the histopathologic response to this regimen. The main theme these pilot protocols are exploring is the efficacy and feasibility of adding ifosfamide to the regimens used to treat osteosarcoma.

STUDY I

Objectives

The main objective of this study was to compare the outcome of patients treated with observation alone and high-dose methotrexate with vincristine and leucovorin following complete excision of a primary nonmetastatic osteosarcoma.

Methods

Treatment protocol. Patients were randomized to either receive no adjuvant chemotherapy or chemotherapy every 3 weeks for 1 year with vincristine 2.0

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 ISBN 0-7923-1905-2

mg/m^2 (maximum dose 2.0 mg) and methotrexate. Methotrexate was given at the dose of 3 g/m^2 on the first course, 6 g/m^2 on the second course, and 7.5 g/m^2 on the third course and thereafter until completion of therapy.

Results

A total of 41 patients were entered onto the protocol, with 20 patients receiving adjuvant chemotherapy. Although the survival and disease-free survival of the two groups were not different, the results of this small pilot study were consistent with a substantial favorable (or unfavorable) effect of chemotherapy. Importantly, the actuarial progression-free survival of the treated group remains 44% and the actuarial survival 45% at 10 years from diagnosis.

Conclusions

Although there was no clear benefit of administering this rather modest chemotherapy regimen to a small group of patients, the overall results of the trial were consistent with a favorable (or unfavorable) effect of chemotherapy. The long-term progression-free survival and survival serve as an important historical baseline to which subsequent studies can be compared.

Study II (OGS Pilot 1)

Objectives

- To assess the histopathologic response of grade III or IV osteosarcoma to a new chemotherapy regimen of ifosfamide, Adriamycin, and high-dose methotrexate
- To assess the feasibility and toxicity of this regimen
- To improve the limb function of patients following resection of osteosarcoma
- To increase the number of patients eligible for limb-salvage procedures
- To improve progression-free survival and survival of the entire group

Methods

Treatment protocol. All patients with high-grade (grade III and IV) osteosarcoma, both with and without metastatic disease, were treated on the same treatment regimen. Three 5-week cycles of chemotherapy were given preoperatively: ifosfamide 1800 mg/m^2/day × 5 days, Adriamycin 25 mg/m^2/day × 3 days, and mesna 2880 mg/m^2/day × 5 days given on week 0 and high-dose methotrexate 12 g/m^2 (maximum 20 g) with leucovorin rescue on weeks 3 and 4. Surgery was performed at week 15.

The postoperative therapy was determined after assessing the histopathologic response to preoperative chemotherapy. If there was 95% or more

necrosis, then five additional 5-week cycles were administered, as in the induction phase, with Adriamycin being omitted in the last two cycles. If there was less than 95% necrosis, then a cisplatin-containing regimen was given.

Results

Sixty-seven patients were formally entered into the trial. Four additional patients were treated with the regimen but not formally entered: two because a painful pathologic fracture at presentation necessitated amputation and two because therapy was begun initially outside a participating institution for medical reasons and not registered prospectively. In every other way they were followed and evaluated like the other patients.

The patient characteristics were typical of an average population of patients with osteosarcoma. Thirty-one females and 40 males with a median age of 14 years were treated. Nine had metastases at diagnosis. Sixty-two percent had osteoblastic morphology, 70% of the tumors arose in the femur or tibia, 47% had a tumor diameter >10 cm, 66% had symptoms for 1–5 months prior to the diagnosis, 81% had a soft tissue swelling, and 51% had a lytic appearance on plain radiograph. Eighty-one percent underwent a limb-sparing procedure; in 64% the joint function was maintained.

The degree of necrosis has now been assessed in 61 patients. Twenty-six (43%) patients have had complete (100%) necrosis of the tumor; 22 (36%) have had 95–99% necrosis; three (5%) have had 90–94% necrosis; seven (11%) have had 70–80% necrosis; and three (5%) have had 50% or less necrosis.

Forty percent of the ifosfamide-Adriamycin courses have been followed by fever and neutropenia; 10% of all courses have been followed by mucositis; three patients have experienced significant transient renal toxicity; and three patients have had transient central nervous system toxicity.

Six of the 61 patients without metastatic disease at diagnosis have relapsed or progressed, whereas 4 of the 9 patients with metastatic disease at diagnosis have relapsed. Two patients clinically progressed in the preoperative phase. The median follow-up of all patients is now 16 months (range 5–32 months). The actuarial progression-free survival for the nonmetastatic patients and survival for the entire cohort are shown in Figures 40-1 and 40-2 respectively. The projected progression-free survival for nonmetastatic patients is greater than 80% at 2 years.

Conclusions

- The 15-week preoperative chemotherapy regimen of ifosfamide, Adriamycin, and high-dose methotrexate is feasible and effective treatment for high-grade osteosarcoma. The initial clinical response is excellent and the percentage of patients having 90% or more necrosis is very high.

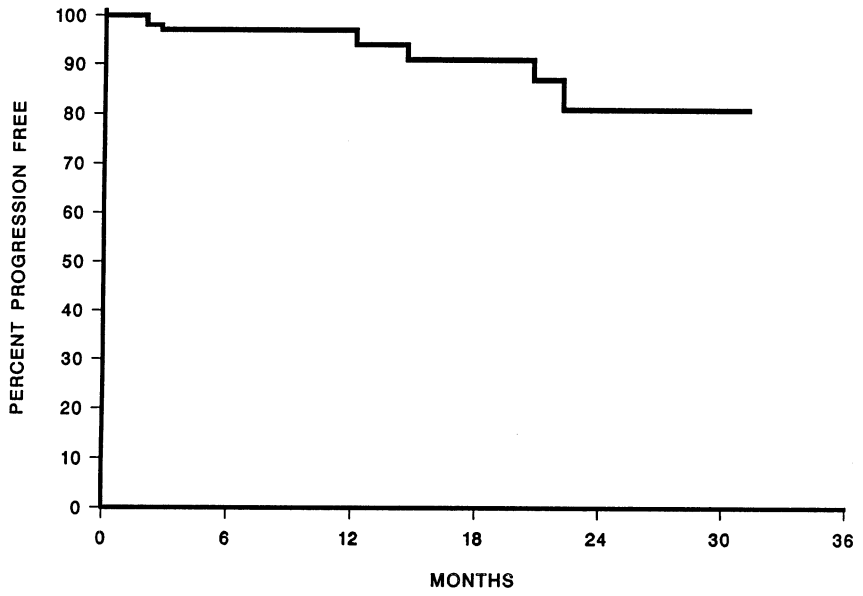

Figure 40-1. Kaplan-Meier plot of the progression-free survival for 71 patients treated on OGS Pilot 1.

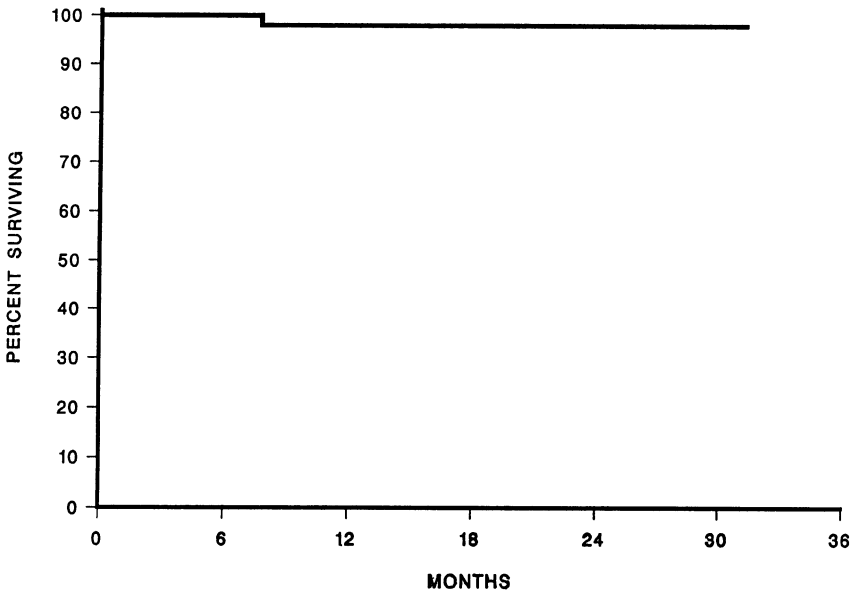

Figure 40-2. Kaplan-Meier plot of the survival for 71 patients treated on OGS Pilot 1.

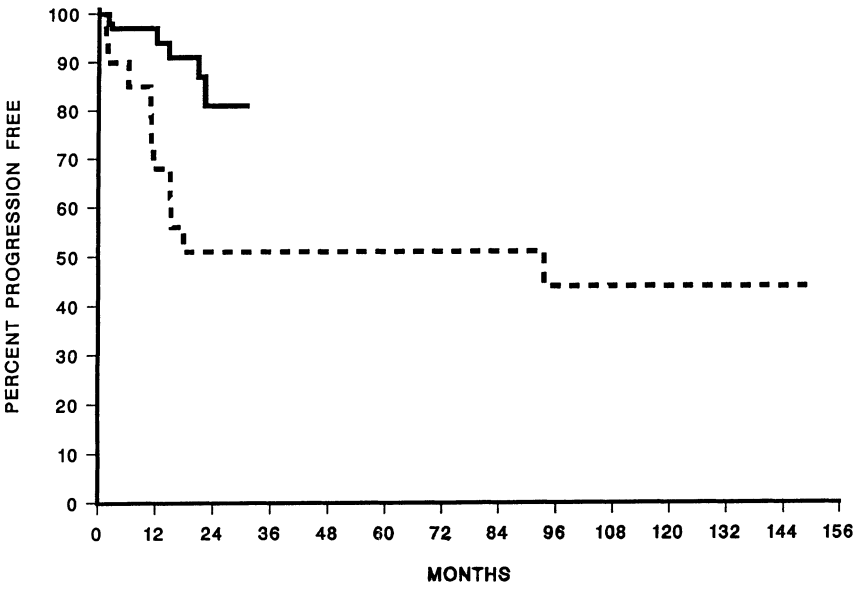

Figure 40-3. Kaplan-Meier plot of the progression-free survival of the 20 patients treated study I with methotrexate and vincristine (---)compared to the 71 patients treated in OGS Pilot 1 (---).

- The progression-free survival of this cohort appears better than that seen with the treatment used in study 1 (Figure 40-3).
- Patients with extensive metastatic disease in the lung or patients with bone metastases respond to this therapy but have relapsed quickly. New therapies will be required before these patients can be reliably cured. Patients who at diagnosis have limited metastatic disease only in the lung are behaving similarly to patients without metastases at diagnosis.

Study III (OGS Pilot 2)

Objectives

- To assess the histopathologic response of grade III or IV osteosarcoma to a new chemotherapy regimen of ifosfamide, Adriamycin, high-dose methotrexate, and cisplatin
- To assess the feasibility and toxicity of this regimen
- To continue to improve the limb function of patients following resection of osteosarcoma
- To continue to increase the number of patients eligible for limb-salvage procedures

- To continue to improve the progression-free survival of patients with osteosarcoma

Methods

Treatment protocol. The treatment protocol is identical to OGS Pilot 1, except cisplatin (120 mg/m^2 is substituted for ifosfamide in the third and sixth 5-week cycle. A third dose of cisplatin is given at week 42.

Results

Patient recruitment began in January 1991 and will continue for approximately 24 months. Thus far, 40 patients have been entered into the trial. The histopathologic response to preoperative chemotherapy is similar to that seen in OGS-Pilot 1. The median follow-up of these patients is still short (less than 1 year).

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41. Neoadjuvant chemotherapy for patients with osteosarcoma: University of Florida studies

John Graham-Pole, Mouhab Ayass, William Cassano, Nancy Dickson, William Enneking, Marj Heare, Travis Heare, Robert Marcus, Ramy Saleh, and Suzanne Spanier

Introduction

We have carried out one trial of adjuvant chemoradiotherapy (ACT) and two trials of neoadjuvant chemotherapy (NCT) followed by definitive surgery and ACT in consecutive patients seen at the University of Florida over a 10-year period [1–4]. The aims of the adjuvant trial were to assess a new chemoradiotherapy regimen, to correlate the outcome with clinicopathological staging, and to compare the results in patients having immediate amputation with those having limb-preserving surgery. The aims of the NCT trials were to assess the initial efficacy of new drug combinations, to see if this predicted freedom from relapse, to increase the proportion of patients whose limbs were preserved, and to assess if the extent of adjacent spread of the primary lesion (E1-E6) [2] retained significance in a neoadjuvant setting.

Methods

Surgery

For the surgical margin to be classified as radical, the entire bone and soft tissue compartments involved by tumor had to have been removed. For a wide surgical margin to be achieved, the whole tumor had to be resected with a cuff of normal tissue. The pathologist and surgeon determined the surgical margin by gross inspection, dissection, and histological examination.

Pathology

One pathologist reviewed the histopathology in all cases. The specimen was sectioned into 1-cm-thick slabs in the plane of greatest soft tissue extent, and the end-slabs were sectioned at a 90° angle to assess tumor extent in the secondary plane. A freehand drawing was made before cutting, and as each tissue block was taken its location was noted on the drawing. Tissues were then decalcified, embedded in paraffin, and stained with hematoxylin and

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 ISBN 0-7923-1905-2

Table 41-1. Definitions of anatomical extent for stage IIB tumors

Maximum extent	Definition
E1	Tumor touches but does not elevate or penetrate the periosteum
E2	Tumor elevates but does not penetrate the periosteum
E3	Tumor penetrates into but not through the periosteum
E4	Minimum extraperiosteal extension, not into a defined structure or space, seen as a nodule of tumor of ≤1 cm in fat just outside the periosteum, where muscle does not insert onto bone; the nodule often lies next to a small artery and may represent a small venous embolus that has destroyed the wall of the vein.
E5	Tumor invades any one of the following: tendon, ligament, periarticular structures (tumor is covered by synovial tissue), joint (tumor is intraarticular), muscle, bone, or space, such as the popliteal fossa or the axilla
E6	Tumor invades two or more structures

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eosin. The local extent of the tumor was classified as E1 through E6, according to the maximum extension on examination of 6- to 7-μm sections (Table 41-1) [2].

For the NCT trials we devised four categories to assess drug effect on the tumor: (1) necrosis—evidenced by "turtle shells," persistent architecture of necrotic tumor, or extensive hyalinization; (2) altered tumor—individual or clumps of typical tumor cells; (3) fibrovascular response—no persistent tumor, but granulation tissue with or without macrophages; (4) live tumor—persistent or regrowing tumor histologically indistinguishable from OS. Each section was scored for each feature and the mean was recorded. Patients having less than 10% live tumor were considered good responders.

Chemoradiotherapy

The ACT used from 1979 to 1984 consisted of whole-lung irradiation (1600 cGy) and five courses of doxorubicin (total dose 450 mg/m^2) over 5 months.

The chemotherapy regimens used as NCT are shown in Figure 41-1. Both groups received four courses over an 8- to 10-week period after diagnosis. After definitive surgery, group A patients were scheduled to receive five more courses of methotrexate, cisplatinum, and doxorubicin; and group B patients four more courses of etoposide, cyclophosphamide, cisplatinum, and doxorubicin.

Reevaluation

We assessed clinical responses and toxicity after both the first two and the latter two NCT courses by history (for subjective improvement and tolerance), physical examination (for lesion size and function), and changes in

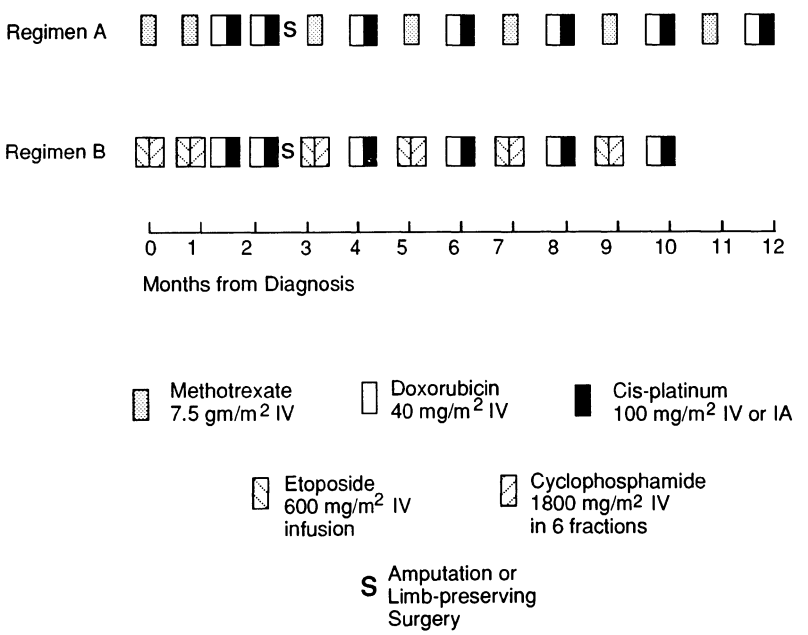

Figure 41-1. Neoadjuvant chemotherapy regimens.

radiographic lesions and serum alkaline phosphatase levels. Plain films and CT were studied pre- and post-chemotherapy for calcification and ossification of the rim of the extraosseous soft tissue mass. CT and MRI scans were examined for change in the size of the mass and development of necrotic foci. These were identified as low-density areas on CT or foci on MRI that were low density on T_1-weighted images and bright on T_2-weighted images. CT scans of the chest were evaluated for changes in metastases.

Results

We accrued 53 patients with nonmetastatic osteosarcoma of an extremity to the adjuvant chemoradiotherapy trial [1]. We excluded patients with tumors arising in areas of Paget's disease or previous irradiation, and those with metastases at diagnosis. The actuarial relapse-free survival of this group is currently just over 50% with a minimum of 6 years follow-up. There is no significant difference in outcome between the 16 who had limb-preserving surgery and the 37 who underwent amputation [1].

We correlated the extent of local disease at diagnosis with outcome in 51 of these patients, dividing them according to amount of invasion of adjacent structures, as shown in Table 41-1 [2]. By univariate analysis the variables having unfavorable prognostic significance were tumor size, extent of invasion,

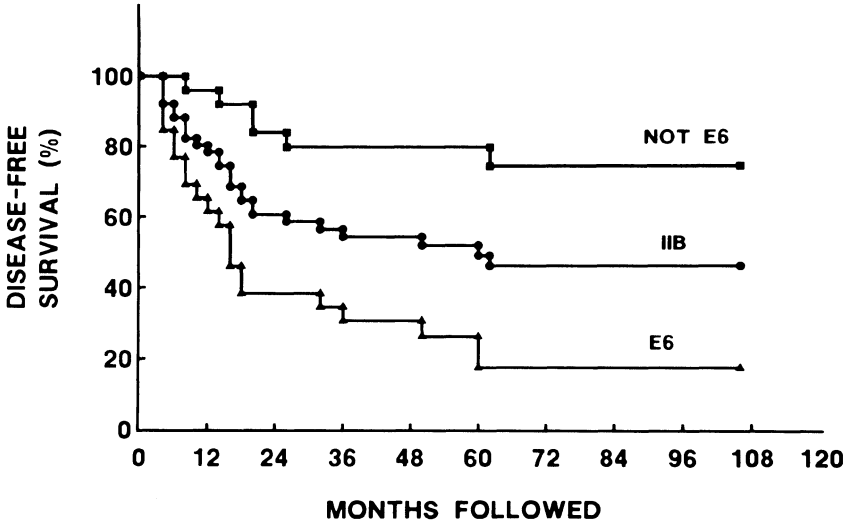

Figure 41-2. Replase-free survival probability of patients receiving adjuvant chemoradiotherapy according to primary tumor extent. Reproduced by permission of the authors and the *Journal of Bone and Joint Surgery*, 72A:643–652, 1990.

and age <10 years. By multivariate analysis only tumor extent retained independent significance for outcome. The estimated relapse-free survival probability at 5 years for patients having extension into two or more adjacent structures (E6) was 18%, compared with 80% for those with less extension (E1-E5) [2]. Figure 41-2 shows the relapse-free survival probability of these patients according to the anatomical extent of the primary tumor.

Since 1984 we have accrued 72 patients in our two NCT trials: 37 in group A and 35 in group B. There is no significant difference between groups A and B in either patient or tumor characteristics. The primary tumors of 77% group A patients showed local invasion of two or more structures (E6); we do not yet have clinicopathological data on group B patients.

We have reported preliminary results of the clinical response of the primary tumor to NCT in the two patient groups [3,4], and a detailed analysis will be reported separately. Briefly, more group B than group A patients had complete or partial responses to NCT (88% vs. 56%, $p < 0.02$) judged by clinical, radiological, and alkaline phosphatase reevaluations. Twenty group A (57%) and 24 (71%) group B patients with extremity lesions had limb-preserving surgery after completing NCT, the remainder requiring amputations. There is no significant difference in frequency of amputation between these two groups, but significantly more limbs were preserved than in our previous ACT trial ($p < 0.01$).

Follow-up is currently 41-76 months for group A and 10–46 months for group B patients. Seventeen (49%) group A and 12 (32%) group B patients

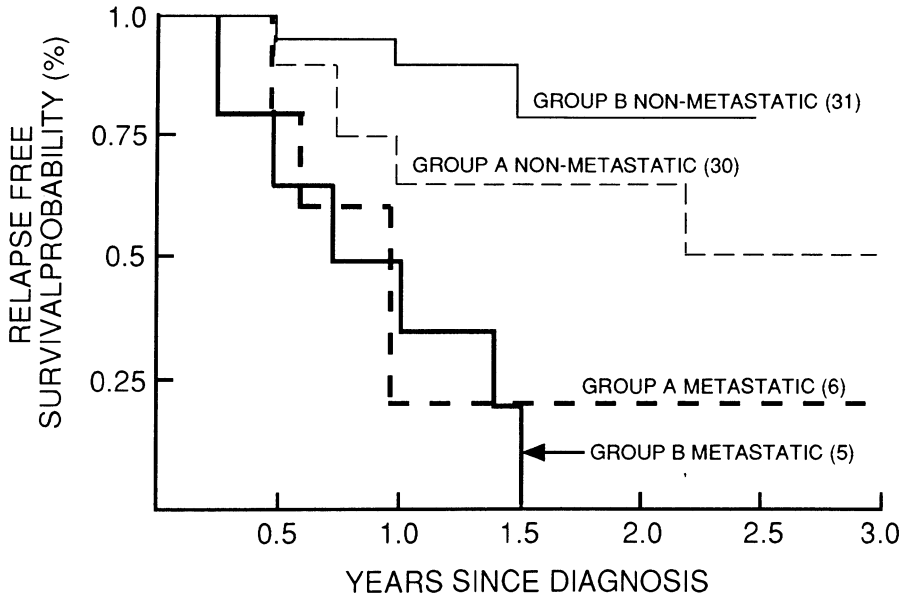

Figure 41-3. Relapse-free probability of group A and B (NCT) patients according to presence of metastases at diagnosis.

have so far relapsed 4–28 (median 13) months after diagnosis, and one group A patient died of sepsis.

Figure 41-3 shows a Kaplan-Meier plot of the relapse-free survival probability for groups A and B patients according to whether or not they had metastases at diagnosis. Ten of 11 patients with metastases have since developed progressive disease, and in both groups there is a significantly better relapse-free survival expectation for those without metastases at diagnosis. We have also plotted the relapse-free survival probability of group A patients according to whether they had limb-preserving surgery or amputation (Figure 41-4). The former have had fewer relapses, perhaps because of the extent of the tumor. Of the 31 patients so far analyzed, 11 of 24 with E6 lesions had amputations, compared with only 1 of 7 with E1-E5 lesions.

Age, sex, race, and primary site were not significant predictors for relapse-free survival in either group. For those without metastases at diagnosis, there is currently no significant difference in the probability of remaining relapse free between group A and B patients. In both groups a level of alkaline phosphatase >300 IU/l was associated with a higher relapse frequency, though the difference is significant only for group A patients.

We have carried out histological analysis of the tumors resected at definitive surgery in 31 group A patients. Fourteen received intravenous (i.v.) and 17 intraarterial (i.a.) cisplatinum. Seventeen (55%) had a good histological response (<10% residual tumor), including 6 of 14 (43%) receiving i.v. and

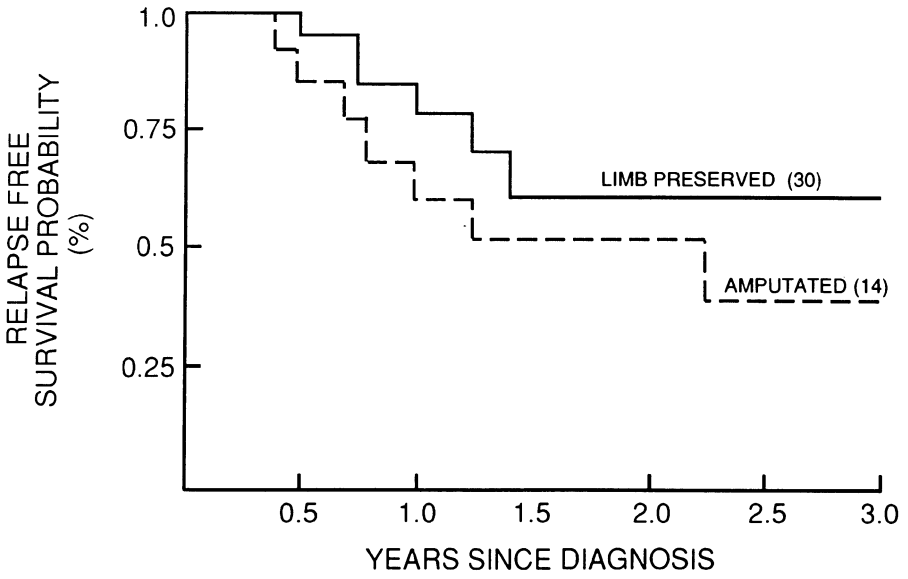

Figure 41-4. Relapse-free survival probability of group A patients according to whether they had amputation or limb-preserving surgery.

Table 41-2. Frequency of relapses according to extent of primary tumor

Tumor extent	No. patients	No. relapsing (%)
E 1–5	7	1 (14)
E 6	24	17 (71)[a]
Total	31	18 (58)

[a] Only 31 group A patients analyzed to date.

11 of 17 (65%) receiving i.a. cisplatinum (not significant). Twenty-four of the 31 (77%) had E6 primary lesions, compared with 26 of 51 (51%) in our previous protocol ($p < 0.02$). Patients with E6 lesions have a significantly higher relapse rate than those with less invasive tumors (Table 41-2). Though patients receiving i.a. cisplatinum tended to have more histological necrosis than those receiving i.v. cisplatinum, this has not been reflected in reduced relapse frequency.

Discussion

The results of these three clinical trials confirm the value of adjuvant and neoadjuvant therapy in treating patients with osteosarcoma. Because they

were single-arm sequential studies, the results are not statistically comparable between groups. Patients on the first (ACT only) trial were selected by having neither axial lesions nor metastases at diagnosis, whereas those receiving NCT were unselected. Perhaps because of this, this group contained significantly fewer patients with E6 lesions than those so far analyzed who received NCT. We have found that the significant association between E6 primaries and unfavorable outcome in the ACT trial was reproduced in our first NCT trial. The presence of metastases at diagnosis has carried an almost uniformly poor prognosis in both NCT groups, even though most of these patients had initial responses to NCT. Thirdly, the presence of a high serum alkaline phosphatase at diagnosis has proved an unfavorable factor, as reported by others [5].

These findings suggest that patients who have E6 lesions at diagnosis with or without metastases or high alkaline phosphatase levels should be stratified separately in future clinical trials. They need different and probably more intensive neoadjuvant and/or adjuvant treatment.

The NCT regimen we used for group B patients was associated with significantly more complete and partial clinical responses than our earlier regimen. This may be partly because we encountered more complications from combining methotrexate and cisplatinum. Nephrotoxicity was dose limiting in almost one third of the group A patients, necessitating non-completion of the prescribed NCT + ACT regimen. The only frequent complication encountered by group B patients was myelotoxicity, which was reversible in all but one, who died of sepsis at another hospital. We did not modify the drug dosages, though sometimes the interval between courses was extended by 5–10 days.

Most patients have shown a rapid response to the neoadjuvant use of etoposide and cyclophosphamide [4]. With the availability of hematopoietic growth factors, it may be possible to use even higher doses, which may be particularly appropriate for those with adverse prognostic features at diagnosis. We now reserve the use of high-dose methotrexate for those patients who fail to respond to our current NCT regimen.

We have not shown a significant advantage for the use of i.a. compared with i.v. cisplatinum, though the patients studied thus far have had more clinical responses and histological necrosis at the time of definitive surgery. We have also not yet shown a significant difference in relapse-free survival between groups A and B. This seems likely to reflect the dominant prognostic effect of characteristics of the tumor at diagnosis, which may have overshadowed any difference in the long-term effect of these different regimens. The use of limb-preserving surgery in most of our patients receiving NCT has not been associated with more frequent relapses, either locally or at distant sites, than the more conservative approach of amputation. This may be because we now reserve amputation for the most invasive lesions and those that are least responsive to NCT.

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42. Chemotherapy in osteogenic sarcoma: The experience of the Pediatric Department of the Gustave Roussy Institute

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Introduction

In 1981, there was strong controversy over the role of chemotherapy in the treatment of osteogenic sarcoma. The most successful approach in terms of disease-free survival was Rosen's T10 protocol [1]. At that time we considered Rosen's results as a challenge and decided to adopt the T10 protocol as a whole.

In the T10 protocol preoperative chemotherapy includes seven courses of high-dose methotrexate (HDMTX); one course of the combination of bleomycin, actinomycin, and cyclophosphamide; and one course of Adriamycin. After surgery the tumor response is defined by the pathologist as *good* in the case of total or subtotal necrosis of the examined specimens and as *poor* in other cases [2]. The postoperative chemotherapic regimen is identical to the preoperative in good responders. In poor responders, cisplatinum replaces HDMTX.

Between April 1981 and December 1986, 76 primary, nonmetastatic osteosarcoma patients were referred to our department. Their ages ranged from 4 to 19 years (median, 12 years), with a sex ratio of 43 boys and 33 girls. The tumor site in our series was "classical": upper femur 4, femoral diaphysis 6, lower femur 37, upper tibia 17, lower tibia 3, fibula 5, humerus 3, and cubitus 1. The height of the tumor, as measured on CT scan or MRI, was 5–29 cm (median, 12 cm). Six patients, presenting with a very large tumor and skin lesions, underwent an initial amputation and received chemotherapy according to the protocol used for poor responders (i.e., an initial phase with HDMTX followed by the arm containing cisplatinum).

Among the 70 patients who received preoperative chemotherapy, conservative surgery was performed in 57 cases, amputation in 12, and rotation plasty in one. On histological grading, 39 responses have been considered as good (56%) and 31 as poor (44%).

The median follow-up is currently 55 months for the whole population. Fifty-two patients are in continuous complete remission and 23 have relapsed (one patient died of toxicity). The actuarial event-free survival of the 70

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Table 42-1.

	Rosen	IGR
Patients	87	76
FU (months)	58	55
CDFS	67 (77%)	52 (68%)
ANED	71 (82%)	56 (74%)

patients is 67% at 7 years. It is 78% for good-responder patients and 57% for poor responders. The difference between both groups is not statistically significant ($p = 0.09$). The overall and disease-free survival of the 76 patients are 68% and 74%, respectively, at 7 years.

HDMTX was usually well tolerated in spite of the usual elevation of hepatic transaminases and some episodes of mucositis. Benign seizures related to this drug occurred during the first weeks of treatment in three patients, but the chemotherapy was continued according to the protocol. The major toxicities observed in 5 of the 76 patients (6%) were one Pneumocystis carinii pneumonitis (which resolved), one fatal pneumonitis of unknown origin, one chronic renal impairment due to cisplatinum, and two cardiac failures that both recovered under medical treatment.

Comparing our results with those of Rosen, the following points can be emphasized:

- This intensive chemotherapy is feasible and safe.
- Primary tumor shrinkage allows a great proportion of limb salvages.
- Total necrosis of the primary is obtained in about half the patients.
- In terms of survival, our results (IGR) are very closed to those of Rosen recently updated [3] as shown on Table 42-1.

In 1987, the French Society of Pediatric Oncology designed a nonrandomized multicentric study that was similar to T10 in its protocol. BCD was replaced by Adriamycin and ifosfamide was added to the treatment for poor responders. A total of 130 patients are included in this study, but the results are not yet available because of the short duration of follow-up at present. While participating in this study we are conducting the following special investigations:

- A pharmacological study of HDMTX to check whether interpatient or intrapatient variations in HDMTX pharmacokinetics and metabolism are major factors in determining tumor response. To our knowledge, until now no data strongly show these pharmacoclinical correlations. We think this is a mandatory step to determine whether an individualized MTX dosage might improve the prognosis of patients with osteosarcoma.
- Imaging studies in order to identify good and poor responders to preoperative chemotherapy after 6 weeks of treatment; these studies include quantitative technetium scintigraphy [4] and gadolinium MRI [5].

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43. Results of therapy in osteosarcoma: Experience in Childrens hospitals in Buenos Aires

Enrique Schvartzman, Marcelo Scopinaro, and
Federico Sackmann Muriel

This is a summary report about the results of the application of therapy in osteosarcoma by the authors' experience during our previous work at the Children's Hospital, the Department of Pediatrics of the Italian Hospital, and the Pediatric Hospital in Buenos Aires, Argentina.

We divided our work into three periods. Three consecutive nonrandomized trials are presented in (Table 43-1). Our historical experience (1965–1978) has also been reported [1]. These results include patients with classic osteosarcoma without metastasis localized in the extremities.

From 1979 to 1983 the first prospective study was conducted in order to determine the disease-free interval and survival. Patients were treated every 3 weeks with adjuvant Adriamycin (30 mg/m^2 daily for 3 days up to a total dose of 540 mg/m^2) and platinum (60 mg/m^2 daily for 2 days, for 1 year) after radical ablation of the tumor [2,3]. Seventeen patients were evaluable. Six are disease free, one was lost to follow-up 12 months after diagnosis, one died of cardiac toxicity at 12 months, and another four patients are disease free up to 120 months after diagnosis.

From 1984 to 1987 a second prospective study was initiated as a pilot study to establish the feasibility of using preoperative intraarterial platinum chemotherapy (100 mg/m^2 every 2 weeks, three times) followed by amputation or limb-salvage and postoperative sequential chemotherapy with Adriamycin (45 mg/m^2 daily for 2 days) and platinum (120 mg/m^2/ day 1) for six courses each [3,4].

Out of 30 total patients, 19 patients relapsed. Eleven patients are disease free at 23–72 months, but two were lost to follow-up at 2 and 16 months. We concluded from this second study that there was a trend toward improvement in the results, although intraarterial catherization was technically complicated.

Finally, from November 1987 until the present time a third prospective study was conducted [5]. This study is the European Osteosarcoma Intergroup Protocol (80862). This is a nonrandomized phase II study, an EORTC/MRC/SIOP/UKCCSG collaborative investigation to evaluate the efficacy of a chemotherapy protocol consisting of a combination of platinum, ifosfamide, and Adriamycin (PIA). This study included patients with primary osteosarcoma who after biopsy had preoperative chemotherapy followed by amputation or

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Table 43-1. Comparison of results with localized osteosarcoma in the extremities

Period	Treatment	Number/pts. (evaluated)	DFS N	DFS (%)	Mean survival (months)[b]
1965/78	Historical[a]	21	2	(9.5)	+144
1979/83	Adjuvant ADRIA + CDDP	17	5	(29.4)	+ 96
1984/87	Neoadjuvant IA CDDP + adjuvant ADRIA + CDDP	30	11	(36.6)	+ 48
1987/91	Neoadjuvant + adjuvant CDDP + ADRIA + IFO	15	12	(80.0)	+ 14

[a] See text.
[b] Patients who have DFS.
DFS = disease-free survival; IA = intraarterial; ADRIA = adriamycin; CDDP = platinum; IFO = ifosfamide.

conservative surgery, patients with osteosarcoma in the trunk, and patients with metastatic disease. However, out of a total 26 patients included in this study thus far, we have evaluated only 15 patients, i.e., 11 patients are not evaluable for different reasons (too early, metastatic disease, etc.).

The chemotherapy regimen consisted of platinum 100 mg/m^2 via 24-hour infusion on days 0 and 42; Adriamycin 25 mg/m^2 on days 0, 1, and 2, and repeated at the same dosage on days 21, 22, and 23, and on days 42, 43, and 44; and ifosfamide 3000 mg/m^2 in a 2-hour infusion on days 21 and 22 (with mesna). Surgery was scheduled for the ninth week (day 63).

Examination of the resected specimen was assessed for the adequacy of resection and to determine the response to chemotherapy using the criteria proposed by Huvos [6]. A good histopathological response was considered to be grade III/>90% tumor necrosis) and grade IV (100% tumor necrosis). A poor response was considered to be grade I (0–50% tumor necrosis) and grade II (50–90% tumor necrosis).

All the patients, regardless of histopathological response, received postoperative chemotherapy with Adriamycin 25 mg/m^2 on days (from the beginning of chemotherapy) 77, 78, 79, 98, 99, 100, and 121, 122, and 123; platinum 100 mg/m^2 in a 24-hour infusion on day 98; and ifosfamide 3000 mg/m^2 in a 2-hour infusion on days 77, 78, 121, and 122. The treatment period lasted about 17 weeks.

Out of a total of 15 patients, 11 (73%) are event free with a mean follow-up period of 13 months, ranging from 4 to 31 months, and four patients relapsed.

With regard to the histopathological response, there were nine patients who had a good response (grades III and IV) and six patients with a poor response (grades I and II). Out of the nine good-response patients, one relapsed despite 90% tumor necrosis in the resected specimen. Out of the six patients with a poor response, three relapsed (0, 20, and 60% tumor necrosis).

This study only has a short follow-up period thus far, so definitive conclusions cannot yet be reached. Nevertheless, we should take note that 3 out of 4 relapsed patients had a limb-salvage procedure. The results thus far indicate that the histopathological response seems to be a valid prognostic predictor and that acute or intermediate toxicity is important but managable with this regimen.

Finally, recapitulating this experience with the management of osteosarcoma, we can say that the prognosis for patients with osteosarcoma has improved over the study period of this report. However, the factors responsible for this improvement remain controversial. Nevertheless, use of chemotherapy is one of the major advances in the treatment of osteosarcoma.

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44. Osteosarcoma: Experience with the Rosen T10 protocol at RCH, Melbourne

Henry Ekert and Karin Tiedemann

Introduction

The poor prognosis of children with osteosarcoma treated with amputation alone or with irradiation followed by amputation has been amply confirmed in the literature. In a retrospective survey in our institution carried out over the years 1965–1975, the 3-year survival rate was 5%.

Because of the limited number of our patients, our protocols have aimed at investigating the reproducibility of the results of Gerald Rosen. Initially we used the T7 protocol [1]. Our experience with the T10 protocol is described in this communication [2]. We paid special attention to the details of administration of high-dose methotrexate, electing to admit patients for at least 72 hours for intravenous hydration and alkalinization of urine until methotrexate levels were below 10^{-7} M.

Careful clinical and radiologic assessment was undertaken during the initial 4 weeks of high-dose methotrexate therapy. In the absence of tumor progression, four weekly doses of methotrexate were given. In the presence of clear evidence of disease progression, patients either proceeded to early surgery or received a course of cisplatinum and Adriamycin while awaiting surgery.

Assessment of the histologic response was by the criteria described by Rosen et al. [2]. Initially all patients received the consolidation phase of the T10 protocol, but from 1987 patients showing a grade I response proceeded immediately to the cisplatinum and Adriamycin arm of the protocol. Patients showing a grade II response continued on consolidation, including high-dose methotrexate, before commencing cisplatinum and Adriamycin.

With this approach we have achieved a disease-free survival rate of 68% and a survival of 80% in patients with nonmetastatic limb primaries. A grade III–IV histologic response to high-dose methotrexate occurred in only 3 of 28 patients. All are long-term survivors. Patients with nonmetastatic disease showing partial tumour sensitivity to methotrexate (grade II response) had a disease-free survival of 75% and a survival of 100%, while those with no response to methotrexate had a disease-free survival of 52% and a survival of 62%.

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Table 44-1. Response to high-dose methotrexate

Clinical response		
Progression	6	
Static or improved	22	
Not evaluable	1	
pathologic response		
Grade	*No. courses HDMTX*	*No. patients*
I	2	2
	3	3
	4	11
II	4	7
III	2	1
	4	1
IV	4	1
Not evaluable	4	1
(no surgery, clinical PD)	5	1
Total		28
Overall response		
No response (clinical failure & grade I)		18 (64%)
Grade II		7 (25%)
Grade III and IV		3 (11%)

Patients

The diagnosis was established by open biopsy in all patients. There were 19 boys and 10 girls, and their ages ranged from 5.5 to 19.5 years. No patients had bony metastases, as demonstrated by a technitium bone scan at presentation.

Results

Chemotherapy using the approach defined above was commenced within 48 hours of histologic confirmation of the diagnosis. Only one patient with a pathological fracture of the lower end of the femoral shaft had immediate amputation and was not evaluable (Table 44-1).

The response to initial chemotherapy was assessed on clinical and pathologic grounds. Two patients showed clinical and radiologic progression after two courses of methotrexate and were treated with Adriamycin and cisplatinum before surgery. Both had a grade I histologic response. A further three patients had clinical progression after three courses and proceeded to surgery. They also showed a grade I response. One patient who developed a pathologic fracture after two courses of methotrexate proceeded to amputation. The histologic response was grade III. Only one of three patients with a pelvic

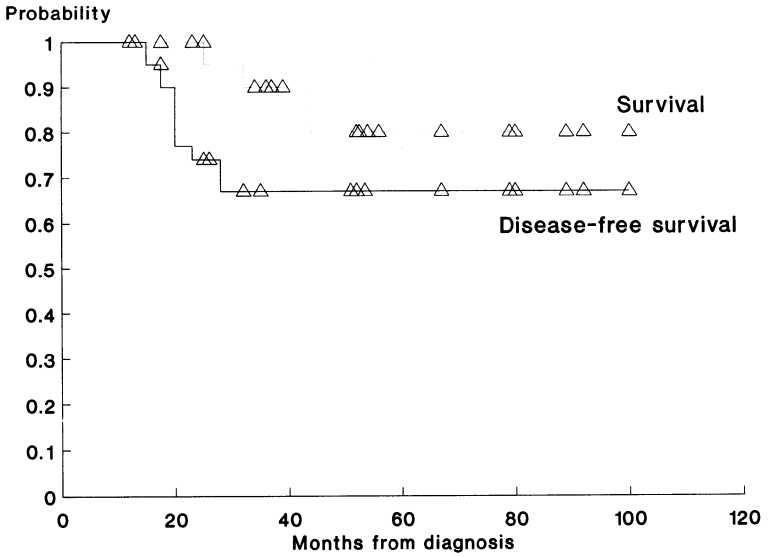

Figure 44-1. Survival and disease-free survival for patients with nonmetastatic limb primaries

primary was considered to be operable after four courses of methotrexate, but showed only a grade I response.

The clinical and histological response to high-dose methotrexate is shown in Table 44-1 and in Figures 44-1 to 44-3.

Disease-Free Survival and Overall Survival

Patients with extremity primaries without metastatic disease at diagnosis had a disease-free survival of 68% and an overall survival of 80%, with a median follow-up of 52 months (Figure 44-1). All patients [3] with grade III and IV responses are disease free at 57, 73, and 96 months. Patients with a grade II response have a disease-free survival of 75% but an overall survival of 100%. Patients with a grade I response have a 52% disease-free survival and a 62% overall survival (Figures 44-2 and 44-3).

Discussion

The disappointing rate of grade III and IV responses in our patients in similar to those reported from other studies [3] but inferior to those of Rosen [2]. One explanation for this discrepancy may be the method of methotrexate administration. The fluid regimen used by Rosen was designed for a lower urine output, particularly in the first 24 hours postmethotrexate. This may result in higher concentrations of methotrexate for a longer period and a

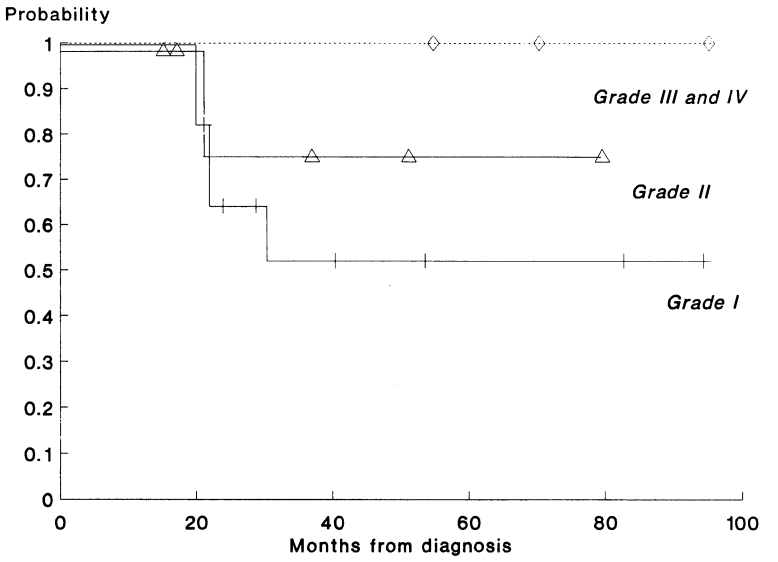

Figure 44-2. Disease-free survival of patients with nonmetastatic limb primaries according to methotrexate responsiveness

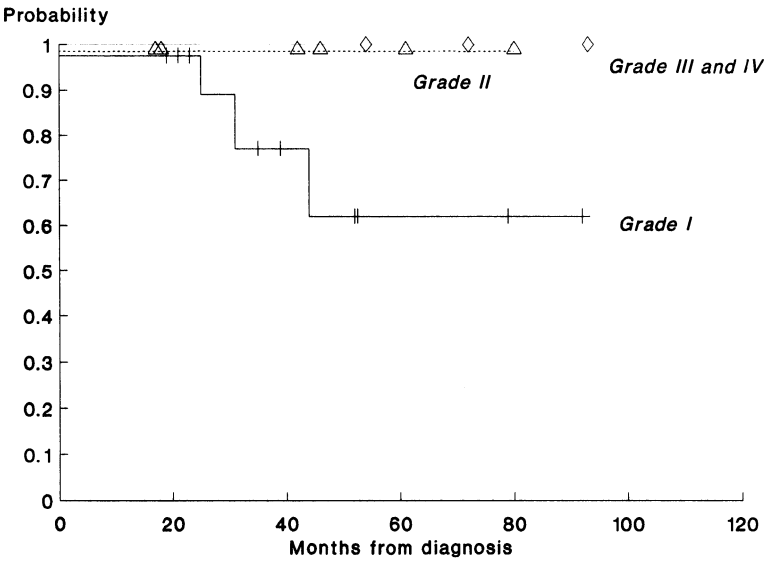

Figure 44-3. Survival of patients with nonmetastatic limb primaries according to methotrexate responsiveness

superior tumoricidal effect, but it also requires greater vigilance to avoid potentially severe methotrexate toxicity. We are now in the process of modifying our fluid replacement protocol in order to make it more in line with that of Rosen. This seems rational because in our patients disease-free survival correlated with histologic response (Figure 44-2).

The policy of early introduction of Adriamycin and cisplatinum in patients showing no significant response to methotrexate seems rational but has not resulted in a disease-free survival in these patients that is equal to that of the methotrexate-responsive group. Presumably the early introduction of Adriamycin and cisplatinum will be beneficial to those patients whose tumors remain drug sensitive, but the fact that almost 50% relapse suggests that a significant proportion of these patients may have had tumors with multiple drug resistance before the commencement of therapy.

While our experience and studies with the Rosen-type approach to treatment differ to an appreciable extent from his original reports, there can be no doubt that this approach can cure the majority of patients with nonmetastatic extremity disease.

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45. Osteosarcoma of the limb: An institutional report of 10 years experience with neoadjuvant chemotherapy and delayed surgery

A. Postma, W.A. Kamps, H. Schraffordt Koops, R.P.H. Veth, L.N.H. Göeken, and W.M. Molenaar

Introduction

Following the initial report of Rosen in 1976 [1], a study of preoperative chemotherapy for treatment for primary osteosarcoma patients was started in our hospital in 1978.

Patients and methods

Patients

Eligibility criteria were (1) histologically confirmed high-grade osteosarcoma of the long bones, (2) no initial metastases, and (3) no previous therapy. Between March 1978 and November 1988, 30 patients were entered into two different studies that were based on Rosen's protocols.

Surgery and pathology

After an open biopsy and diagnostic evaluation, patients were selected for either limb-saving surgery or amputation based upon the presence of tumor involvement of neurovascular structures and soft tissues. Histological grading was done as described by Huvos [3].

Chemotherapy

In the period 1978–1982 all patients received weekly vincristine (VCR) 1.5 mg/m^2 i.v. (maximum 2 mg), followed in 30 minutes by high-dose methotrexate (HDMTX) 8 g/m^2 (adolescents and adults) or 12 g/m^2 (children) in a 6-hour infusion, followed by leucovorin rescue. Patients were to receive a fluid intake greater than that recommended by Rosen (3 l/24 hr). This regimen was continued for 4 weeks and was followed by amputation in patients who were

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 ISBN 0-7923-1905-2

not eligible for limb salvage. Patients who were scheduled for limb salvage had surgical biopsies and continued with another 4 weeks of the same regimen while awaiting the availability of their endoprostheses. If histologic grading of the biopsy specimen still revealed viable tumor cells, the subsequent dose of HDMTX was elevated with increments of 2 g/m^2 on an individual base (maximum 16 g/m^2). Postoperative chemotherapy was resumed 3 weeks after surgery with VCR and HDMTX alternating with Adriamycin 30 mg/m^2 on two consecutive days every 2 weeks; after a cumulative dose of 550 mg/m^2, Adriamycin was replaced by cyclophosphamide 1200 mg/m^2. The total duration of postoperative chemotherapy was 15 months. If on pathological examination the surgical specimen showed an unfavorable response, postoperative chemotherapy was given, consisting of cisplatinum and Adriamycin, as described by Ettinger [4]. From 1982 to 1988 preoperative and postoperative chemotherapy were given to all patients according to Rosen's T10 protocol [2].

Results

As the numbers evaluated are small, the patients in both treatment periods were evaluated as one group.

Response on preoperative chemotherapy

Nineteen patients (63%) showed a histologically unfavorable response, and 11 patients (37%) were favorable responders.

Disease-free survival (DFS)

Nineteen (63%) patients are alive with no evidence of disease at 33–155 months (Figure 45-1). Nine patients showed metastatic disease 5–58 months after diagnosis. Most patients (8/11, i.e., 73%) with a favorable response survived free of disease for 48–51 months. Two patients died due to chemotherapy-related toxicity and one died from pulmonary metastases.

Toxicity and compliance

Compliance with the scheduled treatment was difficult. Seventeen patients received treatment according to the initial outline; 13 patients received incomplete treatment due to either dose-limiting toxicity of chemotherapeutic agents or patient refusal. In this last group 8 patients (61%) developed recurrences, in contrast to only 2 of the 17 patients (12%) who received the full scheduled treatment ($p < 0{,}006$ Fisher's exact probability test).

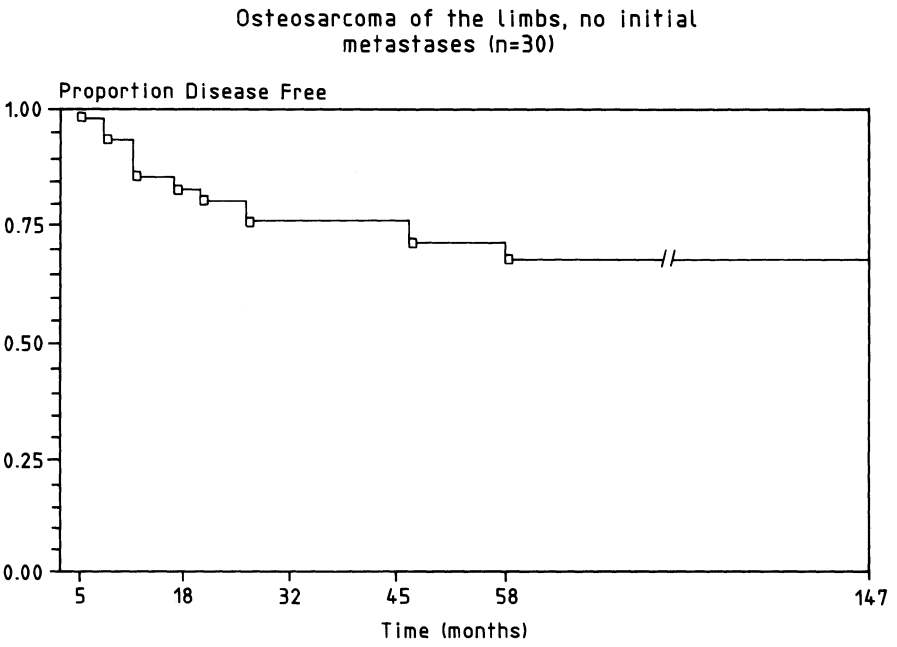

Figure 45-1. Osteosarcoma of the limbs, no initial metastases (n = 30)

Discussion

In this small nonrandomized study on preoperative and postoperative chemotherapy and delayed surgery, survival and DFS are similar to to the results of more extensive studies with the same design [2,7,8]. The prognostic significance of the tumor response to preoperative chemotherapy has been demonstrated by others [7,8,9,12]. The number of patients with a favorable response in our series is only 37%, which is rather low in comparison with some other studies, where response rates as high as 80% were achieved [9,10]. We used excessive hydration before and after the administration of MTX to prevent systemic toxicity. Rosen stressed that under these circumstances efficacy could be reduced [11].

Another prognostic factor in our study was full treatment. It could be argued that incomplete treatment mainly occurs among patients with a poor response on preoperative chemotherapy who subsequently were exposed to the most toxic regimen. However, as 4 of 11 patients with a favorable response and 9 of 19 patients with an unfavorable response had incomplete treatment, an association seems unlikely. The drugs that were particularly responsible for dose-limiting toxicity and late morbidity were Adriamycin and cisplatinum. Unfortunately both are still among the most effective drugs in the treatment of osteosarcoma.

Acknowledgments

This work was supported by the Foundation for Pediatric Oncology, Groningen, The Netherlands (SKOG).

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46. Osteosarcoma: Experience of the Tata Memorial Hospital, Bombay, India

Shabbir S. Susnerwala, Subodh C. Pande, Ketayun A. Dinshaw, Suresh H. Advani, and Jayant N. Suraiya

Introduction

The dramatic improvement in the prognosis of osteosarcoma (OS) achieved in the West is less likely to be emulated in developing countries for a variety of reasons. Foremost among them are advanced disease at presentation, limited access to modern effective chemotherapy, and the dearth of optimal oncologic centers. Basically, this is a reflection on the overall poor socio-economic status of the patient population, coupled with the health priorities of developing nations.

The Tata Memorial Hospital, Bombay, is the country's premier comprehensive cancer center, catering to the needs of about 15,000 biopsy-proven cancer cases annually. The entire experience in the management of OS in our institution over the years 1985–1988 is presented mainly to highlight the problems faced in the management of this formidable cancer in developing countries.

The clinical material

The case records of all biopsy-proven OS of bones presented to our hospital between January 1985 and December 1988 were analyzed retrospectively [1]. A total of 273 cases of OS were found. (0.65% of all cancer cases). A preponderance of males over females was seen, with 198 male and 75 female cases. Patients ranged in age from 5 to 60 years, with a median of 18 years. Two thirds of the patients were in their second decade of life.

A total of 250 cases (92%) presented with primary lesions of the limb bones, and a clustering about the knee joint was the commonest presentation seen in 185 patients (68%). The axial skeleton was involved in 23 cases (8%), among which the primary lesion was in the cranio-facial bones in 15, pelvic in five, ribs in one, and clavicle in two cases. Lung metastases seen in chest roentgenograms were encountered in 22 (8%) patients at presentation.

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Therapeutic rationale

The management of cancer in the developing countries is beset by a variety of constraints. In the specific context of OS, reluctance to readily accept ablative surgery, unaffordability of optimal chemotherapy, and treatment dropout are common phenomena related to the lack of education and financial resources in the patient population at large. Furthermore, patients' limited means for care at the better equipped metropolitian centers, along with poor oncologic awareness among their family practitioners or lack of sophisticated oncologic management facilities at their remote places of birth lead to both noncompliance with therapy as well as follow-up losses. The situation thus calls for gross compromise in management strategies in order to bring the majority of patients within the realm of therapy. This includes modification of therapy protocols with a view to increasing both affordability and compliance, reducing toxicity of their chemotherapy, and to leaving room for necessary exploration of radiotherapy for local control, despite its limited efficacy in OS.

Due to a combination of the above factors, 63 patients (24%) could not complete the prescribed treatment. the 210 (74%) patients who managed to undertake the minimum prescribed treatment have been considered for evaluation. Only the latter are retrospectively analyzed for their treatment responses and the former are excluded from the study. The period of this study represents one of changing attitudes towards the management of OS in our institution. Thus, while on the one hand there seems to be acceptance of the value of adjuvant chemotherapy, radiotherapy is still retained for debulking the primary, albeit as a limited measure and in the context of the problems specific to our setup. This period also heralds the advent of the newer modalities of limb-salvage surgery and neoadjuvant chemotherapy. While these strategies have been applied on an uncontrolled basis, the 210 adequately treated cases were analyzed within four distinct therapy groups (Table 46-1).

I. Surgery alone
II. Surgery plus adjuvant chemotherapy
III. Neoadjuvant chemo-radiotherapy, surgery and adjuvant chemotherapy
IV. Radiotherapy and adjuvant chemotherapy.

Treatment strategies

Surgery alone

A total of 79 patients (38%) underwent surgery as the only mode of therapy.

Surgery plus adjuvant chemotherapy

Chemotherapy in an adjuvant setting was considered in 82 patients (39%) who had primary surgical treatment. During the period of this study there

Table 46-1. Therapy details and survival

Treatment schedule	Total no. of pts	Alive (%)	Dead (%)	Lost to follow-up (%)	Crude survival
Surgery alone	79	09 (11)	47 (60)	23 (29)	11%
Surgery + chemotherapy (unit A)	40	09 (23)	25 (62)	06 (15)	23%
Surgery + chemotherapy (unit B)	42	07 (17)	29 (69)	06 (14)	17%
Chemo-radio therapy + surgery + chemotherapy	30	09 (30)	12 (40)	09 (30)	30%
Radiotherapy + chemotherapy	19	02 (11)	14 (74)	03 (15)	11%
Total (%)	210	36 (17)	127 (61)	47 (22)	17%

were two different chemotherapy schedules in concurrent use by two independent clinicians.

Unit A Schedule. A two-drug combination comprising Adriamycin (ADR) and cisplatin (CDDP) was used as follows:
ADR 60 mg/m^2 i.v. push, day 1
CDDP 100 mg/m^2 i.v./24 hr infusion with adequate hydration on day 22.
The above schedule was repeated every 3 weeks for a total of six cycles until a cumulative dose of ADR of 540 mg/m^2 was reached, at which time CDDP alone was continued at 4-weekly intervals until a total period of 1 year or earlier if severe toxicity resulted.

Unit B schedule. A multidrug combination of methotrexate (MTX), cytoxan (CTX), Adriamycin (ADR), and cisplatin (CDDP) was used as follows:
MTX 200 mg/m^2 as i.v. infusion on day 1 with citrovorum rescue
CTX 600 mg/m^2 as i.v. bolus on day 1
ADR 50 mg/m^2 as i.v. bolus on day 1
CDDP 100 mg/m^2 as i.v. infusion with adequate hydration on day 1.
The above schedule was repeated every 3 weeks for a total of six cycles.

Neoadjuvant chemoradiotherapy, surgery, and adjuvant chemotherapy

A total of 30 patients (14%) were treated with a combination of neoadjuvant chemotherapy, surgery, and adjuvant chemotherapy given sequentially. In nine patients (30%) preoperative irradiation was administered additionally.

Neoadjuvant chemotherapy schedule

This schedule was as follows:
MTX 100 mg/m^2 i.v. infusion with citrovorum factor
ADR 50 mg/m^2 i.v. push
CDDP 100 mg/m^2 i.v. infusion with adequate hydration
CTX 600 mg/m^2 i.v. puswh
Vinblastine (VLB) 6 mg/m^2 i.v. push
All of the above drugs were administered on day 1 and repeated after 3 weeks.

Radiotherapy technique

All patients were treated with megavoltage therapy at a dose of 30–45 Gy over 2–3 weeks by parallel and opposed fields generously encompassing the primary tumor.

Radiotherapy and adjuvant chemotherapy

A total of 19 patients (9%) were treated with radiotherapy and adjuvant chemotherapy due to refusing surgery. Radiotherapy was administered with telecobalt at a dose of 60–70 Gy over 6–7 weeks using the same technique as described earlier. All patients except one received adjuvant chemotherapy, which was given using the same schedule as for the unit B cases in group II.

Response

The responses of these four distinct therapy groups are summarized in Table 46-1.

Summary and conclusions

This is a report on the management of 210 patients with biopsy-proven OS seen at the Tata Memorial Hospital, Bombay, India from January 1985 to December 1988. The treatment administered to these patients reflects the constraints experienced in cancer management by developing nations. The

small number of patients who received neoadjuvant chemoradiotherapy showed the highest survival figures of 30% at 5 years. In the context of the developing countries, limitations of affordability of optimum chemotherapy and the lack of adequate monitoring and support facilities warrant modifications in the currently recommended therapy schedule to suit indigenous needs.

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47. Update of osteosarcoma in Ramathibodi, Thailand

Vorachai Sirikulchayanonta, Lucksana Pochanugool, and Thanya Subhadrabandhu

Introduction

Osteosarcoma is the most common primary malignant bone tumor in Thailand [1,2]. The peak age group and skeletal distributions are not different from those of many countries [1,3,4]. Only a minority of cases have been referred to seven tertiary care centers for radio- or chemotherapy throughout the country. Our institute is one of these, and we admit approximately 5–10 referral cases annually.

Before early 1980, osteosarcomas of the long bones were treated exclusively by amputation; adjuvant chemotherapy was aimed at palliative treatment for advanced and affordable cases. However, the results were poor; all cases died within 2 years [2]. In light of effectiveness of adjuvant chemotherapy for osteosarcoma, as reported in Western countries, our institute pioneered the multidisciplinary treatment of osteosarcoma in this country when our program commenced in 1986 [4,5].

The treatment protocols we adopted were those that had been used successfully in other institutes, and they were adapted partly to suit our socioeconomic conditions [6, 7]. This study was begun in March 1986 and was closed to patient entry at the end of 1989.

Study (1986–1989)

Objectives

- To increase the survival rate and to improve the quality of life of patients
- To determine the efficacy of preoperative intraarterial chemotherapy combined with irradiation by estimating the degree of tumor necrosis of resected or amputated specimens
- To determine the appropriate modality of treatment for osteosarcomas in developing countries

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 ISBN 0–7923–1905–2

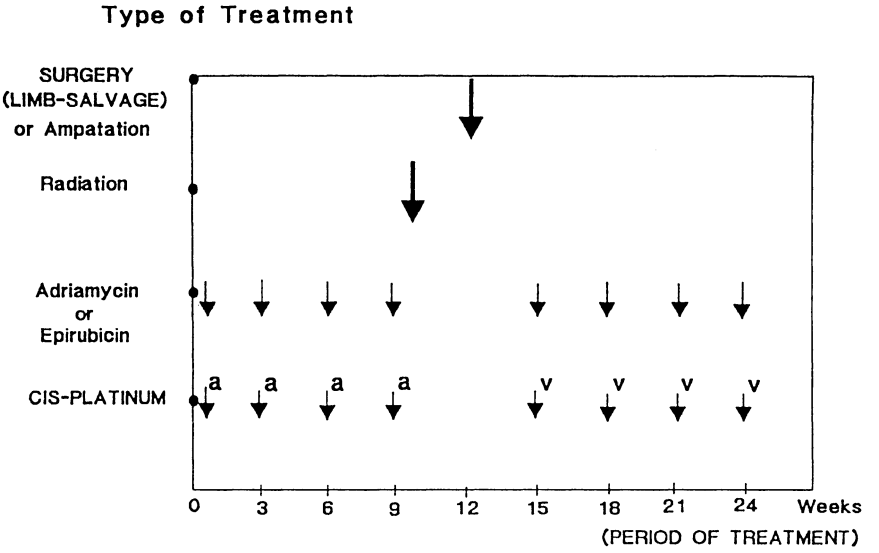

Figure 47-1. Treatment protocol. Note: a = intraarterial infusion; v = intravenous infusion.

Methods

Diagnostic workup. All patients enrolled in the study had only osteosarcoma, stage IIA or IIB according to Enneking's classification [8].

Treatment protocol (Figure 47-1). All patients were given preoperative intraarterial cisplatinum by the Seldinger technique via the femoral or brachial arteries under fluoroscopic guidance [6]. The dose of cisplatinum was 100–120 mg/m^2, and it was completely infused intraarterially over 2 hours via an infusion pump. Doxorubicin (Adriamycin) 60–75 mg/m^2 or epirubicin 75–90 mg/m^2 was given intravenously the following day. The procedures were repeated at 3-week intervals for another three cycles.

Local irradiation of the affected limbs was given the day after the fourth chemotherapy. The total dosage of radiation was 2000–3000 cGy, and it was divided into 10 fractions. The source of radiation was a ^{60}Co machine with 75- and 80-cm SSD.

Limb-salvage procedures were performed 2–4 weeks after radiation treatment was completed [5]. Amputation was performed in the cases that showed an infected biopsy site, tumors involving neurovascular structures, or inadequate skin or normal tissue coverage in all directions. After surgery, intravenous chemotherapy was administered for a total course of eight cycles or to the maximum tolerance of chemotherapy.

Treatment results

From March 1986 to December 1989 26 cases enrolled in the study. The age of patients varied from 9 to 43 years, with a mean of 19.8 years and a standard deviation of 7.6 years. Of 26 cases, 17 cases received the limb-salvage procedure and another nine cases received amputation. Among these 17 cases, two were later amputated: one due to infection and the other due to the presence of tumor in the soft tissue near the resection site. The mean disease-free survival for the limb-salvage group was 23.06 months (SD 15.01 months) and ranged from 8 to 56 months.

Of nine amputated cases, three were still alive without evidence of recurrence or metastasis, whereas six died of metastases. The mean disease-free survival for the amputation group was 16.8 months (SD 11.9 months) and ranged from 1 to 36 months. At 2 years following surgery, more than 50% of cases remained disease-free and were still alive. Chotigavanich et al; studied Thai osteosarcoma patients and reported that all patients who were treated with surgery without adjuvant therapy died within 2 years [2].

This study, therefore, suggests that adjuvant chemoradiotherapy and limb salvage benefit patients in terms of quality of life and disease-free and overall survival rates for at least the first 2 years following treatment. The overall significance in terms of cure rate, however, awaits further follow-up. During treatment of all cases, toxicity and side effects of drugs and radiation were not serious enough to warrant discontinuation of treatment.

Conclusions

Preoperative chemotherapy or radiotherapy and limb-salvage procedures for osteosarcoma in Thailand have been intensively used since 1986 by our group. The outcome shown in our study was satisfactory; it was observed that the disease-free survival, especially in the limb-salvage group, was better than the historical control group reported from other institutes in both Thailand and industrialized countries [2,5]. The cost of the entire treatment was approximately 125,000 baht (US$5000), which might be considered appropriate for the economic status of Thai patients. It is therefore advocated that the limb-salvage procedure with preoperative chemotherapy and radiotherapy be the treatment of choice for early cases of osteosarcoma in this or other developing countries.

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48. Commentary on pathology

Andrew G. Huvos

The epidemiologic comparison of Japanese and English osteosarcoma patients by Machinami and Wikremaratschi attempts to elucidate the true incidence and the manifold clinicopathologic aspects of this relatively rare disease. Whether or not the analysis of these two disparate groups of patients from two countries and from only partially overlapping periods of time is epidemiologically fair remains an open question. Nevertheless, the current study reemphasizes the fact that Paget's disease of bone is practically unknown in Japan and consequently Paget's sarcomas do not occur in that country.

The quest for consistent identification of osteoblastic activity, i.e., osteoid formation by the best means feasible, is many a decade long. In the past, the diagnosis of primitive osteoid matrix by a positive acid phosphatase reaction that is usually tartrate resistant has been used with some measure of success. Similarly, a positive reaction for nonspecific esterase inhibited by fluoride has also been employed. A strong enzymatic reaction with alkaline phosphatase is observed in practically all osteogenic sarcomas. The recognition of minimal osteoid produced directly by sarcoma cells, however often remains elusive and largely controversial. For instance, strong alkaline phosphatase reactivity is seen not only in osteoblastic lesions but also in tumors of endothelial differentiation as well. Fibrous tumors or the glandular components of biphasic synovial sarcomas may also be alkaline phosphatase positive.

Dr. Ushigome and associates address this thorny problem of the role of immunohistochemistry in the differential diagnosis of osteosarcoma. In their effort to identify consistent markers for tumor osteoid, they have examined the immunoreactivity of osteocalcin, which was hoped to be specific for osteoblastic activity, as well as alkaline phosphatase, a well-studied complement to the histologic diagnosis of bone-forming lesions.

These authors also point out the well-known diagnostic difficulties in identifying low-grade intramedullary osteosarcomas, especially if only biopsy material is available for microscopic study. They also invoke the presumed occurrence of "dedifferentiation" to explain the simultaneous presence of a high-grade osteosarcoma in an otherwise low-grade intramedullary bone sarcoma. Some observers, however, consider the coexistence of both low- and

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 ISBN 0-7923-1905-2

high-grade osteosarcoma a manifestation of the diagnostician's inability to adequately assess the true biologic potential of a morphologically complex tumor. The phenomenon of "dedifferentiation" in this context is nothing more nor less than a sign of tumor progression and is not related to a retrograde cell differentiation or to loss of differentiation in an already well-differentiated tumor.

Several authors, including Dr. Ushigome et al. and Ueda et al., discuss the clinicopathologic aspects of the telangiectatic osteosarcoma. By now it is well established that this rare type of osteolytic bone sarcoma does not have a uniformly fatal clinical outcome, as initially found by Matsuno and associates in 1976. As a matter of fact, patients with telangiectatic osteosarcoma do have an unusually favorable chemotherapeutic response to neoadjuvant chemotherapy.

The same authors also analyze the question of whether a malignant fibrous histocytomatous osteosarcoma does or does not exist. Not surprisingly, the two groups come to diametrically opposed results in their analyses. Ushigome and colleagues believe that, based on the positive osteocalcin and alkaline phosphatase reactions in this type of osteosarcoma, the "MFH-mimicking" osteosarcoma is a true histologic entity. In contrast to this finding, Ueda and associates from Münster in Westphalia maintain that it is not a distinct entity at all, but merely an arbitrary histomorphologic viewpoint.

Ueda and associates, in addition, correctly point out that no single type of collagen is specific for tumor osteoid, but the combined immunoreactivity of types I–VI collagen antibodies may be helpful in identifying primitive bone formation in osteosarcoma. Various approaches to a better understanding of the role and composition of noncollagenous bone proteins, including osteocalcin, are currently being explored to better understand the biological makeup of osteosarcoma cells. Unfortunately, osteocalcin may also be detected in chondrosarcoma, clear-cell chondrosarcoma, malignant fibrous histiocytoma, or in several other bone tumors traditionally considered to be non-bone forming. This lack of osteocalcin specificity emphasizes the need for more studies to accurately assess the significance of the antibody.

Monoclonal antibodies specific for human osteosarcoma-associated antigens remain to date a great promise in the practical diagnosis of osteosarcoma. This complex problem is addressed by Ueda et al. in a thoughtful discussion and analysis, the best currently available anywhere.

Four diagnostic radiologists (Dr. Hopper et al.) attempt to analyze the distinctions between "skip" bone lesions as well as metachronous and synchronous metastases in osteosarcoma. The understanding of these entities remains less than clear, especially since the primary focus of this chapter is on osteosarcomatosis. The conclusion that many cases of osteosarcomatosis in fact are forms of metastatic osteosarcoma is worth remembering.

The multidisciplinary team from the M.D. Anderson Cancer Center in Houston (Ayala et al.) updates and extends our understanding of small-cell osteosarcoma, a rare variant of bone-forming sarcoma. The study concludes

that this sarcoma is predominantly of metaphyseal long bone location with preferential femoral involvement. It is slightly more common in females as compared to a male predominance in other types of osteosarcoma. The analysis of the recommended treatment regimen remains inconclusive and awaits further well-controlled prospective studies. Parenthetically, this rare variant of osteosarcoma is currently treated by a Ewing's sarcoma-oriented treatment protocol at Memorial Sloan-Kettering Cancer Center.

Dr. Bauer of Stockholm's Karolinska Sjukhuset reviews the historical background, methodology, and clinical application of DNA cytometric analysis in osteosarcoma. He points out that although Ewing's sarcoma and synovial sarcoma display a diploid cell population, i.e., these sarcomas do not have an increased DNA content, they are still highly malignant in their clinical course. The chapter also discusses the relative usefulness of microspectrophotometry and flow cytometry in studying imprints and paraffin-embedded tissue sections in measuring the DNA content of tumor cells. The author recommends both methods for DNA measurements to be performed in routine analysis, especially in diploid bone and soft tissue sarcomas. If a histologically low-grade sarcoma is nondiploid, a thorough review of histologic material should be undertaken to confirm that this indeed is the case. More than likely the histologic diagnosis is incorrect.

49. Is there a rational role for radiotherapy in the treatment of osteosarcoma?

E.A. Bleher

Up to 1974, The therapy of osteosarcoma consisted of surgery and radiotherapy. With amputation alone, 25–30% of patients could survive for 2 years and 80% of all patients died of distant metastases up to 15 years after the diagnosis [1]. According to Cade [2], local radiotherapy with 7000–8000 cGy was given and the operation was deferred by 6 months in order to obviate unnecessary amputations in patients with distant metastases occurring in these 6 months. A 5-year actuarial survival of 21.8% can be achieved with radiotherapy and delayed surgery. Because of effective prophylaxis or treatment of metastases with methotrexate [3], radiotherapy has since been replaced by even more effective chemotherapy schedules, and therefore in subsequent years radiation therapy was omitted and was only used palliatively or in the rarer locations in the pelvis and the visceral cranium, as well as in elderly patients.

Today, approximately 50–60% 5-year actuarial survival is attained with surgery (amputation or limb saving) and chemotherapy. However, it must be borne in mind, in view of the comparative results from the Mayo Clinic, that the improvement in the results is merely attributable to the more refined diagnostics (such as CT, MRI, etc.) available in recent years [4].

Local recurrences after surgery with and without chemotherapy—10% [5] to 50% [1]—and the toxicity of chemotherapy [6] remain a problem. It hence appears to be justified to reconsider the use of radiotherapy as an additional measure in a multimodal concept. Morton [1] reported on 14 patients who received 3500 cGy fractionated over 12–15 days before the operation subsequent to an intraarterial Adriamycin infusion. This radiotherapy was followed by radical local resection and limb saving after 1 week. Fourteen patients who later received high-dose adjuvant chemotherapy survived without metastases for 4–34 months. Yamamuro [7] described the technique of intraoperative radiotherapy with a single electron dose of 50–60 Gy (or 100 Gy in juxtacortical osteosarcomas) and subsequent adjuvant therapy and provision of a prosthesis after 3 months. Only 1 out of 32 patients developed a local recurrence, and 78% survived for a cumulative 5-years. However, this treatment technique requires great surgical skill, and its use is likely to be confined to a few centers. Takada [8] referred to 38 patients who between

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1975 and 1986 have been treated by fast neutron radiotherapy, systemic chemotherapy, and limb-salvage surgery. Thirty-five of these 38 patients underwent en bloc resection. Only one had evidence of local tumor recurrence. Thirty-one patients were alive without distant metastases after 9–120 months.

Application of percutaneous radiotherapy should also be reevaluated. In view of the results of recent radiobiological investigations [9], better tumor control can be attained in rapidly proliferating tumors with lower dose schedules (hyperfractionated accelerated radiotherapy) applied over a shorter time than conventional treatments with 5×2 Gy/week without increasing short- and long-term toxicity [10].

Adjuvant therapy of microscopic pulmonary metastases with bilateral pulmonary irradiation was investigated in two EORTC studies. Burgers [6,11] reports that whereas lower doses are ineffective [12], 20–22 Gy over the entire lungs is just as effective a prophylaxis with minimal toxicity as with high-dose chemotherapy. Pulmonary metastases that still occur are easier to deal with surgically than after prior chemotherapy. It is conceivable that an improvement of the prognosis can be attained with elective whole-lung irradiation, in particular patients whose tumors have responded less well in histological terms to the preoperative chemotherapy.

In conclusion only few papers have been published in the last decade dealing with radiotherapy in patients with osteosarcoma of the limbs. Therefore the value of radiotherapy should not be overestimated. However, radiotherapy may play a role in the curative treatment of osteosarcoma, even in modern concepts of therapy; both in local tumor and as prophylaxis against lung metastases, the latter being preferable in adolescents after the pubertal growth spurt, and especially in patients with a tumor that is more resistant to chemotherapy or in primaries that are not amenable to surgery.

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50. Commentary on the use of presurgical chemotherapy

Michael P. Link

Refinements in therapy for patients with osteosarcoma are needed. Although the prognosis for children with osteosarcoma has improved dramatically in the past two decades, more than one third of children presenting without metastases will relapse after receiving the therapy currently available. Recently, many investigators have recommended the use of presurgical chemotherapy to deliver systemic treatment against micrometastases early in the course of therapy [1]. Although the approach with presurgical chemotherapy actually evolved to facilitate limb-sparing surgery by providing a window of time during which customized endoprosthetic devices could be fabricated, favorable responses in primary tumors of patients treated with presurgical chemotherapy encouraged investigators to approach all patients with presurgical chemotherapy to improve the overall outcome of these patients, whether or not they are candidates for limb-sparing operations. A number of theoretical advantages of presurgical chemotherapy are attractive. Since children undergoing orthopedic surgery spend several weeks recovering from their operation to allow for wound healing prior to the administration of chemotherapy, a period of several weeks is spent without treatment of their known micrometastatic disease. Such a delay is likely to be most critical early in treatment when the burden of micrometastatic disease is high; the administration of presurgical chemotherapy immediately after biopsy but before definitive surgery of the primary tumor thus allows for the earlier institution of systemic chemotherapy against micrometastases known to exist in virtually all patients at diagnosis, increasing the chance that these micrometastases might be eradicated. Further, earlier treatment would reduce the chance of spontaneous emergence of drug-resistant clones of tumor cells in the micrometastases. Finally, some investigators have utilized presurgical chemotherapy as an in vivo drug trial of drug sensitivity of the primary tumor in order to "customize" the postsurgical adjuvant chemotherapy based upon responsiveness of the primary tumor to presurgical chemotherapy—a strategy based on the belief that responsiveness of the primary will predict the responsiveness of the micrometastases [2,3]. The rationale for the use of presurgical chemotherapy is compelling, and, indeed, preliminary results from early trials of presurgical chemotherapy (especially those utilizing a customized approach to the patient based upon responsiveness of the primary tumor to presurgical chemotherapy) have been excellent [3,4].

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 ISBN 0-7923-1905-2

The theoretical arguments for the *disadvantages* of presurgical chemotherapy are equally compelling. Cell kinetic data suggest that the use of chemotherapy against bulky tumors is not optimal when the majority of tumor cells are not in cycle. Treatment of patients with a high tumor burden also increases the chance of selecting drug-resistant clones in the primary tumor that may then metastasize. In nonresponsive tumors, delay in the surgical approach to the primary neoplasm also increases the opportunity of systemic spread of drug-resistant tumor cells. In light of these considerations, the theoretical arguments that have been used to support immediate surgery followed by the use of chemotherapy adjuvantly after surgery are equally convincing. The reduction of tumor burden by immediate radical surgery increases the growth rate of residual disease, making cycle-specific chemotherapeutic agents more active, and optimizes the conditions for first-order kinetics by which chemotherapy is presumed to work. Moreover, the opportunity for selection of drug-resistant clones is obviously minimized because the surgeon has reduced the tumor burden dramatically. There is, however, a delay in the treatment of micrometastatic disease.

It is noteworthy that presurgical chemotherapy has been utilized in virtually all recently initiated studies for children and adults with osteosarcoma, whether or not the patient is a candidate for limb-sparing surgery. Customizing treatment based upon responsiveness of the primary tumor to presurgical chemotherapy has also been featured in many recent trials. Unfortunately, attempts [5,6] to duplicate results of the initial trials of customized treatment have failed to reproduce the outstanding preliminary results first reported from the Memorial Hospital [1,2]. Moreover, follow-up of the patients treated on the studies from the Memorial Hospital (where studies of preoperative chemotherapy were pioneered) have not confirmed the initial promising results [7]. In several multiinstitutional trials utilizing presurgical chemotherapy with or without customizing treatment, the overall results have not been superior to the results achieved with immediate surgery and postoperative adjuvant chemotherapy in the MIOS [8]. Even more surprising, results reported from single institution trials of presurgical chemotherapy [7,9] are also not substantially better than results of the MIOS—a disappointing finding in view of the fact that results from single institution trials invariably exceed the results that can be achieved in multiinstitutional studies. Thus, the value of presurgical chemotherapy in the treatment of osteosarcoma (independent of its role to enhance limb-sparing surgery) remains to be proven. The role of presurgical chemotherapy is currently under investigation in a study by the Pediatric Oncology Group.

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51. Critique on the use of presurgical chemotherapy: Recent trials as updated in this volume

G. Bennett Humphrey

From the updates published in this volume, is it possible to answer the following question: Does presurgical chemotherapy followed by adjuvant chemotherapy after definitive surgery produce a higher disease-free survival than the traditional approach of definitive surgery followed by adjuvant chemotherapy? We feel that the answer is probably not. Can some insight be gained from what is included in this volume? Possibly.

All of the recently initiated trials or ongoing trials listed in Part II (cooperative groups and institutional reports) of this volume are presurgical chemotherapy. The ongoing trial of the Pediatric Oncology Group is an exception and will be discussed later. Can all of these investigators and clinical oncologists have misread the literature and/or their own experience? That probably depends on the goal of the study. Presurgical chemotherapy may well increase the chance of a patient undergoing limb salvage or make the task of limb salvage easier for the surgeon. The literature here is not clear, and this issue is beyond the scope of this discussion. Presurgical chemotherapy allows the pathologist to evaluate the percent necrosis of the primary tumor. This is an important prognostic factor and follows for a change in therapeutic strategy.

Can some insight be gained from this volume by comparing the slightly older adjuvant trials with the more recent trials that include presurgical therapy? Possibility, but this approach is always risky. Such tabulations, however, can be useful and are used by a number of authors in this volume [1,2]. If we limit this metaanalysis to those trials in which at least 50 patients were evaluated, the range of disease-free survival for patients receiving adjuvant therapy is 46–61% [4–8]. The follow-up on these studies is, in general, at least 5 years, and thus the percentages are not likely to fall.

Using the same limit of at least 50 patients entered into a study, the range of disease-free survival for patients receiving presurgical therapy is 49–88% [4,5,6,9–11]. The figure of 88% is from the Italian group [5] and the 82 patients entered into this trial of presurgical therapy have a minimum follow-up of only 2 years. Their experience with late relapses after 2 years is 9%, and thus they anticipate a cure rate of at least 75%. Another high percentage of 80% is projected by the Mayo group for presurgical therapy [10].

In Meyer's overview of the experience at Memorial Sloan-Kettering [6],

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 ISBN 0-7923-1905-2

there is no significant difference ($p = 0.36$, see Figure 51-1) in disease-free survival for the 55 patients who received no preoperative chemotherapy and the 200 patients who received preoperative therapy. This is an important finding. While not all patients were handled in exactly the same manner and this was not the result of a randomized study, it does represent the experience at a single institution. Two other studies also report a high percentage of disease-free survival for presurgical therapy, but the number of patients under study is small [12,13]. Thus, on the one hand, it can be stated that there is some overlap in the disease-free survival of patients receiving adjuvant therapy and those receiving presurgical therapy as well as adjuvant therapy. On the other hand, it can be stated that the best disease-free survival reported to date for adjuvant therapy is less than that reported for presurgical therapy.

This issue of presurgical therapy vs. adjuvant therapy should be resolved in the future by the current ongoing study of the Pediatric Oncology Group (POG #8651). Patients are randomized at diagnosis to receive either presurgical chemotherapy, definitive surgery at 10 weeks and then adjuvant chemotherapy, or immediate surgery followed by adjuvant chemotherapy. The definitive surgery in either case may be amputation or limb salvage. In either case, the patients receive the same chemotherapy over a 42-week period of time. The same number of courses, the same sequence of agents, and the same total doses of chemotherapeutic agents are given to all patients. (This study should also allow these investigators to determine if preoperative chemotherapy does result in a greater portion of patients undergoing limb-salvage procedures.) This study was opened in 1986 and will probably be closed in 1992. Thus a reasonable statement or projection of results should be available in 1994.

This trial of the Pediatric Oncology Group appears to be a very fair comparison of presurgical therapy to adjuvant therapy in nonmetastatic osteosarcoma of the extremities. This seems to be the only way to clearly answer the issue at hand. Certainly the data available in 1986 seemed to justify the initiation of the trial. It must, however, be remembered that almost all members of the Pediatric Oncology Group participated in the MIOS trial that evaluated adjuvant chemotherapy vs. no therapy until there was evidence of disease. That trial was subjected to an interesting but legitimate critique by Holland, who felt that the trial was not necessary [14]. He felt that the answer was already in the literature. Several months later Bertino came to our rescue by stating that he felt that the trial was justified in order to lay to rest the question of the change in the history of osteosarcoma [14]. Will history repeat itself?

Conclusions

From a theoretical point of view [15] and from a review of the contents of this volume, it must be concluded that the value of presurgical chemotherapy in the treatment of osteosarcoma is an open question.

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