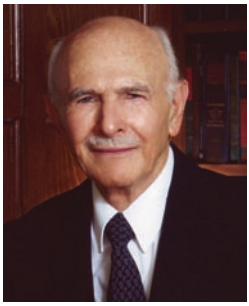

Osteosarcoma: Review of the Past, Impact on the Future. The American Experience

Norman Jaffe

Abstract Major advances have been achieved in the treatment of osteosarcoma with the discovery of several chemotherapeutic agents that were active in the disease. These agents comprise high-dose methotrexate with leucovorin rescue, Adriamycin, cisplatin, ifosfamide and cyclophosphamide. The agents were integrated into various regimens and administered in an effort to destroy silent pulmonary micrometastases which are considered to be present in at least 80% of patients at the time of diagnosis. Their efficacy in achieving this goal was realized and their use was further extended to the application of preoperative (neoadjuvant) chemotherapy to destroy the primary tumor and achieve safe surgical resections. Disease free survival was escalated from <20% prior to the introduction of effective chemotherapy to 55–75% and overall survival to 85%. Further, the opportunity to perform limb salvage was expanded to 80% of patients. Of interest also was an attempt in one series to treat the primary tumor exclusively with chemotherapy, and abrogation of surgery.

Adding to these advances, varieties of subsequently discovered agents are currently undergoing investigations in patients who have relapsed and/or failed conventional therapy. The agents include Gemcitabine, Docetaxel, novel antifolate compounds, and a liposome formulation of adriamycin (Doxil). A biological agent, muramyl tripeptide phosphatidyl ethanolamine (MTPPE) was also recently investigated in a 2×2 factorial design to determine its efficacy in combination with chemotherapy (methotrexate, cisplatin, Adriamycin and ifosfamide).

In circumstances where the tumor was considered inoperable, chemotherapy and radiotherapy were advocated for local control. High dose methotrexate, Adriamycin and cisplatin and Gemcitabine interact with radiation therapy and potentiate its therapeutic effect. This combination is also particularly useful in palliation. Occasionally, the combination of radiation and chemotherapy may render a tumor

N. Jaffe (✉)
Children's Cancer Hospital, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit #87, Houston, TX, 77030-4009, USA
e-mail: njaffe@mdanderson.org

N. Jaffe et al. (eds.), *Pediatric and Adolescent Osteosarcoma*,
Cancer Treatment and Research 152,
DOI 10.1007/978-1-4419-0284-9_12, © Springer Science+Business Media, LLC 2009

suitable for surgical ablation. Samarium,[153] a radio active agent, is also used as palliative therapy for bone metastases.

However, despite the advances achieved with the multidisciplinary application of chemotherapy, radiotherapy and surgical ablation of the primary tumor over the past 3½ decades, the improved cure rate reported initially has not altered. Particularly vexing is the problem of rescuing patients who develop pulmonary metastases after receiving seemingly effective multidisciplinary treatment. Approximately 15–25% of such patients only are rendered free of disease with the reintroduction of chemotherapy and resection of metastases. Extrapulmonary metastases and multifocal osteosarcoma also constitute a major problem. The arsenal of available agents to treat such patients has not made any substantial impact in improving their survival. New chemotherapeutic agents are urgently required to improve treatment and outcome. Additional strategies to be considered are targeted tumor therapy, anti tumor angiogenesis, biotherapy and therapy based upon molecular profiles.

This communication outlines sequential discoveries in the chemotherapeutic research of osteosarcoma in the United States of America. It also describes the principles regulating the therapeutic application of the regimens and considers the impact of their results on the conduct in the design of future investigations and treatment

Introduction

During the past half century, therapeutic research has identified several chemotherapeutic agents that are effective in the treatment of osteosarcoma. These agents were incorporated into a number of therapeutic regimens. With their application in innovative multimodal strategies, cure rates were escalated from <20% prior to the 1907s to current levels of 65–75%. Accompanying this escalation has been the ability to offer limb salvage to approximately 80% of patients.

The principal agents currently in use comprise high-dose methotrexate with leucovorin rescue, doxorubicin (Adriamycin), cisplatin, cyclophosphamide, and ifosfamide. Earlier investigations with nitrogen mustard, mitomycin C, and vincristine had yielded minimal response, and these agents were abandoned.[1]

Co*n*padri/Co*m*padri Series

In the early 1960s, Sutow and coworkers[2] demonstrated anti-tumor activity in osteosarcoma with L-phenylalanine mustard. Temporary regression in 10–43% of patients was achieved.[2] This result prompted an investigation of L-phenylalanine mustard as adjuvant chemotherapy for patients with nonmetastatic osteosarcoma after ablation of the primary tumor, and a disease-free survival rate of 14% was

achieved.[3] In 1969, the combination of vincristine, dactinomycin (actinomycin D), and cyclophosphamide (VAC) was demonstrated to be effective in treating rhabdomyosarcoma.[4] The success achieved in rhabdomyosarcoma prompted Sutow et al[5] to investigate the efficacy of the regimen as adjuvant treatment for osteosarcoma after ablation of the primary tumor. During this same period, osteosarcoma was shown to be responsive to cyclophosphamide (as discussed below).[6] To potentiate the efficacy of the VAC regimen, Sutow administered cyclophosphamide in an intensive intermittent pulse schedule based on studies reported by Finklestein et al.[7] This regimen designated "pulse VAC," was administered to 12 patients and resulted in a 33% disease-free survival rate in all of them.[8]

With the demonstration that doxorubicin was effective in the treatment of osteosarcoma, [9] Sutow elected to substitute doxorubicin for dactinomycin in the pulse VAC regimen and to augment its efficacy with the addition of L-phenylalanine mustard. This regimen [pulsed cyclophosphamide, vincristine (Oncovin), L-phenylalanine mustard, and doxorubicin (Adriamycin)] was designated "Co*n*padri" or Co*n*padri I.[10] It yielded a 55% disease-free survival rate. Sequential changes in the composition and acronym of the Conpadri regimen followed. Methotrexate was incorporated and it was designated "Co*m*padri," commencing with Compadri II. Each successive number indicated an evolution in the regimen.[11] Sutow also observed that pulmonary metastases were appearing later in patients treated with the Compadri regimen.[12] This change in the pattern of development of pulmonary metastases inaugurated new concepts in the treatment of patients with metastatic osteosarcoma. The conceptual development and the evolution of programs designed for this purpose were outlined in two publications.[13,14] Prior to 1970, the survival rate for patients with metastases was considerably less than 2%. After 1970, according to Sutow, it improved to approximately 40%.[13] The best postmetastatic survival rates occurred in patients whose metastatic lesions developed at least 13 months after initial treatment; the worst rates occurred when metastases were present at diagnosis.[11] Sutow's observation was confirmed by Jaffe et al,[15] who noted an alteration in the pattern of relapse in several patients treated with adjuvant chemotherapy: metastases in these patients appeared later than in untreated or inadequately treated patients. They also tended to be single or isolated.[15] This development permitted successful multidisciplinary intervention in an increasing number of patients.

The Compadri II and III regimens yielded disappointing results. It was surmised that their lack of efficacy was due to reduced doses of doxorubicin, and the approach was adjusted in Compadri IV and Compadri V: high-dose methotrexate and doxorubicin were intensified, and aggressive "front loading" was adopted. Unfortunately, Wataru Sutow's untimely death precluded evaluation of the last two Compadri studies. However, before he died, an updated review of the Compadri I, II and III regimens was published: 81 of 200 patients (41%) were alive without evidence of disease, 18 months and longer after diagnosis.[16]

The Compadri regimens represented the first rational attempt to promote the use of combination chemotherapy as adjuvant therapy in osteosarcoma. They comprised different agents with different modes of action and minimal overlapping toxicity. Compadri was later superseded by other chemotherapeutic regimens.

High-Dose Methotrexate with Leucovorin Rescue

The use of methotrexate against osteosarcoma was initiated in the 1970s (see Chap. 11). Methotrexate binds stoichiometrically and irreversibly to dihydrofolate reductase, thereby inhibiting the formation of tetrahydrofolate from dihydrofolate. This inhibition interferes with the de novo biosynthesis of purine and pyrimidine. Ultimately, thymidylate biosynthesis is inhibited; this is the key event leading to cell death. The antidote to methotrexate is leucovorin (5-formyl tetrahydrofolate). Within the cell, leucovorin is converted to 5,10 methylene tetrahydrofolate and 5-methyl tetrahydrofolate, thereby replenishing the product surceased by methotrexate.

The methotrexate-leucovorin "rescue" regimen (MTX-L) took wing following publications by Jaffe et al.[17–20] Methotrexate is usually administered intravenously in doses of 10–12.5 G/m^2 over 4–6 h, with leucovorin "rescue" commencing 24 h after the initiation of the methotrexate infusion. When deployed as single-agent therapy for "intensification" or "consolidation," MTX-L should optimally comprise 4–8 doses administered at 10–14-day intervals. When combined with other agents, the interval between MTX-L and the other agents (generally doxorubicin, which may be administered 8–10 days after MTX-L) is usually extended to 21–28 days before initiating the next MTX-L dose.

MTX-L administered postoperatively as the sole agent to patients with osteosarcoma after ablation of the primary tumor, yielded a 40% disease-free survival.[18] When MTX-L was combined with other agents as pre- and post-operative therapy for osteosarcoma, a disease-free survival rate of 65–75% was achieved.[17–27]

The Children's Cancer Study Group considered high-dose MTX-L and intermediate-dose MTX-L in combination with doxorubicin as adjuvant therapy for nonmetastatic osteosarcoma; no benefit was observed for patients who received MTX-L. The overall outcome in these patients was not superior to that in patients who received doxorubicin alone.[28] Two Osteosarcoma studies, one in Europe and the other in the United Kingdom, found an inferior disease-free survival rate in patients who received a three-drug regimen: doxorubicin, cisplatin and MTX-L as compared with the rate in patients who received the regimen without MTX-L.[29,30] Several factors could possibly account for the inferior results achieved with high- or intermediate-dose MTX-L in these studies. The factors include inadequate tumoricidal concentrations due to substandard doses (vide the Children's Cancer Study Group investigation), and dilution of methotrexate because of the excessive hydration that was designed to eliminate the drug. Other factors, including age and pharmacokinetics, also influence serum methotrexate levels and tumor response.[21,31–33]

It has been suggested that methotrexate levels of 700–1,000 μmol/L at 4–6 h (generally 1,000 μmol/L) after initiation of the infusion are required for optimum results.[31,32] However, for unexplained reasons, inter- and intra-patient variability in methotrexate concentration is often encountered, despite administration of a standard dose, and optimum levels are not constantly obtained. In the author's experience, a methotrexate concentration of 1,500 μmol/L or higher at 4–6 h is desirable. This concentration is more likely to be obtained by limiting pre- and intra-therapeutic intravenous (alkaline) hydration to 3 L/m^2/24 h.

Efforts to increase the therapeutic effect of methotrexate by enhancing the local concentration at the tumor site using the standard dose with intra-arterial, rather than intravenous, administration were unsuccessful.[34] Apparently, response requires a critical dose, and any dose escalation above this level will not enhance therapeutic efficacy. Similarly, in pharmacokinetic and clinical studies of a 24-h infusion of high-dose methotrexate with leucovorin administered after completion of the infusion, the efficacy of the drug did not improve over that of a shorter 4–6-h infusion.[35] These observations indicate that the optimum therapeutic tumoricidal concentration is achieved with the intravenous dosages described above.

With the optimum dosage administered over 4–6 h and the appropriate hydration, the following peak methotrexate levels may be anticipated at specific time intervals after initiation of the infusion (Fig. 1):

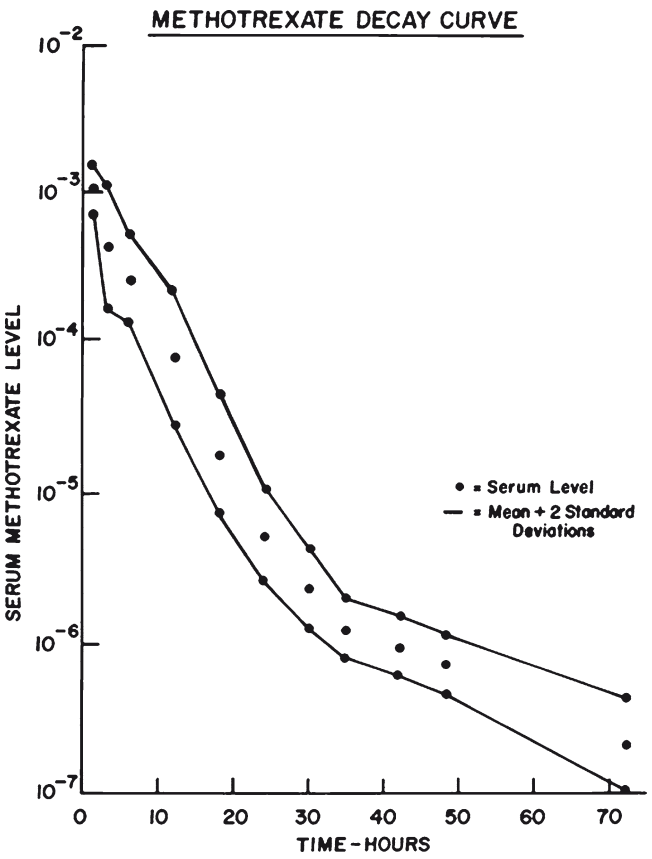

Fig. 1 Methotrexate decay curve following a 6-h infusion at 7.5 G/m^2 dissolved in 600 cc of 5% dextrose water administered over 6 h. Reproduced with permission from Advances in Chemotherapy. Jaffe N. Antifolate rescue use of high-dose methotrexate and citrovorum factor. In: Rossowsky A, ed. *Advances in Chemotherapy*. New York and Basel: Marcel Decker Inc.; 1979:111-141

- 24 h 30–300 μmol/L
- 48 h 3–30 μmol/L
- 72 h <0.3 μmol/L

Values in excess of these concentrations, particularly at the 48-h level, portend potential toxicity.[35–38]

Leucovorin is administered according to specially designed algorithms that are available in most institutions. The algorithm generally advocates an intravenous dose of 10 mg after completion of the methotrexate infusion and a similar dose every 6 h until the methotrexate level is ≤0.1 μmol/L. This usually occurs at 72 h and requires 12 doses. However, it may be necessary to prolong the leucovorin administration if the ≤0.1 μmol/L methotrexate level is not attained at 72 h. In some institutions, a methotrexate level of ≤0.3 μmol/L may be an acceptable endpoint.

Prerequisites for MTX-L therapy include normal renal and hepatic function, a normal hemogram, and absence of infection. The prerequisites are usually determined by obtaining a corrected creatinine clearance rate, a serum electrolyte study, urinalysis, liver function studies, and a complete blood count prior to each course of therapy. Collections of fluid (pleural, pericardial and peritoneal effusions) may cause a delay in methotrexate excretion by sequestering methotrexate into the fluid collection and are contraindicated in MTX-L treatment.

Toxic reactions are infrequent. They are generally induced by incomplete (delayed) renal clearance and are usually associated with methotrexate precipitation in the renal tubules. This reaction manifests with gastrointestinal mucosal ulceration, myelosuppression, and hepatorenal failure. Measures for aborting or treating toxic reactions may comprise any or all (usually the latter) of the following:

1. Increasing fluid intake to 4 L/m^2/24 h.
2. Increasing leucovorin dose to 50–100 mg (or higher) every 6 h, as stipulated by the institution's algorithm.
3. Administering carboxypeptidase G-2 if the serum 24- or 48-h methotrexate level is extremely high and/or anuria or oliguria appears to be developing.
4. Considering high-flux renal dialysis at any time in the above circumstances.

In addition to its efficacy as a pre- and post-operative agent, methotrexate potentiates the tumoricidal effects of radiation therapy.[39–42] The effects are limited to the portals of radiation and include dermatologic reactions. Radiation effects are more likely to occur if the methotrexate administration coincides with radiation or is juxtaposed with the immediate postradiation period. With longer intervals between radiation and methotrexate, response and skin reactions are less observed. The combination of radiation therapy and methotrexate may be used for treatment of resistant pulmonary metastases and inoperable primary tumors. This combination is also extremely useful in alleviating cord compression and relieving bone pain.

Doxorubicin

Doxorubicin came into use for treatment of osteosarcoma in the early 1970s. The agent intercalates into DNA and induces topoisomearase II-mediated single- and double–strand breaks in the DNA. Initial studies in which doxorubicin was administered intravenously, alone or in combination with dacarbazine [dimethyldiethyl triazeno imidazole carboxamide, (DTIC)] produced responses in 35–40% of patients with pulmonary metastases.[43–45] Responses included complete disappearance of lung lesions and a 40% reduction in tumor volume. The onset of the responses occurred within 1–2 months with doses of 30–35 mg/m^2 administered daily for 3 days, at 3–4-week intervals. When administered as the sole agent after ablation of the primary tumor, doxorubicin also improved survival rates in patients with osteosarcoma.[8,43–47]

Doxorubicin can also potentiate the therapeutic effects of radiation therapy.[48] In one study, doxorubicin was administered intra-arterially over 24 h in combination with radiation (3.5 Gy) to treat the primary tumor. More than 75% tumor destruction was reported in 24 of 36 patients.[49] However, the procedure was complicated by erythema and ulceration of the skin and subcutaneous tissue in several patients. Selective entry of the drug into a small vessel was implicated, and it was suggested that the complication could possibly be prevented by positioning the catheter in a large-caliber vessel proximal to the tumor. Ulceration precludes limb-salvage procedures, and consequently intra-arterial doxorubicin is generally not advocated as local treatment for potential limb-salvage candidates.[50]

Doxorubicin may induce cardiac failure. To prevent this complication, the cumulative dose is generally limited to 300 mg/m^2 in children under 6 years of age and to 450–500 mg/m^2 in adolescents. However, based on experiences with adult patients with breast cancer, the cumulative dose may possibly be extended to 600 mg/m^2 (or more) with liposomal formulations of the drug (e.g., Doxil[51]). Dexrazoxane has also been administered in combination with doxorubicin to prevent cardiac failure.[52] The potential salubrious effect of the agent in preventing cardiac complications in long-term survival remains to be determined.

Doxorubicin has been claimed to be the most effective agent for the treatment of osteosarcoma.[53] It is incorporated in most combination chemotherapy regimens used for this disease. A cardiac assessment comprising an electrocardiogram and echocardiogram should optimally be obtained prior to the administration of each course. Cardiac assessments should also be obtained at regular intervals in long term survivors.

Cisplatin

Cisplatin (*cis*-diamminedichloroplatinum II) was first used for treatment of osteosarcoma in the 1970s. It exerts its cytotoxic action by platination of DNA. It may be administered intravenously or intra-arterially. In a series of studies in which

cisplatin was administered intravenously, it produced responses of 30–50%.[54–56] The responses were obtained in patients with unresectable or metastatic disease who received the agent alone or in combination with doxorubicin. In contrast, in studies in which cisplatin was administered intra-arterially as the sole agent for treatment of the primary tumor, response rates were 60–90%.[57,58] The intra-arterial route achieves higher local cytotoxic concentrations which improves penetration across the cell membrane.[57] This strategy was investigated principally at The University of Texas M.D. Anderson Cancer Center.[58–62] The procedure, which involved general anesthesia or conscious sedation of the patient, required placement of an arterial catheter via the Seldinger technique through the brachial or femoral artery (Fig. 2) under fluoroscopic guidance. Concurrently, a systemic intravenous infusion was initiated to provide hydration at 3 L/m^2/24 h and Manitol. The tip of the arterial catheter was positioned into a vessel that supplied the neoplasm. A pulsatile infusion pump was used to induce turbulence of the cisplatin with saline[59]; this turbulence prevented laminar flow and reduced the possibility of a "platinum burn" because of selective entry of high platinum concentrations into small vessels. Occasionally, tumor embolization may be performed to improve the direction and concentration of chemotherapy to the tumor, if excessive neovascularity is present.

In the initial studies, the dosage was 150 mg/m^2 administered with Mannitol over 2 h at 3-week intervals.[57,58] Four preoperative courses were administered. In more recent studies, 120 mg/m^2 cisplatin is administered intra-arterially over 4 h, and concurrently 95 mg/m^2 doxorubicin is administered over 24 h. In a schedule similar to the sole treatment with intra-arterial cisplatin, four preoperative courses are administered at 4-week intervals.

Pharmacokinetic studies were conducted as part of the initial study of 150 mg/m^2 cisplatin administered intra-arterially. Evaluation of the local venous effluent and concurrent systemic venous concentrations demonstrated consistently higher cisplatin concentrations in the local vein than in the peripheral vein[57] (Fig. 3, *left* and *right* respectively). The highest single concentrations in the local vein were 10 and 9 μg/mL at 60 and 90 min, respectively, as opposed to the highest concentrations in the peripheral vein, which were 1.7 and 3.9 μg/mL at 30 and 120 min, respectively. From 90 min, the local venous concentrations plotted on a log scale were linear by curve fitting. The concentration in the systemic circulation was sufficiently tumoricidal to destroy pulmonary metastases. Figure 4 demonstrates complete disappearance of pulmonary metastases after two courses of 150 mg/m^2 intra-arterial cisplatin administered for a primary tumor in the distal femur. These pharmacokinetic and clinical studies contradict the claim that the systemic concentration following intra-arterial administration is insufficient to destroy pulmonary metastases.[63] Systemic concentrations in these studies were also sufficient to cause adverse side effects, including auditory and renal dysfunction.[64,65]

The efficacy of intra-arterial therapy may be demonstrated by angiographic study with the disappearance of tumor neovascularity and staining (Fig. 5). It is also capable of inducing a complete response in patients with pathological fractures (Fig. 6).

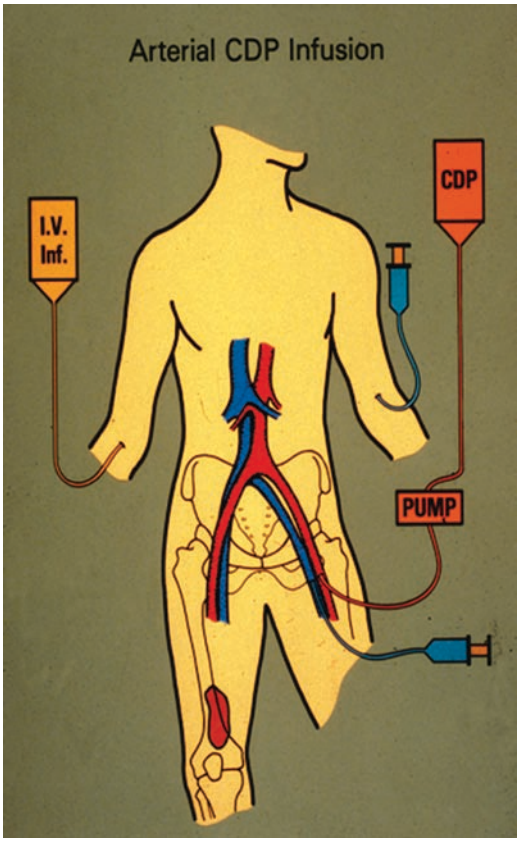

Fig. 2 Arterial catheter containing cisplatin (CDP) attached to pulsatile infusion pump (PUMP) inserted into left femoral artery and directed to the tumor in the contralateral limb via the bifurcation of the aorta. Systemic hydration and Mannitol are provided through a venous catheter (I.V.Inf) in the right antecubital fossa. The diagram also depicts sites of venous catheters inserted to determine cisplatin concentrations in the local tumor draining vein and systemic circulation. I.V. Inf = Intravenous in fusion; CDP = Cisplatin; PUMP = Pusatile Infusion Pump

Tissue determinations of cisplatin levels were obtained in the tumor and the surrounding tissues in some patients (Fig. 7). These revealed that concentrations of 17–40 μg/g were associated with tumor destruction of 60–100%

Figure 8 demonstrates 100% tumor destruction after four courses of intra-arterial cisplatin. This result contrasted sharply with those for concentrations of 12 μg/g or less, which were associated with tumor destruction of less than 60%. The difference in the mean cisplatin tumor concentrations between the groups with greater than 60% tumor destruction and those with less than 60% destruction was 16.7 μg/g. Using the one-tailed *t*-test, this difference was significant at a level of <0.025

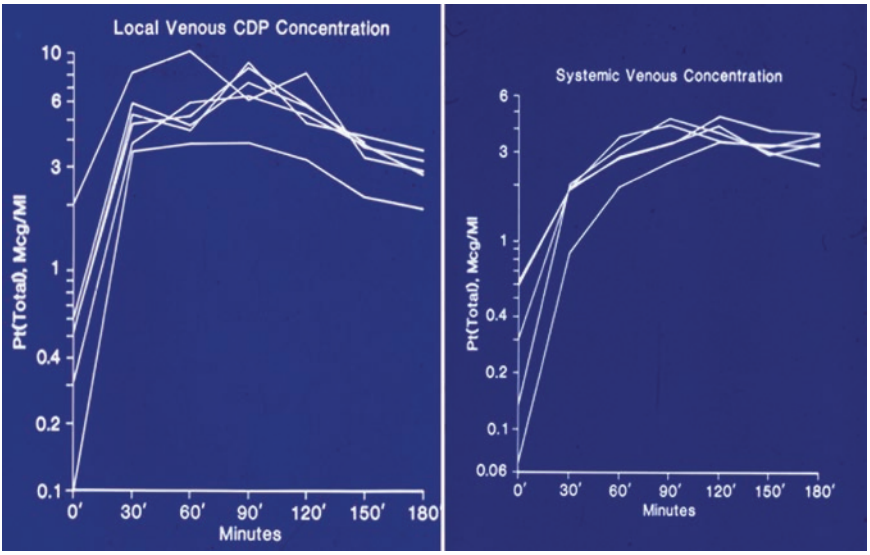

Fig. 3 *Left*: Local venous cisplatin concentrations (tumor draining vein). *Right*: Systemic venous cisplatin concentrations. *CDP* cisplatin; *Pt* total platinum (Mcg/ml)

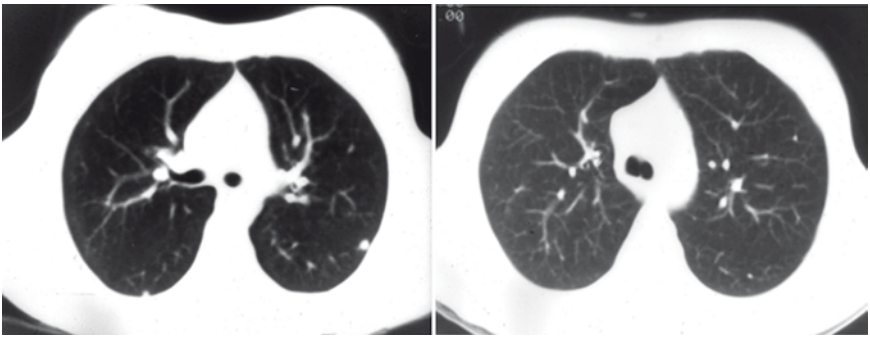

Fig. 4 Computer scan of lungs demonstrating pulmonary metastases (*left*) and disappearance of pulmonary metastases (*right*) following two courses of intra-arterial cisplatin 150 mg/m^2

Cisplatin uptake also varied with tumor subtype in this study. Smaller concentrations were detected in patients with telangiectactic osteosarcoma and malignant fibrocystic histiocytoma, as opposed to chondroblastic osteosarcoma. In patients with malignant fibrocystic histiocytoma, 60% tumor destruction was noted with a cisplatin concentration of 2.4 μg/g.[57] Cisplatin tumor concentration and tumor destruction were also related to the number of infusions: the percentage of tumor destruction was greater with three or more infusions than with two infusions.[58]

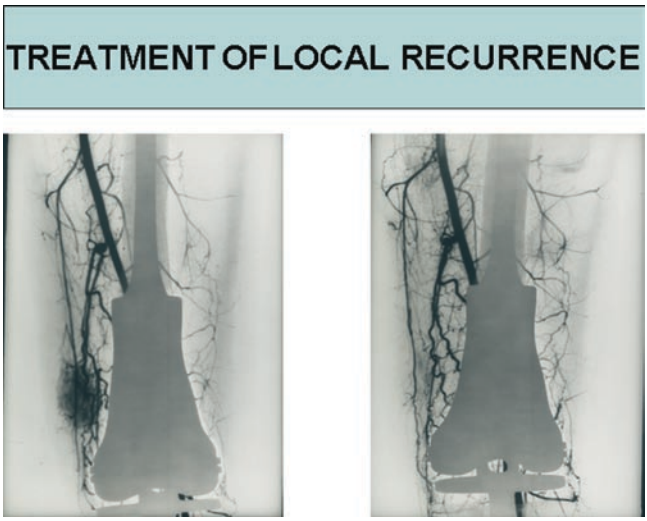

Fig. 5 Arteriogram demonstrating recurrent tumor in the distal end of the femur manifesting with neovascularity and stain (*left*). Complete disappearance of tumor neovascularity was obtained with after four courses of intra-arterial cisplatin, 150 mg/m^2/course (*right*). Pathological examination of resected tissues demonstrated absent tumor or complete tumor necrosis in sites where minimal residual tumor was suspected to be present

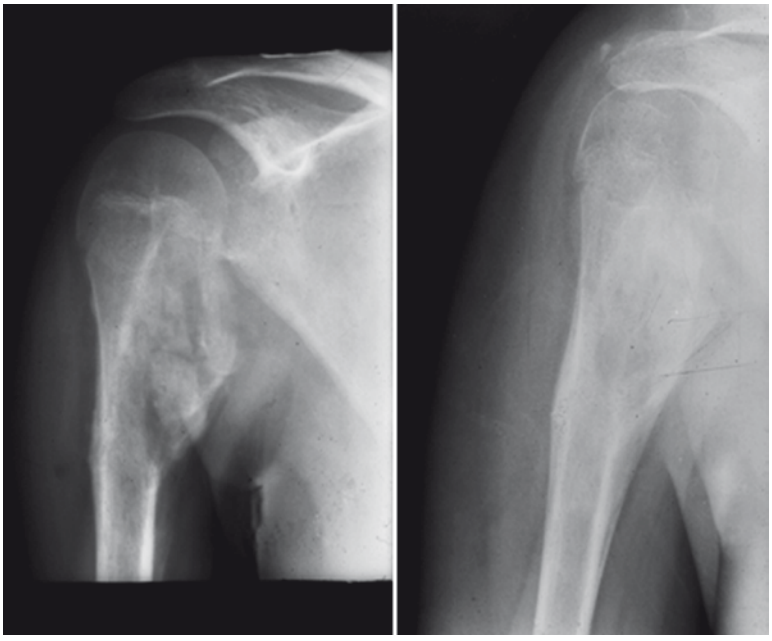

Fig. 6 Pathological fracture of the humerus at diagnosis (*left*) and complete healing after four courses of intra-arterial cisplatin, 150 mg/m^2/course (*right*)

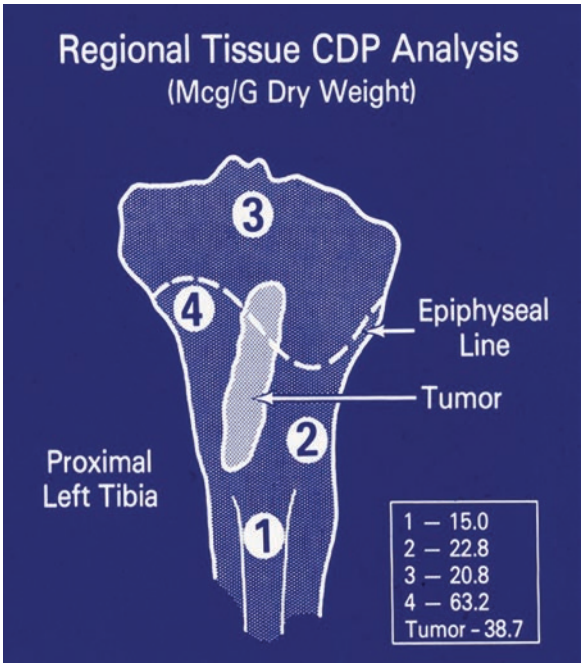

Fig. 7 Tissue cisplatin determinations in the proximal tibia following four course of intra-arterial cisplatin, 150 mg/m^2/course

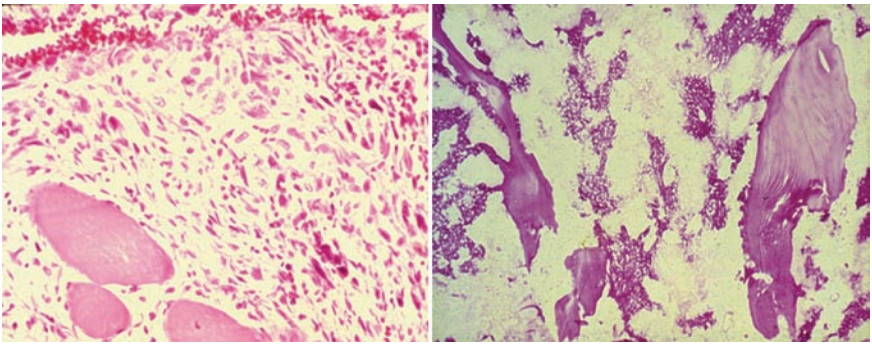

Fig. 8 Photomicrograph of tumor at diagnosis (*left*) and specimen obtained after treatment with four courses of intra-arterial cisplatin, 150 mg/m^2/course (*right*). Complete necrosis was induced. There is a complete absence of tumor cells in the specimen comprising residual bone trabeculae

Patients whose tumors initially respond and later relapse may experience a response again with reinstatement of cisplatin at the same dose (Fig. 5 shows an example). Intra-arterial therapy is extremely useful when an immediate response is desired, particularly in the treatment of pathological fractures[72] (Fig. 6) or with

the threat of tumor invasion and potential imminent compromise to the neurovascular bundle.

A review of the results of treatment with intra arterial cisplatin in several publications revealed an average sensitivity of 95% and a specificity of 87%.[66–71]

The tumoricidal effects achieved with the first or second course of intra-arterial cisplatin may also be achieved with conventional intravenous therapy administered over a more prolonged period (several courses). In contrast, intra-arterial cisplatin is capable of producing a rapid definitive attack on the primary tumor. The rapidity and immediacy of response with intravenous cisplatin are not as impressive as that achieved with the first or second course of intra-arterial cisplatin. In addition, the efficacy of intra-arterial treatment may also be assessed reasonably early on the arteriogram by observing the reduction of tumor neovascularity and staining after the first or second course.

Oxazaphosphorines

The oxazaphosphorines, cyclophosphamide and ifosfamide, are alkylating agents that require hepatic microsomes for activation. They possess moderate to high efficacy in the treatment of osteosarcoma.[5,6,73–75] Cyclophosphamide was probably the first agent discovered to have activity in osteosarcoma[5] (Fig. 9). In 1962, Pinkel[6] stated that he knew of no reports concerning responses of "osteogenic sarcoma to other alkylating agents" at that time.

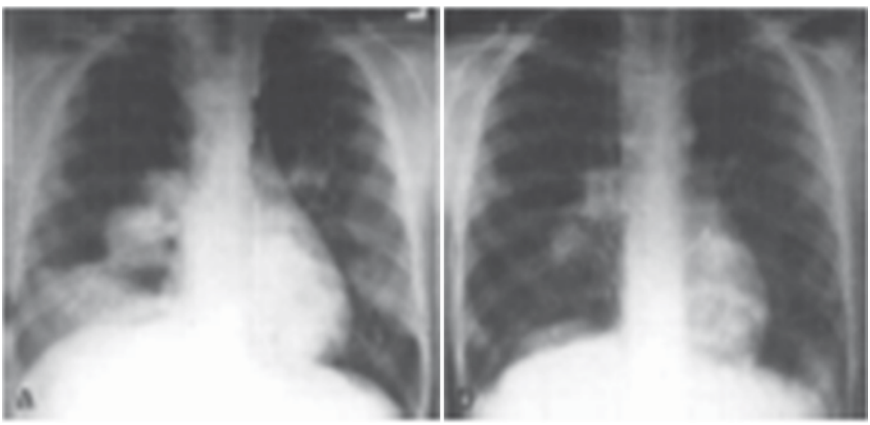

Fig. 9 Chest radiograph demonstrating pulmonary metastases from osteosarcoma (*left*) and partial response to oral cyclophosphamide (*right*). The latter manifested with disappearance and reduction of tumor masses. This material is reproduced with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. (Pinkel[6]: Figs. 3a,b)

Cyclophosphamide may be administered in combination with etoposide (VP-16), which is thought to augment the efficacy of the alkylating agent through a synergistic interaction. An early study of this combination yielded a 58% response rate in various malignant diseases, including osteosarcoma.[76] A follow-up of the study concluded that the combination of cyclophosphamide and etoposide was effective therapy for both primary and metastatic osteosarcoma.[77] Eighty-eight percent of the patients experienced complete or partial responses. Because of its putative synergistic effect, etoposide is also frequently combined with ifosfamide.

Cyclophosphamide and ifosfamide produce a metabolite, acroline, that causes hemorrhagic cystitis. This complication can be prevented with the administration of Mesna. The latter absorbs the acroline, providing uroprotection. This strategy permits the administration of high doses of cyclophosphamide and ifosfamide. The intake of liberal amounts of fluid is another means of preventing hemorrhagic cystitis.

The activity of the alkylating agents, particularly ifosfamide, can be augmented by fractionating the dose. The efficacy can also be enhanced by dose escalation. Investigators have reported initial responses of 10–40% with doses of 6–9 g/m^2 [78–80]; escalating the dose to 12 or 14 g/m^2 yielded enhanced responses of 60%.[81–84] These results were noted in patients who had had relapses or in whom conventional therapy had failed. This experience, however, was not observed by Harris et al,[85] who noted a complete and partial response rate of only 30% with a dose of 12 g/m^2. The alkylating agents also are not cross-resistant: in patients who have relapses after treatment with a specific alkylating agent, responses may again be achieved by substituting an alternative alkylating agent (e.g., ifosfamide for cyclophosphamide or vice versa).

Goorin et al[86] treated patients with newly diagnosed osteosarcoma in a "therapeutic window" at 3–4-week intervals and achieved a 59% response rate with a combination of ifosfamide (3.5 g/m^2/day for 5 days, for a total of 17.5 g/m^2) and etoposide (100 mg/m^2/day for 5 days). This experience was duplicated by investigators at M. D. Anderson Cancer Center. However, in contrast to the patients treated by Goorin et al, patients at M.D. Anderson had been heavily pretreated with high-dose methotrexate, doxorubicin, cisplatin, and ifosfamide (9 g/m^2). Etoposide was usually omitted. The response of one such patient with pulmonary metastases who was treated with ifosfamide only is illustrated in Fig. 10. The total dose of ifosfamide, 17.5 g/m^2, was associated with moderate myelosuppression and mild renal dysfunction. In addition, two other patients developed moderate renal failure following the fifth course of 17.5 g/m^2 ifosfamide. Thus, the use of high-dose ifosfamide (17.5 g/m^2) should probably be limited to four courses. If there is evidence of renal dysfunction, cyclophosphamide, which is unlikely to affect the kidneys, may be substituted for ifosfamide at an equivalent dose. This is determined by dividing the ifosfamide dose (17.5 g/m^2) by 3.5. The resulting dose of cyclophosphamide (3–4 g/m^2) may be administered over two consecutive days (i.e., 1.5–2 g/m^2/day) at 3–4-week intervals with Mesna.

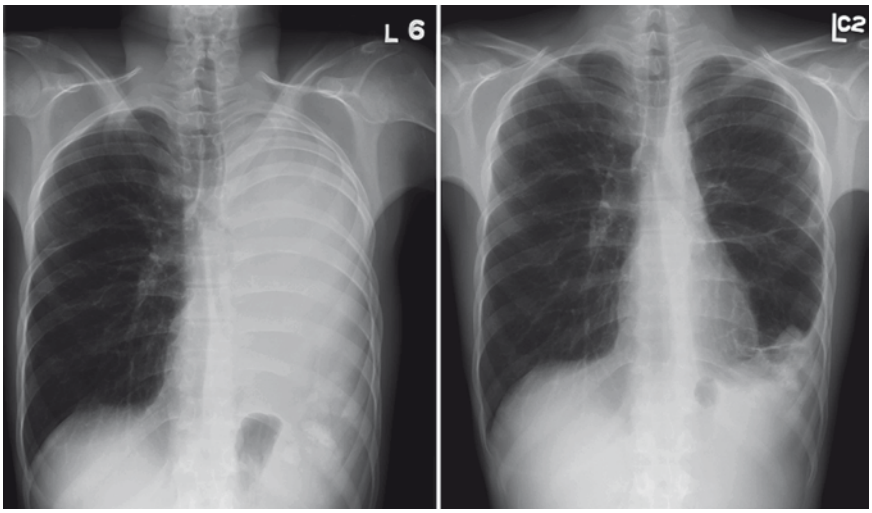

Fig. 10 Chest radiograph of patient who responded to four courses of Ifosafamide 17.5 G/m^2/course administered over 5 days (3.5 G/m^2/dx5). The patient had previously been treated with, and responded to, Ifosfamide 14 G/m^2 and later relapsed

Less Frequently Employed Chemotherapeutic Agents

Carboplatin, has been used in several combination regimens.[87–90] In a study of patients with metastatic lung lesions, a regimen containing 560 mg/m^2/day carboplatin was not as effective as 100 mg/m^2/day cisplatin.[88] Petrilli et al[90] investigated intra-arterial carboplatin (Study III) in a series of patients. They were also treated with epirubicin, ifosfamide and MTX-L. In contrast, in Study IV, intravenous carboplatin in conjunction with cisplatin, doxorubicin and Ifosfamide was employed. The overall survival rate for Study III and Study IV in patients who had nonmetastatic osteosarcoma at the time of their original diagnosis, was 60.5%, and the event-free survival rate, 45.5% at 5 years. Since other agents in addition to carboplatin were employed, the contribution of the latter to the final result cannot be assessed.

Novel antifolate agents, including trimetrexate, have been investigated in patients who have had relapses. Although these agents produced isolated responses, they have not been evaluated in formal clinical trials. In addition, gemcitabine, which has been reported to produce responses in osteosarcoma, awaits further investigation.[91,92]

High-dose radioactive samarium^{153} [^{153}Sm-EDTMP (Quadramet)] has been used to treat bone metastases and has afforded patients appreciable relief with regard to symptoms.[93] However, this treatment is usually complicated by severe myelosuppression and may require peripheral blood or stem-cell support.

A 2×2 factorial design study using two chemotherapy regimens, one standard and one experimental, was employed in conjunction with liposomal muramyl tripeptide phosphatidyl ethanolamine (L-MTPPE).[94] The latter induces infiltration of inflammatory macrophages into lung metastases. The study was designed to evaluate the activity of L-MTPPE in osteosarcoma. As such, each standard and experimental arm included or did not include L-MTPPE. All patients received identical cumulative doses of cisplatin, doxorubicin, and MTX-L. The results published in the initial report found no statistically significant advantage for L-MTPPE in disease-free survival, the primary endpoint, although the trend favored the combination of ifosfamide and L-MTPPE. Overall survival, which was not prespecified in the protocol, showed a 76% six year survival rate for patients who received L-MTPPE with ifosfamide, compared with a rate of 66% for patients who did not receive the combination ($p=0.183$).

The U.S.Food and Drug Administration[95] concluded that the L-MTPPE single study did not provide substantial evidence of effectiveness: the results for the primary endpoint did not reach statistical significance, and the overall survival analysis was not part of the study plan. The report stated: "Follow-up data have not been rigorously collected and are incomplete with insufficient follow-up for a significant proportion of patients."[95] L-MTPPE was not sanctioned for general clinical distribution.

In a follow-up report of the above study the authors confirmed a trend for improved event free survival ($p=0.08$) and improved overall survival ($p=0.03$) for the MTPPE arm.[96] These results were discussed in several letters to the Editor of the Journal of Clinical Oncology.[97–99] It was suggested that additional investigation be performed to substantiate the utility of MTPPE and define its exact role in the treatment of osteosarcoma. The agent is available through a Compassionate Investigational New Drug (CIND) application and in certain investigational trials.

Inhalation therapy with granulocyte-macrophage colony-stimulating factor (GM-CSF) is currently under investigation by the Pediatric Oncology Group for treating pulmonary metastases.

Chemotherapy Regimens

Spawned by the efficacy of chemotherapy, combination regimens were devised for treating patients with osteosarcoma. Currently most regimens, following the principles and example provided by Sutow, comprise agents with different mechanisms of action and minimal or non overlapping toxicity. An important additional principle in the construction of each regimen is the attempt to deliver agents at maximum dose intensity. Chemotherapy is integrated into a multidisciplinary approach to assist surgical extirpation of the primary tumor and resection of pulmonary metastases. Chemotherapy may also potentiate the action of radiation therapy in resistant and recurrent tumors.

The results obtained with most regimens have been similar. Except for an occasional publication, [71] there does not appear to be a regimen which can claim

superiority over those published in most communications. With current strategies, utilizing pre- and postoperative chemotherapy, cure rates of 60–75% in newly diagnosed nonmetastatic patients have been reported.[17–20,22,27,30,71] Limb salvage and, occasionally, rotationplasty have been reported in as many as 80% of the patients in these studies and in several communications devoted specifically to this topic.[100–102] In this context, an attempt was made to perform limb salvage exclusively with chemotherapy and abrogating surgery.[103] It was successful in three patients only: Figs. 11 and 12 demonstrate the result in one of these patients.

Inadequacy of Chemotherapy

While chemotherapy has produced remarkable successes in osteosarcoma, it has also been marred by failures. The survival rate for patients with pulmonary metastases following aggressive multimodal treatment is of the order of 25–30%.[104–113]

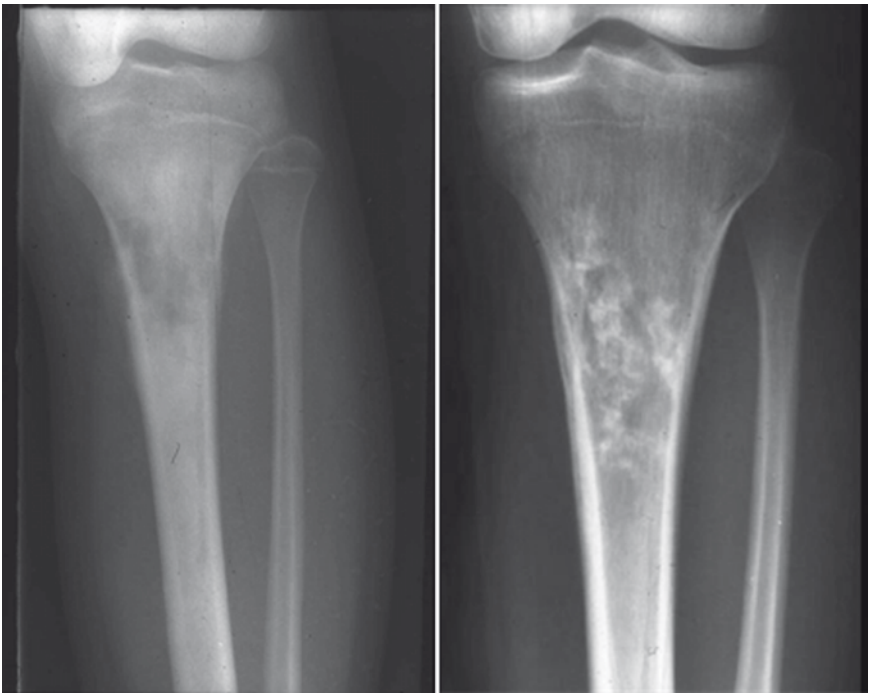

Fig. 11 Initial radiograph of patient with osteosarcoma of the proximal tibia (*left*). There is a mixed osteoblasic and osteolytic lesion. Follow-up after seven courses of high dose methorexate (7.5 G/m^2/course) demonstrates healing by calcification of the medulary lytic lesions and solid periosteal bone formation (*right*). This was accompanied clinically by a complete absence of symptomatology. This material is reproduced with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. (Jaffe N et al: *Cancer* 1985;56:461-466)

Fig. 12 Photograph of patient with lesion depicted in Fig. 11 10 years after completion of treatment exclusively with chemotherapy. The patient has remained alive and well without recurrent tumor 28 years after diagnosis and therapy

It has not improved over the past quarter century. Bielack et al reported that overall and event-free survival rates, respectively, were 16% and 9% for second, 14% and 0% for third, 13% and 6% for fourth, and 18% and 0% for fifth recurrences: "The exact role of retreatment with chemotherapy, particularly in the adjuvant situation, remains to be defined."[114]

Patients with partially treated tumors in whom survival has been prolonged, have also developed extrapulmonary metastases in uncommon sites, notably the kidneys, brain, heart, mediastinum, and epidural space.[115–117] Such metastases may cause severe complications, and considerable pain and discomfort, and require extensive palliative maneuvers. Attempts to prevent and eradicate such extra pul-

monary metastases constitute a major challenge. New therapy for patients with multifocal (sclerosing) osteosarcoma must also be developed.

Conspectus: Impact on the Conduct of Future Investigation and Treatment

Despite major discoveries in the chemotherapeutic pantheon for osteosarcoma over the past 35 years, the survival rate has plateaued. This is due principally to the fact that the arsenal of available effective chemotherapeutic agents over this period has not changed substantially . New agents and alternative strategies for the conquest of this malignancy are urgently required. Employing new paradigm shifts should receive serious consideration, while acquisition of new agents might include biotherapy, gene therapy, anti-angiogenic agents, targeted therapy, and attempts to harness the power of the immune system.

The reviews and reports cited above make no mention of identification markers to detect silent pulmonary micrometastases. Biomarkers or mechanisms with reliable specificity and sensitivity to identify such metastases would constitute a significant saltation in planning new strategies of treatment. Molecular profiles with rigorous characterization, gene expression patterns and phenotypes of osteosarcoma could afford an opportunity for planning risk-adjusted or personalized chemotherapy with reduced toxicity. Identification of patients free of micrometastases would permit treatment with chemotherapy limited to downstaging the primary tumor for surgical extirpation.

Despite the absence of major advances in chemotherapy and of any significant improvement in survival during the past 35 years, the ability to offer limb salvage to approximately 80% of newly diagnosed patients is noteworthy. Further, although the attempt to treat patients exclusively with chemotherapy, avoiding surgical ablation of the primary tumor was not entirely unsuccessful[102], three of the 31 patients were cured with this approach. With new and more effective agents, the ability to cure osteosarcoma without surgical ablation of the primary tumor may yet be realized. The ability to rescue most patients with recurrent pulmonary metastases may also be attained. Considering the phenomenal strides made in the treatment of this disease over the past 35 years, the current lack of new agents notwithstanding, these possibilities may become a reality in the new century.

References

1. Jaffe N, Traggis D, Enriquez C. Evaluation of a combination of mitomycin C (NSC-26980), phenylalanine mustard (NSC-16210) and vincristine (NSC-6757) in the treatment of osteogenic sarcoma. *Cancer Chemother Rep*. 1971;55:181-191.
2. Sullivan MP, Sutow WW, Taylor G. L-phenylalanine mustard as treatment for metastatic osteogenic sarcoma in children. *J Pediatr*. 1963;63:227-237.

3. Sutow WW, Sullivan MP, Fernbach DJ, et al. Adjuvant chemotherapy in primary treatment of osteogenic sarcoma. *Cancer*. 1975;36:1598-1602.
4. Wilbur JR, Sutow WW, Sullivan MP, et al. Successful treatment of rhabdomyosarcoma with combination chemotherapy and radiation therapy. *Proc ASCO*. 1971;7:56.
5. Sutow WW, Sullivan MP, Wilbur JR, et al. The study of adjuvant chemotherapy in osteogenic sarcoma. *J Clin Pharmacol*. 1975;15:530-533.
6. Pinkel D. Cyclophosphamide in children with cancer. *Cancer*. 1962;15:42-49.
7. Finklestein J, Hittle RE, Hammond CD. Evaluation of a high-dose cyclophosphamide regimen in childhood tumors. *Cancer*. 1969;23:1239-1242.
8. Sutow WW. Chemotherapeutic management of childhood rhabdomyosarcoma. In: Taylor G, Sutow WW, eds. *Neoplasia in Childhood*. Chicago: Year Book Medical Publishers; 1969: 201-208.
9. Cortes EP, Holland JF, Wang JJ, et al. Chemotherapy of advanced osteosarcoma. In: Price CHG, Ross FCM, eds. *Colston Paper N.24 Bone-Urban Aspects of Neoplasia*. London: Buttersworth; 1972:265-280.
10. Sutow WW. Combination chemotherapy with Adriamycin (NSC-123127) in primary treatment of osteogenic sarcoma (Part III). *Cancer Chemother Rep*. 1975;6:315-317.
11. Sutow WW, Gehan EA, Dyment PC, et al. Multi-drug adjuvant chemotherapy in osteosarcoma. *Interim report of the Southwest Oncology Group studies. Cancer Treat Rep*. 1978;62: 265-269.
12. Sutow WW. Late metastases in osteosarcoma. *Lancet*. 1976;1:856.
13. Sutow WW, Herson J, Perez C. Survival after metastasis in osteosarcoma. *Natl Cancer Inst Monogram*. 1981;56:227-231.
14. Perez C, Herson J, Kimball JC, et al. Prognosis after metastases in osteosarcoma. *Cancer Clin Trials*. 1978;1:315-320.
15. Jaffe N, Smith E, Abelson HT, Frei E III. Osteogenic sarcoma: Alterations in the pattern of pulmonary metastases with adjuvant chemotherapy. *J Clin Oncol*. 1983;1:251-254.
16. Herson J, Sutow WW, Elder K, et al. Adjuvant chemotherapy in non metastatic osteosarcoma A Southwest Oncology Group Study. *Med Pediatr Oncol*. 1980;8:343-352.
17. Jaffe N, Link M, Traggis D, et al. The role of high-dose methotrexate in osteogenic sarcoma. Sarcomas in soft tissue and bone in childhood. *Natl Cancer Inst Monogr*. 1981;56:201-206.
18. Goorin AM, Delorey M, Gelber RD, et al. The Dana-Farber Cancer Institute/The Children's Hospital adjuvant chemotherapy trials for osteosarcoma: Three sequential studies. *Cancer Treat Symp*. 1986;3:155-159.
19. Rosen G, Marcove RC, Huvos AG, et al. Chemotherapy en bloc resection, and prosthetic bone replacement in the treatment of osteogenic sarcoma. *Cancer*. 1976;37:1-11.
20. Rosen G. Role of chemotherapy in the treatment of primary osteogenic sarcoma. A five-year follow-up of T-10 neoadjuvant chemotherapy. In: Kimura K, Wang Y-M, eds. *Methotrexate in Cancer Therapy*. New York: Raven Press; 1986:227-238.
21. Delepine N, Delepine G, Jasmine C, et al. Importance of age and methotrexate dosage: Prognosis in children and young adults with high-grade osteosarcoma. *Biomed Pharmacother*. 1988;42:257-262.
22. Winkler K, Beron G, Dilling G, et al. Neoadjuvant chemotherapy of osteosarcoma. Results of Randomized Cooperative Trial (COSS-82) with salvage. Chemotherapy based on histologic tumor response. *J Clin Oncol*. 1988;6:329-337.
23. Bacci G, Picci P, Ruggeri P, et al. Primary chemotherapy and delayed surgery (neoadjuvant chemotherapy) for osteosarcoma of the extremities: The Instituto Rizzoli Experience in 127 patients treated preoperatively with intravenous methotrexate (high versus moderate doses) and intra-arterial cisplatin. *Cancer*. 1990;65:2539-2553.
24. Meyers PA, Heller G, Healey JH, et al. Chemotherapy for non-metastatic osteogenic sarcoma: The Memorial Sloan-Kettering experience. *J Clin Oncol*. 1992;10:5-15.
25. Bacci G, Picci P, Avella M, et al. The importance of dose intensity in neoadjuvant chemotherapy of osteosarcoma: A retrospective analysis of high-dose methotrexate, cisplatinum and adriamycin used preoperatively. *J Chemother*. 1990;2:127-135.

26. Provisor AJ, Ettinger LJ, Nachman JB, et al. Treatment of non-metastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: A report from the Children's Cancer Group. *J Clin Oncol*. 1997;15:76-84.
27. Meyers PA, Gorlick R, Heller G, et al. Intensification of preoperative chemotherapy for osteogenic sarcoma: Results of the Memorial Sloan-Kettering (T-12) protocol. *J Clin Oncol*. 1998;16:2452-2458.
28. Krailo M, Ertel I, Makley J, et al. A randomized study comparing high-dose methotrexate with moderate-dose methotrexate as components of adjuvant chemotherapy in childhood nonmetasatic osteosarcoma: A report from the Children's Cancer Study Group. *Med Pediatr*. 1987;15:69-77.
29. Bramwell VH, Berger SM, Sneath R, et al. A comparison of two short intensive adjuvant chemotherapy regimen in operative osteosarcoma of limbs in children and young adults: The first study of the European Osteosarcoma Intergroup. *J Clin Oncol*. 1992;10:1579-1591.
30. Souhami RL, Craft AW, Vander Eijken JW, et al. Randomised trial of two in operable regimens of chemotherapy inoperable osteosarcoma: A study of the European Osteosarcoma Intergroup. *Lancet*. 1997;350:911-917.
31. Grem J, King S, Whittes R, et al. The role of methotrexate in osteosarcoma. *J Natl Cancer Inst*. 1988;80:626-655.
32. Graf N, Winkler K, Betlemovic M, et al. Methotrexate pharmacokinetics and prognosis in osteosarcoma. *J Clin Oncol*. 1994;12:1443-1451.
33. Wang Y-N, Sutow WW, Romsdahl MM, et al. Age related pharmacokinetics of high-dose methotrexate in patients with osteosarcoma. *Cancer Treat Rep*. 1979;63:405-410.
34. Jaffe N, Prudich J, Knapp J, et al. Treatment of primary osteosarcoma with intra-arterial and intravenous high-dose methotrexate. *J Clin Oncol*. 1983;7:428-431.
35. Cohen H, Jaffe N. Pharmacokinetic studies of 24-hour infusions of high-dose methotrexate. *Cancer Chemother Pharmacol*. 1978;1:61-64.
36. Wang YM, Lantin E, Sutow WW. Blood, urinary and cerebral spinal fluid methotrexate levels in children after high-dose methotrexate infusion. *Clin Chem*. 1976;22:1053-1056.
37. Wang YN, Kim PY, Lantin E, et al. Degradation and clearance of methotrexate in children with osteosarcoma receiving high-dose infusion. *Med Pediatr Oncol*. 1978;4:221-229.
38. Perez C, Wang Y-M, Sutow WW, et al. Significance of 48-hour plasma level in high-dose methotrexate regimens. *Cancer Clin Trials*. 1978;1:107-111.
39. Jaffe N, Farber S, Traggis D, et al. Favorable response of metastatic osteogenic sarcoma to past high-dose methotrexate with citrovorin rescue and radiation therapy. *Cancer*. 1973;31:1367-1373.
40. Rosen G, Tefft M, Martinez A, et al. Combination chemotherapy and radiation therapy in the treatment of metastatic osteogenic sarcoma. *Cancer*. 1975;35:622-630.
41. Machak GN, Tkachev SI, Solovyev YN, et al. Neoadjuvant chemotherapy and local radiotherapy for high grade osteosarcoma of the extremities. *Mayo Clin Proc*. 2003;78:147-155.
42. Ozaki T, Flege S, Kevric M, et al. Osteosarcoma of the pelvis: Experience of the Osteosarcoma Study Group. *J Clin Oncol*. 2003;21:334-341.
43. Bonnadonna G, Monfardi S, De Lena M, et al. Phase I and preliminary phase II evaluation of Adriamycin (NSC/123127). *Cancer Res*. 1970;30:2527-2582.
44. Middleman E, Luce L, Frei E. Clinical trials with Adriamycin. *Cancer*. 1971;28:844-850.
45. O'Bryan RN, Luce JK, Talley R, et al. Phase II evaluation of Adriamycin in human neoplasia. *Cancer*. 1973;32:1-7.
46. Cortes EP, Holland JF, Wang JJ, et al. Amputation and Adriamycin in primary osteosarcoma. *New Engl J Med*. 1974;291:998-1000.
47. Cortes EP, Holland JF, Glidewell O. Osteogenic studies by the Cancer and Leukemia Group B. *Natl Cancer Inst Monogr*. 1975;56:207-209.
48. Cassady JR, Richter NP, Piro AJ, et al. Radiation, Adriamycin interactions – Preliminary clinical observations. *Cancer*. 1975;36:946-949.
49. Eilber FR, Grant T, Morton C. Adjuvant chemotherapy of osteosarcoma: Preoperative treatment. *Cancer Treat Rep*. 1978;62:213-216.

50. Jaffe N, Watts H, Fellows KE, et al. Local en bloc resection for limb preservation. *Cancer Treat Rep*. 1978;62:217-273.
51. Basser Rl, Green MD. Strategies for prevention of anthracycline cardiotoxicity. *Cancer Treat Rev*. 1994;19:57-77.
52. Seifert CF, Nesser ME, Thompson DF. Dexrazoxane in the prevention of doxorubicin-induced cardiotoxicity. *Ann Pharmacother*. 1994;28:1063-1072.
53. Smith MA, Ungerleider RS, Horowitz ME, et al. Influence of doxorubicin dose intensity on response and outcome for patients with osteogenic sarcoma and Ewing's sarcoma. *J Natl Cancer Inst*. 1991;83:1460-1470.
54. Baum E, Greenberg L, Gaynon P, et al. Use of *Cis*-diamminedichloroplatinum-II (CPDD) in Osteogenic Sarcoma (OS) in Children (Abstract C-315). *Proc AACR-ASCO*. 1978;19:385.
55. Nitschke R, Starling KA, Vats T, et al. *Cis*-diamminedichloroplatin-II (NSC 119875) In Childhood Malignancies. A Southwest Oncology Group Study. *Med Pedatr Oncol*. 1978;4: 127-132.
56. Ochs JJ, Freeman AR, Douglass HO Jr, et al. *Cis*-diamminedichloroplatinum (II) in advanced osteogenic sarcoma. *Cancer Treat Rep*. 1978;62:239-245.
57. Jaffe N, Knapp J, Chuang VP, et al. Osteosarcoma intra-arterial treatment of the primary tumor with Cis-diamminedichloroplatinum-II (CDP). Angiographic, pathologic and pharmacologic studies. *Cancer*. 1983;51:402-407.
58. Jaffe N, Raymond AK, Ayala A, et al. Effect of cumulative courses of intra-arterial *Cis*-diamminedichloroplatinum II on the primary tumor in osteosarcoma. *Cancer*. 1989;63:63-67.
59. Wright KC, Wallace S, Kim EE, et al. Pulse arterial infusions. Chemotherapeutic complications. *Cancer*. 1986;57:1952-1956.
60. Chuang VP, Benjamin RS, Jaffe N, et al. Radiographic and angiographic changes in osteosarcoma after intra-arterial chemotherapy. *Am J Radiol*. 1982;139:1065-1069.
61. Shirkoda A, Jaffe N, Wallace S, et al. Computed tomography of osteosarcoma following intra-arterial chemotherapy. *Am J Radiol*. 1985;144:95-99.
62. Pan G, Raymond AK, Carrasco CH, et al. Osteosarcoma: MR imaging after preoperative chemotherapy. *Radiology*. 1990;174:517-526.
63. Meyers PA. Osteosarcoma In Pediatric Bone and Soft Tissue Sarcomas. In: Pappo A, ed. *Pediatric Bone and Soft Tissue Sarcomas*. Berlin, Heidelberg, New York: Springer; 2005: 219-233.
64. Ruiz L, Gilden J, Jaffe N, et al. Auditory function in pediatric osteosarcoma patients treated with multiple doses of *cis*-diamminedichloroplatinum-II (CDP). *Cancer Res*. 1989;49: 742-744.
65. Jaffe N, Keifer R III, Robertson R, et al. Renal toxicity with cumulative doses of cis-diamminedichloroplatinum-II in pediatric patients with osteosarcoma. *Cancer*. 1987;59:1577-1581.
66. Kawai A, Sugihara S, Kunisada T, et al. Imaging assessment of the response of bone tumors to preoperative chemotherapy. *Clin Orthop Relat Res*. 1997;Apr(337):216-225.
67. Kunisada T, Ozaki T, Kawai A. Imaging assessment of the responses of osteosarcoma patients to preoperative chemotherapy: Angiography compared with thallium-201 scintigraphy. *Cancer*. 1999;86(6):949-956.
68. Carrasco CH, Charnsangavej C, Raymond AK, et al. Osteosarcoma: Angiographic assessment of response to preoperative chemotherapy. *Radiology*. 1989;170:839-842.
69. Lang P, Vahlensieck M, Matthay KK. Monitoring neovascularity as an indicator to response to chemotherapy in osteogenic and Ewing sarcoma using magnetic resonance angiography. *Med Pediatr Oncol*. 1996;26(5):329-333.
70. Kumpan W, Lechner G, Wittich GR. The angiographic response of osteosarcoma following pre-operative chemotherapy. *Skeletal Radiol*. 1986;15(2):96-102.
71. Wilkins RM, Cullen JW, Odom L, et al. Superior survival in treatment of primary nonmetastatic pediatric osteosarcoma of the extremity. *Ann Surg Oncol*. 2003;10:498-507.
72. Jaffe N, Spears R, Eftekhari F, et al. Pathologic fracture in osteosarcoma: Effect of chemotherapy in primary Tumor and survival. *Cancer*. 1987;59:701-709.

73. Antman KH, Montella D, Rosenbaum C, et al. Phase II Trial of ifosfamide with mesna in previously treated metastatic osteosarcoma. *Cancer Treat Rep*. 1985;69:499-504.
74. Bowman LG, Mayer WN, Douglass EC, et al. Activation of ifosfamide in metastatic and unresectable osteosarcoma (Abstract). *Proc ASCO*. 1987;C-214:844.
75. Meyer WH, Pratt CB, Farham D, et al. The activity of Ifosfamide (IFOS) in previously pretreated patients with osteosarcoma (OS): Preliminary results of SJCRHOS-86 Study. *Proc ASCO*. 1988;7:261.
76. Grana N, Graham-Pole J, Cassano W, et al. Etoposide (VP-16) infusion plus Cyclophosphamide (CY) pulses: An effective combination for refractory cancer. *Proc ASCO*. 1989;8:300.
77. Saleh R, Graham-Pole J, Cassano W, et al. Response of osteogenic sarcoma to the combination of rtoposide and cyclophosphamide as neoadjuvant chemotherapy. *Cancer*. 1990;65:861-865.
78. Pratt CB, Luo X, Fay L, et al. Response of pediatric malignant solid tumors following ifosfamide or ifosfamide/carboplatin/etoposide. *A single hospital experience. Med Pediatr Oncol*. 1996;27:145-148.
79. Harris MB, Cantor AB, Goorin AM. Treatment of osteosarcoma with ifosfamide: Comparison of response in pediatric patients with recurrent disease versus patients previously untreated: A Pediatric Oncology Group study. *Med Pediatr Oncol*. 2002;20:426-433.
80. Pratt CB, Meyer WH, Dauglass EC, et al. A phase I study of ifosfamide with Mesna given daily for 3 consecutive days to children with malignant solid tumors. *Cancer*. 1993;71: 3661-3665.
81. Chawla S, Rosen G, Lowenbraun S, et al. Role of high-dose ifosfamide in recurrent osteosarcoma. *Proc ASCO*. 1990;9:310.
82. Patel S, Vadhan-Raj S, Papadopolous N, et al. High-dose ifosfamide in bone and soft tissue sarcomas: Results of a Phase II and Pilot Studies – dose-response and schedule dependence. *J Clin Oncol*. 1997;15:2378-2384.
83. Berrak S, Pearson P, Berbergoglu S, et al. High-dose ifosfamide in relapsed pediatric osteosarcoma: Therapeutic effects and renal toxicity. *Pediatr Blood Cancer*. 2005;44:215-219.
84. Pratt CB, Meyer WH, Rao BN, et al. Osteosarcoma studies at St Jude Children's Research Hospital from 1968 through 1998. *Cancer Treat Res*. 1993;62:323-326.
85. Harris MB, Gieser P, Goorin AM, et al. Treatment of metastatic osteosarcoma at diagnosis: A Pediatric Oncology Group Study. *J Clin Oncol*. 1998;16:3641-3648.
86. Goorin AM, Harris MB, Bernstein M, et al. Phase II/III trial of etoposide and high-dose ifosfamide in newly diagnosed metastatic osteosarcoma: A Pediatric Oncology Group Trial. *J Clin Oncol*. 2002;20:426-433.
87. Daw NC, Billups CA, Rodroguez-Galindo C, et al. Metastatic osteosarcoma. *Cancer*. 2006;106:403-412.
88. Meyer WH, Pratt CB, Poquette CA, et al. Carboplatin/Ifosfamide window therapy for osteosarcoma: Results of the St Jude Children's Research Hospital OS-91 Trial. *J Clin Oncol*. 2001;19:171-182.
89. Fergusen WS, Harris MB, Goorin AM, et al. Presurgical window of carboplatin and surgery and multi-drug chemotherapy for the treatment of newly diagnosed metastatic or unresectable osteosarcoma: Pediatric Oncology Group trial. *J Pediatr Hematol Oncol*. 2001;23:340-348.
90. Petrilli AS, de Camargo B, Filho VO, et al. Results of the osteosarcoma treatment group studies III and IV: Prognostic factors and impact on survival. *J Clin Oncol*. 2006;24:1161-1168.
91. Merimsky O, Meller I, Flusser G, et al. Gemcitabine in soft tissue or bone sarcoma resistant to standard chemotherapy: A phase II study. *Cancer Chemother Pharmacol*. 2000;45:177-181.
92. Navid F, Willert JR, Mc Carville MB, et al. Combination of gemcitibine and docetaxel in the treatment of children and young adults with refractory bone sarcoma. *Cancer*. 2008;113:419-425.
93. Anderson PM, Wiseman GA, Dispenzieri A, et al. High-dose samarium-153 ethylene diamine tetramethylene phosphonate: Low toxicity of skeletal irradiation in patients with osteosarcoma and bone metastases. *J Clin Oncol*. 2002;20:189-196.

94. Meyers P, Schwartz CL, Krailo M, et al. Osteosarcoma: A randomized prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate. *J Clin Oncol*. 2005;23:2004-2011.
95. Lu L, Lu L. Junovan fails to win ODAC nod for osteosarcoma treatment. *ONI*. 2007;16:1632.
96. Meyers PA, Schwartz CL, Krailo MD, et al. Osteosarcoma: The addition of muramyl tripeptide to chemotherapy improves overall survival – a report from the Children's Oncology Group. *J Clin Oncol*. 2008;26:633-638.
97. Bielack SS, Marina N, Ferrari S, et al. Osteosarcoma: The same old drugs or more? *Journal Clin Oncol*. 2008;26:3192-3193.
98. Hunsberger S, Freidlin B, Smith MA. Complexities in interpretation of osteosarcoma clinical trial results. *Journal Clin Oncol*. 2008;26:3193-3194.
99. Meyers PA, Schwartz CL, Krailo MD, et al. In Reply Journal. *Clin Oncol*. 2008;26:3194-3195.
100. Gitelis S, Neel MD, Wilkins RM, et al. The use of a closed expandable prosthesis for pediatric sarcomas. *Chir Organi Mov*. 2003;88:327-333.
101. Hudson M, Jaffe MR, Jaffe N, et al. Pediatric osteosarcoma: Therapeutic strategies, results and prognostic factors derived from a 10-year experience. *J Clin Oncol*. 1990;8:1988-1997.
102. Hosalkar HS, Dormans JP. Limb sparing surgery for pediatric musculoskeletal tumors. *Pediatr Blood Cancer*. 2004;42(4):295-310.
103. Jaffe N, Carrasco H, Raymond K, et al. Can cure in patients with osteosarcoma be achieved exclusively with chemotherapy and abrogation of surgery? *Cancer*. 2002;95:2202-2210.
104. Kaste SC, Pratt CB, Cain AM, et al. Metastases detected at the time of diagnosis of primary pediatric extremity osteosarcoma at diagnosis: Imaging features. *Cancer*. 1999;86(8):1602-1608.
105. Meyers PA, Heller G, Healy JH, et al. Osteogenic sarcoma with clinically detectable pulmonary metastases at initial presentation. *J Clin Oncol*. 1993;11:449-453.
106. Su WT, Chewning J, Abramson S, et al. Surgical management and outcome of osteosarcoma patients with unilateral pulmonary metastases. *J Pediatr Surg*. 1994;39(3):1849-1858.
107. Meyer WH, Schell MJ, Kumar AP, et al. Thoracotomy for pulmonary metastatic osteosarcoma. An analysis of prognostic indicators of survival. *Cancer*. 1987;59(2):374-379.
108. Skinner KA, Eilber FR, Holmes E, et al. Surgical treatment and chemotherapy for pulmonary metastases from osteosarcoma. *Arch Surg*. 1992;127(90):1065-1070. discussion 1070-71.
109. Chou AJ, Merola P, Wexler LH, et al. Treatment of osteosarcoma at first recurrence after contemporary therapy: The Memorial Sloan-Kettering Cancer Center experience. *Cancer*. 2005;104(10):2214-2221.
110. Harting MT, Blakely ML, Jaffe N, et al. Long-term survival after aggressive resection of pulmonary metastases among children and adolescents with osteosarcoma. *J Pediatr Surg*. 2006;41(1):194-199.
111. Ward WG, Mikaelian K, Dorey F, et al. Pulmonary metastases of stage IIB extremity osteosarcoma and subsequent pulmonary metastases. *J Clin Oncol*. 1994;12(9):1849-1858.
112. Bacci G, Ferrari S, Bertoni F, et al. Long-term outcome for patients with nonmetastatic osteosarcoma of the extremity treated at the Istituto Ortopedico Rizzoli/osteosarcoma-2 protocol: An updated report. *J Clin Oncol*. 2000;18(24):4016-4027.
113. Nathan SS, Gorlick R, Bukata S, et al. Treatment algorithm for locally recurrent osteosarcoma based on local disease-free interval and the presence of lung metastasis. *Cancer*. 2006;107(7):1607-1616.
114. Bielack SS, Kempf-Bielack B, Branscheid D, et al. Second and subsequent recurrences of osteosarcoma: Presentation and treatment outcomes of 249 Consecutive Cooperative Osteosarcoma Study Group patients. *J Clin Oncol*. 2008;27:557-565.
115. McCarten KM, Jaffe N, Kirkpatrick JA. The changing radiographic appearance of osteosarcoma. *Ann Radiol*. 1980;23:203-208.
116. Lockhart SK, Coan JD, Jaffe N, et al. Osteosarcoma metastatic to the kidney. *Clin Imaging*. 1989;13:154-156.
117. Baram TZ, Van Tassel P, Jaffe N. Brain metastases in osteosarcoma: Incidence, clinical and neurological findings and management options. *J Neuro Oncol*. 1988;6:45-52.