1 Adjuvant and Neoadjuvant Chemotherapy for Osteosarcoma: A Historical Perspective

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Abstract

Osteosarcoma was initially resistant to chemotherapy that worked for Ewing sarcoma and rhabdomyosarcoma as well as other chemotherapeutic agents available in the 1960s. In the early 1970s, responses of osteosarcoma to adriamycin were reported, and at about the same time, so were responses of osteosarcoma to high-dose methotrexate. These agents were introduced into adjuvant therapy due to the dire prognosis associated with apparently localized osteosarcoma. After initial questions regarding the role of chemotherapy delayed its uniform acceptance, there is now general agreement that chemotherapy is primarily responsible for the cure of patients with osteosarcoma when combined with surgical elimination of the primary tumor. Advances with combination chemotherapy later adding cisplatin and ifosfamide have improved ultimate survival. The history of the development of effective chemotherapy combinations at Memorial Sloan Kettering Cancer Center, UT MD Anderson Cancer Center, and the Rizzoli Institute are highlighted, and recent large cooperative group studies are reviewed in the context of those findings.

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History

Osteosarcoma was initially resistant to chemotherapy that worked for Ewing sarcoma and rhabdomyosarcoma as well as other chemotherapeutic agents available in the 1960s. In the early 1970s, Wang, Cortes, and Holland reported responses of osteosarcoma to adriamycin (before the name doxorubicin was invented) [70]; and at about the same time, Jaffe reported responses of osteosarcoma to high-dose methotrexate [36]. That was the beginning of the modern era of osteosarcoma chemotherapy. It was also recognized at that time that the vast majority of patients with apparently localized osteosarcoma would die of their disease despite radical amputation, one joint above the level of the tumor [37, 44]. There had even been attempts to delay amputation with radiation of the primary tumor, so that when it was obvious that the patients' lungs were filled with metastases, mutilating surgery could be avoided [18, 42, 55]. With that background, it is easy to see why Jaffe and Cortes pushed the active chemotherapeutic agents that they had discovered into adju-

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vant therapy for patients with localized disease. Their back-to-back publications in the New England Journal of Medicine indicated remarkable improvements in survival and disease-free survival compared with well-established historical control series [23, 37].

So why was there so much controversy regarding the use of adjuvant chemotherapy in the two decades that followed? Several issues contributed. First, as my former mentor, John Murray, frequently said "the worst enemy of a good outcome is long-term follow-up." The initial series of Jaffe and Cortes were published with less than 1 year of median follow-up in the rush to notify the world of a major breakthrough in the treatment of a previously deadly disease. At that time, the vast majority of patients treated with amputation alone had developed metastases and many had died. Further follow-up on the treated patients, however, showed that although the time to the development of metastatic disease was prolonged, the majority of patients ultimately relapsed and died. At the last update of these series, disease-free survival had dropped from 60 to 85% at 1 year to about 40% with 5-years of follow-up [24, 38]. Second, chemotherapy was toxic. High-dose methotrexate was difficult to manage. It involved giving a lethal dose of chemotherapy and then following with an antidote to protect normal cells. Initially, methotrexate levels were not available to monitor drug clearance, and particularly in adults, clearance was not so rapid and predictable as in children. Some patients died. For adriamycin, too, there were infectious complications (there were no hematopoietic growth factors, and antibiotics had limited spectrum), mucositis, and great fear of late congestive heart failure. Third, the statisticians from the Mayo Clinic, a chemotherapeutically conservative institution at that time, showed evidence that their patients treated only with surgery were doing much better than previously and suggested that the improvements claimed by others using chemotherapy were simply due to a change in the natural history of the disease [67, 68]. Fourth, the medical profession, taught to be skeptical and not to believe the results of studies that do not have concurrent randomized controls, believed the illogical assertions of the Mayo Clinic statisticians. Why should the natural history of a cancer change? Was there evidence of that happening in any other cancer? Did the use of plain tomography eliminate such a high proportion of patients with metastatic disease on presentation who would not have been detected with X-rays that the remainder of patients had such a better outcome? Is it not more likely that the referral bias of patients traveling to the Mayo Clinic accounted for their changes? And even if the natural history had improved such that almost 50% of patients were cured with surgery alone as initial therapy, in fact, two-thirds of patients in Taylor's reports relapsed [67, 68]. That some were salvaged by subsequent therapy does not negate the fact that initial surgery was curative in only one-third of patients, and what logical reason is there not to try to improve the lot of those who relapsed despite amputation? How could omitting potentially helpful systemic treatment do that? Were not patients at greater risk of dying from not doing something than from doing too much?

Nonetheless, the medical community was divided. Some, most notably Dr. Gerald Rosen from Memorial Sloan Kettering Cancer Center, chose to build on the activity of high-dose methotrexate and adriamycin by developing combination regimens to increase the cure rate [59–63]. So did both the pediatric [65, 66] and adult groups at MD Anderson [51, 58]. Others chose a more conservative interpretation of the data claiming that until there was a randomized study demonstrating conclusively that adjuvant chemotherapy was beneficial, its use should be considered unproven and experimental. Their view was strengthened by publication of a randomized pilot study from the Mayo Clinic that demonstrated no difference in disease-free survival between patients treated with adjuvant high-dose methotrexate and those treated solely with surgery [28]. A careful examination of that study reveals several issues of concern. First, the population, with a median age of over 21, is not representative of the overall population of patients with osteosarcoma where the peak incidence is in the second decade. Second, and most important, 7 of the 20 patients in the treatment arm never

reached the target therapeutic dose of 7.5 g/m^2, due either to delayed drug excretion or very early disease progression (and few today would ever consider a methotrexate dose as low as 7.5 g/m^2 to be adequate).

Clearly, the most influential studies for the medical community as a whole were the two randomized controlled studies that compared the outcomes of patients treated with adjuvant chemotherapy with those treated by surgery alone[29, 43]. These studies put to rest the controversy as to whether chemotherapy added to the cure of localized osteosarcoma. The answer was a resounding yes. In Link's multi-institutional study [43], 77 of 113 eligible patients declined randomization leaving only 36 patients randomized to receive a complex multidrug adjuvant regimen utilizing high-dose methotrexate at 12 g/m^2, adriamycin, a combination of drugs (now felt not to have much activity) called BCD [50], and the combination of adriamycin and cisplatin (modified from Rosen's T-10 protocol) [59], versus amputation alone. The chemotherapy group had a 2-year disease-free survival of 66% compared with 17% in the control group. Of interest, the 59 patients refusing randomization and selecting to receive chemotherapy had a 67% disease-free survival compared with 9% in the 18 patients selecting amputation. Thus, one might argue that no more was learned from the patients who were randomized than from those studied and observed.

In Eilber's study [29], patients all received one cycle of preoperative chemoradiation therapy and were randomized postoperatively to receive a similar regimen to that used in Link's study with somewhat lower doses and the omission of the four cycles of the adriamycin-cisplatin combination (modified from Rosen's T-10A protocol) [59]. The 32 patients randomized to adjuvant chemotherapy had a 55% 2-year disease-free survival compared with 20% for the 27 patients randomized not to receive adjuvant therapy.

Since both Link and Eilber's studies were based on therapy developed by Rosen, it is worth reviewing the existing data from Rosen's studies at the time of the initiation of those two randomized trials. After studying the sequential use of high-dose methotrexate and adriamycin in patients with metastatic osteosarcoma [62], his group embarked on a series of studies in patients with primary tumors. For chemotherapy, they first utilized high-dose methotrexate and adriamycin, later adding high-dose cyclophosphamide, their T-4 and T-5 protocols [60, 61]. With these protocols, they noted late relapses between 12 and 33 months, so they then substituted the combination of bleomycin, cyclophosphamide, and dactinomycin (BCD)[50] for high-dose cyclophosphamide, but they increased the frequency of high-dose methotrexate administration to weekly, resulting in 18 rather than 6 doses of methotrexate, their T-7 protocol [60].

Rosen's most important contribution was not the regimens he developed but rather the concept of neoadjuvant chemotherapy. During the time it took to develop a custom endoprosthesis so that a tumor involving a portion of a weight-bearing bone could be widely resected while preserving the neurovascular bundle and permitting limb salvage rather than amputation, he gave preoperative chemotherapy [60, 61]. He rightfully observed that tumor shrinkage in a tumor with a bony matrix was not a good indicator of the response to therapy. Since tumors were removed and analyzed histologically, however, it was possible to estimate the effects of chemotherapy by histologic response. Huvos first described the histologic findings in the patients that Rosen treated [35]. He described four grades of response ranging from I, essentially no response, to IV, complete disappearance of tumor. Rosen observed that patients whose tumor was completely or almost completely killed by neoadjuvant chemotherapy (Huvos grade III-IV) had improved disease-free survival compared with those whose tumors demonstrated lesser degrees of tumor kill. That observation added further support to the conclusion that the improved disease-free survival of those treated in the adjuvant situation was a direct result of the chemotherapy administered [60].

Rosen also noted in treating patients with established disease that some patients responded only after escalation of the methotrexate dose above 8 g/m^2. In the T-10 protocol, preoperative therapy was heavily weighted toward methotrexate

and consisted of 4 weeks of high-dose methotrexate at 8–12 g/m^2, one course of BCD, 2 more weeks of methotrexate, one course of adriamycin, and 2 more weeks of methotrexate. Postoperative therapy for good responders was repeating the second portion of the preoperative regimen three times. Poor responders had chemotherapy changed to the combination of adriamycin and cisplatin for two courses followed by BCD and repeating that sequence two more times. By modifying postoperative chemotherapy in poor responders, he converted their prognosis to that of good responders [59].

Rosen's emphasis on escalation of the methotrexate dose in order to obtain a response was studied in terms of peak plasma concentration of methotrexate by Delepine and colleagues in a modified T-10 protocol [25–27]. Patients whose methotrexate dose was adjusted to reach a peak level of ≥1000 μM had a higher rate of good histologic response and better disease-free survival than those whose peak levels were <1000 μM [25]. A subsequent report by Bacci from the Rizzoli Institute (IOR) using multivariate analysis in 336 patients showed no correlation between methotrexate levels and histologic response [7]. It must be emphasized, however, that the protocols at the IOR utilized only two preoperative doses of methotrexate as well as two of adriamycin and cisplatin preoperatively, whereas the T-10 protocol utilized eight doses of methotrexate, one of adriamycin, and one of BCD. Adequate methotrexate levels are critical to the activity of methotrexate, but if much of the preoperative response rate is due to adriamycin and cisplatin, the methotrexate level is irrelevant; as is, perhaps, the administration of methotrexate at all in that regimen. It is clear, however, if one wants methotrexate to work, adequate levels (≥1000 μM) are important.

The activity of cisplatin against osteosarcoma was discovered during phase I clinical trials [21, 41] and confirmed in additional phase II studies [12, 52, 69]. It was put into adjuvant therapy in a regimen alternating with adriamycin by Ettinger and colleagues from Roswell Park [30, 31]. After 3-year median follow-up time, 64% of patients were continuously free of disease [31]. After noting at MD Anderson that cisplatin could be used by intra-arterial infusion in patients with melanoma [57], we expanded our studies to include patients with osteosarcoma [13, 19, 22, 47]. The response rate seen in patients with primary bone tumors (8/15) was substantially higher than the 21% reported in the earlier phase I-II studies of intravenous cisplatin. We also noted in our pharmacologic observations that systemic exposure to cisplatin was the same with intravenous or intra-arterial administration, but the concentration in the vein draining the tumor was 1.5–4 times higher with intra-arterial administration [64]. Thus, intra-arterial cisplatin delivers a full systemic dose plus a boost to the primary tumor.

Jaffe extended those studies to children, confirming the activity [39]. He subsequently compared the activity of intra-arterial cisplatin with intravenous high-dose methotrexate in a randomized study [40]. In the methotrexate arm, 4 of 15 patients responded (3 CR, 1 PR), but in the intra-arterial cisplatin arm, 9 of 15 patients responded (7 CR, 2 PR). In addition, two patients randomized to methotrexate were subsequently treated with and responded to intra-arterial cisplatin. Responses were defined by pathology using the criteria of Ayala, who modified the Huvos grading by quantifying the degree of tumor necrosis [2, 3]. Ayala noted that some degree of tumor necrosis could be seen in the absence of any chemotherapy, but necrosis in excess of 60% represented a definite chemotherapy effect. Most subsequent papers have simply used the 90% necrosis cutoff as a good response and anything less as a poor response. Raymond described in detail the procedures for processing the tumor to get the best estimate of the percent necrosis [58].

While Jaffe was refining the use of intra-arterial cisplatin in pediatric patients, we on the adult sarcoma service at MD Anderson studied the effects of combining systemic adriamycin and intra-arterial cisplatin as preoperative chemotherapy for patients with localized osteosarcoma [14, 58]. Since the dose-limiting toxicities of adriamycin are myelosuppression and mucositis and those of cisplatin are nephrotoxicity and ototoxicity, we reasoned that the two drugs could be given in combination at full single-agent

doses. It is harder, particularly in adults, to add methotrexate to that combination since mucositis and nephrotoxicity overlap the toxicities of the other two drugs. Our studies using different drugs confirmed the observations of Rosen in methotrexate-weighted T-7 and T-10 protocols. Continuous disease-free survival was 58% for the entire group of 40 patients, but it was 91% in those with tumor necrosis ≥90% and only 14% for those with necrosis <90%. Subsequent modification of the postoperative adjuvant regimen in patients with poor necrosis with the addition of high-dose methotrexate and BCD improved disease-free survival to 34%, and with high-dose methotrexate and ifosfamide to 67% [16]. I will return to this subject later as recent studies question the very basic concepts of neoadjuvant therapy.

The group most influenced by our experience with intra-arterial cisplatin, and the group that best developed neoadjuvant and adjuvant therapy for osteosarcoma in the ensuing years was the group from the Rizzoli Institute. Drs. Bacci and Picci spent several months visiting MD Anderson before returning to the IOR where their sequential protocols with large numbers of patients treated at a single institution are landmarks in the history of osteosarcoma therapy. The great advantage of the IOR is that it serves as the referral center for the entire country of Italy for complex orthopedic procedures and thus captures the vast majority of patients with osteosarcoma.

The first adjuvant studies used adriamycin and then added low-intermediate doses of methotrexate. Disease-free survival at 5 years was 45% compared with 10% in their historical control [5, 20]. They then initiated their first neoadjuvant study with intra-arterial cisplatin, initially given 1 week after intermediate- (750 mg/m^2) or high-dose methotrexate (7.5 g/m^2). Patients with good response to initial chemotherapy were randomized to receive only one more cycle of methotrexate and cisplatin versus 24 weeks of therapy that added also adriamycin [10]. Only 5 of 15 patients in the first group remained continuously disease-free compared with 19 of 19 who had the longer treatment with the addition of adriamycin. In the report of the entire series of 127 patients with the same primary chemotherapy, they observed a higher rate of good response (62% vs 42%) in the patients receiving high-dose methotrexate rather than intermediate-dose methotrexate [9]. They also observed superior disease-free survival in the good responders who received prolonged postoperative chemotherapy (62%) to that of those with intermediate response (42%) or poor response (10%). Overall 5-year disease-free survival was 49%. Another conclusion that can be drawn from the study is that five cycles of alternating full-dose adriamycin and BCD were inadequate therapy for patients with truly poor response (< 60% necrosis).

The second neoadjuvant study from the IOR added systemic adriamycin to intra-arterial cisplatin 1 week after high-dose methotrexate for two courses preoperatively and continued the same drugs for three courses postoperatively in good responders [8]. Poor responders (<90% tumor necrosis) received a complex, prolonged postoperative regimen that added three courses of ifosfamide at 10 g/m^2 and substituted three courses of cisplatin plus etoposide for single-agent cisplatin. The regimen was continued for 30 weeks compared with 21 weeks for the good responders [8]. The rate of good necrosis increased to 71% with the addition of preoperative adriamycin (compared with 62% in their previous study). Continuous disease-free survival at 5 years was 63% (compared with 49% in their prior study). Long-term follow-up on these patients confirms disease-free survival of 61% at more than 10 years and no difference between good and poor responders [6]. Importantly, disease-free survival of good responders was 71% and for poor responders was 57% (73% vs. 72% when those with major protocol violations were excluded). This is another study that demonstrates that the addition of an active agent in a prolonged course of postoperative therapy can alter poor prognosis of poor responders.

In the next study from IOR, patients were randomized preoperatively to receive cisplatin intra-arterially or intravenously [11]. This study was prompted in part by the findings of the German Cooperative Osteosarcoma Study Group (COSS) that compared intra-arterial and intravenous

administration of cisplatin in the combination with ifosfamide in the context of a four-drug preoperative protocol and found no difference in the rate of good tumor necrosis between the two routes of administration [71]. In contrast to the COSS study, the IOR group found a higher rate of good necrosis in patients who received intra-arterial cisplatin (78%) than in those who were treated intravenously (46%) [11]. There was no difference in disease-free survival between the groups but fewer local recurrences in the group receiving intra-arterial therapy. Another advantage of intra-arterial cisplatin is the rapidity of the response. Symptomatic improvement is noted usually after the first course of therapy, sometimes in only a few days. Systemic therapy does not usually work so rapidly. So how are we to interpret the COSS study? The more agents that are used in neoadjuvant therapy, the less important optimization of any one is. The COSS study used all of the active agents neoadjuvantly, Bacci used three, MD Anderson uses two. It is not surprising that there is no effect on the ultimate outcome between intra-arterial therapy and intravenous therapy. The ultimate outcome is based on the systemic effects of the drugs, not a local effect. Local control of the tumor is determined by surgery, not chemotherapy, so most groups now use intravenous cisplatin because intra-arterial administration is more complex, expensive, and time-consuming. For patients where limb-salvage surgery can be performed only with marginal margins, however, there may still be a role for intra-arterial therapy, especially if the number of drugs used in the neoadjuvant setting is limited, since there is a high correlation between failure to obtain a good response to initial therapy and risk of local recurrence unless surgery is truly radical [34, 54].

The subsequent study from the IOR modified the preoperative regimen introducing a cycle of ifosfamide-cisplatin and ifosfamide-adriamycin but did not improve overall results from previous studies[4]. Subsequent studies expanded participation to the Italian Sarcoma Group (ISG) and collaborated in one with the Scandinavian Sarcoma Group (SSG). Their study with the SSG added high-dose ifosfamide (15 g/m^2 over 5 days by continuous infusion) in the preoperative phase but did not improve on their prior results [33]. The next study limited to the ISG looked at the addition of ifosfamide either to the preoperative regimen or limiting its use to postoperative therapy only in poor responders [32]. There was no improvement with the addition of ifosfamide preoperatively, but there was increased myelosuppression.

The most controversial drug in the treatment of osteosarcoma is ifosfamide. The activity of ifosfamide against advanced osteosarcoma was noted in the mid-1980s [1, 46, 56]. Further studies suggested not only dose response [15] but also schedule dependency [53]. With that background, its addition to adjuvant and neoadjuvant studies has been extensive. As noted previously, studies from MD Anderson [16] and the IOR [6, 8] demonstrated superior disease-free survival when ifosfamide was added to the postoperative therapy in poor responders. In contrast, the addition of preoperative ifosfamide did not improve disease-free survival [32]. Cooperative group studies with more patients have reached very different conclusions.

A large study from the Children's Oncology Group (COG) studied 662 patients with osteosarcoma and randomized them to receive induction therapy with either methotrexate, adriamycin, and cisplatin (MAP) as their standard regimen or methotrexate, adriamycin, and ifosfamide. Patients were also randomized to receive or not receive mifamurtide (liposomal muramyl tripeptide, MTPPE) [48, 49]. The study showed improved survival and improved (although not statistically significant at the $p < 0.05$ level) event-free survival in the patients randomized to receive mifamurtide, but no advantage to the addition of ifosfamide. On the other hand, there was no difference in the rate of good response (modified Huvos grade III and IV) between MAP and MAI. One could argue that the data from the study suggest that ifosfamide is as active as cisplatin in primary therapy and cisplatin may well be the single most active agent against osteosarcoma.

An even larger cooperative study, the EURAMOS trial, accrued 2260 patients [17, 45].

Good responders were randomized to receive or not receive pegylated interferon alfa-2b after completion of chemotherapy (although poor adherence to randomization and dropout due to toxicity make interpretation of the data difficult) [17], and poor responders were randomized to receive a postoperative regimen containing ifosfamide (at good doses and schedule) or to continue on the same regimen used preoperatively, MAP (including two doses of methotrexate at 12 g/m^{2}per course for two courses) [45]. Only 618 of the 1060 poor responders participated in the randomization. There was no statistically significant benefit from the addition of ifosfamide, but there was a clear separation of the event-free survival curves during the first 2 years. The investigators speculate that some of this difference was an artifact of delayed post-treatment imaging since the patients randomized to ifosfamide finished their therapy after 40 weeks while those who got MAP ended at 29 weeks. An alternate explanation is that there was a guaranteed time while chemotherapy was continued, regardless of when post-treatment imaging started. Another explanation is that ifosfamide delayed but did not eliminate the development of metastases. Either of these last interpretations would suggest that a longer course of postoperative therapy for poor responders would be beneficial. On the other hand, the patients randomized to ifosfamide actually received fewer of their planned doses with a smaller percentage receiving at least 80% of their planned dose than those randomized to the shorter postoperative MAP regimen, so maybe just giving the therapy written into the protocol might have improved the potential cure rate of the poor responders. Nobody will ever know. The study represents real-world experience, but one wonders whether the poor dose intensity reported in the study was observed to the same degree in centers with more experience treating osteosarcoma.

So how should one interpret the data from the large randomized EURAMOS study (or other large cooperative group studies) in the context of much smaller studies from Memorial Sloan Kettering, MD Anderson, and the IOR with regard to modification of postoperative chemotherapy in poor responders? If the induction regimen is MAP as given in EURAMOS, benefit from adding ifosfamide in postoperative therapy of poor responders is questionable at best. If the preoperative regimen uses MAP with less than half the dose intensity of methotrexate than that used in EURAMOS, adding ifosfamide and cisplatin-etoposide postoperatively is beneficial. If preoperative therapy contains mostly methotrexate, adding additional active agents (adriamycin and cisplatin) postoperatively is beneficial. If the preoperative regimen is adriamycin and cisplatin, a postoperative regimen adding methotrexate and ifosfamide is beneficial. The fact that the EURAMOS study failed to show benefit from postoperative ifosfamide with their induction regimen does not mean that it has no value with other induction regimens, despite the size of the study, and since EURAMOS does not show improved disease-free survival to other studies, its size alone does not make it the new standard.

References

1. Antman KH, Ryan L, Elias A, Sherman D, Grier HE (1989) Response to ifosfamide and mesna: 124 previously treated patients with metastatic or unresectable sarcoma. J Clin Oncol 7:126–131
2. Ayala A, Mackay B, Jaffe N, Sutow W, Benjamin R, Murray J (1980) Osteosarcoma: the pathological study of specimens from en bloc resection in patients receiving preoperative chemotherapy. In: van Eys J, Sullivan MP (eds) Status of curability of childhood cancers. Raven Press, New York, pp 127–144
3. Ayala A, Raymond A, Jaffe N (1984) The pathologist's role in the diagnosis and treatment of osteosarcoma in children. Hum Pathol 15:258–266
4. Bacci G, Briccoli A, Ferrari S, Longhi A, Mercuri M, Capanna R, Donati D, Lari S, Forni C, DePaolis M (2001) Neoadjuvant chemotherapy for osteosarcoma of the extremity: long-term results of the Rizzoli's 4th protocol. Eur J Cancer 37(16):2030–2039
5. Bacci G, Campanacci M, Picci P, Pagani P (1979) Adjuvant multiple drug chemotherapy for osteosarcoma of the extremity: a 6 year report. Tumori 65(5):587–600
6. Bacci G, Ferrari S, Bertoni F, Ruggieri P, Picci P, Longhi A, Casadei R, Fabbri N, Forni C, Versari M, Campanacci M (2000) Long-term outcome for patients with nonmetastatic osteosarcoma of the extremity treated at the Istituto Ortopedico Rizzoli according to the Istituto Ortopedico Rizzoli/

Osteosarcoma-2 protocol: an updated report. J Clin Oncol 18(24):4016–4027
7. Bacci G, Loro L, Longhi A, Bertoni F, Bacchini P, Versari M, Picci P, Serra M (2006) No correlation between methotrexate serum level and histologic response in the pre-operative treatment of extremity osteosarcoma. Anti-Cancer Drugs 17(4):411–415
8. Bacci G, Picci P, Ferrari S, Ruggieri P, Casadei R, Tienghi A, Prever ABD, Gherlinzoni F, Mercuri M, Monti C (1993) Primary chemotherapy and delayed surgery for nonmetastatic osteosarcoma of the extremities. Results in 164 patients preoperatively treated with high doses of methotrexate followed by cisplatin and doxorubicin. Cancer 72(11):3227–3238
9. Bacci G, Picci P, Ruggieri P, Mercuri M, Avella M, Capanna R, Brach Del Prever A, Mancini A, Gherlinzoni F, Padovani G, Leonessa C, Biagini R, Ferraro A, Ferruzzi A, Cazzola A, Manfrini M, Campanacci M (1990) Primary chemotherapy and delayed surgery (neoadjuvant chemotherapy) for osteosarcoma of the extremities the istituto rizzoli experience in 127 patients treated preoperatively with intravenous methotrexate (high versus moderate doses) and intraarterial cisplatin. Cancer 65(11):2539–2553
10. Bacci G, Picci P, Gherlinzoni F, Van Horn JR, Orlandi M, Normand AR, Manfrini M, Pignatti G, Campanacci M (1986) Neoadjuvant chemotherapy for high grade osteosarcoma of the extremities: is a good response to preoperative treatment an indication to reduce postoperative chemotherapy? Chemioterapia 5(2):140–143
11. Bacci G, Ruggieri P, Picci P, Mercuri M, Ferraro A, Tella G, Ferrari S, Bertoni F, Comandone A (1996) Intra-arterial versus intravenous Cisplatinum (in addition to systemic Adriamycin and high dose methotrexate) in the neoadjuvant treatment of osteosarcoma of the extremities. Results of a randomized study. J Chemother 8(1):70–81
12. Baum ES, Gaynon P, Greenberg L, Krivit W, Hammond D (1979) Phase II study of cis-dichlorodiammineplatinum(II) in childhood osteosarcoma: children's cancer study group report. Cancer Treat Rep 63(9–10):1621–1627
13. Benjamin R (1989) Regional chemotherapy for osteosarcoma. Semin Oncol 16:323–327
14. Benjamin R, Murray J, Carrasco C, Raymond A, Chawla S, Wallace S, Ayala A, Papadopoulos N, Plager C, Romsdahl M (1984) Preoperative chemotherapy for osteosarcoma: a treatment approach facilitating limb salvage with major prognostic implications. In: Jones S, Salmon S (eds) Adjuvant therapy of cancer IV. Grune & Stratton, Orlando, pp 601–610
15. Benjamin RS, Legha SS, Patel SR, Nicaise C (1993) Single-agent ifosfamide studies in sarcomas of soft tissue and bone: the M. D. Anderson experience. Cancer Chemother Pharmacol 31(Suppl 2):S174–S179
16. Benjamin RS, Patel SR (2010) Pediatric and adult osteosarcoma: comparisons and contrasts in presentation and therapy. In: Jaffe N, Bruland OS, Bielack S (eds) Pediatric and adolescent osteosarcoma. Springer US, Boston, pp 355–363
17. Bielack SS, Smeland S, Whelan JS, Marina N, Jovic G, Hook JM, Krailo MD, Gebhardt M, Pápai Z, Meyer J, Nadel H, Randall RL, Deffenbaugh C, Nagarajan R, Brennan B, Letson GD, Teot LA, Goorin A, Baumhoer D, Kager L, Werner M, Lau CC, Hall KS, Gelderblom H, Meyers P, Gorlick R, Windhager R, Helmke K, Eriksson M, Hoogerbrugge PM, Schomberg P, Tunn P-U, Kühne T, Jürgens H, van den Berg H, Böhling T, Picton S, Renard M, Reichardt P, Gerss J, Butterfass-Bahloul T, Morris C, Hogendoorn PCW, Seddon B, Calaminus G, Michelagnoli M, Dhooge C, Sydes MR, Bernstein M (2015) Methotrexate, Doxorubicin, and Cisplatin (MAP) plus maintenance pegylated interferon alfa-2b versus MAP alone in patients with resectable high-grade osteosarcoma and good histologic response to preoperative MAP: first results of the EURAMOS-1 good response randomized controlled trial. J Clin Oncol 33(20):2279–2287
18. Cade S (1955) Osteogenic sarcoma; a study based on 133 patients. J R Coll Surg Edinb 1(2):79–111
19. Calvo D, Patt Y, Wallace S, Chuang V, Benjamin R, Pritchard J, Hersh E, Body G, Mavligit G (1980) Phase I-II trial of percutaneous intra-arterial cis-diamminedichloro platinum (II) for regionally confined malignancy. Cancer 45:1278–1283
20. Campanacci M, Bacci G, Bertoni F, Picci P, Minutillo A, Franceschi C (1981) The treatment of osteosarcoma of the extremities: twenty years' experience at the Instituto Ortopedico Rizzoli. Cancer 48:1569–1581
21. Chary KK, Higby DJ, Henderson ES, Swinerton KD (1977) Phase I study of high-dose cis-dichlorodiammineplatinum(II) with forced diuresis. Cancer Treat Rep 61(3):367–370
22. Chuang V, Wallace S, Benjamin R, Jaffe N, Ayala A, Murray J, Zornoza J, Patt Y, Mavligit G, Charnsangavej C, Soo C (1981) The therapy of osteosarcoma by intra-arterial cis-platinum and limb preservation. Cardiovasc Intervent Radiol 4:229–235
23. Cortes E, Holland J, Wang J, Sinks L, Blom J, Senn H, A. Bank, Glidewell O (1974) Amputation and adriamycin in osteosarcoma. N Engl J Med 291:998–1000
24. Cortes EP, Holland JF, Glidewell O (1978) Amputation and adriamycin in primary osteosarcoma: a 5-year report. Cancer Treat Rep 62(2):271–277
25. Delepine N, Delepine G, Cornille H, Brion F, Arnaud P, Desbois JC (1995) Dose escalation with pharmacokinetics monitoring in methotrexate chemotherapy of osteosarcoma. Anticancer Res 15(2):489–494
26. Delepine N, Delepine G, Jasmin C, Desbois JC, Cornille H, Mathé G (1988) Importance of age and methotrexate dosage: prognosis in children and young adults with high-grade osteosarcomas. Biomed Pharmacother 42(4):257–262
27. Delepine N, Desbois JC, Delepine G, Cornille H, Astier A, Alkallaf S (1989) Individualization of the dosage of high-dose methotrexate by assaying plasma concentration. Therapeutic value in osteogenic sarcoma. Bull Cancer 76(8):913–918

28. Edmonson J, Fleming T, Irvins J, Burgert EJ, Soule E, O'Connell M, Sim FH, Ahmann D (1984) Randomized study of systemic chemotherapy following complete excision of nonosseous sarcomas. J Clin Oncol 2:1390–1396
29. Eilber F, Giuliano A, Eckardt J, Patterson K, Moseley S, Goodnight J (1987) Adjuvant chemotherapy for osteosarcoma: a randomized prospective trial. J Clin Oncol 5:21–26
30. Ettinger L, Douglass H, Higby D, Mindell E, Nime F, Ghoorah J, Freeman A (1981) Adjuvant adriamycin and cis-diamminedichloroplatinum (cis-platinum) in primary osteosarcoma. Cancer 47:248–254
31. Ettinger L, Douglass H, Mindell E, Sinks L, Tebbi C, Risseeuw D, Freeman A (1986) Adjuvant adriamycin and cisplatin in newly diagnosed, nonmetastatic osteosarcoma of the extremity. J Clin Oncol 4:353–362
32. Ferrari S, Palmerini E, Staals EL, Mercuri M, Franco B, Picci P, Bacci G (2010) The treatment of nonmetastatic high grade osteosarcoma of the extremity: review of the Italian Rizzoli experience. Impact on the future. In: Jaffe N, Bruland OS, Bielack S (eds) Pediatric and adolescent osteosarcoma. Springer US, Boston, pp 275–287
33. Ferrari S, Smeland S, Mercuri M, Bertoni F, Longhi A, Ruggieri P, Alvegard TA, Picci P, Capanna R, Bernini G, Müller C, Tienghi A, Wiebe T, Comandone A, Böhling T, Del Prever AB, Brosjö O, Bacci G, Sæter G (2005) Neoadjuvant chemotherapy with high-dose ifosfamide, high-dose methotrexate, cisplatin, and doxorubicin for patients with localized osteosarcoma of the extremity: a joint study by the Italian and Scandinavian Sarcoma Groups. J Clin Oncol 23(34):8845–8852
34. Gherlinzoni F, Picci P, Bacci G, Campanacci D (1992) Limb sparing versus amputation in osteosarcoma: correlation between local control, surgical margins and tumor necrosis: Istituto Rizzoli experience. Ann Oncol 3(suppl_2):S23–S27
35. Huvos A, Rosen G, Marcove R (1977) Primary osteogenic sarcoma: pathologic aspect in 20 patients treated with chemotherapy, en bloc resection, and prosthetic bone replacement. Arch Pathol Lab Med 101:14–18
36. Jaffe N (1972) Recent advances in the chemotherapy of metastatic osteogenic sarcoma. Cancer 30(6):1627–1631
37. Jaffe N, Frei E, Traggis D, Bisop Y (1974) Adjuvant methotrexate and citrovorum-factor treatment of osteogenic sarcoma. N Engl J Med 291:994–997
38. Jaffe N, Frei E, Watts H, Traggis D (1978) High-dose methotrexate in osteogenic sarcoma: a 5-year experience. Cancer Treat Rep 62:259–264
39. Jaffe N, Knapp J, Chuang V, Wallace S, Ayala A, Murray J, Cangir A, Wang A, Benjamin R (1983) Osteosarcoma: intra-aterial treatment of the primary tumor with cis-diamminedichloroplatinum II (CDP). Angiographic, pathologic, and pharmacologic studies. Cancer 51:402–407
40. Jaffe N, Robertson R, Ayala A, Wallace S, Chuang V, Anzai T, Cangir A, Wang Y-M, Chen T (1985) Comparison of intra-arterial cis-diamminedichloroplatinum II with high-dose methotrexate and citrovorum factor rescue in the treatment of primary osteosarcoma. J Clin Oncol 3:1101–1104
41. Kamalakar P, Freeman AI, Higby DJ, Wallace HJ Jr, Sinks LF (1977) Clinical response and toxicity with cis-dichlorodiammineplatinum(II) in children. Cancer Treat Rep 61(5):835–839
42. Lee ES, Mackenzie DH (1964) Osteosarcoma. A study of the value of preoperative megavoltage radiotherapy. Br J Surg 51(4):252–274
43. Link M, Goorin A, Miser A, Green A, Pratt C, Belasco J, Pritchard J, Malpas J, Baker A, Kirkpatrick J, Ayala A, Shuster J, Abelson H, Simone J, Vietti T (1986) The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. N Engl J Med 314:1600–1602
44. Marcove RC, Miké V, Hajek JV, Levin AG, Hutter RV (1970) Osteogenic sarcoma under the age of twenty-one. A review of one hundred and forty-five operative cases. J Bone Joint Surg Am 52(3):411–423
45. Marina NM, Smeland S, Bielack SS, Bernstein M, Jovic G, Krailo MD, Hook JM, Arndt C, van den Berg H, Brennan B, Brichard B, Brown KLB, Butterfass-Bahloul T, Calaminus G, Daldrup-Link HE, Eriksson M, Gebhardt MC, Gelderblom H, Gerss J, Goldsby R, Goorin A, Gorlick R, Grier HE, Hale JP, Hall KS, Hardes J, Hawkins DS, Helmke K, Hogendoorn PCW, Isakoff MS, Janeway KA, Jürgens H, Kager L, Kühne T, Lau CC, Leavey PJ, Lessnick SL, Mascarenhas L, Meyers PA, Mottl H, Nathrath M, Papai Z, Randall RL, Reichardt P, Renard M, Safwat AA, Schwartz CL, Stevens MCG, Strauss SJ, Teot L, Werner M, Sydes MR, Whelan JS (2016) Comparison of MAPIE versus MAP in patients with a poor response to preoperative chemotherapy for newly diagnosed high-grade osteosarcoma (EURAMOS-1): an open-label, international, randomised controlled trial. Lancet Oncol 17(10):1396–1408
46. Marti C, Kroner T, Remagen U (1985) High dose ifosfamide in advanced osteosarcoma. Cancer Treat Rep 69:115–117
47. Mavligit G, Benjamin R, Patt Y, Jaffe N, Chuang V, Wallace S, Murray J, Ayala A, Johnston S, Hersh E, Calvo D (1981) Intra-arterial cis-platinum for patients with inoperable skeletal tumors. Cancer 48:1–4
48. Meyers PA, Schwartz CL, Krailo M, Kleinerman ES, Betcher D, Bernstein ML, Conrad E, Ferguson W, Gebhardt M, Goorin AM, Harris MB, Healey J, Huvos A, Link M, Montebello J, Nadel H, Nieder M, Sato J, Siegal G, Weiner M, Wells R, Wold L, Womer R, Grier H (2005) Osteosarcoma: a randomized, prospective trial of the addition of ifosfamide and/or muramyl tripeptide to cisplatin, doxorubicin, and high-dose methotrexate. J Clin Oncol 23(9):2004–2011
49. Meyers PA, Schwartz CL, Krailo MD, Healey JH, Bernstein ML, Betcher D, Ferguson WS, Gebhardt MC, Goorin AM, Harris M, Kleinerman E, Link MP, Nadel H, Nieder M, Siegal GP, Weiner MA, Wells RJ, Womer RB, Grier HE (2008) Osteosarcoma:

the addition of muramyl tripeptide to chemotherapy improves overall survival—a report from the Children's Oncology Group. J Clin Oncol 26(4):633–638
50. Mosende C, Gutierrez M, Caparros B, Rosen G (1977) Combination chemotherapy with bleomycin, cyclophosphamide and dactinomycin for the treatment of osteogenic sarcoma. Cancer 40:2779–2786
51. Murphy W, Benjamin R, Eyre H, Thigpen T, Whitecar J, Uribe-Bkotero G, Baker L, Gehan E, Gottlieb J (1977) Adjuvant chemotherapy in osteosarcoma of adults. A Southwest Oncology Group Study. In: Salmon S, Jones S (eds) Adjuvant therapy of cancer. North Holland Press, Amsterdam
52. Ochs J, Freeman A, Douglass H Jr, Higby D, Mindell E, Sinks L (1978) Cis-dichlorodiammine-platinum (II) in advanced osteogenic sarcoma. Cancer Treat Rep 62:239–245
53. Patel S, Vadhan-Raj S, Papadopoulos N, Plager C, Burgess M, Hays C, Benjamin R (1997) High-dose ifosfamide in bone and soft tissue sarcomas: results of phase II and pilot studies – dose response and schedule dependence. J Clin Oncol 15:2378–2384
54. Picci P, Capanna R, Bacci G, Mercuri M, Biagini R, Ruggieri P, Ferraro A, De Cristofaro R, Ferruzzi A, Campanacci M (1990) Margins, necrosis and local recurrence after conservative surgery in osteosarcoma. Chir Organi Mov 75:82–85
55. Platt H, Cade S, Baker SL (1952) Symposium on sarcoma of bone. Proc R Soc Med 45(5):265–265
56. Pratt CB, Horowitz ME, Meyer WH, Etcubanas E, Thompson EI, Douglass EC, Wilimas JA, Hayes FA, Green AA (1987) Phase II trial of ifosfamide in children with malignant solid tumors. Cancer Treat Rep 71(2):131–135
57. Pritchard J, Mavligit G, Benjamin R, Patt Y, Calvo D, Hall S, Bodey G, Wallace S (1979) Regression of regionally confined melanoma with intra-arterial cis-dichloro-diammineplatinum (II). Cancer Treat Rep 63:555–558
58. Raymond A, Chawla S, Carrasco C, Ayala A, Fainning C, Grice B, Armen T, Plager C, Papadopoulos N, Edeiken J, Wallace S, Jaffe N, Murray J, Benjamin R (1987) Osteosarcoma chemotherapy effect: a prognostic factor. Sem Diag Path 4:212–236
59. Rosen G, Caparros B, Huvos AG, Kosloff C, Nirenberg A, Cacavio A, Marcove RC, Lane JM, Mehta B, Urban C (1982) Preoperative chemotherapy for osteogenic sarcoma: selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to preoperative chemotherapy. Cancer 49(6):1221–1230
60. Rosen G, Marcove RC, Caparros B, Nirenberg A, Kosloff C, Huvos AG (1979) Primary osteogenic sarcoma. The rationale for preoperative chemotherapy and delayed surgery. Cancer 43(6):2163–2177
61. Rosen G, Murphy M, Huvos A, Guttierrez M, Marcove R (1976) Chemotherapy, en bloc resection, and prosthetic bone replacement in the treatment of osteogenic sarcoma. Cancer 37:1–11
62. Rosen G, Suwansirikul S, Kwon C, Tan C, Wu SJ, Beattie EJ Jr, Murphy ML (1974) High-dose methotrexate with citrovorum factor rescue and adriamycin in childhood osteogenic sarcoma. Cancer 33(4):1151–1163
63. Rosen G et al (1990) The successful management of metastatic osteogenic sarcoma: a model for the treatment of primary osteogenic sarcoma. In: Oosterom ATV, Muggia FM, Cleton FJ (eds) Therapeutic progress in ovarian cancer, testicular cancer and the sarcomas. Leiden University Press, Hingham, pp 244–265
64. Stewart D, Benjamin R, Zimmerman S, Caprioli R, Wallace S, Chuang V, Calvo D 3rd, Samuels M, Bonura J, Loo T (1983) Clinical pharmacology of intra-arterial cis-diamminedichloroplatinum(II). Cancer Res 43:917–920
65. Sutow W, Gehan E, Dyment P (1978) Multidrug adjuvant chemotherapy for osteosarcoma: interim report of the Southwest Oncology Group studies. Cancer Treat Rep 62:265
66. Sutow W, Sullivan M, Fernbach D, Cangir A, George S (1975) Adjuvent chemotherapy in primary treatment of osteogenic sarcoma. A Southwest Oncology Group study. Cancer 36:1598–1602
67. Taylor W, Ivins J, Dahlin D, Edmonson J, Pritchard D (1978) Trends and variability in survival from osteosarcoma. Mayo Clin Proc 53:695–700
68. Taylor W, Ivins J, Pritchard D, Dahlin D, Gilchrist G, Edmonson J (1985) Trends and variability in survival among patients with osteosardoma: a 7-year update. Mayo Clin Proc 60:91–104
69. Vietti TJ, Nitschke R, Starling KA, van Eys J (1979) Evaluation of cis-dichlorodiammineplatinum(II) in children with advanced malignant diseases: Southwest Oncology Group Studies. Cancer Treat Rep 63(9–10):1611–1614
70. Wang JJ, Cortes E, Sinks LF, Holland JF (1971) Therapeutic effect and toxicity of adriamycin in patients with neoplastic disease. Cancer 28(4):837–843
71. Winkler K, Bielack S, Delling G, Salzer-Kuntschik M, Kotz R, Greenshaw C, Jürgens H, Ritter J, Kusnierz-Glaz C, Erttmann R, Gädicke G, Graf N, Ladenstein R, Leyvraz S, Mertens R, Weinel P (1990) Effect of intraarterial versus intravenous cisplatin in addtion to systemic doxorubicin, high-dose methotrexate, and ifosfamide on histologic tumor response in osteosarcoma (study COSS-86). Cancer 66:1703–1710