CLINICAL INVESTIGATION

Treating pediatric osteosarcoma: recent clinical trial evidence

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Pediatric osteosarcoma is most commonly diagnosed during adolescence and young adulthood and requires treatment with surgical intervention and intensive chemotherapy. While the exact molecular mechanism leading to osteosarcoma has yet to be elucidated, some syndromes and genetic conditions have been associated with development of the disease. Treatment of osteosarcoma has always included surgical intervention with amputation being most common prior to the introduction of multi-agent chemotherapy. The introduction of multi-agent chemotherapy along with improved surgical techniques have allowed increasing use of limb-salvage surgery. Current investigations including the use of modern chemotherapy show no detectable survival difference between amputation and limb salvage surgery. Standard neo-adjuvant and adjuvant regimens using a combination of doxorubicin, cisplatin and methotrexate, with or without ifosfamide have become the standard in the medical management of osteosarcoma. Other newer agents, such as HER2 receptor monoclonal antibody and muramyl tripeptide phosphatidylethanolamine have also been investigated and are included in this review.

Keywords: chemotherapy • clinical trial • limb salvage • osteosarcoma • surgery • treatment

Alexandra K Abrams[1], Raffi S Avedian[2] & Neyssa Marina*[1]

[1]Stanford University & Lucille Packard Children's Hospital, Department of Pediatrics, Division of Hematology/Oncology, Stanford, CA, USA

[2]Stanford University Hospital & Clinics, Department of Orthopedic Surgery, Stanford, CA, USA

*Author for correspondence:
Tel.: +1 650 723 5535
Fax: +1 650 723 5231
E-mail: nmarina@stanford.edu

Malignancies of the bone have an annual incidence of 8.7 cases per million children under the age of 20 years [1]. Of those diagnosed with a malignant primary bone tumor, 56% have osteosarcoma [1]. Pediatric osteosarcoma typically arises during adolescence and is treated with multi-agent chemotherapy and surgical resection [2]. This article will review the epidemiology and treatment of pediatric osteosarcoma, with an emphasis on recent results from both medical and surgical clinical trials.

Primary osteosarcoma of the bone is the sixth most common cancer in children and adolescents with males affected more than females [3,4]. The annual incidence has been estimated to range from approximately 4.4 per 1 million people aged 0–24 years [5] to 5.4 cases per million individuals under the age of 20 years [1]. African Americans have a slightly higher incidence of the disease at 5.2 cases per 1 million people less than 20 years of age, while Caucasians have an incidence of 4.6 cases per 1 million people under 20 years of age. Males also have a slightly higher incidence of the disease (5.2 per million people under 20 for males vs 4.5 per million for females).

Osteosarcoma is most commonly diagnosed during adolescence and young adulthood, likely related to the rapid bone growth seen during this time [3,6]. The highest annual incidence of 8.9 per million is seen in those aged 15–19 years, with ages 10–14 years having a slightly lower rate [1]. A recent investigation examined a cohort of nearly 3000 patients diagnosed with osteosarcoma and only 1% were less than 5 years of age [7].

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The disease typically occurs in the metaphyseal region of the long bones of the extremities, with 78% localized to the lower extremity. Reports have indicated that approximately 64% occur around the knee (distal femur and proximal tibia) while 10% are localized to the humerus [8]. Although rare, primary osteosarcomas of the head and neck, particularly the mandible, have been reported [9]. In addition, primary osteosarcoma also occurs in the pelvis and this location presents a medical challenge since surgical resection tends to be more difficult [1].

Some authors have postulated a relationship between the occurrence of the tumor and rapid bone growth during adolescence [3,6]. The association is thought to be related to the higher rates of cell division during the adolescent growth-spurt and subsequent risk for mitotic error with each cell division cycle. Other conditions associated with osteosarcoma include mutations in the p53 (Li-Fraumeni syndrome) and retinoblastoma (Rb) genes, both of which serve as tumor suppressor genes. Bloom and Rothmund–Thompson syndrome are also associated with the development of osteosarcoma [10]. The *BLM* gene, a member of the RecQ DNA helicase family, is responsible for Bloom syndrome and functions to unwind the DNA during replication, transcription and repair [11]. Mutations in the *RECQL4* gene cause a majority of cases of Rothmund–Thomson syndrome, with this gene functioning in telomere maintenance [12].

Treatment overview

Long-term survival for patients with localized osteosarcoma increased dramatically with the introduction of multi-agent chemotherapy. However, recent reports suggest a plateau in outcome. Prior to the 1980s, surgical resection was the primary treatment modality for children and 5-year survival rates were in the range of 10–20% [3,13]. The introduction of multi-agent chemotherapy along with consistent local control with surgery has resulted in 5-year survival rates of 60–70% [13–16]. Current treatment regimens call for neoadjuvant (preoperative) therapy followed by surgical intervention and adjuvant (postoperative) treatment. The most common chemotherapy protocols include combinations of cisplatin, doxorubicin, ifosfamide, etoposide and high-dose methotrexate (MTX) with leucovorin rescue [17,18].

Treatment response predictors: immunohistochemistry

The histological response of the tumor following neoadjuvant chemotherapy is a reliable indicator for survival in patients with osteosarcoma [19–21]. Tumor necrosis in excess of 90% has been correlated with 5-year survival of 75–90% while necrosis of less than 90% results in 5-year survival of only 20–60% [19,20,22,23]. Unfortunately, the molecular and genetic factors that determine response to neoadjuvant chemotherapy have been difficult to elucidate, but are likely related to errors at the G1 cycle checkpoint that subsequently allows unchecked cell proliferation [24].

One of the most studied factors associated with prognosis for survival in osteosarcoma is HER2. The protein is structurally similar to the epidermal growth factor and overexpression of HER2 has been associated with malignant transformation in culture [25]. Other investigations have shown that HER2 expression was associated with pulmonary metastases, poor response to therapy and decreased survival in osteosarcoma patients [26]. In separate investigations, these findings were supported with results demonstrating higher frequencies of gene expression in patients with metastases at presentation as well as decreased event-free survival (EFS) in those with metastatic disease at presentation [25,27].

Other proteins that have been reported to be prognostic factors include p-glycoprotein and bcl-2 [27–29]. P-glycoprotein is a product of the *MDR-1* gene and is involved in the resistance pathway for a number of chemotherapeutic drugs used to treat osteosarcoma, including doxorubicin [29]. The bcl-2 family of proteins is involved in the regulation of apoptosis. Some proteins within this family prevent cell death (bcl-2) while others promote apoptosis [28]. Ferrari *et al.* investigated the cellular levels of p-glycoprotein and bcl-2 in tumor biopsy samples for patients with osteosarcoma. They found that recurrent pulmonary metastases showed increased levels of p-glycoprotein and bcl-2 compared with the primary tumor [27].

The cyclin-dependent kinase 4 inhibitor p16INK4a (P16) acts at the G1 checkpoint and its expression has been investigated as a prognostic marker for histological response. In their investigation, Borys *et al.* took samples from 40 patients with histologically confirmed osteosarcoma and performed immunohistochemistry using commercially available P16 monoclonal mouse antibody [30]. Percent necrosis was measured in post-resection specimens and correlated with a number of variables, including P16 expression. In both univariate and logistic regression analysis, P16 expression correlated positively with histological response, even after controlling for age, subtype, sex and location [30].

Others have investigated the expression of ezrin and its prognostic value in patients with osteosarcoma. Ezrin is a membrane-cytoskeleton linker protein involved in regulating growth and metastatic behavior of cancer cells [31,32]. Ferrari *et al.* examined the expression pattern of ezrin in a cohort of 67 patients with osteosarcoma [33]. They classified ezrin immunoreactivity as cytoplasmic versus in both the cytoplasm and cell membrane and graded the strength of expression using a numerical

score. While the amount or location of ezrin expression was not related to gender, site, alkaline phosphatase or lactate dehydrogenase serum levels, the 3-year probability of disease-free survival was significantly greater in patients with cytoplasmic immunostaining versus patients with cytoplasmic and membranous immunostaining [33].

Genomic information has also been investigated to determine response to chemotherapy for osteosarcoma [34]. In pretherapy biopsies from 45 osteosarcoma patients, the most frequent genomic alterations included amplifications of chromosome 6p21 (15.6%), 8q24 (15.6%, harboring MYC) and 12q14 (11.1%, harboring CDK4), as well as loss of heterozygosity of 10q21.1 (44.4%). These changes, as well as the total degree of heterozygosity, were significantly associated with an adverse outcome for patients [34]. Furthermore, other genomic data presented by Entz-Werlé *et al.* have demonstrated that *APC, MET* and *TWIST* genes showed correlation with a worse outcome or poor response to chemotherapy in a pediatric population of 91 high-grade osteosarcomas [35,36].

■ Summary

The histological response of the tumor following chemotherapy is a major predictor of survival, with tumor necrosis in excess of 90% portending a more favorable outcome. HER-2 overexpression has been associated with pulmonary metastases as well as decreased survival. Other proteins that have been linked to lower survival include MDR-1 and bcl-2 while those that show a positive effect on clinical outcomes are P16 and ezrin.

Treatment response predictors: clinical factors

While molecular markers may prove useful in determining patient prognosis, many investigations have also been performed on the patient-specific and clinical prognostic factors in osteosarcoma [37–41]. One of the largest studies to date examined clinical factors associated with survival and response to chemotherapy in 1702 patients with high-grade osteosarcoma. Bielack *et al.* found that axial tumor site, male sex and a longer duration of symptoms were associated with a poorer response to chemotherapy [42]. Older patient age, axial tumor site, presence of metastases, increased size of the tumor, proximal location within the limb, as well as poor response to chemotherapy and incomplete surgical resection were all associated with decreased 10-year survival.

Bacci *et al.* examined the records of 789 patients over a 15-year period to determine factors that were associated with local and systemic recurrence as well as overall outcome [37]. Factors examined included gender, age, serum levels of alkaline phosphatase, tumor site and size, pathologic fracture, type of surgery, chemotherapy protocol, surgical margins and histological response to preoperative treatment. They found that an age of less than or equal to 14 years, elevated serum alkaline phosphatase, tumor volume greater than 200 ml, inadequate surgical margins and poor histologic response were all significantly associated with risk of recurrence. In addition, an early two-drug chemotherapy protocol consisting of only high-dose MTX and cisplatin was inferior to multi-agent regimens in terms of risk of recurrence. The 5-year postrecurrence EFS was significantly lower for patients who had a local recurrence and metastases than for those with metastases only, with overall EFS improved with isolated lung lesions versus other locations [37].

In an investigation from the Children's Oncology Group, Janeway *et al.* examined a total of 1054 patients enrolled at a variety of North American sites to investigate the effects of patient age on outcome [43]. They found that an age of 18 years or greater was associated with a significantly poorer EFS. The 10-year EFS in patients <10, 10–17 and ≥18 years of age were 55, 55 and 37%, respectively. Overall survival in these groups were 68, 60 and 41%, respectively. The authors reported that the poorer survival in the over-18 age group was due to a higher rate of relapse. Other factors associated with poorer survival included metastatic disease at diagnosis, poor histologic response and pelvic tumor site [43].

■ Summary

These studies show elevated serum alkaline phosphatase, tumor volume greater than 200 ml, inadequate surgical margins, poor histologic response, metastatic disease at diagnosis and pelvic tumor sites as factors significantly associated with risk of recurrence and/or mortality. Age at diagnosis appears controversial since the Bacci trial concluded that age <14 years was a poor prognostic factor [37], while the Janeway trial concluded age >18 years as significantly associated with a poor outcome [43].

Results of surgical treatment: clinical trials

Surgical treatment for osteosarcoma of the extremities has advanced remarkably since the 1970s, when the only option was amputation. Magnetic resonance imaging became standard in the 1980s and allowed 3D visualization of the tumor and its relationship to critical anatomic structures such as arteries and major nerves [44]. This imaging capability along with refined limb reconstruction techniques such as megaprosthesis joint replacement, osteoarticular allografts and allograft prosthetic composites empowered surgeons to offer the majority of osteosarcoma patients limb salvage surgery. It is, however, important to emphasize that limb salvage

is not necessarily a superior option for all patients. Patients with endoprosthetic replacements or large allograft reconstructions are often not allowed to participate in high-impact activities and are at significant risk for complications such as infection, hardware failure or allograft fracture. In addition, young children who have significant growth remaining require multiple revision surgeries to maintain leg length equality. Ultimately, the choice of which type of surgery to undergo is a personalized decision that takes into account patient expectations, rehabilitation potential, age and risk tolerance for complications. In industrialized countries, the incidence of amputation due to malignancy has fallen from 0.62 per 100,000 people in 1988 to 0.35 per 100,000 people in 1996 [45]. Further evidence of this decreased rate of amputations comes from a large multicenter study that reported that the use of ablative surgery decreased from 60.1% in the 1980s to 31.4% in the 1990s [42].

Although many patients and surgeons may prefer limb salvage over amputation, there are several situations that may preclude a limb-sparing surgery. These conditions include: active infection, tumor encasement of neurovascular structures, tumor location that would make it difficult to achieve margin negative resection, young age and in some cases pathological fracture. The ability to perform limb-sparing surgery also depends on a number of factors. Limb-salvage surgery requires a multidisciplinary team of surgeons, medical oncologists, advanced imaging capabilities, limb reconstruction implants or allografts and appropriate physical therapy resources [46]. Many of these items are not as readily available in developing countries and patients may present with more advanced disease making amputation an important treatment modality [47]. However, limb-sparing surgery has become an important treatment alternative even in developing countries [46,47]. A recent investigation showed successful implementation of a low-cost chemotherapy and low-cost limb-salvage implant protocol in India [46]. In addition, limb-salvage surgery requires increased surgical expertise, with preservation of the neurovascular structures of the extremity needed to ensure a functional segment distal to the tumor location.

With the increasing use of limb-sparing surgery as an option, comparing patient outcomes between amputation and limb-salvage has become an important consideration. Simon *et al.* were some of the first to study these outcomes in patients with osteosarcoma, evaluating a total of 227 patients with tumors of the distal femur from 26 institutions worldwide [48]. The average age of patients in this study was 15.7 years and 73 individuals had limb salvage while 144 underwent amputation (115 above-knee amputation [AKA] and 39 hip disarticulation). The results revealed eight out of 73 (11.0%) local recurrences in the limb-salvage group, nine out of 115 (7.8%) in the AKA group and zero out of 39 (0%) in the hip disarticulation cohort. The rate of detectable synchronous metastatic disease was 43 out of 73 (58.9%) in the limb-salvage group, 65 out of 115 (56.5%) in the AKA cohort and 21 out of 39 (53.8%) in the hip disarticulation group. Caution should be given to strict interpretation of the rate of recurrence and metastatic disease in this study as many of the patients were treated before the consistent use of modern multiagent chemotherapy regimens. Kaplan–Meier survival analysis did not show any difference in overall survival or recurrent disease between the three groups after a mean of 5 years follow up. The authors updated the results in the above patient population at an average of 11 years follow up [49]. Again, there were no reported differences in the overall survival or the duration of postoperative disease-free period between the three groups. There was a significantly higher re-operation rate in the limb-salvage group.

Given these results, limb-salvage surgery has become the accepted form of treatment. It has been traditionally thought that limb-salvage procedures would provide improved psychological function because of maintenance of the extremity with subsequent improved cosmesis. Complications are more frequent in limb-salvage surgery and may affect the psychological function of the patient [50]. These complications can include nonunion, fracture, joint stiffness, leg-length discrepancy, endoprosthesis failure, or aseptic loosening of fixation parts [51–54]. On the other hand, amputations also have complications. Complications from amputation can include bleeding, infection, stump overgrowth, phantom limb pain and prosthetic limb fitting difficulties [55–57].

Further investigation regarding the functional outcomes of patients following amputation versus limb-salvage has been reported. In general, studies have reported improved functional scores for those with lower extremity osteosarcoma following limb salvage as compared with amputation [52,53,55,58–60]. Using two separate functional evaluations, including the Musculoskeletal Tumor Society scoring system, Rougraff *et al.* found that functional scores were higher for those who underwent limb salvage. There was no difference in the ability to walk, pain, or perceived quality of life between the groups [49]. The exception to this, however, was a study by Nagarajan *et al.* that investigated functional outcomes using the Toronto Extremity Salvage Score between amputees and limb-salvage patients. They found that in 528 long-term survivors with a prior lower extremity malignancy, including osteosarcoma, amputees were no more likely to have lower function and quality of life scores or self-perception of disability [61].

Many patients who undergo limb salvage surgery and achieve long-term survival after cancer treatment will likely require some form of revision surgery to repair or maintain their salvaged limb. Schwartz *et al.* reported on the University of California – Los Angeles (CA, USA) experience with distal femur replacements and noted overall implant survival at 10, 20 and 25 years to be 77.2, 57.9 and 50.2%, respectively [62]. The definition of failure was removal of the implant for any reason including infection, loosening or breakage. Similarly, the University of Toronto (Toronto, Canada) group reported on 99 patients who underwent uncemented tumor prosthesis about the knee and found that the mean survival of distal femur and proximal tibia implants was 123.6 and 112.5 months, respectively [63]. The rate of infection was 10% and this was the most common mode of failure.

Allograft reconstructions are another option for limb salvage surgery and although they offer a biological form of limb reconstruction, they carry a significant risk of complications. Mankin *et al.* reported on his extensive experience with 818 allograft reconstructions and noted allograft fracture in 19% of patients, nonunion in 17%, infection in 11% and 6% had unstable joints [64]. They concluded that although the overall complication rate was high in those patients who were able to retain their allografts, after 3 years the complication rate dropped significantly and patients had very good outcomes. More recently Ogilvie *et al.* indicated that in 20 patients who had undergone osteoarticular allograft limb reconstructions and had minimum 10-year follow up (mean 16 years), there was a 70% complication rate [65]. Fracture occurred in nine patients, nonunion in four, infection in two and arthritis in five. However, in those patients who retained their allograft the functional outcome score was very high (95 points) on the Toronto Extremity Salvage Score.

The size of the resection margin is a clinical factor that has important ramifications on the risk of local recurrence and survival. However, determining what constitutes a satisfactory margin varies according to the grade of the tumor, the effect of adjuvant therapy, the type of tissue at the margin (e.g., cortical bone versus cancellous bone) and tumor biological factors. As a result, there is significant controversy in the definition of a 'good' margin. There is a trend however, to decrease the size of the margin in favor of retaining critical anatomy and preserving healthy tissues. For example, many surgeons will dissect a major nerve, leaving the 1–2 mm epineurium as the margin instead of resecting the nerve and leaving the patient with a major functional deficit. Similarly, several authors have indicated that bone cuts around a tumor may be as close as 1 cm without putting the patient at risk of local recurrence [66,67]. This type of bone-preserving tumor resection may lead to improved recovery and improved long-term functional outcomes for patients.

In summary, the surgical treatment for osteosarcoma includes removal of the tumor with clear margins. This can be done with amputation or a limb-sparing surgery. Both of these options have advantages and disadvantages and the decision on which surgery to choose is a personal one that must be tailored to the patient and the clinical scenario. Although the definition of a good margin remains controversial, many surgeons will balance oncological resection with tissue preservation in the hope of maintaining patient function and speeding recovery while minimizing the risk of recurrence.

■ Summary

While amputation remains an option, limb salvage has become the most common method of treatment for osteosarcomas of the extremity. In general, studies have reported improved functional scores for those with lower extremity osteosarcoma following limb salvage as compared with amputation while maintaining equivalent recurrence and mortality. The complication rate following these procedures, however, remains high.

Results of chemotherapy: clinical trials

■ Development of current chemotherapy protocols

The most common chemotherapy protocols worldwide include combinations of cisplatin, doxorubicin and high-dose MTX with or without ifosfamide [17,18]. The goal of this section will be to provide background on how multi-agent chemotherapy treatment for osteosarcoma evolved, as well as to update the reader on new therapies under investigation. Therefore, this section will review previous trials in the treatment of osteosarcoma as well as focus on the more recently published results investigating new therapeutic targets, available in the literature.

Before the introduction of multi-agent chemotherapy, surgical resection was the treatment of choice for primary osteosarcoma. Cores *et al.* were some of the first to investigate the use of chemotherapy for the treatment of osteosarcoma [68]. The multi-institutional osteosarcoma study established the importance of multi-agent chemotherapy in the management of extremity, non-metastatic osteosarcoma. Following that report, multi-institutional efforts continued investigating various multi-agent chemotherapy in a series of studies beginning in 1977 [69,70] and included Link *et al.* who concluded that adjuvant chemotherapy increases the chances of relapse-free survival of patients with high-grade osteosarcoma [71].

During this time, Rosen *et al.* identified the importance of histological response on outcome and reported that altering postoperative treatment based

on histological response improved outcomes [72]. In their study, patients with primary osteosarcoma of the extremity were treated with high-dose MTX and citrovorum factor rescue, Adriamycin® and the combination of bleomycin, cyclophosphamide and dactinomycin (BCD). Patients with greater than 90% tumor necrosis at resection were continued on the same regimen while those with a poor histologic response were treated with cisplatin, adriamycin and BCD. Of the 35 patients with poor histologic response who were subsequently transitioned to the different regimen, 91% remained free of local or metastatic disease at a mean of 20 months [72].

Another multi-institutional study investigated the effects of neo-adjuvant chemotherapy on tumor histological response using cisplatin versus the BCD regimen [73]. In this study, all patients received high-dose MTX and doxorubicin. They were then randomized to receive either cisplatin or the triple-drug combination BCD as the third drug and were randomized a second time to receive or not to receive interferon. While there was no difference in cumulative disease-free survival between the chemotherapy groups, the authors reported that survival rates of patients demonstrating greater than 50% tumor cell destruction following neo-adjuvant chemotherapy was significantly improved versus those with less than 50% tumor cell necrosis [73].

Based on the significant toxicity associated with administration of high-dose MTX, especially in young adults, the European Osteosarcoma Intergroup has performed a series of studies examining the effectiveness of a two-drug regimen consisting of cisplatin and doxorubicin versus multi-agent regimens [74–76]. Souhami *et al.* reported on 407 patients with operable, non-metastatic osteosarcoma who were randomized to receive either cisplatin and doxorubicin or a multi-agent regimen of preoperative vincristine, high-dose MTX and doxorubicin and postoperative regimen of bleomycin, cyclophosphamide, dactinomycin, vincristine, MTX, doxorubicin and cisplatin [76]. Overall survival was 65% at 3 years and 55% at 5 years in both groups with progression-free survival at 5 years of 44% in both groups. The number of patients with a good histological response following neo-adjuvant treatment were not significantly different between groups [76]. Given the increased toxicity noted in the multi-agent group with equivalent survival rates, the authors concluded that the two-drug regimen was preferable.

Even though some members of the European Osteosarcoma Intergroup advocated maintaining a two-drug regimen based on their investigations, other groups in both Europe and the USA reported better outcomes with regimens including MTX, ifosfamide, as well as cisplatin, doxorubicin, bleomycin, cyclophosphamide and Adriamycin [22,23,77–79]. Fuchs *et al.* reported results of a trial in which patients received doxorubicin, cisplatin and high-dose MTX in the preoperative period [78]. Patients received ifosfamide as a fourth agent if they had any of the following criteria: tumor length greater than a third of the bone, greater than 20% chondroid ground substance on histology, or less than 20% reduction in tumor size following initial treatment. The authors included 171 patients in the study and found overall and event-free survival rates at 10 years were 72 and 66%, respectively. Prognosis for the low- and high-risk groups did not differ significantly. The effectiveness of additional agents in the adjuvant setting was further supported by data reported by Smeland *et al.* [22]. In total, 113 patients with classical osteosarcoma received neo-adjuvant chemotherapy consisting of high-dose MTX, cisplatin and doxorubicin. Poor histologic responders were additionally given etoposide/ifosfamide combination. Metastasis-free and overall survival rates at 5 years are 63 and 74%, respectively.

■ Recent clinical trials with standard agents

Ferrari *et al.* investigated the effect of incorporating ifosfamide to the standard chemotherapeutic regimen of MTX, cisplatin and doxorubicin as adjuvant treatment in patients with non-metastatic osteosarcoma of the extremity [80]. Ifosfamide was given postoperatively when pathologic response to the standard regimen was poor (group A) or given in the primary phase of chemotherapy with MTX, cisplatin and doxorubicin (group B). With a total of 246 patients enrolled and a mean follow up of 66 months, 5-year overall survival (73 vs 74%) and EFS (64 vs 55%) did not differ between groups A and B, respectively. Group B was also noted to have a higher incidence of hematologic toxicity [80].

Some investigators have questioned the need for high-dose MTX in the setting of multi-agent chemotherapy as the MTX toxicity may interfere with dose-intensive delivery of other agents [74,76]. A multicenter study by Daw *et al.* examined frontline treatment for newly diagnosed, non-metastatic, resectable osteosarcoma in 72 patients [81]. Treatment entailed 12 cycles of chemotherapy administered over 35 weeks. The regimen consisted of three cycles of carboplatin and ifosfamide daily for 3 days and one cycle of doxorubicin daily for 3 days before surgical resection. This was followed by two additional cycles of the combination of carboplatin and ifosfamide and three cycles each of doxorubicin daily for 2 days combined with ifosfamide or carboplatin. Though this was a single-arm study the authors compared its results with an earlier investigation that had evaluated the combination of carboplatin and ifosfamide given as up-front window therapy, plus doxorubicin and high-dose MTX [82]. There was no difference between groups with respect to 5-year EFS, overall survival or local failure [81]. The authors reported that the chemotherapy was well tolerated with no reports

of grade 3 or 4 serum creatinine toxicity or ototoxicity. Two patients had grade 3 hyperbilirubinemia and six had a grade 3 increase in hepatic transaminase activity [81]. Since this was not a randomized study, but rather a historical control study, the conclusions drawn should be taken with caution especially since a previous study had reported that carboplatin produced inferior responses in patients with metastatic osteosarcoma [83]. Besides the concerns regarding the use of historical controls, one must also consider that the earlier study [82] utilized a 5 cm margin for surgical resection while the most recent investigation used 3 cm [81].

■ Recent clinical trials with novel agents

As HER2 is known to be overexpressed in certain patients with osteosarcoma, there has been interest in blocking the activity of this receptor. Trastuzumab, a HER2 receptor monoclonal antibody, blocks the receptor from binding its ligand and subsequently initiating downstream effects. Ebb *et al.* evaluated 96 patients newly diagnosed with osteosarcoma and found that 41 were positive for HER2 by immunohistochemistry [84]. Those without HER2 expression were given standard treatment of cisplatin, doxorubicin, MTX, ifosfamide and etoposide, while those showing HER2 overexpression also received concurrent therapy with trastuzumab given for 34 consecutive weeks. There was no significant difference between the groups in either 30 month EFS or overall survival [84].

Muramyl tripeptide phosphatidylethanolamine (MTP-PE) has been investigated in the adjuvant setting in the treatment of patients with osteosarcoma. MTP-PE is a synthetic lipophilic analog of the bacterial cell wall. The molecule has been successfully incorporated into liposomes, allowing targeted delivery of MTP-PE to specific tissues, activating cells to become tumoricidal [85]. The effects of liposomal MTP-PE have been investigated in osteosarcoma animal models and has shown antitumor activity [86,87]. Meyers *et al.* examined the effect of MTP-PE on survival outcomes in patients with non-metastatic osteosarcoma [88]. A total of 667 patients were enrolled and randomized into one of four different regimens using a 2 × 2 factorial design. Patients were randomized up front to receive either regimen A consisting of doxorubicin, cisplatin and high-dose MTX or regimen B consisting of doxorubicin, ifosfamide and high-dose MTX. Following neoadjuvant treatment and definitive surgery postoperative chemotherapy was continued and patients were randomly assigned to continue with chemotherapy or to further receive MTP-PE. MTP-PE was initiated at week 12 and monitored for signs of biologic activity. It was administered twice weekly for 12 weeks beginning at week 12 and then weekly (starting at week 24) for an additional 24 weeks. MTP-PE administration was not interrupted for delays in chemotherapy. The authors found that the addition of MTP-PE to standard chemotherapy achieved a 3-year EFS of 68% while the addition of ifosfamide to the standard three-drug regimen resulted in a 3-year EFS of 61%. Interestingly, the addition of ifosfamide and MTP-PE to the standard three-drug regimen produced a 3-year EFS of 78%. The study concluded that the addition of MTP-PE may improve EFS in patients with non-metastatic osteosarcoma, but the addition of ifosfamide in the dose and schedule used in this study did not improve EFS. This publication suggested there was an interaction between the two interventions, which precluded analysis of the study as originally planned.

In a follow-up report with further follow up, there appeared to be no evidence of an interaction [89]. The authors reported that treatment with regimen A without MTP-PE was associated with a 66 and 64% probability of EFS at 4 and 6 years, respectively. The addition of MTP-PE to regimen A did not significantly alter EFS (65 and 63% probability of EFS at 4 and 6 years, respectively). Regimen B without MTP-PE resulted in 60 and 58% probability of EFS at 4 and 6 years, respectively, while the addition of MTP-PE to regimen B resulted in 74 and 71% probability of EFS at 4 and 6 years, respectively. Since there was no evidence of an interaction, the authors conducted the planned statistical analysis and the results indicated an improvement in 6-year overall survival from 70 to 78% ($p = 0.03$; relative risk = 0.71) with the addition of MTP-PE. Given the controversies regarding the possibility of an interaction, the role of MTP-PE in the USA remains unclear (it is not approved by the US FDA). However, MTP-PE is approved in Europe for the treatment of patients with localized, extremity osteosarcoma.

■ Clinical trials in the treatment of metastatic osteosarcoma

Harris *et al.* and the Pediatric Oncology group, evaluated the treatment of metastatic osteosarcoma at diagnosis. A total of 30 patients received two courses of ifosfamide followed by surgery on the primary tumor as well as metastases [90]. In total, 26 patients presented with pulmonary metastases only. Postadjuvant chemotherapy consisted of high-dose MTX, ifosfamide, doxorubicin and cisplatin. The 5-year EFS was 47% and the overall 5-year survival was 53%. Among the patients with bilateral pulmonary metastases, the 5-year EFS was 36% while those with unilateral pulmonary involvement was 75%. The toxicity of the regimen was relatively low, with seven of the 30 patients experiencing renal toxicity in the form of hypophospatemia and/or hypokalemia [90].

Bacci *et al.* reported on a slightly different regimen for 28 patients with metastatic disease at presentation [91]. In their investigation, treatment consisted of cisplatin, Adriamycin, high-dose MTX and ifosfamide followed

by simultaneous resection of primary and metastatic lesions. Six of the patients had complete disappearance of the pulmonary metastases. With a median follow-up of 32 months, 55% remained continuously free of disease, 11 relapsed with new metastases and one died of chemotherapy-related toxicity. The 2-year disease-free survival and overall survival were 36 and 53%, respectively [91].

Chou *et al.* have also reported the results of randomizing metastatic patients to chemotherapy with or without MTP-PE in a separate stratum of the trial mentioned above [92]. The authors reported a 5-year EFS for Regimen A without MTP-PE of 29% while the addition of MTP-PE to regimen A resulted in a 5-year EFS of 41%. The 5-year EFS for Regimen B without MTP-PE was 23% and the addition of MTP resulted in a 5-year EFS of 44%. While a potential trend towards increased EFS existed in the MTP-PE groups, there was no statistically significant difference. Similar results were reported for overall survival, with no significant difference between groups [92]. It is important to consider that this trial was not powered to evaluate this question for the subset of patients with metastatic osteosarcoma.

Treating relapsed and unresectable high-grade osteosarcoma after treatment with standard multi-agent chemotherapy can be a significant challenge. Grignani *et al.* recently investigated the use of sorafenib in this group of patients [93]. Sorafenib is an orally active multi-kinase inhibitor that targets MAPK, VEGF receptor, PDGF receptor and other tyrosine kinase receptors [94]. A total of 35 patients were enrolled all of whom had developed disease progression following standard therapy. At 4 months, progression-free survival was 46% with median progression-free survival of 4 months and median overall survival of 7 months. Response rates included 8% partial responses, 6% minor responses (<30% tumor shrinkage) and 34% stable disease.

■ Summary

The most common chemotherapy protocols worldwide include combinations of cisplatin, doxorubicin and high-dose MTX with or without ifosfamide. Overall 5-year EFS for non-metastatic primary osteosarcoma of the extremity ranges from 70–80%. Studies treating patients with metastatic disease at diagnosis through a variety of multi-modal regimens had a 5-year EFS ranging from 20–50%. Novel agents still under investigation in the treatment of osteosarcoma include trastuzumab, a HER2 receptor monoclonal antibody, as well as MTP-PE.

Future perspective

The understanding and treatment of osteosarcoma has significantly advanced over the last 40 years from a time when surgical resection was the only treatment alternative. We appear, however, to have reached a plateau in outcome and further improvements will require a better understanding of the biology of osteosarcoma with the goal of developing targeted therapy. One area of research focus is that of targeted cell therapy and the identification of cancer stem cells (CSC). CSCs are thought to represent a small subpopulation of cancer cells with unlimited proliferative capacity that drive tumor self-renewal and differentiation. Reports have indicated that they have been identified in certain types of malignancies, such as leukemia [95] as well as bone sarcomas [96]. Current identification of these cells has relied on the presence of unique cell-surface marker combinations, with one investigation demonstrating the existence of a small subpopulation of self-renewing bone sarcoma cells that can form clonal, spherical colonies and expressed genes thought to be associated with embryonic stem cells [96]. The CSC theory has important implications for metastatic disease as it has been shown that these cells are preprogrammed to preferentially spread to specific tissue types (i.e. the pulmonary system in osteosarcoma). Should these cells be identified in osteosarcoma, it may allow for novel targeted therapies to be developed that attack only the CSCs. This may reduce the need for traditional chemotherapy.

In addition, the use of bisphosphonate, in particular the third-generation bisphosphonates zoledronic acid (ZA), is being investigated as a microenvironment regulator in the treatment of osteosarcoma. While ZA has been shown to reduce the rate of skeletal events in many adult cancers [97,98], preclinical data have also shown it to inhibit proliferation, decrease viability and induce apoptosis in various tumor cells [99]. Chang *et al.* investigated the effect of ZA on one particular osteosarcoma cell line. They found that ZA reduced cell viability and increased cell apoptosis [100]. These results suggest that ZA has direct effects on osteosarcoma cell growth and apoptosis and may be a viable adjunct to current treatment regimens. Recently, the Children's Oncology Group published a feasibility and dose discovery analysis for ZA in the treatment of osteosarcoma [101] and the drug is also currently under investigation in a randomized French trial.

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Executive summary

Background

- Osteosarcoma is the most common primary bone malignancy in children.

Pathophysiology

- While certain genetic mutations account for the development of some tumors, most cases of osteosarcoma arise *de novo*.
- Elevated serum alkaline phosphates, tumor volume greater than 200 ml, inadequate surgical margins, poor histologic response and the presence of metastases are significantly associated with risk of recurrence and or decreased overall survival.

Surgical treatment

- Amputation was the initial management of osteosarcoma of the extremity. With the advent of improved prosthesis technology as well as more advanced imaging and surgical techniques, limb-sparing surgery has become common-place for osteosarcoma of the extremity.

Chemotherapeutic treatment

- The most common chemotherapy protocols worldwide include combinations of cisplatin, doxorubicin and high-dose methotrexate with or without ifosfamide.

Conclusion

- Surgical and medical management have led to overall 5-year survival rates of approximately 70–75% for those with primary, non-metastatic, osteosarcoma at presentation.

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