Osteosarcoma: A Review of Diagnosis, Management, and Treatment Strategies

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Abstract: Despite significant advancements in the diagnosis and treatment of osteosarcoma to date, overall survival has remained relatively constant for over 2 decades. The challenge in osteosarcoma stems from the extreme variability from one tumor to the next, making it unlikely that a single target approach would be able to address all or even a majority of patients. Awareness, education, and proper referral patterns serve to minimize avoidable errors in diagnosis and treatment. However, it is unlikely that these efforts alone will significantly improve survival outcomes. Modern multi-agent chemotherapy has resulted in the greatest improvement in overall survival to date, and it is very likely that future improvements in survival will arise from combination-targeted chemotherapy in addition to conventional treatment.

Introduction

Osteosarcoma is a primary mesenchymal tumor that is characterized histologically by the production of osteoid by malignant cells.[1] It is a relatively rare malignancy, with approximately 900 new cases reported in the United States per year.[2] It represents less than 1% of cancers reported within the United States, with a peak incidence of 4.4 cases per million per year in the adolescent and young adult population.[3] Despite their rarity, osteosarcomas are the most common primary malignancy of bone,[4] representing approximately 3.4% of all childhood cancers and 56% of malignant bone tumors in children.[5]

Epidemiology

Osteosarcoma follows a bimodal distribution, with an initial peak in the late adolescent and young adult period and a second peak during or after the 6th decade of life. Although adolescent and young adult osteosarcomas are nearly always considered primary, one-third to one-half of the adult tumors are classified as secondary,[6,7] resulting, for example, from malignant transformation of Paget disease

of bone or, less commonly, another benign bone lesion. A multi-institutional review of the Japanese population reports a lower incidence of Paget disease and a higher incidence of primary adult osteosarcoma, suggesting either a geographic or ethnic influence on incidence.[8]

Adolescent and early adult osteosarcoma (ages 0–24 years) occurs at an age-adjusted incidence of 4.4 per million within the United States.[6] Incidence is higher among males (male:female ratio, 1.43:1), but peaks earlier among females (age 12 vs 16 years). An association between rapid bone growth and osteosarcoma has been argued, given the tumor's typical metaphyseal location and its peak incidence during adolescence and early adulthood. Support for this theory includes a 185-fold risk in large-breed compared with small-breed canines.[9] Patients with osteosarcoma have been found to be significantly taller than the general population[10,11] and the earlier age of onset in the female population also seems to indirectly support this theory, given earlier skeletal growth and maturity in females compared with males. Incidence is highest among Asian/Pacific Islanders (5.3 per million) followed by blacks (5.1 per million), Hispanics (4.9 per million), whites (4.4 per million), and American Indian/Alaskan natives (3.0 per million). The second peak, between the ages of 60 and 85, demonstrates an incidence within the United States of 4.2 per million, and is overall more common among females (male:female ratio, 0.89:1), though Paget-associated osteosarcoma is more frequent among males (male:female ratio, 1.58:1). The greatest incidence within this age group is among blacks (4.6 per million), followed by whites (3.7 per million), Hispanics (3.0 per million), American Indian/Alaskan natives (2.9 per million), and Asian/Pacific Islanders (1.9 per million).

Pathogenesis

Several risk factors for the development of osteosarcoma are well established. The use of ionizing radiation for the treatment of childhood solid cancers has been well implicated in the development of a second malignancy,[12,13] of which osteosarcoma is the most likely to develop within the first 2 decades following treatment.[12,14] A more recent review of 108 secondary sarcomas in patients surviving all types of childhood malignancies found osteosarcoma to be the second most common cancer, occurring in 31 of 100 classifiable tumors.[15] This association has been attributed to high cumulative radiation doses[16] as well as high doses of alkylator or anthracycline-containing chemotherapies.[12,14,17] More recently, it has been shown that even after radiation and chemotherapy treatments are controlled for, primary childhood sarcoma survivors are at an increased risk for a second malignancy, with osteosarcoma occurring approximately one-third of the time.[15] A review of 3,482 cases using the Surveillance, Epidemiology and End Results (SEER) 17 database found the overall incidence of osteosarcoma as a second cancer to be 10%.[3]

A second recognized risk factor for the development of osteosarcoma is Paget disease of bone, or osteitis deformans, which is an uncoupling of bone formation and resorption resulting in an accelerated rate of bone turnover. The incidence of malignant transformation of Paget disease is approximately 1%,[18] relatively unchanged from historical reviews.[19,20] Although histologically the same as spontaneous osteosarcomas, those arising from Paget disease demonstrate a remarkably poor outcome,[21] with no significant improvement in treatment or survival despite the advent of modern adjuvant treatments.[22] There is some evidence that the association between Paget and osteosarcoma is in fact a genetic predisposition, with both demonstrating a loss of heterozygosity involving, to varying extents, the distal end of chromosome 18.[23]

There are a number of inherited genetic conditions that predispose affected individuals to a variety of malignancies, among them osteosarcoma. These include hereditary retinoblastoma, Li-Fraumeni syndrome, Rothmund-Thomson syndrome, and Bloom and Werner syndromes. In patients carrying a germline mutation of the RB gene, osteosarcoma is the second most common malignancy to develop after retinoblastoma,[24] and it occurs at an incidence 500 times that of the normal population.[25] In general, greater than 70% of all osteosarcomas demonstrate an overt form of mutation in the RB gene.[26] Li-Fraumeni is a familial syndrome, which involves the germline mutation of p53, predisposing affected individuals to a multitude of cancers including breast cancer, brain cancers, soft tissue sarcomas, leukemia, adrenocortical tumors, and osteosarcoma. Osteosarcoma has been reported as being the second most common malignancy in this patient population, with an incidence of around 12%.[27] Approximately 71% of cases demonstrate a p53 tumor suppressor gene mutation on chromosome 17p13, implicating this genetic defect as a likely but not exclusive cause of malignancy in this syndrome.[28] Overall, the number of osteosarcoma cases associated with a germline p53 mutation is low, and in the pediatric population has been reported to be involved in only approximately 3% of cases.[29] Although over two-thirds of cases are associated with an overt mutation in the RB and p53 genes, it is conceivable that more elusive defects in their associated pathways exist. The role of RB and p53 may be far understated, and it has been postulated to be essential in the development of this and many other cancers.

Rothmund-Thomson syndrome, or poikiloderma congenitale, is a genomic instability syndrome with *RECQL4* gene mutations identified in approximately 70% of cases. Although sporadic osteosarcoma has not been linked to *RECQL4* gene mutations, osteosarcoma

develops in up to 32% of Rothmund-Thomson syndrome cases.[30] Although these patients present at an earlier age, their clinical course is similar to sporadic osteosarcoma.[31] The *RECQ* gene family has also been implicated in conditions such as Bloom syndrome (RECQL2) and Werner syndrome (RECQL3), both of which are associated with a wide variety of malignancies including osteosarcoma.

Clinical Presentation

Patients typically present with localized pain and swelling of the affected area, with the most frequent sites of disease in descending order being the metaphyseal bone of the distal femur, the proximal tibia, and the proximal humerus. Although mild blunt trauma is often reported as an antecedent event, no convincing evidence to support an association between trauma and osteosarcoma currently exists. Pain may initially be described as activity-related, but over time it often progresses to pain at rest and night pain. Pain is typically reproducible with palpation. Clinical symptoms frequently last for weeks to months prior to presentation and are commonly attributed to "growing pains." The median time from onset of symptoms to diagnosis is 4 months, though significant variability exists. Rarely, pathologic fracture is the presenting sign. Systemic complaints such as fever and weight loss are rare. Laboratory values are of little utility with the exception of alkaline phosphatase (ALP), which is elevated in approximately 40% of cases[32] and lactate dehydrogenase (LDH), which is elevated in approximately 30% of cases.[33] Normal pretreatment ALP levels have been associated with improved 5-year disease-free survival (67% vs 54%) and a longer time to disease recurrence (25 months vs 18 months).[34] LDH also offers prognostic information, with an extreme elevation portending a poor outcome.[35] Despite these and other studies, the clinical utility of these markers is debatable.

Approximately 10–20% of patients present with macroscopic evidence of metastatic disease and approximately 80% of patients present with microscopic metastatic disease, which is subclinical or undetectable using current diagnostic modalities. Metastatic disease typically develops hematogenously, with the most common sites of metastasis being the lungs followed by other bones. Skip metastases, previously described as occurring hematogenously, may represent locoregional events and may occur in a manner distinct from distant hematogenous spread. They are generally thought of as local noncontinuous spread of disease within the same bone as the primary tumor. While it may represent metastatic bone disease, it is currently unclear whether this process is exactly the same as more distant hematogenous spread. Regardless, the presence of skip metastases portends dismal prognosis and may reflect an inherently different biology in this subset of tumors.

The most reliable and important prognostic indicator currently available is the detection of metastatic disease at the time of presentation, with long-term outcomes reduced from 70% to less than 20% in such instances. Metastatic lung disease has a better prognosis than does either metastatic bone disease or skip metastases. Patients with lung disease who have fewer than 3 nodules and unilateral disease may have a survival advantage, probably because surgery can render such individuals free of disease. This advantage remains somewhat controversial, however, and it has been suggested that increased 5-year survival is related to tumor necrosis greater than 98% and a disease-free interval of greater than 1 year rather than nodule number or location.[36,37] Patients with either progressive tumor growth while undergoing systemic treatment or with recurrent disease have a less than 20% rate of long-term survival. Other commonly referenced prognostic indicators include LDH elevation and Huvos tumor necrosis grade,[38] following standard neoadjuvant chemotherapy administration and wide surgical resection. Interestingly, modifications of neoadjuvant treatment regimens to achieve better tumor necrosis thus far have not affected survival outcomes.[39] It has been speculated that Huvos grading simply describes inherent tumor responsiveness to chemotherapy and is not an indicator of systemic chemotherapy effectiveness, and furthermore, that manipulation of chemotherapy to improve local necrosis does not necessarily improve overall patient survival.

Diagnosis and Staging

Accurate diagnosis and staging are fundamental prerequisites for appropriate treatment planning, patient education and guidance, and patient participation in clinical trials. It is important that the treating team be experienced in the diagnosis and treatment of bone sarcomas to minimize iatrogenic morbidity and maximize diagnostic accuracy. Osteosarcomas are often treated at tertiary care facilities, which evaluate and treat these rare malignancies in multidisciplinary settings that serve to improve communication between physicians and coordination of patient care.

Imaging studies include plain radiographs of the involved bone and adjacent joint. Osteosarcoma typically appears as a mixed radiodense and lytic lesion arising in an eccentric manner from the metaphyseal bone. (Figures 1 and 2). There is frequently mass extension into the adjacent tissue. Cortical destruction and periosteal reaction are common, and typically manifest in a sunburst pattern. In addition, a Codman's triangle, or elevation

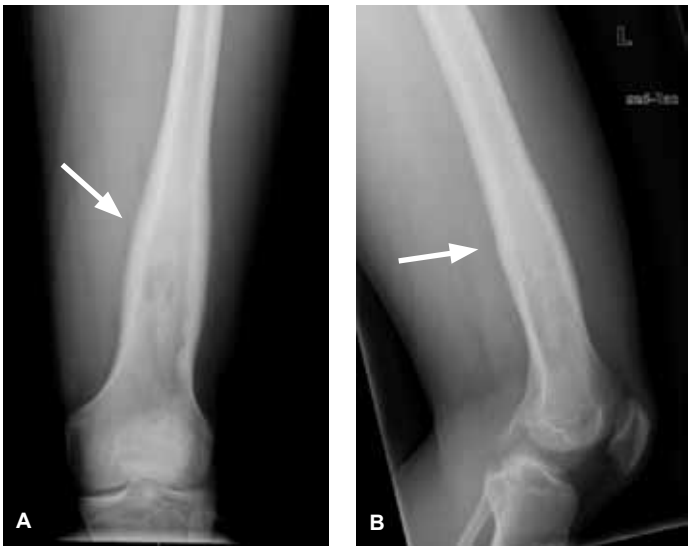

Figure 1. Anterior-posterior (A) and lateral (B) radiographs of a distal femoral osteosarcoma demonstrating a mixed radiodense and radiolucent lesion with associated periosteal reaction.

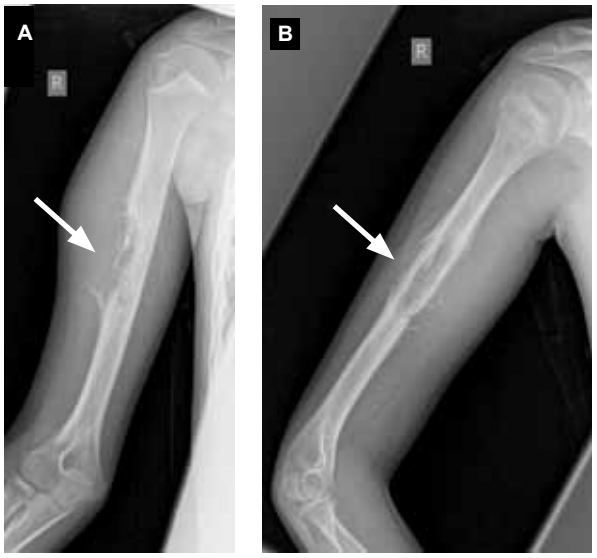

Figure 2. Anterior-posterior (A) and lateral (B) radiographs demonstrating a humeral diaphyseal lesion with extensive cortical destruction, soft tissue mass extension, and periosteal reaction.

of the periosteum at the tumor's periphery, is a classic though nonspecific feature. Osteosarcoma mineralizes in a centrifugal manner and should not be confused with myositis ossificans, which has an overall benign appearance and which ossifies in a centripetal fashion. The plain radiograph is very suggestive, and classic radiographic features should prompt the assumption that the lesion is a primary bone sarcoma until otherwise proven. Pain at rest, night pain, and progressive pain all warrant radiographic examination. Given the relative ease and safety of radiography, clinicians should maintain a low threshold for obtaining plain films.

A magnetic resonance imaging (MRI) study of the entire bone is warranted for anatomic evaluation of soft tissue extension, to assess proximity to surrounding structures, and to identify skip metastases (Figures 3 and 4). MRI studies can also suggest the rare but recognized phenomenon of tumor extension into the adjacent joint, which in the knee more commonly occurs by tumor growth along the cruciate ligaments.

Computed tomography (CT) scans are rarely obtained for the primary tumor, since soft tissue extension, local intramedullary extension, and intramedullary skip metastases are all better visualized using MRI. However, a CT scan of the thorax is currently the most sensitive noninvasive diagnostic modality available for the detection of metastatic disease within the lungs. Current diagnostic limitations preclude accurate detection of metastatic nodules under 5 mm in size, and therefore, a repeat CT scan for assessment of interval change in 6–12 weeks time is often recommended. CT scans have been shown to be inferior to manual tactile examination during open thoracotomies, with metastatic disease identified in up to one-third more cases using manual palpation.[40] In general, florid disease throughout the lungs can be diagnosed with CT scan alone; however, a single or a few small nodules should be histologically confirmed to be metastatic osteosarcoma.

Bone scintigraphy using technetium^{99} is employed for the detection of distant bone disease, which is the second most likely location for metastatic spread. Positive findings on bone scan may warrant additional imag-

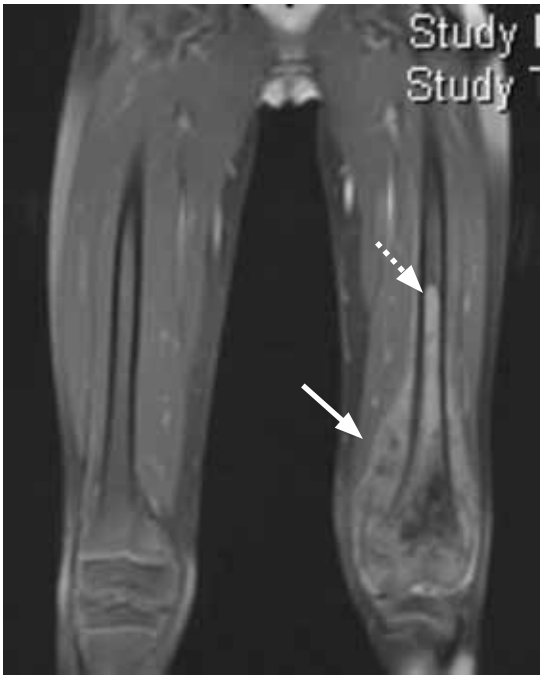

Figure 3. Post-contrast T1 fat suppressed coronal magnetic resonance image demonstrating large extraosseous tumor extension (solid arrow) and intramedullary extent (dashed arrow).

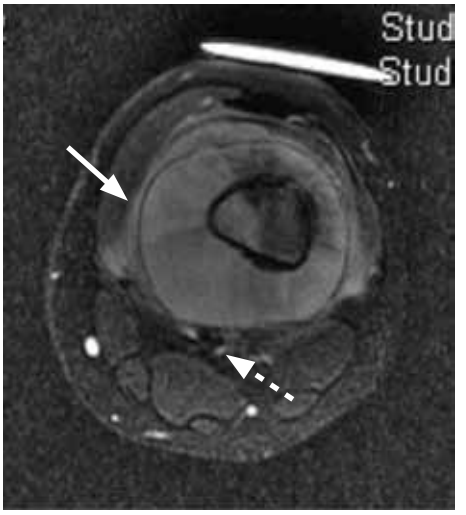

Figure 4. T2 axial magnetic resonance image demonstrating large extraosseous tumor extension with periosteal reaction (solid arrow). The neurovascular bundle is adjacent to but uninvolved in tumor (dashed arrow).

ing of the area of concern and, ultimately, a biopsy may be necessary to prove the definitive presence of distant bone disease.

The role of positron emission tomography (PET) in the setting of osteosarcoma continues to evolve. There has been some interest in using PET technology to assess histologic response to chemotherapy and/or to predict progression-free survival (PFS). At least 1 report has concluded that total lesion glycolysis before chemotherapy correlates with poor overall survival and that an increase in total lesion glycolysis after chemotherapy correlates with worse PFS.[41] The same authors reported that high post-chemotherapy maximum standardized uptake values (SUV), defined as more than 5 g/mL, correlated with poor overall survival, and high pre- and post-chemotherapy SUV (max) correlated with poor PFS. Currently, the exact role for PET imaging within the formal staging scheme remains unclear.

Biopsies should be performed at a tertiary care medical center with experience in sarcoma diagnosis and treatment. Ideally, the biopsy should be obtained either by the surgeon who will ultimately render definitive care or, in some instances, by a radiologist well versed in sarcoma core needle biopsy techniques. This concept is critical to oncologic and functional outcomes, and adverse effects associated with deviation from this approach have been well documented. Biopsies performed at a referring facility were compared with those performed at a treatment center, and results included a higher rate of major error in diagnosis (27% vs 12%), nonrepresentative biopsy results (14% vs 3.5%), alteration in treatment (36 vs 4%), and, most importantly, a change in outcome (17% vs 3.5%).[42] The biopsy should be performed as the final step in the staging process, after imaging studies have been reviewed and considered by the multidisciplinary sarcoma team. Biopsies may be performed in the operating room in an incisional open manner or as an outpatient procedure using a core needle technique. An incisional biopsy yields a large amount of tissue and enjoys the highest rate of diagnostic success, approximately 96%. Careful hemostasis is critical to minimize hematoma formation. Incision and drain site location are vital, as they ultimately need to be resected with the tumor in an en bloc manner. Core needle techniques are also acceptable, and in the setting of malignant bone tumors, yield reasonable, albeit reduced, diagnostic accuracy ranging from 74% to 88%,[43-45] with positive predictive value reported to be above 98%.[46] Fine needle aspiration, while useful for the identification of malignant cytologic features, provides too small a sample with no appreciable histologic architecture, and is not appropriate for the diagnosis of a primary sarcoma.

Surgical staging is performed using the Musculoskeletal Tumor Society staging scheme, originally developed and described by Enneking.[47] It defines tumors as being either low grade or high grade (I vs II), intracompartmental or extracompartmental (A vs B), and

Table 1. Musculoskeletal Tumor Society Staging System for Osteosarcoma

	Intracompartmental	Extracompartmental
Low Grade	IA	IB
High Grade	IIA	IIB
Metastatic	III	III

metastatic (III; Table 1). The American Joint Committee on Cancer (6th edition) has put forth a tumor, lymph nodes, metastases staging system, which arguably is of less surgical utility.

Surgical Treatment

Surgical treatment demands complete extirpation of the tumor together with any previously placed biopsy tract, drain tract, or potentially contaminated tissue. This should be a wide excision, meaning a normal cuff of tissue should surround or envelope the tumor, ensuring complete containment of malignant cells. Wide excisions may be realized through more ablative means such as an amputation or a disarticulation, or through more conservative means. The latter approach spares many of the uninvolved structures and allows for limb-salvage reconstruction. The decision as to whether a limb salvage procedure is appropriate needs to be objectively and accurately stated, and should be considered by the treating sarcoma team in advance of surgery. Though limb salvage surgery is often the preferred choice for many patients and families, it is not always the proper oncologic procedure, and optimizing oncologic outcomes takes priority over functional outcomes.

As most osteosarcomas arise within the metaphyseal bone and do not extend into the joint, intra-articular resections are most commonly offered. In the case of rare intra-articular tumor extension, an extra-articular resection becomes necessary. In the uncommon advent of a small tumor, a hemicortical or partial metaphyseal resection may be possible, which in some instances can yield much better long-term limb function. In the case of a purely diaphyseal lesion, intercalary resection of the involved diaphysis also offers excellent reconstructive and functional outcomes, sparing the adjacent joints and obviating the joint reconstruction typically required (Figure 5).

Intra-articular resections can be reconstructed in a variety of manners. Allograft bone can be obtained from commercial cadaveric bone banks and can be selected to match the patient's size and anatomy (Figure 6). Bulk osteoarticular allograft offers the benefit of restoring bone stock and postponing the need for joint replacement. If and when arthritis necessitates joint replacement, allograft allows for use of conventional joint replacement implants, which have improved longevity compared with that of megaprostheses. In addition, allograft bone is harvested with tendonous and/or ligamentous soft tissue attachments, allowing for a more physiologic soft tissue repair and improved joint function, in particular at the proximal tibia and the proximal humerus where the patellar ligament and the rotator cuff insert, respectively. Despite these advantages, allograft bone does have substantial limitations. Although it will heal to the adjacent host bone and will remodel for several millimeters at the allograft-host junction, it is not viable and does not

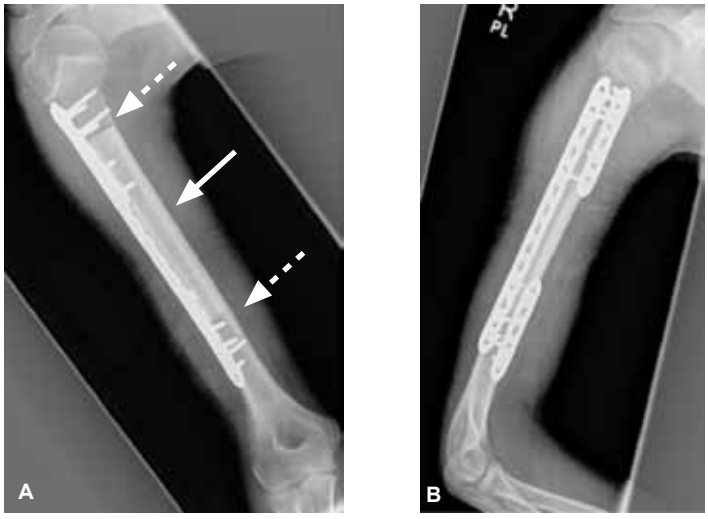

Figure 5. Anterior-posterior (A) and lateral (B) radiographs demonstrating an intercalary reconstruction using a size-matched allograft (solid arrow) and compression plating across the allograft-host junctions (dashed arrows).

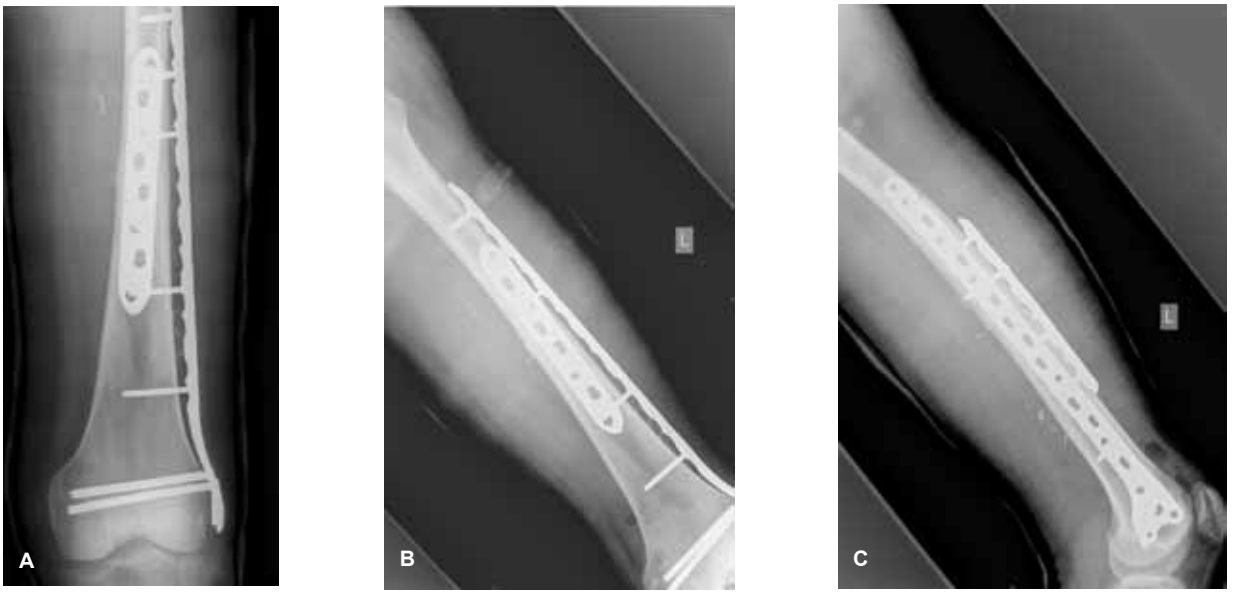

Figure 6. Anterior-posterior (A) and lateral views (B, C) of a distal femoral reconstruction using a sized-matched osteoarticular allograft and locking orthogonal large fragment plates.

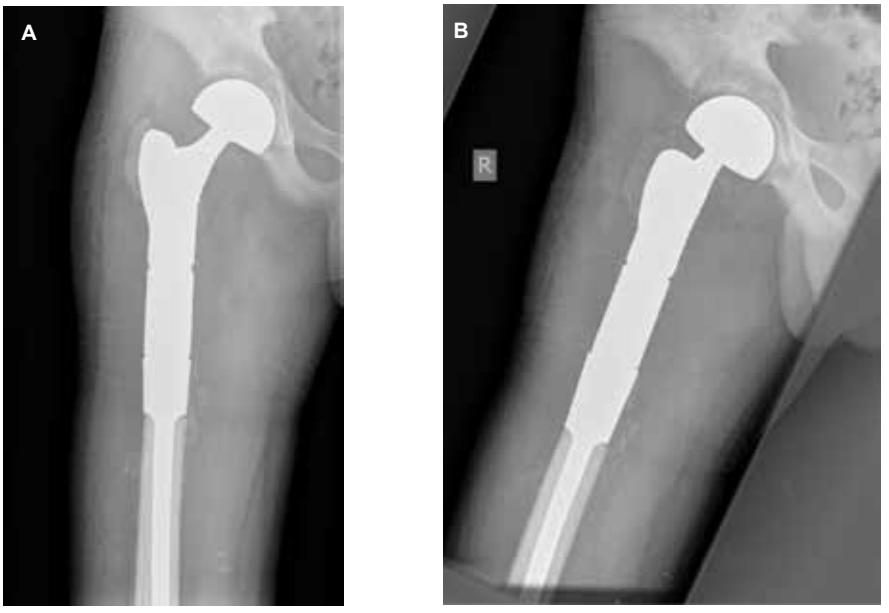

Figure 7. Anterior-posterior (A) and lateral (B) radiographs demonstrating reconstruction of a proximal femoral tumor using a cemented proximal femoral endoprosthesis.

ever fully re-vascularize. For this reason it is subject to non-union, fracture, and infection. Non-union has been shown to increase in the setting of either fracture or infection, and can occur in 11–27% of cases, with the higher rates seen in cases of concomitant chemotherapy administration.[48] Infection can occur in approximately 15% of cases[49] and fracture may occur in up to 27% of cases.[50] These complications are challenging and often require additional surgery, including, at times, amputation.

Intra-articular resections may also be reconstructed using endoprosthetic joint replacements or megaprostheses (Figure 7). Although historically these were custom made for each patient, they are currently modular and typically available as off-the-shelf implants. They can

replace a small segment of bone adjacent to the joint or, at the extreme, an entire bone as well as both adjacent joints. Theses modular systems support intraoperative needs, which may not always be anticipated. They allow for immediate weight bearing and immediate joint stability, resulting in improvement of joint motion and return to functional activity. They preclude additional splinting or casting and facilitate a return to independence for patients. Drawbacks include implant failure such as fracture or aseptic loosening, and infection is always a concern. In addition, the polyethylene articulating components suffer surface wear over time and almost always require replacement at some point.

Allograft-prosthetic composites combine the 2 techniques discussed thus far, marrying the implant benefits of stability, strength, and modularity with the bone-restoring and soft-tissue benefits conferred by the allograft. This reconstruction has particular application in the proximal tibia and in the proximal humerus.

For skeletally immature patients with lower extremity tumors, anticipated limb length inequality beyond 5 cm was historically an indication for amputation. Currently, expandable prostheses are available, which allow for either invasive or non-invasive incremental limb lengthening. These systems are often considered temporary prostheses, lacking the structural strength required for adults' demands, and are often implanted with the understanding that they will need to be revised at some point in the future. They are relatively costly, but address an unusual reconstructive challenge and have made limb salvage for the very young patient possible.

Amputation is always a good oncologic procedure but is often misconstrued to be a procedure with poor functional results. Interestingly, amputations are often preferred over limb-salvage procedures for patients who want to maintain very athletic lifestyles. Patients who undergo amputations can sustain a much higher activity level, including impact activities such as running or skiing. In addition to having a lower risk of recurrence, amputation offers a more definitive solution in the sense that patients are much less likely to require additional surgeries, thereby eliminating the complications of non-union and fracture entirely and greatly decreasing the risk of infection.[51] Replacement or repair of an external prosthesis obviously does not involve surgery or hospitalization and thereby avoids inherently associated complications. Although thought to be less costly in the past, more recent data show that the cost of an amputation and the external prosthesis is more expensive over a patient's lifetime when compared with that of a limb-salvage procedure.[52]

The Van Ness rotationplasty serves to convert an above knee amputation into a below knee amputation and has been characterized as an intercalary amputation with sparing of the sciatic nerve and, when possible, the femoral vessels.[53,54] This is accomplished by resecting the tumor, rotating the lower leg 180 degrees, and reattaching the remaining distal tibia to the remaining proximal femur. This effectively converts the ankle joint into a knee joint. The reconstruction allows for preservation of proprioception and sensation and decreases energy expenditure compared with an above knee amputation. Function is often remarkable, with excellent gait, functional activity, and emotional acceptance reported. The technique is especially appropriate for skeletally immature patients. Although oncologic and functional outcomes are excellent, many parents and patients prefer limb salvage procedures if possible for cosmetic and social reasons. Having a candidate and his or her family meet with a rotationplasty patient often helps to alleviate many initial fears and concerns and offers patients a better appreciation for the procedure and its benefits.

Metastatic disease must be aggressively resected. Complete removal of all known sites of disease confers a survival benefit, and cure is improbable without metastasectomy.[55] Surgical resection should be undertaken via an open thoracotomy, which allows for manual examination of the lung tissue and often identifies small, otherwise unnoticed foci of disease. Up to 30% of lung metastases are too small to detect using current CT scan technology.[40] For this reason, even without radiographic evidence of disease, patients with histologically-proven lung metastases should undergo exploration of the contralateral lung.

Systemic Treatment

Historically, chemotherapy was administered as single-agent treatment. Early studies proved such regimens to be of less benefit, and combination protocols became favored. Doxorubicin and methotrexate in combination provided relapse-free survival rates of up to 60% and, as a result, became central to modern chemotherapy treatment regimens. Although bleomycin, cyclophosphamide, and actinomycin D (BCD) were frequently utilized in the past, this regimen was ultimately abandoned, as it offered little benefit when given in addition to adriamycin and methotrexate.

The value of chemotherapy for the treatment of osteosarcoma has been clearly proven in randomized clinical trials.[56,57] Current systemic chemotherapy treatment typically consists of cisplatin, doxorubicin, and high-dose methotrexate. Neoadjuvant or induction chemotherapy is generally administered for a period of 10 weeks prior to local control. Following surgical resection and a brief lapse to allow for surgical wound healing, maintenance chemotherapy is typically continued for a period of 29 weeks. This treatment regimen yields cure

rates in approximately 70% of patients with localized disease. Unfortunately, it achieves a long-term survival rate of less than 20% in patients presenting with metastatic disease. Patients who responded poorly to frontline 3-agent chemotherapy in prior studies have not enjoyed improved results from second-line or additional chemotherapy regimens. Though some reports suggest a role for added systemic treatment,[58,59] others conclude that these efforts are of minimal benefit at best[55] and, to date, are recognized to be largely ineffective.[60] Rendering a patient surgically clear of disease does confer survival improvement and is currently the most effective means for dealing with recurrence.[55]

Ifosfamide both alone and in combination with etoposide has been controversial and remains under investigation. Response rates of up to 30–40% for patients with either recurrent or metastatic disease have been reported,[59,61] and its efficacy as an addition to first-line treatment has been purported by a number of European studies.[62-64] However, this finding has been called into question by North American studies, which reported a lack of obvious efficacy using ifosfamide alone as an addition to standard first-line treatment.[65] Although the study was designed to evaluate safety and not survival benefit, Schwartz and colleagues noted no statistically significant difference with the intensification of ifosfamide and etoposide for poor responders following induction chemotherapy.[66] To better characterize the efficacy of ifosfamide and etoposide in osteosarcoma, the European and American Osteosarcoma Study Group 1 trial (EURAMOS-1) was undertaken by several European cooperative groups and the Children's Oncology Group (COG) in a collaborative effort and is currently ongoing. This study is designed to evaluate whether ifosfamide plus etoposide offers added benefit to patients demonstrating poor response to induction chemotherapy. In addition, it seeks to identify whether interferon-α offers added benefit for patients demonstrating good response to induction chemotherapy.

Future Strategies

Immunomodulatory Agents

Muramyl tripeptide phosphatidylethanolamine (MTP-PE), a liposomally encapsulated synthetic analog of 1 component of the Bacille Calmette-Guérin bacterial cell wall, is believed to activate monocytes and macrophages against osteosarcoma cells. Initial interest in the drug is rooted in the notion that inflammatory responses, such as those seen with infection, result in improved outcomes in the treatment of malignant tumors. In a nonrandomized retrospective review, postoperative infection had been reported to serve as an independent prognostic factor in patients with osteosarcoma, with 10-year survival increasing to 84.5% from 62.2% in noninfected patients.[67] MTP-PE is intended to cause an inflammatory response, including the activation of macrophages, induction of tumoricidal monocytes, and an increase in levels of cytokines and inflammatory molecules.[68] In vitro work has shown that the drug can enhance activation of murine macrophages and human monocytes[69] and that liposomal packaging further enhances this effect while reducing toxicity.[70] Although activity has been demonstrated in both xenograft and canine models, initial reports of a cooperative Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) phase III study were difficult to interpret due to an interaction between ifosfamide and MTP-PE in the initial report. More recently, a second report with longer follow-up data from the same study demonstrated improved 6-year overall survival (78% vs 70%). This study still showed no significant difference between event-free survivals.[65] Although approval for clinical use has been granted in Europe, approval by the US Food and Drug Administration has not been realized. Tremendous controversy surrounding this topic is ongoing.[71]

Aerosolized granulocyte macrophage colony stimulating factor has primarily been utilized to promote recovery from chemotherapy-induced neutropenia. More recently, its immunomodulatory effects have been investigated. Although phase I results in patients with lung metastases showed no adverse effects,[72] recently presented phase II results were less encouraging.

Signal Transduction Pathway Inhibitors

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase, which plays an important role in mRNA translation, cell growth, and cell proliferation via phosphorylation of downstream targets.[73] It is affected by a variety of signaling factors, including insulin, amino acids, and oxygen levels. It controls advancement of the cell cycle from G1 into S phase via S6K1, which affects ribosomal translation, and via eIF4E, which affects translation. Abnormal mTOR signaling has been implicated in numerous malignancies and, as such, it has been considered a potential therapeutic target. Immunohistochemical expression of mTOR and p70S6 kinase, a downstream target of mTOR, has a significant association with worse survival outcomes.[74] Rapamycin, a macrocyclic lactone antibiotic and its analogs—temsirolimus (Torisel, Wyeth), everolimus (Afinitor, Novartis), and AP23573 (ridaforolimus)—are specific inhibitors of mTOR and may directly effect cancer cell growth and proliferation. In addition, they may also exert an anti-angiogenic effect by decreasing vascular endothelial growth factor (VEGF) production and by inhibiting endothelial response to circulating VEGF. Rapamycin has shown initial promise, inhibiting metastatic disease in murine models[75] and

demonstrating activity in in vivo testing against osteosarcoma xenografts.[76] To date, encouraging phase II data have been reported, with 30% of patients with bone sarcomas treated with AP23573 demonstrating either a partial response or stable disease for 16 weeks.[77] Ongoing evaluation of rapalog use in a variety of malignancies, including sarcomas, is under way.

Tyrosine Kinase Inhibitors

The insulin-like growth factor pathway has been recognized as essential to normal growth, with mutations in either the receptor or the ligand resulting in a multitude of developmental abnormalities.[78] The insulin-like growth factor 1 receptor (IGF-1 R) is a dimeric receptor tyrosine kinase, which binds IGF-1 and IGF-2 to affect the downstream pathways, phosphatidylinositol 3'-kinase (P13K), and mitogen-activated protein kinase (MAPK). There is abundant evidence supporting IGF signal transduction as playing a central role in tumorigenesis. High levels of expression of IGF-1, IGF-2, and IGF-1R in sarcomas have been reported.[79,80] Epidemiologic links between IGF-1 serum levels and the risk of developing a malignancy have been observed,[81,82] and IGF-1R has been reported to transform human fibroblast cells both in vitro and in vivo.[83] Interest in IGF-1R-targeted therapy has developed in 2 ways. The first involves the use of semi-specific small-molecule tyrosine kinase inhibitors such as OSI-906 (OSI Pharmaceuticals, Inc.) or BMS-754807 (Bristol-Myers Squibb). There is some concern that glucose metabolism may be affected due to the cross-reactivity resulting from similarities between the binding sites of the IGF-1R and the insulin receptor. To date, most small molecule inhibitors have not progressed on to clinical trials due to toxicity concerns. The second approach to targeted therapy has been the development of monoclonal antibodies against IGF-1R. Preclinical data have been encouraging, with one agent achieving complete responses in 2 osteosarcoma xenografts.[84] The combination of a second IGF-1R targeting antibody, CP-751,871 (figitumumab, Pfizer), with the mTOR inhibitor rapamycin has been reported to induce significant in vivo reduction in tumor VEGF levels and complete remission in 3 of 4 xenograft osteosarcoma models.[85] Phase I results evaluating the use of CP-751,871 in patients with multiple sarcoma subtypes including Ewing sarcoma demonstrated the drug to be well tolerated with a favorable pharmacokinetic profile.[86] Two patients with Ewing sarcoma showed an objective response, one of whom had a complete response. In addition, 8 patients experienced stabilization of their disease for 4 months or longer. Additional agents have shown variable promise in selected cases of other bone and solid tumors,[87] all with relatively well-tolerated side-effect profiles. Phase II trials are currently under way.

Platelet derived growth factor receptor (PDGFR), another tyrosine kinase protein implicated in the development of osteosarcoma, is thought to inhibit apoptosis through the Akt pathway. Poor prognosis has been linked to the expression of both PDGFR-α and one of the ligand's dimeric forms, PDGF-AA.[88] This finding has been supported in a more recent report, which demonstrated that co-expression of PDGF-AA and PDGF-α receptor correlated with significantly shorter event-free survival, but did not correlate with chemotherapy response.[89] Although preclinical in vitro inhibition of osteosarcoma cell growth using imatinib (Gleevec, Novartis) was achieved, the concentrations required to do so were too high to be clinically relevant. The findings of constitutively active MAPK in 8 of 10 cultures may explain the high concentration needed to inhibit tumor growth. Recent results from a phase II COG study do not support its utility as a single agent.[90]

HER2/neu

The HER2/neu proto-oncogene, located at 17q21, encodes for a transmembrane glycoprotein with tyrosine kinase activity. Its protein shares significant similarity to epidermal growth factor receptor (EGFR) and other members of the EGFR superfamily.[91] Since its description in 1981, the overexpression of HER2 has been implicated in tumorigenicity, and its role is most clearly defined in breast carcinoma, where it is amplified and its gene product is overexpressed in approximately 30% of cases. HER2-targeted treatments have been developed and have yielded improved survival outcomes for patients with HER2 overexpression. In light of HER2-targeted treatment success for breast cancer, interest in HER2-targeted therapy for osteosarcoma has increased. However, the relevance of HER2 expression, even in the context of prognosis, continues to be extremely controversial. Numerous reasons for this controversy exist. To date, published reports have been small, single-institution, retrospective studies with limited size and power. Tissue handling and specimen preparation techniques differ from one institution to the next, which may have variably influenced the interpretation of HER2 expression. Similarly, institutional differences as they relate to treatment, antibody use, storage systems, and scoring schemes all play a role in how HER2 expression is identified and interpreted.

Given the fact that HER2-targeted treatment (trastuzumab [Herceptin, Genentech]) has side effects, it would likely serve as clinically relevant only in patients with proven HER2-positive tumors. Furthermore, if this patient population is, in fact, already responding reasonably well to standard treatment, it is not clear that outcomes would be impacted in any meaningful way. Alternatively, if this patient population showed substantial improvement in overall survival, the argument for

targeted treatment for a small subset of patients could be conceivably supported.

In an effort to better answer HER2-targeted treatment relevance, the COG initiated a phase II clinical trial of trastuzumab plus standard chemotherapy for patients with newly diagnosed metastatic osteosarcoma that was histologically proven to be HER2-positive. The results from this study are not yet available.

Novel Antifolates

Resistance to high-dose methotrexate, one of the current first-line chemotherapies used for osteosarcoma, can occur via a number of mechanisms, including a decrease in reduced folate carrier (RFC) expression, which has been shown to occur in 65% of biopsied tumors.[92] Trimetrexate is a structural analog of methotrexate, which achieves transport into cells independent of RFC and can directly inhibit dihydrofolate reductase. To date, a phase II trial combining refractory acute lymphoblastic leukemia and osteosarcoma patients has shown response in 13% of cases.[93] These results have prompted a phase I trial combining high-dose methotrexate and trimetrexate for patients with recurrent osteosarcoma, with the rationale that trimetrexate would impact the transport-defective osteosarcoma cells and methotrexate would affect the transport-intact osteosarcoma cells. A second novel antifolate, pralatrexate, has been evaluated for use with T-cell lymphoma and lung cancer, with variable results reported. Phase II results demonstrated a response rate in patients with relapsed or refractory lymphoma of 10% for B-cell lymphoma patients and 54% for T-cell lymphoma patients, with an overall response of 31%.[94] Phase II results in non–small-cell lung cancer demonstrated median time to progression of more than 10.5 months and median duration of survival of 13 months.[95] A phase II study in adult patients with unresectable malignant pleural mesothelioma resulted in no partial or complete responses.[96] Currently, the role of pralatrexate in the treatment of osteosarcoma is unclear.

Delivery Mechanisms

Nonconventional delivery mechanisms continue to evolve in an effort to realize improved outcomes, even in the face of relapsed or resistant disease. Aerosolized liposomal cisplatin (sustained release lipid inhalation [SLIT] targeting cisplatin, Transave, Inc.) has been evaluated in patients with pulmonary osteosarcoma in a phase Ib/IIa study.[97] High concentrations within the lungs were achieved in this manner while minimizing systemic side effects. Two of 14 patients were disease free at 1 year from initiation of treatment.

Liposomal doxorubicin has been shown to have increased uptake within osteosarcoma tumor cells.[98] Phase II results yielded objective responses in 3 of 47 patients; however, the authors felt that 15 of 47 patients derived some degree of clinical benefit.[99]

Microenvironment

The importance of tumor microenvironment is gaining recognition, and there is a growing interest in effecting tumor inhibition through the manipulation of local factors and conditions. Bisphosphonates are widely used in the treatment of osteoporosis as well as tumor-related bone pain. The more commonly utilized non-nitrogenous drugs exhibit a much higher potency and act by blocking farnesyl diphosphate synthase (FPPS) within the HMG-CoA reductase pathway.[100] This, in turn, results in inhibition of protein prenylation and, in particular, interferes with the osteoclast's ruffled border. It is speculated that the positive feedback loop of bone resorption, growth factor release, and bone formation may predispose or play a role in the development of osteosarcoma. Therefore, bisphosphonate-dampened bone resorption may be important in the treatment of osteosarcoma. In addition, in vitro and animal studies have shown a more direct effect on tumor cells.[101,102] Currently, a COG feasibility and dose discovery analysis study (COG-AOST06P1) is under way; it is designed to evaluate the use of zoledronic acid (Zometa, Novartis) in combination with cisplatin, high-dose methotrexate, doxorubicin, ifosfamide, and etoposide. It will evaluate the safety profile and event-free survival effects of zoledronic acid in patients with newly diagnosed metastatic osteosarcoma.

VEGF is a glycoprotein involved in the migration of vascular endothelial cells, playing a role in angiogenesis. In addition, its effect on vascular properties, such as permeability, may promote increased migration of tumor cells into and out of the vascular network, leading to more successful metastatic phenomena. The utility of bevacizumab (Avastin, Genentech), an anti-VEGF monoclonal antibody, has been demonstrated in the setting of colorectal carcinoma when used together with conventional chemotherapy. A phase II COG study is under way using bevacizumab in addition to conventional chemotherapy for the treatment of recurrent Ewing sarcoma. Recently, the pediatric preclinical testing program reported results from a second inhibitor of the VEGF receptor family, AZD2171 (cediranib, AstraZeneca), which was shown to exhibit in vivo tumor inhibition in 78% of solid tumor xenografts, including 3 of 3 Ewing sarcomas and 4 of 5 osteosarcomas.[103]

Conclusion

Despite great strides in the diagnosis and treatment of osteosarcoma to date, substantial improvement in overall survival has been elusive and overall survival

has remained relatively constant for over 2 decades. Although awareness, education, and proper referral patterns serve to minimize avoidable errors in diagnosis and treatment, it is unlikely that these efforts alone will significantly improve survival outcomes in the subset of patients who appear to have an inherently more challenging subtype of tumor. It is theoretically possible that a small subset of patients would benefit from upfront surgery, eliminating neoadjuvant treatment cycles and undergoing all of their chemotherapy following local control. These patients—namely those who both present with truly localized disease and who will ultimately also prove to respond poorly to conventional chemotherapy—probably constitute only 5–6% of all patients. Nevertheless, this change in management strategy may result in a small but measurable improvement in overall survival without any new systemic treatments. Ultimately, the greatest potential improvement in outcomes will arise from combination-targeted chemotherapy in addition to conventional treatment. The challenge in osteosarcoma stems from the extreme variability of one tumor to the next, making it unlikely that a single-target approach would be able to address all or even a majority of patients.

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References

1. McKenna RJ, Schwinn CP, Soong KY. Sarcomata of the osteogenic series (osteosarcoma, fibrosarcoma, chondrosarcoma, parosteal osteogenic sarcoma, and sarcomata arising in abnormal bone). An analysis of 552 cases. *J Bone Joint Surg (Amer).* 1966;48-A:1-26.
2. American Cancer Society. http://www.cancer.org/cancer/osteosarcoma/Detailedguide/osteosarcoma-key-statistics.
3. Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer.* 2009;115:1531-1543.
4. Dorfman HD, Czerniak B. In Dorfman HD, Czerniak B, eds. *Bone Tumors*. 1st ed. St. Louis, MO: Mosby; 1998:128.
5. Gurney JG, Swensen AR, Bulterys M. Malignant bone tumors. In: Ries LAG, Smith MA, Gurney JG, et al, eds. *Cancer Incidence and Survival among Children and Adolescents:* United States SEER Program 1975-1995, National Cancer Institute, SEER Program. Bethesda, MD: NIH Pub. No. 99-4649; 1999:99-110.
6. Mirabello L, Troisi RJ, Savage SA. International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. *Int J Cancer.* 2009;125:229-234.
7. Huvos AG. Osteogenic sarcoma of bones and soft tissues in older persons. A clinicopathologic analysis of 117 patients older than 60 years. *Cancer.* 1986;57:1442-1449.
8. Nishida Y, Isu K, Ueda T, et al. Osteosarcoma in the elderly over 60 years: a multicenter study by the Japanese Musculoskeletal Oncology Group. *J Surg Oncol.* 2009;100:48-54.
9. Tjalma RA. Canine bone sarcoma: estimation of relative risk as a function of body size. *J Natl Cancer Inst*. 1966;36:1137-1150.
10. Fraumeni JF Jr. Stature and malignant tumors of bone in childhood and adolescence. *Cancer.* 1967;20:967-973.
11. Cotterill SJ, Wright CM, Pearce MS, Craft AW. Stature of young people with malignant bone tumors. *Pediatr Blood Cancer*. 2004;42:59-63.
12. Le Vu B, de Vathaire F, Shamsaldin A, et al. Radiation dose, chemotherapy and risk of osteosarcoma after solid tumours during childhood. *Int J Cancer.* 1998;77:370-377.
13. Garwicz S, Anderson H, Olsen JH, et al. Second malignant neoplasms after cancer in childhood and adolescence: a population-based case-control study in the 5 Nordic countries. The Nordic Society for Pediatric Hematology and Oncology. The Association of the Nordic Cancer Registries. *Int J Cancer.* 2000;88:672-678.
14. Hawkins MM, Wilson LM, Burton HS, et al. Radiotherapy, alkylating agents, and risk of bone cancer after childhood cancer. *J Natl Cancer Inst.* 1996;88:270-278.
15. Henderson TO, Whitton J, Stovall M, et al. Secondary sarcomas in childhood cancer survivors: a report from the Childhood Cancer Survivor Study. *J Natl Cancer Inst.* 2007;99:300-308.
16. Tucker MA, D'Angio GJ, Boice JD Jr, et al. Bone sarcomas linked to radiotherapy and chemotherapy in children. *N Engl J Med*. 1987;317:588-593.
17. Newton WA Jr, Meadows AT, Shimada H, Bunin GR, Vawter GF. Bone sarcomas as second malignant neoplasms following childhood cancer. *Cancer.* 1991;67:193-201.
18. Hadjipavlou A, Lander P, Srolovitz H, Enker IP. Malignant transformation in Paget disease of bone. *Cancer.* 1992;70:2802-2808.
19. Porretta CA, Dahlin DC, Janes JM. Sarcoma in Paget's disease of bone. *J Bone Joint Surg Am.* 1957;39-A:1314-1329.
20. Barry HC. Neoplastic changes. In: Barry HC, ed. *Paget's Disease of Bone*. Edinburgh, Scotland: Livingstone; 1969:136-193.
21. Grimer RJ, Cannon SR, Taminiau AM, et al. Osteosarcoma over the age of forty. *Eur J Cancer.* 2003;39:157-163.
22. Mankin HJ, Hornicek FJ. Paget's sarcoma: a historical and outcome review. *Clin Orthop Relat Res.* 2005;438:97-102.
23. McNairn JD, Damron TA, Landas SK, Ambrose JL, Shrimpton AE. Inheritance of osteosarcoma and Paget's disease of bone: a familial loss of heterozygosity study. *J Mol Diagn.* 2001;3:171-177.
24. Gurney JG, Severson RK, Davis S, Robison LL. Incidence of cancer in children in the United States. Sex-, race-, and 1-year age-specific rates by histologic type. *Cancer.* 1995;75:2186-2195.
25. Wong FL, Boice JD Jr, Abramson DH, et al. Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk. *JAMA.* 1997;278:1262-1267.
26. Feugeas O, Guriec N, Babin-Boilletot A, et al. Loss of heterozygosity of the RB gene is a poor prognostic factor in patients with osteosarcoma. *J Clin Oncol.* 1996;14:467-472.
27. Siddiqui R, Onel K, Facio F, et al. The TP53 mutational spectrum and frequency of CHEK2*1100delC in Li-Fraumeni-like kindreds. *Fam Cancer.* 2005;4:177-181.
28. Varley JM, Chapman P, McGown G, et al. Genetic and functional studies of a germline TP53 splicing mutation in a Li-Fraumeni-like family. *Oncogene.* 1998;16:3291-3298.
29. McIntyre JF, Smith-Sorensen B, Friend SH, et al. Germline mutations of the p53 tumor suppressor gene in children with osteosarcoma. *J Clin Oncol.* 1994;12:925-930.
30. Wang LL, Levy ML, Lewis RA, et al. Clinical manifestations in a cohort of 41 Rothmund-Thomson syndrome patients. *Am J Med Genet*. 2001;102:11-17.
31. Hicks MJ, Roth JR, Kozinetz CA, Wang LL. Clinicopathologic features of osteosarcoma in patients with Rothmund-Thomson syndrome. *J Clin Oncol.* 2007;25:370-375.
32. Thorpe WP, Reilly JJ, Rosenberg SA. Prognostic significance of alkaline phosphatase measurements in patients with osteogenic sarcoma receiving chemotherapy. *Cancer.* 1979;43:2178-2181.
33. Link MP, Goorin AM, Horowitz M, et al. Adjuvant chemotherapy of high-grade osteosarcoma of the extremity. Updated results of the Multi-Institutional Osteosarcoma Study. *Clin Orthop Relat Res*. 1991;270:8-14.
34. Clark JC, Dass CR, Choong PF. A review of clinical and molecular prognostic factors in osteosarcoma. *J Cancer Res Clin Oncol*. 2008;134:281-297.
35. Bacci G, Longhi A, Ferrari S, et al. Prognostic significance of serum lactate dehydrogenase in osteosarcoma of the extremity: experience at Rizzoli on 1421 patients treated over the last 30 years. *Tumori.* 2004;90:478-484.
36. Huvos AG, Rosen G, Marcove RC. Primary osteogenic sarcoma: pathologic aspects in 20 patients after treatment with chemotherapy en bloc resection, and prosthetic bone replacement. *Arch Pathol Lab Med.* 1977;101:14-18.
37. 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;41:194-199.

38. Ferrari S, Bacci G, Picci P, et al. Long-term follow-up and post-relapse survival in patients with non-metastatic osteosarcoma of the extremity treated with neoadjuvant chemotherapy. *Ann Oncol.* 1997;8:765-771.
39. Gorlick R, Meyers PA. Osteosarcoma necrosis following chemotherapy: innate biology versus treatment-specific. *J Pediatr Hematol Oncol.* 2003;25:840-841.
40. Kayton ML, Huvos AG, Casher J, et al. Computed tomographic scan of the chest underestimates the number of metastatic lesions in osteosarcoma. *J Pediatr Surg.* 2006;41:200-206.
41. Costelloe CM, Macapinlac HA, Madewell JE, et al. 18F-FDG PET/CT as an indicator of progression-free and overall survival in osteosarcoma. *J Nucl Med.* 2009;50:340-347.
42. Mankin HJ, Mankin CJ, Simon MA. The hazards of the biopsy, revisited. Members of the Musculoskeletal Tumor Society. *J Bone Joint Surg Am.* 1996;78:656-663.
43. Hau A, Kim I, Kattapuram S, et al. Accuracy of CT-guided biopsies in 359 patients with musculoskeletal lesions. *Skeletal Radiol.* 2002;31:349-353.
44. Mitsuyoshi G, Naito N, Kawai A, et al. Accurate diagnosis of musculoskeletal lesions by core needle biopsy. *J Surg Oncol*. 2006;94:21-27.
45. Jelinek JS, Murphey MD, Welker JA, et al. Diagnosis of primary bone tumors with image-guided percutaneous biopsy: experience with 110 tumors. *Radiology.* 2002;223:731-737.
46. Altuntas AO, Slavin J, Smith PJ, et al. Accuracy of computed tomography guided core needle biopsy of musculoskeletal tumours. *ANZ J Surg.* 2005; 75:187-191.
47. Enneking WF. A system of staging musculoskeletal neoplasms. *Clin Orthop Relat Res*. 1986;204:9-24.
48. Hornicek FJ, Gebhardt MC, Tomford WW, et al. Factors affecting nonunion of the allograft-host junction. *Clin Orthop Relat Res*. 2001;382:87-98.
49. Mankin HJ, Gebhardt MC, Tomford WW. The use of frozen cadaveric allografts in the management of patients with bone tumors of the extremities. *Orthop Clin North Am.* 1987;18:275-289.
50. Brigman BE, Hornicek FJ, Gebhardt MC, Mankin HJ. Allografts about the knee in young patients with high-grade sarcoma. *Clin Orthop Relat Res.* 2004; 232-239.
51. Ruggieri P, De Cristofaro R, Picci P, et al. Complications and surgical indications in 144 cases of nonmetastatic osteosarcoma of the extremities treated with neoadjuvant chemotherapy. *Clin Orthop Relat Res.* 1993;295:226-238.
52. Grimer RJ, Carter SR, Pynsent PB. The cost-effectiveness of limb salvage for bone tumours. *J Bone Joint Surg Br*. 1997;79:558-561.
53. Hanlon M, Krajbich JI. Rotationplasty in skeletally immature patients. Long-term followup results. *Clin Orthop Relat Res.* 1999;358:75-82.
54. Winkelmann WW. Type-B-IIIa hip rotationplasty: an alternative operation for the treatment of malignant tumors of the femur in early childhood. *J Bone Joint Surg Am*. 2000;82:814-828.
55. Kempf-Bielack B, Bielack SS, Jurgens H, et al. Osteosarcoma relapse after combined modality therapy: an analysis of unselected patients in the Cooperative Osteosarcoma Study Group (COSS). *J Clin Oncol*. 2005;23:559-568.
56. Eilber FR, Rosen G. Adjuvant chemotherapy for osteosarcoma. *Semin Oncol.* 1989;16:312-322.
57. Link MP, Goorin AM, Miser AW, et al. The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. *N Engl J Med*. 1986;314:1600-1606.
58. Saeter G, Hoie J, Stenwig AE, Johansson AK, Hannisdal E, Solheim OP. Systemic relapse of patients with osteogenic sarcoma. Prognostic factors for long term survival. *Cancer.* 1995;75:1084-1093.
59. Chou AJ, Merola PR, Wexler LH, et al. Treatment of osteosarcoma at first recurrence after contemporary therapy: the Memorial Sloan-Kettering Cancer Center experience. *Cancer.* 2005;104:2214-2221.
60. Ferrari S, Briccoli A, Mercuri M, et al. Postrelapse survival in osteosarcoma of the extremities: prognostic factors for long-term survival. *J Clin Oncol*. 2003;21:710-715.
61. Carli M, Passone E, Perilongo G, Bisogno G. Ifosfamide in pediatric solid tumors. *Oncology*. 2003;65(Suppl 2):99-104.
62. Fuchs N, Bielack SS, Epler D, et al. Long-term results of the co-operative German-Austrian-Swiss osteosarcoma study group's protocol COSS-86 of intensive multidrug chemotherapy and surgery for osteosarcoma of the limbs. *Ann Oncol.* 1998;9:893-899.
63. Bacci G, Ferrari S, Bertoni F, et al. Long-term outcome for patients with non-metastatic 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.* 2000;18:4016-4027.
64. 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.
65. 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.
66. Schwartz CL, Wexler LH, Devidas M, et al. Non-metastatic osteosarcoma: response based augmentation of therapy. Paper presented at: Connective Tissue Oncology Society Annual Meeting; November 2–4, 2006; Venice, Italy.
67. Jeys LM, Grimer RJ, Carter SR, Tillman RM, Abudu A. Post operative infection and increased survival in osteosarcoma patients: are they associated? *Ann Surg Oncol*. 2007;14:2887-2895.
68. Meyers PA. Muramyl tripeptide (mifamurtide) for the treatment of osteosarcoma. *Expert Rev Anticancer Ther.* 2009;9:1035-1049.
69. Sone S, Mutsuura S, Ogawara M, Tsubura E. Potentiating effect of muramyl dipeptide and its lipophilic analog encapsulated in liposomes on tumor cell killing by human monocytes. *J Immunol*. 1984;132:2105-2110.
70. Fidler IJ, Brown NO, Hart IR. Species variability for toxicity of free and liposome-encapsulated muramyl peptides administered intravenously. *J Biol Response Mod*. 1985;4:298-309.
71. Hunsberger S, Freidlin B, Smith MA. Complexities in interpretation of osteosarcoma clinical trial results. *J Clin Oncol.* 2008;26:3103-3104.
72. Anderson PM, Markovic SN, Sloan JA, et al. Aerosol granulocyte macrophage-colony stimulating factor: a low toxicity, lung-specific biological therapy in patients with lung metastases. *Clin Cancer Res*. 1999;5:2316-2323.
73. Inoki K, Corradetti MN, Guan KL. Dysregulation of the TSC-mTOR pathway in human disease. *Nat Genet.* 2005;37:19-24.
74. Zhou Q, Deng Z, Zhu Y, Long H, Zhang S, Zhao J. mTOR/p70S6K Signal transduction pathway contributes to osteosarcoma progression and patients' prognosis [published online ahead of print November 20, 2009]. *Med Oncol.* 2009. http://www.springerlink.com/content/kq1220077gr46135/export-citation/.
75. Wan X, Mendoza A, Khanna C, Helman LJ. Rapamycin inhibits ezrin-mediated metastatic behavior in a murine model of osteosarcoma. *Cancer Res.* 2005;65:2406-2411.
76. Houghton PJ, Morton CL, Kolb EA, et al. Initial testing (stage 1) of the mTOR inhibitor rapamycin by the pediatric preclinical testing program. *Pediatr Blood Cancer*. 2008;50:799-805.
77. Chawla SP, Tolcher AW, Staddon AP, et al. Updated results of a phase II trial of AP23573, a novel mTOR inhibitor, in patients with advanced soft tissue or bone sarcomas. *J Clin Oncol*. 2006;24:9505.
78. Abuzzahab MJ, Schneider A, Goddard A, et al. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med.* 2003;349:2211-2222.
79. Ouban A, Muraca P, Yeatman T, Coppola D. Expression and distribution of insulin-like growth factor-1 receptor in human carcinomas. *Hum Pathol.* 2003; 34:803-808.
80. Shimizu C, Hasegawa T, Tani Y, et al. Expression of insulin-like growth factor 1 receptor in primary breast cancer: immunohistochemical analysis. *Hum Pathol.* 2004;35:1537-1542.
81. Chan JM, Stampfer MJ, Giovannucci E, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science*. 1998;279:563-566.
82. Ma J, Pollak MN, Giovannucci E, et al. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. *J Natl Cancer Inst*. 1999;91:620-625.
83. Kaleko M, Rutter WJ, Miller AD. Overexpression of the human insulinlike growth factor I receptor promotes ligand-dependent neoplastic transformation. *Mol Cell Biol.* 1990;10:464-473.
84. Kolb EA, Gorlick R, Houghton PJ, et al. Initial testing (stage 1) of a monoclonal antibody (SCH 717454) against the IGF-1 receptor by the pediatric preclinical testing program. *Pediatr Blood Cancer.* 2008;50:1190-1197.
85. Kurmasheva RT, Dudkin L, Billups C, Debelenko LV, Morton CL, Houghton PJ. The insulin-like growth factor-1 receptor-targeting antibody, CP-751,871, suppresses tumor-derived VEGF and synergizes with rapamycin in models of childhood sarcoma. *Cancer Res.* 2009;69:7662-7671.
86. Olmos D, Postel-Vinay S, Molife LR, et al. Safety, pharmacokinetics, and preliminary activity of the anti-IGF-1R antibody figitumumab (CP-751,871) in patients with sarcoma and Ewing's sarcoma: a phase 1 expansion cohort study. *Lancet Oncol.* 2010;11:129-135.
87. Haluska P, Shaw HM, Batzel GN, et al. Phase I dose escalation study of the anti insulin-like growth factor-I receptor monoclonal antibody CP-751,871 in patients with refractory solid tumors. *Clin Cancer Res*. 2007;13:5834-5840.

88. Sulzbacher I, Traxler M, Mosberger I, Lang S, Chott A. Platelet-derived growth factor-AA and -alpha receptor expression suggests an autocrine and/or paracrine loop in osteosarcoma. *Mod Pathol.* 2000;13:632-637.
89. Kubo T, Piperdi S, Rosenblum J, et al. Platelet-derived growth factor receptor as a prognostic marker and a therapeutic target for imatinib mesylate therapy in osteosarcoma. *Cancer.* 2008;112:2119-2129.
90. Bond M, Bernstein ML, Pappo A, et al. A phase II study of imatinib mesylate in children with refractory or relapsed solid tumors: a Children's Oncology Group study. *Pediatr Blood Cancer*. 2008;50:254-258.
91. Bargmann CI, Weinberg RA. Increased tyrosine kinase activity associated with the protein encoded by the activated neu oncogene. *Proc Natl Acad Sci U S A*. 1988;85:5394-5398.
92. Guo W, Healey JH, Meyers PA, et al. Mechanisms of methotrexate resistance in osteosarcoma. *Clin Cancer Res*. 1999;5:621-627.
93. Trippett T, Meyers P, Gorlick R, Steinherz P, Wollner N, Bertino JR. High dose trimetrexate with leucovorin protection in recurrent childhood malignancies: a Phase II trial. *J Clin Oncol* (ASCO Annual Meeting Abstracts). 1999;9: Abstract 889.
94. O'Connor OA, Horwitz S, Hamlin P, et al. Phase II-I-II study of two different doses and schedules of pralatrexate, a high-affinity substrate for the reduced folate carrier, in patients with relapsed or refractory lymphoma reveals marked activity in T-cell malignancies. *J Clin Oncol.* 2009;27:4357-4364.
95. Krug LM, Azzoli CG, Kris MG, et al. 10-propargyl-10-deazaaminopterin: an antifolate with activity in patients with previously treated non-small cell lung cancer. *Clin Cancer Res*. 2003;9:2072-2078.
96. Krug LM, Heelan RT, Kris MG, Venkatraman E, Sirotnak FM. Phase II trial of pralatrexate (10-propargyl-10-deazaaminopterin, PDX) in patients with unresectable malignant pleural mesothelioma. *J Thorac Oncol.* 2007;2:317-320.
97. Chou AJ, Bell MD, Mackinson C, Gupta R, Meyers PA, Gorlick R. Phase Ib/IIa study of sustained release lipid inhalation targeting cisplatin by inhalation in the treatment of patients with relapsed/progressive osteosarcoma metastatic to the lung. *J Clin Oncol* (ASCO Annual Meeting Abstracts). 2007;25:Abstract 9525.
98. Davies Cde L, Lundstrom LM, Frengen J, et al. Radiation improves the distribution and uptake of liposomal doxorubicin (caelyx) in human osteosarcoma xenografts. *Cancer Res.* 2004;64:547-553.
99. Skubitz KM. Phase II trial of pegylated-liposomal doxorubicin (Doxil) in sarcoma. *Cancer Invest.* 2003;21:167-176.
100. van Beek ER, Cohen LH, Leroy IM, Ebetino FH, Lowik CW, Papapoulos SE. Differentiating the mechanisms of antiresorptive action of nitrogen containing bisphosphonates. *Bone.* 2003;33:805-811.
101. Ashton JA, Farese JP, Milner RJ, Lee-Ambrose LM, van Gilder JM. Investigation of the effect of pamidronate disodium on the in vitro viability of osteosarcoma cells from dogs. *Am J Vet Res*. 2005;66:885-891.
102. Cheng YY, Huang L, Lee KM, Li K, Kumta SM. Alendronate regulates cell invasion and MMP-2 secretion in human osteosarcoma cell lines. *Pediatr Blood Cancer.* 2004;42:410-415.
103. Maris JM, Courtright J, Houghton PJ, et al. Initial testing of the VEGFR inhibitor AZD2171 by the pediatric preclinical testing program. *Pediatr Blood Cancer.* 2008;50:581-587.