Pediatric Malignant Bone Tumors: A Review and Update on Current Challenges, and Emerging Drug Targets

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Osteosarcoma (OS) and the Ewing sarcoma family of tumors (ESFT) are the most common malignant bone tumors in children and adolescents. While significant improvements in survival have been seen in other pediatric malignancies the treatment and prognosis for pediatric bone tumors has remained unchanged for the past 3 decades. This review and update of pediatric malignant bone tumors will provide a general overview of osteosarcoma and the Ewing sarcoma family of tumors, discuss bone tumor genomics, current challenges, and emerging drug targets.

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Introduction

Malignant bone tumors account for approximately 3% of tumors in children and adolescents. Osteosarcoma (OS) and the Ewing sarcoma family of tumors (ESFT) are the most common malignant bone tumors that cumulatively represent the majority of tumors. The remaining 6% of malignant tumors intrinsic to the bone include chondrosarcomas, malignant fibrous histiocytomas, and adamantinomas. Pediatricians rarely encounter patients with these malignancies during clinical practice; however, it is important to note that 60% of patients who are diagnosed with a malignant bone tumor will present initially to their primary practitioner.[1] In addition, the diagnosis of a malignant bone tumor can often be delayed by weeks or months as many adolescents and young adults frequently attribute pain to a nonspecific trauma or an acute sports injury. This review and update of pediatric malignant bone tumors will provide a general overview of the clinical presentation, diagnostic requirements, treatment options, and prognoses of osteosarcoma and the Ewing sarcoma family of tumors, discuss bone tumor genomics, current challenges, and emerging drug targets.

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Osteosarcoma

Incidence and Epidemiology

Primary OS is the most common bone malignancy in children and young adults. It has a worldwide annual incidence of approximately 1–3 cases per million and an age-adjusted incidence of 4.4 per million cases per year.[2] Secondary OS is a malignant neoplasm associated with prior treatment with radiation therapy and/or chemotherapy, which accounts for approximately 1–3% of osteosarcomas.[3–5] Osteosarcomas occur primarily in adolescents and young adults and correlate with a period of rapid bone growth, most commonly the adolescent growth spurt. OS is exceeding rare in children younger than 5 years of age[6] and when it does occur, it is most often associated with the Li–Fraumeni syndrome (LFS), a cancer susceptibility syndrome to be discussed later. OS has a bimodal incidence; in older patients OS is most often associated with prior exposure to radiation and/or chemotherapy or pre-existing Paget's disease of the bone.

Clinical Presentation

Children and adolescents with OS usually present with pain with or without an associated mass. Any

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mass, progressive pain, pain that interrupts the activities of daily living and/or pain that awaken a patient from sleep should always be investigated. Osteosarcoma (OS) can occur in any bone, but has a predilection for the metaphysis of the long bones, most commonly the distal femur, followed by the proximal tibia and the proximal humerus. A small percentage of patients with OS present with a pathological fracture, usually associated with minimal trauma to the affected area. Therefore, it is also important to determine if the history of trauma is sufficient to result in a fracture, or if instead a pathological fracture is likely.

The majority of patients with OS will have localized disease at diagnosis. About 15% of patients will have radiologic evidence of metastatic disease at diagnosis; the most common site of metastasis is the lung, followed by bone. Pulmonary metastases are usually not associated with overt symptoms at diagnosis. Bone metastases are uncommon at initial presentation in patients with OS. These lesions may be solitary or multiple and customarily appear late in the course of the disease. Multifocal osteosarcoma is extremely rare and generally presents with the synchronous appearance of multiple osteosarcoma tumors with or without pulmonary metastases.

Approximately 1% of patients who survive childhood cancer develop treatment-related bone cancer.

Risk Factors

The vast majority of patients with OS have no known risk factors, predisposition syndromes or exposures. Tall stature and male gender are clinical factors that confer a small but higher risk of OS, but these findings stem from a limited number of studies, so they remain controversial.[3,7] However, there are 2 risk factors that have been extensively studied and consistently associated with an increased risk of developing OS: the genetic predisposition syndromes (Table) and exposure to ionizing radiation and/or chemotherapy.

The 2 most common genetic predisposition syndromes are Li–Fraumeni syndrome (LFS) and bilateral retinoblastoma. LFS is an autosomal dominant cancer predisposition syndrome with a de novo or inherited germline mutation in the tumor suppressor gene *TP53*.[8] Individuals with LFS develop a variety of malignancies, including soft tissue sarcoma, osteosarcoma, premenopausal breast cancer, brain tumors, adrenocortical carcinoma (ACC), and leukemia at younger-than-expected years.[9] OS is a sentinel cancer of LFS[9] and is the second most common malignancy in this patient population. Therefore, an in-depth family history is critical in determining whether a patient with a new diagnosis of OS harbors an inherited genetic mutation. A diagnosis of LFS confers a 12–15% risk of developing OS over the patient's lifetime.[9,10] Thus, any patient with a family history suggestive of this syndrome (Table) should be referred to a geneticist, as both the proband and family members who harbor this mutation may have an increased risk of developing malignancy.

The most common intraocular malignancy among children is retinoblastoma (RB). It clinically presents in early childhood with leukocoria or strabismus. RB can either arise spontaneously or be associated with hereditary or de novo germline mutations in the retinoblastoma gene (*RB1*). Patients with RB1 gene abnormalities present at a mean age of 1 year with apparent unilateral disease, but bilateral and/or multifocal tumors are most common. Thus, all patients with a diagnosis of RB must be referred to an ophthalmic oncologist for initial evaluation, treatment, and follow-up examinations including fundoscopic examination under anesthesia as some patients with apparent unilateral disease have bilateral disease recognized on fundoscopic examination under general anesthesia either at the time of diagnosis, or they may develop tumors in the contralateral eye later, that is, metachronous tumors.

Patients with the non-heritable form of RB have a mean onset of 2 years of age and their disease is unilateral. Carriers of an RB1 mutation have an increased incidence of non-retinoblastoma malignancies, especially OS.[11,12] Patients with OS tumors who with this mutation have a lower survival rate, a significantly increased risk of having metastatic disease, and have a poor histological response to chemotherapy.[12] In addition, survivors of hereditary retinoblastoma who received radiation therapy for their primary tumor have an elevated risk of developing a secondary sarcoma lesion in the radiation field.[13]

Approximately 1% of patients who survive childhood cancer develop treatment-related bone cancer within 20 years of their primary therapy.[14] Patients treated with radiation therapy for a prior malignancy have a ninefold higher risk of developing a secondary

TABLE. Sarcoma predisposition syndromes

	Mode of inheritance	Gene mutation	Clinical characteristics	Osteosarcoma Risk
Li–Fraumeni syndrome (LFS)	Autosomal dominant	TP53	Individuals with LFS develop a variety of malignancies including soft tissue sarcoma, osteosarcoma, pre-menopausal breast cancer, brain tumors, adrenocortical carcinoma (ACC), and leukemia. Criteria for diagnosis The patient has been diagnosed with sarcoma at a young age (<45 years of age). A first-degree relative has been diagnosed with any cancer at a young age (≤45 years of age). A first-degree or second-degree relative has been diagnosed with cancer at a young age (<45 years of age), or diagnosed with a sarcoma at any age.	12% Sporadic OS, 3–7%
Retinoblastoma 1 (RB1)	Autosomal dominant	RB1	Retinoblastoma (RB) presents in early childhood with leukocoria or strabismus. Hereditary retinoblastoma usually presents at a mean age of 1 year and can have unilateral disease, but bilateral and/or multifocal tumors are most common.	10%
Neurofibromatosis type 1 (NF1)	Autosomal dominant	NF1	Multisystem genetic disorder characterized by cutaneous findings, skeletal dysplasias, neurofibromas, and an increased risk of cancer.	Eightfold higher risk
Rothmund–Thomson syndrome	Autosomal recessive	RECQL4	Small stature, skeletal dysplasias, sparse hair, or cataracts.	32%
Werner's syndrome (WRN)	Autosomal recessive	RECQL3	Premature aging, abnormal telomere maintenance, and chromosomal rearrangements.	10%
Bloom's syndrome (BLM)	Autosomal recessive	RECQL2	Extremely short stature, pre-natal and post-natal growth retardation, learning disabilities, and high rates of cancers.	3%
RAPADILINO syndrome	Autosomal recessive	RECQL4	Radial hypoplasia/aplasia, patellar hypoplasia/aplasia, cleft or highly arched palate, diarrhea and dislocated joints, little size and limb malformation, and slender nose and normal intelligence.	7–13%
Diamond–Blackfan anemia (DBA)	Autosomal dominant	Ribosomal protein genes: RPS19, RPL5, RPL11, RPL35A, RPS24, RPS17, and RPS7.	Congenital pure red cell aplasia of infancy and childhood with associated congenital abnormalities.	3 reported patients with OS[87] 354 patients registered in the Diamond–Blackfan Anemia Registry of North America (DBAR).

sarcoma compared to the general population.[15] The relative risk of developing a secondary sarcoma is dose-dependent and associated with radiation doses above the threshold of 10 Gy. The sharpest increase in the risk and incidence of OS is noted in patients who receive radiation doses of 50 Gy or higher.[4] The average time between the diagnosis of the primary malignancy and the development of radiation-induced OS is 10 years; however, OS can appear up to 30 years following radiation exposure.[3] Prior treatment with high-dose anthracycline (e.g., doxorubicin) or alkylator (e.g., cyclophosphamide) chemotherapy is also associated with an increased risk of developing secondary sarcomas with estimates between 2- and 5-fold compared to the general population. It is estimated that 66% of the patients who develop a secondary sarcoma do not survive.[15]

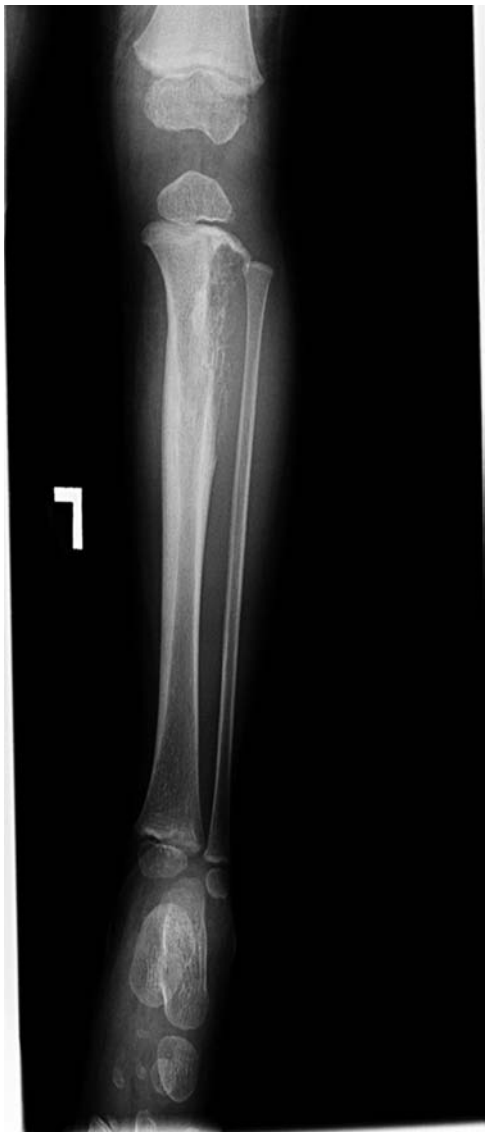

FIG 1. Plain radiograph of the right proximal tibia. Osteosarcoma of the right proximal tibia with periosteal reaction.

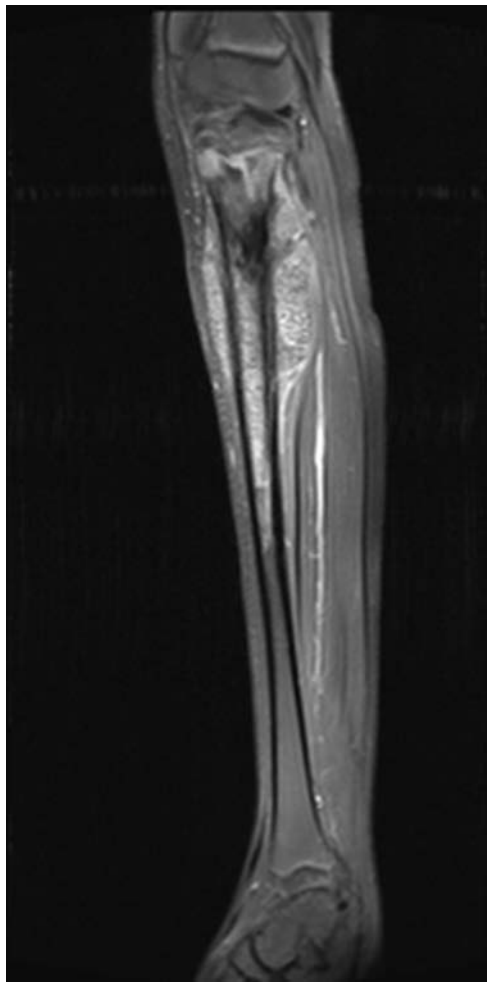

FIG 2. MRI of a right proximal tibia osteosarcoma. 11.7 × 5.5 × 5.5 heterogeneous, partially enhancing mass within the proximal tibia that extends circumferentially through the cortex into the adjacent soft tissues.

Diagnostic Imaging

Plain films of the primary site and adjacent joint are the first steps in evaluating a patient with symptoms suggestive of a bone tumor. The most common radiographic finding associated with OS is a "sunburst" pattern of new bone formation with extension into the adjacent tissue. The "Codman triangle" is also a nonspecific feature seen on the plain radiographs of patients with OS, which is produced by tumor-associated periosteal elevation (Fig 1).

A magnetic resonance imaging (MRI) of the primary tumor is required to define the extent of tumor (Fig 2). MRI is very useful in documenting involvement of neurovascular structures, the extent of marrow involvement, soft tissue extension, and skip metastatic disease, that is, a bone lesion that occurs within the same bone or across the adjacent joint that is not contiguous with the presenting primary tumor. Ideally, the MRI should be obtained prior to biopsy to optimize the surgical approach of the biopsy, as a more extensive resection is required when skip lesions are present.[16]

A non-contrast computerized tomography (CT) scan of the lungs is the modality of choice to detect metastatic disease. The number and distribution of pulmonary nodules on CT are important prognostic factors.[17] Sub-centimeter nodules in particular are unclear as the definition of these pulmonary metastases is not completely codified. Generally any lesion >1 cm or multiple lesions $>$ 5 mm are considered

evidence of metastasis. When unclear, biopsy and/or resection are recommended. A recent study using Fluorine-18-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) suggests that the PET avidity ($SUV_{max} > 1$) of nodules above 6 mm is consistent with malignancy.[18] Traditionally bone scans have been used to detect bone metastasis; however, in the future PET/CT and/or PET/MRI may supplant the routine use of bone scan.[18,19]

Laboratory Testing

Generally, the CBC and blood chemistries are normal in patients with OS and provide a valuable baseline, as they may be perturbed once therapy is initiated. Alkaline phosphatase and/or LDH, markers of increased bone turnover are markedly elevated in most patients. Individuals with very elevated alkaline phosphatase levels at diagnosis generally have higher tumor burdens. A decrease over time in the level of alkaline phosphatase during treatment is often interpreted as a "soft sign" of a response to therapy.

OS Biopsy

The most common approaches in obtaining a diagnostic specimen include open biopsy or core needle biopsy. Generally, open biopsy is preferred because it is more likely to yield sufficient tissue for examination. Biopsies for suspected OS should be performed by an orthopedic surgeon with expertise in oncologic orthopedics and limb-sparing surgical techniques—ideally, the same surgeon who will ultimately render definitive surgical care. The biopsy should be done in a site that can be excised completely at the time of surgical resection and the incision should not cross tissue compartments as that might result in seeding of other compartments with tumor. Tumor biopsies that are done incorrectly often compromise the chance of local control, may complicate reconstructive procedures, and have been shown to increase the rate of local recurrence.[20] If the biopsy is not performed at a medical center with expertise in treating osteosarcoma, there is a greater likelihood of biopsy-associated complications, such as obtaining non-diagnostic tissue, hematoma, infection, and pathologic fracture.[21,22]

Histological Evaluation

OS cells are of mesenchymal origin and are histologically characterized by the abnormal production of osteoid. The diagnosis of OS is made primarily by histological analysis. Immunohistochemical markers and cytogenetic analysis is of limited value in OS as the majority of markers are non-specific. The histological evaluation of OS after definitive surgery is important in order to assess tumor response to pre-operative chemotherapy. The tissue obtained is carefully examined to determine histological response to neoadjuvant chemotherapy. A good histological response to pre-operative chemotherapy is defined as $<10\%$ viable tumor while a poor response has $\geq 10\%$ of viable tumor is present on histological evaluation.

Prognostic Factors

The prognosis for osteosarcoma patients has improved significantly over the last 30 years from roughly 15% survival to 65–70%, secondary to an exponential and cumulative increase in knowledge of prognostic factors, improved surgical techniques, and refinement of chemotherapeutic interventions. A 5-year survival for good responders is 75–80%, compared to 45–55% for poor responders.[23] The strongest independent predictor of overall survival is a good histological response to neoadjuvant combination chemotherapy.[23–25]

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The factors that independently predict a worse outcome are large tumors, inadequate surgical margins, age less than 14 years, male gender, high alkaline phosphatase, local recurrence, and p-glycoprotein expression.[25,26] A poor histological response to pre-operative chemotherapy increases mortality by a factor of 2.4.[24] Individuals with axial primary disease and those with metastatic disease at diagnosis also have a poor prognosis, as they frequently have disease that is not amendable to complete surgical resection. However, these patients have the potential to be cured if a complete surgical remission is attained. Pulmonary metastasis, specifically more than 3 nodules with a bilateral distribution, is associated with an increased likelihood of pulmonary recurrence/progression in the first 3 years after diagnosis.[17] Patients with bony

metastases have the worst overall survival rate compared to patients with pulmonary metastasis.[23–25]

Historical Perspective of Osteosarcoma Treatment

Prior to the era of chemotherapy, amputation was the preferred surgical approach for malignant bone tumors. With the use of surgery alone, 50% of patients developed pulmonary metastases within 6 months and 80% of patients developed metastases within 1 year.[27] The estimated survival rate for patients with OS was thought to be approximately 15–20%. Chemotherapy was introduced as a treatment for OS in the early 1970s, as an adjuvant therapy following biopsy, to provide a bridge therapy prior to definite resection.

In the early 1970s, 2 independent studies reported improvements in the overall survival and event-free survival of metastatic OS with the use of single agent Adriamycin[28] and high-dose methotrexate with citrovorum factor rescue.[29] However, controversy remained regarding whether or not all patients would benefit with chemotherapy as part of the initial therapy. It was not until almost 20 years later that the Multi-Institutional Osteosarcoma Study (MIOS) definitively showed that adjuvant chemotherapy was superior to surgery alone. This study randomly assigned patients after definitive surgery of their primary tumor to receive either multiagent adjuvant chemotherapy or observation without adjuvant treatment. The 6-year event-free survival for the control group was 11% compared to 61% for the chemotherapy group.[30]

OS Treatment: Chemotherapeutic Agents

The standard OS treatment protocol in the United States consists of induction chemotherapy with MAP [high-dose methotrexate, doxorubicin (Adriamycin), and cisplatin] therapy. Induction therapy lasts approximately 10 weeks and is followed by local surgical management during week 11. If surgery is delayed, patients may receive an additional 2 cycles of high-dose methotrexate (maximum of 6 cycles of methotrexate) before surgical resection/amputation. Following definitive resection/amputation, standard protocol directs that patients complete an additional 17 weeks of adjuvant chemotherapy with MAP.

Osteosarcoma Treatment: Surgery

Pediatric orthopedic surgeons have the challenge of performing tumor resection and/or reconstructive surgery on a growing child with an immature skeleton, which can create significant limb-length discrepancy and gait abnormalities.[31] The decision on a surgical approach for a bone tumor is patient-dependent, and factors that must be considered include the tumor location, the presence of metastasis, neurovascular invasion, and patient preference. The objectives for the surgical management of OS are complete resection of the tumor with appropriate surgical margins,[32] while aiming to maintain optimal limb function.

As discussed earlier, prior to the 1970s the preferred surgical approach for malignant bone tumors was amputation. Endoprosthetic reconstruction for patients with OS was introduced in 1970s. Today, amputation is used only in a select number of cases, as the vast majority of malignant tumors of the long bones are treated with a variety of limb-sparing procedures[33–36] that employ the use of prostheses, allografts, or autograft.[37,38] Expandable prostheses are currently used for children with an expected growth of more than 30 mm.[37] (Fig 3).

Today, amputation is used only in a select number of cases, as the vast majority of malignant tumors of the long bones are treated with limb-sparing procedures.

Rotationplasty is another option for patients as an alternative to amputation for skeletally immature patients with tumors of the femur. This procedure consists of excising the distal femur with the objective of obtaining clear surgical margins. The normal portion of the lower distal extremity is then attached to the femoral stump after rotating it 180°. The ankle functions as a knee joint, and the patient is later fitted with a below-the-knee artificial prosthesis. In conjunction with intensive physical therapy, rotationplasty has been associated with excellent functional outcome and a high likelihood of the patient being able to participate in sports.[39,40] The most common long-term effects associated with rotationplasty include local skin and soft tissue changes located in and around the main loading areas of the rotated foot. Asymptomatic adaptive radiographic osseous changes were observed in 25% of patients, but no pathological degenerative

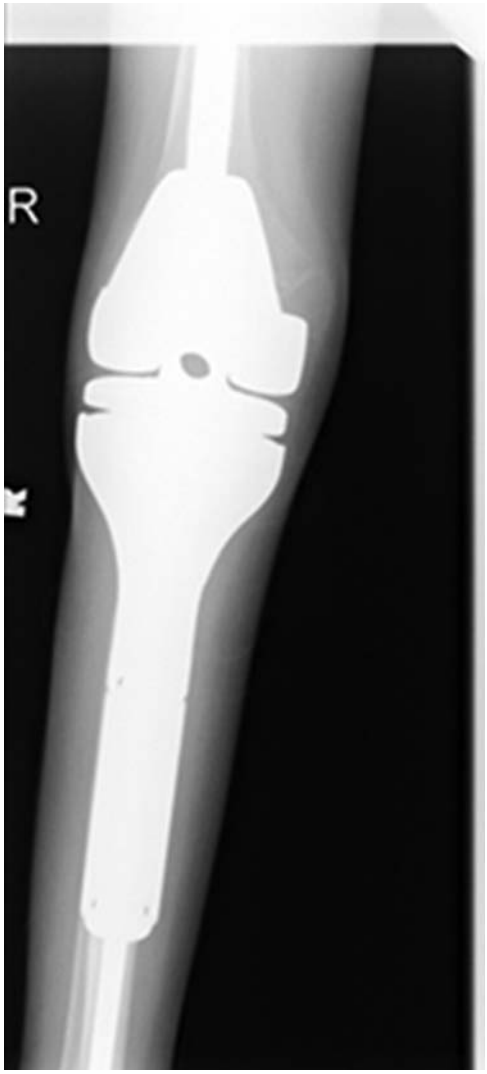

FIG 3. Plain radiograph of the right proximal tibia. Osteosarcoma—status post resection with interval placement of a total right knee prosthesis.

bony or cartilaginous changes secondary to the altered load bearing on the ankle joint have been observed in several long-term studies.[39,41]

Treatment of Recurrent and or Metastatic Disease

Treatment of patients with recurrent osteosarcoma remains challenging, with very few effective therapeutic options. A subset of patients with relapsed disease is able to achieve subsequent surgical remission, but the majority of patients will subsequently relapse. The main predictors of survival after osteosarcoma recurrence include the time to first recurrence, disease burden, and ability to achieve complete surgical remission after recurrence.[42]

In recent years, ifosfamide either alone or in combination with etoposide has been evaluated in recurrent disease. Although the combination in multiagent trials has not been shown to improve survival in patients with poor tumor necrosis, when employed for patients with pulmonary recurrence response rates of 33% or greater have been reported, making this combination the currently recommended therapy for recurrent disease. Local control of OS pulmonary metastasis with thoracotomy and chemotherapy treatment with ifosfamide or ifosfamide plus etoposide chemotherapy is required to effect long-term survival in 30% of patients with recurrence.[43,44]

Complications of Therapy and Late Effects

Treatment of osteosarcoma may be associated with acute and/or chronic toxicities from chemotherapy as well as functional disability. The most common acute treatment-related toxicities of chemotherapy are alopecia, myelosuppression, mucositis, and nausea and vomiting. Young adult survivors of childhood cancer have at least 1 chronic or late effect associated with prior cancer therapy.[45] Treatment-related late effects and toxicities of osteosarcoma therapy may include cardiac toxicity, acute and chronic nephrotoxicity, neurotoxicity, hearing loss, infertility, and second malignant neoplasms.[46] All patients should have baseline renal, cardiac, hematologic, and hepatic function evaluation prior to starting chemotherapy and should be followed in an oncology program focused on late effects of chemotherapy.

Osteosarcoma Tumor Genomics

Unlike many other pediatric and young adult malignancies, there is no single pathognomonic mutation, chromosomal translocation, or common genetic aberration associated with this malignancy.[47] These tumors also have a high mutational burden, comparable to adult malignancies.[48] Chromothripsis is a well-recognized single catastrophic event within a cell characterized by chromosome breakage and inaccurate reassembly that result in genomic alterations and complex karyotypes that lack consistent genetic findings.[49] This is often an early sentinel event in OS tumor development and one-third of tumors exhibit chromothripsis.[50] Next-generation sequencing has

recently verified a variety of somatic mutations in TP53, RB1, CDKN2A, and MYC that drive OS oncogenesis.[48,51]

Emerging Targets and Therapeutic Agents in the Treatment of Osteosarcoma

MTP-PE is an immune modulator and a synthetic analogue of a component of the bacterial cell wall of Bacille Calmette–Guerin that activates monocytes and macrophages to become tumoricidal. The efficacy of MTP-PE was first demonstrated in pre-clinical studies of mouse xenograft models and spontaneous canine osteosarcoma. MTP-PE was evaluated by the Children's Oncology Group in a prospective, randomized, and phase 3 trial of newly diagnosed OS. The patients in this trial were randomized in a factorial manner; first being randomized to a 3-drug chemotherapy regimen (doxorubicin, cisplatin, and high-dose methotrexate) or a 4-drug regimen that added ifosfamide. Then the patients were randomized a second time to receive or not to receive MTP-PE in addition to their assigned chemotherapy. The first analysis of this trial detected a possible interaction between ifosfamide and MTP-PE.[52] A subsequent analysis of data demonstrated that the addition of MTP-PE to chemotherapy resulted in an increase in the 6-year overall survival from 70% to 78% ($P = 0.03$),[53] but this remains an area of controversy. MTP-PE is not currently approved for use in the United States, as clinical evidence of its benefit is inconclusive. This agent is currently approved for use in non-metastatic osteosarcoma in Europe, Mexico, South Korea, Switzerland, and Israel.[54]

Monoclonal antibody therapy against the OS tumor antigen, GD2, is a new emerging therapy that will be investigated by the Children's Oncology Group. GD2 is a surface glycolipid that is expressed at high levels in osteosarcomas. Its expression remains stable and is rarely lost under treatment pressure[48,55] and in some estimates is expressed in 95% of osteosarcomas.[56,57] Anti–GD2 monoclonal antibody therapy has significantly improved survival in neuroblastoma and based on this experience, this approach holds promise for the treatment of osteosarcoma.

The discovery of the primary mechanism involved in bone remodeling, the receptor activator of nuclear factor κB ligand signaling pathway, RANK/RANKL/osteoprotegerin (OPG), has renewed interest in targeting the bone microenvironment to develop new therapeutics.[58] The RANK/RANKL/OPG is not only responsible for normal bone hemostasis; the deregulation of this complex is strongly associated with the development of osteosarcoma.[60,42] In osteosarcoma, RANKL activates downstream signaling and modulates gene expression. The (RANKL) is expressed on osteoblasts and stromal cells and its receptor (RANK) is present on the osteoclast surface.[59] Approximately 69–75% of osteosarcoma tumors express RANK and 9% express RANKL.[59,60] RANK expression occurs more frequently in osteosarcoma of the lower extremity than in any other location and is prognostic for an inferior disease-free survival and a poor response to preoperative chemotherapy in osteosarcoma patients.[61,62] Approximately 69–75% of osteosarcoma tumors express RANK and 9% express RANKL[59,60] Denosumab, a fully human monoclonal antibody to the receptor activator of nuclear factor-κB ligand (RANKL),[62] currently approved by the FDA for the treatment of osteoporosis and bone metastases from solid tumors and is currently being be investigated by the Children's Oncology Group in a prospective single arm, open-label, phase 2 study (AOST1321) for recurrent or refractory osteosarcoma.

Young adult survivors of childhood cancer have at least one chronic or late effect associated with prior cancer therapy.

Osteosarcoma, as discussed earlier, has no pathognomonic mutations, chromosomal translocations, or common genetic aberrations and has a high mutational burden, comparable to adult malignancies. The development of new treatments has focused primarily on the addition of other chemotherapeutic agents to the current backbone, or intensifying the most active chemotherapy drugs. While this strategy has increased the survival of patients with localized disease; the survival rate of patients with metastatic disease remains unchanged. Thus, progress in treating these tumors is unlikely to arise from the discovery of new cytotoxic chemotherapeutics, but rather from the development of targeted therapies. The pediatric oncology community is now focusing on monoclonal antibodies and targeting the bone microenvironment to improve survival in osteosarcoma patients with metastatic and recurrent or refractory disease.

Ewing Sarcoma

Incidence and Epidemiology

Ewing sarcoma of bone (EWS), extraskeletal Ewing sarcoma, peripheral primitive neuroectodermal tumors of bone and soft tissue (PNET), and Askin tumor are malignant tumors that are collectively recognized as the Ewing sarcoma family of tumors (ESFT). Ewing sarcoma is the second most common primary malignant bone and soft tissue tumor in children and adolescents. The ESFT has a incidence of 2.5–3 cases per million per year and accounts for 2.9% of all childhood cancers.[2] This malignancy primarily affects the adolescent population although the age span ranges from pre-adolescents to young adults up to age 30 years. There are also rare documented cases of Ewing Sarcoma in infants and young children.[63–65] Unlike OS, 85–95% of ESFT tumors carry specific genetic aberrations to be discussed below.

The pediatric oncology community is now focusing on monoclonal antibodies and the bone microenvironment to attempt improve survival in osteosarcoma patients.

Clinical Presentation

The clinical presentation of this tumor varies; the vast majority of patients present with pain. A delay in diagnosis is more frequently seen in patients with ESFT compared to osteosarcoma, as a significant number of tumors arise in the axial skeleton and do not become clinically apparent until they grow to an appreciable size. EWS patients may present with constitutional symptoms such as fever and weight loss. Other symptoms are related to the site of tumor.

The majority of ESFT tumors develop in a long bone, followed by the pelvis, chest wall, and the spine; however, unlike other primary bone tumors, approximately 20% arise in soft tissue. However, in contrast to OS, ESFT most frequently involves the diaphyseal or metadiaphyseal regions of long bones rather than the metaphysis. Tumors that arise in the paraspinal region can present with spinal cord compression, a medical emergency. Pelvic tumors may present with urinary retention, sciatic nerve pain, or other neurological symptoms. Askin's tumor originates from the osseous structures of the chest wall including the ribs, scapula, clavicle, or sternum and most often presents with a mass in the chest wall and symptoms suggestive of pneumonia such as cough, fever, dyspnea, weight loss, and pleural effusions.[66]

Risk Factors

Race is a significant clinical factor in the incidence of ESFT. This malignancy occurs predominantly in Caucasians and only rarely in individuals of African or Asian ancestry. There are significant racial and ethnic differences in the age, primary tumor site (soft tissue vs. bone), and tumor size in ESFT patients. Black patients have an inferior overall survival, a predisposition to develop soft tissue tumors as opposed to bone tumors, and a lower proportion of black patients are diagnosed at age <20 years.[67] White Hispanic patients are more likely to have tumors measuring >5 cm.[68]

Intronic Alu retrotransposons, single nucleotide polymorphisms (SNP), and the differential binding of the chimeric protein to microsatellites are the 3 most common mechanisms proposed to explain the racial and ethnic differences in the incidence of the ESFT. Alu elements are discrete pieces of DNA, $\sim$300 base pairs (bp) in length, which can move from site to site within the genome.[69] The ESFT have a balanced chromosomal translocation of the EWSR1 gene on chromosome 22 with a member of the ETS transcription factor family t(11;22)(q24;q12). Alu elements (retrotransposons) located near the chromosomal breakpoint region are more common in Caucasians and occur in only 8% of individuals of African ancestry.[70] These insertions can generate insertion mutations, cause genomic instability, or alter gene expression and may represent an unidentified mechanism of oncogenesis in the ESFT and account for the racial differences in ESFT presentation.

Single nucleotide polymorphisms (SNPs), are variations in a single nucleotide that occur at a specific position in the genome, which produce individual genetic variations. SNPs on chromosome 1 and 10 are more frequently present in Caucasians compared to African populations and is thought to infer an increased risk of developing Ewing Sarcoma. The inherited variation of a locus at 10q21.3 near the transcription factor EGR2 influences Ewing sarcoma susceptibility by altering a binding site for

EWSR1-FLI1, a chimeric fusion oncoprotein created from the chromosomal translocation t(11;22)(q24;q12) between the *EWSR1* gene on chromosome 22 with a member of the ETS transcription factor protein.[71]

Diagnostic Imaging and Staging

The initial studies required for a suspected Ewing sarcoma are plain radiographs of the primary site. Plain radiographic findings associated with Ewing sarcoma include the classic "onion skin" pattern osteolytic lesion, which characteristically extends through cortex into the soft tissue forming multiple thin shells of ossification oriented parallel to the shaft of the bone (Fig 4). Sharpey's fibers are perpendicular periosteal reactions with a "hair on end" appearance and a tumor-associated periosteal elevation; the "Codman triangle" may also be seen in some cases. Similar to OS, magnetic resonance imaging (MRI) of the primary tumor is required to define the extent of tumor. CT scan of the chest with contrast or PET/CT (Fig 5) is required to discern the presence of pulmonary metastases and/or nodal metastases, bone scan or PET/CT or PET/MRI is necessary for the detection of bone metastases. Unlike OS, in ESFT metastases to the bone marrow may occur, thus bilateral bone marrow aspirates and biopsies are required to complete staging.

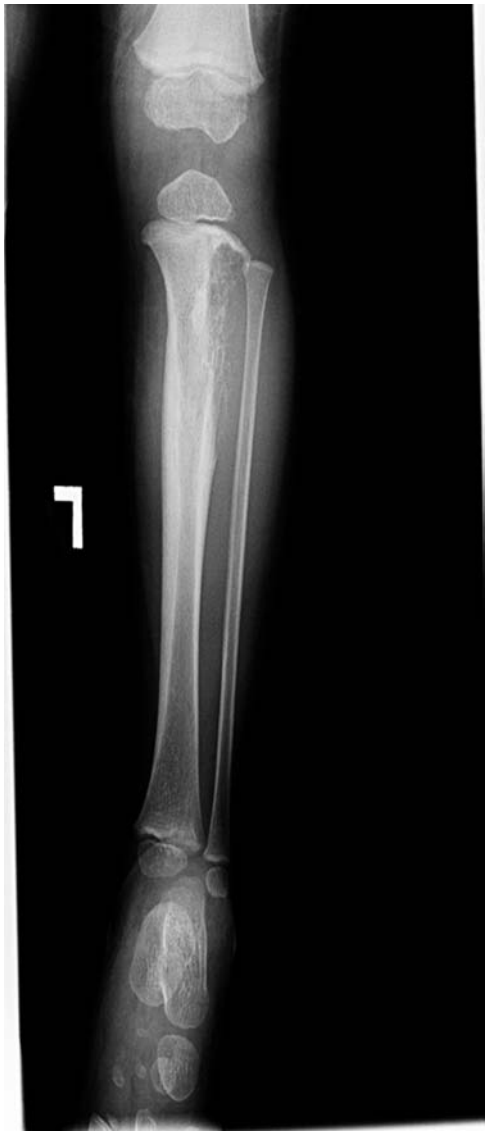

FIG 4. Plain radiograph of the left proximal tibia. Ewing sarcoma of the right proximal tibia.

Laboratory Testing

A complete blood count (CBC), comprehensive metabolic panel (CMP), erythrocyte sedimentation rate (ESR), and lactate dehydrogenase (LDH) are the baseline laboratory studies obtained prior to initiating therapy. Significantly elevated LDH is a common laboratory finding in the ESFT and is associated with a larger tumor burden. Anemia may occur in this malignancy, even in the absence of bone narrow

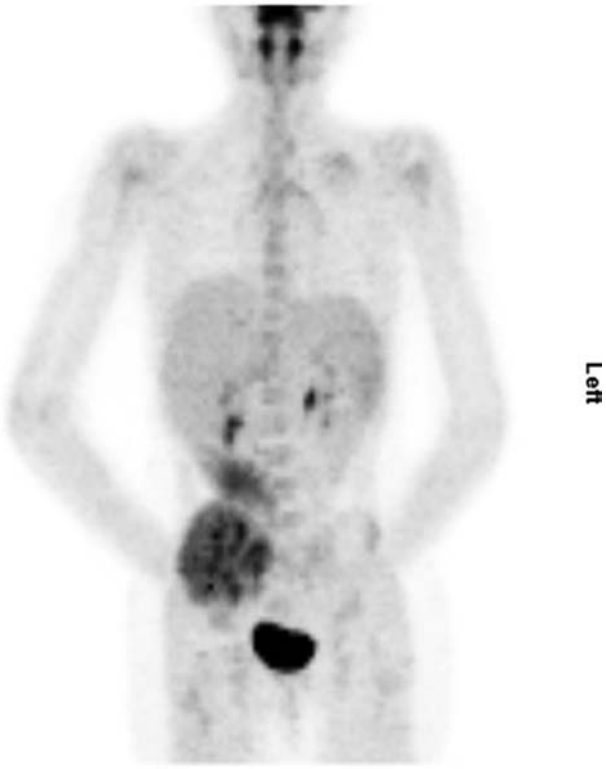

FIG 5. PET/CT—Ewing sarcoma of the right pelvis.

involvement. Rarely the ESFT metastasize to the bone marrow and can be associated with cytopenias; however, bone marrow metastasis occurs more commonly in the absence of peripheral blood findings than with obvious abnormalities in the CBC.

ESFT Biopsy

Fine-needle aspiration is not recommended for these tumors, because ESFT generally have a large amount of tumor necrosis; therefore, as in osteosarcoma core or open biopsy is preferred. It is imperative for tissue to be sent for cytogenetic or FISH evaluation for the known genetic aberrations. As with OS, biopsies for suspected EWS should be performed by an orthopedic surgeon with expertise in oncologic orthopedics and limb-sparing surgical techniques—ideally, the same surgeon who will ultimately render definitive surgical care. The location of the biopsy is carefully planned prior the procedure; as the biopsy tract should be excised completely during definitive surgical resection, and the incision should not cross tissue compartments as that might result in seeding of other compartments with tumor. Ideally, a bilateral bone marrow biopsy and aspiration should be done at the time of biopsy to complete clinical staging and to confirm the presence of ESFT chromosomal translocations.

Histological and Immunohistochemical Techniques

The ESFT are histologically characterized as poorly differentiated, small round blue cell tumors that may or may not have evidence of neural differentiation. This family of tumors is thought to be derived from mesenchymal stem cells; however, the cellular origin of the ESFT remains controversial. Classical EWS has several immunohistochemical markers, which are sensitive, but not specific to Ewing sarcoma; the markers that are consistently positive in EWS/PNET are CD99, FLI1, and NSE. PNET is a well-differentiated ESFT lesion that has both histologic and immunohistochemical evidence of neural differentiation. To date, the varying histologies have not been shown to influence prognosis.

Molecular and Genetic Approaches to Diagnosis

The ESFT have a pathognomonic balanced chromosomal translocation t(11;22)(q24;q12), which creates a chimeric fusion oncoprotein that links domains from the *EWSR1* gene on chromosome 22 with a member of the ETS transcription factor protein. The t(11;22)(q24;q12) translocation is present is approximately 85% of the ESFT. In the remaining 15% of tumors the EWSR1 gene is fused to other ETS transcription factors; ERG, ETV1, ETV4, and FEV.[72] These translocations are identified either by reverse transcription polymerase chain reaction (RT-PCR) or fluorescence in situ hybridization (FISH). Tumors that lack the EWS–ETS fusion translocation or chimeric oncoprotein are classified as translocation-negative Ewing sarcoma.

The surgical techniques employed to treat OS are also used for the ESFT but as more ESFT are axial, other modalities such as internal hemipelvectomy are more commonly required for ESFT.

Historical Perspective of ESFT Treatment

Prior to the 1970s, standard therapy for ESFT consisted of local control directed at the primary tumor. These tumors are radiosensitive, so prior to the chemotherapy era; local control was most often achieved with radiation. As many as 95% of patients treated with the use of local control alone in the absence of overt metastases at diagnosis died as a result of later metastases. This demonstrated that unlike OS, EWS is almost always micrometastatic. Cyclophosphamide was the first chemotherapy agent that showed activity against the ESFT. Combination chemotherapy with VAC (A) (vincristine, actinomycin, cyclophosphamide, and doxorubicin) and later VAC with ifosfamide and etopside was shown to be effective for the ESFT and constitute the backbone of therapy today. Currently the 5-year overall survival for patients with localized disease at presentation is 85% and the 5-year event-free survival was 73%.[73]

Prognostic Factors

The presence of metastatic disease at diagnosis is the strongest adverse clinical prognostic factor. Other independent prognostic factors found by multivariate

analysis to be associated with decreased survival include a large tumor size >8 cm, elevated serum LDH, hypoalbuminemia, older age (14–20 years of age), and axial tumor location. The survival for patients with metastasis only to the bone is marginally better than patients with metastasis to both the bone and bone marrow. Currently the 5-year overall survival rates for patients with localized Ewing sarcoma is 85% and the approximately 27% for patients with metastatic disease.[73]

ESFT Treatment: Chemotherapeutic Agents

Chemotherapy for newly diagnosed ESFT consists of a 5-drug backbone of vincristine, doxorubicin, cyclophosphamide (VDC) alternating with ifosfamide, and etoposide (IE). Standard therapy for patients with localized disease in an extremity is VDC alternating with IE administered on an interval compressed basis (every 2 weeks instead of every 3 weeks) for a total of 14–17 cycles of chemotherapy.[74] The Children's Oncology Group has piloted and is currently evaluating the efficacy of alternating cycles of the combination of vincristine, cyclophosphamide, and topotecan with the standard alternating VDC—IE interval compressed backbone in the treatment of newly diagnosed, non-metastatic Ewing Sarcoma.[75]

The small molecule YK-4-279 binds directly to EWS-FLI1 and inhibits RNA splicing, a critical oncogenic function of the EWS-ETS fusion protein.

Surgical Treatment of ESFT

Surgical resection with reconstruction is the treatment of choice for ESFT tumors that are completely resectable with wide margins. The surgical techniques employed to treat OS are also used for the ESFT, but as more ESFT are axial, other modalities such as internal hemipelvectomy or rib resection and more commonly required for ESFT. The goal of surgery is complete resection not "debulking therapy."

ESFT Radiation Therapy

Patients historically treated with radiation therapy alone were found to have a higher rate of local failure. In addition, secondary sarcomas may occur in as many as 4–30% of irradiated sites. Today, EWS treatment with radiotherapy is most often reserved for tumors with positive surgical margins or for tumors that are unresectable.[76] Most patients will receive a total dose of 60 Gy fractionated over 6 weeks. The short-term toxicities most frequently reported include dermatitis, fatigue, and nausea. Late effects associated with radiation therapy include fracture, growth arrest, joint stiffness, and secondary malignancies (complications and late effects discussed below).

Metastatic and Recurrent Ewing Sarcoma

For those with relapsed disease, time to relapse after initial therapy and location of relapse are important prognostic factors.[77] Patients who recur more than 2 years from initial diagnosis have a better DFS and OS compared to those who recur early.[78] There are limited second-line therapies that improve survival in patients with relapsed and refractory Ewing sarcoma. Combination irinotecan, and temozolomide, is a well-tolerated and active regimen recently described with reported response rates between 63% and 68% and a survival rate of 55% at 2 years.[79,80]

Complications and Late Effects

The most common treatment-related late effects associated with the treatment of the ESFT are cardiac dysfunction, infertility in men, premature menopause in women, and secondary malignant neoplasm. Treatment-related acute myeloid leukemia and myelodysplastic syndrome are the most common hematologic secondary malignant neoplasms reported in ESFT survivors, and occurring in approximately 4% of survivors. ESFT survivors also have an increased risk of developing a sarcoma in a previously irradiated field.

ESFT Tumor Genomics

The ESFT fusion protein is the primary driver of oncogenesis in these tumors; however, recent sequencing efforts have identified tumor variants with somatic mutations that are associated with an inferior outcome.[81,82] The most frequent somatic mutations seen in the ESFT are STAG2 mutations, CDKN2A deletions, and TP53 mutations. An inactivating mutation in

the tumor suppressor gene *TP53* is the second most common mutation in the ESFT.

The STAG family of proteins encode for a component of a multi-protein complex composed of 4 core subunits (SMC1A, SMC3, RAD21, and either STAG1 or STAG2) and are responsible for the cohesion of sister chromatids following DNA replication.[81] STAG2 and TP53 mutations were recently found to coexist in selected tumor populations; these mutations have a synergistic negative effect, which correlates with a more aggressive phenotype and is associated with a poor prognosis.[82]

ESFT Emerging Targets

Insulin-like growth factor 1(IGF-1) and its receptor, insulin-like growth factor 1 receptor (IGF-1R) are mediators of normal linear bone growth and cellular proliferation.[83] The Children's Oncology Group is targeting the insulin-like growth factor pathway with ganitumab, a fully human monoclonal antibody directed against IGF-1R. AEWS1221 in a randomized phase 2 clinical trial that will evaluate the addition of ganitumab to intensively timed VDC/IE for patients with newly diagnosed metastatic Ewing Sarcoma.

The small molecule YK-4-279 that antagonizes EWS–FLI1 induced leukemia has recently been identified in a transgenic mouse model.[84] This molecule binds directly to EWS–FLI1 and inhibits RNA splicing, a critical oncogenic function of the EWS–ETS fusion protein.[85] This will be the first direct target therapy against the EWS–ETS fusion protein evaluated in clinical trials for the ESFT.

The ESFT are a rare and diverse group of mesenchymal malignancies of the bone and soft tissue. Cytogenetic studies characterize these tumors as fusion-positive or fusion-negative. The first direct target therapy against the EWS–ETS fusion protein will be investigated by the COG in the near future. Fusion-negative sarcomas are difficult to distinguish based on histological features alone, owing to frequent phenotypic overlap, and immunohistology is primarily used to exclude morphologically similar tumors. The accurate diagnosis of these tumors is essential because the treatment options, response to therapy and prognoses vary depending on the diagnosis. Epigenetic modifications are now recognized as a primary mechanism of oncogenesis particularly in pediatric cancer.

DNA Methylation and Pediatric Malignant Bone Tumors

In pediatric malignancies, studies of whole exome or whole genome sequence data show that only 5–15% of pediatric tumor types have point mutations, translocations, or copy number alteration in genes compared to adult malignancies. This process is catalyzed by DNA methyltransferases that chemically modifies the promoter region of genes by adding a methyl group to the carbon 5 position of the cytosine ring in CpG dinucleotides that in turn alters the expression and regulation of key genes.[86] Epigenetic alterations of DNA repair or cell-cycle control genes have recently been shown to play an essential role in tumor development. To date, few studies have examined genome-wide DNA methylation in pediatric sarcomas. These studies have been limited by small sample sizes and a restricted number of genes evaluated, but have nonetheless shown that unique methylation patterns correlate with the different subtypes of pediatric sarcomas, including OS and the ESFT.

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