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LEADING ARTICLE

Bone Sarcomas in Pediatrics: Progress in Our Understanding of Tumor Biology and Implications for Therapy

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Abstract The pediatric bone sarcomas osteosarcoma and Ewing sarcoma represent a tremendous challenge for the clinician. Though less common than acute lymphoblastic leukemia or brain tumors, these aggressive cancers account for a disproportionate amount of the cancer morbidity and mortality in children, and have seen few advances in survival in the past decade, despite many large, complicated, and expensive trials of various chemotherapy combinations. To improve the outcomes of children with bone sarcomas, a better understanding of the biology of these cancers is needed, together with informed use of targeted therapies that exploit the unique biology of each disease. Here we summarize the current state of knowledge regarding the contribution of receptor tyrosine kinases, intracellular signaling pathways, bone biology and physiology, the immune system, and the tumor microenvironment in promoting and maintaining the malignant phenotype. These observations are coupled with a review of the therapies that target each of these mechanisms, focusing on recent or ongoing clinical trials if such information is available. It is our hope that, by better understanding the biology of osteosarcoma and Ewing sarcoma, rational combination therapies can be designed and systematically tested, leading to improved outcomes for a group of children who desperately need them.

Key Points

Many of the therapeutic targets important in common adult cancers are also important for osteosarcoma and Ewing sarcoma.

Preclinical and early clinical trial data are available to support the use of many of these agents in children.

Combination therapy has generally been safe in children and should be evaluated further with more agents.

1 Introduction

Osteosarcoma (OS) is the most common type of primary bone cancer [1], occurring primarily in adolescents and young adults, with a peak incidence in the second decade of life. Standard therapy consists of surgical removal of any resectable primary tumor and metastases, combined with 6–9 months of neoadjuvant and adjuvant chemotherapy [2]. Current chemotherapy regimens include four agents: doxorubicin (adriamycin), cisplatin, and high-dose methotrexate with leukovorin rescue [3–5]. Some clinicians have used ifosfamide for patients with high-risk or metastatic disease [6], though the recently completed EURAMOS (European and American Osteosarcoma Study) showed definitively that the addition of ifosfamide to adjuvant MAP (methotrexate, doxorubicin [adriamycin], and cisplatin) chemotherapy for OS patients with poor necrosis increased toxicity without improving survival (results presented at the annual meeting of the Connective

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Tissue Oncology Society Annual Meeting, Berlin, Germany, 2014). Although modern multimodal therapy yields 70 % survival for patients without overt metastasis at diagnosis, outcome for metastatic OS remains poor: fewer than 30 % of patients presenting with metastases survive 5 years after diagnosis [7].

Ewing sarcoma (ES) is the second most common bone malignancy. It is characterized typically by a translocation between chromosomes 22 and 11, generating a fusion between the *EWS* and *FLI1* genes [8]. ES occurs through a broad age range, from infants to older adults, with a peak incidence in the second decade of life and a slightly higher incidence rate in males [9, 10]. ES arises most frequently in bones, but occasionally develops in soft tissues [11]. Intensive multimodal treatment with combination chemotherapy, surgery, and radiation has increased the overall survival rate from less than 10 % to around 50 % [12–15]. The current standard of care for newly diagnosed ES consists of chemotherapy with five drugs: vincristine/doxorubicin/cyclophosphamide alternating with ifosfamide and etoposide [16, 17]. Standard therapy should include 17 cycles of chemotherapy, though 'good-risk' patients with localized disease in an extremity may be safely reduced to 14 cycles. Chemotherapy cycles should be compressed to every 2 weeks rather than every 3 in those patients who can tolerate it—typically younger patients—as compressed timing has a proven survival advantage [18].

While intensive multi-agent chemotherapy has improved survival compared with the pre-chemotherapy era, there have been few recent improvements in outcome for either non-metastatic patients or those who present with metastatic disease, and it has been difficult even for therapies that prove beneficial, such as mifamurtide [19], to obtain regulatory approval. However, in recent years, great advances have been made in understanding the molecular basis of pathogenesis and progression of pediatric bone sarcomas. This new understanding has been achieved in parallel with an explosion of novel therapies developed specifically to inhibit cancer-associated genes and pathways. Identification of key regulatory pathways and molecular biomarkers yielded dramatic changes in outcome for several adult cancers, but childhood cancer, and bone sarcomas in particular, have largely been sidelined in this revolution.

To help make these important discoveries relevant for childhood bone sarcomas, it is important to have an understanding of the role of each signaling pathway in the biology of the disease, as well as the available agents that target these processes. Priority was given to those pathways for which there is good information about the relevance to OS or ES, and those agents for which data are available. Where possible, we describe the reported results of clinical trials completed with novel therapies, especially highlighting those that involve children or are specific for bone sarcoma. We highlight the studies that we have been able to identify that use targeted therapy for bone sarcoma, whether for children or adults, since some treating physicians may choose to apply knowledge gained from these adult studies to their care of children with similar conditions. We have also included the results of some novel therapies that have been proven ineffective in clinical trials. To provide coherence to this broad topic, we have organized this review into sections highlighting processes at the plasma membrane, intracellular signaling pathways, bone metabolism, and the environmental and immune interactions of bone sarcoma.

2 Surface Markers for Osteosarcoma (OS): Receptor Tyrosine Kinases

Receptor tyrosine kinases (RTKs) are cell-surface proteins that act as receptors for various extracellular ligands, including growth factors, hormones, and cytokines. In addition to regulating normal cellular processes, RTKs and the intracellular signaling pathways they activate are critical to oncogenesis for many types of cancer [20]. Deregulation of a variety of RTKs, including insulin-like growth factor receptor type I (IGF-1R), vascular endothelial growth factor receptor (VEGFR), human epidermal growth factor receptor 2 (HER2, also called ERBB2), and platelet-derived growth factor receptor (PDGFR), have been implicated in pediatric bone sarcomas [21, 22]. Note that, because of the biology of the process affected, VEGFR inhibition is discussed in the environmental interactions section, rather than with RTKs, since the target tissue affected by these agents is the tumor vasculature rather than the tumor cells themselves. Since RTKs were the first molecular targets attacked in the current wave of small molecule therapeutics, with the greatest range of drugs approved or in development, each of the RTK pathways relevant to OS and ES biology is considered below.

2.1 Insulin-Like Growth Factor Receptor Type I (IGF-1R)

IGF-1R mediates cell differentiation, proliferation, and apoptosis in human cancer by activating two major oncogenic signaling cascades: the phosphoinositide 3-kinase (PI3K) pathway and the mitogen-activated protein kinase (MAPK) pathway [23, 24]. Elevated expression of IGF-1R has been observed in most OS and ES cell lines and tumor samples [25–27]. Overexpression of IGF-1R and its ligand IGF-1 in pediatric bone sarcomas is correlated with a poorer prognosis, and IGF pathway inhibition impeded tumor growth and metastasis in preclinical models [28, 29].

Current therapeutic approaches directed against the IGF-1R pathway can be grouped into three categories:

monoclonal antibodies targeting IGF-1R, IGF ligand-neutralizing antibodies, and small-molecule tyrosine kinase inhibitors. At present, eight different anti-IGF-1R monoclonal antibodies (mAbs) have been or are currently being evaluated in phase I/II clinical trials, and one of which is being evaluated in pediatric patients (Table 1). Although their safety has been proven in pediatric patients, these IGF-1R antagonists displayed limited or no clinical benefit as monotherapy for patients with advanced bone sarcomas [30–37]. Investigations using IGF-1R-targeted agents in combination are ongoing, though it is not clear what benefit these studies will show. Further, since these agents showed no benefit in common adult malignancies, their development has been abandoned by the pharmaceutical industry, suggesting that they are unlikely to be available for future bone sarcoma patients.

An alternative approach to inhibit IGF signaling is to neutralize the bioactivity of IGF ligands IGF-I and -II with mAbs. In preclinical studies, these agents achieved more effective inhibition of IGF signaling than IGF-1R mAbs by blocking binding of IGF-I and -II ligands to IGF-1R and insulin receptor (IR)-A [38, 39]. Two neutralizing antibodies against IGF-I/II are available: MEDI-573 and BI836845. A phase I clinical trial for MEDI-573 in adult patients with advanced solid tumors demonstrated stable disease in 13 of 39 patients [40]. Currently, five phase I clinical trials of BI836845 are ongoing in adult patients with various solid tumors, but there have been no specific studies in OS or ES patients. Since IGF-II and IGF-2R can also be overexpressed in OS and ES, these patients might benefit from therapies that target both IGF ligands [27, 41].

In addition to mAbs, small-molecule inhibitors of IGF-1R are also being developed. Some of these agents also inhibit IR-A-dependent tumor growth [42]. Novel IGF-1R tyrosine kinase inhibitors include linsitinib, XL-228, INSM-18, GSK1904529A, GSK1838705A, and BMS-554417, all of which have shown promising results in pediatric sarcoma models during preclinical studies [22]. As yet, no pediatric clinical trial data have been reported for these agents.

2.2 Human Epidermal Growth Factor Receptor 2 (HER2) and the ERBB Family

HER2 is one of the four RTKs in the epidermal growth factor receptor (HER/EGFR/ERBB) family and has an essential role in tumor growth. In recent years, targeted therapies against HER2 have achieved significant therapeutic benefits in the treatment of several solid tumors. However, data have been conflicting regarding expression of HER2 in OS and ES and its association with clinical outcome. While some reports demonstrated minimal expression of HER2 in tumor samples of pediatric bone sarcomas or lack of correlation between HER2 expression level and patient outcome [43–48], other studies have suggested that HER2 is highly expressed in up to 40 % of OS cases and 20 % of ES cases and its overexpression is correlated with metastases and poor prognosis [46, 49–55]. One possible reason for these disparate results may be purely technical: the Her-2 antigen is susceptible to oxidative degradation, such that it is essentially undetectable 6 months after slides are cut [56, 57]. The safety of the HER2 mAb trastuzumab in combination with standard chemotherapy has been shown in a phase II clinical trial for OS, but no benefit was seen [58]. There is no clear evidence of therapeutic benefit for this agent in bone sarcoma, and no basis for treating bone sarcoma patients with it except in the context of a clinical trial. In addition to the antibody approach, small-molecule tyrosine kinase inhibitors of the ERBB family such as erlotinib, lapatinib, afatinib, neratinib and dacomitinib are currently in clinical development [59]. Since HER4, another member of the HER family, has emerged in recent years as an essential regulator in OS, ES, and other pediatric solid tumors [60–62], the pan-Her small-molecule inhibitors (afatinib, dacomitinib, and neratinib) may represent a more effective approach in treating pediatric bone sarcomas than EGFR-specific small-molecule inhibitors such as erlotinib [63, 64].

2.3 Platelet-Derived Growth Factor Receptor (PDGFR)

The PDGF family of signaling molecules consists of five ligands (PDGF-AA, -BB, -AB, -CC, -DD), and two RTKs (PDGFR-α and -β) [65]. In OS and ES, PDGF/PDGFR signaling has a central role in tumor growth and metastasis, and overexpression of PDGFR-α and -β is often correlated with poor prognosis [66–69]. Imatinib, a potent inhibitor of c-Kit and PDGFR, has been evaluated in phase II clinical trials for treating bone sarcomas. However, this compound failed to demonstrate significant antitumor activity as a single agent in children with recurrent OS and ES [70, 71]. Since blocking PDGF/PDGFR signaling is not sufficient to inhibit tumor progression in patients, other multi-targeted RTK inhibitors such as dasatinib are currently being studied in phase I/II studies for patients with advanced sarcomas (Table 1).

3 Intracellular Signaling Pathways

Cellular signaling is a complex process by which extracellular events alter intracellular physiology and gene expression. While there is a great diversity of transmembrane receptors and other agents that can initiate signaling,

Table 1 Active clinical trials in osteosarcoma and Ewing sarcoma [180–183]

Target	Class	Drug	Clinical trial	Age (y)
IGF-1R	Anti-IGF-1R antibodies	Cixutumumab with temsirolimus	Phase II: Recurrent or refractory solid tumors in pediatric patients (NCT01614795)	>1 to 30
VEGF/VEGFR	Anti-VEGF antibodies	Bevacizumab with chemotherapy	Phase II: OS (NCT00667342) [180]	Up to 30
		Bevacizumab with chemotherapy	Phase II: ES family of tumor and desmoplastic small round cell tumors (NCT01610570)	>1
	VEGF inhibitors	Endostar (recombinant human endostatin) with chemotherapy	Phase II: OS (NCT01002092)	12–60
	Small-molecule TKIs	Pazopanib	Phase II: OS metastatic to the lung (NCT01759303)	>60
		Pazopanib	Phase II: Refractory solid tumors in children, adolescents, and young adults (NCT01956669)	>1 to 18
		Regorafenib	Phase II: Refractory liposarcoma, OS, and ES (NCT02048371)	>18
		Sorafenib with irinotecan	Phase I: Relapsed or refractory solid tumors in pediatric patients (NCT01518413)	2–22
		Sorafenib with everolimus	Phase II: Relapsed and non-resectable high-grade OS (NCT01804374) [181]	>18
PDGFR	Small-molecule TKIs	Imatinib mesylate	Phase II: Refractory or relapsed solid tumors in children (NCT00030667)	Up to 30
		Dasatinib	Phase II: Advanced sarcomas including ES (NCT00464620)	>13
		Dasatinib with ipilimumab	Phase I: Advanced sarcomas including OS and ES (NCT01643278)	>18
HDACi	Small-molecule inhibitors of histone deacetylase	Vorinostat, docetaxel, and gemcitabine	Phase Ib/II: Advanced sarcoma	>18
		Vorinostat and etoposide	Phase I/II: Relapsed/refractory sarcomas	<4 to 21
		Valproic acid and bevacizumab with gemcitabine and docetaxel	Phase I/II: Locally advanced, unresectable or metastatic sarcoma (NCT01106872) Note: this is a combination of HDACi with VEGF inhibition	>18
Bone metabolism	Bisphosphonates	Zoledronic acid/zoledronic acid with 'standard chemotherapy'	Phase II/III: High-grade OS (NCT00691236)	18–65
		Zoledronic acid with chemotherapy	Phase III: High-grade OS (NCT00470223)	5–50
		Zoledronic acid with busulfan	Phase III: Localized and disseminated ES (NCT00987636)	4–50
	Conjugated radioisotopes	153SM-EDTMP with external beam radiotherapy	Phase II: High-risk OS (NCT01886105)	13–65
		Radium-223 dichloride	Phase I/II: High-risk OS (NCT01833520)	>15
mTOR	Small-molecule inhibitors	Everolimus	Phase II: Refractory or relapsed OS (NCT01216826)	Up to 21
		Sirolimus with chemotherapy	Phase I: Recurrent and refractory solid tumors in children (NCT01331135)	Up to 30
		Sirolimus with cyclophosphamide	Phase II: Advanced sarcomas including OS and ES (NCT00743509)	>16
Notch	Gamma secretase inhibitors	RO4929097 with vismodegib	Phase I/II: Advanced or metastatic sarcoma including OS and ES (NCT01154452)	>18
Hedgehog	Hedgehog signaling antagonists	Vismodegib with RO4929097	See above	>18
Src	Small-molecule inhibitors	Saracatinib	Phase II: Recurrent OS localized to the lung (NCT00752206)	15–74

Table 1 continued

Target	Class	Drug	Clinical trial	Age (y)
PARP	PARP inhibitors/ alkylating agents	Olaparib with temozolomide	Phase I: Recurrent or metastatic ES following failure of prior chemotherapy (NCT01858168)	>18
		Olaparib	Phase II: Recurrent of metastatic ES following failure of prior chemotherapy (NCT01583543) [182]	>18
		Niraparib with temozolomide	Phase I: Previously treated, incurable ES (NCT02044120)	>13
		BMN-673 with temozolomide	Phase I/II: Refractory or recurrent malignancies including ES in younger patients (NCT02116777)	13–30
Immunotherapy	Interferons	Low-dose IFNα-2b with thalidomide	Phase II: Soft tissue sarcoma or bone sarcoma (NCT00026416)	>18
	Immunostimulants	Aerosol IL-2	Phase I/II: Pulmonary metastases of solid tumors including OS and ES (NCT01590069)	12–50
	GD2-based therapies	Activated T cells armed with GD2-bispecific antibody	Phase I/II: OS and neuroblastoma in children and young adults (NCT02173093)	>1 to 29
		Humanized anti-GD2 antibody (HU14.18K233A)	Phase I: OS and ES in children and adolescents (NCT00743496) [183]	Up to 21
		T cells expressing an anti-GD2 chimeric antigen receptor	Phase I: GD2+ solid tumors in children and young adults (NCT02107963)	1–35

ES Ewing sarcoma, *HDACi* histone deacetylase inhibitor, *IFN* interferon, *IGF-1R* insulin-like growth factor receptor type 1, *IL* interleukin, *mTOR* mammalian target of rapamycin, *OS* osteosarcoma, *PARP* poly ADP ribose polymerase, *PDGFR* platelet-derived growth factor receptor, *Src* steroid receptor co-activator, *TKIs* tyrosine kinase inhibitors, *VEGF* vascular endothelial growth factor, *VEGFR* VEGF receptor, *y* years

common pathways often are used by diverse receptors, and therapeutic approaches have been developed to attack the most vital pathways in cancer. In recent years, there have been many advances in our understanding of how these pathways function in OS and ES, and which are essential for the cancer cell. The intracellular signaling pathways important for OS and ES are discussed here.

3.1 Ezrin

As a member of the ezrin/radixin/moesin (ERM) family, ezrin links the actin cytoskeleton to the plasma membrane. Gene microarray studies demonstrated increased Ezrin expression in metastatic OS lesions [72]. Further, high ezrin expression in OS patients, both human and canine, was correlated with poor overall survival [72, 73]. Khanna and colleagues [73, 74] demonstrated that early steps in OS pulmonary metastases are dependent on ezrin-mediated protein kinase B (AKT) and MAPK signaling, and reduction of ezrin expression by a short hairpin RNA (shRNA) decreased the survival of metastatic cells in the lung. The relevance of ezrin in metastatic disease has been validated for other sarcomas, including ES, although in this model ezrin mediates metastasis by signaling through the AKT/mammalian target of rapamycin (mTOR) pathway [75]. Two small-molecule ezrin inhibitors have been successfully studied in vitro and in vivo using OS models, but these agents still await testing in clinical trials [76].

3.2 Mammalian Target of Rapamycin (mTOR)

The mTOR is a serine/threonine kinase and integral effector of the PI3K–AKT signaling pathway. It regulates cell cycle progression and protein synthesis among other steps during carcinogenesis [77]. Rapamycin (sirolimus) and its derivatives have been effective at reducing tumor growth in OS and ES murine models and in clinical trials [78–82] and has been used as a radiosensitizer for OS [83]. A recent phase III clinical trial tested ridaforolimus in adult sarcoma patients who had achieved objective responses with prior chemotherapy [84]. For the 702 patients treated on that study (only 10 % had bone sarcoma), ridaforolimus increased progression-free survival by 28 % ($p < 0.001$), but greatly increased grade 3 or higher toxicities, especially stomatitis, cytopenias, and infection. The report does not provide a subset analysis for the bone sarcoma patients. Several clinical trials using mTOR inhibitors in combination therapies are in progress (Table 1).

3.3 Steroid Receptor Co-Activator (Src)

The steroid receptor co-activator (Src) family of kinases is expressed at high levels and is constitutively active in many cancers, including OS and ES. Pharmacologic inhibition of Src in vitro led to apoptosis and decreased invasion, migration, and adhesion of OS and ES cells; however, these results were not reproducible using OS in vivo

models, pointing at possible redundancy in activation of downstream effectors like focal adhesion kinase (FAK) [85–88]. A dual inhibitor of BCR-Abl and Src, dasatinib has been used in one clinical trial where the maximum tolerated dose was determined but no objective responses were observed [89]. Clinical trials are in progress with either dasatinib alone or in combination therapy or with saracatinib, an Src-specific inhibitor.

3.4 Notch

Signaling via the Notch pathway is essential for the development of most organ systems, including for both neurogenesis [90] and osteoblast maturation [91]. Activation of the Notch pathway is required for vasculogenesis during tumor progression in ES [92]. Notch has been linked to increased invasion and metastasis in OS, in part through promoting a tumor-initiating cell phenotype [93–95]. Membrane-bound Notch activation upon ligand binding occurs through a two-step proteolytic process carried by ADAMs family proteases followed by gamma-secretase cleavage, releasing a soluble intracellular Notch that can regulate transcription [96]. A phase I clinical trial in advanced solid malignancies using a gamma-secretase inhibitor (GSI) showed anti-tumor activity and a low toxicity profile [97]. A phase I/II clinical trial using a GSI in combination with an inhibitor of the hedgehog pathway for the treatment of metastatic sarcomas is currently recruiting patients. In considering the effects of GSI, one should recall that GSIs inhibit the processing of several receptors that effect metastasis, including Her-4, CD44, E-cadherin, and N-cadherin [98].

3.5 Hedgehog

The hedgehog pathway is important for embryonic development and is dysregulated in various cancers. High expression of the hedgehog ligands and targets are observed in both OS and ES models, where this pathway is activated in both a ligand-dependent and a ligand-independent manner [99–101]. Interestingly, EWS-FLI1 signaling is mediated through GLI, an effector and transcription regulator in the hedgehog pathway [102]. Inhibition of the hedgehog pathway in vitro and in ES and OS xenografts has been successful and warrants further research [100, 103]. In a recent clinical trial in adult patients with advanced solid tumors, an oral inhibitor of the hedgehog pathway was fairly well tolerated [104]. While the skeletal abnormalities seen in young mice briefly treated with hedgehog pathway inhibitors might raise concerns about pediatric applications for these agents [105], most OS and ES patients are close to their expected adult size at diagnosis, suggesting that these concerns should not preclude study.

3.6 Histone Deacetylase Inhibitors

Histone deacetylase inhibitors (HDACi) have been studied in cancer due to their effects in promoting transcription of tumor suppressor genes silenced during malignant transformation. Phase I clinical trials in pediatric patients with relapsed or refractory solid tumors using pracinostat or vorinostat monotherapy showed no tumor responses [106, 107]. Patient trials are underway using combination therapy with HDACi and adjuvant chemotherapies and may have greater promise (Table 1). Additionally, treatment with HDACi in preclinical models caused upregulation of natural killer (NK) cell recognition markers and of the apoptosis-promoting Fas receptor, resulting in increased sensitivity to NK-mediated killing [108, 109]. These results warrant further investigation in clinical trials of HDACi plus NK cells.

3.7 Ras

Ras proteins are small GTPases that regulate cell proliferation, apoptosis, and survival by activating multiple downstream signaling pathways, including MAPK. Though constitutively active, Ras mutations are uncommon in pediatric sarcomas; targeting Ras reduced tumor growth, possibly due to the many pathways requiring Ras relay signals [110–113]. Reolysin is an oncolytic virus that selectively targets Ras transformed cells, and xenografts showed tumor growth inhibition by reolysin used alone or with chemotherapy agents [114]. A phase II study in sarcoma patients has been completed. While partial results were presented at the American Society of Clinical Oncology (ASCO) annual meeting in 2009 [115], there have been no peer-reviewed publications for sarcoma since that abstract was presented.

3.8 MDM2

MDM2 is a ubiquitin ligase that regulates p53 activity by targeting this tumor suppressor for proteasomal degradation. Nutlins are small molecules that inhibit MDM2 and p53 binding, leading to increased availability of p53. Treatment using nutlins have been effective in OS and ES models, inducing apoptosis and cell cycle arrest [116–118]. RG7112, a nutlin family member, induced tumor regression in ES models, but no objective response was observed with OS models [119]. Recently, a phase I clinical trial using RG7112 in patients with relapsed or refractory tumors was completed, though results have yet to be reported.

4 Targeting Bone Metabolism

Tumor growth and metastasis often require constant interactions between tumor cells and their surrounding microenvironments [54, 120–123]. Therapeutic agents that target the bone environment and modulate bone metabolism have demonstrated some efficacy in pediatric bone sarcomas.

4.1 Bisphosphonates

Bisphosphonates, which inhibit the mevalonate pathway at high concentrations and impede osteoclast-mediated bone resorption through induction of osteoclast apoptosis, have been shown to suppress tumor growth and pulmonary metastasis of ES in preclinical models [124–128]. To date, several types of bisphosphonates, including zoledronate, pamidronate, and alendronate, displayed significant antitumor activity in vitro and in vivo [129–132]. A phase II study evaluating the combination of chemotherapy and pamidronate for patients with OS demonstrated little impact on patient survival [133]. However, pamidronate has been shown to improve the durability of limb reconstruction [133]. In a recently completed phase I study, the addition of zoledronate to conventional multi-agent chemotherapy was safe but failed to demonstrate statistically significant differences in event-free or overall survival in patients with newly diagnosed metastatic OS [134]. However, our clinical team has treated many patients with bone metastasis of OS with zoledronate, and we have found that patients usually do not develop new bone metastases after receiving four to six doses of monthly zoledronate. We also have the impression that the need for opiates during palliation is reduced after patients receive bisphosphonates, suggesting that the clinical trials performed to date may not have looked at the correct endpoints. Currently, three phase II/III trials that evaluate the efficacy of zoledronate as a single agent or as an adjuvant to chemotherapy in localized and metastatic OS and ES are ongoing (Table 1).

4.2 Conjugated Radioisotopes

Conjugated radioisotopes such as Samarium (^{153}Sm) lexidronam (Samarium-153 EDTMP) and radium-223 dichloride (Xofigo) have high specificity for bone uptake, which allows for the local delivery of high-dose radiation in bone tumors [135, 136]. Standard dose of Samarium-153 EDTMP was originally approved by the US FDA for pain management in patients with bone metastases, and radium-223 was recently approved for the treatment of castration-resistant prostate cancer patients with symptomatic bone metastases. Although radiation therapy is not widely used in treatment for OS, high-dose conjugated radioisotopes are under clinical investigation for their anti-tumor activities against OS. In a follow-up study of 14 patients with osteoblastic OS, Samarium-153 EDTMP in combination with the radiosensitizer gemcitabine induced short-term antitumor response in eight patients [137]. Thus far, conjugated radioisotopes have no clear role in Ewing sarcoma. The ongoing clinical trials for this class of agents include a phase I/II study for radium-223 dichloride and a phase II study for Samarium-153 EDTMP in combination with external radiotherapy in high-risk OS (Table 1).

4.3 Denosumab

Among the signaling molecules that have been associated with worse outcome in OS is the receptor activator of nuclear factor-kb (RANK), along with its ligand (RANKL) and decoy osteoprotegerin (OPG), which normally are essential for regulation of the homeostasis between bone lysis and formation during bone remodeling [138, 139]. High expression of RANKL is associated with reduced survival in OS [140], and some OS cell lines have functional RANK expression [141], allowing for possible autocrine stimulation of this pathway. Inhibition of RANK with shRNA reduced motility and anoikis resistance in OS cell lines, while overexpression of RANK using a retroviral vector increased OS cell motility without affecting proliferation [142].

Denosumab is an mAb specific for human RANKL and was developed initially to treat osteoporosis [143] and was later found effective in treating painful bone metastasis [144–147]. It was subsequently found to be an effective treatment for giant cell tumor of bone [148], a benign but destructive neoplasm in which transformed mononuclear cells secrete RANKL, causing osteoclast hyperactivity. We have found that denosumab can be effective in treating painful bone metastasis in OS, which is in line with the FDA-approved indication for the drug. Whether it will have any direct effect against OS in patients remains to be seen.

5 Environmental and Immune Interactions of Bone Sarcoma

While initial studies of cancer biology took a purely cell-autonomous view of the cancer problem and sought to understand and then target the specific biology of the malignant cell, it is now abundantly clear that all cancers, including bone sarcomas, exist in a complex environment of non-malignant supporting cells like fibroblasts and endothelial cells, non-cellular stromal elements and matrix

proteins, and cellular and protein components of the innate and adaptive immune system [149]. While malignant cells may become resistant to conventional chemotherapy, they still must evade the immune system and continue to recruit a blood supply and engage their environment for tumors to grow and spread [150]. Recent developments seek to better understand these interactions and exploit them for therapy.

5.1 Immunotherapy

5.1.1 Mifamurtide

Muramyl tripeptide phosphatidyl ethanolamine (L-MTP-PE or mifamurtide) is a synthetic peptide derived from the cell wall of the Bacille Calmette-Guerin mycobacterium that has potent immunostimulatory properties [151]. Liposomal encapsulation of MTP-PE with phospholipids that include phosphatidyl serine specifically triggers uptake into macrophages and monocytes [152], which then become activated, increasing phagocytosis and secreting interleukin (IL)-6, tumor necrosis factor (TNF)-α and other cytokines [19, 151, 153]. A phase III clinical trial concluded that addition of mifamurtide to standard chemotherapy leads to an increase in the 6-year overall survival in primary OS patients from 70 to 78 % [19]. Mifamurtide has been approved as an adjuvant for the treatment of primary OS in Europe, Israel, Japan, and Mexico, among other places, but has not been approved by the US FDA [151, 153, 154].

5.1.2 Sargramostim

Sargramostim, the granulocyte macrophage colony-stimulating factor (GM-CSF) is an immune modulator that promotes the activation and recruitment of neutrophils, monocytes, and other immune cells [155]. Promising in vitro and in vivo preclinical studies with sargramostim prompted a phase I clinical trial of inhaled sargramostim, which demonstrated low toxicity [156–158]. However, a phase II clinical trial did not show any survival benefit compared with standard treatment regimes in OS and ES patients [159, 160].

5.1.3 Other Immunomodulators

Conflicting results have been observed using interferon (IFN)-α for OS treatment. Despite some promising early studies [161], the good responder arm of the EURAMOS 1 trial proved there is no benefit of adjuvant IFN in OS patients [162]. Systemic treatment use of IL-2 has limited effects in survival due to life-threatening side effects [163]. Aerosol IL-2 has been demonstrated to target metastatic lung disease by recruiting NK cells to the lungs [164, 165]. A clinical trial using aerosol IL-2 in metastatic lung lesions is underway (Table 1).

5.1.4 Other Immunotherapies

Other immunotherapy approaches currently being investigated in clinical trials include tumor vaccines using tumor antigens or autologous antigen-presenting cells loaded with tumor antigens, T-cell and NK-cell adoptive therapy, and targeted therapy using antibodies for tumor antigens (GD2) or to enhance T-cell activation (ipilimumab) (Table 1). Immunotherapy approaches provide exciting new avenues for pediatric sarcoma treatment.

5.2 Environmental Interactions: Matrix and Vasculature

Part of the pathogenesis of bone sarcomas includes the ability to invade through extracellular matrix tissues and to recruit a new blood supply as tumors grow [166]. As a part of hematogenous metastasis, tumor cells must also gain access to the endovascular space [91]. These activities typically proceed through hijacking normal biological processes that are then exploited by tumor cells to facilitate their growth and spread [167].

5.2.1 Matric Metalloproteases

Matrix metalloproteases (MMPs) are important mediators of invasion and metastatic disease. Expression of MMPs allows tumor cells to effectively degrade extracellular matrix, which in turn allows tumor growth and supplements cancer cells with growth factors [168]. Enhanced expression of MMPs is found in tumors, including pediatric sarcomas [169]. Inhibition of MMP2 and MMP9 affects OS and ES tumor growth and metastasis formation [7, 111, 169, 170]. MMPs inhibition has also been observed in animal models using bisphosphonates [128, 168].

5.2.2 Vascular Endothelial Growth Factor Receptor

VEGF ligands and receptors, as crucial regulators of tumor-associated angiogenesis and vasculogenesis, have been observed to be overexpressed in OS and ES [27, 171], relative to corresponding normal tissues. High levels of VEGF were predictive of pulmonary metastasis and poor prognosis for both diseases in several studies [172–174]. Preclinical efficacy of VEGF-based therapeutics, including anti-VEGF antibodies and small-molecule inhibitors against VEGFR, has been confirmed in pediatric bone sarcomas [27, 175]. The anti-VEGF mAb bevacizumab demonstrated some clinical benefit as monotherapy or in combination with doxorubicin in patients with recurrent ES

[176, 177]. Three phase II trials of bevacizumab in combination with chemotherapy for patients with OS and ES are currently underway. Further, several multi-kinase inhibitors that target VEGFR, including sunitinib, sorafenib, pazopanib, dasatinib, and cediranib, have demonstrated growth inhibition in OS models in preclinical studies [21]. Clinical trials of several of these compounds in bone sarcomas are in progress (Table 1).

6 Discussion

Treatment of pediatric bone sarcomas is complex, requiring multimodal therapy and a comprehensive approach, best delivered in a medical center experienced in caring for children with OS and ES. The field has certainly advanced since chemotherapy became widely accepted in the treatment of these diseases in the 1970s and 1980s, but our inability to improve outcomes in the past 20 or more years underscores the importance of finding new approaches.

It is now clear that cancer therapy, rather than focusing on delivering toxins at maximally tolerated doses, needs to exploit the expanding understanding of tumor biology, both for the signaling within the cells themselves and the interactions between cancer cells and their environment. At the same time, the enthusiasm for novel therapies needs to be tempered by the reality of assessing primarily those agents that are likely to be brought forward for regulatory approval. In this way, as a field, we can avoid the kinds of disappointment that arose from the IGF-1R antibody therapies, which 'died on the vine' not because of a lack of efficacy in bone sarcoma, but because these agents did not have an identified utility for a common adult malignancy and were, therefore, financially non-viable for further development. Even more important, good clinical trial design needs to be supported by excellent preclinical evidence [178] so we can avoid rushing into large, expensive clinical trials in children that result in no improvement in outcome and expose children to unnecessary toxicity [58]. However, what should not impede progress is a misguided effort to 'protect children from the risk' of testing targeted therapies when there is sound basis for the evaluation. For the most part, children tolerate all therapies better than do adults, presumably because they have less 'wear and tear' and are generally more resilient than adults. Even the known child-specific concerns, such as the reduced growth that is known to result from samarium therapy [179] or that may be a concern for hedgehog inhibitors [105], needs to be balanced against the potential benefit to a patient with a poor prognosis. As one parent of a 10-year-old girl with advanced OS seen in our institution articulated, "I would rather have her alive and short than not have her at all".

The investigations most urgently needed now are what treatments to apply during a minimal disease state for patients at high risk of relapse before overt treatment-resistant metastases are identified. An ideal therapy would be relatively non-toxic, allowing its use for a prolonged period after cytotoxic therapy is complete, and would specifically attack the signaling pathways that allow for prolonged survival of treatment-resistant dormant tumor cells. Large genomic studies and personalized therapy may help us to identify those patients at greatest risk of recurrence, but these approaches may not give insight into the biology of dormancy, nor of putative cancer stem cells. The focus of the field now needs to turn to understanding how OS and ES persist in these patients, and which approaches would best eradicate the remaining tumor cells at that stage.

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