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Advances in the Treatment of Pediatric Bone Sarcomas

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OVERVIEW

Bone tumors make up a significant portion of noncentral nervous system solid tumor diagnoses in pediatric oncology patients. Ewing sarcoma and osteosarcoma, both with distinct clinical and pathologic features, are the two most commonly encountered bone cancers in pediatrics. Although mutations in the germline have classically been more associated with osteosarcoma, there is recent evidence germline alterations in patients with Ewing sarcoma also play a significant role in pathogenesis. Treatment advances in this patient population have lagged behind that of other pediatric malignancies, particularly targeted interventions directed at the biologic underpinnings of disease. Recent advances in biologic and genomic understanding of these two cancers has expanded the potential for therapeutic advancement and prevention. In Ewing sarcoma, directed focus on inhibition of EWSR1-FLI1 and its effectors has produced promising results. In osteosarcoma, instead of a concentrated focus on one particular change, largely due to tumor heterogeneity, a more diversified approach has been adopted including investigations of growth factors inhibitors, signaling pathway inhibitors, and immune modulation. Continuing recently made treatment advances relies on clinical trial design and enrollment. Clinical trials should include incorporation of biological findings; specifically, for Ewing sarcoma, assessment of alternative fusions and, for osteosarcoma, stratification utilizing biomarkers. Expanded cancer genomics knowledge, particularly with solid tumors, as it relates to heritability and incorporation of family history has led to early identification of patients with cancer predisposition. In these patients through application of cost-effective evidence-based screening techniques the ultimate goal of cancer prevention is becoming a realization.

Ewing sarcoma (ES) is a small, round blue cell tumor characterized by oncogenic fusions between *EWSR1* or, less often, *FUS* and genes of the *ETS* family (*FLI1* being the most common; Table 1).[1,2] In pediatric patients, ES arises in bone in 80% of patients with occurrence in axial bones slightly more common than occurrence in appendicular bones; conversely, in adults as many as 75% of primary ES arise in soft tissue. The remaining cases of ES arise in soft tissue locations. ES occurs in patients age 0 to 50 with the median age somewhere between age 13 and 17. Poor prognostic factors include presence of metastatic

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disease at diagnosis, age 18 or older at diagnosis, primary site in the pelvis, large tumor, and poor histologic necrosis after induction chemotherapy.[3]

Diagnosis of ES is usually straightforward when biopsy of a typical-appearing mass in a patient of the appropriate age demonstrates a small, round blue cell tumor with intense membranous CD99 staining, and cytogenetics, and fluorescent in situ hybridization, or reverse-transcription polymerase chain reaction demonstrate an associated fusion. It is important to note that fusions involving *EWSR1* and *FUS* are seen in a variety of other sarcomas, as well (Table 1). Thus, a fluorescent in situ hybridization result indicating a fusion involving *EWSR1* is not pathognomonic for ES. In addition, there is increasing recognition of the so-called Ewing-like sarcomas. This ill-defined group of malignancies is characterized by the presence of alternative fusions such as CIC-DUX4 and CCNB3-BCOR and histopathology not entirely classic for ES, including less uniform CD99 immunohistochemistry. The Ewing-like sarcomas appear to represent as many as 5% of the Ewing family of sarcomas, and are thought to occur more often in soft tissue locations and in older patients, and they may have a worse outcome.[2,4]

Successive trials of chemotherapy intensification in ES have resulted in improved outcomes with 5-year overall survival in 1975 to 1977 versus 2002 to 2008 increasing from 58% to 83%. Chemotherapy treatment of ES includes vincristine, doxorubicin, etoposide, and ifosfamide and/or cyclophosphamide. In the United States, all patients receive intensively timed (cycles of every 2 weeks) vincristine, doxorubicin, and cyclophosphamide alternating with ifosfamide and etoposide with growth factor support. In much of Europe, patients receive induction with vincristine, ifosfamide, doxorubicin, and etoposide with consolidation therapy depending on risk factors. Patients with localized disease and poor histologic necrosis after six cycles of vincristine, ifosfamide, doxorubicin, and etoposide induction therapy benefit from consolidation therapy with autologous stem cell transplant with busulfan and melphalan conditioning.[5,6]

THE GERMLINE IN EWING SARCOMA

ES has not classically been thought to be associated with cancer predisposition syndromes, although it has always been of interest that ES rarely occurs in African populations. More recently, links to heritable germline variants and mutations have been proposed.[7] Oncogenic fusions have been found to preferentially bind to GGAA microsatellite repeats,[8] and a large genome-wide association study analysis recently demonstrated three candidate loci associated with ES.[9] Further analysis of one of these candidate loci, *EGR2*, demonstrated cooperation between the susceptibility variant and microsatellite length that regulates a major driver of ES.[10] Whole-genome or whole-exome sequencing has identified pathogenic or likely pathogenic germline mutations in 13.1% of 175 patients with ES. Several of these mutations occurred in genes with a known susceptibility to sarcoma, such as *TP53*; however, several heterozygous carriers of mutations associated with recessive conditions, particularly Bloom syndrome and Fanconi anemia, were identified, suggesting that heterozygous mutation carriers of recessive DNA repair conditions may have increased ES susceptibility.[11] Genetic epidemiology studies such as Project GENESIS (Genetics of Ewing Sarcoma

International Study; Children's Oncology Group trial AEPI10N5) are enrolling patients to further study genetic risk for ES.[12]

USING EWING SARCOMA BIOLOGY TO ADVANCE CARE

The genomic landscape of ES presents a challenge for the development of targeted therapies. The somatic mutation frequency of ES tumors is among the lowest in human cancer.[13,14] There are few, if any, recurrent "actionable" mutations, with the most commonly identified mutations being loss of *STAG2* and *CDKN2A*.[15–17] The one recurrent mutation, the EWSR1-FLI1 transcription factor, found in 85% of tumors is widely appreciated as the oncogenic driver.[1] Unfortunately, EWSR1-FLI1 is a transcription factor and therefore poses challenges as a drug target. Nevertheless, because of the known dependence of the tumor on this target, investigators have approached EWSR1-FLI1–directed therapeutic targeting with two complementary strategies. For example, several groups have sought to develop innovative methods to directly target EWSR1-FLI1, and this has led to the identification of a number of interesting lead molecules. This approach is balanced by efforts that capitalize on the changes in gene expressed induced by EWSR1-FLI1 to target nonmutated but important oncogenes such as *IGF1* and *PARP*.

ES cells depend on the continued activity of EWSR1-FLI1 to maintain the malignant phenotype. An elegant pathway analysis has shown that as a single mutation, EWSR1-FLI1 both blocks differentiation and drives proliferation.[18] Consistent with this finding, silencing of EWSR1-FLI1 expression in ES cells blocks proliferation and places the cell in a dedifferentiated state that no longer clusters with Ewing tumors by gene expression profiling and principal component analysis and instead clusters with mesenchymal stem cells.[19,20] Recent evidence suggests that even the level of EWSR1-FLI1 expression confers adverse properties to subsets of cells within the tumor such as the ability to establish tumors or migrate.[21,22] Nevertheless, the global effect of EWSR1-FLI1 supression on the overall tumor phenotype is clearly supression of growth. Studies that have blocked EWSR1-FLI1 using either small interfering RNA or small molecules clearly demonstrate impressive suppression of ES xenograft growth that is proportional to the degree of EWSR1-FLI1 suppression.[23-25]

To identify EWSR1-FLI1 inhibitors, investigators have used a variety of techniques ranging from mechanism-based approaches, candidate compound methods, and high-throughput screening. This has led to the identification of a number of compounds that fit the following criteria of being EWSR1-FLI1 inhibitors to varying degrees: (1) impact expression of specific downstream targets of EWSR1-FLI1 such as NR0B1; (2) reverse established EWSR1-FLI1 genome-wide signatures using approaches such as gene set enrichment analysis[8,26]; and (3) defined mechanism of action. Given the complexity of transcription, the finer details of the mechanism of EWSR1-FLI1 blockade are not known for even some of the most well-established inhibitors. The first example used a gene signature screening approach to identify cytarabine as an EWSR1-FLI1 inhibitor.[27] This compound translated to the clinic but due to unusual accrual patterns, it is not clear how many patients in the phase II study were patients positive for EWSR1-FLI1.[28] Subsequent to this study, a protein interaction approach led to the identification of YK-4-279 that disrupted an interaction

between EWSR1-FLI1 and DHX9.[29] Shortly thereafter, a cell-based screen led to both the identification of Englerin A and mithramycin,[23,30] the latter an antibiotic that was repurposed for a clinical trial in ES. Unfortunately, liver toxicity prevented the drug from accumulating to serum levels high enough to block EWSR1-FLI1 activity, and the study was prematurely terminated (P.J. Grohar, unpublished data, 2017). This clinical study opened the door to the identification of two second-generation mithramycin analogs EC8042 and EC8105 as less toxic and more potent alternatives, and these continue to be further developed for the clinic.[31] A number of other compounds have been identified as EWSR1-FLI1 inhibitors by a variety of approaches including a screening approach (midostaurin/PKC412),[32] direct interrogation of DNA binding (low-dose actinomycin and shikonin),[33,34] or a candidate compound approach (JQ1).[35-38]

Two of the more established inhibitors are trabectedin and LSD1 inhibitors.[39] In a study that identified EWSR1-FLI1 repressed genes, a direct interaction was found between EWSR1-FLI1 and the nucleosome remodeling and histone deacetylase corepressor complex, of which LSD1 is a member.[40] Subsequent to this study, it was shown that inhibition of LSD1 of the nucleosome remodeling and histone deacetylase complex with a small-molecule inhibitor HCI2509 both induced EWSR1-FLI1–repressed genes and repressed EWSR1-FLI1–induced genes and effectively reversed EWSR1-FLI1 activity on a genome-wide scale.[24] This reversal of EWSR1-FLI1 activity markedly impaired ES xenograft growth.[24] Second-generation inhibitors are now under development, and the clinical translation of these compounds is expected in the near future.

Trabectedin has also been characterized as an EWSR1-FLI1 inhibitor.[39] Early preclinical studies suggested a hypersensitivity of Ewing cells to trabectedin. In the phase I trial of trabectedin, a patient with ES was an extraordinary responder. The patient had widely metastatic disease and achieved a complete response to single-agent therapy.[41,42] Subsequently, trabectedin was shown to block EWSR1-FLI1 activity at the promoter, mRNA, protein, and gene signature levels of expression.[39] More recently, it was shown that the drug redistributes EWSR1-FLI1 within the nucleus to the nucleolus, leading to a marked change in the nuclear distribution of the fusion protein.[43] Interestingly, this effect requires high concentrations of drug perhaps offering a clue why a patient with ES responded in the phase I trial, but the phase II trial in with trabectedin was given over 24 hours did not demonstrate activity in ES.[44] The effect of trabectedin is potentiated by combination with other agents, including insulin-like growth factor 1 receptor (IGF1R)–directed therapies, which mitigates drug resistance or irinotecan via suppression of the WRN helicase.[45,46] Anecdotal responses to trabectedin and irinotecan in combination in the clinic have recently been reported.[47,48] Finally, the second-generation compound lurbinectedin, which has been found to have an improved toxicity profile compared to trabectedin, also suppresses EWSR1-FLI1, causes the same nuclear redistribution of the protein, maintains synergy with irinotecan, and induces the transdifferentiation of ES cells in xenografts.[43] There is clinical interest in further developing lurbinectedin in combination with irinotecan as an EWSR1-FLI1–directed therapy.

Targeting nonmutated oncogenes of high biologic importance in ES is an approach for identifying therapeutic avenues complementary to direct targeting of EWSR1-FLI1. In many

cases, these targets are directly linked to EWSR1-FLI1. For example, a considerable amount of literature supports the therapeutic targeting of *IGF1* in ES, and ES cells have been found to be very sensitive to IGF1 blockade both in vitro and in vivo.[49,50] This sensitivity may be related directly to EWSR1-FLI1 as it is known that EWSR1-FLI1 drives expression of the IGF1R and suppresses expression of the negative regulator of IGF1, IGFBP3.[51-53] Furthermore, EWSR1-FLI1 regulates the expression of a number of micro-RNAs that regulate the IGF1 pathway.[54] It is possible that dysregulation of IGF signaling by EWSR1-FLI1 aids in the process of malignant transformation of ES cells, and this has been suggested by an NIH3T3 model of anchorage-independent growth.[55] Therefore, the Sarcoma Alliance for Research Through Collaboration phase II trial of IGF1R antibody R1507 was met with considerable enthusiasm, and 115 patients with ES accrued in just over 2 years.[56] Impressive clinical responses were seen in a subset of patients, and the overall response rate was 10%. Similar response rates have been seen across a number of different phase II studies of IGF1R inhibition in ES, and one meta-analysis summarizes the response rate for 311 patients with ES treated with all different IGF1R inhibitors as complete response in 0.9%, partial response in 9.9%, and stable disease in 21%.[57] The Children's Oncology Group AEWS1221 trial builds on these phase II results. It is a randomized phase III trial evaluating the combination of the IGF1R antibody with interval compressed therapy in newly diagnosed patients with metastatic ES (NCT02306161).

An alternative approach has focused on targeting the DNA damage response in ES. It is known that there is a baseline level of DNA damage in ES cells that may stem from direct protein interactions with EWSR1-FLI1 or transcriptional changes in expression of targets of EWSR1-FLI1.[58] A screen of 639 cell lines that looked for relationships between cellular sensitivity to 130 agents relative to 64 fully sequenced cancer genes led to interest in PARP inhibitors (PARPi) in ES.[59] Among the most noteworthy findings was a highly important relationship that linked the EWSR1-FLI1 mutation with sensitivity to the PARPi olapirib. A concurrent independent study also identified the sensitivity of ES cells to PARP blockade and suggested a direct protein-protein interaction with EWSR1-FLI1 and PARP as well as DNA-dependent protein kinase.[58] The first clinical evaluation of PARPi in ES had no patients with objective responses and four patients with stable disease.[60] However, combination of PARPi with other therapies, particularly irinotecan and temozolomide, has demonstrated noteworthy activity in preclinical models, and studies translating these combinations to patients are accruing (NCT01858168, NCT02392793, and NCT01286987).[61-64]

In summary, because of the low mutational burden in ES, a number of investigators have focused on directly targeting the recurrent fusion, the EWSR-FLI1 transcription factor. Progress has been made and a number of promising compounds have been identified including the LSD1 inhibitor, trabectedin, and lurbinectedin, which block expression of key downstream targets of EWSR1-FLI1 and reverse expression of the gene EWSR-FLI1 gene signature. As the mechanisms of action are elucidated, these compounds, or second-generation versions, and others will emerge as bona fide EWSR1-FLI1 inhibitors and translate to the clinic in the very near future. In addition, targeting inherent sensitivities in ES established by EWSR1-FLI1 is a promising approach with clinical trials of IGF1R antibody and PARPi underway.

CONSIDERATIONS FOR CLINICAL TRIAL DESIGN IN EWING SARCOMA

Close readers of the section above discussing ES biology for advancing care will note the extent to which the emphasis is on targeting either the EWSR1-FLI1 fusion directly or the downstream consequences of transcriptional activity of the EWSR1-FLI1 fusion. The extent to which the biology of alternative ES fusions such as EWS-ERG and FUS-ERG mirrors that of EWSR1-FLI1 fusion driven ES is not known. Early studies suggest that at least the gene expression pattern of the Ewing-like sarcomas driven by alternative fusions such as CCNB3-BCOR and CIC-DUX4 is distinct from that seen in EWSR1-FLI1-positive ES.[65] Because clinical trials in ES have not required translocation testing for enrollment, it is unclear what proportion of the sarcomas of enrolled patients have alternative fusions. As alternative fusions and Ewing-like sarcomas appear to be more common in older patients, the proportion of patients enrolled on ES clinical trials for which cancer lacks a traditional ES-associated fusion is anticipated to be lower in trials focused on children and adolescents. Notably, in the phase II trial of R1507 led by the Sarcoma Alliance for Research in Cancer, 65% of patients were older than 15 years, and 43% of patients had extraskeletal disease. It has become clear that in the current era of molecular diagnostic tools that it will be essential to define the fusion present in patients enrolled on ES clinical trials.

CLINICAL AND DEMOGRAPHIC FEATURES AND TREATMENT OF OSTEOSARCOMA

Although ES and osteosarcoma are often discussed together due to their similarities, the most obvious being origin in bone, in fact, the two malignancies are markedly different. Similar to ES, osteosarcoma is a malignancy that occurs primarily in children, adolescents, and young adults with a median age of diagnosis of 15 to 19 years. Osteosarcoma has several histologic subtypes, but in all subtypes, the histopathologic hallmark is the presence of malignant osteoid. Difficulties with diagnosis are unusual but do arise when osteoid is sparse in nonosteoblastic subtypes, and as a result, the only matrix seen on the biopsy specimen is fibroblastic or chondroblastic. In addition, determining grade can sometimes be challenging particularly with surface osteosarcomas including the periosteal and parosteal subtypes. Approximately three-quarters of osteosarcoma occurs in appendicular locations. Factors portending a poor prognosis include presence of metastatic disease, axial tumor location, larger tumor size, and older age.[66]

In the 1970s and 1980s, the chemotherapy agents high-dose methotrexate, doxorubicin, and cisplatin (termed MAP) and ifosfamide with or without etoposide (IE) were identified to be active in osteosarcoma. Unlike in ES, intensifying cytotoxic chemotherapy has not, in multiple randomized trials, improved outcome.[67] Also unlike in ES, phase II trials of new cytotoxic chemotherapy agents such as topotecan have not been encouraging. Consequently, therapy and prognosis, 10-year overall survival of 70% for patients with localized disease, has not changed significantly in over three decades,[68] and treatment options for recurrent disease are very limited.

THE GERMLINE IN OSTEOSARCOMA

Osteosarcoma has been associated with cancer predisposition syndromes caused by highly penetrant germline mutations including Li-Fraumeni syndrome (LFS; *TP53* mutations) and retinoblastoma (*RB1* mutations) and has also been reported to occur in rare predisposition syndromes related to the DNA helicase (*REQ4*, *WRN*, and *BLM* mutations) and ribosomal protein pathways (*RPS19*, *RPL5*, *RPL11*, *RPL35A*, *RPS24*, *RPS17*, *RPS7*, *RPS10*, and *RPS26* mutations).[69,70] In osteosarcoma, estimates indicate that as many as 10% of cases diagnosed before age 30 may be due to underlying *TP53* germline mutations or rare variants[71]; some have speculated that this rate may be even higher when osteosarcoma occurs in very young children younger than 5 years. Although these underlying syndromes can be considered relatively rare in general in osteosarcoma, chromosomal aneuploidy remains a frequent finding in this pediatric tumor, suggesting that DNA repair defects leading to chromosomal instability may predispose individuals to development of osteosarcoma.[72,73] Single nucleotide polymorphisms can also be associated with many diseases including cancer. Genome-wide association studies have examined the role of single nucleotide polymorphisms in osteosarcoma with varying results, including the association of germline single nucleotide polymorphisms and risk for metastasis in osteosarcoma significance[74-78]; all of this indicates the genetic causation of osteosarcoma is much greater than previously known.

USING OSTEOSARCOMA BIOLOGY TO ADVANCE CARE

Clear themes about the osteosarcoma genome emerged from investigations using early genomics techniques such as karyotype, array comparative hybridization, and PCR, and these themes have since been confirmed with next-generation sequencing. Whole-exome sequencing from about 100 osteosarcoma tumor normal pairs and whole-genome and RNA sequencing from about 50 osteosarcoma tumor normal pairs has been published in two major sequencing studies.[79,80] The most frequent somatic genomic alterations in osteosarcoma are *TP53* and *RB1* inactivation. When comprehensive approaches to detecting genomic alterations are used, *TP53* loss is present in virtually every tumor. *TP53* alterations are most often structural variants (usually in intron 1), but mutations are also common. On rare occasions, *MDM2* amplification has been observed and reported to be more common in tumors from older patients with osteosarcoma. *RB1* is most often disrupted by deletion, and somatic mutations or structural variations occur only rarely. Osteosarcomas display evidence of chromoplexy and chromothripsis patterns of frequent structural variants resulting from a series of catastrophic genomic events. Copy number alterations and structural variants are the predominant mechanisms by which cancer gene protein function is altered, whereas point mutations and small insertions and deletions appear to be less common. These genomic studies also allude to the fact that osteosarcoma is a cancer with a high degree of intratumoral, intrapatient, and interpatient heterogeneity.[79,80]

Several lines of evidence, reviewed in more detail elsewhere,[81] support a critical role for the phosphoinositide 3-kinase/mTOR pathway in osteosarcoma survival and proliferation, pointing to the possibility of phosphoinositide 3-kinase/mTOR inhibitor activity in osteosarcoma.[82,83] The oncogene *MYC* has been known to be amplified in about 40% of

osteosarcomas for some time. Recent next-generation sequencing studies have confirmed the presence of *MYC* amplification as a common event. *MYC* amplification has been correlated with poor outcome, a finding that needs to be confirmed in a large uniformly treated patient population and in a prospective trial.[84] Until recently, *MYC* has not been a tractable therapeutic target. However, emerging drug classes demonstrating activity in preclinical models of MYC-driven cancers include the bromodomain, aurora kinase, CDK9, and dual P phosphoinositide 3-kinase–histone deacetylase inhibitors.[85-89] The most statistically significant deleted gene region after 13ql4-containing *RBI* (q-value 9.14 × 10-18) and 17pl3-containing *TP53* (q-value 4.94 × 10-11) is 9p21-containing *CDKN2A/B* (q-value 2/43 × 10-6). A total of 10% to 20% of osteosarcomas have been reported to have CDKN2A/B deletion.[90] Other cell cycle gene alterations in osteosarcoma include *CDK4*[91] amplification, which is interesting in that CDKN2A/B inhibits the CDK4-cyclin D (CCND1/2/3) complex. Amplification of both *CCNE1*[92] and *CCND3* have been reported in osteosarcomas.[79]

A number of receptor tyrosine kinase growth factors have been demonstrated to be expressed on osteosarcomas, including MET, PDGFRA, PDGFRB, KIT, IGF1R, ERBB2, the vascular endothelial growth factor receptors, and others.[93] However, activating mutations in these genes are rarely found in osteosarcoma,[79] and at least in the case of IGF1R and ERBB2, responses to targeting antibodies do not seem to be associated with expression.[94,95] That said, it is clear that the broad tyrosine kinase inhibitors have clinical activity in recurrent osteosarcoma, with the phase I trial of cediranib having one out of four patients with osteosarcoma with a response[96] and an osteosarcoma-specific phase II trial of sorafenib[97] demonstrating efficacy. There are case reports of response to pazopanib monotherapy or in combination with immune checkpoint inhibitor nivolumab.[98,99] Phase II clinical trials of pazopanib, regorafenib, and cabozantinib in patient populations, including osteosarcoma, are ongoing.

Other noteworthy biologic processes for which evidence suggests both an important role in osteosarcomagenesis and a potential for therapeutic approaches not discussed in detail in this review are immune evasion and perturbed development. There has been a great interest in exploring enhanced immune surveillance as a therapeutic approach in osteosarcoma, resulting in completed phase III trials of interferon alpha and mifamurtide and ongoing phase II trials of immune checkpoint inhibitors. These trials have produced continued interest in immune modulation but have not yet definitively proven the efficacy of any particular immunotherapy approach. The mifamurtide trial has been variably interpreted, resulting in diverging regulatory decisions and variable use of mifamurtide, whereas the interferon alpha failed to demonstrate improved outcome for those who received this therapy.[100,101] There are ongoing efforts to explore chimeric antigen receptor T-cell therapy (for example, NCT02107963). Osteosarcoma arises from osteoblasts, and the interrelated WNT and NOTCH pathways involved in development have both been implicated.[102,103] Interaction between receptor activator of nuclear factor κB and receptor activator of nuclear factor κB ligand is involved in both bone homeostasis and osteosarcoma and a phase II trial of the receptor activator of nuclear factor κB ligand antibody, denosumab is underway in relapsed osteosarcoma (NCT02470091).

CONSIDERATIONS FOR CLINICAL TRIAL DESIGN IN OSTEOSARCOMA

For a number of reasons, listed below and discussed in further detail elsewhere,[104-106] the osteosarcoma research community is currently focused on conducting phase II trials with the aim of identifying novel agents or combinations of novel agents and chemotherapy active in osteosarcoma.

1. Definitive phase III trials in osteosarcoma require 5 years of patient accrual when conducted internationally and more if conducted nationally.
2. The resources required for a definitive phase III trial in osteosarcoma are considerable, and therefore, previous evidence of activity in the clinic, preferably from an informative II trial, is desirable.
3. There are no standard second-line therapies for relapsed osteosarcoma, and the clinical behavior of recurrent osteosarcoma is predictable, facilitating clinical trial design in the relapsed setting.
4. Phase II trials in patients with recurrent osteosarcoma are needed to provide novel treatment options, and such osteosarcoma-specific phase II trials have been shown to accrue rapidly.

Given the heterogeneity of osteosarcoma, ideally trial enrollment would be based on presence or absence of predictive biomarkers. However, there are challenges to be overcome before it is possible to design such precision trials in osteosarcoma. Although studies published to date suggest which genes or pathways may be most often altered in osteosarcoma, the currently available data are inadequate to clearly delineate the frequencies of cancer gene alterations or the extent to which the identified genomic events are mutually exclusive. Further, preclinical studies in fully characterized (sequenced) osteosarcoma models linking genomic alterations to specific therapeutic vulnerabilities have not yet been published. Basing trial selection on gene alterations (so-called basket trial) in osteosarcoma faces an additional challenge. Given the genomic mechanisms most common in osteosarcoma, copy number, and structural alterations, assays optimized to detect these types of variants are needed. Although currently existing clinical sequencing tests do detect copy number alterations in many of the genes commonly affected in osteosarcoma, standards for calling thresholds predictive of therapy response are lacking. None of the currently existing assays is yet optimized for detecting structural events across a wide variety of cancer genes.

IMPLICATIONS OF GENOMICS FOR PATIENT MANAGEMENT: CLINICAL CANCER GENETIC TESTING FOR PATIENTS WITH PEDIATRIC SARCOMA

Diagnosing ES and osteosarcoma at an earlier stage will facilitate improved cure rates, and prevention is an ultimate goal. The incidence of germline hereditary cancer predisposition mutations is estimated to be approximately 20% for the overall cancer patient population[107] and at least 10% in pediatrics.[14,108-111] When family history is included, the number of children in a survivorship clinic meeting eligibility for genetics referral and testing approaches 30%.[112,113] Moreover, the combination of rare and specific childhood tumors with family history increases the risk for underlying germline mutations substantially (e.g.,

TP53 mutations associated with choroid plexus carcinoma[114,115] or adrenocortical carcinoma[116]). In pediatrics, sarcomas appear to be the disease most closely associated with inherited cancer predisposition. A recent study found that nearly half of (adult) patients with sarcoma have pathogenic monogenic and polygenic variation in known and novel cancer genes, and 5% had pathogenic mutations in genes associated with actionable management guidelines.[117]

Genetic screening for cancer predisposition is based on several characteristics, most importantly, diagnosis, age, and family history.[110,118-122] Assessment of family history must be incorporated into any heritable risk assessment in pediatric patients.[112] However, family history recording and assessment has been a weakness in the oncology community [123,124] challenges for family history collection include lack of time, lack of training, lack of accuracy, and lack of family history tools for clinicians and their patients.[125,126] In many clinical trials and genomic research studies, family history has been omitted or incompletely recorded and/or reported. This, along with de novo mutations and incomplete penetrance, presents barriers when interpreting family history as a risk factor for cancer predisposition. In fact, a recent germline study for cancer predisposition genes in select pediatric cancer subtypes found that recorded family history in the medical record could not be used to predict risk for carrying a cancer predisposition gene mutation.[14,127,128] Given the current limitations of its collection, family history cannot be the only factor in deciding to test pediatric patients with cancer, particularly those with bone sarcomas, for inherited cancer predisposition mutations; a striking family history should support genetic testing, but its absence does not rule against testing. Regardless, the pediatric oncology community should continue to record family history for each patient, and providing regular updates to the history could improve our identification of those patients at high risk for hereditary cancer.

Early identification of hereditary cancer predisposition is important for several reasons. First, it allows clinicians to answer perhaps the most important question from parents: “Why did my son or daughter get cancer?” Although such knowledge does not have a direct effect on patient outcomes, providing an answer to this question goes a long way in setting families’ minds at ease. Secondly, establishing a genetic causation through a newly identified cancer predisposition mutation then alerts other family members to a possible increased risk of malignancy. These family members who would otherwise not have known about a potential health risk can then be referred for genetic counseling regarding the option of being tested for the familial mutation. Finally, and most clinically relevant, through identification of hereditary cancer predisposition syndromes providers can use preventive techniques for early surveillance to identify cancers early, decreasing morbidity and mortality of malignancy. Although many different cancer predisposition syndromes exist that include children,[110,118-122] standardized surveillance protocols have not yet been developed for all of these syndromes. Nevertheless, two recent examples to demonstrate the ability of surveillance to identify early tumors in syndromes affecting children include LFS and hereditary paraganglioma and pheochromocytoma syndrome.[129,130] LFS is caused by germline mutations in *TP53* and associated with breast cancer, brain tumors, adrenal tumors, leukemia, and sarcoma. Surveillance strategy, including rapid sequence whole-body MRI for LFS-associated tumors, has been reported to be advantageous in this patient population and to improve clinical outcome through early tumor detection.[131-133] An analysis model of LFS

surveillance demonstrated its cost-effectiveness (C. R. Tak, BS; E. Biltaji, PhD; W. Kohlmann, MS, CGC; L. Maese, DO; C. M. T. Sherwin, PhD; D. I. Brixner, PhD; J. D. Schiffman, MD; unpublished data, University of Utah, March 2017). A recent workshop sponsored by the Pediatric Cancer Working Group of the American Association of Cancer Research was convened in Boston, Massachusetts in October 2016 (American Association of Cancer Research Special Workshop on Childhood Cancer Predisposition) to provide consensus-driven recommendations for early tumor screening for pediatric patients with cancer predisposition syndromes and manuscripts presenting these recommendations are in development).

A potential for expanded unbiased germline testing of a large number of individuals is the newborn screening program. Currently, the newborn screening program is the largest application of genetic testing in medicine and identifies genetic mutations and protein levels indicative of disease that can be influenced by early intervention. Diseases screened range from the most frequently encountered disease, primary congenital hypothyroidism occurring in 1:1,800 newborns, to the extremely rare inborn errors of metabolism like beta-ketothiolase deficiency and hydroxymethylglutaric aciduria occurring in 1:1 million newborns.[134,135] Pediatric cancer occurs in 1:408 children before age 15 and 1:285 children before the age of 20, with an estimated genetic cause in 10% to 20%; this implies that a mutation in a known cancer predisposition gene potentially could be found in 1:1,500 to 1:3,000 of newborns screened for inherited genetic cancer predisposition, which is within the epidemiologic criteria already established for the newborn screening program.[136] However, the newborn screening program for cancer predisposition cannot be undertaken until issues of genetic counseling, consent, variant interpretation, and follow-up surveillance are incorporated. Until genetic testing for mutations in cancer predisposition genes becomes universal for all healthy children, we recommend the consideration of genetic testing for cancer risk genes in all children diagnosed with sarcoma. Such testing needs to be coordinated through referral to cancer genetics clinics with genetic counselors and oncologists who can discuss the benefits, risks, and subsequent management of genetic risk for the patient being tested and also, importantly, for the patient's family.

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KEY POINTS

- Germline cancer risk mutations are commonly detected in patients with bone sarcomas, particularly osteosarcoma.
- The implications of identifying germline cancer risk mutations are significant enough to warrant a consideration of referral of all patients with bone sarcoma, particularly those with osteosarcoma, to a cancer risk clinic.
- By focusing on the transcriptional activity of the most common fusion found in Ewing sarcoma (ES), EWSR1-FLI1, investigators have identified promising new therapeutic avenues in this disease.
- As trials of new therapeutic approaches derive from research on EWSR1-FLI1, accurate molecular characterization of all patients with ES enrolled in clinical trials is essential.
- Clinical investigation in osteosarcoma is currently focused on conducting phase II trials of novel agents for which preclinical studies and the osteosarcoma genomic landscape suggest potential activity (including immunotherapy, receptor tyrosine kinase inhibitors, and modulators of bone development).

TABLE 1.

Translocations in Ewing and Ewing-like Sarcomas and EWSR1- and FUS-Containing Translocations in Other Sarcomas

Diagnosis	Translocation	Frequency of Ewing (%)
Ewing sarcoma	EWSR1-FLI1	90
	EWSR1-ERG	4
	EWSR1-FEV	< 1
	EWSR1-ETV1	< 1
	EWSR1-ETV4	< 1
	FUS-ERG	< 1
	FUS-FEV	< 1
Ewing-like sarcoma	CIC-DUX4	
	CCNB3-BCOR	
	BCOR-MAML3	
	ZC3H7B-BCOR	
Other sarcomas with EWSR1, TAF15, or FUS fusions		
Desmoplastic small round cell tumor	EWSR1-WT1	
Myoepithelial tumor	EWSR1-PBX1	
	EWSR1-POU5F1	
	EWSR1-ZNF444	
	FUS-KLF17	
	EWSR1-KLF17	
Extraskeletal myxoid chondrosarcoma	EWSR1-NR4A3	
	TAF15-NR4A3	
Myxoid round cell liposarcoma	FUS-DDIT3	
	EWSR1-DDIT3	
Various histologies	EWSR1-ATF1	
Various histologies	EWSR1-CREB1	