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Osteosarcoma

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Abstract

[Osteosarcoma is the most common primary malignant tumour of the bone. Osteosarcoma incidence is bimodal, peaking at 18 and 60 years of age and it is slightly more common in males. The key pathophysiological mechanism involves several possible genetic drivers of disease linked to bone formation, causing malignant progression and metastasis. While there have been significant improvements in the outcome of patients with localized disease, with event-free survival outcomes exceeding 60%, in patients with metastatic disease event-free survival outcomes remain poor at at less than 30%. The suspicion of osteosarcoma based on radiographs still requires pathologic evaluation of a bone biopsy specimen for definitive diagnosis and CT imaging of the chest should be performed to identify lung nodules. So far, population-based screening and surveillance strategies have not been implemented due to the rarity of osteosarcoma and lack of reliable markers. Current screening focuses on high-risk groups only, such as patients with genetic cancer predisposition syndromes. Management of osteosarcoma requires a multidisciplinary team of pediatric and medical oncologists, orthopaedic and general surgeons, pathologists, radiologists and specialist nurses. Survivors of osteosarcoma require specialized medical follow up, as curative treatment consisting of chemotherapy and surgery has long-term adverse effects, which also affect patients' quality of life. The development of osteosarcoma model systems and related research, as well as evaluation of new treatment approaches, are ongoing to improve patient outcomes.

Introduction

Osteosarcoma is a common primary malignant tumour of the bone, with a peak incidence in adolescents and adults >60 years of age.[1]Although Osteosarcoma can present in any bone in the body, the most common sites are around the knee and the proximal humerus (Figure 1).[2] It can also arise in individuals with a history of cancer as a secondary osteosarcoma. The diagnosis of osteosarcoma, which will be discussed further below, is made by the biopsy of mass located most commonly at the metaphysis of the long bones based on the imaging findings of patients who presented with pain, decreased mobility, and often times a palpable mass. Histologically, conventional osteosarcomas most commonly appear as spindle cell tumours hat produce malignant osteoid and, consequently, are thought to derive from the malignant transformation of cells of the mesenchymal lineage at an undefined stage of differentiation towards becoming osteoblasts.[3] Microscopically, based on the predominant matrix being produced, the tumours can be subdivided into chondroblastic, fibroblastic, osteoblastic, and telangiectatic. This suggests that the tumours maintain some of the pluripotency of their early undifferentiated mesenchymal precursors[4]. Osteosarcomas can be also divided into three major groups: low, intermediate, and high-grade.[5] The grade of the tumour serves as a relative indicator of the risk of developing metastatic disease. Low grade, or parosteal, osteosarcomas are typically indolent and are treated by surgical removal alone. High grade tumours have a high-risk of developing metastasis in the lungs, lymph nodes and other bones [6,7], and they require surgery and adjuvant chemotherapy as treatment. High-grade osteosarcomas are the focus of this Primer. Unfortunately, the outcomes for patients with osteosarcomas have remained relatively stagnant since the advent and remarkable improvement in tumour survival associated with modern chemotherapy in the 1980's.[8] However, improvements in our understanding of the biology of the disease has provided the foundation for a new wave of innovative targeted therapy clinical trials using treatment directed at the intrinsic molecular biology of osteosarcomas or antigens ubiquitously expressed on the surface of the tumour[9].

In this Primer, we summarize the epidemiology of osteosarcoma, including known genetic risk factors, influences of age and sex, and discuss current knowledge of disease pathophysiology, highlighting carcinogenesis, clinical progression and development of metastasis, genetic drivers of disease and the identification of potential targets. We summarize osteosarcoma diagnosis and management, which requires a multidisciplinary team approach. Finally, we provide an

overview on patient quality of life, the impact of late effects, and discuss future areas of research.

Epidemiology

Incidence and mortality

Although rare, osteosarcoma is the most common primary malignancy of bone with an incidence in children and adolescents of ~3-4.5 cases per million population per year.[10] In the USA, osteosarcoma accounts for <1% of all new cancer diagnoses with ~1,000 new cases diagnosed per year and half of these cases occurring in children and adolescents.[1] The global incidence rates in younger age groups (individuals ≤24 years) are relatively consistent across the USA, Europe, and Asia. However, higher incidence has been reported in South America (7-7.6 per million young males in Colombia and Ecuador) and in Africa (Sudan and Uganda, relative frequency in childhood 5.3% and 6.4%, respectively) than in Europe (frequency ~2-3%)[10,11]. Data regarding differences between ethnic groups are limited, but higher rates of osteosarcoma have been observed in African American children and young adults in the USA than in white individuals[1]. Greater geographic variation in osteosarcoma incidence in individuals ≥ 60 years has been observed, but data are insufficient to determine whether these differences are due to varying criteria for disease classification in registries, differences in environmental exposures, such as prior radiotherapy for other cancer types, or genetic predisposition.

Approximately 80% of patients with osteosarcoma present with radiographically localized disease[9]. Those patients with radiographically confirmed non-metastatic osteosarcoma have a 5-year event-free survival of ~60%[9]. In patients who present with a primary lesion and an isolated pulmonary nodule that survival is generally <40%[9]. For individuals with a primary lesion and multiple pulmonary nodules or radiographically detectable metastatic disease at other sites, survival prognosis is <20%[9].

Influence of age and sex

Osteosarcoma incidence has a bimodal age distribution with a primary peak in adolescents and young adults and a second smaller peak in the seventh and eighth decade of life[1] (Figure 2A) It is particularly uncommon in young children <10 years of age in whom the genetic etiology may be different to that in adolescents[12]. The incidence rise and peak in adolescents up to the age of

24 years are often attributed to the hormonal changes that occur during puberty with an earlier peak in girls than in boys[13]. Osteosarcoma in adults (>40) and elderly populations (>60) tend to occur secondary to other conditions, such as Paget's disease of bone, transformation of other benign bone conditions, or as a late effect of therapeutic irradiation[14].

Osteosarcoma is slightly more common in males, with an average male-to-female ratio of 1.4:1[15]. A Surveillance, Epidemiology, and End Results (SEER) analysis in a US population provides additional insight into demographic differences that relate to age, sex, and race/ethnicity of patients with osteosarcoma (Figure 2B and 2C)[16]. The age-adjusted incidence rate was 1.9 per million for 0-9 years old, 6.7 per million for 10-24 years old, 1.9 per million for 25-59 years old, and 2 per million in the ≥60 years old age group. In the USA, Hispanic males aged 10-24 had the highest incidence rate compared with any other age group or sex. Data of all age groups combined revealed that the Black population had the highest overall incidence. Notably, the incidence of osteosarcoma in children and adolescents has increased from the 1970s to the 2000s but has declined in adults >60 years of age. Some of the increase in this population may be related to the increase incidence of subsequent osteosarcomas over the past decade which may be attributable to the increasing number of childhood survivors. In regards, the patients >60 years old, the decrease incidence of osteosarcoma in this population may be attributable to the decrease rate of Paget related osteosarcoma. Patient sex does not seem to markedly influence prognosis but reports suggest males may have a slightly worse outcome than females and older patients have a worse outcome than young patients with osteosarcoma[16]. Health disparities do not seem to have a major effect on survival outcomes but data are limited[17].

Risk factors

Genetic predisposition

Most osteosarcoma cases are sporadic; however, a considerable subset of cases occur in the setting of established cancer predisposition syndromes. The frequency of germline mutations in patients with osteosarcoma ranges from 18% to 28% and these mutations are more common in younger patients[12,18]. A growing number of cancer predisposition syndromes are considered risk factors for development of osteosarcoma, including Li-Fraumeni Syndrome, hereditary retinoblastoma, and Diamond-Blackfan anemia, as well as primary DNA helicase disorders involving *RECQ* family of genes, including Rothmund-Thomson Syndrome, RAPADILINO

Syndrome, Bloom Syndrome and Werner Syndrome (Table 1).[19-21] Age of onset for these syndrome-associated tumours can be younger than in individuals with sporadic cases. Patients with retinoblastoma and Rothmund-Thomson Syndrome might present with osteosarcoma in their teens and osteosarcoma associated with Werner Syndrome or Li-Fraumeni Syndrome in middle age[22,23]. The most commonly observed pathogenic or likely pathogenic autosomal dominant germline variants in patients with osteosarcoma are in the tumour suppressor genes *TP53* (associated with Li-Fraumeni Syndrome) and *RB1* (hereditary retinoblastoma). Other likely pathogenic variants have been observed in cancer susceptibility genes including *APC, MSH2, PALB2, CDKN2A, MEN1, VHL, ATRX* and others[12]. In addition, polygenic interactions may explain the association between tall stature and risk of osteosarcoma[24].

Radiation and chemotherapy

Osteosarcomas can occur as secondary cancers in patients that have been previously treated with radiotherapy or chemotherapy. Radiotherapy-associated osteosarcomas tend to occur within the radiation field following a long latency period of >10 years and are more frequent in patients with cancer predisposition syndromes, such as Li-Fraumeni Syndrome or hereditary retinoblastoma[25] Similarly, exposure to alkylating chemotherapy, particularly when given along with radiotherapy, has been associated with an increased incidence of subsequent osteosarcoma in childhood cancer survivors[25].

Paget's disease of bone and other predisposing conditions

Particularly in older adults, osteosarcomas may also arise in the setting of Paget's disease of bone and other bone disorders, suggesting a role of abnormal bone turnover in osteosarcoma pathogenesis[26]. Paget's disease of bone is a benign metabolic bone disorder associated with osteoclast dysregulation. Although the precise incidence is unknown, it is estimated that malignant transformation to osteosarcoma occurs in ~1% of patients with Paget's disease[26] Children or adults with other bone conditions, including fibrous dysplasia (as seen in McCune-Albright syndrome[27]) and several benign bone tumours (such as enchondroma, aneurysmal bone cysts, and giant cell tumour of bone), also have an increased risk of developing osteosarcoma. Whether these benign bone tumours trigger a transformation based on the accumulation of genetic and epigenetic events or the creation of an environment permissive to malignant transformation remains unclear[28].

Mechanisms and Pathophysiology

Osteosarcomagenesis

Cellular origin

Osteosarcoma is defined histologically as a tumour of osteoid-producing cells, which often exist within an admixture of adipogenic, muscle, spindle, fibroblastic, and chondroblastic cells[3]. This microscopic phenotype has long fuelled the assumption that osteosarcoma arises from a multipotent mesenchymal precursor. Epidemiologic observations support this interpretation as tumours were found to arise most frequently within the metaphyses of long bones in children, adolescents, and young adults during times of peak linear growth, suggesting that the bone- and cartilage-producing cells that proliferate rapidly during those growth spurts are those susceptible to transformation giving rise to osteosarcoma[29,30]. Introduction of TP53 mutations into partially differentiated osteogenic stem cells generated osteosarcoma-like cells *in vitro*[31]. Similarly, genetically engineered mouse models have been most successful when introducing TP53 and other mutations using promoters for genes activated along the path that leads from mesenchymal stem cell to osteoblast[29]. Together, these data support the hypothesis that osteosarcomagenesis occurs within a proliferating population of partially-differentiated osteoblast precursor cells (Figure 3). While mesenchymal differentiation is not as well characterized as the hematopoietic system, a number of transcription factors have been identified as key regulators of clusters of genes involved in the development of various cell types. Some of these are highly expressed in the context of osteosarcoma and relate to its osteogenic phenotype and include SOX9, RUNX2 and Osterix. Some of these transcription factors themselves are influenced by tumor suppressor genes and oncogenes such as TP53

and MYC. WWOX a tumor suppressor gene associated with bone tumors and osteosarcoma exerts its effect through RUNX2.

Chromosomal complexity and copy number alterations

The genomic landscape of osteosarcoma tumours is usually dominated by widespread structural rearrangements, suggesting that several different mutational mechanisms, including chromothripsis, chromoplexy, ketaegis and other structure-altering mechanisms, are involved[32-34]. These rearrangements give rise to genome-wide copy number alterations, usually dominated by copy number loss, including of *PTEN, CDKN2A/B*, but with recurrent amplifications of *MYC, VEGFA, CCNE1*[35]. Osteosarcoma tumours often show signs of whole-genome duplication, which probably occurs in response to stresses imposed by pervasive copy number losses[36]. This genomic complexity has long been interpreted as a sign of chromosomal instability, but emerging data suggest that the mechanisms triggering complexity are active early in the process of malignant transformation[35,37]. Of note, the resulting complex genomes are subsequently maintained with some fidelity, even from diagnosis to relapse[35,37].

Recurrent mutations

Aside from these characteristic structural alterations, large-scale sequencing has identified only moderate levels of point mutations with few recurrently mutated genes[33,34,36,38]. The single most frequently altered gene is the tumour suppressor *TP53*, which is lost in >90% of osteosarcoma tumours, with the majority lost through intron 1 rearrangements or deletions rather than through point mutations[33,36,38]. TP53 is an extremely well known tumor suppressor gene which has been referred to as the “guardian of the genome.” Its normal function is to induce apoptosis in cells that acquire mutations. Given the chaotic genome typically present in osteosarcoma, abrogating that guardian function is necessary for cancer cell survival. Deletion of *RB1* also occurs in up to 30% of osteosarcoma tumours, often through loss of heterozygosity (LOH)[33,34]. Using these genomic aberrations to infer the evolution of the tumours, loss of *TP53* and *RB1* likely occurs early in the transformation process (with *TP53* inactivation required to propagate abnormal genomes[39-41]), followed by rapid accumulation of driver lesions such as *MYC* amplification, *PTEN* loss[42], and deletion of *ATRX*, which seems to activate alternative lengthening of telomeres and is associated with decreased survival[36,43].

Malignant progression and metastasis

The stepwise mechanisms that result in osteosarcomagenesis are not well understood, but one can deduce that the process involves loss of *TP53* and a catastrophic event causing widespread chromosomal rearrangements. *TP53* loss likely precedes the mass rearrangement events (via LOH) and/or arises as a consequence of those events (via LOH and intron 1 rearrangements). Cells that inherit patterns of gene copy number changes that endow them with a growth advantage might form the basis of primary tumours, with dominant clones emerging through further acquisition and amplification of growth-promoting alterations.The most likely order of events for osteosarcoma evolution is loss of *TP53* and *RB1* as early events of which *TP53* loss is likely the initiator of the genomic instability[39-41], followed by whole genome doubling, the gain of 8q (*CMYC*), and loss of 10q (*PTEN*)[42].

Osteosarcoma development can be described by the conjunction of multiple factors: oncogenic events that initiate the malignant transformation; progressive increase of genetic aberrations with the increasing proliferation rate of cells committed toward the osteoblast lineage during bone growth[44]; and involvement of a permissive microenvironment which is a prerequisite for the growth of cancer cells (Figure 4). The dialog between osteosarcoma cells and their microenvironment is crucial for tumour growth at the bone site and is associated with direct interaction between mesenchymal, vascular and immune cells (depending on cell differentiation level)[45]. interaction of cells with soluble factors such as chemokines, cytokines[46], and interaction of cells with extracellular vesicles[47]. In early-stage disease, proliferation of osteosarcoma cells disturbs the balance between osteoblasts and osteoclasts and exacerbates osteoclast activity and bone resorption which, in turn, releases pro-tumoural factors from the bone organic matrix. However, the overall role of osteoclasts in osteosarcoma development remains unclear, as they seem to hold a pro-tumour role in early-stage disease[48] but the opposite role in later-stage disease[49]. Osteoclasts, molecularly related to macrophages have been related to reduced metastases perhaps related to immune surveillance and tumor implantation. Mesenchymal stem cells, vascular cells and immune cells complete the landscape of osteosarcoma at the bone site[15] These cells in the context of normal bone provide the cellular scaffold, vascular supply and other critical functions.

The tendency for osteosarcoma to metastasize to the lung is an outcome-defining complication that drives patient mortality and challenges clinicians[50] (Figure 4). This seed and soil phenomenon is driven by a microenvironment that modulates osteosarcoma cell behaviour and

facilitates proliferation, quiescence, invasion, migration and drug resistance[15,51-53], and contributes to their intrinsic heterogeneity[54,55]. Extracellular vesicles released from osteosarcoma cells manipulate the lung environment at a distance and prepare the pre-metastatic niche to host migrating tumour cells[56]. Mesenchymal stem cells (MSCs) have been implicated in osteosarcoma metastasis and therapeutic resistance[57-59]. Osteosarcoma cells educate these MSCs by secreting TGF-β-containing extracellular vesicles[60]. triggering MSC IL-6 release and activating a STAT3-mediated tumour progression program that drives the formation of metastatic foci within the lung[61]The targetable IL-6 and CXCL8 pathways were identified as crucial to lung colonization[62], whereas osteosarcoma- and niche-derived extracellular vesicles were shown to reprogram myofibroblasts[63] and osteosarcoma stem cells[64] toward a fibrogenic phenotype, which seems to be important for metastatic colonization and also provides a targetable process[65].

Tumour education of the innate immune cells was found essential for the maintenance of metastatic lesions. Here, comparative studies (Box 1) have been insightful. Evaluation of samples taken from osteosarcoma-harbouring dogs treated with adjuvant therapy (muramyl tripeptide) suggested that reprogramming of these immune cells, especially macrophages, could prevent metastatic lesions formation[66], a finding that was reproduced to some extent in humans[67,68]. Similar approaches have used engineered Listeria bacteria to reprogram macrophages while also eliciting adaptive responses to the potential tumour antigen HER2, an approach that has suggested increased event free survival in early-phase canine osteosarcoma trials[69].

Several other mechanisms have key roles in osteosarcoma metastasis. Activation of the WNT/β-catenin pathway is important during early steps in the metastatic cascade[70,71]. The cytoskeletal linker ezrin provides a scaffold for PI3K/AKT signalling and facilitates survival through the stresses that disseminated tumour cells first encounter within the lung[72-74]. Evidence further suggests that triggering the hemostatic cascade is important for early survival of disseminated cells[75,76]. Similarly, ANGPTL2[77] and the RANK/RANKL/OPG system[78] have been identified as key contributors to the formation of the pre-metastatic niche in the lung. While epigenomic mechanisms may also play a role in metastases, progression and recurrence, studies thus far are limited for precisely determining the extent to which these mechanisms contribute.

Drivers of disease and potential targets

Investigations of the genomic and immune landscapes of osteosarcoma have suggested several potential precision strategies for patients with osteosarcoma based on somatic gene alterations, copy number alterations, tumour mutational burden, and immune and stromal features. However, each of these approaches comes with important caveats. A major caveat is each of these alterations only apply to a very limited number of patients making clinical trials of these subgroups challenging and many more common alterations are not associated with targetable therapies.

Genetic alterations

Even the most successful molecular matching studies have identified few targetable mutations in patients with osteosarcoma[79,80]. Personalized medicine studies that have included osteosarcoma patients have targeted DNA damage repair pathways, CCNE1, ATR and CDK4 amongst others. If matches were identified, very few responses were observed when patients received the corresponding targeted agent. Copy number amplifications of potentially targetable genes seemed to predict sensitivity to specific agents [35]; however, subsequent work has shown that the picture is much more complicated[9]. Numerous examples of alterations which are not oncogenic drivers related to either redundancy or alternate pathways existing have compromised efficacy of the targeting approaches. As an example, osteosarcoma patients may harbour a CCNE1 amplification at the same time as PDGFR amplification confounding target selection. Further investigation is needed to understand how these genetic lesions identify tumours likely to respond to precision therapies.

Immune approaches

There has been a long standing interest in immune based therapies based on the activity of mifurmatide in osteosarcoma in phase 2 and subsequently a randomized phase 3 trial conducted by the Children's Oncology Group[81]. Compelling evidence suggests this agents acts through its activation of macrophages. This agent has been approved by many drug regulatory bodies but not the Food and Drug Administration in the United States limiting its use. A subsequent international study explored the efficacy of interferon-α and it did not show any activity[82]. Interest in immune based therapies remains high.

The genomic complexity of osteosarcoma might suggest sensitivity to immune modulators such as immune checkpoint inhibitors, but the overall mutational burden within most osteosarcomas is markedly less than that associated with responses to immune checkpoint inhibition (ICI) in other adult tumours[83]. Even in the context of osteosarcoma older patients have a profile that is more associated with response to immune checkpoint inhibitors.[36] Only a few of the mutations occurring in osteosarcoma cells result in protein structure alterations and therefore, possible neo-antigens further limiting the potential immunogenicity of osteosarcoma cells[9]. Indeed, clinical responses to ICI have been generally disappointing[84,85], although several emerging immune-based approaches other than ICI have generated encouraging preclinical results[86-91]

Targeting cell surface antigens is one of those approaches that has received much attention (Figure 5). Several cell surface antigens expressed on osteosarcoma cells are also expressed on other adult tumour cells, making it possible to develop approaches that can be used broadly. Some of these targets include the surface proteins HER2, GD2, GPNMB, LRCC15 and B7H3. The emerging preclinical data for chimeric antigen receptor (CAR)-T and CAR-NK cell therapies are encouraging[88,89] and clinical trials designed to refine those approaches and assess their efficacy are ongoing with those studies including cohorts of osteosarcoma patients Preclinical data evaluating antibody-drug conjugates, such as those targeting B7-H3[90], LRRC15[86], and HER2[92], have been particularly promising and are rapidly moving to and through clinical trials.

Cell-Cycle, transcriptional and translational targets

Several large-scale screening efforts have honed in on drugs that target the cell cycle machinery as agents of particular interest. The most intriguing data has come from a preclinical study of agents that broadly disrupt transcription and translation.[93] The promising preclinical successes seen with CDK12 inhibitors[93] and drugs that block protein elongation[94] may not be surprising, as osteosarcoma cells depend on massive levels of protein production. Recurrent CDK4 alterations have been described in osteosarcoma[35]. There are currently clinical trials evaluating CDK4/6 inhibitors in osteosarcoma[95,96].

Cytokines and growth factors

Osteosarcoma tumours arise during puberty, when many progenitor cells undergo differentiation in response to signalling via, for example, FGF2[97], RANKL, and IGF1[98]. Indeed, IGF1 receptor amplifications occur in up to 14% of osteosarcoma patients[38], which seems to drive activation of the PI3K-AKT-mTOR pathway through the MAPK pathway[99]. Several of the cytokines that mediate metastasis may also constitute therapeutic targets, including IL6, CXCL8[62], CCL2[100],

and β-catenin[101]. These have demonstrated positive data in preclinical studies testing these agents in a variety of osteosarcoma models.

Diagnosis, screening and prevention

Diagnosis

Presentation

Many patients later diagnosed with osteosarcoma first seek medical care with concerns for persisting pain in an extremity[102]. A question frequently asked to assess the severity of pain is whether the pain keeps them up at night, with the answer int the context of osteosarcoma typically answered in the affirmative. The pain is often accompanied with swelling at the same site and patients might misassociate these symptoms with recent minor injuries. The loss of structural integrity due to tumour-related osteolysis puts patients at risk for pathologic fractures, which occur in ~10% of patients and can complicate initial management[103]. Identification of an aggressive lesion should prompt referral to a specialist centre with multidisciplinary experience in caring for patients with skeletal sarcomas to improve outcomes.

Imaging

The work-up for the presenting symptoms usually includes plain-film radiographs, potentially revealing large lesions, which are causing destruction of normal trabecular bone with poorly defined margins[104] (Figure 6) . Lesions often stimulate periosteal new bone formation, which can give rise to the characteristic Codman triangle. The associated soft tissue mass can exhibit variable patterns of ossification, leading to the characteristic radial sunburst pattern often associated with osteosarcoma. Even if conventional radiographs are highly suggestive of osteosarcoma diagnoses, MRI covering the entire length of the affected bone should still be performed[105,106]. MRI can better characterize the associated soft tissue masses and facilitates planning for biopsy and eventual surgical resection. MRI also often reveals skip metastases frequently captured by local site imaging or if more distant suggested by bone scan., which have implications for management and prognosis.

Biopsy and pathology

The diagnosis of osteosarcoma requires the pathologic evaluation of a bone tissue biopsy sample, which can be obtained using either a minimally invasive core needle biopsy approach or an open biopsy. For core needle biopsies, adequate sampling of the tissue has to be ensured, as osteosarcoma lesions can be quite heterogeneous and diagnostic features (such as malignant osteoid) can vary from sample to sample. The biopsy should be performed after consulting with the surgeon that will do the final operation in case the diagnosis is confirmed to ensure the needle track can be removed easily as part of the definitive surgery. For additional downstream molecular diagnostics of fresh or frozen tissue, adequate sampling is even more important. Open biopsies are often preferred, as they provide larger amounts of intact tissue. Fine needle aspirates are usually inadequate for definitive diagnosis of osteosarcoma due to the lack of sufficient histologic context and the resulting difficulty to assess tumour grade and they are not recommended.

The histologic diagnosis of osteosarcoma depends on the identification of malignant cells producing osteoid and irregular woven bone within fields of malignant tumour cells[107] (Figure 7). The tumour cells usually exhibit marked atypia with a high degree of pleiotropism, and multiple morphologies (spindle, epithelioid, small round, and giant cell) may exist within the same tumour. Although SATB2 and osteocalcin immunostaining and negative immunostaining to rule out alternative diagnostic entities can help guide a diagnostic workup, no immunological or molecular marker has yet been identified that confirms a diagnosis of osteosarcoma .

Staging

The post-diagnostic staging work-up aims to identify and to characterize established metastatic disease, whether that is overt (diagnosed synchronously with the primary lesion) or covert (diagnosed metachronously, e.g. after definitive local therapy). All patients presenting with newly diagnosed disease should undergo CT imaging of the chest, which has the highest efficiency for identifying lung nodules (Figure 6G). Skeletal imaging with PET or technetium bone scans is important to identify covert bony disease[108].. Guidelines from the Children's Oncology Group published in 2008 and still widely accepted advocate PET imaging with accompanying whole-body CT or whole-body MRI, with isotope bone scans if these modalities are not available. This workup recommendation reveals lung metastases in 15-20% of patients, and occasionally identifies tumours within other bones or, very rarely, lesions at other sites.

Staging is guided primarily by the Musculoskeletal Tumor Society (MSTS) staging system for sarcomas[109], which defines tumours as being either low or high grade, confined to an anatomic compartment or violating anatomic barriers, and localized or metastatic. Most patients present with high grade lesions that have both bony and soft tissue components, making the presence or absence of metastasis the primary risk-stratifying feature at diagnosis. A small number of patients present with localized, lower grade parosteal and periosteal lesions[110,111]

Stratification systems that categorize patients into subgroups based on prognosis and/or underlying osteosarcoma biology are currently being developed and validated[112]. Future clinical trials will benefit from these systems in patient assignments to either targeted therapy or de-escalation therapy in patients likely to respond well.

Prognosis

At baseline, children and adolescents who present with localized osteosarcoma have an overall survival of ~60%[113]. Patients who present with lung metastasis have the worst prognosis with 3-year survival rates of <30%[114]. Fractures may also be an indicator of more aggressive disease. Patients experiencing fractures have higher rates of lung metastasis, both at presentation and subsequent to treatment[103]. The response to neoadjuvant chemotherapy, assessed in the definitive resection specimen, has clear prognostic value and has been used in previous clinical trials to stratify patients into good responders and poor responders; however, intensification of treatment did not improve outcomes in the poor responder group[115].

The definition of good responder and poor responder to chemotherapy varies depending on the study. For example, necrosis grading had 4 levels but each level was defined descriptively only[116,117] and percentages were added later to facilitate comparison[117]. The improvement in prognosis seems linear with increasing necrosis, with some studies setting the cut-off point between good and poor between grade 2 and grade 3, whereas others have set it between grade 3 and 4. With differences in percentages ascribed to each, the demarcation between good and poor response varied between studies (90% to 98% tumor necrosis in the resection specimen)[118]. Some studies suggested that histologic subtype of osteosarcoma can influence the degree of necrosis, with chondroblastic and telangiectatic subtypes having less necrosis, but those differences have not translated into improvement of survival[1,119]. The dosage of chemotherapy given before surgery shifts the degree of necrosis but it does not change the prognostic value of necrosis grading nor influence survival[120]. Other factors have not been shown to have a consistent effect on the observed degree of necrosis. As therapy changes

based on necrosis grading have not been shown to modify survival outcomes, the use of necrosis grading has declined.

Molecular features that identify patients with higher risk include RB1 loss, MYC amplification, VEGFA amplification, and others. Unfortunately at the moment none of these risk factors are sufficiently validated to serve as a basis for risk stratification in the clinic.

Screening and prevention

Given the rarity of osteosarcoma, broad population-based screening and surveillance strategies have not been developed or implemented. Instead, strategies that focus on identifying patients with cancer predisposition (including osteosarcoma predisposition) in childhood should serve as the basis for osteosarcoma surveillance. These individuals are either identified based on a family member with known cancer predisposition syndrome and subsequent genetic testing or as a result of genetic testing obtained after a cancer diagnosis. Identification of at-risk individuals enables adherence to clinical practice guidelines and early identification and risk reduction for osteosarcoma as well as other cancers[121]. Practical challenge, however, is that nearly half of pathogenic *TP53* germline variants in children with osteosarcoma may be de novo[122]. In these patients, pathogenic germline *TP53* mutations are only identified after the initial diagnosis of osteosarcoma or other Li-Fraumeni Syndrome associated cancers.

Current osteosarcoma screening is focused on high-risk groups, primarily patients with genetic cancer predisposition syndromes. Screening strategies advocate for increased awareness of osteosarcoma risk and annual comprehensive physical examination. Intensive blood and imaging-based surveillance in patients with pathogenic germline *TP53* variants has been shown to be feasible resulted in detection of solid tumours at an earlier stage, and is associated with improved long-term survival, although these findings were not specific to osteosarcoma[123]. Guidelines for patients and families with Li-Fraumeni Syndrome include annual whole-body MRI to screen for multiple possible malignancies including sarcomas and maintaining a high index of suspicion for rare cancers[124].

No specific blood-based biomarkers or routine imaging for screening and early detection of osteosarcoma exist. An additional challenge in osteosarcoma prevention is that most of the cases are sporadic[12]. Efforts to de-escalate cancer treatment by reducing or eliminating the

need for radiotherapy may be beneficial in reducing the incidence of radiation-associated osteosarcomas. Patients with germline *TP53* mutations as well as hereditary retinoblastoma are at particularly high risk for developing radiation-associated sarcomas and, therefore, radiation should be avoided in these at-risk groups[125,126].

Management

The complex multi-modality management of osteosarcoma requires an expert multidisciplinary team that includes pediatric, medical and radiation oncologists, surgeons, pathologists, radiologists and specialist nurses[127]. Combination chemotherapy and complete surgical resection are essential for cure. This applies to both patients with localized disease and those with primary metastatic osteosarcoma, provided complete surgical removal of all known metastatic deposits has been achieved. Current treatment paradigms offer patients with newly diagnosed, resectable osteosarcoma long term survival rates of 60-70%[128]. However, outcomes have hardly improved in the past decades, and the intensive chemotherapy regimens used are associated with important acute and long-term toxic effects and a considerable impact on quality of life. In addition, patients with unresectable primary or metastatic disease at diagnosis and those with disease relapse have extremely poor outcomes[2,129]. New therapies and treatment strategies are, therefore, urgently required for osteosarcoma (Figure 8).

Systemic therapy at diagnosis

Until the 1980s, the extremely high propensity of osteosarcoma to form pulmonary metastases led to an almost universally fatal disease outcome with only local surgical management available. Progress was only made with the introduction of systemic chemotherapy, which was soon administered neoadjuvantly[130]. Multiple studies using a combined approach of neoadjuvant chemotherapy and surgery showed long-term, disease-free survival rates in the range of 60-70% in young patients with apparently localized disease[128].Doxorubicin, high-dose methotrexate with leucovorin rescue, cisplatin, and ifosfamide have since been established as the most active agents in osteosarcoma as both neoadjuvant and adjuvant therapies. The most efficacious regimens employ at least three of these drugs, but adding a fourth agent may not lead to further benefits[131].

Although a minority of international investigators apply other, partially divegent protocols, most experts routinely use the neoadjuvant MAP-regimen of high-dose methotrexate, doxorubicin, and cisplatin as their treatment standard (Figure 9). This choice of regimen is based on the

largest osteosarcoma study ever performed, EURAMOS-1[115]. This prospective, randomized trial, based on the MAP-regimen, unequivocally proved that long-term outcomes could not be further improved by postoperative treatment alterations and augmentations for poor responders. Patients who were and were not randomized to such salvage therapy had event-free survival rates of 53% (95% CI 47–53%) and 55% (95% CI 49–60%), respectively. In addition, maintenance therapy with interferon-α was not of any benefit in those with a good response[82].

Attempts to further improve disease outcomes have generally not been met with undisputed progress. Immunotherapy with the macrophage-activator muramyl tripeptide-phosphatidyl ethanolamine encapsulated in liposomes (L-MTP-PE) was investigated in a US population[67]. The results were hotly disputed at the time and left many questions open[132]. The use of L-MTP-PE in patients with metastatic osteosarcoma was not found to improve event-free or overall survival and should not be used outside of clinical trials[133]. The effectiveness of L-MTP-PE given post-operatively with ifosfamide-containing chemotherapy in patients with high-risk localized and metastatic osteosarcoma is the subject of a small ongoing randomized controlled phase II trial in France[134].

Patients with unresectable or widely metastatic osteosarcoma who are deemed incurable, are generally managed with the same systemic therapy options including MAP chemotherapy and local tumor control, and outcome is very poor with <30% of patients surviving long term[6]. Due to the toxic effects of treatment, quality of life must be balanced against potential treatment benefits for those individuals.

There is no standard-of-care systemic therapy for patients >40 years of age with poor outcome and few clinical trials to inform practice[135]. These guidelines suggest that adult patients (defined as greater than 40) should be treated similar to pediatric and young adult patients. However, adult patients may require tailored regimens especially in regards to high dose methotrexate. Retrospective analysis of the European Musculoskeletal Oncology Society of patients over 40 did demonstrate that adult patients may benefit from aggressive treatment with surgery and chemotherapy, with outcomes possibly being related to decreased chemotherapy administered to some of the elderly patients[135]. The EURO-B.O.S.S study demonstrated a favourable 5-year probability of survival of 66% (95% CI 57–75%) in patients with localized disease receiving intensive multi-agent chemotherapy that included attenuated doses of methotrexate[136]. However, considerable chemotherapy-related toxic effects were observed; neutropenia and

other haematologic adverse effects were most frequent. Randomized studies are required to standardize care for these patients.

Data on lower grade lesion management remains sparse; however, most clinicians agree that grade I localized parosteal tumours can be treated surgically. The general principles of treating subvariants in osteosarcoma is based on their grade. Low grade lesions including low grade central lesions are treated by local control only which is surgical. Intermediate grade lesions which include periosteal lesions and most osteosarcomas that include the jaw similarly need local control that is also typically surgery. In intermediate grade osteosarcomas the role of chemotherapy is controversial and certainly not associated with the same risk:benefit relationship as high grade osteosarcomas. Osteosarcomas in other craniofacial locations can be high grade and are treated with chemotherapy and local control when that is the case. In craniofacial locations local control can become challenging and most often is approached by multidiciplinary surgical oncologists and reconstructive teams.

Surgery

Primary osteosarcoma resection should be carried out by experts in surgical reconstruction to preserve bone function, while achieving a complete resection. Otherwise, intralesional or marginal resections increase local recurrence rate, which is associated with reduced overall survival[2,137]. Limb salvage is feasible for most patients with extremity tumours via reconstruction using an endoprosthetic implant, or allogeneic or autologous bone graft. Minimally invasive and non-invasive growing implants enable limb-salvage reconstruction as well as future limb-length equality for skeletally immature patients[138]. Reconstruction by using the uninvolved part of the limb, for example, by rotationplasty or tibial turn-up may also be beneficial, particularly in children[139]. Amputation remains optimal for some patients with large tumours when limb preservation is not possible, or the expected functional differences between limb-sparing surgery and amputation are small and the risks of limb-sparing surgery high. Technologies such as transosseous suture fixation devices and advances in prosthetics offer the potential for improved functionality for these patients[140]. Local recurrence rates for extremity osteosarcoma is low and generally less than 5% suggesting in most cases complete resection is achieved. In selected patients with osteosarcoma, radiation is considered postoperatively particularly patients with close surgical margins and a poor grade of necrosis in the resection specimen.

Surgery is also an important local control modality for metastatic sites, with long term survival improving with resection of lung metastases. Here, the number of metastases and completeness of excision seems to affect outcomes[6].

Radiotherapy

Although osteosarcoma is regarded as a radio-resistant disease, radiotherapy as local control may be considered if resection of a primary tumour is not possible or anticipated to lead to unacceptable morbidity, such as pelvic, trunk or cranio-facial primary sites of disease[127]. Heavy particles offer a technical advantage to deliver the high doses of 60Gy or ideally 70Gy deemed necessary for disease control[141]. Proton beam therapy (PBT) and carbon ion radiotherapy (CIRT) is, therefore, increasingly used for patients with inoperable disease or disease at challenging primary sites. Five-year local control rates of 62-67% in patients with inoperable pelvic and trunk sarcomas are encouraging[141,142]. The combination of CIRT and PBT for inoperable osteosarcoma, was found to be feasible[143]. A comprehensive evaluation of particle beam therapy, in this setting, is a priority.

Relapsed osteosarcoma

Osteosarcoma recurs most often in the lung followed by bone at a site distant to the primary tumour. Local recurrence is rare; for example, it accounted for only 7% of all events in almost 1,000 patients who had an event in the EURAMOS-1[128]. Surgery to completely remove all sites of recurrent osteosarcoma is recommended (Figure 8). This second complete remission, which is only achievable through surgery, has a strong association with improved outcomes after relapse in retrospective studies[129].

Several chemotherapy regimens are recommended by National Cancer Care Network (NCCN) and European Society of Medical Oncology (ESMO) guidelines at the time of osteosarcoma recurrence[105,144]. In cases of recurrent, surgically resectable osteosarcoma, chemotherapy may be given either prior to or after surgical resection; in select cases with a long disease-free interval, chemotherapy may be omitted. The regimens include high-dose ifosfamide with or without etoposide and gemcitabine and docetaxel. These chemotherapy regimens are recommended based on phase 2 trials or retrospective studies with small numbers of osteosarcoma patients showing moderate response rates of 20-50%[145-147]. One study suggests fractionated cyclophosphamide can replace ifosfamide with similar response rates[148].

As a class, multi-targeted kinase inhibitors (MTKIs) demonstrate activity in recurrent osteosarcoma and are most often utilized in patients with advanced unresectable disease Prospective clinical trials evaluated the MTKIs sorafenib, regorafenib, cabozantinib, lenvatinib, and apatinib as single agents in patients with relapsed or refractory osteosarcoma, with most enrolled patients falling in the adult age range (Table 2)[149-154]. Objective response rates were low at 10-15%. Four-month progression free survival (PFS) ranged from 35% to 70%[149-152,154]. For comparison, a 4-month PFS of 0% in the control arm of the phase 2 trials of regorafenib and 15% for a historical benchmark established by pooled analysis of 96 patients with osteosarcoma and measurable disease enrolled on seven Children's Oncology Group phase 2 trials were observed[155]. Of note, dose interruptions and reductions of MTKIs have been frequent across these trials, secondary to common toxic effects of this drug class including hand-foot rash syndrome (palmar plantar erythrodysesthesia), gastrointestinal toxic effects, and hypertension[149-154]. The mechanism of action of MTKIs in osteosarcoma is still not well understood and correlative translational studies have yet to identify predictive biomarkers of response to MTKIs.

Several different approaches are being taken in recurrent osteosarcoma to identify new, potentially more effective, therapies. Different combinations of MTKIs are currently studied, such as ifosfamide and etoposide plus lenvatinib (randomized phase 2 trial)[156]. The phase 1 trial of this combination demonstrated tolerability and a 4-month PFS of 51%[153]. DNA damage response pathway drugs, such as PARP inhibitors and WEE1 inhibitors, are under investigation in patients with osteosarcoma, based on the genomic features within the patient's tumor. These features include the presence of COSMIC mutational signature possibly representing defective DNA damage response in osteosarcoma, in ~30% of cases, and the frequent presence of genomic events that lead to replication stress, such as *MYC* amplification and *CCNE1* amplification. In addition, PD-1 and PD-L1 ICIs did not show activity in osteosarcoma[157]. Trials combining ICI with other anti-cancer therapies, such as MTKIs and trials of other immune activation approaches such as antibody combinations and cellular therapy, in individuals with osteosarcoma are at early stages [9]. More research is warranted to fully understand the oncogenic and immune response pathways in osteosarcoma that promote cancer development, treatment resistance and metastasis. New trial approaches are expected to emerge as understanding of the disease increases.

Late Effects

Osteosarcoma patients who are long-term, disease-free survivors still require dedicated medical care. Curative therapy has long-term toxic effects. The severity of these late effects may be life-threatening, severe, or mild[158]. Surgery adds its own sequelae, which are usually not life-threatening but may be incapacitating.

Osteosarcoma surgery is usually associated with loss of a major joint, most often the knee. Even the most modern endoprostheses have a limited life-span, as mechanical wear or infections require repeated operations in most patients who undergo limb salvage. Ablative surgery (amputation) may be associated with poor joint function, but usually involves fewer episodes of revision surgery than limb salvage. Numerous studies have been published on both the psychological and functional consequences of surgeries both ablative and limb salvage. The most consistent impairment shown is increased consumption associated with ambulation reflecting the increased work needed to do so[159].

One of the life-threatening late effects of chemotherapy are secondary malignancies such as therapy-related acute myelogenous leukaemia/myelodysplastic syndrome, CNS tumors, or secondary solid tumors, which occur in ~3% of patients[128]. Curative osteosarcoma treatment including anthracyclines, alkylating agents, and/or topoisomerase II inhibitors is known to cause secondary malignancies and the risk is likely increased by individual cancer predisposition and other yet unidentified factors[158,160]. Among all pediatric cancer patients, those with osteosarcoma carry one of the highest rates of genetic cancer predisposition (10-20%)[161]. Li-Fraumeni syndrome is most prominent, but hereditary retinoblastoma, helicase-associated cancers, and others also contribute[160]. The secondary cancers are frequently acute myeloid leukemias[158]. These may be caused by previous exposure to DNA damaging alkylators, often arising after a median of around seven years after the initial treatment [162] Other secondary leukemias are often myelomonoblastic and their lag-time is shorter. These develop after exposure to topoisomerase II inhibitors, including anthracyclines such as doxorubicin[162]. Both forms of secondary leukemia have an extremely poor prognosis despite the most intensive therapies, such as bone marrow transplantation[158,163].

Anthracycline-induced, severe cardiomyopathy is another common, potentially fatal late effect of chemotherapy, with ~2% of non-relapse related deaths amongst childhood cancer survivors attributed to cardiomyopathy or heart failure[164]. The cumulative anthracycline dose is a major risk-factor for severe cardiomyopathy development, but young age at treatment, female sex, peak drug exposure, and additional stress to the heart have also been implicated[165-167]. Importantly, cardiac function may deteriorate over time, even several decades after treatment.

Treatment for anthracycline-induced heart-failure is similar to that of heart failure from any cause[168]. Allogeneic heart transplants may be indicated for severe cases[169,170]. Patients should be screened for signs of cardiac malfunction to detect even subclinical malfunction early to hopefully prevent progression[168,171].

Inner ear damage and permanent hearing loss is a possible incapacitating late chemotherapy effect of cisplatin use with moderate to severe hearing loss occurring in < 30% of osteosarcoma patients[172]. This begins at the highest acoustic frequencies and progresses into the range of speech (225 to 85 Hz) with increasing drug exposure. In addition to the cumulative cisplatin dose, peak drug exposure, young age at treatment, co-administration of other ototoxic drugs, and others are well defined risk factors for more severe auditory damage[171-173]. Hearing aids may be required in those individuals[174].

The renal glomerulus might be affected by cisplatin treatment in 60-80% of children and adolescents and renal tubular function by ifosfamide teatment in 20-25%. Cisplatin-induced glomerular effects are rarely severe enough to require treatment, but ifosfamide-induced renal tubular effects can lead to clinically relevant electrolyte wasting in the form of Fanconi syndrome[175]. Patients affected may require permanent oral electrolyte substitution.

Patients that have received intensive, multi-drug chemotherapy against osteosarcoma may have reduced antibody titers against vaccine-preventable infections for some months after chemotherapy, and some guidelines suggest measuring vaccine-induced antibody titers and repeating vaccinations[176,177]. An increased risk of herpes zoster infection has been found in those individuals and administration of prophylaxis is recommend for at risk patients[178].

Fertility is only modestly affected by standard chemotherapy regimens. Generally, fertility is most impaired by alkylators and more so in males than in females[179]. Oocyte cryopreservation before commencing therapy might be an option for selected young female patients, and sperm-banking should be routine for eligible young male patients[180]. The rate of treatment-related malformations does not seem to be increased in the offspring of former osteosarcoma patients[158].

Quality of life

Few studies have investigated the health-related quality of life (HRQoL) of osteosarcoma survivors[181]. A single institution study evaluating the HRQoL in 80 survivors at least 10 years

after the initial diagnosis of osteosarcoma, revealed that individuals had neurocognitive impairment, with significantly lower mean scores in reading skills ($p = 0.01$), sustained attention ($p = 0.002$), short term memory ($p = 0.01$), and physical processing speed ($p < 0.001$) compared with matched controls[182]. In this group of patients, the burden of physical health conditions was high, with 32% of osteosarcoma survivors self-reporting impaired physical functioning and 16% impaired general health, being considerably worse than in matched controls. Having a grade 3 or grade 4 cardiac, pulmonary, or endocrine toxic effect on chart review was associated with an increased risk of neurocognitive impairment[182]. Surgery for primary site disease control is an important contributor to poor HRQoL in osteosarcoma survivors. A cross-sectional Dutch study compared HRQoL of patients who underwent resection of a malignant bone tumour from the lower extremity to that of healthy controls[183]. Patients who had undergone surgery had lower scores for motor function, cognitive function, pain, and general health.

Given the late toxic effects and their impact on quality of life of osteosarcoma treatments, multi-disciplinary specialized cancer survivor care is recommended for all osteosarcoma patients. Further studies are of importance, as robust data on late effects and HRQoL is required to inform future approaches aimed at minimizing toxic effects and improving quality of life.

[H1] Outlook

Basic Research

In the past 20 years, osteosarcoma research has dramatically changed our understanding of the biology of the disease. Despite being known as one of the most genomically complex pediatric malignancies, many of the alterations that occur in osteosarcoma are translocation events that silence genes rather than create neoantigens[184]. A high proportion of osteosarcoma samples have an increased number of tumour infiltrating lymphocytes, suggesting immune system activation in many osteosarcoma patients[185]. Furthermore, current molecular research continues to classify osteosarcomas not by histologic appearance via classical osteosarcoma pathologic descriptions but by proteo-genomic drivers of disease that provide further insight into disease biology and may have both prognostic and therapeutic implications[35]. To assist this approach, large libraries of PDX models of osteosarcoma have been developed[186,187]. Once fully genomically characterized, these shared resources will be fundamental in expanding our

understanding of the proteo-genomic segmentation of the disease. Combining these large libraries will be necessary to recapitulate the full spectrum of disease in humans. The rational testing of targeted agents in PDX models will provide better understanding of the relevance of the putative disease drivers. Furthermore, analyses of resistant outgrowths may provide further rationale for combination strategies[188,189].

Another major approach to improve treatment of osteosarcoma is the development of agents that target antigens expressed on the tumour cell surface. This strategy, a targeted approach to immunotherapy, is based on the immune cell infiltration known to occur in osteosarcoma, and on the broad development of these types of treatment in a range of other malignancies However, one of the main challenges is to identify antigens that are present in a high proportion of patient tumours but are not expressed on normal tissues to ensure effectiveness and low toxic effects, respectively. he two major areas of active study for this approach are T-cell based therapies and antibody-drug conjugates[9,190]. Further research is required to increase the number of suitable, targetable surface antigens as combinatorial strategies will most likely be required given the intra-tumour and inter-tumour heterogeneity of osteosarcomas[191].

Clinical Research

In the past few years, numerous clinical trials have evaluated new strategies to treat osteosarcoma, with most available for patients with relapsed or refractory disease. In these trials, multi-targeted tyrosine kinase inhibitors (MTKIs) were effective in reducing progression-free survival, becoming the mainstay of treatments for patients with relapsed disease. The Children's Oncology Group is moving forward a clinical trial evaluating MTKIs in combination with standard chemotherapy in patients with newly diagnosed high-risk disease[192]. Another strategy incorporating MTKIs, is evaluating their role as maintenance therapy[193,194]. These trials will hopefully provide a better understanding of how these agents can improve outcomes in patients with newly diagnosed osteosarcoma, at which point most patients have the highest chance of cure.

Trials that evaluate targeting of the genomic complexity of osteosarcoma by inhibiting cell cycle DNA damage regulatory proteins are also ongoing[195,196]. The fundamental premise for these trials being that further inhibition of the regulatory pathways involved in DNA repair [195,196] by targeting Wee 1 kinase or a combination of PARP and ATR inhibition will lead to mitotic catastrophe and cell death.

Personalized medicine targeting somatic alterations in osteosarcoma has become increasingly common as small molecule inhibitors are being developed and tested in various cancers[80]. Due to loosening of FDA restrictions, many of these trials are now also available to patients aged ≥12 years, which includes most patients with osteosarcoma. These trials are histology agnostic, which enables more patients to participate; however, this approach might limit new insights into the biology of the disease given the heterogeneity of actionable mutations in osteosarcoma and the limited number of patients with osteosarcoma treated on any one trial. In addition, because these are new agents, the data is strictly controlled by trial sponsors, which prevents their application to increase understanding of the disease. Once these data enter the public domain, building improved bioinformatic systems to collate and curate the data might be useful to better understand the role of these targeted therapies in osteosarcoma.

Finally, many clinical trials are evaluating agents that target osteosarcoma surface antigens, for example using CAR-T cells targeting GD2[197-199], HER2[200], EGFR[201], and B7-H3[202,203] (Figure 6). Other trials are using antibody-based therapies to target surface proteins either as antibody–drug conjugates or in combination with other immunoregulatory therapies. Tissue sampling of resistant tumours should be an important component of these trials. Future development of these therapies will be, in part, contingent on understanding whether resistance is the result of antigen escape, anergy or resistance to the drug conjugates.

As our understanding of the biology of osteosarcoma continues to improve, new paths are created for innovative clinical trials. In step with these new clinical trials, specimen and bioinformatic data need to be collected and shared with the research community to improve our understanding of the complex biological mechanisms driving osteosarcoma and treatment resistance.

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Author contributions

All authors contributed equally to all sections of the Primer. Overview of Primer (R.G.).

Competing interests

Since 2019, Stefan Bielack has been on Advisory Boards for Eli Lilly, Ipsen, Hoffmann La Roche, Bayer Healthcare, Boehringer Ingelheim, EISAI, and MAP Biopharma. All other authors declare no competing interests.

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Figures

Figure 1 | Anatomical distribution of a primary osteosarcoma tumour

Osteosarcoma can present in any bone in the body but the most common sites are around the knee and the proximal humerus[204].

Figure 2 | Osteosarcoma incidence by age and sex

A) Incidence of primary and subsequent osteosarcoma by age at diagnosis. B) Incidence of primary osteosarcoma by age at diagnosis for males and females. C) Incidence of secondary osteosarcoma according to age at diagnosis for males and females.

Data from the Surveillance, Epidemiology, and End Results 18 database. Adapted from Cole et al. 2022

Figure 3 | Model of osteosarcomagenesis: key role of oncogenetic drivers

Molecular mechanisms of osteoblast differentiation and cell signalling associated with osteosarcomagenesis. Osteoblasts originate from mesenchymal pluripotent progenitors under the control of driver transcription factors, including SOX9, RUNX2 and OSTERIX. The progressive differentiation stages of osteoblasts can be followed by specific temporally regulated protein expression. Osteosarcoma cells are thought to originate from malignant transformation of cells within the osteoblastic lineage at any stage of its differentiation, which is controlled by numerous cellular signaling pathways (e.g. Notch, Wnt and RTK) that can initiate uncontrolled proliferation[205-208] FZD: Frizzled; LRP: Low density lipoprotein receptor-related protein; NCID: Notch intracellular domain; RTK: Receptor tyrosine kinase.

Figure 4 | Model of osteosarcomagenesis: local tumour microenvironment.

osteosarcoma (OS) cells become progressively oligoclonal or polyclonal and form a highly heterogeneous tumour mass. The local microenvironment provides a fertile niche for osteosarcomagenesis and tumour growth. Interaction between cancer and bone cells leads to an increase of OS cell proliferation and altered bone remodeling. In addition, OS cells activate local mesenchymal stem cells by producing extracellular vesicles (EVs) containing TGF-β, which in turn release EVs containing IL-6, facilitating tumour progression. Similarly, cytokine-

containing EVs prepare the lung metastatic niche to receive OS circulating tumour cells. In the metastatic foci, cytokines and growth factors contribute to the local tumour development and EVs seem to be the main messenger between OS cells and the pulmonary parenchyma.

Figure 5 | Osteoblastic osteosarcoma imaging

A) Radiograph of affected left humerus, B) MRI of the left humerus showing an extracortical soft tissue mass and intramedullary infiltration, C) Radiograph of another affected left humerus, D) and E) Bone scintographs showing lytic, metastatic steosarcoma lesions, F) MRI of the humerus showing osteosarcoma soft tissue and intramedullary extensions, G) CT image of lung metastases of varying sizes.

Figure 6 | Potential targets for osteosarcoma treatment

Tyrosine Kinase Inhibitors: Can block multiple tyrosine kinase receptors. With individual difference in binding affinities. Blocking downstream intracellular growth signals

Surface Targets: **HER2:** Antibody, ADC, CAR-T **GD2:** Antibody, CAR-T **B7-H3:** CAR-T **EGFR**: CAR-T

Inhibitors of DNA Damage Repair: Inhibitors of Wee1, PARP, ATR

Figure 7 | Osteosarcoma histology

Representative osteosarcoma histology images of a malignant spindle cell tumour producing osteoid. A) Osteoblastic osteosarcoma. Atypical pleomorphic cells with osteoid. B) Chondroblastic osteosarcoma. Heterogeneous tumor with areas of atypical hyaline cartilage and osteoid-producing malignant cells. C) Fibroblastic osteosarcoma. Atypical spindle cells with osteoid. D) Small cell osteosarcoma. Monotonous round cells with osteoid. E) Telangiectatic osteosarcoma. Blood filled cystic spaces lined by atypical pleomorphic cells with osteoid (magnified inset F) Low-grade central osteosarcoma. Bland spindle cells with thickened neoplastic bone.

Figure 8 | Osteosarcoma treatment algorithm

Patients with suspected osteosarcoma (OS) require referral to a specialist centre with expert pathology, imaging review panel and multi-disciplinary discussion to confirm management. Low and intermediate grade OS are managed with surgery alone. Patients with resectable high-grade osteosarcoma require chemotherapy and resection of all sites of disease. Both neoadjuvant and adjuvant chemotherapy is usually given but surgery may be considered upfront followed by adjuvant chemotherapy in selected cases. Patients with unresectable and/or widely metastatic disease may receive palliative chemotherapy and/or radiotherapy. At relapse, surgery should be considered for resectable disease. The role of adjuvant chemotherapy in this setting is not well-defined but may offer palliative benefit for those with unresectble or systemic relapse. Multi-tyrosine Kinase inhibitors (MTKIs) have demonstrated activity in phase II clinical trials and may offer benefit in this setting. Entry into clinical trials is advised if possible.

*Surgery can be considered upfront, followed by adjuvant chemotherapy. **If available.

Figure 9 | Osteosarcoma MAP chemotherapy example

Traditional MAP chemotherapy involves 10 weeks (2 cycles) of neoadjuvant chemotherapy, followed by local control surgery. After surgery, 18 weeks (4 cycles) of adjuvant chemotherapy are given. Either methotrexate (M), doxorubicin (an anthracycline (A)) or cisplatin (P) may be substituted with ifosfamide, based on toxic effects or practice patterns. In the weeks without any letters, no chemotherapy is administered.

Tables

Table 1 | Cancer predisposition syndromes associated with osteosarcoma .

Syndrome	Gene	Inheritance Pattern	Reference
Li-Fraumeni syndrome	*TP53*	Autosomal dominant	13
Retinoblastoma	*RB1*	Autosomal dominant	123
Rothmund-Thomson syndrome	*RECQL4*	Autosomal recessive	20
Baller-Gerold syndrome	*RECQL4*	Autosomal recessive	20
RAPADILINO	*RECQL4*	Autosomal recessive	20
Werner syndrome	*RECQL2 (WRN)*	Autosomal recessive	21
Bloom syndrome	*RECQL3 (BLM)*	Autosomal recessive	21
Diamond-Blackfan anaemia	>12 different ribosomal protein genes and *GATA1*	Autosomal dominant	19

Adapted from [209]

Table 2 | Response to MTKIs in relapsed or refractory osteosarcoma.

Agent	Number of patients evaluated	Objective response	Median PFS [months] (95% CI)	4-month PFS (95% CI)	Median OS [months] (95% CI)
Sorafenib163	35	3 (8%)	4 (2-5)	0.46 (28-63%)	NR
Apatinib165	37	16 (43%)	4.5 (3.5-6.3)	0.57 (39-71%)	9.9 (8-18.9)
Lenvatinib166	26	2 (7%)	3 (1.8-5.4)	0.29 (14-48%)	10 (5.6-12.3)
Cabozantinib164	42	5 (12%)	6.7 (5.4-7.9)	0.71 (55-83%)	10.6 (7.4-12.5)
Regorafenib (REGOBONE) 167	26 (regorafenib)	2 (8%)	16.4 (8-27)	0.46 (28-63%)	11.3 (5.9-23.9)
	12 (placebo)	0 (0%)	4.1 (3-15.7)	0%	5.9 (1.3-16.4)
Regorafenib (SARCO24) 162	22 (regorafenib)	3 (14%)	3.6 (2-7.6)	44%	11 (4.7-26.7)
	20 (placebo)	NR	1.7 (1.2-1.8)	0%	13.4 (8.5-38.1)

NR; not reported.

Boxes

Box 1 | Relevant osteosarcoma disease models

Cell lines

Well-established cell lines such as SaOS, MG63, KHOS, MNNG-HOS, U2OS, and OS-17 have facilitated investigation into mechanisms of malignancy[210,211] and high-throughput screens to identify osteosarcoma vulnerabilities[93,212]. Companion cell lines with enhanced lung colonization capacity (such as SaOS2-LM7, MG63.3, 143B)[213-215] have enabled studying metastasis driver mechanisms[50,216] Cultures derived from mice with spontaneous osteosarcoma[214] or from genetically engineered mouse models (GEMM)[101,217] are available study disease biology or therapeutics in mice with intact immune systems.

Ex Vivo organ cultures

Ex vivo culture systems are useful to study of tumour cells growing within an intact lung environment[218,219].These techniques have been adapted to study tumour-host interactions, to screen for metastasis-related vulnerabilities, and to validate hits identified in other screens[74,75,93,220-223].

Primary and PDX-derived cultures

Established cell cultures have been the predominant research models of osteosarcoma for decades, but the culture-related alterations that cell lines acquire over tens and sometimes hundreds of passages[224,225] led to the development of systems for propagating osteosarcoma tumours[224,225] by creating libraries of primary tumour cell cultures and patient-derived xenografts (PDXs). These PDX model systems are of particular value in both basic science and potentially personalized medicine research,[185,188,226] as they have clinical and molecular features that are quite representative of the human disease.[227-230] Although prolonged passage in mice can alter the behaviour of tumours maintained as PDXs[231], strategic use of low-passage PDX lines limits this mouse-specific evolution and is a useful tool in the study of osteosarcoma biology,[232] precision medicine approaches, and preclinical validation of therapeutic candidates.

Genetically engineered mouse models (GEMMs)

Many insights into the origins[29] and pathophysiology[216] of osteosarcoma have come from mouse models engineered to develop osteosarcoma[233]. Most of these models incorporate genetic

changes that drive tissue-specific p53 inactivation, together with knockout of other tumour suppressors (such as Rb) or activation of oncogenic pathways such as Myc and c-fos[234].

Comparative studies

Canine companion animals that develop sporadic osteosarcoma present a unique opportunity to study tumour biology and therapy in ways that can accelerate discovery and benefit both species[235]. Canine osteosarcoma has histological, genetic, and clinical features nearly identical to the human disease[236], but has a much higher incidence, with an estimated 25,000 new cases of canine disease occurring each year[236]. Large clinical trial networks facilitating multi-institutional studies are well-established[237]. This integrated approach has been particularly promising for the evaluation of anti-metastatic[100,238] and immune-based therapeutics[239,240].