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## Osteosarcoma

Hannah C. Beird<sup>1</sup>, Stefan S. Bielack<sup>2</sup>, Adrienne Flanagan<sup>3</sup>, Jonathan B. Gill<sup>4</sup>, Dominique Heymann<sup>5</sup>, Katherine Janeway<sup>6</sup>, J. Andrew Livingston<sup>7</sup>, Ryan D. Roberts<sup>8</sup>, Sandra J. Strauss<sup>9</sup>, Richard Gorlick<sup>4,7\*</sup>

<sup>1</sup>Department of Genomic Medicine, University of Texas MD Anderson Cancer Center, Houston, Texas, USA

<sup>2</sup>Pediatric Oncology, Stuttgart Cancer Center, Stuttgart, Germany

<sup>3</sup>Research Department of Pathology, Cancer Institute, University College London, London, UK

<sup>4</sup>Division of Pediatrics, University of Texas MD Anderson Cancer Center, Houston, Texas, USA

<sup>5</sup>Nantes Université, CNRS, UMR6286, US2B, Institut de Cancérologie de l'Ouest, Saint-Herblain, FR

<sup>6</sup>Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, MA, USA

<sup>7</sup>Department of Sarcoma Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA

<sup>8</sup>Center for Childhood Cancer, Nationwide Children's Hospital, Columbus, Ohio, USA

<sup>9</sup>University College London Hospitals NHS Foundation Trust, University College London, UK

\*email: rgorlick@mdanderson.org

## **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 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 less than 30%. The suspicion of osteosarcoma based on radiographs still requires pathological 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 the lack of reliable markers. Current screening focuses only on groups at high risk such as patients with genetic cancer predisposition syndromes. Management of osteosarcoma requires a multidisciplinary team of paediatric 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 the quality of life of patients. The development of osteosarcoma model systems and related research as well as the evaluation of new treatment approaches are ongoing to improve disease outcomes, especially for patients with metastases.

## Introduction

Osteosarcoma is a common primary malignant tumour of the bone, with a peak incidence in adolescents and adults >60 years of age<sup>1</sup>. Although osteosarcoma can present in any bone in the body, the most common sites are around the knee and the proximal humerus<sup>2</sup> (Fig. 1). It can also arise in individuals with a history of cancer as secondary osteosarcoma. The diagnosis of osteosarcoma, which is discussed further below, is made following the biopsy of a mass located most commonly at the metaphysis of the long bones based on imaging findings of patients who presented with pain, decreased mobility and, oftentimes, a palpable mass. Histologically, conventional osteosarcomas most commonly appear as spindle cell tumours that produce malignant osteoid and are consequently thought to derive from the malignant transformation of cells of the mesenchymal lineage at an undefined stage of differentiation towards becoming osteoblasts<sup>3</sup>. Microscopically, based on the predominant matrix being produced, the tumours can be subdivided into chondroblastic, fibroblastic, osteoblastic and telangiectatic tumours, suggesting that they maintain some of the pluripotency of their early undifferentiated mesenchymal precursors<sup>4</sup>. Osteosarcomas can also be divided into three major groups as being low, intermediate and high grade<sup>5</sup>. 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<sup>6,7</sup> and 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 1980s<sup>8</sup>. However, improvements in our understanding of the biology of the disease have provided the foundation for a new wave of innovative targeted therapy clinical trials using treatment directed at the intrinsic molecular biology of osteosarcomas or at antigens ubiquitously expressed on the surface of the tumour<sup>9</sup>.

In this Primer, we summarize the epidemiology of osteosarcoma, including known genetic risk factors and the influence 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 of patient quality of life and the impact of late effects, and we 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<sup>10</sup>. In the USA, osteosarcoma accounts for <1% of all new cancer diagnoses with ~1,000 new cases diagnosed per year, half of these cases occurring in children and adolescents<sup>1</sup>. The global incidence rates in younger age groups (individuals  $\leq 24$  years) are relatively consistent across the USA, Europe and Asia. However, a 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%)<sup>10,11</sup>. 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<sup>1</sup>. Greater geographic variation in osteosarcoma incidence in individuals  $\geq 60$  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<sup>9</sup>. Those patients with radiographically confirmed non-metastatic osteosarcoma have a 5-year event-free survival of ~60%<sup>9</sup>. In patients who present with a primary lesion and an isolated pulmonary nodule, 5-year event-free survival is generally <40%<sup>9</sup>. For individuals with a primary lesion and multiple pulmonary nodules or radiographically detectable metastatic disease at other sites, 5-year event-free survival is <20%<sup>9</sup>.

### ***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<sup>1</sup> (Fig. 2a). It is particularly uncommon in young children <10 years of age in whom the genetic aetiology may be different to that in adolescents<sup>12</sup>. 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<sup>13</sup>. Osteosarcoma in adults (age >40 years) and elderly populations (age >60 years) tends to occur secondary to other conditions, such as Paget disease of bone, as transformation of other benign bone conditions or as a late effect of therapeutic irradiation<sup>14</sup>.

Osteosarcoma is slightly more common in males, with an average male-to-female ratio of 1.4:1 (ref.<sup>15</sup>). 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<sup>16</sup> (Fig. 2b,c). The age-adjusted incidence rate was 1.9 per million for those aged 0–9 years, 6.7 per million for those aged 10–24 years, 1.9 per million for those aged 25–59 years and 2 per million for those aged ≥60 years. In the USA, Hispanic males aged 10–24 had the highest incidence rate compared with any other age group or sex. Data from 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 increased incidence of subsequent osteosarcomas over the past decade, which may be attributable to the increasing number of childhood survivors. In patients >60 years old, the decreased incidence of osteosarcoma may be attributable to a decrease in the rate of Paget-related osteosarcoma. Patient sex does not seem to markedly influence prognosis but reports suggest that males may have a slightly worse outcome than females and older patients have a worse outcome than young patients with osteosarcoma<sup>16</sup>. Health disparities do not seem to have a major effect on survival outcomes but data are limited<sup>17</sup>.

## **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<sup>12,18</sup>. A growing number of cancer predisposition syndromes are considered risk factors for the development of osteosarcoma, including Li–Fraumeni syndrome, hereditary retinoblastoma and Diamond–Blackfan anaemia as well as primary

DNA helicase disorders involving the RECQ family of genes, including Rothmund–Thomson syndrome, RAPADILINO syndrome, Bloom syndrome and Werner syndrome<sup>19,20,21</sup> (Table 1). 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 presents in middle age<sup>22,23</sup>. 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 *RBI* (hereditary retinoblastoma). Other likely pathogenic variants have been observed in cancer susceptibility genes, including *APC*, *MSH2*, *PALB2*, *CDKN2A*, *MEN1*, *VHL*, *ATRX* and others<sup>12</sup>. In addition, polygenic interactions may explain the association between tall stature and risk of osteosarcoma<sup>24</sup>.

### ***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<sup>25</sup>. 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<sup>25</sup>.

### ***Paget disease of bone and other predisposing conditions***

Particularly in older adults, osteosarcomas may also arise in the setting of Paget disease of bone and other bone disorders, suggesting a role of abnormal bone turnover in osteosarcoma pathogenesis<sup>26</sup>. Paget 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 disease<sup>26</sup>. Children or adults with other bone conditions, including fibrous dysplasia (as seen in McCune–Albright syndrome<sup>27</sup>) and several benign bone tumours (such as enchondroma, aneurysmal bone cysts and bone giant cell tumours), 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<sup>28</sup>.

## **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<sup>3</sup>. This microscopic phenotype has long fuelled the assumption that osteosarcoma arises from a multipotent mesenchymal precursor. Epidemiological 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-producing and cartilage-producing cells that proliferate rapidly during those growth spurts are those susceptible to the transformation giving rise to osteosarcoma<sup>29,30</sup>. Introduction of *TP53* mutations into partially differentiated osteogenic stem cells generated osteosarcoma-like cells in vitro<sup>31</sup>. 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 cells to osteoblasts<sup>29</sup>. Together, these data support the hypothesis that osteosarcomagenesis occurs within a proliferating population of partially differentiated osteoblast precursor cells (Fig. 3). While mesenchymal differentiation is not as well characterized as the haematopoietic 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 tumour suppressor genes and oncogenes such as *TP53* and *MYC*. *WWOX*, a tumour suppressor gene associated with bone tumours 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, kataegis and other structure-altering mechanisms, are



involved<sup>32,33,34</sup>. These rearrangements give rise to genome-wide copy number alterations, usually dominated by copy number loss, including of *PTEN* and *CDKN2A/B*, but with recurrent amplifications of *MYC*, *VEGFA* and *CCNE1* (ref.<sup>35</sup>). Osteosarcoma tumours often show signs of whole-genome duplication, which probably occurs in response to stresses imposed by pervasive copy number losses<sup>36</sup>. 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<sup>35,37</sup>. Of note, the resulting complex genomes are subsequently maintained with some fidelity, even from diagnosis to relapse<sup>35,37</sup>.

### ***Recurrent mutations***

Apart from these characteristic structural alterations, large-scale sequencing has identified only moderate levels of point mutations with few recurrently mutated genes<sup>33,34,36,38</sup>. 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<sup>33,36,38</sup>. *TP53* is an extremely well-known tumour suppressor gene that 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 *RBI* also occurs in up to 30% of osteosarcoma tumours, often through loss of heterozygosity (LOH)<sup>33,34</sup>. Using these genomic aberrations to infer the evolution of the tumours, loss of *TP53* and *RBI* likely occurs early in the transformation process (with *TP53* inactivation required to propagate abnormal genomes<sup>39,40,41</sup>), followed by rapid accumulation of driver lesions such as *MYC* amplification, *PTEN* loss<sup>42</sup> and deletion of *ATRX*, which seems to activate alternative lengthening of telomeres and is associated with decreased survival<sup>36,43</sup>.

### ***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 *RBI* as early events, of which *TP53* loss is likely the initiator of genomic instability<sup>39,40,41</sup> followed by whole-genome doubling, gain of 8q (*CMYC*) and loss of 10q (*PTEN*)<sup>42</sup>.

Osteosarcoma development can be described by the conjunction of multiple factors: oncogenic events that initiate malignant transformation, a progressive increase of genetic aberrations with the increasing proliferation rate of cells committed towards the osteoblast lineage during bone growth<sup>44</sup>, and involvement of a permissive microenvironment, which is a prerequisite for the growth of cancer cells (Fig. 4). The dialogue between osteosarcoma cells and their microenvironment is crucial for tumour growth at the bone site and is associated with direct interaction among mesenchymal, vascular and immune cells (depending on cell differentiation level)<sup>45</sup>, the interaction of cells with soluble factors such as chemokines and cytokines<sup>46</sup>, and the interaction of cells with extracellular vesicles<sup>47</sup>. In early-stage disease, proliferation of osteosarcoma cells disturbs the balance between osteoblasts and osteoclasts and exacerbates osteoclast activity and bone resorption, in turn releasing 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-tumorigenic role in early-stage disease<sup>48</sup> but an opposite role in later-stage disease<sup>49</sup>. Osteoclasts, molecularly related to macrophages, have been associated with reduced metastases perhaps related to immune surveillance and tumour implantation. Mesenchymal stem cells, vascular cells and immune cells complete the landscape of osteosarcoma at the bone site<sup>15</sup>. 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<sup>50</sup>. This seed and soil phenomenon is driven by a microenvironment that modulates osteosarcoma cell behaviour and facilitates proliferation, quiescence, invasion, migration, and drug resistance<sup>15,51,52,53</sup> and contributes to their intrinsic heterogeneity<sup>54,55</sup>. Extracellular vesicles released from osteosarcoma cells manipulate the lung environment at a distance and prepare the pre-metastatic niche to host migrating tumour cells<sup>56</sup>. Mesenchymal stem cells (MSCs) have been implicated in osteosarcoma metastasis and therapeutic resistance<sup>57,58,59</sup>. Osteosarcoma cells educate these MSCs by secreting TGF $\beta$ -containing extracellular vesicles<sup>60</sup>, triggering MSC IL-6 release and activating a STAT3-mediated tumour progression programme that drives the formation of

metastatic foci within the lung<sup>61</sup>. The targetable IL-6 and CXCL8 pathways were identified as crucial to lung colonization<sup>62</sup>, whereas osteosarcoma-derived and niche-derived extracellular vesicles were shown to reprogramme myofibroblasts<sup>63</sup> and osteosarcoma stem cells<sup>64</sup> towards a fibrogenic phenotype, which seems to be important for metastatic colonization and also provides a targetable process<sup>65</sup>.

Tumour education of the innate immune cells was found to be essential for the maintenance of metastatic lesions. Here, comparative studies (Box 1) have been insightful. Evaluation of samples taken from osteosarcoma-harboured dogs treated with adjuvant therapy (muramyl tripeptide) suggested that reprogramming of these immune cells, especially macrophages, could prevent metastatic lesion formation<sup>66</sup>, a finding that was reproduced to some extent in humans<sup>67,68</sup>. Similar approaches have used engineered *Listeria* bacteria to reprogramme 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<sup>69</sup>.

Several other mechanisms have key roles in osteosarcoma metastasis. Activation of the WNT- $\beta$ -catenin pathway is important during early steps in the metastatic cascade<sup>70,71</sup>. 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<sup>72,73,74</sup>. Evidence further suggests that triggering the haemostatic cascade is important for the early survival of disseminated cells<sup>75,76</sup>. Similarly, ANGPTL2<sup>77</sup> and the RANK-RANKL-OPG system<sup>78</sup> 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 regarding the precise extent of their contribution.

### **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 that each of these alterations only applies 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<sup>79,80</sup>. Personalized medicine studies that have included patients with osteosarcoma have targeted DNA damage repair pathways as well as *CCNE1*, *ATR* and *CDK4*, among other genes. 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<sup>35</sup>; however, subsequent work has shown that the picture is much more complicated<sup>9</sup>. Numerous examples of alterations that are not oncogenic drivers exist. Redundancy or the presence of alternate pathways have compromised efficacy of the targeting approaches. As an example, patients with osteosarcoma may harbour a *CCNE1* amplification at the same time as a *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 centred on the activity of mifamurtide in osteosarcoma in phase II and subsequently on a randomized phase III trial conducted by the Children's Oncology Group<sup>81</sup>. Compelling evidence suggests that this agent acts through its activation of macrophages. This agent has been approved by many drug regulatory bodies but not the FDA, limiting its use. A subsequent international study explored the efficacy of IFN $\alpha$  and did not show any activity<sup>82</sup>. Interest in immune-based therapies remains high.

The genomic complexity of osteosarcoma might suggest sensitivity to immune modulators such as immune-checkpoint inhibitors (ICIs) but the overall mutational burden within most osteosarcomas is markedly less than that associated with responses to immune-checkpoint inhibition in other adult tumours<sup>83</sup>. Even in the context of osteosarcoma, older patients have a profile that is more associated with response to ICIs<sup>36</sup>. Only a few of the mutations occurring in osteosarcoma cells result in protein structure alterations and, therefore, in possible neoantigens, further limiting the potential immunogenicity of osteosarcoma cells<sup>9</sup>. Indeed,

clinical responses to ICIs have been generally disappointing<sup>84,85</sup>, although several emerging immune-based approaches other than the use of ICIs have generated encouraging preclinical results<sup>86,87,88,89,91</sup>.

Targeting cell surface antigens is one such approach that has received much attention (Fig. 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 natural killer cell therapies are encouraging<sup>88,89</sup>, and clinical trials designed to refine those approaches and assess their efficacy are ongoing and include cohorts of patients with osteosarcoma. Preclinical data evaluating antibody–drug conjugates, such as those targeting B7H3 (ref.<sup>90</sup>), LRCC15 (ref.<sup>86</sup>) and HER2 (ref.<sup>91</sup>), 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 have come from a preclinical study of agents that broadly disrupt transcription and translation<sup>92</sup>. The promising preclinical successes seen with CDK12 inhibitors<sup>92</sup> and drugs that block protein elongation<sup>93</sup> may not be surprising as osteosarcoma cells depend on massive levels of protein production. Recurrent CDK4 alterations have been described in osteosarcoma<sup>35</sup>. There are currently clinical trials evaluating CDK4/6 inhibitors in osteosarcoma<sup>94,95</sup>.

### ***Cytokines and growth factors***

Osteosarcoma tumours arise during puberty, when many progenitor cells undergo differentiation in response to signalling via, for example, FGF2 (ref.<sup>96</sup>), RANKL<sup>96,97</sup> and IGF1 (ref.<sup>98</sup>). Indeed, IGF1 receptor amplifications occur in up to 14% of patients with osteosarcoma<sup>38</sup>, which seems to drive activation of the PI3K–AKT–mTOR pathway through the MAPK pathway<sup>98</sup>. Several of the cytokines that mediate metastasis may also constitute therapeutic targets, including IL-6, CXCL8 (ref.<sup>62</sup>), CCL2 (ref.<sup>99</sup>) and  $\beta$ -catenin<sup>100</sup>. So far, there have been some confirming 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<sup>101</sup>. A question frequently asked to assess the severity of pain is whether the pain keeps them up at night; in the context of osteosarcoma, this is typically answered affirmatively. The pain is often accompanied by 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 pathological fractures, which occur in ~10% of patients and can complicate initial management<sup>102</sup>. 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 workup for the presenting symptoms usually includes plain-film radiographs, potentially revealing large lesions that are causing destruction of normal trabecular bone with poorly defined margins<sup>103</sup> (Fig. 6). Lesions often stimulate periosteal new bone formation, which can give rise to the characteristic Codman triangle (Fig. 6h). 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<sup>104,105</sup>. 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, with implications for management and prognosis.

#### ***Biopsy and pathology***

The diagnosis of osteosarcoma requires the pathological 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 to ensure the needle track is easily removed during 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 a definitive diagnosis of osteosarcoma due to the lack of sufficient histological context and the resulting difficulty to assess tumour grade, and are therefore not recommended.

The histological diagnosis of osteosarcoma depends on the identification of malignant cells producing osteoid and irregular woven bone within fields of malignant tumour cells<sup>106</sup> (Fig. 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 work-up, 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 characterize established metastatic disease, whether it is overt (diagnosed synchronously with the primary lesion) or covert (diagnosed metachronously, for example, after definitive local therapy). All patients presenting with newly diagnosed disease should undergo CT imaging of the chest, which has the highest efficiency in identifying lung nodules (Fig. 6g). Skeletal imaging with PET or technetium bone scans is important to identify covert bony disease<sup>107</sup>. In 2008, guidelines from the Children's Oncology Group were published<sup>107</sup>, and are still widely accepted, advocating PET imaging with accompanying whole-body CT or whole-body MRI, with isotope bone scans if these modalities are not available. This work-up 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 Tumour Society (MSTS) staging system for sarcomas<sup>108</sup>, 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<sup>109,110</sup>.

Stratification systems that categorize patients into subgroups based on prognosis and/or underlying osteosarcoma biology are currently being developed and validated<sup>111</sup>. Future clinical trials will benefit from these systems in patient assignment to either targeted therapy or de-escalation therapy in those likely to respond well.

### ***Prognosis***

At baseline, children and adolescents who present with localized osteosarcoma have an overall survival of ~60%<sup>112</sup>. Patients who present with lung metastasis have the worst prognosis, with 3-year survival rates of <30%<sup>113</sup>. 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<sup>102</sup>. 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<sup>114</sup>.

The definition of good responder and poor responder to chemotherapy varies depending on the study. For example, necrosis grading had four levels but each level was only defined descriptively<sup>115,116</sup> and percentages were added later to facilitate comparison<sup>116</sup>. The improvement in prognosis seems linear with increasing necrosis, with some studies setting the cut-off point between good and poor between grades 2 and 3, whereas others have set it between grades 3 and 4. With differences in percentages ascribed to each, the demarcation between good and poor response varied between studies (90–98% tumour necrosis in the resection specimen)<sup>117</sup>. Some studies suggested that the histological 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 in survival<sup>1,118</sup>. 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<sup>119</sup>. Other factors have not been shown to have a consistent effect on the observed degree of necrosis. Given that



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 at higher risk include *RBI* loss, *MYC* amplification and *VEGFA* amplification, among others. However, none of these risk factors has yet been 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 a known cancer predisposition syndrome and subsequent genetic testing or as a result of genetic testing obtained after a cancer diagnosis. Identification of individuals at risk enables adherence to clinical practice guidelines and early identification and risk reduction for osteosarcoma as well as other cancers<sup>120</sup>. A practical challenge, however, is that nearly half of pathogenic *TP53* germline variants in children with osteosarcoma may be de novo<sup>121</sup>. 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, resulting in the detection of solid tumours at an earlier stage and associated with improved long-term survival, although these findings were not specific to osteosarcoma<sup>120</sup>. 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<sup>122</sup>.

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<sup>12</sup>. 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 individuals<sup>123,124</sup>.

## **Management**

The complex multi-modality management of osteosarcoma requires an expert multidisciplinary team that includes paediatric, medical and radiation oncologists, surgeons, pathologists, radiologists and specialist nurses<sup>104</sup>. 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%<sup>125</sup>. 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 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<sup>2,126</sup>. New therapies and treatment strategies are therefore urgently required for osteosarcoma (Fig. 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<sup>127</sup>. 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<sup>125</sup>. 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, yet adding a fourth agent may not lead to further benefits<sup>128</sup>.

Although a minority of international investigators apply other, partially divergent protocols, most experts routinely use the neoadjuvant MAP regimen of high-dose methotrexate, doxorubicin and cisplatin as their treatment standard (Fig. 9). This choice of regimen is based on the largest osteosarcoma study ever performed, EURAMOS-1 (ref.<sup>114</sup>). 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 IFN $\alpha$  was not of any benefit in those with a good response<sup>82</sup>.

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<sup>67</sup>. The results were hotly disputed at the time and left many questions open<sup>129</sup>. 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<sup>130</sup>. The effectiveness of L-MTP-PE given postoperatively 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<sup>131</sup>.

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 tumour control, and outcomes are very poor, with <30% of patients surviving long term<sup>6</sup>. 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 a poor outcome and few clinical trials to inform practice<sup>132</sup>. These guidelines suggest that adult patients (defined as older than 40) should be treated similarly to paediatric and young adult patients. However, adult patients may require tailored regimens, especially with regards to high-dose methotrexate. Retrospective analysis of the European Musculoskeletal Oncology Society of patients over 40 years 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<sup>132</sup>. 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<sup>133</sup>. However, considerable chemotherapy-related toxic effects were observed; neutropenia and other haematological adverse effects were most frequent. Randomized studies are required to standardize care for these patients.

Data on lower-grade lesion management remain sparse; however, most clinicians agree that grade 1 localized parosteal tumours can be treated surgically. The general principle of treating subvariants in osteosarcoma is based on their grade. Low-grade lesions, including low-grade central lesions, are treated by local surgical control only. Intermediate-grade lesions, including periosteal lesions and most osteosarcomas that affect the jaw, similarly need local control, which is also typically surgery. For intermediate-grade osteosarcomas, the role of chemotherapy is controversial and certainly not associated with the same risk-to-benefit relationship as high-grade osteosarcomas. Osteosarcomas in other craniofacial locations can be high grade, and are treated with chemotherapy and local control in such case. In craniofacial locations, local control can become challenging and is most often approached by multidisciplinary surgical oncologists and reconstructive teams.

## **Surgery**

Primary osteosarcoma resection should be conducted by experts in surgical reconstruction to preserve bone function while achieving complete resection. Otherwise, intralesional or marginal resections increase local recurrence rate, which is associated with reduced overall survival<sup>2,134</sup>. 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 patients with skeletal immaturity<sup>135</sup>. Reconstruction by using the uninvolved part of the limb, for example, by rotationplasty or tibial turn-up, may also be beneficial, particularly in children<sup>136</sup>. 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<sup>137</sup>. Local recurrence rates for extremity osteosarcoma are low and generally less than 5%, suggesting that, in most cases, complete resection is achieved. In selected patients with osteosarcoma,

radiation is considered postoperatively, particularly in 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 the resection of lung metastases. Here, the number of metastases and completeness of excision seem to affect outcomes<sup>6</sup>.

## **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 morbidities such as in pelvic, trunk or craniofacial primary sites of disease<sup>104</sup>. Heavy particles offer a technical advantage to deliver the high doses of 60 Gy or ideally 70 Gy deemed necessary for disease control<sup>138</sup>. Proton beam therapy and carbon ion radiotherapy are, 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<sup>138,139</sup>. The combination of carbon ion radiotherapy and proton beam therapy for inoperable osteosarcoma was found to be feasible<sup>140</sup>. 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 EURAMOS-1 (ref.<sup>125</sup>). Surgery to completely remove all sites of recurrent osteosarcoma is recommended (Fig. 8). This second complete remission, which is only achievable through surgery, has a strong association with improved outcomes after relapse in retrospective studies<sup>126</sup>.

Several chemotherapy regimens are recommended by the National Cancer Care Network (NCCN) and European Society of Medical Oncology (ESMO) guidelines at the time of osteosarcoma recurrence<sup>104,141</sup>. In cases of recurrent, surgically resectable osteosarcoma, chemotherapy may be given either before 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 II trials or retrospective studies with small

numbers of patients with osteosarcoma showing moderate response rates of 20–50%<sup>142,143,144</sup>. One study suggests that fractionated cyclophosphamide can replace ifosfamide, with similar response rates<sup>145</sup>.

As a class, multi-target tyrosine 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<sup>146,147,148,149,150,151</sup> (Table 2). Objective response rates were low at 10–15%. Four-month progression-free survival (PFS) ranged from 35% to 70%<sup>146,147,148,149,151</sup>. For comparison, a 4-month PFS of 0% in the control arm of the phase II trials of regorafenib and of 15% for a historical benchmark established by a pooled analysis of 96 patients with osteosarcoma and measurable disease enrolled on seven Children’s Oncology Group phase II trials were observed<sup>152</sup>. 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<sup>146,147,148,149,150,151</sup>. 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 II trial)<sup>153</sup>. The phase I trial of this combination demonstrated tolerability and a 4-month PFS of 51%<sup>150</sup>. 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 specific tumour. These features include the presence of COSMIC mutational signature 3 (ref.<sup>154</sup>), 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, PD1 and PDL1 ICIs did not show activity in osteosarcoma<sup>84</sup>. Trials combining ICIs with other anticancer therapies, such as MTKIs, and trials of other immune activation approaches, such as antibody combinations and cellular therapy, in individuals with osteosarcoma are in early stages<sup>9</sup>. 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**

Patients with osteosarcoma 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<sup>155</sup>. 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 lifespan 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 both ablative and limb salvage surgeries. The most consistent impairment shown is increased oxygen consumption associated with ambulation reflecting the increased effort needed to walk<sup>156</sup>.

One of the life-threatening late effects of chemotherapy is the development of secondary malignancies, such as therapy-related acute myelogenous leukaemia/myelodysplastic syndrome, central nervous system tumours or secondary solid tumours, which occur in ~3% of patients<sup>125</sup>. 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 as yet unidentified factors<sup>155,157</sup>. Among all paediatric patients with cancer, those with osteosarcoma carry one of the highest rates of genetic cancer predisposition (10–20%)<sup>12</sup>; Li–Fraumeni syndrome is the most prominent but hereditary retinoblastoma, helicase-associated cancers and others also contribute<sup>157</sup>. Secondary cancers are frequently acute myeloid leukaemias<sup>155</sup>, which may be caused by previous exposure to DNA-damaging alkylators, often arising after a median of around 7 years after initial treatment<sup>158</sup>. Other secondary leukaemias are often myelomonoblastic and their lag-time is shorter; these develop after exposure to topoisomerase II inhibitors, including anthracyclines such as doxorubicin. Both forms of secondary

leukaemia have an extremely poor prognosis despite the most intensive therapies such as bone marrow transplantation<sup>155,159</sup>.

Anthracycline-induced, severe cardiomyopathy is another common, potentially fatal late effect of chemotherapy, with ~2% of non-relapse-related deaths among childhood cancer survivors attributed to cardiomyopathy or heart failure<sup>160</sup>. 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<sup>161,162,163</sup>. 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<sup>164</sup>. Allogeneic heart transplants may be indicated for severe cases<sup>165,166</sup>. Patients should be screened for signs of cardiac malfunction to detect even subclinical malfunction early to hopefully prevent progression<sup>164,167</sup>.

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 patients with osteosarcoma<sup>168</sup>. 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<sup>167,168,169</sup>. Hearing aids may be required in those individuals<sup>170</sup>.

The renal glomerulus might be affected by cisplatin treatment in 60–80% of children and adolescents and renal tubular function is affected by ifosfamide treatment in 20–25%<sup>171</sup>. 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<sup>171</sup>. Patients affected may require permanent oral electrolyte substitution.

Patients who have received intensive, multidrug chemotherapy against osteosarcoma may have reduced antibody titres against vaccine-preventable infections for some months after chemotherapy, and some guidelines suggest measuring vaccine-induced antibody titres and repeating vaccinations<sup>172,173</sup>. An increased risk of herpes zoster infection has been found in those individuals and administration of prophylaxis is recommended for patients at risk<sup>174</sup>.



Fertility is only modestly affected by standard chemotherapy regimens. Generally, fertility is most impaired by alkylators and more so in males than in females<sup>175</sup>. 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<sup>176</sup>. The rate of treatment-related malformations does not seem to be increased in the offspring of individuals who formerly had osteosarcoma<sup>155</sup>.

### **Quality of life**

Few studies have investigated the health-related quality of life (HRQoL) of osteosarcoma survivors<sup>177</sup>. A single institution study evaluating 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<sup>178</sup>. 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% reporting impaired general health, which is 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<sup>178</sup>. Surgery for primary site disease control is an important contributor to poor HRQoL in osteosarcoma survivors. A cross-sectional Dutch study compared the HRQoL of patients who underwent resection of a malignant bone tumour from the lower extremity with that of healthy controls<sup>179</sup>. Patients who had undergone surgery had lower scores for motor function, cognitive function, pain and general health.

Given the late toxic effects of treatments and their impact on the quality of life of patients with osteosarcoma, multidisciplinary specialized cancer survivor care is recommended for all patients with osteosarcoma. 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.

### **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 paediatric malignancies, many of the alterations that occur in osteosarcoma are translocation events that silence genes rather than create neoantigens<sup>36</sup>. A high proportion of osteosarcoma samples have an increased number of tumour-infiltrating lymphocytes, suggesting immune system activation in many patients with osteosarcoma<sup>180</sup>. Furthermore, current molecular research continues to classify osteosarcomas not by histological appearance via classical osteosarcoma pathological descriptions but by proteo-genomic drivers of disease that provide further insight into disease biology and may have both prognostic and therapeutic implications<sup>35</sup>. To assist this approach, large libraries of PDX models of osteosarcoma have been developed<sup>181,182</sup>. 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 a better understanding of the relevance of the putative disease drivers. Moreover, analyses of resistant outgrowths may provide further rationale for combination strategies<sup>183,184</sup>.

Another major approach to improve the 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 the identification of antigens that are present in a high proportion of patient tumours but are not expressed in normal tissues to ensure effectiveness and low toxic effects, respectively. The two major areas of active study for this approach are T cell-based therapies and antibody–drug conjugates<sup>9,185</sup>. Further research is required to increase the number of suitable, targetable surface antigens as combinatorial strategies will most likely be required given the intratumour and intertumour heterogeneity of osteosarcomas<sup>186</sup>.

## **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, MTKIs were effective in reducing PFS, 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<sup>187</sup>. Another strategy incorporating MTKIs is evaluating their role as maintenance therapy<sup>188,189</sup>. 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<sup>190,191</sup>. The fundamental premise for these trials is that further inhibition of the regulatory pathways involved in DNA repair<sup>190,191</sup> by targeting WEE1 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<sup>80</sup>. Due to the loosening of FDA restrictions, many of these trials are now also available for patients aged  $\geq 12$  years, which includes most patients with osteosarcoma<sup>192</sup>. 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 (refs.<sup>193,194,195</sup>), HER2 (ref.<sup>196</sup>), EGFR<sup>197</sup> and B7H3 (refs.<sup>198,199</sup>) (Fig. 5). 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.

## References

1. Mirabello, L., Troisi, R. J. & Savage, S. A. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the surveillance, epidemiology, and end results program. *Cancer* 115, 1531–1543 (2009).
2. Bielack, S. S. et al. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J. Clin. Oncol.* 20, 776–790 (2002). A study that affirms tumour site and size, primary metastases, response to chemotherapy, and surgical remission as independent prognostic factors in patients with osteosarcoma.
3. Klein, M. J. & Siegal, G. P. Osteosarcoma: anatomic and histologic variants. *Am. J. Clin. Pathol.* 125, 555–581 (2006).
4. Piperdi, S. et al.  $\beta$ -Catenin does not confer tumorigenicity when introduced into partially transformed human mesenchymal stem cells. *Sarcoma* 2012, 164803 (2012).
5. Bertoni, F. & Bacchini, P. Classification of bone tumors. *Eur. J. Radiol.* 27 (Suppl. 1), S74–S76 (1998).
6. Kager, L. et al. Primary metastatic osteosarcoma: presentation and outcome of patients treated on neoadjuvant Cooperative Osteosarcoma Study Group protocols. *J. Clin. Oncol.* 21, 2011–2018 (2003).
7. Isakoff, M. S., Bielack, S. S., Meltzer, P. & Gorlick, R. Osteosarcoma: current treatment and a collaborative pathway to success. *J. Clin. Oncol.* 33, 3029–3035 (2015).
8. Smith, M. A. et al. Outcomes for children and adolescents with cancer: challenges for the twenty-first century. *J. Clin. Oncol.* 28, 2625–2634 (2010).
9. Gill, J. & Gorlick, R. Advancing therapy for osteosarcoma. *Nat. Rev. Clin. Oncol.* 18, 609–624 (2021).

10. Mirabello, L., Troisi, R. J. & Savage, S. A. International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. *Int. J. Cancer* 125, 229–234 (2009).
11. Parkin, D. M., Stiller, C. A., Draper, G. J. & Bieber, C. The international incidence of childhood cancer. *Int. J. Cancer* 42, 511–520 (1988).
12. Mirabello, L. et al. Frequency of pathogenic germline variants in cancer-susceptibility genes in patients with osteosarcoma. *JAMA Oncol.* 6, 724–734 (2020). This study demonstrates a high frequency of potentially pathogenic germline mutations in patients with osteosarcoma, supporting the role of germline genetic testing.
13. Glass, A. G. & Fraumeni, J. F. Jr. Epidemiology of bone cancer in children. *J. Natl Cancer Inst.* 44, 187–199 (1970).
14. Czerniak, B. *Dorfman and Czerniak's Bone Tumors E-Book* (Elsevier Health Sciences, 2015).
15. Brown, H. K., Schiavone, K., Gouin, F., Heymann, M.-F. & Heymann, D. Biology of bone sarcomas and new therapeutic developments. *Calcif. Tissue Int.* 102, 174–195 (2018).
16. Cole, S., Gianferante, D. M., Zhu, B. & Mirabello, L. Osteosarcoma: a surveillance, epidemiology, and end results program- based analysis from 1975 to 2017. *Cancer* 128, 2107–2118 (2022).
17. Ilcisin, L. A. S. et al. Poverty, race, ethnicity, and survival among U.S. children with non-metastatic osteosarcoma treated on EURAMOS-1: a report from the Children's Oncology Group. *J. Clin. Oncol.* 40, 10004 (2022).
18. Zhang, J. et al. Germline mutations in predisposition genes in pediatric cancer. *N. Engl. J. Med.* 373, 2336–2346 (2015).
19. Vlachos, A., Rosenberg, P. S., Atsidaftos, E., Alter, B. P. & Lipton, J. M. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. *Blood* 119, 3815–3819 (2012).
20. Wang, L. L. et al. Association between osteosarcoma and deleterious mutations in the RECQL4 gene in Rothmund-Thomson syndrome. *J. Natl Cancer Inst.* 95, 669–674 (2003).
21. Lu, L., Jin, W. & Wang, L. L. RECQ DNA helicases and osteosarcoma. *Adv. Exp. Med. Biol.* 1258, 37–54 (2020).
22. Hameed, M. & Mandelker, D. Tumor syndromes predisposing to osteosarcoma. *Adv. Anat. Pathol.* 25, 217–222 (2018).

23. Calvert, G. T. et al. At-risk populations for osteosarcoma: the syndromes and beyond. *Sarcoma* 2012, 152382 (2012).
24. Mirabello, L. et al. Height at diagnosis and birth-weight as risk factors for osteosarcoma. *Cancer Causes Control* 22, 899–908 (2011).
25. Tucker, M. A. et al. Bone sarcomas linked to radiotherapy and chemotherapy in children. *N. Engl. J. Med.* 317, 588–593 (1987).
26. Cundy, T. Paget’s disease of bone. *Metabolism* 80, 5–14 (2018).
27. Ruggieri, P., Sim, F. H., Bond, J. R. & Krishnan Unni, K. Malignancies in fibrous dysplasia. *Cancer* 73, 1411–1424 (1994).
28. Picci, P. et al. Late sarcoma development after curettage and bone grafting of benign bone tumors. *Eur. J. Radiol.* 77, 19–25 (2011).
29. Jones, K. B. Osteosarcomagenesis: modeling cancer initiation in the mouse. *Sarcoma* 2011, 694136 (2011).
30. Mutsaers, A. J. & Walkley, C. R. Cells of origin in osteosarcoma: mesenchymal stem cells or osteoblast committed cells? *Bone* 62, 56–63 (2014).
31. Lin, Y. H. et al. Osteosarcoma: molecular pathogenesis and iPSC modeling. *Trends Mol. Med.* 23, 737–755 (2017).
32. Cortés-Ciriano, I. et al. Comprehensive analysis of chromothripsis in 2,658 human cancers using whole-genome sequencing. *Nat. Genet.* 52, 331–341 (2020).
33. Chen, X. et al. Recurrent somatic structural variations contribute to tumorigenesis in pediatric osteosarcoma. *Cell Rep.* 7, 104–112 (2014). The majority of TP53 loss in osteosarcoma occurs through intron 1 rearrangements or deletions rather than through point mutations.
34. Perry, J. A. et al. Complementary genomic approaches highlight the PI3K/mTOR pathway as a common vulnerability in osteosarcoma. *Proc. Natl Acad. Sci. USA* 111, E5564–E5573 (2014).
35. Sayles, L. C. et al. Genome-informed targeted therapy for osteosarcoma. *Cancer Discov.* 9, 46–63 (2019). A study that defines potentially actionable molecular subtypes of osteosarcoma.
36. Wu, C. C. et al. Immuno-genomic landscape of osteosarcoma. *Nat. Commun.* 11, 1008 (2020). Molecular profiling of samples from patients with osteosarcoma characterizes immune subsets, including immune enrichment among older patients.

37. Rajan, S. et al. Remarkably stable copy-number profiles in osteosarcoma revealed using single-cell DNA sequencing. Preprint at *bioRxiv* <https://doi-org.proxy.insermbiblio.inist.fr/10.1101/2021.08.30.458268> (2021).
38. Behjati, S. et al. Recurrent mutation of IGF signalling genes and distinct patterns of genomic rearrangement in osteosarcoma. *Nat. Commun.* 8, 15936 (2017).
39. Overholtzer, M. et al. The presence of p53 mutations in human osteosarcomas correlates with high levels of genomic instability. *Proc. Natl Acad. Sci. USA* 100, 11547–11552 (2003). One of the first studies to show that p53 mutations correlate with high levels of genomic instability in osteosarcomas.
40. Eischen, C. M. Genome stability requires p53. *Cold Spring Harb. Perspect. Med.* 6, a026096 (2016).
41. Hanel, W. & Moll, U. M. Links between mutant p53 and genomic instability. *J. Cell. Biochem.* 113, 433–439 (2012).
42. Gerstung, M. et al. The evolutionary history of 2,658 cancers. *Nature* 578, 122–128 (2020).
43. Lawlor, R. T. et al. Alternative lengthening of telomeres (ALT) influences survival in soft tissue sarcomas: a systematic review with meta-analysis. *BMC Cancer* 19, 232 (2019).
44. Kovac, M. et al. Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. *Nat. Commun.* 6, 8940 (2015).
45. Tellez-Gabriel, M. et al. Analysis of gap junctional intercellular communications using a dielectrophoresis-based microchip. *Eur. J. Cell Biol.* 96, 110–118 (2017).
46. Bénédicte Brounais, L.-R. & Frédéric, L. In *Bone Cancer* 221–239, Ch. 18 (Academic Press, 2022).
47. Lan, M. et al. Extracellular vesicles-mediated signaling in the osteosarcoma microenvironment: roles and potential therapeutic targets. *J. Bone Oncol.* 12, 101–104 (2018).
48. Cackowski, F. C. et al. Osteoclasts are important for bone angiogenesis. *Blood* 115, 140–149 (2010).
49. Endo-Munoz, L., Evdokiou, A. & Saunders, N. A. The role of osteoclasts and tumour-associated macrophages in osteosarcoma metastasis. *Biochim. Biophys. Acta* 1826, 434–442 (2012).
50. Khanna, C. et al. Toward a drug development path that targets metastatic progression in osteosarcoma. *Clin. Cancer Res.* 20, 4200–4209 (2014).

51. Heymann, M.-F., Lézot, F. & Heymann, D. The contribution of immune infiltrates and the local microenvironment in the pathogenesis of osteosarcoma. *Cell. Immunol.* <https://doi-org.proxy.insermbiblio.inist.fr/10.1016/j.cellimm.2017.10.011> (2017).
52. Brown, H. K., Tellez-Gabriel, M. & Heymann, D. Cancer stem cells in osteosarcoma. *Cancer Lett.* 386, 189–195 (2017).
53. Grunewald, T. G. et al. Sarcoma treatment in the era of molecular medicine. *EMBO Article Google Scholar*
54. Zhou, Y. et al. Single-cell RNA landscape of intratumoral heterogeneity and immunosuppressive microenvironment in advanced osteosarcoma. *Nat. Commun.* 11, 6322 (2020).
55. Guo, J. et al. Single-cell profiling of tumor microenvironment heterogeneity in osteosarcoma identifies a highly invasive subcluster for predicting prognosis. *Front. Oncol.* 12, 732862 (2022).
56. Mazumdar, A. et al. Exploring the role of osteosarcoma-derived extracellular vesicles in pre-metastatic niche formation and metastasis in the 143-B xenograft mouse osteosarcoma model. *Cancers* <https://doi-org.proxy.insermbiblio.inist.fr/10.3390/cancers12113457> (2020).
57. Stamatopoulos, A. et al. Mesenchymal stromal cells for bone sarcoma treatment: roadmap to clinical practice. *J. Bone Oncol.* 16, 100231 (2019).
58. Perrot, P. et al. Safety concern between autologous fat graft, mesenchymal stem cell and osteosarcoma recurrence. *PLoS ONE* 5, e10999 (2010).
59. Cortini, M., Avnet, S. & Baldini, N. Mesenchymal stroma: role in osteosarcoma progression. *Cancer Lett.* 405, 90–99 (2017).
60. Baglio, S. R. et al. Blocking tumor-educated MSC paracrine activity halts osteosarcoma progression. *Clin. Cancer Res.* 23, 3721–3733 (2017).
61. Tu, B., Du, L., Fan, Q. M., Tang, Z. & Tang, T. T. STAT3 activation by IL-6 from mesenchymal stem cells promotes the proliferation and metastasis of osteosarcoma. *Cancer Lett.* 325, 80–88 (2012).
62. Gross, A. C. et al. IL-6 and CXCL8 mediate osteosarcoma-lung interactions critical to metastasis. *JCI Insight* <https://doi-org.proxy.insermbiblio.inist.fr/10.1172/jci.insight.99791> (2018).
63. Mazumdar, A. et al. Osteosarcoma-derived extracellular vesicles induce lung fibroblast reprogramming. *Int. J. Mol. Sci.* <https://doi-org.proxy.insermbiblio.inist.fr/10.3390/ijms21155451> (2020).



64. Zhang, W. et al. Adaptive fibrogenic reprogramming of osteosarcoma stem cells promotes metastatic growth. *Cell Rep.* 24, 1266–1277.e5 (2018).
65. Yui, Y., Kumai, J., Watanabe, K., Wakamatsu, T. & Sasagawa, S. Lung fibrosis is a novel therapeutic target to suppress lung metastasis of osteosarcoma. *Int. J. Cancer* <https://doi-org.proxy.insermbiblio.inist.fr/10.1002/ijc.34008> (2022).
66. Kurzman, I. D. et al. Adjuvant therapy for osteosarcoma in dogs: results of randomized clinical trials using combined liposome-encapsulated muramyl tripeptide and cisplatin. *Clin. Cancer Res.* 1, 1595–1601 (1995).
67. Meyers, P. A. et al. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival—a report from the Children’s Oncology Group. *J. Clin. Oncol.* 26, 633–638 (2008).
68. Kleinerman, E. S. et al. Phase II study of liposomal muramyl tripeptide in osteosarcoma: the cytokine cascade and monocyte activation following administration. *J. Clin. Oncol.* 10, 1310–1316 (1992).
69. Mason, N. J. et al. Immunotherapy with a HER2-targeting listeria induces HER2-specific immunity and demonstrates potential therapeutic effects in a phase I trial in canine osteosarcoma. *Clin. Cancer Res.* 22, 4380–4390 (2016).
70. Chen, K. et al. Wnt10b induces chemotaxis of osteosarcoma and correlates with reduced survival. *Pediatr. Blood Cancer* 51, 349–355 (2008).
71. Goldstein, S. D., Trucco, M., Bautista Guzman, W., Hayashi, M. & Loeb, D. M. A monoclonal antibody against the Wnt signaling inhibitor dickkopf-1 inhibits osteosarcoma metastasis in a preclinical model. *Oncotarget* 7, 21114–21123 (2016).
72. Khanna, C. et al. The membrane-cytoskeleton linker ezrin is necessary for osteosarcoma metastasis. *Nat. Med.* 10, 182–186 (2004).
73. Bulut, G. et al. Small molecule inhibitors of ezrin inhibit the invasive phenotype of osteosarcoma cells. *Oncogene* 31, 269–281 (2012).
74. Ren, L. et al. Dysregulation of Ezrin phosphorylation prevents metastasis and alters cellular metabolism in osteosarcoma. *Cancer Res.* 72, 1001–1012 (2012).
75. Morrow, J. J. et al. Positively selected enhancer elements endow osteosarcoma cells with metastatic competence. *Nat. Med.* 24, 176–185 (2018).
76. Ichikawa, J. et al. Thrombin induces osteosarcoma growth, a function inhibited by low molecular weight heparin in vitro and in vivo. *Cancer* 118, 2494–2506 (2012).

77. Charan, M. et al. Tumor secreted ANGPTL2 facilitates recruitment of neutrophils to the lung to promote lung pre-metastatic niche formation and targeting ANGPTL2 signaling affects metastatic disease. *Oncotarget* 11, 510–522 (2020).
78. Navet, B. et al. The intrinsic and extrinsic implications of RANKL/RANK signaling in osteosarcoma: from tumor initiation to lung metastases. *Cancers* <https://doi-org.proxy.insermbiblio.inist.fr/10.3390/cancers10110398> (2018).
79. Church, A. J. et al. Clinical impact of molecular tumor profiling in pediatric, adolescent, and young adult patients with extra-cranial solid malignancies: an interim report from the GAIN/iCat2 study. *J. Clin. Oncol.* 39, 10005 (2021).
80. Suehara, Y. et al. Clinical genomic sequencing of pediatric and adult osteosarcoma reveals distinct molecular subsets with potentially targetable alterations. *Clin. Cancer Res.* 25, 6346–6356 (2019).
81. Meyers, P. A. et al. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival — a report from the Children’s Oncology Group. *J. Clin. Oncol.* 26, 633–638 (2008).
82. Bielack, S. S. et al. Methotrexate, doxorubicin, and cisplatin (MAP) plus maintenance pegylated interferon Alfa-2b versus MAP alone in patients with resectable high-grade osteosarcoma and good histologic response to preoperative MAP: first results of the EURAMOS-1 Good Response Randomized Controlled Trial. *J. Clin. Oncol.* 33, 2279–2287 (2015).
83. Gröbner, S. N. et al. The landscape of genomic alterations across childhood cancers. *Nature* 555, 321–327 (2018).
84. Tawbi, H. A. et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. *Lancet Oncol.* 18, 1493–1501 (2017).
85. Le Cesne, A. et al. Programmed cell death 1 (PD-1) targeting in patients with advanced osteosarcomas: results from the PEMBROSARC study. *Eur. J. Cancer* 119, 151–157 (2019).
86. Hingorani, P. et al. ABBV-085, antibody–drug conjugate targeting LRRC15, is effective in osteosarcoma: a report by the pediatric preclinical testing consortium. *Mol. Cancer Ther.* 20, 535–540 (2021).
87. Hingorani, P. et al. Trastuzumab deruxtecan, antibody–drug conjugate targeting HER2, is effective in pediatric malignancies: a report by the pediatric preclinical testing consortium. *Mol. Cancer Ther.* 21, 1318–1325 (2022).

88. Lange, S. et al. A chimeric GM-CSF/IL18 receptor to sustain CAR T-cell function. *Cancer Discov.* 11, 1661–1671 (2021).
89. Tullius, B. P., Setty, B. A. & Lee, D. A. In *Current Advances in Osteosarcoma: Clinical Perspectives: Past, Present and Future* (eds Kleinerman, E. S. & Gorlick, R.) 141–154 (Springer International Publishing, 2020).
90. Kendersky, N. M. et al. The B7-H3–targeting antibody–drug conjugate m276-SL-PBD is potently effective against pediatric cancer preclinical solid tumor models. *Clin. Cancer Res.* 27, 2938–2946 (2021).
91. Hingorani, P. et al. Abstract LB-217: Preclinical evaluation of trastuzumab deruxtecan (T-DXd; DS-8201a), a HER2 antibody-drug conjugate, in pediatric solid tumors by the Pediatric Preclinical Testing Consortium (PPTC). *Cancer Res.* 80 (Suppl. 16), LB-217 (2020).
92. Bayles, I. et al. Ex vivo screen identifies CDK12 as a metastatic vulnerability in osteosarcoma. *J. Clin. Invest.* 129, 4377–4392 (2019).
93. Chang, L.-S. et al. Targeting protein translation by rocaglamide and didesmethylrocaglamide to treat MPNST and other sarcomas. *Mol. Cancer Ther.* 19, 731–741 (2020).
94. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT04040205> (2022).
95. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT03242382> (2022).
96. Teven, C. M., Farina, E. M., Rivas, J. & Reid, R. R. Fibroblast growth factor (FGF) signaling in development and skeletal diseases. *Genes Dis.* 1, 199–213 (2014).
97. Ikebuchi, Y. Coupling of bone resorption and formation by RANKL reverse signalling. *Nature* <https://doi-org.proxy.insermbiblio.inist.fr/10.1038/s41586-018-0482-7> (2018).
98. Li, Y. S., Liu, Q., He, H. B. & Luo, W. The possible role of insulin-like growth factor-1 in osteosarcoma. *Curr. Probl. Cancer* 43, 228–235 (2019).
99. Regan, D. P. et al. Losartan blocks osteosarcoma-elicited monocyte recruitment, and combined with the kinase inhibitor toceranib, exerts significant clinical benefit in canine metastatic osteosarcoma. *Clin. Cancer Res.* 28, 662–676 (2022).
100. Nomura, M. et al. Tegavivint and the  $\beta$ -catenin/ALDH axis in chemotherapy-resistant and metastatic osteosarcoma. *J. Natl Cancer Inst.* 111, 1216–1227 (2019).

101. Meltzer, P. S. & Helman, L. J. New horizons in the treatment of osteosarcoma. *N. Engl. J. Med.* 385, 2066–2076 (2021).
102. Zhou, Y. et al. The effect of pathological fractures on the prognosis of patients with osteosarcoma: a meta-analysis of 14 studies. *Oncotarget* 8, 73037–73049 (2017).
103. Papagelopoulos, P. J. et al. Current concepts in the evaluation and treatment of osteosarcoma. *Orthopedics* 23, 858–867 (2000).
104. Strauss, S. J. et al. Bone sarcomas: ESMO-EURACAN-GENTURIS-ERN PaedCan Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann. Oncol.* 32, 1520–1536 (2021).
105. Casali, P. G. et al. Bone sarcomas: ESMO–PaedCan–EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* 29, iv79–iv95 (2018).
106. WHO Classification of Tumours Editorial Board. *WHO Classification of Tumours: Soft Tissue and Bone Tumours* (International Agency for Research on Cancer, 2020).
107. Meyer, J. S. et al. Imaging guidelines for children with Ewing sarcoma and osteosarcoma: a report from the Children’s Oncology Group Bone Tumor Committee. *Pediatr. Blood Cancer* 51, 163–170 (2008).
108. Wolf, R. E. & Enneking, W. F. The staging and surgery of musculoskeletal neoplasms. *Orthop. Clin. North. Am.* 27, 473–481 (1996).
109. Sheth, D. S. et al. Conventional and dedifferentiated parosteal osteosarcoma. Diagnosis, treatment, and outcome. *Cancer* 78, 2136–2145 (1996).
110. Grimer, R. J. et al. Periosteal osteosarcoma — a European review of outcome. *Eur. J. Cancer* 41, 2806–2811 (2005).
111. Roberts, R. D. et al. Provocative questions in osteosarcoma basic and translational biology: a report from the Children’s Oncology Group. *Cancer* 125, 3514–3525 (2019).
112. Gorlick, R., Janeway, K., Lessnick, S., Randall, R. L. & Marina, N. Children’s Oncology Group’s 2013 blueprint for research: bone tumors. *Pediatr. Blood Cancer* 60, 1009–1015 (2013).
113. Aljubran, A. H., Griffin, A., Pintilie, M. & Blackstein, M. Osteosarcoma in adolescents and adults: survival analysis with and without lung metastases. *Ann. Oncol.* 20, 1136–1141 (2009).

114. Marina, N. M. et al. Comparison of MAPIE versus MAP in patients with a poor response to preoperative chemotherapy for newly diagnosed high-grade osteosarcoma (EURAMOS-1): an open-label, international, randomised controlled trial. *Lancet Oncol.* 17, 1396–1408 (2016). An international randomized clinical trial fails to show benefit of addition of I/E to MAP chemotherapy in the primary treatment of osteosarcoma.
115. Rosen, G., Murphy, M. L., Huvos, A. G., Gutierrez, M. & Marcove, R. C. Chemotherapy, en bloc resection, and prosthetic bone replacement in the treatment of osteogenic sarcoma. *Cancer* 37, 1–11 (1976).
116. Rosen, G. et al. Preoperative chemotherapy for osteogenic sarcoma: selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to preoperative chemotherapy. *Cancer* 49, 1221–1230 (1982).
117. Bishop, M. W. et al. Assessing the prognostic significance of histologic response in osteosarcoma: a comparison of outcomes on CCG-782 and INT0133 — a report from the Children’s Oncology Group Bone Tumor Committee. *Pediatr. Blood Cancer* 63, 1737–1743 (2016). This study validates the prognostic significance of pathological treatment response in osteosarcoma following neoadjuvant chemotherapy.
118. Bacci, G. et al. Neoadjuvant chemotherapy for high-grade central osteosarcoma of the extremity. *Cancer* 97, 3068–3075 (2003).
119. Meyers, P. A. et al. Intensification of preoperative chemotherapy for osteogenic sarcoma: results of the Memorial Sloan-Kettering (T12) protocol. *J. Clin. Oncol.* 16, 2452–2458 (1998).
120. Villani, A. et al. Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: 11 year follow-up of a prospective observational study. *Lancet Oncol.* 17, 1295–1305 (2016).
121. Diessner, B. J. et al. Nearly half of TP53 germline variants predicted to be pathogenic in patients with osteosarcoma are de novo: a report from the Children’s Oncology Group. *JCO Precis. Oncol.* 4, 1187–1195 (2020).
122. Kratz, C. P. et al. Cancer screening recommendations for individuals with Li-Fraumeni syndrome. *Clin. Cancer Res.* 23, e38–e45 (2017).
123. Marees, T. et al. Risk of second malignancies in survivors of retinoblastoma: more than 40 years of follow-up. *J. Natl Cancer Inst.* 100, 1771–1779 (2008).

124. Hendrickson, P. G. et al. Radiation therapy and secondary malignancy in Li-Fraumeni syndrome: a hereditary cancer registry study. *Cancer Med.* 9, 7954–7963 (2020).
125. Smeland, S. et al. Survival and prognosis with osteosarcoma: outcomes in more than 2000 patients in the EURAMOS-1 (European and American Osteosarcoma Study) cohort. *Eur. J. Cancer* 109, 36–50 (2019).
126. Kempf-Bielack, B. et al. Osteosarcoma relapse after combined modality therapy: an analysis of unselected patients in the Cooperative Osteosarcoma Study Group (COSS). *J. Clin. Oncol.* 23, 559–568 (2005).
127. Jaffe, N., Puri, A. & Gelderblom, H. Osteosarcoma: evolution of treatment paradigms. *Sarcoma* 2013, 203531 (2013).
128. Anninga, J. K. et al. Chemotherapeutic adjuvant treatment for osteosarcoma: where do we stand? *Eur. J. Cancer* 47, 2431–2445 (2011).
129. Bielack, S. S. et al. Osteosarcoma: the same old drugs or more. *J. Clin. Oncol.* 26, 3102–3103 (2008).
130. Chou, A. J. et al. Addition of muramyl tripeptide to chemotherapy for patients with newly diagnosed metastatic osteosarcoma: a report from the Children’s Oncology Group. *Cancer* 115, 5339–5348 (2009).
131. Brard, C. et al. Sarcome-13/OS2016 trial protocol: a multicentre, randomised, open-label, phase II trial of mifamurtide combined with postoperative chemotherapy for patients with newly diagnosed high-risk osteosarcoma. *BMJ Open* 9, e025877 (2019).
132. Grimer, R. J. et al. Osteosarcoma over the age of forty. *Eur. J. Cancer* 39, 157–163 (2003).
133. Ferrari, S. et al. EURO-B.O.S.S.: a European study on chemotherapy in bone-sarcoma patients aged over 40: Outcome in primary high-grade osteosarcoma. *Tumori* 104, 30–36 (2018).
134. Picci, P. et al. Relationship of chemotherapy-induced necrosis and surgical margins to local recurrence in osteosarcoma. *J. Clin. Oncol.* 12, 2699–2705 (1994).
135. Ruggieri, P. et al. Outcome of expandable prostheses in children. *J. Pediatr. Orthop.* 33, 244–253 (2013).
136. Tate, R., Gerrand, C. & Hale, J. Tibial turn-up procedure as an alternative to rotationplasty in a 4-year-old with osteosarcoma of the distal femur. *J. Pediatr. Orthop. B* 24, 50–55 (2015).

137. Hebert, J. S., Rehani, M. & Stiegelmar, R. Osseointegration for lower-limb amputation: a systematic review of clinical outcomes. *JBJS Rev.* 5, e10 (2017).
138. Ciernik, I. F. et al. Proton-based radiotherapy for unresectable or incompletely resected osteosarcoma. *Cancer* 117, 4522–4530 (2011).
139. Matsunobu, A. et al. Impact of carbon ion radiotherapy for unresectable osteosarcoma of the trunk. *Cancer* 118, 4555–4563 (2012).
140. Seidensaal, K. et al. The role of combined ion-beam radiotherapy (CIBRT) with protons and carbon ions in a multimodal treatment strategy of inoperable osteosarcoma. *Radiother. Oncol.* 159, 8–16 (2021).
141. NCCN. Treatment by cancer type. *NCCN* [https://www.nccn.org/guidelines/category\\_1](https://www.nccn.org/guidelines/category_1) (2022).
142. Gentet, J. C. et al. Ifosfamide and etoposide in childhood osteosarcoma. A phase II study of the French Society of Paediatric Oncology. *Eur. J. Cancer* 33, 232–237 (1997).
143. Lee, J. A. et al. Higher gemcitabine dose was associated with better outcome of osteosarcoma patients receiving gemcitabine-docetaxel chemotherapy. *Pediatr. Blood Cancer* 63, 1552–1556 (2016).
144. Miser, J. S. et al. Ifosfamide with mesna uroprotection and etoposide: an effective regimen in the treatment of recurrent sarcomas and other tumors of children and young adults. *J. Clin. Oncol.* 5, 1191–1198 (1987).
145. Rodríguez-Galindo, C. et al. Treatment of refractory osteosarcoma with fractionated cyclophosphamide and etoposide. *J. Pediatr. Hematol. Oncol.* 24, 250–255 (2002).
146. Davis, L. E. et al. Randomized double-blind phase II study of regorafenib in patients with metastatic osteosarcoma. *J. Clin. Oncol.* 37, 1424–1431 (2019).
147. Grignani, G. et al. A phase II trial of sorafenib in relapsed and unresectable high-grade osteosarcoma after failure of standard multimodal therapy: an Italian Sarcoma Group study. *Ann. Oncol.* 23, 508–516 (2012).
148. Italiano, A. et al. Cabozantinib in patients with advanced Ewing sarcoma or osteosarcoma (CABONE): a multicentre, single-arm, phase 2 trial. *Lancet Oncol.* 21, 446–455 (2020).
149. Xie, L. et al. Apatinib for advanced osteosarcoma after failure of standard multimodal therapy: an open label phase II clinical trial. *Oncologist* 24, e542–e550 (2019).

150. Gaspar, N. et al. Lenvatinib with etoposide plus ifosfamide in patients with refractory or relapsed osteosarcoma (ITCC-050): a multicentre, open-label, multicohort, phase 1/2 study. *Lancet Oncol.* 22, 1312–1321 (2021).
151. Duffaud, F. et al. Efficacy and safety of regorafenib in adult patients with metastatic osteosarcoma: a non-comparative, randomised, double-blind, placebo-controlled, phase 2 study. *Lancet Oncol.* 20, 120–133 (2019).
152. Lagmay, J. P. et al. Outcome of patients with recurrent osteosarcoma enrolled in seven phase II trials through Children’s Cancer Group, Pediatric Oncology Group, and Children’s Oncology Group: learning from the past to move forward. *J. Clin. Oncol.* 34, 3031–3038 (2016).
153. US National Library of Medicine. *ClinicalTrials.gov* <http://www.clinicaltrials.gov/ct2/show/NCT04154189> (2022).
154. Alexandrov, L. B. et al. The repertoire of mutational signatures in human cancer. *Nature* 578, 94–101 (2020).
155. Hecker-Nolting, S., Langer, T., Blattmann, C., Kager, L. & Bielack, S. S. Current insights into the management of late chemotherapy toxicities in pediatric osteosarcoma patients. *Cancer Manag. Res.* 13, 8989–8998 (2021).
156. Mason, G. E. et al. Quality of life following amputation or limb preservation in patients with lower extremity bone sarcoma. *Front. Oncol.* 3, 210 (2013).
157. Kratz, C. P. et al. Predisposition to cancer in children and adolescents. *Lancet Child Adolesc. Health* 5, 142–154 (2021).
158. Leone, G., Pagano, L., Ben-Yehuda, D. & Voso, M. T. Therapy-related leukemia and myelodysplasia: susceptibility and incidence. *Haematologica* 92, 1389–1398 (2007).
159. Boddu, P. et al. Treated secondary acute myeloid leukemia: a distinct high-risk subset of AML with adverse prognosis. *Blood Adv.* 1, 1312–1323 (2017).
160. Armstrong, G. T. et al. Late mortality among 5-year survivors of childhood cancer: a summary from the childhood cancer survivor study. *J. Clin. Oncol.* 27, 2328–2338 (2009).
161. Mancilla, T. R., Iskra, B. & Aune, G. J. Doxorubicin-induced cardiomyopathy in children. *Compr. Physiol.* 9, 905–931 (2019).
162. Bhagat, A. & Kleinerman, E. S. Anthracycline-induced cardiotoxicity: causes, mechanisms, and prevention. *Adv. Exp. Med. Biol.* 1257, 181–192 (2020).



163. Rawat, P. S., Jaiswal, A., Khurana, A., Bhatti, J. S. & Navik, U. Doxorubicin-induced cardiotoxicity: an update on the molecular mechanism and novel therapeutic strategies for effective management. *Biomed. Pharmacother.* 139, 111708 (2021).
164. Armenian, S. H. et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. *Lancet Oncol.* 16, e123–e136 (2015).
165. Bock, M. J. et al. Cancer recurrence and mortality after pediatric heart transplantation for anthracycline cardiomyopathy: a report from the Pediatric Heart Transplant Study (PHTS) group. *Pediatr. Transpl.* <https://doi-org.proxy.insermbiblio.inist.fr/10.1111/petr.12923> (2017).
166. Shugh, S. B. & Ryan, T. D. Heart transplantation in survivors of childhood cancer. *Transl Pediatr.* 8, 314–321 (2019).
167. Curigliano, G. et al. Management of cardiac disease in cancer patients throughout oncological treatment: ESMO consensus recommendations. *Ann. Oncol.* 31, 171–190 (2020).
168. Moke, D. J. et al. Prevalence and risk factors for cisplatin-induced hearing loss in children, adolescents, and young adults: a multi-institutional North American cohort study. *Lancet Child Adolesc. Health* 5, 274–283 (2021).
169. Romano, A. et al. Assessment and management of platinum-related ototoxicity in children treated for cancer. *Cancers* <https://doi-org.proxy.insermbiblio.inist.fr/10.3390/cancers12051266> (2020).
170. Clemens, E. et al. Recommendations for ototoxicity surveillance for childhood, adolescent, and young adult cancer survivors: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group in collaboration with the PanCare Consortium. *Lancet Oncol.* 20, e29–e41 (2019).
171. Skinner, R. Late renal toxicity of treatment for childhood malignancy: risk factors, long-term outcomes, and surveillance. *Pediatr. Nephrol.* 33, 215–225 (2018).
172. Laws, H. J. et al. Impfen bei Immundefizienz : Anwendungshinweise zu den von der Ständigen Impfkommission empfohlenen Impfungen. (III) Impfen bei hämatologischen und onkologischen Erkrankungen (antineoplastische Therapie, Stammzelltransplantation), Organtransplantation und Asplenie [German]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 63, 588–644 (2020).

173. Pittet, L. F. & Posfay-Barbe, K. M. Vaccination of immune compromised children-an overview for physicians. *Eur. J. Pediatr.* 180, 2035–2047 (2021).
174. Bader, M. S. Herpes zoster: diagnostic, therapeutic, and preventive approaches. *Postgrad. Med.* 125, 78–91 (2013).
175. van Santen, H. M. et al. Reproductive complications in childhood cancer survivors. *Pediatr. Clin. North Am.* 67, 1187–1202 (2020).
176. Oktay, K. et al. Fertility preservation in patients with cancer: ASCO clinical practice guideline update. *J. Clin. Oncol.* 36, 1994–2001 (2018).
177. Stokke, J., Sung, L., Gupta, A., Lindberg, A. & Rosenberg, A. R. Systematic review and meta-analysis of objective and subjective quality of life among pediatric, adolescent, and young adult bone tumor survivors. *Pediatr. Blood Cancer* 62, 1616–1629 (2015).
178. Edelmann, M. N. et al. Neurocognitive and patient-reported outcomes in adult survivors of childhood osteosarcoma. *JAMA Oncol.* 2, 201–208 (2016).
179. Bekkering, W. P. et al. Quality of life after bone sarcoma surgery around the knee: a long-term follow-up study. *Eur. J. Cancer Care* <https://doi-org.proxy.insermbiblio.inist.fr/10.1111/ecc.12603> (2017).
180. Koirala, P. et al. Immune infiltration and PD-L1 expression in the tumor microenvironment are prognostic in osteosarcoma. *Sci. Rep.* 6, 30093 (2016).
181. Landuzzi, L., Manara, M. C., Lollini, P. L. & Scotlandi, K. Patient derived xenografts for genome-driven therapy of osteosarcoma. *Cells* <https://doi-org.proxy.insermbiblio.inist.fr/10.3390/cells10020416> (2021).
182. Higuchi, T. et al. Osteosarcoma patient-derived orthotopic xenograft (PDOX) models used to identify novel and effective therapeutics: a review. *Anticancer. Res.* 41, 5865–5871 (2021).
183. Loh, A. H. P. et al. Combinatorial screening using orthotopic patient derived xenograft-expanded early phase cultures of osteosarcoma identify novel therapeutic drug combinations. *Cancer Lett.* 442, 262–270 (2019).
184. Lilienthal, I. & Herold, N. Targeting molecular mechanisms underlying treatment efficacy and resistance in osteosarcoma: a review of current and future strategies. *Int. J. Mol. Sci.* <https://doi-org.proxy.insermbiblio.inist.fr/10.3390/ijms21186885> (2020).
185. DeRenzo, C. & Gottschalk, S. Genetically modified T-cell therapy for osteosarcoma: into the roaring 2020s. *Adv. Exp. Med. Biol.* 1257, 109–131 (2020).

186. Wang, Y. et al. Comprehensive surfaceome profiling to identify and validate novel cell-surface targets in osteosarcoma. *Mol. Cancer Ther.* <https://doi-org.proxy.insermbiblio.inist.fr/10.1158/1535-7163.Mct-21-0836> (2022).
187. Whittle, S. B. et al. Charting a path for prioritization of novel agents for clinical trials in osteosarcoma: a report from the Children's Oncology Group New Agents for Osteosarcoma Task Force. *Pediatr. Blood Cancer* 68, e29188 (2021).
188. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT05135975> (2022).
189. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT04055220> (2022).
190. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT04833582> (2022).
191. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT04417062> (2021).
192. FDA. Considerations for the inclusion of adolescent patients in adult oncology clinical trials: guidance for industry. *FDA* <https://bit.ly/2IMiAdT> (2019).
193. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT03635632> (2021).
194. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT04539366> (2022).
195. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT03721068> (2022).
196. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT00902044> (2021).
197. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT03618381> (2022).
198. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT04483778> (2022).
199. US National Library of Medicine. *ClinicalTrials.gov* <https://clinicaltrials.gov/ct2/show/NCT04897321> (2022).
200. Gianferante, D. M., Mirabello, L. & Savage, S. A. Germline and somatic genetics of osteosarcoma - connecting aetiology, biology and therapy. *Nat. Rev. Endocrinol.* 13, 480–491 (2017).

201. Meyers, P. A. & Gorlick, R. Osteosarcoma. *Pediatr. Clin. North. Am.* 44, 973–989 (1997).
202. Kansara, M., Teng, M. W., Smyth, M. J. & Thomas, D. M. Translational biology of osteosarcoma. *Nat. Rev. Cancer* 14, 722–735 (2014).
203. Jubelin, C. et al. Biological evidence of cancer stem-like cells and recurrent disease in osteosarcoma. *Cancer Drug Resist.* 5, 184–198 (2022).
204. Ségaliny, A. I., Tellez-Gabriel, M., Heymann, M. F. & Heymann, D. Receptor tyrosine kinases: characterisation, mechanism of action and therapeutic interests for bone cancers. *J. Bone Oncol.* 4, 1–12 (2015).
205. Bray, S. J. Notch signalling: a simple pathway becomes complex. *Nat. Rev. Mol. Cell Biol.* 7, 678–689 (2006).
206. Ségaliny, A. I. et al. Interleukin-34 promotes tumor progression and metastatic process in osteosarcoma through induction of angiogenesis and macrophage recruitment. *Int. J. Cancer* 137, 73–85 (2015).
207. Ory, B. et al. Zoledronic acid suppresses lung metastases and prolongs overall survival of osteosarcoma-bearing mice. *Cancer* 104, 2522–2529 (2005).
208. Dharia, N. V. et al. A first-generation pediatric cancer dependency map. *Nat. Genet.* 53, 529–538 (2021).
209. Jia, S.-F., Worth, L. L. & Kleinerman, E. S. A nude mouse model of human osteosarcoma lung metastases for evaluating new therapeutic strategies. *Clin. Exp. Metastasis* 17, 501–506 (1999).
210. Khanna, C. et al. An orthotopic model of murine osteosarcoma with clonally related variants differing in pulmonary metastatic potential. *Clin. Exp. Metastasis* 18, 261–271 (2000).
211. Boyle, D. B. & Coupar, B. E. H. Identification and cloning of the Fowlpox virus thymidine kinase gene using Vaccinia virus. *J. Gen. Virol.* 67, 1591–1600 (1986).
212. Fan, T. M., Roberts, R. D. & Lizardo, M. M. Understanding and modeling metastasis biology to improve therapeutic strategies for combating osteosarcoma progression. *Front. Oncol.* <https://doi-org.proxy.insermbiblio.inist.fr/10.3389/fonc.2020.00013> (2020).
213. Zhao, S. et al. NKD2, a negative regulator of Wnt signaling, suppresses tumor growth and metastasis in osteosarcoma. *Oncogene* 34, 5069–5079 (2015).

214. Mendoza, A. et al. Modeling metastasis biology and therapy in real time in the mouse lung. *J. Clin. Invest.* 120, 2979–2988 (2010).
215. Lizardo, M. M. & Sorensen, P. H. Practical considerations in studying metastatic lung colonization in osteosarcoma using the pulmonary metastasis assay. *J. Vis. Exp.* <https://doi-org.proxy.insermbiblio.inist.fr/10.3791/56332> (2018).
216. Tsai, Y. C. et al. The ubiquitin ligase gp78 promotes sarcoma metastasis by targeting KAI1 for degradation. *Nat. Med.* 13, 1504–1509 (2007).
217. Lizardo, M. M. et al. Upregulation of glucose-regulated protein 78 in metastatic cancer cells is necessary for lung metastasis progression. *Neoplasia* 18, 699–710 (2016).
218. Morrow, J. J. et al. mTOR inhibition mitigates enhanced mRNA translation associated with the metastatic phenotype of osteosarcoma cells in vivo. *Clin. Cancer Res.* 22, 6129–6141 (2016).
219. Yu, P. Y. et al. Target specificity, in vivo pharmacokinetics, and efficacy of the putative STAT3 inhibitor LY5 in osteosarcoma, Ewing’s sarcoma, and rhabdomyosarcoma. *PLoS ONE* 12, e0181885 (2017).
220. Gillet, J.-P. et al. Redefining the relevance of established cancer cell lines to the study of mechanisms of clinical anti-cancer drug resistance. *Proc. Natl Acad. Sci. USA* 108, 18708–18713 (2011).
221. Wilding, J. L. & Bodmer, W. F. Cancer cell lines for drug discovery and development. *Cancer Res.* 74, 2377–2384 (2014).
222. Phan, N. et al. A simple high-throughput approach identifies actionable drug sensitivities in patient-derived tumor organoids. *Commun. Biol.* <https://doi-org.proxy.insermbiblio.inist.fr/10.1038/s42003-019-0305-x> (2019).
223. Stewart, E. et al. Orthotopic patient-derived xenografts of paediatric solid tumours. *Nature* 549, 96–100 (2017).
224. Houghton, P. J. et al. The pediatric preclinical testing program: description of models and early testing results. *Pediatr. Blood Cancer* 49, 928–940 (2007).
225. Morton, C. L. & Houghton, P. J. Establishment of human tumor xenografts in immunodeficient mice. *Nat. Protoc.* 2, 247–250 (2007).
226. Mundi, P. S. et al. Pre-clinical validation of an RNA-based precision oncology platform for patient-therapy alignment in a diverse set of human malignancies resistant to standard treatments. Preprint at *bioRxiv* <https://doi-org.proxy.insermbiblio.inist.fr/10.1101/2021.10.03.462951> (2021).

227. Ben-David, U. et al. Patient-derived xenografts undergo mouse-specific tumor evolution. *Nat. Genet.* 49, 1567–1575 (2017).
228. Woo, X. Y. et al. Conservation of copy number profiles during engraftment and passaging of patient-derived cancer xenografts. *Nat. Genet.* 53, 86–99 (2021).
229. Jacques, C. et al. Murine models of bone sarcomas. *Methods Mol. Biol.* 1914, 331–342 (2019).
230. Wang, Z. Q., Liang, J., Schellander, K., Wagner, E. F. & Grigoriadis, A. E. c-fos-induced osteosarcoma formation in transgenic mice: cooperativity with c-jun and the role of endogenous c-fos. *Cancer Res.* 55, 6244–6251 (1995).
231. Fenger, J. M., London, C. A. & Kisseberth, W. C. Canine osteosarcoma: a naturally occurring disease to inform pediatric oncology. *ILAR J.* 55, 69–85 (2014).
232. Gardner, H. L. et al. Canine osteosarcoma genome sequencing identifies recurrent mutations in DMD and the histone methyltransferase gene SETD2. *Commun. Biol.* 2, 266 (2019).
233. LeBlanc, A. K. et al. Perspectives from man’s best friend: National Academy of Medicine’s Workshop on Comparative Oncology. *Sci. Transl Med.* 8, 324ps325 (2016).
234. Paoloni, M. et al. Canine tumor cross-species genomics uncovers targets linked to osteosarcoma progression. *BMC Genomics* 10, 625 (2009).
235. Dow, S. A role for dogs in advancing cancer immunotherapy research. *Front. Immunol.* 10, 2935 (2020).

## Figure legends

**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<sup>201</sup>.

**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. Reprinted with permission from ref.<sup>16</sup> (Wiley).

**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 the malignant transformation of cells within the osteoblastic lineage at any stage of differentiation, which is controlled by numerous cellular signalling pathways (for example, Notch, Wnt and RTK) that can initiate uncontrolled proliferation<sup>202,203,204,205</sup>.

**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 in OS cell proliferation and altered bone remodelling. In addition, OS cells activate local mesenchymal stem cells (MSCs) by producing extracellular vesicles (EVs) containing TGF $\beta$ , 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 : Potential targets for osteosarcoma treatment.** Tyrosine kinase inhibitors (TKIs) can block multiple tyrosine kinase receptors with individual differences in binding affinities,

ultimately blocking downstream intracellular growth signals. Surface targets for the following proteins include HER2: Antibody, ADC, CAR T; GD2: Antibody, CAR T; B7H3: CAR T; EGFR: CAR T. Inhibitors of DNA damage repair include inhibitors of WEE1, PARP and ATR. These all represent therapeutic approaches in clinical development, ongoing clinical trials or completed clinical trials. ADC, antibody–drug conjugate; CAR, chimeric antigen receptor.

**Figure 6 : 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,e**, Bone scintigraphy showing lytic, metastatic osteosarcoma lesions. **f**, MRI of the humerus showing osteosarcoma soft tissue and intramedullary extensions. **g**, CT image of lung metastases of varying sizes. **h**, A ‘sunburst’ and multilaminar ‘onion skin’ periosteal reaction is present along the length of the lesion with Codman triangles, consistent with osteosarcoma.

**Figure 7 : Osteosarcoma histology.** Representative haematoxylin and eosin-stained osteosarcoma histology images of a malignant spindle cell tumour producing osteoid. **a**, Osteoblastic osteosarcoma. Atypical pleomorphic cells with osteoid. **b**, Chondroblastic osteosarcoma. Heterogeneous tumour 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. Magnification: **a**,  $\times 200$ ; **b**,  $\times 100$ ; **c**,  $\times 200$ ; **d**,  $\times 200$ ; **e**,  $\times 40$  (inset  $\times 200$ ); and **f**,  $\times 100$ .

**Figure 8 : Osteosarcoma treatment algorithm.** Patients with suspected osteosarcoma require referral to a specialist centre with expert pathology, imaging review panel and multidisciplinary discussion to confirm management. Low-grade and intermediate-grade osteosarcomas 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 unresectable or systemic relapse. Multi-target tyrosine kinase inhibitors (TKIs) have demonstrated activity in phase II clinical trials and may offer benefit in this setting. Entry into clinical trials is advised if possible. FDG, fluorodeoxyglucose. <sup>a</sup>Surgery can be considered upfront, followed by adjuvant chemotherapy. <sup>b</sup>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.

### **Box 1 : 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<sup>206,207</sup> and high-throughput screens to identify osteosarcoma vulnerabilities<sup>92,208</sup>. Companion cell lines with enhanced lung colonization capacity, such as SaOS2-LM7, MG63.3 and 143B<sup>209,210,211</sup>, have enabled the study of metastasis-driving mechanisms<sup>50,212</sup>. Cultures derived from mice with spontaneous osteosarcoma<sup>210</sup> or from genetically engineered mouse models<sup>100,213</sup> are available to study disease biology or therapeutics in mice with intact immune systems.

#### **Ex vivo organ cultures**

Ex vivo culture systems are useful to study tumour cells growing within an intact lung environment<sup>214,215</sup>. These techniques have been adapted to study tumour–host interactions, to screen for metastasis-related vulnerabilities and to validate hits identified in other screens<sup>74,75,92,216,217,218,219</sup>.

#### **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<sup>220,221</sup> led to the development of systems for propagating osteosarcoma tumours<sup>220,221</sup> 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<sup>180,183,222</sup> as they have clinical and molecular

features that are quite representative of the human disease<sup>223,224,225,226</sup>. Although prolonged passage in mice can alter the behaviour of tumours maintained as PDXs<sup>227</sup>, strategic use of low-passage PDX lines limits this mouse-specific evolution and is a useful tool in the study of osteosarcoma biology<sup>228</sup>, precision medicine approaches and preclinical validation of therapeutic candidates.

### **Genetically engineered mouse models**

Many insights into the origins<sup>29</sup> and pathophysiology<sup>212</sup> of osteosarcoma have come from mouse models engineered to develop osteosarcoma<sup>229</sup>. 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 Fos<sup>230</sup>.

### **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<sup>231</sup>. Canine osteosarcoma has histological, genetic and clinical features nearly identical to the human disease<sup>232</sup> but has a much higher incidence, with an estimated 25,000 new cases of canine disease occurring each year<sup>232</sup>. Large clinical trial networks facilitating multi-institutional studies are well established<sup>233</sup>. This integrated approach has been particularly promising for the evaluation of anti-metastatic<sup>99,234</sup> and immune-based therapeutics<sup>69,235</sup>.

**Table 1 Cancer predisposition syndromes associated with osteosarcoma**

Syndrome	Gene	Inheritance pattern	Ref.
Li–Fraumeni syndrome	<i>TP53</i>	Autosomal dominant	13
Retinoblastoma	<i>RBI</i>	Autosomal dominant	121
Rothmund–Thomson syndrome	<i>RECQL4</i>	Autosomal recessive	20
Baller–Gerold syndrome	<i>RECQL4</i>	Autosomal recessive	20
RAPADILINO	<i>RECQL4</i>	Autosomal recessive	20
Werner syndrome	<i>RECQL2 (WRN)</i>	Autosomal recessive	21
Bloom syndrome	<i>RECQL3 (BLM)</i>	Autosomal recessive	21
Diamond–Blackfan anaemia	>12 different ribosomal protein genes and <i>GATA1</i>	Autosomal dominant	19

Adapted from ref.200 (Springer Nature Limited).

**Table 2 Response to MTKIs in relapsed or refractory osteosarcoma**

Agent	Patients evaluated, number	Objective response, number (percentage)	Median PFS, months (95% CI)	4-month PFS (95% CI)	Median OS, months (95% CI)	Ref.
Sorafenib	35	3 (8%)	4 (2–5)	0.46 (28–63%)	NR	12
Apatinib	37	16 (43%)	4.5 (3.5–6.3)	0.57 (39–71%)	9.9 (8–18.9)	159
Lenvatinib	26	2 (7%)	3 (1.8–5.4)	0.29 (14–48%)	10 (5.6–12.3)	160
Cabozantinib	42	5 (12%)	6.7 (5.4–7.9)	0.71 (55–83%)	10.6 (7.4–12.5)	158
Regorafenib (REGOBONE)	26 (regorafenib)	2 (8%)	16.4 (8–27)	0.46 (28–63%)	11.3 (5.9–23.9)	161
	12 (placebo)	0 (0%)	4.1 (3–15.7)	0%	5.9 (1.3–16.4)	
Regorafenib (SARCO24)	22 (regorafenib)	3 (14%)	3.6 (2–7.6)	44%	11 (4.7–26.7)	157
	20 (placebo)	NR	1.7 (1.2–1.8)	0%	13.4 (8.5–38.1)	

MTKIs, multi-target tyrosine kinase inhibitors; NR, not reported; OS, overall survival; PFS, progression-free survival.