Translational biology of osteosarcoma

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Abstract | For the past 30 years, improvements in the survival of patients with osteosarcoma have been mostly incremental. Despite evidence of genomic instability and a high frequency of chromothripsis and kataegis, osteosarcomas carry few recurrent targetable mutations, and trials of targeted agents have been generally disappointing. Bone has a highly specialized immune environment and many immune signalling pathways are important in bone homeostasis. The success of the innate immune stimulant mifamurtide in the adjuvant treatment of non-metastatic osteosarcoma suggests that newer immune-based treatments, such as immune checkpoint inhibitors, may substantially improve disease outcome.

Metaphyseal growth plate
The wide portion of the long bone between the narrow diaphysis and the epiphysis that grows during childhood.

Osteoid
This is the organic un-mineralized portion of the bone matrix composed primarily of type I collagen that is secreted by osteoblasts prior to maturation of bone tissue.

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Osteosarcoma, the most common primary malignant tumour of bone, has an incidence worldwide of approximately one to three cases annually per million. It arises primarily in children and adolescents, with a second peak in incidence in those over the age of 50 (REF. 1). Risk factors and established genetic syndromes associated with osteosarcoma are shown in BOX 1. Osteosarcomas occur in the long bones of the limbs, near the metaphyseal growth plate. Common sites include the femur, the tibia and the humerus, and less commonly the skull, the jaw or the pelvis. Osteosarcomas are composed of malignant osteoblasts producing immature bone or osteoid tissue and can be subdivided histologically into conventional, low-grade central, periosteal, parosteal, telangiectatic, chondroblastic and small cell forms. Some of these histological forms have distinct molecular and biological behaviour[1].

The mainstay of curative osteosarcoma treatment is surgery. However, the survival of patients with osteosarcoma treated with surgery alone is approximately 15–17% (REFS 2,3). In the early 1970s, high-dose methotrexate (HDMTX), and vincristine followed by folinic acid, was introduced as adjuvant chemotherapy to facilitate surgical resection, tripling survival rates for patients with non-metastatic disease[4]. Current therapies incorporate surgical resection and combinational chemotherapy (doxorubicin and cisplatin with or without methotrexate), which cures ~70% of patients. In patients with localized disease, response to preoperative combination chemotherapy is the strongest predictor of overall survival[5]. However, survival for patients with metastatic or relapsed osteosarcoma has remained virtually unchanged over the past 30 years, with an overall 5-year survival rate of about 20% (REFS 3,6). New therapies are needed. In this Review, we discuss normal bone biology relevant to osteosarcoma, including the immunobiology of bone, model systems for studying osteosarcoma, genetic and genomic studies on germline predisposition and tumour landscapes, and recent clinical trials.

Bone development

Bone is a specialized connective tissue that supports and protects muscles and vital organs, allows leverage and mobility, provides a microenvironment for haematopoietic tissue and stores minerals[7]. Bone is composed of two cell types, which are responsible for bone formation and remodelling. Bone-forming cells, or osteoblasts, are mesenchymal cells that are located within bone marrow stroma and at periosteal surfaces. Mesenchymal stem cells (MSCs), which are capable of giving rise to multiple connective tissue lineages, give rise to preosteoblasts, which express osteoblast markers, such as alkaline phosphatase, parathyroid hormone receptor and type I collagen[8]. These cells exhibit a limited capacity for self-renewal *in vitro*. The mature osteoblasts, which express RUNX-related transcription factor 2 (RUNX2), osterix (OSX; also known as SP7), osteopontin, bone sialoprotein and osteocalcin[9], synthesize and lay down precursors of type I collagen, which comprises 95% of the organic matrix of bone osteoid. The terminal stage of the bone cell lineage is the post-mitotic osteocyte embedded within mineralized osteoid. Osteosarcomas have many characteristics of immature osteoblasts[10]. Bone resorption is accomplished by osteoclasts, which can be considered to be highly specialized macrophages. Osteoclasts are typically large, multinucleated cells that are located on bone surfaces. They are derived from the monocyte lineage, and, like macrophages, have phagocytic-like mechanisms[11]. Osteoclast differentiation and function is tightly regulated by local signals that are secreted by osteoblasts, the most important of which are receptor

Box 1 | **Risk factors for osteosarcoma**

Bone turnover, age, height and gender

- Rapid bone turnover and growth, particularly during puberty (10–14 years old)[153].
- Taller stature has also been correlated with increased risk[154].
- Slightly higher incidence in boys (56%) compared with girls (42%), but tumours occur earlier in girls than in boys, corresponding to differences in rate of skeletal growth[154].

Environment

- Radiation to bone. Most secondary osteosarcomas arise owing to ionizing radiation, with or without chemotherapy. Radiation is thought to cause up to 3% of osteosarcomas, some of which can appear up to 30 years after radiation exposure[155]. Radiation-induced osteosarcoma is dose-dependent and its incidence is increasing as more patients survive following irradiation for the treatment of other primary tumours[156].

Bone diseases

- Paget's disease of bone is characterized by extensive bone remodelling that results in enlarged and weakened bone tissue and mostly affects those older than 50 years of age[157]. Approximately 1% of individuals with Paget's disease will develop osteosarcoma[158], and although usually sporadic, familial cases have been linked to mutations in sequestosome 1 (*SQSTM1*)[159].

Heritable syndromes that predispose to osteosarcoma

- Li–Fraumeni syndrome germline mutations in the tumour suppressor *TP53* result in a rare autosomal dominant disorder[160], in which up to 12% of patients develop osteosarcoma[161].
- Germline mutations in the *RB1* tumour suppressor gene, a key regulator of cell cycle progression, increase the incidence of osteosarcoma several hundred-fold[162].
- Rare autosomal recessive syndromes caused by mutations in the RecQ DNA helicase family also predispose to osteosarcoma. DNA helicases are required for DNA replication and repair and are important for genomic stability[163]. Rothmund–Thomson syndrome (RTS) is caused by mutations in the helicase RecQ protein-like 4 (*RECQL4*)[164] and, of the four syndromes that involve RecQ helicases, is the most strongly associated with osteosarcoma predisposition, with up to 32% of affected individuals developing osteosarcoma[165]. Werner's syndrome (WRN; also known as progeria) is a premature aging syndrome caused by mutations in the WRN helicase *RECQL2* and is characterized by abnormal telomere maintenance and chromosomal rearrangements[166]. Approximately 10% of patients (19 of 189) with WRN syndrome developed osteosarcoma[167]. Bloom's syndrome (BLM) is a disorder characterized by extremely short stature and is caused by mutations in BLM 3′–5′ DNA helicase belonging to the RecQ family. Approximately 3% of patients (2 of 168) developed osteosarcomas[168]. RAPADILINO syndrome results from mutations in *RECQL4*. Of the 15 cases of RAPADILINO syndrome reported, 13.3% (2 of 15) developed osteosarcomas[169].

Conventional
Conventional osteosarcomas are primary intramedullary high-grade malignant tumours in which neoplastic cells produce osteoid.

Low-grade central
Low-grade central osteosarcomas arise from the medullary cavity of bone and are composed of hypo-cellular to moderately cellular fibroblastic stroma with variable amounts of osteoid.

Periosteal
Periosteal osteosarcoma is an intermediate-grade chondroblastic osteosarcoma that occurs on the surface of the metaphysis of long bone.

activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG; see below). Osteoclast differentiation and function is also indirectly modulated by circulating hormones, such as the insulin-like growth factors (IGFs), parathyroid hormone and parathyroid hormone-like protein[12] (FIG. 1).

Genome-wide association studies

Several heritable genetic predisposition syndromes are associated with a small percentage of osteosarcomas. Genes identified as having a role include *TP53*, *RB1* and RecQ DNA helicase family members (BOX 1). Genomic technologies are starting to give more insights into the genetic basis for osteosarcoma. Genome-wide association studies (GWAS) using single nucleotide polymorphism (SNP) arrays have mapped the contribution of common variants to cancer risk, typically with lower penetrance (<1.5-fold relative risk) than observed in familial cancer syndromes[13]. The first large-scale GWAS applied to osteosarcoma included 941 affected individuals and 3,291 cancer-free controls. The investigators identified two novel susceptibility loci in osteosarcoma: glutamate receptor, metabotropic 4 (*GRM4*) located at 6p21.3 and a gene desert at chromosome 2p25.2 (rs7591996 and rs10208273)[14]. GRM4 has a role in cyclic AMP (cAMP) signalling, which could be relevant to bone tumorigenesis. Glutamate signalling has been described in regulating bone physiology[15], and GRM4 is overexpressed in colorectal cancer and is associated with increased tumour recurrence[16]. In support of a role for glutamate signalling, a GWAS study carried out in large-dog breeds identified glutamate receptor ionotropic, kainite 4 (*GRIK4*) as being associated with osteosarcoma development[17]. Mouse studies recently showed that cAMP-dependent protein kinase A (*Prkar1a*) functions as a tumour suppressor in osteosarcoma[18]. Parathyroid hormone is used to increase bone mass via cAMP signalling, and induced osteosarcoma in Fisher 344 rats[19]. Although to date there is no evidence for an increased risk of osteosarcoma in patients treated with recombinant parathyroid hormone, these observations led the US Food and Drug Administration to voice its concern and to make recommendations limiting the use of parathyroid hormone in patients with osteoporosis[15,16].

Somatic genetic and epigenetic features

Rarity and genomic complexity, as well as intratumoural and intertumoural heterogeneity, have presented challenges to the molecular characterization of osteosarcomas. Apart from parosteal osteosarcoma — an indolent subtype of osteosarcoma that is characterized by episomal ring neochromosomes containing high-level amplification of *MDM2* and cyclin-dependent kinase 4 (*CDK4*)[20] — conventional high-grade osteosarcomas are generally genomically unstable tumours with complex chaotic karyotypes[21]. Osteosarcomas are characterized by chromosomal instability, in which chromosomes or parts of chromosomes are duplicated or deleted, with high levels of somatic structural variations and copy number alterations[22–26]. Recurrent regions of amplification and DNA copy number gain, as well as of DNA deletion or loss of heterozygosity, are described in TABLE 1. Some of these regions contain candidate driver genes with biological evidence for a role in osteosarcoma development, and they represent potential therapeutic targets (TABLE 1). Somatic mutations in both *TP53* (REF. 27) and *RB1* (REF. 28) are the most frequently reported and have recently been verified again using whole-genome sequencing (WGS)[23]. Both *TP53* alleles were mutated in up to 80% of tumours examined and, interestingly, most *TP53* mutations were structural variations in intron 1 (REF. 23). Other mutated genes include RecQ protein-like 4 (*RECQL4*; which encodes a RecQ helicase) and *RUNX2* (TABLE 1). Another contributor to genomic instability is the alternative lengthening of telomeres (ALT) — a homologous recombination-dependent mechanism that prevents telomere shortening and induces senescence[29]. Longer telomeres are

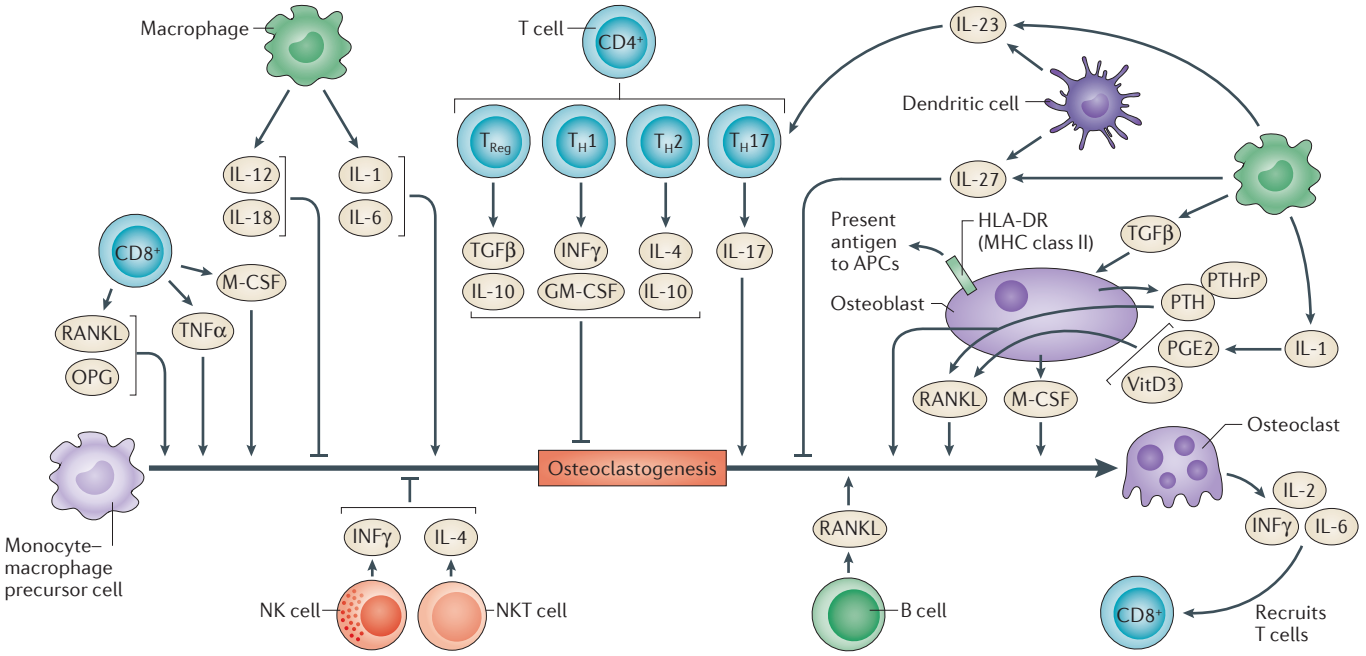

Figure 1 | **Osteoclast, osteoblast and immune cell crosstalk.** Crosstalk between cells of bone lineage and immune cells is common. Osteoclast formation requires receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). RANKL is produced by osteoblasts in response to 1,25 dihydroxy vitamin D3 (VitD3) and prostaglandin E2 (PGE2) and by activated T cells to regulate osteoclast differentiation. Osteoblasts express the parathyroid hormone (PTH) receptor when bound to the ligand parathyroid hormone related protein (PTHrP). The PTH receptor can activate osteoclast activity by increasing RANKL expression. M-CSF is produced by immune cells and stimulates RANK expression in monocyte–macrophage osteoclast precursor cells. Factors secreted by immune cells can promote or suppress osteoclast formation. Osteoclasts can secrete cytokines to facilitate T cell recruitment and activation. Transforming growth factor-β (TGFβ) produced by macrophages can induce osteoblast proliferation, block apoptosis and recruit osteoblast precursors. Osteoblasts can also express major histocompatibility complex II (MHC class II) to present antigen. APCs, antigen-presenting cells; GM-CSF, granulocyte–macrophage colony stimulating factor; HLA-DR, human leukocyte antigen d-related; IL, interleukin; IFNγ, interferon-γ; NK, natural killer; NKT, natural killer T; OPG, osteoprogerin; T_H, T-helper; TNFα, tumour necrosis factor-α; T_{Reg}, regulatory T.

Parosteal
Parosteal osteosarcoma is a low-grade tumour that originates from the outer surface of the periosteum.

Telangiectatic
Telangiectatic osteosarcoma occurs in the metaphyseal portion of the long bones. It is characterized by dilated blood-filled vascular spaces lined by malignant osteoblasts.

Chondroblastic
In chondroblastic osteosarcoma, chondroid matrix is predominant, with minimal amounts of osseous matrix.

Small cell
Small cell osteosarcoma is composed of small cells with variable degrees of osteoid production.

Periosteal surfaces
Thick membranes composed of fibrous connective tissue that wraps around all bone except for the articulating surfaces in joints.

common in osteosarcoma, and ALT has been found in 85% of tumours (12 of 14 tumours)[23] and is associated with poor clinical outcomes[30].

To date, the search for common molecular therapeutic targets in osteosarcoma has been disappointing. However, the genomic chaos that is characteristic of osteosarcoma is shedding light on new mutation patterns through recent WGS studies. Approximately 33% of primary osteosarcomas (3 of 9) examined showed evidence of chromothripsis, compared to 2–3% of cancers overall[31]. One-half of all osteosarcomas exhibit patterns of localized hypermutation that are termed kataegis[23]. The regions that are affected by kataegis are not recurrent, and the majority of mutated genes are not located in these regions[23]. Taken together, it is likely that emerging data from next-generation sequencing studies will uncover new patterns within the apparent disorder of the osteosarcoma genome and may even identify new potential therapeutic targets.

Epigenetic modifications undoubtedly also have a role in the development of osteosarcoma, with both hypermethylation and hypomethylation observed (TABLE 1). The importance of histone modifications is suggested by the response of osteosarcoma cell lines to histone deacetylase inhibitors (HDACis)[32,33], and treatment with demethylating agents and/or deacetylating agents has been suggested[34] for use in radiation-resistant tumours[34,35]. A Phase I and II clinical trial (ClinicalTrials.gov Identifier NCT01422499) is currently investigating safety and efficacy of the HDACi vorinostat in several paediatric tumours, including osteosarcoma. MicroRNAs (miRNAs) are non-coding RNAs that regulate gene expression. Similar to protein-coding genes, the expression of miRNA is regulated by both genetic and epigenetic mechanisms. Several miRNAs, including miR-133b, miR-100, miR-340 and miR-214, have been reported to be differentially regulated in osteosarcoma cell lines and/or primary tumours compared with normal bone (Supplementary information S1 (table)). However, evidence for mutations affecting miRNAs that are relevant in osteosarcoma development is limited.

Translational biology and clinical trials

Several pathways that have been targeted in osteosarcoma are outlined in FIG. 2 and discussed below. Recently completed and current clinical trials in osteosarcoma are listed in TABLE 2. A discussion of model systems for

studying osteosarcoma preclinically is beyond the scope of the current manuscript but is briefly summarized in Supplementary information S2, S3 (tables).

Targeting the bone microenvironment. The role of osteoclasts in driving osteosarcoma tumorigenesis is still controversial[36]. Normally, bone is removed by osteoclasts and new bone is synthesized by osteoblasts. The balance of bone homeostasis is disturbed in osteosarcomas, and most bone lesions are osteolytic. Osteosarcomas secrete osteoclast-stimulating cytokines that stimulate bone resorption, while tumour growth is supported by factors that are released during osteolysis. Two key factors that are required for osteoclast differentiation are RANKL, a tumour necrosis factor (TNF) receptor family member, and macrophage colony-stimulating factor (M-CSF). RANKL is required for osteoclast formation and function[37]. In a small study, high expression of RANKL by osteosarcoma cells was associated with poor response to preoperative chemotherapy and inferior patient survival[38]. In rodent models of osteosarcoma, small interfering RNAs (siRNAs) targeting RANKL had no effect on tumour growth, but they may potentiate the use of chemotherapy[39].

Bisphosphonates are a class of drugs that prevent bone loss by inhibiting osteoclast development and function. Functionally, bisphosphonates have diverse growth inhibitory effects on tumour cells and can induce apoptosis, inhibit cell proliferation and downregulate vascular endothelial growth factor A (VEGFA) and VEGF receptor 1 (VEGFR1) expression. However the role of these drugs in suppressing osteosarcoma is controversial. The bisphosphonate zoledronic acid suppressed tumour growth and lung metastasis, and it prolonged overall survival in osteosarcoma-bearing mice[40]; it also enhanced tumour regression when combined with chemotherapeutics[41]. Bisphosphonates can also suppress tumour-induced angiogenesis in mouse models of osteosarcoma and inhibit expression of VEGFR2 expression by endothelial cells[42]. A Phase II clinical trial (ClinicalTrials.gov Identifier NCT00586846) investigated the bisphosphonate pamidronate and found that it could be safely incorporated with chemotherapy and may improve the durability of limb reconstruction following surgical resection of osteosarcoma[6]. The role of bisphosphonates in the adjuvant treatment of osteosarcoma is currently the subject of an ongoing study investigating zoledronic acid in combination with chemotherapy (ClinicalTrials.gov Identifier NCT00691236). Osteosarcomas are also being clinically targeted using bone-seeking radiopharmaceuticals. Samarium-153 lexidronam (153Sm-EDTMP) may improve local control of unresectable osteosarcoma. A Phase II study using 153Sm-EDTMP and peripheral blood stem cell support in 22 patients with high-risk osteosarcoma (ClinicalTrials.gov Identifier NCT00245011) found that progression free survival (PFS) was 60 days with no overall survival benefit[43]. This limited response was probably due to the stage of the disease. Phase I and II clinical trials are currently active in treating patients with osteosarcoma with intravenous radium-223 dichloride (ClinicalTrials.gov Identifier NCT01833520).

Hedgehog, Notch and WNT pathways. Signalling pathways that are involved in normal bone development, such as Hedgehog (Hh), Notch and WNT pathways, have been implicated in osteosarcoma development. Hh signals through the Patched (PTCH) receptor to relieve inhibition of the smoothened (SMO) receptor, activating the GLI family of transcriptional regulators. Indian hedgehog (IHH), PTCH1 and glioma-associated oncogene homologue 1 (GLI1) are highly expressed in many primary osteosarcomas[44]. Expression of GLI2, a transcriptional target of Hh signalling, correlates with poor outcome in patients, and siRNA knockdown of GLI2 increased the sensitivity of osteosarcoma cell lines to chemotherapeutic drugs[45]. Preclinical investigation in mice of the SMO antagonist IPI-926 (saridegib) found that treatment decreased the growth of one of four patient-derived xenografts (PDXs) that were tested[46]. The expression of Notch genes (*NOTCH1*, *NOTCH2* and *NOTCH3*) has been associated with a more aggressive metastatic osteosarcoma phenotype[47]. Preclinical testing of RO4929097 (a γ-secretase Notch pathway inhibitor) in six PDXs consistently inhibited osteosarcoma growth[48]. Crosstalk between the Hh, Notch and WNT pathways might underpin therapeutic resistance. With this rationale, a Phase I and II clinical trial (ClinicalTrials.gov Identifier NCT01154452) is currently underway, investigating targeting both the Notch pathway with RO4929097 and the Hh pathway with vismodegib, a cyclopamine-based competitive antagonist of SMO. WNTs have increased activity in human sarcomas, including osteosarcoma[49,50]. Both dickkopf 3 (DKK3) and WNT inhibitory factor 1 (WIF1) are secreted antagonists of the WNT pathway and are downregulated in osteosarcoma. Re-expression of DKK3, or treatment with recombinant DKK3 protein, suppressed tumour growth in a mouse model of osteosarcoma[51]. *WIF1* is epigenetically silenced in osteosarcoma and preclinical studies show that treatment with recombinant WIF1 can suppress osteosarcoma tumour cell growth *in vitro* and *in vivo*[50,52,53]. However, targeting signalling pathways such as Hh, Notch and WNT may be problematic in children, given the role of these pathways in skeletal development.

Targeting receptor tyrosine kinases. Several therapeutically targetable kinases or their ligands are overexpressed in osteosarcoma, including VEGF, IGF1, platelet-derived growth factor (PDGF), HER2 (also known as ERBB2) and MET. High levels of VEGF correlate with progression and poor survival in osteosarcoma[54], and a recent Phase II study (ClinicalTrials.gov Identifier NCT00889057) using sorafenib, a pan receptor tyrosine kinase inhibitor whose targets include VEGFR2 and VEGFR3, in unresectable high-grade osteosarcoma yielded some durable responses[55]. PDGF and PDGF receptors (PDGFRs) are overexpressed in 70–80% of osteosarcomas[56]. Pazopanib, a multikinase inhibitor targeting KIT, VEGF receptors, fibroblast

Alternative lengthening of telomeres
(ALT). A mechanism used by 10–15% of cancer cells to counteract telomere attrition that accompanies DNA replication and finite replicative potential. ALT uses homologous recombination to maintain telomere length throughout many cell doublings in the absence of telomerase activity.

Chromothripsis
A genomic phenomenon in which a single catastrophic event results in massive genomic rearrangements and remodelling of a chromosome.

Kataegis
Kataegis is defined by patterns of localized hypermutation colocalized with regions of somatic genome rearrangements.

Table 1 | **Selected candidate oncogenes and tumour suppressor pathways in human osteosarcoma***

Genes	Names	Location	Event	Frequency	Refs
Tumour suppressors					
TP53	Tumour protein p53	17p13.1	Del	29–42%	23,27,170
			LOH	29–42%	
			Mut	80–90%	23
RB1	Retinoblastoma 1	13q14.2	LOH	19–67%	23,28
			Mut	10–39%	
RECQL4	RecQ protein-like 4	8q24.4	Mut	<5%	171,172
			Gain	33%	
P14 (also known as *CDKN2A* and *ARF*)	Cyclin-dependent kinase inhibitor p14	9p21	Del	5–21%	173,174
			Hypermeth	NA	
P15 (also known as *CDKN2B* and *INK4B*)	Cyclin-dependent kinase inhibitor p15	9p21	Del	5–21%	175
			Hypermeth	NA	
P16 (also known as *CDKN2A* and *INK4A*)	Cyclin-dependent kinase inhibitor p16	9p21	Hypermeth	NA	176
BUB3 and *FGFR2*	Budding uninhibited by benzimidazoles 3 homologue and fibroblast growth factor receptor 2	10q26	LOH	60%	177,178
APC	Adenomatous polyposis coli	5q21	LOH	62%	177
LSAMP	Limbic system-associated membrane protein	3q13.31	Del or LOH	54%	23,179, 180
			Hypermeth	NA	
WWOX	WW domain containing oxidoreductase	16q23.1-q23.2	Del	30%	181
ATRX	α-thalassaemia/mental retardation syndrome X-linked	Xq21.1	Mut	30%	23
DLG2	Discs large homologue 2	11q14.1	Mut	52%	23
PTEN	Phosphatase and tensin homologue	10q23.3	Del	44%	23,182
HIC1	Hypermethylated in cancer 1	NA	Hypermeth	17%	183,184
WIF1	WNT inhibitory factor 1	NA	Hypermeth	NA	52
TSSC3	Pleckstrin homology-like domain, family member A	NA	Hypermeth	NA	185
RASSF1A	RAS association (RalGDS/AF-6) domain family member 1	NA	Hypermeth	NA	186
GADD45	Growth arrest and DNA damage-inducible protein 45	NA	Hypermeth	NA	187
ESR	Oestrogen receptor 1	NA	Hypermeth	NA	174
AKAP12, *CXCL5*, *EFEMP1* and *IL11RA*	A kinase (PRKA) anchor protein 12, CXC-chemokine ligand 5, EGF-containing fibulin-like matrix protein 1 and interleukin-11 receptor-α	NA	Hypermeth	NA	188
Oncogenes					
CDK4	Cyclin-dependent kinase 4	12q14	Amp	8%	189
			Hypermeth	NA	
MDM2	MDM2 p53 binding protein homologue	12q15	Amp	3–25%	170
PRIM1	Primase DNA polypeptide 1	12q13	Amp	41%	190
MYC	V-MYC myelocytomatosis viral oncogene homologue	8q21.4	Amp	7–10%	23
MET	MET protooncogene	7q31	Del	41%	177
TWIST	Twist homologue 1	7p21	Amp	14%	191
			Del	32%	
PMP22, *MAPK7* and *TOP3A*	Peripheral myelin protein 22, mitogen-activated protein kinase 7 and topoisomerase 3A	17p11.2	Amp	13–29%	192
RUNX2	RUNX-related protein 2	6p21.1	Amp, Mut or Hypermeth	18–55%	23,25, 188,193
VEGFA	Vascular endothelial growth factor A	6p21.1	Amp	60%	193,194
CDC5L and *CCND3*	Cell division cycle 5-like and cyclin D3	6p21.1	Amp	18%	195

Table 1 (cont.) | **Selected candidate oncogenes and tumour suppressor pathways in human osteosarcoma***

Genes	Names	Location	Event	Frequency	Refs
Oncogenes (cont.)					
CCNE1	Cyclin E1	19q12	Amp	NA	23,196
NCOR1	Nuclear receptor co-repressor 1	17p11.2	Amp	NA	23
UBB	Ubiquitin B	17p11.2	Amp	NA	23
COPS3	COP9 signalosome subunit 3	17p11.2	Amp	31%	23,197
H19 and *PHLDA2*	H19 imprinted maternally expressed transcript and pleckstrin homologue-like domain, family member A	NA	Hypometh	NA	198,199
IGF2	Insulin-like growth factor 2	NA	Hypometh	NA	199

Amp, amplified; Del, deleted; Hypermeth, hypermethylated; Hypometh, hypomethylated; LOH, loss of heterozygosity; Mut, mutated; NA, not applicable.
*We apologize to those investigators whose work on the genes described was not cited owing to space limitations.

growth factor receptor (FGFR) and PDGFR, has shown activity in preclinical mouse models[57], and a Phase II clinical trial of pazopanib (ClinicalTrials.gov Identifier NCT01759303) is currently recruiting patients with primary osteosarcoma and metastatic osteosarcoma.

Overexpression of HER2 protein has been noted in osteosarcoma using immunohistochemistry, and it seems to be associated with worse clinical outcomes[58]. However, unlike HER2-positive breast cancers, which respond well to trastuzumab (a monoclonal antibody that binds to HER2), *HER2* is not amplified in osteosarcomas[59]. Furthermore, other studies have challenged the prognostic value of HER2 in osteosarcoma[60]. This disparity may account for the outcome of a Phase II clinical trial (ClinicalTrials.gov Identifier NCT00023998) incorporating trastuzumab. Despite intensive chemotherapy and treatment with trastuzumab, the outcome for patients with metastatic disease was poor and did not correlate with HER2 expression[61]. The IGF1 receptor (IGF1R) has a role in osteoblast proliferation[62]. The dual insulin receptor and IGF1R inhibitor OSI-906 inhibited growth of osteosarcoma cell lines[63], and cixutumumab, a human monoclonal antibody that binds to IGF1R, inhibited the growth of osteosarcoma xenografts[64]. A Phase II clinical trial (ClinicalTrials.gov Identifier NCT00831844) that was carried out using cixutumumab in a range of tumours, including 11 osteosarcomas, showed limited objective single agent activity[65]. Signalling via the MET receptor tyrosine kinase seems to be upregulated in some human osteosarcomas[66]. Preclinical studies in mice showed that the small molecule MET inhibitor crizotinib suppressed the growth of osteosarcoma xenografts and that targeting this pathway in human tumours may be of use[67].

Targeting intracellular signalling molecules. Aberrant activation of the SRC kinase has been implicated in various cancers, including osteosarcoma. Dasatinib, a dual SRC and ABL kinase inhibitor, suppressed the adhesion and migration of osteosarcoma cells *in vivo*[68], and the novel SRC inhibitor SI-83 induced apoptosis in osteosarcoma cell lines *in vitro* and decreased tumour growth *in vivo*[69]. The SRC kinase inhibitor AZD0530 (also known as saracatinib) is currently being investigated in patients with pulmonary metastatic osteosarcoma (ClinicalTrials.gov Identifier NCT00752206).

mTOR has also been of interest[70]. The selective mTOR inhibitor ridaforolimus has been studied in Phase II and III trials as a single agent. Ridaforolimus improved PFS in heavily pretreated advanced sarcomas, including osteosarcoma, with 61 of 214 patients (28.8%) achieving a complete or partial response, or stable disease for more than 16 weeks[71]. However, the objective response rate using Response Evaluation Criteria In Solid Tumours (RECIST) criteria was low, with only two patients with osteosarcoma showing partial responses. These findings were confirmed in a recent international Phase III trial (SUCCEED: Sarcoma Multi-Center Clinical Evaluation of the Efficacy of ridaforolimus; ClinicalTrials.gov Identifier NCT00538239), which aimed to determine whether maintenance therapy with ridaforolimus could prolong PFS in patients with metastatic soft-tissue or osteosarcomas who had previously responded to chemotherapy. The PFS following treatment with ridaforolimus was 17.7 weeks, compared with 14.6 weeks in the placebo group, although overall survival was not significantly different at 15 months[72]. Ridaforolimus seemed to control tumour growth but, as a single agent, intracellular compensatory pathways could limit optimal antitumour activity of mTOR inhibition. Co-targeting mTOR along with other kinases that are known to drive osteosarcoma growth may represent a strategy to address drug resistance. Recent preclinical studies in mice using sorafenib (a small molecular inhibitor of RAF kinase, VEGFR2, VEGFR3, KIT and PDGFR) in combination with the rapamycin analogue everolimus showed complete inhibition of mTOR signalling and impaired tumour growth[73]. A Phase II study investigating these two agents in metastatic and relapsed osteosarcoma is ongoing (ClinicalTrials.gov Identifier NCT01804374).

The Aurora family of protein kinases (Aurora A, B and C) are key regulators of mitosis and the cell cycle and are frequently overexpressed in human cancers[74]. Aurora A and B seem to be overexpressed in human osteosarcomas, and knockdown of these genes using siRNAs in osteosarcoma cell lines reduced cell growth[75]. Osteosarcoma cell lines were also found to be sensitive to the Aurora-targeting drugs VX-680 and ZM447439, which induced hyperploidy and apoptosis[75,76]. A Phase II trial is currently underway investigating

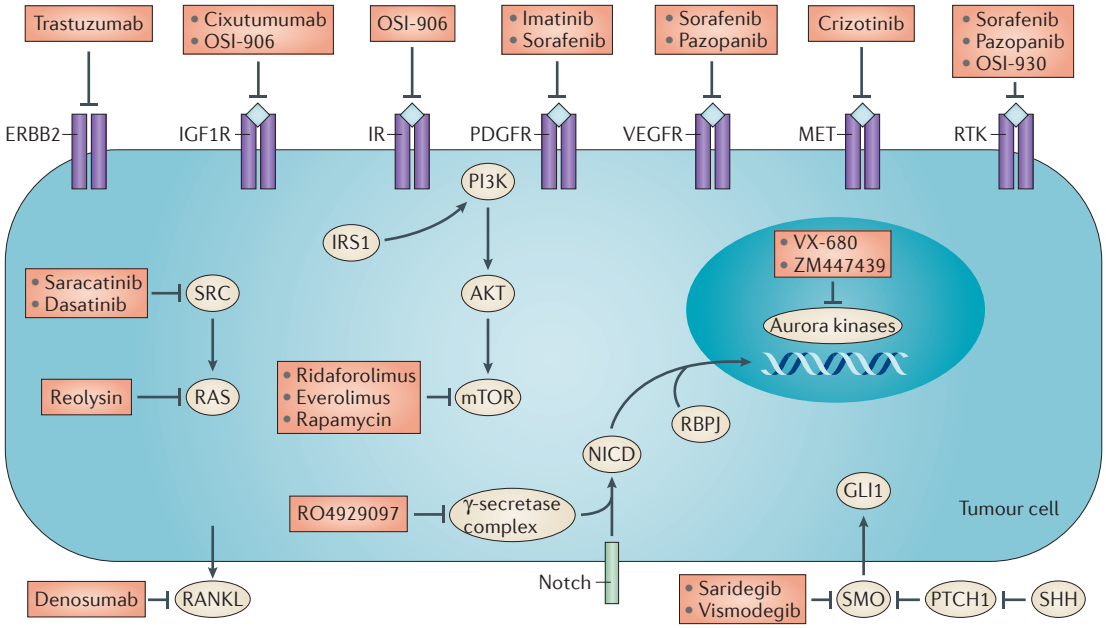

Figure 2 | **Pathways for targeted therapies in osteosarcoma.** This figure schematically shows molecular targets and associated drugs identified for therapeutic intervention in osteosarcoma. Therapeutic targets include specific cell surface receptor tyrosine kinases (RTKs): ERBB2, insulin-like growth factor 1 receptor (IGF1R), insulin receptor (IR), platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and hepatocyte growth factor (HGF) receptor (also known as MET). Alternatively, pan-RTK inhibitors, such as sorafenib, pazopanib and OSI-930, could be used. Other potential targets include PI3K, insulin receptor substrate 1 (IRS1), AKT, mTOR, sonic hedgehog (SHH), smoothened (SMO), Patched 1 (PTCH1), glioma-associated oncogene homologue 1 (GLI1), Notch, Notch intracellular domain (NICD) and recombination signal binding protein for immunoglobulin κJ region (RBPJ). Aberrant activation of these signalling molecules and pathways in osteosarcoma may promote tumour cell proliferation, survival, migration, angiogenesis and/or metastasis.

inhibition of Aurora kinase A in children with a variety of recurrent or refractory solid tumours and leukaemias, including osteosarcoma (ClinicalTrials.gov Identifier NCT01154816).

Immune-targeted therapies. Immunotherapies have recently generated tremendous enthusiasm in the cancer community owing to clinical trials using adoptive cellular therapy for melanoma, cancer vaccines, such as sipuleucel-T, for castration-resistant prostate cancer[77], and the gp100 vaccine in combination with interleukin-2 (IL-2) for advanced melanoma. These therapies exploit the ability of the innate and adaptive immune system to collectively constrain the growth of transformed cells[78,79]. Immunomodulatory strategies, including mifamurtide, have shown clinical promise in the treatment of osteosarcoma (FIG. 3).

Osteosarcomas may represent a special case with respect to immunotherapy. There is crucial crosstalk between bone cells and cells of the immune system, leading to the new interdisciplinary field of osteoimmunology. Many signalling pathways (RANK–RANKL signalling and cytokines such as IL-1, IL-6, IL-17 and transforming growth factor-β (TGFβ)) have roles in both bone and the immune system (FIG. 1). Mice that lack immune-related genes, such as *Tnfsf11* (which encodes RANKL)[80], interferon (α and β) receptor 1 (*Ifnar1*)[81], nuclear factor-κB (*Nfkb*)[82] and interferon-γ (*Ifng*)[83], have altered bone phenotypes[84,85]. Understanding the crosstalk between osteosarcoma cells, osteoclasts and cells of the immune system and how they might drive tumorigenesis is still in its infancy.

Perhaps surprisingly, immunotherapies have a long history in osteosarcoma. More than 100 years ago, the surgeon William Coley used a mixture of heat-killed *Streptococcus pyogenes* and *Serratia marcescens* (Coley's toxin) to treat patients with bone and soft-tissue sarcomas, with debatable success[86,87]. More recently, a study of the treatment of patients with resectable osteosarcoma with adjuvant immunotherapy consisting of bacillus Calmette–Guerin and allogeneic tumour cell vaccine found that 18% (3 of 17) of the patients who received immunotherapy remained alive and disease-free, compared with 0 of the 12 patients who did not receive immunotherapy[88]. Both Coley's toxin and Calmette–Guerin induce systemic expression of several pro-inflammatory cytokines, including IL-6, IL-1β, TNF and IFNγ, and result in acute activation of cytotoxic immune cells and tumour regression[89]. Moreover, patients with osteosarcoma who develop postoperative infections have significantly increased survival rates compared to those without infection[90]. Chemotherapies that are commonly used in the treatment of osteosarcoma (doxorubicin, cisplatin and alkylating agents) elicit immune antitumour activity by killing immunosuppressive regulatory T cells and myeloid-derived suppressor

Table 2 | **Current and recently completed clinical trials in osteosarcoma**

Targets	Treatment	Study*	Phase	Tumour	Status or outcome	Refs
Targeting the bone microenvironment						
Osteoclast	Pamidronate	NCT00586846	II	11 metastatic	EFS (5 years) 45%	6
				29 non-metastatic	EFS (5 years) 72%	
Osteoclast	Zoledronic acid (single agent or adjuvant)	NCT00691236	II and III	High grade	Active	
Osteoclast	Zoledronic acid and combination chemotherapy	NCT00470223	III	High-grade osteosarcoma	Active	
Bone seeking	Samarium (153Sm-EDTMP)	NCT00245011	II	22 relapsed metastatic	• PFS (60 days) 45% • No improved outcome	43
Bone seeking	Radium-223 chloride	NCT01833520	I and II	Recurrent or metastatic	Active	
Notch and Hedgehog pathways	RO4929097 and vismodegib	NCT01154452	I and II	Recurrent osteosarcoma	Active	
Targeting receptor tyrosine kinases						
VEGFR	Sorafenib	NCT00889057	II	35 relapsed unresectable	• Response rate 14% • Disease control rate 49%	55
KIT, VEGFR, FGFR and PDGFR	Pazopanib	NCT01759303	II	Primary and metastatic osteosarcoma	Active	
HER2	Trastuzumab	NCT00023998	II	96 metastatic osteosarcoma	No improved outcome	61
IGF1R	Cixutumumab	NCT00831844	II	11 refractory or relapsed osteosarcomas	Limited single agent activity	65
Targeting intracellular signalling pathways						
SRC	Saracatinib (AZD0530)	NCT00752206	II	Recurrent osteosarcoma localized to lung all resected	Active	
mTOR	Ridaforolimus	NCT00538239	II	Recurrent osteosarcomas	2 partial response	71
			III	Metastatic soft tissue or osteosarcoma	• PFS in treated group 17.7 compared to 14.6 weeks • OS not different at 15 months	
RAF, VEGFR, KIT and mTOR	Sorafenib and everolimus	NCT01804374	II	Relapsed and non-resectable osteosarcoma	Active	
Aurora kinase A	MLN8237	NCT01154816	II	Recurrent osteosarcoma	Active	
Histone deacetylase inhibitor	Vorinostat	NCT01422499	II and III	Paediatric tumours, including osteosarcoma	Active	
Immune-targeted therapies						
Immune system	Mifamurtide	NCT00631631	III	662 non-metastatic	EFS (6 years) 70% to 78%	200
				91 metastatic	OS (5 years) 40% to 53%	99
				205 metastatic recurrent	OS (2 years) 45.9%	200
Immune system	Interferon	Pilot	III	19 non-metastatic	DFS (5 years) 63%	201
	PEG-interferon 2β	NCT00134030	III	1,400 patients	Awaiting results	
Immune system	Inhaled sagramostim (GM-CSF)	NCT00066365	II	43 lung metastasis recurrent	• EFS (2 years) 12.9% • No improved outcome	147
Immune system	Aerosolized proleukin (IL-2)	NCT01590069	I and II	Lung metastasis	Active	
Immune system	Ipilimumab (anti-CTLA4 antibody)	NCT01445379	I	Osteosarcoma, <20 years of age	Active	
Immune system	Anti -GD2 antibody	NCT00743496	I	Relapsed and/or refractory osteosarcoma	Active	

CTLA4, cytotoxic T lymphocyte antigen 4; DFS, disease-free survival; EFS, event-free survival; FGFR, fibroblast growth factor receptor; GD2, disialyl ganglioside; GM-CSF, granulocyte–macrophage colony stimulating factor; IGF1R, insulin-like growth factor 1 receptor; IL-2, interleukin-2; OS, overall survival; PDGFR, platelet-derived growth factor receptor; PEG, pegylated; PFS, progression-free survival; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor. *See ClinicalTrials.gov.

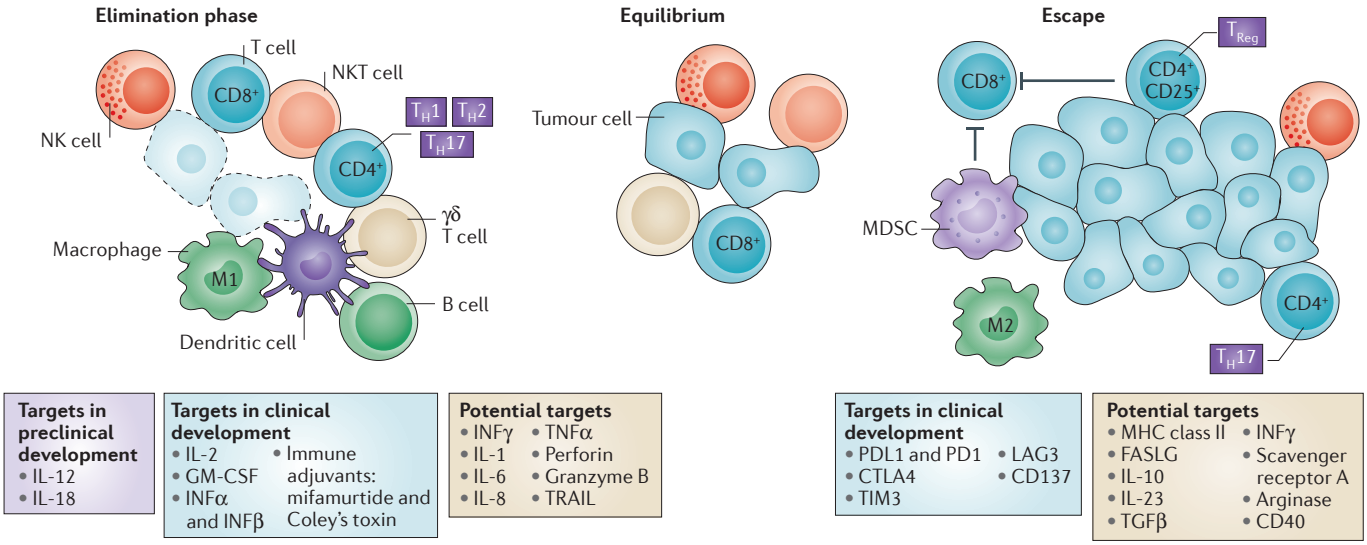

Figure 3 | **Targeting immune modulators in osteosarcoma.** During the elimination phase, cells of the innate and adaptive immune system work to detect and destroy tumour cells and include $CD4^+$ T-helper (T_H1, T_H2 and T_H17) cells, $CD8^+$ cytotoxic T cells, γδT cells, natural killer (NK) cells, natural killer T (NKT) cells; M1 macrophages and dendritic cells. Immune molecules and adjuvants currently being targeted to activate an immune response against osteosarcoma in preclinical or clinical development, as well as potential targets, are listed below. In the equilibrium phase, the tumour is kept in check by the immune system. In the escape phase, the balance between tumour growth and immune response shifts towards tumour growth. Immune cells conferring tolerance to tumour include myeloid derived suppressor cells (MDSCs), regulatory T (T_{Reg}) cells, T_H17 cells and M2 macrophages. Immune molecules being targeted clinically in osteosarcoma to break immune tolerance to tumour, as well as potential targets, are listed below. CTLA4, cytotoxic T cell lymphocyte antigen 4; CD40, TNF receptor superfamily 5; CD137, TNF receptor super family 9; FASLG, FAS ligand; GM-CSF, granulocyte–macrophage colony stimulating factor; LAG3, lymphocyte activation gene 3 protein; IFN, interferon; IL, interleukin; MHC class II, major histocompatibility complex II; TGFβ, transforming growth factor-β; TIM3, hepatitis A virus cellular receptor 2; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; PD1, programed cell death 1; PDL1, programmed cell death ligand 1.

cells (MDSCs) and by activating immune effector cells[91]. In paediatric patients with osteosarcoma, rapid recovery of lymphocyte numbers following chemotherapy was associated with a significantly better prognosis[92].

An immunoadjuvant therapy, mifamurtide, is the most important recent therapeutic advance in osteosarcoma. Mifamurtide is a synthetic lipophilic analogue of muramyl dipeptide, the minimal peptidoglycan motif common to Gram-negative and Gram-positive bacteria that can activate the innate immune system[93]. Mifamurtide-activated monocytes and macrophages are associated with increased serum levels of TNFα, IL-1α, IL-1β, IL-6 and IL-8, as well as with the engagement of other immune cells[94,95]. As noted above, these cytokines have important roles in normal and pathological processes within the bone microenvironment, and IL-6 is rate-limiting for development of osteosarcoma in mouse models[96]. Mifamurtide first demonstrated antitumour activity in dogs with osteosarcoma, with a median survival of 222 days, compared with 77 days for controls[97]. To determine whether the addition of mifamurtide to adjuvant chemotherapy improved outcomes, 662 patients with localized osteosarcoma were treated preoperatively with cisplatin, methotrexate and doxorubicin. Patients were then randomly assigned to receive ifosfamide and/or mifamurtide (INT-0133; ClinicalTrials.gov Identifier NCT00631631). The addition of mifamurtide without ifosfamide trended towards better event-free survival ($p = 0.08$). However, the addition of mifamurtide significantly improved 6-year overall survival from 70% to 78% ($p = 0.03$)[98]. Unexpectedly, this effect was not observed in the arm containing ifosfamide, perhaps owing to drug interaction. The addition of mifamurtide to chemotherapy also trended towards improved event-free and overall survival in patients with metastatic osteosarcoma, without reaching statistical significance[99]. Patients receiving adjuvant mifamurtide had an average additional 2.58 years of life and 2.20 quality-adjusted life years compared with patients receiving chemotherapy alone[99,100]. A combination of the discrepancy between the event-free survival and the overall survival in the adjuvant study, the unexplained interaction with ifosfamide, the costs of therapy and the effect size has led to mixed views from the clinical community, and mifamurtide is currently only approved for use in the European Union.

Type 1 interferons (that is, IFNα and IFNβ) have shown antitumour activity in a range of malignant tumours[101]. IFNα inhibits osteosarcoma cells *in vitro* and enhances sensitivity of osteosarcoma cells to chemotherapeutic agents such as doxorubicin[102,103]. In mouse models of osteosarcoma, IFNα arrested tumour growth[104,105]. A small clinical trial using INFα seemed to improve survival and resulted in partial tumour regression in patients with metastatic osteosarcoma[106]. A recently closed Phase III clinical trial

Quality-adjusted life years
This measure takes into account both the quantity (life expectancy) and the quality of the remaining life years generated by health care interventions.

(The European American Osteosarcoma Study (EURAMOS); ClinicalTrials.gov Identifier NCT00134030) investigated the efficacy of pegylated IFNα that was added to standard chemotherapeutics in treating 1,400 patients with osteosarcoma who had shown good responses to preoperative chemotherapy. Early reports suggest little effect of adjuvant IFNα on survival, but definitive conclusions are limited by short follow up, poor uptake of patients who were randomized to receive IFNα2, and the anticipated poor tolerance by patients of long-term treatment with IFNα2 (REF. 107). Importantly, this trial represented the first transatlantic collaborative clinical trial in osteosarcoma, engaging multiple national trial organizations. This kind of collaboration is crucial for rapid progression in the treatment of rare diseases.

A plethora of immune checkpoints are 'hard-wired' into the immune system and are crucial for the normal maintenance of self-tolerance and for limiting physiological immune responses in peripheral tissues to minimize collateral damage. These same immune checkpoints may allow immune tolerance to tumours. Immune checkpoint blockade inhibitors, such as ipilimumab, a monoclonal antibody (mAb) against cytotoxic T lymphocyte antigen 4 (CTLA4), and mAbs targeting anti-programmed cell death protein 1 (PD1) or PD1 ligand (PDL1), are showing promise in the clinic[77,108]. These inhibitors increase endogenous antitumour activity and might increase the tumour immunogenicity that is induced by treatment with chemotherapy, radiotherapy and targeted therapies[109,110]. In particular, there are opportunities to rationally combine immune checkpoint inhibitors with first-line therapy.

Targeting immune checkpoint pathways may be potentiated by the presence of high-level genomic instability, as observed in osteosarcoma. A significant association between high mutational load and overall response rates to agents targeting PD1 and PDL1 was observed in several tumour types, including melanoma and non-small-cell lung cancer[111]. Exome-guided immunomonitoring of patients treated with immune checkpoint-targeting agents has revealed that genomically complex tumours are producing a large panel of neo-antigens that can drive immune response once tumour tolerance pathways are removed. These neo-antigens may arise from the tumour 'mutanome', as mutations accumulate during tumour development[112]. Initial data suggest that immune checkpoints might be relevant to osteosarcoma. *CTLA4* polymorphisms are associated with higher risk of developing osteosarcoma[113,114]. Tumour lysate-pulsed dendritic cells in combination with an antibody against CTLA4 decreased immunosuppressive regulatory T cells and increased cytotoxic T cells in a mouse model of metastatic osteosarcoma, and this correlated with increased survival[115,116]. Depletion of $CD25^+$ regulatory T cells also suppressed mouse osteosarcoma metastasis[117]. Ligation of PD1 (a TNF receptor family member that is expressed on tumour-specific cytotoxic T lymphocytes (CTLs)) by PDL1 on tumours inhibits CTL proliferation, cytokine production and cytotoxicity, thereby leading to tumour progression[118]. PD1 is expressed on CTLs that infiltrate osteosarcomas, and osteosarcoma cells express PDL1. The inhibition of PD1–PDL1 interactions markedly improves survival outcomes in mouse models of metastatic osteosarcoma[119], and mAbs against PD1 suppress osteosarcoma growth *in vivo*[120]. Other preclinical T cell targets might also be useful. An agonist of T cell activation, CD137 (also known as TNFRSF9)[121], also suppressed osteosarcoma growth in mice, and growth inhibition was increased when antibodies against PD1 and CD137 were used together[120]. To date, blockade of CTLA4, PD1 or PDL1 has not been used in patients with osteosarcoma.

Osteosarcoma-specific antigens have been difficult to identify[122]. Mesenchymal cells lack specific markers and tend to be particularly non-immunogenic[123]. Several antigens are expressed on osteosarcomas, most of which are found in normal tissues[124–127]. As noted above, HER2 is expressed at low levels in osteosarcomas and may be amenable to targeting with genetically modified T cells expressing HER2-specific chimeric antigen receptors[128]. Disialyl ganglioside (GD2) is expressed in 50% of osteosarcomas[129] and might correlate with increased tumour aggressiveness[130]. Systemic tumour immunotherapy using antibodies targeting GD2 has been investigated in neuroblastoma during the past two decades, with proven safety and efficacy[131]. Tumour cells are killed as a result of GD2-specific antibody-dependent cell-mediated cytotoxicity, and this involves natural killer cells[132]. A Phase I trial (ClinicalTrials.gov Identifier NCT00743496) is currently underway to investigate the use of a humanized GD2-specific mAb in children and adolescents with relapsed or refractory osteosarcoma, neuroblastoma or melanoma[133]. Other potential antigens that are overexpressed in osteosarcoma include folate receptor-α (FOLR1)[134] and CD146 (REF. 135). Dendritic cells loaded with osteosarcoma fragments eliminated implanted and metastatic tumours in mice and rats[136,137]. However, a Phase I trial in patients with relapsed osteosarcoma using dendritic cells presenting peptides from an osteosarcoma tumour lysate and activated by keyhole limpet haemocyanin (KLH) induced a limited immune response and showed no evidence of clinical benefit[138]. To date, vaccination strategies using autologous dendritic cells or osteosarcoma-specific antigens remain limited to preclinical or early phase clinical research.

Although a small trial found that high doses of IL-2 induced complete responses in a subset of patients with metastatic osteosarcoma, major toxicities were observed[139]. This is likely to be a general problem with the systemic use of potent cytokines. IL-12 inhibits osteosarcoma growth, in part by upregulation of CD95 (also known as APO1 and FAS) receptor expression[140–142]. Many tumours downregulate CD95 to evade immune surveillance[143]. CD95 expression in osteosarcoma is inversely correlated with metastatic potential and low expression of CD95 correlates with poor prognosis in osteosarcoma[144,145]. Inhaled granulocyte–macrophage colony stimulating factor, which induces differentiation and apoptosis of the human osteosarcoma cell line SAOS2 *in vitro*[146], was investigated in patients with osteosarcoma who relapsed

Chimeric antigen receptors (CARs). These are engineered receptors that consist of an antibody-derived targeting domain fused with a T cell signalling domain that, when expressed by T cells, confers T cell antigen specificity governed by the targeting domain of the CAR.

Keyhole limpet haemocyanin (KLH). This is a large, multi-subunit metalloprotein that is found in the haemolymph of the giant keyhole limpet (*Megathura crenulata*), which is a type of gastropod, and is used extensively as a carrier protein to generate a substantial immune response in the production of antibodies.

with pulmonary metastases, but it was not associated with improved outcomes or immunomodulatory effects (ClinicalTrials.gov Identifier NCT00066365)[147].

Conclusions and perspectives

The recent accelerated development of techniques to rapidly assess the genetic and epigenetic status of tumour biopsies has led to the concept of personalized medicine[148,149]. Osteosarcomas present a challenge to personalized medicine, because the absence of pathognomonic mutations, together with the rarity and heterogeneity of the disease, may account for the disappointing results of recent trials of targeted therapies. However, a considerable amount is being learnt about the details of genomic instability itself. It is possible that future therapeutic opportunities will emerge from a growing understanding of the role of key DNA damage pathways in facilitating and tolerating genomic instability[150,151].

Importantly, the next few years will see the results of collaborative multi-centre international deep-sequencing efforts in this disease, increasing numbers of tumours sequenced and, potentially, the discovery of new therapeutic targets. Aside from opportunities in personalizing treatment for osteosarcoma, genomic information is likely to shed important light on the genetic determinants of risk. The recent GWAS will inevitably be succeeded by whole-exome and whole-genome studies. These approaches are likely to uncover the contribution of a new set of rare disease-causing variants with larger effect sizes and involving hitherto unsuspected genes[149]. Clinically, this information may be used to explore opportunities for risk-stratified screening and early detection, and perhaps for enhanced secondary prevention opportunities in young survivors of osteosarcoma.

Immunotherapies are creating a renaissance in oncology, finally harnessing the immune system in ways that are more nuanced, improving both tolerability and efficacy. There is reason to believe that the bone microenvironment represents a unique compartment of the immune system, in which immunological cytokines form part of an intercellular crosstalk that is relevant to bone development and homeostasis. The general principle underpinning recent advances in immunotherapies has been to remove tumour-induced immune suppression and to reactivate T cell-specific immunity that constitutes the natural immune reaction to a tumour. Newer agents, including the T cell checkpoint inhibitors, antibodies against PD1, PDL1 or CTLA4, are yet to be fully explored in osteosarcoma but are likely to present further opportunities to improve survival in certain patient groups, particularly if biomarkers of response are discovered. If bone represents a special immunological microenvironment, a better understanding of therapeutic opportunities for immunomodulation will require immunocompetent model systems, as well as screening of human samples for suitable targets. High levels of genomic instability may make immunotherapies particularly suitable, because of the constant generation of neo-epitopes that are the substrate for immune-mediated killing of cancer cells[152]. Certainly, the cancers that have shown the greatest promise for newer immunotherapies have been characterized by high rates of genomic instability[108,111]. In the future, interventions will ideally shift to adjuvant therapy, as it is thought that immunotherapies will have their greatest effect in the setting of micrometastatic disease. Whatever the future holds, surgery and chemotherapy will probably remain the backbone of conventional treatments for non-metastatic disease. An interesting feature of the integration of immunotherapies with standard cytotoxic agents is an emerging role for the immune system in the clinical activity of drugs such as doxorubicin and cisplatin. Perhaps the greatest challenge for the future will be developing effective options for patients with osteosarcoma with advanced disease at diagnosis, whose outlook remains grim. Fortunately, the existence of a global collaborative network supporting clinical trials for osteosarcoma will be a key asset in addressing the needs of patients who are affected by this rare disease.

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Competing interests statement
The authors declare no competing interests.

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