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Genomic Complexity of Osteosarcoma and Its Implication for Preclinical and Clinical Targeted Therapies

Courtney Schott, Avanthi Tayi Shah, and E. Alejandro Sweet-Cordero

Abstract

Osteosarcoma is a genomically complex disease characterized by few recurrent single-nucleotide mutations or in-frame fusions. In contrast, structural alterations, including copy number changes, chromothripsis, kataegis, loss of heterozygosity (LOH), and other large-scale genomic alterations, are frequent and widespread across the osteosarcoma genome. These observed structural alterations lead to activation of oncogenes and loss of tumor suppressors which together contribute to oncogenesis. To date, few targeted therapies for osteosarcoma have been identified. It is likely that effectiveness of targeted therapies will vary greatly in subsets of tumors with distinct key driver events. Model systems which can recapitulate the genetic heterogeneity of this disease are needed to test this hypothesis. One possible approach is to use patient-derived xenograft (PDX) models characterized with regards to their similarity to the human tumor samples from which they were derived. Here we review evidence pointing to the genomic complexity of osteosarcoma and how this is reflected in available model systems. We also review the current state of preclinical testing for targeted therapies using these models.

Keywords

Patient-derived xenografts · Targeted therapy · Combination therapy · MYC · CCNE1 · AKT · PTEN · VEGFR · CDK4

General Introduction

The genome of osteosarcoma is highly complex, and tumors are extraordinarily heterogeneous between patients. This heterogeneity may be one reason why it has been difficult to successfully identify targeted therapies for this disease. Osteosarcomas are characterized primarily by structural rearrangements, aneuploidy, and copy number alterations, whereas recurrent point mutations are few. Whether the observed structural alterations are truly targetable vulnerabilities remains to be fully explored, and it is almost certain that combination approaches need to be developed, since single agents are unlikely to lead to significant tumor regression. Given that osteosarcoma is a rare disease, the development of new therapies will depend heavily on preclinical testing in appropriate models. In this chapter,

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we review the current understanding of the complex genome of osteosarcoma and discuss how this complexity is reflected in available cell lines and patient-derived xenografts (PDXs). We also review efforts to evaluate possible targeted therapies in these models and briefly discuss the impact of this testing on clinical trial development. We focus here only on human model systems and defer discussion of mouse models and the canine patient to other chapters.

Genomics of Human Osteosarcoma

Osteosarcoma has long been known to be characterized by a complex karyotype. An early cytogenetic analysis of 73 samples from 53 patients identified widespread aneuploidy, recurrent numerical abnormalities, and recurrent chromosomal breakpoints [1]. The authors concluded that the majority of osteosarcomas were characterized by complex chromosomal abnormalities with marked cell-to-cell heterogeneity. This study was one of the first to determine that chromosomal breakpoints were nonrandom, with increased frequency occurring in defined regions of chromosomes 1, 2, 3, 4, 11, 12, 14, 16, 17, 19, and 22. Loss of whole chromosomes was also noted to be more common than whole chromosome gains, highlighting the importance of loss of heterozygosity (LOH) in this disease. In particular, frequent LOH of chromosome 17 was observed, a region which contains the tumor suppressor TP53. This early pioneering work noted a distinct cytogenetic profile in parosteal osteosarcoma, which they observed to be characterized by the presence of ring chromosomes. This provided an early indication that there may be molecular subtypes of osteosarcoma with unique features. Applying spectral karyotyping (SKY) to refine these prior cytogenetic studies, Bayani and colleagues analyzed 14 primary tumors and 4 established osteosarcoma cell lines [2]. They quantified a high rate of structural rearrangements, with an average of 38.5 breakpoints per tumor with those involving chromosomes 8 and 20 being disproportionately prevalent. Gains in 8q23–24 and 17p11–13 were also frequently observed. In addition to these and other studies evaluating the landscape of alterations in osteosarcoma [3, 4], a large body of literature has highlighted specific alterations in tumor suppressors and oncogenes including CDK4 [5], p53 and MDM2 [6], MYC [7, 8], Rb [9], and others. Other comprehensive molecular profiling studies of osteosarcoma have demonstrated that copy number amplification and overexpression of genes in chromosome 8 and chromosome 17 strongly correlate with osteosarcoma progression and relapse [10, 11]. Summarizing the work of several laboratories, Martin et al. concluded that conventional osteosarcoma is characterized by losses of portions of chromosomes 3q, 6q, 9, 10, 13, 17p, and 18q and gains of portions of chromosomes 1p, 1q, 6p, 8q, and 17p [9]. 6p gains commonly involve RUNX2, VEGFA, E2F3, PIM1, and CCND3, all of which could potentially play oncogenic roles in this disease. 8q contains MYC, which has been reported to be frequently amplified in osteosarcoma by many groups [12, 13]. 8q also contains RECQL4. While germ line loss of RECQL4 causes Rothmund-Thomson syndrome and predisposes patients to osteosarcoma development, in sporadic osteosarcoma tumors, RECQL4 has been reported to be upregulated at the level of protein expression or amplified at the gene level [14].

Cheng et al. evaluated copy number changes in a large panel of 117 osteosarcomas using Affymetrix SNP arrays [15]. They noted frequent gains in chromosomes 8, 12, 21, and X and frequent deletions in chromosomes 2, 10, and 13. These authors also correlated copy number alterations with therapy response in sarcoma cell lines. For example, they noted a correlation between IGFIR copy number gain and sensitivity to clofarabine. Similarly, Smida et al. profiled 45 osteosarcomas using Affymetrix SNP arrays and identified frequent alterations in 6p21 (including VEGFA, CCND2, and RUNX2), 8q24 (including MYC), and 12q14 (including CDK4) as well as loss of 10q21.1. They noted that a high LOH score (greater than 1500 loci with LOH) was predictive of a poor response to chemotherapy and a higher risk of recurrence. This was one of the first papers to correlate specific alterations

with poor event-free survival [16]. In a more recent study, Smida et al. evaluated a larger cohort of 160 pretreatment osteosarcoma samples using high-density arrays. They described frequent loss of tumor suppressors WWOX (31%), DLG2 (27%), and LSAMP (8%), in addition to Rb and Trp53. They also described the frequency of "chromothripsis-like pattern" (CTLP) and noted that the presence of CTLP in osteosarcoma was associated with a worse outcome [17]. Overall, it should be emphasized that loss of the tumor suppressors Trp53 (17p13.1) and Rb (13q14.2) is very common in sporadic osteosarcoma and germ line deletions are strongly associated with increased incidence [18, 19]. In addition to loss of function of Rb itself, osteosarcomas often exhibit deregulation of this pathway through other means, such as loss of the tumor suppressor CDKN2A/p16/INK4A or amplification of cyclin-dependent kinase 4 (CDK4).

The widespread availability of next-generation sequencing (NGS) has allowed further refinement of our understanding of the genomics of osteosarcoma. Two initial studies using NGS confirmed the prediction that these tumors are characterized by recurrent structural rearrangements. A very interesting observation was that Trp53 is more commonly altered by structural rearrangements in intron 1, rather than by deep deletion or mutation [20]. DLG2, ATRX, and RB alterations were also frequently noted. Another study identified frequent alterations in the PI3K pathway and suggested that this may represent a targetable opportunity for a subset of patients [21]. More recently, Sayles and Breese et al. also identified a number of subsets of osteosarcoma patients with potentially targetable alterations using whole-genome sequencing [22]. Alterations in MYC, CDK4, VEGFA, AKT or PTEN, and CCNE1 were frequently observed across a cohort of 63 tumors profiled using whole-genome sequencing. These and other studies suggest that some subsets of osteosarcoma may contain gains or losses in genes that represent potential therapeutic vulnerabilities. However, further identification and validation of such therapy biomarkers will likely require a combination of preclinical and clinical studies.

A critical issue that remains to be fully explored is the prognostic and therapeutic value of specific genomic alterations in osteosarcoma. To address this, Suehara et al. evaluated 66 patients with osteosarcoma using a clinical grade panel sequencing assay and found a fraction of patients with targetable or potentially targetable alterations [23]. Twenty-one percent of patients had a genomic alteration suggestive of an actionable alteration including CDK3, MDM2, BRCA2, PDGFRA, and VEGFR. In another study, Kovac et al. sequenced the exomes of 31 tumors and identified 14 genes as the main drivers in osteosarcoma. They also suggested that a large percentage of osteosarcomas have genome instability signatures characteristic of BRCA1/2-deficient tumors [24]. Whether this will translate into a therapeutic opportunity as it has for BRAC1/2-deficient breast and ovarian cancers remains to be explored.

To date, relatively few studies have systematically evaluated the evolution of osteosarcoma using matched samples; thus, we know little regarding how this disease progresses over time in individual patients, which is a significant gap in knowledge with regard to the development of targeted therapies. Negri and colleagues used whole-genome and whole-exome sequencing to deeply characterize a set of 13 primary and metastatic matched pairs [25]. High conservation of copy number in the matched pairs was seen, suggesting that perhaps the concept of osteosarcoma as a "genomically unstable" cancer needs to be further refined. Alternatively, it is possible that many of the genomic events seen in osteosarcoma are early events that remain stable over time. These authors also identified a recurrent amplification in the gene KDR in metastatic osteosarcoma. In another recent study, Brady and colleagues performed a deep analysis of tumor evolution in four osteosarcoma patients. They described a pattern of "branching evolution" shaped by treatment with cisplatin [26]. In general, these studies point to the need for analysis of more longitudinal samples, so we can begin to understand the evolutionary dynamics of osteo-

sarcoma and how they may define therapeutic vulnerabilities at relapse.

Overall, a great deal of knowledge has been gained regarding the genomic alterations present in osteosarcoma. Here we have emphasized studies evaluating structural alterations in the genome of osteosarcoma. However, many studies have also pointed to an important role for epigenetic mechanisms including DNA methylation. For example, promoter methylation may be an important mechanism of silencing the p16INK4A locus in osteosarcoma [27]. Studies using osteosarcoma models for preclinical therapeutic development will need to take the above summarized complexity of this disease into account. It is our belief that because of this complexity, osteosarcoma should be considered to include subsets of tumors with different drivers and thus will require an evaluation of specific genomic and epigenomic alterations to define appropriate subgroups for targeted therapy. Below we discuss the current understanding of the genomics of available preclinical models followed by a summary of some therapeutic studies with an emphasis on those that have addressed genomics while evaluating response.

Cell Line Genomics

Cell lines are the most widely utilized model system to study osteosarcoma due to their ease of use and wide availability. Several authors have attempted to evaluate the genomic characteristics of available cell lines to correlate the observed alterations with those found in patient samples. Ottaviano et al. used multiplex ligation-dependent probe amplification (MLPA) to screen for loss of 38 tumor suppressors across 19 osteosarcoma cell lines, including HOS, Saos2, U2OS, and other commonly used cell lines [28]. Loss of CDKN2A (42%) and TP53 (47%) was the most frequently observed alteration. Notably, these percentages are higher than what has been reported when evaluating tumors in vivo [22]. Lorenz et al. performed whole-genome sequencing of osteosarcoma cell lines including IOR/OS15, IOR/ OS18, MG63, and ZK-58. They found that inactivating genomic rearrangements, most commonly involving TP53, were frequent in both osteosarcoma patient samples (10/25) and cell lines (7/11). Interestingly, they also reported that osteosarcoma cell lines had numerous deletions, tandem duplications, inversions, and interchromosomal translocations at frequencies similar to human tumor samples [29]. They suggest that involvement of nonhomologous end-joining (NHEJ) and microhomology-mediated end-joining (MMEJ) DNA repair contribute to the generation of structural alterations in osteosarcoma. Additionally, RB1 rearrangements were similarly common between cell lines (3/11) and patient samples (5/25). Lastly, the presence of multiple fusion transcripts in osteosarcoma was also reported, including a previously undescribed fusion of *PMP22-ELOVL5*.

Cell Line Models of Metastatic Disease

Most osteosarcoma patients who do not survive die from metastatic disease; therefore, there has been a strong emphasis on developing metastatic cell line models. Several studies have evaluated the tumorigenicity and metastatic capacity of established osteosarcoma cell lines [30–33]. Some, but not all, commerical cell lines are capable of forming tumors after subcutaneous implantation [31]. Formation of spontaneous metastasis from those subcutaneous xenografts is less common [31, 34, 35]; however, formation of metastatic nodules after intravenous injection does occur with many cell lines [30, 35–39] (Table 1.1). Notably, there is discordance with respect to the metastatic capacity of certain cell lines between published studies, perhaps reflective of genetic drift in sublines expanded in different laboratories (Table 1.1).

To help overcome the variation in metastatic capacity observed in commercially available cell lines, in vivo passaging or transformation of established cell lines has been used to create metastatic derivatives (e.g., MG63.2 [71], MG63.3 [30], 143B, SaOS2-LM2 to LM7 [37, 38], KRIB [67] etc.). These metastatic derivatives can

Table 1.1 Genomic characteristics of cell line models for osteosarcoma

Cell line model (derivation)				Genomic characteristics	Tumorigenicity	Metastatic capacity
MG63 [40, 41]				MTAP-BCN2 fusion transcript (inversion ch9) [42]; fusion transcript with TP53 [42]; P53 arrangement intron 1 [43]; homozygous deletion CDKN2A [42, 44]; p53 wildtype [44]	*Yes*: SQ [45]; OT [46, 47] *No*: SQ [48]	*No*: IV [47, 49]; SQ/IM [48]; OT [47, 50]; *Yes*: OT [46]
	MG63.2 [46]				*Yes*: OT [46, 47]	*Yes*: IV [47]; OT [46, 47]
	MG63.3 [47]				*Yes*: OT [47]	*Yes*: IV [47]; OT [47]
U20S [51]				PMP22-ELOVL5 fusion [42]; P53 [44, 52]; single gene deletion P53 [43]; hemizygous deletion CDKN2A MLPA normal aCGH [44]	*Yes*: SQ [45, 48, 53]; OT [47]	*Yes*: IV [53] *No*: IV [49]; SQ [48, 53];IM [48]
Saos2				Fusion transcript involving TP53, RB1 transcript truncated after exon 20 [42]; P53 deletion [43, 44, 54–56]; normal CDKN2A [44]	*Yes*: SQ [45, 57]; OT [47, 50, 57–60] *No*: SQ [48]	*Yes*: OT [47, 58–60] *No*: IV [37, 47, 49]; SQ/IM [48]; OT [50]
	SaOS-LM2 [37]					*No*: IV [37, 61]
	SaOS-LM3–6 [37]					Yes: IV [37, 61]
	SaOS-LM7 [61]				*Yes*: OT [47, 50]	*Yes*: IV [47, 61];OT [47, 50]
TE85					*Yes*: OT [47] *No*: OT [50]	*No*: IV [47]; OT [47]
	HOS [62]			TP53 mutant, Rb wildtype [63]; homozygous CDKN2A deletion, TP53 p.Arg156Pro (c.467G > C) [44]	*Yes*: SQ [45]; OT [47] *No*: SQ [48]	*No*: IV [47, 49]; SQ/IM [48]; OT [47]
		MNNG/HOS [64]		KRAS [64]	*Yes*: OT [47, 65]; SQ [45, 48]; IM [48]	*Yes*: IV [47]; OT [47, 65] *No*: SQ/IM [48]
			KHOS			
		143B			*Yes*: SQ [45, 48]; IM [48]; OT [47, 50, 65, 66]	*Yes*: IV [47, 66]; SQ/IM [48]; OT [47, 50, 65, 66]
		KRIB [67]			*Yes*: OT [47, 68]	*Yes*: IV [47]; OT [47, 68]
G292					*Yes*: SQ [45] *No*: OT [50]	*No*: IV [49]; SQ/IM [48]
SJSA (OSA)				MDM2 amplification [42]; hemizygous deletion CDKN2A, P53 wildtype [44]	*Yes*: SQ [45, 48]	*No*: SQ/IM [48]
OHS [69]				Gain CKN2A aCGH, P53 mutant p.Glu286Lys [44]	*Yes*: SQ [45, 48, 70]; OT [70]	*No*: SQ/IM [48]

then be compared to their cell line of origin to investigate possible mechanisms of metastatic progression [30, 72, 73]. For example, Muff et al. showed that metastatic derivatives of established osteosarcoma cell lines have increased Hedgehog and WNT signaling pathways compared to their cell line of origin [73].

Orthotopic implantation is becoming increasingly common, despite the increased technical challenges [30, 33, 39, 71, 72, 74–85]. In general, osteosarcoma cell line xenografts more consistently form tumors at orthotopic sites than at subcutaneous or intramuscular sites (Table 1.1). To facilitate metastatic progression in orthotopic models, limb amputation is frequently performed as it allows for more time for metastasis development before the mice need to be sacrificed [30, 33, 39, 76]. Amputations are typically performed when the orthotopic tumor reaches 1–2 cm in diameter, and the mice are sacrificed 2–8 weeks later or when metastasis-associated morbidity is observed [30, 33, 39, 76]. A thorough investigation by Berlin et al. using KRIB cells demonstrated that a minimum of 2 weeks post orthotopic implantation is required for formation of pulmonary nodules [84]. Amputation models are arguably the ones that most accurately recapitulate the natural disease.

The phenotypic and genomic stability of continuously passaged cells has been evaluated. One commercial osteosarcoma cell line, SaOS2, exhibited increased proliferation and matrix mineralization with passage number, although many other phenotypic properties were stable over at least 100 passages and no significant changes in the expression of various growth factors were observed [86]. The use of cell lines to model osteosarcoma has certain pitfalls and limitations. Cross contamination with other cell lines and mycoplasma infection are frequent problems and must be considered [87, 88]. Additionally, when comparing cell line gene expression profiles to primary tumor samples of the same cancer type, not all cell lines have gene expression profiles that correlate with samples from their presumed tumor of origin [89]. Finally, cancer cell line xenografts lack complexity in both cellular heterogeneity and with regard to their tumor microenvironment [90]. The need for a more representative and reliable model to study osteosarcoma has led to increased utilization of the PDXs, which may more faithfully recapitulate features of the primary disease, and their derivative cell lines.

An increasingly utilized approach to model metastasis is the use of the ex vivo pulmonary metastasis assay (PuMA) [91]. Recently, this assay was used to carry out a screen for over 100 potential therapies for metastatic osteosarcoma, with CDK12 inhibition emerging as a strong candidate [92]. In another powerful approach, Morrow et al. recently mapped putative enhancer elements in matched human osteosarcoma tumors and in metastatic/nonmetastatic cell line pairs and identified metastasis-associated variant enhancer loci. This led them to identify a potential role for individual genes as key metastatic drivers [93].

PDX Models

To produce a PDX model, tumor tissue, as a fragment or after digestion into a single-cell suspension, is transplanted directly into an immunodeficient mouse [90]. PDX establishment was initially made possible by the introduction of nude mice and, more recently, the more immunodeficient NSG mouse [94]. In 1982, Ishii et al. reported that 24 of 30 patient-derived osteosarcoma samples established subcutaneous tumors in mice and they were able to continuously passage and maintain 2 of these PDXs in nude mice for 3 years [95]. Bauer et al. described establishment of six models in nude mice and noted that one line became polyploid after extended passage [34]. Meyer et al. described establishing eight transplantable osteosarcoma lines in mice made immunodeficient by whole-body irradiation [96].

Reported success rates vary widely for the initial establishment of osteosarcoma PDXs in mice (20–100%) [34, 95, 97–101]. Success rates can vary with tumor type, tumor stage (biopsy of a primary tumor vs. metastatic tumor), mouse strain, and implantation site [90]. PDX

maintenance requires continuous passaging through immunodeficient mice. Published osteosarcoma PDX models report widely variable implantation sites, including bone (femur [98], intratibial [102]), lung [103], muscle [104], subrenal capsule [22], and subcutis [34, 79, 101, 105–109]. Overall osteosarcoma PDXs maintain similar gross and histologic features to their primary tumor of origin [22, 96, 97, 102, 109–111], with preservation of the patient's tumor transcriptome [112] and tumor microenvironment [113]; however, some reports have described divergence [114]. Using microarray-based comparative genomic hybridization, Kresse et al. analyzed nine matched osteosarcoma patient/xenograft pairs over multiple xenograft passages. They concluded that most alterations were maintained, although some drift was identified [108]. Other authors have also found close similarity of their PDX models to the primary tumor and stable characteristics of PDX models in serial passage [109].

Several authors have reported the development of orthotopic osteosarcoma models (PDOX). This model is ideal because the transplanted tissue grows at a site that is most similar to its natural environment [90]. The orthotopic location could, in theory, more closely recapitulate the biology of osteosarcoma, including the metastatic phenotype [115]. Many researchers have developed osteosarcoma PDOX models [98, 102, 104, 110, 111, 115–120], including some with amputation protocols that facilitate the development of metastasis [84, 104, 116]. Goldstein et al. showed that metastatic spread was not observed after subcutaneous implantation of two osteosarcoma PDXs, whereas after orthotopic implantation, pulmonary metastases developed with both PDXs [104].

Cell lines can also be derived from PDXs, and several groups have used this approach [22, 101, 121, 122]. An important consideration is whether the PDX-derived cell lines are obtained from pretreatment biopsies, localized recurrence, or distant metastasis. For example, some lines obtained from pretreated tumor type have been reported to be cisplatin-resistant and can therefore be useful in the evaluation of combination therapies for relapsed disease such as trabectedin and temozolomide [115].

In perhaps the most comprehensive analysis to date, Stewart et al. evaluated 31 osteosarcoma patient-derived orthotopic models, compared them to their tumor of origin, and characterized the alterations that occur over time. This work also evaluated a large number of models for other pediatric solid tumors. Extensive evaluation determined there was preservation of clonal complexity between the primary tumors and the corresponding PDX. Furthermore, evolution of the PDX over subsequent passages was also evaluated. Notably, among all the tumors examined, osteosarcomas had the best preservation of the primary tumor's clonal complexity [98]. These results suggest that the use of osteosarcoma PDX models matched to the primary tumor may be a particularly fruitful strategy for identification of biomarker-driven therapeutic opportunities.

Preclinical Models for Drug Testing

Many authors have tested individual targeted therapies using cell lines. We will not make a comprehensive effort to evaluate all of these due to space limitations. Importantly, very few studies have systematically evaluated a large number of drugs in screens using cell lines. A recent notable effort utilized short-term cultures from PDX models, representing one possible approach [110]. Perhaps the most comprehensive effort to utilize osteosarcoma PDX models for preclinical evaluation of targeted agents has been done through the Pediatric Preclinical Testing Consortium (PPTC), formerly known as the Pediatric Preclinical Testing Program (PPTP). The PPTC is a National Cancer Institute (NCI)-sponsored initiative for the investigation, consideration, and prioritization of drugs for early-phase pediatric clinical trials. This multi-institutional consortium uses cell line and xenograft tumor panels to evaluate the antitumor activity of agents in osteosarcoma, as well as other sarcomas, renal tumors, neuroblastoma, CNS tumors, and hematologic malignancies [123].

The PPTC has evaluated a significant number of potential therapeutics for osteosarcoma. Here we highlight a few of these rather than attempting a comprehensive review. Glycoprotein NMB (GPNMB), also known as osteoactivin, is a transmembrane glycoprotein that is expressed in many nonmalignant cells, including osteoblasts, and is overexpressed in many malignancies, including osteosarcoma, making it a good candidate for targeting [124]. Glembatumumab vedotin is an antibody-drug conjugate that combines an anti-GPNMB antibody with the antimitotic agent vedotin [125]. Upon binding of the antibody to GPNMB, the drug is internalized and active glembatumumab vedotin is released, causing cell-cycle arrest and death. Because transmembrane expression of GPNMB is required for binding and downstream cytotoxicity of glembatumumab vedotin, the PPTC tested it in a subset of osteosarcoma xenografts that were known to express GPNMB. It yielded high activity in some xenografts and response seemed to be related to GPNMB expression [126].

The insulin-like growth factor-1 receptor (IGF1R) is a transmembrane receptor tyrosine kinase. Upon binding of insulin-like growth factor-1 or -2, IGF1R becomes autophosphorylated and activates multiple downstream signaling pathways that regulate cell growth and development, including the PI3K/Akt pathway [127]. The IGF1R pathway has been implicated in the pathogenesis of both pediatric and adult sarcomas [128, 129]. The PPTC evaluated anti-IGF1R monotherapies, including the monoclonal antibodies robatumumab and cixutumumab, and the small molecule inhibitor BMS-754807. Robatumumab demonstrated in vitro and in vivo activity [130]. These results were reproduced by other investigators, who also showed that combining robatumumab with cisplatin or cyclophosphamide further enhanced its activity [131]. Cixutumumab only demonstrated in vivo activity in osteosarcoma [132]. Furthermore, cixutumumab in combination with rapamycin resulted in increased antitumor activity, compared to either agent alone [133]. BMS-754807 also demonstrated in vitro and in vivo activity [134]; however, this drug is no longer in development for pediatric use. All three anti-IGF1R agents demonstrated varying degrees of antitumor activity; however, IGF1R copy number (assessed by PCR, FISH, and dot blot analysis), IGF1R mRNA expression (determined by RT-PCR), and IGF1R surface antibody expression (measured by flow cytometry) were not correlated with response to therapy [135].

The vascular endothelial growth factor (VEGF) pathway plays a critical role in angiogenesis, and activation of this pathway has been described in a variety of inflammatory disease as well as cancer [136]. Several tyrosine kinase inhibitors with activity against the VEGF receptor (VEGFR) family have been investigated by the PPTC. For example, cediranib, a highly potent VEGFR inhibitor, demonstrated promising antitumor activity [137]. Subsequently, phase I clinical studies with this receptor tyrosine kinase (RTK) inhibitor have been performed in a variety of advanced pediatric solid tumors, including osteosarcoma [138]. Sunitinib and sorafenib, which are multi-RTK inhibitors with high activity for VEGFRs, also demonstrated tumor growth inhibition [139, 140]. In a phase I trial of sunitinib, one osteosarcoma patient demonstrated stable disease, but no objective responses were observed in any tumor type [141]. Although a phase II Children's Oncology Group (COG) trial of sorafenib in refractory pediatric solid tumors did not demonstrate objective responses [142], a phase II trial by the Italian Sarcoma Group did demonstrate activity, when administered as a combination therapy, in relapsed and unresectable high-grade osteosarcoma [143]. Another multi-RTK, regorafenib, which targets VEGFR1–3, as well as BRAF, FGFR1, KIT, PDGFR-β, RAF-1, and RET, demonstrated modest tumor inhibition in osteosarcoma xenograft models [144]. The SARC024 phase II trial of regorafenib demonstrated improved progression-free survival in progressive, metastatic osteosarcoma patients [145].

SRC is overexpressed in osteosarcoma and results in increased cell proliferation and decreased apoptosis [146]. Dasatinib, a multi-RTK inhibitor that also has activity against the Src family of kinases, demonstrated intermedi-

ate tumor growth inhibition in osteosarcoma models when tested by the PPTC [147]. Interestingly, in another study, dasatinib altered metastatic potential of osteosarcoma in vitro, but not in vivo [148]. Phase I testing of dasatinib monotherapy in pediatric solid tumor patients demonstrated poor activity [149]; however, investigations of dasatinib combination therapies in both pediatric and adult advanced solid tumors are ongoing (NCT00788125, NCT03041701, NCT02389309).

mTOR activation has also been implicated in osteosarcoma tumorigenesis. The mTOR inhibitor rapamycin demonstrated intermediate to high activity in osteosarcoma xenografts as a single agent [150] and in combination with cytotoxic chemotherapy [151]. However, a phase I trial of temsirolimus, another mTOR inhibitor, did not result in significant antitumor activity in osteosarcoma [152]. Furthermore, phase II testing of rapamycin in combination with cyclophosphamide failed to meet its primary endpoint [153]. In contrast, phase II and III studies of ridaforolimus, another mTOR inhibitor, have had more promising results [154, 155]. Treatment with dual mTORC1 and mTORC2 inhibitors may help circumvent some of the resistance that develops after mTOR inhibition; however, the dual inhibitor AZD8055 demonstrated poor activity in vivo [156].

PI3K and Akt activation are thought to contribute to tumorigenesis in osteosarcoma [157]. MK-2206, a highly selective, non-ATP-competitive, pan-Akt inhibitor, and GSK690693, an ATP-competitive, pan-Akt inhibitor, both demonstrated prolonged event-free survival, but did not result in any objective responses in osteosarcoma xenografts [158, 159]. As tumor cells often respond to inhibition of this pathway by activating feedback loops, a large challenge to therapeutic targeting of the PI3K/Akt pathway is the development of resistance. Several other groups have investigated targeting of the PI3K and Akt pathways with other agents, and these results will be discussed below.

The MAPK/ERK pathway is activated by many different growth factor receptors, including IGF1R, VEGFR, and PDGFR, which are often overexpressed in osteosarcoma, and thus it has been considered a promising candidate for inhibition [160]. Unfortunately, selumetinib, a potent MEK1/2 (a.k.a MAP2K1/2) inhibitor, had poor in vitro and in vivo antitumor activity, as a single-agent therapy, despite demonstrating significant pathway inhibition [161]. Though MEK inhibitors hold much potential, clinical application of these inhibitors can prove challenging due to the complex signaling cascades and cross talk between pathways. To this end, clinical trials have investigated combination therapies incorporating MEK inhibitors for refractory sarcoma patients, but did not demonstrate significant antitumor activity [162, 163].

As mentioned above, cell-cycle aberrations occur frequently in osteosarcoma, with amplifications in CDK4, CDK6, CCND, and CCNE and deletions in CDKN2A/B. Dinaciclib, a potent inhibitor of CDK1, CDK2, CDK5, and CDK9, was tested by the PPTC, and stable disease was the best response that was seen [164]. In contrast, when tested in PDXs harboring copy number changes in cell-cycle checkpoint genes [22], dinaciclib and palbociclib, a CDK4/6 inhibitor, demonstrated significant inhibition of tumor growth. This suggests that CDK inhibition may be efficacious in a subset of osteosarcoma patients who harbor genomic aberrations in cell-cycle genes. Several cell-cycle inhibitors are available, and these results support further preclinical testing in a genomically informed manner.

The Aurora kinase family members are key regulators of mitosis and cell-cycle progression, and amplifications in Aurora kinase A and B have been described in osteosarcoma [2]. Testing of the Aurora kinase A inhibitor alisertib by the PPTC resulted in high antitumor activity in one osteosarcoma xenograft and intermediate activity in the remaining five xenografts [165]. However, phase II testing of alisertib in relapsed and refractory solid tumors demonstrated very poor response rates (<5%) [166]. The serine/threonine kinase Chk1 directs the DNA damage response (DDR) and cell-cycle checkpoint response. Prexasertib, a CHK1 inhibitor, demonstrated poor single-agent activity when tested by the

PPTC; however, when combined with irinotecan, a topoisomerase I inhibitor, it showed prolonged event-free survival in osteosarcoma xenograft studies [167].

As described above, inactivating structural alterations in TP53 occur in the majority of osteosarcoma tumors. In a subset of osteosarcoma, inactivation can occur through amplification of MDM2. Serdemetan is an MDM2 antagonist that reactivates p53 and results in apoptosis. It resulted in tumor growth inhibition when tested by the PPTC. Unfortunately, a phase I trial in adults with advanced solid tumors did not demonstrate significant clinical activity [168].

Finally, several other targeted agents have been tested by the PPTC that failed to demonstrate significant antitumor activity. These include navitoclax [169], a potent Bcl-2 inhibitor; lapatinib [170], an EGFR inhibitor; RG7112 [171], an MDM2 inhibitor; pevonedistat [172], a NEDD8 inhibitor; RO4929097 [173], a gamma-secretase inhibitor that targets the NOTCH pathway; and seclidemstat, an LSD1 inhibitor [174].

Other PDX Studies

Several other investigators have evaluated targeted agents in in vivo osteosarcoma models. The Italian Sarcoma Group investigated inhibition of the mTOR pathway and showed that sorafenib monotherapy resulted in decreased mTORC1 signaling but resulted in mTORC2 activation as an escape mechanism. However, when sorafenib was combined with everolimus, another mTOR inhibitor, it caused increased antitumor activity and complete inhibition of the mTOR pathway in osteosarcoma xenograft models [175]. As mentioned above, a nonrandomized phase II trial investigating the utility of this combination in patients with unresectable, progressive, high-grade osteosarcoma was subsequently activated and showed promise. Although the combination therapy demonstrated activity, it failed to achieve the prespecified outcome of a 6-month progression-free survival of >50% [176].

One group investigated PI3K inhibition in osteosarcoma using two different agents, NVP-BEZ235, a dual PI3K/mTOR inhibitor, and BYL719, a PI3K inhibitor that specifically targets the alpha isoform. Both agents slowed osteosarcoma tumor growth in allograft and xenograft mouse models; however, neither drug induced in vivo tumor shrinkage [177, 178]. Interestingly, administration of ifosfamide in combination with BYL719 resulted in a synergistic effect [178]. Combining NVP-BEZ235 with cisplatin also enhances its antitumor effects in osteosarcoma xenografts [179]. Furthermore, in other in vivo sarcoma models, vincristine and NVP-BEZ235 combination therapy decreased metastasis and slowed tumor growth [180]. These studies highlight the importance of considering combination therapy to help potentiate an agent's efficacy. There are several ongoing phase I/II clinical trials investigating these two agents in adult cancers as monotherapies or in combination with other agents.

Multi-RTKs have also been an avenue of investigation for groups outside of the PPTC. Gobin et al. showed that imatinib, which targets PDGFRα, PDGFR-β, EGFR, IGF1R, and several other receptors, inhibits tumor growth in mouse models of osteosarcoma [181]. Sampson et al. demonstrated that crizotinib, which is FDA-approved for ROS1-positive non-small cell lung cancer and ALK-positive solid tumors, had significant antitumor effects in vitro and in vivo, at least in part, through inhibition of Met [182].

Other investigators have also studied MDM2 inhibition. Treatment with Nutlin-3 caused disruption of p53-MDM2 binding, resulting in decreased degradation of p53. Of note, efficacy of the agent is dependent on wild-type p53, which is seen in only a small subset of osteosarcoma. Osteosarcoma xenografts with wild-type p53 were treated with Nutlin-3 and experienced significant tumor growth inhibition [183].

Beyond the therapeutic targets already mentioned, several groups have identified targets of interest that have not been investigated by the PPTC. One such target is ezrin, a cytoskeletal linker protein that connects actin cytoskeleton to plasma membrane proteins [184]. High ezrin

expression in an osteosarcoma mouse model resulted in pulmonary metastasis and has been associated with poor survival in dogs with naturally occurring osteosarcoma, as well as pediatric patients [185]. When an ezrin-dependent metastatic mouse model was treated with NSC305787 and NSC66839, both small molecule inhibitors of ezrin, overall survival was increased with both drugs, though it was only statistically significant for NSC305787 [186]. Additionally, targeting of the ezrin-regulated mTOR/S6K1/4E-BP1 pathway with rapamycin and CCI-779 resulted in pulmonary metastasis inhibition and prolonged survival in vivo [187]. Another group demonstrated that sorafenib treatment in vivo resulted in decreased tumor volume and metastasis, in part through downregulation of the ezrin pathway [188].

Another approach to inhibit osteosarcoma metastasis is to target chemokines and their receptors. CXCR3 expression has been described in a variety of malignancies, including osteosarcoma [189], and is thought to play a role in metastasis [190, 191]. Treatment with AMG487, a small molecule inhibitor of CXCR3, resulted in a significant reduction in metastatic burden in a metastatic model utilizing the SaO2-LM7 cell line [192].

Heat shock protein 90 (HSP90) stabilizes and activates a multitude of proteins. Many of these proteins are involved in constitutive signaling and responses to stress [193, 194]. Cancer cells use this complex to protect mutated and overexpressed oncoproteins from misfolding and degradation. When osteosarcoma xenograft models were treated with single-agent alvespimycin, an HSP90 inhibitor, or in combination with imetelstat, a telomerase inhibitor, there was significant reduction in tumor volume [195]. Similarly, Ory et al. demonstrated that treatment with the HSP90 inhibitor PF4942847, alone, or in combination with zoledronic acid, resulted in significant tumor growth inhibition and decreased metastases [196].

Although recurrent Wnt/β-catenin mutations have not been described in osteosarcoma, the role of this pathway in osteosarcoma biology is an area of investigation. Tegavivint, a small molecule inhibitor of β-catenin, had strong antitumor effects in both primary and metastatic tumors in a osteosarcoma xenografts [197]. Interestingly, treatment with BHQ880, a monoclonal antibody against dickkopf-1 (DKK-1), which is an inhibitor of Wnt signaling, resulted in decreased tumor growth and metastases [116].

A subset of osteosarcoma is characterized by MYC overexpression. Historically, MYC has been considered undruggable; however, recent studies have demonstrated various strategies for indirect inhibition. In osteosarcoma PDXs, treatment with AT7519 resulted in significant tumor growth inhibition in MYC-amplified tumors, likely through inhibition of CDK9 [22]. Another strategy is to target the transcriptional activity of MYC through bromodomain and extra-terminal domain (BET) inhibition; however, in osteosarcoma models, Baker et al. showed that treatment with the BET inhibitor JQ1 induced apoptosis independent of MYC [198].

STAT3 activation is thought to play a role in tumor cell survival and proliferation in human and canine osteosarcoma [199]. Toosendanin, a STAT3 inhibitor, suppressed osteosarcoma cell growth, invasion, and angiogenesis in vitro. Furthermore, toosendanin treatment resulted in decreased tumor growth, reduction of metastasis, and prolonged survival of osteosarcoma xenografts [200]. In another study, administration of pectolinarigenin to osteosarcoma xenografts blocked STAT3 activation and impaired tumor growth and metastasis [79].

Wee1 is a mediator of the G2/M cell-cycle checkpoint, and inhibition of Wee1 by adavosertib has been reported to enhance the effects of cytotoxic chemotherapy [201, 202]. Kreahling et al. demonstrated that adavosertib had significant antitumor activity in osteosarcoma xenografts, both as a single agent and in combination with gemcitabine [105]. Most of the studies done to date in preclinical models have not specifically matched targeted therapies to the subsets of osteosarcomas that have specific alterations. Given the heterogeneity of osteosarcoma described previously, it is possible that

stratification for targeted therapy based on genomic characteristics of a tumor could increase the rate of response. Such a "genome-informed" approach was tested recently [22]. Whole-genome sequencing was used to identify recurrent copy number alterations in subsets of osteosarcoma PDX models. Based on this analysis, six candidate pathways were identified for targeting: MYC amplification, with the CDK9 inhibitor AT7519; CCNE amplification with the CDK2 inhibitor dinaciclib; VEGFA amplification with the VEGFR inhibitor sorafenib; CDK4 amplification or FOXM1 amplification with the CDK4/6 inhibitor palbociclib; AURKB amplification with the AURKB inhibitor AZD1152; and AKT gain or PTEN loss with the AKT inhibitor MK2206. PDX models treated with the matched drug based on copy number analysis demonstrated significantly higher tumor growth inhibition compared to a "nonmatched" approach. Translation of this approach to clinical trials has still not been done but may represent a promising new approach to treatment of this disease. Another potentially highly effective strategy is to leverage the availability of mouse and canine models together with human osteosarcoma. Such an approach has recently been described [203]. Lastly, it should be noted that the high mutational burden of osteosarcomas could potentially make them susceptible to immunotherapies. To date, such approaches have not been successful, but it is possible that future studies could find ways to reactivate the immune system for therapeutic benefit in osteosarcoma.

References Cited

1. Bridge JA et al (1997) Cytogenetic findings in 73 osteosarcoma specimens and a review of the literature. Cancer Genet Cytogenet 95(1):74–87
2. Bayani J et al (2003) Spectral karyotyping identifies recurrent complex rearrangements of chromosomes 8, 17, and 20 in osteosarcomas. Genes Chromosomes Cancer 36(1):7–16
3. Tarkkanen M et al (1995) Gains and losses of DNA sequences in osteosarcomas by comparative genomic hybridization. Cancer Res 55(6):1334–1338
4. Hoogerwerf WA, Hawkins AL, Perlman EJ, Griffin CA (1994) Chromosome analysis of nine osteosarcomas. Genes Chromosom Cancer 9(2):88–92
5. Wei G et al (1999) CDK4 gene amplification in osteosarcoma: reciprocal relationship with INK4A gene alterations and mapping of 12q13 amplicons. Int J Cancer 80(2):199–204
6. Lonardo F, Ueda T, Huvos AG, Healey J, Ladanyi M (1997) p53 and MDM2 alterations in osteosarcomas: correlation with clinicopathologic features and proliferative rate. Cancer 79(8):1541–1547
7. Ikeda S et al (1989) Amplification of both c-myc and c-raf-1 oncogenes in a human osteosarcoma. Jpn J Cancer Res 80(1):6–9
8. Gamberi G et al (1998) C-myc and c-fos in human osteosarcoma: prognostic value of mRNA and protein expression. Oncology 55(6):556–563
9. Miller CW, Aslo A, Won A, Tan M, Lampkin B, Koeffler HP (1996) Alterations of the p53, Rb and MDM2 genes in osteosarcoma. J Cancer Res Clin Oncol 122(9):559–565
10. Martin JW, Squire JA, Zielenska M (2012) The genetics of osteosarcoma. Sarcoma 2012:627254
11. Morrow JJ, Khanna C (2015) Osteosarcoma genetics and epigenetics: emerging biology and candidate therapies. Crit Rev Oncog 20(3–4):173–197
12. Ladanyi M, Park CK, Lewis R, Jhanwar SC, Healey JH, Huvos AG (1993) Sporadic amplification of the MYC gene in human osteosarcomas. Diagn Mol Pathol 2(3):163–167
13. Tarkkanen M et al (1999) DNA sequence copy number increase at 8q: a potential new prognostic marker in high-grade osteosarcoma. Int J Cancer 84(2):114–121
14. Maire G, Yoshimoto M, Chilton-MacNeill S, Thorner PS, Zielenska M, Squire JA (2009) Recurrent RECQL4 imbalance and increased gene expression levels are associated with structural chromosomal instability in sporadic osteosarcoma. Neoplasia 11(3):260–268., 3p following 268
15. Cheng L et al (2019) Integration of genomic copy number variations and chemotherapy-response biomarkers in pediatric sarcoma. BMC Med Genet 12(Suppl 1):23
16. Smida J et al (2010) Genomic alterations and allelic imbalances are strong prognostic predictors in osteosarcoma. Clin Cancer Res 16(16):4256–4267
17. Smida J et al (2017) Genome-wide analysis of somatic copy number alterations and chromosomal breakages in osteosarcoma. Int J Cancer 141(4):816–828
18. Malkin D et al (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science (80-) 250(4985):1233–1238
19. Wong FL et al (1997) Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk. JAMA 278(15):1262–1267
20. Chen X et al (2014) Recurrent somatic structural variations contribute to tumorigenesis in pediatric osteosarcoma. Cell Rep 7(1):104–112
21. Perry JA et al (2014) Complementary genomic approaches highlight the PI3K/mTOR pathway as

a common vulnerability in osteosarcoma. Proc Natl Acad Sci 111(51):E5564–E5573
22. Sayles LC et al (2019) Genome-informed targeted therapy for osteosarcoma. Cancer Discov 9(1):46–63
23. Suehara Y et al (2019) Clinical genomic sequencing of pediatric and adult osteosarcoma reveals distinct molecular subsets with potentially targetable alterations. Clin Cancer Res 25(21):6346–6356
24. Kovac M et al (2015) Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. Nat Commun 6:1–9
25. Negri GL et al (2019) Integrative genomic analysis of matched primary and metastatic pediatric osteosarcoma. J Pathol 249(3):319–331
26. Brady SW et al (2019) The clonal evolution of metastatic osteosarcoma as shaped by cisplatin treatment. Mol Cancer Res 17(4):895–906
27. Oh JH, Kim H-S, Kim H-H, Kim WH, Lee SH (2006) Aberrant methylation of p14ARF gene correlates with poor survival in osteosarcoma. Clin Orthop Relat Res 442:216–222
28. Ottaviano L et al (2010) Molecular characterization of commonly used cell lines for bone tumor research: a trans-European EuroBoNet effort. Genes Chromosomes Cancer 49(1):40–51
29. Lorenz S et al (2016) Unscrambling the genomic chaos of osteosarcoma reveals extensive transcript fusion, recurrent rearrangements and frequent novel TP53 aberrations. Oncotarget 7(5):5273–5288
30. Ren L et al (2015) Characterization of the metastatic phenotype of a panel of established osteosarcoma cells. Oncotarget 6(30):29469–29481
31. Mohseny AB et al (2011) Functional characterization of osteosarcoma cell lines provides representative models to study the human disease. Lab Investig 91(8):1195–1205
32. Lauvrak SU et al (2013) Functional characterisation of osteosarcoma cell lines and identification of mRNAs and miRNAs associated with aggressive cancer phenotypes. Br J Cancer 109(8):2228–2236
33. Yuan J et al (2009) Osteoblastic and osteolytic human osteosarcomas can be studied with a new xenograft mouse model producing spontaneous metastases. Cancer Investig 27(4):435–442
34. Bauer HCF, Brosjö O, Broström LÅ, Nilsson OS, Reinholt FP, Tribukait B (1986) Growth and ploidy of human osteosarcoma xenografts in serial passage in nude mice. Eur J Cancer Clin Oncol 22(7):821–830
35. Kimura K et al (2002) Establishment of human osteosarcoma cell lines with high metastatic potential to lungs and their utilities for therapeutic studies on metastatic osteosarcoma. Clin Exp Metastasis 19(6):477–485
36. Manara MC et al (2000) Reversal of malignant phenotype in human osteosarcoma cells transduced with the alkaline phosphatase gene. Bone 26(3):215–220
37. Jia SF, Worth LL, Kleinerman ES (1999) A nude mouse model of human osteosarcoma lung metastases for evaluating new therapeutic strategies. Clin Exp Metastasis 17(6):501–506
38. Duan X, Jia S-F, Zhou Z, Langley RR, Bolontrade MF, Kleinerman ES (2004) Association of alphavbeta3 integrin expression with the metastatic potential and migratory and chemotactic ability of human osteosarcoma cells. Clin Exp Metastasis 21(8):747–753
39. Zhang Y et al (2018) Down-regulation of Skp2 expression inhibits invasion and lung metastasis in osteosarcoma. Sci Rep 8(1):14294
40. Billiau A, Edy VG, Heremans H et al (1977) Human interferon: mass production in a newly established cell line, MG-63. Antimicrob Agents Chemother 12:11–15
41. Heremans H, Billiau A, Cassiman JJ et al (1978) In vitro cultivation of human tumor tissues. II. Morphological and virological characterization of three cell lines. Oncology 35:246–252
42. Lorenz S, Barøy T, Sun J et al Unscrambling the genomic chaos of osteosarcoma reveals extensive transcript fusion, recurrent rearrangements and frequent novel TP53 aberrations. Oncotarget 7. Epub ahead of print 2016. https://doi.org/10.18632/oncotarget.6567
43. Masuda H, Miller C, Koeffler HP et al (1987) Rearrangement of the p53 gene in human osteogenic sarcomas. Proc Natl Acad Sci U S A 84:7716–7719
44. Ottaviano L, Schaefer K-L, Gajewski M et al (2010) Molecular characterization of commonly used cell lines for bone tumor research: a trans-European EuroBoNet effort. Genes Chromosomes Cancer 49:40–51
45. Lauvrak SU, Munthe E, Kresse SH et al (2013) Functional characterisation of osteosarcoma cell lines and identification of mRNAs and miRNAs associated with aggressive cancer phenotypes. Br J Cancer 109:2228–2236
46. Su Y, Luo X, He B-C et al (2009) Establishment and characterization of a new highly metastatic human osteosarcoma cell line. Clin Exp Metastasis 26:599–610
47. Ren L, Mendoza A, Zhu J et al (2015) Characterization of the metastatic phenotype of a panel of established osteosarcoma cells. Oncotarget 6:29469–29481
48. Mohseny AB, Machado I, Cai Y et al (2011) Functional characterization of osteosarcoma cell lines provides representative models to study the human disease. Lab Investig 91:1195–1205
49. Kimura K, Nakano T, Park Y-B et al (2002) Establishment of human osteosarcoma cell lines with high metastatic potential to lungs and their utilities for therapeutic studies on metastatic osteosarcoma. Clin Exp Metastasis 19:477–485
50. Yuan J, Ossendorf C, Szatkowski JP et al (2009) Osteoblastic and osteolytic human osteosarcomas can be studied with a new xenograft mouse model producing spontaneous metastases. Cancer Investig 27:435–442
51. Pontén J, Saksela E (1967) Two established in vitro cell lines from human mesenchymal tumours. Int J Cancer 2:434–447

52. Zhang Y, Xiong Y, Yarbrough WG (1998) ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell 92:725–734
53. Manara MC, Baldini N, Serra M et al (2000) Reversal of malignant phenotype in human osteosarcoma cells transduced with the alkaline phosphatase gene. Bone 26:215–220
54. Diller L, Kassel J, Nelson CE et al (1990) p53 functions as a cell cycle control protein in osteosarcomas. Mol Cell Biol 10:5772–5781
55. Kubbutat MH, Jones SN, Vousden KH (1997) Regulation of p53 stability by Mdm2. Nature 387:299–303
56. Moneo V, Serelde BG, Fominaya J et al (2007) Extreme sensitivity to Yondelis (Trabectedin, ET-743) in low passaged sarcoma cell lines correlates with mutated p53. J Cell Biochem 100:339–348
57. Tiram G, Segal E, Krivitsky A et al (2016) Identification of dormancy-associated MicroRNAs for the design of osteosarcoma-targeted dendritic polyglycerol nanopolyplexes. ACS Nano 10:2028–2045
58. Gvozdenovic A, Arlt MJE, Campanile C et al (2013) CD44 enhances tumor formation and lung metastasis in experimental osteosarcoma and is an additional predictor for poor patient outcome. J Bone Miner Res 28:838–847
59. Sabile AA, Arlt MJE, Muff R et al (2012) Cyr61 expression in osteosarcoma indicates poor prognosis and promotes intratibial growth and lung metastasis in mice. J Bone Miner Res 27:58–67
60. Sabile AA, Arlt MJE, Muff R et al (1832) Caprin-1, a novel Cyr61-interacting protein, promotes osteosarcoma tumor growth and lung metastasis in mice. Biochim Biophys Acta 2013:1173–1182
61. Duan X, Jia S-F, Zhou Z et al (2004) Association of alphavbeta3 integrin expression with the metastatic potential and migratory and chemotactic ability of human osteosarcoma cells. Clin Exp Metastasis 21:747–753
62. Rhim JS, Cho HY, Huebner RJ (1975) Non-producer human cells induced by murine sarcoma virus. Int J Cancer 15:23–29
63. Botter SM, Arlt MJE, Born W et al (2015) Mammalian models of bone sarcomas. In: Heymann D (ed) Bone cancer. Elsevier Inc., Amsterdam, Boston, Heidelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo, pp 347–364
64. Rhim JS, Park DK, Arnstein P et al (1975) Transformation of human cells in culture by N-methyl-N'-nitro-N-nitrosoguanidine. Nature 256:751–753
65. Luu HH, Kang Q, Jong KP et al (2005) An orthotopic model of human osteosarcoma growth and spontaneous pulmonary metastasis. Clin Exp Metastasis 22:319–329
66. Zhang Y, Zvi YS, Batko B et al (2018) Down-regulation of Skp2 expression inhibits invasion and lung metastasis in osteosarcoma. Sci Rep 8:14294
67. Samid D, Mandler R (1989) Human osteosarcoma cells transformed by ras-oncogenes: a new model for in vivo studies of pulmonary metastasis. Clin Biotechnol 1:21–26
68. Berlin O, Samid D, Donthineni-Rao R et al (1993) Development of a novel spontaneous metastasis model of human osteosarcoma transplanted orthotopically into bone of athymic mice. Cancer Res 53:4890–4895
69. Fodstad O, Brøgger A, Bruland O et al (1986) Characteristics of a cell line established from a patient with multiple osteosarcoma, appearing 13 years after treatment for bilateral retinoblastoma. Int J Cancer 38:33–40
70. Eikenes L, Tari M, Tufto I et al (2005) Hyaluronidase induces a transcapillary pressure gradient and improves the distribution and uptake of liposomal doxorubicin (Caelyx) in human osteosarcoma xenografts. Br J Cancer 93:81–88
71. Su Y et al (2009) Establishment and characterization of a new highly metastatic human osteosarcoma cell line. Clin Exp Metastasis 26(7):599–610
72. Luu HH et al (2005) An orthotopic model of human osteosarcoma growth and spontaneous pulmonary metastasis. Clin Exp Metastasis 22(4):319–329
73. Muff R et al (2015) Genomic instability of osteosarcoma cell lines in culture: impact on the prediction of metastasis relevant genes. PLoS One 10(5):e0125611
74. Tiram G et al (2016) Identification of dormancy-associated MicroRNAs for the Design of Osteosarcoma-Targeted Dendritic Polyglycerol Nanopolyplexes. ACS Nano 10(2):2028–2045
75. Nomura M et al (2019) Tegavivint and the β-catenin/ALDH axis in chemotherapy-resistant and metastatic osteosarcoma. J Natl Cancer Inst 111:1–12
76. Majzner RG et al (2019) CAR T cells targeting B7-H3, a pan-cancer antigen, demonstrate potent preclinical activity against pediatric solid tumors and brain tumors. Clin Cancer Res 25(8):2560–2574
77. Husmann K et al (2013) Matrix Metalloproteinase 1 promotes tumor formation and lung metastasis in an intratibial injection osteosarcoma mouse model. Biochim Biophys Acta 1832(2):347–354
78. Butch ER et al (2019) Positron emission tomography detects in vivo expression of Disialoganglioside GD2 in mouse models of primary and metastatic osteosarcoma. Cancer Res 79(12)3112–3124
79. Zhang T et al (2016) Natural product pectolinarigenin inhibits osteosarcoma growth and metastasis via SHP-1-mediated STAT3 signaling inhibition. Cell Death Dis 7(10):e2421
80. Sabile AA et al (2012) Cyr61 expression in osteosarcoma indicates poor prognosis and promotes intratibial growth and lung metastasis in mice. J Bone Miner Res 27(1):58–67
81. Broadhead ML, Dass CR, Choong PFM (2011) Systemically administered PEDF against primary and secondary tumours in a clinically relevant osteosarcoma model. Br J Cancer 105(10):1503–1511

82. Igarashi K et al (2017) Effective metabolic targeting of human osteosarcoma cells in vitro and in orthotopic nude-mouse models with recombinant Methioninase. Anticancer Res 37(9):4807–4812
83. Xu WT, Bian ZY, Fan QM, Li G, Tang TT (2009) Human mesenchymal stem cells (hMSCs) target osteosarcoma and promote its growth and pulmonary metastasis. Cancer Lett 281(1):32–41
84. Berlin O, Samid D, Donthineni-Rao R, Akeson W, Amiel D, Woods VL (1993) Development of a novel spontaneous metastasis model of human osteosarcoma transplanted orthotopically into bone of athymic mice. Cancer Res 53(20):4890–4895
85. Eikenes L, Tari M, Tufto I, Bruland OS, de Lange Davies C (2005) Hyaluronidase induces a transcapillary pressure gradient and improves the distribution and uptake of liposomal doxorubicin (Caelyx) in human osteosarcoma xenografts. Br J Cancer 93(1):81–88
86. Hausser H-J, Brenner RE (2005) Phenotypic instability of Saos-2 cells in long-term culture. Biochem Biophys Res Commun 333(1):216–222
87. Masters JRW (2000) Human cancer cell lines: fact and fantasy. Nat Rev Mol Cell Biol 1(3):233–236
88. Drexler HG, Uphoff CC, Dirks WG, MacLeod RAF (2002) Mix-ups and mycoplasma: the enemies within. Leuk Res 26(4):329–333
89. Wang H et al (2006) Comparative analysis and integrative classification of NCI60 cell lines and primary tumors using gene expression profiling data. BMC Genomics 7:166
90. Jung J, Seol HS, Chang S (2018) The generation and application of patient-derived xenograft model for cancer research. Cancer Res Treat 50(1):1–10
91. Mendoza A et al (2010) Modeling metastasis biology and therapy in real time in the mouse lung. J Clin Invest 120(8):2979–2988
92. Bayles I et al (2019) Ex vivo screen identifies CDK12 as a metastatic vulnerability in osteosarcoma. J Clin Invest 129(10):4377–4392
93. Morrow JJ et al (2018) Positively selected enhancer elements endow osteosarcoma cells with metastatic competence. Nat Med 24(2):176–185
94. Shultz LD et al (2005) Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol 174(10):6477–6489
95. Ishii S et al (1982) Analysis of osteoid-forming activity of human osteosarcoma implanted into nude mice. Int Orthop 6(4):215–223
96. Meyer WH, Houghton JA, Houghton PJ, Webber BL, Douglass EC, Look AT (1990) Development and characterization of pediatric osteosarcoma xenografts. Cancer Res 50(9):2781–2785
97. Meohas W, Granato RA, Guimarães JAM, Dias RB, Fortuna-Costa A, Duarte MEL (2018) Patient-derived xenografts as a preclinical model for bone sarcomas. Acta Ortop Bras 26(2):98–102
98. Stewart E et al (2017) Orthotopic patient-derived xenografts of paediatric solid tumours. Nature 549(7670):96–100
99. Bruheim S, Bruland OS, Breistol K, Maelandsmo GM, Fodstad Ø (2004) Human osteosarcoma xenografts and their sensitivity to chemotherapy. Pathol Oncol Res 10(3):133–141
100. Llombart-Bosch A, Carda C, Boix J, Pellin A, Peydro-Olaya A (1988) Value of nude mice xenografts in the expression of cell heterogeneity of human sarcomas of bone and soft tissue. Pathol Res Pract 183(6):683–692
101. Fujisaki T, Wada T, Takahashi M, Yamawaki S, Ishii S (1995) In vitro chemosensitivity assay for human osteosarcoma using tumor xenografts. Clin Orthop Relat Res 313:279–285
102. Blattmann C et al (2015) Establishment of a patient-derived orthotopic osteosarcoma mouse model. J Transl Med 13:136
103. Igarashi K et al (2018) Tumor-targeting Salmonella typhimurium A1-R combined with recombinant methioninase and cisplatinum eradicates an osteosarcoma cisplatinum-resistant lung metastasis in a patient-derived orthotopic xenograft (PDOX) mouse model: decoy, trap and kill chemothe. Cell Cycle 17(6):801–809
104. Goldstein SD, Hayashi M, Albert CM, Jackson KW, Loeb DM (2015) An orthotopic xenograft model with survival hindlimb amputation allows investigation of the effect of tumor microenvironment on sarcoma metastasis. Clin Exp Metastasis 32(7):703–715
105. Kreahling JM et al (2013) Wee1 inhibition by MK-1775 leads to tumor inhibition and enhances efficacy of gemcitabine in human sarcomas. PLoS One 8(3):e57523
106. Kuijjer ML et al (2011) mRNA expression profiles of primary high-grade central osteosarcoma are preserved in cell lines and xenografts. BMC Med Genet 4:66
107. Broström LA, Crnalic S, Löfvenberg R, Stenling R, Boquist L (1996) Structure, growth and cell proliferation of human osteosarcoma and malignant fibrous histiocytoma xenografts in serial transplantation in nude mice. APMIS 104(11):775–783
108. Kresse SH, Meza-Zepeda LA, Machado I, Llombart-Bosch A, Myklebost O (2012) Preclinical xenograft models of human sarcoma show nonrandom loss of aberrations. Cancer 118(2):558–570
109. Kito F et al (2018) Establishment and characterization of novel patient-derived osteosarcoma xenograft and cell line. In Vitro Cell Dev Biol Anim 54(7):528–536
110. Loh AHP et al (2019) Combinatorial screening using orthotopic patient derived xenograft-expanded early phase cultures of osteosarcoma identify novel therapeutic drug combinations. Cancer Lett 442:262–270
111. Crnalic S, Hakansson I, Boquist L, Lofvenberg R, Brostrom LA (1997) A novel spontaneous metastasis model of human osteosarcoma developed using orthotopic transplantation of intact tumor tissue into tibia of nude mice. Clin Exp Metastasis 15(2):164–172

112. Whiteford CC et al (2007) Credentialing preclinical pediatric xenograft models using gene expression and tissue microarray analysis. Cancer Res 67(1):32–40
113. Monsma DJ et al (2012) Genomic characterization of explant tumorgraft models derived from fresh patient tumor tissue. J Transl Med 10(1):1–18
114. Delgado-Baeza E, Miralles-Flores C, Gonzalez-Medievo W (1991) Heterogeneity of xenografted osteosarcoma: a human sarcoma transplanted into nude mice. Acta Orthop 62(5):410–414
115. Igarashi K et al (2018) Temozolomide combined with irinotecan regresses a cisplatinum-resistant relapsed osteosarcoma in a patient-derived orthotopic xenograft (PDOX) precision-oncology mouse model. Oncotarget 9(8):7774–7781
116. Goldstein SD, Trucco M, Bautista Guzman W, Hayashi M, Loeb DM (2016) A monoclonal antibody against the Wnt signaling inhibitor dickkopf-1 inhibits osteosarcoma metastasis in a preclinical model. Oncotarget 7(16):21114–21123
117. Igarashi K et al (2017) A patient-derived orthotopic xenograft (PDOX) mouse model of a cisplatinum-resistant osteosarcoma lung metastasis that was sensitive to temozolomide and trabectedin: implications for precision oncology. Oncotarget 8(37):62111–62119
118. Higuchi T et al (2019) Trabectedin and irinotecan combination regresses a cisplatinum-resistant osteosarcoma in a patient-derived orthotopic xenograft nude-mouse model. Biochem Biophys Res Commun 513(2):326–331
119. Murakami T et al (2017) Tumor-targeting Salmonella typhimurium A1-R regresses an osteosarcoma in a patient-derived xenograft model resistant to a molecular-targeting drug. Oncotarget 8(5):8035–8042
120. Brennecke P et al (2014) CXCR4 antibody treatment suppresses metastatic spread to the lung of intratibial human osteosarcoma xenografts in mice. Clin Exp Metastasis 31(3):339–349
121. Rainusso N et al (2019) Generation of patient-derived tumor xenografts from percutaneous tumor biopsies in children with bone sarcomas. Pediatr Blood Cancer 66(4):e27579
122. Houghton PJ, Cheshire PJ, Myers L, Stewart CF, Synold TW, Houghton JA (1992) Evaluation of 9-dimethylaminomethyl-10-hydroxycamptothecin against xenografts derived from adult and childhood solid tumors. Cancer Chemother Pharmacol 31(3):229–239
123. Houghton PJ et al (2007) The pediatric preclinical testing program: description of models and early testing results. Pediatr Blood Cancer 49(7):928–940
124. Halim A, Bagley RG, Keler T (2016) Abstract 5032: glycoprotein NMB (gpNMB) overexpression is prevalent in human cancers: pancreatic cancer, non-small cell lung cancer, head and neck cancer, and osteosarcoma. Cancer Res 76(14 Supplement):5032–5032
125. Tse KF et al (2006) CR011, a fully human monoclonal antibody-auristatin E conjugate, for the treatment of melanoma. Clin Cancer Res 12(4):1373–1382
126. Kolb EA et al (2014) Initial testing (stage 1) of glembatumumab vedotin (CDX-011) by the pediatric preclinical testing program. Pediatr Blood Cancer 61(10):1816–1821
127. Maki RG (2010) Small is beautiful: insulin-like growth factors and their role in growth, development, and cancer. J Clin Oncol 28(33):4985–4995
128. Scotlandi K, Picci P (2008) Targeting insulin-like growth factor 1 receptor in sarcomas. Curr Opin Oncol 20(4):419–427
129. Rikhof B, de Jong S, Suurmeijer AJH, Meijer C, van der Graaf WTA (2009) The insulin-like growth factor system and sarcomas. J Pathol 217(4):469–482
130. Kolb EA et al (2008) Initial testing (stage 1) of a monoclonal antibody (SCH 717454) against the IGF-1 receptor by the pediatric preclinical testing program. Pediatr Blood Cancer 50(6):1190–1197
131. Wang Y et al (2010) A fully human insulin-like growth factor-I receptor antibody SCH 717454 (Robatumumab) has antitumor activity as a single agent and in combination with cytotoxics in pediatric tumor xenografts. Mol Cancer Ther 9(2):410–418
132. Houghton PJ et al (2010) Initial testing of a monoclonal antibody (IMC-A12) against IGF-1R by the Pediatric Preclinical Testing Program. Pediatr Blood Cancer 54(7):921–926
133. Kolb EA et al (2012) Combination testing (Stage 2) of the Anti-IGF-1 receptor antibody IMC-A12 with rapamycin by the pediatric preclinical testing program. Pediatr Blood Cancer 58(5):729–735
134. Kolb EA et al (2011) Initial testing (stage 1) of the IGF-1 receptor inhibitor BMS-754807 by the pediatric preclinical testing program. Pediatr Blood Cancer 56(4):595–603
135. Cao Y et al (2014) Insulin-like growth factor 1 receptor and response to anti-IGF1R antibody therapy in osteosarcoma. PLoS One 9(8):e106249
136. Kieran MW, Kalluri R, Cho Y-J (2012) The VEGF pathway in cancer and disease: responses, resistance, and the path forward. Cold Spring Harb Perspect Med 2(12):a006593
137. Maris JM et al (2008) Initial testing of the VEGFR inhibitor AZD2171 by the pediatric preclinical testing program. Pediatr Blood Cancer 50(3):581–587
138. Fox E et al (2010) A phase 1 trial and pharmacokinetic study of cediranib, an orally bioavailable pan-vascular endothelial growth factor receptor inhibitor, in children and adolescents with refractory solid tumors. J Clin Oncol 28(35):5174–5181
139. Maris JM et al (2008) Initial testing (stage 1) of sunitinib by the pediatric preclinical testing program. Pediatr Blood Cancer 51(1):42–48
140. Keir ST et al (2010) Initial testing (stage 1) of the multi-targeted kinase inhibitor sorafenib by the pediatric preclinical testing program. Pediatr Blood Cancer 55(6):1126–1133

141. Dubois SG et al (2011) Phase I and pharmacokinetic study of sunitinib in pediatric patients with refractory solid tumors: a children's oncology group study. Clin Cancer Res 17(15):5113–5122
142. Kim A et al (2015) Phase 2 trial of sorafenib in children and young adults with refractory solid tumors: a report from the Children's Oncology Group. Pediatr Blood Cancer 62(9):1562–1566
143. Grignani G et al (2012) 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 Off J Eur Soc Med Oncol 23(2):508–516
144. Harrison DJ et al (2019) Evaluation of the multikinase inhibitor regorafenib in the Pediatric Preclinical Testing Consortium osteosarcoma, rhabdomyosarcoma, and Ewing sarcoma in vivo models. J Clin Oncol 37(15_suppl):10038
145. Davis LE et al (2019) Randomized double-blind phase II study of Regorafenib in patients with metastatic osteosarcoma. J Clin Oncol 37(16):1424–1431
146. Chen Q, Zhou Z, Shan L, Zeng H, Hua Y, Cai Z (2015) The importance of Src signaling in sarcoma. Oncol Lett 10(1):17–22
147. Kolb EA et al (2008) Initial testing of dasatinib by the pediatric preclinical testing program. Pediatr Blood Cancer 50(6):1198–1206
148. Hingorani P, Zhang W, Gorlick R, Kolb EA (2009) Inhibition of Src phosphorylation alters metastatic potential of osteosarcoma in vitro but not in vivo. Clin Cancer Res 15(10):3416–3422
149. Aplenc R et al (2011) Pediatric phase I trial and pharmacokinetic study of dasatinib: a report from the children's oncology group phase I consortium. J Clin Oncol 29(7):839–844
150. Houghton PJ et al (2008) Initial testing (stage 1) of the mTOR inhibitor rapamycin by the pediatric preclinical testing program. Pediatr Blood Cancer 50(4):799–805
151. Houghton PJ et al (2010) Stage 2 combination testing of rapamycin with cytotoxic agents by the Pediatric Preclinical Testing Program. Mol Cancer Ther 9(1):101–112
152. Spunt SL et al (2011) Phase I study of temsirolimus in pediatric patients with recurrent/refractory solid tumors. J Clin Oncol 29(21):2933–2940
153. Schuetze SM et al (2012) Results of a phase II study of sirolimus and cyclophosphamide in patients with advanced sarcoma. Eur J Cancer 48(9):1347–1353
154. Chawla SP et al (2011) Results of the phase III, placebo-controlled trial (SUCCEED) evaluating the mTOR inhibitor ridaforolimus (R) as maintenance therapy in advanced sarcoma patients (pts) following clinical benefit from prior standard cytotoxic chemotherapy (CT). J Clin Oncol 29(15_suppl):10005
155. Chawla SP et al (2012) Phase II study of the mammalian target of rapamycin inhibitor ridaforolimus in patients with advanced bone and soft tissue sarcomas. J Clin Oncol 30(1):78–84
156. Houghton PJ et al (2012) Initial testing (stage 1) of the mTOR kinase inhibitor AZD8055 by the pediatric preclinical testing program. Pediatr Blood Cancer 58(2):191–199
157. Zhang J, Yu X-H, Yan Y-G, Wang C, Wang W-J (2015) PI3K/Akt signaling in osteosarcoma. Clin Chim Acta 444:182–192
158. Gorlick R et al (2012) Testing of the Akt/PKB inhibitor MK-2206 by the Pediatric Preclinical Testing Program. Pediatr Blood Cancer 59(3):518–524
159. Carol H et al (2010) Initial testing (stage 1) of the Akt inhibitor GSK690693 by the pediatric preclinical testing program. Pediatr Blood Cancer 55(7):1329–1337
160. Chandhanayingyong C, Kim Y, Staples JR, Hahn C, Lee FY (2012) MAPK/ERK signaling in osteosarcomas, Ewing sarcomas and chondrosarcomas: therapeutic implications and future directions. Sarcoma 2012:1–8
161. Kolb EA et al (2010) Initial testing (stage 1) of AZD6244 (ARRY-142886) by the Pediatric Preclinical Testing Program. Pediatr Blood Cancer 55(4):668–677
162. Eroglu Z et al (2015) A randomised phase II trial of selumetinib vs selumetinib plus temsirolimus for soft-tissue sarcomas. Br J Cancer 112(10):1644–1651
163. Subbiah V et al (2017) Phase Ib/II study of the safety and efficacy of combination therapy with multikinase VEGF inhibitor Pazopanib and MEK inhibitor Trametinib in advanced soft tissue sarcoma. Clin Cancer Res 23(15):4027–4034
164. Gorlick R et al (2012) Initial testing (stage 1) of the cyclin dependent kinase inhibitor SCH 727965 (dinaciclib) by the pediatric preclinical testing program. Pediatr Blood Cancer 59(7):1266–1274
165. Maris JM et al (2010) Initial testing of the aurora kinase A inhibitor MLN8237 by the Pediatric Preclinical Testing Program (PPTP). Pediatr Blood Cancer 55(1):26–34
166. Mossé YP et al (2019) A phase II study of alisertib in children with recurrent/refractory solid tumors or leukemia: Children's oncology group phase I and pilot consortium (ADVL0921). Clin Cancer Res 25(11):3229–3238
167. Lowery CD et al (2019) Broad Spectrum activity of the checkpoint kinase 1 inhibitor Prexasertib as a single agent or Chemopotentiator across a range of preclinical pediatric tumor models. Clin Cancer Res 25(7):2278–2289
168. Smith MA et al (2012) Initial testing of JNJ-26854165 (Serdemetan) by the pediatric preclinical testing program. Pediatr Blood Cancer 59(2):329–332
169. Lock R et al (2008) Initial testing (stage 1) of the BH3 mimetic ABT-263 by the pediatric preclinical testing program. Pediatr Blood Cancer 50(6):1181–1189
170. Gorlick R et al (2009) Initial testing (stage 1) of lapatinib by the pediatric preclinical testing program. Pediatr Blood Cancer 53(4):594–598

171. Carol H et al (2013) Initial testing of the MDM2 inhibitor RG7112 by the Pediatric Preclinical Testing Program. Pediatr Blood Cancer 60(4):633–641
172. Smith MA et al (2012) Initial testing of the investigational NEDD8-activating enzyme inhibitor MLN4924 by the pediatric preclinical testing program. Pediatr Blood Cancer 59(2):246–253
173. Kolb EA et al (2012) Initial testing (stage 1) by the pediatric preclinical testing program of RO4929097, a γ-secretase inhibitor targeting notch signaling. Pediatr Blood Cancer 58(5):815–818
174. Houghton P, Erickson SW, Teicher B, Smith MA, Han R, Kurmasheva R (2019) In vivo evaluation of the LSD1 inhibitor SP-2577 against Ewing sarcoma and rhabdomyosarcoma preclinincal models: A report from the Pediatric Preclinical Testing Consortium (PPTC) [abstract], in Proceedings: AACR Annual Meeting 2019
175. Pignochino Y et al (2013) The combination of Sorafenib and Everolimus abrogates mTORC1 and mTORC2 upregulation in osteosarcoma preclinical models. Clin Cancer Res 19(8):2117–2131
176. Grignani G et al (2015) Sorafenib and everolimus for patients with unresectable high-grade osteosarcoma progressing after standard treatment: a non-randomised phase 2 clinical trial. Lancet Oncol 16(1):98–107
177. Gobin B et al (2014) NVP-BEZ235, a dual PI3K/mTOR inhibitor, inhibits osteosarcoma cell proliferation and tumor development in vivo with an improved survival rate. Cancer Lett 344(2):291–298
178. Gobin B et al (2015) BYL719, a new α-specific PI3K inhibitor: single administration and in combination with conventional chemotherapy for the treatment of osteosarcoma. Int J Cancer 136(4):784–796
179. Huang J-C et al (2018) NVP-BEZ235 synergizes cisplatin sensitivity in osteosarcoma. Oncotarget 9(12):10483–10496
180. Manara MC et al (2010) NVP-BEZ235 as a new therapeutic option for sarcomas. Clin Cancer Res 16(2):530–540
181. Gobin B et al (2014) Imatinib mesylate exerts anti-proliferative effects on osteosarcoma cells and inhibits the tumour growth in immunocompetent murine models. PLoS One 9(3):e90795
182. Sampson ER et al (2011) The orally bioavailable met inhibitor PF-2341066 inhibits osteosarcoma growth and osteolysis/matrix production in a xenograft model. J Bone Miner Res 26(6):1283–1294
183. Vassilev LT et al (2004) In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 303(5659):844–848
184. Bretscher A, Reczek D, Berryman M (1997) Ezrin: a protein requiring conformational activation to link microfilaments to the plasma membrane in the assembly of cell surface structures. J Cell Sci 110(24):3011–3018
185. Khanna C et al (2004) The membrane-cytoskeleton linker ezrin is necessary for osteosarcoma metastasis. Nat Med 10(2):182–186
186. Bulut G et al (2012) Small molecule inhibitors of ezrin inhibit the invasive phenotype of osteosarcoma cells. Oncogene 31(3):269–281
187. Wan X, Mendoza A, Khanna C, Helman LJ (2005) Rapamycin inhibits ezrin-mediated metastatic behavior in a murine model of osteosarcoma. Cancer Res 65(6):2406–2411
188. Pignochino Y et al (2009) Sorafenib blocks tumour growth, angiogenesis and metastatic potential in preclinical models of osteosarcoma through a mechanism potentially involving the inhibition of ERK1/2, MCL-1 and ezrin pathways. Mol Cancer 8:118
189. Laverdiere C et al (2005) Messenger RNA expression levels of CXCR4 correlate with metastatic behavior and outcome in patients with osteosarcoma. Clin Cancer Res 11(7):2561–2567
190. Kawada K et al (2004) Pivotal role of CXCR3 in melanoma cell metastasis to lymph nodes. Cancer Res 64(11):4010–4017
191. Walser TC et al (2006) Antagonism of CXCR3 inhibits lung metastasis in a murine model of metastatic breast cancer. Cancer Res 66(15):7701–7707
192. Pradelli E et al (2009) Antagonism of chemokine receptor CXCR3 inhibits osteosarcoma metastasis to lungs. Int J Cancer 125(11):2586–2594
193. Wandinger SK, Richter K, Buchner J (2008) The Hsp90 chaperone machinery. J Biol Chem 283(27):18473–18477
194. Zhao R et al (2005) Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone. Cell 120(5):715–727
195. Hu Y, Bobb D, He J, Hill DA, Dome JS (2015) The HSP90 inhibitor alvespimycin enhances the potency of telomerase inhibition by imetelstat in human osteosarcoma. Cancer Biol Ther 16(6):949–957
196. Ory B et al (2016) Blocking HSP90 addiction inhibits tumor cell proliferation, metastasis development, and synergistically acts with Zoledronic acid to delay osteosarcoma progression. Clin Cancer Res 22(10):2520–2533
197. Nomura M et al (2019) Tegavivint and the β-catenin/ALDH axis in chemotherapy-resistant and metastatic osteosarcoma. J Natl Cancer Inst 111(11):1216–1227
198. Baker EK et al (2015) BET inhibitors induce apoptosis through a MYC independent mechanism and synergise with CDK inhibitors to kill osteosarcoma cells. Sci Rep 5:10120
199. Fossey SL et al (2009) Characterization of STAT3 activation and expression in canine and human osteosarcoma. BMC Cancer 9:81
200. Zhang T et al (2017) Toosendanin demonstrates promising antitumor efficacy in osteosarcoma by targeting STAT3. Oncogene 36(47):6627–6639
201. Hirai H et al (2010) MK-1775, a small molecule Wee1 inhibitor, enhances anti-tumor efficacy of various DNA-damaging agents, including 5-fluorouracil. Cancer Biol Ther 9(7):514–522
202. Rajeshkumar NV et al (2011) MK-1775, a potent Wee1 inhibitor, synergizes with gemcitabine to

achieve tumor regressions, selectively in p53-deficient pancreatic cancer xenografts. Clin Cancer Res 17(9):2799–2806

203. Davis LE et al (2017) Integration of genomic, transcriptomic and functional profiles of aggressive osteosarcomas across multiple species. Oncotarget 8(44):76241–76256