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REVIEW

Targeted therapies for bone sarcomas

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Bone sarcomas include a very large number of tumour subtypes, which originate form bone and more particularly from mesenchymal stem cell lineage. Osteosarcoma, Ewing's sarcoma and chondrosarcoma, the three main bone sarcoma entities develop in a favourable microenvironment composed by bone cells, blood vessels, immune cells, based on the 'seed and soil theory'. Current therapy associates surgery and chemotherapy, however, bone sarcomas remain diseases with high morbidity and mortality especially in children and adolescents. In the past decade, various new therapeutic approaches emerged and target the tumour niche or/and directly the tumour cells by acting on signalling/metabolic pathways involved in cell proliferation, apoptosis or drug resistance. The present review gives a brief overview from basic to clinical assessment of the main targeted therapies of bone sarcoma cells.

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Introduction

Current treatment of malignant primary bone tumours consists of excision of the tumour, associated with high toxicity chemotherapy. Unfortunately, in many cases, an absence of response to anti-tumour drugs is observed, leading to the development of metastases and the death of the patient. Survival is closely correlated to the response of tumour cells to anti-mitotic drugs, reaching 70% in 5 years for osteosarcomas in the best series and only 30% when the pulmonary metastases are detected at the time of diagnosis. Ewing's sarcomas also give a poor prognosis in their metastatic form. In fact, the prognosis of patients with bone or medullary metastases and that of patients who relapse is very poor and $<25\%$ of them are cured. Tumours found at the time of diagnosis but that resist to initial chemotherapy also give a poor prognosis. Whether the main cause of most bone sarcomas are unknown, the close relationship between tumours cells and their local microenvironment strongly contributes to their survival and proliferation.[1] This 'seed and soil' theory leads to define the notion of 'niche', which is a specialized environment, which promotes the emergence of tumour stem cells and provides all the factors required for their development. Consequently, a vicious cycle established between the niche and tumour cells is now well recognised for bone sarcomas[2–4] and has been used as therapeutic targets.[5,6] For instance, bone resorption component has been targeted by bisphosphonates and in combination with conventional chemotherapy has shown promising efficacy by enhancing tumour regression and tissue repair and by decreasing lung metastases.[7–11] The most recent knowledge on the biology of bone sarcomas has identified new therapeutic targets expressed by tumour cells, opening a new era of the therapeutic development.[1,12] Targeted therapies could be defined as more specific than conventional chemotherapeutic agents, which target tumour cell proliferation as a whole. The advent of targeted therapies is related to the development of more sophisticated techniques of molecular biology allowing the clinicians to gain insight into genomic and transcriptional data on specific genes whose expression is modulated during tumourigenesis. These new targets constitute the basis for the development of new therapeutic options in many types of cancers including bone sarcomas. Promising data have been published on preclinical studies, some being confirmed at the clinical level. The present review gives a brief overview from basic to clinical assessment of the main targeted therapies recently developed for bone sarcomas.

Inhibition of Growth Factor/Cytokine Signalling Pathways

Most of the signalling pathways are implicated in cell proliferation and apoptosis resistance. They are mediated by proteins with kinase activity, both outside (at the cell membrane) or inside the cells (cytoplasm or nucleus). These proteins may be inhibited by monoclonal antibodies directed against extramembrane receptor or small molecule inhibitors of the intracellular kinase domain (**Figure 1**; **Table 1**).

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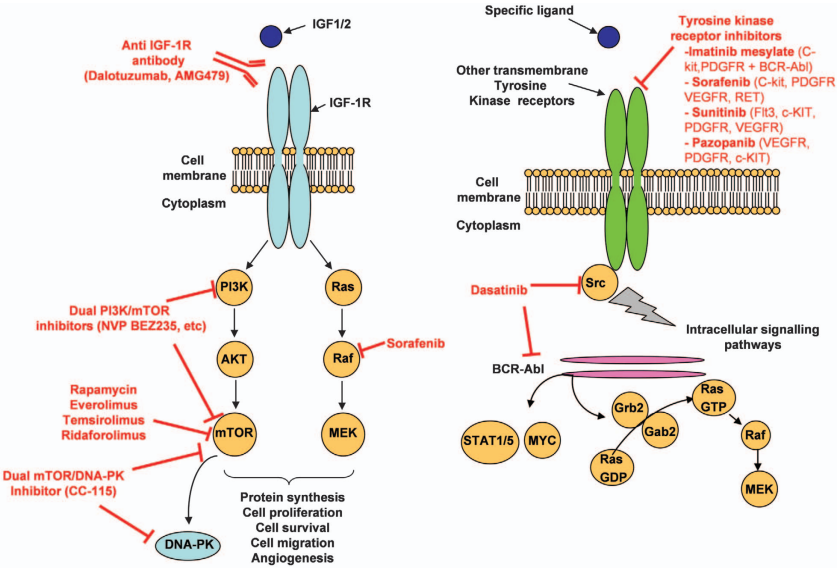

Figure 1 Targeting of signalling pathways. Tyrosine kinase receptors (IGF1-R (right panel) and others such as VEGFR, PDGFR, c-MET and so on (left panel)) are activated upon binding of their respective ligands to their extracellular domain. It subsequently leads to activation of various signalling pathways (PI3K/Akt/mTOR, Ras/RAF/MEK and so on) promoting malignancies. In red, are mentioned the main therapies based on targeting of tyrosine kinase proteins and on associated downstream signalling pathways. Multi-target inhibitors has been also developed (dual PI3K/mTOR, dual mTOR/DNA-PK, dasatinib and so on).

Therapies based on targeting of IGF1-R and associated downstream signalling pathways. The insulin-like growth factor-1 receptor (IGF1-R) pathway has an important role in osteosarcoma and Ewing's sarcoma.[13] As both tumours have a peak incidence at puberty and because osteosarcoma occurs in zone of high bone remodelling rate at long bone metaphyses, a role of growth hormone and insulin-like growth factor is suggested. Concerning Ewing's sarcoma, the IGF axis has been also shown to be a direct target of the EWS–FLI-1 fusion gene.[14] Indeed, gene profiling of Ewing cells in which the EWS/FLI-1 fusion gene had been inactivated allowed the identification of downstream targets. Among these targets, the IGF-binding protein 3 (*IGFBP-3*) gene, a major regulator of IGF-1 proliferation and survival signalling, was strongly induced upon treating Ewing cells with EWS/FLI-1-specific small interfering RNAs. Ligand binding to IGF-1 receptor activates the downstream PI3K/Akt/mTOR pathways, stimulates osteosarcoma and Ewing's sarcoma cell survival and angiogenesis through hypoxia inducible factor-1α and vascular endothelial growth factor (VEGF) secretion. Preclinical data using IGF-R1 inhibitors against xenograft models of paediatric sarcomas, coupled with responses in adults with Ewing sarcoma, have generated significant excitement about the clinical potential of this class of drugs and have driven the rapid development of numerous clinical trials now under way. In contradiction with the everlasting antagonist concept, it has been shown that they can induce receptor downregulation rather than inhibition of the IGF1 effect.[15] With different anti-IGF1-R monoclonal antibodies, children and adolescents suffering of relapsed or refractory Ewing's sarcoma had stable disease (SD) in phase I trials[16] and 10–15% of objective responses in paediatric/adult phase II trials.[17–19] SDs were observed in relapsed/refractory osteosarcoma (SCH 717454, ongoing study P04720, NCT00617890).[20] Predictive factors of the response remain, however, largely unknown. A reduced activity of the IGF system might associate with tumour progression and poor response to treatment,[21] high expression levels of IGF-IR, insulin-like receptors (IR) and IGF-I mRNAs with increased survival, and high circulating IGF-1 levels with low progression risk.[22] Unfortunately, the median duration of Ewing's sarcoma response was very low,[17,18] probably because tumour cells escape through AKT or other feedback loops of signalling pathway. These observations lead to consider using either combination of mono-targeted inhibitors or multi-targeted inhibitors.

mTOR being a downstream pathway activated by IGF-1 binding to its receptor IGF1-R, its targeting has been also largely studied. The mTOR inhibitor rapamycin was first used in children to prevent graft rejection. mTOR, an intra-cytoplasmic serine kinase regulated by AKT has been envisaged to treat osteosarcoma.[23,24] In osteosarcoma cells, rapamycin inhibits proliferation through ezrin,[25] a protein implicated in intracellular signal transduction and migration. In paediatric Ewing's sarcoma, phospho-mTOR overexpression is correlated with survival.[26] Paediatric phase I trials of everolimus and temsirolimus have shown a good tolerance profile.[27,28] One osteosarcoma patient treated by everolimus out of 5 treated by mTOR inhibitors had prolonged SD.[27] Ridaforolimus phase II in sarcomas shows a low response rate $<2\%$ (2/4 patients with responses had osteosarcoma), but 28% of clinical benefit.[29] An everolimus phase II study in children and adolescents with refractory or relapsed osteosarcoma (NCT01216826) is ongoing. A double-blind phase III comparing ridaforolimus against placebo (SUCEED trial) in sarcoma maintenance treatment after stabilisation or regression under chemotherapy, have included 50 bone sarcomas and showed an increased progression free survival (PFS) with mTOR inhibitor.[30] A paediatric phase II is ongoing in refractory or relapsed osteosarcoma in Brazil (NCT01216826). Strategies targeting simultaneously at several levels the IGF1-R/PI3K/AKT/mTOR pathways are being evaluated in preclinical models.[31] A phase I–II of Ridaforolimus combined with the

Table 1 New therapeutic approaches for osteosarcoma and Ewing's sarcoma

Targets	*Therapeutic agents*	*Preclinical (P)/clinical assessment*	*Clinical trials n°*	*References*
IGF1-R inhibitors	R1507; SCH 717454; CP-751871; IMC-A12	Phase I/II paed	NCT00617890 SCH717454 P04720	16–20
mTOR inhibitors	Everolimus (RAD001, Afinitor) Temserolimus (Torisel) Ridaforolimus RAPIRI	II Paed (OS) I paed (EWS) I/II paed Phase II Phase III ad Phase I paed	NCT01216826 NCT01216826 SUCCEED ongoing	27 27 28 29 30
Multitarget inhibitors PDGFR, c-Kit, BCR-ABL Src, BCR-ABL BRaf, c-KIT, PDGFR, VEGFR, RET Flt3, c-KIT, PDGFR, VEGF VEGFR1-3, PDGFRα/β, c-KIT	Imatinib mesylate (Glivec) Dasatinib (Spryvel) Sorafenib (Nexavar) Sunitinib (Sutent) Pazopanib	I I Paed (OS/EWS) Phase II paed Phase I Paed Phase II (OS) P Phase I paed		36,37 38 42 44 45 46 47,48
Cell cycle inhibitors	CDK inhibitors (Dinaciclib) Regin-G Aurora A inhibitor (MLN8237) Aurora A inhibitor (AT9283) PLK1 selective inhibitor (BI 2536) MDM2 inhibitors (Nutlin-3) MDM2 inhibitor (RO5503781)	P Phase I/II ad P Paed phase I Phase I paed P P Adult Phase I	NCT01154816 NCT00985868 NCT01431664 NCT01462175	49 50 51 52 53
Apoptosis	BCL-2 inhibitor (Navitoclax) TRAIL Smac mimetic (LCL161) X-linked IAP antisense	Phase I ad + docetaxel P P Phase I adults + paclitaxel P	NCT01098838 NCT01240655	56 57–61 62 63
Telomerase inhibitors	TMPyP4	P		65–68
Hedgehog pathway inhibitors (SHH/PATCH/Smo/GLI)	Cyclopamine, arsenic trioxide LDE225	P Phase I paed	NCT01125800	73–75
HDAC inhibitors	HDAC inhibitors (SNDX-275) FK228 Vorinostat, valproic acid	P P Phase I paed		79–83 86–88 77,78
HSP90 inhibitors	17-AAG	P Phase I paed		89,90
c-Met inhibitors	PF-2341066	P		94,95

Abbreviations: Ad, adult patients; EWS, Ewing's sarcoma; HSP, heat shock protein; IGF, insulin-like growth factor; OS, osteosarcoma; Paed, paediatric patients; PDGFR, platelet-derived growth factor receptor; TRAIL, TNF-related apoptosis-inducing ligand; VEGFR, vascular endothelial growth factor.

anti-IGF1-R antibody Dalotuzumab is ongoing (NCT01431547) in children in Europe and United States. Dual PI3K/mTOR inhibitors (NVP-BEZ235 and so on) are in adult phase I trial and dual mTOR/DNA-PK inhibitor (CC-115) in adolescent/adult phase I trial (NCT01353625). To bypass resistance to mTOR inhibitors, which have been observed in some patients, combined treatment with bisphosphonate showed promising efficacy in preclinical models of osteosarcoma.[32]

Multi-target inhibitors for bone sarcomas. As several signalling pathways are activated during tumour growth, the development of drugs that have several targets (mostly with kinase activity) has recently emerged in many types of cancers, including osteosarcoma and Ewing's sarcoma (**Table 1**).

Imatinib mesylate inhibits PDGFR, c-KIT and BCR-ABL. High expression of c-KIT (stem cell factor receptor) and platelet-derived growth factor receptor (PDGFR) is observed in Ewing's sarcoma and osteosarcoma[33] and associated with low event free survival but not with poor response to chemotherapy.[33] Imatinib appeared to have an anti-Ewing's sarcoma activity *in vitro* and in xenografts.[34] However, expression of imatinib targets is not sufficient to confer drug sensitivity.[35] Several phase II trials have shown stabilisation of bone sarcomas (3/20 Ewing's sarcoma, 7/26 osteosarcoma) with a median PFS <2 months.[36,37] In a COG paediatric phase II trial, only 1/24 Ewing's sarcoma had partial response (PR).[38] Preclinical data showed increased anti-tumour activity of imatinib mesylate when combined with doxorubicin and vincristin[39] in Ewing's sarcoma or ifosfamide in osteosarcoma.[32]

Dasatinib inhibits Src and BCR-ABL. Dasatinib shows *in vitro* cytostatic and anti-migration effect and no apoptosis in Ewing's sarcoma.[40] Src has a role in osteosarcoma cell adhesion/migration through FAK decrease, but its inhibition does not prevent metastasis,[41] suggesting a secondary role for Src in this process. Paediatric phase I trial showed similar dasatinib pharmacokinetic in children and adults.[42]

Sorafenib inhibits BRaf, c-KIT, PDGFR, VEGFR, RET. In osteosarcoma, sorafenib inhibits tumour growth, angiogenesis (by VEGF inhibition), invasion (by MMP2 inhibition) and pulmonary

metastases formation (via inhibition of the Ezrin/β4-integrin/PI3K signalling pathway), and induced apoptosis.[43] A phase II trial of 35 osteosarcoma patients aged more than 14 years treated in second or third line showed 14% of objective response (3 PRs, 2 minor responses and 29% of tumour control (12 additional SDs). Tumour control lasted ⩾6 months for 8 patients. The median PFS and survival were 4 and 7 months, respectively.[44]

Sunitinib inhibits Flt3 (fms-related tyrosine kinase-3), c-KIT, PDGFR, VEGF. Efficacy was observed in *in vivo* models of most paediatric tumours, including 4/5 Ewing's sarcoma xenografts.[45] In paediatric phase I trial, the main toxicities were haematological and cardiac for children previously treated with anthracyclins.[46]

Pazopanib inhibits VEGFR1-3, PDGFRα/β, c-KIT. Pazopanib appeared active in paediatric *in vivo* tumour models, used as single agent in Ewing's sarcoma[47] or combined with metronomic topotecan in osteosarcoma.[48] A phase II study of pazopanib in bone sarcoma is ready to begin in Europe.

Inhibition of Cell Growth Depending on Cell Cycle Regulators

The CDK (cyclin-dependent kinase) inhibitor dinaciclib induces *in vitro* osteosarcoma cell apoptosis.[49] Phase I/II of rexin-G, a pathotropic nanoparticle bearing acytocidalcyclin G1 construct showed low toxicity in relapsed osteosarcoma, 2/3 SD and 7 months survival[50] (**Figure 2**). Aurora A has a crucial role during mitosis. Aurora A inhibitor, MLN8237, leads to prolonged complete response in *in vivo* Ewing's sarcoma and osteosarcoma models.[51] Two aurora A inhibitors, MLN8237 (NCT01154816) and AT9283 (NCT00985868 and NCT01431664), are in paediatric phase I development. The polo-like kinase 1 (PLK1) selective inhibitor, BI 2536 had antiproliferative effects and induces mitotic death in osteosarcoma cell lines[52] (**Figure 2**). Mouse double minute 2 homologue (*Mdm2*/E3 ubiquitin-protein ligase Mdm2) is an oncoprotein that negatively regulates p53, overexpressed in p53 wild-type cancers. Mdm2 inhibitors such as nutlin-3, activate p53 signalisation pathway leading to important tumour regressions in osteosarcoma xenografts through apoptotsis[53] (**Figure 2**). This effect is also seen in p53 wild-type Ewing's sarcoma and can be increased by either nuclear factor kappa B inhibition through tumour necrosis factor-α (TNF-α),[54] or histone desacetylase (HDAC) inhibitors.[55] An adult phase I study with an oral MDM2 inhibitor (RO5503781) is ongoing in solid cancers (NCT01462175) (**Table 1**).

Targeting of Cell Death Resistance

Resistance to apoptosis is a key element in tumour progression and chemoresistance. The mechanisms involved increased survival signals (growth factors/TK receptors, downstream pathways), anti-apoptotic molecule overexpression (Bcl-2, Bcl-XL and FAK in osteosarcoma), pro-apoptotic molecule under expression (Bim in osteosarcoma), or resistance to cell death receptors Fas/FasL (Fas ligand) or TRAIL (TNF-related apoptosis-inducing ligand) (**Figure 2**; **Table 1**).

1. The BCL-2 inhibitors navitoclax is developed in adult refractory tumours in combination with docetaxel, with acceptable toxicity and few responses (2 PR, 5 SD).[56]
2. TRAIL is a pro-apoptotic cytokine belonging to the TNF-a superfamily that inhibits several EWS cell lines *in vitro*.[57,58] Experimental data reported that TRAIL inhibits Ewing's sarcoma tumour growth in mice models, decreases osteolysis, prolongs survival and decreases pulmonary metastatic spread.[59] Combination with Imatinib further increased TRAIL effect on tumour growth and metastases in *in vivo* Ewing's sarcoma models.[60] In a recent study, van Valen *et al.*[61]

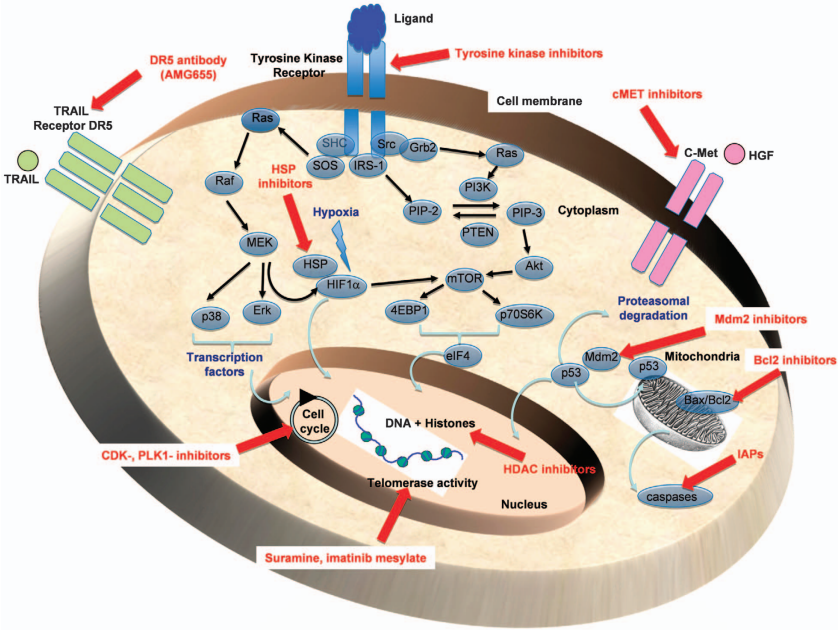

Figure 2 Targeting of key factors associated to cell cycle, cell death resistance, autophagy and other metabolic activities. Genomic, transcriptional and functional analyses carried out on bone sarcoma cells identified numerous targets, which constitute the basis for the development of sophisticated and promising therapies (in red). These targets are involved in the key mechanisms controlling cell biology (cell cycle, apoptosis, replicative immortality, autophagy, histone deacetylation and acetylation, and so on).

showed that anti-IGF1-R antibody sensitise Ewing's sarcoma cells to apoptosis induced by TRAIL. In addition, a multi-center 2-part phase 1b/2 study is ongoing using AMG655, the antibody agonist of TRAIL death receptor 5 in combination with AMG479 (antibody agonist of IGF1-R)(NCT00819169) (**Table 1**).

3. Inhibitors of apoptosis proteins (IAPs) inhibit caspase-dependent apoptosis. Smac, a mitochondrial protein binds to IAPs, impedes the formation of the protective complex IAP/caspase and facilitates caspase degradation by the proteasome. Smac mimetic LCL161 increases event free survival of paediatric *in vivo* models, including 5/6 osteosarcoma and glioblastomas.[62] LCL161 adult phase I trial in solid tumours has just finished (NCT01098838) and a combination trial with paclitaxel is ongoing (NCT01240655). X-linked IAP antisense oligonucleotide (XIAP, ASO-AEG35156) decreases XIAP in paediatric osteosarcoma, rhabdomyosarcoma and Ewing's sarcoma cell lines, and sensitises osteosarcoma to doxorubicin, etoposide and vincristin[63] (**Table 1**).
4. Replicative immortality through telomerase activity restoration in cancer cells induces resistance to cell death. Telomerase activity is present in 85% of metastases (100% in Ewing's sarcoma and 75% in osteosarcoma), but only in 12% of primary bone tumours and associates with shortened telomeres and decreases patient survival.[64] Telomerase is inhibited by suramine in osteosarcoma[65] and imatinib mesylate, doxorubin or irradiation in Ewing's sarcoma.[66–68]

Other Exploitable Therapeutic Pathways

Targeting of autophagy. Autophagy, a cell survival program implicated in tumourogenesis and chemoresistance,[69] participates through HMGB1 (high-mobility group protein B1) to osteosarcoma resistance to doxorubicin, cisplatin and methotrexate. HMGB1 inhibition by small interfering RNA restores chemosensitivity.[70] HMGB1 binds to Beclin1 that regulates the Beclin1–PI3KC3 complex formation and favours autophagy. The 2-*O*,3-*O*-disulfateheparine is a low weight anticoagulant with anti-inflammatory activity but low anticoagulant activity.[71] It might exert its anti-tumour action through inhibition of heparinase (invasion), selectins (pulmonary metastatic spread) and RAGE (receptor for advanced glycation end products), which is no more able to bind to HMGB1 (pro-inflammatory and pro-autophagy roles).

Hedgehog pathway inhibitors (SHH/PATCH/Smo/GLI). Hedgehog signalling pathway has an important role in growing organisms (embryogenesis, morphogenesis) and is activated in osteosarcoma and Ewing's sarcoma (GLI is a EWS–FLI1 target).[72,73] Its inhibition by cyclopamine in osteosarcoma[74] and arsenic trioxide, a GLI inhibitor in Ewing's sarcoma,[73] limits tumour growth. Arsenic trioxide inhibits the growth of chemotherapy-resistant osteosarcoma cells through inducing apoptosis.[75] A paediatric phase I study with smoothen inhibitor LDE225 is ongoing (NCT01125800). Itraconazole, another inhibitor of the Hedgehog pathway, is a commonly used antifungal that inhibits cancer growth[76] (**Table 1**).

HDAC inhibitors. HDAC and histone acetyl transferase (HAT) are enzymes that catalyse histone deacetylation and acetylation, respectively, modifying chromatin access to transcription factors and gene transcription. Two paediatric phases I trials have been completed with two HDAC inhibitors (vorinostat and valproic acid).[77,78] In osteosarcoma models, HDAC inhibitors decrease DNA reparation ability,[79] sensitise cells to irradiation[80] and doxorubicine,[81] and decreases FLIP expression, a caspase 8 negative regulator.[82] Another HDAC inhibitor, SNDX-275, given by nasal administration has a preventive action against pulmonary metastases in murine osteosarcoma model.[83] But HDAC inhibitors are suspected to exert negative effects in osteosarcoma through induced-Notch expression and invasion, which might facilitate osteosarcoma metastatic potential.[84] In Ewing's sarcoma cells, EWS–FLI1 represses HAT and activates HDAC.[85] HDAC inhibition restores HAT activity, inhibits cell growth and induces apoptosis. Another HDAC inhibitor (FK228) decreases EWS–FLI1 expression and Ewing's sarcoma proliferation and induces TRAIL-dependent apoptosis.[86] Acquired resistances to the cyclic tetrapeptide HDAC inhibitor family (FK228) are mediated by the drug efflux P glycoprotein and the MAPK pathway, and might be reverted by verapamil in Ewing's sarcoma,[87] and MEK inhibitors in osteosarcoma.[88]

Heat shock protein 90 (HSP90) inhibitors. HSP90 is a chaperone protein implicated in numerous cancers, overexpressed in 21/54 Ewing's sarcoma patient samples.[89] Sera anti-HSP90 antibodies associate with osteosarcoma poor response to chemotherapy.[90] HSP90 inhibitors induce proteasome-mediated degradation of many oncogenic proteins involved in all hallmark characteristics of cancer. 17-AAG induces apoptosis *in vitro*[24] and osteosarcoma growth retardation *in vivo* as single agent and in combination with cisplatin,[90] and restores efficacy of IGF1-R inhibitor and Imatinib in Ewing's sarcoma models.[89] No objective response was observed in two paediatric phase I trials (SD in 1/3 Ewing's sarcoma patients, 0/7 in osteosarcoma). However, acquired resistance to 17-AAG is rapid,[91] and new generations of HSP90 inhibitors might be more promising (adult phase I/II trials ongoing) (**Table 1**).

c-Met inhibitors. c-Met belongs to the receptor tyrosine kinases and is strongly involved in the control of mitosis, cell motility and cell survival and consequently alterations (overexpression, mutation and so on) of c-Met signalling induced by its ligand, the hepatocyte growth factor lead to the proliferation, invasiveness and metastasis of numerous cancer cell types including osteosarcoma. hepatocyte growth factor receptor/c-Met has been shown to be overexpressed and activated in osteosarcoma cells.[12,92] Very recently, it has been shown that c-Met overexpression by primary culture of human bone-derived cells drives the cell differentiation into osteosarcoma.[93] The corresponding transformed cells exhibited both mesenchymal and stemness markers and authors suggest that c-Met initiates the transformation of bone cells by regulating self-renewal of osteosarcoma cells (894). Overall, these data identify c-Met as a potential target in osteosarcoma. Oral inhibitor of c-Met (PF-2341066) showed promising clinical responses in non-small-lung cancer and its efficacy has been addressed in preclinical models of osteosarcoma.[94] The results

revealed that PF-2341066 inhibits the survival, proliferation, invasiveness and clonogenicity of osteosarcoma cells. In addition, this drug inhibits the *in vivo* tumour growth as well as associated-tumour bone remodelling.[94] Combined treatments with c-Met has been also assessed and showed that inhibition of c-Met pathway enhances chemosensitivity.[95]

Conclusion

The multiplicity of targets in primitive malignant bone tumours of children and adolescents and the experience with anti-IGF1-R antibodies suggest that therapeutic future in these tumours will reside in the way of combining these therapies targeting different characteristics of the malignant cells and their environment. The development of therapies targeting founder genetic abnormalities such as EWS-FLI in EW appears crucial. More efforts remain necessary to understand biological processes implicated in osteosarcoma oncogenesis. An increasing number of new molecular therapies becoming available and the rarity of these tumours also require developing relevant preclinical models and new methodologies for therapeutic trials.

Conflict of Interest

The authors declare no conflict of interest.

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