Receptor Tyrosine Kinases in Osteosarcoma: 2019 update

Running Title: RTKs in Osteosarcoma

Edward M. Greenfield, Christopher D. Collier, and Patrick J. Getty

E. M. Greenfield, PhD
Department of Orthopaedic Surgery, Indiana University School of Medicine, FH 115 ORTS, Indianapolis, IN 46202, USA
email: egreenf@iu.edu

C. D. Collier, MD
Department of Orthopaedics, University of Chicago Pritzker School of Medicine, 5841 Maryland Avenue, MC 3079, Chicago, IL 60637, USA
email: collier.christopher.d@gmail.com

P.J. Getty, MD
Department of Orthopaedics, Case Western Reserve University, Cleveland, OH 44106, USA
Seidman Cancer Center, University Hospitals – Cleveland Medical Center, Cleveland, OH 44106, USA
Email: Patrick.Getty@UHhospitals.org

Corresponding Author: Edward M. Greenfield PhD; egreenf@iu.edu

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Abstract

The primary conclusions of our 2014 contribution [1] to this series were:

- Multiple receptor tyrosine kinases (RTKs) likely contribute to aggressive phenotypes in osteosarcoma and therefore inhibition of multiple RTKs are likely necessary for successful clinical outcomes [2, 3].
- Inhibition of multiple RTKs may also be useful to overcome resistance to inhibitors of individual RTKs as well as resistance to conventional chemotherapies [2, 3].
- Different combinations of RTKs are likely important in individual patients.
- AXL, EPHB2, FGFR2, IGF1R, and RET were identified as promising therapeutic targets by our in vitro phosphoproteomic/siRNA screen of 42 RTKs in the LM7 and 143B highly-metastatic human osteosarcoma cell lines [4].

This chapter is intended to provide an update on these topics as well as the large number of osteosarcoma clinical studies of inhibitors of multiple tyrosine kinases (multi-TKIs) that were recently published.

AXL

AXL, from ανεξελεκτός the Greek word for uncontrolled, was originally identified as a transforming gene in chronic myelogenous leukemia. It is the primary member in the mesenchymal lineage of the TAM family of RTKs that also includes TYRO3 and MER. GAS6 is the primary ligand for the TAM RTKs. The initial evidence suggesting that AXL might be important in osteosarcoma was that AXL is the most highly upregulated (~40-fold) of the 637 measured cancer-related mRNAs in highly-metastatic subclones of the HuO9 human osteosarcoma cell line [5]. Osteosarcoma cell lines also had the second highest level of AXL mRNA of the 37 types of cancer cell lines included in the Broad Institute Cancer Cell Line Encyclopedia [1]. A phosphoproteomics study found abundant AXL phosphorylation in all four human osteosarcoma cell lines that were studied [6]. AXL expression may be higher in tumors than in those cell lines as its transcription is induced by hypoxia, at least in epithelial cancers [7]. In that regard, AXL was detected by immunohistochemistry in 30 out of 40 human osteosarcomas but in only 8 out of the 40 adjacent non-cancerous tissues [8]. Most importantly, high levels of AXL mRNA correlated with poor clinical outcomes in a study of 68 osteosarcoma patients [9]. Osteosarcoma cell lines also had the seventh highest level of
GAS6 mRNA of the human cancer cell lines included in the Broad Institute Cancer Cell Line Encyclopedia [1]. In contrast, GAS6 mRNA is down-regulated in primary osteosarcoma biopsies and human osteosarcoma cell lines compared with both bone marrow derived stromal cells and osteoblasts [10]. Moreover, low levels correlated with poor clinical outcomes in that study of 83 osteosarcoma patients [10]. A high level of immunostaining for active phosphorylated AXL was also reported to correlate with poor clinical outcomes in osteosarcoma patients [11]. However, we (unpublished data) found that the anti-phospho-AXL antibody used in that study is not specific when used for immunohistochemistry.

Our in vitro phosphoproteomic/siRNA screen identified AXL as contributing to migration, invasion and non-adherent colony formation, but not to cell growth, by the highly-metastatic 143B human osteosarcoma cell line [4]. More recently, we found that AXL shRNA also inhibits migration, non-adherent colony formation, growth of spherospheres generated from highly-metastatic human osteosarcoma cell lines [12]. Other investigators reported that AXL shRNA inhibits proliferation and induces apoptosis of the MG63 human osteosarcoma cell line [8] and GAS6 inhibits apoptosis and increases migration by the MG63 and U2OS human osteosarcoma cell lines [11]. All of those in vitro results are consistent with our finding that stable transfection of two different AXL shRNA constructs reduced tumor growth by ~70% and the number of metastases by ~90% by the 143B cell line in orthotopic murine xenografts [12]. A miR-199a-3p mimic down-regulates AXL mRNA and inhibits in vitro migration by the MG63 and U2OS human osteosarcoma cell lines [13]. Moreover, high levels of that miR correlated with better clinical outcomes in a study of 30 osteosarcoma patients [13]. The same group of investigators went on to show that overexpression of the lncRNA DANCR upregulates AXL, increases proliferation, migration, invasion, and expression of stemness genes by the HOS and 143B human osteosarcoma cell lines in vitro, and increases tumor growth and the number of metastases formed by the 143B cell line in subcutaneous murine xenografts [9]. Moreover, high levels of DANCR correlated with poor clinical outcomes in osteosarcoma patients [9].

Multiple small molecule inhibitors that target the ATP-binding domain of AXL are in development [14, 15]. Most, if not all, of them target multiple RTKs [14, 15]. More specific inhibition can be achieved by targeting the extracellular domain of AXL and the other TAM family RTKs with small molecules [16], neutralizing antibodies [17], decoy receptors [18], or nucleic acid aptamers [19]. However, the polypharmacology of the
more common inhibitors that target the intracellular ATP-binding domain may contribute to their potential clinical efficacy [2, 3]. For example, BGB324 (previously known as R428), which is often considered to be specific for AXL, also potently inhibits a number of other RTKs, including RET [16, 20]. Indeed, BGB324 inhibits growth in our in vitro 3D sarcosphere platform [21] by both AXL-dependent and AXL-independent mechanisms [12].

AXL and the other TAM RTKs can cause resistance to conventional chemotherapeutics and kinase inhibitors in many other cancers [15, 22, 23]. Molecular mechanisms responsible for that resistance include feedback loops that increase expression of the TAM RTKs or their ligand, GAS6, crosstalk with other kinases or other oncogenes, and induction of dormancy [15, 22-28]. AXL and the other TAM RTKs also repress innate immunity [29] and targeting their activity might therefore be especially useful in combination therapy with liposomal muramyl tripeptide, a macrophage activator approved for osteosarcoma therapy in Europe [30]. Activation of innate immunity by targeting AXL or the other TAM RTKs may also increase the efficacy of T cell-mediated immune checkpoint therapy [31, 32]. The discovery of T cell-mediated cancer immunotherapy received the 2018 Nobel Prize in Physiology or Medicine [33] and has also received considerable attention as a potential therapy for osteosarcoma [34, 35].

**EPHB2**

EPHs were originally discovered in an Erythropoietin-producing hepatocellular carcinoma cell line as a homologue of the viral oncogene v-fps. The 14 mammalian EPHs comprise the largest RTK family [36]. EPHA3, EPHB2, and EPHB3 mRNAs were highly expressed in human osteosarcoma tissue samples when compared to primary human osteoblasts [37]. Proteomic studies showed that cell surface levels of EPHA2, EPHB2, and EPHB4 are respectively 12-, 43-, and 20-fold more abundant on five human osteosarcoma cell lines than on primary human osteoblasts [38] and found abundant EPHB2 phosphorylation in one of the four tested human osteosarcoma cell lines [6]. Our in vitro phosphoproteomic/siRNA screen detected higher levels of EPHA2, EPHA4, and EPHB2 in the highly-metastatic LM7 human osteosarcoma cell line than in its non-metastatic parental SAOS-2 cell line and identified EPHB2 as contributing to migration and non-adherent colony formation, but not to cell growth or invasion, by the LM7 cell line [4]. We confirmed the siRNA results
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with EPHB2 antisense experiments [4]. Other investigators showed that mRNAs encoding EFNA5 and EFNB1, two of the ligands that activate EPHB2 as well as a number of other EPH RTKs, are upregulated in human osteosarcomas and EFNB1 mRNA level was prominent in samples from patients with poor clinical outcomes [39]. EPHB2 is also highly expressed in SYT-SSX2-positive synovial sarcoma tissues and SYT-SSX2–induced stabilization of the microtubule network is blocked by soluble forms of the extracellular domain of EPHB2 that bind and inactivate its ligands [40]. Given that osteosarcomas arise from relatively immature members of the osteoblast lineage [41], it is intriguing that EPHB2 and the other EPH RTKs modulate differentiation at multiple steps in that lineage [36, 42, 43].

FGFR2

FGFRs were originally identified biochemically on fibroblasts and muscle cells as membrane receptors that bind Fibroblast Growth Factors. All four of the FGFRs are amplified in human osteosarcomas [44-47]. Those amplifications can predict responsiveness to NVP-BGJ398, a fairly specific inhibitor of FGFR1-3, and are associated with a poor response to conventional osteosarcoma chemotherapy [45, 46]. A phosphoproteomics study found abundant FGFR1 phosphorylation in all four human osteosarcoma cell lines that were studied, and abundant phosphorylation of FGFR2 and FGFR4 in two of them [6]. A separate study found abundant FGFR1 phosphorylation in ~70% of human osteosarcomas but did not examine the other FGFRs [48]. Moreover, the intensity of total FGFR immunostaining in primary osteosarcomas correlated with metastasis and reduced survival [49]. Both FGFR1 and FGFR2 were identified as contributing to viability of human osteosarcoma cell lines in a kinome-wide siRNA screen [50]. Our in vitro phosphoproteomic/siRNA screen detected higher levels of FGFR2 and FGFR3 in the highly-metastatic LM7 human osteosarcoma cell line than in its non-metastatic parental SAOS-2 cell line and identified FGFR2 as contributing to migration and non-adherent colony formation, but not to cell growth or invasion, by the LM7 cell line [4]. We confirmed the siRNA results with FGFR2 antisense experiments [4].

Signalling by FGFR2 can support stemness in many cancers, including osteosarcoma [51, 52]. An elegant study recently showed that FGFR2 signalling induces fibrogenic reprogramming in human osteosarcoma cell line-derived stem cells, which, in turn, induces growth of metastases in the lung microenvironment without affecting growth of the primary tumor [49]. Those results led to experiments in which nintedanib, an inhibitor
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of FGFR1-3, reduced stemness and the fibrogenic reprogramming, and increased apoptosis, in the human osteosarcoma cell line-derived stem cells as well as in stem cells derived from all six of the primary human osteosarcomas that were studied [49]. Moreover, a preventive regimen of nintedanib blocked lung metastasis following tibial or tail vein injection of the Well5 human osteosarcoma cell line, and even more impressively, a therapeutic regimen of nintedanib caused regression of lung metastases [49]. A preventive regimen of another FGFR inhibitor, AZD4547, reduced metastasis from an orthotopic human osteosarcoma xenograft model [53]. PD173074, in combination with doxorubicin inhibited growth and stemness of the primary tumors in a murine syngeneic subcutaneous model, while neither agent had detectable effects as monotherapies [52]. It should however be noted that nintedanib, AZD4547, and PD173074 inhibit multiple tyrosine kinases with similar or greater potency than the FGFRs [54, 55].

IGF1R

IGF1R was originally identified biochemically as the type 1 membrane receptor that binds Insulin-like Growth Factor-I and -II. Amplification of IGF1R occurs in 14-31% of osteosarcomas, depending on the threshold used to define amplification [56, 57]. Those studies also found other genetic events predicted to activate IGF1R (amplifications of IGF1 or IGF2 and deletions of either IGF2R, IGFBP3, or IGFBP5) in an additional 4.5-19% of the osteosarcomas. IGF1R mRNA and IGF1R protein levels are substantially increased in human osteosarcomas compared with adjacent non-cancerous tissues [58] and a phosphoproteomics study found abundant IGF1R phosphorylation in three of the four human osteosarcoma cell lines that were studied [6]. IGF1R mRNA and IGF1R protein levels are substantially increased in human osteosarcomas compared with adjacent non-cancerous tissues [58]. Moreover, higher IGFIR protein levels in the tumors associate with poor clinical outcomes in both human [58, 59] and canine osteosarcomas [60]. At least eight miR’s have been reported to inhibit proliferation and other in vitro measures of osteosarcoma aggressiveness in part by targeting IGF1R [61-68]. IGF2 siRNA substantially reduced growth of the MG63 human osteosarcoma cell line in low-serum cultures [69] and exogenous IGF2 can induce dormancy in both human and murine osteosarcoma cell lines and thereby induce resistance to methotrexate, doxorubicin, and cisplatin [70]. Consistent with those in vitro findings, elevated IGF2 serum levels associate with decreased event-free survival in osteosarcoma patients [69] and IGF2 mRNA tumor levels were reduced
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post-chemotherapy in the five osteosarcoma patients who responded well to chemotherapy but were either unchanged or increased 13-fold in the two osteosarcoma patients who did not respond [70].

IGF1R is one of the most studied RTKs in osteosarcoma [71]. We therefore consider the identification of IGF1R as contributing to cell growth, migration, invasion, and non-adherent colony formation by the highly-metastatic LM7 human osteosarcoma cell line as validation of our in vitro phosphoproteomic/siRNA screen [4]. We confirmed the siRNA results with an IGF1R neutralizing antibody [4]. Other investigators found that stable transfection of IGF1R shRNA reduced adhesion, migration and invasion in vitro as well as the number of metastases and increased survival of mice following tail vein injection of the U2OS human osteosarcoma cell line [58]. A recent study showed that IGF1R upregulation is responsible for the increased in vitro measures of osteosarcoma aggressiveness that are induced by overexpression of CYR61/CCN1 [72]. We [73] and other investigators [74] found that picropodophyllin, which was originally described as an IGF1R inhibitor [75], reduced growth, migration, and non-adherent colony formation, and induced apoptosis, by multiple human osteosarcoma cell lines. However, subsequent studies showed that the effects of picropodophyllin are primarily due to microtubule destabilization, rather than inhibition of IGF1R [76, 77].

IGF binding proteins (IGFBPs) can inhibit IGF1R activity by sequestering IGFs [78]. In that regard, IGFBP3, IGFBP4, IGFBP6, and IGFBP7 mRNA levels were down-regulated in primary osteosarcomas and in two osteosarcoma patient-derived xenografts compared with mesenchymal stem cells before and after osteogenic differentiation [37, 79]. Similarly, IGFBP5 mRNA and IGFBP5 protein levels were substantially reduced in highly-metastatic human osteosarcoma cell lines compared with isogenic, but weakly-metastatic, cell lines and immunostaining for IGFBP5 was reduced in metastases compared with matched primary osteosarcomas from the same patients [80]. Low levels of IGFBP4 mRNA correlated with poor clinical outcomes in the study of 83 osteosarcoma patients described above in the section on AXL [10]. Moreover, IGFBP5 overexpression induced apoptosis and inhibited primary tumour growth and metastasis by the highly-metastatic cell lines in orthotopic murine xenografts, and IGFBP5 siRNA had the opposite effects [80].

An IGF1R neutralizing antibody inhibited primary tumor growth in subcutaneous xenografts of multiple human osteosarcoma cell lines [81, 82]. In a similar xenograft model, the combination of two neutralizing antibodies
that bind to different epitopes on IGF1R inhibited primary tumor growth more effectively than either agent as monotherapy [83]. Three different IGF1R neutralizing antibodies in combination with a mTOR inhibitor reduced primary tumor growth more effectively than either agent as monotherapy in multiple subcutaneous xenograft osteosarcoma models [84-86]. Nonetheless, multiple IGF1R neutralizing antibodies showed little clinical efficacy against osteosarcoma in phase II studies, either alone [87, 88] or in combination with a mTOR inhibitor [89, 90]. Targeting IGF1R along with other RTKs might be more effective as dual IGF1R/IR inhibitors resensitized doxorubicin-resistant and cisplatin-resistant subclones of human osteosarcoma cell lines in vitro [91, 92]. Moreover, the combinations of IGF1R siRNA and insulin receptor siRNA or neutralizing antibodies against IGF1R and HER2 were more effective in combination than alone at reducing in vitro growth of human osteosarcoma cell lines [69, 93]. A bispecific IGF1R/EGFR neutralizing antibody inhibited both tumor growth and the number of metastases from the 143B human osteosarcoma cell line in an orthotopic murine xenograft model [94]. Antibodies against either of those RTKs had less effect, either alone or in combination, and the authors suggest that the recruitment of Natural Killer (NK) cells by the bispecific antibody may account for its increased efficacy [94]. The EGFR neutralizing antibody used in that study stimulates NK cell-mediated cytotoxicity against the SJSA-1 human osteosarcoma cell line in vitro [95] but we are unaware of similar studies with the bispecific IGF1R/EGFR neutralizing antibody.

Identification of biomarkers that predict which osteosarcoma patients will respond robustly is another approach that could increase the clinical efficacy of IGF1R inhibitors [56, 96]. In the osteosarcoma clinical studies, however, responses to IGF1R neutralizing antibodies, either alone or in combination with the mTOR inhibitor, did not correlate with IGF1R mutations or amplifications or with levels of IGF1R mRNA or IGF1R protein [89, 97, 98]. However, nuclear immunostaining for IGF1R in the absence of cytoplasmic staining associated with 6-fold longer progression-free survival and 4-fold higher overall survival in a study of soft tissue sarcoma (n = 9), Ewing sarcoma (n = 3), and osteosarcoma (n = 4) patients treated with IGF1R neutralizing antibodies [97]. In that regard, a number of recent studies found that nuclear IGF1R can contribute to in vitro measures of aggressiveness in epithelial cancers [99-101].
RET

RET (rearranged during transfection) was originally identified as a transforming gene in lymphoma. Translocation-induced RET fusion genes are well known oncogenes in epithelial cancers such as thyroid and non-small-cell lung cancer [102, 103]. Although RET fusion proteins have not been identified in osteosarcoma [56], RET point mutations or overexpression can also be oncogenic in the absence of translocations [103, 104]. Our in vitro phosphoproteomic/siRNA screen detected higher levels of RET in the highly-metastatic LM7 and 143B human osteosarcoma cell lines than in their non-metastatic parental SAOS-2 and HOS-TE85 cell lines and identified RET as contributing to migration, and to a lesser extent non-adherent colony formation, but not to cell growth or invasion by the LM7 cell line [4]. We confirmed the siRNA results with RET antisense experiments [4]. Chen and colleagues reported that RET siRNA can also decrease migration, invasion and colony formation by other human osteosarcoma cell lines [105]. Most importantly, high levels of RET mRNA associated with poor clinical outcomes in studies of 68 and 19 osteosarcoma patients [105, 106].

Overexpression of the IncRNA MALAT1 upregulates RET in human osteosarcoma cell lines in vitro, at least in part, by inhibiting miR-129-5p [105]. MALAT1 overexpression increases, and MALAT1 knockdown decreases, proliferation, invasion and colony formation by multiple human osteosarcoma cell lines in vitro as well as tumor growth in subcutaneous or peritoneal murine xenografts [105, 106]. Moreover, MALAT1 expression correlated with RET expression and negatively correlated with expression of miR-129-5p and survival in the study of 68 osteosarcoma patients [105].

multi-TKIs

This section will focus on the multi-TKIs evaluated in clinical studies that included patients with osteosarcoma (Table 1). All eleven of those multi-TKIs can inhibit at least one of the RTKs identified in our original phosphoproteomic/siRNA screen [4]. For example, AXL and IGF1R were among the eight RTKs inhibited by imatinib in the HOS human osteosarcoma cell line, as assessed by phospho-RTK arrays [107]. Moreover, live cell, biochemical and proteomic profiling as well as X-ray crystallography revealed that, among many other RTK targets, sunitinib can potently inhibit AXL, EPHB2, FGFR2, IGF1R and RET; dasatinib can potently inhibit AXL, EPHB2, FGFR2 and RET; cabozantinib can potently inhibit AXL, EPHB2 and RET;
sorafenib can potently inhibit AXL, FGFR2 and RET; pazopanib can potently inhibit FGFR2, IGF1R and RET; cediranib can potently inhibit AXL and RET; axitinib and regorafenib can potently inhibit FGFR2 and RET; crizotinib can potently inhibit AXL; and apatanib can potently inhibit RET [2, 54, 103, 108-112]. The polypharmacology of the multi-TKIs likely contributes to their potential clinical efficacy [2, 3] but also can contribute to serious “off-target” toxicities [103, 113].

Cediranib, dasatinib, and sunitinib were among the most effective drugs in a screen that measured viability of monolayer cultures obtained from four primary canine osteosarcomas [114]. Sorafenib, the only other multi-TKI in Table 1 included in that screen, had no detectable effects on viability of cultures from any of the canine osteosarcomas. Those results led to dasatinib treatment of four canines with osteosarcoma following limb amputation and carboplatin chemotherapy, which is a standard-of-care chemotherapy for canine osteosarcoma [115]. In two of the four canines, initial results suggest that dasatanib led to stable disease or partial remission [115]. Many multi-TKIs are more effective against epithelial cancers in hypoxic conditions [116]. Similarly, gefitinib is substantially more potent against human osteosarcoma cell lines in low-serum cultures, and in the presence of doxorubicin or methotrexate (but not cisplatin), compared with cultures containing 10% serum without chemotherapeutics [117]. Since 3D cultures mimic the oxygen, nutrient, and drug gradients found in sarcomas and other solid tumors [41], it is therefore not surprising that multi-TKIs were one of the most effective drug classes in our screen of FDA-approved oncology drugs that measured effects on the in vitro growth of 3D sarcospheres in both the absence and presence of MAP (methotrexate, doxorubicin, and cisplatin) standard-of-care chemotherapeutics [118]. Moreover, six (cabozantinib, crizotinib, dasatinib, pazopanib, regorafenib, and sunitinib) of the nine multi-TKIs in Table 1 that were included in our screen were among the top hits in at least one of the three tested highly-metastatic human osteosarcoma cell lines [118]. The three other multi-TKIs in Table 1 that were included in our screen (axitinib, imatinib, sorafenib) had modest effects. Regorafenib was also the fourth most effective drug in a screen that measured viability of monolayer cultures of five human osteosarcoma cell lines [119].

To evaluate the potential clinical relevance of the in vitro screening results described in the previous paragraph, it is important to determine whether the drugs are effective in vivo. Imatinib reduced growth of primary osteosarcomas in a syngeneic murine model [107]. Moreover, preventive regimens of cediranib,
dasatinib, sorafenib, and sunitinib each had intermediate to high activity in multiple subcutaneous xenograft primary osteosarcoma models evaluated by the Pediatric Preclinical Testing Program [120], and crizotinib, pazopanib, and regorafenib reduced tumor growth in similar xenograft models [121-123]. However, none of those studies [107, 120-123] determined whether the multi-TKIs also block growth of osteosarcoma metastases – the life-threatening process in osteosarcoma. In contrast, a therapeutic regimen of sorafenib caused regression in a subcutaneous xenograft primary tumor model and reduced the number and size of lung metastases in mice after tail vein injections of the SJSA-1 and MMNG human osteosarcoma cell lines [124, 125] and a therapeutic regimen of pazopanib reduced the number of lung metastases in mice after subcutaneous injection of the LM8 murine osteosarcoma cell line and resection of the resultant primary tumor [126]. Similarly, a therapeutic regimen of sunitinib reduced primary tumor growth and the number of detectable metastases derived from intratibial injection of the 143B human osteosarcoma cell line in mice [127] but no effect was seen in response to dasatinib [128], imatinib [129], or sorafenib [130] as monotherapies in similar models. In the later studies however, combinations of doxorubicin with either sorafenib or imatinib were more effective than the monotherapies [129, 130]. Given the potential translational relevance [131], it is surprising that none of the multi-TKIs have been tested in animal models in combination with all three components of MAP chemotherapy. In other combinations, sorafenib either with the mTOR inhibitor everolimus or with the CDK inhibitor palbociclib blocked growth in a MNNG human osteosarcoma cell line subcutaneous xenograft primary tumor model and in a patient-derived osteosarcoma orthotopic xenograft model [125, 132, 133]. More importantly, the therapeutic regimen of sorafenib with everolimus inhibited the number and size of lung metastases more effectively than either agent as monotherapy following tail vein injection of the MNNG human osteosarcoma cell line [125]. To maximize clinical relevance, it will be important for future murine studies to focus on therapeutic rather than preventive regimens.

Although the available clinical trials are limited in size, some of multi-TKIs appear promising as monotherapies (Table 1). The most encouraging are the Phase II studies of apatanib [134, 135], regorafenib [136, 137], and sorafenib, both alone [138] and in combination with everolimus [139]. Those studies recently led to designation of regorafenib as a category 1 recommendation by the National Comprehensive Cancer Network for second-line therapy of osteosarcoma patients with relapsed/refractory
or metastatic disease (NCCN Guidelines Version 1.2020, Bone Cancer). Sorafenib alone and in combination
with everolimus are included respectively as category 2A and 2B recommendations. Multi-TKIs in on-going
clinical trials listed in ClinicalTrials.gov for osteosarcoma patients include apatinib plus gemcitabine and
docetaxel (Phase II, NCT03742193), apatinib plus anti-PD1 (Phase II, NCT03359018),
cabozantinib (Phase II, NCT02243605 and NCT02867592), dasatinib plus ifosfamide, carboplatin and
etoposide (Phase II, NCT00788125), famitinib plus anti-PD1 (Phase I/II, NCT04044378), lenvatinib plus
ifosfamide and etoposide (Phase I/II, NCT02432274), pazopanib plus topotecan (Phase II, NCT02357810),
regorafenib (Phase II, NCT02048371 and NCT03277924), sunitinib plus anti-PD1 (Phase I/II,
NCT03277924), and sunitinib plus losartan (Phase I, NCT03900793). In addition, the Pediatric
MATCH (Molecular Analysis for Therapy Choice) screening trial (NCT03155620) includes osteosarcoma
patients in sub-studies of ensartinib, erdafitinib, larotrectinib, ulixertinib, and vemurafenib. Future studies will
be needed to determine whether the multi-TKIs are more effective in combination with other agents and
whether a subset of osteosarcoma patients can be identified that will respond to individual multi-TKIs. For
example, levels of RTKs or their ligands might serve as biomarkers to predict responsiveness to appropriate
multi-TKIs [45-47, 56, 96].

Systemic toxicities are a major limitation regarding multi-TKI therapies. Strategies are therefore being
developed to target multi-TKIs and other drugs to the involved tissue. For example, intranasal administration
can directly target multi-TKIs to osteosarcoma metastases in the lung [140, 141]. Another potential approach
is to target the multi-TKIs to the tumor and/or metastases following systemic administration. For example, a
liposomal formulation of ponatinib inhibited primary tumor growth by the K7M2 murine osteosarcoma cell line
in a subcutaneous syngeneic model more effectively than a ten-fold higher dose of free ponatinib without
inducing the systemic toxicity caused by the free drug [142]. A high-dose but pulsatile (once every two
weeks) regimen has also shown promise to increase efficacy and decrease toxicity of multi-TKIs in epithelial
cancers [143, 144].

Much work, both pre-clinical and clinical, remains to be done to identify optimal multi-TKIs, optimal regimens,
and the most responsive patients for each multi-TKI. We are nonetheless cautiously optimistic that multi-TKIs
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will ultimately improve survival for osteosarcoma patients and/or will allow use of lower doses of conventional
chemotherapeutics and thereby reduce their systemic toxicity.
<table>
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<tr>
<td></td>
<td>Retrospective</td>
<td>6 / advanced, after 1-4 lines of therapy</td>
<td>Stable Disease (2, 33%)</td>
<td>[163]</td>
</tr>
<tr>
<td></td>
<td>Case report</td>
<td>15 / refractory and metastatic</td>
<td>Partial Response (1, 7%)</td>
<td>Stable Disease (8, 53%)</td>
</tr>
<tr>
<td></td>
<td>Phase I</td>
<td>4 / recurrent or refractory</td>
<td>Stable Disease (1, 25%)</td>
<td>[165]</td>
</tr>
<tr>
<td>Regorafenib</td>
<td>Randomized Phase II</td>
<td>22 + 10 in placebo group who crossed over after progression / progressive and either advanced or metastatic, after 1 lines of therapy</td>
<td>Improved mean Progression-Free Survival (3.6 months vs 1.7 months w/ placebo group)</td>
<td>[136]</td>
</tr>
<tr>
<td></td>
<td>Randomized Phase II</td>
<td>26 / progressive and metastatic, after 1-2 lines of therapy</td>
<td>Increased Stable Disease (7, 27% vs 0% w/ placebo)</td>
<td>[137]</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Case report</td>
<td>1 / refractory, progressive, and metastatic</td>
<td>Partial Response (1, 100%)</td>
<td>[167]</td>
</tr>
<tr>
<td></td>
<td>Case report</td>
<td>4 / refractory and relapsed</td>
<td>Stable Disease (3, 75%)</td>
<td>[159]</td>
</tr>
<tr>
<td></td>
<td>Case report</td>
<td>8 / metastatic (6 patients) or local (2 patients)</td>
<td>Partial Response (6, 75%)</td>
<td>[168]</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>Case report, combo w/ denosumab</td>
<td>1 / relapsed and unresectable</td>
<td>Stable disease (1, 100%)</td>
<td>[169]</td>
</tr>
<tr>
<td></td>
<td>Phase I</td>
<td>10 / refractory</td>
<td>No objective response</td>
<td>[170]</td>
</tr>
<tr>
<td></td>
<td>Phase I, combo w/ bevacizumab and cyclophosphamide</td>
<td>2 / recurrent or refractory</td>
<td>Stable Disease (2, 100%)</td>
<td>[171]</td>
</tr>
<tr>
<td></td>
<td>Phase II</td>
<td>35 / metastatic, relapsed, unresectable, and progressive</td>
<td>Progression-free survival at 6 months (10, 29%)</td>
<td>[138]</td>
</tr>
<tr>
<td></td>
<td>Phase II, combo w/ everolimus</td>
<td>38 / progressive and either locally advanced, unresectable, or metastatic</td>
<td>Progression-free survival at 6 months (17, 45%)</td>
<td>[139]</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Case report</td>
<td>5 / refractory and relapsed</td>
<td>Partial Response (1, 20%)</td>
<td>Stable Disease (1, 20%)</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>Phase I</td>
<td>2 / refractory</td>
<td>Stable Disease (1, 50%)</td>
<td>[172]</td>
</tr>
</tbody>
</table>

* CBR: Dasatinib: Objective Response within 6 months or Stable Disease for > 6 months
** CBR: Imatinib: Complete or Partial Response at 2 or 4 months or Stable Disease at 2 & 4 months
RTKs in Osteosarcoma

References


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