Targeting the IGF/PI3K/mTOR pathway and AXL/YAP1/TAZ pathways in primary bone cancer

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ABSTRACT

Primary bone cancers (PBC) belong to the family of mesenchymal tumors classified based on their cellular origin, extracellular matrix, genetic regulation, and epigenetic modification. The three major PBC types, Ewing sarcoma, osteosarcoma, and chondrosarcoma, are frequently aggressive tumors, highly metastatic, and typically occur in children and young adults. Despite their distinct origins and pathogenesis, these sarcoma subtypes rely upon common signaling pathways to promote tumor progression, metastasis, and survival. The IGF/PI3K/mTOR and AXL/YAP/TAZ pathways, in particular, have gained significant attention recently given their ties to oncogenesis, cell fate and differentiation, metastasis, and drug resistance. Naturally, these pathways – and their protein constituents – have caught the eye of the pharmaceutical industry, and a wide array of small molecule inhibitors and antibody drug-conjugates have emerged. Here, we review how the IGF/PI3K/mTOR and AXL/YAP/TAZ pathways promote PBC and highlight the drug candidates under clinical trial investigation.

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1. Introduction

Osteosarcoma (OS) and Ewing sarcoma (ES) are the most frequently occurring malignant primary bone cancers (PBC) of childhood and adolescence[1]. Chondrosarcoma (CS) tends to occur later in the 4th-7th decades. Standard treatment with chemotherapy, radiation, and surgery has variable success across these tumor types, but the cure rate for those with metastatic or relapsed disease has changed little over the last five decades[2].

Fortunately, molecular characterization of these tumors has revealed potential pathways for drug intervention. Though IGF-1 regulates osteogenesis and bone homeostasis in normal bones [3,4], it also instigates an aberrant IGF/PI3K/mTOR pathway signaling cascade in PBC[5]. Downstream of IGF-1R, PI3K activation has been shown in several solid tumors to affect cell metabolism, cell survival, proliferation, and protein synthesis [6,7]. Due to the frequent activation of the IGF/PI3K/mTOR pathway in PBC and other solid tumors, which intersects a range of mechanical and chemical signaling mechanisms in cancer, this pathway has been identified as an attractive target in OS, ES, and CS[8–10]. Though IGF-1R-targeted therapies have been the subject of many clinical trials

for sarcoma, with or without mTOR inhibitors [11,12], their impact on cell differentiation, plasticity, and drug resistance mechanisms remain largely unexplored. The high inter-patient heterogeneity among PBC patients also suggests the need for response biomarkers to select and steer patients toward the agents most likely to provide clinical benefit [13,14].

The dysregulated AXL-ABL2-YAP/TAZ feedback loop – comprised of Yes-associated protein 1 (YAP), transcriptional coactivator with PDZ-binding motif (TAZ), and the oncogenic receptor tyrosine kinase (RTK) AXL – have been observed in ES and OS. Activation of YAP, TAZ, and AXL can induce cell proliferation, resistance to biologically targeted therapy, and metastasis of bone sarcoma [7,15–19]. The importance of this pathway in tumorigenesis has naturally led scientists to investigate whether its protein constituents are druggable targets in bone sarcomas [19]. Interestingly, Dupont et al. showed that YAP and TAZ also regulate biomechanical signals independent of the Hippo pathway, which in turn have striking effects on cell fate determination, stem cell properties, and proliferation rates. [20] Colocalization of nuclear YAP and TAZ in mesenchymal stem cells (MSCs) correlates with the environment's tensegrity. An increased propensity toward osteogenic lineage commitment is induced through high tensegrity. In contrast, less stiff environments favor an adipogenic phenotype and cytosolic YAP/TAZ expression[21]. In human OS cell lines, knockdown of AXL leads to decreased proliferation and increased

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apoptosis[22]. In addition, the clinical activity of cabozantinib, an inhibitor of AXL and other kinases, has been recently reported in phase 2 clinical trials for patients with OS and ES[23].

Though other pathways almost certainly contribute to bone sarcomagenesis, this review focuses exclusively on two important ones, IGF/PI3K/mTOR and AXL/YAP/TAZ, that have immediate clinical relevance given the exponential rise of new investigational agents that have reached the clinic.

2. Clinical description

PBCs are included in the broader category of sarcomas and correspond to 0.2% of diagnosed cancers, which are primarily composed of osteosarcoma (OS), Ewing sarcoma (ES), and chondrosarcoma (CS) [24]. These malignant bone tumors are clinically aggressive and often need extensive multi-modality treatment. Despite their low incidence, PBCs are associated with excessive morbidity and mortality, greatly affecting the children and young adult population[25]. The lack of specific symptoms at disease onset can delay the diagnosis and allow time for local tumor invasion and distant metastasis to bone and lung. Though the 5-year survival rate for those presenting with localized OS and ES reaches 50–70%, only 20–30% of those presenting with lung or bone metastases at diagnosis survive[26]. While slower growing, CS is notoriously resistant to chemotherapy and often recurs locally[27].

2.1. Osteosarcoma

OS is the most common PBC that usually affects children and adolescents[25]. The etiology of OS is complex and may occur due to widespread chromosomal errors or specific gene mutation in p53, RB1, or the RECQL4 genes that, respectively, cause Li-Fraumeni syndrome, hereditary retinoblastoma, and Rothmund-Thomas syndrome[28]. Interestingly, whole-genome sequencing data suggests that one-third of primary OSs – compared with just 2%–3% of other cancer types – have high mutation burdens thought to result from chromothripsis[29]. The phenomenon of chromothripsis (i.e., shattered DNA) occurs when one or more chromosomes are broken into many pieces and then chaotically rejoined [29,30]. Since the resulting DNA damage is unpredictable, one frequently observes marked inter-patient genetic and phenotypic heterogeneity [29,30].

From a clinical perspective, the World health organization classifies OS subtypes by their location relative to the bone cortex and by their grade (high, intermediate, or low). High-grade OS can occur in any bone, but usually it originates near the distal femur (43%) or proximal tibia (23%). These bones experience rapid cell division during the adolescent growth spurt, though it remains to be determined if the stimulatory signals during puberty contribute to tumorigenesis[31]. The main prognostic factor in OS is the presence or absence of metastatic disease. Though beyond the scope of this review, treatment often relies upon chemotherapy (mainly cisplatin, doxorubicin, methotrexate, or ifosfamide) and surgery[28]. Complementing traditional chemotherapy, several molecularly and pathway-targeted therapies have been developed, including immune modulators [32,33], bone signaling regulators, receptor activator of nuclear factor k-B ligand blockade [34,35], and receptor or non-receptor tyrosine kinases inhibitors [14,31,36–37].

2.2. Ewing sarcoma

ES can occur in the bone (mainly in the pelvis, femur, tibia, and ribs) or soft tissue sites (e.g., thoracic wall, gluteal muscle, cervical muscles, and pleura cavities). The cell of origin remains unknown, but many suspect this sarcoma subtype emerges from neural crest cells or mesoderm-derived mesenchymal stem cells[38–40]. Phenotypically, ES is characterized by a small round cell appearance, positive expression of the surface marker CD99[41], and chromosomal translocation of the EWSR1 gene to ETS family genes[42]. This fusion protein acts as an oncogenic transcription factor that controls ES progression. 70–80% of patients with localized ES, and ~30% for those with metastatic disease, survive. All patients receive chemotherapy, which typically consists of vincristine, doxorubicin, cyclophosphamide alternated with ifosfamide and etoposide [38,43]. When practical, surgery is preferred, though unresectable tumors in the spine or pelvis can successfully be treated with radiation.

Several next-generation EWSR1-FLI1 targeted therapeutics are in preclinical development, with at least one compound (TK-216) that has entered phase clinical testing in patients (NCT02657005) [44]. In addition, several other genes were also reported to promote the recurrence and progression of ES, such as VEGF, IGF-1, CAV1, GLI1, RB, and p53, which might be used as therapeutic targets for ES. Several proteins appear to enable resistance to IGF-1R/mTOR-directed treatment, including IRS1, PI3K, STAT3, YAP-1, and TAZ[7].

2.3. Chondrosarcoma

CS is a malignant tumor of bone characterized by cartilage matrix production and a diverse histopathological and clinical behavior[45]. The exact etiology of CS is not known. There may be a genetic or chromosomal component that predisposes certain individuals to this type of PBC. However, the somatic mutations of isocitrate dehydrogenase (IDH) genes that encode for proteins catalyzing the oxidative decarboxylation of isocitrate, producing αKG and CO2 in the Krebs cycle [46,47], are present in 50% of primary conventional CS [48,49]. The CS is subclassified in primary central, secondary peripheral, and periosteal (aka juxtacortical) suptypes [14,50]. The most common primary location is the pelvis, followed by the femur, humerus, and ribs[51]. Conventional CS is a low or intermediate grade (90%), characterized by a slow clinical course and low metastatic potential.

In contrast, the high-grade CS (10%) is associated with high metastatic potential and poor prognosis [48,52]. Among patients with primary CS of bone and metastasis at presentation, low tumor grade, surgical treatment, tumor size <10 cm, and first primary tumor predict prolonged survival[53]. CS is characterized by a resistance to chemo- and radiotherapies mainly due to a high ECM deposition and low neovascularization, which blocks drug diffusion and activity[54]. The *IDH1/2* mutations in CS make the development of IDH targeted therapy a promising treatment option, and there are several ongoing clinical trials in Phase I/II assessing the clinical activity of IDH blockades (NCT02273739, NCT02481154/NCT02073994, and NCT02496741).

3. IGF1/PI3K/mTOR pathway

Insulin-like growth factor 1 (IGF-1) is important for several different growth and differentiation processes of normal bone physiology through endocrine mechanisms[55]. IGF-1 receptor (IGF-1R) blockade in chondrocytes, osteoblasts, and osteocytes has shown that IGF-1 signaling is required for controlling cell proliferation and differentiation.

IGF1/PI3K/mTOR pathway activation begins when IGF-1 binds to IGF1R, prompting phosphorylation at several tyrosine residues in the kinase domain (e.g., 1131, 1135, 1136) or membrane domain (e.g., tyrosine 950). IGF-1R phosphorylation, in turn, activates downstream substrates, such as insulin receptor substrate (IRS)

and Shc[56]. IRS1 activates phosphatidylinositol 3 kinase (PI3K) [57] and the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) network by binding to Shc and Grb2 (Fig. 1) [14,58–59]. PI3K phosphorylates phosphatidylinositol 4,5 bisphosphate, which leads to phosphatidylinositol 3,4,5, trisphosphate. Ultimately, serine/threonine kinase (PDK1) is recruited and AKT is partially activated at threonine-308 (Fig. 1). Full AKT activation is accomplished by Ser-473 phosphorylation by mTORC [28,60]. At the terminal end of this cascade, the mammalian target of rapamycin (mTOR) regulates several processes critical for cell proliferation and protein synthesis.

mTOR subsists in two distinct entities, mTORC1 and mTORC2, a serine/threonine tyrosine kinase that regulates normal cell growth, development, metabolism, and angiogenesis. In response to nutrients and growth factor receptor signals, the downstream effectors (p70S6K and 4E-BP1) profoundly affect cellular growth, proliferation, and protein synthesis (translation) [8]. mTORC1 is sensitive to rapamycin and other so-called rapalogs (e.g., temsirolimus, everolimus, ridaforolimus, or rapamycin) [8]. mTORC2, which is not sensitive to rapamycin, can enhance AKT activity [61,62].

The IGF/PI3K/mTOR pathway is linked to the pathogenesis and progression of PBC [63,64]. Comparing PBCs with normal bone cells at the gene expression levels validated a loss of all the intracellular IGF inhibitors, IGFBPs [7,14,65]. This pathway actively modulates cell migration, cell cycle progression, EMT, and tumor growth in preclinical models of PBCs [7,14,66]. Increased IGF1/IGF1R expression has been demonstrated in OS, ES, and CS patients' tumors, and it was associated with a poor prognosis [14,67–68]. Despite this evidence, IGF-1R blockade in PBC cell lines with mAb against IGF/IGF1R was rarely successful when used as monotherapy [7,69]. Activation of the downstream PI3K/Akt pathway has also been demonstrated in OS and CS cells, though again, single-agent targeting was generally unsuccessful [10,70]. More encouraging

results were achieved in combination studies, and partial responses were shown in PBC cases treated with dual IGF1R/mTOR blockade [12,14].

4. Therapeutic opportunities targeting the IGF/PI3K/mTOR pathway in PBC

The IGF/PI3K/mTOR pathway can be therapeutically targeted conceptually at three different levels: 1) blocking its proximal components (either IGF ligands or IGF-1R using antagonistic humanized monoclonal antibodies), 2) impeding its mid constituents at either PI3K or AKT using small molecules tyrosine kinase inhibitors, and 3) blocking its distal mTOR components using rapamycin and its analogs. Given the prominent role this pathway has on vital cellular processes, feedback loops abound and quickly counter the therapeutic effect of most drugs. For that reason, it is increasingly common to see combination strategies employed in the preclinical and clinical setting to block the IGF/PI3K/mTOR pathway at two or more levels in an attempt to mitigate acquired drug resistance [7,14].

4.1. IGF ligand inhibitors

IGF ligand-blockade demonstrated a complementary therapeutic approach that overcame the upregulation of insulin receptors as a mechanism of resistance to IGF-1R neutralization. Humanized monoclonal antibodies against IGF-1 and IGF-2 have been developed by Medimmune (MEDI-573, dusigitumab) and Boehringer Ingelheim (BI836845, xentuzumab). Both antibodies bind and neutralize IGF-1 and IGF-2, thereby preventing activation of IGF-1R and IR-α while avoiding unwanted effects upon insulin[71]. These ligand-directed antibodies were generally well tolerated within
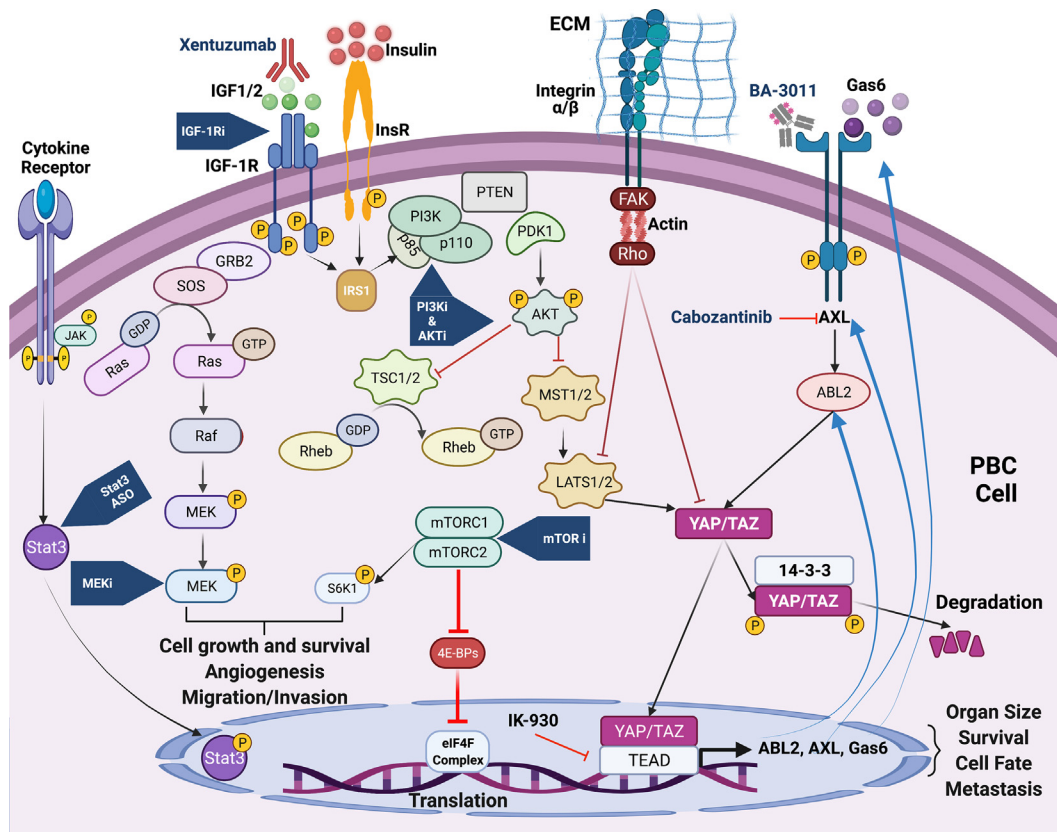

Fig. 1. Activation of the IGF/PI3K/mTOR and AXL/YAP/TAZ pathways in PBC.

phase 1 trials in patients with solid tumors. To date, the sole clinical experience treating PBC patients with ligand-targeted drugs includes a ES patient who received a subtherapeutic dose of MEDI-573 while enrolled in an early dose cohort[72]. Xentuzumab, which also binds and neutralizes murine IGFs, has shown strong preclinical activity in ES. Despite this, both IGF ligand inhibitors (dusigitumab and xentuzumab) have been discontinued due to poor activity in common cancer types.

4.2. IGF-1R blockade

The IGF-1R pathway has been studied extensively in PBCs [7,65–66]. IGF-1R antagonists have generally consisted of antibodies or small molecule TKIs, as summarized in Table 1 [73–75]. To date, six mAbs had been investigated in clinical trials: R1507 (robatumumab, Roche), cixutumumab (IMC-A12, ImClone), SCH-717454 (19D12, Schering-Plough), MK-0646 (dalotuzumab, Merck), AMG479 (Ganitumab, Amgen), figitumumab (CP-751-871, Pfizer). Single-agent activity ranged between 9 and 14%, and most responses lasted <2 months [73,75–76]. Small molecule IGF-1R antagonists, including NVP-AEW541, BMS-536924, GSK-183870A, and OSI-906 showed promising preclinical activity. However, cross-reactivity to the insulin receptor led to unacceptable toxicity[77–80], and these compounds were subsequently abandoned. Table 2.

4.3. IRS-1 inhibitors

The IGF-1R/PI3K/mTOR pathway has been the target of preclinical studies for OS PBC[87] and has a vital role in various mitogenic and antiapoptotic signaling through other components like insulin receptor substrates 1 and 2 (IRS1/2), which play central roles in cancer cell proliferation, resistance to anticancer drugs, and tumor metastasis[88–90]. A selective inhibitor of IRS1/2, NT157 was evaluated as a dose-dependent inhibitor of growth in several OS cell lines by downregulating the expression of IRS1/2 and principal downstream mediators of the IGF pathway. NT157 affected the OS cell migratory ability. In addition, the same IRS1/2 inhibitor

was combined with mTOR and PI3K/mTOR inhibitors, and significant synergistic effects were obtained[87]. This IRS1/2 inhibitor is not yet been tested clinically.

4.4. PI3Ks inhibitors

These targets are downstream of the IGF-1R/IR but also of other RTKs. Their effective targeting can eliminate the activation of downstream signaling, reduce cell proliferation and survival that are inducted by other RTKs, and downgrade cell activation caused by PI3K mutations. Impaired PI3K signaling can trigger compensatory alterations at the cellular and whole-body level, the latter occurring via drug-induced hyperglycemia and hyperinsulinemia that activates IR-A[91]

There are two broad types of PI3K inhibitors, the pan-PI3K and the isoform-specific inhibitors. Pan-PI3K inhibitor has been reported to inhibit autophagy and enhance OS cell apoptosis [92,93]. Despite the effective inhibition of the PI3K pathway and PBC tumor growth in PBC preclinical studies, these pan-inhibitors may never be fully developed as clinical therapeutic drugs because of their poor pharmacokinetic parameters, including low solubility, instability, and excessive toxicity. However, the development of isoform-specific inhibitors of PI3K p110 α,β,δ or γ catalytic subunits have emerged and might offer the opportunity to lower these side effects, thus granting their tolerability. For example, alpelisib, a specific PI3Kα inhibitor that blocks the ATP site, is proposed to reduce cell proliferation and block tumor bone formation in murine preclinical models of OS[94]. Another PI3Kα inhibitor, BKM120, can decrease OS cell invasion and survival[95].

4.5. mTOR inhibitors

As a central hub of cell biology, mTOR profoundly affects whole-organism carbohydrate physiology. mTORC1 and mTORC2 contribute to tumorigenesis differently [99,100] and elicit unique behaviors when targeted. The rapamycin analogs ridaforolimus, everolimus, and temsirolimus, which inhibit mTORC1, were first approved to treat renal malignancies[101].

Table 1
IGF-1R blockade strategies in ES and OS.

	IGF-1R Blockade	Phase	Company	Clinical activities (ORR & SD)
Monoclonal Antibodies	SCH-717454	II	Schering-Plough	PRs seen in ES patients, Phase II in relapsed OS & ES[81]
	Figitumumab (CP-751871)	I-II	Pfizer	ES (14% CR + PR & 24% SD) [82]
	Cixutumumab (IMC-A12)	I-II	ImClone	Phase II with ES (PD: 66.7%) [83]
	Dalotuzumab (MK-0646)	II	Merck	Phase I- dose escalations in 1 ES that had a mixed response[84]
	Teprotumumab (R1507)	II	Roche	Several PRs in ES (11, CR/PR) [75] & OS (PR: 2, SD: 10, PD: 26) [85] patients
	Ganitumab (AMG-479)	II	Amgen	ES (6% CR + PR & 49% SD) [86]
TKIs	Linsitinib (OSI-906)	II	Astellas Pharma	ES (16 patients; no CR/PR) (NCT02546544)
	BSM-754807	Preclinical	BMS	No Clinical testing
	BMS-554417	Preclinical	BMS	No Clinical testing
	NVP-AEW541	Preclinical	Novartis	No Clinical testing
	GSK1904529A	Preclinical	GSK	No Clinical testing

ORR: overall response rate; SD: stable disease; CR: complete response; PR: partial response; PD: progressive disease; ES: Ewing sarcoma; OS: osteosarcoma.

Table 2
PI3K blockade strategies in PBCs.

PI3K Blockade	Name	Phase	Company	MOA on PBC
PI3Kα	Alpelisib (BYL719)	Preclinical	Novartis	Cell migration inhibition Tumor progression inhibition[94]
Pan-PI3K	BKM120 Buparlisib	Preclinical	Novartis	Inhibition of Cell proliferation inhibits activation of Akt[96]
	LY294002	Preclinical	Eli Lilly	Inhibition of Cell proliferation[97]
	Copanlisib (BAY80-6946)	I/II	Bayer	Inhibition of cell survival[98]. Ongoing trial to investigate the safety and feasibility in pediatric patients with recurrent or refractory OS, ES (NCT03458728)

A downstream target of the IGF/PI3K/mTOR pathway, activated S6 kinase is readily detectable in PBC specimens. In a subgroup analysis, ridaforolimus led to a non-statistically significant improvement PFS for bone tumors (HR 0.70, 95% CI upper limit >1); this study was not adequately powered for subgroup analyses [102]. Everolimus has demonstrated some activity in osteosarcoma; within a pediatric phase 1 study, one of two enrolled osteosarcoma patients showed prolonged stable disease for several courses[103]. Several drug combinations, rapamycin with cyclophosphamide and temsirolimus with cixutumumab, failed to demonstrate significant activity in OS [12,104]. Ten CS patients were treated with a sirolimus/cyclophosphamide combination, which was well tolerated and had modest clinical activity[105]. Temsirolimus may also potentiate the cytotoxicity of liposomal doxorubicin[106]. Last, neoadjuvant everolimus has been tried in CS (NCT02008019) was suspended due to limited activity.

4.6. Cotargeting IGF/PI3K/mTOR pathway

Given the limited or short-lived antineoplastic response observed when individual proteins are targeted, many have hypothesized that effective blockade of the IGF/PI3K/mTOR will require co-targeting two or more proteins concurrently to avoid counter-productive feedback loop activation. Preclinical data indicate that the combination of mTOR with IGF-1R blockade results in greater Akt downregulation and enhanced antiproliferative effects [7]. This therapeutic approach guided the design of numerous early phase clinical trials assessing mTORC1 and IGF-1R blockade in sarcoma patients [14,107–109]. Dual IGF-1R/mTOR therapies were generally more successful than monotherapy and were reasonably well tolerated compared to traditional cytotoxic chemotherapy [11,14].

While single-agent IGF-1R targeted monoclonal antibodies delivered a clear activity signal, neither the response rate (10–14%) nor response duration (2 months) offers enough clinical benefit to expand into the next clinical trial[75]. During the last decade, our research team has demonstrated that combined IGF-1R/mTOR-targeted therapy was synergistic, with a 29% response rate and, significantly, with a standard response duration enduring more than one year[11]. Later, other trials developed by Schwartz et al. and COG (Children's Oncology Group) were unsuccessful when trying to replicate these promising results, principally because the mTOR dose was decreased in a sizable fraction for patients due to mucositis or hepatotoxicity [104,110]. Since those studies were conducted, investigators have become adept at palliating mucositis and other mTOR-associated symptoms and thereby have extended the safety profile of mTOR combinations [111,112].

Harmonizing these clinical experiences, our lab and others throughout the country have made considerable advances shaping how to efficiently cotarget the IGF-1/PI3K/mTOR pathway to significantly succeed in managing PBC clinically. Despite exhibiting little single-agent activity, our results suggest mTOR suppression is central to meaningful clinical action. Of critical importance, because mTOR inhibition quickly upregulates IRS-1 and PI3K (due to derepression of the p70S6K → IRS-1 feedback loop) [113–116], it is imperative to simultaneously block the proximal portion of the IGF-1/PI3K/mTOR pathway (Fig. 1).

In our experience, this can be achieved by co-targeting mTOR together with the IGF-1/IGF-2 ligands (plus the hybrid IR-α/IGF-1R), IGF-1R, or PI3K. As shown in our preclinical studies, IGF-1R targeting in animal models leads to compensatory changes in Smad3, STAT3, and other proteins that can be explored for additional synergy[7]. In the clinic, data from one ES patient who had responded to therapy and then progressed 14 months later on IGF-1R/mTOR-based therapy revealed similar compensatory changes[7]. Preliminary data using alpelisib (a p110α-selective PI3K inhibitor) in ES patient-derived tumor explants (PDX) models suggest a xentuzumab/alpelisib two-drug combination might also be effective. They could serve to expand future clinical trial options if neutralizing antibody programs are revived.

Though antagonists of IGF, IGF-1R, IRS-1, and mTOR have largely been abandoned as a single-agent treatment for PBC, PI3K inhibitors remain under clinical investigation. Alpelisib has been FDA-approved for specific PI3K-mutant breast cancer subtypes. Pan-PI3K inhibitors, such as copanlisib with a broader affinity for the p110-α p110-δ catalytic PI3K subunit, have received approval for lymphoma subtypes. To date, PI3K inhibitors have not been studied in PBC. If and when they are, Hopkins et al. have suggested in other cancers that their antineoplastic effect can be magnified by reducing hyperinsulinemia via adherence to a ketogenic diet [117]. Whether that strategy proves beneficial in PBC remains to be determined.

5. AXL-YAP/TAZ positive feedback loop

5.1. The Hippo and YAP/TAZ pathway is conserved across species.

YAP (encoded by Yes-associated protein 1) [118–119] and TAZ (encoded by WWTR1) [118–120] are transcriptional activators that regulate cell proliferation, survival, and differentiation [121,122]. The biological response is directly tied to the location of YAP/TAZ in the cell. For instance, activation leads to the shuttling of YAP/TAZ into the nucleus, and suppression of YAP/TAZ maintains its presence in the cytoplasm. When YAP and TAZ were first discovered, the biological function was unclear. Later, key insights regarding YAP and TAZ function were inferred from the effects of Yorkie, a homolog within the Drosophila Hippo signaling pathway[123].

The Hippo signaling pathway is a conserved cascade regulating the growth and size of tissues, involving the translocation of Yorkie, the YAP/TAZ homolog, between the cytoplasm and nucleus. When Hippo signaling is inactivated in Drosophila, it causes enlargement of larval tissue and tumor initiation, indicating the Hippo signaling pathway is a key tumor suppressor[124]. Similar findings emerged in mammalian cells, where YAP/TAZ was shown to regulate organ size[125] and tumor progression. The Hippo signaling pathway is mediated through the kinases MST1/2 and LATS1/2 in mammals. When activated, MST1/2 kinases form a complex with SAV1 to phosphorylate and activate LATS1/2. Subsequently, LATS1/2 phosphorylates YAP and TAZ, marking them for ubiquitination and subsequent degradation[124]. Since the nuclear-localized YAP/TAZ paralogs act as transcription factors, upstream Hippo signaling can inhibit cell proliferation.

Conversely, Hippo inactivation promotes YAP/TAZ nuclear shuttling. Notably, because YAP/TAZ lack DNA-binding domains themselves, they must first complex with one of several TEA-binding domains (TEAD) to exert their diverse epigenetic effects. As discussed shortly, obligate binding to TEAD1 or other TEAD proteins has created a therapeutic opportunity to target TEAD as a potential antineoplastic agent.

5.2. Diverse regulation of YAP/TAZ via mechanotransduction

Though canonical YAP/TAZ activation was first linked to Hippo mutations, Hippo downregulation can have similar effects. Notably, key findings from the regenerative medicine field revealed that YAP/TAZ activity is also critically dependent upon biomechanical cues imparted on cells by their surroundings. In vivo, this mostly occurs through cell-cell adhesion, mediated at the subcellular level by adherens junctions (AJ), and cell-ECM associated focal adhesions (FA) that rely upon integrin/ECM pairings[126]. Besides their

role as structural anchors that preserve tissue shape and polarity, AJ and FA serve as critical macro-molecular hubs that regulate cytoskeletal architecture and recruit signaling proteins. Through both direct and indirect interactions with the Hippo pathway, cytoskeleton, FAK, and cadherin-catenin complexes, YAP/TAZs acts as a cell's molecular 'rheostat' of mechanotransduction.

Cadherin-catenin complexes, FAK-Src kinases, and cytoskeletal tension influence Hippo signaling [127,128]. In normal cells and tissues, cell-cell contact inhibition gradually reduces the rate of proliferation[129]. Cadherins, such as E-cadherin, participate in homophilic binding that initiates a cascade through β-catenin and α-catenin, leading to the interaction with the Merlin and Kibra complex. This complex activates MST1/2 initiating the canonical Hippo signaling. Apical and basolateral cell polarity can regulate the Hippo signaling pathway via AJs, tight junctions (TJ), and gap junctions (GJ) [130]. When correctly engaged, AJs and TJs bind and retain YAP and TAZ at the cell membrane, thereby preventing their activity.

Cell shape and cytoskeletal dynamics also regulate YAP/TAZ [131]. Mechanical cues and activation of RhoA promote increased F-actin polymerization, which inhibits LATS1/2 and activates YAP/TAZ. For example, when cells are placed on larger two-dimensional surfaces or micropatterned culture substrates that promote increased cytoskeletal tension, YAP/TAZ becomes activated. Conversely, rounded cells with low cytoskeletal tension maintain YAP/TAZ within the cytoplasm in an inactive state. This mechanism is influenced by the adhesive area, substrate stiffness, cell density, and shear stress. Taken together, by modulating YAP/TAZ activity, physical cues strongly influence cell proliferation, differentiation, and survival.

5.3. The critical role of YAP/TAZ in connective tissues and PBC

Given the impact of YAP/TAZ and TEAD[118–120] upon stemness[132], cell migration[133], organ size[125], and epithelial-to-mesenchymal transition (EMT) [119], it is not surprising that YAP/TAZ plays a vital role in osteogenesis [134,135] and chondrogenesis[136–138]. Human mesenchymal stem cells (hMSCs), when grown on substrates of varying stiffnesses, adopted distinct cell fates dictated by the specific stiffness. For instance, hMSCs on rigid substrate differentiated toward an osteogenic cell fate while hMSCs on soft substrate differentiated toward an adipocytic cell fate[21].

YAP and TAZ are overexpressed and activated in several cancers and are linked to sustained proliferation, cell survival, EMT[119], and tumor progression. In OS, a recent study showed that 75% of patient histology samples (n = 175) had high YAP expression, with 46% of patients demonstrating YAP nuclear localization[139]. YAP nuclear localization and B1-integrin expression have been linked to adverse metastatic events and worse prognosis [140,141]. Verteporfin, a small molecule TEAD inhibitor, impaired the growth and migration of OS cell lines. ROCK2 silencing in the preclinical setting had a similar effect[139]. While knockdown of YAP in OS cell lines suppressed in vitro tumor cell proliferation, invasion, and tumor formation in mice [142,143], the role of TAZ in these PBCs requires further investigation. To that end, recent work showed that U2OS and HOS human OS cell lines cultured under a migratory ability had increased expression in TAZ and EMT-TFs, including N-cadherin, vimentin, and SNAIL[144]. This EMT-TF phenotypic induction was reversed through inhibition of TAZ[144]. Thus, YAP and TAZ might play divergent roles in OS pathogenesis and progression.

The precise role of YAP and TAZ in ES is less well understood. A recent study comparing YAP expression between ES and normal tissue demonstrated only a moderate increase in ES[145]. Another study found an association of YAP/TAZ expression with disease progression. Knockdown of YAP led to decreased cell proliferation in ES cell lines and decreased tumor growth in an ES xenograft [146]. Further, in ES cell lines, YAP and TAZ regulated the expression of secreted ECM proteins proteoglycan four and tenascin C downstream of CDC42 signaling[147]. Counterintuitively though, in OS, tenascin C complexed to α9β1 integrin to foster metastasis by blocking YAP nuclear translocation and target gene activation [148]. Tenascin C also complexed to α5β1 integrin in ES promoted metastasis by triggering YAP[149] and tyrosine phosphorylation through SRC kinase[150].

Elevated YAP/TAZ expression has been reported in some CS. A recent study showed that less than half of CS specimens harbored activated YAP/TAZ[151]. While little is known of the role of YAP/TAZ in CS, recent studies of chondrogenesis showed that TAZ-deficient mice have impaired chondrogenic differentiation and development[152]. In that regard, the dysregulation of YAP/TAZ may contribute to pathogenesis and aggressiveness in CS[153]. In addition, blockade of the YAP/TAZ-activating kinases SRC and RAC was described to impede chondrosarcoma cells migration [154], and nuclear accumulation of YAP as a consequence of LATS1 inactivation by protein arginine methyltransferase one was shown as the worst prognostic factor in chondrosarcoma[155]. Finally, downregulation of YAP/TAZ and LATS1 in chondrosarcoma cells treated with BRD4 inhibitor, JQ1, led to cell cycle arrest, senescence, and apoptosis[156]. It is likely that the function of YAP/TAZ in the migration or metastasis of bone cancers at least partially recapitulates their role during normal development of the respective tissues of the origin.

5.4. AXL and its impact upon YAP/TAZ

In addition to the YAP/TAZ transcriptional activators, the AXL protein can profoundly affect cell viability, dedifferentiation, cell fate, and metastasis in human bone sarcoma [19,157]. As a TAM (Tyro3, AXL, and MER) RTK family member, AXL also regulates cell survival, proliferation, migration, invasion, and angiogenesis[158–166]. In carcinomas, including hepatocellular carcinoma[167] and lung adenocarcinoma brain metastasis[168], AXL promotes tumorigenesis. In addition to activating YAP/TAZ via ABL2 in some cancers, AXL – along with CTGF, CYR61, and MYC1 – is also a downstream YAP/TAZ gene target [169,170]. In lung adenocarcinomas, for instance, YAP was overexpressed and positively correlated with AXL expression[171]. In vitro YAP knockdown significantly reduced AXL expression[171].

6. Therapeutic opportunities targeting AXL, YAP, or TAZ in PBC

Though IGF, PI3K, and mTOR inhibitors have been extensively tested as anticancer agents, drug candidates targeting the AXL/YAP/TAZ pathway remain in their infancy; see clinical trial data summarized in Table 3. In the preclinical setting, Fleuren et al. showed for the first time that AXL is a potential novel and druggable therapeutic target in ES[157]. They demonstrated that AXL and Gas6 are abundantly expressed in ES tumors and that high AXL protein expression is an independent prognostic marker of poor overall survival[157]. They also blocked AXL function using BGB324, which reduced ES cell viability and migration in all cell lines in vitro[157]; however, there have not been any animal preclinical efficacy studies of the AXL inhibitor, BGB324. Preclinical acellularized lung (ACL) models have been used to investigate how these proteins affect OS metastasis[19]. In that system, AXL inhibition attenuated proliferation, migration, and metastatic potential in vitro and in vivo.

In the clinic, the recently opened sarcoma-specific phase 1/2 trial testing BioAtla's AXL-targeted therapy (NCT03425279) is cur-

Table 3

AXL blockade strategies in PBCs.

AXL Blockade	Name	Company	Sarcoma type	Phase	Clinical Activities
Antibody-drug Conjugate	BA3011	BioAtla, Inc.	Osteosarcoma Ewing Sarcoma	I-II	No results. (NCT03425279)
TKIs	Cabozantinib	Exelixis	Osteosarcoma Ewing Sarcoma	II	ES (26% PR + 49% SD) OS (12% PR + 33% SD) (NCT02243605)[174]
			Osteosarcoma	II	No results. (NCT05019703)
			Osteosarcoma Ewing Sarcoma	I	No results. (NCT04661852)
			Osteosarcoma	II	No results. (NCT02867592)

SD: stable disease; PR: partial response; ES: Ewing sarcoma; OS: osteosarcoma.

rently enrolling patients with ES, OS, CS, and several other sarcoma subtypes. Small molecule oral TEAD inhibitors such as IK-930 are completing IND-enabling studies and are expected to reach the clinic in early 2022. Several multi-targeted FDA-approved TKIs known to antagonize AXL, such as cabozantinib, have shown promising activity in ES and OS (NCT02867592) [23,172–173].

7. Crosstalk between IGF-1R/PI3K/mTOR pathway and AXL-YAP/TAZ positive feedback loop

Our group has previously shown that mechanical stress and culture architecture can affect PBC drug sensitivity in vitro to chemotherapy and IGF-1R/mTOR targeted therapy[175–177]. Increased mechanical stimulation through focal adhesions and actin stress fibers interface with the IGF/PI3K/mTOR pathway and AXL/YAP/TAZ feedback loop in at least two ways. First, FAK activates Akt through the canonical IGF/PI3K/mTOR pathway by stimulating the activity of PI3K [178,179] (Fig. 1). Downstream, Akt then negatively regulates MST1/2, leading to a downregulation of the cytosolic YAP/TAZ and afterward boosted nuclear YAP[180]. Second, a substrate stiffness signal cascade is initiated by Rho GTPases through its interaction with the distal AXL-YAP/TAZ feedback loop by suppressing LATS1/2 and directly inhibiting the phosphorylation and subsequent degradation of YAP and TAZ [20,181]. Nuclear YAP/TAZ then potentiate the IGF/PI3K/mTOR pathway through a PTEN inhibition caused by miRNA-29[182]. Thus, the mechanical cues can potentiate the IGF-1R/mTOR cascade either by promoting the activity of PI3K through FAK-mediated signaling or by inhibiting PTEN through nuclear YAP induction [20,178–182]. Additionally, recent gain-of-function screens identified connections between AXL-YAP/TAZ and the MAPK and PI3K signaling pathways[183]. Further research is needed to determine whether cotargeting the MAPK or PI3K pathways and AXL/YAP/TAZ will prove synergistic as an anticancer strategy for treating patients with high-risk PBCs.

8. Conclusion and future perspectives

Recent research investigating the molecular drivers of PBCs has suggested that the IGF/PI3K/mTOR and AXL/YAP/TAZ pathways are critically important. Nevertheless, despite strong antitumor activity in the preclinical setting, anticancer agents directed against IGF-1R or mTOR have had limited clinical utility when used individually. Optimal studies co-targeting the IGF/PI3K/mTOR pathway at two or more levels within the pathway are still being explored to prevent the activation of feedback loops that promote acquired tumor drug resistance. Multi-targeted TKIs with partial AXL activity, like cabozantinib, have demonstrated early success in PBCs, and confirmatory studies are ongoing. However, newer selective inhibitors of AXL, YAP, or TAZ have just reached the clinic, and their effect on PBCs remains to be seen.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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University of Michigan, Ann Arbor, MI; H. Lee Moffitt Cancer Canter & Research Institute, Tampa, FL; Mayo Clinic, Rochester, MN; University of Texas M. D. Anderson Cancer Center, Houston, TX; Vanderbilt University, Nashville, TN; Albert Einstein Cancer Center, Bronx, NY; Medical University of South Carolina Hollings Cancer Center, Charleston, SC; Washington University, St. Louis, MO; NorthShore University HealthSystem, Chicago, IL; Mount Sinai School of Medicine, New York, NY. A phase II multicenter study of the IGF-1 receptor antibody cixutumumab (A12) and the mTOR inhibitor temsirolimus (TEM) in patients (pts) with refractory IGF-1R positive (+) and negative (-) bone and soft tissue sarcomas (STS). American Society of Clinical Conference Annual Meeting. Chicago: ASCO; 2012.

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