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Chondrosarcoma models : understanding chemoresistance mechanisms for use in targeted treatment

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Chapter 2

Update on targets and novel treatment options for high grade chondrosarcoma

This chapter is based on the review: van Oosterwijk JG, Anninga JK, Gelderblom H, Cleton-Jansen AM, Bovée JVMG, Update on Update on targets and novel treatment options for high grade osteo- and chondrosarcoma, *Hem/Onc Clinics of North America*, 2013

Introduction

Primary bone tumors are rare and have a very specific age distribution (fig 2.1). Conventional osteosarcoma (OS) is the most frequent primary high-grade bone tumor in humans with 4 new cases per 10^6 population and year with the highest incidence in adolescence (1). The second most frequent primary bone malignancy, chondrosarcoma, accounts for approximately 3 new cases per 10^6 population and year predominantly affecting adults (2). The clinical management of unresectable and metastatic disease as well as therapy resistance remain a clinical challenge (3). This review will discuss the molecular pathways that have been identified as a result of intensive genome wide and basic biology analysis and rationale to current clinical and pre-clinical targets for therapy of these two most frequent bone sarcomas

Chondrosarcoma

Clinicopathological features

Chondrosarcomas are hyaline cartilaginous tumors most often arising in bones which develop during endochondral ossification. Incidence and location are shown in figures 1 and 2. Conventional chondrosarcoma accounts for approximately 85% of all primary chondrosarcomas (3) and prognosis is strongly correlated with histological grading. Grade I chondrosarcoma, now reclassified as an atypical cartilaginous tumor, shows low cellularity and is locally aggressive, but typically does not metastasize (2). Grade II and grade III conventional chondrosarcomas show increased cellularity with mitoses and reduced cartilaginous matrix, and a corresponding increase in metastasizing capacity alongside poor patient survival (2;4). Amongst the rare chondrosarcoma subtypes, dedifferentiated chondrosarcoma accounts for up to 10% of all chondrosarcomas and shows a dismal prognosis. Dedifferentiated chondrosarcoma is comprised of two histologically well distinctive components: a high grade dedifferentiated component, and a seemingly low grade cartilaginous component (5). Mesenchymal chondrosarcoma is considered high grade and accounts for approximately 3% of primary chondrosarcoma histologically showing undifferentiated small round cells admixed with well differentiated cartilage (6). Clear cell chondrosarcoma is considered low grade and comprises about 2% of all primary chondrosarcomas, demonstrating tumor cells with a clear, empty cytoplasm (7).

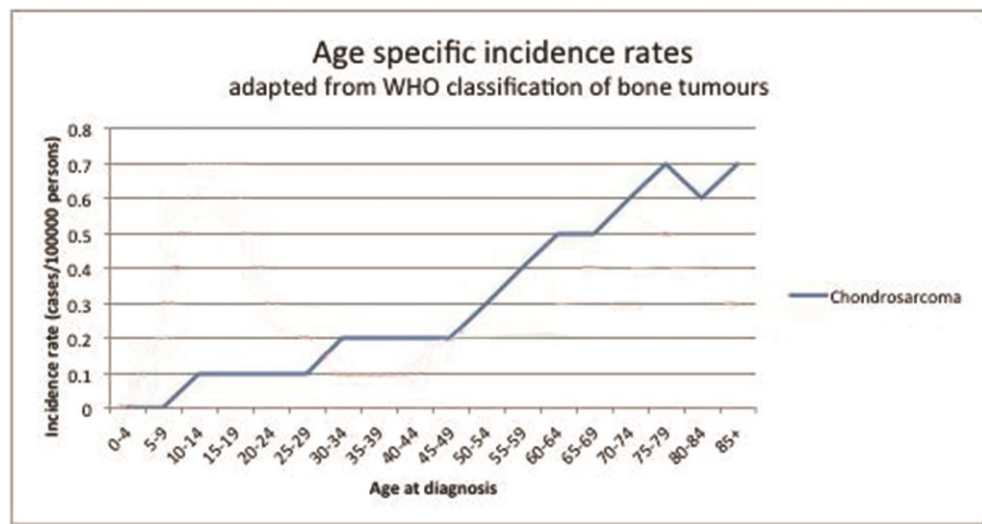


Figure 2.1. Incidence of chondrosarcoma stratified by age group. Chondrosarcoma (CS) is the second most common primary bone malignancy in humans and occurs predominantly between the 3rd and 6th decade of life. The increase in incidence observed after the 6th decade is attributed to recurrences. Adapted from WHO 2013

Current management of chondrosarcoma and resistance to therapy

The first line of treatment for chondrosarcoma is surgical resection with local adjuvant treatment such as phenol or cryosurgery, followed by filling the cavity with bone graft, showing long term local control in atypical cartilaginous tumor / grade I chondrosarcoma (8). Due to the necessity of wide resection margins to prevent recurrence in grade II and III chondrosarcoma, the patient often needs to undergo mutilating surgery. In the event of tumor location at a nonresectable site, such as in the skull or pelvis, or metastatic disease, there is still no curative treatment (3;9). Chondrosarcoma is notorious for its resistance to conventional chemo- and radiotherapy (3). Recently, a phase II study including 25 patients with chondrosarcoma using the nucleoside analog gemcitabine (657 mg/m² on day 1 and day 8) followed by the anti-mitotic docetaxel (75 mg/m² on day 8) over a course of 21 days, was aborted as only 2 patients showed partial response (10). In a recent study including 9 patients with dedifferentiated chondrosarcoma treated with surgery and chemotherapy (adriamycin, ifosfamide, cisplatin, and methotrexate) all patients died of metastatic disease (11). These results illustrate the high need for new targeted treatments in chondrosarcoma, as the conventional chemotherapeutics targeting the DNA machinery are not effective.

Primary chemoresistance of chondrosarcoma has long been ascribed to the phenotypic properties, such as hyaline cartilaginous matrix surrounding the cells prohibiting access to the cells, poor vascularization, and a slow division rate (12;13). As these properties are less prominent in high grade chondrosarcoma,

which typically shows less matrix, increased vascularization and increased mitotic rate, the resistance to therapy could also be due to activated anti-apoptosis or pro-survival pathways (12). Moreover, nuclear accumulation of doxorubicin was shown despite the presence of matrix and multidrug resistance pump activity. In addition, inhibition of the anti-apoptotic Bcl-2 family members was found to overcome resistance to doxorubicin and cisplatin in chondrosarcoma cell lines (14).

Targets and novel treatment options in chondrosarcoma

Over the past years advances have been made identifying multiple active pathways in chondrosarcoma, and preclinical work has led to the identification of potential targets for clinical trials (table 1). Here the recent identification of *IDH* mutations will be discussed in relation to active survival pathways and HIF1 α expression found in high grade chondrosarcomas, as well as growth plate signaling pathways including anti-apoptotic signaling, and retinoblastoma pathway alterations.

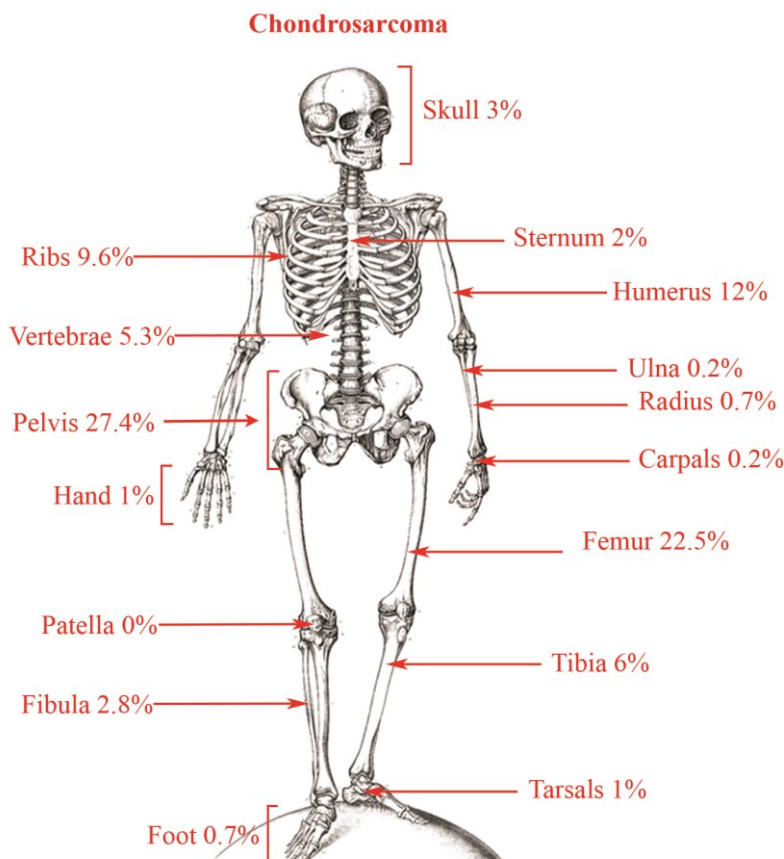


Figure 2.2. Distribution of chondrosarcoma across the skeleton.

Survival pathways: IDH mutations

Mutations in the isocitrate dehydrogenases (IDH) are found in 87% of benign enchondromas, 38-70% of primary conventional central chondrosarcoma, and 54% of dedifferentiated chondrosarcomas, but not in clear cell or mesenchymal chondrosarcomas (15-20). IDH is involved in the tricarboxylic acid cycle (Kreb's cycle) (21) and mutations in IDH1/2 lead to a diminished capacity to convert isocitrate to α -ketoglutarate (α -KG) and an acquired ability to convert α -KG to D-2-hydroxyglutarate (D2HG), which is considered an oncometabolite (19;21-25).

The exact mechanism through which D2HG causes tumor formation is unknown although increasing evidence points towards epigenetic mechanisms (26-31). D2HG impairs the function of the α -KG dependent dioxygenase TET2, leading to inhibition of DNA demethylation causing CpG island hypermethylation (27;32;33). Indeed, enchondromas carrying *IDH* mutations were hypermethylated (17). In addition, D2HG was shown to impair histone demethylation (33). Moreover, mutations in IDH are postulated to inhibit the prolyl/lysyl/hydroxylation of collagen proteins and thereby their maturation as an *IDH1* R132H conditional knock-in mouse model showed a reduction in collagen IV maturation (34). Finally, D2HG was postulated to induce pseudohypoxia (fig3) by inhibition of the HIF proline hydroxylases although this is controversial (22;34;35).

HIF-1 α is upregulated by a multitude of malignancies to cope with reduced perfusion, and is associated with increased proliferation, VEGF production, and resistance to chemo- and radiotherapy (36-40). High grade conventional chondrosarcoma shows activation of the hypoxia pathway through HIF1 α (41). Most drugs targeting hypoxia, are designed either to target VEGF, the downstream target of HIF1 α , or to target the PI3K/AKT/mTOR pathway, which can induce HIF1 α independent of oxygen conditions (fig 3) (36;42).

Survival pathways: PI3K, AKT, mTOR, VEGF

The PI3K/AKT pathway is often upregulated in cancer and can either inhibit apoptosis, or promote cell proliferation (fig 3) (43). Active AKT signaling was shown in chondrosarcoma(44) and the PI3K/AKT pathway has been shown to be involved in proliferation in mesenchymal chondroprogenitor cells (45). In chondrocytes, the PI3K/AKT can be activated by the chondrogenic transcription factor SOX9 (46), which is also expressed in chondrosarcoma (47;48). SOX9 siRNA in a chondrosarcoma cell line (SW1353) induced apoptosis which could be rescued by PTEN expression (46). Mutations in the tumor suppressor *PTEN* are rare in chondrosarcoma (49). Perifosine, an AKT inhibitor inhibiting AKT membrane recruitment, showed 17% decrease in tumor size in one chondrosarcoma patient after two cycles (Steinert, CTOS 2006). A larger phase II study was conducted including patients with chemoinensitive sarcomas but has not posted results (NCT00401388).

Mechanistic TOR (mTOR) is a point of convergence of many pathways involved in protein synthesis and cell proliferation, including the PI3K/AKT pathway (fig

3). The first suggestion of activation of the mTOR pathway was in mesenchymal chondrosarcoma, showing strong cytoplasmic p-AKT, p-mTOR, and PDGFR- α staining (50). In an adjuvant rat orthotopic Swarm Rat chondrosarcoma model, everolimus alone or in combination with doxorubicin after curettage showed inhibition of mTORC1 and decreased cell proliferation, however, the combination with doxorubicin showed an antagonistic effect with activation of the mTORC2 pathway (51). Allosteric inhibitors of the mTOR pathway, rapalogs, (rapamycin (sirolimus), everolimus, and temsirolimus) have limited efficacy in the clinic, but show high synergy with dual PI3K/mTOR inhibitors such as BEZ235 (52). A clinical trial with temsirolimus and liposomal doxorubicin included chondrosarcoma patients (NCT00949325). While awaiting the results of this trial, a study including ten patients with unresectable chondrosarcoma who were treated with sirolimus and cyclophosphamide showed a disease control rate of 70% (53). However, the resistance to rapalogs observed in other malignancies is suggestive that in chondrosarcoma a strategy including dual PI3K/mTOR inhibitors such as BEZ235 should be considered for future clinical trials.

Activated Src signaling can also lead to HIF1 α expression (fig 3) (12;54;55) and promote cell survival. Src signaling was shown to be elevated in chondrosarcoma (44), and the tyrosine kinase inhibitor dasatinib showed a decrease in cell proliferation in 7 out of 9 cell lines (44). However, in a phase II study no objective response was obtained with dasatinib single agent (70mg bid as starting dose) in chondrosarcoma patients (Schuetze CTOS 2010).

Activation of survival pathways can be through stimulation of the receptor tyrosine kinases by IGF-1 or PDGF. IGF-1 pathway activation was shown to be involved in chondrosarcoma proliferation, migration, apoptosis (56) (57;58), as well as progression to malignancy (58). Activation of the PDGF pathway has been shown to be related to worse prognosis in chondrosarcoma (59-61). Inhibition with imatinib, however, showed no effect in vitro in four chondrosarcoma cell lines (44), and in a clinical study including 26 patients no objective response was measured (62). HIF1 α expression is suggested to result in increased VEGF expression in chondrosarcoma (40). Sunitinib and pazopanib are tyrosine kinase inhibitors, targeting multiple kinases including both PDGF and VEGF. In combination with proton beam radiation, sunitinib was reported to achieve complete symptomatic relieve and durable response in a patient with metastatic clear cell chondrosarcoma (63). A clinical study with pazopanib is currently recruiting chondrosarcoma patients (NCT01330966).

Developmental pathways: Hedgehog

In osteochondroma, a benign cartilaginous tumor at the surface of bone that can give rise to secondary peripheral chondrosarcoma, mutations in the genes encoding either *exostosin -1 (EXT1)* or *-2 (EXT2)* have been identified (64). *EXT1* and *EXT2* are involved in the biosynthesis of heparan sulfate proteoglycans, which are necessary for the diffusion of the morphogen Indian Hedgehog (IHH) (65).

Recently, osteochondromas were shown to contain a mixture of both *EXT* mutant as well wildtype tumor cells (with functional *EXT*), and the latter were shown to be the precursor cells of peripheral chondrosarcoma (66) since peripheral chondrosarcoma have functional *EXT*, pointing towards a pathogenesis in chondrosarcoma independent of *EXT*.

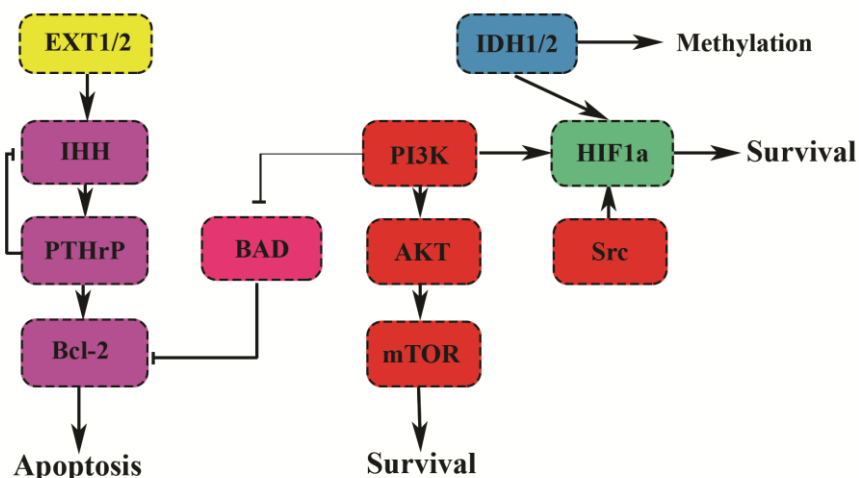


Figure 2.3. Apoptosis and survival pathways. EXT1/2: exostosin 1/2, IHH: Indian hedgehog, PTHrP: parathyroid protein, Bcl-2: B-cell lymphoma 2, BAD: Bcl-2 associated protein 2, IDH1/2: isocitrate 1/2, PI3K: phosphoinositide 3-kinase, AKT (PKB: Protein kinase B), mTOR: mammalian target of rapamycin, HIF1a: hypoxia inducible factor 1a, Src: sarcoma.

IHH is part of a negative feedback loop with parathyroid hormone-related protein (PTHrP), creating a tight balance between proliferation and differentiation (fig 3) (for review see (67;68)). Aberrant hedgehog signaling is also found in central chondrosarcoma (69;70), despite absence of *EXT* mutations. Blocking of the hedgehog pathway with triparanol was shown to be effective (70), but reports on the effect of cyclopamine are conflicting (69-71).

A recent randomised phase II clinical trial with IPI-926 (saridegib), a potent cyclopamine analogue (72), for patients with metastatic or locally advanced conventional chondrosarcoma was terminated as the primary endpoint, progression-free survival, was not met (NCT01609179). A second trial is currently ongoing with vismodegib, a cyclopamine-competitive SMO-inhibitor

(NCT01267955). Preliminary results show stable disease in 4 out of 17 patients (Italiano, ASCO 2012). In osteochondroma, primary cilia were found to retain their normal length but lose their orientation contributing to loss of chondrocyte directionality (73) while 70-100% of human enchondromas and chondrosarcomas were found to lack primary cilia (74). In *lft88*^{-/-} mice lacking primary cilia increased hedgehog signaling and enchondroma and chondrosarcoma formation, was observed. As cyclopamine depends on the primary cilia for SMO accumulation, chondrosarcoma cells lacking primary cilia were unresponsive to cyclopamine treatment (74). These results support the role for IHH in initiation of chondrosarcoma, and suggest that when inhibiting the hedgehog pathway in chondrosarcoma targets should be carefully selected.

Developmental pathways: Anti-apoptosis

The anti-apoptotic protein Bcl-2 is under direct regulation of PTH1R and is upregulated in chondrosarcoma (fig 3) (75). Moreover, Bcl-xl, another anti-apoptotic protein belonging to the Bcl-2 family was shown to be overexpressed in 18 chondrosarcoma tissues (76), indicating a specific defense mechanism contributing to chemoresistance in chondrosarcoma. siRNA against Bcl-2, Bcl-xl, and XIAP showed an enhanced sensitivity to doxorubicin and radiation (77;78), and treatment with the BH-3 mimetic ABT-737, was shown to synergistically overcome resistance to doxorubicin and cisplatin (14). Another anti-apoptotic protein, not related to the Bcl-2 family, survivin, was also found to be highly expressed in chondrosarcoma (79;80) and inhibition experiments in 2 cell lines resulted in overcoming resistance to doxorubicin (79). These data point towards an effective defense mechanism in which chondrosarcoma cells prevent programmed cell death in response to stress signals such as DNA damage.

Treatment with dulanermin (rhApo2L/TRAIL), a death receptor 4 (DR4) and 5 (DR5) agonist, showed complete remission in one patient (81), and treatment with apomab, a DR5 agonist, showed a 20% reduction in measureable disease in one chondrosarcoma patient (82), but showed no efficacy in a follow up phase 2 trial with 90 chondrosarcoma patients (NCT00543712). These (pre-) clinical results combined with this promising result with dulanermin show that restoring the defect in the apoptotic machinery could prove strong therapeutic potential in chondrosarcoma. However, since multiple anti-apoptotic proteins are upregulated in chondrosarcoma, a multi-targeted approach may be more effective, considering that dulanermin, targeting both DR4 and DR5, was more effective than apomab, targeting only DR5.

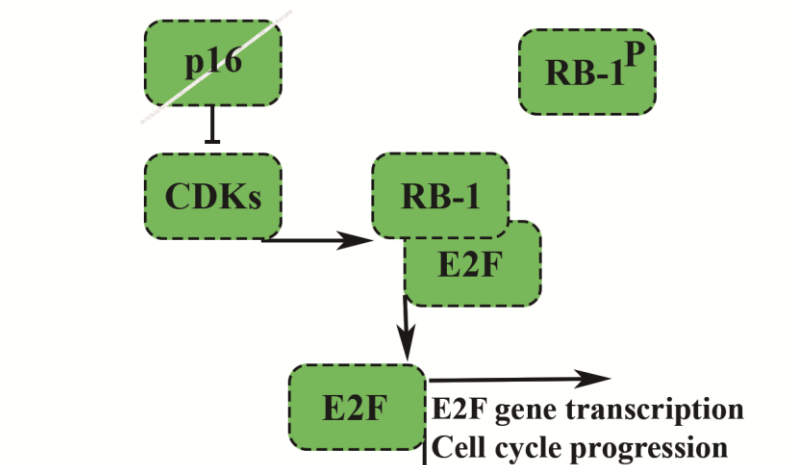


Figure 2.4. RB-1 pathway: p16 is a tumor suppressor and inhibits the cyclin dependent kinases (CDKs). Upon loss of p16, active CDKs phosphorylate RB-1 and release it from the E2F transcription factors, allowing for E2F target gene transcription and uncontrolled cell cycle progression.

Retinoblastoma signaling

The retinoblastoma protein pRb is a tumor suppressor controlling the cell cycle. In the absence of p16^{INK4A}, RB-1 is released from E2F transcription factors such as histone deacetylases (HDAC) and cell cycle progression and gene transcription can occur (fig 4) (83). Recently *Rb* was shown to be required for hypertrophic chondrocyte differentiation, and *Rb*^{c/c}/*p107*^{-/-} mice were shown to develop enchondromas, indicating an important role for cell cycle regulation during tumor development (84).

Ninety-six percent of conventional central high grade chondrosarcoma show alterations in the retinoblastoma pathway (85); not only through loss of the tumor suppressor *CDKN2A/p16* (86;87) along with elevated *CDK4* (88) but also through amplifications of *CDK6* and *E2F3* (89). In dedifferentiated chondrosarcoma p16 aberrations were found to be common (85%) and associated with loss of chromosome 9p (16) or promoter methylation (90). In mesenchymal and clear cell chondrosarcoma p16 alterations are found in 70% and 95% of the cases, respectively (16). Inhibition of CDK4 using shRNA against CDK4 was found to inhibit cell proliferation in three central chondrosarcoma cell lines (85). In a phase I dose defining study of the HSP90 inhibitor alvespimycin, one chondrosarcoma

patient showed CR (>6months stable disease) with reduction in CDK4 levels (91), supporting further exploration of HSP90 or CDK4 inhibitors in chondrosarcoma. On close proximity to the *CDKN2* locus on chromosome 9 is the methylthioadenosine phosphorylase (*MTAP*), an enzyme vital for the recycling of adenine and methionine synthesis. Deletions involving the *MTAP* locus (9p21) have been reported in 50% of chondrosarcoma cases (89;92-94). In *MTAP* deficient cells, adenine and methionine are not metabolized rendering these cells more sensitive to selective inhibition of de novo purine synthesis. Permetrexed disodium is a multitargeted anti-folate preventing the formation of precursor purine and pyrimidine nucleotides (95). A phase II trial with permetrexed disodium, has been performed in patients with metastatic or locally advanced chondrosarcoma (NCT00107419). No results have been posted yet.

Other therapies: COX-2 and aromatase inhibitors

Estrogen signaling plays a role in skeletal maturation and was found to be active in all chondrosarcoma subtypes (96-98). Even though initial results were promising (96;99), a recent retrospective series including 6 patients with locally advanced or metastatic chondrosarcoma treated with aromatase inhibitors did not show an increase in PFS compared to historically untreated patients (98). The prostaglandin synthase cyclooxygenase-2 (COX-2) is upregulated during inflammation, but also in for example colorectal, breast and prostate cancer (100). COX-2 upregulation was shown in chondrosarcoma (101;102) and to be associated with poor survival (103). COX-2 inhibition with celecoxib showed decreased cell viability in 4 chondrosarcoma cell lines, however, in chondrosarcoma xenografts, a relapse was observed after 6 weeks (102). The negative results obtained with aromatase inhibitors in patients and COX-2 inhibitors in mice do not support clinical implementation of these therapeutic strategies.

Table 2.1 Overview of Targets and Selected Trials in chondrosarcoma

Target	Drug	Mechanism	Clinical results	Clinical trial identifier or reference
				(10).
DNA synthesis	gemcitabine	Nucleoside analog	Phase II (n=53) combination with docetaxel. Terminated due to lack of evidence of efficacy	
	permetrexed	Prevents formation of DNA and RNA	Study completed, no results posted	NCT00107419
AKT/PI3K	perifosine	Inhibits AKT membrane recruitment	Phase I (n=10) combination with gemcitabine CS patient showed 17% decrease in tumor size after two cycles	NCT00401388 (Steinert CTOS 2006)
mTOR	sirolimus	mTOR inhibitor	Combination with cyclophosphamide in 10 patients disease control rate of 70%	(53)
SRC	dasatinib	Small molecule kinase inhibitor	Phase II, ongoing, NOR in CS	NCT00464620 (Schuetze CTOS 2006)

PDGF	sunitinib (SU11248)	Multi-targeted receptor tyrosine kinase inhibitor	Phase II, completed, no results posted Case study: Antitumor activity in 2 patients with extraskeletal myxoid CS Case study: Durable response after combination with proton beam radiation in 1 patient with metastatic clear cell CS	NCT00474994 (104) (63)
	imatinib	Competitive tyrosine kinase inhibitor	Phase II (n=26), NOR	(62)
	pazopanib	Blocks autophosphosphorylation of PDGF receptors, VEGF receptors, FGF receptors 1 and 3; inhibits Kit and Lck	Recruiting	NCT01330966
Hedgehog	saridegib (IPI- 926)	Smoothened inhibitor	Study terminated due to lack of evidence of efficacy Ongoing	NCT01609179 NCT01310816
	vismodegib (GDC-0449)	Smoothened inhibitor	Ongoing	NCT01267955

Apoptosis	Dulanermin rhAPO2L/TRAIL (AMG 951)	induces apoptosis through binding to DR4 and DR5	Phase I study n=71 2 CS patients durable PR Case study: near CR over 78 months in one patient with metastatic disease	(81;105)
	apomab	Mono-clonal IgG1 anti- antibody that triggers extrinsic apoptotic pathway through DR5	Phase I study n=50, terminated due to lack of evidence of efficacy CS patient 20% reduction in measurable disease	(82)
Rb pathway	alvespimycin	HSP90 inhibitor	Phase I study n=25 CS patient CR with reduction in CDK4 levels	(9)

CS: chondrosarcoma, NOR: no objective response, CR: complete response, PR: partial response.

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