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).

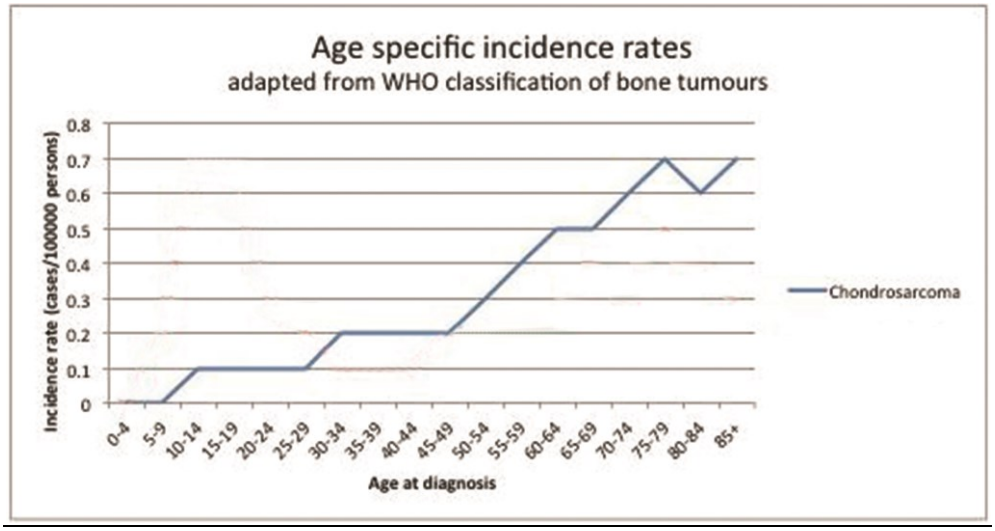

Figure 2.1. Incidence of chondrosarcoma stratified by age group. Chondrosarcoma (CS) is the second most common primary bone malignancy in humans and occurs predominantly between the 3rd and 6th decade of life. The increase in incidence observed after the 6th decade is attributed to recurrences. Adapted 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 HIF1α expression found in high grade chondrosarcomas, as well as growth plate signaling pathways including anti-apoptotic signaling, and retinoblastoma pathway alterations.

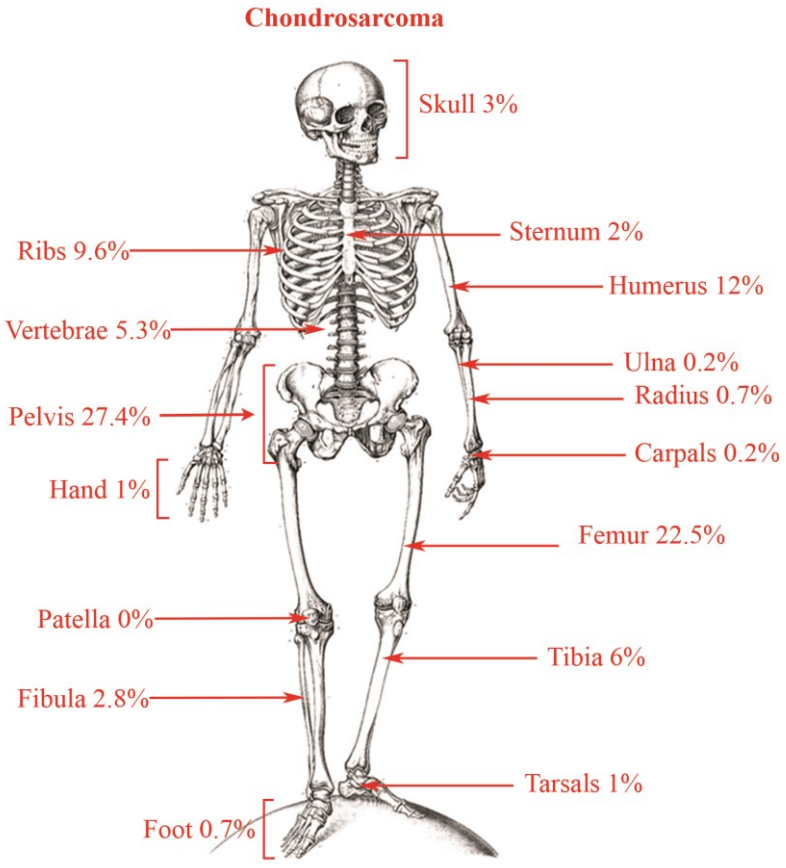

Figure 2.2. Distribution of chondrosarcoma across the skeleton.

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 IHD1/2 lead to a diminished capacity to convert isocitrate to α-ketoglutarate (α-KG) and an acquired ability to convert αKG 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 αKG 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-1α 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 HIF1α (41). Most drugs targeting hypoxia, are designed either to target VEGF, the downstream target of HIF1α, or to target the PI3K/AKT/mTOR pathway, which can induce HIF1α 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 chemoinsensitive 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-alpha 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 HIF1α 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). HIF1α 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.

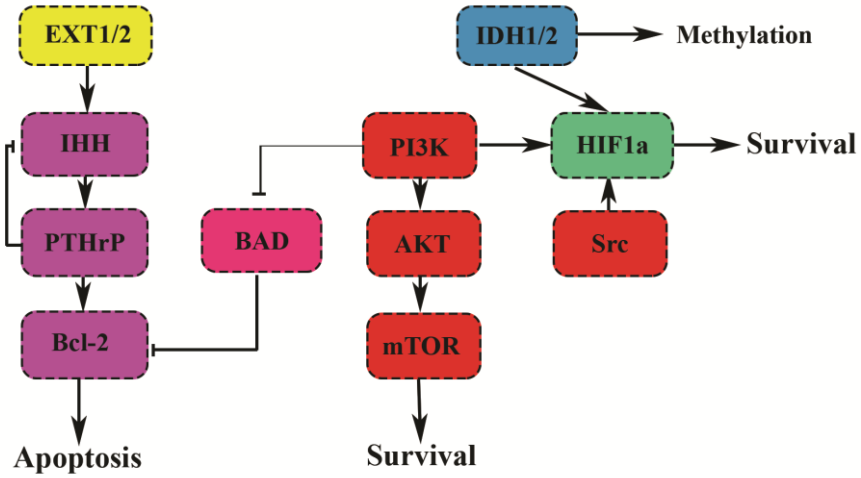

Figure 2.3. Apoptosis and survival pathways. EXT1/2: exostosin 1/2, IHH: Indian hedgehog, PTHrP: parathyroid protein, Bcl-2: B-cell lymphoma 2, BAD: Bcl-2 associated protein 2, IDH1/2: isocitrate 1/2, PI3K: phosphoinositide 3-kinase, AKT (PKB: Protein kinase B), mTOR: mammalian target of rapamycin, HIF1a: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

(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.

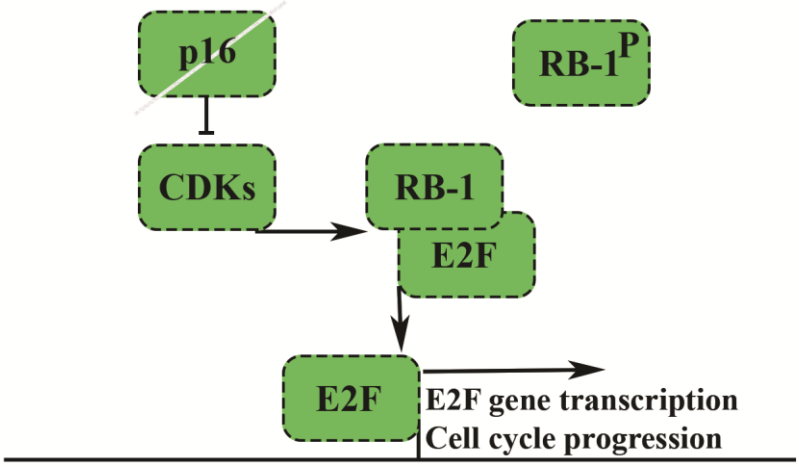

Figure 2.4. RB-1 pathway: p16 is a tumor suppressor and inhibits the cyclin dependent kinases (CDKs). Upon loss of p16, active CDKs phosphorylate RB-1 and release it from the E2F transcription factors, allowing for E2F target gene transcription and uncontrolled cell cycle progression.

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 identifyer or reference
DNA synthesis	gemcitabine	Nucleoside analog	Phase II (n=53) combination with docetaxel. Terminated due to lack of evidence of efficacy	(10).
	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.

References

(1) Fletcher C.D.M., Bridge JA, Hogendoorn PCW, Mertens F. WHO Classification of Tumours of Soft Tissue and Bone. 4 ed. 2013.

(2) Hogendoorn PCW, Bovée JVMG, Nielsen GP. Chondrosarcoma (grades I-III), including primary and secondary variants and periosteal chondrosarcoma. In: Fletcher C.D.M., Bridge JA, Hogendoorn PCW, Mertens F, editors. World Health Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. 4 ed. 2013. p. 264-8.

(3) Gelderblom H, Hogendoorn PCW, Dijkstra SD, van Rijswijk CS, Krol AD, Taminiau AH, Bovee JV. The clinical approach towards chondrosarcoma. Oncologist 2008;13(3):320-9.

(4) Evans HL, Ayala AG, Romsdahl MM. Prognostic factors in chondrosarcoma of bone. A clinicopathologic analysis with emphasis on histologic grading. Cancer 1977;40:818-31.

(5) Inwards CY, Hogendoorn PCW. Dedifferentiated Chondrosarcoma. In: Fletcher C.D.M., Bridge JA, Hogendoorn PCW, Mertens F, editors. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. 4 ed. 2013. p. 269-70.

(6) Nakashima Y, de Pinieux G, Ladanyi M. Mesenchymal Chondrosarcoma. In: Fletcher C.D.M., Bridge JA, Hogendoorn PCW, Mertens F, editors. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. 2013. p. 271-2.

(7) McCarthy EF, Hogendoorn PCW. Clear Cell Chondrosarcoma. In: Fletcher C.D.M., Bridge JA, Hogendoorn PCW, Mertens F, editors. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. 4 ed. 2013. p. 273-4.

(8) Verdegaal SH, Brouwers HF, van Zwet EW, Hogendoorn PC, Taminiau AH. Low-grade chondrosarcoma of long bones treated with intralesional curettage followed by application of phenol, ethanol, and bone-grafting. J Bone Joint Surg Am 2012;94(13):1201-7.

(9) Bone sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2012;23 Suppl 7:vii100-vii109.

(10) Fox E, Patel S, Wathen JK, Schuetze S, Chawla S, Harmon D, Reinke D, Chugh R, Benjamin RS, Helman LJ. Phase II Study of Sequential Gemcitabine Followed by Docetaxel for Recurrent Ewing Sarcoma, Osteosarcoma, or Unresectable or Locally Recurrent Chondrosarcoma: Results of Sarcoma Alliance for Research Through Collaboration Study 003.

Oncologist 2012;17(3):321-e329.

(11) Yokota K, Sakamoto A, Matsumoto Y, Matsuda S, Harimaya K, Oda Y, Iwamoto Y. Clinical outcome for patients with dedifferentiated chondrosarcoma: a report of 9 cases at a single institute. J Orthop Surg Res 2012;7(1):38.

(12) Bovée JVMG, Hogendoorn PCW, Wunder JS, Alman BA. Cartilage tumours and bone development: molecular pathology and possible therapeutic targets. Nat Rev Cancer 2010;10(7):481-8.

(13) David E, Blanchard F, Heymann MF, De PG, Gouin F, Redini F, Heymann D. The Bone Niche of Chondrosarcoma: A Sanctuary for Drug Resistance, Tumour Growth and also a Source of New Therapeutic Targets. Sarcoma 2011;2011:-932451.

(14) van Oosterwijk JG, Herpers B, Meijer D, Briaire-de Bruijn IH, Cleton-Jansen AM, Gelderblom H, van de Water B, Bovée JVMG. Restoration of chemosensitivity for doxorubicin and cisplatin in chondrosarcoma in vitro: BCL-2 family members cause chemoresistance. Ann Oncol 2012;23(6):1617-26.

(15) Schaap FG, French PJ, Bovee JVMG. Mutations in the Isocitrate Dehydrogenase Genes IDH1 and IDH2 in Tumors. Adv Anat Pathol 2013;20(1):32-8.

(16) Meijer D, de JD, Pansuriya TC, van den Akker BE, Picci P, Szuhai K, Bovee JV. Genetic characterization of mesenchymal, clear cell, and dedifferentiated chondrosarcoma. Genes Chromosomes Cancer 2012;51(10):899-909.

(17) Pansuriya TC, van ER, d'Adamo P, van Ruler MA, Kuijjer ML, Oosting J, Cleton-Jansen AM, van Oosterwijk JG, Verbeke SL, Meijer D, van WT, Nord KH, Sangiorgi L, Toker B, Liegl-Atzwanger B, San-Julian M, Sciot R, Limaye N, Kindblom LG, Daugaard S, Godfraind C, Boon LM, Vikkula M, Kurek KC, Szuhai K et al. Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. Nat Genet 2011;43(12):1256-61.

(18) Amary MF, Bacsi K, Maggiani F, Damato S, Halai D, Berisha F, Pollock R, O'Donnell P, Grigoriadis A, Diss T, Eskandarpour M, Presneau N, Hogendoorn PC, Futreal A, Tirabosco R, Flanagan AM. IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. J Pathol 2011;224(3):334-43.

(19) Amary MF, Damato S, Halai D, Eskandarpour M, Berisha F, Bonar F, McCarthy S, Fantin VR, Straley KS, Lobo S, Aston W, Green CL, Gale RE, Tirabosco R, Futreal A, Campbell P, Presneau N, Flanagan AM. Ollier disease and Maffucci syndrome are caused by somatic mosaic

mutations of IDH1 and IDH2. Nat Genet 2011.

(20) Damato S, Alorjani M, Bonar F, McCarthy SW, Cannon SR, O'Donnell P, Tirabosco R, Amary MF, Flanagan AM. IDH1 mutations are not found in cartilaginous tumours other than central and periosteal chondrosarcomas and enchondromas. Histopathology 2011.

(21) Leonardi R, Subramanian C, Jackowski S, Rock CO. Cancer-associated isocitrate dehydrogenase mutations inactivate NADPH-dependent reductive carboxylation. J Biol Chem 2012;287(18):14615-20.

(22) Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, Yu W, Li Z, Gong L, Peng Y, Ding J, Lei Q, Guan KL, Xiong Y. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science 2009;324(5924):261-5.

(23) Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM, Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, Su SM. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 2009;462(7274):739-44.

(24) Luchman HA, Stechishin OD, Dang NH, Blough MD, Chesnelong C, Kelly JJ, Nguyen SA, Chan JA, Weljie AM, Cairncross JG, Weiss S. An in vivo patient-derived model of endogenous IDH1-mutant glioma. Neuro Oncol 2012;14(2):184-91.

(25) Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Coller HA, Cross JR, Fantin VR, Hedvat CV, Perl AE, Rabinowitz JD, Carroll M, Su SM, Sharp KA, Levine RL, Thompson CB. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. Cancer Cell 2010;17(3):225-34.

(26) Dang L, Jin S, Su SM. IDH mutations in glioma and acute myeloid leukemia. Trends Mol Med 2010;16(9):387-97.

(27) Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li Y, Bhagwat N, Vasanthakumar A, Fernandez HF, Tallman MS, Sun Z, Wolniak K, Peeters JK, Liu W, Choe SE, Fantin VR, Paietta E, Lowenberg B, Licht JD, Godley LA, Delwel R, Valk PJ, Thompson CB, Levine RL et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell 2010;18(6):553-67.

(28) Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, Felsberg J, Wolter M, Mawrin C, Wick W, Weller M, Herold-Mende C, Unterberg A, Jeuken JW, Wesseling P, Reifenberger G, von DA. Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study

of 1,010 diffuse gliomas. Acta Neuropathol 2009;118(4):469-74.

(29) Kang MR, Kim MS, Oh JE, Kim YR, Song SY, Seo SI, Lee JY, Yoo NJ, Lee SH. Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. Int J Cancer 2009;125(2):353-5.

(30) Murugan AK, Bojdani E, Xing M. Identification and functional characterization of isocitrate dehydrogenase 1 (IDH1) mutations in thyroid cancer. Biochem Biophys Res Commun 2010;393(3):555-9.

(31) Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD. IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360(8):765-73.

(32) Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 2010;17(5):510-22.

(33) Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, Edwards CR, Khanin R, Figueroa ME, Melnick A, Wellen KE, O'Rourke DM, Berger SL, Chan TA, Levine RL, Mellinghoff IK, Thompson CB. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature 2012;483(7390):474-8.

(34) Sasaki M, Knobbe CB, Itsumi M, Elia AJ, Harris IS, Chio II, Cairns RA, McCracken S, Wakeham A, Haight J, Ten AY, Snow B, Ueda T, Inoue S, Yamamoto K, Ko M, Rao A, Yen KE, Su SM, Mak TW. D-2-hydroxyglutarate produced by mutant IDH1 perturbs collagen maturation and basement membrane function. Genes Dev 2012;26(18):2038-49.

(35) Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S, Losman JA, Joensuu P, Bergmann U, Gross S, Travins J, Weiss S, Looper R, Ligon KL, Verhaak RG, Yan H, Kaelin WG, Jr. Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. Nature 2012;483(7390):484-8.

(36) Greer SN, Metcalf JL, Wang Y, Ohh M. The updated biology of hypoxia-inducible factor. EMBO J 2012;31(11):2448-60.

(37) Robey IF, Lien AD, Welsh SJ, Baggett BK, Gillies RJ. Hypoxia-inducible factor-1alpha and the glycolytic phenotype in tumors. Neoplasia 2005;7(4):324-30.

(38) O'Donnell JL, Joyce MR, Shannon AM, Harmey J, Geraghty J, Bouchier-Hayes D. Oncological implications of hypoxia inducible factor-1alpha (HIF-1alpha) expression.

Cancer Treat Rev 2006;32(6):407-16.

(39) Fang J, Yan L, Shing Y, Moses MA. HIF-1alpha-mediated up-regulation of vascular endothelial growth factor, independent of basic fibroblast growth factor, is important in the switch to the angiogenic phenotype during early tumorigenesis. Cancer Res 2001;61(15):5731-5.

(40) Lin C, McGough R, Aswad B, Block JA, Terek R. Hypoxia induces HIF-1alpha and VEGF expression in chondrosarcoma cells and chondrocytes. J Orthop Res 2004;22(6):1175-81.

(41) Boeuf S, Bovee JVMG, Lehner B, Hogendoorn PCW, Richter W. Correlation of hypoxic signalling to histological grade and outcome in cartilage tumours. Histopathology 2009.

(42) Agani F, Jiang BH. Oxygen-independent regulation of HIF-1: novel involvement of PI3K/AKT/mTOR pathway in cancer. Curr Cancer Drug Targets 2013.

(43) Maddika S, Ande SR, Panigrahi S, Paranjothy T, Weglarczyk K, Zuse A, Eshraghi M, Manda KD, Wiechec E, Los M. Cell survival, cell death and cell cycle pathways are interconnected: implications for cancer therapy. Drug Resist Updat 2007;10(1-2):13-29.

(44) Schrage YM, Briaire-de Bruijn IH, de Miranda NFCC, van Oosterwijk JG, Taminiau AHM, van Wezel T, Hogendoorn PCW, Bovée JVMG. Kinome profiling of chondrosarcoma reveals Src-pathway activity and dasatinib as option for treatment. Cancer Res 2009;69(15):6216-22.

(45) Akiyama H, Furukawa S, Wakisaka S, Maeda T. Cartducin stimulates mesenchymal chondroprogenitor cell proliferation through both extracellular signal-regulated kinase and phosphatidylinositol 3-kinase/Akt pathways. FEBS J 2006;273(10):2257-63.

(46) Ikegami D, Akiyama H, Suzuki A, Nakamura T, Nakano T, Yoshikawa H, Tsumaki N. Sox9 sustains chondrocyte survival and hypertrophy in part through Pik3ca-Akt pathways. Development 2011;138(8):1507-19.

(47) Cajaiba MM, Jianhua L, Goodman MA, Fuhrer KA, Rao UN. Sox9 expression is not limited to chondroid neoplasms: variable occurrence in other soft tissue and bone tumors with frequent expression by synovial sarcomas. Int J Surg Pathol 2010;18(5):319-23.

(48) Wehrli BM, Huang W, De CB, Ayala AG, Czerniak B. Sox9, a master regulator of chondrogenesis, distinguishes mesenchymal chondrosarcoma from other small blue round cell tumors. Hum Pathol 2003;34(3):263-9.

(49) Lin C, Meitner PA, Terek RM. PTEN Mutation Is Rare in Chondrosarcoma. Diagn Mol Pathol 2002;11(1):22-6.

(50) Brown RE, Boyle JL. Mesenchymal chondrosarcoma: molecular characterization by a proteomic approach, with morphogenic and therapeutic

implications. Ann Clin Lab Sci 2003;33(2):131-41.

(51) Perez J, Decouvelaere AV, Pointecouteau T, Pissaloux D, Michot JP, Besse A, Blay JY, Dutour A. Inhibition of chondrosarcoma growth by mTOR inhibitor in an in vivo syngeneic rat model. PLoS ONE 2012;7(6):e32458.

(52) Yang S, Xiao X, Meng X, Leslie KK. A mechanism for synergy with combined mTOR and PI3 kinase inhibitors. PLoS ONE 2011;6(10):e26343.

(53) Bernstein-Molho R, Kollender Y, Issakov J, Bickels J, Dadia S, Flusser G, Meller I, Sagi-Eisenberg R, Merimsky O. Clinical activity of mTOR inhibition in combination with cyclophosphamide in the treatment of recurrent unresectable chondrosarcomas. Cancer Chemother Pharmacol 2012;70(6):855-60.

(54) Aligayer H, Boyd DD, Heiss MM, Abdalla EK, Curley SA, Gallick GE. Activation of Src kinase in primary colorectal carcinoma: an indicator of poor clinical prognosis. Cancer 2002;94(2):344-51.

(55) Fizazi K. The role of Src in prostate cancer. Ann Oncol 2007;18(11):1765-73.

(56) Matsumura T, Whelan MC, Li XQ, Trippel SB. Regulation by IGF-I and TGF-beta1 of Swarm-rat chondrosarcoma chondrocytes. J Orthop Res 2000;18(3):351-5.

(57) Wu CM, Li TM, Hsu SF, Su YC, Kao ST, Fong YC, Tang CH. IGF-I enhances alpha5beta1 integrin expression and cell motility in human chondrosarcoma cells. J Cell Physiol 2011;226(12):3270-7.

(58) Ho L, Stojanovski A, Whetstone H, Wei QX, Mau E, Wunder JS, Alman B. Gli2 and p53 cooperate to regulate IGFBP-3- mediated chondrocyte apoptosis in the progression from benign to malignant cartilage tumors. Cancer Cell 2009;16(2):126-36.

(59) Masui F, Ushigome S, Fujii K. Clear cell chondrosarcoma: a pathological and immunohistochemical study. Histopathology 1999;34(5):447-52.

(60) Sulzbacher I, Birner P, Trieb K, Muhlbauer M, Lang S, Chott A. Platelet-derived growth factor-alpha receptor expression supports the growth of conventional chondrosarcoma and is associated with adverse outcome. Am J Surg Pathol 2001;25(12):1520-7.

(61) Franchi A, Baroni G, Sardi I, Giunti L, Capanna R, Campanacci D. Dedifferentiated peripheral chondrosarcoma: a clinicopathologic, immunohistochemical, and molecular analysis of four cases. Virchows Arch 2012;460(3):335-42.

(62) Grignani G, Palmerini E, Stacchiotti S, Boglione A, Ferraresi V, Frustaci S, Comandone A, Casali PG, Ferrari S, Aglietta M. A phase 2 trial of imatinib mesylate in patients with recurrent nonresectable chondrosarcomas expressing platelet-derived growth factor receptor-alpha or -beta: An Italian Sarcoma

Group study. Cancer 2011;117(4):826-31.

(63) Dallas J, Imanirad I, Rajani R, Dagan R, Subbiah S, Gaa R, Dwarica WA, Ivey AM, Zlotecki RA, Malyapa R, Indelicato DJ, Scarborough MT, Reith JD, Gibbs CP, Dang LH. Response to sunitinib in combination with proton beam radiation in a patient with chondrosarcoma: a case report. J Med Case Rep 2012;6:41.

(64) Jennes I, Pedrini E, Zuntini M, Mordenti M, Balkassmi S, Asteggiano CG, Casey B, Bakker B, Sangiorgi L, Wuyts W. Multiple osteochondromas: mutation update and description of the multiple osteochondromas mutation database (MOdb). Hum Mutat 2009;30(12):1620-7.

(65) Stickens D, Brown D, Evans GA. EXT genes are differentially expressed in bone and cartilage during mouse embryogenesis. Dev Dyn 2000;218(3):452-64.

(66) de Andrea CE, Reijnders CM, Kroon HM, de JD, Hogendoorn PC, Szuhai K, Bovee JV. Secondary peripheral chondrosarcoma evolving from osteochondroma as a result of outgrowth of cells with functional EXT. Oncogene 2011.

(67) Chung U-I, Lanske B, Lee K, Li E, Kronenberg HM. The parathyroid hormone/parathyroid hormone-related peptide receptor coordinates endochondral bone development by directly controlling chondrocyte differentiation. Proc Natl Acad Sci USA 1998;95:13030-5.

(68) Chung UI, Schipani E, McMahon AP, Kronenberg HM. Indian hedgehog couples chondrogenesis to osteogenesis in endochondral bone development. J Clin Invest 2001;107(3):295-304.

(69) Schrage YM, Hameetman L, Szuhai K, Cleton-Jansen AM, Taminiau AHM, Hogendoorn PCW, Bovée JVMG. Aberrant heparan sulfate proteoglycan localization, despite normal exostosin, in central chondrosarcoma. Am J Pathol 2009;174(3):979-88.

(70) Tiet TD, Hopyan S, Nadesan P, Gokgoz N, Poon R, Lin AC, Yan T, Andrulis IL, Alman BA, Wunder JS. Constitutive hedgehog signaling in chondrosarcoma up-regulates tumor cell proliferation. Am J Pathol 2006;168(1):321-30.

(71) Oji GS, Gomez P, Kurriger G, Stevens J, Morcuende JA. Indian hedgehog signaling pathway differences between swarm rat chondrosarcoma and native rat chondrocytes. Iowa Orthop J 2007;27:9-16.

(72) Tremblay MR, Lescarbeau A, Grogan MJ, Tan E, Lin G, Austad BC, Yu LC, Behnke ML, Nair SJ, Hagel M, White K, Conley J, Manna JD, Alvarez-Diez TM, Hoyt J, Woodward CN, Sydor JR, Pink M, MacDougall J, Campbell MJ, Cushing J, Ferguson J, Curtis MS, McGovern K, Read MA et al. Discovery of a potent and orally active hedgehog pathway antagonist (IPI-926). J

Med Chem 2009;52(14):4400-18.

(73) de Andrea CE, Wiweger M, Prins F, Bovee JVMG, Romeo S, Hogendoorn PCW. Primary cilia organization reflects polarity in the growth plate and implies loss of polarity and mosaicism in osteochondroma. Lab Invest 2010;90(7):1091-101.

(74) Ho L, Ali SA, Al-Jazrawe M, Kandel R, Wunder JS, Alman BA. Primary cilia attenuate hedgehog signalling in neoplastic chondrocytes. Oncogene 2012.

(75) Rozeman LB, Hameetman L, Cleton-Jansen AM, Taminiau AHM, Hogendoorn PCW, Bovée JVMG. Absence of IHH and retention of PTHrP signalling in enchondromas and central chondrosarcomas. J Pathol 2005;205(4):476-82.

(76) Shen ZN, Nishida K, Doi H, Oohashi T, Hirohata S, Ozaki T, Yoshida A, Ninomiya Y, Inoue H. Suppression of chondrosarcoma cells by 15-deoxy-Delta 12,14-prostaglandin J2 is associated with altered expression of Bax/Bcl-xL and p21. Biochem Biophys Res Commun 2005;328(2):375-82.

(77) Kim DW, Kim KO, Shin MJ, Ha JH, Seo SW, Yang J, Lee FY. siRNA-based targeting of antiapoptotic genes can reverse chemoresistance in P-glycoprotein expressing chondrosarcoma cells. Mol Cancer 2009;8:28.

(78) Kim DW, Seo SW, Cho SK, Chang SS, Lee HW, Lee SE, Block JA, Hei TK, Lee FY. Targeting of cell survival genes using small interfering RNAs (siRNAs) enhances radiosensitivity of Grade II chondrosarcoma cells. J Orthop Res 2007;25(6):820-8.

(79) Lechler P, Renkawitz T, Campean V, Balakrishnan S, Tingart M, Grifka J, Schaumburger J. The antiapoptotic gene survivin is highly expressed in human chondrosarcoma and promotes drug resistance in chondrosarcoma cells in vitro. BMC Cancer 2011;11:-120.

(80) Machado I, Giner F, Mayordomo E, Carda C, Navarro S, Llombart-Bosch A. Tissue microarrays analysis in chondrosarcomas: light microscopy, immunohistochemistry and xenograft study. Diagn Pathol 2008;3 Suppl 1:S25.

(81) Subbiah V, Brown RE, Buryanek J, Trent J, Ashkenazi A, Herbst R, Kurzrock R. Targeting the Apoptotic Pathway in Chondrosarcoma Using Recombinant Human Apo2L/TRAIL (Dulanermin), a Dual Proapoptotic Receptor (DR4/DR5) Agonist. Mol Cancer Ther 2012;11(11):2541-6.

(82) Camidge DR. Apomab: an agonist monoclonal antibody directed against Death Receptor 5/TRAIL-Receptor 2 for use in the treatment of solid tumors. Expert Opin Biol Ther 2008;8(8):1167-76.

(83) Witkiewicz AK, Knudsen KE, Dicker AP, Knudsen ES. The meaning of p16(ink4a) expression in tumors: functional

significance, clinical associations and future developments. Cell Cycle 2011;10(15):2497-503.

(84) Landman AS, Danielian PS, Lees JA. Loss of pRB and p107 disrupts cartilage development and promotes enchondroma formation. Oncogene 2012.

(85) Schrage YM, Lam S, Jochemsen AG, Cleton-Jansen AM, Taminiau AHM, Hogendoorn PCW, Bovee JVMG. Central chondrosarcoma progression is associated with pRb pathway alterations; CDK4 downregulation and p16 overexpression inhibit cell growth in vitro. J Cell Mol Med 2008;13(9A):2843-52.

(86) Hallor KH, Staaf J, Bovée JVMG, Hogendoorn PCW, Cleton-Jansen AM, Knuutila S, Savola S, Niini T, Brosjo O, Bauer HCF, Vult von Steyern F., Jonsson K, Skorpil M, Mandahl N, Mertens F. Genomic Profiling of Chondrosarcoma: Chromosomal Patterns in Central and Peripheral Tumors. Clin Cancer Res 2009;15(8):2685-94.

(87) van Beerendonk HM, Rozeman LB, Taminiau AHM, Sciot R, Bovée JVMG, Cleton-Jansen AM, Hogendoorn PCW. Molecular analysis of the INK4A/INK4A-ARF gene locus in conventional (central) chondrosarcomas and enchondromas: indication of an important gene for tumour progression. J Pathol 2004;202(3):359-66.

(88) Asp J, Inerot S, Block JA, Lindahl A. Alterations in the regulatory pathway involving p16, pRb and cdk4 in human chondrosarcoma. J Orthop Res 2001;19(1):149-54.

(89) Niini T, Scheinin I, Lahti L, Savola S, Mertens F, Hollmen J, Bohling T, Kivioja A, Nord KH, Knuutila S. Homozygous deletions of cadherin genes in chondrosarcoma-an array comparative genomic hybridization study. Cancer Genet 2012;205(11):588-93.

(90) Ropke M, Boltze C, Neumann HW, Roessner A, Schneider-Stock R. Genetic and epigenetic alterations in tumor progression in a dedifferentiated chondrosarcoma. Path Res Pract 2003;199(6):437-44.

(91) Pacey S, Wilson RH, Walton M, Eatock MM, Hardcastle A, Zetterlund A, Arkenau HT, Moreno-Farre J, Banerji U, Roels B, Peachey H, Aherne W, de Bono JS, Raynaud F, Workman P, Judson I. A phase I study of the heat shock protein 90 inhibitor alvespimycin (17-DMAG) given intravenously to patients with advanced solid tumors. Clin Cancer Res 2011;17(6):1561-70.

(92) Jagasia AA, Block JA, Qureshi A, Diaz MO, Nobori T, Gitelis S, Iyer AP. Chromosome 9 related aberrations and deletions of the CDKN2 and MTS2 putative tumor suppressor genes in human chondrosarcomas. Cancer Lett 1996;105:91-103.

(93) Jagasia AA, Block JA, Diaz MO, Nobori T, Gitelis S, Inerot SE, Iyer AP. Partial deletions of the CDKN2 and MTS2 putative tumor suppressor genes in a

myxoid chondrosarcoma. Cancer Lett 1996;105:77-90.

(94) Chow WA, Bedell V, Gaytan P, Borden E, Goldblum J, Hicks D, Slovak ML. Methylthioadenosine phosphorylase gene deletions are frequently detected by fluorescence in situ hybridization in conventional chondrosarcomas. Cancer Genet Cytogenet 2006;166(2):95-100.

(95) Bertino JR, Waud WR, Parker WB, Lubin M. Targeting tumors that lack methylthioadenosine phosphorylase (MTAP) activity: current strategies. Cancer Biol Ther 2011;11(7):627-32.

(96) Cleton-Jansen AM, van Beerendonk HM, Baelde HJ, Bovée JVMG, Karperien M, Hogendoorn PCW. Estrogen signaling is active in cartilaginous tumors: implications for antiestrogen therapy as treatment option of metastasized or irresectable chondrosarcoma. Clin Cancer Res 2005;11(22):8028-35.

(97) Grifone TJ, Haupt HM, Podolski V, Brooks JJ. Immunohistochemical expression of estrogen receptors in chondrosarcomas and enchondromas. Int J Surg Pathol 2008;16(1):31-7.

(98) Meijer D, Gelderblom H, Karperien M, Cleton-Jansen A-M, Hogendoorn PCW, Bovee JVMG. Expression of aromatase and estrogen receptor alpha in chondrosarcoma, but no beneficial effect of inhibiting estrogen signaling both *in vitro* and *in vivo*. Clinical Sarcoma Research 2011.

(99) Fong YC, Yang WH, Hsu SF, Hsu HC, Tseng KF, Hsu CJ, Lee CY, Scully SP. 2-methoxyestradiol induces apoptosis and cell cycle arrest in human chondrosarcoma cells. J Orthop Res 2007;25(8):1106-14.

(100) Rizzo MT. Cyclooxygenase-2 in oncogenesis. Clin Chim Acta 2011 April 11;412(9-10):671-87.

(101) Sutton KM, Wright M, Fondren G, Towle CA, Mankin HJ. Cyclooxygenase-2 expression in chondrosarcoma. Oncology 2004;66(4):275-80.

(102) Schrage YM, Machado I, Meijer D, Briaire-de Bruijn I, van den Akker B, Taminiau AHM, Kalinski T, Llombart-Bosch A, Bovée JVMG. COX-2 expression in chondrosarcoma: a role for celecoxib treatment? Eur J Cancer 2010;46:616-24.

(103) Endo M, Matsumura T, Yamaguchi T, Yamaguchi U, Morimoto Y, Nakatani F, Kawai A, Chuman H, Beppu Y, Shimoda T, Hasegawa T. Cyclooxygenase-2 overexpression associated with a poor prognosis in chondrosarcomas. Hum Pathol 2006;37(4):471-6.

(104) Stacchiotti S, Dagrada GP, Morosi C, Negri T, Romanini A, Pilotti S, Gronchi A, Casali PG. Extraskeletal myxoid chondrosarcoma: tumor response to sunitinib. Clin Sarcoma Res 2012;2(1):22.

(105) Herbst RS, Eckhardt SG, Kurzrock R, Ebbinghaus S, O'Dwyer PJ, Gordon MS, Novotny W, Goldwasser MA, Tohnya TM, Lum BL,

Ashkenazi A, Jubb AM, Mendelson DS. Phase I dose-escalation study of recombinant human Apo2L/TRAIL, a dual proapoptotic receptor agonist, in patients with advanced cancer. J Clin Oncol 2010;28(17):2839-46.