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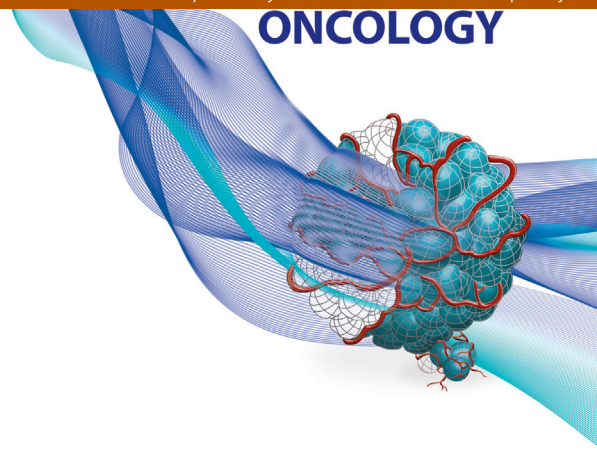

Novel therapeutic approaches in chondrosarcoma

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Chondrosarcoma is a malignant tumor of bones, characterized by the production of cartilage matrix. Due to lack of effective treatment for advanced disease, the clinical management of chondrosarcomas is exceptionally challenging. Current research focuses on elucidating the molecular events underlying the pathogenesis of this rare bone malignancy, with the goal of developing new molecularly targeted therapies. Signaling pathways suggested to have a role in chondrosarcoma include Hedgehog, Src, PI3k–Akt–mTOR and angiogenesis. Mutations in *IDH1/2*, present in more than 50% of primary conventional chondrosarcomas, make the development of IDH inhibitors a promising treatment option. The present review discusses the preclinical and early clinical data on novel targeted therapeutic approaches in chondrosarcoma.

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KEYWORDS

• chondrosarcoma • HDACIs • Hedgehog pathway • *IDH1/2* • novel agents • Src pathway • treatment

Chondrosarcoma is a malignant tumor of bone that is characterized by the production of cartilage matrix by tumor cells but displays diverse histopathology and clinical behavior [1]. Following osteosarcoma, it is the second most frequent primary malignant tumor of bone [2], accounting for 20–27% of primary malignant bone neoplasms [3]. The most common anatomical location of origin is pelvis, followed by the proximal femur, proximal humerus, distal femur and ribs [4]. The vast majority (90%) are conventional chondrosarcomas, either presenting *de novo* in the medulla of bone, or arising as a secondary tumor from pre-existing benign lesion such as enchondromas and osteochondromas. The remaining 10% are rare variants of chondrosarcoma, which include dedifferentiated, clear cell and mesenchymal chondrosarcomas. It remains a matter of debate whether myxoid chondrosarcoma represents a higher grade variant of conventional chondrosarcoma or should be considered a distinct disease entity [5]. Conventional chondrosarcomas are typically low or intermediate grade and are characterized by indolent clinical course and low metastatic potential. High-grade chondrosarcomas account for 5–10% of conventional chondrosarcomas and are associated with high metastatic potential and poor prognosis. The main site of metastatic disease is lung, while the regional lymph nodes and liver are much less commonly involved [6,7].

The cornerstone of the management of localized chondrosarcomas is surgical resection. Low-grade tumors confined to the bone, in selected cases can be managed by extensive intralesional

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curettage, with the aim of minimizing functional disability [8,9], while high-grade tumors require wide, en-bloc local excision with negative margins [10]. The adequate surgical margin for low-grade intracompartmental chondrosarcomas is a matter of controversy, as some surgeons propose wide resection, while others believe that intralesional resection, followed by adjuvants, such as liquid nitrogen, phenol, cryotherapy, electrocautery and argon-beam laser is adequate [11]. Chondrosarcomas are inherently resistant to conventional chemo and radiation therapy. Proposed mechanisms of chemoresistance include low mitotic fraction and restricted drug penetration into tumor microenvironment as a result of poor vascularity and abundant hyaline-dense extracellular matrix [1,12]. The possible activation of multidrug resistance pumps and the increased expression of antiapoptotic factors have also been described to contribute to chemotherapy resistance [12,13]. However, some of the rare subtypes may be more responsive [12], for example, mesenchymal chondrosarcomas have been demonstrated to be sensitive to doxorubicin-based combination chemotherapy, based on modest clinical evidence from nonrandomized trials [14]. Stereotactic radiosurgery has been successfully employed for skull-base and spinal chondrosarcomas and proton radiotherapy has been used for chondrosarcoma of the skull base and cervical spine [15]. Likewise, retrospective evidence suggests that dedifferentiated chondrosarcoma managed with combination of surgery and chemotherapy may have a better outcome than those treated with surgery alone [16].

Due to the limited efficacy of available treatments, the clinical management of chondrosarcomas is challenging, and new therapeutic approaches are urgently needed. Current research focuses on elucidating the molecular events underlying the pathogenesis of this rare bone malignancy, aiming at the identification of new molecularly-targeted therapies, especially for chemotherapy refractory, inoperable or metastatic chondrosarcomas. Our objective in this review is to discuss the current research of pharmaceutical targets for novel therapeutic interventions **(Figure 1)**.

The emerging role of *IDH* mutations in chondrosarcoma

Over the last few years, growing evidence suggests that certain oncogenic alterations in pathways directly reprogram the metabolic activity of the cancer cell [17,18]. One of the best described genetic alterations linking oncogenesis and metabolism are mutations in the *IDH1* and *IDH2* genes. IDH proteins, encoded by the *IDH* genes, catalyze the oxidative decarboxylation of isocitrate, producing αKG and CO2 in the Krebs cycle. Mutations in the *IDH* family of genes have been described in patients with a variety of cancers [19,20]. These mutations are known to produce 2HG from αKG conversion. 2HG accumulates in the serum of the patients and inhibits the function of enzymes that are dependent on αKG, leading to a hypermethylated state of DNA and histones, which results in different gene expression associated with tumorigenesis [20]. 2HG inhibits TET2 activity *in vitro*, which is a member of the TET family of αKG-dependent DNA modifying enzymes, and a DNA demethylation mediator. Thus, 2HG results in DNA hypermethylation, by inhibiting TET2-mediated DNA demethylation. 2HG also inhibits αKG-dependent JHKDMs. JHKDMs modify chromatin to regulate gene expression epigenetically. These enzymes, considered as tumor suppressors, have been associated with oncogenesis in various types of cancer [21].

Somatic mutations of the *IDH* genes are present in around 87% of benign enchondromas and more than 50% of primary conventional chondrosarcomas [5,22]. These mutations have been linked to the enchondromatosis-associated nonhereditary Maffucci and Ollier syndromes. The high mutation frequency in enchondromas and the fact that they are early events suggest a causal role for *IDH1* or *IDH2* mutations in tumorigenesis in Ollier disease and Maffucci syndrome [23,24].

*IDH*2 mutation has been shown to induce 2HG-dependent DNA hypermethylation in chondrosarcoma cells, resulting in inhibition of mesenchymal differentiation through epigenetic dysregulation. Treatment with the demethylating agent 5-azacytidine reversed this differentiation block, suggesting a potential route for therapeutic development [25]. Treatment of cells with AGI-5198, a specific inhibitor of mutant *IDH1*, has been shown to reduce production of 2-HG by up to 90% across a number of chondrosarcoma cell lines harboring endogenous *IDH1* mutation. However, the effect of IDH1 inhibition on viability, proliferation and migration on these cells has been inconsistent between studies, suggesting there is further

Figure 1. Novel targets in chondrosarcoma.

complexity to the role of epigenetic dysregulation in chondrosarcoma [26,27].

Ongoing clinical trials are evaluating the clinical activity of novel IDH inhibitors. AG-221, an oral IDH2 inhibitor, is currently being tested in a Phase I/II, multicenter trial enrolling patients with advanced solid tumors, including glioma, angioimmunoblastic T-cell lymphoma and chondrosarcoma with an *IDH2* mutation (NCT02273739). The IDH inhibitors AG-881 and AG-120 are also under clinical evaluation in Phase I studies of advanced solid tumors that harbor an *IDH1* and/or *IDH2* mutation, including gliomas, cholangiocarcinomas and chondrosarcomas (NCT02481154/NCT02073994). Moreover, there is an ongoing Phase Ib, open-label, single-center, nonrandomized clinical trial that evaluates the toxicity and efficacy of metformin in combination with chloroquine in *IDH1/2* mutated patients with a glioma, intrahepatic cholangiocarcinoma or chondrosarcoma (NCT02496741). Based on the data described above, the development of IDH inhibitors is emerging as a promising treatment option on the horizon for patients with chondrosarcoma.

Targeting the hedgehog pathway

The Hedgehog signaling pathway is an important regulator of cell growth and differentiation during embryogenesis and is also involved in the maintenance of homeostasis in postembryonic tissues by regulating the differentiation of stem cells [28]. When extracellular hedgehog ligands binds to PTCH1, a transmembrane receptor, PTCH1-mediated inhibition of signaling by SMO is prevented [29]. Activation of the SMO results in the activation of transcription factors encoded by *GLI* family zinc finger and consequent expression of hedgehog target genes, including *GLI1* and *PTCH1* [30].

The Indian Hedgehog (IHH)/parathyroid hormone-related peptide pathway (PTHrP) has a crucial role to chondrocyte differentiation and emerging evidence proposes that constitutive IHH signaling has a key role in the pathogenesis of chondrosarcomas [31]. Aberrant activation

of this pathway leads to constant signals from IHH, which induce chondrocyte proliferation and secretion of PTHrP from chondrocytes to the perichondrial space. There, PTHrP mediates inhibition of chondrocyte differentiation and apoptosis, therefore maintaining the cells in their proliferative state. The deregulation of the pathway also leads to high expression levels of its intramembrane receptor PTCH1 and its downstream transcription factor GLI [31].

A recent study evaluated in primary chondrosarcoma xenografts the activity of IPI-926, a potent oral Hh-inhibitor, on tumor formation and growth. The results demonstrated that IPI-926 had significant antitumor effects against human chondrosarcoma cell lines [32]. IPI-926 had previously shown activity in xenograft models of basal cell carcinoma and medulloblastoma, tumors which are also dependent on constitutive Hh signaling [32,33]. In another study, treatment of human chondrosarcoma SW1353 cells with the HH pathway inhibitor-4 (HPI-4) significantly decreased proliferation, invasion and migration capacity [34]. An additional study demonstrated that knockdown of *GLI1* expression by siRNA downregulated the expression of key Hh pathway members, including PTCH1 and SMO, and restrained the growth and survival of the treated chondrosarcoma cells [35]. The results of these studies provide the rationale for investigating Hh pathway blockade as a novel therapeutic option for patients with chondrosarcoma presenting aberrant activation of the HH signaling pathway.

While preclinical data of IPI-926 (saridegib) have demonstrated activity in a wide range of malignancies, the clinical data of a Phase II randomized placebo-controlled trial in patients with advanced chondrosarcoma have been discouraging [36]. Likewise, treatment with GDC-0449 (vismodegib) tested in a single-arm Phase II trial, although suggesting some activity in a subset of patients with progressive grade 1 or 2 conventional chondrosarcoma, did not meet the primary end point of 6-month clinical benefit rate [37]. These disappointing clinical results may indicate a ligand-independent activation of the Hh pathway in chondrosarcoma, which might occur with loss-of-function mutation of PTCH or gain-of-function mutation of SMO. Surrogate markers for selection of patients more likely to benefit from such targeted treatments could lead to more favorable outcomes in future studies [37].

The SRC pathway

Src and other members of the Src family of protein tyrosine kinases play key roles in regulating the transduction of signals originating from cell surface receptors [38,39]. The activation of the c-Src pathway has been observed in several tumors and has been linked to intratumoral signaling involved in cell survival, angiogenesis, proliferation and migration [39,40]. The Src was found to be activated in human sarcoma tissues (leiomyosarcoma, high-grade osteosarcoma) and sarcoma cell lines (osteosarcoma, Ewing's sarcoma, rhabdomyosarcoma) [41], while in synovial sarcoma cells it has been identified as one of the most strongly phosphorylated kinases [42]. Thereby, Src has been proposed to play an important role in signal transduction in human sarcomas, including osteosarcoma, rhabdomyosarcoma, leiomyosarcoma, fibrosarcoma and Ewing sarcoma [43].

Based on these findings, Src became an enticing target for drug development and a number of Src inhibitors, including dasatinib (BMS354825) are currently at various stages in the development process [44]. Dasatinib is a small molecule inhibitor that targets a broad range of tyrosine kinases, including ABL, SRC family kinases, c-KIT and PDGFR-α and -β [45]. Inhibition of the Src pathway by dasatinib in sarcoma cell lines demonstrated therapeutic benefit, preventing the growth and metastasis [41,46], while in another study it resulted in decreased cell viability in seven of nine chondrosarcoma cell cultures [46].

Although dasatinib as a single agent has shown some preclinical activity in chondrosarcomas, there is an accumulating body of evidence to support the use of dasatinib in combination with other antineoplastic drugs [47–50]. Oosterwijk *et al.* showed that dasatinib was effective in overcoming chemoresistance and successfully sensitized chondrosarcoma for doxorubicin treatment, especially in *TP53* mutant chondrosarcoma cell lines [51]. On the contrary, the SARC009 trial, a Phase II study of dasatinib in patients with previously treated, high-grade, advanced sarcomas, including chondrosarcomas concluded that dasatinib was inactive as a single agent [52]. Thus, Src inhibitors might offer a new approach in the treatment of sarcoma in combination with other chemotherapeutic regimens.

Targeting the PI3K–Akt–mTOR pathway

The PI3K–Akt–mTOR pathway has a significant role in various normal cellular processes

including cell proliferation, growth and survival [53]. The activation of the pathway is initiated by ligand activation of receptor tyrosine kinases (RTKs), such as the IGF-1R and PDGFR-α and -β. Aberrant activation of the PI3K pathway has been implicated in tumorigenesis of various cancers, and increased activation of this pathway is often associated with resistance to cytotoxic therapies [54,55]. Due to these observations and its critical contribution in cell growth and survival, this pathway became a promising target for pharmacologic intervention. The rapamycin analogs everolimus and temsirolimus, which inhibit mTORC1, were the first PI3K pathway-targeted agents approved for the treatment of renal cancer. Subsequently, an increasing number of PI3K pathway inhibitors have undergone clinical evaluation [56].

In a recent study, multiple RTKs were found to be activated in chondrosarcoma cells and to exhibit crucial roles in mediating cell growth [57]. Strong phosphorylation of S6 kinase, a surrogate of PI3K–mTOR pathway activity, was detected in 69% of conventional chondrosarcoma and 44% of dedifferentiated chondrosarcoma clinical samples, suggesting that activation of the PI3K–Akt–mTOR pathway in cell lines is clinically relevant. The study links RTK activation in chondrosarcoma cells to PI3K–Akt–mTOR signaling by showing that RTK inhibitors suppress Akt and S6 kinase phosphorylation. Treatment with BEZ235, a dual PI3K/mTOR inhibitor, significantly reduced the growth of chondrosarcoma cell lines and in a xenograft model of chondrosarcoma, suggesting that inhibition of the PI3K/mTOR pathway represents a rational therapeutic strategy [57].

Another study investigated the antitumor effect of doxorubicin and/or everolimus as a single agent or in combination in a rat chondrosarcoma model, both in macroscopic and in microscopic residual disease following R1 resection of the implanted tumor [58]. Doxorubicin as a single agent was inactive, in terms of inhibiting tumor growth, while everolimus had a major suppressant effect on tumor progression in macroscopic tumors and significantly delayed or prevented tumor recurrence in rats with microscopic residual disease. The combination with doxorubicin did not exhibit additive synergistic effect. These preclinical data support the use of mTOR inhibitors as a single agent in treating chondrosarcoma patients, and moreover the use of everolimus as adjuvant long-term therapy in chondrosarcoma patients following surgery [58]. A retrospective study of ten patients with unresectable chondrosarcoma, who were treated with the mTOR inhibitor sirolimus in combination with cyclophosphamide, showed that the regimen was well tolerated with disease control rate of 70%, implying meaningful clinical activity [59]. A recently published Phase I/II clinical trial evaluated the ability of temsirolimus to potentiate the cytotoxic effect of liposomal doxorubicin [60]. Reported Phase I data have demonstrated safety of the combination in adult and pediatric patients. The Phase II expansion part of this study is ongoing [60]. A Phase II study of everolimus in patients with primary or relapsed chondrosarcomas is ongoing, aiming to evaluate the efficacy and safety of everolimus as neo-adjuvant therapy (NCT02008019).

The IGF-1R-mediated activation of the PI3K–Akt pathway is a recognized mechanism of intrinsic mTORC1 inhibitor resistance described in a range of malignancies, including sarcoma [61–63]. IGF-1R overexpression has been reported in a number of sarcoma subtypes [64,65]. Preclinical data indicate that combination of mTOR with IGF-1R inhibitors results in suppression of Akt activation and enhancement of drug-induced antiproliferative effects [63]. This knowledge led to the design of several early phase clinical trials evaluating the combined mTORC1 and IGF-1R inhibition in sarcoma patients [66–68], with early results indicating that the combination may have greater clinical efficacy than treatment with each agent alone [67]. PDGFR-α is another RTK that can mediate rapamycin-induced Akt phosphorylation in sarcoma cell lines in a subset of tumors, and therefore, could be an attractive therapeutic target to inhibit in combination with mTORC [69]. Moreover, kinome analysis of chondrosarcoma tumors has shown that among others, the PDGFR pathway is overactive in chondrosarcomas [46].

A Phase II trial of the R1507, an IGF-1R inhibitor, enrolled patients with recurrent or refractory bone and soft tissue sarcoma and demonstrated that R1507 is safe and well tolerated but has limited clinical activity [70]. Another Phase II study investigated patients with previously treated advanced or metastatic bone and soft tissue sarcomas, treated with the IGF-1R inhibitor cixutumumab (IMC-A12), a fully human IgG1 monoclonal antibody. The results indicated that cixutumumab was well tolerated and patients with adipocytic sarcoma may

benefit from the treatment [65]. An additional Phase I clinical trial is exploring the safety, efficacy and best dose of cixutumumab given in combination with doxorubicin in patients with unresectable, locally advanced or metastatic soft tissue sarcoma (NCT00720174).

The Italian Sarcoma Group enrolled 26 patients in a Phase II trial of imatinib, a multi tyrosine-kinase inhibitor including inhibition of PDGFR, in patients with recurrent nonresectable chondrosarcomas, which all demonstrated immunohistochemical expression of PDGFR. Although imatinib was well-tolerated, the trial failed to demonstrate meaningful clinical activity [71]. The efficacy of imatinib is also under evaluation in the NCT00928525 open-label trial of Patients with Desmoid Tumors and Chondrosarcoma, expressing PDGFR-α and -β.

Histone deacetylase inhibitors

Modification of histones by acetylation is a key mechanism for the regulation of gene expression and plays a central role in determining cellular differentiation state. Dysregulation of histone modification is commonly found across a broad range of cancer types and has emerged as an important therapeutic target. The established knowledge that epigenetic alterations can change gene expression and consequently phenotype, disturb homeostasis and contribute to tumor growth has made the histone deacetylase inhibitors (HDACIs) an attractive class of anticancer therapy [72,73].

The acetylation status of histones, as it alters chromatin architecture, has an important role in modifying gene expression [72]. Abnormal histone acetylation status has been linked to the development of various diseases, including cancer. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 constitutes a common finding in cancer in a variety of cancers [73].

Currently, there are four HDACIs approved by the US FDA, for the treatment of refractory cutaneous T-cell lymphoma (vorinostat/ZOLINZA and romidepsin/ISTODAX®), peripheral T-cell lymphoma (belinostat/Beleodaq and romidepsin) [74–77], relapsed and/or refractory multiple myeloma (panobinostat/FARYDAK®), as well as several clinical trials investigating novel HDACIs in various malignancies **(Table 1)**.

Based on preclinical data showing that HDACIs induce growth arrest, apoptosis and differentiation in sarcoma cancer stem cells [78], as well as in chondrosarcoma cells [79], there is a Phase II clinical trial of a single agent Romidepsin in metastatic or unresectable soft tissue sarcomas (NCT00112463). Compelling preclinical work and early signals of clinical efficacy from early phase clinical trials indicate the likely worth of continued investigation of this class of drugs in chondrosarcoma [80].

The role of angiogenesis

Angiogenesis is a hallmark trait required for cancer development, progression and metastasis [81], and has proven to be an important therapeutic target over the past two decades. There is growing evidence from preclinical data, implicating angiogenesis with the pathogenesis of chondrosarcomas [82–86]. In addition, vascularization increases with increasing histological grade [87]. SU6668 is an inhibitor of the RTKs Flk-1/KDR (VEGR2), PDGFR-β and FGFR1. SU6668 induced a growth inhibition of chondrosarcoma animal models, which appears to be linked to the antiangiogenic effects of SU6668 [82]. Inhibition of COX-2 by celecoxib, a COX-2 inhibitor, which is another mediator of angiogenesis, has been tested in four high-grade chondrosarcoma cell lines. Treatment with celecoxib minimized cell viability *in vitro*. Conventional central and peripheral cartilaginous tumors from 66 patients were immunohistochemically assessed for COX-2 protein expression and 65% were found positive. The initial response in tumor growth supported a role for celecoxib, although a relapse in tumor growth was noticed after 6 weeks of treatment [88].

The NCT01330966 Phase II study is investigating the efficacy and safety of the single agent pazopanib in patients with unresectable or metastatic chondrosarcoma. Pazopanib is a potent and selective multitargeted RTK inhibitor, which inhibits c-KIT, FGFR, PDGFR and VEGFR among other enzymes [89]. Additionally, the NCT02066285 Phase II trial is also testing pazopanib in patients with unresectable or metastatic solitary fibrous tumor and extraskeletal myxoid chondrosarcoma. Regorafenib is another oral multikinase inhibitor, which targets angiogenic, stromal and oncogenic RTKs and is currently being tested in a Phase II study in patients with metastatic bone sarcomas (NCT02389244).

Other targeted treatments

Estrogen receptors are key regulators of the longitudinal skeletal growth that is initiated from

Table 1. Registered clinical trials in chondrosarcoma.

Study ID	Agent	Mechanism of action	Study design	Study population	Status
Targeting* IDH *mutations					
NCT02273739	AG-221	Oral IDH2 inhibitor	Phase I/II	Advanced solid tumors, including chondrosarcoma, and angioimmunoblastic T-cell lymphoma, with an *IDH2* mutation	Ongoing, but not recruiting participants
NCT02481154	AG-881	Oral IDH inhibitor	Phase I	Advanced solid tumors, including chondrosarcoma, with an *IDH1* and/or *IDH2* mutation	Recruiting
NCT02073994	AG-120	Oral IDH inhibitor	Phase I	Advanced solid tumors, including chondrosarcoma, with an *IDH1* mutation	Recruiting
NCT02496741	Metformin plus chloroquine	Oral antidiabetic (metformin) and oral antimalarial	Phase Ib	*IDH1*/2 mutated patients with a glioma, intrahepatic cholangiocarcinoma or chondrosarcoma.	Recruiting
PI3K–Akt–mTOR pathway					
NCT02008019	Everolimus	mTOR inhibitor	Phase II	Neo-adjuvant therapy in patients with primary or relapsed chondrosarcomas	Recruiting
NCT00720174	Cixutumumab plus doxorubicin hydrochloride	IGF-1R inhibitor	Phase I	Patients with unresectable, locally advanced, or metastatic soft tissue sarcoma	Completed
NCT00928525	Imatinib	A multi tyrosine kinase inhibitor including inhibition of PDGFR	Phase II	Patients with advanced desmoid tumor and chondrosarcoma expressing the PDGFR-α and -β	Ongoing, but not recruiting participants
HDAC inhibitors					
NCT00112463	Romidepsin	Histone deacetylase inhibitor	Phase II	Patients with metastatic or unresectable soft tissue sarcoma	Unknown
Targeting angiogenesis					
NCT01330966	Pazopanib	Selective multi-targeted receptor tyrosine kinase inhibitor	Phase II	Unresectable or metastatic chondrosarcoma	Ongoing, but not recruiting participants
NCT02066285	Pazopanib	Selective multi-targeted receptor tyrosine kinase inhibitor	Phase II	Patients with unresectable or metastatic solitary fibrous tumor and extraskeletal myxoid chondrosarcoma	Recruiting
NCT02389244	Regorafenib	Multi-kinase inhibitor, which targets angiogenic, stromal and oncogenic receptor tyrosine kinase	Phase II	Patients with metastatic bone sarcomas, including intermediate or high-grade chondrosarcomas	Recruiting

chondrocyte differentiation and proliferation in the epiphyseal growth plate of long bones [90]. Expression of estrogen and aromatase receptors has been identified in chondrosarcomas [90,91], suggesting that these tumors could be susceptible to estrogen and aromatase signaling inhibition [90]. However, in one study, *in vitro* and pilot *in vivo* studies showed no effect of any of the aromatase inhibitors on proliferation of conventional chondrosarcomas [91].

The monoclonal antibody PRO95780 (Genentech) is a proapoptotic receptor agonist, which specifically targets DR5, it activates the extrinsic apoptotic pathway and found to induce apoptosis in a plethora of human cancer cell lines and xenograft models [92,93]. PRO95780 targets DR5, which is expressed in a broad spectrum of hematologic malignancies and solid tumors [94,95]. A Phase I study of PRO95780 in patients with advanced malignancies reported a minor response in a chondrosarcoma case [96], although an another Phase II study of PRO95780 in patients with advanced chondrosarcoma has been terminated

due to lack of efficacy in this population (NCT00543712).

INI-1/hSNF5 is a tumor suppressor gene, encoding a subunit of the SWI/SNF chromatin remodeling complex and is widely expressed in most normal cells. Mutations or deletions of *INI-1/hSNF5* have been described in malignant rhabdoid tumors and epithelioid sarcomas, including extraskeletal myxoid chondrosarcomas [97]. The histone-lysine N-methyltransferase enzyme EZH2 is encoded by the *EZH2* gene and is involved in suppressing gene expression through methylation of H3K27. *EZH2* mutations or overexpressions are implicated in tumorigenesis of various cancers and correlate with poor prognosis thus, EZH2 has been an attractive target for anticancer therapy [98]. The EZH2 inhibitor, Tazemetostat is under clinical evaluation in a Phase II study, enrolling adult patients with *INI1*-negative tumors – including extraskeletal myxoid chondrosarcomas or relapsed/refractory synovial sarcoma (NCT02601950). Additionally, tazemetostat is being tested in a Phase I study of pediatric population with relapsed or refractory *INI1*-negative tumors or synovial sarcoma (NCT02601937).

Immunotherapy with immune checkpoint inhibition is an area of interest that is currently being elucidated in numerous malignancies, including sarcoma. PD-1 and its ligand (PD-L1) are key suppressors of the cytotoxic immune response, and their interaction results in a down-regulation of the T-cell response. Blockade of the interaction between PD-1 and PD-L1 has been reported to have impressive antitumor effect. Kostine *et al.* reported an analysis of PD-L1 protein expression in a series of conventional, mesenchymal, clear cell and dedifferentiated chondrosarcomas [99]. Only dedifferentiated chondrosarcomas displayed PD-L1 positivity, which in association with immune-infiltrating cells and HLA class I expression in nearly 50% of the dedifferentiated chondrosarcomas provided rationale for testing PD-1/PD-L1-targeted therapies in these patients [99]. Preliminary results of a Phase II study (SARC028) of the anti-PD1 antibody pembrolizumab (MK-3475) in patients with advanced soft tissue and bone sarcomas showed that 33% of patients with undifferentiated pleomorphic sarcoma and dedifferentiated liposarcoma had a tumor size shrinkage, while one out of the six patients enrolled with dedifferentiated chondrosarcoma exhibited partial tumor remission. These results imply that further investigations are warranted in these sarcoma types [100].

Conclusion

The past few years research focused on elucidating the molecular events underlying the pathogenesis of chondrosarcoma has led to the identification of several new potential therapeutic targets. Most of these targets demonstrated meaningful antitumor activity in preclinical studies, although the results in early phase clinical studies has been inconsistent. Future studies should further explore the utility of these candidate molecularly targeted therapies in the different subgroups of chondrosarcoma patients.

Future perspective

As previously described, chondrosarcoma has a diverse pattern behavior, ranging from slow-growing nonmetastasizing lesions to aggressive metastasizing sarcomas. In the past, the modalities that were used to treat these rare tumors were surgical resection, as the mainstay of treatment and chemo-radiotherapy with rather discouraging results. In our site (The Royal Marsden Hospital, Sarcoma Unit), we have treated ten patients with antiangiogenic agents with durable disease stabilization, in patients with previously progressing metastatic disease. We are currently in the process of publishing this case series, although further work with these agents is clearly needed. As it is crucial to identify and develop effective adjuvant treatments, the results of the current research are being awaited with great interest, so as to achieve in the future a better clinical outcome to this rare entity.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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EXECUTIVE SUMMARY

- Chondrosarcoma displays diverse histopathology and clinical behavior. The clinical management is exceptionally challenging as they are inherently resistant to conventional chemo and radiation therapy, therefore new therapeutic approaches are urgently needed.

The emerging role of IDH mutations in chondrosarcoma

- Preclinical data indicate antitumor activity of IDH inhibitors in chondrosarcoma cell lines. Ongoing early phase clinical trials are evaluating the clinical activity of novel IDH inhibitors in patients with advanced solid tumors, including chondrosarcoma with an *IDH1* and/or *IDH2* mutation.

Targeting the hedgehog pathway

- Emerging evidence proposes constitutive Indian Hedgehog signaling to have a key role in the pathogenesis of chondrosarcomas. However, the clinical data of a Phase II trial of IPI-926 (saridegib) in patients with advanced chondrosarcoma have been discouraging, although preclinical data have demonstrated activity in a wide range of malignancies.

The SRC pathway

- Src has been proposed to play an important role in signal transduction in human sarcomas and a number of Src inhibitors, including dasatinib (BMS354825) are currently at various stages in the development process.
- Dasatinib has shown some preclinical activity in chondrosarcomas as a single agent, but there is, also, evidence to support the use of dasatinib in combination with other antineoplastic drugs, for example, doxorubicin.

Targeting the PI3K–Akt–mTOR pathway

- Preclinical data indicate that inhibition of the PI3K/Akt/mTOR pathway has suppressant effect on chondrosarcoma tumor progression.
- Several early phase clinical trials are evaluating the combined mTORC1 and IGF-1R inhibition in sarcoma patients, with early results indicating that the combination may have greater clinical efficacy than treatment with each agent alone.

Histone deacetylase inhibitors

- Preclinical data show that histone deacetylase inhibitors induce growth arrest, apoptosis and differentiation in sarcoma cancer stem cells, as well as in chondrosarcoma cells. There is an ongoing Phase II clinical trial of the histone deacetylase inhibitors Romidepsin in metastatic or unresectable soft tissue sarcomas (NCT00112463).

The role of angiogenesis

- Evidence from preclinical data implicates angiogenesis with the pathogenesis of chondrosarcomas, while angiogenesis inhibitors show tumor growth inhibition in chondrosarcoma animal models and cell lines.

Future perspective

- A plethora of molecularly targeted therapies are under clinical evaluation in patients with chondrosarcoma, aiming to a more personalized and effective treatment approach.

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•• **Preliminary results of a Phase II trial of pembrolizumab in patients with advanced soft tissue and bone sarcomas.**