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REVIEW

Biology and management of clear cell sarcoma: state of the art and future perspectives

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ABSTRACT

Introduction: Clear cell sarcoma (CCS) is an aggressive tumor, typically developing in tendons or aponeuroses. The outcome of this orphan disease is poor, with 5-year and 10-year survival rates of localized CCS around 60–70% and 40–50%. Once the disease has metastasized, it is usually fatal due to its chemotherapy-resistant nature. Systemic treatment options are poorly standardized and the use of chemotherapy is based on weak scientific evidence.

Areas covered: In this review, we systematically discuss the current scientific evidence for the systemic treatment of CCS, including tyrosine kinase inhibitors, immunotherapy and MET inhibitors.

Expert commentary: Recent insights in the biology of CCS have identified new potential therapeutic targets, which should be tested in prospective clinical trials. Whenever possible, patients with metastatic CCS should be included in clinical trials with good biological rationale. Innovative trial methodology and new regulatory mechanisms are required to provide patients with uncommon cancers with active drugs.

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1. Introduction

Clear cell sarcoma (CCS) was first described in 1965 by Enzinger as a very rare, morphologically distinct soft tissue tumor likely originating from tendons and aponeuroses [1]. Since then, around 500 cases have been reported in literature (Table 1) [1–13]. Within the heterogeneous family of rare mesenchymal malignancies, CCS accounts for less than 1% of cases [14]. The primary tumor site of CCS is commonly the extremities, with around 40% of tumors detected in foot or ankle [1–5,8–10,12,13]. More rare localizations are the retroperitoneal space, viscera, bone and the gastro-intestinal tract. CCS typically presents in the second to fourth decade of life as a slowly growing mass with limited symptoms at initial diagnosis [1–3,6,8–11,13]. This indolent early course of the disease can lead to a significant delay in diagnosis and treatment. Despite their prolonged clinical course, CCSs tend to be aggressive tumors, mainly due to their rapid dissemination. At the diagnosis, around one-third of patients present with locally advanced disease or with synchronous metastatic disease [8,13]. While soft tissue sarcomas typically disseminate by hematogenous spread, up to 50% of patients with CCS develop lymph node metastasis [8, 9,12,13], which is a distinct feature of this disease. The 5- and 10-year survival rates of localized CCS are around 60–70% and 40–50%, respectively [8, 13,15]. Rates of local recurrence, synchronous, and metachronous metastases are up to 84%, 63%, and 30%, respectively [1,3–5,15,16]. A relapse of CCS can occur either very early after initial treatment or even 29 years after surgery [12]. In multivariate analyses, primary tumor size ≥5 cm, tumor located in the trunk, and the development of metastatic disease at any time during the disease course have been identified as negative prognostic factors for overall survival (OS) [3,8,9,12,13]. Taking into account the young age of many patients and the risk of a late relapse, a long follow-up is mandatory, aiming especially to detect local relapse at an early stage in which local treatment with curative intent can still be provided to these patients. However, patients relapsing with metastatic disease will uniformly die from their disease, and strict follow-up will unfortunately not alter the natural history of the disease in these patients.

Macroscopically, CCSs are commonly located in close proximity to tendons [6,8,10,12–14], and they typically show a characteristic growth pattern in nests, separated by collagenous bands. Another morphological feature is the presence of multinucleated giant cells with a wreath-like nuclear appearance [10,13,14,17]. Immunohistochemically, CCS cannot be distinguished from malignant melanoma, explaining the former name 'malignant melanoma of soft parts'. Both entities show strong and consistent staining for S100, human melanoma black 45 (HMB45), melan A, neuron-specific enolase, CD57, and vimentin. CCSs are often (but variable) also positive for tyrosinase, microphtalmia-associated transcription factor (MITF), and CD117 (KIT), but negative for keratins, epithelial membrane antigen, muscle actin and desmin [10,12–14].

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Table 1. Published series of clear cell sarcoma.

Reference	Year	No. of patients	M:F ratio	Age at diagnosis, median (range), y	Tumor size, median (range), cm	Follow-up, median (range), mo	Local recurrence (% of patients)	Metastasis (% of patients), median time to metastasis	Disease-related death (%), median time to death
Enzinger [1]	1965	21	3:4	26 y (1–65)	4 cm (2–6)	48 mo (1–432)	84	63, 7 y	74, 8 y
Chung and Enzinger [2]	1983	141	6:7	27 y (7–83)	3.3 cm (1–15)	68 mo (1–432)	39	50, 96 mo	50, 96 mo
Sara et al. [3]	1990	17	1:1	28 y (70–90)	4.5 cm (2–9.5)	49 mo (3–158)	24	59, 25 mo	59, 27 mo
Lucas et al. [4]	1992	35	2:3	30 y (10–64)	4.5 cm (1–14)	74 mo (7–258)	14	63, 60 mo	54, 67 mo
Finley et al. [5]	2001	8	1:1	33 y (16–55)	5 cm (1.7–10)	85 mo (6–144)	13	63, 32 mo	63, 42 mo
Kuiper et al. [6]	2003	8	5:3	30 y (12–57)	4.8 cm (1.5–14)	85 mo (14–198)	0	13, 4 mo	0
Coindre et al. [7]	2006	44	13:9	32 y (5–66)	4 cm (1–12)	32 mo (9–264)	39	43, 33 mo	57, 36 mo
Kawai et al. [8]	2007	75	41:34	36 y (10–71)	4 cm (1–11)	44 mo (2–243)	21	69, 13 mo	45, 20 mo
Clark et al. [9]	2008	35	24:11	38 y (12–76)	ND	41 mo (1–321)	23	63, 14 mo	43, 32 mo
Hisaoka et al. [10]	2008	33	20:13	30 y (13–73)	4 cm (1–15)	38 mo (3–171)	7	52, 27 mo	38, 28 mo
Stacchiotti et al. [11]	2010	35	18:17	45 y (20–79)	ND	15 mo	26	40	ND
Hocar et al. [12]	2012	52	15:11	33 y (6–81)	4.9 cm (1–15)	120 mo (11–348)	56	63, 55 mo	54, 66 mo
Bianchi et al. [13]	2014	31	16:15	38 y (9–73)	3 cm (0.7–25)	ND	26	32, 24 mo	ND

F, female; M, male; mo, months; ND, not determined; y, years.

The genetic hallmark of CCS is a t(12;22)(q13;q12) translocation, which can be detected in more than 90% of the cases [18–22]. This translocation leads to a fusion of the genes *activating transcription factor 1 (ATF1)* in 12q13 and *Ewing sarcoma breakpoint region 1 (EWSR1)* in 22q12, producing the EWSR1-ATF1 fusion protein [23]. Less frequently the t(2;22)(q34;q12) translocation is detected in CCS, resulting in the *EWSR1-CREB1* fusion transcript [24]. These chimeric oncoproteins induce expression of the melanocyte-specific MITF promoter, leading to proliferation and melanocytic differentiation of the tumor cells, which explains the histological resemblance to malignant melanoma [25,26]. Since these genetic markers are absent in malignant melanoma, the detection of *EWSR1* rearrangement by fluorescence *in situ* hybridization (FISH) or reverse-transcription polymerase chain reaction is an important component for establishing the diagnosis of CCS and differentiating it from melanoma, which has important prognostic and therapeutic implications [7].

2. Treatment of localized disease

2.1. Surgery

As in the case of most other soft tissue sarcomas, wide surgical excision remains the only curative treatment for localized CCS. Unfortunately, many patients with this disease have suboptimal surgery (often referred to as 'whoops surgery') with positive resection margins at first diagnosis. Nevertheless, the aim of the surgery should always be a macro- and microscopically complete resection with negative margins, even if this can only be achieved through aggressive, sometimes mutilating excision, as positive resection margins emerged as unfavorable prognostic factors in multiple series [8,12]. More aggressive surgical strategies, however, do not improve the rate of local recurrence or distant metastases [4], and therefore should only be considered when limb sparing surgery is technically not feasible.

2.2. Adjuvant treatment

It remains unclear whether adjuvant radiotherapy adds a survival benefit after surgical resection. The European Society for Medical Oncology clinical practice guidelines recommend adjuvant radiotherapy in case of high grade (grade 2–3) and deep and large (>5 cm) soft tissue sarcomas (level IIB) [27]. Grading of soft tissue sarcomas is based on the Federation Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system, which distinguishes three malignancy grades based on differentiation, necrosis and mitotic rate. CCSs, however, typically have low mitotic activity and necrosis is only seen occasionally in these tumors [28], classifying most CCSs as grade 1 tumors. However, additive radiotherapy should be considered whenever negative resection margins have not been achieved and additional surgery is not feasible or refused by the patient.

In general, the data on the adjuvant use of chemotherapy in soft tissue sarcoma are controversial, and it cannot be recommended in CCS, which is known to be a very chemotherapy-resistant histological subtype of sarcoma.

3. Treatment of metastatic disease

3.1. Palliative chemotherapy

Systemic treatment is the treatment of choice in case of inoperable and/or metastatic CCS. Unfortunately, results are discouraging as CCS is typically resistant to established chemotherapeutic agents. The scientific evidence for the use of systemic agents in CCS is based on retrospective series and experience of sarcoma teams in high-volume centers. A retrospective analysis of 24 patients with metastatic CCS treated with palliative first-line chemotherapy documented an objective response rate of only 4% [29]. Regimens in this small series included anthracyclines given as single agent or in combination with ifosfamide or platinum and other cytotoxic or targeted compounds. Only one patient achieved a partial

response (PR) and the overall median progression-free survival (PFS) was only 11 weeks [29]. An Italian retrospective series reported the outcome of 11 patients with advanced CCS treated with doxorubicin + dacarbazine ± ifosfamide. At 3 months, two patients achieved PR, three patients had stable disease (SD) and six patients presented with progressive disease (PD) according to Response Evaluation Criteria in Solid Tumors (RECIST). In all patients, the PR and SD lasted less than 6 months [11], confirming the aggressive and chemotherapy-resistant nature of CCS. Isolated limb perfusion has been shown to have utility in the setting of unresectable soft tissue sarcomas of the extremities [30]. However, experience with isolated limb perfusion in CCS is scarce [31,32], and the limited data suggests even a worse long-term outcome when isolated limb perfusion is administered to patients with in-transit metastases [32], as is often the case in locally advanced CCS. Taking into account the very aggressive, rapidly disseminating nature of CCS, systemic chemotherapy is likely the treatment of choice for the majority of patients with this tumor type, although no clinical trial specifically designed to assess the efficacy of a drug intervention in CCS has been published so far.

3.2. Reports on treatment with tyrosine kinase inhibitors

Objective tumor responses have been observed in patients with advanced CCS upon treatment with sorafenib and sunitinib, tyrosine kinase inhibitors that both target the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) signaling pathways. The rationale for the use of such agents is based on the expression and activation of PDGF receptor (PDGFR) beta in a large proportion of CCS tumors [33]. A patient treated with sorafenib 400 mg twice daily after progression under doxorubicin, ifosfamide and cisplatin chemotherapy achieved an objective tumor regression and pain relief, with a response duration of 8.2 months [34]. Two patients with disseminated CCS progressing under conventional chemotherapy experienced PR upon treatment with sunitinib 37.5 mg daily [35,36]. Taken together, the role of oral multikinase inhibitors targeting VEGF and PDGF signaling pathways should be further investigated, ideally in prospective clinical trials. Of note, the case reports summarized here are potentially subject to publication bias; it is unknown what the actual response rate to the drugs mentioned above would be in a prospective setting.

Pazopanib, a multitargeted tyrosine kinase inhibitor of VEGFR 1, 2, and 3, PDGFR alpha and beta, fibroblast growth factor receptor (FGFR) 1 and 2 and KIT, was found to delay tumor growth in a newly established CCS cell line (Hewga-CCS) and in an orthotopic CCS xenograft model [37]. Antitumor effects of pazopanib in this tumor model were attributed to the inhibition of hepatocyte growth factor receptor (MET), but not of VEGF and PDGF signaling. Although pazopanib mainly targets VEGFR, PDGFR, FGFR, and KIT in a cell-free assay system [38], authors argue that kinase activity might be different in cell growth assays. There are no available data about treatment of CCS patients with pazopanib, which is approved for advanced soft tissue sarcoma after failure of chemotherapy. Pazopanib might thus be a reasonable alternative to the antiangiogenic agents described earlier, which are used off label.

3.3. Therapies under clinical investigation

3.3.1. MET inhibitors

The demonstration of direct activation of the MITF promoter by the pathognomonic EWSR1-ATF1 fusion protein [25] sheds new light on the oncogenic pathways implied in CCS. The expression of several genes are regulated by MITF. Interestingly, the oncogenic receptor tyrosine kinase MET has also been identified as one of the downstream targets of MITF [39]. The expression of MET and activation of its downstream PI3K/AKT and ERK pathways have subsequently been shown in CCS preclinical models and in patient samples [33,40]. Moreover, blocking MET activity led to significantly reduced CCS cell growth *in vitro* and significantly suppressed tumor growth in preclinical CCS xenograft models [40].

Based on these observations, MET inhibition is currently being investigated as a therapeutic strategy in MITF-associated tumors. Due to the rarity of these tumors, published studies are cross-tumoral trials. They include families of seemingly unrelated tumors, which employ distinct strategies to oncogenically activate the MITF family proteins, such as translocation-associated renal cell carcinoma, alveolar soft part sarcoma or CCS [26]. A multi-center phase 2 trial with tivantinib (ARQ 197), a selective MET inhibitor, included 47 patients with advanced MITF-associated tumors, including 11 CCS cases (NCT00557609). The trial included a mostly young and fit patient population with a median age of 25 years (range: 11–73 years) and Eastern Cooperative Oncology Group performance status 0 or 1. Although baseline MET expression, as assessed by immunohistochemistry, was strongly or focally positive in 74% of archived tumor samples, the primary endpoint of overall response rate was not met [41]. Single-arm treatment with tivantinib was safe and tolerable, but only 9% and 27% of patients with CCS achieved a PR and SD, respectively, according to RECIST version 1.0. The median PFS among the CCS subgroup was only 1.9 months and 55% of CCS patients progressed at the first radiological evaluation, confirming the aggressive nature of CCS [41]. However, later on in clinical development the presumed MET-targeting agent tivantinib was found to be a cytotoxic agent acting on microtubule dynamics [42], questioning the rationale and results of this phase 2 trial.

Currently, a cross-tumoral multi-tumor phase 2 clinical trial explores the use of the MET/ALK inhibitor crizotinib in patients with six different types of advanced tumors, including disease-specific cohorts of MET-positive and MET-negative CCS cases (EORTC 90101, NCT01524926). Between January 2013 and December 2014, 16 investigational sites in 8 European countries recruited 43 patients, of whom 32 had a centrally confirmed diagnosis of metastatic CCS. Final results of this largest prospective CCS study are expected in 2016.

3.3.2. Histone deacetylase (HDAC) inhibitors

Whereas acetylation of histones leads to relaxation of chromatin condensation and activation of transcriptional activity, HDAC inhibits transcription by condensing chromatin.

Deregulation of HDAC recruitment seems to play an important role in tumorigenesis, as overexpression of HDACs has been shown to induce cell proliferation [43]. Yokoyama et al. showed that multiple HDAC inhibitors are able to potently repress MITF expression level in CCS cell lines [44]. In line with these *in vitro* results, treatment with the HDAC inhibitors MS-275 and romidepsin led to a significant induction of apoptosis in CCS cell lines. A phase 1b/2 study of the HDAC inhibitor vorinostat in combination with gemcitabine and docetaxel in advanced sarcoma is currently recruiting patients (NCT01879085). However, this study is not restricted to patients with CCS or other MITF-associated tumors as patients with evidence of metastatic or unresectable soft tissue sarcoma, regardless of the sarcoma subtype, are eligible. It is currently unknown whether patients with CCS have been included in this trial.

3.3.3. Immunotherapy

It was shown that the EWSR1-ATF1 fusion protein may be considered as an immune stimulant [45]. However, there are very limited data on the use of immune-modulating therapies in soft tissue sarcoma, and even less in CCS. There is anecdotal evidence of clinical activity of interferon-alpha 2b in the setting of metastatic CCS. One patient achieved SD during 17 months after simultaneous treatment with subcutaneous interferon-alpha 2b and six courses of cyclophosphamide, vincristine, doxorubicin, and dacarbazine (CYVADIC) [46]. However, the contribution of the cytokine to the outcome cannot be assessed as the patient also received systemic chemotherapy. Administration of perilesional interferon-alpha 2b induced complete response (CR) during 17 months in another heavily pretreated patient [47]. A recently published phase 1 clinical trial of the anti-CTLA-4 antibody and immune checkpoint modulator ipilimumab in pediatric patients with advanced tumors (NCT01445379) included two children with metastatic CCS. Ipilimumab was safely administered and one CCS patient experienced SD for a total duration of six cycles (=24 weeks) of this treatment [48]. An anecdotal SD for 2 years under ipilimumab and PR under pembrolizumab (anti-PD1 antibody) in a 6-year old patient with metastatic CCS has also been reported [49]. It is unknown whether a deregulation of the immune system plays a role in the pathophysiology of CCS, so such early clinical data from non-controlled trials have to be interpreted with caution. Goldberg et al. recently published data of a phase 1 clinical trial exploring vaccination with irradiated, autologous tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients with metastatic or locally advanced CCS and alveolar soft part sarcoma (NCT00258687) [50]. Three patients with CCS were included. Although vaccination elicited a strong dendritic cell reaction and humoral immunity, no tumor regressions were observed. Two CCS patients died of their disease after 4 and 24 months. Another CCS patient with lung metastases was still alive 103 months after the enrollment in the study, which is a remarkable survival in the setting of metastatic CCS [50].

In summary, prospective clinical trial data for patients with advanced CCS are very limited. The pending results of EORTC 90101 (NCT01524926) will likely be the benchmark for future clinical research in this field. Figure 1 summarizes the cellular pathogenesis of CCS, highlighting particular molecular targets of interest.

3.4. Preclinical identification of potential therapeutic targets

One of the most potent activators of the PI3K/AKT axis is ERBB3, which is upregulated in many types of cancer, in particular following treatment with epidermal growth factor receptor (EGFR, ERBB1) and HER2 (ERBB2) inhibitors [51]. The *ERBB3* gene is known to be overexpressed in CCS [33,52]. Apart from ERBB3,

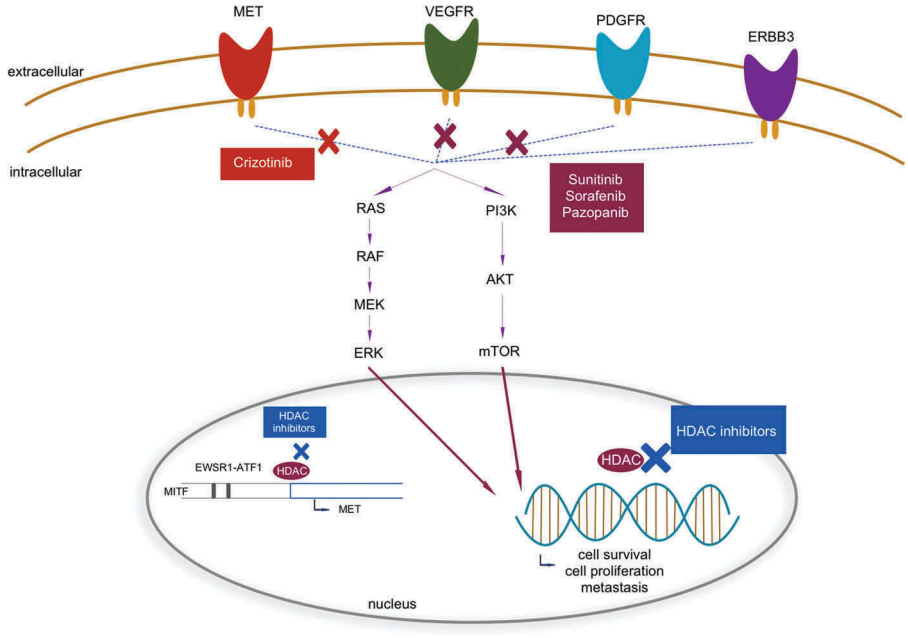

Figure 1. Schematic diagram of clear cell sarcoma cellular pathogenesis and possible therapeutic targets.

cell lines derived from CCS also express the co-receptors HER2 or ERBB4 [53]. *In vivo* data suggest a role for autocrine stimulation of ERBB3 by neuroregulin-1, the most prominent ligand of the oncogenic HER2-ERBB3 heterodimer [53]. This makes ERBB3 an interesting therapeutic target for CCS. However, no *in vivo* or clinical data on targeting CCS with HER2 or ERBB3 monoclonal antibodies or inhibitors have been published till date.

4. Conclusion

At present, the only curable treatment for localized, resectable CCS remains complete surgical tumor excision with negative margins. Additive radiotherapy is warranted when resection margins are positive, radical surgery is not feasible or refused by the patient. There is no indication for adjuvant chemotherapy. Tumor size and site are relevant prognostic factors for survival.

Inoperable and metastatic cases of CCS are incurable with currently available soft tissue sarcoma treatments, as the disease is intrinsically insensitive to palliative chemotherapy. However, recent insights in the biology of CCS have identified new potential therapeutic targets, such as MET, PDGFRA/B, and HDAC. The identification of these targets has led to the first clinical trials with small molecules and monoclonal antibodies in CCS, offering new hope to improve outcome for patients suffering from this aggressive orphan disease.

5. Expert commentary

CCS is a rare malignancy which accounts for only 1% of all soft tissue sarcomas and is commonly misdiagnosed mainly due to its morphological resemblance to melanoma. It is an aggressive tumor that typically occurs in children and young adults, presenting as a slowly growing mass but with the potential to rapidly lead to death due to metastatic spread. Clinical outcome of patients with CCS has remained static over the years. Despite its intrinsic resistance to established chemotherapeutical agents, systemic therapy still remains the standard of treatment in the advanced or metastatic setting. However, these systemic treatment options are poorly standardized and the use of chemotherapy is based on weak scientific evidence.

Recent insights in the biology of CCS have identified new potential therapeutic targets such as MET, PDGFRA/B, HDAC, and ERBB3. Off-label use of tyrosine kinase inhibitors and immune checkpoint modulators can induce responses in individual patients. Furthermore, the increased knowledge on the pathogenesis of CCS has led to the first clinical trials with small molecules and monoclonal antibodies in CCS, although clinical studies in this orphan disease are compromised due to the rarity of the disease. When treating a patient with a rare and chemotherapy-resistant malignancy as CCS, the physician should actively pursue to include the patient in an ongoing clinical trial whenever possible, even as first line therapy. Participation in clinical trials, including phase 1 trials with a good biological rationale, might be the only way to give such patients access to active agents.

The pending final results of EORTC 90101 (NCT01524926) with the MET inhibitor crizotinib in six different types of advanced tumors including disease-specific cohorts of CCS with or without MET activation, will likely be leading for future clinical research in CCS.

6. Five-year view

Considering the rarity of CCS, multicenter and international cooperation is required to advance the knowledge on the biology of CCS and to improve patient outcome. Due to its intrinsic resistance to chemotherapy, efforts should be focused on a better understanding of the mechanisms leading to cell proliferation, in order to identify new potential therapeutic targets.

Modulation of these targets should be tested in well-designed, prospective clinical trials, but the rarity of CCS makes such studies hard to run. However, the EORTC 90101 trial (NCT01524926) proves that it is possible to conduct a prospective trial in orphan diseases, such as CCS, with a cross-tumoral design in which patients are not included based on tumor type, but on the presence of a specific target. Innovative trial methodology and new regulatory mechanisms are thus required to provide patients with uncommon cancers with active drugs.

Key issues

- Clear cell sarcoma of tendons and aponeuroses (CCS) is an orphan disease that accounts for less than 1% of soft tissue sarcomas.
- In over 90% of CCS cases the t(12;22)(q13;q12) translocation is present, resulting in the formation of a EWSR1-ATF1 fusion protein. This fusion regulates the melanocytic differentiation of the CCS tumor cells, explaining the former name 'malignant melanoma of soft parts' as well as abnormal expression of oncogenes including *MET*.
- Clinical outcome of patients with CCS has remained static over the past decades with 5-year overall survival rates between 40 and 60% for localized disease. Once the disease has metastasized, it is usually fatal due to its intrinsic resistance to chemotherapy.
- Wide surgical resection is the only curative treatment for localized CCS.
- Adjuvant radiotherapy should be considered whenever negative resection margins have not been achieved and additional surgery is not feasible. Adjuvant chemotherapy is not recommended.
- Systemic treatment remains the standard treatment in case of inoperable/metastatic CCS, nevertheless its intrinsic resistance to chemotherapy. The use of systemic agents in CCS is based on retrospective series and experience of sarcoma teams in high-volume centers. An anthracycline based chemotherapeutic regimen is most commonly used in the first line.
- Recent insights in the biology of CCS have identified new potential therapeutic targets, such as MET, platelet-derived growth factor (receptor) [PDGR(R)], histone deacetylase (HDAC) and ERBB3.
- These insides have led to the first prospective clinical trials in CCS. MET-inhibitors, vaccination therapy and immunotherapy are currently being tested in clinical trials. Long-lasting disease control was observed in a subset of

patients treated with the MET/ALK inhibitor crizotinib. Data for immunotherapy in CCS are still limited.

- Physicians treating patients with rare and chemotherapy-resistant malignancies such as CCS should actively try to include their patients in ongoing clinical trials, including phase 1 trials with a good biological rationale, as this might be the only way to give such patients access to active agents.

Declaration of interest

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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••of considerable interest

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