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Role of immunotherapy in chondrosarcoma: A case report and review of the literature

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***Abstract*:** Chondrosarcomas (CSs) consist of a heterogenous group of primary bone cancers arising from malignant cells which produce cartilaginous matrix. As the second most common primary bone cancer, CS are often resistant to systemic chemotherapy due to poor vascularization, slow proliferation, and expression of multidrug-resistant pumps. Immune checkpoint inhibitors have transformed the field of oncology and are now designated as frontline therapy for many solid tumor cancers. Several studies have demonstrated increased expression of programed cell death 1 (PD-1) and PD-L1 in CS tissue *in vitro*, which has led to the development of multiple clinical trials for immunotherapy in patients with aggressive CS. In this review, we highlight the ongoing investigation into the role for immunotherapy in CS. We also report the case of a 44-year-old female with a history of stage IV primary CS of the right shoulder who underwent radical resection with recurrence and demonstrated a spectacular sustained response to pembrolizumab at our center. Our review highlights the need for further studies investigating the role of immunotherapy in the treatment of aggressive bone sarcomas that are resistant to standard surgical resection, chemotherapy, and radiation treatment.

Plain language summary

Chondrosarcoma is a cancer of the cells that make cartilage and is often removed surgically. However, when the cancer spreads to other organs such as the lungs or are in areas unreachable by surgeons, there are not many effective treatments. While targeted treatments are in development, many of them have unclear effectiveness. A new and rapidly growing area of cancer treatment is known as immunotherapy, which uses the body's own immune system to kill cancer cells. In this review, we discuss trials in using immunotherapy against aggressive forms of chondrosarcoma. We also present the case of a patient where an immunotherapy agent called pembrolizumab was highly effective in preventing disease progression.

Keywords: anti-PD-1, checkpoint inhibitor, chondrosarcoma, drug resistance, immunotherapy, tumor microenvironment

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Introduction

As a collection of generally indolent and primary bone sarcomas, chondrosarcoma (CS) represents the second most common bone tumor after osteosarcoma, accounting for 30% of all cases.[1,2] CS can be a primary tumor or secondary if they present from a preexisting benign bone tumor such as enchondroma or osteochondroma. CS are classified based on histologic grade, using a scale of 1–3. Conventional CS is the most prevalent subtype, comprising up to 90% of all cases. The majority of conventional CS are histologic

grade 1–2 and have favorable prognosis due to their quiescent nature. A minority of cases (5–10%), however, present as high-grade (grade 3), aggressive tumors with metastatic potential. The so-called 'unconventional CSs' are considered rare. This class includes myxoid, mesenchymal, dedifferentiated, juxtacortical, and clear cell variants. Dedifferentiated CS (DD-CS), which begins as low grade and later transforms to a high grade, portends the lowest 5-year survival rate (11%).[3] Mesenchymal CS is highly malignant, with histology similar to Ewing's sarcoma.[4] CS is further subclassified based on location – central, peripheral, or periosteal.[5] Central tumors arise from the medullary cavity, whereas peripheral tumors arise from the bone's surface. Staging (Stages I–III) is based on histologic grade, anatomic compartmental status, and presence of metastatic disease. Grade is divided into two categories, high and low. Anatomic compartmental status is based on whether the tumor has grown beyond the cortex of the bone or not. Presence of metastatic disease is classified as stage III.

The standard of care for conventional CS remains serial imaging and surgical resection, which can be limited by the location of the tumor and are associated with significant morbidity. There are no evidence-based systemic therapies for the treatment of CS. Conventional CS tends to be resistant to chemotherapy, owing to multidrug-resistant pumps, poor vascularization, slow proliferative index, and expression of Bcl2 proteins.[6–9] With an annual estimated incidence of 1/200,000, conducting clinical trials requires a dedicated multidisciplinary sarcoma team.

There are currently some agents available for systemic treatment of patients with CS, which include targeted therapies directed at biomarkers such as mutant isocitrate dehydrogenase (IDH) and tyrosine kinases. IDH-1 or IDH-2 mutations have been detected in enchondroma, central conventional CS as well as DD-CS.[10] A small phase I study demonstrated that ivosidenib, a mutant IDH-1 inhibitor, and lower plasma oncometabolite levels were associated with stable disease in CS patients.[11] Tyrosine kinase inhibitors (TKIs) and multi-kinase inhibitors have also been studied in CS. A prospective study by Chow *et al.*[12] demonstrated pazopanib, an oral TKI, was able to mildly prolong progression-free survival (PFS) in patients with metastatic or unresectable CS, with one patient achieving partial response for 72 weeks.[12] Sunitinib, regorafenib, and dasatinib have been found to have some efficacy in extra-skeletal myxoid CS, CS, and low to intermediate-grade CS, respectively.[13–15]

Immune checkpoint inhibitors (ICIs), a type of immunotherapy, have transformed the field of solid tumor oncology and are now considered front line in the treatment of several solid tumor malignancies, including melanoma, non-small cell lung cancer, and renal cell carcinoma.[16] Given the limited options for effective systemic therapies in recurrent, unresectable, or metastatic CS, several clinical trials have begun to investigate the efficacy of immunotherapy in CS. This review aims to discuss the current evidence on immunotherapy in CS, drawing from both preclinical and clinical case studies. Further, we present a case of a 44-year-old female with a history of stage IV primary CS of the right shoulder who underwent radical resection with recurrence and demonstrated a spectacular sustained response to pembrolizumab.

Cancer immunotherapy and the tumor microenvironment

Immunotherapy, a class of antineoplastic therapy which relies on activating the immune system to target cancer cells, has led to tremendous breakthroughs in the treatment of several cancers. ICIs, a type of immunotherapy, work to inhibit negative regulatory pathways which cancer exploits as a mechanism to evade the immune response. Programed cell death 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA4) are the two examples of immune checkpoint proteins which regulate the immune response, promoting immune tolerance, and preventing hyperactivation.[17] Medications such as nivolumab and pembrolizumab target PD-1, a protein present on the surface of activated T-cells. Programmed Cell Death Protein 1 (PD-1), when activated by its ligand Programmed Cell Death Ligand 1 (PD-L1), induces anergy and blunts the T-cell-mediated immune response in an Interleukin-2 (IL-2) dependent manner.[18] This pathway promotes the development of Tregs and protects against self-reactive T-cells and autoimmunity.[19] Thus, blocking PD-1 activation leads to upregulation of the immune response. Ipilimumab, another ICI that targets CTLA4, similarly prevents activation of inhibitory signaling involved in immune cell suppression.

The tumor microenvironment (TME) of bone sarcomas is rich in immune infiltrates, including lymphocytes (CD3/CD4/CD8), neutrophils, natural

killer cells, and macrophages. Tumor-associated macrophages (TAMs), are a heterogenous group of immune effector cells which play a role in regulating the TME.[20] Two classes of TAMs have been identified: M1, which promotes antitumor immune activity, and M2, which promotes tumor growth and metastasis. M2 TAMs promote angiogenesis and immunosuppression through the production of suppressive cytokines such as IL-10 and Transforming growth factor beta (TGF-β).[21] Large numbers of M2 TAMs have been shown to correlate with tumor growth, invasion, immune evasion, metastasis, larger tumor size, and poorer overall survival (OS).[22,23] Therapies that target T-cell activation may heighten immune response and tumor infiltration in aggressive CS despite the presence of TAMs.

Independent of this immune interplay, one well-studied predictor of ICI therapy response is the tumor mutational burden (TMB), or the number of somatic mutations per 1 million DNA bases.[24] Higher TMBs tend to be associated with greater response and PFS in patients receiving ICI therapy, although most of the data are from trials studying TMB in patients with lung cancer, not sarcomas. Interestingly, TMB seems to be relatively low in most sarcomas except for undifferentiated and high-grade sarcomas.[25] Additionally, bone sarcomas tend to exhibit microsatellite stability without mismatch repair defects common to other solid tumors such as colorectal adenocarcinoma.[26] It will be important to explore other predictive biomarkers for ICI outcomes in patients with CS and other bone sarcomas.

Preclinical studies in CS

Checkpoints. Tumor PD-1 or PD-L1 expressivity is a promising predictor of the efficacy of ICI therapy.[27] Tissue immunohistochemistry (IHC) studies have demonstrated robust PD-L1 expression in DD-CS but not conventional CS, with PD-L1 expression detected in 52% of tissue DD-CS specimens and associated with infiltrating lymphocytes and Human leukocyte antigens (HLA) Class I expression.[28] PD-L1 expression in CS has also been positively correlated with expression of Ki-67 and TP53 in CS.[29] In another study, tissue analysis demonstrated PD-1 expression in 9/10 samples from CS tumors but a lack of PD-L1 expression.[30] Further, Zhang *et al.*[31] demonstrated that while the majority of tumor cells from CS samples are PD-L1 negative, positive expression was associated with higher histologic grade, either grade 3 or dedifferentiated. While lack of tissue PD-L1 expression detectable by IHC does not preclude conventional CS responses to anti-PD-L1 agents, these studies do suggest anti-PD-1 agents such as pembrolizumab may be effective in most low-grade subtypes of CS.[32,33]

Immune infiltrate. Tumor-infiltrating lymphocytes (TILs) have been shown to be predictive of patient outcomes in many solid cancers, including ovarian, colorectal, lung, hepatocellular, and renal cell.[34] For DD-CS tumors in particular, Iseulys *et al.*[35] found that a high density of $CD3^+$ and $CD8^+$ TIL is predictive of better OS and high $CD68^+/CD8^+$ ratio is associated with poor OS and metastases at time of diagnosis.[35] However, the main population of immune cells in the TME among DD-CS and conventional CS in this study was TAMs. M2 class CD163+ TAMs are associated with disease progression and metastatic disease in CS, with tumors characterized by high $CD8^+$ and low $CD163^+$ tending to be less invasive and smaller in size.[36,37] Similarly, a high $CD163^+$ TAM infiltrate inversely correlates with PFS and is associated with a higher density of intratumor microvessels in both conventional and DD-CS.[38]

Clinical studies in CS

Immune checkpoint inhibitors. Given the rarity of disease, there are few clinical trials investigating the safety and efficacy of ICIs in the treatment of CS. SARC028, a phase II trial investigating anti-PD-1 antibody pembrolizumab in soft-tissue and bone sarcoma, including those with advanced CS, found that one patient had an objective response, one patient had stable disease, and three patients had progression.[39] In another trial investigating pembrolizumab in combination with doxorubicin hydrochloride, three patients with CS had tumor regression with one patient having shrinkage of 26%.[40] Interestingly, authors found that tumors with histologic evidence of TIL (21%) were inversely associated with PFS, although it is unclear whether the tumors included were CS. A new European single-arm phase Ib/II trial investigating anti-PD-1 nivolumab in combination with anti-angiogenesis TKI sunitinib in patients with advanced bone and soft-tissue sarcomas demonstrated a partial response in one patient out of four with extra-skeletal myxoid CS.[41] Unfortunately, patients with conventional CS were not included in the phase II study.

Dendritic cells. Dendritic cells (DCs) are a small population of antigen-presenting cells in the peripheral blood that are integral to T-cell lymphocyte

activation. DCs have demonstrated the ability to activate the γδ T-cells subclass that exhibits potent antitumor activity *in vitro*.[42] However, while Miwa *et al.*[43] demonstrated that DC therapies are associated with upregulation in serum Interferon–gamma (IFN–γ) and IL-12 levels in patients with advanced bone and tissue sarcomas, only 1 patient had partial response and the 75% of patients (28/37) had disease progression.[43] Other clinical trials have begun investigating DC therapies in patients with bone sarcomas, with disappointing preliminary results.[44,45]

CAR-T cell therapy. Chimeric antigen receptor (CAR)-T cell therapy is an exciting new targeted therapy that employs the use of synthetic receptors to evoke a T-cell response to common tumor antigens and has demonstrated remarkable success with the CD19 antigen in B-cell malignancies.[46] Nota *et al.*[47] have designed and studied the use of CAR-T cell therapy directed at chondroitin sulfate proteoglycan 4, a tumor antigen highly expressed in conventional CS, with notable *in vitro* success. Multiple clinical trials are underway to investigate the safety and efficacy of CAR-T cell therapy with multiple targets including CD276 and GD2 in bone sarcomas (NCT05312411, NCT03721068, NCT03356782, NCT04864821).

TAMs. Trabectedin, a drug which is poisonous to macrophages, has been investigated in soft-tissue sarcomas with FDA approval as therapy for metastatic liposarcoma or leiomyosarcoma.[48] Some innovative efforts to target macrophages include a melanoma study using *Mycobacterium indicus pranii* (Mw), an immunomodulator that held some promise *in vitro* but failed to have antitumor effect *in vivo*.[49] Inhibiting the pro-tumor activity of TAMs remains an area of interest in drug development (Table 1).

Case report

Our patient is a 44-year-old woman who presented in November 2019 with a T1N0M0G2 stage IIA, $7.8 \times 7.5 \times 6.3\,cm^3$ right scapula tumor. She underwent a radical resection in January 2020, with reconstruction of right shoulder hemiarthroplasty with replacement of the glenoid, local muscle rotation flaps to the right upper extremity, osteoarticular allograft of the right scapula, including the articular surface and contiguous bone, and neuroplasty of the right brachial plexus. Pathology was consistent with a grade 2 CS with negative margins.

By March 2020, evidence of metastatic disease was seen with a 3.5 cm lesion in the shoulder and multiple lung nodules seen in MRI and CT. Patient was initiated on pazopanib (800 mg oral daily). Imaging (9/2020) showed an increase in the scapular tumor size to $4.9 \times 3.2 \times 3.2\,cm^3$, as well as increase of lung nodules. Presence of disease progression led to the discontinuation of pazopanib after 2 months of therapy. Radical resection of the growing sarcoma was performed, with the tumor measuring 7 cm in size. Next generation sequencing revealed genomic alteration in IDH-1 (R132G) and TP53 (L111P), TMB status low and microsatellite status stable. Patient was started on ivosidenib (500 mg oral daily). After two cycles, restaging CT imaging showed progression of lung metastases and treatment was discontinued (Figure 1 and Table 2).

Patient started on a clinical trial (NCT04340843) of guadecitabine and belinostat (2/2021). Again, progression of disease was noted after two cycles and patient discontinued the clinical trial. She began another clinical trial (NCT04553692) of IGM-8444. Worsening pulmonary metastasis were noted after six cycles, and the clinical trial was discontinued.

After failure of four prior lines of treatment, the patient reported marked shortness of breath and unrelenting cough. She was started on pembrolizumab (200 mg IV q3 weeks), off label. The patient underwent imaging to assess response to therapy after every four cycles of pembrolizumab. Imaging showed a generalized decrease in size of pulmonary nodules and metastatic mediastinal and hilar lymph nodes. As well, patient reported resolution of cough and dyspnea. As her tumor volume decreased significantly, a right supraclavicular tumor grew, prompting a radical resection of the 6.1 cm right shoulder CS. Pathology reported approximately 40–50% necrosis of CS. Follow-up CT of the neck, chest, abdomen-pelvis showed marked improvement of the disease, with a significant decrease in right lung metastases. After 20 cycles of pembrolizumab, MRI imaging of her right shoulder showed an increase in the size of the tumor posterior to the right clavicle with a likely new tumor lateral to this, and the remainder of the metastatic disease grossly stable. Another surgical resection removed a 3.1 cm right

Table 1. Clinical trials of immunotherapy in sarcoma.

Phase	Drug(s)	Mechanism(s)	NCT	Status	*N*	Bone sarcomas studied	Results
I	Pembrolizumab	Anti-PD-1	02301039 (SARC028)	Completed	86	CS (5), OS, and EWS	1/5 CS patients had PR, >50% tumor size reduction lasting >6 months
I/II	Pembrolizumab and doxorubicin HCl	Anti-PD-1 and anthracycline	02888665	Completed	37	CC-CS (1), C-CS (3), and ESM-CS (1)	3/5 patients had tumor regression, 1 C-CS with 26% size reduction
I	HuMax-IL8	Anti-IL-8	02536469	Completed	15	CS (1) and chordoma (5)	CS patient had disease progression
I	Toripalimab	Anti-PD-1	03474640	Active	198	CS	–
II	Nivolumab and ipilimumab	Anti-PD-1 and anti-CTLA4	02982486	Recruiting	–	CS, EWS, and OS	–
II	Atezolizumab	Anti-PD-L1	04458922	Active	19	CS and CC-CS	–
II	INBRX-109	DR5 agonistic ab	04950075	Recruiting	–	CS	–
II	LN-145, LN-145-S1, aldesleukin, cyclophosphamide, fludarabine, ipilimumab, nivolumab	Autologous TILs, IL-2, alkylating agent, antimetabolite, and anti-PD-1/ anti-CTL4	03449108	Recruiting	–	CS, OS, and UPS	–
II	Nivolumab and sunitinib	Anti-PD-1 and TKI	03277924	Recruiting	–	DD-CS, ESM-CS, and CC-CS	–

C-CS, conventional CS; CC-CS, clear cell CS; CS, chondrosarcoma; CTLA4, cytotoxic T lymphocyte associated antigen 4; DD-CS, dedifferentiated CS; DR5, death receptor 5; ESM-CS, extra-skeletal myxoid CS; EWS, Ewing's sarcoma; OS, osteosarcoma; PD-1, programed cell death 1; PR, partial response; TIL, tumor-infiltrating lymphocyte; TKI, tyrosine kinase inhibitor; UPS, undifferentiated pleomorphic sarcoma.

subclavicular CS and 1.4 cm right shoulder CS. She has resumed her treatment of pembrolizumab, which is ongoing with marked response to therapy after 23 cycles. She is planned for 2 years of immunotherapy (Figure 2).

Conclusion and future directions

Recurrent or metastatic CS poses a unique problem to medical oncologists, as there are few evidence-based systemic agents that target aggressive disease. Repeated surgical resection and radiation increases morbidity and leads to loss of functionality. Furthermore, some tumors are unresectable, depending on their location. As an added challenge, CS is an orphan disease making it difficult to investigate through clinical trials. Thus, much of the data available on the efficacy of immunotherapy is based on small studies and case reports.

There is preliminary evidence drawing from preclinical studies, case reports, and ongoing clinical trials that immunotherapy may be effective in CS. The SARC028 phase II trial, while focused on bone and soft-tissue sarcomas, did

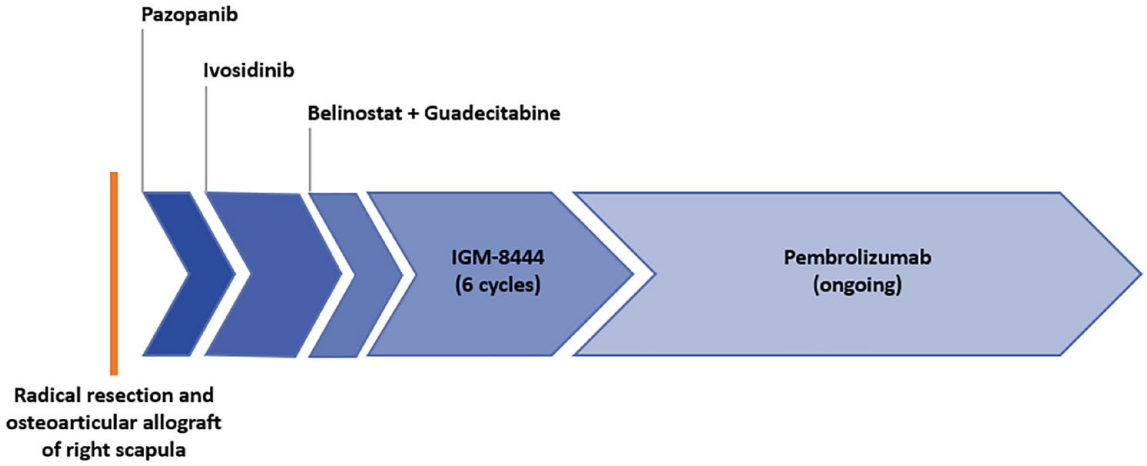

Figure 1. Treatment course. Sizing proportional to time on each treatment.

Table 2. Summary of the five lines of treatment and a description of the radiologic features of the right scapula throughout treatment.

Therapy	Clinical trial (if applicable)	Cycles until progression	PFS ratio	Radiologic features of right scapula	
				Treatment response	Mass at progression
Pazopanib	–	2		Disease progression	Shoulder tumor increased to $4.9 \times 3.2 \times 3.2\,cm^3$
Ivosidenib	–	4		Disease progression	
Belinostat and guadecitabine	NCT04340843	2		Disease progression; presence of several small bilateral pulmonary nodules	
IGM-8444	NCT04553692	6			
Pembolizumab	–	Ongoing with 23 previous cycles		Increase in the size of tumor posterior to the right clavicle, with a likely new tumor visualized laterally. Metastatic disease stable	n/a

PFS, progression-free survival.

include five patients with CS. One of the five CS patients had a partial response to pembrolizumab with >50% tumor reduction lasting 6 months. In a non-randomized phase I/II trial funded by the Fred Hutchinson Cancer Center looking at a combination regimen of doxorubicin with pembrolizumab, patients with clear cell CS (1), conventional CS (3), and extraskeletal myxoid CS (1) were included. Three CS patients had tumor regression including one conventional CS with a 26% size reduction. Interestingly, IHC studies of tumor samples from 66% of the participants demonstrated a low level of expression of PD-L1, and tumor expression of PDL-1 did not correlate with PFS or OS after treatment with PD-1 blockade.

Indeed, we report the case of a patient with metastatic CS who had a remarkable sustained response to treatment with pembrolizumab after failing multiple lines of systemic therapy. While pazopanib and ivosidenib have demonstrated some

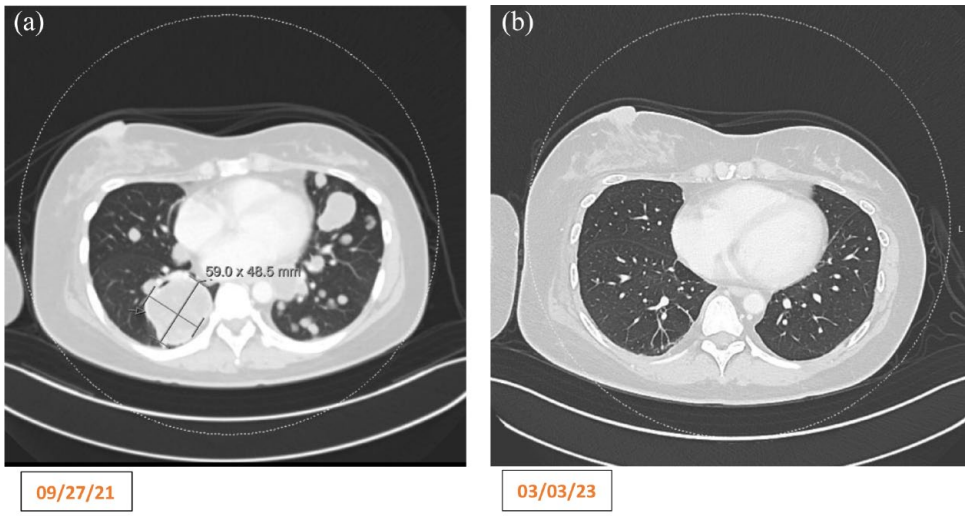

Figure 2. CT imaging of pulmonary metastases before immunotherapy (a) and after cycle 24 of pembrolizumab (b).

success in CS, our patient had disease progression on both, despite the presence of an IDH-1 mutation. Formal guidelines do not discuss the use of ICIs in the treatment of metastatic or unresectable CS. Pembrolizumab was chosen as a last-line agent to attempt to induce a response in this patient with a disease resistant to standard therapies. While she had initial growth of a new tumor on pembrolizumab she was able to maintain response to metastatic disease after resection. This likely represents pseudo-progression after immunotherapy, which is well documented in the literature.[50] Wagner *et al.*[51] also reported the case of a patient with grade III conventional CS with pulmonary metastases, who after four doses of treatment with nivolumab initially demonstrated increase in size of pulmonary nodules on CT scan. These nodules regressed 3 months later and the patient achieved a near complete response.

Currently, there are several ongoing trials assessing the use of ICI therapy in CS. The PD-L1 inhibitor, atezolizumab, is currently being evaluated in a phase II trial of pediatric patients with clear cell sarcoma and advanced CS (NCT04458922). Immunosarc, a phase I/II trial assessing the value of the TKI, sunitinib, in combination with the PD-1 inhibitor, nivolumab in advanced soft-tissue and bone sarcomas, is currently recruiting (NCT03277924).

While there are ongoing clinical trials studying immunotherapy in patients with bone and soft-tissue sarcomas, metastatic or unresectable CS is a rare and heterogeneous disease for which there is unlikely to be robust evidence for systemic treatments in the future. Interestingly, some patients with conventional CS appear to have sustained responses in clinical trials with immunotherapy but there are currently no reliable biomarkers predictive of response and no consensus statement on choice of therapy. More research is needed to study the aggressive form of this orphan disease.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Written consent was obtained for publication of de-identified medical information.

Author contributions

Adam J. Cohen-Nowak: Investigation; Writing – original draft; Writing – review & editing.

Danielle B. Dressler: Resources; Writing – review & editing.

Adam Rock: Writing – review & editing.

Katherine Mojica: Writing – original draft.

Doni Woo: Investigation; Methodology; Resources.

Lee M. Zuckerman: Conceptualization; Resources.

Warren Chow: Resources; Validation; Writing – review & editing.

Mark Agulnik: Conceptualization; Investigation; Resources; Supervision; Validation; Writing – review & editing.

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