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Efficacy of Gemcitabine-based Chemotherapy in Clear Cell Sarcoma of Soft Tissue

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Abstract. *Background/Aim: Clear cell sarcoma (CCS) is an aggressive sarcoma subtype, resistant to conventional anthracycline-based chemotherapy and radiation. The diagnosis is often challenging due to similarities with malignant melanoma. Patients and Methods: We aimed to analyse the activity of gemcitabine-based chemotherapy in a cohort of patients with CCS treated at the Royal Marsden Hospital. Results: Five patients with metastatic CCS received gemcitabine as first- or second-line systemic therapy. The median time-to-progression was 10 weeks. The median number of cycles of gemcitabine-based therapy was 3 (range=2-7 cycles). Median overall survival in our cohort was 66 months from the initial diagnosis but in the metastatic setting, the overall survival was reduced to 28 months. Conclusion: Gemcitabine-based therapy has modest activity in CCS. There remains a significant unmet medical need for novel, effective therapies for this disease.*

Clear cell sarcoma (CCS), previously known as malignant melanoma of the soft tissues, is an extremely rare and aggressive type of sarcoma, frequently diagnosed in young adults. The prognosis is poor, with 5-year survival rates between 40 and 60% (1). At diagnosis, about 30% of cases present with locally advanced or metastatic disease (1, 2). The majority of CCSs arise in the lower and upper extremities and are localised deep to the fascia. The diagnosis is often challenging due to similarities with the classical malignant melanoma, such as predilection for lymph node metastases and immunohistochemical positivity for melanoma-specific markers. Contrary to melanoma, CCS is characterised by the translocation t(12; 22)(q13; q12) that fuses the Ewing sarcoma gene *EWSR1* with cyclic AMP (cAMP)–regulated transcription factor *ATF1*, resulting in the *EWSR1-ATF1* gene fusion (3-5). Less frequently, the t(2;22)(q34;q12) translocation resulting in the *EWSR1-CREB1* (Cyclic AMP-Responsive Element-Binding Protein) fusion, is present in CCS (3).

Histologically, CCS is characterised by nests of uniform, rounded or fusiform ovoid cells with abundant eosinophilic to clear cytoplasm, separated by dense collagenous septa. Immunohistochemistry is usually positive for S100 protein and melanocyte-specific markers such as HMB-45 and Melan-A, hence the similarities with melanoma tumour cells (6). Keratin, epithelial-membrane antigen (EMA), smooth muscle actin and desmin are negative in CCS. Microphthalmia-associated transcription factor (MITF) is positive in 81-97% of CCS samples (6). Alongside alveolar soft-part sarcoma, CCS is part of the family of rare mesenchymal tumours that are driven by chromosomal translocations that activate the MITF, which leads to upregulation of the *c-Met* proto-oncogene, with subsequent promotion of growth factor signals for tumoral cell proliferation, survival and invasion (7).

Standard management of localised disease consists of complete surgical resection with or without radiation. The role of adjuvant chemotherapy is unclear. Even with optimal treatment, about 50% of patients will have a local or distant relapse, showing the aggressive character of CCS (1). The most common sites of metastases are lymph nodes and lungs, and most surgical centres will perform lymph node dissection with the initial surgery if lymph node involvement is suspected (8).

In general, conventional cytotoxic chemotherapy has minimal benefit, as reported by various published case-series. In the metastatic setting, anthracyclines are widely used with very few partial responses reported (9). In a retrospective

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series of metastatic CCS treated with conventional chemotherapy, the objective response rate was 4% and the median progression-free survival was 11 weeks (9). Therefore, targeted therapies have been evaluated in CCS.

A phase 2 trial of crizotinib, a MET tyrosine kinase inhibitor, resulted in only one objective response amongst 26 treated patients with MET positive CCS (10). Tivantinib, another selective inhibitor of MET, was administered to 11 patients with CCS in a phase 2 study (11). One patient achieved a partial response after four cycles of treatments. The median progression-free survival (PFS) for tivantinib in the CCS population in this trial was only two months (11).

Platelet derived growth factor receptor beta (PDGFRb) seems to be expressed in CCS on immunohistochemistry; the tyrosine kinase inhibitors sorafenib and sunitinib have shown antitumoral activity with variable degrees of disease regression in CCS (12, 13). *In vitro*, histone deacetylase inhibitors (HDACi) suppressed the growth of CCS cell lines, however monotherapy with HDACi has modest results in sarcomas (14, 15). Other agents active in CCS in pre-clinical experiments include trabectedin (16). In a cohort of soft tissue sarcoma patients treated with trabectedin, 2 patients with CCS were enrolled and best response was stable disease in one of these 2 patients (17).

Anecdotal reports of responses to programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) blockade or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) with or without radiotherapy in patients with CCS have been published in the last years (18). A phase 1 trial published by Goldberg and colleagues, explored the vaccination with irradiated, autologous sarcoma cell engineered to secrete granulocyte-macrophage colony-stimulating factor (19). This is the first study to analyse the host immunity response to CCS. Even though there were no objective tumour reductions, vaccination elicited local dendritic cell infiltrates and stimulated T cell–mediated immunoreactivity (19).

In aggregate, despite occasional responses to systemic therapy, the data indicate that chemotherapy and targeted therapies have minimal activity in advanced CCS. There have been anecdotal reports of radiological responses to gemcitabine-based therapy in advanced CCS. Therefore, the aim of this study was to document the efficacy and safety of gemcitabine-based therapy in a series of CCS patients treated at a sarcoma referral centre.

Patients and Methods

Local institutional approval was obtained to perform this study (SE 935). We performed a retrospective search of the prospectively maintained Royal Marsden Sarcoma Unit database to identify patients with CCS treated with gemcitabine-based therapy between January 2000 and January 2020. In all cases, the diagnosis of CCS was confirmed by an experienced soft tissue sarcoma pathologist (KT, CF). The *EWSR1-ATF1* fusion transcript was assessed by real-time quantitative polymerase chain reaction (RT-qPCR). We performed immunohistochemistry for S100 protein, Melan-A, SSRY-related HMG-box 10 (SOX10) protein, HMB45, h-caldesom, CD34, cytokeratin AE1/AE3, desmin and SMA.

Patient demographics, date of diagnosis, disease location, systemic and local treatment, follow-up and survival data were obtained from the database and electronic patient records.

Patients on active surveillance underwent repeat imaging every 3-6 months, with either computed tomography (CT) or magnetic resonance imaging (MRI). For patients treated with local therapy, response was evaluated 2-3 months following the procedure. For patients with metastatic disease receiving systemic chemotherapy, the response to therapy was assessed every two cycles with either CT or MRI scans. Descriptive statistics were used. Time-to-progression (TTP) was defined as the time between treatment initiation and disease progression, as defined by Response Evaluation Criteria in Solid Tumours versions 1.1 (RECIST 1.1) or by clinical progression. Toxicity data were assessed according to Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. Local disease progression was defined as progression of tumour sites present at staging, and distant progression was progression of distal sites not involved at the time of diagnosis. Overall survival (OS) was defined as time from diagnosis to date of death. Outcomes were presented as median TTP and OS.

Results

Five patients diagnosed with metastatic clear cell sarcoma with confirmed *EWSR1-ATF1* translocation and treated with gemcitabine-based therapy were identified from our database. No patients in our cohort harboured an *EWSR1-CREB1* translocation. All five patients were treated at our hospital during the course of their disease and two patients had also received previous treatments at other hospitals. In our cohort, two patients were female and three patients were male. The median age at diagnosis was 31 years old.

Case 1 (Figure 1). The first patient, aged 29 years at diagnosis, was a male who presented with a tumour on the left buttock. After an initial resection of the tumour, he had multiple local recurrences resected and he developed distant progression in the lungs 13 years after the initial diagnosis. Due to the limited efficacy of anthracyclines, he was initially treated within a phase 1 trial with metalloprotease inhibitor and paclitaxel. The best response was stable disease and he remained on treatment for 18 weeks before his disease progressed, when the patient stopped the trial. He then received gemcitabine-dacarbazine for seven cycles between the 2nd of November 2018 and the 3rd of May 2019. The best response was stable disease by RECIST 1.1, with a reduction in tumour size being noted on CT (Figure 1). After seven cycles, the patient opted for surveillance and the latest CT scan has shown ongoing stability 17 months (66 weeks) since starting gemcitabine-chemotherapy.

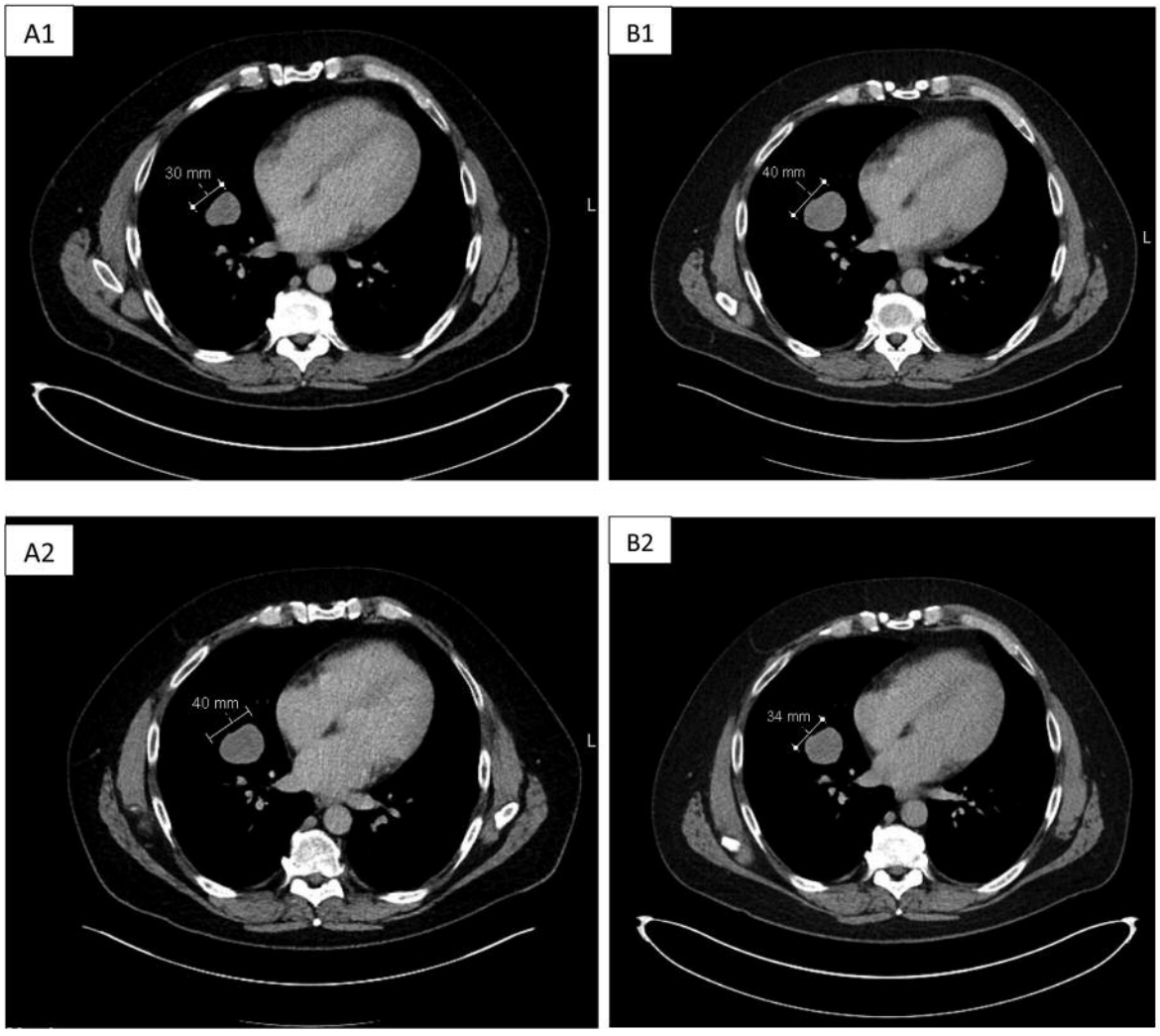

Figure 1. *Computer-tomography (CT) images from patient 1. A) Baseline images prior to treatment with gemcitabine-dacarbazine chemotherapy demonstrating increase in size of the tumour (A1 – baseline scan, A2 – most recent scan). B) Images during treatment with gemcitabine-dacarbazine chemotherapy showing a response to treatment with a reduction of the tumour (B1 – baseline scan at the start of treatment with gemcitabine; B2 – most recent scan).*

Case 2. The second patient was a 31-year-old woman, diagnosed with a CCS of the right tendo-calcaneous. She had received pre-operative dacarbazine, vindesine and cisplatin for 3 cycles, followed by amputation at the referring hospital. A CT scan after surgery raised the suspicion of lung metastases and she thus received two cycles of post-operative chemotherapy. Three months after surgery, the CT scan confirmed lung metastases and the patient received two cycles of gemcitabine plus docetaxel. A restaging CT scan after two cycles showed recurrence at the amputation site, with a TTP of six weeks. The patient's care was then transferred to our institution where she received a further 12 cycles of pazopanib and died of disease 30 months after the initial diagnosis.

Case 3. The third patient was a 48-year-old male who presented with a 3-year history of a neglected swelling in the posterior thorax. The initial CT scan showed extensive lymphadenopathy, therefore he received first line chemotherapy with gemcitabine-dacarbazine. A CT scan after two cycles demonstrated multifocal disease progression, therefore treatment was stopped, and the patient died soon after. Time-to-progression and overall survival were nine weeks and eleven months respectively.

Case 4. The fourth patient was a 52-year-old man, who presented with a mass in the right knee. He underwent surgical excision and gastrocnemius flap followed by adjuvant radiotherapy (60 Gy in 30 Fractions). Three years after the initial diagnosis, he developed lung metastases and recurrence

in the right groin. He received radiotherapy to the right groin, following which he developed further progression in the lung metastases and received pazopanib for five months. In view of the disease progression on pazopanib, he was treated with six cycles of gemcitabine and docetaxel, with stable disease being the best response. The time-to-progression was 25 weeks. Upon development of progressive disease in the lungs and groin he was referred to a phase 1 trial of combination radiotherapy and immunotherapy and was treated within that trial for a total duration of 11 weeks, with the best response achieved being stable disease. He died of disease six years after initial presentation.

Case 5. The fifth patient was aged 25 years at diagnosis. She presented with a right ankle mass and underwent wide excision and post-operative radiotherapy consisting of 60 Gy in 30 fractions. She developed local recurrence and lung metastases 20 months after completion of adjuvant radiotherapy. She received local treatments in the form of isolated limb perfusion of the lower leg and resection of lung metastases. Gemcitabine and dacarbazine were given for a total of 3 cycles for recurrent disease. The best response was progressive disease by RECIST 1.1. The time-to-progression on gemcitabine-based therapy was 10 weeks. The patient went on to receive sunitinib for a total of six weeks, followed by best supportive care and death due to the disease at 5 years and 4 months since the initial presentation.

Concluding remarks. The activity of gemcitabine-based therapy in CCS is modest. In our cohort of five patients, the median time-to-progression was 10 weeks (95%CI=7.8-12.1) and only one patient had a prolonged disease stability with gemcitabine-dacarbazine. The overall survival in our cohort was 66 months (95%CI=0.0-162.6) from initial diagnosis but in the metastatic setting, the overall survival was reduced to 28 months (95%CI=10.8-45.1). In our cohort, two patients received gemcitabine-dacarbazine as first-line therapy for metastatic disease, and the other three patients received gemcitabine-docetaxel (n=2) or gemcitabine-dacarbazine (n=1) as second-line therapy. One patient only received one line of systemic therapy before dying of disease, four patients received second-line therapy and two patients received third-line treatment, with pazopanib and avelumab. The median number of cycles of gemcitabine-based regimens in our cohort was 3 (range=2-7 cycles). None of the patients in our cohort experienced grade 3, 4 or 5 toxicities during their treatment with gemcitabine-based chemotherapy.

Discussion

Our study suggests that gemcitabine-based therapy has limited efficacy in advanced CCS. One patient had durable disease control (17 months) following gemcitabine plus dacarbazine, whereas the other four patients did not derive benefit from gemcitabine-based therapy. Consequently, the management of advanced CCS remains a considerable challenge with a significant unmet need for novel, effective systemic therapies. Gemcitabine-based regimens are relatively well tolerated. For patients with a good performance status, participation in a phase 1 trial should be offered whenever possible. The results of the phase 1/2 trial with the HDACi vorinostat, gemcitabine and docetaxel are eagerly awaited, and it might prove that gemcitabine in combination with epigenetic drugs might be beneficial in this orphan disease, as suggested by the preclinical data demonstrating synergistic interaction between gemcitabine and HDAC inhibition, resulting in increased DNA damage response and apoptosis in lymphoma (NCT NCT01879085) (15, 20). The study by Goldberg *et al*. is the first to evaluate the role of host immunity in CCS, and even though vaccination itself did not result in tumour shrinkage, it brings insights into the immune response in CCS (19). VEGFR and PDGFR inhibitors have demonstrated antitumoral activity in retrospective case reports and in our own case series, however, none of these are approved for the treatment of CCS, and their clinical activity warrants further investigations in the context of a clinical trial (12, 13). Targeting the MET receptor tyrosine kinase in CCS had very modest results in phase 2 trials with crizotinib and tivantinib, two selective MET inhibitors (11, 13).

The similarities with malignant melanoma raise the question as to whether treatments currently used in melanoma could also be effective in CCS. The immune-mediated response to autologous vaccinations, as described by Goldberg and colleagues (19), also provides new treatment opportunities. However, we must emphasise that CCS is an aggressive subtype of soft tissue sarcoma and better treatments are urgently needed (21).

The limitations of this study include its retrospective design, small number of patients and heterogeneity of gemcitabine chemotherapy regimens (combination of gemcitabine with either dacarbazine or docetaxel).

Conflicts of Interest

R L Jones: Receipt of grants/research support: MSD, GSK. Receipt of consultation fees: Adaptimmune, Athenex, Blueprint, Boehringer-Ingelheim, Clinigen, Eisai, Epizyme, Daichii, Deciphera, Immunedesign, Lilly, Merck, Pharmamar, UptoDate. All other Authors: no conflicts of interest to declare.

Authors' Contributions

Elena Cojocaru- project design, data curation, writing original draft, review and editing; KHIN THWAY- review of the histopathology, review and editing of the manuscript; Cyril Fisher- review of the histopathology, review and editing of the manuscript; Christina Messiou- radiological review, review and editing of the manuscript; Shane Zaidi- supervision and critical review of the article; Aisha B. Miah- supervision and critical review of the article; Charlotte Benson- supervision and critical review of the article; Spyridon Gennatas- data curation, supervision, and critical review of the article; Paul Huang-

supervision, and critical review of the article; Robin L. Jones-project design, supervision, final approval of the manuscript.

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