Axitinib plus pembrolizumab in patients with advanced sarcomas including alveolar soft-part sarcoma: a single-centre, single-arm, phase 2 trial

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Summary

Background VEGF promotes an immunosuppressive microenvironment and contributes to immune checkpoint inhibitor resistance in cancer. We aimed to assess the activity of the VEGF receptor tyrosine-kinase inhibitor axitinib plus the anti-PD-1 immune checkpoint inhibitor pembrolizumab in patients with sarcoma.

Methods This single-centre, single-arm, phase 2 trial was undertaken at a tertiary care academic medical centre in Miami, FL, USA, and participants were recruited from all over the USA and internationally. Patients were eligible if they were aged 16 years or older, and had histologically confirmed advanced or metastatic sarcomas, including alveolar soft-part sarcoma (ASPS); measurable disease with one site amenable to repeated biopsies; an ECOG performance status of 0–1; and progressive disease after previous treatment with at least one line of systemic therapy (unless no standard treatment existed or the patient declined therapy). The first five patients were enrolled in a lead-in cohort and were given axitinib 5 mg orally twice daily and pembrolizumab 200 mg intravenously for 30 min on day 8 and every 3 weeks for cycles of 6 weeks for up to 2 years. Thereafter, patients received escalating doses of axitinib (2–10 mg) plus flat dose pembrolizumab according to the schedule above. The primary endpoint was 3-month progression-free survival. All patients were evaluable for survival and safety analyses. This study is registered with ClinicalTrials.gov, number NCT02636725, and is closed to accrual.

Findings Between April 19, 2016, and Feb 7, 2018, of 36 patients assessed for eligibility, 33 (92%) were enrolled and given study treatment (intention-to-treat population and safety population), 12 (36%) of whom had ASPS. With a median follow-up of 14·7 months (IQR 10·1–19·1), 3-month progression-free survival for all evaluable patients was 65·6% (95% CI 46·6–79·3). For patients with ASPS, 3-month progression-free survival was 72·7% (95% CI 37·1–90·3). The most common grade 3 or 4 treatment-related adverse events included hypertension (five [15%] of 33 patients), autoimmune toxicities (five [15%]), nausea or vomiting (two [6%]), and seizures (two [6%]). Serious treatment-related adverse events occurred in seven (21%) patients, including autoimmune colitis, transaminitis, pneumothorax, haemoptysis, seizures, and hypertriglyceridemia. There were no treatment-related deaths.

Interpretation Axitinib plus pembrolizumab has manageable toxicity and preliminary activity in patients with advanced sarcomas, particularly patients with ASPS, warranting further investigation in randomised controlled trials.

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Introduction

Sarcomas are a rare and heterogeneous group of over 100 different cancers of bone and soft tissue, comprising approximately 1% of adult and 15% of paediatric cancers.[1] Several soft tissue sarcoma subtypes are inherently resistant to cytotoxic chemotherapy, including alveolar soft-part sarcoma (ASPS).[2] ASPS usually affects adolescents and young adults, and presents early with widespread metastases that are ultimately fatal. The conserved translocation of the *ASPSCR1-TFE3* fusion gene in ASPS leads to aberrant transcription of downstream target genes including *HIF-1α*, which upregulates proangiogenic factors including VEGF.[3] Tyrosine-kinase inhibitors are the most active treatment to date for patients with ASPS, although most patients ultimately develop resistance and die as a result of the disease.[4–7] In other sarcoma subtypes, high expression of VEGF and hypoxia have been associated with poor prognosis and resistance to chemotherapy, and anti-VEGF receptor tyrosine-kinase inhibitors such as pazopanib, regorafenib, and axitinib have shown modest antitumour activity.[8–10]

Immune checkpoint inhibitors that disrupt PD-1 and PD-L1 activity have led to remarkable outcomes in many chemotherapy-refractory solid cancers. Anti-PD-1 antibodies as monotherapy and in combination with anti-CTLA-4 antibodies have shown antitumour activity in advanced sarcomas, but the proportion of patients who achieve a response remains modest, at less than 20%.[11,12] Many sarcomas exhibit characteristics of immunologically

Research in context

Evidence before this study

We searched PubMed for articles published in English from database inception until Dec 1, 2018, using the search terms "metastatic sarcoma", "alveolar soft part sarcoma", and "immune checkpoint inhibitors". We excluded review articles and meta-analyses. Immune checkpoint inhibitors against CTLA-4, PD-1, and PD-L1 have shown modest activity in several phase 2 trials, including various sarcoma subtypes, with the proportion of participants who showed an objective response being less than 20%. Alveolar soft-part sarcoma (ASPS) is resistant to traditional cytotoxic chemotherapy regimens, with extended stability of disease being achievable with tyrosine-kinase inhibitors, but with the proportion of patients with a response being less than 40%. Responses to immune checkpoint inhibitors in ASPS have been noted in case reports, and a preliminary report at the 2018 Annual Meeting of the Connective Tissue Oncology Society observed eight (42%) of 19 patients with ASPS had an objective response with the PD-L1 inhibitor atezolizumab. Lack of response to immune checkpoint inhibitors in other sarcomas might be associated with restricted mutational burden and few neoantigens, particularly in translocation-dependent sarcoma subtypes. Low cytotoxic T-cell infiltration, low PD-L1 expression, and the presence of suppressive immune cell phenotypes such as macrophages and regulatory T cells have also been postulated to contribute to inert immune microenvironments in sarcomas. Immune cell infiltration and activation has been improved in melanoma and renal cell carcinoma with the addition of anti-VEGF receptor antibodies or tyrosine-kinase inhibitors to immune checkpoint blockade.

Added value of this study

To our knowledge, this is the first reported clinical trial to investigate combination therapy with an anti-VEGF receptor tyrosine-kinase inhibitor and an immune checkpoint inhibitor in sarcomas. Patients with ASPS treated with axitinib plus pembrolizumab had meaningful and durable objective responses. In the non-ASPS population, axitinib plus pembrolizumab had clinical benefit akin to other active sarcoma chemotherapy regimens including tyrosine-kinase inhibitors in the second-line or further lines of treatment.

Implications of all the available evidence

A combination of VEGF and PD-1 blockade is feasible in patients with advanced sarcomas and shows promising preliminary activity in a subset of patients, including those with ASPS. To confirm our hypothesis that a VEGF blockade improves immune response over immune checkpoint inhibitor monotherapy, further investigation with a randomised trial is needed. Additional analyses are required to identify mechanisms of resistance to immunotherapy and biomarkers for patients with sarcomas that are likely to benefit from this approach.

cold tumours—ie, restricted immune infiltrates largely composed of suppressive T cells and macrophages, low tumour mutational burden, and infrequent PD-L1 tumour expression.[13–16] The contribution of tumour angiogenesis in maintaining the immunosuppressive tumour microenvironment has been well established in melanoma and renal cell carcinoma, but the specific effects of VEGF and related pathways on the sarcoma tumour microenvironment are not well understood.[17,18]

VEGF not only promotes tumour angiogenesis, leading to growth and metastasis, but also suppresses immune responses within the tumour microenvironment by inhibiting dendritic cell maturation and antigen presentation, restricting migration of lymphocytes across endothelium into the tumour compartment, and accumulating suppressive tumour-associated macrophages, regulatory T cells, and myeloid-derived suppressive cells.[19] Supporting the crucial role of VEGF in antitumour immunity, combination therapies with VEGF blockade and immune checkpoint inhibitors have shown favourable outcomes in melanoma and renal cell carcinoma.[20] On the basis of the central role of VEGF in maintaining the suppressive immune microenvironment, we sought to determine the activity of the anti-VEGF receptor tyrosine-kinase inhibitor axitinib combined with the anti-PD-1 immune checkpoint inhibitor pembrolizumab in patients with advanced sarcomas.

See Online for appendix

Methods

Study design and participants

In this investigator-initiated, single-arm, phase 2 trial patients were recruited from a tertiary care academic medical centre in Miami, FL, USA, but patients came from all over the USA and internationally to participate. Most patients self-referred after locating the trial through ClinicalTrials.gov, or were referred by treating sarcoma physicians. Since this trial was the first to our knowledge that incorporated intrapatient axitinib dose escalation with pembrolizumab, we designed an early stopping rule for toxicity using Bayesian methods, including a safety lead-in cohort of five patients (appendix p 123). In the event of treatment-related deaths, or if four of the five lead-in patients had pre-defined treatment-related toxic effects, the study would be halted for safety review. This lead-in phase was based on the Bayesian stopping rule that the frequency of treatment-related toxic effects should not exceed 40%, with a posterior probability of 90% or higher. Treatment-related toxic effects that were considered under the stopping rule occurred during the first 49 days (cycle 1) of study therapy and included grade 4 anaemia or thrombocytopenia, grade 3 neutropenic fever, grade 3 acute kidney injury, proteinuria, transaminitis, nausea, vomiting, diarrhoea, constipation, oedema, and hypertension not resolving within 7 days with medical management, or other grade 3 or greater

toxicity events. After the main cohort had been recruited and during analysis, we designed an expansion cohort to enrol up to ten additional patients with ASPS (protocol amendment 4, March 20, 2018); however, the study sponsor later declined this additional expansion cohort (on Oct 1, 2018) and the study was closed. No additional patients were accrued under this amendment.

Patients were eligible if they were aged 16 years or older, and had histologically confirmed sarcoma (we did not predefine the number of patients to be enrolled with various subtypes); measurable, progressing disease as defined by Response Evaluation Criteria In Solid Tumors (RECIST; version 1.1) within 6 months before enrolment, confirmed by a musculoskeletal radiologist (TKS), and one site of disease amenable to repeated biopsies; and an ECOG performance status score of 0 or 1. Eligible patients had at least one line of chemotherapy or targeted therapy appropriate for their sarcoma subtype, unless no standard treatment existed or the patient had refused standard treatment (see protocol for complete details; appendix p 18). Previous treatment with tyrosine-kinase inhibitors other than axitinib or immune checkpoint inhibitors was permitted. All adverse events from previous treatments were required to have resolved to grade 1 or less before enrolment, except for alopecia or neuropathy.

Patients also had to have adequate renal, liver, and bone marrow function; and no active or untreated brain metastases, underlying haematological or bleeding diatheses, or cardiac abnormalities. Patients were excluded if they had a history of autoimmune disorders, concurrent malignancies, or viral infections. Before initiating therapy, we required patients to have at least 2 weeks without previous chemotherapy, radiation, and targeted therapies, and 4 weeks without receiving monoclonal antibodies or investigational products.

The study protocol is available in the appendix (pp 18–165) and was approved by the US Food and Drug Administration (FDA) and the University of Miami (Miami, FL, USA) institutional review board. All participants provided written, informed consent, including paediatric assent. An institutional review board exception was granted to enrol one patient aged 15 years with ASPS who was not a candidate for other clinical trials and whose disease had progressed while on two previous tyrosine-kinase inhibitors.

Procedures

Study therapy consisted of axitinib 5 mg orally twice daily continuously, and pembrolizumab 200 mg via 30-min intravenous infusion on day 8 and then every 3 weeks thereafter for up to 2 years (appendix p 3). Study cycles were 6 weeks in duration except cycle 1, which included a 7-day axitinib lead-in period. The FDA guidelines[21] for axitinib permit dose escalation above 5 mg twice daily because of increased benefit with higher axitinib exposure and variable patient tolerance in those with renal cell carcinoma. Thus, we adopted intrapatient dose escalation and de-escalation with doses ranging from 2 mg to 10 mg twice daily (appendix p 4). The initial safety lead-in cohort of five patients was not escalated above 5 mg twice daily. Subsequent patients were escalated by one dose level on day one of each cycle beginning at cycle 2 (1 mg twice daily per cycle) if they did not exhibit pre-specified grade 2 or greater toxicities, including fatigue, hand-foot (erythrodysesthesia) syndrome, mucositis, or diarrhoea.

Dose reductions, interruptions, or both were permitted at any time for toxicities not relieved by supportive care. Reasons permitted for treatment discontinuation included radiographic or clinical progression, intolerable toxicity, indication for surgery, withdrawal of consent, or non-compliance with study procedures.

Patients were evaluated weekly during the first two cycles of treatment for adverse events and safety assessments, including complete blood counts and chemistries, and thereafter every 3 weeks. Adverse events were graded according to Common Terminology Criteria for Adverse Events version 4.0. Causality for all adverse events was attributed by investigators to axitinib, pembrolizumab, or both drugs. For adverse events attributable to either or both study drugs, particularly possible immune-related adverse events, interruption of axitinib was done first because most axitinib-related toxic effects resolve promptly with drug withdrawal. Autoimmune toxic effects were managed with pembrolizumab delayed for up to 12 weeks, and corticosteroids, infliximab, or both, if indicated. Serious adverse events were those which resulted in deaths, disability, or incapacity; were life-threatening; required treatment as an inpatient in hospital; or were considered a medically important event.

Patients underwent imaging with contrast-enhanced CT, MRI, or both of all sites of disease at baseline and every two cycles thereafter. If pembrolizumab was delayed, imaging was also delayed to correspond with treatment cycles. Tumour responses were determined by investigators with a musculoskeletal radiologist (TKS) by RECIST, with responses confirmed by a second scan at least 4 weeks after criteria for objective response were met. Patients could remain on treatment beyond RECIST-defined progression if symptomatic benefit was noted by the patient and investigator. No central review of radiographic imaging was done. Correlative procedures included peripheral blood collection, tumour core-needle biopsies, and combination PET-CT scanning, which were all done at baseline, day 1 of cycle 3, and at the time of progression. Patients were followed up for adverse events for 90 days from last dose of study therapy, and survival after discontinuation of study treatment until death or withdrawal of consent.

Pseudoprogression is commonly noted in sarcomas treated with tyrosine-kinase inhibitors and is characterised by a paradoxical increase in tumour size due to early tumour necrosis and inflammation before eventual RECIST responses. Pseudoprogression with emergence of new lesions has also been noted in some solid tumours

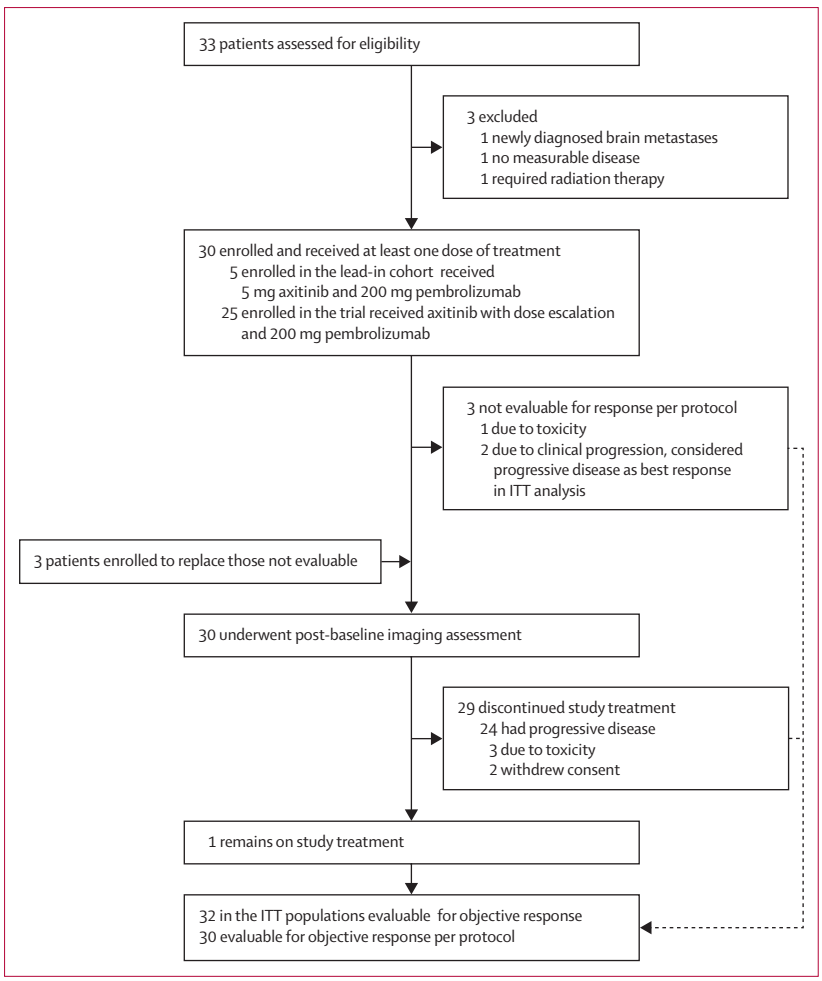

Figure 1 **Trial profile**
ITT=intention-to-treat.

treated with immune checkpoint inhibitors, which led to development of immune-related response criteria (irRC). To assess for pseudoprogression in this study, as part of the prespecified exploratory endpoints, we compared the proportion of patients who achieved a RECIST response with the proportion who achieved response by Choi criteria (which incorporates a decrease in tumour density from necrosis), irRC, and PERCIST (which incorporates decreased tumour metabolic activity by combined PET-CT).[22–24]

Outcomes

The primary endpoint was progression-free survival at 3 months, with progression-free survival defined as the time from treatment initiation until radiographic disease progression, clinical progression requiring treatment discontinuation, or death.

Secondary endpoints were incidence of treatment-related adverse events, the proportion of patients who achieved an objective response (ie, complete response or partial response), the proportion of patients who achieved a clinical benefit (ie, complete response, partial response, or stable disease), time to progression (defined as time from treatment initiation to first occurrence of progression or death) and overall survival (time from beginning treatment to death from any cause).

Exploratory endpoints reported here include tumour PD-L1 expression, lymphocyte infiltration score, plasma angiogenic activity, and circulating neutrophil-to-lymphocyte ratio, which were correlated with best response and progression-free survival. Other pre-specified exploratory endpoints of immune phenotyping of tumour-infiltrating and circulating lymphocytes, cytokine profiles, expression of immune-related genetic signatures, quantity of circulating tumour cells, and responses by MRI volumetrics will be reported elsewhere.

Statistical analysis

Clinical and demographic characteristics of study participants were summarised using descriptive statistics. In this trial, clinically meaningful activity would be considered if 3-month progression-free survival reached 40% compared with 19% 3-month progression-free survival in a historical control cohort of patients with metastatic or locally advanced sarcoma refractory to or intolerant of doxorubicin.[25] This benchmark for 3-month progression-free survival is often used in single-arm, phase 2 trials of patients with advanced sarcoma to support further investigation of a therapy in randomised studies,[9] but does not prove therapeutic value. Assuming 15 months for enrolment and 12 months of minimum follow-up, we estimated that enrolment of 30 evaluable patients would provide 93·5% power to reject 3-month progression-free survival of 19% in favour of 3-month progression-free survival of 40% using a single-arm survival design with one-sided 5% significance level.[26]

For response, as per protocol, patients were evaluable if they received at least one dose of both axitinib and pembrolizumab and had at least one post-treatment radiographic imaging assessment. Patients who discontinued therapy before the 3-month imaging assessment due to clinical progression or toxicity were replaced to ensure at least 30 patients reached the 3-month imaging timepoint. To avoid bias in the survival analyses by the exclusion of patients who had early disease progression, we report outcomes by intention-to-treat and include the patients who discontinued therapy early for clinical progression as progressive disease.

Patients were evaluable for safety if they received at least one dose of both axitinib and pembrolizumab. We had no planned interim or sensitivity analyses.

We used the Kaplan-Meier method to generate progression-free survival and overall survival curves, with prespecified point estimates at 3, 6, and 12 months with 95% CIs, and we determined median progression-free survival and overall survival using Greenwood's variance and the log-log transform method.[27] Patients who withdrew consent or discontinued treatment

because of toxicity were censored at the date of last imaging. We used the log-rank test to compare Kaplan-Meier curves between the study population and historical controls.[27,28] We tabulated the proportion of patients who achieved an objective response (ie, complete or partial response) and who achieved a clinical benefit (ie, complete response, partial response, or stable disease) with 95% CIs. If imaging was not obtained in the setting of clinical progression, these patients were considered to have progressive disease as best response. Duration of response was measured from the time that criteria were met for response until progressive disease. We summarised adverse events as the proportion of the total number of patients treated. Post hoc, we compared primary and secondary endpoints between ASPS and non-ASPS patient subsets, using two sample tests to compare progression-free survival at 3, 6, and 12 months, and two sample proportion tests to compare the proportions of patients who achieved an objective response and a clinical benefit.[28]

We did all statistical tests for clinical outcomes using SAS (version 9.4) and software R (version 3.3.1), and we used STPLAN, MD Anderson Biostatistics software, version 4.5, for the power calculation. All tests were two-sided and p values of less than 0·05 was considered statistically significant. Statistical tests for exploratory endpoints were done using Graphpad Prism, version 8. p values associated with post-hoc and exploratory analyses were descriptive in intent.

The study is registered with ClinicalTrials.gov, number NCT02636725.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Funders reviewed the manuscript before submission. The corresponding author had full access to all the data in the study and BAW, MMT, DKw, and JCT had access to the raw data. The corresponding author had final responsibility to submit for publication.

Results

Between April 19, 2016, and Feb 7, 2018, 36 participants were recruited and enrolled, of whom 33 (92%) were given study therapy and were evaluable for toxicity (figure 1). 30 patients underwent radiographic imaging at 3 months and were evaluable for per-protocol analysis. Three (9%) of 33 patients discontinued treatment before the 3-month imaging assessment, two due to clinical progression who were included in the ITT analysis of the primary endpoint, and one due to toxicity. Baseline characteristics of the study population are in table 1. Complete details of specific patient's histological subtypes, previous therapies received, and best overall response to study therapy are in the appendix (pp 9–11). Notably, our patients with non-ASPS histologies had refused doxorubicin therapy.

	Study population (n=33)
Demographics	
Age, years	44 (27–62)
Sex	
Male	18 (55%)
Female	15 (45%)
Self-reported race or ethnicity	
White non-Hispanic or non-Latino	17 (52%)
Black non-Hispanic or non-Latino	1 (3%)
Hispanic or Latino	12 (36%)
White	6 (18%)
Black or African-American	1 (3%)
Other	5 (15%)
Asian	3 (9%)
Clinical	
Histology	
Alveolar soft part sarcoma	12 (36%)
High-grade pleomorphic sarcoma	5 (15%)
Uterine leiomyosarcoma	4 (12%)
Non-uterine leiomyosarcoma	2 (6%)
De-differentiated liposarcoma	2 (6%)
Other*	8 (24%)
Disease stage	
Locally advanced	2 (6%)
Metastatic	31 (94%)
Brain metastases	
Yes	5 (15%)
No	28 (85%)
Histological grade	
1	1 (3%)
2	3 (9%)
3	15 (45%)
Not applicable†	14 (42%)
ECOG performance status	
0	20 (61%)
1	13 (39%)
Lines of systemic therapy	
0	6 (18%)
1	4 (12%)
2	12 (36%)
3 or more	11 (33%)
Previous tyrosine-kinase inhibitor	
Yes	17 (51%)
No	16 (48%)
Previous immunotherapy	
Yes	5 (15%)
No	28 (85%)

Data are median (IQR) or n (%). Complete clinical and pathological data are in the appendix (pp 9–11). *Other histologies included one patient with each of the following: malignant peripheral nerve sheath tumour (radiation associated), angiosarcoma (visceral), de-differentiated chondrosarcoma, Ewing sarcoma, gastrointestinal stromal tumour, synovial sarcoma, epithelioid haemangioendothelioma, epithelioid sarcoma. †Patients with alveolar soft part sarcoma, epithelioid haemangioendothelioma, and gastrointestinal stromal tumour are not graded.

Table 1: **Baseline characteristics**

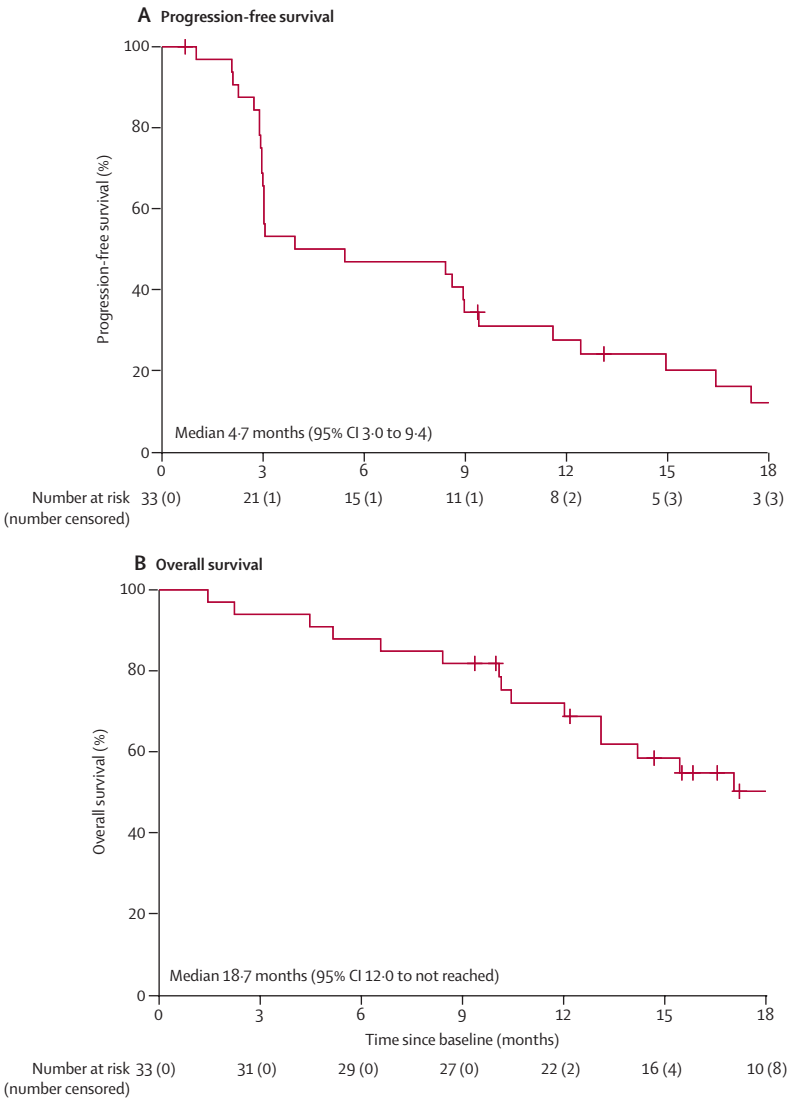

Figure 2: **Progression-free survival (A) and overall survival (B)**
Kaplan-Meier estimates show the distribution of progression-free survival and overall survival over time for all patients by intention-to-treat analysis (n=33). Patients who remained event free were censored at time of data cutoff (Dec 5, 2019). Crosses indicate censored patients.

As of data cutoff (Dec 5, 2018), median follow-up was 14·7 months (IQR 10·1 to 19·1). 3-month progression-free survival for all patients by intention-to-treat analysis was 65·6% (95% CI 46·6 to 79·3), and median progression-free survival was 4·7 months (95% CI 3·0 to 9·4; figure 2A). 6-month progression-free survival was 46·9% (95% CI 29·2 to 62·8) and 12-month progression-free survival was 27·5% (13·4 to 43·6). 17 (52%) of 33 patients died due to disease progression by data cutoff, including two who died during the reporting window (figure 1). Median overall survival for all 33 patients was 18·7 months (95% CI 12·0 to not reached) with 1-year overall survival of 72% (95% CI 53·0 to 84·4; figure 2B). In the per-protocol analysis (n=30), median progression-free survival was 6·9 months (95% CI 3·0 to 9·4), 3-month progression-free survival was 70·0% (50·3 to 83·1), 6-month progression-free survival was 50·0% (31·3 to 66·1), and 12-month progression-free survival was 29·3% (14·4 to 46·1; appendix pp 7–8).

Of 32 patients evaluable for objective response, none achieved a complete response. Eight (25·0%, 95% CI 12·1–43·8) achieved a partial response at any point during treatment, and nine (28·0%) achieved stable disease, so the proportion of patients who achieved clinical benefit was 53·1% (n=17; 95% CI 35·0–70·5; figure 3). In the per-protocol analysis, eight of 30 evaluable patients achieved a partial response (26·7%, 95% CI 13·0–46·2) with nine (30%) of 30 patients achieving stable disease, so the proportion of patients who achieved clinical benefit was 56·7% (17 of 30; 95% CI 37·7–74·0; appendix p 7). Follow-up imaging at least 4 weeks after initial response confirmed all partial responses. Median duration of response was 29 weeks (IQR 21·8–76·5), and median time to achieve partial response was 19·4 weeks (IQR 12·8–31·4; figure 4). Most responses occurred in patients with ASPS, with six of 11 evaluable patients with ASPS achieving a partial response (54·5%, 95% CI 24·6–81·9), and two (18%) of 11 achieving stable disease, so the proportion of patients who achieved a clinical benefit was 72·7% (n=8; 95% CI 32·3–92·7) by intention-to-treat analysis throughout the study period (figure 3). Median time to partial response in patients with ASPS was 25·1 weeks (IQR 12·7–34·3). We also observed partial responses in two patients, one with conventional-type epithelioid sarcoma and one with soft tissue leiomyosarcoma, and minor responses (decrease in size of target lesion of less than 30%) in three patients, one with soft tissue leiomyosarcoma, one with synovial sarcoma, and one with high-grade undifferentiated pleomorphic sarcoma.

The toxicity profile of axitinib plus pembrolizumab therapy was consistent with previous clinical trials of the drugs as monotherapy. Treatment-related toxicity occurred in only two (40%) of five patients in the safety lead-in cohort, and no application of the early stopping rule was needed throughout the study. Treatment-related grade 3 or 4 adverse events occurred in 13 (39%) of 33 patients, and grade 3 or 4 autoimmune toxic effects in five (15%) patients. The most common treatment-related adverse events of any grade included fatigue (26 [79%]), oral mucositis (23 [70%]), hypothyroidism or hyperthyroidism (21 [64%]), nausea or vomiting (22 [67%]), nasopharyngeal congestion (18 [55%]), and diarrhoea (19 (58%]; table 2). Serious treatment-related adverse events occurred in seven (21%) of 33 patients, including autoimmune colitis, transaminitis, pneumothorax, haemoptysis, seizures, and hypertriglyceridemia. Determination of causality of each adverse event was subjective, but most adverse events were determined by the investigator to be associated with axitinib on the basis of resolution with supportive care and axitinib dose

interruptions or reductions. Fatigue, thyroid dysfunction, tumour pain, and arthralgias or myalgias were thought to be associated with one or both drugs. Two (40%) of five patients with ASPS who enrolled with previous irradiated brain metastases had grade 3 seizures. Seizures have been reported with axitinib but in the context of uncontrolled hypertension or reversible posterior leukoencephalopathy syndrome, which were not observed in these patients. Since seizures are also rare with pembrolizumab monotherapy, we considered our patients' seizures as possibly associated with one or both drugs.

31 patients received at least one complete cycle of axitinib; one patient discontinued study therapy due to axitinib-related hepatotoxicity, and another patient clinically progressed before completing the first cycle. Based on protocol-defined, grade 2 toxic effects, axitinib dose reduction was required in 18 (58%) of 31 patients, nine (29%) required two or more dose reductions, and 15 (48%) required one or more dose interruptions. Only five (16%) patients tolerated the axitinib intrapatient dose escalation above 5 mg twice daily (appendix p 4). No associations were observed between axitinib doses and best response or incidence of grade 3 or 4 axitinib-related adverse events (appendix pp 4–5).

Pembrolizumab-related autoimmune toxic effects occurred in eight (24%) of 33 patients, with five grade 3 or 4 adverse events, three of which required corticosteroids and discontinuation of study treatment (one each of autoimmune hepatitis, colitis, and arthritis). Patients with autoimmune hepatitis and colitis had complete resolution of symptoms with corticosteroids. The patient with autoimmune arthritis has been off study therapy for 11 months to date with persistent arthritis requiring methotrexate and hydroxychloroquine. Despite these immunosuppressive medications, this patient with ASPS continues to be in partial response without additional cancer treatment.

Several clinical and immunological exploratory enpoints were correlated with progression-free survival longer than or shorter than 6 months, best objective response, and ASPS versus non-ASPS histology (appendix p 12). Tumour cell PD-L1 expression was positive in 15 (52%) of 29 patients with evaluable tumour biopsy samples, and all tested biopsy samples from patients with ASPS showed PD-L1 expression. Additionally, tumour lymphocyte infiltration score was 2 or higher or 3 or higher by immunohistochemistry in most patients, including six (66%) of nine patients with ASPS, and 12 (63%) of 19 without ASPS. However, neither PD-L1 positivity nor increased tumour-infiltrating lymphocyte score correlated with progression-free survival of longer than 6 months or achieving a partial response (appendix p 12). Baseline neutrophil-to-lymphocyte ratio of more than 5 was associated with progressive disease (p=0·027). Increased baseline plasma angiogenic activity correlated with best response of partial response or stable disease (p=0·042) and progression-free survival for longer than 6 months

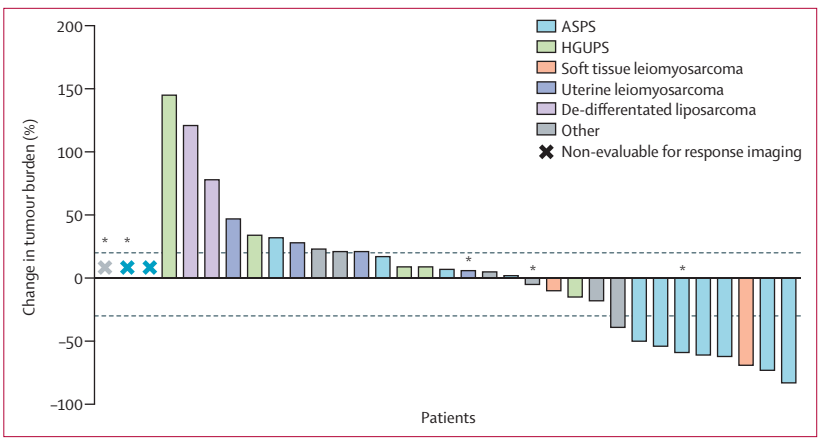

Figure 3: **Change from baseline in tumour burden**
Each bar represents one patient (n=30). Three patients were removed from the study before radiographic imaging assessments; two for clinical progression, and one for toxicity. Dashed lines indicate RECIST criteria for progressive disease (+20%) or partial response (–30%). ASPS=alveolar soft part sarcoma. HGUPS=high-grade undifferentiated pleomorphic sarcoma. RECIST=response evaluation criteria in solid tumors. *These patients met criteria for progression due to non-target lesion progression, emergence of new lesions, or clinical deterioration; bars represent best change in dimension of target lesions.

(p=0·0083; appendix p 12). Previous tyrosine-kinase inhibitor exposure did not affect responses to axitinib plus pembrolizumab (appendix pp 13–14).

To assess whether pseudoprogression or changes in metabolic activity could be early indicators of treatment response, we compared concordance of the proportion of patients who achieved an objective response by Choi criteria, irRC, and PERCIST to the proportion when assessed using RECIST (appendix pp 15–16). Ten (48%) of 21 patients without ASPS showed necrotic or cystic changes on CT imaging along with increased tumour size, of whom eight met Choi criteria for partial response. The proportion who achieved an objective response was significantly higher using Choi criteria (13 [65%] of 20; 95% CI 43·2–82·0) than when using RECIST (five [25%] of 20; 95% CI 10·8–47·2; p=0·026) with poor concordance (linear weighted κ=0·18). No patients with a partial response determined by Choi criteria achieved RECIST responses despite these early necrotic changes. The proportion of patients who achieved an objective response determined by RECIST was highly concordant with that determined by irRC (κ=0·89; appendix pp 15–16), and consistent with previous studies.[11,12] We did note discrepancies between RECIST and PERCIST at 3 months (κ=0·42; appendix p 15). Importantly, six (60%) of ten patients with ASPS with PET scans had progressive metabolic disease as determined by PERCIST at 3 months, while three (30%) had stable metabolic response and one (10%) had a partial metabolic response. Four of six patients with ASPS with progressive metabolic disease and one of three with stable metabolic response ultimately achieved partial response as defined by RECIST. By contrast, all patients without ASPS with progressive metabolic disease as determined by PERCIST at 3 months also had RECIST progressive disease or

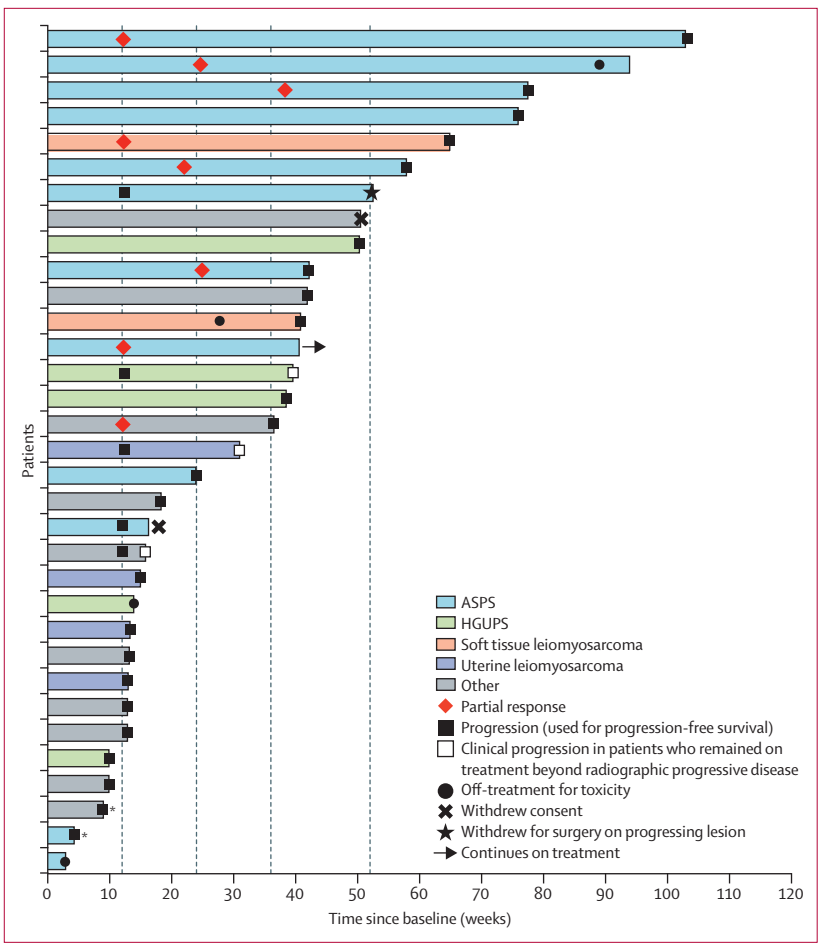

Figure 4: **Duration of responses**
Each bar represents one patient on study (n=33) and dashed lines indicate 12, 24, 36, and 52 weeks. Black boxes are the timepoint at which radiographic progression occurred that was used in analysis. Three patients continued on therapy after radiographic progression due to clinical benefit or changes in tumour appearance suggestive of necrosis. Patients without partial response or progression noted achieved stable disease during interim scans. *Did not have radiographic confirmation of progression. ASPS=alveolar soft part sarcoma. HGUPS=high-grade undifferentiated pleomorphic sarcoma.

clinical progression, and five of six patients who achieved PERCIST partial metabolic response also achieved partial response or stable disease as defined by RECIST (appendix pp 15–16).

ASPS comprises only 1% of soft tissue sarcomas and often exhibits more indolent progression than more common sarcoma subtypes.[2] To assess whether the high proportion of patients with ASPS in our study population was skewing our primary endpoint analysis compared with historical controls, we did a post-hoc analysis to assess progression-free survival, overall survival, and the proportion of patients who achieved an overall response among patients with ASPS and without ASPS (figure 5; appendix pp 6–8). By intention-to-treat analysis, 3-month progression-free survival in patients with ASPS (n=12) was 72·7% (95% CI 37·1–90·3); however, patients without ASPS (n=21) still achieved a 3-month progression-free survival of 61·9% (95% CI 38·1–78·8). 6-month and 12-month progression-free survival in the subset of patients without ASPS were also favourable at (6 month 38·1%, 95% CI 18·3–57·8, and 12 month 14·3%, 3·6–32·1). Median progression-free survival was 12·4 months (95% CI 2·7–22·3) in patients with ASPS compared with 3·0 months (3·0–8·6) in those without ASPS (figure 5). A lower proportion of patients with non-ASPS subtypes achieved an objective response during the study treatment (ie, best response), with partial response in two of 21 patients (9·5%, 95% CI 1·7–31·8), compared with six of 11 patients with ASPS (54·5%, 95% CI 24·6–81·9). However, seven of 21 patients without ASPS achieved stable disease, such that the proportion who achieved a clinical benefit as best response was 42·9% (n=9; 95% CI 22·6–65·6), and at 6 months this proportion was 35·0% (seven of 20, 95% CI 16·3–69·1). Point estimates of progression-free survival and objective responses by ITT and per-protocol analysis are reported in appendix (pp 6–8). Per protocol, 3-month progression-free survival in patients with ASPS (n=10) was 80·0% (95% CI 40·9–90·3) compared with 65·0% (n=20, 95% CI 40·3–81·5) in patients without ASPS. Best objective response was partial response in six (60·0%, 95% CI 27·4–86·3) of ten evaluable patients with ASPS, compared with two (10·0%, 95% CI 18·0–33·1) of 20 without ASPS.

Discussion

To our knowledge, this is the first reported trial of a combined VEGF and PD-1 blockade in sarcoma. 3-month progression-free survival for all patients who were given treatment was 65·6% (95% CI 46·6–79·3), exceeding the historical benchmark (19%) required to suggest meaningful clinical activity of a second-line regimen and so warranting future investigation.[25] Accrual of patients with ASPS was higher than anticipated (n=12) given the rarity of the disease, accounting for 36% of the enrolled patients. The median progression-free survival of the study population without ASPS who were treated with axitinib plus pembrolizumab was similar to the median progression-free survival of axitinib monotherapy (3·0 months in leiomyosarcoma and other sarcoma cohorts [ie, high-grade undifferentiated pleomorphic sarcoma, liposarcomas, and ASPS])[9] and similar to other chemotherapy regimens used in the second-line setting, such as pazopanib (median progression-free survival 4·6 months, 95% CI 3·7–4·8).[10] The proportion of patients in the non-ASPS subset who were treated with axitinib plus pembrolizumab who achieved an objective response was slightly higher than among similar patients treated with axitinib monotherapy (0% [leiomyosarcoma] and 4% [other soft tissue sarcomas]),[9] nivolumab monotherapy (5%),[12] and pazopanib (6%).[10]

Although our observed median progression-free survival and modest proportion of patients who achieved an objective response argued against the benefit of addition of pembrolizumab over axitinib monotherapy, we were

intrigued to note that the patients without ASPS who did not rapidly progress by 3 months appeared to derive meaningful benefit at later timepoints. Progression-free survival at 6 months in patients in the non-ASPS subset who were given axitinib plus pembrolizumab was 38·1%, and compared favourably with similar patients who have undergone axitinib monotherapy (25–30% in leiomyosarcoma and other soft tissue sarcoma cohorts).[9] Similarly, a favourable proportion of patients in the non-ASPS subset who were given axitinib plus pembrolizumab had a clinical benefit at 6 months (35%) compared with the proportion of patients with sarcomas treated with nivolumab monotherapy (10% at 6 months) and nivolumab plus ipilimumab (12% at 6 months) in a previous study.[12] Increasing evidence supports that traditional endpoints designed to assess efficacy of cytotoxic drugs, such as median progression-free survival, might underestimate clinical benefit in immunotherapy clinical trials because antitumour effects from immune checkpoint inhibitors can take time to manifest.[29] Although these observations and cross-study comparisons are speculative, we believe that consideration of a future randomised trial is warranted.

Axitinib plus pembrolizumab was most effective in patients with progressive ASPS. The dependency of ASPS on VEGF is well documented, with median progression-free survival ranging from 10·8 months to 18·2 months with different broad-spectrum tyrosine-kinase inhibitors.[4–7,30] Although the median progression-free survival of patients with ASPS given axitinib plus pembrolizumab (12·4 months) was similar to when analogous patients have been given other tyrosine-kinase inhibitors, the proportion who achieved an objective response with combination therapy exceeded the highest previously reported proportion of 35% with cediranib.[4] No objective responses were reported in all four patients with ASPS who were given axitinib monotherapy,[9] making it less likely that our observed outcomes could be attributed to axitinib alone. Additionally, four of five patients with ASPS who achieved partial response in this study had not achieved partial response with at least one previous broad-spectrum tyrosine-kinase inhibitor (appendix p 13). Further supporting the contribution of the PD-1 blockade, preliminary data from an ongoing study[31] of 19 patients with ASPS treated with the PD-L1 inhibitor atezolizumab reported that 42% (n=8) of patients achieved an objective response. Although our results are promising, benefit from combination VEGF and PD-1 inhibition for ASPS can only be confirmed with a randomised study including a monotherapy group.

Despite the apparent sensitivity of ASPS to immune checkpoint inhibitors, the mechanism behind this response remains unknown. All patients with ASPS who had biopsy samples in our study expressed PD-L1, and most had high tumour lymphocyte infiltration scores, consistent with the so-called inflamed phenotype observed in melanoma and other cancers that respond to immune checkpoint inhibitors. High tumour mutational burden (as observed in cancers deficient in mismatch repair) has been associated with high proportions of patients with a response to immune checkpoint inhibitors. However, in our clinical experience and reported by others,[32] patients with ASPS have minimal mutations apart from the conserved translocation. A neo-epitope arising from the ASPL-TFE3 fusion protein itself has been speculated to be immunogenic.[33] Overall, ASPS remains a puzzling example of an immunoresponsive tumour that appears to lack mutation-associated neo-antigen complexity.

Biomarkers to identify patients likely to respond to immune checkpoint inhibitors remain elusive in sarcomas. We observed that a high baseline neutrophil-to-lymphocyte ratio was associated with progression of disease. Increased neutrophil-to-lymphocyte ratio has been associated with increased post-surgical recurrence in extremity sarcomas and progression of metastatic

	Grade 1 or 2	Grade 3	Grade 4
Fatigue	26 (79%)	..	..
Oral mucositis	22 (67%)	1 (3%)	..
Hypothyroidism or hyperthyroidism	21 (64%)	..	..
Nausea or vomiting	20 (61%)	2 (6%)	..
Nasopharyngeal congestion	18 (55%)	..	..
Diarrhoea	18 (55%)	1 (3%)	..
Elevated ALT, AST, or AP	17 (51%)	..	1 (3%)
Abdominal pain or dyspepsia	16 (48%)	1 (3%)	..
Tumour pain	15 (45%)	..	..
Arthralgia or myalgia	15 (45%)	..	..
Palmar-plantar erythrodysesthesia syndrome	15 (45%)	..	..
Hypertension	11 (33%)	5 (15%)	..
Anorexia or weight loss	12 (36%)	..	..
Cough	11 (33%)	..	..
Rash or pruritis or dry skin	9 (27%)	..	..
Constipation	9 (27%)	..	..
Mucositis rectal or vaginal inflammation	6 (18%)	..	..
Creatinine or BUN increased	6 (18%)	..	..
Haemoglobin increased	5 (15%)	..	..
Headache	5 (15%)	..	..
Haemoptysis	2 (6%)	1 (3%)	..
Hypertriglyceridemia or hyperlipidaemia	2 (6%)	..	1 (3%)
Pneumothorax	..	1 (3%)	..
Seizures*	..	2 (6%)	..
Autoimmune toxic effects	4 (12%)	5 (15%)	..
Hyperglycaemia	4 (12%)	1 (3%)	..
Autoimmune hepatitis	..	1 (3%)	..
Autoimmune colitis	..	1 (3%)	..
Autoimmune arthritis	..	2 (6%)	..

Data are n (%). Grade 1 and 2 events are reported here if they occurred in over 10% of patients. All grade 3 and 4 and autoimmune adverse events are shown. No treatment-related deaths occurred. Nasopharyngeal congestion includes nasal congestion, rhinorrhea, ear pain, or hoarseness. ALT=alanine aminotransferase. AST=aspartate aminotransferase. AP=alkaline phosphatase. BUN=blood urea nitrogen. *Two patients with alveolar soft part sarcoma and brain metastases previously treated with radiation therapy had seizures on therapy.

Table 2: **Treatment-related adverse events**

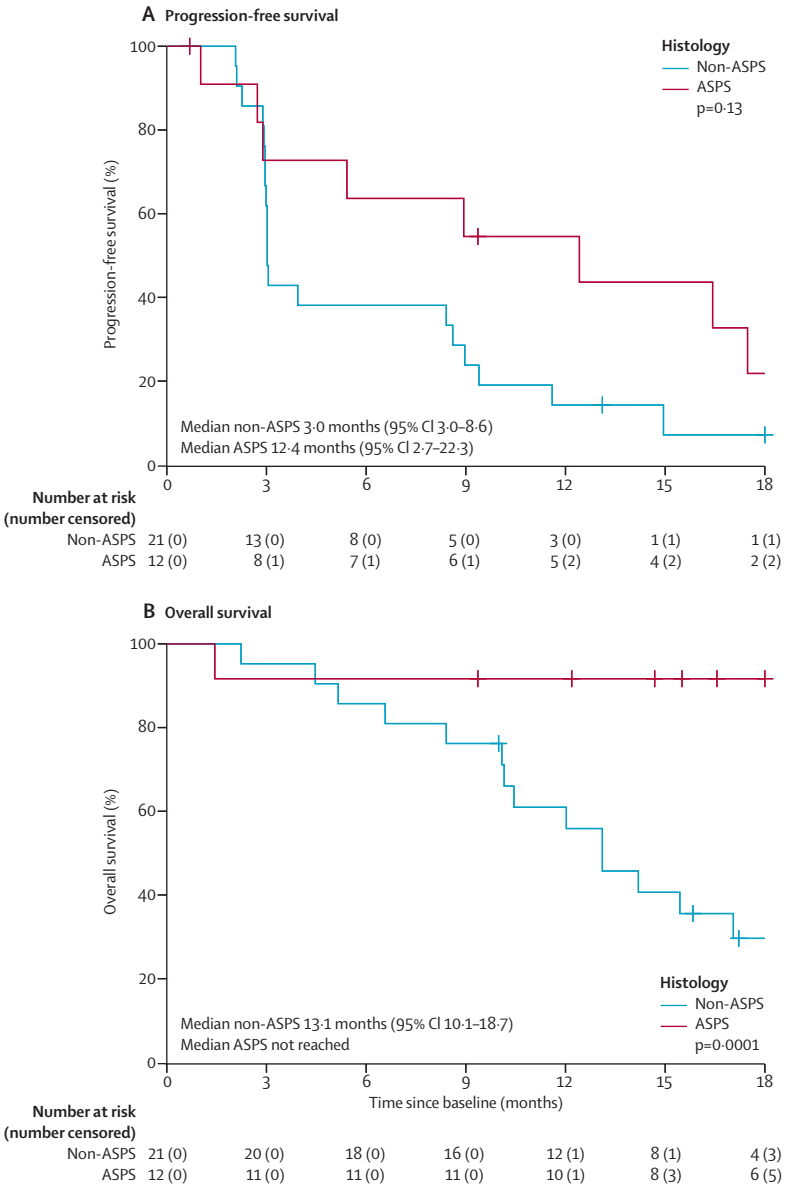

Figure 5: **Post-hoc analysis of progression-free survival (A) and overall survival (B) by ASPS status**
Kaplan-Meier estimates by intention-to-treat analysis (n=33). Patients who remained event-free were censored at the time of last imaging or withdrawal of consent. We compared curves from ASPS and non-ASPS populations using the log-rank test. Crosses indicate censored patients. ASPS=alveolar soft part sarcoma. NE=non-estimable.

disease, but also with inferior progression-free survival and overall survival in patients with sarcoma treated with immune checkpoint inhibitors.[34–36] Further investigation will be required to determine if neutrophil-to-lymphocyte ratio is predictive specifically for immunotherapy or prognostic regardless of chosen therapy. We also noted that patients with higher baseline plasma angiogenic activity were more likely to respond to axitinib plus pembrolizumab than those with lower baseline plasma angiogenic activity. Without monotherapy controls, we cannot confirm a link between sarcoma neovascularity and antitumour immunity, or whether plasma angiogenic activity is simply a marker for sensitivity to VEGF blockade. Preclinical experiments in sarcoma mouse models are planned to determine the effects on the tumour microenvironment of VEGF blockade with and without PD-1 inhibition, to provide an additional rationale for future randomised studies. Additionally, analysis of tumour infiltrating and circulating immune components, cytokines, and gene expression in patients treated with axitinib plus pembrolizumab are ongoing and might help elucidate additional mechanisms of response and resistance.

Although several of our patients showed substantial tumour density changes with axitinib plus pembrolizumab suggestive of pseudoprogression, we did not find evidence that alternative radiographic criteria could predict later RECIST responses in patients without ASPS. However, we did note that metabolic progression by combination PET-CT imaging at 3 months was frequent in patients with ASPS, and did not preclude subsequent RECIST defined responses. This finding suggests that early inflammatory changes that occur in responding ASPS tumours or even in new lesions could be mistaken for tumour progression, particularly given the extended time required to reach response.

Our study had several important limitations. Our sample size was small, and was calculated on the basis of outcomes from a historical database[25] of patients with sarcoma refractory to or intolerant of doxorubicin. Our study population might have selection bias because it contained many patients with ASPS, lacked patients with other indolent histologies such as chordoma or clear cell sarcoma, and included several patients without ASPS who had refused doxorubicin therapy previously. Generalisability of the study population is further limited by the nuances of specific inclusion and exclusion criteria (eg, requirement for disease amenable to biopsy, optimal performance status, and exclusion of patients with previous autoimmune, infectious, or inflammatory conditions). As a result, the primary and post-hoc analyses are probably underpowered, and our observations must be viewed as preliminary and as a means to generate hypotheses. Moreover, this was a single-institution, single-arm study without central radiology review, and without a randomised study we cannot attribute observed outcomes to the combination over what could be expected from either drug alone.

Overall, the combination of VEGF receptor and PD-1 blockade showed acceptable toxic effects and preliminary activity in ASPS and other advanced sarcomas that warrant additional investigation through a randomised trial. Although intrapatient dose escalation of axitinib was feasible, only a few patients tolerated doses higher than 5 mg twice daily without an obvious increase in tumour response. To avoid additional toxic effects, we conclude that axitinib dosing at 5 mg twice daily with dose reductions as required is probably sufficient

for future investigations. Since most patients with sarcoma who respond to axitinib plus pembrolizumab ultimately progressed, combining immune checkpoint inhibitors with chemotherapy, radiation, adoptive cellular therapies, or metabolic therapies to counteract other resistance mechanisms might be required for optimal and durable immune response in sarcoma. Further investigation of the sarcoma immune microenvironment will be crucial to better understand resistance mechanisms and biomarkers of response and to better tailor the next generation of immunotherapy clinical trials for sarcomas.

Contributors

BAW, DKw, JCT, EDW, KVK, and JRM designed the study. BAW, MMT, TKS, DKw, ES, MF, and JCT analysed the clinical data and interpreted the data. BAW, TKS, VF, WP, JRM, AER, DKw, DAK, DKo, EDW, and KVK participated in design, collection, analysis, or a combination of these, of correlative samples. BAW, MMT, JCT, WP, TKS, and DKw wrote the manuscript. All authors reviewed and gave approval for the final manuscript.

Declaration of interests

BAW reports research funding from Merck and Pfizer pertaining to this study, and research funding from Agenus Bio and personal fees from Lilly and Immune Design outside of the submitted work. TKS reports research support from Merck and Pfizer pertaining to this study and personal fees from iiCME, Agios Pharmaceuticals, Arog Pharmaceuticals, and Toshiba America Medical Systems outside of the submitted work. ES reports research funding from Merck pertaining to this study. JRM reports grants from Genentech, Novartis, Eisai, Peloton, Calithera, Vyriad, Tocagen, REPlimune, Sillagen, Seattle Genetics, Astellas, Rexahn, Lilly, Corvus, and Exelixis outside of the submitted work. KVK reports personal fees from Merck, Kite/Gilead, Novartis, Atara, Kiadis, Celgene/Juno, and Incyte outside of the submitted work. All other authors declare no competing interests.

Data sharing

De-identified individual participant data that underlie the results reported in this Article and the study protocol will be available from 3 years after publication due to ongoing research, and subject to sponsor approval. Data will be made available to investigators who develop methodologically sound proposals for comparison with other immunotherapy studies. For access, contact Breelyn Wilky at breelyn.wilky@ucdenver.edu.

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