Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial

Hussein A Tawbi, Melissa Burgess, Vanessa Bolejack, Brian A Van Tine, Scott M Schuetze, James Hu, Sandra D'Angelo, Steven Attia, Richard F Riedel, Dennis A Priebat, Sujana Movva, Lara E Davis, Scott H Okuno, Damon R Reed, John Crowley, Lisa H Butterfield, Ruth Salazar, Jaime Rodriguez-Canales, Alexander J Lazar, Ignacio I Wistuba, Laurence H Baker, Robert G Maki, Denise Reinke, Shreyaskumar Patel

Summary

Background Patients with advanced sarcomas have a poor prognosis and few treatment options that improve overall survival. Chemotherapy and targeted therapies offer short-lived disease control. We assessed pembrolizumab, an anti-PD-1 antibody, for safety and activity in patients with advanced soft-tissue sarcoma or bone sarcoma.

Methods In this two-cohort, single-arm, open-label, phase 2 study, we enrolled patients with soft-tissue sarcoma or bone sarcoma from 12 academic centres in the USA that were members of the Sarcoma Alliance for Research through Collaboration (SARC). Patients with soft-tissue sarcoma had to be aged 18 years or older to enrol; patients with bone sarcoma could enrol if they were aged 12 years or older. Patients had histological evidence of metastatic or surgically unresectable locally advanced sarcoma, had received up to three previous lines of systemic anticancer therapy, had at least one measurable lesion according to the Response Evaluation Criteria In Solid Tumors version 1.1, and had at least one lesion accessible for biopsy. All patients were treated with 200 mg intravenous pembrolizumab every 3 weeks. The primary endpoint was investigator-assessed objective response. Patients who received at least one dose of pembrolizumab were included in the safety analysis and patients who progressed or reached at least one scan assessment were included in the activity analysis. Accrual is ongoing in some disease cohorts. This trial is registered with ClinicalTrials.gov, number NCT02301039.

Findings Between March 13, 2015, and Feb 18, 2016, we enrolled 86 patients, 84 of whom received pembrolizumab (42 in each disease cohort) and 80 of whom were evaluable for response (40 in each disease cohort). Median follow-up was 17·8 months (IQR 12·3–19·3). Seven (18%) of 40 patients with soft-tissue sarcoma had an objective response, including four (40%) of ten patients with undifferentiated pleomorphic sarcoma, two (20%) of ten patients with liposarcoma, and one (10%) of ten patients with synovial sarcoma. No patients with leiomyosarcoma (n=10) had an objective response. Two (5%) of 40 patients with bone sarcoma had an objective response, including one (5%) of 22 patients with osteosarcoma and one (20%) of five patients with chondrosarcoma. None of the 13 patients with Ewing's sarcoma had an objective response. The most frequent grade 3 or worse adverse events were anaemia (six [14%]), decreased lymphocyte count (five [12%]), prolonged activated partial thromboplastin time (four [10%]), and decreased platelet count (three [7%]) in the bone sarcoma group, and anaemia, decreased lymphocyte count, and prolonged activated partial thromboplastin time in the soft-tissue sarcoma group (three [7%] each). Nine (11%) patients (five [12%] in the bone sarcoma group and four [10%] in the soft-tissue sarcoma group) had treatment-emergent serious adverse events (SAEs), five of whom had immune-related SAEs, including two with adrenal insufficiency, two with pneumonitis, and one with nephritis.

Interpretation The primary endpoint of overall response was not met for either cohort. However, pembrolizumab showed encouraging activity in patients with undifferentiated pleomorphic sarcoma or dedifferentiated liposarcoma. Enrolment to expanded cohorts of those subtypes is ongoing to confirm and characterise the activity of pembrolizumab.

Funding Merck, SARC, Sarcoma Foundation of America, QuadW Foundation, Pittsburgh Cure Sarcoma, and Ewan McGregor.

Introduction

Sarcomas, which are broadly categorised as soft-tissue sarcomas or bone sarcomas, represent a heterogeneous group of mesenchymal malignancies with more than 50 histological subtypes.[1] Studies of sarcomas and therapeutic outcomes are limited by their rarity and heterogeneity. The median overall survival is around 2 years for advanced leiomyosarcoma but less than 1 year for most other advanced soft-tissue sarcomas, and only about 10% of patients are alive at 5 years.[2] Similarly, adult patients with metastatic bone sarcomas have a 5-year overall survival of less than 25%.[3,4]

Treatment options are few and generally palliative, while the expected benefits are tempered by a high rate of severe side-effects. Response to conventional chemotherapy and radiotherapy is dependent on the

Lancet Oncol 2017; 18: 1493–1501

Published Online October 4, 2017 http://dx.doi.org/10.1016/S1470-2045(17)30624-1

This online publication has been corrected. The corrected version first appeared at thelancet.com/oncology on November 29, 2017

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University of Texas MD Anderson Cancer Center, Houston, TX, USA (H A Tawbi MD, R Salazar MD, J Rodriguez-Canales MD, Prof A J Lazar MD, Prof I I Wistuba MD, Prof S Patel MD); **University of Pittsburgh, Pittsburgh, PA, USA** (M Burgess MD, Prof L H Butterfield PhD); **Cancer Research and Biostatistics, Seattle, WA, USA** (V Bolejack MPH, Prof J Crowley PhD); **Washington University School of Medicine, St Louis, MO, USA** (B A Van Tine MD); **University of Michigan, Ann Arbor, MI, USA** (S M Schuetze MD, Prof L H Baker DO); **University of Southern California, Los Angeles, CA, USA** (J Hu MD); **Memorial Sloan Kettering Cancer Center, New York, NY, USA** (S D'Angelo MD); **Mayo Clinic, Jacksonville, FL, USA** (S Attia DO, Prof S H Okuno MD); **Duke University, Durham, NC, USA** (R F Riedel MD); **Washington Cancer Institute, MedStar Washington Hospital Center, Washington, DC, USA** (D A Priebat MD); **Fox Chase Cancer Center, Philadelphia, PA, USA** (S Movva MD); **Oregon Health & Science University, Portland, OR, USA** (L E Davis MD); **Moffitt Cancer Center, Tampa, FL, USA** (D R Reed MD); **Hofstra Northwell School of Medicine, Hempstead, NY, USA**

(Prof R G Maki MD); **and Sarcoma Alliance for Research Through Collaboration, Ann Arbor, MI, USA** (D Reinke NP)

Correspondence to:
Dr Hussein A Tawbi, Department of Melanoma Medical Oncology and Department of Investigational Cancer Therapeutics, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
htawbi@mdanderson.org

Research in context

Evidence before this study

We searched PubMed up to May 5, 2017, with the terms "metastatic sarcoma", "clinical trials", "PD-1", "PD-L1", "immune checkpoint blockade", "immune response", "pembrolizumab", and "nivolumab" for articles published in English. Patients with metastatic soft-tissue sarcoma or bone sarcoma have a poor prognosis and low survival. First-line treatment of soft-tissue sarcoma with single-agent chemotherapy remains the standard of care and leads to a response in 10–15% of patients. Combination therapies have little or no effect on overall survival in patients with soft-tissue sarcoma, while consistently increasing the incidence of adverse events. Treatment of bone sarcoma with combination chemotherapy in the neoadjuvant and adjuvant setting can be curative despite the substantial toxicity. However, in patients with advanced bone sarcomas, no existing therapeutic options offer a survival advantage. Sarcoma has not traditionally been considered an immunogenic tumour; however, several studies showed PD-L1 to be expressed in up to 30–40% of certain sarcoma subtypes. Anecdotal reports and retrospective series studies have described responses to immune checkpoint blockade in select sarcomas. One phase 2 study of nivolumab in leiomyosarcoma was stopped early for futility, but no prospective studies have been published to date in other subtypes of soft-tissue sarcoma or bone sarcoma. Treatment with antibodies targeting the PD-1/PD-L1 axis led to durable responses in several cancers.

Added value of this study

To our knowledge, this prospective, multicentre, phase 2 study is the first to investigate the use of immune checkpoint blockade with anti-PD-1 antibodies in the treatment of soft-tissue sarcoma and bone sarcoma. We considered the heterogeneity of sarcomas and limited the study to specific histological subtypes to identify potential subtype-specific efficacy signals. We observed an objective response in seven (18%) of 40 patients with soft-tissue sarcoma, and the benefit was limited to patients with undifferentiated pleomorphic sarcoma and dedifferentiated liposarcomas. The characteristics of the responses were consistent with the durable benefit observed with checkpoint blockade in other cancers. We observed little to no benefit in patients with synovial sarcoma or leiomyosarcoma. Similarly, we observed infrequent responses to therapy in patients with bone sarcomas, suggesting the need for combination approaches in this population.

Implications of all the available evidence

These findings suggest that pembrolizumab is clinically active in patients with undifferentiated pleomorphic sarcoma or dedifferentiated liposarcoma. Expanded cohorts of these subtypes, which represent more than 30% of all soft-tissue sarcomas, are ongoing. Further investigation is required to determine the utility of predictive biomarkers for response and to understand the mechanisms of resistance in the other subtypes of soft-tissue sarcoma and bone sarcoma, in which rational combination therapies could be considered.

specific histology because some subtypes are chemoresistant. The past decade has seen collaborative investigation of novel drugs for the treatment of sarcoma in large randomised controlled clinical trials,[5–8] leading to approval of several drugs by the US Food and Drug Administration (FDA), including pazopanib, trabectedin, eribulin, and olaratumab. However, these therapies still do not have a substantial cure rate, prompting the need for development of other novel drugs.

Immunotherapy with adjuvant mifamurtide, a non-specific immune stimulator that was shown to improve overall survival in osteosarcoma in a phase 3 trial,[9] is already approved in some countries. The appeal of immunotherapy has increased as studies[10–14] of pembrolizumab, an anti-PD-1 antibody, have shown the benefits of immune checkpoint inhibition beyond melanoma; for example, pembrolizumab has shown therapeutic benefit in non-small-cell lung cancer, renal cell carcinoma, bladder cancer, Hodgkin's lymphoma, and Merkel cell carcinoma. However, except for mifamurtide, immunotherapy has had little therapeutic benefit in patients with soft-tissue sarcoma or bone sarcoma, with studies[9,15–17] using cytokines or immune adjuvants not achieving their primary endpoints.

Therefore, we aimed to determine the safety and activity of immune checkpoint blockade with pembrolizumab in patients with advanced soft-tissue sarcoma or bone sarcoma.

Methods

Study design and participants

SARC028 was a multicentre, two-cohort, open-label, phase 2 trial of pembrolizumab monotherapy, done at 12 academic medical centres in the USA that were members of the Sarcoma Alliance for Research through Collaboration (SARC; appendix p 11). Patients with soft-tissue sarcoma had to be aged 18 years or older to enrol; patients with bone sarcoma could enrol if they were aged 12 years or older. Eligible patients had histological evidence of metastatic or surgically unresectable locally advanced sarcoma, including one of several histological subtypes: leiomyosarcoma, poorly differentiated or dedifferentiated liposarcoma, undifferentiated pleomorphic sarcoma, synovial sarcoma, Ewing's sarcoma, osteosarcoma, and dedifferentiated or mesenchymal chondrosarcoma. The histological subtypes were chosen on the basis of prevalence; we decided to study the most common subtypes of soft-tissue sarcoma and bone

See Online for appendix

sarcoma given limitations of the available funding. Eligible patients also had disease measurable by CT or MRI (according to Response Evaluation Criteria In Solid Tumors [RECIST] version 1.1), at least one site of disease that was safely accessible for core biopsies before and during treatment, a life expectancy of more than 12 weeks, and an Eastern Cooperative Oncology Group performance status score of 0 or 1. Patients could have received up to three previous lines of systemic anticancer therapy.

Eligible patients had adequate kidney, liver, and bone marrow function tested within 14 days before first pembrolizumab administration. Key exclusion criteria were active brain metastases or any serious or uncontrolled medical disorder affecting study participation. Patients with an active autoimmune disease or syndrome, except for vitiligo or resolved childhood asthma or atopy, or requiring chronic use of steroids or immunosuppressive drugs, were also excluded. Previous treatment with anti-PD-1 or anti-PD-L1 antibodies was also not allowed. Treatment with chemotherapy, radiotherapy, biologics, or investigational therapy was not permitted within 28 days of first administration of pembrolizumab, and previous palliative radiotherapy had to have been completed at least 2 weeks (or 4 weeks if wide field) before pembrolizumab administration. This trial is registered with ClinicalTrials.gov, number NCT02301039.

The protocol (appendix p 10) was approved by the institutional review boards or independent ethics committees of each site and done according to Good Clinical Practice guidelines as per the International Conference on Harmonisation. Patients provided written informed consent on the basis of the Declaration of Helsinki principles.

Procedures

Patients received 200 mg pembrolizumab via 30 min intravenous infusion every 3 weeks until disease progression or unacceptable toxicity. Population pharmacokinetic studies have shown that a fixed dose of pembrolizumab has similar pharmacokinetic and pharmacodynamic profiles to weight-based dosing, and fixed dosing is the FDA-approved regimen for non-small-cell lung cancer, head and neck cancer, and other indications.

Disease was assessed using CT or MRI at baseline, after 8 weeks, and then every 12 weeks until disease progression. Response was determined by investigators using RECIST version 1.1. No central radiology review was done. Responses were confirmed with a second scan at least 4 weeks after criteria for objective response were met. Treatment beyond RECIST-defined progression was permitted if pembrolizumab was tolerated, and clinical benefit was noted on the basis of the investigator's assessment. No dose modifications were allowed, but dose delays up to 12 weeks were permitted for adverse events. Patients requiring treatment discontinuation because of adverse events were followed up until disease progression or initiation of subsequent therapy and at 30 days after the last dose of pembrolizumab. Safety assessments, including laboratory monitoring, were done during screening and on the first day of each cycle of therapy. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 during treatment and up to 30 days after treatment discontinuation.

Biopsies before and during treatment were required and obtained during screening (before the first drug administration) and after 8 weeks of therapy; biopsies were optional at the time of disease progression. We obtained blood for correlative analyses during screening, at the time of subsequent disease assessments (eg, after 8 weeks of treatment and then every 12 weeks), and at progression.

Haematoxylin and eosin slides from all tissue specimens were reviewed by three pathologists (RS, JR-C, and AJL) to identify the presence of malignant cells and to select the best representative tumour block from each patient. The slides were stained in a Leica Bond Max stainer (Leica Biosystems Nussloch GmbH, Nussloch, Germany). The primary antibody was PD-L1, clone 22C3 (Dako, Santa Clara, CA, USA; catalogue number M365329-1, dilution 1:50). PD-L1 staining was detected with diaminobenzidine counterstained with haematoxylin (Leica Bond Polymer Refine kit; Leica Biosystems). The slides were scanned in an Aperio AT2 scanner (Leica Biosystems). PD-L1 staining was assessed by three pathologists (RS, JR-C, and AJL), and the immunohistochemistry score was expressed as percentage of tumour cells positive for PD-L1 (ie, showing a distinct membranous staining). A tumour was considered positive for PD-L1 expression if more than 1% of its cells showed membranous staining. The final immunohistochemistry score was reviewed by a pathologist expert in soft-tissue tumours (AJL).

Outcomes

The primary endpoint was investigator-assessed objective response according to RECIST version 1.1. Objective response was defined as the proportion of patients in each cohort with a best overall response of complete or partial response. The duration of an objective response was measured from the time criteria were met for complete or partial response until the first date that recurrent or progressive disease was objectively documented. Secondary endpoints included objective response according to Immune-Related Response Criteria (irRC), incidence of adverse events and immune-related adverse events, progression-free survival (calculated from the first date of study treatment to the earliest date of progression or death; data for patients who did not have disease progression or died were censored at their date of last contact), and overall survival (first date of study treatment to death from any cause). Exploratory objectives included the correlation of PD-L1

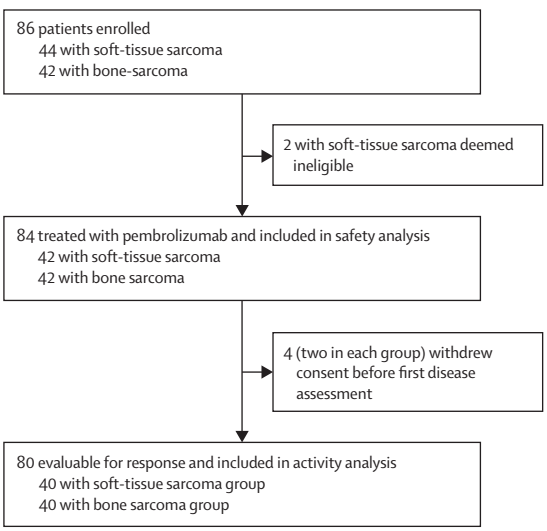

Figure 1: **Trial profile**

expression with clinical outcome and other biomarker analyses, including immune monitoring in peripheral blood and tumour tissues, for which data are still being analysed.

Statistical analysis

We hypothesised that pembrolizumab would lead to clinical benefit in patients with advanced soft-tissue sarcoma or bone sarcoma. A treatment success was defined as a partial response or better according to RECIST version 1.1. For each disease group, an objective response in 25% of patients was considered clinically meaningful, and a response in less than 10% of patients was considered ineffective. Patients with soft-tissue sarcoma were assigned to one of four cohorts on the basis of sarcoma subtype, with a preplanned goal of enrolment of ten patients per cohort. Enrolment of 40 patients with bone sarcoma was planned, but the number of patients with Ewing's sarcoma, osteosarcoma, or chondrosarcoma was not prespecified. Treatment was considered successful if eight or more of the 40 enrolled patients in each disease group had a partial response or better. This design had a one-sided type I error of 4·2% and a power of 82% to detect a difference in objective response between 10% and 25%. In advanced soft-tissue sarcoma, a regimen that improves the 12-week progression-free survival from 20% of patients to greater than 40% of patients is considered an active therapy.[18] We estimated that 40 patients would provide 87% power to detect an improvement in the 12-week progression-free survival from 20% to 40% of patients, with a one-sided type I error of 4%.

Patients who received at least one dose of pembrolizumab were included in the safety analysis. Patients who received at least one dose of treatment and had either disease progression or had undergone at least one disease assessment were considered evaluable and included in assessment of the primary endpoint.

We summarised the objective response on the basis of the best response while on study treatment. We used the Kaplan-Meier method to estimate overall and progression-free survival.[19] We calculated two-sided 95% CIs using the method described by Brookmeyer and Crowley[20] for median values and using standard methods for point estimates. Two-sided 95% CIs for objective response and progression-free survival were calculated with Fisher's exact method. Analyses by histological subtype, as well as correlative analyses, were exploratory. All statistical analyses were done with SAS version 9.4.

This trial is registered with ClinicalTrials.gov, number NCT02301039.

Role of the funding source

The study drug and partial funding was provided by Merck. The funder otherwise had no role in study design, data collection, data analysis, data interpretation, or in the writing of this report. The funder reviewed the manuscript before submission. Raw data were accessible to all authors. All authors made the decision to submit the report for publication.

	Bone sarcoma (n=42)	Soft-tissue sarcoma (n=42)
Age (years)	33 (16–70; 22–48)	53 (18–81; 45–63)
Sex		
Female	16 (38%)	15 (36%)
Male	26 (62%)	27 (64%)
Previous therapies		
One	8 (19%)	8 (19%)
Two	16 (38%)	17 (41%)
Three	18 (43%)	17 (41%)
Previous treatment in metastatic setting		
No	16 (38%)	20 (48%)
Yes	26 (62%)	22 (52%)

Data are n (%) or median (range; IQR).

Table 1: **Demographic and clinical characteristics of 84 eligible, treated patients**

Results

Between March 13, 2015, and Feb 18, 2016, 86 patients were enrolled in the study and 84 patients were treated with pembrolizumab (figure 1). 40 patients with soft-tissue sarcoma were evaluable for response and assigned to one of four disease subtypes (n=10 per subtype): undifferentiated pleomorphic sarcoma, dedifferentiated liposarcoma, synovial sarcoma, and leiomyosarcoma. 40 patients with bone sarcoma were evaluable for response, of whom 22 had osteosarcoma, 13 had Ewing's sarcoma, and five had dedifferentiated chondrosarcoma. The data cutoff date was March 1, 2017, and median follow-up was 17·8 months (IQR 12·3–19·3); 19·1 months

(13·4–20·5) for patients with soft-tissue sarcoma and 16·6 months (7·6–18·8) for patients with bone sarcoma. Demographic and clinical characteristics are shown in table 1.

Activity data are presented for 80 patients evaluable for the primary endpoint (figure 1). Seven (18%, 95% CI 7–33) of 40 patients with soft-tissue sarcoma achieved an objective response (figure 2, table 2). One (10%) patient with undifferentiated pleomorphic sarcoma—a woman aged 50 years with primarily pulmonary lesions whose response lasted for longer than 13 months—achieved a confirmed complete response. Responses in patients with soft-tissue sarcoma were generally durable, with a median duration of 33 weeks (IQR 23–49) and with some responses still ongoing at the time of this analysis (figure 3; appendix p 2).

In the bone sarcoma group, a confirmed partial response was observed in one (5%) of 22 patients with osteosarcoma and in one (20%) of five patients with chondrosarcoma (table 2, figure 4). No patient with Ewing's sarcoma had an objective response. The median duration of response was 43 weeks (IQR 25–61; figure 5; appendix p 2).

Response assessment with irRC was generally concordant with RECIST in the soft-tissue sarcoma cohort; however, two patients with stable disease according to RECIST were classified as having a partial response by irRC and two patients classified as having a partial response by RECIST had stable disease according to irRC (appendix p 2). The number of patients who had an objective response remained the same after assessment with irRC, and no patient who discontinued therapy secondary to disease progression according to RECIST was subsequently determined to be responding by irRC. Only one patient with Ewing's sarcoma who was classified as having stable disease with RECIST was reclassified as having a partial response with irRC (appendix p 2).

37 (93%) of 40 evaluable patients with soft-tissue sarcoma had a progression event (ie, progressed or died), and median progression-free survival was 18 weeks (95% CI 8–21; appendix p 3). 12-week progression-free survival was 55% (95% CI 40–70), which was significantly higher than the threshold of 40% expected from an active regimen in patients with soft-tissue sarcoma (p=0·039). Median progression-free survival was 30 weeks (95% CI 8–68) for patients with undifferentiated pleomorphic sarcoma (seven [70%] of whom had a progression event), and 12-week progression-free survival was 70% (42–98). In ten patients with liposarcoma (all of whom had a progression event), the median progression-free survival was 25 weeks (95% CI 8–42), and the 12-week progression-free survival was 60% (30–90). The median overall survival for patients with soft-tissue sarcoma was 49 weeks (95% CI 34–73); 25 patients died because of disease progression (appendix p 3). The median overall survival for patients with undifferentiated pleomorphic sarcoma had not been reached at the time of this analysis; four patients had died.

38 (95%) of 40 patients with bone sarcoma had a progression event; data for one (3%) patient has been censored. The median progression-free survival was 8 weeks (95% CI 7–9; appendix p 5). 25 (63%) patients with bone sarcoma died, all because of disease progression; the median overall survival was 52 weeks (95% CI 40–72). The median overall survival was not reached in patients with chondrosarcoma. Overall survival and progression-free survival for all subtypes are shown in the appendix (pp 3–6).

Tumour biopsies were safely obtained from 78 (93%) of 84 patients before treatment and from 68 (81%) of 84 patients during treatment (all of whom had matching pretreatment samples). Of the 78 samples obtained before treatment, 70 (90%) passed quality control for presence of tumour tissue and were successfully analysed for pretreatment PD-L1 expression. PD-L1 was positive at the 1% threshold in only three (4%) of the 70 samples; all three were from patients with undifferentiated

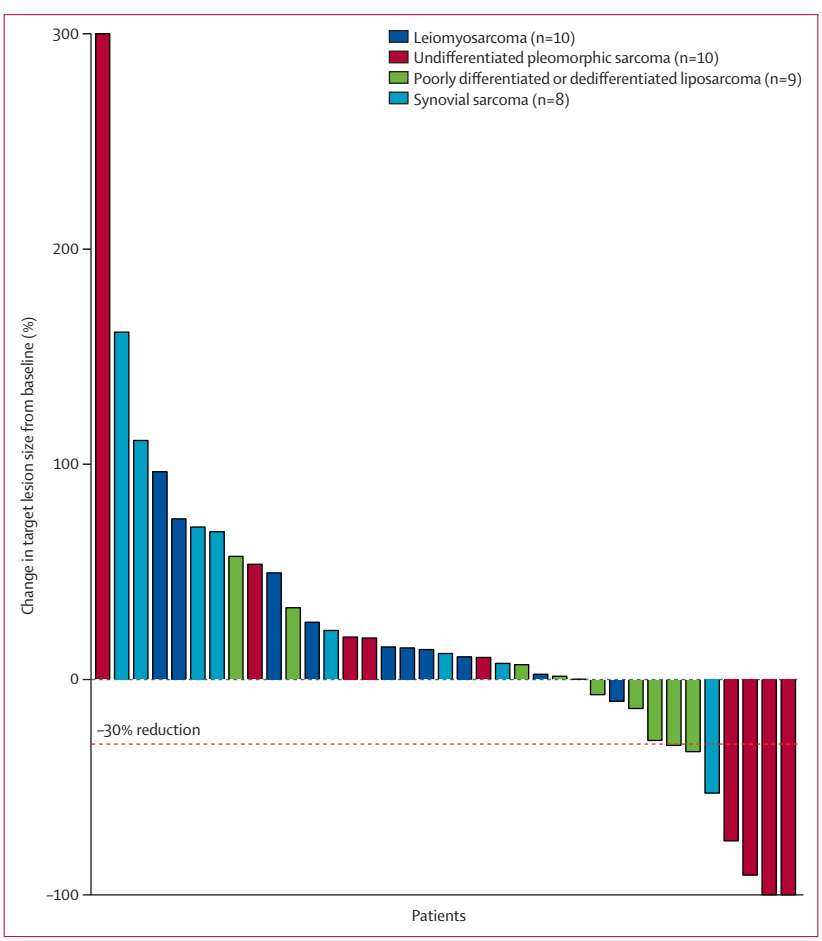

Figure 2: **Best percentage change from baseline in size of target lesions in patients with soft-tissue sarcoma**
Tumour sizes were calculated as the sum of target lesion diameters. Data for two patients with synovial sarcoma and one patient with liposarcoma are not shown because they did not have a second scan.

	Complete response	Partial response	Stable disease	Progressive disease
Soft-tissue sarcomas (n=40)	1 (3%)	6 (15%)	15 (38%)	18 (45%)
Leiomyosarcoma (n=10)	0 (0%)	0 (0%)	6 (60%)	4 (40%)
Undifferentiated pleomorphic sarcoma (n=10)	1 (10%)	3 (30%)	3 (30%)	3 (30%)
Liposarcoma (n=10)	0 (0%)	2 (20%)	4 (40%)	4 (40%)
Synovial sarcoma (n=10)	0 (0%)	1 (10%)	2 (20%)	7 (70%)
Bone sarcomas (n=40)	0 (0%)	2 (5%)	9 (23%)	29 (73%)
Chondrosarcoma (n=5)	0 (0%)	1 (20%)	1 (20%)	3 (60%)
Ewing's sarcoma (n=13)	0 (0%)	0 (0%)	2 (15%)	11 (85%)
Osteosarcoma (n=22)	0 (0%)	1 (5%)	6 (27%)	15 (68%)

Data are n (%).

Table 2: **Best response in 80 evaluable patients by sarcoma histological subtype**

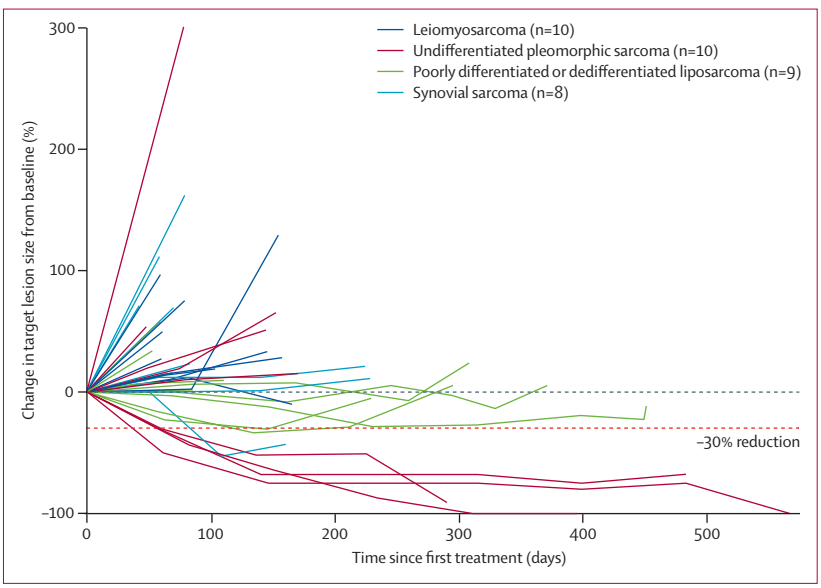

Figure 3: **Percentage change in tumour size from baseline at each assessment for patients with soft-tissue sarcoma**

Tumour sizes were calculated as the sum of target lesion diameters. Data for two patients with synovial sarcoma and one patient with liposarcoma are not shown because they did not have a second scan.

pleomorphic sarcoma. Of the three patients, only two were evaluable for response: one had a complete response and the other had a partial response.

At the time of the analysis, only two (2%) of 84 patients were still receiving pembrolizumab, including one patient with osteosarcoma who discontinued the study but is receiving pembrolizumab on a compassionate-access basis. The most common reason for treatment discontinuation was disease progression or death (35 [83%] of 42 patients with soft-tissue sarcoma and 33 [79%] of 42 patients with bone sarcoma). Five (6%) patients discontinued treatment secondary to toxicity, including two patients with bone sarcoma (one had interstitial nephritis and one had pneumonitis) and three patients with soft-tissue sarcoma (two had adrenal insufficiency and one had pneumonitis). All patients had an adverse event; the most frequent grade 3 or worse adverse events were anaemia (six [14%]), decreased lymphocyte count (five [12%]), prolonged activated partial thromboplastin time (four [10%]), and decreased platelet count (three [7%]) in the bone sarcoma group and anaemia, decreased lymphocyte count, and prolonged activated partial thromboplastin time in the soft-tissue sarcoma group (three [7%] each; appendix pp 7–10). Nine (11%) of 84 treated patients (five [12%] with bone sarcoma and four [10%] with soft-tissue sarcoma) had treatment-related serious adverse events, of which only pneumonitis was seen in both groups (table 3). None of these treatment-related serious adverse events were fatal.

Discussion

To our knowledge, SARC028 is the first multicentre, open-label, phase 2 study of immune checkpoint blockade in patients with advanced soft-tissue sarcoma or bone sarcoma. Pembrolizumab monotherapy was associated with clinically meaningful and sustained objective responses in seven (18%) of 40 patients with soft-tissue sarcoma and in two (5%) of 40 patients with bone sarcoma. Although eight or more of the 40 patients in each group had to have an objective response for treatment to be considered a success, the 12-week progression-free survival in the soft-tissue sarcoma group was 55%, which was significantly higher than the 40% expected for an active regimen, suggesting meaningful clinical activity in this population.

In the soft-tissue sarcoma group, six of seven objective responses and improvements in 12-week progression-free survival were observed in patients with undifferentiated pleomorphic sarcoma or liposarcoma. The median duration of response (49 weeks) and the median overall survival (not reached) for patients with undifferentiated pleomorphic sarcoma suggested that immune checkpoint blockade induces durable responses and has meaningful clinical activity in patients with this subtype. This cohort is being expanded to confirm and further characterise the clinical activity of pembrolizumab.

PD-L1 expression has been correlated with T-cell infiltration in undifferentiated pleomorphic sarcoma.[21–23] This finding suggests that undifferentiated pleomorphic sarcoma might fit the model of an inflamed tumour and could explain the activity of single-agent anti-PD-1 antibodies in this disease.[24] PD-L1 expression was observed in only two (5%) of 40 samples in which tumour response was evaluable; both tumours were undifferentiated pleomorphic sarcoma and responded to therapy. This finding is consistent with a previous report[21] in which PD-L1 expression was found in only two (5%) of 36 non-gastrointestinal stromal soft-tissue sarcomas, both of which were undifferentiated pleomorphic sarcoma. In our study, responses were seen even in the absence of PD-L1 expression, consistent with other tumour types, including melanoma, in which

PD-L1-negative tumours might respond to checkpoint blockade.[14] The role of PD-L1 expression in soft-tissue sarcoma remains unclear;[22,23,25] however, ongoing analyses with multicolour immunohistochemistry using samples collected from our study will help to elucidate the role of PD-L1 and will be reported separately.

We also observed clinical activity in the absence of PD-L1 expression in patients with liposarcoma. The liposarcoma cohort included several high-grade liposarcoma subtypes. However, because of the absence of responses in patients with myxoid or round cell liposarcoma, only patients with dedifferentiated liposarcoma will be included in an expansion cohort.

The results for leiomyosarcoma were consistent with a phase 2 study[26] of nivolumab that was stopped early because of futility, suggesting that single-agent anti-PD-1 therapy might not elicit an immune response in these patients. The mechanisms underlying resistance to immunotherapy in patients with leiomyosarcoma remain unclear, although a report implicated loss of PTEN expression as a potential mechanism of resistance. Such investigations could yield targetable pathways—in the case of PTEN, the PI3K-AKT pathway—that could be pursued in combination with checkpoint blockade.[27]

Synovial sarcoma, which has high expression of cancer testis antigens, was anticipated to be responsive to pembrolizumab given reports of objective reponses with T-cell therapy in patients with advanced synovial sarcoma.[28] However, most patients with synovial sarcoma in this study had rapid progression. A phase 2 pilot study investigating use of ipilimumab to treat synovial sarcoma expressing the NY-ESO-1 antigen in six patients was terminated early because of poor activity and absence of immune responses.[29] In our study, only one patient with synovial sarcoma had a short-lived partial response and another had prolonged stable disease and is still deriving clinical benefit while on therapy for almost 2 years. The response of the patient still on therapy was classified as a partial response according to irRC, confirming that this pattern of response can correlate with clinical benefit.

Pembrolizumab had low activity in patients with bone sarcoma, although the two responses observed (one in a patient with osteosarcoma and the other in a patient with chondrosarcoma) were substantial (>50% tumour shrinkage) and durable (>6 months). Although immunotherapy has shown promise as an adjuvant therapy for osteosarcoma, only one patient with metastatic osteosarcoma had a response to pembrolizumab. Osteosarcoma has been shown to have variable PD-L1 expression and frequent loss of MHC class I, potentially facilitating immune evasion.[30–32] Mifarmutide was shown to increase immune-cell infiltration into osteosarcoma metastases,[33] a crucial step to improve the efficacy of anti-PD-1 antibodies.

Pembrolizumab had no activity in the 13 patients with Ewing's sarcoma, which could be associated with the

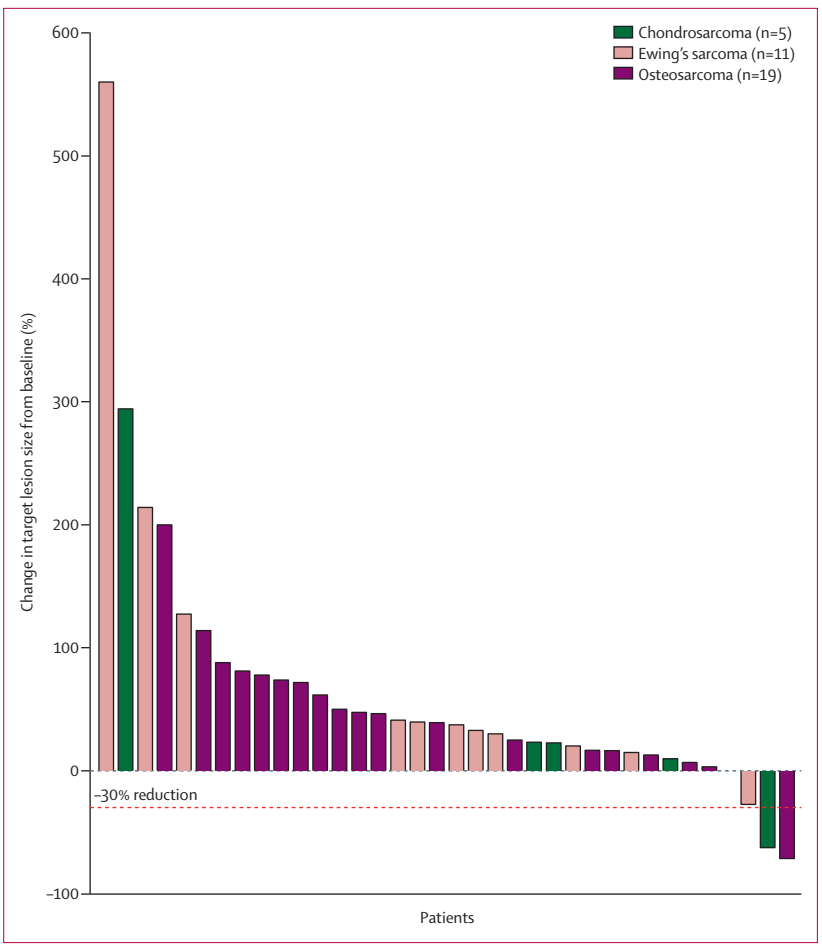

Figure 4: **Best percentage change from baseline in size of target lesions in patients with bone sarcoma**
Tumour sizes were calculated as the sum of target lesion diameters. Data for two patients with Ewing's sarcoma and three patients with osteosarcoma are not shown because they did not have a second scan.

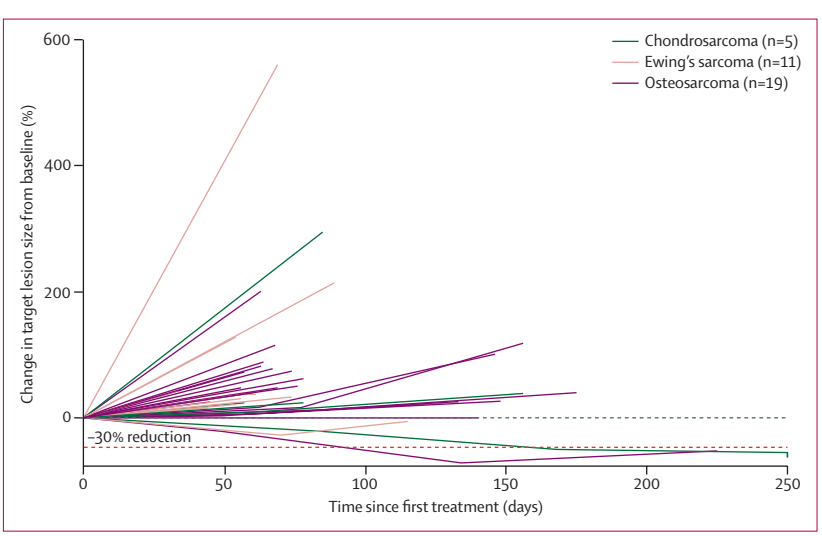

Figure 5: **Percentage change in tumour size from baseline at each assessment for patients with bone sarcoma**
Tumour sizes were calculated as the sum of target lesion diameters. Data for two patients with Ewing's sarcoma and three patients with osteosarcoma are not shown because they did not have a second scan.

	Bone sarcoma (n=42)			Soft-tissue sarcoma (n=42)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Overall	1 (2%)	3 (7%)	1 (2%)	1 (2%)	3 (7%)	0 (0%)
Pulmonary embolism	..	..	..	..	1 (2%)	..
Adrenal insufficiency	..	..	..	1 (2%)	1 (2%)	..
Interstitial nephritis	..	1 (2%)	..	..	..	..
Infectious pneumonia	..	1 (2%)	..	..	..	..
Bone pain	..	1 (2%)	..	..	..	..
Hypoxia	..	..	1 (2%)	..	..	..
Pleural effusion	..	1 (2%)	..	..	..	..
Pneumonitis	1 (2%)	..	..	..	1 (2%)	..

No grade 5 serious adverse events related to treatment were reported during the course of the trial.

***Table 3*: Serious adverse events related to treatment**

highly immunosuppressive microenvironment of the tumour. Only one patient with Ewing's sarcoma had a partial response according to irRC, suggesting that a small subset of patients with Ewing's sarcoma could benefit from single-agent therapy. Nevertheless, combination approaches will clearly be needed for clinical activity in this rapidly growing, aggressive malignancy. In a retrospective study[34,35] high regulatory T-cell counts were seen in the tumour tissues of patients who presented with metastatic Ewing's sarcoma, which might contribute to inhibition of cytotoxic CD8-positive T lymphocytes and promotion of tumour escape.

Five patients with chondrosarcoma were enrolled in this study, one of whom achieved a partial response. This finding was consistent with one retrospective series study,[36] in which one (25%) of four patients with dedifferentiated chondrosarcoma responded to nivolumab. The finding of a response to treatment is important given the few therapeutic options available for chondrosarcoma, which is refractory to most if not all anticancer therapies.[37] In a retrospective report,[38] PD-L1 expression was associated with the number of tumour-infiltrating lymphocytes and HLA class I expression in 11 (52%) of 21 dedifferentiated chondrosarcomas. These results suggest that further evaluation of immune checkpoint blockade in chondrosarcoma is warranted.

The safety profile of pembrolizumab in the study population was consistent with what has been observed for other approved indications; specifically, no increase was seen in the incidence of pneumonitis despite most patients with advanced sarcomas having lung metastases.

Sarcoma is generally considered a non-immunogenic tumour. However, we found sarcomas to be highly variable in their biology, suggesting that each histological subtype should be considered a separate therapeutic challenge requiring a distinct understanding of its immune and molecular biology. The role of PD-L1 and other potential biomarkers in the observed clinical differences between individual sarcoma subtypes will be explored as we continue to analyse the serial blood and tumour samples collected during this study.

Our study was limited by its non-randomised design and small sample size. However, if the clinical activity of pembrolizumab can be confirmed in other, larger studies, our findings could change practice given that undifferentiated pleomorphic sarcoma and liposarcoma are two of the three most common soft-tissue sarcomas (together representing more than 30% of all soft-tissue sarcomas). The results of planned correlative analyses will hopefully improve our understanding of the immune response and mechanisms of resistance to immunotherapy in sarcoma, and help prioritise combination strategies with chemotherapy, radiotherapy,[39] targeted agents, or other immune checkpoint inhibitors.

Our study was a testament to the collaborative efforts of SARC investigators, pharmaceutical companies, and philanthropic organisations, which allowed rapid accrual of patients and collection of high-quality data and biospecimens. The results of this study will lay the foundation for the future of immunotherapy in sarcoma.

Contributors

HAT, MB, and JC led the study design with support from Merck. The study was managed by SARC. VB, JC, HAT, and MB did the data analysis. HAT, MB, VB, BAVT, SMS, JH, SD, SA, RFR, DAP, SM, LED, SHO, DRR, and JC interpreted the data. HAT and VB wrote the manuscript. All versions of the manuscript were reviewed by HAT, MB, VB, BAVT, SMS, JH, SD'A, SA, RFR, DAP, SM, LED, SHO, DRR, JC, LHBu, RS, JR-C, AJL, IIW, LHBa, RGM, DR, and SP. All authors gave final approval of the manuscript. The investigators who contributed to recruitment, treatment, and follow-up of patients are listed in the appendix (p 2).

Declaration of interests

HAT reports Bristol-Myers Squibb consulting and research support to his institution and consulting for Novartis and EMD-Serono, outside the submitted work. MB reports personal fees from Immune Design, EMD-Serono, and Eisai, and other from Eli Lilly, outside the submitted work. BAVT reports grants from Merck, outside the submitted work. SMS reports grants from SARC and non-financial support from Merck, during the conduct of the study, and personal fees from Janssen and Daiichi Sankyo, outside the submitted work. SD'A reports other from Nektar Therapeutics and Amgen, outside the submitted work. RFR reports other from AADi Bioscience, Threshold, Arog, Immune Design, Karyopharm, Merck, SARC, and Plexxlkon; personal fees and other from Daiichi Sankyo, Novartis, Eli Lilly, Tokalas, Ignyta, and Eisai; and personal fees from Janssen and EMD-Serono, outside the submitted work. LED reports grants from Novartis and personal fees from Eisai, outside the submitted work. IIW reports grants and personal fees from AstraZeneca/Mediimune, Roche/Genentech, Merck, and HTG Molecular Diagnostics; grants from Adaptimmune and EMD-Serono; and personal fees from Ariad, Pfizer, and Asuragen, outside the submitted work. RGM reports personal fees from Novartis, Eli Lilly, Gem Pharma, Karyopharm, Bayer, and Arcus; personal fees and other from Tracon, Eisai, and SARC; and other from UpToDate, Springer, GlaxoSmithKline, Tracon, and Bayer, outside the submitted work. SP reports grants and personal fees from Janssen, Eisai, and Morphotek, and personal fees from EMD-Serono, CytRx, Bayer, Eli Lilly, Epizyme, and Novartis, outside the submitted work. All other authors declare no competing interests.

Acknowledgments

The study was funded in part by Merck. Additional funding was obtained from the SARC, the Sarcoma Foundation of America, the QuadW Foundation, Ewan McGregor, and Pittsburgh Cure Sarcoma. We thank the patients and their families, the investigators, and participating study teams.

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