Histologic and Immunologic Factors Associated with Response to Immune Checkpoint Inhibitors in Advanced Sarcoma

Alex Q. Lee, Clara Hao, Minggui Pan, Kristen N. Ganjoo, and Nam Q. Bui

ABSTRACT

Purpose: To characterize factors associated with response to immune checkpoint inhibitors (ICI) in advanced sarcoma.

Experimental Design: This is a retrospective study with a cohort of 216 patients with advanced sarcoma treated with ICI between 2016 and 2023 at Stanford Health Care. Overall survival, progression-free survival (PFS), objective response rates (ORR) per RECIST criteria, and reason for ICI discontinuation were analyzed across histologic subtypes, ICI regimens, tumor mutational burden, and PD-L1 expression.

Results: The overall ORR in the cohort was 16.7%. The histologic subtypes with the highest ORR were Kaposi sarcoma (KS, 66.7%), alveolar soft part sarcoma (ASPS, 50%), angiosarcoma (33.3%), myxofibrosarcoma (MFS, 28.6%), and undifferentiated pleomorphic sarcoma (UPS, 27.8%). The subtypes with the lowest ORR were osteosarcoma (0%), synovial sarcoma (0%), and liposarcoma (3.7%). The subtypes with the highest median PFS were KS (median not reached), ASPS (median not reached), MFS (27.4 months), and UPS (11.3 months). The ORR for sarcomas with PD-L1 ≥ 1% was 27.8% ($P = 0.02$), whereas the ORR for sarcomas with tumor mutational burden ≥10 mutations per megabase of DNA was 28.6% ($P = 0.20$).

Conclusions: ORR and PFS were highly variable across sarcoma histologic subtypes. In this large analysis, KS, ASPS, angiosarcoma, MFS, and UPS demonstrated the highest ORR and longest PFS while osteosarcoma, synovial sarcoma, and liposarcoma had the lowest ORR and shortest PFS. PD-L1 expression was also associated with increased ORR. Our findings provide further insight into understanding the sarcoma histologic and immunologic factors that correspond with response to ICI.

Introduction

Sarcomas are uncommon mesenchymal neoplasms of the bone or soft tissue with more than 100 different histologic subtypes, posing many challenges to management (1, 2). The risk of relapse is high for patients with intermediate- and high-grade sarcomas, and chemotherapy yields only modest response rates and survival benefits in advanced disease (3, 4).

Recently, immune checkpoint inhibitors (ICI) have emerged as an efficacious therapeutic strategy in a wide range of solid tumors, including melanoma, renal cell cancer, and lung cancer (5). However, ICI have only shown modest efficacy in certain sarcoma histologic subtypes due to the tumor microenvironment (TME) of most sarcomas being immune cold (6, 7). In this study, we analyzed a large cohort of patients with advanced sarcoma treated with ICI at Stanford Health Care. Our data provide additional insight into the efficacy of ICI in advanced sarcoma and the clinical factors that may be associated with response.

Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford, California.

Corresponding Author: Nam Q. Bui, Medicine/Oncology, Stanford University, 780 Welch Road, Room CJ250J, Palo Alto, Stanford, CA 94304. E-mail: nambui@stanford.edu

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Materials and Methods

This is a retrospective study of 216 patients with advanced sarcoma treated with ICI between 2016 and 2023 at Stanford Health Care. This study was approved by the Stanford University Institutional Review Board.

Data collection

Patients were identified in the Stanford Research Repository cohort finder, and patient data were collected from the electronic medical record. Baseline characteristics included date of birth, gender, race, ethnicity, vital status, sarcoma histology, tumor size, date of diagnosis, local or metastatic disease at diagnosis, site of origin, and histologic grade. Treatment data consisted of the surgery date, surgical margins, adjuvant chemotherapy or radiation, immunotherapy regimen given, duration of immunotherapy, and response to therapy as defined by RECIST. The authors calculated RECIST responses based on the sum of long-axis measurements reported by radiology for the primary tumor and/or any confirmed metastatic lesion(s). For Kaposi sarcoma (KS), the response was defined by PET/CT and/or clinical skin exam by dermatology. Patients who received immunotherapy for less than 21 days were excluded from the cohort unless they had confirmed progressive disease by RECIST within that duration. Follow-up data included the reason for discontinuation, date and sites of recurrence, and date and sites of metastasis. There was no randomization.

Molecular profiling

Targeted exome molecular profiling of tumors was performed using Stanford Actionable Mutation Panel for Solid Tumors, Foundation One, Tempus, and Natera. Tumor mutational burden

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Translational Relevance

We have studied a cohort of 216 patients with advanced sarcoma treated with immune checkpoint inhibitors and found that the histologic subtypes with the highest objective response rate were Kaposi sarcoma, alveolar soft part sarcoma, angiosarcoma, myxofibrosarcoma, and undifferentiated pleomorphic sarcoma. PD-L1 expression ≥1% was associated with a higher response rate ($P = 0.02$). High tumor mutational burden ≥10 mutations per megabase of DNA showed a trend toward higher response rates as well ($P = 0.2$). These findings suggest to clinicians which patients with sarcoma, based on histology and immunologic biomarkers, may preferentially respond to immunotherapy. Due to the high variability in response rates across different sarcoma subtypes, further identification of useful biomarkers is also necessary to improve immune checkpoint inhibitor response rates and survival in sarcoma.

(TMB), microsatellite instability status, and PD-L1 expression by tumor proportion score (TPS) or combined positive score (CPS) were included. Data cutoff date was December 21, 2023.

Statistical methods

The primary endpoints in this study were overall survival (OS) calculated from the date of immunotherapy initiation until death from any cause, progression-free survival (PFS) calculated from the date of immunotherapy initiation until radiographic progression of disease or death from any cause, and objective response rates (ORR) as defined by RECIST. Kaplan–Meier curves were generated to estimate the probability of survival without death or progression of disease. Hypothesis testing to determine whether there was an increased ORR in TMB ≥ 10 or PD-L1 ≥ 1% groups compared with sarcomas with TMB < 10 or PD-L1 < 1%, respectively, was performed using a one-tailed two-proportion z-test with a significance level of 0.05. Hypothesis testing to evaluate increased prevalence of specific mutations in sarcoma responders compared with all sarcoma patients was also performed using a one-tailed two-proportion z-test with a significance level of 0.05. The same test and significance level were used to investigate whether TMB or PD-L1 positivity was more common in patients with prolonged stable disease (SD) >6 months. All statistical analyses were performed using code written in Python (RRID: SCR_008394) by the authors or in Microsoft Excel (RRID: SCR_016137). Kaplan–Meier curves were analyzed via the lifelines package.

Data availability

The human data generated in this study are not publicly available due to patient privacy requirements but are available upon reasonable request from the corresponding author.

Results

Demographics

A total of 216 patients treated with ICI between 2016 and 2023 at Stanford Health Care were identified and included in the study. Patient demographics and tumor characteristics are provided in **Table 1**. The median age at diagnosis was 54.6 years. Of the total patients, 53.2% were females and 46.8% were males. Most of the patients in the study were White (51.4%), with a smaller proportion of Asian (23.2%), Hispanic (11.6%), and Black (6%) patients. Approximately one-third (32.9%) of the patients were alive at the time of statistical analysis.

Histologic characteristics

The most prevalent sarcoma histologic subtypes in this patient cohort were leiomyosarcoma (LMS, 25%, $n = 48$), liposarcoma (LPS, 14.1%, $n = 27$), and undifferentiated pleomorphic sarcoma (UPS, 9.4%, $n = 18$), whereas the least common were osteosarcoma (3.1%, $n = 6$), gastrointestinal stromal tumor (2.6%, $n = 5$), and alveolar soft part sarcoma (ASPS, 2.1%, $n = 4$). Among LMSs, half were uterine LMSs ($n = 24$). Among LPS, the majority were dedifferentiated LPS ($n = 24$), whereas the remainder were well-differentiated LPS ($n = 3$). One of six patients with KS had human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS)–associated KS. Fifteen percent of patients presented with metastatic disease at diagnosis. Most tumors originated in the extremity (31%), pelvis (24%), or retroperitoneum (14.5%). The median tumor size at diagnosis was 8.9 cm. Most sarcomas were graded as 2 to 3 (35.7%) or 3 (27.9%) at diagnosis.

Treatment

Less than half of the patients received adjuvant chemotherapy (43.1%) or radiation (38.4%). The most common immunotherapy regimen was ipilimumab and nivolumab (77.8%), followed by pembrolizumab (14.8%). The median duration of immunotherapy was 3.6 months, and a small proportion of patients ($n = 12/216$ or 5.6%) were actively undergoing immunotherapy at the time of analysis. Immunotherapy was discontinued most often due to disease progression (62.3%) or adverse effects (18.6%).

Correlation between histologic subtypes and response

The median OS for the entire cohort was 12.8 months. The ORR across all histologic subtypes was 16.7%, and responses included a complete response (CR) rate of 3.2%, partial response (PR) rate of 13.4%, SD rate of 30%, and progressive disease rate of 53.2%. The ORR in patients treated with pembrolizumab (21.9%) was not statistically different from the ORR in patients treated with ipilimumab and nivolumab (14.3%, $P = 0.28$). The ORR of all sarcoma histologic subtypes with $n > 3$ is demonstrated in **Fig. 1**. The histologic subtypes with the highest ORR were KS (66.7%), ASPS (50%), angiosarcoma (AS, 33.3%), myxofibrosarcoma (MFS, 28.6%), and UPS (27.8%). The subtypes with the lowest ORR were osteosarcoma (0%), synovial sarcoma (SS, 0%), and LPS (3.7%). Among LMSs, uterine LMS (uLMS) had an ORR of 4.2% ($n = 24$), whereas all other LMSs had an ORR of 12.5% ($n = 24$). Within LPS, dedifferentiated LPS (DDLPS) had an ORR of 4.2% ($n = 24$), whereas all other LPS (myxoid/round cell LPS and well-differentiated LPS) had an ORR of 0.0% ($n = 3$). The median PFS across all sarcoma subtypes was 4.5 months (95% confidence interval, 3.6–5.4 months). The subtypes with the highest median PFS were KS (median not reached), ASPS (median not reached), MFS (27.4 months), and UPS (11.3 months; **Fig. 2**). The median PFS for all subtypes is shown in **Table 2**.

Correlation between immunologic factors and response

Molecular profiling by next-generation sequencing was available for 159 of the 216 total patients. The most common alterations across all patients were *TP53*, *RB1*, *CDKN2A/B*, *MDM2*, and *CDK4* (Supplementary Table S1). These alterations were also common

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Table 1. Baseline characteristics of patients.

Characteristic	Overall (*N* = 216)
Age at sarcoma diagnosis, median, years (range)	54.6 (16.5–99.3)
Sex	
Female	115 (53.2)[a]
Male	101 (46.8)
Race	
White	111 (51.4)
Asian	50 (23.2)
Hispanic/Latino	25 (11.6)
Black	13 (6)
Other, non-Hispanic/Latino	12 (5.6)
Pacific Islander	4 (1.9)
Native American	1 (0.5)
Alive	71 (32.9)
Histology	
LMS	48 (22.2)
Uterine LMS	24 (11.1)
LPS	27 (12.5)
Dedifferentiated LPS	24 (11.1)
Well-differentiated LPS	3 (1.4)
UPS	18 (8.3)
Angiosarcoma	15 (6.9)
MFS	14 (6.5)
Chondrosarcoma	10 (4.6)
Sarcoma (not otherwise specified)	9 (4.2)
Hemangiopericytoma	8 (3.7)
Malignant peripheral nerve sheath tumor	8 (3.7)
SS	7 (3.2)
Spindle cell sarcoma	7 (3.2)
KS	6 (2.8)
Osteosarcoma	6 (2.8)
Gastrointestinal stromal tumor	5 (2.3)
ASPS	4 (1.9)
Malignant perivascular epithelial cell tumor	3 (1.4)
Malignant phyllodes tumor	3 (1.4)
Rhabdomyosarcoma	3 (1.4)
Carcinosarcoma	2 (0.9)
Endometrial stromal sarcoma	2 (0.9)
Desmoplastic small round cell tumor	2 (0.9)
Dermatofibrosarcoma protuberans	1 (0.5)
Epithelioid sarcoma	1 (0.5)
Fibrosarcoma	1 (0.5)
Inflammatory myofibroblastic tumor	1 (0.5)
Intimal sarcoma	1 (0.5)
Malignant rhabdoid tumor	1 (0.5)
Malignant Triton tumor	1 (0.5)
Uterine sarcoma	1 (0.5)
Clear cell sarcoma	1 (0.5)
Metastatic at diagnosis	33 (15.3)
Site of origin	
Extremity	62 (28.7)
Pelvic	48 (22.2)
Retroperitoneum	29 (13.4)
Trunk	17 (7.9)
Head and neck	16 (7.4)
Abdominal	11 (5.1)
Breast	8 (3.7)
Lung	5 (2.3)
Liver	4 (1.9)
Bone	3 (1.4)
Peritoneum	3 (1.4)
Spine	3 (1.4)
Cardiac	2 (0.9)
Mediastinum	1 (0.5)
Nasopharyngeal	1 (0.5)
Pancreas	1 (0.5)
Uterus	1 (0.5)
Vascular	1 (0.5)
Size at diagnosis, median, cm	8.9
Grade	
1	21 (13.6)
2	35 (22.7)
2/3	55 (35.7)
3	43 (27.9)
Adjuvant therapy	
Adjuvant chemotherapy	93 (43.1)
Adjuvant radiation	83 (38.4)
Immunotherapy regimen given	
Ipilimumab and nivolumab	168 (77.8)
Pembrolizumab	32 (14.8)
Nivolumab	13 (6)
Envafolimab	1 (0.5)
Investigatory drug	1 (0.5)
Ipilimumab	1 (0.5)
Duration of immunotherapy, median, months (range)	3.6 (0.2–54.7)
Reason for discontinuation	
Disease progression	123 (62.3)
Adverse effects	38 (18.6)
Comfort care	27 (13.2)
Quiescent disease	7 (3.4)
Other	3 (1.5)
No evidence of disease	1 (0.5)

[a]*n* (%).

among patients with an objective response (PR or CR) to ICI (Supplementary Table S2). Five alterations were statistically significantly more common in patients who exhibited an objective response to ICI compared to their prevalence in all sarcoma patients: *TET2* (4/28 or 14.3% in responders vs. 5/159 or 3.1% in all sarcoma patients with molecular profiling available, $P = 0.006$), *FGF3/FGF19* (3/28 or 10.7% in responders vs. 3/159 or 1.9% in all sarcoma patients, $P = 0.007$), *KMT2C* (4/28 or 14.3% in responders vs. 6/159 or 3.8% in all sarcoma patients, $P = 0.01$), *CDKN2A* (4/28 or 14.3% in responders vs. 7/159 or 4.4% in all sarcoma patients, $P = 0.02$), and *DNMT3A* (3/28 or 10.7% in responders vs. 5/159 or 3.1% in all sarcoma patients, $P = 0.03$); $n \geq 3$ only (Supplementary Fig. S1). *FGF3* and *FGF19* were 100% comutated, whereas *CDKN2A* was 0% comutated with the four other alterations. Otherwise, except for *TET2* and *KMT2C* (50% comutation rate), no two alterations had a comutation rate greater than 25%. The overall TMB ($n = 141$) was low with a median of two mutations per megabase of DNA (mut/MB). Only seven patients ($n = 7/141$ or 5.0% of all patients with TMB data available) had a TMB ≥10 mut/MB, with a corresponding ORR of 28.6%. The histologies of these patients were LMS ($n = 3$), malignant perivascular epithelial cell tumor ($n = 1$), osteosarcoma ($n = 1$), sarcoma not otherwise specified ($n = 1$), and UPS ($n = 1$). Overall PD-L1 expression ($n = 93$ with TPS or CPS) was also low with a median of 0%. Thirty-six ($n = 36/93$ or 38.7% of all patients with PD-L1 data available) had positive PD-L1

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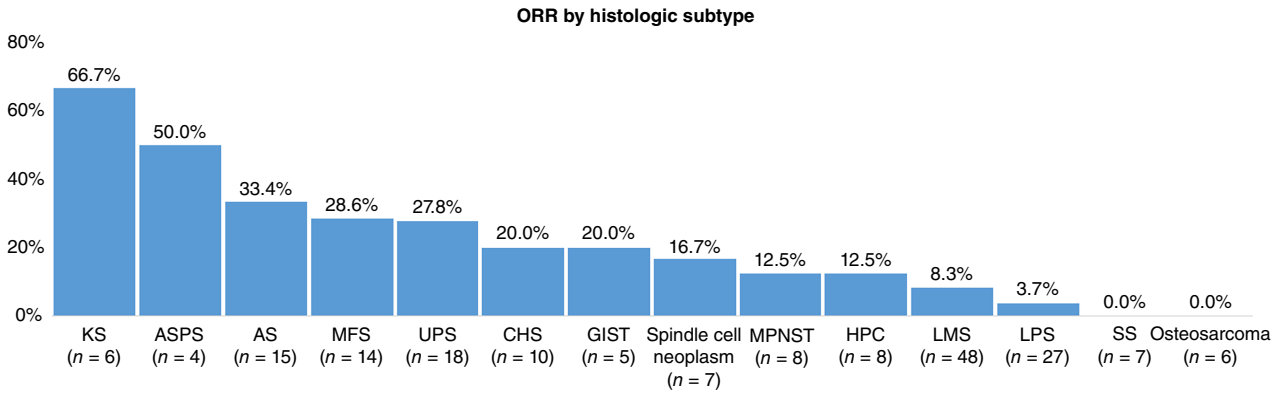

Figure 1.

The histologic subtypes with the highest ORR as per the RECIST criteria were KS (66.7%), ASPS (50%), AS (33.3%), MFS (28.6%), and UPS (27.8%). Overall sarcoma ORR was 16.6%. CHS, chondrosarcoma; GIST, gastrointestinal stromal tumor; HPC, hemangiopericytoma; MPNST, malignant peripheral nerve sheath tumor.

expression, defined as ≥1% by TPS or CPS. Positive PD-L1 expression was associated with an ORR of 27.8% (10/36), which was significantly higher compared with negative PD-L1 expression which had an ORR of 10.5% (6/57, $P = 0.02$). Although there was a trend toward increased response rate with TMB ≥10 mut/MB, there was no statistical difference in ORR when compared with patients with TMB <10 mut/MB (2/7 or 28.6% vs. 22/134 or 16.4%, $P = 0.20$), as shown in **Fig. 3**. TMB and PD-L1 expressions were also analyzed in a subset of patients with prolonged SD (defined as SD > 6 months). In 24 patients with prolonged SD with TMB data available, 0% (0/24) of these patients had TMB ≥10 mut/MB, compared with 5.0% (7/141) in all patients with TMB data available ($P = 0.13$). In 15 patients with prolonged SD with PD-L1 data available, 53.3% (8/15) had

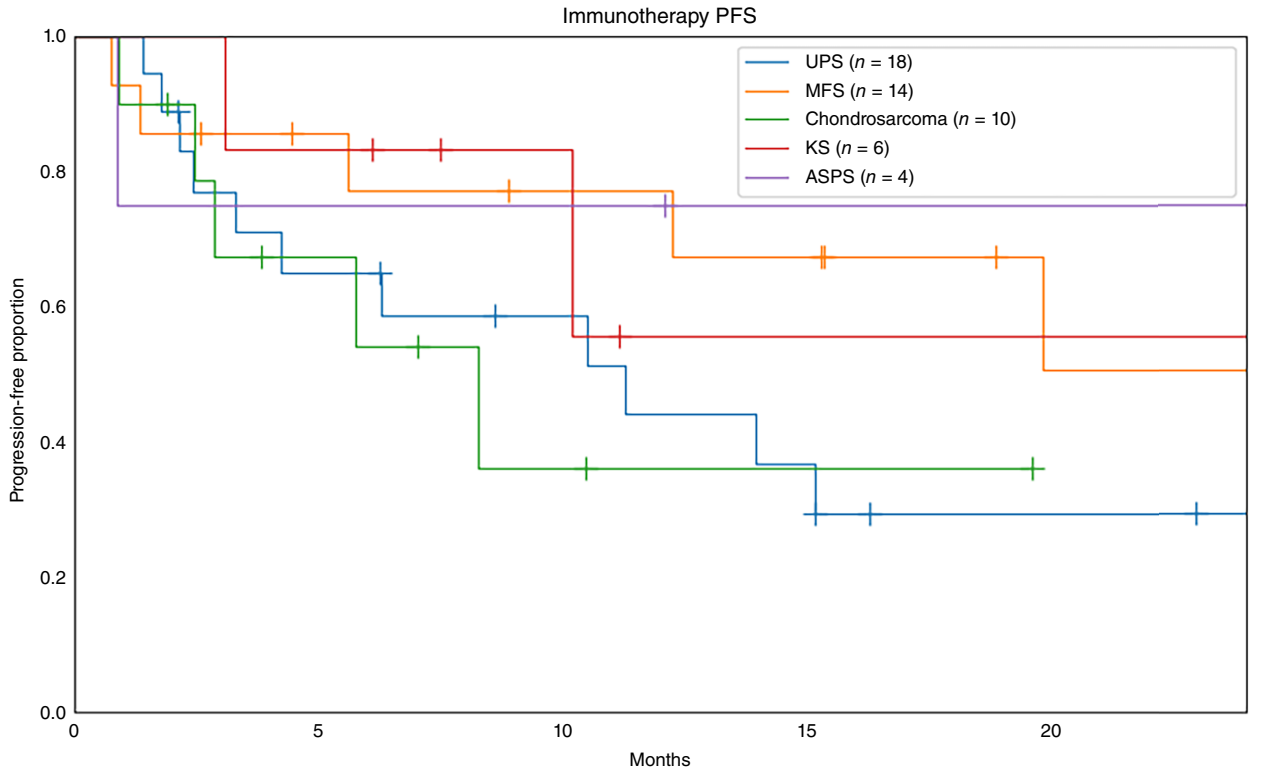

Figure 2.

The histologic subtypes with the highest median PFS include KS (median not reached), ASPS (median not reached), MFS (27.4 months), UPS (11.3 months), and chondrosarcoma (8.3 months). Overall sarcoma median PFS was 4.5 months.

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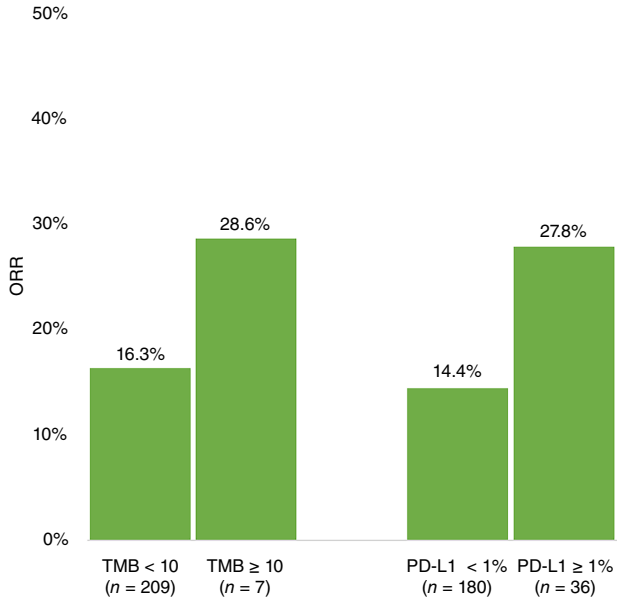

Figure 3.

Sarcomas with TMB ≥10 mut/MB (n = 7, 5.0% of all sarcomas with TMB data available) demonstrate an ORR of 28.6%, whereas sarcomas with TMB <10 mut/MB (n = 134) show an ORR of 16.4%. This difference was not statistically significant (P = 0.20). On the other hand, sarcomas with PD-L1 ≥ 1% (n = 36, 38.7% of all sarcomas with PD-L1 data available) demonstrate a significantly higher ORR compared with sarcomas with PD-L1 < 1% (n = 57, 27.8% vs. 10.5%, P = 0.02). Median TMB (n = 141) was 2 mut/MB and median PD-L1 expression (n = 93) by TPS or CPS was 0%.

PD-L1 ≥ 1% compared with 38.7% (36/93) in all patients with PD-L1 data available (P = 0.14).

Adverse effects

A significant benefit of immunotherapy when considered as a therapeutic option is a different side effect profile compared with conventional chemotherapy, which allows immunotherapy to be offered to patients who may not be candidates for chemotherapy. In our study, we investigated the impact of ICI-related toxicity on discontinuation of therapy and found that this occurred in 18.6% of patients. This percentage included a wide range of manifestations as illustrated in Supplementary Table S3 such as pneumonitis, colitis, arthritis, hepatitis, cutaneous toxicity, anemia, apophysitis, cranial nerve inflammation, edema, demyelinating syndromes, hearing loss, hypercalcemia, hypophysitis, infection, myasthenia gravis, myocarditis, nephritis, and thrombosis. The most common adverse effects leading to discontinuation were autoimmune pneumonitis (3.2% of all patients), autoimmune colitis (2.8%), autoimmune arthritis (2.3%), and autoimmune hepatitis (2.3%).

Discussion

This retrospective study of 216 sarcoma patients treated with ICI represents one of the largest published samples to date of immunotherapy in sarcoma. In the study cohort, the sarcoma subtypes closely resemble the known, nationwide epidemiology of sarcomas, with the most common being LMS and LPS (8). The study population included 30 different subtypes, including six subtypes with a sample size of at least 10 patients.

The median OS in this study population was 12.8 months, which is consistent with previous studies of ICI in sarcoma (9). The total sarcoma ORR in our study was 16.6%; however, this varied significantly across histologic subtypes, ranging from more than 67% for KS and 50% for ASPS to 0% for SS and osteosarcoma. Of note, the ORR for DDLPS is 4.2%, which is surprisingly lower than seen historically. Furthermore, the median PFS for LPS was 4.2 months, which is essentially equivalent to the overall sarcoma median PFS of 4.5 months. Initially, the SARC028, a phase 2 trial evaluating pembrolizumab in advanced soft tissue and bone sarcomas, was highly promising for LPS with an ORR of 20% (2/10), but a subsequent expansion of 30 patients revealed only 2/29 additional PR in evaluable patients for a total ORR of 10% (10). This finding is consistent with response rates seen in our real-world clinical practice, in which most DDLPS that require systemic treatment are large retroperitoneal tumors in which a radiographic partial response would require significant shrinkage, which is difficult to attain. Using a cutoff of at least 10% reduction in tumor size, rather than 30% as defined by RECIST for PR, we found that 4/24 or 16.7% of patients with DDLPS demonstrated at least a modest regression in tumor size with ICI. Further research is needed to determine whether certain subtypes of DDLPS respond better to ICI, such as LPS that have a significant inflammatory infiltrate (11). The significant response seen in KS in our cohort is consistent with results from clinical trials, in which an ORR of 87% has been seen with ipilimumab and nivolumab, and an ORR of 62% has been shown with pembrolizumab (12, 13). It is also particularly notable when compared with the ORR in KS treated with chemotherapy, which has been shown to be approximately 43% to 46% (14, 15). In ASPS, a classically chemotherapy-resistant disease, the response to ICI is even more striking. Studies of PD-L1 inhibitor atezolizumab in ASPS have shown a high ORR of 37% (16), leading to its FDA approval in 2022. In comparison, prior studies with chemotherapy show an ORR in ASPS of 7% (17).

There are several frameworks that aim to describe the TME associated with immunotherapy response in sarcoma. Petitprez and colleagues (18) established an immune-based classification of sarcoma-immune classes and showed that the immune-high group, which was characterized by the presence of B-cell–rich tertiary lymphoid structures, demonstrated high response to PD-1 immunotherapy. Subramanian and colleagues (19) describe distinct, transcriptionally defined sarcoma ecotypes and show that one ecotype characterized by tumor-associated macrophages and epithelial-like malignant cells is associated with response to ICI. The results described in our study are consistent with these frameworks. For example, the TME in KS is known to be composed of activated B cells and tumor-associated macrophages (20). Similarly, ASPS has been shown by transcriptomic profiling to be highly lymphocyte-infiltrated, with dense cytotoxic T-lymphocyte tumor infiltrates (16, 21). On the other hand, studies of SS demonstrate that tumor-infiltrating lymphocytes are rare and a high number of infiltrating anti-inflammatory $CD163^{+}$ macrophages is associated with poor prognosis (22, 23). The minimally immunologic environment of SS may underlie our finding of an ORR of 0% with ICI.

Sarcoma is known to have a low tumor mutation rate (24). However, the heterogeneity of sarcoma also extends to variability in diverse genomic alterations across histologic subtypes. Subtype-dependent variability in TMB has previously been reported, with

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Table 2. Median PFS by sarcoma histologic subtype.

Sarcoma subtype	Median PFS, months (95% confidence interval)
KS ($n = 6$)	NR[a] (3.1–NR)
ASPS ($n = 4$)	NR (0.9–NR)
MFS ($n = 14$)	27.4 (5.6–NR)
UPS ($n = 18$)	11.3 (2.4–30.1)
Spindle cell sarcoma ($n = 7$)	9.2 (0.4–37.8)
Chondrosarcoma ($n = 10$)	8.3 (0.9–NR)
Hemangiopericytoma ($n = 8$)	7.7 (1.2–NR)
Angiosarcoma ($n = 15$)	6.4 (1.9–20.4)
LMS ($n = 48$)	4.6 (3.3–5.4)
Gastrointestinal stromal tumor ($n = 5$)	4.5 (1.9–7.1)
LPS ($n = 27$)	4.2 (2.7–9.1)
Osteosarcoma ($n = 6$)	4 (0.7–NR)
SS ($n = 7$)	2.4 (0.8–9.3)
Malignant peripheral nerve sheath tumor ($n = 8$)	2.1 (0.6–4.0)

[a]NR, median not reached.

cutaneous AS shown to carry TMB >20 mut/MB, whereas MFS, LPS, and SS carry TMB <2 mut/MB (25). In our study, AS demonstrates one of the highest ORR, which may reflect its high TMB. In contrast, MFS is known to have low TMB, yet in our study, ICI treatment of MFS results in a relatively high ORR of 28.6%. Gross analysis of the correlation between TMB and response to ICI in our study is consistent with this, as high TMB ≥10 shows a nonstatistically significant trend toward increased response rate, with a difference of 12.2% (28.6% vs. 16.%, $P = 0.20$). As such, based on our exploratory analysis in a very small sample ($n = 7$ for TMB ≥10), TMB does not seem to be a clinically useful biomarker to guide the utility of immunotherapy in sarcoma, as the histology and corresponding TME seem to have a greater impact on ICI response. On the other hand, PD-L1 expression ≥1% in our study is associated with an improved response rate by 17.3% (27.8% vs. 10.5%, $P = 0.02$), suggesting there may be utility in PD-L1 expression level as a biomarker for response. Moreover, an exploratory analysis investigating specific mutations and their associations with response revealed that *TET2*, *FGF3*, and *FGF19* (which were 100% comutated), as well as *KMT2C*, *CDKN2A*, and *DNMT3A*, were all significantly more prevalent in sarcoma responders than in all patients ($P < 0.05$). This is consistent with other studies linking alterations in *TET2*, *FGF/FGFR*, and *KMT2C* with increased antitumor immune activity and ICI response (26–28). On the other hand, *DNMT3A* alterations have not yet been shown to correlate with ICI response, whereas *CDKN2A* alterations seem to confer resistance, improve response, or have no association with ICI treatment depending on the tumor type (29, 30). However, as sample sizes in our analysis were small, ultimately further characterization of the sarcoma genetic microenvironment is necessary to determine its significance in predicting responses to immunotherapy.

One limitation of our study is that despite the overall sample size of 216 patients, analysis of response rates and survival in our cohort focused on the 14 sarcoma subtypes ($n > 3$), whereas the 15 additional subtypes ($n \leq 3$) had sample sizes that were too low to meaningfully study. As our analysis of genetic and immunologic factors was drawn from the entire sample of all sarcomas, these findings are skewed toward the more common subtypes, with limited application toward the underrepresented subtypes by sample size. Another limitation is that several key variables such as performance status and number of prior lines of therapy were not available. Future studies can expand on our findings by incorporating gene expression profiling to identify additional biomarkers and patterns that may predict response rates to immunotherapy. In addition, as seven patients achieved CR in our study, further analysis of these outstanding response patients and their genetic and immunologic profiles may yield added insight into the strongest predictors of response. In an analysis of the subset of patients with prolonged SD > 6 months, neither TMB ≥10 nor PD-L1 ≥ 1% were significantly more likely to occur than in all patients with TMB and PD-L1 data available ($P = 0.13$ and $P = 0.14$, respectively), suggesting against the utility of TMB and PD-L1 as predictive biomarkers in this population. Further analysis of these patients to elicit predictive biomarkers will be valuable, as durable disease control despite not achieving PR or CR is still a clinically meaningful result.

In one of the largest published retrospective studies of ICI use in sarcoma, we show that KS, ASPS, AS, MFS, and UPS demonstrated the highest response rates and longest survival periods and that PD-L1 ≥ 1% was associated with increased response to ICI. Our findings can inform clinical practice on which patients with sarcoma to preferentially offer immunotherapy as well as provide direction for future studies to continue to characterize the immunologic factors that predict response.

Authors' Disclosures

N.Q. Bui reports grants and personal fees from SpringWorks Therapeutics and personal fees from Rain Therapeutics, Boehringer Ingelheim, Immunome, and AADi outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

A.Q. Lee: Conceptualization, data curation, formal analysis, investigation, visualization, methodology, writing–original draft. **C. Hao:** Data curation. **M. Pan:** Supervision, writing–review and editing. **K.N. Ganjoo:** Supervision, writing–review and editing. **N.Q. Bui:** Conceptualization, resources, data curation, formal analysis, supervision, project administration, writing–review and editing.

Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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