PD-L1 (CD274) copy number gain, expression, and immune cell infiltration as candidate predictors for response to immune checkpoint inhibitors in soft-tissue sarcoma

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ABSTRACT
Soft-tissue sarcomas (STS) are rare malignancies that account for 1% of adult cancers and comprise more than 50 entities. Current therapeutic options for advanced-stage STS are limited. Immune checkpoint inhibitors targeting the PD-1/PD-L1 signaling axis are being explored as new treatment modality in STS; however, the determinants of response to these agents are largely unknown. Using the sarcoma data set of The Cancer Genome Atlas (TCGA) and an independent cohort of untreated high-grade STS, we analyzed DNA copy number status and mRNA expression of PD-L1 in a total of 335 STS cases. Copy number gains (CNG) were detected in 54 TCGA cases (21.1%), of which 21 (8.2%) harbored focal PD-L1 CNG and that were most prevalent in myxofibrosarcoma (35%) and undifferentiated pleomorphic sarcoma (34%). In the untreated high-grade STS cohort, we detected CNG in six cases (7.6%). Analysis of co-amplified genes identified a 5.6-Mb core region comprising 27 genes, including JAK2. Patients with PD-L1 CNG had higher PD-L1 expression compared with STS without CNG (fold change, 1.8; p = 0.02), an effect that was most pronounced in the setting of focal PD-L1 CNG (fold change, 3.0; p = 0.0027). STS with PD-L1 CNG showed a significantly higher mutational load compared with tumors with a diploid PD-L1 locus (median number of mutated genes; 58 vs. 40; p = 3.6E-06), and PD-L1 CNG were associated with inferior survival (HR = 1.82; p = 0.025). In contrast, T-cell infiltrates quantified by mRNA expression of CD3Z were associated with improved survival (HR = 0.88; p = 0.024) and consequently influenced the prognostic power of PD-L1 CNG, with low CD3Z levels conferring poor survival in cases with PD-L1 CNG (HR = 1.8; p = 0.049). These data demonstrate that PD-L1 CNG and elevated expression of PD-L1 occur in a substantial proportion of STS, have prognostic impact that is modulated by T-cell infiltrates, and thus warrant investigation as response predictors for immune checkpoint inhibition.

Introduction
Soft-tissue sarcomas (STS) are a heterogeneous group of rare tumors comprising more than 50 different entities. STS may be curable when presenting as localized disease; however, local recurrence and metastatic spread are frequent despite multimodal therapy. In patients with advanced-stage STS, conventional chemotherapy is generally administered with palliative intent, as reflected by a median survival after development of distant metastases of 12 months, and associated with substantial toxicity. With the notable exception of gastrointestinal stromal tumors, these data emphasize the urgent need for new therapies beyond palliation and disease control.

Pioneering work by Rosenberg and colleagues demonstrated that the immune system can be harnessed for cancer therapy. Specifically, administration of interleukin-2, either alone or in combination with tumor-infiltrating lymphocytes expanded ex vivo and chemotherapy, resulted in objective responses and even long-term survival in subsets of patients with melanoma and renal cell carcinoma. However, treatment-related toxicity limited the widespread use of these regimens. Underlining the potential of immune-mediated therapy, Robbins et al. observed partial responses in four of six patients with metastatic synovial sarcoma (SS) treated with T-cells harboring a T-cell receptor that was genetically modified to detect the NY-ESO-1 antigen.
With an increasingly better understanding of the immune system and its interactions with tumor cells, monoclonal antibodies targeting immune checkpoints involved in regulating T-cell activation were added to the arsenal of therapeutic options. For example, there is evidence from multiple clinical trials that blockade of the PD-1/PD-L1 axis can lead to durable responses in a considerable proportion of patients with advanced-stage melanoma, non-small cell lung cancer, renal cell carcinoma, bladder cancer, head and neck cancer, and Hodgkin lymphoma.12 In contrast to these insights, the role of immune checkpoint inhibition in STS is largely unexplored. However, in a recent phase 2 trial of the anti-PD-1 antibody pembrolizumab, partial response or disease stabilization were observed in 19% and 40% of 40 STS patients, respectively, indicating that further investigation of this approach is warranted.13

To select patients who potentially benefit from checkpoint blockade, PD-L1 expression levels on tumor cells and/or immune cells, as measured by immunohistochemistry, were introduced as predictive biomarker, although their predictive value remains controversial.14,15 This prompted several investigations of PD-L1 expression across different tumor types, including STS. For example, Kim et al. reported that PD-L1 and PD-1 expression were associated with poor overall and progression-free survival in patients with leiomyosarcoma (LMS), SS, and undifferentiated pleomorphic sarcoma (UPS).16 Subsequent studies corroborated the expression of PD-L1 in different STS subtypes and confirmed, in part, the association with outcome parameters.17-19

A potential molecular mechanism underlying aberrant PD-L1 expression are acquired DNA copy number gains (CNG) comprising the PD-L1 locus, which have been observed in several tumor types20-23 and are being used as response predictor in clinical trials of PD-1/PD-L1 antagonists in Hodgkin lymphoma.24

In contrast to epithelial and lymphoid malignancies, the prevalence of PD-L1 CNG and their association with PD-L1 expression and clinical outcome among the major STS subtypes are currently unknown. To address these issues, we interrogated the sarcoma data set of The Cancer Genome Altas (TCGA) and an independent in-house cohort of untreated high-grade STS for changes in PD-L1 copy number and mRNA expression and correlated the results with overall survival. Furthermore, we delineated the genes that are co-amplified with PD-L1 in STS and searched for an association between PD-L1 alterations and overall mutational load.

Data retrieval, data analysis, and graphics generation were performed using the statistical programming language R.25 Putative copy number alterations (GISTIC), mRNA expression data (RNA Seq V2 RSEM), and clinical data, including overall and disease-free survival were retrieved from the Cancer Genomic Data Server (http://www.cbioportal.org/public-portal) using the R package cgdsr.26

Classification of copy number alterations as focal, arm, or chromosome-scale was based on genomic information retrieved from the R annotation package org.Hs.eg.db.27 CNG were classified as arm alterations if, 90% or more genes on the chromosome arm were amplified. CNG were classified as chromosome alterations if, 90% or more genes on the chromosome were amplified. Differences in gene expression were assessed using fold changes, and significance was assessed by Welch’s t-test.

Somatic mutation data (level 2) from STS cases were retrieved from the TCGA Data Portal (https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm). To assess mutational load, the number of genes carrying at least one nonsynonymous mutation in the coding region was computed for each tumor. The significance of the difference in mutational load between tumors with and without PD-L1 CNG was computed using the Wilcoxon test. Univariate survival analysis was performed using the Kaplan–Meier method and proportional hazards modeling as implemented by the functions survfit and coxph of the R package survival.28 Significance of hazard ratios (HR) was assessed using the log-rank test.

RNA sequencing data of immune genes were put to log2 scale and centered to mean = 0 and scaled to variance = 1. Data were visualized using the function heatmap from the R package stats. Hierarchical clustering was performed using Pearson correlations as similarity measure and the average linkage method to calculate distances between clusters. The CD4/CD8 ratio was calculated as 2×CD4/(CD8A+CD8B). Analysis of overall survival was performed using continuous data of mRNA expression. HR refer to a doubling of gene expression. Significance was assessed using the log-rank test. A combined analysis of immune gene expression and PD-L1 copy number status was performed by stratifying patients in four subgroups defined by dichotomized CD3Z (CD247) mRNA expression (cutoff, median) and presence or absence of PD-L1 CNG.

Analysis of the untreated high-grade STS cohort

The second cohort comprised samples from 79 patients with untreated high-grade STS (UPS, LMS, SS, MPNST, DDLs, MFS, myxoid liposarcoma (MLS), and pleomorphic liposarcoma (PLS)) that were collected at the Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany, between 1989 and 2008. Tumors were categorized according to current World Health Organization criteria,1 and grading was performed using the Fédération Nationale des Centres de Lutte Contre le Cancer system.29 Cases of MLS, SS, and DDLs were also verified by detection of the FUS-DDIT3 and SS18-SSX fusion genes and MDM2 amplification, respectively.

Array-based comparative genomic hybridization (aCGH) was performed as described previously.30 In brief, 1.5 μg tumor or gender-matched control DNA were labeled with the Genomic DNA ULS Labeling Kit (Agilent Technologies, Waldbronn.

Material and methods

Analysis of the TCGA sarcoma cohort

Analysis of PD-L1 (CD274) was performed using copy number, gene expression, mutation, and clinical data from the TCGA cohort of 256 STS cases, which comprises six STS subtypes. Specifically, we analyzed dedifferentiated liposarcoma (DDLs, 58 cases), leiomyosarcoma (LMS, 103 cases), malignant peripheral nerve sheath tumors (MPNST, 9 cases), myxofibrosarcoma (MFS, 23 cases), SS (10 cases), undifferentiated pleomorphic sarcoma (UPS, 50 cases), desmoid tumors (2 cases), and STS not otherwise specified (1 case). In line with TCGA publication guidelines, we included only data sets that are publicly available without restrictions (http://cancergenome.nih.gov/).
Germany). Microarray processing, including hybridization, washing, and scanning steps, was performed according to the manufacturer’s recommendations (Agilent Technologies). Data extraction was done using Feature Extraction Software v10 (Agilent Technologies). The R package DNAcopy was used for normalization of profiles, and outliers were smoothed. The R package CGHcall was used for calling of copy number profiles. Gene expression profiling was performed using HumanHT-12 v3 Expression BeadChip technology (Illumina, San Diego, USA) as described previously,31 and the data set is available at NCBI’s Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo; accession number GSE52392). The study was approved by the Ethics Committee of the Medical Faculty Heidelberg (ethics votes 206/2005 and 207/2005). Differences in protein expression and CNG in STS. (A) Percentages of focal gains, 9p gains, and chromosome 9 gains in different STS subtypes in the TCGA cohort. (B) Percentages of PD-L1 CNG in six tumors (7.6%; Fig. 1B), including SS, UPS, LMS, and DDLS. No PD-L1 CNG were observed in MFS, PLS, MLS, and MPNST. For six cases, we validated PD-L1 CNG and associated protein expression by FISH and immunohistochemistry, respectively. Three representative cases are shown in Fig. 2A.

In the TCGA sarcoma cohort, analysis of co-amplified genes located on chromosome arm 9p identified a 5.6-Mb core region comprising 27 genes (Fig. 1C). In addition to PD-L1, this region included PD-L2, encoding the second PD1 ligand, and JAK2, encoding a cytoplasmic tyrosine kinase.

**PD-L1 mRNA and protein expression**

In the TCGA data set (Fig. 3A), PD-L1 expression was significantly higher in MFS compared with DDLS (fold change = 2.2; \( p = 0.036 \)) and SS (fold change = 12.2; \( p = 0.00012 \)). Likewise, PD-L1 expression was significantly higher in LMS compared with DDLS (fold change = 1.5; \( p = 0.039 \)) and SS (fold change = 8.6; \( p = 0.00047 \)). PD-L1 expression in SS was significantly lower compared with each of the other subtypes. In the independent cohort of primary untreated high-grade STS (Fig. 3B), PD-L1 expression was highest in LMS and UPS, whereas PD-L1 expression was significantly lower (\( p < 0.05 \)) in MLS compared with all other subtypes except MPNST. As observed in the TCGA data set, SS showed significantly lower PD-L1 expression compared with MFS (\( p = 0.0012 \)), UPS (\( p = 0.013 \)), and MFS (\( p = 0.025 \)). To verify these results at the protein level, we performed PD-L1 immunohistochemistry in 48 cases (60.1% of the untreated high-grade STS cohort) for which the same FPPE tissue block was available that was used for aCGH (Fig. 2B, for details see Table S1). We detected PD-L1-positive tumor cells in 10 (20.8%) and PD-L1-positive lymphocytes in 19 (39.6%) of 48 tumors. Seven tumors were PD-L1-positive for both tumor cells and immune cells, resulting in a significant positive correlation between tumor cell and immune cell PD-L1 protein.

**Results**

**Landscape of PD-L1 copy number gains**

We detected PD-L1 CNG in 54 of 256 cases (21.1%) from the TCGA sarcoma cohort. Of these, 21 (8.2%) harbored focal PD-L1 CNG. As shown in Fig. 1A, the frequency of PD-L1 CNG varied among the different STS subtypes and was highest in MFS (35%) and UPS (34%), followed by DDLS (21%), LMS (16%), MPNST (11%), and SS (10%). In an independent cohort of 79 patients with primary untreated high-grade STS, we detected focal PD-L1 CNG in six tumors (7.6%; Fig. 1B), including SS, UPS, LMS, and DDLS. No PD-L1 CNG were observed in MFS, PLS, MLS, and MPNST. For six cases, we validated PD-L1 CNG and associated protein expression by FISH and immunohistochemistry, respectively. Three representative cases are shown in Fig. 2A.
While the correlation between PD-L1 protein expression of tumor cells and PD-L1 mRNA expression, as determined by microarray analysis of bulk tissues, was not significant (fold change = 1.1; p = 0.63), we noted a borderline significant correlation between PD-L1 protein expression in immune cells and PD-L1 mRNA levels (fold change = 1.2; p = 0.075).

Association between PD-L1 CNG, mRNA expression, and mutational load

In the TCGA cohort, samples with PD-L1 CNG were characterized by higher PD-L1 expression compared with samples without PD-L1 CNG (fold change = 1.8; p = 0.02). As expected, the effect of focal PD-L1 CNG on PD-L1 expression was even more pronounced (fold change = 3.0; p = 0.0027), particularly in LMS (fold change = 3.1; p = 0.04), and UPS (fold change = 4.9; p = 0.055). The median number of mutated genes (Fig. 4) was highest in MFS (64), followed by MPNST (56), UPS (53), LMS (46), DDLS (42), and SS (36). Samples with PD-L1 CNG showed a significantly higher mutational load compared with samples without PD-L1 amplification (median number of mutated genes: 58 vs. 40; p = 3.6E-06). This was especially pronounced in the subgroup of DDLS (55 vs. 34; p = 0.0020). Among the 48 cases from the untreated high-grade STS cohort that were immunostained for PD-L1 (Table S1), detection of PD-L1-positive tumor cells correlated significantly with PD-L1 CNG (p = 0.0011). Of the 10 tumors with PD-L1 immunoreactivity in tumor cells, four showed PD-L1 CNG. The remaining 38 cases showed no PD-L1 CNG and were negative for PD-L1 protein expression.

Prognostic impact of PD-L1 copy number gains

PD-L1 CNG were associated with significantly inferior overall survival across the entire TCGA sarcoma cohort (HR = 1.82; p = 0.025; Fig. 5A) and in the subgroup of LMS (HR = 2.84; p = 0.013; Fig. 5B). Separate analysis of focal, chromosome 9p, and chromosome 9 alterations revealed that only chromosome 9p gains were significantly associated with poorer outcome in the entire TCGA sarcoma cohort (HR = 5.12; p = 2.2E-05; Fig. 5C) and in LMS (HR = 6.01; p = 0.00053; Fig. 5D).

Expression of immune genes in the tumor microenvironment

To explore the role of immune cells, we investigated the expression of T-cell- (CD3, CD4, CD8) and macrophage (CD4, CD68) markers as well as PD-L1 and PD-L1 transcript levels in the TCGA cohort (Fig. 6A and B). CD3 chains (CD3D, CD3E, CD3G, and CD3Z (CD247) were strongly co-expressed with CD8 (CD8A, CD8B) with Pearson correlations, whereas the expression of macrophage markers only partly overlapped with that of T-cell-specific genes. PD-L1 showed a distinctly different expression...
pattern compared with the other immune genes \((R < 0.55\) for all pairwise Pearson correlations). The described expression patterns were observed in the entire TCGA cohort and specifically in LMS.

**Prognostic impact of immune genes**

The mRNA levels of the different T-cell-related genes were positively correlated with overall survival both in the entire TCGA cohort and specifically in LMS. For example, the HR per doubling of mRNA expression of **CD3D** were **0.89** (confidence interval \([CI]\): 0.82–0.97; \(p = 0.0095\)) in the entire cohort and **0.85** (CI: 0.74–0.97; \(p = 0.017\)) in LMS. There was no significant correlation between **CD4** or **CD68** expression and prognosis in the entire cohort or in LMS. The **CD4/CD8** ratio was not prognostic in the entire cohort, but associated with significantly inferior outcome in LMS (HR = 1.34; CI, 1.09–1.65; \(p = 0.0053\)).

**Prognostic impact of PD-L1 CNG and CD3Z levels**

Finally, we sought to investigate the combined impact of immune gene expression and **PD-L1** copy number status on prognosis. To this end, we stratified patients from the TCGA cohort by combining **CD3Z** mRNA expression and **PD-L1** copy number status. This analysis demonstrated that low **CD3Z** expression in combination with **PD-L1** CNG was a negative prognostic factor (Fig. 6C and D). Specifically, the outcome of tumors with **PD-L1** CNG and low **CD3Z** levels was inferior compared with that of **CD3Z** low/ **PD-L1** normal/loss (HR = 1.8; \(p = 0.049\)), **CD3Z** high/ **PD-L1** normal/loss (HR = 3.08; \(p = 0.00019\)), or **CD3Z** high/ **PD-L1** CNG tumors (HR = 3.29; \(p = 0.0098\)). Similar associations of these two markers with prognosis were found in the subgroup of patients with LMS (HR = 2.92 (\(p = 0.024\)), HR = 6.8 (\(p = 0.0001\)), and HR = 6.16 (\(p = 0.057\)).

**Discussion**

We here report the first analysis of **PD-L1** CNG across major STS subtypes and analyzed associations with **PD-L1** expression levels, mutational load, immune infiltrates, as well as survival. We observed a significant correlation of **PD-L1** CNG with increased **PD-L1** expression across all STS subtypes and for UPS and LMS in particular. These data are in line with our previous analysis of 22 major cancer types,23 and in the light of

Figure 3. **PD-L1** mRNA expression in the TCGA cohort (A) and in the independent cohort of high-grade STS (B). In the TCGA cohort, sarcomas with **PD-L1** CNG showed higher **PD-L1** expression than STS without **PD-L1** CNG (fold change, 1.8; \(p = 0.02\)). **PD-L1** expression was significantly higher in MFS compared with DDLS (fold change, 2.2; \(p = 0.036\)) and SS (fold change, 12.2; \(p = 0.00012\)). Furthermore, **PD-L1** expression in LMS was significantly higher than in DDLS (fold change, 1.5; \(p = 0.039\)) and SS (fold change, 8.6; \(p = 0.00047\)). In the high-grade STS cohort, LMS and UPS cases showed highest **PD-L1** expression levels, whereas **PD-L1** expression was significantly lower (\(p < 0.05\)) in MLS compared with all other subtypes except MPNST.

Figure 4. Analysis of mutational load in STS (TCGA cohort). (A) The median number of mutated genes was highest in MFS, followed by MPNST, UPS, LMS, DDLS, and SS. (B) Sarcomas with **PD-L1** CNG and **PD-L1** copy number losses showed a significantly higher mutational load compared with tumors without **PD-L1** CNG.
recent trial data of patients with relapsed or refractory Hodgkin lymphoma, it is reasonable to argue that PD-L1 CNG could also be predictive of response toward immune checkpoint blockade in STS. In line with this notion, a recent study from our group identified a patient with metastatic cancer of unknown primary and PD-L1 amplification, who achieved a durable response to PD-1 blockade.33 As the predictive power of PD-L1 expression varies considerably due to a multitude of reasons, including diagnostic assays, antibody clones, and tumor types,34,35 these data collectively open up new avenues for identifying adjunct parameters that may improve response prediction.

In keeping with a recent report from Roemer et al. on the prognostic significance of PD-L1 and PD-L2 alterations in classical Hodgkin lymphoma,36 we found that chromosome 9p gains were associated with poor survival in the entire set of STS patients investigated and particularly in patients with LMS. These observations underscore the biological relevance of 9p amplification and, in particular, focal amplification of PD-L1 and framing genes,23,32 which likely confer oncogenic properties by facilitating evasion of 9p/PD-L1-amplified cancer cells from attack by the immune system.

We also identified 27 genes framing PD-L1 that are co-amplified in more than 80% of STS cases with PD-L1 CNG. Hence, it is likely that other genes beyond PD-L1 contribute to a specific tumor biology that confers a dismal prognosis in patients with 9p-amplified STS. Importantly, some of these putative “co-drivers” might represent therapeutic targets. For instance, a recent report by Balko et al.37 showed that triple-negative breast cancers harboring 9p24 amplifications are dependent on JAK2 signaling. KDM4C, encoding a lysine demethylase, was recently reported to facilitate leukemic transformation, and combined inhibition of PRMT1 and KDM4C abrogated aberrant transcriptional programs mediating leukemia development in mice.38 In addition, KDM4C appears to be implicated in regulating chromosome segregation, thereby influencing genomic instability.39 Conversely, the chromosome 9p gains observed here might also be the result of a more general mechanism of genetic instability surgical in STS.

The varying incidence of PD-L1 CNG between the TCGA data set and the high-grade STS cohort may be attributed to differences in the respective patient populations. For example, the histologic subtypes most commonly associated with PD-L1 CNG (UPS, DDLS, and LMS) account for 82% and 44% of cases in the TCGA cohort and in the high-grade STS cohort, respectively. Furthermore, the high-grade STS cohort is exclusively composed of untreated high-grade sarcomas, whereas the TCGA cohort also includes lower-grade tumors and possibly pre-treated cases. Of particular clinical relevance, immunohistochemistry analysis of 48 cases from the untreated high-grade STS cohort revealed a significant association between PD-L1 CNG and protein expression. As expected, we also observed cases without CNG that express PD-L1, reflecting other mechanisms of PD-L1 upregulation described in the literature.15 In this context, it is important to note that we cannot exclude some bias in the immunohistochemical results as the untreated high-grade STS cases had been selected for high tumor cell content and particularly low immune cell levels to facilitate optimal aCGH analysis.31 Additionally, it is well known that different antibody clones yield different staining results that may influence immunohistochemistry-based expression analysis, and PD-L1 levels may show spatial intratumoral heterogeneity,40,41 a point that we addressed by using whole-tissue slides instead of a tissue microarray.

Mutational load has gained considerable attention as surrogate marker for neoantigens that stimulate the immune system, and several tumor types with particularly high mutational burden have been identified.42,43 Several clinical studies showed that assessment of mutational load can be used to predict response to immunotherapies in a variety of solid tumors, including

Figure 5. Prognostic impact of PD-L1 CNG in STS (TCGA cohort). PD-L1 CNG were associated with shortened overall survival in (A) the entire TCGA cohort (162 patients, 56 events) and (B) in the LMS subcohort 63 patients, 22 events). Chromosome 9p gains were associated with shortened overall survival (C) in the entire TCGA cohort (162 patients, 56 events) and (D) in the LMS subcohort (63 patients, 22 events).
microsatellite instability-driven colorectal cancer, melanoma, non-small cell lung cancer, and possibly viral antigen-negative Merkel cell carcinoma. While we noted statistically significant differences in mutational load among STS subtypes, with SS showing fewer mutations and MFS exhibiting a relatively high mutational burden, our analysis suggests that the number of non-synonymous mutations per se might not be an ideal predictor of response to immune checkpoint inhibition, as the range of mutational loads across subtypes is rather narrow and only few cases harbored 100 or more mutated genes. However, as some STS subtypes in the TCGA data set, e.g. MPNST and SS, are represented by very few cases, the data might be too preliminary to draw definite conclusions. The same notion might apply to the prevalence of PD-L1 CNG in MPNST, SS, and MFS, where only 9, 10, and 23 cases were available.

In the TCGA cohort, we found T-cell infiltrates in STS to correlate with improved survival, a phenomenon that has been observed in several tumor entities. Interestingly, T-cell infiltrates also appear to modify the prognostic value of PD-L1 CNG in STS, a finding that is in keeping with the concept that both the levels and composition of immune cell infiltrates are key components in the multifaceted interaction between the immune system and cancer cells, which shapes the biological behavior of the tumor. Immune infiltrates have also been reported to predict therapy response, which raises the intriguing question whether STS cases with a "hot" microenvironment may respond differently to immune checkpoint blockade compared with "cold" tumors.

Our analysis might be of particular interest in light of preliminary data from SARC028, a phase II trial investigating the efficacy and safety of pembrolizumab in advanced STS, which demonstrated that a substantial proportion of patients with UPS, LMS, SS, and liposarcoma either responded or had stable disease. Considering that current therapeutic options for advanced STS are limited, these results suggest that immune checkpoint inhibitors might be a viable therapeutic option in a relevant fraction of STS patients. However, an important challenge will be to identify patients most likely to benefit from PD-1/PD-L1 blockade. Our results suggest that studies such as SARC028 might be ideally suited to assess the predictive value of PD-L1 CNG and the composition of immune infiltrates in STS, a tempting question that we could not answer by our analysis as therapy data for patients included in the TCGA sarcoma cohort were not available and immune checkpoint inhibition was no standard of care at initiation of the TCGA study.
In summary, together with the encouraging data on the efficacy of the PD-1 blocker pembrolizumab in advanced STS, our results support further exploration of the predictive power of PD-L1 CNG, mutational load, and immune cell infiltrates in STS to broaden the therapeutic armamentarium for this challenging group of diseases.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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