Original Article

Prognostic attributes of immune signatures in soft tissue sarcomas show differential dependencies on tumor mutational burden

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BACKGROUND: Cellular and intrinsic markers of sarcoma immunogenicity are poorly understood. To gain insight into whether tumor–immune interactions correlate with clinical aggressiveness, the authors examined the prognostic significance of immune gene signatures in combination with tumor mutational burden (TMB) and cancer–testis antigen (CTA) expression. **METHODS:** RNA sequencing and clinical data of 259 soft tissue sarcomas from The Cancer Genome Atlas project were used to investigate associations between published immune gene signatures and patient overall survival (OS) in the contexts of TMB, as computed from whole-exome sequencing data, and CTA gene expression. Multivariate Cox proportional hazards regression models and log-rank tests were used to assess survival associations. **RESULTS:** Immune signature scores that reflected in part the intratumoral abundance of cytotoxic T cells showed significant positive associations with OS. However, the prognostic power of the T-cell signatures was highly dependent on TMB-high status, consistent with protective effects of tumor-infiltrating T cells in tumors with elevated antigenicity. In TMB-low tumors, a signature of infiltrating plasma B cells was significantly and positively associated with OS, independent of T-cell signature status. Although tumor subtypes based on differential expression patterns of CTA genes showed different survival associations within leiomyosarcoma and myxofibrosarcoma histologies, neither CTA nor histologic subtype interacted with the T-cell–survival association. **CONCLUSIONS:** Signatures of T-cell and plasma B-cell infiltrates were associated with a survival benefit in soft tissue sarcomas. TMB, but not CTA expression, influenced the prognostic power of T-cell–associated, but not plasma B-cell–associated, survival. ***Cancer* 2022;128:3254-3264**.

LAY SUMMARY:

- Clinical data and RNA analysis of 259 soft tissue sarcomas from The Cancer Genome Atlas project were used to investigate associations between five published gene immune cell expression signatures and survival in the context of tumor mutations.
- Activated T cells had a significant positive association with patient survival.
- Although high tumor mutation burden was associated with good survival, the prognostic power of T-cell signatures was highly dependent on tumor mutational status, consistent with protective effects of tumor-infiltrating T cells in tumors with high levels of antigens.
- In low tumor mutation-bearing tumors, plasma B cells were positively associated with survival.

KEYWORDS: burden, immune, mutational, prognosis, signatures, tumor.

INTRODUCTION

Soft tissue sarcomas are refractory malignancies characterized not only by local recurrence but also by metastasis. Over 50 histologic subtypes have been identified. Despite several recent advances, the outcome for patients with recurrence and/or metastasis remains poor, with a reported median overall survival (OS) from 14 to 17 months.[1–3] Immunotherapy has been an attractive approach to refractory cancers, and sarcoma is considered to be the first cancer for which immunotherapy was effectively applied.[4] However, the efficacy of immunotherapy in soft tissue sarcomas has been limited. Immune checkpoint blockade with antibodies that target cytotoxic T-lymphocyte–associated antigen 4 and the programmed cell death protein 1 (PD-1) pathway is leading to durable clinical responses in an increasing number of cancers. Although clinical responses in patients with soft tissue sarcoma have been observed, they are infrequent.[1–9] Identifying patients who

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may benefit has been difficult. Biomarkers that can be applied for patient selection and for developing approaches to treatment resistance are needed.

Tumor-infiltrating lymphocytes have been recognized as a positive prognostic marker in several cancers and as a predictive marker for checkpoint inhibitor response.[10,11] The presence of tumor-infiltrating lymphocytes in soft tissue sarcomas has been associated with an improved prognosis in some studies[12,13]; in others, an association has not been observed.[6,14,15] Gene expression profiling studies have increasingly been applied to characterize the abundance and function of tumor-infiltrating immune cells in solid tumors and their relation to prognosis and treatment response.[16,17] Petitprez et al. identified a B-lineage gene signature in soft tissue sarcomas that was associated with improved survival and clinical response to PD-1 blockade.[18]

Mutations and the neoantigens they generate are among the tumor-intrinsic alterations that promote adaptive immunity, and tumor mutational burden (TMB) has been associated with treatment response to immune checkpoint inhibitors.[19–22] Although soft tissue sarcomas overall are in the low range of solid tumors with regard to TMB, some with relatively high numbers of mutations have been identified.[23–25] Soft tissue sarcomas express cancer–testis antigens (CTAs), which are immunogenic because they are not normally expressed by somatic tissue cells in adults.[26–28] Immune responses to the CTA NY-ESO1 have been associated with clinical response to checkpoint inhibitors in patients with melanoma.[29,30] High levels of several CTAs, including NY-ESO-1, have been observed in soft tissue sarcoma.[31] How TMB and CTA expression levels influence soft tissue sarcoma immunogenicity is not known.

We have described clusters of coordinately expressed genes, referred to as *immune metagenes*, that reflect tumor-infiltrating populations of various effector-immune cell types, including T cells, natural killer (NK) cells, B cells, macrophages, and dendritic cells; multivariate analyses have demonstrated that these signatures are significantly and independently associated with survival in breast cancer.[32–34] We examined the prognostic associations of multiple published T-cell–based gene signatures using RNA sequencing (RNA-Seq) expression profiles of soft tissue sarcomas. We furthered hypothesized that TMB and CTAs may influence the prognostic power of the immune subclasses. In this report, we demonstrate that TMB expression, but not CTA expression, can be used to discern beneficial versus nonbeneficial immune configurations in soft tissue sarcomas and that a plasma B-cell gene signature is associated with survival benefit even in the context of TMB-low tumors.

MATERIALS AND METHODS

RNA-Seq data acquisition, processing, and TMB annotation

The Cancer Genome Atlas (TCGA) sarcoma data set (SARC), consisting of 259 de-identified RNA-Seq tumor expression profiles and corresponding clinical data from a multi-institutional cohort of patients with soft tissue sarcoma from the TCGA project, was used in this study.[35] Level 3 Illumina Hi-Seq RNA-Seq V2 data were accessed from the Firebrowse resource.[36] RNA-Seq data were processed by using MapSplice alignment,[37] normalization using RSEM software,[35] and $\log_2$ transformation (with pseudocount +1). Patient survival data were updated according to the TCGA clinical data resource.[38] Updated TCGA TMB calculations from two studies,[39,40] computed from whole-exome sequencing data as the rate of nonsynonymous mutations per megabase (Mb) of sequenced DNA, were integrated by averaging ($n = 251$ tumors with TMB annotation). To investigate the prognostic power of the T-cell signatures as a function of TMB status, the mean TMB score in the population was used to divide patients into TMB-low and TMB-high groups, thereby categorizing patients within the upper 17.5% of TMB scores as the TMB-high group. To investigate the appropriateness of using the mean TMB as a cutoff point, we considered the impact of using alternative TMB cutoff points based on five-percentile increments either side of the population's mean and using Cox regression to evaluate the prognostic significance of the T-cell/NK cell (T/NK) signature (see Table S1). We observed that, whereas the T/NK signature remains significant at all cutoff points tested, the hazard ratios (HRs) increase (relative to the HR from the mean cutoff point) as the size of the TMB-high group increases in number. This corresponds to a decrease in prognostic effect size because patients with TMB below the mean are added to the TMB-high group. As the TMB-high group becomes smaller in size (i.e., more selective for patients with above-mean TMB), the HR of greatest effect (0.39) is achieved at 5% above the mean (equating with 12.4% of the population). Beyond this cutoff point, sample size becomes limiting. These data indicate the general suitability of using a cutoff point at the mean or within five-percentile increments either side of the mean.

Derivation of immune gene signatures

T/NK, B-cell/plasma cell (B/P), and myeloid/dendritic cell (M/D) immune subclasses were defined by using a gene-expression–based classification system. Continuous scores corresponding to these and to

published immune gene signatures, including effector T cells (T-eff),[41] cells with immune cytologic activity (CYT),[21] CD8-positive T cells (CD8 + T),[42] and type 1 T-helper cells (Th1),[43] were calculated for each tumor using the geometric mean of the $\log_2$-normalized RNA-Seq counts. Tumors/patients corresponding to low, intermediate, or high signature scores were assigned to groups by a ranking method using tertile cutoff points (for the signature scores) in the full population. For the B/P signature, immunoglobulin-related gene annotations were not available for the TCGA RNA-Seq data from the Firebrowse resource; therefore, B/P signature scores were computed for the TCGA cohort using the high-throughput (HT)-Seq–derived, FPKM-UQ–normalized TCGA RNA-Seq data and gene annotations downloaded from the National Institutes of Health Genomic Data Commons data portal (https://portal.gdc.cancer.gov/; accessed February 2, 2020).

Cancer–testis antigen gene analysis

In total, 276 CTA genes selected from the CTDatabase of the Ludwig Institute for Cancer Research (http://www.cta.lncc.br) as of April 12, 2020 were analyzed in this study. We observed that CTA gene expression patterns varied widely but with some correlation structure and thus were used to cluster tumors into putative CTA subtypes using the k-means clustering algorithm implemented in Cluster version 2.11.[44] Through repeated assessment using different hyperparameters, three CTA clusters gave the most common solution.

Statistical analyses

Kaplan–Meier (log-rank test) and multivariate Cox proportional hazards regression were used to assess associations between OS and gene signature scores or various patient or tumor characteristics. Relations among categorical variables used to define patient subgroups were compared by using nonparametric statistics and χ^2 or Fisher exact tests (2-sided).

RESULTS

T-cell signatures are robustly associated with OS in sarcoma

To investigate the prognostic attributes of tumor-infiltrating T cells in soft tissue sarcoma, five previously described T-cell–based gene signatures reflecting CD8 + T cytolytic activity, T-eff,[43] CYT,[21] and CD8 + T[43] or the combination of cytolytic activity and Th1 adaptive immunity, Th1,[44] and T/NK,[45] were analyzed in the TCGA RNA-Seq data set consisting of tumor expression profiles from 259 patients.

TABLE 1. Demographic and Clinical Variables, Tumor Mutational Burden Status Groups, and Cancer–Testis Antigen Molecular Subtypes Associated with The Cancer Genome Atlas cohort

Variable	No. of patients, $n = 259$
Histology	
Leiomyosarcoma	104
Dedifferentiated liposarcoma	58
Undifferentiated pleomorphic sarcoma	51
Myxofibrosarcoma	25
Synovial sarcoma	10
Malignant peripheral nerve sheath tumor	9
Desmoid tumor	2
Sex	
Men	118
Women	141
Age, years	
20–39	22
40–59	93
60–79	117
80–89	26
NA	1
Tumor size, cm	
0.5–10.0	123
10.1–20.0	95
20.1–40.0	30
NA	11
Residual disease	
R0	154
R1	69
R2	9
NA	27
Mutation rate per Mb DNA	
0.05–0.20	6
0.21–0.59	66
0.60–0.79	46
0.8–1.09	65
1.1–1.7	54
2.0–10.0	10
11.0–20.0	3
>20.0	1
NA	8
CTA subtype	
CTA1	189
CTA2	21
CTA3	49

Abbreviations: CTA, cancer–testis antigen; Mb, megabase; NA, not applicable/unknown.

Demographic and clinical variables as well as TMB status groups and CTA molecular subtypes associated with the TCGA cohort are displayed in Table 1. A continuous score based on the geometric mean of the normalized expression values of the gene sets comprising each of the T-cell signatures (ie, a signature score) was calculated (Fig. 1A). Pairwise comparisons of T-cell signature scores showed a high degree of cross-signature correlation (Spearman $\rho > 0.92$ for all pairwise comparisons; Fig. 1B), consistent with their representation of T-cell relative abundance in tumors. In multivariate Cox models, each signature remained independently associated with OS ($p < .05$) in the presence of clinical

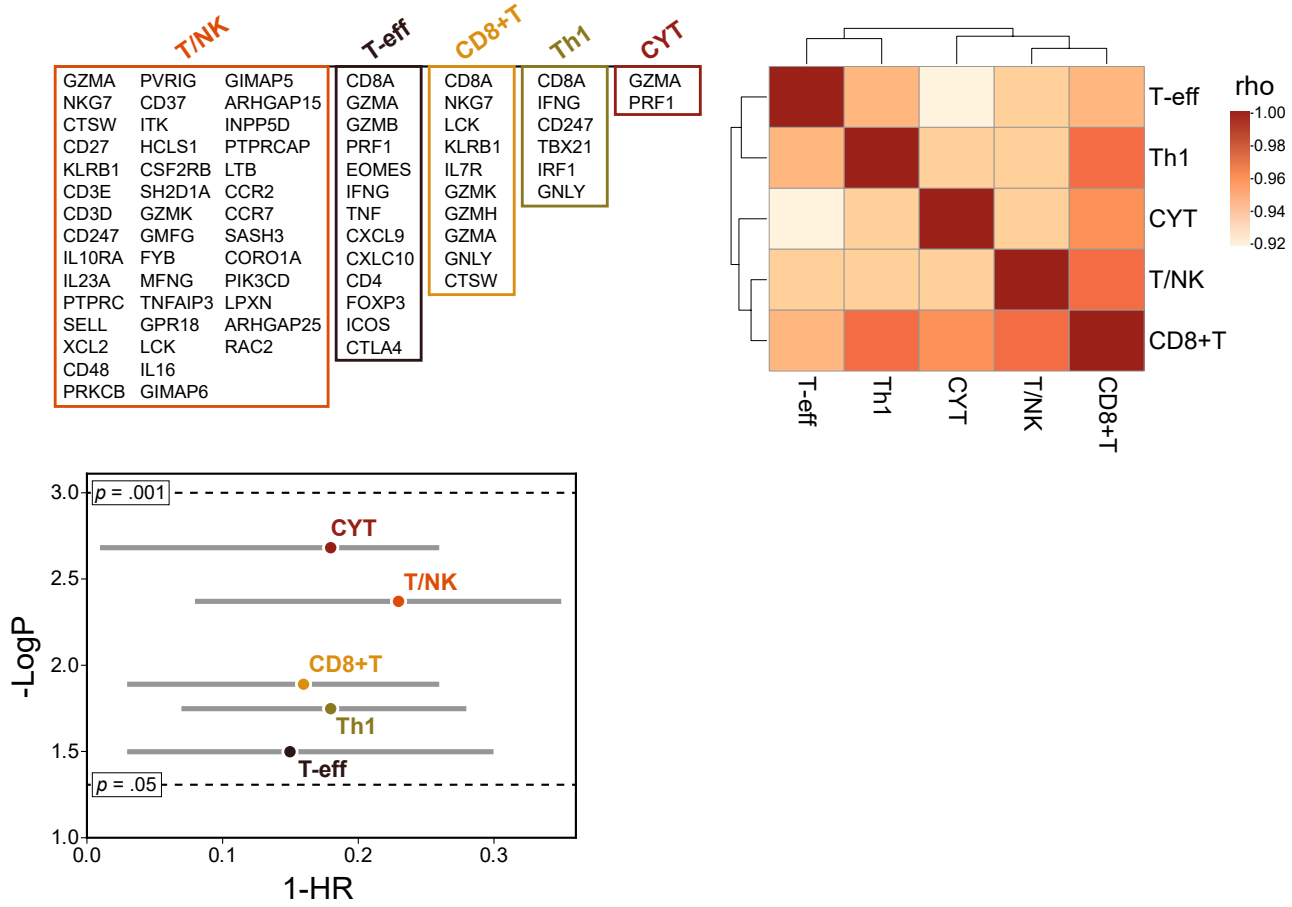

Figure 1. Correlative and prognostic attributes of T-cell signatures in soft tissue sarcomas. (A) Reported T-cell–related gene expression signatures and the genes that comprise them. (B) Correlation matrix heatmap based on Spearman analysis of expression signature scores in The Cancer Genome Atlas data set. Scores were computed for each tumor (n = 259) from the geometric mean of the signature gene expression values. (C) Plot of T-cell signature results from multivariate Cox regression models. Shown for each signature are hazard ratios (HRs) plotted against Wald test-negative log p values (–LogP). CD8+T indicates CD8-positive T-cells; CYT, cells with immune cytolytic activity; T/NK, T-cells/natural killer cells; T-eff, effector T cells; Th1, type 1T-helper cells.

variables, including patient age, sex, residual disease, tumor length, and histologic type (Fig. 1C). Among the five signatures, CYT, T/NK, and CD8+T showed the highest degree of positive correlation (Fig. 1B) and had the most significant associations with OS (Fig. 1C).

TMB is a determinant of immune-associated survival

We next sought to determine whether TMB could influence the prognostic significance of the T-cell signatures, as previously reported for immune effector gene signatures in breast cancer.[24] The TMB for the TCGA patient population ranged from 0.05 to 40.9 nonsynonymous mutations per Mb of DNA, with a mean TMB of 1.29. This mean value was used as a threshold for assigning patients to low TMB (TMB-low; n = 207) or high TMB (TMB-high; n = 44) categories (see Materials and Methods, above). Then we evaluated the association between TMB and the OS of patients stratified by T-cell signature scores into low, intermediate, and high score tertiles (based on the entire population). Strikingly, the significance of the associations between T-cell signature tertiles and survival was lost in TMB-low tumors (Fig. 2A,C,E) but remained highly significant in TMB-high tumors (Fig. 2B,D,F), indicating that the significance of the signature scores achieved by Cox regression analysis (Fig. 1C) depended largely on TMB status. Furthermore, we observed that, among patients who had high T cell-signature scores, those associated with TMB-high status exhibited more favorable survival than those classified as TMB-low. We therefore examined the

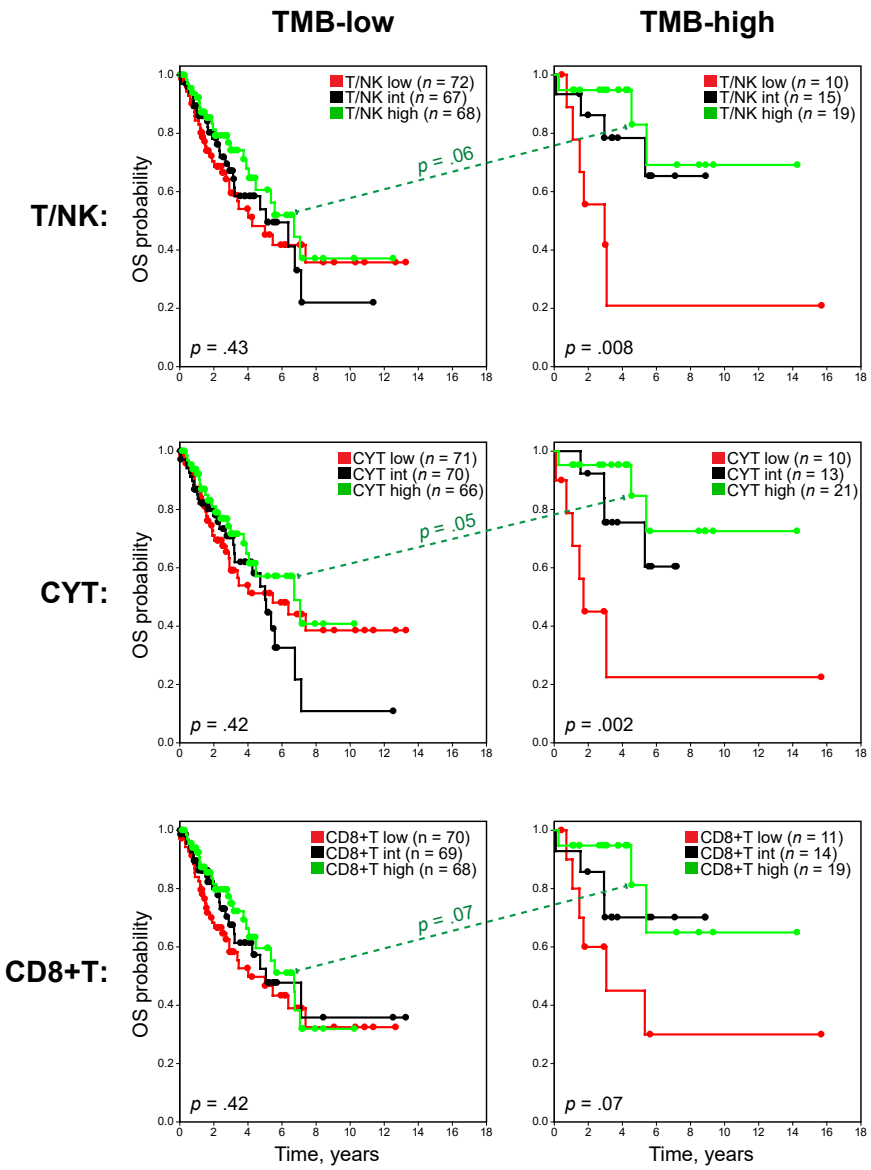

Figure 2. T-cell signature tertiles are prognostic in tumor mutational burden (TMB)-high tumors but not in TMB-low tumors. T-cell signature scores were used to partition patients from The Cancer Genome Atlas cohort into signature tertiles for Kaplan–Meier analyses. Results for the T-cell/natural killer cell (T/NK), cells with immune cytolytic activity (CYT), and CD8-positive T-cell (CD8+T) signatures are shown. Overall survival (OS) rates are compared among patients classified in the full cohort with low (red), intermediate (int; black), and high (green) signature tertiles in the context of (A,C,E) TMB-low tumors and (B,D,F) TMB-high tumors. Log-rank *p* values are shown for each plot (black) and for comparisons of signature-high tertiles between TMB-low and TMB-high strata (green).

prognostic status of TMB itself. As a continuous score, TMB exhibited no significant survival association in univariate Cox regression ($p = .25$; HR, 0.91; 95% CI, 0.77–1.07) or multivariate Cox regression ($p = .11$; HR, 0.71; 95% CI, 0.46–1.09; see Table S2). As a categorical variable, however, TMB-high (relative to TMB-low) status exhibited a near-significant association with improved survival ($p = .08$; Fig. 3D); and, in the presence of histologic type, sex, age, tumor size, and residual disease, TMB-high status achieved a significant association with improved survival ($p = .008$; HR, 0.39; 95% CI, 0.19–0.78; see Table S3). These findings suggest the possibility that high TMB may be moderately associated with improved survival in soft tissue sarcoma, although further analysis of this association using alternative TMB cutoff points in larger populations is warranted.

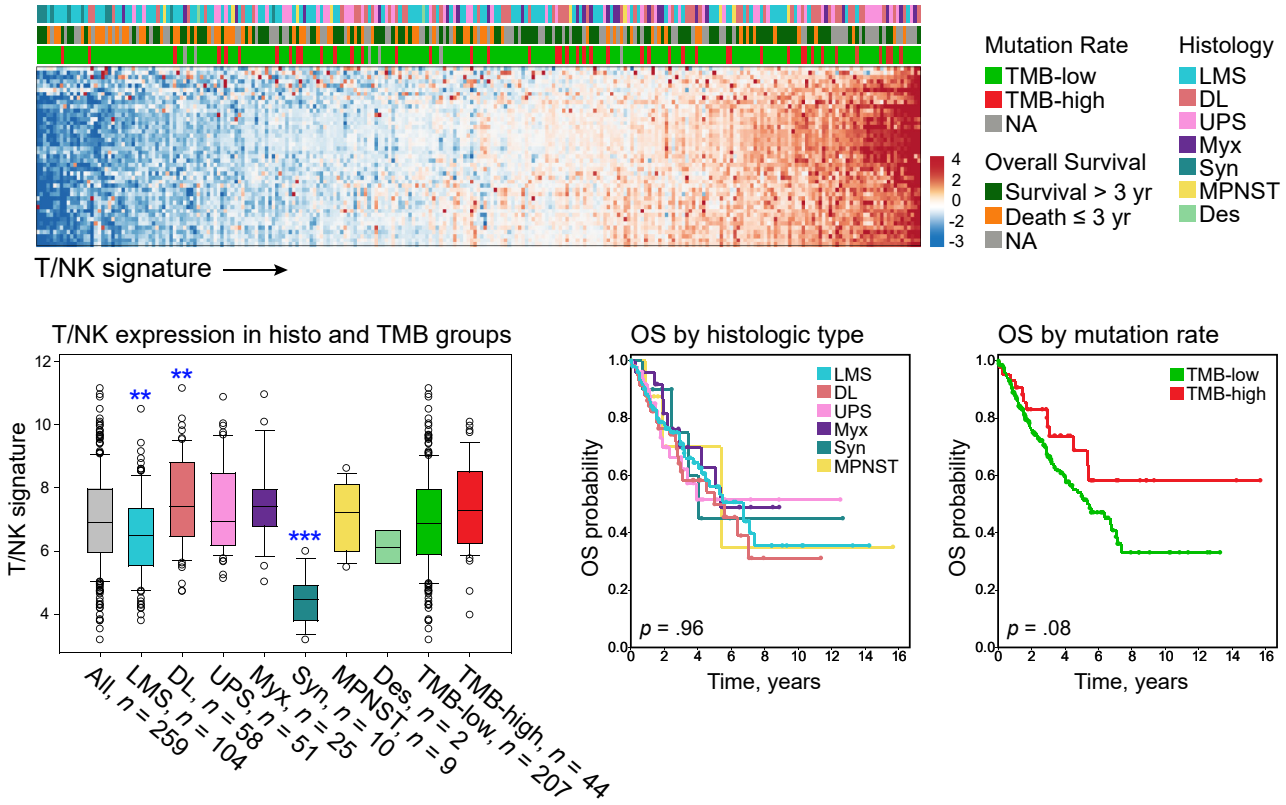

Figure 3. Analysis of T-cell signature scores, sarcoma histology, and survival associations. (A) Heatmap of The Cancer Genome Atlas (TCGA) cohort tumor expression profiles (columns) and T-cell/natural killer cell (T/NK) signature genes (rows) with cases stratified (from left to right) by T/NK score. Annotations for TCGA histologic type, overall survival (OS) outcome, and mutation rate status are indicated in the top three rows. (B) Comparison of T/NK signature score distributions among histologic types and tumor mutational burden (TMB) status groups. Statistical comparisons were made between individual histologic groups and the whole cohort (All; $n = 259$ cases). TMB-low was compared with TMB-high (two asterisks indicate $p < .01$; three asterisks, $p < .001$). (C) Kaplan–Meier analysis of patient OS by histologic type. (D) Kaplan–Meier analysis of patient OS by TMB status (low vs. high). Log-rank test p values are shown. Des indicates desmoid tumor; DL, dedifferentiated liposarcoma; LMS, leiomyosarcoma; MPNST, malignant peripheral nerve sheath tumor; Myx, myxofibrosarcoma; NA, not available; Syn, synovial sarcoma; UPS, undifferentiated pleomorphic sarcoma.

T-cell signature score distributions vary by histologic subtype but not by TMB

Pathologic annotation of the TCGA series consisted of seven common histologic subtypes: leiomyosarcoma, dedifferentiated liposarcoma, undifferentiated pleomorphic sarcoma, myxofibrosarcoma, synovial sarcoma, malignant peripheral nerve sheath tumor, and desmoid tumor. Although T-cell signature–survival associations could not be adequately evaluated within histologic subtypes because of small sample sizes, the distribution of T-cell signature scores (as represented by the T/NK signature) were evaluated as a function of histologic subtype or TMB group (Fig. 3A,B). Among the histologic subtypes, although no intersubtype survival differences were observed (Fig. 3C), significantly different T/NK distributions were noted (Fig. 3B), with lower T/NK scores occurring in the synovial sarcoma and leiomyosarcoma subtypes ($p < .001$ and $p < .01$, respectively) and higher T/NK scores occurring in the dedifferentiated liposarcoma subtype ($p < .01$). T/NK scores did not vary significantly between TMB-low and TMB-high groups.

The B/P signature is prognostic of survival independent of TMB status

The activation and infiltration of effector T cells is associated with productive antitumor immunity and is the target of efficacious immunotherapy. This association is believed to depend largely on TMB status and likely underlies our observation of improved survival among patients who had sarcoma with TMB-high tumors and with

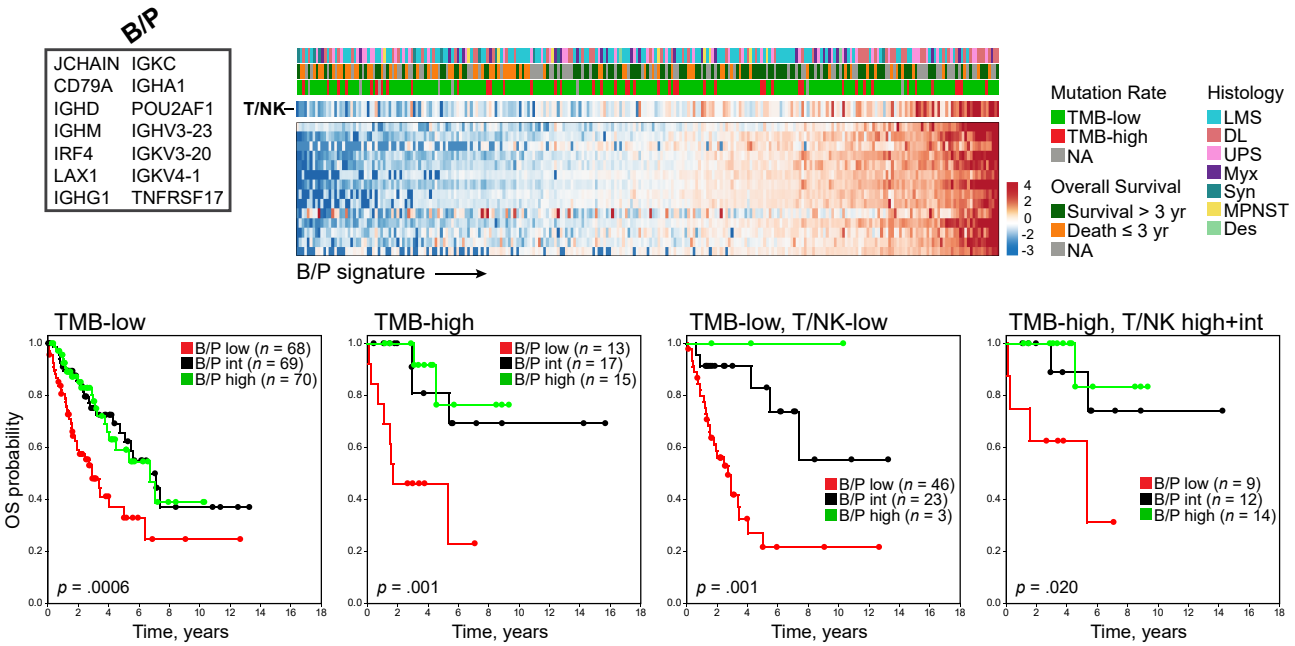

Figure 4. Prognostic significance of a B-cell signature in the The Cancer Genome Atlas (TCGA) sarcoma database (SARC). (A) Genes comprising the previously described B-cell/plasma cell (B/P) signature are shown. (B) Heatmap of the TCGA cohort tumor expression profiles (columns) and B/P signature genes (rows) with cases stratified (from left to right) by B/P signature score. Annotations for TCGA histologic type, overall survival outcome, mutation rate status, and T-cell/natural killer cell (T/NK) score are indicated in the top four rows. Survival rates of patients in the TCGA cohort stratified into B/P score low, intermediate (int), and high tertile groups were analyzed by Kaplan–Meier analysis in (C) cases with low TMB, (D) cases with high TMB, (E) cases with low TMB and low T/NK tertile scores, and (F) cases with high TMB and high-to-intermediate T/NK tertile scores. Log-rank test *p* values are shown. Des indicates desmoid tumor; DL, dedifferentiated liposarcoma; LMS, leiomyosarcoma; MPNST, malignant peripheral nerve sheath tumor; Myx, myxofibrosarcoma; NA, not available; Syn, synovial sarcoma; UPS, undifferentiated pleomorphic sarcoma.

high T-cell signature scores. Therefore, we asked whether other mediators of antitumor immunity may affect prognosis in a T-cell–dependent or TMB-dependent manner. In previous studies in breast cancer, we identified the T/NK signature in conjunction with prognostic signatures of B/P cells marked by IgG antibody isotype-related genes, and M/D cell populations marked by myeloid markers and major histocompatibility complex Class II antigen-presenting molecules; in multivariate models, the prognostic power of these signatures remained largely independent of one another.[33] Therefore, we asked whether the B/P or M/D signatures exhibited prognostic associations in the TCGA series relative to TMB and the T/NK signature. Although the M/D signature was nonsignificant, the B/P signature exhibited marked prognostic power in TCGA that, in contrast to the T/NK signature, exhibited prognostic significance that was independent of TMB status (Fig. 4A–D). Intermediate-to-high B/P score tertiles were associated with improved survival regardless of TMB status, but with the greatest effect in TMB-high tumors (Fig. 4C,D). Remarkably, in TMB-low tumors with low T/NK scores, intermediate-to-high B/P scores were associated with significantly enhanced patient survival (Fig. 4E); whereas, in TMB-high tumors with intermediate-to-high T/NK scores, the B/P signature added further prognostic strength (Fig. 4F).

CTA subtypes show histology-dependent survival associations

CTAs are aberrantly expressed in some tumors, including sarcomas, in which they can initiate and sustain immune responses. We examined the expression patterns of 276 documented CTAs in the TCGA series for survival associations. Individually, no notable CTA gene–survival associations could be observed after false-discovery correction. Therefore, we considered a more global approach to investigate CTA expression–survival associations based on CTA gene expression profiles. By using k-means clustering, we identified three predominating CTA tumor expression profiles, or CTA subtypes, in TCGA which we termed CTA-1, CTA-2 and CTA-3 (Fig. 5A). We asked whether or not the CTA subtypes showed interactions with the T/NK signature–survival association or whether the subtypes

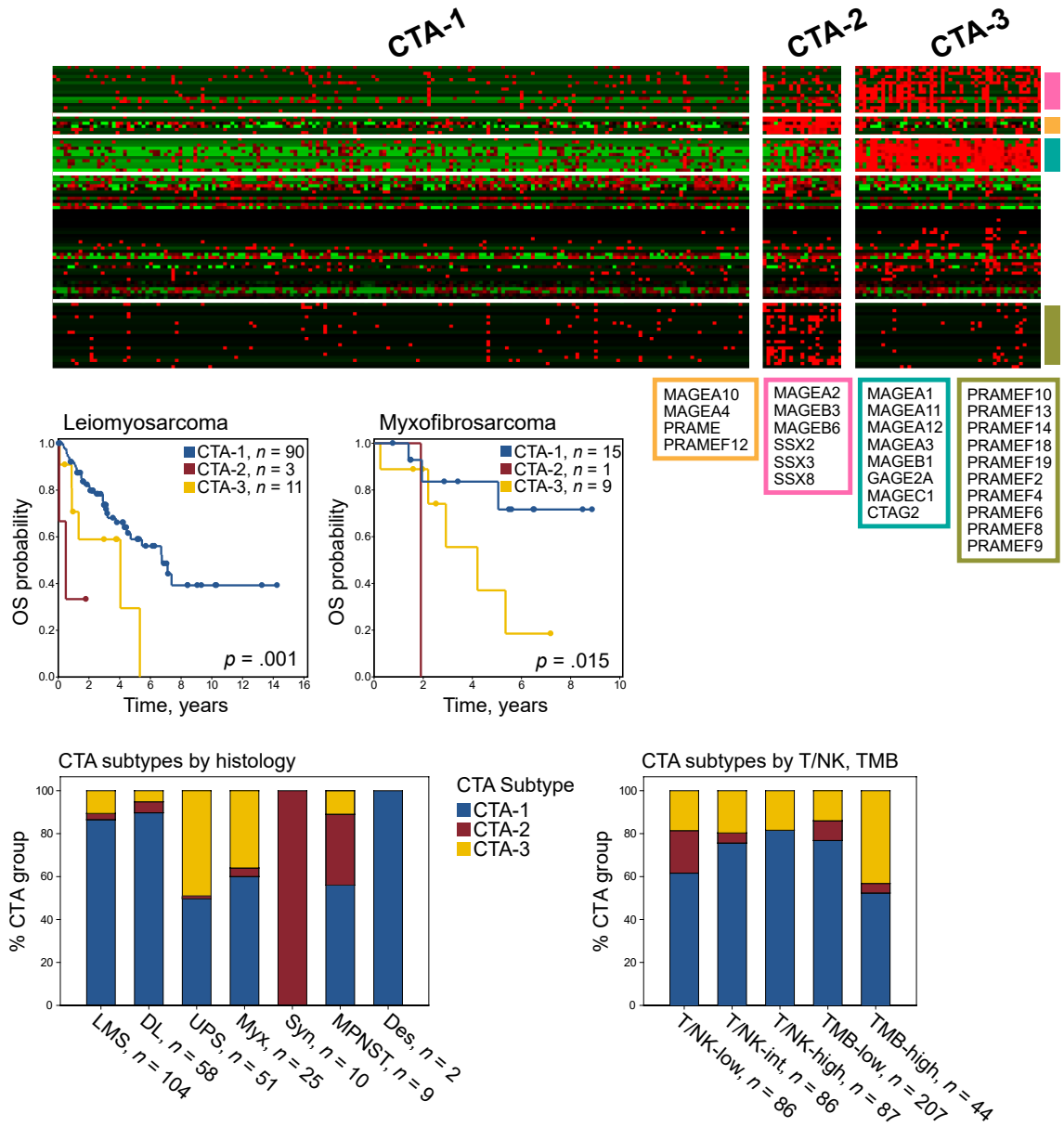

Figure 5. Analysis of cancer–testis antigen (CTA) molecular subtypes as a function of histologic type, T-cell/natural killer cell (T/NK) tertile, and tumor mutational burden (TMB) status. (A) CTA gene expression profiles in The Cancer Genome Atlas (TCGA) cohort were used to identify sarcoma CTA molecular subtypes (CTA-1, CTA-2, and CTA-3) by k-means clustering. Genes that contributed most to subtype differentiation are indicated in colored boxes. Kaplan–Meier plots and log-rank *p* values comparing overall survival rates among TCGA CTA subtypes are shown for (B) leiomyosarcoma and (C) myxofibrosarcoma histologies, in which significant differences were observed. Relative proportions of cases representing the three CTA subtypes are shown by (D) histologic type and (E) T/NK or TMB strata. Des indicates desmoid tumor; DL, dedifferentiated liposarcoma; LMS, leiomyosarcoma; MPNST, malignant peripheral nerve sheath tumor; Myx, myxofibrosarcoma; OS, overall survival; Syn, synovial sarcoma; UPS, undifferentiated pleomorphic sarcoma.

were associated with sarcoma histologic types, patient survival, or T/NK or TMB groups. We did not observe evidence of survival-related interactions between the CTA subtypes and the T/NK signature. However, within the leiomyosarcoma and myxofibrosarcoma histologies, the CTA-2 and CTA-3 subtypes were associated with worse OS compared with the CTA-1 subtype (Fig. 5B,C). Furthermore, the CTA subtypes were significantly disproportionally represented in undifferentiated pleomorphic sarcomas (49% CTA-3)

and synovial sarcoma tumors (100% CTA-2), whereas CTA-2 was overrepresented in T/NK-low tumors (compared with T/NK-high tumors), and CTA-3 was overrepresented in TMB-high tumors (compared with TMB-low tumors; Fig. 5D,E).

DISCUSSION

The complex tumor–host interactions regulating immune response in solid tumors in general, and soft tissue sarcomas in particular, remain incompletely understood. Several intratumoral gene-based markers of T-cell activation and antigen presentation have been associated with clinical outcomes, but such markers have not been shown to be sufficiently prognostic or predictive to be clinically useful. The Cancer Genome Atlas Research Network analyzed 28 different immune cell signatures in 206 soft tissue sarcomas.[24] Improved survival was correlated with NK cell scores in leiomyosarcoma and in undifferentiated pleomorphic sarcoma, with CD8 cell scores in leiomyosarcoma, and with dendritic cell scores in undifferentiated pleomorphic sarcoma. In dedifferentiated liposarcoma, a Th2 signature was correlated with shorter survival. Pollack et al. could not demonstrate an association between genes related to T-cell infiltration and antigen presentation and survival in a study of 81 soft tissue sarcomas.[26] Petitprez. et al.[18] demonstrated five distinct immune-gene phenotypes in a study of 608 soft tissue sarcomas, and a B-lineage signature that correlated with an improved survival was identified in tumors with both high and low infiltration of CD8-positive T cells. It that report, the B-lineage signature was a hallmark of an *immune-high class* associated with clinical response to PD-1 blockade in a phase 2 soft tissue sarcoma clinical trial.

Here, we investigated the clinical relevance of prognostic T-cell–related signatures in patients who had soft tissue sarcoma by evaluating five expression signatures with previously reported survival associations. Among the five signatures, all associated positively with OS independent of conventional risk variables, whereas the CYT, T/NK, and CD8+T signatures showed the highest degree of positive correlation as well as the most significant associations with OS.

We next examined how measures of tumor antigenicity affect this observed T-cell–related survival. Although TMB has been associated with proinflammatory gene expression signatures in some studies, there are reports that the expression of mutational neoantigens and CTAs are comparable in T-cell–inflamed and noninflamed melanoma.[46] TMB appeared to be similar across all five sarcoma immune classes reported in Petitprez et al.[18] In our study, TMB for the patient population ranged from 0.05 to 40.9 nonsynonymous mutations per Mb of DNA, with a mean TMB of 1.29. Although TMB as a continuous score showed no survival association, TMB-high status (relative to TMB-low status) trended toward a univariate association with improved survival that became significant in the presence of histologic type as well as patient sex, age, tumor size, and residual disease. The difference in our study is that we determined that the prognostic power of a measure of tumor-infiltrating T-cell abundance depends on TMB status: T-cell gene signatures were associated with OS in TMB-high tumors, but not in TMB-low tumors, consistent with a possible immune-protective effect in tumors that exhibit both higher mutational load and higher T-cell abundance.

In histologic subtypes, although no intersubtype survival differences were observed, significantly different T-cell signature score distributions were noted, with lower T/NK scores in the synovial cell sarcoma and leiomyosarcoma subtypes and higher T/NK scores in the dedifferentiated liposarcoma subtype. T/NK scores did not vary significantly between TMB-low and TMB-high groups.

Several other antigenic determinants that may be targets for antitumor T cells, including CTAs, have been targeted in several clinical trials, including those in patients with soft tissue sarcoma.[47,48] A gene signature reflective primarily of Th1 adaptive immunity and the interferon-γ pathway was identified in patients who responded to MAGE-A3–specific cancer immunotherapy.[49] High expression of CTAs is very frequent in synovial sarcoma, but not in myxofibrosarcoma or liposarcoma, with very low TMB.[27,31] In our analysis, although tumor subtypes based on differential expression patterns of CTA genes showed different survival associations within leiomyosarcoma and myxofibrosarcoma histologies, neither CTA nor histologic subtypes were found to interact with the T/NK signature–survival association.

Our results also support the role of plasma B cells in the regulation of antitumor immune responses in soft tissue sarcomas. B cells in the tumor microenvironment can enhance antitumor immune response by activating cytotoxic T cells and producing antitumor antibodies and cytokines. In contrast, they can also participate in cancer immune evasion.[50] We observed that, in patients who had TMB-high tumors with moderate-to-high T/NK scores, the B/P signature added further

prognostic power, consistent with a role for plasma B cells in augmenting T-cell–mediated antitumor immunity. Furthermore, in patients who had TMB-low tumors with low T/NK scores, moderate-to-high B/P scores were associated with significantly enhanced survival. This observation suggests clinical potential for therapeutic strategies in sarcoma that target antitumor functions of plasma B cells. Our study does have limitations. Soft tissue sarcomas are rare, heterogeneous malignancies, and the sample sizes investigated were small. Patient outcomes-related research based on historic data sets like TCGA may result in observations that do not fully apply to the current clinical landscape because of progressive changes in clinical practice. In addition, these data sets were not specifically designed or powered, a priori, to answer our research questions. The prognostic relevance of TMB, CTA, and immune subclass is correlative only and does not account for unmeasured or poorly measured variables that may otherwise significantly affect our interpretations.

CONCLUSIONS

The findings presented here illuminate beneficial and nonbeneficial immunologic configurations in soft tissue sarcoma. We provide evidence that TMB may influence immunologically driven clinical outcomes, a previously unappreciated role for TMB in soft tissue sarcoma. The classification system described herein provides a basis for distinguishing immunogenic subtypes of soft tissue sarcoma that may offer opportunities for therapeutic stratification. Further investigation of the genomic alterations and molecular pathways that underlie these immunologic configurations could also shed light on mechanisms of tumor immune escape and reveal new opportunities for immunotherapeutic targeting in soft tissue sarcoma.

AUTHOR CONTRIBUTIONS

Shailaja K. S. Raj: Conceived of the study and analytical strategies, performed data analyses, wrote the article, critically reviewed the findings, edited the article, and approved the final version to be published. **Eric D. Routh:** Performed data analyses, critically reviewed the findings, edited the article, and approved the final version to be published. **Jeff W. Chou:** Performed data analyses, critically reviewed the findings, edited the article, and approved the final version to be published. **Konstantinos I. Votanopoulos:** Critical input and technical guidance in writing the article, critically reviewed the findings, edited the article, and approved the final version to be published. **Pierre L. Triozzi:** Conceived of the study and analytical strategies, wrote the article, critically reviewed the findings, edited the article, and approved the final version to be published. **Lance D. Miller:** Conceived of the study and analytical strategies, wrote the article, critically reviewed the findings, edited the article, and approved the final version to be published.

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CONFLICTS OF INTEREST

The authors made no disclosures.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new data were created or analyzed in this study.

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