Immune profiles of desmoplastic small round cell tumor and synovial sarcoma suggest different immunotherapeutic susceptibility upfront compared to relapse specimens

Mary Frances Wedekind1,2 | Kellie B. Haworth3 | Michael Arnold4,5 | Joseph R. Stanek1 | Dean Lee1,2 | Timothy P. Cripe1,2

1Division of Hematology, Oncology, Blood and Marrow Transplant, Department of Pediatrics, Nationwide Children’s Hospital, Columbus, Ohio
2Center for Childhood Cancer and Blood Diseases, The Research Institute at Nationwide Children’s Hospital, Columbus, Ohio
3Division of Neuro-Oncology, Department of Oncology, St Jude Children’s Research Hospital, Memphis, Tennessee
4Department of Pathology and Laboratory Medicine, Nationwide Children’s Hospital, Columbus, Ohio
5Department of Pathology, The Ohio State University College of Medicine, Columbus, Ohio

Correspondence
Timothy P. Cripe, Nationwide Children’s Hospital, 700 Children’s Drive, Columbus, OH 43205.
Email: timothy.cripe@nationwidechildrens.org

This work was presented previously at AACR Pediatric Cancer Research: From Basic Science to the Clinic, December 3–6, 2017.

Abstract

Background: Desmoplastic small round cell tumor (DSRCT) and synovial sarcoma are rare tumors with dismal outcomes requiring new therapeutic strategies. Immunotherapies have shown promise in several cancer types, but have not been evaluated in DSRCT and synovial sarcoma. Because the immune microenvironment can provide indications of the inflammatory nature of tumors, immunohistochemical staining is able to assess the tumor immune infiltrates in both tumor types.

Procedure: Using tissue microarrays of DSRCT and synovial sarcoma tumor samples, we detected tumoral HLA-A/B/C, beta-2-microglobulin (B2M), and PD-L1 expression, and quantified tumor-infiltrating lymphocytes expressing CD4, CD8, CD56, CD45RO, or FOXP3 by immunohistochemistry. We used staining intensity on a scale of 0–3 and percentage of tumor stained to determine HLA, B2M, and PD-L1 scores. We calculated the cytotoxic T lymphocyte (CTL) target score as HLA score \times B2M score/100.

Results: In diagnostic samples, we found high HLA and CTL target scores and low PD-L1 expression with decreased scores in recurrence for both tumor types. We found an increase in CD56+ natural killer cells in DSRCT samples from diagnosis to recurrence.

Conclusions: We found similar immunostimulatory profiles in DSRCT and synovial sarcoma. Our findings suggest that DSRCT and synovial sarcoma may be amenable to immunotherapies, albeit there was significant heterogeneity. Interestingly, HLA and CTL target scores decreased at recurrence, possibly reflecting immune evasion. Our findings suggest both tumor types may be amendable to CTL-based therapies at diagnosis but less so at relapse. Our results support further investigation into the prognostic and predictive value of these findings in a larger dataset.

KEYWORDS

DSRCT, immune profile, immunohistochemistry, synovial sarcoma

1 INTRODUCTION

Desmoplastic small round cell tumor (DSRCT) and synovial sarcoma are aggressive and rare cancers. DSRCT is a soft-tissue sarcoma that primarily occurs in the abdomen and mainly affects adolescents with a male predominance. Survival rates are extremely poor with 5-year overall survival of 15%.1,2 Standard treatment for DSRCT consists of surgical resection, radiation therapy, and chemotherapy. Synovial sarcoma is also a soft-tissue sarcoma with variability in anatomic location, which primarily affects older children and younger adults. Survival outcomes are also poor with a 10-year overall survival rate of 50% and half of patients developing metastasis within 5 years of diagnosis, primarily to the lung or pleura.3,4 Other than surgery for localized disease, conventional treatments such as chemotherapy and
radiotherapy have not had significant impacts on outcomes. Due to the current limitations in treatment for DSRCT and synovial sarcoma, there is a need to find new therapeutic options.

Immunotherapies have shown promise in numerous cancer types with FDA approval of immune checkpoint inhibitors for recurrent Hodgkin's lymphoma, non–small cell lung cancer, metastatic renal cell carcinoma, and melanoma.\(^5\)-\(^9\) Within the pediatric population, the FDA approval of anti-GD2 antibodies for neuroblastoma and anti-CD19 chimeric antigen receptor T cells for the treatment of relapsed acute lymphoblastic B cell leukemia suggests immune therapy can also be important for children.\(^10\),\(^11\)

As immunotherapy options show increasingly more significant and durable responses, there are still many unanswered questions. Despite much research, no reliable biomarker has been found to indicate whether a patient will respond to these therapies. Checkpoint inhibition successes have shown that there is an association of high expression of programmed cell death ligand 1 (PD-L1) with poor prognosis; however, it did not predict therapeutic benefit from cognate inhibitors.\(^12\)-\(^16\) There have been a number of studies detailing the expression of PD-L1 and PD-1 on numerous sarcoma subtypes, including DSRCT and synovial sarcoma. Most of these studies show conflicting data on expression levels and clinical outcomes.\(^17\)-\(^20\)

Whether immunotherapies for DSRCT and synovial sarcoma will be useful is currently unknown. Numerous results from other cancer subtypes suggest that expression of relevant immunologic molecules and intratumoral immune cell infiltrates may be predictive of prognosis and therapeutic response.\(^20\)-\(^25\) In order to explore which immunotherapies may be useful for patients with DSRCT and synovial sarcoma, we sought to describe the immunologic profiles of specimens from each tumor type. We characterized tumoral expression of human leukocyte antigen (HLA)-A/-B/-C and \(\beta\)-2-microglobulin (B2M) and calculated a "cytotoxic T-lymphocyte (CTL) target score."\(^26\) We also assessed tumoral PD-L1 expression and the presence of intratumoral CD4\(^+\), CD8\(^+\), FOXP3\(^+\), CD45RO\(^+\), and CD56\(^+\) lymphocytes via immunohistochemistry (IHC). Our data suggest that immune profiling of DSRCT and synovial sarcoma may aid in the selection of which type of immunotherapy may be most useful for these patients.

2 | METHODS

2.1 | Tissue microarray construction

The Institutional Review Board at Nationwide Children’s Hospital approved this study. Nine DSRCT and 12 synovial sarcoma samples were obtained through the Nationwide Children’s Hospital Pathology Department. Three of the nine DSRCT samples were collected at diagnosis with each sample from a different patient while the other five samples were collected at recurrence with three different patients represented. One DSRCT sample was not analyzed with the other samples when separated in to diagnosis or recurrence categories as there was no documentation about when in the treatment time course the sample was obtained. Nine of the 12 synovial sarcoma samples were collected at diagnosis with 6 different patients represented while the other 3 samples were collected at recurrence representing 2 patients. One occurrence of the same patient having samples from diagnosis and through relapse with synovial sarcoma. These samples were included onto tissue microarrays constructed from 2 mm punches of formalin-fixed paraffin-embedded tissue blocks.

2.2 | Immunohistochemical staining

All stages of the IHC procedure were automated on the Bond III IHC system (Leica Microsystems) using the Refine polymer detection system, with 3,3’-diaminobenzidine visualization and counterstain with hematoxylin.

We used an antibody to HLA class I ABC (clone EMR8-5, Abcam Inc, catalog number ab70328) at a dilution factor of 1:6,000, after on-line antigen retrieval in a citrate buffer, pH 6 (ER1, Leica Microsystems, catalog number AR9961) for 20 minutes at 100°C and \(\beta\)-2-microglobulin antibody (HPA006361, rabbit polyclonal, Sigma-Aldrich, catalog number HPA 006361) at a dilution of 1:6,000 with EDTA retrieval (ER2, Leica Microsystems, catalog number AR9640) for 20 minutes at 100°C. We used an antibody to PD-L1 (clone B55, Sino Biological Inc., catalog number 10084-H08H) at a dilution factor of 1:200, after on-line antigen retrieval in an EDTA buffer, pH 9 (ER2, Leica Microsystems, catalog number AR9640) for 20 minutes at 100°C.

We used prediluted antibodies to CD4 (clone SP35, Cell Marque, catalog number 104R-18) and CD8 (clone 4B11, Leica Microsystems, catalog number PA0183) after on-line antigen retrieval in an EDTA buffer, pH 9 (ER2, Leica Microsystems, catalog number AR9640) for 20 minutes at 100°C. We used prediluted antibodies to CD56 (clone 1B6, Leica Microsystems, catalog number NCL-L-CD56-1B6) and CD45RO (clone UCHL1, Leica Microsystems, catalog number PA0146) after on-line antigen retrieval in a citrate buffer, pH 6 (ER1, Leica Microsystems, catalog number AR9961) for 20 minutes at 100°C. We diluted an antibody to FOXP3 (clone 236A/E7, Abcam Inc., catalog number ab20034) to a factor of 1:100 after on-line antigen retrieval in an EDTA buffer, pH 9 (ER2, Leica Microsystems, catalog number AR9640) for 20 minutes at 100°C.

3 | EVALUATIONS

3.1 | Scoring of HLA-A/B/B, B2M, and PD-L1 expression in DSRCT and synovial sarcoma tumor samples by immunohistochemical staining

A single pathologist (MAA) scored immunohistochemical stainings for HLA-A/B/C, B2M, and PD-L1 based upon maximal staining intensity on a scale of 0 to 3 (absent = 0, weak = 1, moderate but less than normal cells = 2, or strong = 3) and percentage of tumor cells stained (range 0 to 100%), as previously described.\(^21\)

3.2 | Calculation of CTL target scores

For tumor samples where HLA-A/B/C and B2M expression were detected by immunohistochemical staining, we calculated CTL target scores as previously described\(^21\): average HLA-A/B/C score (range,
0~300) = % positive staining × staining intensity/n. Average B2M score (range, 0~300) = % positive staining × staining intensity/n. CTL target score (range, 0~900) = average HLA score × average B2M score/100.

### 3.3 Scoring of CD4+, CD8+, CD56+, FOXP3+, and CD45RO+ lymphocytic cellular infiltrates

The pathologist (MAA) scored immunohistochemical staining of intra-tumoral CD4+, CD8+, CD56+, FOXP3+, and CD45RO+ cellular infiltrates. Counts were based upon the number of positively stained lymphocytes per square millimeter area of tumor present.

### 3.4 Statistical analyses

All statistical analyses were performed using GraphPad Prism, version 7 (GraphPad Software, La Jolla, California). Tumor subtype immunohistochemical staining scores and cellular infiltrates were compared using Mann–Whitney nonparametric tests. Results were considered to be significant when P ≤ 0.05.

Correlation of immunologic marker IHC staining and intratumoral immune cellular infiltrates was calculated using Spearman correlation coefficient. Sample score values were determined to positively correlate with cellular infiltrates when Spearman R = 0.00 to 0.39 (weak), 0.40 to 0.59 (moderate), 0.6 to 0.79 (strong), and 0.8 to 1.0 (very strong). Sample score values were determined to negatively correlate with cellular infiltrates when Spearman R = 0.00 to −0.39 (weak), −0.40 to −0.59 (moderate), −0.6 to −0.79 (strong), and −0.8 to −1.0 (very strong). Results were considered to be statistically significant when P ≤ 0.05.

### 4 RESULTS

#### 4.1 Recurrent DSRCT and synovial sarcoma show decreased HLA scores and CTL target scores compared to diagnostic samples

To assess the tumor immune infiltrates, we performed immunohistochemical staining for immunologic markers of interest (HLA-A/B/C, B2M, and PD-L1) on patient DSRCT and synovial sarcoma samples. We scored their staining intensity and percentage as previously described.21 In general, synovial sarcoma and DSRCT displayed greater median HLA-A/B/C staining (Figure 1A) and CTL target scores (Figure 1B) at diagnosis than at recurrence. For synovial sarcoma, B2M scores decreased from diagnosis to recurrence, whereas DSRCT B2M scores increased from diagnosis to recurrence (Figure 1C).

#### 4.2 DSRCT and synovial sarcoma exhibit low PD-L1 expression

To further assess the potential for CTL inhibition, we assessed tumoral PD-L1 expression. Both DSRCT and synovial sarcoma had minimally positive PD-L1 scores at diagnosis. Both tumor types showed a decrease in PD-L1 scores in the recurrence samples compared with diagnostic samples (Figure 1D).

### 4.3 Synovial sarcoma has high CTL scores compared with DSRCT

We previously reported a CTL target score that takes into account both HLA class I and B2M expression to predict which tumors might be susceptible to CTLs.21 We found synovial sarcoma displayed a greater median HLA-A/B/C, B2M, and thus greater CTL target scores at diagnosis and recurrence compared with DSRCT. Utilizing Mann–Whitney test, there was a statically significant difference between HLA scores and CTL scores comparing DSRCT to synovial sarcoma (Supplementary Information Table S1).

### 4.4 Low HLA scores with increase in CD56+ demonstrated in DSRCT

We quantified CD8+, CD4+, FOXP3+, CD45RO+, and CD56+ lymphocytes within the tumors. We found that DSRCT had an increased CD8+, and thus total tumor-infiltrating T cells, than synovial sarcoma at diagnosis. However, at recurrence, DSRCT had fewer T-cell numbers than synovial sarcoma (Figure 2A–B). DSRCT showed an increase in CD56+ cells from diagnosis to recurrence (Figure 2A).

### 4.5 Immunologic marker staining scores do not correlate with cellular infiltrates

We used Spearman correlation coefficients to determine whether the expression of immunologic cells correlated with each other or the lymphocytic cellular infiltrates we observed in the tumors. The only “very strong” correlation we found was a positive correlation between the HLA expression and the CD8+ T-cell infiltrates (r = 0.81, P = 0.002) and a “strong” with B2M (r = 0.78, P = 0.003) in synovial sarcoma (Figure 3A). We also observed HLA expression and CTL target score correlated “strongly” with CD45RO+ infiltrates (r = 0.74, P = 0.007; r = 0.61, P = 0.04, respectively), likely reflective of the increase in CD8+ T cells. The only correlation seen in the DSRCT tumors was a “strong” correlation between HLA scoring and the presence of CD4+ cells (r = 0.70, P = 0.04) (Figure 3A–D). We did not detect any correlations for synovial sarcoma or DSRCT with PD-L1 (Figure 3D).

### 4.6 Cellular infiltrates correlate with other cellular infiltrates in synovial sarcoma

We performed Spearman correlation calculations to determine whether intratumoral lymphocytic cellular infiltrates correlated with other lymphocytic cellular infiltrate types. In DSRCT, we did not detect any significant correlations (Figure 4A). In synovial sarcoma, we observed a “very strong” positive correlation between CD45RO+ infiltrates with both CD8+ (r = 0.87, P < 0.001) and CD4+ (r = 0.85, P < 0.001) (Figure 4B), which likely represent memory CD8+ and CD4+ infiltrates. We also observed a “strong” positive correlation between Foxp3+ infiltrates and CD4+ cells (r = 0.70, P = 0.015), representing T regulatory cells. We also observed a “very strong” correlation between CD8+ and CD4+ infiltrates (r = 0.88, P < 0.001), which is likely indicative of a strong Th1 immune environment.
4.7 | Evaluation of immunologic marker expression and immune cellular infiltrates in synovial sarcoma tumors from the same patient reveals that immunosuppressive infiltrates progressed with each relapse accompanied by wide tumor heterogeneity

We assessed a few tumor samples obtained from the same patient with synovial sarcoma beginning at diagnosis and through two recurrences. We had two samples from the patient’s initial diagnosis at 18 years of age followed by one sample from lung recurrences 3 years and 4 years after diagnosis. The patient received chemotherapy and radiation after diagnosis. We observed a decrease in HLA, B2M, and CTL target scores (Figure 5A–C) from diagnosis to relapse. At first relapse, the PD-L1 score increased by 4.5-fold, but by the patient’s second relapse, tumoral PD-L1 expression was no longer present (Figure 5D). CD8+ cells, CD4+ cells, total T cells, and CD45RO+ cells all decreased with each relapse (Figure 6). Also, CD56+ lymphocytic infiltrates initially decreased in the first relapse, but then increased dramatically in the second relapse (Figure 6).

5 | DISCUSSION

Here, we describe cellular immune profiles of a cohort of human DSRCT and synovial sarcoma tissue samples. We included specimens at diagnosis and, when available, at relapse. We calculated the CTL target score, which takes into account both HLA-A/B/C and B2M expression, as both are vital components for functional antigen
FIGURE 3  Correlations between immunohistochemical staining scores and immune cellular infiltrates. A. HLA correlations; B. B2M correlations; C. CTL Target correlations; D. PD-L1 correlations. Number of samples per tumor type listed in parentheses below tumor type. \( r \) = Spearman correlation coefficient. Positive correlations are defined as: \( r \) = 0.00–0.39 “weak,” \( r \) = 0.40–0.59 “moderate” (lightest red), \( r \) = 0.60–0.79 “strong” (light red), \( r \) = 0.80–1.0 “very strong” (dark red). Negative correlations are defined as: \( r \) = 0.00 to –0.39 “weak,” \( r \) = –0.40 to –0.59 “moderate” (lightest blue), \( r \) = –0.60 to –0.79 “strong” (light blue), \( r \) = –0.80 to –1.0 “very strong” (dark blue). Yellow shading indicates results that were not statistical significant.

FIGURE 4  Correlations between immunohistochemical immune cellular infiltrates. A. DSRCT tumor samples (n=9). B. synovial sarcoma samples (n=12). \( r \) = Spearman correlation coefficient. Positive correlations are defined as: \( r \) = 0.00–0.39 “weak,” \( r \) = 0.40–0.59 “moderate” (lightest red), \( r \) = 0.60–0.79 “strong” (light red), \( r \) = 0.80–1.0 “very strong” (dark red). Negative correlations are defined as: \( r \) = 0.00 to –0.39 “weak,” \( r \) = –0.40 to –0.59 “moderate” (lightest blue), \( r \) = –0.60 to –0.79 “strong” (light blue), \( r \) = –0.80 to –1.0 “very strong” (dark blue). Yellow shading indicates results that were not statistical significant.

In original diagnostic samples, we found higher HLA and CTL target scores and low PD-L1 expression compared with recurrent sample counterparts. Interestingly, HLA and CTL target scores decreased at recurrence in both tumor types. In addition, we found an increase in CD56+ natural killer (NK) cells in DSRCT samples from diagnosis to recurrence, which may reflect the reduced HLA expression. In the one synovial sarcoma patient, we assessed with multiple relapses, we found different immunologic profiles at each relapse.

Exactly what biomarkers predict response to immunotherapies is not well defined, but most likely there will be numerous parameters that in combination will be predictive. For example, Galon et al. described the combination of CD8+ cellular infiltrates and CD45RO+ cellular infiltrates to formulate a prognostically significant “immunoscore” in colon cancer.27 In fact, naturally occurring tumor-infiltrating lymphocytes have been associated with better patient survival in numerous cancer types28–30 and may also correlate with better responsiveness to checkpoint inhibition.31

In addition, predictive biomarkers of response are likely to depend on the type of immunotherapy used. MHC class I expression is important for antigen presentation, necessitating its consideration when planning for use of strategies which target neoantigens. Immunotherapies that require MHC class I expression on target cells for recognition include autologous T-cell transfer with or without genetically modified T-cell receptors, adoptively transferred T cells such as donor lymphocyte infusions, use of immune checkpoint inhibitors, and tumor vaccines.26 We found that CTL target scores in our synovial sarcoma samples were significantly higher than in those from DSRCT. Thus, synovial sarcoma may be a particularly suitable candidate for CTL-based immunotherapeutics at diagnosis, but as we found decreasing CTL target scores in recurrence, these strategies may be less beneficial at relapse. Despite synovial sarcoma demonstrating a higher CTL target score compared with DSRCT, neither tumor type has a high target score to begin with. Whether the lower scores in synovial cell sarcoma are sufficient to enable neoantigen presentation is unknown. In this regard, it is interesting to note that NY-ESO-1, a cancer--testis antigen expressed on 80% of all synovial sarcomas, is being explored as a therapeutic CTL target.32

Alternatively, some immunotherapeutic approaches do not rely on neoantigen presentation by MHC class I such as chimeric antigen receptor T cells, bispecific T-cell engagers, and NK cell therapies. Given the low CTL target scores for both tumor types at relapse, we
We hypothesize that such approaches are more likely to be effective for relapsed synovial cell sarcoma and DSRCT patients than those which rely on targeting class I-presented antigens.

Interestingly, we found that the number of tumor-infiltrating CD56+ NK cells increased from diagnosis to recurrence in DSRCT. This phenomenon is likely due to decreased HLA I expression resulting in a subsequent decreased T-cell response, which allows the NK cells to infiltrate the tumor; however, the implications for immunotherapeutic efficacy are not known. There are numerous conflicting reports regarding the benefit of resident intratumoral CD56+ cells, whether their presence can predict response to NK-based therapy, or whether increased CD56+ cells intratumorally is a poor prognostic sign.33–35

Numerous studies have evaluated the expression of PD-L1 in DSRCT and synovial sarcoma with conflicting results. While some authors reported high levels of PD-L1 in DSRCT tissue20,36 and in synovial sarcoma,18 in contrast, and consistent with our findings, two other groups reported very low or no expression of PD-L1 in either tumor type.19,37 We observed extensive heterogeneity of PD-L1 expression within each tumor type, which might explain the disparate reports. Despite an association between high PD-L1 expression and poor prognosis in other cancers, PD-L1 expression has not accurately predicted outcomes following PD-1 or PD-L1 blockade therapies.12–16 We were unable to determine prognostic value of tumoral PD-L1 expression due to our small sample size.

In one synovial sarcoma patient, we tested multiple tumor tissue samples during different treatment stages. Over the course of two relapses, the intratumoral cellular infiltrates appeared to be different each time. This observation is consistent with other published reports of the immune infiltrates shifting through treatment and relapse.38,39 From this one patient as well as looking at all our samples, we can conclude that a possible challenge for immunotherapies to be efficacious is heterogeneity not only within the same tumor in one patient but also the generalizability of findings to other patient’s tumors. The two diagnostic samples of our one patient revealed a wide range among many of the cellular infiltrates (Figure 6), demonstrating that even within the same tumor, there is a potential the entire tumor may not respond uniformly to immunotherapies. Whether tumor heterogeneity is a significant challenge, whether therapies can cause tumors to become more homogeneous, or whether therapies can overcome this heterogeneity is still unknown.

Both synovial cell sarcoma and DSRCT have unacceptably poor outcomes. Our findings suggest immunotherapies may be promising, albeit
strategies may need to differ at diagnosis with CTL-based therapies compared with relapse which perhaps need non-CTL-based therapies. Our conclusions are limited by small sample size, but the wide heterogeneity and case-to-case variability we observed even in these few cases suggest the need to develop and validate predictive biomarkers for individual patients. Indeed, patient-specific immune scoring using IHC may enable rational selection of immunotherapeutic treatments which could be tested in a clinical trial. Such biomarkers will have utility for more personalized medicine and, hopefully, better patient outcomes.

ACKNOWLEDGMENTS
This work was funded by the Research Institute at Nationwide Children’s Hospital.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

ORCID
Mary Frances Wedekind http://orcid.org/0000-0001-9707-7175
Kellie B. Haworth http://orcid.org/0000-0001-9813-4290
Joseph R. Stanek http://orcid.org/0000-0003-1630-2102
Dean Lee http://orcid.org/0000-0001-6693-5392
Timothy P. Cripe http://orcid.org/0000-0002-8595-3577

REFERENCES
1. Thway K, Nourjain J, Zaidi S, et al. Desmoplasic small round cell tumor: pathology, genetics, and potential therapeutic strategies. Int J Surg Pathol. 2016;24:672–684.
2. Bent MA, Padilla BE, Goldsby RE, DuBois SG. Clinical characteristics and outcomes of pediatric patients with desmoplasic small round cell tumor. Rare Tumors. 2016;8:6145.
3. Thway K, Fisher C. Synovial sarcoma: defining features and diagnostic evolution. Ann Diagn Pathol. 2014;18:369–380.
4. Krieg AH, Hefti F, Speth BM, et al. Synovial sarcomas usually metastasize after >5 years: a multicenter retrospective analysis with minimum follow-up of 10 years for survivors. Ann Oncol. 2011;22:458–467.
5. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin’s lymphoma. N Engl J Med. 2015;372:311–319.
6. Ornstein MC, Rini BI. The safety and efficacy of nivolumab for the treatment of advanced renal cell carcinoma. Expert Rev Anticancer Ther. 2016;16:577–584.
7. Younes A, Santoro A, Shipp M, et al. Nivolumab for classical Hodgkin’s lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. Lancet Oncol. 2016;17:1283–1294.
8. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387:1540–1550.
9. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-Positive non-small-cell lung cancer. N Engl J Med. 2016;375:1823–1833.
10. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378:439–448.
11. Yu AL, Gilm AN, Ozkaynak MF, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N Engl J Med. 2010;363:1324–1334.
12. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. Lancet Oncol. 2016;17:e542–e551.
13. Antonia S, López-Martin JA, Bendell J, et al. Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. Lancet Oncol. 2016;17:883–895.
14. Larkin J, Hodi FS, Wolchok JD, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373:23–34.
15. Postow MA, Chesney J, Pavlick AC, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med. 2015;372:2006–2017.
16. Ficarra L, Botti G, Lorigan P, et al. Cancer treatment with anti-pd-1/pd-l1 agents: Is PD-L1 expression a biomarker for patient selection?. Drugs. 2016;76:925–945.
17. Paydas S, Bagir EK, Deveci MA, Gonlusen G. Clinical and prognostic significance of PD-1 and PD-L1 expression in sarcomas. Med Oncol. 2016;33:93.
18. Kim C, Kim EK, Jung H, et al. Prognostic implications of PD-L1 expression in patients with soft tissue sarcoma. BMC Cancer. 2016;16:434.
19. Inaguma S, Wang Z, Lasota J, et al. Comprehensive immunohistochemical study of programmed cell death ligand 1 (PD-L1): analysis in 5536 cases revealed consistent expression in trophoblastic tumors. Am J Surg Pathol. 2016;40:1133–1142.
20. van Erp AEM, Verselejen-Jonkers YMH, Hillebrandt-Roeffen MHS, et al. Expression and clinical association of programmed cell death-1, programmed death-ligand-1 and CD8(+) lymphocytes in primary sarcomas is subtype dependent. Oncotarget. 2017;8:71371–71384.
21. Haworth KB, Arnold MA, Pierson CR, et al. Characterization of MHC class I and beta-2-Microglobulin expression in pediatric solid malignancies to guide selection of immune-based therapeutic trials. Pediatr Blood Cancer. 2016;63:618–626.
22. Li B, Severson E, Pignon JC, et al. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. Genome Biol. 2016;17:174.
23. Nowicki TS, Akiyama R, Huang RR, et al. Infiltration of CD8 T cells and expression of PD-L1 in sarcomas. Cancer Immunol Res. 2017;5:118–126.
24. Chifman J, Pullikuth A, Chou JW, Bedognetti D, Miller LD. Conserved tumor immune gene signatures in solid tumors and prognostic implications. BMC Cancer. 2016;16:911.
25. Hsu DS, Kim MK, Balakumaran BS, et al. Immune signatures predict prognosis in localized cancer. Cancer Invest. 2010;28:765–773.
26. Haworth KB, Leddon JL, Chen CY, Horwitz EM, Mackall CL, Cripe TP. Going back to class I: mHC and immunotherapies for childhood cancer. Cancer Invest. 2016;34:177–190.
27. Galon J, Fox BA, Bifulco CB, et al. Immunoscore and immunoprofiling in cancer: an update from the melanoma and immunotherapy bridge 2015. J Transl Med. 2016;14:273.
28. Adams S, Goldstein LJ, Sparano JA, Demaria S, Badve SS. Tumor infiltrating lymphocytes (TILs) improve prognosis in patients with triple negative breast cancer (TNBC). Oncotarget. 2015;4:e985930.
29. Mina M, Boldrini R, Citti A, et al. Tumor-infiltrating T lymphocytes improve clinical outcome of therapy-resistant neuroblastoma. Oncoimmunology. 2015;4:e1019981.
30. Vassilakopoulou M, Avgeris M, Velchel V, et al. Evaluation of PD-L1 expression and associated tumor-infiltrating lymphocytes in laryngeal squamous cell carcinoma. Clin Cancer Res. 2016;22:704–713.
31. Tang H, Wang Y, Chlewicki LK, et al. Facilitating T cell infiltration in tumor microenvironment overcomes resistance to PD-L1 blockade. Cancer Cell. 2016;29:285–296.
32. Jungbluth AA, Antonescu CR, Busam KJ, et al. Monophasic and biphasic synovial sarcomas abundantly express cancer/testis antigen NY-ESO-1 but not MAGE-A1 or CT7. Int J Cancer. 2001;94:252–256.
33. Georgiannos SN, Renault A, Goode AW, Sheaff M. The immunophenotype and activation status of the lymphocytic infiltrate in human breast cancers, the role of the major histocompatibility complex in cell-mediated immune mechanisms, and their association with prognostic indicators. Surgery. 2003;134:827–834.
34. Vgenopoulou S, Lazaris AC, Markopoulos C, et al. Immunohistochemical evaluation of immune response in invasive ductal breast cancer of not-otherwise-specified type. Breast. 2003;12:172–178.
35. Lefebvre S, Antoine M, Uzan S, et al. Specific activation of the non-classical class I histocompatibility HLA-G antigen and expression of the ILT2 inhibitory receptor in human breast cancer. J Pathol. 2002;196:266–274.
36. Movva S, Wen W, Chen W, et al. Multi-platform profiling of over 2000 sarcomas: identification of biomarkers and novel therapeutic targets. Oncotarget. 2015;6:12234–12247.
37. Pollack SM, He Q, Yearley JH, et al. T-cell infiltration and clonality correlate with programmed cell death protein 1 and programmed death-ligand 1 expression in patients with soft tissue sarcomas. Cancer. 2017;123:3291–3304.
38. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168:707–723.
39. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991–998.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Wedekind MF, Haworth KB, Arnold M, Stanek JR, Lee D, Cripe TP. Immune profiles of desmoplastic small round cell tumor and synovial sarcoma suggest different immunotherapeutic susceptibility upfront compared to relapse specimens. Pediatr Blood Cancer. 2018;65:e27313. https://doi.org/10.1002/pbc.27313