



## PD-L1 expression in inflammatory myofibroblastic tumors

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### Abstract

Inflammatory myofibroblastic tumor is a rare mesenchymal tumor occurring at many anatomic sites, with a predilection for children and young adults. Often indolent, they can be locally aggressive and can metastasize, resulting in significant morbidity and mortality. Therapeutic options are often limited. The identification of underlying kinase mutations has allowed the use of targeted therapy in a subset of patients. Unfortunately, not all tumors harbor mutations and resistance to tyrosine kinase inhibitor therapy is a potential problem. We hypothesized that these tumors may be amenable to PD-L1 therapy given the immune nature of the tumor. PD-L1 expression in inflammatory myofibroblastic tumors has not yet been defined. The purpose of this study was to explore PD-L1 expression in inflammatory myofibroblastic tumors, as adaptive PD-L1 expression is known to enrich for response to anti-PD-1/PD-L1 therapies. Expression of PD-L1 (clone SP142) was assessed in 35 specimens from 28 patients. Positivity was defined as membranous expression in  $\geq 5\%$  of cells and evaluated separately in tumor and immune cells. Adaptive vs. constitutive patterns of tumor cell PD-L1 expression were assessed. PD-L1 status was correlated with clinicopathologic features. CD8<sup>+</sup> T cell infiltrates were quantified by digital image analysis. ALK status was assessed by immunohistochemistry and/or FISH. Twenty-four (69%) tumors had PD-L1(+) tumor cells and 28 (80%) showed PD-L1(+) immune cells. Most recurrent and metastatic tumors (80%) and ALK(−) tumors (88%) were PD-L1(+). Adaptive PD-L1 expression was present in 23 (96%) of PD-L1(+) tumors, which also showed a three–four fold increase in CD8<sup>+</sup> T cell infiltration relative to PD-L1(−) tumors. Constitutive PD-L1 expression was associated with larger tumor size ( $p = 0.002$ ). Inflammatory myofibroblastic tumors show frequent constitutive and adaptive PD-L1 expression, the latter of which is thought to be predictive of response to anti-PD-1. These data support further investigation into PD-1/PD-L1 blockade in this tumor type.

Inflammatory myofibroblastic tumors are rare mesenchymal tumors, which can arise at any age and throughout the body. While often indolent in their behavior a subset of tumors are

locally aggressive and metastases have been reported in up to 5% of cases [1, 2]. Up until recently, surgery was the treatment of choice with its associated morbidity. The recent identification of underlying kinase mutations in many, if not all, of these tumors has allowed for the use of targeted therapy. The most common mutation identified is rearrangement in anaplastic lymphoma kinase (*ALK*) [3] present in ~50–70% of cases. A smaller subset of inflammatory myofibroblastic tumors have been associated with alternate gene fusions, including *ROS1* (5–10%), *NTRK3*, *RET*, and *PDGFRB* [3–6]. Good response to tyrosine kinase inhibitors has been reported. However, as has been seen in other tumors treated with tyrosine kinase inhibitor therapy, the development of acquired resistance in inflammatory myofibroblastic tumors [3, 7] has also been noted. In addition, not all inflammatory myofibroblastic tumors harbor actionable mutations. Thus, novel treatment options are highly desirable. The immunologic nature of inflammatory myofibroblastic tumors demonstrated by the prominent

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tumor-associated inflammatory infiltrate and the constitutional symptoms, including fever, anemia, and weight loss, which can afflict some patients suggests an immunologic-based therapeutic approach, specifically PD-1/PD-L1 blockade, may be worth exploring. PD-L1 expression in inflammatory myofibroblastic tumors has not yet been defined. The purpose of this study was to explore PD-L1 expression in inflammatory myofibroblastic tumors, as PD-L1 is known to enrich for response to anti-PD-1/PD-L1 therapies in many other tumor types [8, 9].

## Materials and methods

### Clinical data

The study was approved by the Johns Hopkins Medicine institutional review board. Thirty-five inflammatory myofibroblastic tumor specimens obtained from 28 patients between 1998 and 2015 were retrieved from the surgical pathology archives. A surgical pathologist (DAB) reviewed the hematoxylin and eosin (H&E)-stained slides to confirm the diagnosis. Clinicopathologic information was obtained from the medical records (Tables 1 and 2). Tumor size was based on gross specimen examination if completely excised and intact or radiographic assessment at the time of tissue sampling. For patients with multiple tumor foci, only the size corresponding to the sampled tumor was assessed. For patients with multiple specimens, only those obtained from different time points or locations were studied. ALK status was assessed by immunohistochemistry and/or FISH. One activating *ROS1* fusion (*TGF-ROS1*) was identified by genomic sequencing. The remaining ALK-negative specimens were assessed for *ROS1* translocation by FISH. Fusion-positive inflammatory myofibroblastic tumors, defined as those with *ALK* or *ROS1* genetic rearrangements, were assessed as a group.

### Assessment of histopathologic features

The histologic subtypes were classified as: (1) fascicular or leiomyomatous-like, (2) fasciitis-like, (3) desmoid-like, and (4) epithelioid [1]. Tumor cellularity, tumor cell morphology and pleomorphism, mitotic rate, and necrosis were also scored. The composition (lymphocytes, plasma cells, eosinophils, and/or neutrophils) of the inflammatory cell infiltrate and the presence of lymphoid aggregates (defined as at least 100 lymphocytes with no germinal center) were assessed on H&E.

### Immunohistochemical staining and scoring

Immunohistochemistry for PD-L1 (clone SP142) was performed using a laboratory-derived manual assay as previously described [10]. The percentage of tumor and

intratumoral immune cells demonstrating membranous (cell surface) PD-L1 staining were scored separately as ~0%, 5%, 10%, and then at increasing 10% intervals. Cases were scored independently by two board-certified pathologists (JMT and DAB), and cases with >10% discordance were adjudicated by consensus. A threshold of  $\geq 5\%$  was used independently for both tumor and immune cells when a case was designated as PD-L1(+). The geographic association of PD-L1 expression with tumor infiltrating lymphocytes was assessed. Patterns of PD-L1 expression were classified as constitutive (PD-L1 expression in absence of tumor infiltrating lymphocytes), adaptive (PD-L1 geographically associated with tumor infiltrating lymphocytes), or adaptive and constitutive (broad PD-L1 expression independent of tumor infiltrating lymphocytes, further accentuated in areas of lymphocyte infiltration).

Immunohistochemistry for CD8 (clone c8/c8144B) was performed using standard automated methods. CD8-immunostained slides were scanned using the NanoZoomer-XR (Hamamatsu, Hamamatsu City, Japan). Halo Image Analysis Software (Indica Labs, Corrales, NM) was used to annotate tumor and exclude necrotic, folded, and fragmented areas. Intratumoral CD8<sup>+</sup> cell densities were quantified with the Halo Immune Cell Module using best-fit parameters [10].

### Statistical analysis

The relationships between clinicopathologic features and PD-L1 status were assessed using Fisher's exact test for categorical variables. Correlations between two continuous variables were assessed by Spearman's correlation coefficient. All tests were two-sided and statistical significance was determined with an alpha level of 0.05.

## Results

### Clinicopathologic characteristics

The clinicopathologic features of each individual case are presented in Table 1. Patients ranged in age from <1 month to 77 years, and 10 (36%) were under 20 years old. The most common sites of origin were the lung and abdomen. Tumor specimens included 32 (91%) resections and 3 (9%) biopsies from 26 (74%) primary tumors, 2 (6%) recurrences, and 7 (20%) metastases. Multiple specimens were available from three patients. Two patients had therapy prior to tissue sampling (Table 1), including one patient who received neoadjuvant radiation therapy and one patient who was treated with chemotherapy for another malignancy. The lack of paired pretreatment and post-treatment specimens preclude analysis regarding whether prior treatment

**Table 1** Summary of clinical characteristics and PD-L1 status for the study cohort

Patient	Tumor	Age (years)	Description <sup>a</sup>	Location	Size (cm)	Alk	Tumor <sup>d</sup> PD-L1	IC PDL1
1	T1	0.06	Primary	Heart	2.5	Neg	Mixed	Pos
2	T2	0.42	Primary	Mediastinum	4.0	Pos	Adaptive	Pos
3	T3	1.08	Primary	Extremity	5.5	Pos	Adaptive	Pos
4	T4	2	Primary	Mesenteric mass	9.5	Pos	Neg	Neg
5	T5	2.3	Primary	Liver	12.5	Pos	Mixed	Pos
	T6	2.7	Metastasis	Omentum	4.5	Pos	Mixed	Pos
	T7	2.7	Metastasis	Bowel serosa	1.7	Pos	Adaptive	Pos
6	T8	4	Primary	Trachea	1.7	Pos	Neg	Pos
7	T9	9	Primary	Abdomen	20	Pos	Mixed	Pos
	T10	9	Recurrence	Abdomen	6.5	Pos	Mixed	Pos
8	T11	9	Primary	Lung	1.3	Pos	Neg	Pos
9	T12	10	Primary	Extremity	3	Neg <sup>b</sup>	Adaptive	Pos
10	T13	12	Primary	Brain	1.9	Neg <sup>c</sup>	Adaptive	Neg
11	T14	26	Primary	Pulmonary artery	5	Neg	Mixed	Pos
12	T15	27	Primary	Lung	1.2	Pos	Neg	Pos
13	T16	28	Primary	Placenta	3.2	Pos	Neg	Pos
14	T17	30	Primary	Pelvic mass	10	Neg <sup>b</sup>	Neg	Pos
15	T18	35	Metastasis (lung)	Rectus sheath	9	Pos	Neg	Neg
16	T19	36	Metastasis (lung)	Brain	3.1	Pos	Neg	Neg
17	T20	36	Primary	Lung	4.5	Pos	Neg	Neg
18	T21	37	Primary	Bladder	4	Pos	Mixed	Pos
19	T22	39	Primary	Bladder	3.6	Pos	Mixed	Pos
20	T23	42	Primary	Lung	1	Pos	Neg	Pos
21	T24	43	Primary	Bladder	2.2	Pos	Mixed	Pos
22	T25	44	Primary	Lung	1.5	Pos	Adaptive	Pos
23	T26	55	Primary	Liver	17.5	Neg <sup>b</sup>	Mixed	Pos
24	T27	57	Primary	Lung	0.7	Neg <sup>b</sup>	Adaptive	Pos
25	T28	61	Recurrence <sup>e</sup>	Pelvic mass	6.8	Neg	Mixed	Pos
26	T29	61	Primary <sup>f</sup>	Lung	NA	Pos	Adaptive	Pos
	T30	61	Primary <sup>f</sup>	Lung	9.5	Pos	Mixed	Pos
	T31	65	Metastasis <sup>f</sup>	Chest wall	7	Pos	Mixed	Pos
	T32	66	Metastasis <sup>f</sup>	Kidney	9	Pos	Const.	Neg
	T33	67	Metastasis <sup>f</sup>	Chest wall	1.5	Pos	Adaptive	Pos
27	T34	74	Primary	Epiglottis	0.5	Pos	Neg	Neg
28	T35	77	Primary	Bladder	2	Pos	Adaptive	Pos

IC immune cell, *Pos* positive, *Neg* negative

<sup>a</sup>When only the metastasis is present, the primary location is noted in parentheses

<sup>b</sup>ROS1 rearrangement negative

<sup>c</sup>ROS1 rearrangement positive

<sup>d</sup>For specimens positive for tumor cell PD-L1 expression, the pattern is noted as adaptive, constitutive (Const.), or mixed

<sup>e</sup>Prior radiation

<sup>f</sup>Prior chemotherapy for another malignancy

**Table 2** Clinical characteristics by IMT PD-L1 status

Characteristics	PD-L1 positive <i>n</i> (%)	PD-L1 negative <i>n</i> (%)	<i>p</i> value <sup>a</sup>
All patients	17	11	
Age at resection			
<20 years	7 (41)	3 (27)	0.69
≥20 years	10 (59)	8 (73)	
Median (years)	37	30	0.72 <sup>b</sup>
Range (years)	0.06–77	2–74	
Sex <sup>c</sup>			
Female	10 (59)	6 (55)	1
Male	7 (41)	5 (46)	
Race			
Caucasian	11 (65)	8 (73)	0.69
African American	4 (24)	3 (27)	
Other	2 (12)	0 (0)	
Other malignancy <sup>d</sup>	2 (15)	0 (0)	0.49
Multifocal disease <sup>d</sup>	3 (23)	2 (25)	1
Clinical outcome			
No evidence of disease	11 (65)	6 (55)	0.70
Alive with disease	4 (24)	2 (18)	1
Died of disease	1 (6)	2 (18)	0.54
Lost to follow up	1 (6)	1 (9)	1

<sup>a</sup>Fisher's exact test unless otherwise noted<sup>b</sup>Wilcoxon rank-sum test<sup>c</sup>Percentages may not add up to 100 due to rounding<sup>d</sup>Data not available for all patients

potentially altered the tumor microenvironment. The median follow-up time was 3.8 years. Nine patients had persistent disease following treatment, including six living with disease and three who died of metastatic disease. Twenty (71%) of patients' tumors were ALK(+). Among all ALK(–) tumors, one was positive for *ROS1* rearrangement, four were negative, and three could not be assessed due to technical failure.

### PD-L1 expression on tumor and immune cells in inflammatory myofibroblastic tumor

Membranous PD-L1 expression was observed on both tumor and immune cells (Fig. 1). Of the 35 specimens analyzed, 24 (69%) had PD-L1(+) tumor cells and 28 (80%) showed PD-L1(+) immune cells. Concurrent tumor and immune PD-L1 expression was observed in 22 (63%) specimens and there was a positive correlation between the proportion of tumor and immune cells expressing PD-L1 ( $r = 0.48$ ,  $p = 0.0035$ ), supporting a component of adaptive PD-L1 expression. Tumor size was positively correlated with the proportion of PD-L1

(+) tumor cells ( $r = 0.38$ ,  $p = 0.029$ ), but not PD-L1(+) immune cells ( $p = 0.7$ ). The remaining clinicopathologic features assessed did not correlate with tumor cell (Tables 2 and 3) or immune cell (data not shown) PD-L1 expression. Multiple specimens were available from three patients and were concordant for PD-L1 staining (Table 1 and Fig. 2).

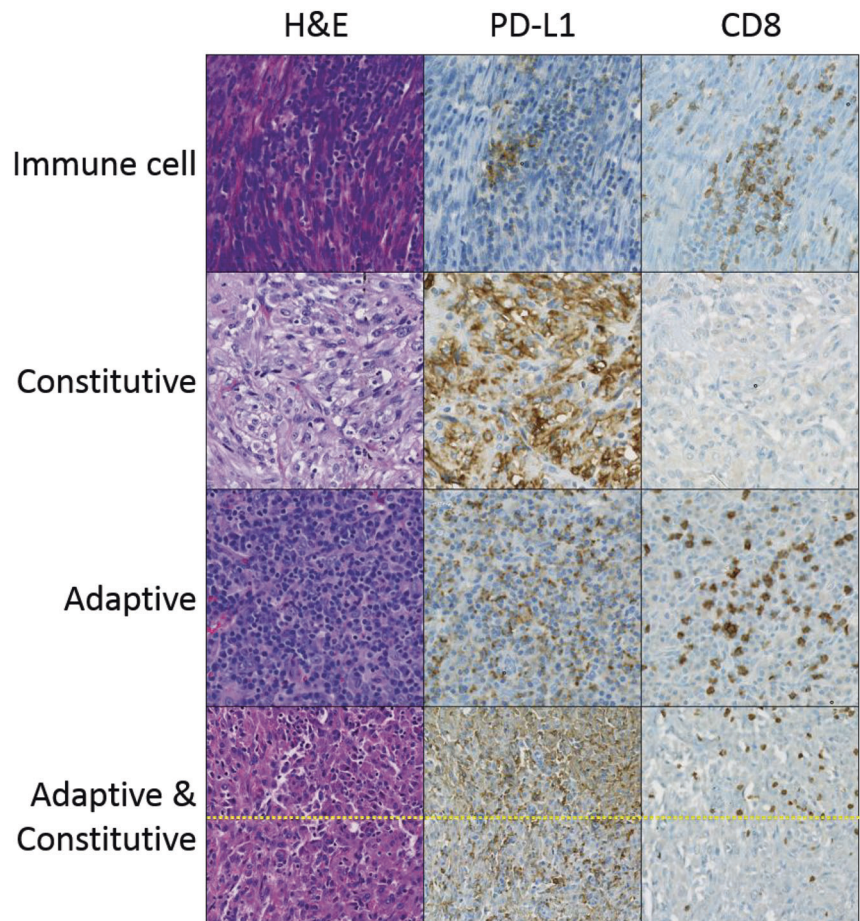
### PD-L1 expression and the tumor immune microenvironment

We next sought to determine the association between PD-L1 expression and infiltrating immune cells in inflammatory myofibroblastic tumors. Tumors with brisk lymphocytic infiltrates were more likely to express PD-L1 on tumor and immune cells, although the trend was not significant for tumor cell PD-L1 ( $p = 0.13$  and  $p = 0.03$ , respectively) (Table 4). PD-L1 expression was not associated with lymphoid aggregates or non-lymphocytic immune infiltrates. Quantification of tumor infiltrating CD8<sup>+</sup> T cells by digital image analysis showed a nearly threefold increase in cases with PD-L1(+) tumor cells ( $p = 0.085$ ) and a fourfold increase in cases with PD-L1(+) immune cells ( $p = 0.008$ ) (Table 4). The CD8<sup>+</sup> T cell density positively correlated with the proportion of PD-L1(+) immune cells ( $r = 0.36$ ,  $p = 0.03$ ), but not the proportion of PD-L1(+) tumor cells ( $p = 0.4$ ). There was no correlation between CD8<sup>+</sup> T cell density and tumor size.

Three patterns of tumor cell PD-L1 expression were observed (Fig. 1). Ten (42%) cases showed an adaptive (tumor infiltrating lymphocyte-associated) expression pattern only, and 1 (4%) case showed a purely constitutive (non-tumor infiltrating lymphocytes-associated) expression pattern. The majority of cases ( $n = 13$ , 54%) showed evidence of both adaptive and constitutive PD-L1 expression (Fig. 3). In total, 23 (96%) of PD-L1(+) tumors showed a component of an adaptive expression pattern.

The pathologist-assessed patterns of PD-L1 expression were supported by CD8<sup>+</sup> T cell quantification. Tumors with adaptive tumor cell PD-L1 expression (adaptive only and adaptive+ constitutive) showed a threefold increase in CD8 density over those without (379 vs. 126 cells/mm<sup>2</sup>,  $p = 0.03$ ). When tumors with adaptive tumor cell PD-L1 are combined with those showing immune cell PD-L1 expression, a 4.5-fold increase in CD8 density is observed (377 vs. 83 cells/mm<sup>2</sup>,  $p = 0.005$ ). In contrast, constitutive tumor cell PD-L1 expression is not associated with CD8 density (230 vs. 365 cells/mm<sup>2</sup>,  $p = 1.0$ ). Inflammatory myofibroblastic tumors with a component of constitutive PD-L1 expression were significantly larger than those without (6.7 cm vs. 2 cm,  $p = 0.002$ ), while there was no association between tumor size and adaptive PD-L1 expression ( $p = 0.5$ ).

**Fig. 1** PD-L1 expression patterns in inflammatory myofibroblastic tumors. H&E, PD-L1, and CD8 immunohistochemistry stains are shown. Patterns of PD-L1 expression observed include: (row 1) immune cell expression only; (row 2) constitutive (non-tumor infiltrating lymphocyte-associated) tumor cell expression; (row 3) adaptive (tumor infiltrating lymphocyte-associated) tumor expression ( $\pm$ immune cell PD-L1). Row 4 shows a combination of adaptive and constitutive expression. Note low-level PD-L1 expression in the absence of tumor infiltrating lymphocytes (bottom) that is further enhanced in association with tumor infiltrating lymphocytes (top). Rows 1–3 are at a magnification of  $\times 200$ , row 4 is at  $\times 100$ . Figure 3 shows additional images of combined adaptive and constitutive PD-L1 expression



**PD-L1 expression and inflammatory myofibroblastic tumor fusion status**

Genetic fusion status, defined as ALK overexpression or *ROS1*-rearrangement, did not associate with PD-L1 status or PD-L1 expression pattern. However, fusion-negative inflammatory myofibroblastic tumors were enriched for PD-L1(+) immune cells (median of 20% in fusion-negative vs. 10% in fusion-positive tumors,  $p = 0.005$ ). In contrast, the median percentage of PD-L1(+) tumor cells and median CD8<sup>+</sup> T cell density were not significantly different in fusion-positive vs. fusion-negative inflammatory myofibroblastic tumors (50 vs. 25% and 468 vs. 218 cells/mm<sup>2</sup>,  $p = 0.11$  and  $p = 0.13$ , respectively).

**Discussion**

Our findings demonstrate a high prevalence of PD-L1 expression on both tumor and infiltrating immune cells in inflammatory myofibroblastic tumors. Among recurrent and metastatic inflammatory myofibroblastic tumors evaluated, 80% were PD-L1(+). PD-L1 expression was also prevalent

in ALK(-) inflammatory myofibroblastic tumors (88%). Although increasing tumor size was associated with constitutive PD-L1 expression, 96% of PD-L1(+) tumors showed a component of adaptive PD-L1 expression. This latter point is noteworthy, as this pattern of PD-L1 expression is thought to be predictive of response to anti-PD-1 [11]. Collectively, these data support further investigation into PD-1/PD-L1 blockade in this tumor type.

Conflicting reports exist about the potential relationship between ALK overexpression in other tumor types and increased constitutive PD-L1 expression [12–15]. In the present study, we found no difference in constitutive (or adaptive) tumor cell PD-L1 expression in ALK(+) vs. ALK(-) inflammatory myofibroblastic tumors. However, among tumors with PD-L1(+) immune cells, ALK(-) tumors were noted to have a significant increase in the proportion of PD-L1(+) immune cells. Consequently, PD-1/PD-L1 blockade may be of particular benefit in this subset.

Approximately one-third of patients diagnosed with inflammatory myofibroblastic tumor develop a paraneoplastic syndrome characterized by fever, leukocytosis, and hematologic abnormalities [16]. This syndrome has been ascribed to elevated levels of interleukin 6, a pro-

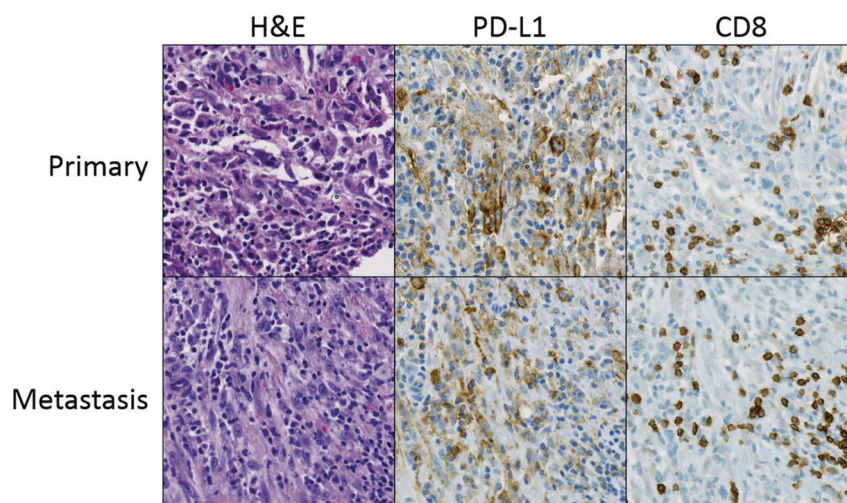
**Table 3** Histopathologic characteristics by IMT PD-L1 status

Characteristic	PD-L1+ (n = 24)	PD-L1- (n = 11)	p value <sup>a</sup>
<b>Tumor size (cm)</b>			
Median	4.0	3.1	0.25 <sup>b</sup>
Range	0.7–20	0.5–10	
<b>Location, n (%)</b>			
Lung	4 (17)	4 (36)	0.23
Abdomen	6 (25)	2 (18)	1
Bladder	4 (17)	0 (0)	0.28
Extremity/Soft tissue	2 (8)	0 (0)	1
Other	8 (33)	5 (45)	0.71
<b>Specimen type, n (%)</b>			
Primary	17 (71)	9 (82)	0.69
Recurrence	2 (8)	0 (0)	1
Metastasis	5 (21)	2 (18)	1
<b>Histologic subtype, n (%)</b>			
Fasciitis-like	18 (75)	7 (64)	0.69
Fascicular	6 (25)	3 (27)	1
Epithelioid	0 (0)	1 (9)	0.31
Hypercellularity	21 (88)	10 (91)	1
Spindled tumor cells	24 (100)	10 (91)	0.31
Cellular pleomorphism	13 (54)	4 (36)	0.47
Mitoses (>2/10HPF)	5 (21)	2 (18)	1
Necrosis	8 (33)	3 (27)	1

<sup>a</sup>Fisher's exact test unless otherwise noted<sup>b</sup>Wilcoxon rank-sum test

inflammatory cytokine secreted by T cells and macrophages to stimulate immune response. Here, we found that tumor or immune cell PD-L1 was associated with a 3-4-fold increase in infiltrating CD8<sup>+</sup> T cells, which is consistent with the finding that T cell secretion of interferon gamma can stimulate PD-L1 expression [17]. Notably, several cases of inflammatory myofibroblastic tumor spontaneous regression have been reported [18]. Collectively, these data support the hypothesis that at least a subset of infiltrating lymphocytes may possess the capacity for anti-tumor activity in inflammatory myofibroblastic tumors. Most patients with inflammatory myofibroblastic tumor are cured by complete surgical excision. However, a subset of inflammatory myofibroblastic tumors recur and, in rare instances, progress to metastatic disease. In particular, tumors with *RANBP2-ALK* fusions and epithelioid morphology have been associated with more aggressive behavior [19, 20]. Studies in other soft tissue sarcomas have showed that prevalence of PD-L1 expression varies between histologic subtypes and may be an unfavorable prognostic feature, although reports conflict regarding the latter point, depending on assay used and scoring system [21–25]. Two phase 2 clinical trials of Pembrolizumab (anti-PD-1) in advanced soft tissue sarcomas have reported objective response rates up to 18% with the few patients with PD-L1-positive tumors among the responders [26, 27].

This study has several limitations. Inflammatory myofibroblastic tumors are rare and only 28 patients had tissue available for study. This cohort was skewed toward adult patients (64%), so these results may not be generalizable in



**Fig. 2** PD-L1 expression is concordant in paired primary and metastatic inflammatory myofibroblastic tumors. Primary and metastatic specimens from three patients were included in the study and all were concordant for PD-L1 expression. Representative examples, including a primary inflammatory myofibroblastic tumor of the lung (row 1) and

chest wall metastasis from the same patient (row 2), stained with H&E, PD-L1, and CD8 are shown. In both tumors, membranous PD-L1 expression is observed on tumor cells and is associated with CD8<sup>+</sup> T cell infiltration. Original magnification  $\times 200$

**Table 4** Features of the tumor immune microenvironment and PD-L1 expression in IMTs

Characteristic	Tumor cell PD-L1			Immune cell PD-L1		
	Positive (n = 24)	Negative (n = 11)	p value <sup>a</sup>	Positive (n = 28)	Negative (n = 7)	p value <sup>a</sup>
Fusion status <sup>b</sup>						
Positive	18 (75)	10 (91)	0.31	21 (75)	7 (100)	0.30
Negative	6 (25)	1 (9)		7 (25)	0 (0)	
Lymphoid aggregates	10 (42)	3 (27)	0.48	12 (43)	1 (14)	0.22
Brisk IC infiltrates						
Lymphocytes	18 (75)	5 (45)	0.13	21 (75)	2 (29)	0.03
Plasma cells	13 (54)	4 (36)	0.47	15 (54)	2 (29)	0.40
Eosinophils	4 (17)	0 (0)	0.28	4 (14)	0 (0)	0.56
Neutrophils	1 (4)	0 (0)	1	1 (4)	0 (0)	1
CD8 density (cells/mm <sup>2</sup> )	378	134	0.085 <sup>c</sup>	378	94	0.008 <sup>c</sup>

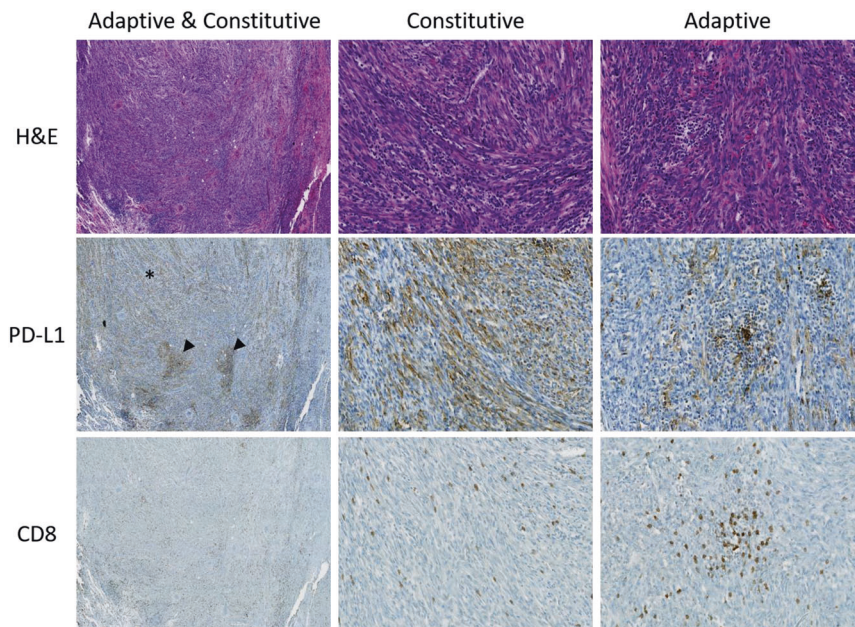
IC immune cells

<sup>a</sup>Fisher’s exact test unless otherwise noted

<sup>b</sup>Includes ALK-positive and ROS1-rearranged tumors

<sup>c</sup>Wilcoxon rank sum test

**Fig. 3** Mixed adaptive and constitutive PD-L1 expression in inflammatory myofibroblastic tumors. H&E, PD-L1, and CD8 stains of an inflammatory myofibroblastic tumor showing a combination of adaptive and constitutive expression. Heterogeneous PD-L1 expression can be seen at low power (first column), including diffuse constitutive expression (asterisk) and focal higher intensity adaptive expression (arrowheads). Higher magnification images highlight areas of constitutive expression (second column) and adaptive expression (third column). Original magnification  $\times 40$  (first column) and  $\times 200$  (second and third columns)



the pediatric population. The majority of specimens studied were primary tumors, although PD-L1 expression in recurrent and metastatic lesions was concordant with the primary ( $n = 3$  patients). Finally, assessment of PD-L1 status by histologic subtype and specific genomic alterations was limited by the small sample size. Reproduction of the study findings, ideally in a larger patient cohort, will be important in confirming the significant associations identified in this report. It is also worth emphasizing that the

manual PD-L1 immunohistochemistry staining protocol using the SP142 antibody clone in this study is distinct from the FDA-approved automated SP142 PD-L1 immunohistochemistry assay. We and others have previously shown that the SP142 clone itself can demonstrate a similar sensitivity for the detection of PD-L1, depending on assay conditions [28–32]. In summary, we found that PD-L1 expression in inflammatory myofibroblastic tumors is prevalent and associated with tumor infiltrating CD8<sup>+</sup> T cells,

supporting an adaptive immune resistance mechanism of expression. PD-L1 status appears to be independent of ALK status, suggesting that these two features could be considered independent biomarkers. This could be particularly useful clinically in ALK(−) inflammatory myofibroblastic tumors or those resistant to tyrosine kinase inhibitor therapy. Our findings provide a rationale for further investigation of the utility of checkpoint blockade therapy in treatment refractory inflammatory myofibroblastic tumors.

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### Compliance with ethical standards

**Conflict of interest** JMT receives grant funding from Bristol-Myers Squibb (BMS) and is a consultant/advisory board member for BMS, Merck, and Astra-Zeneca. The remaining authors declare that they have no conflict of interest.

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