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ARTICLE

V-domain Ig-containing suppressor of T-cell activation (VISTA), a potentially targetable immune checkpoint molecule, is highly expressed in epithelioid malignant pleural mesothelioma

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Abstract

V-domain Ig-containing suppressor of T-cell activation (VISTA) is an immune checkpoint gene that inhibits anti-tumor immune responses. Since most malignant pleural mesotheliomas do not respond to anti-programmed cell death(-ligand)1 (PD-(L)1)/cytotoxic T-lymphocyte-associated protein 4 (CTLA4) therapy and given the recent finding of The Cancer Genome Atlas Study that pleural mesothelioma displays the highest expression of VISTA among all cancers studied, we examined VISTA expression in a large pleural mesothelioma cohort. VISTA and PD-L1 immunohistochemistry were performed on tissue microarray of immunotherapy-naive pleural mesotheliomas (254 epithelioid, 24 biphasic and 41 sarcomatoid) and ten whole-tissue sections of benign pleura (VISTA only). Percentages of tumor and inflammatory cells with positive staining were assessed. Optimal prognostic cutoff percentages were determined using maximally selected rank statistics. Overall survival was evaluated using Kaplan–Meier methods and Cox proportional hazard analysis. All benign mesothelium expressed VISTA. Eighty-five percent of 319 and 38% of 304 mesotheliomas expressed VISTA and PD-L1 (88% and 33% of epithelioid, 90% and 43% of biphasic, and 42% and 75% of sarcomatoid), respectively. Median VISTA score was significantly higher in epithelioid (50%) (vs. biphasic [20%] and sarcomatoid [0]) ($p < 0.001$), while median PD-L1 score was significantly higher in sarcomatoid tumors (20%) (vs. biphasic and epithelioid [both 0%]) ($p < 0.001$). VISTA and PD-L1 were expressed in inflammatory cells in 94% ($n = 317$) and 24% ($n = 303$) of mesothelioma, respectively. Optimal prognostic cutoffs for VISTA and PD-L1 were 40% and 30%, respectively. On multivariable analysis, VISTA and PD-L1 expression in mesothelioma were associated with better and worse overall survival ($p = 0.001$ and $p = 0.002$), respectively, independent of histology. In a large cohort of mesothelioma, we report frequent expression of VISTA and infrequent expression of PD-L1 with favorable and unfavorable survival correlations, respectively. These findings may explain poor responses to anti-PD-(L)1 immunotherapy and suggest VISTA as a potential novel target in pleural mesothelioma.

Introduction

Given the high lethality of malignant pleural mesothelioma and the limited efficacy of currently commercially available checkpoint inhibitors [1–16], a new area of exploration is

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immune checkpoints other than programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1). V-domain Ig suppressor of T-cell activation (VISTA), also known as c10orf54, PD-1H and B7-H5 and a member of the B7-CD28 family of ligands and receptors, is a novel immune checkpoint gene structurally similar to PD-L1 [12]. VISTA is expressed on myeloid cells and T-lymphocytes and, when overexpressed, suppresses early T-cell activation and proliferation and reduces cytokine production [12, 13]. VISTA is unique in that it acts as both a ligand on antigen-presenting cells and as a receptor on T-cells [14, 15]. VSIG-3 was recently discovered as the ligand for VISTA [16]. Increased VISTA expression has been observed in tumor cells and/or immune microenvironment of some malignant tumors, including, but not limited to, oral squamous cell carcinoma, gastric carcinoma, hepatocellular carcinoma, prostate carcinoma, and melanoma [17–21].

The Tumor Cancer Genome Atlas reported strong expression of VISTA in benign mesothelium by immunohistochemistry and increased mRNA expression of VISTA in malignant pleural mesothelioma compared to other tumor types [22], suggesting that VISTA may be a potential therapeutic target in malignant pleural mesothelioma. Additionally, the utility of PD-L1 immunohistochemistry as a biomarker for predicting response to anti-PD(-L)1 therapies in malignant pleural mesothelioma is not well understood. Most malignant pleural mesothelioma are negative for PD-L1 by immunohistochemistry [23–27]. In the few trials in which PD-L1 immunohistochemistry was tested, PD-L1 expression in tumor cells did not correlate with response to immunotherapy [10]. Yet, tumor-infiltrating lymphocytes have been shown to be a positive prognostic factor in malignant pleural mesothelioma, suggesting the immune system may have an important function in malignant pleural mesothelioma [28, 29].

In an effort to expand our understanding of the immune microenvironment and potential checkpoint targets in malignant pleural mesothelioma, we characterized both VISTA and PD-L1 expression by immunohistochemistry in a large malignant pleural mesothelioma cohort.

Materials and methods

Study cohort

Following Institutional Review Board approval (IRB #19–001), tissue (tumor samples procured from 1989 to 2010) was retrieved from surgical pathology archives at Memorial Sloan Kettering Cancer Center, including tissue microarrays containing a total of 319 immunotherapy-naive malignant pleural mesothelioma (Table 1) and ten whole-tissue sections of benign mesothelium (two previously included in The Cancer Genome Atlas study) [22]. Tissue microarray construction has been previously described [30–32]. Briefly, representative areas of tumor were selected from hematoxylin and eosin-stained slides to construct Tissue microarrays from 0.6 mm tissue cores (1–9/case) of corresponding formalin-fixed, paraffin-embedded tissue.

Table 1 Demographic, clinical, and histopathologic data for MPM patient cohort

Characteristic	$n = 319$ (%)
Sex, male/female	237/82 (74/26)
Age (yr) at diagnosis, median (range)	64 (29–85)
Procedure	
Biopsy	21 (7)
Pleurodesis/decortication	121 (38)
Extrapleural pneumonectomy	177 (55)
Tumor histology	
Epithelioid	254 (80%)
Biphasic	41 (13%)
Sarcomatoid	24 (7%)
Pathologic stage[a]	
I	10 (3)
II	75 (24)
III	187 (60)
IV	39 (13)
Overall survival (mo), median (range)	12 (0–246.7)

[a]A subset that were not resected were staged clinically

MPM malignant pleural mesothelioma, *mo* month, *n* number, *yr* year

Two thoracic pathologists (JLS and SM) re-reviewed all malignant pleural mesothelioma cases to adjudicate tumor classification and histologic subtyping. A third thoracic pathologist (WDT) was consulted in a subset of cases. Tumors were classified according to 2015 WHO Classification of Tumors of the Lung, Pleura, Thymus, and Heart (4th edition) [33]. When possible, tumors with epithelioid histology were further assessed for the presence of pleomorphic features using criteria established by Kadota et al. [34].

Clinical data including age, gender, and survival was extracted from the electronic medical record. Survival was defined from date of diagnosis to database lock (3/2018). Median follow-up for malignant pleural mesothelioma cohort was 12 months. Staging was performed according to 8th edition of AJCC TNM staging manual [35]. Patient demographics, stage, and survival are provided in Table 1.

Immunohistochemistry

Immunohistochemistry was performed on 4 µm formalin-fixed, paraffin-embedded tissue sections using VISTA

(D1L2G; dilution 1:200), and PD-L1 (E1L3N; dilution 1:100) antibodies (Cell Signaling Technologies, Danvers, MA) and an automated immunostaining platform (Bond III, Leica, Buffalo Grove, IL) using heat-based antigen retrieval employing high pH buffer (Epitope retrieval solution-2, Leica) for 30 min. A polymeric secondary kit (Refine, Leica) was used for detection of primary antibody.

Positive staining for VISTA in tumor cells was defined as the presence of any cytoplasmic and membranous staining, and for PD-L1, the presence of any partial or complete membranous staining. For both VISTA and PD-L1, the percentage of tumor cell staining was scored (0 to 100%). The percentage of tumor-associated inflammatory cells staining for each antibody was also scored (0 to 100%). Positive scores were defined as positive staining in at least 1% of cells, and a negative score was defined as staining in <1% or 0 cells. Multiple tumor cores of each case from tissue microarray were scored independently, and average score was used.

Statistical analysis

Continuous variables were summarized by median and range. Categorical variables were summarized by frequency and percentage. PD-L1 and VISTA scores were compared between tumor types using Wilcoxon rank-sum test. Correlation between these two scores was assessed by Pearson correlation coefficient. Median overall survival (OS) was estimated by Kaplan–Meier method. Association between immunohistochemistry scores and overall survival was assessed by Cox proportional hazard regression. To find the optimal cutoff of PD-L1 and VISTA score for overall survival, a grid search was conducted overall, the observed range of expression scores and the cutoff that gave the maximal log-rank statistic was selected as the optimal cutoff. A univariate Cox proportional hazard regression was then conducted to compare overall survival between the two groups defined by the identified optimal cutoff. Since an exhaustive search was used to identify the cutoff, the univariate p-values needed to be adjusted for inflated false-positive rate. The method proposed by Lausen and Schumacher [36] was used for the adjustment. All statistical analyses were conducted using R 3.5.2 [37].

Results

VISTA immunohistochemistry

All benign mesothelium in whole-tissue sections showed strong diffuse positive staining with VISTA.

Positive VISTA staining in tumor cells was seen 270 (85%) of 319 malignant pleural mesotheliomas, more frequently in epithelioid (223 [88%] of 254) and biphasic (37 [90%] of 41) compared to sarcomatoid malignant pleural mesotheliomas (10 [42%] of 24) (Fig. 1 and Supplemental Fig. 1A). In biphasic malignant pleural mesotheliomas, VISTA was generally positive in epithelioid components and negative in sarcomatoid components.

Quantity of VISTA expression varied, but two-thirds of malignant pleural mesotheliomas showed ≥50% of tumor cells staining for VISTA (1–5% of tumor cells, 11 malignant pleural mesotheliomas [3%]; 6–25%, 77 [24%]; 26–49%, 19 [6%], 50–75%, 66 [21%] and 76–100%, 97 [30%]). Median VISTA score was higher in tumors with epithelioid histology (50%) compared to those with biphasic (20%) and sarcomatoid (0%) histology ($p < 0.001$) (Table 2).

Pleomorphic features were seen at least focally in 41 (17%) of 247 epithelioid malignant pleural mesotheliomas. VISTA expression was significantly higher (median score of 60% [range 0–100%] vs. 20% [0–95%]; $p < 0.001$) in epithelioid malignant pleural mesotheliomas without pleomorphic features compared to tumors with pleomorphic features.

VISTA expression was seen in tumor-associated inflammatory cells in most cases (94% of 317 malignant pleural mesotheliomas with tumor-associated inflammatory cells present; range 1–20% of inflammatory cells showing positive staining).

PD-L1 immunohistochemistry

PD-L1 was expressed by immunohistochemistry in less than half of malignant pleural mesotheliomas (115 [38%] of 304), but more frequently in sarcomatoid (75% of 24) compared to epithelioid (33% of 240) and biphasic (43% of 40) tumors (Fig. 1 and Supplemental Fig. 1B). Median score for PD-L1 was significantly greater in sarcomatoid (20%) malignant pleural mesotheliomas compared to biphasic and epithelioid (both 0) tumors ($p < 0.001$) (Table 2).

Pleomorphic features were present in 39 (17%) of 233 epithelioid malignant pleural mesotheliomas in which PD-L1 immunohistochemistry was assessed. There was no significant difference in the median score of PD-L1 between epithelioid malignant pleural mesotheliomas (median score 30% [range 0–90%]) with pleomorphic features and tumors without pleomorphic features (35% [0–90%]) ($p = 0.013$).

In most cases, PD-L1 immunohistochemistry was not expressed in tumor-associated inflammatory cells (76% of 303 malignant pleural mesotheliomas with tumor-associated inflammatory cells present).

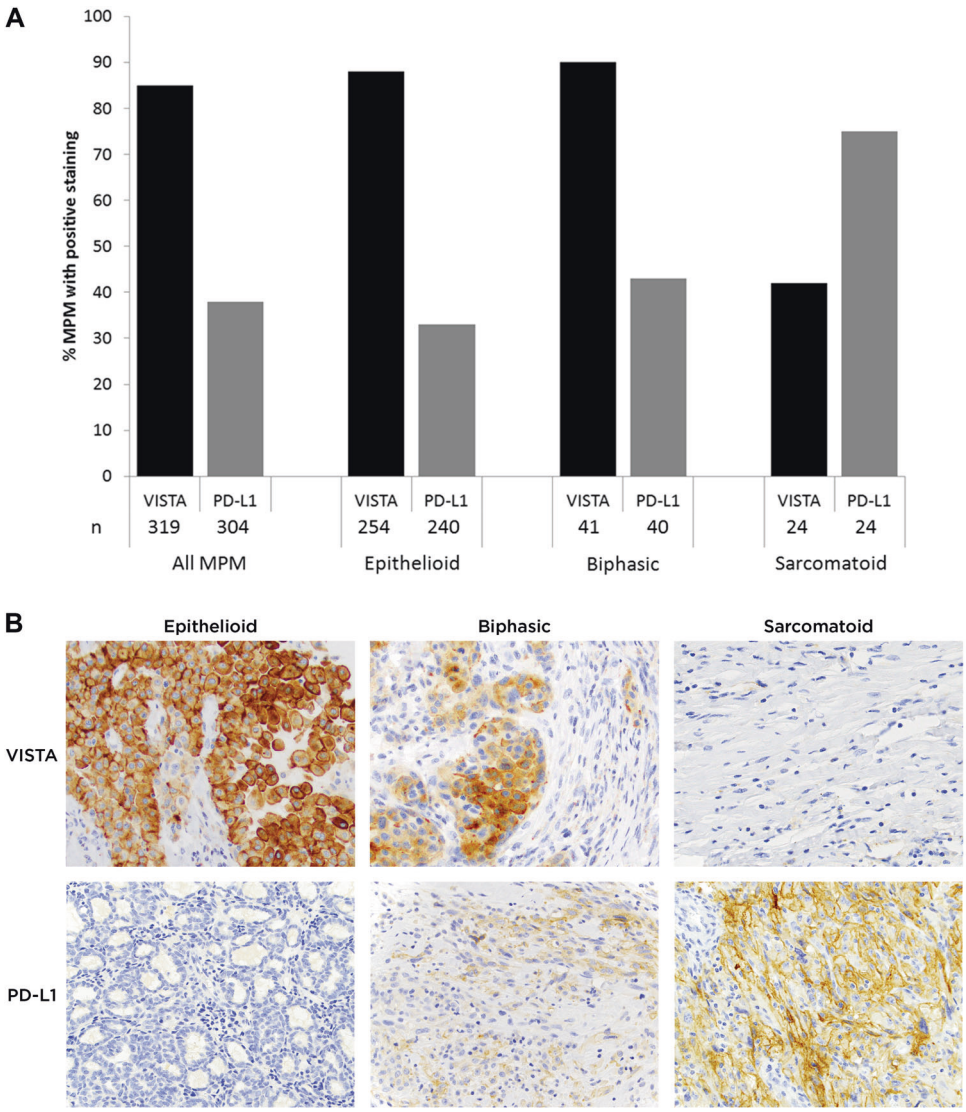


Fig. 1 Expression of V-domain Ig-containing suppressor of T-cell activation (VISTA) and programmed death-ligand 1 (PD-L1) by immunohistochemistry in malignant pleural mesothelioma. (**a**) Percentage of tumors in tissue microarray with positive staining for VISTA and/or PD-L1. (**b**) VISTA and PD-L1 immunohistochemistry in epithelioid, biphasic and sarcomatoid malignant pleural mesothelioma (original magnifications ×200)

Table 2 Clinicopathologic and histologic characteristics and the expression of VISTA and PD-L1 by immunohistochemistry in MPM TMA cohort

	Overall $n = 319$	Epithelioid $n = 254$	Biphasic $n = 41$	Sarcomatoid $n = 24$	p-value*
VISTA raw score, median (range)	50 (0 to 100)	50 (0 to 100)	20 (0 to 95)	0 (0 to 50)	<0.001
PD-L1 raw score, median (range)	0 (0 to 95)	0 (0 to 95)	0 (0 to 95)	20 (0 to 95)	<0.001
Age, median (range)	64 (29 to 85)	63 (29 to 85)	66 (41 to 79)	66 (46 to 78)	0.016
Female, *n* (%)	82 (25.7)	72 (28.3)	5 (12.2)	5 (20.8)	0.073
Stage, *n* (%)					
1	10	10	0	0	0.023
2	75	67	4	4	
3	187	147	27	13	
4	47	30	10	7	

*p-values compare rows among the three histology groups

MPM malignant pleural mesothelioma, *n* number, *PD-L1* programmed cell death-ligand 1, *TMA* tissue microarray, *VISTA* V-domain Ig suppressor of T-cell activation

Relationship of VISTA and PD-L1 expression

VISTA expression was seen more frequently in epithelioid and biphasic malignant pleural mesotheliomas; while PD-L1 expression was seen more frequently in sarcomatoid tumors (Fig. 1). An inverse negative relationship between VISTA and PD-L1 scores in tumor cells was observed (Pearson correlation coefficient, $r = -0.16$; $p = 0.004$) (Supplemental Fig. 2).

Clinical significance of VISTA and PD-L1 expression in malignant pleural mesothelioma univariate analysis

VISTA expression in malignant pleural mesotheliomas by immunohistochemistry was associated with significantly better overall survival ($p < 0.001$), while PD-L1 expression was associated with significantly worse overall survival ($p < 0.001$). For every 10% increase in VISTA expression within a tumor, there was a 6% decreased risk of death (hazard ratio [HR] 0.94; [95% confidence interval [CI], 0.91–0.97]), and for every 10% increase in PD-L1 expression within a tumor, there was a 10% increased risk of death (HR 1.10; [95% CI, 1.05–1.15]).

Multivariate analysis

In final multivariate models, percentage of tumor cells in malignant pleural mesotheliomas with positive staining for VISTA immunohistochemistry was associated with better overall survival (HR 0.94, [95% CI, 0.91–0.98]; $p = 0.001$), and percentage of tumor cells with positive staining for PD-L1 immunohistochemistry was associated with worse overall survival (HR 1.1, [95% CI, 1.0–1.1]; $p = 0.002$), independent of histology, stage, age and gender (Tables 3 and 4).

Optimal cutoff percentages of VISTA and PD-L1 expression by immunohistochemistry for prognostic significance in malignant pleural mesothelioma

Using a grid search over all possible VISTA scores, a cutoff of 40% of tumor cells with positive staining provided the most statistically significant difference in overall survival

Table 3 Multivariate Cox proportional hazards model on overall survival using raw VISTA scores in MPM ($n = 317$)

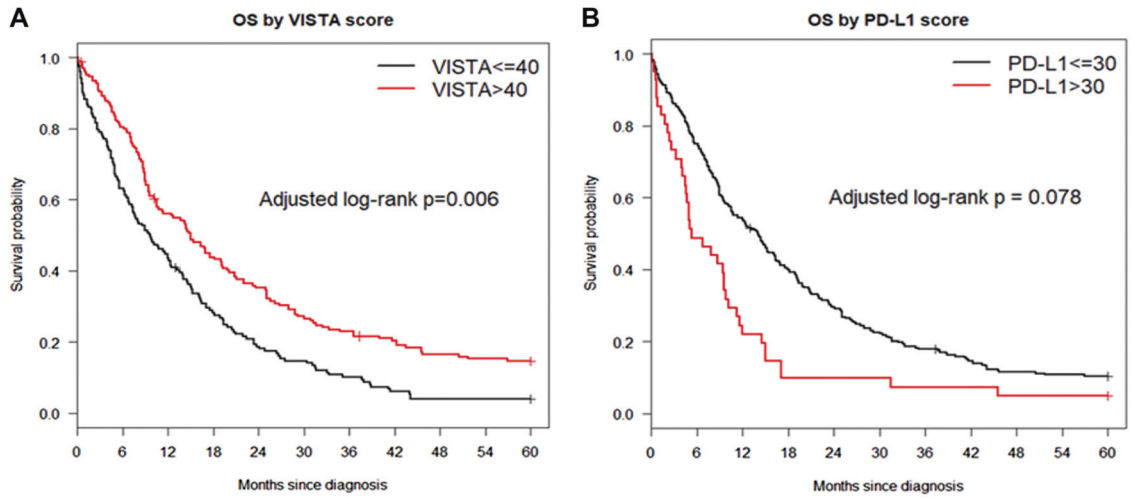
	Hazard ratio	95% CI of HR	*p*-value	Concordance index
VISTA raw score (10 percent increment)	0.94	0.91 to 0.98	0.001	0.661
Histology				
Biphasic vs. epithelioid	1.5	1.1 to 2.2	0.020	
Sarcomatoid vs. epithelioid	1.8	1.1 to 2.9	0.011	
Stage				
2 vs. 1	1.8	0.92 to 3.6	0.084	
3 vs. 1	3.0	1.5 to 5.9	0.001	
4 vs. 1	3.6	1.8 to 7.4	<0.001	
Age	1.0	1.0 to 1.0	0.406	
Gender, male vs. female	1.6	1.2 to 2.1	<0.001	

CI confidence interval, *HR* hazard ratio, *MPM* malignant pleural mesothelioma, *n* number, *vs.* versus, *VISTA* V-domain Ig suppressor of T-cell activation

Table 4 Multivariate Cox proportional hazards model on overall survival using raw PD-L1 scores in MPM ($n = 302$)

	Hazard ratio	95% CI of HR	*p*-value	Concordance index
PD-L1 raw score (10% increment)	1.1	1.0 to 1.1	0.002	0.654
Histology				
Biphasic vs. epithelioid	1.8	1.2 to 2.5	0.002	
Sarcomatoid vs. epithelioid	1.9	1.2 to 3.0	0.007	
Stage				
2 vs. 1	2.1	1.1 to 4.2	0.035	
3 vs. 1	3.2	1.6 to 6.3	<0.001	
4 vs. 1	3.6	1.7 to 7.3	<0.001	
Age	1.0	1.0 to 1.0	0.415	
Gender, male vs. female	1.5	1.2 to 2.0	0.002	

CI confidence interval, *HR* hazard ratio, *MPM* malignant pleural mesothelioma, *n* number, *PD-L1* programmed cell death-ligand 1, *vs.* versus



	n	events	median OS	0.95LCL	0.95UCL
VISTA ≤ 40	152	151	9.65	7.4	12.5
VISTA > 40	165	160	15.00	11.2	19.1

	n	events	median OS	0.95LCL	0.95UCL
PD-L1 ≤ 30	261	256	14.0	10.8	16.3
PD-L1 > 30	41	41	5.3	4.6	9.8

Fig. 2 (**a**) Malignant pleural mesothelioma-specific cumulative incidence of death and overall survival comparing patients with tumors with high V-domain Ig-containing suppressor of T-cell activation (VISTA) expression (≥ optimal cutoff of 40%) vs. patients with low VISTA expression (< optimal cutoff of 40%) and (**b**) patients with tumors with high programmed death-ligand 1 (PD-L1) expression (≥ optimal cutoff of 30%) vs. patients with low PD-L1 expression (< optimal cutoff of 30%)

(HR 0.64, [95% CI, 0.51–0.80], adjusted $p = 0.006$.) and best stratified patients with better and worse overall survival (Fig. 2a). On univariate analysis, patients whose tumors demonstrated positive VISTA expression in at least 40% of tumor cells ($n = 165$) had significantly better overall survival compared to those whose tumors demonstrated VISTA expression in <40% of tumor cells ($n = 152$) (HR 0.64; adjusted $p = 0.006$) (Fig. 2a). Two- and 3-year overall survival for patients whose tumors showed positive VISTA expression in >40% of tumor cells was 35.1% and 22.8%, respectively, compared to 18.7% and 10.0%, respectively, in patients whose tumors expressed VISTA in 40% or fewer tumor cells (Supplemental Table 1).

On multivariate analysis, including histology, stage, age and gender, overall survival was significantly longer in patients whose tumors showed expression in >40% of tumor cells for VISTA compared to those whose tumors exhibited fewer tumor cells with positive VISTA expression (HR 0.68, [95% CI, 0.54–0.87]; $p = 0.002$) (Supplemental Table 2).

Using the same method, the optimal cutoff of percentage of tumor cells with positive staining for PD-L1 was 30% (HR 1.38, [95% CI, 1.31–2.56], adjusted $p = 0.078$), providing marginal evidence that this cutoff identifies two groups of patients with distinct overall survival (Fig. 2b). Patients whose tumors demonstrated >30% of tumor cell staining for PD-L1 ($n = 41$) had marginally worse overall survival compared to patients whose tumors expressed PD-L1 in 30% or fewer tumor cells ($n = 261$) (adjusted $p =$ 0.078) (Fig. 2b). Two- and 3-year overall survival for patients whose tumors showed positive PD-L1 expression in >30% of tumor cells was 9.8% and 7.3%, respectively, compared to 29.5% and 17.8%, respectively, in patients whose tumors expressed PD-L1 in 30% or fewer tumor cells (Supplemental Table 1).

Although only marginal difference in survival was observed in patients whose tumors expressed PD-L1 above and below the optimal cutoff percentage, when the a priori chosen 50% was used as a cutoff for PD-L1 expression to place tumors into two categories for PD-L1 expression (i.e., low [<50%] and high [≥50%] expression), patients with malignant pleural mesothelioma with high PD-L1 expression had significantly worse overall survival (2- and 3-year overall survival of 10.8% and 8.1%, respectively) compared to those whose tumors demonstrated low expression for PD-L1 (2- and 3-year OS of 29.0% and 17.6%, respectively) ($p < 0.001$) (Supplemental Fig. 3 and Supplemental Table 3).

Discussion

Consistent with The Cancer Genome Atlas [22], our data demonstrate that VISTA is expressed in benign mesothelium, as well as a high percentage of malignant pleural mesotheliomas and more commonly in epithelioid malignant pleural mesotheliomas compared to other histologic subtypes. In addition, we found that although overall PD-L1 expression is infrequent in malignant pleural mesothelioma,

it is expressed more frequently in sarcomatoid compared to other histologic subtypes. Survival analysis showed VISTA expression was associated with better overall survival, whereas PD-L1 expression was associated with worse overall survival.

The Cancer Genome Atlas reported that *VISTA* mRNA was expressed in malignant pleural mesothelioma at levels higher than in any other tumor type [22]. Increased VISTA expression has also been identified in tumor-infiltrating immune cells in oral squamous cell carcinomas, in tumor and immune cells of gastric carcinomas and in the immune microenvironment of prostate carcinoma and melanoma following treatment with immunotherapy, suggesting that VISTA may play a role in resistance to anti-PD-(L)1 antibodies. [17–19, 21] In non-small cell lung cancer where VISTA expression occurs in 21% of tumors and 98% of tumor-infiltrating lymphocytes, VISTA expression is higher in cytotoxic T-lymphocytes compared to T-helper cells and macrophages and is associated with low mutational burden, as well as increased 5-year survival [38].

Interestingly, unlike other tumors where VISTA is preferentially expressed in infiltrating inflammatory cells, VISTA expression in malignant pleural mesotheliomas occurs in both tumor and infiltrating inflammatory cells. VISTA expression in epithelioid malignant pleural mesothelioma may be related to retention of antigen presenting properties in these tumor cells [22]. Of note, in the current study, VISTA was expressed significantly less in epithelioid tumors that had at least focal pleomorphic features (median score of 20% vs. 60% in epithelioid malignant pleural mesothelioma without and with pleomorphic features, respectively). Despite this difference in the location of VISTA expression, as with non-small cell lung cancer, VISTA expression in our malignant pleural mesothelioma cohort was also associated with longer overall survival, even when multivariate analysis included histologic subtype.

Additionally, our study confirms prior reports that PD-L1 is infrequently expressed in malignant pleural mesotheliomas but is more common in sarcomatoid malignant pleural mesotheliomas compared to other histologic subtypes. [23–27, 39] In contrast to VISTA, PD-L1 expression was associated with shorter overall survival. The utility of PD-L1 immunohistochemistry as a biomarker for selecting patients with malignant pleural mesothelioma who will respond to immunotherapy is controversial. Many studies do not require PD-L1 immunohistochemistry for inclusion. Preliminary results of one phase II trial with PD-L1 expression data, KEYNOTE-139, showed pembrolizumab activity in PD-L1-unselected malignant pleural mesotheliomas. In this cohort, PD-L1 expression did not correlate with response to anti-PD-1 therapy [11]. Similarly, in a trial investigating nivolumab in patients with recurrent malignant pleural mesothelioma, there was no correlation between PD-L1 expression in pre-treatment nor on-treatment biopsy specimens and outcome [10]. While PD-L1 immunohistochemistry may not be a predictive biomarker for immunotherapy response, it is prognostic, and PD-L1 expression in our cohort of malignant pleural mesothelioma was associated with shorter survival, even when histologic subtype was included in multivariate analysis.

There are some important limitations to our work. Tumor heterogeneity is a known confounder in analyses of PD-L1 expression [23, 40] and may also be an issue with VISTA expression. Additionally, the use of tissue microarrays precludes complete tumor assessment. Additional large studies using whole-tissue sections are needed to better characterize temporal and spatial heterogeneity of PD-L1 and VISTA expression by immunohistochemistry in malignant pleural mesotheliomas. The impact of VISTA expression in benign mesothelial cells on the utility of VISTA as a target for anti-cancer therapies is unknown. However, PD-L1 is also expressed on surrounding non-tumor cells and successfully targeted with acceptable and manageable toxicity. Preliminary data from a Phase 1 study of CA-170 (NCT02812875), the first oral small molecule dual inhibitor of immune checkpoints PD-L1 and VISTA, shows evidence of peripheral T-cell activation and no dose limiting toxicity to date in 19 patients with advanced solid tumors or lymphomas [41].

Taken together, these data provide a strong rationale for exploring VISTA as a novel immunotherapy target in malignant pleural mesotheliomas. Specifically, anti-VISTA therapies may be particularly effective in better differentiated epithelioid malignant pleural mesothelioma given the high frequency of expression of VISTA in both tumor and tumor-associated inflammatory cells in these tumors. We have also shown that VISTA expression is an independent prognostic factor for longer survival. In contrast, PD-L1 is not frequently expressed in malignant pleural mesothelioma, with the exception of tumors with sarcomatoid histology, and is an independent prognostic factor for shorter overall survival. We also show that VISTA is frequently expressed in tumor-associated inflammatory cells in malignant pleural mesothelioma, while PD-L1 is not frequently expressed in these cells. Prospective studies analyzing VISTA as a predictive marker for patients whose tumors may respond to anti-VISTA antibodies will be important as trials with anti-VISTA therapies are expanded to malignant pleural mesotheliomas.

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Compliance with ethical standards

Conflict of interest The authors disclose the following conflict of interest: MGZ reports personal fees from Epizyme, personal fees from Sellas Life Sciences, personal fees from Aldeyra, grants from NIH, grants from Department of Defense, grants from MedImmune, grants from Epizyme, grants from Polaris, from Sellas Life Sciences, from BMS, from Millenium, non-financial support from Roche, grants from Curis, outside the submitted work, and Chair, Board of Directors, Mesothelioma Applied Research Foundation (uncompensated); VL reports serving on advisory boards for G1 Therapeutics and Pharma-Mar; ML reports personal fees from Bayer, personal fees from Astra-Zeneca, personal fees from Bristol Myers Squibb, personal fees from Takeda, grants from Helsinn Therapeutics, grants from Loxo Oncology, personal fees from Merck, outside the submitted work; JLS reports stock ownership in the following companies: Pfizer, Thermo Fischer Scientific, Inc., Merck & Co Inc and Chemed Corp; The other authors declare that they have no conflict of interest.

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