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Original contribution

Tumor PD-L1 expression in malignant pleural and peritoneal mesothelioma by Dako PD-L1 22C3 pharmDx and Dako PD-L1 28-8 pharmDx assays☆,☆☆,★

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Summary Malignant mesothelioma (MM) is an aggressive neoplasm with poor prognosis. The Dako PD-L1 22C3 and 28-8 pharmDx assays are approved by the US Food and Drug Administration (FDA) as companion and complementary diagnostics for the anti–PD-1 drugs pembrolizumab and nivolumab, respectively. Data from multiple clinical trials indicate that immunotherapy has antitumor activity in advanced malignant pleural (MPM) and peritoneal mesothelioma (MPeM). However, large studies of PD-L1 expression in MM using the FDA-approved anti–PD-L1 assays are lacking. We stained tissue microarray sections (N = 125; 112 MPM and 13 MPeM) using 2 FDA-approved clinical immunohistochemical makers for PD-L1 expression: Dako PD-L1 22C3 pharmDx and Dako PD-L1 28-8 pharmDx. Overall, 22% (27/125) of MMs were positive using the 22C3 assay, whereas 27% (32/117) were positive using the 28-8 assay, using a tumor proportion score cutoff of 1%. Tumor cell PD-L1 expression was strongly correlated with PD-L1 expression on tumor-associated immune cells. No significant difference in PD-L1 expression was observed by patient sex, age, treatment history, pathologic stage, or histologic subtype. However, the proportion of cases positive for PD-L1 expression was higher among MPeM compared to MPM ($P = .007$ for 22C3 assay; $P = .04$ for 28-8 assay). PD-L1 is expressed in a substantial proportion of MM cases, as measured by FDA-approved companion assays for widely used immunotherapeutic drugs. PD-L1 expression is particularly prevalent in MPeM. These findings support large clinical studies to further examine PD-L1 as a biomarker for a subset of MM patients that may benefit from immunotherapy.

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1. Introduction

Malignant mesothelioma (MM) is a highly aggressive neoplasm arising from the serosal lining of body cavities. Most cases arise in the pleural cavities, but up to 10%-15% of cases occur in the peritoneum [1]. The prognosis for MM is poor, particularly in pleural MM, with only 5% of patients surviving 5 years after diagnosis [2]. The majority of patients with MM die of complications of local disease. MM is considered an incurable tumor. First-line treatment consists of pemetrexed and platinum-based chemotherapy, with or without bevacizumab, but outcomes are poor, and second- and third-line therapies are experimental only [3-5].

Expression of programmed cell death protein 1 (PD-1) on activated T cells, as well as B cells and myeloid cells, provides a key immune checkpoint. PD-L1, the principal ligand of PD-1, is normally expressed on antigen-presenting cells and epithelial cells, including a proportion of cells in some tumors. The binding of PD-1 by PD-L1 suppresses activated T cells, providing a critical check on immune activity under physiologic conditions. However, in the neoplastic setting, inactivation of intratumoral T cells is thought to play a role in tumor evasion of immune surveillance. Some studies have reported poorer survival in MM cases with tumoral PD-L1 expression [6,7], whereas others reported no significant difference in survival between MM cases with and without PD-L1 expression [8].

Immune checkpoint inhibitors, including anti–PD-1 and anti–PD-L1 therapies, have been developed to lift the PD-L1–mediated blockade on the antitumor immune response and have shown promise in treating aggressive neoplasms such as non–small cell lung cancer (NSCLC) [9] and advanced solid tumors [10]. Importantly, tumor cell expression of PD-L1 by immunohistochemistry (IHC) is a Food and Drug Administration (FDA)–approved biomarker for predicting response to anti–PD-1 targeted therapies [11]. A fundamental element of the development and clinical evolution of these immunotherapies has been collaboration between pharmaceutical and in vitro diagnostics companies to develop drug-specific IHC assays, each with a specific antibody clone and testing platform. The Dako pharmDx assay using anti–PD-L1 clone 22C3 has gained FDA approval as a companion diagnostic test for pembrolizumab in NSCLC, gastric and gastroesophageal junction adenocarcinoma, cervical carcinoma, and urothelial carcinoma [12], whereas Dako pharmDx using anti–PD-L1 clone 28-8 is the approved complementary/co-diagnostic for nivolumab in nonsquamous NSCLC, melanoma, urothelial carcinoma, and head and neck squamous cell carcinoma [13]. These assays, in addition to some laboratory-developed tests, are currently used in routine tumor pathologic workup to determine eligibility for immune checkpoint inhibitor therapy.

Studies of mesothelioma cell lines have shown that these tumors are susceptible to anti-PD-L1–dependent cellular cytotoxicity [8]. Preliminary results from KEYNOTE-028, a nonrandomized, multicohort phase Ib trial, demonstrated that the immune checkpoint inhibitor pembrolizumab has robust antitumor activity in patients with advanced malignant pleural mesothelioma (MPM) and is generally well tolerated [14]. Other studies have supported the clinical efficacy of PD-L1 blockade in mesothelioma [15]. But although immune checkpoint inhibition may be a viable therapeutic option in MPM, the proportion of MPM patients whose tumors express PD-L1 remains unclear. Although a limited number of studies have examined PD-L1 expression in both MPM and peritoneal mesothelioma (MPeM), most of these studies have not used the specific FDA-approved anti–PD-1 clones or IHC protocols for currently available immunotherapies [3,6-8,16-18]. These studies have reported variable PD-L1 expression in MPM, ranging from 18% to 63% of cases. Interestingly, one small clinical trial using the Dako PD-L1 IHC 28-8 pharmDx assay reported PD-L1 expression data in 9 of 33 (27%) mesothelioma patients [15]—a figure significantly lower than those generally reported using nonapproved antibody clones. This result suggests the need for reevaluation of existing mesothelioma PD-L1 expression data, testing a large number of tumors using specific FDA-approved IHC assays, to guide further clinical and pathologic studies.

We sought to characterize PD-L1 expression in both MPM and MPeM using the FDA-approved Dako 22C3 and 28-8 pharmDx anti–PD-L1 antibodies and to characterize clinical and pathologic features associated with tumor PD-L1 expression in MM.

2. Materials and methods

This study was carried out under Institutional Review Board approval (University of Utah 00091019; University of Chicago 16-00057). To determine the proportion of MM cases expressing PD-L1, we stained 5 tissue microarrays (TMAs; N = 125, including 112 MPM and 13 MPeM) using 2 FDA-approved clinical IHC markers for PD-L1 expression: Dako PD-L1 22C3 pharmDx and Dako PD-L1 28-8 pharmDx (Dako, Carpinteria, CA), using the FDA-approved protocols and platforms, as per manufacturer's specifications [19-22]. Four TMAs contained MPM tissue, constructed with four 1-mm tissue cores per case. The fifth TMA contained MPeM tissue, constructed with four 2-mm tissue cores per case. Positive and negative controls stained appropriately. These included manufacturer-provided positive and negative control cell lines, as well as in-house positive and negative NSCLC controls, and an in-house tonsil tissue intensity control.

IHC-stained TMA slides were scored for tumor cell PD-L1 expression independently by 2 board-certified pathologists (L. F. and G. D.) who were blinded to clinical variables; discordances were resolved by a third pathologist (R. L. S.). Scoring was dichotomized into positive (≥1% tumor proportion score) and negative (<1% tumor proportion score) per current interpretation guidelines [14]. Only cytoplasmic membrane staining was considered positive, irrespective of completeness and intensity (as per manufacturer specifications [19-22]); nuclear or

cytoplasmic staining was considered nonspecific and disregarded. Missing or damaged tissue cores were excluded from analysis, as was any case with <100 total tumor cells for scoring (per clinical interpretation guidelines [19-22]).

For immune cell PD-L1 expression, cytoplasmic and membranous staining in lymphocytes and macrophages was considered for scoring. For a case to be considered positive for immune cell expression, 1 of 2 conditions needed to be met: presence of PD-L1 staining in at least 1 immune cell for every 100 tumor cells present or presence of at least 1 lymphoid aggregate showing PD-L1 expression, irrespective of PD-L1 expressing immune cells to total tumor cell relation.

For each case, age at diagnosis, sex, history of asbestos exposure, administration of neoadjuvant chemotherapy, tumor pathologic stage (for resected cases), and overall survival were collected from the electronic medical record. Relationship between tumor cell PD-L1 expression and immune cell PD-L1 expression, sex, treatment history, pathologic stage (American Joint Committee on Cancer, eighth edition [23]), primary site (pleural or peritoneal), and histotype (epithelioid, biphasic, or sarcomatoid) was evaluated by Fisher exact test. Relationship between PD-L1 expression and age was evaluated by Student *t* test, and that between PD-L1 expression and overall survival was evaluated by the log-rank test. All parameters were compared to outcomes of the 22C3 anti–PD-L1 assay and the 28-8 anti–PD-L1 assay independently and then compared to "dual-positive" cases that showed PD-L1 expression by both assays. Tumor PD-L1 expression and immune cell PD-L1 expression between the 22C3 and 28-8 clones were compared using McNemar test.

3. Results

This study included 125 cases of MM, including 112 MPMs (56 epithelioid, 48 biphasic, and 10 sarcomatoid) and 13 MPeMs (all epithelioid). The MPeM cohort was significantly younger than the MPM cohort (median age at diagnosis: 64 and 69 years, respectively; $P = .02$) and significantly more female ($P = .003$). Overall survival in MPeM was significantly longer than in MPM (median survival 65 versus 15 months, $P < .0001$). The MPM and MPeM cohorts did not differ significantly in asbestos exposure ($P = .12$) (Table 1).

With the Dako 22C3 pharmDx assay ("22C3 assay"), all 125 cases yielded evaluable staining, of which 27 were positive for tumor PD-L1 expression (20/112 MPMs [18%] and 7/13 MPeMs [54%]) and 29 were positive for immune cell PD-L1 expression (22/112 MPMs [20%] and 7/13 MPeMs [54%]). With the Dako 28-8 pharmDx assay ("28-8 assay"), 117 cases (52 epithelioid MPMs, 45 biphasic MPMs, 9 sarcomatoid MPMs, and 13 MPeMs) yielded evaluable staining (4 epithelioid MPMs, 3 biphasic MPMs, and 1 sarcomatoid MPM damaged or missing), of which 32 were positive for tumor PD-L1 expression (25/104 MPMs [24%] and 7/13 MPeMs [54%]) and 37 were positive for immune cell PD-L1 expression (30/104 MPMs [29%] and 7/13 MPeMs [54%]) (Figure).

Of the 117 cases with evaluable tumor and immune cell staining on both the 28-8 assay and the 22C3 assay, 107 (91%) showed concordant tumor cell staining (24 concordant positive, 83 concordant negative, 8 positive by 28-8 assay alone, and 2 positive by 22C3 assay alone), whereas 105 (90%) showed concordant immune cell staining (27

Table 1 Demographic and clinicopathologic characteristics of the study cohort

	All MMs (N = 125)	MPMs (n = 112)	MPeMs (n = 13)
No. of cases			
28-8 positive	32	25	7
22C3 positive	27	20	7
PD-L1 negative [a]	83	77	6
All cases	125	112	13
Age (median, y)			
28-8 positive	67	68	63
22C3 positive	66	68.5	63
PD-L1 negative [a]	70	70	67
All cases	69	69	64
Sex			
28-8 positive	8 F, 24 M	5 F, 20 M	3 F, 4 M
22C3 positive	5 F, 22 M	2 F, 18 M	3 F, 4 M
PD-L1 negative [a]	22 F, 61 M	17 F, 60 M	5 F, 1 M
All cases	31 F, 94 M	23 F, 89 M	8 F, 5 M
Asbestos exposure			
28-8 positive	19	15	4
22C3 positive	19	15	4
PD-L1 negative [a]	57	55	2
All cases	84	78	6
Stage (AJCC 8th edition)			
I	42	42	0
II	4	4	0
III	52	52	0
IV	0	0	0
N/A	27	14	13
Histotype			
Epithelioid	69	56	13
Biphasic	47	47	0
Sarcomatoid	9	9	0
Treatment			
Treated	3	3	0
Nontreated	122	109	13
Overall survival			
28-8 positive	N/A	15 mo	Not reached
22C3 positive	N/A	16 mo	Not reached
PD-L1 negative	N/A	15 mo	65 mo
All cases	N/A	15 mo	65 mo

Abbreviations: AJCC, American Joint Committee on Cancer; F, female; M, male; N/A, not applicable.
[a] By both assays.

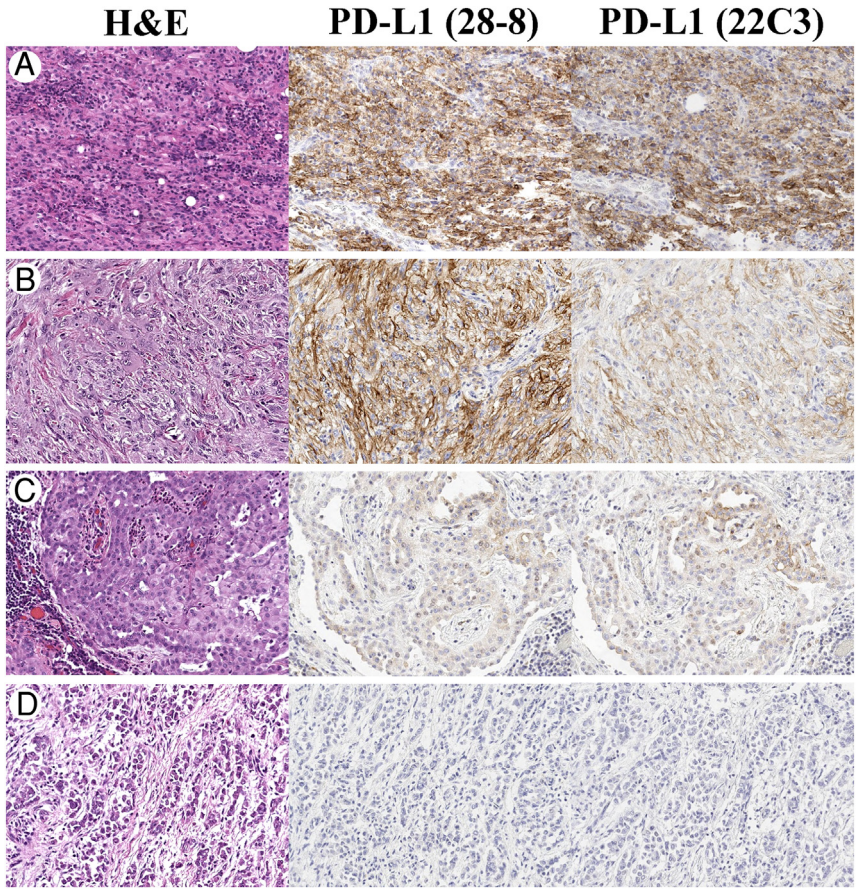

Figure PD-L1 expression in malignant mesothelioma. A, An epithelioid peritoneal mesothelioma, with 90%-100% of cells expressing PD-L1 by both the 28-8 and 22C3 assays. B, The sarcomatoid element of a biphasic pleural mesothelioma, with 80%-90% of cells expressing PD-L1 by the 28-8 assay but only 10%-20% cells expressing PD-L1 by the 22C3 assay. C, An epithelioid peritoneal mesothelioma, with 1%-10% of cells expressing PD-L1 by both the 28-8 and the 22C3 assays. D, This epithelioid pleural mesothelioma is negative for PD-L1 expression by both assays (hematoxylin and eosin; 22C3, Dako PD-L1 22C3 pharmDx assay; 28-8, Dako PD-L1 28-8 pharmDx assay; original magnification ×100 for all photomicrographs).

concordant positive, 78 concordant negative, 10 positive by 28-8 only, 2 positive by 22C3 only). Among MPM cases only, evaluable tumor and immune cell staining was obtained by both assays in 104/112 cases (8 cases not evaluable by 28-8 assay), of which 94 (90%) showed concordant tumor cell staining (17 concordant positive, 67 concordant negative, 8 positive by 28-8 alone, 2 positive by 22C3 alone), whereas 92 (88%) showed concordant immune cell staining (20 concordant positive, 72 concordant negative, 10 positive by 28-8 only, 2 positive by 22C3 only). All 13 MPeM cases yielded evaluable tumor and immune cell staining for both assays, of which 7/7 showed concordant positive PD-L1 expression and 6/6 showed concordant negative PD-L1 expression for both tumor and immune cells.

Detection of tumor cell PD-L1 expression by the 22C3 assay and 28-8 assay was not statistically significant, either among all cases or among MPM only ($P = .11$ for both comparisons). However, there was significantly higher detection of immune cell PD-L1 expression with the 28-8 assay than with the 22C3 assay, both for all cases and for MPM only ($P = .04$ for both comparisons).

By the 28-8 assay, 8 cases showed only tumor cell PD-L1 expression, 13 cases showed only immune cell PD-L1 expression, 24 showed both tumor and immune cell PD-L1 expression, and 72 were negative for PD-L1 on both tumor and immune cells. By the 22C3 assay, 7 cases showed only tumor cell PD-L1 expression, 9 cases showed only immune cell PD-L1 expression, 20 showed both tumor and immune cell PD-L1 expression, and 89 were negative for PD-L1 on both tumor and immune cells. Tumor cell PD-L1 expression was strongly correlated with immune cell PD-L1 expression for both assays ($P < .001$).

Tumor cell PD-L1 expression was significantly more common in MPeM than in MPM ($P = .007$ for 22C3 assay, $P = .04$ for 28-8 assay, $P = .009$ for dual-positive cases). In the MPM cohort, there was no significant relationship between tumor cell PD-L1 expression (as detected by the 28-8 assay, 22C3 assay, or dual positivity) and age at diagnosis, sex, asbestos exposure, treatment history, or pathologic stage (Tables 2 and 3). In the 22C3 assay, tumor cell PD-L1 expression was significantly higher in biphasic MPM than in epithelioid or sarcomatoid MPM ($P = .03$), but this

Table 2 Comparison of PD-L1 expression to pathologic stage and histotype in malignant pleural mesothelioma.

	Total cases	28-8 positive	22C3 positive	PD-L1 negative (by both assays)
Pathologic stage (AJCC 8th edition): MPM only				
I	42	9	7	31
II	4	2	0	2
III	52	11	10	34
IV	0	0	0	0
Histotype: MPM only				
Epithelioid	56	9	6	43
Biphasic	47	14	12	28
Sarcomatoid	9	2	2	6

Table 3 Comparison of PD-L1 to asbestos exposure history in malignant pleural and peritoneal mesothelioma

Asbestos exposure	MPMs (n = 112)			MPeMs (n = 13)		
	28-8 positive	22C3 positive	PD-L1 negative	28-8 positive	22C3 positive	PD-L1 negative
Exposed	15	15	55	4	4	2
Nonexposed	10	5	22	3	3	4

relationship was not seen with the 28-8 assay (P = .25). Overall survival was not significantly associated with tumor cell PD-L1 expression in MPM (P = .73 for 28-8, P = .65 for 22C3) or MPeM (P = .80).

4. Discussion

This is the largest reported study of tumor PD-L1 expression in MM to use the Dako 22C3 and 28-8 pharmDx assays, which are FDA-approved as companion and complementary diagnostic tests for administration of pembrolizumab and nivolumab, respectively.

We found tumor PD-L1 expression in 18% of MPMs using the 22C3 assay and in 24% of MPMs using the 28-8 assay. This degree of discordance between the 2 tests was not statistically significant. The levels of tumor PD-L1 expression that we detected fall at the lower end of the spectrum of reported values for PD-L1 expression by MM but are in keeping with the results of a recent clinical study, which used the 28-8 assay and detected PD-L1 expression in 27% of MPM cases [15]. Although studies have shown generally good concordance between the 28-8 assay, 22C3 assay, and other anti–PD-L1 clones commonly used in laboratory-developed IHC tests [24], no concordance studies involving the FDA-approved 28-8 or 22C3 assays have been done in MM. Furthermore, some studies in lung cancer have reported more accurate PD-L1 detection with the 28-8 assay as compared to the E1L3N clone, particularly in conjunction with the FDA-approved IHC platform and scoring methods [25]. The broad variability in reported prevalence of tumor PD-L1 expression in MM across studies using different anti–PD-L1 antibodies and the generally higher prevalence reported in MM when measured with anti–PD-L1 clones other than 28-8 or 22C3 suggest a need for further studies of concordance between all clones currently in clinical use to ensure appropriate pathologic reporting of tumor PD-L1 expression in cases where anti–PD-1 or anti–PD-L1 immunotherapy is considered. This is particularly important when evaluating expression cutoffs to predict tumor response to immune checkpoint inhibitor treatment in MM.

Compared with MPM, there are considerably less data available on PD-L1 expression patterns and response to anti–PD-1 or anti–PD-L1 therapy in MPeM, with reported prevalence of PD-L1 expression ranging from 22% to 64% of tumors [8,18]. No reports of MPeM have assessed PD-L1 with the Dako 22C3 or 28-8 pharmDx assays. Our study identified PD-L1 expression in 54% of MPeM cases—significantly higher than the prevalence of PD-L1 expression in our MPM cohort. Furthermore, the 28-8 and 22C3 assays showed 100% concordance for the 13 MPeM cases in this study, suggesting strong reproducibility between these 2 assays, although verification of this result in a larger MPeM cohort is needed.

Our data indicate a strong correlation between PD-L1 expression on tumor cells and PD-L1 expression on tumor-associated immune cells. Although the precise therapeutic implications of this finding remain to be elucidated, our data may suggest similar mechanisms governing PD-L1 expression in tumor and immune cells in MM. Interestingly, compared to the 22C3 assay, the 28-8 assay yielded a statistically higher rate of immune cell PD-L1 expression, whereas the trend toward higher detection of tumor cell PD-L1 expression with the 28-8 assay did not reach statistical significance. This finding may be related to increased nonspecific background staining with the 28-8 assay, which is likely amplified in immune cells when cytoplasmic staining is considered in addition to membranous staining.

Across tumor types, the precise correlation of PD-L1 expression with tumor response to immunotherapy remains poorly defined and appears to depend on factors specific to both the tumor and immunotherapeutic in question. Immunotherapy efficacy in melanoma and renal cell carcinoma appears to be independent of tumor PD-L1 expression [26,27]. In contrast, early data in nonsquamous NSCLC appeared to indicate improved immunotherapy outcomes among patients with at least 1% tumor PD-L1 expression compared to those with no tumor PD-L1 expression [28]. However, other studies in nonsquamous NSCLC show improved survival among patients treated with immunotherapy (versus those treated with standard chemotherapy), irrespective of tumor PD-L1 expression [29,30]. Multiple clinical trials of immunotherapy in MM have indicated that PD-L1 expression levels did not correlate with patient outcomes when administered therapeutic PD-L1 blockade [3,15]. However, these results stem from small clinical studies of 34 [15] and 29 [3] patients, underpowered to detect potentially clinically significant differences in survival by PD-L1 expression levels. Calabrò and colleagues [3] specifically noted power only to detect at least 40% difference in survival outcomes between patients with and without tumor PD-L1 expression. Accordingly, studies of larger MM cohorts may find that PD-L1 can indeed predict clinically significant differences in outcome on immunotherapy. At the University of Chicago, PD-L1 tumor proportion score is currently reported for all MM cases, and positive cases (tumor proportion score (TPS) $\geq$1%) are considered on a case-by-case basis for primary or salvage immunotherapy.

Our study has some limitations that warrant consideration. The number of MPeM cases tested was relatively low, and although both the rate of PD-L1 expression and the expected clinicopathologic distinctions between MPeM and MPM reached clinical significance, additional larger studies of PD-L1 in MPeM are warranted. The use of TMAs may be regarded as a shortcoming, particularly given the documented intratumoral heterogeneity of PD-L1 expression. However, the use of amply sized TMA cores sampled from 4 sites across each tumor provides a sufficient representation of potential tumor heterogeneity. Finally, our MPM cohort did include 3 patients who had received neoadjuvant chemotherapy, which may raise concern for posttreatment changes in PD-L1 expression. Published data have documented increased tumor PD-L1 expression in MM following treatment with vinorelbine but not with pemetrexed [17]. All 3 posttreatment cases in our cohort had been exposed only to standard first-line cisplatin plus pemetrexed, and it is unclear how this might have affected their posttreatment PD-L1 expression.

Measured by the FDA-approved Dako PD-L1 22C3 and 28-8 pharmDx assays, tumor PD-L1 expression is present in approximately one-fifth to one-quarter of MPMs and approximately half of MPeMs. In this large cohort, tumor PD-L1 expression was not significantly associated with patient age, sex, asbestos exposure, pathologic stage, or overall survival. These data may provide an important pathologic benchmark for future clinical studies of anti–PD-1 and anti–PD-L1 therapies in MM.

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