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Prevalence of *PDL1* Amplification and Preliminary Response to Immune Checkpoint Blockade in Solid Tumors

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IMPORTANCE Copy number alterations in programmed cell death ligand 1 (*PDL1* or *CD274*), programmed cell death 1 ligand 2 (*PDCD1LG2* or *PDL2*), and Janus kinase 2 (*JAK2*) genes (chromosome 9p24.1) characterize Hodgkin lymphoma, resulting in high response rates to programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) blockade. The prevalence and utility of *PDL1* amplification as a response biomarker to PD-1/PD-L1 blockade are unknown in other tumors.

OBJECTIVES To examine the prevalence of *PDL1* amplification and its utility as a response biomarker to PD-1/PD-L1 blockade in solid tumors.

DESIGN, SETTING, AND PARTICIPANTS This retrospective study (October 1, 2012, to October 1, 2017) used a deidentified tumor database from a commercial company and annotated clinical records from a subset of patients treated at a university tertiary referral center. The study analyzed 118 187 tumors from the deidentified database, including a clinically annotated subgroup of 2039 malignant tumors.

INTERVENTIONS Comprehensive genomic profiling was performed on all samples to determine *PDL1* amplification, microsatellite instability, and tumor mutational burden (TMB). A subset of patients was treated with PD-1/PD-L1 blockade.

MAIN OUTCOMES AND MEASURES The prevalence of *PDL1* amplification was determined among 118 187 patient samples that underwent next-generation sequencing. Solid tumors treated with checkpoint blockade were evaluated for response and progression-free survival (PFS).

RESULTS Of the 118 187 deidentified tumor samples, *PDL1* amplifications were identified in 843 (0.7%), including more than 100 types of solid tumors. Most *PDL1*-amplified tumors (84.8%) had a low to intermediate TMB. *PDL1* amplification did not always correlate with high-positive PD-L1 expression by immunohistochemical analysis. Six of 9 patients (66.7%) from 1 center with *PDL1*-amplified solid tumors had objective responses after checkpoint blockade administration. The median PFS among all treated patients was 15.2 months. Responders included 1 patient with glioblastoma (PFS, ≥5.2 months), 2 patients with head and neck squamous cell cancer (PFS, ≥9 and 15.2 months), 2 patients with metastatic basal cell cancer (PFS, 3.8 and ≥24.1 months), and 1 patient with urothelial cancer (PFS, ≥17.8 months).

CONCLUSIONS AND RELEVANCE The results of this study suggest that *PDL1* amplification occurs in a small subset of malignant tumors. Additional large-scale, prospective studies of *PDL1*-amplified cancers are warranted to confirm the responses to checkpoint blockade described herein, even in the absence of microsatellite instability, high PD-L1 expression, and a high TMB.

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Author Audio Interview

Supplemental content

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Checkpoint blockade with anti-programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antibodies has revolutionized the treatment of solid and hematologic malignant tumors. However, immune checkpoint inhibitors are only effective in a subset of patients. Biomarkers for determining response to PD-1/PD-L1 blockade include PD-L1 expression,[1,2] microsatellite instability (MSI),[3] and a high tumor mutational burden (TMB).[4-6]

Response rates of 65% to 87% have been reported in patients with refractory classic Hodgkin lymphoma treated with checkpoint inhibitors.[7,8] In nodular sclerosing Hodgkin lymphoma, amplification of the chromosomal region 9p24.1, which contains the genes programmed cell death ligand 1 (*PDL1* or *CD274*) (OMIM 605402), programmed cell death ligand 2 (*PDCD1LG2* or *PDL2*) (OMIM 605723), and Janus kinase 2 (*JAK2*) (OMIM 147796), is directly correlated with increased expression of these proteins on Reed-Sternberg cells.[9] Overall, 105 of 108 biopsy specimens (97.2%) from patients with newly diagnosed classic Hodgkin lymphoma[10] have had increased *PDL1* and *PDCD1LG2* copy numbers. This increase is attributable to 9p24.1 amplifications, copy number alterations (CNAs), or polysomy of chromosome 9p. In addition, expression and activation of JAK2, which is also encoded by a gene residing on the 9p24.1 locus, are increased in Hodgkin lymphoma Reed-Sternberg cells, further augmenting transcription of the *PDL1* gene.[9]

The CNAs of the 9p24.1 locus have also been detected in 63% of primary mediastinal large B-cell lymphomas and 50% of primary central nervous system large B-cell lymphomas and are associated with high PD-L1 and PD-L2 expression on immunohistochemical analysis.[9,11,12] Recently, a study[13] found that all 5 patients with relapsed or refractory primary central nervous system large B-cell lymphoma or testicular large B-cell lymphoma treated with PD-1 blockade experienced an objective response, and 60% remained progression free at 13 to 17 months. Taken together, in certain lymphomas, chromosome 9p24.1 alterations, which include *PDL1*, are relatively common and are associated with high susceptibility to PD-1 blockade.

In contrast, data are limited regarding *PDL1* amplifications in solid tumors. To date, such amplifications have only been detected in small studies of head and neck squamous cell carcinoma,[14] cervical squamous cell carcinoma,[15] triple-negative breast cancer,[16-18] and non-small cell lung cancer.[19] Consistent with the aforementioned data on lymphomas, recent case reports found responses to PD-1 blockade in patients with *PDL1*-amplified, microsatellite-stable colon cancer[20] and metastatic basal cell carcinoma,[21] suggesting the need for further interrogation of the potential utility of *PDL1* amplifications as a biomarker for immune checkpoint blockade response. We describe, to our knowledge, the largest cohort of tumor samples (N = 118 187) evaluated for *PDL1* CNAs and report the frequency of *PDL1* amplification across a variety of solid tumors.

Key Points

Question What is the prevalence and utility of programmed cell death ligand 1 (*PDL1*) gene amplification as a response biomarker to programmed cell death/programmed cell death ligand 1 blockade in solid tumors?

Findings In this study of 118 187 tumor samples from a deidentified database, including a subset of 2039 samples from a clinically annotated database, the prevalence of *PDL1* amplification was 0.7%. The objective response rate for patients with solid tumors that harbored *PDL1* amplification was 66.7%, with a median progression-free survival of 15.2 months.

Meaning The results of this study suggest that *PDL1* amplification occurs in a small subset of malignant tumors; however, testing for this alteration may be warranted because of the frequent and durable responses to programmed cell death/programmed cell death ligand 1 blockade.

Methods

Patients and Samples

We analyzed 118 187 deidentified tumor samples from the Foundation Medicine (https://www.foundationmedicine.com/) database, including a subset of 2039 clinically annotated patient tumors from the University of California, San Diego (UCSD) Moores Center for Personalized Cancer Therapy from October 1, 2012, to October 1, 2017 (eFigure 1 in Supplement 1). This study was performed in accordance with UCSD Institutional Review Board guidelines for data analysis[22,23] and for any investigational treatments for which patients gave written informed consent.

Profiling and Assessment of *PDL1* Amplification, MSI, and TMB

Comprehensive Genomic Profiling and *PDL1* (*CD274*) Assessment

Comprehensive genomic profiling was performed using the FoundationOne and FoundationOneHeme assay (Foundation Medicine), as previously described in detail.[24,25] In brief, the pathologic diagnosis of each case was confirmed by review of hematoxylin-eosin-stained slides, and all samples that advanced to DNA extraction contained a minimum of 20% tumor cells. The fail rate was approximately 1%. Hybridization capture of exonic regions from 315, 327, or 405 cancer-related genes was applied to 50 ng or more of DNA extracted from formalin-fixed, paraffin-embedded cancer specimens. These libraries were sequenced to high, uniform median coverage (>500 times) and assessed for base substitutions, short insertions and deletions, CNAs, and gene fusions and rearrangements. Sequencing was performed from October 1, 2012, to October 1, 2017.[24] *PDL1* amplification was performed for 6 or more CNAs.

TMB Evaluation

For TMB (mutations per megabase), the number of somatic mutations detected on comprehensive genomic profiling (interrogating 1.2 Mb of the genome) were quantified, and that value was extrapolated to the whole exome using a validated

algorithm.[26] Alterations likely or known to be oncogenic drivers and germline polymorphisms were excluded. A TMB of 5 mutations per megabase or more was designated as low; 6 to 19, intermediate; and 20 or more, high.

MSI Assessment

The MSI status was calculated using 114 loci determined to be useful in detecting evidence of polymerase slippage and therefore MSI.[27] The information from these loci were then used in principal component analysis to produce an MSI score. Ranges of MSI scores were assigned as high MSI (MSI-H), microsatellite stable, or intermediate or ambiguous MSI.

Database Analysis for *PDL1* Amplification and TMB

To understand the large-scale prevalence of *PDL1* amplification and its relevant associations, we analyzed 118 187 patient samples with cancer from the Foundation Medicine deidentified database. Only patients with chromosome 9p24.1 alterations in *PDL1*, *PDL2*, and/or *JAK2* alterations were further reviewed (eFigure 1 in Supplement 1 and Supplement 2). We focused on patients with solid tumors.

Patient and Sample Selection

To retrieve data that would provide clinical correlations of *PDL1* CNAs with checkpoint inhibitor response, we evaluated 2039 consecutive cancer samples from patients at the UCSD Moores Center for Personalized Cancer Therapy (October 1, 2012, to October 1, 2017). All patients had undergone comprehensive genomic profiling (Foundation Medicine; https://www.foundationmedicine.com/).

Pathology for TILs and Immunohistochemistry for PD-L1

Tumor samples, when available, were reviewed by a pathologist (H.-Y.W.) for enumeration of tumor-infiltrating lymphocytes (TILs) as described by Salgado et al.[28,29] The mean percentage of TILs was quantified from evaluating 3 high-power fields (original magnification, ×400). Macrophages were excluded from the TIL count. Immunohistochemical analysis for PD-L1 expression was performed using commercially available assays (eTable 1 and eTable 2 in Supplement 1).

Outcomes

Responses were assessed based on Response Evaluation Criteria In Solid Tumors (RECIST) criteria.[30] Progression-free survival (PFS) and overall survival (OS) were calculated using the Kaplan-Meier method (*P* values by log-rank test) (starting from the first day of immunotherapy). The PFS and OS are censored at the date that the patient was last seen provided that the patient's cancer had not progressed (for PFS) and the patient had not died (for OS).

Statistical Analysis

The Fisher exact test was used to assess categorical variables. Bonferroni correction was applied as a multitesting correction. Statistical analyses were performed using GraphPad Prism, version 7.0. A 2-sided $P \leq .05$ and $Q \leq .05$ were considered to be statistically significant.

Results

Patient Characteristics

Overall, 843 of 118 187 patient samples (0.7%) that had undergone comprehensive genomic profiling had 6 or more CNAs in *PDL1* (**Table**). A total of 405 gene panels were performed on 15 982 tumor samples, 327 gene panels on 450 tumor samples, and 315 gene panels on 101 755 tumor samples. *PDL1* amplification was identified in 88 samples from the 405 gene panels, 5 samples from the 327 gene panels, and 750 samples from the 315 gene panels (total of 843 samples). *PDL1* CNAs were identified in more than 100 solid tumor histologic types (eTable 3 in Supplement 1). The tumor type with the highest percentage of *PDL1* amplification was mixed hepatocellular cholangiocarcinoma (10.5% of samples). Solid tumors with a significantly increased percentage of *PDL1* amplification included breast carcinoma (111 [1.9%]; $P < .001$), head and neck squamous cell carcinoma (39 [3.1%]; $P < .001$), lung squamous cell carcinoma (50 [1.7%]; $P < .001$), undifferentiated soft-tissue sarcoma (13 [3.9%]; $P < .001$), thyroid anaplastic carcinoma (9 [5.1%]; $P < .001$), unknown primary squamous cell carcinoma (16 [2.0%]; $P = .01$), nasopharyngeal carcinoma (5 [5.1%]; $P = .03$), and kidney sarcomatoid carcinoma (4 [6.1%]; $P = .04$) (Table). Neoplasms notable for having a lower frequency of *PDL1* CNAs included colorectal, pancreatic, and prostate cancer and melanoma (Table).

TMB and MSI

The mean TMB for *PDL1*-amplified tumors was 13.3 mutations per megabase, and the median was 6.3 mutations per megabase. For unamplified tumors, the mean was 7.4 mutations per megabase and the median was 3.6 mutations per megabase (eTable 3 in Supplement 1). Overall, 128 PDL1-amplified tumors (15.2%) were classified as having high TMB compared with 7510 unamplified tumors (6.4%). Most PDL1-amplified tumors had a low to intermediate TMB (84.8%). For some tumors (ie, kidney sarcomatoid carcinoma [n = 4], pancreas ductal carcinoma [n = 1], and prostate cancer [n = 5]), 100% of PDL1-amplified tumors had a low TMB (≤5 mutations per megabase). The MSI-H and *PDL1* amplification were not mutually exclusive. Five of 741 patients (0.7%) with *PDL1* amplification (2 gastrointestinal tumors and 3 carcinomas of unknown primary) who were tested for microsatellite status were MSI-H; 1435 of 103 373 patients (1.4%) who did not have *PDL1* amplification and were tested for microsatellite status were MSI-H. In the UCSD cohort (n = 13), the median TMB for *PDL1*-amplified tumors was 9 mutations per megabase vs 4 mutations per megabase for non-*PDL1*-amplified tumors ($P = .007$). Nine of the 13 patients (69.2%) had an intermediate to high TMB, whereas 4 patients (30.8%) had a low TMB (1-5 mutations per megabase). Finally, 11 of 13 tumors (84.6%) tested for MSI were stable.

Clinical Characteristics of the Cohort With *PDL1* CNAs

Thirteen patients were identified with *PDL1* CNAs from the 2039 patients who had undergone comprehensive genomic profiling (eTable 4 and eFigure 1 in Supplement 1). All 13

Table. Frequency of *PDL1* Amplifications[a]

Diagnosis	Total Patients, No. (%) (N = 118 187)	Patients With *PDL1* Amplification	
		Q Value[b]	OR (95% CI)
Tumors with the highest prevalence of *PDL1* amplification			
Breast carcinoma (NOS)	111/5838 (1.9)	<0.0001	3.0 (2.4-3.6)
Head and neck squamous cell carcinoma	39/1275 (3.1)	<0.0001	4.6 (3.3-6.3)
Lung squamous cell carcinoma	50/2952 (1.7)	<0.0001	2.5 (1.9-3.3)
Undifferentiated soft-tissue sarcoma	13/330 (3.9)	<0.0001	5.8 (3.3-10.1)
Thyroid anaplastic carcinoma	9/177 (5.1)	0.0004	7.5 (3.8-14.8)
Soft-tissue sarcoma (NOS)	18/903 (2.0)	0.0069	2.9 (1.8-4.6)
Unknown primary squamous cell carcinoma	16/788 (2.0)	0.0119	2.9 (1.8-4.8)
Cervix squamous cell carcinoma	10/374 (2.7)	0.0188	3.9 (2.1-7.3)
Nasopharyngeal carcinoma	5/99 (5.1)	0.0293	7.4 (3.0-18.3)
Renal sarcomatoid carcinoma	4/66 (6.1)	0.0449	9.0 (3.3-24.9)
Bladder squamous cell carcinoma	3/40 (7.5)	0.0905	11.3 (3.5-36.8)
Liver mixed hepatocellular cholangiocarcinoma	2/19 (10.5)	0.1807	16.41 (3.8-71.2)
Lung sarcomatoid carcinoma	5/187 (2.7)	0.2226	3.8 (1.6-9.4)
Tumors with the lowest prevalence of *PDL1* amplification			
Colorectal adenocarcinoma	18/9851 (0.18)	<0.0001	0.2 (0.1-0.4)
Pancreatic cancer	1/3294 (0.03)	<0.0001	0.04 (0.01-0.3)
Multiple myeloma	2/2707 (0.07)	<0.0001	0.1 (0.03-0.4)
Acute myeloid leukemia	0/1273	0.0133	0 (0-0.9)
Prostate cancer	5/2461 (0.2)	0.0337	0.3 (0.1-0.7)
Myelodysplastic syndromes	0/861	0.0984	0 (0-1.3)
Cutaneous melanoma	1/1090 (0.09)	0.1426	0.1 (0.02-0.9)
PDL1 amplification in common tumor histologic types			
Lung adenocarcinoma	90/14 910 (0.6)	1.0000	0.8 (0.4-1.7)
Glioblastoma	11/3199 (0.3)	0.2095	0.5 (0.3-0.9)
Gastroesophageal junction adenocarcinoma	5/1956 (0.3)	0.2095	0.4 (0.2-0.9)
Lung small cell carcinoma	14/1071 (1.3)	0.4373	1.9 (1.1-3.2)
Ovarian epithelial carcinoma	14/1052 (1.3)	0.4100	1.9 (1.1-3.2)
Renal cell carcinoma	4/766 (0.5)	1.0000	0.7 (0.3-2.0)
Stomach adenocarcinoma	8/1325 (0.6)	1.0000	0.8 (0.4-1.7)
Endometrial adenocarcinoma	6/1223 (0.2)	1.0000	0.2 (0.03-1.7)
Hepatocellular carcinoma	3/691 (0.4)	1.0000	0.6 (0.2-1.9)
Cholangiocarcinoma	7/1867 (0.4)	1.0000	0.5 (0.3-1.1)

Abbreviations: NOS, not otherwise specified; OR, odds ratio; *PDL1*, programmed cell death ligand 1.

[a] Data are provided for solid tumors with the highest percentile of *PDL1* amplification. Nonsolid tumors, such as Hodgkin lymphoma, also had *PDL1* amplification in 97% of patients.[10] Tumors with a significant percentage of *PDL1* amplification and all tumor types with a low-percentile *PDL1* amplification are reported.

[b] Calculated using Fisher exact test. $Q \leq 0.05$ is considered to be significant when using Bonferroni correction.

patients had coamplification of *PDCD1LG2* (*PDL2*), and all but 1 (92.3%) had coamplification of *JAK2*. All 13 (100%) had locally advanced (3 [23.1%]) or metastatic (10 [76.9%]) disease (2 with hematologic malignant tumors and 11 with solid tumors). The median time alive with locally advanced or metastatic disease was 26.5 months (range, 7.9-65.5 months). Nine different malignant tumors were identified that harbored *PDL1* CNAs, including head and neck tumors (3 patients) and a glioblastoma (eTable 4 in Supplement 1). Nine patients (69.2%) (all with solid tumors) received therapy with a PD-1/PD-L1 inhibitor.

Genomics, PD-L1 Expression, and TILs in the Cohort With *PDL1* CNAs

A total of 70 genes with 143 alterations were identified among the 13 patients with *PDL1* CNAs (**Figure 1** and eTable 5 in Supplement 1). Among the 13 patients, only 5 samples from 5 patients were available for pathologic evaluation for TILs (4 of them were stromal TILs and 1 [B-cell lymphoma] was intratumoral TILs). The TILs ranged from a mean of 10% to 60% per high-power field (original magnification, ×400). Four of 6 tumors (66.7%) tested expressed PD-L1 (immunohistochemical analysis) (eTable 1 in Supplement 1). Of note,

one patient with glioblastoma and another patient with metastatic basal carcinoma had undetectable PD-L1 expression by immunohistochemical analysis, but both responded to checkpoint blockade.

Additional Alterations in *PDL1* and *PDCD1L2G (PDL2)*

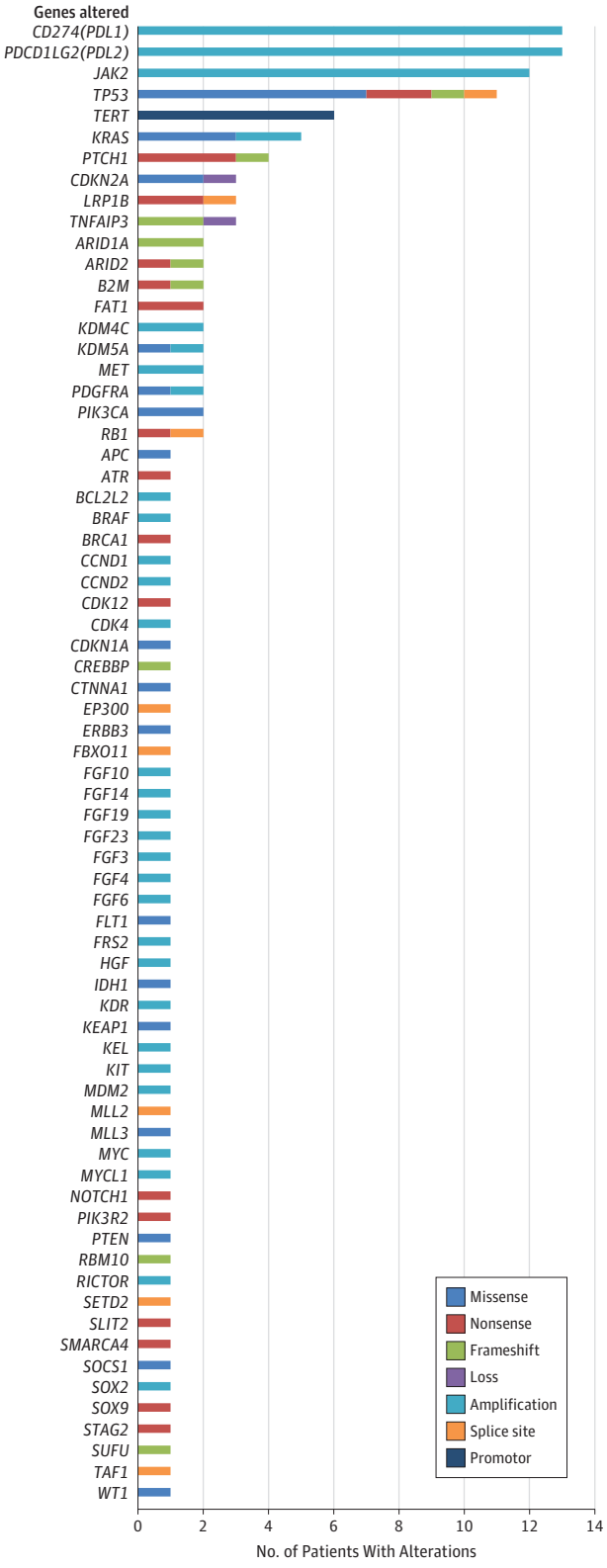
Eleven (0.001%) of the 118 187 samples harbored *PDL1* exon 7 truncations. Two individuals in the UCSD cohort had other alterations that involved *PDL1* and *PDCD1LG2* (eTable 6 in Supplement 1). One patient had metastatic head and neck squamous cell carcinoma that harbored a *PDL1* exon 7 truncation. This alteration disrupts the 3′ untranslated region of PD-L1.[31] The patient achieved a partial response to treatment with durvalumab, a PD-L1 inhibitor. The other patient had metastatic cholangiocarcinoma with a *PKD1P1-PDCD1LG2* rearrangement (but was not treated with checkpoint blockade). This alteration was not identified in any of the other 118 186 samples.

Response to Checkpoint Blockade

Nine of the 13 patients (69.2%) with *PDL1* amplification were treated with checkpoint blockade (all solid tumors) (eTable 5 in Supplement 1). The median number of prior therapies in these 9 patients was 4 (range, 1-7). Five patients were treated with PD-1/PD-L1 inhibitor monotherapy, 3 with a PD-1/PD-L1 inhibitor plus an investigational agent, and 1 with anti–PD-1 and anti-CTLA4 combination therapy. The response rate was 66.7%. The median PFS among the 9 patients was 15.2 months (range, 1.6 to ≥24.1 months); median OS was not reached from the start of checkpoint blockade (range, 1.6 to ≥24.1 months) (**Figure 2**). Responders included 1 patient with glioblastoma (PFS, ≥5.2 months), 2 patients with head and neck squamous cell cancer (PFS, ≥9 and 15.2 months), 2 patients with metastatic basal cell cancer (PFS, 3.8 and ≥24.1 months), and 1 patient with urothelial cancer (PFS, ≥17.8 months). In addition, a patient with primary mediastinal lymphoma that was refractory to chemotherapy, including high-dose chemotherapy followed by autologous stem cell rescue, had an ongoing complete response to allogeneic stem cell transplantation at 24.1 months.

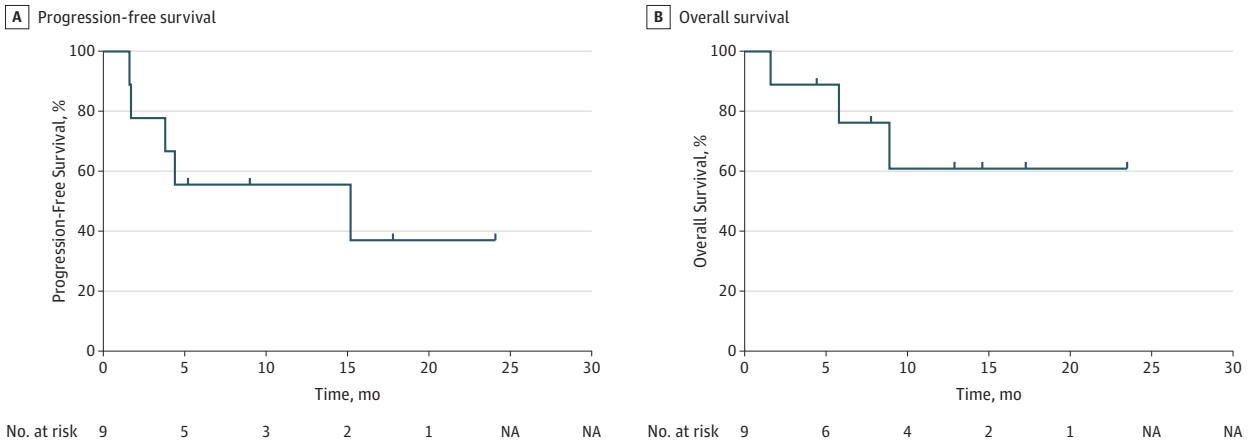
One patient had progressive glioblastoma after tumor resection followed by adjuvant radiation therapy with concurrent temozolamide.[32] Comprehensive genomic profiling identified 12 characterized alterations, including *PDL1*, *PDCD1LG2*, and *JAK2* amplifications. MET protooncogene (*MET*) (OMIM 164860) and mouse double minute homolog 2 (*MDM2*) (OMIM 164785) amplifications were also identified. The case was presented at the molecular tumor board, and treatment with checkpoint inhibition was debated because of the presence of *MDM2* amplification, which has been associated with hyperprogression.[33] However, because of the grave prognosis of glioblastoma, the patient was prescribed combination therapy with nivolumab and the MET inhibitor cabozantinib (after signing consent for an institutional review board–approved protocol [Study of Molecular Profile-Related Evidence to Determine Individualized Therapy for Advanced or Poor Prognosis Cancers (I-PREDICT)[23]]). Brain magnetic resonance imaging (eFigure 2 in Supplement 1) performed 4 weeks after therapy initiation demonstrated a partial response with decreased enhancement within the primary mass and decreased mass ef-

Figure 1. Genomic Alterations



Total genomic alterations in 13 patients with cancer with alterations that involve programmed cell death ligand 1 gene (*PDL1*).

Figure 2. Patient Survival



Progression-free survival among 9 patients with *PDL1* amplification treated with checkpoint blockade (median, 15.2 months; range, 1.2 to ≥24.1 months). Median overall survival among patients with *PDL1* amplification was not reached from start of checkpoint blockade (range, 1.6 to ≥24.1 months). NA indicates not applicable.

fect. Cabozantinib (weeks 14-22) and nivolumab (weeks 11-23) were given secondary to the development of a transaminitis. Subsequent brain magnetic resonance imaging demonstrated an improving and ongoing response at 5.2 months.

Discussion

In our study, the prevalence of *PDL1* CNAs in a large cohort of diverse tumors was 0.7%. These alterations were identified in a small subset of multiple solid tumor types, including rare neoplasms, such as bladder squamous cell carcinoma, undifferentiated soft-tissue sarcomas, and sarcomatoid renal cell carcinoma. Furthermore, we found that *PDL1* CNAs can be associated with responses to checkpoint blockade across a diverse spectrum of tumors (eTable 4 in Supplement 1). Six of 9 patients (66.7%) with *PDL1* amplification responded to immunotherapy vs 45 of 151 patients (29.8%) in the overall UCSD-treated cohort (P = .03).[5]

Although rare outside certain lymphomas, identification of amplifications in *PDL1* is important because this subset of tumors appears to have a high likelihood of responding to checkpoint blockade. This situation is analogous to that in patients with lung cancer that harbors anaplastic lymphoma kinase (*ALK*) (OMIM 105590) and V-ROS avian UR2 sarcoma virus oncogene homolog 1 *ROS1* (OMIM 165020) alterations, which both confer sensitivity to ALK inhibitors.[34,35] Regarding histologic agnostic responsiveness, MSI-H confers response to checkpoint inhibitors across cancers and neurotrophic tyrosine receptor kinase fusions respond to neurotrophic tyrosine receptor kinase targeting in a tissue-agnostic fashion.[3,36]

Infection has been implicated in certain types of neoplasms identified to have a higher prevalence of *PDL1* amplifications. These neoplasms include bladder squamous cell carcinoma associated with *Schistosoma hematobium*, nasopharyngeal carcinoma, Epstein-Barr virus, head and neck squamous cell carcinoma, human papillomavirus, and mixed cellularity variant of Hodgkin lymphoma, which is associated with Epstein-Barr infection.[37] Viral-associated malignant neoplasms may be susceptible to tumor immune responses, perhaps through upregulation of APOBEC (apolipoprotein B messenger RNA editing enzyme, catalytic polypeptide-like), a family of cytidine deaminases that help protect from viral infections. APOBEC upregulation in turn correlates with high levels of PD-L1.[2,38] It is plausible that these tumors are using *PDL1* amplification as a mechanism of immune escape from an endogenous immune response.

Sarcomatoid renal cell carcinoma (6.5% of which had *PDL1* amplification—one of the highest rates for solid tumors) (Table) is a rare subtype of renal cell carcinoma. Although only accounting for approximately 5% of renal cell carcinomas, the aggressive nature of this variant results in many patients having metastatic disease at diagnosis.[39] In addition, these tumors are responsive to checkpoint blockade at least in a small series, with 2 of 6 patients achieving objective response to atezolizumab (PD-L1 inhibitor).[40] PD-L1 expression in sarcomatoid renal cell carcinoma appears to be higher compared with standard renal cell carcinoma without sarcomatoid differentiation.[41]

Glioblastoma is a lethal tumor with limited effective treatment options. Outside MSI-H glioblastoma, checkpoint blockade has not been effective.[42] In this report, we demonstrate, for the first time to our knowledge, a response to nivolumab in a *PDL1*-amplified glioblastoma.

Expression of PD-L1 was identified by immunohistochemical analysis in 4 of 6 patients who were tested. Of interest, 2 of the patients who lacked PD-L1 protein expression (1 with glioblastoma and 1 with metastatic basal cell carcinoma) (eTable 5 in Supplement 1) responded to PD-1 blockade. A recent report[14] in head and neck squamous cell carcinoma also found that *PDL1* CNAs were concordant with PD-L1 expression by immunohistochemical analysis only 73% of the time. Presence of gene am-

plification with no or low-level PD-L1 protein expression should make immune checkpoint blockade inhibitors less effective. Post-transcriptional splicing and methylation could be mechanisms that limit expression. However, insufficient sampling of tumor and other technical problems with immunohistochemical analysis, in part related to tumor heterogeneity and the presence of stroma or attributable to differences in affinity of distinct anti-PD-L1 antibodies, may limit the accuracy of the protein expression methods and may explain responses in patients who lacked PD-L1 expression on immunohistochemical analysis. Other mechanisms, such as expression of PD-L2 rather than PD-L1, may also be operative when patients respond to anti-PD1 agents in the absence of PD-L1 expression (because PD-L1 and PD-L2 interact with PD-1).[43] All these issues merit in-depth exploration in larger cohorts of treated patients to better understand the association among PD-L1 expression, *PDL1* amplification, and response to checkpoint blockade.

Of interest, in addition to the 13 patients in the UCSD cohort who had *PDL1* amplification, 2 patients harbored alterations that involved *PDL1* and *PDCD1LG2* (eTable 4 in Supplement 1) that were not CNAs. The first alteration, a *PDL1* exon 7 truncation, is predicted to disrupt the 3′ untranslated region of *PDL1*. Similar alterations have been observed in many tumor types and correlate with increased PD-L1 expression, presumably via loss of inhibitory microRNA binding sites.[31,44,45] This patient achieved a partial response that lasted 9 months with a durvalumab (anti-PD-L1)–based regimen. The other alteration, with a *PKD1P1-PDCD1LG2* rearrangement, has not been previously reported or characterized. However, translocations that involve *PDCD1LG2* and numerous partners have been highly characterized in primary mediastinal large B-cell lymphoma and result in increased PD-L2 expression.[2]

Limitations

The small number of patients precludes definitive conclusions regarding response rates, PFS, or OS except to suggest that further additional prospective clinical trials of checkpoint blockade in *PDL1*-amplified cancers are warranted. In addition, the current assay was validated for 6 or more copy numbers of *PDL1*, and future studies should determine the frequency of CNAs that are less than 6. This study also did not assess features of the tumor microenvironment, such as the presence of transforming growth factor β, which can have profound influences on the response to checkpoint blockade.[46,47] Thus, application of checkpoint blockade and comparison to standard-of-care chemotherapy require properly designed randomized clinical trials with both PFS and OS end points.

Conclusions

Our data suggest that *PDL1* CNAs are found in a small subgroup of diverse solid tumors and may correlate with responses to checkpoint blockade. Additional prospective studies are needed to validate this finding and to determine whether routine testing for this alteration is warranted.

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