

# Comparing VIP and PD-L1 expression as cancer biomarkers

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## Abstract

Immune checkpoint molecules are critical targets of cancer therapies due to their ability to modulate immune responses to cancer. Vasoactive intestinal peptide (VIP) has been proposed as an immune checkpoint molecule, but its predictive and prognostic values have not been established. We evaluated expression levels of VIP and programmed death-ligand 1 (PD-L1) across different cancer types and identified specific cancer histologies in which the expression of these markers is elevated. We conducted systematic analyses of the prognostic and predictive values of VIP and PD-L1 in various cancers using publicly available patient databases and analysis tools including the Gene Expression Profiling Interactive Analysis, PrognoScan, Protein Atlas, cBioportal, and Timer2.0. We also assessed the relationship of PD-L1 and VIP expression levels with survival and the frequencies of tumor-infiltrating immune cells in various cancers. We observed a negative correlation between PD-L1 and VIP expression across cancer types, suggesting the functional redundancy of VIP and PD-L1 immunosuppressive pathways as mechanisms of immune escape. High expression levels of VIP and the association of VIP expression with immune cell infiltrates in the pancreatic adenocarcinoma tumor microenvironment suggest that VIP may be a predictive biomarker for treating pancreatic adenocarcinoma patients with drugs that inhibit the VIP signaling pathway.

## KEYWORDS

cancer genome atlas, protein death-ligand 1, vasoactive intestinal peptide

## 1 | INTRODUCTION

A wide spectrum of genetic and epigenetic alterations in cancers leads to a diverse set of antigens that the immune system recognizes to differentiate tumor cells from normal cells. The amplitude and quality of antigen-specific T cell responses are regulated by a signaling network of costimulatory and inhibitory immune checkpoints. Under normal physiological conditions, immune checkpoints preserve self-tolerance to prevent autoimmunity. However, aberrantly high

expression of immune checkpoint proteins by cancer cells mediates immune suppression and evasion of anticancer immune surveillance.

T cells regulated by immune checkpoint pathways can be targeted by drugs that block immune checkpoint signaling. Currently, available immune checkpoint inhibitors (ICI) are monoclonal antibodies that block immune checkpoint ligand and receptor interactions. Clinical trials with ICIs revealed the power of reinvigorating cancer-exhausted immune systems and revolutionized cancer therapy.<sup>1</sup> CTLA-4 blockade was the first ICI to successfully enhance T cell expansion and enhance

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antitumor immunity. More recently, the expression of PD-L1 by cancer cells and the cognate PD1 receptor on T cells have been identified as clinically relevant immune checkpoint molecules in cancers. Antibodies targeting this pathway have been approved for numerous malignancies including melanoma, non-small cell lung cancer, renal cell carcinoma, Hodgkin lymphoma, bladder cancer, head, and neck squamous cell carcinoma, and Merkel cell carcinoma, and are currently being evaluated for the treatment of other cancers.<sup>2</sup>

Recent preclinical studies have identified vasoactive intestinal polypeptide (VIP), a 28-amino acid neuropeptide, as a potential ICI target. VIP has previously been shown to be a potent suppressor of T cell activation and proliferation. As such, VIP antagonists were found to enhance adaptive immunity to cytomegalovirus infection in murine models.<sup>3</sup> Past studies also show that VIP and its receptors were over-expressed in breast, prostate, and lung cancer, leading to the growth and metastasis of tumors.<sup>4–8</sup> T cells have been found to upregulate VIP receptors during T cell activation. In response to this VIP receptor signaling, T cell activation and proliferation become inhibited while Treg and Th2 cells were generated.<sup>9–11</sup> Concerning cancer immunotherapy VIP antagonists enhanced anti-leukemia T cell response and down-regulated myeloid-derived suppressor cells in acute myeloid leukemia murine models.<sup>12</sup> Furthermore, VIP expression by pancreatic ductal adenocarcinoma cells was identified as a possible immunomodulator that protects cancer from immune surveillance by decreasing T cell proliferation, trafficking, activation, and function. Thus, VIP production by cancer cells has been proposed as an immune escape pathway used by some cancers. A multitude of clinical studies has proved the therapeutic value of anti-PD-L1/PD-1 antibody treatments in cancers with overexpressed PD-L1.<sup>13</sup> However, treatment targeting PD-L1 and PD-1 are traditionally believed to have low efficacy in cancers with low PD-L1 expression such as pancreatic adenocarcinoma.<sup>14</sup> The objective of this paper is to evaluate the relative expression levels of PD-L1 and VIP, compare the prognostic values of PD-L1 and VIP expression in cancers, and identify cancers that might be responsive to agents that target VIP signaling pathways. The over-arching hypothesis motivating this study is that drugs targeting VIP signaling might be effective in cancers that express high levels of VIP but low levels of PD-L1.

## 2 | METHODS

### 2.1 | Gene expression profiling interactive analysis

Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn>)<sup>15</sup> uses data from The Cancer Genome Atlas (TCGA) and Gene Tissue Expression (GTEx) to analyze the RNA expression of 9736 tumors and 8587 normal samples. We used GEPIA to compare the expression of VIP and PD-L1 in normal tissue and across various cancers. A  $p$ -value  $<0.05$ , determined through one-way ANOVA, was used as an indicator for significance in expression between normal and

tumor tissue. Additionally, a  $p$ -value  $<0.05$ , determined through a Student  $t$ -test, was used as an indicator of significance for cancers with high VIP or PD-L1. Cancers with high VIP or PD-L1 expression in tumor tissue were further analyzed using GEPIA-generated survival curves to investigate the prognostic values of cancer patients with high and low expression of PD-L1 and VIP. A median expression level was used as a cutoff to stratify patients.

### 2.2 | PrognScan database analysis

The PrognScan database (<http://dna00.bio.kyutech.ac.jp/PrognScan/index.html>)<sup>16</sup> uses patient prognostic values including overall survival (OS) and disease-free survival (DFS) from a large group of public cancer datasets. The PrognScan database was used to evaluate the prognostic values of the immune checkpoint molecules VIP and PD-L1 in various cancers. A  $p$ -value  $<0.05$  was used as an indicator for significance and further analysis.

### 2.3 | Protein atlas database analysis

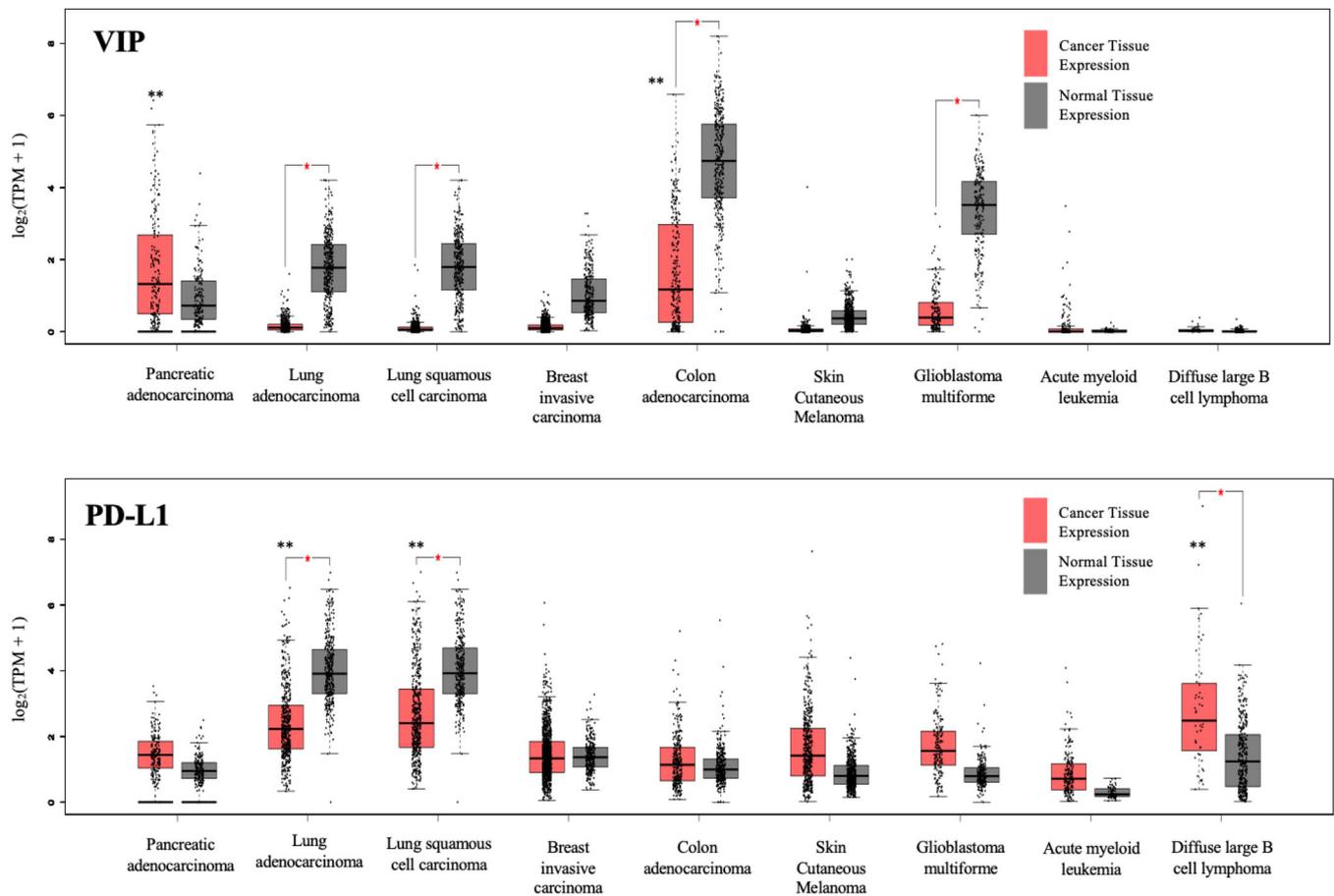
The Protein Atlas (<https://www.proteinatlas.org>)<sup>17</sup> uses data from 8000 cancer patients from TCGA to characterize the miRNA expression for proteins in 17 main cancers. The Protein Atlas database was used to evaluate the prognostic values of the immune checkpoint molecules VIP and PD-L1 in various cancers. A  $p$ -value  $<0.05$  was used as an indicator for significance and further analysis.

### 2.4 | cBioportal database analysis

The cBioportal (<https://www.cbioportal.org>)<sup>18,19</sup> is an interactive web server for analyzing RNA sequencing data of 9736 tumors and 8587 normal samples from the TCGA and GTEx projects. The PD-L1 and VIP RNA-Seq by Expectation Maximization (RSEM) data from the TCGA were plotted against each other to compare the expression for TCGA cancer subtypes. A  $p$ -value of 0.05 was used as a threshold for significance.

### 2.5 | TIMER2.0 database analysis

The TIMER2.0 (<http://timer.comp-genomics.org>)<sup>20</sup> is a comprehensive resource for analyzing tumor-infiltrating immune cells across cancers using robust algorithms to characterize data from the TCGA. TIMER2.0 was used to analyze the association of VIP and PD-L1 with immune infiltrates in lung, pancreatic, and colon cancers. A  $p$ -value  $<0.05$ , determined through Spearman's correlation, was used to determine statistical significance for immune infiltrates with specific cancers.



**FIGURE 1** Expression of VIP and PD-L1 in cancer tissues and corresponding normal adjacent tissues.

VIP and PD-L1 expression based on RNA-seq Transcripts Per Million (TPM) data from the TCGA and GTEx were analyzed across various cancers. A red star is used to show statistical significance in expression levels by comparing cancer tissues to the corresponding normal tissues using one-way ANOVA:  $*p < 0.05$ . Two black stars are used to show statistical significance in overexpression of VIP and PD-L1 when comparing stated cancer tissues to all other cancer tissues presented in the figure using student t-test:  $**p < 0.05$ . The graph was generated with GEPIA. Unique tissue samples in the analysis included 179 pancreatic adenocarcinomas and 171 normal pancreatic tissue; 483 lung adenocarcinoma and 347 normal adjacent tissues; 486 lung squamous cell carcinoma and 347 adjacent normal tissue; 1085 breast invasive carcinoma and 291 adjacent normal tissue; 275 colon adenocarcinoma and 349 normal adjacent tissue; 461 cutaneous melanoma and 558 normal adjacent tissue; 163 cases of glioblastoma multiforme with 207 normal adjacent brain tissue samples; 173 acute myeloid leukemia and 70 adjacent normal tissue; 47 diffuse large B cell lymphoma and 337 adjacent normal tissue

### 3 | RESULTS

#### 3.1 | Differential expression of VIP and PD-L1 across multiple human cancers

To assess the aberrant expression of VIP and PD-L1, VIP and PD-L1 RNA-seq transcripts per million (TPM) data from the TCGA and GTEx were analyzed across various cancer histology. We used GEPIA analysis to compare PD-L1 and VIP expression in cancer tissues to adjacent normal tissues across various cancer types. Data on gene expression in cancer tissue was obtained from the TCGA. Additionally, data on gene expression in adjacent normal tissue in cancer patients was obtained from both the TCGA and GTEx databases, as GEPIA did not have a sufficient sample size of normal tissue data. As shown in Figure 1, lung adenocarcinoma, lung squamous cell carcinoma, breast

invasive carcinoma, colon adenocarcinoma, skin cutaneous melanoma, and glioblastoma multiforme tissue had lower levels of VIP expression than adjacent normal tissues. In contrast, pancreatic adenocarcinoma had higher VIP expression compared to adjacent normal tissue. Acute myeloid leukemia and diffuse large B cell lymphoma had similar VIP expression between cancer and normal tissue. In contrast, PD-L1 expression was higher in pancreatic adenocarcinoma, colon adenocarcinoma, skin cutaneous melanoma, glioblastoma multiforme, acute myeloid leukemia, and diffuse large B cell lymphoma when compared to corresponding normal tissues, while lower in lung adenocarcinoma, lung squamous cell carcinoma, and breast carcinoma when compared to corresponding adjacent normal tissues. Additionally, compared with other cancer types, VIP is relatively overexpressed in pancreatic adenocarcinoma and colon adenocarcinoma, while PD-L1 is overexpressed in lung adenocarcinoma, lung squamous cell carcinoma, and diffuse

large B cell lymphoma. Overall, both VIP and PD-L1 expression differ across different cancers, and some cancers had higher levels of VIP or PD-L1 compared to adjacent normal tissues. These results suggest that cancers may be selectively targeted by VIP or PD-L1 pathway inhibitors.

### 3.2 | Prognostic values of VIP and PD-L1 expression

Protein Atlas analysis of TCGA data was used to evaluate the prognostic values of VIP and PD-L1 expression in cancers. The distinction between expression of biomarkers (VIP vs. PD-L1) in cancer tissues versus expression in adjacent normal tissues was addressed by analyzing expression patterns in 15 cancers defined by histopathological classification in the Protein Atlas. The results from the Protein Atlas showed that VIP expression levels were not of prognostic value when all cancers were analyzed in aggregate, whereas high PD-L1 expression was favorable for survival and of significant prognostic value in breast and colorectal cancer patients.

However, in cancer microarray datasets with clinical annotation collected from the public domain (Mizuno 2009), PrognosScan meta-analysis showed that VIP expression was prognostic in colorectal cancer (three cohorts comprised of 167 patients), lung cancer (three cohorts comprised of 493 patients), and ovarian cancer (one cohort of 133 patients). The prognostic value of higher VIP levels differed by histologic subtype of lung cancer, where higher VIP levels portend a worse prognosis in lung squamous cell carcinoma but a better prognosis in lung adenocarcinoma. PD-L1 expression levels were prognostic in bladder cancer (two cohorts comprised of 330 patients), breast cancer (one cohort of 60 patients), colorectal cancer (one cohort of 49 patients), and ovarian cancer (one cohort of 278 patients).

These variable results indicate that levels of PD-L1 and VIP are not uniformly prognostic across all cancer histology. Differences between cancer types might be due to variation between cohorts from the Protein Atlas and the PrognosScan analysis or the lack of underlying mechanism by which overexpression of a specific immune checkpoint molecule confers prognosis in the absence of specific therapies targeting that pathway. Indeed, results from multiple clinical trials and clinical studies have shown that the efficacy of PD-L1/PD-1 ICIs in cancer patients is roughly proportional to the degree of PD-L1 expression.<sup>21</sup>

We next used GEPIA to characterize the overall survival of patients with different cancer histology to further validate the prognostic values of VIP and PD-L1 expression in cancers screened with the PrognosScan, TIMER2.0, and Protein Atlas analysis (Figure 2). VIP was not shown to have significant prognostic value in any cancer. In pancreatic adenocarcinoma, high expression levels of PD-L1 were associated with decreased overall survival ( $p = 0.019$ , HR High = 0.022) (Figure 2C). These results suggest that the expression of the immune checkpoint molecules PD-L1 might be used as a prognostic indicator in pancreatic ductal adenocarcinoma. However, the results of clinical trials indicate the level of PD-L1 expression did not predict response to anti-PD-

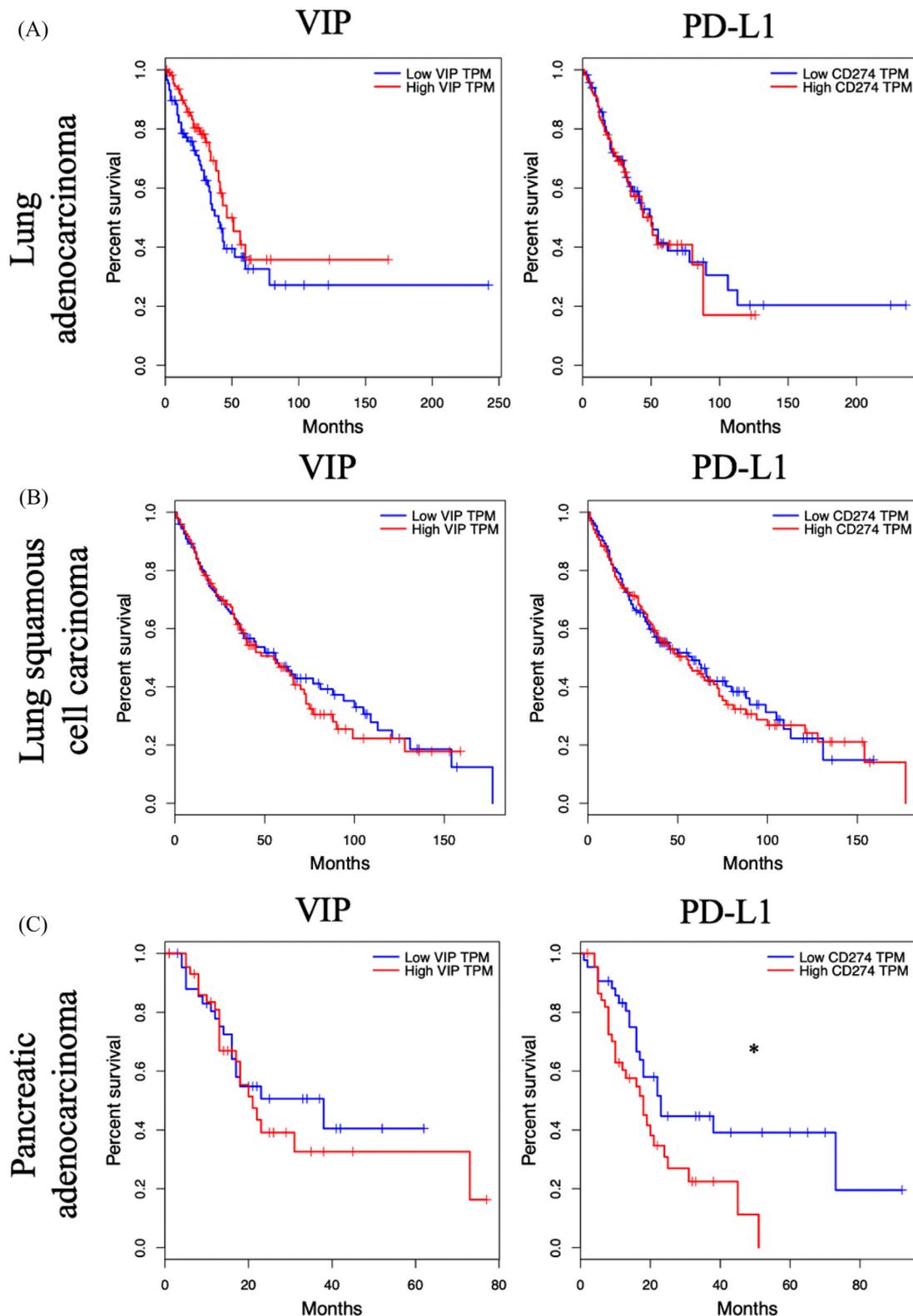
1/PD-L1 antibodies in pancreatic ductal adenocarcinoma.<sup>14</sup> PD-L1 expression by cancer cells exhausts T cells through PD-1 signaling and renders them useless to attack cancers. However, PD-L1 expression levels have not been shown to predict the efficacy of PD-1 antagonists in patients with pancreatic ductal adenocarcinoma. The dissociation of the prognostic and predictive value of PD-L1 expression in pancreatic ductal adenocarcinoma may be due to the relative paucity of effector T cells in cancer in which the tumor microenvironment has been characterized as an “immune desert.”<sup>22</sup> PD-L1 expression levels were not shown to have significant prognostic value in other cancers.

### 3.3 | Association of VIP expression with PD-L1 expression

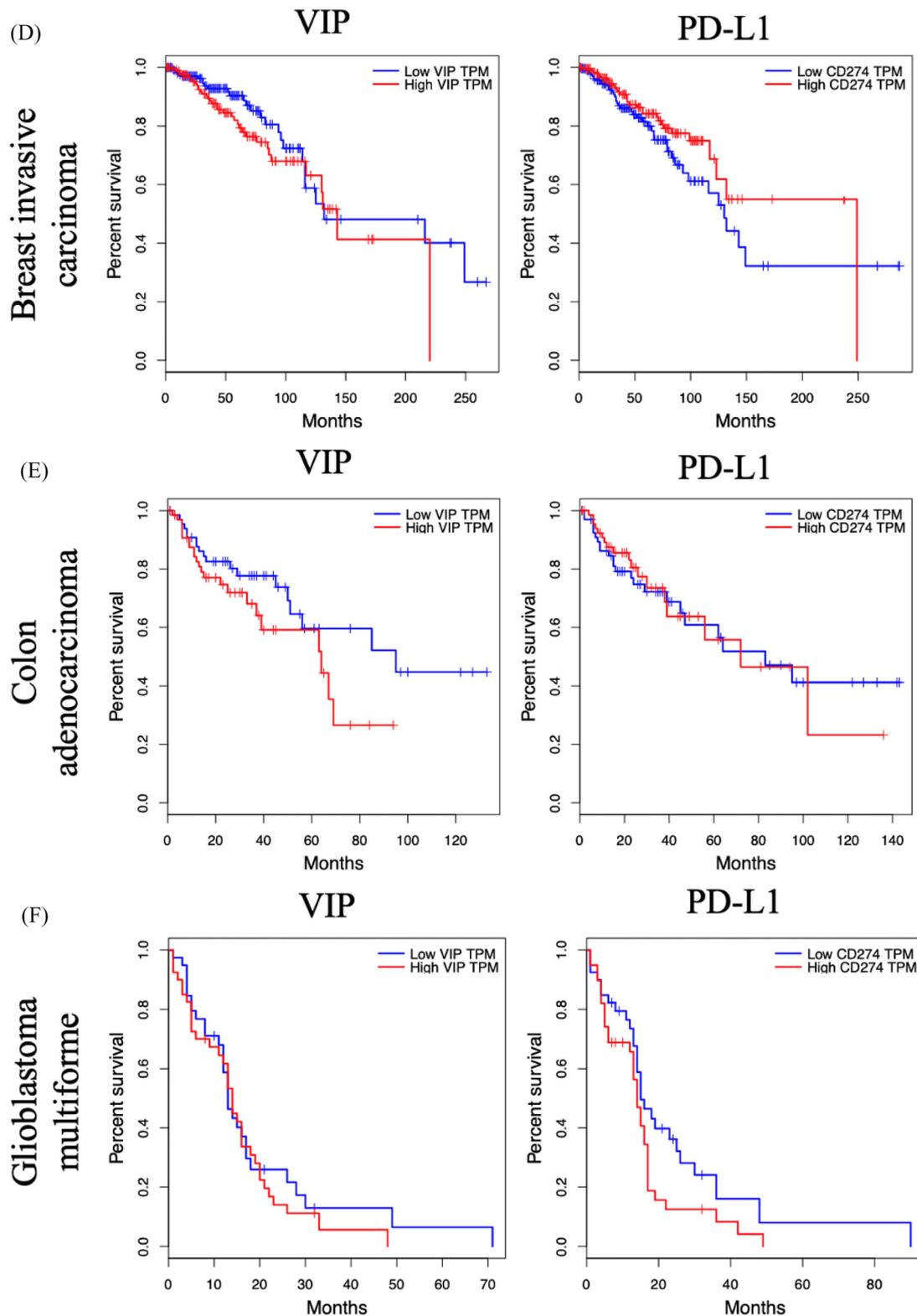
Next, we correlated PD-L1 and VIP expression in 17 cancers using the TCGA RNA-seq dataset and RNA-seq by expectation maximization (RSEM). Among the 17 cancers, lung adenocarcinoma, lung squamous cell carcinoma, colorectal adenocarcinoma, and pancreatic adenocarcinoma, showed interesting associations between VIP and PD-L1 expression (Figure 3). Including adenocarcinoma and lung squamous cell carcinoma, PD-L1 expression tended to be high while VIP expression was low (Figure 3A and B). In both colorectal adenocarcinoma and pancreatic adenocarcinoma, VIP expression was generally high while PD-L1 was low (Figure 3C and D). These results suggest that VIP and PD-L1 expression might be negatively correlated, and even mutually exclusive as immune checkpoint pathways are overexpressed in cancers (Figure 3F).

### 3.4 | Association of VIP and PD-L1 expression with immune cell infiltrates in the tumor

High levels of immune cells infiltrating the tumor microenvironment have positive prognostic values in many cancers, including ovarian cancer.<sup>23</sup> It is hypothesized that local expression of PD-L1 and VIP in the tumor microenvironment mediates an immunosuppressive state and hampers anti-cancer immunity mediated by T cells. Therefore, we correlated the levels of immune cells in the TME with VIP and PD-L1 expression levels across lung adenocarcinoma, lung squamous cell cancer, pancreatic adenocarcinoma, and colorectal cancer histology. Analysis of immune cell infiltration in the TME included the content of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells (Figure 4 and Table 1). In lung adenocarcinoma, PD-L1 expression positively correlated with the infiltration of CD8+ T cells, macrophages, neutrophils, and dendritic cells whilst negatively correlated with CD4+ T cells. In colorectal cancer, PD-L1 expression was positively correlated with CD8+ T cells, macrophages, neutrophils, and dendritic cell infiltration, and negatively correlated with B cell infiltration. In lung adenocarcinoma and lung squamous cell carcinoma, VIP expression was positively correlated only with CD4+ T cell infiltration. In colorectal cancer, VIP expression was positively correlated with CD4+ T cells, macrophage, neutrophil, and dendritic cell



**FIGURE 2** Overall survival of cancer patients with high and low levels of PD-L1 and VIP expression. (A) lung adenocarcinoma (VIP  $n = 203$ , PD-L1  $n = 239$ ), (B) lung squamous cell carcinoma (VIP  $n = 437$ , PD-L1  $n = 482$ ), (C) pancreatic adenocarcinoma (VIP  $n = 90$ , PD-L1  $n = 90$ ), (D) breast invasive carcinoma (VIP  $n = 484$ , PD-L1  $n = 539$ ), (E) colon adenocarcinoma (VIP  $n = 135$ , PD-L1  $n = 136$ ), and (F) glioblastoma multiforme (VIP  $n = 82$ , PD-L1  $n = 81$ ). The survival curves were generated by GEPIA, dividing cases by median levels of PD-L1 or VIP expression based upon data from TCGA. In all analyzed cancers, only high expression of PD-L1 was associated with a decreased survival in pancreatic adenocarcinoma ( $p < 0.05$ ). The log rank test was used to calculate statistical significance with  $*p < 0.05$

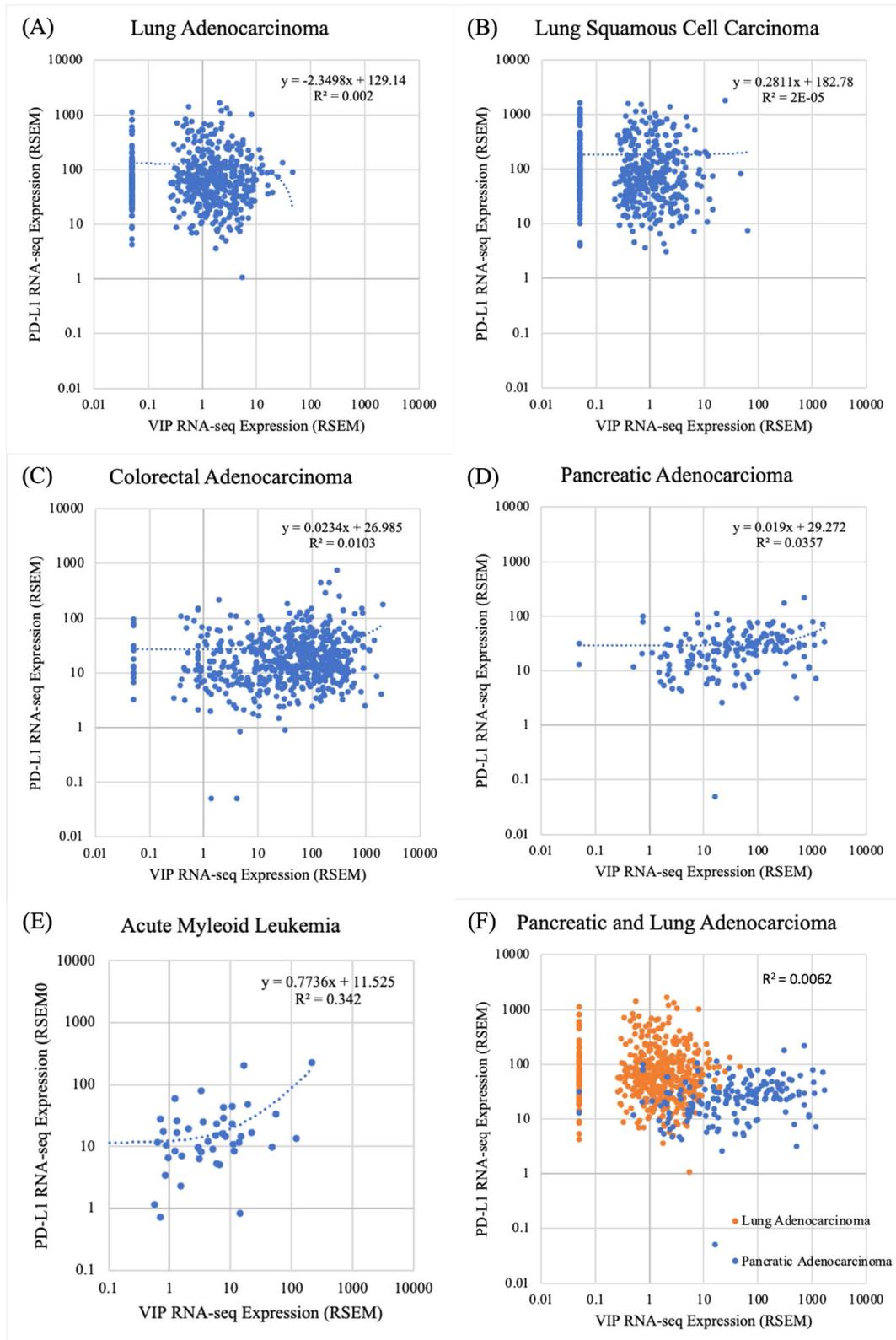


**FIGURE 2** Continued

infiltration. Lastly, in pancreatic adenocarcinoma, VIP expression was positively correlated with CD8+ T cells, macrophages, neutrophils, and dendritic cells in the TME. Overall, VIP and PD-L1 expression are both positively correlated with immune cell infiltration in the TME of non-small cell lung cancers and pancreatic adenocarcinomas.

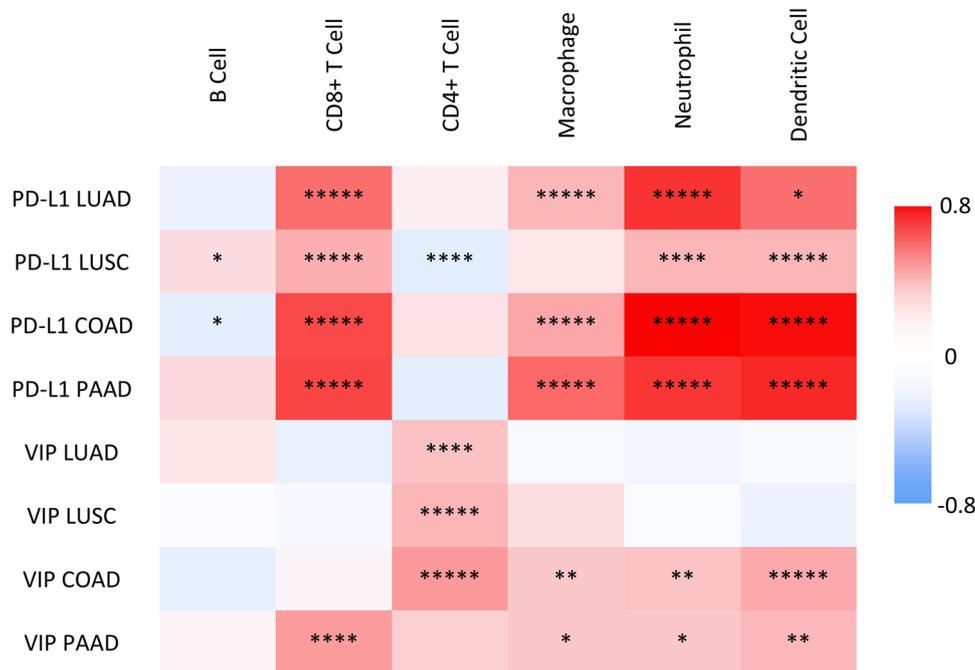
#### 4 | DISCUSSION

Monoclonal antibodies that target immune checkpoints represent a paradigm-changing breakthrough in cancer therapeutics. PD-L1/PD-1 and CTLA4 inhibitors have demonstrated significant efficacy and



**FIGURE 3** The expression of VIP and PD-L1 is mutually exclusive in some lung adenocarcinoma, lung squamous cell carcinoma, pancreatic adenocarcinoma, colorectal adenocarcinoma, and acute myeloid leukemia cancer patients.

VIP versus PD-L1 expression of individual cancer patients was plotted based upon cohorts of (A) lung adenocarcinoma cancer patients ( $n = 510$ ), (B) lung squamous cell carcinoma patients ( $n = 484$ ), (C) colorectal adenocarcinoma patients ( $n = 592$ ), (D) pancreatic adenocarcinoma cancer patients ( $n = 177$ ), (E) acute myeloid leukemia patients ( $n = 173$ ). (F) VIP and PD-L1 expression were compared between lung adenocarcinoma and pancreatic adenocarcinoma. Expression levels are based upon RNA-Seq by Expectation Maximization (RSEM) for PD-L1 and VIP expression taken from the cBioportal which uses data from the TCGA



**FIGURE 4** Positive correlation of immune cells in the TME with PD-L1 and VIP expression in cancers.

The heat map shows the correlations between RNA-seq expression of PD-L1 and VIP and immune cell infiltrates, including B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in lung adenocarcinoma (LUAD;  $n = 515$ ), lung squamous cell carcinoma (LUSC;  $n = 501$ ), colorectal adenocarcinoma (COAD;  $n = 458$ ), and pancreatic adenocarcinoma (PAAD;  $n = 179$ ). The TIMER2.0 analysis was used to determine correlations of data from the TCGA. Partial Spearman's correlation was used to perform association analyses. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ ; \*\*\*\*\* $p < 0.00001$

have been approved by the Food and Drug Administration for several malignancies. More recently, several pre-clinical studies have explored the immunomodulatory role of VIP in cancers and used VIP as a potential target for the development of next-generation ICIs.<sup>12</sup> Indeed, the analysis from the TCGA and GTEx demonstrated that VIP and PD-L1 were overexpressed in different cancers (Figure 1). The biggest obstacles to incorporating immunotherapy into routine treatment regimens for cancer patients are the low response rates and immune-related adverse events in some patients. Thus, there is an urgent need for identifying the predictive biomarkers to match patients with the ICI that has the greatest potential for therapeutic benefit.

Because immune suppression plays an important part in driving cancer growth and progression, the expression of immune checkpoints may have prognostic value. Based on an analysis of public databases, the prognostic values of PD-L1 and VIP expression levels are inconsistent across multiple cancer types. High PD-L1 expression was associated with better survival in subsets of bladder, ovarian, colorectal, and breast cancer patients. Previous studies reported that PD-L1 is prognostic in breast cancer, gastric cancer, hepatocellular carcinoma, non-small cell lung cancer, and renal cell carcinoma (Hu, 2019). Prognoscan suggested that VIP was of prognostic value in colorectal, lung, and ovarian cancer. However, GEPIA survival curves did not show VIP expression to be prognostic among any cancers (Figure 2). Although some databases show VIP and PD-L1 expression have prog-

nostic value, the prognostic values of VIP and PD-L1 expression remain variable across different cancers.

PD-L1 expression has been reported to be a positive predictive biomarker for ICI efficacy and survival in non-small cell lung cancer patients such as lung adenocarcinoma and lung squamous cell carcinoma.<sup>24,25</sup> However, current studies show that PD-L1 ICIs have low efficacy in pancreatic adenocarcinoma. In cancer like lung adenocarcinoma where PD-L1 inhibitors are effective, PD-L1 is overexpressed relative to other cancers. Our results showed that PD-L1 has lower expression in pancreatic adenocarcinoma than in lung adenocarcinoma and lung squamous cell carcinoma. This low expression of PD-L1 suggests that PD-L1 expression plays a limited role in immune evasion in pancreatic adenocarcinoma and is consistent with the limited activity of ICIs that target PD1-PD-L1 signaling in this malignancy. In the same way, drugs that inhibit the VIP signaling pathway may have low efficacy in cancers with low expression of VIP but could be effective in cancers that have high expression of VIP. While pre-clinical data indicated that VIP receptor inhibition with VIP receptor antagonist peptides has therapeutic benefits in murine pancreatic ductal adenocarcinoma mouse models, the clinical relevance of these findings is unknown. Thus, we selectively analyzed PD-L1 and VIP expression in lung and pancreatic adenocarcinoma patients. The data showed that expression of VIP is high in pancreatic adenocarcinoma and colorectal adenocarcinoma while expression of PD-L1 is high in lung adenocarcinoma and lung squamous cell carcinoma (Figure 3).

**TABLE 1** Spearman's coefficients and p values and for associations of immune cells in the tumor microenvironment with levels of PD-L1 and VIP mRNA expression. Correlations between RNA-seq expression of PD-L1 and VIP and immune cell infiltrates, including B cells, CD8<sup>+</sup> T cells, CD4<sup>+</sup> T cells, macrophages, neutrophils, and dendritic cells in lung adenocarcinoma (LUAD; *n* = 515), lung squamous cell carcinoma (LUSC; *n* = 501), colorectal adenocarcinoma (COAD; *n* = 458), and pancreatic adenocarcinoma (PAAD; *n* = 179). The TIMER2.0 analysis was used to determine correlations of data from the TCGA. Partial Spearman's correlation was used to perform association analyses

Spearman's coefficients	B Cell	CD8+ T Cell	CD4+ T Cell	Macrophage	Neutrophil	Dendritic cell
PD-L1 LUAD	-0.086	0.457	0.048	0.225	0.648	0.451
PD-L1 LUSC	0.102	0.248	-0.131	0.07	0.225	0.228
PD-L1 COAD	-0.122	0.573	0.085	0.278	0.847	0.768
PD-L1 PAAD	0.115	0.589	-0.119	0.477	0.638	0.682
VIP LUAD	0.073	-0.095	0.187	-0.021	-0.045	-0.02
VIP LUSC	0.005	-0.029	0.228	0.096	-0.006	-0.077
VIP COAD	-0.113	0.024	0.32	0.169	0.186	0.269
VIP PAAD	0.029	0.312	0.147	0.172	0.172	0.222
P values	B Cell	CD8+ T Cell	CD4+ T Cell	Macrophage	Neutrophil	Dendritic cell
PD-L1 LUAD	0.055	9.07E-07	0.283	4.71E-07	6.33E-60	0.031
PD-L1 LUSC	0.026	4.14E-08	9.72E-5	0.126	1.16E-05	3.12E-22
PD-L1 COAD	0.044	2.32E-25	0.158	2.83E-06	5.25E-12	1.78E-28
PD-L1 PAAD	0.134	6.21E-6	0.122	4.42E-11	4.57E-09	2.32E-19
VIP LUAD	0.105	0.366	2.86E-05	0.637	0.894	-0.655
VIP LUSC	0.916	0.93	4.58E-7	0.123	0.459	-0.0949
VIP COAD	0.062	0.092	5.73E-08	0.0058	0.00195	6.15E-15
VIP PAAD	0.706	1.92E-5	0.055	0.024	0.0249	0.0035

Thus, VIP could be a predictive biomarker for treatment inhibiting the VIP signaling pathway in pancreatic adenocarcinoma and colorectal adenocarcinoma.

To further investigate the potential interaction between the two molecules, we found that the expression of VIP and PD-L1 is mutually exclusive in colorectal adenocarcinoma, lung adenocarcinoma, lung squamous cell carcinoma, and pancreatic adenocarcinoma (Figure 3F). The results showed that VIP expression was high and PD-L1 expression was low in individual tumors from patients with pancreatic adenocarcinoma and colorectal adenocarcinoma. In contrast, PD-L1 expression was high and VIP expression was low in individual tumor specimens from lung adenocarcinoma and lung squamous cell carcinoma patients. These results may indicate functional redundancy between the two immune-suppressive molecules. Thus, it may be possible that some cancers may use either VIP or PD-L1 as a primary means of immune escape. We also found higher levels of PD-L1, and VIP expression was associated with an increase of immune effector cells in the TME (Figure 4). These results support the relevance of the VIP and PD-L1 pathways in the immune control of cancer.

This paper has several limitations. When using the Protein Atlas, Prognoscan, and GEPIA, there was heterogeneity among the prognostic values for PD-L1 and VIP in various cancers due to variability among different patient cohorts. Further analysis of the variability among patient cohorts may be needed to further investigate the reliability of the prognostic values of PD-L1 and VIP. There may also be biases in

sample collection for the publicly available datasets resulting from the availability of tissues, institutional research interests, the structure of operation, and the patient population. Furthermore, the datasets only evaluated primary tumors from untreated patient populations. Thus, there is limited availability of data collection from tumors that commonly undergo neoadjuvant therapy. In turn, conclusions based upon analyses of these datasets may not translate to modern clinical practices for cancer.<sup>26</sup> Although the findings herein may suggest that VIP could function like PD-L1 as a key immune checkpoint molecule in cancer, further laboratory experiments and clinical trials are needed to explore the prognostic and predictive value of VIP expression in cancer.

## 5 | CONCLUSION

In conclusion, PD-L1 expression levels are prognostic biomarkers for breast and colorectal cancer patients. PD-L1 is a proven predictive biomarker in non-small cell lung cancer. VIP expression levels were not a consistent prognostic biomarker when analyzed across multiple cancers. However, the high expression of VIP in pancreatic adenocarcinoma and the association of VIP levels with immune cell infiltrates in the TM suggest that, like PD-L1 expression and anti-PD1 antibody therapy, VIP could be a predictive biomarker for pancreatic adenocarcinoma patients treated with drugs that block signaling through the VIP receptor. In addition, the negative correlation between

PD-L1 and VIP expression suggests functional redundancy of the two immunosuppressive pathways. Preclinical data suggest synergy between anti-PD1/PD-L1 antibodies and anti-VIP drugs in mouse models of pancreatic adenocarcinoma (unpublished data), but the activity of anti-VIP and anti-PD1 therapies in human PDAC remains to be established. Mechanistic studies that link the action of immune checkpoint drugs in specific cancer patients with the activation of anti-cancer immune responses are needed.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

E.K.W. has intellectual property covering VIP antagonists. E.K.W. and T.K.O are founders and have equity ownership in Cambium Oncology which has licensed intellectual property related to VIP antagonists as immune checkpoint drugs and cancer therapeutics. The other authors declare no financial conflict of interest.

## REFERENCES

- Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12(4):252-64. <https://doi.org/10.1038/nrc3239>
- Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. *Immunity*. 2018;48(3):434-52. <https://doi.org/10.1016/j.immuni.2018.03.014>
- Li J-M, Darlak KA, Southerland L, Hossain MS, Jaye DL, Josephson CD, et al. VIPhyb, an antagonist of vasoactive intestinal peptide receptor, enhances cellular antiviral immunity in murine cytomegalovirus infected mice. *PLoS ONE*. 2013;8(5):e63381-e. <https://doi.org/10.1371/journal.pone.0063381>
- Fernández-Martínez AB, Bajo AM, Valdehita A, Isabel Arenas M, Sánchez-Chapado M, Carmena MJ, et al. Multifunctional role of VIP in prostate cancer progression in a xenograft model: suppression by curcumin and COX-2 inhibitor NS-398. *Peptides*. 2009;30(12):2357-64. <https://doi.org/10.1016/j.peptides.2009.09.018>
- Moody TW, Gozes I. Vasoactive intestinal peptide receptors: a molecular target in breast and lung cancer. *Curr Pharm Des*. 2007;13(11):1099-104. <https://doi.org/10.2174/138161207780619000>
- Moody TW, Leyton J, Chan D, Brenneman DC, Fridkin M, Gelber E, et al. VIP receptor antagonists and chemotherapeutic drugs inhibit the growth of breast cancer cells. *Breast Cancer Res Treat*. 2001;68(1):55-64. <https://doi.org/10.1023/A:1017994722130>
- Moody TW, Nuche-Berenguer B, Jensen RT. Vasoactive intestinal peptide/pituitary adenylate cyclase activating polypeptide, and their receptors and cancer. *Curr Opin Endocrinol Diabetes Obes*. 2016;23(1):38-47. <https://doi.org/10.1097/med.0000000000000218>
- Moody TW, Zia F, Draoui M, Brenneman DE, Fridkin M, Davidson A, et al. A vasoactive intestinal peptide antagonist inhibits non-small cell lung cancer growth. *Proc Natl Acad Sci U S A*. 1993;90(10):4345-9. <https://doi.org/10.1073/pnas.90.10.4345>
- Delgado M, Ganea D. Cutting edge: is vasoactive intestinal peptide a type 2 cytokine? *J Immunol*. 2001;166(5):2907-2912. <https://doi.org/10.4049/jimmunol.166.5.2907>
- Anderson P, Gonzalez-Rey E. Vasoactive intestinal peptide induces cell cycle arrest and regulatory functions in human T cells at multiple levels. *Mol Cell Biol*. 2010;30(10):2537-51. <https://doi.org/10.1128/mcb.01282-09>
- Gonzalez-Rey E, Delgado M. Vasoactive intestinal peptide and regulatory T-cell induction: a new mechanism and therapeutic potential for immune homeostasis. *Trends Mol Med*. 2007;13(6):241-51. <https://doi.org/10.1016/j.molmed.2007.04.003>
- Petersen CT, Li J-M, Waller EK. Administration of a vasoactive intestinal peptide antagonist enhances the autologous anti-leukemia T cell response in murine models of acute leukemia. *Oncoimmunology*. 2017;6(5):e1304336-e. <https://doi.org/10.1080/2162402X.2017.1304336>
- Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372(21):2018-28. <https://doi.org/10.1056/NEJMoa1501824>
- O'Reilly EM, Oh D-Y, Dhani N, Renouf DJ, Lee MA, Sun W, et al. Durvalumab with or without tremelimumab for patients with metastatic pancreatic ductal adenocarcinoma: a phase 2 randomized clinical trial. *JAMA Oncol*. 2019;5(10):1431-8. <https://doi.org/10.1001/jamaoncol.2019.1588>
- Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucl Acids Res*. 2017;45(W1):W98-W102. <https://doi.org/10.1093/nar/gkx247>
- Mizuno H, Kitada K, Nakai K, Sarai A. PrognoScan: a new database for meta-analysis of the prognostic value of genes. *BMC Med Genom*. 2009;2(1):18. <https://doi.org/10.1186/1755-8794-2-18>
- Uhlen M, Zhang C, Lee S, Sjöstedt E, Fagerberg L, Bidkhorji G, et al. A pathology atlas of the human cancer transcriptome. *Science*. 2017;357(6352). <https://doi.org/10.1126/science.aan2507>
- Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, & Schultz N. (2012). The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discovery*, 2(5), 401-404. <https://doi.org/10.1158/2159-8290.cd-12-0095>
- Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C, & Schultz N. (2013). Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science Signaling*, 6(269). <https://doi.org/10.1126/scisignal.2004088>
- Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res*. 2017;77(21):e108-e10. <https://doi.org/10.1158/0008-5472.CAN-17-0307>
- Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. *J Immuno Therapy Cancer*. 2019;7(1):278. <https://doi.org/10.1186/s40425-019-0768-9>
- Li H-B, Yang Z-H, Guo Q-Q. Immune checkpoint inhibition for pancreatic ductal adenocarcinoma: limitations and prospects: a systematic review. *Cell Commun Signal*. 2021;19(1):117. <https://doi.org/10.1186/s12964-021-00789-w>
- Barnes TA, Amir E. HYPE or HOPE: the prognostic value of infiltrating immune cells in cancer. *Br J Cancer*. 2017;117(4):451-60. <https://doi.org/10.1038/bjc.2017.220>
- Tashima Y, Kuwata T, Yoneda K, Hirai A, Mori M, Kanayama M, et al. Prognostic impact of PD-L1 expression in correlation with neutrophil-to-lymphocyte ratio in squamous cell carcinoma of the lung. *Sci Rep*. 2020;10(1):1243. <https://doi.org/10.1038/s41598-019-57321-x>
- Vrankar M, Kern I, Stanic K. Prognostic value of PD-L1 expression in patients with unresectable stage III non-small cell lung cancer treated

- with chemoradiotherapy. *Radiat Oncol.* 2020;15(1):247. <https://doi.org/10.1186/s13014-020-01696-z>
26. Cooper LA, Demicco EG, Saltz JH, Powell RT, Rao A, Lazar AJ. Pan-Cancer insights from The Cancer Genome Atlas: the pathologist's perspective. *J Pathol.* 2018;244(5):512-24. <https://doi.org/10.1002/path.5028>

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