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Expression of the Vesicular Monoamine Transporter Gene Solute Carrier Family 18 Member 1 (*SLC18A1*) in Lung Cancer

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Abstract. *Background: One aspect of smoking and lung cancer that has not been closely examined, is that regarding genes that may predispose to tobacco dependence. Smoking and mental illness are tightly linked, apparently the result of smokers using cigarettes to self-medicate for mental problems. The gene for solute carrier family 18 member A1 (vesicular monoamine transporter; SLC18A1) is of particular interest in this regard because of its association with schizophrenia, autism and bipolar illness as well as with cancer. In the current study, the relationship of SLC18A1 expression with smoking and lung cancer was analyzed. Materials and Methods: The association between smoking, SLC18A1 expression and overall survival in the lung cancer dataset in The Cancer Genome Atlas was evaluated using the Genomic Data Commons Data Portal (https://portal.gdc.cancer.gov), as well as CbioPortal for Cancer Genomics (http://www.cbioportal.org) and the University of California Santa Cruz Xena browser (https://xenabrowser.net). Results: Increased expression of SLC18A1 was found to be associated with a significantly increased survival in patients with adenocarcinoma (p=0.0058), but not those with squamous carcinoma (p=0.96). Lifelong never-smokers had the highest SLC18A1 expression. In the Pan Cancer Atlas, increased expression of SLC18A1 places such a tumor in group C5, among immunologically- quiet tumors. Conclusion: Most never-smokers with lung cancer do not respond to immune checkpoint inhibitors (ICIs). But for unknown reasons, a small proportion do show clinical benefit from the ICI pembrolizumab. Because of the good response of this group, it may be worthwhile assessing their SLC18A1 expression pre-treatment as a marker for potential clinical benefit. If SLC18A1 expression is low, a never-smoker may respond well to ICIs. High levels of expression would indicate a C5 tumor less likely to respond to ICIs. SLC18A1 might complement other biomarkers currently under study in relation to programmed cell death protein 1/programmed cell death protein ligand 1 inhibition.*

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Lung cancer is the most common malignancy and the primary cause of cancer-related deaths worldwide. The genetics of lung cancer has been the subject of intense investigation. Epidermal growth factor receptor (*EGFR*), Kirsten rat sarcoma virus (*KRAS*), phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*), and P53 or tumor protein 53 (*TP53*) genes are frequently mutated (1). Inhibitors of programmed death 1 (PD1) and its ligand PD-L1 are effective therapies for metastatic non-small-cell lung cancer (NSCLC) lacking sensitizing *EGFR* or anaplastic lymphoma kinase (*ALK*) mutations. Pembrolizumab (Keytruda, Merck), nivolumab (Opdivo, Bristol-Myers Squibb), and atezolizumab (Tecentriq, Genentech) are effective in smokers with stage IV disease, but not in never-smokers (2, 3).

Cigarette smoking significantly increases lung cancer risk and results in genetic changes. Tobacco smoke contains more than 60 mutagens capable of binding to and chemically modifying DNA, and these alterations leave characteristic mutational patterns seen in lung cancers. For example, distinctive point mutation patterns in *KRAS* and *TP53* have been observed in patients with lung cancer with a history of smoking *versus* their non-smoking counterparts (1).

One aspect of smoking and lung cancer that has not been closely examined is that regarding genes which may predispose to tobacco dependence. Smoking and mental illness are tightly linked. In an Israeli study, the rate of smoking did not appear to differ between bipolar (43.0%) and schizophrenic (45.0%) patients, whereas the rate for both patient groups was higher than that for the general Israeli population (27.5%) (4). The link to smoking is apparently the result of smokers using cigarettes to self-medicate for mental problems (5).

The solute carrier family 18 member A1 (vesicular monoamine transporter) gene *SLC18A1* is of particular interest because of its association with schizophrenia (6-10), autism (11) and bipolar illness (9) as well as with cancer (12, 13). In the current study, the relationship of *SLC18A1* expression with smoking and lung cancer was analyzed.

Materials and Methods

The association between smoking, *SLC18A1* expression and overall survival in the lung cancer dataset in The Cancer Genome Atlas (TCGA) was evaluated. To access and analyze the data the following were used: Genomic Data Commons Data Portal (https://portal.gdc.cancer.gov); University California Santa Cruz Xena browser (https://xenabrowser.net); and CbioPortal for Cancer Genomics (http://www.cbioportal.org/)

SLC18A1 expression is quantified as RNAseq count. The normalized unit for count is log2(norm_count+1). The normalization calculation is described elsewhere (14).

Data selection. The entire TCGA lung cancer data set was analyzed (1,299 cases).

The following features were assessed: Effect of *SLC18A1* on survival; expression of *SLC18A1* by smoking status; frequency of genetic alteration of *SLC18A1* in lung cancer according to histology; *SLC18A1* expression in the TCGA PanCan Atlas. Endpoints were death or last time-point the patient was known to be alive. Survival analyses were carried out by the method of Kaplan and Meier (15).

Results

A total of 1,299 patients were included in the sample; 43.5% were women, 56.5% were men. Their mean age was 66±9 years. Table I lists the pathological stages of the tumors included.

The effect of *SLC18A1* on survival is shown in Figure 1. Note that higher expression of *SLC18A1* was found to be associated with significantly increased survival of patients with adenocarcinoma (p=0.0058) but not of those with squamous carcinoma (p=0.96). Number of patients in each group is shown above corresponding error bar. The high/low survival cutoff was identified by methods described in the R2 web-based application (http://r2.amc.nl); the method divides the sample, in ascending order of gene expression, into two equal-sized groups.

In those with adenocarcinoma, increased *SLC18A1* expression and increased survival (284 patients out of 564 total) were probably due to the fact that never-smokers reportedly have better survival than smokers (16), as well as higher *SLC18A1* expression (Figure 2). In a separate analysis, *SLC18A1* was more highly expressed in 527 squamous cell carcinomas compared to 364 adenocarcinomas (mean±SEM=4.5±0.08 *versus* 3.5±0.14, respectively).

Expression of *SLC18A1* according to smoking status is shown in Figure 2 (p=0.035 one-way ANOVA). Lifelong never-smokers had the highest expression.

Table I. *Pathological stage of tumors analyzed.*

	Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	82	6.3	6.3	6.3
Stage I	8	0.6	0.6	6.9
Stage IA	266	20.5	20.5	27.4
Stage IB	354	27.3	27.3	54.7
Stage II	5	0.4	0.4	55
Stage IIA	131	10.1	10.1	65.1
Stage IIB	203	15.6	15.6	80.8
Stage III	3	0.2	0.2	81
Stage IIIA	169	13	13	94
Stage IIIB	36	2.8	2.8	96.8
Stage IV	42	3.2	3.2	100
Total	1299	100	100	

Frequency of alteration of *SLC18A1* in 1,299 lung cancers according to histology is shown in Figure 3. *PDCD1* (the PD1 gene) and *SLC18A1* have four significantly co-occurent alterations (p=0.011) in lung adenocarcinoma, suggesting that these two genes might play a similar role or need to interact (17). *PDCD1* alterations are shown in Table II.

SLC18A1 expression in the TCGA PanCan Atlas was examined. The Pan Cancer Atlas includes the analyses of over 11,000 tumors from 33 of the most prevalent forms of cancer. Expression of *SLC18A1* places it in group C5, immunologically quiet tumors (Figure 4). This group consists mostly of lower grade brain gliomas, exhibiting the lowest lymphocyte and highest macrophage responses, dominated by M2 macrophages (18). *SLC18A1* is most highly expressed in pheochromocytoma and paraganglioma (Figure 5). *SLC18A1* expression in 7,903 PanCan Atlas samples is shown in Figure 6. This gene is not highly expressed in most tumors but might represent a biomarker and a therapeutic target. Information on gene program score is included in the figure (19).

Discussion

SLC18A1 acts to accumulate cytosolic monoamines into vesicles, using the proton gradient maintained across the vesicular membrane. The transporter is a site of action of drugs, including reserpine and tetrabenazine (20). Its normal function is essential for the correct activity of the monoaminergic systems that have been implicated in neuropsychiatric disorders. *SLC18A1* maps to chromosome 8p23.1, a region of genes associated with personality traits (21). *SLC18A1* rs1390938/Thr136Ile is associated with mood, depression, personality, and alcohol use in the general population (22, 23). Individuals homozygous for the 'hyperfunction' allele (AA; Ile/Ile) appear to be more resilient to these disorders (24).

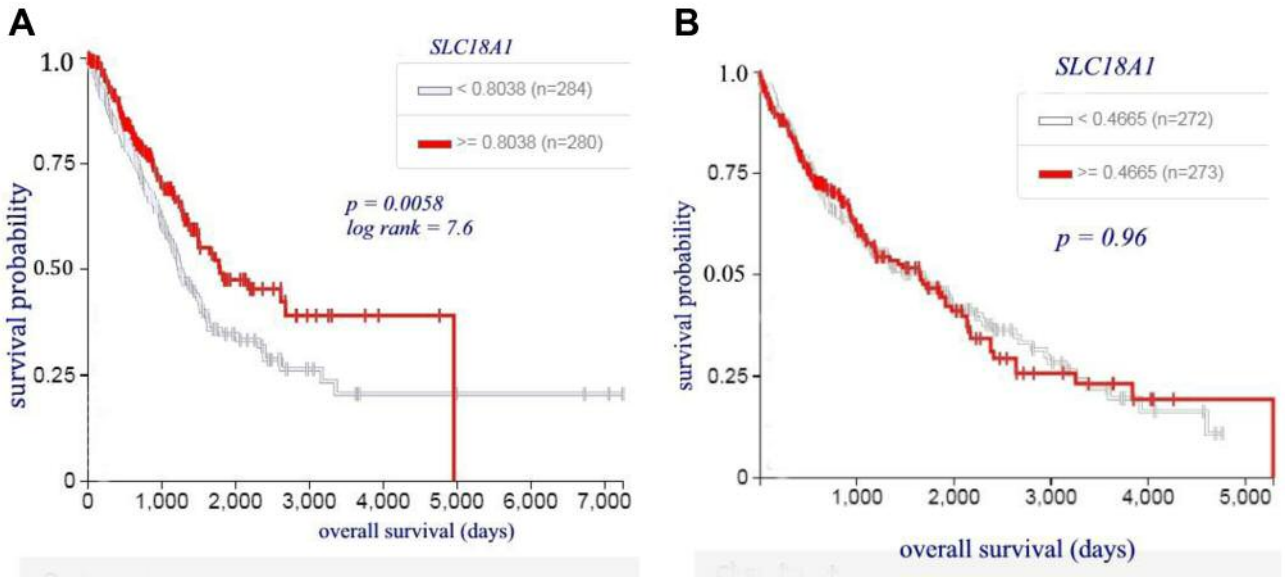

Figure 1. *Survival analysis of patients with lung cancer. Note the significant effect of higher solute carrier family 18 member 1 (SLC18A1) expression [log2(norm_count+1)] on survival of those with adenocarcinoma (A), but the absence of such effect in those with squamous cell carcinoma (B).*

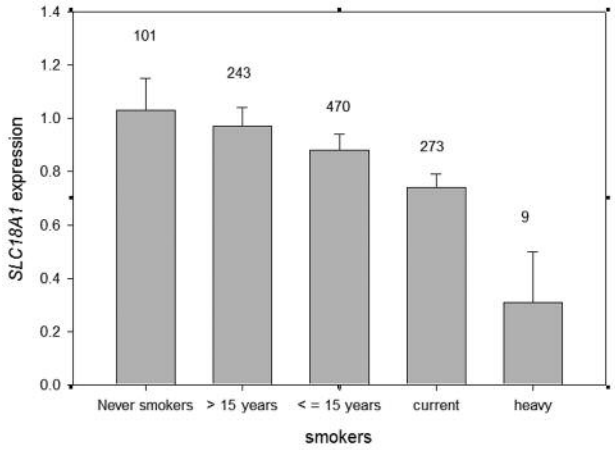

Figure 2. *Solute carrier family 18 member 1 (SLC18A1) expression (mean+SEM) stratified by smoking status in The Cancer Genome Atlas lung cancer data. The variation was significant (p=0.035 one-way ANOVA). Number of patients in each group is shown above corresponding error bar.*

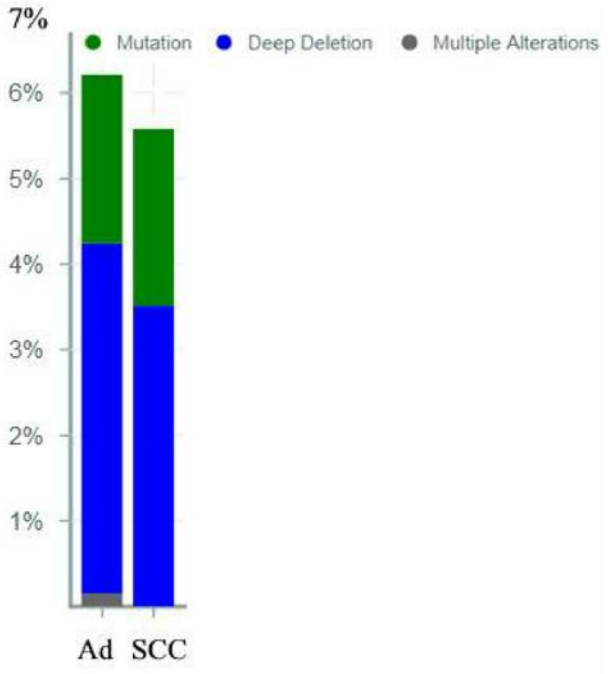

Figure 3. *Frequency of alteration of solute carrier family 18 member 1 (SLC18A1) gene in lung cancer by histology. Ad: Adenocarcinoma; SCC: squamous cell carcinoma.*

Many genes are related to personality, mental illness, and smoking (25, 26). But of these genes, only *SLC18A1* is known to be also involved in cancer (12, 13, 27).

Immune checkpoint inhibitors (ICIs) have emerged as a new treatment option for patients with cancer, especially advanced NSCLC. ICIs can prolong survival, compared to chemotherapy alone, in ever-smokers. However, ICIs mostly fail to improve survival in never-smokers (28).

Rizvi *et al*. assessed the effects of smoking on mutations and pembrolizumab response in NSCLC (29). According to the tumor molecular signature of smoking (frequency of C→A transversions in lung cancer exomes), Rizvi *et al*.

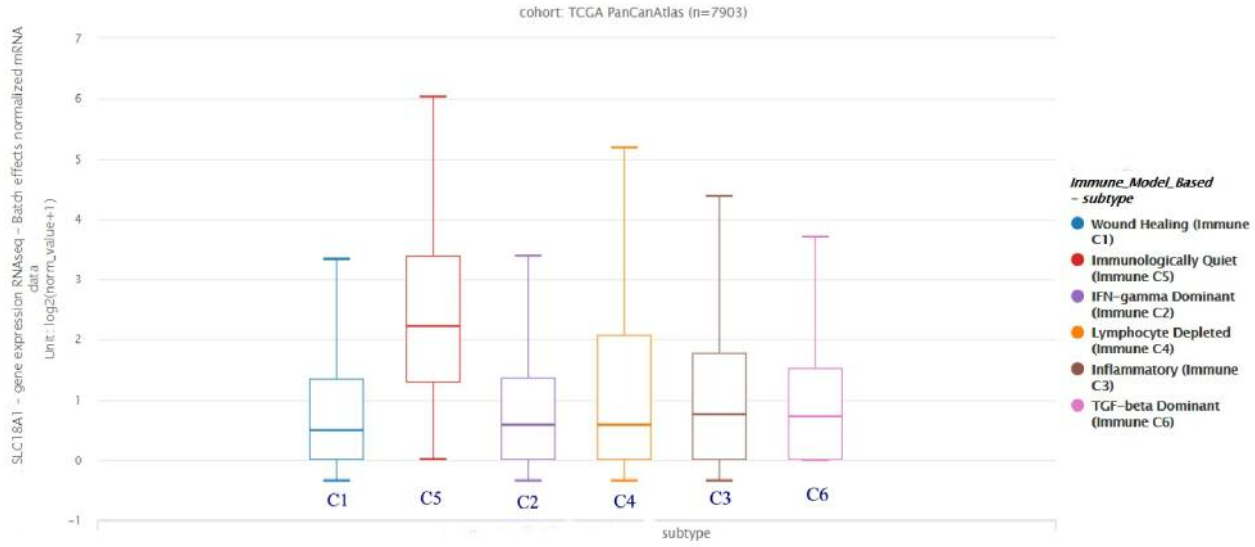

Figure 4. *Solute carrier family 18 member 1 (SLC18A1) expression by immune model-based subtypes in The Cancer Genome Atlas PanCan Atlas ($p<0.001$, one-way ANOVA). Expression is higher in immune subtype C5, immunologically-quiet tumors.*

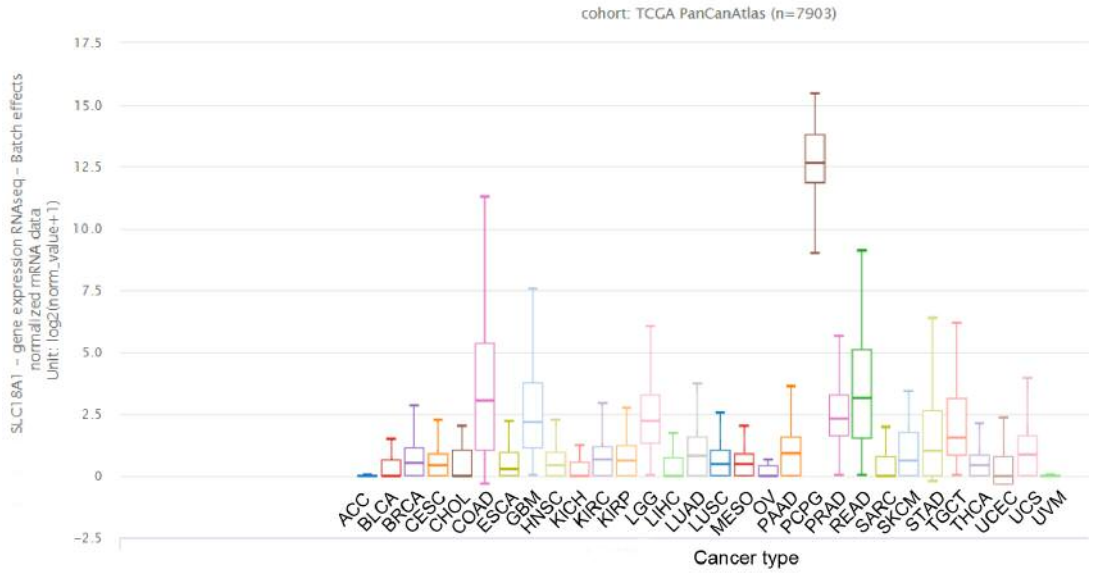

Figure 5. *Solute carrier family 18 member 1 (SLC18A1) expression by cancer type ($p<0.001$, one-way ANOVA). SLC18A1 is most highly expressed in pheochromocytoma and paraganglioma, PCPG.*

Table II. *Programmed cell death 1 (PDCD1) gene mutations in 1,299 tumors. The oncogenic function of these mutations is unknown.*

Sample ID	Protein change	Mutation type	Copy number	COSMIC allelic frequency (T)	Number of mutants in sample
TCGA-55-8616-01	R104L	Missense	Diploid	0.24	432
TCGA-86-8073-01	S109I	Missense	Diploid	0.19	1337
TCGA-97-81 79-01	S137R	Missense	Gain	0.2	151
TCGA-78-7536-01	T205N	Missense	Diploid	0.26	594

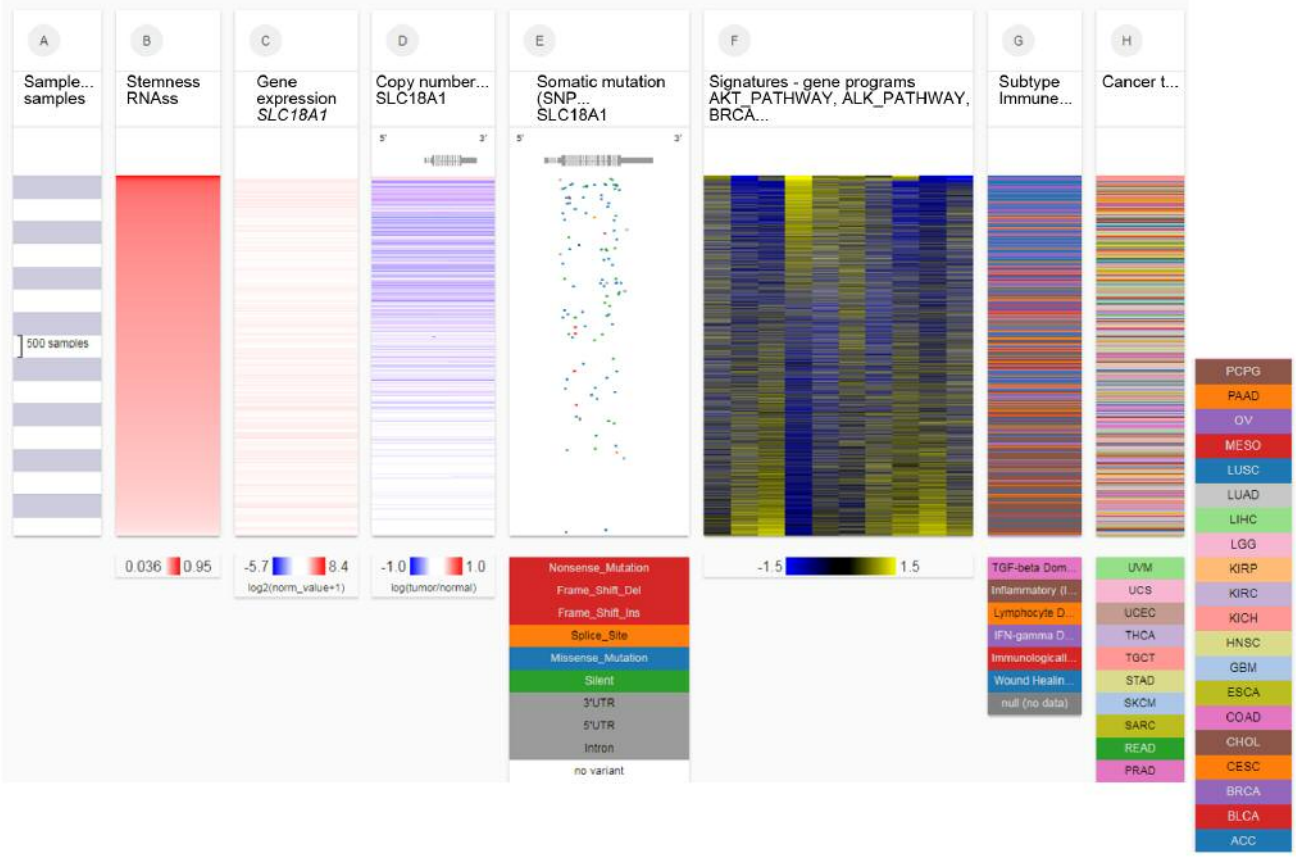

Figure 6. *A total of 7,903 PanCan Atlas samples (rows) by seven genomics columns. Each row corresponds to a single sample. The first sample column is followed by the seven data columns. The rows are sorted on the left-most data column and sub-sorted on subsequent columns. Data columns, starting at the left, are stemness score, SLC18A1 gene expression, copy numbers, and mutations, scores for various gene programs, immune subtype, and cancer type. Higher stemness is indicated with brighter red. The mutations column E shows a gene diagram at the top with exons in alternating light and dark grey boxes. For each sample, the position of the mutation is marked in relation to the gene diagram at the top. Mutations are colored by their functional impact with deleterious mutations in red, and missense mutations and in-frame indels (an insertion or deletion of bases) in blue. Gene expression is colored red to blue for high to low expression. The gene program score is colored yellow to blue for high to low scores. The immune-based subtype and cancer type are colored by their values (https://xenabrowser.net). Stemness is defined by the ability to self-renew and differentiate. Unlike normal stem cells, which differentiate into healthy, mature cell types, cancer stem cells differentiate into cancer cells.*

defined tumor samples as transversion high (TH, ever-smoking signature) or transversion low (TL, never-smoking signature). Patients with TH molecular signature had higher mutational burden and showed better clinical benefit from pembrolizumab. This suggests that smoking status might be a predictive marker for clinical benefit from ICIs. Indeed, the expression of PD1 and its ligands PD-L1 and PD-L2, which predict response to pembrolizumab and nivolumab, is heterogeneous within kirsten rat sarcoma virus (*KRAS*)-mutant NSCLC and suggests an inducible expression of PD-L1 by smoking (30).

Both Keytruda® (pembrolizumab) and Opdivo® (nivolumab) are monoclonal antibodies that bind to the PD1 receptor found on T-cells and block its interaction with PD-L1 and PD-L2 (31). Two of the approved indications for Keytruda are based on a US Food and Drug Administration-approved test for tumor expression of PD-L1, namely NSCLC and gastric cancer. Opdivo is indicated for both these cancer types without this limitation. Interestingly, most types of cancer for which these drugs are indicated are associated with smoking (NSCLC, gastric cancer, head and neck squamous cell carcinoma, renal cell carcinoma, urothelial carcinoma, hepatocellular carcinoma, but not melanoma) (https://dailymed.nlm.nih.gov/dailymed/index.cfm Search "Keytruda" and "Opdivo").

Smoking may be related to cancer in two distinct ways: i) Systemic suppression of the immune response producing cancer that is susceptible to Keytruda and Opdivo. ii) Direct irritation producing tumors not susceptible to these agents. Where both mechanisms are present, there might be intermediate susceptibility.

For unknown reasons, a small proportion of TL never-smokers show clinical benefit from pembrolizumab. This small proportion may correspond with the frequency of alteration of *SLC18A1* in lung cancer (Figure 3). Because of the good response of this small group, it may be worthwhile assessing *SLC18A1* expression prior to treatment and gene alteration as markers for potential clinical benefit from ICIs in never-smokers. If *SLC18A1* expression is low or *SLC18A1* is mutated, a never-smoker may respond well to ICIs, despite TL never-smoker mutation status. Higher *SLC18A1* expression levels would cause the tumor to fall into the C5 immunologically-quiet group, as is the case with squamous cell carcinoma, and it would, therefore, be less likely to respond to ICIs (Figure 1). *SLC18A1* might complement other biomarkers currently under study in relation to PD1/PD-L1 inhibition (32).

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