# Genomic Profiling of Rare Undifferentiated Sarcomatoid Subtypes of Pancreatic Carcinomas: In Search of Therapeutic Targets

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## ABSTRACT

**PURPOSE** The highly aggressive undifferentiated sarcomatoid carcinoma (USC) subtype of pancreatic ductal adenocarcinoma (PDAC) remains poorly characterized because of its rarity. Previous case reports suggest that immune checkpoint inhibitors could be a promising treatment strategy, but the prevalence of established predictive biomarkers of response is largely unknown. The objective of this study was to leverage comprehensive genomic profiling of USC PDAC tumors to determine the prevalence of biomarkers associated with potential response to targeted therapies.

**METHODS** USC tumors (n = 20) underwent central pathology review by a board-certified gastrointestinal pathologist to confirm the diagnosis. These samples were compared with non-USC PDAC tumors (N = 5,562). Retrospective analysis of DNA and RNA next-generation sequencing data was performed.

**RESULTS** USC PDACs were more frequently PD-L1+ by immunohistochemistry than non-USC PDAC (63% *v* 16%, respectively, $P < .001$). Furthermore, USC PDAC had an increase in neutrophils (8.99% *v* 5.55%, $P = .005$) and dendritic cells (1.08% *v* 0.00%, $q = 0.022$) and an increased expression of *PDCD1LG2* (4.6% *v* 1.3%, $q = 0.001$), *PDCD1* (2.0% *v* 0.8%, $q = 0.060$), and *HAVCR2* (45.9% *v* 21.7%, $q = 0.107$) than non-USC PDAC. Similar to non-USC PDAC, *KRAS* was the most commonly mutated gene (86% *v* 90%, respectively, $P = 1$).

**CONCLUSION** To our knowledge, this work represents the largest molecular analysis of USC tumors to date and showed an increased expression of immune checkpoint genes in USC tumors. These findings provide evidence for further investigation into immune checkpoint inhibitors in USC tumors.

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## INTRODUCTION

As one of the deadliest cancers, pancreatic ductal adenocarcinoma (PDAC) is notorious for its characteristics of early invasion and metastasis.[1] Undifferentiated sarcomatoid carcinoma (USC) is a particularly aggressive but rare subtype, comprising 2%-3% of all PDACs.[2,3] Histologically, USC is notable for poor differentiation, including lack of glandular differentiation and the presence of mesenchymal-like, spindle-shaped cells.[2] Because of the rarity of this subtype, the genomic landscape and tumor microenvironment (TME) have been poorly characterized as most information about USC is from individual case reports and small cohort studies.[4,5]

Previous case reports of USC have suggested that immune checkpoint inhibitors may be effective.[4] In comparison, immunotherapy approaches have largely fallen short in the treatment of PDAC.[6] Furthermore, a previous cohort study of six patients with USC found a positive correlation between PD-L1 and neurogenic locus notch homolog protein 1 (Notch) gene expression.[5] In other cancer types, there have been reported correlations between Notch expression and predictive clinical response to the immune checkpoint blockade, suggesting a potential avenue for exploration of targeting USC using this type of immunotherapy.[7,8]

In this work, we retrospectively reviewed genomic profiles of the largest data set of USC patient samples to date (n = 20). First, the prevalence of common genetic mutations found in non-USC PDAC tumors was compared with their prevalence in USC. Next, tumor-associated features of USC including

## CONTEXT

**Key Objective**

Because of its rarity, the highly aggressive undifferentiated sarcomatoid carcinoma (USC) subtype of pancreatic ductal adenocarcinoma (PDAC) remains poorly characterized, and thus, therapies targeting this subtype have been underexplored. Using comprehensive genomic profiling of the largest number of USC PDACs to date, we sought to identify biomarkers affiliated with particular targeted therapies in this subtype.

**Knowledge Generated**

USC PDACs were more frequently PD-L1–positive compared with non-USC PDAC and had increased expression of various genes associated with immunosuppression. The prevalence of common drivers of PDAC including *KRAS* and *TP53* were not significantly different between USC and non-USC PDAC.

**Relevance**

To our knowledge, this is the largest molecular analysis of the rare USC subtype of PDAC to date. Our data suggest that the use of immune checkpoint inhibitors should be further explored in USC PDAC.

PD-L1 expression, mismatched repair enzyme status, microsatellite instability, and tumor mutational burden were investigated. Finally, the immunologic landscape of the USC TME was investigated to better understand the relative prevalence of immune cell populations and expression of immune-related genes compared with non-USC PDAC tumors.

## METHODS

### Cohort

A total of 20 USC and 5,562 non-USC PDAC tumors were submitted to Caris Life Sciences (Phoenix, AZ). This study was conducted in accordance with guidelines of the Declaration of Helsinki, Belmont report, and US Common rule. In line with 45 CFR 46.101(b)(4), this study was performed using retrospective, deidentified clinical data. Therefore, this study is considered institutional review board exempt.

### Pathology Review

Tumors were resected or biopsied at multiple institutions between 2016 and 2022. Histopathologic evaluation of scanned H&E-stained tumor sections was performed by a board-certified gastrointestinal pathologist (K.A.) using the HALO AP platform (Indica Labs, Albuquerque, NM). All cases were reviewed to ensure that the histology of the tumors included were consistent with USC criteria. The tumors that morphologically did not meet the diagnostic criteria were excluded. Specifically undifferentiated tumors with predominant osteoclast-like giant cell morphology and solid poorly differentiated adenocarcinomas with overlapping features were excluded. Several cases of USC also had a minor anaplastic component; these cases were included in the study.

### DNA Next-Generation Sequencing

Tumors were microdissected with a scalpel to enrich for the tumor fraction. A targeted 592-gene panel or whole-exome sequencing (WES) was performed using genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples. The 592-gene panel was sequenced using the NextSeq platform (Illumina, Inc, San Diego, CA). A custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, CA). Next-generation sequencing (NGS) detects variants with >99% confidence on the basis of allele frequency and amplicon coverage, with an average sequencing depth of coverage of >500 and an analytic sensitivity of 5%.

WES was performed using the Illumina NovaSeq 6000 sequencers (Illumina, Inc). A hybrid pull-down panel of baits designed to enrich for more than 700 clinically relevant genes at high coverage and high read depth was used, along with another panel designed to enrich for an additional >20,000 genes at lower depth. The performance of the WES assay was validated for sequencing variants, copy number alteration, tumor mutational burden, and microsatellite instability. The test was validated to 50 ng of input and has a positive predictive value of 0.99 against a previously validated NGS assay. WES can detect variants with tumor nuclei as low as 20% and will detect variants down to 5% variant frequency with an average depth of at least 500×. Matched normal tissue was not sequenced.

### Identification of Genetic Variants

Genetic variants identified were interpreted by board-certified molecular geneticists and categorized as pathogenic, likely pathogenic, variant of unknown significance, likely benign, or benign, according to the American College of Medical Genetics and Genomics standards. When

 

assessing mutation frequencies of individual genes, pathogenic and likely pathogenic were counted as mutations, whereas benign and likely benign variants and variants of unknown significance were excluded. TP53 mutants that have annotation in the literature as gain-of-function (GOF) mutations were annotated as such.[9]

### TME Immunologic Cell Fractions

Immune cell fractions of the TME were estimated by RNA deconvolution analysis using quanTIseq.[10] QuanTIseq is an immune deconvolution algorithm that uses RNA transcripts that are known to be expressed in specific immune cell types to deconvolute bulk RNA sequencing data and predict the different immune cell fractions that were present in the bulk RNA lysate.

### Tumor Mutational Burden

Tumor mutational burden (TMB) was measured by counting all nonsynonymous missense, nonsense, in-frame insertion/deletion, and frameshift mutations found per tumor that had not been previously described as germline alterations in dbSNP151 and Genome Aggregation Database databases, or benign variants were identified. A cutoff point of ≥10 mutations per MB was used on the basis of the KEYNOTE-158 trial, which showed that patients with a TMB of ≥10 mt/MB across several tumor types had higher response rates to pembrolizumab than patients with a TMB of <10 mt/MB.[1] Caris Life Sciences is a participant in the Friends of Cancer Research TMB Harmonization Project.

### Microsatellite Instability/Mismatch Repair Deficiency Status

A combination of multiple test platforms was used to determine microsatellite instability/mismatch repair deficiency (MSI-H/dMMR) status of the tumors profiled, including fragment analysis (FA, Promega, Madison, WI), immunohistochemistry (IHC; MLH1, M1 antibody; MSH2, G2191129 antibody; MSH6, 44 antibody; and PMS2, EPR3947 antibody [Ventana Medical Systems, Inc, Tucson, AZ]), and NGS (for tumors tested with NextSeq platform, 7,000 target microsatellite loci were examined and compared with the reference genome hg19 from the University of California). The three platforms generated highly concordant results as previously reported, and in the rare cases of discordant results, the MSI-H or MMR status of the tumor was determined in the order of IHC, FA, and NGS.

### IHC

IHC was performed on full FFPE sections of glass slides. Slides were stained using automated staining techniques, per the manufacturer's instructions, and were optimized and validated per Clinical Laboratory Improvement Amendments/cyclophosphamide, doxorubicin, and cisplatin and ISO requirements. Staining was scored for intensity (0 = no staining; 1+ = weak staining; 2+ = moderate staining; 3+ = strong staining) and staining percentage (0%-100%). PD-L1 (SP142)–positive (+) staining was defined as ≥2+ and ≥5%.

### Data and Statistical Analyses

Descriptive analyses were conducted using Mann-Whitney *U* (SciPy V.1.9.3) and $X^2$/Fisher's exact tests (R v.3.6.1) for continuous and categorical variables. *P* values were adjusted for multiple comparisons, with $q < 0.05$ considered significant when appropriate.

## RESULTS

From a cohort of 5,582 patients, 20 (0.36%) were classified as USC. The median age at diagnosis was similar in the USC and the non-USC PDAC cohorts; however, the USC cohort had a slightly higher prevalence of male patients (Table 1).

Of the 20 USC tumors analyzed, 16 had whole transcriptome data available and 19 had the 592-gene panel or whole-exome data available. There were significantly more tumors derived from the primary site as compared with the metastatic site in the USC cohort. The USC tumors showed predominantly sarcomatoid and anaplastic components (Fig 1). In a small subset of USC cases, a separate conventional ductal carcinoma component was also identified alongside the USC histology.

The three most commonly altered genes among USC tumors were *TP53* (95% [18 of 19] USC *v* 77% [3,917 of 5,071] non-USC, $q = 1$, $P = .07$), *KRAS* (84% [16 of 19] *v* 90% [4,545 of 5,052] non-USC, $q = 1$, $P = .41$), and *CDKN2A* (21% [4 of 19] USC *v* 23% [1,178 of 5,022] non-USC, $q = 1$, $P = .80$; Fig 2). *KRAS* variants among USC tumors included G12D (56% [9 of 16]), G12V (31% [5 of 16]), and G12R (13% [2 of 16]); no G12C variants were detected. No significant differences in the prevalence of *TP53* GOF mutants were observed between USC (16.7%, 3 of 18) and non-USC tumors (24.6%, 963 of 3,917).

Among the non-USC tumors, *KRAS* mutations included G12D (43% [1,944 of 4,545]), G12V (31% [1,430 of 4,545]), G12R (15% [680 of 4,545]), and G12C (1.7% [77 of 4,545]). Also noted were significant minor differences in gene

**TABLE 1.** Demographic Data on USC PDAC and Non-USC Tumors

| Cohort Information | USC | Non-USC | *P* |
|---|---|---|---|
| Count | 20 | 5,562 | NA |
| Median age, years (range) | 71.5 (40-80) | 68 (13-89) | .9887 |
| Sex (male), % (n/N) | 65 (13/20) | 53 (2,941/5,562) | .053 |
| Primary, % (n/N) | 80 (16/20) | 43 (2,330/5,454) | < .001 |

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; USC, undifferentiated sarcomatoid carcinoma.

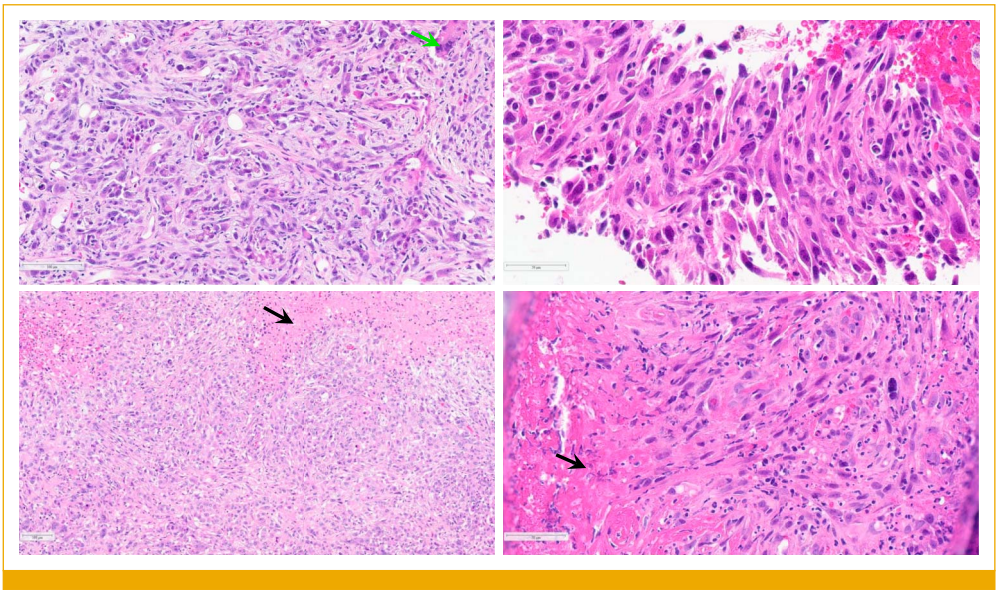

**FIG 1.** Representative images of four different cases of undifferentiated pancreatic carcinoma with the predominant sarcomatoid component characterized by irregular bundles of spindle shaped tumor cells with marked nuclear pleomorphism. Occasional bizarre tumor giant cells (green arrow) and tumor necrosis (black arrows) were identified. H&E stain, magnification scale bar included with each image.

alterations including a higher prevalence in *MSH6* (11% [2 of 19] USC *v* 0.65% [33 of 5,087] non-USC, $q = 0.00077$), *MLH3* (8% [1 of 13] USC *v* 0.27% [9 of 3,381] non-USC, $q = 0.00333$), *CTCF* (5.3% [1 of 19] USC *v* 0.04% [2 of 5,109] non-USC, $q < 0.001$), *MET* (5.3% [1 of 19] USC *v* 0.02% [1 of 5,121] non-USC, $q < 0.001$), *NF2* (5.3% [1 of 19] USC *v* 0.20% [10 of 5,108] non-USC, $q = 0.008$), *TCF7L2* mutations (5.3% [1 of 19] USC *v* 0.0% [0 of 5,108] non-USC, $q < 0.001$), and *RAF1* gene fusion (6% [1 of 16] USC *v* 0.18% [10 of 5,558] non-USC, $q = 0.00019$).

The prevalence of immune cell infiltrate in USC tumors was examined via RNA deconvolution (Fig 3). Compared with non-USC tumors, USC samples had a significant decrease in B cells (3.40% *v* 4.36% mean percent infiltrate, $q = 0.024$) and a significant increase in neutrophils (8.99% *v* 5.55% mean percent infiltrate, $q = 0.005$; Fig 3B). In addition, there was an increased prevalence of M1 and M2 macrophages in USC tumors (not significant, $q > 0.05$).

Investigating the prevalence of biomarkers of response to immunotherapy, a significantly higher percentage of USC tumors were PD-L1+ by IHC (59% [10 of 17] *v* 16% [810 of 5,224], $q < 0.001$). There was a low prevalence of dMMR/MSI-High (5.3% [1 of 19] USC *v* 1.1% [59 of 5,444] non-USC, $q = 0.08$) and TMB-High tumors (5.3% [1 of 19] *v* 2.0% [84 of 4,139], $q = 0.32$; Fig 4A). Quantitatively stated, these data are as follows: Median MSI: USC = 77, non-USC = 76; $P = .307$, unit: number of microsatellite loci; Median TMB: 4 versus four $P = .932$, unit: mutations per Mb. Among USC tumors, PD-L1 expression was not associated with differences in *NOTCH1*, *NOTCH2*, or *NOTCH3* expression (Figs 4B-4D).[5]

Finally, we hypothesized that the transcriptional landscape of immune-related genes would be different between USC and non-USC tumors. There was no difference in the expression of immunoglobulins between USC and non-USC tumors (Fig 4E, Table 2). The same was true for human leukocyte antigen genes (Fig 4F, Table 2). Finally, the expression of emerging immuno-oncologic targets was examined (Fig 4G, Table 2). Most of these targets showed no significant differences between USC and non-USC tumors, except for the increased mean expression of *PDCD1LG2* (PD-L2; 4.6 *v* 1.3 transcripts per million [TPM], $q = 0.001$). Other targets that did not reach statistical significance include *PDCD1* (PD-1; 2.0 *v* 0.8 TPM, $q = 0.060$) and *HAVCR2* (45.9 *v* 21.7 TPM, $q = 0.107$).

## DISCUSSION

In this article, to our knowledge, we describe the largest molecular analysis of USC tumors to date. The most commonly mutated genes in USC tumors were *KRAS*, *TP53*, and *CDKN2A*, which are the same driver mutations observed in non-USC pancreatic cancers.[11] Furthermore, the top three most common *KRAS* mutations were the same between USC and non-USC. The frequency of G12C mutations was very low (<2%) for both tumor types. The other common driver gene of conventional pancreatic cancer, *SMAD4*, was mutated in two of 19 cases in this USC series, and this finding is in line with a recent molecular investigation on the same entity.[2] Beyond driver mutations, significant differences were observed in USC tumors, including an increased prevalence of mutations in tumor suppressor genes *MSH6* and *MLH3* and an increased prevalence of fusions in *RAF1*, a kinase involved in the MAPK oncogenic signaling pathway.[12-14] However, the

 

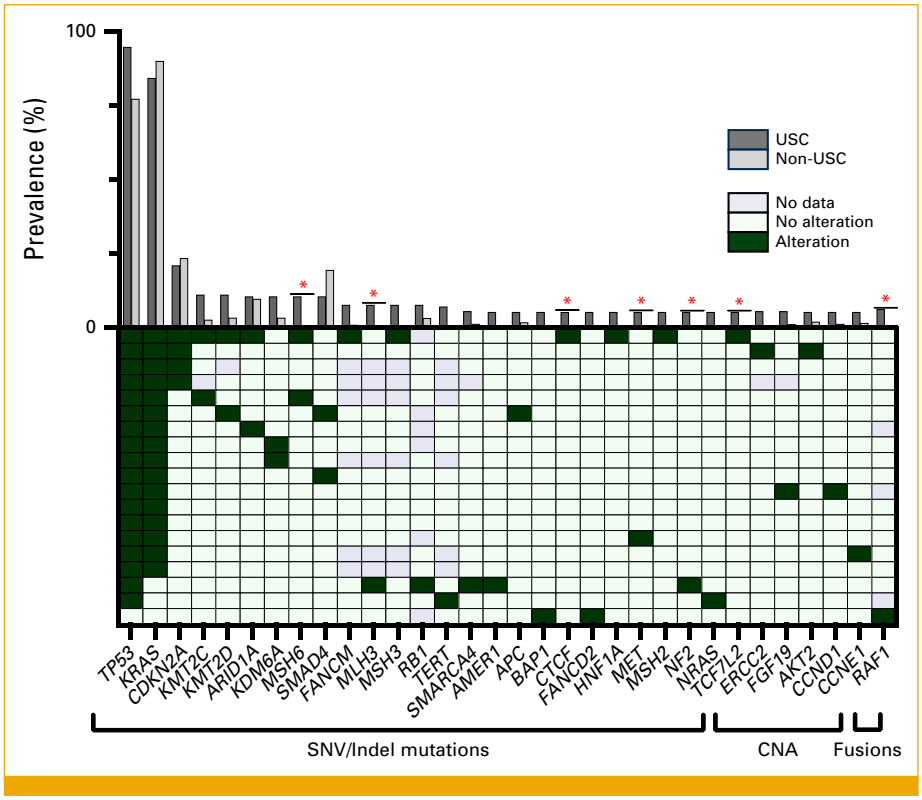


**FIG 2.** Genomic landscape of PDAC with USC and non-USC subtype mutations via next-generation sequencing (NGS; SNV/indel mutations), copy number amplification (CNA), and fusions (red asterisk denotes significance $q < 0.05$, chi-squared). Each row of oncoprint represents one USC tumor. PDAC, pancreatic ductal adenocarcinoma; SNV, single-nucleotide variant; USC, undifferentiated sarcomatoid carcinoma.

clinical significance of these differences is unclear as the genomic landscape of non-USC is relatively sparse with most detectable mutations occurring at a prevalence of <2%. In addition, a low prevalence of dMMR/MSI-High and TMB-High tumors was observed in both non-USC and USC, further bringing into question the pathogenic nature of the *MSH6* and *MLH3* mutations observed as only one patient with USC had dMMR.[8] These results suggest that although there are

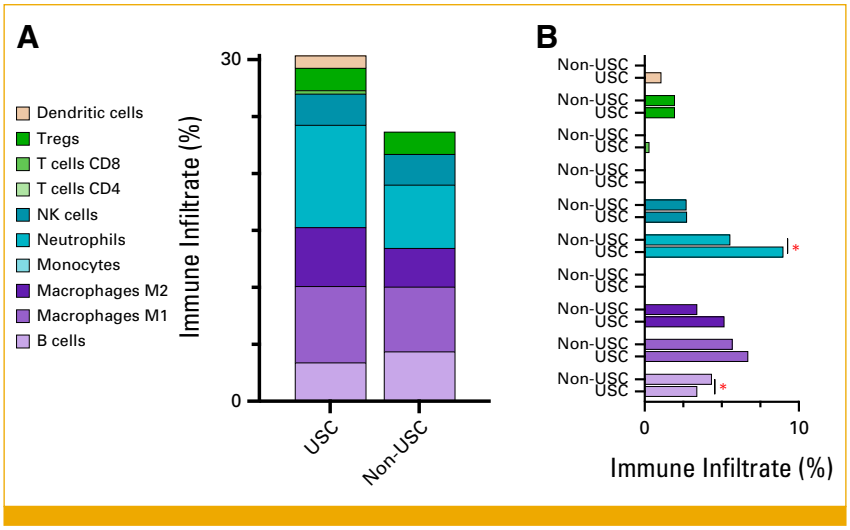


**FIG 3.** (A) Immune cell populations in USC and non-USC cases. (B) Difference in % immune cell infiltrate between USC versus non-USC cases ($q < 0.05$, Mann-Whitney *U*). USC, undifferentiated sarcomatoid carcinoma.

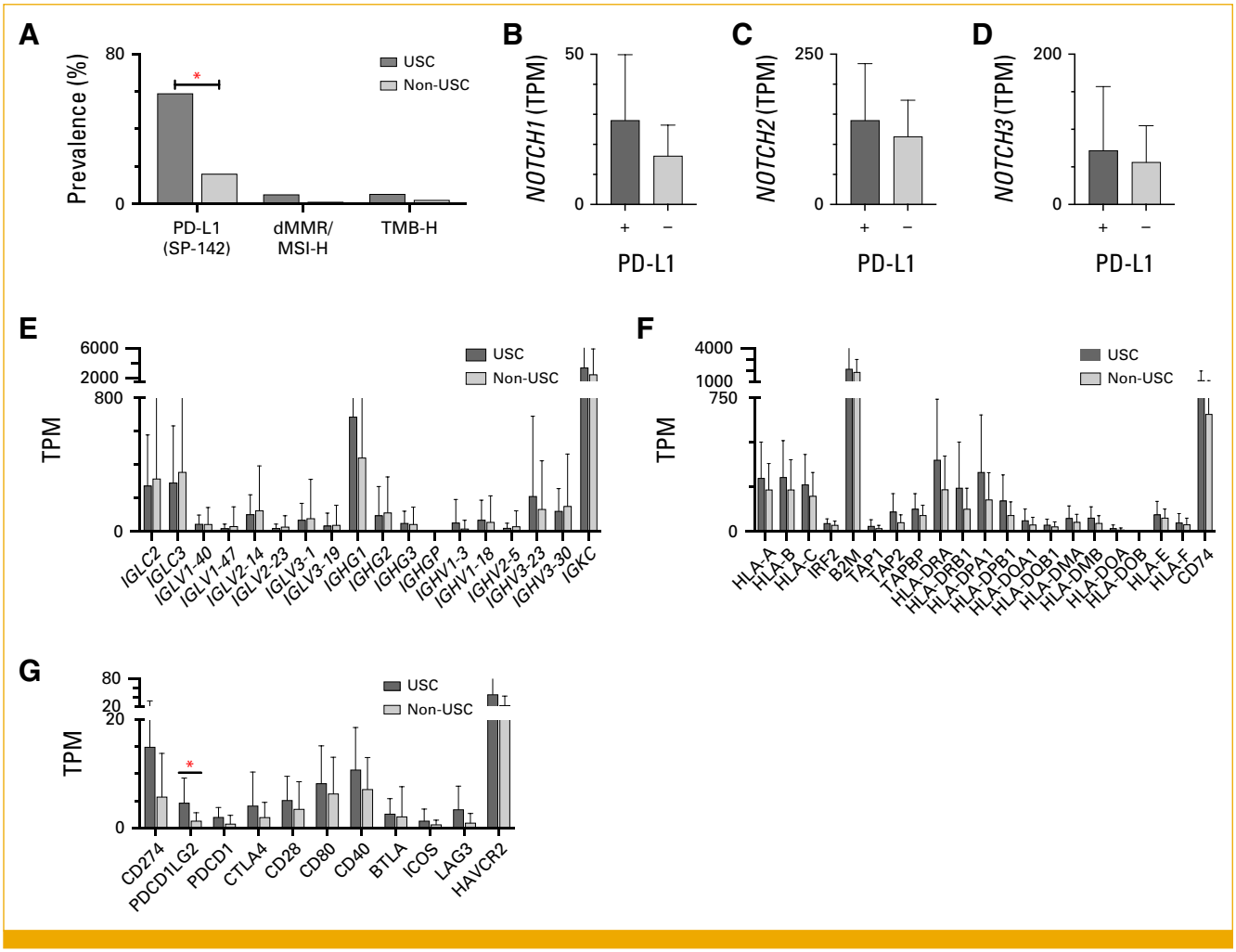


**FIG 4.** (A) Prevalence of biomarkers: PD-L1+ (63% *v* 16%, $q < 0.001$), dMMR/MSI (5% USC *v* 1% non-USC, $P = .08$, $q = 1$), or tumor mutational burden-high (≥10 mut/megabase, 5% *v* 2%, $P = .89$, $q = 0.32$). Expression of (B) *NOTCH1*, (C) *NOTCH2*, and (D) *NOTCH3* for PD-L1+ and PD-L1– tumors, TPM. (E-G) Gene expression of immune regulatory-related genes ($q < 0.05$, Mann-Whitney *U*). dMMR/MSI, Mismatch Repair Deficiency/ Microsatellite Instability; TPM, transcripts per million; USC, undifferentiated sarcomatoid carcinoma.

slight differences in the genomic landscape between USC and non-USC, there is not one set of mutations that defines the non-USC subtype on the basis of our current understanding of cancer-driving mutations.

While other solid tumor types like melanoma and non–small cell lung cancer have benefited from advances in immunologic targeting agents, multiple small trials of immune-targeting therapies have not shown benefit against the majority of PDAC tumors.[15] Thus, there remains interest in finding a subset of patients that may respond to these types of treatments. We find that despite similar genetic mutations in USC tumors compared with other non-USC, the significantly increased prevalence of PD-L1+ IHC and *PDCD1LG2* gene expression raises interest into the exploration of immune-targeting agents in this rare but aggressive PDAC subtype. Furthermore, *PDCD1* expression was increased in USC tumors, suggesting that these programmed death receptor immunoregulatory axes could be therapeutic targets for this particularly aggressive PDAC subtype. Finally, the previously described association between PD-L1 and Notch expression in a previous study of patients with USC (n = 6) was not seen in our analysis of this larger cohort (n = 20).[5] However, the range of expression in our analysis was large, making sampling error a possible reason for this discrepancy. The TME of USC had fewer B cells but increased neutrophils and M2 macrophages compared with non-USC PDAC, suggesting that USC tumors might have a more immunosuppressive TME compared with other subtypes of PDAC.[16] However, there were also more immunostimulatory M1 macrophages, and the functions of these immune cells within this particular subtype require further exploration.

In this study, a lower prevalence of USC histology (0.36% [20 of 5,582]) was reported compared with the previously observed 2%-3% prevalence rate.[2,3] However, other reports have indicated this subtype has a prevalence of <1%.[17]

 

**TABLE 2.** Transcriptional Landscape of Immune-Related Genes Would Be Different Between USC and Non-USC Tumors

| | USC | | | Non-USC | | | | |
|---|---|---|---|---|---|---|---|---|
| Gene | Mean | SD | No. | Mean | SD | No. | *P* | *q* |
| Immune targets | | | | | | | | |
| CD274 | 14.9 | 16.8 | 16 | 5.7 | 8 | 5,488 | .006 | 0.304 |
| PDCD1LG2 | 4.6 | 4.6 | 16 | 1.3 | 1.5 | 5,488 | 0 | 0.001 |
| PDCD1 | 2 | 1.8 | 16 | 0.8 | 1.6 | 5,488 | .001 | 0.06 |
| CTLA4 | 4.1 | 6.2 | 16 | 2 | 2.8 | 5,488 | .237 | 1 |
| CD28 | 5.1 | 4.4 | 16 | 3.5 | 5 | 5,488 | .038 | 1 |
| CD80 | 8.2 | 6.9 | 16 | 6.3 | 6.7 | 5,488 | .128 | 1 |
| CD40 | 10.7 | 7.8 | 16 | 7.1 | 5.8 | 5,488 | .04 | 1 |
| BTLA | 2.6 | 2.8 | 16 | 2.1 | 5.5 | 5,488 | .214 | 1 |
| ICOS | 1.3 | 2.2 | 16 | 0.6 | 0.8 | 5,488 | .545 | 1 |
| LAG3 | 3.4 | 4.3 | 16 | 0.9 | 1.7 | 5,488 | .019 | 0.929 |
| HAVCR2 | 45.9 | 36.1 | 16 | 21.7 | 20.8 | 5,488 | .002 | 0.107 |
| HLA | | | | | | | | |
| HLA-A | 299.8 | 202.9 | 16 | 235.1 | 148.1 | 5,488 | .259 | 1 |
| HLA-B | 305.1 | 205.4 | 16 | 235.6 | 169 | 5,488 | .128 | 1 |
| HLA-C | 263.5 | 169.6 | 16 | 200.1 | 131.8 | 5,488 | .125 | 1 |
| IRF2 | 46.9 | 25 | 16 | 37.3 | 22.7 | 5,488 | .105 | 1 |
| B2M | 2,158.7 | 2,300 | 16 | 1,873.8 | 1,135.7 | 5,488 | .934 | 1 |
| TAP1 | 31.5 | 36.6 | 16 | 19 | 16.6 | 5,488 | .502 | 1 |
| TAP2 | 111.7 | 102 | 16 | 52 | 43.6 | 5,488 | .002 | 0.101 |
| TAPBP | 127.2 | 86.1 | 16 | 92.4 | 56.8 | 5,488 | .133 | 1 |
| HLA-DRA | 401.8 | 341.1 | 16 | 237.1 | 186.9 | 5,488 | .066 | 1 |
| HLA-DRB1 | 245.8 | 257 | 16 | 128 | 115.5 | 5,488 | .042 | 1 |
| HLA-DPA1 | 334 | 320.4 | 16 | 180.3 | 150.4 | 5,488 | .053 | 1 |
| HLA-DPB1 | 174.1 | 144.7 | 16 | 91.8 | 76.3 | 5,488 | .016 | 0.818 |
| HLA-DQA1 | 62.8 | 64.3 | 16 | 38.9 | 42.8 | 5,488 | .09 | 1 |
| HLA-DQB1 | 38.8 | 31.3 | 16 | 28.9 | 26.9 | 5,488 | .166 | 1 |
| HLA-DMA | 75.2 | 69.5 | 16 | 53.8 | 44.3 | 5,488 | .127 | 1 |
| HLA-DMB | 78 | 62.4 | 16 | 48.3 | 41.9 | 5,488 | .036 | 1 |
| HLA-DOA | 18.2 | 20 | 16 | 10.7 | 10.9 | 5,488 | .081 | 1 |
| HLA-DOB | 2.1 | 2.7 | 16 | 2.2 | 3 | 5,488 | .519 | 1 |
| HLA-E | 96.3 | 74 | 16 | 77.6 | 50 | 5,488 | .61 | 1 |
| HLA-F | 50.2 | 52.5 | 16 | 40.1 | 36.2 | 5,488 | .952 | 1 |
| CD74 | 1,145.9 | 839.4 | 16 | 658.9 | 462.9 | 5,488 | .016 | 0.814 |
| Immunoglobulins | | | | | | | | |
| IGLC2 | 273.9 | 305 | 16 | 316.1 | 546.2 | 5,488 | .809 | 1 |
| IGLC3 | 292.6 | 340.6 | 16 | 356 | 565.1 | 5,488 | .671 | 1 |
| IGLV1-40 | 45.7 | 53.1 | 16 | 44.3 | 100.1 | 5,488 | .906 | 1 |
| IGLV1-47 | 18.6 | 25.7 | 16 | 31.5 | 116.9 | 5,488 | .527 | 1 |
| IGLV2-14 | 102.4 | 118.4 | 16 | 125.5 | 267.9 | 5,488 | .877 | 1 |
| IGLV2-23 | 20.1 | 24.2 | 16 | 28.9 | 66.2 | 5,488 | .677 | 1 |
| IGLV3-1 | 69.1 | 100.7 | 16 | 78.4 | 234.2 | 5,488 | .945 | 1 |
| IGLV3-19 | 34.7 | 76 | 16 | 39.4 | 118.8 | 5,488 | .456 | 1 |
| IGHG1 | 686.1 | 804.6 | 16 | 441.1 | 743.1 | 5,488 | .477 | 1 |
| IGHG2 | 96.2 | 173.8 | 16 | 113.3 | 213.8 | 5,488 | .242 | 1 |
| IGHG3 | 50.5 | 71.3 | 16 | 43.8 | 102.1 | 5,488 | .958 | 1 |
| IGHGP | 1.2 | 1.9 | 16 | 0.8 | 2 | 5,488 | .632 | 1 |
| IGHV1-3 | 52.5 | 139.6 | 16 | 17.3 | 50.4 | 5,488 | .391 | 1 |

(continued on following page)

**TABLE 2.** Transcriptional Landscape of Immune-Related Genes Would Be Different Between USC and Non-USC Tumors (continued)

| | USC | | | Non-USC | | | | |
|---|---|---|---|---|---|---|---|---|
| Gene | Mean | SD | No. | Mean | SD | No. | *P* | *q* |
| IGHV1-18 | 69.6 | 118.8 | 16 | 56.5 | 157.9 | 5,488 | .742 | 1 |
| IGHV2-5 | 20.3 | 29.5 | 16 | 30.8 | 93.9 | 5,488 | .813 | 1 |
| IGHV3-23 | 211.5 | 478.3 | 16 | 134.2 | 289.7 | 5,488 | .799 | 1 |
| IGHV3-30 | 122.7 | 134.5 | 16 | 151.4 | 313.3 | 5,488 | .734 | 1 |
| IGKC | 3,408.9 | 4,085.3 | 16 | 2,470.8 | 3,428.5 | 5,488 | .668 | 1 |

Abbreviations: SD, standard deviation; USC, undifferentiated sarcomatoid carcinoma.

The prevalence of USC in this study might be different from that previously reported because tumors that undergo NGS, like those in our study, tend to be recurrent or of an advanced stage. In addition, the rarity of the USC subtype makes its prevalence difficult to accurately determine although the number of cases analyzed in this study is greater than previous cohort studies examining the USC subtype with a sample size of 20.

Finally, the genomic analysis of this subtype requires microscopic separation of tumor versus surrounding stroma, which may be complicated for USC histology given the similar histology of the tumor itself versus the surrounding connective tissue. Despite these reservations, the methods used were rigorous and the gold standard for this type of analysis. The paucity of data surrounding the USC subtype makes this work provocative for further study and testing of the TME and immunologic landscape for patients with this aggressive PDAC subtype. Further investigation could include comparing outcomes for patients with USC tumors treated with and without immunotherapy and if immunotherapy-treated patients with USC had different outcomes compared with patients with non-USC PDAC.

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## DATA SHARING STATEMENT

The data used in this study are not publicly available but can be made available upon reasonable request.

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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