# Uncovering actionable genetic alterations and immune predictive biomarkers for anal squamous cell carcinomas in the era of immunotherapy: PD-L1 and Beyond

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## Background

Squamous cell carcinoma of the anal canal (SCAC) is a rare cancer with limited effective treatments. Immune checkpoint inhibitors (ICIs) use is routine for recurrent/refractory disease, but predictive biomarkers remain elusive. We analyzed the largest dataset of SCAC to date, focusing on PD-L1 expression.

## Methods

Next-generation sequencing of DNA (592 genes or WES) and RNA (WTS) was tested at Caris Life Sciences (Phoenix, AZ). PD-L1 was tested by immunohistochemistry (IHC) (SP142) and grouped as high (2+ and ≥5%), low (1-2 and 1-5%), and negative (0). dMMR/MSI-H was tested by IHC/NGS and tumor mutation burden (TMB)-High was defined as ≥10 mt/MB. RNA expression data was used to estimate the tumor microenvironment (TME) using QuantiSEQ. RNA signatures predictive of ICI response (interferon gamma [IFNγ]; T-cell inflamed signature [TIS]) were tested. $X^2$/Fisher-Exact was used with significance shown as *P*-value adjusted for multiple comparisons (Q < 0.05). Real-world overall survival (rwOS) was from insurance claims and calculated from tissue collection to last contact; time-on-treatment (TOT) was from start to finish of ICI.

## Results

Table 1: Patient demographics

| | High | Low | Negative | p-value High vs Low | p-value High vs Negative | p-value Low vs Negative |
|---|---|---|---|---|---|---|
| **Count (N)** | 518 | 280 | 444 | | | |
| **Median Age** | 64 | 62 | 63 | 0.011 | 0.124 | 0.269 |
| **Female (N)** | 71.2% (369) | 77.5% (217) | 70.7% (314) | 0.056 | 0.861 | 0.045 |
| **Male (N)** | 28.8% (149) | 22.5% (63) | 29.3% (130) | 0.056 | 0.861 | 0.045 |

Figure 1: PD-L1 distribution in anal squamous carcinoma

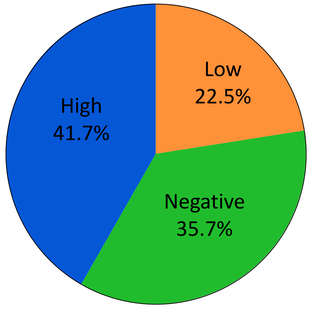



Figure 2: TMB-H was higher in PD-L1 low vs negative (p=0.036)

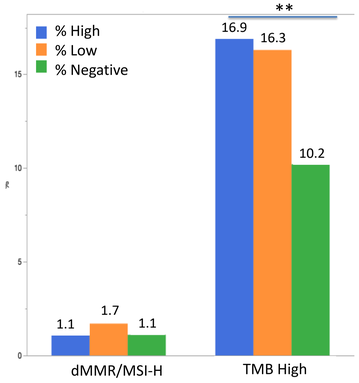



Table 2: Expression of IO markers decreased with PD-L1 expression (all p<0.001)

| Gene | Median (High) | Median (Low) | Median (Negative) |
|---|---|---|---|
| *PDCD1* | 0.66 | 0.50 | 0.48 |
| *IDO1* | 9.45 | 5.97 | 3.29 |
| *CD274* | 14.89 | 7.36 | 4.84 |
| *IFNG* | 0.98 | 0.55 | 0.38 |
| *HAVCR2* | 17.77 | 12.28 | 10.52 |
| *CTLA4* | 2.96 | 2.38 | 1.86 |
| *LAG3* | 1.21 | 0.83 | 0.82 |
| *CD80* | 6.55 | 4.90 | 3.94 |
| *CD86* | 9.31 | 7.05 | 5.89 |
| *PDCD1LG2* | 1.92 | 1.12 | 0.94 |

Table 3: Molecular alteration differences observed in PD-L1 High vs Negative (p<0.05)

| Test-Gene | % (High) | % (Negative) | p value |
|---|---|---|---|
| NGS-*PIK3CA* | 38.8 | 21.7 | **<0.001** |
| NGS-*CASP8* | 5.3 | 0.3 | **<0.001** |
| CNA-*FGFR3* | 0.0 | 2.2 | **0.002** |
| NGS-*JAK2* | 0.0 | 2.6 | **<0.001** |
| NGS-*NF1* | 2.4 | 0.4 | **0.049** |
| NGS-*BAP1* | 1.1 | 3.6 | **0.019** |
| NGS-*ARID1A* | 2.7 | 0.6 | **0.029** |

Figure 4 : RNA signatures show TIS and IFN scores decreased with PD-L1 (p<0.001)

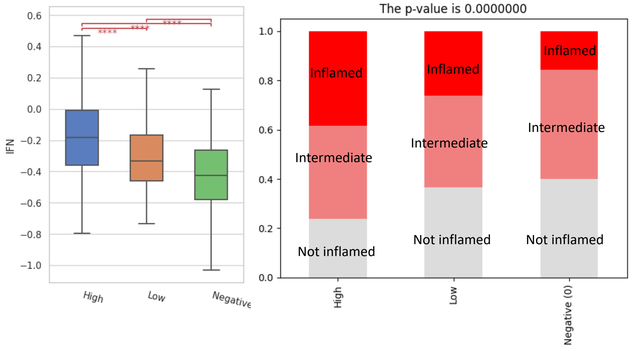



Figure 3: For PD-L1 high vs negative, infiltration of Tregs, M1 macrophages, neutrophils, CD8+ cells, and cancer-associated fibroblasts decreased with PD-L1 expression (p<0.001).

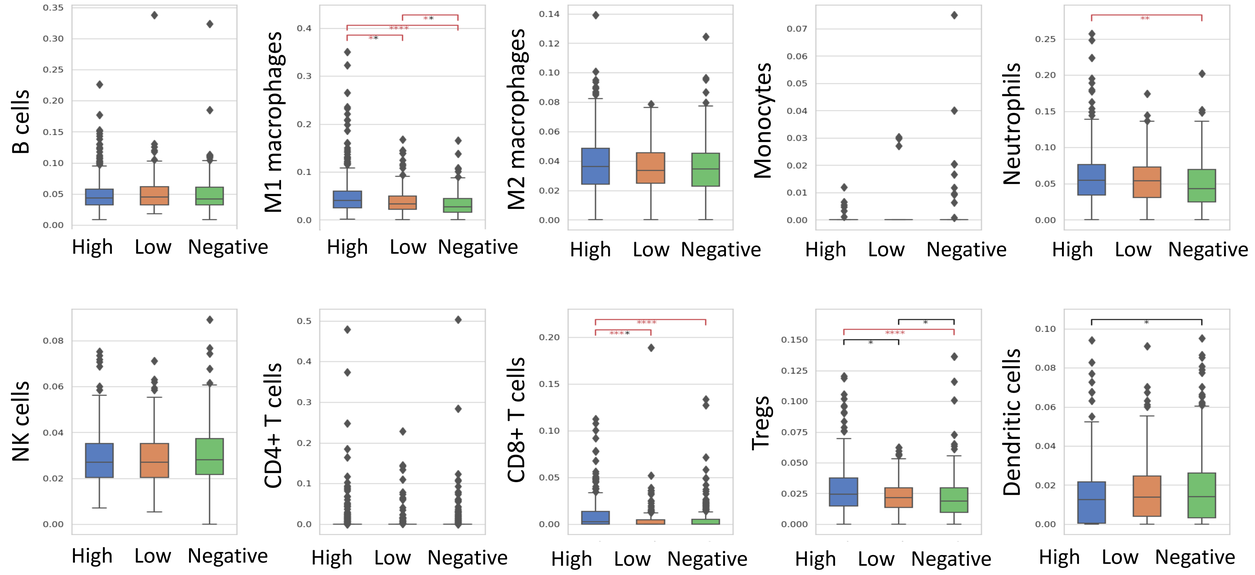
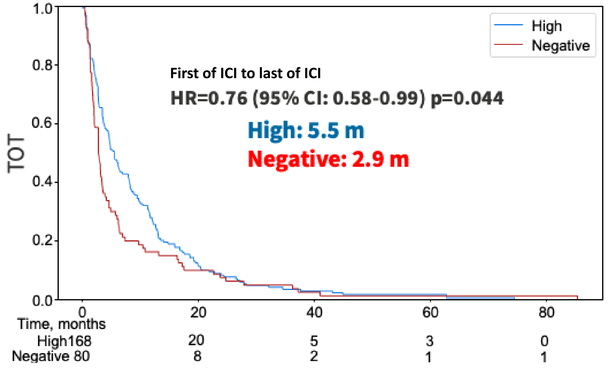



Figure 5: In 316 SCAC patients treated with ICIs, PD-L1 high had longer TOT compared to PD-L1 negative




## Conclusions

PD-L1 is expressed in over 50% of SCACs, while dMMR/MSI-H is rare. High PD-L1-expressing tumors have higher *PIK3CA*and *CASP8* mutations and overexpress IO markers, are inflamed, and have prolonged treatment times with ICIs. This is the largest study to date reporting SCAC genomic profiles and identifies the utility of PD-L1 as a predictive biomarker of IO efficacy. This discovery needs to be confirmed in prospective trials.

ASCO June 2024