Transcriptomic immune profiling: A precision path forward for immunotherapy in patients with cervical cancer?

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Background

Immunotherapy has emerged as a promising intervention in metastatic or recurrent cervical cancer, but response rates have been modest, albeit with some durable responses. To date, immune profiling and pathway characterization via whole transcriptome sequencing (WTS) has been limited to sampled tumors from newly diagnosed, mostly early-stage disease. In the current study, we sought to use WTS-based immune profiling to develop a more representative analysis of the cervical cancer population receiving immunotherapy.

Methods

Cervical cancer tumor samples were analyzed (Caris Life Sciences, Phoenix, AZ) using:

- Next-generation sequencing (NGS: NextSeq, 592 Genes and NovaSEQ, WES)
- Immunohistochemistry (IHC)
- Whole Transcriptome Sequencing (WTS: NovaSeq)

PD-L1 expression was tested by IHC using standard protocol (positive: CPS ≥1).

Microsatellite instability (MSI) was tested by fragment analysis, IHC and NGS.

Tumor mutational burden (TMB) was measured by counting all somatic mutations found per tumor (TMB-high: ≥ 10 mutations per MB).

Immune cell infiltration was calculated by QuantiSeq.

TP53 mutations were used as a proxy indicator for non-HPV tumors.

Statistical significance was determined using chi-square and Wilcoxon rank sum test and adjusted for multiple comparisons using Benjamini & Hochberg and Bonferroni, respectively (significance threshold: adjusted p-value < 0.01).

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Results

930 patients with cervical cancer underwent molecular profiling. Median age was 52 years, and 449 (48.3%) patients had metastatic disease.

Table 1. Immune-related markers in cervical cancer by age and histology. (*adjusted p-value<0.05)

Marker		Age (% or median TPM)		adjusted P-value	Histology (% or median TPM)		adjusted P-value
		19-62	63-90		Adenocarcinoma	Squamous	
IO Therapy Biomarkers (%)	*PD-L1 (22c3)*	62.70%	82.20%	0.820	70.30%	89.10%	<0.001*
	TMB	18.10%	16.10%	0.820	8.50%	21.50%	<0.001*
	dMMR/MSI-H	3.90%	3.90%	0.991	3.40%	2.90%	0.709
Immune Cell Infiltration (%)	*B Cells*	4.24%	4.46%	0.579	4.41%	4.14%	1
	M1 Macrophages	3.33%	3.08%	0.172	3.14%	3.31%	1
	M2 Macrophages	3.20%	3.32%	1	3.60%	3.05%	<0.001*
	Monocytes	0.00%	0.00%	1	0.00%	0.00%	1
	Neutrophils	5.59%	4.42%	0.025*	3.57%	6.07%	<0.001*
	NK Cells	2.64%	2.66%	1	3.15%	2.46%	<0.001*
	CD4+ T Cells	0.00%	0.00%	1	0.00%	0.00%	1
	CD8+ T Cells	0.59%	0.51%	1	0.31%	0.70%	<0.001*
	Regulatory T Cells	2.24%	2.18%	1	1.99%	2.33%	<0.001*
	Dendritic Cells	1.11%	1.50%	<0.001*	1.33%	1.12%	0.275
Immune Checkpoint Genes (median TPM, normalized to median 19-62 or adenocarcinoma TPM)	*CD274 (PD-L1)*	1	1.084	1	1	2.179	<0.001*
	PDCD1LG2 (PD-L2)	1	0.995	1	1	1.447	<0.001*
	IFNG	1	1.078	1	1	1.634	<0.001*
	IDO1	1	1.126	0.850	1	1.872	<0.001*
	LAG3	1	1.069	0.405	1	1.305	<0.001*
	CTLA4	1	0.921	1	1	1.641	<0.001*
	HAVCR2	1	0.959	1	1	1.210	<0.001*
	PDCD1	1	0.985	1	1	1.581	0.002*

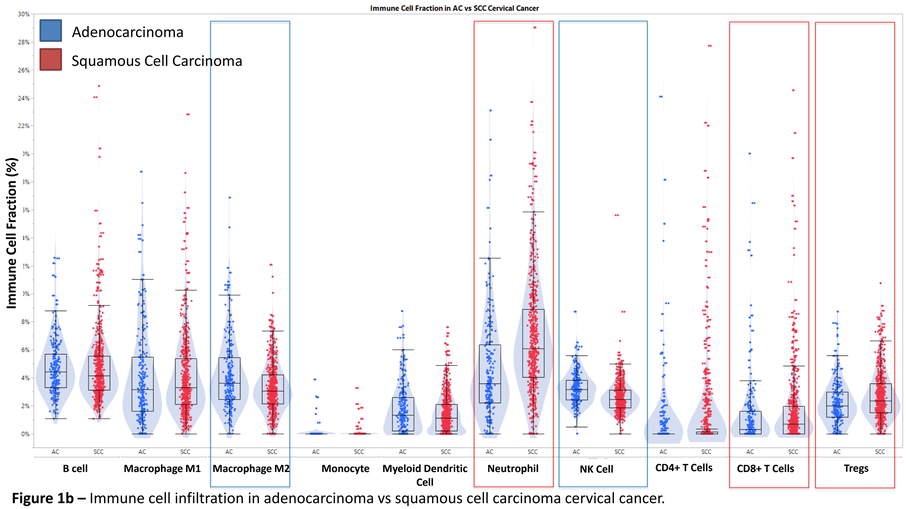

Figure 1b – Immune cell infiltration in adenocarcinoma vs squamous cell carcinoma cervical cancer.

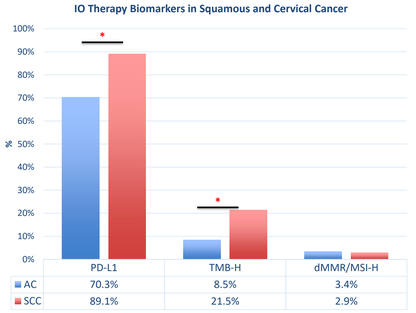

Figure 1a – IO Therapy related biomarkers—PD-L1, TMB and dMMR/MSI-H—in adenocarcinoma and squamous cell carcinoma cervical cancer.

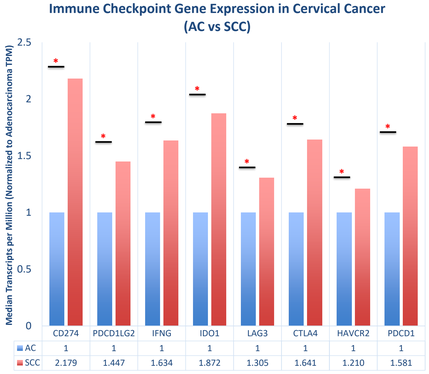

Figure 1c – Immune checkpoint (IC) gene expression in adenocarcinoma (AC) vs squamous cell carcinoma (SCC) cervical cancer. IC gene expression was normalized to median IC gene expression in adenocarcinoma tumors. All adjusted p-values <0.01.

Study Highlights

Compared to adenocarcinoma, squamous cell carcinoma (SCC) had a more robust immune signal, with increased

- PD-L1 positivity (89.1% vs 70.3%; adjusted p<0.001)
- TMB-high status (21.5% vs 8.5%; adjusted p<0.001)
- Infiltration of multiple types of immune cells (neutrophils, CD8+ T cells, regulatory T cells)
- Increased expression of immune checkpoint genes

PD-L1+ status was significantly associated with increased

- Macrophage M1 (3.51% vs. 2.04%)
- NK (2.6% vs. 3.2%)
- CD8+ T (0.7% vs. 0%)
- Regulatory T (2.4% vs. 1.3%) cell infiltration

TMB-high was associated with significantly increased infiltration of neutrophils and CD8+ T cells

Older (≥63 yrs) patients had significantly more

- Somatic *TP53* mutations (25.2% vs. 10.1%, indicating more non-HPV tumors with increasing age)
- Dendritic cell infiltration compared to younger patients

Conclusions

Cervical SCC had a higher immune signal than adenocarcinoma: increased immunotherapy biomarkers (PD-L1 and TMB), immune cell infiltration, and upregulation of immune checkpoint genes. Non-HPV status and dendritic cell infiltration increased with advanced age. **The variety of signals noted in this analysis suggests that WTS immune profiling should be further investigated in the push to better predict which cervical cancer patients might benefit most from immuno-therapy.**