Sensitivity and concordance of *CD274* (PD-L1) expression by RNA sequencing (RNA-seq) in comparison with three PD-L1 immunohistochemistry methods in head and neck squamous cell carcinoma (HNSCC)

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Introduction

PD-L1 expression by immunohistochemistry (IHC) is associated with HNSCC immunotherapy response [1]. The performance of different PD-L1 IHC clones however, has shown variability and poor concordance for immune vs. tumor cell scoring in HNSCC. Crucially, this leads to poor reproducibility in the combined positive score (CPS) method by the PD-L1 IHC 22C3 companion diagnostic [2]. Alternatively, PD-L1 by mRNA next generation sequencing is objective and assesses both tumor and inflammatory background cells in the tumor microenvironment, potentiating a more robust assay than PD-L1 immunohistochemistry Here, we explored the clinical sensitivity and concordance of *CD274* (PD-L1) expression by RNA-sequencing compared to three PD-L1 IHC methods.

Methods

- A retrospective cohort of HNSCC patient FFPE tumor specimens (n=258) was tested by comprehensive immune profiling (2017-2022), including both PD-L1 by IHC and RNA-seq (*CD274*)[3]. Testing was performed in a CAP and CLIA certified lab as part of standard care.
- IHC was performed by 28-8 or 22C3 PD-L1 antibody. The 28-8 assay was scored based on % tumor cells stained (TC, n=34), while the 22C3 assay was scored based either on tumor proportion score (TPS, n=61) or combined positive score (CPS, n=163); the FDA-approved companion diagnostic method for frontline pembrolizumab immunotherapy in HNSCC.
- Receiver operator characteristics (ROC) models for each PD-L1 IHC method were constructed for 5 sets of patients with different pairwise interpretation groups, and used to determine RNA-seq cutoffs and assess the clinical sensitivity of PD-L1 (*CD274*) by RNA-seq.
- Concordance between standard PD-L1 IHC assay and scoring methods vs. *CD274* by RNA-seq was assessed.

Table 1. HNSCC patient characteristics by PD-L1 IHC antibody and scoring method

		IHC 22C3 CPS ≥20 (High) 1-19 (Low) <1 (Negative) (n=163)	IHC 22C3 TPS ≥50 (High) 1-49 (Low) <1 (Negative) (n= 61)	IHC 28-8 TC ≥50 (High) 1-49 (Low) <1 (Negative) (n=34)	Total (n=258)
Age	Average	66	64	66	65
Sex	Female	36 (21.5)	10 (16.4)	5 (14.7)	50 (19.4)
	Male	128 (78.5)	51 (83.6)	29 (85.3)	208 (80.6)
PD-L1 IHC interpretation	High	77 (47.2)	15 (24.6)	9 (26.5)	101 (39.1)
	Low	79 (48.5)	28 (45.9)	19 (55.9)	126 (48.8)
	Negative	7 (04.2)	18 (29.5)	6 (17.6)	31 (12.0)
Tissue Site	Distant Metastasis	26 (16.0)	11 (18.0)	6 (17.6)	43 (16.7)
	Larynx	17 (10.4)	0 (00.0)	2 (05.9)	19 (07.4)
	Lymph Node	29 (17.8)	12 (19.7)	7 (20.6)	48 (18.6)
	Oral Cavity	29 (17.8)	4 (06.6)	5 (14.7)	38 (14.7)
	Other Local	8 (04.9)	3 (04.9)	4 (11.8)	15 (05.8)
	Paranasal sinuses	1 (00.6)	2 (03.3)	1 (02.9)	4 (01.6)
	Pharynx	27 (16.6)	17 (27.9)	3 (08.8)	47 (18.2)
	Salivary gland	4 (02.5)	6 (09.8)	0 (00.0)	10 (03.9)
	Soft tissue	22 (13.5)	6 (09.8)	6 (17.6)	34 (13.2)
Tissue status	Primary	73 (49.1)	27 (44.3)	19 (55.9)	119 (49.1)
	Metastatic	80 (49.1)	33 (54.1)	15 (44.1)	128 (49.6)
	Missing	10 (06.1)	1 (01.6)	0 (00.0)	9 (03.4)
Tumor cell %	Average	56	59	75	59

REFERENCES

Validation of a Next-Generation Sequencing Assay to Monitor Immune Responses in Solid Tumors," J. Mol. Diagnostics, vol. 20, no. 1, pp. 95–109, Jan. 2018. [1] NCCN, "NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Head and Neck Cancers, Version 2.2022," National Comprehensive Cancer Network, Fort Washington, Pennsylvania, Version 2.2022, Mar.

[2] J. Ribbat-Idel et al., "Performance of Different Diagnostic PD-L1 Clones in Head and Neck Squamous Cell Carcinoma," Front. Med., vol. 8, no. April, pp. 1–8, 2021.

[3] J. M. Conroy et al., "Analytical

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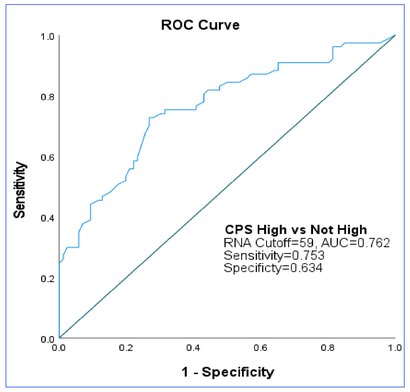
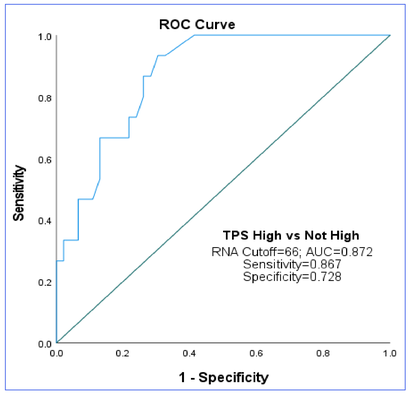
Results

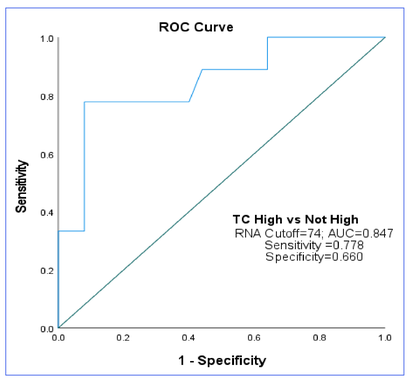
Table 2. Receiver Operator Characteristics (ROC) analysis to identify *CD274* (PD-L1) RNA-seq cutoffs

METHOD	PD-L1 IHC Model*	RNA-Seq Cutoff*	AUC	Sensitivity	Specificity	Youden Index	p-value
CPS (n=163) ≥20 (High) 1-19 (Low) <1 (Negative)	High vs Negative	68	0.809	0.636	0.785	0.421	0.007
	Low vs Negative	51	0.646	0.443	0.714	0.157	0.201
	High vs Low	60	0.758	0.753	0.646	0.399	<.001
	Positive (High + Low) vs Negative	59	0.727	0.561	0.714	0.275	0.043
	High vs Not High (Low + Negative)	59	0.762	0.753	0.634	0.387	<.001
TPS (n=61) ≥50 (High) 1-49 (Low) <1 (Negative)	High vs Negative	73	0.924	0.733	0.889	0.622	<.001
	Low vs Negative	55	0.741	0.571	0.778	0.329	0.006
	High vs Low	74	0.838	0.700	0.714	0.414	<.001
	Positive (High + Low) vs Negative	66	0.805	0.512	0.888	0.400	<.001
	High vs Not High (Low + Negative)	66	0.872	0.867	0.728	0.595	<.001
TC (n=34) ≥50 (High) 1-49 (Low) <1 (Negative)	High vs Negative	56	0.981	1.000	1.000	1.000	0.002
	Low vs Negative	58	0.877	0.605	1.000	0.605	0.006
	High vs Low	82	0.804	0.778	0.684	0.462	0.011
	Positive (High + Low) vs Negative	68	0.911	0.607	1.000	0.607	0.002
	High vs Not High (Low + Negative)	74	0.847	0.778	0.660	0.438	0.002

** Based on maximum J statistic for Youden index (sensitivity + specificity) - 1

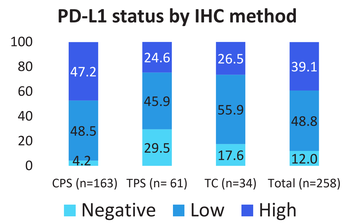


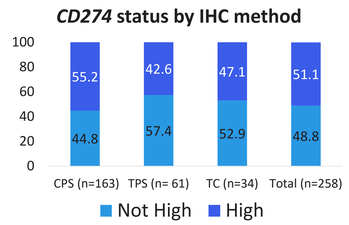




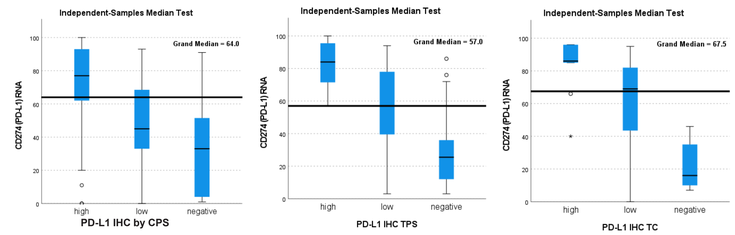
CD274 (PD-L1) sensitivity by RNA-seq

- For all three IHC antibody and scoring approaches, PD-L1 RNA-seq classified IHC high vs negative, high vs low, and high vs not high status with at least fair range for AUC (0.758-0.981), sensitivity (0.636-1.00), and specificity (0.785-1.00).
- RNA-seq could not accurately discern between IHC low vs negative or for positive (high + low) vs negative status for any antibody or scoring method.
- RNA-seq ROC models with low diagnostic accuracy for negative vs low or positive had poor sensitivity vs specificity.

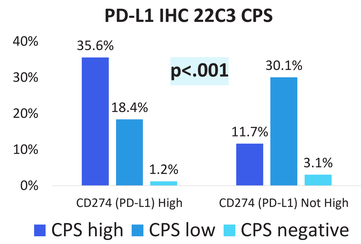


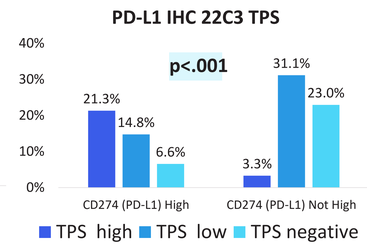


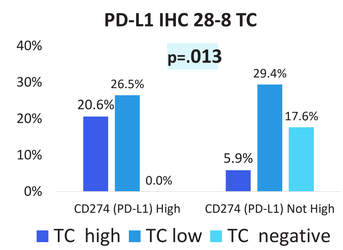
- IHC 22C3 by CPS, the frontline pembrolizumab companion diagnostic method, had the least PD-L1 negative cases (4.2%) and the most PD-L1 high cases (47.2%).
- The proportion of cases identified as CD274 (PD-L1) by RNA-seq increased from 47% to 55% (CPS), 24.6% to 42.6% (TPS), and 26.5% to 47.1% (TC) based on ROC cutoffs for RNA high vs not high status.



- Median *CD274* (PD-L1) measured by RNA-seq was significantly different between PD-L1 high vs not high status for all 3 IHC method pairwise comparisons.







CD274 (PD-L1) RNA concordance with IHC

- The proportion of patients in each PD-L1 IHC group was significantly associated with the proportion of patients in each corresponding *CD274* (PD-L1) RNA-seq group based on ROC cutoffs for RNA high vs not high status.
- A low % of PD-L1 IHC high patients were also classified as high by RNA-seq.

Conclusions

- RNA-seq accurately discerns PD-L1 high vs. not high HNSCC tumors based on standard IHC scoring methods and may more reliably select patients for first line immunotherapy.
- RNA-seq does not distinguish PD-L1 low vs. negative HNSCC tumors, suggesting there may be no difference between these patient groups.

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