173 SENSITIVITY AND CONCORDANCE OF *CD274* 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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Background PD-L1 expression by immunohistochemistry (IHC) is associated with HNSCC immunotherapy response.[1] The performance of different PD-L1 IHC clones 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] 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 patients (n=258) with FFPE tissue was tested by comprehensive immune profiling[3](2017–2022), including *CD274* by RNA-seq (normalized percentile rank 0–100). IHC was performed with either the 28–8 or 22C3 PD-L1 clones. 28–8 was scored with% tumor cells stained (TC, n=34), while 22C3 was scored with either tumor proportion score (TPS, n=61) or combined positive score (CPS, n=163). For 22C3, CPS$\geq$1 is low positive, and $\geq$20 is high positive. For 28–8 TC and 22C3 TPS, $\geq$1 is low positive, and $\geq$50 is high positive. ROC models for each IHC method were constructed for 5 sets of patients with different pairwise interpretation groups and used to determine RNA-seq cutoffs based on individual PD-L1 IHC scoring methods and accuracy at those cutoffs. Concordance between standard IHC scoring methods and *CD274* by RNA-seq was also assessed.

Results PD-L1 IHC results varied depending on the clone and scoring method used. Not surprisingly, CPS had the fewest negative cases (2.7%) and most high cases (47.2%). Tumor cell scoring by TPS (29.5% negative, 24.6% high) and TC (17.6% negative, 26.5% high) was similar for high vs. not high. For all three IHC approaches, PD-L1 RNA-seq classified IHC high v negative, high v low, and high v not high status with at least fair range of AUC (0.758–0.981), sensitivity (0.636–1.00), and specificity (0.785–1.00). RNA-seq could not discern between IHC low v negative status for any method. Pairwise comparisons showed significant concordance between median RNA-seq percentile ranks for IHC high v low and high v negative status for all IHC methods. By RNA-seq, the frequency of PD-L1 high cases for each scoring method increased from 47% to 55% (CPS), 24.6% to 42.6% (TPS) and 26.5% to 47.1% (TC) based on modeled RNA-seq cutoffs.

Conclusions RNA-seq accurately discerns PD-L1 high vs. not high HNSCC tumors based on IHC scoring methods and may more reliably identify patients for frontline immunotherapy.

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