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)

1Mary Nesline, 1Sarabjot Pabla, 1Jeffrey Conroy, 1Paul DePietro, 1Shengle Zhang, 1Roger Klein, 2Achyut Bhagelu, 2Rebecca Previs, 2Prasanth Reddy, 2Shakti Ramkissoon, 2Eric Severson. 1OmniSeq, Inc., Buffalo, NY, USA; 2Labcorp, Durham, NC, USA

Background: PD-L1 expression by immunohistochemistry (IHC) is associated with HNSCC immunotherapy response. 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. 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, 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 ≥1 is low positive, and ≥20 is high positive. For 28–8 TC and 22C3 TPS, ≥1 is low positive, and ≥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 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.

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.

REFERENCES