143 CORRELATING RNA-SEQ DETECTION AND IHC STAINING OF POTENTIAL ANTIBODY-DRUG CONJUGATE (ADC) TARGETS: HER3, HER2, TROP2, NECTIN4, AND AFLR

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Background The growing interest in antibody-drug conjugates (ADCs) as promising therapies for multiple cancer types highlights the need for accurate approaches to detect ADC targets.[1] [2] While the conventional method to identify ADC targets is immunohistochemistry (IHC), using RNA sequencing (RNA-seq) could help improve detection by minimizing the diagnostic variability often seen between IHC assays.[3] With the increasing use of RNA-seq in precision oncology, the relationship between IHC scores and the ADC target gene expression is important to assess. Here, we show that the IHC scores of five promising ADC targets (HER3, HER2, TROP2, Nectin4, and aFLR) correlate with their gene expression values.

Methods We compared gene expression for all 5 targets with IHC scores in an internal cohort (n=299) of breast, gastrointestinal, renal cancers, non-small cell lung cancer (NSCLC), neuroendocrine carcinomas, squamous cell carcinomas of the head and neck and the esophagus, sarcomas, skin melanoma, and glioma. IHC scores from 503 slides were assessed by two pathologists, using an intensity scale where 1+ was weak, 2+ was moderate, and 3+ was strong staining. Samples with more than 10% positively stained 1+ tumor cells (TC) were considered positive.The percentage of stained TC and mRNA expression in log2(TPM+1) were compared using Spearman's rank correlation coefficient. Based on the expression of a single gene, RNA-seq thresholds were developed that maximize the F1-score in predicting IHC positivity.

Results RNA-based biomarker positive and negative cut-offs for HER3, HER2, TROP2, Nectin4, and aFLR were determined by comparing each target's RNA-seq gene expression values with their IHC clinical threshold (table 1). Although they were statistically significant, we found variations in the correlations between IHC and RNA-seq data for the ADC targets. While we observed a strong correlation ($R = 0.81$; $p < 0.001$) for TROP2, with an F1-score of 0.98 that reflects high accuracy in detecting this target, for aFLR, we found a moderate correlation ($R = 0.53$; $p < 0.001$) between the IHC score and RNA-seq data.

Conclusions Using our internal cohort, we established RNA-seq cut-offs for HER3, HER2, TROP2, Nectin4, and aFLR, suggesting the potential use of RNA-seq for ADC target detection. The variation in correlation underscores the need for ongoing refinement of these methods to optimize the detection and quantification of ADC targets, leading to a better understanding of their expression profiles that could enable more effective personalized treatment decisions.

Abstract 143 Table 1 Correlation between IHC and RNA-seq data

Protein / Gene	Tumor Type	Spearman correlation of RNA-seq and IHC	Number of patients	IHC threshold	Corresponding RNA-seq threshold, $\log_2$(TPM+1)	F1-score
TROP2 / TACSTD2	Solid carcinoma	0.81 ($p < 0.001$)	94	≥ 10% TC with 1+	> 5	0.98
Nectin4 / PVRL	Solid carcinoma	0.79 ($p < 0.001$)	91	≥ 10% TC with 1+	> 4	0.90
aFLR / FOLR1	Solid carcinoma	0.51 ($p < 0.001$)	91	≥ 10% TC with 1+	> 2	0.76
HER3 / ERBB3	Solid carcinoma	0.70 ($p < 0.001$)	44	≥ 10% TC with 1+	> 6	0.92
HER2 / ERBB2	Solid carcinoma	0.65 ($p < 0.001$)	183	≥ 10% TC with 1+ and 2+	> 6	0.75
				≥ 10% TC with 3+	> 7.5	> 0.99

IHC – immunohistochemistry; TC – tumor cells

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