38 CLINICAL VALIDATION OF AN IMAGE ANALYSIS ASSAY FOR DETERMINING PROGRAMMED DEATH-LIGAND 1 (22C3) IN NON-SMALL CELL LUNG CANCER

Roberto Gianani*, Will Paces, Elliott Ergon, Kristin Shotts, Vitria Adisetiyo, Charles Caldwell. *Flagship Biosciences, Inc., Lakewood, CO, USA*

Background Determination of programmed death-ligand 1 (PD-L1) level in tumor by immunohistochemistry (IHC) is widely used to predict response to check point inhibitor therapy. In particular, the Dako PD-L1 (22C3) antibody is a common companion diagnostic to the monoclonal antibody drug Keytruda® (pembrolizumab) in non-small cell lung cancer (NSCLC).[1] However, for the practicing pathologist, interpretation of the PD-L1 (22C3) assay is cumbersome and time consuming. Manual pathologist scoring also suffers from poor intra- and inter-pathologist precision, particularly around the cut-off point.[2] In this clinical validation study, we developed an image analysis (IA) based solution to accurately and precisely score digital images obtained from PD-L1 stained NSCLC tissues for making clinical enrollment decisions.

Methods 10 NSCLC tissue samples were purchased from a qualified vendor and IHC stained for PD-L1; 4 of these samples had serial sections stained on two separate days. Stained slides were scanned at 20X magnification and analyzed using Flagship Biosciences' IA solutions that quantify PD-L1 expression and separate tumor and stromal compartments. Resulting image markups of cell detection and PD-L1 expression were reviewed by an MD pathologist for acceptance. PD-L1 staining was evaluated by digital IA in the sample's tumor compartment for Total Proportion Score (TPS,%). Assay specificity was defined by $\geq$ 90% of the tissue cohort exhibiting appropriate cell recognition ($\geq$ 90% cells correctly recognized as determined by the pathologist), with $\leq$ 10% false positive rate for staining classification. Sensitivity was defined by $\geq$ 90% of the cohort exhibiting appropriate cell identification ($\geq$ 90% cells correctly identified), with $\leq$ 10% false negative rate for staining classification. Accuracy was defined by the combination of sensitivity and specificity and precision was defined by concordance of the binned TPS (<1%, $\geq$ 1%, $\geq$ 50%) in $\geq$ 80% of the samples stained on multiple days.

Results The preliminary results show that IA can yield high analytical sensitivity, specificity, accuracy, and precision in the determination of the PD-L1 score. 100% of the tissue cohort met criteria for analytical specificity, sensitivity, and accuracy and 100% of the samples stained on multiple days met the precision criteria.

Conclusions This data demonstrates the feasibility of an IA approach as applied to PD-L1 (22C3) scoring. Ongoing experiments include application of the developed 22C3 algorithm on a separate cohort of 20 NSCLC samples to determine the correlation of digital scoring and scoring obtained by three pathologists. Additionally, we will evaluate the precision obtained by digital scoring in relation to the intra- and inter-pathologist concordance.

REFERENCES

1. Incorvaia L, Fanale D, Badalamenti G, *et al*. Programmed death ligand 1 (PD-L1) as a predictive biomarker for pembrolizumab therapy in patients with advanced non-small-cell lung cancer (NSCLC). *Adv Ther* 2019;**36**:2600–2617.
2. Rimm DL, Han G, Taube JM, *et al*. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non–small cell lung cancer. *JAMA Oncol* 2017;**3**:1051–1058.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0038

39 SPATIAL SINGLE-CELL QUANTITATIVE ANALYSES OF HUMAN HEAD AND NECK SQUAMOUS CELL CARCINOMAS

Katie Blise*, Shamilene Sivagnanam, Lisa Coussens, Jeremy Goecks. *Oregon Health and Science University, Portland, OR, USA*

Background While the quantities and types of immune, tumor, and structure-related cells present in the tumor-immune microenvironment (TiME) are important for understanding aspects of cancer progression and potential responses to therapy, spatial locations and relationships of these cells also play a critical role. Emerging single-cell imaging modalities, such as multiplex immunohistochemistry (mIHC), provide phenotypic and functional state information for each cell present in the TiME while maintaining the spatial context of tissue architecture. We performed a quantitative analysis of mIHC images to characterize the cellular composition and spatial organization of human head and neck squamous cell carcinomas (HNSCC) and identified features correlated with patient survival.

Methods mIHC is an immunoassay-based imaging platform that evaluates sequentially stained immune lineage epitope-specific antibodies for immunodetection on FFPE tissue sections to phenotype single cells as tumor, stromal (mesenchymal), or one of more than 20 different immune cell lineages, all while maintaining the Cartesian coordinates of each cell.[1 2] Matched primary and recurrent HNSCC tumors from nine patients were assayed via mIHC. Using unsupervised hierarchical clustering and principal component analysis, we interrogated the heterogeneity in cellular composition of each tumor section. We further quantified the spatial organization of tumors and identified prognostic tumor and immune cell architectures,[3] as well as cellular neighborhoods that clustered together based on similar compositions and physically grouped together to reveal common spatial features across tumors.

Results Regions from the same tumor and tumors from the same patient clustered together more in their cellular composition than tumors from different patients. We also observed a decrease in the fraction of B cells present in recurrent tumors following therapy for all patients ($p=0.024$). While common biomarkers for HNSCC, such as CD8+ T cell density and tumor cell abundance were not associated with outcome, the tumor-immune spatial relationship was prognostic. Tissue regions of compartmentalization between immune and tumor cells were associated with higher fractions of αSMA+ stromal cells and had a greater proportion of Ki-67+ lymphocytes present, as compared to mixed regions. Patients with more compartmentalization in their primary tumors demonstrated longer progression free survival than those with more mixing between these cell types ($p=0.027$).

Conclusions Our results provide insight into the spatial organization of HNSCCs, highlighted by the result that compartmentalization between immune and tumor cells is associated with improved outcomes. This study provides spatial analysis methods and hypotheses that can be used as a framework for analysis of larger cohorts.

Ethics Approval This study was approved by Oregon Health and Science University's IRB (protocol #809 and #3609), and written informed consent was obtained.

REFERENCES

1. Tsujikawa T, *et al*. Quantitative multiplex immunohistochemistry reveals myeloid-inflamed tumor-immune complexity associated with poor prognosis. *Cell Rep* 2017;**19**:203–217.