Flagship BIOSCIENCES

BUILDING PREDICTIVE MODELS OF CLINICAL OUTCOMES IN IMMUNE ONCOLOGY USING COMPUTATIONAL TISSUE ANALYSIS SCORECARDS

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ABSTRACT

Several studies have shown that the location and expression of infiltrating immune cells in patient tumors can better identify which patients are more likely to respond to anti- PD-1/PD-L1 therapy. In particular, immunohisto-chemistry-based studies have shown that the spatial location of PD-L1 expression has particular biological relevance. Here, we use Flagship's digital pathology platform (cTA®) to investigate IHC based PD-L1 and CD8 staining patterns in Non-Small Cell Lung (NSCLC) tissue biopsies. The cTA platform creates thousands of per-cell Biofeatures™ derived from the scanned images of the IHC stained tissue, and applies Artificial Intelligence (AI) to the data to score endpoints for patient and cohort classification. In this approach, each tissue's IO landscape is represented using an "IO Scorecard", which summarizes the IHC biomarker data and captures a comprehensive analysis of the tissue sample. The scorecard models can be used to monitor changes before and after drug treatment and/or create predictive models for patient response outcomes. In this study, NSCLC samples were sectioned and stained using either Dako 22C3 or SP263 PD-L1 IHC assays. Serial sections of each tissue specimen were also stained for CD8 expression. The cTA process detected all cells, assigned them to the tumor or TME compartments, and recorded the Biofeatures™ data which characterized PD-L1 or CD8 staining in the Tumor or TME compartments. The method was validated by its ability to reproduce pathologist scoring for PD-L1 and CD8. The AI Scorecard approach demonstrated that certain PD-L1 staining Biofeatures™ may also predict the CD8 status of a tumor, suggesting that additional CD8 staining may not be necessary to understand important expression patterns pertaining to cytotoxic T-cells.

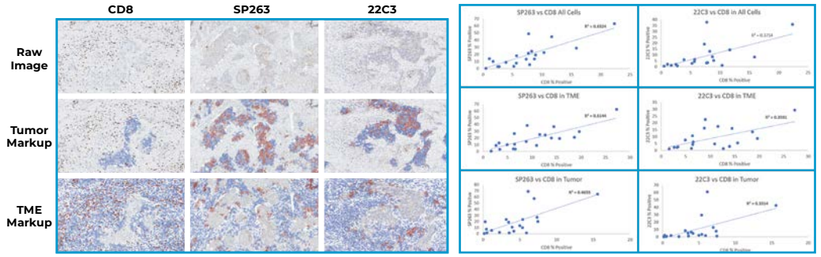
MATERIALS & METHODS

20 Formalin-Fixed Paraffin Embedded (FFPE) Non-Small Cell Lung Cancer (NSCLC) sections tissue blocks were cut in to 4µm-thick tissue sections on to glass slides. NSCLC serial tissue sections were stained for PD-L1 using either SP263 or 22C3 antibody clones according to their specified protocols. An additional serial section of NSCLC was stained for CD8 expression using the C8/144B antibody clone. Images of all stained slides were scanned at 20x magnification using Aperio AT2 slide scanners. Scanned images were analyzed using Flagship Biosciences' cTA® platform. In brief, cTA® analysis first detects all cells in a slide section, then measures hundreds of features (Biofeatures™) characterizing every single cell, which includes morphometric, spatial, and IHC-related data points. Additional machine-learning and data-science based workflows allow the cTA® platform to separate the tumor and TME-associated cells to assign biomarker scoring in those compartments. Flagship Phenotype analysis identifies specific cellular phenotypes within the tissues to understand biomarkers in the context of specific cellular subtypes. All data generated are fed in to Flagship's Scorecard modeling system to build predictive models around specified endpoints, in this case CD8 positivity. The top cellular Biofeatures™ associated with predicting CD8 expression in were identified as the features which explained the most variance in a regression analysis. These features were normalized and displayed as visual representations of the top features in each data set, along with the values of each identified Biofeature™.

FLAGSHIP cTA: PATHOLOGY ENDPOINTS

PD-L1 assays SP263 and 22C3 were analyzed using Flagship cTA® in 20 serial sections of NSCLC samples. An additional serial section was also stained and analyzed for CD8 expression. Each sample was analyzed for All Cells expression as well as expression in the Tumor and TME compartments of the tissue. PD-L1 expression as measured by SP263 correlated higher with CD8 expression than with the 22C3 assay in all compartments (SP263:CD8 R^2= 0.632, 22C3: CD8 R^2 = 0.3754)

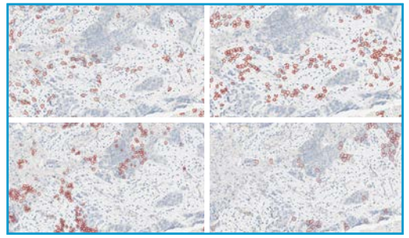
Assay	% Positive All Cells	% Positive Tumor	% Positive TME
CD8	7.29%	4.41%	10.20%
SP263	18.52%	18.98%	18.97%
22C3	8.77%	9.69%	8.81%



FLAGSHIP cTA: PHENOTYPE ANALYSIS

As part of the scorecard analysis workflow, cellular subtypes are separated beyond just tumor/TME designation into phenotypic designations. Each phenotype is described by hundreds of cell morphology and location-based Biofeatures™. Biomarker expression and any pathology analysis (Biomarker positivity, Tumor/TME separation, spatial relations, margin analysis, leukocyte counts, etc.) can be analyzed on a per-phenotype basis. Differences between patient groups such as responder/non-responder, or biomarker expression such as CD8 positivity (shown here) can be assessed in discrete phenotypes to help understand biomarkers in biological, cell-derived context.

PD-L1 changes in specific cell phenotypes in CD8 High and Low Tissues



Pathology review of phenotypes marked up in Flagship Slide Viewer provides context for generated phenotype data. Clockwise from top left: small TME cells with convoluted nuclei, small round TME cells, small round intra-tumoral cells, large tumor cells with convoluted nuclei

FLAGSHIP SCORECARD: PREDICTIVE MODEL OF CD8⁺ INFILTRATE BASED ON PD-L1 STAINING

Flagship Scorecard Workflow

Cohort With Clinical Outcomes

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Measure Cell Biofeatures™
Separate Tumor/TME
Identify and Mark Up Cell Phenotypes
Generate Biofeature™ Model
Predictive of Clinical Outcomes

↓

Stratify New Patients in to Outcomes Groups

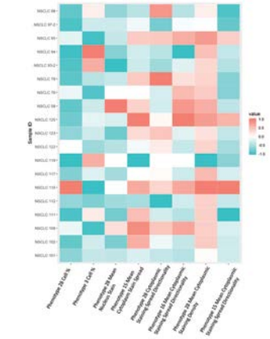
Accuracy of PD-L1 Based CD8⁺ Prediction

Assay	Measure	CD8⁺ Prediction Accuracy (R^2)
SP263	Flagship Scorecard	0.9012
	All Cells % Positive	0.6324
	Tumor % Positive	0.4655
	TME % Positive	0.6144
22C3	Flagship Scorecard	0.8833
	All Cells % Positive	0.3754
	Tumor % Positive	0.3314
	TME % Positive	0.3591

Predictive models were established using random forest regression analysis with 1000 trees and an unconstrained number of nodes. R2 values represent the ratio of variation explained by the regression model to the total variation in the dataset.

SP263:CD8 Predictive Features

22C3:CD8 Predictive Features



All data generated from image analysis of pathology endpoints and cellular phenotyping data are combined and assessed via Flagship's machine learning models. This method selects the top endpoints, phenotypes, and BioFeatures™ that correspond with clinical outcomes, such as survival or drug response, or biomarker expression such as CD8+ infiltration (shown here). The models built using this method are trained and tested thousands of times to assess accuracy of the models. Once a predictive model is trained on a statistically significant number of tissues, new tissues can be assessed using this model as a predictor of clinical outcomes or biomarker positivity.

CONCLUSIONS

Biofeatures generated by Flagship's cTA provide contextual information about biomarker staining and expression that can be used to predict clinical outcomes and endpoints.

- Flagship AI separates All Cells, Tumor, and Stroma (TME) in whole tissue sections to quantify biomarker heterogeneity and give context to the tumor microenvironment
- Quantified CD8 expression in NSCLC samples showed higher correlation with SP263 PD-L1 expression on TME-associated cells (R^2= 0.6144). PD-L1 22C3 did not correlate as highly with CD8 expression alone (R^2 All Cells = 0.3754)
- Biofeature profiles of each individual cell describe different phenotype presences in tissues. PD-L1 staining of individual cell types in $CD8^{high}$ and $CD8^{low}$ tissues shows larger fluctuations in SP263 staining in certain cell subtypes based on CD8 expression levels
- Predictive outcome models can be built using the Flagship IO Scorecard approach, which takes in to account hundreds of data points from each cell in a tissue
- Flagship IO Scorecard-derived endpoints built a model to predict $CD8^+$ infiltration using only PD-L1 staining in NSCLC tissues. IO Scorecard models increase CD8+ infiltration predictive accuracy of SP263 (R^2= 0.9012) and 22C3 (R^2=0.8833) over tumor/TME specific biomarker quantification methods