Flagship BIOSCIENCES

ANALYSIS OF COMPANION DIAGNOSTIC POTENTIALS FOR MULTIFACETED PD-L1 ASSAYS

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ABSTRACT

In vitro diagnostic (IVD) approvals of qualitative immunohistochemical (IHC) assays offer unique patient selection strategies by equipping pathologists with new tools to assess tumor status including tumor immune landscape. PD-L1/PD-1 checkpoint therapies require accurate diagnostic tools for optimal patient selections, however the interpretation of these tests may be very complicated upon manual assessment. As assessment criteria have increased in complexity, an accurate quantitative approach is needed to objectively and consistently interpret challenging paradigms. Recent IVD tests have multifaceted paradigms which assess both tumor and immune positivity. Although the Ventana PD-L1 SP263 and the Dako PD-L1 22C3 qualitative diagnostics are guiding immunotherapy decisions, interpretation of the results may be subjective and challenging in many cases. This creates a further level of complexity as tumor cell positivity, and immune cell presence and positivity must be assessed in combination.

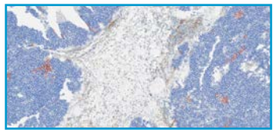
In this study, we have used Flagship Biosciences' image analysis platform (cTA®) to explore the use of artificial intelligence (AI) and machine learning in the context of complex PD-L1 assays to provide accurate and precise quantification that allows for objective interpretation of the IVD. Non-small cell lung cancer (NSCLC) and urothelial carcinoma (UC) samples were stained with the SP263 and 22C3 PD-L1 assays and image analysis was used to provide an interpretation status based upon the IVD scoring paradigms. A comparison of the cTA results across both tests and tissue indications was also performed to explore how we may further support cross-platform PD-L1 solutions that provide adaptable and objective quantification. The use of Flagship's cTA platform to identify per-cell biofeatures, using machine learning, may successfully quantify PD-L1 staining in various cell populations. The ability to independently assess these populations allows for a consistent and unbiased method for the assessment tumor status for PD-L1 immunotherapy treatment decisions.

SP263 ASSAY ANALYSIS

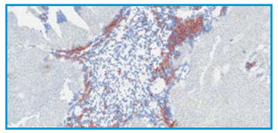
Urothelial Carcinoma

PD-L1 is quantified in tumor cells and immune cells, separately, to identify tumor cell positivity (TC+), immune cells present (ICP), and immune cell positivity (IC+).
PD-L1 High status is achieved if any of the following are met:

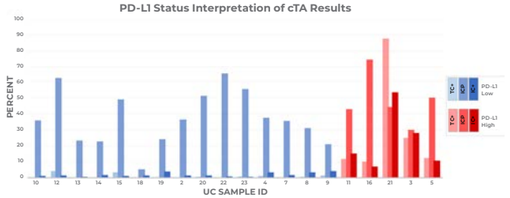
- ≥ 25% TC+
- ICP > 1% & IC+ ≥ 25%
- ICP = 1% & IC+ = 100%



cTA Tumor Cell Markup



cTA Immune Cell Markup

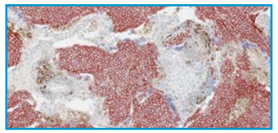


Non-Small Cell Lung Cancer

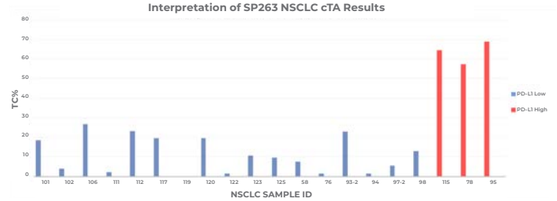
PD-L1 status is determined by calculating the positive tumor cell percentage (TC%). A PD-L1 High status is achieved if the TC% is ≥ 50%. The TC% is calculated using the following equation:

$$TC\% = \frac{\text{PD-L1 positive tumor cells}}{\text{Total tumor cells}} \times 100$$

Native Image



cTA Tumor Cell Markup

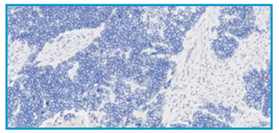


22C3 ASSAY ANALYSIS

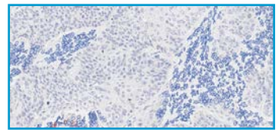
Urothelial Carcinoma

PD-L1 status is determined using a Combined Positive Score (CPS). A PD-L1 High status is achieved if a CPS is ≥ 10. The CPS is calculated using the following equation:

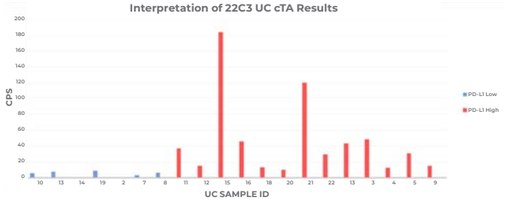
$$CPS = \frac{\text{PD-L1 positive cells (tumor \& immune)}}{\text{Total tumor cells}} \times 100$$



cTA Tumor Cell Markup

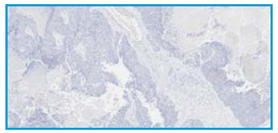


cTA Immune Cell Markup

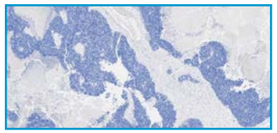


Non-Small Cell Lung Cancer

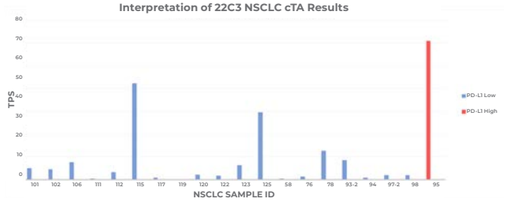
PD-L1 status is determined by calculating a Tumor Proportion Score (TPS). The TPS is calculated in the same manner as the TC%, where the proportion of tumor cells expressing membrane staining is measured. If the TPS does not reach 50%, the sample will receive a PD-L1 Low status.



Native Image



cTA Immune Cell Markup



CROSS-PLATFORM COMPARISONS

PD-L1 Status Per Assay

PD-L1 statuses for each assay may be compared to determine concordance of status assignment across IHC assays.

When comparing the SP263 and 22C3 assays, a concordance rate of 60% is found for UC and 90% is found for NSCLC.

IVD Scoring Paradigm

An examination was performed in which the UC IVD scoring paradigms were applied to the alternate IHC assay; the CPS to SP263 and TC+, ICP, IC+ method to 22C3.

Each SP263 stained sample achieved the same PD-L1 status using both paradigms. Of the 22C3 stained samples, 8 High status specimen obtained a Low status when the alternative paradigm was used.

Sample Status Distribution per Assay

IHC Assay	PD-L1 High Samples	PD-L1 Low Samples
SP263 UC	5	15
22C3 UC	13	7
SP263 NSCLC	3	17
22C3 NSCLC	1	19

Concordance of Alternative Paradigms

IHC Assay	Alternative Paradigm	Per-Sample Concordance
SP263 UC	CPS	100%
22C3 UC	TC+, ICP, IC+	60%

CONCLUSIONS

Flagship's cTA may be used to create companion diagnostic models for multidimensional PD-L1 assays.

- CDx models may be successfully constructed to allow for quantitative data which mimics an approved paradigm, producing consistent and qualitative PD-L1 status interpretations.
- PD-L1 status effected by subjectivity during manual evaluation may be further predisposed to inaccurate reporting due discordance between IHC assays.
- The ability to successfully apply alternative IVD paradigms to PD-L1 stained samples may allow for a scoring method which best suites a patients immune profile, as the influence of the peritumoral stroma impacts the paradigms differently.

*Author is also employed by Roche and research was abided by requisite confidentiality for both Roche and Flagship Biosciences.