Virtual Centralized Pathology = More Accurate Patient Data

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Background

Historically, immunohistochemistry (IHC) assays have been designed for simplicity of manual pathology, however, this is not always ideal to acquire the appropriate data for patient selection and outcomes. Due to various challenges within the current pathology scoring paradigms and the increasing complexity of biomarker strategies for patient enrollment, advancements in this field is required. Particularly the movement of clinical diagnostics (CDx) into the digital age is needed to allow for data driven approaches. Flagship Biosciences, Inc. provides a tissue and biomarker analysis platform that gives an advantage to drug development and clinical diagnostics. Flagship Biosciences, Inc. has an in-house developer team that is at the forefront of innovation with the capability to provide end-to-end biomarker assays and analysis for clinical trials and regulatory management. Our central site allows for custom and complex assay design, as well as, reliable and validated imaging criteria in normal tissues and many disease types (e.g. oncology, muscle, liver, and neuronal). Our proprietary image analysis (IA) platform allows for the collection of many data endpoints, from simple to complex with the flexibility and adaptability to create new capabilities when required. Operation of the IA platform is performed by dedicated engineers intricately familiar with the platform to get the most of out of it, ensuring high quality data. Furthermore, IA data yields comparable results to pathologist interpretation but eliminates the intra-pathologist and inter-pathologist variability associated with manual scoring.

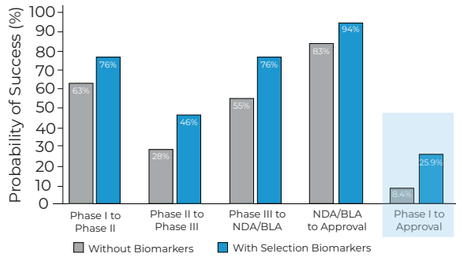


Figure 1. Probability of therapeutic successfully approved with and without the use of biomarkers[1].

Materials & Methods

FFPE blocks were stained for various biomarkers, including PD-L1 (Figure 2), DKK1 RNAscope (Figure 3), a General Immuno-oncology (IO) multiplex panel (CD3, CD8, CD56, CD68, CD163, and PDL1) (Figure 4), Mersin, 2C1, and GFP (Figure 5). Slides were scanned at 20X magnification. Whole-tissue image analysis (IA) was performed via Flagship Biosciences' proprietary IA platform. All cells in each tissue section were identified via AI processes that generated hundreds of morphology, spatial, and staining related features per-cell. Machine learning algorithms stratified cells as belonging to the tumoral or stromal space based on their cellular features. Core level expression data was pulled and represented on a whole-cohort basis. All staining and image analysis outputs were reviewed by a board-certified, MD pathologist.

Summary

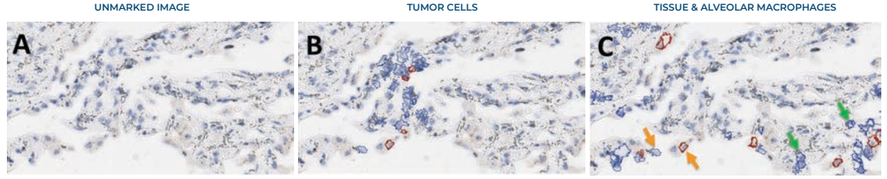
Current standards of diagnostic assay development for ease of pathologist read and global implementation can result in high variability of assay reads across interpreters. With the ever-increasing complexity of new biomarkers being introduced into clinical trials, the need for accuracy and precision across global sites is more important than ever. Coupled with the exponential increase in costs to get a drug to market and the competition over patients, making faster, more accurate decisions is critical.

Presented here is a discussion of the novel approach provided by Flagship Biosciences, a data centric, CLIA certified tissue imaging partner, which generates centralized assay reads virtually through cell-based image analysis and machine learning from images that can be uploaded from any location globally. As a virtual central site diagnostic lab, assay interpretations are standardized and reproduced by image analysis reads verified by medical pathologists. By design, this virtual central imaging clinical support system eases the pathologist burden of reproducing complex assay reads and allows for rapid development and deployment of cutting-edge research and diagnostic tools.

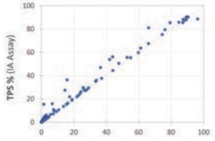
References

1. Carroll A (2016) Biomarkers in Medicine 10:9; 939-941.
2. Caldwell C (2021) Scientific Reports 11:9920.

PD-L1 Staining Analysis in NSCLC Samples



D. IA Assay Digital TPS (without and with Macrophage Solution)



E. Manual Pathology TPS vs. IA Assay Digital TPS (without and with Macrophage Solution)

Pathologist	Manual TPS vs. Digital TPS (IA Assay)	Manual TPS vs. Digital TPS (IA Assay w/Macrophage Solution)
#1 (Flagship)	r = 0.900 (p < 0.00001)	r = 0.901 (p < 0.00001)
#2 (Outside)	r = 0.884 (p < 0.00001)	r = 0.859 (p < 0.00001)
#3 (Outside)	r = 0.858 (p < 0.00001)	r = 0.838 (p < 0.00001)

Figure 2. Digital Analysis of PD-L1-Stained NSCLC Samples. The 22C3 IVD PD-L1 antibody staining assessment is widely used to predict the efficacy of these interventions in NSCLC. The pathologist interpretation of this assay is, however, cumbersome, time consuming, and suffers from relatively poor intra-pathologist and inter-pathologist variability as well as a potential lack of accuracy in less experienced readers. Often, there are failures in PD-L1 assay harmonization and therefore do not select a high number of patients or responders. Thus, the implementation of Flagship Biosciences, Inc. proprietary IA platform as a pathologist support tool aids in patient selection and can ease the burden of pathologist scoring.

A) DAB-conjugated PD-L1 stained NSCLC tissue (brown) without IA markups. B) The clinically validated PD-L1 (22C3) IA assay detected and quantified PD-L1 cellular expression within the tumor compartment to calculate the Tumor Proportion Score (TPS) (Blue IA markups: PD-L1 negative cells. Red IA markups: PD-L1 positive cells). C) Application of the macrophage solution to the PD-L1 (22C3) IA assay differentiates tissue macrophages (green arrows) and alveolar macrophages (orange arrows) from tumor cells and excludes these macrophages from the TPS calculation. D) Comparing the digital TPS from the same samples calculated with the IA assay with versus without the macrophage solution reveals very similar values. However, without applying the macrophage solution, there was a consistent shift towards higher TPS values. Overall, by removing the confounding contribution of macrophages in calculating the TPS, application of the macrophage solution to the IA assay resulted in similar or reduced TPS values. E) Digital-to-manual PD-L1 (22C3) scoring agreement was assessed by Pearson's correlation analysis of the TPS from the clinically validated IA assay and from each pathologist. All three pathologists (1 internal pathologist [Flagship], two outside pathologists) demonstrated a significant correlation with the digital TPS from the IA assay without and with the macrophage solution.

Validation of a DKK1 RNA Scope chromogenic in situ hybridization assay

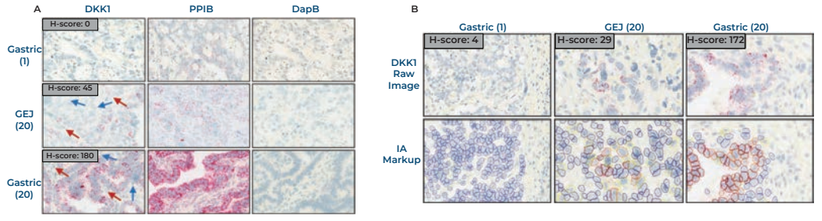


Figure 3. Patient Enrollment Based on IA of Samples Stained with DKK1 RNAscope[2]. Utilizing an appropriate IA solution eliminates the error associated with manual slide reads, providing truly reproducible, quantitative results, particularly with samples stained with complex assays, such as RNAscope. The development of the IA solution is guided and reviewed by pathologists as a novel pathologist support tool. Furthermore, CAP/CLIA validation of IA solutions, such as this DKK1 IA solution, can be performed to support patient enrollment in clinical trials, which ultimately helps to accelerate timelines and minimize budgets.

A) The DKK1 RNAscope assay in tumor resections. Representative images from 3 tumor resections with no (top row), moderate (middle row) and high (bottom row) DKK1 signal are shown with the PPIB control for RNA integrity and the DapB background control. DKK1 H-scores (range 0-300) were semi-quantified manually as described in the methods. Red arrows denotes non tumoral cells without DKK1 signal. Blue arrows denote tumor cells with low levels of DKK1 signal (one dot per cell). Scale bar: 50 µm B) Representative images from 3 tumor resections with no, low, and high DKK1 signal and the image analysis markup are shown. Markup of colors correspond to the following; blue indicates tumor cells with no DKK1 signal, represents tumors cells with low signal (1–3 dots), orange selected tumors cells have medium signal (4–9 dots), and red identifes tumors cells with high signal (10+dots). Stromal cells which are not highlighted by the algorithm are not scored. Image analysis (IA). Scale bar: 10 µm.

Complex Multiplex Immunoflourescnece Staining

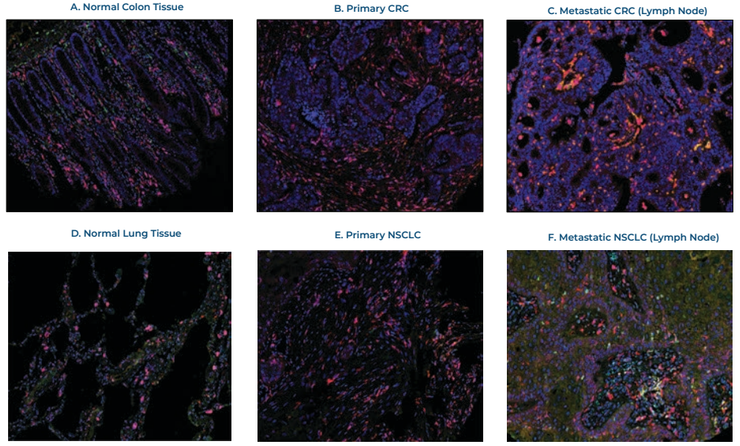


Figure 4. As the drug development landscape evolves, it brings with it a need for more complex biomarker assays to answer questions about the drug mechanism of action and efficacy. Flagship Biosciences, Inc. provides off-the-shelf multiplex immunoflourescnece (IF) assays as well as custom multiplex panel development to fit the needs of the drug or clinical trial. We have the capabilities to perform complex immune phenotyping to determine the immune microenvironment and potential immune infiltration into tumor nests. We also have extensive work with bispecific antibodies to monitor the colocalization of two molecules on patient samples.

Above is an example of matched (A) normal colon tissue, (B) primary colorectal cancer (CRC) tissue, and (C) metastatic CRC tissue from the same CRC patient as well as D) normal lung tissue, E) primary NSCLC, and F) metastatic NSCLC from the same NSCLC stained with our General (immunoncology) IO panel to measure expression levels of CD3, CD163, CD56, CD8, and CD68. The data provided through Flagship Biosciences' proprietary image analysis platform can distinguish the presence, location (tumor vs stroma vs margin), and amount of T cells, cytotoxic T cells, helper T cells, NK cells, M1 and M2 macrophages, as well as PDL1 positive tumor and immune cells.

Image Analysis Capabilities in Muscle and Other Tissues

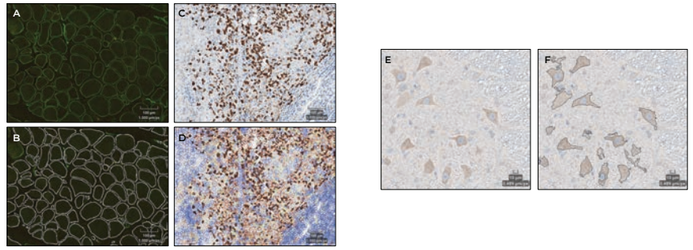


Figure 5. IA in Muscle, Liver, and Neurons. Flagship Biosciences, Inc. routinely works in the muscle, liver, and neuronal space as well. We have validated assays for typical biomarkers in these indications, such as (A,B) Merosin in muscle samples, (C,D) 2C1 in liver biopsies, and (E,F) GFP in neurons. Our proprietary IA software can detect individual muscle fibers (B) or hepatocytes (D) using routine histological stains, chromogenic stains or immunofluorescence labeling. Each cell can be precisely quantified, and images can then be digitally marked to illustrate positivity in expression of a particular marker(s). These data support clinical trial efficacy comparing pre- and post-treatment samples. Our proprietary IA software can also be trained to identify neurons or trained to identify specific neuronal subtypes (F). Staining of each individual neuron can be quantified and thresholds can be set to differentiate positive and negative cells. These data are used for evaluating viral vector transduction.

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