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Getting the Whole Picture for Immuno-Oncology Therapies

Key concepts:

- *Immuno-oncology (IO) therapies have changed the way we think about patient selection for precision medicine.*
- *The complexity of tumor/immune interactions creates an opportunity to leverage multiple diagnostic approaches for understanding patient-specific responses to IO therapies.*
- *The biological complexity of these therapies requires technologically complex solutions.*
- *Technological solutions must have a sound regulatory pathway to be implemented as predictive diagnostic tests to support use of an IO therapy.*

IO therapies are changing our approach to medicine

Diagnostics that identify the targets of a drug have been a cornerstone in conveying the value of precision medicine approaches. In the past, patient selection for classic targeted therapeutics was based on a single biomarker using a single assay involving a single methodology (e.g., HercepTest™); however, a lot has changed since the approval of Herceptin® nearly 20 years ago. The first-generation immune checkpoint inhibitors such as YERVOY®, OPDIVO®, KEYTRUDA®, TECENTRIQ®, and BAVENCIO® have transformed the way we approach cancer therapy. Now, the profile of the patient's immune system and its interaction with the tumor is considered as much as the target of the therapy itself.

In the case of checkpoint inhibitors, the response to therapy is influenced by multiple other aspects of both the tumor and the tumor microenvironment, and precision medicine requires the ability to deeply characterize these influences for each patient. The uniqueness and individuality of each patient's immune system combined with the specific genetic profile of an individual patient's tumor will require more comprehensive diagnostic approaches, likely utilizing a combination of diagnostic methodologies, to select the right patient at the right time for advanced treatment strategies.

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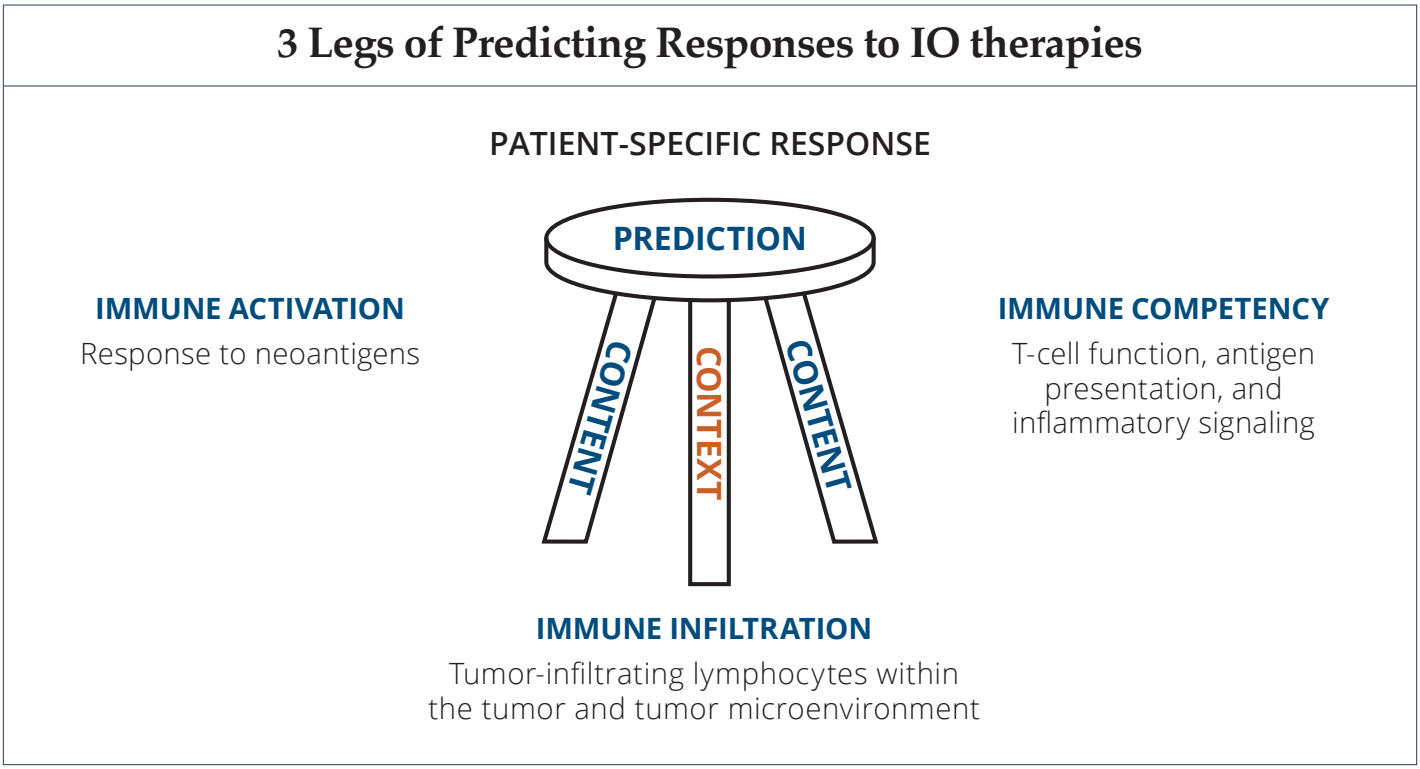
This combination diagnostic approach will require both "content"-based methods, which measure specific DNA, RNA, or protein components of the tumor as a whole, and "context"-based methods, which examine the spatial relationships of specific cell types within the tumor microenvironment (Figure 1). In this way, content and context will capture the full biological repertoire of both the tumor and the tumor microenvironment to create sufficient understanding of the patient-specific biology driving the disease and enable a successful precision medicine approach appropriate for IO.

Figure 1: Three Legs of Predicting Response to IO Therapies



The Need for Stronger Patient Selection and Prediction

Medical research continues to identify successful therapeutic strategies, including combination approaches for treatment. The unexpected efficacy and toxicity profiles being observed, however, suggest that a more selective approach to administering combination therapies is required. As learned in the monotherapy space, a biomarker-driven approach to drug development can identify responsive patients and improve the risk-benefit profile of a drug. Combination therapies, which rely on 2 distinct mechanisms of action for each drug, however, make a biomarker strategy that supports patient selection for these drugs more ambiguous. To fully characterize a patient profile, more sophisticated biomarker strategies are required to create predictors of response to particular drugs. Methodologies may have to support consideration of biomarkers for the drug target, immune cell constituents, mutational changes, and inflammatory cell signatures, all required to elucidate this patient-specific biology. To support this, patient care will require a holistic, integrated diagnostic

approach that leverages multiple approaches for genomic-, blood-, and pathology-guided decision-making. A broader understanding of a patient's profile can be important when considering which aspect of the cancer/immune interaction a given immunotherapy is designed to affect, either alone or in combination.

Capturing Biological Complexity

The mechanism of action of an immunotherapy can be designed to affect different aspects of cancer/immune interactions. An article published in 2016 by Blank and colleagues explores the concept of a "cancer immunogram" in which 7 parameters that characterize aspects of cancer/immune interactions could be used as a framework for making more individualized treatment decisions. Within the discussed parameters, 3 primary biological considerations for the cancer/immune interaction seem to emerge: (1) tumor foreignness, (2) immune competence and (3) local tumor/immune interactions. The concepts of tumor foreignness, immune competency, and tumor/immune interactions can now be measured with supportive testing, as evidenced by the data collected in numerous clinical trials that are evolving IO through the use of biomarker-driven therapeutic strategies. These biological considerations are being applied to development strategies and patient selection criteria for immunotherapies, both alone and in combination (Table 1).

Table 1: Application of Immunotherapies for Certain Immune Profiles

Biological Immune Consideration	Therapeutic (Sponsor)	Tumor Indication	Biomarker	FDA-Approved Diagnostic Testing Method
Tumor Foreignness	**KEYTRUDA® (Merck)**	Solid tumors	MSI-H and dMMR*	Laboratory IHC test for dMMR tumors and laboratory PCR test for MSI-H tumors
	OPDIVO® (Bristol-Meyers Squibb)	Colorectal cancer	MSI-H and dMMR*	Laboratory IHC test for dMMR tumors and laboratory PCR test for MSI-H tumors
Immune Competency	**KYMRIAH® (Novartis)**	Leukemia	N/A	N/A
Tumor/Immune Interactions	**KEYTRUDA® (Merck)**	Multiple	PD-L1	Companion Dx PD-L1 IHC test (Dako 22C3)
	OPDIVO® (Bristol-Meyers Squibb)	Multiple	PD-L1	Complementary Dx PD-L1 IHC test (Dako 28-8)
	IMFINZI® (AstraZeneca)	Urothelial carcinoma	PD-L1	Complementary Dx PD-L1 IHC test (Ventana SP263)
	BAVENCIO® (Pfizer)	Merckel cell carcinoma	N/A	Prototype PD-L1 IHC test (Dako 73-10)
	TECENTRIQ® (Roche)	Urothelial carcinoma	PD-L1	Complementary PD-L1 IHC test (Ventana SP142)

*The FDA approved FoundationOne CDx™ is seeking approval for this indication also.

Abbreviations: BMS, Bristol-Myers Squibb; dMMR, mismatch repair deficient; Dx, diagnostic; IHC, immunohistochemistry; MSI-H, microsatellite instability–high; NA, not applicable; PCR, polymerase chain reaction; PD-L1, programmed death ligand 1.

Tumor Foreignness is defined as the presence or absence of neoantigens that would elicit an immune response against tumor cells. The concept of neoantigens playing a role in IO has emerged as a common theme, since the presentation of tumor antigens and stimulation of an immune response is often central to immunotherapy mechanisms of action. As such, tumor signatures are being investigated to capture tumor foreignness. They include genetic information on the tumor as well as tumor microenvironment signatures. Comprehensive genomic profiling (CGP) using next-generation sequencing approaches enables the evaluation of microsatellite instability (MSI) and tumor mutational burden (TMB), surrogate measurements of mutation load, signifying an increase in tumor neoantigens and increased recognition of the tumor by the immune system.

Immune Competency is defined as the ability of a patient's immune system to mount an attack against the tumor. While relevant to all therapies, this is most relevant to immunotherapeutic approaches that activate T-cell–mediated immune responses, such as interleukin 2 (IL-2) therapies, chimeric antigen receptor (CAR) T-cell therapies, dendritic cell (DC)–based vaccines, and antibody-based therapies that target negative regulatory molecules, cytotoxic T-lymphocyte–associated antigen (CTLA) 4, or programmed death receptor (PD) 1. Diagnostic tests that provide information about the total number of leukocytes, detailed quantitative and qualitative changes in the composition of lymphocyte subsets, cytokine levels in the serum, and functional properties of T cells, natural killer (NK) cells, and monocytes are required. The interferon gamma (IFN-γ) signature uses a mulitiplex gene RNA expression panel (e.g., NanoString nCounter®) to measure an immune-related gene expression signature composed of genes associated with T-cell cytotoxic function, antigen presentation machinery, and IFN-γ signaling, which together represent the profile of immune activity against the tumor.

Tumor/immune interactions are defined as the direct interactions between tumor cells and immune cells that modulate immune response to tumors, including the specific interactions with effector, helper, and regulatory T cells, dendritic cells, and macrophages. Discrete changes within the tumor microenvironment and direct receptor/coreceptor binding interactions between immune cells and tumor cells can (a) produce cytokines that skew macrophages from having a tumor-eliminating M1 phenotype to a tumor-promoting M2 phenotype, (b) produce inhibitory molecules such as indoleamine 2,3-dioxygenase (IDO) to inhibit T-cell responses, (c) dysregulate antigen-presenting cells such as dendritic cells, (d) induce T-cell tolerance and anergy, and (e) increase expression of coinhibitory signals and receptors, all of which function to decrease the amplitude of the antigen-specific T-cell response against the tumor.

To be inclusive of these 3 aspects of IO biology, approaches to predicting patient responses to immunotherapies are changing dramatically to be inclusive, wide-encompassing biomarker strategies that measure a tumor's genetic variability, assess the tumor/immune microenvironment, and analyze the patient's individual immune system. The uniqueness and individuality of each patient's immune system combined with the specific genetic profile of an individual patient's tumor requires more comprehensive diagnostic approaches, likely utilizing a combination of methodologies, to select the right patients for a therapy.

Enabling technologies

The need for tissue content as well as tissue context information to describe a patient's localized immune system and the intricate, multifaceted interactions between tumors and the immune system is driving new approaches to designing patient selection criteria and treatment strategies. The drive to develop treatment strategies that revolve around a patient's immune, genetic, and health profile rather than a given tumor type has been initially validated by the recent FDA approval of Keytruda for an unprecedented tumor-agnostic indication, granted on May 23, 2017. This approval signals a major shift in cancer treatment strategies that are based on a tumor signature rather than a tumor type. This pan-tumor indication goes beyond the typical programmed death ligand 1 (PD-L1) immunohistochemistry (IHC) approaches and predicts the response to tumors with a high mutational burden as measured by MSI or mismatch repair deficiency (dMMR).

Data continue to demonstrate that tumor foreignness, immune competency, and tumor/immune interactions are all related but biologically distinct concepts whose significance varies dramatically between disease types and therapeutic approaches. Additionally, the specific weighting of these factors is expected to vary significantly between individual patients in a cohort. Thus, there is no one-size-fits-all approach to profiling a patient, and patient testing will most likely include a number of content methods to determine the tumor foreignness and immune competency of specific patients.

Tumor signatures being investigated include genetic information on the tumor as well as tumor microenvironment signatures to describe "hot" tumors, which have active inflammation. For example, CGP using next-generation sequencing approaches enables the evaluation of TMB (FoundationOne CDx) as a surrogate to MSI or dMMR. The newly developed IFN-γ signature can be delivered by many methods of RNA expression panel profiling (eg, NanoString nCounter).

IHC, however, remains the only method capable of delivering the context-specific information required to deduce tumor/immune interactions. This approach examines tumor-infiltrating immune cells in a variety of ways, such as measuring their quantity and, most importantly, measuring the spatial relationships between these cells with tumor cells and other specific types of immune cells. Currently there are approximately 90 active or enrolling IO trials utilizing tumor-infiltrating immune cells as biomarkers. The number of trials describing the use of this end point has increased tenfold in the last 5 years. According to DeciBio, of these trials, approximately half are early phase 1 and phase 2 trials, with the other half being later-stage phase 2 and phase 3 trials.

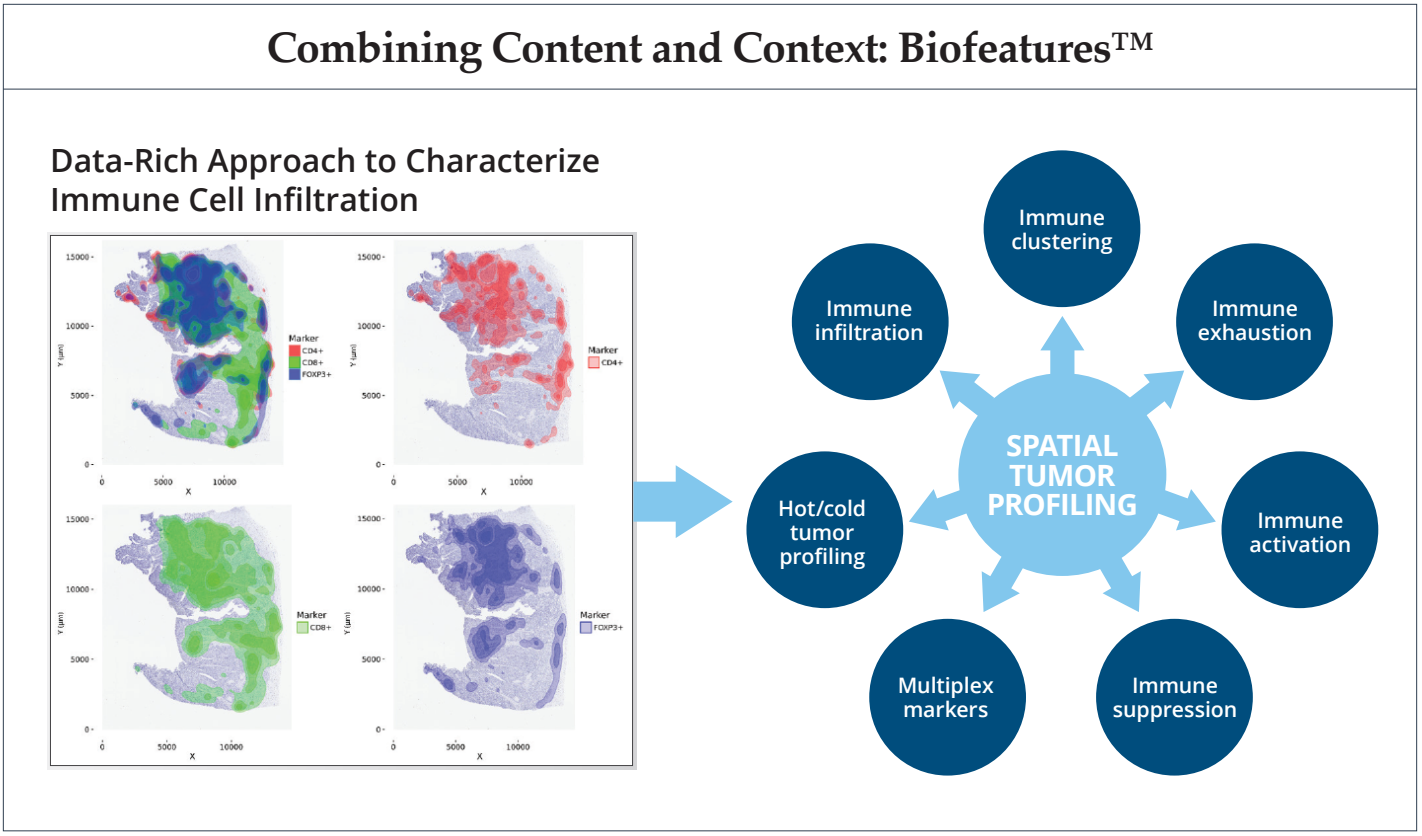
To meet the challenges of measuring tumor/immune interactions to support companion diagnostics, a simpler, more unifying approach to IHC tissue context biomarker analysis is needed. Flagship Biosciences's Computational Tissue Analysis (cTA®) platform provides a computational approach for solving the key problems of variability, lack of precision, and insufficient clarity in the relationship between test results and clinical outcomes. Flagship developed the cTA platform to simplify tissue context biomarker analysis and determine the cutoff values that define a positive result.

Flagship's cTA uses proprietary computer algorithms that quantify IHC-based biomarker content from whole-slide images of patient biopsy samples. By combining traditional IHC methods and stains with digital pathology approaches, cTA can provide more precise data that can better predict contextual relationships between biomarker expression and treatment outcomes. A more precise value for biomarker content enables more accurate patient selection for IO drugs without the complexity limitations imposed by manual pathology approaches. The cTA platform can also elucidate more complex biomarker expression patterns

and create multivariate signatures that increase the predictive ability of the biomarker tests that accommodate the emerging diagnostic strategies required to support the next generation of IO drugs.

By integrating the cTA tools into existing clinical pathology workflows, methods to support IO drugs can be executed in ways that are impossible using conventional pathologist interpretation (Figure 2).

Figure 2: Combining Content and Context: Biofeatures



Enabling regulatory paths

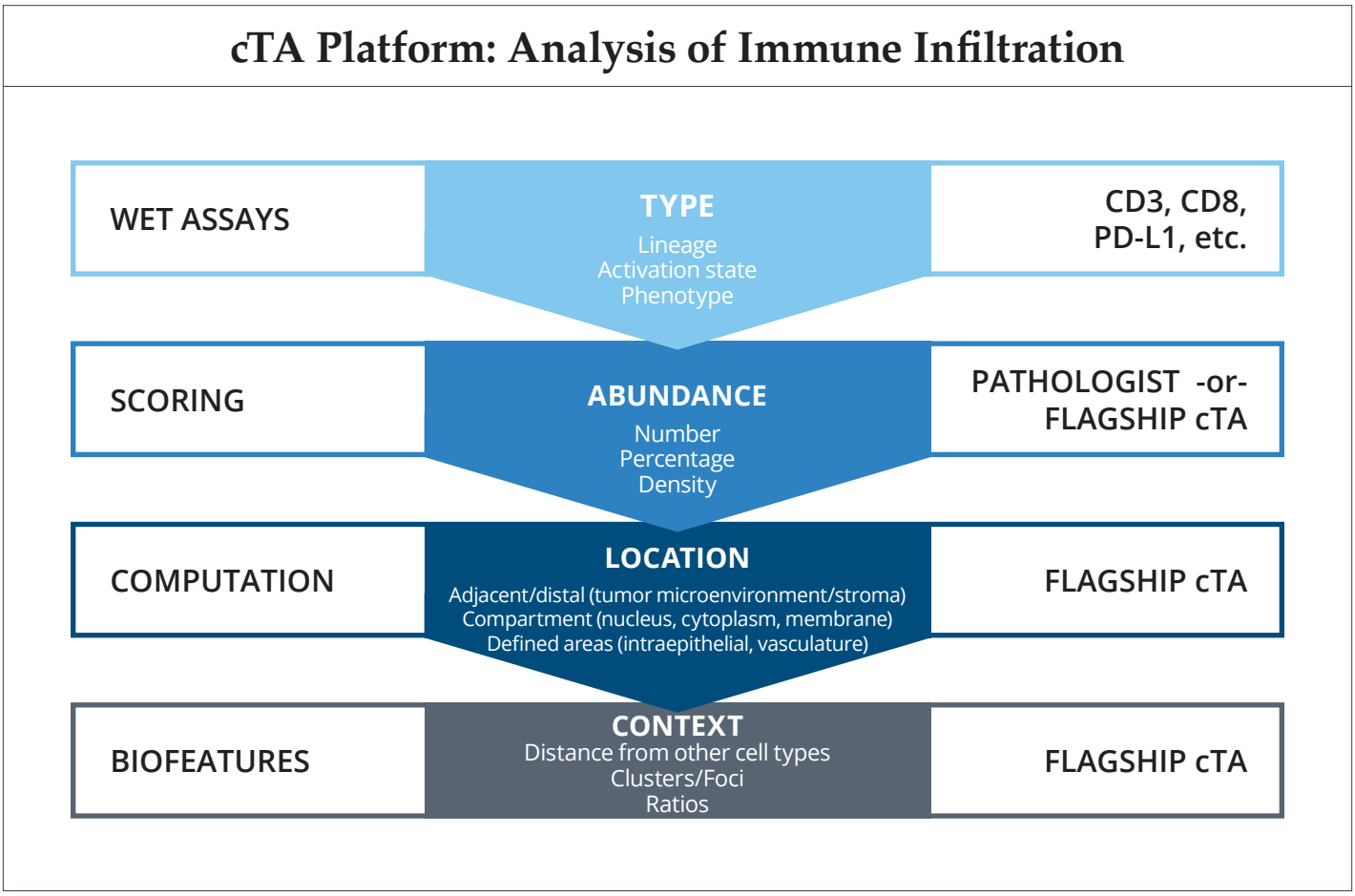
Technologies that simplify readouts of complex biology will be essential to the practice of medicine. While many research applications for these approaches exist today, bringing these types of tools into a regulated diagnostic setting to support the patient care decision process will require the adoption of new technologies that can objectively, quantifiably, and reproducibly report data from patient samples that reflect the biological complexity of the disease and the immunotherapy target.

To date, only PD-L1 IHC tests have earned FDA approval as a companion/complementary diagnostic to support immunotherapy treatment decisions. While the MSI/dMMR testing is included as part of the Keytruda tumor agnostic indication labeling, the actual tests used have not gone through the process of FDA approval yet and are used as laboratory-derived tests. Support of FDA-approved diagnostic tests, however, are postmarketing commitments for the approval. The biological complexity that

these tests evaluate will certainly bring regulatory and product development complexities as these sophisticated technologies meet new challenges and bring new unknowns in front of regulatory agencies.

In contrast to the newer genomic methods, the cTA platform developed by Flagship is more familiar to regulatory agencies. The cTA platform builds on standard methods for IHC analysis of a tumor biopsy sample on a glass slide and uses a conventional slide scanner to obtain a high-resolution image of the entire slide. There are already over 20 digital pathology/tissue image analysis clearances. Similarly, Flagship's cTA platform does not require any special IHC workflow or complex imaging, enabling a smooth workflow that can be directly validated and used in the clinical setting. Flagship's patented approaches are applied to the common IHC staining and whole-slide imaging methods that are part of the traditional pathology laboratory workflows of chromogenic/bright-field staining and slide scanning. The cTA platform then accesses the image automatically and collects data from every cell to create a data-rich profile of the entire tissue slide, delivering a summary score. This approach has the benefit of improving simplicity, efficiency, and reproducibility and simplifying validation methods for clinical trials (Figure 3).

Figure 3: cTA Platform: Analysis of Immune Infiltration



Conclusion

The shift in focus from a single biomarker to a complex combination of biomarkers that affect multiple biological pathways in IO requires patient selection approaches that integrate sophisticated diagnostic methodologies with scientific expertise and interpretation. This integrated scientific approach across multiple technologies and methods can be consolidated into meaningful reported endpoints associated with clinical outcome, which is primed to be the standard for the clinical development of IO clinical approaches. It will be important to understand and evaluate which technologies alone and in combination are best suited to appropriately select patients who will benefit from a specified therapeutic strategy. Flagship's cTA platform can unlock the full potential of existing (eg, PD-L1) and future (eg, CD8) IHC testing to augment and improve the predictive performance of these assays as part of comprehensive patient profiling for precision medicine.
