Executive Summary:

- The explosion of immuno-oncology (I/O) therapies has created a complicated, dynamic, and competitive landscape that is focused on patient selection for drug efficacy and differentiation.

- Understanding both immune content and immune context is critical for differentiating patient response to enable a patient selection strategy.

- A novel Computational Tissue Analysis (cTA™) platform can deliver data-rich tissue context information to support I/O drug development and provide clarity to diagnostic decisions, despite the complexity of tissue biomarkers.

- The use of standard immunohistochemistry (IHC) assays and standard slide scanning to support cTA-based diagnostics simplifies the approach and provides for a clear regulatory pathway.
Personalizing the Cancer Diagnosis

The success of immuno-therapeutics in oncology has introduced a truly novel, targeted, and personalized approach to treating cancer. The promise of this new class of drugs for immuno-oncology (I/O) is generating much hope and excitement among clinicians and patients, and in the pharmaceutical and diagnostics industries. Immunotherapy’s novelty lies in the fact that it does not directly target tumor cells, but instead harnesses the cell-killing power of the immune system to combat cancer. This approach makes it possible to overcome cancer’s long-elusive ability to avoid immune detection and empowers a patient’s T cells to identify a tumor and target it for destruction. The result can be a potent, long-lasting anti-tumor response.

A critical aspect of this new targeted I/O treatment paradigm involves the use of biomarker-based companion or complementary diagnostics (CDx) to determine the probability that an individual patient will respond to treatment. Clinicians can not only use these diagnostic tools to select patients who are most likely to be good candidates for a particular immunotherapeutic drug, they can also use them to identify patients that have a low probability of benefiting from a particular immune checkpoint inhibitor. This spares probable “non-responders” from exposure to these powerful immune-modulating agents, and saves the cost of treatment.

The FDA-approved biomarker-based diagnostic assays that currently support the use of I/O drugs are useful tools for stratifying patients as probable high or low responders. However, this heterogeneous disease also requires comprehensive knowledge of the complexity of each individual patient’s immune system to improve predictive tests and realize the full potential of therapy. In contrast to the classic paradigm of targeted therapies, which relies on a single target/single biomarker approach, the patient response associated with expression of the biological target of I/O therapy is subject to many other factors that are dependent on tumor-host immune interactions.

The patient response profiles observed with the first-generation checkpoint inhibitors that target PD-L1 or PD-1 clearly reflect this. Patients that are diagnostically positive have widely varying responses to treatment; whereas treatment of diagnostically negative patients with the same drug can still frequently achieve unexpected efficacy. Thus, the contribution of immune system status to treatment outcome encumbers the ability to rely on any one single predictive testing approach. Instead, it requires that for each individual drug and disease there is a clinically proven, specific patient selection strategy using a unique testing and scoring approach, regardless of the similarity between therapies and tumor types.

Putting the Tumor in “Immune” Context

The first generation I/O drugs have shown us that no single biomarker or diagnostic approach will be sufficient to support cancer immunotherapy, as the factors that contribute to tumorigenesis, different features of the disease, and possible treatment approaches are too numerous. Understanding key characteristics of the tumor micro-environment that regulate the immune response is crucial to be able to predict patient response. This is an essential part of understanding what drugs to use in an individual patient, and how to use them to modulate the immune system for optimal response.
As we move into a new era of treating cancer using immunomodulatory drugs to stimulate a patient’s immune system to destroy a tumor, the current “immune content” diagnostic model no longer provides enough information to support treatment decision-making. The components of the immune content model, outlined in Table 1, contribute information derived from genomic and fluid-based analyses. These can identify cancer-associated mutations as well as gene and protein expression profiles of tumor and immune cells using an array of molecular, phenotypic, and omics-based diagnostic strategies. These diagnostics can only provide a portion of the information necessary to make effective treatment decisions.

A new strategy is to use tissue context-based biomarker assays in parallel with tissue content profiling assays to deliver measures of immune content and immune context that best predict a patient’s likelihood of responding to a specific therapy. Analysis of tumor-derived and immune cell-derived biomarkers needs to encompass a broad range of test strategies that deliver key information about immune-related content (such as neoantigens, cytokines, and general immune response) as well as the immune context (immune cell phenotyping, infiltration, and proximity) of each patient’s tumor (Figure 1). Importantly, thought leaders emphasize the use of immunohistochemistry (IHC) assays to measure immune checkpoint proteins. The context in which proteins are expressed provides the understanding to determine key cell-cell co-receptor interactions that drive the patient-specific immune response.

To meet the demand for supplying tissue context data to match data-rich tissue content approaches, Flagship Biosciences has developed the Computational Tissue Analysis (cTA™) platform as the core of its interdisciplinary, team-based solution. When this proprietary technology is applied to IHC-stained tissue biopsies, it delivers a Biofeatures™ profile, which is a data-rich description of tissue context measurements that describe the tissue and its cellular components. In the I/O setting, the Biofeatures profile provides a detailed tissue-based portrait of the tumor and tumor microenvironment. This profile can be used to characterize the relationships between immune and tumor cells and their associated biomarkers. The high-complexity, cell-based data profile and analytical platform improve biological interpretation to realize the full breadth of information captured in a tissue biopsy. This patient-specific profile characterizes the tumor-immune interactions that are predictive of response to a particular cancer immunotherapy.

### Table 1: Diagnostic Assays to Support Immune Content Assessments

| • Genome sequencing to identify cancer-related mutations |
| • Transcriptomics to assess multigene signatures and epigenomic changes |
| • Functional tumor markers |
| • Detection of circulating tumor cells (CTCs) and cell-free tumor DNA, RNA, and exosomes |
| • Phenotypic and functional characterization of T cells |

**Targeted Therapy with Twists and Turns**

The positive clinical trial results and drug approvals achieved with the first crop of PD-1/PD-L1-based drugs for different tumor types are tempered...
by the tremendous uncertainty surrounding the diagnostic strategies needed to deliver the precision medicine approach itself to fulfill the promise of I/O therapies. The field of I/O is rapidly commanding new treatments and associated diagnostic products to match the scientific discoveries of the hundreds, if not thousands, of I/O therapeutic trials underway. The regulatory landscape is struggling to understand how to meet the diagnostic needs of clinicians who must be able to differentiate between and select from the immunotherapies and companion and complementary diagnostics best suited to provide the comprehensive solution needed to help them navigate the path from diagnosis to treatment for each patient.

Therapies that target new checkpoints for immune regulation (such as LAG3, Tim3, GITR, CD40, OX40, 4-1BB, and others) and rational combination therapies (such as CTLA4/PD-L1, IDO-1/PD-L1, and PARP/PD-L1) are creating a playing field that is changing so quickly that decision-makers on the drug development side struggle to define an optimal path forward for their drug portfolios. Increasingly, drug developers are trimming their development portfolios in areas where a competitive landscape has raised the bar for efficacy in the largest indications. They are instead seeking narrow, opportunistic indications based on unmet need (such as Merck KGaA's FDA-approved drug avelumab for the rare disease Merkel cell carcinoma); or are pursuing combination therapies in specific indications that stand to show more promise than PD-1/PD-L1 monotherapy (such as AstraZeneca's pursuit of durvalumab and tremelimumab in bladder cancer).

While researchers and developers are optimistic about these strategies, there is much confusion as to how to use combinations of therapies to safely and effectively administer immunomodulatory drugs. This can require a tricky balancing act involving “stepping on the gas” with co-stimulatory targets (such as 4-1BB or OX40) in conjunction with “releasing the brake” with co-inhibitory targets (such as PD-1/PD-L1). The ability to successfully implement an effective strategy is confounded by the importance of distinguishing “hot” vs “cold” tumors in each patient, and the ability to manipulate this state in the patient.

For a more complete picture of a patient's immune status as it relates to the cancer, again, immune context information is needed. Together, immune content and immune context data can give clinicians the knowledge they need to understand what is going on within the tumor microenvironment and to determine how best to leverage immunotherapeutics to launch a targeted attack on the
cancer. The tumor microenvironment contains both tumor and immune cells and is influenced by the effects of chemokines and various other signaling molecules and by the infiltration of cells from surrounding tissues. The current use by I/O CDx of IHC approaches to measure PD-L1 in tissue context, and the varied interpretation of tumor versus tumor microenvironment expression of PD-L1, validates the need to rely on context-based measurements for optimal patient selection.

Immune context comprises three main factors: checkpoint inhibition; immune infiltration; and immune effector trafficking. As these are all mediated by interactions between tumor and immune cells and by signaling events that take place within the tumor microenvironment, they can be analyzed by detecting and measuring the levels of relevant tissue context biomarkers in tumor biopsy samples using traditional IHC techniques. The recent rapid increase in both the number of I/O tissue context biomarkers and of clinical trials for cancer immunotherapeutics coupled with tissue biomarker-based diagnostic assays is clear evidence of the expanding role these biomarkers are playing in cancer treatment decision-making.

Over the next 10 years, we expect immunotherapeutic drugs and their CDx to become more diverse. In 2017, we will likely see approvals in more types of cancer, with many new drugs still relying on detection of PD-L1. As the numbers of I/O therapies and indications continue to climb, though, based on current trends in the clinical trial setting, we can expect that a greater percentage of new drugs will be part of combination approaches and will also target newer second-generation checkpoints rather than PD1/PD-L1. Consequently, the range and diversity of tissue biomarker-based CDx will multiply dramatically.

An analysis of the clinical trials database, clinicaltrials.gov, over the past two years reveals that the number of I/O late stage (phase 3/4) trials using tissue context biomarkers nearly doubled, while the number of early stage (phase 1/2) trials tripled. This difference reflects the growing importance of this approach and indicates that tissue context biomarkers are increasingly becoming an intrinsic element in I/O clinical trial design. In North America, PD-L1 is the basis for the biomarker assays used in 55% of ongoing phase 3/4 studies, compared to only 22% of phase 1/2 studies. Once again, this suggests that the repertoire of I/O biomarkers is expanding and that novel biomarkers are being used in many early-stage trials. Globally, of the approximately 60 phase 3/4 clinical trials using tissue context I/O biomarkers, more than half rely on PD-1/PD-L1. In contrast, only about a quarter of the 600 phase 1/2 trials underway are using PD-L1 as a biomarker to predict treatment response.

Adding Clarity to the Complexity of Tissue Biomarkers

As our understanding of how to modulate the patient’s immune system for better response improves, so will diagnostic measures for predicting patient-specific responses. Application of these
diagnostics to patient care will certainly further increase complexity, but will also yield benefits for patients. The pace and magnitude at which this is likely to occur will undoubtedly overwhelm the FDA’s current review paradigm and the agency’s capabilities to support timely regulatory oversight.

A simpler, unifying approach to tissue context biomarker analysis is needed. Flagship Biosciences’ 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 to determine the cutoff values that define a positive result.

The cTA platform uses proprietary computer algorithms to quantify IHC-based biomarker content from whole slide images of patient biopsies. By combining traditional IHC methods and stains with digital pathology approaches it can provide more precise data that can better predict contextual relationships between biomarker expression and treatment outcomes. A more precise understanding of biomarkers in context enables more accurate patient selection for I/O drugs without complexity limitations imposed by manual pathology approaches. The cTA platform can also illuminate more complex biomarker expression patterns and elucidate multivariate signatures that increase the predictive ability of the biomarker tests that accommodate the emerging diagnostic strategies required to support the next generation of I/O drugs. By integrating the cTA tools into existing clinical pathology workflows, methods to support I/O drugs can be executed in ways that are impossible using conventional pathologist interpretation.

The method developed by Flagship uses standard methods for IHC analysis of a tumor biopsy sample on a glass slide, and a conventional slide scanner to obtain a high-resolution image of the entire slide. No special IHC techniques or complex imaging is required, enabling a smooth workflow that can be directly validated and used in the clinical setting. The cTA software 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. Flagship’s patented approaches are applied to the common IHC staining and whole slide imaging methods that are part of the traditional pathology lab workflows of chromogenic/brightfield staining and slide scanning. This has the benefit of improving efficiency and reproducibility while it simplifies validation methods for clinical trials.

Flagship’s integrated team of pathologists, biologists, and application scientists works with drug developers to leverage the cTA platform to acquire quantitative, reproducible data that can provide a clear understanding of the biological mechanism of action of a drug. This information is crucial for validating a patient selection strategy in clinical trials. This, in turn, enables drug developers to populate a clinical trial with subjects that are much more likely to respond to an experimental treatment and can greatly increase the chances of demonstrating efficacy.
Conclusions

Cancer therapy continues to evolve, with I/O offering a new approach to treatment that holds significant promise. When cancer is detected, patients undergo a variety of tests to aid in diagnosis, prognosis, and treatment decision-making. The need for multiple, diverse diagnostic approaches to guide treatment decision-making in oncology is not likely to change, and the emergence of groundbreaking immunotherapeutic drugs that work by stimulating the immune system to attack a tumor is only adding to the diagnostic complexity. Biomarker-based CDx are essential to guide treatment decisions and are a valuable tool to aid in patient selection for clinical testing of new I/O drugs. Tissue-based biomarkers are proving to be invaluable for helping to stratify patients based on the immune context of a tumor and the patient-specific characteristics of the tumor microenvironment as well as interactions between tumor and immune cells and between a tumor and surrounding tissues. Tissue context biomarkers can capture this diversity and complexity, and biomarker-based CDx can drive individualized treatment decisions.

Flagship Biosciences’ cTA platform can help leverage and clarify that data-rich complexity of tissue context biomarkers. cTA quantifies cell-based biomarker content, interprets the data using an objective and consistent scoring method, and delivers more precise, actionable results to help guide patient selection and treatment decisions in immuno-oncology. The platform can analyze and interpret the data in light of the full range of available assays across assay platforms, regardless of the specific test used.

Flagship’s scientific expertise in clinical oncology, diagnostics, and computational analysis, combined with an interdisciplinary team and its experience navigating a challenging and rapidly evolving regulatory environment, can support a successful I/O strategy and deliver a path to commercialization. Flagship’s team works closely with clinical decision-makers so they can benefit from an innovative solution that helps them complete the path from diagnosis to treatment, matching patients with the best available therapeutic option. Flagship has collaborated with over 100 pharma customers who are utilizing its expertise to explore cTA-based strategies to enable their drug approval.