DEFINIENS®
the tissue phenomics company

The Power of Predictive Quantitative Tissue-Based Diagnostics in Cancer Immunotherapy




The American Society of Clinical Oncology named Immunotherapy 2.0 "Advance of the Year" at its 2017 Annual Meeting in Chicago, IL. This recognition comes after decades of incremental progress in our understanding of how the human immune system interacts with cancerous lesions. It also is the culmination of recent successes with checkpoint inhibitor therapy that promised dramatic changes in clinical practice and the approach to cancer treatment. What started with cutaneous melanoma in 2011 is now rapidly showing remarkable results also in other cancer types. Just over the past year, the Food and Drug Administration (FDA) approved the use of checkpoint inhibitors in five additional cancer indications: lung, head and neck, bladder, kidney, and classical Hodgkin lymphoma.

Despite the recent advances, many patients will not benefit from immunotherapy because of their current immunoprofile, with some experiencing a rather short-lived treatment effect or even no change at all in the clinical course of their disease. At the same time, some treated patients are at risk of developing severe immune-related adverse events leading to potentially life-threatening situations.

The identification of patients that are likely to respond to immunotherapy while sparing others from its intolerable toxic effects is therefore imperative for oncologists to make evidence-based decisions and increase the predictive effectiveness of the chosen treatment regimen. Efforts have been focused on finding disease markers that help predict the likelihood of triggering a response to treatment. One of such markers that quickly gained interest due to its association with clinical outcome is "programmed cell death ligand-1" (PD-L1). The importance of PD-L1 testing of a patient's tumor sample was soon realized and led to the first FDA-approved pairing of a companion diagnostic with the therapeutic checkpoint inhibitor Keytruda® (pembrolizumab) for 2nd-line treatment of non-small cell lung cancer (NSCLC) in October 2015. Within just one week, the FDA approved a second option for this indication and treatment line, Opdivo® (nivolumab), yet this time without the restriction of mandatory PD-L1 testing. As a result, the necessity for PD-L1 testing came under intense scrutiny by oncologists, pathologists and patients alike.

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The dispute about the usefulness of PD-L1 testing in lung cancer was settled in the fall of 2016 when the CheckMate-026 clinical trial of 1st-line treatment with Opdivo failed to improve progression-free survival (PFS) over physician's choice of chemotherapy. In contrast, Keytruda proved to be superior to chemotherapy in the 1st-line setting in both PFS and overall survival (OS) and gained FDA approval for the treatment of NSCLC patients that had been tested for PD-L1 with the companion diagnostic test for Keytruda. It was now evident that PD-L1 testing and selecting the right patients for immunotherapy was essential to predict clinical benefit and improve therapy success. Development programs for other checkpoint inhibitors also had to come to grips with this reality. In early 2017, the IMvigor211 clinical phase III trial of Tecentriq® (atezolizumab) in patients with urothelial carcinoma missed its primary endpoint of improving overall survival – again in a cohort that had not undergone rigorous testing to include only patients with high PD-L1 expression levels in their tumors.

The discussion about the most suitable predictive biomarkers for immunotherapies has so far been dominated by PD-L1 and testing of its expression levels by immunohistochemistry (IHC) in tumor tissue and sometimes in conjunction with immune cells. However, PD-L1 as a robust biomarker has many limitations, most of all its poor Negative Predictive Value (NPV), i.e. patients tested negative for PD-L1 still benefit to some extent from checkpoint inhibitor therapy. And even in the population of patients with advanced NSCLC and very high PD-L1 expression levels (i.e. a PD-L1 tumor proportion score of 50% or greater), response rates to Keytruda hover only slightly above 52%.

Clearly PD-L1 alone does not represent all of the relevant predictive parameters within tumor tissue when it comes to immunotherapy. In the ongoing debate over which tumor and immune features need to be analyzed when selecting patients for checkpoint inhibitors, several suggestions have been made such as an interferon gamma (IFNγ) gene expression signature. Despite its association with overall survival [1], this gene signature serves as a surrogate marker for overall effector T-cell activation status in the tumor and has limited utility in capturing the complex tumor microenvironment.

We know today that different types of tumor-infiltrating immune cells such as T-cells, B-cells, natural killer cells (NK cells), dendritic cells and mast cells interact with tumor cells but also the stromal components of the tumor, i.e. cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs) and endothelial cells. The complex interactions within the tumor tissue architecture and the spatial relationship (e.g. proximity) of its immune components harbor indispensable contextual information that is not adequately represented by an expression signature. This is also true for intra-tumoral heterogeneity and local micro-niches within the tumor environment that can only be assessed in the context of tumor tissue [Figure 1]. The comprehensive analysis of tissues with immunohistochemistry (IHC-) based multiplex image analysis technology such as Definiens' Tissue Phenomics® is able to capture and extract complex distribution patterns of immune components within the tissue architecture, resulting in the emergence of entire regional context maps – so-called "heatmaps" - that harbor a wealth of predictive information. Consequently, tissue-based diagnostics that can capture the detail of the tumor microenvironment will continue to play a role in immunotherapy treatment decisions.

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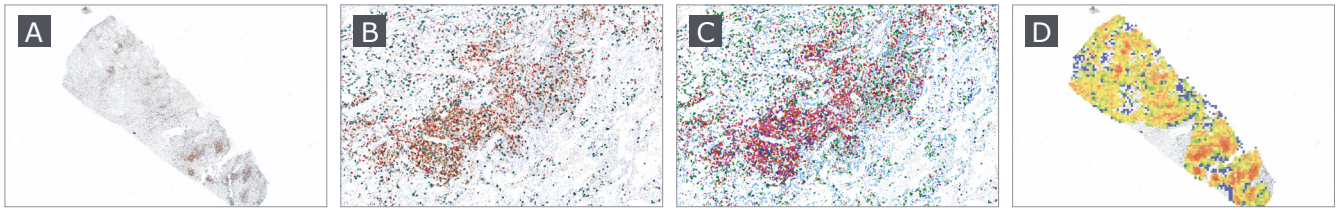

Figure 1: Quantitative image analysis of tumor tissue reveals the complex interaction of its immune components
Original multiplex IHC image of resected tumor tissue (A) and higher magnification image of area outlined in red (B). CD8-positive cytotoxic T-lymphocytes are labeled green, FoxP3-positive regulatory T-cells are labeled purple (B). Automated image analysis of B and classification of cells. CD8+ cells are shown in red, FoxP3+ cells are shown in green (C). Resulting regional context map for CD8/FoxP3 reflecting the inhibition status of cytotoxic T-lymphocytes (D). Distance of CD8+ cells to the nearest 3 neighboring FoxP3+ cells is depicted in a color gradient ranging from close contact (red) to a distance of 300 µm (green) and beyond (blue).
Images courtesy of Mosaic Laboratories

Tissue Diagnostics and Immunotherapy

Tissue analysis provides rich contextual information which is obscured by molecular techniques. From the earliest FDA approved CDx tests such as Herceptest® in 1998, tissue and cell compartment context has played a critical role in constructing accurate and reproducible test interpretation paradigms. As shown in Figure 2, the approval of this test marked the beginning of a paradigm shift towards personalized medicine, with CDx tests playing a critical role in securing first-line therapy designations and identifying patient populations most likely to benefit from targeted therapeutics. An example of this is the DAKO PD-L1 PharmDx® test for the Merck drug Keytruda, which has underscored the economic impact of tissue based CDx tests and played a critical role in the growing number of indications secured for Keytruda vs. competitive compounds. Maximizing the performance of CDx tests for IO therapies is therefore of tremendous interest to all parties involved, a task for which image analysis is uniquely suited. Image analysis can quantify biomarkers reproducibly on a continuous scale, making determination of cut-points more precise and less subject to visual bias effects, search satisfaction bias, and other limitations of manual assessments including technical inconsistencies and pre-analytical variabilities.

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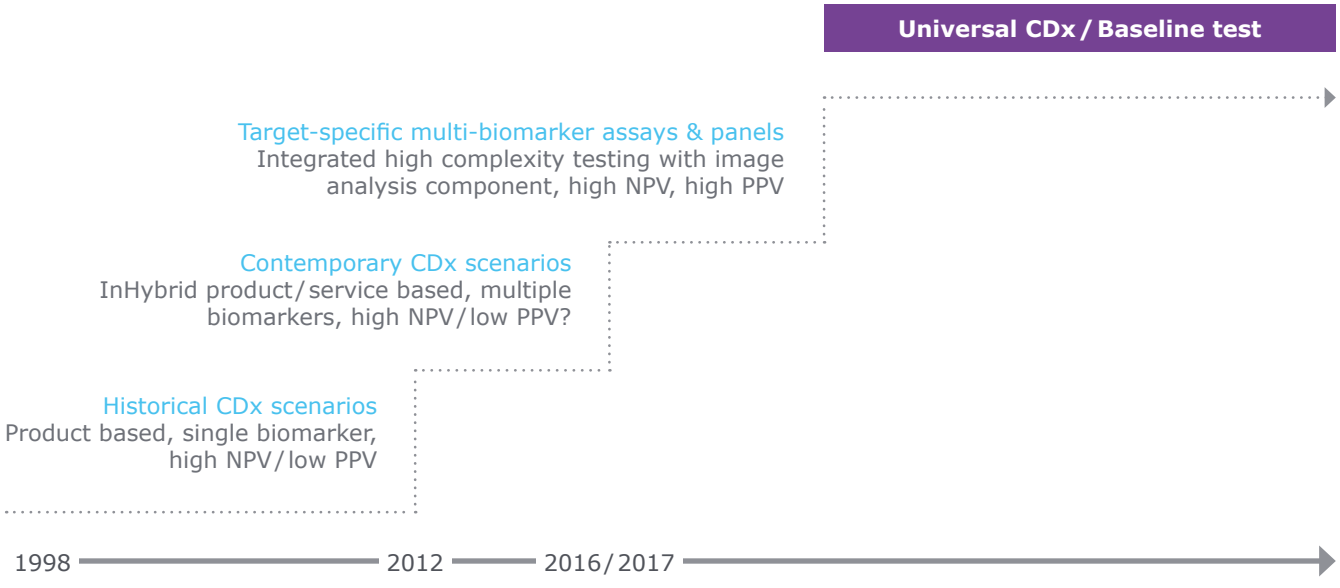

Figure 2: The Evolution of CDx Approaches in Oncology. Beginning with the approval of Herceptest in 1998 as a diagnostic test for Herceptin, the trend in oncology diagnostics has been towards increasing sophistication, to enable the best possible identification of patient populations for targeted therapies. The future of diagnostic tests are likely to grow in complexity still further, to generate tests which cover multiple drugs and indications with a single panel-based test. [2]

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Additionally, quantitative spatial measurements such as cell density and cell-cell distances are difficult if not impossible to determine without image analysis techniques. They have proven clinical utility in tests such as the Immunoscore®, which outperforms standard TNM staging as a prognostic test for colorectal cancer. Furthermore, Definiens' Tissue Phenomics approach, a combination of advanced image analysis, data mining, and machine learning techniques, has already demonstrated the ability to out-perform manual interpretations of the diagnostic test for Imfinzi (an anti-PD-L1 antibody developed by MedImmune/AstraZeneca) in NSCLC. Tissue Phenomics was also successful in identifying responders to the Bristol-Myers-Squibb anti-CTLA4 drug Yervoy® in melanoma patients, where a manual interpretation of the tissue-based test proved impossible.

As the IO field continues to move in the direction of combination therapies, this lack of a viable manual interpretation paradigm will likely become the rule rather than the exception. Multiplexed tests will have to be created anew for each combination, or multiple tests run and interpreted serially, either of which has serious implementation drawbacks in terms of time, cost, and availability of sufficient tissue for analysis. How will physicians effectively choose from the broad menu of possible IO therapeutic approaches available in 5 or 10 years? Clearly, the information content needed to select from such a menu points to multiplexed tests, and tissue availability and cost constraints will determine their adoption and use. While gene expression analyses could cover almost an endless number of biomarkers to capture a broad range of possible combination therapies, they would be blind to tissue context which however is central to identifying responders to IO monotherapies, and determines the prognostic capabilities of IO cell population markers such as in the Immunoscore. Therefore, a standardized panel of IO-relevant tissue biomarkers analyzed by image analysis, alone or in combination with gene expression analyses and other clinical information, will form the basis of best-in-class tests designed to select patients for a broad range of therapeutics [Figure 1] By combining knowledge gained in monotherapy trials and continuously aggregating new trial results made comparable through the use of such a standardized panel, the test could be built and refined over time into one capable of supporting clinical decision platform for an entire drug portfolio.

Next Generation of Companion Diagnostics

As shown in Figure 3, the majority of CDx tests for targeted therapies follow a one-drug/one-test paradigm. While this level of precision medicine represents an improvement over the SOC being determined solely by population-level results, it cannot continue to meet the needs of physicians and patients who in the coming years will be faced with dozens of monotherapy options and geometrically increasing numbers of potential combination therapies. Two recent regulatory approvals signal that the FDA is looking ahead to this future with an understanding that the paradigm must evolve: The accelerated approval of Keytruda for MSI-H or dMMR solid tumors regardless of the tissue of origin, and the approval of the Oncomine® Dx test for 4 different lung cancer therapies. These approvals show that the FDA is willing to consider tests which cross indications and cover multiple therapies, as long as the test is sufficiently robust and provides patient benefit. It is not difficult to envision an extension of the MSI-H test which currently includes a gene expression signature to guide combo therapy selection, or further refinement of the dMMR selection approach with other IHC biomarkers or image analysis. Combining these two tests would already seem to have value in lung cancer for exploring combinations of Keytruda with small molecule inhibitors, and suggest a core IHC panel with an indication-specific gene expression or mutation analysis component could be a viable approach for de-mystifying combo therapy decisions in both exploratory and clinical settings.

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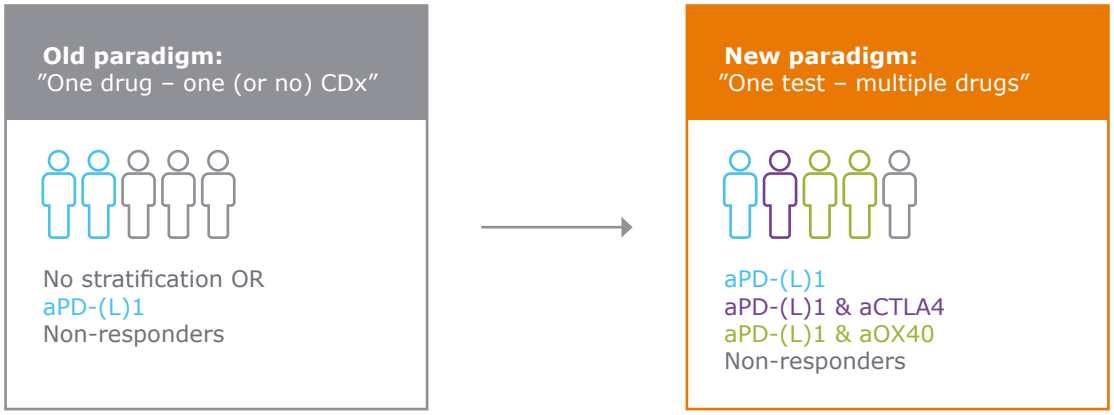

Figure 3: Details of universal CDx. With several therapy options in the market patients and physicians will benefit from new universal CDx to identify treatment options simultaneaously.

Use of a standard panel also allows sufficient comparable data to be compiled for use of machine learning techniques to further refine the selection paradigm, and potentially to deliver actionable biological insights such as patient profiles associated with resistance to IO therapies, recurrence, or DLTs. Thus, the more forward looking a company is with their study design, data collection, and biomarker strategy also for combination therapies, the sooner all available information to develop comprehensive tests can be leveraged which eventually give them a competitive advantage and patient benefit compelling enough to garner regulatory approval. While no image analysis-based class III IVD has yet achieved FDA approval, this is likely to change in the near future with the proven value of tissue context in identifying responders to IO therapies and the need for high dimension tests to cover a growing number of therapeutic options. Besides the obvious benefit to patients and physicians, the economic benefits of standardized diagnostic panels able to cover an entire drug company's portfolio, or even cross company portfolios, would be considerable.

The Value of Immunoprofiling

The advances in the development of new cancer therapies that utilize the anti-tumor immune response hold a great deal of promise for patients and has generated a great deal of excitement among life science industry professionals and investors. Checkpoint inhibitors have successfully been used to treat patients with a number of different cancer types, but the currently approved therapies only work in a subset of patients: those in which the tumors are already "inflamed". Pharma pipelines are full of new drug candidates meant to remove the "brakes" from the immune system in hopes of broadening the patient pool for PD-1 and PD-L1 therapies.

While the explosion in the number of potential future treatment options is positive news for patients, it causes challenges amongst health care providers and payers. Providers will soon have a number of treatment options available to their patients that are indicated only for a select patient subpopulation, and the only way to identify the right patient is through a companion diagnostic. If the CDx testing paradigm of today still exists in the future, it will be a step-wise approach (e.g., single gene test for EGFR, then if negative, a single gene test for ALK, then if negative, a single IHC test for PD-L1) that is unsustainable for reasons related to feasibility (tissue fatigue), logistics (increased time to treatment), and cost. Some diagnostic companies have developed more comprehensive tests that allow providers to obtain the greatest amount of information as quickly and as cost-effectively as possible, such as Thermo Fisher's Oncomine and Foundation Medicine's FoundationOne®. However, while these tests are great for identifying the best small molecule therapy option for patients, they do little to profile the immune system to select a patient for mono or combo immunotherapies (though some evidence is being generated to support genomic sequencing of tumor samples to predict response to immunotherapies).

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Image analysis-based companion diagnostics pose a number of advantages over manual IHC diagnostic tests, and given the ability to better contextually profile the tumor microenvironment, it has advantages over other -omics tests:

- **Automated**
  The automated analysis of multiplexed IHC allows the lab to increase their volumes and be more efficient by optimizing workflows
- **Objective**
  Manual IHC reads are inherently subjective, and image analysis removes any subjectivity from the read, allowing the quality and accuracy of the report to increase
- **Higher reimbursement (compared to manual IHC)**
  Broader applicability of computer-assisted IHC biomarkers support value based re-imbursement discussions with payers

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Advancements of other -omics tests, specifically comprehensive genomic profiling, both maximize and standardize data output resulting from the testing of tumor samples. Many would argue that there is a great deal of value in this maximization and standardization, and the high reimbursement is evidence of that value. This appears equally true for assessment of the tumor microenvironment, particularly for the high-cost immunotherapies that are beginning to flood the market. Many of these drugs cause therapy costs of ~$150,000 per year. Thus, payers may be more willing to pay a certain fraction of this amount for immunoprofiling and genomic profiling to assure physicians have the greatest amount of information for treatment decisions and to avoid unnecessary costs and safety risks of immunotherapies. Furthermore, and most importantly, this information may allow patients to begin with the right IO-treatment earlier in hopes of increasing survival and quality of life.

References

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