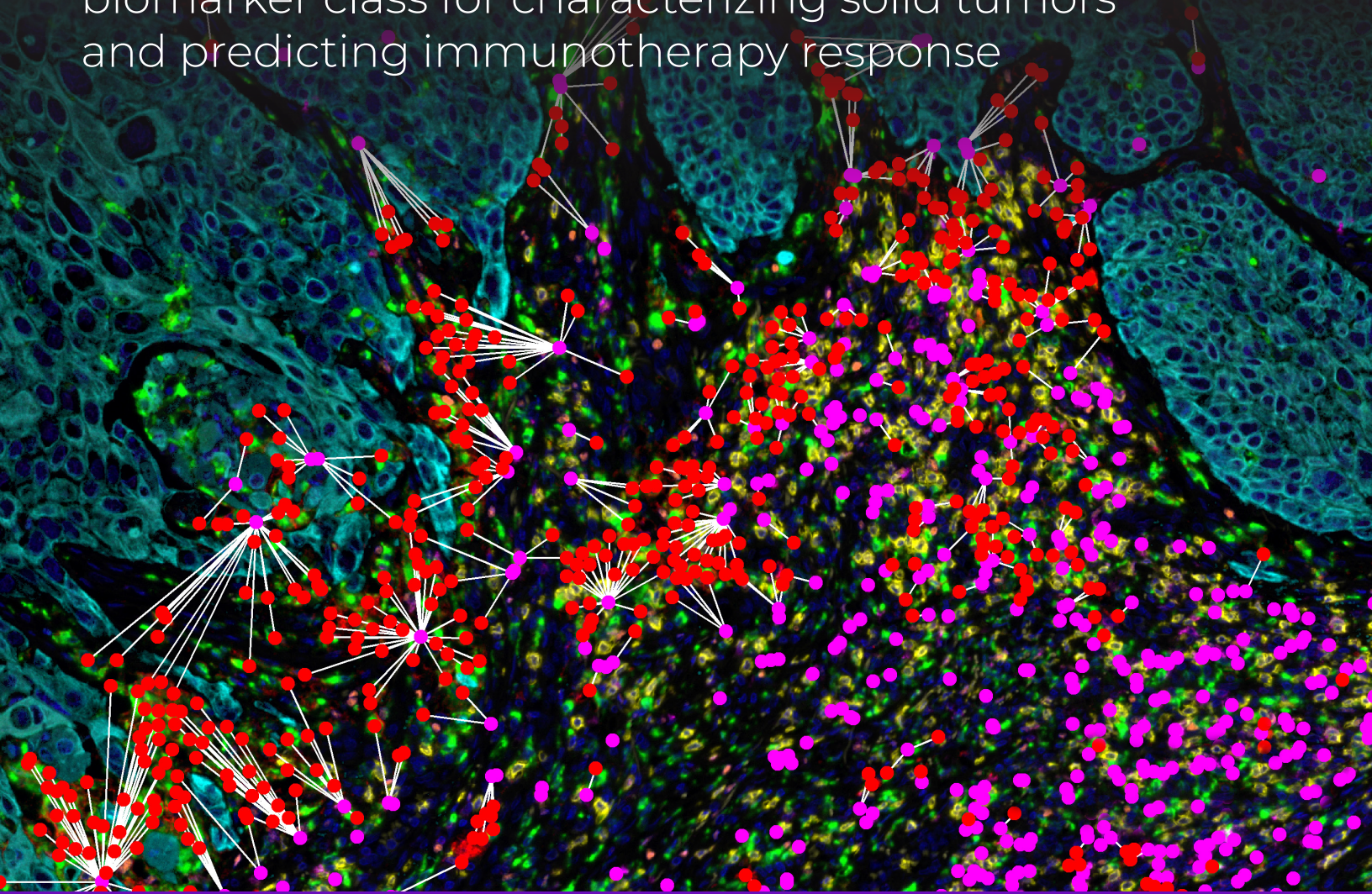# Spatial Phenotypic Signatures: A novel biomarker class for characterizing solid tumors and predicting immunotherapy response

Today, only a minority of patients identified by FDA-approved biomarkers show a positive response to PD-1/PD-L1 checkpoint inhibitor therapy, and not everyone who tests negative ends up doing poorly.[1] This uncertainty is keeping treatment from patients who could benefit, adding to the cost of cancer care for therapy that is not working, and slowing the development of next-generation immunotherapies.

Recent research suggests that the answer may lie in a new type of biomarker, the *spatial phenotypic signature*, which uses multiplex antibody panels to characterize tumor and immune cells by function and state in the context of an intact tissue sample. The different patterns of cell densities and interactions resulting in cell-by-cell maps of the tumor microenvironment (TME) have been shown to be highly predictive of disease outcome and response to immunotherapy.

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SETTING A NEW STANDARD FOR PREDICTIVE VALUE

At the core of the spatial phenotyping process is a technique known as multiplexed immunofluorescence (mIF), a next-generation pathology workflow that generates quantitative and reproducible insights about tumor samples.

A meta-analysis published in *JAMA Oncology* by Lu et al.[2] showed that mIF outperformed the three most widely used techniques – PD-L1 IHC, tumor mutational burden (TMB) based on next-generation sequencing, and gene expression profiling (GEP) – in predicting who will respond to PD-1/PD-L1 immunotherapy.

The study looked at analyses of tumor samples from over 10 different tumor types in 8,135 patients and calculated the performance and predictive value of each type of biomarker. The resulting weighted sROC (summary receiver operating characteristic) curve evaluation showed mIF/mIHC (multiplex immunohistochemistry) to have significantly higher diagnostic predictive value (AUC: 0.79) compared with PD-L1 immunohistochemistry (AUC: 0.65, p<0.001), as well as GEP (AUC: 0.65, p=0.003) and TMB (AUC: 0.69, P=0.049). **FIGURE 1**.

The authors also plotted the success rate seen in individual studies for both predicting responders (y-axis) and identifying likely nonresponders (x-axis). **mIF was the only technique for which the majority of studies, 6 out of 7, fall in the upper right quadrant, indicating high predictive value across both dimensions. FIGURE 2**.[2]
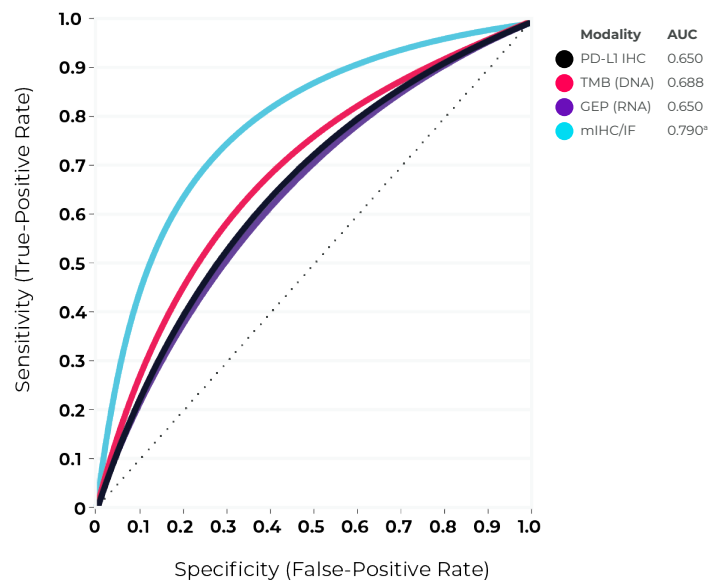

Modality	AUC
PD-L1 IHC	0.650
TMB (DNA)	0.688
GEP (RNA)	0.650
mIHC/IF	0.790[a]

FIGURE 1:
A meta-analysis of different diagnostic techniques for predicting response to PD-1/PD-L1 checkpoint inhibitor immunotherapy showed that multiplexed mIF/mIHC methods had higher predictive value than currently used assays, including PD-L1 immunohistochemical staining (IHC), tumor mutational burden (TMB), and gene expression profiling (GEP).[2]
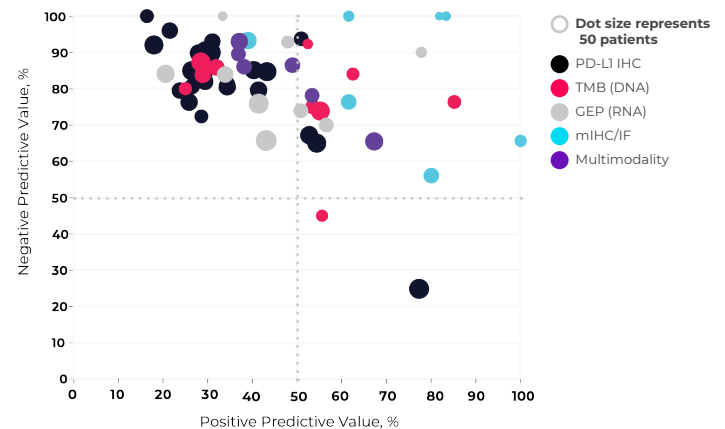

FIGURE 2:
This plot of all studies in the meta-analysis shows that mIF studies (light blue) had the highest combined positive and negative predictive values for response to checkpoint inhibitor immunotherapy.[2]

1. Duffy MJ, Crown J. Biomarkers for predicting response to immunotherapy with immune checkpoint inhibitors in cancer patients. *Clinical Chemistry*. 2019;65(10):1230.
2. Lu S, Stein JE, Rimm DL, et al. Comparison of biomarker modalities for predicting response to PD-1/PD-L1 checkpoint blockade: a systematic review and meta-analysis. *JAMA Oncol*. 2019;5(8):1195–1204.

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mIF: REVEALING NEW INSIGHTS INTO PD-1/PD-L1 BLOCKADE RESPONSE

Multiple studies show that using mIF-based platforms for measuring PD-L1 expression can produce more quantitative and accurate results, which can improve predictive capability. In addition, mIF allows for the simultaneous measurement of PD-L1 expression with other markers to produce a higher predictive value than is produced by each biomarker alone.[3,4,5]

Vanhersecke et al.[6], demonstrated this in a pan-cancer study across 11 different tumor types where analysis of PD-L1 expression was paired with measurements of tertiary lymphoid structures (TLS) and CD8+ lymphocyte density. An mIF assay combining CD4, CD8, CD20, CD21, and CD23 was used to identify TLS and distinguish mature (mTLS) from immature (iTLS) forms.

While the presence of mTLS+ correlated with improved objective response rates, progression-free survival, and overall survival in all patient groups, the results from combining mTLS with other values were revealing. In patients with PD-L1+ tumors, the objective response rates to immunotherapy were 69.2% in mTLS+ patients and 40.3% in mTLS- patients. In patients with PD-L1- tumors, the overall response rate dropped to 35.6% in mTLS+ patients and 14.1% in mTLS- patients. Similar differences were seen in progression-free survival and overall survival. **FIGURE 3**.
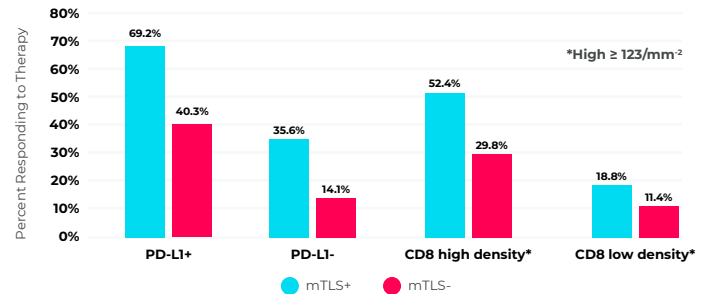

FIGURE 3:
Presence of tertiary lymphoid structures (TLS) was shown to have a high predictive value for response to PD-1/PD-L1 blockade therapy across multiple cancer types, particularly when combined with PD-L1 expression and CD8 T-cell density.[6]

Looking at CD8+, where a T-cell density of $>123/mm^{-2}$ appeared to confer an advantage in terms of all endpoints, significant differences were seen between the mTLS+ and mTLS- cohorts. Among patients exhibiting high CD8 density, response rates were 52.4% when patients were mTLS+ versus 29.8% when patients were mTLS-. In those exhibiting low CD T-cell density ($<123/mm^{-2}$), mTLS+ patients had an 18.8% objective response versus 11.4% in mTLS- patients. **These findings underscore the potential value, in terms of both predictive value and clinical insight, achievable with mIF-based multi-dimensional analyses.**

3. Hofman P, et al. Multiplexed immunohistochemistry for molecular and immune profiling in lung cancer—just about ready for prime time? *Cancers*. 2019;11(3):283.
4. Nguyen QH, et al. Profiling human breast epithelial cells using single-cell RNA sequencing identifies cell diversity. *Nature Communications*. 2018.
5. Berry S, et al. Analysis of multispectral imaging with AstroPath platform informs efficacy of PD-1 blockade. *Science*. 2021;372(6547):eaba2609.
6. Vanhersecke L, et al. Mature tertiary lymphoid structures predict immune checkpoint inhibitor efficacy in solid tumors independently of PD-L1 expression. *Nature Cancer*. 2021;2:794-802.

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A QUEST FOR BETTER PREDICTIVE TOOLS

There are seven FDA-approved cancer immunotherapies targeting the PD-1/PD-L1 pathway at the time of this writing:

MOLECULE	BRAND	COMPANION DIAGNOSTICS*
Nivolumab	Opdivo®	None – one prior patient regimen
Pembrolizumab	Keytruda®	PD-L1 IHC expression ≥ 1% TPS MSI-H or dMMR TMB-H > 10 mutations/megabase
Atezolizumab	Tecentriq®	PD-L1 IHC – percentage TC or IC stained
Avelumab	Bavencio®	None – metastatic or advanced diagnostics
Durvalumab	Imfinzi®	None – prior radiation or patient regimen
Cemiplimab	Libtayo®	PD-L1 IHC expression ≥ 50% TPS
Dostarlimab	Jemperli®	dMMR mutation

TABLE 1:
For immunotherapies with multiple indications, diagnostic requirements may differ between indications. This lists companion diagnostics that appear anywhere on the label and may not apply to all approved indications. All information is taken from the product label on file with the FDA.[7]

Three of these include companion diagnostic values based on mIHC staining for PD-L1 expression in their indications for use, and two require DNA or RNA sequencing to identify microsatellite instability, tumor mutational burden, or mismatch repair errors.

In each case, patients screened using these tools show higher response rates than would be expected from patients in general, but the link between the screening diagnostic and response is far from definitive.[7]

In addition, as the number of anti-PD-L1 therapies grows, so do the different testing requirements. As can be seen in **TABLE 1** above, there are four different scoring algorithms using PD-L1 IHC

staining, some focusing on TCs (tumor cells) and others on ICs (immune cells), and all requiring some subjective operator judgment. Rimm et al. note that interobserver concordance is particularly poor for pathologists when attempting to score PD-L1 expression on ICs, particularly at low expression ranges.[8]

All of this points to the need for new approaches to tumor tissue analysis that reduce interlaboratory and inter-user variability and elevate the predictive value of each sample.

7.   Opdivo, Keytruda, Tecentriq, Bavencio, Imfinzi, Libtayo, Jemperli package inserts: https://www.accessdata.fda.gov/scripts/cder/daf/
8.   Rimm DL, Han G, Taube JM, et al. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer. *JAMA Oncol* 2017;1051-1058.

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THE TUMOR MICROENVIRONMENT HAS MORE TO REVEAL

Increasingly, oncologists and pathologists are recognizing that predicting therapeutic response and understanding tumor heterogeneity are best done in the context of the TME. Observations that get lost when cells are dissociated from a sample, such as clustering and interaction between cell types and variations in biomarker expression across the TME, have all been shown to have potential prognostic value.
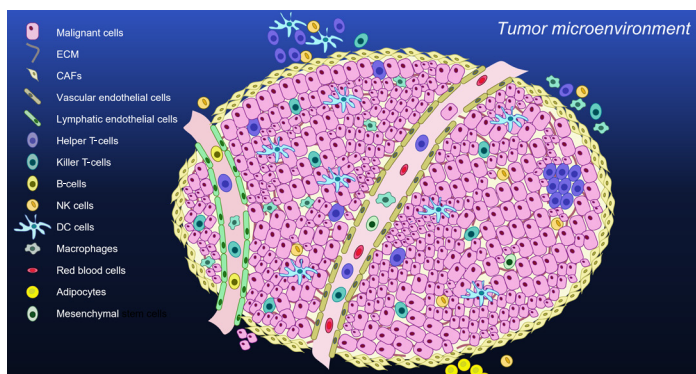

FIGURE 4:
This simplified illustration of the tumor microenvironment shows just 14 of the many different cell types typically present, including tumor cells, vascular cells, immune cells, and others that may or may not be related to the tumor or have a role in tumor promotion or suppression. The patterns of relationships between these different cell types have been shown to correlate with different disease prognoses, with specific "spatial phenotypes" being recognized as characteristic of response to therapy or mortality risk.[9]

These different spatial phenotypic signatures can be associated with different courses of disease progression, response to therapy, and even mortality across tumor types.[10] Binnewies et al. characterize the immune tumor microenvironment (iTME) as "a complex assembly of tumor, immune, stromal and extracellular components," and add that the "organization of these components at the cellular and tissue levels plays a crucial role in the effectiveness of antitumor immunity."[11]

Schürch et al.[12] from Stanford and Nguyen et al.[13] from UC Irvine have shown the TME across tumor types to be a dynamic ecosystem where selective pressures shape which types of cells are present and how they organize, and help define the "immunosuppressive barrier" where TCs and ICs interact to determine the balance between immune-stimulating and -inhibiting factors.

Giraldo et al.[14] showed this in a retrospective analysis of studies across tumor types that established a clear correlation between the density of infiltrating immune cells and a patient's clinical outcome. They reported that TMEs rich in the T-cells, TLSs, and dendric cells responsible for orchestrating the cytotoxic antitumor immune response are widely associated with good clinical outcomes, while TMEs characterized by high densities of macrophages and regulatory T-cells are predictive of poor prognoses.

Most recently, researchers at Johns Hopkins University described a novel platform called AstroPath™ that is used to discover and validate a first-of-its-kind biomarker signature to predict immunotherapy response in advanced melanoma cases.[15]

They showed how algorithms originally developed for analyzing telescopic images of deep space could be applied to generate operator-independent spatial analyses and immuno-architectural characterizations of mIF-labeled tissue specimens.

Working from a standardized panel of six markers (PD-1, PD-L1, CD8, FOXP3, CD163, and SOX10), they were able to map relatively rare cells to the tumor stromal boundary and identify high-density clusters of cells distinguished by different levels of PD-1 and PD-L1 expression to predict response to therapy (or lack thereof) and overall survival.[15]

9.   Akoya Biosciences, 2021. https://www.akoyabio.com/webinar/know-thy-neighborhood-spatial-phenotyping-of-cells-at-the-neighborhood-level/
10. Schürch CM, et al. Coordinated cellular neighborhoods orchestrate antitumoral immunity at the colorectal cancer invasive front. *Cell*. 2020;182(5):1341-1359.e19.
11. Binnewies, M. et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nature Medicine*. 2018;24:541-550.
12. Schürch CM, et al. 2020.
13. Nguyen QH, et al. Profiling human breast epithelial cells using single-cell RNA sequencing identifies cell diversity. *Nature Communications*. 2018;9:2028.
14. Giraldo NA, et al. The clinical role of the TME in solid cancer. *BJC*. 2019;120:45-53.
15. Berry S, et al. 2021.

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mIF-BASED SPATIAL PHENOTYPIC SIGNATURES IN THE CLINICAL PATHOLOGY LAB

In the landmark Multi-Institutional TSA-amplified Multiplexed Immunofluorescence Reproducibility Evaluation, or MITRE study, the mIF solution was used across six institutions to demonstrate and validate an automated end-to-end workflow that characterizes PD-1/PD-L1 immune checkpoint signaling in tumor tissue samples. The study findings demonstrated high intralaboratory and interlaboratory concordance for measurements of IC densities, coexpression, and proximity parameters. **FIGURE 5**.

Of particular interest were the results for two analyses that have proven challenging with current lab methods – relative expression of PD-L1 in different CK+ and CD68+ cell phenotypes, and proximity measures between PD-1+ and PD-L1+ cells. For both, the study showed "strong concordance" between labs with R^2 values of 0.82–0.88 across the four assays. **The MITRE results represent an important step toward standardizing an automated mIF-based spatial biology workflow that provides the level of performance needed to support clinical trials and that can be applied to clinical testing in the future.**
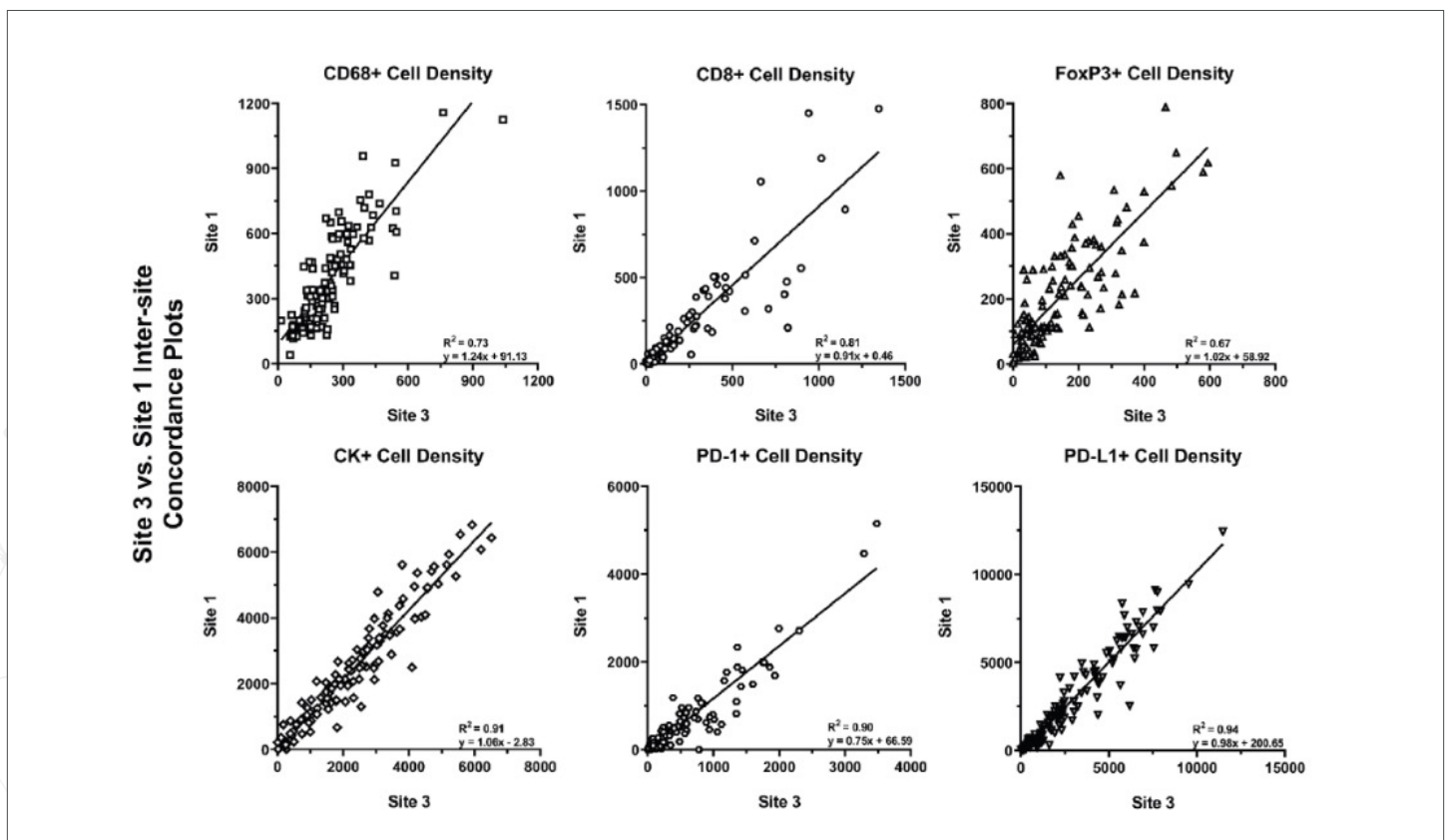

FIGURE 5:

Representative inter-site cell density concordance plots for each marker – CD68, CD8, FOXP3, CK (tumor cells), PD-1, and PD-L1 – produced from two different runs at six separate sites show the high level of concordance achieved using the optimized protocols developed by Taube et al. on the Phenoptics™ platform.[16]

16. Taube JM, Roman K, Engle EL, et al. Multi-institutional TSA-amplified Multiplexed Immunofluorescence Reproducibility valuation (MITRE) Study. *J Immunother Cancer*. 2021;9(7):e002197.
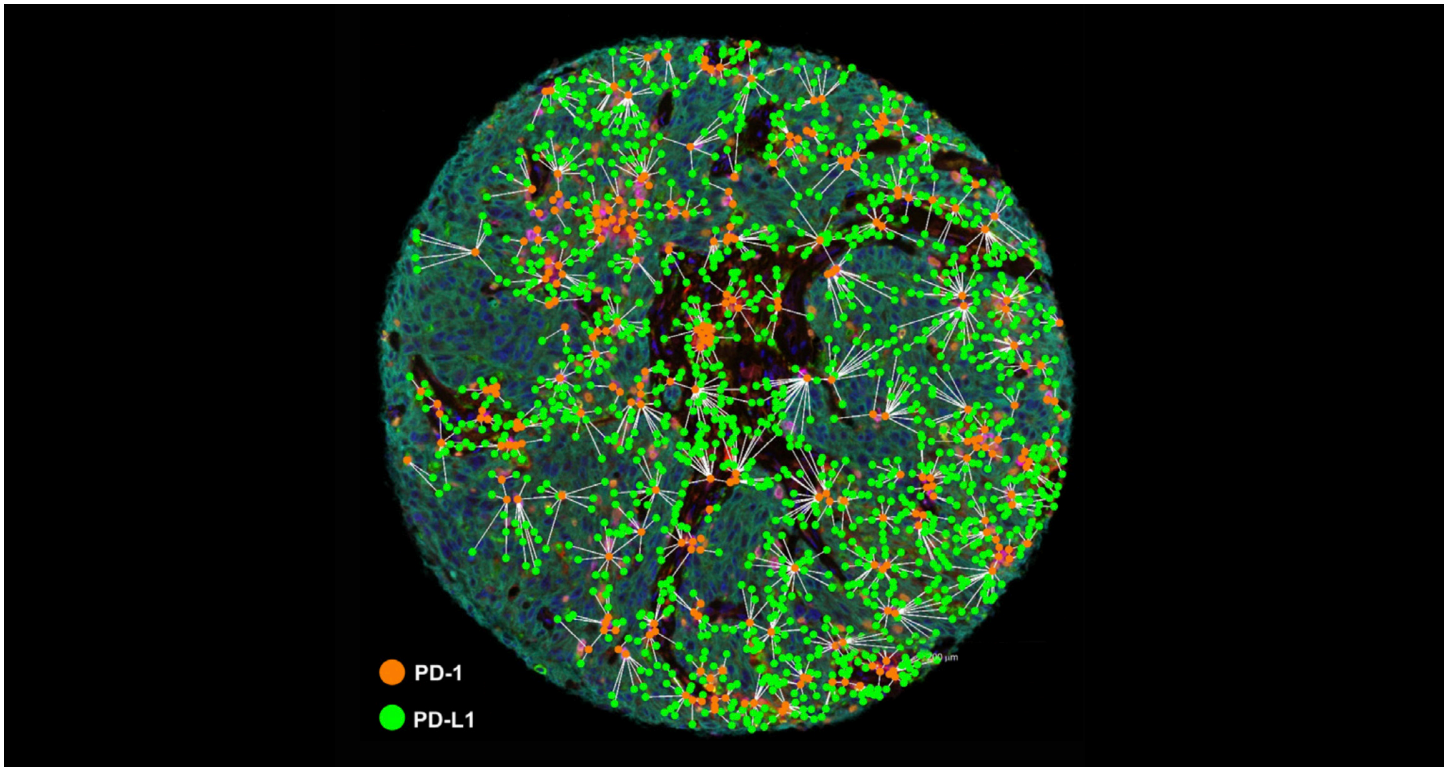
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FIGURE 6:
This analysis of the proximity of PD-1+ and PD-L1+ cells in a sample of breast cancer tissue is shown to have higher value in predicting response to immunotherapy than does traditional PD-L1+ expression using IHC. Taube et al. showed that this relatively complex analysis can be performed with high reproducibility at multiple labs across the US and by multiple operators within each lab.[17]

Simultaneously, we have seen the publication of a growing number of standardized methods for employing spatial phenotypic biomarkers to predict immunotherapy response for oncology patients.

In a publication from the Cancer Immune Monitoring and Analysis Centers and Cancer Immunologic Data Commons (CIMAC-CIDC), labs from the Icahn School of Medicine at Mt. Sinai, MD Anderson Cancer Center, and Dana-Farber Cancer Institute described their multistep harmonization of image analysis algorithms, image acquisition platforms, and multiplex staining protocols to achieve concordant data across sites.[17]

Separately, a task force assembled by the Society for Immunotherapy of Cancer (SITC) made up of pathologists and other experts from academia and pharmaceutical and diagnostic manufacturers is evaluating the use of mIF tools in routine clinical testing. Calling mIF technologies "standard tools… that are likely to enter routine clinical practice in the near future," the task force is in the process of establishing best practices "to help ensure outputs are robust and comparable across laboratories."[18]

17. Akturk G, et al. Multiplex tissue imaging harmonization: a multicenter experience from CIMAC-CIDC immuno-oncology biomarkers network. *Clin Cancer Res.* 2021;27(18):5072-5083.
18. Taube JM, et al. The Society for Immunotherapy of Cancer statement on best practices for multiplex immunohistochemistry (IHC) and immunofluorescence (IF) staining and validation. *J Immunother Cancer.* 2020;8(1):e000155.

Join the labs adding spatial phenoptyping capabilities with the **Akoya Phenoptics™ platform**

These are just a few examples of the growing number of studies showing how mIF-based spatial phenotypic signatures are creating a new generation of biomarkers bridging the worlds of single-cell transcriptomics and cellular biology. Groundbreaking studies like AstroPath™ and MITRE were conducted using standard Phenoptics instrumentation and commercially available biomarkers and reagents.

They demonstrate how any lab can gain novel spatially-informed insights into the structure, progress, and potential response to immunotherapy of almost any tumor from an intact tissue sample. The Phenoptics Solution integrates staining, imaging, and analysis using existing workflows and skill sets to quantitatively capture details of cell organization and integration across an entire sample for rich insights into the mechanisms and vulnerabilities of disease.

Our solutions have been proven in clinical and translational research and in drug development applications around the world, and are supported by a dedicated team of scientists, medical professionals, and technicians with firsthand research and clinical experience, ready to partner with you to take the next step in immuno-oncology analysis.

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