Quantitative Spatial Profiling of TILs as the Next Step beyond PD-L1 Testing for Immune Checkpoint Blockade

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SUMMARY

Analysis of tumor-infiltrating lymphocyte (TIL) functional states, particularly tumor-reactive PD-1^T TILs, within specific spatial context, can serve as a biologically informed predictive marker of immunotherapy that may be superior to standard clinical biomarkers. High-plex quantitative immune cell phenotyping within their spatial context has tremendous potential in immuno-oncology.

See related article by Hummelink et al., p. 4893

In this issue of *Clinical Cancer Research*, Hummelink and colleagues report on programmed cell death protein 1 (PD-1^T) TILs, a tumor-reactive tumor-infiltrating T lymphocyte (TIL) pool, as a predictive biomarker for immunotherapy in non–small cell lung cancer (NSCLC; ref. 1). PD-1^T TILs represent an intratumoral CD8$^+$ T-cell population with high PD-1 expression, distinct transcriptional profiles, and increased tumor recognition capacity (2). This subset of PD-1+ tumor-infiltrating T cells is preferentially recruited in tertiary lymphoid structures (TLS) and can be identified by bright PD-1 expression that can be digitally quantified and distinguished from other PD-1+ cells (1, 2). Hummelink and colleagues report their findings on the predictive accuracy of PD-1^T TILs in the context of immune checkpoint inhibitor (ICI) therapy for patients with NSCLC receiving nivolumab or pembrolizumab. Following a digital workflow for PD-1^T TIL quantification in formalin-fixed paraffin-embedded tissue, the authors evaluated the association of PD-1^T TIL density with clinical outcomes, focusing on disease control at 6 months (a surrogate endpoint also known as durable clinical benefit; ref. 3) as their primary endpoint. The predictive accuracy of PD-1^T TIL density (AUC ROC, 0.72–0.79) was superior to that of programmed death-ligand 1 (PD-L1) TPS score, commonly used in NSCLC to identify tumors more likely to regress with ICIs (AUC ROC, 0.58). Notably, the predictive nature of PD-1^T TILs may be enhanced for determining long-term clinical outcome and sustained clinical response past 6 months (ROC AUC, 0.79–0.89 for prediction of disease control at 12 months). As PD-1^T TILs were predominantly found in TLSs (1, 2) and the role of mature TLS in antitumor immune responses in the context of immune checkpoint blockade (4), the authors investigated the incremental value of assessing PD-1^T TILs over the number of TLS within the analyzed tumors; these analyses showed that the predictive value of PD-1^T TILs was not driven by TLS density alone (ROC AUC for the latter 0.62). Taken together, these findings build on the previously reported role of this functionally distinct subset of CD8$^+$ intratumoral T cells (2) and support PD-1^T TILs as a putative determinant of response to immune checkpoint blockade and suggest that prospective validation in larger cohorts should be prioritized.

The study of Hummelink and colleagues, emphasizes that a nuanced spatially informed quantitative analysis, that captures T-cell populations with unique functional properties and tumor recognition capacities, may more accurately identify individuals more likely to respond to immune checkpoint blockade compared with conventionally used biomarkers. Currently established predictive biomarkers of ICI response include microsatellite instability (MSI; ref. 5), which is detected in <5% of human cancers, as well as PD-L1 expression and tumor mutation burden (TMB), that both suffer from technical and biological limitations. The clinical utility of PD-L1 testing varies on the basis of the cancer type evaluated and the ICI therapy considered (6), with several phase III trials failing to reproduce the association between PD-L1 expression and ICI response (7, 8). Similarly, with the exception of MSI-high tumors, the predictive value of TMB is cancer-lineage dependent (9) and not consistently predictive of ICI response (10, 11). In contrast to PD-L1 expression or TMB that serve as surrogates of an antitumor immune response, PD-1^T TILs are an indicator that an effective tumor-specific T-cell response has occurred and can therefore serve as a biologically relevant measure of clinical outcomes. Furthermore, as PD-1^T TIL density was largely independent from PD-L1 TPS in the study by Hummelink and colleagues; it is conceivable that PD-1^T TIL density may be informative for PD-L1 negative tumors as well as tumors with PD-L1 TPS in the gray zone of 1% to 50% (**Fig. 1**). Conceptually, PD-1^T TILs can be used as a footprint for active tumor-specific adaptive immune responses and therefore might enable patient selection for ICIs in cancers with marginal anti–PD-1 response rates, for example ovarian and breast cancer.

The value of TILs in reflecting adaptive antitumor immune responses and ultimately clinical responses with ICI therapy has been previously demonstrated (12), with emerging studies supporting the additive benefit of considering TIL functional profiles and their spatial localization within the tumor microenvironment (TME). To this end, spatially resolved multiplex immunofluorescence analyses have uniquely enabled spatial mapping of immune cells and assessment of their heterogeneity in the TME (13–15), revealing relationships among TIL subpopulations that are linked with differential ICI clinical outcomes (16). Furthermore, evaluation of PD-1/PD-L1 proximity rather than PD-L1 expression alone may more optimally distinguish tumors more likely to regress with ICI therapy (17). In addition to evaluation of the PD-1/PD-L1 axis, spatially resolved quantitative immunofluorescence approaches have the potential to interrogate interactions and localization of

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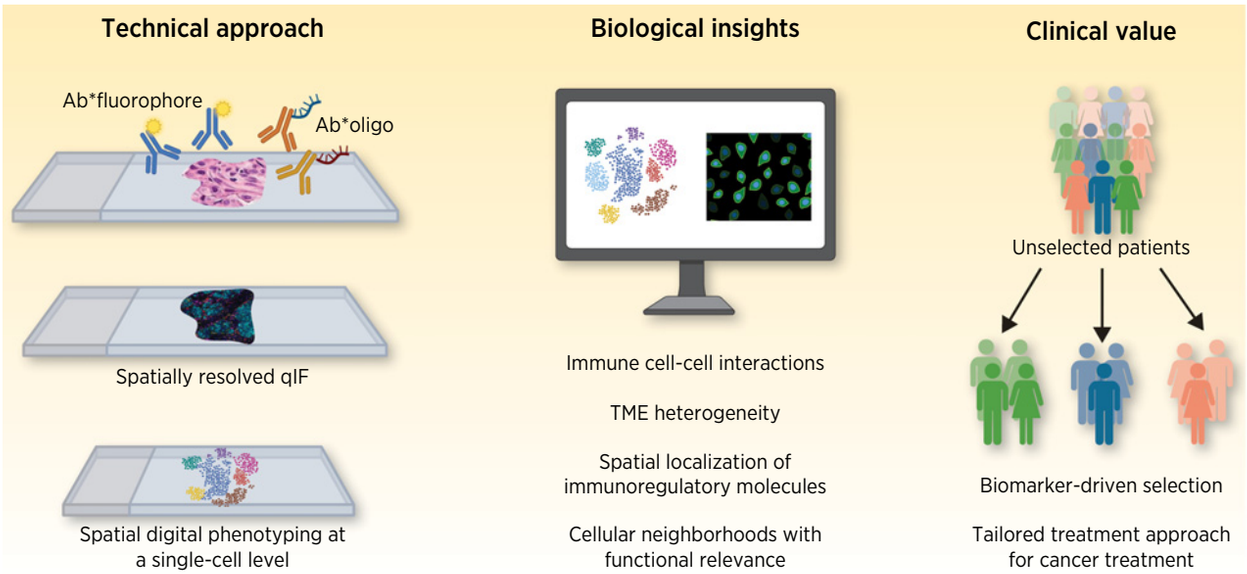

Figure 1.
Impact and roles for spatially resolved high-plex assays in immunotherapy treatment and biomarker discovery. Spatially resolved high-plex methods, including quantitative immunofluorescence and digital spatial profiling, are high-throughput approaches that allow for simultaneous identification of multiple biomarkers in their spatial context. These methods have the unique potential to provide insights in the phenotype and spatial localization of immune cell subsets and thus serve as biology-informed biomarkers reflecting the quality and architecture of antitumor immune responses. As such, they can be incorporated in patient selection strategies for cancer immunotherapy and be used as a platform for novel biomarker discovery. (Adapted from an image created with BioRender.com.)

immunoregulatory molecules such as IDO-1, LAG-3, TIGIT, TIM-3, and VISTA, providing a unique opportunity to understand mechanisms of response and resistance to novel checkpoint inhibitors currently tested in clinical trials. Overall, these approaches have been shown to more accurately predict ICI response compared with PD-L1 expression and TMB (18).

Similarly, spatial phenotyping by reconstruction of cellular neighborhoods has pointed towards local enrichment in immune cell subpopulations and differential organization of the TME that is reflective of distinct antitumor immunity states (19). Implementation of photo-cleavable oligonucleotide tags attached to antibodies or RNA probes has further increased the multiplexing capacity, dynamic range and level of detection of digital spatial profiling approaches. Spatial transcriptomics represent another avenue of interrogation of immune cell spatial heterogeneity, with neoantigen-reactive T-cell clones shown to harbor unique transcriptomic profiles that are further differentiated in the TME of ICI responsive tumors (20). While spatially resolved and high-plex assays may uniquely assess the immune contexture of tumors at a single-cell resolution, further standardization is required to generate analytical platforms that allow for measurement of complex spatial associations. Notably, these approaches are more likely to succeed when representative of spatial and functional interactions, following the paradigm of the study by Hummelink and colleagues that relied on interrogation of a TIL subset previously functionally characterized and found to be tumor-reactive (2).

Collectively, high-plex quantitative evaluation of immune cell subpopulation phenotypes, in their spatial context, holds unique promise as a near-term improved biomarker of treatment response and has tremendous potential for ICI biomarker discovery, especially for the subset of tumors with low PD-L1 expression and/or low TMB.

Authors' Disclosures

V. Anagnostou reports grants from AstraZeneca, Bristol-Myers Squibb, and Delfi Diagnostics outside the submitted work; in addition, V. Anagnostou has a patent for 63/276,525 issued, a patent for 17/779,936 issued, a patent for 16/312,152 issued, a patent for 16/341,862 issued, a patent for 17/047,006 issued, and a patent for 17/598,690 issued. J.J. Luke reports DSMB participation with AbbVie, Immutep, and Evaxion; scientific advisory board participation (no stock) with 7 Hills, Bright Peak, Exo, Fstar, Inzen, RefleXion, Xilio (stock), Actym, Alphamab Oncology, Arch Oncology, Duke Street Bio, Kanaph, Mavu, NeoTx, Onc.AI, OncoNano, Pyxis, Saros, STipe, and Tempest; consultancy with compensation from AbbVie, Alnylam, Atomwise, Bayer, Bristol-Myers Squibb, Castle, Checkmate, Codiak, Crown, Cugene, Curadev, Day One, Eisai, EMD Serono, Endeavor, Flame, G1 Therapeutics, Genentech, Gilead, Glenmark, HotSpot, Kadmon, KSQ, Janssen, Ikena, Inzen, Immatics, Immunocore, Incyte, Instil, IO Biotech, Macrogenics, Merck, Mersana, Nektar, Novartis, Partner, Pfizer, Pioneering Medicines, PsiOxus, Regeneron, Ribon, Roivant, Servier, STINGthera, Synlogic, and Synthekine; and research support (all to institution for clinical trials unless noted) from AbbVie, Astellas, AstraZeneca, Bristol-Myers Squibb, Corvus, Day One, EMD Serono, Fstar, Genmab, Ikena, Immatics, Incyte, Kadmon, KAHR, Macrogenics, Merck, Moderna, Nektar, Next Cure, Numab, Palleon, Pfizer, Replimmune, Rubius, Servier, Scholar Rock, Synlogic, Takeda, Trishula, Tizona, and Xencor; in addition, J.J. Luke has patents (both provisional) Serial #15/612,657 (Cancer Immunotherapy) and PCT/US18/36052 (Microbiome Biomarkers for anti–PD-1/PD-L1 Responsiveness: diagnostic, prognostic, and therapeutic uses thereof). No other disclosures were reported.

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