Editorial Commentary

Are PD-1T TILs merely an expensive and unuseful whim as biomarker?

Esther Garcia-Lorenzo, Victor Moreno^

START Madrid-FJD, Early Phase Clinical Trials Unit, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain

Correspondence to: Dr. Victor Moreno, MD, PhD. START Madrid-FJD, Early Phase Clinical Trials Unit, Hospital Fundación Jiménez Díaz, Av. Reyes Católicos, 2 28040 Madrid, Spain. Email: victor.moreno@startmadrid.com.

Comment on: Hummelink K, Tissier R, Bosch LJW, *et al.* A Dysfunctional T-cell Gene Signature for Predicting Nonresponse to PD-1 Blockade in Non-small Cell Lung Cancer That Is Suitable for Routine Clinical Diagnostics. Clin Cancer Res 2024;30:814-23.

Keywords: Programmed cell death-ligand 1 (PD-L1); biomarker; immunotherapy; non-small cell lung cancer (NSCLC)

Submitted Mar 18, 2024. Accepted for publication Jul 03, 2024. Published online Aug 23, 2024.
doi: 10.21037/tlcr-24-255
View this article at: https://dx.doi.org/10.21037/tlcr-24-255

Non-small cell lung cancer (NSCLC) represents the second most prevalent cancer in the world and the leading cause of mortality (1). The advent of drugs targeting Programmed cell death 1 (PD-1), such as pembrolizumab and nivolumab, and programmed cell death-ligand 1 (PD-L1), like atezolizumab, has significantly improved outcomes (2-5). These drugs have demonstrated efficacy in NSCLC through various clinical trials, showing superiority over the previously stablished standard platinum-based treatment (6,7). Despite these advancements, not all patients benefit from immunotherapy (8,9). The current standard for predicting response is assessing PD-L1 expression using immunochemistry (IHC). Nonetheless, the predictive accuracy of PD-L1 is compromised by technical variables such as the choice of detection antibody and cutoff values, as well as biological factors, including the widespread expression of PD-L1 on cell membranes due to oncogene activation and the variability within the tumor microenvironment (8).

Considering all the uncertainties about PD-L1 determination and its value as a predictor of response, recent studies have shown a myriad of promising alternative biomarkers. These include PD-L1 T-cell immunohistochemistry (assessing PD-L1 expression on tumor-infiltrating T cells), the co-expression of additional immunological markers [such as PD-L2 and fractalkine (CX3CL1), T-cell immunoglobulin-3 (Tim-3), lymphocyte-activation gene (LAG-3) and co-stimulatory molecules like OX40 and 41BB] and T-cell receptor (TCR) clonality (indicating a more clonal T-cell population surrounding or infiltrating the tumor) (8).

In this context, there has been considerable effort to identify new markers capable of distinguishing patients who are less likely derive benefit from immunotherapy. Hummelink *et al.* have identified a specific subpopulation of $CD8^+$ tumor-infiltrating lymphocyte (TIL), named PD-1T TILs, whose presence was associated with higher response and longer survival in patients with NSCLC treated with anti-PD-1 therapy (10). PD-1T TILs are a subset of $PD\text{-}1^+$ T cells that are characterized by their high expression of PD-1 (>90 PD-1T TILs/mm^2) and are transcriptionally and functionally distinct from other TIL populations with low or no expression of PD-1 (<90 PD-1T TILs/mm^2) (10). These PD-1T TILs show high tumor reactivity and are predominantly located in tertiary lymphoid structures (TLS), recognized as predictors of response to immune-checkpoint inhibitors in various tumor types, such as melanoma (10). Consequently, the absence of PD-1T TILs suggest a deficiency in tumor-reactive T-cell populations, thereby facilitating the identification of patients less likely to respond to immunotherapy (10).

Hummelink *et al.* described a methodology for

^ ORCID: 0000-0001-6099-4236.

quantifying PD-1T TILs via IHC aimed at reducing human error stemming from manual processes throughout the procedure. Despite these advancements, the technique still necessitates significant user involvement, which could hinder its practical application in routine clinical settings due to interobserver variability that undermines the test's predictive accuracy. However, it merits acknowledgement that, according to the investigators, this novel biomarker yielded a sensitivity of 77% and a specificity of 67% to detect patients in response after 6 months of treatment with pembrolizumab or nivolumab. These results improved to 93% and 65% respectively for identifying those in response after 12 months, with a negative predictive value (NPV) of 88% at 6 months and 98% at 12 months. Additionally, a high presence of PD-1T TILs was associated with significantly longer progression-free survival (PFS) and overall survival (OS). Notably, the predictive performance of PD-1T TILs was superior to that of PD-L1 and TLS in the same cohort (10).

In an effort to standardize this biomarker, the research team now introduces an updated version of their proposed biomarker. In this new article, they aim to stablish a robust RNA signature capable of determining a tumor's PD-1T TILs status using NanoString nCounter platform technology (9). The nCounter technology provides a robust method for multiplexing up to 800 gene expression targets using direct detection technology requiring 15 minutes hands-on time and producing highly reproducible data (11). The researchers use two cohorts: one to develop the PD-1T signature and another to validate it independently (9).

Of the 94 patients initially included in the signature development cohort, only 58 of them were assessable (almost 30% of patients were excluded due to low RNA yield and/or low RNA quality, and 8.5% were excluded due to quality control failure) (9). Some patients were also not included in the validation cohort (27.5%) due to antiquity of samples (over two years old, accounting for 24%) and the procurement of tumor samples from lymph nodes (3.5%) (9). In addition, this cohort encompassed patients from two distinct locations, each employing different antibodies for PD-L1 assessment (9). Among the 58 patients in the development cohort, 12 maintained response after 12 months on treatment with nivolumab, all exhibiting high levels of IHC-PD-1T TILs (9). Conversely, among the 46 patients experiencing disease progression within 12 months, 29 (63%) had absence of IHC-PD-1T TILs. A differential gene expression analysis was conducted on these 41 patients (12 "responders" and 29 "non-responders") to derive a unique mRNA expression gene set. Subsequent regularized regression analysis [least absolute shrinkage and selection operator (LASSO)] yielded a set of 12 genes: 11 were elevated in patients with high IHC-PD-1T TILs (*STAT1*, *OAS1*, *TAP1*, *CXCL13*, *IFIT2*, *IL6*, *TDO2*, *CD6*, *CTLA4*, *CD274*, and *LAG3*), and one was decreased (*HEY1*). This gene set proved equivalent ($P<0.0001$) to the IHC detection of PD-1T TILs and effectively differentiated patients with high IHC-PD-1T TILs and disease control from those with low IHC-PD-1T TILs and progressive disease. The resulting technique showcased a sensitivity of 92%, specificity of 93%, a positive predictive value (PPV) of 85%, and a negative predictive value (NPV) of 96%.

In the context of comparing this new technique to the current gold standard in clinical practice, the PD-L1 status assessed by IHC, the validation cohort revealed a diminished capacity to differentiate patients with extended disease control, demonstrating a sensitivity of 50%, a specificity of 82%, a PPV of 33%, and a NPV of 90%. Additionally, a PD-L1 expression level of ≥50% was not correlated with prolonged PFS and OS.

Despite these promising outcomes, several aspects concerning its clinical application warrant attention. These concerns arise both from the methodology employed in the development of the technique and its practicality for routine clinical use.

The first concern is the limited number of patients included in both cohorts, attributable not only to the study's design but also to the substantial attrition of patients. Reasons for this attrition include inadequate sample quality or quantity, outdated samples (older than two years), and samples derived from lymph nodes. This latter issue is particularly concerning for clinical applicability, as patients often present with limited samples primarily obtained from lymph node biopsies (notably mediastinal). Furthermore, the use of differing antibodies for PD-L1 assessment—NAT105 (Roche Diagnostics), 22C3 DAKO (Agilent), and E1L3N (Cell Signaling Technology)—might have introduced analytical variability. Lastly, while these findings aim to discern patients' responsiveness to PD-L1 monotherapy, they overlook the prevalent clinical practice of combining immunotherapy with chemotherapy, especially in NSCLC patients with PD-L1 expression below 50%, a strategy known to influence both PFS and OS significantly.

In terms of its practical application, the authors point out the complexity of implementing this method through IHC, which led to the adoption of the NanoString

nCounter platform for gene set development. While this gene set has been demonstrated to have statistical equivalence to IHC, its utilization in routine clinical settings invites scrutiny. Despite identifying eleven genes as upregulated and one as downregulated, the classification of tumors lacking this precise gene expression configuration—whether they are immunoresistant or immunosensitive—remains uncertain (9).

In conclusion, while immunotherapy has emerged as a transformative therapy in oncology, significant challenges remain in accurately identifying patients who stand to benefit from these treatments (8,12,13). Currently, the only biomarkers prospectively validated in clinical trials include PD-L1 expression by IHC, tumor mutational burden, and microsatellite instability/mismatch repair deficiency (MSI/MMRd) (12). Other promising biomarkers, such as PD-1T TILs identified through IHC or the gene signature panel by Hummelink *et al.* discussed herein, are yet to demonstrate their generalizability and their potential to enhance the predictive power of existing validated biomarkers in terms of efficiency and efficacy (9).

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Translational Lung Cancer Research*. The article has undergone external peer review.

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-255/prf

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-255/coif). V.M. reports receiving consulting fees from Roche, Bayer, Pieris, BMS, Janssen, Basilea, Regeneron/Sanofi, and Nanobiotix; serving as principal investigator and receiving Institutional Funding from AbbVie, Achilles, AbbVie, AceaBio, Adaptimmune, ADC Therapeutics, Arcus, Ascendis Pharma, Aduro, Agenus, Amcure, Amgen, Astellas, AstraZeneca Bayer, Beigene, Biomea, BioInvent International AB, BMS, Boheringer, Boston Therapeutics, Celgene, Daichii Sankyo, Debiopharm, Eisai, e-Terapeutics, Exelisis, Forma Therapeutics, Genmab, GSK, Harpoon, Hutchison, Immutep, Incyte, Inovio, Iovance, Janssen, Kyowa Kirin, Lilly, Loxo, Monta Bioscience, MedSir, Menarini, Merck, Merus, Millennium, MSD, Nanobiotix, Nektar, Novartis, Odonate Therapeutics, Pfizer, Pharma Mar, PharmaMar, Principia, PsiOxus, Puma, Regeneron, Relay Therapeutics, Revolution Medicines, Rigontec, Roche, Sanofi, Sierra Oncology, Synthon, Taiho, Takeda, Tesaro, Transgene, Turning Point Therapeutics, and Upshersmith. The other author has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Siegel RL, Miller KD, Wagle NS, et al. Cancer statistics, 2023. CA Cancer J Clin 2023;73:17-48.
2. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med. 2016;375:1823-33.
3. Paz-Ares L, Ciuleanu TE, Cobo M, et al. First-line nivolumab plus ipilimumab combined with two cycles of chemotherapy in patients with non-small-cell lung cancer (CheckMate 9LA): an international, randomised, open-label, phase 3 trial. Lancet Oncol 2021;22:198-211.
4. Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med 2018;378:2078-92.
5. Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. N Engl J Med 2018;378:2288-301.
6. Hanna N, Johnson D, Temin S, et al. Systemic Therapy for Stage IV Non-Small-Cell Lung Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. J Clin Oncol 2017;35:3484-515.

7. Planchard D, Popat S, Kerr K, et al. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2018;29:iv192-237.
8. Chae YK, Pan A, Davis AA, et al. Biomarkers for PD-1/PD-L1 Blockade Therapy in Non-Small-cell Lung Cancer: Is PD-L1 Expression a Good Marker for Patient Selection? Clin Lung Cancer 2016;17:350-61.
9. Hummelink K, Tissier R, Bosch LJW, et al. A Dysfunctional T-cell Gene Signature for Predicting Nonresponse to PD-1 Blockade in Non-small Cell Lung Cancer That Is Suitable for Routine Clinical Diagnostics. Clin Cancer Res 2024;30:814-23.
10. Hummelink K, van der Noort V, Muller M, et al. PD-1T TILs as a Predictive Biomarker for Clinical Benefit to PD-1 Blockade in Patients with Advanced NSCLC. Clin Cancer Res 2022;28:4893-906.
11. Veldman-Jones MH, Brant R, Rooney C, et al. Evaluating Robustness and Sensitivity of the NanoString Technologies nCounter Platform to Enable Multiplexed Gene Expression Analysis of Clinical Samples. Cancer Res 2015;75:2587-93.
12. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. Lancet Oncol 2016;17:e542-51.
13. Presti D, Dall'Olio FG, Besse B, et al. Tumor infiltrating lymphocytes (TILs) as a predictive biomarker of response to checkpoint blockers in solid tumors: A systematic review. Crit Rev Oncol Hematol 2022;177:103773.

Cite this article as: Garcia-Lorenzo E, Moreno V. Are PD-1T TILs merely an expensive and unuseful whim as biomarker? Transl Lung Cancer Res 2024;13(8):2087-2090. doi: 10.21037/tlcr-24-255