Editorial Commentary

Predictive biomarkers for immune checkpoint efficacy: is multi-omics breaking the deadlock?

Alice Mogenet[1,2], Laurent Greillier[1,2], Pascale Tomasini[1,2]

[1]Multidisciplinary Oncology and Therapeutic Innovations Department, Assistance Publique – Hôpitaux de Marseille, Marseille, France; [2]Aix Marseille University, CNRS, INSERM, Predictive Oncology Department, CRCM, Marseille, France

Correspondence to: Alice Mogenet, MD, MSc. Multidisciplinary Oncology and Therapeutic Innovations Department, 6th Floor, CHU Nord, Assistance Publique, Hôpitaux de Marseille, Chemin des Bourrely, 13015 Marseille, France; Aix Marseille University, CNRS, INSERM, Predictive Oncology Department, CRCM, Marseille, France. Email: Alice.Mogenet@ap-hm.fr.

Comment on: Parra ER, Zhang J, Duose DY, *et al*. Multi-omics Analysis Reveals Immune Features Associated with Immunotherapy Benefit in Patients with Squamous Cell Lung Cancer from Phase III Lung-MAP S1400I Trial. Clin Cancer Res 2024;30:1655-68.

Keywords: Lung cancer; immunotherapy; biomarkers

Submitted Jul 11, 2024. Accepted for publication Sep 18, 2024. Published online Oct 17, 2024.
doi: 10.21037/tlcr-24-594
View this article at: https://dx.doi.org/10.21037/tlcr-24-594

Introduction

The progressive understanding of lung cancer biology led to the development of various biotherapies such as antiangiogenic agents, targeted therapies and immune checkpoint inhibitors (ICIs), revolutionizing lung cancer treatment and prognosis. ICIs targeting the programmed cell death protein 1 (PD-1)-programmed cell death ligand 1 (PD-L1) axis to restore anticancer immunity were first approved as single agents for pre-treated patients and then in the first line setting, for patients with a high PD-L1 expression (1,2). They were also approved in combination with chemotherapy regardless of PD-L1 expression (3). However, ICI objective response rate in real life remains disappointing and does not exceed 20% when given alone and 50% when combined with chemotherapy. The biological features underlying the benefit of a combination of systemic anticancer drugs are certainly various and complex. PD-L1 expression on tumor cells is to date the only approved routine biomarker for ICI in non-small cell lung cancer (NSCLC), although imperfect and insufficient because of operator variability and spatial heterogeneity (4,5). Several other predictive biomarkers were already studied, but none of them appeared to be reliable and reproducible enough to overcome PD-L1 expression. Tumor mutational burden (TMB), thought to reflect the amount of neoantigens by quantifying non-synonymous mutations in coding areas, failed to show a clear survival difference when used alone and remains difficult to standardize for routine use (6). In a same way, quantification of tumor infiltrating lymphocytes, interferon gamma pathway signatures, methylation profile, transcriptomic signature and microbiota assessment failed to confirm their predictive role (7-11). Considering existing literature, genomic, transcriptomic and proteomic data alone did not succeed to predict ICIs efficacy, thus justifying multi-omics strategy.

Herein, samples used for multi-omics analyses were provided from the Lung-MAP S1400I trial, a phase III study comparing nivolumab alone (N) to nivolumab and ipilimumab (NI) for advanced squamous NSCLC after a first line of platinum-based chemotherapy. Ipilimumab adjunction did not show any survival benefit and standard of care remains nowadays ICIs alone in this situation. Indeed, objective response rate was 18% in the NI arm and 17% in the N arm, and no overall survival difference was observed [hazard ratio (HR) =0.87, 95% confidence interval (CI): 0.66–1.16, P=0.34]. However, some patients actually derived long-term benefit from the ICI combination (12). In this paper, we will discuss ancillary analysis of the Lung-MAP S1400I study in which multiplex immunofluorescence (mIF), gene expression profiling, whole exome sequencing (WES) and circulating serum analyte measurements were performed to set up a multi-omics signature associated with

Table 1 Gene overexpression significantly associated with overall survival or progression free survival, by Nanostring technology

Molecule	Immune response	Inflammation	Apoptosis	Proliferation
Nivolumab	*CLEC4C*, *PIN1*	*CLEC4C*, *IL19*		*CREB5*
Nivolumab + ipilimumab	*CCL22*, *CD163*, *CXCL10*, *CXCL11*, *C1S*, *IL32*, *STAT2*	*C1R*, *ETS1*	*IFI27*, *ITGB3 PRKCD*	*MAPK8*, *IL15RA*
Both arms	*BLNK*, *CD163*, *FCGR2A*, *IRF1*	*F12*	*FADD*	*MAPK11*

Table 2 Spatial relationship between cell type associated with longer progression free survival

Treatment arm	Shorter distance between cell type	P value
Nivolumab + ipilimumab	Granzyme B CTc to malignant cells	0.02
Both arms	CTc to malignant cells	0.045
	CTc to PD-L1+ malignant cells	0.02

CTc, cytotoxic T cells; PD-L1, programmed cell death ligand 1.

ICIs benefit (13). Among the 252 patients included in the study, 160 longitudinal liquid biopsies were available for Olink analysis, 82 baseline tumor samples for mIF, 38 for trancriptomic analysis and 50 for WES.

Coherent data flow

Results of this ancillary study are concordant with the current scientific data emphasizing the importance of a "hot" immune tumor microenvironment (TME) to support ICI efficacy. Indeed, mIF on baseline samples showed that clinical benefit was associated to immune cell density in the stroma (14), especially PD-1 cytotoxic T-cells (CTc) (P=0.04) and Granzyme B CTc in the tumor compartment (P=0.01) in both arms. However, T regulatory lymphocytes presence was negatively associated with OS in the nivolumab and ipilimumab arm but surprisingly not in the nivolumab arm (15). This result may be explained by the small sample size but also highlight the lack of functional information of mIF analysis, knowing that some data showed an association between nivolumab benefit and receptor occupancy on effector regulatory T cells (16). Furthermore, transcriptomic analysis highlighted the association between ICI benefit and main cellular pathways, immune response as expected but also inflammation, apoptosis and proliferation gene expression, highlighting the complexity of underlying ICI pathways (*Table 1*). Existing literature already highlighted the overexpression of genes involved in immune response and proliferation but with various genes significantly associated with ICIs efficacy (10,17). Indeed, identification and validation of a unique and reproducible transcriptomic signature seems unlikely, standing for the multi-omics approach. After multivariate analysis adjusting on PDL1 expression and TMB, overexpression of *CD163*, *BLNK*, *IRF1* and *FCGR2A* was still associated with better outcome (P<0.005), which support the fundamental role of immune response gene expression in ICI response after all and support their utilization for further predictive signatures.

Concerning WES analysis, high copy number variation was associated with a cold TME and immune evasion in this population, in line with previously published data (18,19), but this relation seems to be related to methylation profile specificities, unfortunately not explored in this multi-omics approach (20).

Innovative data flow

In this paper, the special attention paid to features of exceptional response (response >18 months and survival >24 months) compared to early progression (death <6 months) give originality to this subject already widely covered even considering the small sample-size. Despite this specificity, mIF data are consistent with overall population results with a higher density of CTc and memory T-cells in the total compartment in exceptional responders (respectively P=0.03 and P=0.04). Moreover, the focus on spatial cell organization adds a new interest to mIF data. Despite the small sample size (n=12), authors highlighted higher immune infiltration and CTc density among exceptional responder TME. Going further, spatial segregation analysis showed higher segregation between immune cells and tumor cells among early progressors confirmed by distance based hierarchical clustering, confirmed in the whole cohort analysis (*Table 2*). These data remain preliminary and seem difficult to transfer within daily care but artificial intelligence tools are currently developed to extrapolate segmentation and qualification of

multiple histologic components with promising results (21).

Another original result in this study is the predictive role of *LRP1B* mutation, associated with less CTc infiltration, significantly negatively correlated with response and survival. *LRP1B* mutation are not described in the paper but previous studies in other tumor types rather highlighted a positive association between *LRP1B* mutation and ICI efficacy (22,23). This surprising association may result of the small sample size but we cannot exclude a particular role of *LRP1B* mutation in squamous NSCLC considering specific tobacco exposure in this population.

Finally, the adjunction of liquid-biopsies analysis in this multi-omics vision allows a non-invasive and dynamic approach to overcome the limits of baseline solid sample for biomarkers analysis. Despite the originality of the multi-sampling approach, results from the Olink proteomic analysis are consistent with data based on baseline solid sample. Indeed, immune activation and priming markers (ICOS-L, LAMP3/DC-LAMP, IL4, IL13, NRC1, CD5) appeared to be increased at baseline or early during the treatment among responders whereas macrophages, hyperinflammation and stromal markers (IL6, IL8, CXCL13, CSF-1, TNF-SF14, CCL23, VEGFA, HGF) appeared to be increased among non-responders.

Relevance of multi-omics analysis

Multi-omics analysis by recursive partitioning highlighted the ability of Olink analysis alone to predict ICI efficacy and the decision tree survival prediction model was able to identify two population with different survival relying on CTc mIF identification and IL6, LAG3 and MICA.B Olink identification. Both analyses did not find any relevant features in Nanostring and WES data, maybe because of small sample size but their relevance will have to be verified in larger validation cohorts considering these techniques are the most expensive and difficult to transpose in daily care. To confirm this viewpoint, a recent paper explored a multi-omic approach to overcome anti-PD-1 and anti-LAG3 resistance and this identified a negative regulation signature of TCR signal, but these data requested various cutting-edge methods and are not validated so far on a routine representative population (24).

Conclusions

This paper offers a multi-omics approach of immune features underlying ICI efficacy in a population of pretreated squamous NSCLC patients. Despite a good coherence with the existing literature on the topic considering CTc infiltration and immune TME as the main requirement for ICI efficacy, the focus on extreme responders and spatial cell repartition reinforces the results and give originality to the study. Some results remain unexpected such as the identification of *LRP1B* mutation as a negative predictive biomarker of ICI efficacy. In addition, mIF, spatial, trancriptomic, genomic and proteomic data all find almost the same biomarkers in the nivolumab arm and in the nivolumab plus ipilimumab arm. Indeed, this study does not provide innovative key to ICI intensification, especially since chemo-immunotherapy is the new first-line standard in NSCLC, making the study population rarer. To finish, this paper is reinforcing knowledge about various immune predictive biomarkers but failed to integrate a real and reproducible multi-omics approach, thus there is still a need for innovative and especially non-invasive and longitudinal technique, considering Olink proteomic analysis on liquid biopsies is already showing promising results.

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-24-594/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-594/coif). A.M. received honoraria from Amgen, Oseus, Viatris, and AstraZeneca; received support for attending meetings from BMS and Takeda; and took part in the advisory board of Takeda, AstraZeneca and Pfizer. L.G. received honoraria from Amgen, BMS, Takeda, Sanofi, Pfizer, MSD, and AstraZeneca; and took part in the advisory board of Amgen, AstraZeneca, Takeda, Sanofi, Roche, Pfizer, Novartis, MSD and BMS. P.T. received honoraria from Lilly, Amgen, Janssen, and AstraZeneca; received support for attending meetings from BMS and Daiichi Sankyo; and took part in the advisory board of Amgen, Janssen, and AstraZeneca.

The authors have no other 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. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med 2015;373:1627-39.
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. Garassino MC, Gadgeel S, Speranza G, et al. Pembrolizumab Plus Pemetrexed and Platinum in Nonsquamous Non-Small-Cell Lung Cancer: 5-Year Outcomes From the Phase 3 KEYNOTE-189 Study. J Clin Oncol 2023;41:1992-8.
4. Aguiar PN Jr, De Mello RA, Hall P, et al. PD-L1 expression as a predictive biomarker in advanced non-small-cell lung cancer: updated survival data. Immunotherapy 2017;9:499-506.
5. Kerr KM, Tsao MS, Nicholson AG, et al. Programmed Death-Ligand 1 Immunohistochemistry in Lung Cancer: In what state is this art? J Thorac Oncol 2015;10:985-9.
6. Greillier L, Tomasini P, Barlesi F. The clinical utility of tumor mutational burden in non-small cell lung cancer. Transl Lung Cancer Res 2018;7:639-46.
7. Mezquita L, Auclin E, Ferrara R, et al. Association of the Lung Immune Prognostic Index With Immune Checkpoint Inhibitor Outcomes in Patients With Advanced Non-Small Cell Lung Cancer. JAMA Oncol 2018;4:351-7.
8. Ayers M, Lunceford J, Nebozhyn M, et al. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. J Clin Invest 2017;127:2930-40.
9. Duruisseaux M, Martínez-Cardús A, Calleja-Cervantes ME, et al. Epigenetic prediction of response to anti-PD-1 treatment in non-small-cell lung cancer: a multicentre, retrospective analysis. Lancet Respir Med 2018;6:771-81.
10. Mogenet A, Finetti P, Denicolai E, et al. Immunologic constant of rejection as a predictive biomarker of immune checkpoint inhibitors efficacy in non-small cell lung cancer. J Transl Med 2023;21:637.
11. Routy B, Gopalakrishnan V, Daillère R, et al. The gut microbiota influences anticancer immunosurveillance and general health. Nat Rev Clin Oncol 2018;15:382-96.
12. Gettinger SN, Redman MW, Bazhenova L, et al. Nivolumab Plus Ipilimumab vs Nivolumab for Previously Treated Patients With Stage IV Squamous Cell Lung Cancer: The Lung-MAP S1400I Phase 3 Randomized Clinical Trial. JAMA Oncol 2021;7:1368-77.
13. Parra ER, Zhang J, Duose DY, et al. Multi-omics Analysis Reveals Immune Features Associated with Immunotherapy Benefit in Patients with Squamous Cell Lung Cancer from Phase III Lung-MAP S1400I Trial. Clin Cancer Res 2024;30:1655-68.
14. Shum B, Larkin J, Turajlic S. Predictive biomarkers for response to immune checkpoint inhibition. Semin Cancer Biol 2022;79:4-17.
15. Attias M, Piccirillo CA. The impact of Foxp3(+) regulatory T-cells on CD8(+) T-cell dysfunction in tumour microenvironments and responses to immune checkpoint inhibitors. Br J Pharmacol 2024. [Epub ahead of print]. doi: 10.1111/bph.16313.
16. Hosonuma M, Hirasawa Y, Kuramasu A, et al. Nivolumab receptor occupancy on effector regulatory T cells predicts clinical benefit. Cancer Sci 2024;115:752-62.
17. Thompson JC, Davis C, Deshpande C, et al. Gene signature of antigen processing and presentation machinery predicts response to checkpoint blockade in non-small cell lung cancer (NSCLC) and melanoma. J Immunother Cancer 2020;8:e000974.
18. Davoli T, Uno H, Wooten EC, et al. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. Science 2017;355:eaaf8399.
19. Roh W, Chen PL, Reuben A, et al. Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance. Sci Transl Med 2017;9:eaah3560. Erratum

in: Sci Transl Med 2017;9:eaan3788.
20. Jung H, Kim HS, Kim JY, et al. DNA methylation loss promotes immune evasion of tumours with high mutation and copy number load. Nat Commun 2019;10:4278.
21. Park S, Ock CY, Kim H, et al. Artificial Intelligence-Powered Spatial Analysis of Tumor-Infiltrating Lymphocytes as Complementary Biomarker for Immune Checkpoint Inhibition in Non-Small-Cell Lung Cancer. J Clin Oncol 2022;40:1916-28.
22. Cheng Y, Tang R, Li X, et al. LRP1B is a Potential Biomarker for Tumor Immunogenicity and Prognosis of HCC Patients Receiving ICI Treatment. J Hepatocell Carcinoma 2022;9:203-20.
23. Brown LC, Tucker MD, Sedhom R, et al. LRP1B mutations are associated with favorable outcomes to immune checkpoint inhibitors across multiple cancer types. J Immunother Cancer 2021;9:e001792.
24. Chocarro L, Blanco E, Fernandez-Rubio L, et al. PD-1/LAG-3 co-signaling profiling uncovers CBL ubiquitin ligases as key immunotherapy targets. EMBO Mol Med 2024;16:1791-816.

Cite this article as: Mogenet A, Greillier L, Tomasini P. Predictive biomarkers for immune checkpoint efficacy: is multi-omics breaking the deadlock? Transl Lung Cancer Res 2024;13(10):2856-2860. doi: 10.21037/tlcr-24-594