

Multimodal stratification of predictive biomarker in lung cancer: A focus on immune checkpoint inhibitor

AUTHORS: CAMILLE LATAPY, ADELE PONZONI, VICTOR SENECHAL, CORINNE RAMOS

Non-small cell lung cancer (NSCLC) accounts for about 85% of all lung cancers. There is a strong rationale for incorporating immunotherapy into the treatment of early-stage NSCLC, given the breakthrough results with PD-1 checkpoint inhibitors in advanced-stage NSCLC. How immunotherapy should be implemented in patients who are operable is still unclear. Most of the efforts so far to identify clinically useful biomarkers do not preserve spatial information and leave us blind to the critical source of information revealed in the cell-to-cell biology of the tumor microenvironment (TME). In order to overcome these limitations, we used spatial biomarkers assays that preserve this critical information about which cells are influencing treatment response.

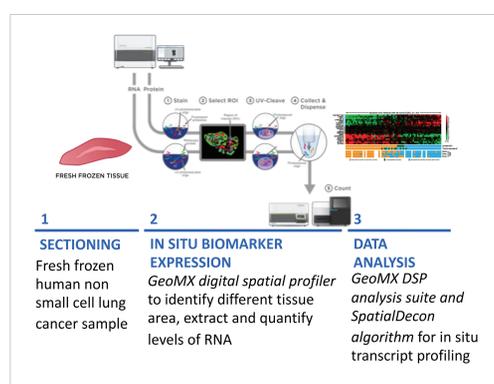
INTRODUCTION

Immunotherapy has reshaped the field of lung cancer therapeutics but, despite positive results, only a minority (<20%) of the patients derived a very long-term benefit from Immune Checkpoint Inhibitor¹ (ICI) therapy warranting a companion diagnostic. In this work, total expression of relevant transcripts and spatially resolved proteins was quantitated using multiplexed methods. This analysis identified numerous differentially expressed transcripts associated with the response to ICI. In addition, significant alterations in the expression and spatial distribution of immunologically relevant proteins in different regions of the tumor microenvironment provided additional insight into the predictive immunological effects of clinically relevant adjuvant ICI therapy for resectable lung cancer. Taken together, these results identify features within the tumor microenvironment and provide preliminary evidence on biomarkers that may influence the suitability of ICIs.

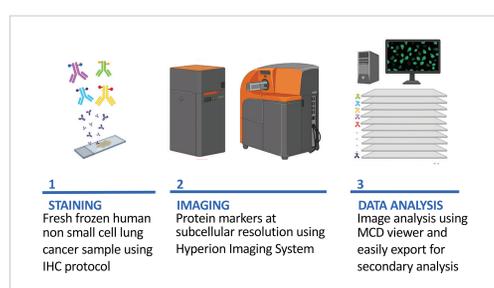
MATERIALS AND METHODS

Frozen sections from retrospectively collected surgically resected NSCLC (adenocarcinoma and squamous cell carcinoma) tumors treated with adjuvant Pembrolizumab therapy were used. Patients were classified in two groups according to their Objective Response Rate (ORR): Complete Response (CR) and Progression Disease (PD) for spatial transcriptomic and proteomics assays.

GeoMx[®] DSP RNA assay (Nanostring[®]) was performed by applying the GeoMx[®] RNA Immune Pathways Panel. The statistical analysis was performed through the GeoMx[®] DSP analysis suite. Cell deconvolution using the SpatialDecon[®] algorithm (Nanostring[®]) was then used to estimate the cell-type abundance in the spatially-resolved region of interest.



Results were validated with single cell proteomic spatial analysis. In brief, samples were stained using a panel of 20 immune or tumor-related antibodies. Deep learning (StarDist QuPath extension) was used for cell segmentation and cells classification was performed using in-house Python scripts. Basic morphometry algorithms were applied to identify proximity and relative spatial distribution of cells.



TRANSCRIPTIONAL PHENOTYPE

The analysis of the non-responder patients highlighted the overexpression of inhibitory ligands CD86 and B7H3 (CD276). Interestingly, TIGIT, CTLA4 and TIM-3 were significantly overexpressed on the surface of the CD8a+ T cells.

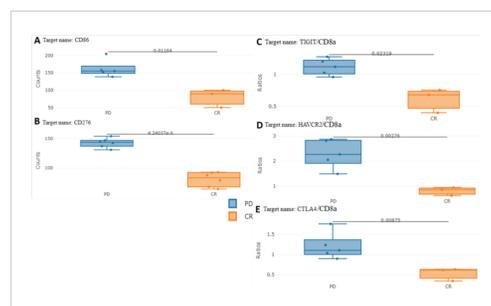


FIGURE 1. Bar graph showing the significant overexpression of co-inhibitory ligands and receptors

A higher expression of the drug targets, PD1 (PDCD1) and PD-L1 (CD274), and genes related to T lymphocytes cytotoxicity (GZMB, CD8a) and activation (CD44, CD27, TNFRSF9) were detected in the tumor microenvironment of the responder patient.

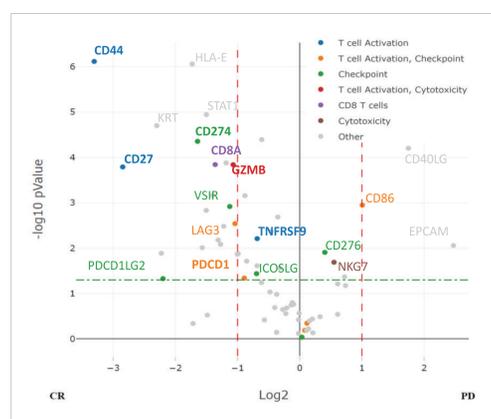


FIGURE 2. Volcano plot highlighting the differences in mRNAs differentially regulated

SPATIAL DECONVOLUTION

The differentiation founded between CR and PD by performing cell deconvolutions was subtle: 2/3 of CR have plasma cells versus 1/6 of PD, all PD segments presented NK cells while only one segment in CR patient.

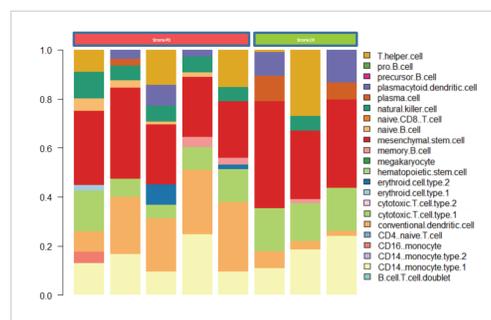


FIGURE 3. Barplot outcome of the cell deconvolution

CORRELATION TME AND ORR

The previous results were validated by investigating the drug targets and immunosuppressive cells in the tumor microenvironment of patient samples. Each immune subpopulation was infiltrating in larger percentage the TME of the responder patient. Notably, in the CR sample the CD8+ T-cytotoxic lymphocytes, responsible for the anti-tumor activity, were the immune subset mostly represented.

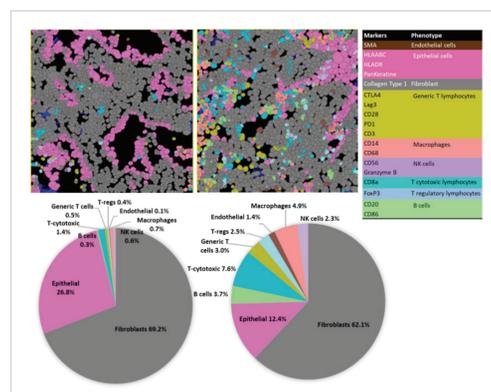


FIGURE 4. Classified immune cell infiltrating the patient's TME

Furthermore, by investigating the abundance of the marker PD-1, target of the therapy, a higher value was found on the cytotoxic T cell infiltrating the tumor of the CR patient.

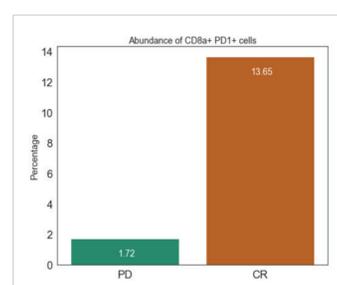


FIGURE 5. Percentage of immune population co-expressing cytotoxicity activity marker and drug target

CELL-CELL PROXIMITY

Spatial distribution of epithelial cancer cells relative to immune cells was studied. The nearest neighbor analysis showed that CD8+ T lymphocytes were located at a minimum distance from tumor cells in the patient that responded to ICI therapy. Proximity analysis between CD8+ T Cells and other cells was studied. The spatial proximity of CD8+ T cells to tumor cells functions as an independent biomarker for response to anti-PD1 therapy.

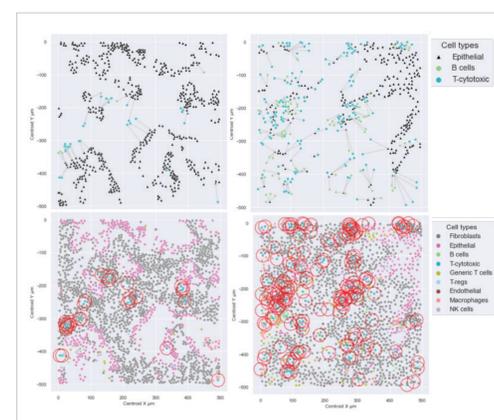


FIGURE 6. Nearest neighbor and proximity analysis representing spatial distribution of CD8+ T cell relative to others

CONCLUSION

These findings highlight the relevance of considering a set of spatial biomarkers involved in immune suppression pathways to obtain a comprehensive portrait of the tumor microenvironment for personalized therapy selection.

With a wide set of probes to investigate the spatial transcriptome, we better defined the overall pre-treatment phenotype to render the tumor suitable for therapy effectiveness.

High-multiplexed proteomics analyses using the Hyperion imaging system helped identifying and analyzing the tumor microenvironment heterogeneity to decipher mechanisms of ICI response.

Our results suggest that for patients who did not respond to monotherapy, it would have been preferable to resort to a combined immune checkpoint inhibitors treatment strategy, aimed at the complete inhibition of all the immune-suppressive pathways.

References

- Haslam A, Prasad V. Estimation of the Percentage of US Patients With Cancer Who Are Eligible for and Respond to Checkpoint Inhibitor Immunotherapy Drugs. *JAMA Netw Open*. 2019;2(5):e192535. Published 2019 May 3. doi:10.1001/jamanetworkopen.2019.2535
- Vansteenkiste J, Wauters E, Reymen B, Ackermans CJ, Peters S, De Ruyscher D. Current status of immune checkpoint inhibition in early-stage NSCLC. *Ann Oncol*. 2019;30(8):1244-1253. doi:10.1093/annonc/mdz1175
- Croci DO, Zacarias Fluck MF, Rico MJ, Matar P, Rabinovich GA, Scharovsky OG. Dynamic cross-talk between tumor and immune cells in orchestrating the immunosuppressive network at the tumor microenvironment. *Cancer Immunol Immunother*. 2007;56(11):1687-1700. doi:10.1007/s00262-007-0343-y
- Baghban R, Roshangar L, Jahanban-Esfahlan R, et al. Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Commun Signal*. 2020;18(1):59. Published 2020 Apr 7. doi:10.1186/s12964-020-0530-4