SELECTING THE RIGHT THERAPY:

Immunologic alterations in the lung cancer environment of patients treated with immune checkpoint inhibitor

SUMMARY

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 Inhibitor (ICI) therapy warranting a companion diagnostic. In this work, total expression of relevant transcripts and spatially resolved proteins was quantitated using multiplexed methods (NanoString nCounter and GeoMx platforms). This analysis identified numerous differentially expressed transcripts associated with the response to ICI. In addition, significant alterations in the expression and/or spatial distribution of immunologically relevant proteins in different regions (tumor cell rich versus immune cell rich) of the tumor microenvironment provide additional insight into the predictive immunological effects of clinically relevant adjuvant ICI therapy for resectable lung cancer. Taken together, these results identify gene expression profiles within the tumor microenvironment and provide preliminary evidence on biomarkers that may influence the suitability of ICIs.

APPROACH

The objective response to immune checkpoint inhibitors (ICI) is limited to 40%. A key limitation of immunotherapy is the lack of reliable biomarkers to guide treatment to the patients who are most likely to benefit from it. The mechanisms by which ICI treatment approaches, affect the individual cellular components of this tumor microenvironment in patients are poorly understood. In particular, uncovering signaling pathways and actionable immune targets in distinct tumor or immune cell compartments could generate data to better inform the use of targeted or immune-based therapy.

In the present study, we assessed the power of both spatially protein profiling combined with gene expression on retrospectively collected non small cell lung cancer (NSCLC) patients that received treatment with anti-PD1 (pembrolizumab)(Table 1). Several actionable signaling and immune biomarkers were identified in tumor and immune cell compartments of these lung tumors that may inform how ICI therapy can be maneuvered for improved efficacy.

Overall, these results represents a comprehensive analysis of numerous biomarkers conducted on the lung cancer microenvironment to guide strategic new combination therapies for lung cancer.

FIGURE 1. Workflow of the complementary strategy to quantify biomolecules within a targeted ROI.

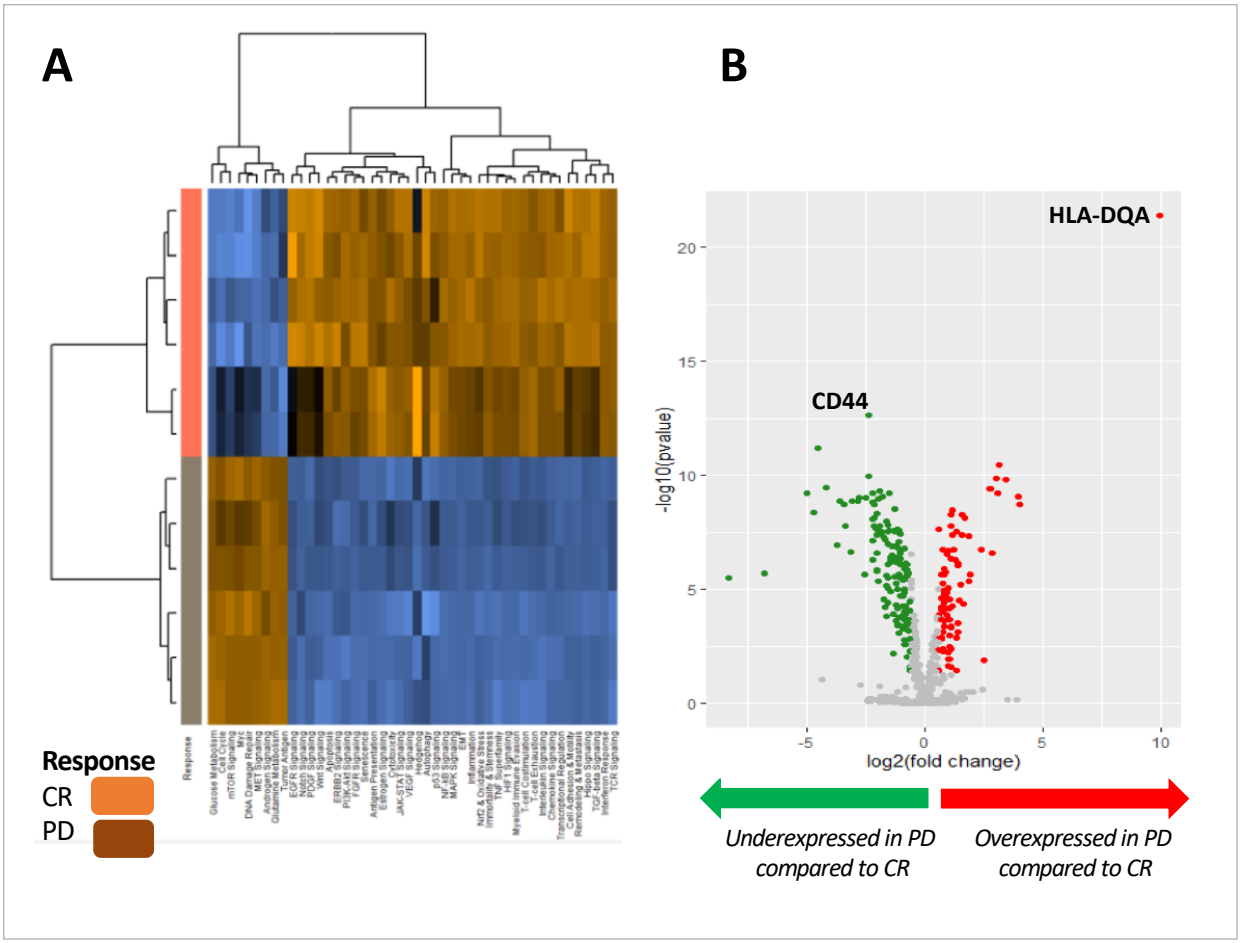
ICI Therapy	Pembrolizumab (n=2)
Age at diagnosis (yr)	
Mean	77
Range	76-78
Sex	
Female	1
Male	1
Race	
White	2
Tumor Location	
Lung	2
Initial Stage	
Resectable	2
Lymphovascular Invasion	
No	2

TRANSCRIPTS ASSOCIATED TO ICI RESPONSE

To assess the treatment predictivity on gene expression patterns related to immunological function, transcript levels of 780 predefined immunologically relevant and signaling tumor pathways genes (Tumor Signaling 360 Panel) from surgically resected frozen NSCLC were assessed using the NanoString nCounter platform.

Unsupervised hierarchical clustering (Figure 1A) revealed that the gene expression data clustered into objective response rate (ORR) with pathways such as tumor immunogenicity being overexpressed in the complete response (CR) patient. In comparison, T cell exhaustion phenotype is highly represented in the progression disease (PD) cohort with the statistically significant downregulation of the marker CD44 in the PD patient that identify activated T cell (Figure 1B).

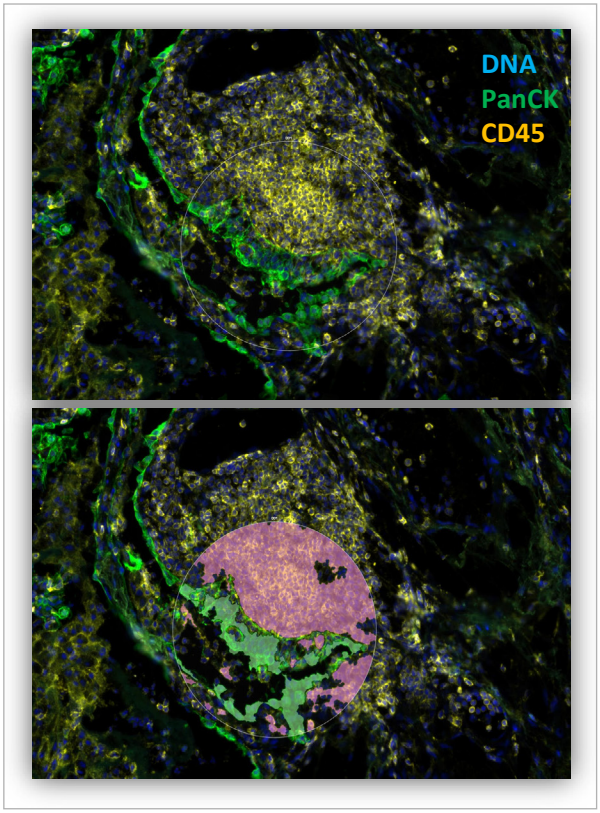
FIGURE 1. Response to ICI is associated with substantial alterations in immunologically relevant expression A-Heatmap clustering of gene expression in NSCLC samples resected from patients who received adjuvant ICI therapy (n = 1 patient/ORR, 6 replicates per patient). Each column represents one functional pathway, and each row represents 1 patient replicate. Unsupervised hierarchical clustering of genes and samples was carried out by uncentered Pearson correlation. Color indicates normalized counts of each gene, with yellow/orange representing higher expression and blue relatively lower expression. B- Volcano plot depicting differentially expressed gene P value as a function of fold change between the PD response compared to CR response.



IMMUNE CELLS COMPOSITIONS ASSOCIATED TO ICI RESPONSE

Changes in the quantity and spatial distribution of various immune-related protein markers in the lung tumor micro-environment were next assessed using the GeoMx platform (NanoString Inc.). This platform measures protein abundance in a multiplexed and spatially resolved manner. We selected 4 target regions per lung tumor on the basis of fluorescently labeled anti-CD45 and anti–pan-cytokeratin which were used essentially to “map” the tissue (Figure 2). Based on these fluorescent labels, we selected “immune cell–rich” regions that were enriched for CD45 staining, and “tumor rich” regions that were enriched for pan-cytokeratin.

FIGURE 2. Selection of tumor-rich and immune cell–rich regions within lung tumors. Selection of tumor-rich and immune rich regions within NSCLC tumors. As part of the GeoMx workflow, frozen slides of lung tumors from patients who received ICI therapy were stained with fluorescently labeled anti–pan-cytokeratin (green) and anti-CD45 (yellow). A representative region of interest (ROI) is shown in the below picture.



Within the immune cell-rich regions, 21 proteins differed significantly across the ORR (Figure 3). Response to ICI therapy was associated with high immune infiltration within the vicinity of the tumor. Indeed, CD8 and CD45 markers as well as the target of the drug PD-1 were highly expressed in the CR patient. In contrast, in the PD patient, Fibronectin was highly expressed and no immunosuppression marker was identified in this patient.

Among the statistically significant target that were differentially expressed across ORR in the immune cell-rich regions; CD4, HLA-DR, Granzyme B and CD40 were upregulated in the patient that responded to ICI (Figure 4). This emphasized the importance of activation of T cell and the antigen presenting cells in ICI response. Notable differences between the responder versus the non-responder were also evident in the tumor rich regions (Figure 3). Indeed the costimulatory marker CD40 is highly expressed in this region in the CR patient.

FIGURE 3. Expression levels of immunologically relevant proteins in immune and tumor cells-rich regions. Heatmap clustering of expression of the indicated proteins. Individual regions of interest were derived from each patient tumor and stroma from a total of n = 2 patients.

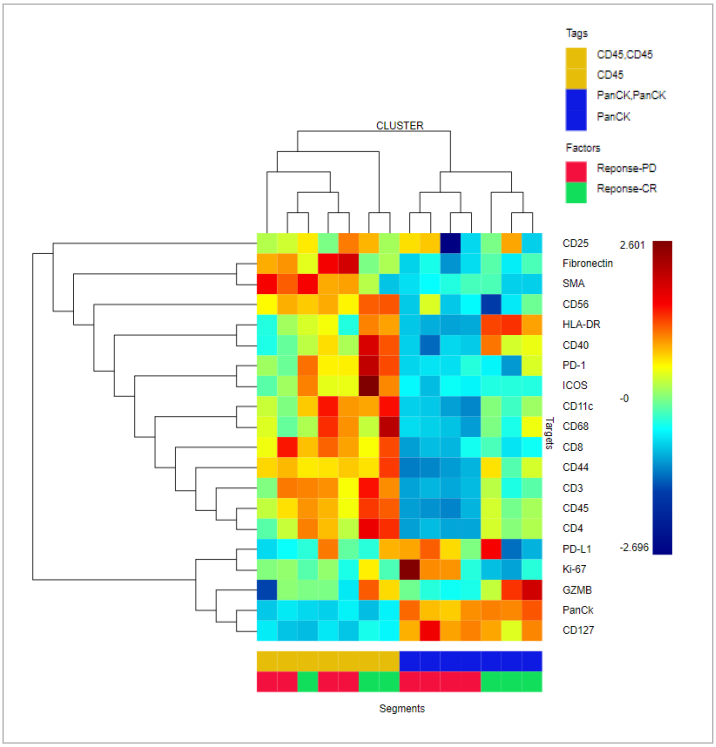
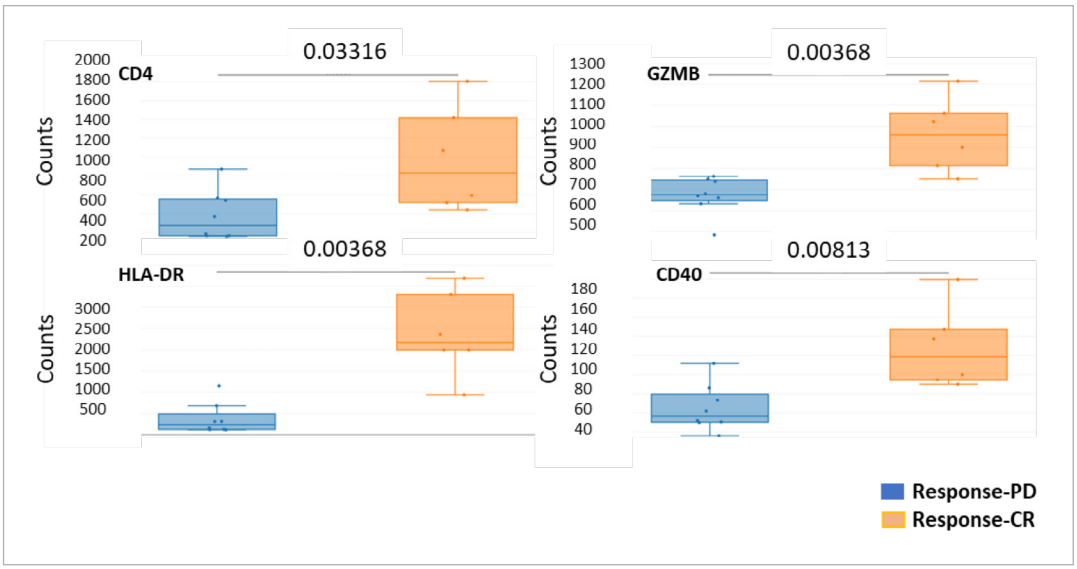


FIGURE 4. Response to ICI is associated with elevated expression of T cell activation and antigen presenting cell activation markers. Expression levels of the indicated proteins, CD4, HLA-DR, GZMB and CD40 are represented.



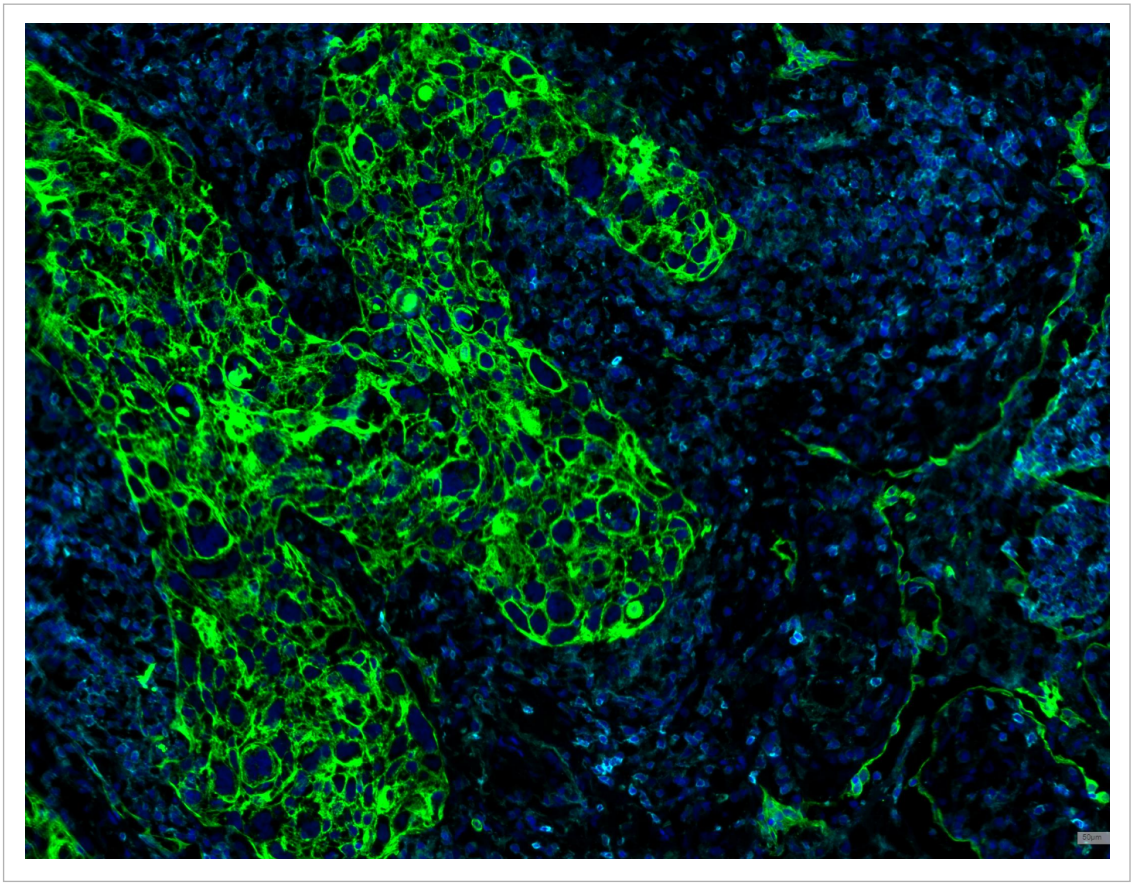
CONCLUSION

Thanks to Aliri's platform, we have been able to identity spatial biomarkers in the context of the lung cancer disease that help the further classification of patient that could benefit from ICI therapy.

Lung cancer, despite marginal improvements in survival over the last several years, continues to have a very poor prognosis. This resistance to therapeutic approaches extends to immunotherapy with very few patients responding to immune checkpoint blockade. In this white paper we examined the effect of spatial immune biomarkers in the microenvironment of lung tumor has on response to ICI. These results directly identified that the alterations in gene expression in the CR patient were sufficiently durable that unsupervised hierarchical clustering of the resulting gene expression profiling data distinguished tumor from patient that responded to ICI from the patient that did not respond. These spatial gene expression profiling analyses also identified several immunologically relevant gene families that belong to inflammation or immune responses. These observations argue that ICI therapy is associated with differential modulation of immune-related genes and there may be opportunity to leverage these changes to potentiate immunotherapy.

Taken together, these results identify gene expression profiles within the tumor microenvironment associated with response to ICI and provide preliminary evidence on biomarkers that may influence the suitability of biomarkers to be combined with other immune-based treatment strategies. Overall, these results provide insight into how tumor and immune components of the NSCLC tumor microenvironment modulate the response to ICI.

FIGURE 5. Expression of CD45 and PanCK in Lung Cancer that responds to ICI therapy.



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