Unveiling the diversity of melanoma immunotherapy response biomarkers between lymph node and non-lymph node biopsies

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Introduction

- Immunotherapy revolutionized the treatment of melanoma, however, a significant proportion of patients fail to respond. Thus, reliable biomarkers are needed to predict treatment response in order to improve patient outcomes.
- Spatial biology, which investigates the spatial distribution and interactions of cells within tissues, has emerged as a powerful approach to unravel the complexity of the tumor microenvironment (TME).
- In this study, we analyzed multiplex immunofluorescence (mIF) and H&E images from melanoma patients treated with immunotherapy, aiming to identify spatially resolved biomarkers associated with treatment response.

Methods

- We assembled a cohort of pretreatment biopsies of 47 melanoma patients who received PD-1 blockade, as a single-agent, or combined with ipilimumab. From each patient, two consecutive slides were stained with two 6-plex mIF antibody panels using PhenoImager, followed by hematoxylin and eosin (H&E) stain on the same slide.
- Utilizing our deep learning-based multiplex analysis pipeline[1] we identified 6 cell types from panel 1 and 5 cell types from panel 2, which were enriched with 3 additional cell types from the corresponding H&E image such as fibroblasts and granulocytes (Figure 1).
- mIF single-channel predictions and cell typing performance was evaluated against >10,000 annotations by expert pathologists to assess F1 scores and balanced accuracy (Figure 2)
- More than 1,300 spatial features were calculated using a combination of cell type, marker positivity and area assignments, as well as cell-cell interactions, and were compared between responders and non-responders to immunotherapy using Mann–Whitney U test in lymph node (LN) and non-LN tumor biopsies.

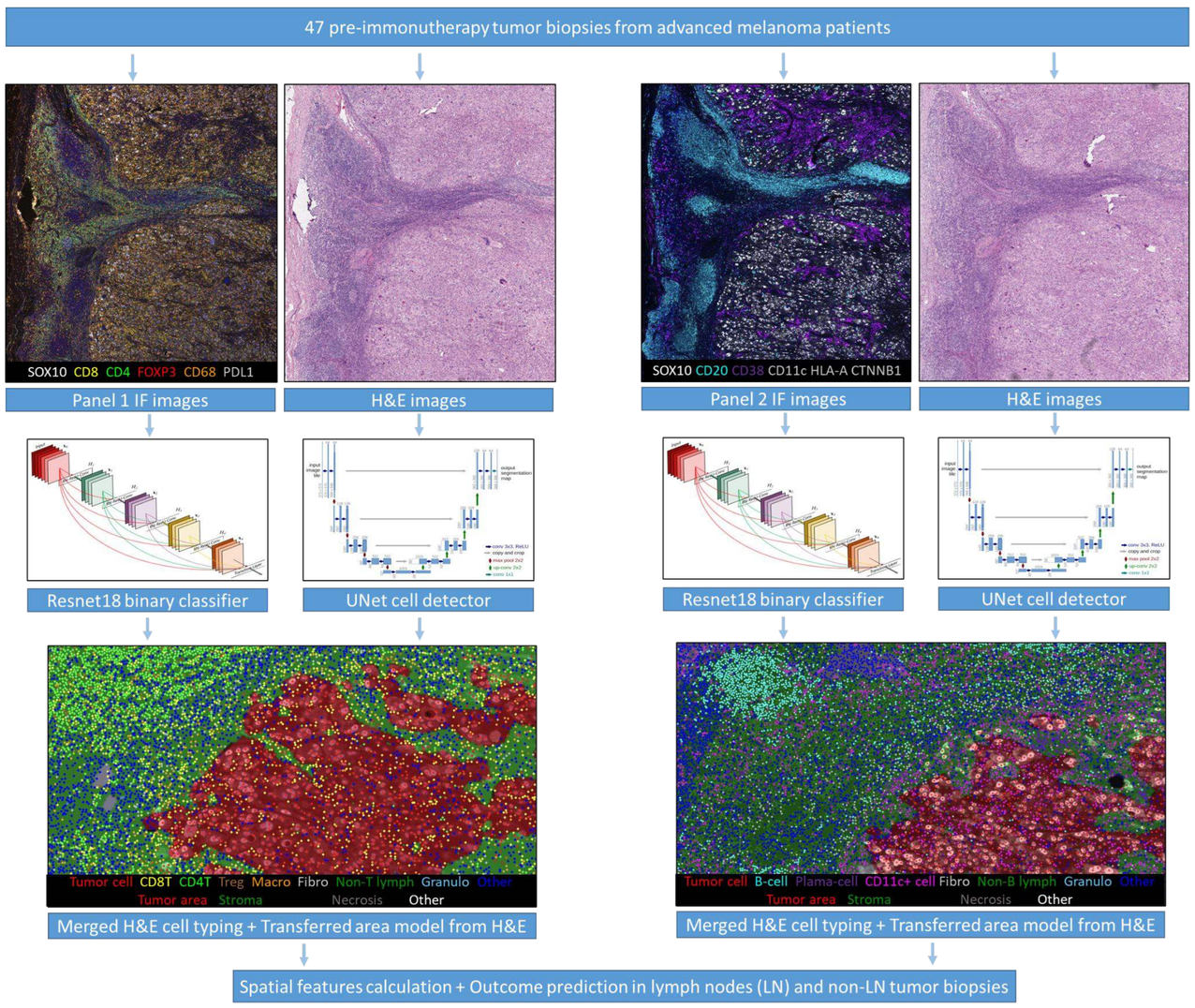

Figure 1. Study design and analysis workflow

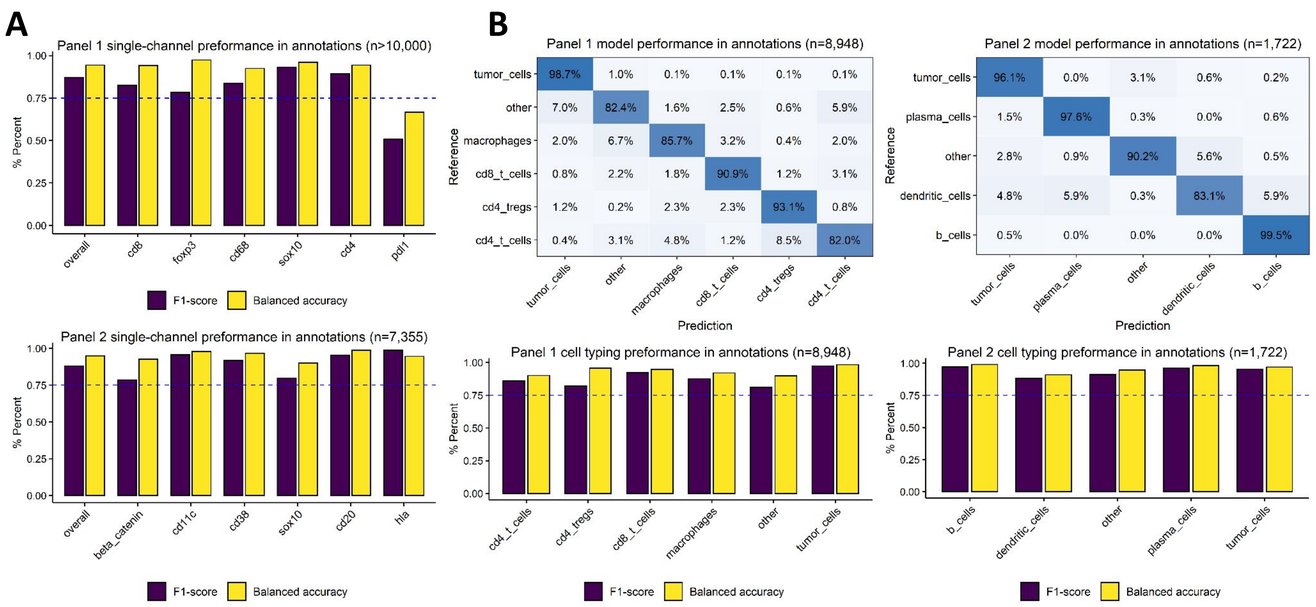

Figure 2. Deep learning-based multiplex imaging analysis pipeline performance in single-markers positivity classification (A) and cell typing (B)

Results

- Our analysis pipeline demonstrated excellent performance both in accurately defining cell types and single-markers positivity (Figure 2).
- Given the high stromal density of lymphocytes in LNs, the resulting TME composition in LN biopsies is vastly different. As a result, we aimed to characterize different spatial features which are associated with response to treatment in each biopsy type.
- In LN biopsies, we observed CD8+ FOXP3+ T-cell upregulation in the TME of responders to PD-1 blockade, while fibroblast and granulocyte interactions in the TME correlated with poor response (Figure 3).
- However, these associations were not observed in non-LN biopsies, where a high number of B-cell and T-cell interactions in the tumor invasive margin, and tumor cells and lymphocyte interactions in the tumor area were associated with treatment response. Additionally, a high number of granulocytes in the TME of non-LN biopsies was associated with treatment resistance (Figure 4).

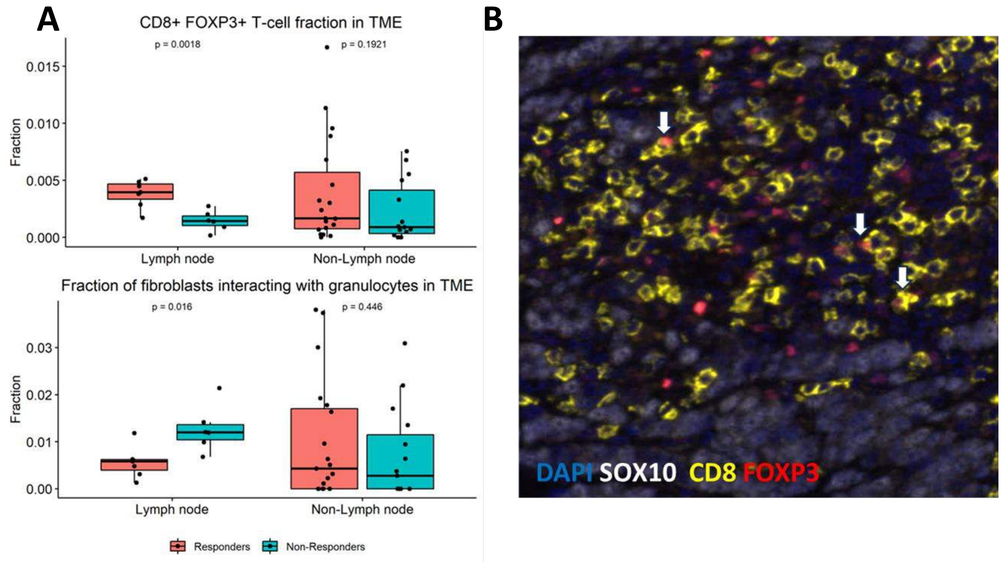

Figure 3. (A) The top differentially expressed features in between responders (n=7) and non responders (n=6) to PD-1 blockade in lymph node biopsies and their distribution in responders (n=19) and non responders (n=15) in non-lymph node biopsies. (B) A representative image of a LN biopsy with CD8+ FOXP3+ T-cells enrichment n the TME (white arrows).

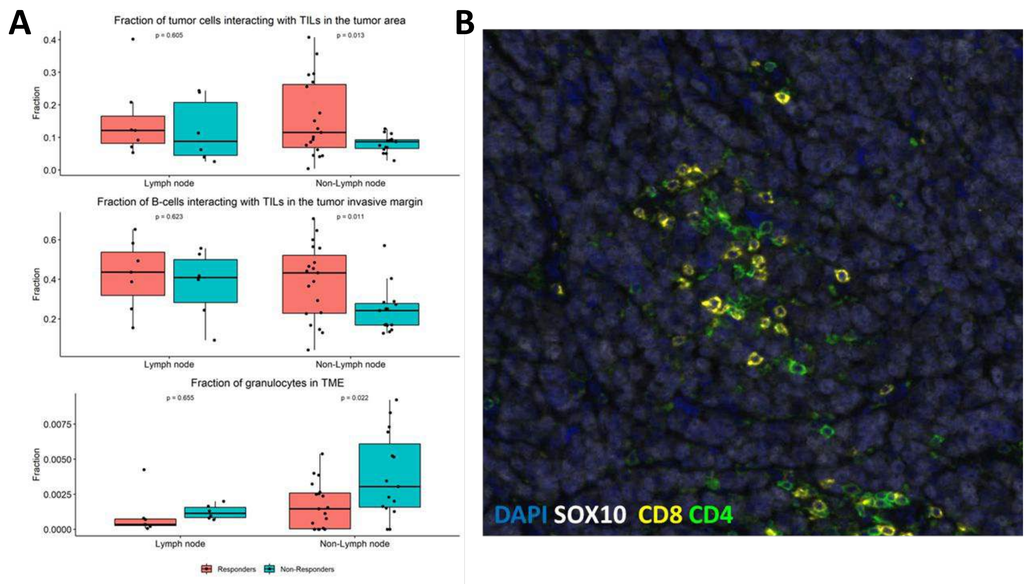

Figure 4. (A) The top differentially expressed features in between responders (n=19) and non-responders (n=15) to PD-1 blockade in non-lymph node biopsies, and their distribution in responders (n=7) and non-responders (n=6) in lymph node biopsies. (B) A representative image of a tumor infiltrating T-cells in non-LN biopsy.

Conclusions

- Our study demonstrates that the tumor-microenvironment in LN and non-LN melanoma biopsies are distinct and as a result, exhibit different spatial features associated with immunotherapy response depending biopsy site.
- Additionally, we reaffirm the critical role that T and B cells play in and around the tumor microenvironment
- These findings highlight the importance of spatial features, cell composition, and biopsy site when identifying spatial biomarkers for immunotherapy response prediction.

References

1. Markovits, E. et al. A novel deep learning pipeline for cell typing and phenotypic marker quantification in multiplex imaging. bioRxiv 2022.11.09.515776 (2022) doi:10.1101/2022.11.09.515776.