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Circulatory Plasma Proteomic Biomarkers Predict Response to Immunotherapy in Melanoma Patients and Reveal Biological Insights into the Tumor Microenvironment

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Background

Immune checkpoint blockade (ICB) has revolutionized the treatment of many immunogenic tumors, but a significant fraction of patients relapse or do not respond to treatment. Early identification of non-responders is essential to avoid adverse reactions to treatment and for timely identification of alternative treatments. However, predictive biomarkers for treatment response are incompletely characterized and are not representative of the entire patient population. Several approaches have leveraged clinical data and tissue biomarkers including tumor mutational burden, PD-L1 abundance, fraction of copy number alterations, HLA-I loss of heterozygosity, microsatellite status, interferon signatures, and immune composition from bulk RNA-sequencing. While these have proven fruitful, their performance has limited clinical utility due to suboptimal predictive performance, the need for adequate tissue, and a failure to capture a global measure of host response. Circulating protein biomarkers provide easy access for serial monitoring essential to deliver further insight into the mechanisms of response to ICB. However, the use of plasma proteomics to reveal biological insight into immune responses has been limited by small sample sizes or targeted approaches focused on either individual or small subsets of cytokines. Advanced proteomic technologies enabling large scale discovery of circulating protein biomarkers promise to identify tumor and immune changes associated with response to anti-PD1 treatment and uncover biological insights underlying primary resistance and toxicity. Combining protein biomarkers with other 'omics' approaches and sophisticated data analysis holds enormous potential to establish predictive tools for cancer immunotherapy.

Methods

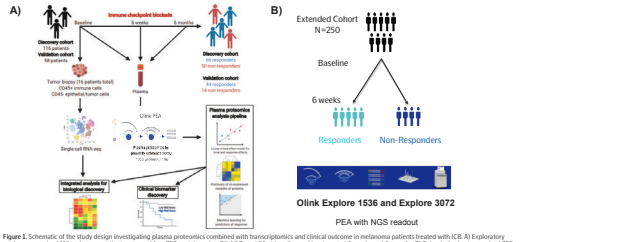

Figure 1. Schematic of the study design investigating plasma proteomics combined with transcriptomics and clinical outcome in melanoma patients treated with ICB. A) Exploratory analyses comprised 116 melanoma patients screened for >700 proteins using Olink® Target 96 at baseline and treatment at 6-weeks and 6-months. B) Extended cohort comprised 250 patients analyzed using the Olink® Explore 3072 high throughput proteomic platform at baseline and 6-weeks on treatment. Group differences and treatment effects were evaluated using a linear model with maximum likelihood estimation for model parameters with Benjamini and Hochberg multiple hypothesis correction.

Conclusions

- Plasma proteomics identified baseline predictive biomarkers for immunotherapy response.
- Circulatory protein biomarkers revealed important immune and tumor changes associated with resistance during checkpoint blockade treatment.
- Plasma response proteins were associated with primary myeloid cells and provided a roadmap for how circulatory proteins can generate biological insight on a tumor-intrinsic response to immunotherapy.
- Future work leveraging both plasma and tumor features holds great promise to establish predictive tools for cancer immunotherapy and is essential to expand clinical impact.

Results

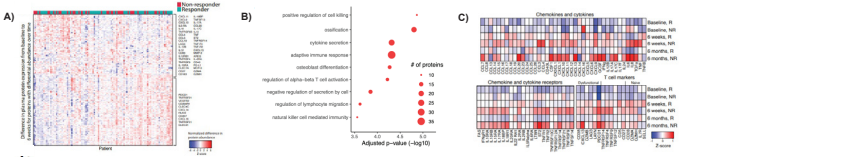

Figure 2. Differentially expressed plasma proteins over time during ICB treatment. A) Heatmap showing differentially expressed proteins at 6-weeks during ICB treatment compared to baseline. In total, 247 proteins were identified as significant over time using a linear mixed-effect model during ICB treatment in the discovery cohort. B) Gene-ontology analysis of time-effect significant proteins showed enrichment in regulators of cytotoxicity, cytokine secretion, lymphocyte migration and T cell and NK cell activation. C) Heatmaps demonstrate normalized mean expression of cytokines, cytokine receptors and markers of T cell substates in plasma over time within treatment responders and non-responders.

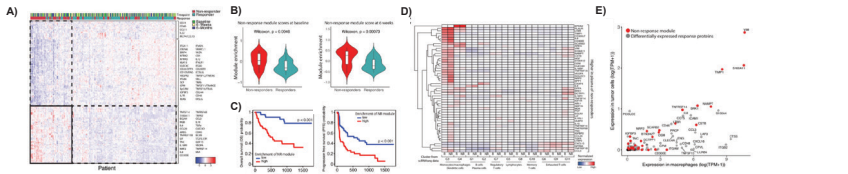

Figure 3. Plasma proteins are predictive of response to ICB treatment. A) Heatmap showing normalized plasma expression for all proteins significant for response-effect in all 116 patients and at all timepoints. B) Violin plots stratified by response status showing enrichment level of the non-response module set of plasma proteins of patients at baseline and at 6-weeks on treatment. C) Kaplan-Meier survival curves for overall survival and progression free survival for patients stratified by expression of the non-response module enrichment score at baseline. D) Plasma proteomic profiling vs scRNASeq. Plasma proteins differentially expressed in responders vs non-responders are most highly expressed in the myeloid compartment within tumor microenvironment. E) A suppressive tumor macrophage subset and tumor epithelial cells have enriched gene expression for the plasma-derived non-response module of proteins. Data represented by scatter plot were obtained by scRNAseq analysis of biopsies, derived from the fraction of patients analyzed by plasma proteomics, either from immune cell subsets or tumor epithelial cells.

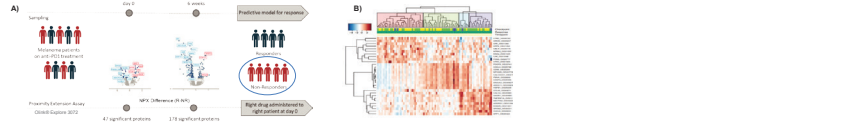

Figure 4. NGS Olink® Explore 3072 analyses in extended melanoma cohort on ICB treatment. A) Analyses of 1500 proteins in a fraction of 200 annotated melanoma patients identified set of baseline predictive biomarkers of response with changes profoundly enhanced at 6-weeks on treatment. NPX = Normalized Protein Expression; R-NR: Responders compared to Non-responders in log2 scale. B) Distinctive proteomic profile observed among non-responding patients.

References
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