neoadaptigen were evaluated. RNA analysis of the solid tumor enabled the investigation of the mTME.2 3

Results The average number of somatic SNVs in plasma samples was 100.5 (Range 50–250). KRAS, APC, PIK3CA, SMAD4, FBXW7, ARID1A were identified. Specifically, two components of SWI/SNF complex, ARID1A and BRD9, were both mutated in plasma samples, suggesting the potential dysregulation of epigenetic pathways. RTK-RAS and Notch pathways were also frequently mutated. Further, 1,195 somatic events were found in genes not covered by commercially available targeted panels. 27 of these SNVs are in immuno-oncology related genes, which highlight the importance of somatic evidence observable through an exome-scale cfDNA approach. In solid tumor, the average number of detected somatic SNVs was 133.4 (Range 69–230), with similar mutation landscape. Concordance is observed between tumor and plasma samples (mean: 40.6%; range: 15.13%–94.2%). However, a number of variants are plasma-specific, suggesting that cfDNA WES detects tumor mutations that might be missed by a single site biopsy. We evaluated neoantigen and determined that the fraction of variants predicted as neoantigens are similar between plasma and tumor. Importantly, several of the top neoepitopes are uniquely predicted in plasma, suggesting the potential clinical value of using WES cfDNA. RNA-sequencing of solid tumor samples enabled mTME profiling. CD8 T cell immune infiltration, TCR beta clonality and clone counts were low, suggesting these patients have cold tumors. Myeloid dendritic cells and macrophages demonstrated uniform abundance across samples, while B and T regulatory cells showed variable tumor infiltration.

Conclusions Results demonstrate potential clinical utility and highlight the advantages of whole-exome scale profiling of plasma and matched tumor samples, which enables a systematic interrogation of tumor biology, including mTME. Notably, a whole-exome based liquid biopsy assay offers indispensable insights that might be otherwise missed by a single site tumor biopsy or targeted liquid biopsy panels.

Ethics Approval The study protocol was in accordance with the tenets of the Declaration of Helsinki. Commercial samples used in this study were procured from Bioreclamation IVT and BioChain following protocols approved by the local Institutional Review Board (IRB) committee. Informed consent forms were obtained from all the human subjects in this study.

Consent N/A

REFERENCES

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response in non-small cell lung cancer. Here, we present initial results of a host response-based machine learning classifier that predicts clinical outcome in melanoma patients treated with immune checkpoint inhibitors (ICIs).

**Methods** Plasma samples from melanoma patients (training set; n=32) treated with anti-PD-1 or anti-PD-1 and anti-CTLA-4 combination were obtained at baseline and early on treatment. Response was based on RECIST criteria. Proteomic profiling of the plasma samples was performed using ELISA-based antibody arrays. Machine learning algorithms were used to identify a predictive signature that stratifies between responders and non-responders. The signature was validated on an independent cohort of melanoma patients (validation set; n=14). In addition, advanced bioinformatic analysis was performed in order to identify biological pathways unique to responders and non-responders.

**Results** A 3-protein signature was identified as a predictor of clinical outcome following immunotherapy with an area under the curve (AUC) of the receiver operating characteristics (ROC) plot of 0.88 (p-value 5.84E-05; confidence interval 0.76 – 1.0), and sensitivity and specificity of 0.65 and 0.95, respectively. This signature was successfully validated with AUC of 0.85 (p-value 0.03; confidence interval 0.63 – 1.0), and sensitivity and specificity of 0.75 and 0.9, respectively. To further explore the biological basis of resistance to immunotherapy, we performed a pathway enrichment analysis. Multiple mechanisms for resistance were identified in the non-responder group, including immunosuppression and inflammation associated pathways. Comparison between the two treatment modalities revealed pathways unique to each treatment that involve extracellular modulation, immunosuppression and processes associated with tumor progression, which may imply important differences between the two regimens.

**Conclusions** Our results demonstrate that analyzing the host response to IC therapy using plasma-based proteomic profiling combined with machine learning algorithms serves as a successful approach for predictive biomarker discovery in melanoma. This bioinformatics-based functional analysis provides insights into mechanisms of resistance and may be used to identify potential strategies for improving clinical outcomes.

**Ethics Approval** The study was approved by the Yale University Institutional Review Ethics Board, approval number 0609001869.

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**Abstracts**

**CIRCULATING TUMOR DNA (CTDNA) SERIAL ANALYSIS DURING PROGRESSION ON PD-1 BLOCKADE AND LATER CTLA4 RESCUE IN PATIENTS WITH MISMATCH REPAIR DEFICIENT METASTATIC COLORECTAL CANCER**

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**Background** Patients with mismatch repair deficient/microsatellite instability high (dMMR/MSI-High) tumors respond well to immune checkpoint blockade. Pembrolizumab was the first drug to be approved by the FDA in an agnostic fashion for any tumor type with dMMR/MSI-High for the very same reason. The responses in dMMR/MSI-High tumors tend to be brisk, dramatic and durable to the point that the word ‘cure’ is being used for patients who do respond to PD-1 blockade. This year, pembrolizumab now got approval as 1st line therapy for dMMR/MSI-High metastatic colorectal cancers as well.

**Methods** Plasma samples from melanoma patients (training set; n=32) treated with anti-PD-1 or anti-PD-1 and anti-CTLA-4 combination were obtained at baseline and early on treatment. Response was based on RECIST criteria. Proteomic profiling of the plasma samples was performed using ELISA-based antibody arrays. Machine learning algorithms were used to identify a predictive signature that stratifies between responders and non-responders. The signature was validated on an independent cohort of melanoma patients (validation set; n=14). In addition, advanced bioinformatic analysis was performed in order to identify biological pathways unique to responders and non-responders.

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