neoantigens 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 neoantigens 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 Bio-Chain 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

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20 QUANTIFICATION OF SBCMA IN HUMAN PLASMA USING A HIGH-THROUGHPUT MASS SPECTROMETRY WORKFLOW FOR EXPLORATORY, CAP/CLIA OR REGULATED STUDIES

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Background Bioanalytically validated methods are needed for reliable measurement of B cell maturation antigen (BCMA) in clinical samples. BCMA contributes to multiple myeloma (MM) pathophysiology and is targeted by various novel immunotherapies. Soluble BCMA (sBCMA) is a promising novel biomarker for disease monitoring and prediction. To address the need for sBCMA measurement, we developed a mass spectrometry-based assays for the quantitation of total sBCMA in plasma.

Methods Immunoaffinity enrichment of sBCMA from human plasma was performed on a Agilent AssayMap Bravo platform using streptavidin cartridges. Recovered sBCMA protein was digested to peptides using trypsin, spiked with a fixed level of stable isotope labeled (SIL) peptide standard, and analyzed by multiple-reaction-monitoring (MRM) mass spectrometry. The MRM assay targeted a BCMA-specific endogenous peptide used as a surrogate measure of the protein, as well as the corresponding spiked SIL peptide at known concentration. An 8-point external calibration curve was prepared by spiking varying amounts of recombinant BCMA and fixed amounts of SIL peptides in surrogate matrix. Endogenous sBCMA levels were determined by back-calculating against the curve. To assess the performance of the method, ≥ 2 precision and accuracy runs were performed. To assess the impact of ligand or drug binding on the developed assay, performance of the assay was tested using plasma spiked at a range of concentrations with rhAPRIL, rhBAFF or simulated mAb drug.

Results The developed assay allowed the quantitation of sBCMA from 1 to 1000 ng/mL. The precision and accuracy at different QC levels was within 20% CV and 20% bias. The presence of binding proteins (rhAPRIL, rhBAFF, simulated mAb) did not interfere with the measurement of sBCMA indicating that the assay measures total sBCMA.

Conclusions We have developed an assay for the absolute quantitation of total sBCMA in human plasma. The assay can be analytically validated and deployed for clinical studies with a $\sim$ 3-day turnaround time.

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21 PLASMA-BASED PROTEOMIC PROFILING AS A TOOL FOR PREDICTING RESPONSE TO IMMUNOTHERAPY IN MELANOMA PATIENTS

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Background In recent years, studies have indicated that in response to almost any type of anti-cancer therapy, the patient (the host) may generate pro-tumorigenic and pro-metastatic effects. This phenomenon, called host-response, counteracts the anti-tumor activity of the treatment. We have previously shown that machine learning-based plasma proteomic analysis of the host response may serve as a predictive tool for

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 ICI 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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23 | 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.[1][2] 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.
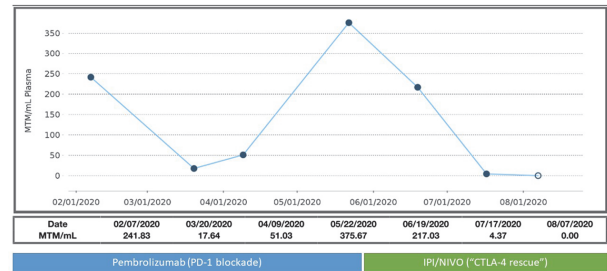
Abstract 23 Figure 1 Example of a patient with serial tumor-informed ctDNA monitoring showing initial response and subsequent progression on PD-1 blockade followed by 'CTLA-4 rescue'.

However, a third of patients do not respond.[3] Predictive markers and data for subsequent therapy options are lacking. Here we present for the first time a series of dMMR/MSI-High patients who not only had serial circulating tumor DNA (ctDNA) monitoring during PD-1 blockade/progression, but also were able to get anti-CTLA4 in conjunction with an anti-PD1 ('CTLA4-rescue'), with ctDNA trends predicting responses weeks ahead of standard imaging.

Methods Metastatic colorectal cancer patients enrolled in the expanded access program for tumor informed circulating tumor DNA monitoring (Signatera 16-plex bespoke mPCR NGS assay) who were noted to be dMMR/MSI-High colorectal cancers were identified. Serial monitoring results while they were receiving immune checkpoint blockade therapy is presented. This only includes patients who had progression on PD-1 blockade whereby CTLA-4 rescue was done as part of their treatment strategy.

Results Serial monitoring and trends of progression followed by responses are depicted in the patients who had CTLA-4 rescue post PD-1 progression (figure 1). This correlated with radiographic responses in all the patients. The ctDNA decreases in patients showing responses as well as ctDNA increases earlier during progression on PD-1 blockade happened within administration of a single dose.

Conclusions To date there is only 1 case report published earlier this year showing the value of 'immunotherapy after immunotherapy' in patients with dMMR/MSI-High tumors. Here we not only present a series of patients but also in parallel provide a snapshot on serial ctDNA trends whereby this could serve as a dynamic predictive marker of early response or progression to therapy.[4][5] Finally, 'CTLA4-rescue' needs to be formally included in NCCN and other respective guidelines. Even though nivolumab/ipilimumab is listed as an option for dMMR/MSI-High tumors in addition to single agent pembrolizumab or nivolumab, it is not listed as an option post-PD-1 progression. For all the patients, we have had to fight to get peer to peer approval.

Ethics Approval The study is approved at University of Iowa and part of IRB#201202743.

Consent Written informed consent was obtained from the patients for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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