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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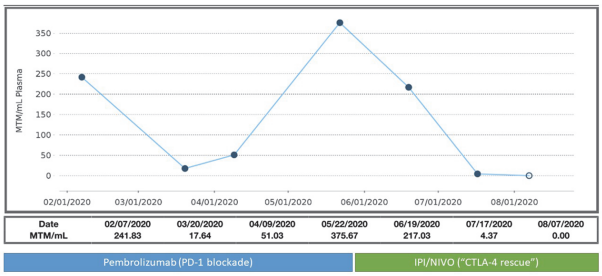
Background Patients with mismatch repair deficient/microsatellite instability high (dMMR/MSI-High) tumors respond well to immune checkpoint blockade.[1 2] Pembrolizumab was the first drug to be approved by the FDA in an agnostic fashion for any tumor type with dMMR/MSI-High for the very same reason. 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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24 UTILITY OF TUMOR-INFORMED MOLECULAR RESIDUAL DISEASE ASSAYS IN PATIENTS WITH COMPLETE RESPONSE TO IMMUNE CHECKPOINT BLOCKADE

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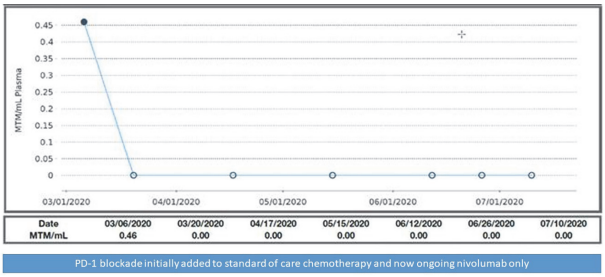
Background Recent data suggests that responses in patients with mismatch repair deficient (dMMR) tumors tend to be durable and potentially curative. Typically immunotherapy is employed and approved only in metastatic settings. However, it is not uncommon now to consider usage in patients with dMMR tumors secondary to the hypermutated nature of these malignancies alongside the concerns that these do not respond to therapy either through a clinical trial or off-label compassionate access programs. As noted, in patients who have these dramatic and durable responses, the question of foregoing surgery and/or radiation comes up. There is no great test to help predict or guide who are these complete responders. Assessment of molecular residual disease or minimal residual disease through tumor-informed assays is one potential test that can be employed in this setting. We here show the feasibility of such an approach in patients with dMMR tumors who got immunotherapy in the advanced but not metastatic setting.

Methods We identified patients who were enrolled in the serial tumor informed molecular residual disease BESPOKE expanded access ctDNA testing program (Signatera 16-plex bespoke mPCR NGS assay) who got immunotherapy in the neoadjuvant setting and were also enrolled in our biobanking program.

Results We were able to serially do ctDNA analysis in 2 patients (1 with advanced but not metastatic esophageal adenocarcinoma and another patient with advanced but not metastatic nearly obstructing rectal adenocarcinoma with extensive nodal metastases in both situations) who got immunotherapy off-label per physician discretion and tumor board discussion. Of note both these patients also had germline lynch syndrome. Both of them had robust, dramatic and ongoing responses to immune checkpoint blockade. Of note, while they both had some radiographic question of residual disease, repeated endoscopic ultrasounds and random biopsies have yielded no tumor and all just scarred tissue. Circulating tumor DNA in these instances quickly declined to become undetectable and has remained not detectable to date (figure 1). Surgery and radiation is deferred and close follow up alongside serial endoscopic/radiographic assessment in addition to novel usage of ctDNA MRD assays is being employed.



Abstract 24 Figure 1 Ongoing response and negative ctDNA minimal residual disease to PD-1 blockade added to standard of care chemotherapy for a patient with advanced rectal cancer

Conclusions Data regarding neoadjuvant usage of immunotherapy is scarce pertaining to mismatch repair deficient tumors. There is a case series of 3 patients with rectal cancer that was just reported this year.[1] Here we not only report a case of an advanced rectal cancer, but also a case of an advanced esophageal cancer who have achieved dramatic responses with no evidence of disease on repeated sampling. It is rare for surgery or radiation to be deferred in these situations. However, with added utility of ctDNA MRD assays, it gives us one more tool in our toolbox in terms of applying it to patients suitable for these 'watch-&-wait' approaches.

Ethics Approval The study has been approved by University of Iowa's Institutional Review Board IRB#201202743.

Consent Written informed consent was obtained from the patient 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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25 LINE OF THERAPY ADJUSTMENT IN A PATIENT WITH ADVANCED TRIPLE-NEGATIVE BREAST CANCER (TNBC) BY USING PERSONALIZED CTDNA TEST FOR TREATMENT RESPONSE MONITORING

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Background Triple negative breast cancer (TNBC) is an aggressive form of breast cancer that is most difficult to treat due to the absence of hormone/growth factor receptors.[1 2] Metastatic TNBC (mTNBC) is particularly challenging, given the limited efficacy and duration of response to chemotherapy.[3] The repertoire of therapeutic options for mTNBC patients continues to increase with chemotherapeutic and immuno oncology based treatments and now includes sacituzumab govitecan, a novel antibody-chemotherapy conjugate.[4]

Methods Here we present a case study of a 40-year-old female who on biopsy of her left breast mass was diagnosed with TNBC. The patient underwent neoadjuvant chemotherapy with weekly administration of paclitaxel and carboplatin followed by dose-dense doxorubicin with cyclophosphamide. Following one-month, the patient underwent bilateral mastectomy, showing pathological staging ypT2 pN0. The patient underwent periodic radiological imaging along with the assessment of circulating tumor DNA in blood using a personalized and tumor-informed multiplex PCR, next-generation sequencing assay (Signatera bespoke, mPCR NGS assay) to identify the minimal residual disease (MRD) and treatment response.

Results After surgery, MRD assessment revealed ctDNA positive status (0.41 MTM/mL) prompting PET/CT scan that revealed liver metastasis. Continued ctDNA monitoring