array, to determine whether we could detect treatment-related changes.

Results We demonstrated that this peptide array yielded highly reproducible measurements of serum IgG levels. We found that the overall number of antibody responses did not increase with disease burden. However, the composition of recognized proteins shifted with clinical stage of disease. Our analysis revealed that the largest difference was between patients with castration-sensitive and castration-resistant disease. Patients with castration-resistant disease recognized more proteins associated with nucleic acid binding and gene regulation compared to men in other groups. Our longitudinal data showed that vaccine-treated patients developed increased responses to more proteins over the course of treatment than did ADT-treated patients, consistent with the development of antigen spread.

Conclusions This study represents the largest survey of prostate-cancer associated antibodies to date. We have been able to characterize the classes of proteins recognized by patients and determine how they change with disease burden. Our findings demonstrate the potential of this platform for measuring antigen spread and studying responses to immunomodulatory therapies.

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Ethics Approval Study protocols that permitted collection and use of human blood samples were reviewed and approved the University of Wisconsin Human Subjects' Review Board (IRB). All patients gave written informed consent for use of blood products for research.

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17 ACTIVITY SENSORS FOR NONINVASIVE MONITORING OF IMMUNE RESPONSE AND TUMOR RESISTANCE DURING IMMUNE CHECKPOINT BLOCKADE THERAPY

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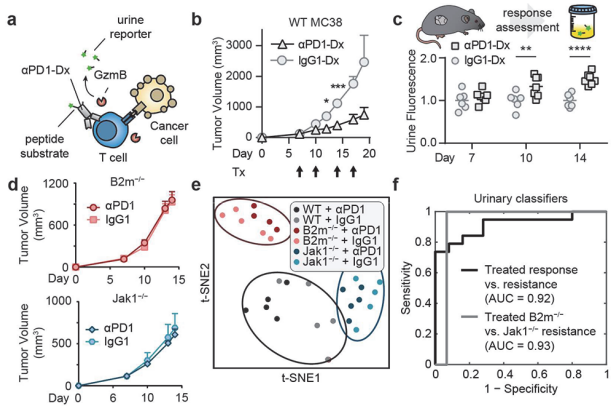
Background Despite the curative potential of immune checkpoint blockade (ICB) therapy, only small subsets of patients achieve tumor regression while many responders relapse and acquire resistance. Monitoring treatment response and detecting the onset of resistance are critical for improving patient prognoses. Here we engineered ICB antibody-sensor conjugates known as ICB-Dx by coupling peptides sensing the activity of granzyme B (GzmB), a T cell cytotoxic protease, directly on αPD1 antibody to monitor therapeutic responses by producing a fluorescent reporter into urine. To develop biomarkers that indicate mechanisms of resistance to ICB, we generated B2m-/- and Jak1-/- tumor models and performed transcriptomic analyses to identify unique protease signatures of these resistance mechanisms. We then built a multiplexed library of αPD1-Dx capable of detecting early therapeutic response and illuminating resistance mechanisms during ICB therapy.

Methods FITC-labeled GzmB substrates were synthesized (CEM) and conjugated to αPD1 antibody. B2m-/- and Jak1-/- tumors were generated from WT MC38 cells using CRISPR/Cas9. For tumor studies, 10^6 cells were inoculated s.c. in B6 mice. Tumor mice were treated with αPD1 or IgG1 isotype conjugates (0.1 mg), and urine was collected at 3 hours. Tumor RNA was isolated with RNEasy kit (Qiagen) and prepared for sequencing with TruSeq mRNA kit (Illumina).

Results To synthesize αPD1-Dx, we coupled FITC-labeled GzmB substrates to αPD1 antibody (figure 1a). In MC38 tumors, systemic administration of αPD1-Dx lowered tumor burden relative to control treatment while producing significantly elevated urine signals that preceded tumor regression (figure 1b, c). To investigate the ability to monitor tumor resistance to ICB, we developed knockout tumors to model B2m and Jak1 mutations, which are observed in human patients. in vivo, B2m-/- and Jak1-/- MC38 tumors were resistant to αPD1 monotherapy (figure 1d). Tumor RNA sequencing revealed that gene expression was altered during αPD1 treatment only in WT tumors. Importantly, B2m-/- tumors showed very different expression profiles than Jak1-/- tumors during αPD1 treatment, indicative of unique regulation of resistance (figure 1e). We used differential expression analyses to discover unique protease signatures associated with these two resistance mechanisms. Finally, a multiplexed library of αPD1-Dx engineered to monitor both tumor and immune proteases detected early on-treatment responses and stratified B2m-/- from Jak1-/- resistance with high diagnostic validity (figure 1f).



Abstract 17 Figure 1 Monitoring response and resistance with ICB-Dx (a) αPD1-Dx can reinvigorate T cell response and monitor protease activities in the tumor microenvironment. (b) Growth curves of WT MC38 tumors treated with αPD1- or IgG1-Dx (ANOVA). (c) Urine signals detect treatment response to αPD1 monotherapy (ANOVA). (d) Growth curves of B2m-/- and Jak1-/- tumors treated with αPD1- or IgG1-Dx (ANOVA). (e) TSNE plot showing RNA profiles of WT, B2m-/-, Jak1-/- tumors treated with αPD1 or isotype control. (f) ROC curves of random forest classifiers built from urine signals that differentiate on-treatment response from on-treatment resistance and B2m-/- from Jak1-/- resistance.

Conclusions We have engineered activity sensors that accurately detect therapeutic responses and stratify resistance mechanisms noninvasively from urine, thereby potentially expanding the precision of ICB therapy to benefit cancer patients.

Ethics Approval All animal studies were approved by Georgia Tech IACUC (A100193)

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