DISCOVERY OF GANGLIOSIDE GM2 ACTIVATOR AS A NOVEL PROTEOMIC BIOMARKER ASSOCIATED WITH RESPONSE TO TREATMENT IN FIRST-LINE MELANOMA SUBJECTS TREATED WITH PD-1 IMMUNOTHERAPY

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Background Immune checkpoint inhibitors (ICI) have greatly improved the treatment options for patients with metastatic melanoma. Yet, a large percentage of melanoma patients do not respond to ICIs, there is a need for biomarkers that can predict patients’ clinical benefit thereby identifying the patient population most likely to respond. Here, we apply unbiased discovery proteomics to deeply characterize global tumor proteomes and associate proteins and pathways at baseline with clinical response to anti-PD-1 immunotherapy.

Methods Unbiased, data-independent acquisition (DIA) mass spectrometry was used to analyze Formalin Fixed Paraffin Embedded (FFPE) tumor tissue from subjects with stage III-C IV melanoma which were resected prior to initiation of first-line anti-PD-1 therapy. The selected samples represent two distinct clinical subgroups; those who received clinical benefit (CR or PR by RECIST criteria or OS >1 year with SD by RECIST criteria, n = 13), and those with no clinical benefit (PD by RECIST criteria or OS <1 year with SD by RECIST criteria n = 9). Previously, the sample cohort had been analyzed by a 2-hour LC-MS/MS gradient setup operated in DIA mode. In this study, all samples were analysed with a longer gradient of 4-hours which enabled the quantification of 1K proteins more proteins and enabled an updated analysis with a deeper level of characterization.

Results 8548 proteins were quantified across all samples, with 7416 quantified on average per sample. Univariate statistical testing between groups identified 285 proteins that were significantly regulated in subjects who received clinical benefit. Through partial least squares discriminant analysis (PLS-DA) a set of 25 proteins was identified that describe the variance between the two sample groups. Ganglioside GM2 activator (GM2A) and other members of its interaction network such as HEXB, HRNR and CPPE1 were identified to be upregulated in the non-responder group.

Conclusions Global profiling of the baseline tumor proteome provides a unique characterization of melanoma tumor biology. A signature of 25 protein markers was identified as a driver of separation between responder and non-responder patients to PD-1 blockade. Among the protein markers, GM2A and its interactors, were previously shown to perturb T cell function, which might explain their enrichment in the non-responder group and provide an attractive target for improving patient response to immunotherapy.
A NOVEL DISCOVERY PIPELINE IDENTIFIES MELANOMA-SPECIFIC ANTIBODIES IN PATIENTS RESPONDING TO IMMUNE CHECKPOINT INHIBITORS

1Daniel Delitto*, 1Evans Lipson, 1Laura Cappelli, 2Klaus Busam, 1Antony Rosen, 1Suzanne Topalian, 1Livia Casciola-Rosen. 1Daniel Delitto*, 1Evan Lipson, 1Laura Cappelli, 2Klaus Busam, 1Antony Rosen, 1Suzanne Topalian, 1Livia Casciola-Rosen.

Background Tumor-specific antibodies have been reported in patients with cancers responding to immune checkpoint inhibitors (ICI), and there is an increasing appreciation for the potential role of B cells in mediating ICI responses. However, the humoral immune response to melanoma remains incompletely defined. We hypothesized that screening sera for antibodies by immunoprecipitation with lysates of cultured melanoma cells would increase the likelihood of detecting circulating antibodies in melanoma patients receiving ICI, and potentially identify novel antibody targets associated with treatment response and/or immune-related adverse events (IRAEs).

Methods Pre-and on/post-treatment sera or plasma from 12 clinically-annotated patients with advanced metastatic melanoma receiving ICI were assayed for tumor-specific antibodies. Our comprehensive screening platform detected circulating antibodies specific to multiple melanoma-associated and cancer testis antigens in patients deriving clinical benefit from ICI. Expanded investigations of the evolution of antibody production over the course of ICI therapy, associated with tumor response to treatment and development of IRAEs, are warranted.

Conclusions Our comprehensive screening platform detected circulating antibodies specific to multiple melanoma-associated and cancer testis antigens in patients deriving clinical benefit from ICI. Expanded investigations of the evolution of antibody production over the course of ICI therapy, associated with tumor response to treatment and development of IRAEs, are warranted.

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Ethics Approval This study was approved by the Johns Hopkins Institutional Review Board, approval #NA_00090257.

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232 THE EPITHELIAL-TO-MESENCHYmal TRANSITION (EMT) CONTRIBUTES TO IMMUNOSUPPRESSION IN BREAST CARCINOMAS AND REGULATES THEIR RESPONSE TO IMMUNE CHECKPOINT BLOCKADE

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Background Immune checkpoint blockade (ICB) has generated some dramatic responses in certain types of human tumors, most notably, melanomas. However, the response of breast tumors has been largely limited. We have previously demonstrated that the residence of breast cancer cells in the epithelial or mesenchymal phenotypic states can itself be used as an important determinant of the success or failure of ICB. Specifically, we have shown that while epithelial tumors are sensitive of 1 (100%) patient with a complete response, 2 of 4 (50%) with a partial response, 1 of 1 (100%) with stable disease, and 0 of 6 (0%) with progressive disease. Antibody levels varied over the course of therapy, with previously undetectable specificities arising during treatment response in patients #1–3. Patient #1 with a complete tumor regression developed antibodies to SSX2 and MageA10 that were absent before treatment. Further, detection of these antibodies coincided with diagnosis of IRAEs (anti-SSX2 with pancreatitis and anti-MageA10 with dermatitis). In contrast, patient #3, initially with a partial tumor regression, demonstrated a loss of detectable anti-NY-ESO-1 antibodies upon disease progression, and subsequent metastasectomy demonstrated loss of NY-ESO-1 protein expression in the progressing tumor. Testing sera from all 12 patients with IVTT products for NY-ESO-1, SSX2 and MageA10 did not reveal additional humoral responses.

Abstract 232 Table 1 Antibodies detected in the serum or plasma of patients with metastatic melanoma treated with ICI therapy. Treatment response indicates best overall response according to RECIST v1.1. Post-treatment blood collections were drawn during or after ICI therapy.

<table>
<thead>
<tr>
<th>Antibody Specificity</th>
<th>Tumor Response</th>
<th>Tumor-Associated Specificity</th>
<th>Post-treatment Specificity</th>
<th>Specificity</th>
<th>% of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>NY-ESO-1</td>
<td>Complete</td>
<td>NY-ESO-1</td>
<td>NY-ESO-1</td>
<td>NY-ESO-1</td>
<td>100%</td>
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<tr>
<td>SSX2</td>
<td>Stable</td>
<td>SSX2</td>
<td>SSX2</td>
<td>SSX2</td>
<td>100%</td>
</tr>
<tr>
<td>MageA10</td>
<td>Progressive</td>
<td>MageA10</td>
<td>MageA10</td>
<td>MageA10</td>
<td>0%</td>
</tr>
</tbody>
</table>

Conclusions Our comprehensive screening platform detected circulating antibodies specific to multiple melanoma-associated and cancer testis antigens in patients deriving clinical benefit from ICI. Expanded investigations of the evolution of antibody production over the course of ICI therapy, associated with tumor response to treatment and development of IRAEs, are warranted.

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