Consent NA.

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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 IIIc-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 1'000 more proteins and enabled an updated analysis with a deeper level of characterization.

Results 8'548 proteins were quantified across all samples, with 7'416 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 CPED1 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.

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SINGLE CELL PI3K GENE EXPRESSION PATTERNS SUPPORT DUVELISIB (PI3K-DELTA, GAMMA INHIBITOR) TREATMENT OF MELANOMA AND OTHER TUMORS AFTER CHECKPOINT INHIBITOR THERAPY

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Background Duvelisib, an FDA-approved oral phosphoinositide 3-kinase (PI3K)- δ,γ inhibitor, targets tumor cells of B/T cell malignancies, but may modulate non-malignant immune cells in the tumor microenvironment (TME) of many cancers. PI3K- δ and PI3K- γ downmodulate immunosuppressive Tregs and myeloid cells in solid tumors.¹⁻³ We used single-cell RNA analysis of PIK3CD and PIK3CG to explore resistance mechanisms to checkpoint inhibitors (CPI).

Methods Single-cell melanoma (SKCM) RNAseq datasets: GSE120575;⁴ CD45+ cells from 48 CPI responders and non-responder tumors, and GSE115978;⁵ 33 treatment-naïve and CPI-progressing (resistant) tumors. Cancer cells and CD45+ TME subpopulations, specified by gene expression signatures and tSNE plots, had PI3K gene expressions profiled. Differential gene expression (DE) was gated in MAST/Seurat. Fishers test Odds Ratio (OR) was calculated for 'high' expression.

Results PIK3CD expression is higher in SKCM than most cancers (10.8 median RSEM log 2).⁷ By single-cell analysis, PIK3CD (> 0.3 log2 TPM) occurs in 68.2% of cancer cells, with PIK3CB, PIK3CA, and PIK3CG expressed in 32.3%, 12.0%, and 7.2% respectively. PIK3CD-high cancer cells (>4 log2 TPM) have a 711-gene DE gene signature mostly related to immune processes. A higher proportion of cancer cells in CPI resistant tumors express PIK3CD, than untreated tumors (OR 2.02, 95% CI 1.65–2.48, p=3.04 × 10⁻¹²), as do PIK3CD+PIK3CG-expressing cancer cells (OR 2.14, 95% CI 1.47–3.13, p=4.2 × 10⁻⁵). Additionally, in PI3K- δ or PI3K- γ high melanoma cell lines duvelisib inhibited proliferation, p-AKT and c-myc.⁷ PIK3CD and PIK3CG are prominently expressed in many SKCM CD45+ TME cells (84.5% and 31.7% respectively). PIK3CD (>0.3 log2 TPM) occurs in a high fraction of T (85.7%), CD8+ T (86.3%), CD4+ T (86.9%), B (78.5%), macrophages (88%), and NK (85%). PIK3CG is highest in B, dendritic, cycling lymphocytes and plasma cells. Strikingly, a significantly higher proportion of PIK3CD+ cells occur in resistant tumors compared to untreated for all CD45+ cells, (OR 1.64, 95% CI 1.40–1.94, p=4.79 × 10⁻¹⁰), CD8+ T (OR 2.15, 95% CI 1.61–2.86, p=6.5 × 10⁻⁸), and an exhausted C8+ T subpopulation (OR 3.17, 95% CI 1.89–5.37, p=2.95 × 10⁻⁶). PIK3CD +PIK3CG-expressing CD45+ cells are significantly increased in CPI-resistant tumors (OR 1.22, 95% CI 1.07–1.39, p=0.002).

Conclusions These findings support a mechanism where CPI therapies may contribute to modulation of PI3K δ expression in cancer cells and the immune TME. The PI3K- δ,γ inhibitor duvelisib is being investigated in combination with CPI and evaluated in the context of CPI resistance in clinical trials: pembrolizumab (HNSC, NCT04193293), and nivolumab (Richter's Syndrome, NCT03892044).

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A NOVEL DISCOVERY PIPELINE IDENTIFIES MELANOMA-SPECIFIC ANTIBODIES IN PATIENTS RESPONDING TO IMMUNE CHECKPOINT INHIBITORS

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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 with an established immunoprecipitation platform. 35S-methionine-labeled lysates from cultured 624Mel cells were used for immunoprecipitation. 624Mel expresses several shared non-mutated melanoma antigens (e.g., MAGEA3, tyrosinase, MART-1/Melan-A, gp75, and gp100). Antigen identity was determined using on-bead digests followed by mass spectrometry, and was confirmed by immunoprecipitation with in vitro transcription/translation (IVTT) products.

Results Antibodies reactive against 624Mel proteins were detected in 4 of 12 (33%) patients (table 1). Mass spectrometric sequencing performed on proteins captured with sera from 3 of 4 patients identified several putative antigens. Immunoprecipitation with IVTT candidate proteins confirmed antibodies against melanoma-associated and cancer testis antigens NY-ESO-1, SSX2 and MAGEA10. Antibodies were observed in 1

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 231 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.

Subject ID	Sex	Treatment response	Pre-treatment antibody specificities	Post-treatment antibody specificities	IRAEs
1	F	CR	NY-ESO-1	NY-ESO-1, SSX2, MAGEA10	Pancreatitis, dermatitis
2	F	PR	None	Unidentified 80 kd band	Thyroiditis, pneumonitis, enteritis
3	F	PR	None	NY-ESO-1	Hepatitis, thyroiditis, adrenalitis, enteritis, arthritis
4	M	PR	None	None	Arthritis
5	F	PR	None	None	Meningitis, dermatitis, thyroiditis
6	F	SD	NY-ESO-1	NY-ESO-1	None
7	M	PD	None	None	None
8	F	PD	None	None	Dermatitis, colitis
9	F	PD	None	None	Polymyalgia rheumatica
10	F	PD	None	None	Hepatitis
11	F	PD	None	None	Thyroiditis, pancreatitis, vitiligo, arthritis
12	M	PD	None	None	Pancreatitis, diabetes

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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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