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231 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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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

to anti-CTLA4, mesenchymal tumors are highly resistant. Most strikingly, in tumors arising from a mixture of both cell types, a minority population (10%) of mesenchymal cells can cross-protect the vast majority (90%) of their epithelial neighbors from immune attack.[1] However, the mechanisms underlying such cross-protection remain elusive. This is particularly important as most human breast cancers contain minority populations of such mesenchymal cells which can protect the tumor as a whole from immune attack.

Methods Using a combination of transcriptomic and CRISPR/Cas9 approaches, we first identified that mesenchymal carcinoma cells express high levels of CD73, an ecto-enzyme that catalyzes the production of adenosine. Additionally, we used digital spatial profiling to determine whether CD73 expression differs across distinct epithelial and mesenchymal sectors in mixed tumors.

Results Abrogation of CD73 from mesenchymal carcinoma cells prevented the assembly of an immunosuppressive tumor microenvironment and resulted instead in increased numbers of tumor-infiltrating lymphocytes and cross-presenting dendritic cells. Most strikingly, abrogation of CD73 sensitized previously refractory mesenchymal tumors completely to ICB. In the context of mixed tumors comprised of both epithelial and mesenchymal carcinoma cells, gradients in expression of CD73 were observed corresponding to the epithelial or mesenchymal sectors of these mixed tumors. Importantly, mixed tumors in which the minority population of mesenchymal carcinoma cells lacked the expression of CD73, were also sensitized partially to ICB. Thus, these mesenchymal carcinoma cells knocked out for CD73 could no longer protect their epithelial neighbors from immune attack.

Conclusions Taken together, our work suggests that mesenchymal carcinoma cells exert immune-suppressive effects which are also prominent in heterogeneous tumors. Furthermore, targeting the adenosinergic signaling pathway in mesenchymal carcinoma cells can potentiate the efficacy of ICB.

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233 GENE EXPRESSION ANALYSIS OF IMMUNE CHECKPOINT THERAPY IN MOUSE TUMOR MODELS REVEALS SIMILARITIES AND DIFFERENCES IN IMMUNE CELL POPULATIONS AND FUNCTIONAL PROCESSES THAT REFLECT RESPONSE TO TREATMENT

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Background Experimental therapies that target the immune system have expanded greatly in recent years due to the success of immune checkpoint inhibitory antibodies such as ipilimumab and pembrolizumab. Preclinical development of these novel immune-oncology drugs requires the availability of well characterized mouse models to evaluate therapeutic mode of action, efficacy, and safety. Syngeneic mouse tumor models provide robust systems in which to evaluate novel immune-oncology therapies. Efficacy in these models can be measured by tumor volume changes in subcutaneous implants or by impacts on survival for orthotopic implants. Mode of action can be assessed by identifying changes in the tumor microenvironment following dosing. Multiple analytical methods can be used to track changes in immune populations and activation status from flow cytometry to immunohistochemistry to gene expression analysis.

Methods We endeavored to characterize the functional tumor microenvironment changes for two syngeneic models following treatment with anti-PD-1 and anti-CTLA-4 antibodies The syngeneic models used for the study were both colon adenocarcinomas, MC38 and CT26. Mice bearing subcutaneous tumors were dosed intraperitoneally with either vehicle alone, anti-CTLA-4, anti-PD-1, or a combination of the two immune checkpoint inhibitors on days 1, 4, and 8. Tumors were harvested on day 9 and assessed for gene expression by microarray analysis. The gene expression results were evaluated for the relationship between treatment regimen and tumor volume change by expression level association, functional set enrichment analysis, and immune cell population gene set variation analysis.

Results For each of the tumor models, >10,000 genes were found to be significantly differentially expressed. Functional set enrichment analysis showed notable changes in cell cycle and mitotic markers as well as immune response markers in MC38. In contrast, CT26 showed principally changes in immune response markers. Immune cell population set analysis revealed differential impacts on numerous immune cell populations between the models which correlate with therapy induced changes in tumor growth. These include expected changes in CD8+ T-cell populations for both models but also differential changes in other populations including CD56dim NK cells, eosinophils and B cells in MC38 and neutrophils in CT26. We also conducted a genomic analysis by whole exome sequencing. Both tumor models have relatively high tumor mutational burden; CT26 TMB = 377 MB and MC38 TMB = 69/MB. These data show the value of robust bioinformatics analysis of gene expression data sets to provide insights into the mode of action and model responses to investigational immune-oncology drugs.

Conclusions N/A

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235 ANTIGEN PRESENTATION PATHWAYS PRIME MELANOMA PATIENTS FOR MORE DURABLE RESPONSE TO ANTI–PD-1 CHECKPOINT BLOCKADE THERAPY

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Background Favorable outcomes utilizing anti–PD-1 based immune therapies for unresectable melanoma patients are hypothesized to be dependent on antigen processing and presentation mechanisms. The present study utilizes multiomics to examine the contribution of antigen presentation pathways in metastatic melanoma tumors either responsive or resistant to anti-PD-1 therapy.

Methods To unveil the mechanisms that predispose unresectable, stage III/IV melanoma patients to respond to anti–PD-1-based therapies, we conducted expression proteomics as well as employed the mRNA immune oncology panel (HTG Molecular Diagnostics, Inc., Tucson, AZ). Formalin-fixed, paraffin-embedded tissues collected from 27 patients prior to anti-PD-1 therapy (previously consented and enrolled in the