Cohen EE, Varner JA. PI3Kγ is a molecular switch that controls immune suppression. *Nature* 2016; **539**(7629):437–442.
3. De Henau O, Rausch M, Winkler D, Campesato LF, Liu C, Cymerman DH, Budhu S, Ghosh A, Pink M, Tchaicha J, Douglas M, Tibbitts T, Sharma S, Proctor J, Kosmider N, White K, Stern H, Soglia J, Adams J, Palombella VJ, McGovern K, Kutok JL, Wolchok JD, Merghoub T. Overcoming resistance to checkpoint blockade therapy by targeting PI3Kγ in myeloid cells. *Nature* 2016; **539**(7629):443–447.
4. Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, Jenkins RW, Lieb DJ, Chen JH, Frederick DT, Barzily-Rokni M, Freeman SS, Reuben A, Hoover PJ, Villani AC, Ivanova E, Portell A, Lizotte PH, Aref AR, Eliane JP, Hammond MR, Vitzthum H, Blackmon SM, Li B, Gopalakrishnan V, Reddy SM, Cooper ZA, Paweletz CP, Barbie DA, Stemmer-Rachamimov A, Flaherty KT, Wargo JA, Boland GM, Sullivan RJ, Getz G, Hacohen N. Defining T Cell States Associated with Response to Checkpoint Immunotherapy in Melanoma. *Cell* 2018; **175**: 998–1013.
5. Jerby-Arnon L, Shah P, Cuoco MS, Rodman C, Su MJ, Melms JC, Leeson R, Kanodia A, Mei S, Lin JR, Wang S, Rabasha B, Liu D, Zhang G, Margolais C, Ashenberg O, Ott PA, Buchbinder EI, Haq R, Hodi FS, Boland GM, Sullivan RJ, Frederick DT, Miao B, Moll T, Flaherty KT, Herlyn M, Jenkins RW, Thummalapalli R, Kowalczyk MS, Cañadas I, Schilling B, Cartwright ANR, Luoma AM, Malu S2, Hwu P, Bernatchez C, Forget MA, Barbie DA, Shalek AK, Tirosh I, Sorger PK, Wucherpfennig K, Van Allen EM, Schadendorf D, Johnson BE, Rotem A, Rozenblatt-Rosen O, Garraway LA, Yoon CH, Izar B, Regev A. A Cancer Cell Program Promotes T Cell Exclusion and Resistance to Checkpoint Blockade. *Cell* 2018; **175**: 984–997.
6. Firebrowse Gene Expression Viewer http://firebrowse.org/viewGene.html.
7. Coma S, Weaver DT, Pachter JA. [Poster #663] The dual PI3K-δ/PI3K-γ inhibitor duvelisib inhibits signaling and proliferation of solid tumor cells expressing PI3K-δ and/or PI3K-γ. *AACR*. 2020.

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

[1]Daniel Delitto*, [1]Evan Lipson, [1]Laura Cappelli, [2]Klaus Busam, [1]Antony Rosen, [1]Suzanne Topalian, [1]Livia Casciola-Rosen. *[1]Johns Hopkins University, Baltimore, MD, USA; [2]Memorial Sloan Kettering Cancer Center, New York, NY, USA*

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

[1]Anushka Dongre*, [1]Robert Weinberg, [2]Mohammad Rashidian, [1]Elinor Eaton, [1]Ferenc Reinhardt, [1]Prat Thiru, [3]Maria Zagorulya, [1]Sunita Nepal, [1]Tuba Banaz, [4]Anna Martner, [3]Stefani Spranger. *[1]Whitehead Institute for Biomedical Research, Cambridge, MA, USA; [2]Dana Farber Cancer Institute, Boston, MA, USA; [3]Koch Institute/MIT, Cambridge, MA, USA; [4]University of Gotherburg, Gothenburg, Sweden*

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