cell activity. KIT mutation was seen in 10 of 147 patients with high MS4A1/CD20 expression, and 10 of 135 patients with low MS4A1/CD20 expression. Overall survival was 15 months for the patients with KIT mutation and low MS4A1/CD20 expression, and significantly lower when compared with other groups despite low number of patients. (P<0.0001) (figure 1).

Conclusions B cells have significant role in immune response to tumor. Lower expression of MS4A1/CD20 is known to be associated with poor prognosis in melanoma and other solid tumors.[3] We demonstrated that a concurrent KIT mutation in melanoma with lower expression of MS4A1/CD20 contributes to poor prognosis in melanoma. Therefore, this small subset of aggressive tumors may need combination strategies involving targeting driver pathways with a kinase and immune checkpoint inhibitor.

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526 **INHIBIGEN™-SPECIFIC RESPONSES SUPPRESS ANTI-TUMOR IMMUNITY AND PROMOTE TUMOR GROWTH**

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Background Personalized cancer immunotherapies can generate potent antitumor responses yet finding the right targets remains challenging. The ATLAS™ platform employs ex vivo functional screening of tumor mutations using autologous cells to identify patient-specific neoantigens. Stimulatory neoantigens are identified by upregulation of inflammatory cytokine secretion and can be employed in vaccines or cell therapies. Conversely, ATLAS also identifies inhibitory neoantigens (termed Inhibigens) that lead to cytokine downregulation, and in murine models accelerate tumor growth and abrogate the efficacy of otherwise-protective vaccines. Here we further explore Inhibigen mechanism of action in humans and mice including whether checkpoint inhibition (CPI) can ameliorate Inhibigen-accelerated tumor growth.

Methods Human and mouse ATLAS screens were performed as previously described.[1] ATLAS-identified stimulatory or Inhibigen peptide vaccines were evaluated in a therapeutic B16F10 melanoma tumor model ± CPI. Immune responses were measured using ELISPOT, flow cytometry, and immunohistochemistry (IHC).

Results In the GEN-009 personalized neoantigen vaccine trial (NCT03633110), Inhibigens were observed in 92% of patients (N=39). Of total mutations screened, 16% (1.8 - 47.5%) were classified as Inhibigens, which were found more often in the CD4 + (mean 10.3%; 0.5 - 42%) versus CD8 + T cell subset (mean 6.1%; 1.2–23%). No relationship between Inhibigen-specific responses and tumor type or mutational burden were observed. To study the functional effects of Inhibigen vaccination in vivo, a B16F10 mouse

melanoma model was employed. Inclusion of Inhibigens in an otherwise protective vaccine abrogated efficacy and correlated with decreased T cell responses to vaccine antigens as well as a global depression of T cell cytokine secretion. Early experiments suggest that these decreases are not due to MHC competition. In addition, administration of a therapeutic vaccine containing an Inhibigen led to reduced tumor infiltration of CD8 + T cells and myeloid populations. A corresponding increase of classical Tregs in the tumor or periphery was not observed. Surprisingly, preliminary data show combination therapy with anti-CTLA4 partially ameliorated Inhibigen-accelerated tumor growth but anti-PD1 provided no additional benefit.

Conclusions The nearly ubiquitous presence of Inhibigens in human cancer patients and the demonstrated pro-tumor effects in mice suggest that ATLAS-identified Inhibigens must be considered and omitted in the design of cancer immunotherapies. Furthermore, in mice, CPI co-administration has a modest (anti-CTLA4) or no (anti-PD1) effect on Inhibigen-accelerated tumor growth suggesting that Inhibigen profiling could guide CPI selection or predict clinical outcome. These data confirm the benefits of the ATLAS platform for neoantigen and Inhibigen identification.

Ethics Approval All animal studies were undertaken in conformity with the Cambridge, MA City Ordinance 1086 of the city's Municipal Code and in accordance with the policies and protocols approved by Genocea's Institutional Animal Care and Use Committee (IACUC).

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527 **TUMOR ORGANOID AND IMMUNE CELL CO-CULTURE SYSTEM POTENTIATES IMMUNO-ONCOLOGY DRUG DEVELOPMENT**

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Background Patient-derived organoids (PDOs) are derived from adult epithelial stem cell with self-renewal, organisation and differentiation properties, reflecting the original 3D organ-like or tissue-like structure and morphology in vitro. PDOs also faithfully recapitulate the genetic modifications and phenotypical features of original tumors, making them an attractive preclinical models for oncology drug development. However, modeling the tumor microenvironment (TME) in vitro remains a challenge due to the lack of stromal and immune cells. In this study, we reconstituted component of the TME through co-culture of tumor organoids with various immune cells in vitro to assess the immune modulatory and tumor killing effects of immuno-oncology (IO) drug candidates such as therapeutic monoclonal antibodies, bispecific T cell engagers and CAR-T cells.

Methods Using the Hubrecht organoid technology (HUB) protocols we have established a biobank of tumor and normal organoids, which closely resemble the genetic and morphologic features of original organs from multiple different tissue types. This large and diverse biobank of organoids can act as

surrogates for individual patients making them suitable for patient population studies including evaluating the response to IO drug candidates in vitro.

Results We co-cultured organoids expressing tumor associated antigen (TAA) of interest with bispecific T cell engagers and CAR-T cells recognizing the TAAs. Our data demonstrated antigen-specific T cell killing of tumor organoids and tumor antigen reactivity of bispecific antibody activated T cells and CAR-T. We engineered tumor organoids to express CD19 and a luciferase reporter gene and measured luciferase activity to monitor the growth and killing of tumor organoids by CD19 CAR-T cells. The luciferase activity in organoids reflected the killing efficiency in a very sensitive, robust and high through-put manner. Immune checkpoint molecules are differentially expressed on individual tumor organoids and we evaluated the potency of immune check blockade using tumor organoids cocultured with allogenic T cells. Killing of tumor organoids and T cell activation was enhanced by PD-1/PD-L1 blockade. We profiled the expression of immune check point molecules on our banked tumor organoids which will provide a valuable resource to choose tumor models and cancer types for preclinical testing of IO drugs.

Conclusions In conclusion, we demonstrated the feasibility of in vitro patient-derived model system in the field of IO research using tumor organoid co-culture with immune cells, and their application in IO target and drug discovery.

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528 SEXUAL DIMORPHISM IN MYELOID-DERIVED SUPPRESSOR CELLS PROMOTE GBM PROGRESSION IN FEMALES VIA IL-1B

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Background A potently immunosuppressive tumor microenvironment facilitates progression of glioblastoma (GBM). Immunotherapies have had variable success in improving the outcome of GBM patients, suggesting that there is a need to gain insight into the mechanisms of immunosuppression. Our findings indicated that proliferating monocytic MDSCs (mMDSCs) accumulate in tumors of male mice and patients, while female tumor-bearing mice had an increase in circulating granulocytic MDSC (gMDSC) frequency, and a high gMDSC gene signature correlated with worse outcome of female patients.

Methods To investigate the basis and prognostic value of sex differences in MDSC profile, we analyzed the role of sex hormones, determined gene expression signatures of MDSCs and preclinically tested the therapeutic benefit of candidate drugs predicted to be effective against individual MDSC subsets.

Results In line with the differential MDSC accumulation pattern, targeting the systemic gMDSCs with the anti-Ly6G neutralizing antibody extended the lifespan of female mice without affecting males. These differences were not driven by sex steroids, as castration or ovariectomy failed to alter MDSC subset accumulation patterns in GBM-bearing mice. Drug-prediction algorithms using the differential MDSC gene expression profiles predicted IL-1 inhibitors are effective against gMDSCs. Correspondingly, IL-1β was highly expressed in female but not male gMDSCs. Single-cell sequencing revealed that circulating but not tumor-infiltrating gMDSCs were the primary source of IL-1β and that its neutralization provided a female-specific survival advantage by reducing circulating gMDSCs. This was accompanied by declines in tumor infiltration of microglia, microglia activation status and tumor cell proliferation. In vitro, IL-1β inhibition reduced viability and expression of activation markers by primary microglia.

Conclusions These findings highlight a novel peripheral gMDSC-microglia IL-1β mediated communication axis in female GBM and indicate expression differences in MDSC subsets can be leveraged for improved immunotherapy efficacy in a sex-specific, precision medicine strategy.

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529 CANCER CELLS EDUCATE NATURAL KILLER CELLS TO A METASTASIS PROMOTING CELL STATE

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Background Metastatic disease drives breast cancer mortality. We recently discovered that leading cells at the invasive edge of mammary tumor organoids retain a conserved basal epithelial program defined by their expression of keratin-14 (K14), establishing K14 as a good marker of invasive breast cancer cells. K14-positive invasive cells also exhibit characteristics that make them targets of immunosurveillance by natural killer (NK) cells. While NK cells are key immune mediators in the control of metastasis, our understanding of the specific mechanisms behind this regulation and its eventual evasion by metastatic cells remains incomplete.

Methods We have developed a novel preclinical 3D co-culture assay to discover mechanisms behind interactions between K14 + invasive breast cancer cells and NK cells. Combined with in vivo assays of metastasis, we are able to determine how NK cells limit the early stages of metastasis and also how tumor cells can influence key NK cell properties.

Results In ex vivo co-culture assays of NK cells isolated from healthy mouse donors and mammary tumor organoids from MMTV-PyMT and C31T mouse models of breast cancer, we demonstrate that NK cells limit the early stages of metastasis. Antibodies to invasive K14+ cells were able to enhance the ability of NK cells to limit colony formation, suggesting antibody-dependent cell mediated cytotoxicity. Surprisingly, when isolated from tumor bearing mice, NK cells did not limit invasion and instead promoted colony formation. The in vivo adoptive transfer of NK cells from healthy donors prevents the progression of early lung metastatic seeds to macrometastases, while the adoptive transfer of cells isolated from tumor bearing donors promotes macrometastatic development. Transcriptomic analysis of reprogrammed NK cells demonstrate they have similar profiles to resting NK cells. This growth promoting phenotype can be reversed with antibodies targeting inhibitory cell surface receptors or the epigenome.