analysis. The initial analysis focused on CD8 T cells as a primary mediator of antitumor immunity.

Results Matched samples revealed enrichment of effector memory (EM) and central memory (CM) CD8 T cells in tumors compared to PBMCs, as expected. EM cells represented on average 63.36% of the CD8 T cells in tumors vs 30.31% in PBMCs (p=0.0067), and CM cells 12.11% vs 5.58% respectively (p=0.1558). Non-linear dimensionality reduction mapping of these CD8 EM and CM cell subtypes among tumors displayed an activated but potentially dysfunctional phenotype, characterized by substantially higher expression of multiple coinhibitory receptors (PD-1, LAG-3, TIM-3, TIGIT) and activation markers (HLA-DR, ICOS) compared to PBMCs. Among these cells, a PD-1+/LAG-3+ subset, observed in 17/28 TIL samples, expressed TIM-3, TIGIT, HLA-DR, and ICOS at significantly higher levels compared to other PD1/LAG3 expression subsets. Interestingly, CD137 (4-1BB), a marker of potentially tumor-reactive cells, is expressed predominantly in PD-1+ memory CD8 T cells, with the most intense expression levels observed in the PD-1+/LAG-3+ subset.

Conclusions The present results provide insight into the relative (co)expression of potentially targetable immunological pathways, and suggest a biological basis for informing approaches to combination checkpoint inhibition therapy.

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Ethics Approval This study was approved by Bristol Myers Squibb's Global Data Repository (Biological Assessment of Risk (BAR) number LVL_2020_12339). Samples were provided by Discovery Life Sciences (CA), MT Group (CA), Avaden BioSciences (WA), or BioOptions (CA). All patients gave written informed consent at the time of sample collection according to the IRB protocols of each provider.

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497 LONGITUDINAL IMMUNE PROFILING REVEALS UNIQUE MYELOID AND T CELL PHENOTYPES ASSOCIATED WITH SPONTANEOUS IMMUNOEDITING IN A NOVEL PROSTATE TUMOR MODEL

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Background The theory of cancer immunoediting, which describes the dynamic interactions between tumors and host immune cells that shape the character of each compartment, is foundational for understanding cancer immunotherapy. Few models exist that facilitate in-depth study of each of the three canonical phases of immunoediting: elimination, equilibrium, and escape. Here, we perform high dimensional longitudinal immune profiling of NPK-C1, a transplantable prostate tumor model that recapitulates the three phases of immunoediting spontaneously in immunocompetent C57BL/6 animals.

Methods We generated a 28-color immune phenotyping panel to interrogate the NPK-C1 microenvironment using a Cytek Aurora spectral flow cytometer. We analyzed NPK-C1 tumors on days 10, 15, 20 and 24 post-implantation, representing elimination, equilibrium, early escape, and late escape phases, respectively. These data were analyzed by both traditional gating and with an optimized dimensionality reduction and unsupervised clustering workflow. We additionally performed in vivo depletion studies of T cell and granulocyte subsets at early and late time points to determine if these bulk populations are required for immunoediting during elimination and equilibrium/escape.

Results We found that a cluster of activated CD4 effector T cells were enriched early during elimination phase and were overrepresented in NPK-C1 tumors which regress rather than progress to escape. CD4 in vivo depletion studies validated a functional role for CD4 T cells in suppressing NPK-C1 progression at these phases. Additionally, a central memory-like cytotoxic CD8 T cell cluster was enriched in regressing NPK-C1 tumors, and CD8 depletion allowed NPK-C1 progression throughout immunoediting. Regulatory T cells (Tregs) as a whole were counterintuitively enriched in regressing tumors, however high dimensional analysis revealed their significant phenotypic diversity, with a number of Treg subpopulations enriched in progressing tumors. In the myeloid compartment, we found that iNOS+ DC-like cells were enriched in regressing tumors, while CD103+ DCs were counterintuitively associated with late stage tumor progression.

Conclusions These data introduce a new model – NPK-C1 – to study immunoediting and suggest both CD8 and CD4 T cells are required to suppress tumor outgrowth throughout each phase of cancer immunoediting, while myeloid populations exhibit significant phenotypic and functional diversity throughout this process. Further, our identification of unique sub-populations of myeloid and T cells correlating with either regression or progression to escape highlights a role for multi-dimensional flow-based analyses to more deeply understand immune cell dynamics in the tumor microenvironment.

Ethics Approval All experiments and procedures for this study were approved by the Columbia University Medical Center Institutional Animal Care and Use Committee (IACUC)

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498 DOWNREGULATION OF CD5 IN CD8+ T TUMOUR-INFILTRATING LYMPHOCYTES ASSOCIATES WITH INCREASED LEVEL OF ACTIVATION AND EXHAUSTION

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Background CD5, a member of the scavenger receptor cysteine-rich superfamily, is a marker for T cells and a subset of B cells (B1a). CD5 associates with T-cell and B-cell receptors and impair TCR signaling[1 2] and increased CD5 is an indication of B cell activation. Furthermore, CD5 levels on CD8+ T cell splenocytes were significantly increased after TCR/CD3 stimulation using ex vivo treatment with anti-CD3/anti-CD28 MAbs compared to non-stimulated CD8+ T splenocytes.[3] Previous studies have shown a correlation between CD5 and anti-tumour immunity where CD5 knockout mice inoculated with B16F10 melanoma cells had delayed tumour growth compared to wild type mice.[4] In tumour-infiltrating lymphocytes (TILs) isolated from lung cancer patients, CD5 levels were negatively correlated with anti-tumour activity and tumour-mediated activation-induced T cell death,[5] suggesting that CD5 could impair activation of anti-tumour T cells. However, the correlation between CD5 level expression and T cell activation and exhaustion in the tumour microenvironment and in peripheral organs is ill-defined and requires further investigation.

Methods We determined CD5 levels in T cell subsets in different organs in mice bearing syngeneic 4T1 breast tumour