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 immunoeediting, 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 immunoeediting: 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 immunoeediting 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 immunoeediting 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 repressing NPK-C1 tumors, and CD8 deletion allowed NPK-C1 progression throughout immunoeediting. Regulatory T cells (Tregs) as a whole were counterintuitively enriched in repressing 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 repressing tumors, while CD103+ DCs were counterintuitively associated with late stage tumor progression.

Conclusions These data introduce a new model – NPK-C1 – to study immunoeediting and suggest both CD8 and CD4 T cells are required to suppress tumor outgrowth throughout each phase of cancer immunoeediting, 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 multidimensional flow-based analyses to more deeply understand immune cell dynamics in the tumor microenvironment.

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Ethics Approval This study was approved by Bristol Myers Squibb’s Global Data Repository (Biological Assessment of Risk (BAR) number EVL_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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homographs and assessed the relationship between CD5 and increased CD69 and PD-1 (markers of T cell activation and exhaustion) by flow cytometry.

**Results** We report that T cell CD5 levels were higher in CD4 + T cells than in CD8+ T cells in 4T1 tumour-bearing mice, and that high CD5 levels on CD4+ T cells were maintained in peripheral organs (spleen and lymph nodes). However, both CD4+ and CD8+ T cells recruited to tumours had reduced CD5 compared to CD4+ and CD8+ T cells in peripheral organs. In addition, CD5highCD4+ T cells and CD5highCD8+ T cells from peripheral organs exhibited higher levels of activation and associated exhaustion compared to CD5lowCD4+ T cell and CD5lowCD8+ T cell from the same organs. Interestingly, CD8+ T cells among TILs and downregulated CD5 were activated to a higher level, with concomitantly increased exhaustion markers, than CD8+CD5+ TILs.

**Conclusions** Thus, differential CD5 levels among T cells in tumours and lymphoid organs can be associated with different levels of T cell activation and exhaustion, suggesting that CD5 may be a therapeutic target for immunotherapeutic activation in cancer therapy.

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**Ethics Approval** This study was approved by the Animal Use Subcommittee of the University of Western Ontario

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**Background** Colorectal cancer (CRC) associated with Lynch syndrome is characterized by an abundance of infiltrating lymphocytes. To study whether tumor-specific antibodies with therapeutic potential can be isolated from these patients, the B-cell repertoire from a patient with Lynch syndrome who recovered from a stage IV colon carcinoma was screened. Here we describe an antibody, AT1636 that recognizes a previously unidentified O-mannosylated 70kDa variant of E-cadherin. The intercellular interactions by E-cadherin on tumor cells have for long been recognized as protective in cancer metastasis, and deregulation of E-cadherin is a hallmark for epithelial-mesenchymal transition (EMT).

**Methods** The study protocol was approved by the Medical Ethical Committee of the Academic Medical Centre, Amsterdam, The Netherlands (NL42718.018.12). AIMM’s BCL6 and Bcl-XL immortalization method was used to interrogate the human antibody repertoire. From a carrier of a pathogenic gene variant in the MSH6 gene diagnosed with stage IV CRC and liver metastasis that had been treated with avastin, capecitabine and oxaliplatin, peripheral-blood memory B cells were obtained 9 years after last treatment. Antibodies-containing supernatant of cultured B-cells were screened for binding to 3 different CRC cell lines (DLD1, LS174T and COLO205) and absence of binding to fibroblast by flow cytometry. A high-affinity variant of AT1636 (AT1636IYN) was sorted from the original AT1636, AID-expressing B-cell clone.2

**Results** Antibodies that demonstrated differential binding to CRC cells were characterized and targets recognized by such antibodies were identified using immunoprecipitation and mass-spectrometry. One of the antibodies, AT1636, recognized a previously unidentified O-mannosylated 70kDa E-cadherin variant (ECV). Although the 70kDa ECV is found in all full-length E-cadherin expressing cells, tumor-specific binding of AT1636 is dependent on the O-mannosylation pattern in the antibody epitope on ECV. Using shRNA knock-down AT1636 binding was shown to depend on the transmembrane O-mannosyltransferase targeting cadherins 3 (TMTC3). 3 In accordance, coexpression of TMTC3 and E-cadherin in tumor cells is predictive for AT1636 binding. In addition, we observed that (over)expression of ECV results in a strong de-adhesive, EMT-like phenotype. Although AT1636 by itself is not able to induce ADCP, the CD3-bispecific antibody (single-chain UCHT1) AT1636 format specifically killed CRC cell lines.

**Conclusions** The AT1636 antibody retrieved from a patient with Lynch syndrome binds a previously unidentified cancer-specific O-mannosylated 70kDa form of E-cadherin. This variant might play a role in tumor-cell invasion and metastasis. More importantly, we provide a rationale to advance AT1636 based therapeutics for treatment of CRC.

**Ethics Approval** The study protocol was approved by the Medical Ethical Committee of the Academic Medical Centre, Amsterdam, The Netherlands (NL42718.018.12)

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**Background** Extracellular adenosine triphosphate (eATP) is a ‘danger signal’ used to sense cellular damage, and recognized by purinergic receptors in mammals. Among those...