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

Casey Age*, Aleksander Obradovic, Juan Ariaga, Matthew Chaimowitz, Cory Abate-Shen, Andrea Califano, Charles Drake. Columbia University, New York, NY, USA

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 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.

Acknowledgements We thank Paul Fischer for his contributions in acquiring the CyTOF data and performing initial data QC and analysis.

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.

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

Faizah Alotaibi*, Mark Vincent, Weiping Min, James Koropatnick. Western university, London, Canada

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 signaling1-2 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 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.

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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 form 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 oxalplatin, 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 (AT1636YRN) was sorted from the original AT1636, AID-expressing B-cell clone.

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 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). 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 previous 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.

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