homografts 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 method1 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, capeci-tabine 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 (DDL1, LS174T and COLO205) and absence of binding to fibroblast by flow cytometry. A high-affinity variant of AT1636 (AT1636YIN) 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).1 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 ADC, 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 therapies 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)

REFERENCES

receptors, P2RX7 is preferentially expressed in immune cells. Notably, we recently discovered that P2RX7 is crucial for the generation and maintenance of long-lived tissue-resident and circulating memory CD8+ T cells.1 2 CD8+ T cell function is fundamental for tumor control, and therapies to harness protective CD8+ T cells that overcome exhaustion are currently in the limelight of anticancer strategies. Given our previous data, and the fact that eATP is abundantly present inside the melanoma microenvironment, we tested whether (a) P2RX7 is required for activated CD8+ T cells to infiltrate and control melanoma upon adoptive cell therapy, and (b) P2RX7 agonism can boost the anticancer capacity of CD8+ T cells.

Methods (a) We in vitro-activated WT or P2rx7-/- CD8+ T cells (transgenic for the LCMV epitope gp33-P14 or for the ovalbumin SIINFEKL peptide-OTI) with anti-CD3/CD28/IL-2, ± IL-12, for 72h. Cells were adoptively transferred (single transfer of WT or P2rx7-/- cells) into mice with 7 days after subcutaneous transfer of B16 melanoma encoding gp33 or SIINFEKL. We tracked tumor growth until 60 days or at the appropriate endpoint. In some experiments, we sacrificed recipient mice 7 days after adoptive T cell transfer for immune cell phenotyping. Some parameters (cytokine production, mitochondrial respiration via Seahorse) were measured in in vitro-activated cells. (b) WT and P2rx7-/- cells were activated with anti-CD3/anti-CD28/IL-2, ± Bz-ATP, a P2RX7 agonist. Tumor growth was tracked over time until 60 days or at the appropriate endpoint.

Results WT and P2RX7-deficient (P2rx7-/-) CD8+ T cells in the absence of IL-12 do not differ in tumor infiltration and/or control. However, P2rx7-/- CD8+ T cells activated in response to IL-12 tertiary stimulus do not control B16 melanomas as well as their WT counterparts. Phenotypically, IL-12-P2rx7-/- CD8+ T cells do not profoundly differ from IL-12-WT CD8+ T cells, except for diminished mitochondrial respiration levels in vitro, and diminished mitochondrial membrane potential (e.g. mitochondrial health) among tumor-infiltrating cells. Strikingly, Bz-ATP treatment increased the mitochondrial activity of WT CD8+ T cells in vitro and in vivo and led to increased B16 infiltration and control, in a P2RX7-dependent manner.

Conclusions We are currently studying the mechanisms behind the ability of P2RX7 agonists to increase the antitumor function of CD8+ T cells; these are promising results that can lead to a new alternative in immune cell therapies against melanoma.

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