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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**AT1636, A COLON CANCER SURVIVOR-DERIVED ANTIBODY RECOGNIZES A PREVIOUSLY UNIDENTIFIED TRUNCATED, O-MANNOSYLATED 70kDA VARIANT OF E-CADHERIN**

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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, capcitabine 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 different CRC cell lines (DLD1, LS174T and COLO205) and absence of binding to fibroblast by flow cytometry. A high-affinity variant of AT1636 (AT1636YN) 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 (TMT3C). In accordance, coexpression of TMT3C 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.

**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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**P2RX7 AGONIST TREATMENT BOOSTS THE ABILITY OF IL-12-ACTIVATED CD8+ T CELLS TO INFILTRATE AND CONTROL MURINE MELANOMA**

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