

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, CD5^{high}CD4+ T cells and CD5^{high}CD8+ T cells from peripheral organs exhibited higher levels of activation and associated exhaustion compared to CD5^{low}CD4+ T cell and CD5^{low}CD8+ 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.

Acknowledgements The author thanks Rene Figueredo and Ronak Zareardalan for their assistance in animal work

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

REFERENCES

1. Azzam HS, *et al.*, Fine tuning of TCR signaling by CD5. *The Journal of Immunology* 2001; **166**(9): p. 5464–5472.
2. Voisinne GA, Gonzalez de Peredo and Roncagalli R. CD5, an undercover regulator of TCR signaling. *Frontiers in Immunology* 2018; **9**:p. 2900.
3. Alotaibi, F., *et al.*, CD5 blockade enhances ex vivo CD8+ T cell activation and tumour cell cytotoxicity. *European journal of immunology* 2020; **50**(5): p. 695–704.
4. Tabbekh, M., *et al.*, Rescue of tumor-infiltrating lymphocytes from activation-induced cell death enhances the antitumor CTL response in CD5-deficient mice. *The Journal of Immunology*, 2011. **187**(1): p. 102–109.
5. Dorothee, G., *et al.*, In situ sensory adaptation of tumor-infiltrating T lymphocytes to peptide-MHC levels elicits strong antitumor reactivity. *The Journal of Immunology* 2005; **174**(11): p. 6888–6897.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0498>

499

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

¹Tim Beaumont*, ¹Martijn Kedde, ¹Sabrina Merat, ¹Mark Kwakkenbos, ¹Lina Bartels, ¹Dorien van der Berg, ¹Koen Wagner, ¹Arjen Bakker, ¹Kelly Majoor, ¹Martino Bohne, ¹Camille Bru, ¹Veronika Kattler, ¹Yvonne Claassen, ¹Gemma Moiset, ¹Hans Van Eenennaam, ²Victorine Roos, ²Frank Kallenberg, ²Jan Medema, ³Paul Hensbergen, ¹Pauline van Helden, ²Evelien Dekker, ¹Hergen Spits. ¹*AIMM Therapeutics, Amsterdam, Netherlands*; ²*Cancer Centre Amsterdam, Amsterdam, Netherlands*; ³*Leiden University Medical Center, Leiden, Netherlands*

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 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, 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 ADCC, 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)

REFERENCES

1. M.J. Kwakkenbos, *et al.* Generation of stable monoclonal antibody-producing B cell receptor-positive human memory B cells by genetic programming. *Nature Medicine* 2010; **16**:123–128.
2. K. Wagner, *et al.* Bispecific antibody generated with sortase and click chemistry has broad antiinfluenza virus activity. *Proc Natl Acad Sci USA* 2014; **111**: 16820–16825.
3. I.S.B. Larsen, *et al.* Discovery of an O-mannosylation pathway selectively serving cadherins and protocadherins. *Proc Natl Acad Sci USA* 2017; **114**:11163–11168.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0499>

500

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

¹Kelsey Wanhainen, ¹Stephen Jameson, ²Henrique Borges Da Silva*. ¹*University of Minnesota, Minneapolis, MN, USA*; ²*Mayo Clinic Arizona, Scottsdale, AZ, USA*

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

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.

Acknowledgements We would like to thank Jane Ding and Lily Qian for technical assistance, and Kristin Hogquist for scientific input.

Ethics Approval This study was approved by the IACUC board at the University of Minnesota (IACUC number A3456-01)

REFERENCES

- Borges da Silva H, Beura LK, Wang H, Hanse EA, Gore R, Scott MC, Walsh DA, Block KE, Fonseca R, Yan Y, Hippen KL, Blazar BR, Masopust D, Kelekar A, Vulchanova L, Hogquist KA, Jameson SC. The purinergic receptor P2RX7 directs metabolic fitness of long-lived memory CD8⁺ T cells. *Nature*. 2018; **559**(7713):264–268.
- Borges da Silva H, Peng C, Wang H, Wanhainen KM, Ma C, Lopez S, Khoruts A, Zhang N, Jameson SC. Extracellular ATP sensing via P2RX7 promotes CD8⁺ tissue-resident memory T cells by enhancing TGF-β sensitivity. *Immunity* 2020;**53**(1):158–171.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0500>

501

VISTA REGULATES THE DIFFERENTIATION AND SUPPRESSIVE FUNCTION OF MYELOID-DERIVED SUPPRESSOR CELLS

Juan Dong*, Cassandra Gilmore, Hieu Ta, Keman Zhang, Sarah Stone, Li Wang. *Cleveland Clinic Foundation, Lerner Research Institute, Cleveland, OH, USA*

Background V-domain immunoglobulin suppressor of T cell activation (VISTA) is a B7 family inhibitory immune checkpoint protein and is highly expressed on myeloid cells and T cells.¹ VISTA acts as both an inhibitory ligand when expressed on antigen-presenting cells and a receptor when expressed on T cells. Our recent study has shown that VISTA is a myeloid cell-specific immune checkpoint and that blocking VISTA can reprogram suppressive myeloid cells and promote a T cell-stimulatory tumor microenvironment.² In this study, we further demonstrate that VISTA blockade directly alters the differentiation and the suppressive function of myeloid-derived suppressor cells (MDSC).

Methods Flow cytometry was performed to examine VISTA expression on MDSCs in multiple murine tumor models including the B16BL6 melanoma model, MC38 colon cancer model, and the KPC pancreatic cancer models. To examine the role of VISTA in controlling the differentiation and suppressive function of MDSCs, we cultured wild type (WT) and VISTA.KO bone marrow progenitor cells with GM-CSF and IL-6 to induce BM-derived MDSCs.

Results Our preliminary results show that VISTA is highly expressed on M-MDSCs in B16BL6, MC38 and KPC tumors. In BM-derived MDSCs, VISTA deletion significantly altered the signaling pathways and the differentiation of MDSCs. Multiple inflammatory signaling pathways were downregulated in VISTA KO MDSCs, resulting in decreased production of cytokines such as IL1 and chemokines such as CCL2/4/9, as well as significantly impaired their ability to suppress the activation of CD8⁺ T cells. The loss of suppressive function in VISTA KO MDSCs is correlated with significantly reduced expression of iNOS. To validate the results from BM-MDSCs, we sorted CD11b+CD11c-Ly6C+Ly6G- M-MDSCs and CD11b+CD11c-Ly6G+ G-MDSCs from B16BL6 tumor tissues and tested the ability of a VISTA-blocking mAb to reverse the suppressive effects of tumor-derived MDSCs. Our results show that blocking VISTA impaired the suppressive function of tumor-derived M-MDSC but not G-MDSCs.

Conclusions Taken together, these results demonstrate a crucial role of VISTA in regulating the differentiation and function of MDSCs, and that blocking VISTA abolishes MDSC-mediated T cell suppression, thereby boosting.

Ethics Approval All in vivo studies were reviewed and approved by Institutional Animal Care and Use Committee (Approval number 2019-2142).

REFERENCES

- Xu W, Hire T, Malarkannan, S. *et al.* The structure, expression, and multifaceted role of immune-checkpoint protein VISTA as a critical regulator of anti-tumor immunity, autoimmunity, and inflammation. *Cell Mol Immunol* 2018;**15**:438–446.
- Xu W, Dong J, Zheng Y, *et al.* Immune-checkpoint protein VISTA regulates antitumor immunity by controlling myeloid cell-mediated inflammation and immunosuppression. *Cancer Immunol Res* 2019;**7**:1497–510.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0501>