

- combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* 2017;**377**(14):1345-1356.
3. Motzer RJ, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, Plimack ER, Barthélémy P, Porta C, George S, Powles T, Donskov F, Neiman V, Kollmannsberger CK, Salman P, Gurney H, Hawkins R, Ravaud A, Grimm MO, Bracarda S, Barrios CH, Tomita Y, Castellano D, Rini BI, Chen AC, Mekan S, McHenry MB, Wind-Rotolo M, Doan J, Sharma P, Hammers HJ, Escudier B; CheckMate 214 Investigators. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. *N Engl J Med* 2018 Apr 5;**378**(14):1277-1290.
 4. Teng MW, Ngiow SF, von Scheidt B, McLaughlin N, Sparwasser T, Smyth MJ. Conditional regulatory T-cell depletion releases adaptive immunity preventing carcinogenesis and suppressing established tumor growth [published correction appears in *Cancer Res*. 2010; **70**(23):10014]. *Cancer Res* 2010;**70**(20):7800-7809.
 5. Simpson TR, Li F, Montalvo-Ortiz W, Sepulveda MA, Bergerhoff K, Arce F, Roddie C, Henry JY, Yagita H, Wolchok JD, Peggs KS, Ravetch JV, Allison JP, Quezada SA. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J Exp Med* 2013;**210**(9):1695-710.
 6. Plitas G, Konopacki C, Wu K, Bos PD, Morrow M, Putintseva EV, Chudakov DM, Rudensky AY. Regulatory T cells exhibit distinct features in human breast cancer. *Immunity* 2016;**45**(5):1122-1134.
 7. De Simone M, Arrighi A, Rossetti G, Gruarin P, Ranzani V, Politano C, Bonnal RJP, Provasi E, Sarnicola ML, Panzeri I, Moro M, Crosti M, Mazzara S, Vaira V, Bosari S, Pallechi A, Santambrogio L, Bovo G, Zucchini N, Totis M, Gianotti L, Cesana G, Perego RA, Maroni N, Pisani Ceretti A, Opocher E, De Francesco R, Geginat J, Stunnenberg HG, Abrignani S, Pagani M. Transcriptional landscape of human tissue lymphocytes unveils uniqueness of tumor-infiltrating T regulatory cells. *Immunity* 2016;**45**(5):1135-1147.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0861>

862

TARGETING PSGL-1, A NOVEL MACROPHAGE CHECKPOINT, REPOLARIZES SUPPRESSIVE MACROPHAGES, INDUCES AN INFLAMMATORY TUMOR MICROENVIRONMENT, AND SUPPRESSES TUMOR GROWTH

¹Phuong Nguyen*, ¹Ryan Phenicie, ¹Kevin Kauffman, ¹Dominika Nowakowska, ¹Mohammad Zafari, ¹Veronica Komoroski, ¹Steve Sazinsky, ¹Joe Wahle, ¹Denise Manfra, ²Shantashri Vaidya, ²Michael Brehm, ¹Igor Feldman, ¹Tatiana Novobrantseva. ¹Verseau Therapeutics, Bedford, MA, USA; ²UMass Medical School, Worcester, MA, USA

Background Macrophages play an important role in cancer by modulating both the innate and adaptive parts of the immune system. In non-pathological conditions, multiple subsets of macrophages balance the immune response. In cancer, M2-like immune-suppressive tumor-associated macrophages (TAMs) dominate the tumor microenvironment (TME). TAMs promote tumor growth, support neo-angiogenesis and enable metastasis formation. Macrophage modulators driving macrophage repolarization from the M2-like to a pro-inflammatory M1-like phenotype are an attractive novel class of cancer immunotherapy. Here we present identification, validation, and pre-clinical data of a novel macrophage checkpoint, PSGL-1, which supports targeting this molecule for immune-oncology.

Methods To assess the therapeutic potential of using anti-PSGL-1 antibodies to convert macrophage phenotype and the tumor microenvironment toward a more inflammatory state, we employed in vitro primary macrophage and multi-cellular assays, ex vivo patient-derived tumor cultures, and a humanized mouse PDX model.

Results Within the multiple subsets of macrophages, PSGL-1 is expressed at high levels on immune-suppressive TAMs and in vitro differentiated M2 macrophages. We show that targeting PSGL-1 via an antagonistic antibody repolarized M2 macrophages to a more M1-like state, both phenotypically and functionally as assessed in primary in vitro macrophage assays. Further, these repolarized M1-like macrophages enhanced the

inflammatory response in complex multi-cellular assays, including SEB stimulated PBMC assays and mixed-lymphocyte reactions (MLRs).

To establish a pre-clinical proof-of-concept for targeting PSGL-1, we turned to ex vivo cultures of fresh patient-derived primary tumors, where the complexity of the TME can be most preserved. RNA-seq data show that ex vivo cultures treated with anti-PD-1 antibody recapitulate TME changes in anti-PD-1 treated patients, including a strong T-cell IFN-gamma signature and a reduction in oncogenic pathway activation. Blocking PSGL-1 resulted in a robust pro-inflammatory signature driven by TNF-alpha/NF-kappa-B and chemokine-mediated signaling. The increase in TNF-alpha signaling was accompanied by reduction in oxidative phosphorylation and fatty acid metabolism. The increase in pro-inflammatory cytokine and chemokine production was confirmed by measuring secreted protein levels, further confirming the re-polarization of macrophages within a tumor setting.

Lastly, we employed a humanized mouse PDX model of melanoma and show that anti-PSGL-1 treatment resulted in suppression of tumor growth favorably compared to anti-PD-1. At the cellular and molecular levels, anti-PSGL-1 treatment lead to a more enhanced inflammatory microenvironment, including a reduced M2:M1 macrophage ratio, increased antigen presentation, pro-inflammatory mediators, and effector T cell infiltration and activation.

Conclusions Our data support anti-PSGL-1 as a macrophage repolarizing agent and an effective macrophage-targeted therapy for Immuno-Oncology.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0862>

Other

863

IDENTIFICATION AND CHARACTERIZATION OF AN IMMUNODOMINANT SARS-COV-2-SPECIFIC CD8 T CELL RESPONSE

Anastasia Gangaev*, Pia Kvistborg. *Netherlands Cancer Institute, Amsterdam, Netherlands*

Background Global efforts are ongoing to develop vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease (COVID-19). While there is accumulating information on antibody responses against SARS-CoV-2, less is known about CD8 T-cell recognized SARS-CoV-2 epitopes and the functional state of SARS-CoV-2-specific CD8 T cells.

Methods We analysed samples from 18 patients with ongoing severe and critical COVID-19 disease for CD8 T-cell recognition of 500 peptide human leukocyte antigen (HLA) class I complexes, restricted by 10 common HLA alleles. In addition we carried out an in-depth characterisation of the functional state of identified SARS-CoV-2-specific CD8 T cell responses based on peptide stimulation assays, ex vivo flow cytometry and transcriptome analysis.

Results Several epitopes derived from the open reading frame 1ab polyprotein (ORF1ab) were identified, including an immunodominant epitope restricted by HLA-A*01:01. The immunodominance was further supported by high T cell receptor (TCR) diversity within the CD8 T cells specific for this epitope. In-depth characterisation the

immundominant SARS-CoV-2-specific CD8 T cell response revealed a regulated activation program that maintains CD8 T cell survival while halting their effector function and migratory capacity.

Conclusions The ORF1ab, that was found to be the source of an immunodominant SARS-Cov-2-specific CD8 T cell epitope, is not included in the majority of vaccine candidates in development, which may influence their clinical activity. Furthermore, these data may be a cautious indication that SARS-CoV-2 specific CD8 T cells – unlike CD4 T cells – are less likely to contribute to the immunopathology observed in severely and critically ill COVID-19 patients.

Ethics Approval The samples from both COVID-19 patients were collected in accordance with the Declaration of Helsinki after approval by the institutional review boards.

Consent Each participant signed informed consent.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0863>

Tumor and stromal cell biology

864

THE MESENCHYMAL STROMAL COMPARTMENT IN COLORECTAL CANCER GREATLY ALTERS THE INNATE TUMOUR IMMUNE MICROENVIRONMENT BOTH IN 2D AND 3D CULTURE SYSTEMS

¹Niamh Leonard*, ¹Oliver Treacy, ¹Hannah Egan, ¹Grace O'Malley, ²Elena Tomas Bort, ²Eleonora Peerani, ¹Thomas Ritter, ³Daniela Loessner, ¹Laurence Egan, ¹Aideen Ryan. ¹National University of Ireland Galway, Galway, Ireland; ²Queen Mary University London, London, UK; ³Monash University, Melbourne, Australia

Background Colorectal cancer is the fourth most common occurring cancer and despite new treatment options it remains the third leading cause of cancer related deaths worldwide.¹ Of the four Consensus Molecular Subtypes (CMS), the mesenchymal stromal rich CMS4 tumours are shown to have the worst disease free progression survival. However the role mesenchymal stromal cells (MSC) play in the tumour immune microenvironment has yet to be fully elucidated. Understanding the complex communication in this stromal cell rich multicellular environment is challenging but may reveal novel targets for the treatment of colorectal cancer patients.

Methods Tumour cell secretome (TCS) was generated from colon cancer cells with/without the addition of TNF- α , an inflammatory stimulus using both human and mouse cell lines. MSCs were then conditioned with the TCS and inflammatory TCS and changes in surface and secreted immunomodulatory molecules were assessed using RNA-seq, flow cytometry and ELISA analysis. Macrophage antigen processing and migration following co-culture with the TCS conditioned MSCs was observed using DQ-ova and transwell experiments. A Gelatin Methacryloyl hydrogel, 3D triple culture systems was established to study the role of MSC in the colon tumour immune microenvironment. HCT116 colon cancer cell line with THP1 monocytic cell line and primary bone marrow derived MSCs were embedded in the hydrogel and incubated for 10 days, changing the media on Day 8 with/without the addition of TNF- α . Cell proliferation viability and protein secretion were assessed from the 3D CRC system.

Results Bioplex analysis revealed secretion of potent chemokines and cytokines from the cancer cells. This inflammatory TSC resulted in increased expression of cell surface MSC immunomodulatory markers PD-L1 and CD47 and a variety of secreted molecules. These conditioned MSCs reduced macrophage-mediated antigen processing and increase monocyte migration. A triple culture 3D model of CRC was successfully developed, and while the addition of MSC to the system did not alter spheroid size they increased the release of potent chemokines (CCL2, CXCL12), cytokines (IL-6, IL8) and growth factors (GM-CSF) from the culture system.

Conclusions The inflammatory tumour cell secretome can alter MSC surface expression and secretion of a variety of immunomodulatory makers. These tumour conditioned MSCs can alter innate immune cell antigen processing and migration. When MSCs are combined in 3D with monocytes and colon cancer cells the MSC significantly alter the secretion of immune modulating and tumour promoting factors from the culture system. Targeting MSC immune suppression in the colon tumour microenvironment could be a novel therapeutic target.

Ethics Approval Human MSC (hMSC) were isolated from the bone marrow of three healthy volunteers at Galway University Hospital under an ethically approved protocol (NUIG Research Ethics Committee, Ref: 08/May/14) according to a standardized procedure.

REFERENCE

1. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol* 2019;**14**(2):89-103.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0864>

865

SUGAR HIGH: DOES THE SIALIC ACID PROFILE OF CANCER-ASSOCIATED FIBROBLASTS INDUCE A MORE TUMOUR-PERMISSIVE MICROENVIRONMENT?

Hannah Egan*, Oliver Treacy, Kevin Lynch, Niamh Leonard, Amir Nader, Margaret Sheehan, Sean Hynes, Laurence Egan, Thomas Ritter, John Daly, Aisling Hogan, O Michael, Aideen Ryan. *National University of Ireland, Galway, Galway, Ireland*

Background Immunosuppressive tumour microenvironments (TME) inhibit the effectiveness of cancer immunotherapies. Sialic acids, which exist as terminal sugars of glyco-conjugates, are highly expressed on cancer cells and are involved in various pathological processes including increased immune evasion, tumour invasiveness and tumour cell metastasis.¹ Siglecs (Sialic acid-binding immunoglobulin-type lectins) are expressed on immune cell surfaces and bind sialic acid. Siglec binding to hypersialylated tumour glycans blocks immune cell activation to promote immunosuppression.¹ ²Intestinal stromal cells (iSCs), precursors to cancer-associated fibroblasts (CAFs), are a key component of the TME and play a vital role in tumour progression by enhancing a tumour-promoting microenvironment. The aim of this study was therefore to investigate if iSC/CAF sialylation contributes to enhanced immunosuppression in the TME.

Methods iSCs were isolated from colorectal cancer patient biopsies and cultured ex vivo. Informed consent was obtained from all patients prior to sampling. Tumour-derived iSCs were termed CAFs while control iSCs, isolated from tumour-adjacent non-cancerous tissue, were termed normal-associated