CRISPR library screen for key modulators of T cell-induced cytotoxicity against cancer cells in vitro. This customized library contains sgRNAs targeting nearly all membrane proteins expressed in both murine and human T cells. For our in vitro screen, mouse colorectal cancer cell line MC38 expressing chicken ovalbumin (Ova) were co-cultured with Ova-specific CD8+ T cells isolated from OT-I transgenic mice. The proliferation and function of CD8+ T cell were dampened by tumor cells in an antigen-dependent way. On the other hand, we successfully developed a genome-scale CRISPR screen platform on the difficult-to-transduce DLBCL cells. The platform is currently deployed to validate modulators involved in bispecific antibody-mediated tumor cell killing by T cells.

Conclusions We have established CRISPR Cas9 pooled screen platforms for identification of modulators of tumor-immune interaction by either target primary T cells or difficult-to-transduce DLBCL cells.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0841

### 842 A NOVEL AGONISTIC ANTI-CD40 TARGETING STRATEGY WITH AN AFFINITY PEPTIDE BINDING FEATURE FOR ANTIGEN CARGO FUNCTIONALITY: IMPROVING Peptide STABILITY AND T CELL PROLIFERATION

Ida Olsson*, Mohamed Ethisir, Sara Mangsbo. Uppsala University, Uppsala, Sweden

Background To induce a prominent anti-tumor T-cell response, a viral or tumor derived antigen epitope imbedded in a longer synthetic peptide (SLP) can be used, which also requires internalization and processing by antigen presenting cells (APCs) to enable T cell priming. Herein we present the design and evaluation of a CD40 targeting tetravalent bispecific antibody, binding peptides through affinity as an antibody-drug conjugate. APC activation as well as in vitro and in vivo T-cell proliferation studies demonstrate retained agonistic activity as well as improved T cell proliferation/expansion in vitro and in vivo, compared to non-linked peptide/antibody mixes.

Methods T-cell priming was evaluated with B3Z assay or a cytomegalovirus (CMV) model and displayed superior uptake to non-bound peptide in the co-stimulatory independent B3Z assay. In addition, intracellular peptide release in APCs was analysed using a unique quenching strategy displaying peptide release after around 4-6 hour post antigen.

Results Peptide stability in vitro, when bound to the antibody, was analysed by mass spectrometry and displayed prolonged peptide stability in serum, increasing the peptide half-life by 15 times in vitro (http://dx.doi.org/10.1136/jitc-2020-SITC2020.0842

### 843 REPRODUCIBLE, MOA-REFLECTING REPORTER-BASED BIOASSAYS TO ENABLE DRUG DEVELOPMENT OF BIOSIMILARS AND BIOBETTERS

Jeffrey Nelson*, Richard Moravec, Dun Li, Jennifer Wilkinson, Frank Fan, Mei Cong. Promega, Madison, USA

Background Cytokines and growth factors are small immunomodulatory proteins secreted by a wide variety of cells (e.g. lymphocytes in cancer cell areas and in surrounding stroma). P3028 was graded (semi-quantitatively) as high or low. Quantitative flow cytometry was performed focusing on CD8+, CD3+, and CD4+ T-cells, macrophage and dendritic cells.

The study was approved by the Swedish Ethical Review Authority (no. 2017/380). Written informed consent was obtained from the patients. A copy of the written consent is available for review by the Editor of this journal.

Results Based on immunohistochemistry focusing on presence and distribution of CD8+ T-cells, most TC lesions were found to be of an ‘inflamed’ immune phenotype. This particular phenotype also featured low expression of immunosuppressive peptides P3028 (cf. other immune phenotypes). Flow cytometry verified that ‘immune excluded’ and ‘inflamed’ cancer lesions were associated with high levels of CD8+ T-cells (cf. desert lesions). The presence of CD3+ and CD4+ T-cells as well as macrophages and dendritic cells in relation to immune phenotypes were indicated.

Conclusions TC lesions may be classified into ‘inflamed’, ‘immune excluded’, and ‘desert’ phenotypes based on presence and distribution of CD8+ T-cells. Other immune cells may be associated with these immune phenotypes, including CD3+ and CD4+ T-cells, macrophages, and dendritic cells. P3028 is present in TC lesions: low levels of this immunosuppressive peptide are observed in the ‘inflamed’ phenotype. Arguably, P3028 prevents successful recruitment of immune cells in TC. Inferentially, presence and distribution of P3028 may be considered as a prognostic marker as well as a treatment target of this condition.

Ethics Approval The study was approved by the Swedish Ethical Review Authority (no. 2017/380).

Consent Written informed consent was obtained from the patients. A copy of the written consent is available for review by the Editor of this journal.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0840

### Immune-stimulants and immune modulators

**841 CRISPR CAS9 LIBRARY SCREEN IN PRIMARY T CELLS AND DIFFUSE LARGE B CELL LYMPHOMA CELLS TO IDENTIFY MODULATORS IN TUMOR-IMMUNE INTERACTION**

Qian Li, Zhengang Peng*. WXi ApTec (Shanghai) Co., Ltd., Shanghai, China

**Background** Immunotherapy, especially checkpoint blockers targeting programmed cell death protein 1 (PD-1) pathways, has transformed cancer treatment. Current checkpoint blockers are limited by low response rate, side effect and treatment relapse. The emergence of CRISPR Cas9-based screen provides a superior and powerful tool in gene function profiling. The application of CRISPR Cas9 screen in primary immune cells and tumor cells such as diffuse large B cell lymphoma (DLBCL) cells will accelerate the identification of key regulators in tumor-immune interaction.

**Methods** CRISPR screen using membrane protein-focused sgRNA library and genome-scale sgRNA library; primary T cell and tumor cell co-culture.

**Results** First of all, we developed a CRISPR-Cas9 gene targeting method that can achieve efficient gene disruption in primary CD8+ T cells isolated from mouse (~60% efficiency) or human (~70% efficiency). We have applied this method to a pooled
IMMUNOMODULATORY ACTIVITY OF EPIGENETIC DRUGS COMBINATIONS IN MESOTHELIOMA: LAYING THE GROUND FOR NEW IMMUNOTHERAPEUTIC STRATEGIES

Sara Carnito, Health Biology*, Ornella Cutaia, Carolina Fazio, Maria Fortunata Logi, Francesca Piazzini, Laura Salmonese, Luana Calabrò, Michele Maio, Alessia Covre. Center for Immuno-Oncology, Siena, Italy

Background Growing evidence are demonstrating the therapeutic efficacy of immune checkpoint inhibitors (ICI) in mesothelioma; however, a limited percentage of patients benefits from this therapeutic approach. Epigenetic modifications play a relevant role in negatively regulating the cross-talk between neo-plastic and immune cells, and in contributing to the highly immunosuppressive mesothelioma microenvironment. A better understanding of mesothelioma epigenetic landscape could open the path to novel and potentially more effective approaches combining ICI and epigenetic drugs. We investigated the immunomodulatory potential of epigenetic agents by comparing the activity of DNA hypomethylating agents (DHA) with histone deacetylases inhibitors (HDACi) and EZH2 inhibitors (EZH2i), alone or combined with DHA, in mesothelioma cells.

Methods Four mesothelioma cell lines were treated with the DHA guadecitabine 1μM, or with the HDACi, Valproic Acid (VPA) 1mM, or the EZH2i, EPZ-6438 1μM, alone or combined with guadecitabine. We investigated the expression of HLA class I molecules by flow-cytometry and of PD-L1, cancer testis antigens (CTA: NY-ESO, MAGE-A1), Natural Killer Group 2 member D (NKG2DLs: MIC-A, MIC-B, ULBP2) and EMT-regulating cadherins (CDH1, CDH2) by quantitative Real-Time PCR. Fold change (FC) expression for each treatment vs untreated cells was reported as mean values (FCm) among investigated cell lines. A positive modulation of the expression was considered if FCm>1.5.

Results Guadecitabine upregulated the expression of HLA class I antigens (FCm=1.73), PD-L1 (FCm=2.38), NKG2DLS (MIC-A FCm=1.96, MIC-B FCm=2.57, and ULBP2 FCm=3.56), and upregulated/induced CTA expression. Similarly, VPA upregulated HLA class I antigens (FCm=1.67), PD-L1 (FCm=3.17), NKG2DLS (MIC-A FCm=1.78, MIC-B FCm=3.04, and ULBP2 FCm=3.75) expression; however, CTA expression was modulated only in 1 mesothelioma cell line. Conversely, EPZ-6438 up-regulated only NY-ESO-1 and MIC-B expression in 1 mesothelioma cell line.

The addition of both VPA and EPZ-6438 to guadecitabine strengthened its immunomodulatory activity. Specifically, guadecitabine plus VPA or EPZ-6438 upregulated the expression of HLA class I antigens FCm=2.53 or 2.69, PD-L1 FCm=8.04 or 2.65, MIC-A FCm=3.81 or 2.26, MIC-B FCm=8.00 or 3.03, ULBP2 FCm=6.24 or 4.53, respectively. Higher levels of CTA upregulation/induction were observed with combination treatments vs guadecitabine alone.

Conclusions Combination of DHA-based immunotherapies with other classes of epigenetic drugs could be an effective strategy to be pursued in the mesothelioma clinic.

DEVELOPING MORE POTENT INHIBITORS OF VASOACTIVE INTESTINAL PEPTIDE SIGNALING WITH ENHANCED EFFICACY IN MOUSE MODELS OF LEUKEMIA

Shriha Ravindranathan*, Jin-ming Li, Yiwen Li, Passang Tenzin, Anish Majumdar, Edmund Waller. Emory University, Tucker, GA, USA; Cambium Oncology, Menlo Park, CA, USA

Background Vasoactive intestinal peptide (VIP) is an immunosuppressive neuropeptide that significantly affect proliferation and anti-tumor properties of T cells.1,2 VIP overexpression is a potential mechanism of immune escape in solid tumors with paracrine VIP production. Our published work shows that inhibiting VIP receptor (VIP-R) signaling via VIPyhb, an antagonist fusion peptide between neurotensin and VIP, improves T cell dependent anti-tumor response in mouse models of acute myeloid leukemia (AML) and T lymphoblastic leukemia

Abstracts