

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, macrophages and dendritic cells.

The study was approved by the Swedish Ethical Review Authority (no. 2017/580). 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 peptide 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. 2017/580).

**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.

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## 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

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**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

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.

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### 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

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**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 (

**Conclusions** Data support that the novel delivery system can improve antigen targeting to dendritic cells, but can also provide a prolonged peptide half-life as well as a peptide delivery to APCs. Combined this improves the efficiency of both antigen delivery and CD40 agonistic functionality.

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

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**Background** Cytokines and growth factors are small immunomodulatory proteins secreted by a wide variety of cells (e.g.