independent manner. We previously studied $\gamma\delta$ T cells, that also express checkpoint molecules, in patients in the pre-checkpoint blockade era and thereafter, and identified correlations between subset frequencies of these unconventional T cells and patients' overall survival (OS). Here, we present a detailed phenotyping and functional investigation of tumor-resident as well as peripheral $\gamma\delta$ T cells.

Methods Phenotyping was performed in stage IV melanoma patients before and under PD-1+/-CTLA-4 blockade using as basis our published OMIP-20 protocol.¹ Cytokine expression patterns and proliferative capacities were determined as described according to our established protocols.² Primary flow cytometry data analysis was performed using FlowJo (BD) and correlations with clinical meta data were determined using Prism (GraphPad) and SPSS (IBM).

Results We found previously that low frequencies of peripheral V δ 1 $\gamma\delta$ T cells were associated with prolonged OS. Here, we investigated functional aspects and abundance of $\gamma\delta$ T cells within the tumor as well as in the blood. The peripheral V δ 1 but not the V δ 2 differentiation signature revealed significantly lower proportions of naive and effector cells as well as an accumulation of late differentiated cells in patients with high V δ 1 frequencies. The cytokine expression pattern (IFN γ , TNF and IL-17) and the degranulation marker CD107a were different in patients with high versus low peripheral V δ 1 frequencies. The proliferative capabilities of V δ 1 cells in melanoma were limited in comparison to healthy subjects. Both V δ 1 and V δ 2 cells were found in tumor tissues, and these analyses are ongoing, including analyses of replicative senescence through CD57 expression.

Conclusions Our data provide novel insights into the role of $\gamma\delta$ T cells in cancer rejection. The previously found negative correlation of V δ 1 T cells with OS is likely due to an accumulation of mal-functioning, probably exhausted V δ 1 T cells in patients with poor outcome of ICB. Thus, we suggest that V δ 1 T cells are promising candidates for future exploitation in novel ICB-approaches.

Ethics Approval This study was approved by K. Wistuba-Hamprecht's Ethics Committee (approval nos. 490/2014BO1 and 792/2016BO2).

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TRANSCRIPTOMIC PROFILING OF T-CELL POPULATIONS IN NON-MUSCLE INVASIVE AND MUSCLE INVASIVE BLADDER CANCER

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Background Bladder cancer is categorized as non-muscle invasive (NMIBC) or muscle invasive (MIBC). NMIBC makes up around 70% of the cases and although it is less aggressive, the recurrence rate is 50-70%, thus requiring extensive monitoring. Additionally, there is a risk of progression into MIBC

with a 5-year survival of only 50% even when treated with radical cystectomy. Immune checkpoint inhibitors have shown promising results for treatment of bladder cancer; however, only around 30% of patients have a therapeutic effect and novel therapies are thus required. With the aim of pinpointing novel targets for T-cell based therapy, we have performed transcriptomic profiling of specific T cell populations in MIBC and NMIBC, as well as in control bladder tissue.

Methods Muscle-invasive (n=7) as well as non-muscle invasive (n=13) bladder tumor biopsies were obtained from untreated patients and control bladder tissue (n=7). Upon digestion, cells were stained with an antibody panel to enable sorting of CD8+ cytotoxic T-cells (CD8T), CD4+ T-helper cells (Th) and regulatory T-cells (Treg) using fluorescence activated cell sorting. RNA was extracted and subject to sequencing. Differential gene expression analysis was performed, using DESeq2 (genes with padj)

Results Principal component analysis demonstrated that CD8T, unlike Th and Tregs, cluster according to the invasiveness of the disease. Accordingly, many genes were significantly differentially expressed between CD8T in MIBC and NMIBC compared to control, and also between CD8T in MIBC compared to NMIBC. Several genes associated with CD8 T-cell exhaustion were significantly upregulated in MIBC compared to both NMIBC and control. Further, GSEA results indicated biological differences of the CD8T compartment between different tumor stages.

Conclusions The gene expression profiles of CD8 T-cells were significantly different in NMIBC, MIBC and control. The transcriptional profiles give clues on biological differences and disease progression and can be relevant for development of novel treatment strategies.

Ethics Approval The study was approved by the Regional Ethics Committee (EPN - Regionala Etikprövningsnämnden i Lund), approval number 2017/34.

Consent Written informed consent was obtained from all patients included in the study.

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PRESENCE AND DISTRIBUTION OF IMMUNOSUPPRESSIVE PEPTIDE P3028 IN RELATION TO IMMUNE PHENOTYPE OF TONSILLAR CANCER

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Background A cancer lesion may avoid detection by the immune system through a variety of immunosuppressive mechanisms involving myeloid-derived suppressor cells and regulatory T-cells. One such mechanism recently discovered is the immuno-suppressive effect of a specific peptide, i.e., P3028, produced through degradation of albumin. In this study, involving biopsies obtained from patients with tonsillar cancer (TC), P3028 is assessed in relation to overall immune phenotype, as indicated by presence and distribution of CD8+ T-cells as well as to other specific immune cells.

Methods Immunohistochemistry was performed on fresh frozen biopsies. CD8+ T-cells were used to classify the cancer lesions into immune phenotypes: 'inflamed' (lymphocytes infiltrating cancer cell areas), 'immune excluded' (lymphocytes in surrounding stroma, but few within cancer cell areas) and 'desert' (few

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.