cell induction was positively correlated with ΔASA values, while cross-reactivity of induced T cells was inversely correlated.

**Conclusions** Our results indicate that dissimilarity is key for both T-cell induction and discrimination from self. ΔASA may help predict immunogenic non-anchor type neoantigens inducing specific T-cell response from a variety of cancer mutation pools.

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**837**  
**RELEASING THE RESTRAINTS OF VγVδ2 T-CELLS IN CANCER IMMUNOTHERAPY**

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**Background** VγVδ2 T-cells are a subset of cells with a crucial role in immunosurveillance which can be activated and expanded by multiple means to stimulate effector responses, often exploited in cancer immunotherapy. Little is known about the expression of checkpoint molecules on this cell population and whether the ligation of these molecules can regulate their activity. The aim of this study was to assess the expression of activatory and inhibitory markers on VγVδ2 T-cells to assess potential avenues of regulation to target with immunotherapy.

**Methods** PBMCs were isolated from healthy donors and the expression of activatory and inhibitory receptors was assessed on VγVδ2 T-cells by flow cytometry at baseline, following 24 hours activation and 14 days expansion using zoledronic acid (ZA) and Bacillus Calmette-Guerin (BCG), both with IL-2. Activation and expansion of Vδ2 cells was assessed by expression of CD69 and by frequency of Vδ2 cells, respectively. Production of effector molecules was also assessed following coculture with various tumour cell targets. The effect of immune checkpoint blockade on VγVδ2 T-cells was also assessed.

**Results** VγVδ2 T-cells constitutively expressed high levels of NK-associated activatory markers NKG2D and DNAM1 which remained high following stimulation with ZA and BCG. VγVδ2 T-cells expressed variable levels of checkpoint inhibitor molecules at baseline with high levels of BTLA, KLRL1 and NKG2A and intermediate levels of PD1, TIGIT and VISTA. Expression of checkpoint receptor were modulated following activation and expansion with ZA and BCG with decreased expression of BTLA and upregulation of numerous markers including PD1, TIGIT, TIM3, LAG3 and VISTA. Expression of these markers is further modulated upon coculture with tumour cell lines with changes reflecting activation of these cells with VγVδ2 T-cells expressing inhibitory receptors PD1 and NKG2A producing the highest level of TNF.

**Conclusions** Our data reveals unique characteristics of Vδ2 in terms of their expression of immune checkpoints, which provide a mechanism which may be utilised by tumour cells to subvert VγVδ2 T-cell cytototoxicity. Our work suggests different profiles of immune checkpoints dependent on the method of stimulation. This highlights importance of expansion method in the function of VγVδ2 T-cells. Furthermore, this work suggests important candidates for blockade by immune checkpoint therapy in order to increase the successful use of VγVδ2 T-cells in cancer immunotherapy.

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**838**  
**PHENOTYPIC AND FUNCTIONAL SIGNATURES OF PERIPHERAL AND TUMOR-RESIDENT γδ T CELLS ARE INFORMATIVE FOR OUTCOME OF CHECKPOINT BLOCKADE IN MELANOMA**

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**Background** Immune checkpoint blockade (ICB) set a milestone in cancer immunotherapy, but still only a fraction of patients responds. Thus, there is an urgent need for biomarkers predicting outcome, and also for understanding the responsible mechanisms. γδ T cells constitute a numerically minor subset of 1-10% of the peripheral T cell compartment in healthy people and have a major role in defense against multiple microbial and non-microbial challenges. Unlike the majority of T cells, γδ T cells bind their ligands in an MHC-
independent manner. We previously studied γδ 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 γδ T cells.

Methods Phenotyping was performed in stage IV melanoma patients before and under PD-1+/-CTLA-4 blockade as basis our published OMIP-20 protocol. Cytokine expression patterns and proliferative capacities were determined as described according to our established protocols. 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 Vδ1 γδ T cells were associated with prolonged OS. Here, we investigated functional aspects and abundance of γδ T cells within the tumor as well as in the blood. The peripheral Vδ1 but not the Vδ2 differentiation signature revealed significantly lower proportions of naive and effector cells as well as an accumulation of late differentiated cells in patients with high Vδ1 frequencies. The cytokine expression pattern (IFNγ, TNF and IL-17) and the degranulation marker CD107a were different in patients with high versus low peripheral Vδ1 frequencies. The proliferative capabilities of Vδ1 cells in melanoma were limited in comparison to healthy subjects. Both Vδ1 and Vδ2 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 γδ T cells in cancer rejection. The previously found negative correlation of Vδ1 T cells with OS is likely due to an accumulation of mal-functioning, probably exhausted Vδ1 T cells in patients with poor outcome of ICB. Thus, we suggest that Vδ1 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).

REFERENCES

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840 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 phenotypes, 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 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 pad)

Results Principal component analysis demonstrated that CD8T, unlike Th and Tregs, cluster according to the invasiveness of the disease. Accordingly, many genes were significantly differently 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 Ethikprövningsnämnden i Lund), approval number 2017/34.

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

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839 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.