**Background** Head and neck squamous cell carcinomas (HNSCCs) arise in the mucosal linings of the upper aerodigestive tract. Risk factors are smoking, excessive alcohol consumption, and infection with human papillomavirus (HPV). Tumors are classified according to stage, HPV status, and to anatomical site: oral cavity, hypopharynx, larynx and oropharynx. Immune checkpoint inhibitors belong to the treatment arsenal for HNSCC but are effective in only a minority of patients. Treatment response and prognosis are influenced by the tumor immune microenvironment (TIME). Generally, HPV-related HNSCCs are associated with increased immune infiltration, but for different HNSCC anatomical sites such data are lacking.

**Materials and Methods** Using flow cytometry, we investigated the TiME of 36 fresh HNSCC samples. We further examined the spatial distribution of CD8+ subsets using multiplex immunohistochemistry (mIHC) on 20 samples. Additionally, single cell RNA-sequencing (scRNA-seq) was performed on five samples to obtain a comprehensive understanding of individual cells in the TIME of HNSCC.

**Results** Increased T cell frequencies were observed with flow cytometry in oral cavity squamous cell carcinoma (OCSCC) in comparison to HPV-unrelated HNSCC at other anatomical sites. Using mIHC, no significant differences were found in tissue-resident or proliferating CD8+ densities between sites. However, a trend towards increased proliferating CD8+ densities was observed in OCSCC. Moreover, the number of infiltrating CD8+ cells within 30μm from tumor cells was highest in OCSCC. Using scRNA-seq, we were able to annotate cell types and perform subclustering. This enabled us to distinguish among others CD8+ exhausted T cells.

**Conclusions** Our data indicate that, compared to other anatomical sites, oral cavity SCCs are more often populated by T cells. This suggests that particularly OCSCC may more easily respond to immunotherapy strategies aimed at activating T cells.


### A CAR-T CELL-BASED APPROACH FOR THE TREATMENT OF MALIGNANT T CELL DISEASES

**Abstract**

**Background** The T cell receptor (TCR) is uniformly expressed in monoclonal T cell lymphoma (TCL) populations and would thus represent an ideal target antigen for a CAR-based immunotherapy. However, the TCR is present on all T cells including malignant, healthy, and CAR-T cells. Strategies to increase tumor specificity of TCR-specific CAR-T cells are therefore required. To address this issue, CARs targeting one of the two TCR-β constant chains and CARs targeting the CDR3 region of a malignant clone have been designed. However, in the first case the off-tumor activity would affect about 50% of T cells, which could lead to impaired T cell immunity in the patient. The latter approach is highly specific to the malignant T cell clone but would require creation of individual CDR3-specific CARs for every patient which is currently impracticable. The TCR V-segment chains can be grouped into families and targeting TCR malignancies via V-family-specific CARs would spare the majority of healthy T cells. Targeting the TCR V-segments would unite high tumor specificity with the limited effort of creating a panel of CAR molecules that could be used for all patients.

**Materials and Methods** The TCR sequence of a malignant clone from a patient with γδ-TCL was obtained and cloned into a lentiviral expression plasmid. This γδ-TCR was expressed in Jurkat E.6.1 TCL cells together with a panel of other γδ-TCRs to create target cells with different variable chain usage. A Vδ1-specific CAR molecule was designed and the CAR was expressed in a previously described Jurkat E.6.1 based T cell reporter cell line, that allows to measure T cell activation by flow cytometry.

**Results** A panel of γδ-TCR expressing target cells with different variable chain usage was created. This panel included a lymphoma-patient derived Vγ2Vδ1 TCR and several other TCRs consisting of Vδ1 chains with different CDR3 regions paired to various Vγ chains. Vγ9Vδ2- and γδ-TCR expressing target cells served as control cells. The Vδ1-specific CAR was highly expressed in Jurkat E.6.1 reporter cells. Co-culture experiments revealed strong activation of reporter cells by all Vδ1 chain expressing target cells whereas no reactivity towards Vδ1 negative TCRs was detected.

**Conclusion** We show highly specific activation of Vδ1-specific CAR T cells in a reporter cell based in-vitro model of γδ-TCL. Provided that a panel of V-segment specific CARs would be created, these CAR-T cells would be able to specifically kill malignant T cells with very limited collateral damage to the non-malignant T cell repertoire. Unlike CDR3 regions the V-segments are largely unaffected by V(D)J-recombination and thus represent an ideal target antigen for a CAR-based immunotherapy approach. We therefore propose the TCR V-segments as ideal targets for immunotherapies against TCLs.


### DYSREGULATED EXPRESSIONS OF INHIBITORY CHECKPOINT MOLECULES AND THEIR LIGANDS ON T-CELLS AND BLASTS IN AML RELAPSES AFTER STEM CELL TRANSPLANTATION (SCT)

**Abstract**

**Background** Dysregulated expressions of inhibitory checkpoint molecules and their ligands are associated with T-cell dysfunction and resistance to immunotherapies in myeloid malignancies. In AML, T cells and blasts are known to express higher levels of inhibitory checkpoint molecules and their ligands compared to normal T cells.

**Materials and Methods** Using flow cytometry and quantitative PCR, we analyzed the expression of inhibitory checkpoint molecules and their ligands on T-cells and blasts in AML relapses after stem cell transplantation (SCT).

**Results** A significant dysregulation of inhibitory checkpoint molecules and their ligands was observed on T-cells and blasts in AML relapses after SCT.

**Conclusion** The dysregulation of inhibitory checkpoint molecules and their ligands on T-cells and blasts in AML relapses after SCT may contribute to T-cell dysfunction and resistance to immunotherapies. Targeting these molecules may improve the efficacy of immunotherapies in AML relapses after SCT.