compared to non-loaded CAR-T cells or CSGP4-negative tumor cells by flow cytometry. Additionally, tumor cell lysis was investigated via impedance-based monitoring of cell viability and microscopic analysis of the dissolution of three-dimensional tumor spheroids.

**Results** We observed that SPION-loading did not affect the expression of activation markers, differentiation, or proliferation of CAR-T cells. Furthermore, SPION-loaded CAR-T cells retained their capability for antigen-specific tumor cell lysis over multiple days. Additionally, these CAR-T cells demonstrated the ability to be controlled by an external magnetic field, as well as infiltrating and dissolving tumor spheroids.

**Conclusions** In summary, we demonstrated that SPION-loading did not compromise the functionality of CAR-T cells, as they were still able to perform the investigated effector functions with similar efficacy as the non-loaded control CAR-T cells. These findings underscore the potential of SPIONs in enhancing site-specific anti-tumor responses of CAR-T cells in the therapy of solid cancers in the future.

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## P09.03 LOADING OF T CELLS WITH SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES GIVES THEM MAGNETIC CONTROLLABILITY WHILE RETAINING ANTIGEN-SPECIFIC EFFECTOR FUNCTIONS

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**Background** The composition of the tumor microenvironment in solid tumors is of crucial importance for the prognosis and clinical outcome of patients with solid cancers (1). Infiltration of CD8+ T cells into the tumor can improve the prognosis and treatment options of patients. Adoptive T cell therapy is intended to increase the number of CD8+ T cells in the tumor. However, only a fraction of cancer patients benefit from this option, partially because the T cells do not effectively reach the tumor (2). We developed citrate-coated superparamagnetic iron oxide nanoparticles (SPIONs) for the loading of T cells to make them magnetically controllable (3,4). After intra-arterial application and magnetic enrichment in the tumor region, SPION-loaded T cells must pass through the vessel wall to reach the tumor and they must retain antigen-specific effector functions to fight the tumor. This study investigated the effects of SPION loading on primary human T cells, particularly on antigen-specific effector functions and their cellular migration capacity (5).

**Materials and Methods** T cells were freshly isolated from human whole blood and subsequently loaded with SPIONs for 4 h. Unloaded T cells served as controls. Using a Boyden-Chamber-based assay, we acquired information about the ability of T cell to migrate towards a CXCL12-gradient. Furthermore, the tethering and attachment of T cells on an endothelial cell monolayer was investigated by fluorescence microscopy. The deformability upon SPION-loading was investigated using Real-Time Deformability Cytometry (RT-DC). Antigen-specific effector functions were examined after stimulation via an introduced exogenous T cell receptor (TCR) specific for the melanoma antigen MelanA or the endogenous TCR specific for the cytomegalovirus antigen pp65.

**Results** SPION-loading had no effect on the attachment of T cells to an endothelial monolayer, however, the chemotactic migration was reduced by SPIONs, which was cancelled out by magnetic attraction. RT-DC ruled out stiffening of the cells due to nanoparticle loading, which is important for squeezing through the vessel walls during transmigration. Lastly, we observed no alterations in antigen-specific effector functions regarding proliferation, expression of activation markers, cytokine secretion, or tumor cell killing after antigen-specific activation mediated by endo- or exogenous TCRs.

**Conclusions** In sum, we showed that SPION loading did not impair cellular mechanics or antigen-specific effector functions. With regard to cell transmigration, possible negative effects of SPION-loading on the T cells were compensated by magnetic attraction. These results underline the potential of SPIONs for the enrichment of T cells in the tissue of solid tumors through magnetic attraction.

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## P09.04 ISOLATION OF A HIGH AVIDITY TCR TARGETING A NEWLY IDENTIFIED EPITOPE OF A COMMON CANCER TESTIS ANTIGEN EXPRESSED BY SOLID TUMORS

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**Background** T cell receptor (TCR)-based immunotherapy is a promising cancer treatment particularly as the TCR antigen repertoire detects both intracellular and extracellular tumor antigens. Our newly identified cancer-testis antigen (CTA) is an intracellular antigen exclusively expressed by cancer and reproductive tissue making it an ideal target for TCR-T cell therapy. Here, we aim to isolate a TCR against an HLA-A*02:01 restricted CTA epitope from the human HLA-A*02:01 negative repertoire to evaluate its safety and efficacy *in vitro* and *in vivo*.

**Materials and Methods** CTA expression and epitope presentation is analyzed in hematological and solid neoplasms by immunohistochemistry, qRT-PCR, and mass spectrometry. TCRs are isolated using a co-culture of naive HLA-A*02:01 negative CD8+ T cells with antigen-presenting cells. Epitope-specific cells are identified by multimer staining and sorted by fluorescence-activated cell sorting followed by sequencing of TCR alpha and beta chains to identify mutation-specific TCRs. Functional avidity of TCR transduced CD8+ T cells is determined by measuring IFNγ release (ELISA). Cross-reactivity is evaluated by the Alanine- and Glycine-Scan-Assay, and alloreactivity is determined by co-culturing immortalized B lymphoblastoid cell lines holding different haplotypes with TCR-transduced T cells.
**Results** CTA expression was detected in patient samples of colorectal, breast, and head and neck cancer by immunohistochemistry and qRT-PCR. Epitope presentation was quantified by mass spectrometry and PDX models of breast cancer and is ongoing in PDX models of colorectal cancer. Thus far, four potential CTA-specific TCRs containing minimally murinized constant regions have been identified and constructed, awaiting further safety evaluation and efficacy testing in established PDX models.
**Conclusions** We have identified an undescribed epitope of a CTA not yet targeted by immunotherapy and isolated four potential high avidity epitope-specific TCRs from human HLA-A*02:01 negative donors. We confirmed the expression of the target CTA as well as epitope presentation in several common solid neoplasms. This indicates a huge therapeutic potential of our identified TCRs in a wide patient population with relapsed/refractory solid tumors in a tumor agnostic manner.

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## P09.05 NOVEL A20 BASED THERAPEUTIC STRATEGIES TO FIGHT LUNG CANCER

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**Background** Lung cancer still accounts for the most cancer-related deaths worldwide. Although immune checkpoint blockade therapy is now the frontline therapy for lung adenocarcinomas with non-targetable oncogenic KRAS mutations, patients still have poor prognosis, which highlights the need to improve immunotherapy-based treatment strategies. Recently we identified that the systemic downregulation of the anti-inflammatory protein A20, induces a tumor-suppressive microenvironment in mouse models of lung cancer. This indicates A20 as a potential target for immune modulation to enhance the efficacy of immune-based therapies.
**Materials and Methods** Using mouse models of KRAS-driven lung tumorigenesis, we evaluated the response of the tumor immune microenvironment to partial A20 knockdown in the stroma. *In vitro* and *in vivo* evaluations of A20 heterozygous and wild type $CD8^+$ T cells were conducted using flow cytometry, RT-qPCR, tumor size measurement, survival assay and RNA sequencing.
**Results** We discovered that systemic reduction in expression of the immune-modulatory enzyme A20, as seen in A20 heterozygous mice, induced modifications in the tumor microenvironment. This led to the formation of an anti-tumorigenic milieu, facilitating heightened infiltration of $CD4^+$ and $CD8^+$ T lymphocytes, as well as dendritic cells. In our *in vitro* experiments, we discovered that downregulation of A20 in $CD8^+$ T cells in an increased capacity for proliferation and demonstrated anti-tumor activity in *in vitro* killing assays compared to wild-type $CD8^+$ T cells. Moreover, adoptive transfer of antigen-specific $CD8^+$ T cells led to a more pronounced reduction in tumor cell growth in A20 heterozygous recipients compared to wild-type recipients. This enhanced effectiveness of T cell transfer can be attributed to the potent anti-tumorigenic microenvironment present in the A20 heterozygous mice.
**Conclusions** Our preliminary results demonstrated that systemic downregulation of the anti-inflammatory protein A20 in immune cells of the stroma, enhances the anti-tumor capability of $CD8^+$ T cells and impedes tumor growth in mice. Additional experiments are required to investigate whether a combination therapy approach based on controlled A20 knockdown in immune cells and immune checkpoint blockade is efficacious in limiting tumor growth in KRAS driven lung adenocarcinoma.

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## P09.06 ESTABLISHING A MOUSE MODEL TO VISUALIZE LIMITATIONS OF CAR-T CELLS ACTIVITY IN SOLID TUMORS

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**Background** The use of CAR-T cells (chimeric antigen receptor T cells) has shown promising success in the treatment of B hematological malignancies, but their therapeutic performance in solid tumors remains more limited. Our objective is to decipher the dynamics of CAR-T cells in solid tumors *in vivo* at the single-cell level and to compare it to hematological tumor models to identify limitations to CAR-T cells efficacy.
**Materials and Methods** For this purpose, we developed a murine subcutaneous solid tumor model to study the effect of CAR-T cells therapy. We generated tumor cell lines expressing a fluorescent probe to monitor apoptosis (the DEVD probe) and expressing the CD19 antigen. Response to CAR-T cells is then analyzed by flow cytometry and intravital imaging.
**Results** We found that CAR-T cells therapy in this solid tumor model prolong mouse survival and delay tumor growth but could not induce complete remission. Moreover, we could visualize CAR-T cells infiltration at the tumor site. Therefore, our model is suitable to visualize potential limitations of CAR-T cells therapy. We are currently using intravital two-photon imaging to decipher CAR-T cells behavior and killing potential during the course of CAR-T cells therapy.