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.