P06 Cell Therapy in Solid Tumors

P06.01 $\alpha\beta$ -T CELLS ENGINEERED TO EXPRESS $\gamma\delta$ -T CELL RECEPTORS CAN KILL NEUROBLASTOMA ORGANOIDS INDEPENDENT OF MHC-I EXPRESSION

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Background Currently ~50% of patients with the diagnosis of high-risk neuroblastoma will not survive due to relapsing or refractory disease. Recent innovations in immunotherapy for solid tumors are highly promising, but the low MHC-I expression of neuroblastoma represents a major challenge for T cell-mediated immunotherapy. Here, we propose a novel T cell-based immunotherapy approach for neuroblastoma, based on the use of TEG002, $\alpha\beta$ -T cells engineered to express a defined $\gamma\delta$ -T cell receptor, which are thought to recognize and kill target cells independent of MHC-I. In this pilot project we have tested the potential efficacy of TEG002 therapy as a novel treatment for neuroblastoma, with tumor organoids.

Materials and Methods Effector cells were created from healthy donor peripheral blood T cells. The TEG002 cells were engineered by transducing $\alpha\beta$ -T cells with a defined $\gamma\gamma$ V δ 2-T cell receptor. Both the untransduced $\alpha\beta$ -T cells and the endogenous $\gamma\gamma$ V δ 2-T cells from the same healthy donor were used as controls in all experiments. Activation and killing of TEG002 was tested in a co-culture setting with neuroblastoma organoids. Supernatant of the co-culture was collected at 24 hours for IFN γ ELISA to measure activation of TEG002. The dynamics of cytotoxicity were analyzed over time from 0 till 72 hours, using the live-cell imaging system IncuCyte from Sartorius®. Killing was quantified using a Caspase3/7 Green dye and the IncuCyte software. Transcriptional profiling of the neuroblastoma organoids was done by RNA sequencing and MHC-I expression of the neuroblastoma organoids was determined by flow cytometry.

Results We showed that 3 out of 6 neuroblastoma organoids could activate TEG002 as measured by IFN γ production. Transcriptional profiling of the neuroblastoma organoids showed that this effect correlates with an increased activity of processes involved in interferon signaling and extracellular matrix organization. Analysis of the dynamics of organoid killing by TEG002 over time confirmed that organoids which induced TEG002 activation were efficiently killed independently of their MHC-I expression. Of note, efficacy of TEG002 treatment was superior to donor-matched untransduced $\alpha\beta$ -T cells or endogenous $\gamma\delta$ -T cells.

Conclusions We demonstrated that 50% of tested neuroblastoma organoids can effectively activate TEG002 and that killing of the organoids is independent of MHC-I expression. Hence, this pilot study identified TEG002 as a promising novel cellular product for immunotherapy for a subset of neuroblastoma tumors, warranting further investigations into its clinical application.

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P06.02 CANCER-SPECIFIC DIFFERENCES OF TERTIARY LYMPHOID STRUCTURES AND CELLULAR RESPONSES AGAINST FREQUENTLY EXPRESSED CANCER TESTIS ANTIGENS

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Background Tertiary lymphocyte structures (TLS) can be detected in the tumor microenvironment across a wide range of cancer types and are associated with increased patient survival and susceptibility to immune checkpoint inhibition. However, evidence for the functional significance of TLS on humoral and cellular immunity is scarce. In this study, we combine assessment of abundance and spatial distribution of TLS with expression levels of 10 tumor associated antigens (TAAs) and functional analyses of T cell responses to these antigens.

Materials and Methods 52 treatment naïve cancer patients across 5 tumor types (NSCLC, CRC, RCC, HCC and BCA) were included. Presence and localization of TLS was assessed in immunohistochemical stainings (CD20) of whole section slides from FFPE embedded tumor samples. B cell clusters were quantified in the whole tumor region and in two different tumor margins (300 μ m, 2000 μ m). A panel of 30 cancer testis antigens was selected via GEPIA software (TCGA Database) and their expression in our cohort was determined using NanoString based RNA expression analysis of tumor samples and patient-matched healthy tissue. The 10 peptide pools with the largest cross-cancer overlap were selected based on our NanoString results. 2-color Fluorospot assays (IFN- γ and IL-2) were applied to assess the frequency of tumor-specific T-cell responses in patient PBMCs (triplicates for each TAA).

Results CD20 immunohistochemistry and enumeration of intra- and peritumoral TLS revealed different distribution patterns of TLS/mm² with the largest proportion in the 300 μ m margin ($p < 0.01$) in most of the cancer types. This effect was particularly observed in patients with non-small cell lung cancer (NSCLC). The 10 tumor antigens CEP55, CT83, GAGE1, IGF2BP3, MAGEA1, MAGEA3/6, PBK, PRAME, Survivin and TTK were selected as they showed the highest overlap across different cancer types and the most pronounced differential expression between tumor and matched normal tissue. While 31/52 (59.6%) patients showed an IFN- γ , only 11/52 (21.2%) patients exhibited an IL-2 response against at least one of the tested CTAs. Survivin was the CTA presenting the highest frequency of responses (18/52 IFN- γ and 5/52 IL-2 responses). PBMCs of patients with NSCLC showed the highest frequency of T-cell responses (83.3% with at least one IFN- γ response)

and patients with HCC and BCA the lowest proportion (40.0%).

Conclusions The observed findings underline the importance of TLS as a novel biomarker and a possible association to cellular responses may even enhance their prognostic value. Our planned analyses of combined humoral immune responses will further elucidate the role of TLS in anti-tumor immune response of the analyzed cancer types. A combined targeting of a predefined or personalized set of included TAAs appears promising across the different cancer types.

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P06.03

BISPECIFIC ANTIBODY-DRIVEN SYNTHETIC AGONISTIC RECEPTOR ENGINEERED T CELLS LEAD TO SPECIFIC AND CONDITIONAL THERAPY IN MELANOMA CANCER MODELS

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Background Immunotherapeutic approaches, including immune checkpoint blockade and adoptive T cell therapy (ACT) in the form of tumor-infiltrating lymphocytes (TIL), have had marked success in the treatment of melanoma. Despite these successes, many patients are refractory to treatment or relapse with therapy-resistant disease. To overcome said limitations, we propose a controlled ACT approach, where T cells are armed with synthetic agonistic receptors (SAR) that are conditionally activated only in the presence of a target melanoma-associated antigen, and a cross-linking bispecific antibody (BiAb) specific for both SAR T cell and tumour cell.

Materials and Methods A SAR composed of an extracellular EGFRvIII, trans-membrane CD28, and intracellular CD28 and CD3z domains was fused via overlap-extension PCR cloning. T cells were retrovirally transduced to stably express our SAR construct. We validated our approach in two murine as well as two human cancer models expressing our melanoma-associated target antigens TYRP (murine) and MCSP (human). We confirmed conditional and specific stimulation and proliferation of our T cells, as well as their tumour-antigen-directed cytotoxicity, in vitro and in vivo.

Results Crosslinking TYRP-EGFRvIII (murine) and MCSP-EGFRvIII (human) BiAb, monovalently selective for our SAR, induced conditional antigen-dependent activation, proliferation of SAR-T cells and directed tumour cell lysis with specificity towards two TYRP-expressing murine melanoma and two MCSP-expressing human melanoma cancer models. In vivo, anti-tumoural activity was mediated by the co-administration

of SAR-T cells and BiAb, in A375 and MV3 melanoma xenograft models. Further, we could show that SAR T cells exhibited resistance to MDSC-induced suppression of activation and proliferation.

Conclusions Here we apply the SAR x BiAb approach in efforts to deliver specific and conditional activation of SAR transduced T cells, and targeted tumour cell lysis. The modularity of our platform is key for a targeting approach in a tumor entity with a high mutational load such as melanoma and is fundamental in our drive towards personalised immunotherapies. Further, the SAR approach has demonstrated resistance to MDSC-induced suppression, an interesting axis that requires further investigation.

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P06.04

TRANSCRIPTOME-WIDE NETWORK ANALYSIS PREDICTS THE ROLE OF LACTATE DEHYDROGENASE C IN BREAST CANCER CELL SURVIVAL AND IMMUNE DYSFUNCTION

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Background Cancer testis antigens (CTAs) are lucrative anti-cancer targets given their restricted expression patterns and known roles as mediators of cancer hallmarks, including cancer metabolism, proliferation, survival, and cell motility. Lactate dehydrogenase C (LDHC) is a CTA with upregulated expression in poor prognosis subtypes of breast cancer, however its tumorigenic role is less understood. We recently reported that silencing LDHC reduces breast cancer cell survival through a dysregulated DNA damage response, thus highlighting its potential as an anti-cancer target with limited off-target effects. This study aimed to explore the changes in the transcriptome of breast cancer cells and immune-related mediators upon *in vitro* LDHC targeting.

Materials and Methods We silenced *LDHC* expression in breast cancer cell lines and investigated the downstream effects on the tumor cell transcriptome. Differentially expressed genes were subjected to regulatory network analyses. We further assessed the secretory profile of cytokines and immune checkpoint expression in *LDHC*-silenced cells and used the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm to determine the effect of the interaction between *LDHC* expression and cytotoxic T lymphocyte (CTL) infiltration in the METABRIC breast cancer cohort.

Results Network analysis to investigate the effects of silencing *LDHC* on the tumor cell transcriptome identified 47 up- and 55 down-regulated transcripts (2.0-fold change, adj p<0.05). Differentially expressed genes in the *LDHC*-silenced cells were particularly enriched in canonical pathways regulating cell cycle checkpoint control, BRCA1-mediated DNA damage response and NF-kb signaling in response to infection. Upstream regulator analyses revealed the altered expression profile was associated with mTOR (p=1.27e-5, z=2.242) and CASP3 (p=3.2e-4, z=2.250) pathways, which in the presence of LDHC are predicted to activate TP53, Myc, NF-KB complex, STAT1/3, PRKC, CDK2, FOXO3 and HIF-1a while