statistical robustness. Pattern confluency is reliably measured with trained AI software allowing for processing of high-throughput live-cell imaging data which is essential for selecting T cell variants which best perform in given conditions like the complex tumor microenvironment.

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P12.08 TARGETING TUMOR-REDUCED DRUG ACCESSIBILITY TO COUNTERACT ORGAN-SPECIFIC RESISTANCE TO ANTI-CD22 IMMUNOTOXINS

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Background The tumor microenvironment (TME) not only depends on tumor type but also on tumor location and tissue type. Therefore, it may interfere with drug accessibility and response to antibody-based therapies in an organ-specific manner. We have established a human CD22-transgenic, systemic B cell lymphoma mouse model representing local resistance to CD22-targeted, recombinant immunotoxins (rIT) in lymph nodes. Hence, the aim of this study was to analyze drug accessibility in different organs and screen for possible combination therapies to overcome organ-specific resistance.

Materials and Methods Primary murine, MYC-driven B cell lymphoma positive for human CD22 (MyC22-1/-2/-3) were injected i.v. in syngeneic mice. Mice were treated with unlabeled or fluorochrome-labeled rIT. Organ-specific tumor infiltration, immune infiltration, and drug homing was determined *ex vivo* in bone marrow (BM), spleen (SPL), and lymph nodes (LN) by flow cytometry.

Results I.v. injection of three distinct B cell lymphoma clones MyC22-1/-2/-3 resulted in diffuse organ infiltration of BM, SPL, and LN showing organ-specific immune infiltration and histology. Despite phenotypic similarity among the lymphoma clones, anti-CD22 immunotoxins induced tumor clone- and organ-specific treatment responses. In contrast to major tumor regression in BM and SPL, sensitivity to rIT differed between LNs of the three models. While LNs of MyC22-1 showed strong response to rIT, LNs of MyC22-2 and MyC22-3 were resistant. To measure organ-specific drug distribution, a fluorochrome-labeled rIT was injected i.v. and maximal fluorochrome intensity was reached 6 h after injection. The overall signal was lower in LN compared to SPL and BM of tumor-bearing and tumor-free mice indicating reduced drug accessibility in LN *per se*. Despite similarly high signal intensity in SPL among all models, signal in LN of rIT-resistant MyC22-2 and MyC22-3 was strongly reduced compared to rIT-sensitive MyC22-1 and tumor-free mice suggesting tumor response correlates with organ-specific drug accessibility. To counteract local resistance, we screened potential rIT enhancers and identified two drugs that achieved complete remissions in combination with rIT also in LNs of resistant mice. Although both drugs enhanced tumor response in LN, only one of them increased drug accessibility. Substituting the enhancer of rIT accessibility with a second drug of the same mechanistic family similarly reproduced the observed effects.

Conclusions Tumor responses to anti-CD22 immunotoxins strongly varied in a tumor clone- and also in an organ-specific manner. Response correlated with reduced drug accessibility in LN emphasizing the importance to study drugs in systemic tumor models. Impaired accessibility was reversed in combination with rIT enhancers. Distinct accessibility of other antibody-based therapeutics is currently being evaluated.

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P12.09 EXPANDED T CELL CLONES IN NEUROBLASTOMA PERSIST THROUGHOUT CHEMOTHERAPY AND DISPLAY A DRUGGABLE DYSFUNCTIONAL PROFILE

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Background Currently, patients with high-risk neuroblastoma are treated with an intense multimodal regimen including chemotherapy, leading to severe side effects. Still, many patients relapse, which indicates the need for new treatment options. Immunotherapy represents a promising solution. However, neuroblastoma tumors exploit many immunosuppressive mechanisms to evade immune cell recognition. Targeting these immunosuppressive pathways may enhance tumor killing and increase patient survival.

Materials and Methods To unveil T cell dynamics, (dys)functionality and immunosuppressive mechanisms, tumors from 3 patients at diagnosis, after chemotherapy and at relapse were dissociated into single cells and enriched for $CD45^+CD3^+$ T cells. Single-cell RNA and single-cell αβ and γδ T-cell receptor sequencing were performed in parallel with the 10x platform. Data were analyzed in R (Seurat package) and validated with high-dimensional spectral flow cytometry.

Results In tumors, clonally expanded tumor-infiltrating T lymphocytes are expected to be largely responsible for the anti-tumor response. Preliminary TCR sequencing data from matched tumor and blood before and after chemotherapy of n=3 high-risk neuroblastoma patients, revealed the presence of clonally expanded T cells in the tumor samples at diagnosis. Some of these expanded clones at diagnosis persisted throughout the course of chemotherapy and were found in the resected tumor as expanded clones. Many of the expanded clones in the tumors could be traced back in the blood of the same patients (as non-expanded clones), possibly suggesting T cell recirculation between the blood stream and

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the tumor site. Moreover, the same expanded clones present in the tumor were found in the adjacent normal adrenal gland tissue, suggesting that tumor-reactive T cells may reside in tumor-adjacent tissues, and that these tissues may serve as reservoir for tumor-reactive cells. Expanded clones were particularly enriched in the $CD8^+$ central memory and terminally differentiated effector memory (TEMRA) subsets, suggesting tumor-reactivity of the CD8 T cell compartment. Functionally, the top expanded clones displayed high levels of the immune checkpoint molecule LAG-3 and of the immunosuppressive cytokine TGF-β, both at transcriptional and protein level, compared to non-expanded clones. Expression of these markers may be indicative of a dysfunctional/exhausted phenotype in the expanded tumor-reactive clones. Further in-depth characterization of expanded clones, and analysis of an additional n=7 patients, is currently ongoing.

Conclusions These preliminary results give first insights into the dynamics of tumor-reactive T cells in neuroblastoma throughout the course of therapy. Our understanding of the dysfunctionality mechanisms specifically in the hyperexpanded, likely tumor-reactive, clones will enable us to target them, e.g. by immune checkpoint inhibition, to promote tumor killing. These findings will be at the base for the generation of new immunotherapies to implement in clinic with the final goal to increase survival of high-risk neuroblastoma patients.

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P12.10 *IN VIVO* DYNAMICS AND ANTI-TUMOR EFFECTS OF B7-H3-DIRECTED CAR T-CELLS IN AN ORTHOTOPIC MEDULLOBLASTOMA MOUSE MODEL

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Background The majority of solid tumors in pediatric patients occurs in the central nervous system, with medulloblastoma accounting for approximately 20% of pediatric brain tumors. Current treatment, involving resection, radiotherapy, and chemotherapy, improves long-term survival but is associated with significant adverse effects. Hence, there is an urgent need for novel therapeutic approaches. Chimeric Antigen Receptor (CAR) T-cell therapy has shown promise in treating hematologic malignancies. Its potential suitability for medulloblastoma remains uncertain. Initial preclinical and clinical studies suggest efficacy, but the dynamics of CAR-T recruitment, their interactions with tumor cells, optimal administration routes (intravenous, intraventricular, intratumoral), and mechanisms of potential treatment failure are unclear. To address these gaps, we aimed to establish an *in vivo* model for further investigation of CAR T-treatment in medulloblastoma.

Materials and Methods Three weeks after the microsurgical implantation of a chronic cerebellar window in immunodeficient Fox^{N1} mice, red fluorescent human medulloblastoma cells ($DAOY^{tdt}$; SHH) were injected into the cerebellum. After 20 days solid tumor had formed, and CAR $T\text{-cells}^{GFP}$ directed against the surface antigen B7-H3 were injected at a distance of 1mm adjacent to the tumor. As control, we used antiCD-19 CAR $T\text{-cells}^{GFP}$. Intravital two-photon laser scanning microscopy was used to longitudinally monitor tumor growth and CAR T-cells at a cellular level.

Results We successfully established a xenogeneic orthotopic medulloblastoma model which allows repetitive *in vivo* microscopy on a cellular resolution. Our preliminary results show, that following intracranial injection CAR T-cells were recruited to the tumor site and reduced tumor growth.

Conclusions Our mouse model can be used to evaluate the efficacy of CAR T-treatment for medulloblastoma and analyze CAR T-biology at a cellular level. Preliminary data showed a possible efficacy of antiB7-H3 CARs. In further experiments, we plan to dissect the efficacy of different application routes on the treatment efficacy.

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P12.11 RECQL4 PROMOTES IMMUNE EVASION AND LIMITS RESPONSE TO ANTI-PD-1 THERAPY AND SURVIVAL IN MELANOMA PATIENTS

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Background The DNA helicase RECQL4 is involved in DNA replication, recombination, transcription and damage repair by unwinding various DNA structures. Components of the DNA repair machinery have been shown to influence response to immune checkpoint inhibitor (ICI) therapy in cancer patients. Therefore, we defined the impact of RECQL4 for melanoma progression and ICI efficacy.

Materials and Methods We investigated how *RECQL4* copy number levels affect patient progression using whole exome sequencing data in a pan-cancer cohort of 25,775 patients. In addition, gene set enrichment analysis revealed the pathways modulated by *RECQL4* amplification. We also used the Proteome Profiler Human XL Cytokine Array and performed

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