pronounced inhibition of cell proliferation (p = 0.0046). PI3K inhibition suppressed cancer cell growth, migration and colony formation *in vitro*. Pan-PI3K inhibition, anti-programmed death 1 (PD1) therapy and combination improved the overall survival (OS) of syngeneic mice with PTEN-deleted tumors from 27 days of the control to 48, 37 and 65 days, respectively. In mice with tumors not containing a PI3K pathway alteration, OS was prolonged by the combination, but not single treatments. Pan-PI3K inhibition significantly upregulated CD80, CD86, MHC-I and MHC-II in dendritic cells, and downregulated the transforming growth factor beta pathway with a false discovery rate (FDR)-adjusted q-value of 0.001. Interferon alpha response was significantly upregulated with anti-PD1 therapy (q value: < 0.001) and combination (q value: 0.027). Compared to the control, combination treatment increased $CD8^+$ T cell infiltration (p = 0.005), decreased T_{reg} cell infiltration (p = 0.036), and upregulated the expression of multiple immunostimulatory cytokines and Granzyme B (p< 0.01). Secondary resistance was associated with upregulation of the mammalian target of rapamycin (mTOR) pathway and multiple *Sprr* family genes.

Conclusions The combination Pan-PI3K inhibition and ICB has significant anti-tumor effects in aUC with or without activated PI3K pathway and warrant further clinical investigation. This combination creates an immunostimulatory tumor milieu. Secondary resistance is associated with upregulation of the mTOR pathway and *Sprr* family genes. Base on this study, a Phase II clinical trial has been designed.

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Plenary symposium 8: 'lost in translation'

08.03 MHJC-BASED LARGE-SCALE SCREENING OF ANTI-TUMOR T CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA REVEALS CD8+ T CELLS WITH SPECIFICITY AGAINST THE CLONOTYPIC B-CELL RECEPTOR IMMUNOGLOBULIN

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Background Chronic lymphocytic leukemia (CLL) remains incurable, indicating a need for novel strategies towards disease eradication, including reinvigoration of anti-tumor immune responses. T cells in CLL appear selected by restricted antigens, with recent evidence suggesting that the selecting epitopes may lie within the clonotypic B-cell receptor immunoglobulins (BcR IGs). Here, we present a large-scale evaluation of T cell recognition towards BcR IGs. We predicted MHC-I binding peptides from such clonotypic regions and determined the presence of T cell recognition towards such sequences, using DNA-barcoded multimers of peptide-major histocompatibility complexes (MHC).

Materials and Methods We evaluated 653 peptides derived from the clonotypic BcR IGs of 25 CLL patients across 13 MHC-I alleles based on the MHC-I typing of the patient. We constructed patient-specific peptide-MHC dextran multimers labeled with a unique DNA barcode and a fluorochrome. MHC-multimer binding T cells from PBMC samples where sorted and evaluated through amplification and sequencing of the MHC-attached DNA barcode, to determine the presence of neoepitope reactive T cells.

Results and Conclusion Across the 25 patients we observe T cell reactivity towards 3 peptide-MHC specificities, among the 653 evaluated. The T cell responses observed are listed below:

Peptide sequence	MHC-I allele association	Peptide-associated region in somatically hypermutated clonotypic BcR IG	Somatic hypermutation (SHM) position
VTVADTAVYY	A03*01	IGHV4-34 FR3	A to V at position 96
INLNPSLKRR	A03*01	IGHV4-39 FR2-FR3	T to I at position 65, Y to L at position 67, S to R at position 74
YSFTSYWINW	A24*02	IGHV5-10-1 CDR1-FR2	S to N at position 40

These response where further validated using conventionally fluorescence labelled pMHC tetramers. This demonstrates that cancer-specific somatic mutation in the BcR IG can be targets of T cell recognition of CLL, and hence serve as targets for novel immunotherapeutic strategies. The level of such T cell recognition was sparse in the cohort evaluated, but could potential be boosted with immunotherapy.

The data to be presented, was in-part presented at the European Hematology Association (EHA) annual meeting.

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Plenary session 9: young researcher session

09.01 ARMORING ANTI-HER2 CAR-T CELLS WITH C-C-MOTIVE RECEPTOR 8 (CCR8) AND A DOMINANT NEGATIVE TGF-B RECEPTOR (DNR) TO ENABLE EFFICACY IN SOLID TUMOR MODELS

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Background Chimeric antigen receptor (CAR) T cells have shown great efficacy in treating hematological malignancies. Nonetheless, in solid tumors CAR T cells have yet to demonstrate significant clinical efficacy. In solid tumors, CAR T cells are frequently prevented access to tumor tissue and face profound suppression at the tumor site. To overcome this issue,

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our group could previously demonstrate that arming CAR T cells with C-C-motive-receptor 8 for improved tumor-directed migration along the C-C-chemokine ligand 1 - CCR8 axis and a dominant-negative receptor against TGF-β for resistance to suppression enable activity in pancreatic cancer models. The value of this approach for other entities was however unclear. We now investigated the potential of this combination for treatment of HER2-positive cancer models in conjunction with a HER2-targeted CAR.

Materials and Methods Primary murine and human T cells were isolated and activated. T cells were retrovirally transduced. Phenotype, activation, exhaustion and proliferation were monitored *in vitro*. Cytokine production was assessed with ELISA. *In vivo*, survival and tumor growth of mice that were subcutaneously injected with tumor cells and treated with CAR T cells carrying either CCR8, DNR or both receptors were measured. To look at chemokine expression in tumor material, mRNA was isolated from tumor material and RT-qPCR was performed.

Results We found that expression of CCR8 can redirect CAR T cells to the tumor and a DNR can prevent immunosuppression of CAR T cells in the tumor microenvironment. The improved functionality of CAR-CCR8-DNR T cells compared to CAR T cells against the HER2 antigen could be demonstrated *in vitro* and *in vivo* in human HER2+ tumor models.

Conclusions Equipping CAR T cells with CCR8 and DNR emerges as a strategy not only limited to certain antigens, but as a potential universal approach to render cellular therapies more effective. The modularity of this concept promises further preclinical and perhaps clinical development to improve personalized immunotherapy.

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09.02 BISPECIFIC ANTIBODIES ENABLE SYNTHETIC AGONISTIC RECEPTOR T CELL THERAPY IN MELANOMA

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Background Immunotherapies, like immune checkpoint inhibition and tumor infiltrating lymphocytes, have had remarkable success in treating melanoma. However, many patients do still not respond or relapse with therapy-resistant disease. To overcome said limitations, we propose a controlled adoptive cell therapy approach, where T cells are armed with EGFRvIII synthetic agonistic receptors (E3 SAR) that are selectively activated by a cross-linking bispecific antibody (BiAb) specific for both SAR T cell and melanoma-associated antigens.

Materials and Methods Murine as well as human SAR constructs were generated and T cells were retrovirally transduced to stably express the SAR constructs. We validated our approach in murine, human and patient-derived cancer models expressing the melanoma-associated target antigens TYRP1 and MCSP. SAR T cells were functionally characterised by proving specific activation and proliferation of SAR T cells, as well as their tumor-directed cytotoxicity, in vitro and in vivo.

Results Both on a mRNA and protein level, MCSP and TYRP1 were shown to be differentially expressed in treatment-naive as well as treatment-resistant melanoma patients compared to samples from healthy donors. Crosslinking anti-TYRP1 x anti-E3 and anti-MCSP x anti-E3 BiAb mediated conditional antigen-dependent activation, proliferation of SAR-T cells and lead to tumor cell lysis in all models tested. *In vivo*, anti-tumoral activity and tumor-free survival was mediated by the co-administration of SAR T cells and BiAb in a syngeneic tumor model and was further confirmed in several xenograft models.

Conclusions Here, we apply the SAR x BiAb approach in an effortto deliver specific and conditional activation of SAR transduced T cells, and targeted tumor cell lysis in melanoma models. The modularity of our approach is key for targeting melanoma and is essential towards personalised immunotherapies addressing cancer heterogeneity. Due to variations of antigen expression in primary melanoma tissues, we propose that a dual-targeting approach, either simultaneous or sequential, could mitigate issues of heterogeneity and deliver therapeutic benefit to patients.

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