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 Treg 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 Sppr 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 Sppr family genes. Base on this study, a Phase II clinical trial has been designed.


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 were sorted and evaluated through amplification and sequencing of the MHC-attached DNA barcode, to determine the presence of neoeptitope 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:

<table>
<thead>
<tr>
<th>Peptide sequence</th>
<th>MHC-I allele association</th>
<th>Peptide-associated region in somatically hypermutated clonotypic BcR IG</th>
<th>Somatic hypermutation (SHM) position</th>
</tr>
</thead>
<tbody>
<tr>
<td>YTVADTAVYY</td>
<td>A03*01</td>
<td>IGHV4-34 FR3</td>
<td>A to V at position 96</td>
</tr>
<tr>
<td>INLIPSUKRR</td>
<td>A03*01</td>
<td>IGHV3-39 FR2-3FR3</td>
<td>T to I at position 65, Y to L at position 67, S to R at position 74</td>
</tr>
<tr>
<td>YSFTSYINNW</td>
<td>A24*02</td>
<td>IGHV5-10-1 CD81 FR2</td>
<td>S to N at position 40</td>
</tr>
</tbody>
</table>

These response where further validated using conventionally fluorescence labelled pHMC 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.


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,
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.

Disclosure Information

T.J. Strzalkowski: None. B.L. Cadilha: A. Employment (full or part-time); Significant; University Hospital, LMU Munich. I. Dalloull: A. Employment (full or part-time); Significant; University Hospital, LMU Munich. K. Manske: A. Employment (full or part-time); Significant; University Hospital, LMU Munich. S. Endres: A. Employment (full or part-time); Significant; University Hospital, LMU Munich. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; TCR2 Inc, Bio-M, Munich, Germany. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Significant; Else Kröner-Fresenius Stiftung, Paul-Martini-Stiftung. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Carina Biotech Ltd, Mawson Lakes, Australia. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; TCR2, Cambridge, MA, USA. F. Consultant/Advisory Board; Significant; Gilde Healthcare, Utrecht, Netherlands. S. Kobold: A. Employment (full or part-time); Significant; University Hospital, LMU Munich. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; TCR2 Inc, Arcus. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Significant; GlaxoSmithKline. D. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Carina Biotech Ltd, Mawson Lakes, Australia. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; TCR2, Cambridge, MA, USA. F. Consultant/Advisory Board; Significant; Tabby therapeutic ltd. F. Consultant/Advisory Board; Significant; TCR2 Inc, Novartis.