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 an A375 melanoma xenograft model. Further, overexpression of IDO (a key immunosuppressive enzyme implicated in the suppression of T cell function in the tumor microenvironment) in a melanoma model did not influence the killing kinetics of SAR T cells.

Conclusions Here we apply the SAR x BiAb approach in efforts to deliver specific and conditional activation of synthetic agonistic receptor 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 IDO-mediated inhibition in the context of melanoma, an interesting axis that requires further investigation.

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P06.02 ENHANCING CAR T CELL PERSISTENCE AND MEMORY THROUGH MODULATING MITOCHONDRIAL FUNCTION

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Background CAR T cell therapy for solid tumours has achieved limited success compared to its application to B cell malignancies. One reason for this failure is the low differentiation rate to memory subsets and low persistence of CAR T cells due to activation-induced cell death (AICD) in lymphoid tissue and the tumour microenvironment. In this study, we have expressed the MCL1 gene within CAR T cells to overcome losses by AICD in adoptively transferred T cells. The MCL1 gene expresses two isoforms; the long isoform localises to the outer membrane of mitochondria and inhibits the CD95 signalling death pathway, while the short isoform localises to the inner membrane of mitochondria to enhance mitochondrial oxidation, phosphorylation and fusion. In addition, we have also utilized a microRNA (miR) 429 to promote memory T cell formation through the suppression of genes such as T-cell-restricted intracellular antigen-1 (TIA-1), T cell activation inhibitor, mitochondrial (TCAIM) and mitochondrial fission factor (MFF).

Materials and Methods Overexpression of MCL1 was confirmed at both mRNA and protein level by real time RT-PCR (qPCR) and western blot. Similarly, overexpression of miR-429 was measured by qPCR and specific binding of miR-429 to the 3′ UTR of target genes was confirmed by luciferase reporter assay. Mitochondrial depolarization and cell viability were assessed by TMRE mitochondrial membrane potential assay (flow-cytometry) and resazurin assay. The effect of MCL1 or miR429 overexpression on HER2-CAR T cells was determined by flow cytometry. Soluble leucine-zipper CD95L (https://www.addgene.org/104349/) was expressed and purified from Expi293 cells.

Results Overexpression of MCL1 in both Jurkat T cells and primary human T cells protected cells against mitochondria depolarization as well as the loss of cell viability in response to CD95L-triggering. Expression of miR429 downregulated TIA1, TCAIM and MFF. A HER2-CAR construct with either MCL1 or miR429 in a lentiviral system was successfully designed and transduced into primary T cells. Mitochondria in transduced T demonstrated enlarged and fusion morphology - a classic feature of memory T cells.

Conclusions Overexpressing MCL1 or miR429 significantly improves mitochondrial function in T cells. This approach will be used to increase persistence of adoptively transferred CAR T cells.

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P06.03 C-C CHEMOKINE RECEPTOR 8 TUMOR-DIRECTED RECRUITMENT ENABLES CAR T CELLS TO REJECT SOLID TUMORS

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Background CAR T cell therapy is remarkably successful in patients with hematological malignancies, in some cases inducing durable remissions. However, it remains ineffective in solid tumors, in part due to poor T cell infiltration into the tumor mass. Determinants of successful T cell infiltration to the tumor site remain to be defined. In contrast, tumors actively attract T regulatory (T_{reg}) cells for immune suppression through the C-C chemokine receptor 8 (CCR8) - CCL1 axis. As this axis is functional across cancer entities, we postulated that CCR8 could also be used to target tumor-ablating T cells to the tumor site.

Material and methods Murine and human CCR8 have been cloned in a retroviral expression vector. CCR8 can be expressed in murine and human T cells upon transduction. A chimeric antigen receptor (CAR) targeting the murine epithelial cell adhesion molecule (EpCAM) was used for syngeneic pancreatic tumor models and a CAR targeting human mesothelin was used for a xenograft pancreatic tumor model.Mechanistically, we use flow cytometry and multi-photon intra-vital microscopy to interrogate infiltration of CCR8-transduced CAR T cells.

Results Here we show that genetically engineering CAR T cells to express CCR8 improves their migration into solid tumors and allows rejection of tumors that are otherwise resistant to CAR T cell therapy. We demonstrate the capacity of these enhanced CAR T cells to stunt solid tumor growth and improve survival in both murine syngeneic and human xenograft tumor models.

Conclusion Our results demonstrate the viability of using CCR8 to confer T_{reg}cell trafficking-properties in CAR T cells to enable their effectiveness in solid tumors. This receptor may be combined with other promising strategies to improve the efficacy of cellular approaches.