presence of a bispecific antibody (BiAb) cross-linking the receptor with a tumor-associated antigen. While we could show efficacy of the SAR platform in different models, limited infiltration and immune suppression still hamper its function. We could previously demonstrate that T cell infiltration can be enhanced by transduction with carefully chosen chemokine receptors like CXCR6, CCR4 and CCR8. At the same time, gene silencing of checkpoint molecules like PD-1 can make T cells more resistant to immunosuppression, thus we assumed that combining these approaches might generate a desired T cell product.

Materials and Methods All constructs had been generated previously by overlap-extension cloning. The EGFRvIII (E3) SAR consists of extracellular EGFRvIII, transmembrane CD28 and intracellular CD28 and CD3ζ. Human CXCR6-GFP, CCR4-GFP and CCR8-GFP are composed of the chemokine receptors fused to GFP via a 2A sequence. Primary human T cells were retrovirally transduced to stably express the SAR and chemokine receptors. We analyzed migration, cytotoxicity and activation of the single and double (E3 SAR and chemokine receptor) transduced T cells. In addition, PD-1 was knocked out using CRISPR-Cas9 and killing kinetics of target cells and T cell activation were assessed.

Results Co-transduction with chemokine receptors significantly increased migration of E3 SAR T cells to their respective ligand while lysis of target-expressing tumor cell and T cell activation in the presence of BiAb were not affected in vitro. Additionally knocking out PD-1 enhanced killing kinetics and activation of E3 SAR and E3 SAR + CXCR6-GFP transduced T cells compared to corresponding mock electroporated T cells.

Conclusions Using the controllable and modular SAR – BiAb platform SAR T cell activation can be limited by stopping BiAb dosing if adverse events occur. In addition, SAR T cells can be redirected to an alternative tumor-associated antigen by exchanging the BiAb in the case of antigen escape. Here we present add-ons to this approach for increased tumor infiltration and resistance to immunosuppression. Since migration is enhanced upon co-transduction with chemokine receptors and target cell lysis is accelerated upon PD-1 knockout in vitro these two additional modifications seem very promising options to further improve tumor control in vivo.

Disclosure Information M. Schwerdtfeger: None. M. Benmebarek: None. F. Märkl: None. C.H. Karches: A. Employment (full or part-time); Significant; Daiichi Sankyo Deutschland GmbH. A. Öner: None. M. Geiger: A. Employment (full or part-time); Significant; Roche. B. Cadilha: None. S. Endres: None. V. Desiderio: None. C. Klein: A. Employment (full or part-time); Significant; Roche. S. Kobold: None.

P06.07 IN VIVO STUDIES OF IMMUNOMODULATORY A-CTLA-4 ANTIBODY IN A HUMANIZED MOUSE MODEL

C. Reitinger*, F. Nimmerjahn. University Erlangen, Erlangen, Germany

10.1136/jitc-2021-ITOC8.41

Background Recent findings in cancer immunotherapy have reinforced the hypothesis that the immune system is able to control most cancers. Immunomodulatory antibodies can enhance immune responses, having the potential to generate anti-cancer immunity.1-4

Materials and Methods Most current studies addressing this question are performed in murine mouse model systems or use in vitro culture systems, which do not reflect the human in vivo situation, potentially leading to results that cannot be fully translated into human cancer therapy. Therefore, it is necessary to establish a new mouse model, which allows the study of cancer immunotherapy in the context of a human immune system. We focused on the establishment of a humanized mouse model, in which different immunomodulatory antibodies can be tested in the presence of a human immune system.

Results First experiments concerning the suitability to test immunomodulatory antibodies in the humanized mouse model, revealed that effects of checkpoint-control antibody a-CTLA-4 were similar to the effects seen in patients of clinical studies. To analyse the anti-tumor activities of immunomodulatory antibodies in vivo we are establishing a human melanoma-like tumor model in humanized mice.

Conclusions This enables us to test the efficacy of immunomodulatory agonistic antibodies (such as CP-870,893) and checkpoint control antibodies (such as anti-CTLA-4) in eliminating a melanoma-like tumor. Furthermore, parameters like tumor infiltrating human cells and cytokine/chemokine production can be analysed.

REFERENCES

Disclosure Information C. Reitinger: None. F. Nimmerjahn: None.

P07 Cell therapy in haematologic diseases

P07.01 A MODULAR AND CONTROLLABLE T CELL THERAPY PLATFORM FOR AML

1M Benmebarek*, 1B Loureiro Cadilha, 1M Hermann, 1S Schmitt, 1S Lesch, 1S Stoiber, 2A Darwin, 2C Augsberger, 2B Brauchle, 1M Schwerdtfeger, 1A Gottschlich, 1Rataj, 3NC Fenn, 3C Klein, 5M Subklewe, 5S Endres, 5K Hopfer, 5S Kobold. 1Center of Integrated Protein Science Munich (CIPS-M) and Division of Clinical Pharmacology, Munich, Germany; 2Department of Medicine III, Klinikum der Universität München, LMU, Munich, Germany; 3Department of Medicine III, Klinikum der Universität München, Munich, Germany; 4Mucosal Immunology and Microbiota Lab, Humanitas Clinical and Research Center, Milan, Italy; 5Roche Innovation Center Zurich, Schlieren, Switzerland; 6Gene Center, LMU, Munich, Germany

10.1136/jitc-2021-ITOC8.42

Background Targeted immunotherapies have shown limited success in the context of acute myeloid leukemia (AML). The mutational landscape, heterogeneity attributed to this malignancy and toxicities associated with the targeting of myeloid lineage antigens, it has become apparent that a modular and controllable cell therapy approach with the potential to target multiple antigens is required. We propose a controlled ACT
approach, where T cells are equipped with synthetic agonistic receptors (SARs) that are selectively activated only in the presence of a target AML-associated antigen, and a cross-linking tandem single chain variable fragment (taFv) specific for both (SAR) T cell and tumour cell.

**Materials and Methods** A SAR composed of an extracellular EGFR\textsubscript{vIII}, trans- membrane CD28, and intracellular CD28 and CD3\textsubscript{z} domains was fused via overlap- extension PCR cloning. T cells were retrovirally transduced to stably express our SAR construct. SAR-specific taFvs that target AML-asso- ciated antigens were designed and expressed in Expi293FTM cells and purified by nickel affinity and size exclusion chrom- atography (SEC). We validated our approach in three human cancer models and patient-derived AML blasts expressing our AML-associated target antigens CD33 and CD123.

**Results** Anti-CD33-EGFR\textsubscript{vIII} and anti-CD123 EGFR\textsubscript{vIII} taFv, monovalently selective for our SAR, induced conditional antigen-dependent activation, proliferation and differentiation of SAR-T cells. Further, SAR T cells bridged to their target cells by taFv could form functional immunological synapses, resulting in efficient tumor cell lysis with specificity towards CD33-expressing AML cells. SAR-taFv combination could also mediate specific cytotoxicity against patient-derived AML blasts and leukemic stem cells whilst driving SAR T cell activation. In vivo, treatment with SAR-taFv combination could efficiently eradicate leukemia and enhance survival in an AML xenograft models. Furthermore, we could show selective activation of SAR T cells, as well as a controllable reversibility and modularity of said activation upon depletion of the T cell engaging molecule, both in vitro and in vivo.

**Conclusions** Here we apply the SAR-taFv platform in efforts to deliver specific and conditional activation of SAR-trans- duced T cells, and targeted tumor cell lysis. The modularity of our platform will allow for a multi-targeting ACT approach with the potential to translate the ACT successes of B cell malignancies to AML. With a lack of truly specific AML anti- gens, it is invaluable that this approach possesses an intrinsic safety switch via its taFv facet. Moreover, we are able to circumvent pan-T cell activation due to the specific targeting and activation of SAR T cells.