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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Functional Characterization and Optimization of Switchable Allogeneic Chimeric Antigen Receptor T Cells for Targeting CD19 and CD20 in B Cell Malignancies

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Chimeric antigen receptor (CAR) T cell therapy has demonstrated significant efficacy in B cell malignancies. However, despite clinically manageable on-target/off-tumor toxicity when targeting antigens like CD19 and BCMA, severe cytokine release and neurotoxicity remain a clinical challenge. Additionally, patients may require immunoglobulin replacement therapy, and antigen-loss is recognized as a cause of relapse (PMID: 30275569, 31798590, 36263838, 37051246 and 37122700).
To overcome these limitations, we developed a novel switchable CAR-T cell therapy that simultaneously targets both CD19 and CD20 in B cell malignancies. This innovative therapy is based on our reverse universal chimeric antigen receptor platform (RevCAR), a 2-component CAR-T technology (Figure 1; PMID: 34638268 and 32923149). The first component is a universal CAR-T cell, incorporating a CAR based on a short, non-immunogenic peptide motif derived from the human nuclear La/SSB autoantigen. The CAR itself does not recognize any human cell surface antigen. However, it is specifically targeted by an antibody-based binder in the second component, a soluble adaptor called targeting module (TM). This TM confers specificity against the desired cancer antigens of choice. Consequently, the availability of the TM effectively controls the activity of RevCAR-T cells.
We have recently reported preclinical development of an allogeneic RevCAR-T cell therapy for CD123-positive hematologic malignancies (AVC-201; Ehninger et al. 2023, AACR Abstract 4095; NCT05949125). To target CD123, which is also expressed on normal hematopoietic progenitor cells, a TM with a short half-life was selected (R-TM123), enabling a rapid switch-off of the RevCAR system by TM withdrawal to avoid acute toxicity and continued aplasia.
As CD19 and CD20 have a substantially more favorable safety profile and autologous conventional CAR-T cells and bispecific T cell engagers with respective target-specificities are approved in certain B cell malignancies, a half-life extended TM was developed to allow for dosing every 1-2 weeks. After an extensive screen for an optimized TM targeting CD19 and CD20, we screened multiple RevCAR variants differing in hinge, transmembrane and costimulatory domains for optimal anti-tumor potency using both preclinical *in vitro* and *in vivo* models. Profound efficacy and complete tumor control could be observed for optimized RevCAR constructs (Figure 2). While short-term *in vitro* cytotoxicity assays already gave a small hint towards *in vivo* efficacy, surprisingly, long-term *in vitro* rechallenge assay results did not correlate with *in vivo* efficacy. A summary of functional characterization data will be presented at the meeting with a focus on the lack of predictability of certain *in vitro* models and underlying hypotheses. Insights from these studies are now flowing into preclinical development of a novel switchable allogeneic CAR-T cell product candidate targeting CD19 and CD20 in B cell malignancies engineered to fully overcome graft-versus-host disease as well as graft rejection by host T and NK cells.

Disclosures Spehr: *AvenCell Europe GmbH:* Current Employment; *AvenCell Therapeutics, Inc.:* Current holder of *stock options* in a privately-held company. **Meyer:** *AvenCell Europe GmbH:* Current Employment; *AvenCell Therapeutics, Inc.:* Current holder of *stock options* in a privately-held company. **Loff:** *AvenCell Europe GmbH:* Current Employment; *AvenCell Therapeutics, Inc.:* Current holder of *stock options* in a privately-held company. **Langer:** *AvenCell Europe GmbH:* Current Employment; *AvenCell Therapeutics, Inc.:* Current holder of *stock options* in a privately-held company. **Langer:** *AvenCell Europe GmbH:* Current Employment; *AvenCell Therapeutics, Inc.:* Current holder of *stock options* in a privately-held company. **Boyerinas:** *AvenCell Therapeutics, Inc.:* Current Employment, Current holder of *stock options* in a privately-held company. **Le Mercier:** *AvenCell Therapeutics, Inc.:* Current Employment, Current holder of *stock options* in a privately-held company. **Lescarbeau:** *AvenCell Therapeutics, Inc.:* Current Employment, Current holder of *stock options* in a privately-held company. **Cartellieri:** *AvenCell Europe GmbH:* Current Employment; *AvenCell Therapeutics, Inc.:* Current holder of *stock options* in a privately-

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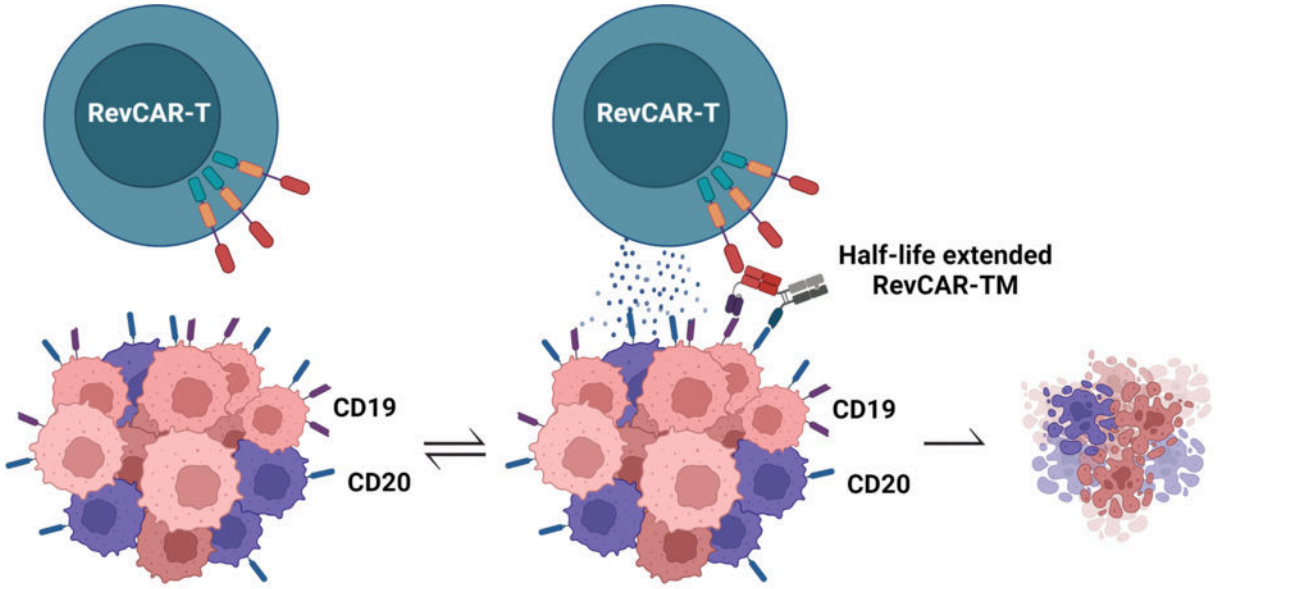

Figure 1

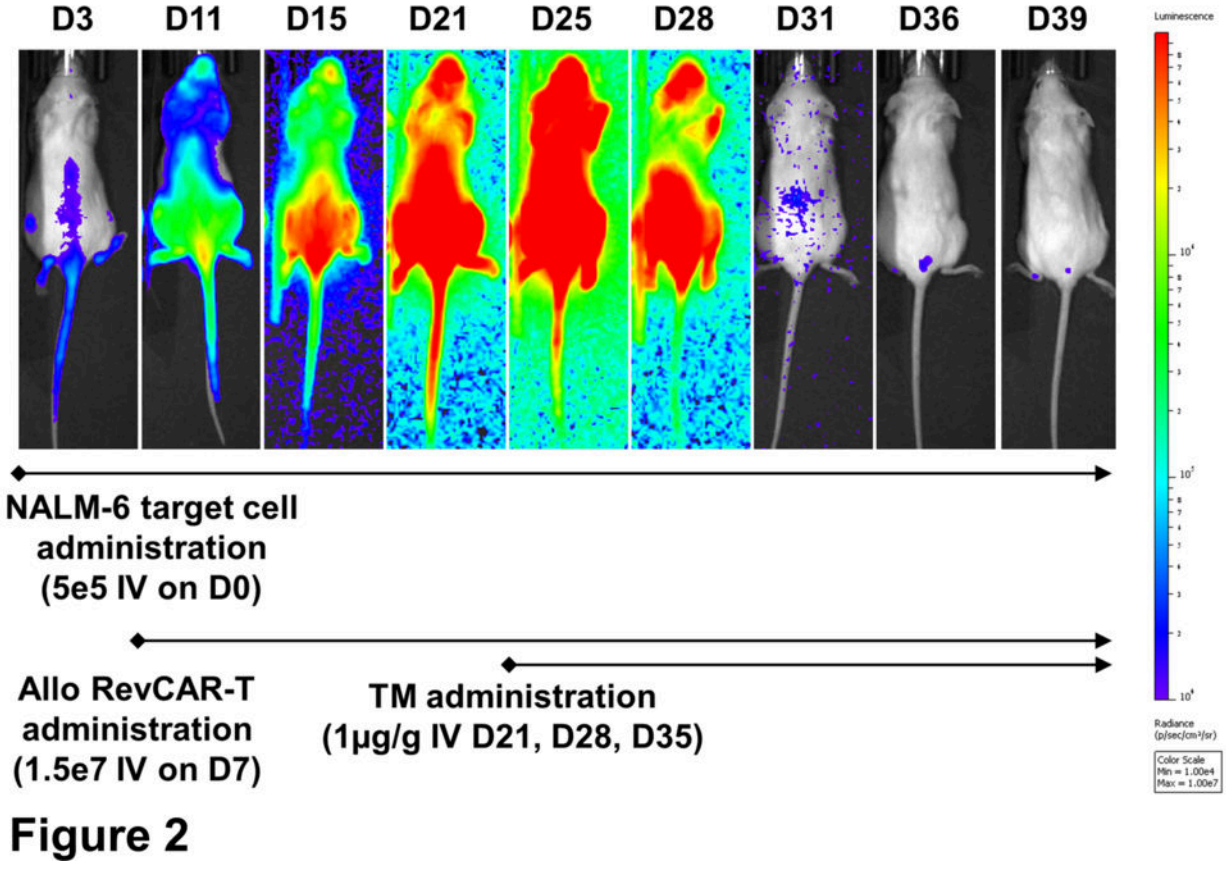

Figure 2

Figure 1

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