

## The 67th ASH Annual Meeting Abstracts

## POSTER

**711. CELL COLLECTION AND MANUFACTURING OF HSPCS, CAR-T CELLS, AND OTHER CELLULAR THERAPY PRODUCTS****Preclinical development of an optimized manufacturing, CRISPR-edited, fully non-viral 1XX-enhanced anti-BCMA CAR-T therapy for multiple myeloma**

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**Abstract Background:** B-cell maturation antigen (BCMA)-targeted chimeric antigen receptor T cell (CAR-T) therapies have transformed treatment for relapsed/refractory multiple myeloma (RRMM); however, durable remissions remain limited by poor CAR-T persistence, T-cell exhaustion, and manufacturing challenges. Currently approved BCMA CAR-T products rely on lentiviral or retroviral vectors, leading to random genomic integration, variable expression, and increased risk of insertional mutagenesis. Additionally, lentiviral supply shortages and high costs hinder global accessibility. Strategies that improve CAR-T efficacy, safety, and scalability are urgently needed.

**Aims:** We developed a novel, fully non-viral, CRISPR/Cas9-edited BCMA CAR-T therapy using precise TRAC locus integration and a 1XX-modified CD3 $\zeta$  signaling domain to enhance persistence and reduce exhaustion. The goal was to create a safer, more potent, and scalable next-generation CAR-T platform for multiple myeloma.

**Methods:** Human T cells were engineered using CRISPR/Cas9-mediated insertion of a BCMA CAR construct (bb2121 scFv, CD28 costimulatory domain, and 1XX CD3 $\zeta$ ) at the TRAC locus via single-stranded DNA (ssDNA) templates. This approach ensures uniform CAR expression, eliminates endogenous TCR expression to reduce GvHD risk in a mouse model, and minimizes tonic signaling. Manufacturing was performed under GMP-compatible conditions using the Maxcyte electroporation system and G-Rex expansion. In vitro cytotoxicity assays and in vivo efficacy studies were performed using NSG xenograft models (MM1.S and OPM2-luc). CAR-T performance was directly compared to FDA-approved BCMA CAR-T constructs (idecabtagene vicleucel and ciltacabtagene autoleucel). Off-target activity was assessed via GUIDE-seq and rhAmpSeq. Genomic stability and safety were evaluated by karyotyping, aCGH, ddPCR for translocations, and proliferation assays.

**Results:** TRAC-targeted BCMA CAR-T cells achieved high knock-in efficiency (37.7–72.7%) and >99% TCR knockout across manufacturing runs. In vitro cytotoxicity assays showed potent anti-myeloma activity, comparable to lentivirally transduced CARs, with robust killing at low effector-to-target ratios. In vivo, TRAC-integrated CD28-1XX CAR-T cells outperformed both wild-type CD3 $\zeta$  CD28 and 41BB constructs, achieving complete tumor clearance in MM1.S and OPM2 xenografts. Importantly, mice treated with CD28-1XX CAR-T cells remained tumor-free after multiple rechallenges, while all control groups relapsed. Overall survival was significantly prolonged, with no tumor-related deaths observed in 1XX-treated mice.

Direct comparison with idecabtagene vicleucel and ciltacabtagene autoleucel constructs demonstrated superior in vivo expansion (up to 1000-fold greater CAR-T: tumor cell ratios in bone marrow) and sustained tumor control with the TRAC-targeted 1XX CAR-T, even at reduced doses (50,000 CAR+ cells per mouse). In contrast, FDA-approved constructs showed tumor regression followed by relapse in all mice.

Off-target analysis revealed only two low-frequency (<0.2%) editing events in non-pathogenic intronic regions, with no evidence of clonal expansion or genomic instability. No balanced translocations were detected by ddPCR. Karyotyping and

aCGH confirmed genomic integrity. CAR-T products exhibited minimal residual Cas9 protein and no autonomous proliferation in cytokine-free conditions.

Optimization of cryopreservation media (10% human serum albumin) preserved post-thaw viability and cytotoxicity, supporting clinical readiness. In pharmacology and toxicology studies, CAR-T cells demonstrated dose-dependent tumor clearance without observed toxicities, weight loss, or GvHD-like events in mice. Biodistribution studies confirmed expected expansion in lymphoid organs without off-target tissue accumulation or damage.

**Conclusions:** These findings support the development of a fully non-viral, CRISPR-edited BCMA CAR-T therapy incorporating TRAC-targeted integration and a 1XX CD3 $\zeta$  design to enhance persistence, efficacy, and safety. This next-generation platform addresses key limitations of currently approved CAR-T products and enables scalable, cost-effective manufacturing without reliance on viral vectors. A first-in-human clinical trial is planned.

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