409 DEVELOPMENT OF *EX VIVO* PRECISION GENE ENGINEERED B CELL MEDICINES THAT PRODUCE HIGHLY ACTIVE AND SUSTAINED LEVELS OF TRANSGENIC ANTI-TUMOR BIOLOGICS

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Background Bispecific T Cell Engagers (BiTEs), consisting of an anti-CD3 scFv fused to an anti-tumor antigen scFv, are highly effective in the treatment of relapsed/refractory Acute Lymphoblastic Leukemia (ALL). However, the short half-life of BiTEs necessitates continuous intravenous administration at high doses for four-week increments. To overcome these pharmacokinetic shortcomings, we developed a method to engineer plasma cell precursors to continuously secrete transgenic biologics. Plasma cells were chosen for their high antibody production capacity (thousands of Ig molecules/cell/sec) and long-term survival (persisting for decades), making them a highly attractive cell-based platform for continuous biologic delivery.[1]

Methods To demonstrate proof-of-concept, we integrated a transgene coding for a bispecific non-Ig anti-CD3:CD19 scFv into the CCR5 safe-harbor locus of primary human B cells with CRISPR/Cas9, then initiated differentiation into plasma cells using a modified feeder-free culture system. Cells were characterized by flow cytometry, indel frequency, and droplet digital PCR. Edited cell supernatant was tested for therapeutic protein production with a timed ELISA and in vitro function with a cytolytic activity assay incorporating co-cultured T effector and CD19 expressing tumor cells. We also assessed *in vivo* anti-tumor activity of anti-CD3:CD19 scFv engineered B cell medicines (BeCMs) in NSG mice harboring a patient-derived xenograft (PDx). To avoid targeting of the BeCM by CD3+ cells, CD19 was knocked-out via a multiplexed CRISPR/Cas9 editing protocol. Mice were then inoculated with a luciferized B-ALL PDx line. Autologous T cells were delivered 24 hours and 72 hours following tumor transfer, and IVIS imaging was performed over the course of 17 days. A control GFP-engineered BeCM arm and PBS-dosed cohort were monitored in parallel, with n=6 mice per group.

Results Flow cytometric analysis confirmed robust differentiation of BeCMs along the plasma cell lineage. We observed >85% cutting efficiency and 40–50% targeted integration, which translated to secretion rates (0.6–0.8 μg BiTE/10^6 cells/day) that were sufficient for in vitro functional assay tumor cytolytic activity. Significant reduction in tumor burden (bioluminescent flux, area under the curve) was observed *in vivo* in the anti-CD3:CD19 scFv cohort compared to the controls, which was in concordance with heightened *in vivo* T cell activation. The ~1000 pg/mL BiTE detected in mouse plasma demonstrates that BeCM-derived biologics can meet or even exceed the steady-state plasma concentrations achieved with clinically relevant doses.[2]

Conclusions These findings underscore the clinical potential of BeCMs as an emerging platform for sustained delivery of anti-tumor biologics.

REFERENCES

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Ethics Approval Deidentified human PBMCs were acquired under informed consent from the Fred Hutch Specimen Processing and Research Cell Bank (protocol #3942).

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