Development of an *ex vivo* gene engineered B cell medicine platform with precision, modularity, and broad therapeutic utility

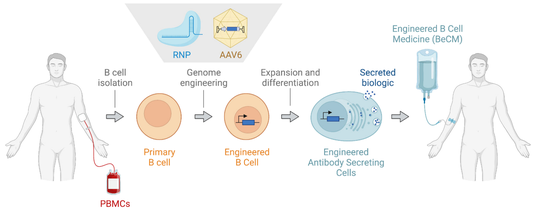
Hanlan Liu, Tim Mullen, Swati Singh, Jasmine Edelstein, Caroline Bullock, Sean Keegan, Sarah Leach, Troy Patterson, Kate Molloy, Amy Lundberg, Michael Leiken, Ishara Datta, Wayne Bainter, Sakshisingh Thakur, Charuta Yadav, Claudia Fiorini, Sean Arlauckas, Anja Hohmann, Adam Lazorchak, and Richard A Morgan

Be Biopharma, One Kendall Square, Cambridge, MA 02139

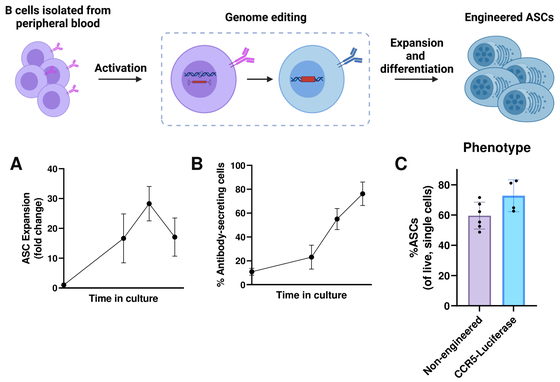
Introduction

Ex vivo gene and cell therapies have produced transformative medicines to treat previously intractable diseases. Still, there are barriers to the broad adoption of these efficacious therapies as current platforms lack high biosynthetic capacity, durability, and cannot be administered as an off-the-shelf medicine without conditioning. Terminally differentiated human plasma cells derived from genetically engineered B cells (termed B Cell Medicines, BeCMs), offer natural longevity (persisting up to decades), capacity for high levels of protein secretion (up to 10,000 Ig molecules/cell/sec), and naturally allogeneic properties, which make them an attractive platform for the sustained supply of biologics, where continuous dosing is required to achieve therapeutic benefit. Herein we describe a modular and scalable platform for the generation of BeCMs capable of stable expression and continuous secretion of biologically active therapeutic cargos. BeCMs are produced via CRISPR-Cas9 based homology-directed repair (HDR)-mediated transgene insertion followed by *ex vivo* differentiation into long-lived plasma cells.

Engineered B Cell Medicine (BeCM)



*Ex Vivo C*ell Culture System with Precision Engineering, Expansion and Differentiation



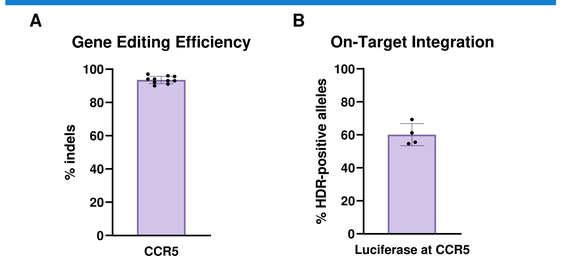
B cells engineered as described above were cultured according to a proprietary process to promote differentiation into antibody-secreting cells (ASCs). Quantification of **(A)** ASC fold change and **(B)** percentage of ASCs over the time course of the culture process (n=3 healthy blood donors). **(C)** Quantification of the percentage of ASCs from non-engineered cells (n=6 healthy blood donors) and cells engineered with a luciferase transgene inserted at CCR5 (n=4 healthy blood donors) at the end of the culture process.

References

1. Hung KL, Meitlis I, Hale M, Chen CY, Singh S, Jackson SW, Miao CH, Khan IF, Rawlings DJ, James RG. Engineering Protein-Secreting Plasma Cells by Homology-Directed Repair in Primary Human B Cells. Mol Ther. 2018 Feb 7;26(2):456-467.
2. Cheng, R.YH., Hung, KL, Zhang, T, Stoffers, CM, Ott, AR, Suchland, ER, Camp, ND, Khan, IF, Singh, S, Yang, Y-J, Rawlings, DJ, James RG. Ex vivo Engineered Human Plasma Cells Exhibit Robust Protein Secretion and Long-Term Engraftment *In Vivo*. Nat Commun. 13, 6110 (2022). https://doi.org/10.1038/s41467-022-33787-8.

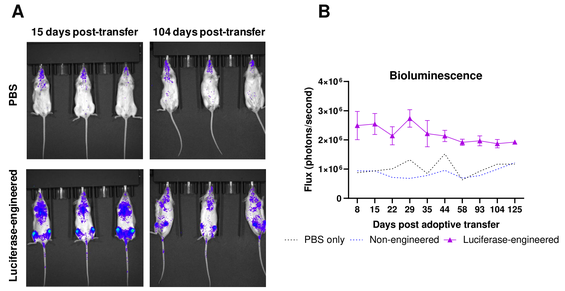
Diagrams created with BioRender.com

Highly Efficient B Cell Engineering Platform



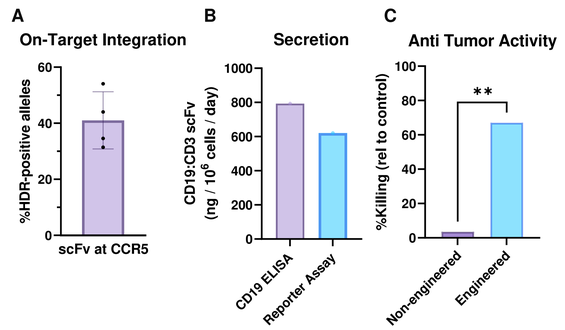
Human B cells were engineered using CRISPR-Cas9 system. Cas9 ribonucleoprotein (RNP) complexed with a guide RNA targeting the CCR5 locus was delivered to B cells via electroporation to introduce a DNA double-strand break. Transduction with a recombinant AAV6 vector was used to deliver a repair template encoding a luciferase expression cassette for HDR-mediated insertion at the on-target site. Quantification of **(A)** on-target indels (n=10 healthy blood donors) and **(B)** on-target integration frequency measured by droplet digital PCR (n=4 healthy blood donors).

Engineered B Cells Engraft in a NOG-hIL6 Mouse Model and Persist for >100 days



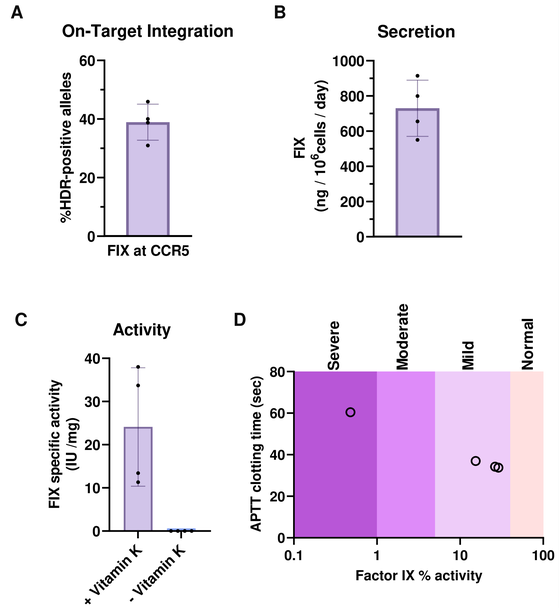
B cells engineered as described above were transferred into NOG human IL-6 (NOG-hIL6) transgenic mice. **(A)** Representative image of mice injected with PBS or Luciferase-engineered B cells 15 and 104 days post-adoptive transfer. **(B)** Engraftment and persistence were tracked via bioluminescence imaging across 125 days.

Anti-CD19/CD3 scFv for Acute Lymphocytic Leukemia (ALL)

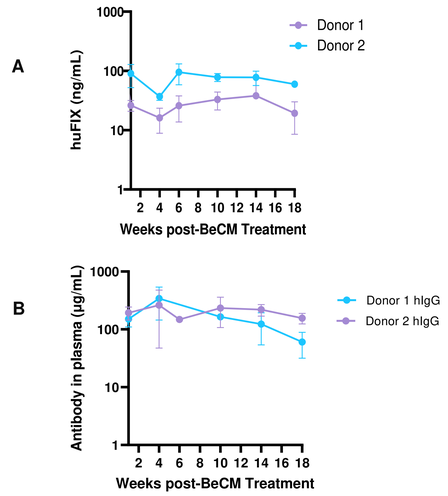


B cells were engineered to secrete an anti-CD19/CD3 single chain variable fragment (scFv), then analyzed for **(A)** on-target integration efficiency by droplet digital PCR. For one representative donor, B cells were incubated in fresh media for 2 hours to generate supernatant for quantification of **(B)** secretion rate by CD19 sandwich ELISA and Promega Jurkat Reporter. To assess **(C)** anti-tumor activity, supernatant was added to a co-culture of human primary CD8⁺ T and GFP⁺ Raji cancer cells. After a 72-hour incubation, Raji cell death was quantified by flow cytometry.

FIX for Hemophilia B

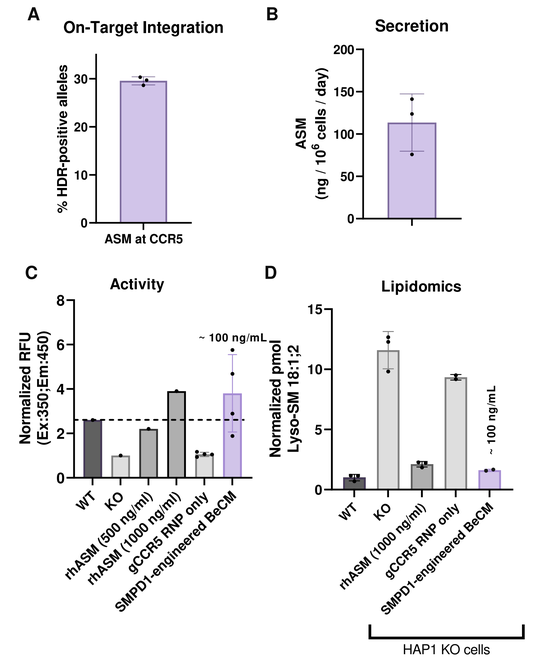


Healthy human B cells were engineered using Cas9 ribonucleoprotein (RNP) complexed with a guide targeting the CCR5 locus, delivered via electroporation followed by recombinant AAV6 transduction, to deliver repair template encoding *F9* expression cassette for HDR-mediated insertion. **(A)** On-target integration frequency measured by droplet digital PCR (n=4 healthy donors). **(B)** FIX secretion from engineered B cells was measured by ELISA (n=4 healthy donors). **(C)** To determine the FIX activity, engineered B cells were cultured with Vitamin K for 24 hr. Chromogenic activity assays were used to quantify the enzymatic activity of secreted FIX (n=4 healthy donors). **(D)** FIX was purified from engineered B cells cultured in the presence of Vitamin K (n=3 healthy donors) and in the absence of Vitamin K (n=1 healthy donor) and clotting activity was determined by activated partial thromboplastin time (aPPT) assay.

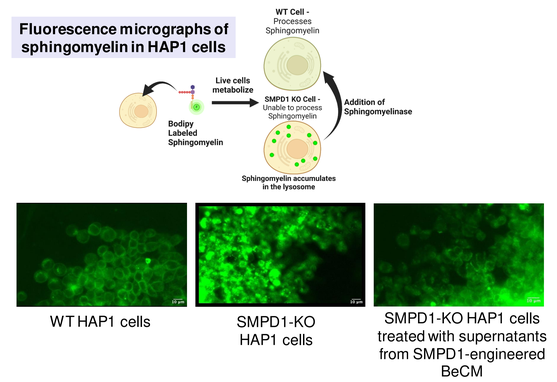


Engraftment and persistence of FIX engineered B cell in NOG-hIL6 mouse model. B cells engineered with construct A were transferred into NOG-hIL6 mice. Plasma was collected at different time interval post a single dose intravenous (IV) administration; level of hFIX, hIgG and hIgM was determined by ELISA. **(A)** hFIX, **(B)** hIgG and hIgM in mouse plasma post a single dose IV administration.

Acid Sphingomyelinase for Niemann-Pick Disease



B cells were engineered to secrete Acid Sphingomyelinase (ASM). **(A)** SMPD1-encoding constructs showed >30% HDR. **(B)** Human ASM secretion (ng/1e6 cells/24hr) determined by ELISA. **(C)** Activity assay was used to quantify ASM secreted from BeCM. **(D)** Lipidomic analysis using LC-MS to quantify lyso-sphingomyelin (18:1;2) in normal (WT) or SMPD1 knockout (KO) HAP1 cells treated with supernatants from SMPD1-engineered BeCM.



Conclusions

- We demonstrated the ability to transform the power of B cells into a platform for the advancement of engineered B cell medicines (BeCMs).
- BeCMs were able to produce highly divergent and functional biologics such as Lysosomal Storage Disease (LSD) enzymes, serum proteases, and anti-tumor bispecific engagers.
- BeCMs showed rapid bone-marrow-homing and exhibited durable engraftment (>100 days).
- BeCM platform has demonstrated therapeutic versatility and modularity.
- BeCMs have the potential for broad and meaningful therapeutic utility in rare diseases, cancer, and beyond.