



JANUARY 22 - 25, 2024 | SANTA FE, NEW MEXICO

## KEYSTONE SYMPOSIA: EMERGING CELLULAR THERAPIES

Joint Meeting with [Stem Cells and Regeneration](#)

Organizers: Dan Kaufman, Hans-Peter Kiem, and Sonja Schrepfer

# Development of an *Ex Vivo* Precision Gene Engineered B Cell Medicine Platform that Produces Active and Sustained Levels of Therapeutic Proteins with Broad Utility

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Hanlan Liu, Ph.D., MBA  
SVP, Pipeline and Nonclinical Development  
Be Biopharma

### Poster:

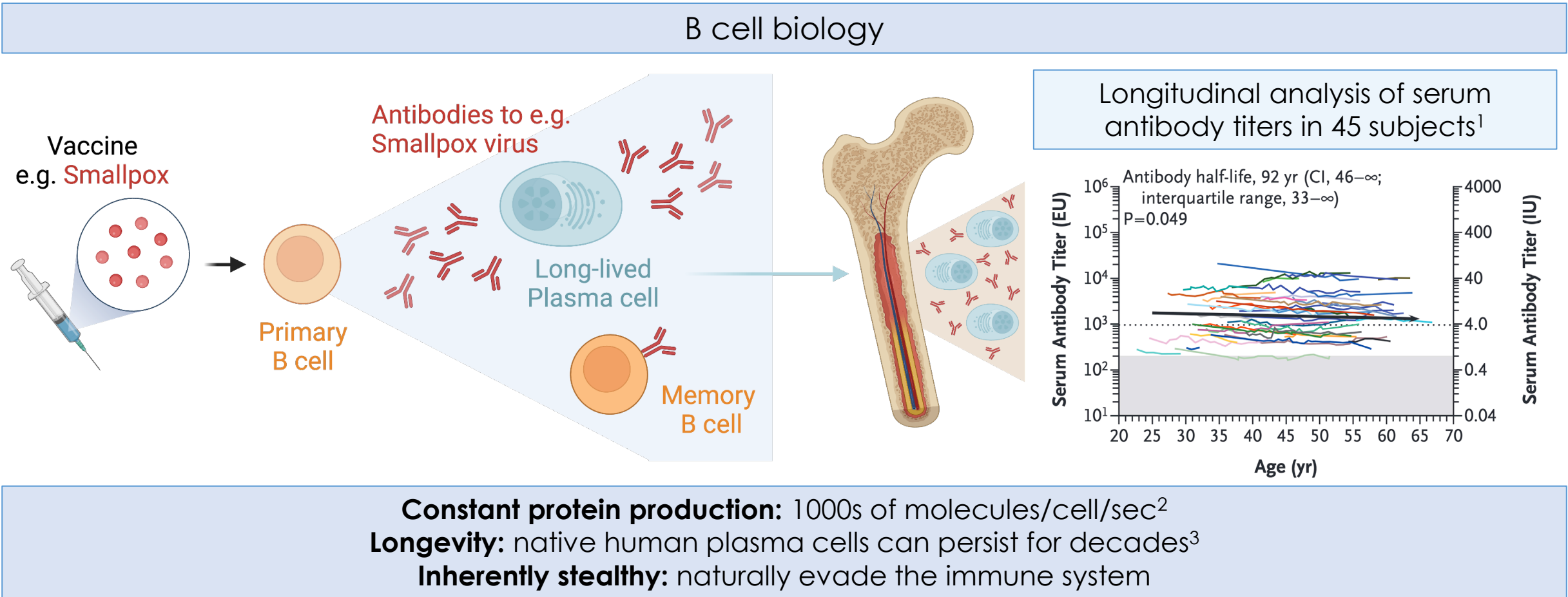
Hanlan Liu<sup>1</sup>, David J Young<sup>2</sup>, Swati Singh<sup>1</sup>, Timothy Mullen<sup>1</sup>, Caroline Bullock<sup>1</sup>, Sean Keegan<sup>1</sup>, Abigail Edwards<sup>1</sup>, Jasmine Edelstein<sup>1</sup>, Ella Liberzon<sup>1</sup>, Troy Patterson<sup>1</sup>, Ishara Datta<sup>1</sup>, Amy Lundberg<sup>1</sup>, Sakshisingh Thakur<sup>1</sup>, Charuta Yadav<sup>1</sup>, Shamael Dastagir<sup>1</sup>, Lily Li<sup>1</sup>, Sarah Leach<sup>1</sup>, Sogun Hong<sup>2</sup>, Noriko Sato<sup>3</sup>, Tyler F Hill<sup>4</sup>, Katherine Molloy<sup>1</sup>, Michael Leiken<sup>1</sup>, Wayne Bainter<sup>1</sup>, Megan L Brophy<sup>1</sup>, Madison Clements<sup>1</sup>, Shalini Chilakala<sup>1</sup>, Anja Hohmann<sup>1</sup>, Adam S Lazorchak<sup>1</sup>, Sean Arlauckas<sup>1</sup>, Monika Musial-Siwiek<sup>1</sup>, Chris Scull<sup>1</sup>, David J Rawlings<sup>4</sup>, Richard G James<sup>4</sup>, Cynthia E Dunbar<sup>2</sup>, and Richard A Morgan<sup>1</sup>

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# Disclosures

- Employee is the stock owner at Be Biopharma

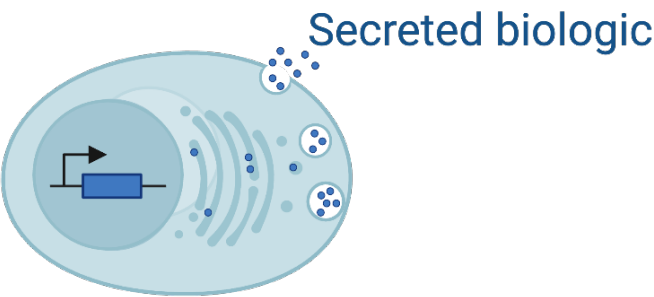
# B Cell Biology: Natural Sustained Protein Production and Bone Marrow Engraftment Without Conditioning



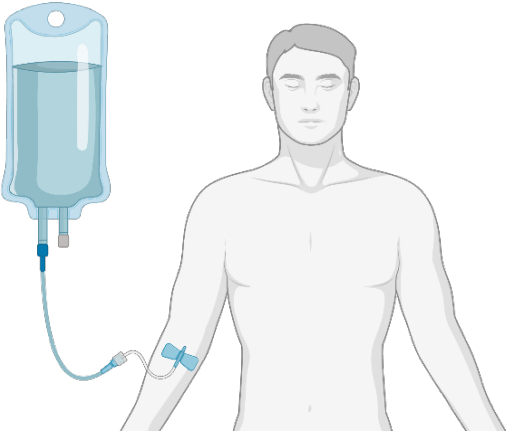
1) Amanna, Carlson, and Slifka (2007) *NEJM*  
2) Hibi and Dosch (1986) *Eur J Immunol*; Eyer et al (2017) *Nat Biotech*  
3) Landsverk et al (2017) *J Exp Med*

# BCMs Are Uniquely Suited for Sustained Supply of Biologics

## Engineered B Cell Medicines (BCMs)



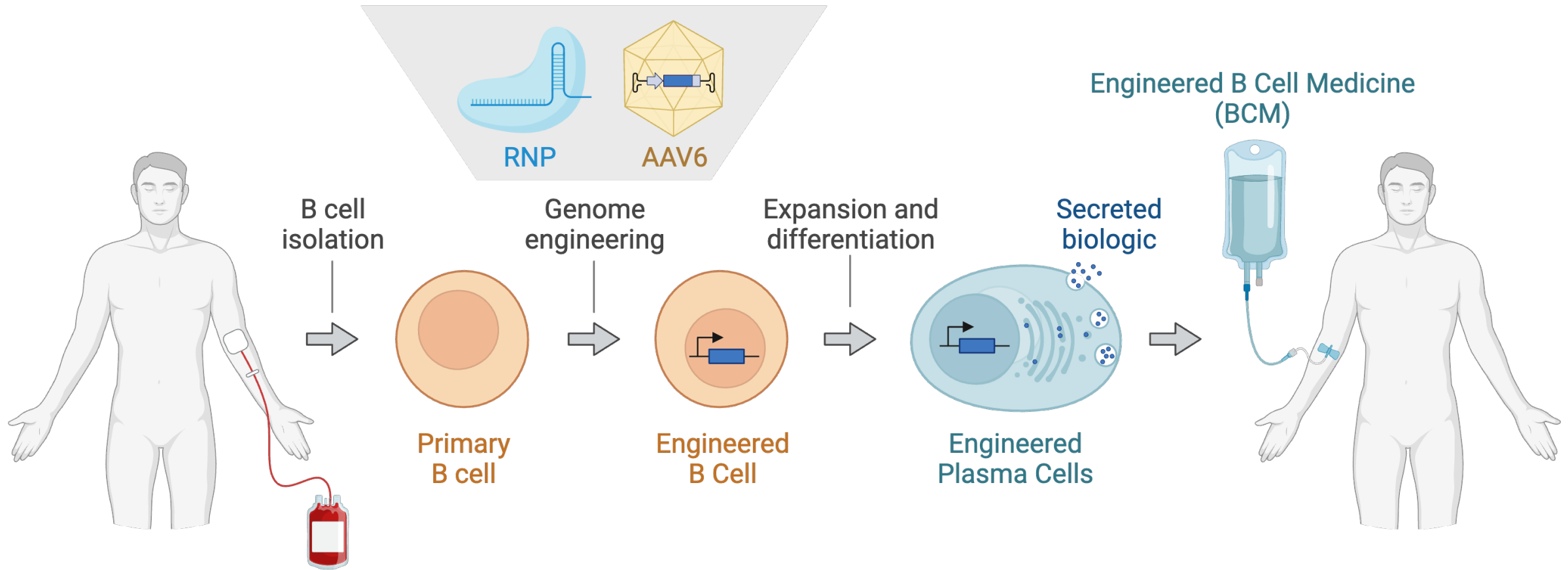
Engineered B Cell Medicine (BCM)



<b>Favorable attributes</b>	Capable of making versatile biologics Stable protein expression Continuous secretion Durable	Engraftment without pre-conditioning Long-term persistence Redosable Inherently immunologically stealthy
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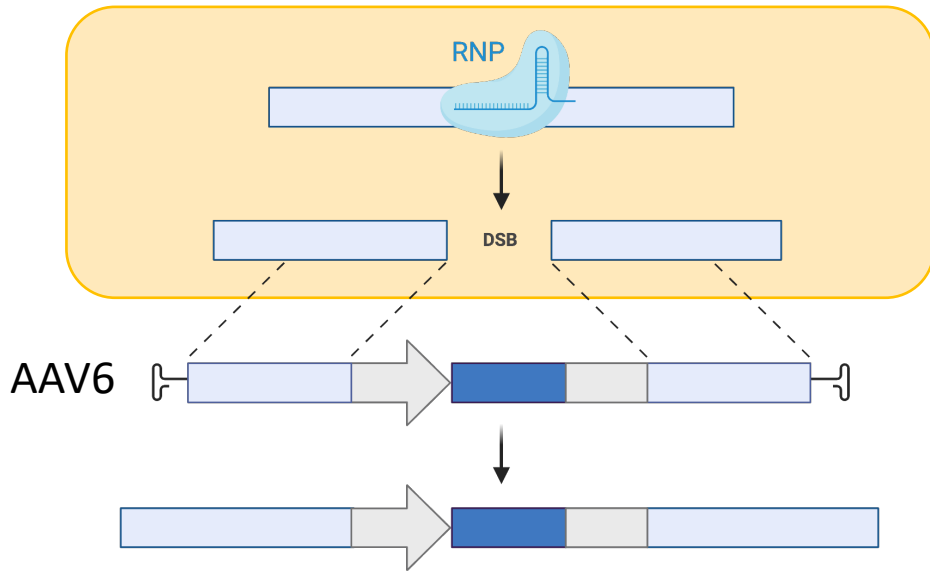
**The challenge:** culturing, engineering, and differentiating B cells.

# BCMs Are Engineered and Differentiated *Ex Vivo* to Serve as Protein Factories in the Body Upon Infusion



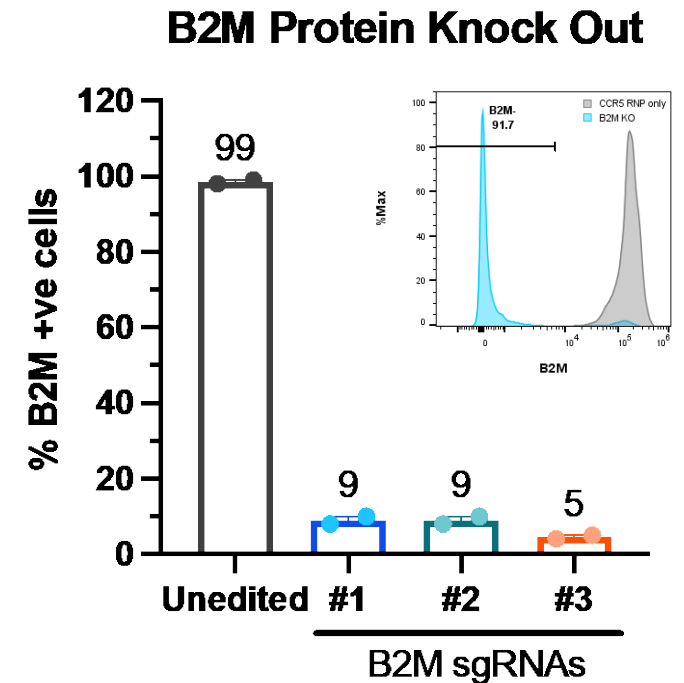
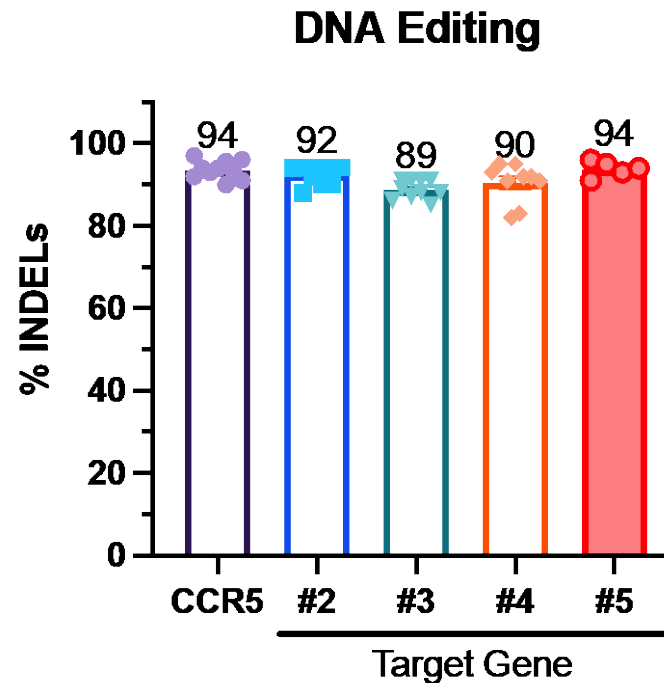
# >90% Gene Editing Efficiency in Primary B Cells

## Optimization of DNA editing and gene **KNOCK OUT**



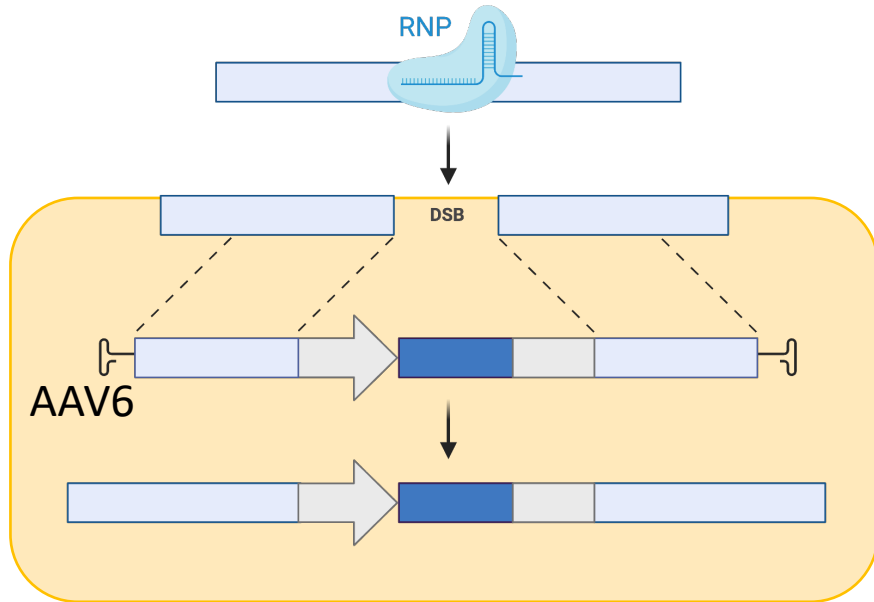
### Optimization parameters

Electroporator & electroporation code  
Guide:Cas9 ratio  
RNP concentration  
Cell concentration during electroporation  
Day of electroporation



# Up to 60% Transgene insertion in Primary B Cells

## Optimization of transgene **KNOCK IN**



### Optimization parameters

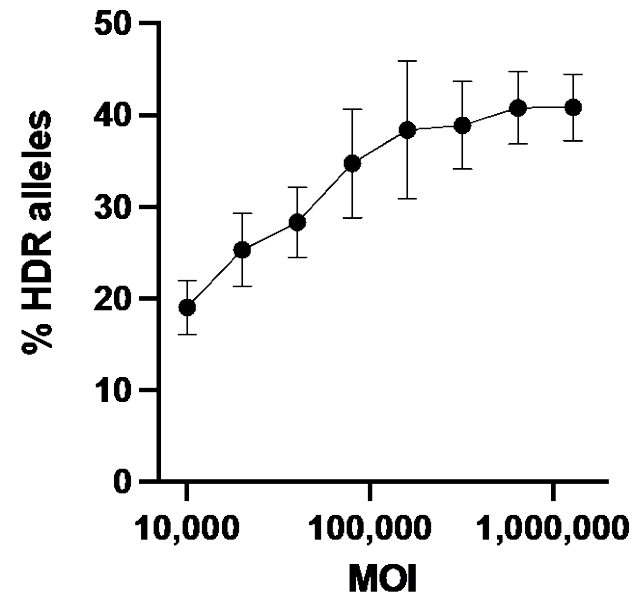
AAV MOI

Timing and duration of AAV transduction

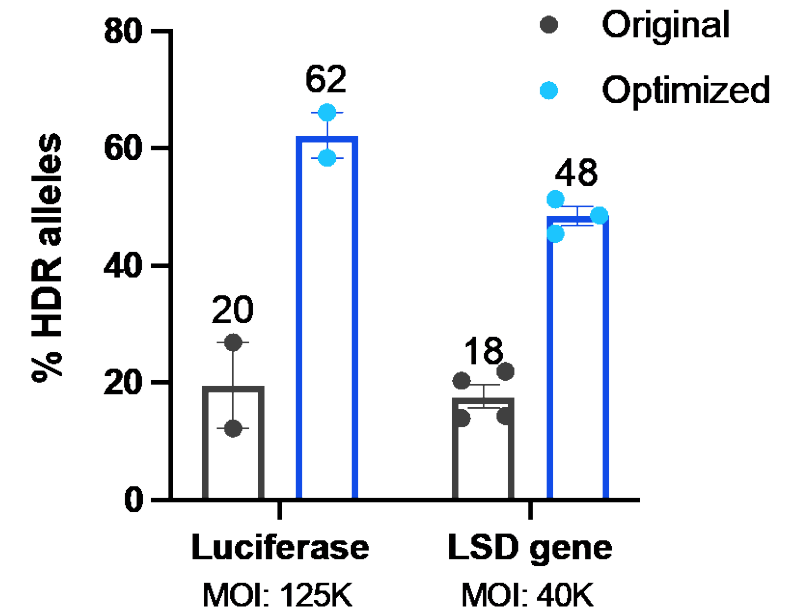
Transduction volume

Media composition

### AAV Titration

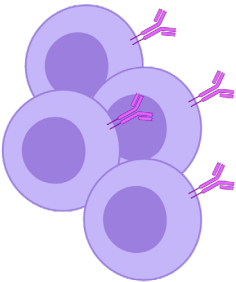


### Transgene insertion at CCR5



# Efficient Production of Engineered BCMs in Less Than Two Weeks\*

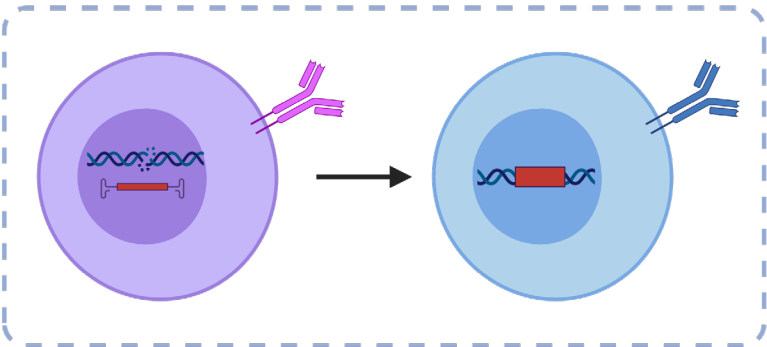
B cells isolated from peripheral blood



Activation



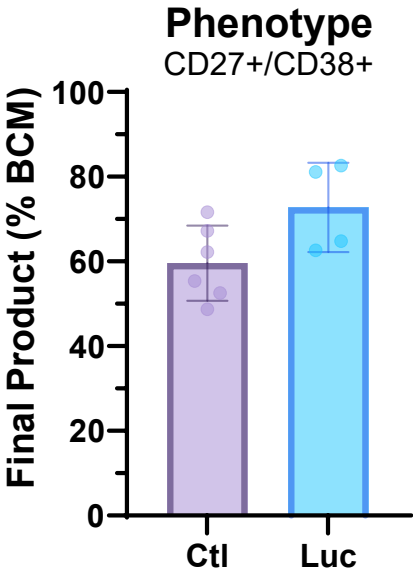
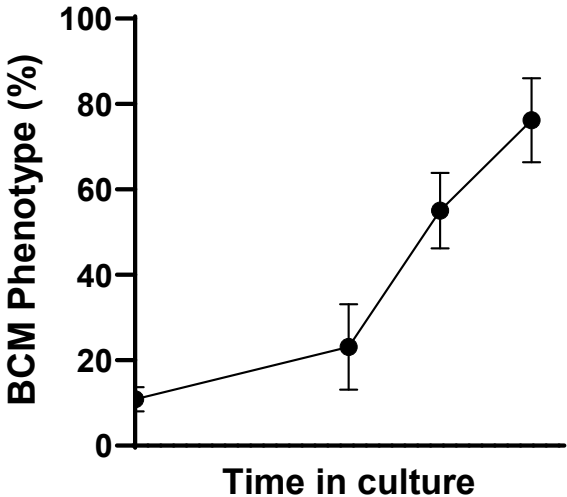
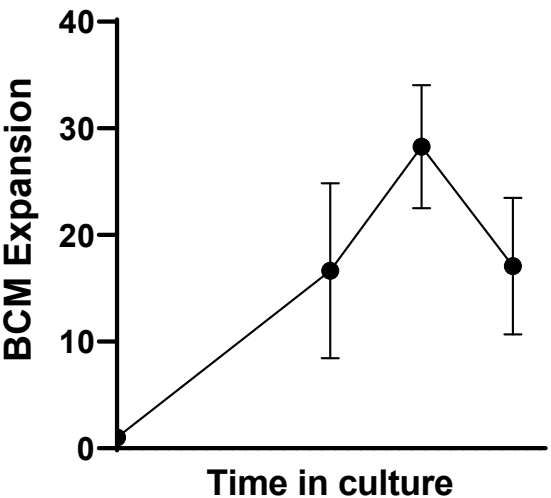
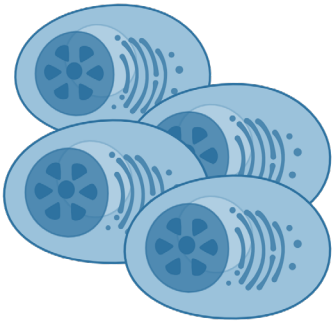
Genome editing



Expansion and differentiation



Engineered BCM

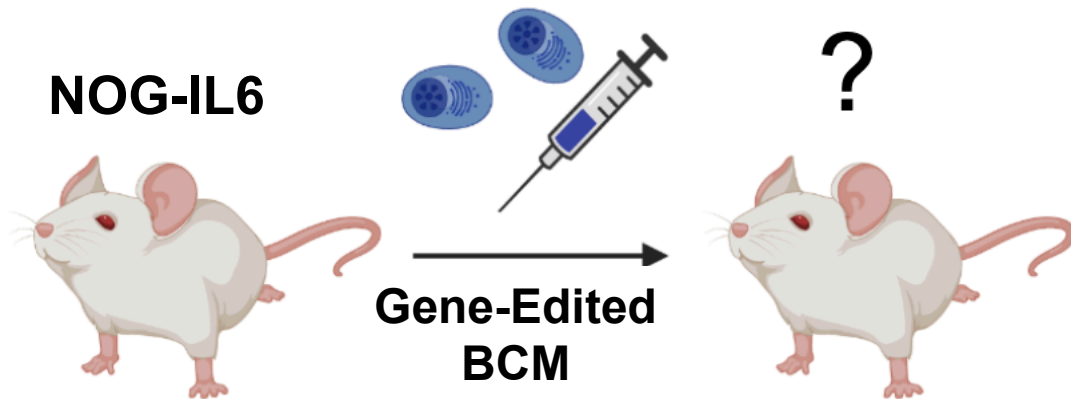
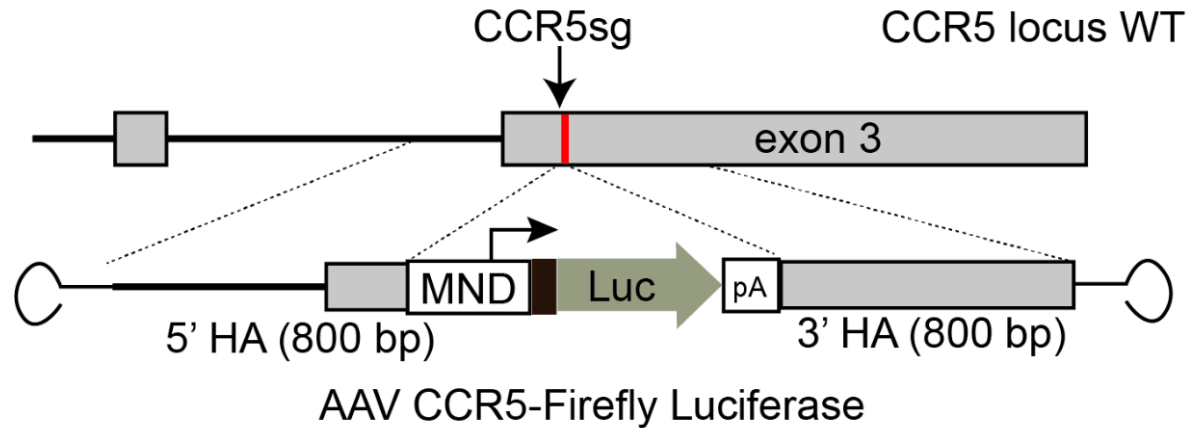


\*Nat Commun 2022 Oct 16;13(1):6110. doi: 10.1038/s41467-022-33787-8

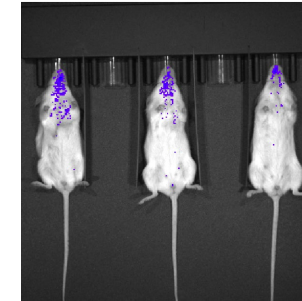


# Demonstration of Long-term Engraftment of Engineered BCM in Mice, >100 days

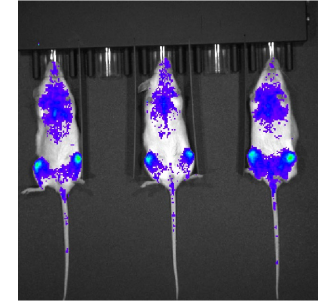
## Engineering strategy



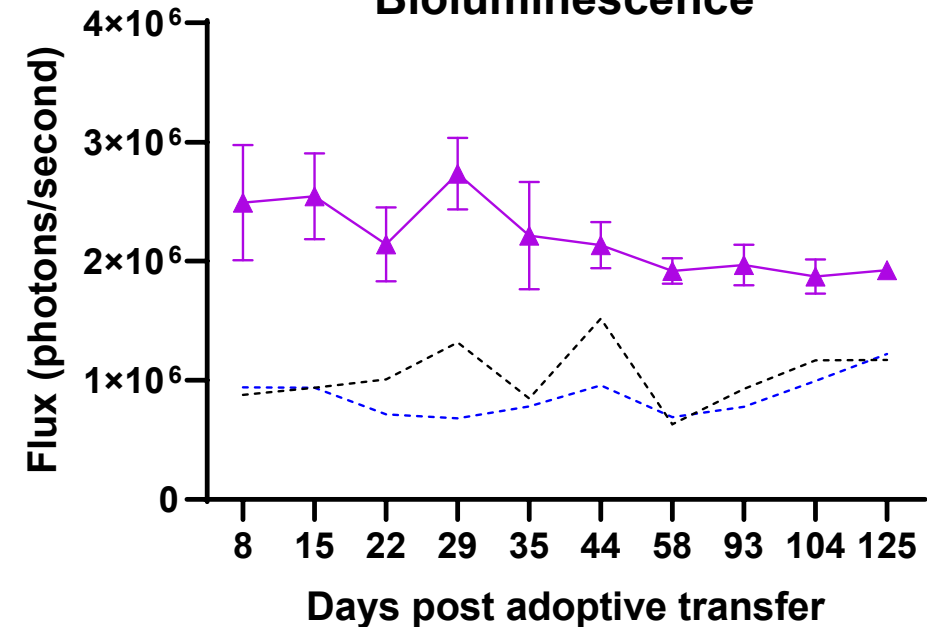
PBS



Luc-BCM



## Bioluminescence



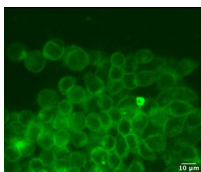
----- PBS only    ..... Non-engineered    ▲ Luciferase-engineered

# Activity of Therapeutic Proteins from Engineered BCM with Broad Utility in Rare Diseases and Cancer

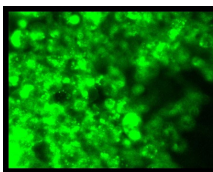
## Engineered BCM for Niemann Pick B Disease

SMPD1-KO HAP1 cells

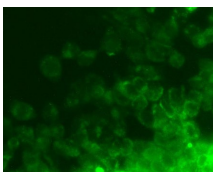
(+) WT HAP1 cells



(-) SMPD1-KO HAP1 cells



BCM + SMPD1-KO HAP1 cells



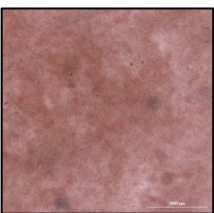
Bodipy labelled sphingomyelin

ASM BCM demonstrated *in vitro* phenotypic correction by reducing sphingomyelin in SMPD1-KO HAP1 cells

## Engineered BCM for Hypophosphatasia

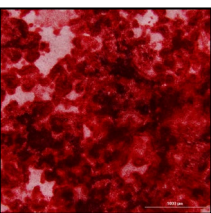
MC3T3 cells under Osteogenic Conditions

(-) Control



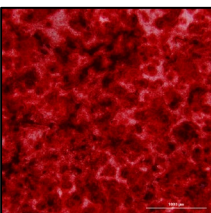
Treatment with PPI inhibits mineralization

(+) Control



No PPI demonstrate robust mineralization

BCM HPP

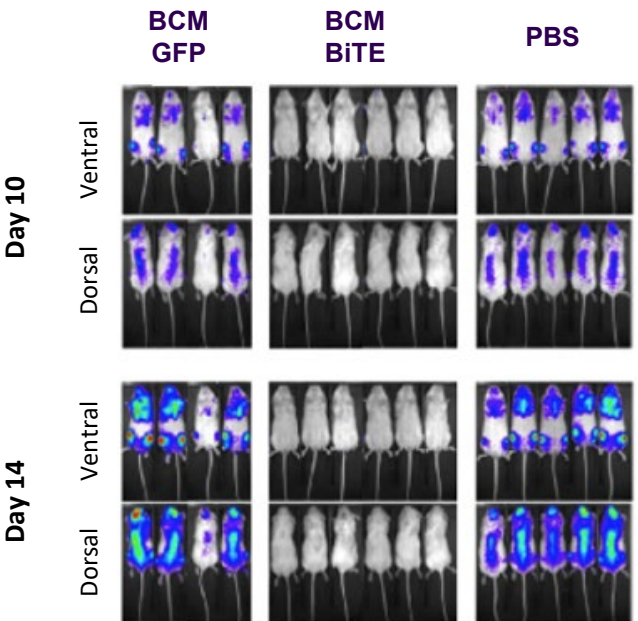


Treatment with PPI and BCM demonstrates robust mineralization

ALP BCM demonstrates ability to abolish PPI-induced mineralization defect

## Engineered BCM BiTE™ (CD19 x CD3) for ALL\*

PDX Model of ALL

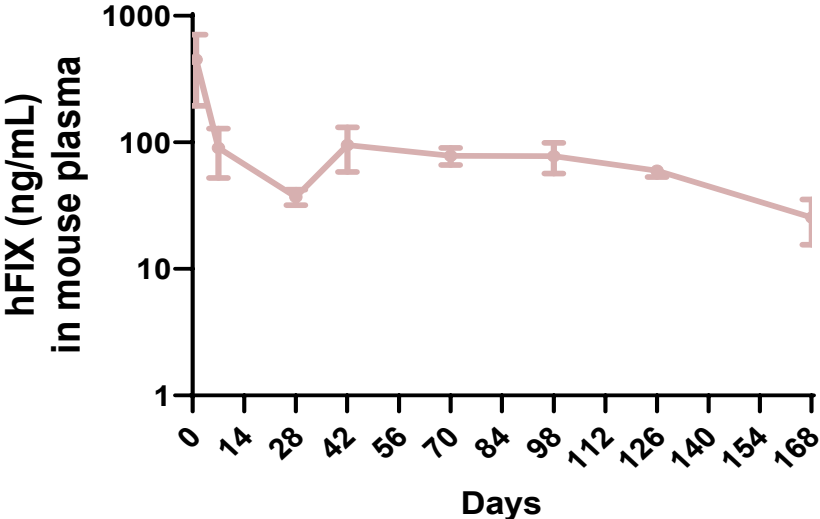


\*In collaboration with Seattle Children's Research Institute, David J Rawlings & Richard G James

Reference: Edelstein et al., Development of ex vivo precision gene engineered B cell medicines that produce highly active and sustained levels of transgenic anti-tumor biologics, SITC 38th Annual Meeting, November 1-5, 2023

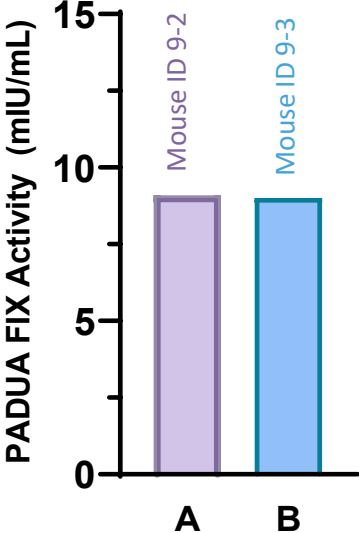
# BE-101, Be Bio's 1<sup>st</sup> development BCM program, Produces Active and Sustained Levels of FIX for the Treatment of Hemophilia B

Sustained hFIX plasma level for at least 168 days



n = 3 mice per time point dosed with BCMs from a single donor

Padua FIX activity using immune-capture chromogenic assay



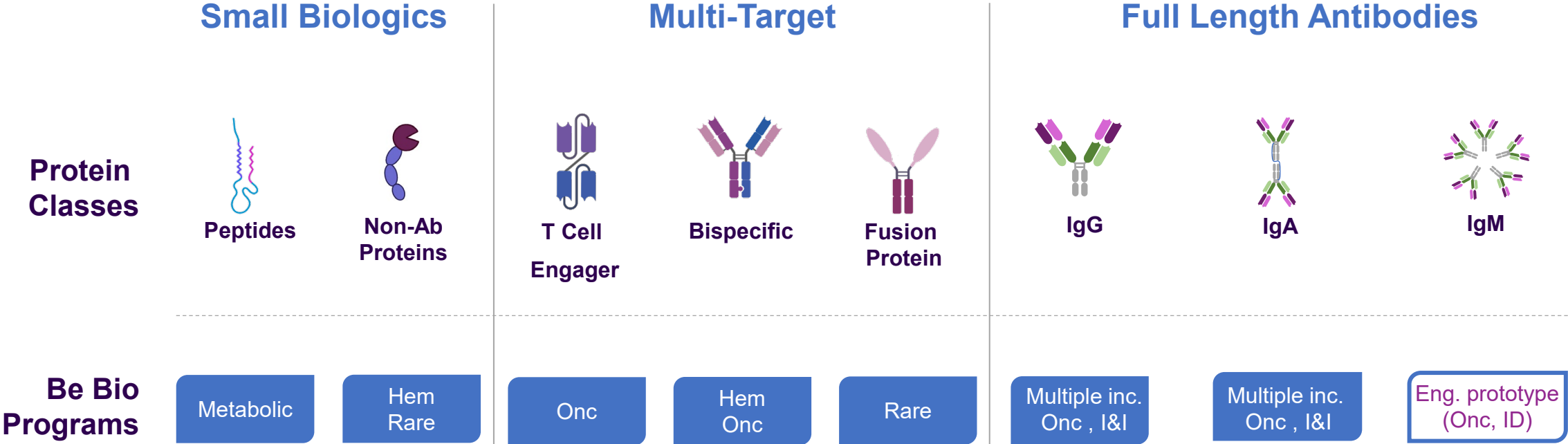
2 independent measurements at days 126 (A) and pooled sample from d98/d126 (B)



BE-101

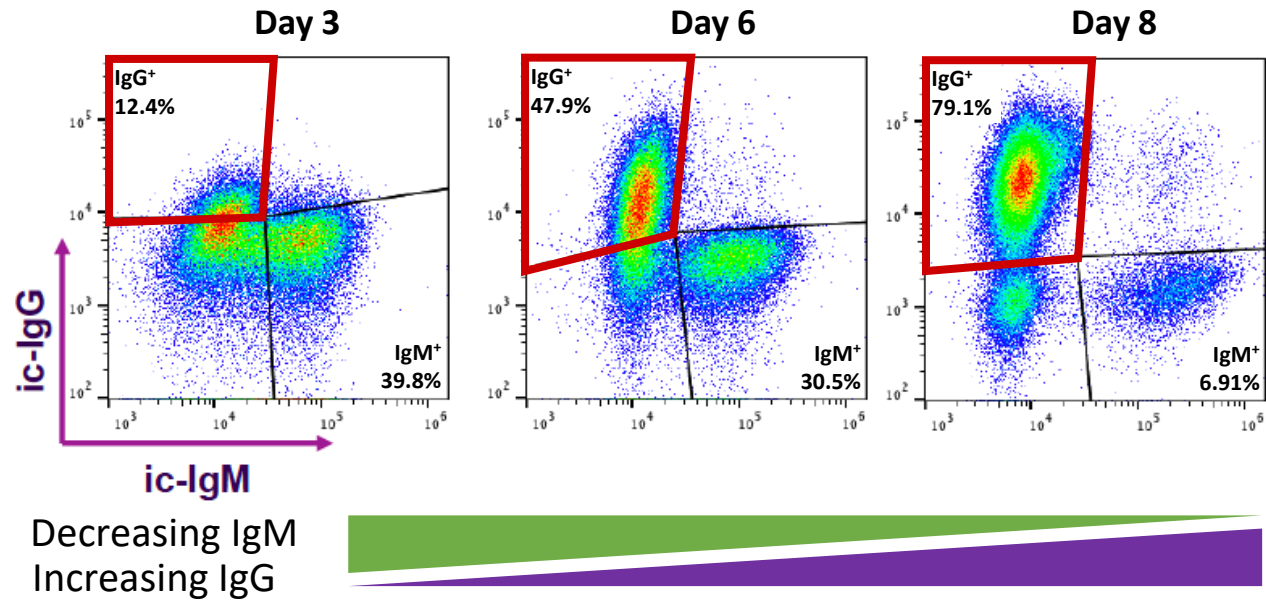
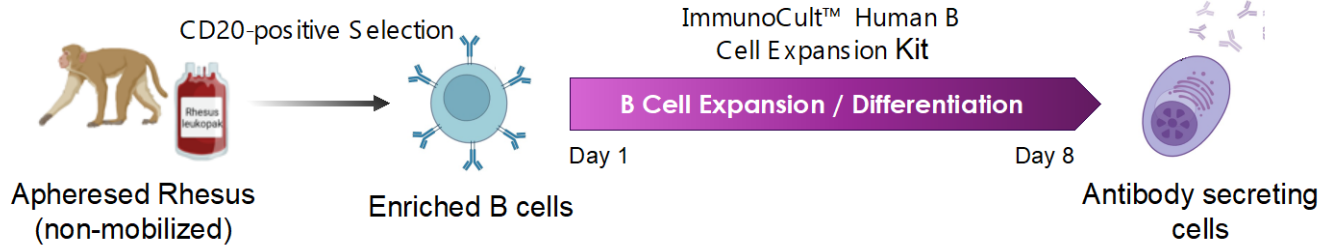
A Pre-IND meeting has been completed and a robust package of preclinical studies is nearing completion in anticipation of a **first-in-human** clinical trial in **2024** for people with moderately severe to severe hemophilia B.

# Versatile Plasma Cell Biosynthetic Capacity: BCMs Created Across Protein Classes and Applications

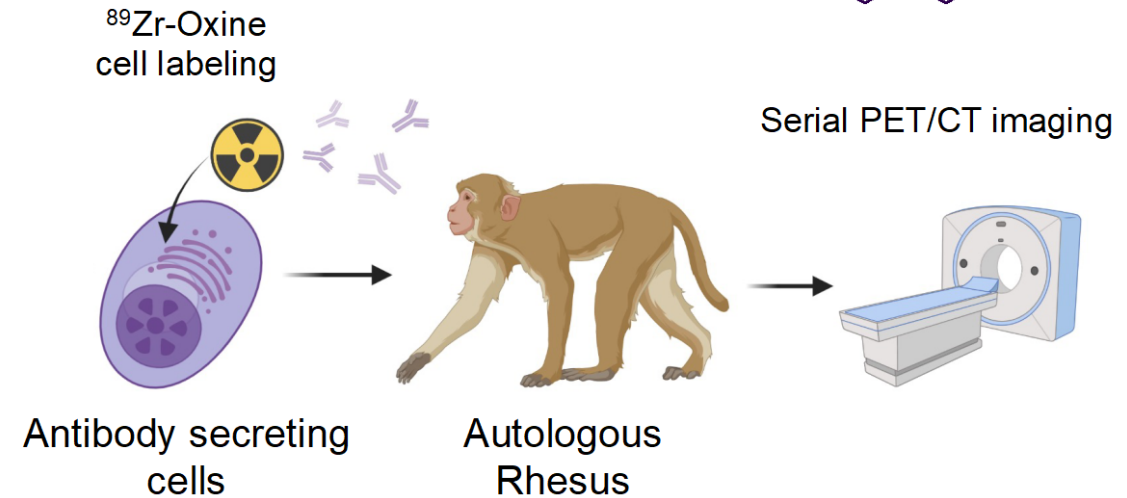
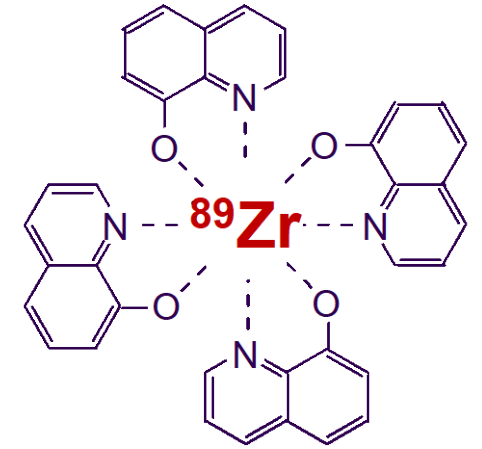


# Fundamental Validation: Engraftment without preconditioning demonstrated using NHP BCMs\*

## Cell Culture Process:

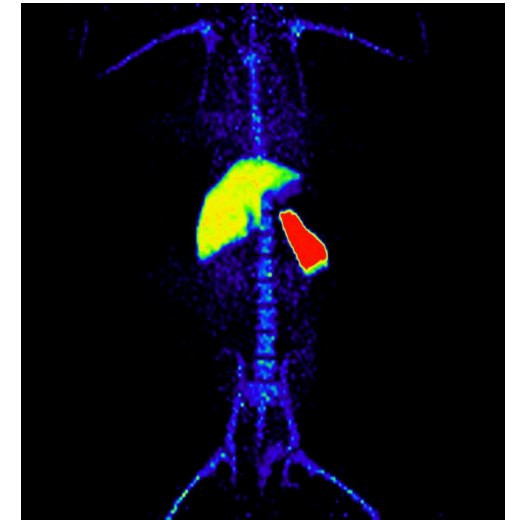
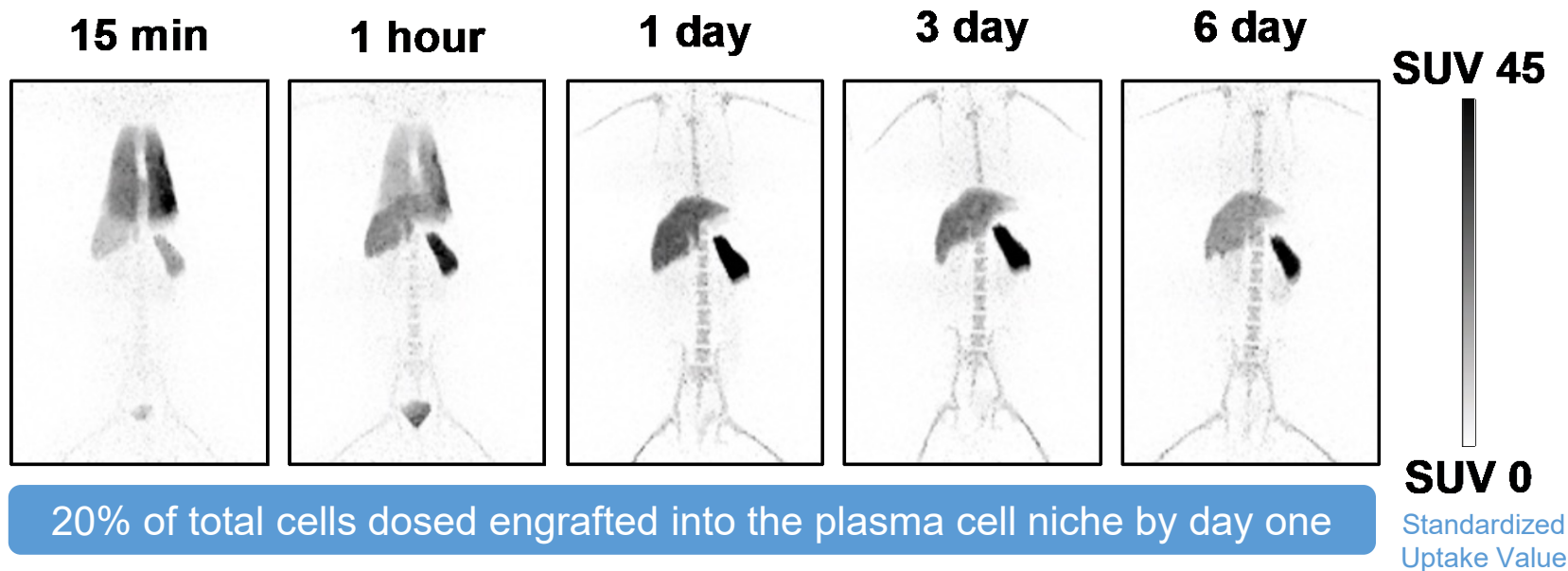


## Zirconium-89-oxine $[^{89}\text{Zr}](\text{oxinate})_4$



# Imaging of *Ex Vivo* Expanded NHP Plasma Cells

## $^{89}\text{Zr}$ Labeled Rhesus Plasma Cells



# Conclusion

Transformed the power of B cells into a platform for the advancement of B cell-based medicines

Demonstrated the versatility and modularity of BCM by successful production of highly divergent biologics

Demonstrated the potential of BCM with broad and meaningful therapeutic utility in rare diseases, cancer, and beyond

Will file the 1st IND in 2024 for persons with hemophilia B (BE-101)

## BCM

- Durable
- Redosable
- Without Preconditioning
- Autologous and allogenic



# Acknowledgements



We thank all our  
Be Bio team  
members for  
their support

## NIH Team

Cynthia Dunbar  
David Young  
Shiqin Judy Yu  
Sogun Hong  
Aylin Bonifacino  
Seth Linde  
Allen Krouse  
Theresa Engles  
Justin Golomb  
Krystal Allen-Worthington  
Kevan Keyvanfar

## SCRI Team

David J Rawlings  
Richard G James  
Tyler F Hill  
Parnal Narvekar  
Gregory Asher  
Nathan Camp  
Kerri Thomas  
Sarah Tasian



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# Questions?

Accelerating Life Science Discovery

And please see our poster at Poster Session 2  1/24/2024 7:30:00 PM

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<sup>1</sup>Beigene Biopharma, Cambridge, MA 02139, USA; <sup>2</sup>National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA; <sup>3</sup>National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA; <sup>4</sup>Seattle Children's Research Institute, Seattle, WA 98101, USA