-Oltre l'editing genomico-
"Ex vivo and in vivo therapies for hemoglobinopathies"

Laura Breda, PhD: bredal@chop.edu

Children's Hospital of Philadelphia℠

XIV CONGRESSO NAZIONALE SITE

Roma, Pontificia Università Urbaniana

11-13 Settembre 2025

Team members/projects portfolio

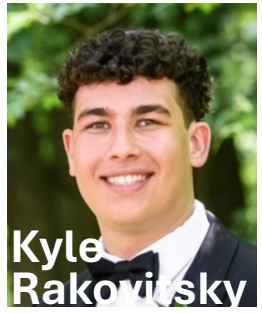
Ex vivo/in vivo
Cell and Gene Therapy - Disease models (Gene):

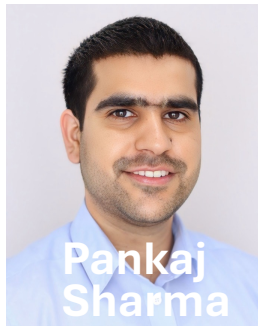
- α/β-thalassemia(α/β-globin)
- SCD (β-globin)

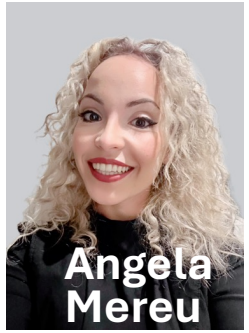
- XLSA(ALAS2)
- CDA1(CDAN1)
- CDA2(SEC23B)
- CSA(SCL25A38)

- SCID(IL2Rγ)
- SCID(IL7Rα)

- MLD(ARSA) (**pre-IND phase**)







Pathways and drugs regulating erythropoiesis and iron metabolism

Genes/Pathways

- Hepcidin, Ferroportin, ERFE
- JAK2
- TF/TFR2
- SMAD/TNFa/IL6

Drugs

Hepcidin agonists or inducers
Luspatercept

Comprehensive Center for the CUre of Sickle Cell Disease and other Red Cell Disorders: CuRED

Frontier Program

Laura Breda
Naoto Tanaka
Lucas Tricoli
Carlo Castruccio Castracani
Osheiza Abdulmalik

Janet Kwiatkowski
Alexis Thompson
Catherine Hamilton

Tim Olson
Steve Grupp
Yonping Wang

Gregory Podsakoff
Hyeon Kim
Camille Jolly Tornetta

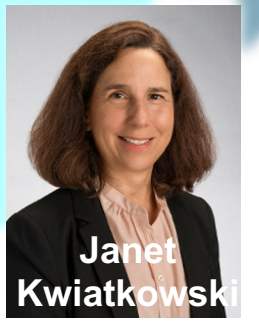
The path of the patient to the cure

Pre-Treatment

Treatment

Gene Therapy Trial
HSCT
Gene Therapy
New Drugs

Post-Treatment



IND approved study (NCT06364774) with ALS20: a lentivector for the cure of β-thalassemia, recruited patient #1

CHOP-ALS20

β-globin unit

Breda et al, Molecular Therapy, 2021

Clinical vectors (CV)

Globe **ALS20 +157%**

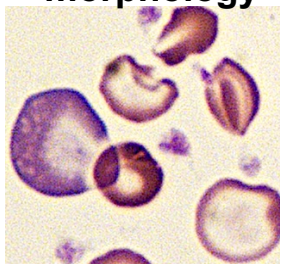
BB305 +40%
(Zyntheglo/Lyfgenia)

In vivo

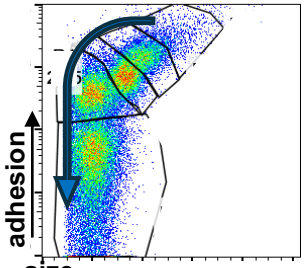
βthal *mouse*

+ ALS20

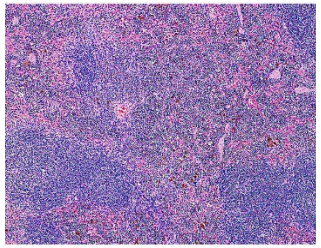
Impaired blood morphology



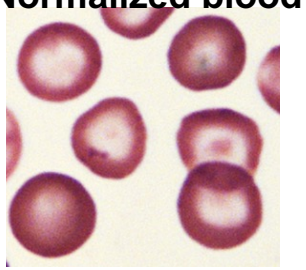
Impaired Spleen RBC maturation



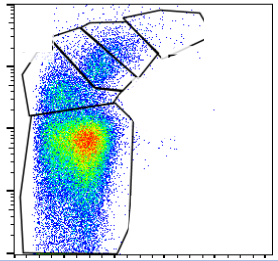
architecture

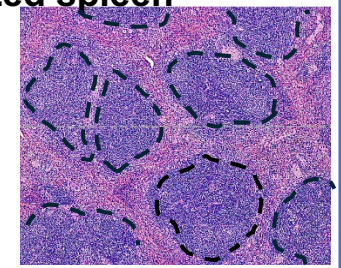


Normalized blood

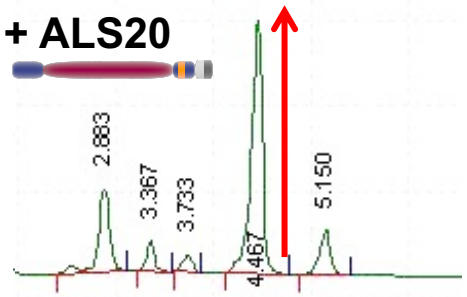


Normalized spleen



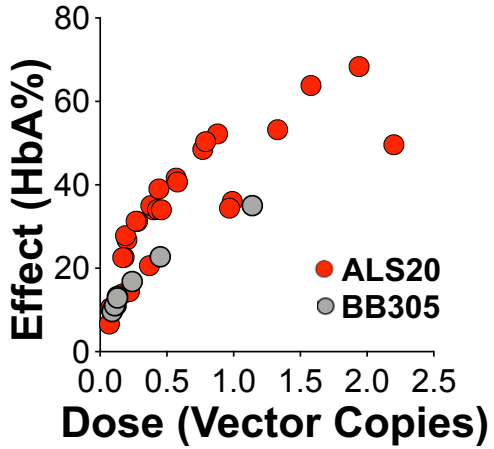


+ ALS20



Thalassemic 30/0 RBC lack adult hemoglobin

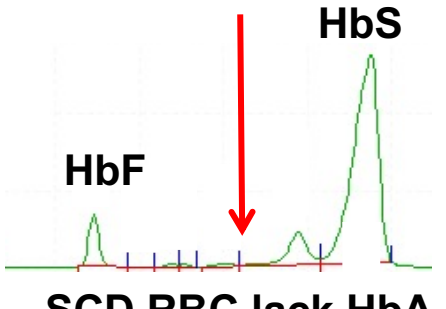
30% RBC+65% HbA

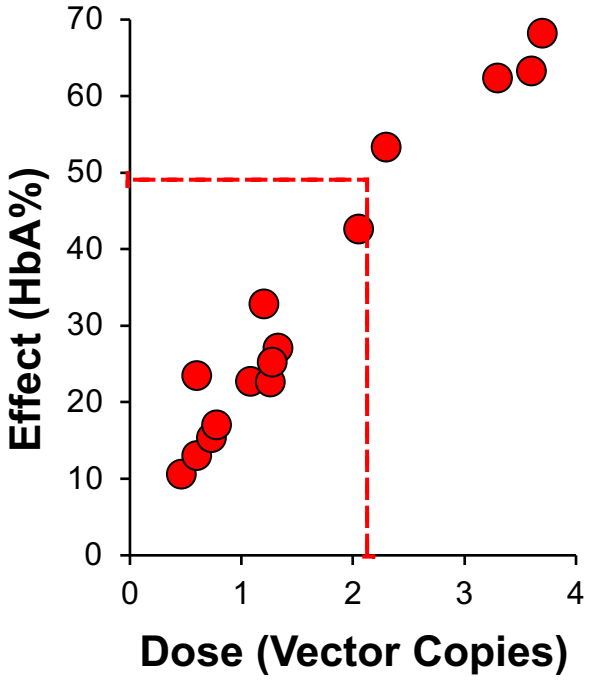


- First patient enrolled ✓
- Mobilized HSC product+AL20 produced ✓
- Quality control assays ✓
- Release assays ✓

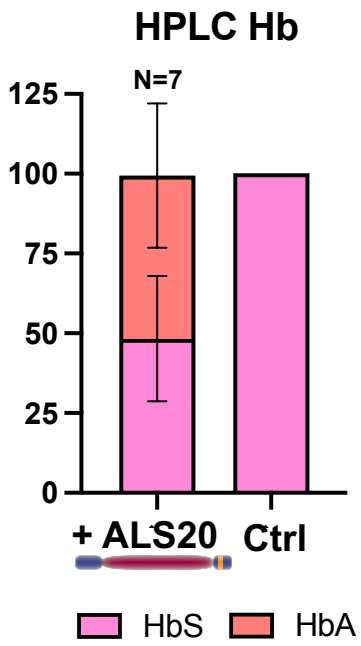
IND submission for GT of SCD with ALS20 in progress

Ex vivo

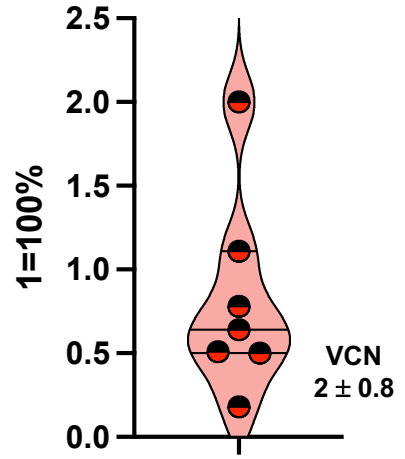




In vivo (S/S-Townes model)



LC-MS/MS on
GTFA**T**LSELHCDK
vs.
GTFA**Q**LSELHCDK
Molar ratio Q/T



*Chappell, Breda et al, Blood, 2024 (**ALS20-α**)*

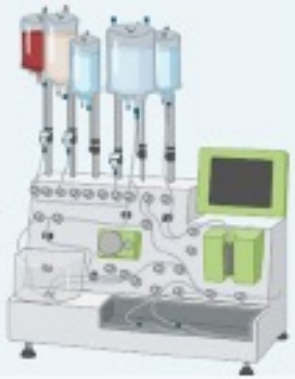
α-globin unit

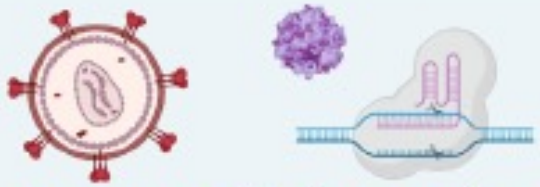
Unpublished results

Phases/bottlenecks of ex-vivo gene therapy process

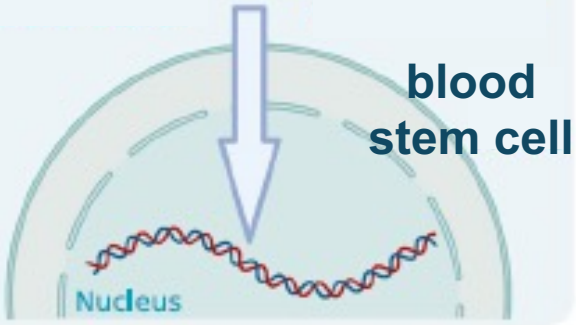
Mobilization and apheresis

Isolation of blood stem cells



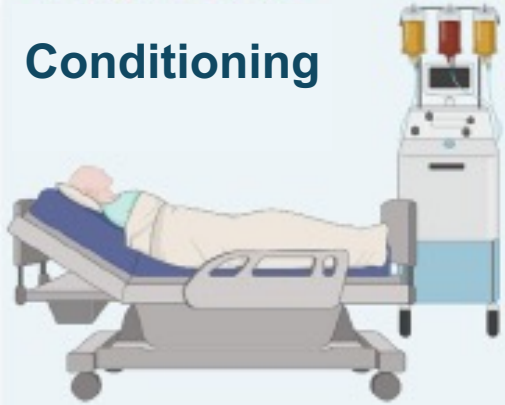


Vector/Gene editing material and drug product (DP) manufacturing and QC

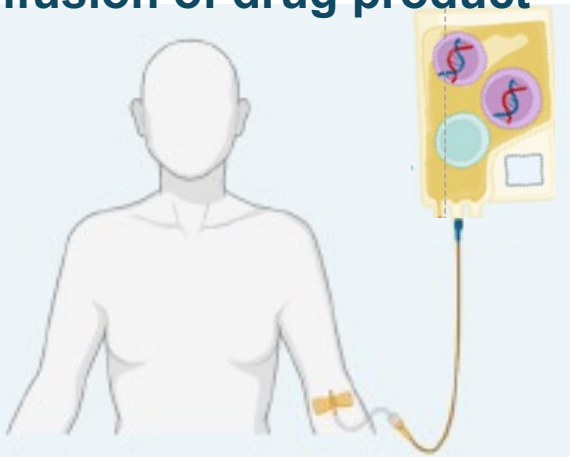


blood stem cell

Conditioning



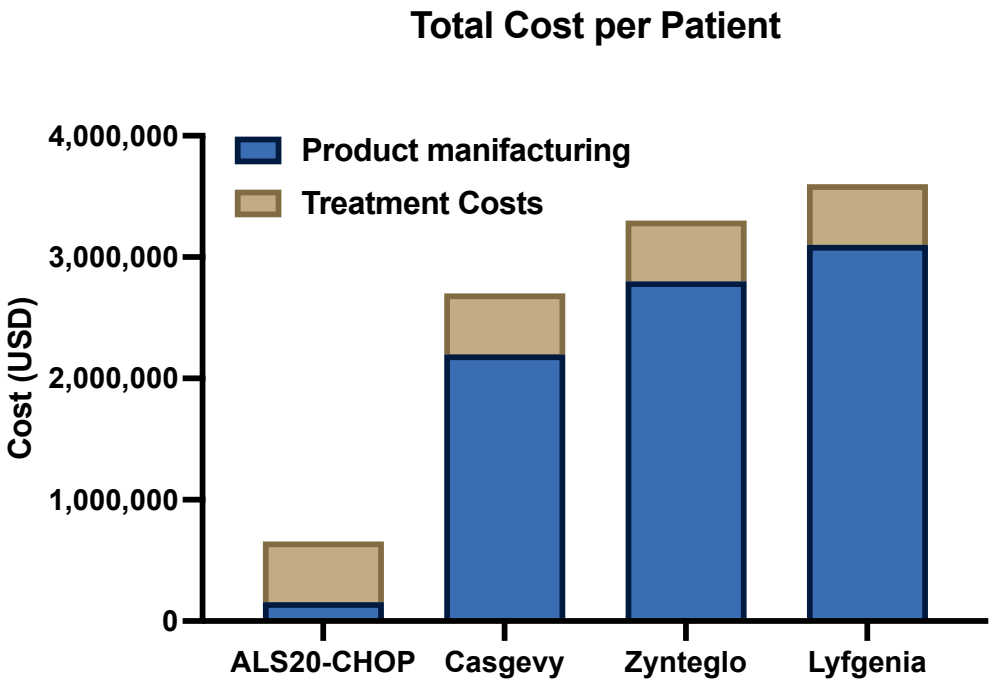
Infusion of drug product



Product manufacturing

Patient treatment

Cost comparison of ex-vivo gene therapy process



Treatment	Product manufacturing and QC	Treatment costs*	Cost/patient	Estimated saving
ALS20-CHOP	$156,000	$500,000	**$656,000**	-
Casgevy	$2,200,000	$500,000	$2,700,000	**$2,044,000**
Zynteglo	$2,800,000	$500,000	$3,300,000	**$2,644,000**
Lyfgenia	$3,100,000	$500,000	$3,600,000	**$2,994,000**

*including fertility preservation, conditioning, BMT, and post-BMT treatments, follow-up, and analysis costs.

Cost Efficiency and Benefits of All-In-House Therapy

- ALS20-CHOP is ~20–45% less expensive than commercial alternatives
- Control over vector production, use and quality standards
- Enables iterative improvements, customization and outcomes measures
- CHOP patient hub access
- Facilitates deeper scientific collaboration and training
- Streamlines IRB/IACUC alignment and compliance

What's Included: Cost Element Differences

Gene Therapy Product:

- ALS20-CHOP: Lentiviral vector treated autologous cells (manufacturing at CHOP)
- Casgevy: CRISPR-edited autologous cells (manufacturing outsourced)
- Lyfgenia: Lentiviral vector treated autologous cells, (manufacturing outsourced)

Additional Costs:

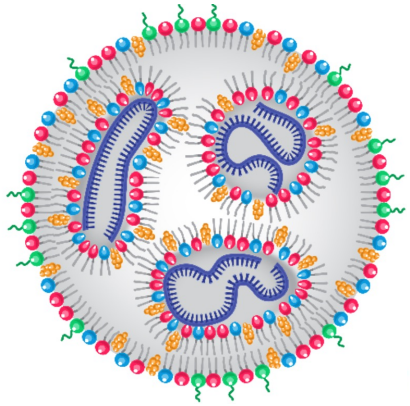
- Hospitalization (4–6 weeks), conditioning, labs, follow-up
- Often excluded from commercial list prices
- In-house budget explicitly includes real-world clinical costs

Limitations of ex-vivo gene therapy process

- Untethered lentiviruses/gene editors are not amenable to target blood stem cells
- Cost of research/clinical procedures, specialized personnel, manufacturing of drug product
- Centralization of GT in very specialized centers
- Inclusion/exclusion criteria
- Toxicity of conditioning

High drug cost and limited patient access

Lipid Nanoparticles clinically approved/in development



Cholesterol

Helper lipid

Support integrity of LNP structure

PEG-Lipid

Provides colloidal stability and stealth properties (hydrophilic and no charge) to avoid accumulation in RES

Cationic ionizable lipid

Complexation with nucleic acids
Only protonated at non physiological pH-> not charged in circulation (cationic LNP are toxic!!!)

Hamideh Parhiz

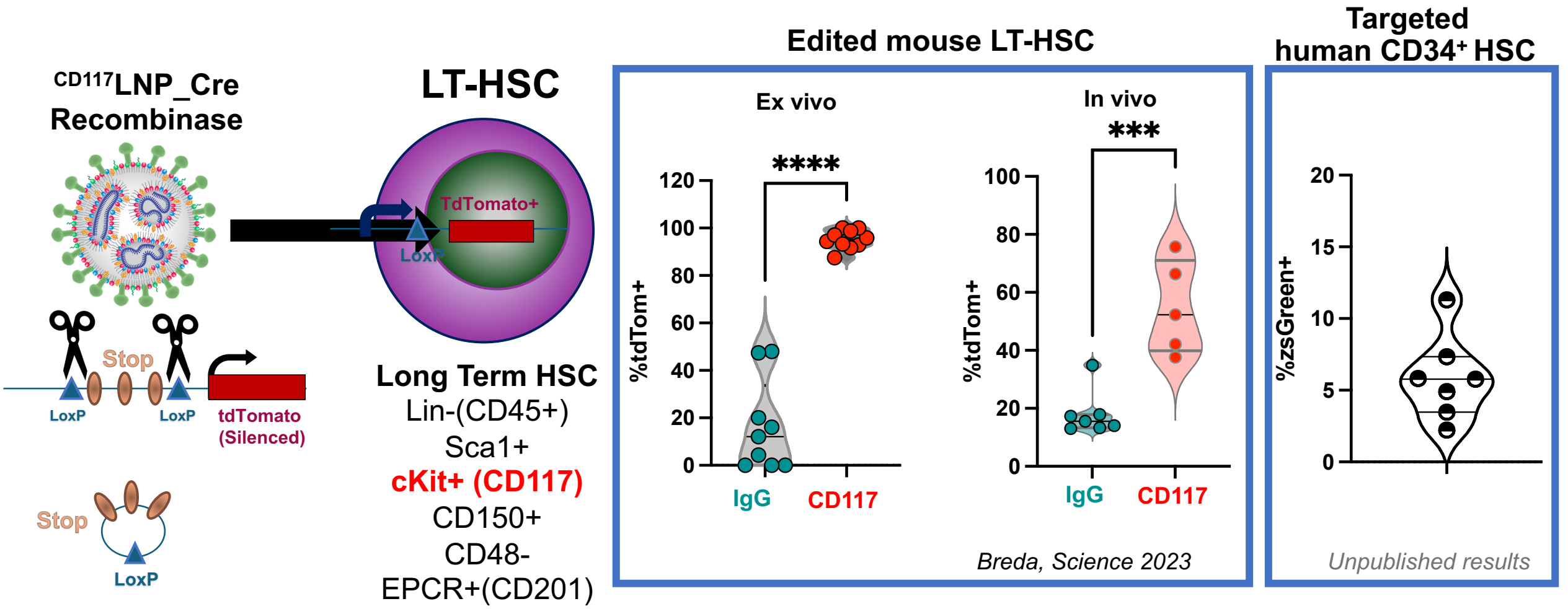
Drew Weissman

Tyler Papp

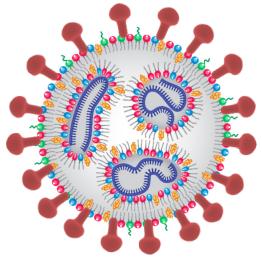
Institute for RNA Innovation

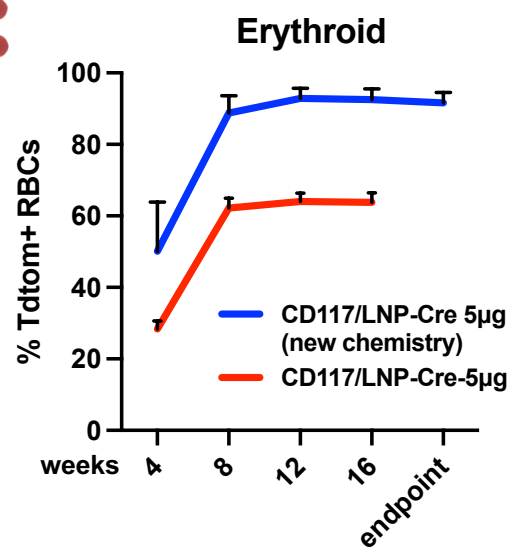
- 2018 siRNA therapeutic, Patisiran (ONPATTRO®): lower transthyretin protein in the liver for the treatment of hereditary transthyretin-mediated amyloidosis
- 2019 siRNA therapeutic, Givosiran, ALN-AS1 (GIVLAARITM) : target the ALAS1 gene in hepatocytes for adult patients with the hereditary disease acute hepatic porphyria
- 2020 COVID-19 vaccines (EUA) (Pfizer and Moderna): deliver mRNA encoding for the SARS-CoV-2 spike protein
- 2025 First personalized therapy for CPS-1 deficiency (CHOP) deliver mRNA encoding a base editor to correct the allelic defect that causes disruptions of the urea cycle, leading to elevated ammonia levels
  - Currently >20 CT for vaccines, immunotherapy, protein replacement therapy, and genomic editing

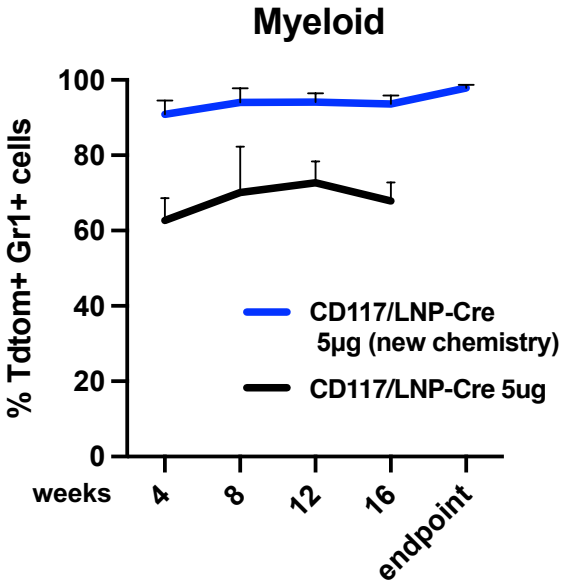
Ex vivo/in vivo editing of mouse HSC with targeted LNP

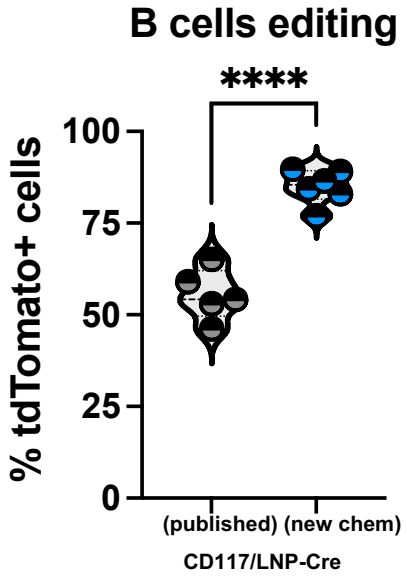


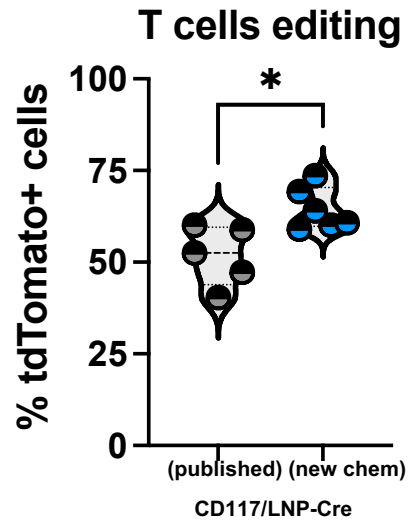
Increased BM targeting with improved Ab complexation

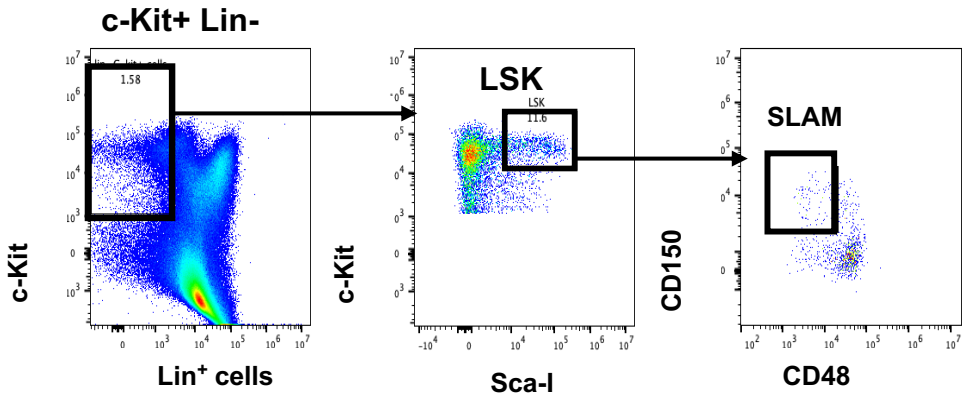


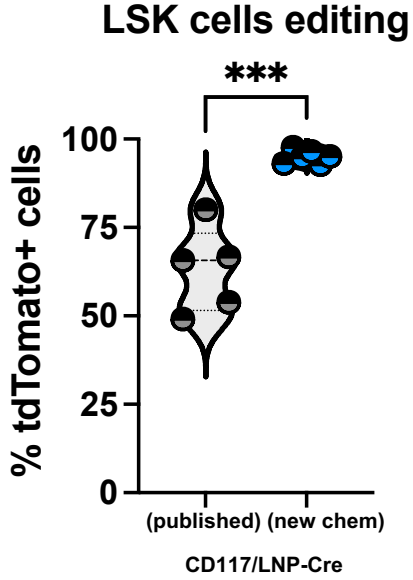


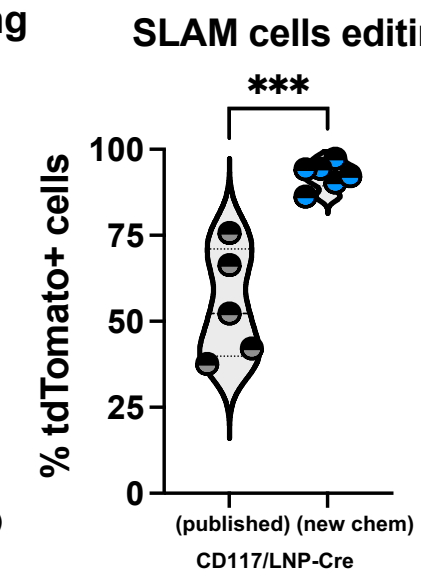








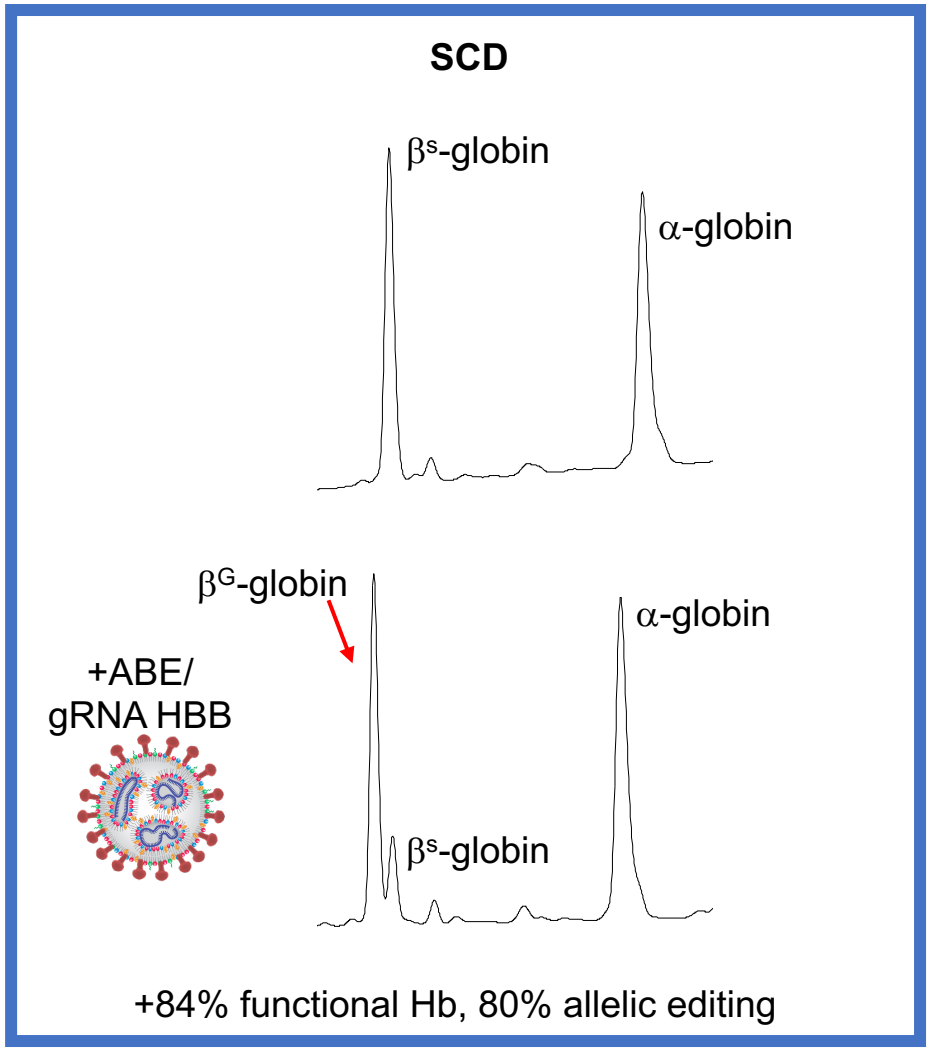


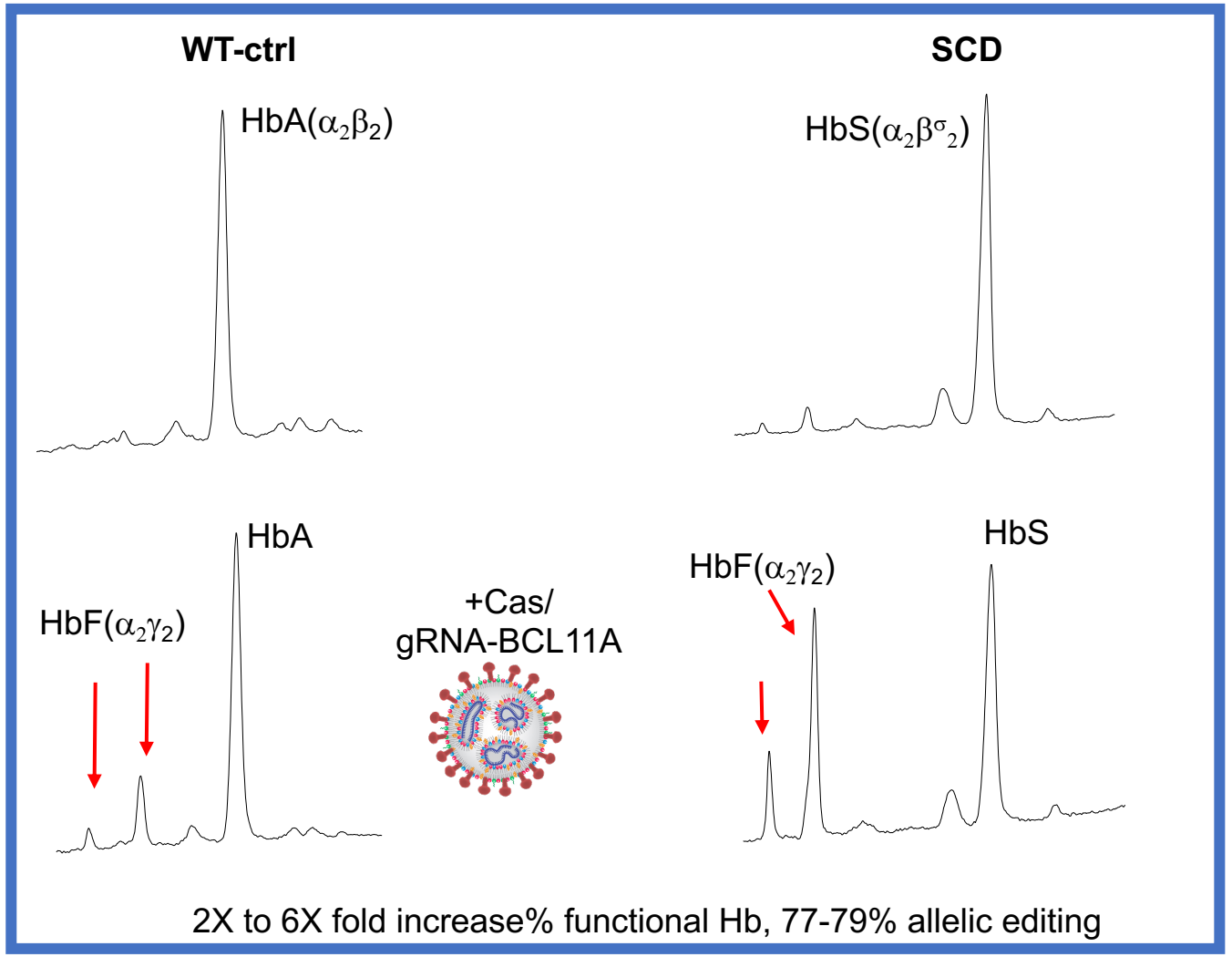


M.-G. Alameh

Unpublished results

LNP mRNA increase functional Hb synthesis via HSC gene editing mediated by ABE and CRISPR-Cas mRNA

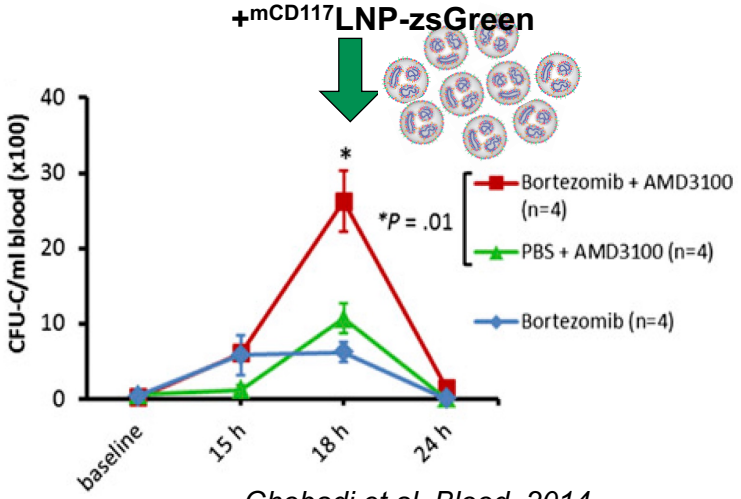




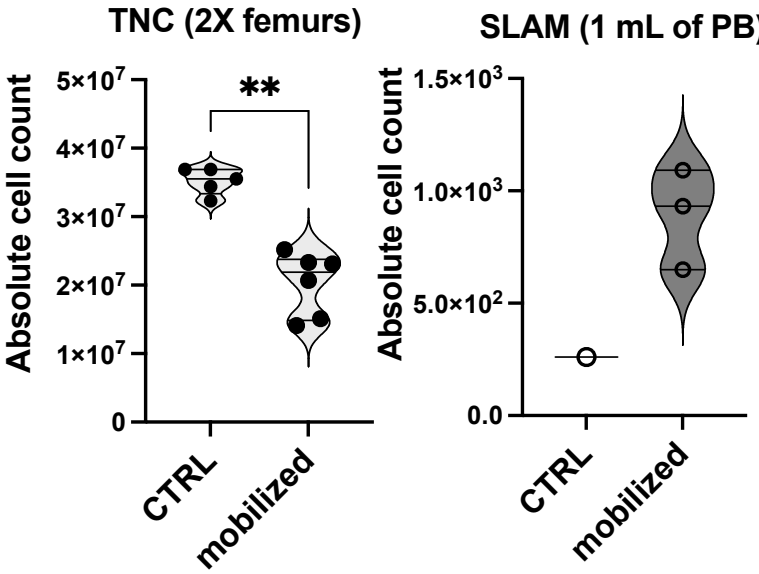
Single globin chain separation (reversed-phase HPLC)

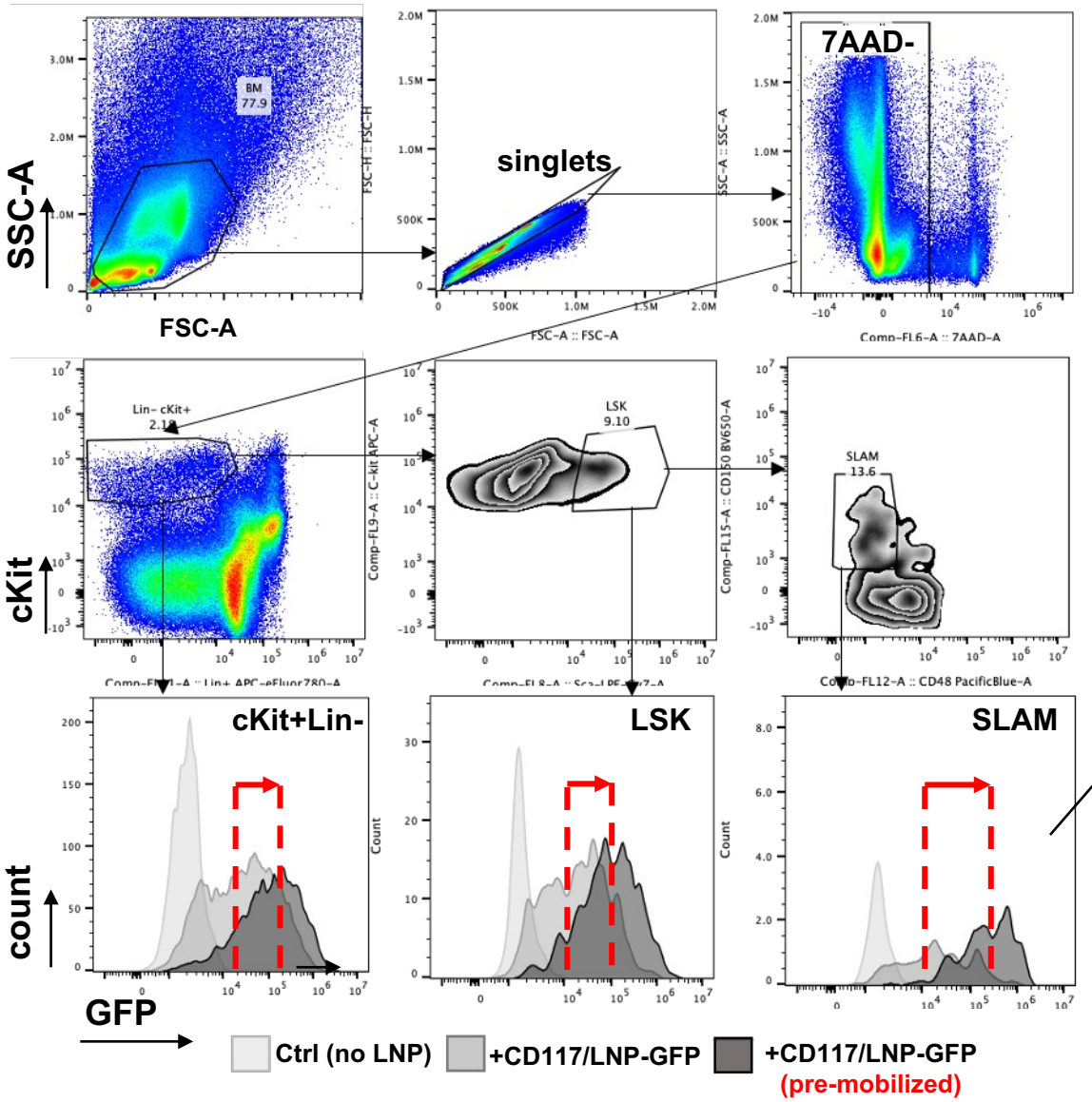
Tetrameric hemoglobin (Hb) separation (cation-exchange HPLC)

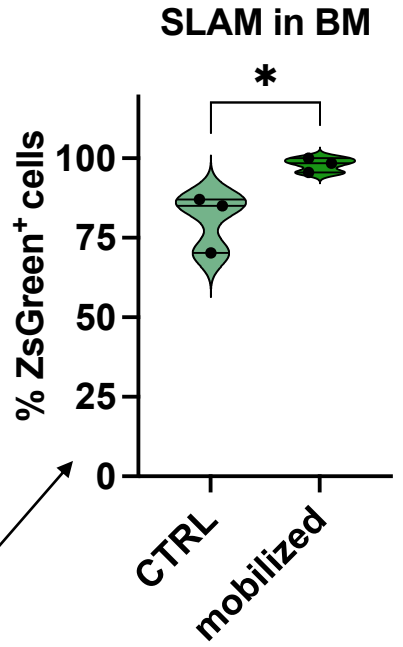
Use of HSC mobilizing agents to higher HSC targeting



Ghobadi et al, Blood, 2014



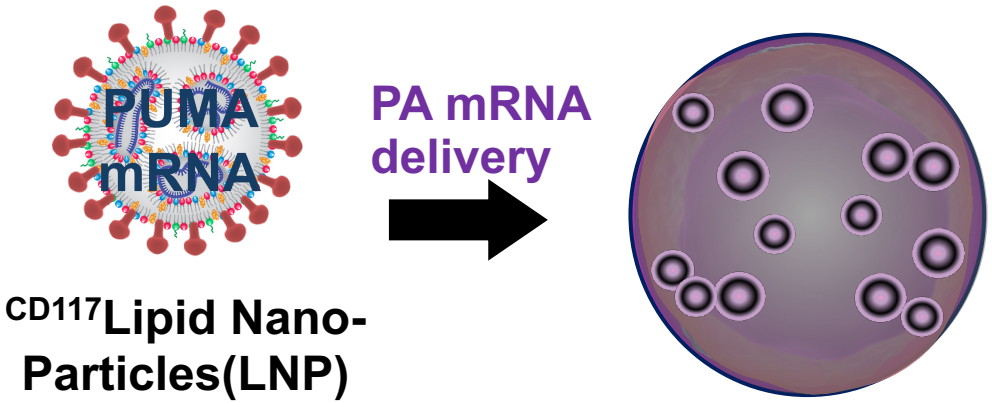


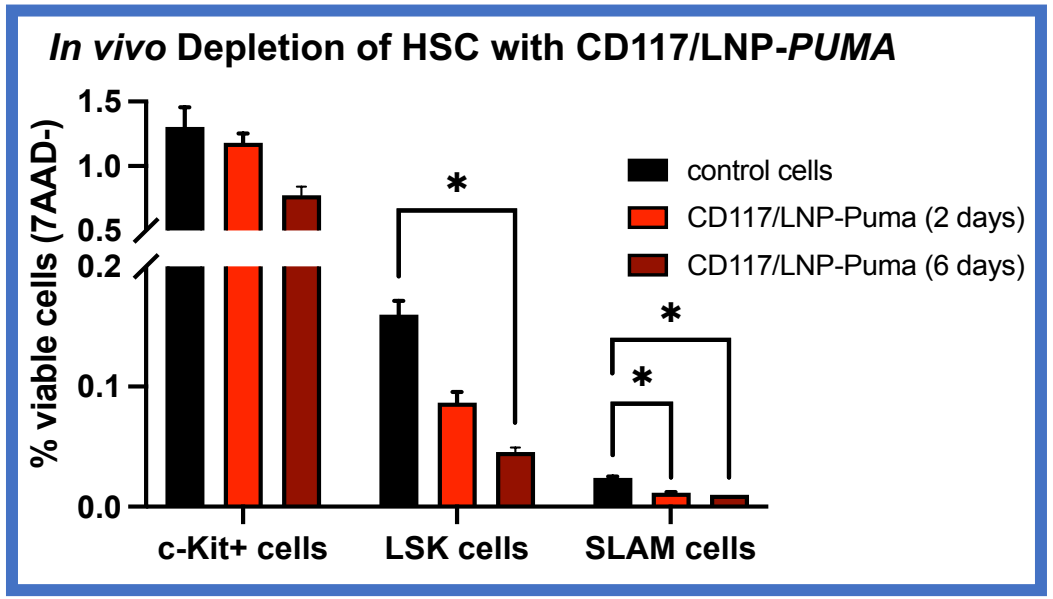


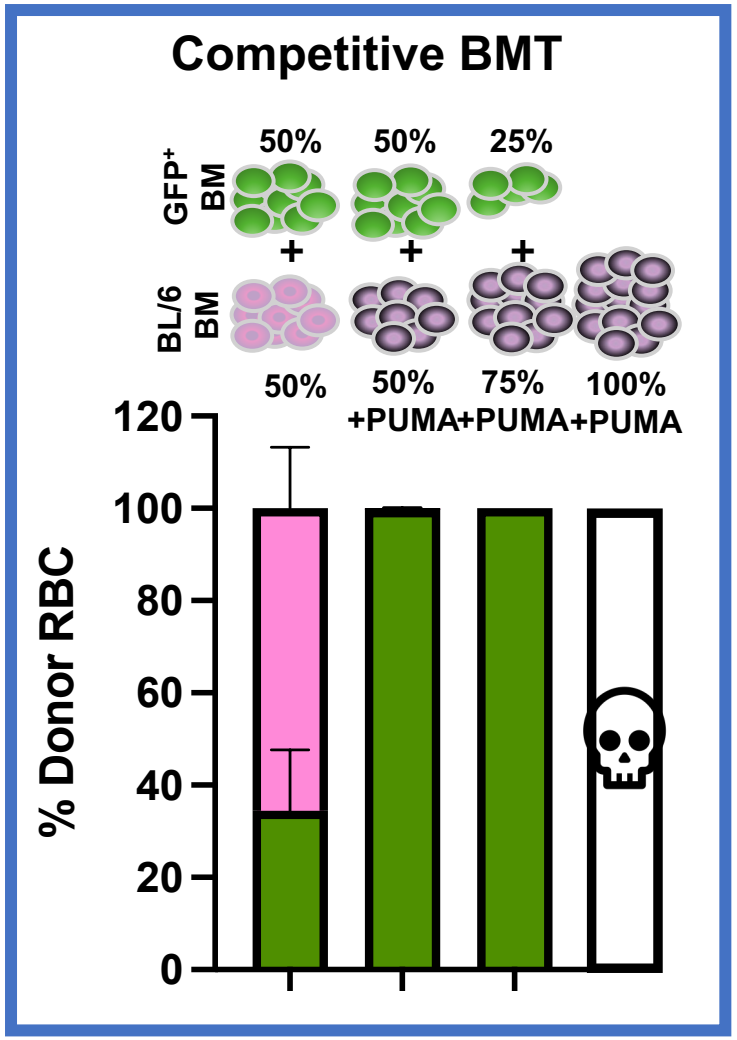
Unpublished results

Pro-apoptotic (PA) mRNA delivery to deplete HSC for allo-BMT

Non alkylating HSC depletion





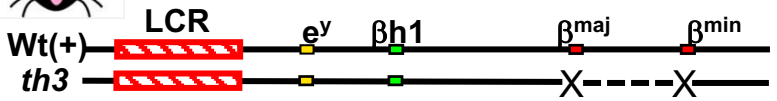


Breda, Science 2023

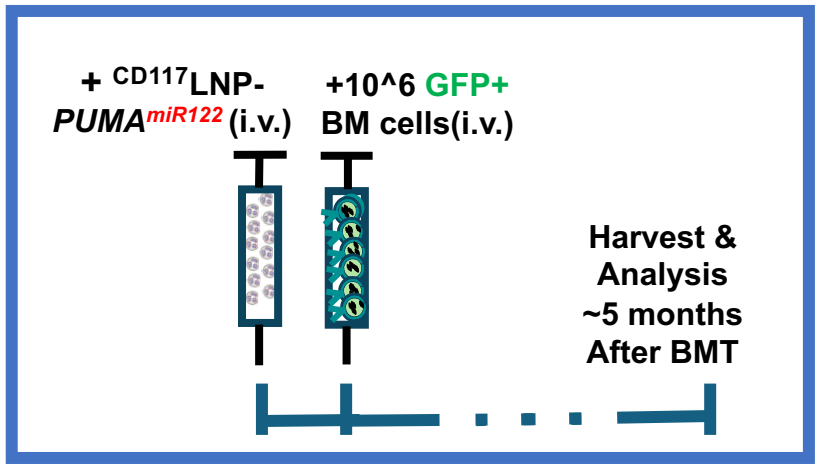
Successful allo-BMT in thalassemia model-*Hbb*$^{th3/+}$ after PA-LNP conditioning: amelioration of BM features

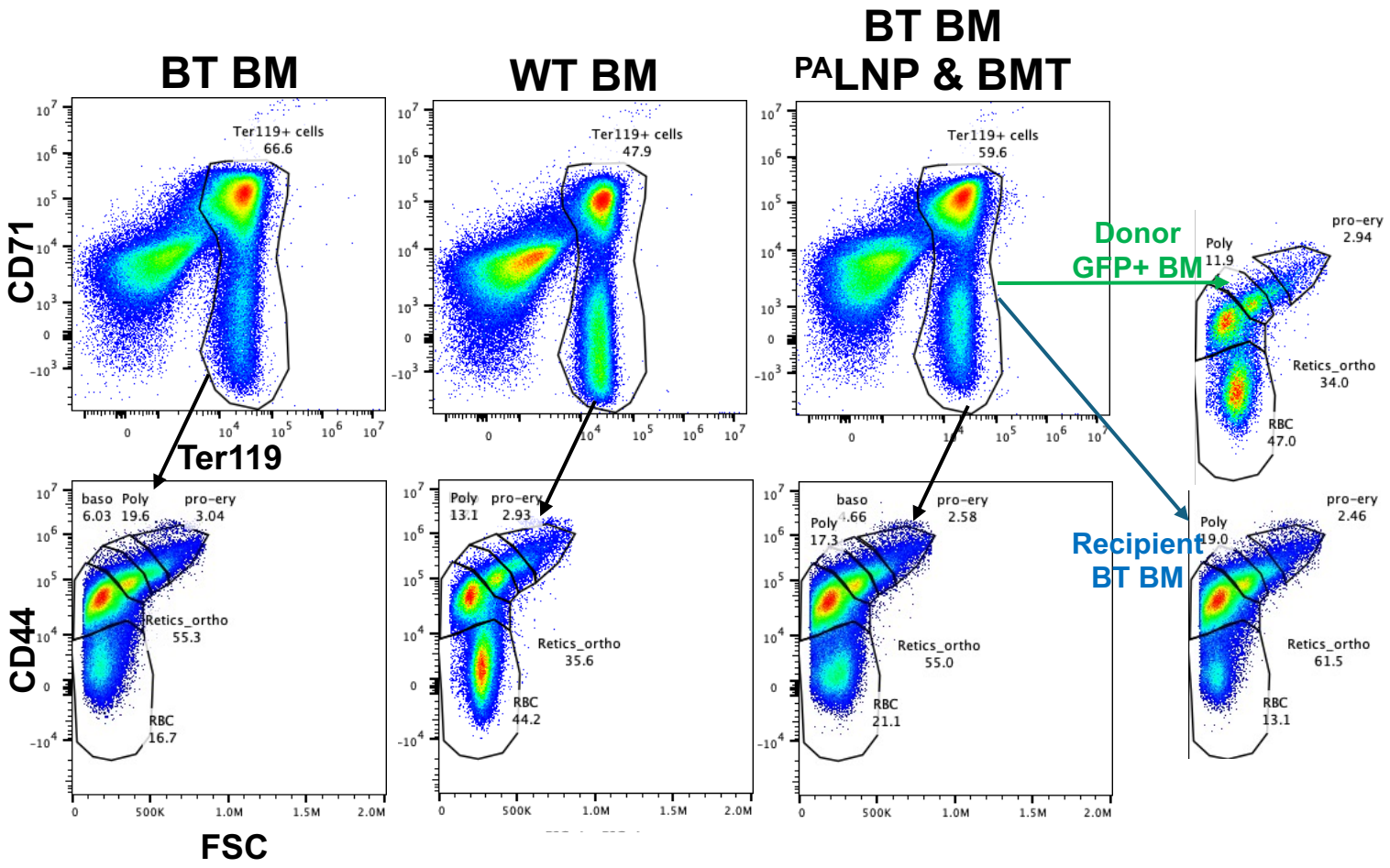
***Hbb*$^{th3/+}$ BT model**

Hb: 7.5 g/dL

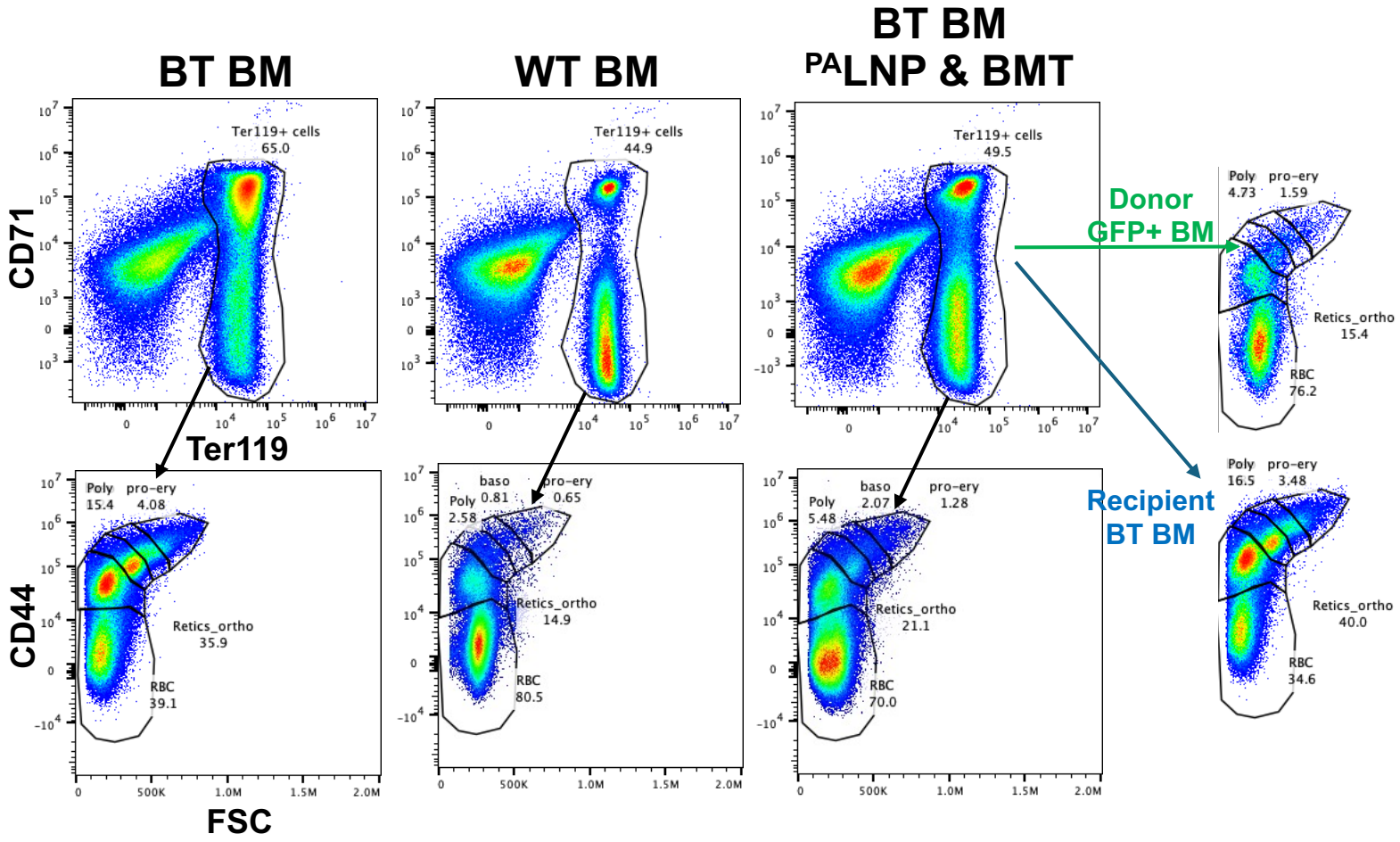


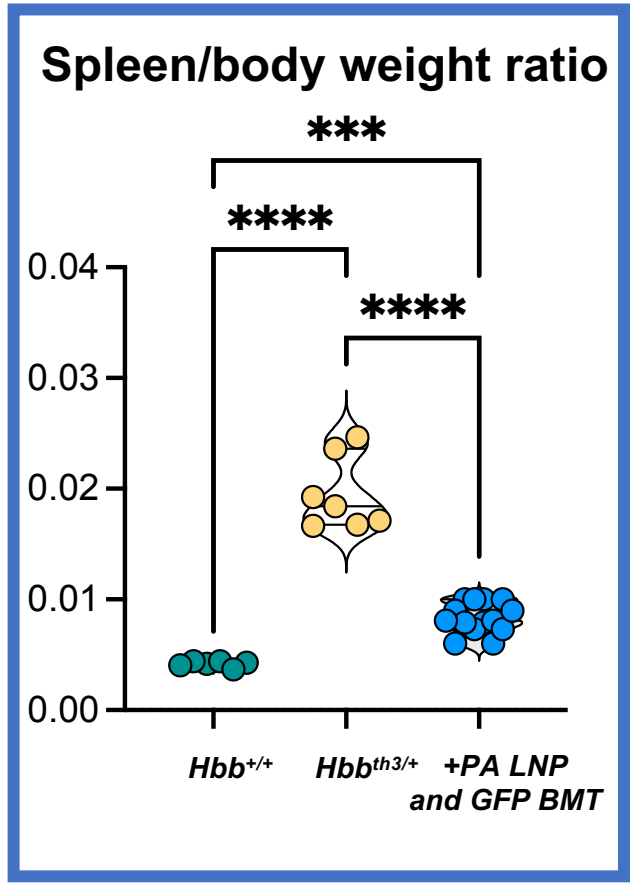
Yang B, et al. PNAS. 1995 Dec 5; 92:11608-12.
Ciavatta DJ, et al. PNAS 1995; 92: 9259-63.



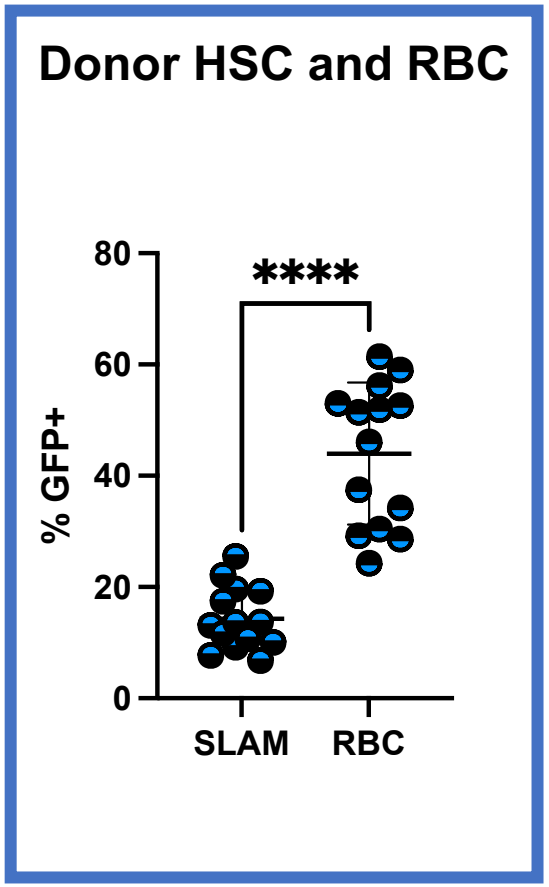


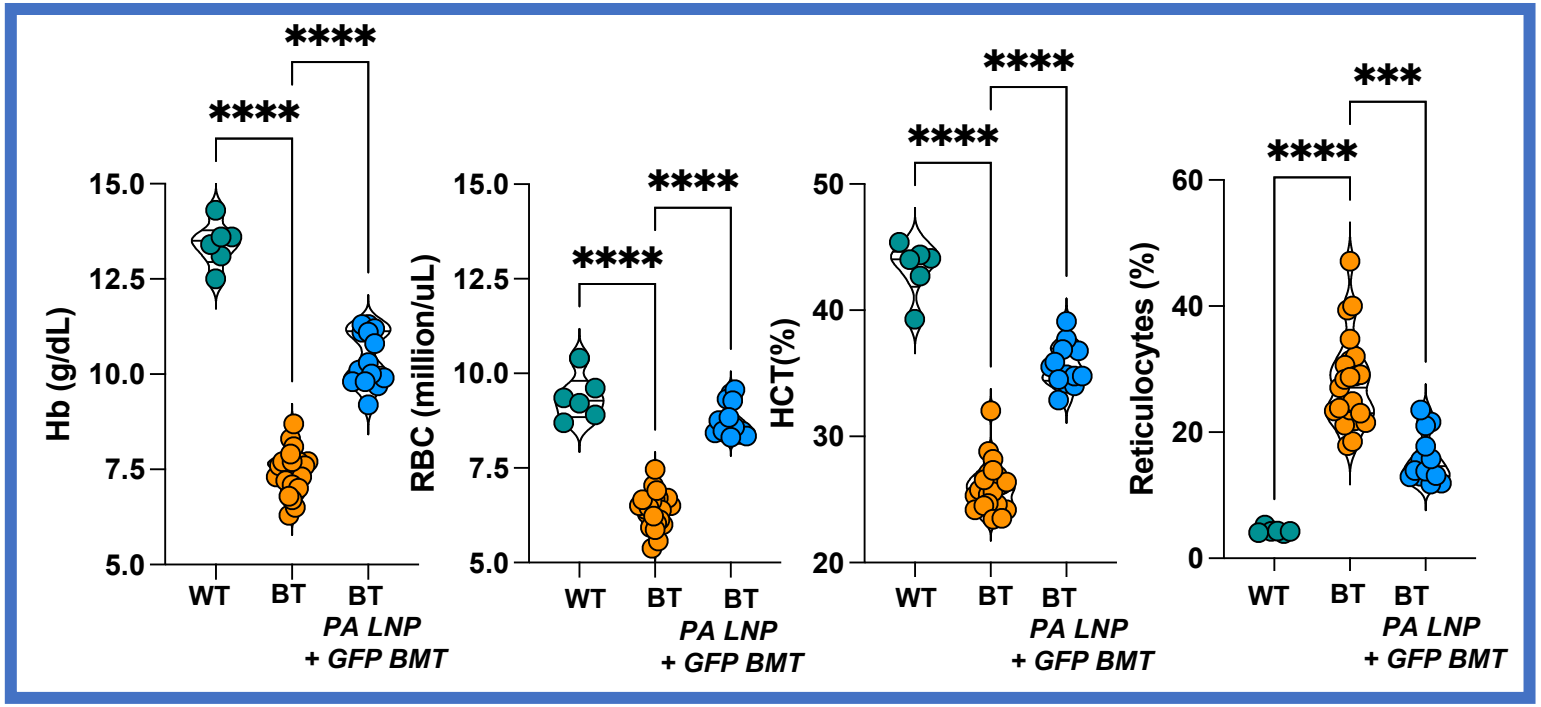
Successful allo-BMT in thalassemia model-*Hbb*$^{th3/+}$ after PA-LNP conditioning: hematopoietic/erythroid recovery leads to reduced splenomegaly

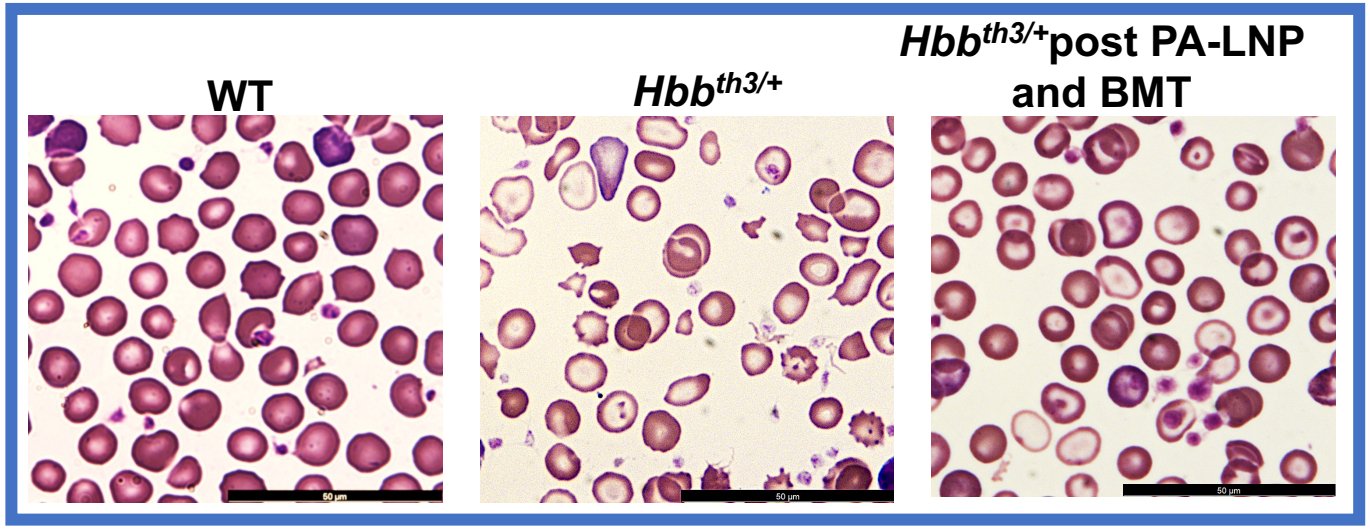




Amelioration of erythroid features







Summary

CHOP is committed to increase access to curative and lower cost treatments to patients with BT and SCD (ALS20)

Our goal is to gather data-driven financial momentum to get FDA approval

Our pre-clinical work with targeted LNP show that these vehicles can transfer curative genetic material into blood stem cells

Unpublished pre-clinical study in mice show that LNPmRNA-based conditioning allows healthy bone marrow engraftment can ameliorate thalassemia

In progress

In vivo gene editing with improved formulations (xenograft)

PA-LNP in human HSC and to selectively deplete them in vivo (xenograft)

Complete characterization of

LNP distribution in healthy and disease models

Exploring the use of new formulations with higher dissolution in BM environment