Base Editing of Gamma Globin Gene Promoters Generates Durable Expression of Fetal Hemoglobin for the Treatment of Sickle Cell Disease

Adrian P. Rybak, Elsie Zahr Akrawi, Conrad Rinaldi, Scott J. Haskett, Ling Lin, Jeffrey Marshall, Alexander Liquori, Luis Barrera, Jenny Olins, S. Haihua Chu, Jeremy Decker, Minerva Sanchez, Yeh-Chuin Poh, Matt Humes, Michael S. Packer, Nicole M. Gaudelli, Sarah Smith, Adam Hartigan and Giuseppe Ciaramella.

May 13, 2020

DISCLOSURE

- I am a Beam employee and shareholder

Normal Erythropoiesis, Sickle Cell Disease and Hereditary Persistence of Fetal Hemoglobin (HPFH)

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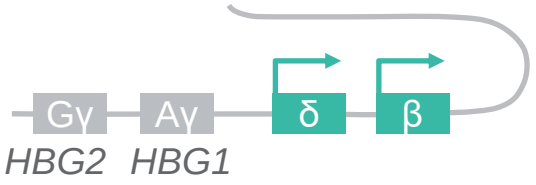
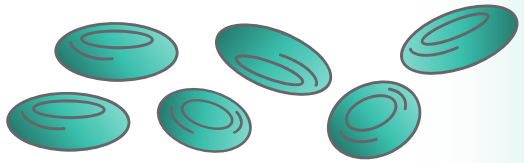
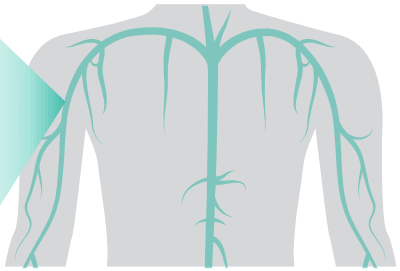
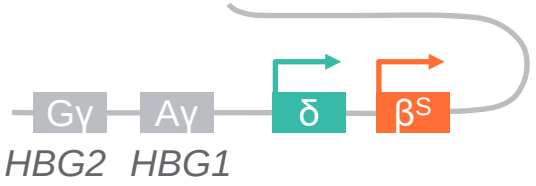
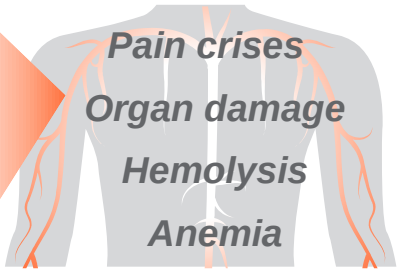
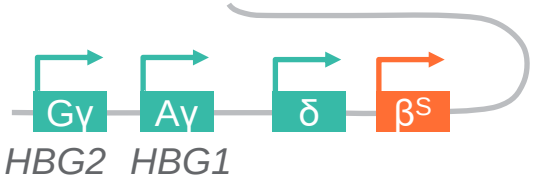
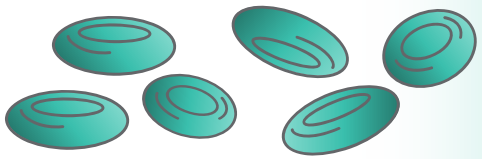
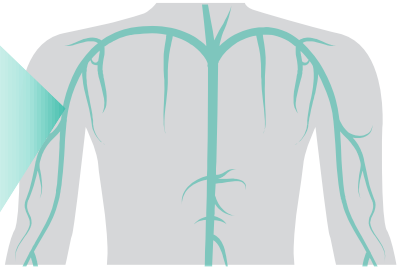
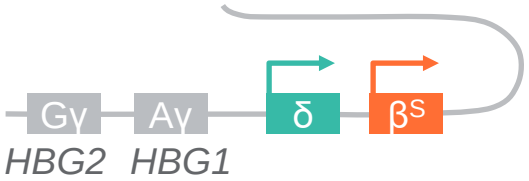
	β-like Globin Genes	Hemoglobin Proteins	Red blood cells	System
Normal (Adult)	 After birth, γ-globin is repressed and wild-type β-globin is expressed	 Normal β:α hemoglobin tetramers form	 Red blood cells form and function normally	
Sickle Cell Disease (Adult)	 After birth, γ-globin is repressed and sickle-causing β^S-globin is expressed	 β^S-globin tetramers form polymers under hypoxia	 Polymers cause red blood cells to sickle under hypoxia	
Hereditary Persistence of Fetal Hemoglobin (HPFH)	 γ-globin expression persists after birth and through adulthood	 Normal γ:α hemoglobin tetramers form	 Red blood cells form and display decreased sickling phenotype	

Base Editing at *HBG1 and HBG2 (HBG1/2)* Gene Promoters

Beam THERAPEUTICS

Sickle Cell Disease (Adult)

β-like Globin Genes



After birth, γ-globin is repressed and sickle-causing β^S-globin is expressed

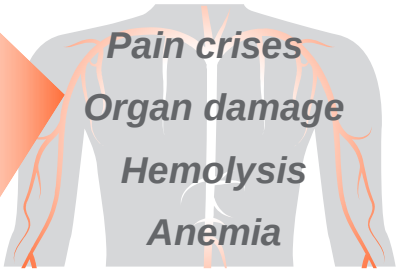
Hemoglobin Proteins

β^S-globin tetramers form polymers under hypoxia

Red blood cells

Polymers cause red blood cells to sickle under hypoxia

System



Base Editing at *HBG1 and HBG2 (HBG1/2)* Gene Promoters

Base Editing recreates naturally occurring HPFH

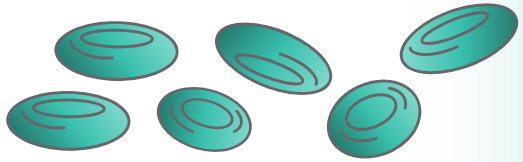
β-like Globin Genes

Guide RNA-targeting ABE produces A-to-G edits in *HBG1/2* promotor regions and derepresses γ-globin expression

Hemoglobin Proteins

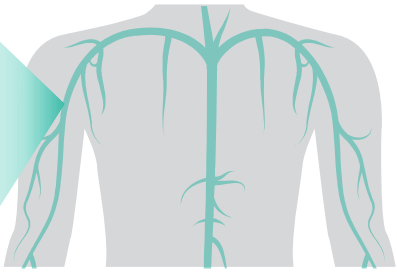
Re-expression of γ-globin decreases β^S-globin and inhibits hemoglobin polymer formation

Red blood cells

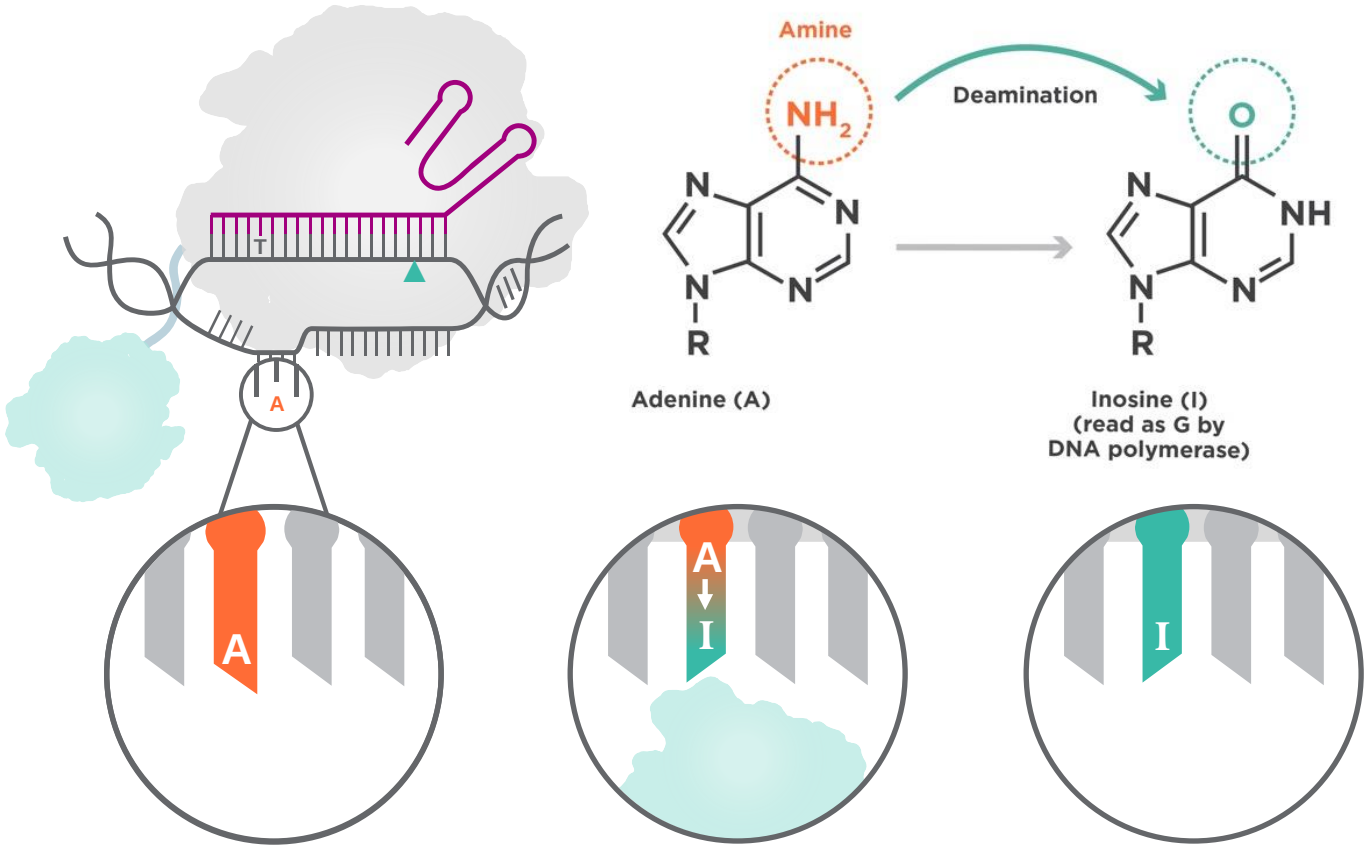


Red blood cells with increased γ-globin have decreased sickling phenotype

System



Adenine Base Editing Technology



- Adenine Base Editor (ABE) comprises a deaminase enzyme fused to catalytically impaired CRISPR protein.
- Guide RNA (gRNA) directs the ABE to a target genomic DNA sequence and exposes the editing window.
- Deaminase chemically converts target adenine (A) to inosine (I) via deamination.
- Guanine (G) subsequently replaces inosine during DNA repair or replication.

Gaudelli NM *et al*., *Nature* (2017); Gaudelli NM *et al*., *Nature Biotechnology* (2020).

Base Editing at *HBG1 and HBG2 (HBG1/2)* Gene Promoters

Base Editing recreates naturally occurring HPFH

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Guide RNA-targeting ABE produces A-to-G edits in *HBG1/2* promotor regions and derepresses γ-globin expression

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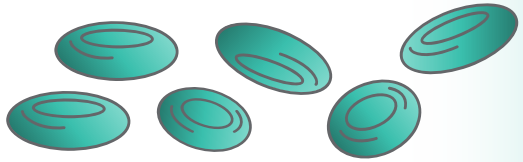
Red blood cells



Red blood cells with increased γ-globin have decreased sickling phenotype

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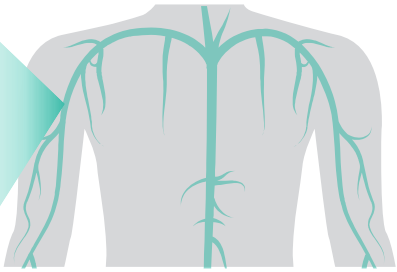


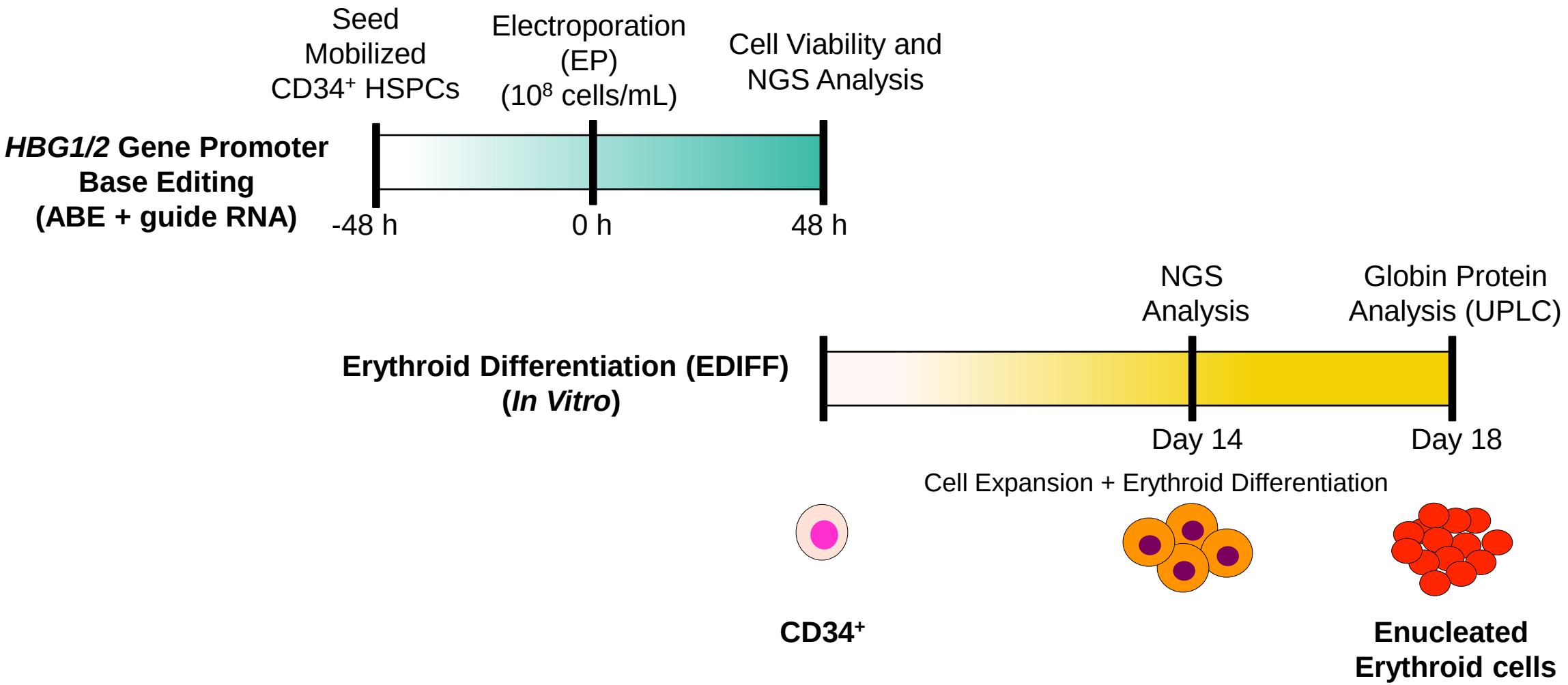
Today's Agenda:

1. ***In vitro* optimization**
   Optimize A-to-G base editing in mobilized human CD34+ hematopoietic stem/progenitor cells (HSPCs) by titrating ABE mRNA and guide RNA.

2. **γ-globin upregulation**
   Maximize γ-globin protein levels produced in erythroid cells.

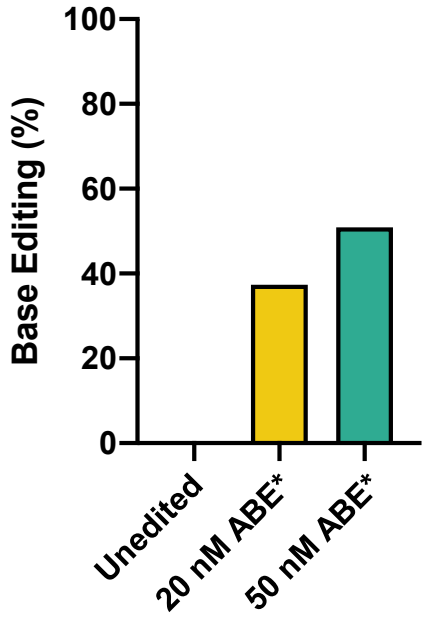
3. ***In vivo* performance**
   Long-term engraftment, retention of editing, γ-globin protein upregulation, and multi-lineage hematopoietic reconstitution.

Experimental Outline

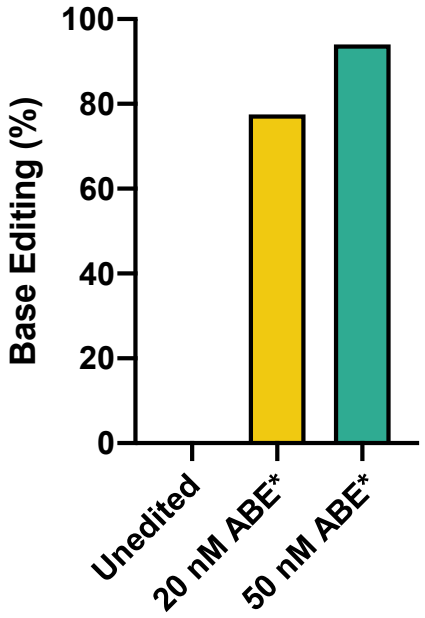


Maximizing A-to-G Base Editing at *HBG1/2* Gene Promoters

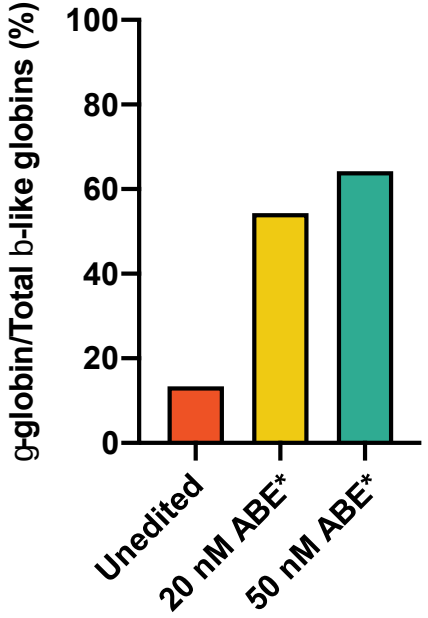
***HBG1/2* Promoter Base Editing (%) (48 h Post-EP)**



***HBG1/2* Promoter Base Editing (%) (Day 14 EDIFF)**



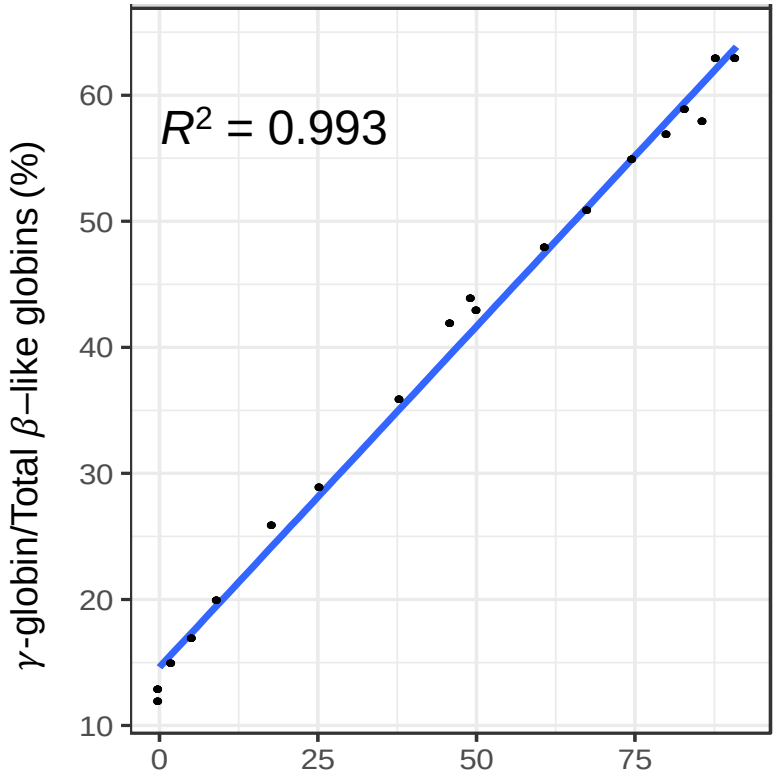
Gamma Globin Protein Levels (%) (Day 18 EDIFF)



*Next Generation Variant

- Base editing at *HBG1/2* promoters in CD34+ HSPCs is dependent on ABE mRNA and guide RNA concentration.
- Base editing levels increase following *in vitro*-mediated erythroid differentiation (EDIFF).
- Increased gamma globin protein levels with increasing A-to-G base editing at *HBG1/2* promoters in erythroid cells.

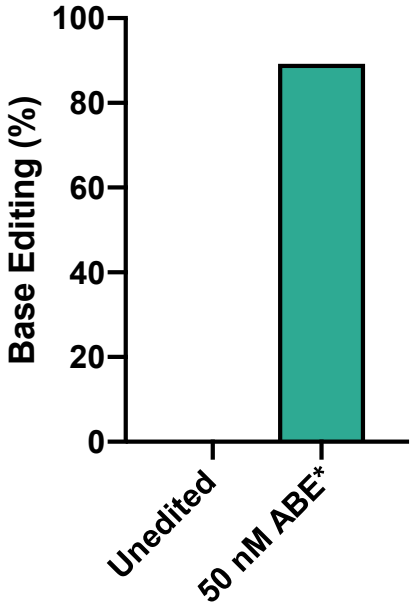
HBG1/2 Gene Promoter Base Editing is Tightly Correlated with Gamma Globin Protein Induction *In Vitro*



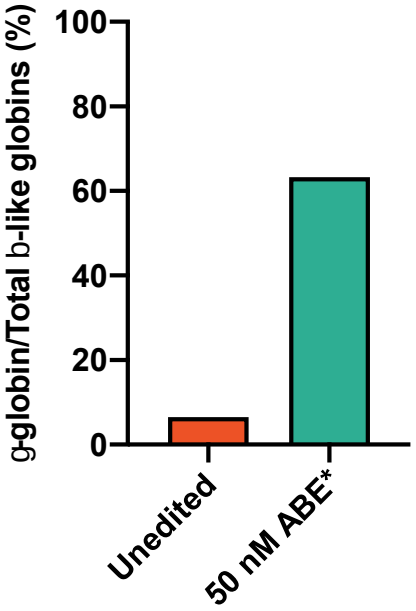
- Linear regression analysis demonstrates that *HBG1/2* gene promoter base editing and gamma globin protein levels in human erythroid cells *in vitro* are tightly correlated.
- Analysis is consistent with achieving >60% gamma globin protein levels at high base editing levels.

Base Editing at *HBG1/2* Gene Promoters in SCD Patient Cells Increases Gamma Globin Levels in Erythroid Cells *In Vitro*

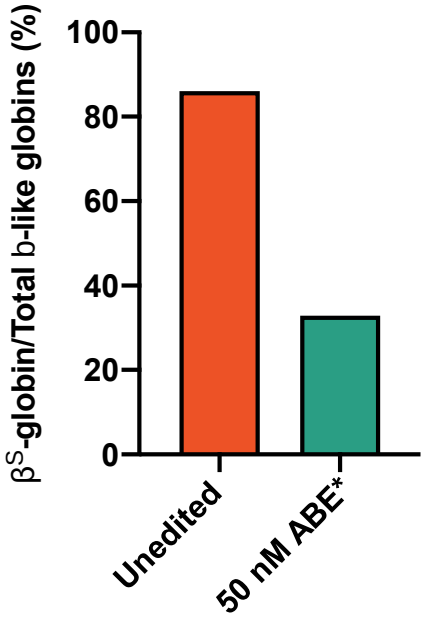
HBG1/2 Promoter Base Editing (%)



Gamma Globin Protein Levels (%)



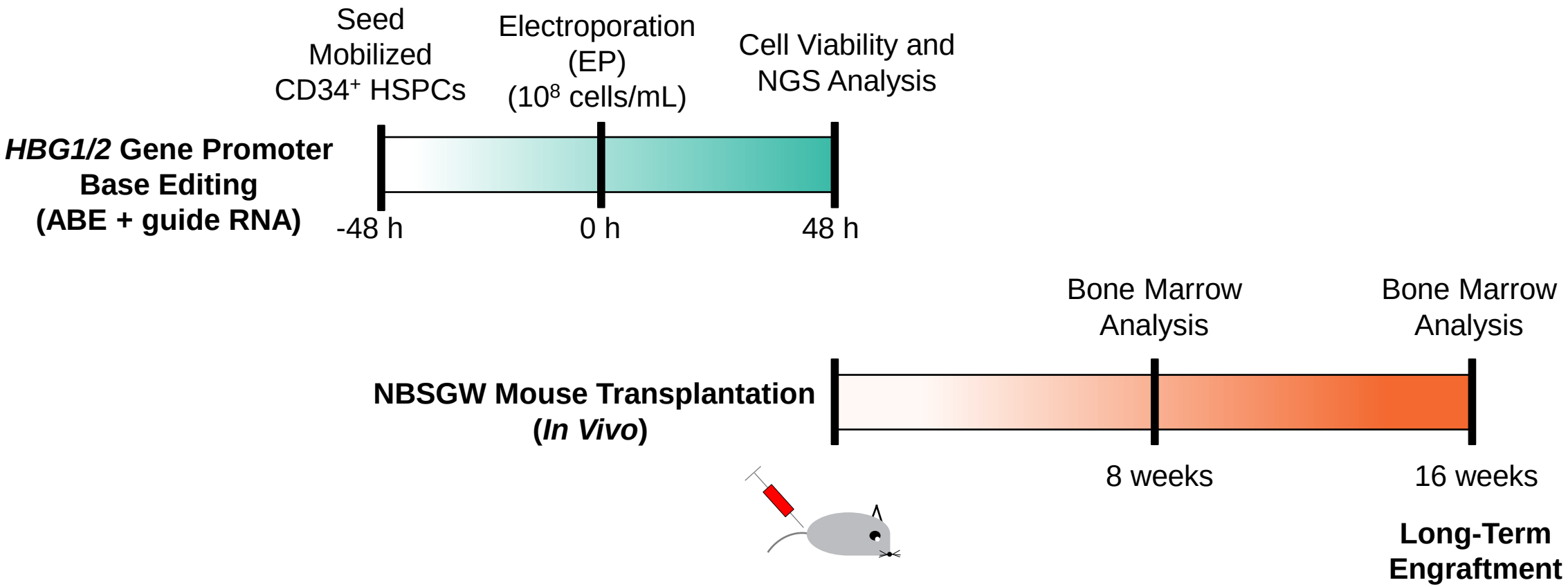
Sickle β-Globin Protein Levels (%)



*Next Generation Variant

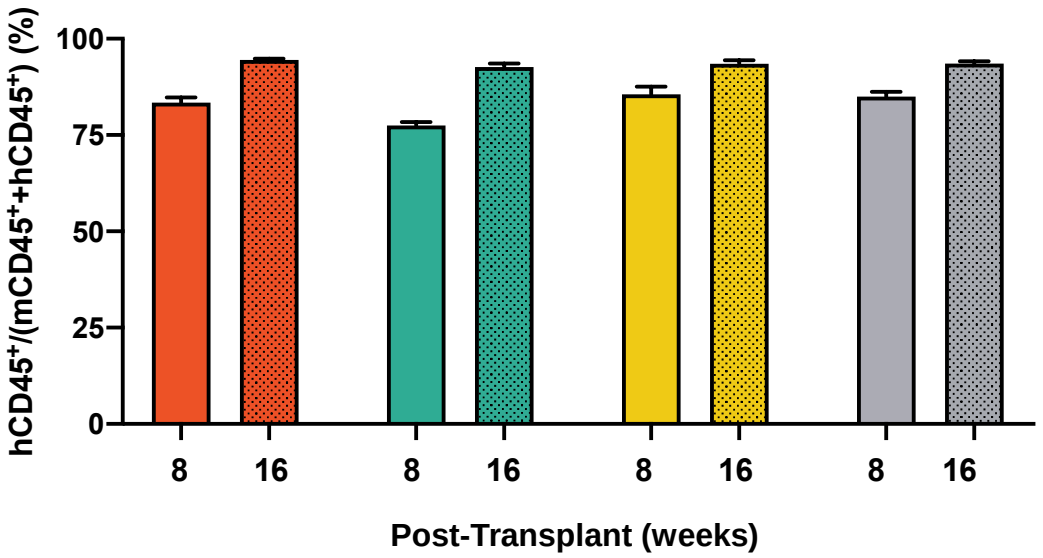
- >80% base editing at *HBG1/2* gene promoters achieved in *in vitro*-derived erythroid cells from a homozygous sickle cell disease (SCD) donor.
- >60% gamma globin protein levels (relative to total β-like globins) with a concomitant decrease in sickle β-globin.

Experimental Outline

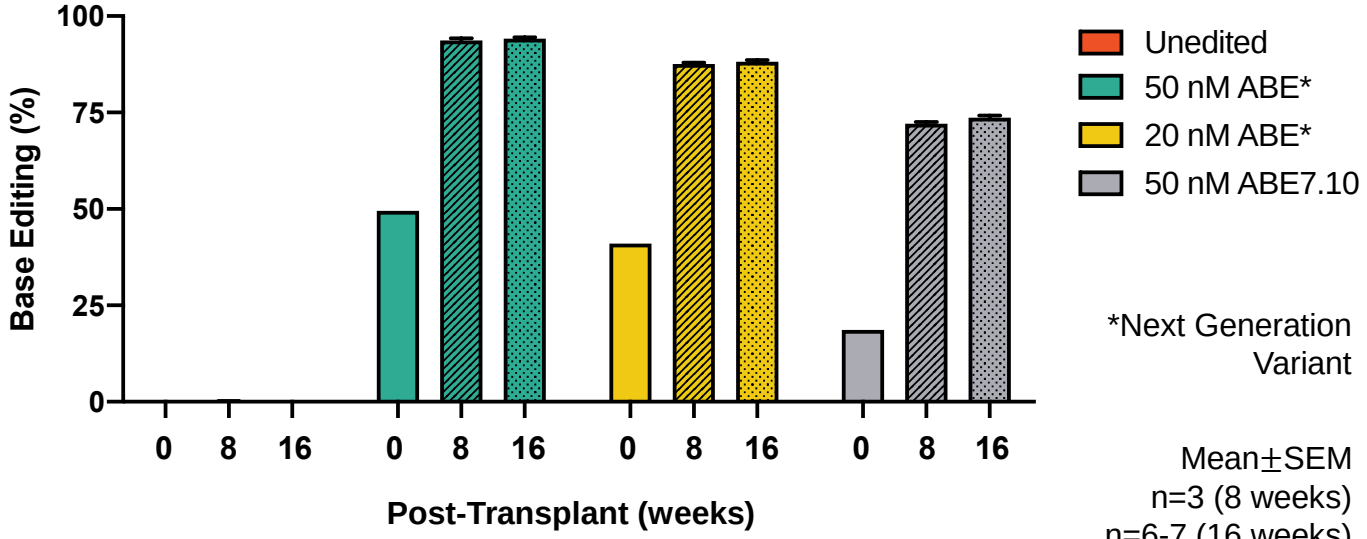


Human CD34+ HSPCs Retain Long-term Engraftment and *HBG1/2* Gene Promoter Base Editing *In Vivo*

Human Chimerism (%)



HBG1/2 Promoter Base Editing (%)

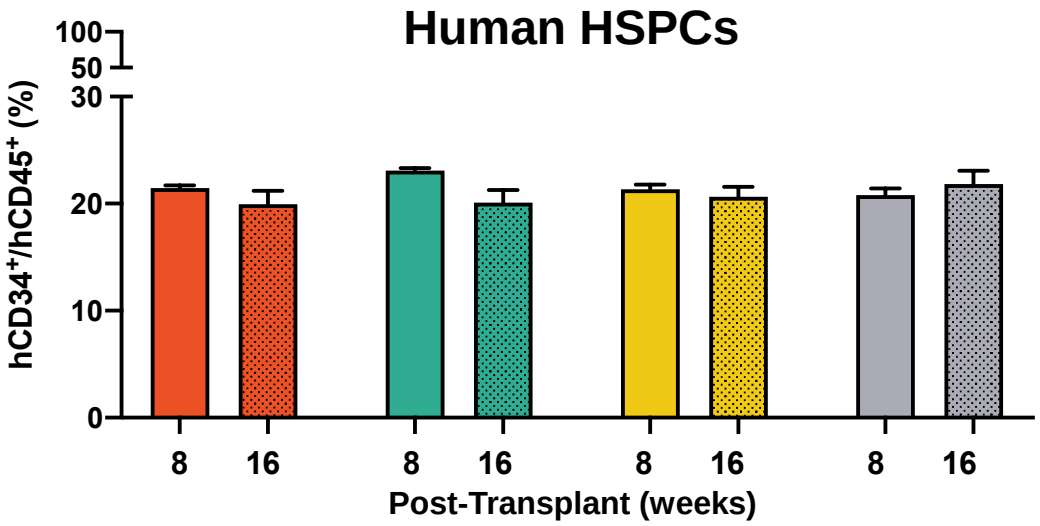


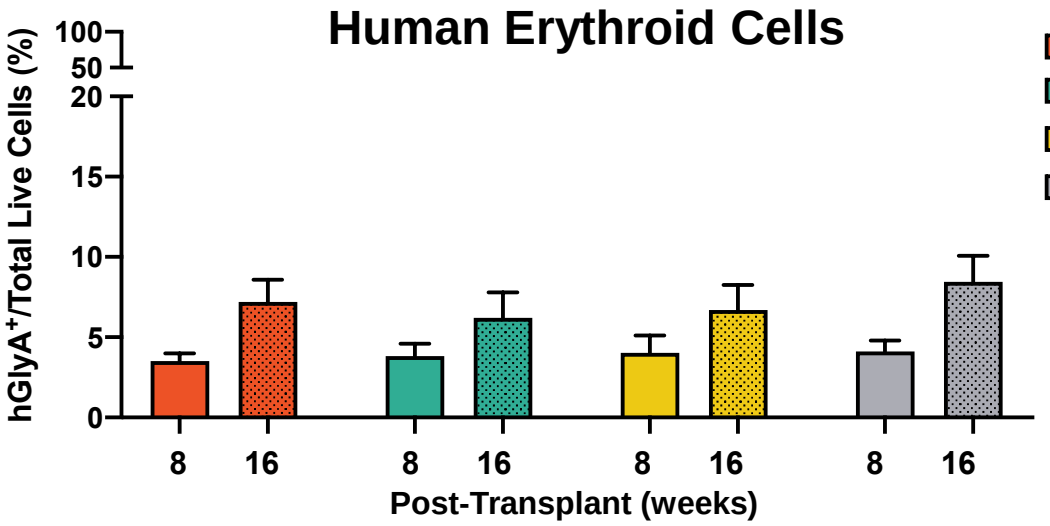
*Next Generation Variant

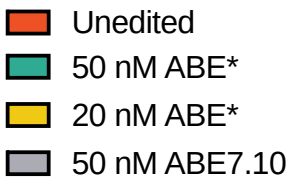
Mean±SEM
n=3 (8 weeks)
n=6-7 (16 weeks)

- >90% human chimerism and >90% base editing at *HBG1/2* gene promoters achieved in bone marrow samples at 16 weeks post-transplantation.

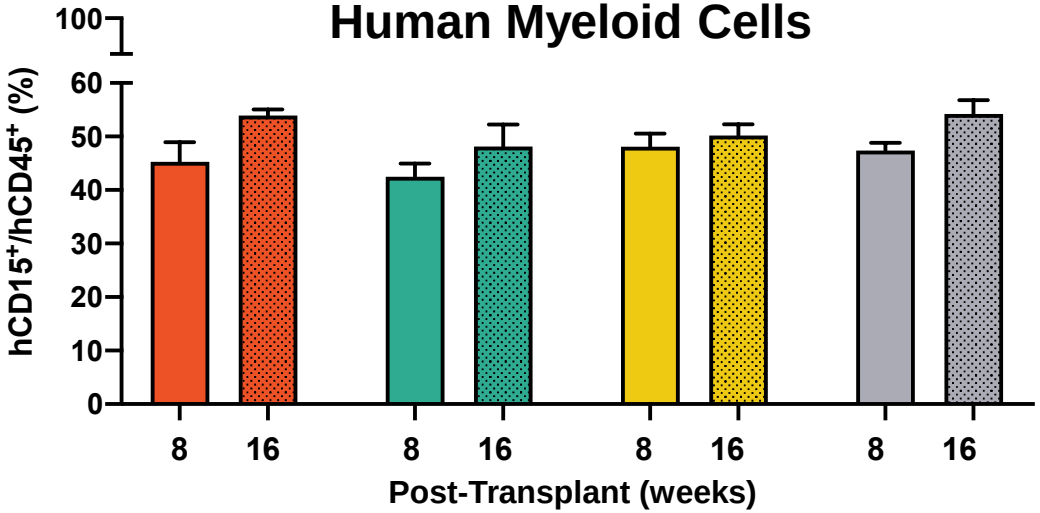
HBG1/2 Gene Promoter Edited CD34+ HSPCs Display Long-Term Multi-Lineage Hematopoietic Reconstitution *In Vivo*

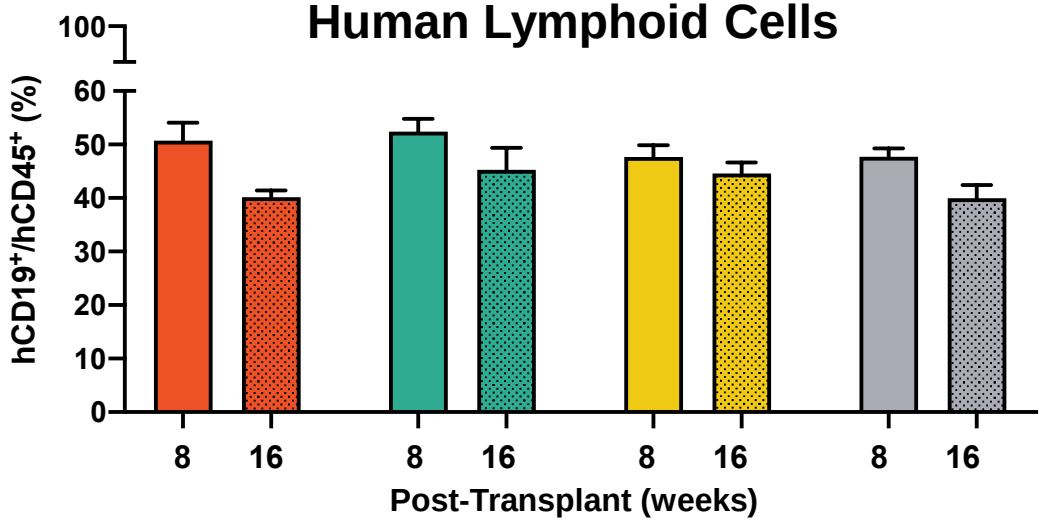






*Next Generation Variant

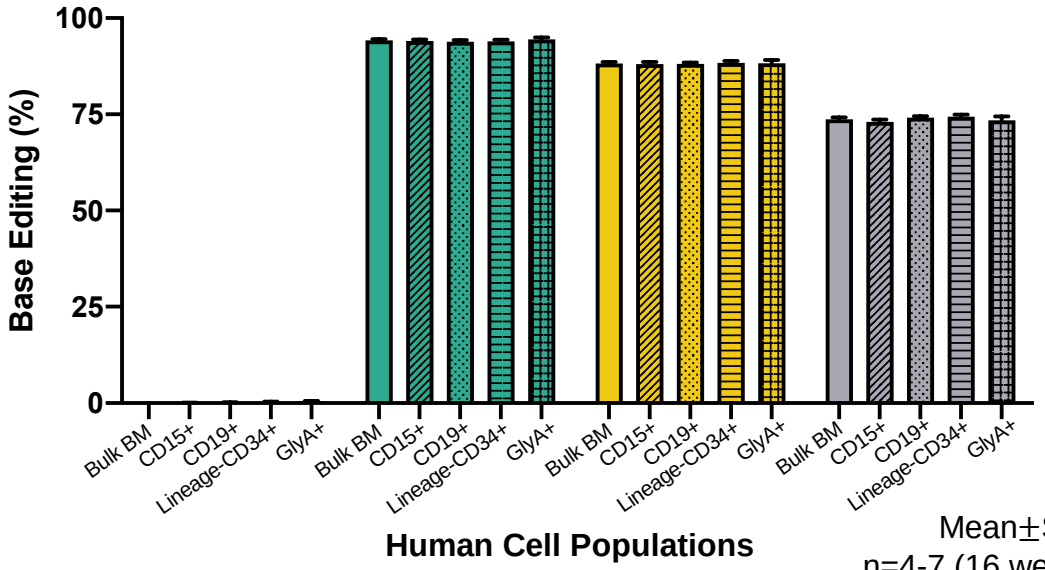




Mean±SEM
n=3 (8 weeks)
n=6-7 (16 weeks)

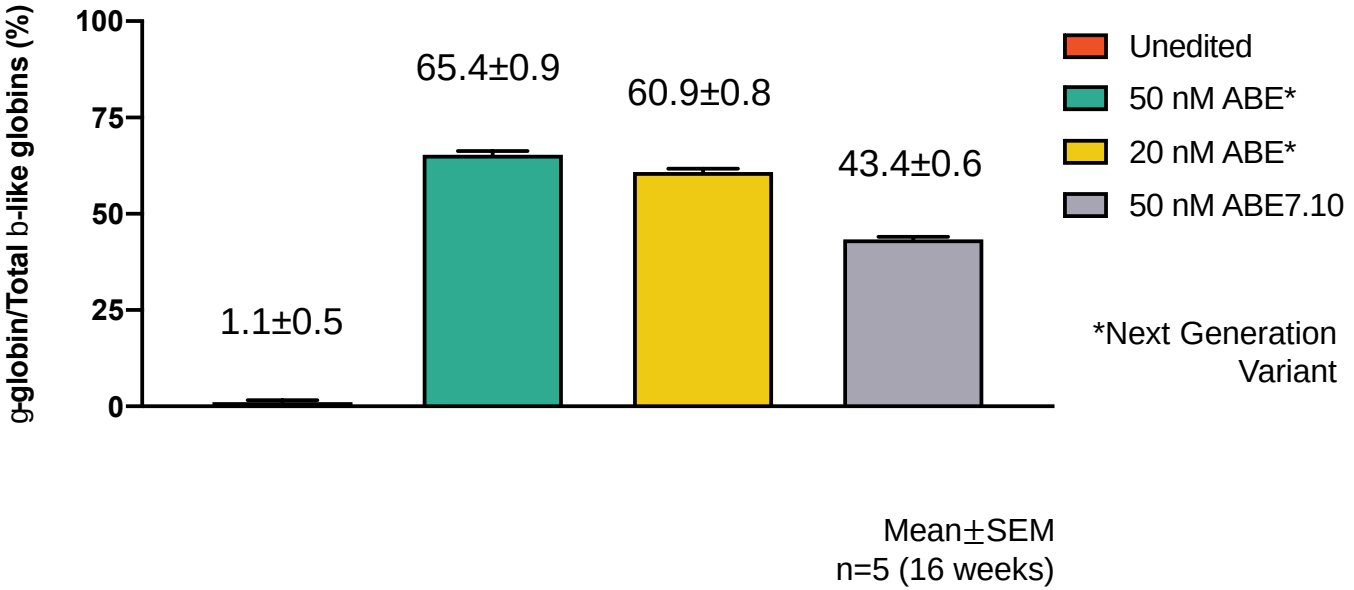
HBG1/2 Gene Promoter Base Editing is Maintained Long-Term Post-Engraftment with Elevated Gamma Globin Levels *In Vivo*

HBG1/2 Promoter Base Editing (%) (Sorted Bone Marrow Cells)



Mean±SEM
n=4-7 (16 weeks)

Gamma Globin Protein Levels (%) (Sorted Human Erythroid Cells)



Mean±SEM
n=5 (16 weeks)

- >90% base editing achieved in sorted human HSPCs, myeloid, lymphoid and erythroid cells at 16 weeks post-transplantation.
- >65% gamma globin protein levels expressed in sorted base edited erythroid cells compared to unedited cells.
- Similar human chimerism, *HBG1/2* promoter base editing and gamma globin protein upregulation has been achieved in a second mobilized CD34+ HSPC donor at 18 weeks post-transplantation.

Key Takeaways

✓ *In vitro* optimization

- Increased base editing of the *HBG1/2* gene promoters was achieved in mobilized CD34+ HSPCs in a dose-dependent manner using ABE mRNA and guide RNA.

✓ γ-globin upregulation

- Base editing highly correlated with γ-globin production (R^2=0.99) and suggests that >60% γ-globin protein induction could be achieved *in vitro*.

- SCD Patient Cells: >80% base editing was observed in erythroid cells *in vitro*, resulting in upregulation (>60%) of γ-globin protein levels with a concomitant decrease in sickle β-globin.

✓ *In vivo* performance

- Human CD34+ HSPCs retained long-term engraftment and >90% human chimerism, maintained >90% base editing at *HBG1/2* gene promoters, and displayed multi-lineage hematopoietic reconstitution.

- Base edited erythroid cell progeny produced high (>65%) γ-globin levels compared to unedited cells (<1.5%).

Thank you!

Please Visit Our Poster:

“A Novel Base Editing Approach to Directly Edit the Causative Mutation in Sickle Cell Disease”

- **Session Date:** Wednesday, May 13, 2020
- **Presentation Time:** 5:30pm - 6:30pm
- **Abstract number:** 808

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