Applied Base Editing to Treat Beta-Hemoglobinopathies

Nicole Gaudelli, PhD
Director, Head of Gene Editing Platform Technologies
ASH 2021

Disclosure

- I am a Beam employee and shareholder

Cautionary note regarding forward-looking statements

This presentation contains forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995. Such forward-looking statements include statements regarding: the initiation, timing, progress and results of preclinical studies and research and development programs, including the advancement of our pipeline, including the advancement of BEAM-101 and BEAM-102; and the therapeutic applications and potential of our technology, including our potential to develop life-long, curative, precision genetic medicines for patients through base editing, all of which are subject to known and unknown important risks, uncertainties and other factors that may cause our actual results, performance or achievements, market trends, or industry results to differ materially from those expressed or implied by such forward-looking statements. Therefore, any statements contained herein that are not statements of historical fact may be forward-looking statements and should be evaluated as such. Without limiting the foregoing, the words “anticipate,” “expect,” “suggest,” “plan,” “vision,” “believe,” “intend,” “project,” “forecast,” “estimates,” “targets,” “projections,” “potential,” “should,” “could,” “would,” “may,” “might,” “will,” and the negative thereof and similar words and expressions are intended to identify forward-looking statements.

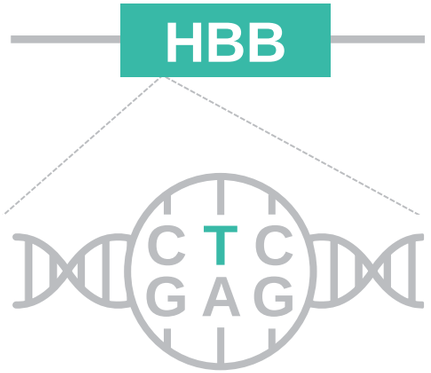
Each forward-looking statement is subject to important risks and uncertainties that could cause actual results to differ materially from those expressed or implied in such statement, including, without limitation, risks and uncertainties related to: our ability to develop, obtain regulatory approval for, and commercialize our product candidates, which may take longer or cost more than planned; our ability to raise additional funding, which may not be available; our ability to obtain, maintain and enforce patent and other intellectual property protection for our product candidates; the potential impact of the COVID-19 pandemic; that preclinical testing of our product candidates and preliminary or interim data from preclinical studies and clinical trials may not be predictive of the results or success of ongoing or later clinical trials; that initiation and enrollment of our clinical trials may take longer than expected; that our product candidates may experience manufacturing or supply interruptions or failures; risks related to competitive products; and the other risks and uncertainties identified under the headings “Risk Factors Summary” and “Risk Factors” and elsewhere in our annual report on Form 10-K for the year ended December 31, 2020, our Quarterly Report on Form 10-Q for the quarter ended March 31, 2021, our Quarterly Report on Form 10-Q for the quarter ended June 30, 2021, our Quarterly Report on Form 10-Q for the quarter ended September 30, 2021, and in any subsequent filings with the Securities and Exchange Commission (the “SEC”) which are available on the SEC’s website at www.sec.gov. Additional information will be made available by our annual and quarterly reports and other filings that we make from time to time with the SEC. These forward-looking statements speak only as of the date of this presentation. Factors or events that could cause our actual results to differ may emerge from time to time, and it is not possible for us to predict all of them. We undertake no obligation to update any forward-looking statement, whether as a result of new information, future developments or otherwise, except as may be required by applicable law.

.

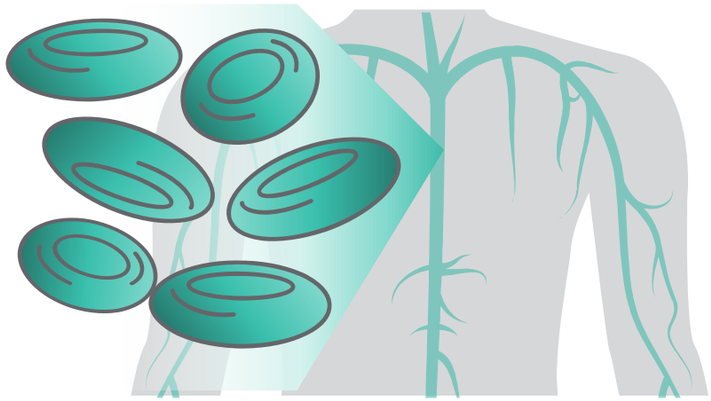
Sickle Cell Disease (SCD)

β-globin gene

Adult β-globin gene



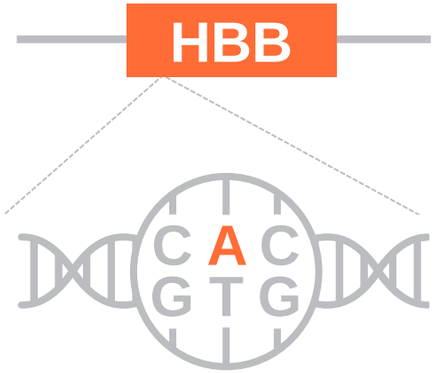
Glutamic acid at 6th amino acid (HbA)



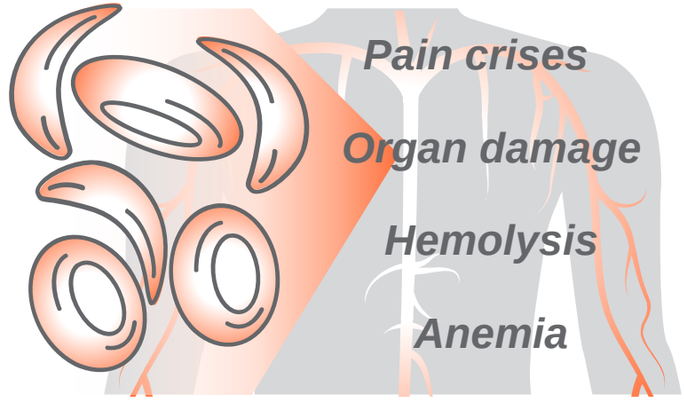
Normal red blood cells

T-to-A mutation causes sickling

Sickle β-globin gene



Valine at 6th amino acid (HbS)



Sickling red blood cells

Approximately 100,000 sickle cell disease patients in the US

Spectrum of challenges for SCD patients

Transplant or cell therapy

Primary disease impact

Pain crises, hemolysis, anemia, organ damage, diminished quality of life, early mortality.

Non-genotoxic conditioning

Conditioning toxicities & risks

Risks caused by alkylating agents, possibility of graft failure, transplant related mortality.

In vivo delivery to HSCs

Overall transplant challenges

Transplants not widely available, require long hospital stay, involve cumbersome process.

Can we create precision genetic medicines to address these challenges?

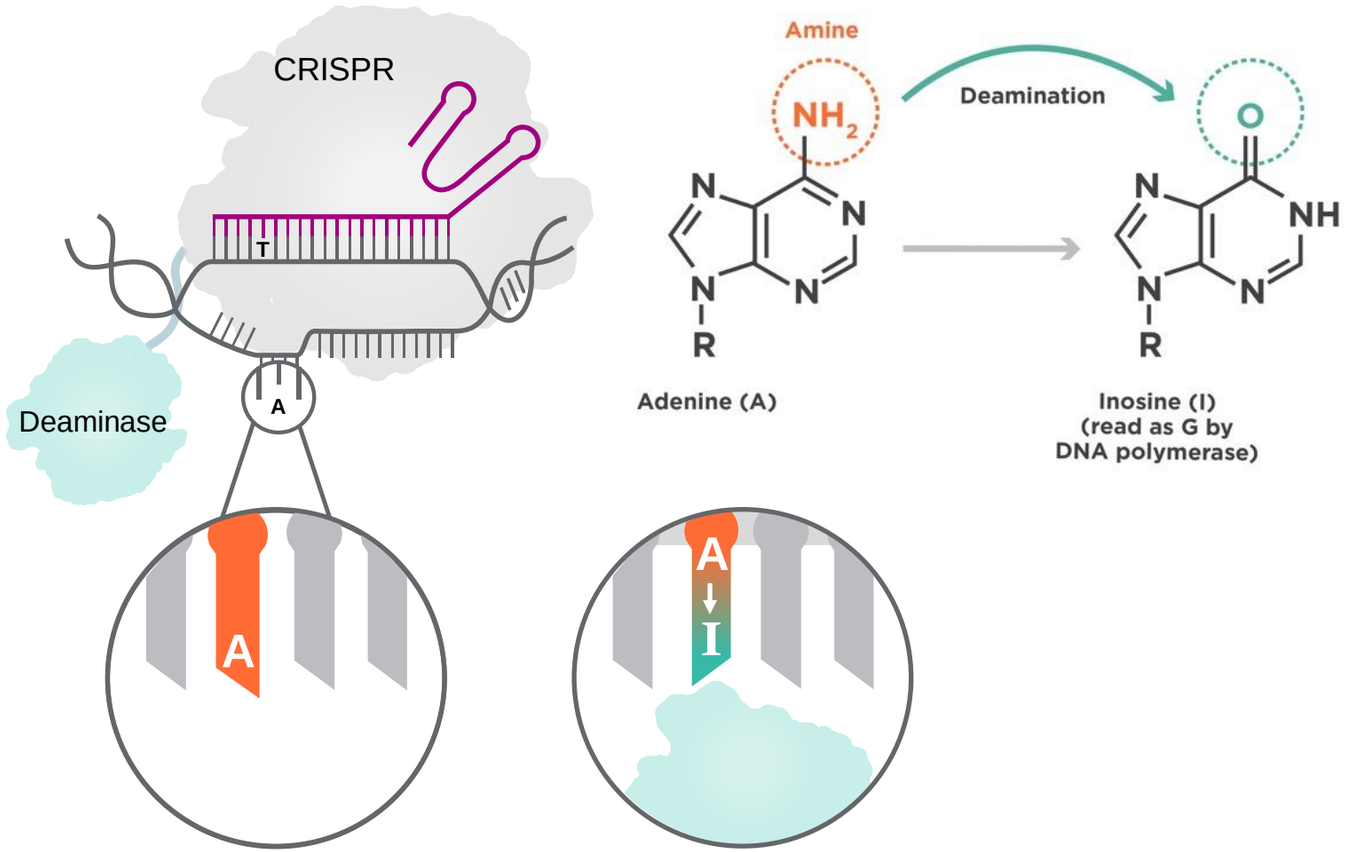
Long term development strategy to potentially cure SCD

	Wave 1 Base Editing + HSCT	Wave 2 Improved Conditioning	Wave 3 In vivo LNP Delivery
Goals	Non-dsDNA break cutting, non-viral, precise genotype correction	Less toxic, targeted conditioning	In vivo editing (infusion) replaces transplant
Required technologies	BEAM-101/BEAM-102 Base editing (ex vivo)	Antibody conditioning	HSC-targeted LNP (Please see poster 2931)

Well-positioned to potentially create improved regimens for patients, now and in the future

LNP = Lipid Nanoparticle; HSC = Hematopoietic Stem Cell; HSCT = Hematopoietic Stem Cell Transplant;

Adenine Base Editing Technology

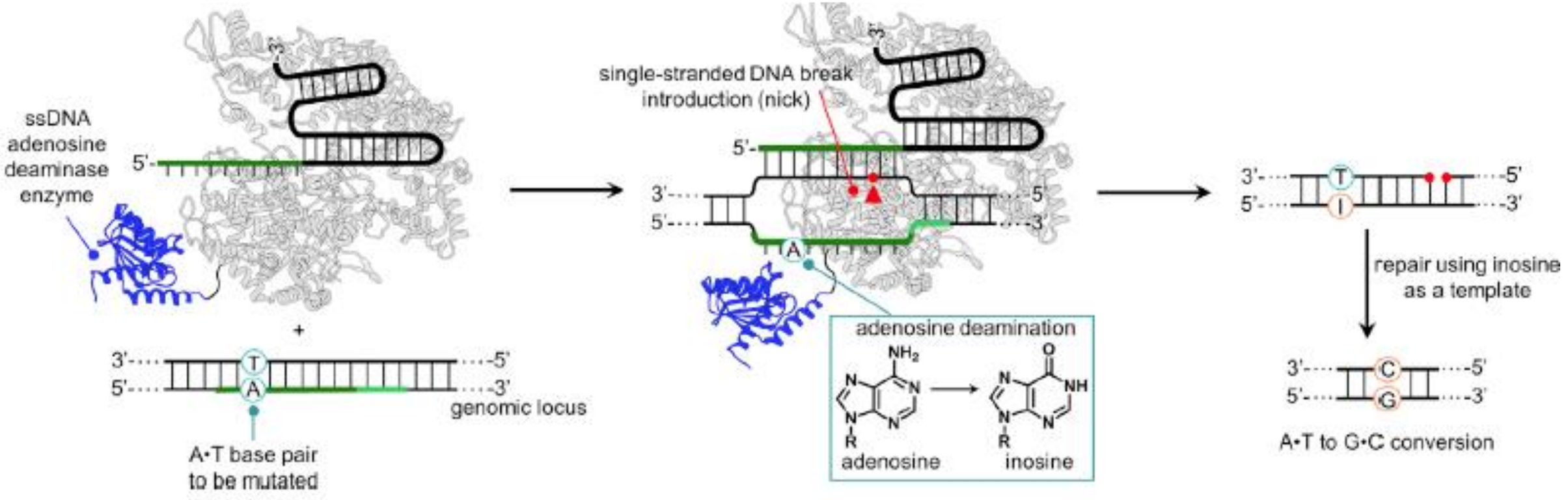


- Adenine Base Editor (ABE) comprises a deaminase enzyme fused to catalytically impaired CRISPR protein.
- Guide RNA (gRNA) directs the ABE to a target genomic DNA sequence and exposes the editing window.
- Deaminase chemically converts target adenine (A) to inosine (I) via deamination.
- Two types of off-target events possible that we must characterize: guide-dependent and guide-independent

Komor, A. C. et. al. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* **533**, 420-424 (2016)

Gaudelli, N. M. *et al.* Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. *Nature* **551**, 464-471 (2017)

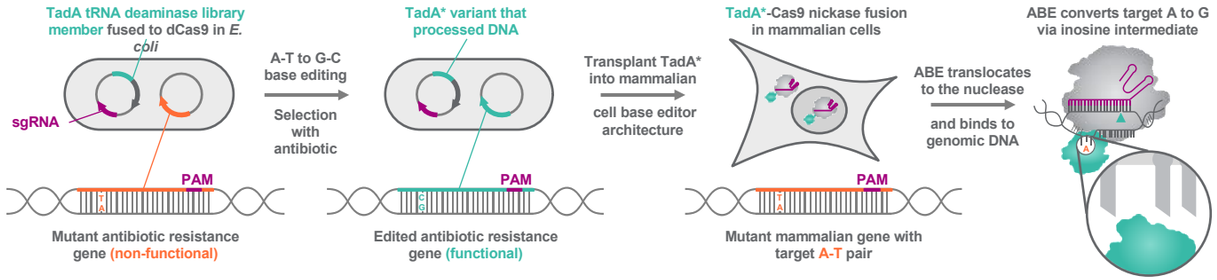
Adenine Base Editing: programmable single base editing without double-stranded breaks

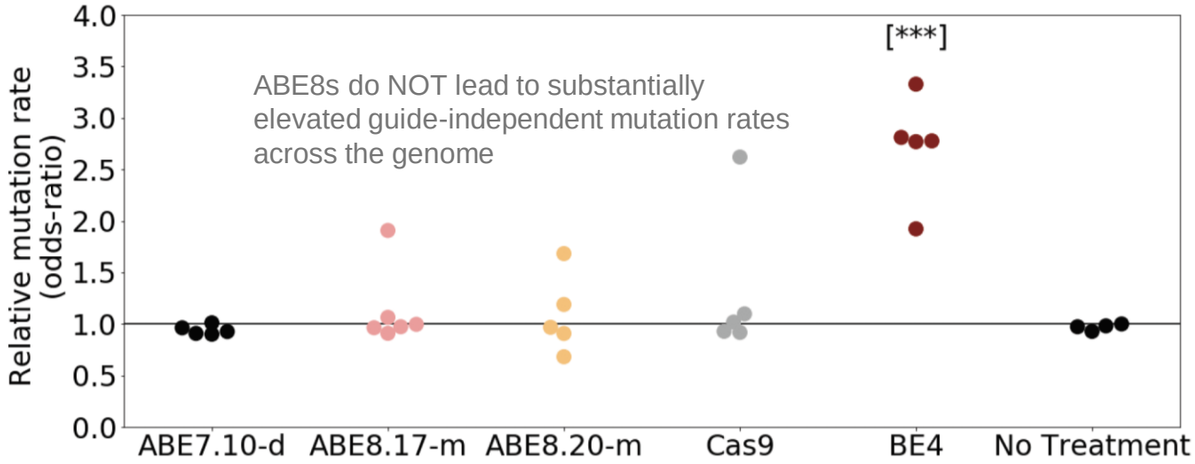


Base editors require a nearby PAM recognition sequence, catalyze deamination on ssDNA, and operate within an activity window

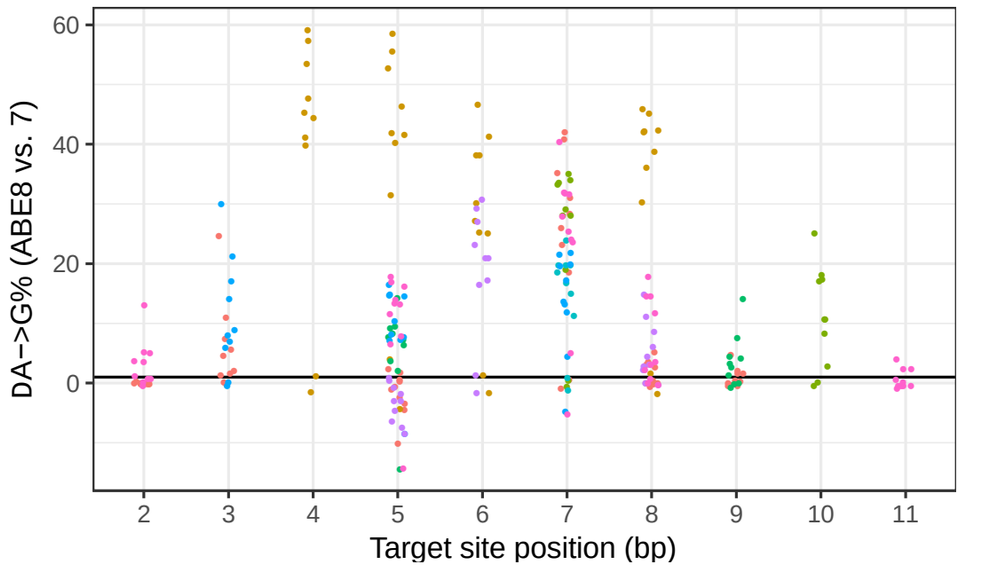
Gaudelli, N. M. *et al.* Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. *Nature* **551**, 464-471 (2017)
Rees, et/ al. CRISPR-derived genome editing therapies: progress from bench to bedside. *Molecular Therapy* **29**, (2021)

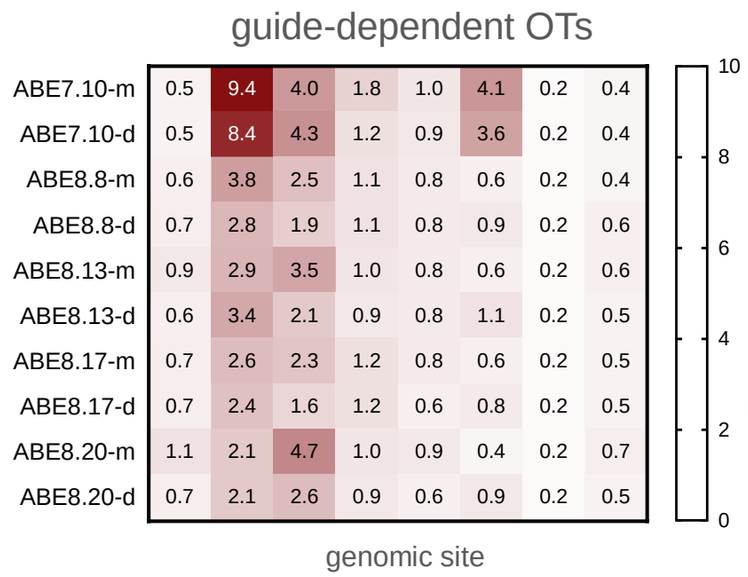
Next-generation ABEs (ABE8s) evolved to have higher on-target activity than ABE7.10 and maintained no observable guide-independent off-targets genome-wide

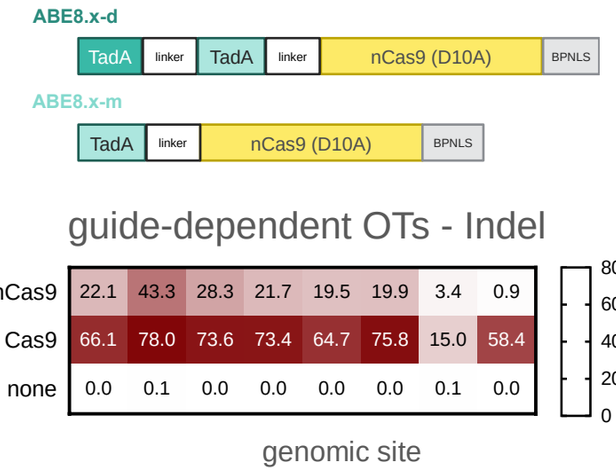




ABE8s all significantly outperform ABE7.10 at all genomic sites tested (P-value = 0.0006871, two-tailed Wilcoxon rank sum test)



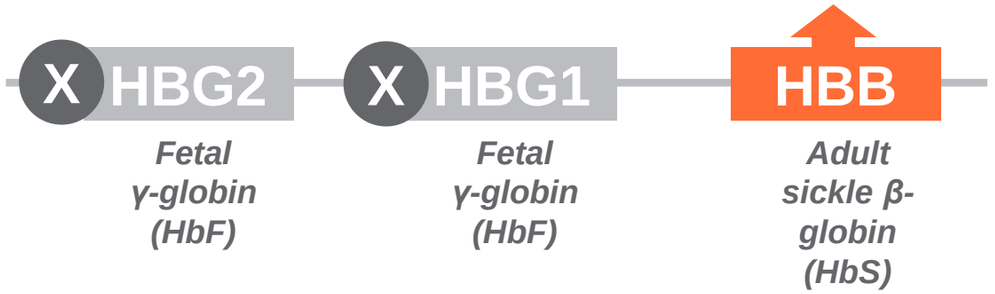




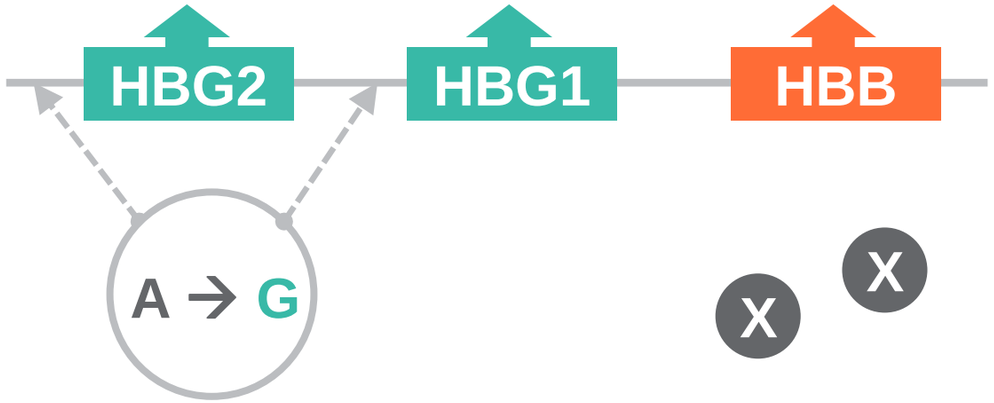
Gaudelli, N.M., Lam, D.K., Rees, H.A. *et al.* *Nat Biotechnol* **38,** 892–900 (2020).

BEAM-101: Recreating Hereditary Persistence Of Fetal Hemoglobin (HPFH) With Base Editing

Sickle cell disease patient



Reactivating fetal hemoglobin genes



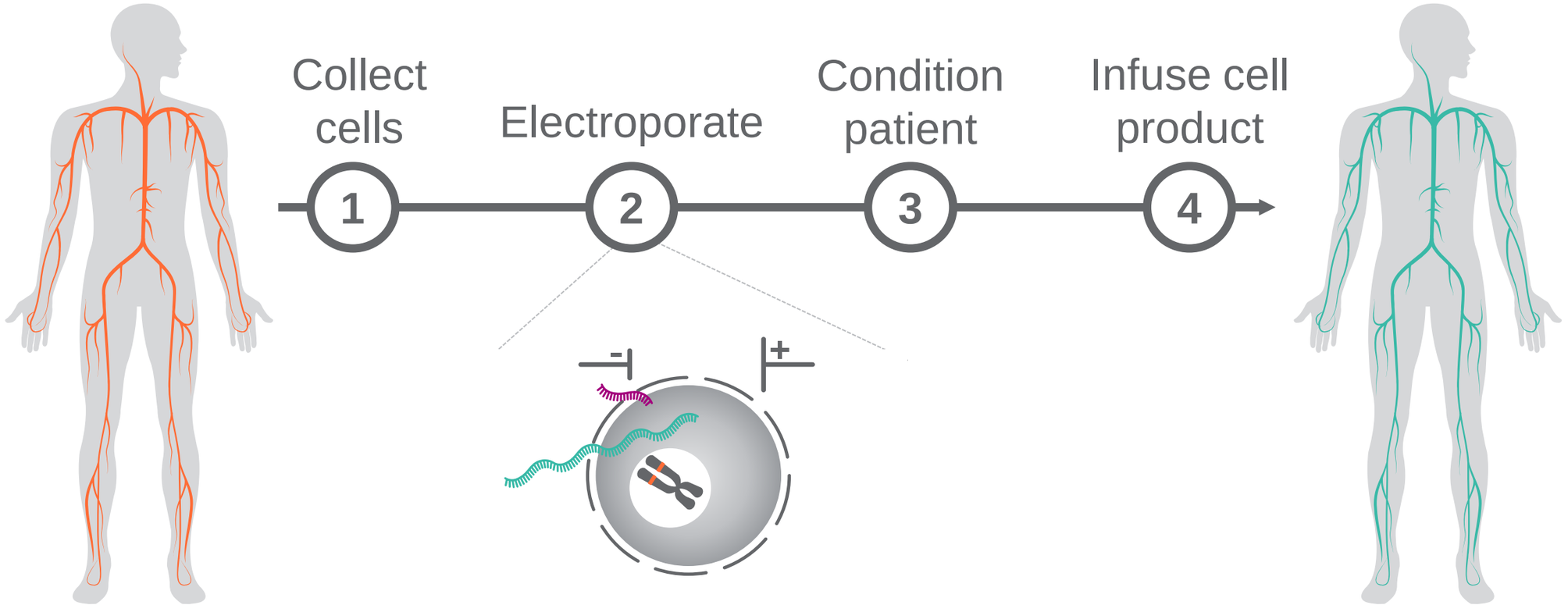
ABE edits regulatory element of <u>both</u> fetal hemoglobin genes, without cutting DNA

Repressors unable to bind but are otherwise unaffected

- Naturally-occurring base changes cause Hereditary Persistence of Fetal Hemoglobin (HPFH), which protect patients from SCD/B-Thal
- Base editors can be designed to reproduce these changes, leading to elevated levels of fetal hemoglobin
- Higher fetal hemoglobin likely to correlate with further reductions in disease activity by inhibiting HbS polymerization

Mussallam, et al. 2012. *Blood.*

Autologous *ex vivo* cell process for editing hematopoietic stem cells



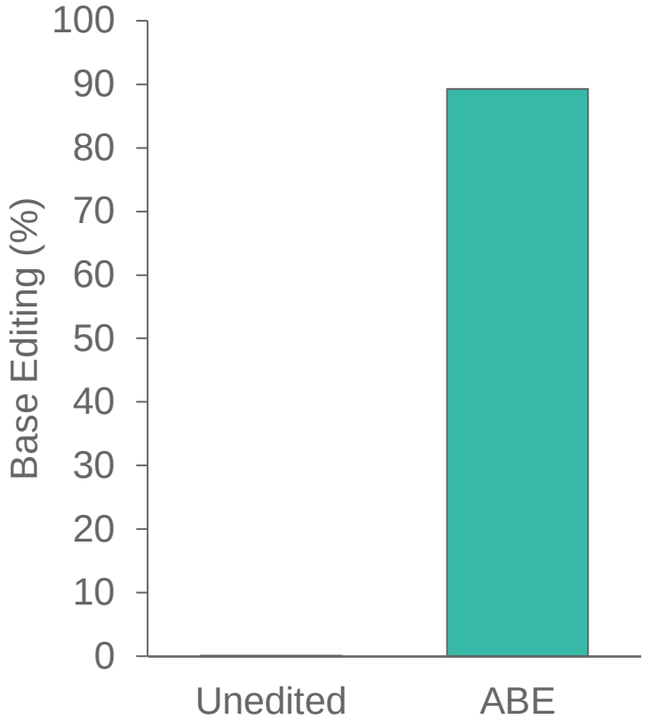
Sickle Cell Disease Patient

Program-specific gRNA and ***base editor mRNA***

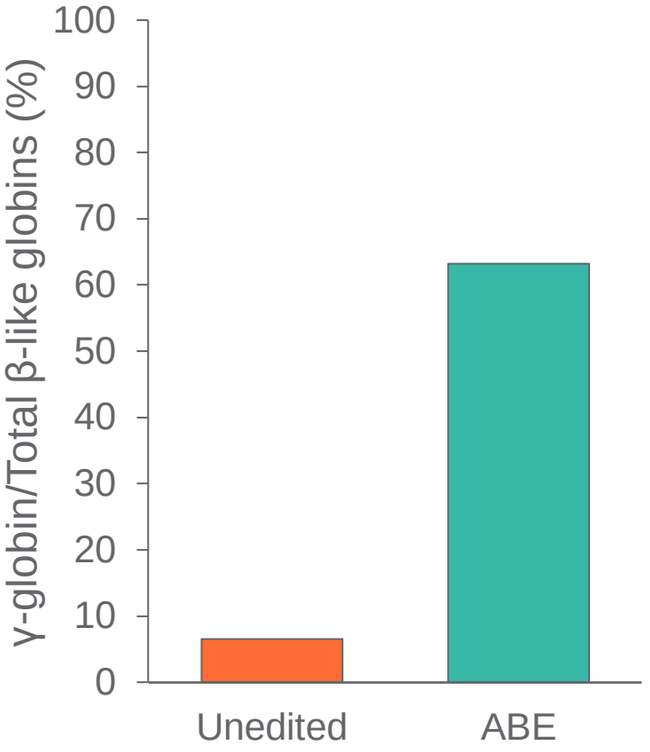
Sickle Cell Disease Patient

BEAM-101: Robust base editing at HBG1/2 gene promoters in sickle cell disease patient cells

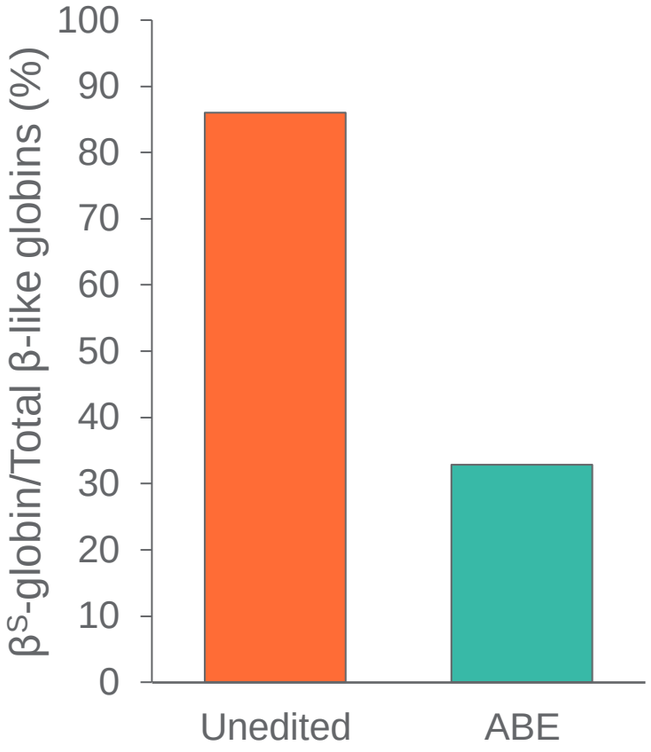
>80% base editing at HBG1/2 promoters



>60% levels of HbF gamma globin protein



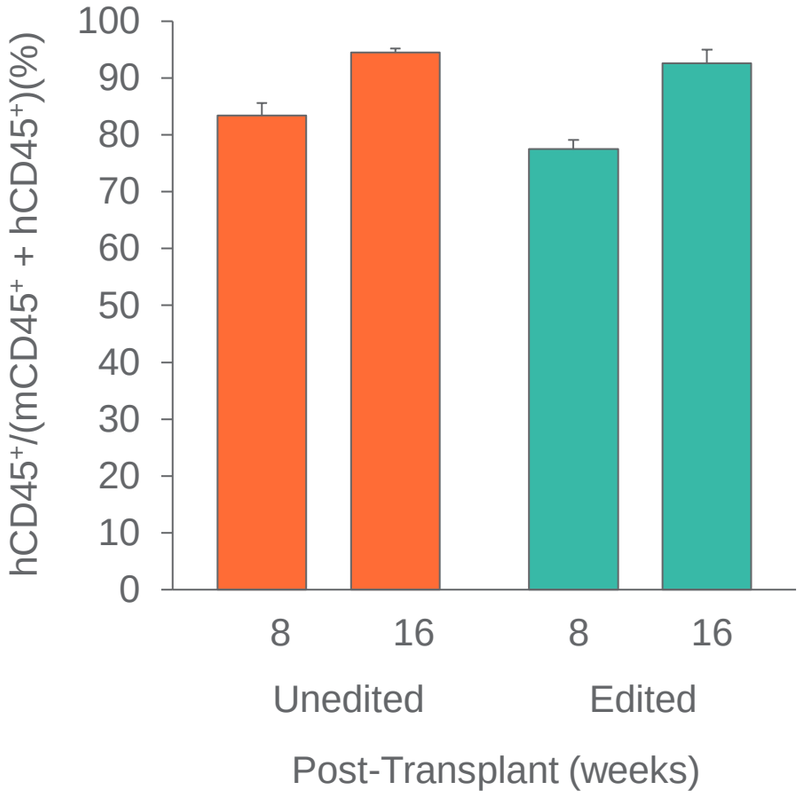
<40% levels of HbS sickle protein



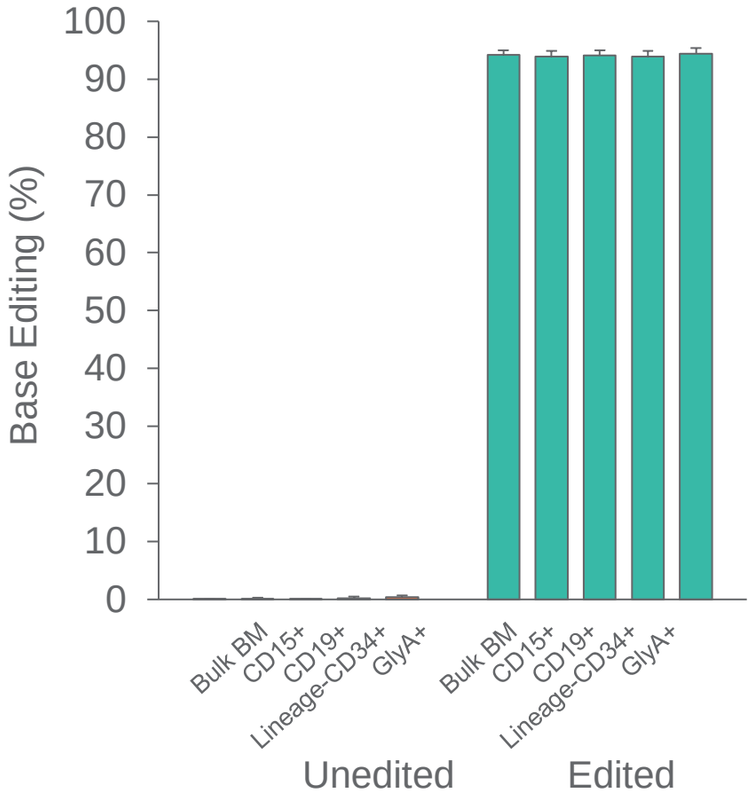
Experiments shown are *in vitro*-derived erythroid cells

BEAM-101: High levels of editing and robust HbF induction after long-term *in vivo* engraftment

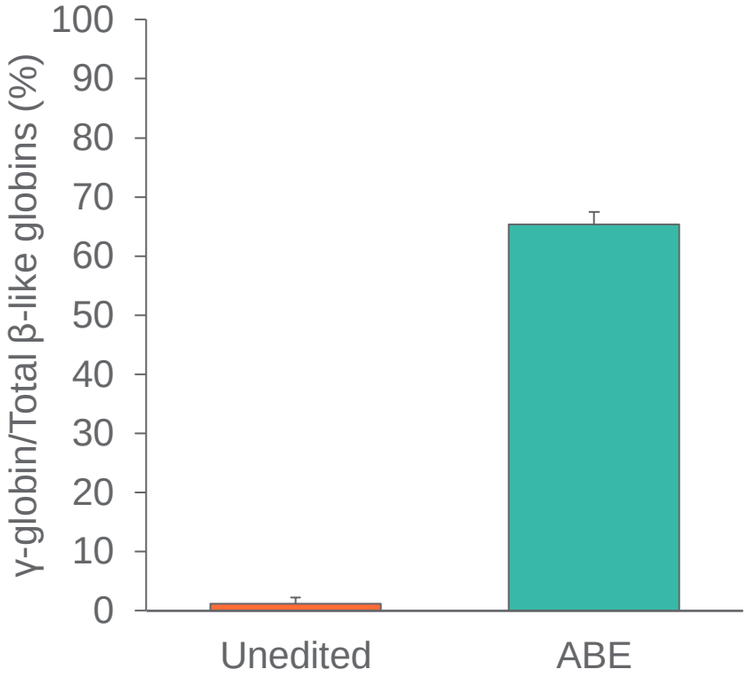
>90% human chimerism in bone marrow 16 weeks post-transplant[a]



>90% base editing at *HBG1/2* promoters in multilineage cells[b]

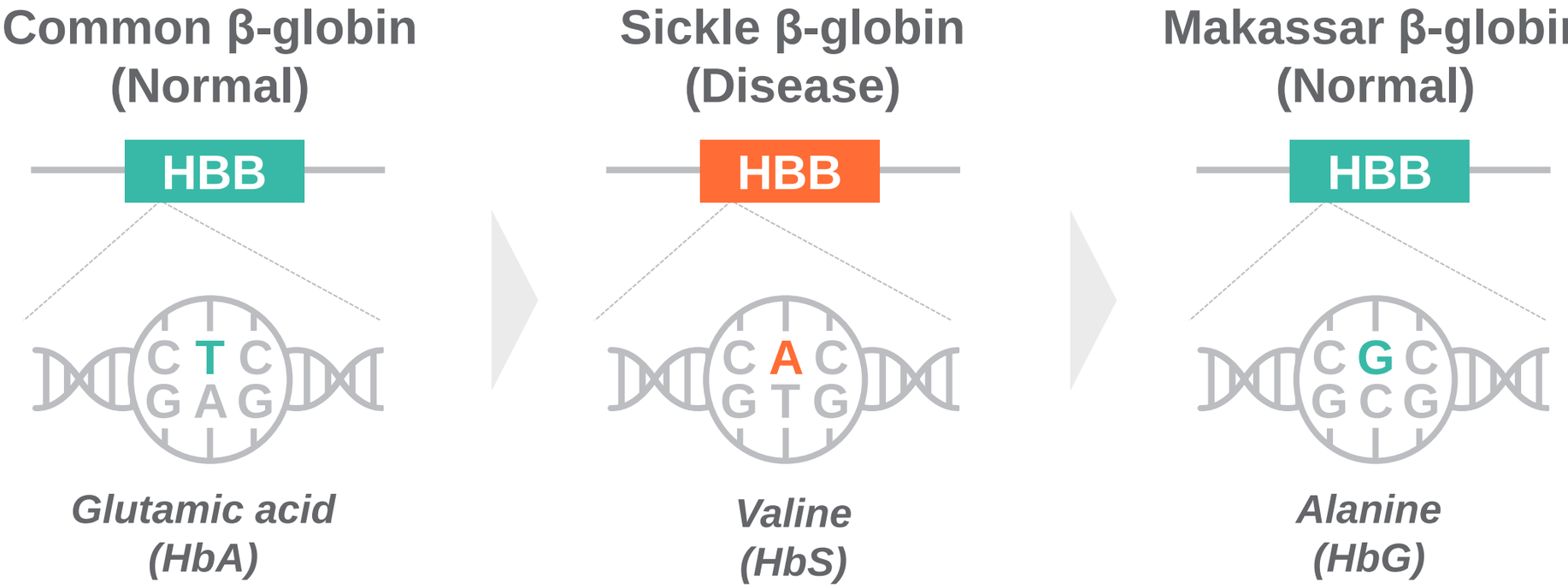


>65% gamma globin protein levels in sorted erythroid cells[c]



*Next Generation Variant; a. Mean±SEM; n=3 (8 weeks); n=6 (16 weeks); b. Mean±SEM; n=4-6 (16 weeks); Sorted human HSPCs (Lineage-CD34+), myeloid (CD15+), lymphoid (CD19+) and erythroid (GlyA+) cells (derived from BM samples) at 16 weeks post-transplantation; c. Mean±SEM; n=5 (16 weeks)

BEAM-102: Direct correction of the sickle causing mutation

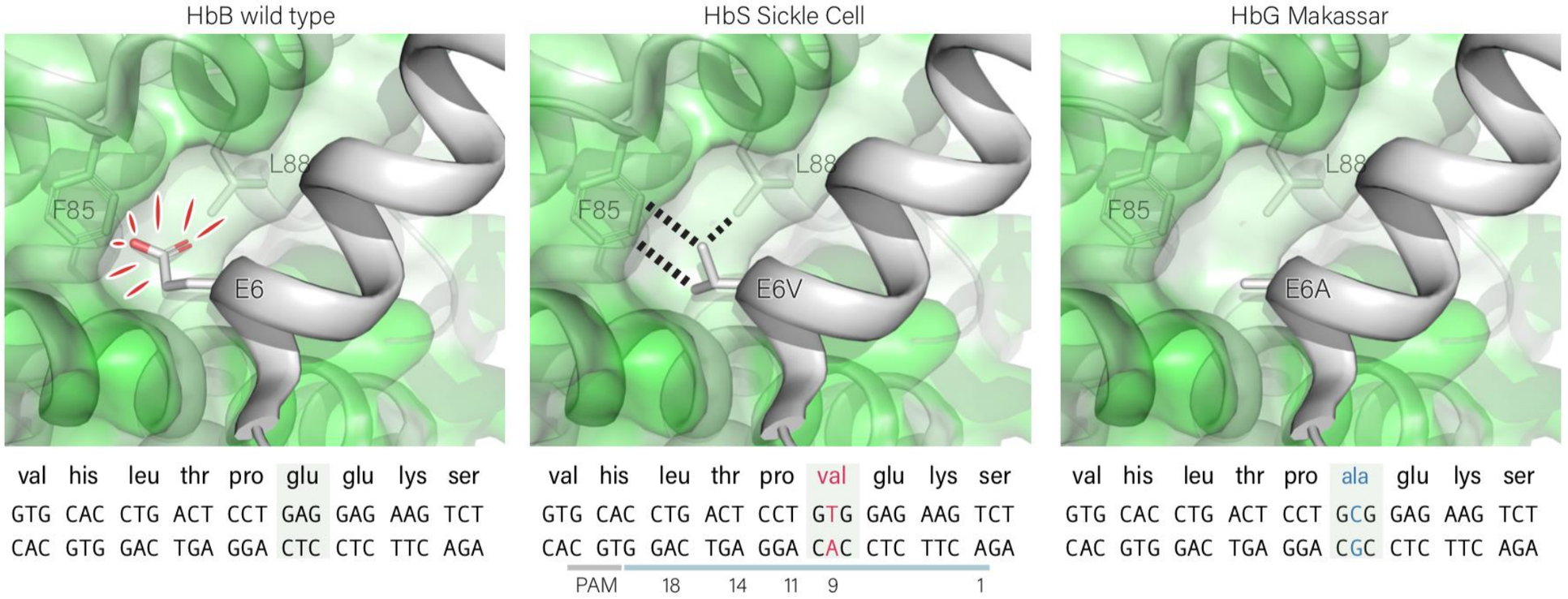


- Base editing recreates naturally-occurring human variant Hb-G Makassar which has alanine (E6A) instead of sickle-causing valine (E6V)[1]
- Hb-G Makassar is a normal β-globin variant and does not cause sickle disease, e.g., blood smear shows negative for sickle cells[2]

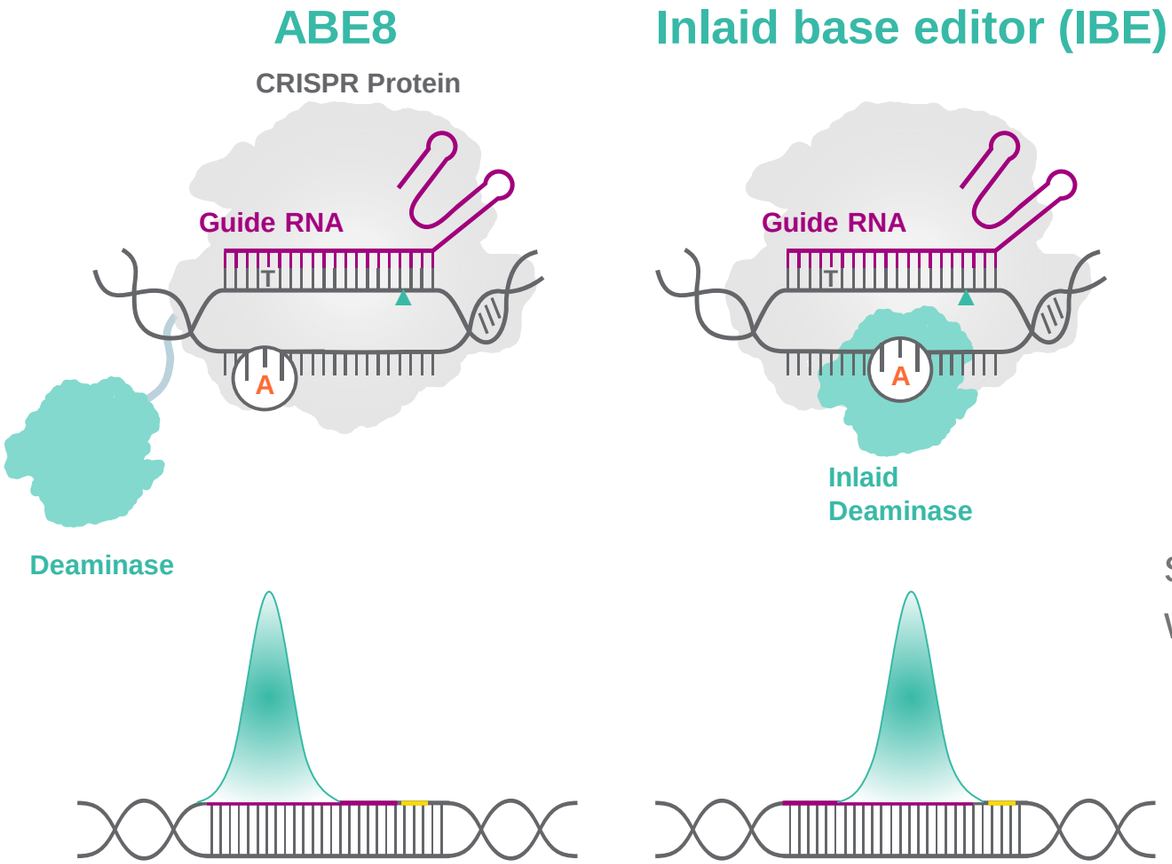
1. Cummings, MR. 2006. Human Heredity: Principles and Issues, Updated Edition.; 2. Mohamad, et al. 2018. Hematology Reports.

Three Major Challenges for a Base Editing Strategy to convert HbS to HbG-Makassar

1. There exists no NGG sequence proximal to the target A — Engineered and evolved PID to create an “NGC” tolerant PAM
2. Off-the-shelf ABE7.10 didn’t yield desirable levels of editing in preliminary studies — Directed Evolution to create ABE8
3. Needed flexibility in ABE activity window to unlock the use of NG PAM landing pads — Re-arrange ABE architecture – “IBEs”



BEAM-102 editor is a structural variant of ABE that shifts editing window to enable editing of the sickle allele



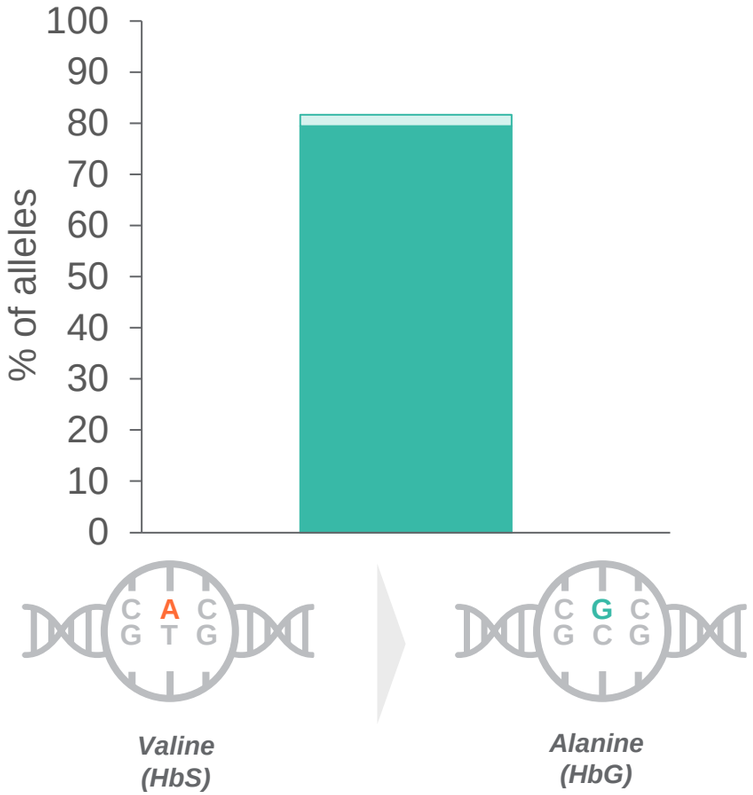
sickle adenine at position 9 in activity window of NGC PAM

5′-ACTTCTCCACAGGAGTCAGGTGCACCATG-3′

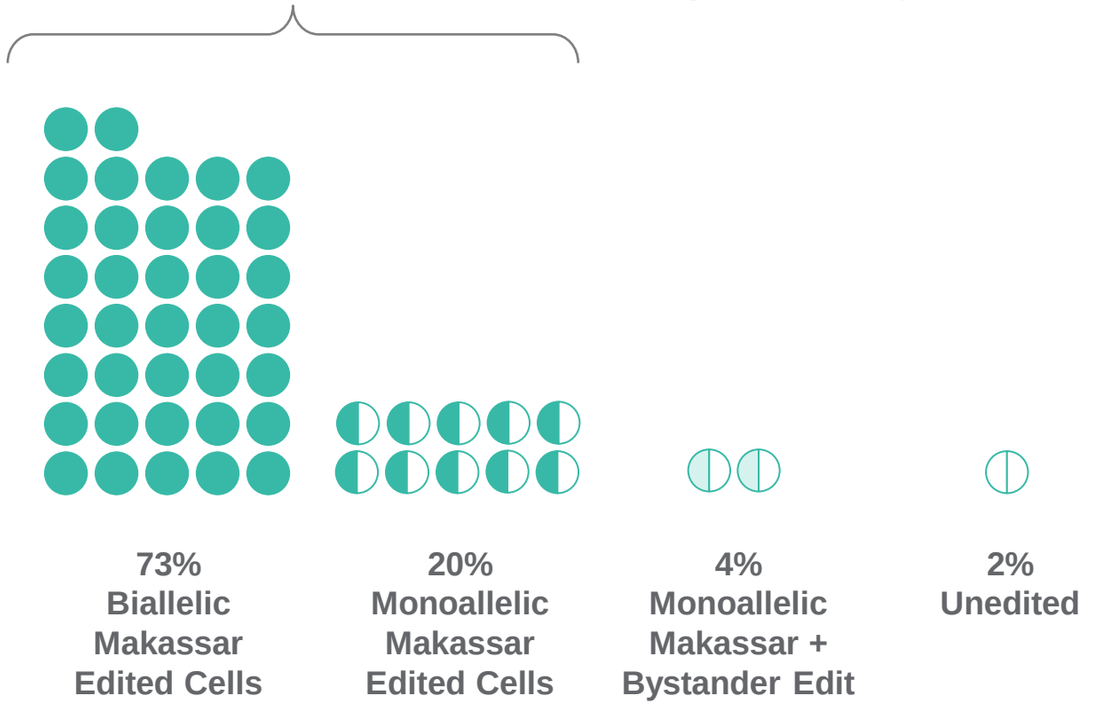
Chu, S. H. *et al.* Rationally Designed Base Editors for Precise Editing of the Sickle Cell Disease Mutation. *CRISPR J* **4**, 169-177 (2021).

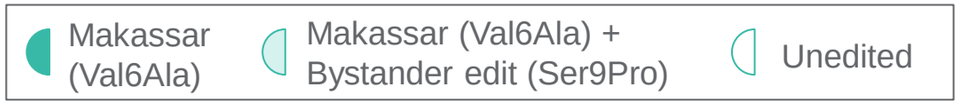
BEAM-102: Highly efficient, novel direct correction of sickle mutation in sickle patient cells

80% Sickle → Makassar correction in sickle patient CD34 cells with ABE (N=100)



~93% of cells have at least one sickle allele converted to Makassar and are potentially cured



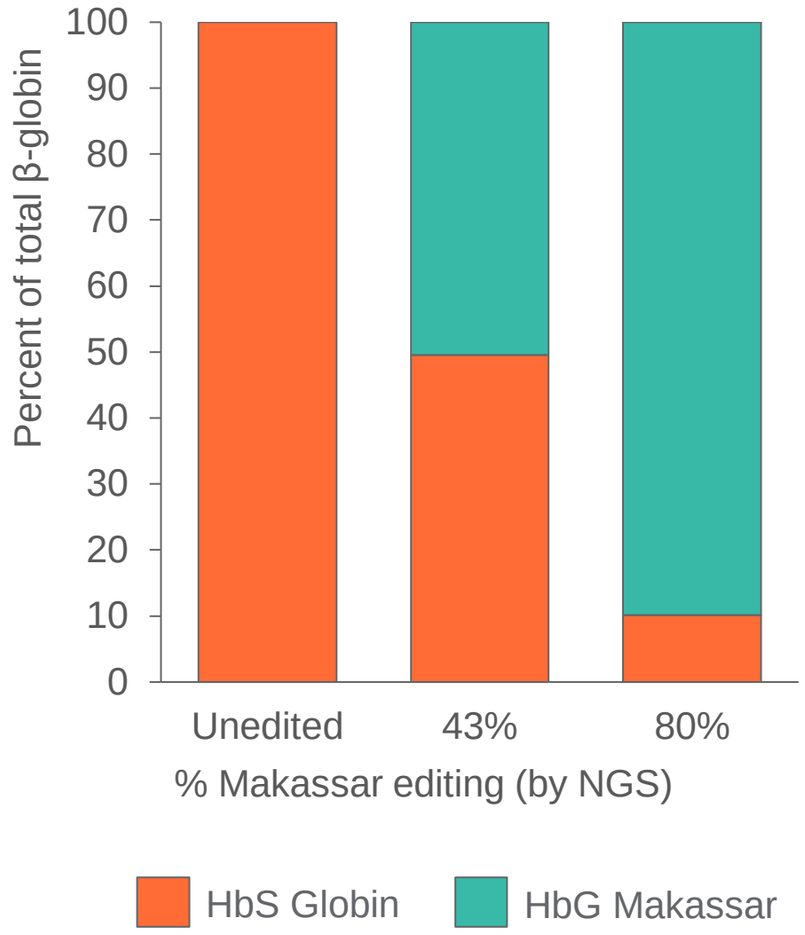


Allelic editing of 100 individual erythroid colonies post-electroporation was assessed for the Makassar edit by NGS using cells from HbSS donor.

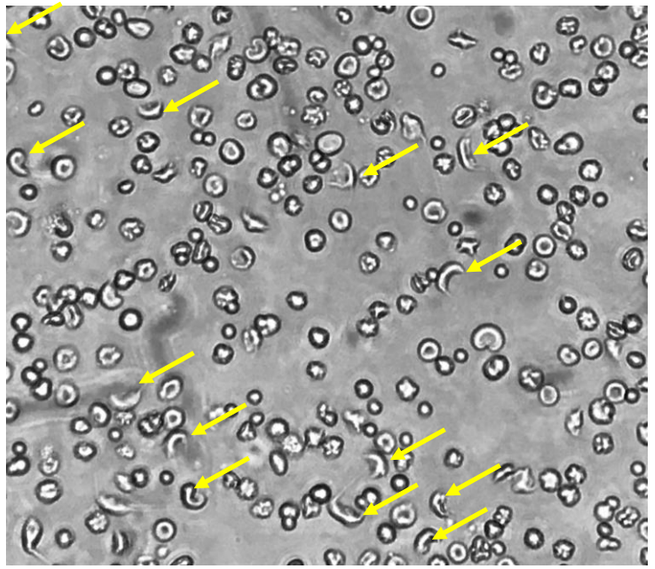
Makassar editing leads to reduced HbS in a dose-dependent manner and reduced sickling under hypoxia

Beam THERAPEUTICS

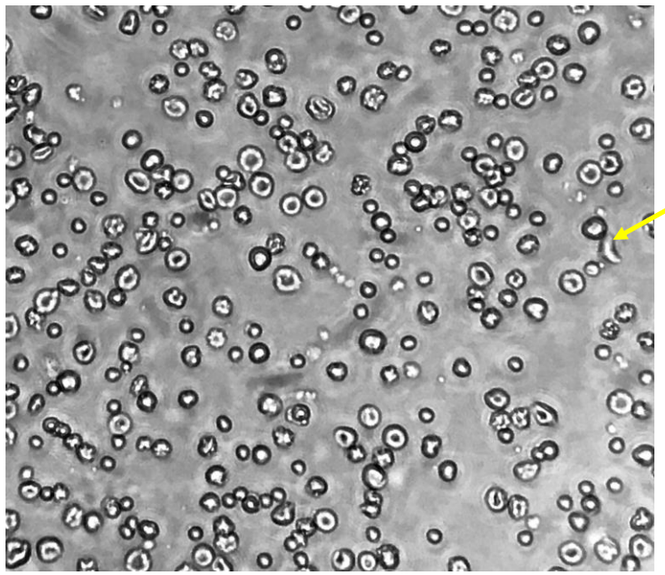
Elimination of HbS globin



Sickling in unedited HbSS cells

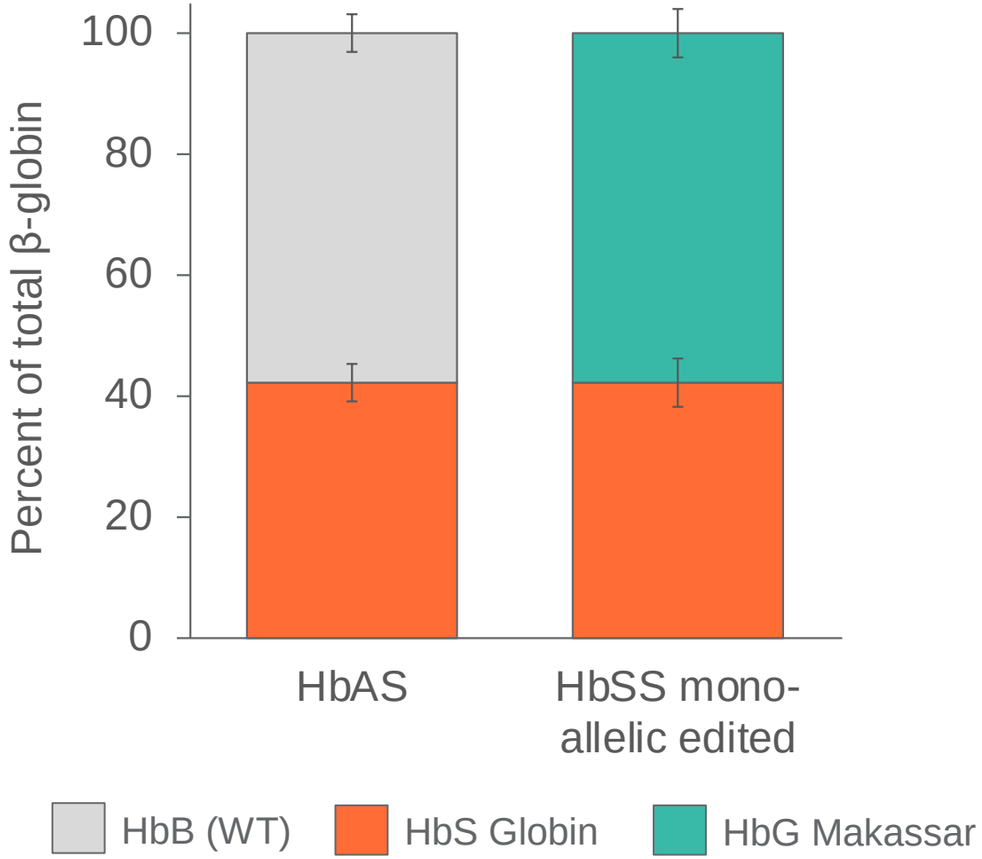


~89% Hb G-Makassar by UPLC

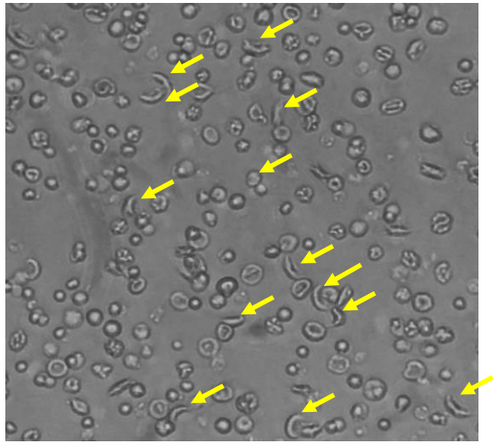


*UPLC and LC-MS peptide mapping assays to measure abundance have been developed

Mono-allelically Makassar edited HbSS IVED cells have similar sickle globin protein levels to HbAS

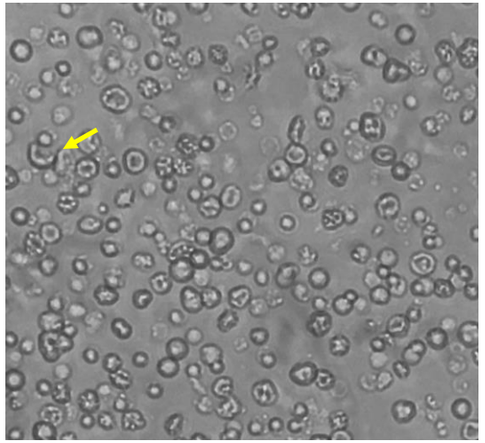


Unedited HbSS

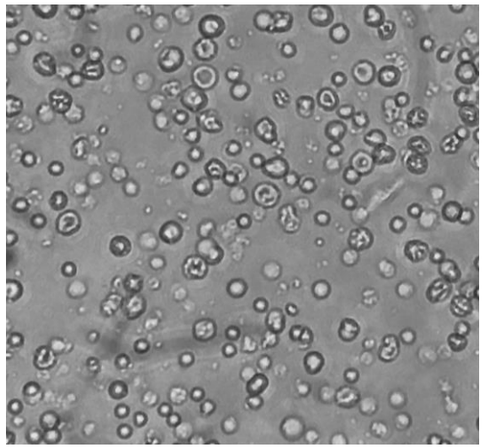


IVED cells derived from HbSS CD34+ exposed to 2% hypoxia (single clones)

mono-allelic edited

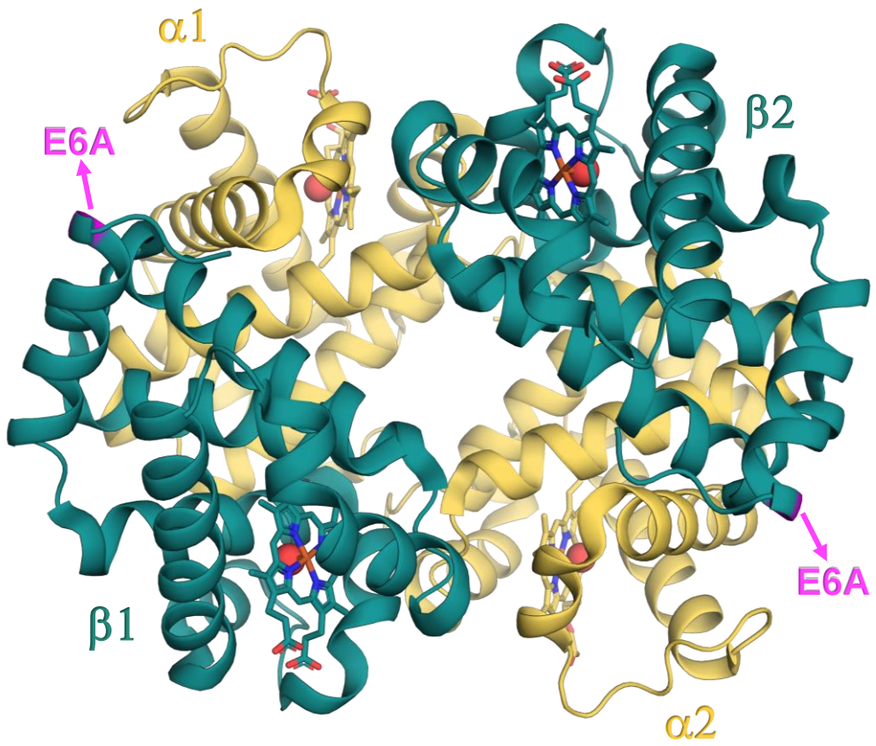


bi-allelic edited

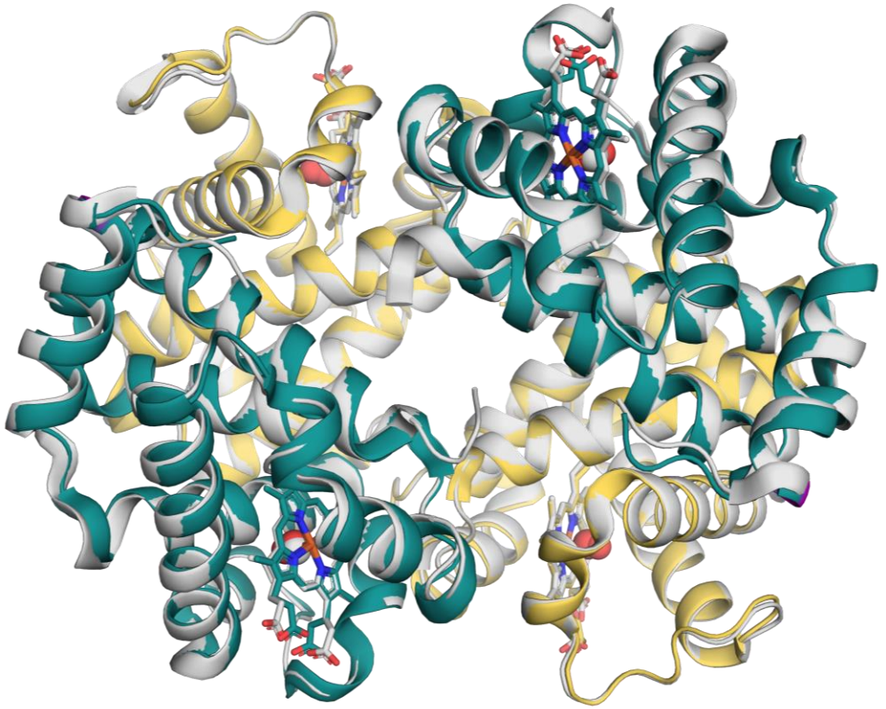


≤ 40% HbS on a per cell basis in >90% of erythroid cells → average per cell HbS level of ~10%

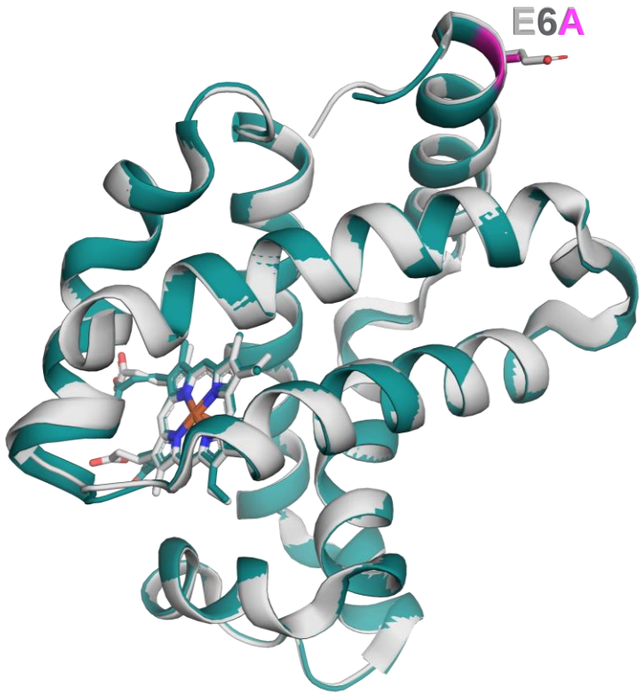
Overall structure of HbG is similar to HbA



Hb G-Makassar structure at 2.2-Å resolution



Superposition of Hb G-Makassar (yellow and green) and R2-state HbA (PDB 1BBB; gray)
RMSD = 0.385 Å



Superposition of Hb G-Makassar β subunit (green) and HbA β subunit (PDB 1BBB; gray)
RMSD = 0.254 Å

HbG β-E6A substitution does not affect the protein structure and, consequently, its function

(Please see poster 951 for additional characterization details)

Long term development strategy to potentially cure SCD

	Wave 1 Base Editing + HSCT	Wave 2 Improved Conditioning	Wave 3 In vivo LNP Delivery
Goals	Non-dsDNA break cutting, non-viral, precise genotype correction	Less toxic, targeted conditioning	In vivo editing (infusion) replaces transplant
Required technologies	BEAM-101/BEAM-102 Base editing (ex vivo)	Antibody conditioning	HSC-targeted LNP (Please see poster 2931)

Well-positioned to potentially create improved regimens for patients, now and in the future

LNP = Lipid Nanoparticle; HSC = Hematopoietic Stem Cell; HSCT = Hematopoietic Stem Cell Transplant;

Thank you.

VISIT **BEAMTX.COM** FOR MORE INFORMATION.

Hematology:
Conrad Rinaldi
Ka Tat Siu
Sarah Thomas
Adrian Rybak
Kavitha Sompalli
Haihua Chu
Alex Harmon
Shawn Jennings
Kathy Zhang
Nandini Mondal
Lili Yu
Molly Foster
Adam Hartigan
Alex Minella
Stephen Huang

Program Manager:
Shane Larson
Rahim Merchant

Bioinformatics:
Luis Barrera
Lauren Young
Maya Sen
David Born
Tanggis Bohnuud

Flow Cytometry:
Scott Haskett
Lisa Hardy

Automation:
Bob Gantzer
Jerry Decker

Analytical Development:
Alexander Liquori
Boriana Tzvetkova
Valerie Winton
Bo Yan
Calvin Lee
Darren Begley
Ling Lin
Lisa Schlehuber
Jeffrey Marshall
Carlo Zambonelli
Jenny Olins
Mano Singh

Gene Editing Technologies:
Dieter Lam
Yi Yu
Thomas Leete

Liver:
Michael Packer
Genesis Lung
Lo-I Cheng

Liver "Plus":
Holly Rees
Shelby Tonelli

mRNA:
Aaron Larsen
Valentina McEneany
Mark Naniong

Cell Biology:
Jamie Durbin

Synthetic Biology
Ian Slaymaker

PSSB:
Matt Lee
Manny Ortega
Paty Feliciano
Kindo Lee
Chavonna Xu

***In Vivo*:**
Kyle Rehberger
Sarah Smith
Elsie Zahr Akrawi
Franklin Lime Ma

Cell Processing:
Tao Bai
Lillian Ye
Brent Coisman
Andrew Ryan
Corrina Lucini
Tao Bai
Paul Kopesky

MS&T:
Jessica Hoyer
Chris Oesterling

Off-target:
Ka Wai Mok
Seda Gyonjyan
Yingying Zhang

sgRNA:
Brian Cafferty

Strategy:
Steve Cavnar

Regulatory Affairs:
Chris Swenson
Daisy Lam

Leadership Team
Pino Ciaramella
John Evans
Terry-Ann Burrell
Brian Riley
Courtney Wallace
Christine Bellon
Amy Simon
Sunita Goyal

And many more!

Beam
THERAPEUTICS