Adenine Base Editing of the Sickle Allele in CD34^{+} Hematopoietic Stem and Progenitor Cells Eliminates Hemoglobin S

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DISCLOSURE

- I and all authors are Beam employees and shareholders

Sickle cell disease (SCD)

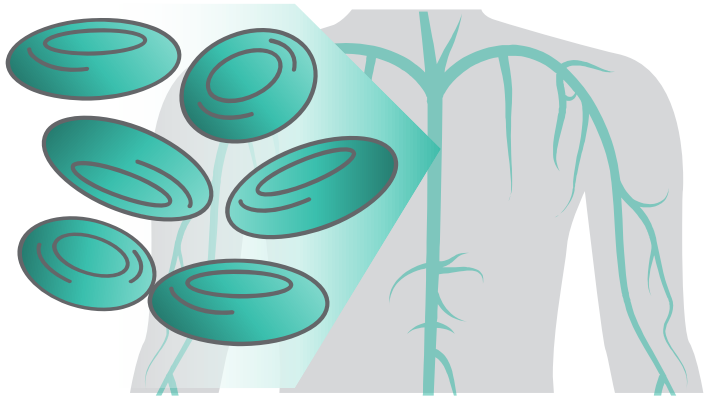
β-globin gene

Adult β-globin gene

HBB

Glutamic Acid at 6th amino acid (HbA)

C T C
G A G



Normal red blood cells

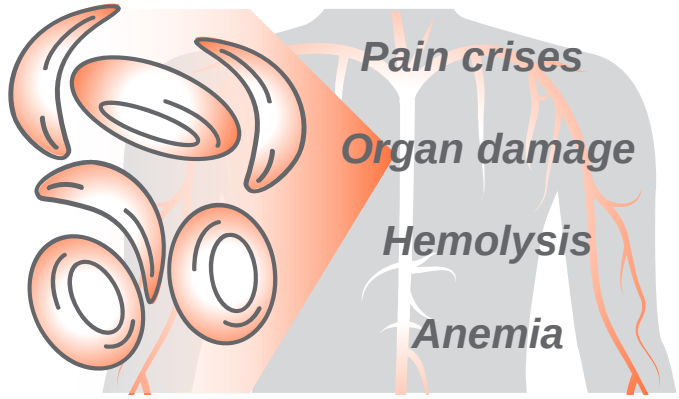
T-to-A mutation causes sickling

Sickle β-globin gene

HBB

Valine at 6th amino acid (HbS)

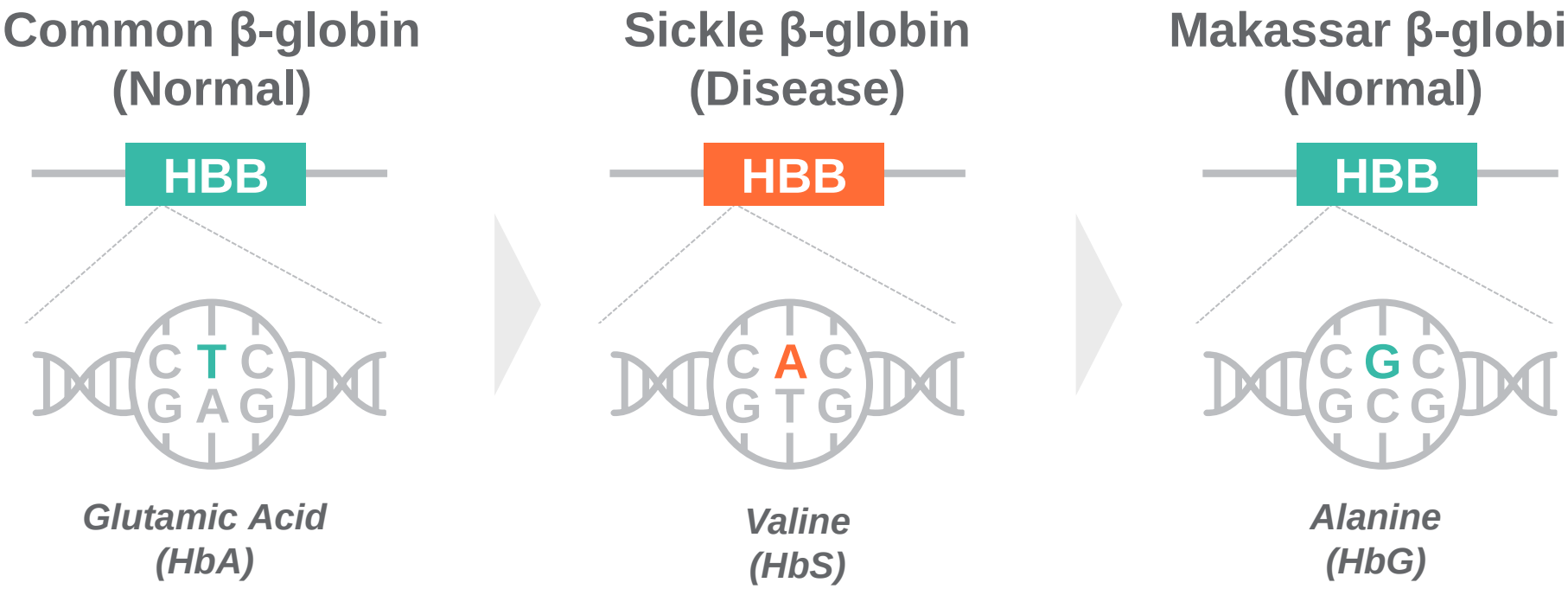
C A C
G T G



Sickling red blood cells

Approximately 100,000 sickle cell disease patients in the US

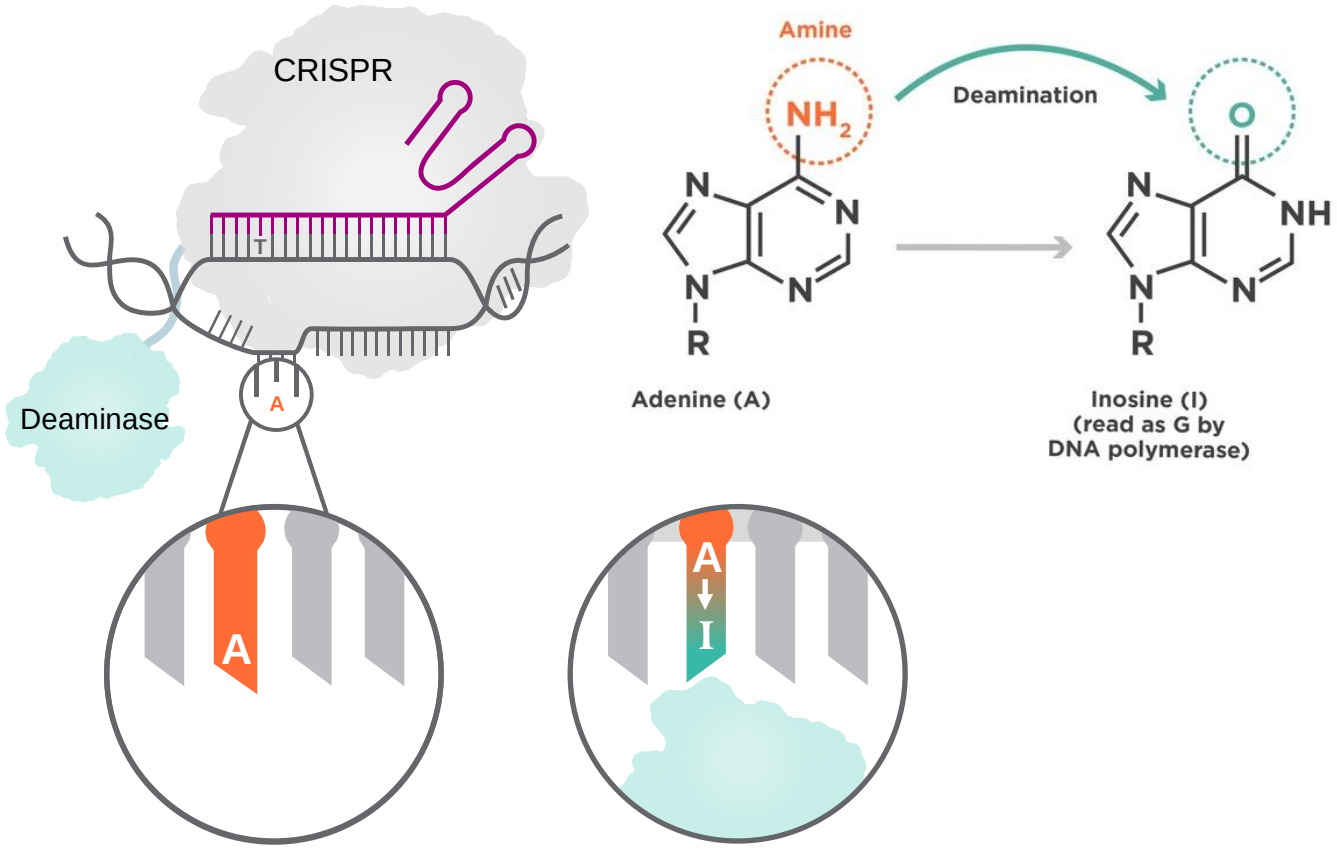
Base editing of the sickle allele to a naturally occurring, non-pathologic hemoglobin, Hb G-Makassar



- 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]
- E6A substitutions in β-globin do not contribute to polymer formation in vitro[3-5].

1. Cummings, MR. 2006. Human Heredity: Principles and Issues, Updated Edition.; 2. Mohamad, et al. 2018. Hematology Reports. 3. Pagnier et al., 1993; 4. Prehu, et al., 1999;

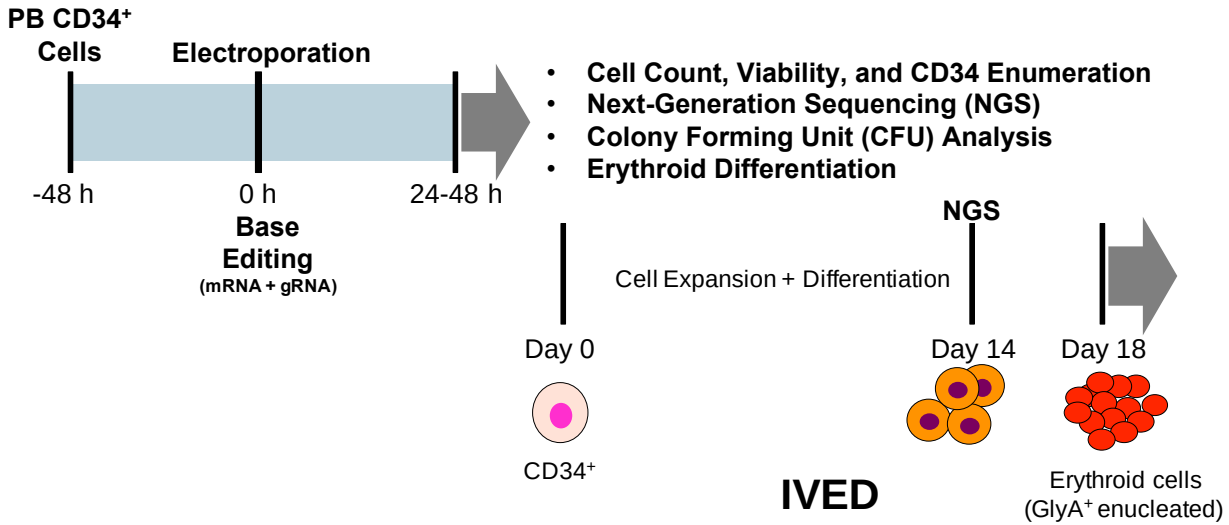
Adenine Base Editing Technology

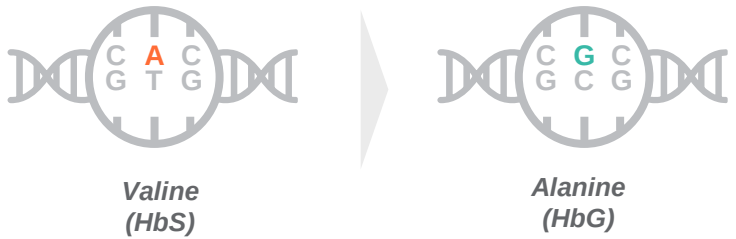


- Adenine Base Editor (ABE) comprises a deaminase enzyme fused to catalytically impaired CRISPR protein.
- Guide RNA (gRNA) directs the ABE to a target genomic DNA sequence and exposes the editing window.
- Deaminase chemically converts target adenine (A) to inosine (I) via deamination.

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

High efficiency Makassar editing of HbSS CD34+ HSPCs

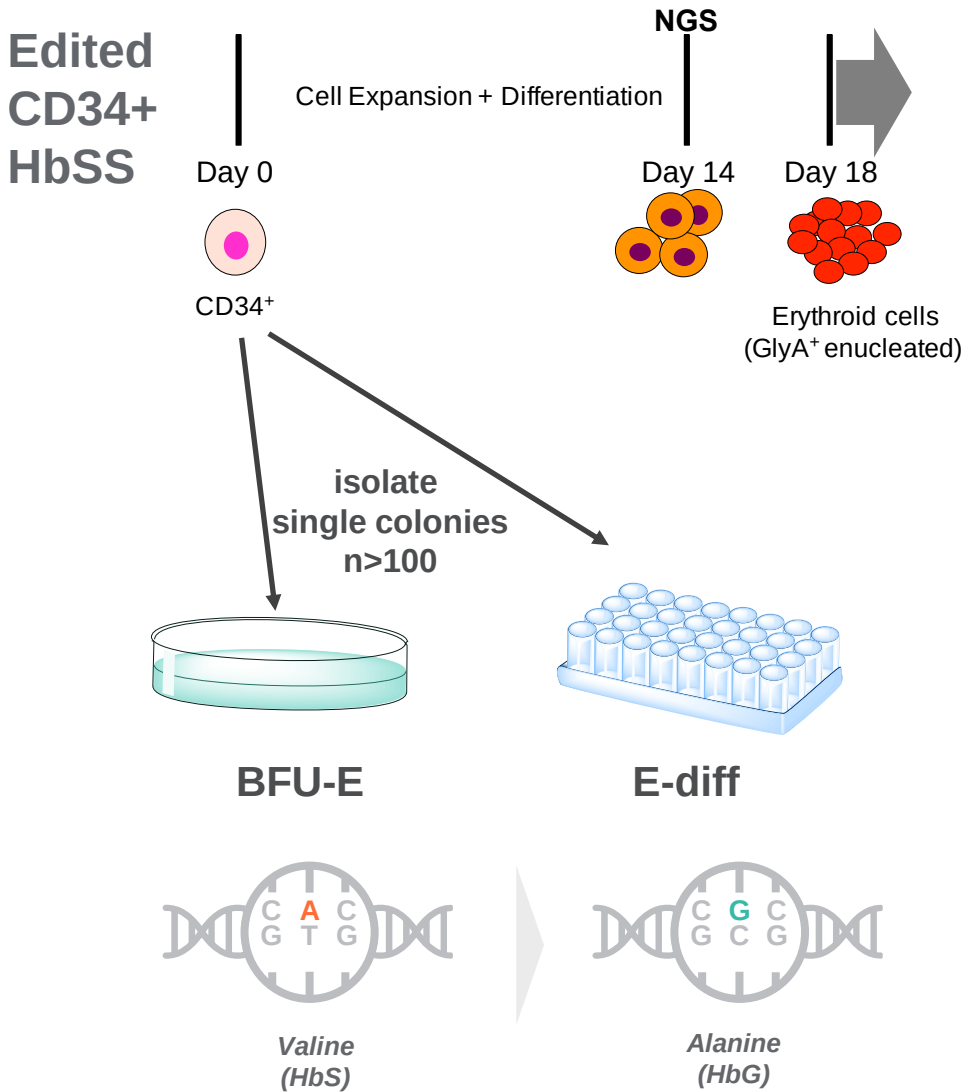


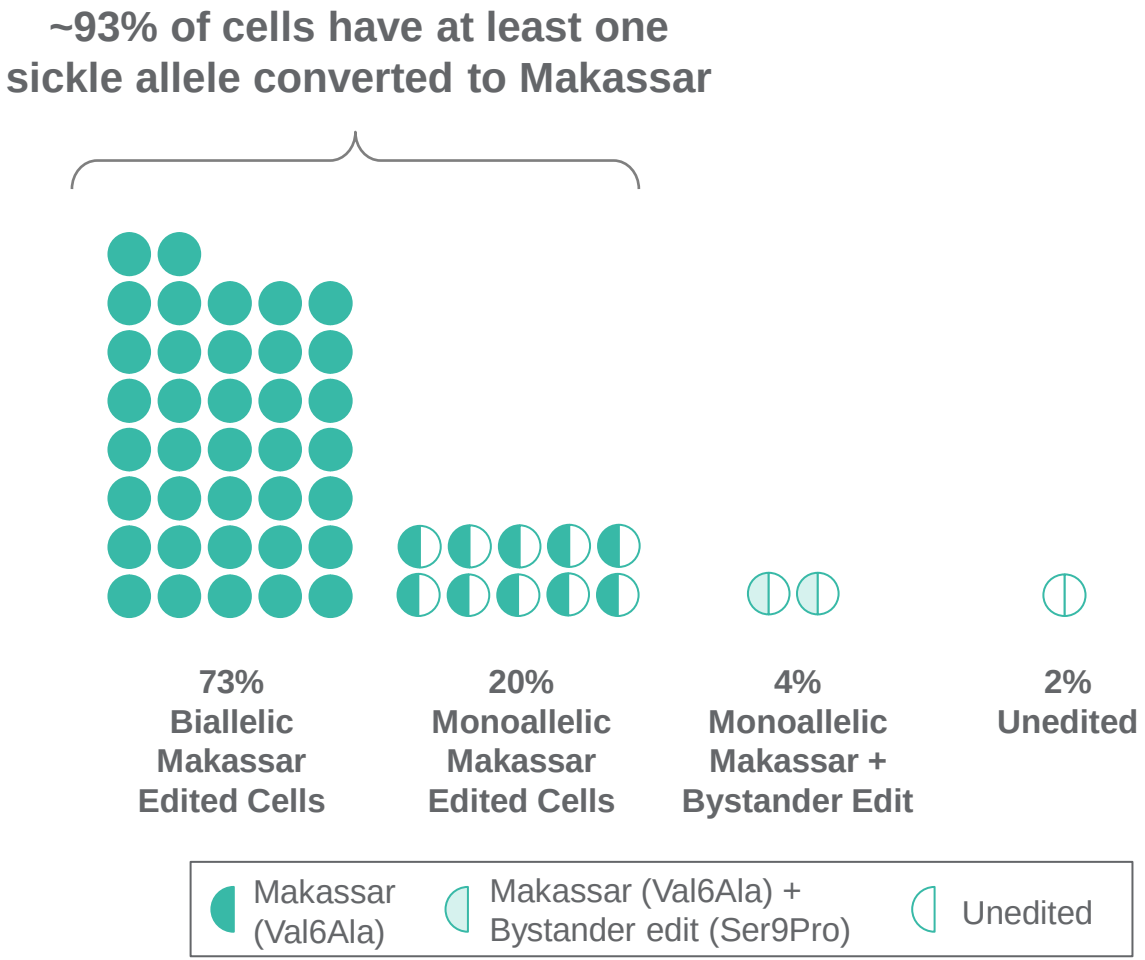


...ACTTCTCCACAGGAGT...

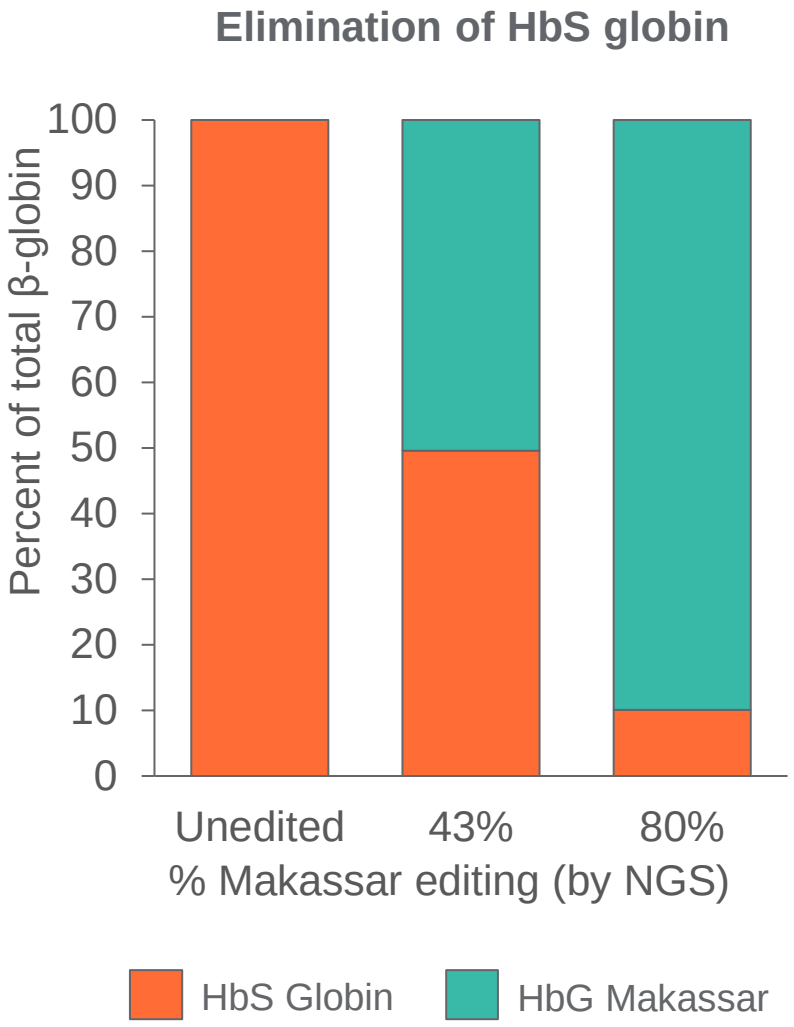
Ser9 Lys8 Glu7 Val6 Pro5 Thr4

Base editing achieves >90% mono- and bi-allelic Makassar editing

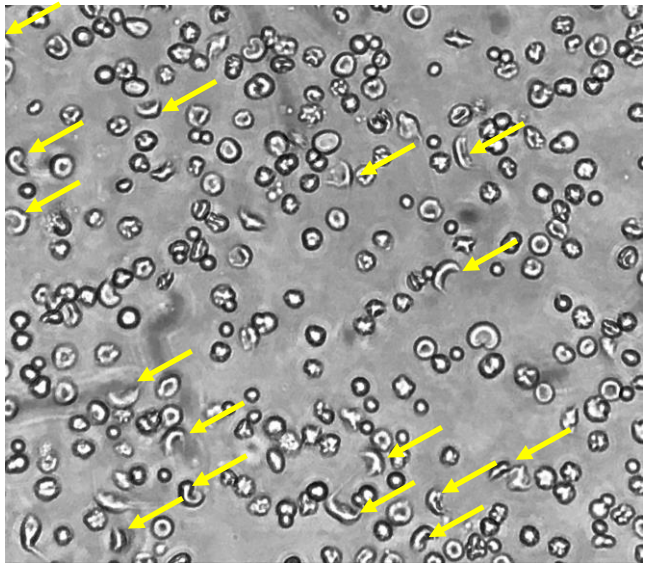




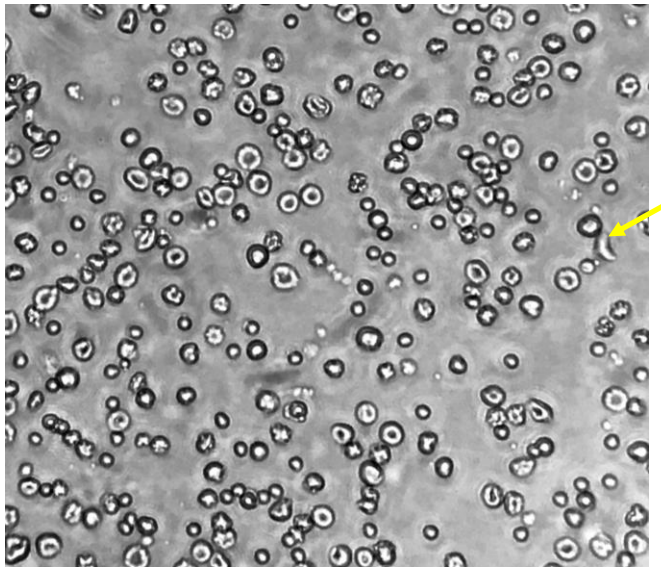
Makassar editing leads to decrease in pathogenic HbS and reduced sickling in response to hypoxia



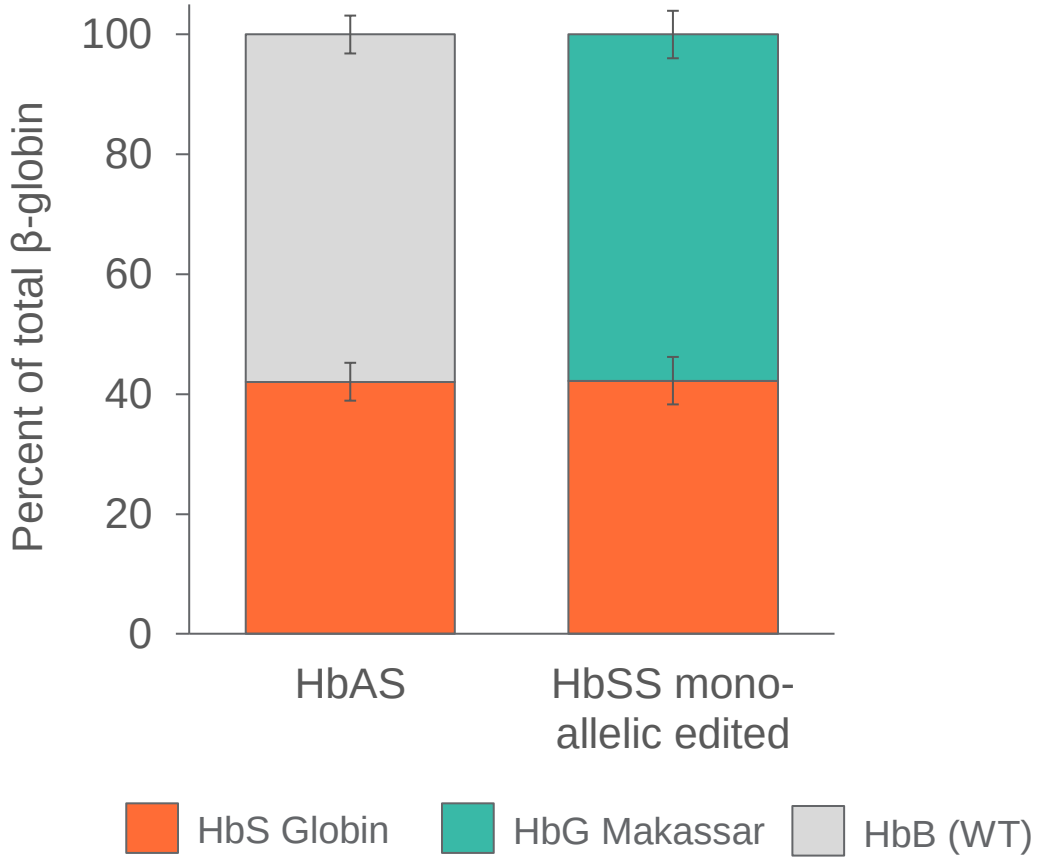
Sickling in unedited HbSS cells



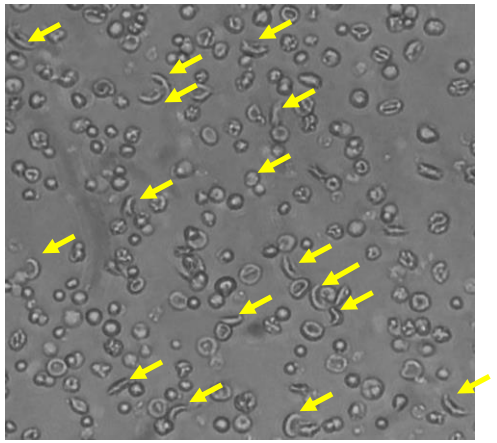
~89% Hb G-Makassar by UPLC



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

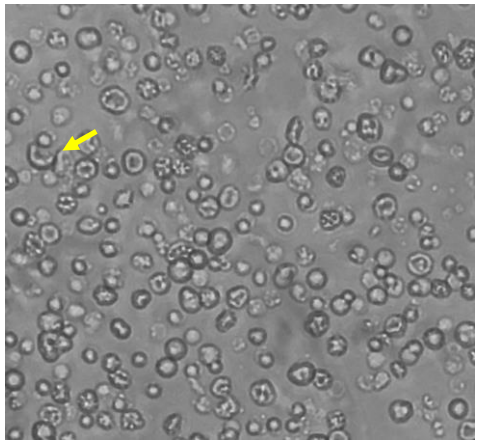


Unedited HbSS

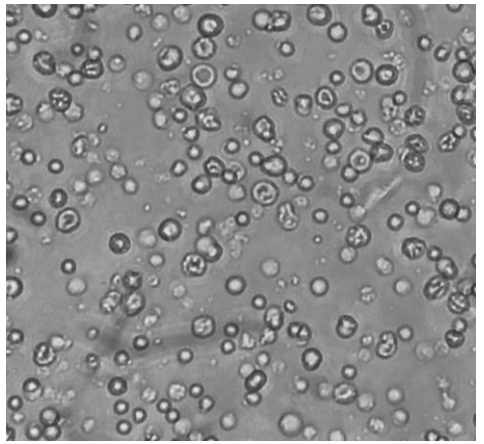


IVED cells derived from HbSS CD34+ exposed to 2% hypoxia (single clones)

mono-allelic edited

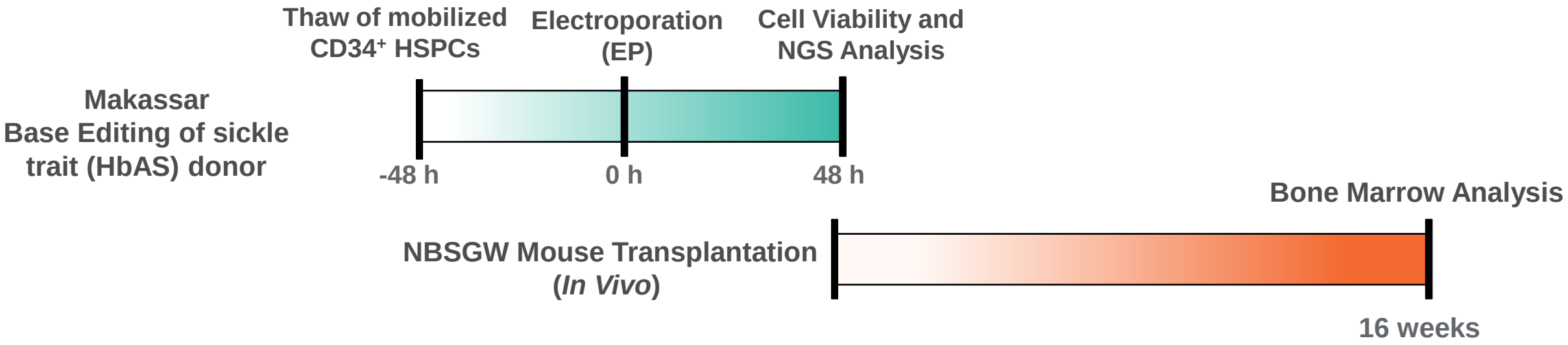


bi-allelic edited



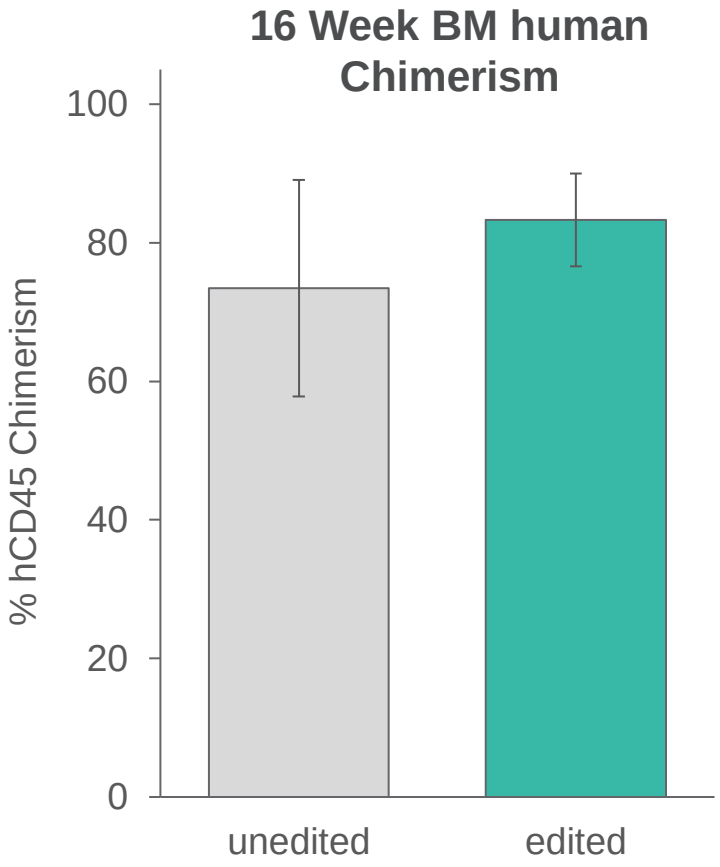
≤ 40% HbS on a per cell basis in >90% of erythroid cells → average per cell HbS level of ~10%

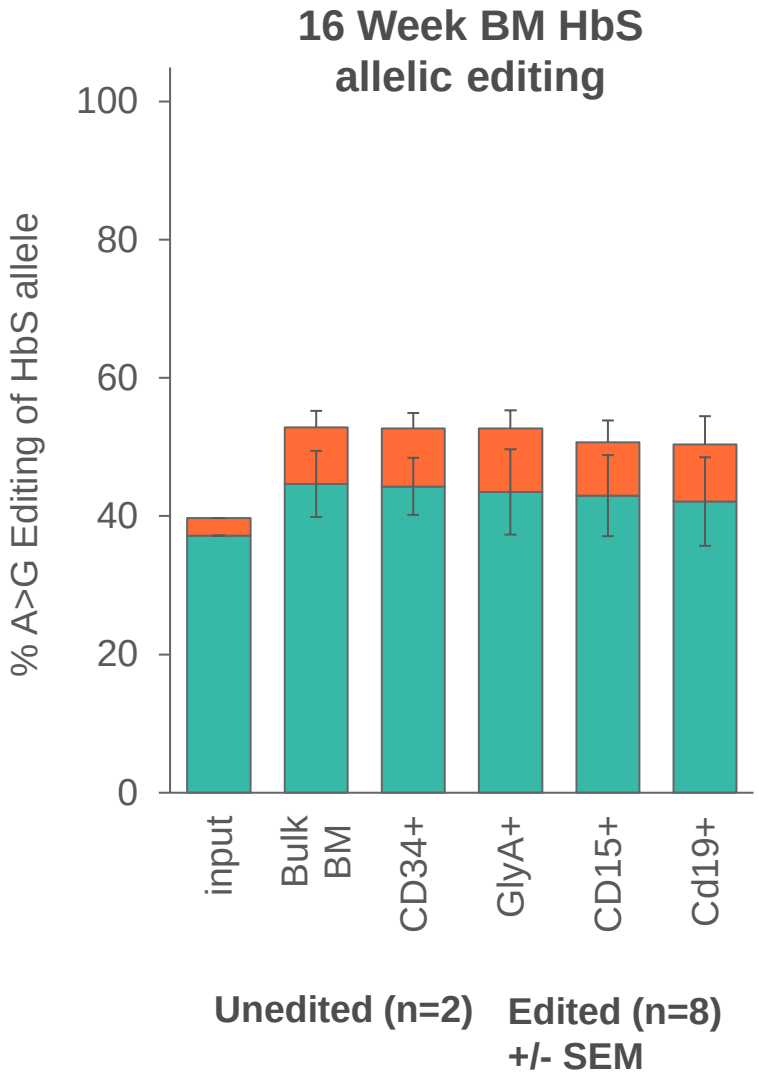
In vivo retention of editing and detection of Hb G-Makassar globin by UPLC

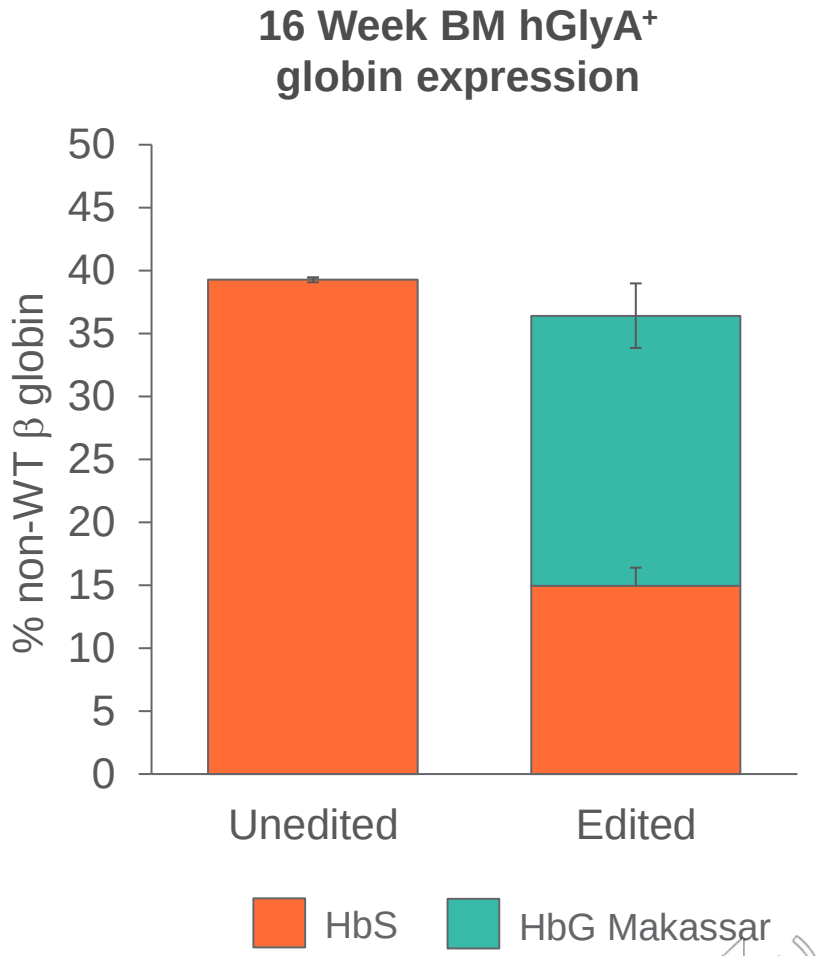


*** Editing conditions (mRNA and sgRNA concentrations) that yielded ~50% Makassar editing *in vitro* were used**

16 week long-term engraftment and *in vivo* retention of editing and detection of Hb G-Makassar globin by UPLC







Key Takeaways

- Highly efficient base editing of the sickle causing allele in SCD cells was achieved in $CD34^+$ HSPCs, converting HbS to a non-pathogenic globin variant, Hb G-Makassar.

- >90% bi- and mono-allelic Makassar editing can be achieved in HbSS *in vitro* erythroid differentiated (IVED) cells, resulting in a reduction of HbS globin levels to <10% on an average per cell basis with reduced hypoxia-induced sickling *in vitro*.

- Edited mobilized sickle trait individual (HbAS) $CD34^+$ HSPCs not only retained long-term engraftment and multilineage human hematopoietic reconstitution, but also Makassar editing of the sickle allele to generate Makassar beta globin protein *in vivo.*

Thank you!