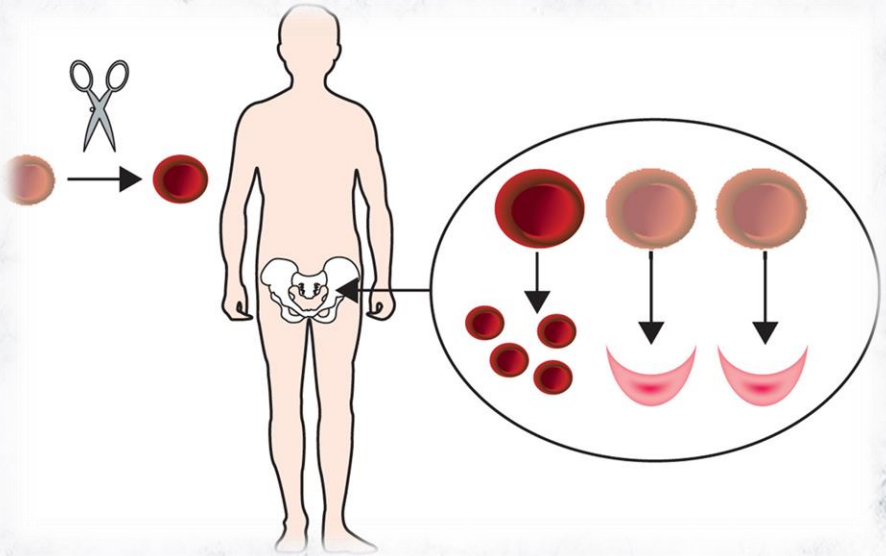
Therapeutic genome editing



Yuxuan Wu

East China Normal University

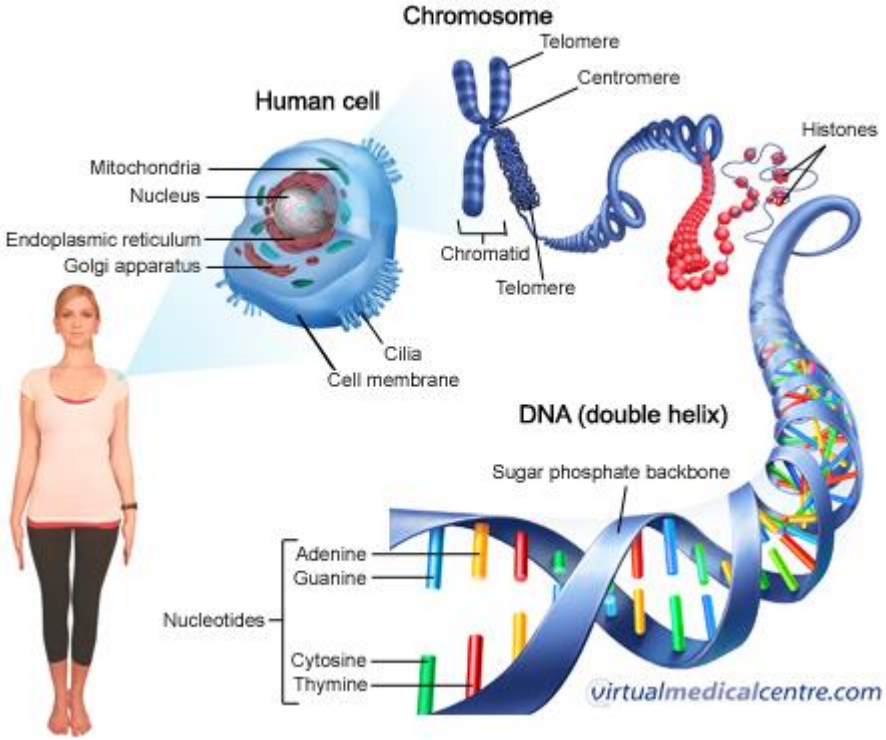
Therapeutic genome editing

- **Principle and applications of CRISPR genome editing**
- Therapeutic genome for β-hemoglobin disorders
- Future?

The CRISPR revolution

1. Eukaryotic genomes contain billions of DNA bases

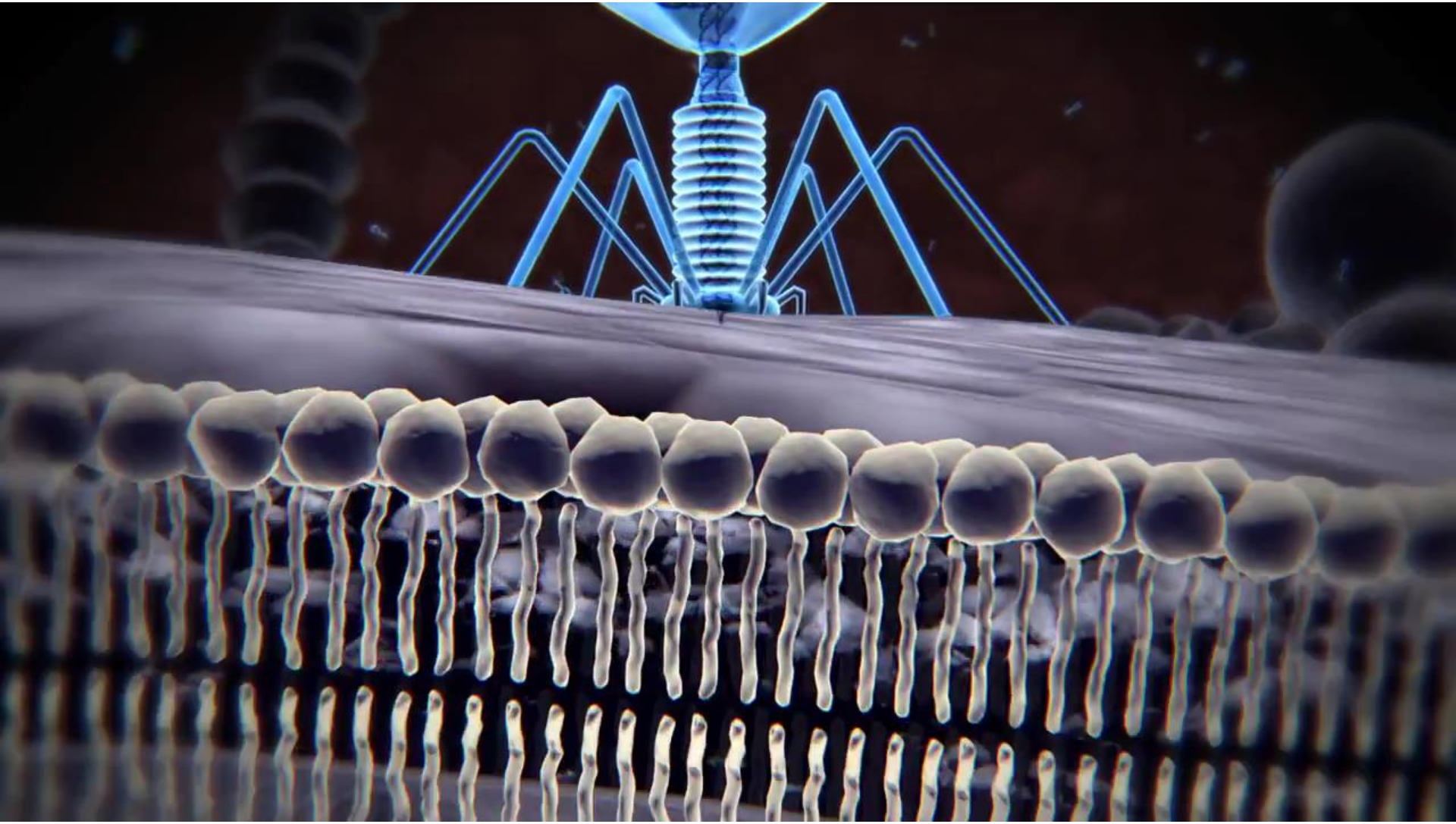
2. Abnormalities in an individual's genetic makeup cause genetic disease.



https://edgy.app/

CRISPR/Cas—Clustered regularly-interspaced short palindromic repeats

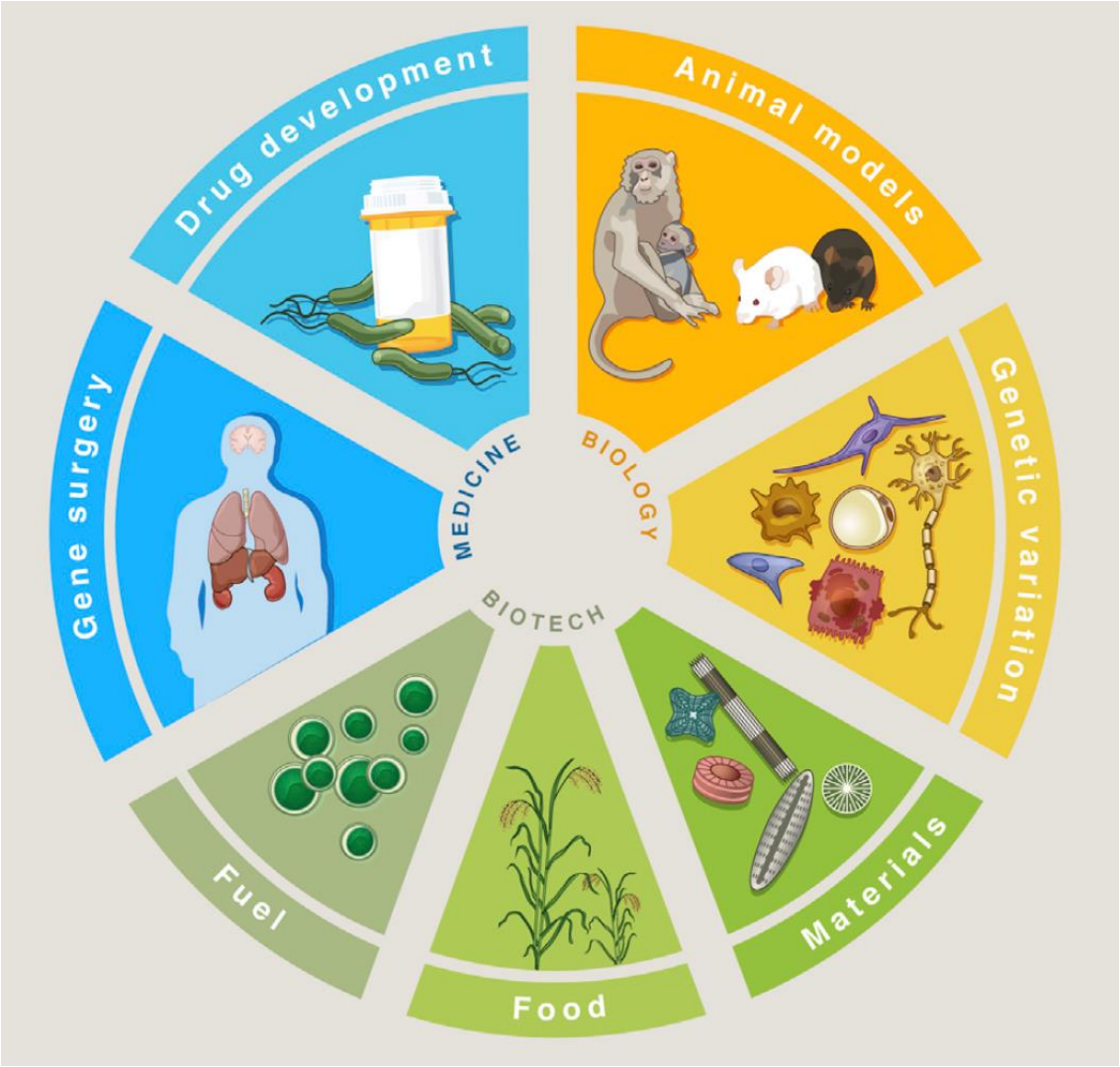
The weapon of bacteria fight against virus



CRISPR/Cas9 - the new genome editing tool

Engineering CRISPR/Cas system to cut genome wherever we want

Applications of CRISPR genome editing

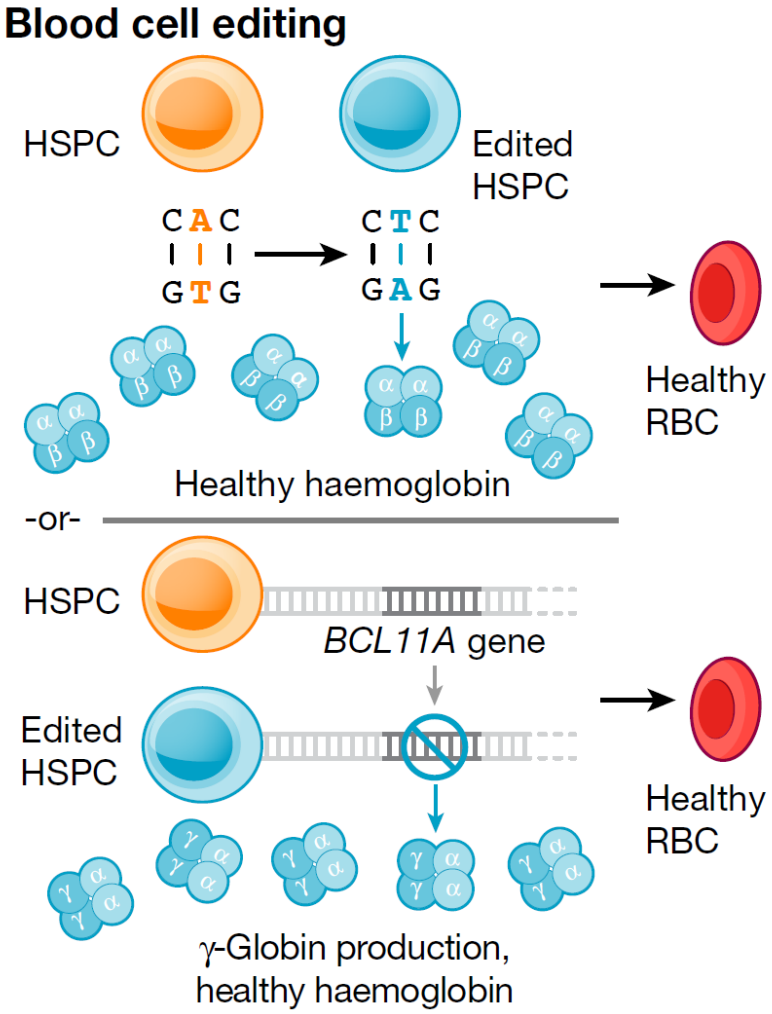


Therapeutic genome editing

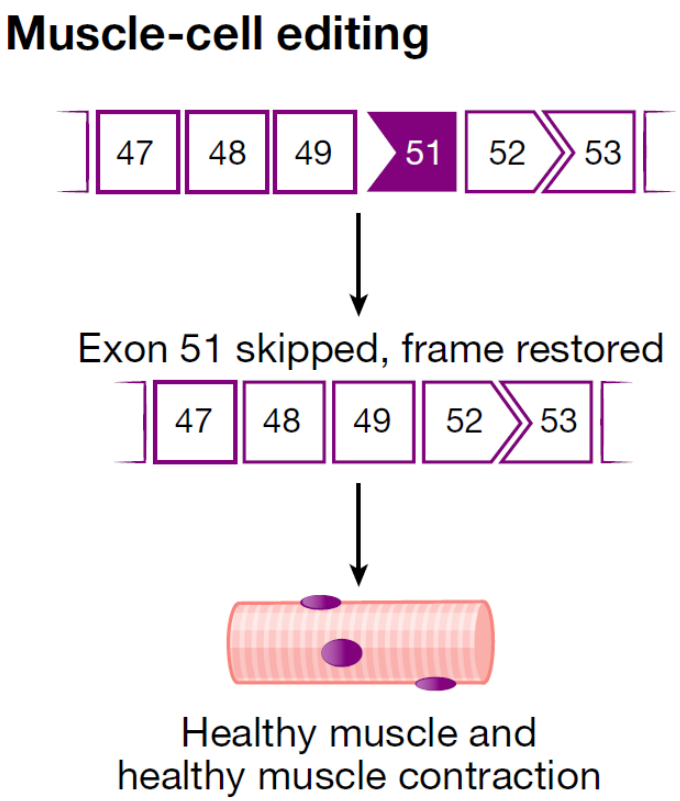
- Principle and applications of CRISPR genome editing
- **Therapeutic genome editing for β-hemoglobin disorders**
- Future?

Ex vivo or in vivo gene editing

Ex vivo

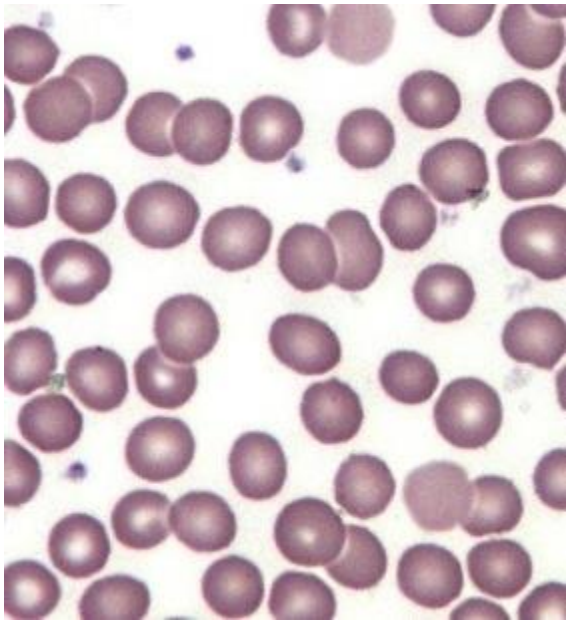


In vivo

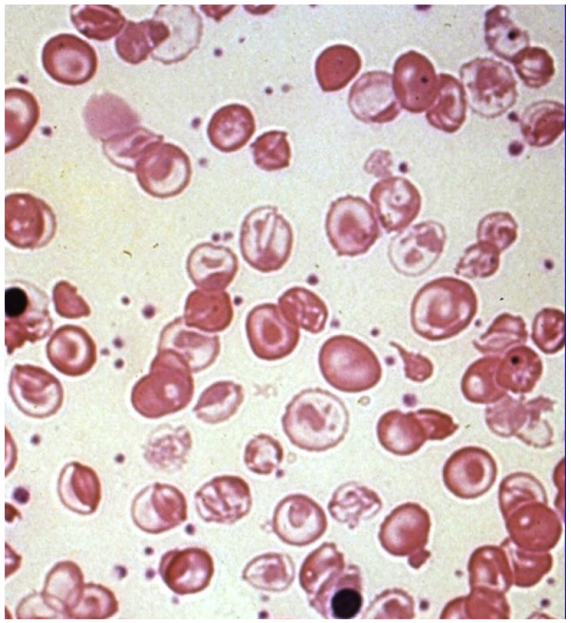


β-hemoglobin disorders, the most common monogenic diseases, remain a global public health challenge

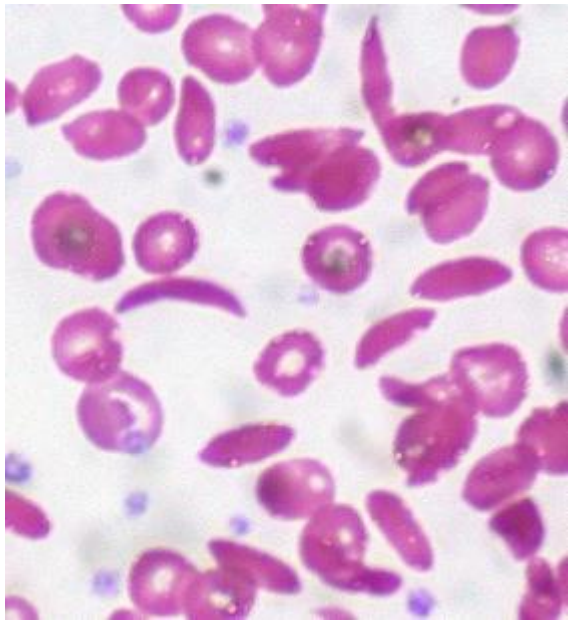
Normal

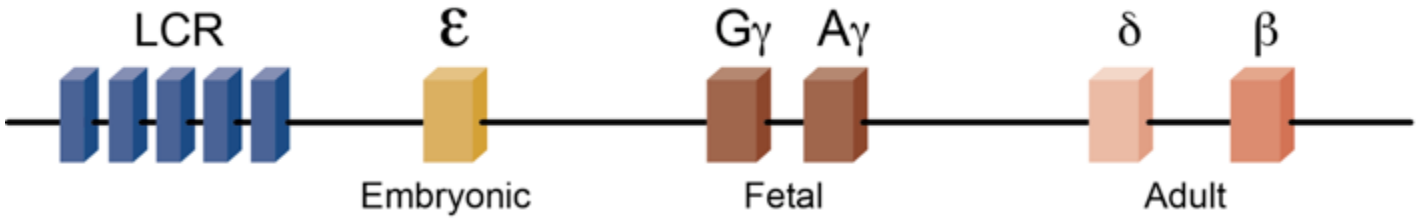


β-thalassemia

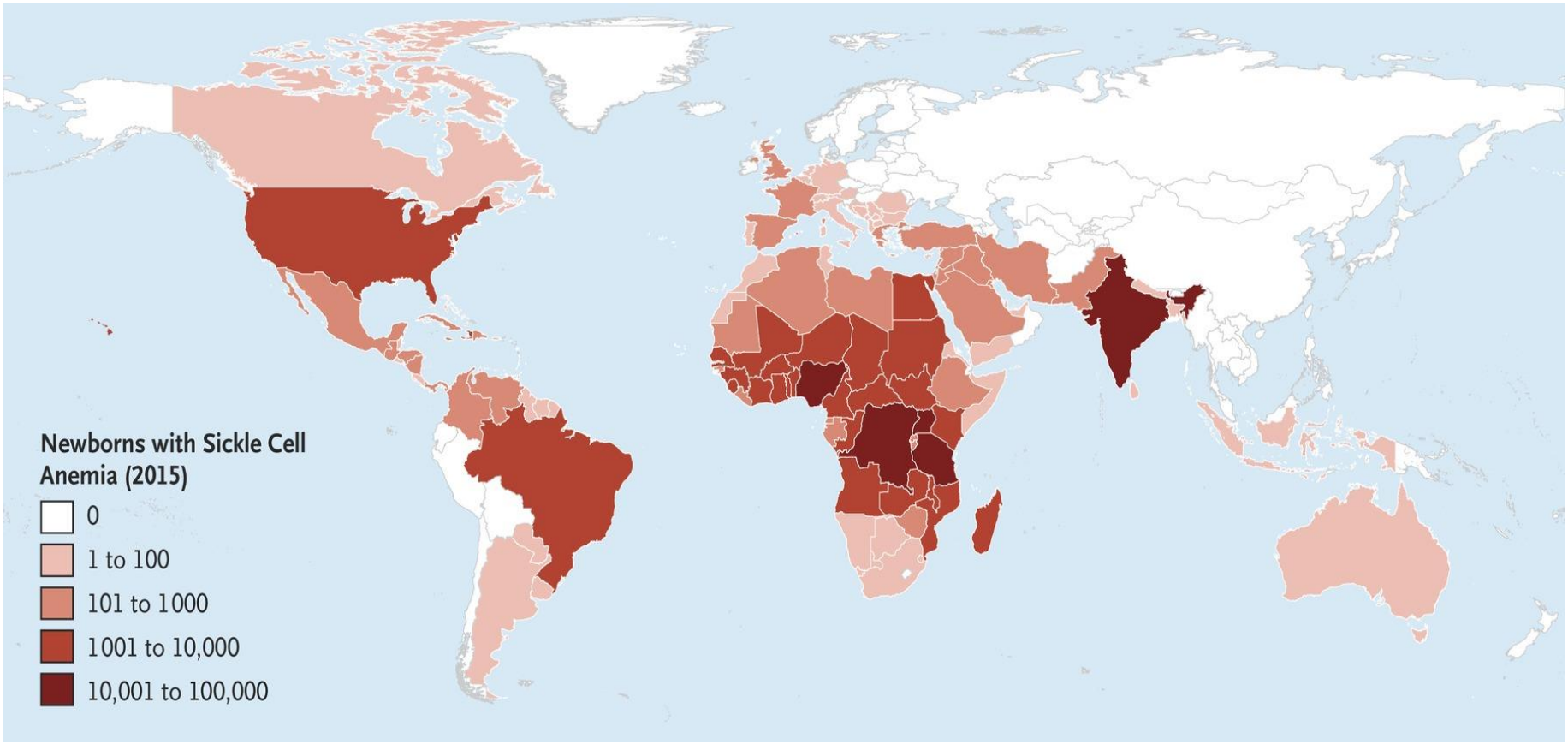


Sickle cell disease



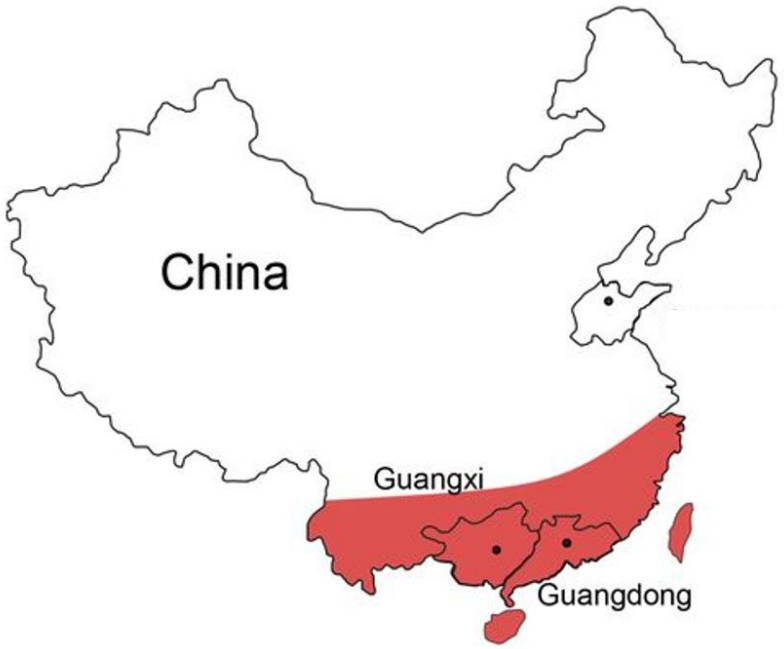


β-hemoglobin disorders, the most common monogenic diseases, remain a global public health challenge



Piel FB *et al*. NEJM (2017) 376:1561.

β-thalassemia in China



Blood disorders caused by mutations in the β-globin gene

Sickle Cell | Normal Cell | ß-thalassemia

Significant worldwide burden

ANNUAL BIRTHS

300k
Sickle cell disease

60k
ß-thalassemia

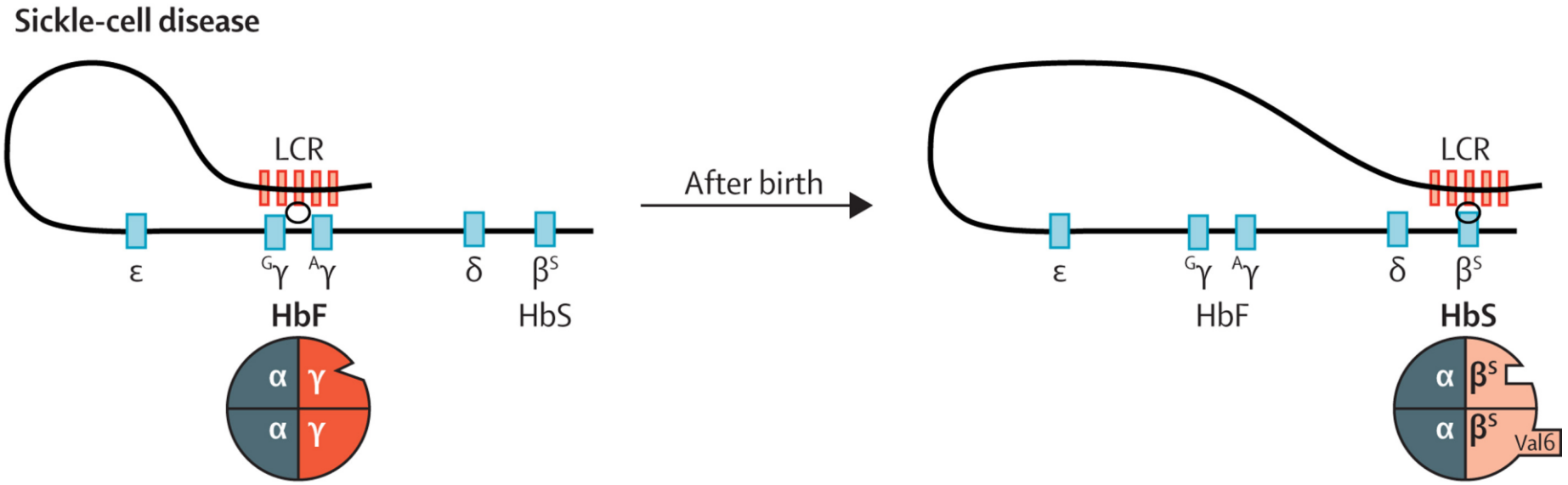
High morbidity and mortality

Anemia and transfusions | Pain | Early death

Heavy burden of patient care

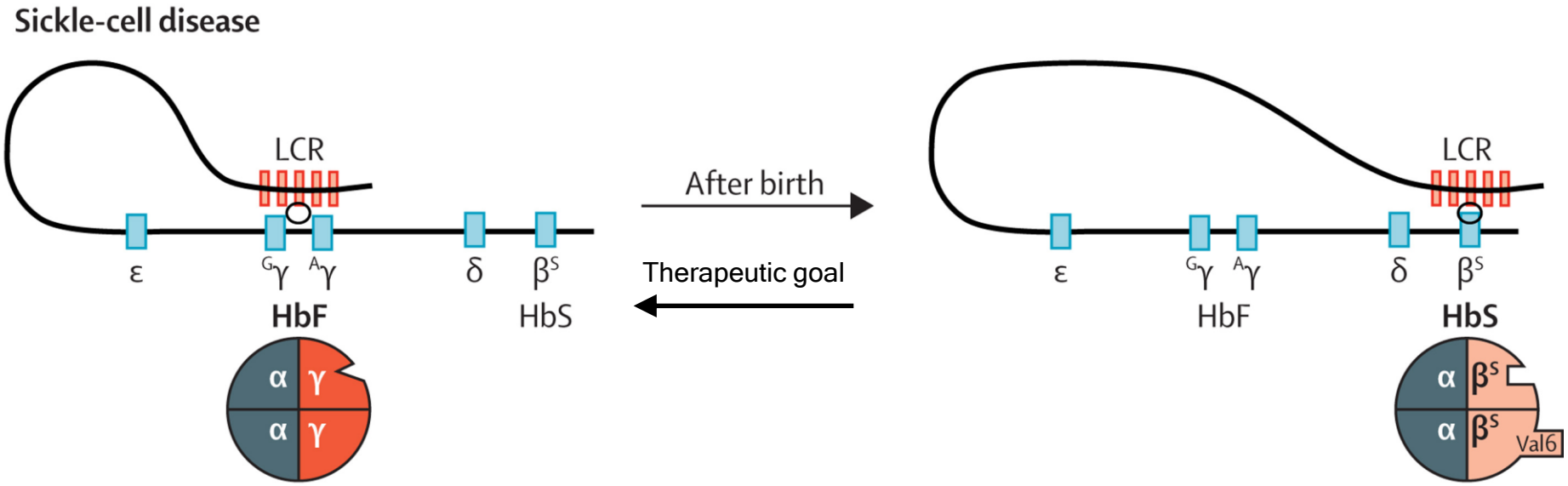
Frequent transfusions & hospitalizations

Fetal hemoglobin (HbF) induction



Lettre and Bauer. Lancet (2016) 387:2554.

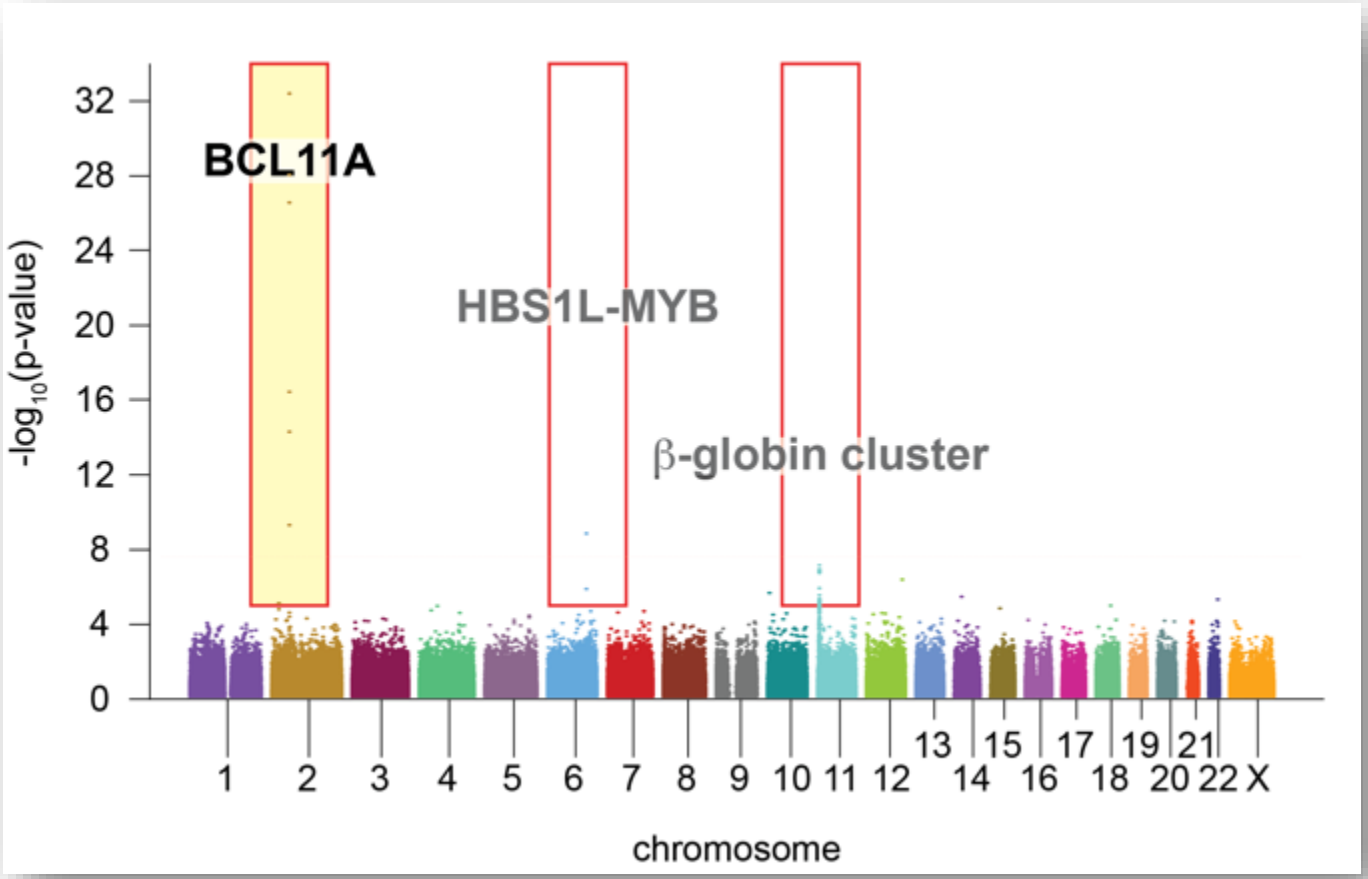
Fetal hemoglobin (HbF) induction



Clinical induction of HbF production holds tremendous promise to ameliorate the clinical symptoms of sickle cell disease (SCD) and β-thalassemia.

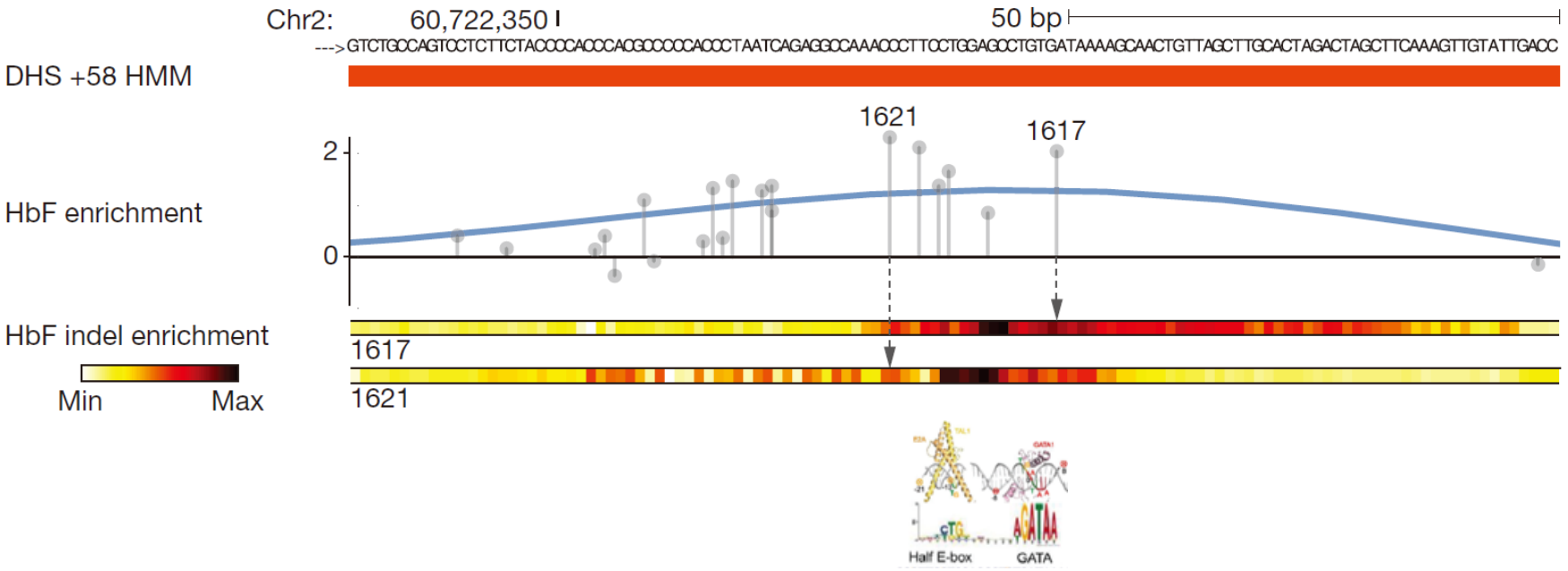
Lettre and Bauer. Lancet (2016) 387:2554.

GWAS demonstrate genetic variation at *BCL11A* modifies HbF level and β-hemoglobin disorder clinical severity



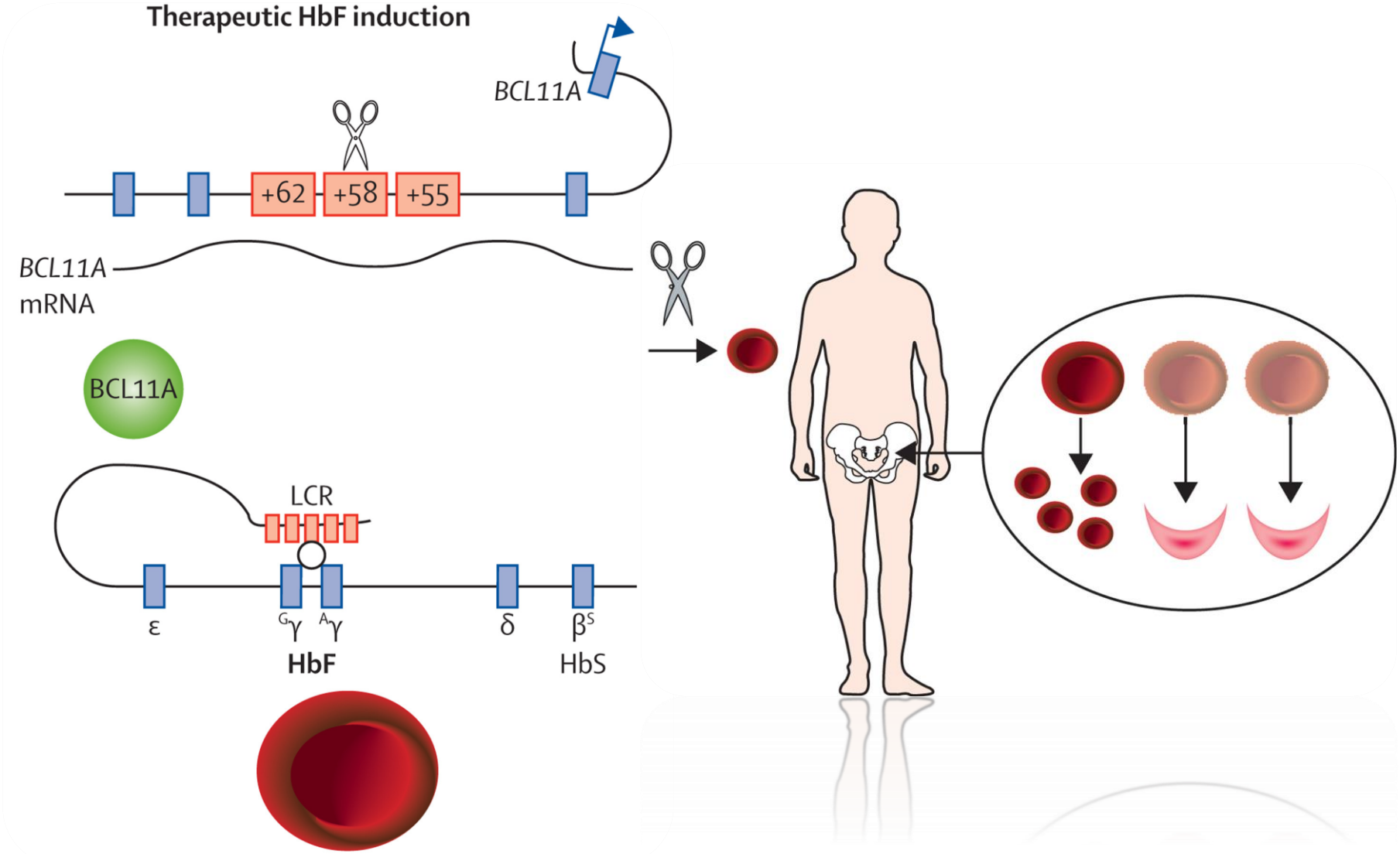
Manhattan plot from CSSCD. Representative of: Menzel *et al*. Nat Genet (2007) 39:1197; Uda *et al*. PNAS (2008) 105:1620; Lettre *et al*. PNAS (2008) 105:11869; Nuinoon *et al*. Hum Genet (2010) 127:303; Solovieff *et al*. Blood (2010) 115:1815; Bhatnagar *et al*. J Hum Genet (2011) 56:316.

Identifying critical sequences within *BCL11A* erythroid enhancer



Canver *et al*. Nature (2015) 527:192. Half E-box/GATA composite motif from Han *et al*. MCB (2016) 36:157.

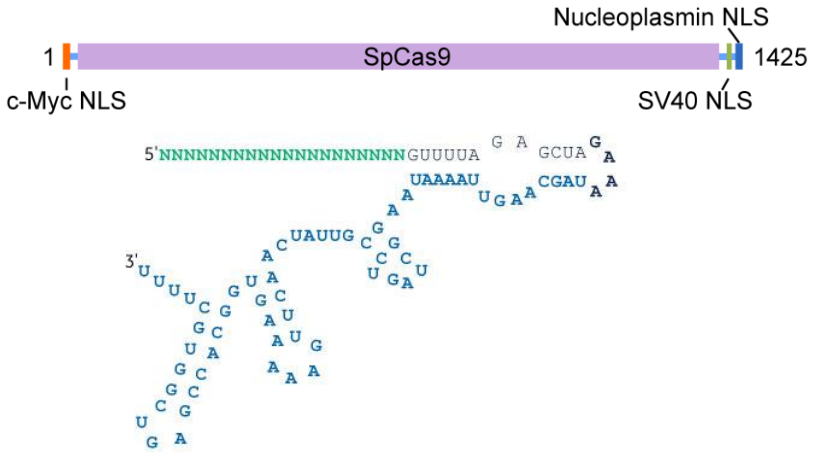
Therapeutic vision

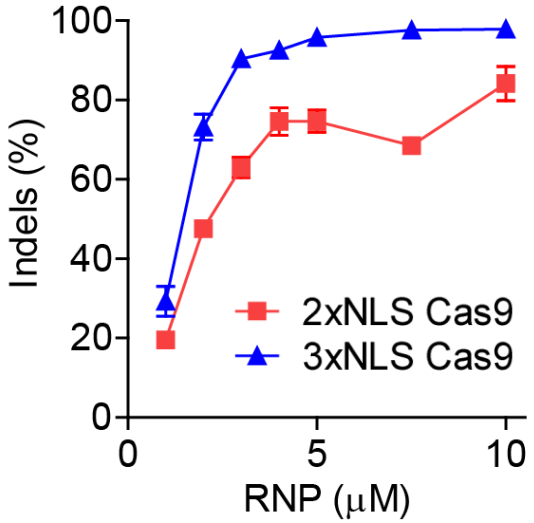


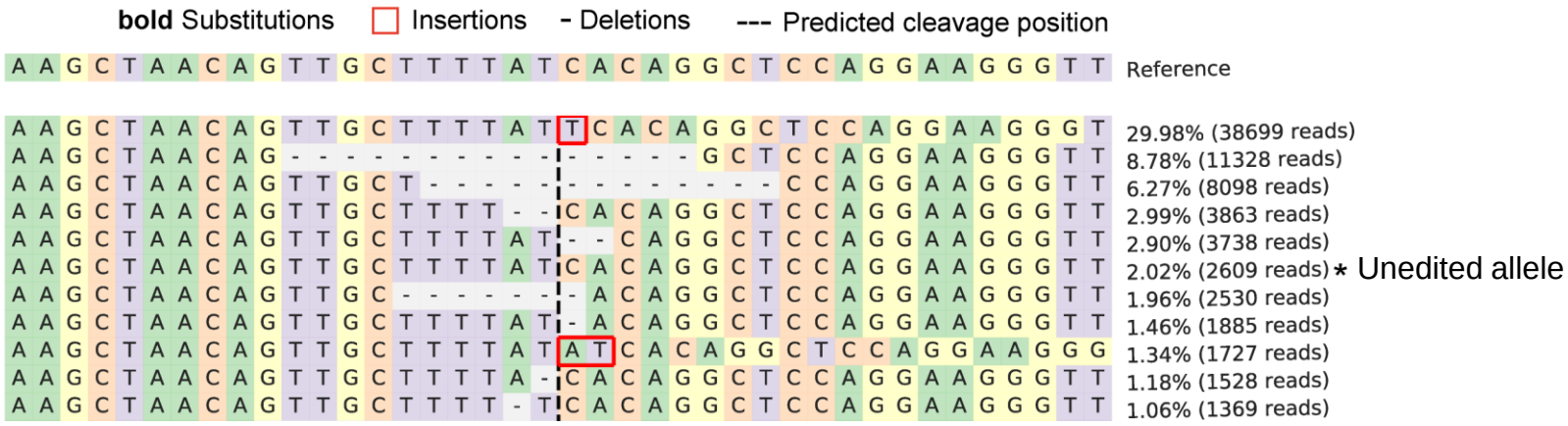
Lettre and Bauer. Lancet (2016) 387:2554.

Highly efficient *BCL11A* enhancer editing in human HSPCs

- *Optimized editing protocol with electroporation of 3xNLS Cas9 protein*
- *Deep sequencing confirms that the edited alleles are 98%*



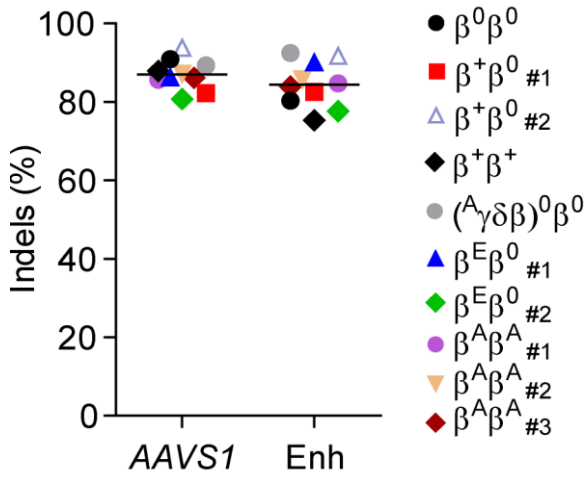


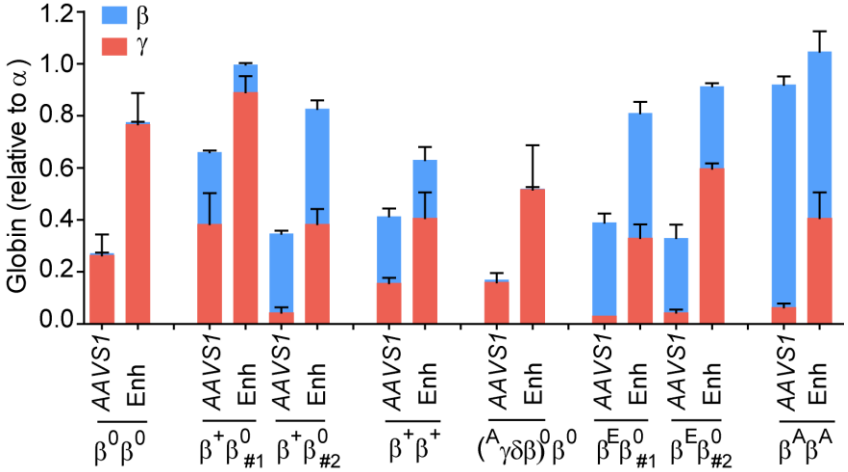


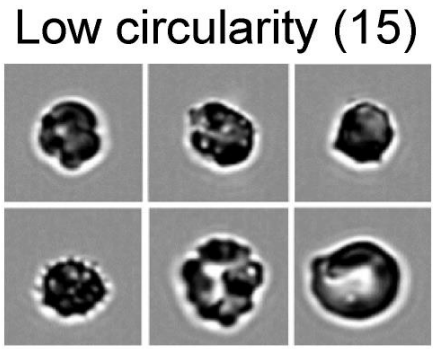
Wu et al. Nature Medicine (2019)

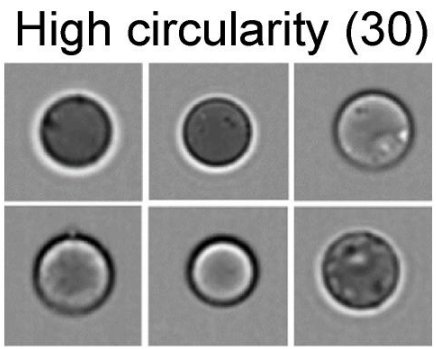
Therapeutic *BCL11A* enhancer editing of patient cells

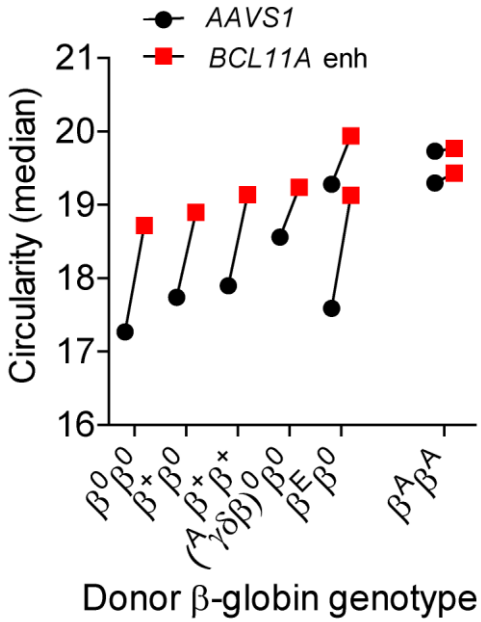
β-thalassemia donor CD34+ hematopoietic stem and progenitor cells, RNP electroporation



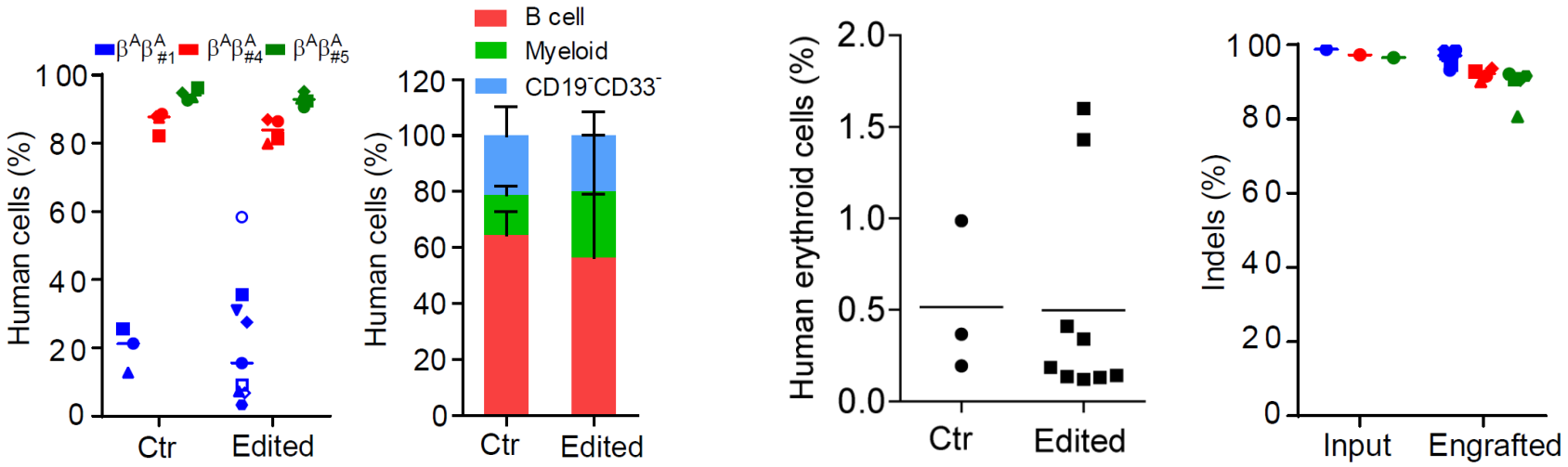








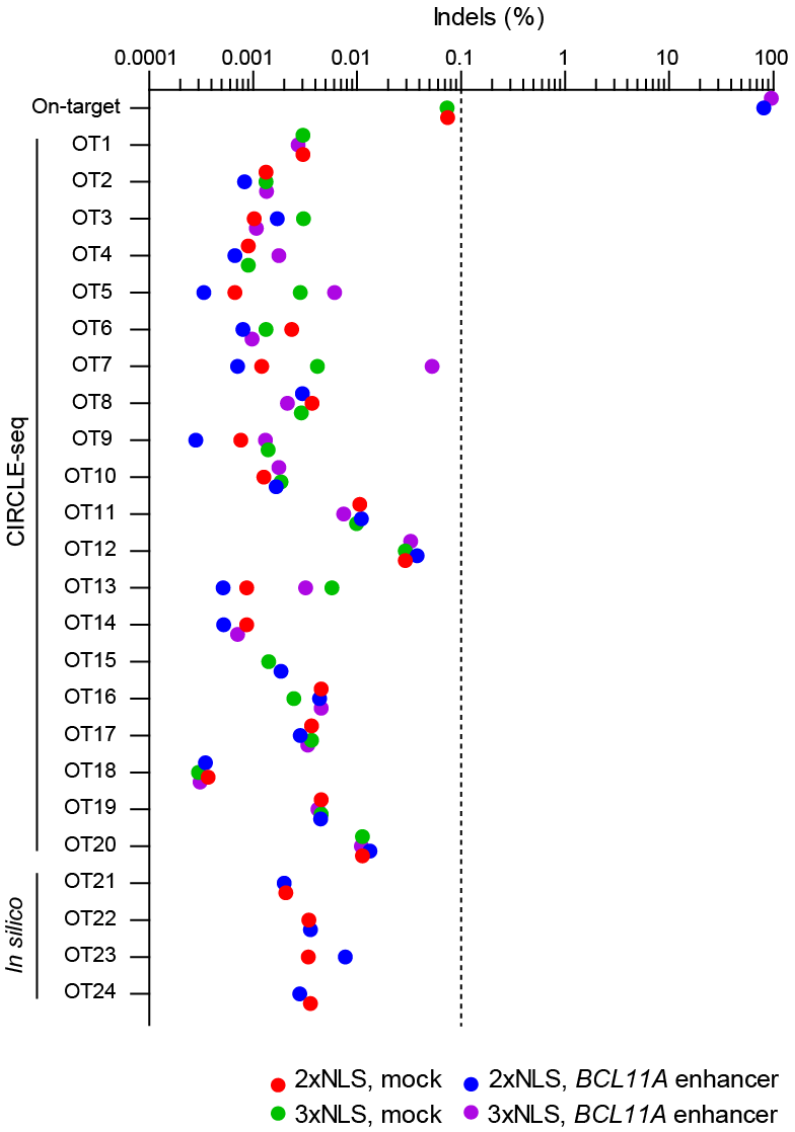
Long-term multi-lineage engraftment of Cas9 edited HSPCs in immunodeficient mice



Lack of detectable genotoxicity of *BCL11A* enhancer editing

- *Top 24 possible off-target (OT) sites evaluated as defined by genome-wide in vitro cleavage (CIRCLE-seq) or computational prediction (in silico)*

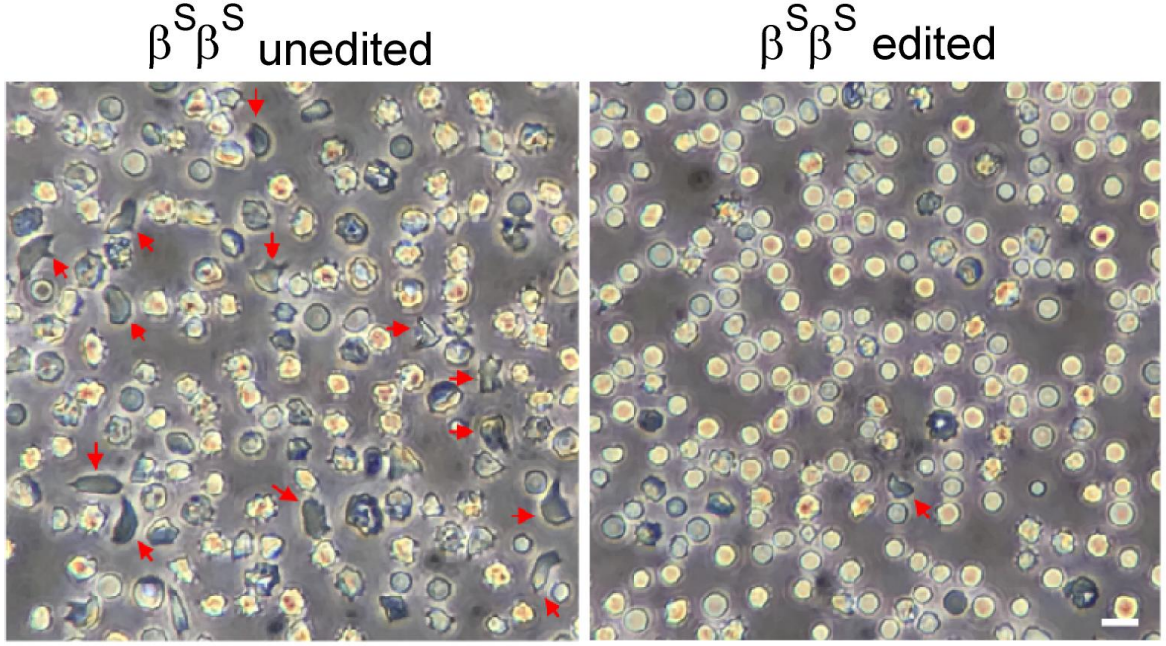
- *No off-target mutations detected in CD34+ HSPCs despite highly efficient on-target BCL11A enhancer editing (limit of detection for indels by deep sequencing is ~0.1%)*

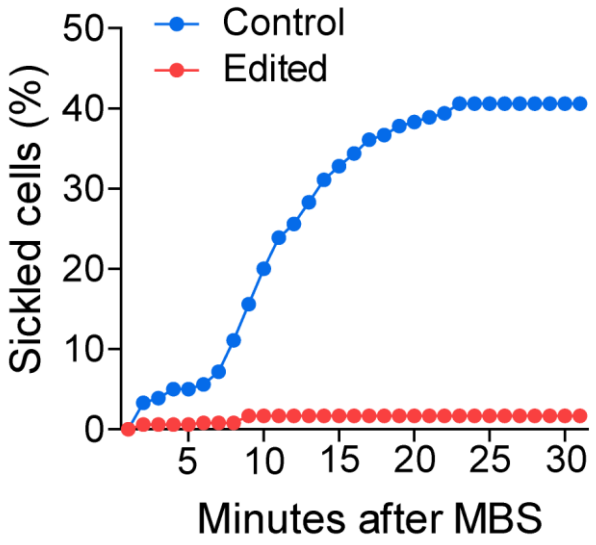


with Shengdar Tsai

Therapeutic *BCL11A* enhancer editing of SCD patient cells

- *Reversal of sickling propensity in edited SCD primary cells*

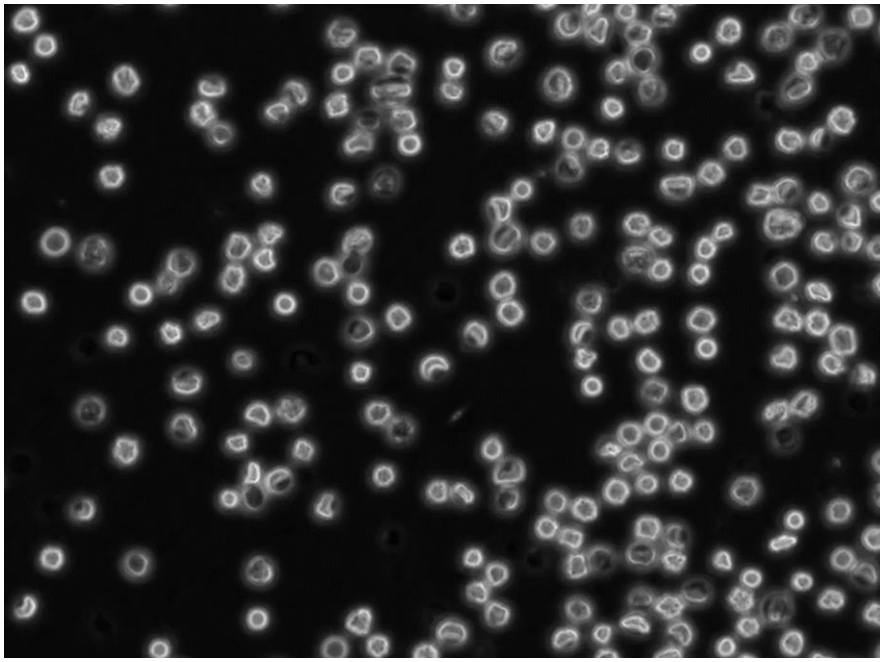




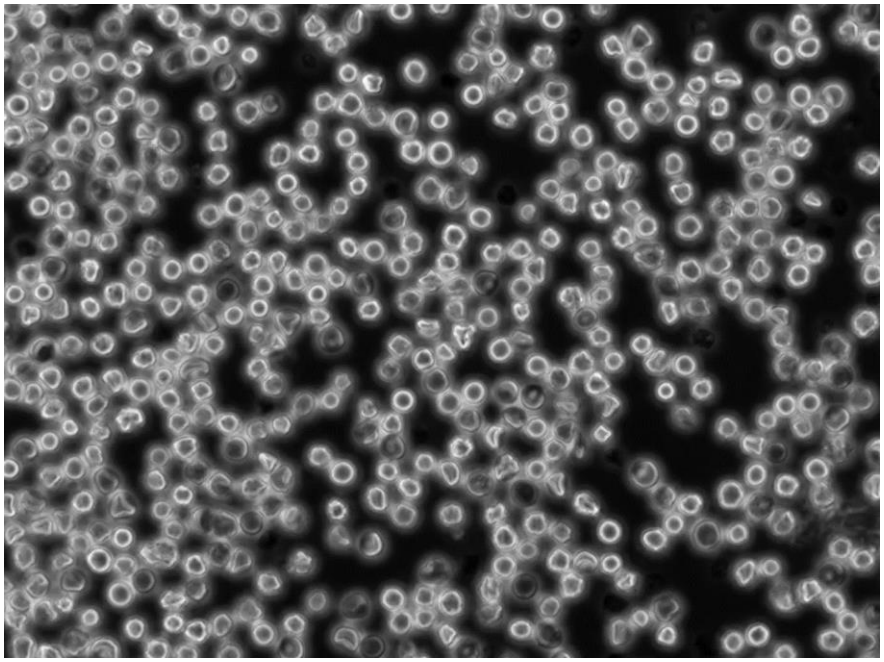
Unedited SCD enucleated erythroid cells derived from engrafting HSCs demonstrated robust in vitro sickling following sodium metabisulfite (MBS) treatment, edited SCD cells were resistant to sickling.

Editing *BCL11A* enhancer in SCD patient HSCs prevents sickling

$\beta^S\beta^S$ unedited

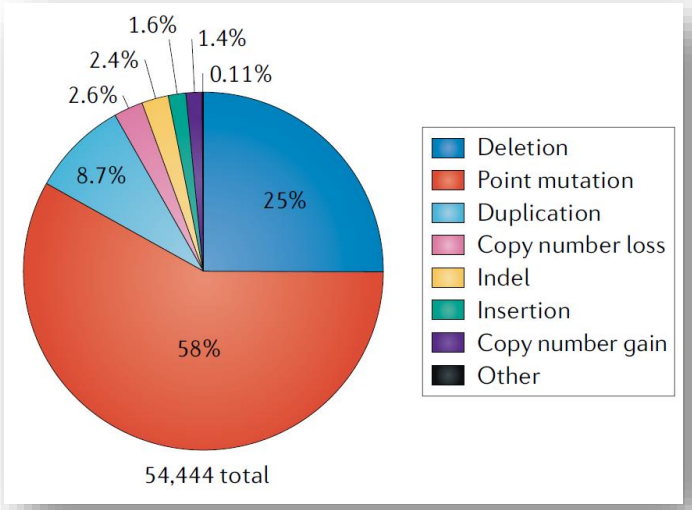


$\beta^S\beta^S$ edited



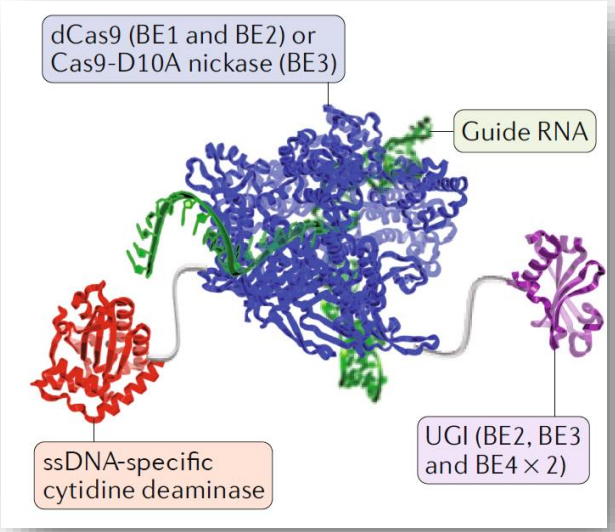
Therapeutic base editing of hematopoietic stem cells

58%: point mutations



Rees et al. Nature Reviews Genetics (2018)

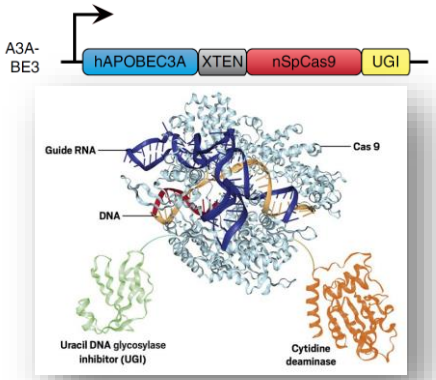
Base editor

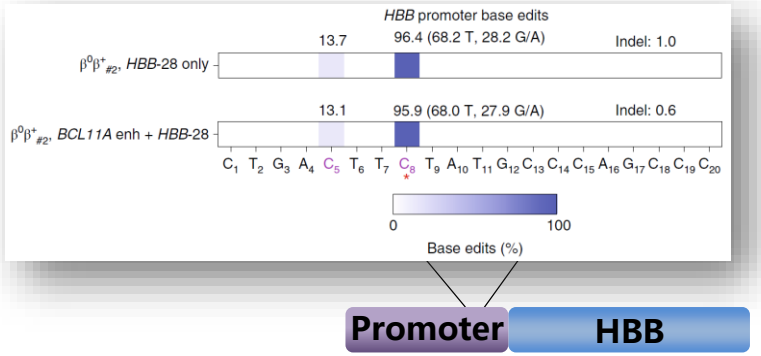


The feasibility of base editing in HSCs to enable durable therapeutic modification of blood

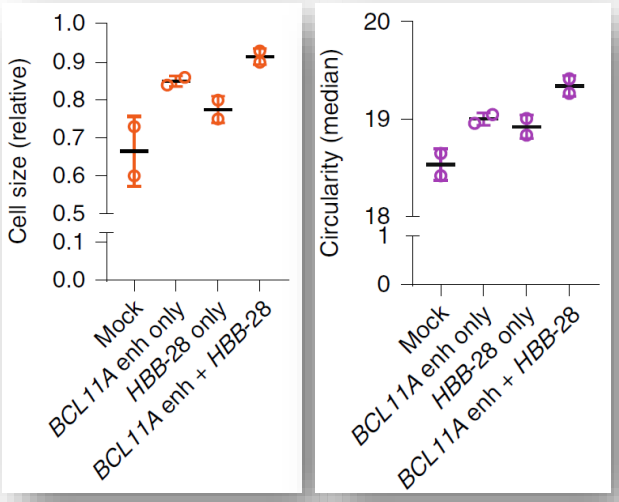
Therapeutic and multiplex base editing

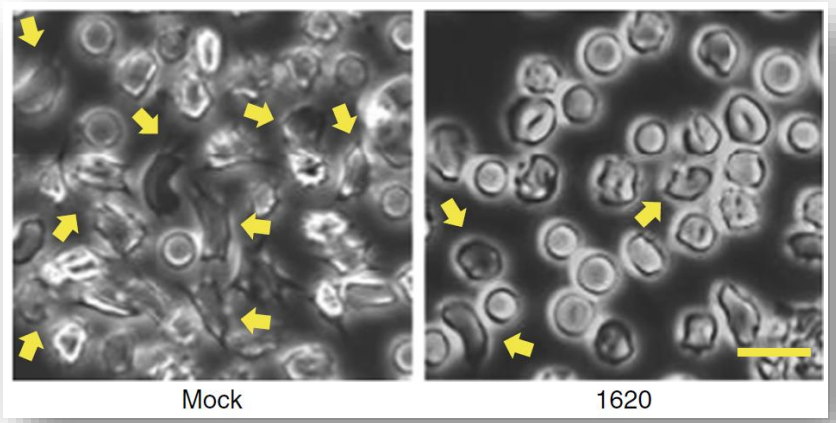
Application of A3A(N57Q)-BE3 base editor in HSC





Cure β-thalassemia or sickle cell disease





Zeng and Wu et al. Nature Medicine (2020)

Therapeutic genome editing

- Principle and applications of CRISPR genome editing
- Therapeutic genome for β-hemoglobin disorders
- **Future?**

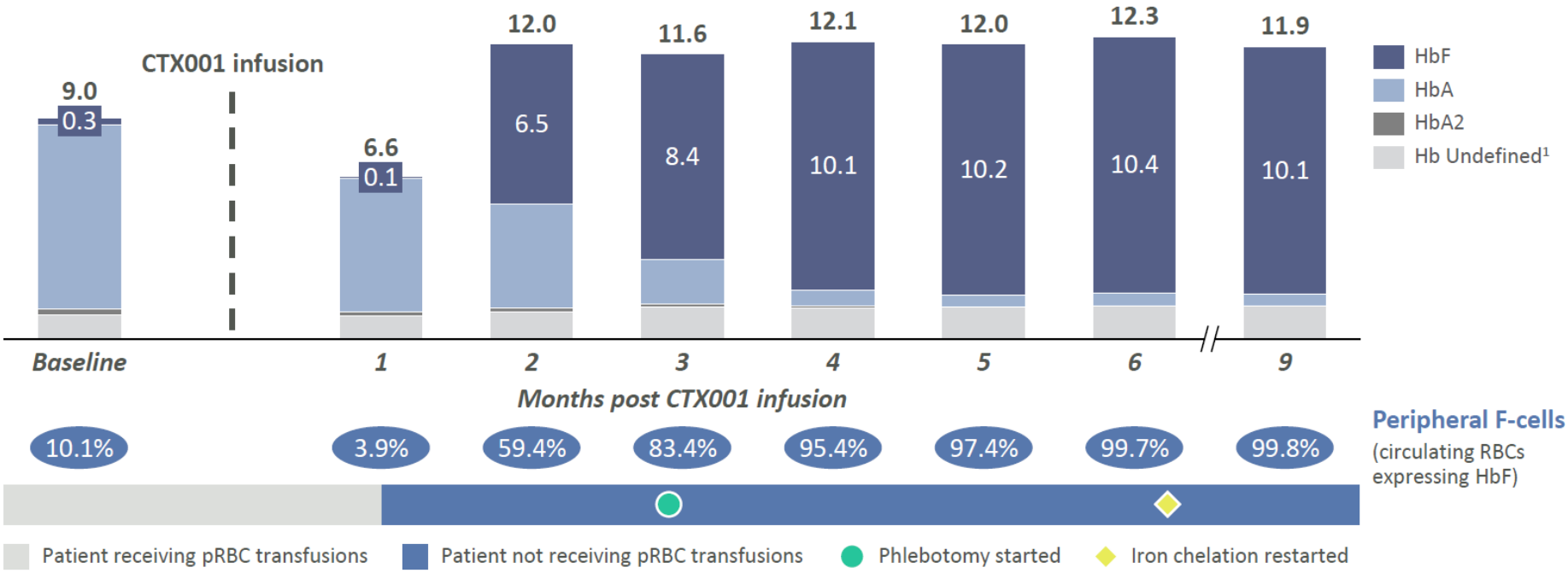
Clinical Trial

In 2018, Vertex and CRISPR Therapeutics initiated a Phase 1/2 study evaluating CTX001 in subjects with transfusion-dependent beta thalassemia and sickle cell disease.

The first attempt to use the gene-editing technique CRISPR to treat a genetic disorder in the U.S.

First TDT Patient Treated is Transfusion Free with Sustained HbF > 10 g/dL

Hemoglobin fractionation over time pre and post CTX001 infusion, Hemoglobin (g/dL)



http://www.crisprtx.com/

Thanks