Gene Therapy for Sickle Cell Disease

—Targeting HbS Polymerization —

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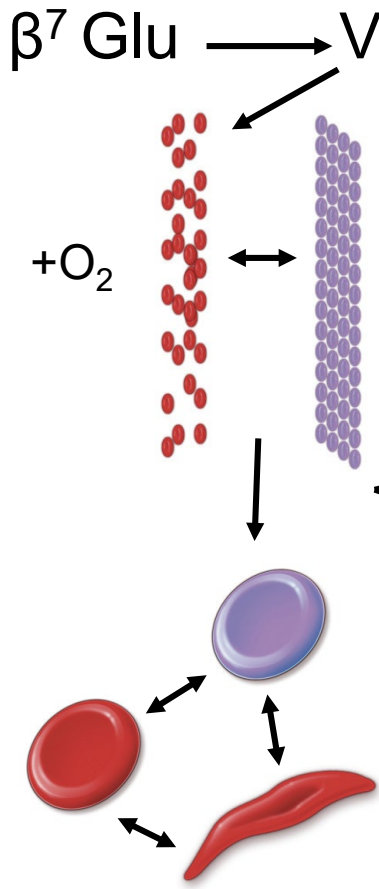
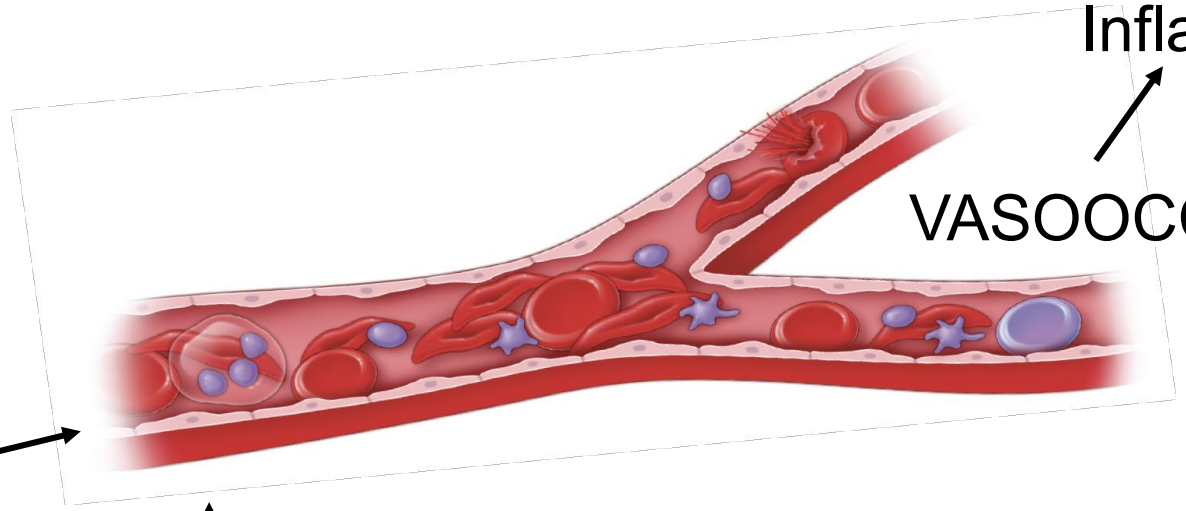
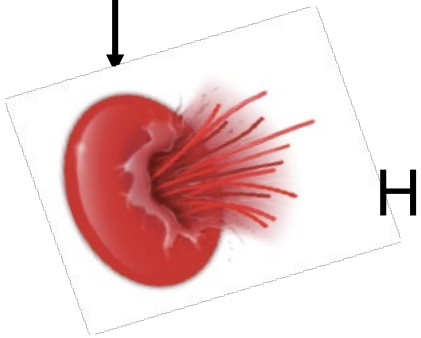
Pathophysiology of Sickle Cell Disease







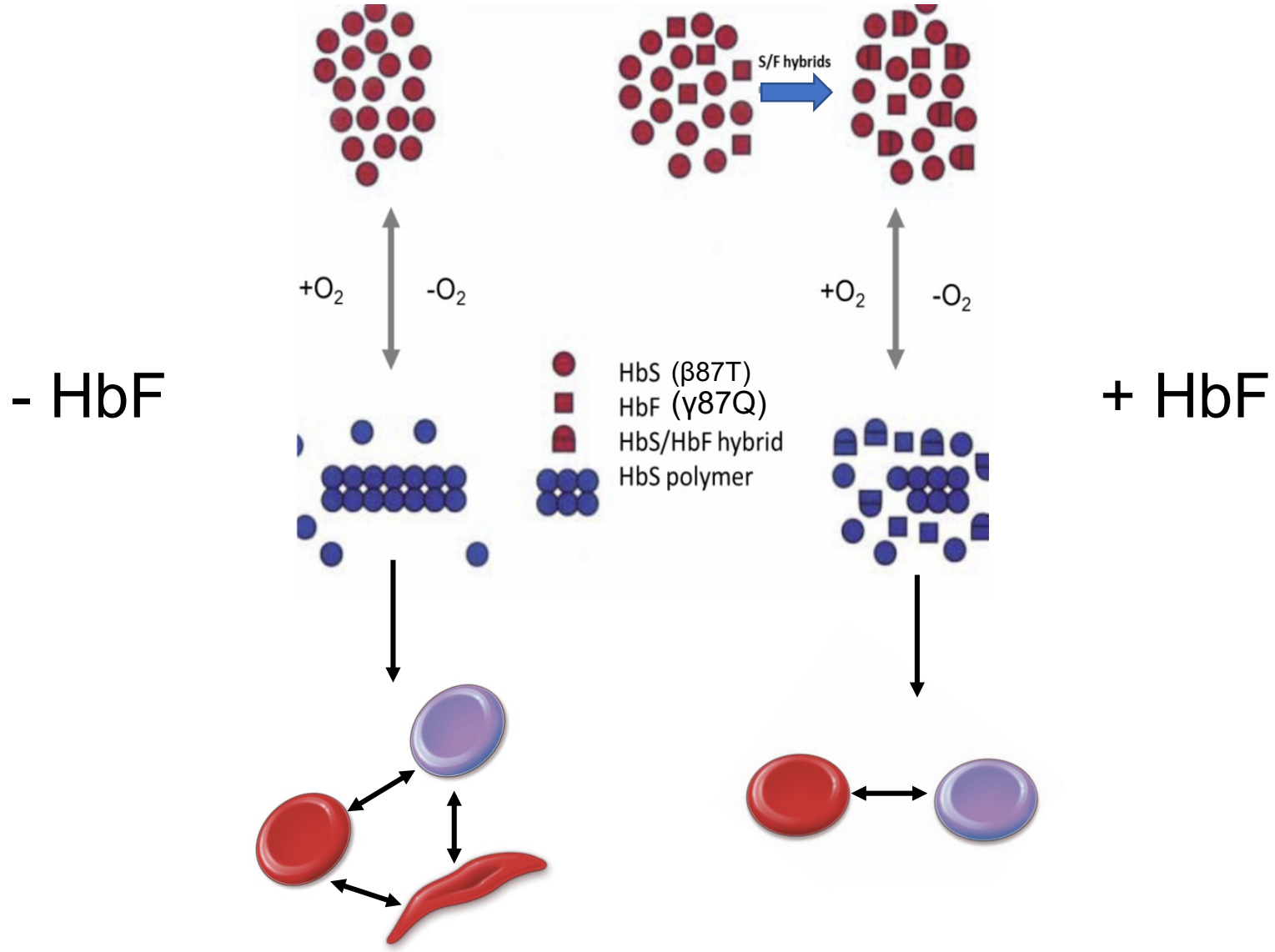
VASOOCCLUSION

HEMOLYSIS

Reperfusion injury
Inflammation

NO scavenging
Endothelial dysfunction
Vasculopathy

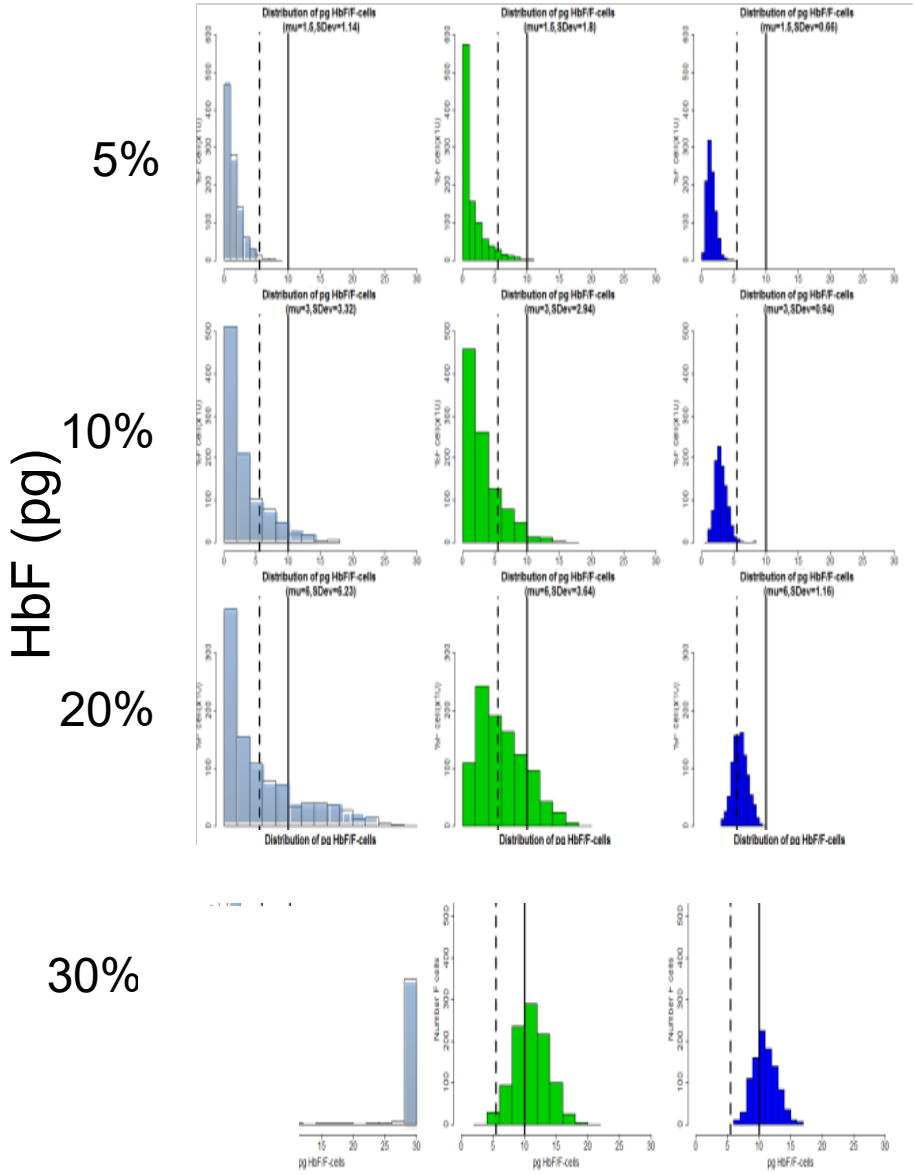
HbF interferes with deoxyHbS polymerization



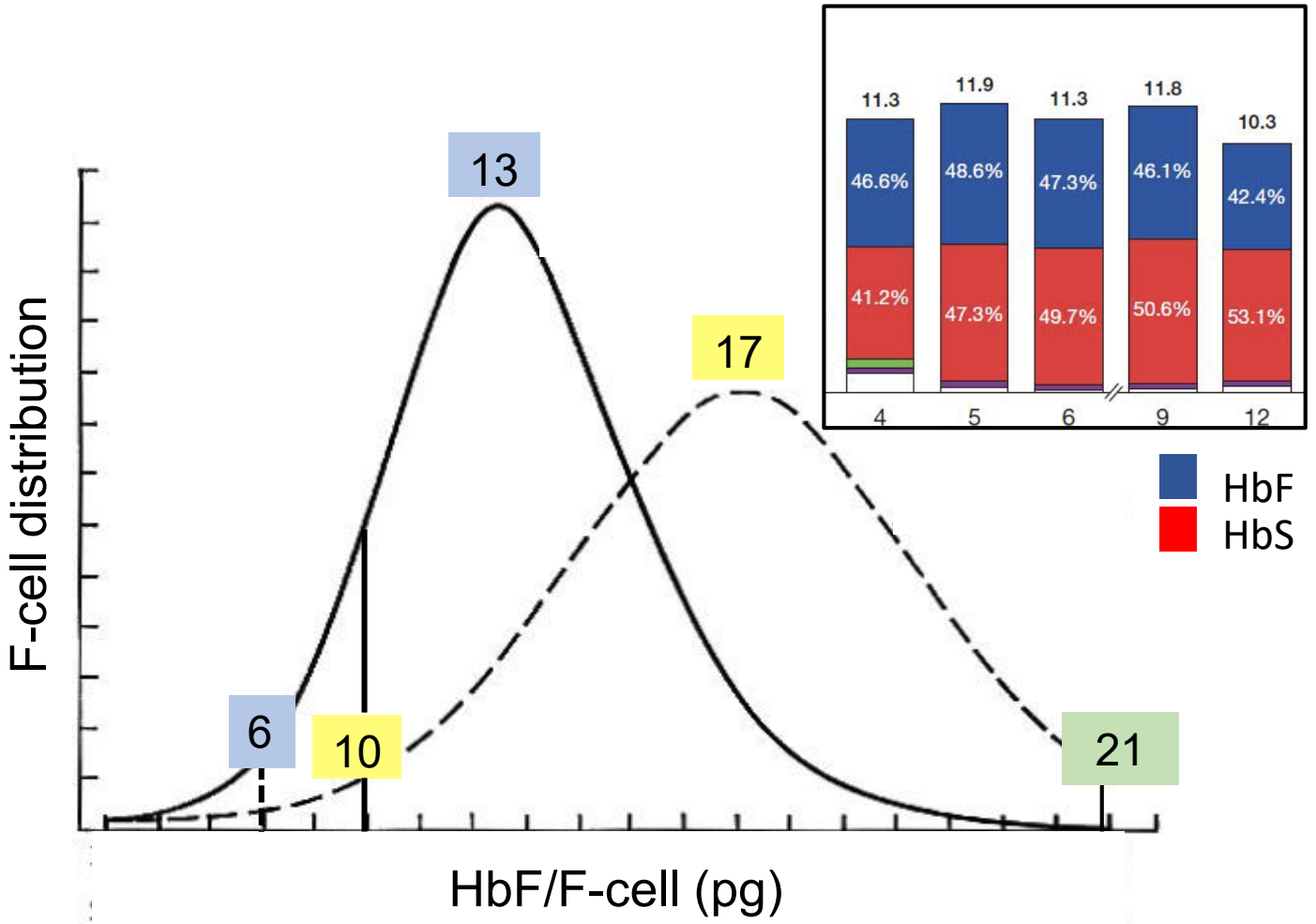
HbF

- HbF $\alpha_2\gamma_2$ (HbA $\alpha_2\beta_2$; HbS $\alpha_2\beta^s_2$)
  - γ- differs from β-globin in 39 or 40 amino acids
- ~80% HbF in newborns
  - <1% by 12 months in people without hemoglobinopathies
- In SCD, HbF levels fall with time, but remain elevated
  - 4-10% HbF in most patients of African descent
  - 16-23% HbF in many patients of Arab and Indian descent
- F-cells are RBCs with FACS-detectable HbF
  - 4-6 pg. HbF/cell is needed for detection
- HbF is heterogeneously distributed amongst F-cells

HbF in F-cells



Distribution of HbF concentrations

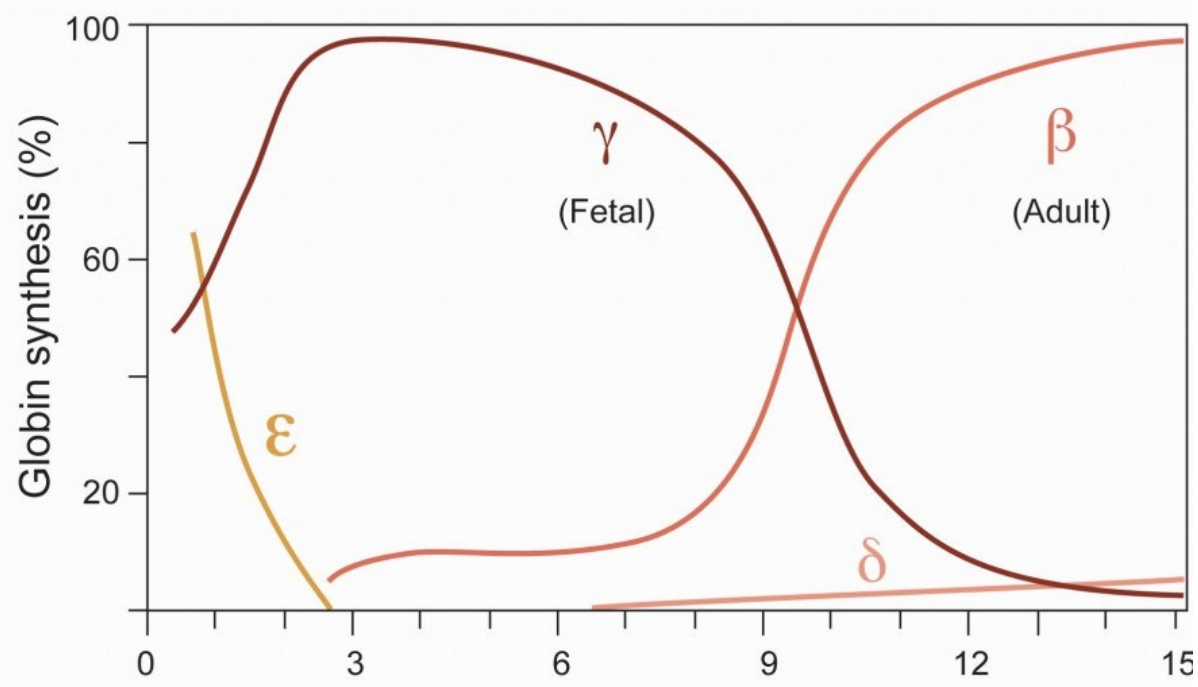


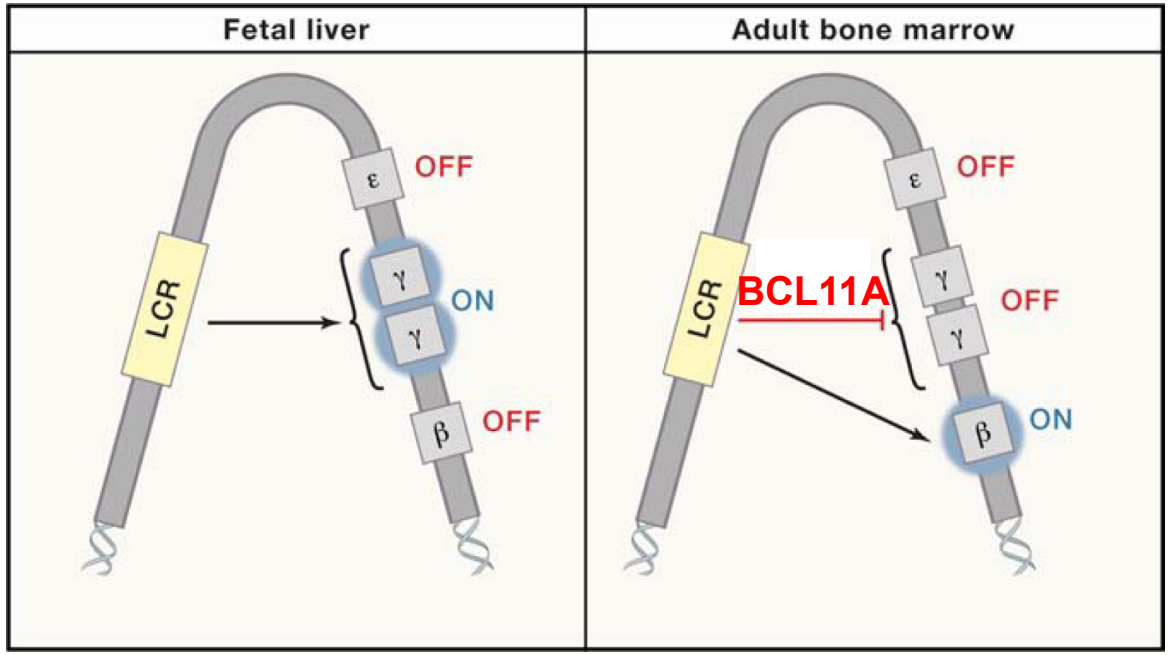
——— Lower limit of HbF "total" protection

- - - - Lower limit of HbF detection

(Steinberg et al, Blood 2014)

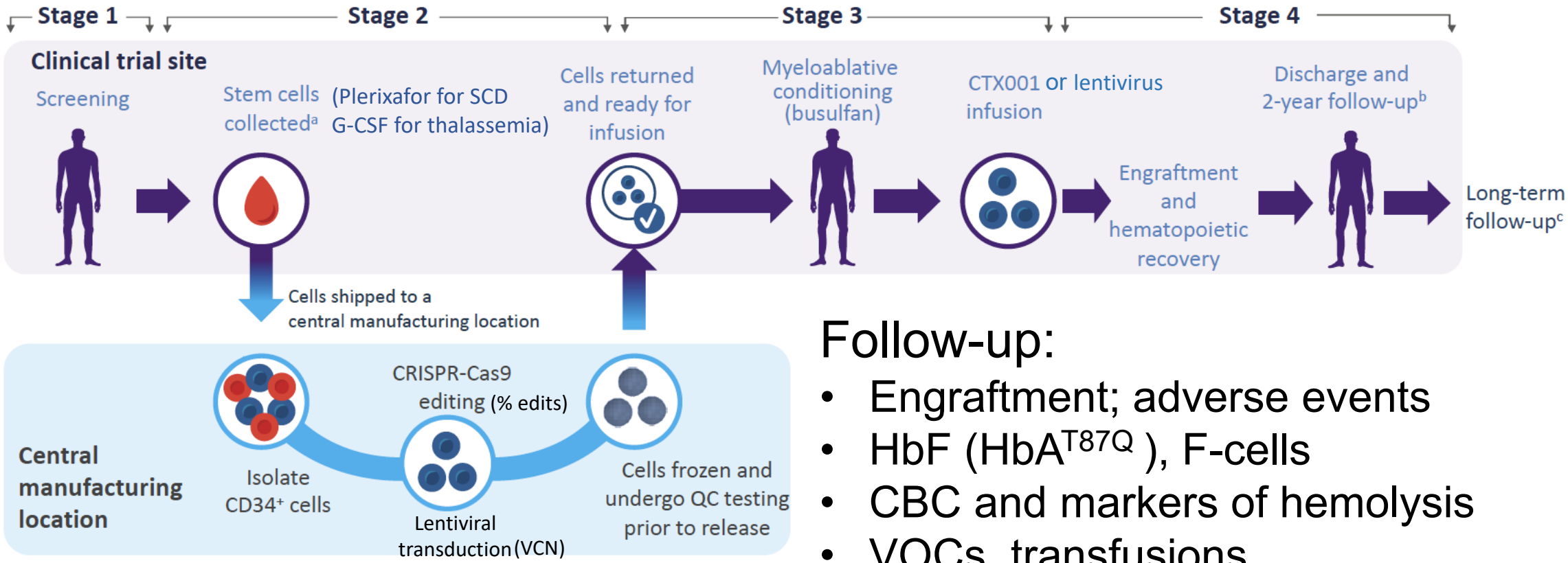
Hemoglobin switching





(after Orkin, Med, 2021)

Approaches to gene therapy using CD34+ HSPCs

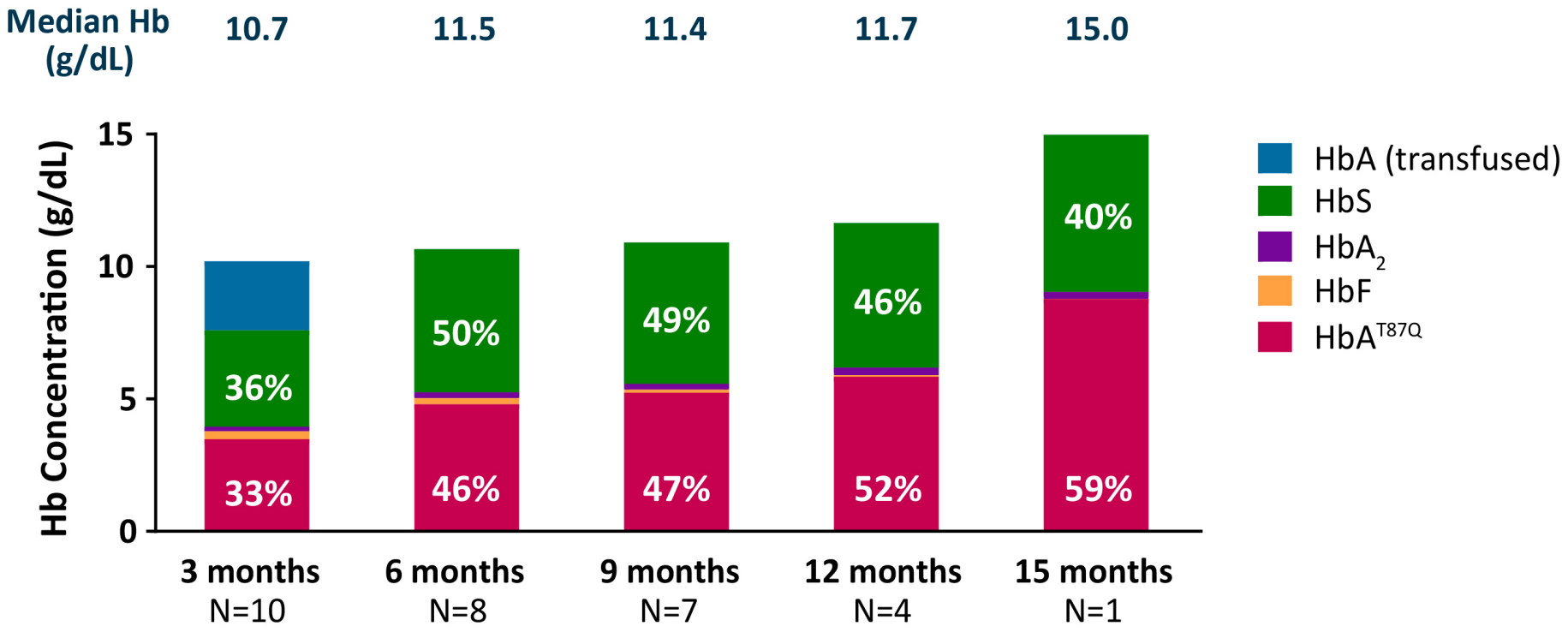


Follow-up:

- Engraftment; adverse events
- HbF (HbA^{T87Q}), F-cells
- CBC and markers of hemolysis
- VOCs, transfusions
- Allelic editing; VCN
- Off-target edits

(after Frangoul et al, ASH 2020)

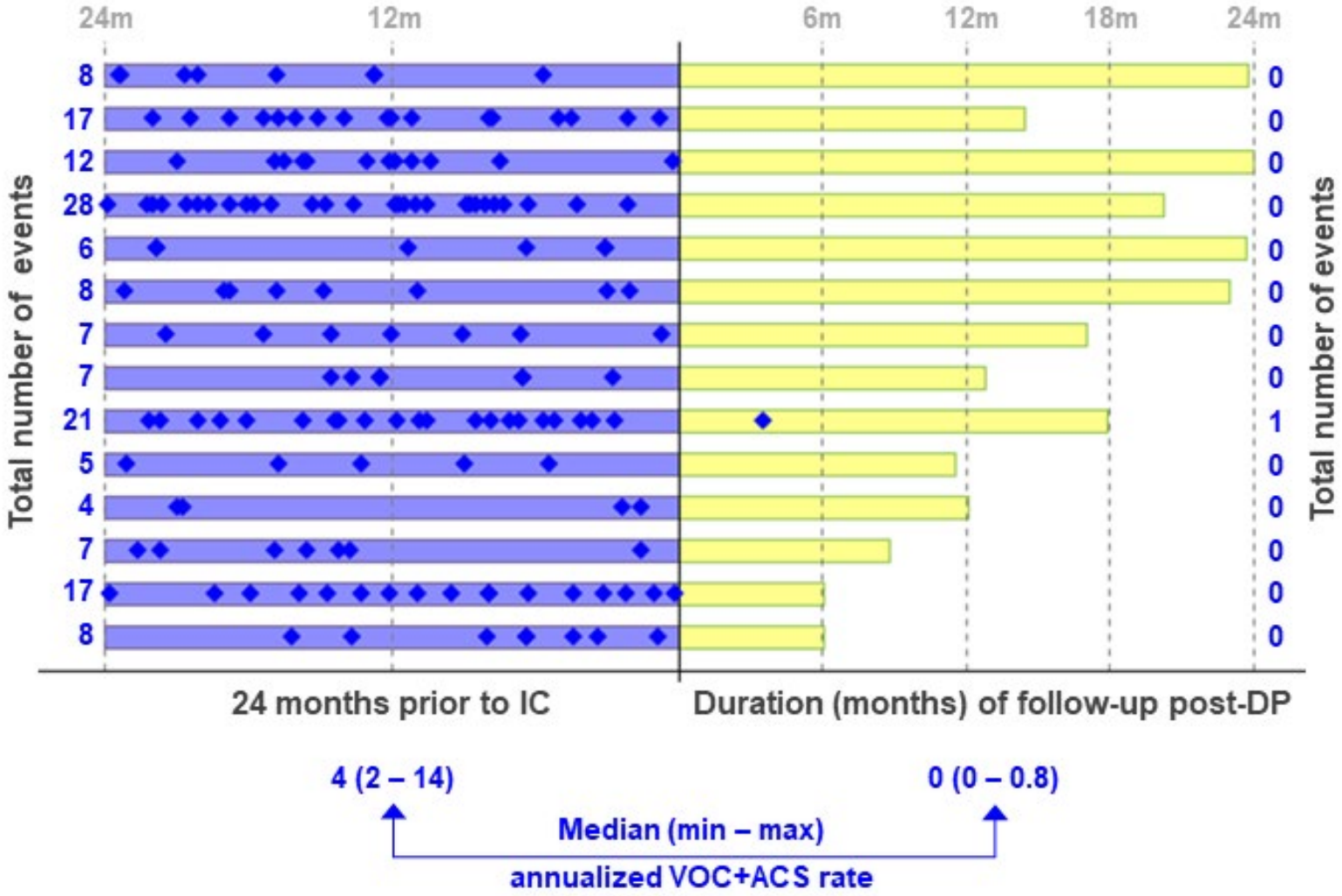
Lentivirus-mediated (β^{T87Q}) gene therapy: SCD



- LDH 225.0 (130.0–337.0) U/L; Reticulocytes 150.0 (42.1–283.0) 109/L; bilirubin 1.3 (0.2–2.0) mg/dL
- No VOC or ACS
- *MDS, AML reported 2/16/21*

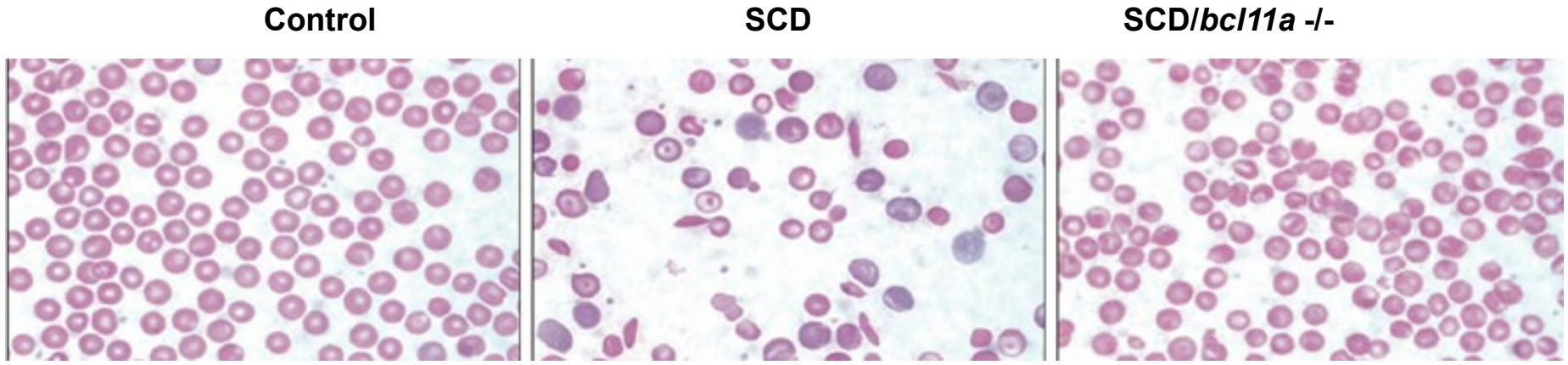
(Kanter et al, ASH 2019; bluebird bio 2021)

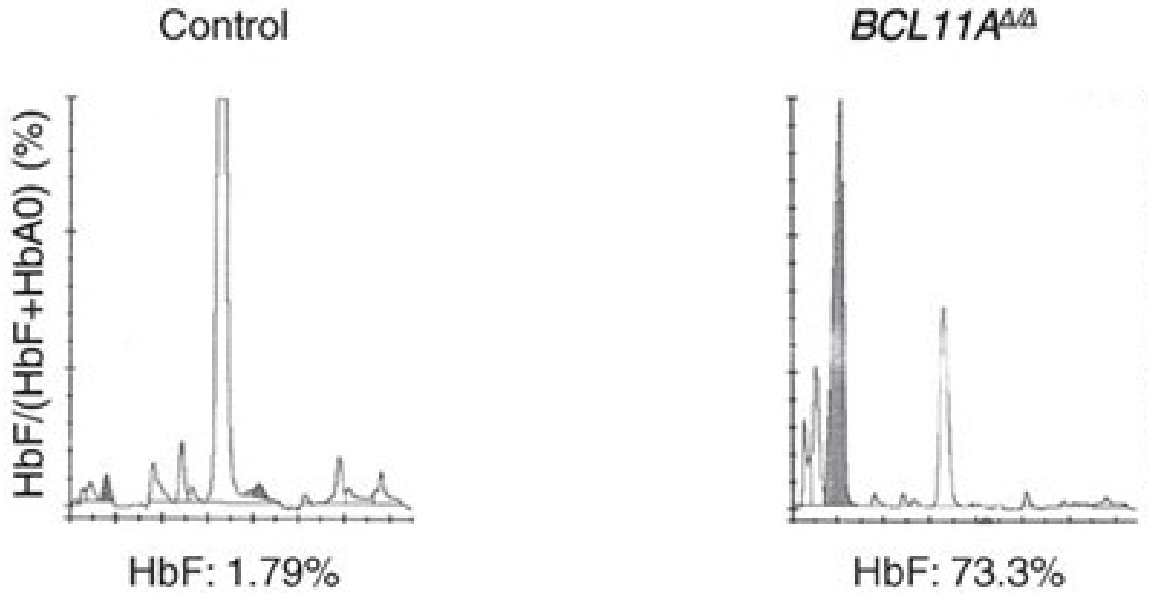
Sickle vasoocclusion following gene therapy with LentiGlobin



(Thompson et al, ASH 2020;

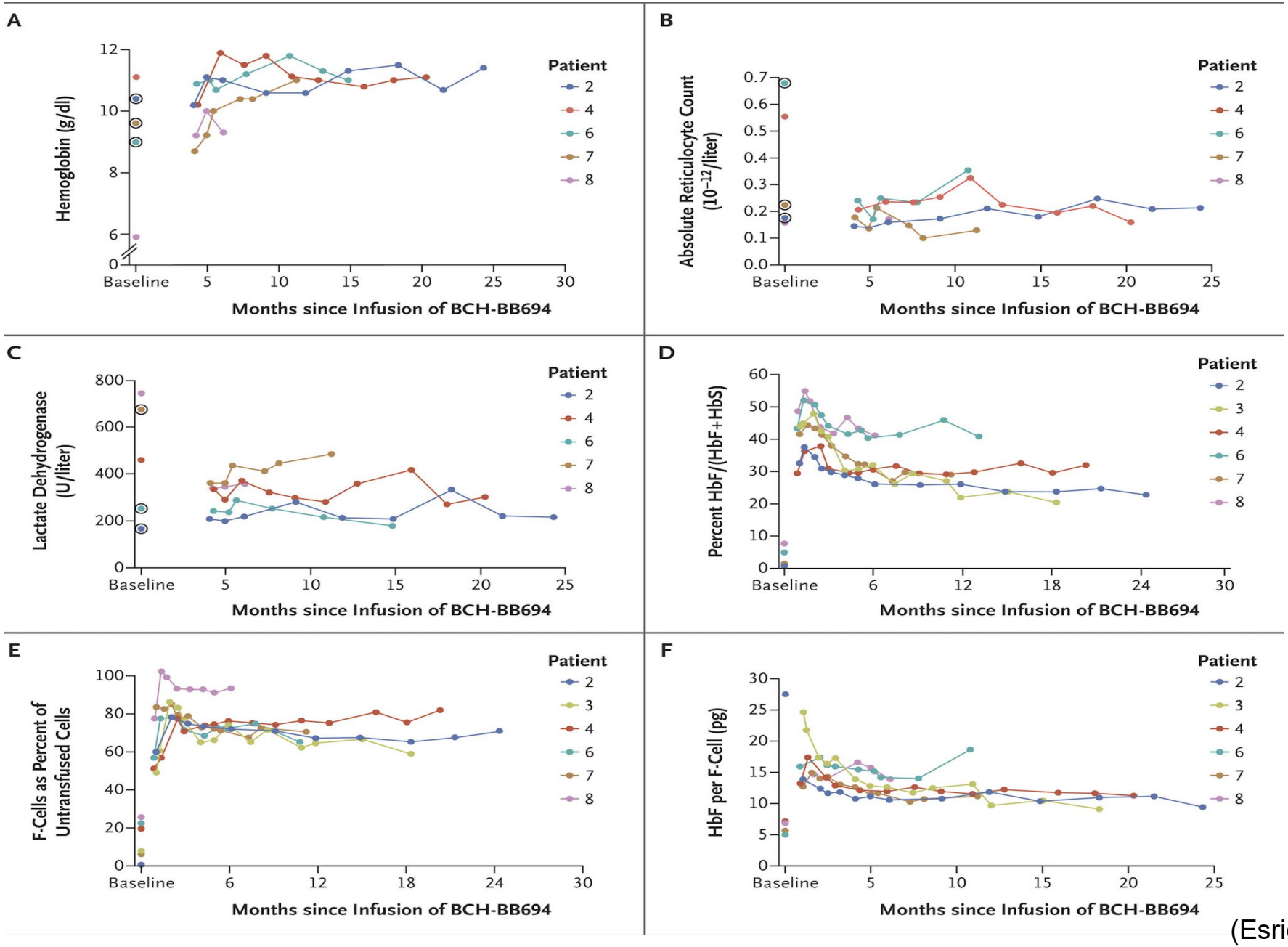
Trans-acting regulation of *HBG* expression: *bcl11a* represses *HBG2/1* in transgenic mice and HUDEP2 cells





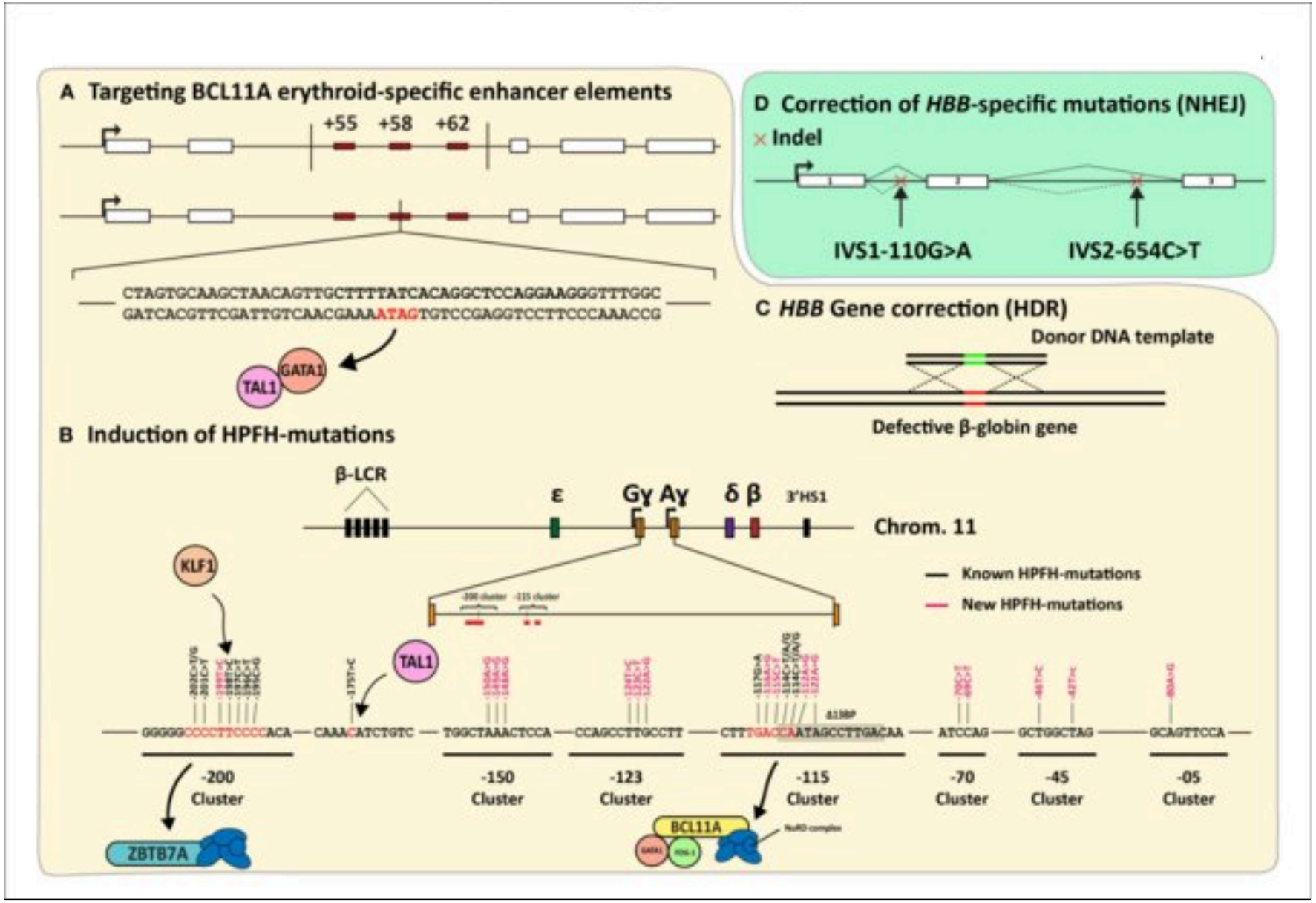
(Xu et al, Science 2011; Bauer et al, Science 2013; Masuda et al, Science 2016)

shmRNA directed to the erythroid enhancer of *BCL11A* increases HbF



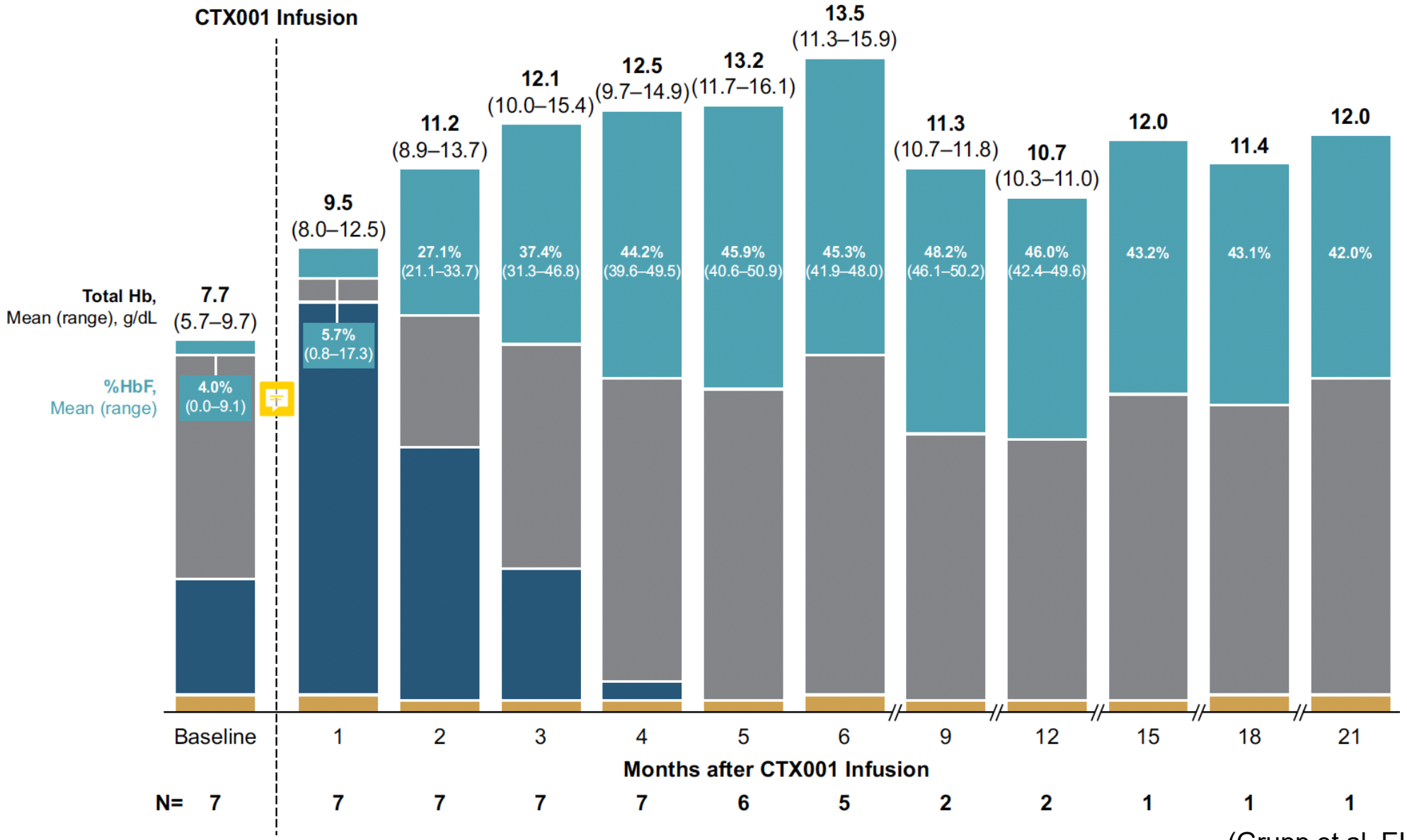
(Esrick et al, N Engl J Med 2021)

Genome editing to “cure” β hemoglobinopathies



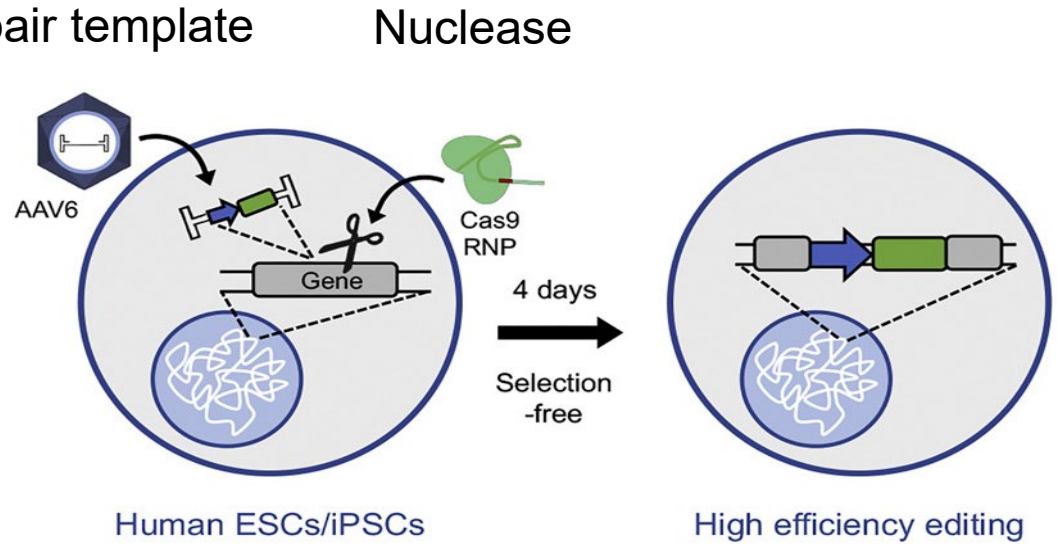
(Zittersteijn et al, Front Genome Ed 2021)

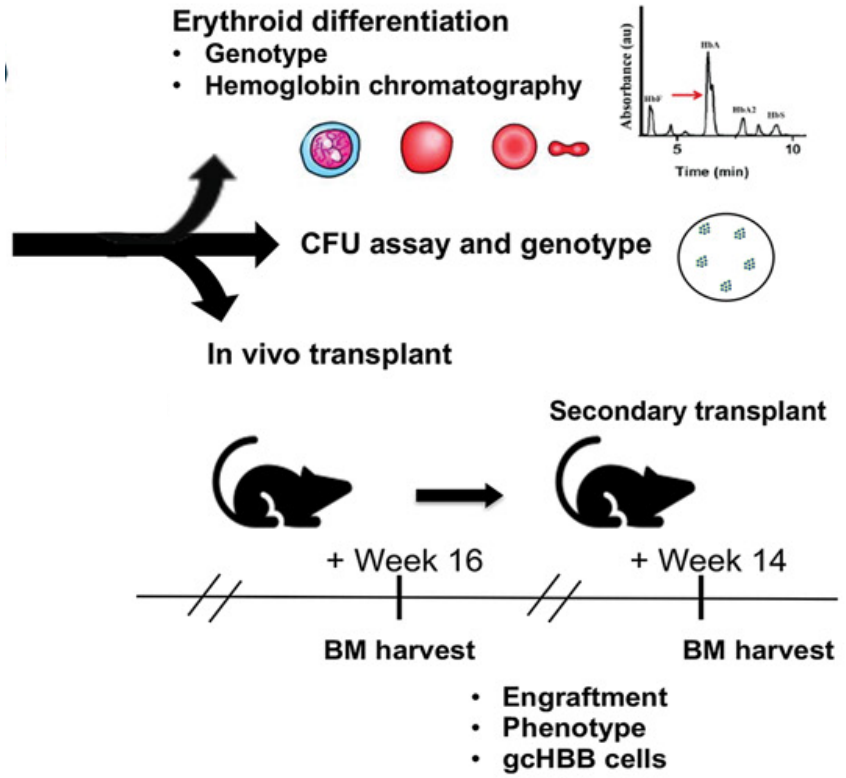
Hemoglobin fractions after *BCL11A* enhancer disruption in sickle HPSCs



(Grupp et al, EHA 2021)

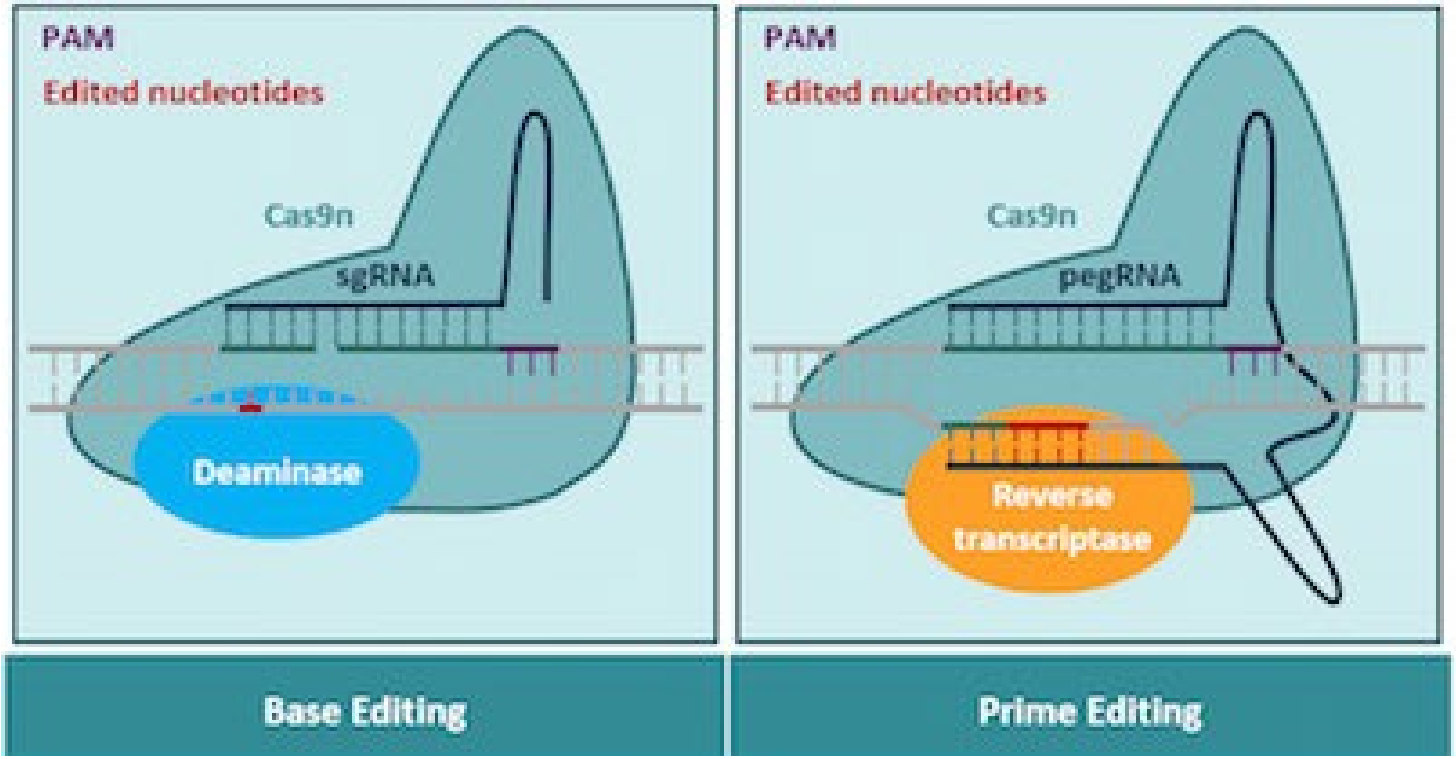
An efficient approach to HDR; correcting the HbS gene





(Martin et al, Cell Stem Cell, 2019; Lattanzi et al, Sci Transl Med 2021)

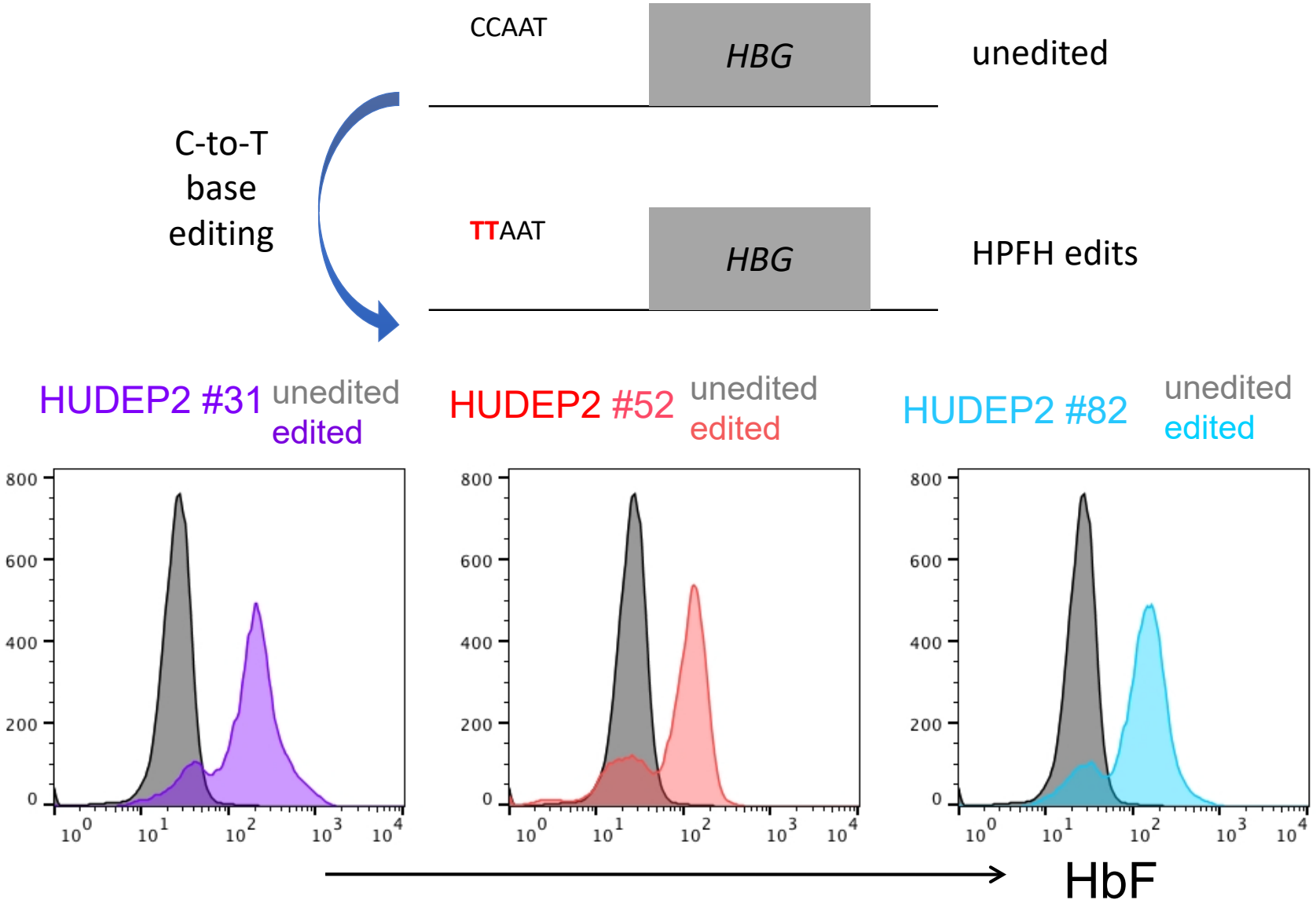
Other gene editing technologies



Base Editing	Prime Editing
Cas and deaminase	Cas and reverse transcriptase
Only transitions (C-T; G-A)	All possible modifications
Requires nearby PAM	Less restrictive PAM
Off-target effects	Off-target effects

(after Razza & Armazzani, genOway, 2019)

BCL11A binding motif base-editing increases HbF

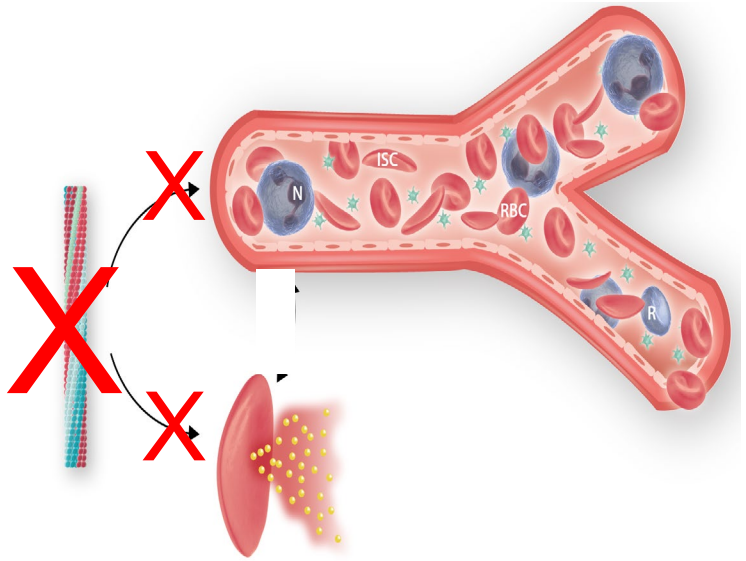


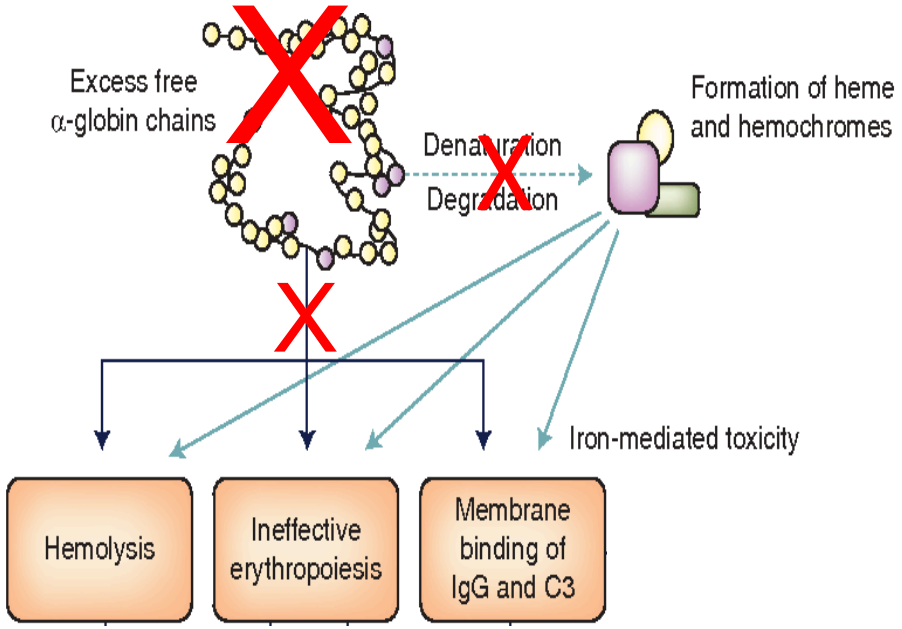
(Vanuytsel et al, 2020)

Summary

cellular therapeutics → induce ~50% HbF

cellular therapeutics → correct (replace) HbS (β thalassemia) gene





- Benefits depend on amount of HbF induced and HbF distribution amongst F-cells
- With ~50% HbF β hemoglobinopathies are “cured”

Can too much HbF be harmful?

- Fetal development is normal
- Homozygotes for deletion HPFH have100% HbF and:
  - low P_{50}
  - mild erythrocytosis
  - low MCV
  - α/γ biosynthesis ratio ~1.5, ≈ β thalassemia trait
- HbF >70% has been associated with IUGR
- Pregnant patients with HbSS had fewer complications when HbF >15%
- In HbS-HPFH (30% HbF), P_{50} is nearly normal as the predominant tetramer is $\alpha_2\beta^S\gamma$, not $\alpha_2\gamma_2$

(Murji et al, J Maternal-Fetal Neonat Med, 2012; Mikobi et al, Mediterr J Hematol Infect Dis. 2019)

Issues

- Genotoxic conditioning regimens
  - single dose melphalan vs. busulfan?
  - non-genotoxic antibody-based regimens
- MDS, AML
  - complication of SCD ?
  - complication of conditioning?
  - complication of random vector insertion?
  - complication of lentiviral vectors?
- Off-target editing effects
  - depends on editing approach?
  - induces thalassemia?

Future of gene-based therapy

- Non-myeloablative, non-genotoxic conditioning
- Improved methods of stem cell collection
- Use of iPSC-derived HPSCs
- In vivo delivery of “editors” to HPSCs
- Small, orally available molecules that induce high concentrations of pancellularly distributed HbF