Sickle cell disease (SCD)

MSCRF-CIRM collaboration to reduce levels of sickle hemoglobin (HbS) in the red blood cells of SCD patients via cooperative development of the following 2 strategies:

- **CIRM grant (Don Kohn, UCLA):** Correct the classic β-globin chain gene codon 6 GAG->GTG mutations of SCD via ZFN technology

- **MSCRF grant:** Disrupt the erythroid enhancer of BCL11A via CRISPR/Cas9 technology

Both strategies target hematopoietic stem-progenitor cells

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MSCRF project: Genetic Modification of Sickle Cell Disease in Hematopoietic Stem Cells

Disrupt the DNA encoding an erythroid-specific enhancer that regulates levels of BCL11A via CRISPR/Cas9 genome editing technology of human HSCs

Curt Civin, Wen-Chih Cheng, Tami Kingsbury, MinJung Kim

SNPs in BCL11A revealed that disruption of BCL11A can rescue SCD phenotypes via producing γ-globin

BCL11A (transcriptional repressor)

⊣

γ-globin

↓

fetal hemoglobin, HbF, $\alpha_2\gamma_2$

↓

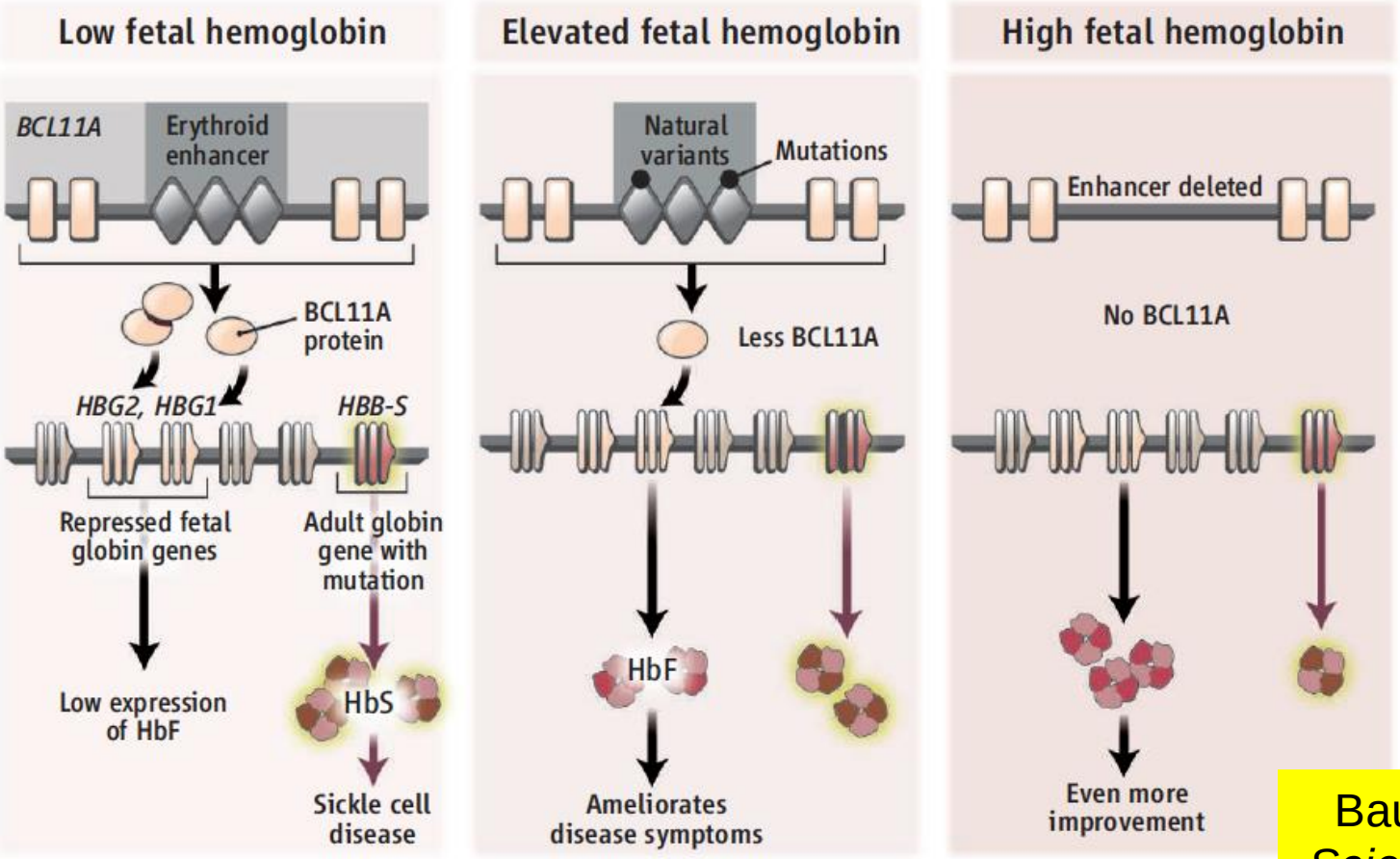
Patients with HPFH have reduced severity of SCD because HbS polymerization is inhibited by HbF

HPFH patients have rare SNPs in the enhancer of BCL11A that specifically disrupt BCL11A expression in erythroid cells

*Bauer, D.E. et al. Science **342**, 253-257 (2013).*

GWAS to Therapy by Genome Edits?

Genetic and epigenetic studies of gene variants reveal a potential genomic target for treating hemoglobin disorders. Hardison & Blobel editorial, *Science* 341:206. 2013



Bauer (Orkin group), *Science* 342:253, 2013

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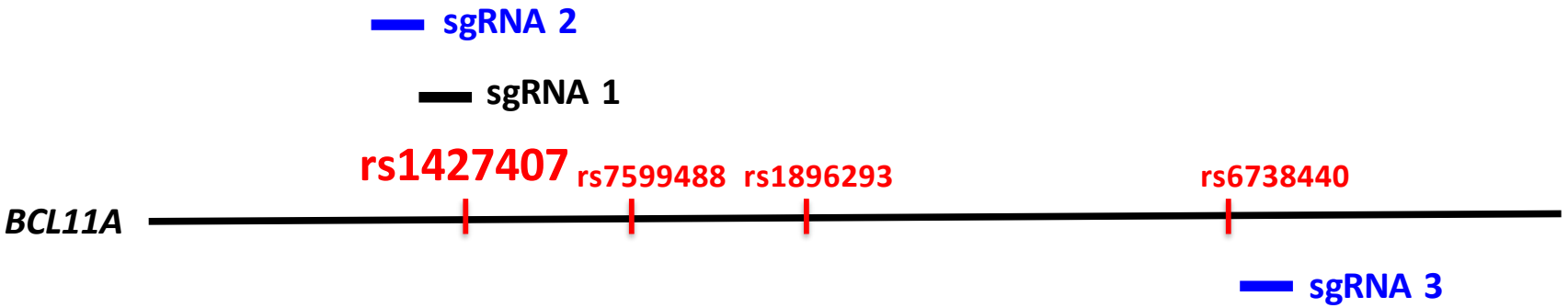
CRISPR/Cas9 strategies to disrupt the erythroid-specific enhancer of BCL11A



- **Strategy #1:** remove a small regulatory element surrounding the most significant SNP in the erythroid-specific enhancer
  - Small deletions **~100 bp**

- **Strategy #2:** remove a **~4kb** region from the regulatory element including the most significant SNPs and other SNPs also linked to HPFH

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The big problem will be the stem cell biology, not the gene editing!

MaxCyte flow electroporation technology

- Madhusudan Peshwa, PhD
- Linhong Li, PhD

Genetic Modification of Sickle Cell Disease in Hematopoietic Stem Cells

Targeting BCL11A regulatory region via CRISPR/Cas9 genome engineering technology

Sickle cell disease (SCD)

Correct sickle hemoglobin (HbS) in the red blood cells of SCD patients with the following strategies:

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- MSCRF grant: Disrupt the erythroid enhancer of BCL11A via CRISPR/Cas9 technology

Both strategies targets hematopoietic stem-progenitor cells

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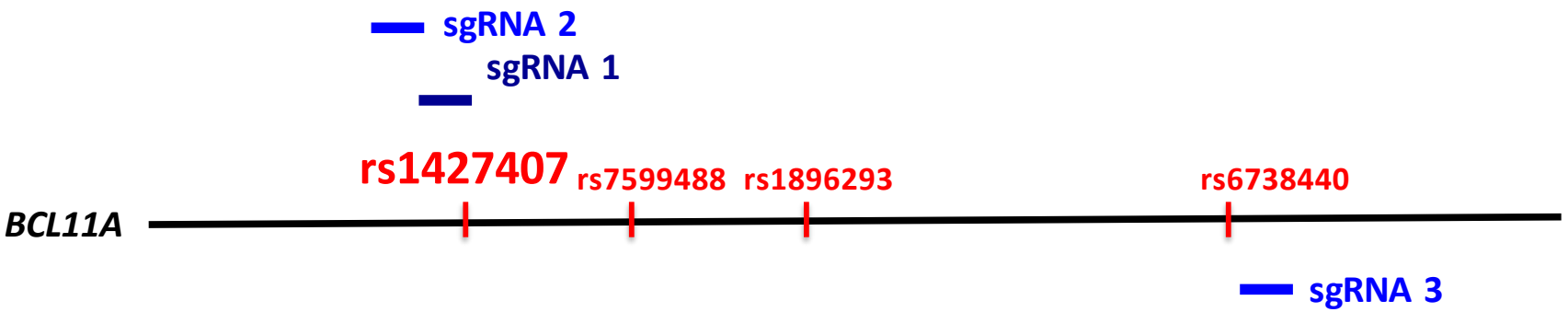
CRISPR/Cas9 strategies to disrupt the erythroid-specific enhancer of BCL11A



- Strategy #1: remove a small regulatory element surrounding the most significant SNP in the erythorid-specific enhancer. Small deletions ~100 bp.

- Strategy #2: remove a ~4kb regulatory element including the most significant SNPs and other SNPs also linked to HPFH.