Understanding Gene Therapy and Addressing Health Disparities in Sickle Cell Disease

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THE FOUNDATION FOR SICKLE CELL DISEASE RESEARCH

CHANGE THE CONVERSATION.

RESHAPE THE FUTURE.

New England Journal of Medicine

"..a primary challenge to many patients with sickle cell disease remains a **social** one: being seen and treated as individuals who deserve relief, and being supported rather than stigmatized in a highly charged atmosphere" (Wailoo, 2017: 807)

Wailoo, K (2017) Sickle cell disease: a history of peril and progress *New England Journal of Medicine* 376(9): 805-807

Sickle Cell Disease – The American Saga

Azfar-E-Alam Siddiqi, MD, PhD, Lanetta B. Jordan, MD,MPH,MSPH, and **Christopher S. Parker, PhD**
National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, Georgia (AS, CSP); University of Miami, Miller School of Medicine, Miami, Florida (LBJ)

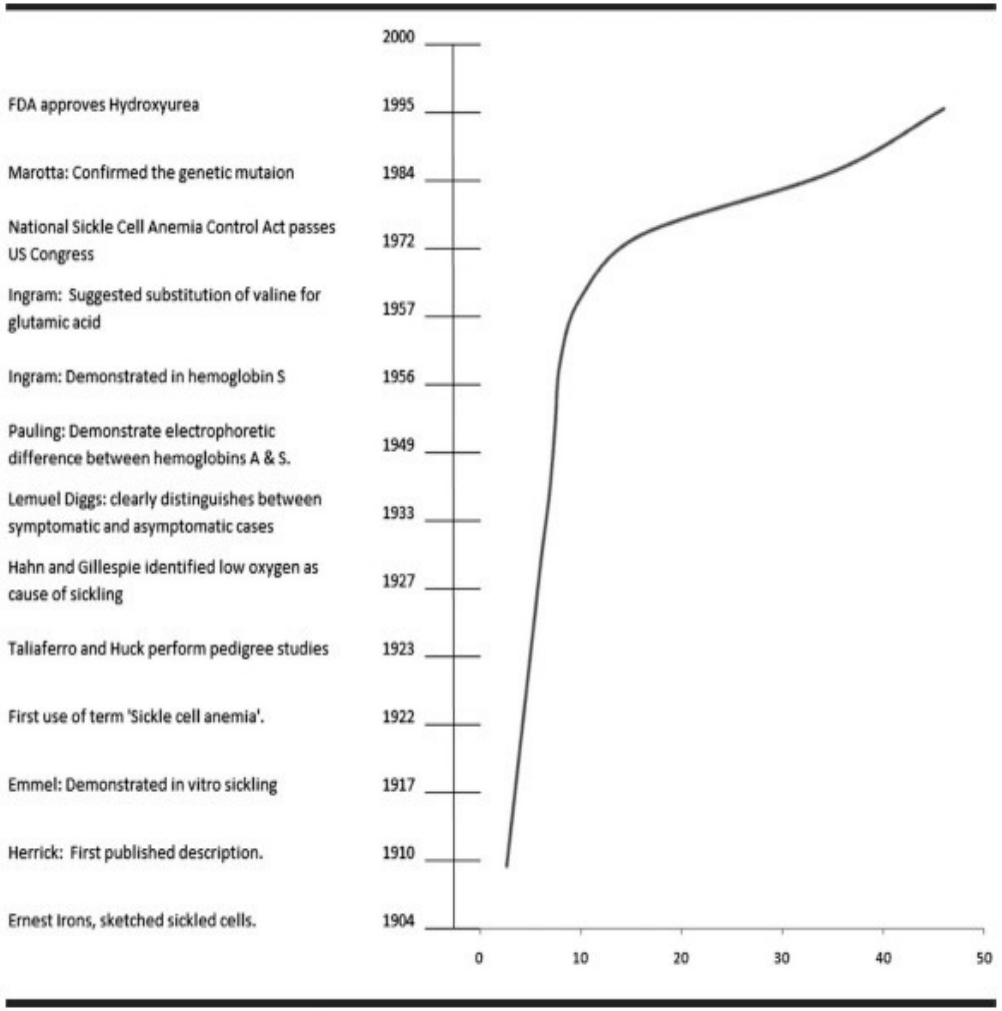

Fig 1.
Timeline of major scientific events in the history of sickle cell disease and life expectancy at birth of persons with sickle cell disease in the United States

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The New York Times | https://nyti.ms/2DeGjfG

HEALTH

How One Child's Sickle Cell Mutation Helped Protect the World From Malaria

The genetic mutation arose 7,300 years ago in just one person in West Africa, scientists reported on Thursday. Its advantage: a shield against rampant malaria.

Carl Zimmer

MATTER MARCH 8, 2018

NIH and National Foundation Expenditures For Sickle Cell Disease and Cystic Fibrosis Are Associated With Pubmed Publications and FDA Approvals

John J. Strouse, Katie Lobner, Sophie Lanzkron, MHS, and Carlton Haywood Jr.

Blood 2013 122:1739;

Table

Funding and Research Productivity for Sickle Cell Disease and Cystic Fibrosis by Year.

	Sickle Cell Disease	Cystic Fibrosis
NIH Funding 2010	$85,023,144	$99,201,078
NIH Funding 2011	$65,094,922	$78,861,688
NIH Career Development Awards 2010	15	14
NIH Career Development Awards 2011	16	18
Foundation Funding 2010	$1,399,062	$122,135,426
Foundation Funding 2011	$1,185,023	$176,209,849
Per Person Funding 2010	$970	$7,378
Per Person Funding 2011	$744	$8,502
PubMed Publication 2010	801	1553
PubMed Publication 2011	867	1556
Clinical Trials 2010	24	37
Clinical Trials 2011	26	25

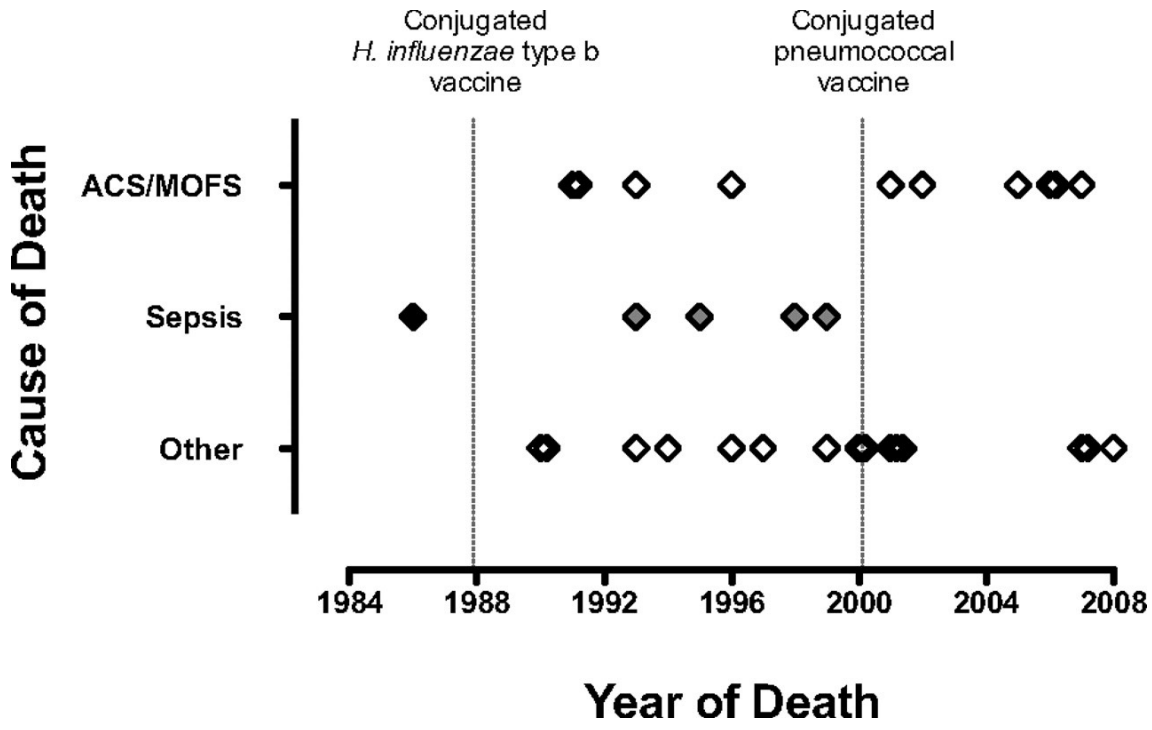
Survival in SCD

- Newborn screening for hemoglobinopathies mandated in all states in 2006[1]
- Prior to universal screening and intervention, infection was the most common cause of death in children with SCD[1]
- Penicillin prophylaxis and pneumococcal vaccines have reduced the rate of invasive pneumococcal disease by up to 90%[1]
- >98% of children with SCD now live to become adults[2]

1. Meier ER, Rampersad A. *Pediatr Res.* 2017;81(1-2):249-258. 2. Quinn CT, et al. *Blood*. 2010;115(17):3447-3452. 3. Saulsberry A, et al. *Hematology Am Soc Hematol Educ Program*. 2019;2019(1):496-504.

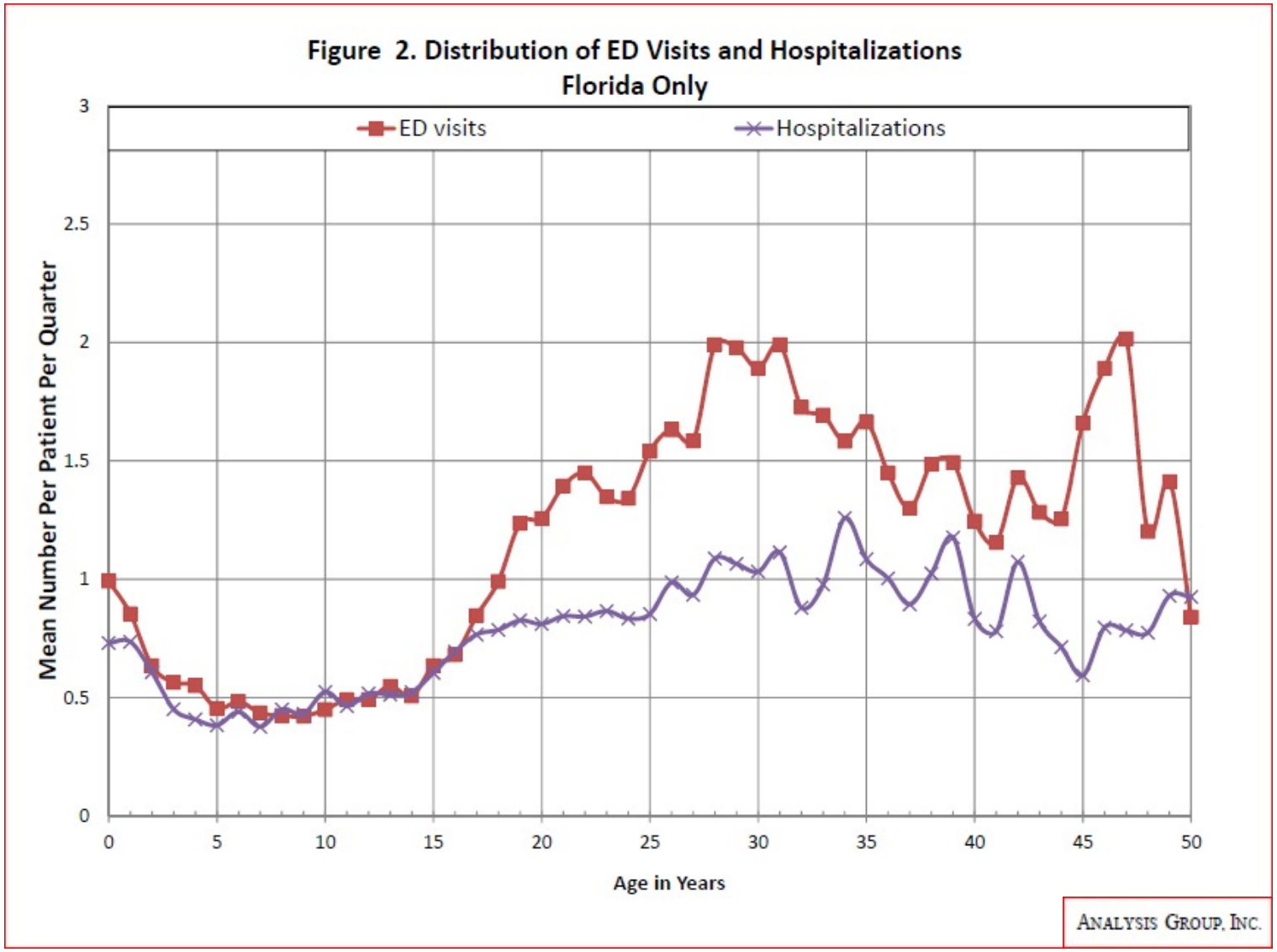
Causes of Death in Children with SCD: Then and Now

- Deaths due to acute chest syndrome (ACS) and multiorgan failure syndrome (MOFS) are now more common than fatal sepsis



Quinn CT, et al. *Blood*. 2010;115(17):3447-3452.

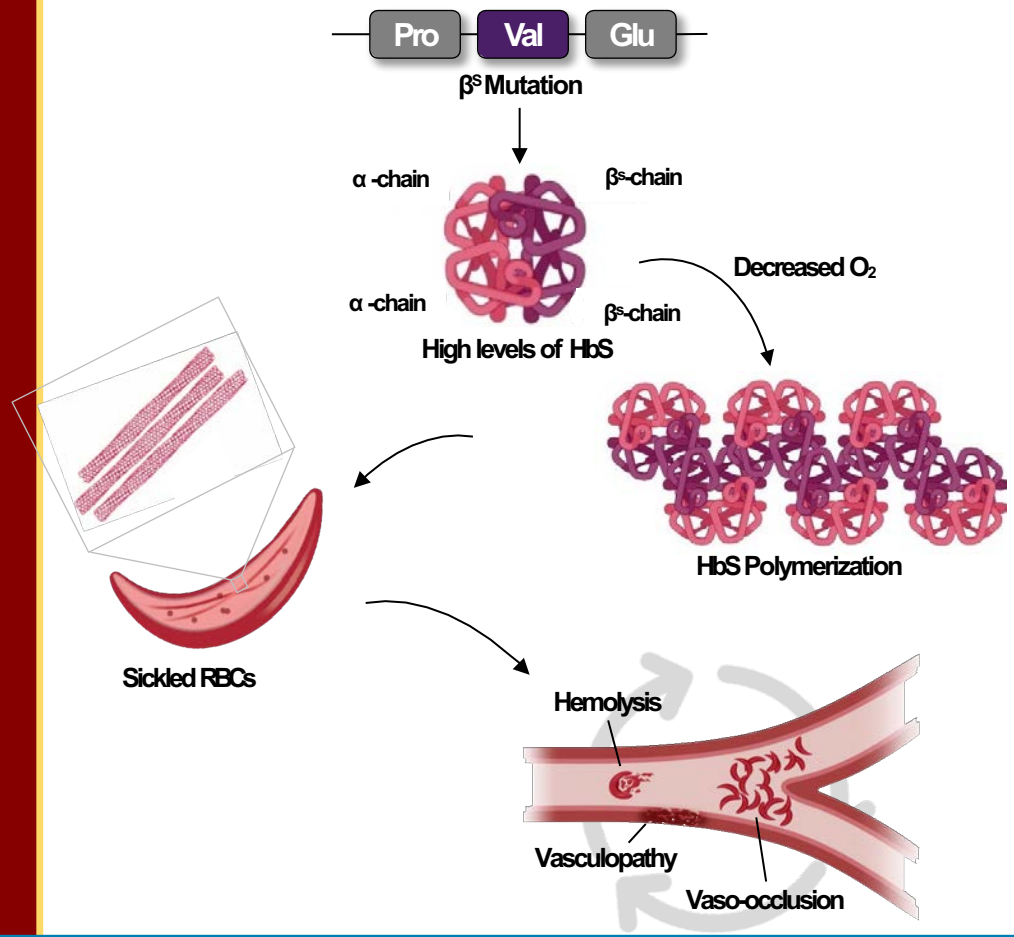
ED and Hospitalizations



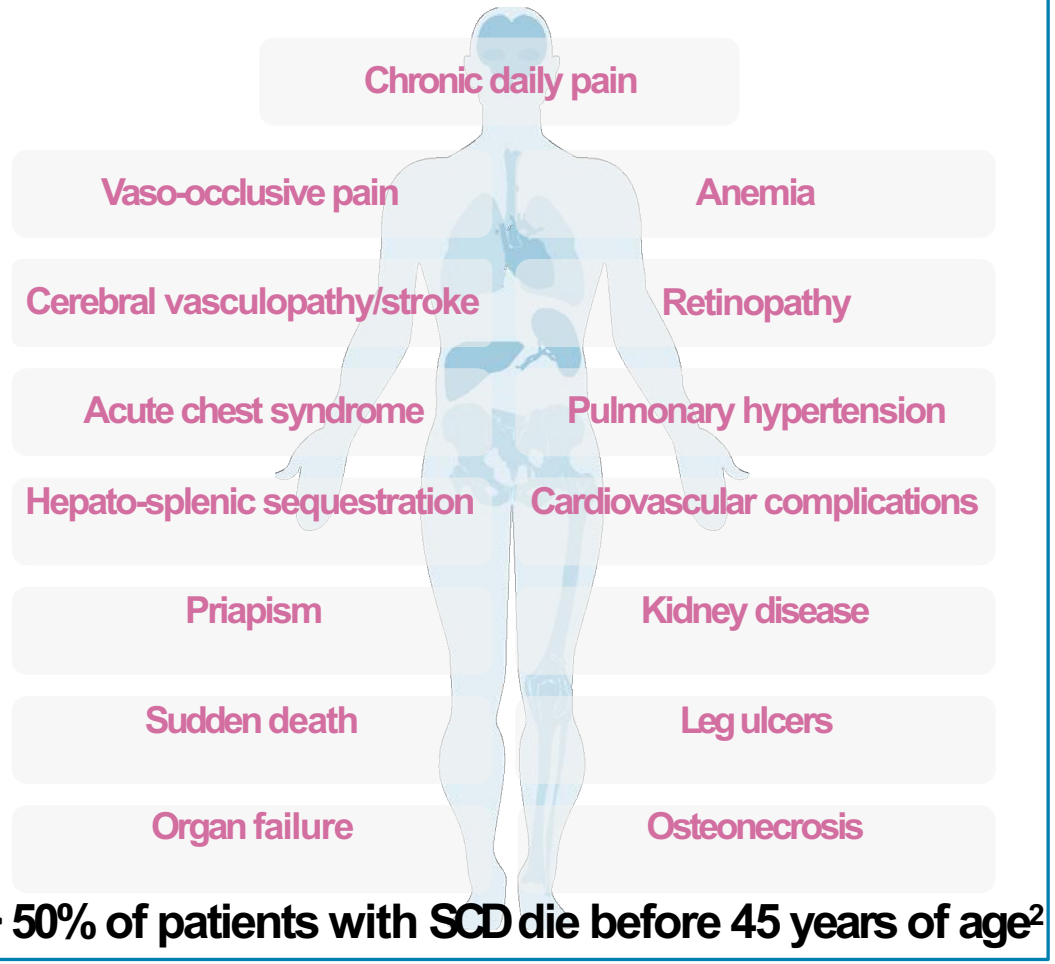
SCD is characterized by high morbidity and early mortality

For video

Pathophysiology of SCD[1]



Complications[2,3]



> 50% of patients with SCD die before 45 years of age[2]

Glu, glutamic acid; Hbs, sickle hemoglobin; Pro, proline; RBC, red blood cell; SCD, sickle cell disease; Val, valine.

1. Kato GJ, et al. *Nat Rev Dis Primer.* 2018;4:18010; 2. Hassell K., *Am JPrev Med.* 2010; 3. Kanter, et al. *Blood Rev.* 2013;27(6):279- 287.

THE FOUNDATION FOR SICKLE CELL DISEASE RESEARCH CHANGE THE CONVERSATION. RESHAPE THE FUTURE.

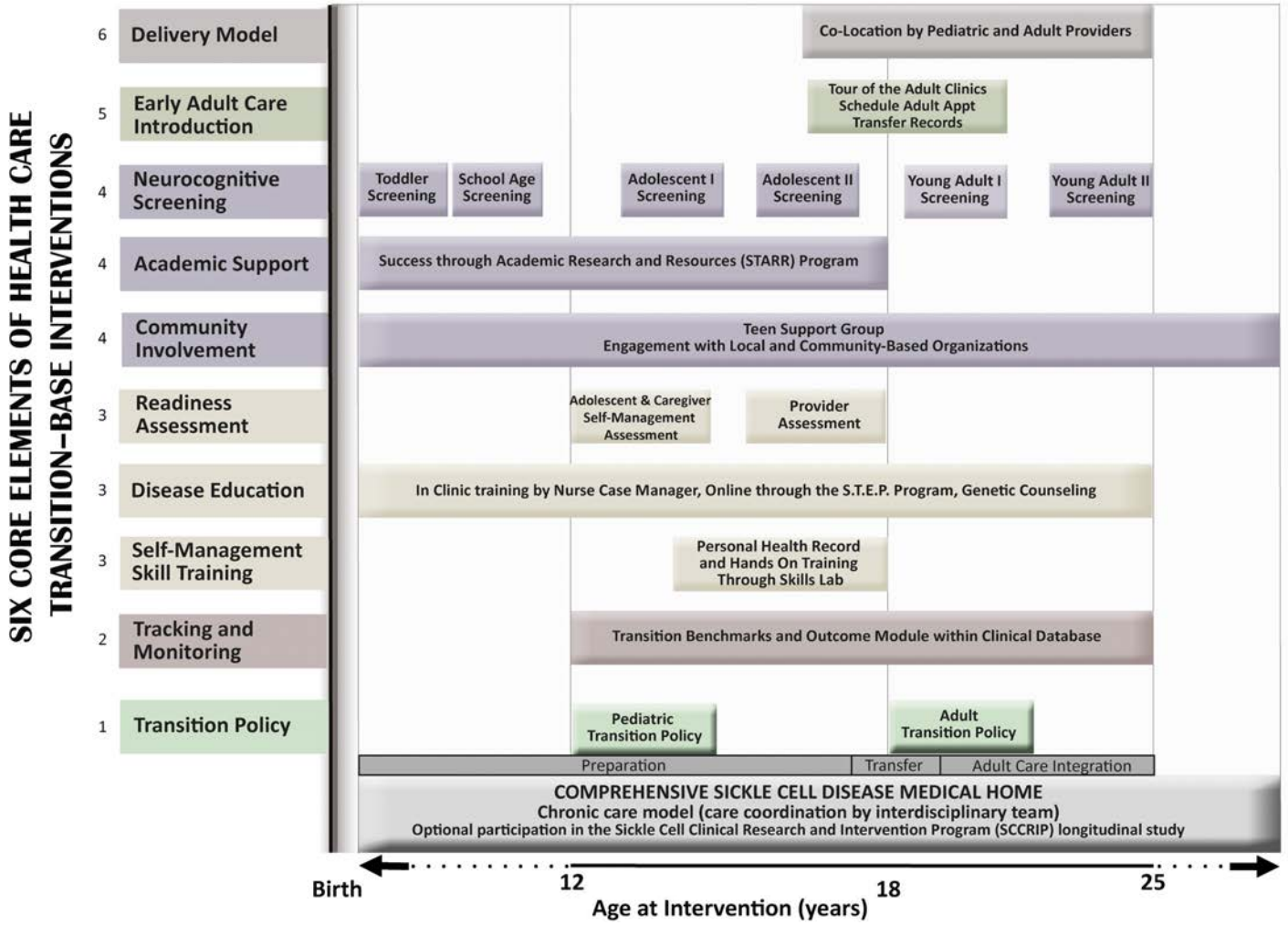
Treatment Evolution for in children and adults with SCD

- Patients have lacked FDA-approved drug therapies for almost 100 years
- Blood transfusion and supportive therapies (penicillin, folic acid) have the primary treatments for SCD patients[1]
- The first two FDA-approved drugs in SCD were in 2017 with L-glutamine (July)[2] and hydroxyurea in 1998 for adults and now in pediatric patients from 2 years of age and older (December)[3]
- Two more drugs were approved in November 2019 for adolescents with SCD with voxelotor[4] in patients aged 12 years and older and crizanlizumab[5] in patients 16 years and older

1. NHLBI Publications and Resources. Evidence-based management of sickle cell disease: Expert panel report, 2014. 2. US Food and Drug Administration. FDA approved L-glutamine powder for the treatment of sickle cell disease [press release]. July 2, 2017. 3. US Food and Drug Administration. FDA approves hydroxyurea for treatment of pediatric patients with sickle cell anemia [press release]. December 21, 2017. 4. US Food and Drug Administration. FDA approves novel treatment to target abnormality in sickle cell disease [press release]. November 25, 2019. 5. US Food and Drug Administration. FDA approves crizanlizumab-tcma for sickle cell disease. [press release]. November 19, 2019.

Sickle Cell Disease is Chronic and Complex

A program of transition to adult care for sickle cell disease



Saulsberry A, et al. *Hematology Am Soc Hematol Educ Program*. 2019;2019(1):496-504.

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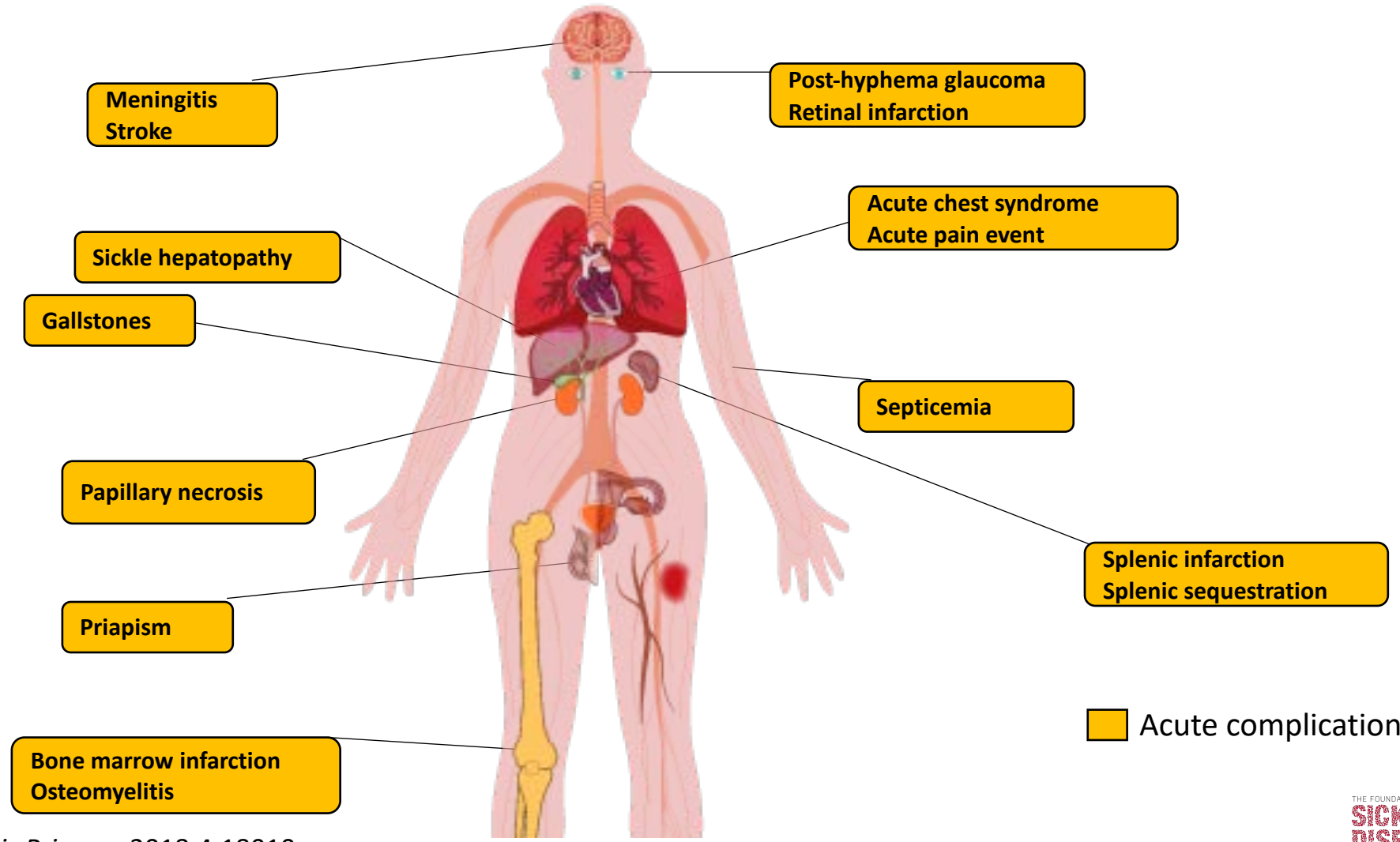
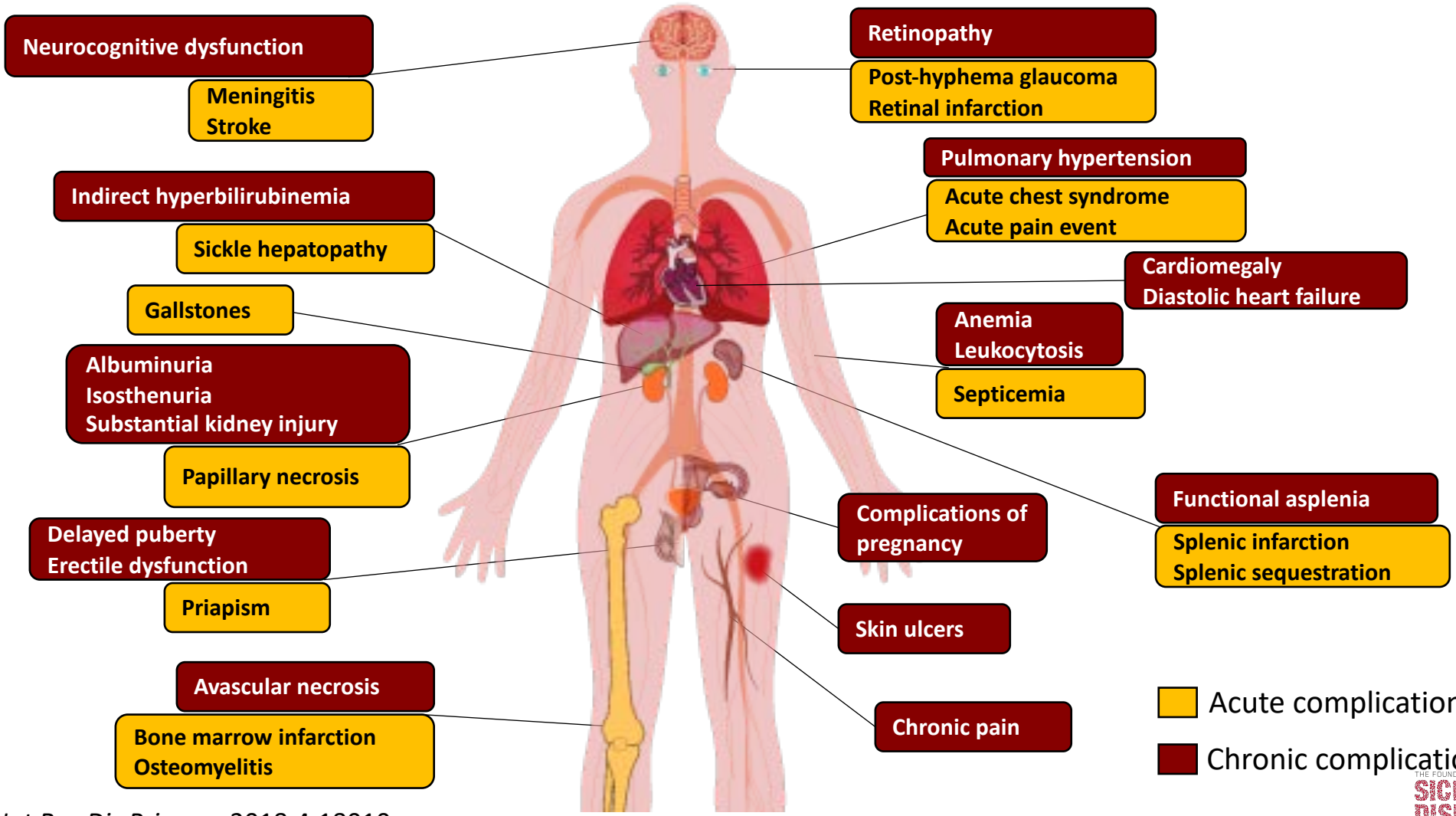
SCD Clinical Complications



Kato G, et al. *Nat Rev Dis Primers*. 2018;4:18010.

SCD Clinical Complications



Kato G, et al. *Nat Rev Dis Primers*. 2018;4:18010.

Vaso-occlusive Episodes (VOEs)

- Pain is the hallmark feature of SCD in patients
  - Complex, multidimensional pain
  - Nociceptive or neuropathic
  - Visceral or somatic
- Pain from VOE starts as early as the first 6-12 months of life
  - Dactylitis is often the first indicator of SCD
  - Involvement shifts to arms, leg, back, and pelvis as child ages
- VOEs typically last 3-9 days

Challenges in the Management of Pediatric Pain in Sickle Cell Disease. https://cme.dannemiller.com/articles/activity?id=310&f=1

Pain Management

- Acetaminophen
  - Exceeding recommended daily dose can result in hepatic toxicity
- NSAIDs
  - Pain relief and peripheral anti-inflammatory activity
  - Conflicting evidence of efficacy in children
- Opioids
  - Benefits
    - Potent, centrally-mediated analgesic action
    - Multiple routes for delivery
    - Lack of ceiling effect
  - Drawbacks
    - Side effects (nausea, vomiting, pruritus, constipation, urinary retention, respiratory depression, oversedation)
    - Tolerance = higher and higher doses necessary
    - Risk for dependence

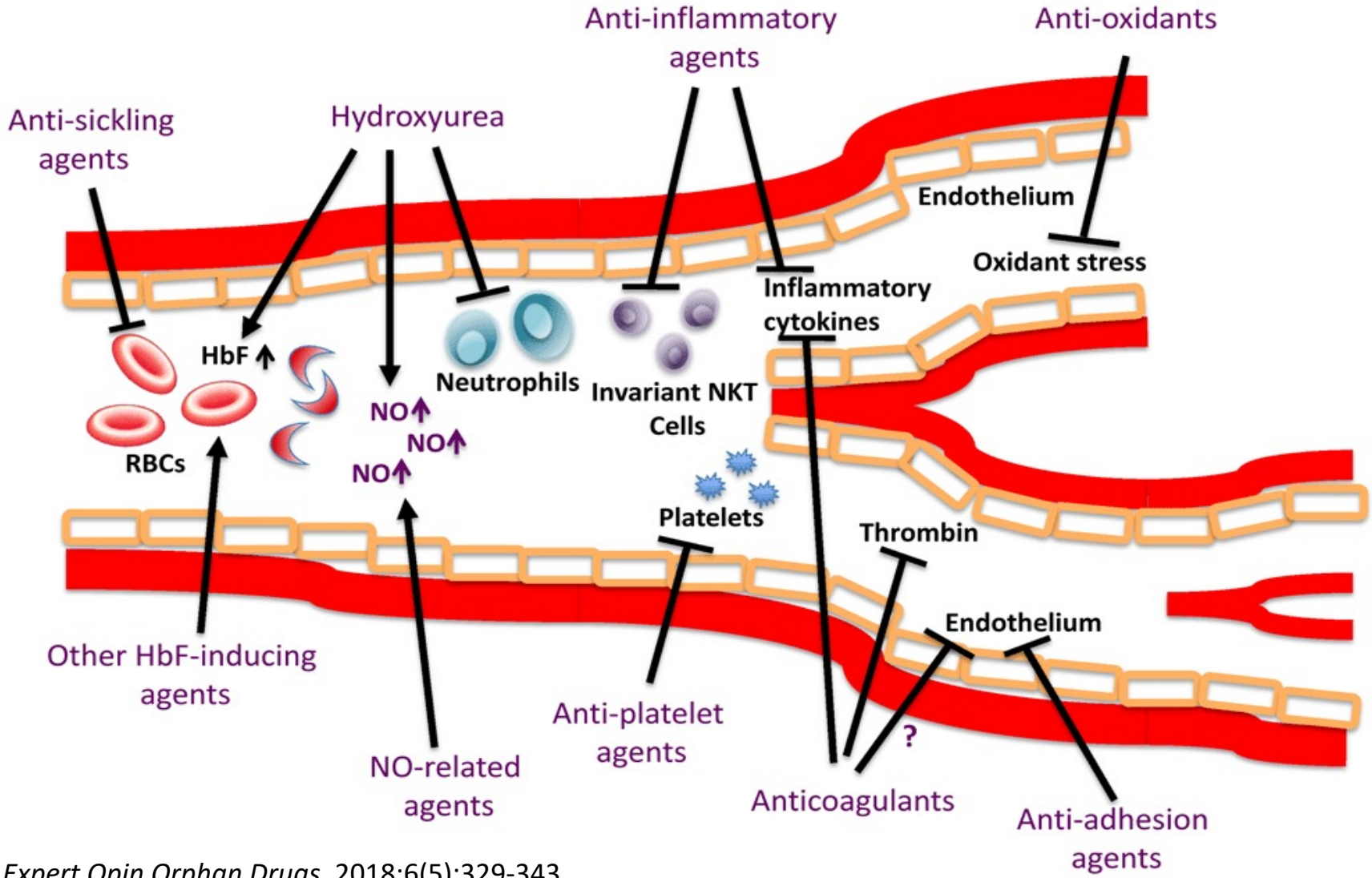
Challenges in the Management of Pediatric Pain in Sickle Cell Disease. https://cme.dannemiller.com/articles/activity?id=310&f=1

Sickle Cell FDA-approved Drugs (4)

Agent	Mode of Action	FDA Approved	Indicated Pediatric Age
Hydroxyurea[1]	Increases fetal hemoglobin Anti-inflammatory Anti-adhesion	Adults: 1998 Children: 12/2017	18 y/o and older 2 years and older
L-glutamine[2]	Anti-oxidant	2017	5 years and older
Crizanlizumab[3]	Anti-adhesion	Children and adults: 11/2019	16 years and older
Voxelotor[4]	Increases hemoglobin Red blood cell allosteric modifier (increases O_2 to sickle cells)	Children and adults: 11/2019	12 years and older

1. US Food and Drug Administration. FDA approves hydroxyurea for treatment of pediatric patients with sickle cell anemia [press release]. December 21, 2017.; 2. US Food and Drug Administration. FDA approved L-glutamine powder for the treatment of sickle cell disease [press release]. July 2, 2017.; 3. US Food and Drug Administration. FDA approves crizanlizumab-tcma for sickle cell disease. [press release]. November 19, 2019.; 4. US Food and Drug Administration. FDA approves novel treatment to target abnormality in sickle cell disease [press release]. November 25, 2019.

How Do New Sickle Cell Treatments Work?



Ataga KI, Desai PC. *Expert Opin Orphan Drugs.* 2018;6(5):329-343.

Considerations for the Type of Treatment: Age, How is it Given, Side Effects, Indications

- Considerations
  - Age eligibility (2 y/o, 5 y/o, 12 y/o, 16, y/o, 18 y/o)
  - Route of delivery (oral vs IV); tablet vs solution
  - Side-effect profile
- Indications
  - Prevention of ongoing end organ damage
  - Decrease the frequency of symptoms/complications
    - Pain episodes
    - Acute chest syndrome
    - Priapism
    - Lack of energy

Sickle Cell Disease - Types of Therapies

- Categories
  - Disease modifying
    - Change the course of the disease without cure
      - Hydroxyurea
      - Crizanlizumab
      - Voxelotor
      - L-glutamine
  - Curative therapies
    - Bone marrow (stem cell) transplantation
      - Sibling match
      - Haplo (half match, ie, parent) identical match
      - Match unrelated donor
  - Gene therapy

Onimoe G, Rotz S. *Cleve Clinic J Med.* 2020;87(1):19-27.

Sickle Cell Disease - Types of Therapies

- Quality of life
  - Effective in improving or maintaining an acceptable quality of life
- Risks and benefits
  - Side effects
  - Short-term risks
  - Long-term risks
    - Development of another chronic disease (ie, GVHD) or worsening the health of another organ (ie, kidney)
    - Mortality (chance of dying from this therapy)
    - Reproductive health
      - Spermatogenesis/sperm count
      - Oocyte (eggs) damage
      - Sperm collection pre BMT
      - Oocyte (eggs) preservation pre-BMT/?

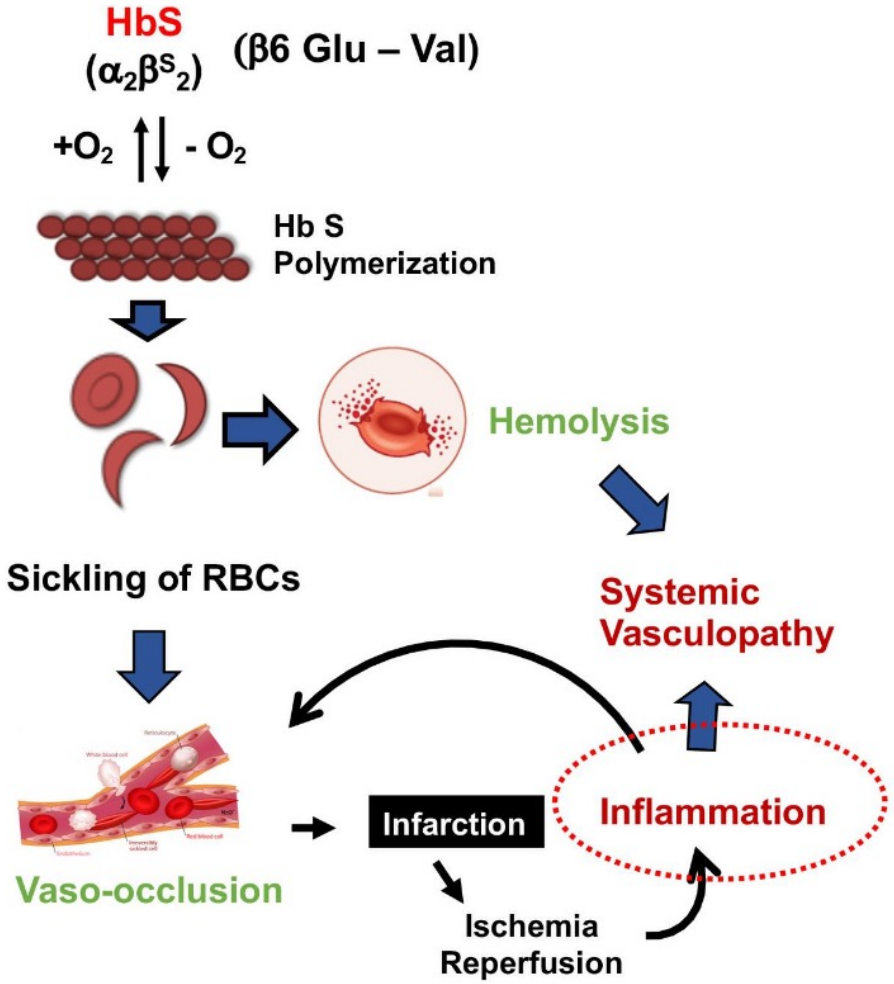
Recommended Treatment Approaches

Treatment Approach	Dose and Frequency	Duration	Recommendation	Evidence Quality	Availability in Low Resource Areas
Prevention of infection					
Penicillin V	62.5-250 mg twice daily	At least until 5 yr of age	Strong	Moderate	Available
Pneumococcal vaccines	Every 5 yr, starting at 2 yr of age	Lifelong	Strong	Moderate	Limited availability
Malarial prophylaxis when appropriate	Daily (eg, proguanil), weekly (eg, pyrimethamine), or intermittent (eg, mefloquine-artesunate or sulfadoxine-pyrimethamine plus amodiaquine)	Lifelong (in malarious area)	Strong	Low	Available
Blood transfusion					
Acute care					
Treatment of anemia	Simple transfusion; target Hb level 10 g/dl	Limited	Strong	Low	Limited availability
Preoperative transfusion (if Hb <8.5 g/dl)	Simple transfusion, performed once; target Hb level 10 g/dl		Strong	Moderate	Limited availability
Ongoing care					
Primary stroke prevention	Target HbS <30%; transfusions every 3-6 wk	Indefinite	Strong	High	Very limited availability
Secondary stroke prevention	Target HbS <30% or <50%; transfusions every 3-6 wk	Indefinite	Moderate	Low	Very limited availability
Prevention of additional silent cerebral infections	Target HbS <30%; transfusions every 3-6 wk	Indefinite	Moderate	Moderate	Very limited availability
Hydroxyurea					
Universal use	20-35 mg/kg/day	Indefinite	Moderate	Moderate	Limited availability
Prevention of acute complications	15-35 mg/kg/day	Indefinite	Strong	High	Limited availability
Primary stroke prevention	15-35 mg/kg/day	Indefinite	Strong	Moderate	Limited availability

*Data on recommended treatments, the strength of the recommendation, and the quality of the evidence are from DeBaun MR, et al. *N Engl J Med.* 2014;371:699-710., Ware RE, et al. *Lancet.* 2016;387:661-670.; and Yawn BP, et al. *JAMA*. 2014;312:1033-1048. Data availability in low resource area are from Bello-Manga H,et al. *Expert Rev Hematol.* 2016;9:1031-1042. HbS denotes sickle hemoglobin

Piel FD, et al. *N Engl J Med.* 2017;376(16):1561-1573.

Targets of Treatments for SCD



- Change the genotype
  - Allogeneic BMT
  - Autologous HSCT modification
- Target HbS polymerization
  - Increase fetal hemoglobin
    - Genetic and genomic approaches
      - Suppressing BCL11A
      - Simulate HPFH variants
    - Pharmacologically (eg, hydroxyurea)
  - Hb O2 affinity
- Targeting vaso-occlusion
  - Inhibiting adhesive interactions between cells and endothelium
- Targeting inflammation
  - Feedback loop of sterile inflammation that promotes further vaso-occlusion
  - L-glutamine
  - Inflammasome inhibition

Salinas Cisneros G, Thein SL. *Front Physiol.* 2020;11:435.

Categories of Treatment Options

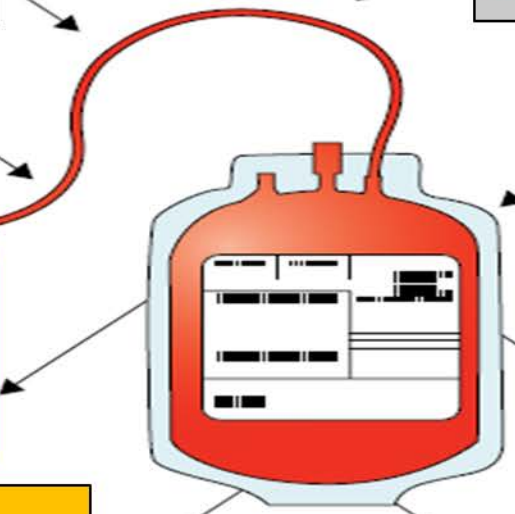
	Hydroxyurea (FDA approved)	Ribonucleotide diphosphate reductase inhibitor
Hemoglobin S polymerization	LBH589/panobinostat (NCT01245179)	Pan histone deacetylase inhibitor
	Voxelotor/GBT440 (NCT03036813) (FDA approved)	α-Globin reversible binding
	Decitabine/THU (NCT01685515)	DNMT1 inhibition
	Sanguinate (NCT02411708)	Targeting carbon monoxide delivery
Vaso-occlusion	IMR-687 (NCT04053803)	Phosphodiesterase 9 inhibitor
	L-Glutamine (FDA approved)	Increase NADH and NAD redox potential
	Crizanlizumab (NCT03264989) (FDA approved)	P-selectin inhibitor
	Heparinoids: Sevuparin (NCT02515838)	P-selectin and L-selectin inhibitor
	Poloxamer and Vepoloxamer	Nonionic block copolymer surfactant
Inflammation	Prasugrel, ticagrelor (NCT02482298)	P2Y2 inhibitors
	Intravenous immunoglobulin (NCT01783691)	Effects on neutrophils and monocytes activation
	Simvastatin (NCT03599609)	Vascular endothelium
	Rivaroxaban (NCT02072668)	Anti factor Xa
	N-Acetylcysteine (NCT01800526)	Oxidative stress reduction

THU, tetrahydrouridine; DNMT1, DNA methyltransferase type 1

Salinas Cisneros G, Thein SL. *Front Physiol.* 2020;11:435.

Blood Transfusions

A Mainstay of Treatment in SCD Patients



Indications for simple transfusion
- Acute splenic sequestration
- Transient aplastic crisis
- Symptomatic severe anemia
- Severe acute chest syndrome
- Preoperative preparation

Indications for chronic transfusion
- Clinical stroke
- Abnormal TCD ≥200 cm/sec
- Multisystem organ failure

Possible indications for transfusion
- Pregnancy
- Hepatic sequestration
- Recurrent splenic sequestration

Complications not warranting transfusion
- Uncomplicated painful crisis
- Priapism
- Acute kidney injury
- Asymptomatic anemia
- Avascular necrosis

Goals of transfusion
- Increased oxygen carrying capacity
- Restoration of blood volume
- Prevention of acute vaso-occlusion
- Reduction of sickle cell erythropoiesis

Short-term side effects
- Volume overload
- Acute non-hemolytic reactions
- Acute hemolytic reactions

Management and monitoring
- ABO, Rh, and C/E/K antigen matching
- Target hemoglobin level of ≤10 g/dL
- Target <30% HbS with chronic transfusions
- Assessment of transfusional iron overload

Long-term complications
- Erythrocyte alloimmunization
- Delayed hemolytic reactions
- Transfusional iron overload
- Transmission of infections

TCD, transcranial doppler

Ware RE, et al. *Lancet.* 2017;390(10091):P311-P323.

Benefits of Each Therapy

Hydroxyurea	
Anemia/hemolysis	⬇
Vaso-occlusion	⬇
Acute chest syndrome	⬇
Stroke	?
Nephropathy	?
Pulmonary hypertension	?
Fatigue and QOL	⬇ for some patients
Mortality	⬇

L-Glutamine	
Anemia/hemolysis	➡
Vaso-occlusion	⬇
Acute chest syndrome	⬇
Stroke	No evidence
Nephropathy	No evidence
Pulmonary hypertension	No evidence
Fatigue and QOL	➡
Mortality	No evidence

Voxelotor	
Anemia/hemolysis	⬇
Vaso-occlusion	➡
Acute chest syndrome	➡
Stroke	No evidence
Nephropathy	No evidence
Pulmonary hypertension	No evidence
Fatigue and QOL	➡
Mortality	No evidence

Crizanlizumab	
Anemia/hemolysis	➡
Vaso-occlusion	⬇
Acute chest syndrome	➡
Stroke	No evidence
Nephropathy	No evidence
Pulmonary hypertension	No evidence
Fatigue and QOL	➡
Mortality	No evidence

Current Curative Therapies/Strategies

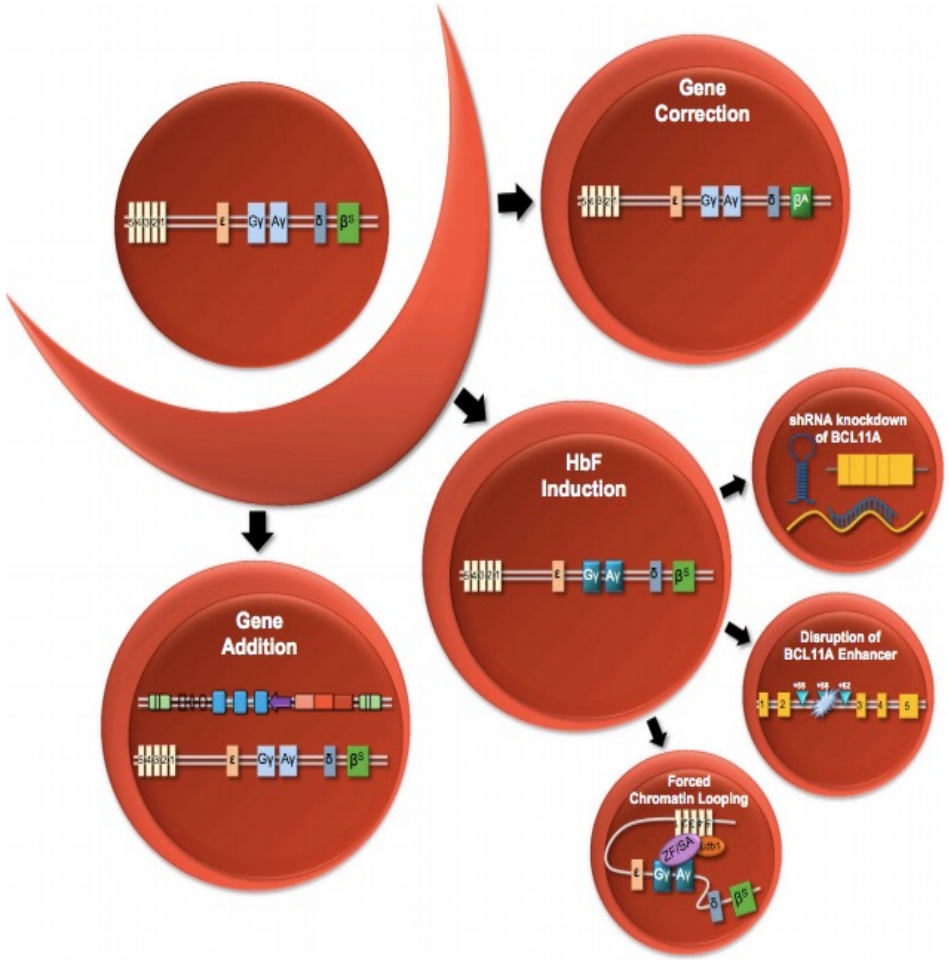
Changing the genotype		
(1) Allogeneic stem cell transplant	Myeloablative regimens (MAC), reduced intensity regimens (RC), and non-myeloablative regimens (NMA)	50 clinical trials listed in ClinicalTrials.gov
(2) Autologous transplant		10 clinical trials listed in ClinicalTrials.gov
	a) <u>Gene therapy</u> Lentiviral strategies (NCT02247843, NCT02140554, NCT02186418)	
	Inducing fetal hemoglobin	Downregulation of *BCL11A* (NCT03282656) Globin chromatin structure manipulation Downregulating beta globin expression
	b) <u>Gene editing</u> Using zinc finger nucleosomes (ZFN), transcription activator-like effector nucleases (TALENs), CRISPR/Cas9 techniques (NCT03745287)	Downregulation of *BCL11A* Reactivation of HbF by HPFH mutations Globin gene repair

Salinas Cisneros G, Thein SL. *Front Physiol.* 2020;11:435.

Gene Therapies
"Disease Modifying" vs "Curative-Like Results"

- Approaches to Gene Therapy:

1. Addition of a helpful gene (Gene Addition)→ the level of production of this" new hemoglobin" determines how well it changes the course of the SCD
2. Gene knockdown (eg, Bcl11A) to Improve hemoglobin F levels → level of production fetal Hgb determines how well it changes the course of disease

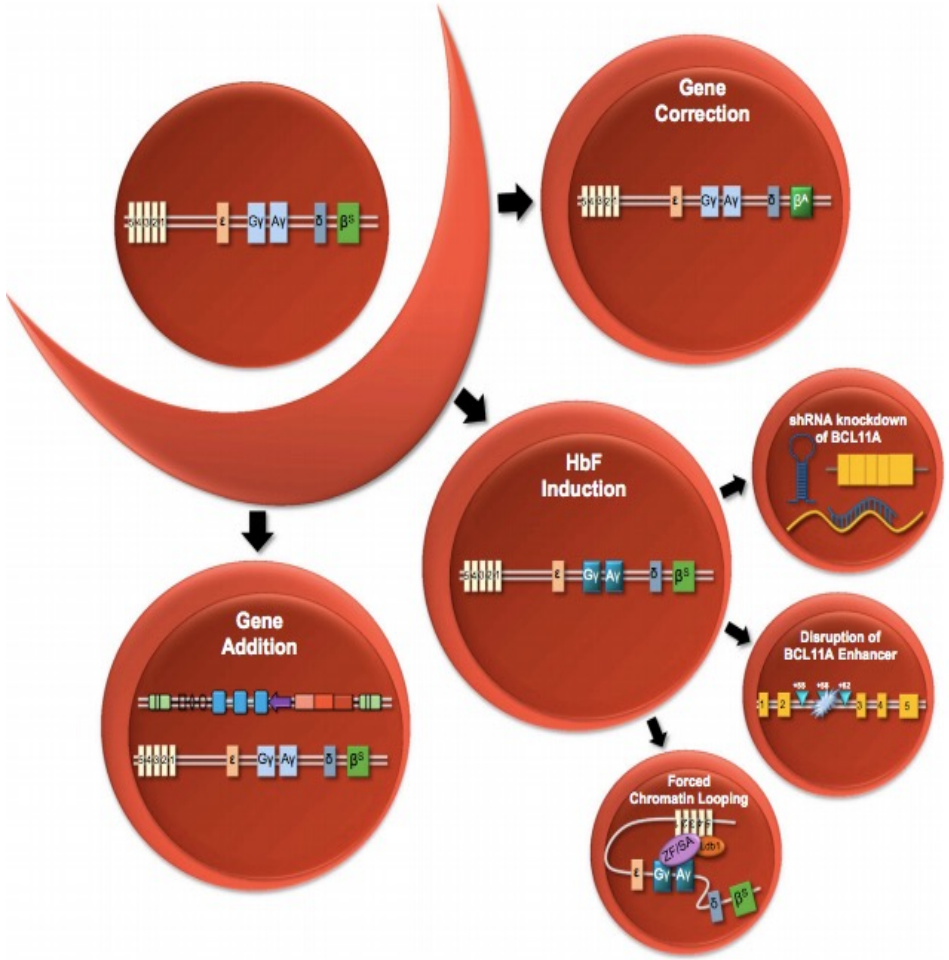


Salinas Cisneros G, Thein SL. *Front Physiol.* 2020;11:435.

Gene Therapies
"Disease Modifying" vs "Curative-Like Results"

- Approaches to Gene Therapy:

3. Direct globin gene editing to "correct" the mutation present (eg, changing a hemoglobin S [HbS]–encoding gene to one encoding hemoglobin A)
4. Gene editing of globin regulatory elements, to at least partially reverse the normal hemoglobin switching from fetal to adult hemoglobin



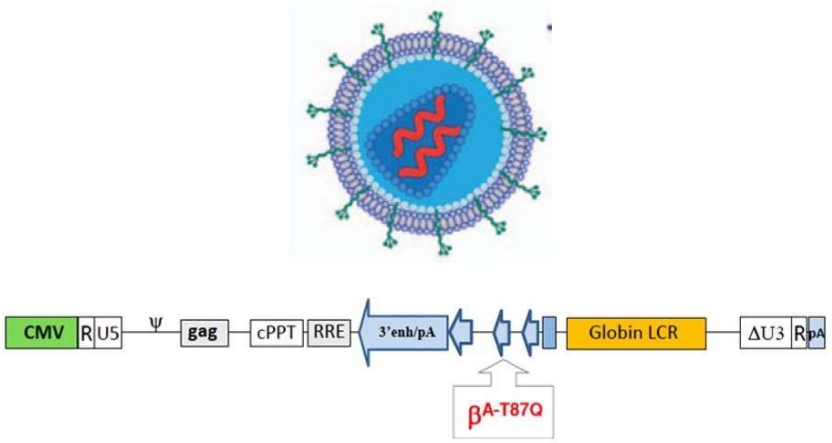
Salinas Cisneros G, Thein SL. *Front Physiol.* 2020;11:435.

Efficiency and Safety of CRISPR/Cas9 Gene Edited Therapy to Treat Sickle Cell Disease

CRISRP/Cas9 Gene Editing

CRISRP/Cas9 gene editing can lead to broad spectrum of unintended genomic modifications and unforeseen side effects

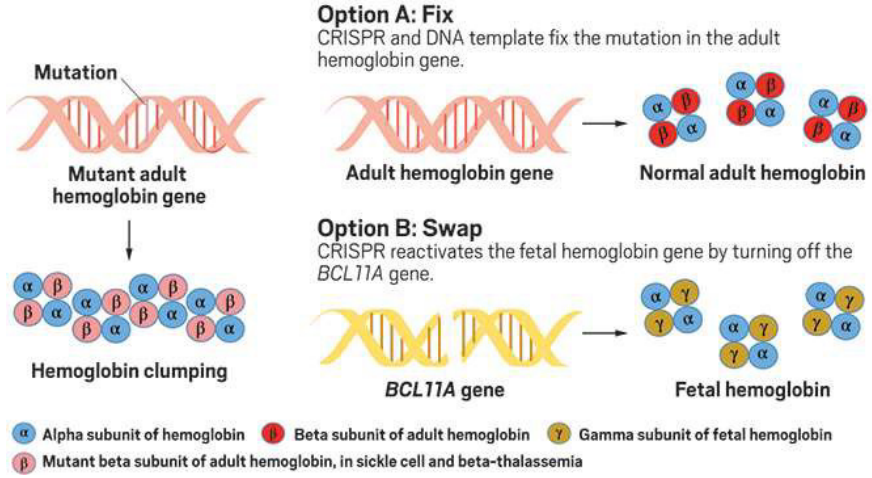
LentiGlobin Gene Therapy



- Randomly inserting copies of a gene into the target cell using a lentivirus
- Make functioning proteins despite the presence of a faulty
- VCN, insertional mutagenesis

Image Credit: Bluebird bio

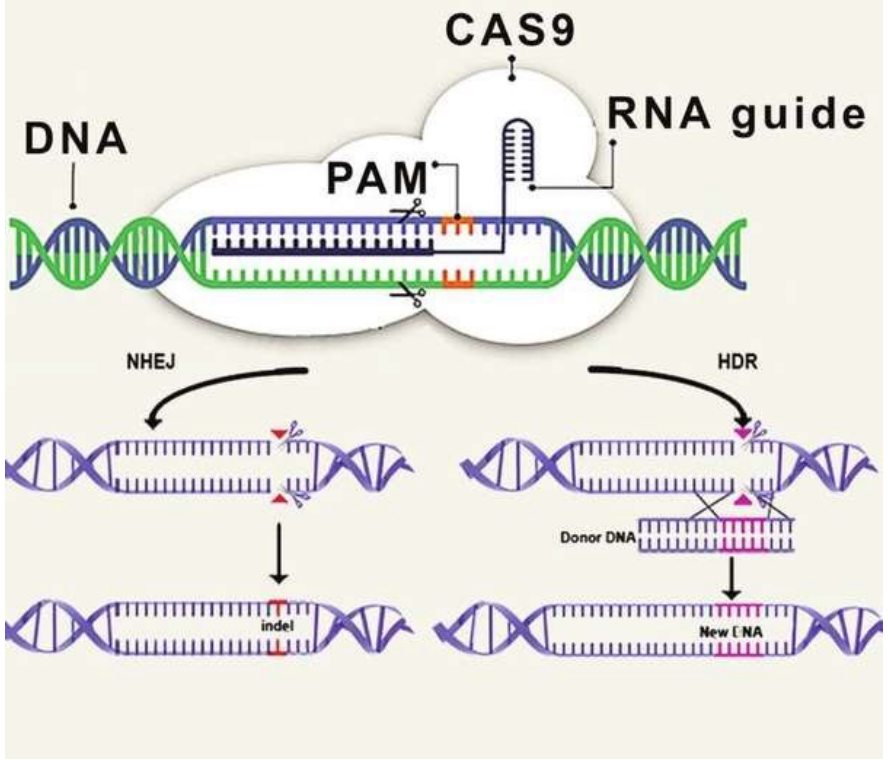
CRISPR/Cas9 Gene Editing



- Disrupt, Insert or Correct genes by targeting specific sequences
- Therapeutic gene editing efficiency, off-target effect

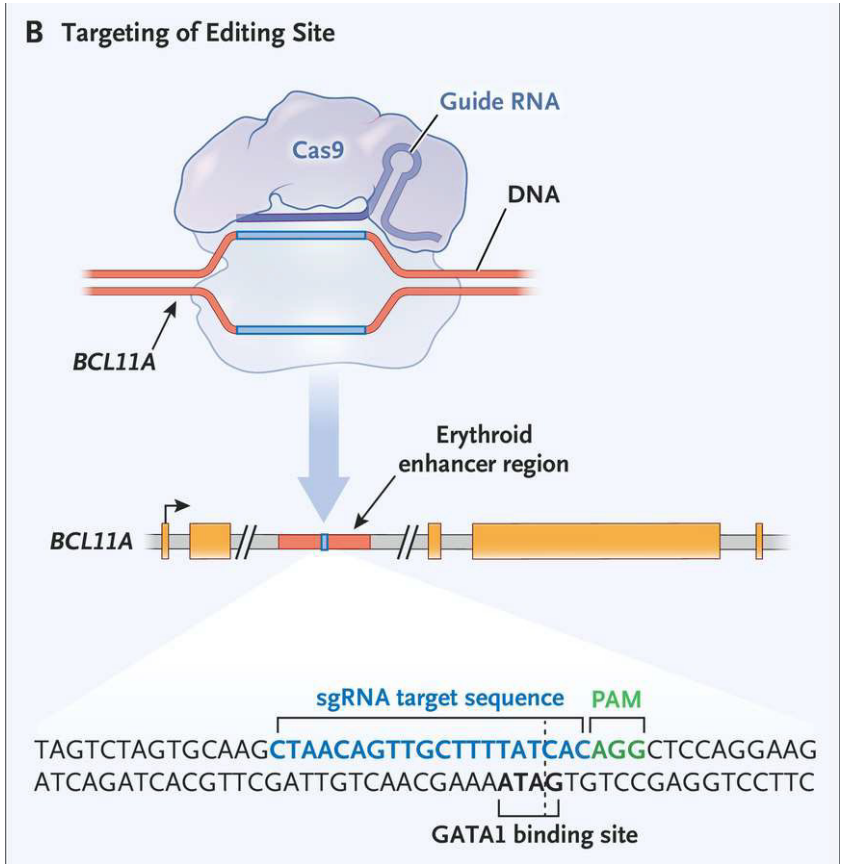
Image Credit: Shutterstock/C&EN

Gene Editing with *S. pyogenes* CRISPR/Cas9



- Cas9+gRNA=Ribonucleoprotein (RNP)
- **DNA repair pathway choices**
  - Non Homologous End Joining (**NHEJ**) Insertion/deletion (INDEL)
  - Homology Directed Repair (**HDR**) Gene correction
- **Challenge**: Improve therapeutic gene editing rate over unintended editing

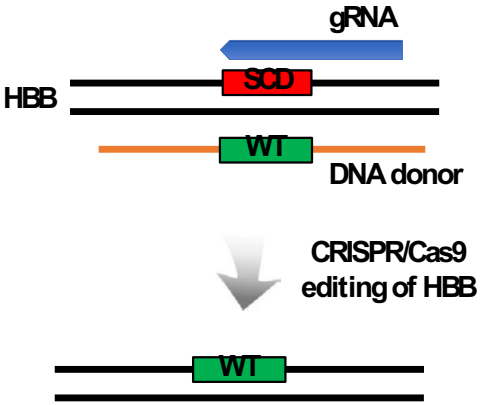
CRISPR/Cas9 Gene Edited Therapy for SCD

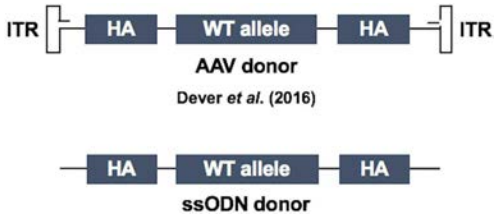


- Produce fetal hemoglobin
  - Disrupt HBG repressor (BCL11A)
    - CTX001 clinical trial

Frangoul *et al*. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. N Engl J Med (2021)

CRISPR/Cas9 Gene Edited Therapy for SCD

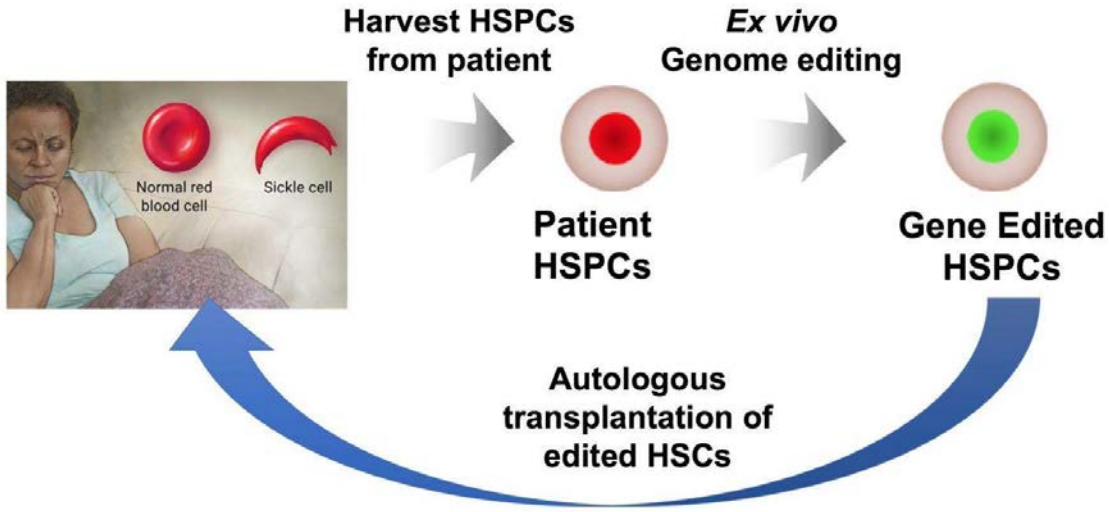




- Directly correct sickle mutation
  - AAV6 donor
    - High HDR efficiency, risk of AAV integration, espisomal AAV
    - GPH101 IND approved
  - Single stranded DNA (ssODN) donor

Dever et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. Nature (2016)
Park, Lee et al. Highly efficient editing of the β-globin gene in patient-derived hematopoietic stem and progenitor cells to treat sickle cell disease. NAR (2019)
Pattabhi et al. In Vivo Outcome of Homology-Directed Repair at the HBB Gene in HSC Using Alternative Donor Template Delivery Methods. Mol ther. Nucleic acids (2019)
Hanlon et al. High levels of AAV vector integration into CRISPR-induced DNA breaks. Nat Commun (2019)

Ex vivo gene edited therapy for treating SCD



1. Isolate HSPCs from a patient
2. Deliver gene editing reagents into HSPCs, cells tested for efficiency/safety
3. Remove the remaining HSPCs in the patient using chemotherapy
4. Infuse the gene-edited HSPCs
5. A few percent of gene-edited hematopoietic stem cells (HSCs) can re-generate the blood system

Ex vivo gene editing research workflow

Harvest CD34+ HSPCs from patient

Ex vivo
Gene editing

Patient HSPCs

Edited HSPCs

1. Electroporation of Cas9/ gRNA+ssODNs
2. On/ off-target analysis by targeted amplicon sequencing

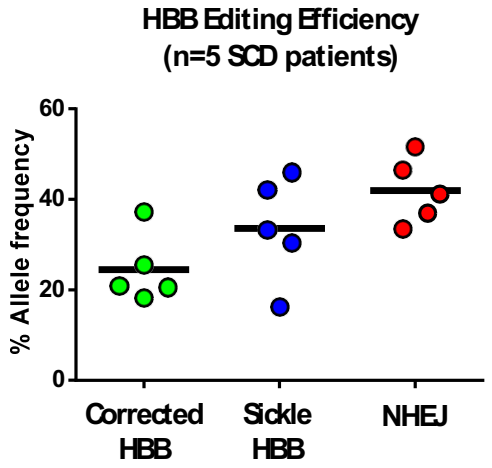
In vitro differentiation into red blood cells

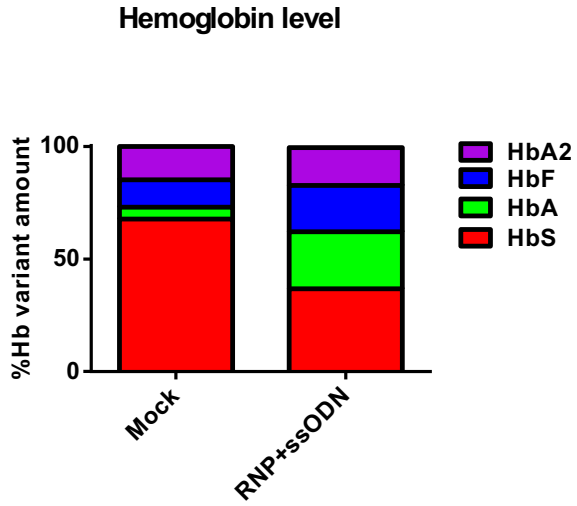
1. Hemoglobin profile
2. Erythropoiesis
3. Sickling assay

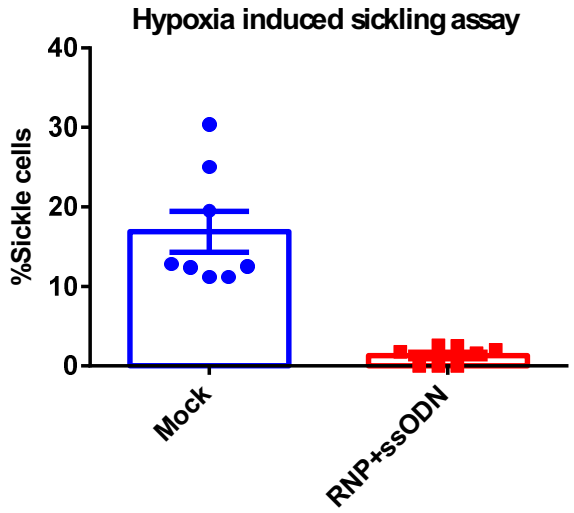
Xeno-transplantation

1. Engraftment efficiency
2. Post-engraftment on/off-target analysis

Characterization of gene edited SCD HSPCs





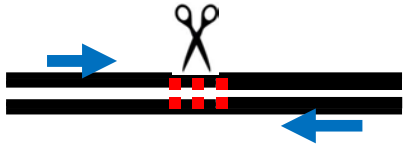


- High level (upto 39%) sickle mutation correction in patient HSPCs
- Reduced Sickle Hemoglobin (HbS), increased Normal Hemoglobin (HbA)
- Reduced sickling
- Detected gene corrected cells after long-term (16 weeks) engraftment

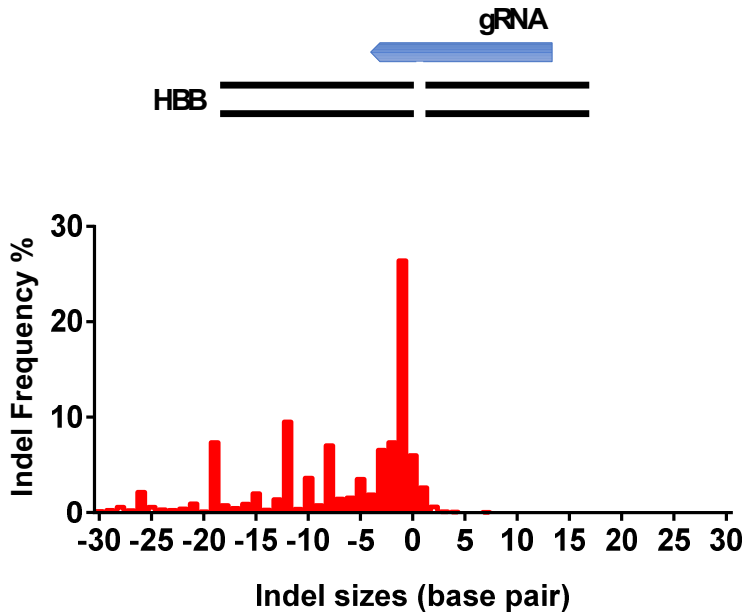
Park, Lee *et al*. Highly efficient editing of the β-globin gene in patient-derived hematopoietic stem and progenitor cells to treat sickle cell disease. NAR (2019

Unintended genomic modifications with unforeseen side effects

Small INDEL quantification by NGS



Targeted Amplicon Sequencing
Short-Range PCR (300bp)
+
Illumina Next Generation Sequencing (NGS)

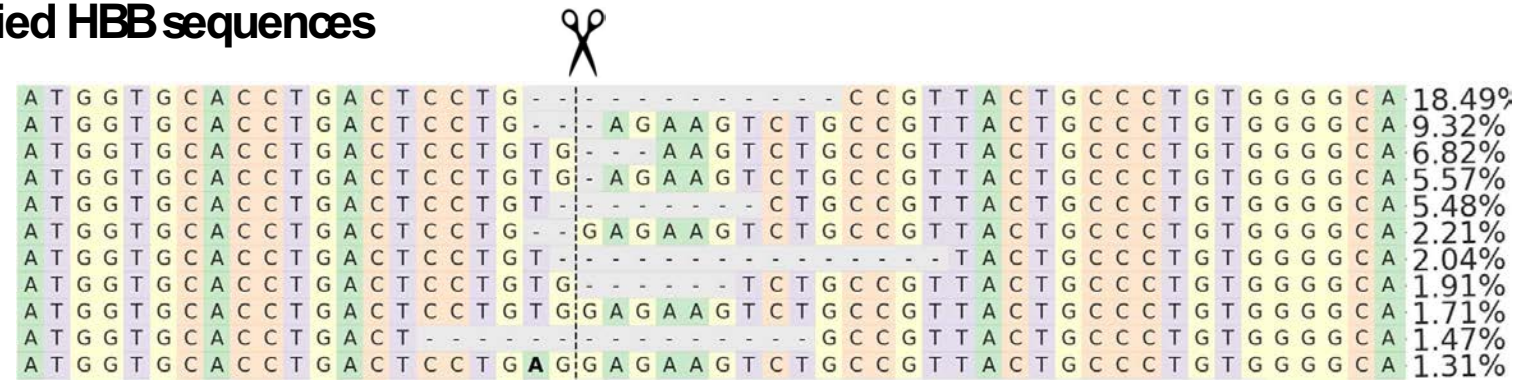


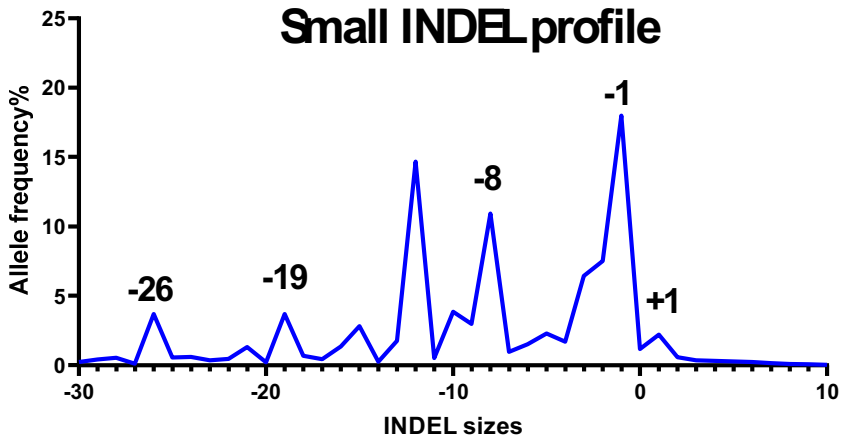
- Successful genome editing relies on the accurate quantification of the repair outcomes
- **Challenge**: Fail to detect large genomic modifications and rare INDELs (<0.1%),

Bennett, E. et al. INDEL detection, the 'Achilles heel' of precise genome editing: a survey of methods for accurate profiling of gene editing induced indels, NAR (2020)

Frameshift INDELs lead to HBB Knockout

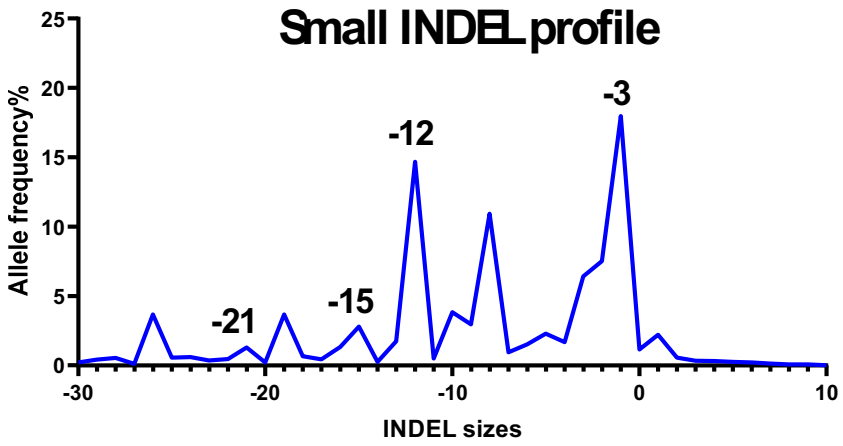
Modified HBB sequences





- **Frameshift INDEL**
  - HBB knockout
  - β-thalassemia

In-frame deletions lead to HBB variants



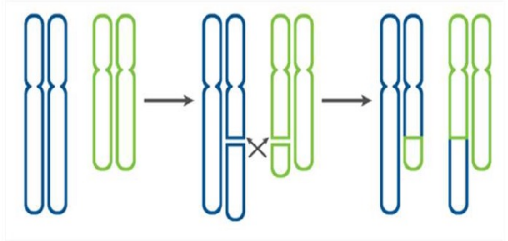
INDEL length (bp)	Amino acid change
-12	p.V7_T9del
-3	p.V6del
-3	p.E7del
-15	p.E8_V12del or p.V7_A11del
-6	p.E7_K8del
-15	p.P5_S9del
-21	p.V7_T12del

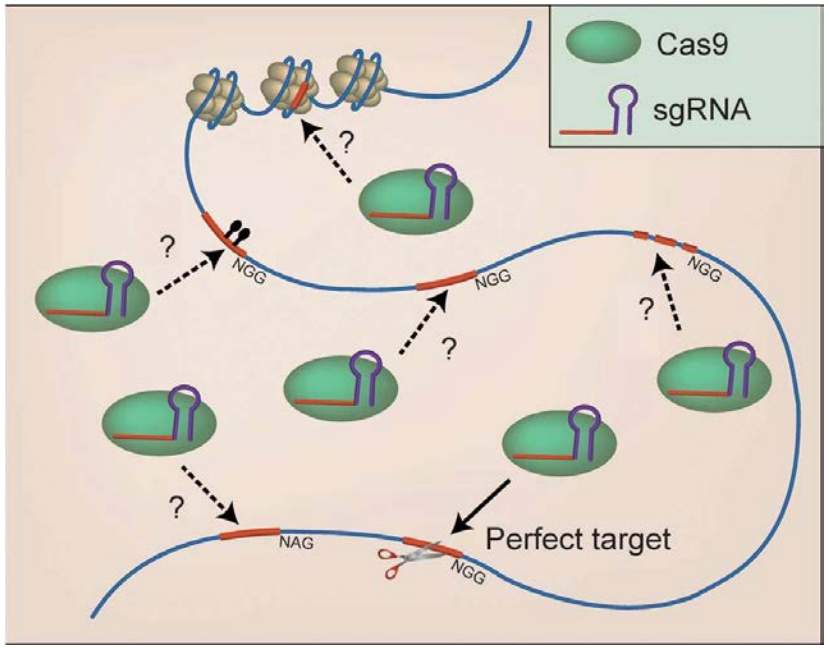
Hb Leiden

- **In-frame deletion**
  - Hemoglobin variants with unknown function
  - *ex* P.V7del > Hb Leiden: slightly unstable, no clear symptom in carrier
  - Other Hb variants with larger deletions not in HbVar database

Bonaventura et al., "Functional Properties of Hemoglobin Leiden (A2Aβ26 Or7 Glu Deleted)."

CRISPR/Cas9 Off-target Effects





- Cas9 could generate a DSB at off-target sites with sequence homology
- Gross chromosomal rearrangements between DSBs
- **Challenge**: identification, quantification, reduction of off-target effects

Wu et al. Quantitative Biology (2014)

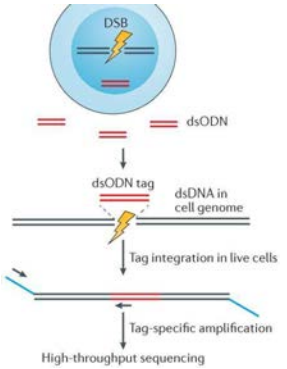
Off-target discovery and quantification workflow

Off-target prediction

Computational prediction

- A Web-based Tool for identifying CRISPR/Cas off-target sites based on sequence homology

Cell-based discovery by GUIDE-seq



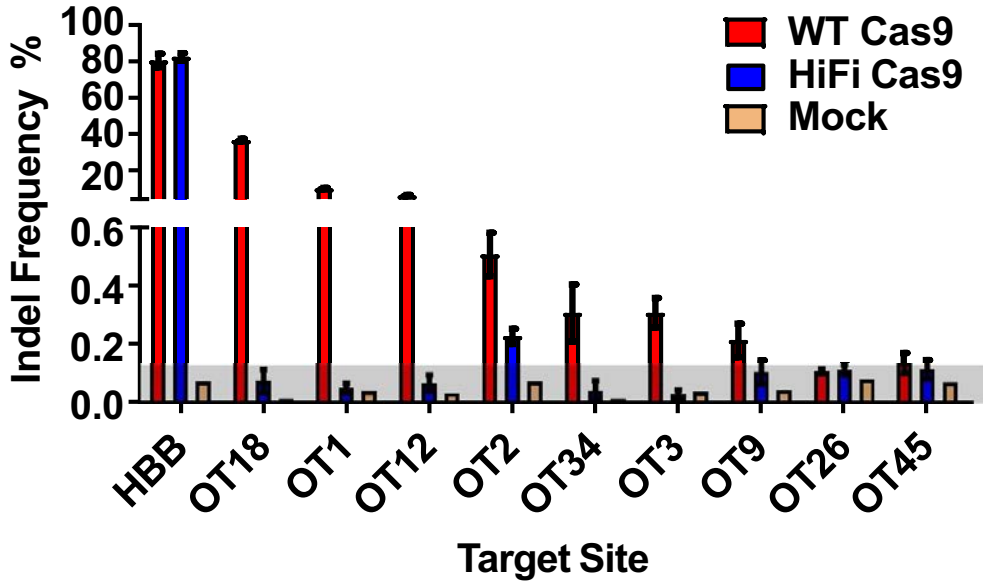
- **G**enome-wide **U**nbiased **I**dentifications of **D**SBs **E**valuated by **Seq**uencing
- Off-target detection relies on the integration of dsODN tag into DSBs

Off-target validation

- Quantification of off-target editing rate by targeted amplicon sequencing

Tsai, S. et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nat Biotechnol* (2015).
Tsai & Joung, Nat Rev Genet. (2016)

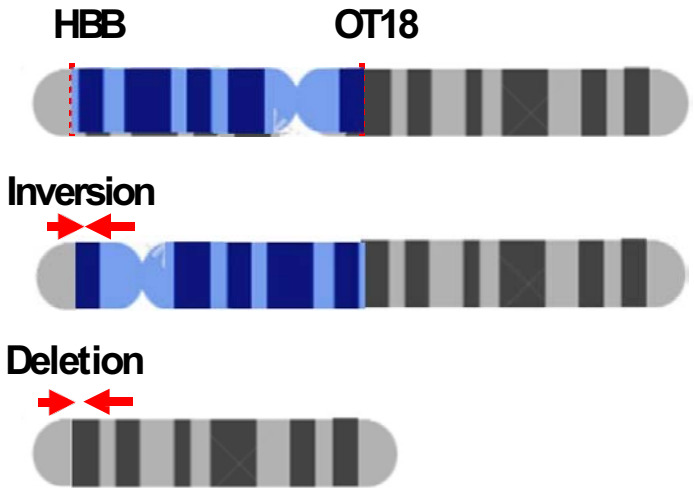
HiFidelity Cas9 Reduced Off-Target Activity

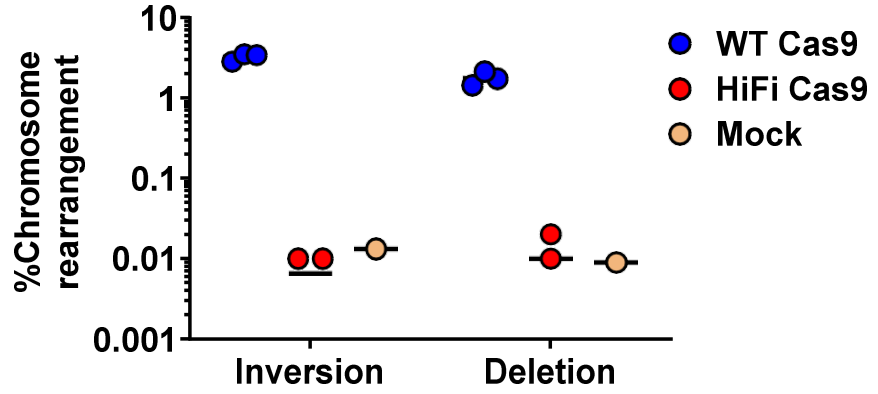


- Quantified INDELs frequency at 57 predicted OT sites
- 9 active OT sites >0.1% INDEL rate
- HiFi Cas9 with improved specificity
- Reduced off-target effects while preserving high on-target activity

Vakulskas et al. Nat Med. (2018)
Park, Lee *et al*. Nucleic Acids Research (2019)

Large genomic rearrangement between on and off-target sites

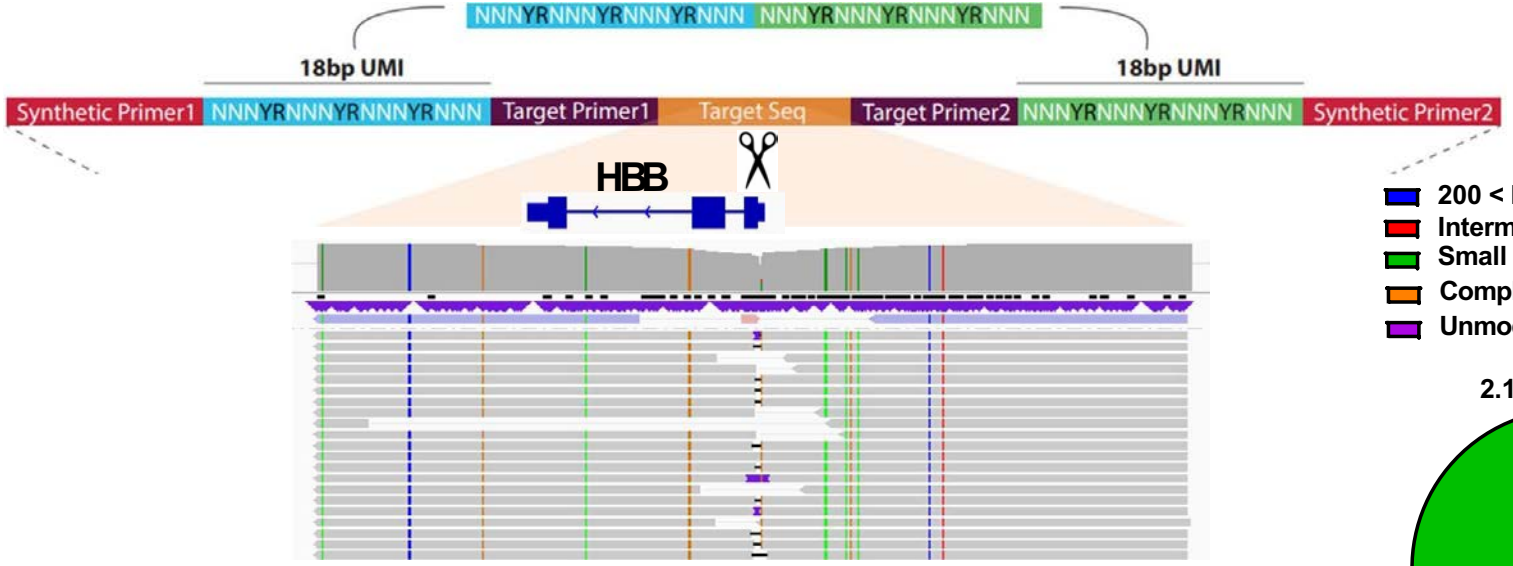


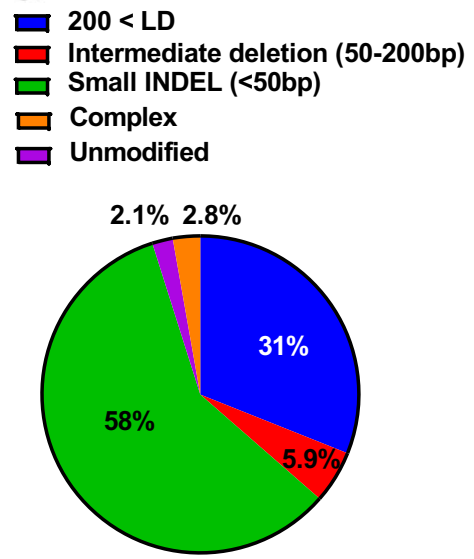


- Optimized droplet digital PCR(ddPCR) assay to quantify intra-chr inversion and deletion between on-target and OT18
- Reduced Chr rearrangement by using HiFi Cas9
- **Difficult to predict and quantify unintended repair outcomes**

Park, Lee *et al*. Nucleic Acids Research (2019)

CRISPR/Cas9 induced large gene modifications

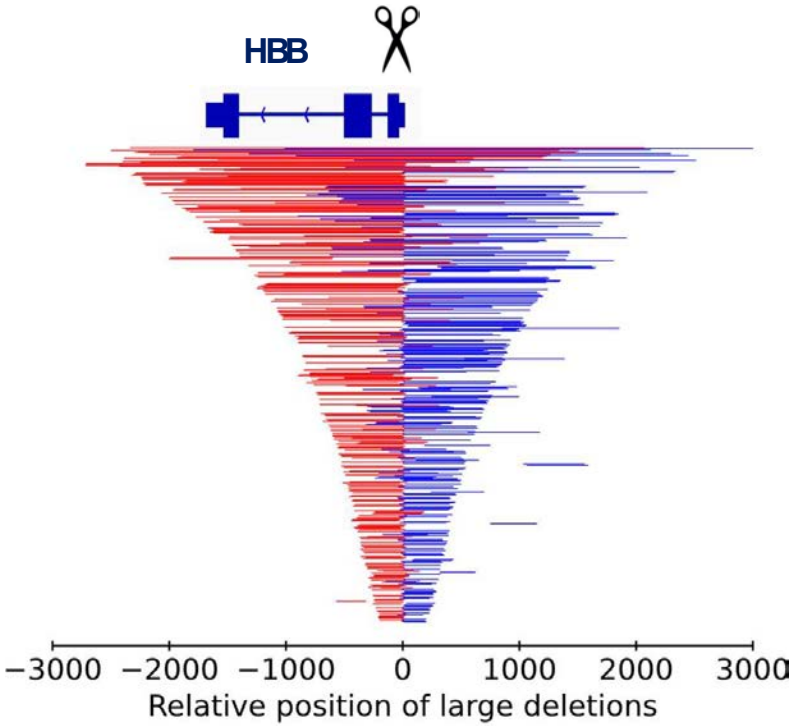


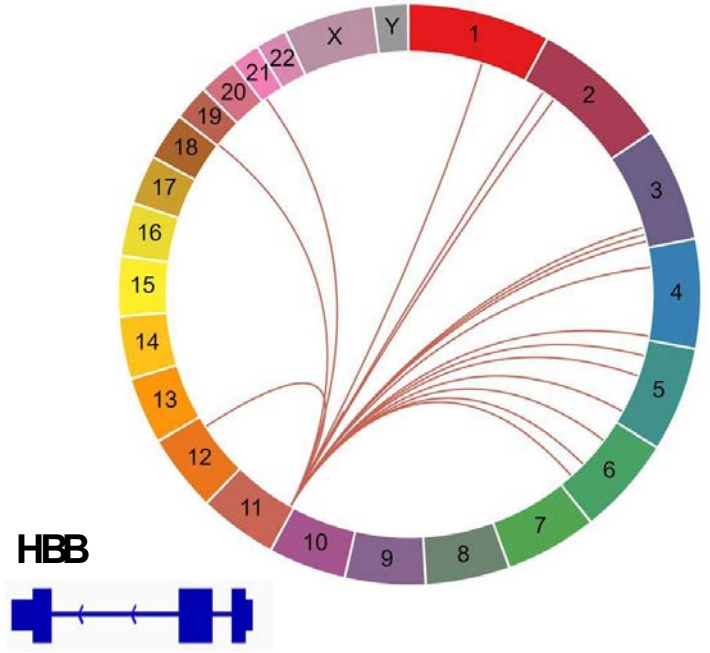


- **SMRT-sequencing with Unique molecular Identifier (UMI)**
  - Unmodified, Small INDELs
  - Large deletion and insertions
  - Complex local rearrangement

Karst et al. High-accuracy long-read amplicon sequences using unique molecula identifiers with Nanopore or PacBio sequencing. Nat Methods (2021).

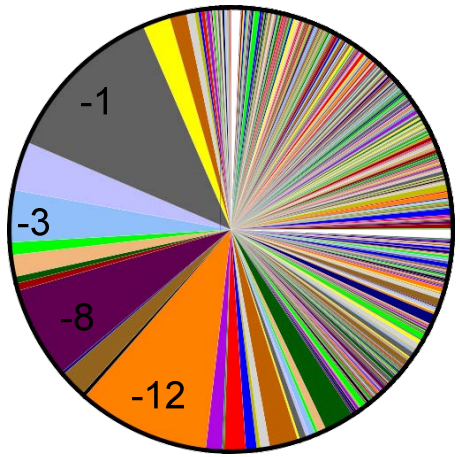
Large genomic modification on HBB

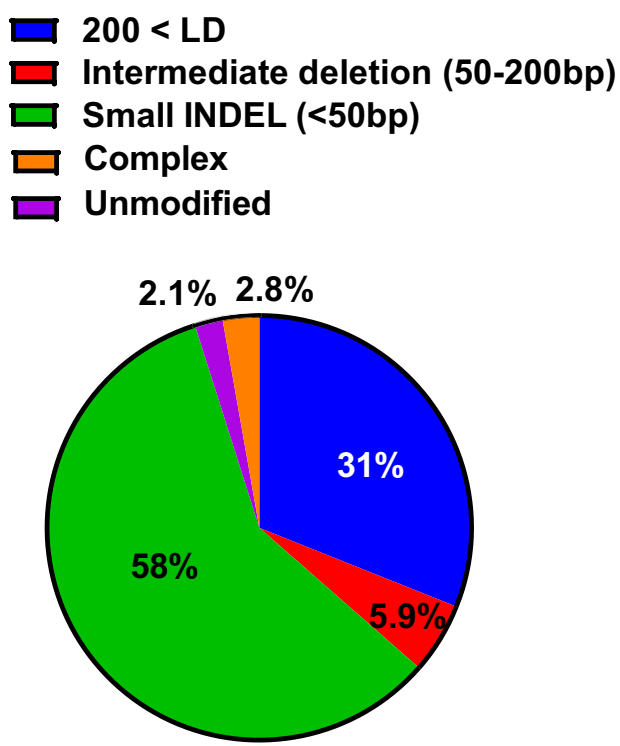




- **Large deletions:** Diverse, each deletion rare, make up high total frequency
- **Large insertions:** HBB local rearrangements, sequences donated from other loci

Broad spectrum of CRISPR/Cas9 gene edited outcomes in HSPCs





- Broad spectrum of unintended genomic modifications at the CRISPR/Cas9 target site
- Limitation of sequencing technology to detect rare genomic modifications
- Better understand gene editing outcomes in preclinical studies to design as safe clinical trials as possible

Myeloablative Transplant for SCD

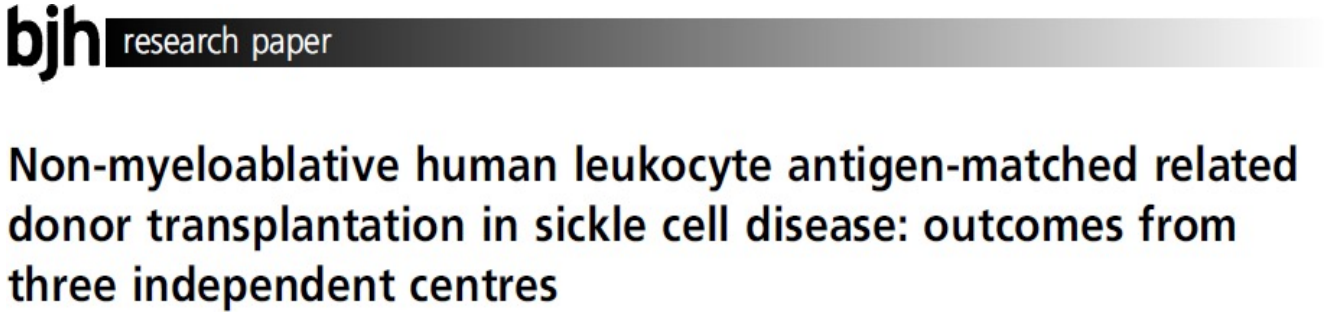
- **HLA-matched sibling transplant is an established curative treatment for SCD**

- **Historically has involved myeloablative conditioning, but myeloablation is**
  - **associated with significant toxicity (i.e. mucositis, GVHD, infertility)**
  - **not necessary as mixed donor chimerism can achieve hematologic cure of SCD**

Can we cure SCD with less intensive conditioning?

Nonmyeloablative Transplant for SCD in adults

- **Regimen of alemtuzumab, TBI, and sirolimus first studied in <u>adults</u> with SCD at the NIH**



- **n=122**
- **Sickle-free survival 85%**
- **No chronic GVHD**

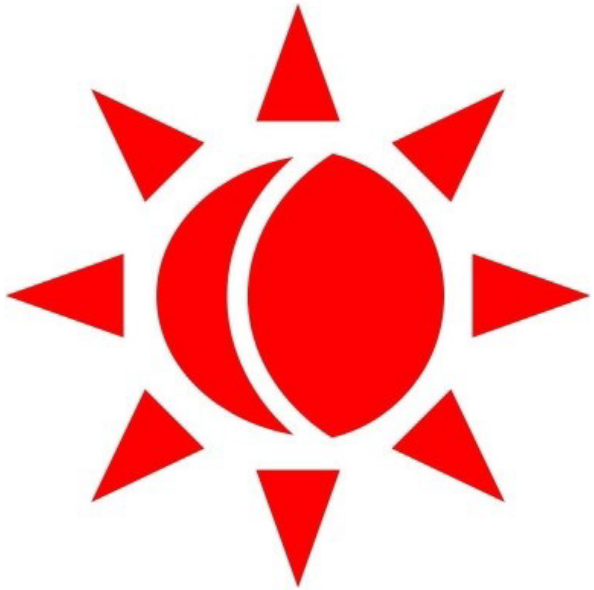
Can we use this approach to effectively cure children?

Alzahrani M et al. *Br J Haematol* 2021;192(4):761-8.

THE FOUNDATION FOR SICKLE CELL DISEASE RESEARCH
CHANGE THE CONVERSATION. RESHAPE THE FUTURE.

SUN Trial

Sickle transplant
Using a
Nonmyeloablative approach

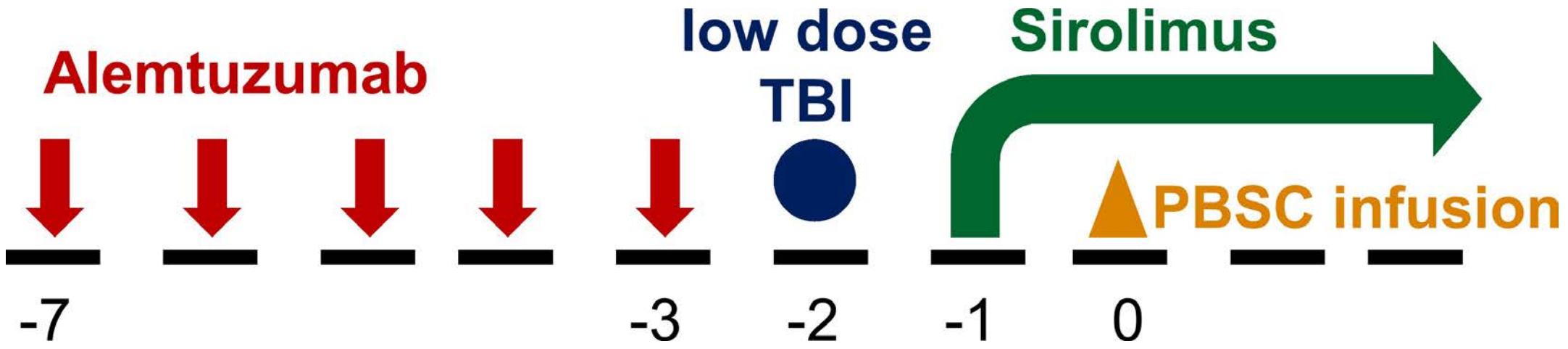


- **Prospective Phase 2 multicenter trial (NCT03587272)**
- **Inclusion:**
  - **Age 2-24.99**
  - **HLA-identical sibling able to donate peripheral blood stem cells**
  - **All SCD genotypes**
  - **At least one clinical complication from SCD**

Disease Severity Eligibility

Hb SS / $S\beta^0$	Hb SC / $S\beta^+$ / Others
abnormal TCD, any infarct on brain MRI	overt stroke
ACS x 2 (lifetime)	ACS x 2 in previous 2 years
pain event x 3 (lifetime)	pain event x 3 in previous year
hospitalization for any SCD complication	hospitalization for ACS or pain on hydroxyurea
priapism x 2	priapism x 2
chronic transfusion	chronic transfusion
splenic sequestration x 2 or splenectomy	splenic sequestration x 2 or splenectomy

SUN Transplant Regimen



- Total 1 mg/kg IV Alemtuzumab
- 300 cGY TBI
- Sirolimus adjusted to trough 5-15 ng/ml for one year, then wean off if CD3 donor chimerism >50%
- Unmanipulated PBSCs, target CD34+ 10 x10^6/kg

SUN Trial Enrollment

- July 2018 first patient transplanted on study

- March 2020 enrollment paused due to COVID-19 pandemic, May 2020 enrollment resumed

- Current enrollment: 28 consented, 24 transplanted

- Target n=30, expanded to 40 patients

► Results on first 15 patients transplanted

Baseline Patient Characteristics (n=15)

Age, years median (range)	 13.7 (2-21)
Male sex, n (%)	9 (60%)
Genotype, n (%) Hb SS Hb $S\beta^0$ thalassemia Hb $S\beta^+$ thalassemia Hb SD-Punjab	 12 (80%) 1 (7%) 1 (7%) 1 (7%)
Pre-HSCT treatment Hydroxyurea Chronic Transfusion	 13 (87%) 5 (33%)
# RBC units transfused pre-HSCT median (range)	 10 (0-224)
# hospitalizations in 2 yrs pre-HSCT median (range)	 2 (0-8)

Most common disease severity eligibility criteria met:

- ≥3 episodes of pain requiring treatment with opiate or IV medication (n=9)
- Hospitalization for pain or ACS on hydroxyurea treatment (n=8)
- ≥2 ACS events (n=6)
- Silent stroke (n=5)

THE FOUNDATION FOR SICKLE CELL DISEASE RESEARCH
CHANGE THE CONVERSATION. RESHAPE THE FUTURE.

Aim 1: Disease-Free Survival

- Median follow-up 580 days (291-1049)
- 11/15 (73%) >1 year post-transplant

- 15/15 (100%) survival

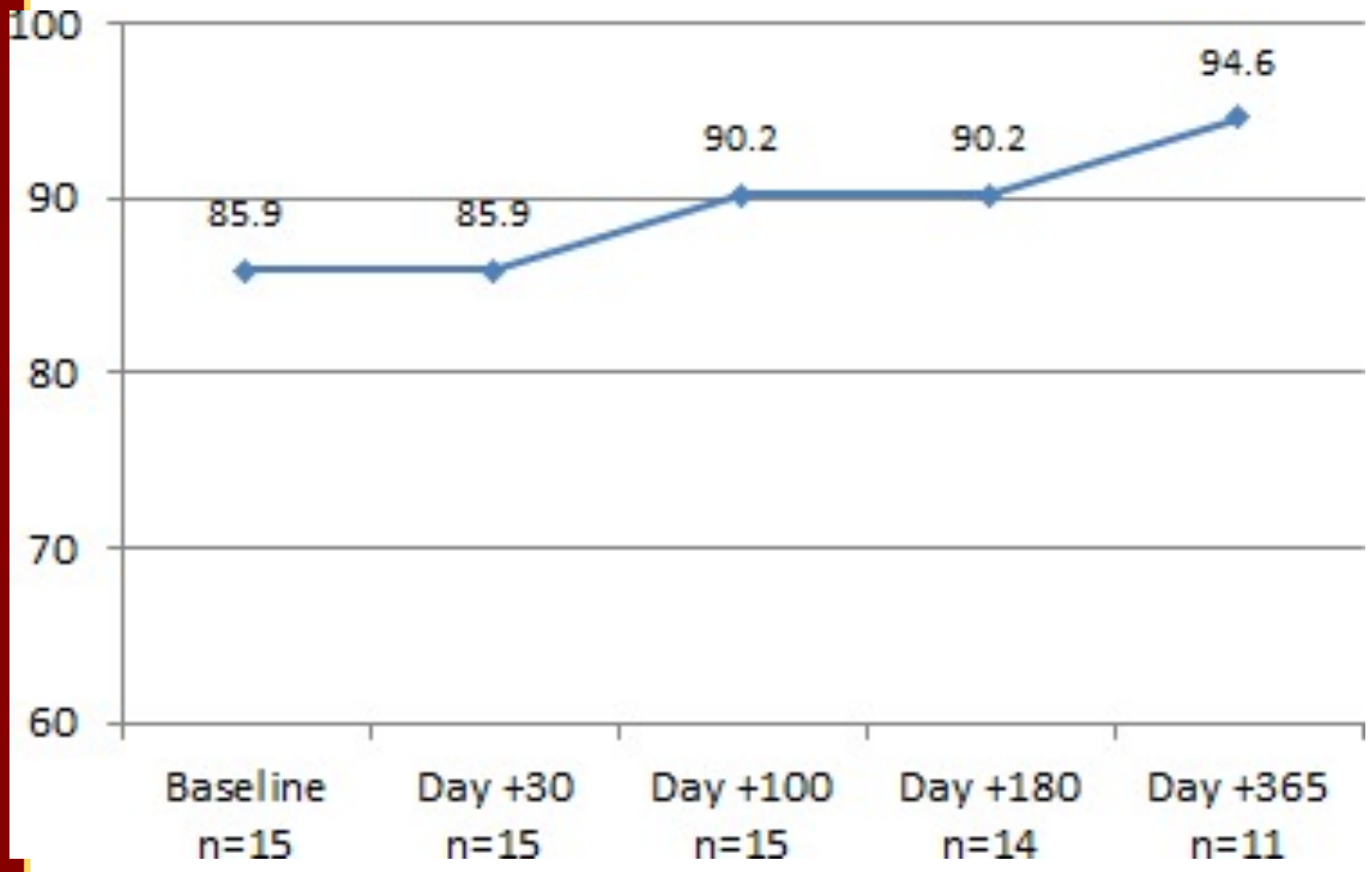
- No acute or chronic GVHD

- 1 graft rejection, return of sickle cell disease

- 14/15 (93%) disease-free survival

Aim 2: Quality of Life

Median PedsQL Scores during Transplant

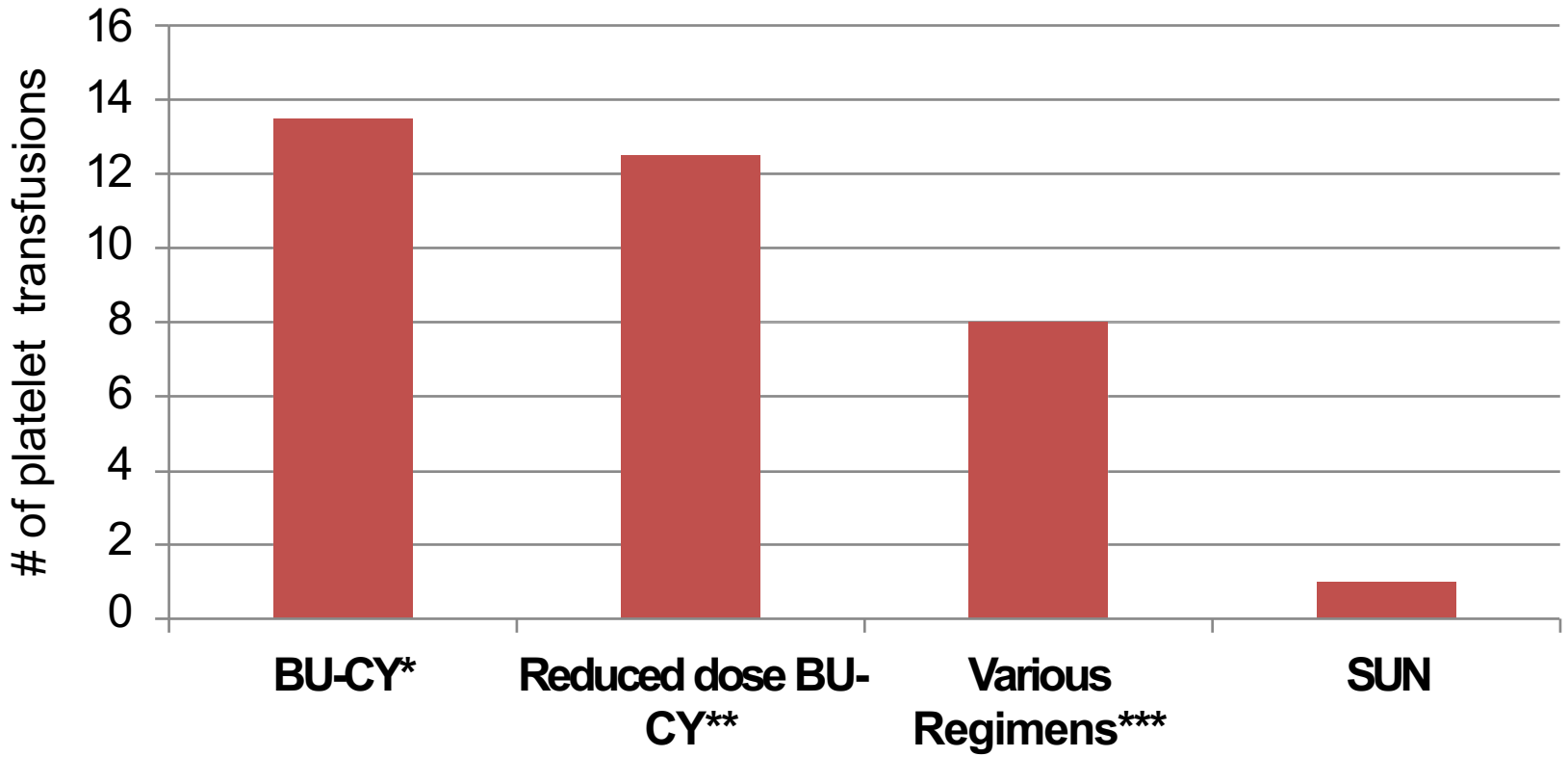


- No decline in QOL during transplant
- Improvement in QOL 1 year post-transplant

THE FOUNDATION FOR SICKLE CELL DISEASE RESEARCH
CHANGE THE CONVERSATION.
RESHAPE THE FUTURE.

Aim 3: Transfusions

- **6/15 (40%) patients required no platelet transfusions**
- **# of platelet transfusions: median 1 (range 0-6)**



* McPherson ME et al. Transfusion 2009;49(9):1977-86.

** Horan JT et al. *Biol Blood Marrow Transplant* 2015;21(5):900-5

*** Nickel RS et al. *Br J Haemtol* 2020;189(1):162-70.

Future directions

- Evaluation of fertility (hormone levels, semen analyses)
- Immune reconstitution studies
- Impact on cognition
- Long term outcomes
- Decrease graft rejection, expand eligibility

HGB-206 Group C: Treatment and drug product characteristics

N=32 Infused Patients

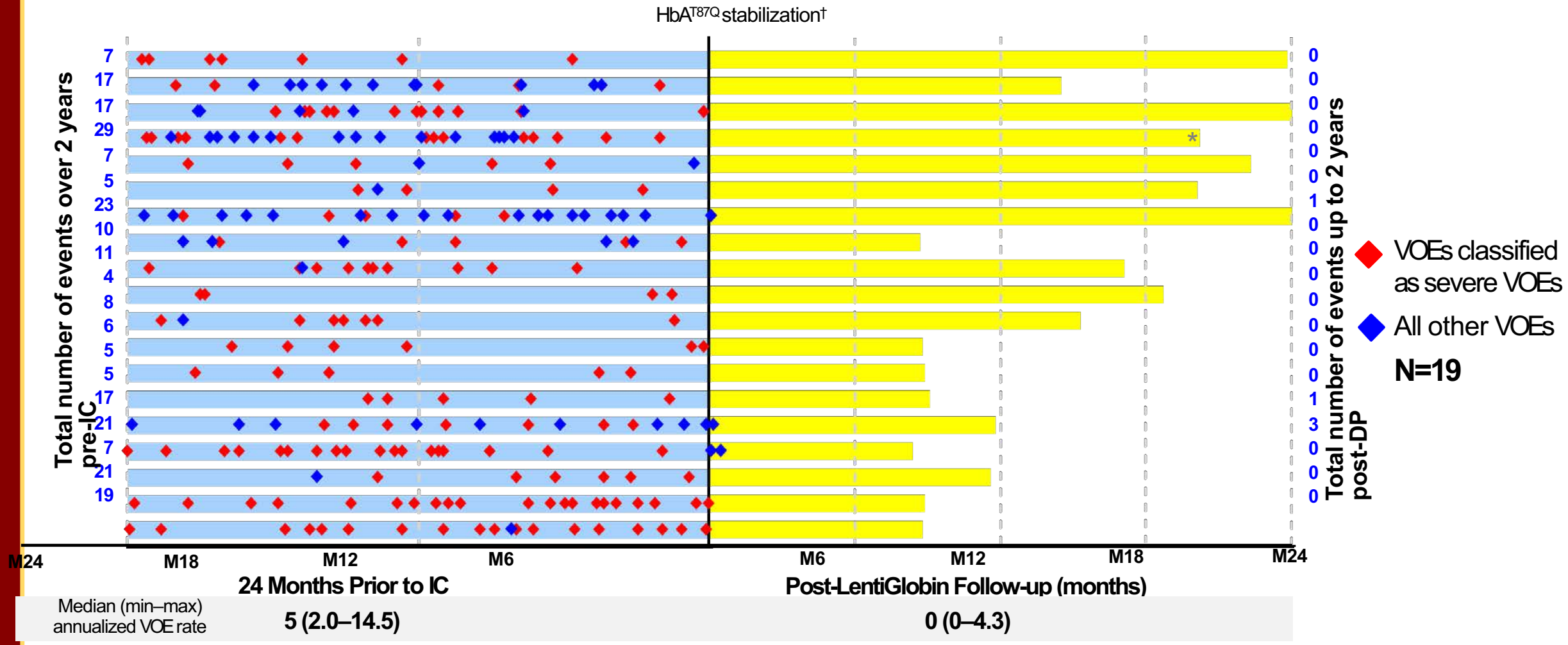
Parameter	N=32 Median (min–max)
Treatment characteristics	
No. of mobilization cycles	**2** (1–4)
CD34+ cells collected per mobilization cycle, x10^6 cells/kg	**10.4** (3.9–55.4)
Estimated average busulfan AUC, min*µmol†	**4843** (1445•–7322)
Neutrophil engraftment, ANC ≥ 500 /µl x 3 days, days	**19.5** (12–35)
Platelet engraftment, platelets > 50k /µl x 3 days, days‡	**30** (18–136)
Duration of hospitalization§, days	**35** (26–65)
Drug product characteristics (per patient)	
Vector copy number, copies/diploid genome	**3.8** (2.3–5.7)
CD34+ cells transduced, %	**80.2** (63–93)
CD34+ cell dose, x10^6 cells/kg	**6.8** (3.0–24.0)

†5 patients pending AUC result; • Data error is being corrected; ‡3 patients pending platelet engraftment at days 29, 30, and 39 post-DP infusion, but on their way to achieving engraftment; § Duration of hospitalization from conditioning to discharge.

ANC, absolute neutrophil count; AUC, area under the curve; DP, drug product; max, maximum; min, minimum; no., number

Data as of 20 August 2020

HGB-206 Group C: Complete resolution of VOEs ≥ 6 months post-LentiGlobin treatment



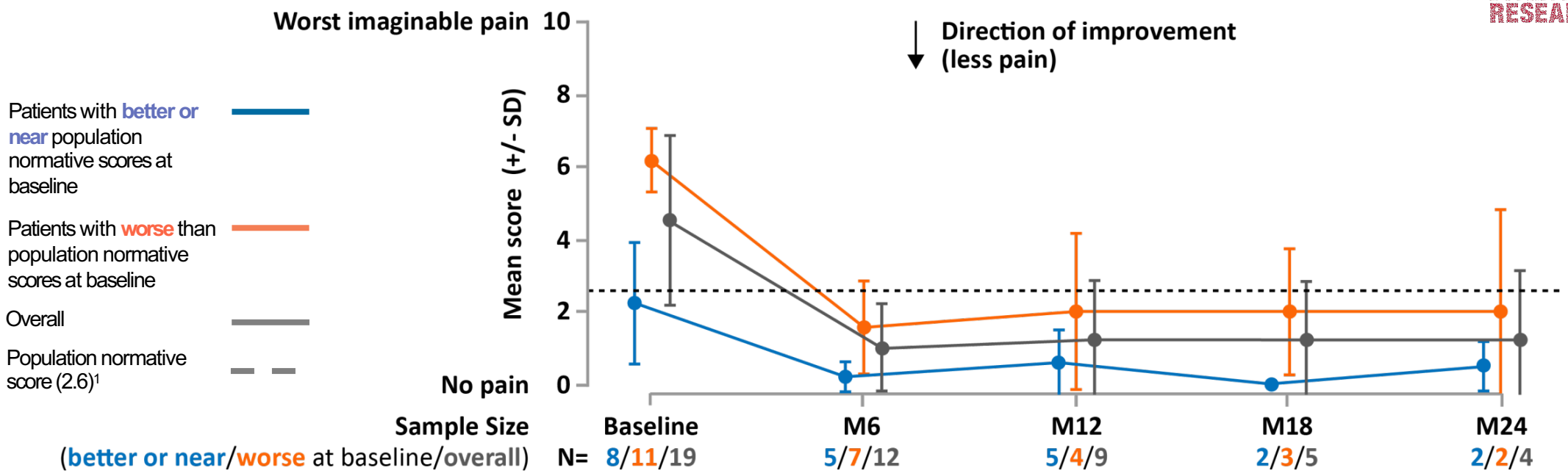
Protocol VOEs are shown; Patients with ≥ 4 sVOEs at baseline before IC and with ≥ 6 months of follow-up post-DP infusion are included. A VOE includes episodes of acute pain with no medically determined cause other than a vaso-occlusion, lasting more than 2 hours and severe enough to require care at a medical facility, a VOE includes acute episodes of pain, acute chest syndrome, acute hepatic sequestration, and acute splenic sequestration; †HbA^{T87Q} expression stabilizes within 6 months; *One death, unlikely related to LentiGlobin, > 18 months post treatment in a patient with significant baseline SCD-related cardiopulmonary disease.
Note: In the last datacut, one patient had a non-serious VOC at Day 107. The event is recorded as an investigator reported VOE but does not meet the definition of a protocol VOE.

DP, drug product; IC, informed consent; max, maximum; min, minimum; sVOEs, severe VOEs; VOC, vaso-occlusive crisis; VOE, vaso-occlusive

Data as of 20 August 2020

HGB-206 Group C: Decrease in patient-reported pain intensity

PROMIS-57 Pain Intensity NRS



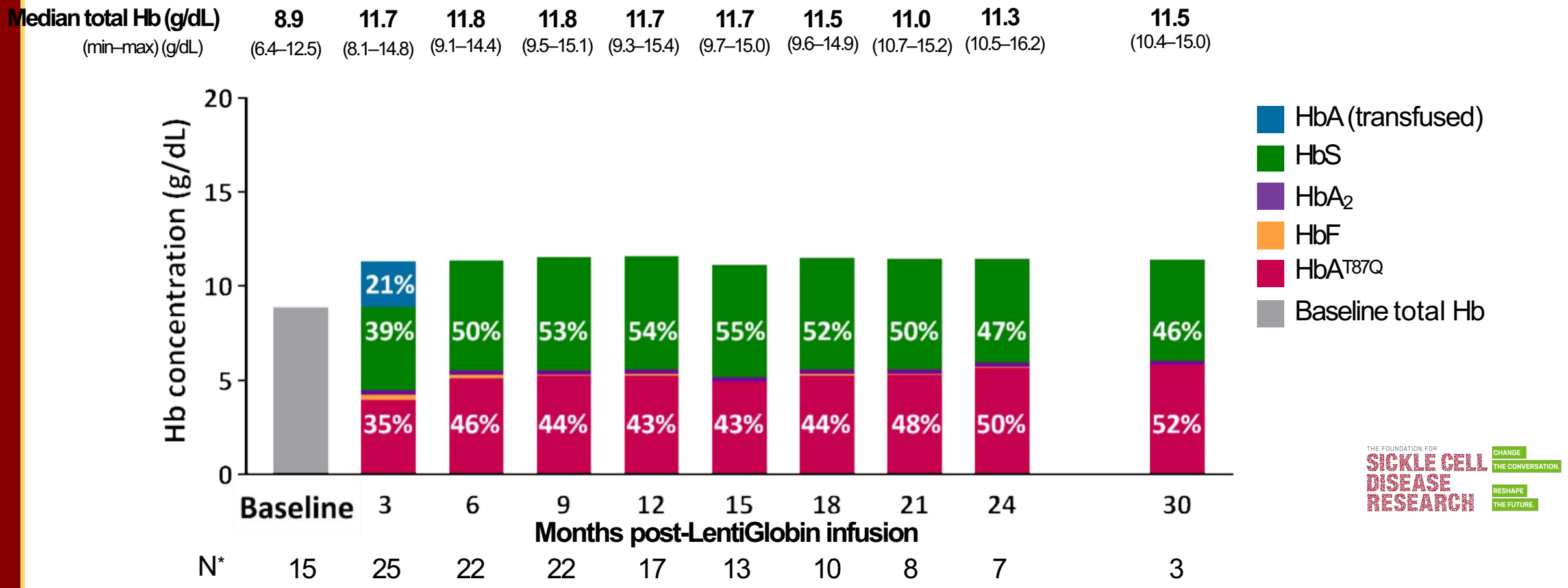
Patients with baseline values (n):	Baseline though M24
Better or near population normative values	Improvement noted at M6 and were generally sustained through M24
Worse than population normative values	Improvement in pain to levels better than population norm values were observed at M6 and were sustained through M24
Overall	Improvement in pain intensity was observed irrespective of baseline values relative to population norm

Average pain (0–10) over the past 7 days; 1. PROMIS Cooperative Group. 2020. PROMIS Reference Populations. Available at https://www.healthmeasures.net/score-and-interpret/interpret-scores/promis/reference-populations. Accessed Nov, 2020

NRS, Numeric Rating Scale; PROMIS, Patient Reported Outcomes Measurement Information System; SD, standard deviation

Data as of 20 August 2020

HGB-206 Group C: Median HbA^{T87Q} ≥ 40% at ≥ 6 months post-LentiGlobin treatment

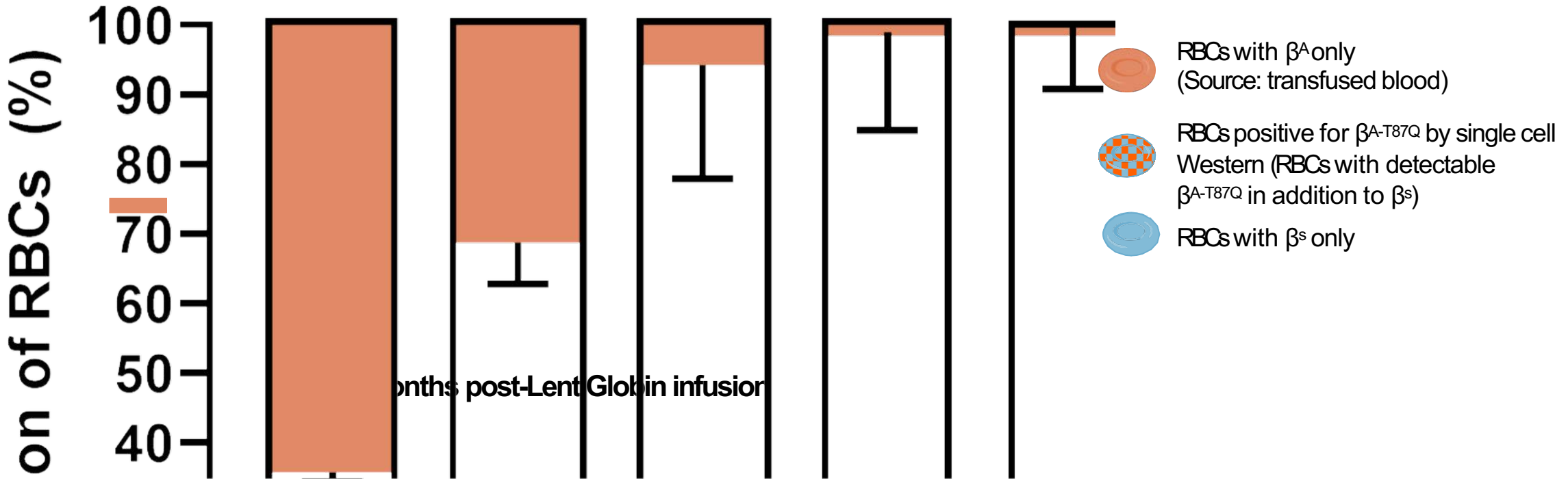


- In patients with ≥ 6 months of follow-up, median total Hb increased from 8.9 g/dL at baseline to ≥ 11.8 g/dL at Month 6
- At last visit in adolescents with ≥ 6 months of follow-up (n=6), median total Hb and HbA^{T87Q} were 13.5 g/dL and 6.1 g/dL, respectively

%represents median Hb fraction as % of total Hb; *Number of patients with data available. Hb, hemoglobin; max, maximum; min, minimum.

Data as of 20 August 2020

HGB-206 Group C: Near pancellular expression of HbA^{T87Q} ≥ 6 months post-LentiGlobin treatment



- Median (min–max) HbA^{T87Q}/RBC was 15.3 (11.7–20)† pg in patients with ≥ 6 months follow-up, which is comparable to the 13–18 pg of HbA/RBC in individuals with sickle cell trait‡ and higher than 10 pg of HbF/RBC in those with HPFH§

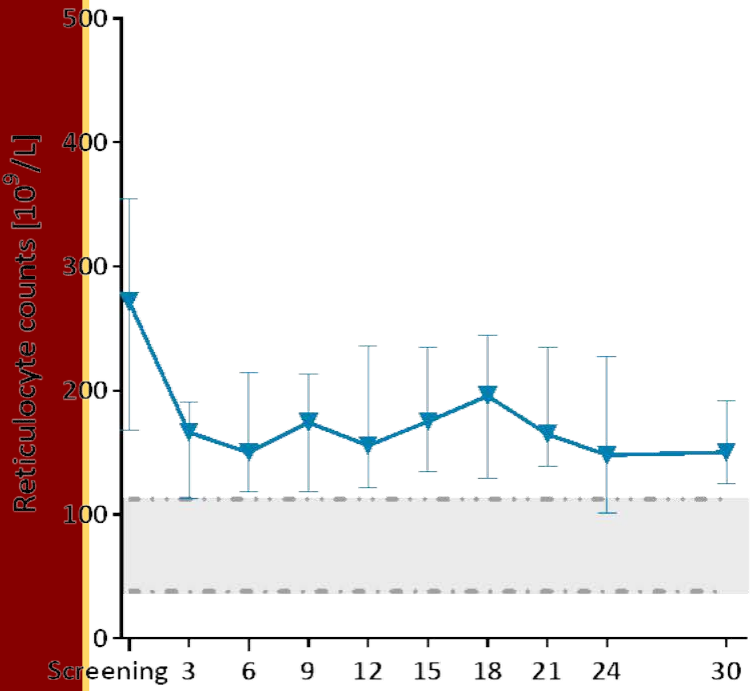
Mean & SD are depicted; Reducing HbS to < 30% is recommended by guidelines for exchange RBC transfusions for patients with SCD (indicated by dashed line); *Number of patients with data available; †Calculated as (% HbA^{T87Q} of total Hb/% RBCs containing βA-T87Q) x MCH; ‡Calculated to 13–18 pg HbA/RBC using 50% HbA/RBC for the lower end of the range and 60% HbA/RBC for the upper end of the range; §Estimated in Steinberg MH et al., Blood 2014.

Data as of 20 August 2020

Hb, hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; max, maximum; MCH, mean corpuscular hemoglobin; min, minimum; pg, picogram; RBCs, red blood cells; SD, standard deviation.

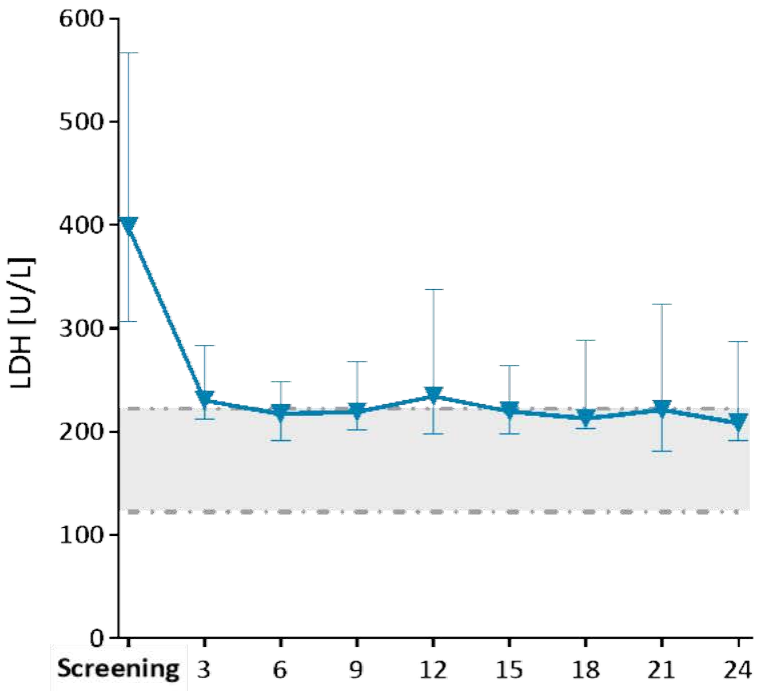
HGB-206 Group C: Hemolysis markers approaching near-normal levels post-LentiGlobin treatment

Reticulocyte counts



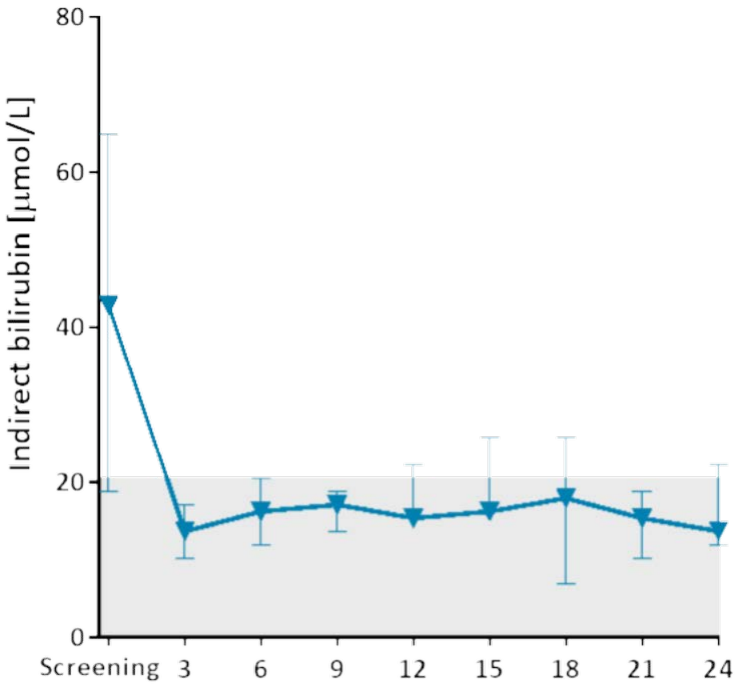
N*	31	26	23	22	17	14	10	9	7		3

Lactate dehydrogenase



N*	31	25	21	23	17	14	10	7	7

Indirect bilirubin



N*	26	20	22	17	14	10	9	7

Median (Q1, Q3) depicted; Dot-dash lines denote lower and upper limits of normal values; *Number of patients with data available; Q1, quartile 1; Q3, quartile 3.

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Data as of 20 August 2020

HGB-206 Group C: Safety profile post-LentiGlobin treatment

Treatment-emergent ≥ Grade 3 AEs *Reported in ≥ 2 patients**	N=32 n (%)
Stomatitis	21 (65.6)
Febrile neutropenia	14 (43.8)
Increased ALT	4 (12.5)
Increased AST	4 (12.5)
Increased GGT	4 (12.5)
Nausea	4 (12.5)
Increased blood bilirubin	2 (6.3)
Premature menopause	2 (6.3)
Upper abdominal pain	2 (6.3)
Serious treatment-emergent AEs *Reported in ≥ 2 patients*	
Abdominal pain	2 (6.3)
Nausea	2 (6.3)
Drug withdrawal syndrome	2 (6.3)
Vomiting	2 (6.3)

*Hematologic AEs commonly observed post-transplantation have been excluded;
AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

- 1 patient with a nonserious Grade 2 DP-related neutropenic fever (resolved)
- No cases of veno-occlusive liver disease, graft failure
- No vector-mediated RCL and no insertional oncogenesis
- One death, attributed to cardiopulmonary disease and unlikely related to LentiGlobin, > 18 months post treatment in a patient with significant baseline SCD burden

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†Occurred on study day 10 and resolved on study day 19

Data as of 20 August 2020

ACS, acute chest syndrome; AE, adverse event; DP, drug product; LVH, left ventricular hypertrophy; PIs, principal investigators; RCL, replication competent lentivirus; SCD, sickle cell disease; VOC, vaso-occlusive crisis.

Clinical hold on bluebird bio studies on LentiGlobin for sickle cell disease (bb1111) – *hold uplifted*

Data in this presentation are accurate as of 20 August 2020
Beyond this data cutoff, two SUSARs were reported from the Phase 1/2 HGB-206 study

Initially reported MDS diagnosis revised to transfusion-dependent anemia in a patient treated in Group C

- Patient had persistent anemia 6 months after transplant and was found to have trisomy 8 in 6% of cells scored on a 6-month bone marrow aspirate but no blasts or dysplastic cells
- Investigator assessed as serious, Grade 3, ongoing, and possibly related to LentiGlobin for SCD
- A follow-up bone marrow aspirate revealed no genetic or chromosomal abnormalities and no evidence of myeloid neoplasm, and the diagnosis was changed to transfusion-dependent anemia with investigations ongoing

bluebird bio remains committed to patient safety and is maintaining close contact with the treating physicians who are monitoring the patients
As of Feb 2021, the FDA has placed the clinical studies of LentiGlobin for SCD on clinical hold, and bluebird bio is in dialogue with the FDA to resume all clinical studies currently on hold

SUSAR suspected unexpected serious adverse reactions; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; VAMP4, vesicle-associated membrane protein 4; BM, bone marrow.

Clinical hold on bluebird bio studies on LentiGlobin for sickle cell disease (bb1111)

Data in this presentation are accurate as of 20 August 2020

Beyond this data cutoff, two SUSARs were reported from the Phase 1/2 HGB-206 study

Patient in Group A diagnosed with AML (treated 5.5 years ago)

- Lab analyses showed significant chromosomal abnormalities and mutations in genes typically associated with the development of AML, specifically, monosomy 7 and mutations in *RUNX1* and *PTPN11*
- Vector insertion in the AML cells took place in the *VAMP4* gene, which has no known role in the development of AML or with any cellular process related to cancer, and this insertion into the *VAMP4* gene had no impact on gene expression or gene regulation
- Case is unlikely related to vector-mediated insertional oncogenesis. Investigator assessed it as serious, Grade 4, and possibly related to LentiGlobin for SCD
    - This safety event is separate from and in addition to the patient who was diagnosed with MDS in 2018 and passed away due to relapsed AML in 2020. The 2018 MDS patient had a monosomy 7 mutation frequently associated with myeloid cancer and did not have vector integration present in cancer cells. The development of the 2018 MDS case was determined to be unlikely related to LentiGlobin for SCD and attributed to busulfan conditioning by the independent data monitoring committee and primary investigator treating the patient[1]
- The underlying increased risk of hematologic malignancies in SCD, combined with the transplant procedure and associated proliferative stress, as well as continued hematopoietic stress due to minimal clinical benefit in these Group A patients (DP manufactured using stem cells collected via BMH and using an earlier version of the manufacturing process which has since been discontinued) may have contributed to the development of AML

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As of Feb 2021, the FDA has placed the clinical studies of LentiGlobin for SCD on clinical hold, and bluebird bio is in dialogue with the FDA to resume all clinical studies currently on hold

[1]Hsieh et al, 2020 *Blood Advances*. SUSAR, suspected unexpected serious adverse reactions; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; VAMP4, vesicle-associated membrane protein 4; BM, bone marrow.

HGB-206 Group C: Summary

- Complete resolution of severe VOEs with up to 24 months of follow-up
  - Complete resolution of VOEs after stabilization of HbA^{T87Q} expression†, with up to 24 months of follow-up
- Improvement in patient-reported pain intensity sustained over 24 months of follow-up
- Median total Hb is consistently ≥ 11 g/dL ≥ 6 months post-LentiGlobin treatment, with a median anti-sickling HbA^{T87Q} ≥ 40%
- Near pancellular expression of HbA^{T87Q} ≥ 6 months post-LentiGlobin, with, on average, ~90% of RBCs containing HbA^{T87Q} at ≥ 18 months post treatment
- Key markers of hemolysis approaching near-normal levels post-LentiGlobin treatment
- The safety profile post-LentiGlobin for SCD remains generally consistent with the risks of autologous stem cell transplant, myeloablative single-agent busulfan conditioning, and underlying SCD

†HbA^{T87Q} expression stabilizes within 6 months.

Hb, hemoglobin; MDS, myelodysplastic syndrome; RBC, red blood cell; SUSAR, Suspected Unexpected Serious Adverse Reaction; SCD, sickle cell disease; VOE, vaso-occlusive event.

ARU-1801 has demonstrated meaningful clinical benefit for patients with severe SCD using reduced intensity conditioning

- ARU-1801 is an investigational gene therapy to induce expression of novel HbF^{G16D}
- No serious adverse events related to ARU-1801 or chemotherapy have been reported
- Long-term engraftment for up to 36 months without the use of myeloablative chemotherapy
- Clinically meaningful long-term reductions in disease burden was observed with ARU-1801
  - Significant reductions in VOEs
- Process improvements correlated with improved efficacy in patient 3
  - >37% total HbF and highest HbF^{G16D} to date
  - Near-pancellular Hb F distribution
  - No VOEs through 12 months post treatment

ARU-1801 has demonstrated that a gene therapy for SCD with reduced intensity conditioning is possible, and an important future option for patients

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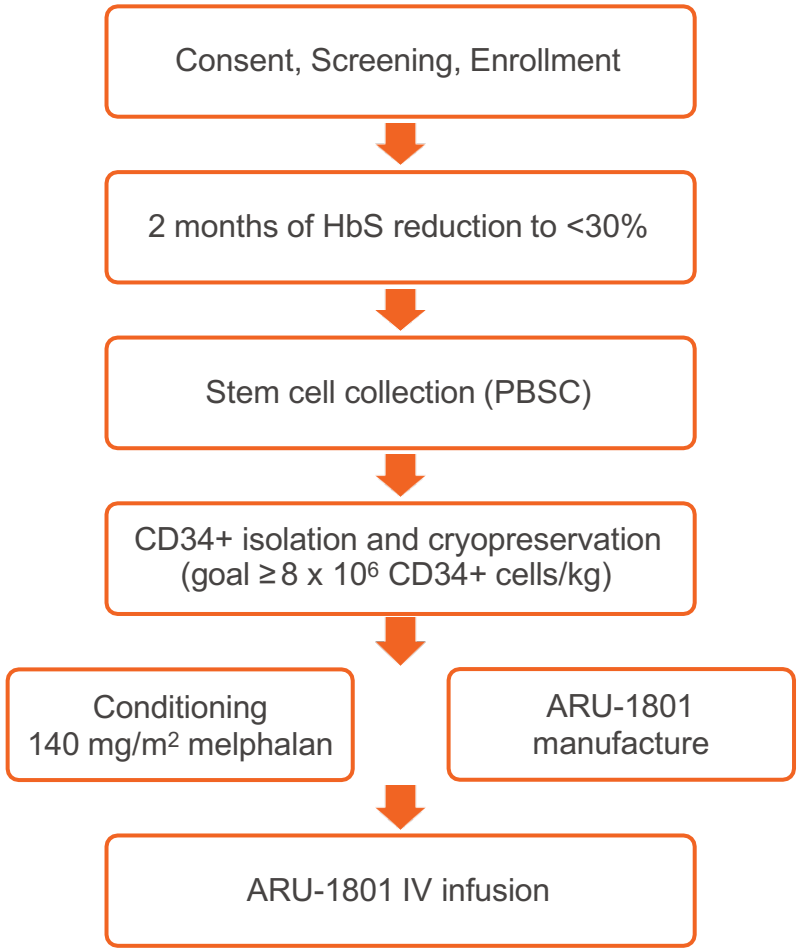
The MOMENTUM study is a Phase 1/2 trial of ARU-1801 utilizing reduced-intensity conditioning (RIC) in patients with severe SCD

Key Inclusion Criteria

- HbSS / HbSβ^0 / HbSβ$^+$ thalassemia
- 18-45 years of age
- Patients with severe SCD (frequent painful VOEs, 2 or more lifetime ACS, or one ACS requiring ICU admission or requiring chronic transfusions)
- Failed hydroxyurea, actively refused to take it, or have no access
- No matched sibling donor or refused allogeneic transplant

Key Exclusion Criteria

- Hx of stroke or on disease modifying therapy for moderate to high risk for stroke
- Patients with alpha thalassemia (2 or more deletions)



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Questions and Contact

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