Novel therapeutic approaches and patient-directed care in sickle cell disease: past, present, and future

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Medical Alumni Council Symposium

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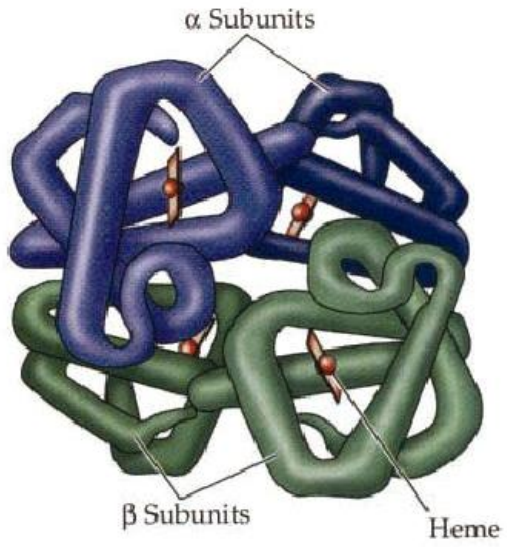
Learning objectives

- **Primary goal** - To increase understanding of the identification, diagnosis, and current and emerging treatments of sickle cell disease via recognition of prior SCD treatment challenges, current novel effective therapies and management strategies, and future ongoing SCD-related trials.

- **Objectives**:
  1. Recognize the scientific and patient care-based challenges that SCD patients have faced in the past including lack of effective treatments, difficulties with transition to adult care, and lack of medical home.
  2. Identify recent advances in mechanistic understanding of the SCD that have led to several new approved therapies, as well as new advances in patient care such as multimodal care in the outpatient setting and defined care pathways in emergency and inpatient settings.
  3. Describe future SCD treatment approaches to gene therapy and drug discovery, as well as ways to integrate these treatments into multimodal care and education of SCD patients.

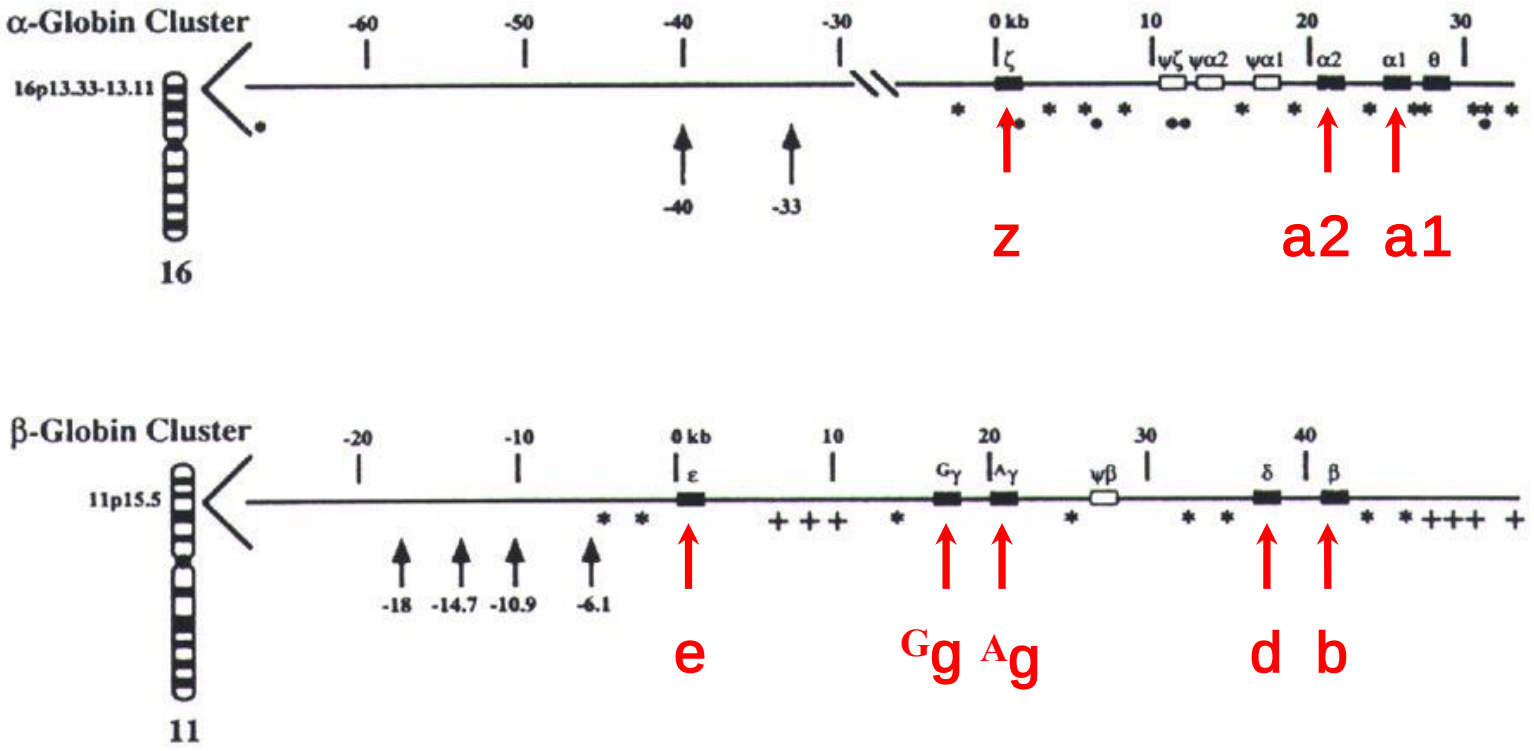
Hemoglobin structure and genes



HEMOGLOBIN = HEME + GLOBIN

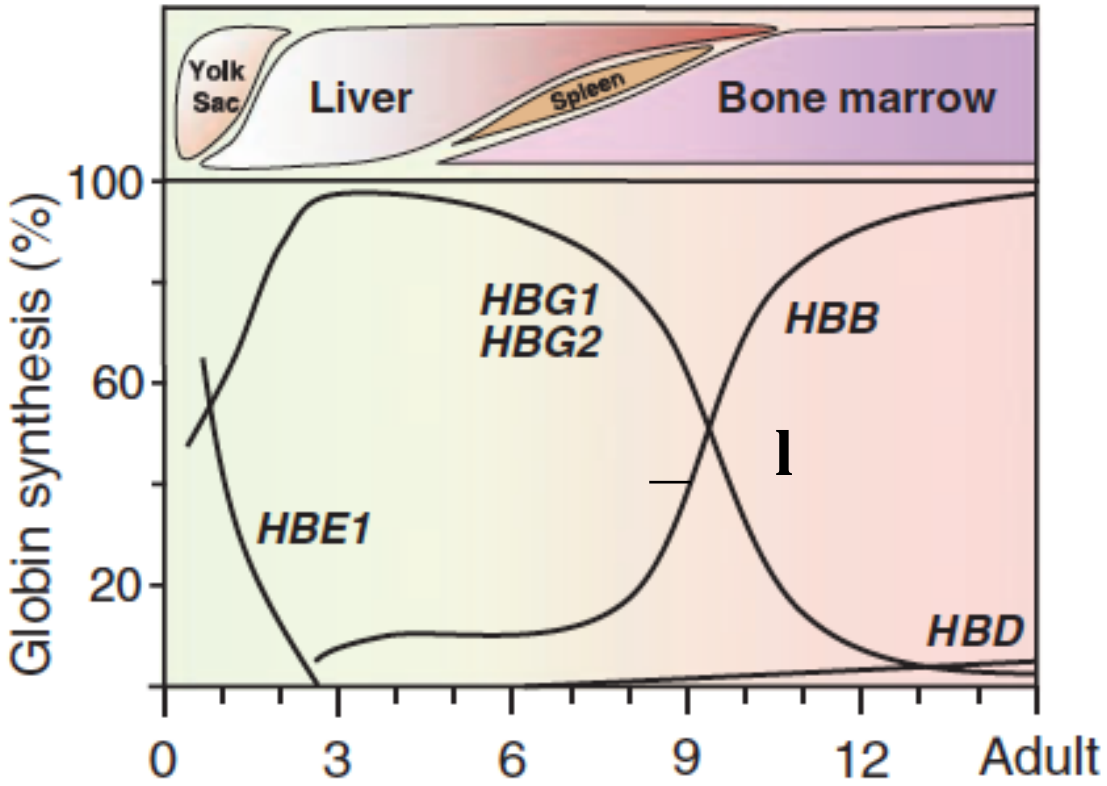
HETEROTETRAMERIC

2 α-like subunits + 2 β-like subunits

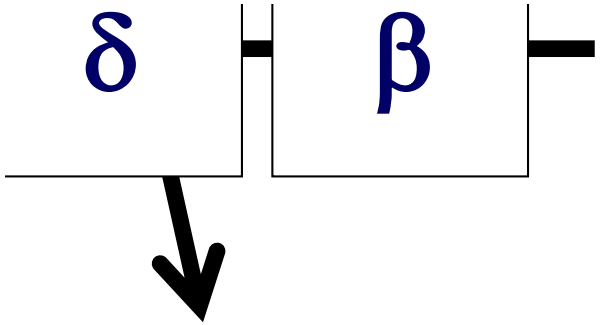


There are 3 functional α-like globin genes and 5 functional β-like globin genes, arranged in clusters on different chromosomes.

The various α- and β- like globin genes are expressed in a developmental stage-specific sequence



Sankaran and Orkin (2010)



Adult

$\alpha_2\beta_2 + \alpha_2\delta_2$

(Hb A + Hb A2)

Sickle cell disease overview

A congenital genetic chronic hemolytic disorder that is frequently interrupted by acute life-threatening events

- **SCD affects approximately 100,000 Americans**
- **SCD occurs among about 1 out of every 365 Black or African-American births**
- **About 1 in 13 Black or African-American babies is born with sickle cell trait (SCT)**

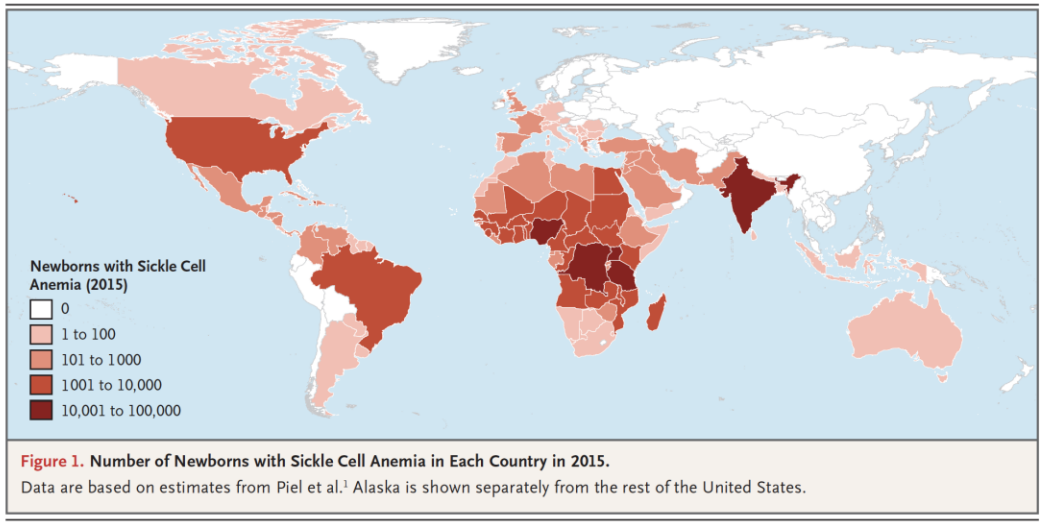


Figure 1. Number of Newborns with Sickle Cell Anemia in Each Country in 2015.
Data are based on estimates from Piel et al.[1] Alaska is shown separately from the rest of the United States.

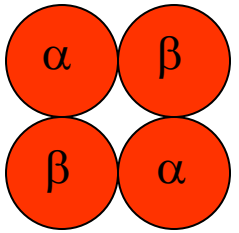
Piel et al. *NEJM* (2017)

<u>Genetic basis of SCD</u> - A G<u>A</u>G->G<u>T</u>G missense mutation at β-globin codon 6 that results in a Glu->Val substitution

GAG

β^A Val-His-Leu-Thr-Pro-Glu-Glu-...

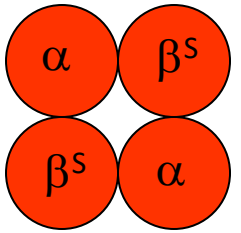
Hb A



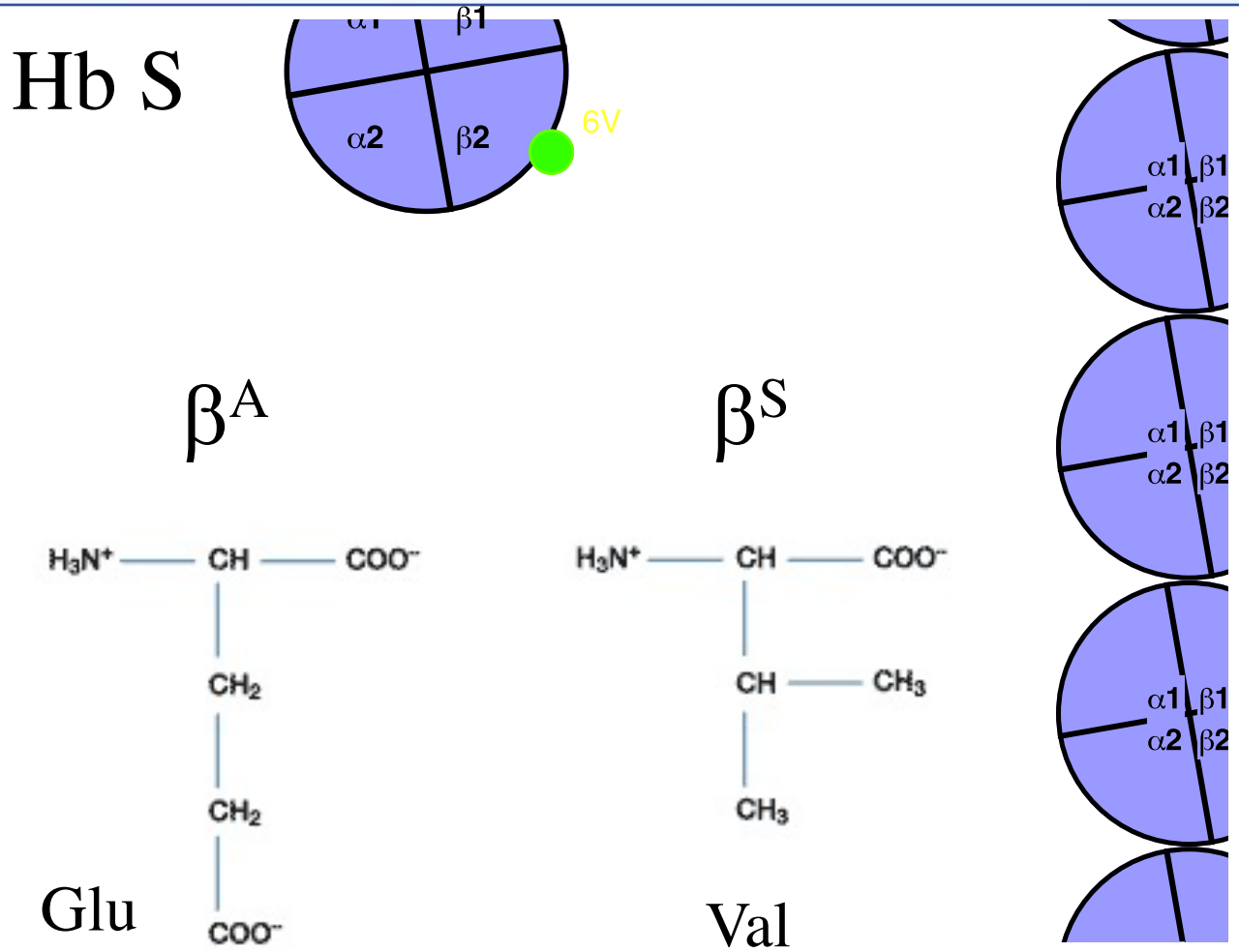
β^S Val-His-Leu-Thr-Pro-Val-Glu-...

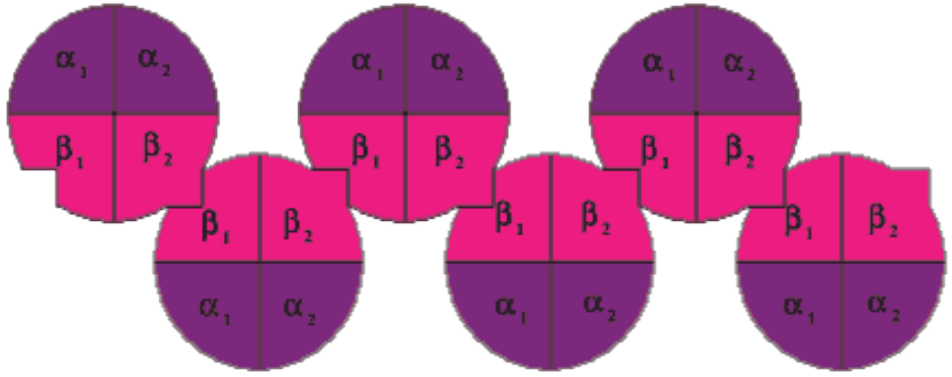
GTG

Hb S

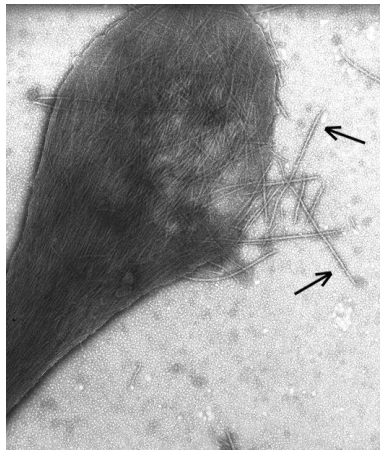


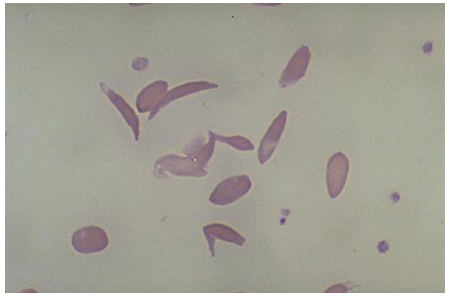
HbS mutation + deoxygenation leads to polymerization of hemoglobin





Deoxyhemoglobin S polymerizes into filaments

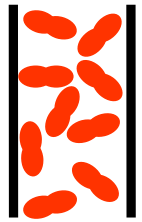
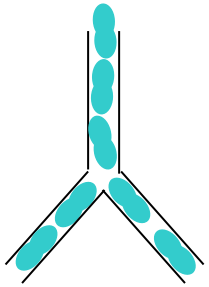
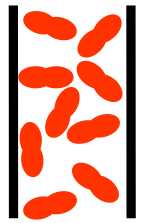
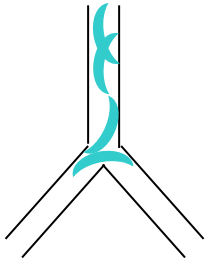




Non-polar valine (V6) on the surface of one protein embeds itself in a hydrophobic pocket of an adjacent hemoglobin (85F/88L), forming a hemoglobin dimer

Evolving pathophysiologic models of sickle cell disease

Historic "Log-Jam" Model

	Large vessels (Oxy)	Terminal vessels (Deoxy)	
Hb A			Normal flow
Hb S			Vaso-occlusion

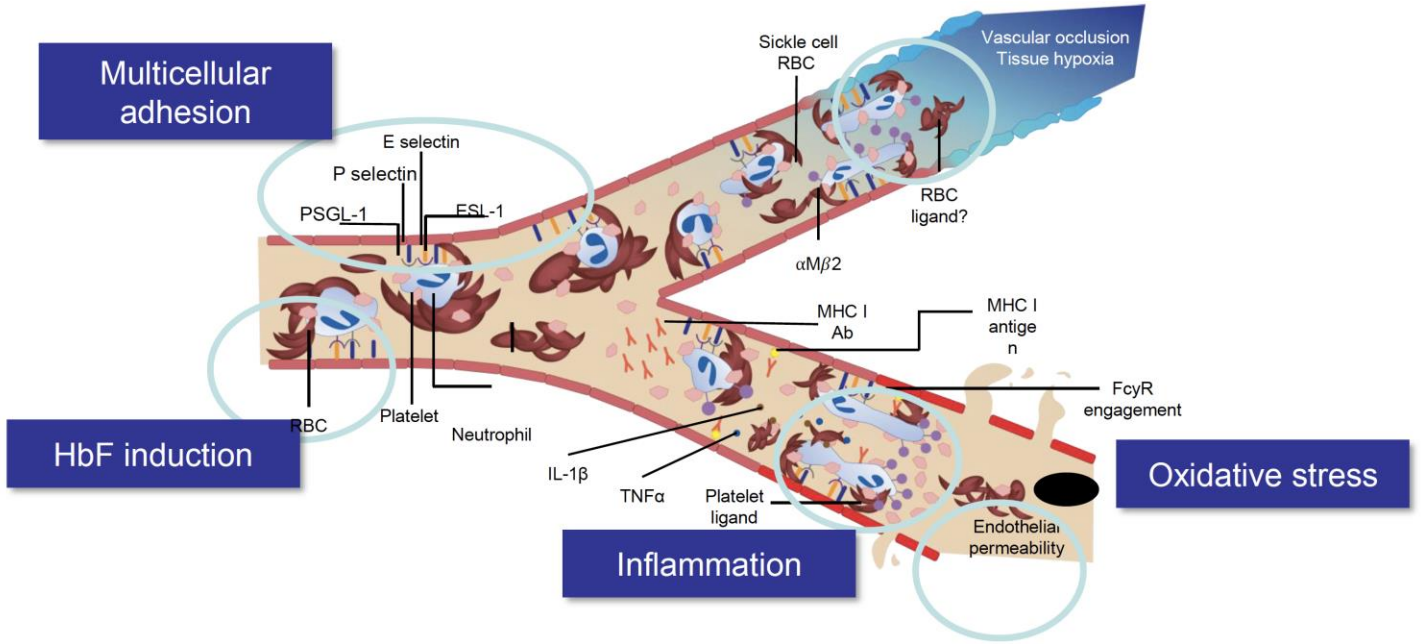
Model fails to explain:

How does process initiate?
How does process terminate?

Evolving pathophysiologic models of sickle cell disease

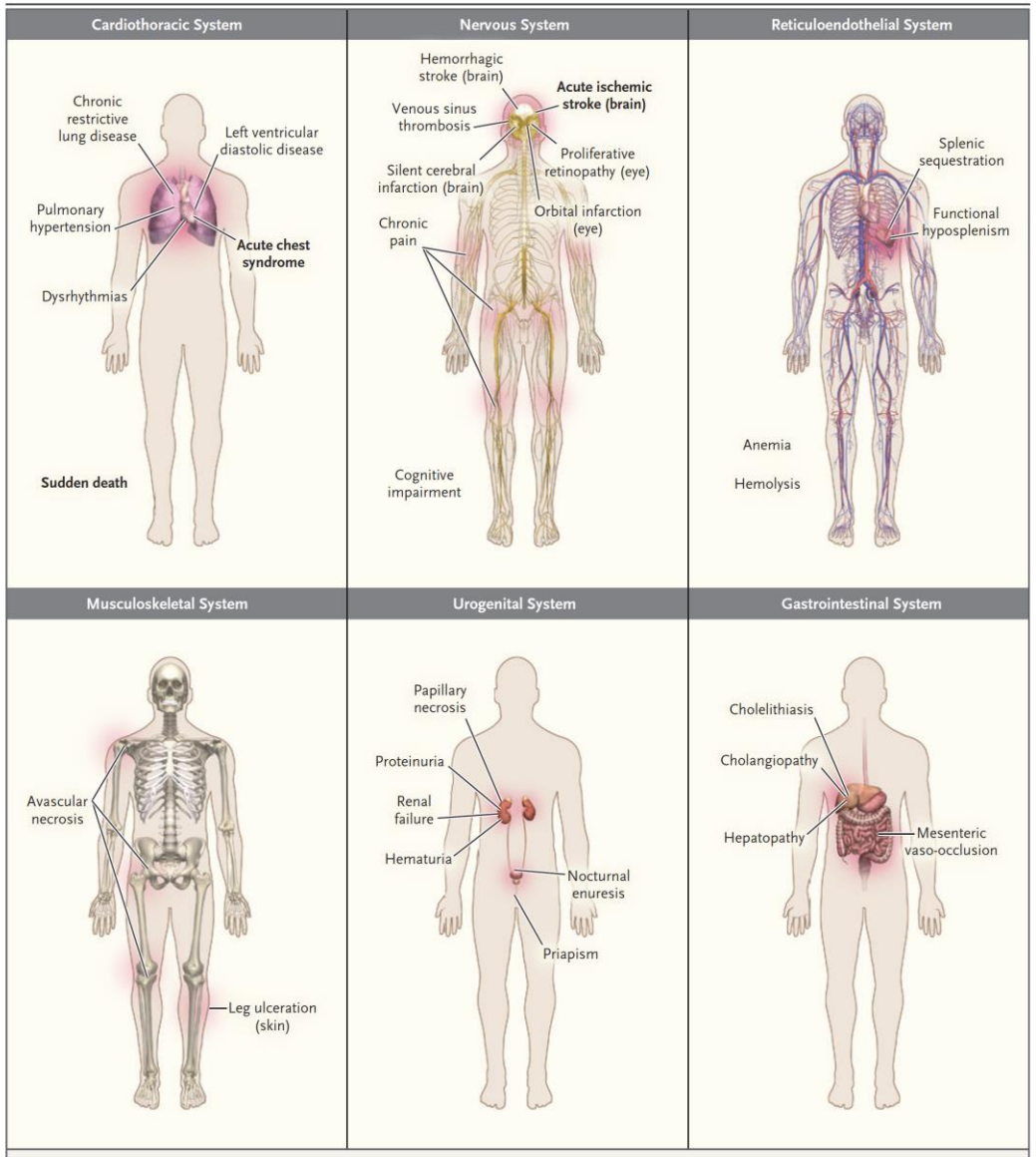
Evolved Model - Multifactorial

- **Rheology**
  - Increased vasoocclusion in low-oxygen tissues (renal medulla, trabecular bone, small intracranial vessels, etc.)
- **Cellular Adhesion**
  - Due to tissue hypoxia and inflammation
    - RBC $\alpha 4\beta 1$ to endothelium VCAM-1
    - Leukocyte P-selectins to PSGL-1 (crizanlizumab)
- **RBC Dehydration and reduced NO Bioavailability**
  - Leads to increased hemolysis
- **Oxidative stress and reperfusion injury, hypercoagulability, and platelet activation**



Adapted from Looney MR, Matthay MA. *Nature Medicine*. 2009;15:364-366.
Zhang D, Xu C, et al. *Blood*. 2016;127(7):801-809.

Sickle cell disease is a systemic disorder that requires multimodal treatment strategies



TREATMENT OPTIONS FOR ACUTE CRISES

Pain control

Treat precipitating event

Dehydration->hydrate
Infection->antipyretics, antibiotics
Pregnancy->manage

Oxygen

If hypoxic

PRBC transfusion

Generally not required for vaso-occlusive crisis
useful in other situations:

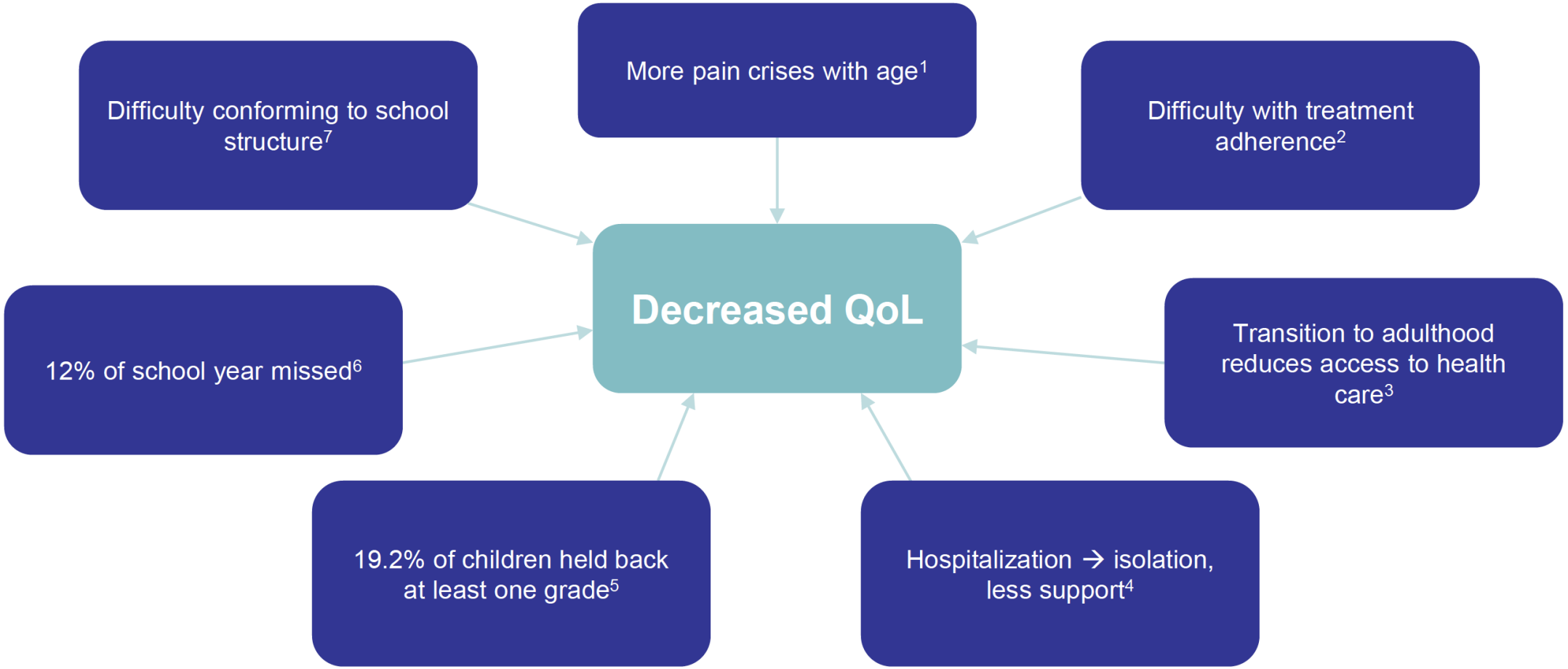
stroke	sickle cell intrahepatic cholestasis
acute chest syndrome	pre-operative
aplastic crisis	pregnancy-related pain
splenic sequestration	multiorgan failure

Piel et al. *NEJM* (2017)

Factors affecting SCD severity

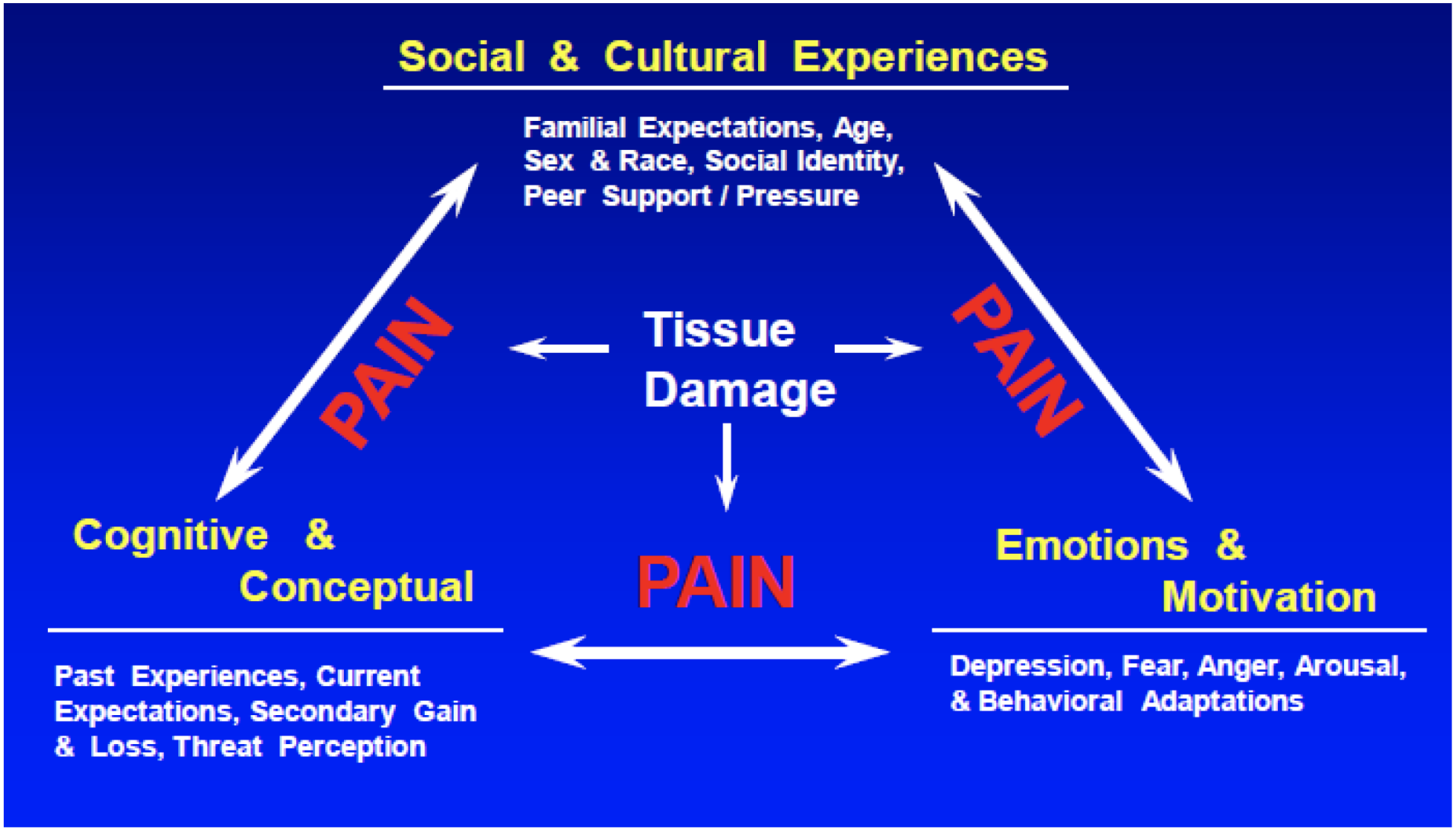
- Although influenced by genotype (HbSS vs HbSC), disease severity is variable
- Variability is partly explained by genetic modifiers such as HbF level and co-inheritance of α-thalassemia
- Disease severity is influenced by other factors
  - Geographical/home environment
  - Psycho-social factors
  - Nutrition
  - Access to care

Factors affecting SCD severity



Kanter J et al. *Blood Rev*. 2013;27(6):279-287; Fisak B et al. *Child Care Health* Dev; 2011;38(2):204-210; Blinder MA et al. *PaediatrBlood Cancer*. 2013;60(5):828-835; Weisberg D et al. *J Hosp Med*. 2013;8(1):42-46; 5Ladd RJ et al. *PaediatrBlood Cancer*. 2014;61(7):1252-1256; Schwartz LA et al. *PaediatrBlood Cancer*. 2009;52(1):92-96; Dyson SM et al. *SociolHealth Illn*. 2011;33(3):465-483.

Multiple dimensions of pain in SCD



Current treatment of vaso-occlusive pain

- For adults and children with SCD presenting with acute pain related to SCD, recommendations include:
  - *R*apid (within 1 hour of ED arrival) assessment and administration of analgesia with frequent reassessments (every 30-60 minutes) to optimize pain control
  - Tailored opioid dosing based on consideration of baseline opioid therapy and prior effective therapy
  - Short course (5 to 7 days) of NSAIDs in addition to opioids for acute pain management
  - DO NOT USE: corticosteroids for acute pain

- Use a standardized protocol (for example, PCA in a pain management pathway) to treat acute pain in the acute care setting, with individualized pain plans for more severe phenotypes
  - Receive care (when possible) at SCD-specific hospital-based facilities

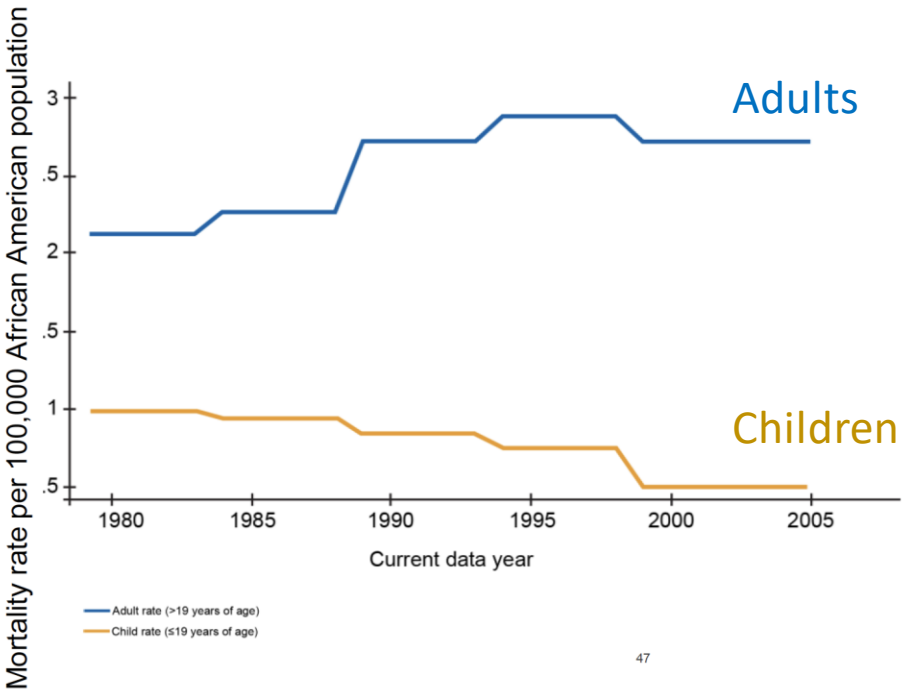
- Optimal treatment of chronic pain requires an individualized approach that involves interdisciplinary care and cognitive/behavioral pain strategies
  - Guidelines suggest adjunct therapy in addition to medical and pharmacologic therapy: massage, yoga, transcutaneous electrical nerve stimulation (TENS), virtual reality (VR), and guided audiovisual (AV) relaxation in addition to standard pharmacological management

Improved SCD outcomes in children, but significant need for improved treatments in older populations

- Relative to the rate for the period 1983 through 1986, the SCD mortality rate for the period 1999 through 2002 decreased by
  - 68% at age 0 through 3 years;
  - 39% at age 4 through 9 years; and
  - 24% at age 10 through 14 years

- Recent decreases in SCD mortality in children under age 4 years coincided with the introduction of the 7-valent pneumococcal conjugate vaccine in 2000

- Essentially no reduction in SCD mortality at older ages (in pediatric study) or in adults (Lanzkron et al 2013)
  - The lack of significant recent reduction in SCD mortality in older children and adults indicates the need for new treatment approaches



Yanni et al. Trends in Pediatric Sickle Cell Disease-Related Mortality in the United States, 1983-2002, *J. Pediatrics* (2009)
Lanzkron S et al. *Public Health Rep*. 2013;128(2):110-116.

Recommended and future treatments in SCD

- Infection prevention
  - PenVK, pneumococcal vaccination, malaria prophylaxis
- Stroke prevention
  - Primary prevention - Transcranial Doppler (TCD) screening
  - Secondary prevention – exchange transfusion
- Disease–modifying therapies
  - Hydroxyurea
    - Vaso-occlusive episode reduction
    - Stroke risk reduction, reduction in CKD progression
    - Additional benefits?
  - Crizanlizumab (P-selectin inhibitor)
  - Voxelotor (HbS polymerization inhibitor, binds high-oxygen affinity state)
- Future therapies
  - Novel HbF inducers (heme-regulated inhibitor, HRI)
  - Gene therapy (Lentiglobin gene addition)

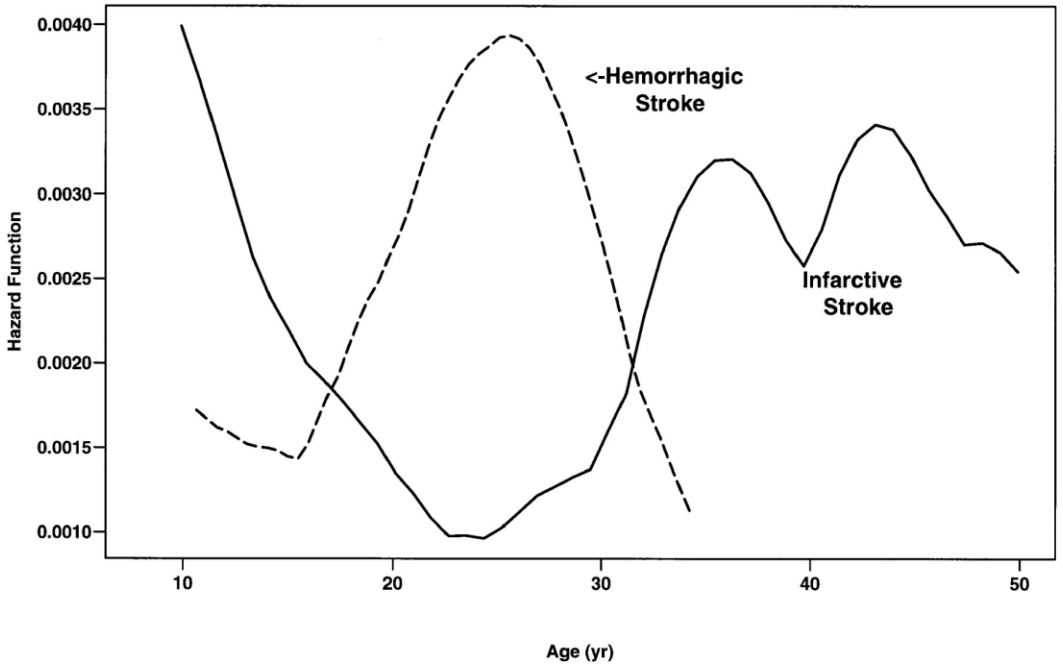
↑CBF, ↓Cerebrovascular reserve

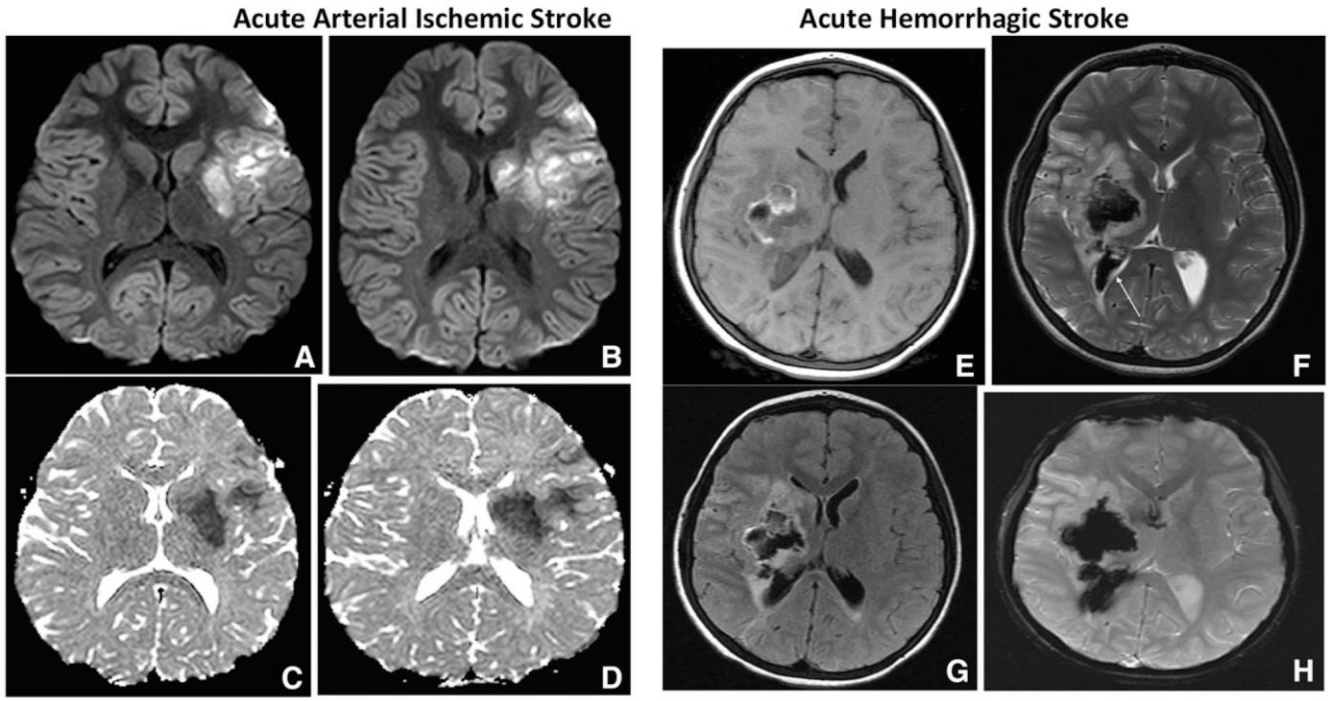
- Low baseline hemoglobin
- High % HbS increases CBF independent of hemoglobin, especially at HbS levels >40%

Relative and acute anemia

- Low baseline hemoglobin
- Relative acute drop in hemoglobin (<5.5 g/dl)

Overview of Stroke in SCD





Ohene-Frempong K et al. Blood 1998;91:288.

Kassim et al. Blood 2015;125:3401-10.

Overview of Stroke in SCD

First stroke in Hb SS, by age:

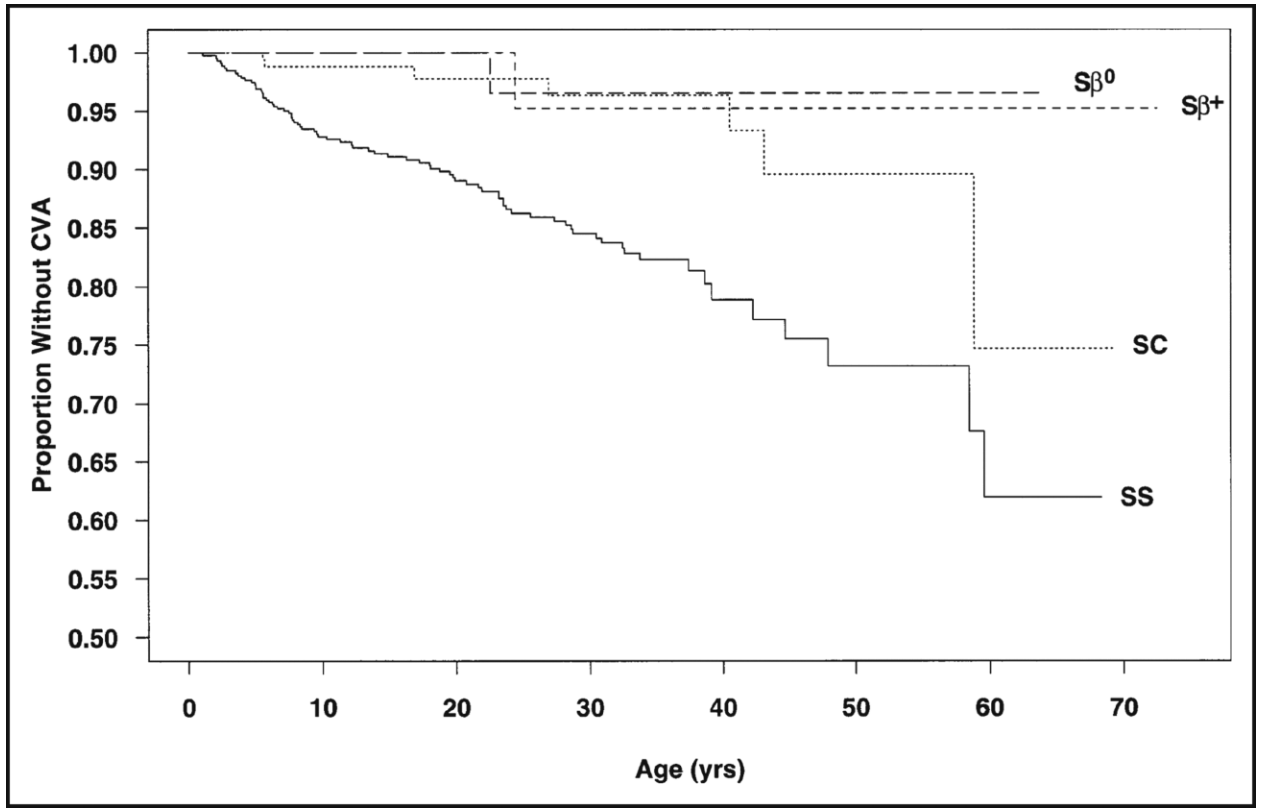
20 yo	11%
30 yo	15%
45 yo	24%

First stroke in Hb SC, by age:

20 yo	2%
30 yo	4%
45 yo	10%

Risk factors for stroke:

- Prior TIA (RR=56)
- Low Hb (RR 1.9 for each 1 g/dL decrease)
- ACS frequency (RR 2.4 for each event/year)
- ACS proximity (RR 7.0 for ACS within 2 weeks)
- HTN (RR 1.3/10 torr increase in SBP)



Ohene-Frempong K et al. Blood 1998;91:288

Acute Management Recommendations

CLINICAL GUIDELINES

blood advances

American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults

M. R. DeBaun,[1,*] L. C. Jordan,[2,*] A. A. King,[3] J. Schatz,[4] E. Vichinsky,[5] C. K. Fox,[6,7] R. C. McKinstry,[8,9] P. Telfer,[10] M. A. Kraut,[11] L. Daraz,[12] F. J. Kirkham,[13-15] and M. H. Murad[12]

- Acute and timely treatment of suspected or confirmed ischemic stroke/TIA:
  - For children or adults with SCD and acute neurological deficits, including transient ischemic attack (TIA), the ASH guideline panel recommends prompt blood transfusion.
    - The transfusion should be given immediately upon recognition of symptoms <u>without delay beyond 2 hours of acute neurological symptom presentation.</u>
    - The type of transfusion (simple, modified exchange, or apheresis) is dependent on individual patient factors and local transfusion resources.

  - The ASH guideline panel suggests exchange transfusion vs simple transfusion
    - When exchange transfusion is not available within 2 hours of presentation for medical care and hemoglobin is < 8.5 g/dL, simple transfusion can be performed to avoid delays in treatment while a manual exchange transfusion or an automated apheresis is planned

DeBaun et al., *Blood Adv* (2020) 4 (8): 1554–1588.

IV tPA Acute Management Recommendations

CLINICAL GUIDELINES

blood advances

American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults

M. R. DeBaun,[1,*] L. C. Jordan,[2,*] A. A. King,[3] J. Schatz,[4] E. Vichinsky,[5] C. K. Fox,[6,7] R. C. McKinstry,[8,9] P. Telfer,[10] M. A. Kraut,[11] L. Daraz,[12] F. J. Kirkham,[13-15] and M. H. Murad[12]

- For adults with SCD presenting with symptoms of acute ischemic stroke who are being evaluated for IV tissue plasminogen activator (tPA; age >18 years, no hemorrhage on computed tomography [CT] scan, within 4.5 hours of onset of symptoms/signs and without contraindications for thrombolysis):

1. For all patients, the administration of tPA should not delay prompt simple or exchange blood transfusion therapy.
2. Patients may be considered for IV tPA based on its established inclusion and exclusion criteria detailed in stroke management algorithms.
3. The following factors suggest likely benefit from IV tPA: older age, atrial fibrillation, diabetes, hypertension, and hyperlipidemia. Management of younger patients without these risk factors should emphasize early transfusion.
4. There are no validated risk stratification or reliable age cutoff criteria to guide the choice of initial therapy.
5. IV tPA is not recommended for children with SCD (<18 years of age).

DeBaun et al., *Blood Adv* (2020) 4 (8): 1554–1588.

Stroke and Sickle Cell Disease

- Management of acute stroke in SCD
  - Exchange transfusion is the mainstay of therapy
- Primary prevention
  - Transcranial doppler (velocities > 200 cm/s) (Age 2-16y)
    - Role of long-term blood transfusions to HbS < 30% (STOP 1/STOP 2)
    - Transition to HU following 12 months transfusions (TWiTCH)
- Secondary prevention
  - Standard of care is a chronic transfusion program targeting HbS < 30%
  - Inability to transition to HU following overt stroke (SWiTCH)
  - Consideration of HU in place of transfusions in select situations

Table 3. Characteristics of the Patients with Strol

Patient No.	Sex	Age at Entry	Age at Stroke	Time from Entry to Stroke	MRI Findings	Blood-Flow Velocity†	Time from Ultrasonography to Stroke‡
		yr		*mo*		*cm/sec*	*mo*
1	M	7	7	4	Right MCA distribution	245	4
2	M	5	6	4	Left BZ, MCA distribution	251	4
3	M	6	7	6	Right BZ	172	6
4	M	7	10	41	Left BZ	230	1
5	F	12	14	20	Left BZ	174	12
6	M	5	8	35	Left BZ, right BG	145	35
7	M	7	11	51	Left MCA	215	11

(TCD>170 cm/sec)

- In general, higher TCD velocities are associated with more severe cerebral arterial narrowing.
- "Our overall positive results were not greatly affected by choosing cutoff values between 140 and 190 cm/sec."
- Velocities of >200 cm/sec may indicate more proximate risk."

Adams R et al. N Engl J Med 1992;326:605.

Primary Stroke Prevention
(STOP I, 1998)

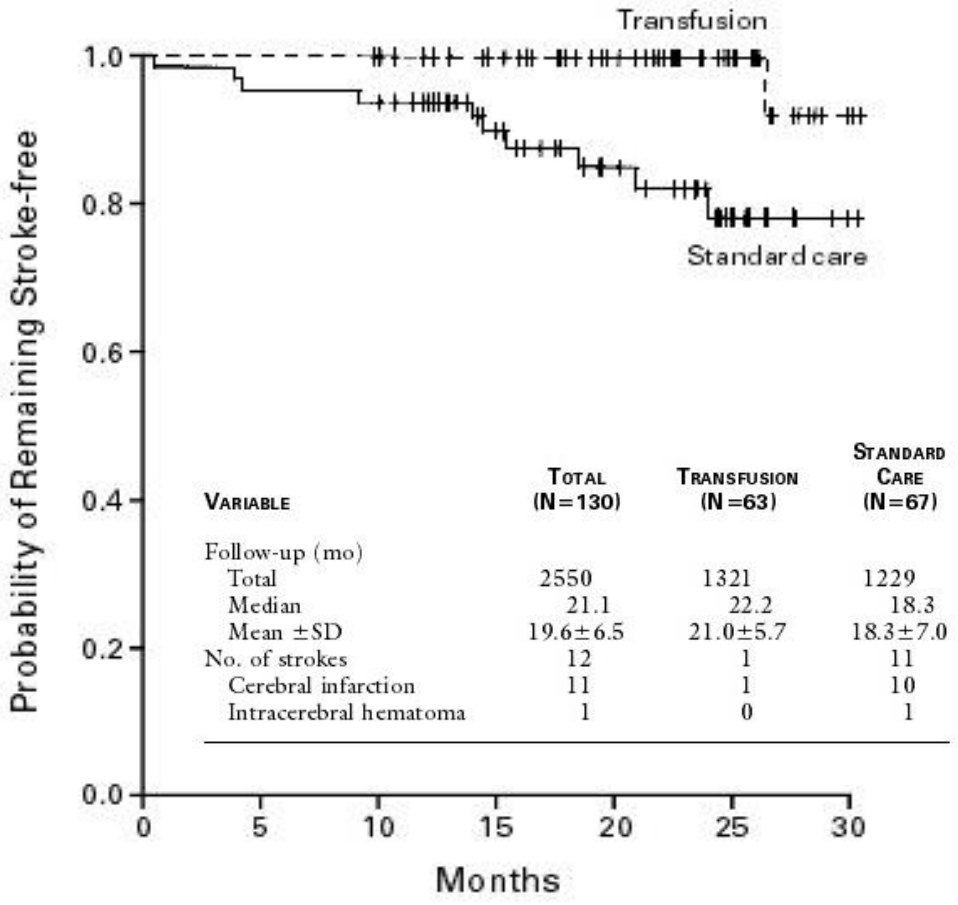
Goal: Efficacy of transfusion *versus* standard care for children with SCD at high stroke risk

Criteria: SCD (HbSS, HbSβ^{o}thal)
TCD ≥ 200 cm/sec
No previous stroke
No hydroxyurea

Method: Straight transfusion (sTx) or red cell exchange (RCE) to HbS < 30% *versus* standard care

Results: Children screened by TCD 1934
Rate of abnormal results 9.7%
Randomized / sTx or RCE 63
(63% sTx; 12% RCE; 25% combination)
Randomized / standard care 67
Age = 8.3+/-3.3 yr

Conclusion: Transfusion greatly reduces the risk of a first stroke in children with SCD who have abnormal results on TCD (>200 cm/sec).



Variable	Total (N=130)	Transfusion (N=63)	Standard Care (N=67)
Follow-up (mo)			
Total	2550	1321	1229
Median	21.1	22.2	18.3
Mean ±SD	19.6±6.5	21.0±5.7	18.3±7.0
No. of strokes	12	1	11
Cerebral infarction	11	1	10
Intracerebral hematoma	1	0	1

Adams RJ et al. N Engl J Med 1998;339:5.

Discontinuing Prophylactic Transfusions in SCD (STOP II, 2005)

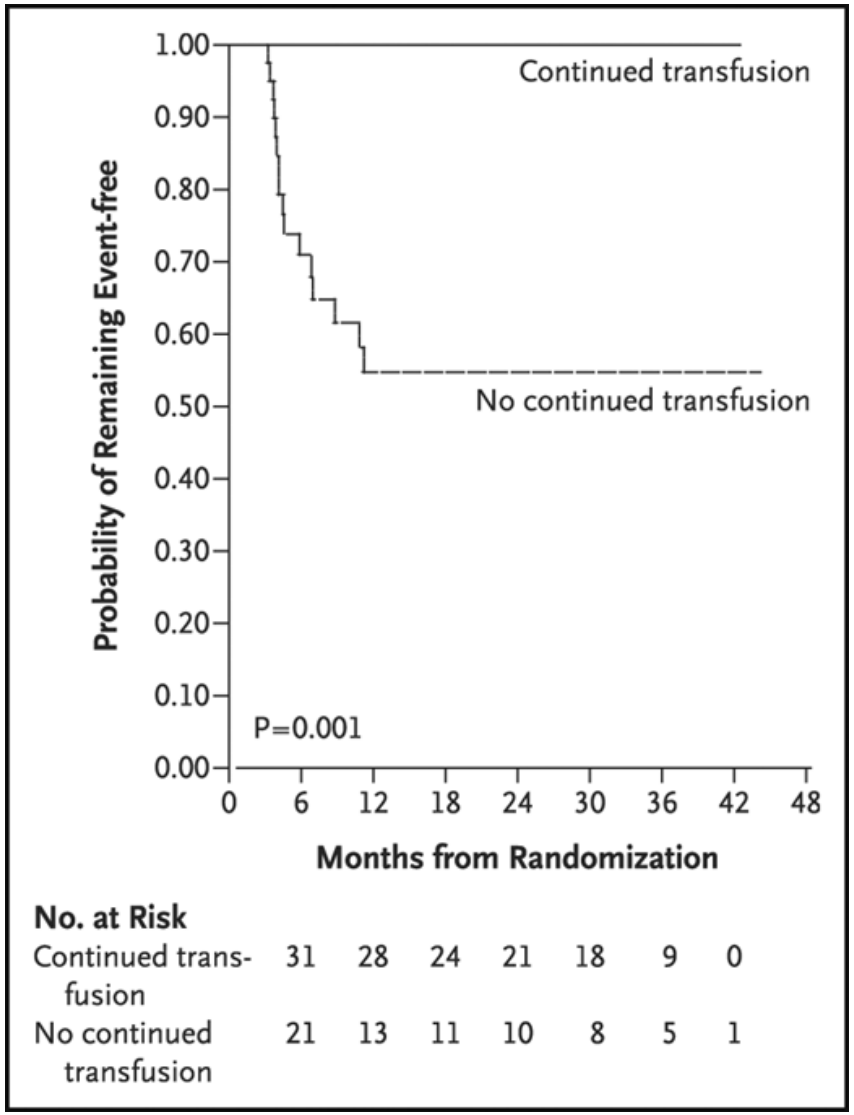
Goal: Required duration of transfusion in at-risk children with SCD and elevated TCD

Criteria: SCD (HbSS, HbSβ^{o}thal)
High-risk TCD that normalized with sTx/RCE
30-month transfusion history
No stroke
No hydroxyurea

Method: Continued transfusion *versus* no transfusion
Composite end point: stroke *or* reversion to abnormal TCD

Results: Enrolled 79
Continued transfusion 38
Stroke n=0; high-risk TCD n=0
Discontinued transfusion 41
Stroke n=2; high-risk TCD n=14; 4.5+/-2.6 mo
Age = 12.2+/-3.2 yr

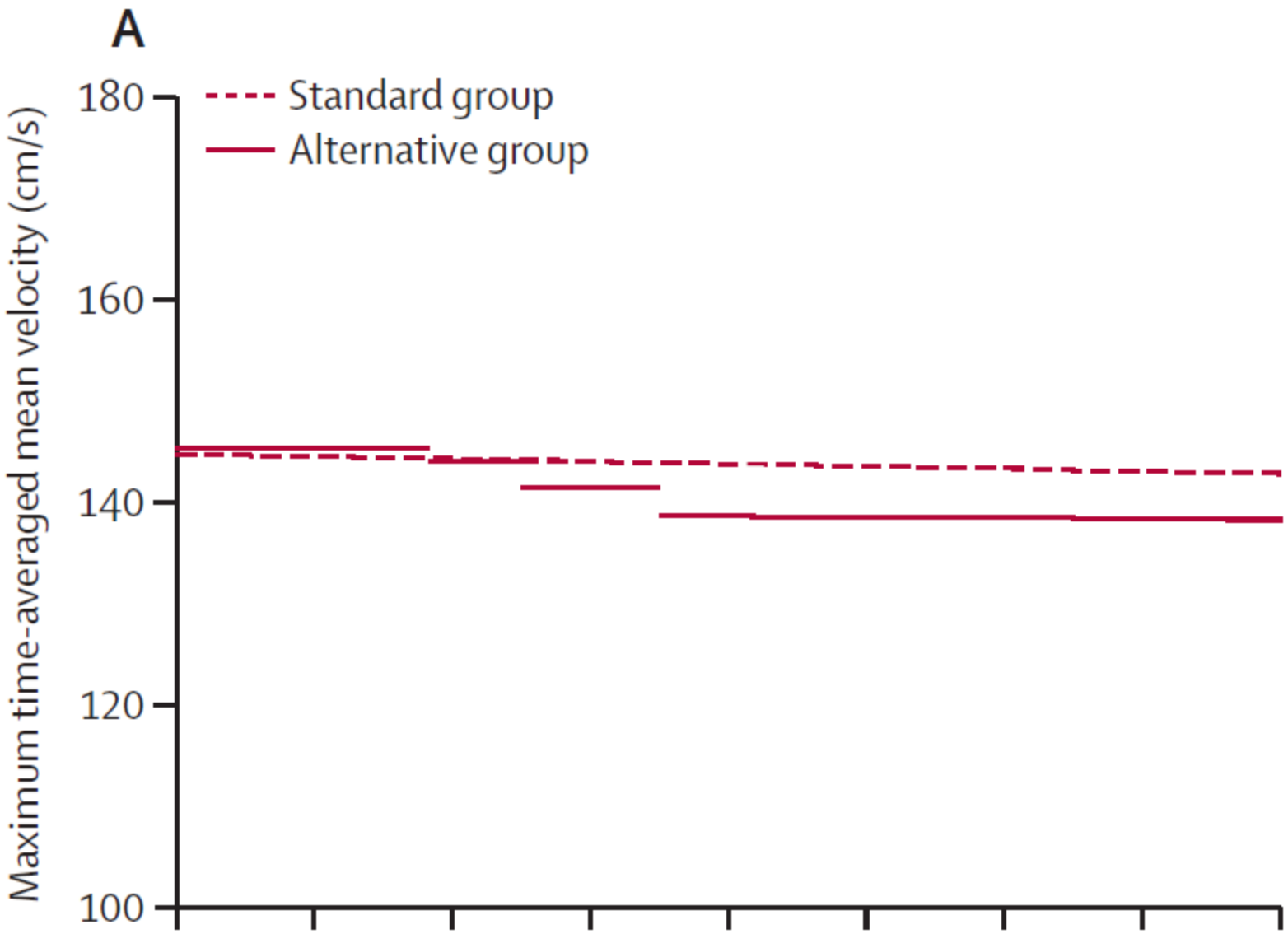
Conclusion: Discontinuation of transfusion for the prevention of stroke in children with SCD cell disease results in a high rate of reversion to abnormal blood-flow velocities on Doppler studies and stroke.



N Engl J Med 2005;353:2769.

urea

stroke risk



Ferritin = 2674
Ferritin = 1276

Ware RE et al. Lancet 2016;387:661.

have
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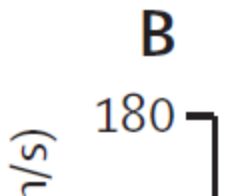
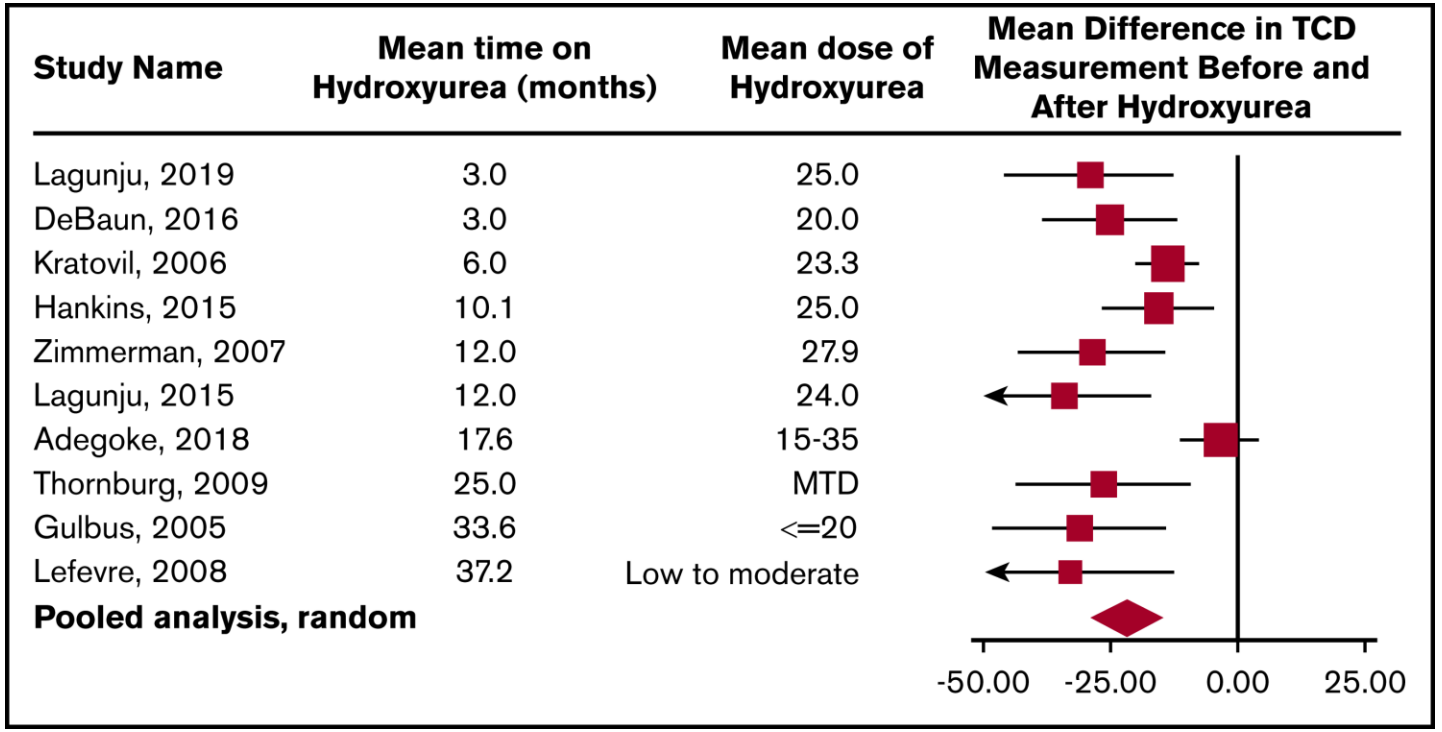
velocities and



Effect of HU on TCD (low-income countries)

- Pooled analysis of the 10 studies documenting TCD measurement before and after hydroxyurea therapy in children with HbSS or HbSβ0thalassemia.
- This meta-analysis demonstrates the average drop in TCD measurement after starting hydroxyurea therapy of 21 cm/s (95% confidence interval [CI], 14.8-29.0).
- The plot also suggests that the decrease in TCD measurements can be seen as early as 3 months after the start of hydroxyurea therapy with a sustained impact of hydroxyurea therapy on decreasing TCD measurements for at least 36 months.



DeBaun et al., *Blood Adv* (2020) 4 (8): 1554–1588.

Primary Prevention Recommendations

CLINICAL GUIDELINES

blood advances

American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults

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- For children with HbSS or HbS-beta^0 thalassemia (ages 2-16 years): annual TCD screening
  - High-income setting
    - Regular blood transfusion for at least a year (vs no transfusion) with the goal of keeping maximum HbS levels below 30% and maintaining hemoglobin levels > 9.0 g/dL to reduce the risk of stroke
    - After 1 year transfusions and are interested in stopping transfusion, hydroxyurea treatment at the maximum tolerated dose can be considered to substitute for regular blood transfusions
  - Low-income setting
    - Where regular blood transfusion therapy and chelation therapy are not available or affordable), suggests hydroxyurea therapy with at least 20 mg/kg per day at a fixed dose or the maximum tolerated dose

DeBaun et al., *Blood Adv* (2020) 4 (8): 1554–1588.

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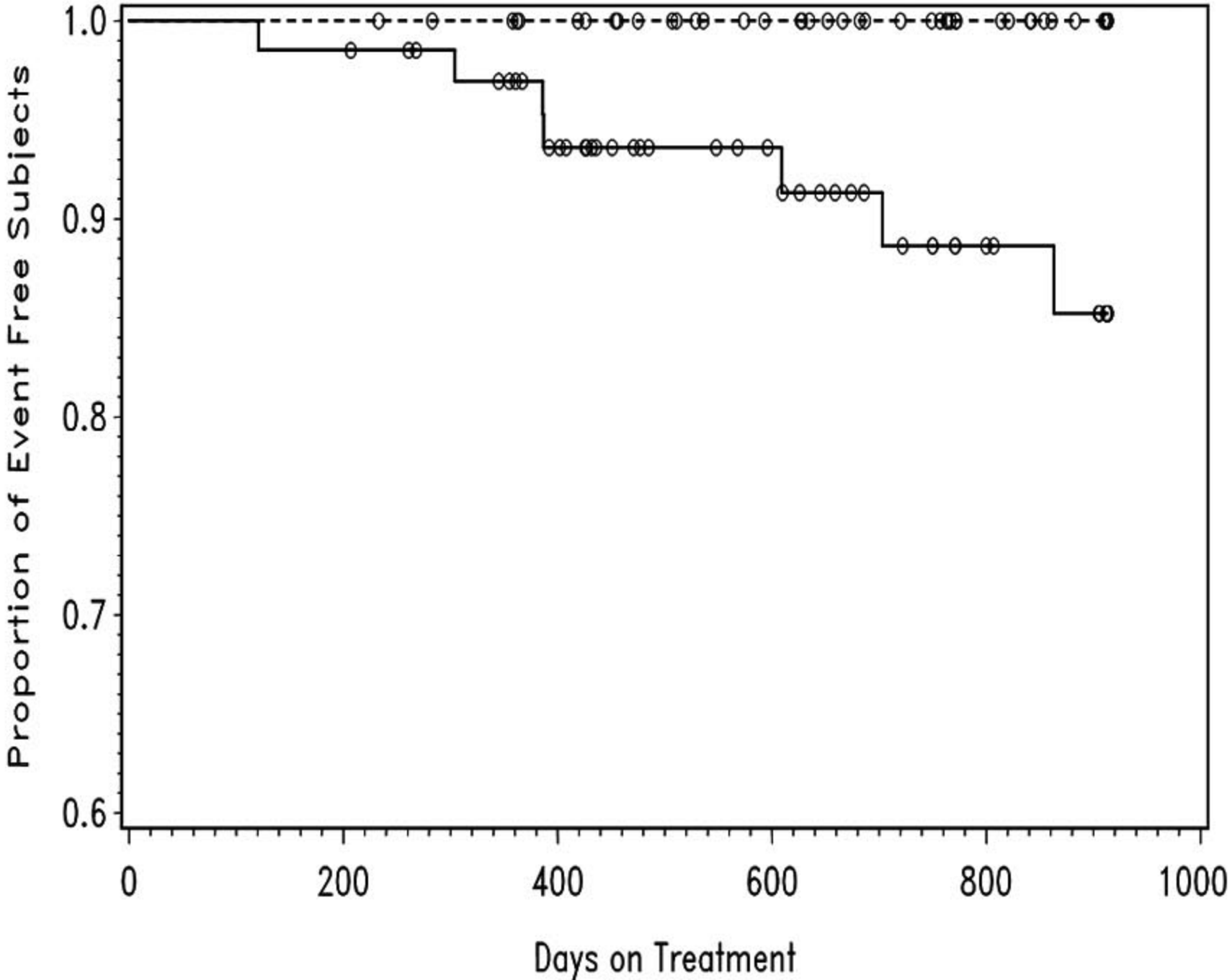


Standard

Alternate

Stroke
P<0.05

(LIC)

Ware RE, Helms RW.
Blood 2012;119:3925.

usions

Recommended and future treatments in SCD

- Infection prevention
  - PenVK, pneumococcal vaccination, malaria prophylaxis
- Stroke prevention
  - Primary prevention - Transcranial Doppler (TCD) screening
  - Secondary prevention – exchange transfusion
- Disease–modifying therapies
  - Hydroxyurea
    - Vaso-occlusive episode reduction
    - Stroke risk reduction, reduction in CKD progression
    - Additional benefits?
  - Crizanlizumab (P-selectin inhibitor)
  - Voxelotor (HbS polymerization inhibitor, binds high-oxygen affinity state)
- Future therapies
  - Novel HbF inducers (heme-regulated inhibitor, HRI)
  - Gene therapy (Lentiglobin gene addition)

Characteristics of agents FDA-approved for SCD

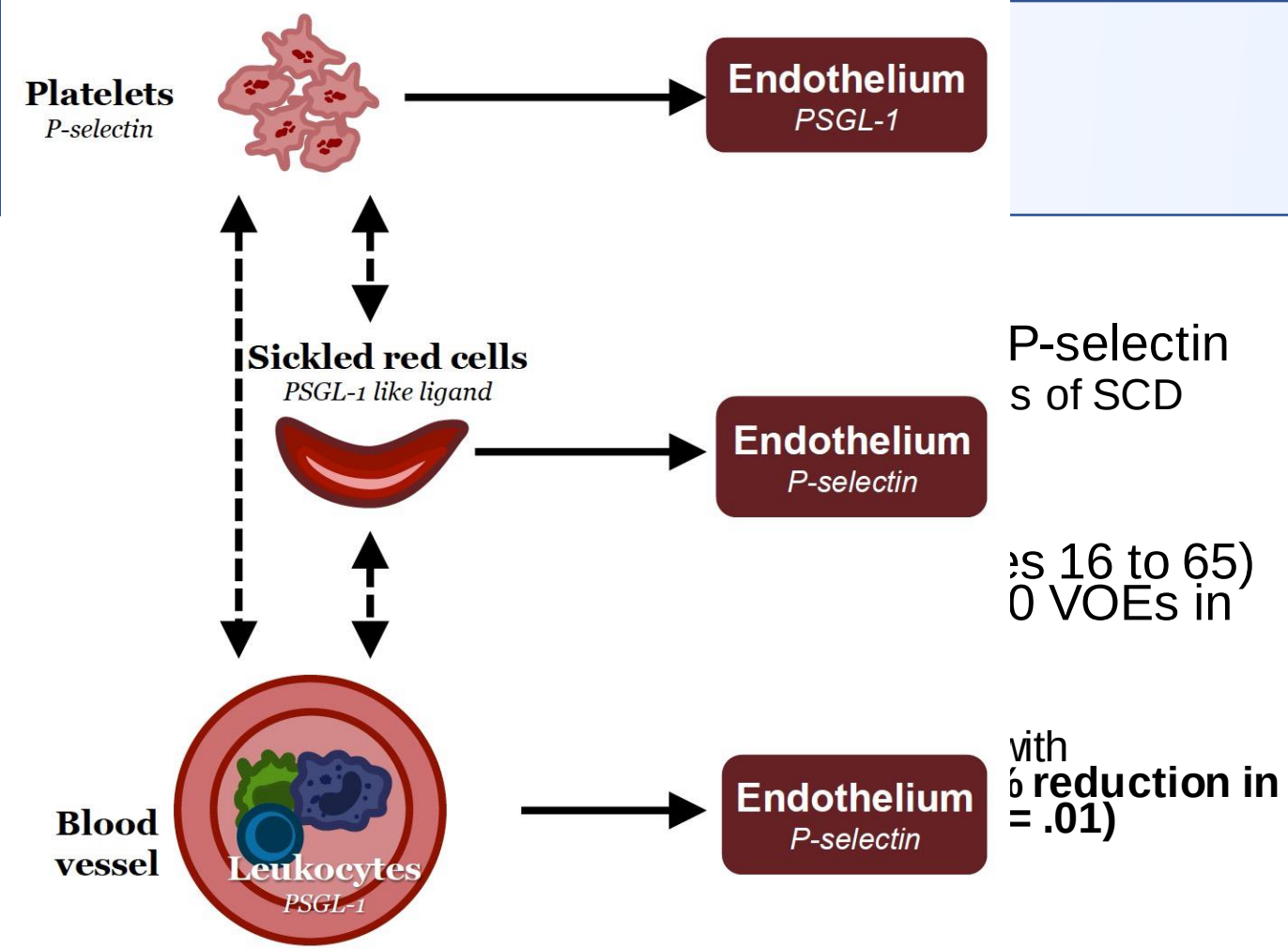
	Hydroxyurea	L-Glutamine	Crizanlizumab	Voxelotor
Age (years)	≥2	≥5	≥16	≥12
Genotypes	HbSS, HbSβ^0 thalassemia	All genotypes (only studied in HbSS, HbSβ^0 thalassemia)	All genotypes	All genotypes
Mechanism of action	Multiple, but primarily by increasing HbF production	Uncertain, but thought to reduce NAD redox potential, possible decrease in cell adhesion	Anti P-selectin inhibitor (decreases adhesion of WBCs and RBCs to endothelium)	Decreases HbS polymerization by increasing Hb–oxygen affinity
Route of administration	Oral (capsules/tablets)	Oral (powder)	Intravenous	Oral (tablets)
Clinical effects of therapy	Decreased frequency of VOC, decreased frequency of ACS, decreased hospitalization, decreased RBC transfusion requirement, decreased stroke risk	Decreased frequency of VOC, decreased frequency of ACS, decreased hospitalization	Decreased frequency of VOC	Increased hemoglobin

Rai P, Ataga KI. *F1000Res*. 2020;9:F1000 Faculty Rev-592.

Hydroxyurea

- First FDA-approved medication for SCD
  - Has been shown to increase lifespan in adults
- Original studies (Charache et al. *NEJM* 1995) showed a decrease in VOC by 40% and average increase in hemoglobin by 0.6g/dL
- MSH study: VOC improved median, 4.5 vs. 2.5 crises per year, P < 0.001 for patients on HU and the median times to the first crisis increased to (3.0 vs. 1.5 months, *P*= 0.01)
  - Fewer patients assigned to hydroxyurea had chest syndrome (25 vs. 51, *P*< 0.001)
  - Fewer underwent transfusions (48 vs. 73, *P*= 0.001).
  - Max tolerated doses may not be necessary to achieve therapeutic effect
- Pediatric studies have proven similar safety and efficacy
- <u>Target population</u> – ALL HbSS and HbS-beta thalassemia patients starting at age 2

Charache S et al. N Engl J Med. 1995;332:1317-1322.
Heeney MM et al. Hematol Oncol Clin North Am. 2010;24:199-214.



P-selectin
s of SCD

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0 VOEs in

with
% reduction in
= .01)

- Target population – patients with vaso-occlusive phenotype (particularly patients with frequent VOE and acute chest syndrome) or patients who have continued severe complications despite HU or who cannot tolerate HU

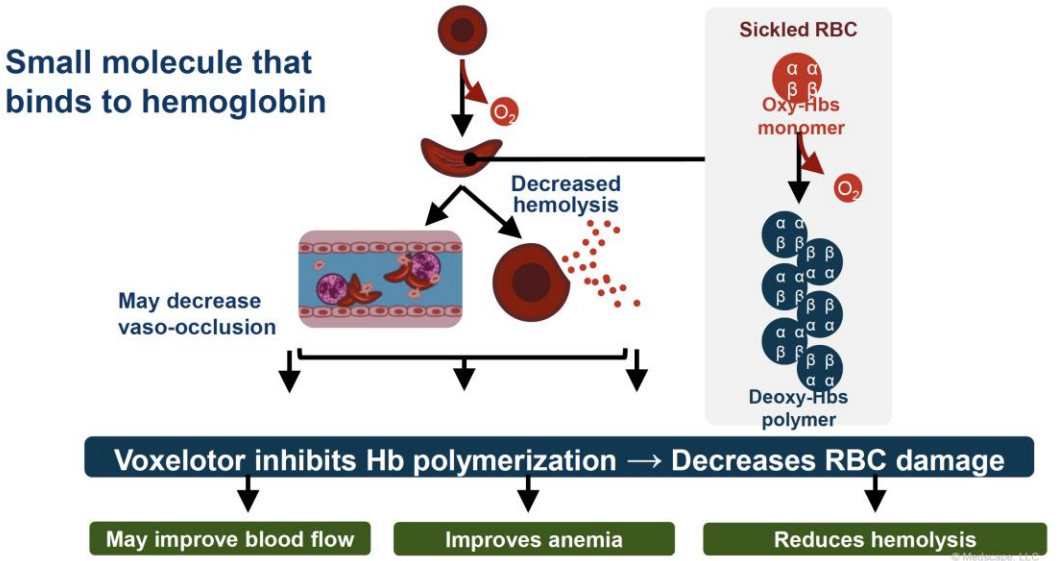
Ballas SK, Lusardi M. *Am J Hematol*. 2005;79:17-25
Wagner DD et al. *Blood*. 2008;111:5271-5281.3
Matsui NM et al. *Blood*. 2001;98:1955-1962
Ataga KI et al. *N EnglJ Med*. 2017;376:429-439
Kanter J et al. *Blood*. 2017.

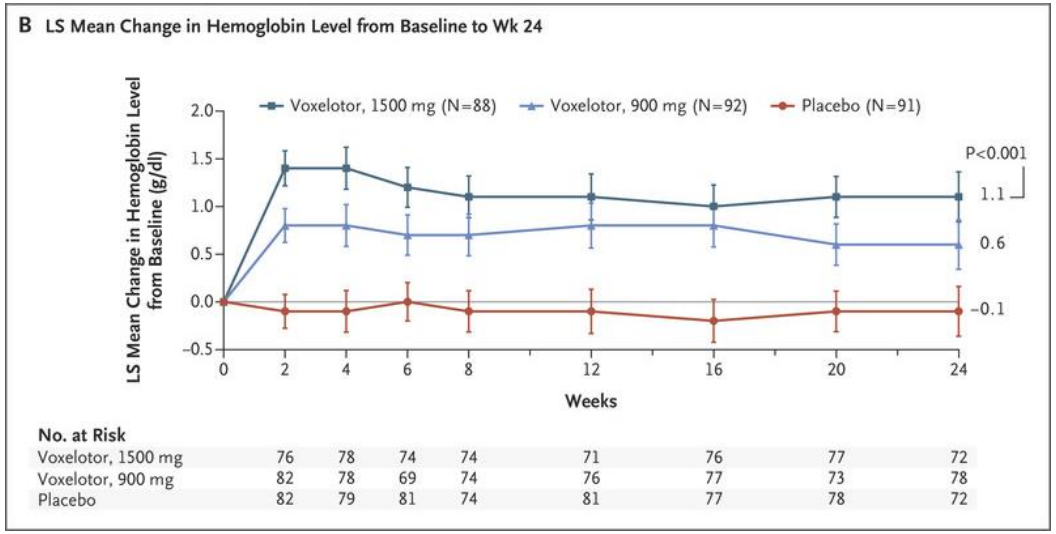
Voxelotor

- Deoxygenated HbS polymerization drives the molecular pathogenesis of sickle cell disease
  - Because the rate of HbS polymerization is extremely sensitive to deoxygenated HbS concentration, small changes in concentration can have substantial effects on polymerization
- Voxelotor is an HbS polymerization inhibitor that stabilized the high-oxygen affinity binding state of hemoglobin S

- HOPE trial (2009, NEJM)
  - Primary end point was the percentage of participants who had a hemoglobin response, which was defined as an increase of more than 1.0 g per deciliter from baseline at week 24 in the intention-to-treat analysis.
  - Significantly higher percentage of participants had a hemoglobin response in the 1500-mg voxelotor group (51%; 95% confidence interval [CI], 41 to 61) than in the placebo group (7%; 95% CI, 1 to 12).

- <u>Target population</u> – SCD patients with CKD or ESRD, patients with low-normal baseline hemoglobins (Hgb 5.5-7.0)





Vickinsky et al. *NEJM* 2019

Ongoing studies and future treatments in SCD

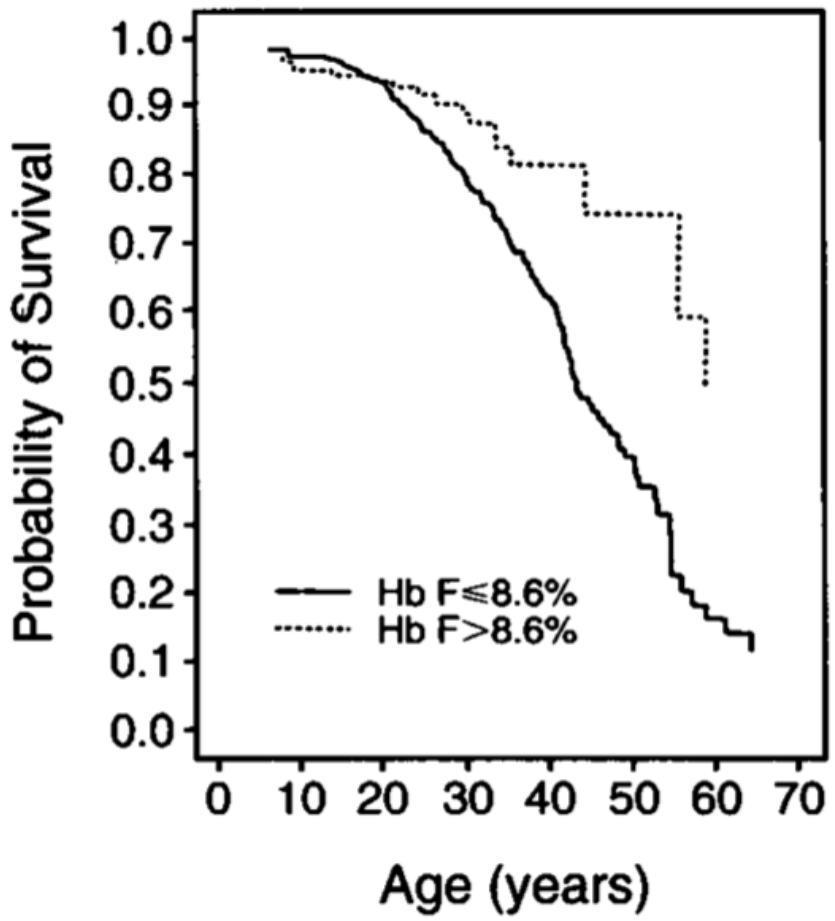
- Future therapies
  - Novel HbF inducers (heme-regulated inhibitor, HRI)
  - Gene therapy (Lentiglobin gene addition)

Mechanism	Drug	Sponsor	NCT number (study acronym)	Clinical phase/ status	Intervention	Number/age	Objective
HbF induction	Nicotinamide vs. THU and decitabine	EpiDestiny, Inc; NIH; NHLBI	NCT04055818	Phase I Recruiting	Oral nicotinamide vs. THU + decitabine for 12 weeks followed by combination for a further 12 weeks	20/≥18 years	Compare effect of oral nicotinamide vs. THU-decitabine and in combination on hemoglobin level at week 12
	Panobinostat (LBH589)	Abdullah Kutlar	NCT01245179	Phase I Active, not recruiting	Oral, for 12 weeks	18/≥18 years	Evaluate safety, HbF, F cells, total hemoglobin, markers of inflammation, QOL measures
	Metformin	Baylor College of Medicine	NCT02981329 (FITMet)	Phase I Recruiting	Hydroxyurea + metformin vs. metformin	56/10–60 years	Change in HbF or total hemoglobin, QOL, RNA sequencing
Allosteric modifier (to the R-state)	Voxelotor (formerly GBT440)	Global blood therapeutics	NCT03943615 (expanded access)	Approved for marketing	Oral	≥12 years	To provide early access to patients without alternative treatment options
			NCT04247594	Phase II Recruiting	Oral, open-label	45/18–60 years	Dose escalation study to evaluate safety and tolerability of doses, 1,500 mg to 3,000 mg daily
			NCT02850406	Phase IIa Recruiting	Part A: single dose Part B: 24 weeks Part C: 48 weeks	125/4–17 years	Pharmacokinetics, change in hemoglobin, effect on hemolysis, TCD velocity, safety
			NCT03573882	Phase III Active, not recruiting	Oral, daily	179/≥12 years	Open-label extension study, adverse events, frequency of SCD-related complications
			NCT04218084 (HOPE kids 2)	Phase III Not yet recruiting	Oral voxelotor vs. placebo	224/2–14 years	Evaluate effect on TCD in children
			NCT04188509	Phase III Enrolling by invitation	Oral, open-label	50/4–18 years	Evaluate safety and tolerability, SCD-related complications
Allosteric activator of RBC pyruvate kinase-R	AG-348 (Mitapivat sulfate)	NHLBI	NCT04000165	Phase I Recruiting	Oral, with two dose escalations after 2 weeks	25/≥18 years	Safety and tolerability, pharmacokinetics, and pharmacodynamics
	FT-4202	Forma therapeutics	NCT03815695	Phase I Recruiting	Single and multiple ascending oral doses of FT-4202 vs. placebo	130/12–60 years	Safety, pharmacokinetics, pharmacodynamics
RBC ion transport channels	SCD-101	Invenux	NCT02380079	Phase Ib Recruiting	Part A: open-label, dose-escalation study Part B: randomized, placebo-controlled, cross-over study	60/18–55 years	Safety, change in hemoglobin, markers of hemolysis, QOL measures, functional capacity
	Memantine (NMDAR antagonist)	HaEmek Medical Center, Israel	NCT03247218	Phase IIa/b Recruiting	Oral, once daily for 1 year	40/≥10years	Safety, frequency of hospitalizations, duration of hospitalizations, analgesic use, transfusion requirement, QOL measures

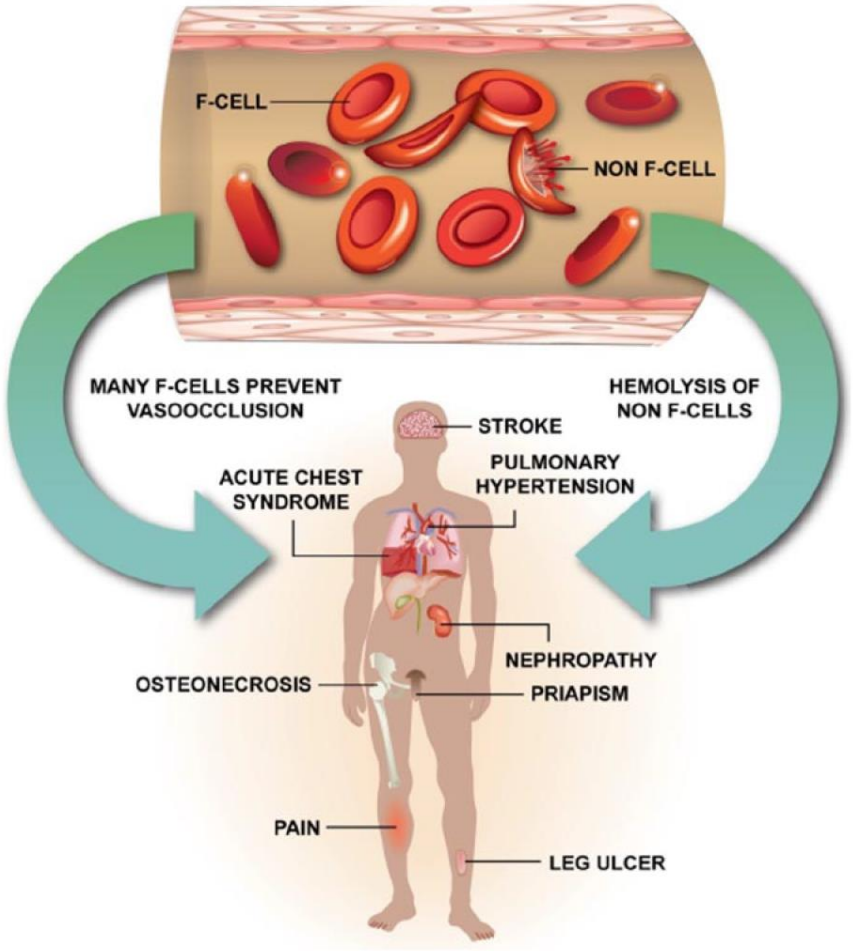
Mechanism	Drug	Sponsor	NCT number (study acronym)	Clinical phase/status	Intervention	Number/age	Objective
P-selectin antagonist	Crizanlizumab	Novartis Pharmaceuticals	NCT03264989 (SOLACE-adults)	Phase II Active, not recruiting	IV infusion, open-label	57/16–70 years	Pharmacokinetics, pharmacodynamics, safety, and efficacy
			NCT03814746 (STAND)	Phase III Recruiting	IV infusion every 2 weeks for 1st month and then monthly for 1 year	240/≥12 years	Compare efficacy and safety of 5 mg/kg and 7.5 mg/kg doses with placebo
			NCT04053764 (STEADFAST)	Phase II Recruiting	IV infusion every 2 weeks for 1st month and then every 4 weeks for 51 weeks + SoC vs. SoC alone	170/≥16 years	Evaluating effect on kidney function (albumin-creatinine ratio, protein-creatinine ratio, estimated glomerular filtration rate)
			NCT03938454 (SPARTAN)	Phase II Recruiting	IV infusion every 2 weeks for 1st month and then every 4 weeks x 51 weeks	56/≥16 years	Evaluate efficacy in priapism, uncomplicated VOC events
			NCT03474965	Phase II Recruiting	IV infusion every 2 weeks for 1st month and then every 4 weeks	100/6 months–<18 years	Evaluate pharmacokinetics, pharmacodynamics, safety, and effect on VOC events
Blockade of fcγrIII receptors	IVIG	Albert Einstein College of Medicine	NCT01757418	Phase I–II Recruiting	Single dose of IVIG vs. placebo given within 24 hours of hospitalization	94/12–65 years	Length of VOC, total opioid use, time to end of VOC, *in vitro* adhesion studies
Antioxidant (increased glutathione)	NAC	Bloodworks	NCT01800526	Phase I/II Enrolling by invitation	IV or oral, NAC Part 1: two doses of IV infusion over 8 hours 4 weeks apart or oral NAC for 4 weeks Part 2 (during VOC): IV infusion every 6 hours for 5 days	20/≥18 years	Evaluate effect on vWF activity, measures of hemolysis and oxidation In Part 2, evaluate efficacy during VOC

Rai P, Ataga KI. *F1000Res*. 2020;9:F1000 Faculty Rev-592.

Persistent levels of fetal hemoglobin lead to improved outcomes in sickle cell disease

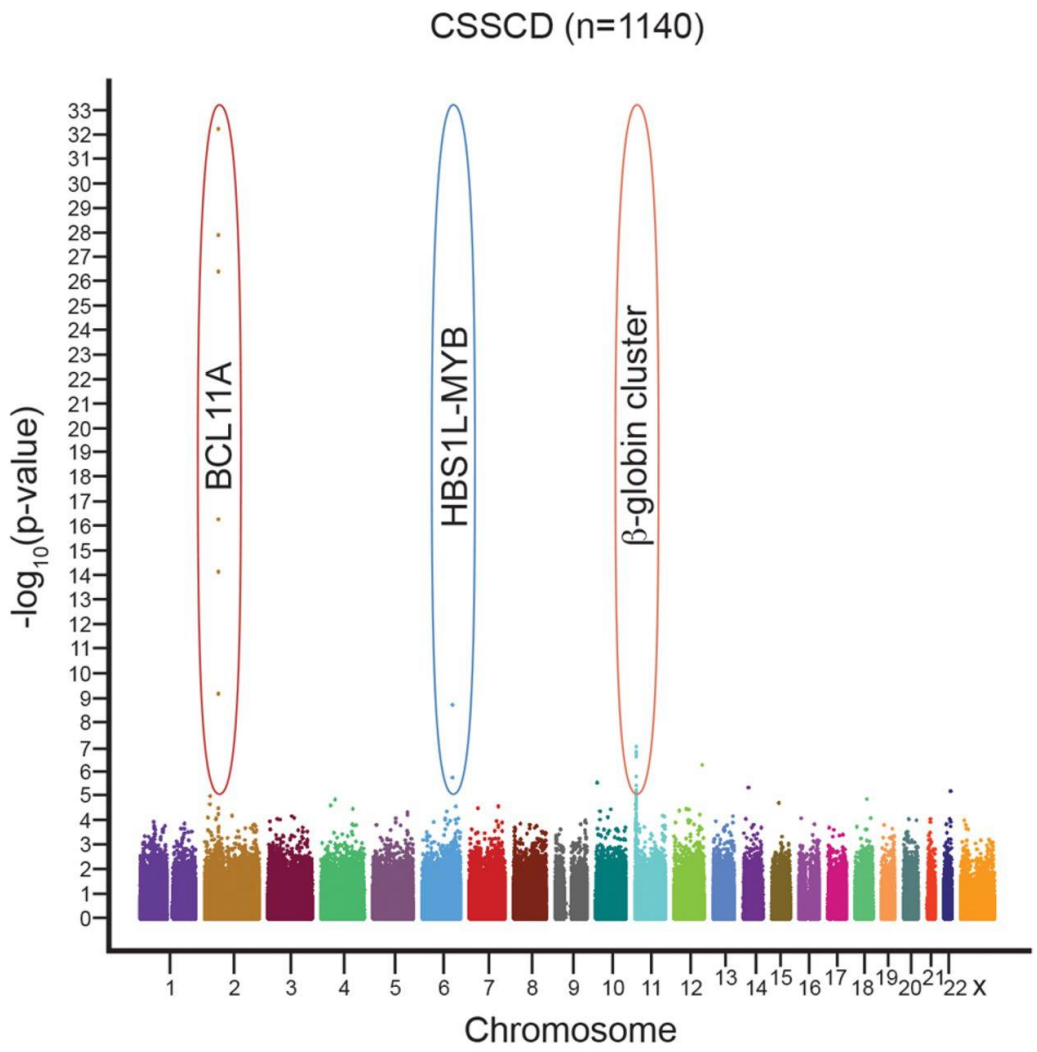


Platt et al. (1994)

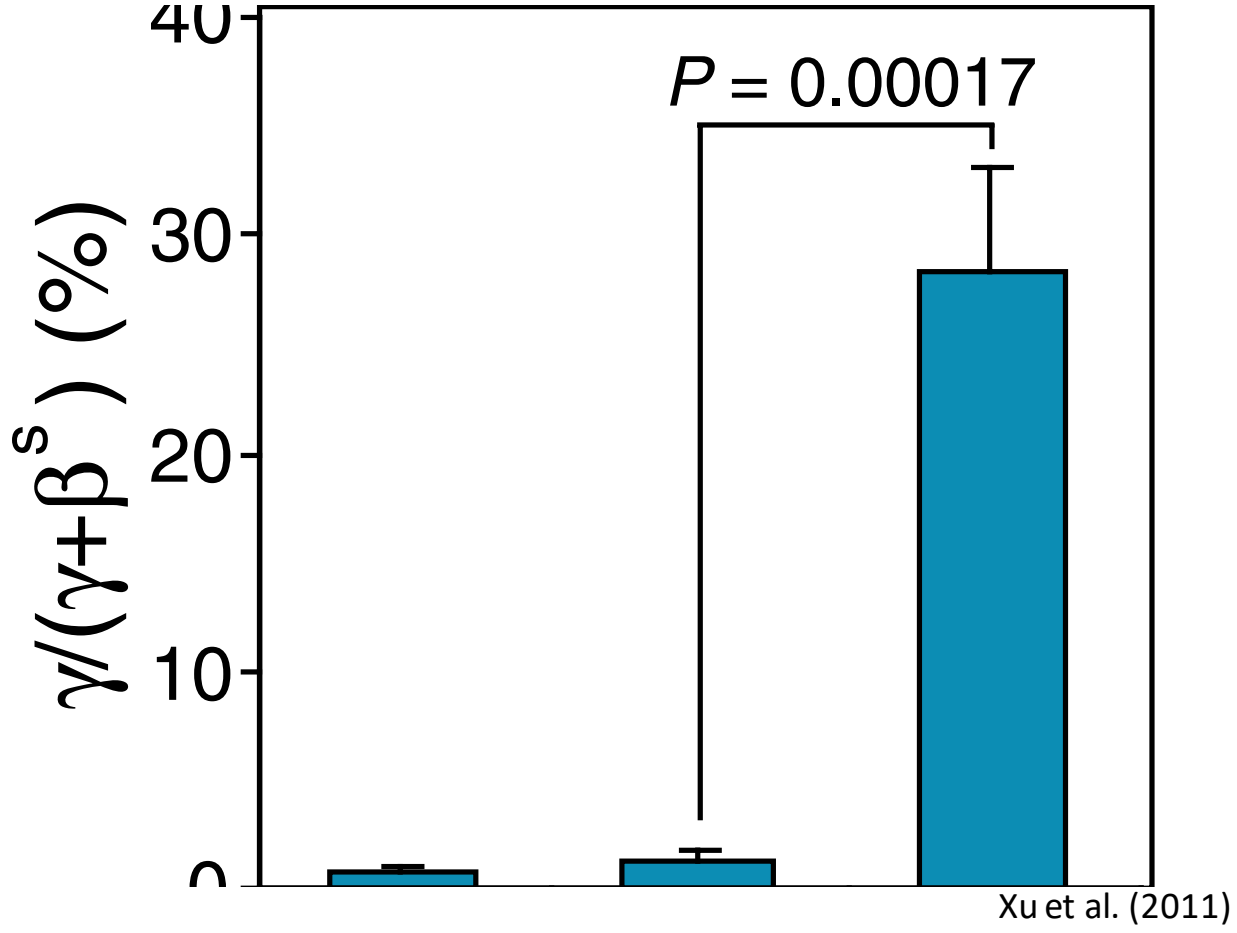


Akinsheye et al. (2011)

Bcl11A is a major repressor of fetal hemoglobin

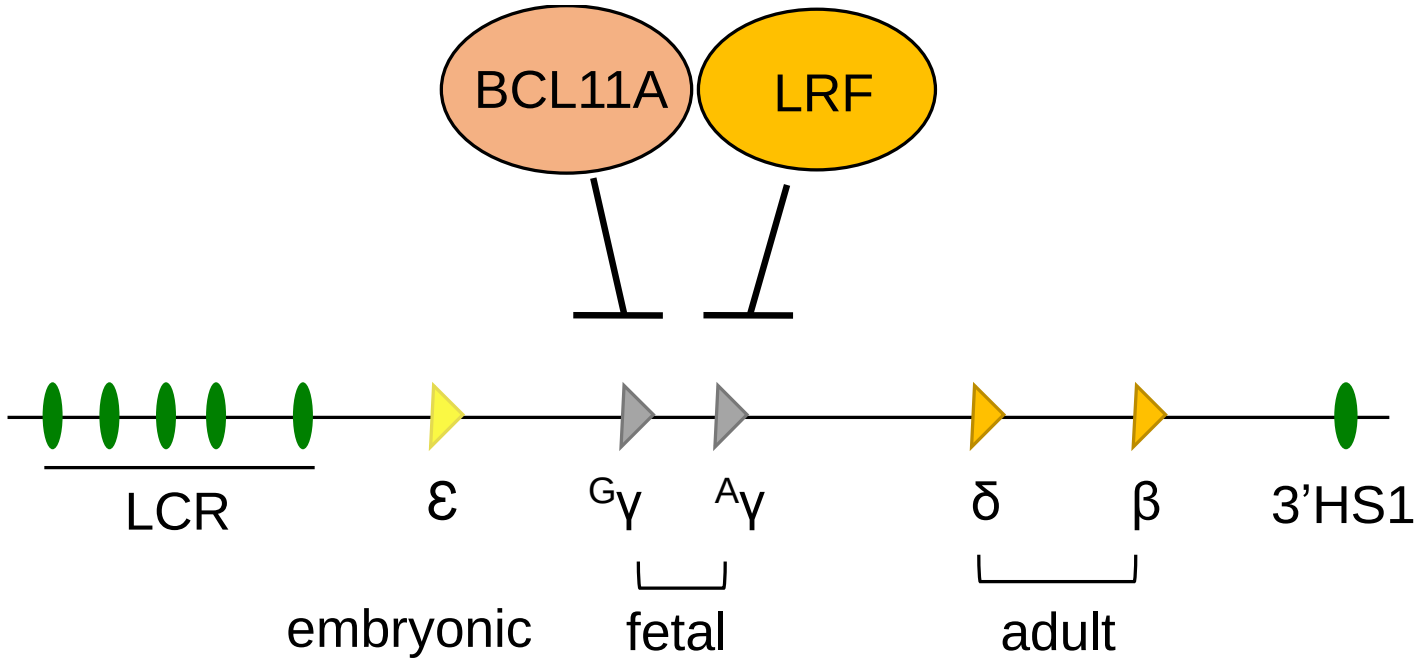


Bauer et al. (2012)



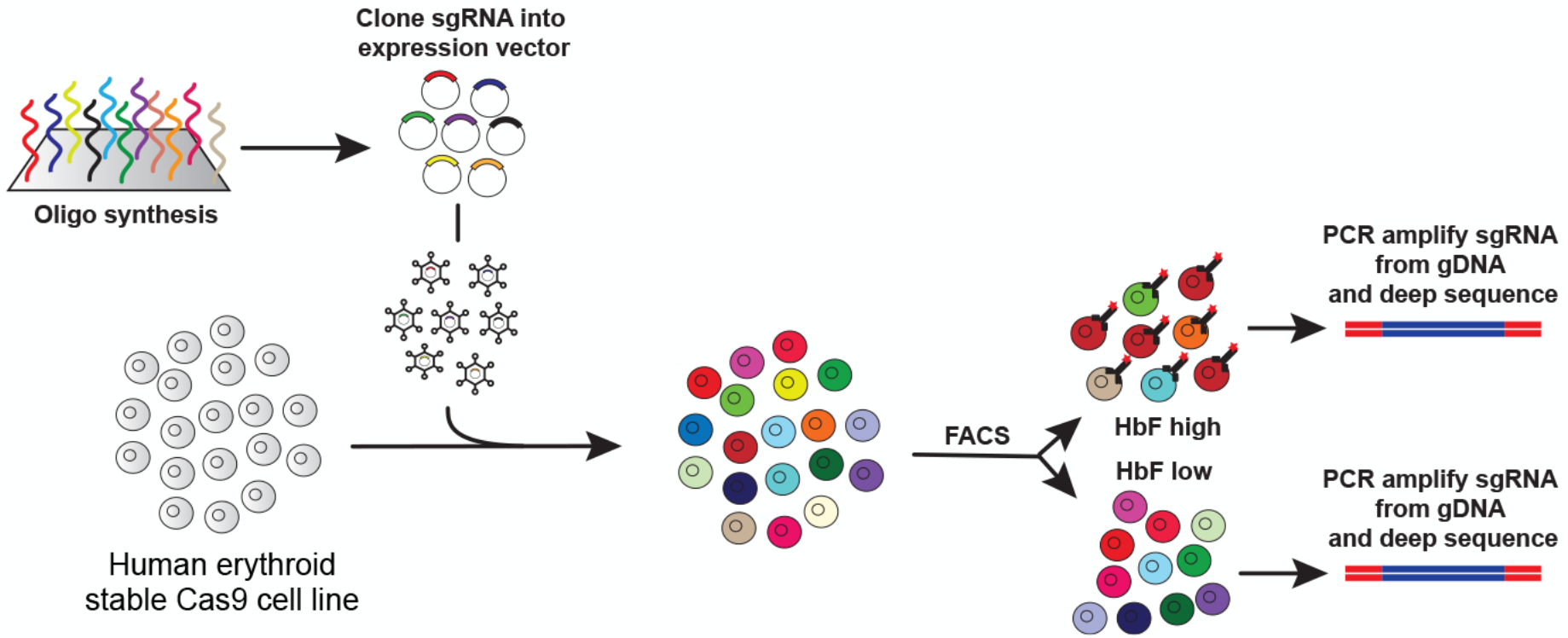
Xu et al. (2011)

Bcl11A and LRF are the major direct transcriptional repressors of HbF



- Knockout of LRF and BCL11A each result in ~60% HbF levels in vitro
- Double KO of LRF and BCL11A results in HbF levels of 91-94
  - Suggest that LRF and BCL11A silence HbF through distinct mechanisms
- However, BCL11A and LRF are transcription factors, <u>making targeting of these factors challenging therapeutically</u>

Identification of genes and associated protein domains involved in fetal globin repression via domain-targeted CRISPR screening



EIF2AK1 (HRI, heme-regulated inhibitor)

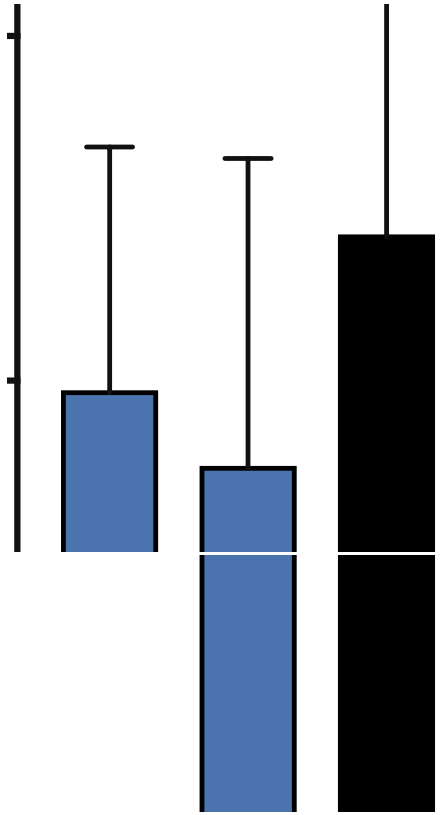
HRI is a novel regulator of fetal hemoglobin

Science

RED BLOOD CELLS

Domain-focused CRISPR screen identifies HRI as a fetal hemoglobin regulator in human erythroid cells

Jeremy D. Grevet[1,2*], Xianjiang Lan[1*], Nicole Hamagami[1], Christopher R. Edwards[1], Laavanya Sankaranarayanan[1], Xinjun Ji[2], Saurabh K. Bhardwaj[1], Carolyne J. Face[1], David F. Posocco[1], Osheiza Abdulmalik[1], Cheryl A. Keller[3], Belinda Giardine[3], Simone Sidoli[4], Ben A. Garcia[4], Stella T. Chou[1], Stephen A. Liebhaber[2], Ross C. Hardison[3], Junwei Shi[5†], Gerd A. Blobel[1,2†]



HRI is a potential drug target for raising fetal hemoglobin levels

- **HRI (heme-regulated inhibitor, EIF2AK1)** phosphorylates the translation initiation factor eIF2α
- HRI **regulates protein translation**, particularly during times of erythropoietic stress (JBC 2000, 2008)
- HRI is an **erythroid specific kinase** (GTEx, BloodSpot)
- However, maximal HbF induction by HRI knockdown requires **85%-90% HRI depletion**, which is challenging therapeutically

Hydroxyurea, pomalidomide, and UNC0638 induce HbF production

- **Hydroxyurea**
  - Multifactorial mechanism of HbF induction
  - Only FDA-approved therapy to raise HbF levels

Charache et al. (1995)

- **Pomalidomide**
  - Third-generation imide
  - Decreases levels of BCL11A and other regulators

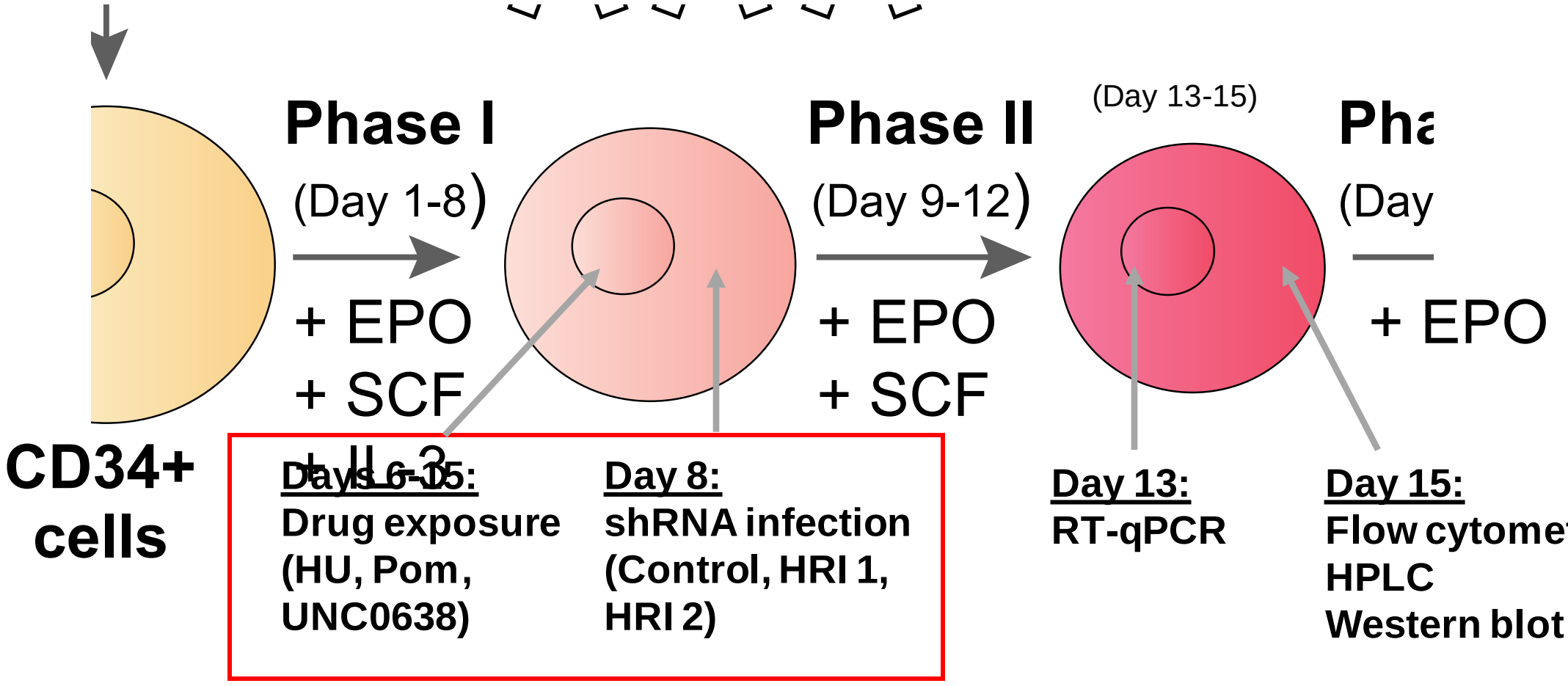
Moutouh-de Parseval et al. (2008)
Dulmovits et al. (2016)

- **UNC0638**
  - G9a/EHMT 1/2 methyltransferase inhibitor
  - Epigenetic de-repression of gamma-globin gene region

Renneville et al. (2015)
Krivega et al. (2015)

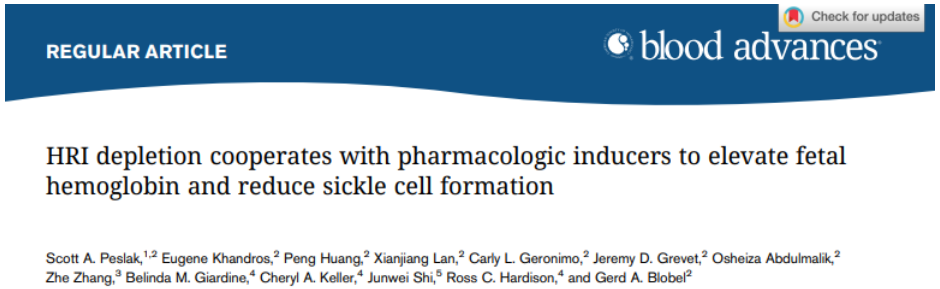
CD34+ Primary Human Culture
PBMCs

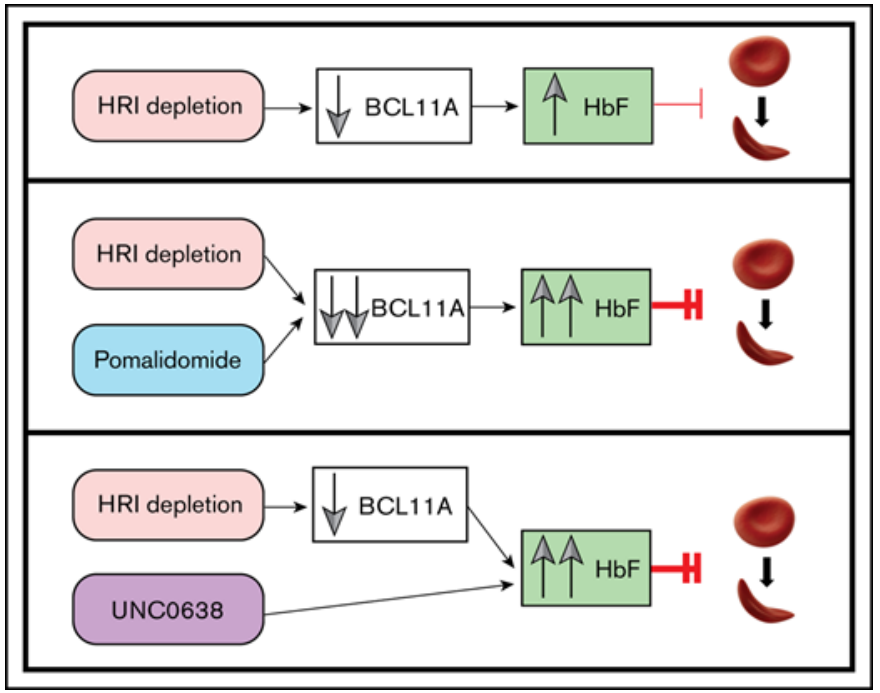
Combined HRI knockdown and HbF pharmacologic induction



HbF Combinatorial Induction

- Maximal HbF induction by HRI knockdown requires 85%-90% HRI depletion
- HRI depletion cooperates with pomalidomide and UNC0638 to markedly increase HbF with relatively minimal effects on erythroid maturation or the erythroid transcriptome
- Bcl11A plays a key role in the cooperativity between HRI knockdown and pomalidomide but not UNC0638
- HRI-mediated HbF regulation is species-specific and primarily mediated by ATF4/BCL11A interactions at the +55 BCL11A enhancer
- Combination of HRI knockdown and pomalidomide or UNC0638 significantly reduces in vitro sickling
- Future small molecule inhibition of HRI may be combined with other pharmacotherapies to increase HbF and reduce sickling





Recommended and future treatments in SCD

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  - PenVK, pneumococcal vaccination, malaria prophylaxis
- Stroke prevention
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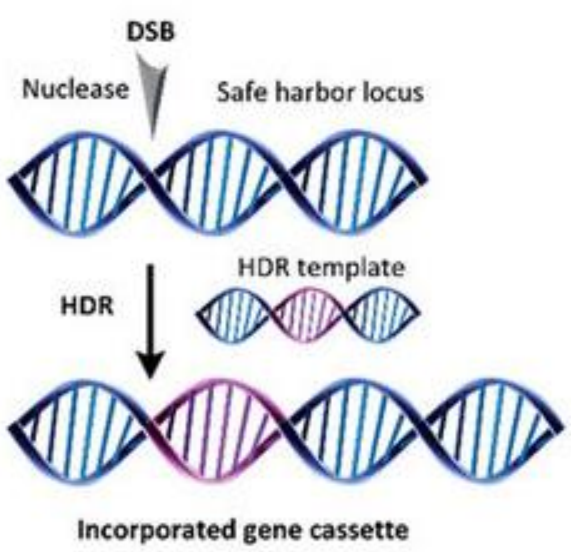
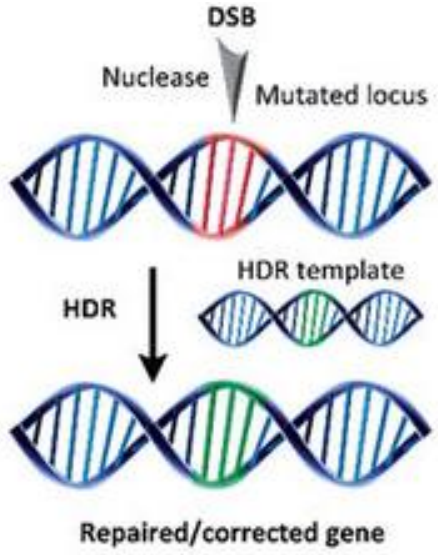
- Future therapies
  - Novel HbF inducers (heme-regulated inhibitor, HRI)
  - **Gene therapy (Lentiglobin gene addition)**

Gene addition



- Lentiglobin (Bluebird Bio) – addition of HbA^{T87Q} beta-globin locus
- MOMENTUM (ARU-1801, Cincinnati) – addition of G16D-mutated gamma-globin locus BCL11A
- shMiR Trial (Boston) – addition of miRNA-directed erythroid-specific shRNA-mediated knockdown of gamma-globin repressor BCL11A

Gene editing



- CLIMB-121 (CTX001, Vertex) – BCL11A erythroid-specific enhancer editing

- Not yet precise enough to specifically fix SCD mutation (E6V), although base editors and PRIME editing pending
  - Anzalone et al. 2019 *Nature* (David Liu group) - targeted insertions, deletions, and all 12 types of point mutation, without requiring double-strand breaks or donor DNA templates.

HGB-206: Study of LentiGlobin gene therapy for severe sickle cell disease (SCD)

HGB-206

Key Enrollment Criteria

- 18+ years of age
- History of symptomatic SCD
- Adequate organ function
- No previous HSCT or gene therapy

Target enrollment: up to 29

Study Objectives

- Primary objective: Safety
- Key Secondary Objectives:
  - Frequency of VOCs and ACS
  - HbA^{T87Q} production
  - Total Hb and Hb fractions
  - Vector copies in peripheral blood

Study initiated August 2014

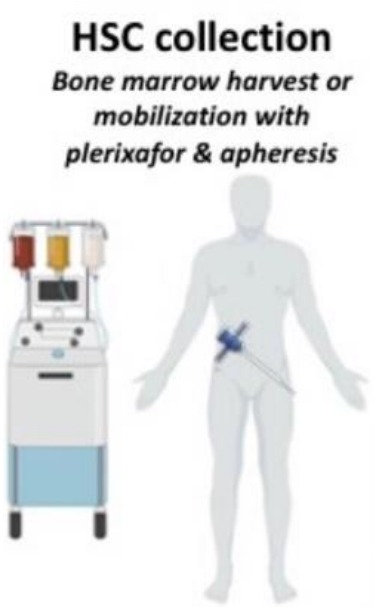
LentiGlobin gene therapy overview in patients with SCD

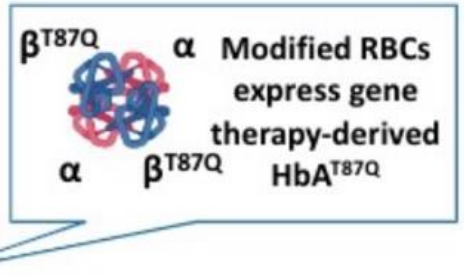
HSC collection
Bone marrow harvest or mobilization with plerixafor & apheresis

Busulfan myeloablative conditioning

DP infusion

Transduced HSCs engraft and contribute to reconstitution of functional RBCs





2-yr follow-up

Long-Term Follow-Up Study

	Group A	Group B	Group C
Pre-collection transfusion regimen	Optional	Required	Required
HSC source	Bone marrow	Bone marrow	Mobilized PB
Manufacturing process	Original	Orig → Refined	Refined

LentiGlobin DP centralized manufacturing

Select CD34+ cells → *Transduce with BB305 lentiviral vector* → *Cryopreserve, test, release DP*

DP, drug product; HSCs, hematopoietic stem cells, PB, peripheral blood; RBC, red blood cell

HGB-206 Group C: Patient characteristics

N=19 patients who started cell collection

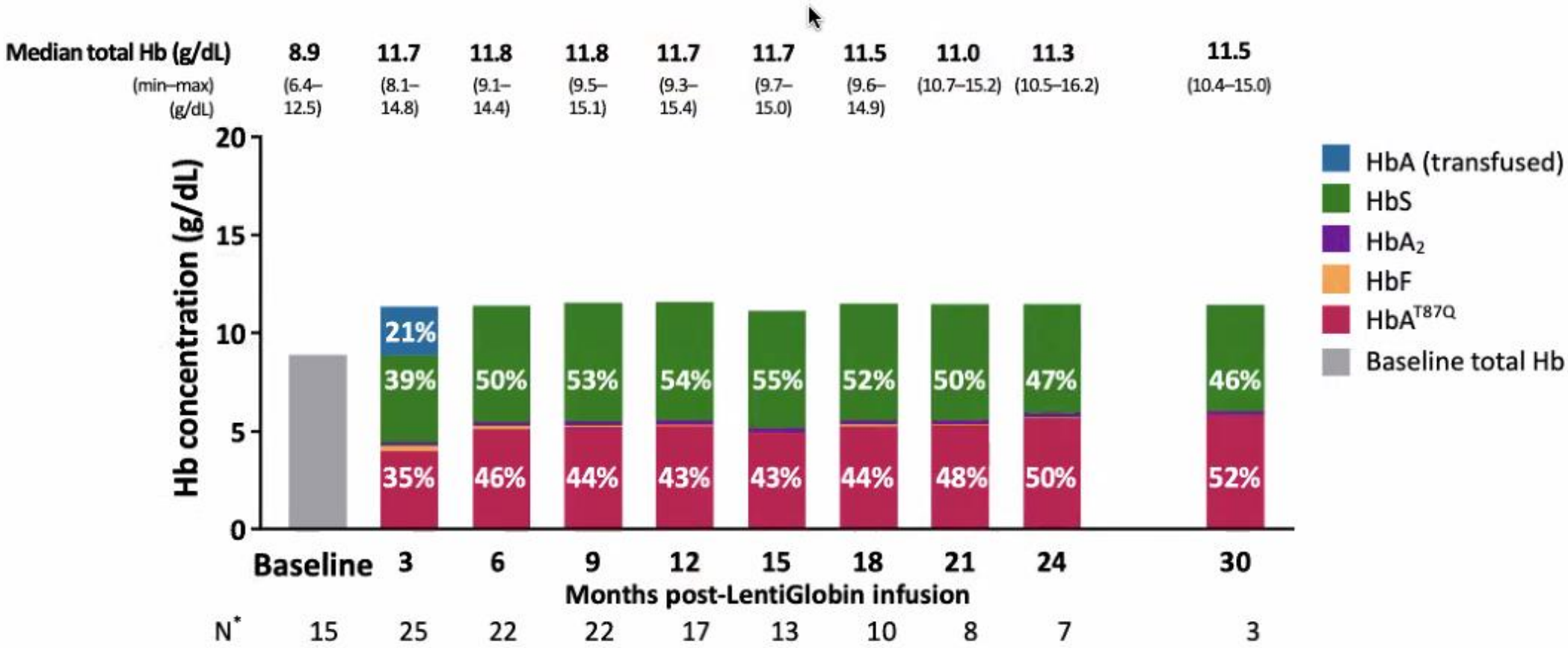
Parameter	Group C N=19
Age at consent, years median (min – max)	**26** (18 – 36)
Gender	**8F 11 M**
Genotype, β^S/β^S	**19**
SCD History	
Hydroxyurea[#], n	**11**
VOCs[*], n Annualized no. of events, median (min – max)	**15** **4.0** (2.0 – 13.5)
ACS[†], n Annualized no. of events, median (min – max)	**2** **1** (1 – 1)
Stroke, n	**3**
TRJV > 2.5 m/s, n	**1**

[*]≥ 2 events/year in preceding 2 years; [†]≥ 2 episodes in preceding 2 years, with ≥ 1 episode in the past year or in the year prior to the initiation of regular transfusions;
[#]Within 30 days prior to informed consent

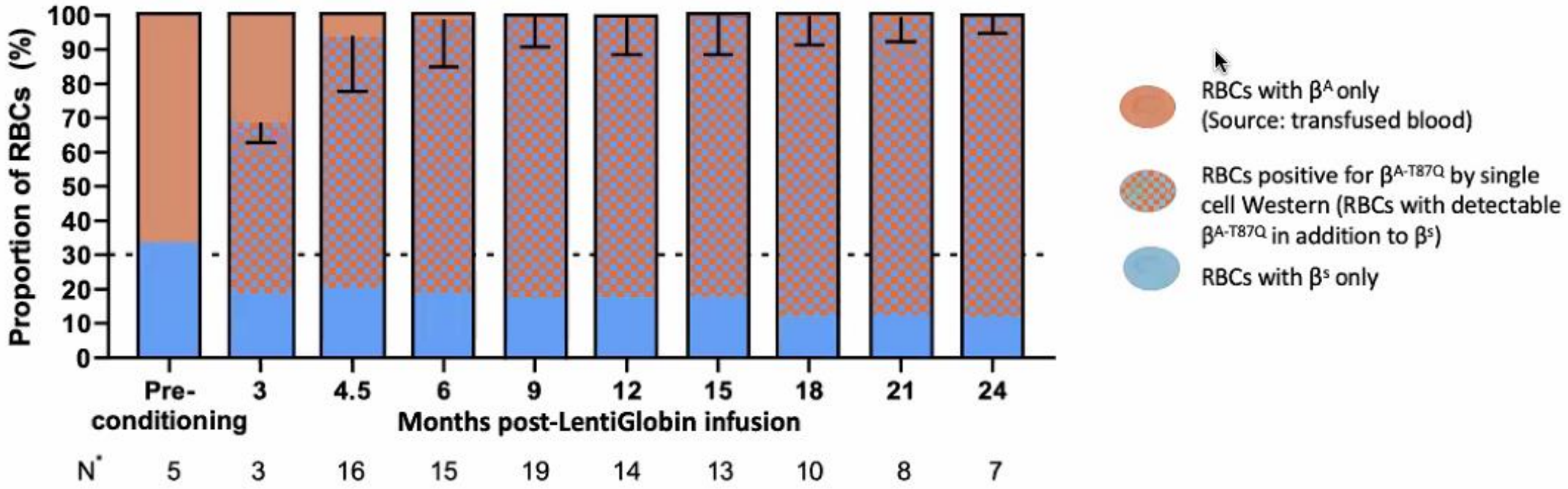
Definitions: ACS, acute chest syndrome; F, female; M, male; TRJV, tricuspid regurgitant jet velocity; VOC, vaso-occlusive crisis

Data as of 7 March 2019

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

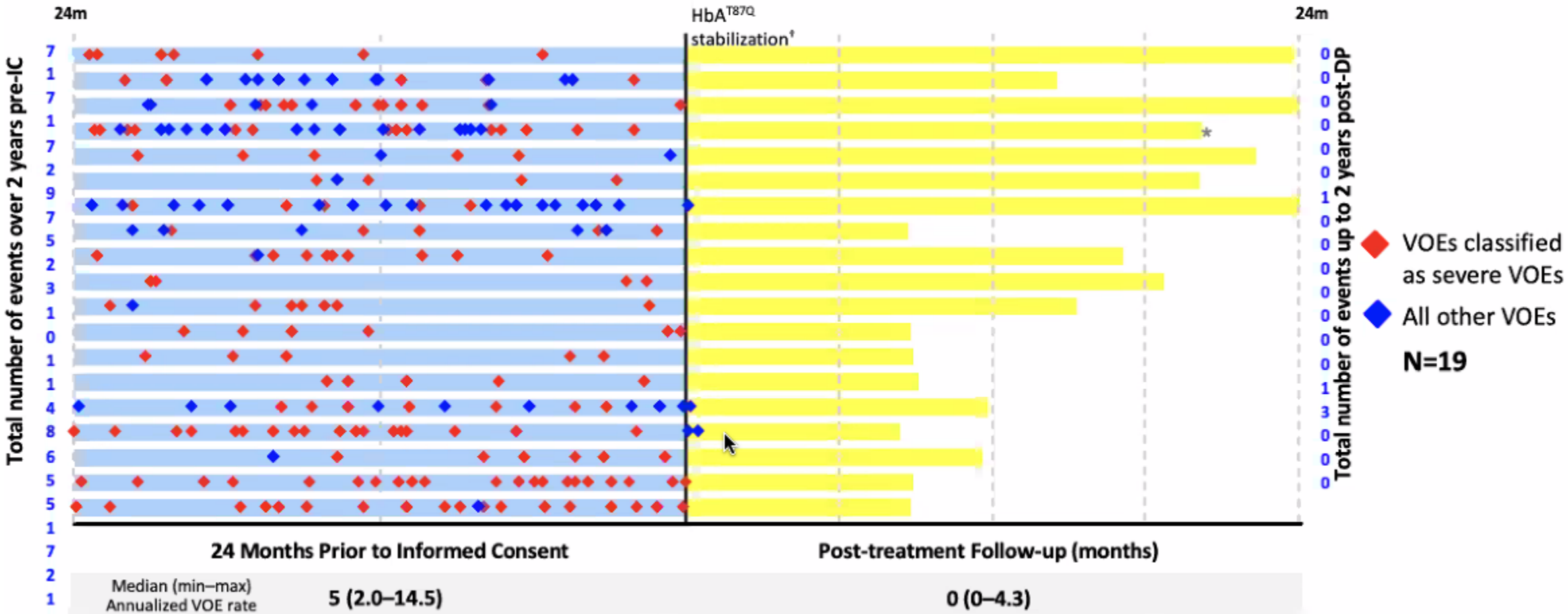


HGB-206 Group C: Near pancellular expression of HbA^{T87Q} ≥ 6 months post-treatment



- 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§

HGB-206 Group C: Complete resolution of severe and other VOEs ≥6 months post-treatment



A second case of AML in Group A results in a pause in gene therapy trials

	Patient 1303	Patient 1301
Baseline	No mutations or cytogenetic abnormalities (microarray, NGS)	No mutations or cytogenetic abnormalities (microarray, NGS)
Post-treatment prior to AML diagnosis		No mutations M3, M6, M18, M24 (microarray, NGS)
At AML diagnosis	• **Monosomy 7** • Abnormal 19p • **RUNX1** (NP_001745.2:p.Asp198Gly), • **PTPN11** (NP_002825.3:p.Phe71Leu), • **KRAS** NP_203524.1:p.Gly12Ala	• **Monosomy 7** • Partial loss of 11p • **RUNX1** Exon 5 stop gained p.A149*fs • **PTPN11** Exon 3 missense: p.A72V
Vector in blasts?	No	Yes; insertion in 4th intron of VAMP4

MDS/AML documented in patients following transplantation with different conditioning regimens and with different donor sources in SCD

	Matched 0170 TBI/Campath	Matched 0077 TBI/Campath Pentostatin/Cy	Matched (Chicago, Riyadh) TBI/Campath	Haplo 0225 TBI/Campath -/+ Cy	Haplo 0069 TBI/Campath/Cy Pentostatin/Cy	Eapan CIBMTR Many types	Gene therapy SCD Busulfan	Gene therapy thal
N in study	58	26	64	21	19	900	47	63
MDS, AML	2 (3.5%)	1 (3.8%)	1 (1.6%)	3 (14%)*	0	6**(0.7%)	2(4.3%)	0
	1 graft failure (MDS)	1 low engraft (AML)	1 graft failure (MDS)	2 graft failure MDS, AML		Details not available	2 in Group A	

- MDS/AML more common in those with graft failure
- Improved engraftment in NIH haplo trial appears to have improved risk of MDS/AML
- Median follow up not sufficient to be conclusive

* Two patients with preexisting TP53 mutations

**Overlap with NIH reporting in n=2

National Institutes of Health

SUMMARY: Vector insertion not likely contributing to leukemogenesis

✓ Classical driver alterations consistent with AML	Monosomy 7, partial loss of 11p, RUNX1, PTPN11
✓ No substantial change in gene expression around IS	No remarkable expression changes in 10 MB region around VAMP4 IS
✓ Vector is NOT transcriptionally active in tumor cells	Very low level HBB detected in CD34+ cells
✓ Transcriptional profile consistent with properties of known AML driver alterations	RNAseq data consistent with monosomy 7 and contains PTPN11 and RUNX1 mutations
✓ IS(s) found in other patients without sequelae	VAMP4 IS common and 1301 is only subject with VAMP4 IS >0.05% at any point
✓ IS(s) unremarkable with respect to cancer-associated genes	VAMP4 has no known association with cellular proliferation or oncogenesis
✓ IS(s) does not disrupt genomic elements	VAMP4 IS in subject 206-113-1301 does not disrupt mapped genomic features

Most likely cause of oncogenesis: Combination of underlying increased risk of hematologic malignancy in patients with SCD exacerbated by the replicative stress of autologous transplant and persistent hematopoietic stress due to uncorrected sickle cell disease

Learning Objectives

- **Primary goal** - To increase understanding of the identification, diagnosis, and current and emerging treatments of sickle cell disease via recognition of prior SCD treatment challenges, current novel effective therapies and management strategies, and future ongoing SCD-related trials.

- **Objectives**:
  1. Recognize the scientific and patient care-based challenges that SCD patients have faced in the past including lack of effective treatments, difficulties with transition to adult care, and lack of medical home.
  2. Identify recent advances in mechanistic understanding of the SCD that have led to several new approved therapies, as well as new advances in patient care such as multimodal care in the outpatient setting and defined care pathways in emergency and inpatient settings.
  3. Describe future SCD treatment approaches to gene therapy and drug discovery, as well as ways to integrate these treatments into multimodal care and education of SCD patients.

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