Current and Novel Preventive and Curative Interventions for the Treatment of Patients with Sickle Cell Disease

2018 AABB Annual meeting

Boston MA October 13

Topics and Speakers

- Medical Therapies
  - Samir K. Ballas MD
- Curative Therapies
  - Stem Cell Transplantation

    Lakshmanan Krishnamurti MD
    Professor of Pediatrics, Director BMT
    Aflac Cancer and Blood Disorders Center
    Emory University, Atlanta GE
  - Gene Therapy

    John F. Tisdale MD
    Senior Investigator
    Molecular and Clinical Hematology Branch, NHLBI

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Samir K. Ballas, MD, FACP, FASCP, DABPM, FAAPM

Emeritus Professor of Medicine and Pediatrics

Cardeza Foundation for Hematologic Research

Thomas Jefferson University

Philadelphia, PA USA

Medical Therapy Beyond Palliative Treatment

- Pharmacotherapy
- Transfusion Therapy

Pharmacotherapy

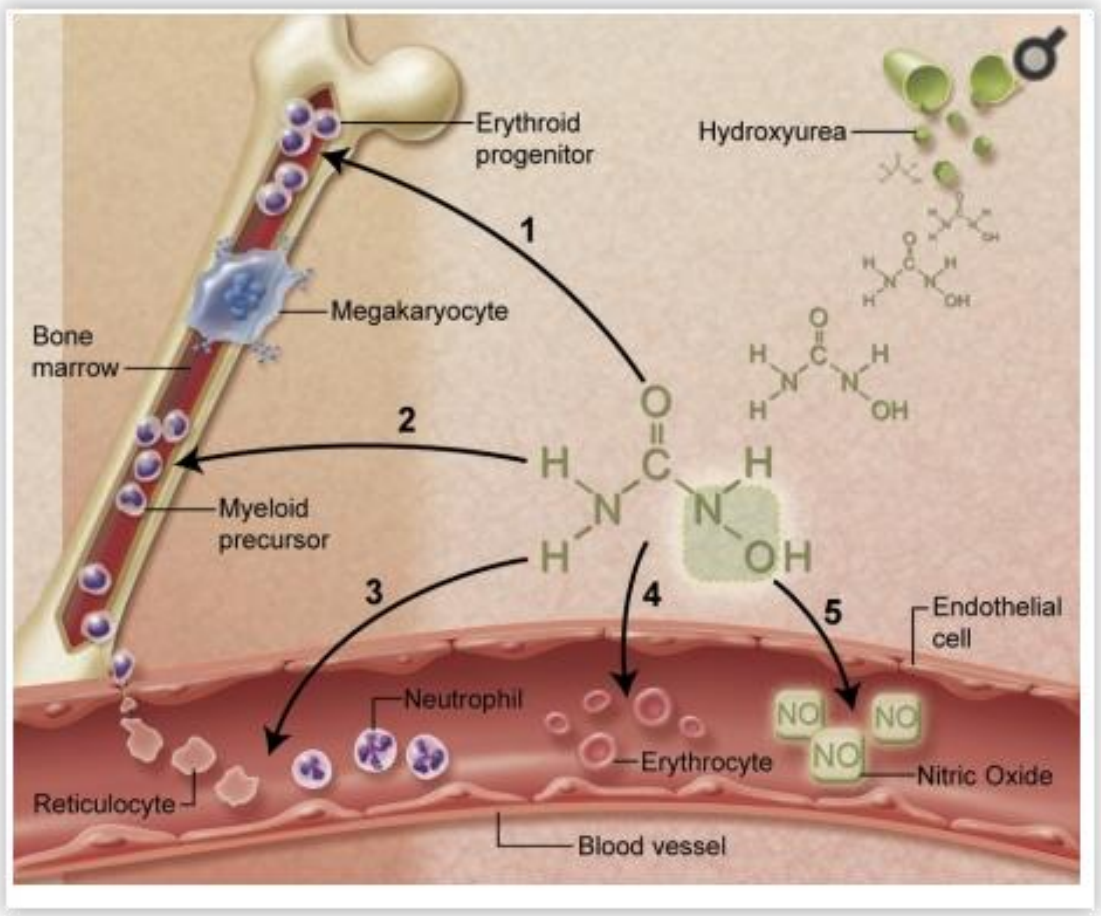
- **Preventive**
  - Agents that decrease the frequency of vaso-occlusive crises (VOCs), other complications and hospital admissions
- **Therapeutic**
  - Agents that abort or decrease the duration of VOCs, hospital days and the amount of analgesics used

Hydroxyurea (Hydroxycarbamide)
A long history for a reference product in SCD

	1869	**Hydroxyurea**: Synthesis of Hydroxylated analogue of urea
	1967	First indication in **Adult patients with myeloproliferation of cancerous disease** **500mg**
	1974	**Hemoglobin F (Hb F) inducer in SCD**
	1995	**MSH: Pivotal clinical trial showing efficacy in Adult SCD** + Several clinical trials in both children and adults
	1998	FDA Approval (Orphan drug) in **Adult SCD** **200, 300 & 400mg capsules**
	2007	European MA (Orphan drug) in both **Children and Adults with recurrent SCD VOCs** **1000mg & 100mg tablets**
	2017	FDA Approval (Orphan drug) for use in Pediatric patients with SCD **1000mg & 100mg tablets**

Medicine 1996; 75:300-26

Multiple beneficial effects of hydroxyurea for Sickle Cell Anemia (SCA)



1) Hb F Induction

2) Myelosuppression

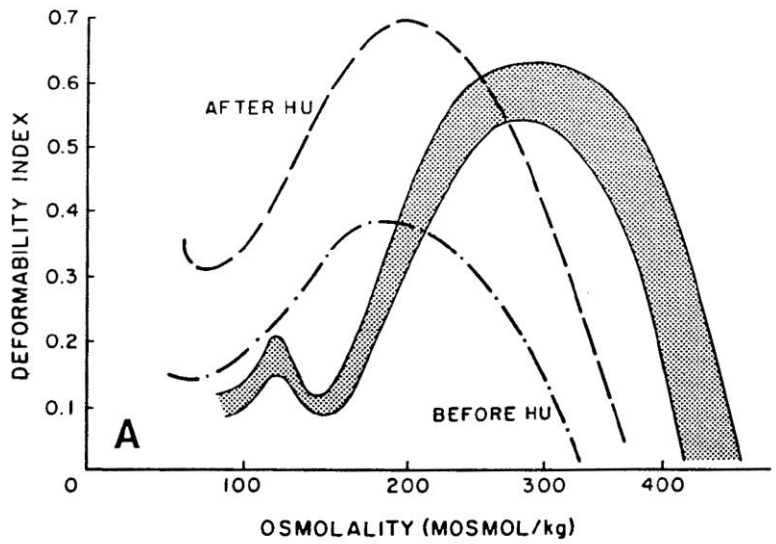
3) Less adhesion & Endothelial effects

4) Better rheology

5) Nitric Oxide

Blood 2010;115(26):5300-5311

Osmotic Deformability Profile Before and After Treatment with HU



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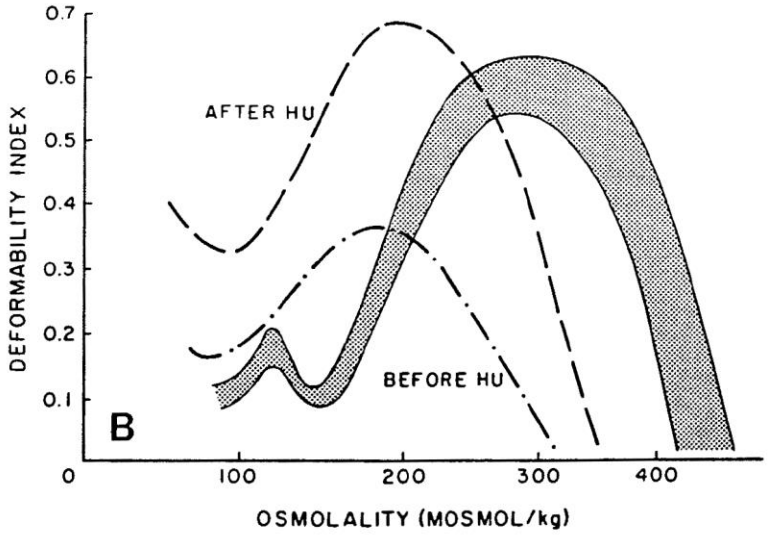

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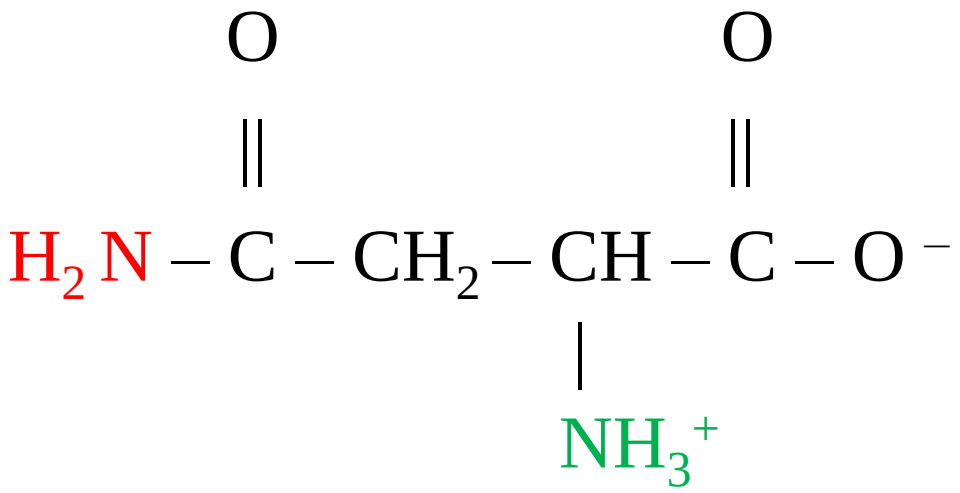
L-glutamine



Increases NADH levels with antioxidant activity

Pivotal Phase 3 Trial Design

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- ≥5 years old
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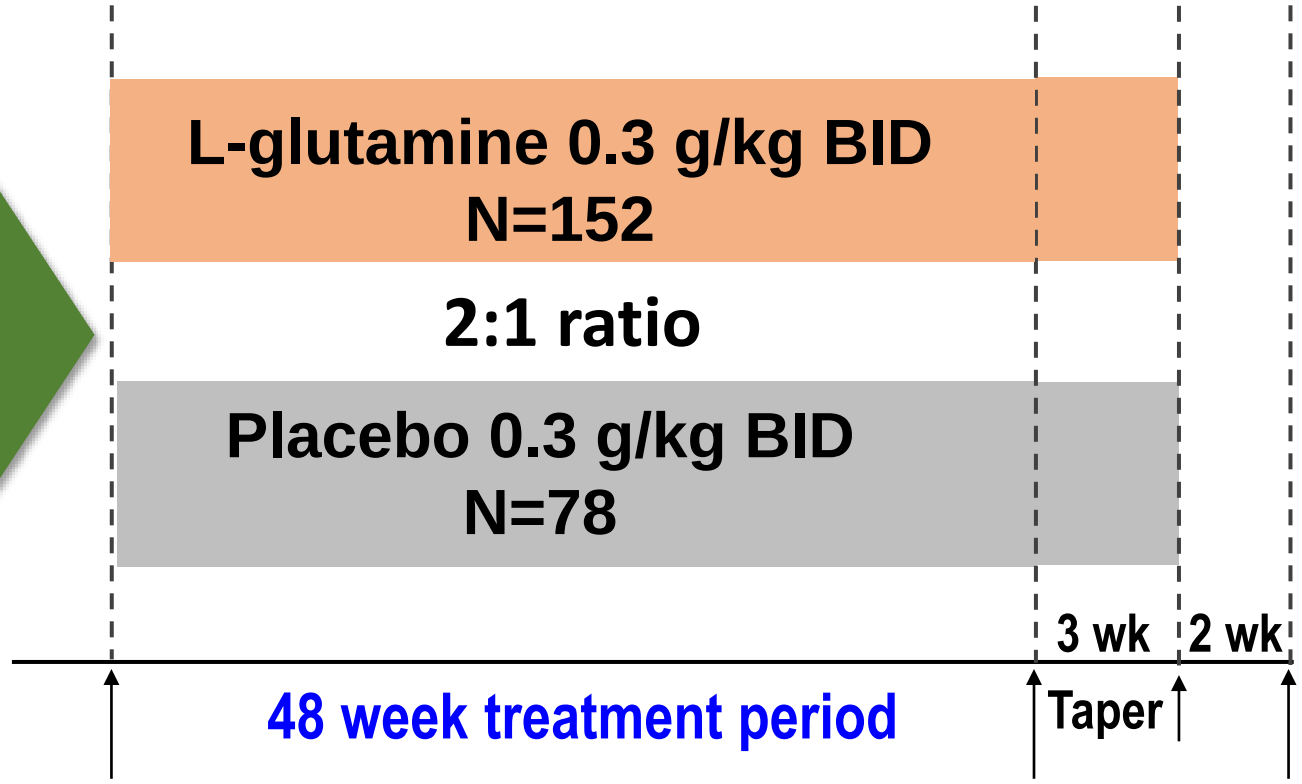
- HU use permitted
- Transfusions allowed



Phase 3, Placebo controlled, randomized, double-blind to evaluate difference between L-glutamine and placebo arms

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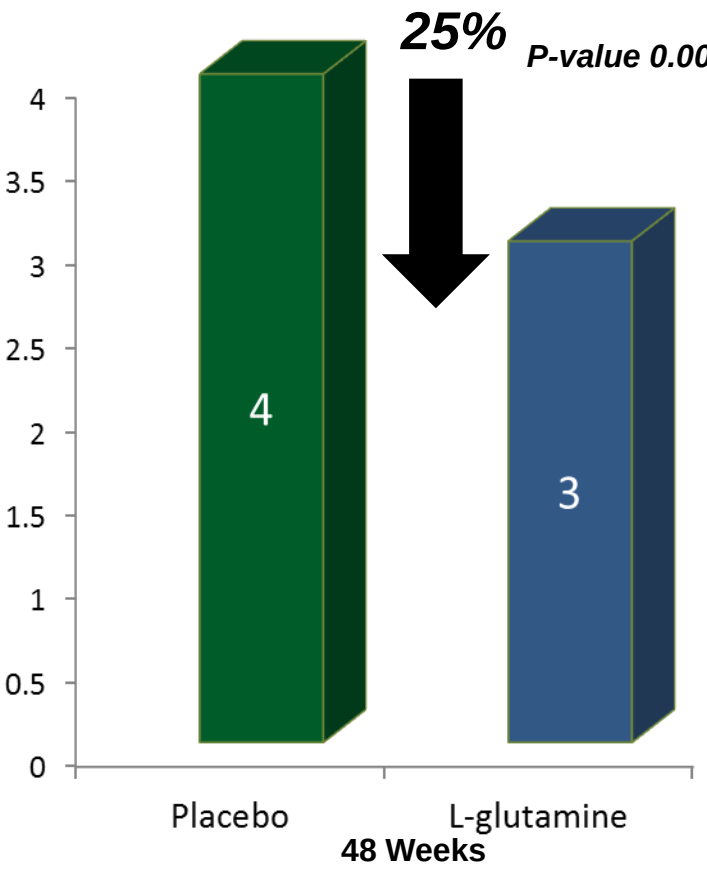
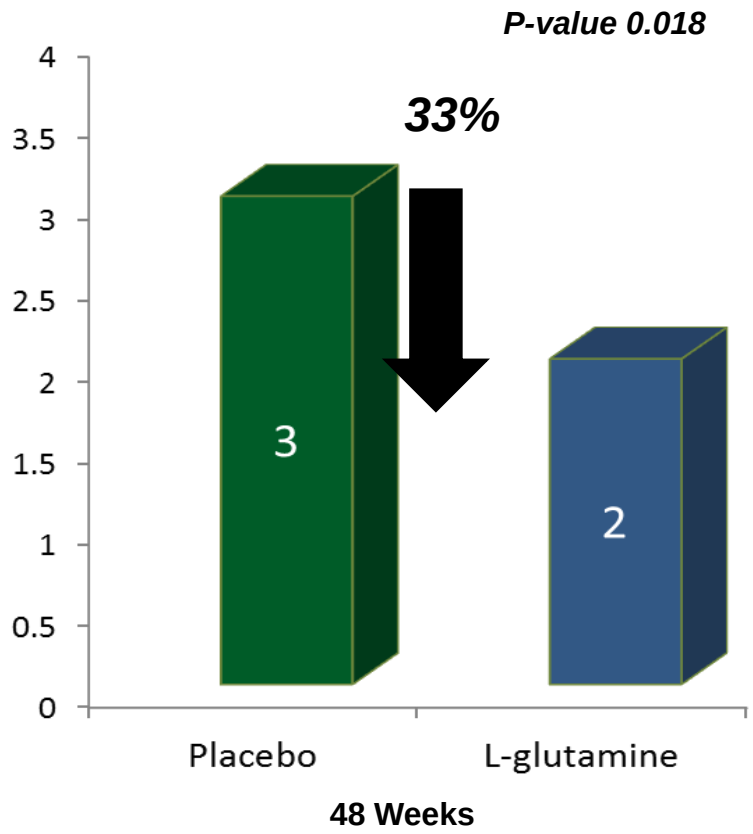
N Engl J Med. 2013;368:1489-97

Phase III Study Results
After 48 Weeks of Treatment

Median Frequency of Sickle Cell Painful Crises



Median Frequency of Hospitalizations



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***P-value per Cochran-Mantel-Haenszel controlling for hydroxyurea use only**

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Difference in Cumulative Days Hospitalized

Cumulative Days Hospitalized	L-glutamine N=152	Placebo N=78
Wilcoxon Rank Sum Test difference between the 2 treatment arms	p = 0.022	
Descriptive Statistics		
Mean (SD)	12.1 (16.6)	18.1 (27.4)
Median (min, max)	6.5 (0, 94)	11 (0, 187)

41% fewer days hospitalized

Observed Data

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Acute Chest Syndrome (ACS)	L-glutamine N=152	Placebo N=78
CMH analysis difference between the 2 treatment arms	p = 0.0028	
Descriptive Statistics		
Mean (SD)	0.1 (0.4)	0.3 (0.6)
Number of occurrences of acute chest syndrome, n (%)		
≥1	13 (8.6)	18 (23.1)
0	139 (91.4)	60 (76.9)
1	10 (6.6)	13 (16.7)
2	3 (2.0)	4 (5.1)
3	0	1 (1.3)

63% lower incidence of ACS

Observed Data

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Efficacy Evidence for L-glutamine

When compared with placebo:

- ✓ Lower frequency of VOCs
- ✓ Lower frequency of hospitalizations
- ✓ Longer time to first and second crisis
- ✓ Lower cumulative event rates*
- ✓ Lower occurrence of ACS
- ✓ Lower cumulative days in hospital
- ✓ L-glutamine +HU is more effective then HU

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Emerging Preventive & Therapeutic Approaches to Treat SCA - Pharmacotherapy

Hb F Induction	• Oral Decitabine & tetrahydrouridine • Pomalidomide
Inhibition of Hb S Polymerization	• Voxelotor (GBT-440) • SCD 101
Anti-oxidants/anti-inflammatories	• Omega-3 fatty acids
Nitric Oxide (NO) production	• L-Arginine – Phase 3 ongoing
Anti-Adhesion (Pan-selectin inhibitors)	• Crizanlizumab (SelG1) – Phase 2 complete • Rivipansel (GMI-1070) – Phase 3 on going
Other Agents – Limited Data	• IMR-687 • Sevuparin

Decitabine (5-aza-2’-deoxycytidine)

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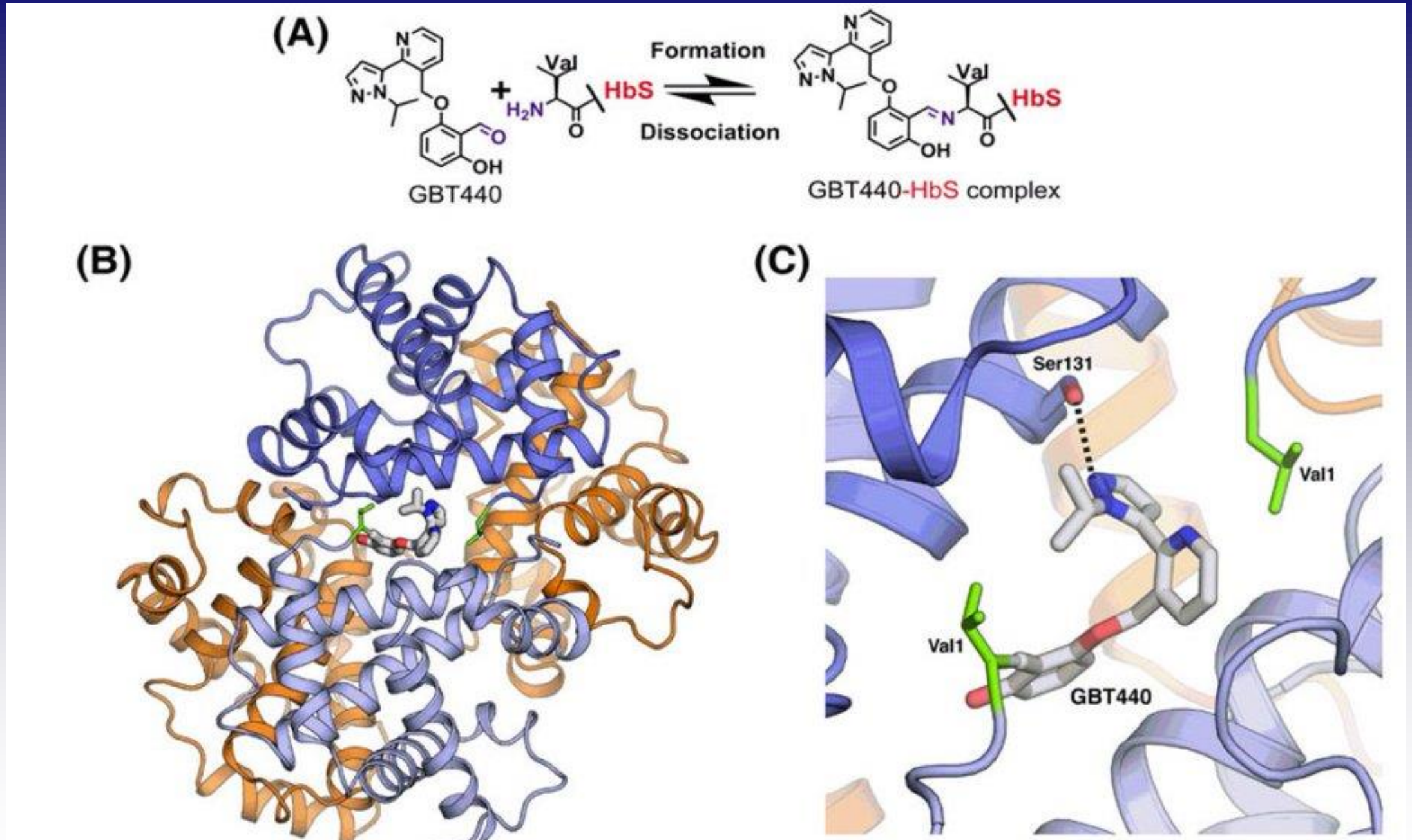
- Decitabine demethylates DNA, resulting in an induction of Hb F production
- To be effective, decitabine has to be given IV
- Oral Decitabine is ineffective: it is rapidly inactivated by cytidine deaminase
- A formulation of oral tetrahydrouridine and decitabine was reported to re-induce Hb F by demethylation of DNA in patients with SCA (randomized phase 1 study)

PLoS Med 2017; 14(9):e1002382

Voxelotor (GBT-440)


(A)
Formation
Dissociation
HbS
Val
GBT440
GBT440-HbS complex
(B)
(C)
Ser131
Val1
Val1
GBT440

Original Phase 3 HOPE Study Design and Status

SCD Patient Population:

- 1-10 VOCs in prior year
- Baseline Hb ≤ 10.5g/dL
- ≥ 12 years old
- Concomitant HU allowed

Endpoints:

- Primary: Proportion of patients who achieve a >1 g/dL Hb improvement at week 24

- Key Secondaries:
  PRO exacerbation days and/or Total Symptom Score
  VOC requiring a HCP interaction, hospitalizations

Part A

Randomize up to 150 Patients

- Voxelotor 1500 mg
- Voxelotor 900 mg
- Placebo

Three month treatment

- Select doses
- Finalize secondary endpoints
- Announce top-line data

Part B

Randomize up to 250 SCD Patients

- Voxelotor selected dose
- Placebo

Eight months treatment

Hemasphere 2018; 2:PS1461;670; Blood 2017;130:689

Efficacy And Safety Summary For Part A

- **Improvement in hemolytic anemia**: statistically significant and dose-dependent improvements in Hb, reticulocytes and bilirubin occurred with both Voxelotor doses

  - Improvements were similar in patients with or without background use of HU. Approximately 64% of patients enrolled in Part A used HU.

- **Numerically fewer VOC episodes** in both Voxelotor groups than in the placebo group.

- **Voxelotor was generally safe and well tolerated** with similar safety profiles between the two doses. There was no evidence of tissue hypoxia at either dose.

Hemasphere 2018; 2:PS1461;670; Blood 2017;130:689

Omega-3 Fatty Acids

Double-blind, randomized, multicenter phase 2 study of Omega-3 fatty acids in children with SCA (SCOT trial)

- Investigated the effect of 3 different doses of SC411 on clinical and biochemical endpoints in 67 children with SCD (5-17 years old) ± HU
- Results showed:
  - Significant increase in RBC membrane omega 3 fatty acids at 4 weeks
  - Significant increase in D-dimer and soluble E-selectin at 8 weeks in subjects taking 36 mg/kg dose
  - Significant increase in Hb in patients receiving 20 mg/kg/day
  - No significant difference in the clinical rate of VOCs between the study group and placebo

Blood Adv 2018; 2(15): 1969-1979

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Emerging Preventive & Therapeutic Approaches to treat SCA - Transfusion

Apheresis	• Red Blood Cell Exchange • RBCX + Plasma Exchange • Whole Blood Exchange
Sanguinate and SCD	• Therapeutic role for VOC

Definition of Erythrocytapheresis

- **Red blood cell exchange,** also known as therapeutic erythrocytapheresis, is a nonsurgical therapy that removes and replaces a patient's **red blood cells** with **red blood cells** provided by a **blood** donor

- The plasma, platelets and white blood cells are returned to the patient

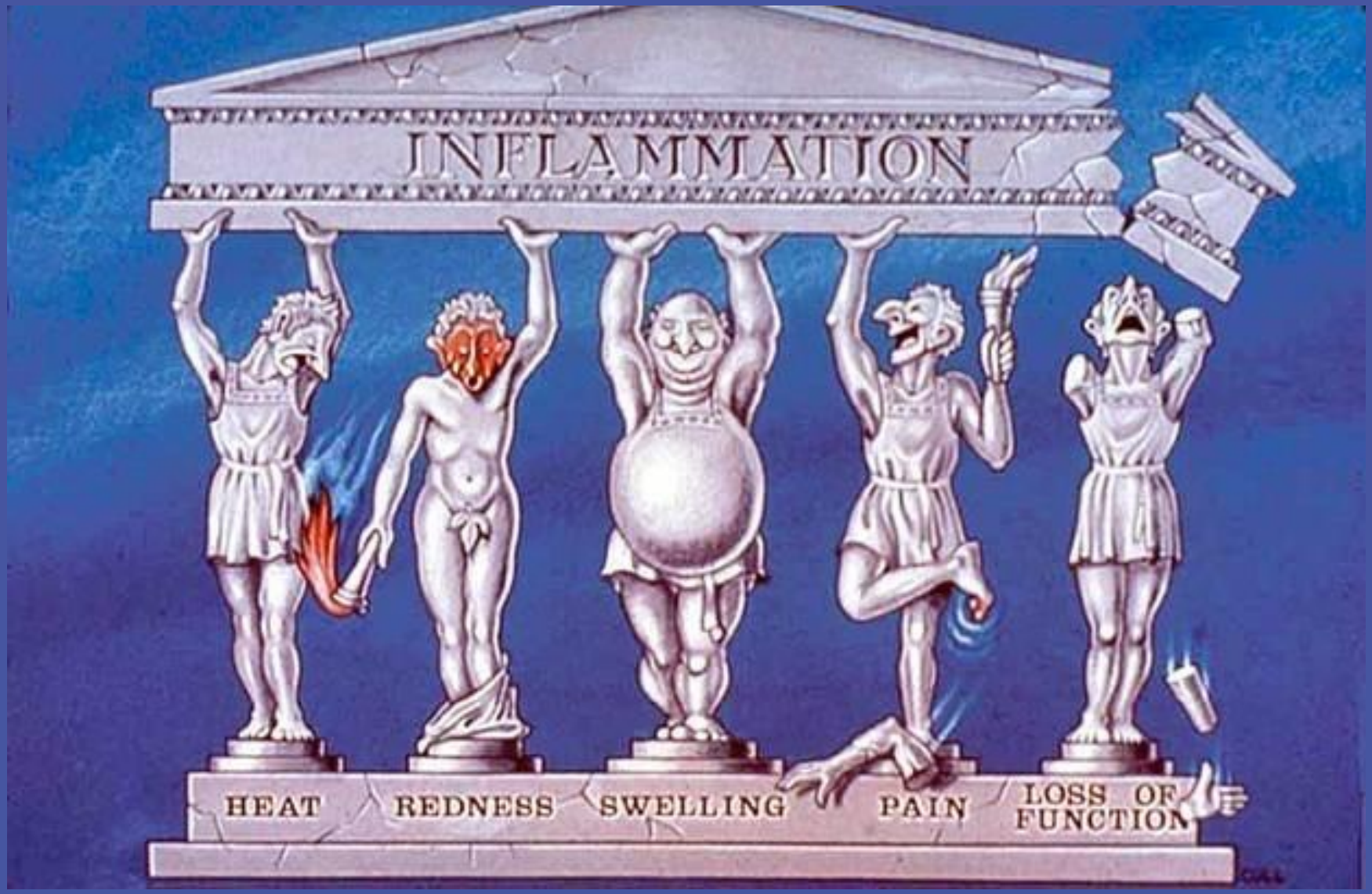
The Plasma Metabolomics Profile of Patients with SCA is Abnormal

Components that are increased in plasma of patients with SCA:

- Fibrinogen
- von Willebrand
- Coagulation factors
- Immunoglobulins (IgG, IgA)
- Cytokines
- Chemokines
- D-dimer
- VCAM-1

- Increased Thrombin and Fibrin Generation
- Increased Tissue Factor Activity
- Triglycerides
- Degradation of old proteins (Inflammasomes, Autophagy)
- Nonesterified Fatty Acids (NEFA)

Hematology Am Soc Hematol Educ Progam 2013; 2013:362-369


INFLAMMATION
HEAT
REDNESS
SWELLING
PAIN
LOSS OF FUNCTION

Indications for Apheresis in Patients with Sickle Cell Anemia

A. Preventive Indications (Non-inflammatory)

a. Red Cell Exchange

i. Stroke, primary and secondary

ii. Recurrent ACS

B. Therapeutic Indications (with Inflammatory component)

a. Red Cell Exchange ± Plasma Exchange

i. Acute stroke

ii. ACS

iii. Multi-organ Failure

iv. Leg ulcers

Brief History of Human Blood Transfusion

The first human to human blood transfusion was performed on Sep 25, 1818 by Dr James Blundell

Brief History of Non-Human Blood Transfusion

- History repeats itself: what is old becomes new again
- **1628**: William Harvey discovered the circulation of blood
- **1665**: Richard Lower kept dogs alive by transfusion of blood from other dogs
- **1667** Jean-Baptiste Denis in France and Richard Lower in England separately reported successful transfusions from lambs to humans

Blood: An Epic History of Medicine and Commerce by Douglas Starr

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Brief History of Non-Human Blood Transfusion Continued

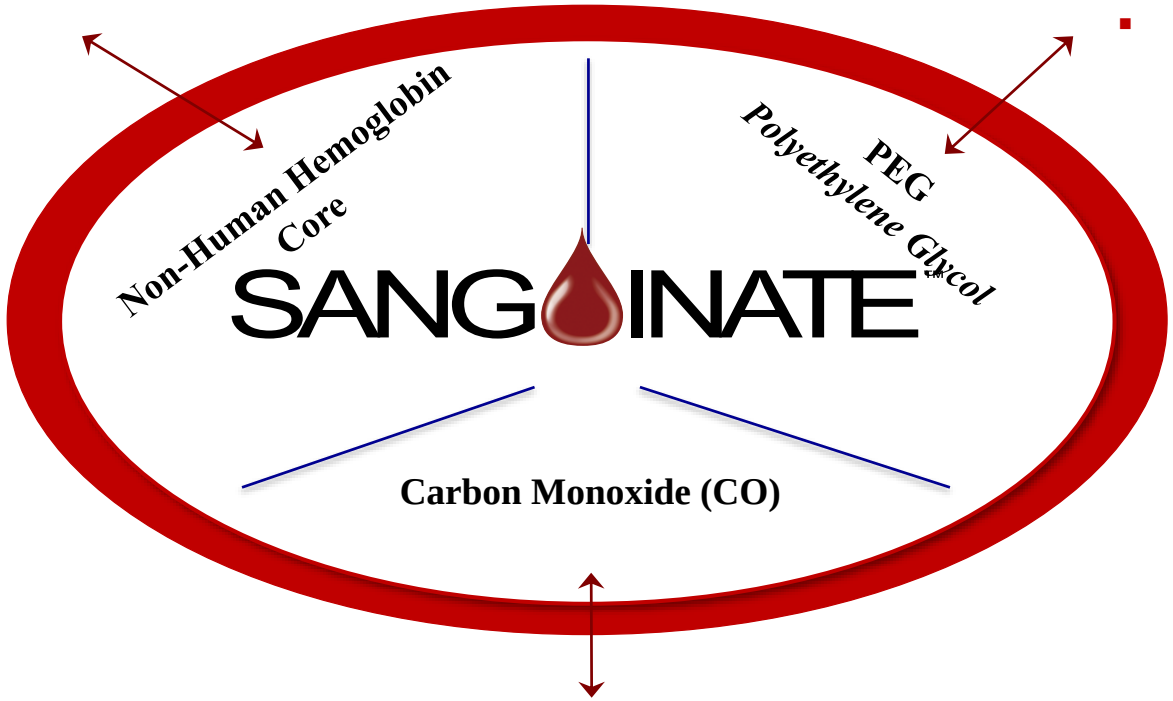
- **1668-1669**: The British Royal Society and the Vatican prohibited transfusions from animals to humans
- **20th-21 century**: Use of modified bovine Hb (Sanguinate) instead of bovine RBC may be useful.

Blood: An Epic History of Medicine and Commerce by Douglas Starr

SANGUINATE®
(PEGylated Bovine Carboxyhemoglobin; PEG-COHb)

- **Transfers oxygen to hypoxic RBC/tissues**

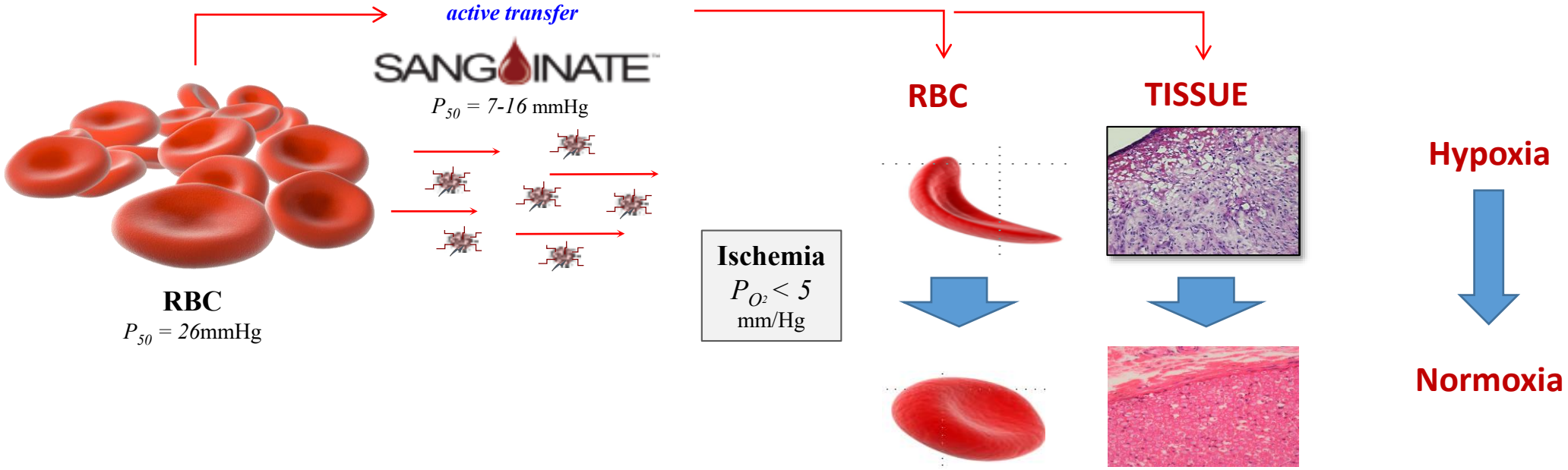
- **Hydrophilic "Shell"**
  - **Increased circulating life**
  - **Decreased immunogenicity**
  - **Inhibition of extravasation**



***Multiple* Modes of Action to Treat *Multiple* Factors in SCD**

Artif Organs 2014 38(8): 702-707; Artif Organs 2017 41(4):346-350; Rev Bras Hemoter 2017; 39(1): 20-27

SANGUINATE® Targeted O_2 Delivery – p50 Dependency



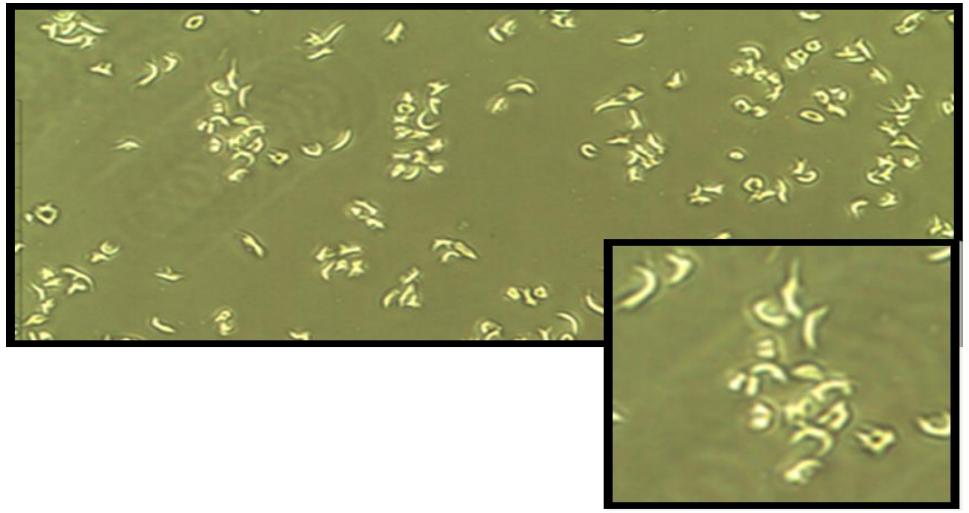
P_{50} of SANGUINATE® is key to targeted delivery of oxygen to hypoxic tissue

- *Can enhance oxygenation during an occlusive event* - **blocked vessel(s)** ***[delivery defect]***
- *Can enhance oxygenation during chronic or acute anemia* - **low hemoglobin** ***[capacity defect]***

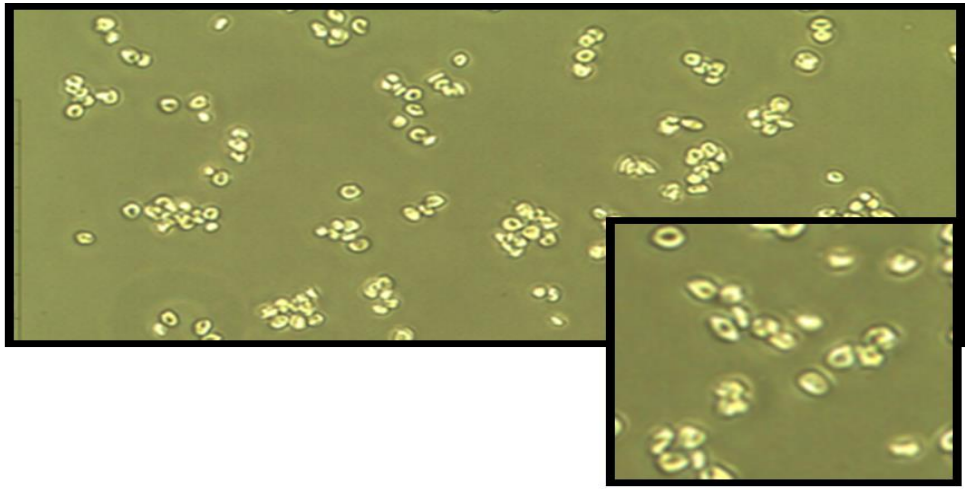
Artif Organs 2014 38(8): 702-707; Artif Organs 2017 41(4):346-350; Rev Bras Hemoter 2017; 39(1): 20-27

Depolymerization Effects of SANGUINATE® on HbSS RBC

PEG-BSA Control



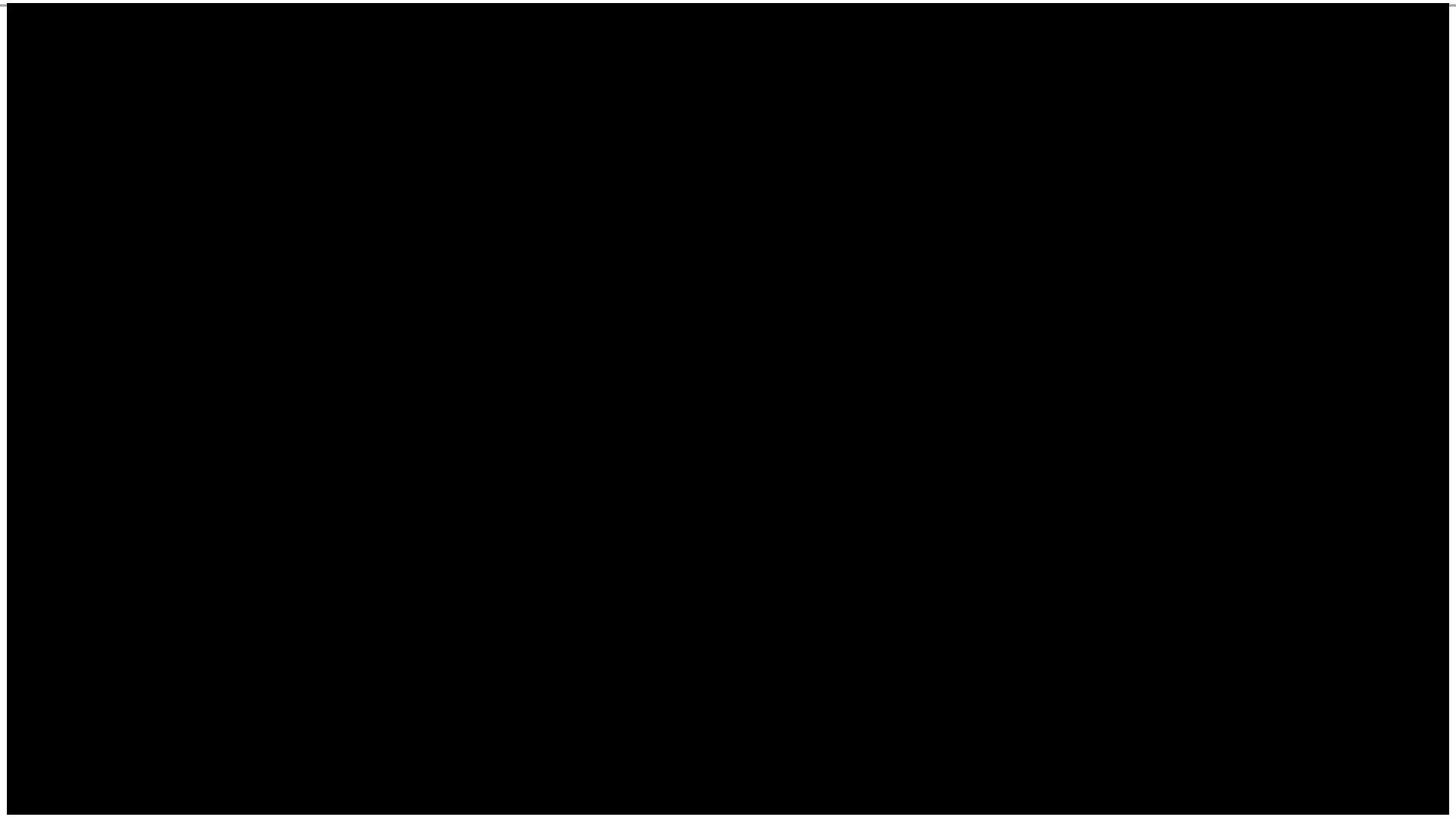
SANGUINATE®



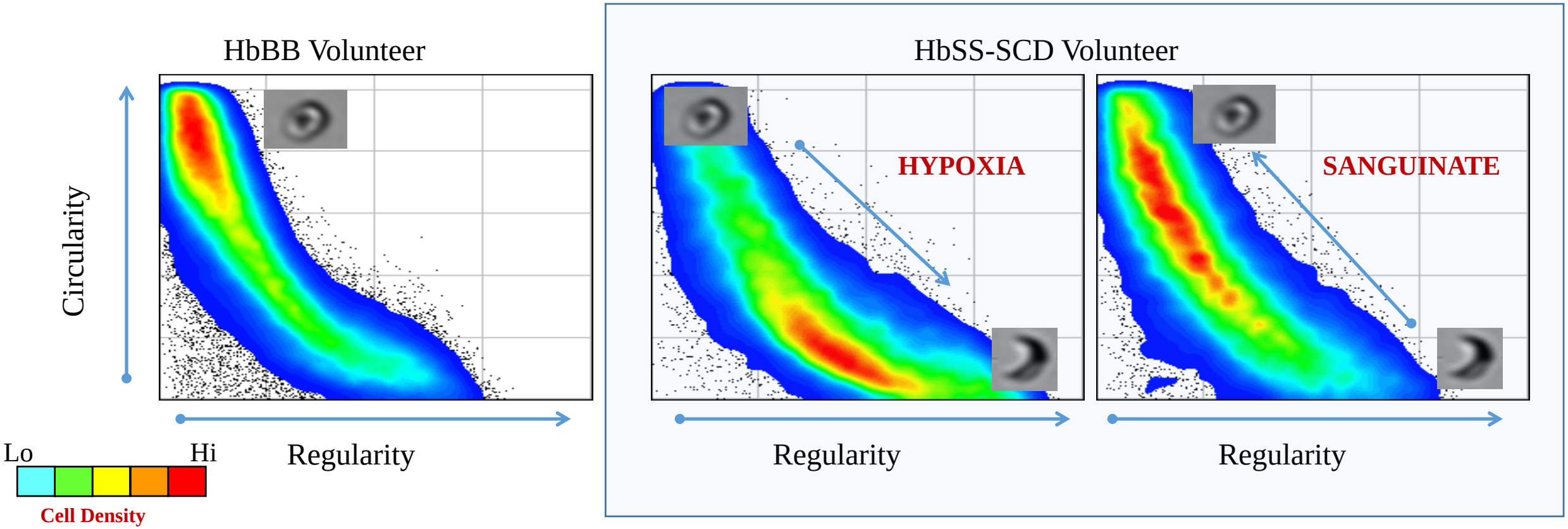
SANGUINATE® promotes reversal of deoxygenated HbSS RBC

Artif Organs 2014 38(8): 702-707

SANGUINATE® Visualizing HbSS RBC Un-sickling - in vitro



Ex vivo HbSS RBC Depolymerization Monitored by Imaging Cytometry

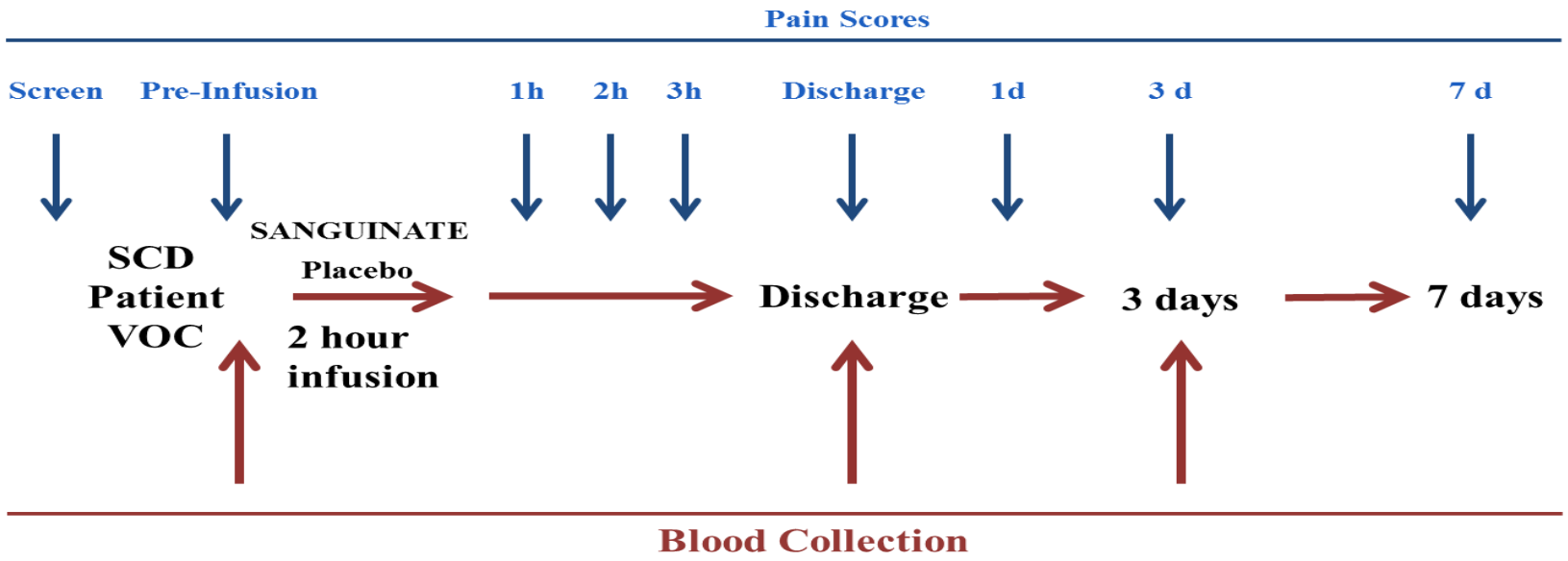


SANGUINATE® Treatment Rapidly Shifted the Sickle/Unsickle Ratio

Artif Organs 2014 38(8): 702-707; Artif Organs 2017 41(4):346-350; Rev Bras Hemoter 2017; 39(1): 20-27

SGSC-005 Basic Trial Design

Data Collection: Pain Scores and Blood Samples



Blood was collected at pre-infusion, 2 hours post infusion and at 3 days post treatment for RBC shape, RNA, and Plasma cytokines

NCT02411708

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Summary

- **The last 20 years witnessed significand advances in preventive and therapeutic therapies of SCD beyond palliative therapies**
- **Role of plasma exchange in addition to RBCX (WBX) in patients with SCD needs further investigation**
- **The role of non-human hemoglobin (Sanguinate) in the management of SCD seems promising and requires further controlled trials**

Thank you for Your Attention

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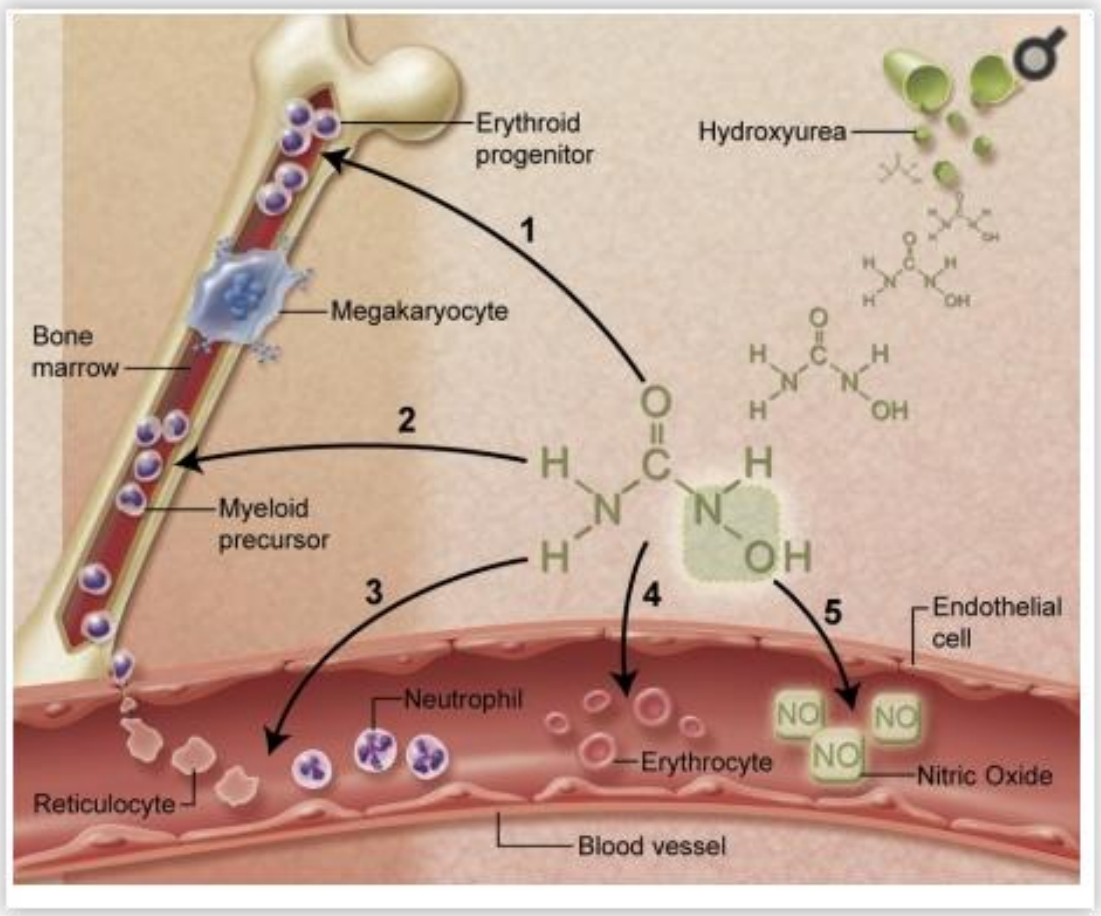

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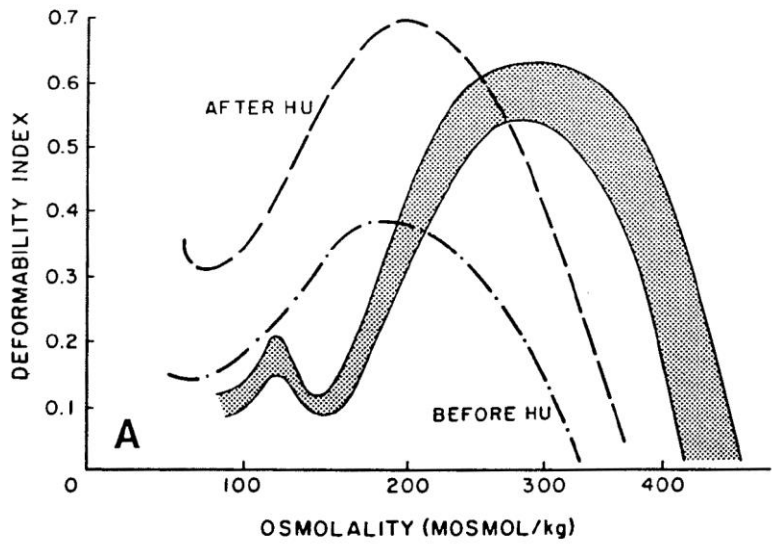

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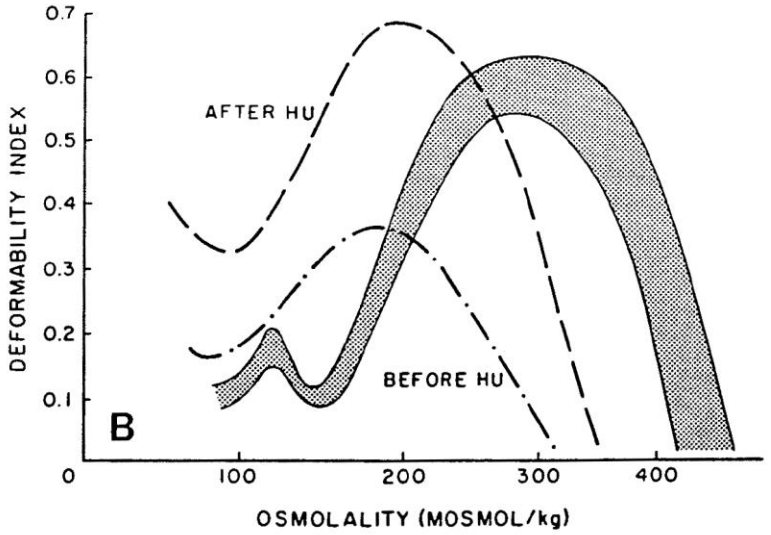

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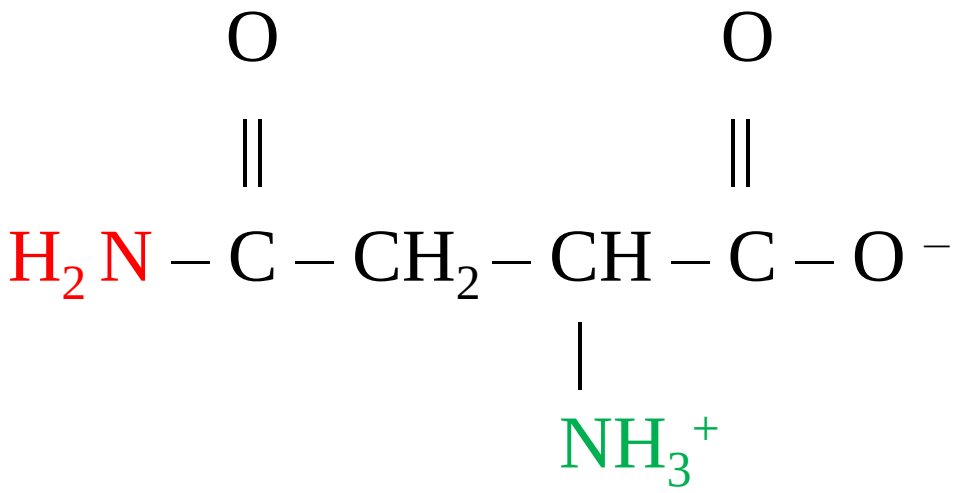

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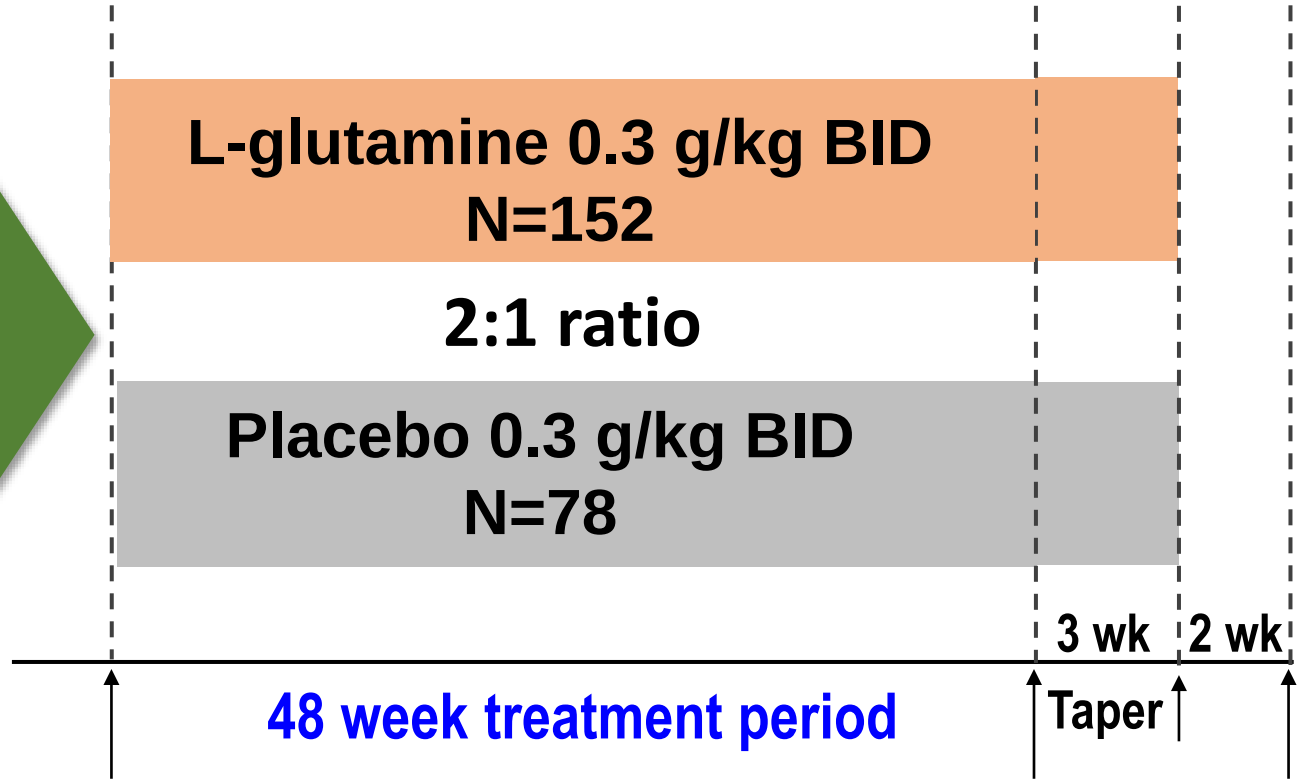

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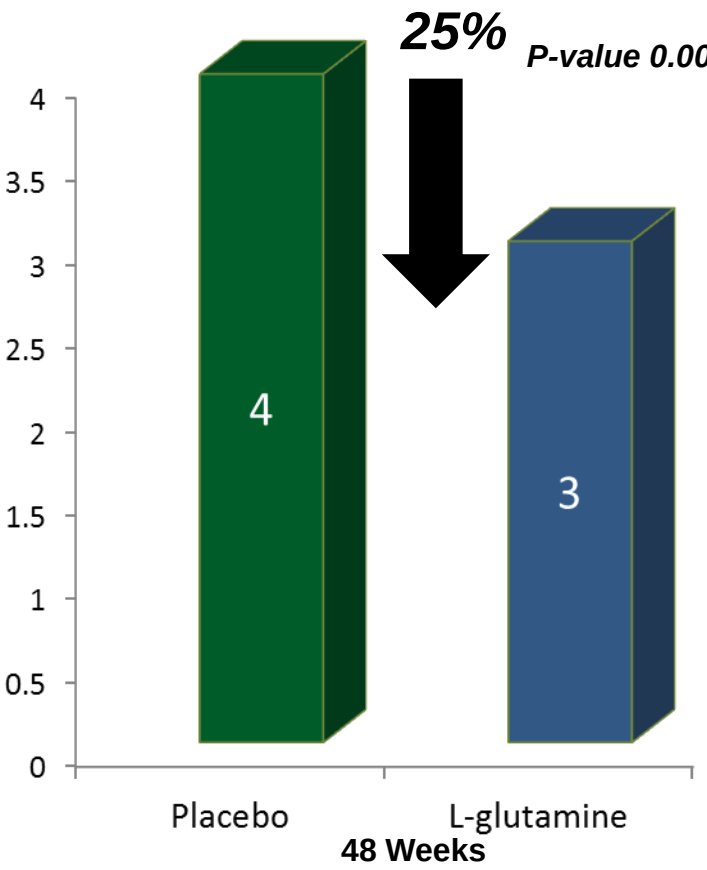

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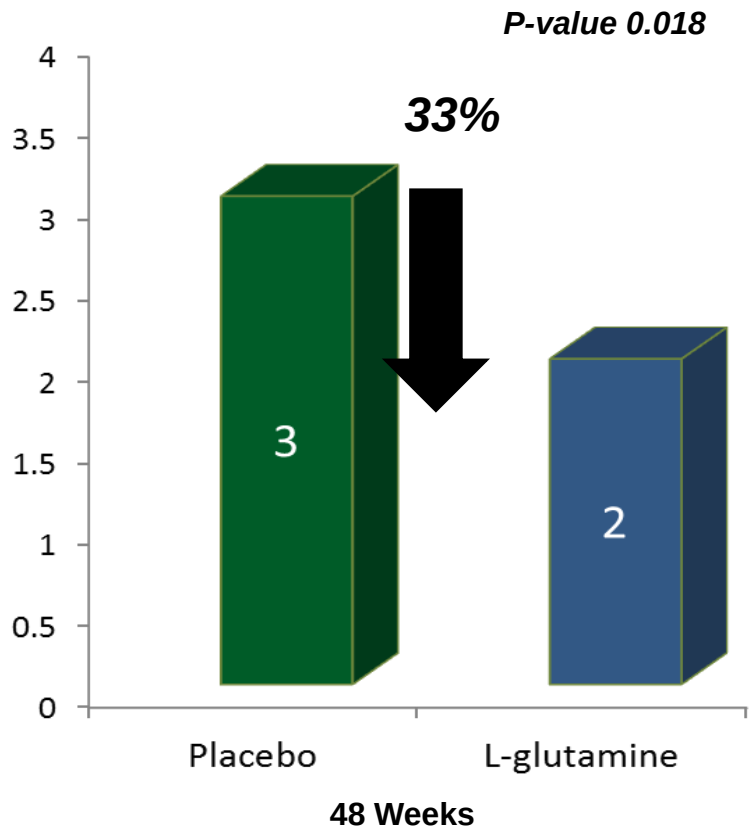

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≥1	13 (8.6)	18 (23.1)
0	139 (91.4)	60 (76.9)
1	10 (6.6)	13 (16.7)
2	3 (2.0)	4 (5.1)
3	0	1 (1.3)

63% lower incidence of ACS

Observed Data

N Engl J Med. 2013;368:1489-97

Efficacy Evidence for L-glutamine

When compared with placebo:

- ✓ Lower frequency of VOCs
- ✓ Lower frequency of hospitalizations
- ✓ Longer time to first and second crisis
- ✓ Lower cumulative event rates*
- ✓ Lower occurrence of ACS
- ✓ Lower cumulative days in hospital
- ✓ L-glutamine +HU is more effective then HU

N Engl J Med. 2013;368:1489-97

Emerging Preventive & Therapeutic Approaches to Treat SCA - Pharmacotherapy

Hb F Induction	• Oral Decitabine & tetrahydrouridine • Pomalidomide
Inhibition of Hb S Polymerization	• Voxelotor (GBT-440) • SCD 101
Anti-oxidants/anti-inflammatories	• Omega-3 fatty acids
Nitric Oxide (NO) production	• L-Arginine – Phase 3 ongoing
Anti-Adhesion (Pan-selectin inhibitors)	• Crizanlizumab (SelG1) – Phase 2 complete • Rivipansel (GMI-1070) – Phase 3 on going
Other Agents – Limited Data	• IMR-687 • Sevuparin

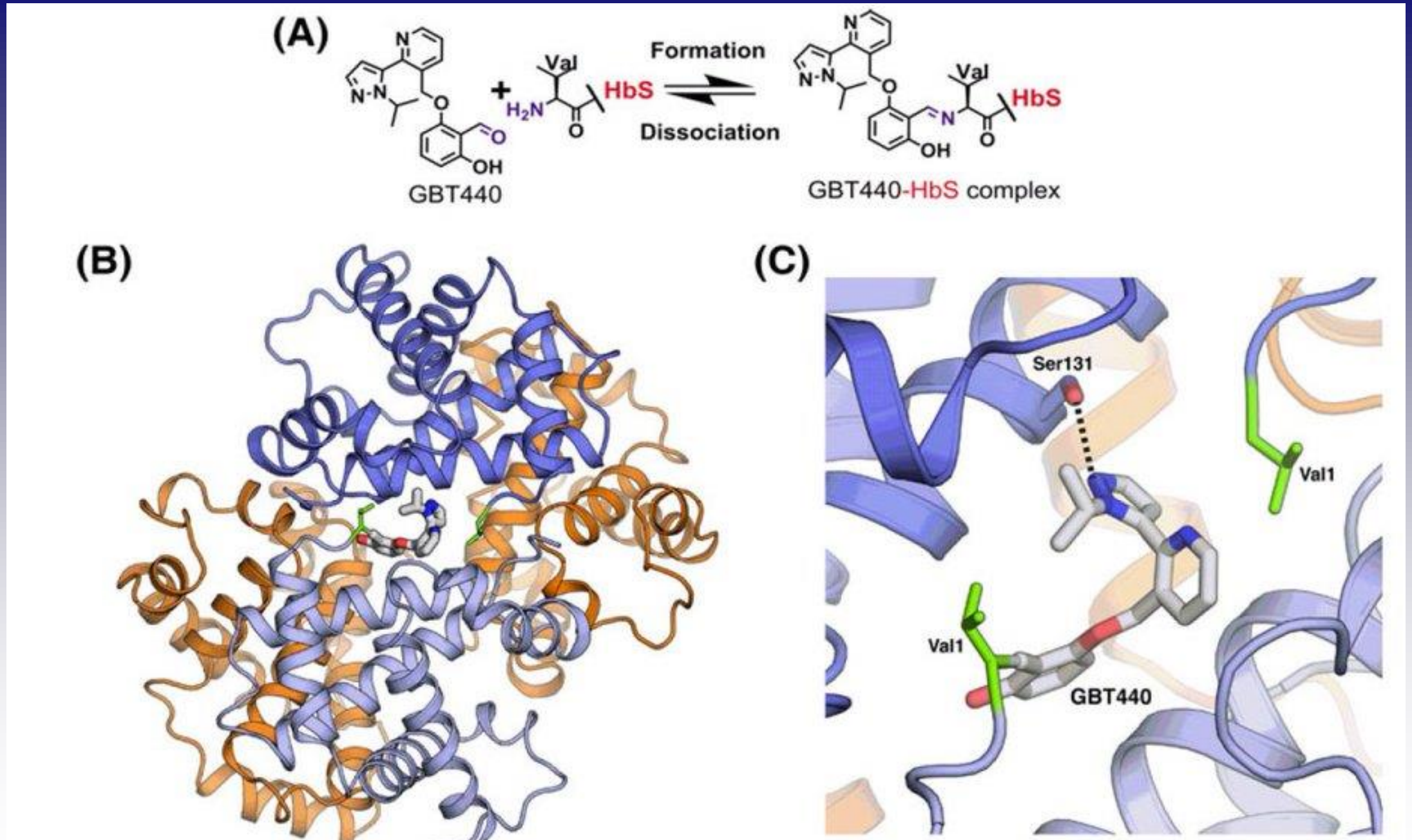
Decitabine (5-aza-2’-deoxycytidine)

Decitabine (5-aza-2’-deoxycytidine)

- Decitabine demethylates DNA, resulting in an induction of Hb F production
- To be effective, decitabine has to be given IV
- Oral Decitabine is ineffective: it is rapidly inactivated by cytidine deaminase
- A formulation of oral tetrahydrouridine and decitabine was reported to re-induce Hb F by demethylation of DNA in patients with SCA (randomized phase 1 study)

PLoS Med 2017; 14(9):e1002382

Voxelotor (GBT-440)


(A)
Formation
Dissociation
HbS
Val
GBT440
GBT440-HbS complex
(B)
(C)
Ser131
Val1
Val1
GBT440

Original Phase 3 HOPE Study Design and Status

SCD Patient Population:

- 1-10 VOCs in prior year
- Baseline Hb ≤ 10.5g/dL
- ≥ 12 years old
- Concomitant HU allowed

Endpoints:

- Primary: Proportion of patients who achieve a >1 g/dL Hb improvement at week 24

- Key Secondaries:
  PRO exacerbation days and/or Total Symptom Score
  VOC requiring a HCP interaction, hospitalizations

Part A

Randomize up to 150 Patients

- Voxelotor 1500 mg
- Voxelotor 900 mg
- Placebo

Three month treatment

- Select doses
- Finalize secondary endpoints
- Announce top-line data

Part B

Randomize up to 250 SCD Patients

- Voxelotor selected dose
- Placebo

Eight months treatment

Hemasphere 2018; 2:PS1461;670; Blood 2017;130:689

Efficacy And Safety Summary For Part A

- **Improvement in hemolytic anemia**: statistically significant and dose-dependent improvements in Hb, reticulocytes and bilirubin occurred with both Voxelotor doses

  - Improvements were similar in patients with or without background use of HU. Approximately 64% of patients enrolled in Part A used HU.

- **Numerically fewer VOC episodes** in both Voxelotor groups than in the placebo group.

- **Voxelotor was generally safe and well tolerated** with similar safety profiles between the two doses. There was no evidence of tissue hypoxia at either dose.

Hemasphere 2018; 2:PS1461;670; Blood 2017;130:689

Omega-3 Fatty Acids

Double-blind, randomized, multicenter phase 2 study of Omega-3 fatty acids in children with SCA (SCOT trial)

- Investigated the effect of 3 different doses of SC411 on clinical and biochemical endpoints in 67 children with SCD (5-17 years old) ± HU
- Results showed:
  - Significant increase in RBC membrane omega 3 fatty acids at 4 weeks
  - Significant increase in D-dimer and soluble E-selectin at 8 weeks in subjects taking 36 mg/kg dose
  - Significant increase in Hb in patients receiving 20 mg/kg/day
  - No significant difference in the clinical rate of VOCs between the study group and placebo

Blood Adv 2018; 2(15): 1969-1979

Emerging Preventive & Therapeutic Approaches to Treat SCA - Pharmacotherapy

Hb F Induction	• Oral Decitabine & tetrahydrouridine • Pomalidomide
Inhibition of Hb S Polymerization	• Voxelotor (GBT-440) • SCD 101
Anti-oxidants/anti-inflammatories	• Omega-3 fatty acids
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Other Agents – Limited Data	• IMR-687 • Sevuparin

Emerging Preventive & Therapeutic Approaches to treat SCA - Transfusion

Apheresis	• Red Blood Cell Exchange • RBCX + Plasma Exchange • Whole Blood Exchange
Sanguinate and SCD	• Therapeutic role for VOC

Definition of Erythrocytapheresis

- **Red blood cell exchange,** also known as therapeutic erythrocytapheresis, is a nonsurgical therapy that removes and replaces a patient's **red blood cells** with **red blood cells** provided by a **blood** donor

- The plasma, platelets and white blood cells are returned to the patient

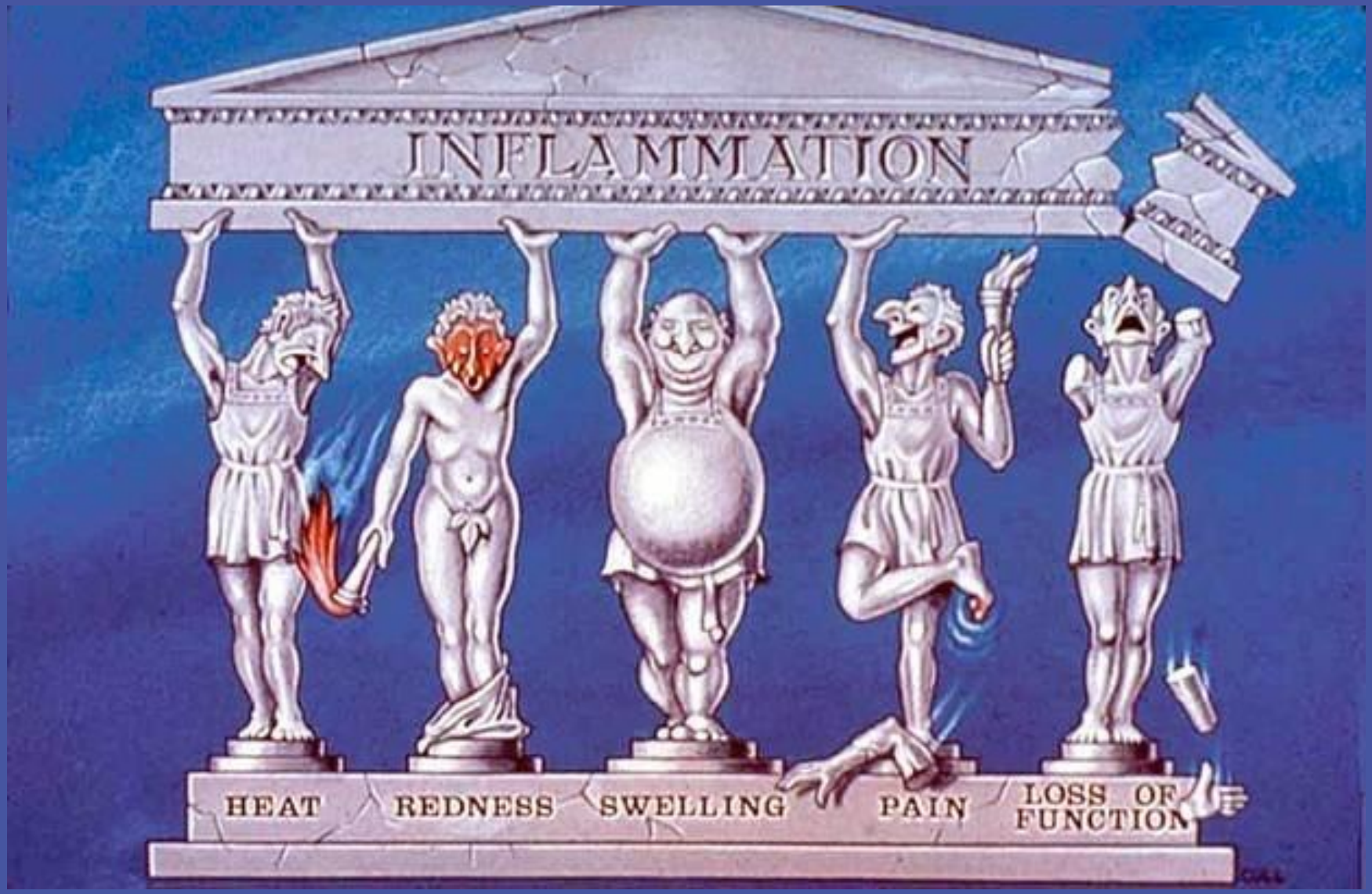
The Plasma Metabolomics Profile of Patients with SCA is Abnormal

Components that are increased in plasma of patients with SCA:

- Fibrinogen
- von Willebrand
- Coagulation factors
- Immunoglobulins (IgG, IgA)
- Cytokines
- Chemokines
- D-dimer
- VCAM-1

- Increased Thrombin and Fibrin Generation
- Increased Tissue Factor Activity
- Triglycerides
- Degradation of old proteins (Inflammasomes, Autophagy)
- Nonesterified Fatty Acids (NEFA)

Hematology Am Soc Hematol Educ Progam 2013; 2013:362-369


INFLAMMATION
HEAT
REDNESS
SWELLING
PAIN
LOSS OF FUNCTION

Indications for Apheresis in Patients with Sickle Cell Anemia

A. Preventive Indications (Non-inflammatory)

a. Red Cell Exchange

i. Stroke, primary and secondary

ii. Recurrent ACS

B. Therapeutic Indications (with Inflammatory component)

a. Red Cell Exchange ± Plasma Exchange

i. Acute stroke

ii. ACS

iii. Multi-organ Failure

iv. Leg ulcers

Brief History of Human Blood Transfusion

The first human to human blood transfusion was performed on Sep 25, 1818 by Dr James Blundell

Brief History of Non-Human Blood Transfusion

- History repeats itself: what is old becomes new again
- **1628**: William Harvey discovered the circulation of blood
- **1665**: Richard Lower kept dogs alive by transfusion of blood from other dogs
- **1667** Jean-Baptiste Denis in France and Richard Lower in England separately reported successful transfusions from lambs to humans

Blood: An Epic History of Medicine and Commerce by Douglas Starr

Blood: An Epic History of Medicine and Commerce by Douglas Starr

Brief History of Non-Human Blood Transfusion Continued

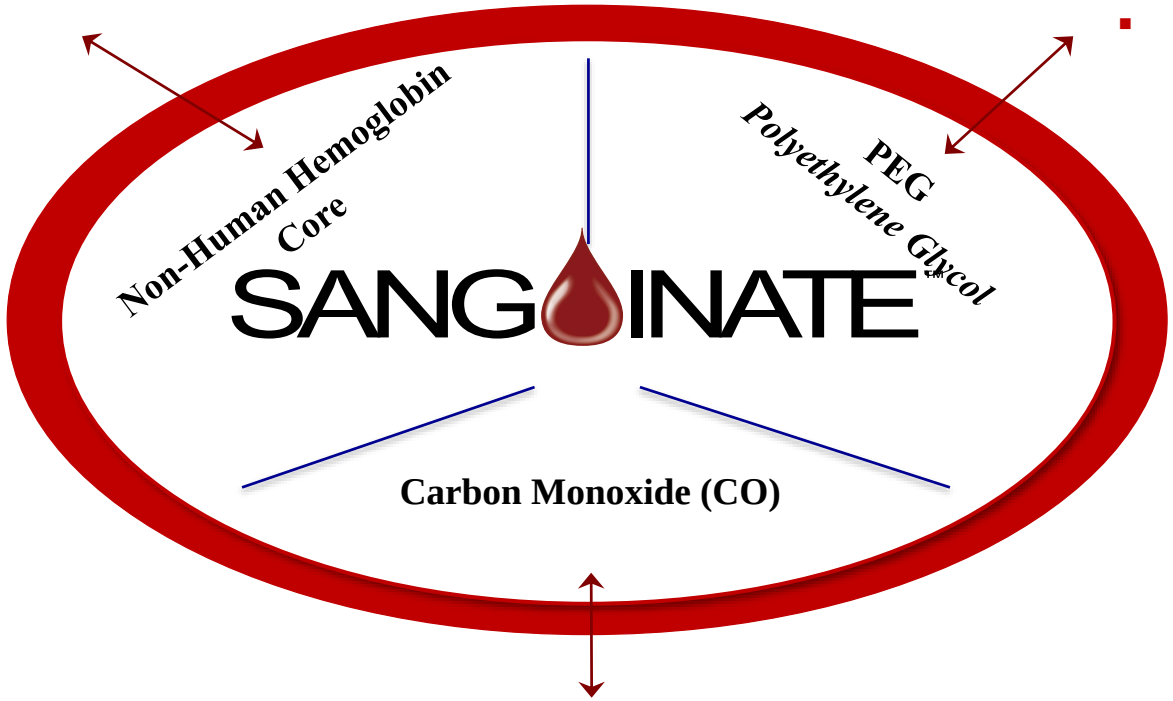
- **1668-1669**: The British Royal Society and the Vatican prohibited transfusions from animals to humans
- **20th-21 century**: Use of modified bovine Hb (Sanguinate) instead of bovine RBC may be useful.

Blood: An Epic History of Medicine and Commerce by Douglas Starr

SANGUINATE®
(PEGylated Bovine Carboxyhemoglobin; PEG-COHb)

- **Transfers oxygen to hypoxic RBC/tissues**

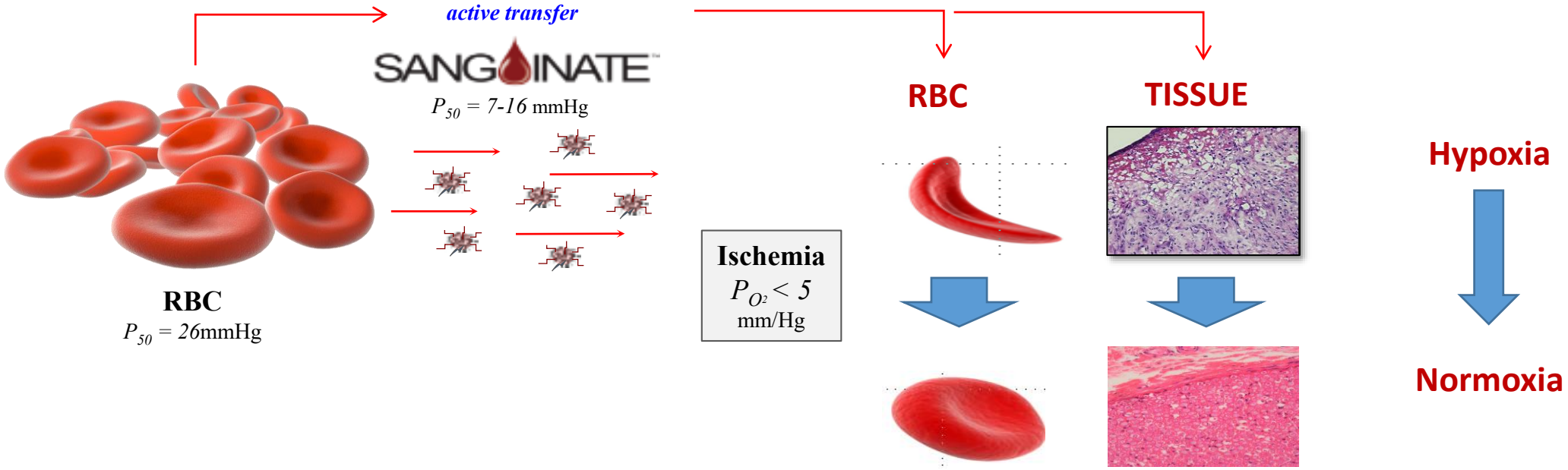
- **Hydrophilic "Shell"**
  - **Increased circulating life**
  - **Decreased immunogenicity**
  - **Inhibition of extravasation**



***Multiple* Modes of Action to Treat *Multiple* Factors in SCD**

Artif Organs 2014 38(8): 702-707; Artif Organs 2017 41(4):346-350; Rev Bras Hemoter 2017; 39(1): 20-27

SANGUINATE® Targeted O_2 Delivery – p50 Dependency



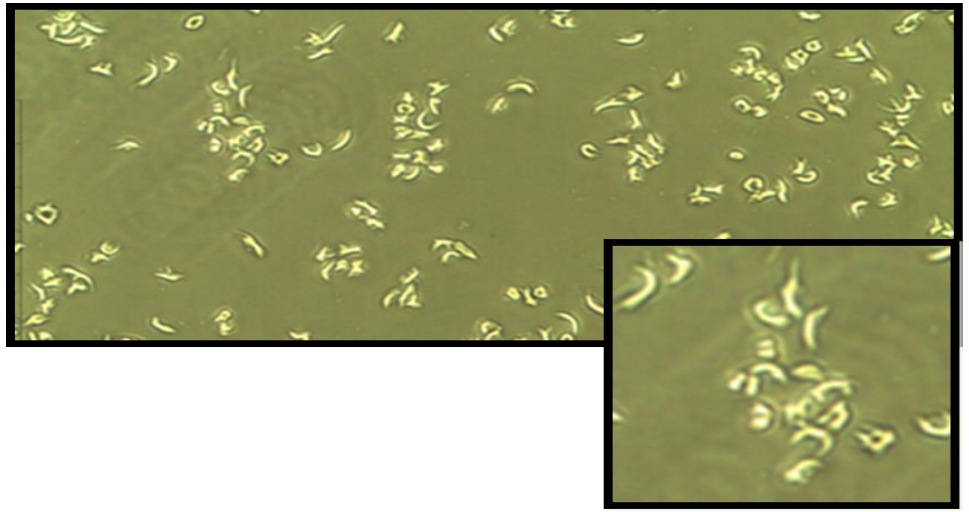
P_{50} of SANGUINATE® is key to targeted delivery of oxygen to hypoxic tissue

- *Can enhance oxygenation during an occlusive event* - **blocked vessel(s)** ***[delivery defect]***
- *Can enhance oxygenation during chronic or acute anemia* - **low hemoglobin** ***[capacity defect]***

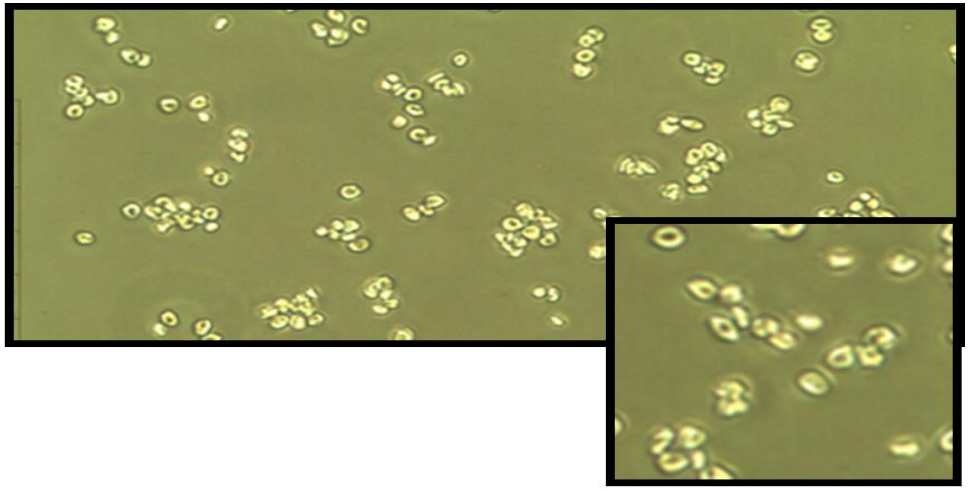
Artif Organs 2014 38(8): 702-707; Artif Organs 2017 41(4):346-350; Rev Bras Hemoter 2017; 39(1): 20-27

Depolymerization Effects of SANGUINATE® on HbSS RBC

PEG-BSA Control



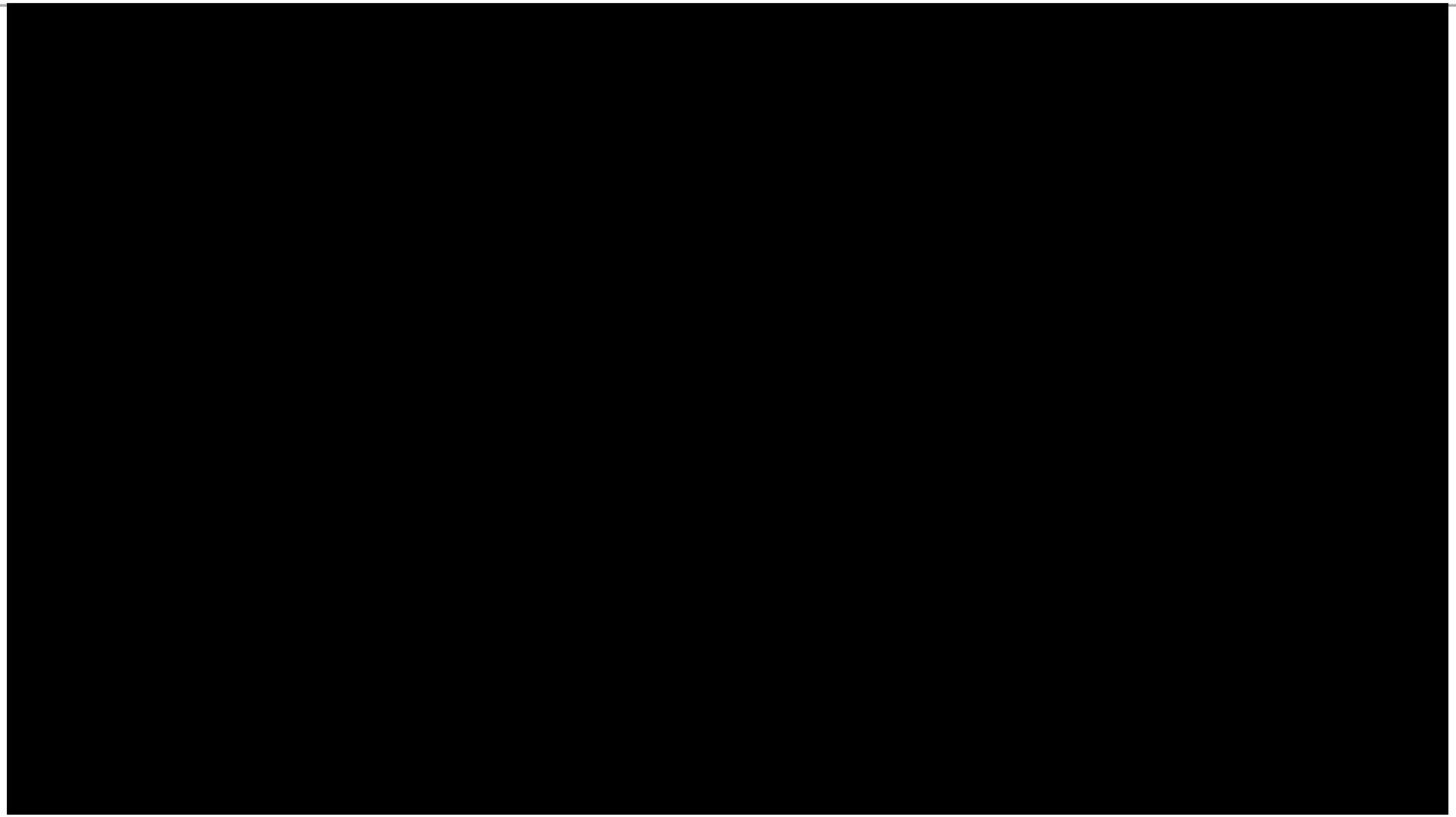
SANGUINATE®



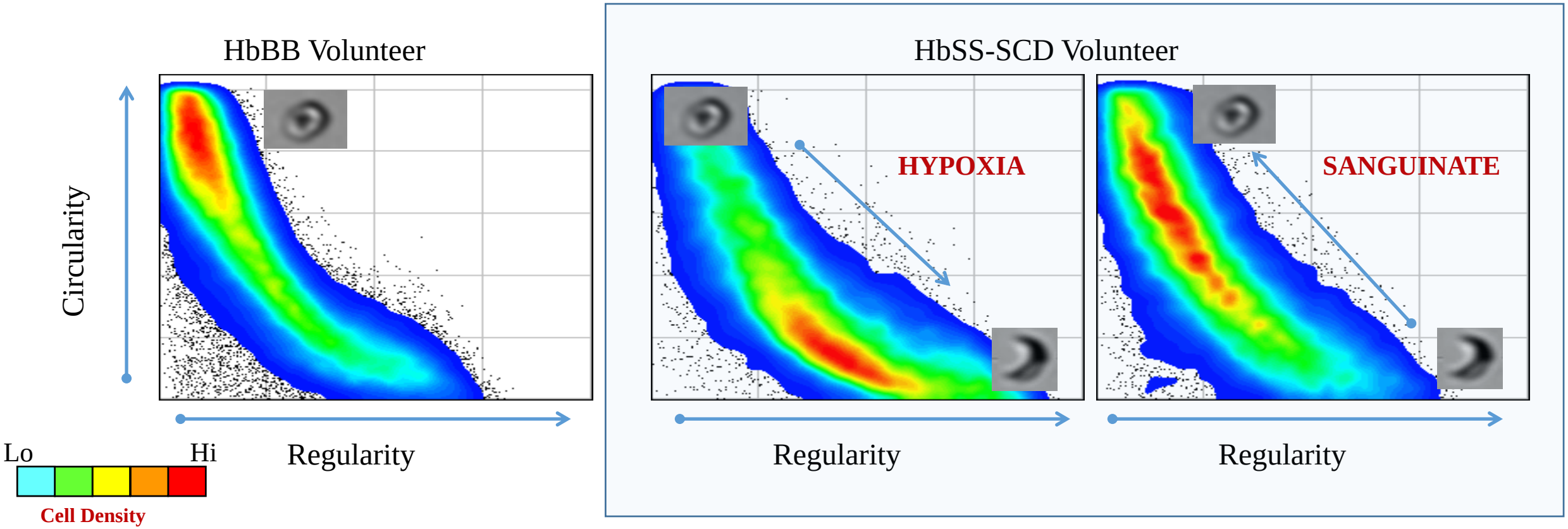
SANGUINATE® promotes reversal of deoxygenated HbSS RBC

Artif Organs 2014 38(8): 702-707; Artif Organs 2017 41(4):346-350; Rev Bras Hemoter 2017; 39(1): 20-27

SANGUINATE® Visualizing HbSS RBC Un-sickling - in vitro



Ex vivo HbSS RBC Depolymerization Monitored by Imaging Cytometry

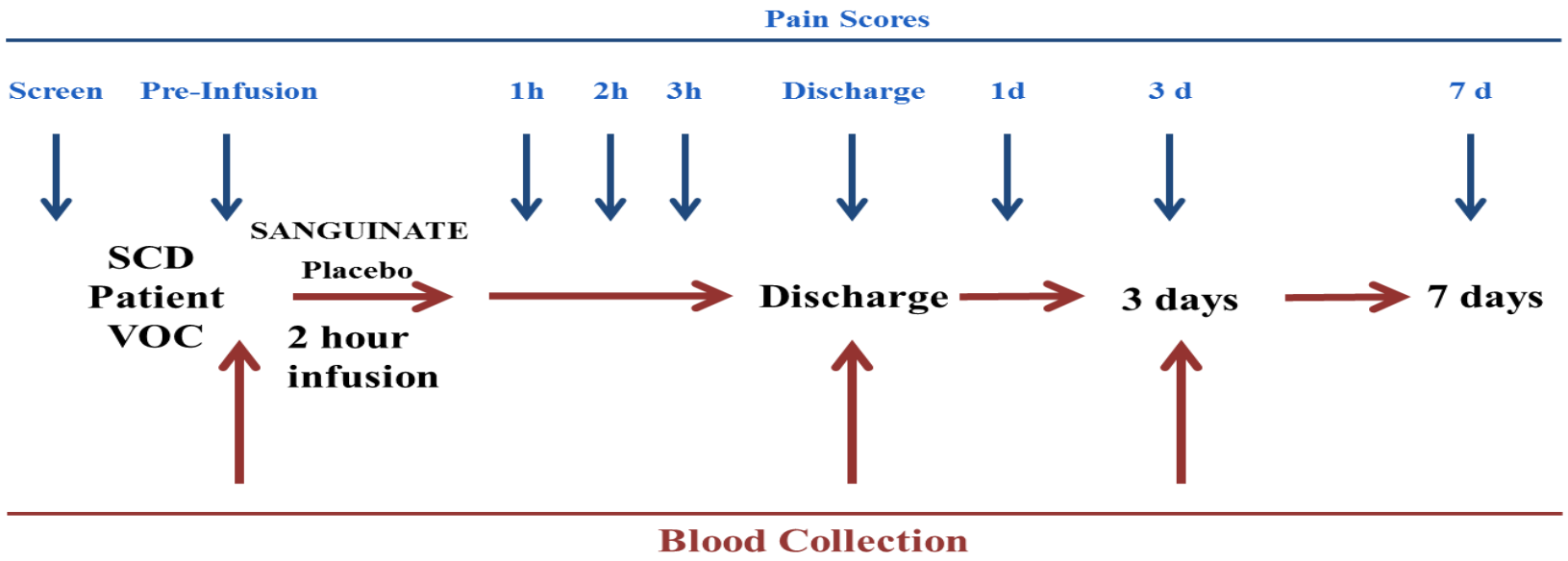


SANGUINATE® Treatment Rapidly Shifted the Sickle/Unsickle Ratio

Artif Organs 2014 38(8): 702-707; Artif Organs 2017 41(4):346-350; Rev Bras Hemoter 2017; 39(1): 20-27

SGSC-005 Basic Trial Design

Data Collection: Pain Scores and Blood Samples



Blood was collected at pre-infusion, 2 hours post infusion and at 3 days post treatment for RBC shape, RNA, and Plasma cytokines

NCT02411708

Artif Organs 2014 38(8): 702-707; Artif Organs 2017 41(4):346-350; Rev Bras Hemoter 2017; 39(1): 20-27

Summary

- **The last 20 years witnessed significand advances in preventive and therapeutic therapies of SCD beyond palliative therapies**
- **Role of plasma exchange in addition to RBCX (WBX) in patients with SCD needs further investigation**
- **The role of non-human hemoglobin (Sanguinate) in the management of SCD seems promising and requires further controlled trials**

Thank you for Your Attention

Samir.Ballas@Jefferson.edu

Hematopoietic Cell Transplantation for Sickle Cell Disease: Then, Now, and What is Around the Corner

Lakshmanan Krishnamurti, MD
Professor Pediatrics,
Joseph Kuechenmeister/Aflac Field Force Chair
Director BMT

Aflac
Cancer & Blood Disorders Center
CHILDREN'S HEALTHCARE OF ATLANTA

EMORY UNIVERSITY SCHOOL OF MEDICINE

- No Relevant Conflicts of Interest

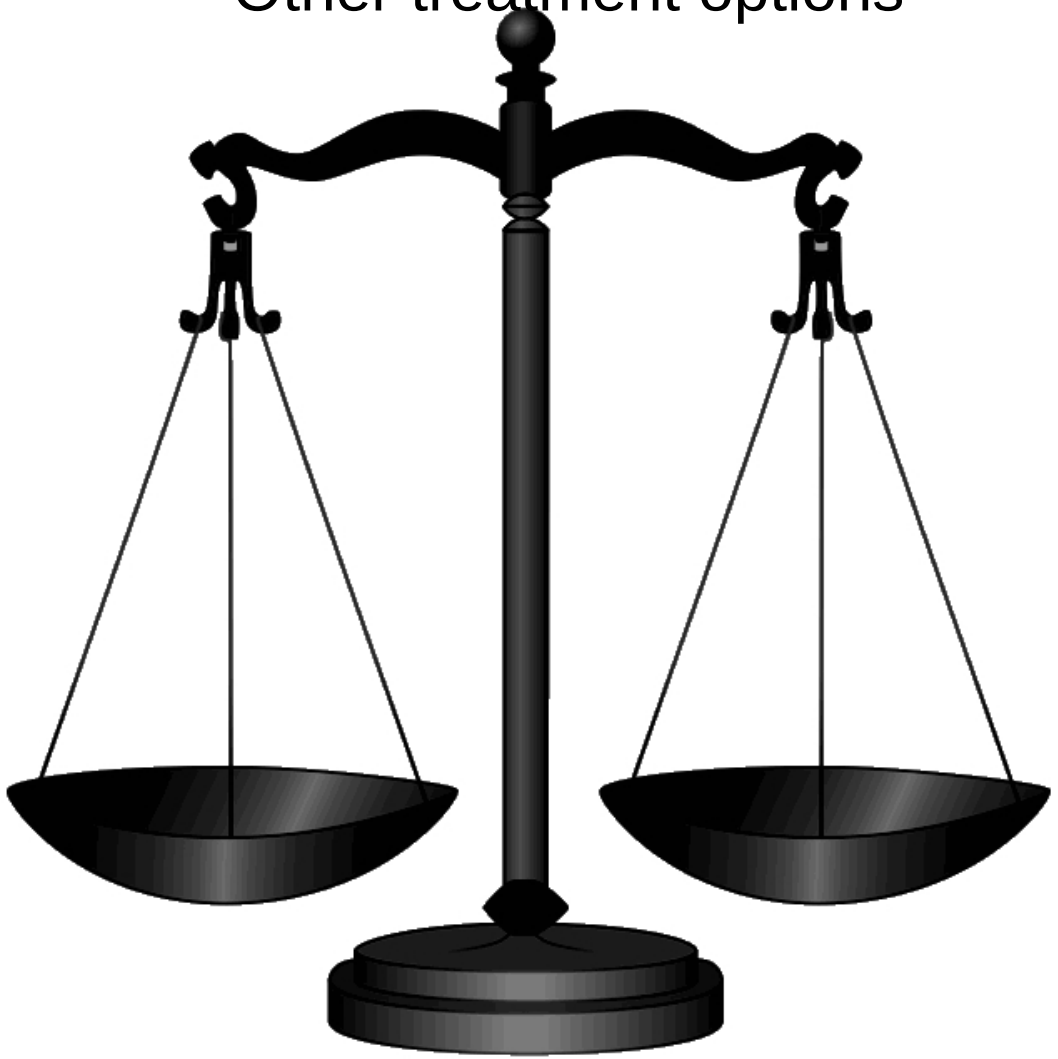
What we will talk about today

- Excellent results in children with HLA identical sibling donor
- Challenge: donor availability, awareness/access, gonadal toxicity, need to study long term outcomes
- BMT from unrelated donors feasible in children.
- Additional challenge: Rate of CGVHD. knowledge and service gap in adults
- BMT from Haploidentical family donors feasible, expands donor pool
- Challenge: Donor specific HLA antibodies, Engraftment
- Is BMT superior to standard of care. No comparison data
- STRIDE, a comparison trial of BMT vs. standard of care. Pilot data suggest that conditioning regimen safe, effective. comparison study ongoing
- Increase awareness and acceptance of HCT. 50% of HCTs since 2007
- Patients not aware of option. Medicaid coverage may be problematic. www.sickleoptions.org is a useful resource.
- Gene therapy studies open. Early results promising

Risk-Benefit Paradigm for Curative Therapy for SCD

- Age
- Donor options
- Other treatment options

- Potential for cure
- Freedom from risk of sickle complications
- Improved survival



- Risk for death
- Treatment related complications
- Long term effects

BONE-MARROW TRANSPLANTATION IN A PATIENT WITH SICKLE-CELL ANEMIA

F. Leonard Johnson, M.B.B.S.,
A. Thomas Look, M.D., Jon Gockerman, M.D.,
Mary R. Ruggiero, P.N.P.,
Luciano Dalla-Pozza, M.B.B.S.,
and Frederic T. Billings III, M.D.

SICKLE-CELL anemia affects 1 in 600 of the U.S. black population and accounts for 80,000 deaths annually throughout the world.[1,2] Current therapy is

From the Division of Marrow Transplantation, St. Jude Children's Research Hospital, Memphis, Tenn.; the University of Alabama Hospital, Birmingham, Ala.; and Our Lady of the Lake Regional Medical Center, Baton Rouge, La. Address reprint requests to Dr. Johnson at St. Jude Children's Research Hospital, 332 North Lauderdale, P.O. Box 318, Memphis, TN 38101.

Supported in part by a Cancer Center Support (CORE) Grant (CA 21765), a Leukemia Program Project Grant (CA 20180), and the American Lebanese Syrian Associated Charities.

Presented in part at the 25th Annual Meeting of the American Society of Hematology, San Francisco, 1983.

First report of BMT for sickle cell 1984
Patient transplanted for Leukemia but also happened to have sickle cell disease

VOLUME 335 AUGUST 8, 1996 NUMBER 6

BONE MARROW TRANSPLANTATION FOR SICKLE CELL DISEASE

Mark C. Walters, M.D., Melinda Patience, R.N., M.S.N., Wendy Leisenring, Ph.D., James R. Eckman, M.D., J. Paul Scott, M.D., William C. Mentzer, M.D., Sally C. Davies, M.D., Kwaku Ohene-Frempong, M.D., Françoise Bernaudin, M.D., Dana C. Matthews, M.D., Rainer Storb, M.D., and Keith M. Sullivan, M.D.

Twenty-one patients with sickle cell anemia and one patient with sickle β^+-thalassemia received marrow allografts from HLA-identical siblings between September 1991 and April 1995 at 15 collaborating transplantation centers. The 8 girls and 14 boys ranged in age from 3.3 to 13.9 years (median, 10.4) (Table 2). The indications for transplantation included a history of stroke (12 patients), recurrent acute chest syndrome (5 patients), and recurrent painful episodes (5 patients). The pretransplantation

First clinical trial of BMT for sickle cell 1996
12 of 22 patients were transplanted for stroke

Indications for BMT (Gluckman et al 2017)

One or more complications for each patient	% of the evaluable patients
Recurrent vaso-occlusive crises	**77%**
Stroke or CNS event	48%
Recurrent chest syndrome	32%
Elevated Cerebral Arterial Velocity	13%
Osteonecrosis of multiple joints	12%
Red-cell alloimmunization	11%
Growth impairment	7%
Cardiac insufficiency	6%
Sickle nephropathy	5%
Priapism	2%
Retinopathy	2%
Gonadal dysfunction	2%
Other	10%

Survival	Cure
91%[92-95]	73%[74-87]

Children	Adults
93%[92-95]	81%[74-87]

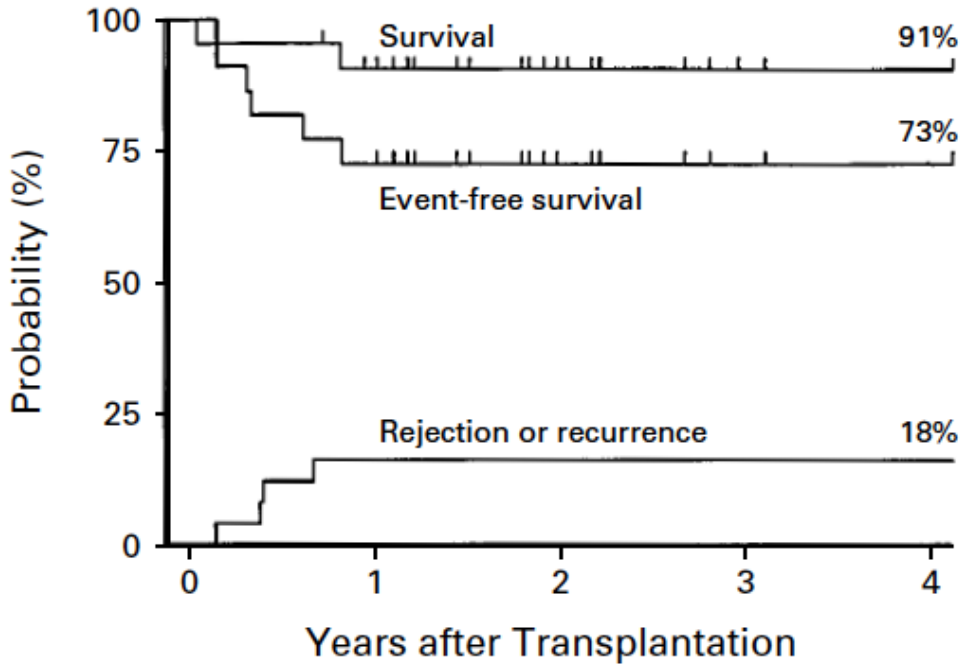
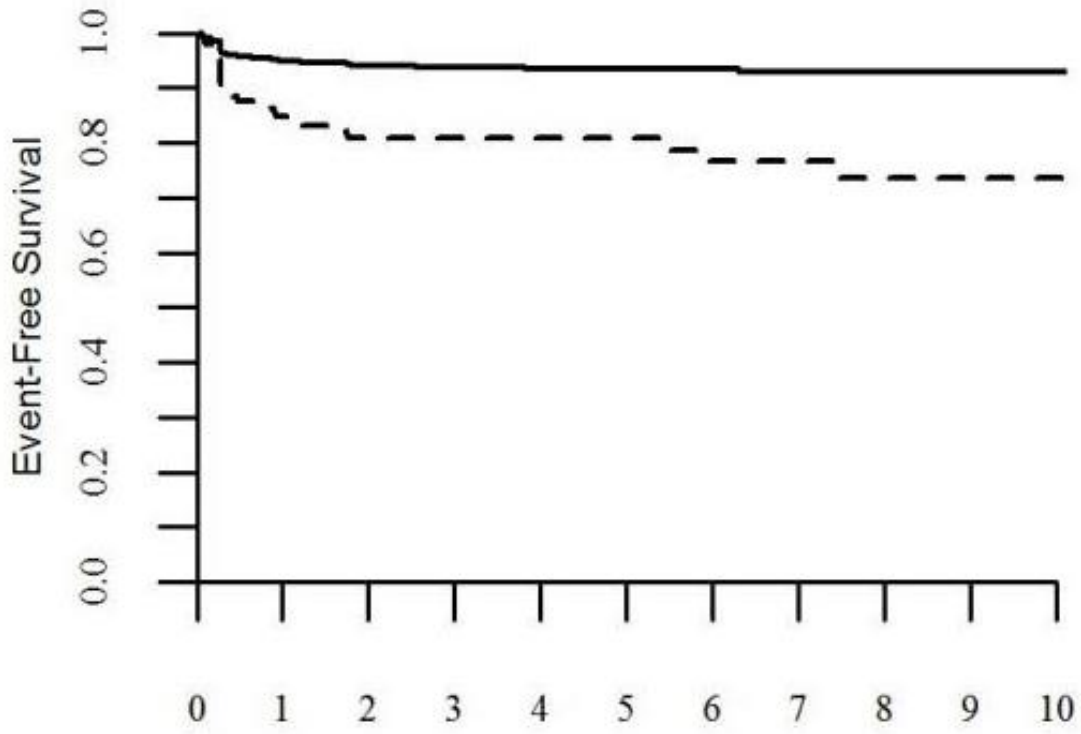


Figure 1. Kaplan–Meier Estimates of Survival and Event-free Survival after Bone Marrow Transplantation in 22 Patients with Sickle Cell Disease.

Walters et al 1996



Gluckman 2017

Time from transplant (years)
number of at-risk patients

Children	812	697	620	531	452	368	296	243	204	165	13
Adults	148	115	98	81	61	48	34	30	22	20	18

Increasing Survival and Cure Rate with Time

Cure Rates Depend on Patient Age and the Year of Transplant

	HR	95% CI	p-value
PBSC vs BM	1.93	0.87 – 4.26	0.104
CB vs BM	0.55	0.13 - 2.31	0.412
Patient age	1.09	1.05 - 1.12	<0.001
Year of Tx	0.95	0.90 - 0.99	0.013
RIC vs MAC	1.13	0.46-2.81	0.793
in vivo TCD	1.34	0.63 – 2.82	0.445

Younger age at BMT and year of transplant ≥2006 were independently associated both with a better cure rate

Gluckman et al 2017 BLOOD

Outcomes	Age 0-5 years	Age 6-15 years	Age >15 years	p
Neutrophil engr aftment (only for BM) @60d	97%±2	98%±1	98%±2	0.432
acute GvHD @ 100d	9%±2	18%±2	17%±4	0.022
chronic GvHD @3 yrs	9%±2	12%±2	20%±4	0.006
Chimerism(%):				
§	65	65	46	0.006
Full donor	32	32	49	**Cappelli et al BLOOD 2017**
Mixed chimera	3	3	5	
Autologous				
3- year EFS	96±2%	92±1%	84±4%	0.001
3- year OS	99±1%	95±1%	88±3%	<0.001

Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel

Recommendations

- *Young patients with symptomatic SCD who have an HLA-matched sibling donor should be transplanted as early as possible, preferably at pre-school age.*
- *Unmanipulated BM or UCB (whenever available) from matched sibling donors are the recommended stem cell source.*

Emerging Trends in BMT for SCD

- Increasing acceptance of BMT
- BMT for Milder disease/younger patients, fewer or no complications/silent infarcts/ with abnormal TCD
- BMT from unrelated donors
- BMT in Adults
- BMT from Haploidentical donors
- Centers for Medicare services (CMS) approves coverage in the context of prospective comparative clinical trials & study of QOL

Unrelated Donor BMT for SCD Results of a BMT CTN 0601:

- Umbilical cord blood arm of the study closed because of unacceptable graft rejection
- Of patients receiving BMT:
  - Overall survival at 1 year 86%
  - Survival and cured at 1 year 76%
  - Graft rejection: 10%
  - Chronic graft versus host disease extensive 38%; primary reason for mortality
  - No major organ toxicity
  - Reversible neurological complications 34%

Shenoy, BLOOD 2016

Non Ablative HCT from matched sibling donors for Adults with Severe Disease: the NIH Experience

- Median follow-up: 6 yrs (3 mo to 13 yrs)
- Overall survival 42 of 44 patients 95%, sickle-free survival 89%
- Low intensity, well tolerated, simple regimen
- Mixed chimerism in all, and is stable with or without immunosuppression
- Very low or no GvHD
- Replicated at UIC (adults), Alberta (children), and many other single patient experiences

Hsieh et al 2018

PROMIS outcomes (quality of life measures)

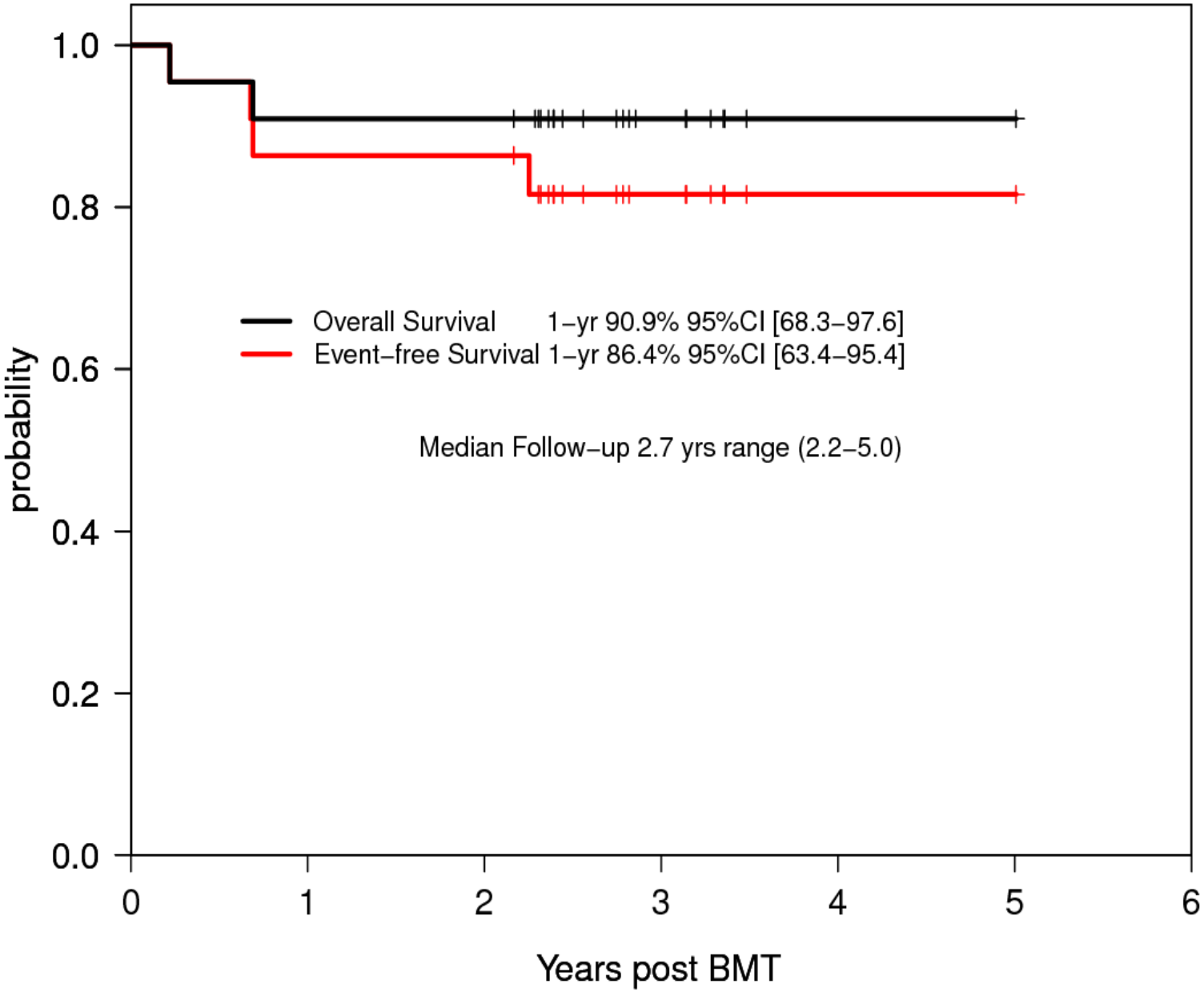
PROMIS Measures (n=20)	Pre-HSCT	Post-HSCT	Outcome
Pain intensity (NRS 0-10)	4.5 (0-8)	0.5 (0-7)	Improved (p<0.05)
Pain impact	60 (41-77)	41 (35-77)	Improved (p<0.05)
Anxiety	49 (37-67)	45 (37-74)	Unchanged (n/s)
Depression	46 (38-65)	46 (31-81)	Unchanged (n/s)
Satisfaction with social role	50 (27-66)	56 (31-66)	Improved (p<0.05)
Physical function	44 (32-59)	59 (32-59)	Improved (p<0.05)
Fatigue	53 (33-65)	43 (33-72)	Unchanged (trend)
Sleep disturbance	53 (31-78)	48 (31-74)	Unchanged (n/s)

Darbari et al, BJH 2018 14

STRIDE Pilot study of HCT for Young Adults with SCD

- Cure rate is ~90-95% after HLA-ID sibling HCT in pediatricSCD
- Toxicity observed after BMT in adults with SCD in early trials
- HLA-ID sib BMT in adults with SCD using non-myeloablative conditioning effective
- Alternate donor BMT will require higher intensity prep Bu+Flu+ATG conditioning effective in unrelated donor BMT for other conditions
- Is BMT with Bu+Flu+ATG in adults with SCD safe and effective?

Survival and Cure Rate at 1 year in Pilot Study.



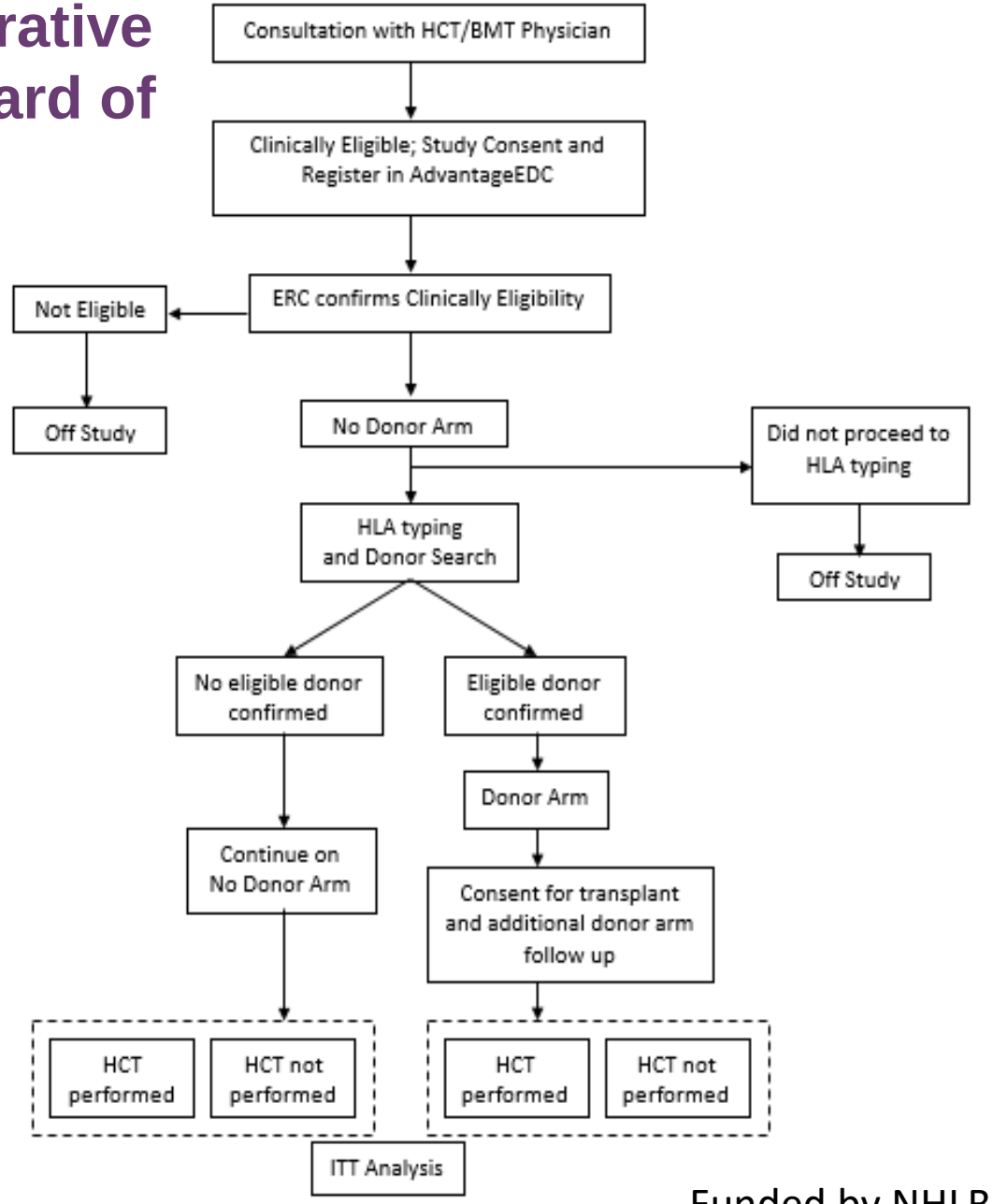
BMT CTN 1503

A Study to Compare Bone Marrow Transplantation to Standard Care in Adolescents and Young Adults with Severe Sickle Cell Disease

BLOOD AND MARROW TRANSPLANT CLINICAL TRIALS NETWORK

STRIDE the First Comparative Study of BMT and Standard of Care

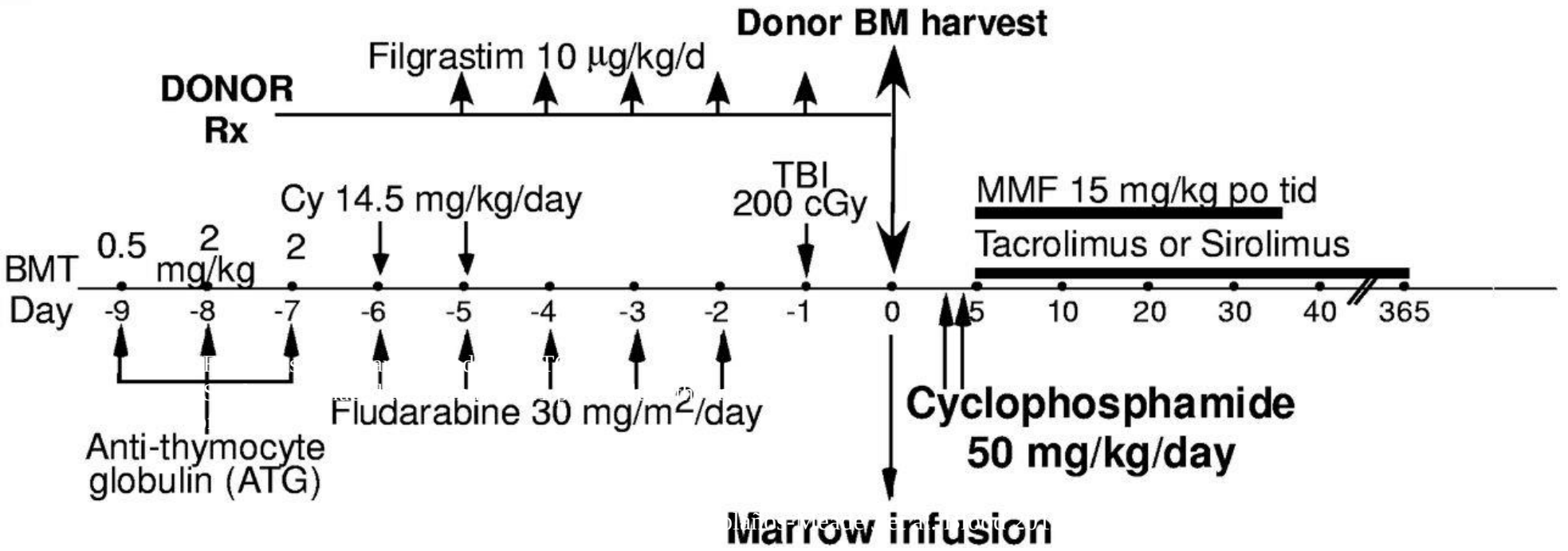
- Primary endpoint: overall survival at 2 years post biologic assignment
- Survival 3-10 years post HCT
- Impact of BMT on sickle-related events, organ function (pulmonary, renal), health related quality of life and pain assessments (via e-diary)



Funded by NHLBI-U01 (LK)

BMT from Haploidentical Donors Johns Hopkins Pioneer Giving Immunosuppression with Cyclophosphamide After Giving Stem Cells

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY



Addition of Thiotepa 10mg ± HU/Aza or TBI 400cGy

Experience with Haploidentical Transplantation

Cohort/ Institution	Lead-in therapy	Conditioning	Post-tx immune supp	Graft Source	N	Engraft-ment	Survival
St. Mary's, London	HU/Aza	Flu, CPM, rATG, TT, TBI 2Gy	CPM 50 x2, sirolimus	G-stim Marrow	12	11/12	92%
JHU	None	Flu, CPM, rATG, TBI 2-4 Gy	CPM 50 x 2, tacro or sirolimus	Marrow	31	22/31	97%
NY Med	HU/Aza	Flu, BU, CY, TT, rATG/ TLI	TCD, tacro	PBSC	8	8/8	7/8
VanderbiltConsort.	None	Flu, CPM, rATG, TBI 2 Gy	CPM 50 x2, sirolimus	G-stim Marrow	5	2/5	100%
VanderbiltConsort.	None	Flu, CPM, rATG, TT, TBI 2 Gy	CPM 50 x2, sirolimus	G-stim Marrow	6	6/6	100%

BMT CTN 1507 Haploidentical HCT for SCD

- Primary Endpoint: Disease-free survival (DFS) at 1 year with each stratum
- DFS is defined as survival with stable donor erythropoiesis

Events that count to DFS are:

- primary/late graft rejection or 2^{nd} transplant
- death

Haploidentical Stem Cell Transplantation with Ex-vivo CD3+/CD19+ Depleted Peripheral Stem Cells

- 10 patients with SCD transplanted with a $CD3^{+}/CD19^{+}$ depleted T-haplo-SCT
- Conditioning thiotepa, fludarabine, treosulfan and ATG-F.
- Long-term engraftment was achieved in nine of ten patients with a complete or stable mixed chimerism
- one patient succumbed to CMV pneumonitis.
- five patients limited Grade I aGvHD
- one patient presented a steroid sensitive mild chronic GvHD.

Selim Corbacioglu et al BBMT 2017

Unmanipulated UCB Co-Transplantation of Ex Vivo Expanded UCB Progenitor Cells with Nicotinamide (Nicord)

- engrafted neutrophils at a median of 7 (range 6-20)
- long term engraftment from NiCord (n = 2), UM unit (n = 4) One secondary graft failure on day 13 and died after a second
- transplant.
- Six patients had acute GVHD (grade II = 3, IIIIV
- = 3) ; 3 chronic GVHD. One patient died on day +241 from
- liver GVHD. Six of the 7 patients with sustained full donor
- cell engraftment are currently alive disease free at a median
- follow up of 37 (range 5.5-48) months.

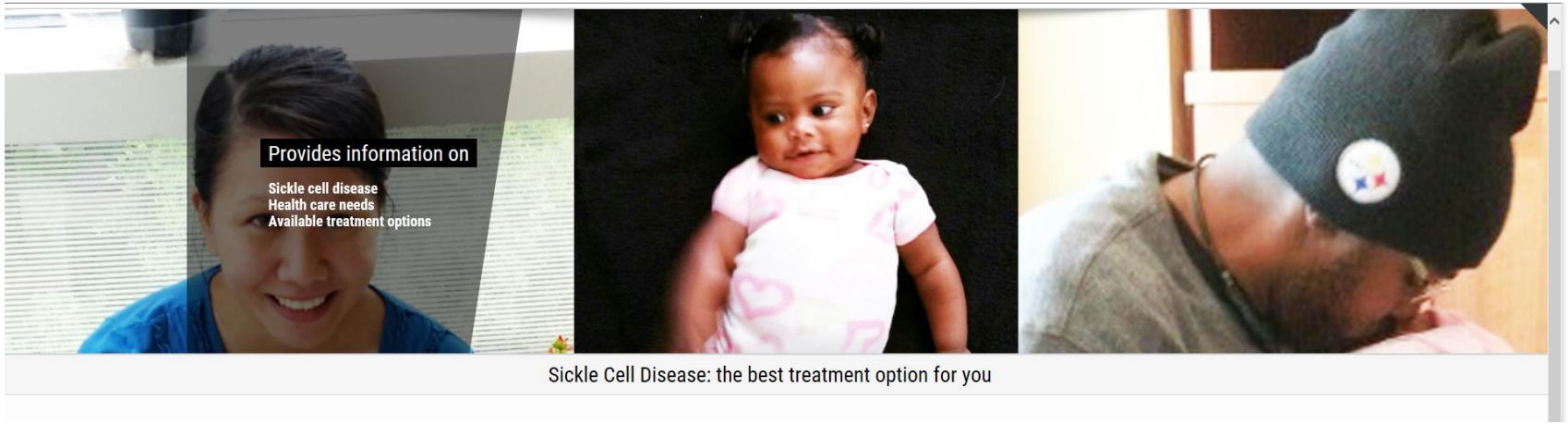
Suhag Parikh et al BBMT 2017

The Gap Between Availability and Acceptability of HCT for SCD: A Single Center Experience

- Large volume of pediatric SCD patients (> 1900), HCT for SCD (>90)
- In a two year look back period:
- Proportion of Adolescents meeting disease severity criteria 18%
- Proportion undergoing HLA typing 6.5%
- Proportion undergoing donor search 2.9%
- Proportion undergoing BMT 0.4%
- Proportion of all patients with Medicaid 80%
- Proportion of SCD patients undergoing HCT with Medicaid 59%. No difference in SES

Krishnamurti, 2018

Sickleoptions.org : Sickle Cell Decision Aid



- Provides disease-related information
- Assists to identify what is important to you, preferences and how to talk with health care providers
- Options for SCD treatment
- Treatment risks, benefits, outcomes
- Questions? Email Diana Ross (diana.ross @emory.edu)

What We Talked About Today

- Excellent results in children with HLA identical sibling donor
- Challenge: donor availability, awareness/access, gonadal toxicity, need to study long term outcomes
- BMT from unrelated donors feasible in children.
- Additional challenge: Rate of CGVHD. knowledge and service gap in adults
- BMT from Haploidentical family donors feasible, expands donor pool
- Challenge: Donor specific HLA antibodies, Engraftment
- Is BMT superior to standard of care. No comparison data
- STRIDE, a comparison trial of BMT vs. standard of care. Pilot data suggest that conditioning regimen safe, effective. comparison study ongoing
- Increase awareness and acceptance of HCT. 50% of HCTs since 2007
- Patients not aware of option. Medicaid coverage may be problematic. www.sickleoptions.org is a useful resource.
- Gene therapy studies open. Early results promising

Current and Novel Preventive and Curative Interventions for the Treatment of Patients with Sickle Cell Disease

John F. Tisdale, M.D.

Cellular and Molecular Therapeutics Branch,
NHLBI, National Institutes of Health

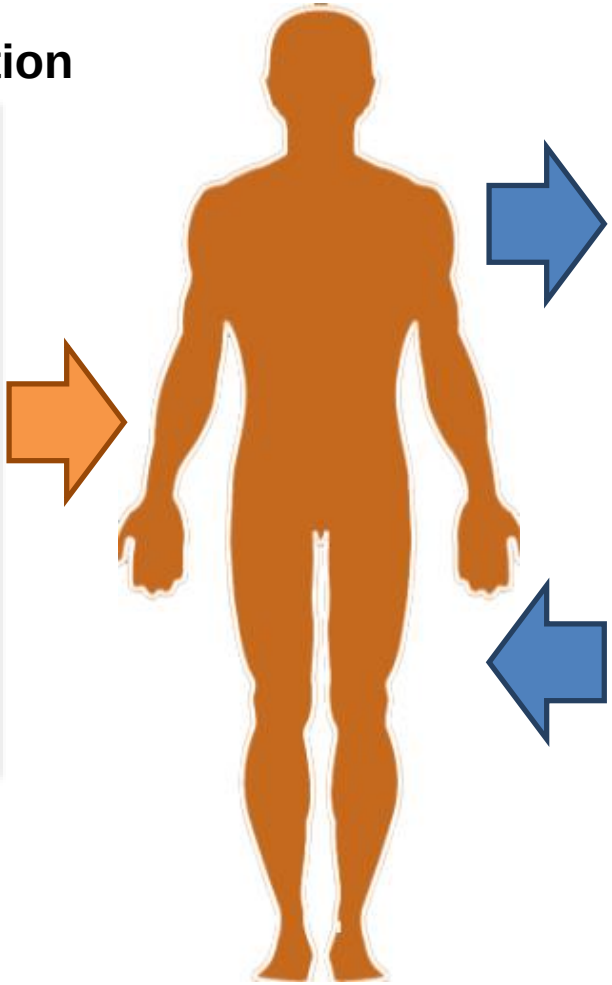
Bone marrow stem cell transplant strategies for SCD

National Institutes of Health

1. Allogeneic transplantation

Bone marrow transplant from someone who does not have SCD

Donor is usually an HLA-matched sibling, but could include cord blood or half-matched family member



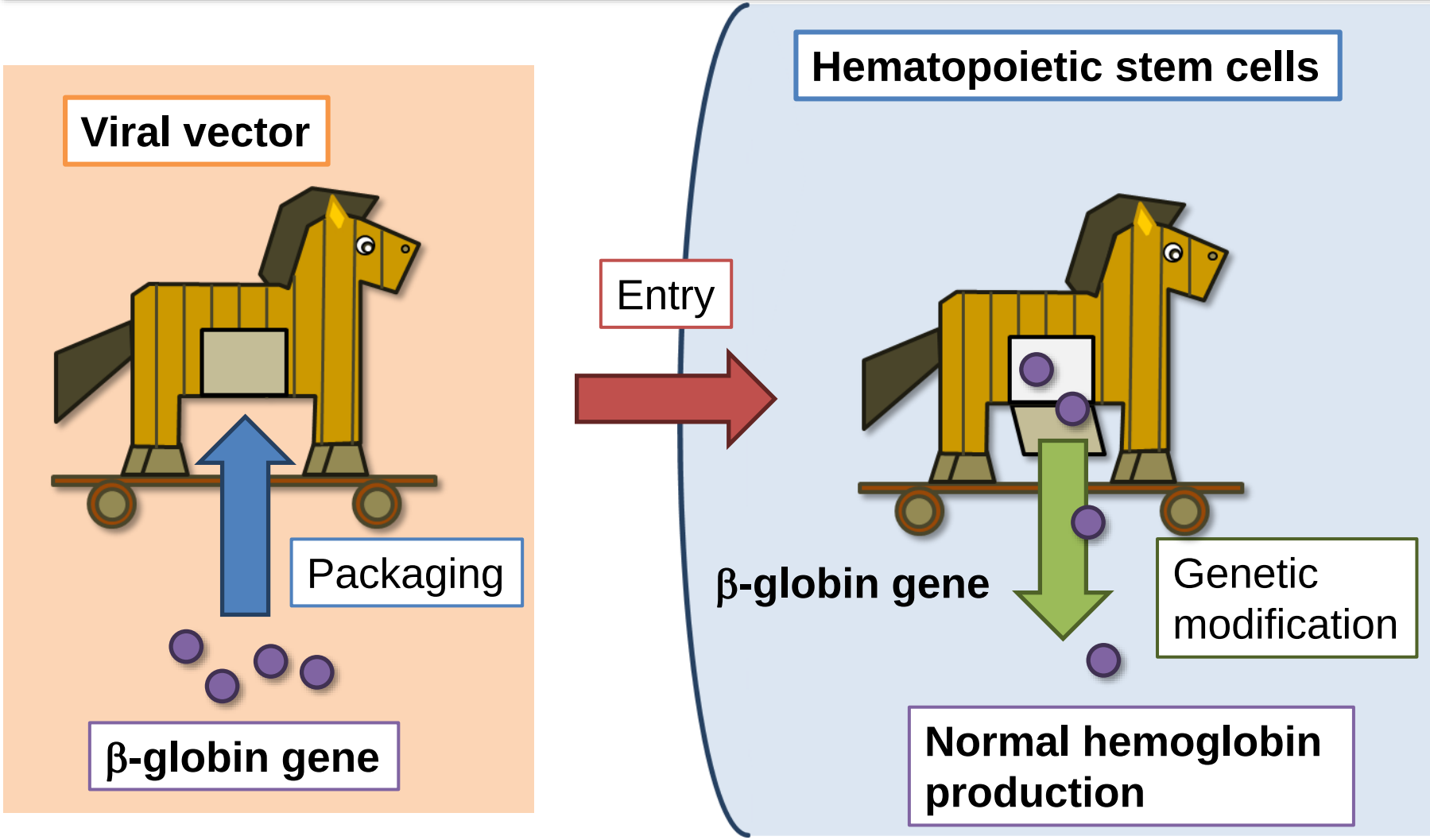
Sickle cell disease patients

2. Autologous gene therapy

Bone marrow transplant from patient's own bone marrow

β-globin gene transfer with an engineered virus to transfer or gene editing with an engineered endonuclease

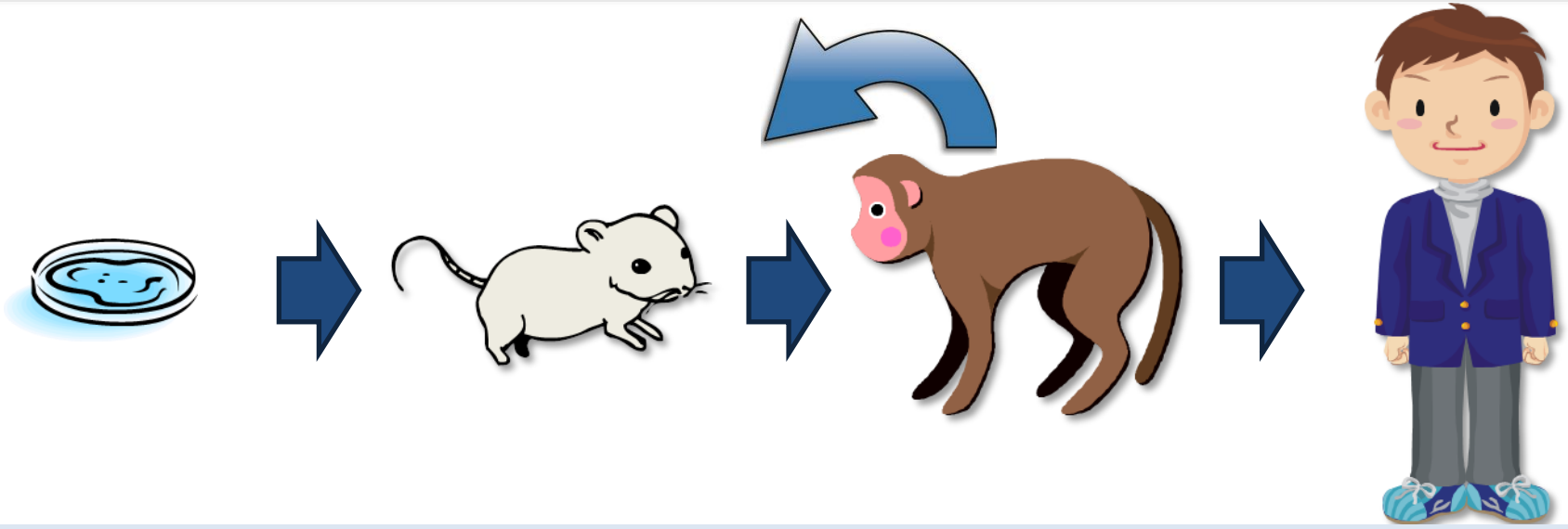
Gene transfer for “gene addition” therapies



Stringent requirements for the hemoglobinopathies including sustained, high-level, lineage restricted expression of therapeutic globin sufficient to overcome HbS

Translational research strategy to develop gene therapy for sickle cell disease

National Institutes of Health



Cell culture	Small animal	Large animal	Clinical trial
Cell lines iPS cells	Mice Disease model mice Humanized mice	Non-human primates	Phase I Phase II Phase III Phase IV

Efficiency

Cell lines $>$ Mouse HSCs $>>$ Rhesus HSCs $\approx$ Human HSCs

HGB-206: study of LentiGlobin gene therapy for severe sickle cell disease

HGB-206

Key Enrollment Criteria

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

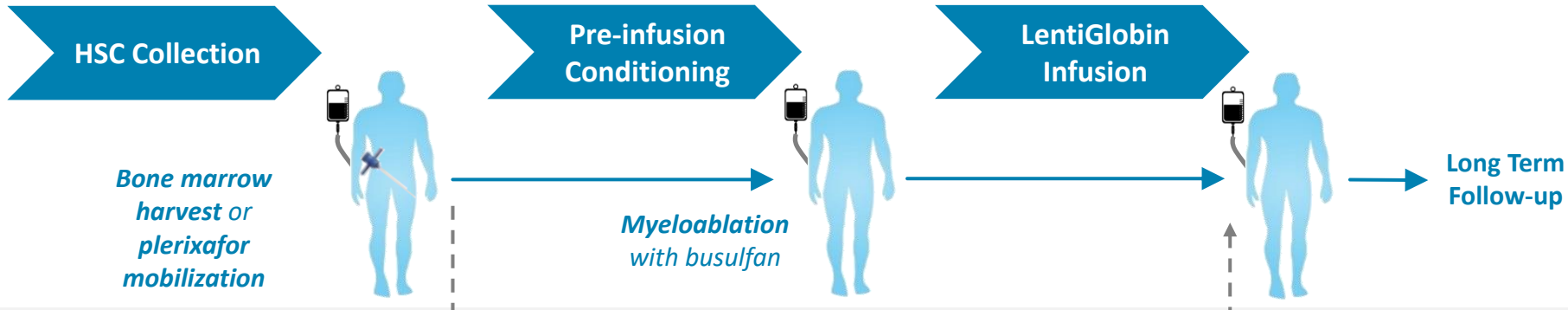
Target enrollment: up to 29

Study Objectives

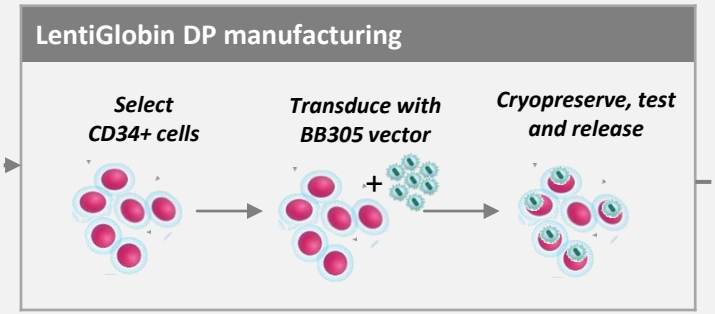
- Primary objective: Safety
- Key Secondary Objectives:
  - Frequency of VOCs and ACS
  - HbA^{T87Q} production
  - Total Hb and Hb fractions
  - Vector copies in peripheral blood

Study initiated August 2014

Evolution of HGB-206: Protocol and manufacturing changes promise improved outcomes

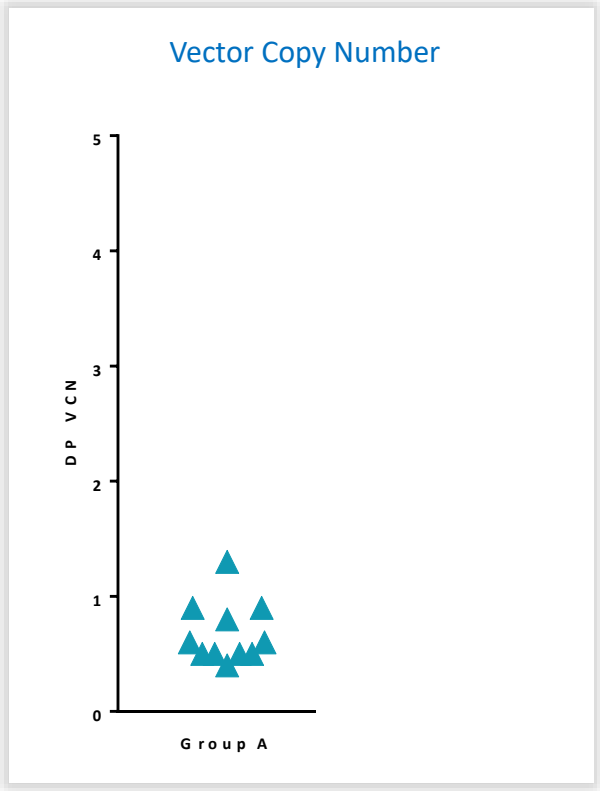


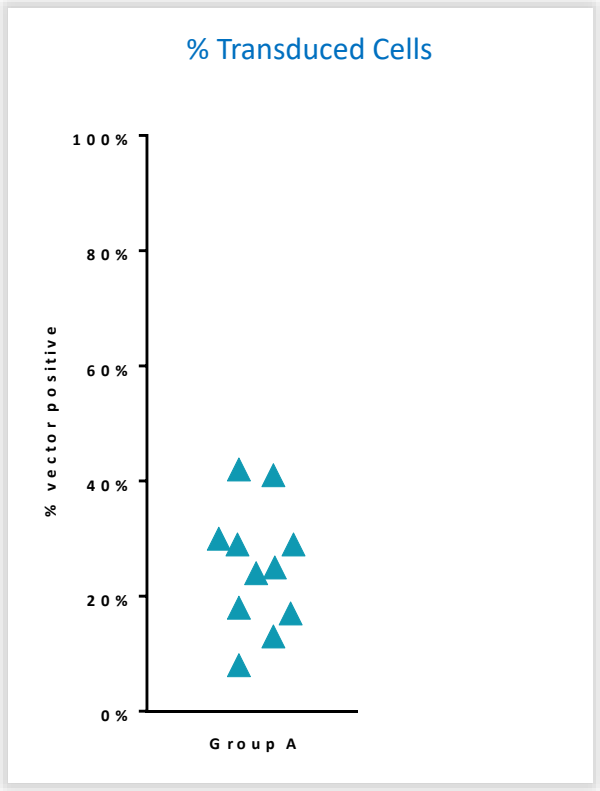
	Group A	Group B	Group C
Pre-collection transfusion regimen	Optional	Required	Required
HSC source	Bone marrow	Bone marrow	Mobilized PB
Manufacturing process	Original	Orig → Refined	Refined
	N=7	N=2	N=1



[a]Protocol was modified to increase DP VCN, require pre-harvest transfusions, increase target busulfan levels, and explore the use of plerixafor for mobilization and apheresis for cell collection. [b]Patients underwent plerixafor mobilization & apheresis for exploratory analysis
BMH, bone marrow harvest; DP, drug product; HSC, hematopoietic stem cell; VCN, vector copy number.

Enhancements to manufacturing lead to improved cell product characteristics

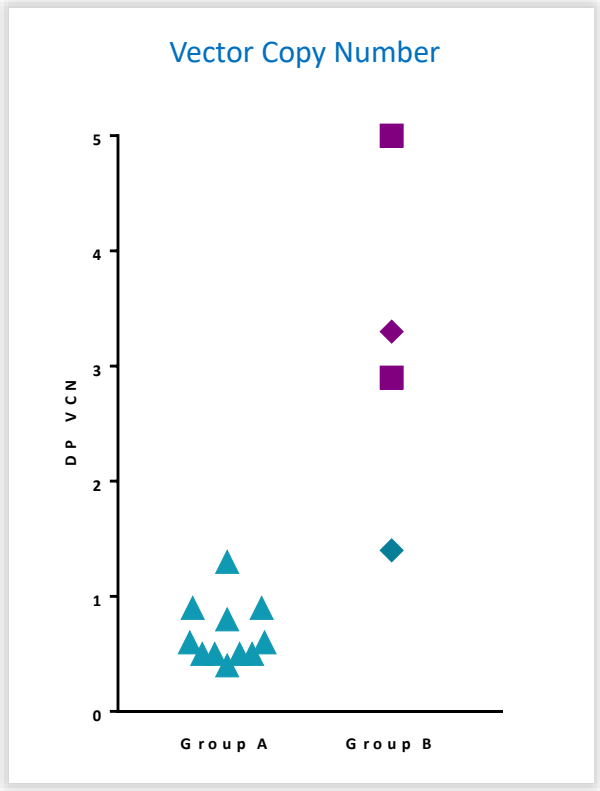


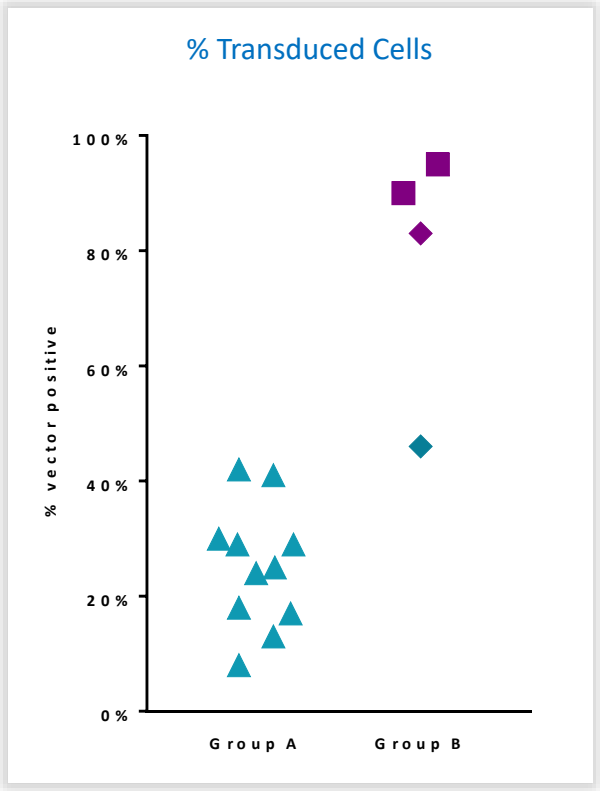


LentiGlobin manufacturing process
Original
Refined

Data as of November 30, 2017

Enhancements to manufacturing lead to improved cell product characteristics



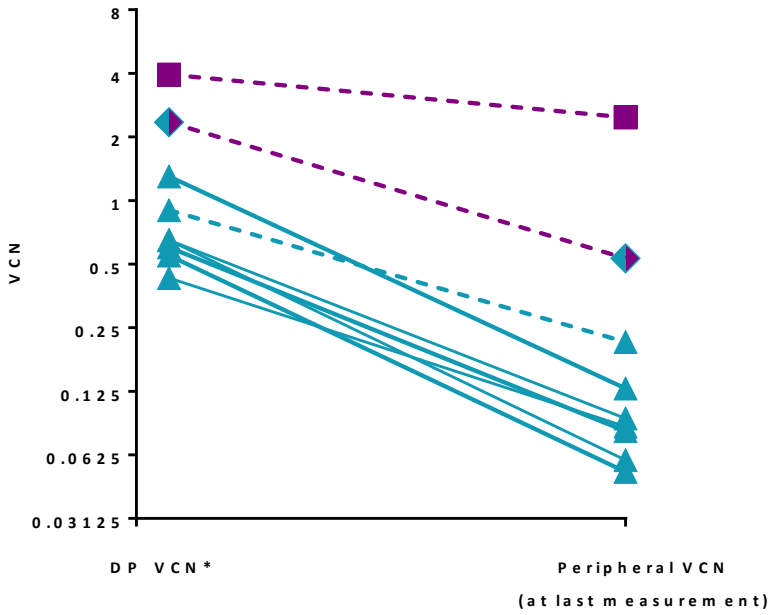


LentiGlobin manufacturing process
Original
Refined

Data as of November 30, 2017

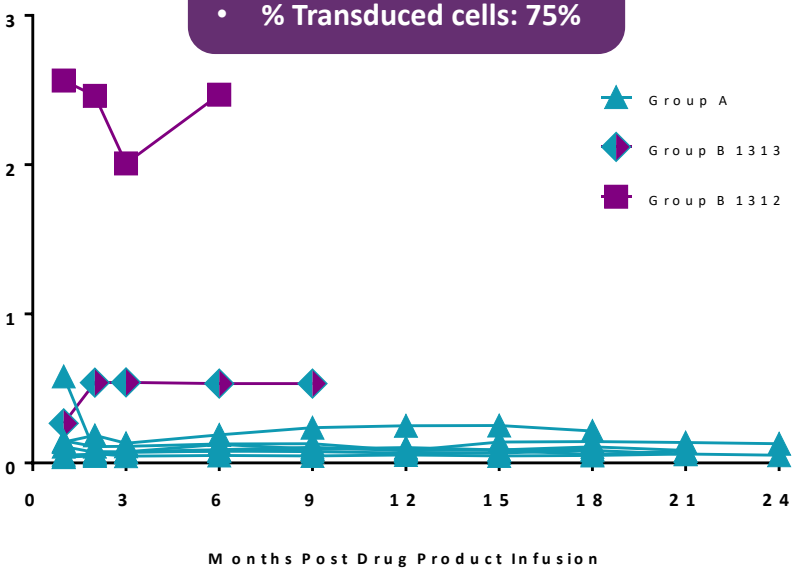
Product VCN and peripheral blood VCN are higher in patients in Group B than Group A

VCN drop from drug product to peripheral blood



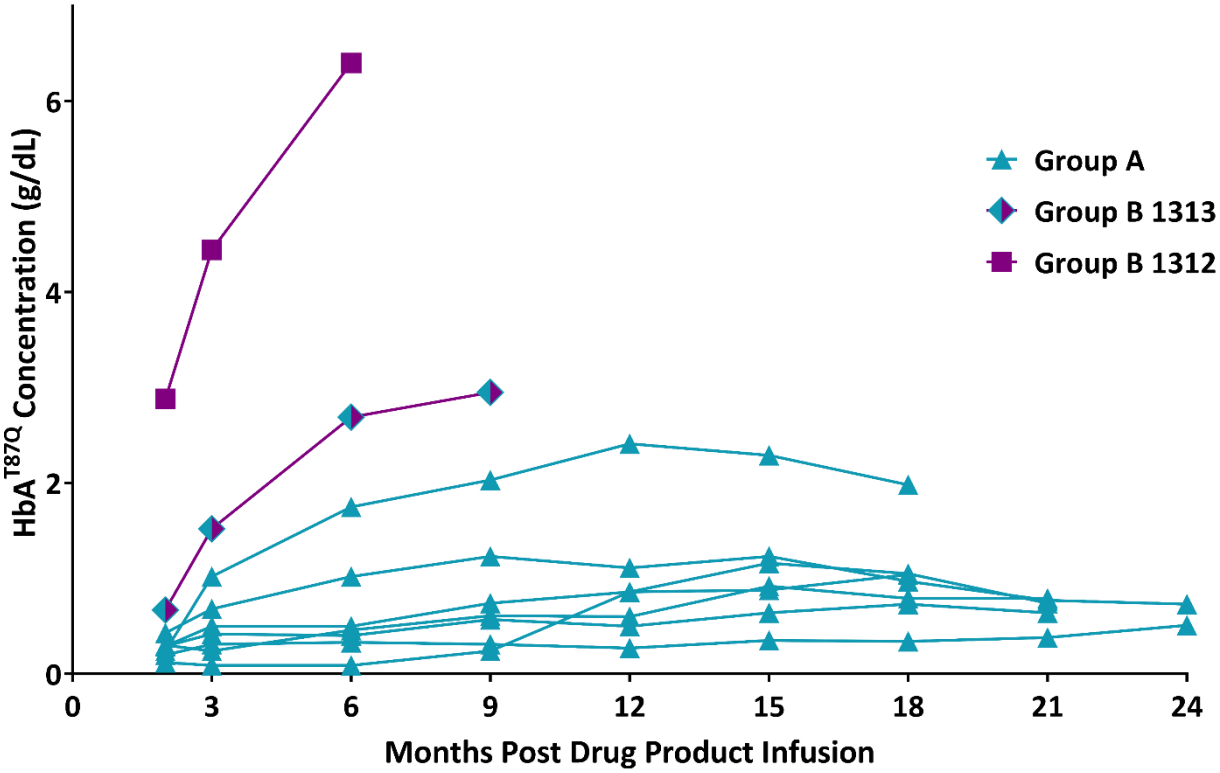
Peripheral blood VCN over time

Pt. 1312 6 month marrow
- **VCN: 2.78**
- **% Transduced cells: 75%**



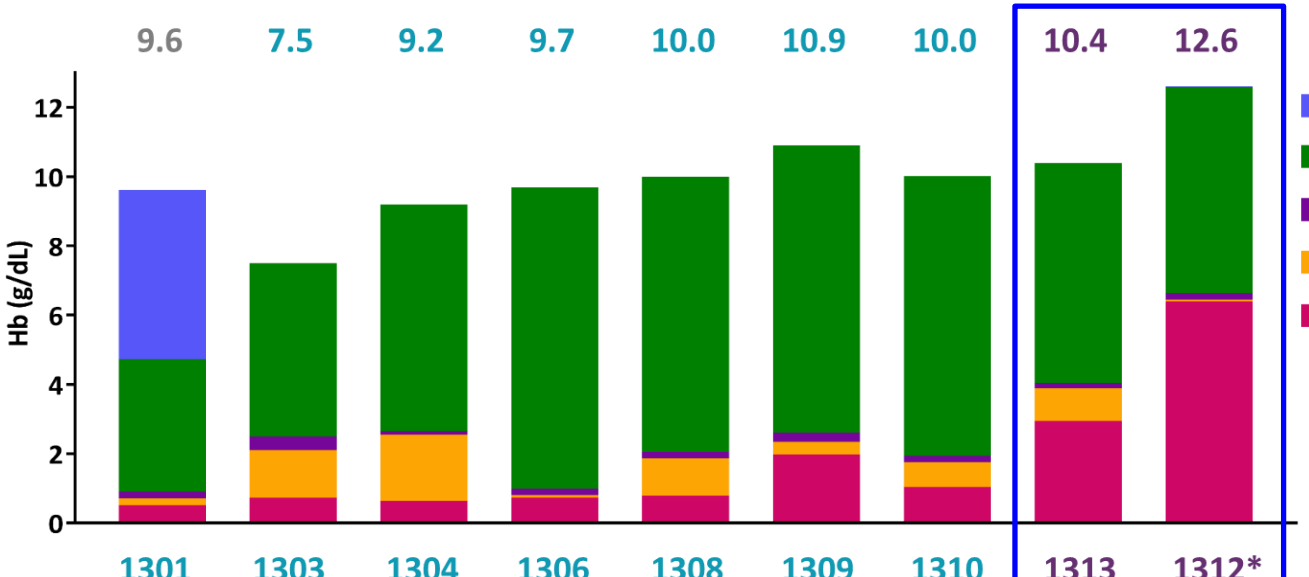
Data as of Oct 26, 2017 for Grp A; Nov 30 for Grp B Pt 1312

Patients in Group B demonstrate higher HbA^{T87Q} production than Group A



Data as of Oct 26, 2017 for Grp A; Nov 30 for Grp B Pt 1312

Improvements in manufacturing have resulted in normalization of Hb patient 1312



	1301	1303	1304	1306	1308	1309	1310	1313	1312*
Follow up (m)	24	24	21	21	21	18	18	9	6
% HbA^{T87Q}	5	10	7	8	8	18	10	28	51
% HbF	2	18	21	1	11	3	7	9	0
% HbS	40	67	71	90	79	76	81	61	47

Data as of Oct 26, 2017 for Grp A; Nov 30 for Grp B Pt 1312

Safety profile consistent with myeloablative conditioning After LentiGlobin DP infusion

Non-hematologic grade ≥3 AEs post DP infusion reported in ≥2 patients	Incidence n[#] (%)
Sickle cell anemia with crisis	5 (55.6)
Febrile neutropenia	5 (55.6)
Stomatitis	7 (77.8)
Bacteremia	2 (22.2)
Pyrexia	2 (22.2)
Pharyngeal inflammation	3 (33.3)

[#]In 9 patients; 7 Group A and 2 Group B

- Grade ≥3 hematologic AEs post infusion consistent with myeloablative busulfan conditioning
- SAEs in 8 patients, most common being sickle cell anemia with crisis (n=4)
- 1 patient in Group C: no unexpected Grade ≥3 AEs, no SAEs or DP-related AEs*
- **1 AE (hot flush, Grade 1) considered related to LentiGlobin DP**
- **No replication competent lentivirus detected**
- **Continued highly polyclonal repopulation**

Data as of Oct 26, 2017 for Grp A; Nov 30 for Grp B Pt 1312

$CD34^{dim}$ and $CD34^{bright}$ populations in BM

Figure 4. Imagestream Data Show Lower Purity in CD34+ Cells in Subjects with SCD

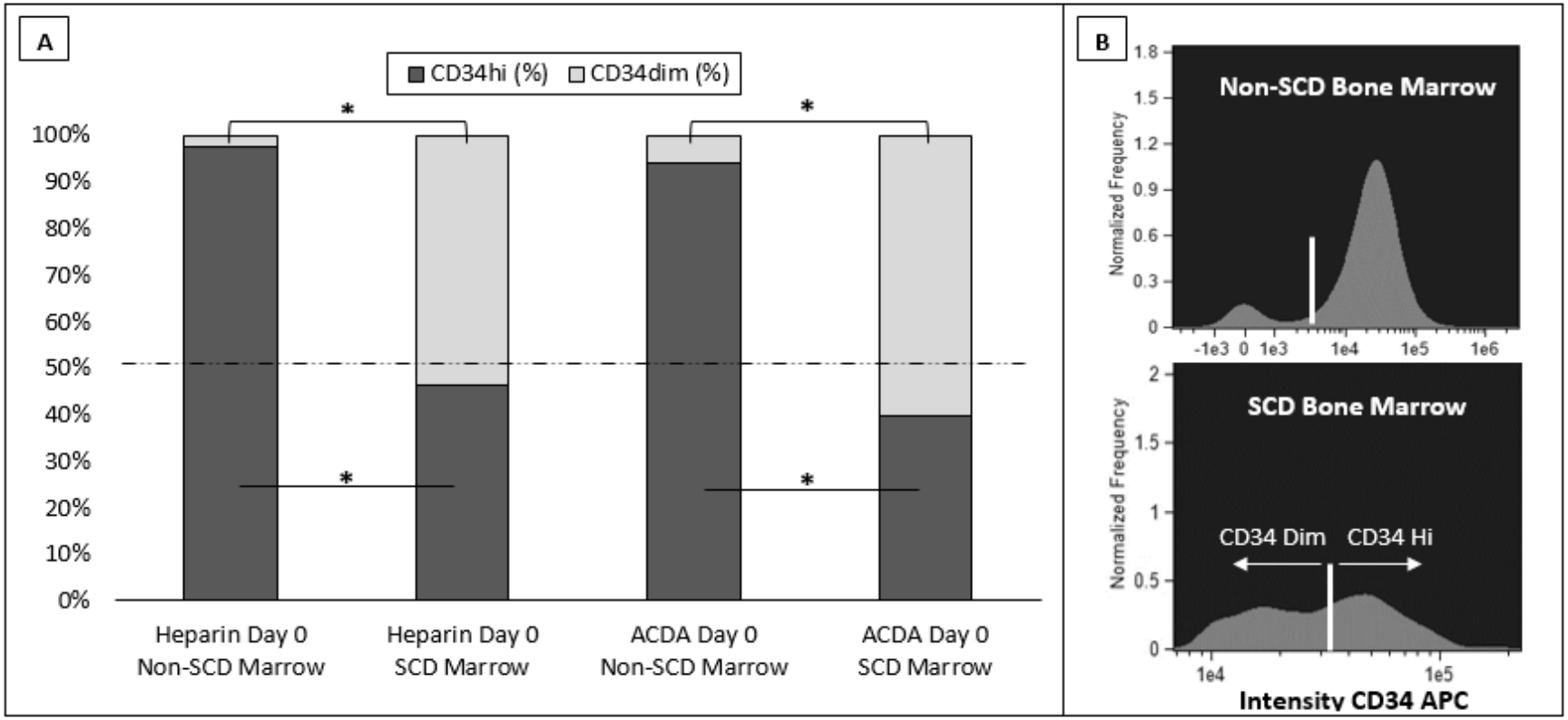
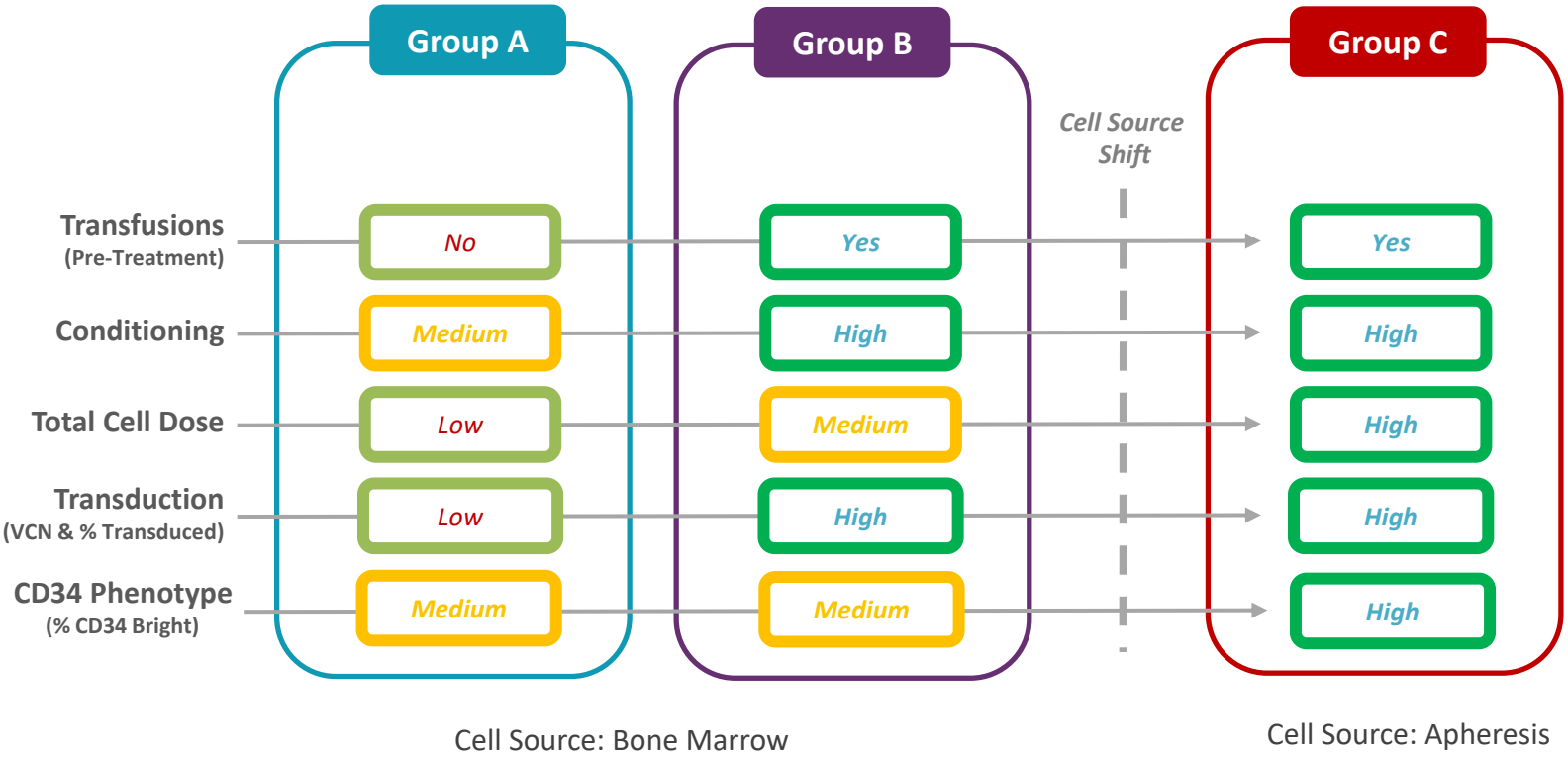


Figure 4. CD34+ Antibody Intensity in SCD vs. Non-SCD Bone Marrow. Image Flow Cytometry performed on CD34+ selected HSCs collected in two different anticoagulants (Heparin and ACD-A) and processed immediately after collection demonstrated a significantly lower proportion of CD34hi HSCs in SCD marrow compared to non-SCD marrow. (A) Greater than 50% of SCD HSCs are characterized as CD34dim. (B) ImageStream histogram plot displays two populations of CD34+ antibody intensity corresponding to CD34hi and CD34dim populations. *p<0.001

Alexis Leonard and John Pierciey

Evolution of HGB-206: Protocol and manufacturing changes promise improved outcomes



** 7 patients mobilized*

DP manufactured for 5 patients

*Tisdale J et al, ASBMT Abstract # 188

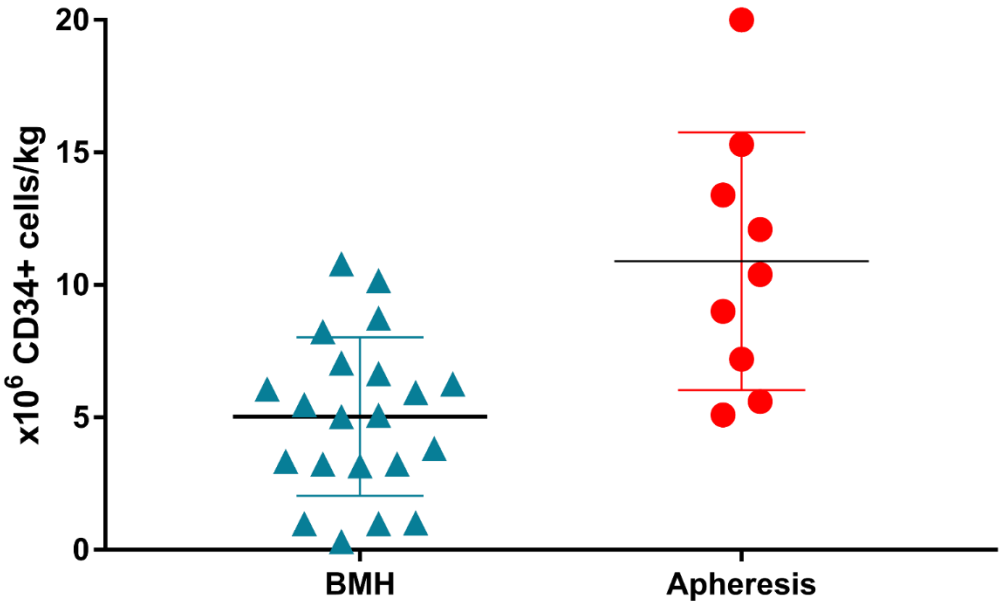
Safety with bone marrow harvest vs plerixafor mobilization and apheresis

- In 26 BMHs in 9 patients, 17 ≥ grade 3 AEs were reported in 5 patients
  - 10 grade 3 AEs of procedural pain in 5 patients, including 1 SAE
  - 3 grade 3 AEs of anemia in 2 patients
  - 3 grade 3 SAEs of SCD-related pain crisis in 2 patients
  - 1 event of decreased lymphocyte count

- In 7 patients who underwent mobilization and apheresis, 5 ≥ grade 3 AEs were reported in 3 patients
  - 2 non-serious grade 3 AEs in 1 patient each: hypomagnesemia and non-cardiac chest pain
  - 3 grade 3 SAEs of SCD-related pain crisis in 3 patients
  - Pain crises were non-severe and were consistent with patients' histories of vaso-occlusive events. The affected patients were hospitalized, or hospitalization was prolonged, for standard management. All 3 patients recovered without sequelae.

AE, adverse event; SAE, serious adverse event; SCD, sickle cell disease

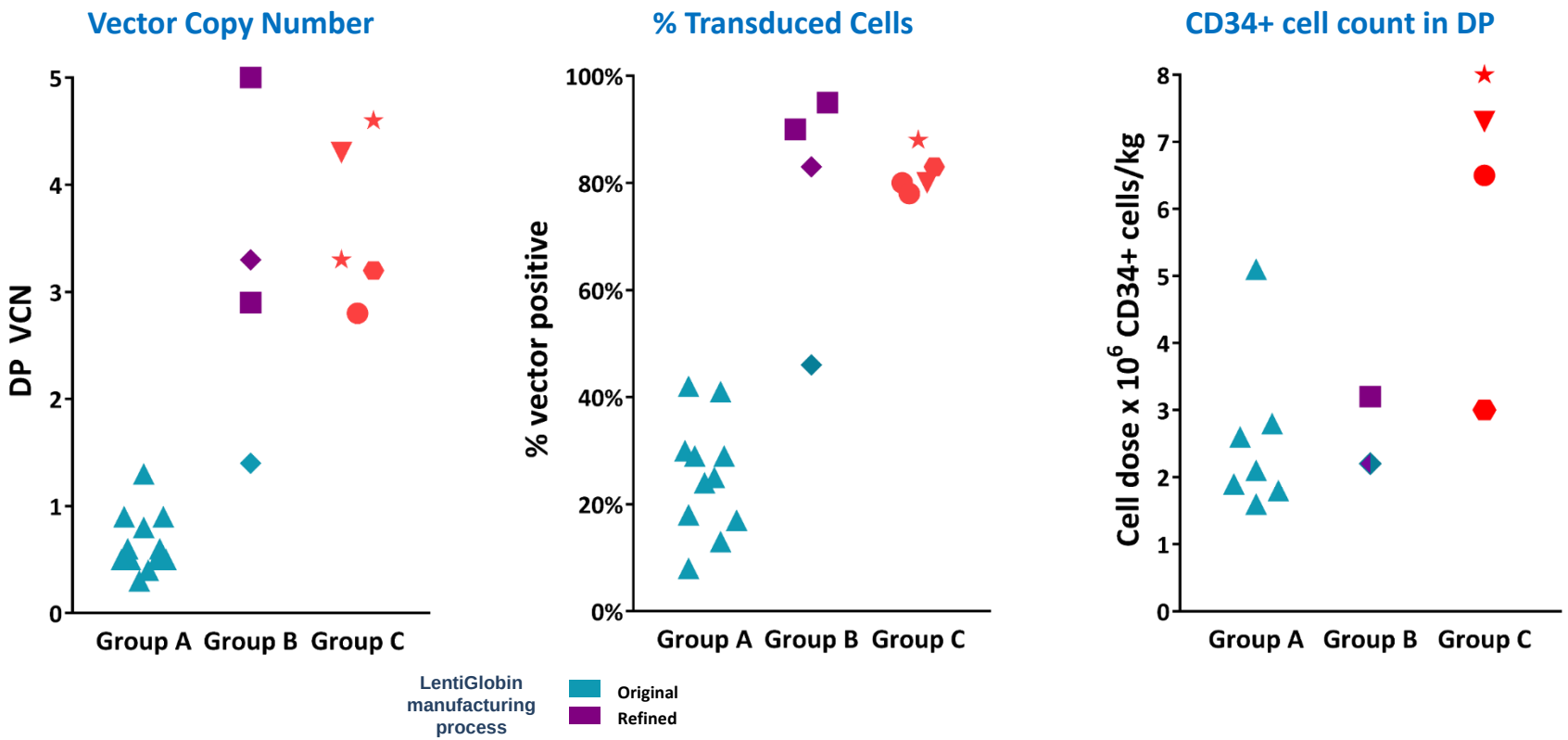
Total CD34+ cells collected per collection cycle higher after mobilization with plerixafor



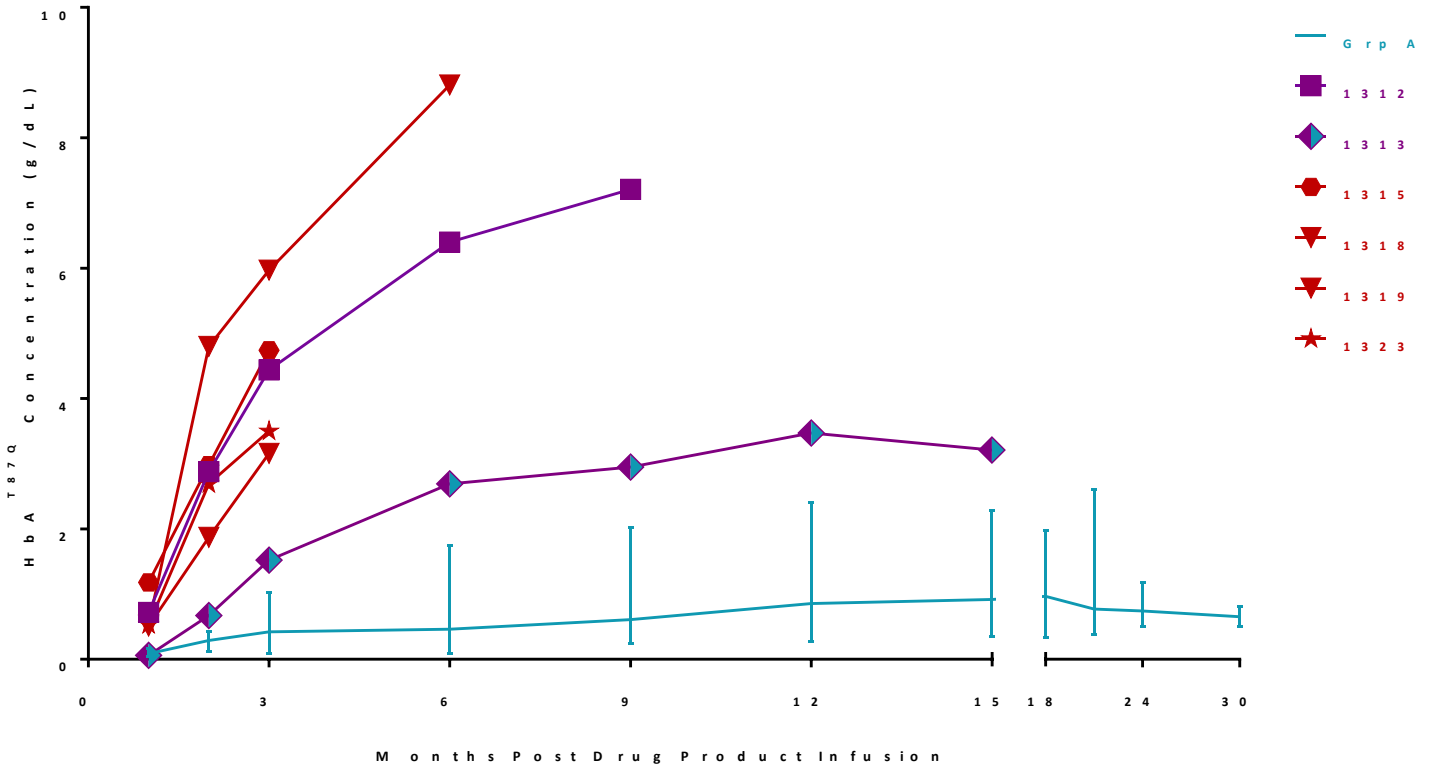
- **A median of 5.0** (0.3 – 10.8) x 10^6 CD34+ cells/kg were collected per BMH.
- **A median of 10.4** (5.1 – 20.0) x 10^6 CD34+ cells/kg were collected per apheresis cycle.
- Sufficient cells for both DP manufacture and back-up were collected in 1 apheresis cycle in 3/6 patients.
- A median of 2 (1 – 4) BMH were required to collect adequate cells for both DP manufacture and back-up.

BMH, bone marrow harvest; DP, drug product

Peripheral blood CD34+ cells transduce comparably to bone marrow-derived CD34+ cells, while enabling higher cell doses



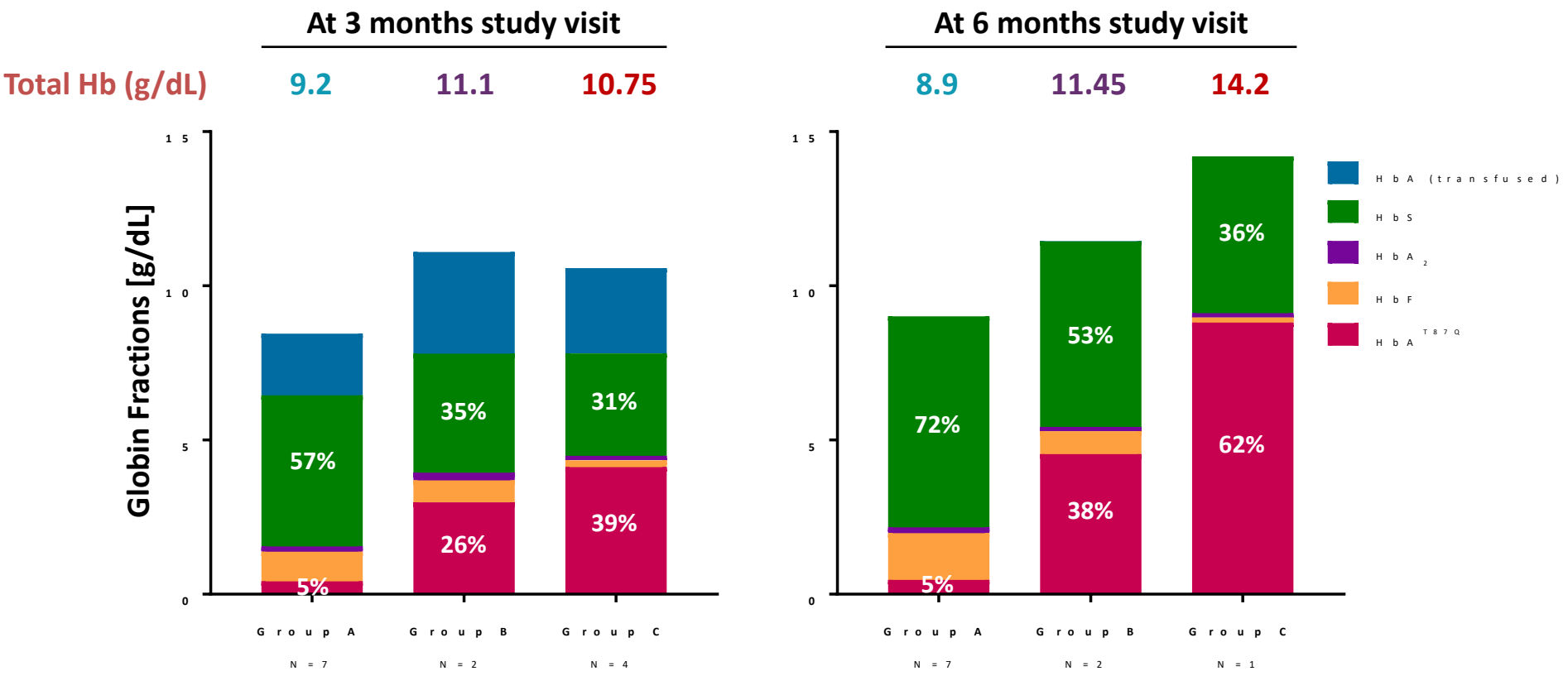
Patients in Group B and C demonstrate higher HbA^{T87Q} production



For Group A patients, medians (min, max) depicted; Group A patients with month 30 study visit (N=3)

Data as of May 15, 2018

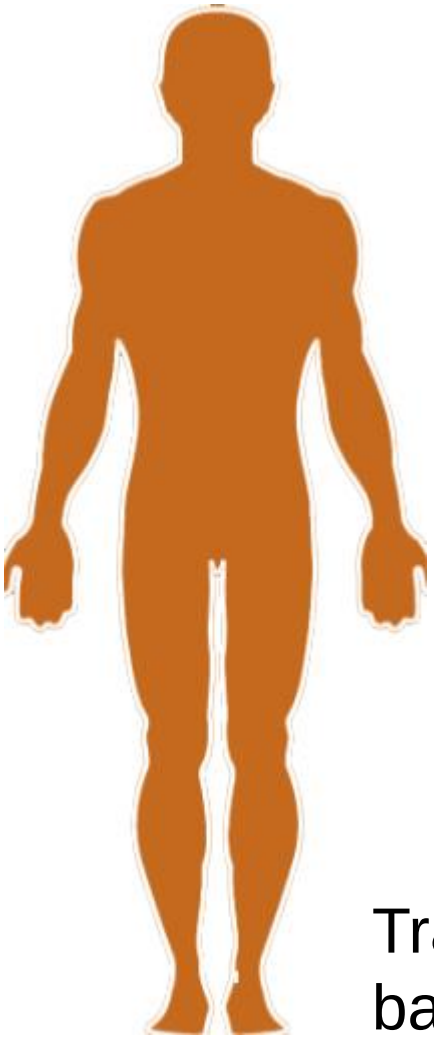
Higher vector-derived Hb in Group B and C patients at 3 and 6 months



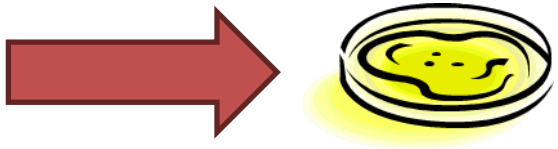
Median for DP-infused patients depicted, except for Group C at 6 months given N=1

Data as of May 15, 2018

Autologous bone marrow stem cell-targeted gene editing



Harvest



Patients' own bone marrow stem cells

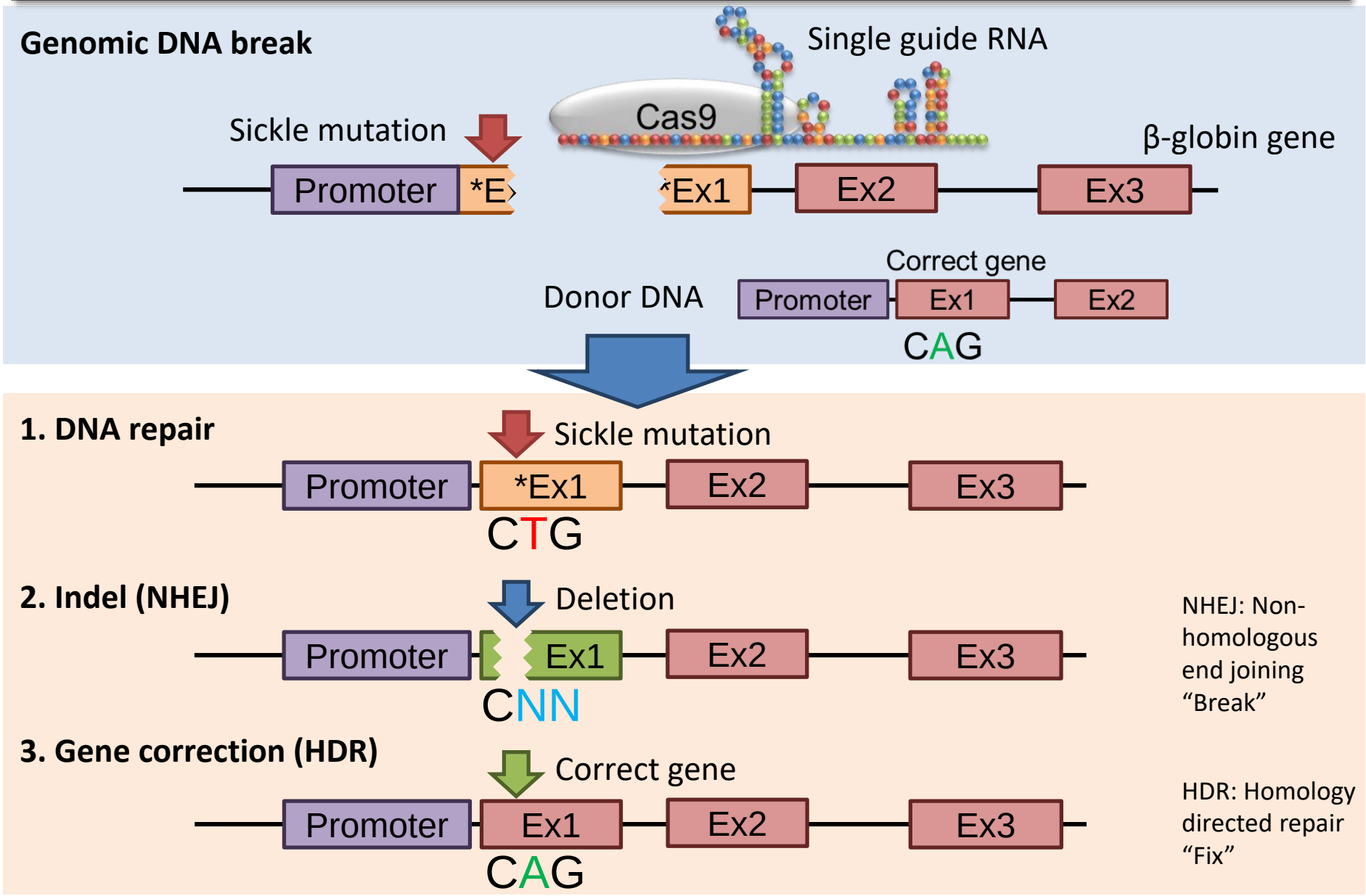
Reactivate fetal hemoglobin by cutting repressor genes or correct the mutation by cutting and repairing

Transplantation back to patients

- Patients serve as their own donor
- Available for all patients
- No need for immunosuppression
- No risk of GVHD

SCD patients

CRISPR/Cas9 system for genome editing

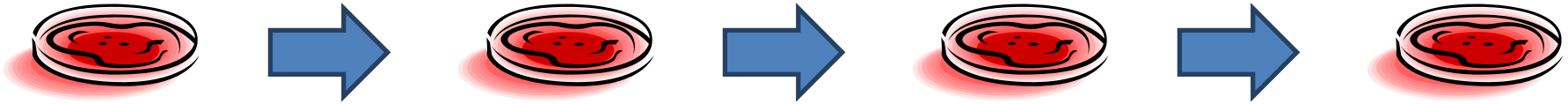


NHEJ: Non-homologous end joining "Break"

HDR: Homology directed repair "Fix"

Gene correction with CRISPR/Cas9 in SCD CD34+ cells

Guide RNA targeting the β-globin gene
Cas9 mRNA or Cas9 protein
Donor ssDNA : 80, 120, or 200 µg/ml



SCD CD34+ cells

MaxCyte electroporation

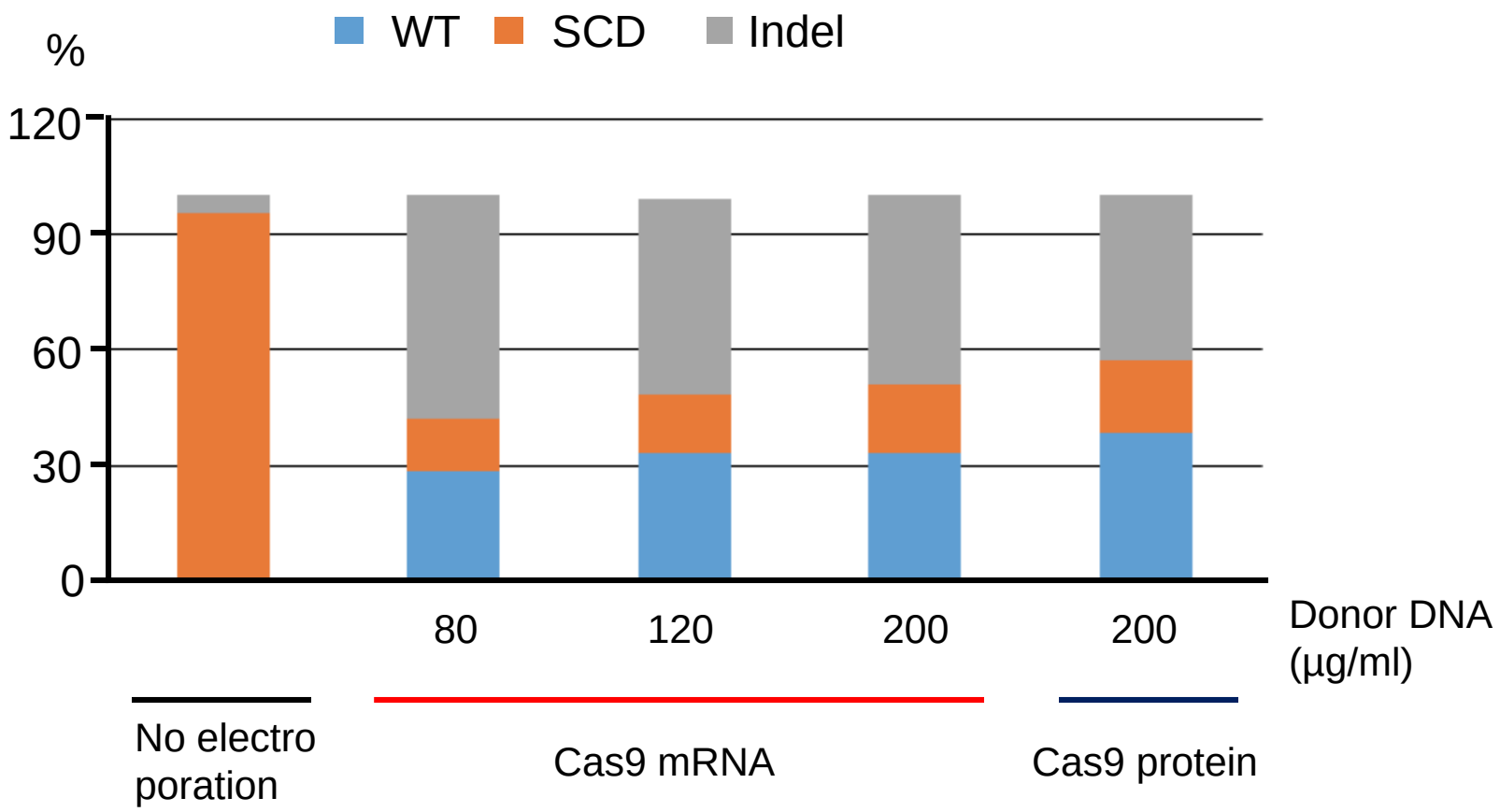
Erythroid differentiation

Electrophoresis
RP-HPLC
Targeted sequence

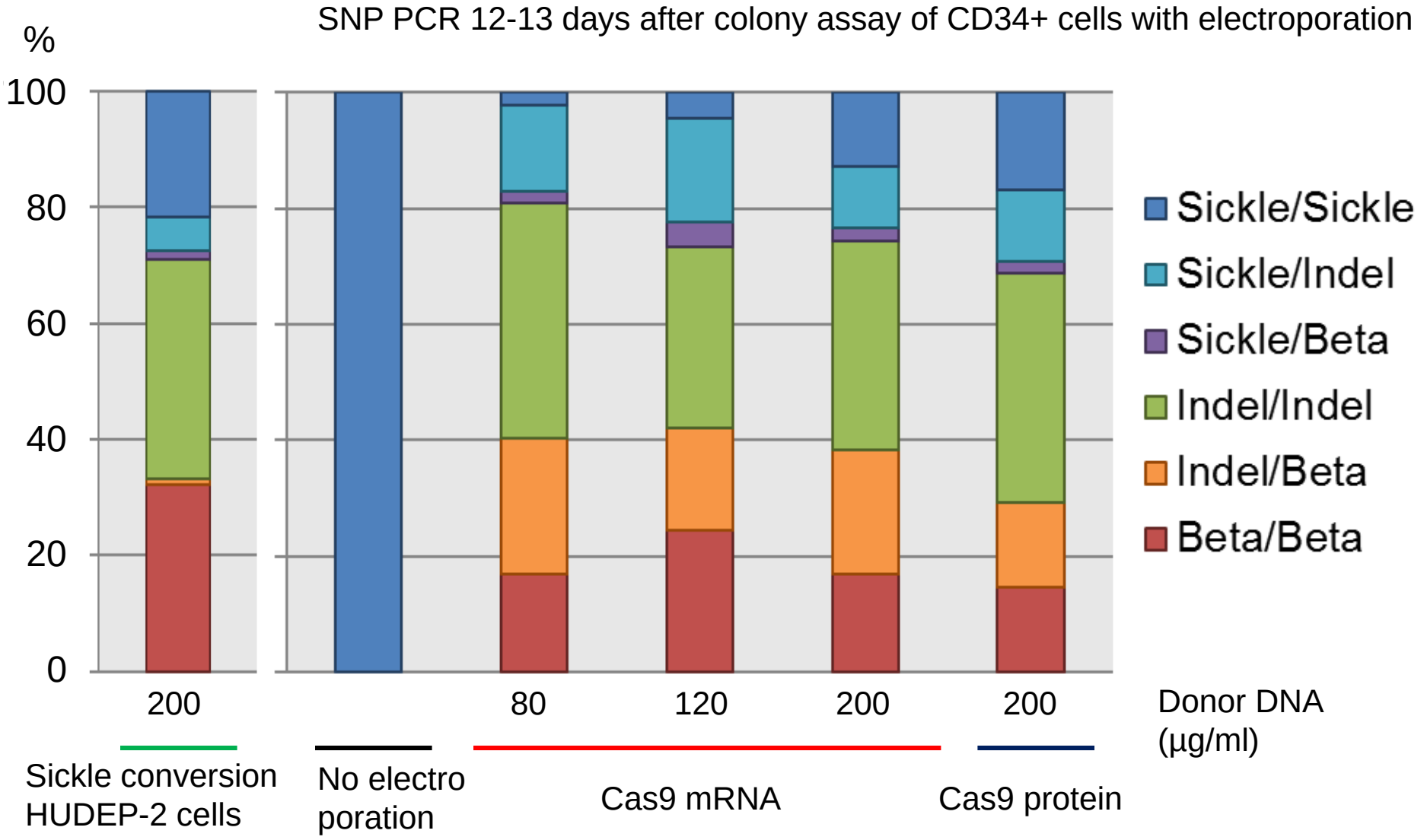
Colony assay

SNP PCR

~30% of gene correction evaluated by targeted sequencing



~20% of homologous β-globin gene correction in CFUs

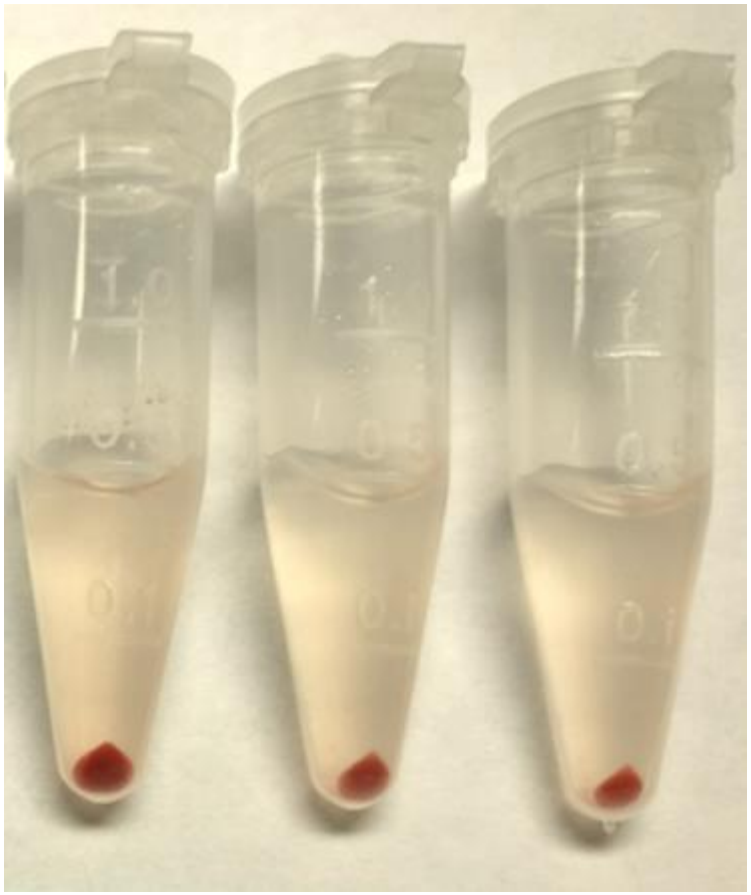


Robust hemoglobinization following erythroid differentiation

National Institutes of Health

SCD CD34+ cell gene correction

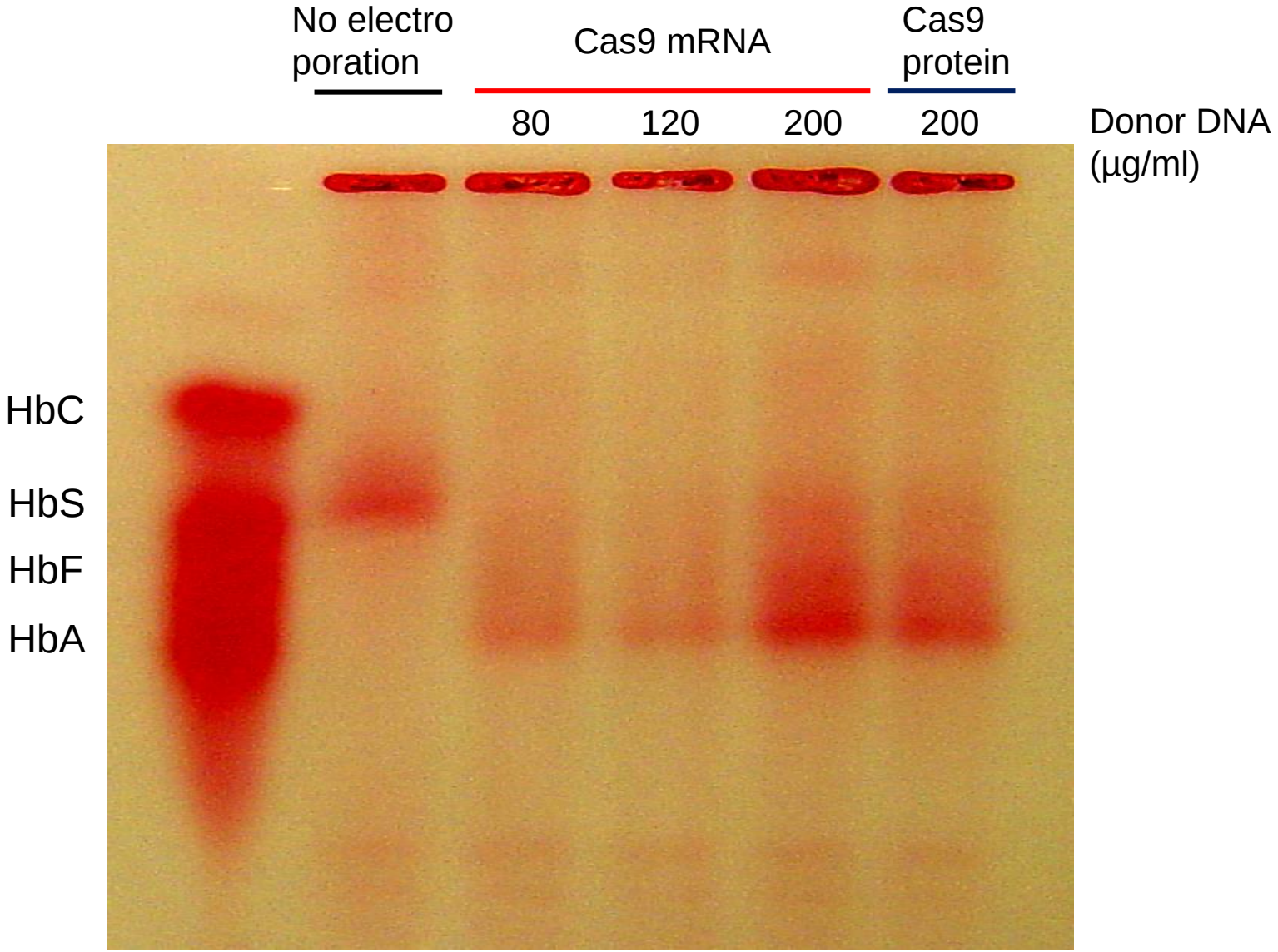
No electro poration	Gene correction 120 µg/ml DNA	Gene correction 200 µg/ml DNA



High-efficiency gene correction from βs-globin to β-globin

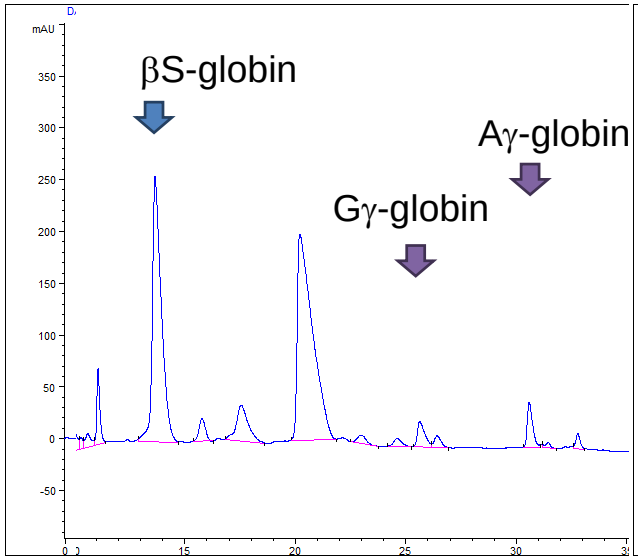
National Institutes of Health

Electrophoresis after erythroid differentiation of CD34+ cells with electroporation

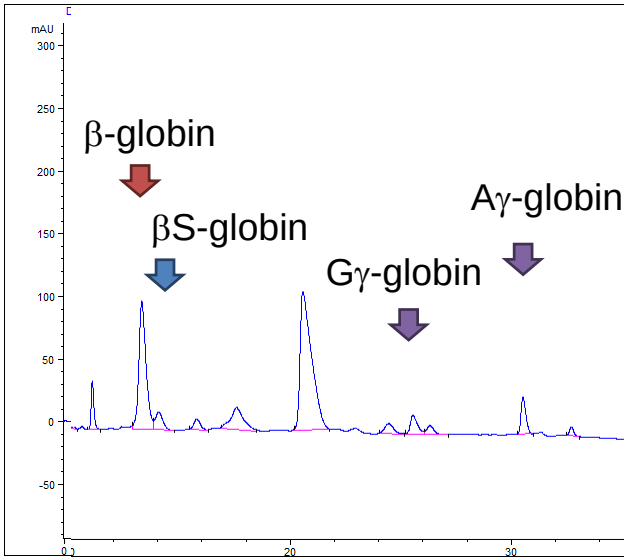


Detection of globin peaks in differentiated erythroid cells by HPLC

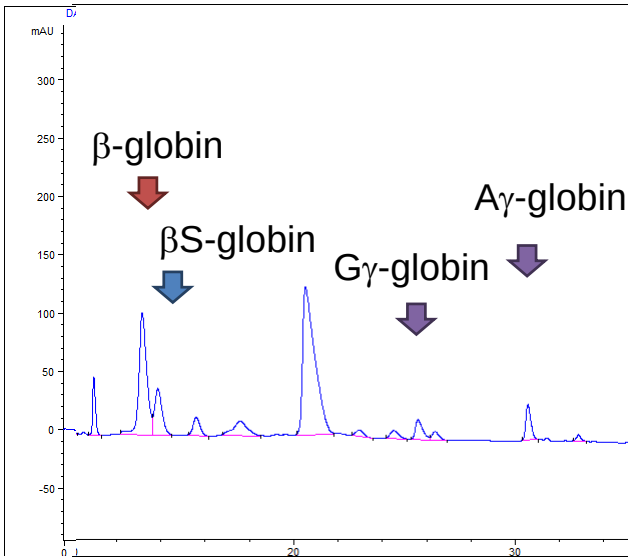
National Institutes of Health



No electroporation



Gene correction
120 µg/ml DNA

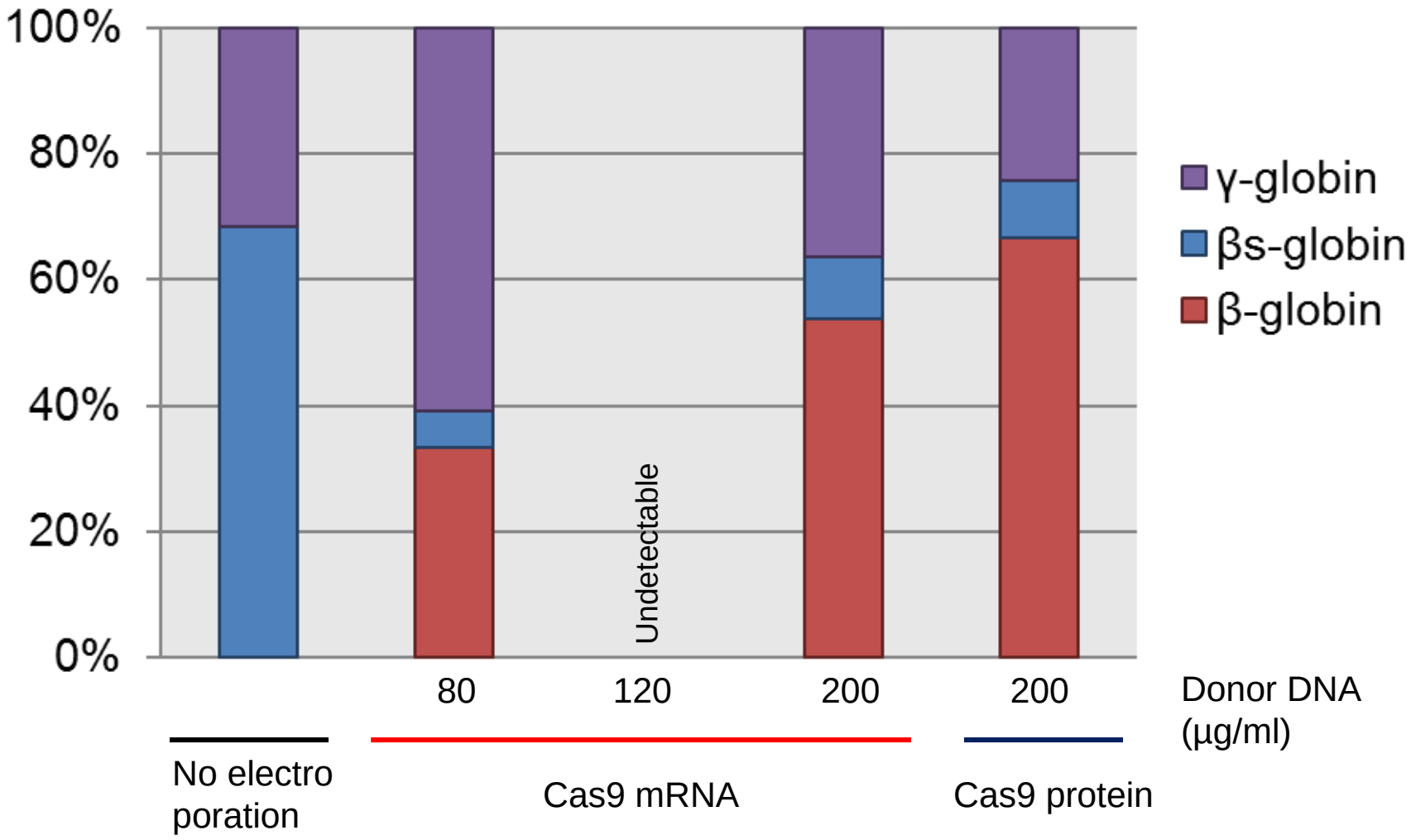


Gene correction
200 µg/ml DNA

SCD CD34+ cell gene correction

~60% of β-globin production in gene-corrected erythroid cells

RP-HPLC 17 days after erythroid differentiation of CD34+ cells with electroporation



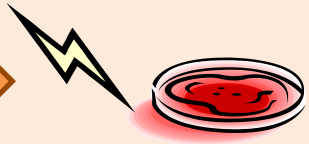
Xenograft transplantation of gene-corrected SCD CD34+ cells

National Institutes of Health

Plerixafor-mobilized sickle cell disease CD34^{+} cells

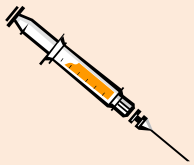
3 SCD donors

Electroporation



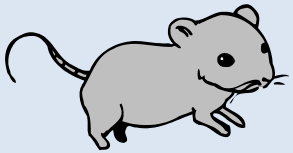
Guide RNA targeting β-globin
Cas9 mRNA
Donor ssDNA

Gene-corrected CD34^{+} cells

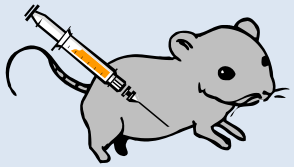


Infusion

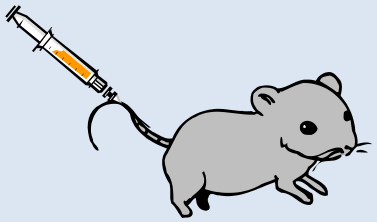
(Busulfan 25mg/kg ip)



NOD/SCID/IL2rγ$^{-/-}$ /Kit$^{W41/W41}$ mice

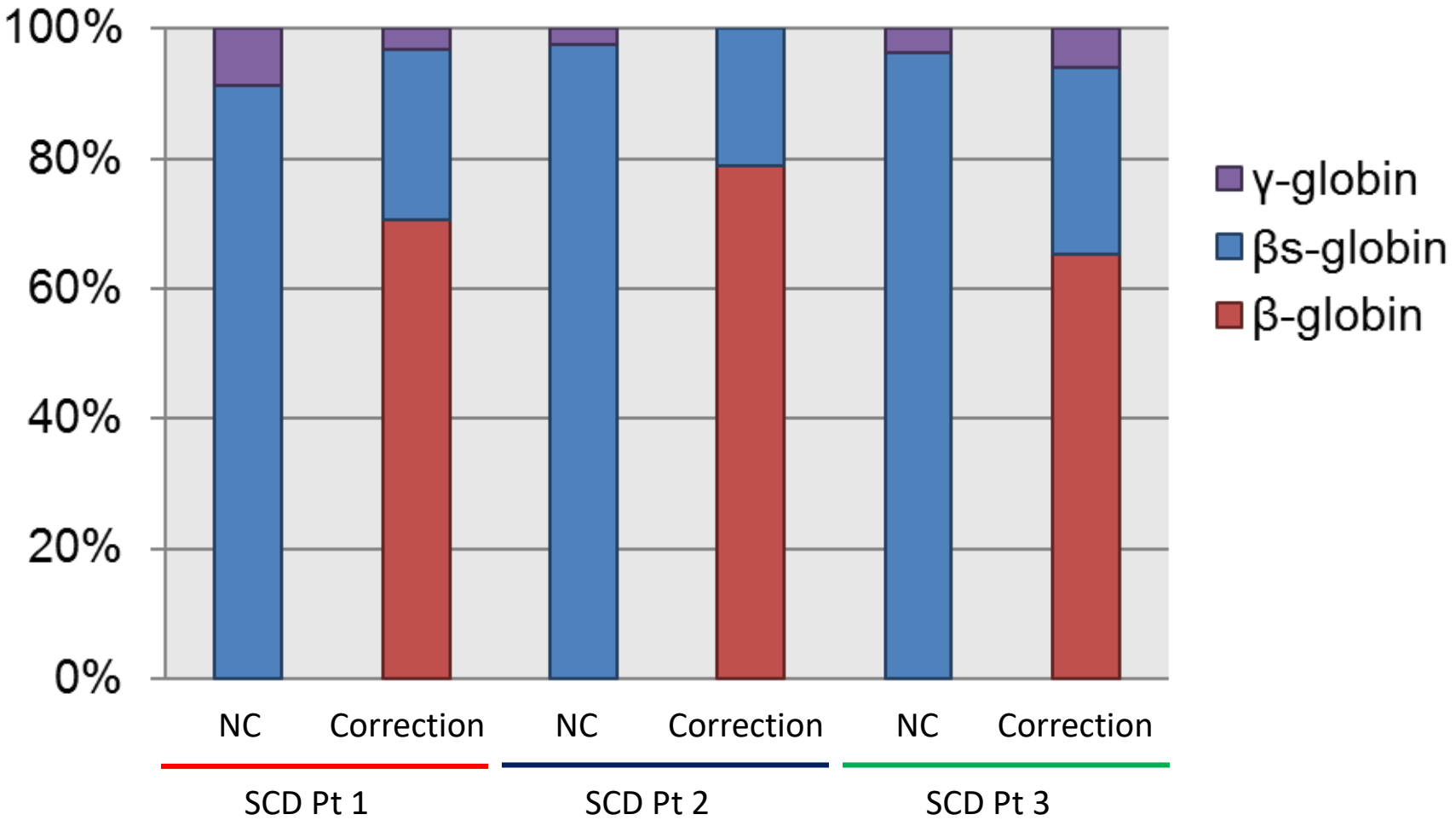


Non-myeloablative conditioning



Transplantation

~70% β-globin production in gene-corrected erythroid cells *in vitro*



Control: no electroporation

Summary

1. Sickle cell disease is a single-gene disorder.

2. Clinical trials have established bone marrow transplant as a one time cure for SCD.
   - Bone marrow transplantation can cure >90% of SCD patients; however, it requires suitable donor (~10%).

3. Gene addition and gene editing strategies provide hope for those without a suitable donor.

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