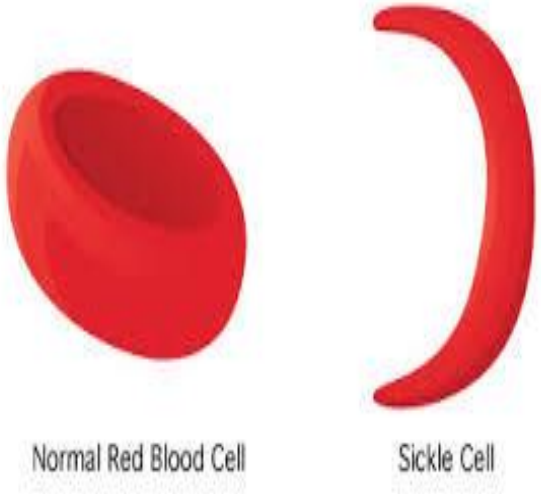
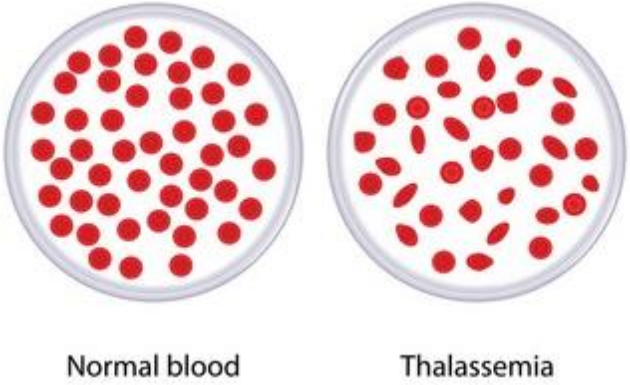
Update on Genome Modifications and Gene Therapies in Hemoglobinopathies

Mehran Karimi MD,

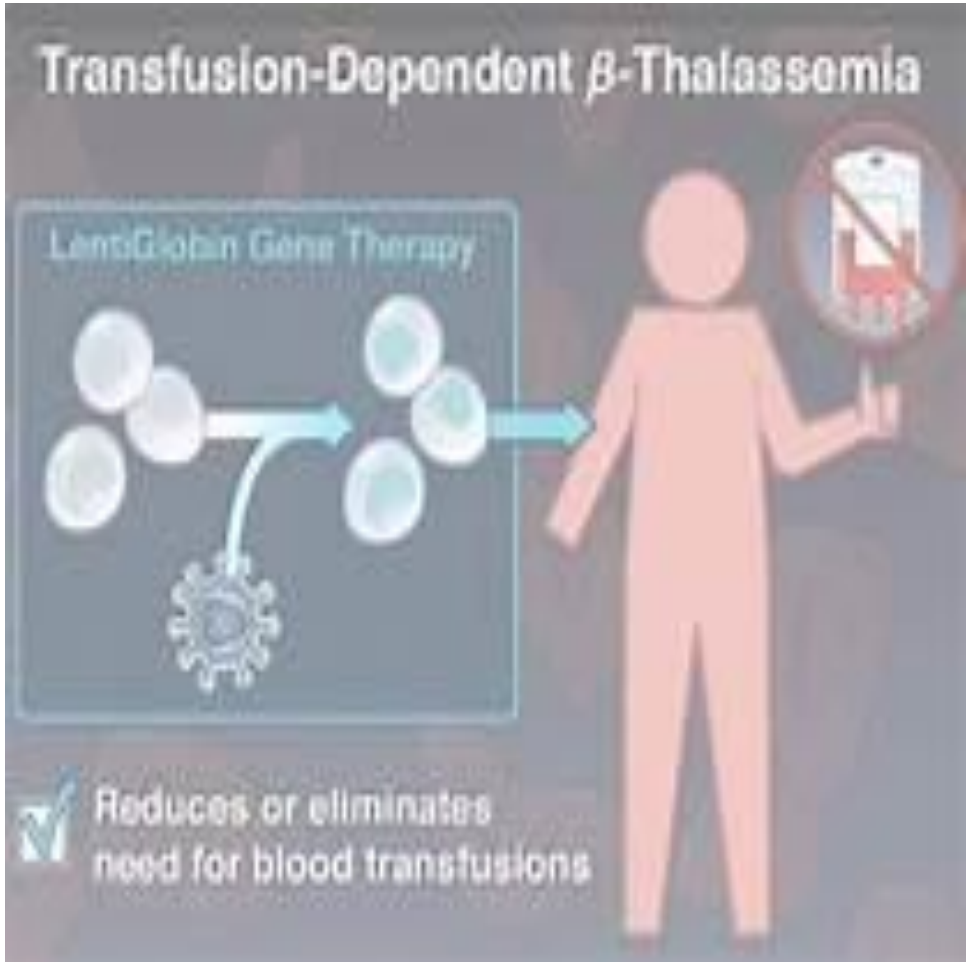
IRAN, March 3, 2022





Disclosures

- I have received Honoraria for speaking by Novo Nordisk, CSL Behring, Cinnagen, Roche and Novartis
- I have received research grants from CSL Behring, OctaPharma , Novo Nordisk and Kedrion
- **My commitment to patients**
  - Treatment for all
  - Support patients
  - Further the science



Agenda

- **Case vignette**
- **Background of hemoglobinopathies**
- **Sickle cell anemia**
- **Thalassemia**
- **Treatment options**
  - Gene therapy
  - Gene editing
- **Conclusion**

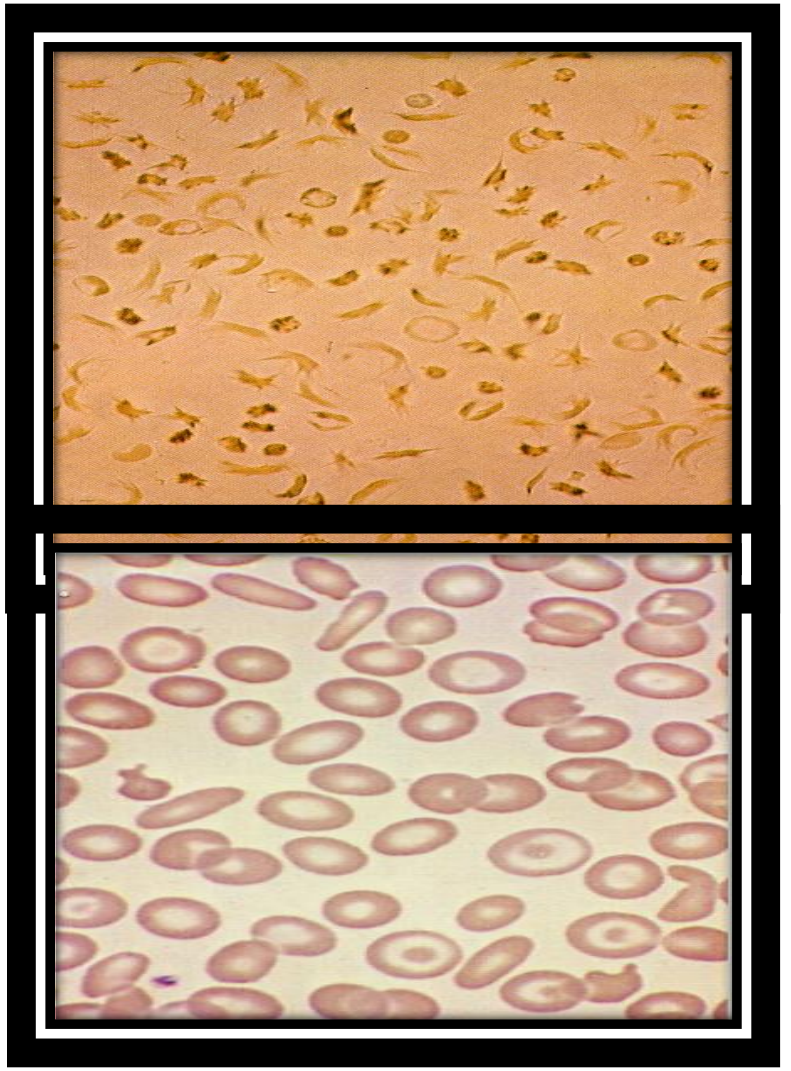
Case vignette:

- 34-year-old female was diagnosed as sickle cell disease at age of 2 years
  - History of severe pain and acute chest syndrome
  - She takes Hydroxyurea and occasional blood transfusion
  - She got married and has four kids
- She had to drop out of school, quit work and spend weeks in the hospital away from her family.
- Since many sickle cell patients don't survive past their 40s, Gray worries whether she'll live to see her children grow up
- "It's horrible, knowing that I could have a stroke or a heart attack...at any time because I have these cells in me that are misshapen, "She says. "Who wouldn't worry?"

➢ What is the treatment plan ?

Background: Sickle cell anemia and Thalassemia

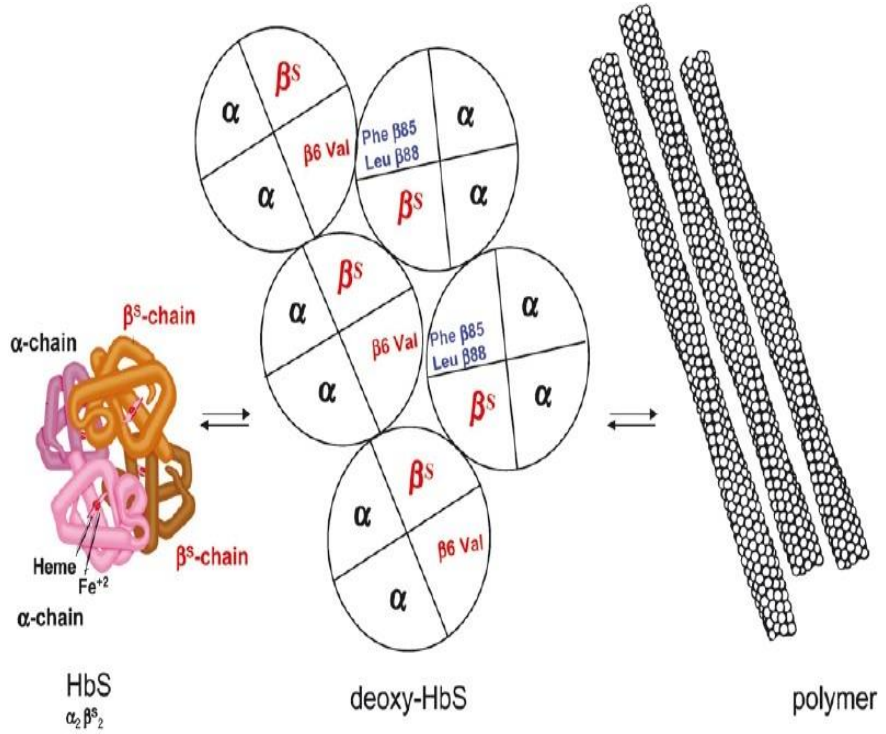
- Sickle cell disease (SCD) and B-thalassemia are a group of inherited hemoglobin disorders (autosomal codominant trait and autosomal recessive respectively)
- These disorders characterized by mutations in the β gene
- SCD is the most prevalent genetic blood disorder worldwide
  - 250,000 children born annually with Sickle Cell worldwide
- Thalassemia syndromes involve 7% of world population
- Patients with hemoglobinopathies present multiple challenges due to the complexity of their condition, associated comorbidities, and need for frequent medical interventions
- The only curative treatment is hematopoietic stem cell transplantation but recent clinical trials in gene therapy/editing have been showing very promising results



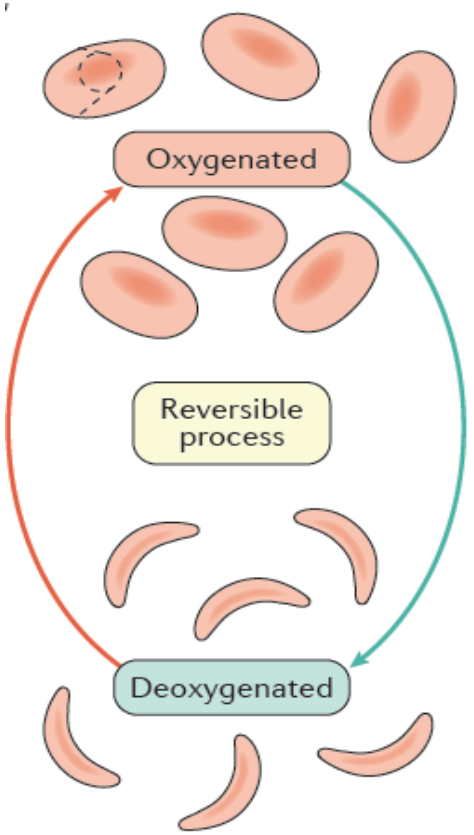
1. Lancet. 2010;376(9757):2018–2031.
2. Gardner et al. Blood 2016; 128:1436.
3. De Baun et al. Blood 2019; 133:615

Molecular pathology of SCD: A single amino acid substitution in B-Globin

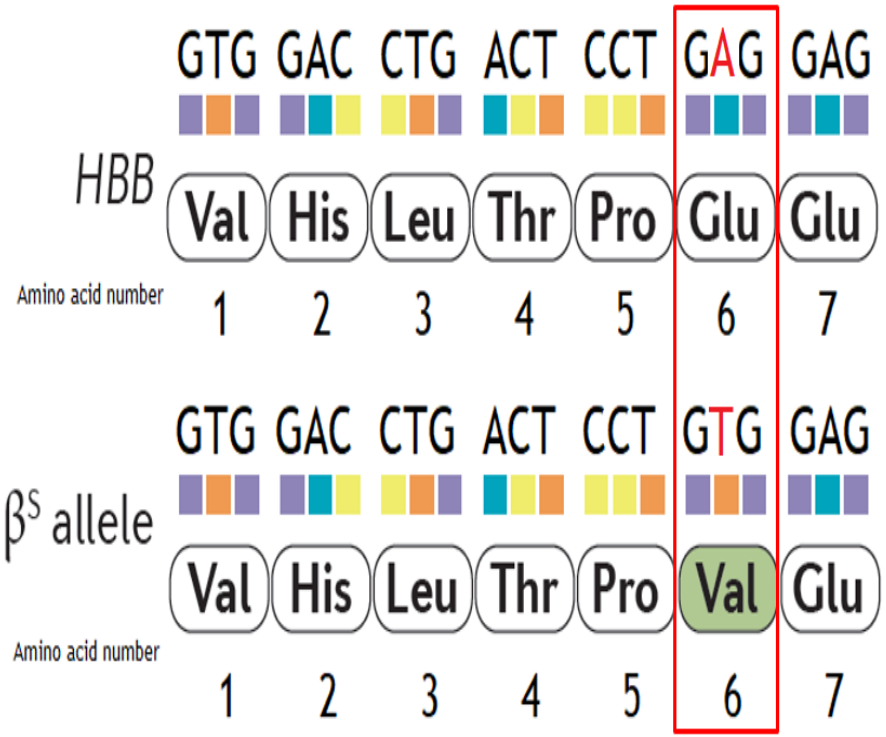
- In normal HBB allele an adenine-to-thymine (A→T) substitution results in the replacement of glutamic acid with valine at position 6 in the mature β-globin chain
- Deoxygenated Hb S causes for hemoglobin polymers to form, and HbS polymers can stiffen the erythrocyte that forms sickle shape



Deoxygenation of HbS leading to HbS polymers



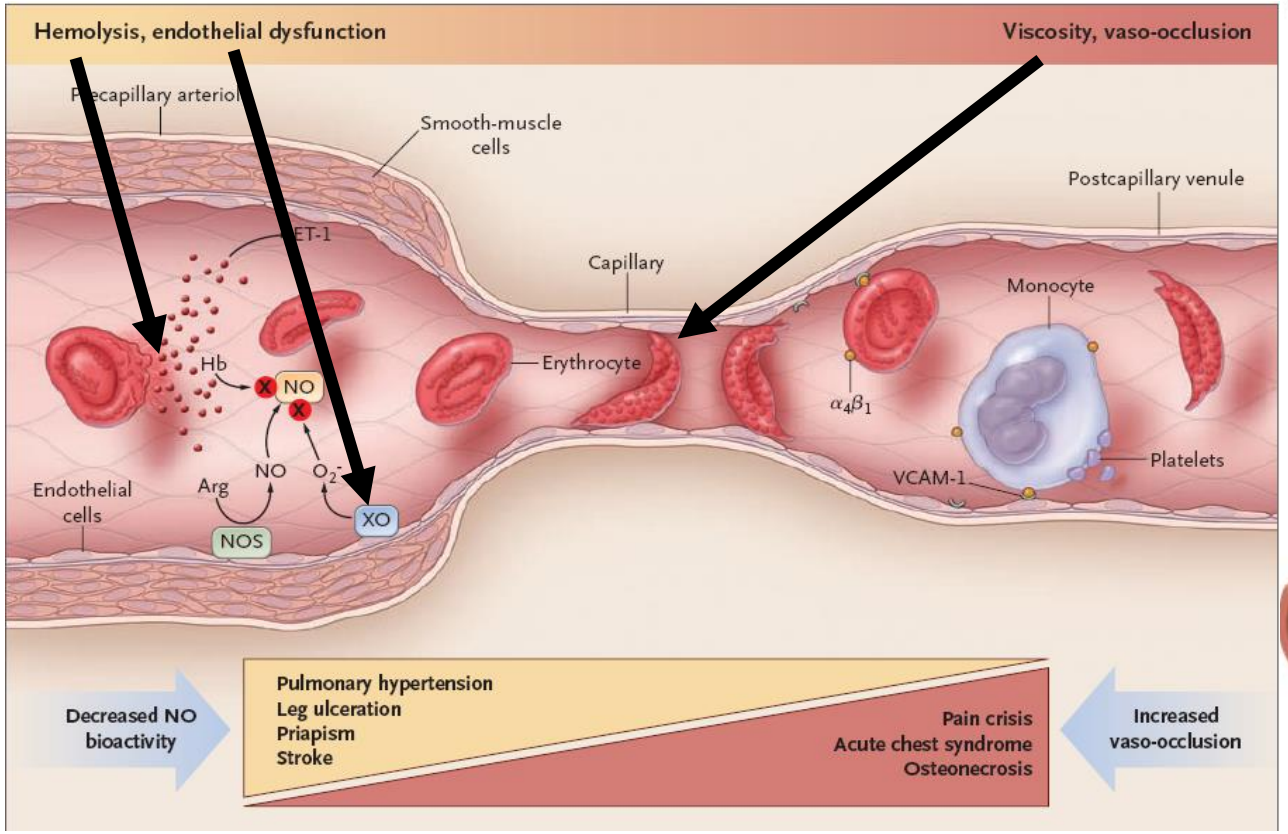
Deoxygenation of HbS leading to sickle cell

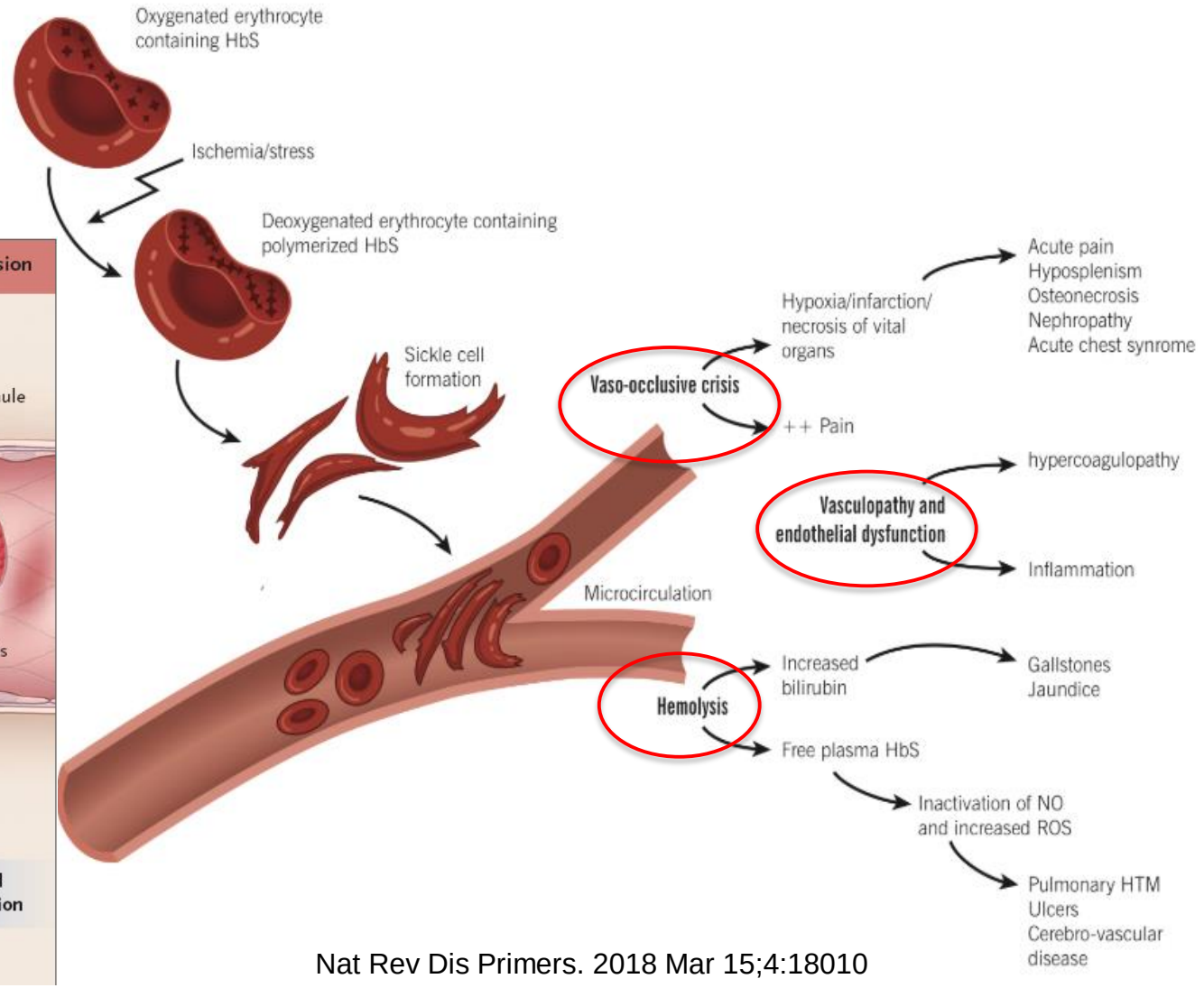


Nat Rev Dis Primers. 2018 Mar 15;4:18010

Clinical pathology of SCD

- **The pathology of SCD are based on three problems:**
  - Hemolysis and chronic anemia
  - Vaso-occlusive complications
  - Chronic organ damage

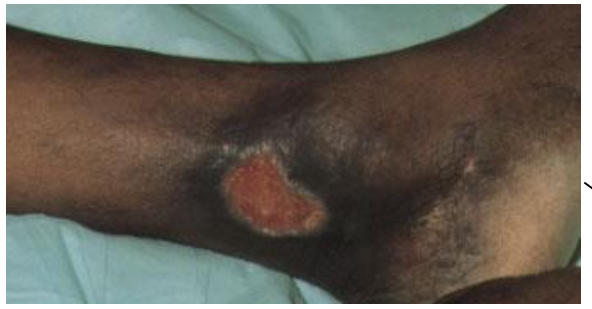


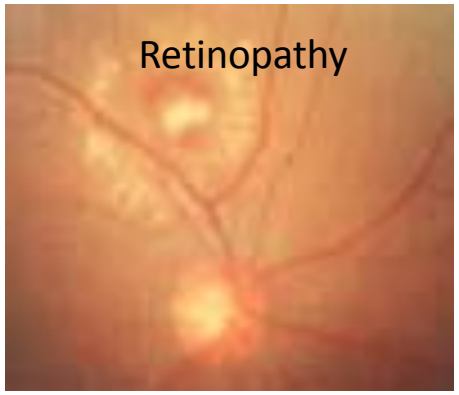


Nat Rev Dis Primers. 2018 Mar 15;4:18010

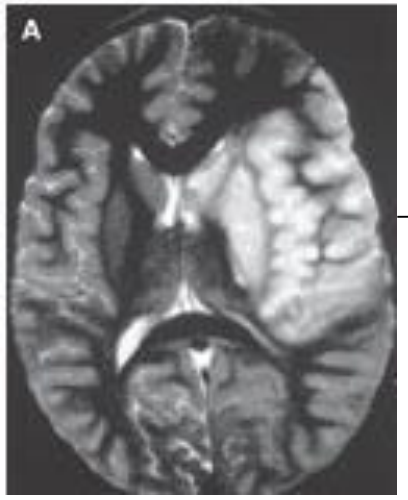
Sickle cell manifestations

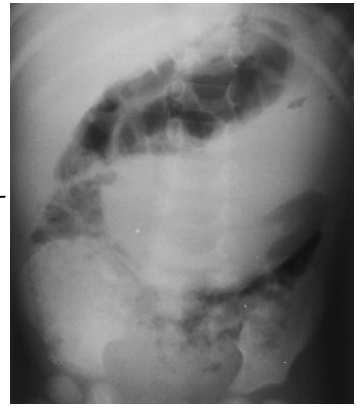
Leg ulcers





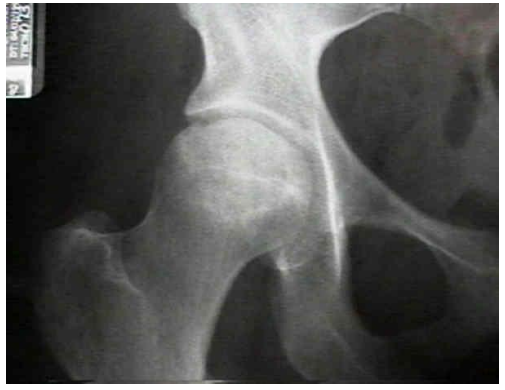
Stroke

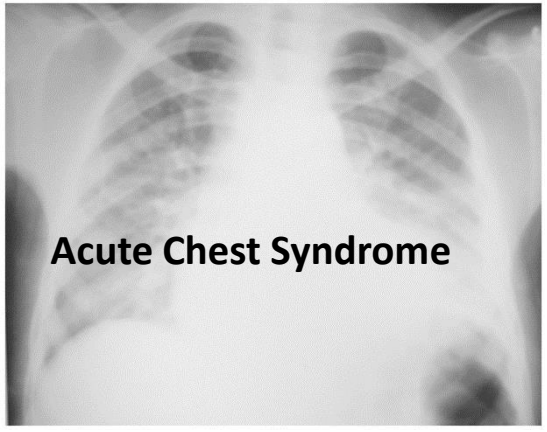




Splenic Sequestration

AVN



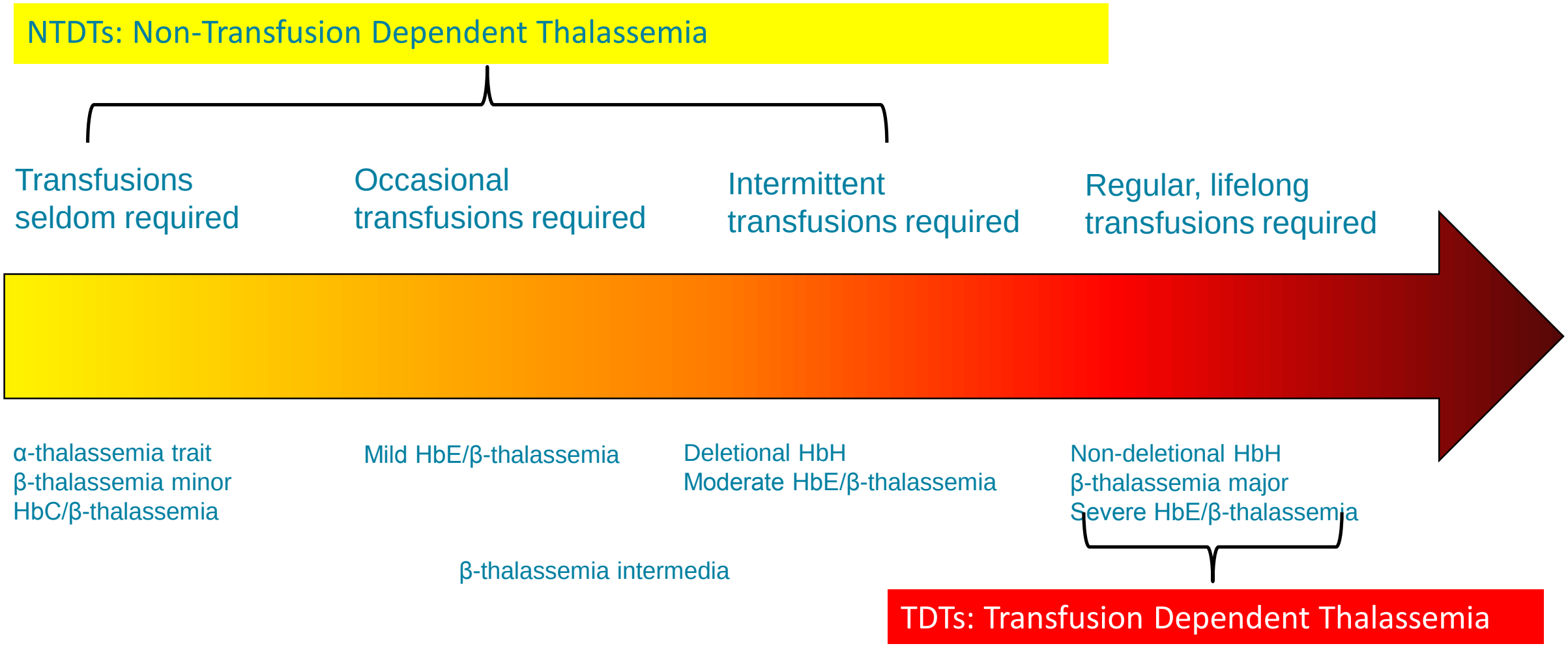


Pain-Dactylitis

Management of SCD: A comprehensive supportive care

- **The mainstay of treatment include:**
  - Adequate pain control and management
  - Hydration and O2 if needed
  - Prophylactic Antibiotics (Penicillin) and fever education
  - Simple or exchange transfusion (1997)
  - Hydroxyurea (1998) and +/- Folic Acid
  - Novel therapies
    - ❖Glutamine (2017)
    - ❖Crizanlizumab (2019)
    - ❖Voxelotor (2019)
  - Hematopoietic stem cell transplantation
    - ❖1996, Only curative option
  - Gene therapy or editing

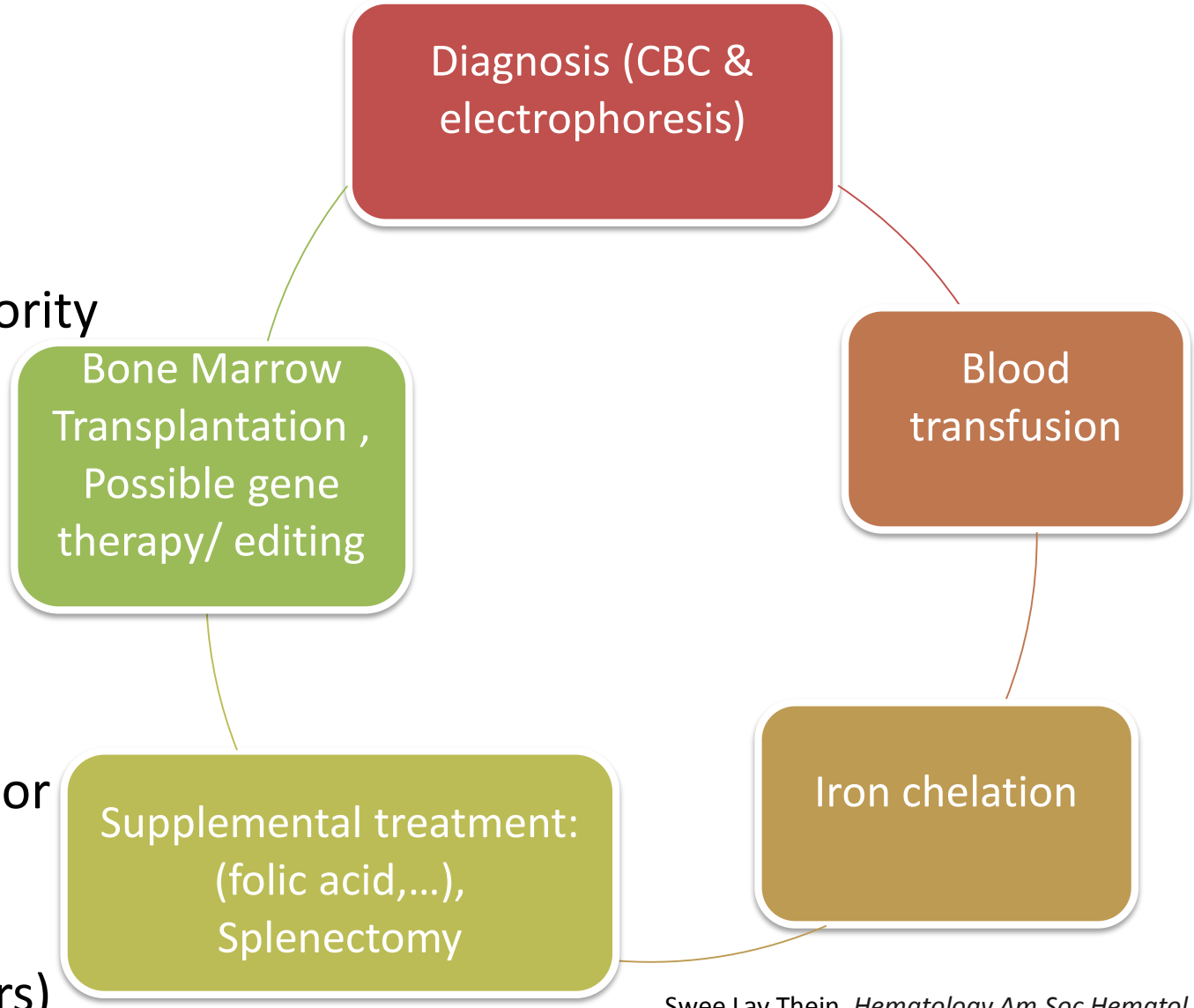
Spectrum of transfusion requirements in thalassemia



Muncie HL & Campbell JS. Am Fam Physician 2009;80:339–344; Galanello & Origa. Orphanet Journal of Rare Diseases 2010, 5:11; Harteveld & Higgs. Orphanet Journal of Rare Diseases 2010, 5:13; Cohen AR et al. Hematology Am Soc Hematol Educ Program 2004;14–34.

Clinical manifestations and management of β-thalassemia Major

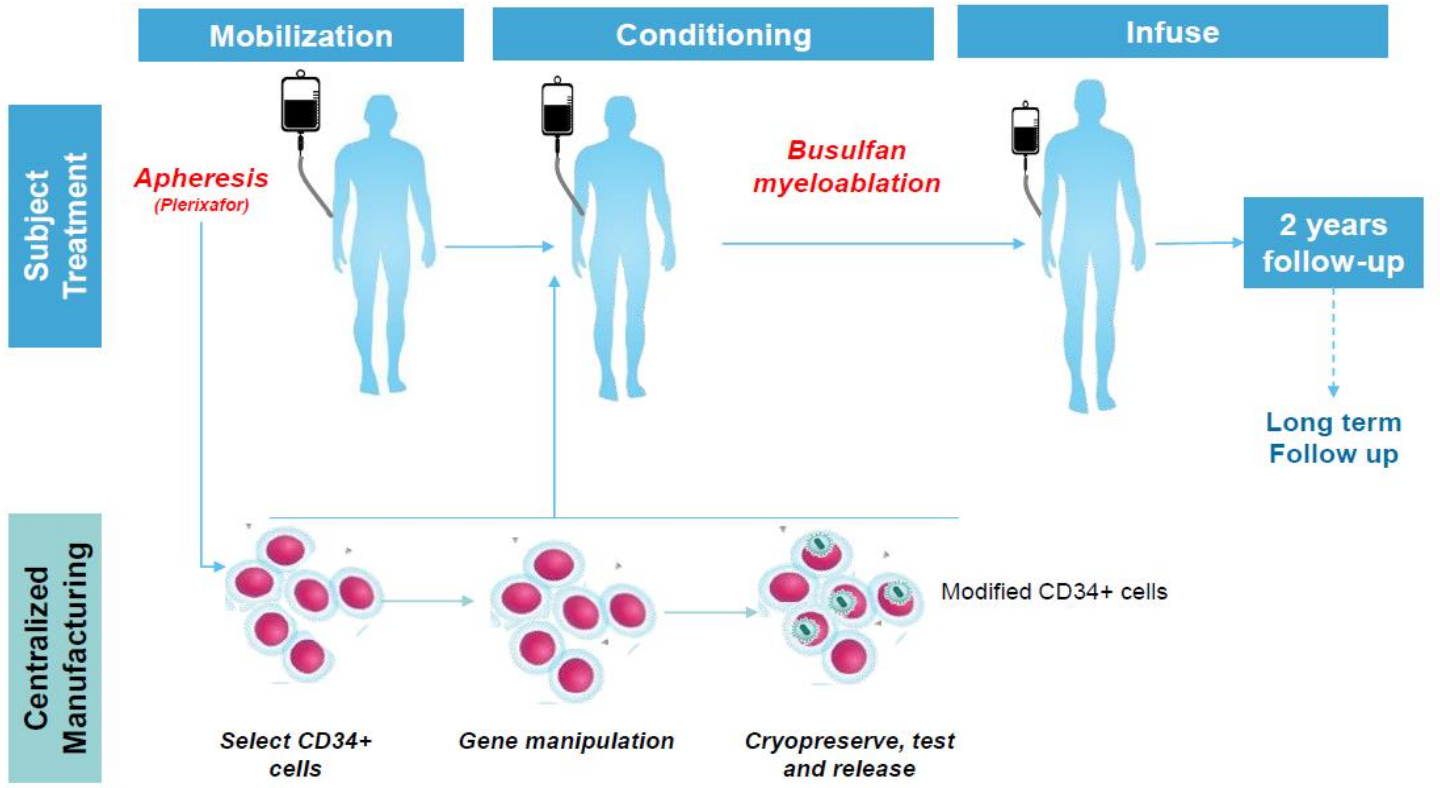
- **Wide clinical variation**
  - More than 200 mutations causing β thalassemia have been described and, except for a few deletions, the vast majority of β thalassemia are caused by point mutations
  - Anemia (Hb < 7-8 gr/dl)
  - Skeletal deformity
  - Both parents: typical β-thalassemia minor
  - Splenomegaly, hepatomegaly
  - Age of diagnosis: infant (less than 2 years)

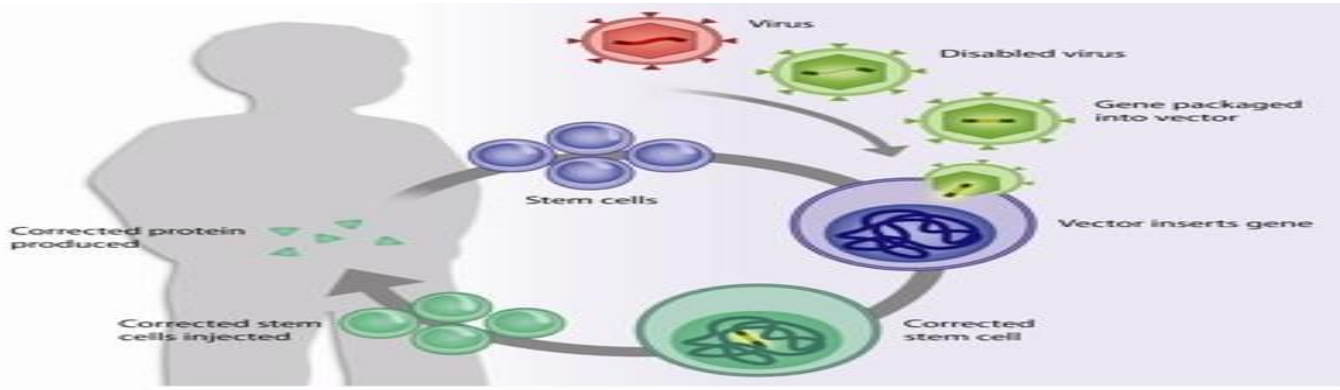


Swee Lay Thein. *Hematology Am Soc Hematol Educ Program*, 2005 (1): 31–37

Gene Therapy/editing: Overview of the treatment plan

- Gene therapy is a potential treatment option for patients lacking an allogenic compatible hematopoietic stem cell (HSC) donor.
- Globin gene addition
  - New-generation lentiviral vectors (LVs) carrying a functional B-globin-like gene by allowing effective HSC transduction
  - Functional Gamma globin gene
- Genome-editing approaches
  - Correct the B-globin mutation
  - Fetal hemoglobin (HbF) induction
    - ❖ Novel LV-based strategies for reactivating endogenous HbF
    - ❖ BCL11a enhancer editing
    - ❖ BCL11a binding site editing
    - ❖ Gama chain promoter editing



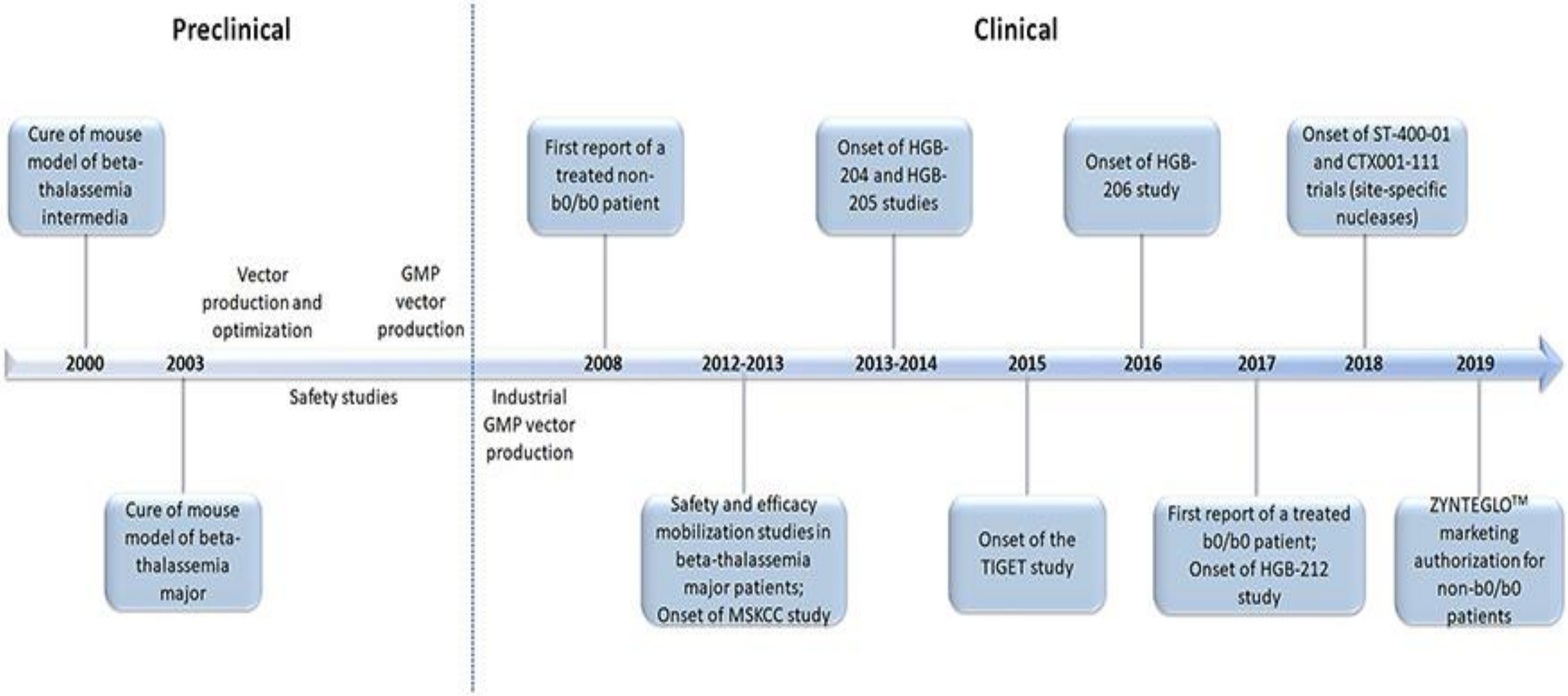


Gene therapy versus Hematopoietic stem cell transplantation

- Gene therapy is a promising approach with some advantages over HSCT
- However, Chemo-myeloablative regimen is like allogenic HSCT
- Gene therapy is not suitable in patients with pre-existing severe organ damage

Allogeneic HSCT	Autologous Gene Therapy
Toxicity: conditioning + immunosuppression	Toxicity: related to intensity of busulfan
Immunosuppression required	None
Risk of immune-mediated rejection	None
GvHD	No risk
Donor availability	No donor required
Long-term risks: organ toxicities	Potential risk of oncogenesis or 'off-target' activity

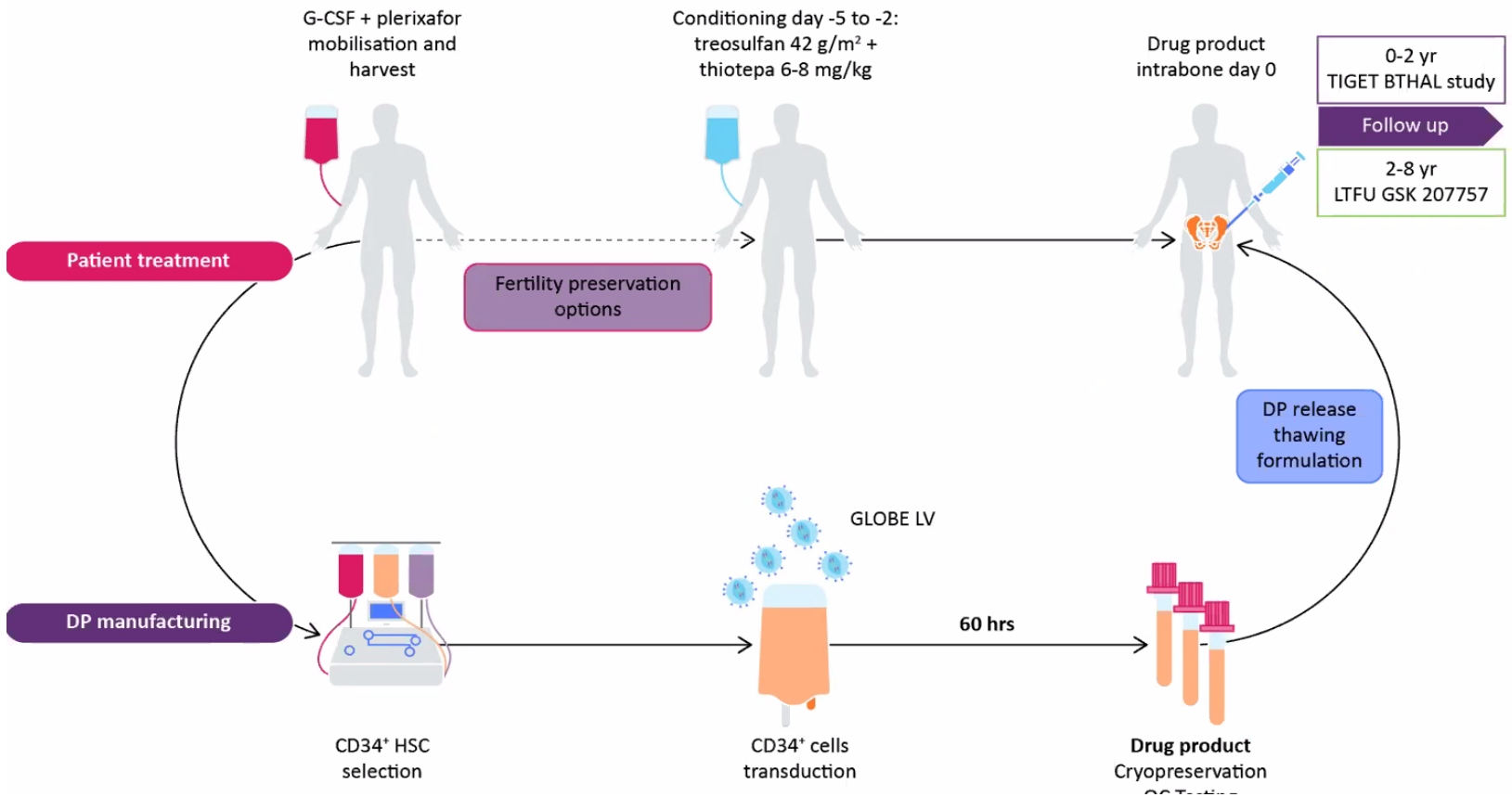
History of gene therapy for Beta-thalassemia



https://doi.org/10.2147/TACG.S178546

The TIGET-BTHAL study: phase I/II study using the GLOB lentiviral vector in transfusion dependent beta-thalassemia for two years post gene-therapy

- Actual Enrollment: 9 participants
- Actual Start Date: October 4, 2017
- Status: Active, not recruiting
- Study Completion Date: June 1, 2026
- Location: Italy-Milan
- This study is designed to follow patients for an additional six years (for a total of eight years)
- The three adult patients had a reduction of transfusion requirement but are still transfusion dependent at the last follow-up (22, 18 and 16 months respectively)
- Among the 4 pediatric patients, 3 have discontinued transfusion shortly after gene therapy and are transfusion independent at the last follow-up (13, 10 and 8 months respectively).
- One pediatric patient is still receiving regular blood transfusions



112. THALASSEMIA AND GLOBIN GENE REGULATION II | DECEMBER 7, 2017

Gene Therapy for Beta Thalassemia: Preliminary Results from the PHASE I/II Tiget-Bthal Trial of Autologous Hematopoietic Stem Cells Genetically Modified with GLOBE Lentiviral Vector

Sarah Marktel, MD, Maria Pia Cicalese, Fabio Giglio, MD, Samantha Scaramuzza, Valeria Calbi, Miriam Casiraghi, Francesca Ciotti, Maria Rosa Lidonnici, Claudia Rossi, Nicoletta Masera, Emanuela D'Angelo, Nadia Mirra, Raffaella Origa, MD, Immacolata Tartaglione, Giacomo Mandelli, Raffaella Milani, Salvatore Gattillo, Milena Coppola, Gianluca Viarengo, Luca Santoleri, Andrea Calabria, Silverio Perrotta, Eugenio Montini, Giovanna Graziadei, MD, Luigi Naldini, MD PhD, Maria Domenica Cappellini, MD, Fabio Ciceri, MD, Alessandro Aiuti, Giuliana Ferrari

Sponsor:IRCCS San Raffaele

Synopsis of beti-cel Clinical Trials in Patients with B-Thalassemia Major

HGB-205

non-β^0/β^0 genotypes

Phase 1/2, single-center study

Location
- France

Primary outcomes
- Engraftment, survival, safety

Status
- Complete
  - All patients enrolled in LTF-303
- N = 4 patients with TDT
  - 16 – 19 years old
- Median follow-up: 49.6 months (min-max: 40.5 – 60.6)[1]

NCT02151526

NorthStar (HGB-204)

non-β^0/β^0 and β^0/β^0 genotypes

Phase 1/2, multi-center study

Locations
- US, Australia, Thailand

Primary efficacy outcomes
- Transfusion Independence*
- ≥ 2 g/dL HbA^{T87Q} Month 18 – 24

Status
- Complete
  - All patients enrolled in LTF-303
- N = 18 patients
  - 12 – 35 years old
- Median follow-up: 44.9 months (min – max: 34.8 – 61.3)[2]

NCT01745120

NorthStar 2 (HGB-207)

non-β^0/β^0 genotypes

Phase 3, multi-center study

Locations
- Italy, US, UK, France, Germany, Thailand

Primary outcome
- Transfusion independence*

Target enrollment
- N = 23 patients ≤ 50 years old

Status
- Ongoing
- 23 patients treated[3]
- Median follow-up: 19.4 months (min – max: 1.2 – 36.2)

NCT02906202

NorthStar 3 (HGB-212)

β^0/β^0; IVS1-110/IVS1-110; β^0/IVS1-110 genotypes

Phase 3, multi-center study

Locations
- Italy, US, UK, France, Germany, Greece

Primary outcome
- Transfusion reduction†

Target enrollment
- N = 18 patients ≤ 50 years old

Status
- Ongoing
- 15 patients treated[4]
- Median follow-up: 14.4 months (min – max: 1.1 – 24.0)

NCT03207009

Results of phase 3 clinical studies of NorthStar 2 and NorthStar 3 and side effects across all 4 studies (Total number of patients#60)

- **NorthStar 2 (HGB-207):**
  - The primary end point was transfusion independence (average Hb level of ≥9 g/dl for ≥12 months)
  - A total of 23 patients were enrolled with a median follow-up of 29.5 months (range, 13.0 to 48.2).
  - 91% (20/22) of patients (6 of 7 patients (86%) were younger than 12 years) have stopped transfusion
  - The average Hb level during transfusion independence was 11.7 g per deciliter (range, 9.5 to 12.8)
  - Twelve months after beti-cel infusion, the median level of adult hemoglobin (HbA) was 8.7 g/dl (range, 5.2 to 10.6) in patients who had transfusion independence.
  - Treatment with beti-cel resulted in a sustained HbA level that was high enough to enable transfusion independence in most patients with a non–β0/β0 genotype, including those younger than 12 years
- **NorthStar 3 (HGB-212):**
  - 85% (11/13) of patients have been off transfusion for more than 6 months
- **Side effects**
  - No death or graft failure
  - One severe adverse event
    - ❖ Thrombocytopenia (in NorthStar2)
  - No vector-mediated replication-competent Lentivirus
  - No evidence of clonal dominance (cancer)
  - Five serious veno-occlusive liver disease events
    - ❖ All events resolved following defibrotide

- All 10 Italian patients (7 from NorthStar 2 and 3 from NorthStar3) achieved and maintained transfusion independence with Hb levels ≥ 9 gr/dl over 12 months
- Transfusion reduction: ≥ 60% in blood transfusion over 12-24 months post gene therapy

The New England Journal of Medicine, 386;5 nejm.org February 3, 2022
(Funded by Bluebird Bio; HGB-207 ClinicalTrials.gov number,

Gene Therapy/Editing Clinical Trials in SCD

Trial numbers	Sponsor	Phase	Site	Start date	Number of Patients	Vector and Transgene	Cell source
NCT02151526 (HGB205)	Bluebird bio	1/2	France	July2013/active, not recruiting	3	BB305(bA-T87Q-globin)	BM
NCT04443907	Intellia therapeutics/Novartis,	1/2	US	August 2020	30	CRISPR (OTQ923/HIX763)	genome-edited HSPC
NCT04853576	Editas Medicine	1/2	US	April 2021	40	HIX763, EDIT-301	HSCT

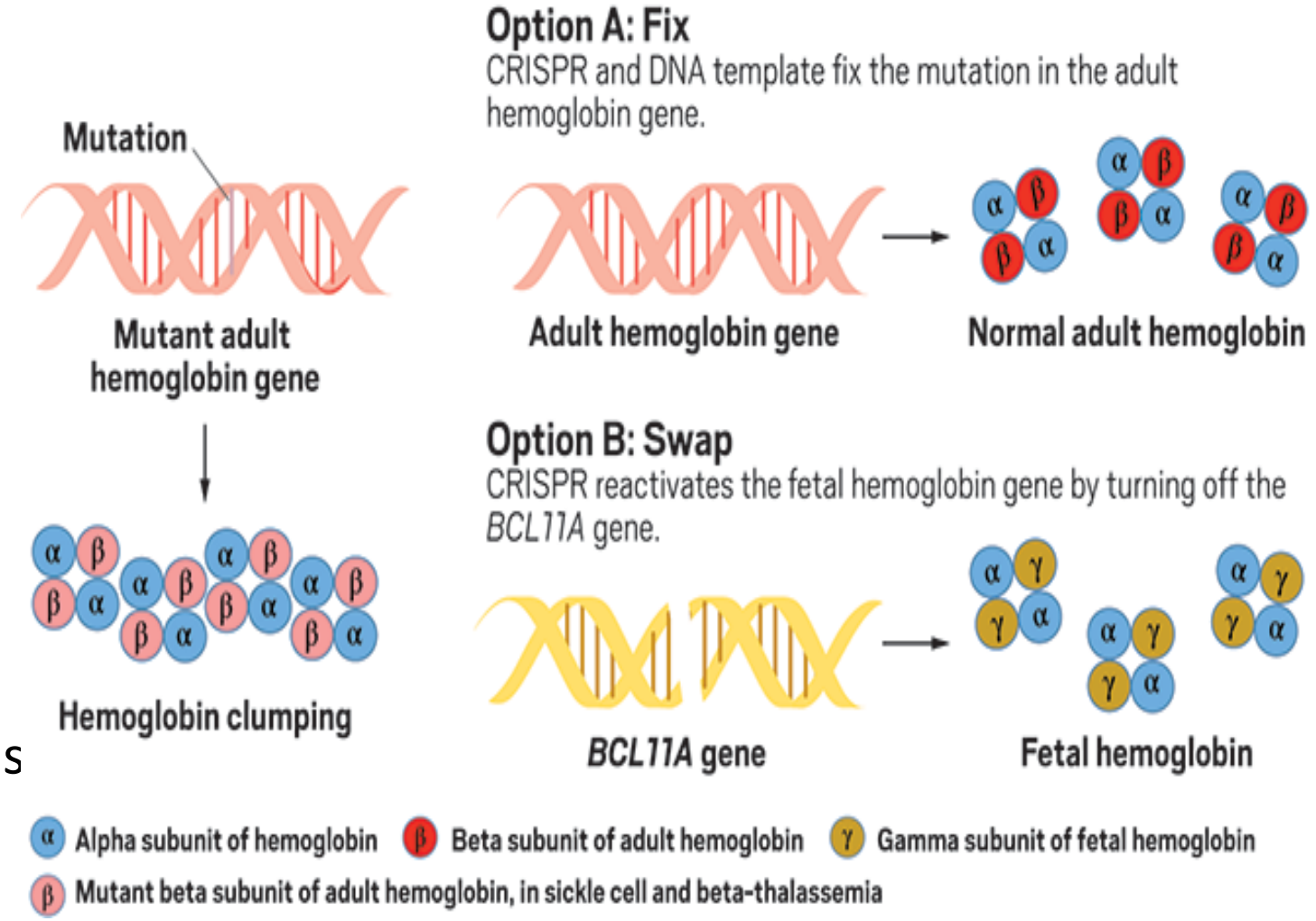
- Bluebird stops gene therapy trials (phase 1/2 HGB-206 trial and its Phase 3 HGB-210 study) after 2 sickle cell patients develop cancer(AML and MDS) both of which were testing LentiGlobin in patients with SCD (**Feb 16, 2021**)
- At the same time, the company paused two other Phase 3 trials (HGB-207 and HGB-212) which were testing another gene therapy that uses the same viral vector as LentiGlobin in patients with transfusion-dependent beta-thalassemia.
- FDA has lifted the clinical holds on the Phase 1/2 HGB-206 and Phase 3 HGB-210 studies of LentiGlobin for SCD, and the Phase 3 Northstar-2 (HGB-207) and Northstar-3 (HGB-212) studies of gene therapy transfusion-dependent β-thalassemia (**June 07, 2021**)

ClinicalTrial.gov. Margin E et al. Blood.2019; 134:1203-1213

CRISPR Based-Gene Editing Strategies

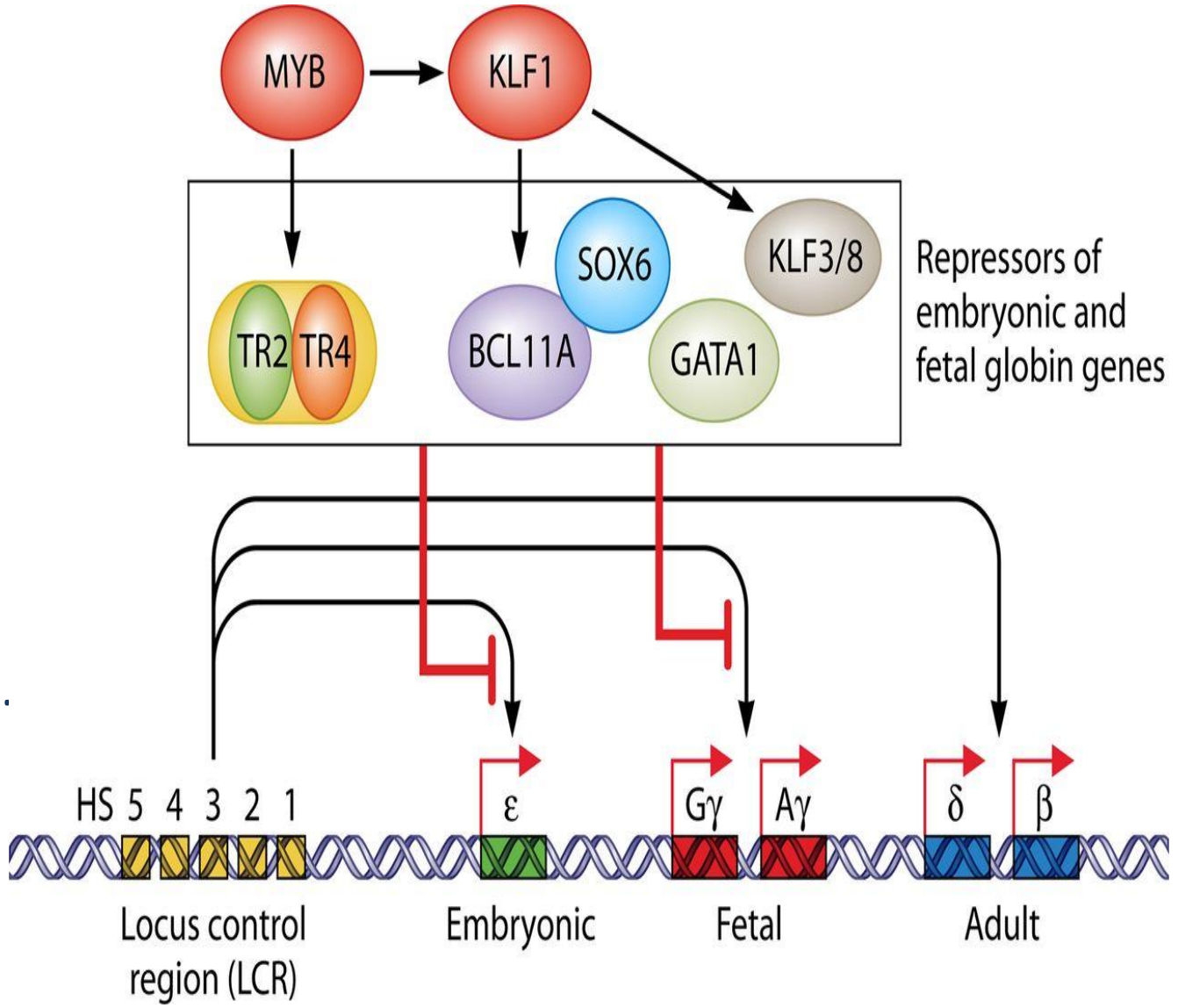
- **Direct mutation editing**
  - Suitable for specific mutations
  - Need set up for each patient
  - Suitable for Sickle cell disease

- **Gamma globin activation**
  - Fetal hemoglobin induction
  - Suitable for Sickle cell disease
  - Suitable for all hemoglobinopathies



Gamma Globin Gene Repressors Reverse Engineering for Reactivation

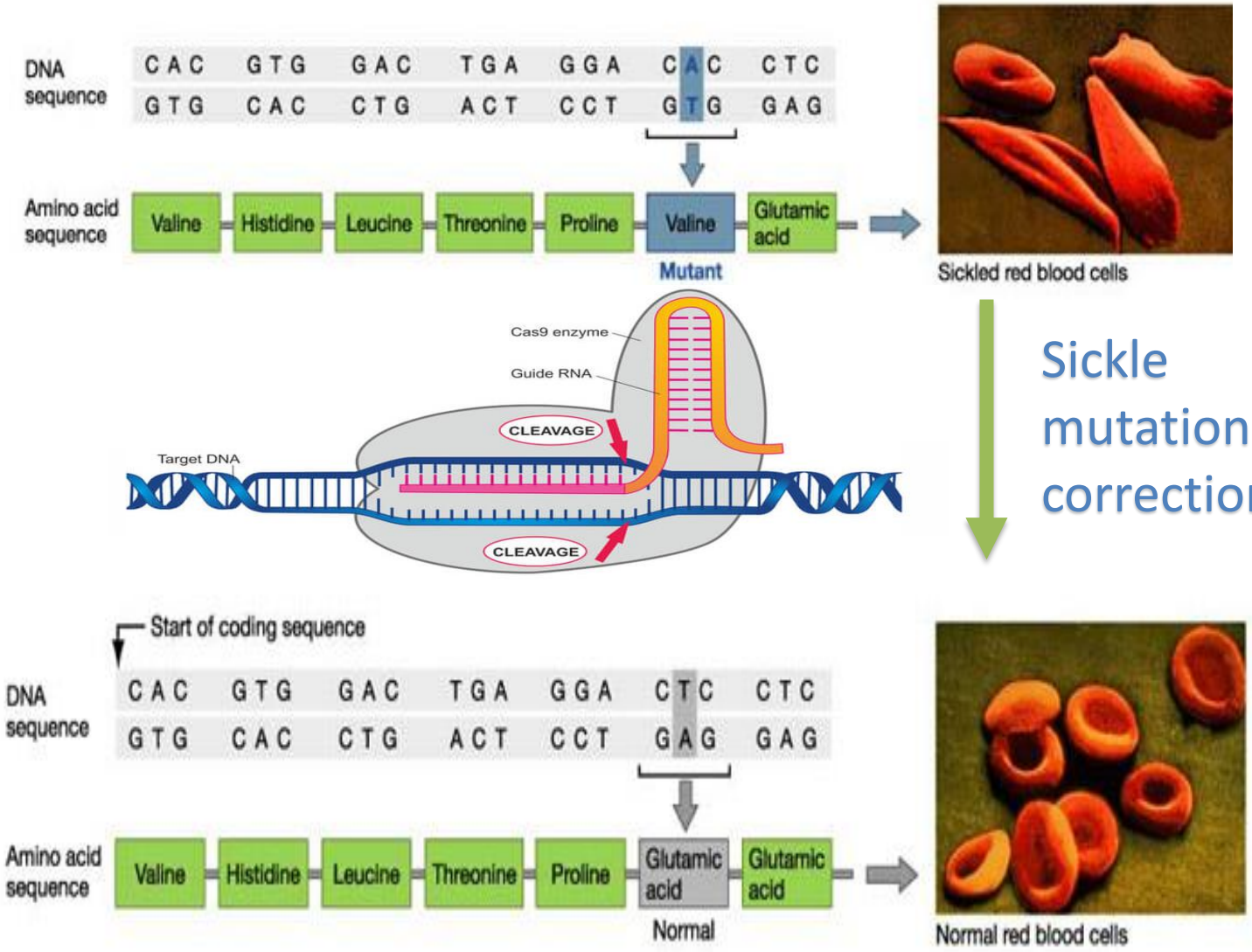
- BCL11a Is a Goal Target for Gene Editing in Hemoglobinopathies
  - TR2/TR4 bind to DR elements in promoters.
    - ❖ Represses directly
  - MYB by activating TR2/TR4 and KLF1.
    - ❖ Represses indirectly
  - KLF1 activates BCL11A
  - BCL11A in collaboration with SOX6 & KLF3/8.
    - ❖ Represses directly
  - GATA1 contribute to BCL11A.
    - ❖ May repressed directly



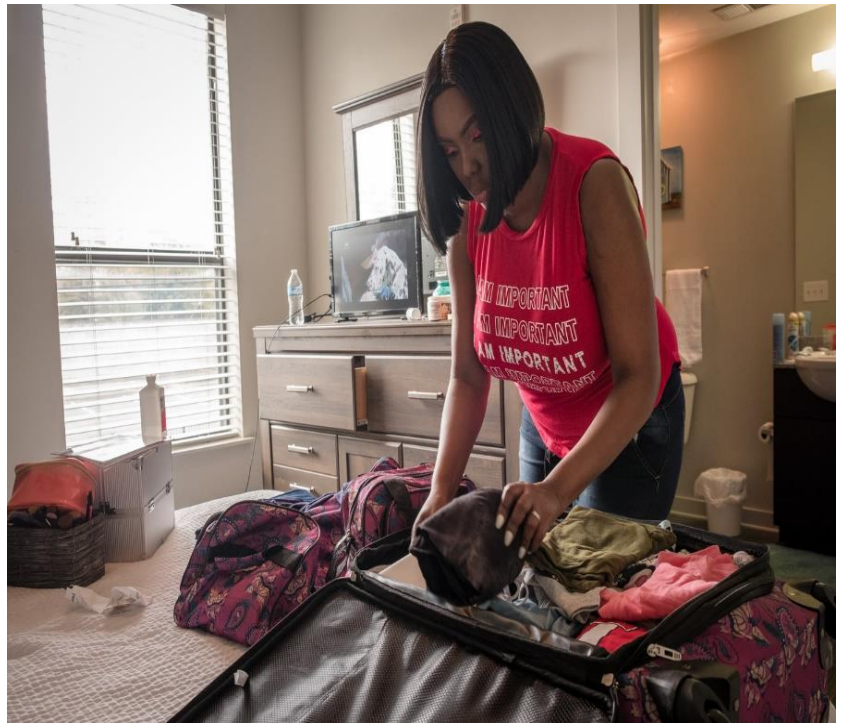
FDA Approved first CRISPR-based Therapy for sickle cell disease

- CRSPR-Cas9 directly corrects the mutation in the beta-globin gene responsible for sickle cell disease

- UCSF Benioff Children's Hospital Oakland and UCLA's Broad Stem Cell Research Center



Case Vignette: Sickle cell treated by gene editing (HbF reactivation)



The patient says: " I'm just genetically modified now; I can't imagine the lives that could be saved if this thing works. Yeah, oh my God. Just to not have to deal with that pain anymore is enough. I feel hopeful for the future,"

cells back into her body, after they had been edited using the gene-editing technique CRISPR.

Nashville. she is the first patient using the gene-editing technique CRISPR.

marrow biopsy. But, she says, the hardest part is over.

Follow up: A Year In, 1st Patient To Get Gene Editing For Sickle Cell Disease Is Progressing (June 23, 2020)

- The one-year anniversary of her landmark treatment approaches:
  - Victoria Gray, who underwent a landmark treatment for SCD last year, has been at home in Forest, Miss., with her three kids.
  - She's the first person with a genetic disorder to get treated in the US with the revolutionary gene-editing technique called CRISPR.

- Dr. Haydar Frangoul, medical director of pediatric hematology-oncology in Nashville.
- Victoria Gray was a part of the study at Medical Center in Nashville, one of eight sites recruiting patients for the research in the U.S., Canada and Europe.
  - Up to 45 patients ages 18 to 35 will eventually be enrolled.

Sponsor: Vertex Pharmaceuticals, in Boston, and CRISPR Therapeutics, in Cambridge, Massachusetts

CLIMB THAL-111/SCD-121: Phase I/II Studies of CTX001 for Transfusion-Dependent β-Thalassemia (TDT) and Sickle Cell Disease

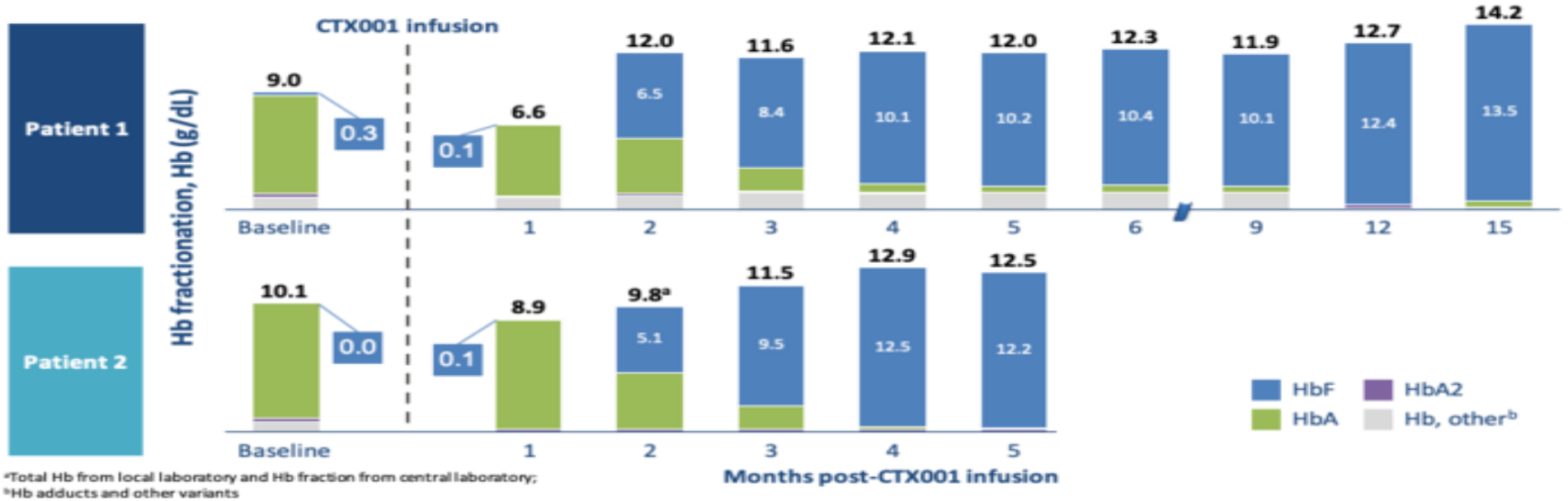
	CLIMB THAL-111	CLIMB SCD-121
Design	Phase 1 / 2, international, multicenter, open-label, single-arm study (NCT03655678)	Phase 1 / 2, international, multicenter, open-label, single-arm study (NCT03745287)
Target enrollment	45 patients aged between 12 and 35 years with TDT, including β^0 / β^0 genotypes, defined as a history of at least 100 mL/kg/year or 10 units/year of packed RBC transfusions in the previous 2 years	45 patients aged between 12 and 35 years with severe SCD and a history of ≥2 vaso-occlusive crises (VOCs)/year over the previous 2 years
Primary endpoint	Proportion of patients achieving sustained transfusion reduction of 50% for at least 6 months starting 3 months after CTX001 infusion	Proportion of patients with HbF ≥20% sustained for at least 3 months starting 6 months after CTX001 infusion

Among the first 10 study participants with ≥ 3 months of follow-up, CTX001 infusion achieved cessation of pRBC transfusions for TDT patients and eliminated veno-occlusive events for severe SCD patients.

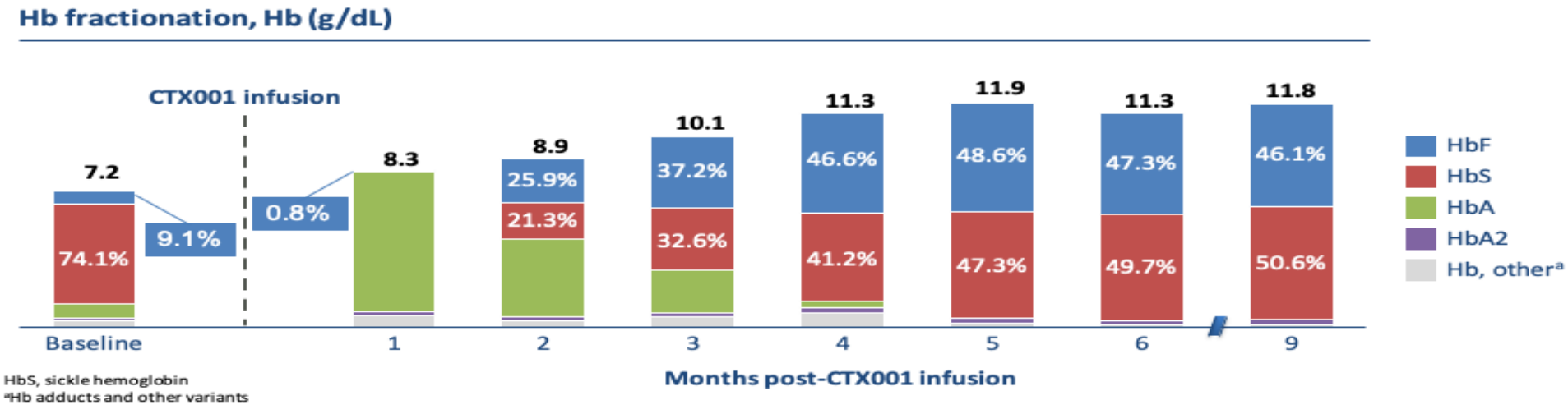
1-ASH meeting, December 16, 2020
2-CRISPR Therapeutics

CRISPR-Cas9 gene editing may help SCD ,thalassemia and other hemoglobinopathies patients

- Initial safety profile of CTX001 is consistent with myeloablative busulfan conditioning and autologous HSCT
- First patient with TDT and β0/IVS-I-110 genotype in CLIMB THAL-111 has stopped pRBC transfusions
  - HbF sustained >10 g/dL at 9 months post infusion
- First patient with severe SCD in CLIMB SCD-121 has had no VOC since CTX001 treatment and has stopped pRBC transfusions
  - HbF of 46.6% at 4 months post infusion



[a]Total Hb from local laboratory and Hb fraction from central laboratory;
[b]Hb adducts and other variants
The value at the top of each bar represents total Hb (g/dL)



HbS, sickle hemoglobin
[a]Hb adducts and other variants
The value at the top of each bar represents total Hb (g/dL)

H. Frangoul et al. NEJM, 384;3 nejm.org January 21, 2021

Our Project: A therapeutic approach for Thalassemia and sickle cell disease by genome editing tools and Fetal hemoglobin induction

- **Hypothesis:**
  - Genome editing in gamma globin promoter can create a new transcription factor site and induce gamma globin expression
- **General Objectives:**
  - Production of edited HSCs with high expression of gamma globin for treatment of beta thalassemia and sickle cell/ direct mutation correction for SCD
- **Methods:**
  - Autologous genome editing of gamma and beta globin genes by base editor in $CD34^{+}$ HSCs
  - Investigate the rate of globin enhancements in edited HSCs
  - Finding the most efficient ex-vivo globin induction strategy
  - Apply delivery, efficiency safety
  - Apply for clinical trial approval
  - Start the clinical study, monitoring and follow up

Mehran Karimi and Shiva Nickaria

Conclusions

- Management of hemoglobinopathies requires the ability to offer appropriate quality care
- Less than 15% of patients with SCD/thalassemia have HLA-matched donors
- Gene therapy eliminates the problems of
  - Donor availability
  - GVHD
  - Prolong immunosuppression
- Several barriers to gene therapy seem to be overcome and promising results have been reported in the initial trials on Beti-cel-driven gene correction
  - The maximization of transgene expression in hematopoietic cells with long term self renewal potential remains a major challenge
- CRISPR technology has a lot of potential use in the future, not only in blood disorders," Doctors have already started using it to try treat cancer, mostly in China. At least two patients in the U.S. have been treated for cancer, in a study at the University of Pennsylvania in Philadelphia
- Gene editing by inducing HbF could be another valuable option and ideal treatment in near future

Take home message: We have still gap in gene therapy/editing, but future is brilliant

Thank you for listening

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