THALASSEMIA

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Disclosures

- **Consultant**
  - **Bluebird bio**
  - **Celgene Bristol Myers Squibb Acceleron**
  - **Agios**
  - **Chiesi**
- **Steering**
  - **CRISPR/ Vertex CTX001**
- **Will discuss therapeutics not yet FDA approved – results from clinical trials**

HEMOGLOBIN DISORDERS

Qualitative Hemoglobinopathies

Globin gene mutations that result in structural abnormalities of the globin chain:

Hb S, Hb C, Hb E and other Hb variants

Quantitative Hemoglobinopathies (disorders of ineffective erythropoiesis)

Globin gene mutations that result in decreased production of globin chains:

Thalassemias (Alpha, Beta, Gamma, Delta)

THE THALASSEMIA SYNDROMES

ALPHA: Decreased or Absent α globin chains

BETA: Decreased or Absent β globin chains

DELTA: Decreased or Absent δ globin chains

GAMMA: Decreased or Absent γ globin chains

STRUCTURAL VARIANTS:

Hb Constant Spring (α)

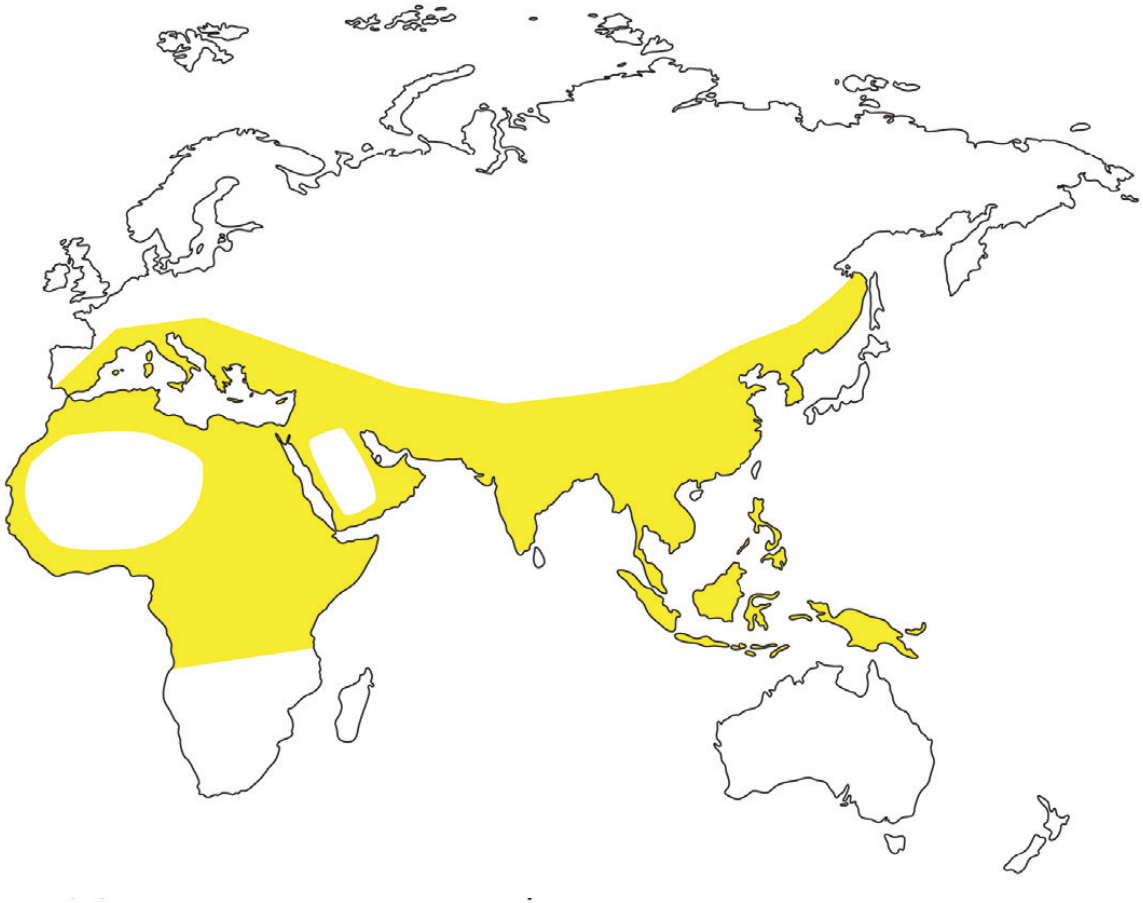
Hb Hasharon (α)

Hb E ($\beta^{26Glu\text{-}Lys}$)

Hb Lepore ($\delta\beta$ fusion)

Adams and Steinberg. *Prog Clin Biol Res.* 1981;55:81.
Clarke and Higgins. *Clin Chem.* 2000;46:1284.

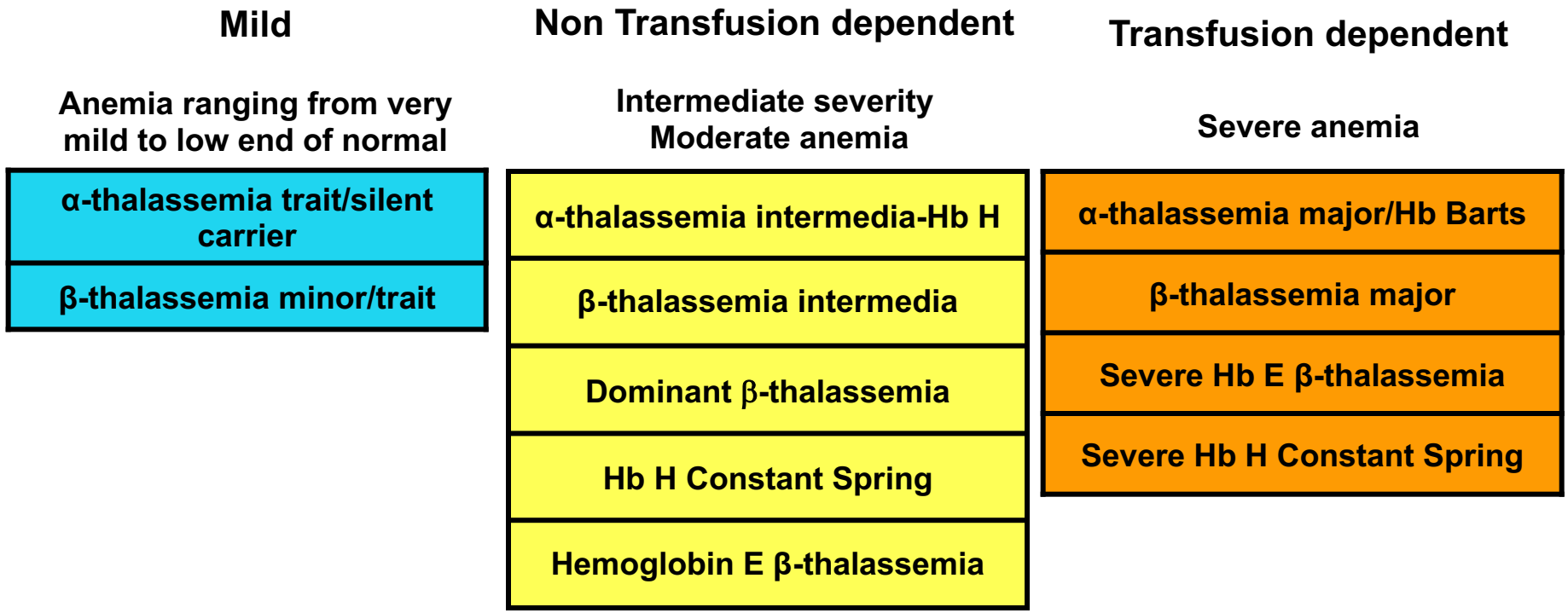
DISTRIBUTION



- 80-90 million carriers worldwide (~ 1.5% of population)[1]
- 60,000 affected individuals born annually[1]
- Exact prevalence in US unknown; estimated to be ~ 2000 individuals[2]
- Estimated prevalence in Italy: ~ 6000[3]
- High prevalence in Asia—South and Southeast Asia, China[1]
- Immigration patterns

1. Weatherall. *BMJ*. 1997;314:1675;
2. Cohen et al. *Hematology* (*Am Soc Hematol Educ Program*). 2004:14.

GENOTYPE-PHENOTYPE



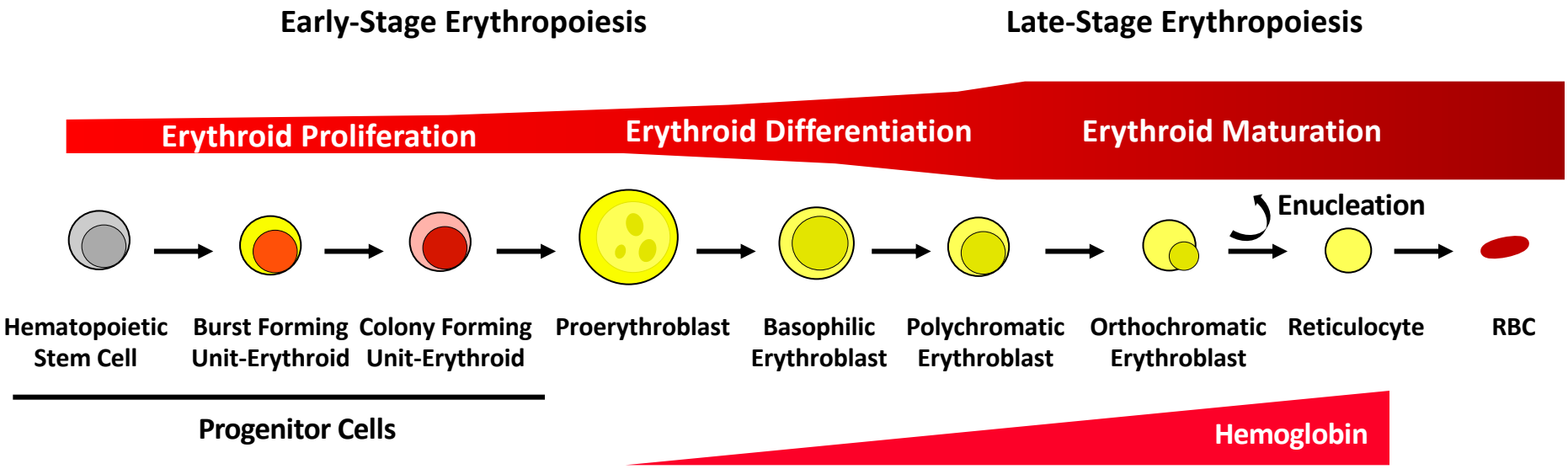
NOT SO BENIGN

β THALASSEMIA

MUTATIONS

- β^0 - nonsense, frameshift or splicing
- β^+ - promoter area CACCC or TATA box, polyadenylation signal, 5’ or 3’ UTR, or splicing defects
- Complex $\delta\beta$ or $\gamma\delta\beta$ thalassemias - deletions of part of β globin gene cluster
- Deletion of LCR with intact β globin gene
- Silent - distal CACCC box, 5' unbalanced region, polyadenylation signal, some splicing defects

NORMAL ERYTHROPOIESIS



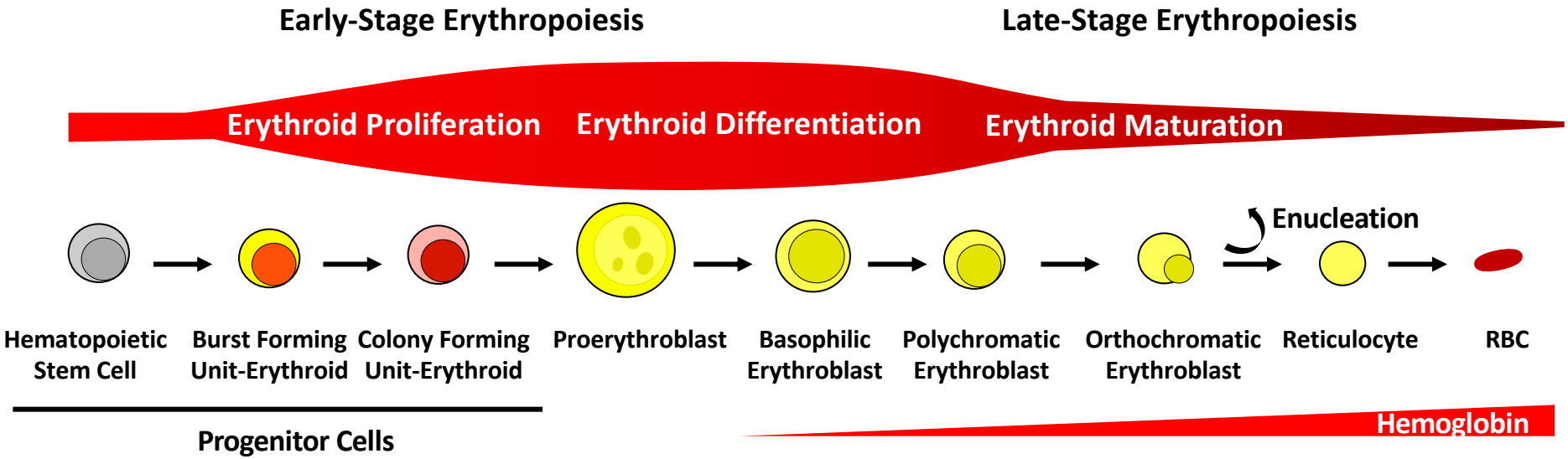
- Characterized by proliferation of progenitor cells
- Promoted by EPO

- Characterized by differentiation of erythroblasts, maturation of reticulocyte precursors into RBCs
- Regulated by TGF-β ligands

HEMOGLOBIN ABNORMALITY

- Imbalance between normal ratio of α : β chain production
- Insufficient upregulation of complementary genes
- Precipitation of tetramers, formation of hemichromes
- Cell disruption - apoptosis
- Ineffective erythropoiesis

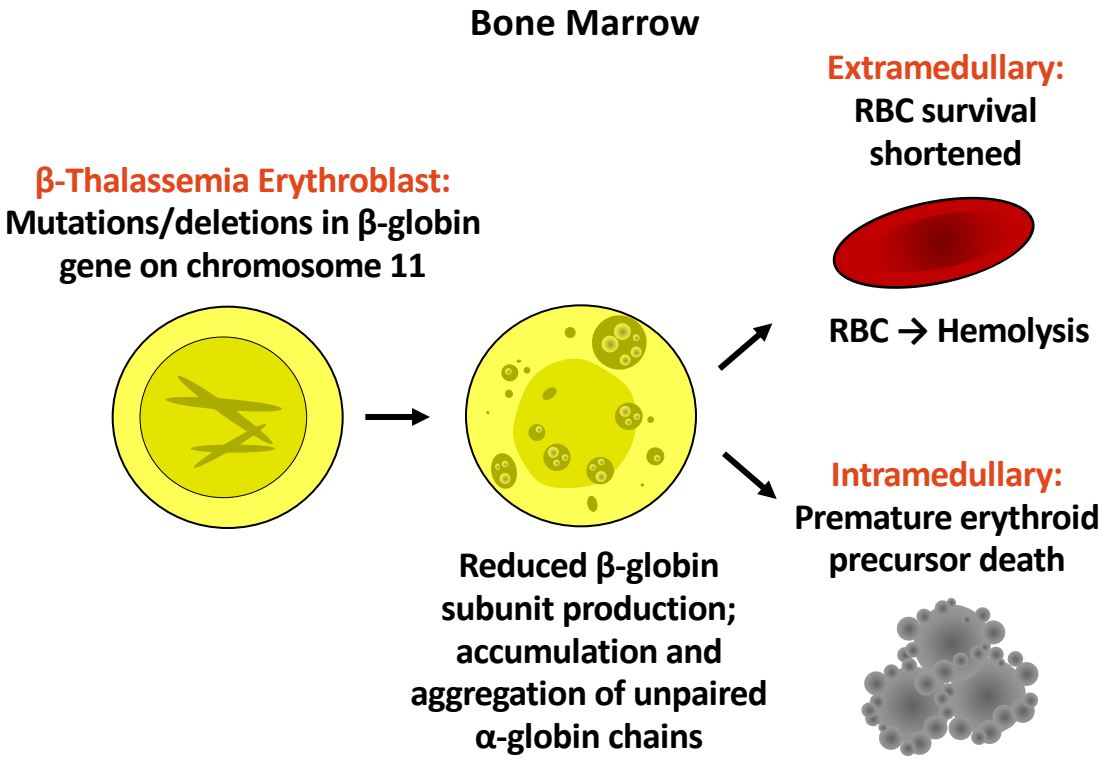
INEFFECTIVE ERYTHROPOIESIS



- Characterized by expansion of early erythroid precursors

- Characterized by accelerated differentiation, maturation arrest in polychromatic erythroblast stage, and apoptosis
- Accumulation of TGF-β ligands

FEATURES OF INEFFECTIVE ERYTHROPOIESIS



- Impaired erythroid precursor maturation
- α and β chain imbalance
- Formation of toxic hemichromes from precipitation of unpaired α-globin chains
- Apoptosis of erythroid precursors
- Reduced RBC survival
- Anemia
- Increased erythropoietic drive
- Extramedullary hematopoiesis
- Dysregulated iron metabolism

CLINICAL PHENOTYPES IN BETA THALASSEMIA HETEROZYGOUS

Silent carrier [1]	Carrier with normal phenotype:
Trait	Normal CBC, retic, electrophoresis
	Requires DNA testing for detection of mutations
	Genotypes: β^{+}/β
Minor [1]	Carrier of the classic trait
Trait	Slight anemia at worst with low MCV
	Quantitative Electrophoresis: Elevated A2, elevated F
	Genotypes: β^{+}/β or β^{0}/β

1. Xu et al. *J Clin Pathol*. 2004;57:517

CLINICAL PHENOTYPES IN BETA THALASSEMIA HOMOZYGOUS OR COMPOUND HETEROZYGOUS

Intermedia [1]

Inherits two Thalassemia mutations
Diagnosis usually at 2-5 years of age
Moderate anemia; Hb >7-10 g/dL
Elevated Ret, ct: 2-10%; NRBCs on smear
Hepatosplenomegaly, Extramedullary Hematopoietic Masses
Minimal or periodic transfusions
Daily Folic Acid supplementation: 1 mg daily
May benefit from splenectomy
Genotypes: β^+/β^+, β^+/β^0, β^0/β^0, β^E/β^+, β^E/β^0, $\beta^+/\alpha\alpha\alpha\alpha$, $\beta^0/\alpha\alpha\alpha\alpha$

Major [1, 2]

Inherits two Thalassemia mutations
Diagnosis in first year
Severe anemia; Hb <7 g/dL
Ret. Ct: 10-15%; many NRBCs on smear
Lifelong transfusions
Genotypes: β^0/β^0, β^+/β^+, β^E/β^0, β^E/β^+

1. Camaschella and Cappellini. *Haematologica.* 1995;80:58;
2. 2. Weatherall et al. *Ciba Found Symp.* 1979;66:147.

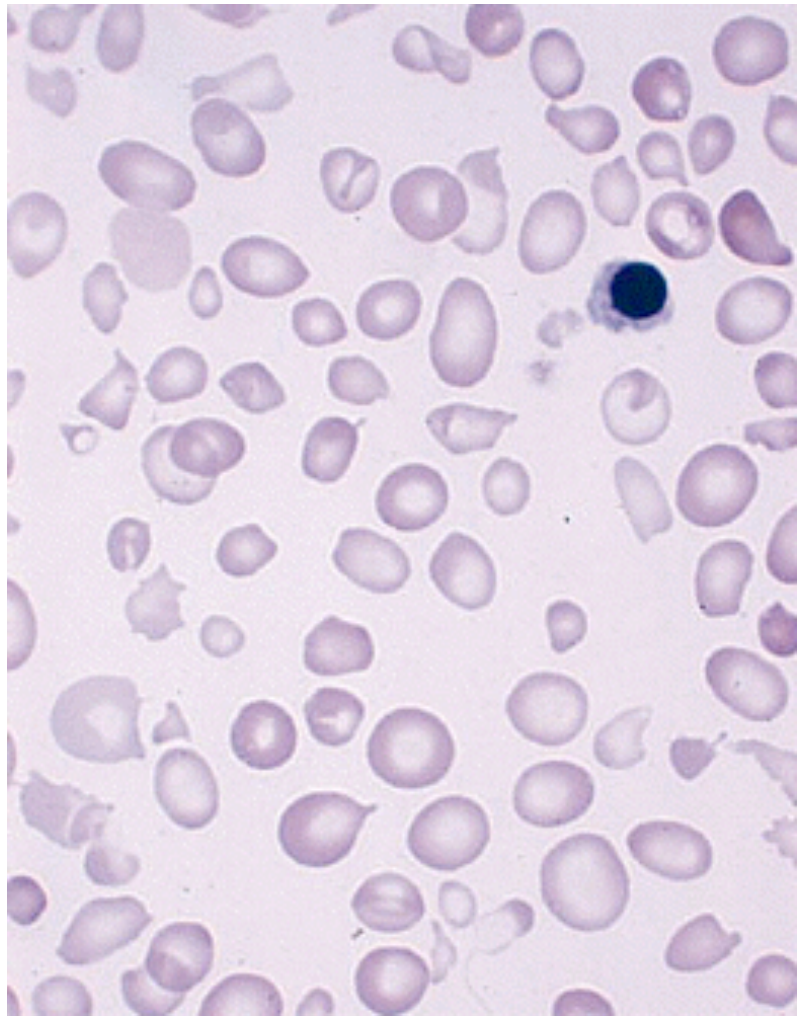
SPECTRUM OF DISEASE

Syndrome	Genotype	Hematology	Disease Severity
Thalassemia major	β^0/β^0	▪ Complete absence of Hb A ▪ Severe anemia requiring transfusions from infancy	▪ **TD** ▪ Lifelong supportive care required
Thalassemia intermedia	β^+/β^+ or β^0/β^+	▪ Diminished production of Hb A ▪ Mild to moderate anemia	▪ **NTD** ▪ May need occasional transfusions or may become **TD** ▪ Significant variability in disease severity
Thalassemia minor	β^+/β or β^0/β	▪ Mild or no anemia	▪ **NTD** ▪ May be asymptomatic

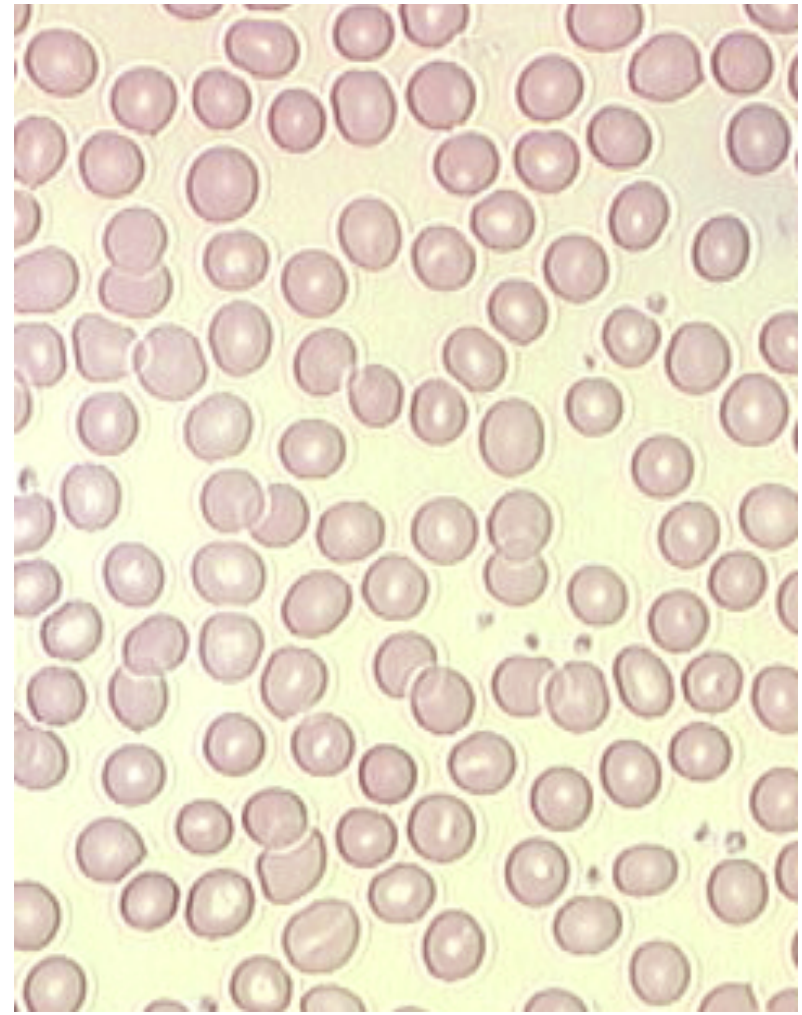
DIAGNOSIS

- History - family history, ethnicity
- Clinical syndrome - anemia, hepatosplenomegaly, facies, skeletal abnormalities
- CBC - anemia with low MCV, low MCH, smear
- Hemoglobin electrophoresis
- Genetic testing

THE BLOOD SMEAR

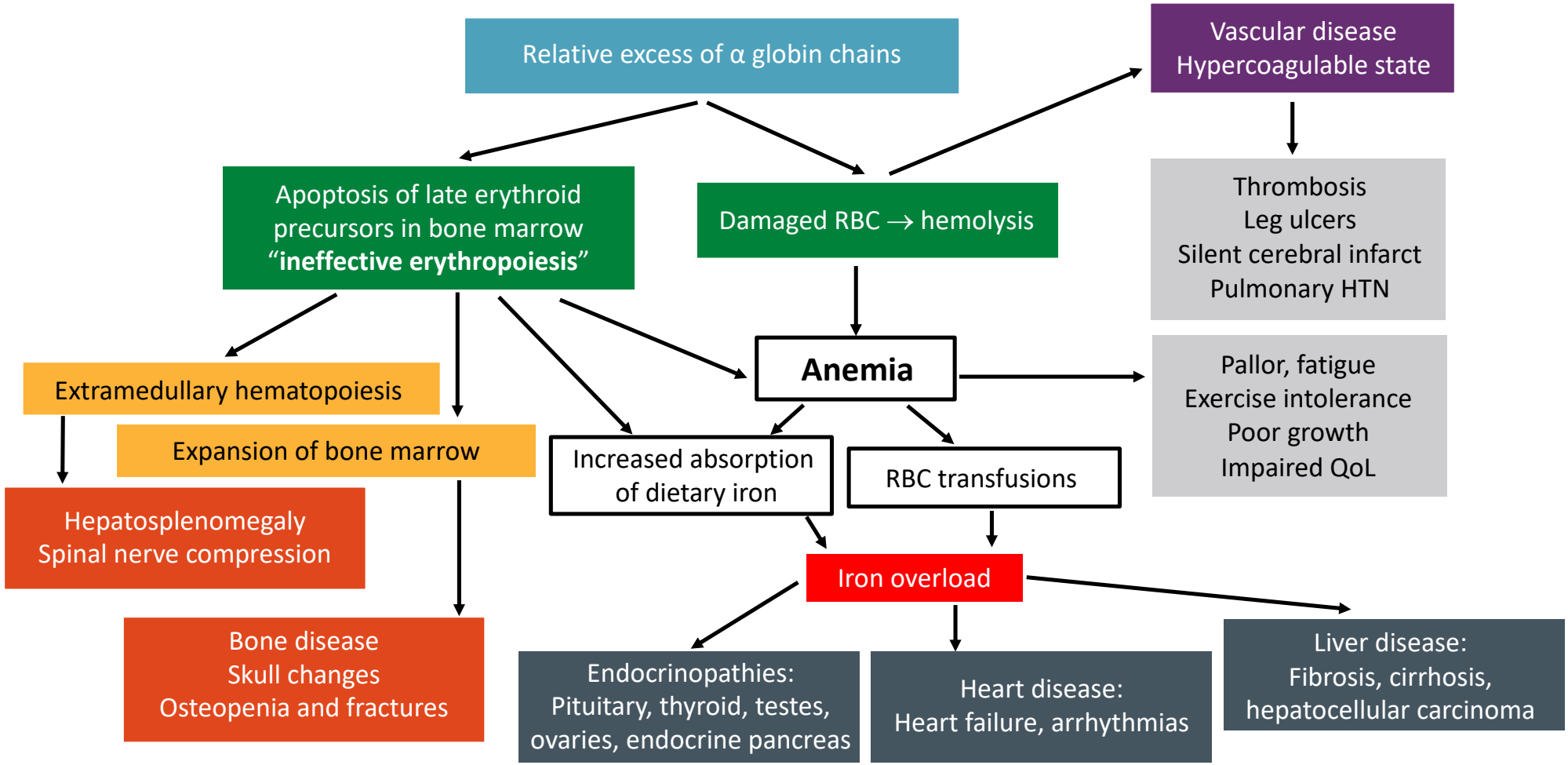


Major



Minor

PATHOLOGY AND CLINICAL FEATURES



CLINICAL MANIFESTATIONS

- **Anemia:** Impaired function, impaired growth, impaired QoL
- **Iron overload:** Increased GI absorption, transfused iron
- **Cardiac disease:** Anemia, iron deposition
- **Endocrinopathy:** Pituitary, thyroid, endocrine pancreas, gonads
- **Bone disease:** Erythroid hyperplasia, endocrinopathy
- **Gallbladder disease:** Increased red cell turnover
- **Extramedullary hematopoiesis:** Hepatosplenomegaly, spinal nodules
- **Vascular disease:** Leg ulcers, pulmonary hypertension, stroke

COURSE - TDT

- Regular blood transfusions: Every 2-4 wks
- Splenectomy as needed
- Iron chelation therapy: Oral vs parenteral
- Monitoring for iron overload: MRI (annually)
- Monitoring for side effects of chelation (monthly)
- Monitoring for complications of disease and treatments (annually or more frequently if present)
- Hematopoietic stem cell transplantation

COURSE - NTDT

- Supportive care: Bone health, vitamin D and folic acid supplementation, thromboprophylaxis
- Splenectomy: If severe anemia and splenomegaly
- Transfusions (periodic): Leg ulcers, splenomegaly, pregnancy, surgery
- Iron chelation
- Induction of Hb F: Hydroxyurea, 5’azacytidine, decitabine, butyrate
- Other: Luspatercept, ruxolitinib
- Stem cell transplantation, gene therapy

MANAGEMENT ISSUES

- Transfusions, splenectomy
- Transfusion complications
- Complications of Iron overload
- Chelation - Side effects and monitoring
- Organ dysfunction
- Stem cell transplantation
- Newer therapies

TRANSFUSION GOALS

- Correction of anemia Hgb > 10 gm/dl
- Suppression of (ineffective) erythropoiesis
- Prevention of bony changes, hepatosplenomegaly
- Inhibition of GI iron absorption
- Minimization of transfusional iron overload
    - Splenectomy
    - 15 mL/kg monthly approx. 0.3 – 0.6 mg/kg/day Fe
    - Short transfusion intervals q 2 wks

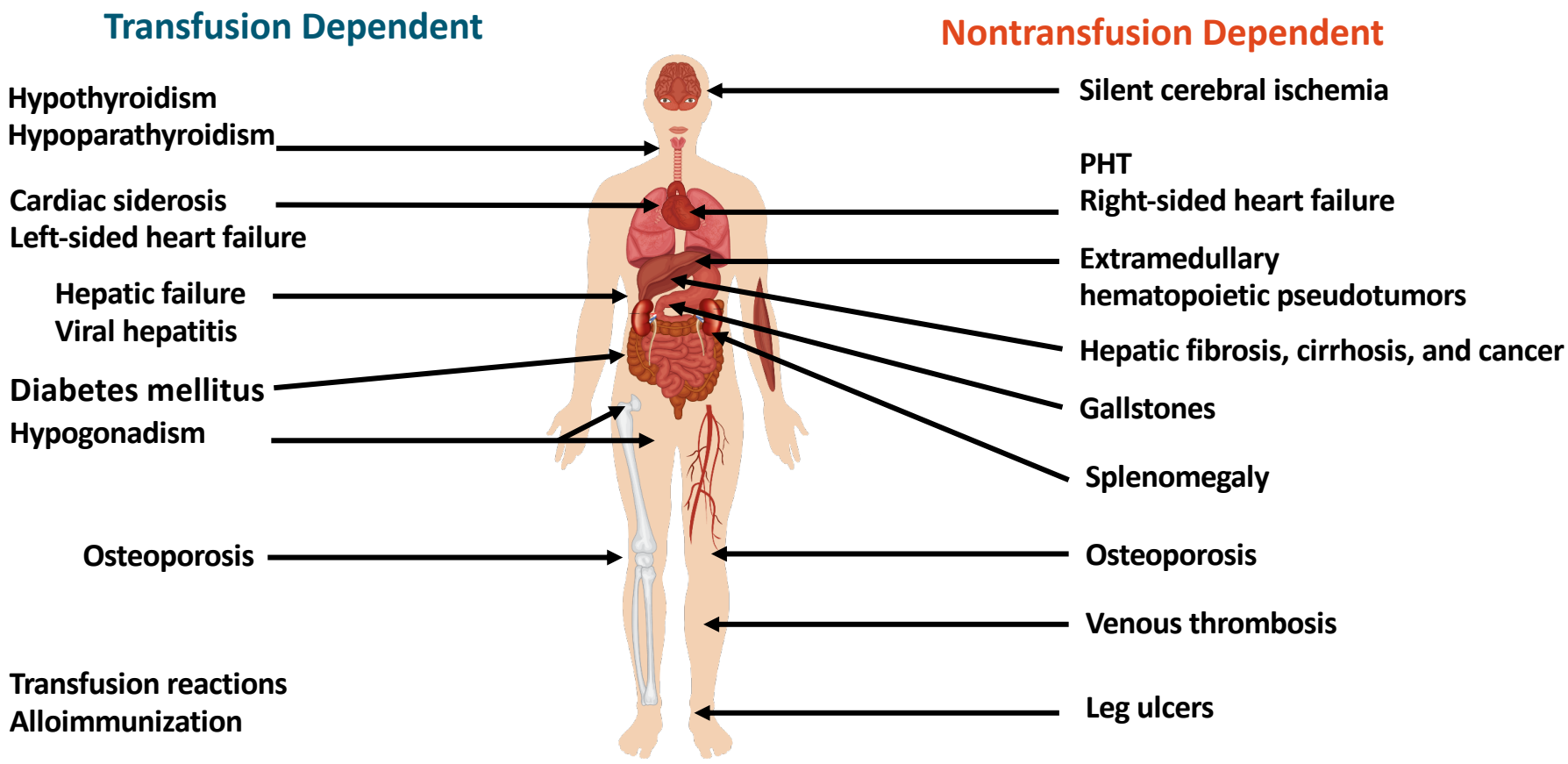
TRANSFUSION COMPLICATIONS

- Complications related to transfusions
  - Alloimmunization
  - Infections
  - Iron overload
- Complications related to iron overload
  - Cardiac failure
  - Liver cirrhosis/fibrosis/cancer
  - Diabetes mellitus
  - Infertility
  - Arthritis

SPLENECTOMY

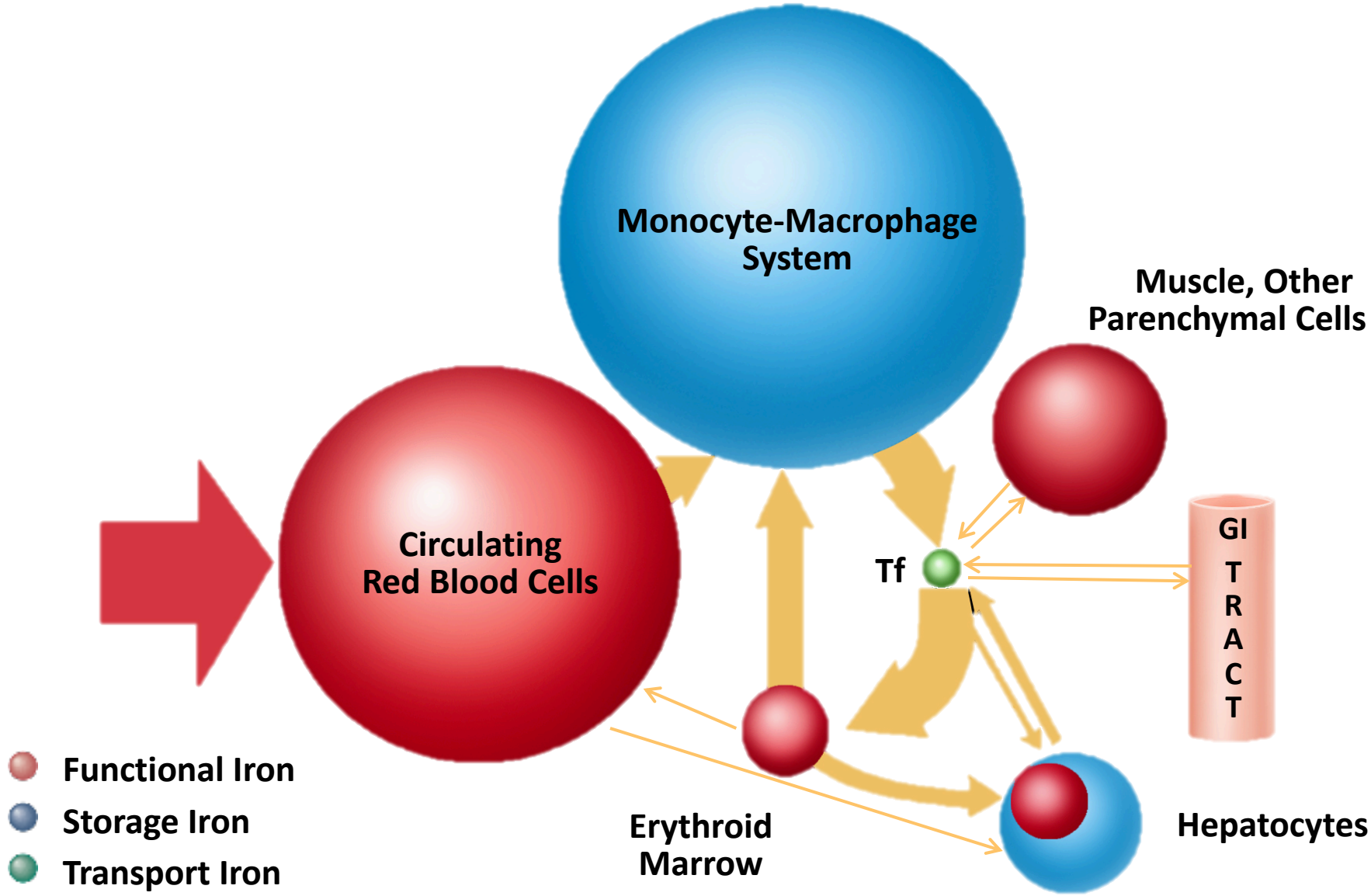
- TDT
  - Hypersplenism with increasing transfusion requirement - >200 ml/kg/year PRBCs
- NTDT
  - Massive splenomegaly
  - Hypersplenism – extramedullary hematopoiesis
- Risks – infection, vascular disease
- Benefits – minimize transfusions and iron loading

COMPLICATIONS

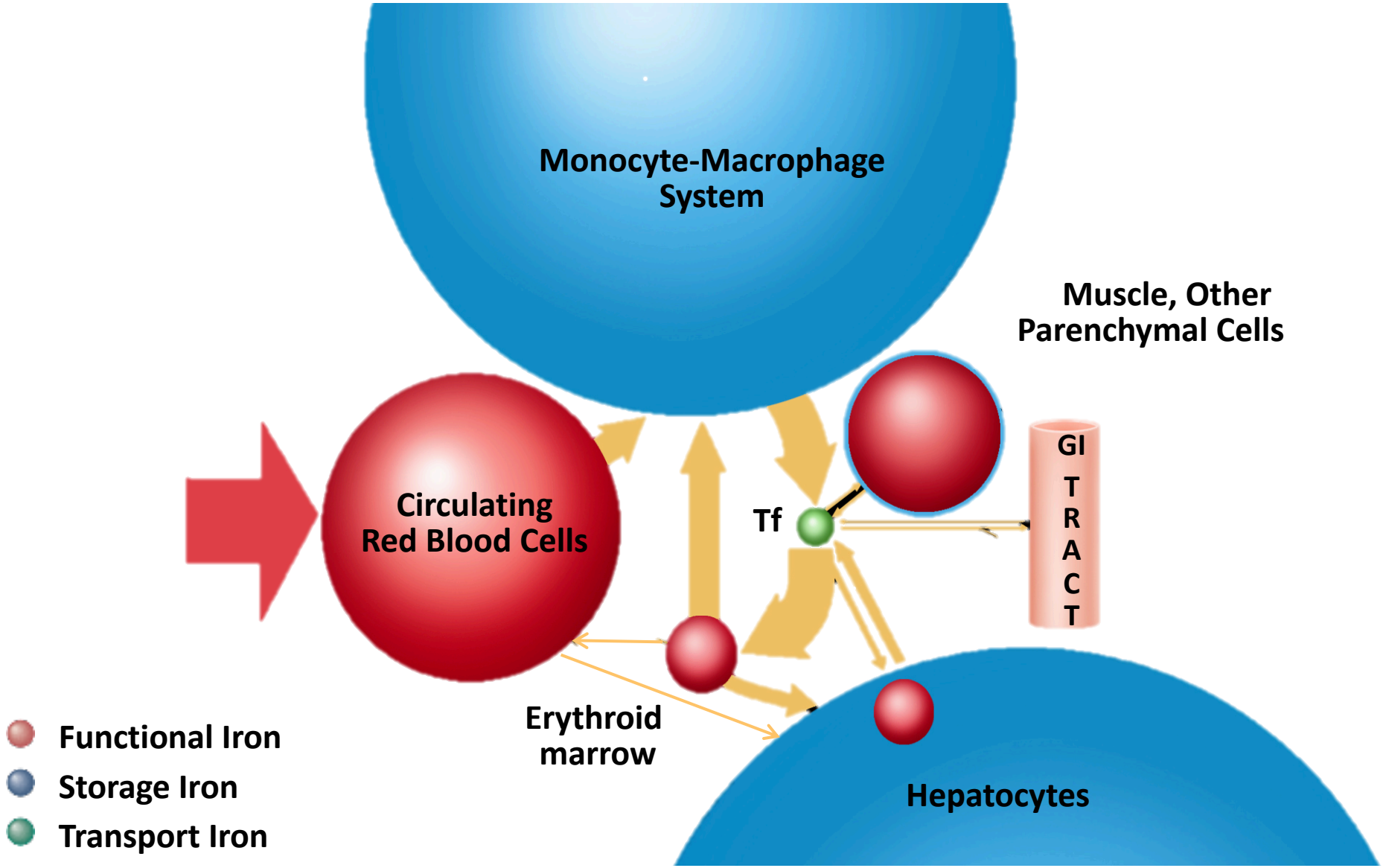


Musallam. Acta Haematol. 2013;130:64. Musallam. Haematologica. 2013;98:833.

TRANSFUSIONAL IRON OVERLOAD: 16 Units PRBC



TRANSFUSIONAL IRON OVERLOAD: 100 Units PRBC



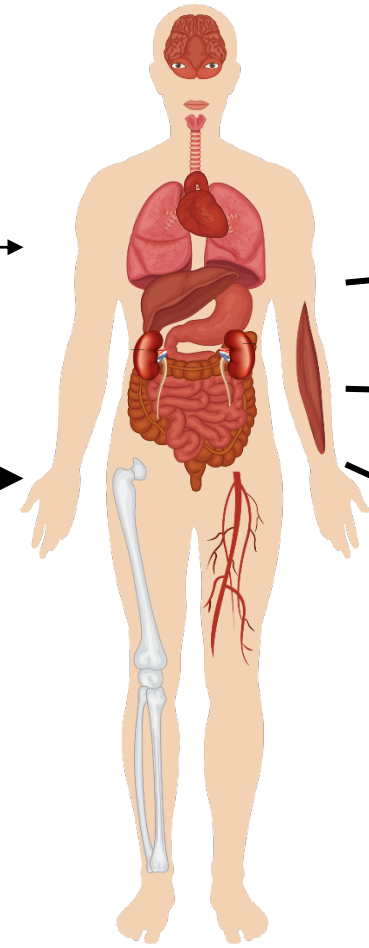
IRON LOADING

Iron Intake

Dietary iron: 0-5 mg/day

Iron received as transfused erythrocytes: 20-25 mg/day

Total:
0.3-0.6 mg/kg/day
20-30 mg/day



Iron Excretion

Sweat: 0.2-0.3 mg/day

Urine: ~ 1 mg/day

Feces: 0-3 mg/day

Total:
1-4 mg/day

Toxicity = tissue iron x tissue sensitivity x time

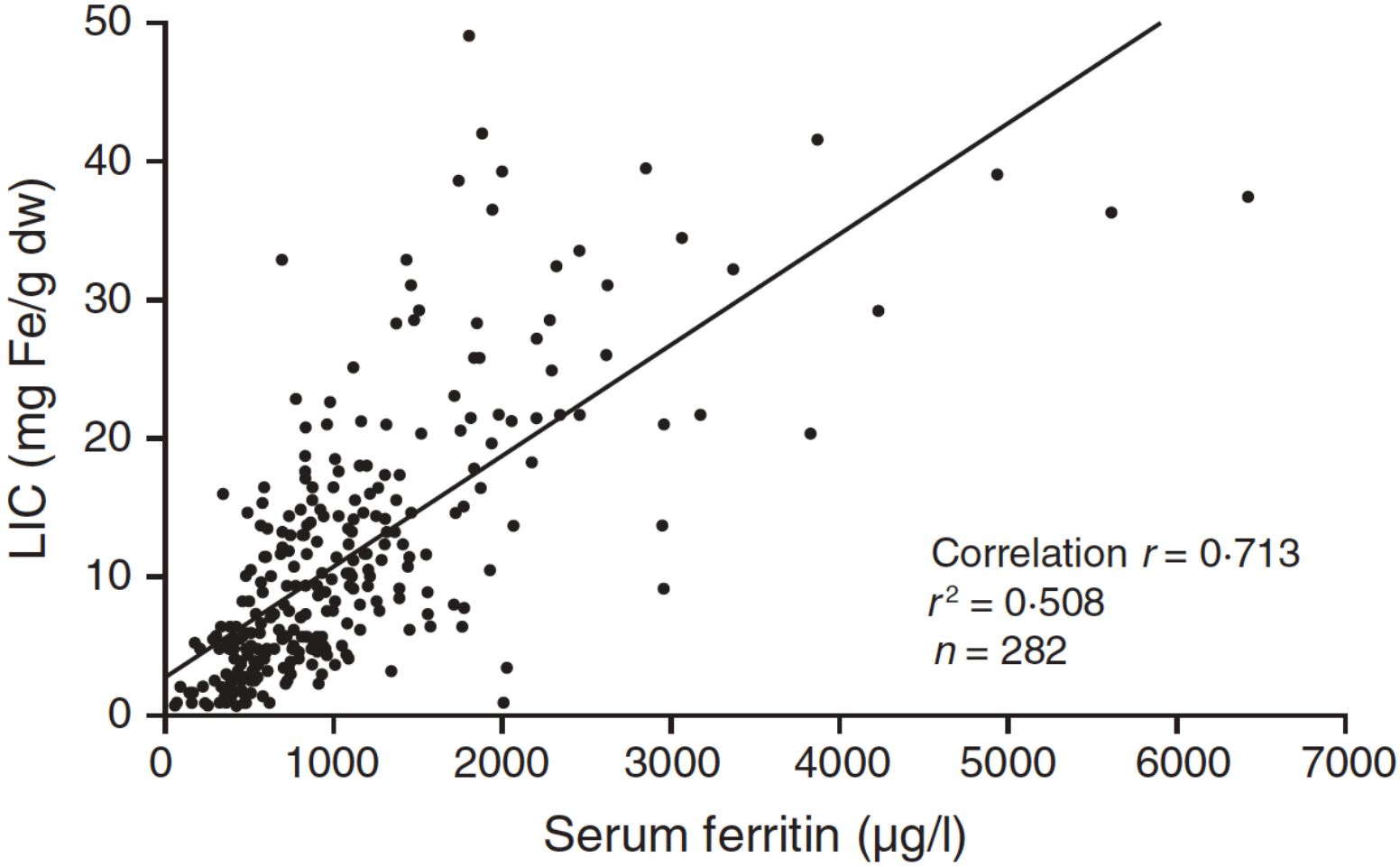
RISK OF ORGAN DAMAGE

- Liver – all patients load the liver
  - Fibrosis, cirrhosis, risk of hepatocellular carcinoma
  - Hepatitis C independent risk factor
- Heart – patients with inappropriately low hepcidin
  - Contractile dysfunction - diastolic
  - Electrophysiologic dysfunction
- Endocrine – patients with inappropriately low hepcidin
  - Pituitary dysfunction
  - Diabetes
  - Gonadal dysfunction
  - Osteopenia

MEASURING IRON BURDEN

- Serum Ferritin
- Liver Biopsy LIC
- SQUID LIC
- MRI LIC R2/R2*
- MRI Cardiac T2*

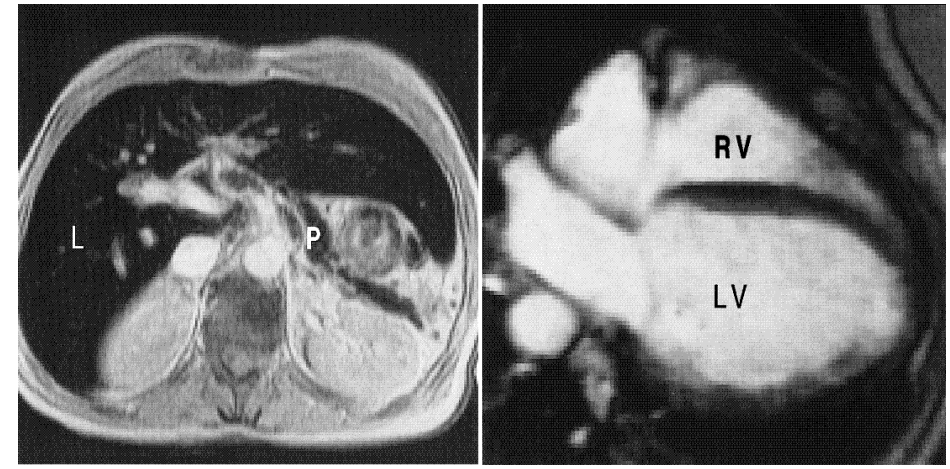
SERUM FERRITIN

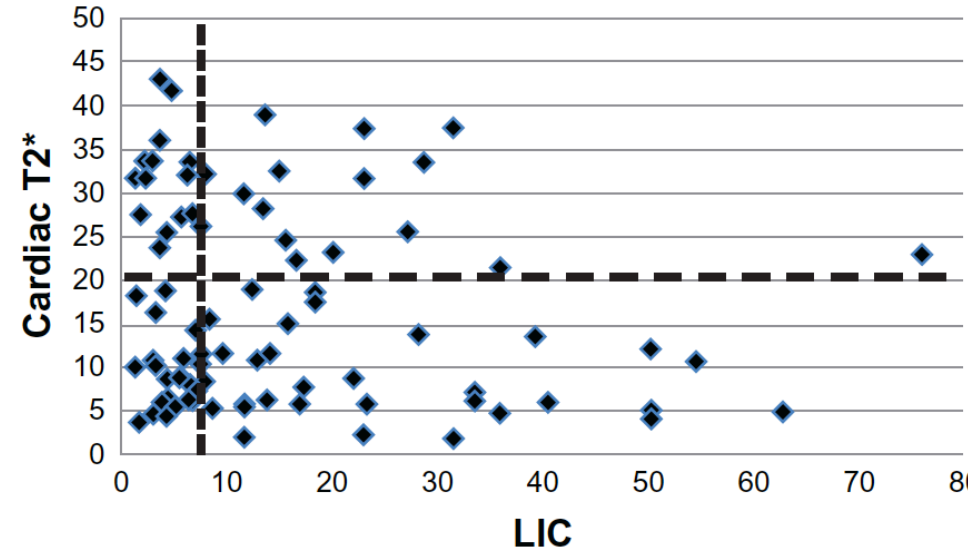


Taher et al., Br J Haematol 2015

MRI - IRON QUANTIFICATION

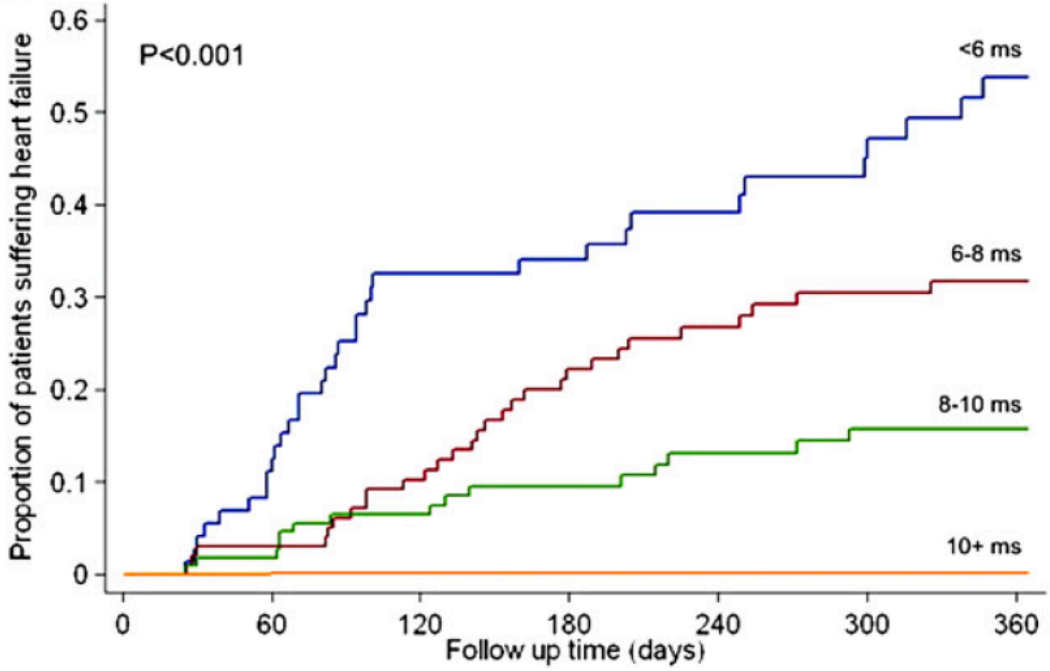
- Utility
  - Non-invasive
  - No irradiation
  - Easily accessed
- Caveats
  - Technique variability
  - Sensitivity
  - Not correlated between tissues

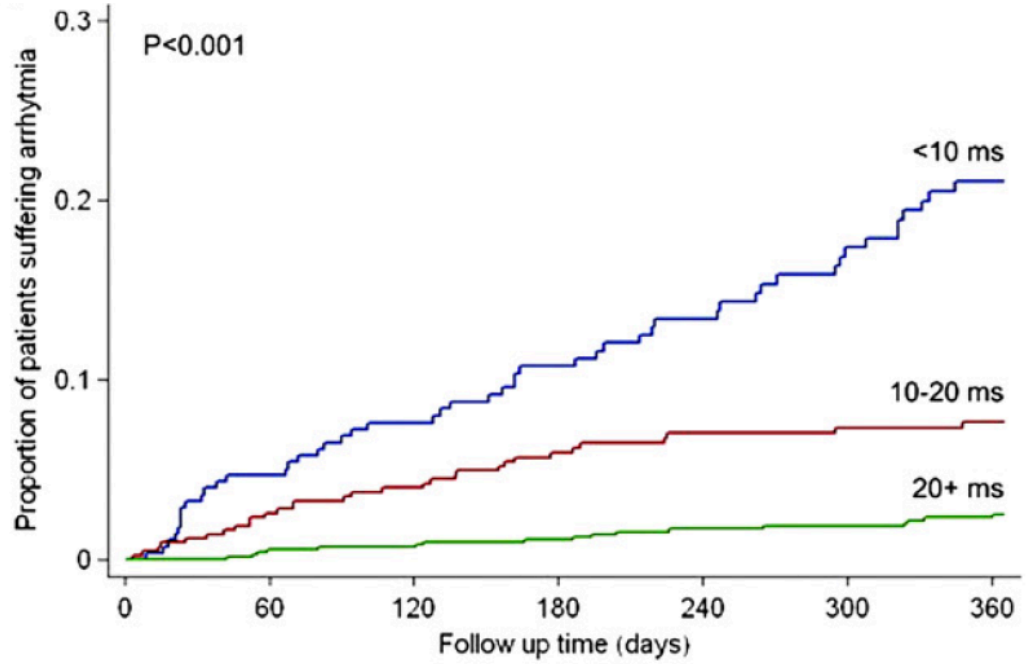




CARDIAC T2* - PROGNOSTIC VALUE

(Thalassemia Major)



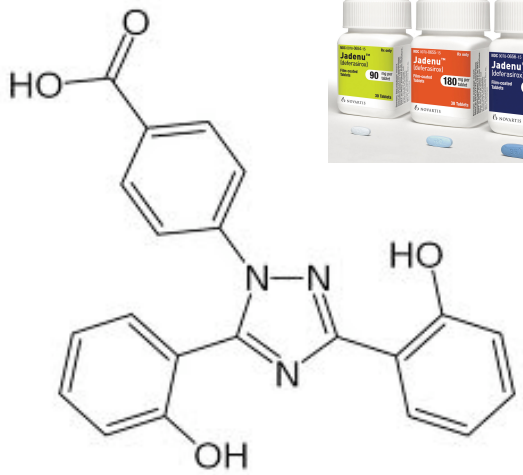


Kirk P et al. Circulation 2009.

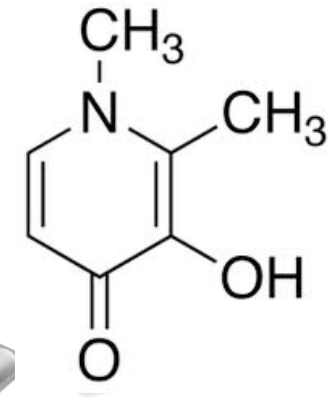
CHELATION

- Which Chelator
  - Iron binding
  - Route of administration
  - Efficacy
  - Toxicity
- When to start
- Monitoring
- COMPLIANCE

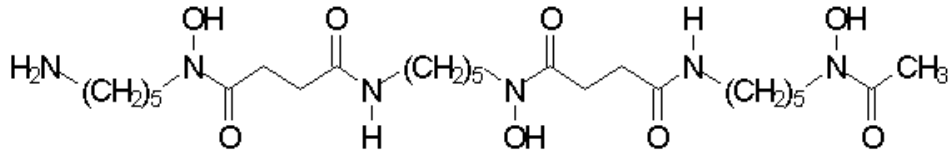
DEFERASIROX
(Exjade®, Jadenu®)

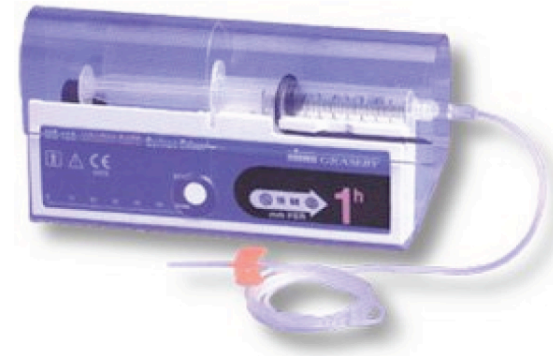


DEFERIPRONE
(Ferriprox®)



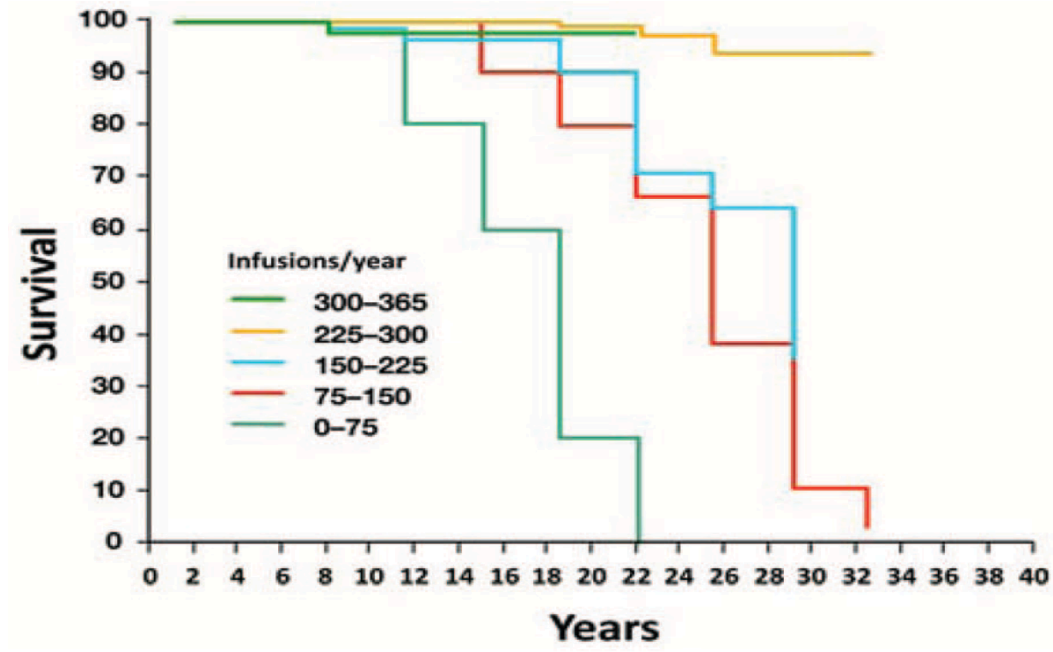
DEFEROXAMINE
(Desferal®)





DEFEROXAMINE (Desferal®)

- Longest used approved effective iron chelator
- Challenges
  - Subcutaneous slow infusion 5 to 7 nights/week
  - Infusion-site reactions and pain
  - High degree of noncompliance
- Survival correlated with compliance in thalassemia



Wood JC et al. Blood 2008

DEFERIPRONE (Ferriprox®)

- FDA approved November 2011
  - Second line and combination use
  - Less effective than deferoxamine in reducing LIC
  - More effective in removing cardiac iron
- Side effects
  - Nausea, vomiting, abdominal pain
  - Arthralgia
  - Neurologic syndrome
  - Reports of increased risk of liver fibrosis
  - Neutropenia/Agranulocytosis

    Weekly neutrophil count recommended

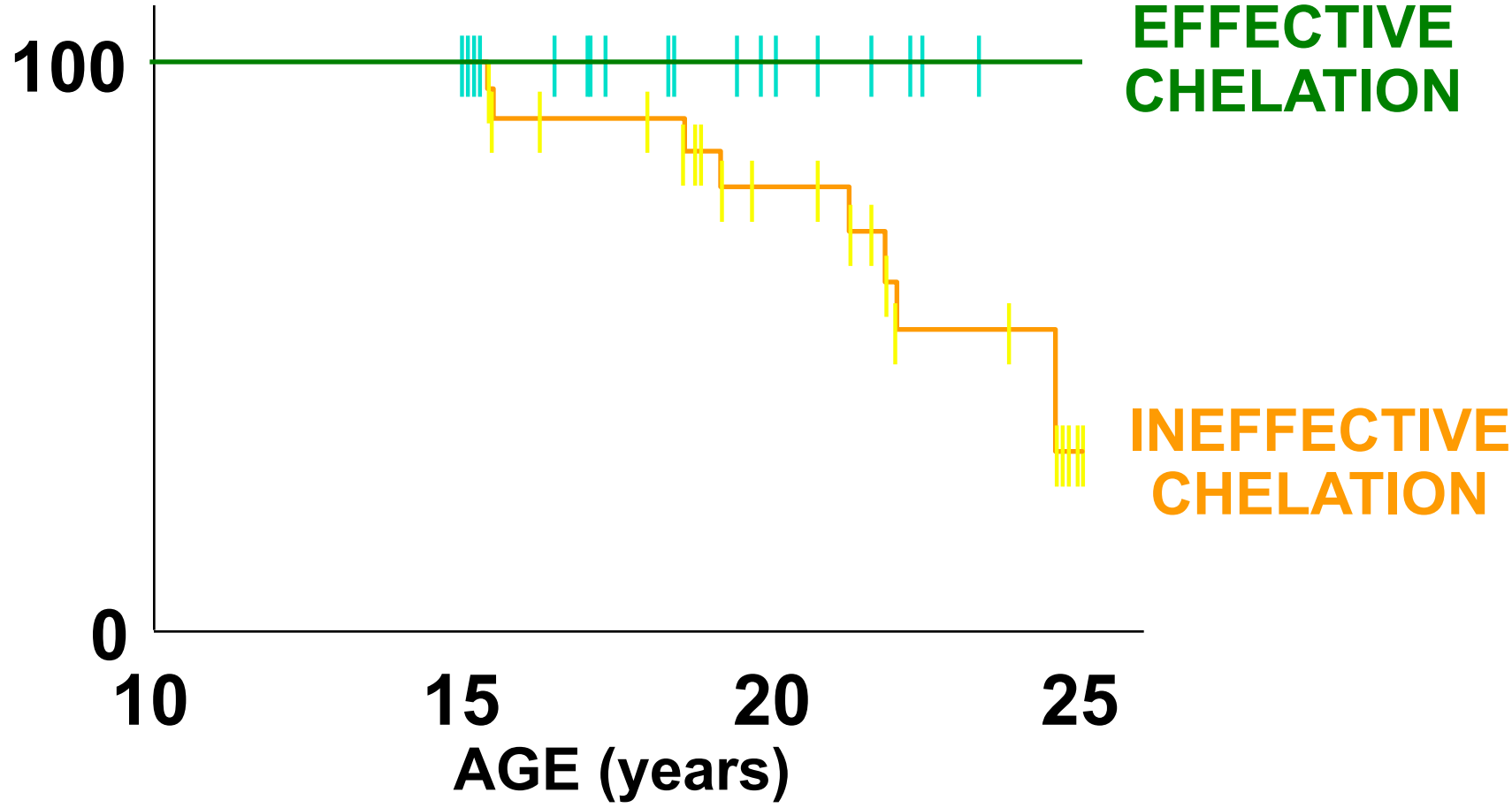
DEFERASIROX (Exjade®, Jadenu®)

- FDA approved November 2005, 2015
  - Orally effective
  - Once a day only
  - Wide therapeutic index, dose range
- Side effects
  - Nausea, vomiting, abdominal pain
  - Liver and kidney toxicity
  - Rare reports of Neutropenia/Agranulocytosis
- Good long term safety and efficacy data
- Can be used in Combination therapy
- Demonstrated efficacy in cardiac iron removal

MONITORING IRON OVERLOAD

Level of Iron Overload	Iron Overload Measurements	Frequency of MRI Testing
Target	• LIC 2-5 mg/g DW • Ferritin <1,000 ng/mL • T2*>20 msecs	• Check LIC when chelation is first initiated and every year thereafter • Check cardiac T2* at age 10 and every 2 years thereafter
Moderately Elevated	• LIC 5-10 mg/g DW • Ferritin 1,000 to 2,500 ng/mL • T2*>20 msecs	• Check LIC when chelation is first initiated and every year thereafter • Check cardiac T2* at age 10 and every 1-2 years thereafter based on LIC trends
Seriously elevated	• LIC >10 mg/g DW • Ferritin >2,500 ng/mL • T2*<20 msecs	• Check LIC when chelation is first initiated and every 6 months thereafter, if on intensive chelation • Check cardiac T2* at age 10 and every year thereafter based on LIC trends
Mild cardiac iron overload with normal cardiac function	• T2* 10-20 msecs	• Check LIC and cardiac T2* when chelation is first initiated and every 6-12 months thereafter while on intensive chelation. • Monitor cardiac function (MRI/ECHO) every 6 months
Severe cardiac iron overload with or without cardiac dysfunction	• T2* <10 msecs	• Check LIC and Cardiac T2*when chelation is first initiated and every 6 months thereafter on intensive chelation. • Monitor cardiac function (MRI/ECHO) every 6 months with cardiac specialist

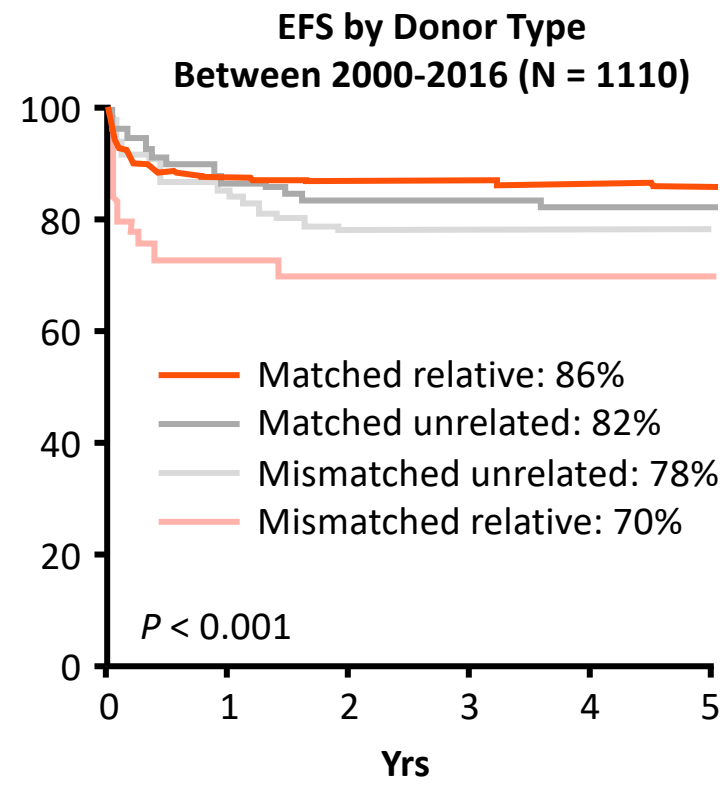
SURVIVAL IN THALASSEMIA MAJOR WITH EFFECTIVE OR INEFFECTIVE CHELATION



Brittenham et al, New Engl J Med 1994;331:567-573

STEM CELL TRANSPLANTATION

- Only currently available curative option
- Pretransplant organ function and iron status important; younger patients do better
- Excellent outcomes with matched sibling donors (including umbilical cord blood): 85% to 95% TFS
  - Matched unrelated donors: 68% to 80% TFS
- Nonmyeloablative regimens in clinical trials
- Alternative donor sources in clinical trials
  - Unrelated PBSC, UCB
  - Haploidentical donors

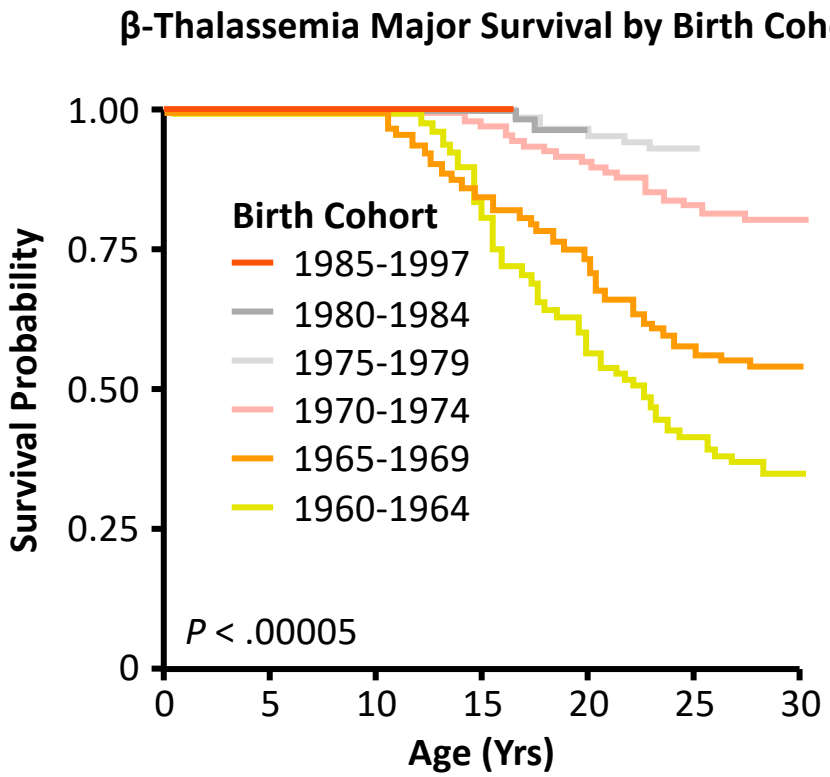


Li. Blood Adv. 2019;3:2562. Baronciani. ASH 2018. Abstr 168. Mohamed. Hematol Oncol Stem Cell Ther. 2017;10:290.

QUALITY OF LIFE

- Issues related to:
  - Symptoms – fatigue
  - Physical appearance (NTDT)
  - Frequent visits to the hospital
  - Need for chelation compliance
  - Pain - Bone disease, extramedullary hematopoiesis (NTDT)
  - Endocrine – growth, development, fertility
  - Financial issues
- Psychosocial issues
  - Chronic illness
  - Reduced life expectancy with complications

SURVIVAL



- Without treatment
  - β^0/β^0: Die in first 2-5 yrs
  - Non- β^0/β^0 : Variable clinical spectrum with complications
- β^0/β^0 and non-β^0/β^0 with treatment
  - Improving survival
  - Significant morbidity but with decreasing incidence
  - Deaths related to complications

*Kaplan-Meier analysis included 977 patients who survived beyond first decade of life.

Borgna-Pignatti. Haematologica. 2004;89:1187. Galanello. Orphanet J Rare Dis. 2010;5:11.

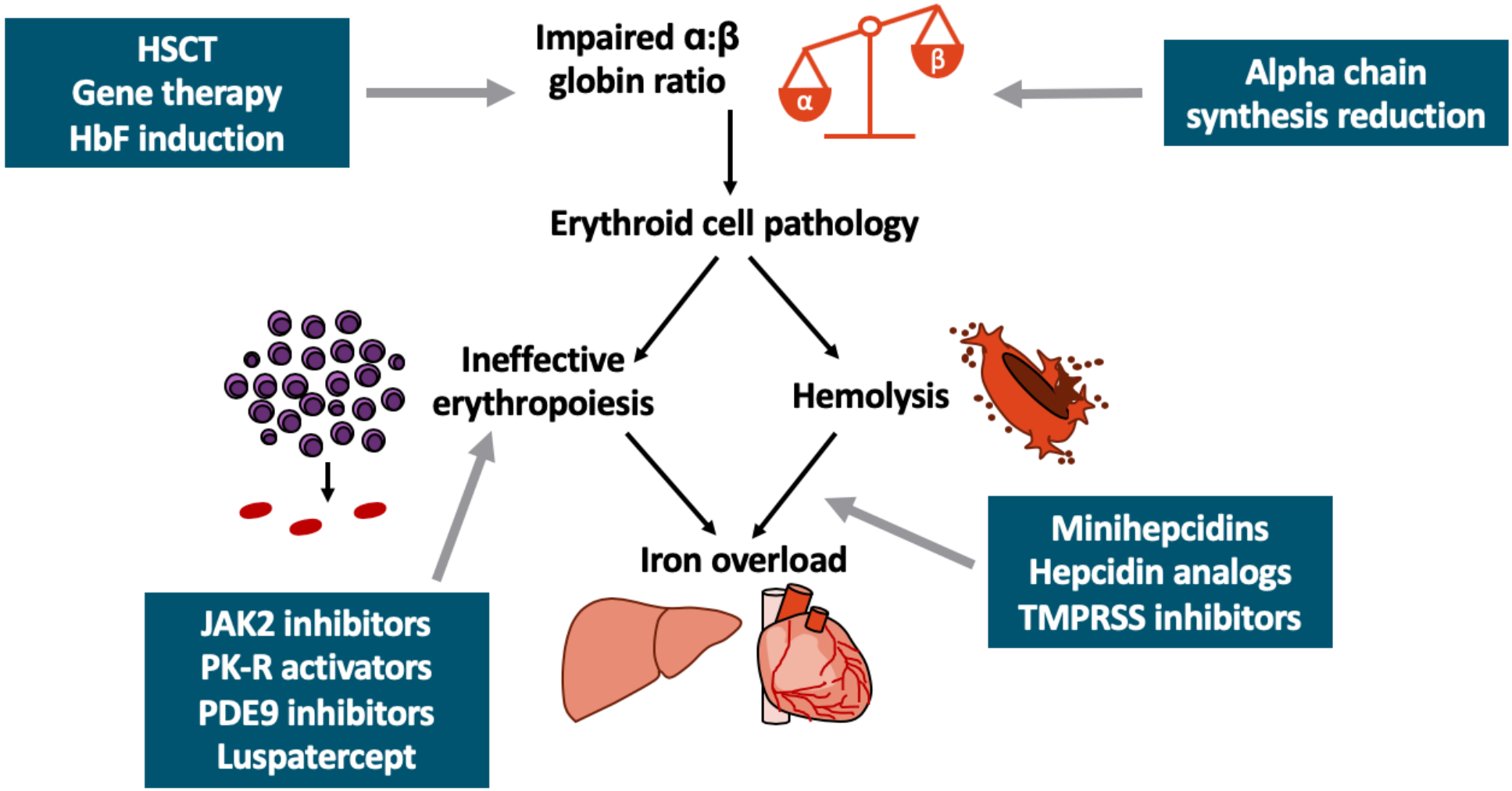
IMPROVED CARE

- Improved safety of blood supply
- Reduced incidence of alloimmunization
- Oral iron chelation
- Improved monitoring of iron overload to enable individualized tailoring of treatment regimen
- Improved treatment for hepatitis
- Improved outcomes for stem cell transplantation

UNMET NEEDS

- Curative options for those without matched sibling donors
- Means of ameliorating ineffective erythropoiesis
  - This could reduce/eliminate transfusion requirement
  - In turn, reducing iron loading
    - From gut absorption
    - From transfusions
- Prevent iron overload and its complications
- Reduce bone disease
- Improve quality of life

NOVEL THERAPIES



Cappellini. Hematology Am Soc Hematol Educ Program. 2017;2017:278.

NOVEL THERAPIES

- Targeted therapies
  - Activin traps
  - Ruxolitinib
  - Hepcidin manipulation
  - Mitapivat
- Gene therapy
  - Gene insertion
  - Gene editing

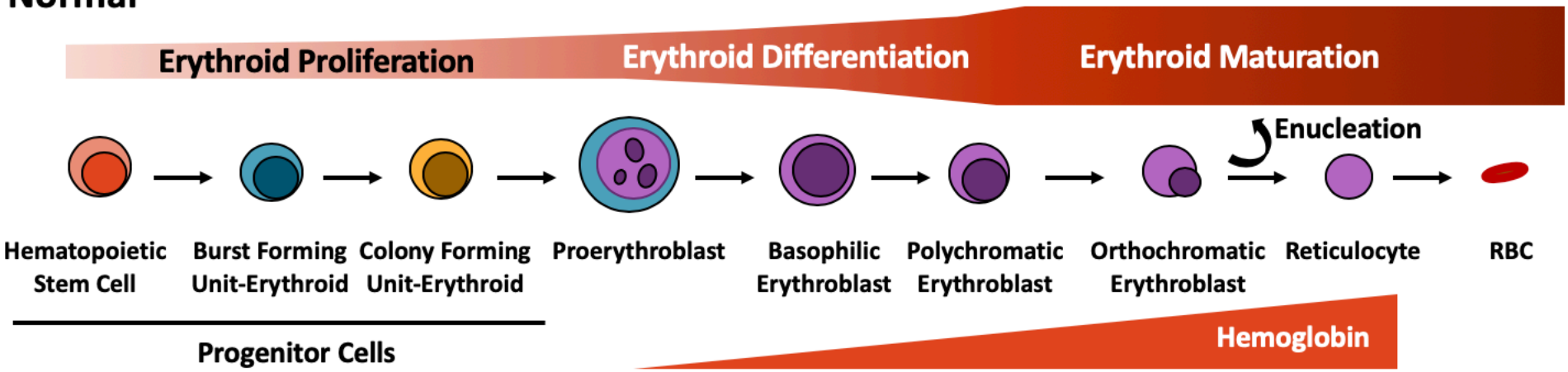
LUSPATERCEPT

- Recombinant fusion protein containing a modified extracellular domain of ActRIIB[1]
- Binds to GDF11 and other TGF-β superfamily ligands, inhibits Smad2/3 signaling, and promotes RBC differentiation/maturation[1]
- Early data
  - Animal studies[1]
  - Phase I study of healthy human volunteers[2]
  - Phase II clinical trial in patients with β-thalassemia showed improved Hb levels (NTDT) and RBC transfusion burden (TDT) with luspatercept[3]

1. Martinez. ASH 2015. Abstr 751. 2. Attie. Am J Hematol. 2014;89:766. 3. Piga. Blood. 2019;133:1279.

LUSPATERCEPT

Normal

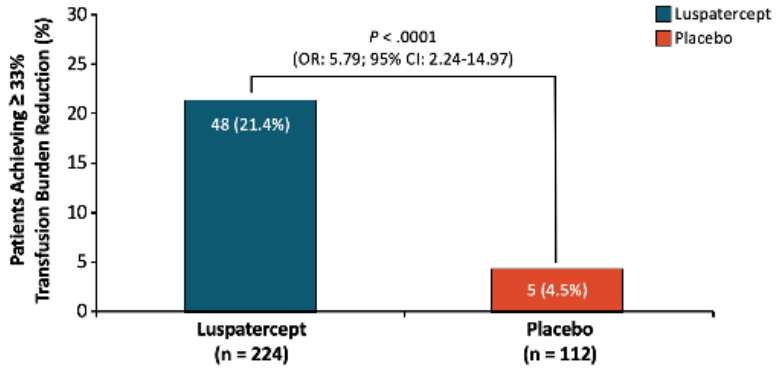


Ineffective in β-Thalassemia

Luspatercept: blocks TBF-β ligands to promote RBC differentiation and maturation

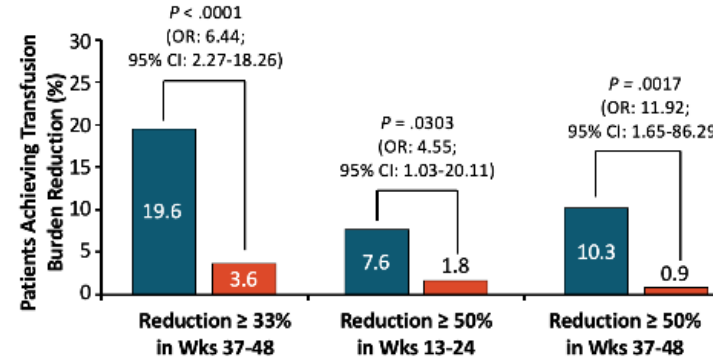
BELIEVE: ENDPOINTS

Primary Endpoint

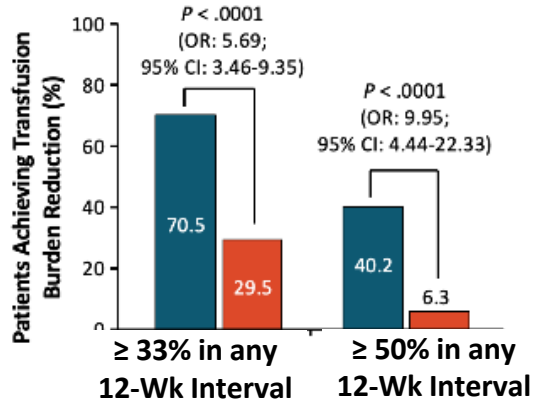


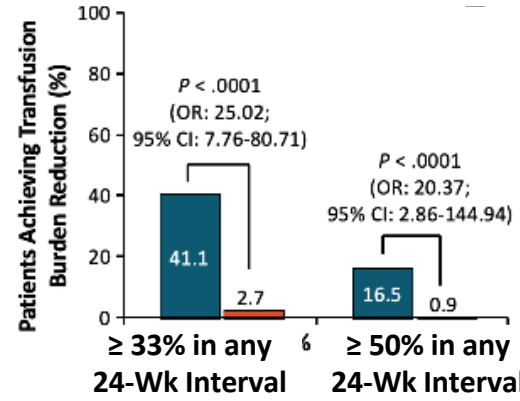
≥ 33% in Wks 13-24

Secondary Endpoints



Additional Endpoints





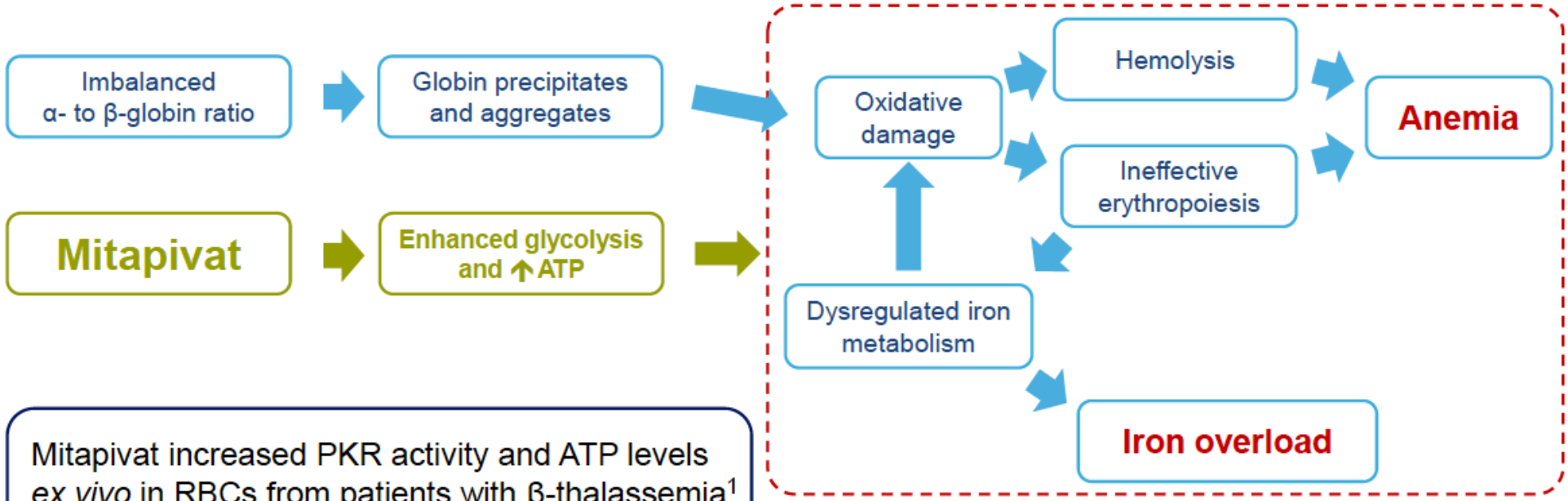
Cappellini. ASH 2018. Abstr 163.

BELIEVE: CONCLUSIONS

- BELIEVE met its primary endpoint, demonstrating statistically significant improvement, with a ≥ 33% reduction in RBC transfusion burden with luspatercept vs placebo
- Key secondary endpoints showed statistically significant improvement with luspatercept vs PBO, including ≥ 33% and ≥ 50% RBC transfusion burden reduction
- Luspatercept demonstrated a statistically significant, clinically meaningful reduction in RBC transfusion burden vs placebo during
  any 12-wk or 24-wk interval of the study period
- Luspatercept was generally well tolerated

Cappellini. ASH 2018. Abstr 163.

Mitapivat – Mechanism in thalassemia



Mitapivat increased PKR activity and ATP levels *ex vivo* in RBCs from patients with β-thalassemia[1]

Mitapivat ameliorated ineffective erythropoiesis, iron overload, and anemia in the $Hbb^{th3/+}$ mouse model of β-thalassemia[2]

Results

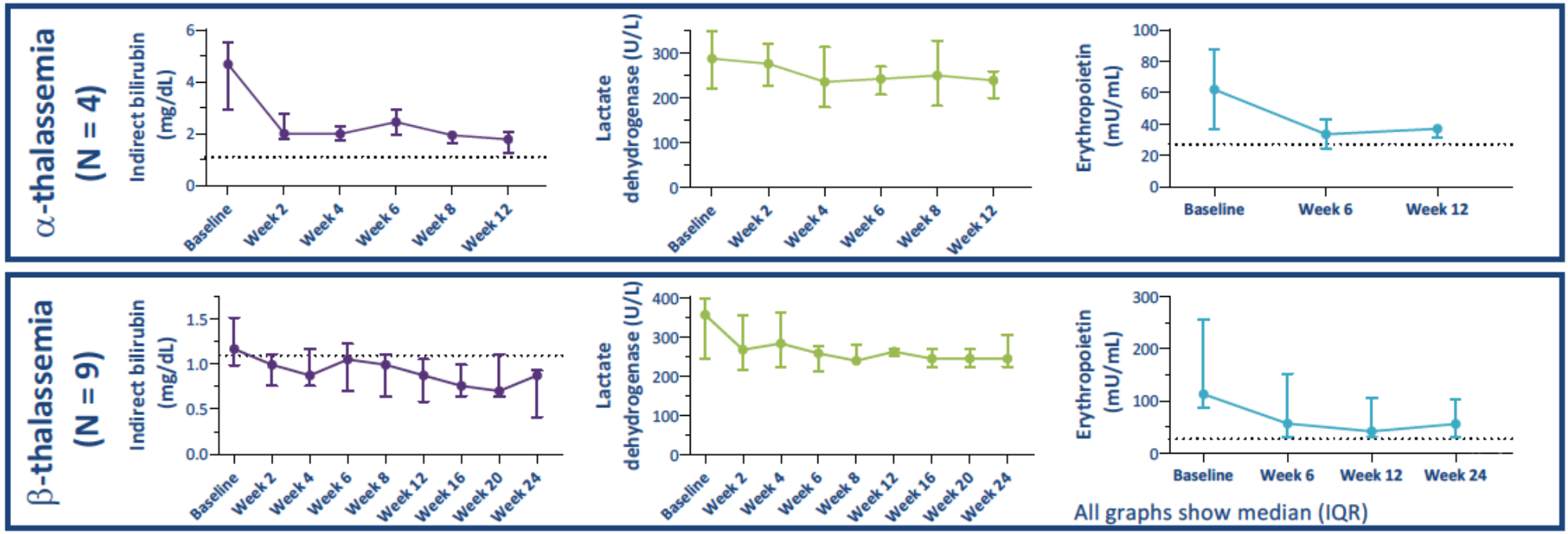
- Primary endpoint was met in 92.3% of patients

Endpoint	Genotype	n/N	%	90% CI
Hb responders during Weeks 4–12 (completed 12 weeks)	All	12/13	92.3	68.4, 99.6
	α	4/4	100	47.3, 100
	β	8/9	88.9	57.1, 99.4
Hb responders during Weeks 12–24 (completed 24 weeks)	β[a]	8/9	88.9	57.1, 99.4
Sustained responders: primary response and ≥ 2 Hb responses during Weeks 12–24	β[a]	7/8	87.5	52.9, 99.4

Patient population	N	Weeks	Mean (SD) change from baseline Hb, g/dL
All patients	13	4–12	1.34 (0.7)
α-thalassemia	4	4–12	1.17 (0.4)
β-thalassemia	9	4–24	1.43 (0.8)

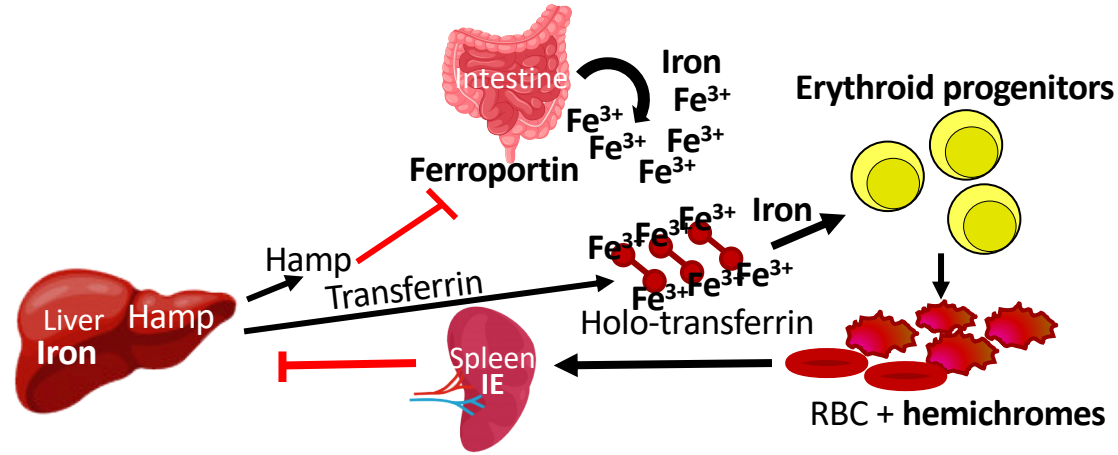
- Median (range) time to Hb increase of ≥ 1 g/dL among responders was 3.1 (1.4–7.1) weeks

Results – Increased Hb, decreased hemolysis



TARGETING THE HEPCIDIN PATHWAY

- In thalassemia, IE results in low levels of hepcidin leading to increased iron absorption, iron redistribution to organs
- Hepcidin mimetics function to:
  - Improve IE
  - Reduce iron absorption
  - Reduce iron to organs
- Trials of hepcidin mimetics
  - Trial of LJPC-401 did not meet endpoint
  - TRANSCEND trial of PTG-300 for TD or TDT and chronic anemia ongoing



- TMPRSS6 is another potential target to decrease iron overload, improve RBC survival by increasing hepcidin levels
  - Inhibition of TMPRSS6 ameliorated iron overload and IE in a mouse model of β-thalassemia

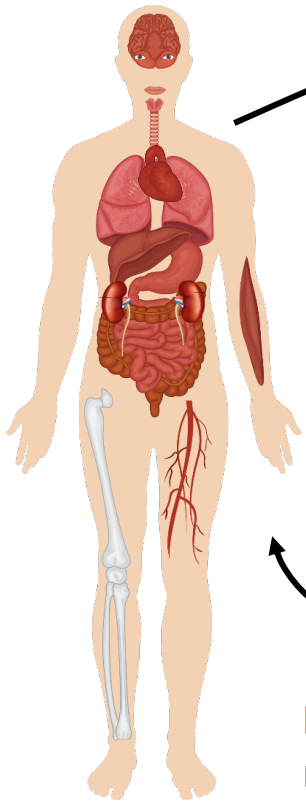
Casu. Blood. 2018;131:1790. NCT03802201. Guo. J Clin Invest. 2013;123:1531.

GENE THERAPY APPROACHES

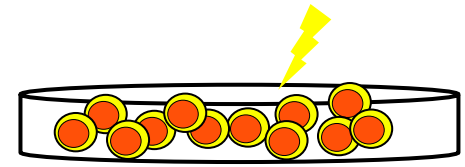
- Globin gene addition
  - Functional β-globin gene
  - Functional γ-globin gene
- Gene editing
  - Reverse fetal hemoglobin repression
    - » BCL11A
  - Correct the β-globin mutation

GENE THERAPY

HSC Collection: HSCs harvested from bone marrow or by mobilization/apheresis



Hematopoietic stem cells



Transduction or Gene Editing: HSC modified by transduction (gene addition) or gene editing

Modified hematopoietic stem cells

Reinfusion: Conditioning (partial or full myeloablation) and reinfusion of genetically modified HSC into bone marrow

Rivella. Haematologica. 2015;100:418.

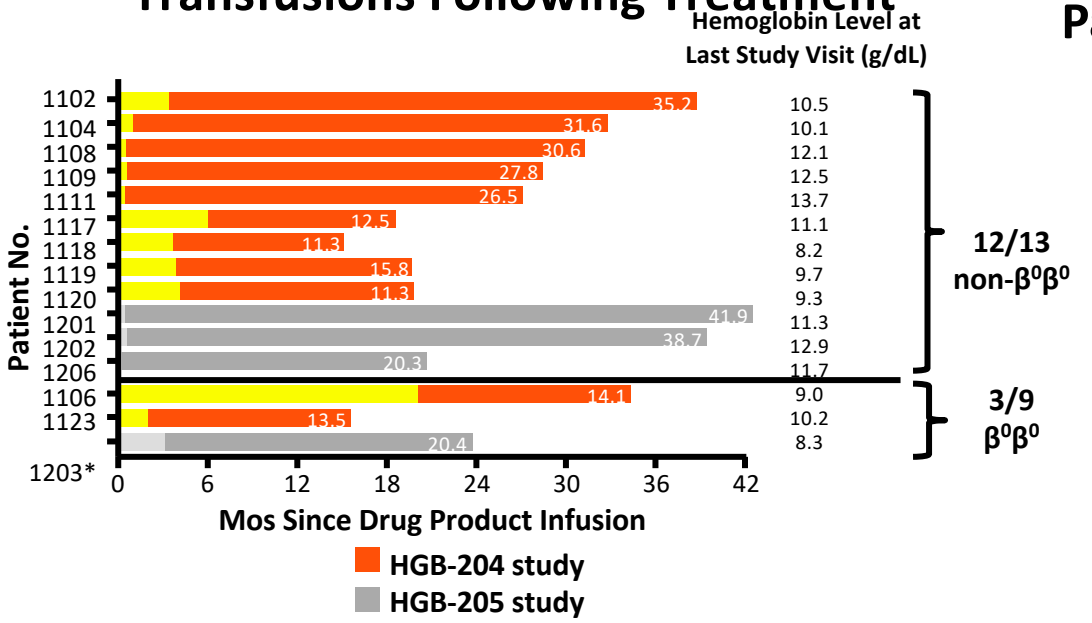
GENE THERAPY – CURRENT TRIALS

- Gene addition
  - LentiGlobin BB305
    - » Northstar trials
      - Early studies: HGB-204 and HGB-205[1]
      - More recent phase III studies: HGB-207 and HGB-212[2,3]
  - GLOBE lentiviral vector
    - » Phase I/II trial[4]
- Gene editing
  - Phase I/II Thales study of ST-400[5]
  - Phase I/II study of CRISPR/CAS9 gene-editing therapy CTX001[6]

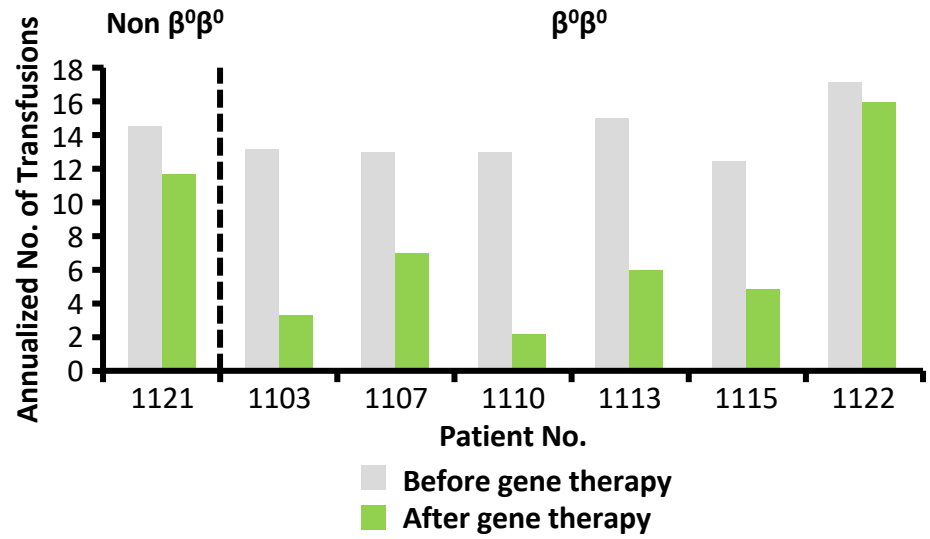
1. Thompson. NEJM. 2018;378:1479. 2. Locatelli. EHA 2019. Abstr S1632. 3. Kulozik. EHA 2019. Abstr S140.
4. Marktel. Nat Med. 2019;25:234. 5. NCT03432364. 6. NCT03655678.

HGB-204/205: Early Studies of LentiGlobin BB305

15 of 22 Patients Discontinued Transfusions Following Treatment



Reduced Transfusion Requirements in Patients Not Achieving Transfusion Independe

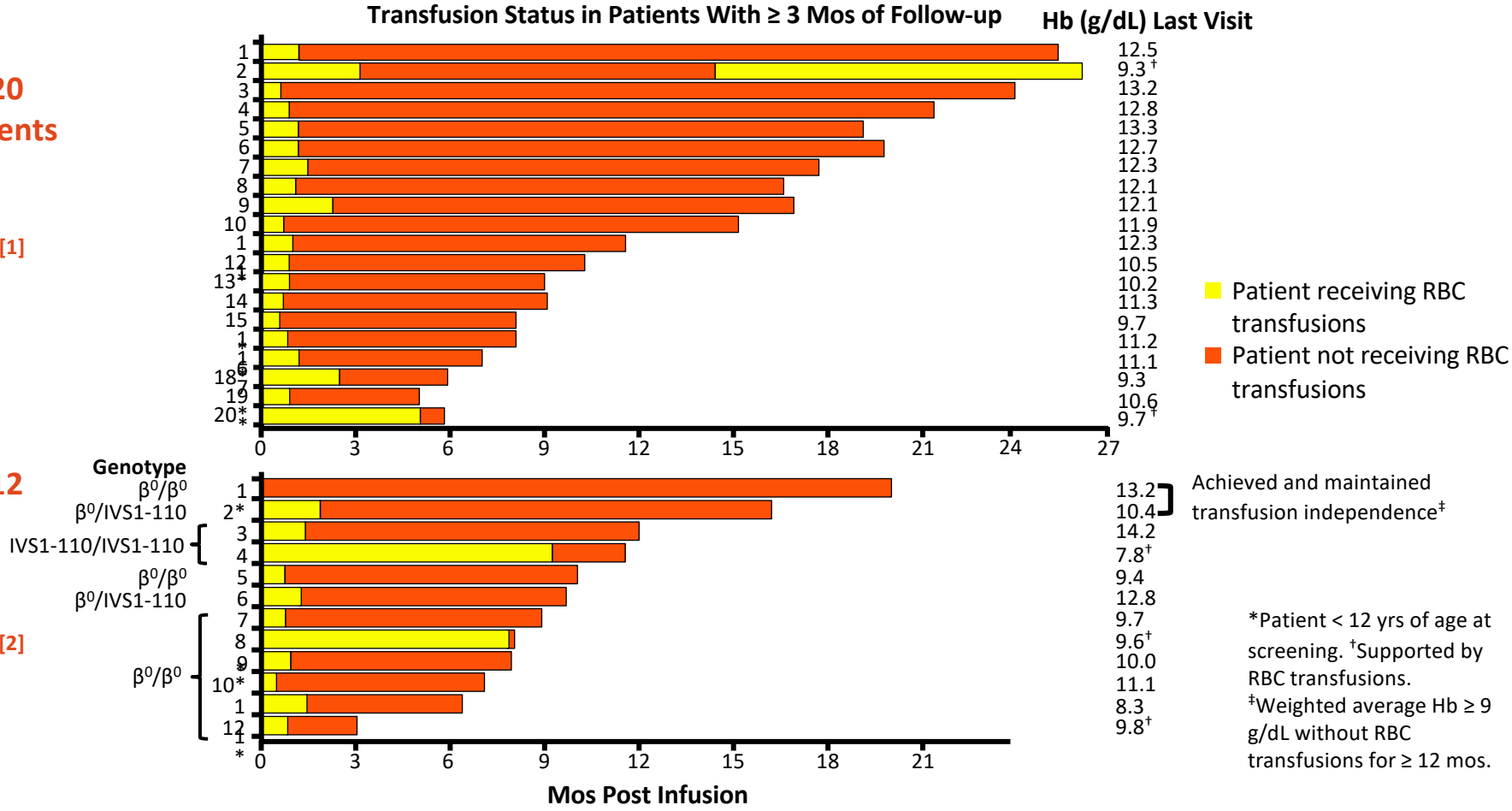


Thompson. NEJM. 2018;378:1479.

HGB-207/212: Phase III Trials of LentiGlobin BB305

HGB-207: 18/20 non-β^0/β^0 Patients Achieved Transfusion Independence[1]

HGB-212: 11/12 β^0/β^0 Patients Achieved Transfusion Independence[2]

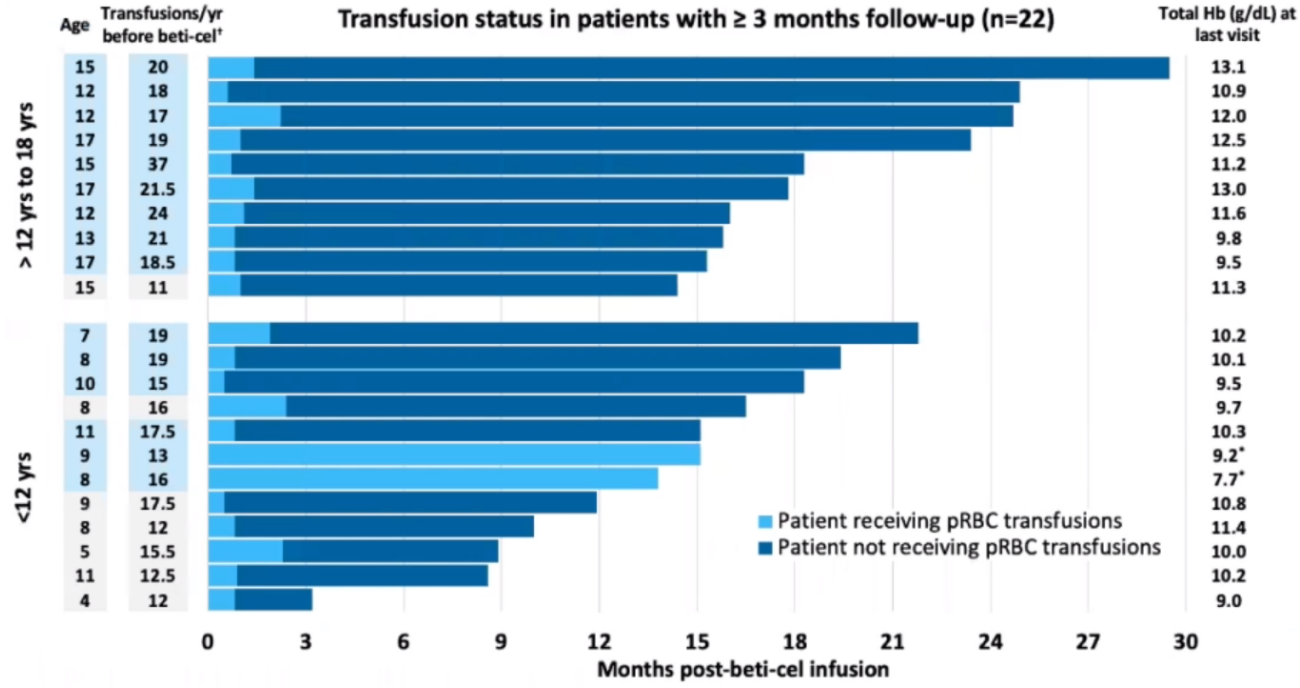


*Patient < 12 yrs of age at screening. †Supported by RBC transfusions. ‡Weighted average Hb ≥ 9 g/dL without RBC transfusions for ≥ 12 mos.

1. Thompson. ASH 2019. Abstr 3543. 2. Lal. ASH 2019. Abstr 815.

Gene Therapy in Pediatric Patients (Subgroup Analysis of 2 Phase 3 Studies)

- **Safety (N = 24)**
  - **AEs considered related or possibly related to the drug product**
    - » **Day of infusion: tachycardia and abdominal (one each, both grade 1)**
    - » **Post-infusion: 1 non-serious grade 3 event of thrombocytopenia**
  - **Veno-occlusive liver disease occurred in 3 patients (2 serious grade 4 and 1 grade 2); all events resolved with defibrotide**
  - **No vector-derived replication competent lentivirus**
- **Efficacy (N = 15)**
  - **87% of evaluable pediatric patients achieved TI**
  - **Ineffective erythropoiesis improved after beti-cel gene therapy**



100% of patients between 12 and 18 years achieved TI

GENE THERAPY: SAFETY

- No deaths to date
- Most toxicities associated with myeloablative conditioning including stomatitis, febrile neutropenia, thrombocytopenia, bleeding, elevated LFTs, hypotension, sepsis, transfusion reactions, lower respiratory infection
- Venoocclusive disease seen in initial cohort (all treated successfully)
  - Now all subjects prophylaxed, reduced incidence in subsequent trials

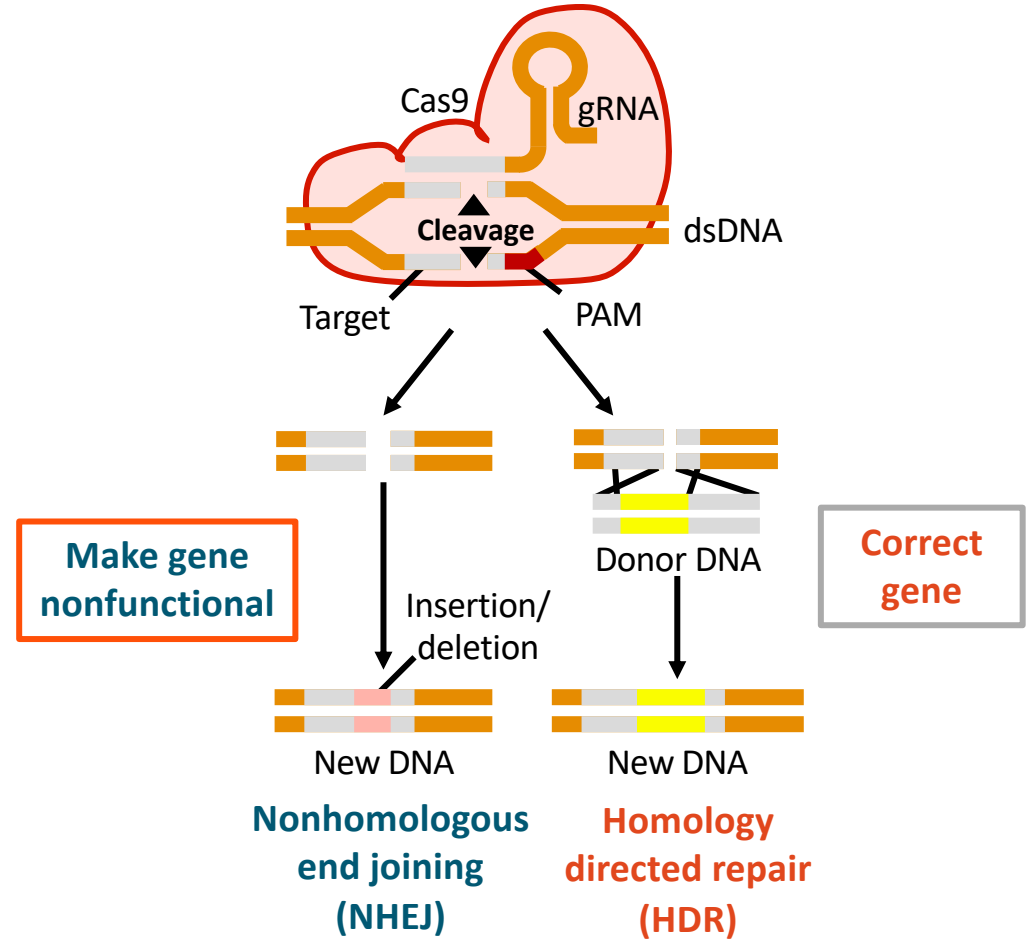
Thompson. NEJM. 2018;378:1479. Kulozik. EHA 2019. Abstr S140.

FACTORS AFFECTING SUCCESS GENE ADDITION

- Transduction efficiency
  - But does increasing VCN increase risk of insertional mutagenesis?
- Transgene expression levels
  - Improved β-globin expression per copy
- Genotype: β^+ vs β^0
- Quality of HSC
  - Mobilized vs bone marrow harvest
  - Pediatric vs adult
- Bone marrow microenvironment
  - Hypertransfusion
  - Myeloablation

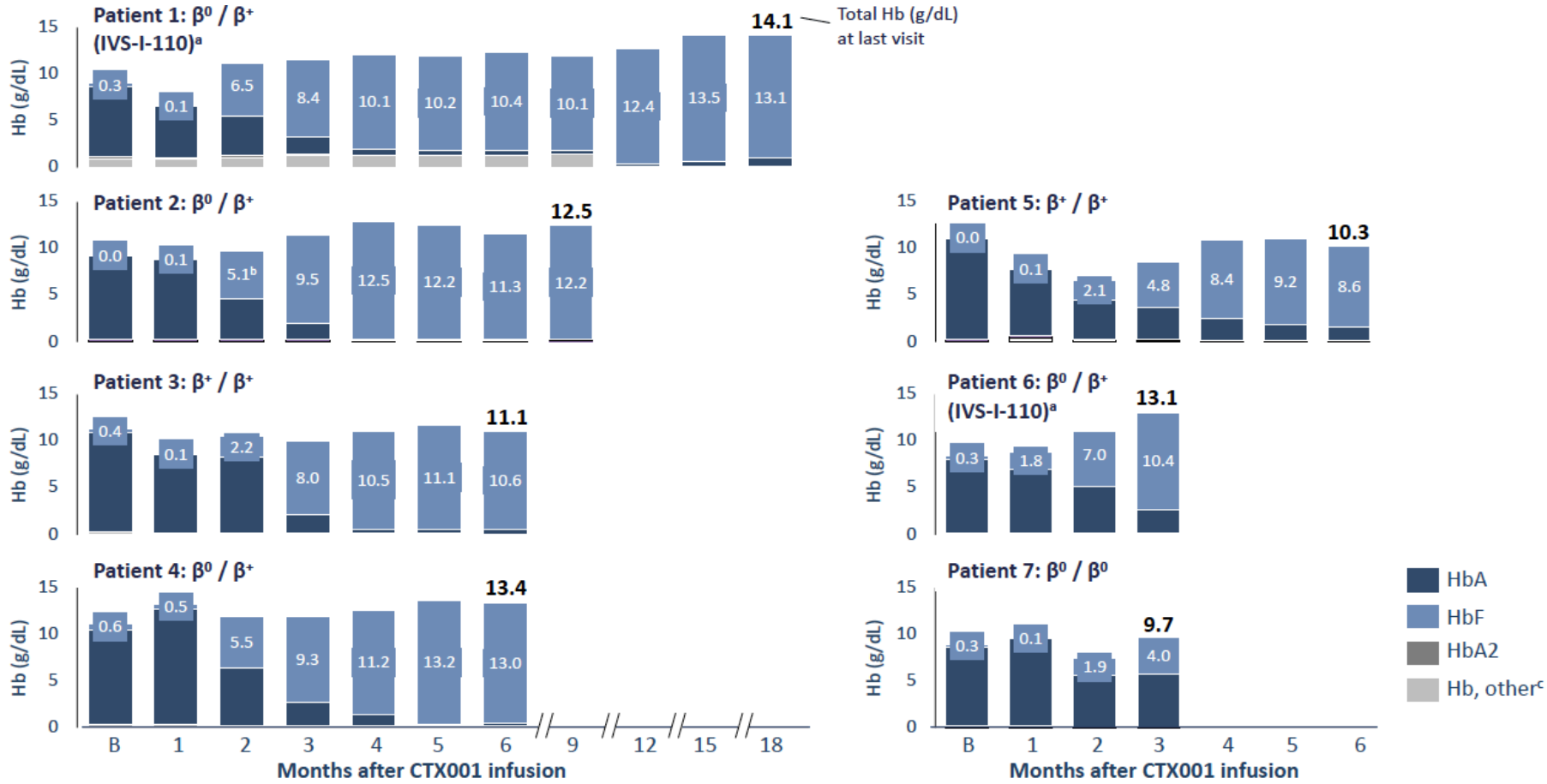
GENE EDITING

- Targets
  - Disruption of BCL11A using zinc finger nuclease or CRISPR/Cas9
  - Editing β-globin locus using CRISPR/CAS9
- Delivery of edited gene
  - HSC mobilization and myeloablation
  - Nonviral delivery
    - » Reduce risk of insertional mutagenesis
    - » Risk of off-target editing
- Phase I clinical trials underway



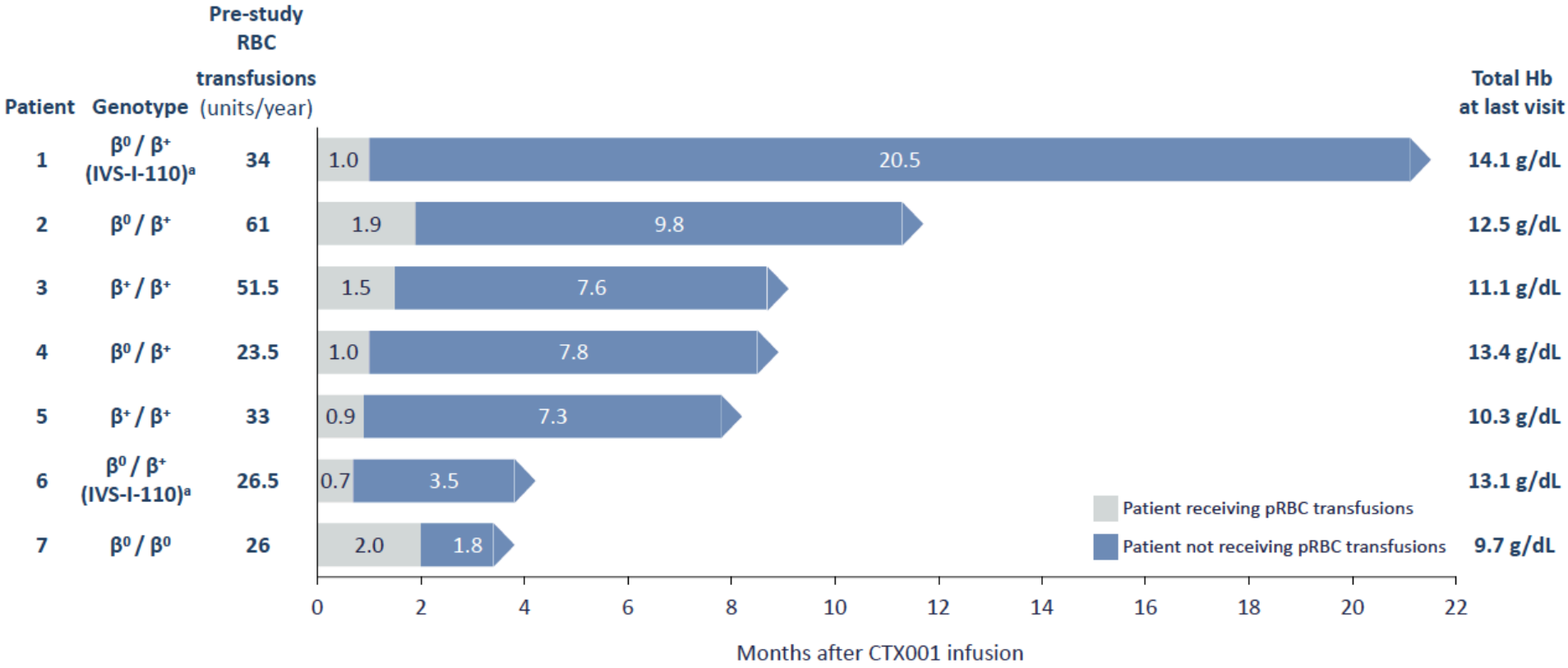
Morgan. Cell Stem Cell. 2017;21:574. **NCT03432364. NCT03655678.**

Results: TDT



B: Baseline, Hb: hemoglobin; HbA: adult hemoglobin; HbF: fetal hemoglobin; TDT: transfusion-dependent β-thalassemia. [a]Total Hb from local laboratory and Hb fraction from central laboratory.
[b]IVS-I-110 phenotype is severe and similar to β⁰ / β⁰ [c]Hb adducts and other variants

Results: TDT – transfusion free



[a]IVS-I-110 phenotype is severe and similar to β⁰ / β⁰.
Hb: hemoglobin; pRBC: packed red blood cell; RBC: red blood cell; TDT: transfusion-dependent β-thalassemia.

GENE THERAPY: CONCLUSIONS

- β-globin gene addition trials have achieved transfusion independence in patients with β-thalassemia, especially with less severe genotypes
  - AEs typical of myeloablative conditioning observed; well tolerated in pediatric and adult patients
  - Long-term data lacking (especially with high VCN)
  - Some patients have poor response
- Gene editing targeting *BCL11A* to raise fetal hemoglobin levels in
  early clinical trials
- Expensive treatment; availability to patients needs to be addressed

OVERALL SUMMARY

- β-thalassemia is a chronic condition with significant morbidity and impact on QoL
- Monitoring and treating iron overload has significantly improved
- HSCT outcomes are better
- Targeted therapies have great potential to alter natural history of the disease and improve QoL for patients
- Gene therapy is on the horizon