THALASSEMIA

Sujit Sheth, M.D.
Pediatric Hematology Oncology

Weill Cornell Medicine

NewYork-Presbyterian Kids
Komansky Children's Hospital

Disclosures

- Consultant
  - Novonordisk
  - Celgene / Bristol Myers Squibb
  - Agios
  - BEAM
- Steering Committee
  - CRISPR/ Vertex CTX001
- Will discuss therapeutics not yet FDA approved – results from clinical trials

Learning Objectives

- Understand the inheritance and epidemiology of thalassemia
- Know the wide spectrum of disease and its pathophysiology
- Learn the clinical features and diagnosis
- Understand the rational management and complications
- Be aware of novel therapies in development

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)

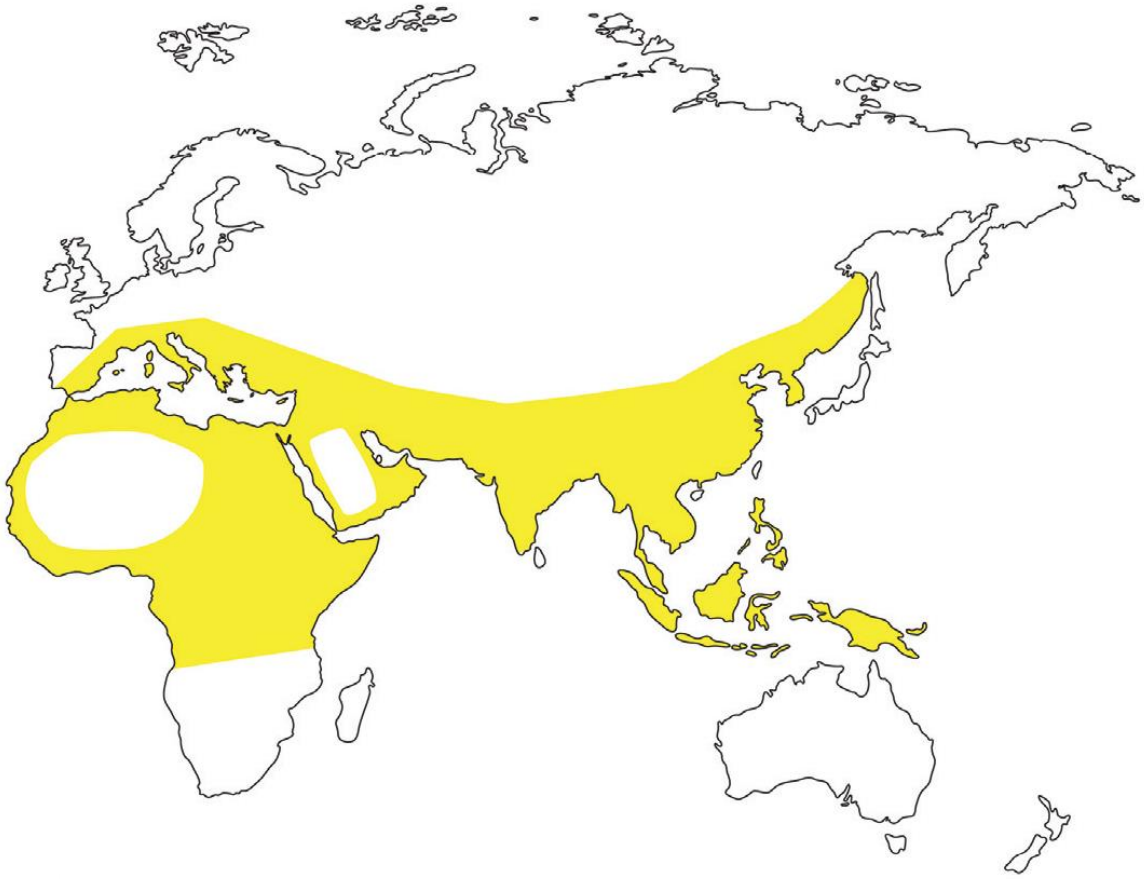
Hb Hasharon (α)

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

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]

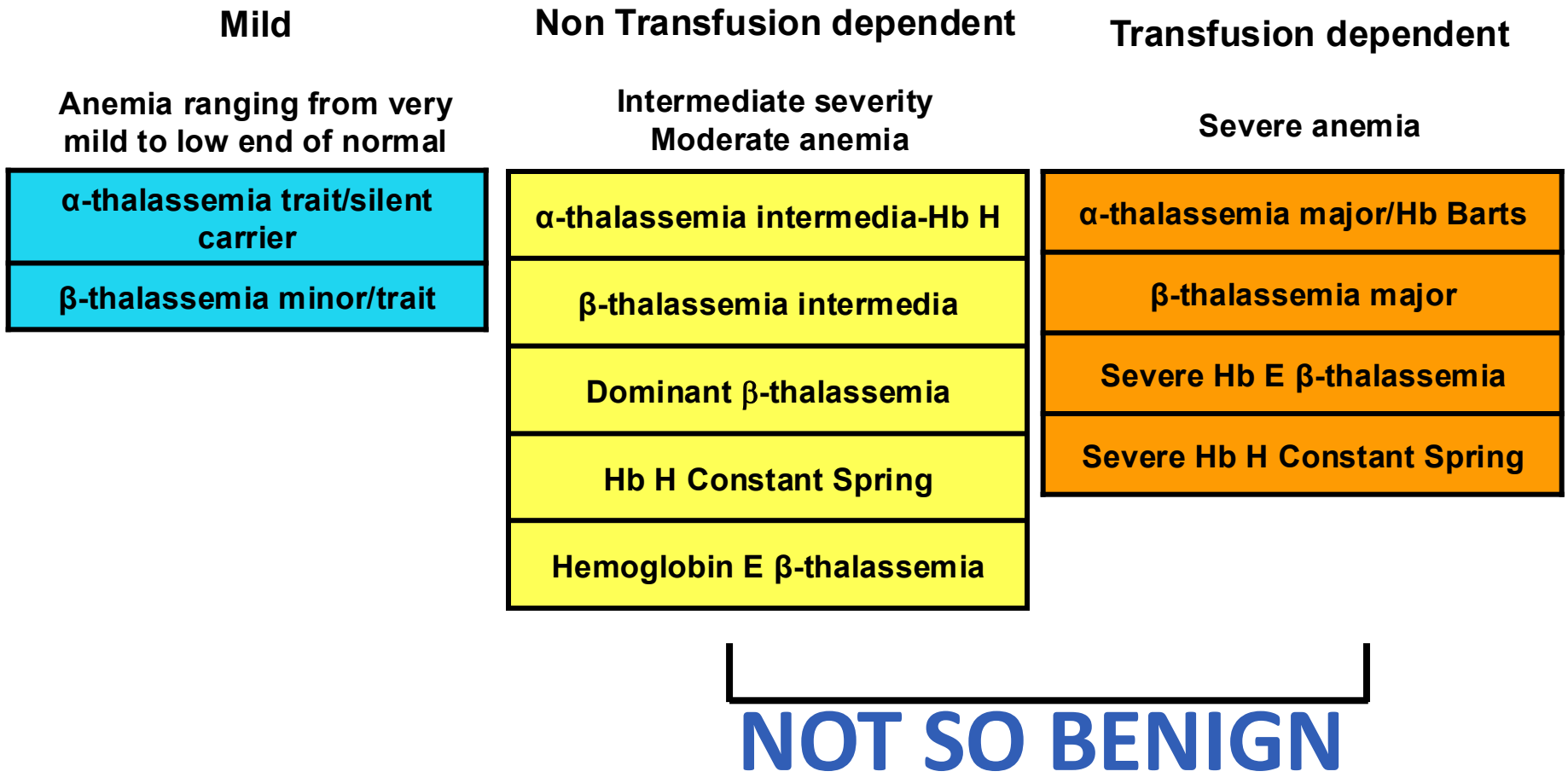
Estimated prevalence in Italy: ~ 6000[3]

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

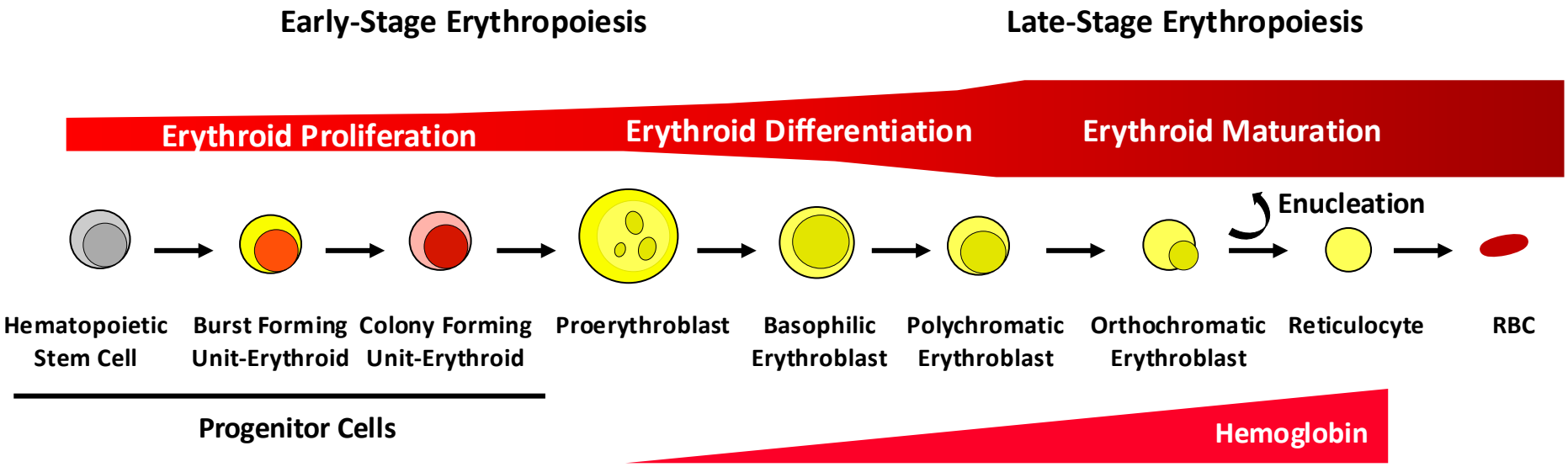


β THALASSEMIA

MUTATIONS

- ~350 mutations described
- 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



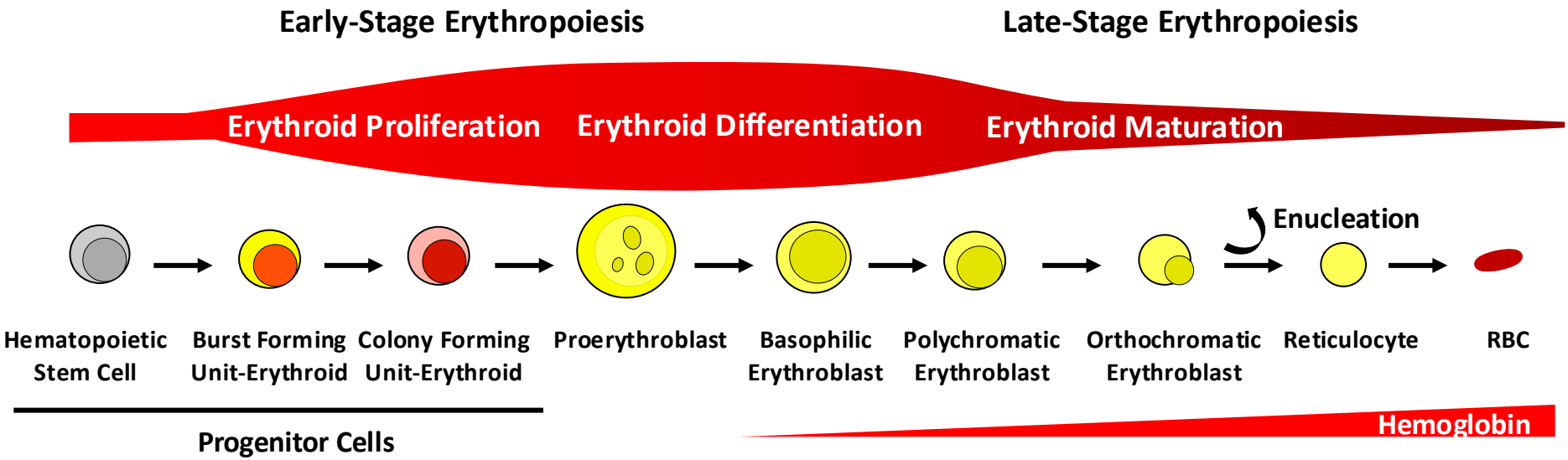
- Characterized by proliferation of progenitor cells
- Promoted by EPO

- 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

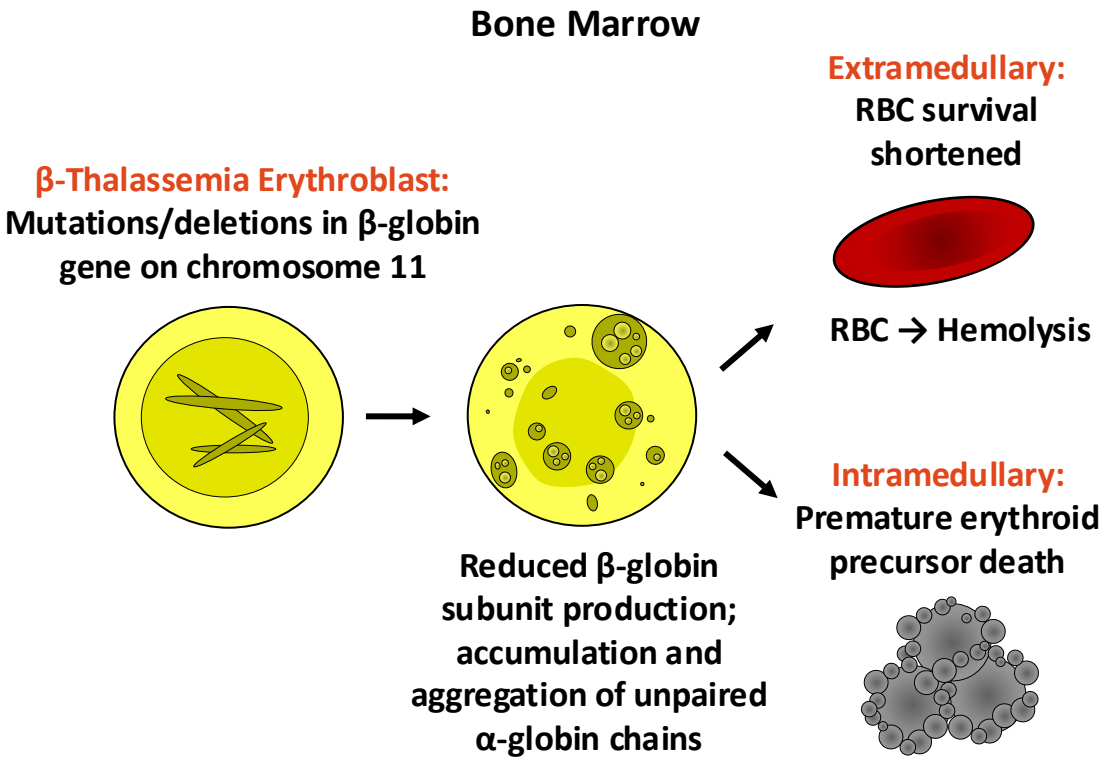
INEFFECTIVE ERYTHROPOIESIS



- 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	Mild anemia 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.

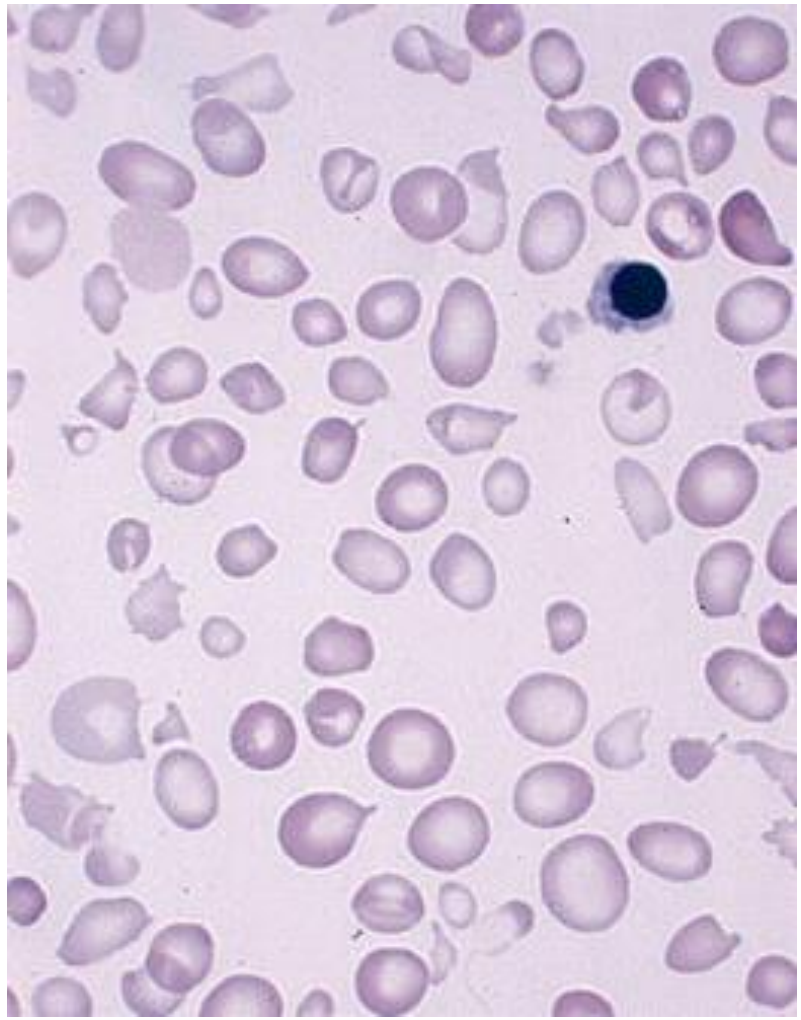
SPECTRUM OF DISEASE

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

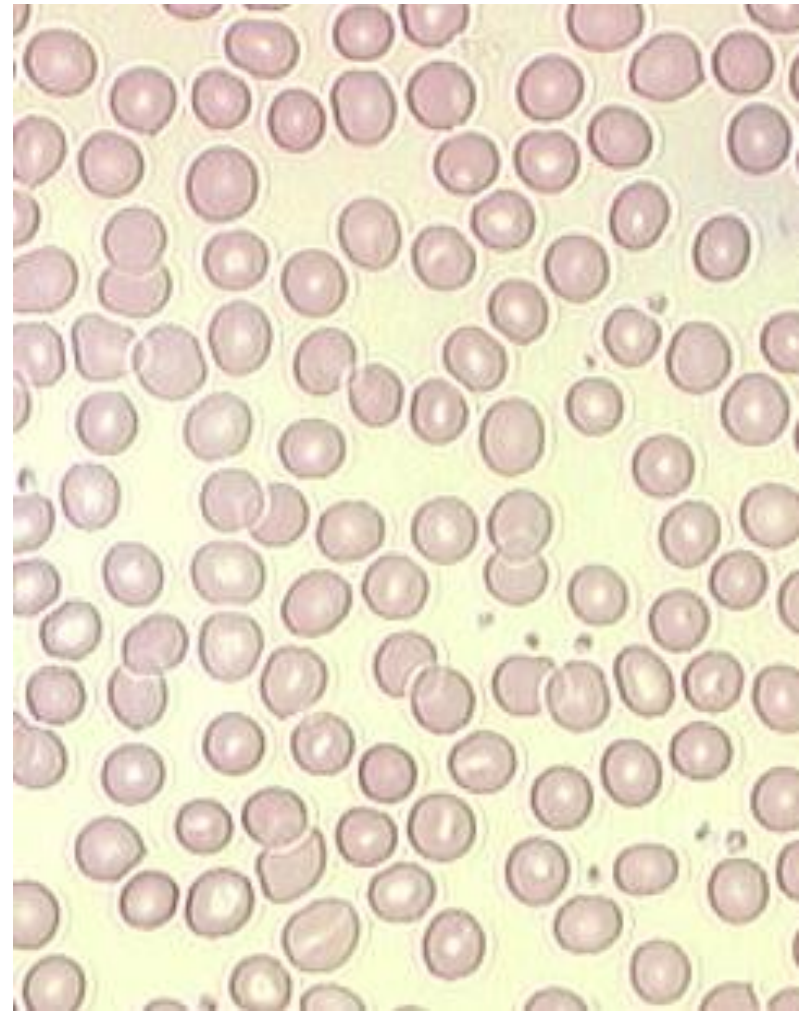
DIAGNOSIS

- 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

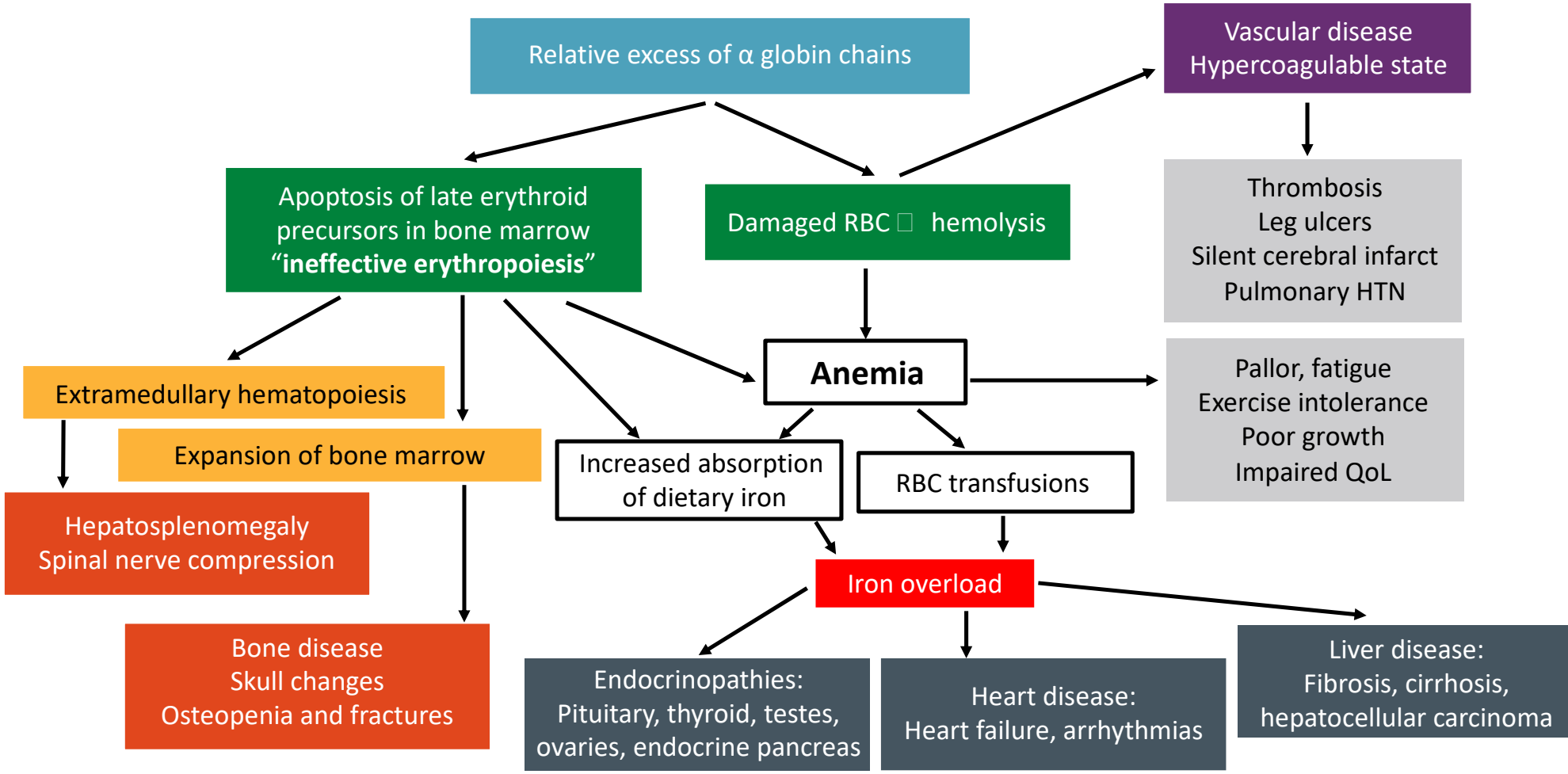


Major



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)
- Luspatercept
- Hematopoietic stem cell transplantation / gene therapy

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
- Luspatercept
- 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
    - Short transfusion intervals q 2 wks
    - Erythroid maturation agent

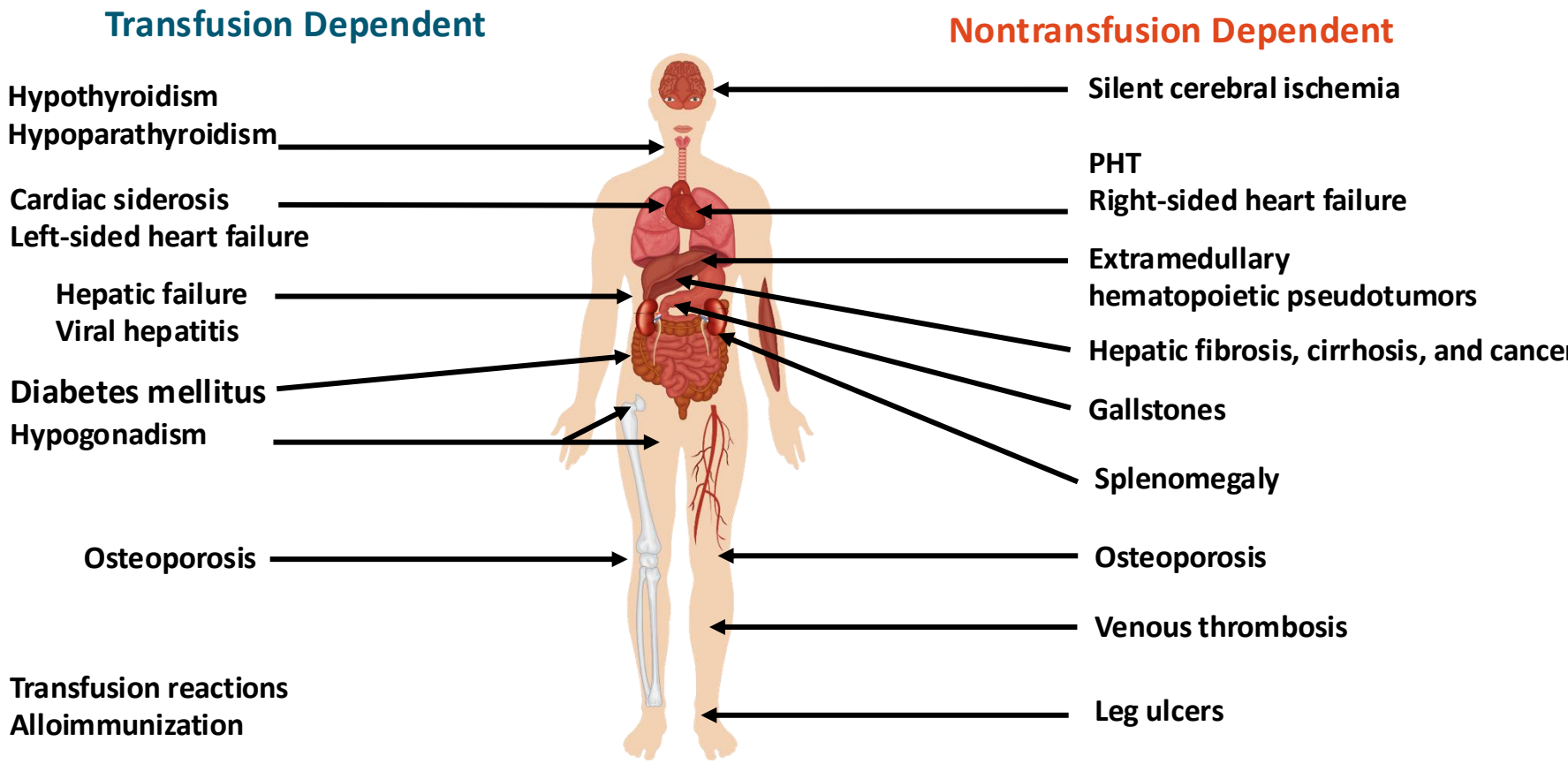
TRANSFUSION COMPLICATIONS

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

SPLENECTOMY

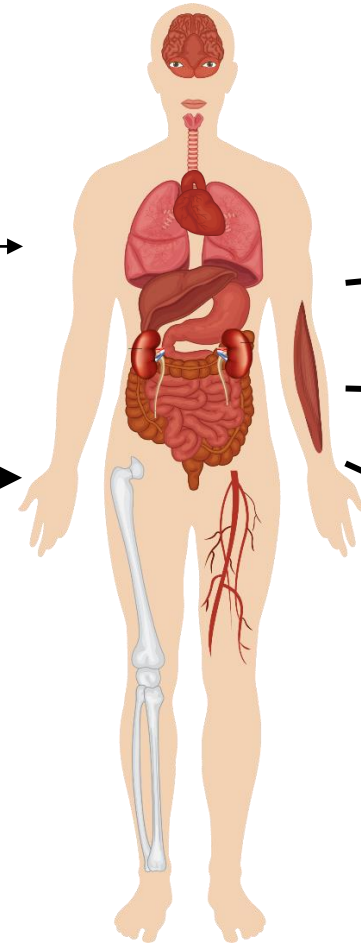
- TDT
  - Hypersplenism with increasing transfusion requirement - >200 ml/kg/year PRBCs
- NTDT
  - Massive splenomegaly
  - Hypersplenism – extramedullary hematopoiesis
- Risks – infection, vascular disease
- Benefits – minimize transfusions and iron loading

COMPLICATIONS



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

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

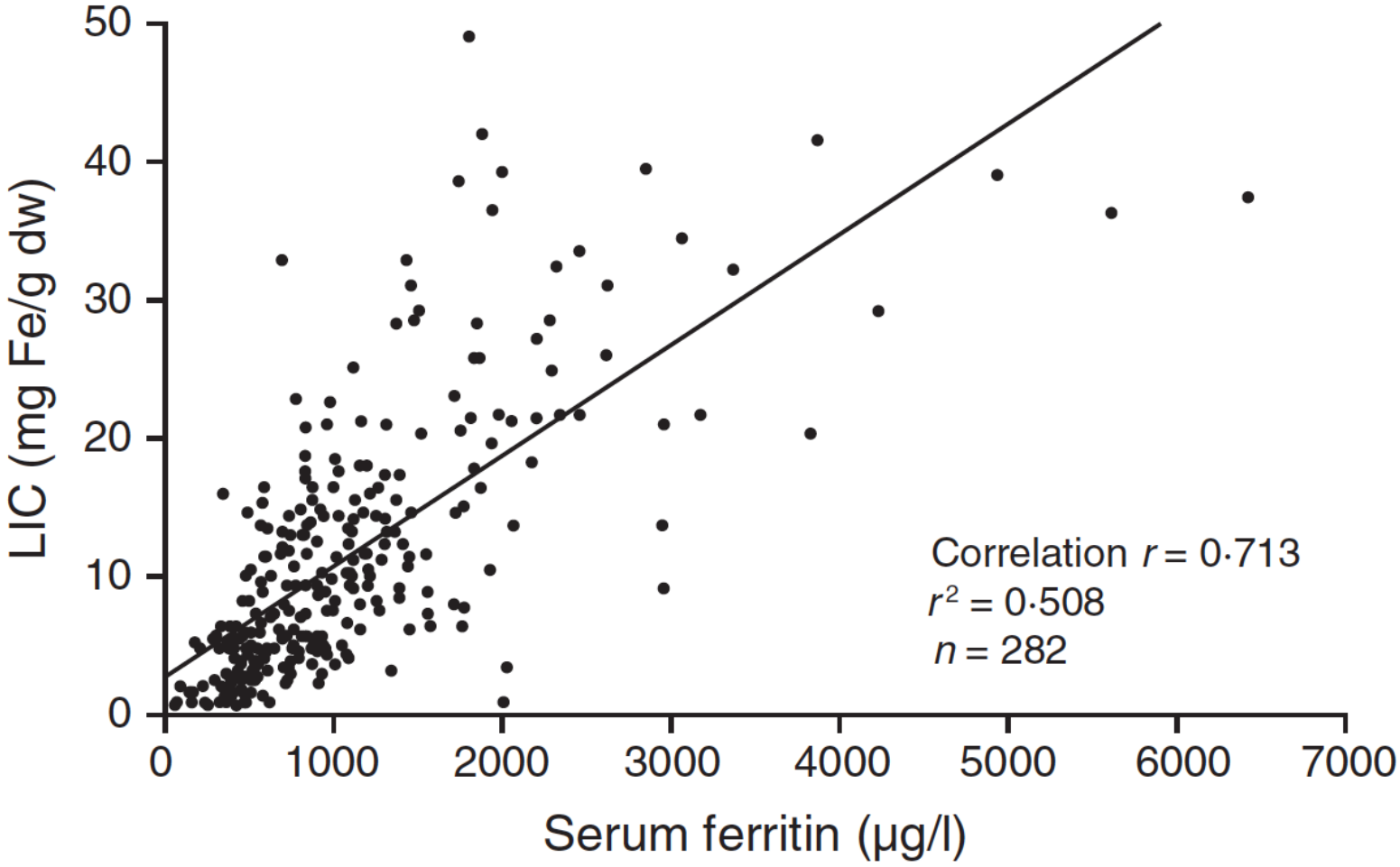
RISK OF ORGAN DAMAGE

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

MEASURING IRON BURDEN

- Serum Ferritin
- Liver Biopsy Liver Iron Concentration (LIC)
- MRI LIC R2/R2*
- MRI Cardiac T2*

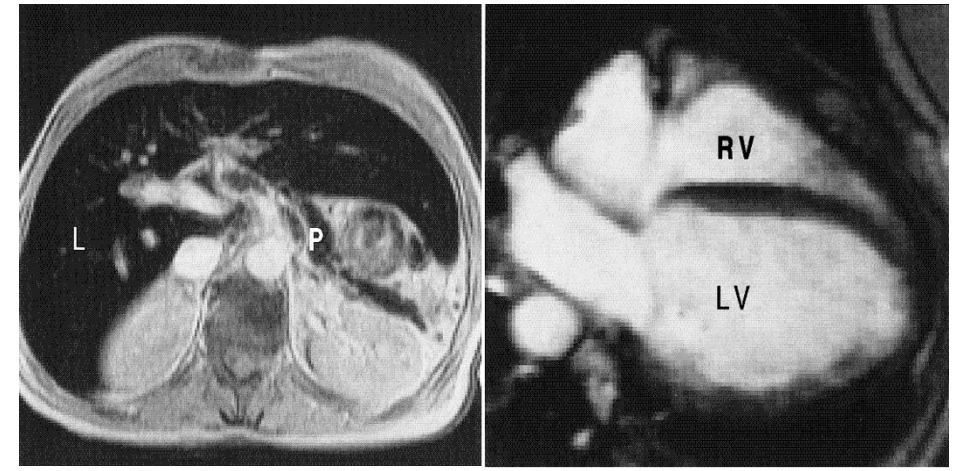
SERUM FERRITIN

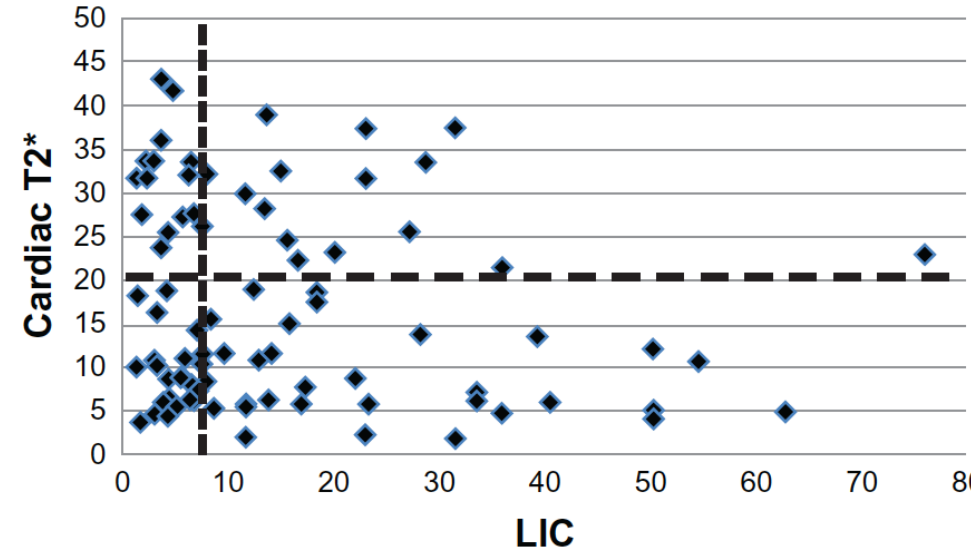


Taher et al., Br J Haematol 2015

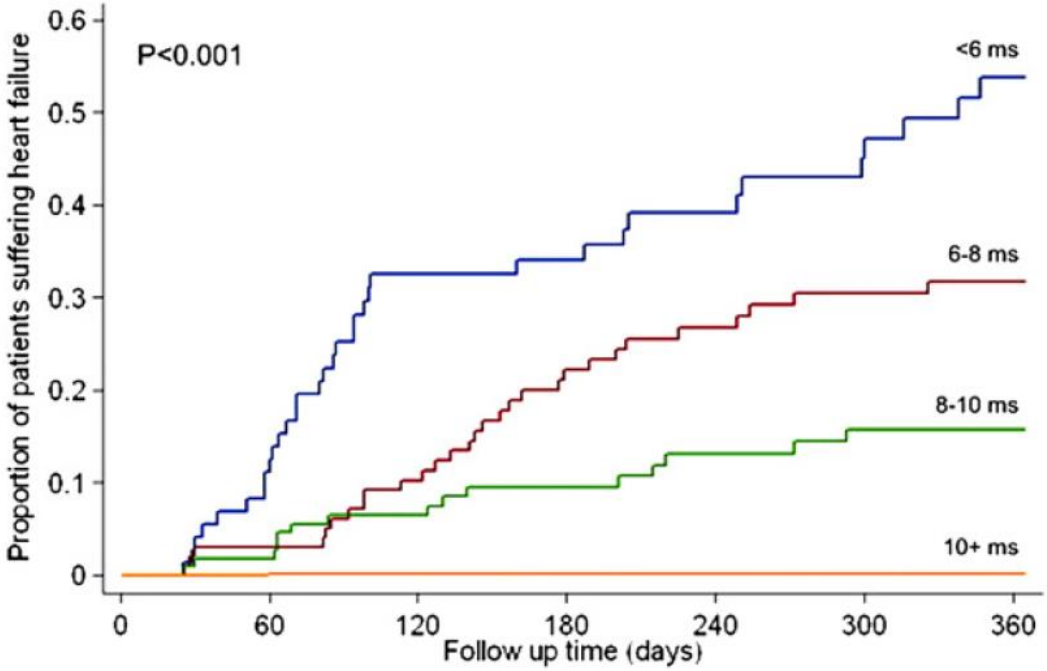
MRI - IRON QUANTIFICATION

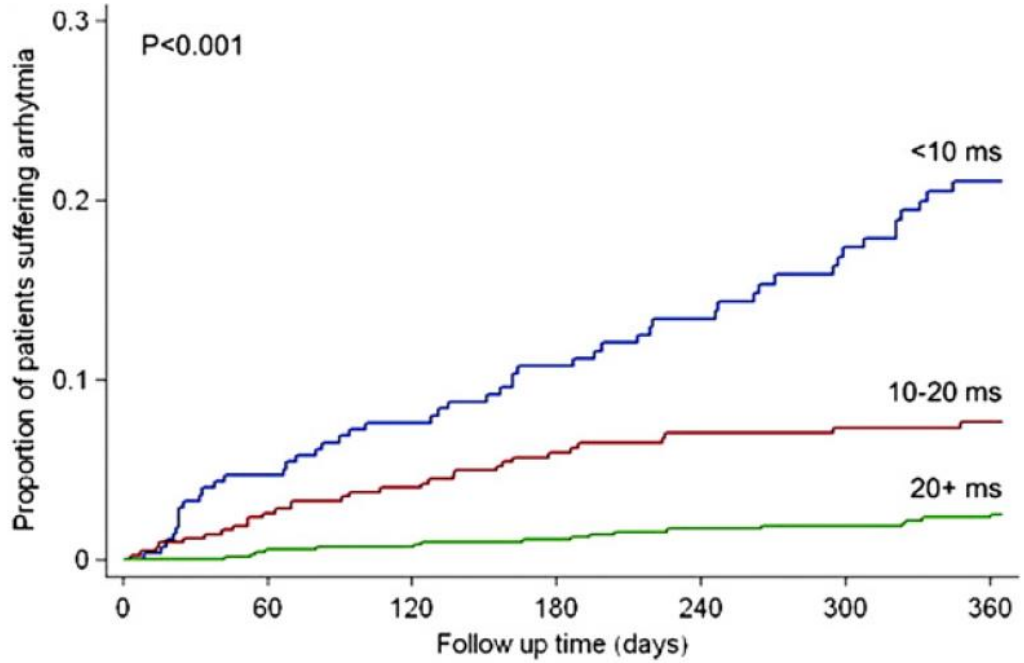
- Utility
  - Non-invasive
  - No irradiation
  - Easily accessed
- Caveats
  - Technique variability
  - Sensitivity
  - Not correlated between tissues





CARDIAC T2* - PROGNOSTIC VALUE
(Thalassemia Major)



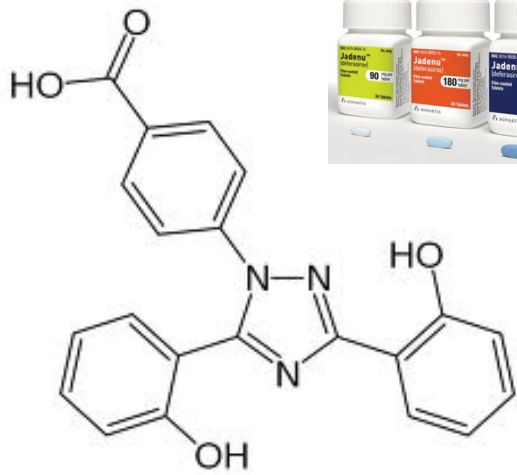


Kirk P et al. Circulation 2009.

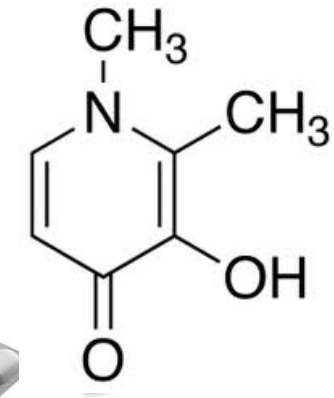
CHELATION

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

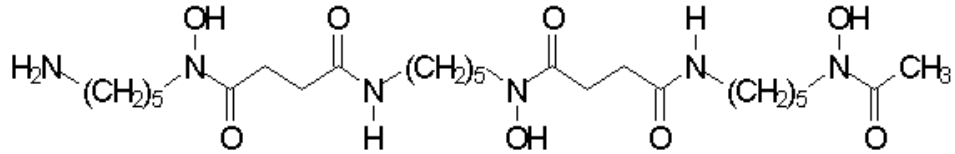
DEFERASIROX
(Exjade®, Jadenu®)

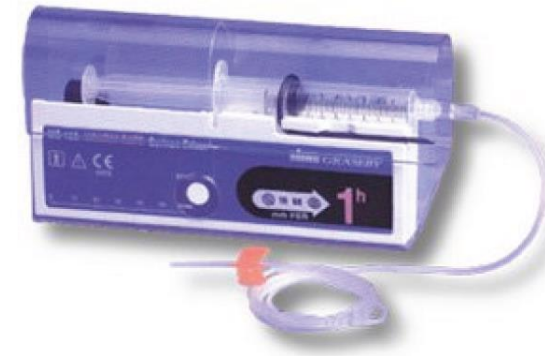


DEFERIPRONE
(Ferriprox®)



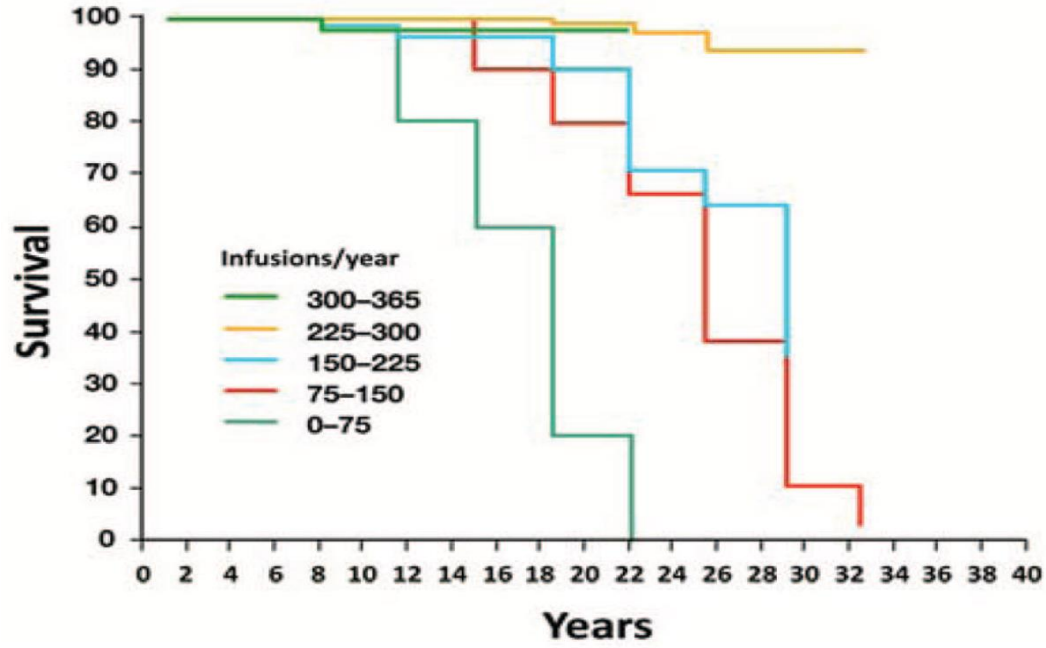
DEFEROXAMINE
(Desferal®)





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®)

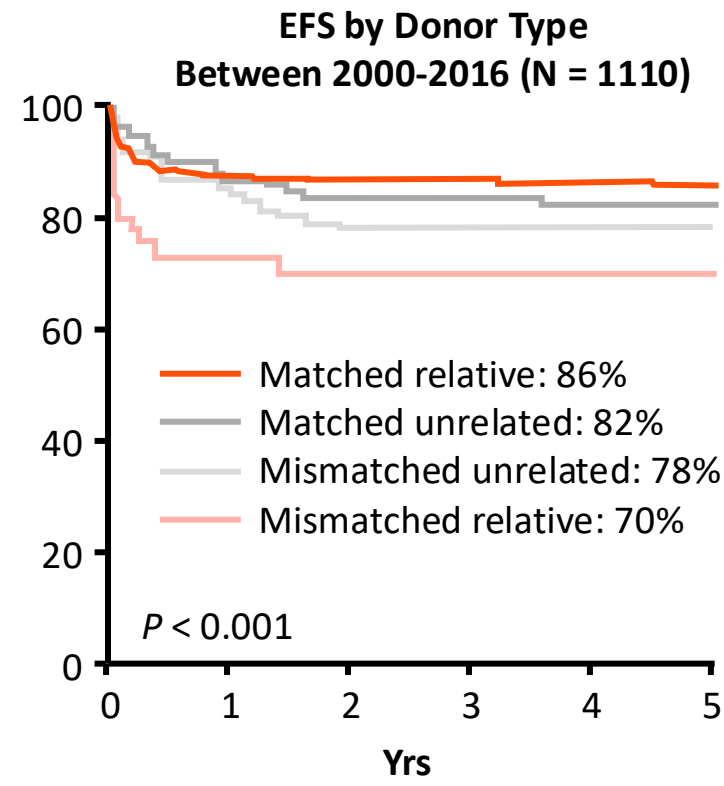
- FDA approved November 2005, 2015
  - Orally effective
  - Once a day only
  - Wide therapeutic index, dose range
- Side effects
  - Nausea, vomiting, abdominal pain
  - Liver and kidney toxicity
  - Rare reports of Neutropenia/Agranulocytosis
- Good long term safety and efficacy data
- Can be used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

STEM CELL TRANSPLANTATION

- Until recently - 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

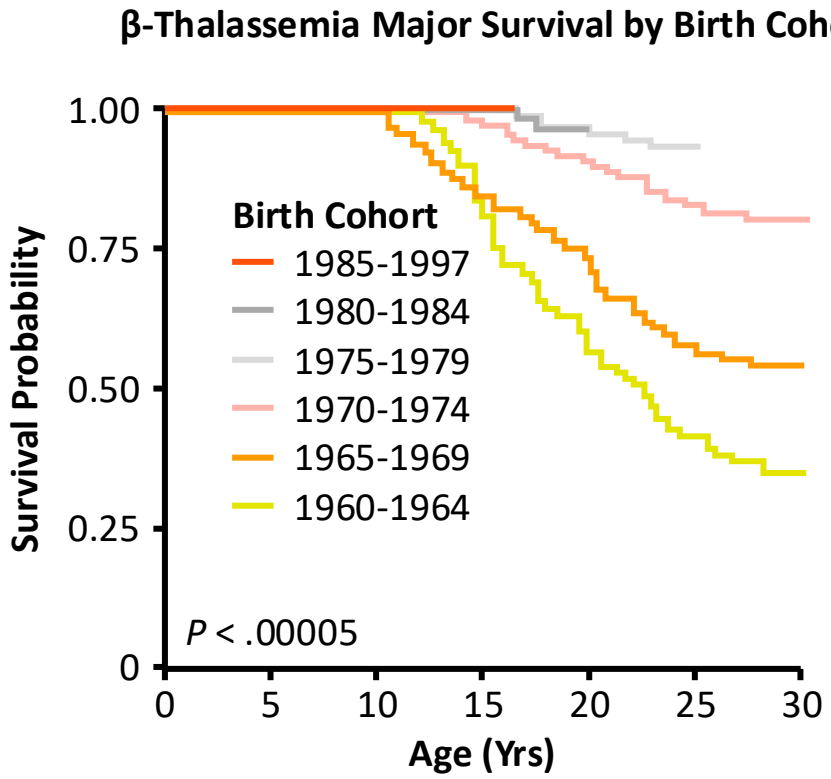


Li. Blood Adv. 2019;3:2562. Baronciani. ASH 2018. Abstr 168. Mohamed. Hematol Oncol Stem Cell Ther. 2017;10:290.

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.

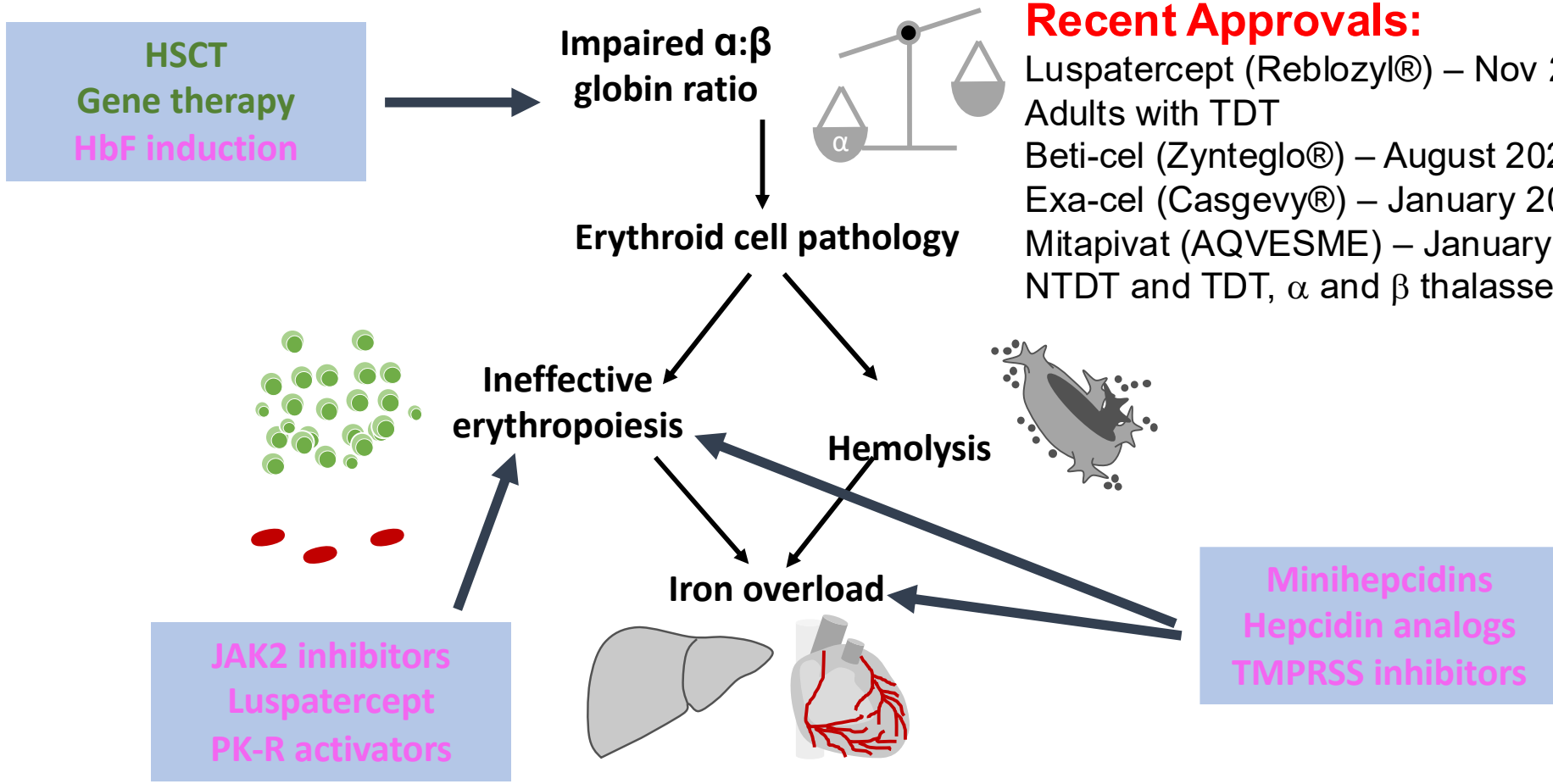
IMPROVED CARE

- 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
- Novel therapies

UNMET NEEDS

- 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
- Until recently – lack of curative options for those without matched sibling donors

NOVEL THERAPIES



Recent Approvals:

Luspatercept (Reblozyl®) – Nov 2019 – Adults with TDT

Beti-cel (Zynteglo®) – August 2022 – TDT

Exa-cel (Casgevy®) – January 2024- TDT

Mitapivat (AQVESME) – January 2026 – NTDT and TDT, α and β thalassemia

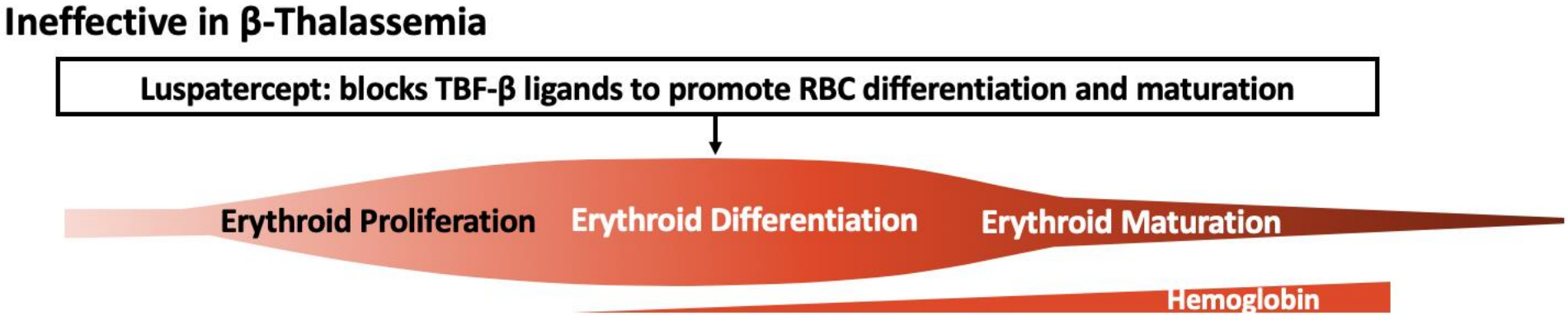
Adapted from: Cappellini. Hematology Am Soc Hematol Educ Program. 2017;2017:278.

Clinical trials of Novel Therapies for β-Thalassemia

Drug	Mechanism of Action	Route & Frequency of administration	Phase of Development	Current target patient population
PTG-300	Hepcidin mimetic	Subcutaneous, once weekly	Phase 2 trial NCT03802201	NTDT, TDT
TMPRSS6-LRx	TMPRSS6 inhibitor (ASO)	Subcutaneous, every 4 weeks	Phase 2 trial NCT04059406	NTDT
SLN124	TMPRSS6 inhibitor (siRNA)	Subcutaneous injections	Phase 1 trial NCT04718844	NTDT
VIT-2763 (vamifeport)	Ferroportin inhibitor	Oral, 1-2x per day	Phase 2 trials NCT04938635 NCT04364269	 TDT NTDT
Apotransferrin	Hepcidin upregulation	Intravenous, every 2 weeks	Phase 2 trial	NTDT
AG-348 (Mitapivat)	Pyruvate kinase activator	Oral, twice daily	Phase 3 trials NCT04770779 NCT04770753	 TDT NTDT
FT-4202 (Etavopivat)	Pyruvate kinase activator	Oral, once daily	Phase 2 trial NCT04987489	TDT, NTDT (and SCD)
Benserazide	HbF induction	Oral, daily	Phase 1 trial NCT04432623	NTDT adult

LUSPATERCEPT

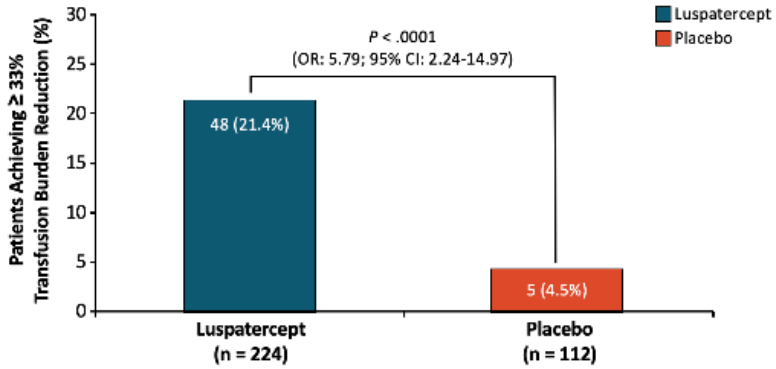
- Recombinant fusion protein containing a modified extracellular domain of ActRIIB
- Binds to GDF11 and other TGF-β superfamily ligands, inhibits Smad2/3 signaling, and promotes RBC differentiation/maturation



Phase 3 trials

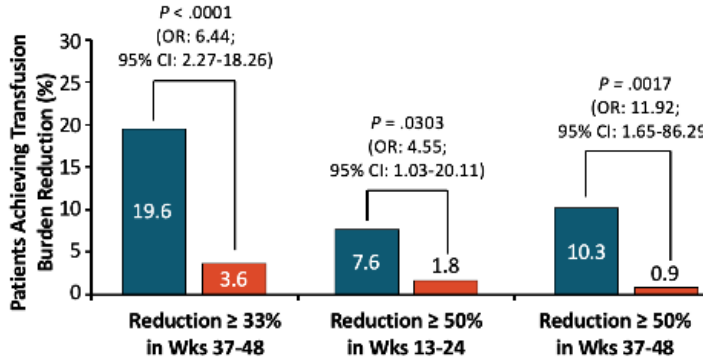
BELIEVE: TDT

Primary Endpoint



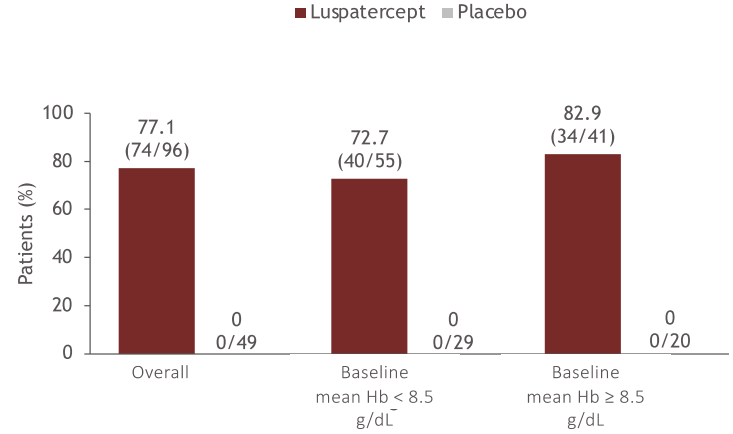
≥ 33% in Wks 13-24

Secondary Endpoints

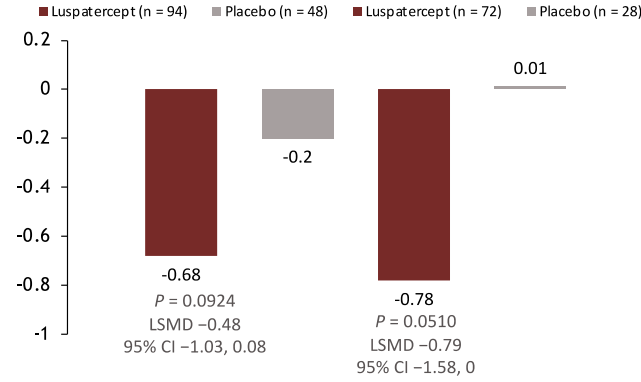


BEYOND: NTDT

Primary Endpoint – increase in hemoglobin



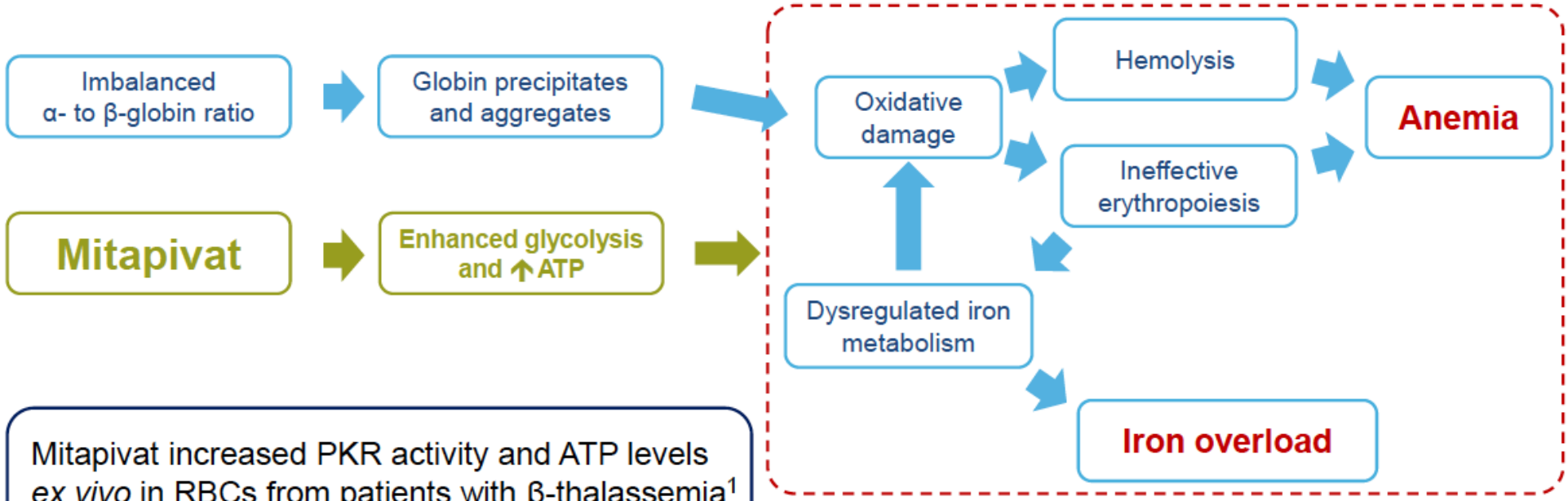
Secondary Endpoint – improvement in PRO



LUSPATERCEPT: CONCLUSIONS

- BELIEVE met its primary endpoint, demonstrating statistically significant improvement, with a ≥ 33% reduction in RBC transfusion burden with luspatercept vs placebo, and key secondary endpoints
- BEYOND met its primary endpoint, demonstrating a ≥1g/dl increase in hemoglobin, but did not meet its key secondary endpoint related to PRO
- Luspatercept was generally well tolerated
- **Approved for adults with TDT, pediatric trial underway**

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]

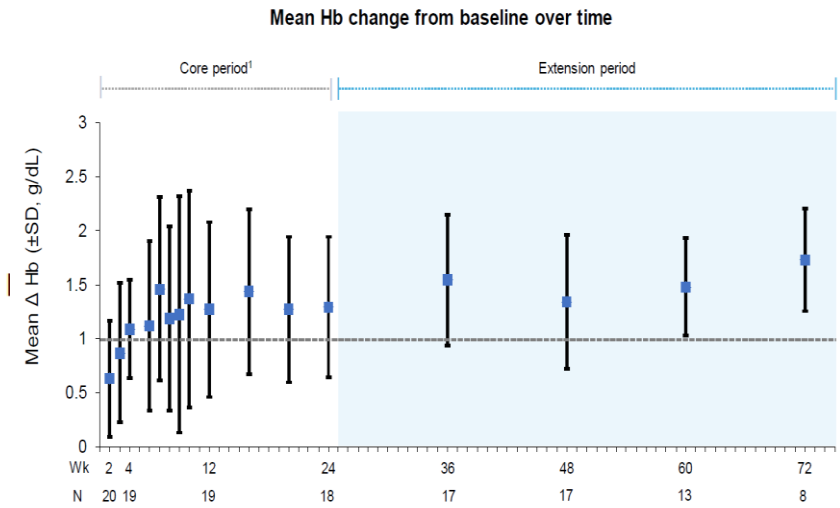
Results – Phase 2

- Primary endpoint was met in 92.3% of patients

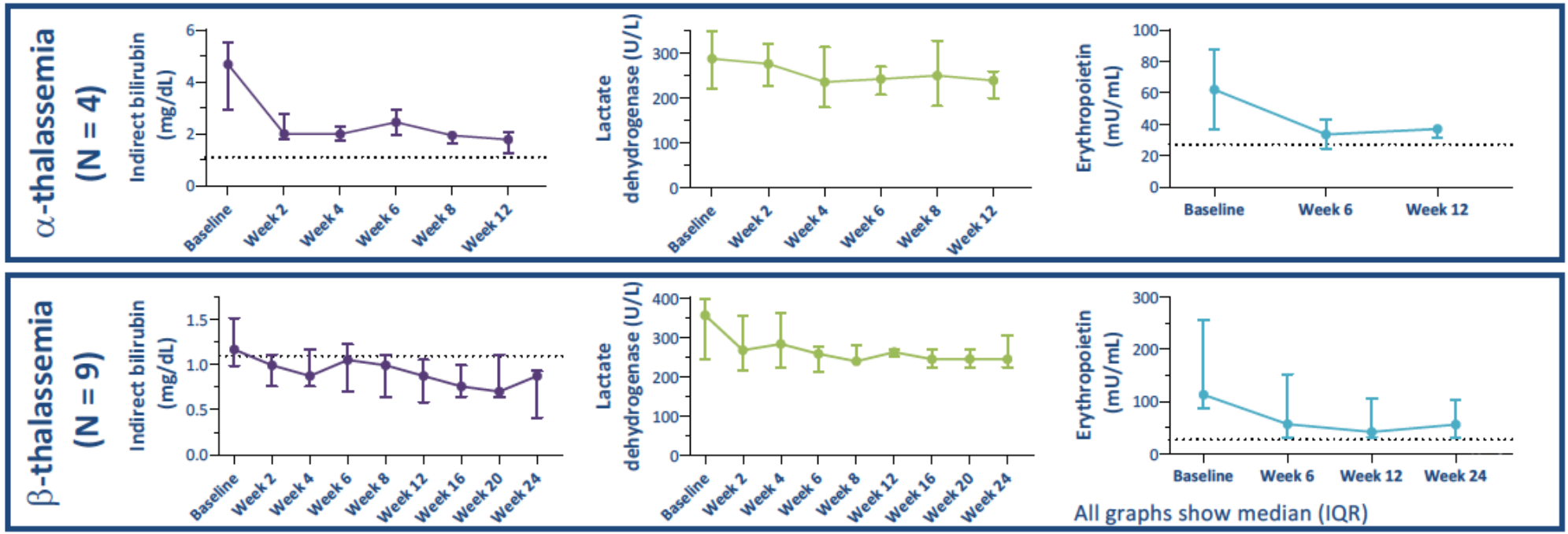
Endpoint	Genotype	n/N	%	90% CI
Hb responders during Weeks 4–12 (completed 12 weeks)	All	12/13	92.3	68.4, 99.6
	α	4/4	100	47.3, 100
	β	8/9	88.9	57.1, 99.4
Hb responders during Weeks 12–24 (completed 24 weeks)	β[a]	8/9	88.9	57.1, 99.4
Sustained responders: primary response and ≥ 2 Hb responses during Weeks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

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

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)

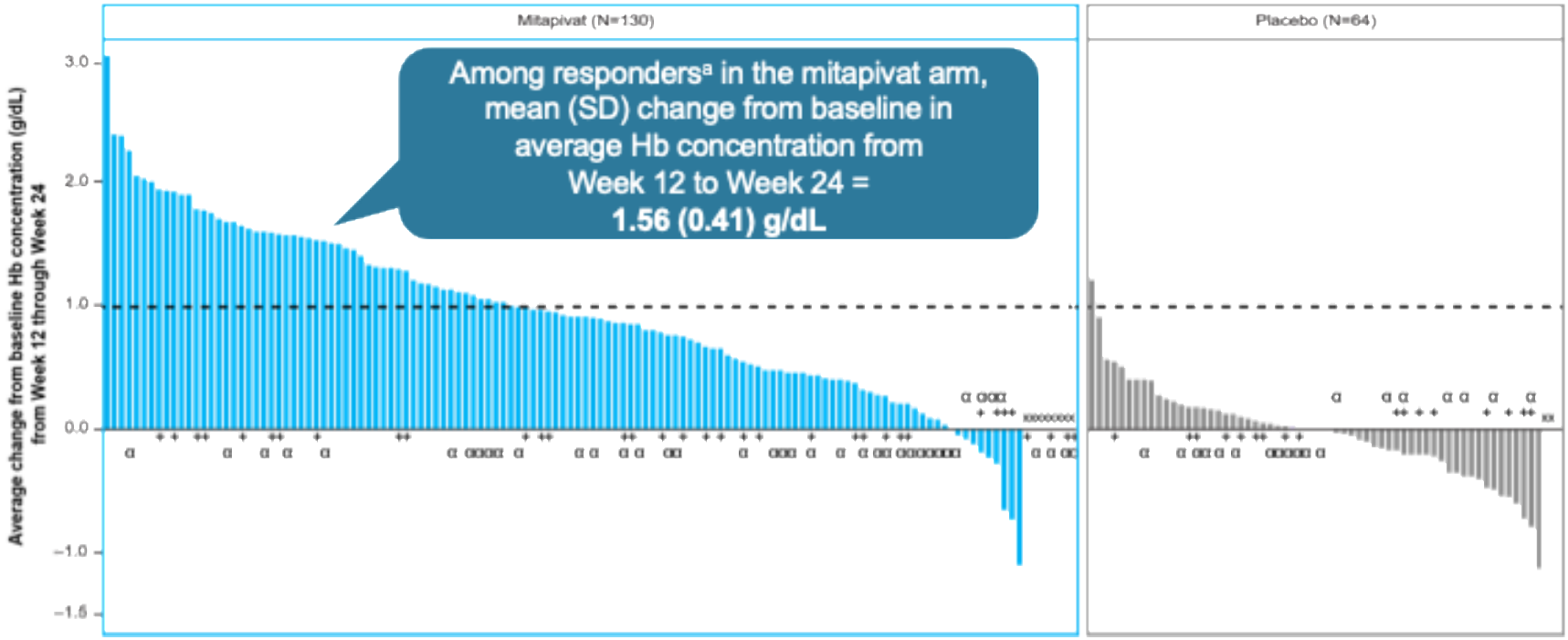


Results – Increased Hb, decreased hemolysis



ENERGIZE TRIAL – Primary Endpoint

	Mitapivat N=130	Placebo N=64	2-sided p-value
Hb response, n (%)	55 (42)	1 (2)	p<0.0001

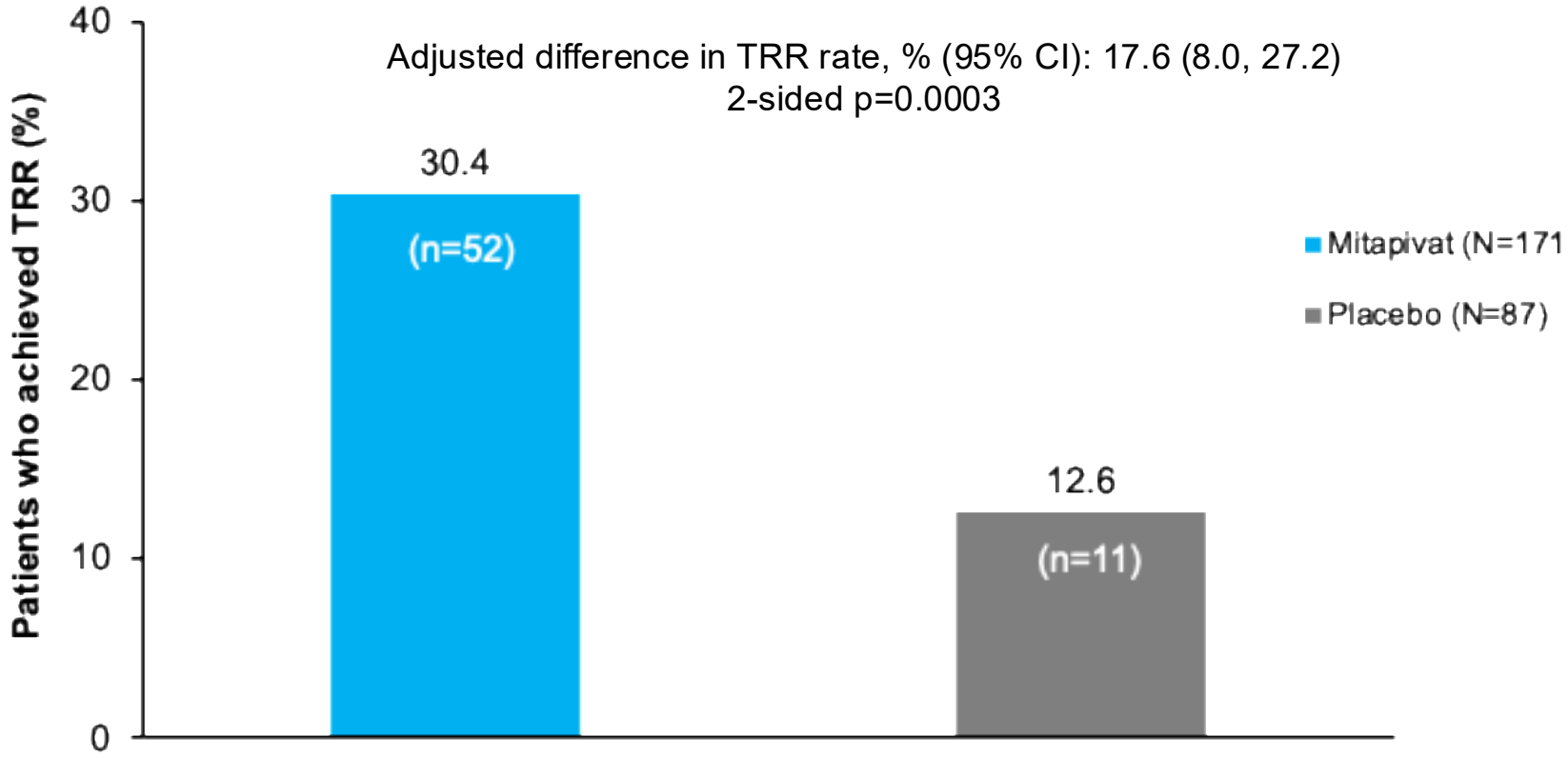


α = α-thalassemia/HbH disease
\+ = Baseline Hb category: 9.1–10 g/dL
x = Patient with missing baseline or no assessments from Week 12 - Week 24

Mitapivat demonstrated a statistically significant improvement in Hb response vs placebo

Taher AT et al. Lancet 2025;406:33–42.

ENERGIZE-T TRIAL – Primary endpoint



Transfusion Reduction Rate (TRR) was defined as a ≥50% reduction in transfused RBC units and a reduction in ≥2 units of transfused RBCs in any consecutive 12-week period through Week 48 compared with baseline

Mitapivat demonstrated a statistically significant reduction in transfusion burden vs placebo

Results – Phase 3

ENERGIZE - NTDT

-met primary endpoint

~42% patients had 1 g/dl or greater increase in Hb

-Greater proportion of beta compared with alpha

-Improved FACIT-Fatigue

-Improved 6 minute walk test

-Well tolerated with few AEs

-Increased AST/ALT – would need monitoring

ENERGIZE-T - TDT

-met primary endpoint

~30% patients had 50% or greater decrease in transfusion requirement in any 12 week interval

-Met key secondary endpoints as well

-Well tolerated with few AEs

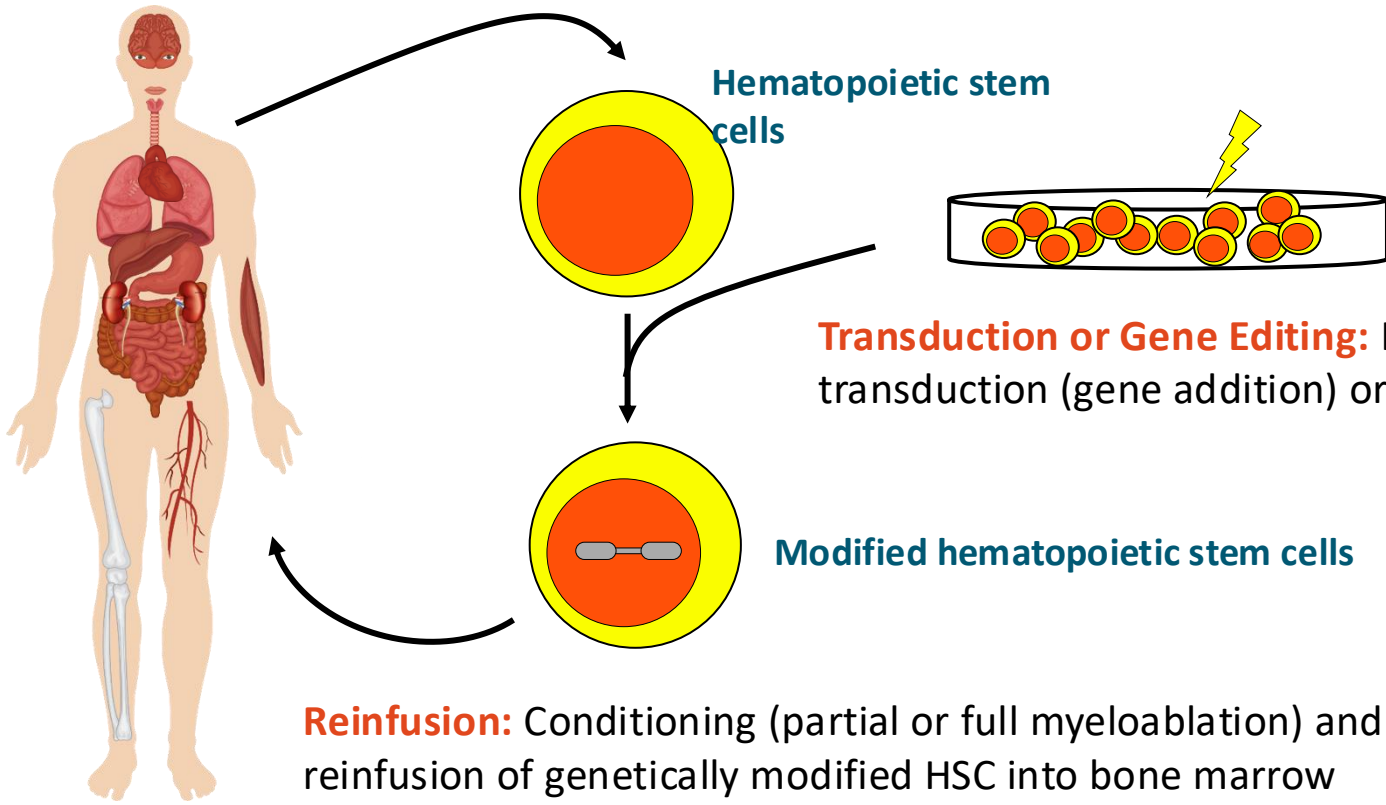
-Increased AST/ALT – would need monitoring

GENE THERAPY APPROACHES

- Globin gene addition
  - Functional β-globin gene
    - » LentiGlobin BB305
    - » GLOBE lentiviral vector
  - Functional γ-globin gene
- Gene editing
  - Reverse γ-globin repression
    - » Targeting BCL11A
      - • CRISPR/CAS9 gene-editing - CTX001
    - » Targeting γ-globin gene promoters
      - • CRISPR-Cas12a editing – EDIT-301
  - Correction of the β-globin mutation

GENE THERAPY

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



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 (lovo-cel, beti-cel)
    - » HGB 205, 206, 210 (SCD)
    - » HGB 204, 205, 207, 212 (thal)
  - GLOBE lentiviral vector (thal)
    - » Phase I/II trial
- Gene editing
  - Phase I/II Thales study of ST-400 (thal)
  - Phase I/II study of CRISPR/CAS9 gene-editing therapy CTX001 - SCD and thal (exa-cel)
  - CRISPR-Cas12a editing of gamma globin promoters *HBG1* and *HBG2* – SCD and thal (reni-cel)

LentiGlobin BB305 – Results

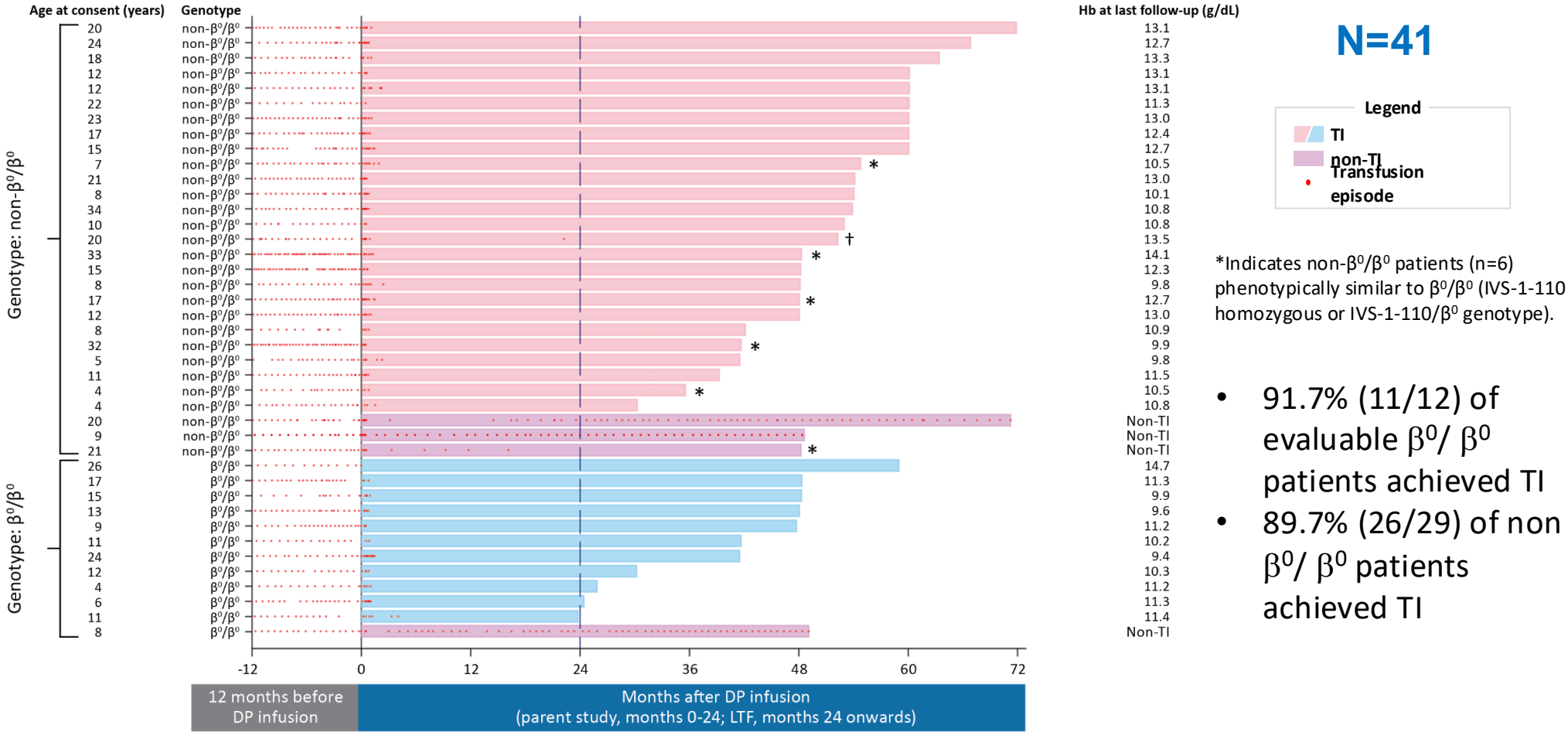
	N	TI evaluable, n	TI rate, n/N (%)	Weighted average Hb during TI, median (min, max), g/dL
By study				
Phase 3 patients	41	41	**37/41 (90.2)**	11.03 (9.6, 13.7)
HGB-207	23	23	**21/23 (91.3)**	12.06 (9.8, 13.0)
HGB-212	18	18	**16/18 (88.9)**	10.47 (9.6, 13.7)
By genotype				
Non-β^0/β^0	29	29	**26/29 (89.7)**	11.92 (9.8, 13.4)
β^0/β^0	12	12	**11/12 (91.7)**	10.54 (9.6, 13.7)
By age				
Adult, ≥18 y	14	14	**12/14 (85.7)**	12.65 (9.6, 13.7)
Adolescent, ≥12 to <18 y	11	11	**11/11 (100)**	11.80 (10.0, 13.4)
Pediatric, <12 y	16	16	**14/16 (87.5)**	10.41 (9.8, 11.4)

- In the phase 3 studies, in which the commercial DP manufacturing process was used, 90.2% (37/41) of patients achieved and maintained TI through last follow-up (up to 6 years)
  - TI rate and median weighted average Hb were generally similar across study, genotype, and age subgroups
- Those who did not achieve TI had substantial reduction in transfusion burden

TI was defined as a weighted average Hb ≥9 g/dL without packed red blood cell transfusions for ≥12 months.
DP, drug product; Hb, hemoglobin; TI, transfusion independence.

Thompson AA, et al. American Society of Hematology Annual Meeting, 2023. Poster 1102.

Phase III Trials of Beti-cel – by genotype



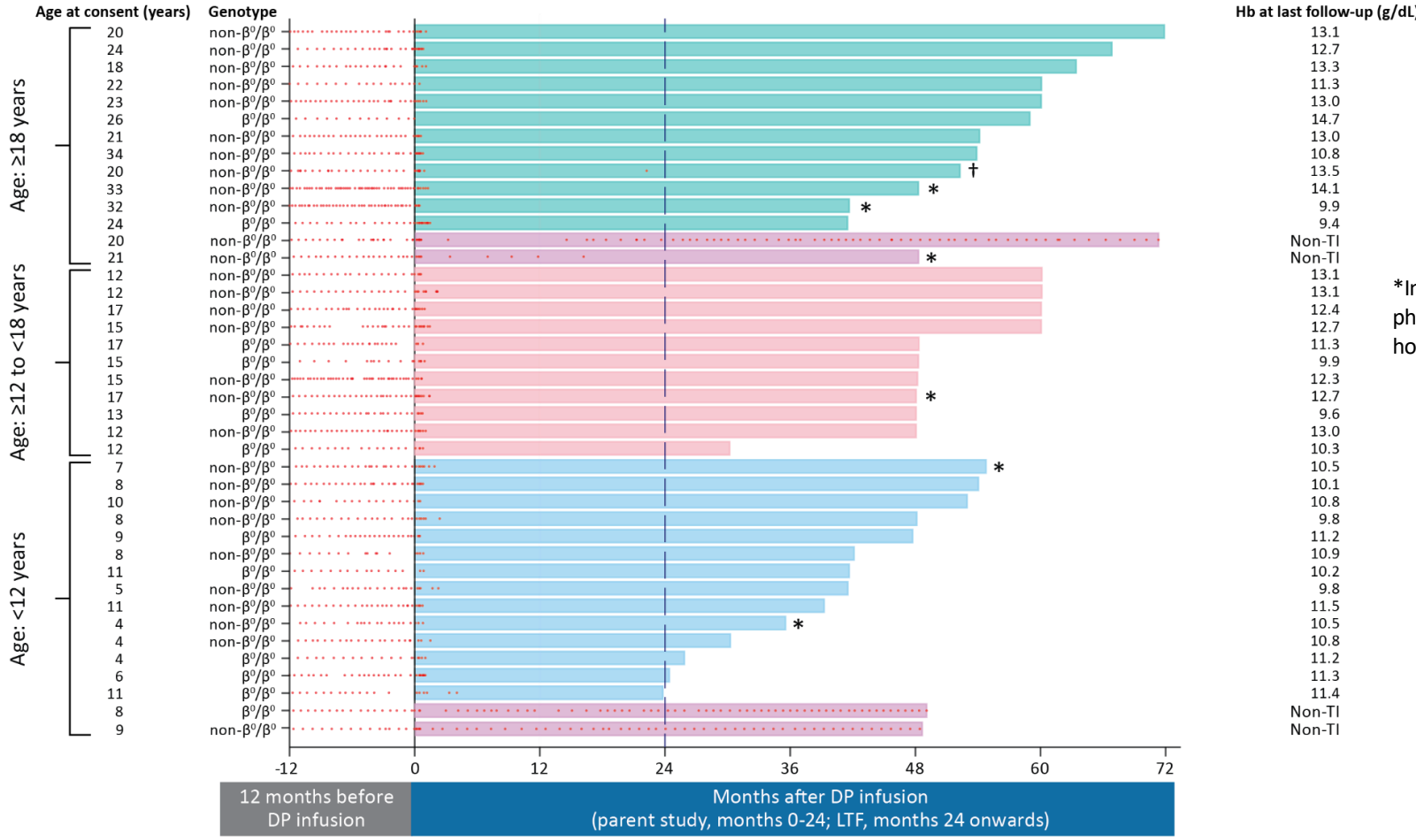
N=41

*Indicates non-β⁰/β⁰ patients (n=6) phenotypically similar to β⁰/β⁰ (IVS-1-110 homozygous or IVS-1-110/β⁰ genotype).

- 91.7% (11/12) of evaluable β⁰/ β⁰ patients achieved TI
- 89.7% (26/29) of non β⁰/ β⁰ patients achieved TI

Presented as a Poster at 2024 Tandem Meetings | Transplantation & Cellular Therapy (TCT) Meetings of ASTCT and CIBMTR | Febru ary 21-24, 2024| San Antonio, TX

Phase III Trials of Beti-cel – by age



N=41

Legend
- TI
- non-TI
- Transfusion episode

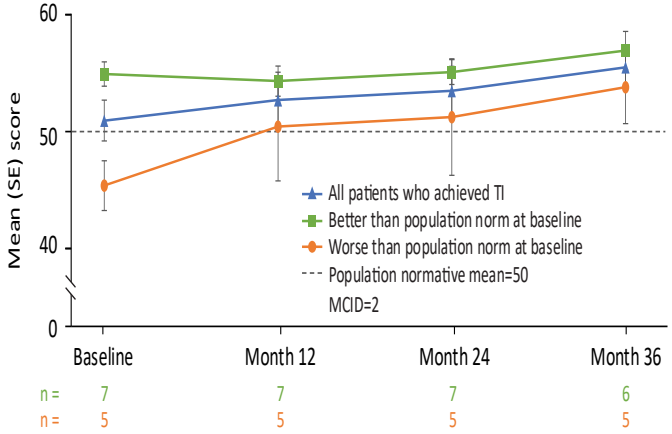
*Indicates non-β⁰/β⁰ patients (n=6) phenotypically similar to β⁰/β⁰ (IVS-1-110 homozygous or IVS-1-110/β⁰ genotype).

- 87% of evaluable pediatric patients achieved TI
- 100% of patients between 12 and 18 years achieved TI

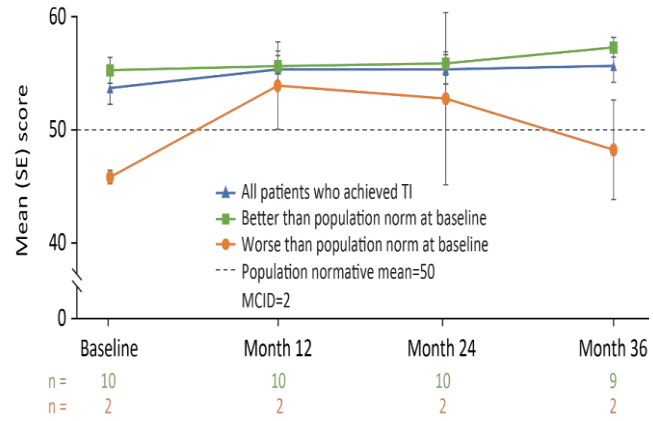
Presented as a Poster at 2024 Tandem Meetings | Transplantation & Cellular Therapy (TCT) Meetings of ASTCT and CIBMTR | Febru ary 21-24, 2024| San Antonio, TX

Beti-cel - HRQoL

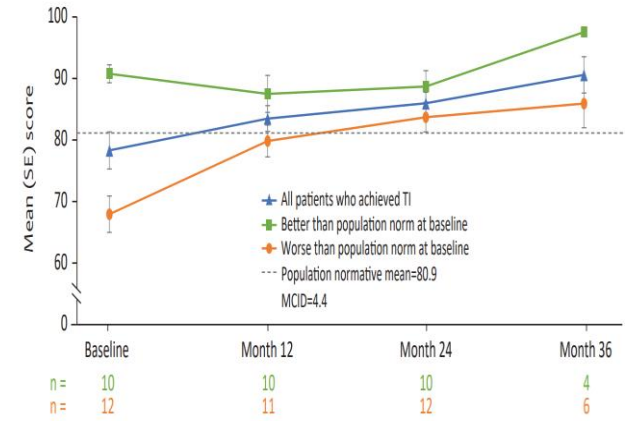
SF-36 mental component summary



SF-36 physical component summary



Pediatric HRQoL



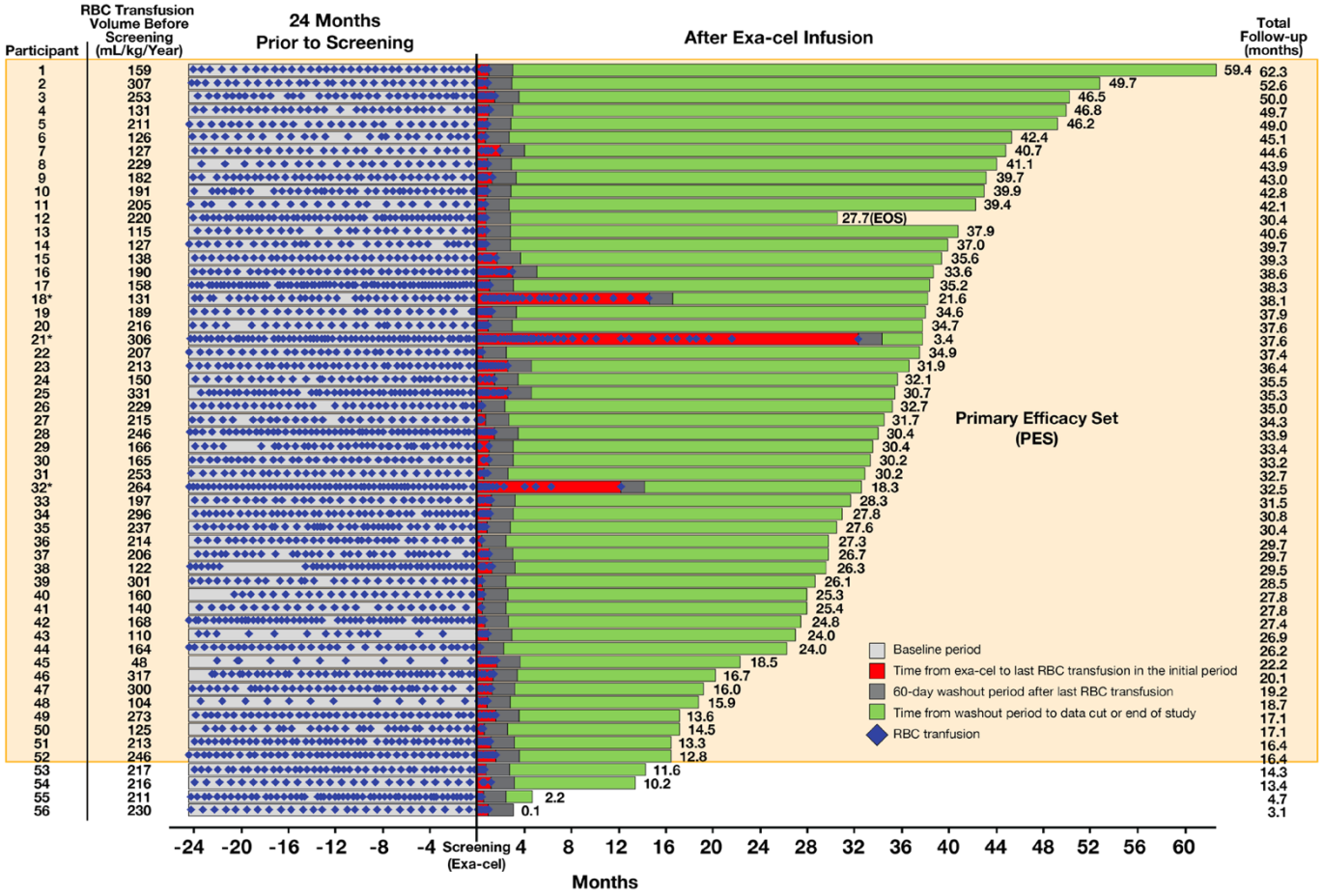
- Clinically meaningful improvements (mean score increase >2) were reported in Short Form-36 Health Survey Questionnaire mental and physical component summary scores at month 36
- Mean pediatric HRQOL scores also showed clinically meaningful improvement at month 36

Presented as a Poster at 2024 Tandem Meetings | Transplantation & Cellular Therapy (TCT) Meetings of ASTCT and CIBMTR | Febru ary 21-24, 2024| San Antonio, TX

CTX-001 – CLIMB THAL-111 Phase 3 trial

Primary Efficacy Endpoint	Proportion of participants transfusion independent for ≥12 consecutive months while maintaining a weighted average hemoglobin ≥9 g/dL (TI12)
Key Secondary Efficacy Endpoint	Proportion of participants transfusion independent for ≥6 consecutive months while maintaining a weighted average hemoglobin ≥9 g/dL (TI6)

Thal trial of Exa-cel – Transfusion Independence (TI)

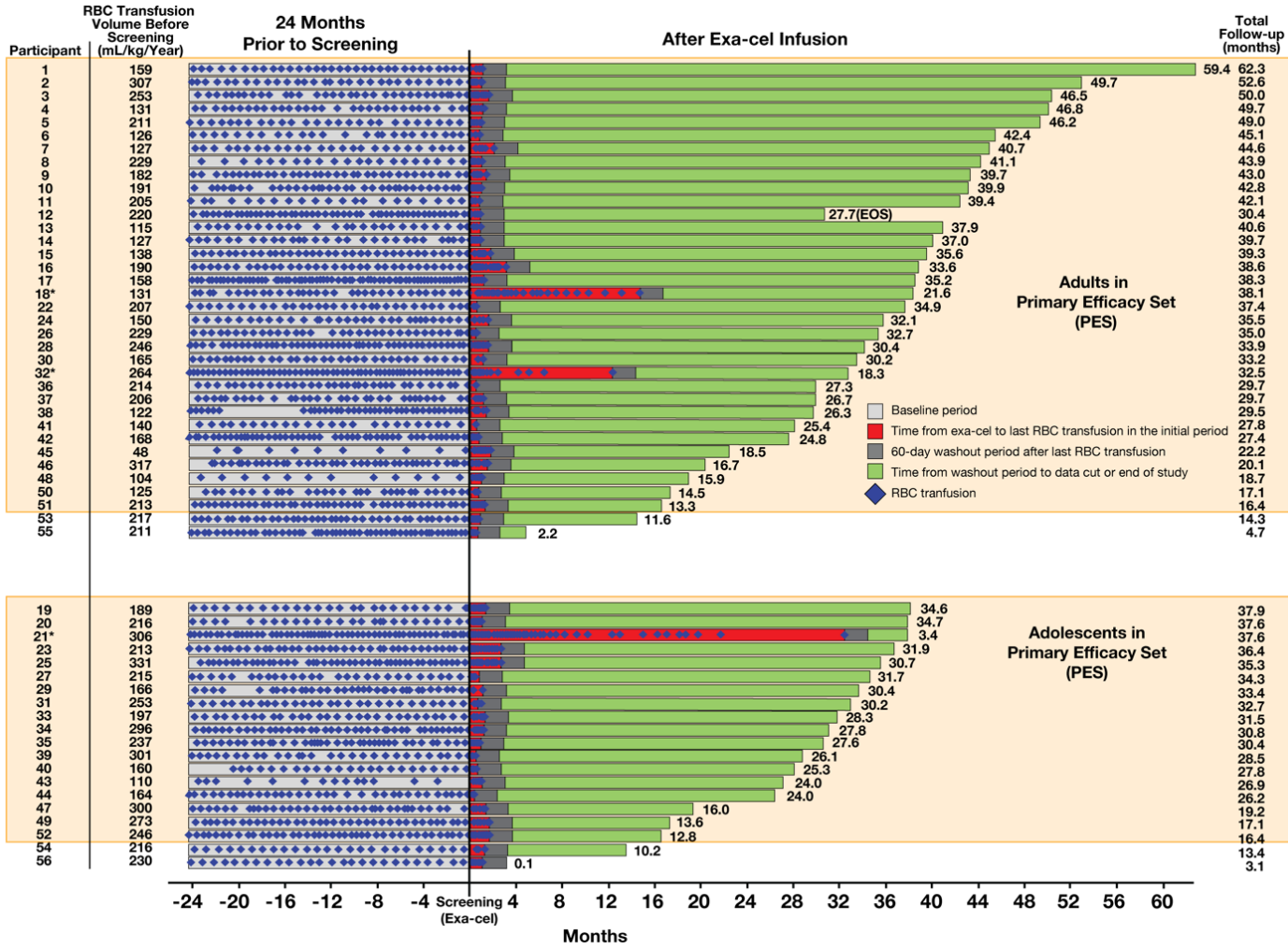


N=52

- **94.2%** (49/52) patients achieved transfusion independence for 12 months
- Mean duration of TI was 31.0 months (range 12.8 to 59.4 or 1-5 years)
- 2 participants stopped RBC transfusions and achieved TI later with a duration of 21.6 and 14.3 months
- 1 participant stopped RBC transfusions for 10.6 months, had a transfusion for a transient episode of anemia, and now transfusion free for 3.4 months since this event

Locatelli et al. EHA 2024

Thal trial of Exa-cel – TI by age

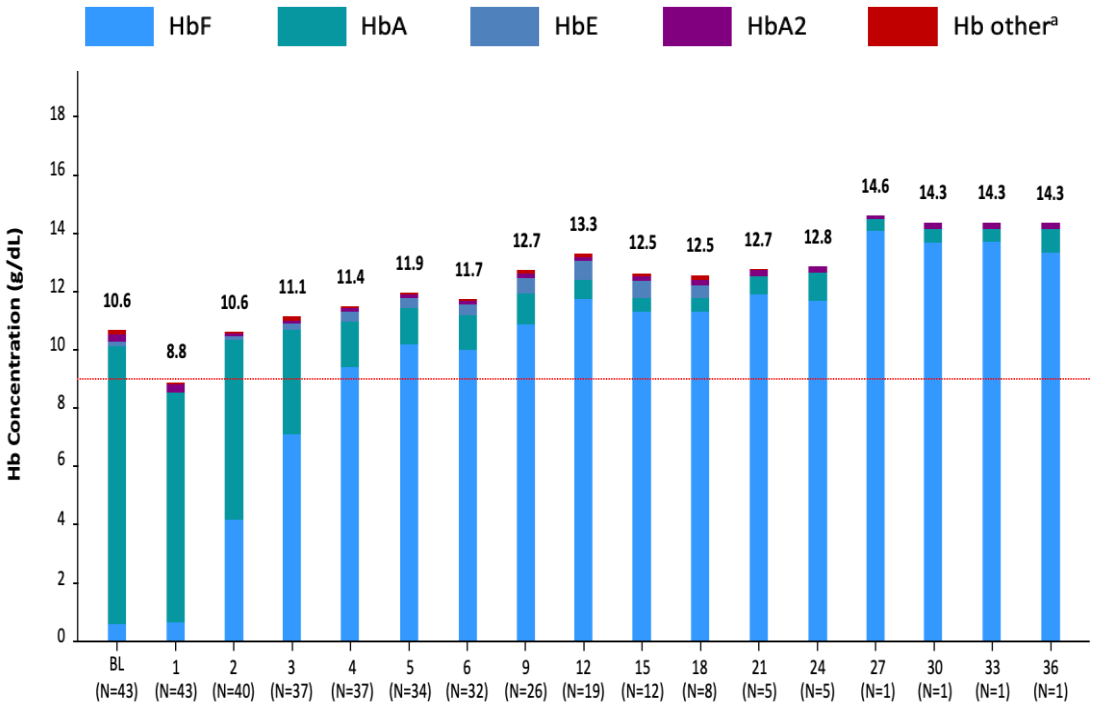


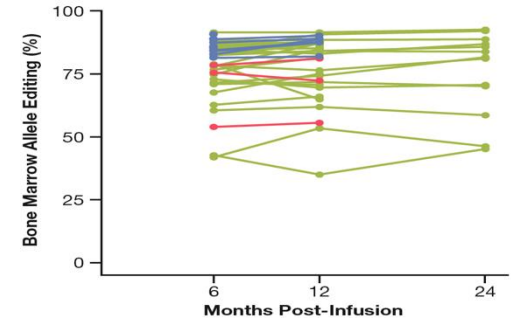
Locatelli et al. EHA 2024

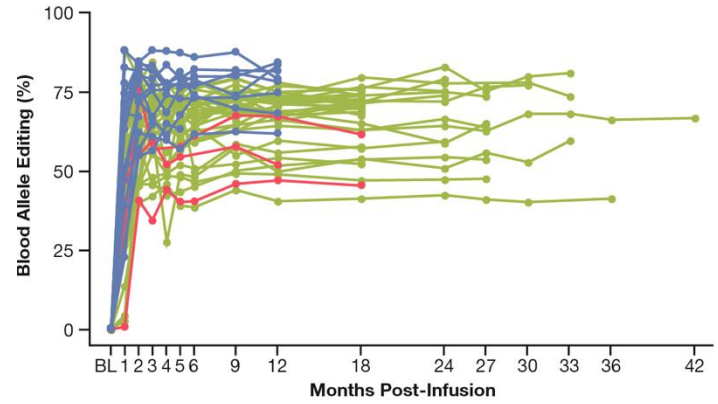
N=52

- **94.1%** (32/34) evaluable adults achieved TI for 12 months
- **94.4%** (17/18) evaluable adolescents achieved TI for 12 months

CTX-001 – HbF and Allelic editing







Locatelli, et al. American Society of Hematology Annual Meeting, 2023.

Thal trial of Exa-cel – HRQoL

- PROs demonstrated substantial and clinically meaningful improvements in health-related quality of life
  - Clinically meaningful improvements seen by month 6 to month 12 and sustained over time
- Improvements seen across all instruments
  - PRO tools specific to general well-being, HSCT, and Transfusion dependent thalassemia
- Improvements seen across all domains
  - Assessments of general health, physical, emotional, social, and functional well-being

Locatelli et al. EHA 2024

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
- No evidence of clonal disease in thalassemia population

GENE THERAPY: CONCLUSIONS

- β-globin gene addition and editing trials have shown positive results
  - High rate of transfusion independence across all genotypes
  - Long-term data with high VCN / high degree of editing
  - Hemoglobin levels are in normal or near normal ranges
  - Response is durable
  - Neutrophil (29d) and platelet (44d) engraftment longer than allo-HSCT
- No deaths to date, generally well tolerated in pediatric and adult patients
- Toxicity mostly related to myeloablative conditioning
- Risk for insertional mutagenesis (addition) or off-target effects (editing) need careful follow-up

Allo HSCT vs Gene Therapy

Parameter	HSCT	Gene Therapy
Chemotherapy	Yes	Yes
Immunosuppression	Yes	None
Graft-vs-host disease	Yes	None
Medication burden	Higher	Lower
Donor availability	Allogeneic – limited	Autologous – available
Speed of Engraftment	Faster	Slower, especially platelets
Insertional mutation/off target effects	None	Possible
Degree of phenotype correction	Complete (if full chimerism)	Partial/Sufficient
Long term data	Available	Limited/still novel

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 here!