ACCADEMIA di FORMAZIONE

SIMTI

Emoglobinopatie: supporto trasfusionale e nuovi approcci terapeutici

Talassemie: approccio diagnostico e terapeutico

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La sottoscritta, in qualità di Relatrice
dichiara che nell'esercizio della Sua funzione e per l'evento in oggetto, NON È in alcun modo portatore di interessi commerciali propri o di terzi; e che gli eventuali rapporti avuti negli ultimi due anni con soggetti portatori di interessi commerciali non sono tali da permettere a tali soggetti di influenzare le mie funzioni al fine di trarne vantaggio.

EMOGLOBINOPATIA

Alterazione ematologica, talvolta associata ad anemia, dovuta ad un difetto genetico di una o più globine, costituenti il tetramero dell'emoglobina

Circa il 7% della popolazione mondiale è portatore di anomalie ereditarie dell'emoglobina, rendendole le più comuni malattie monogeniche "life-threating"

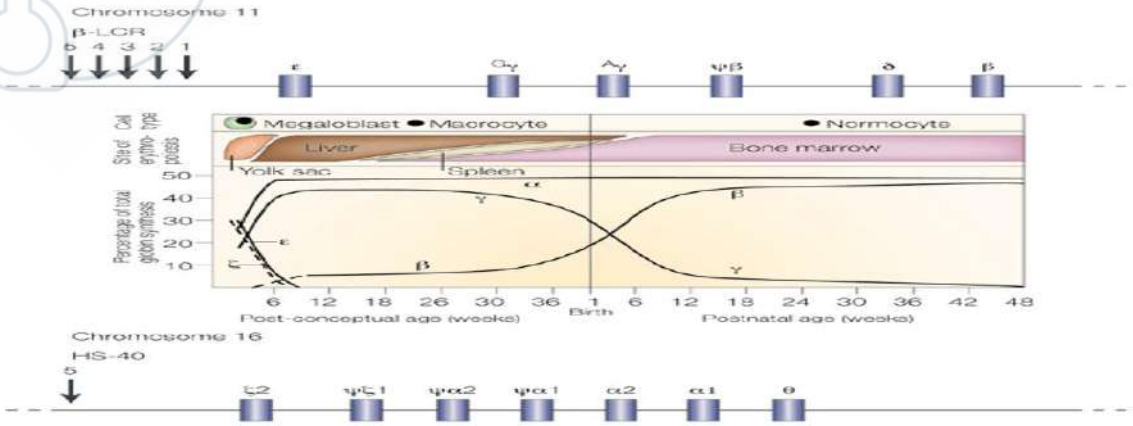
3 gruppi

- varianti strutturali dell'emoglobina
- talassemie: difetti di sintesi delle catene globiniche
- difetto della normale accensione della produzione di emoglobine da fetali ad adulte, chiamato «persistenza ereditaria di emoglobina fetale (HPFH)»

Emery and Rimoin, 2007

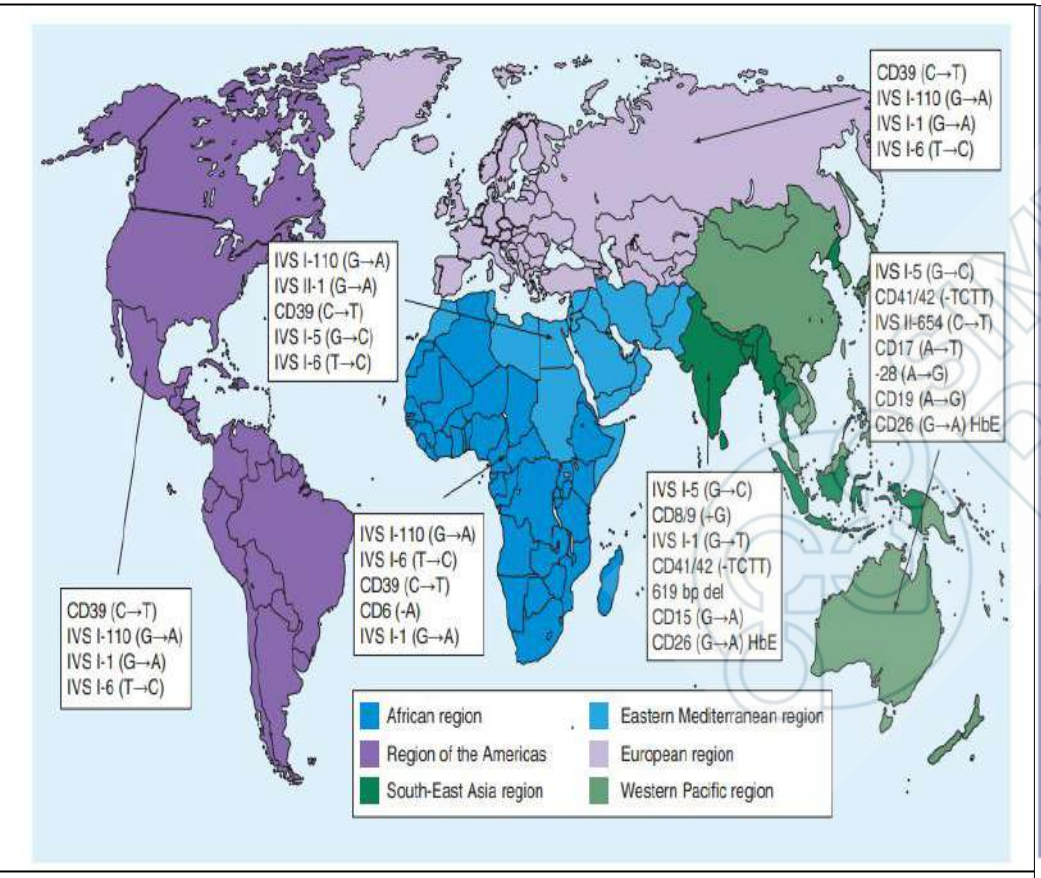
Le Hb umane

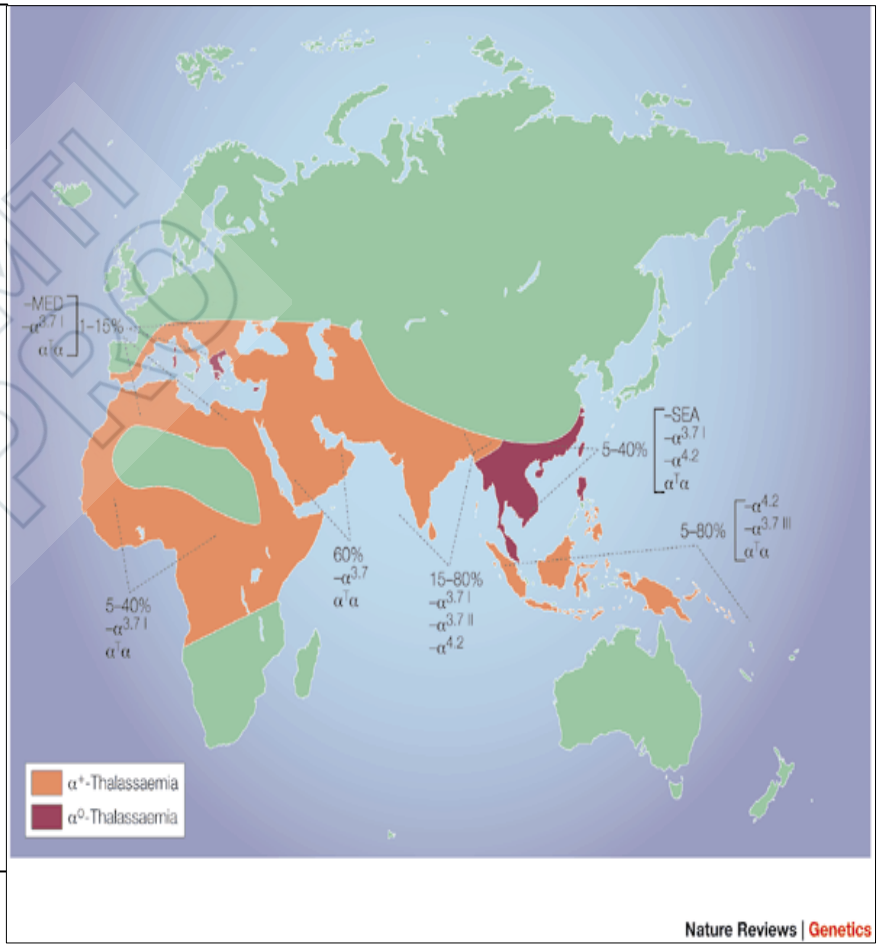
Hb	struttura	% adulto
A	α2β2	97-98
A2	α2δ2	2-3
F	α2γ2	< 1
Gower1	ζ2ε2	0
Gower2	α2ε2	0
Portland	ζ2γ2	0



- I geni per le catene globiniche sono situati sui **cromosomi 11 e 16**
- **I geni della FAMIGLIA β** (catene **ε γ δ β**) sono linearmente situati sul **cromosoma 11**
- **I geni della FAMIGLIA α** (catene **ζ α**) sono linearmente situati sul **cromosoma 16**

Common beta and alpha thalassemia mutations in different regions of the world





Colah R et al. Global burden, distribution and prevention of b-thalassemias and hemoglobin E disorders
Expert Rev. Hematol. 3(1), 103–117 (2010)

- Distribuzione geografica simile alla β –thalassemia maggiormente diffusa in SE asiatico ed in Africa
- α^{+}: 3.7Kb del: Africa, Mediterraneo, Bangladesh, India, Pakistan, Malesia
- α^{+}: 4.2Kb del: Sud Est Asia
- α^{0}: Mediterraneo (**Cipro, Grecia, Turchia, Sud Italia);** Asia (Cina, Tailandia, Cambogia,Filippine, Vietnam)

Alpha and Beta Thalassemia

Variant	*Chromosome 16*	*Signs and symptoms*
Alpha thalassemia silent carrier	One of four gene deletions	Asymptomatic
Alpha thalassemia trait	Two of four gene deletions	Asymptomatic
Hemoglobin Constant Spring	Reduced output of alpha globin	Silent or mildly symptomatic
Alpha thalassemia intermedia with significant hemoglobin H (hemoglobin H disease)	Three of four gene deletions	Moderate to severe hemolytic anemia, modest degree of ineffective erythropoiesis, splenomegaly, variable bone changes[4]
Alpha thalassemia major with significant hemoglobin Bart's	Four of four gene deletions	Causes nonimmune hydrops fetalis, usually fatal[5]

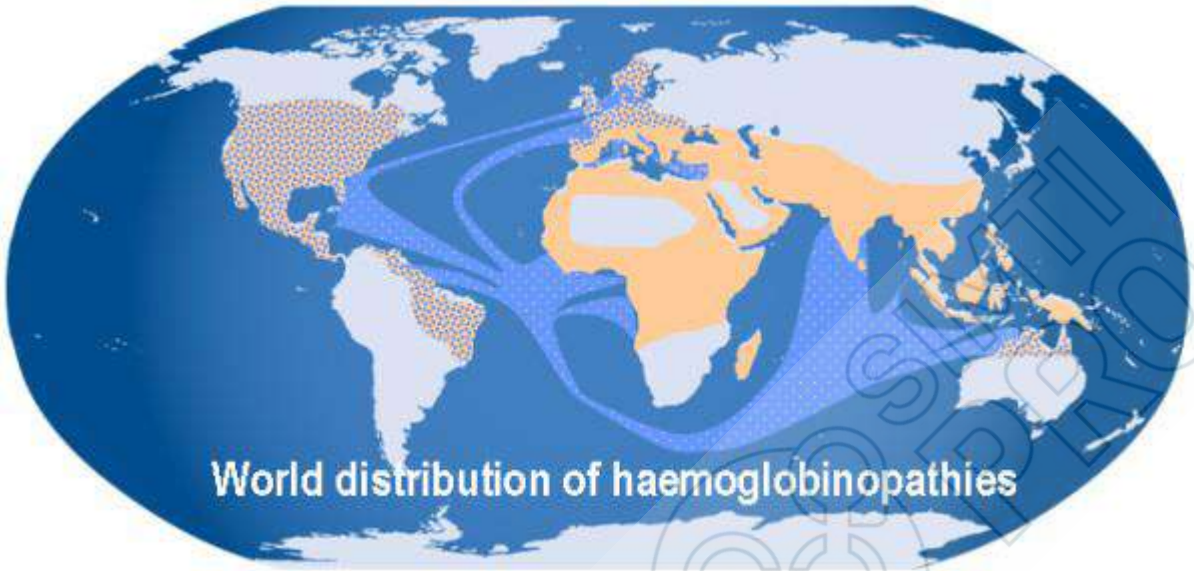
HERBERT L. MUNCIE, JR., MD, and JAMES S. CAMPBELL, MD
Am Fam Physician. 2009;80(4):339-344, 371

Variant	*Chromosome 11*	*Signs and Symptoms*
Beta thalassemia trait	One gene defect	Asymptomatic
Beta thalassemia intermedia	Two genes defective (mild to moderate decrease in beta globin synthesis)	Variable degrees of severity of symptoms of thalassemia major
Beta thalassemia major	Two genes defective (severe decrease in beta globin synthesis)	Abdominal swelling, growth retardation, irritability, jaundice, pallor, skeletal abnormalities, splenomegaly; requires lifelong blood transfusions[6]

Beta-thalassemia with associated Hb variants:
HbC/Beta-thalassemia • HbE/Beta-thalassemia
HbS/Beta-thalassemia

Galanello and Origa Orphanet Journal of Rare Diseases 2010, 5:11

EPIDEMIOLOGY OF THALASSEMIA



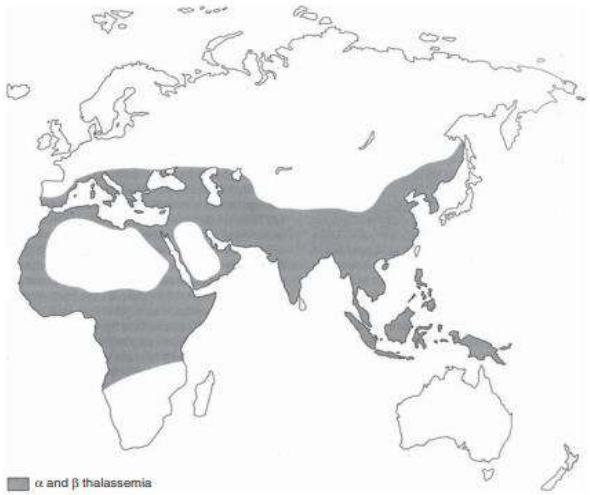
β thalassemia is most common in Mediterranean, African and Southeast Asian countries

α thalassemia occurs most often in African and Southeast Asian countries

The annual births of thalassemic disorders is estimated to be nearly 70,000
The highest prevalence occurs where malaria was, or still is, endemic

Muncie et al. Am Fam Physician 2009;80:339-344;Review
Williams TN and Wheatherall DJ. Cold Spring Harb Perspect Med 2012;2:a011692.Review

EVOLVING GLOBAL BURDEN DUE TO MIGRATION



Annals of Human Biology, March–April 2005; 32(2): 117–122

The global problem of genetic disease

D. J. WEATHERALL

Weatherall Institute of Molecular Medicine, University of Oxford, UK

Abstract
Inherited haemoglobin disorders will undoubtedly cause an increasing health burden in many developing countries. Although much is known about their molecular pathology and the mechanisms for their phenotypic diversity, many important questions remain, not least the role of the environment in modifying the clinical course. Methods for screening these conditions are now well established and inexpensive and it is vital that they are applied to define the magnitude of the problem that will be posed by these conditions in the future. Similarly, they form the basis for widespread screening and counselling programmes directed at developing prenatal diagnosis expertise where this is not available. Answers to some relatively simple questions about the role of the environment could also make a major difference to the management of the haemoglobin disorders. There is a major case for the development of regional networks to apply such technology as has been developed for the control and prevention of the important haemoglobin disorders, particularly in Asian countries.

Public health reviews

Global epidemiology of haemoglobin disorders and derived service indicators

Bernadette Modell[I] & Matthew Darlison[II]

Abstract To demonstrate a method for using genetic epidemiological data to assess the needs for equitable and cost-effective services for the treatment and prevention of haemoglobin disorders. We obtained data on demographics and prevalence of gene variants responsible for haemoglobin disorders from online databases, reference resources, and published articles. A global epidemiological database for haemoglobin disorders by country was established, including five practical service indicators to express the needs for care (indicator 1) and prevention (indicators 2–5).

Haemoglobin disorders present a significant health problem in 71% of 229 countries, and these 71% of countries include 89% of all births worldwide. Over 330 000 affected infants are born annually (83% sickle cell disorders, 17% thalassaemias). Haemoglobin disorders account for about 3.4% of deaths in children less than 5 years of age. Globally, around 7% of pregnant women carry β or α zero thalassaemia, or haemoglobin S, C, D Punjab or E, and over 1% of couples are at risk. Carriers and at-risk couples should be informed of their risk and the options for reducing it. Screening for haemoglobin disorders should form part of basic health services in most countries.

Bulletin of the World Health Organization 2008;86:480–487.

Public Health Reviews

Inherited haemoglobin disorders: an increasing global health problem

D.J. Weatherall[1] & J.B. Clegg[2]

Abstract Despite major advances in our understanding of the molecular pathology, pathophysiology, and control and management of the inherited disorders of haemoglobin, thousands of infants and children with these diseases are dying through lack of appropriate medical care. This problem will undoubtedly increase over the next 20 years because, as the result of a reduction in childhood mortality due to infection and malnutrition, more babies with haemoglobin disorders will survive to present for treatment. Although WHO and various voluntary agencies have tried to disseminate information about these diseases, they are rarely mentioned as being sufficiently important to be included in setting health care priorities for the future. It takes considerable time to establish expertise in developing programmes for the control and management of these conditions, and the lessons learned in developed countries will need to be transmitted to those countries in which they occur at a high frequency.

Keywords Hemoglobinopathies/mortality/therapy/epidemiology; Anemia, Sickle cell/mortality/therapy/epidemiology; Thalassemia/mortality/therapy/epidemiology; Malaria/complications/blood; Genetic techniques; Child; Cost of illness; Forecasting (*source: MeSH*).

Mots clés Hémoglobinopathie/mortalité/thérapeutique/épidémiologie; Anémie cellule falciforme/mortalité/thérapeutique/épidémiologie; Thalassémie/mortalité/thérapeutique/épidémiologie; Paludisme/complication/sang; Technique génétique; Enfant; Coût maladie; Prévision (*source: INSERM*).

Palabras clave Hemoglobinopatias/mortalidad/terapia/epidemiologia; Anemia de células falciformes/mortalidad/terapia/epidemiologia; Talasemia/mortalidad/terapia/epidemiologia; Paludismo/complicaciones/sangre; Técnicas genéticas; Niño; Costo de la enfermedad; Predicción (*fuente: BIREME*).

Bulletin of the World Health Organization, 2001, **79**: 704–712.

- Osservazione nella popolazione di un maggior numero di difetti già noti e comparsa di nuovi e maggiore frequenza di composti emoglobinici

- Incremento di soggetti con emoglobinopatie in zone non tradizionalmente coinvolte

- Necessità di percorsi diagnostici che prevedano il momento del "counselling", supporto alla diagnosi pre-natale e post-natale da parte di Centri specialistici e diagnostica in laboratori specializzati e dedicati

Diagnostica di I e II livello delle Emoglobinopatie

Buone Pratiche SITE

SOCIETA' ITALIANA TALASSEMIE ED EMOGLOBINOPATIE

www.site-italia.org

PERCORSO DIAGNOSTICO PER L'IDENTIFICAZIONE DEL PORTATORE DI EMOGLOBINOPATIA

Prelievo venoso	Informazioni pre-test Informativa sul test e i limiti del percorso diagnostico di I livello	Raccolti direttamente dal paziente o ricevute dal clinico Fornita dal laboratorio
Assetto Hb	Mediante HPLC Elettroforesi capillare	I due metodi risultano parzialmente sovrapponibili nella valutazione dell'HbF, dell'HbA e delle varianti più frequenti
Indici emocromocitometrici	Relativamente a G.R., Hb, Ht, MCV, MCH, Reticolociti (in presenza di varianti)	
Assetto marziale	Sideremia, transferrina, ferritina, IS	
Eventuali altri test di conferma	Elettroforesi capillare	
Altri possibili test	Aptoglobina, bilirubina, P50, metHb	
Conclusione certa o presuntiva	Eventuale coinvolgimento del clinico o del Laboratorio di II livello	In gravidanza eventuali esami ai partner possono essere previsti contemporaneamente ad esami si conferma molecolare
Referto	Per quanto riguarda l'assetto Hb va riportato il valore dell'HbA2 oltre a quello dell'HbF e delle eventuali altre frazioni HB	L'HbA va riportata come dato dell'assetto qualitativo ma non va riportato il valore quantitativo

PRINCIPALI VARIABILI PREANALITICHE INDIVIDUALI CHE POSSONO MODIFICARE IL VALORE DELL'HbA2

Cause di aumento	Cause di diminuzione
Ipertirodismo	Anemia sideropenica grave
Anemia megaloblastica	Anemia siedeoblastica
Terapia retrovirale per HIV	Presemza di Delta Talassemia (b)
Alcube Hb varianti instabili	Presenza di Varianti delle catene Delta (c)
Geni alfa sovranbnumerati (es. $\alpha\alpha\alpha/\alpha\alpha$)	Presenza di Varianti delle catene Alfa (d)
Componente glicata della beta Variante eventualmente presente (a)	Alcune forme di HpFH da difetto del promotore dei geni Gamma (e)
Epatopatia/alcool	Delta-Beta Talassemie
Osteoartropatia ipertrofica	Alfa Talassemie: marginale in Alfa+ o Alfa0 mentre è marcata nell'Emoglobinosi H
Mutazioni del gene KLF 1	Hb Lepore (f)
	HbD (a) e C (f)

(a) Nel caso di quantificazione con sistemi HPLC
(b) Il valore dell'Hb A2 potrebbe essere normale
(c) Il valore reale dell'Hb A2 equivale a circa il doppio rispetto a quella dosata e definita tale dalla strumentazine. Nel caso in cui l'Hb A2x è rilevata, l'Hb A2 reale è data dalla somma delle due frazioni.
(d) In tale circostanza il valore dell'Hb A2 va sommato al valore dell'Hb A2x.
(e) È ciò che si verica nella cosidetta "delta-beta talassemia sarda" con la mutazione -196C>T del promotore del gene Ay.
(f) Rilevabile solo nel caso di quantificazione con CE.

CONDIZIONI NELLE QUALI SI PUÒ RISCONTRARE UN AUMENTO DELLA HBF NON RICONDUCIBILE A DIFETTI GAMMA GLOBINICI

Anemie congenite o acquisite da primitiva insufficienza midollare con o senza displasia	Neoplasie	Condizioni associate a specifici trattamenti terapeutici	Altre condizioni
Anemia aplastica congenita o acquisita	Epatocarcinoma	Chemioterapie per leucemie	Gammopatia monoclonale di incerto significato
Anemia megaloblastica da carenza vitaminica	Leucemie acute mieloidi	Terapia con idrossiurea, butirrati ed agenti stimolanti dell'eritropoiesi	Gravidanza
Anemia di Diamond-Blackfan	Mielofibrosi primitiva		Insufficienza renale cronica
Alcune forme di anemia normoblastica	Leucemia mielomonocitica cronica giovanile		Ipertirodismo
Anemie sideroblastiche congenite			Trisomia 13
Anemie sideroblastiche acquisite			
Emoglobinuria parossistica notturna			

Ematologia

Emocromo

Esame emocromo-citometrico

Leucociti (WBC)	5,89	x1000/µL	[4,00 - 11,00]
Eritrociti (RBC)	4,57	milioni/µL	[4,10 - 5,10]
Emoglobina (Hb)	13,00	g/dL	[12,30 - 15,30]
Ematocrito (Ht)	37,2	%	[36,0 - 45,0]
Volume eritrocitario medio (MCV)	81,6	fl	[80,0 - 96,0]
Hb media eritrocitaria (MCH)	28,5	pg	[27,5 - 33,2]
Conc. Hb media eritrocitaria (MCHC)	34,9	g/dL	[32,0 - 35,5]
Distribuz. volume eritrocitario (RDW)	12,9	CV (%)	[11,5 - 16,0]
Piastrine (PLT)	257	x1000/µL	[150 - 400]
Volume medio piastrinico (MPV)	8,40	fl	[6,00 - 12,00]

Formula leucocitaria

Neutrofili %	54,90	%	[40,00 - 75,00]
Linfociti %	35,10	%	[20,00 - 45,00]
Monociti %	5,90	%	[1,00 - 10,00]
Eosinofili %	2,10	%	[1,00 - 6,00]
Basofili %	0,20	%	[0,00 - 1,00]
Neutrofili	3,23	x1000/µL	[2,00 - 8,00]
Linfociti	2,07	x1000/µL	[1,50 - 4,00]
Monociti	0,35	x1000/µL	[0,20 - 1,00]
Eosinofili	0,12	x1000/µL	[0,04 - 0,35]
Basofili	0,01	x1000/µL	[0,00 - 0,20]

Assetto Emoglobinico	**Hb A + Hb A2 +Hb F + Hb S**			(*)
Dosaggio HbF (*)	**1.6**	%	v.n. Inf. 1.0%	(*) Dosaggio e assetto ottenuti mediante HPLC
Dosaggio HbA_2 (*)	**3.2**	%	v.n. Inf. 3.2%	
Dosaggio Hb S (*)	**39.6**	%		
Morfologia eritrocitaria				
ZnPP		(**)	v.n.20-40	(**) µmolZnPP/mole eme Dosaggio mediante ematofluorimetro. Valori elevati di ZnPP sono indicativi di carenza marziale e/o intossicazione da piombo.
Reticolociti		%	v.n.0.8-2%	
Test di sickling	**POSITIVO**			
Ricerca inclusi eritrocitari		%		
Test di Termolabilità				
Dosaggio metHb		%	v.n. Inf. 3%	
Pink Test(T.lisi al glicerolo modificato)		%	v.n. Inf.25%	

Ematologia

Emocromo

Esame emocromo-citometrico

Leucociti (WBC)	*	11,87	x1000/µL	[4,00 - 11,00]
Eritrociti (RBC)	*	5,77	milioni/µL	[4,10 - 5,10]
Emoglobina (Hb)		14,50	g/dL	[12,30 - 15,30]
Ematocrito (Ht)		42,9	%	[36,0 - 45,0]
Volume eritrocitario medio (MCV)	*	74,4	fl	[80,0 - 96,0]
Hb media eritrocitaria (MCH)	*	25,1	pg	[27,5 - 33,2]
Conc. Hb media eritrocitaria (MCHC)		33,7	g/dL	[32,0 - 35,5]
Distribuz. volume eritrocitario (RDW)		14,6	CV (%)	[11,5 - 16,0]
Piastrine (PLT)		171	x1000/µL	[150 - 400]
Volume medio piastrinico (MPV)		10,60	fl	[6,00 - 12,00]

Indagini per Emoglobinopatie

Assetto Emoglobinico	**Hb A + (Hb A2 + Hb X)**			(*)
Dosaggio HbF (*)		%	v.n. Inf. 1.0%	(*) Dosaggio e assetto ottenuti mediante HPLC
Dosaggio HbA_2 (*)	**non dosabile**	%	v.n. Inf. 3.2%	
Dosaggio Hb X (*)	**+ Hb A2= 29.4**	%		

CONCLUSIONE: **Il soggetto esaminato è risultato portatore allo stato eterozigote della Variante Emoglobinica HbE (Beta 26 Glu> Lys)**

ESEMPI DI VARIANTI EMOGLOBINICHE CHE COELUISCONO CON O VICINO A HBA2 IN HPLC

Hb Abruzzo	Hb Kenya
Hb Akron	Hb Korle Bu*
Hb Boras	Hb Lepore Baltimore
Hb Bethesda*	Hb Lepore Boston
Hb Chandigarth	Hb Lepore Hollandia
Hb Deer Lodge	Hb Loves Park*
Hb D Iran*	Hb M Saskatoon
Hb Denver*	Hb Muravera
Hb D-Ouled Rabah	Hb Nebraska
Hb E	Hb Ocho Rios
Hb Ethiopia*	Hb Osu Christiansborg*
Hb Fort Worth	Hb Paddington
Hb G Copenhagen	Hb Rocky Mountain
Hb G Coushatta*	Hb San Bruno*
Hb G Ferrara	Hb Santa Juana*
Hb G Galveston	Hb SId (the aged adduct of Hb due to glutathione)
Hb G Honolulu*	Hb Spanish Town
Hb G Taipei	Hb Toulon
Hb Hoshida	Hb Tubingen
Hb Hamadan	Hb Zuri
HPLC, high-performance liquid chromatography.	
*Queste varianti emoglobiniche sono talvolta etichettate come Hb A2 e talvolta non evidenziate a seconda dello strumento e lievi variazioni nei tempi di conservazione. Anche quando la variante non coeluisce esattamente con Hb A2, può comunque interferire con la misurazione dell'Hb A2 e quindi in questa situazione dovrebbe essere utilizzato un metodo diverso per misurare l'Hb A2.	

ESEMPI DI VARIANTI EMOGLOBINICHE CHE MIGRANO CON O VICINO A HBA2 IN ELETTROFORESI CAPILLARE

Hb Chad
Hb E-Saskatoon
Hb O-Arab
Hb C Harlem*
*Separazione insufficiente per una quantificazione accurata

oltre **700 varianti Hb** sono state identificate, ma solo **3** sono presenti ad alta frequenza in popolazioni differenti

HbS	Africa sub Sahariana Mediterraneo Medio-oriente India
HbC	Africa occidentale Mediterraneo
HbE	India Sud-Est Asia

- Hb S
- Hb C
- Hb E
- Hb D Punjab
- Beta tal.° Cd 6
- Beta tal.° del -619 bp
- Beta tal.° Cd 8-9
- Beta tal.$^{+}$ -28
- Beta tal.$^{+}$ -30
- Alfa tal. --Fil
- Alfa tal. --SEA

Emoglobinopatie più frequenti e clinicamente rilevanti soprattutto allo stato omozigote o in associazione a trait Talassemici o con altre varianti

- Hb O Arabia
- Hb G Philadelphia
- Hb D Ouled Rabah
- Hb D Iran
- Hb J Habana
- Hb J Meerut
- Hb G Copenhagen
- Hb A_2 X (B_2)
- Beta tal.$^{+}$ -92
- Beta tal.$^{+}$ IVSII-654
- Beta tal.$^{+}$ IVSI-5
- Alfa tal. -4.2kb

Emoglobinopatie più rare, clinicamente meno rilevanti anche allo stato omozigote o in associazione a trait Talassemici ma che necessitano comunque una caratterizzazione per differenziarle da quelle clinicamente rilevanti

Classical methods utilised for Hemoglobinopathies characterization

For **Beta gene (HBB)** characterization:

- Sequencing with sanger method (different set of primers)
- Reverse Dot Blot : different kits for Thalassaemia and most frequent variants
- MLPA for cluster deletions
- Sickling test to conferm the presence of HbS seen in HPLC

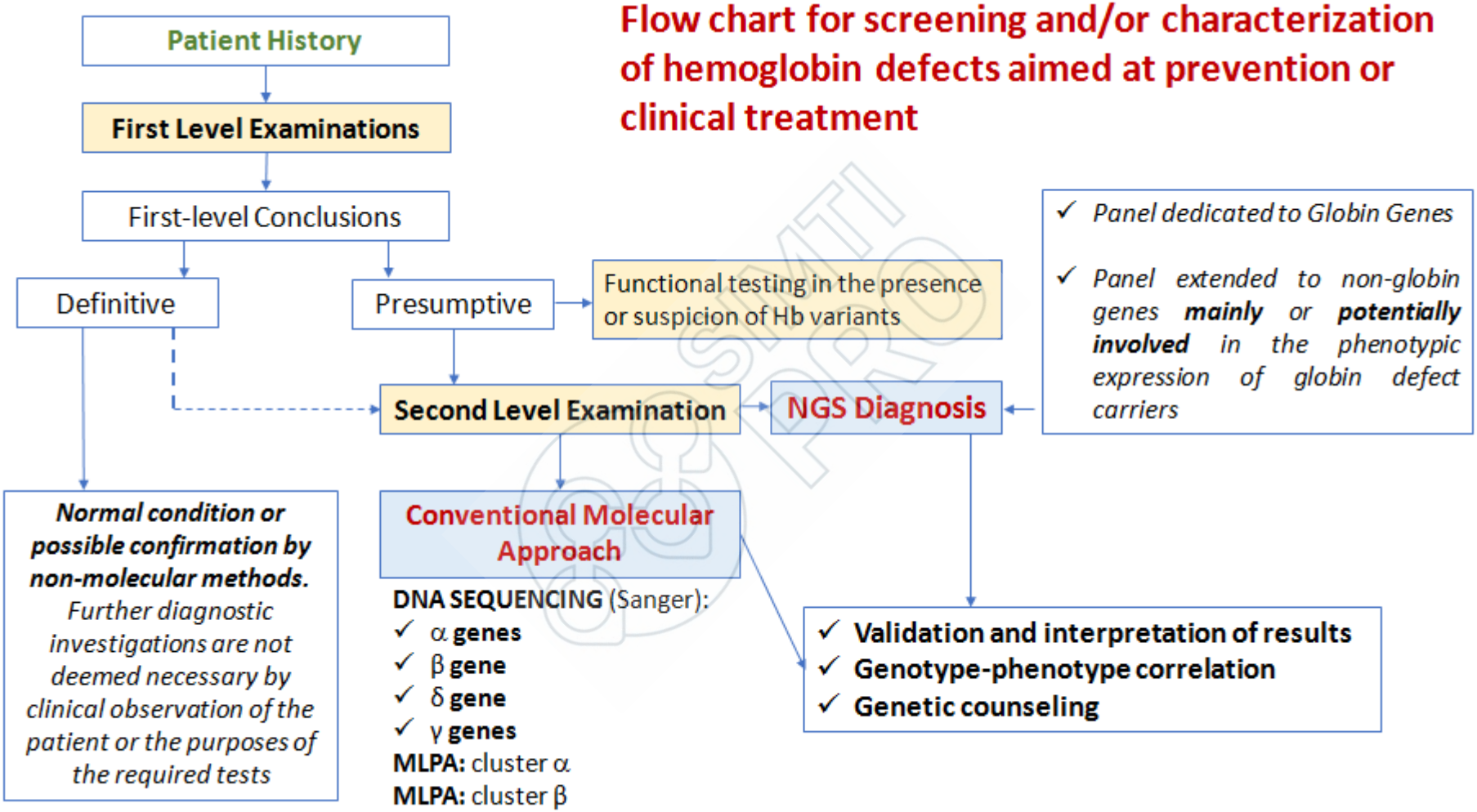
For **Alfa genes (HBA1, HbA2)** characterization:

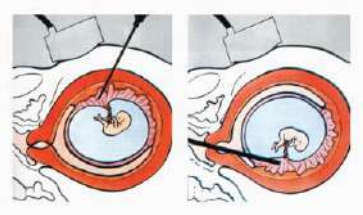
- Sequencing with sanger method (different set of primers that differenziate the two genes **or variants presents on 3.7Kb deletion fusion gene**)
- Reverse Dot Blot : different kits for Thalassaemia and most frequent variants
- MLPA for cluster deletions

For **Delta gene (HBD) and Gamma genes (HBG1, HBG2)** characterization:

- Sequencing with sanger method :primers that differenziate the two gamma genes or the promoter region in case of the HPFH.
- Reverse Dot Blot : only a few deletions present on cluster BETA
- MLPA for cluster deletions (big as HPFH or little as Corfù deletion)

Flow chart for screening and/or characterization of hemoglobin defects aimed at prevention or clinical treatment





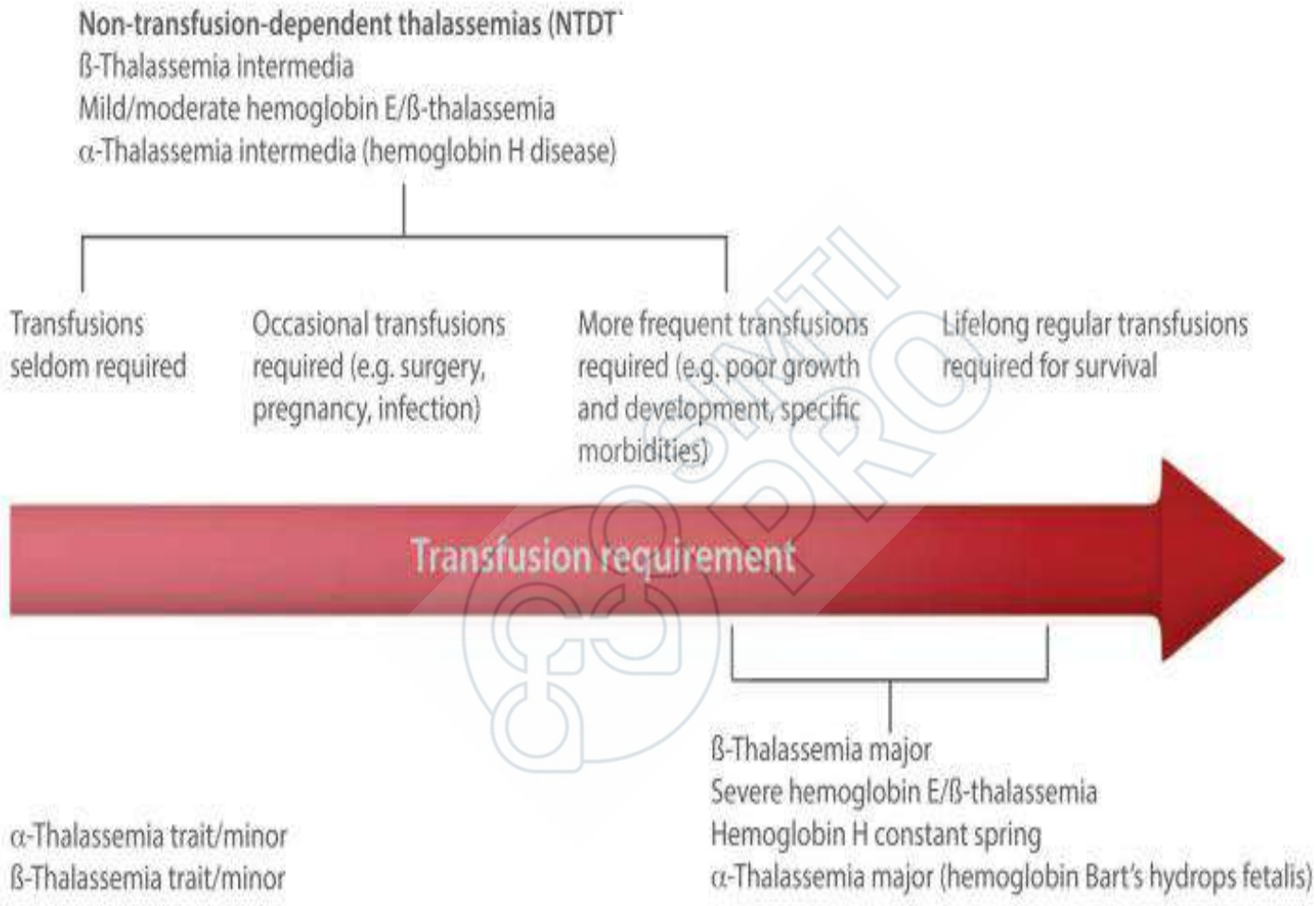
DIAGNOSTICA MOLECOLARE PRENATALE

È la scelta prevalente delle coppie a rischio (fatta soprattutto nel 1° trimestre, ma anche in 2° trimestre con ricerca delle mutazioni parentali su DNA estratto da villi coriali o da amniociti)

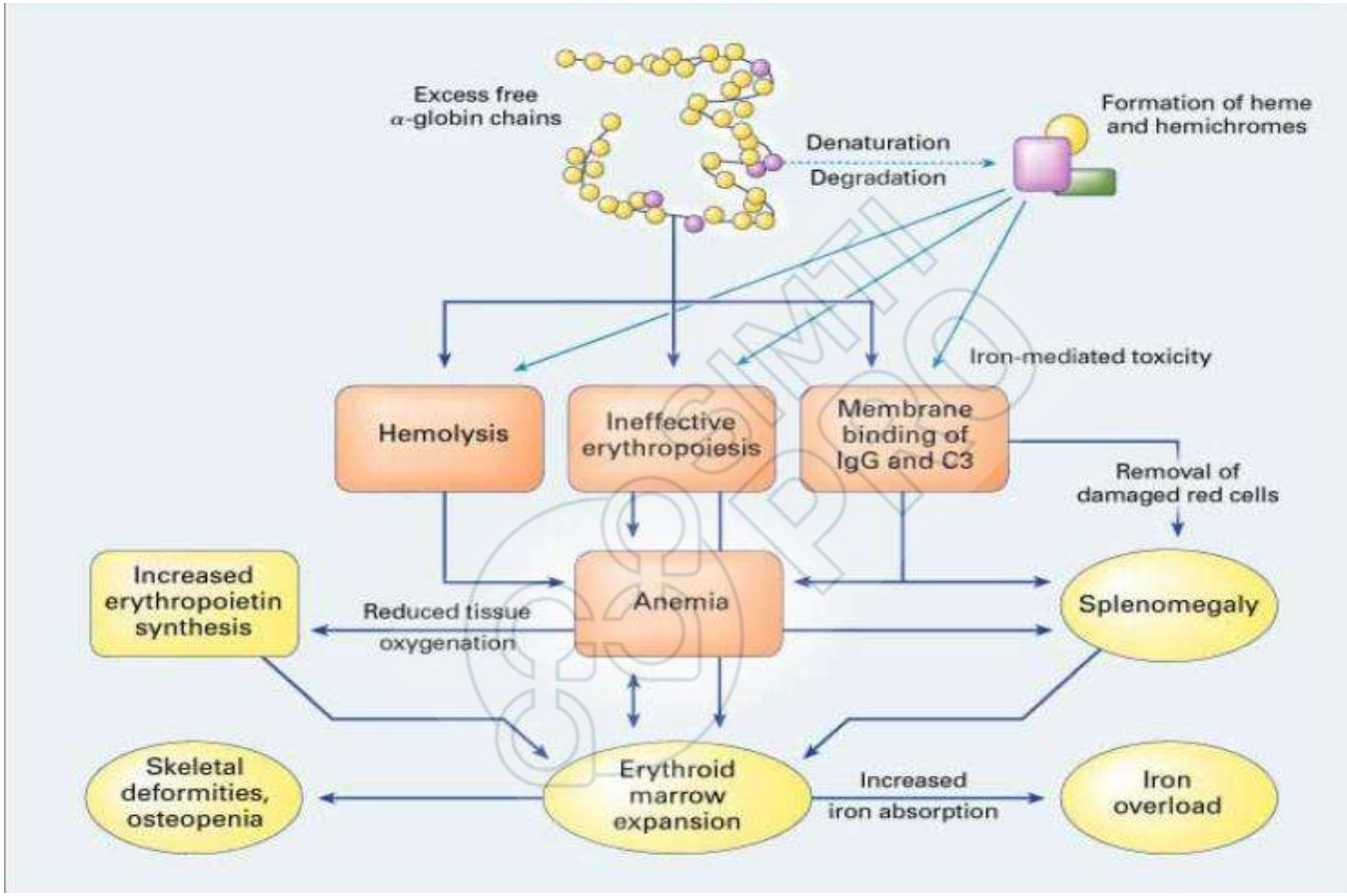
Previa somministrazione di specifico consenso informato con dettagliati i rischi di occorrenza della patologia, il quadro clinico atteso in base ai difetti molecolari e i rischi di abortività connessi alla metodiche proposte

Alle coppie con precedente figlio affetto offerta la possibilità di caratterizzazione HLA fetale in caso di feto sano o portatore

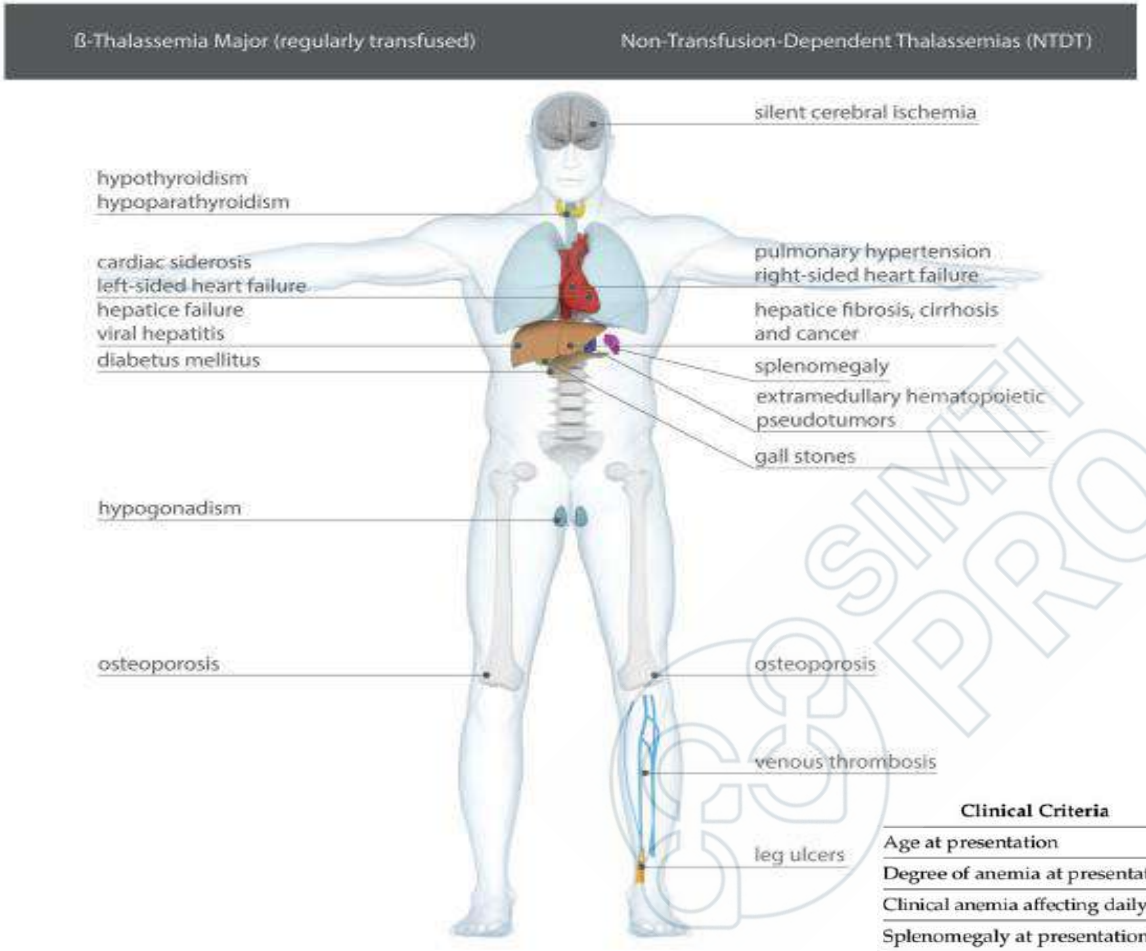
Transfusion requirement is commonly used to distinguish phenotypes



Musallam Km et al .Haematologica. 2013 Jun;98(6):833-44



Olivieri et al. N Engl J Med. 1999 Jul 8;341(2):99-109

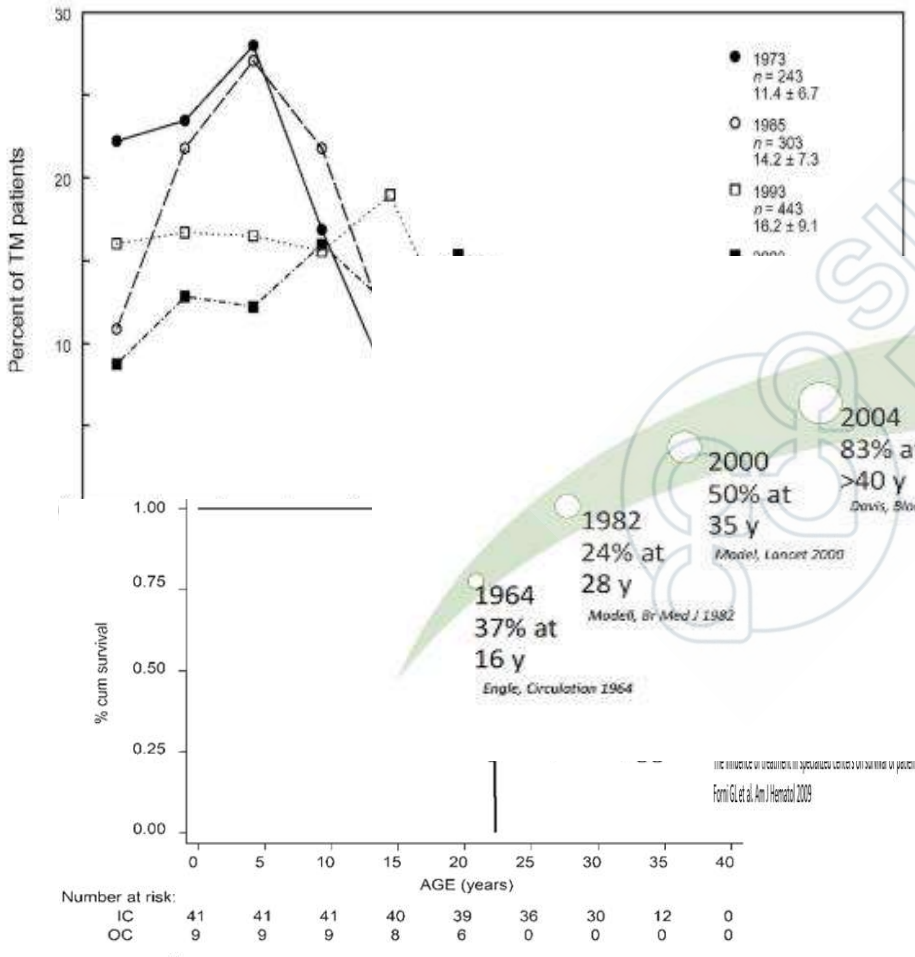


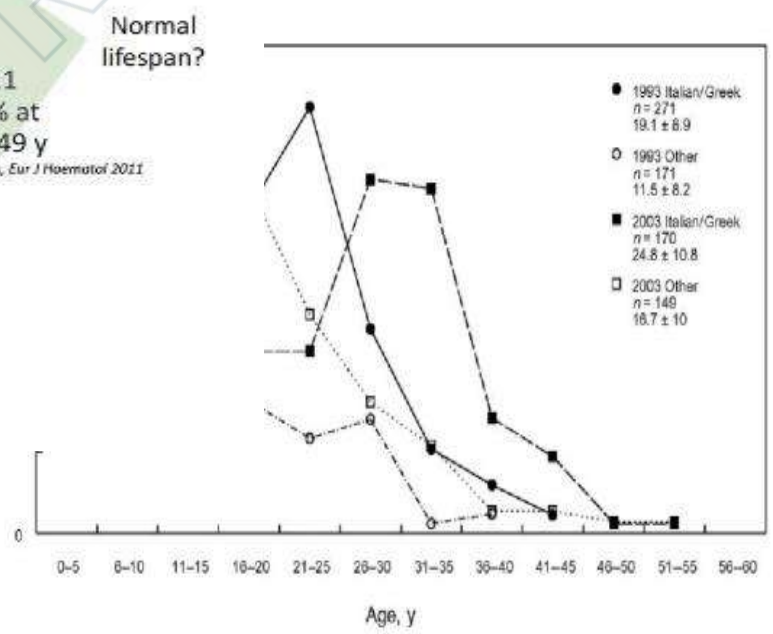
Clinical Criteria	TDT More Likely	NTDT More Likely
Age at presentation	≤2 years	>2 years
Degree of anemia at presentation	Severe	Mild to severe
Clinical anemia affecting daily living	Yes	No
Splenomegaly at presentation	Severe	Mild to severe
Jaundice	No	Mild
Skeletal deformities	Yes	Negative to mild
Growth retardation	Moderate to Severe	Negative to moderate
Transfusion requirements	Lifelong, dependence for survival	None, occasional, or frequent but temporary
Hb levels	6–7 gm/dL	8–10 gm/dL
Nucleated RBCs	Numerous	Negative to few
Reticulocytes	>10%	<10%

Musallam Km et al .Haematologica. 2013 Jun;98(6):833-44

1960 → 1980 → 1984 → 1999 → 1999 → 2006

REGULAR TRANSFUSION | **DFO THERAPY** | **BMT** | **RMN T2*** | **DFP** | **DFX**

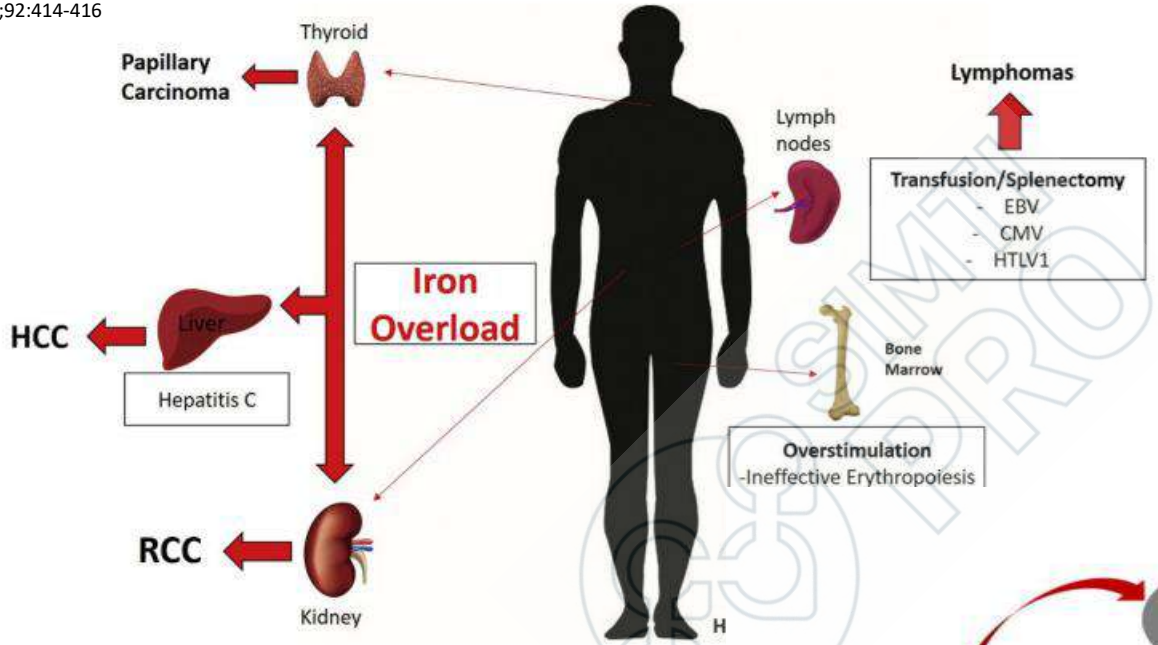




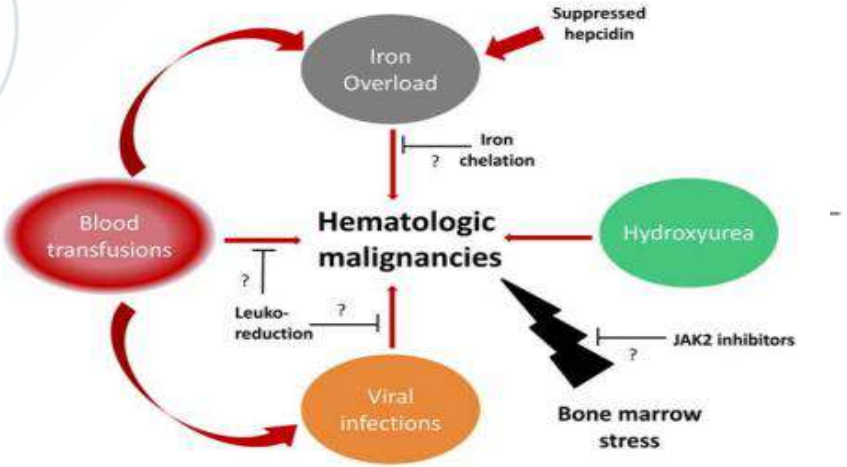
Vichinsky EP et al. Pediatrics Vol 116 N6 Dec 2005

….Causes of morbidity and mortality….

Halawi R et al. Am J Hematol 2017;92:414-416



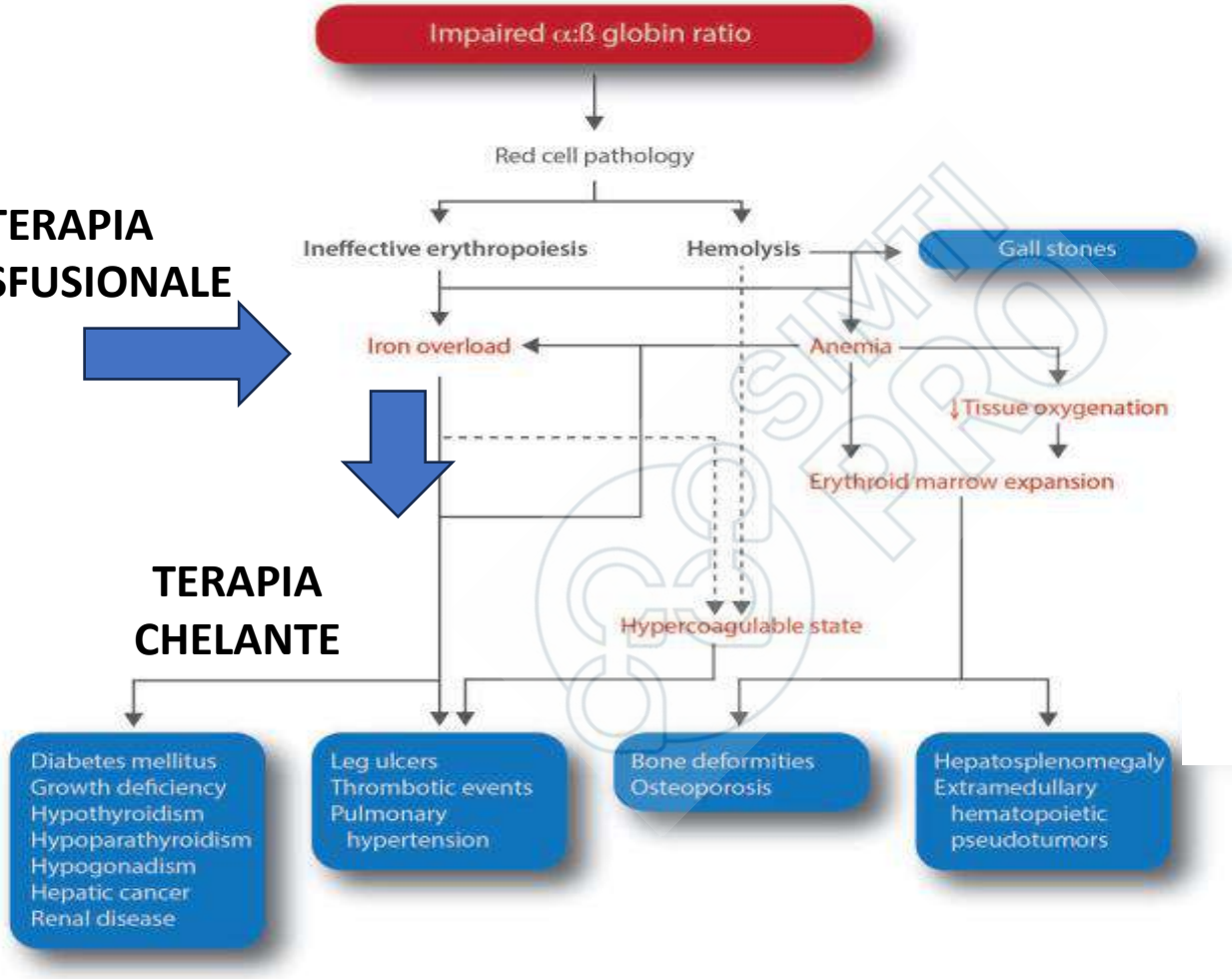
All patients (N=1073)		*Patients born after 1970 (N=720)*	
N	*%*	*N*	*%*
133	60.2	31	50.8
15	6.8	9	14.8
15	6.8	4	6.6
4	1.8		
9	4.1		
9	4.1	2	3.3
8	3.6	2	3.3
7	3.2	2	3.3
4	1.8	1	1.6
3	1.4		



TERAPIA STANDARD

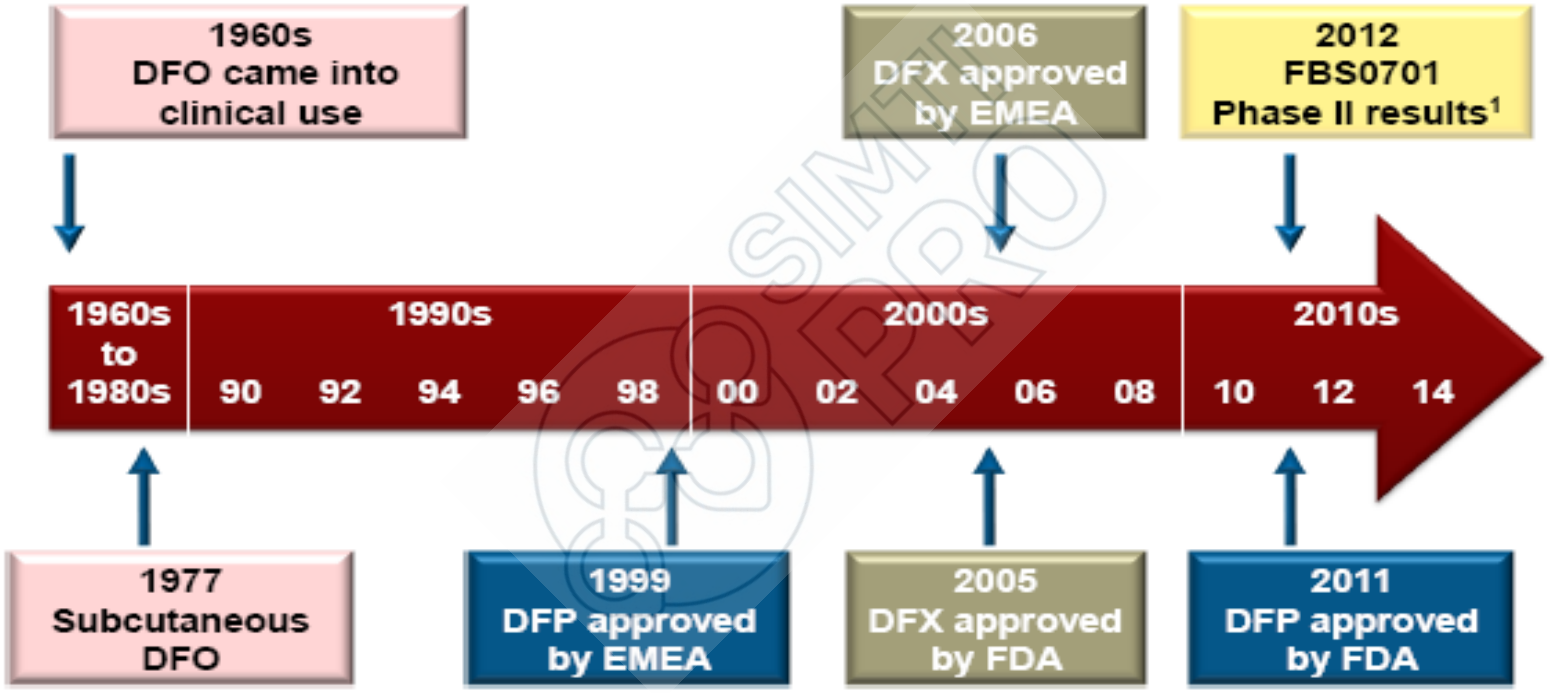
TERAPIA TRASFUSIONALE

TERAPIA CHELANTE



Musallam KM, et al. Haematologica. 2013;98:833-4

Evolution of iron chelators

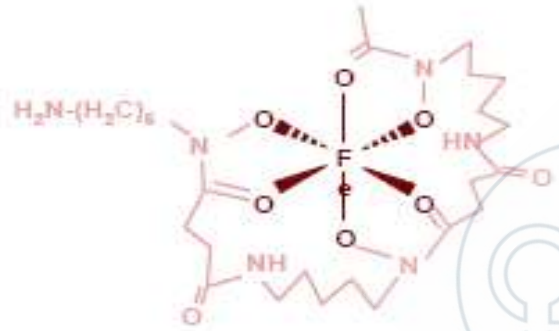


DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; EMEA, European Medicines Agency; FDA, US Food and Drug Administration

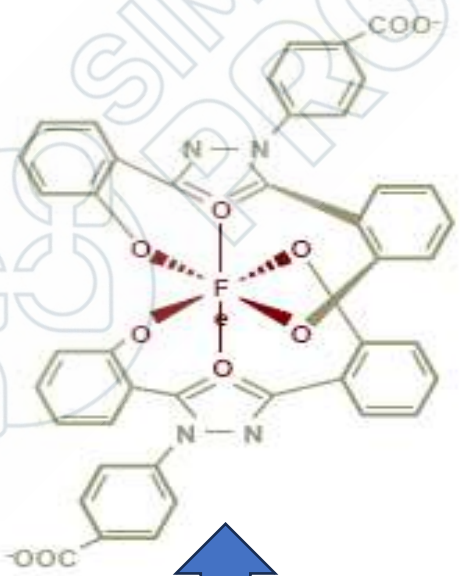
1. Neufeld EJ *et al. Blood* 2012;119:3263–3268. Multicenter, open-label, randomized, Phase II study; n=73.

Chelators and coordination with iron

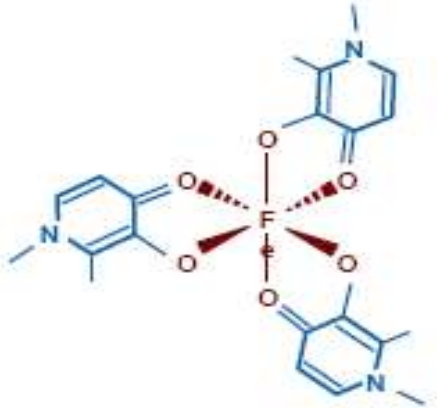
DFO
Hexadentate (1:1)
High MW



DFX
Tridentate (2:1)
Low MW



DFP
Bidentate (3:1)
Low MW



MW, molecular weight

Nick H *et al. Curr Med Chem* 2003;102:1065–1076. Review.

Active ingredient	DFO	DFP	DFX[a]	DFX[a]
Administration	Parenteral	Oral tablets or solution	Oral, tablets dispersible in liquid	Oral, film-coated tablets
Usual dose and frequency	30–50 mg/kg/day over 8 h of infusion for 5 or 7 days/week	75–100 mg/kg/day in three times divided doses daily	10–40 mg/kg/day once daily	7–28 mg/kg/day once daily
Excretion	Urinary, fecal	Mainly urinary	Fecal	Fecal
Adverse effects	Reactions at site of infusion Bone anomalies Auditory ophthalmologic	Gastrointestinal Arthralgia Increase in liver enzymes neutropenia/ agranulocytosis	Gastrointestinal disturbances Rash Renal insufficiency Increase in liver enzymes	Rash Increase in liver enzymes Renal insufficiency
Advantages/ disadvantages	Long-standing experience/ parenteral	Robust evidence on improving cardiac siderosis/weekly monitoring of CBC	Once-daily dosing, oral / high cost	Once-daily dosing, oral / high cost
Licensed use	Treatment of chronic iron overload resulting from transfusion-dependent anemia	Treatment of iron overload in thalassemia major where DFO is contraindicated or inadequate	US: treatment of transfusional iron overload in patients 2 years or older. Europe: treatment of transfusional iron overload 6 years and older, and when DFO is contraindicated or inadequate, in patients 2–5 years	US: treatment of transfusional iron overload in patients 2 years or older. Europe: treatment of transfusional iron overload 6 years and older, and when DFO is contraindicated

DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox.
[a]Same active ingredient but with different peak serum concentration and bioavailability; CBC, complete blood count; TM, thalassemia major.

DFX compresse rivestite con film

- **EFFETTO CIBO RIDOTTO** (non obbligo di assumere farmaco a stomaco vuoto o 30 minuti prima del pasto, maggiore flessibilità e comodità)
- **MIGLIOR PALATABLITA'**: (possono essere deglutite intere, o frantumate e mescolate a yougurth o purea di mele ; non più necessaria dispersione in acqua che conferiva gusto riferito calcareo e consistenza limacciosa che comportava problemi gastrici)
- **MIGLIORE PROFILO DI TOLLERABILITA' GI**: esclusione di lattosio e sodio lauril solfato che hanno consentito di ridurre effetti GI
- **INFERIORE VARIABILITA' INTER-SOGGETTO IN TERMINI DI ESPOSIZIONE**: con la nuova formulazione si puo' raggiungere una relazione dose-esposizione più prevedibile nella pratica clinica sia per la migliorata compliance sia per il sicuro assorbimento della dose totale giornaliera prescritta
- **MAGGIORE BIODISPONIBILITA'** (> 36%)→ DOSE RIDOTTA DEL 30%

Pediatr. Blood Cancer 2011;56:420–424

Improved Efficacy and Tolerability of Oral Deferasirox by Twice-Daily Dosing for Patients With Transfusion-Dependent β-Thalassemia

Hsiu-Hao Chang, MD,[1,2] Meng-Yao Lu, MD,[1] Yu-Mei Liao, MD,[3] Pei-Chin Lin, MD,[3] Yung-Li Yang, MD,[1,2,4] Dong-Tsamn Lin, MD,[1,4] Shyh-Shin Chiou, MD,[3,5] Shiann-Tarng Jou, MD, PhD,[1] Kai-Hsin Lin, MD,[1] and Tai-Tsung Chang, MD,[3,5]*

Background. Deferasirox is an oral iron-chelating agent taken once-daily by patients with transfusion-dependent iron overload. However, some patients are unresponsive or unable to tolerate once-daily deferasirox. The current study evaluated whether twice-daily deferasirox treatment showed increased efficacy or tolerability in unresponsive or intolerant patients. ***Procedure.*** Patients from two Taiwanese hospitals with transfusion-dependent β-thalassemia, including those who showed increasing serum ferritin levels for six consecutive months, with at least one level >2,500 ng/dl, while treated with >30 mg/kg/day of once-daily deferasirox (unresponsive) or developed deferasirox-related adverse events (AEs) at the dosage required to maintain the iron burden balance (intolerant) and were treated twice-daily with the same total daily dose of deferasirox since 2008, were enrolled in the study and evaluated retrospectively by medical record review. ***Results.*** Eighteen patients were included for analysis. A statistically significant median decrease in serum ferritin levels was detected in the 11 unresponsive patients after 6 months of continuous twice-daily deferasirox treatment. Five out of the seven intolerant patients experienced either no deferasirox-related AEs or less severe AEs. The 12 patients from both groups (11 unresponsive, 1 intolerant) who received continuous twice-daily deferasirox for 6 months showed a mild but significant median increase in serum creatinine levels. ***Conclusions.*** Twice-daily deferasirox dosing is effective in iron chelation and improves tolerability in transfusion-dependent β-thalassemia patients who are unresponsive to or intolerant of once-daily deferasirox. Future studies with greater patient numbers will be required to confirm the results reported herein. Pediatr Blood Cancer 2011;56:420–424 © 2010 Wiley-Liss, Inc.

reported [6]. Dividing the once-daily deferasirox dose into two smaller daily doses should have pharmacokinetic advantages because peak concentrations will be lower and may be associated with decreased intolerance, and trough concentrations should be higher and increase the period during which drug concentrations are at efficacious levels. These pharmacokinetic benefits were dis-

INDICAZIONI TERAPEUTICHE DEFERASIROX

- Trattamento del sovraccarico cronico di ferro dovuto a frequenti emotrasfusioni (> 7 ml/Kg/mese di GR) in pz affetti da TM di età >6 aa
- Trattamento del sovraccarico cronico di ferro dovuto a emotrasfusioni quando la terapia con DFO è controindicata o inadeguata nei seguenti pz:

a) pz pediatrici con TM con sovraccarico di ferro dovuto a frequenti emotrasfusioni (> 7 ml/Kg/mese di GR) di età compresa tra 2 e 5 aa

b) in pz adulti e pediatrici di età> 2 aa con TM con sovraccarico cronico di ferro dovuto a emotrasfusioni non frequenti (< 7 ml/Kg/mese di GR)

c) In pz adulti e pediatrici di età > 2 aa con altre anemie

- Trattamento del sovraccarico cronico di ferro quando la terapia con DFO è controindicata o inadeguata in paziente di età> 10 aa affetti da sindromi talassemiche non trasfusione dipendenti (NTDT)

DOSI RACCOMANDATE PER LE TDT

	Compresse rivestite con film/granulato	Compresse dispersibili	Trasfusioni	Ferritina sierica
Dose iniziale	14 mg/kg/die	20 mg/kg/die	Dopo 20 unità (circa 100 ml/kg) di GRC	oppure > 1,000 µg/l
Dosi iniziali alternative	21 mg/kg/die	30 mg/kg/die	>14 ml/kg/ mese di GRC (circa >4 unità/mese per un adulto)	
	7 mg/kg/die	10 mg/kg/die	<7 ml/kg/ mese di GRC (circa <2 unità/mese per un adulto)	
Per i pazienti adeguatamente trattati con deferoxamina	Un terzo della dose di deferoxamina	Metà della dose di deferoxamina		
Monitoraggio				**Mensile**
Intervallo di riferimento				**500-1,000 µg/l**
Intervalli di aggiustamento della dose (ogni 3-6 mesi)	**Aumento** 3.5-7 mg/kg/die Fino a 28 mg/kg/die	5-10 mg/kg/die Fino a 40 mg/kg/die		>2,500 µg/l
	Riduzione 3.5-7 mg/kg/die Nei pazienti trattati con dosi > 21mg/kg/die	5-10 mg/kg/die Nei pazienti trattati con dosi >30 mg/kg/die		< 2,500 µg/l 500-1,000 µg/l
	- Quando il valore di riferimento è raggiunto			
Dose massima	**28 mg/kg/die**	**40mg/kg/die**		
Considerare l'interruzione				**<500 µg/l**

TERAPIE ALTERNATE O SEQUENZIALI

1) **DFO e DFX** : un report retrospettivo con tp sequenziale del 2010→ DFX 20-30 mg/Kg per 4 giorni+ DFO 20-40 mg/Kg per 3 giorni (24 mesi)

→ Riduzione ferritina (nessun dato di LIC e T2* cuore)

→ No AE

2) **DFP E DFX SEQUENZIALE** : DFP 75 mg/kg/die per 4 giorni e DFX 30 mg/Kg/die per 3 giorni → riduzione ferritina

3) **DFP E DFX ALTERNATA:** DFP 75 mg/kg/die- DFX 25 mg/kg/die→ riduzione ferritina e LIC

- **BETTER ACCESSIBILITY TO VARIOUS IRON POOLS**
- **BETTER CONTROL ON NTBI**
- **BETTER TOLERABILITY**
- **IMPROVED COMPLIANCE**
- **REDUCED MYOCARDIAL IRON AND IMPROVED CARDIAC DYSFUNCTION IN IRON OVERLOAD CARDIOMIOPATHY**

COMBINED THERAPY WITH MORE THAN ONE CHELATOR

DFO-DFX

-REDUCED SERUM FERRITIN
-DECREASED LIC
-BENEFICAL EFFECTS ON LIVER AND HEART HEMOSIDEROSIS

DFO-DFP

-REDUCED SERUMFERRITN, LIVER IRON AND MYOCARDIAL SIDEROSIS
--ENHANCED CARDIAC FUNCTION
--REVERSAL ENDOCRINE DISORDER
--REDUCED CARDIAC MORTALITY

DFX-DFP

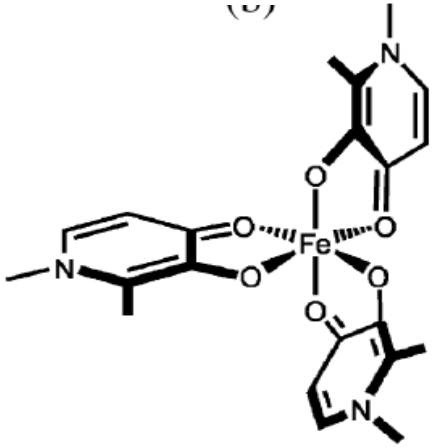
-BEST EFFICIENCY IN REDUCING CARDIAC IRON OVERLOAD
-REDUCED LIC

Dosi raccomandate per NTDT

	Compresse rivestite con film/granulato	Compresse dispersibili	Concentrazione del ferro epatico (LIC)*		Ferritina sierica
Dose iniziale	**7 mg/kg/die**	**10 mg/kg/die**	≥5 mg Fe/g peso secco	o	>800 µg/l
Monitoraggio					**Mensile**
Intervalli di aggiustamento della dose (ogni 3-6 mesi)	**Aumento**		≥7 mg Fe/g peso secco	o	>2.000 µg/l
	3,5 - 7 mg/kg/die	5-10 mg/kg/die			
	Riduzione		<7 mg Fe/g peso secco	o	≤2.000 µg/l
	3,5 - 7 mg/kg/die	5-10 mg/kg/die			
Dose massima	**14 mg/kg/die**	**20 mg/kg/die**			
	7 mg/kg/die	**10 mg/kg/die**			
	Per adulti Per pazienti pediatrici		non valutata	e	≤2.000 µg/l
Interruzione			**<3 mg Fe/g/peso secco**	o	**<300 µg/l**
Ritrattamento			**Non raccomandato**		

DEFERIPRONE

Ferrochelante bidentato
(3 molecole di farmaco legano un atomo di Fe)
PM: 139
75 -100 mg/kg/die (tre somministrazioni)



Deferiprone

Compresse 500 -1000 mg
Sciroppo 100 mg/ml

Cibo riduce velocità ma non capacità di assorbimento

Ferro chelato escreto quasi esclusivamente per via urinaria

Emivita plasmatica media 3 - 4 hr

Picco plasmatico 45'-60'

1999 è stato approvato con l'indicazione " in casi **eccezionali**, per pazienti affetti da thalassemia major per i quali la terapia con DFO sia controindicata o che presentino grave tossicità con detta terapia".

L'EMEA nel **2004** ha allargato l'indicazione "il deferiprone è indicato per il trattamento del sovraccarico di ferro nei pazienti affetti da talassemia e per i quali la terapia a base di deferoxamina è controindicata o inadeguata".

DEFERIPRONE E CUORE

- Borgna-Pignatti 2006
- Galanello 2006
- Pennell 2006
- Smith 2011
- Maggio 2012
- Filosa 2013
- Kuo 2014

DFP TREATMENT IS ASSOCIATED WITH SIGNIFICANT INCREASE IN CARDIAC PROTECTION COMPARED WITH DFO

TERAPIA ALTERNATA O SEQUENZIALE

- DFP - DFX

- **DFP - DFO**

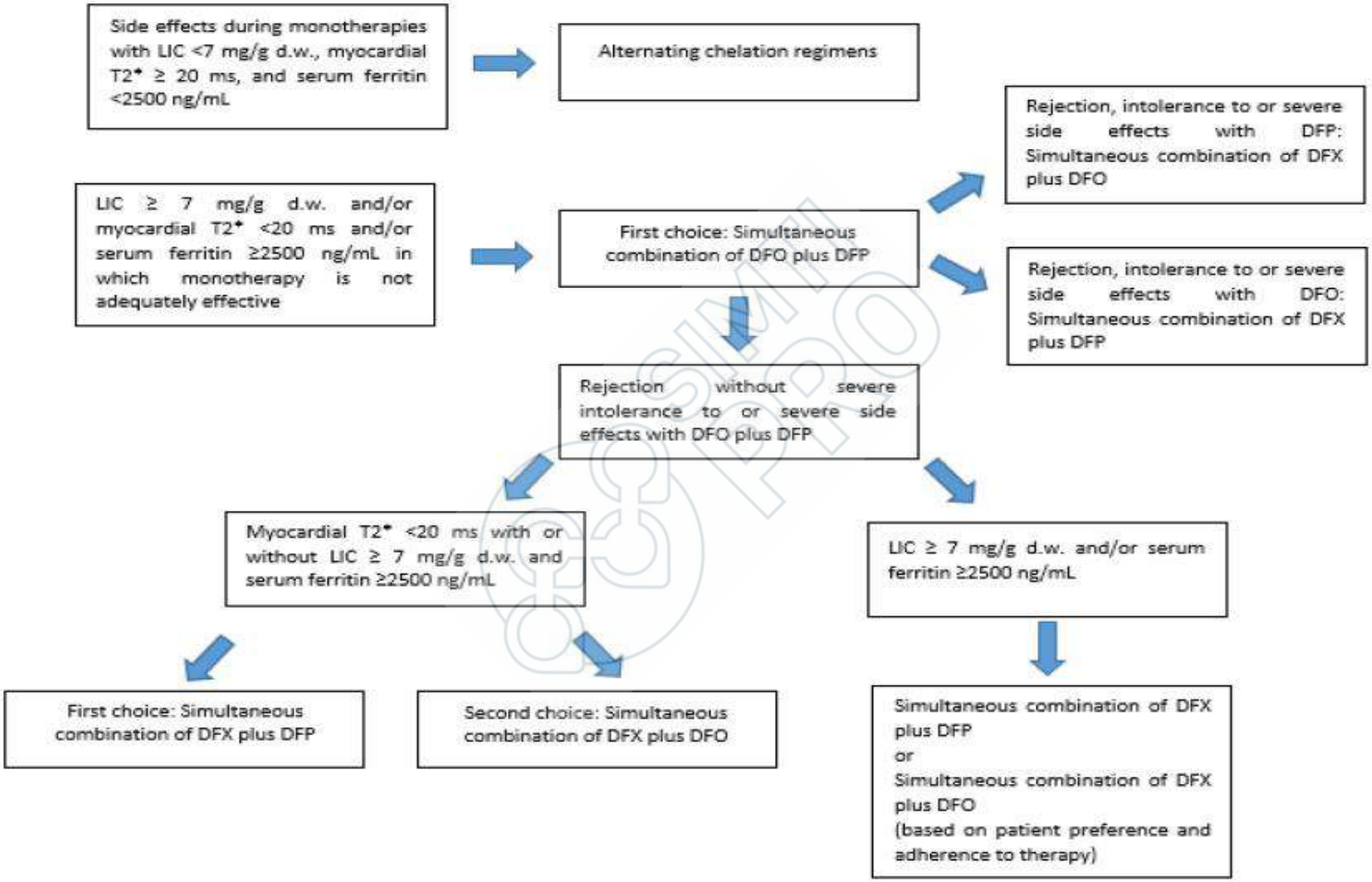
TERAPIA ALTERNATA→ RIDUZIONE SIGNIFICATIVA FERRITINA E LIC EPATICA → NESSUNA DIFFERENZA AE e SOPRAVVIVENZA

TERAPIA COMBINATA

- DFP+DFX
- **DFO + DFP**

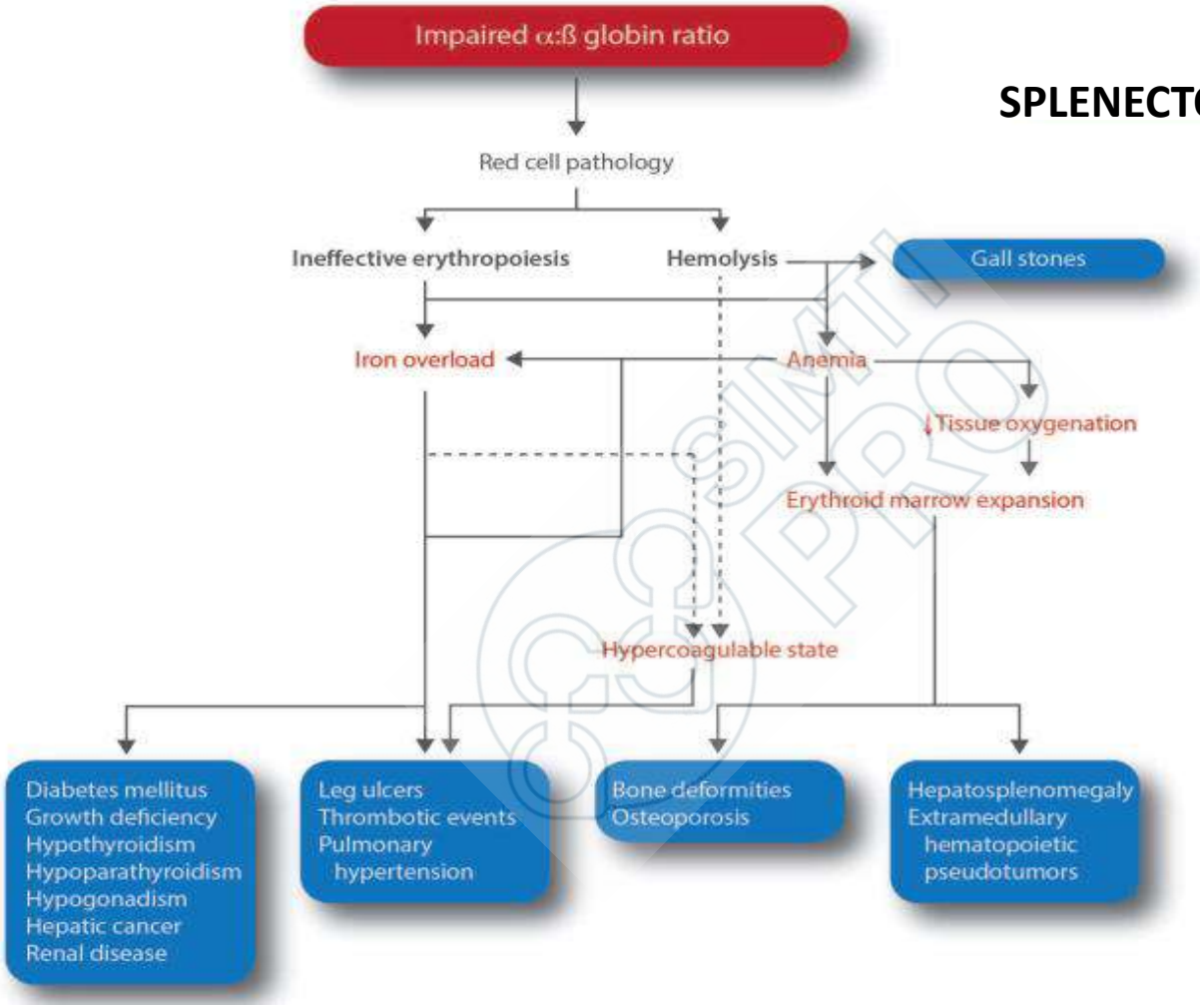
- Un aumento additivo o sinergico nell'escrezione del ferro comparata ad ognuno degli altri chelanti dato da solo.
- Il Deferiprone può, entrando nelle cellule, rapidamente rimuovere dagli organi il ferro che viene rapidamente escreto con l'aiuto della desferroxamina
- E' una terapia di EMERGENZA e TEMPORANEA per trattare un GRAVE sovraccarico di ferro soprattutto a livello cardiaco

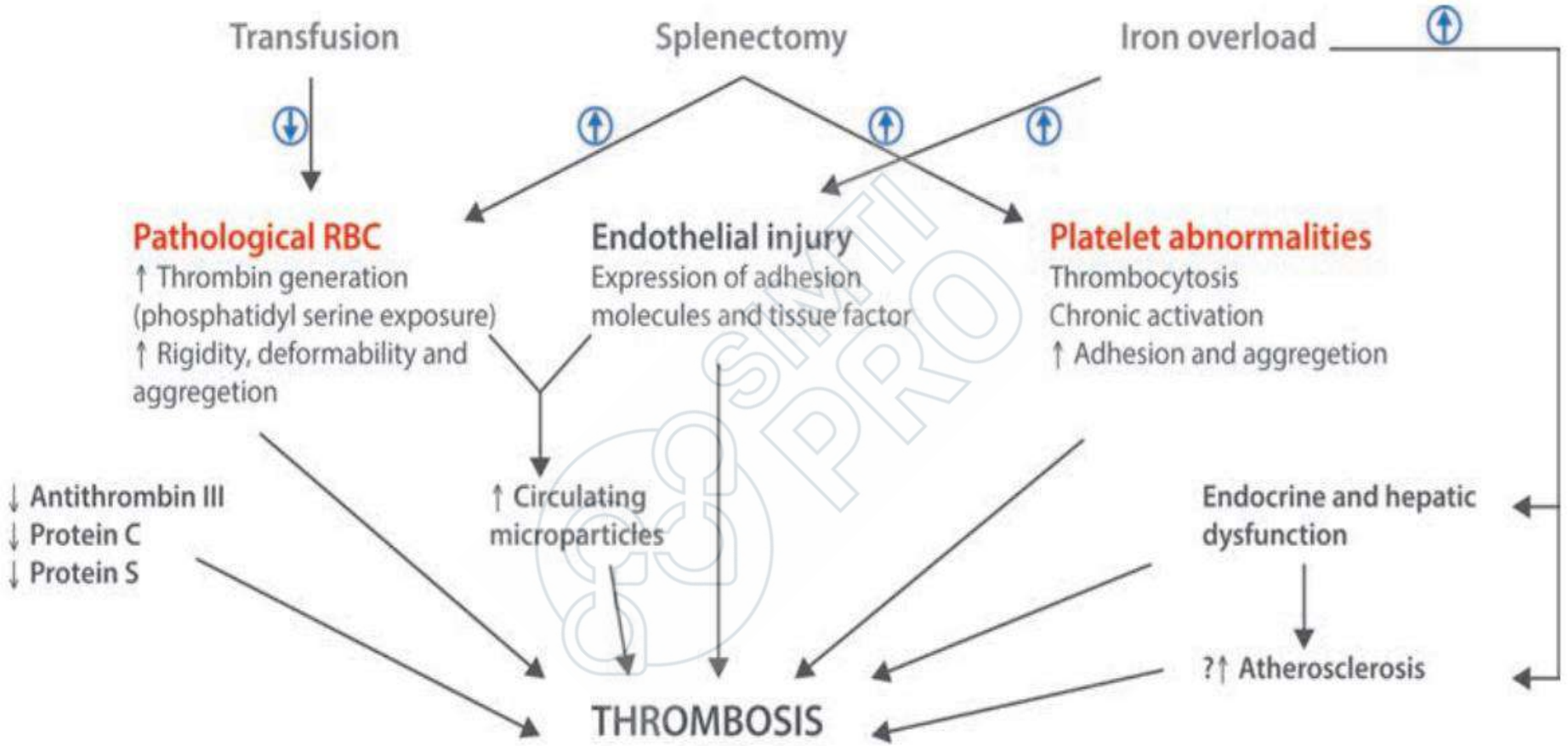
Alternative possibili



Safety and efficacy of the new combination iron chelation regimens in patients with transfusion-dependent thalassemia and severe iron overload

SPLENECTOMIA





Musallam KM et al. Haematologica 2013;98(6)

OPTIMAL CARE STUDY

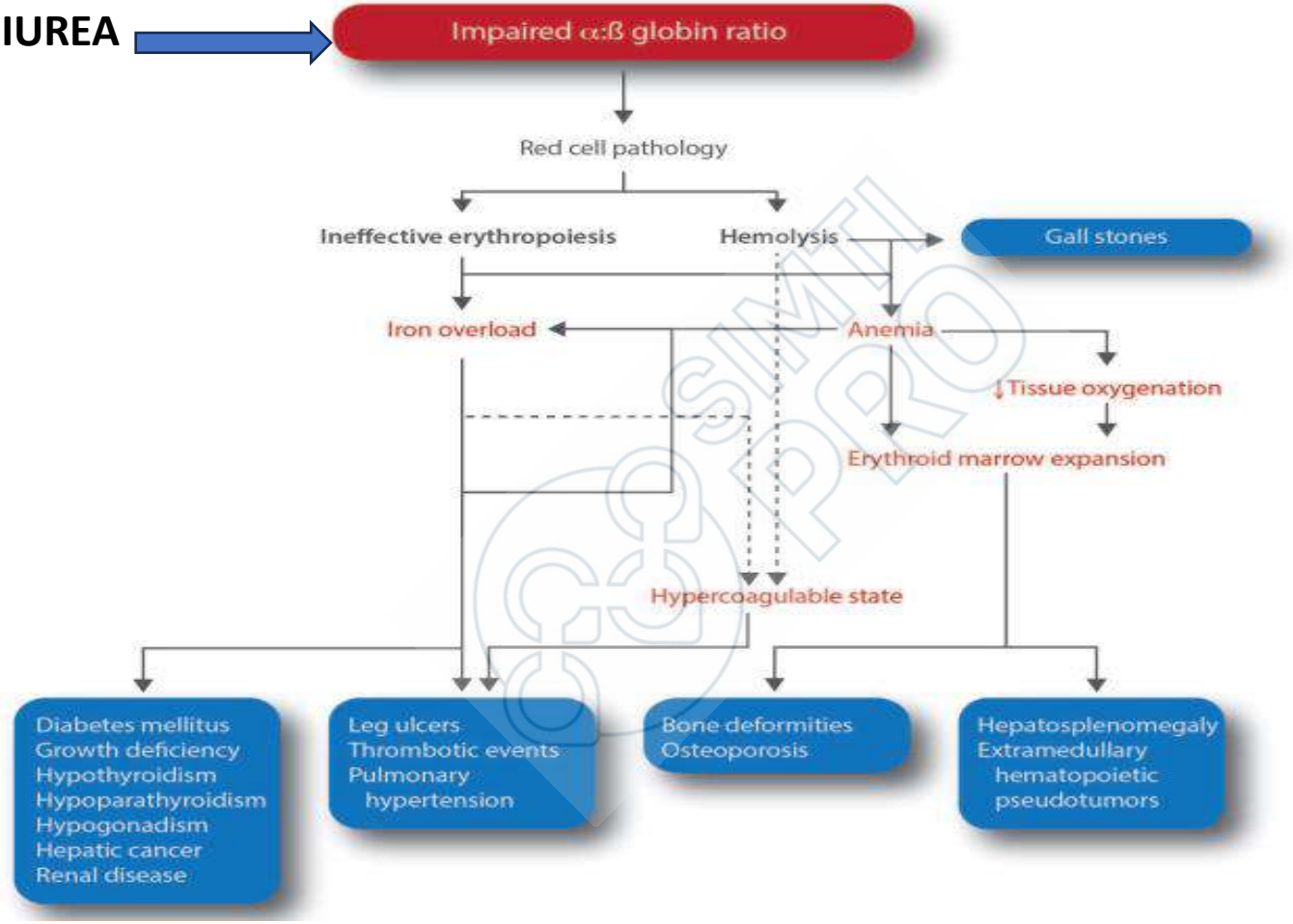
Parameter	Group	OR	95% CI	*P*-value
NRBC count ≥ 300 × 10^6 L^{-1}	Group III	1.00	Referent	< 0.001
	Group II	5.35	2.31–12.35	
Platelet c				< 0.001
	Group I	76.92	22.22–250.00	
PHT	Group III	1.00	Referent	0.020
	Group II	4.00	0.99–16.13	
	Group I	7.30	1.60–33.33	
Transfusion naivety	Group III	1.00	Referent	0.001
	Group II	1.67	0.82–3.38	
	Group I	3.64	1.82–7.30	

NRBC, nucleated red blood cell; PHT, pulmonary hypertension; OR, adjusted odds ratio; CI, confidence interval.

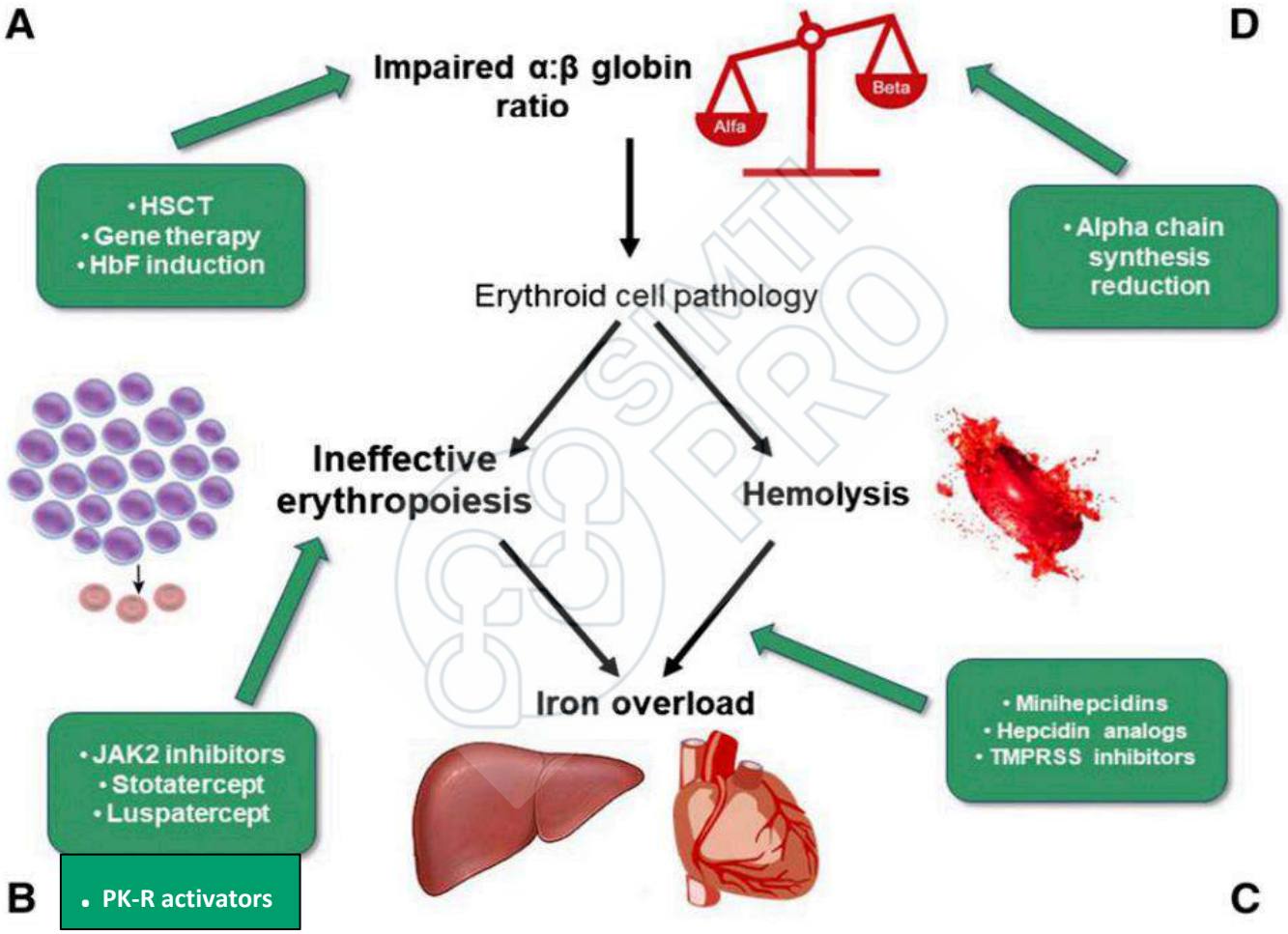
GROUP 1 PATIENTS HAD SIGNIFICANTLY HIGHER NRBC, PLT, PTH, AND WERE MOSTLY NON TRANSFUSED

Taher At et al. J Thromb and haemost 2010;8:2152-2158

IDROSSIUREA



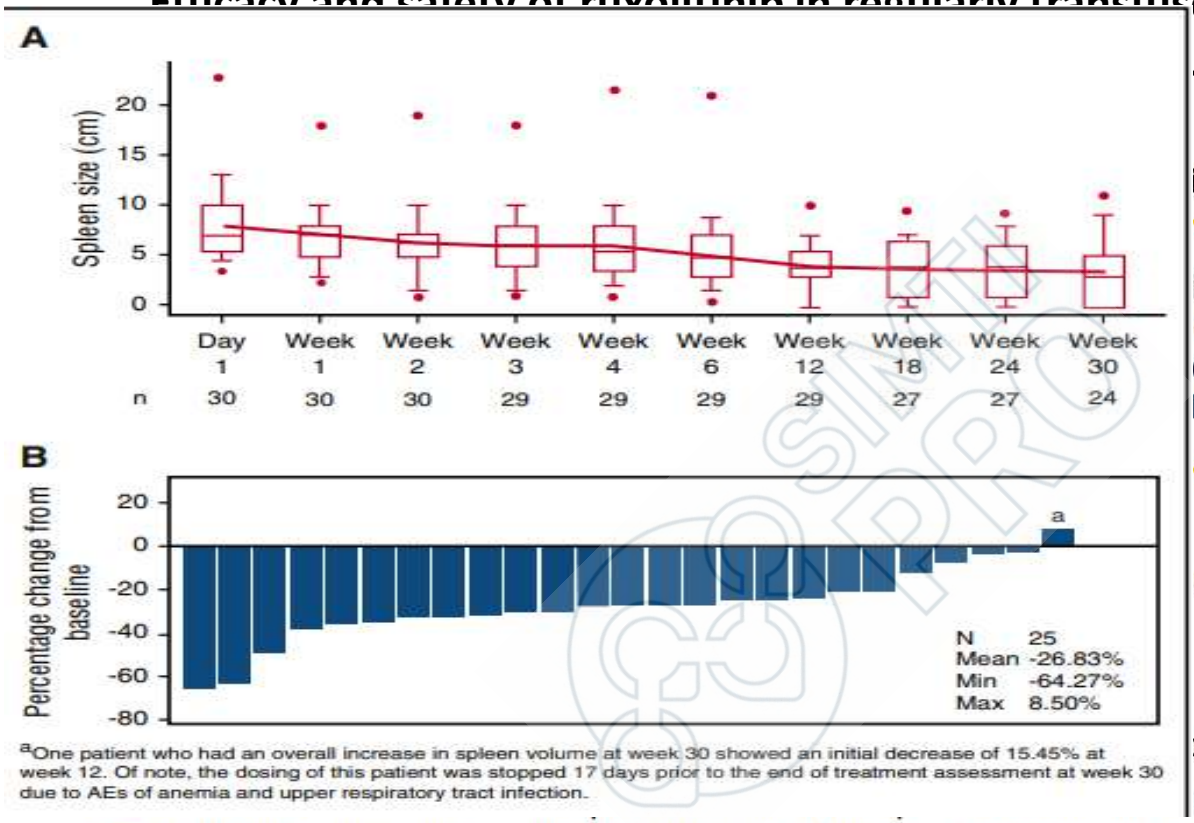
INNOVATIVE THERAPIES



New therapeutic targets in b-thalassemias: (A,D) impaired a:b-globin ratio, (B) ineffective erythropoiesis, and (C) iron metabolism and hemolysis. TMPRSS6, transmembrane protease serine 6

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

Efficacy and safety of ruxolitinib in regularly transfused patients with



[a]One patient who had an overall increase in spleen volume at week 30 showed an initial decrease of 15.45% at week 12. Of note, the dosing of this patient was stopped 17 days prior to the end of treatment assessment at week 30 due to AEs of anemia and upper respiratory tract infection.

udy

imane

tinib
mg x 2 volte/dì
se: da 5 a 25 mg
te/dì

6 e 30 rispetto al

on RMN o TC

ogni visita post

baseline

- Farmacocinetica (PK)
- Safetv

.Mean % change of transfusion rate: −5.9 (95% CI: −14.7, 2.83).
. Median pre-transfusion Hb levels: • pre-Tx = 8.4 g/dl; • end of study = 8.9 g/dl (week 24-30)
.Mean Spleen Volume reduction from BL : • at week 12 (N = 26): −19.7% • at week 30 (N = 25): −26.8%

Taher AT. Blood 2018

• In addition to a noticeable reduction in spleen volume over time with ruxolitinib treatment, a trend for improvement in transfused red cells and a slight improvement in pretransfusion hemoglobin was noted with ruxolitinib treatment.

• A majority of patients continued with the treatment beyond the core study.

• Ruxolitinib was **well tolerated** in the study population with modest incidences of grade 3 or 4 and serious AEs, with no new safety findings.

• Given the sustained decrease in spleen volume, ruxolitinib treatment may serve **as an alternative option in patients with TDT who are potential candidates for splenectomy.**

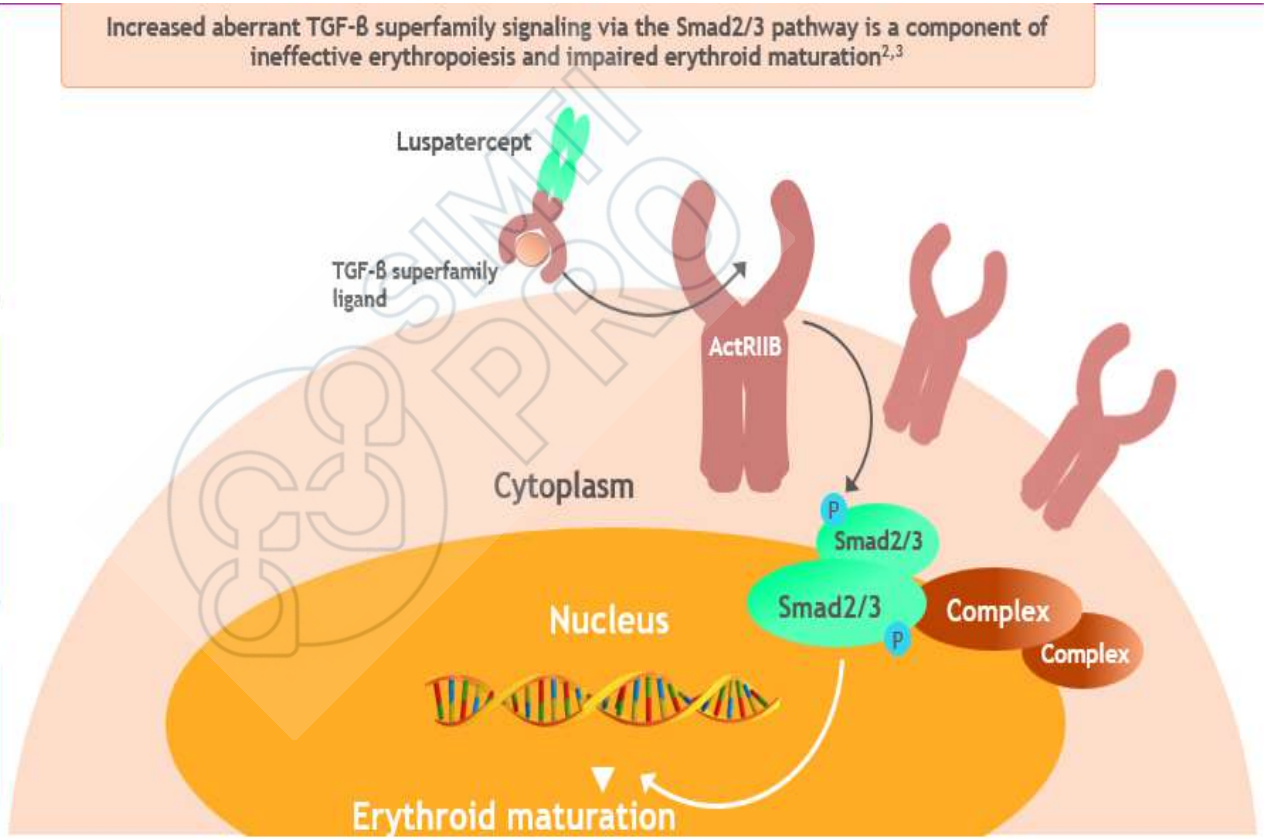
LUSPATERCEPT

Luspatercept is a first-in-classe erythroid maturation agent: a recombinant fusion protein consisting of a modified form of the extracellular domain of the human ActRIIB linked to the human IgG1 Fc domain[1]

Luspatercept binds to select TGF-β superfamily ligands[2-4]...

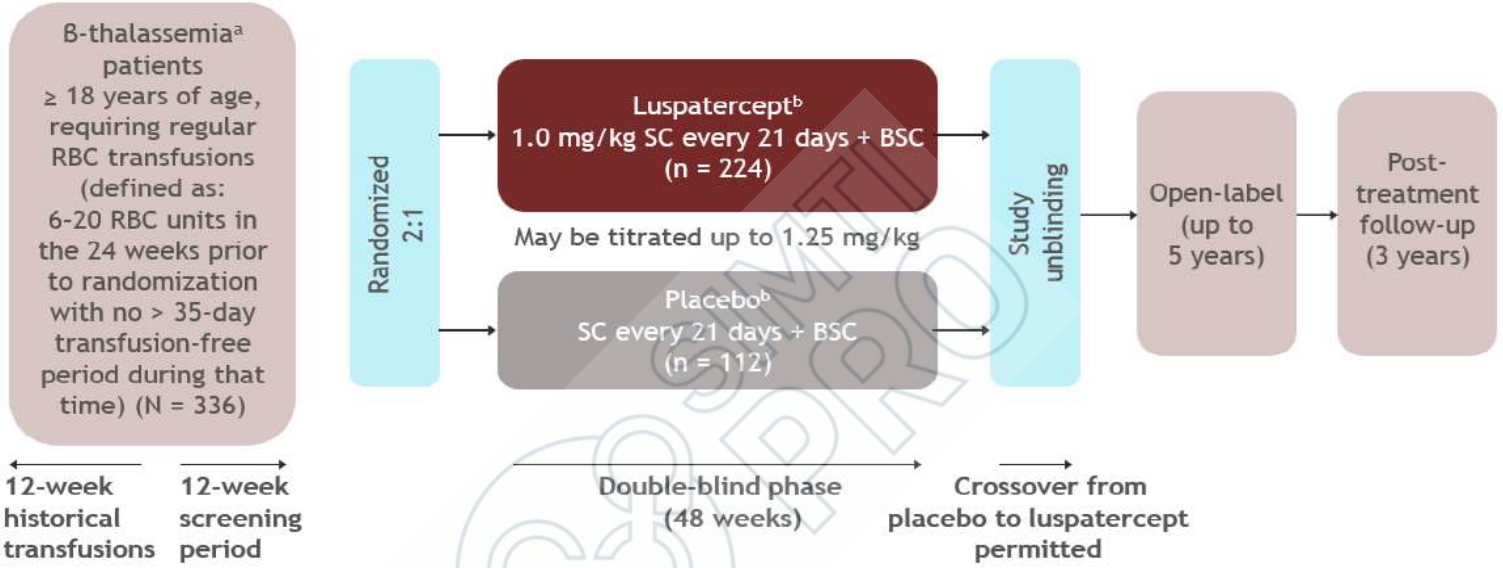
... inhibiting ActRIIB activation and decreasing Smad2/3 signaling[2]

Decreasing Smad2/3 signaling enhances erythroid maturation in late-stage erythropoiesis[3]



ActRIIB, activin receptor type IIB; IgG1 Fc, immunoglobulin G1 fragment crystallizable; P, phosphorylation; Smad, small mothers against decapentaplegic; TGF-β, transforming growth factor β.
1. Attie KM et al. *Am J Hematol* 2014;89:766-770. 2. Suragani RN et al. *Nat Med* 2014;20:408-414. 3. Suragani RN et al. *Blood* 2014;123:3864-3872. 4. Cappellini MD et al. *Blood* 2018;132:163.
Included with permissions Blank U et al, 2015, *Blood*.

BELIEVE TRIAL: a randomized, double-blind, placebo-controlled, phase 3 study



Primary endpoint

- ≥ 33% reduction in RBC transfusion burden (plus reduction of ≥ 2 RBC units) from baseline during weeks 13-24

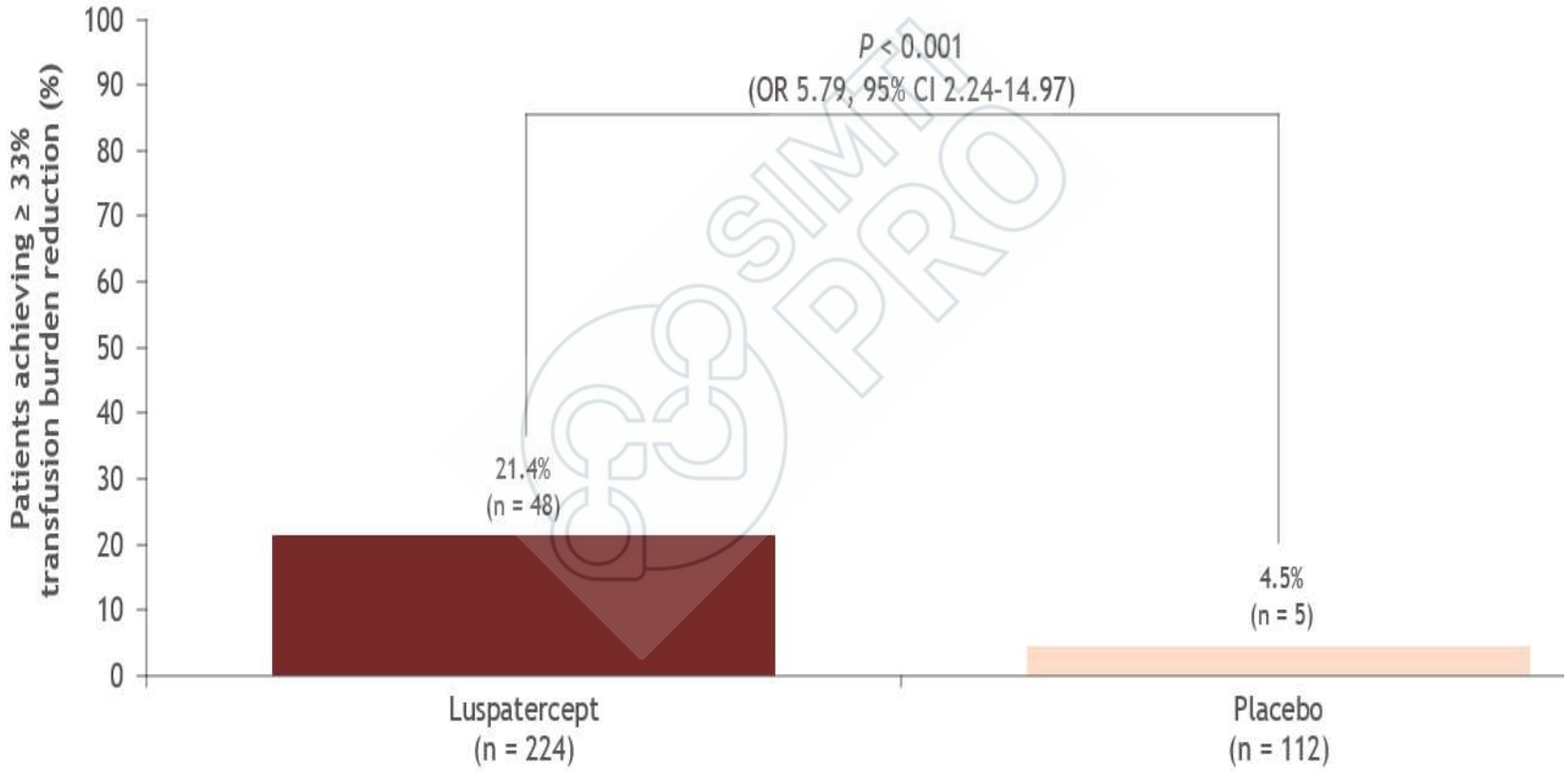
Key secondary endpoints

- ≥ 33% reduction (plus reduction of ≥ 2 RBC units) in RBC transfusion burden from baseline during weeks 37-48
- ≥ 50% reduction (plus reduction of ≥ 2 RBC units) in RBC transfusion burden from baseline during weeks 13-24
- ≥ 50% reduction (plus reduction of ≥ 2 RBC units) in RBC transfusion burden from baseline during weeks 37-48
- Mean change from baseline in RBC transfusion burden during weeks 13-24

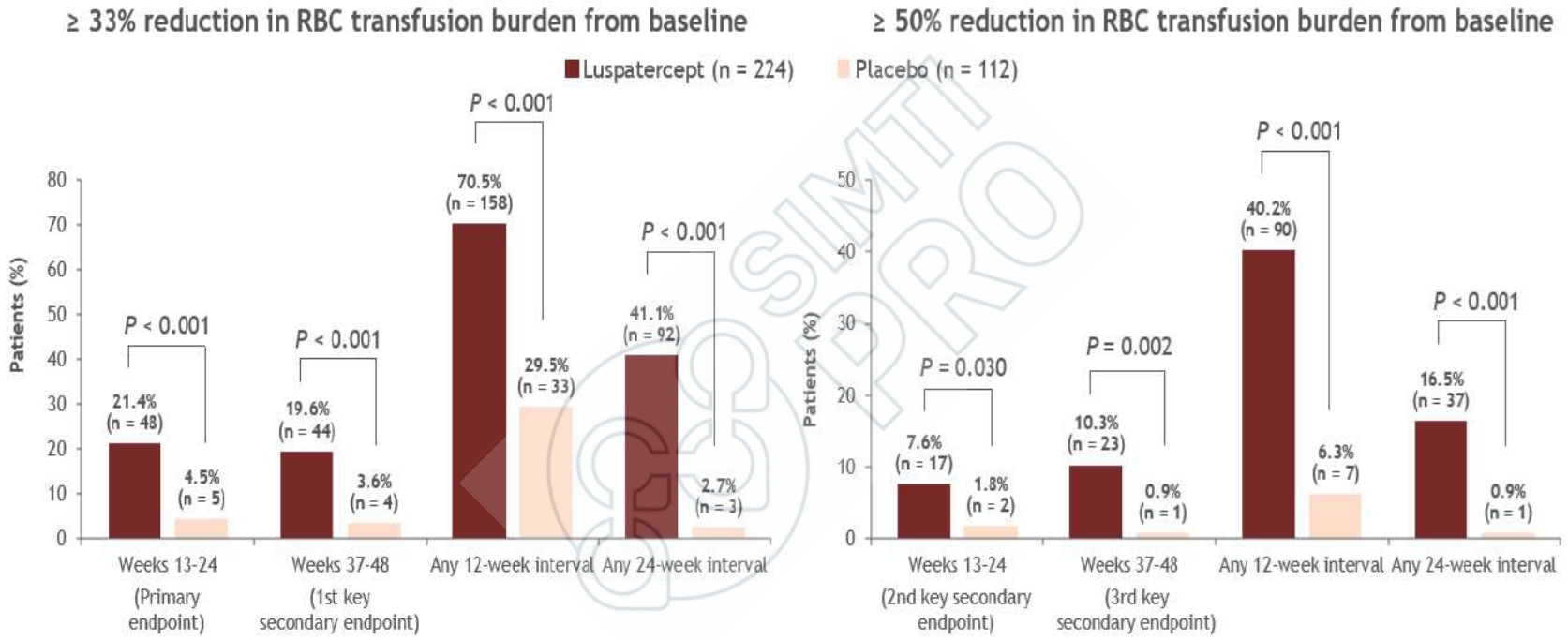
Additional endpoints

- ≥ 33% and ≥ 50% reduction (plus reduction of ≥ 2 RBC units) from baseline in RBC transfusion burden during any 12-week interval
- ≥ 33% and ≥ 50% reduction from baseline in RBC transfusion burden during any 24-week interval
- Duration of longest continuous erythroid response and time to first erythroid response (during any 12-week study interval)
- RBC-TI and duration of RBC-TI during any 8-week or 12-week study interval
- Mean reduction from baseline in transfusion burden over any 24-week study interval
- Mean change from baseline in pretransfusion Hb level (in defined 12-week study intervals)
- Serum ferritin level during weeks 37-48 or the 12-week period before discontinuation
- LIC and myocardial iron deposition at week 48
- Safety analyses

Efficacy: primary endpoint

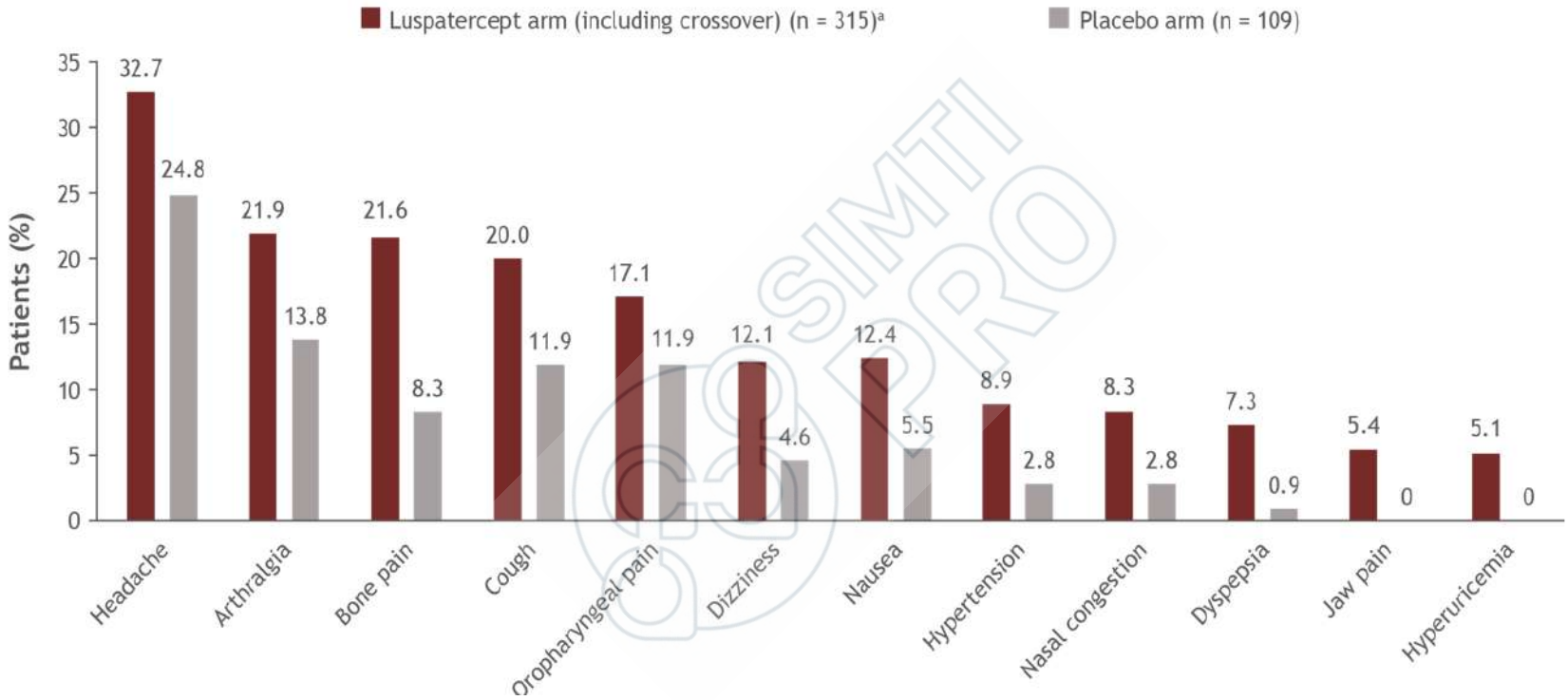


EFFICACY: reduction in RBC transfusion burden from baseline



- A statistically significant LS mean difference in mean change in transfusion burden from baseline during weeks 13-24 was observed for luspatercept versus placebo: −1.35 units/12 weeks (95% CI, −1.77 to −0.93; $P < 0.001$)
- For patients achieving ≥ 33% and ≥ 50% reduction in transfusion burden, a reduction from baseline of 6.55 RBC units and 8.27 units, respectively, per patient per 24 weeks was estimated

TEAEs (any grade) with ≥ 5% higher incidence in the luspatercept arm (including crossover) than in the placebo arm



Bone pain was reported more frequently during the first 24 study weeks versus the last 24 weeks in both arms, was generally of short duration, low grade, and managed using analgesic medications

Clinically confirmed thrombotic TEAEs were reported in 8 (3.6%) luspatercept and 1 (0.9%) placebo patients

Additional grade ≥ 3 TEAEs (with “any grade” occurrence < 5%) reported in ≥ 3% of patients in the luspatercept arm: anemia (n = 7 [3.1%])

Treatment discontinuation due to TEAEs was reported in 12 (5.4%) luspatercept patients and 1 (0.9%) placebo patient

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

A Phase 3 Trial of Luspatercept in Patients with Transfusion-Dependent β-Thalassemia

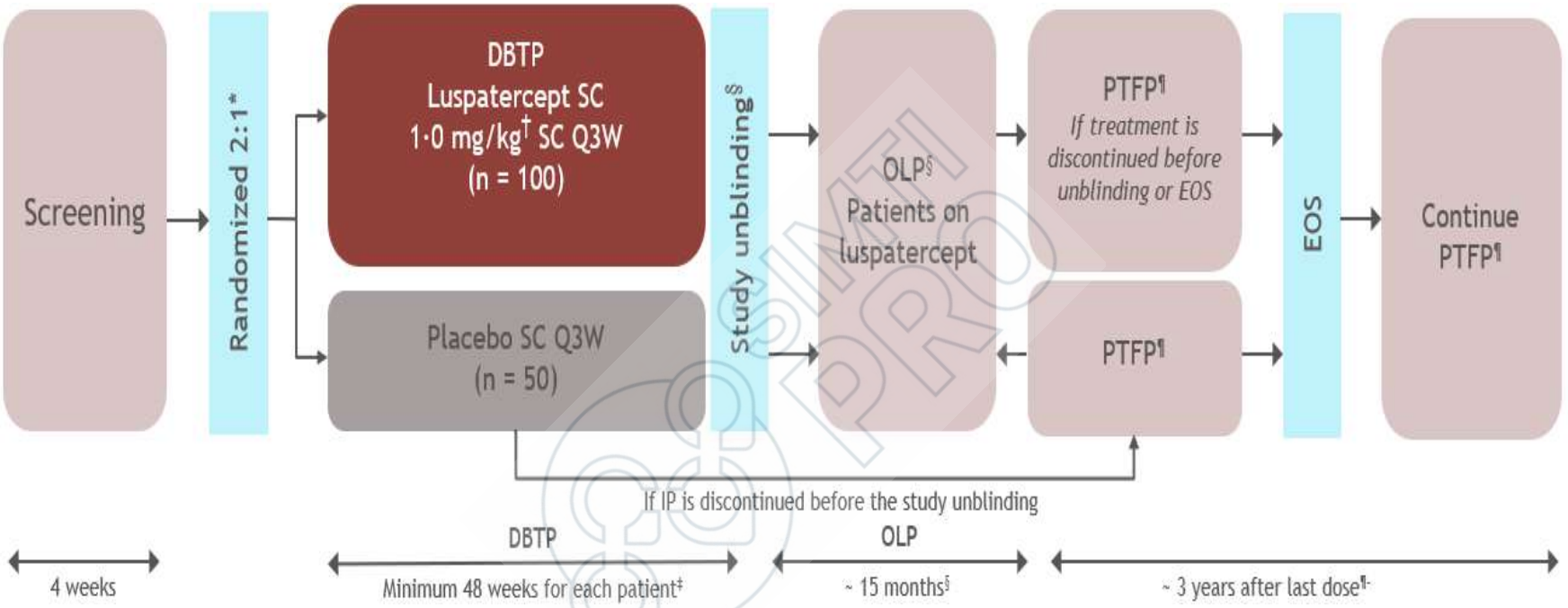
M.D. Cappellini, V. Viprakasit, A.T. Taher, P. Georgiev, K.H.M. Kuo, T. Coates, E. Voskaridou, H.-K. Liew, I. Pazgal-Kobrowski, G.L. Forni, S. Perrotta, A. Khelif, A. Lal, A. Kattamis, E. Vlachaki, R. Origa, Y. Aydinok, M. Bejaoui, P.J. Ho, L.-P. Chew, P.-C. Bee, S.-M. Lim, M.-Y. Lu, A. Tantiworawit, P. Ganeva, L. Gercheva, F. Shah, E.J. Neufeld, A. Thompson, A. Laadem, J.K. Shetty, J. Zou, J. Zhang, D. Miteva, T. Zinger, P.G. Linde, M.L. Sherman, O. Hermine, J. Porter, and A. Piga, for the BELIEVE Investigators*

N Engl J Med 2020, 382: 1219-31

LUSPATERCEPT REGISTRATION

Luspatercept has been approved by the US Food and Drug Administration in 2019 and by the European Medicines Agency in 2020 to treat anemia in adult patients with beta thalassemia who require regular blood cell transfusions.

Phase II RCT trial of luspatercept in adults with NTDT: the BEYOND trial

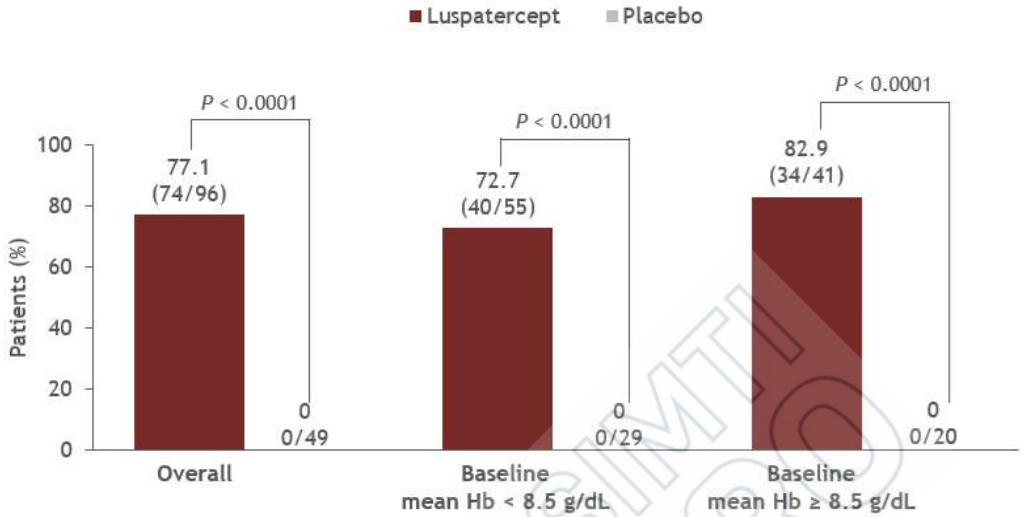


Primary efficacy endpoint
Proportion of patients with mean hemoglobin increase of at least 1.0 g/dL from baseline over a continuous 12-week interval (weeks 13–24) in the absence of RBC transfusions

Key secondary efficacy endpoints
Mean change from baseline in NTDT-PRO T/W domain score over a continuous 12-week interval (weeks 13–24)
Mean change from baseline in hemoglobin level over a continuous 12-week interval (weeks 13–24)
Proportion of patients with a mean hemoglobin increase of at least 1.0 g/dL from baseline over a continuous 12-week interval (weeks 37–48)

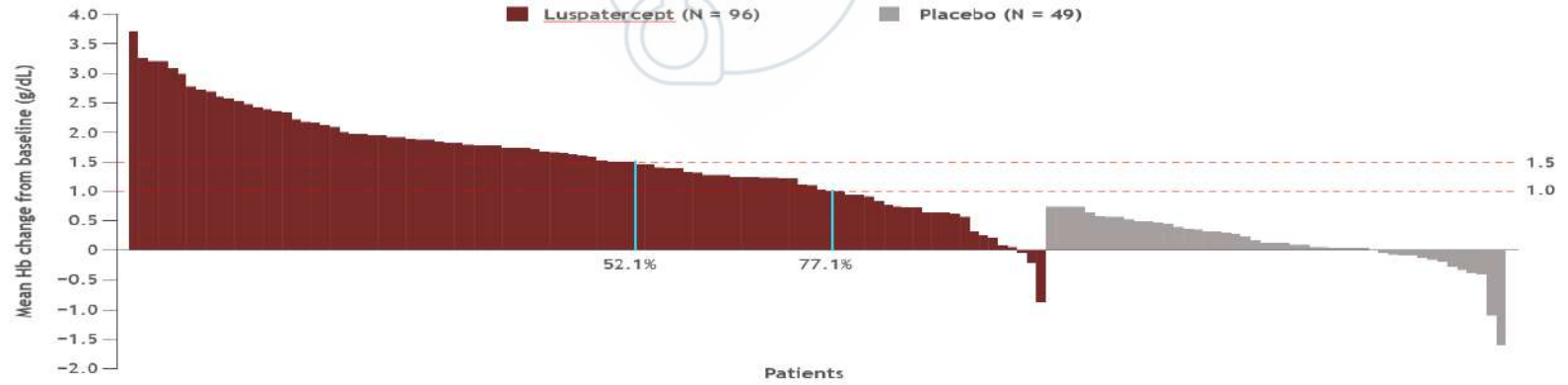
Taher AT, et al. *Lancet Haematol 2022*

Primary endpoint



- The study met its primary endpoint
  - 74 (77.1%) of patients in the luspatercept arm vs 0 placebo patients achieved a mean Hb increase of ≥ 1.0 g/dL from baseline[a] over a continuous 12-week interval during weeks 13-24 in the absence of RBC transfusions

Mean Hb change from baseline during weeks 13-24

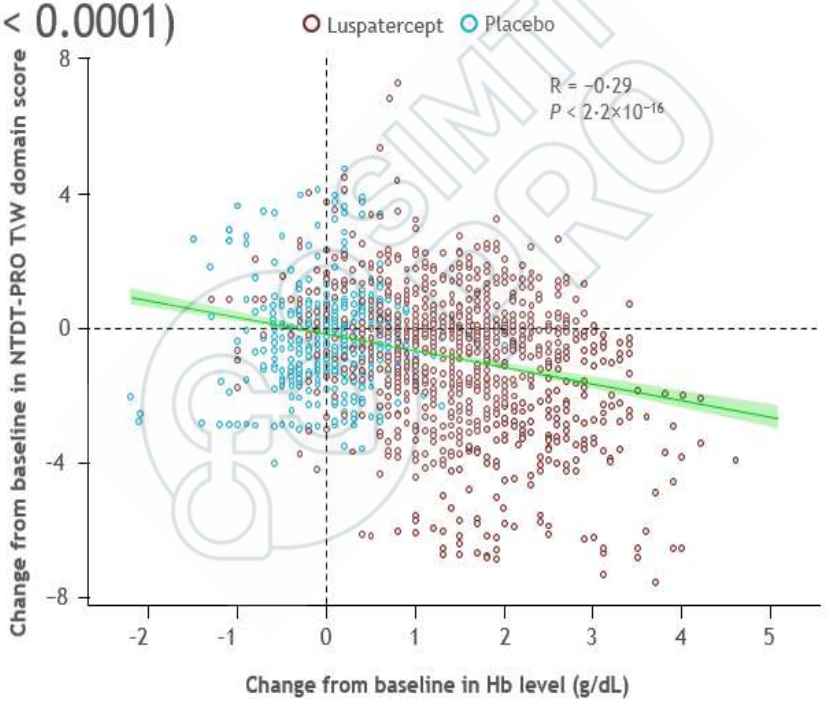


- During weeks 13-24, 50 (52.1%) patients in the luspatercept arm achieved a mean Hb increase of ≥ 1.5 g/dL from baseline

Taher AT et al. EHA 2021; Oral S101

NTDT-PRO T/W domain score improvement and Hb decrease

The correlation analysis showed that as hemoglobin levels increased, NTDT-PRO T/W domain scores decreased, suggesting improvement in patient-reported tiredness and weakness (R = −0.29; $P < 0.0001$)



Taher AT, et al. *Lancet Haematol 2022*

Long-term erythroid response data from patients with non-transfusion-dependent beta-thalassemia receiving luspatercept in the BEYOND trial

No. of doses received per patient[a]

Luspatercept (n = 96)

Mean (SD): 38.9 (15.1)

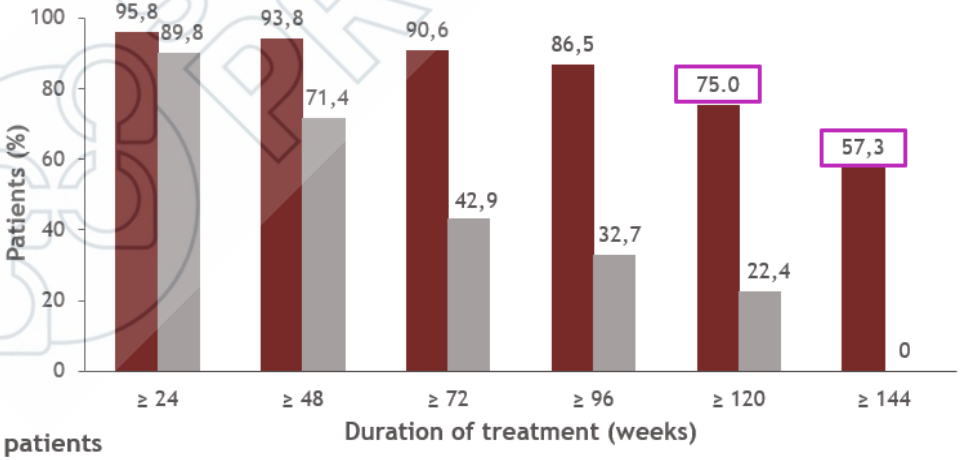
Median (range): 42.0 (3.0-61.0)

Placebo (n = 49)

Mean (SD): 22.5 (13.2)

Median (range): 20.0 (1.0-46.0)

Treatment duration,[b] weeks	Luspatercept (n = 96)	Placebo (n = 49)
Mean (SD)	137.1 (43.0)	71.9 (41.8)
Median (range)	150.1 (15.0-185.4)	61.1 (3.0-138.0)



No. of patients						
Luspatercept, n	92	90	87	83	72	55
Placebo, n	44	35	21	16	11	0

Data cut: September 22, 2021. [a]Defined as the total number of doses the patient received, a non-zero dose; [b]Defined as (treatment end date – date of first dose + 1)/7; where treatment end date = min ([date of last dose + 20], death date). SD, standard deviation.

Taher AT, et al. EHA 2023 [Abstract #S273]

Ali T. Taher, et al. EHA 2023

Erythroid response

- With a longer follow up:
  - More than 90% of patients treated with luspatercept experienced an erythroid response
  - Mean total duration of the hemoglobin increase was considerably longer

Patients with NTDT receiving long-term luspatercept treatment continued to experience increases in their Hb level

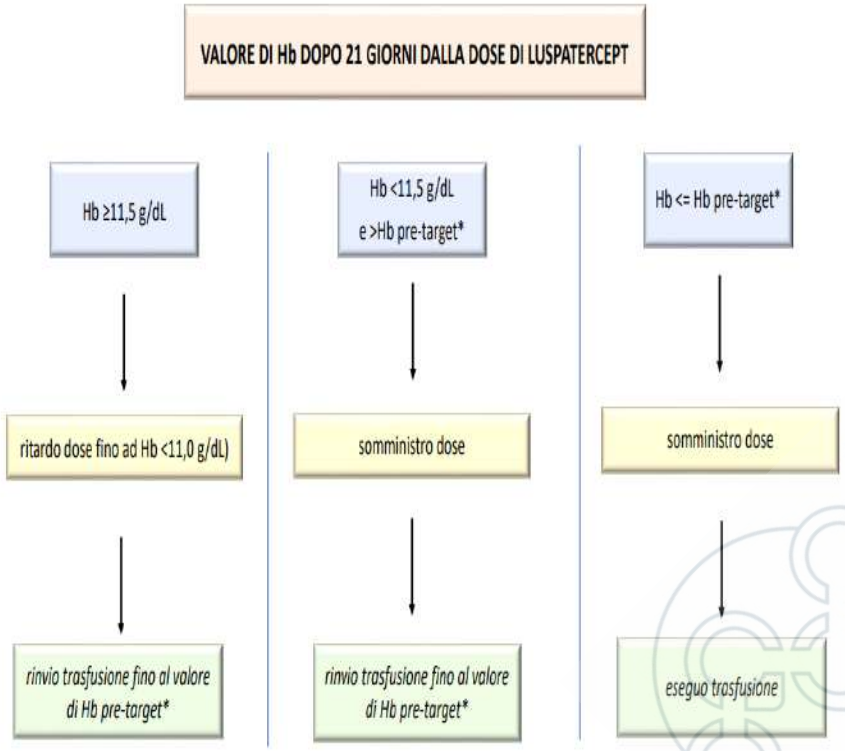
	Current data cut (September 22, 2021)		Primary data cut (September 14, 2020)[1]	
	Luspatercept (n = 96)	Placebo (n = 49)	Luspatercept (n = 96)	Placebo (n = 49)
Patients with mean Hb increase ≥ 1.0 g/dL during any 12-week interval, n (%)	90 (93.8)	11 (22.4)	88 (91.7)	11 (22.4)
Total duration of mean Hb increase ≥ 1.0 g/dL, days				
Mean (SD)	873.1 (363.4)	203.3 (170.8)	611.7 (243.3)	176.5 (132.9)

Erythroid response was defined as mean Hb change from baseline of ≥ 1.0 g/dL over rolling 12-week intervals.

1. Taher AT, et al. *Lancet Haematol* 2022;9:e733-e744.

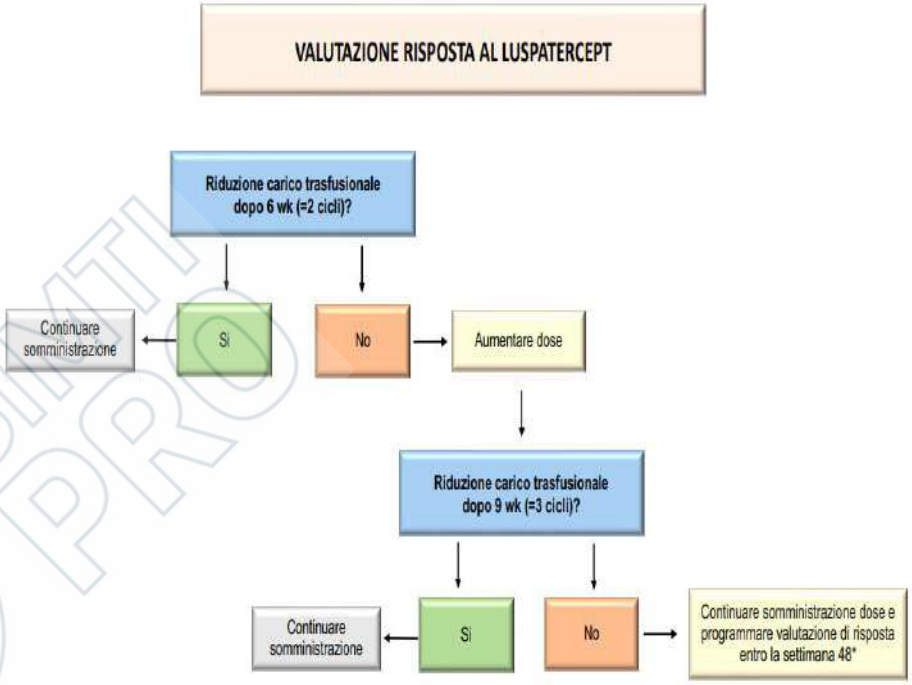
Taher AT, et al. EHA 2023 [Abstract #S273]

Flowchart 1 - TIMING DOSAGGIO LUSPATERCEPT/TRASFUSIONE



*Hb pre-target: il valore medio dell'Hb pre-trasfusionale delle 24 settimane precedenti l'inizio della terapia.

Flowchart 2 - TIMING VALUTAZIONE RISPOSTA AL LUSPATERCEPT



* risposta poco significativa dopo aumento di dose e/o con genotipo β0/β0.

Journal of Clinical Medicine

MDPI

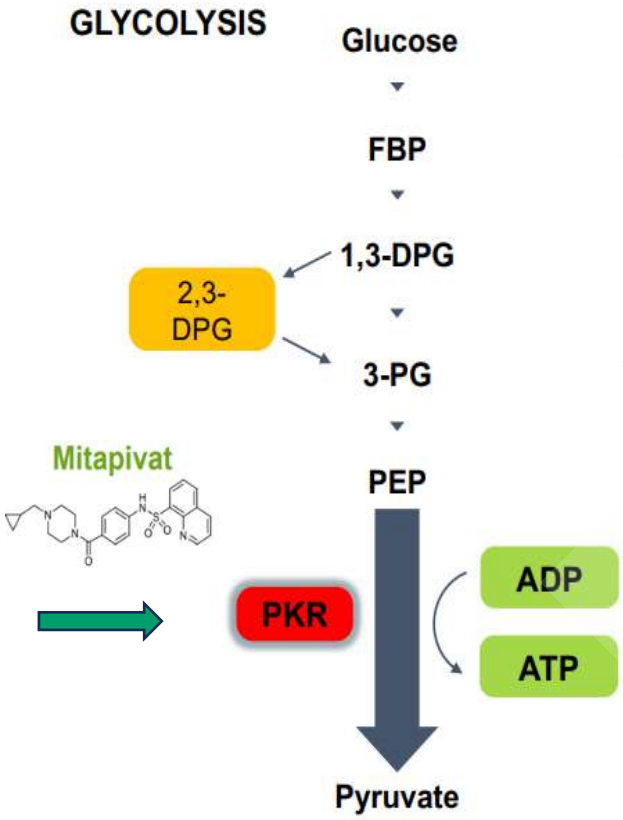
Opinion

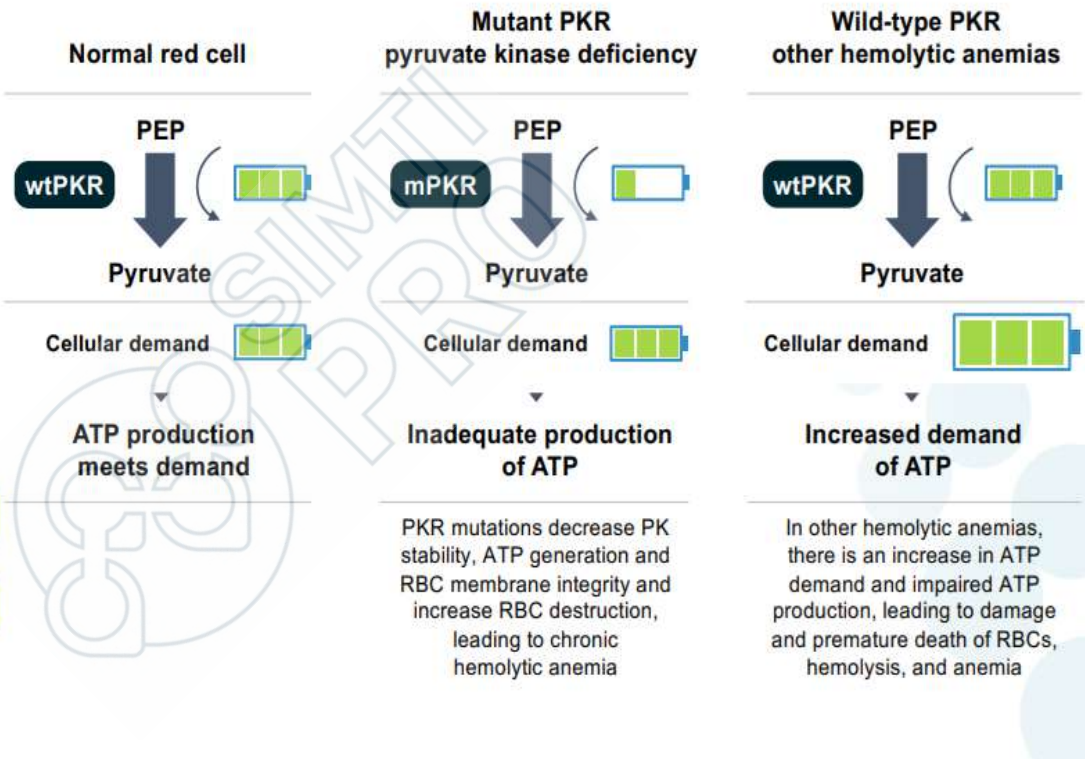
Treating Thalassemia Patients with Luspatercept: An Expert Opinion Based on Current Evidence

Filomena Longo [1,2,*], Irene Motta [3,4], Valeria Pinto [5], Andrea Piolatto [2], Paolo Ricchi [6], Immacolata Tartaglione [7] and Raffaella Origa [8]

J. Clin. Med. 2023, 12, 2584. https://doi.org/10.3390/jcm12072584

PKR activation represents a unique mechanism of action with the potential to address a broad range of hemolytic anemias

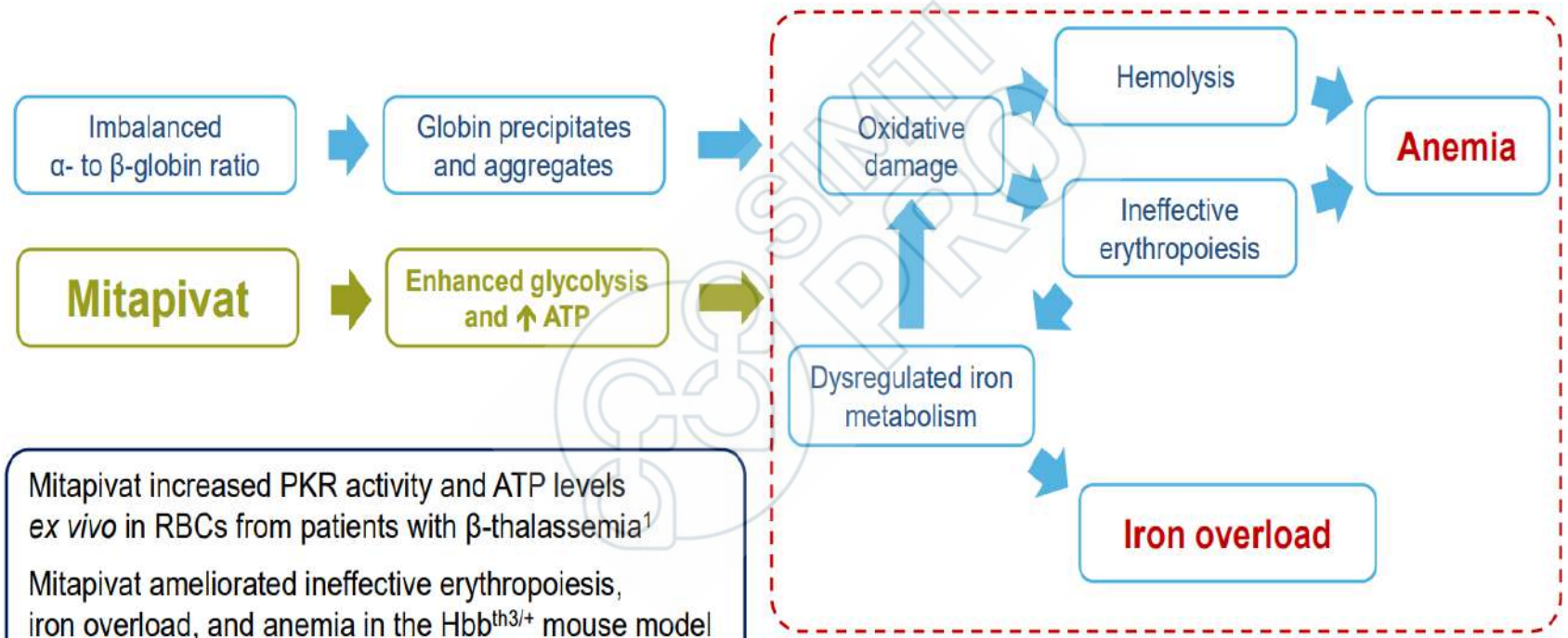




ADP = adenosine diphosphate; ATP = adenosine triphosphate; DPG = diphosphoglycerate; FBP = fructose bisphosphate; m = mutant; PEP = phosphoenolpyruvate; PG = phosphoglycerate; PK = pyruvate kinase; PKR = RBC-specific PK; RBC = red blood cell; wt = wild-type.

Date of preparation: October 2021 [MIT-ALL-0068]

Hypothesis: mitapivat mechanism in thalassemia via activation of wild-type PKR



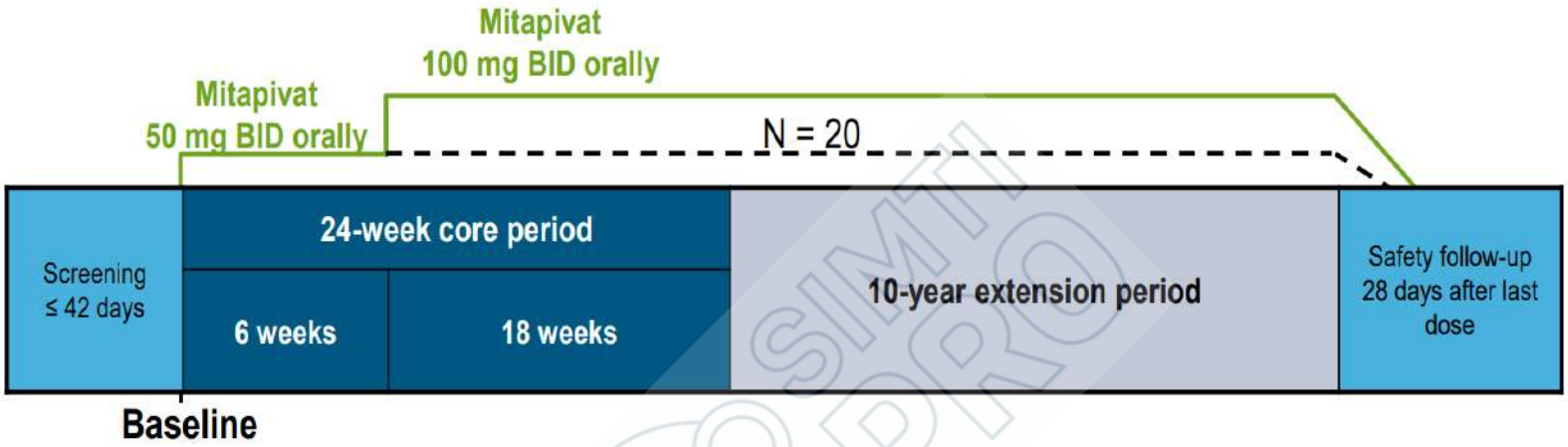
Mitapivat increased PKR activity and ATP levels *ex vivo* in RBCs from patients with β-thalassemia[1]

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

ATP = adenosine triphosphate; PK = pyruvate kinase; PKR = PK in RBCs; RBC = red blood cell.

1. Rab MAE et al. ASH Congress 2019, Abstract 3506; 2. Matte A et al. J Clin Invest 2021;144206

This phase 2, open-label, multicenter study investigated the efficacy and safety of mitapivat in non–transfusion-dependent α- and β-thalassemia[a]



Key inclusion criteria:

- β-thalassemia ± α-globin gene mutations, HbE β-thalassemia, or α-thalassemia (HbH disease)
- Hb ≤ 10.0 g/dL
- Non–transfusion-dependent[b]

Primary endpoint[c]

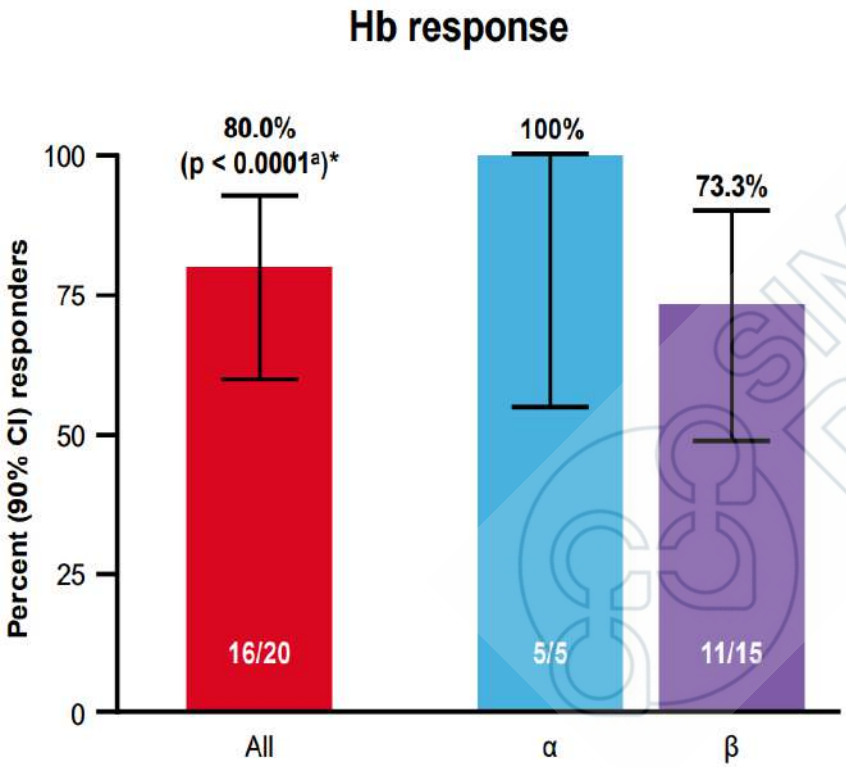
- Hb response, defined as increase of ≥ 1.0 g/dL from baseline at any time between Weeks 4–12, inclusive

Secondary and exploratory endpoints

- Sustained Hb response; delayed Hb response; markers of hemolysis and erythropoiesis; safety

aEudraCT 2018-002217-35, ClinicalTrials.gov: NCT03692052; b≤ 5 RBC units transfused in the preceding 24 weeks and none in the 8 weeks prior to study drug; cWith the originally planned sample size of 17 patients, the study would have 80% power to reject a ≤ 30% response rate at a 1-sided 0.05 type 1 error rate. BID = twice daily; dL = deciliter; Hb = hemoglobin; HbE = hemoglobin E; HbH = hemoglobin H; RBC = red blood cell.

Mitapivat met the primary endpoint of a Hb response in 80% of patients

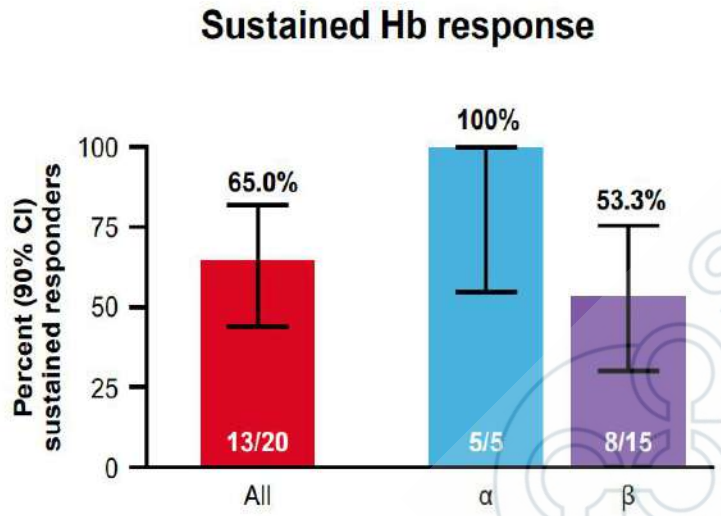


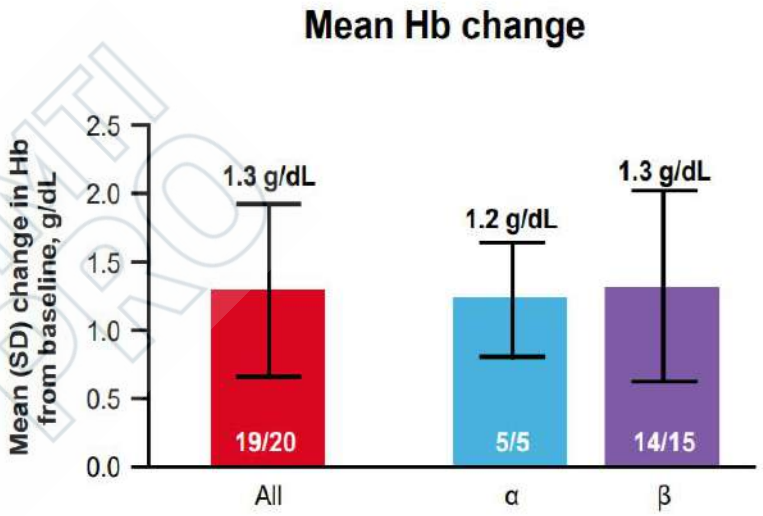
Primary endpoint

Hb response:
≥ 1.0 g/dL increase in Hb concentration from BL at ≥ 1 assessments between Weeks 4–12 (inclusive)

NB: Primary endpoint; Hb response, defined as a ≥1.0 g/dL increase in Hb concentration from baseline at 1 or more assessments between Week 4 and Week 12 (inclusive). a1-sided p-value based on Clopper-Pearson method. BL = baseline; CI = confidence interval; Hb = hemoglobin

Secondary endpoints: sustained Hb response and consistent increases in mean Hb



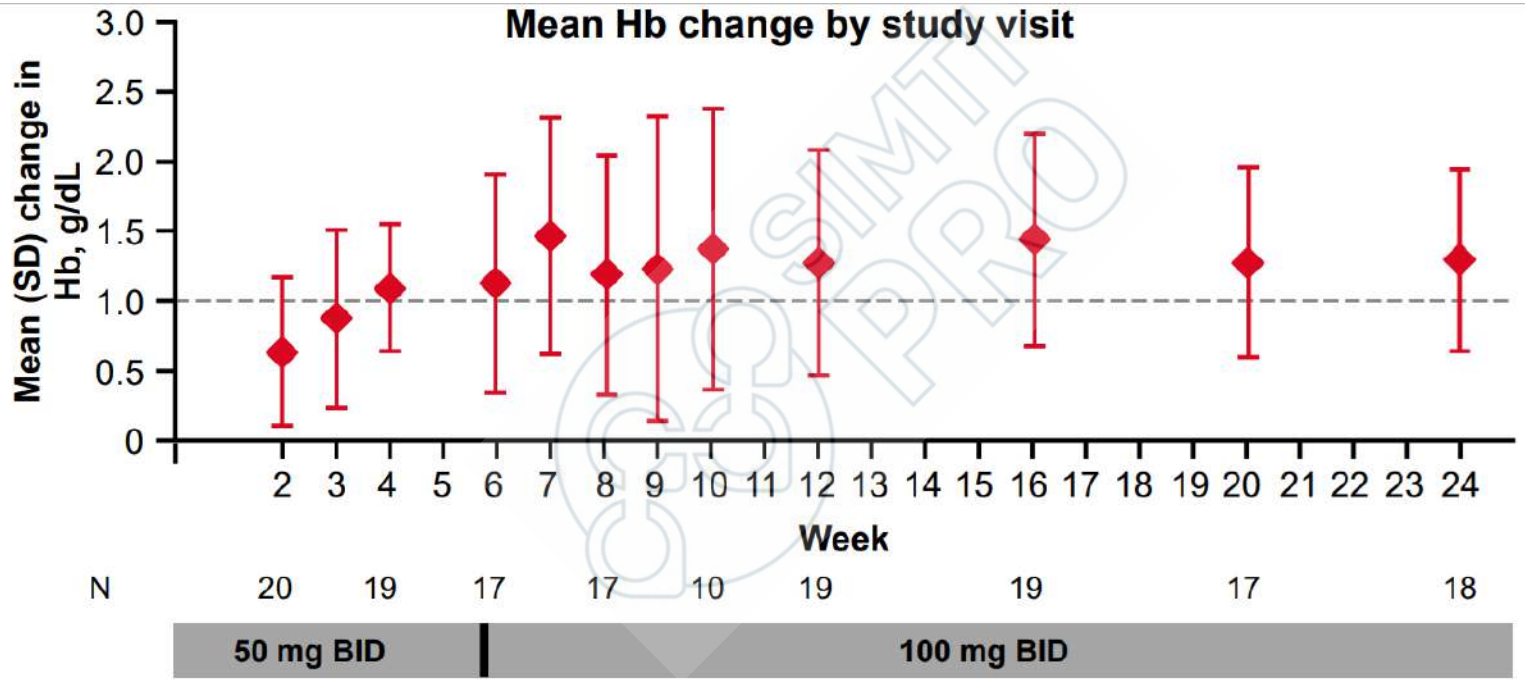


Sustained Hb response:
A primary endpoint response during Weeks 4–12 and a ≥ 1.0 g/dL increase in Hb concentration at ≥ 2 assessments between Weeks 12 and 24

Mean Hb change:
Mean change from BL in Hb concentrations over a 12-week interval from Weeks 12 and 24

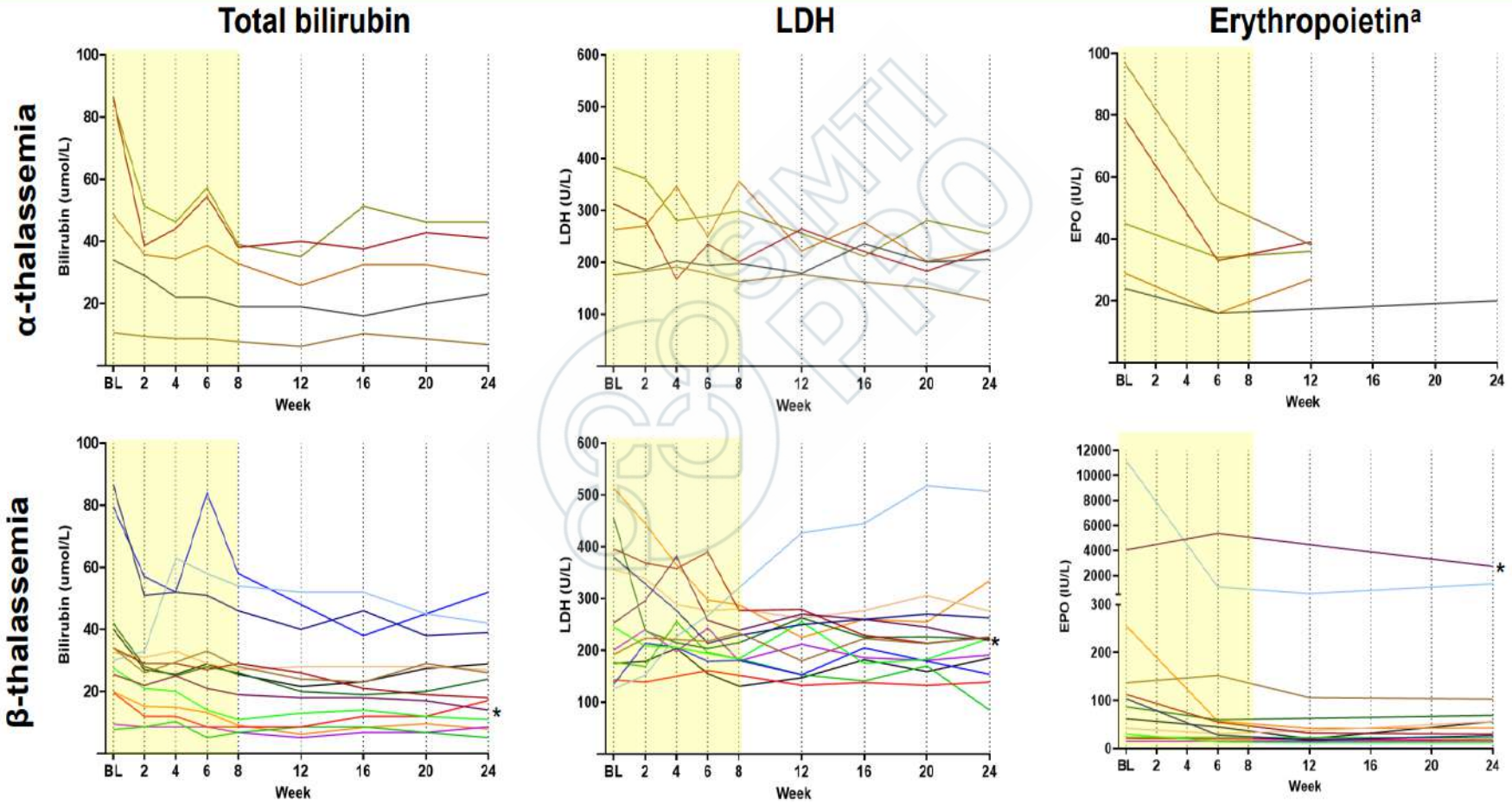
BL = baseline; CI = confidence interval; Hb = hemoglobin; SD = standard deviation

Improvements in Hb were rapid and maintained over the duration of the core treatment period



- Mean (SD) time to first Hb increase of ≥ 1 g/dL among responders was 4.5 (3.2) weeks

Treatment with mitapivat improved markers of hemolysis and erythropoiesis in both α- and β-thalassemia

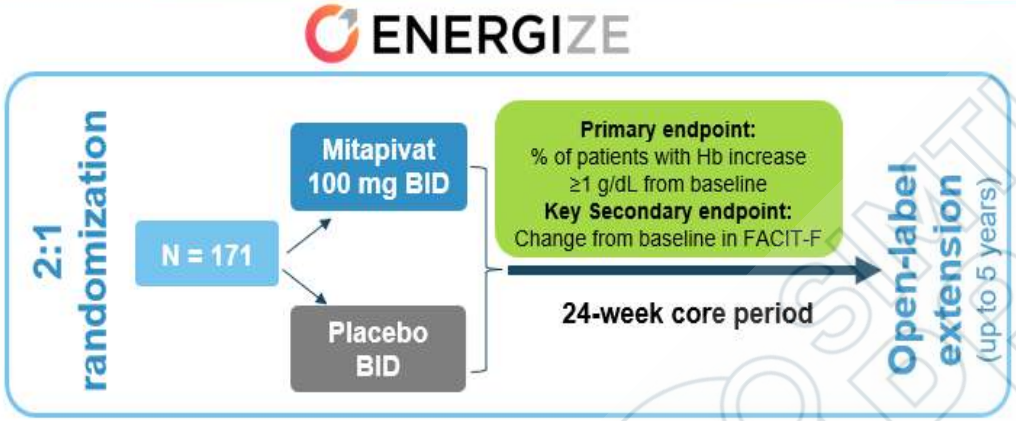


Safety summary

All patients (n = 20)	Patients, n (%)	TEAEs[a]
Treatment-related TEAEs	13 (65.0)	Initial insomnia (n = 10), diarrhea (n = 3), dyspepsia (n = 3), abdominal distension (n = 3), nausea (n = 3)
Grade ≥ 3 TEAEs	5 (25.0)	Initial insomnia (n = 1), arthralgia (n = 1), renal impairment (n = 1), anemia (n = 1), vertigo positional (n = 1)
Grade ≥ 3 treatment-related TEAEs	1 (5.0)	Initial insomnia (grade 3)
Serious TEAEs	1 (5.0)	Renal impairment (grade 3)
TEAEs leading to study drug:		
Dose reduction	3 (15.0)	Abdominal distension and dyspepsia (both grade 2), initial insomnia (grade 3), renal impairment (grade 3)
Interruption	1 (5.0)	Vertigo positional (grade 3)
Discontinuation	1 (5.0)	Renal impairment (grade 3) Patient discontinued after the Week 4 visit

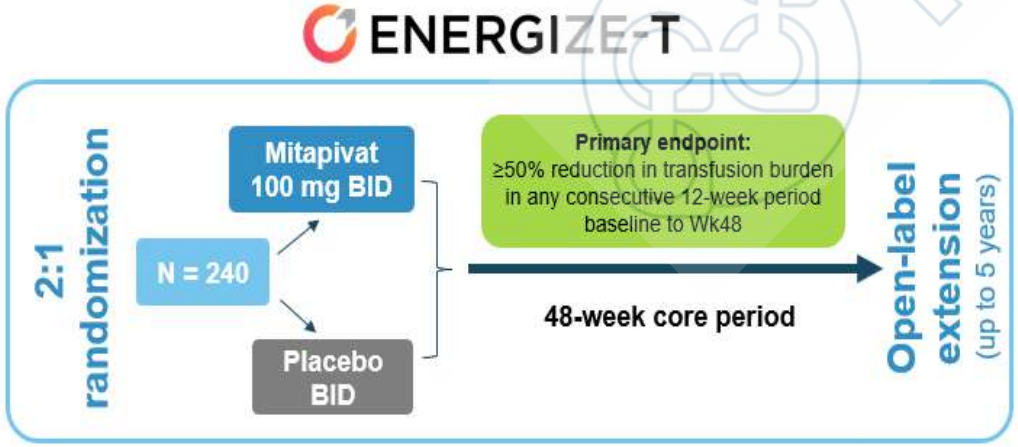
Patients with multiple adverse events within a PT are counted only once in that PT; for patients with multiple occurrences of an adverse event, the adverse event with the worst CTCAE grade is included in the summary; MedDRA version 23.0 and CTCAE version 4.03 were used. aTEAEs ≥ 20% listed for 'any TEAEs'; ≥ 20% listed for 'treatment-related TEAEs'; all TEAEs listed for other sections. CTCAE = Common Terminology Criteria for Adverse Events; MedDRA = Medical Dictionary for Medical Regulatory Activities; PT = preferred term; TEAE = treatment-emergent adverse event.

Phase 3 randomized, placebo-controlled, trials of mitapivat in thalassemia



Key inclusion criteria

- ≥ 18 years
- β-thalassemia ± α-globin mutations, HbE β-thalassemia, or α-thalassemia (HbH disease)
- Non–transfusion-dependent; ≤5 units prior 24 weeks before randomization
- Hb ≤ 10.0 g/dL



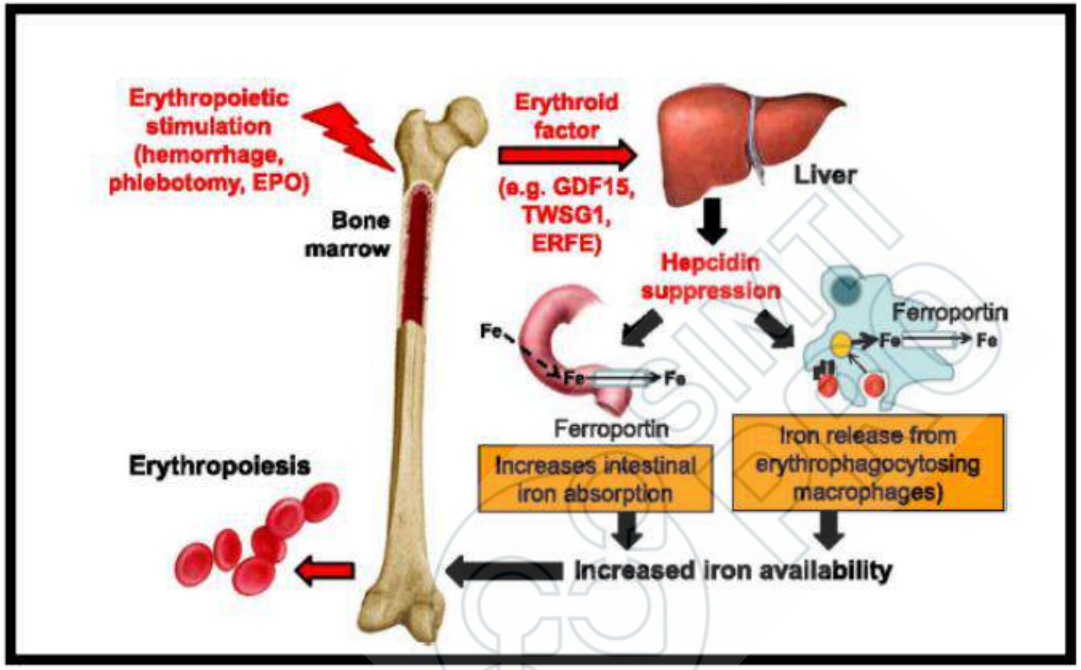
Key inclusion criteria

- ≥ 18 years
- β-thalassemia ± α-globin mutations, HbE β-thalassemia, or α-thalassemia (HbH disease)
- Transfusion-dependent; 6-20 units prior 24 weeks before randomization

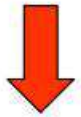
BID = twice daily; Hb = hemoglobin; HbE = hemoglobin E; HbH = hemoglobin H.

Date of preparation: November 2020 [MIT-ALL-0035]

Metabolismo del ferro ed epcidina



Epcidina

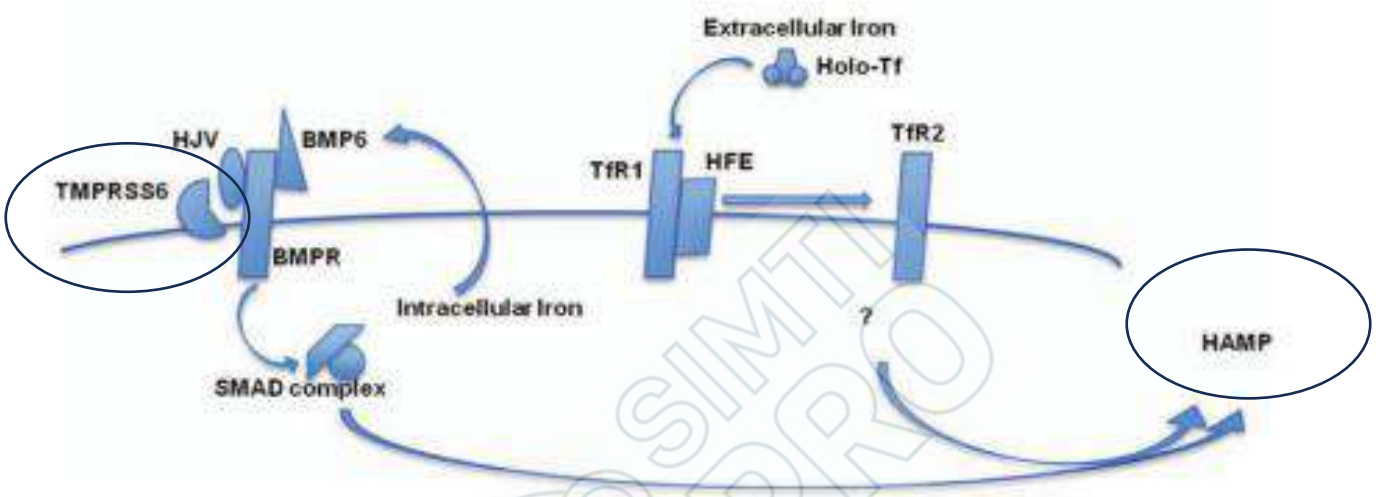


Ferroportina

Bassi livelli di epcidina nella beta Talassemia Intermedia e Major

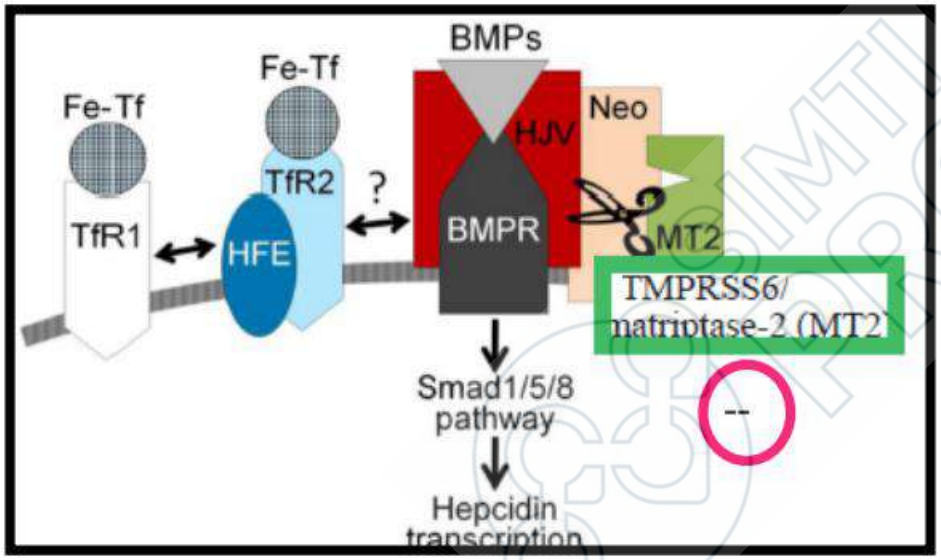
Papanikolaou, et al, Hepcidin in iron overload disorders, **Blood. 2005;105:4103-4105)**
Pasricha et al. Transfusion suppresses erythropoiesis and increases hepcidin in adult patients with β-thalassemia major: a longitudinal study. Blood. 2013;122:124–33.

Studi pre-clinici hanno dimostrato che aumentando l'espressione di epcidina (HAMP) si riduce il sovraccarico marziale



Casu C. Hematology 2014

Matriptase -2 (TMPRSS6) Antagonist



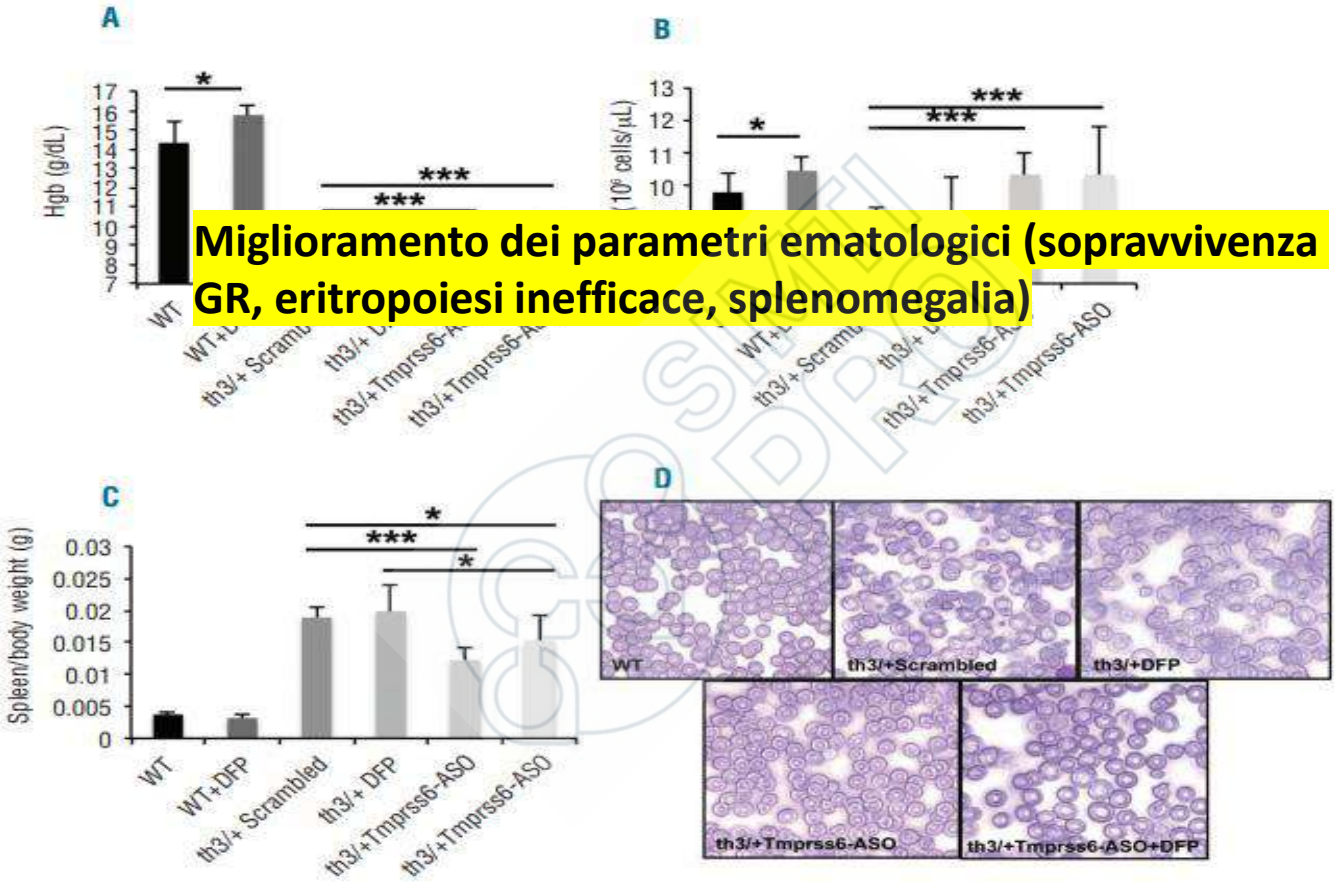
Regulation of hepcidin transcription by extracellular iron

TMPRSS6 is a transmembrane serine protease mainly produced by hepatocytes that negatively regulates hepcidin expression

TMPRSS6 inhibition to induce hepcidin transcription and syntesis

Nai A et al. Deletion of TMPRSS6 attenuates the phenotype in a mouse model of beta-thalassemia. Blood. 2012;119:5021–5029.

Tmprss6-ASO



Miglioramento dei parametri ematologici (sopravvivenza GR, eritropoiesi inefficace, splenomegalia)

Casu C. Haematologica 2015

MINI-EPCIDINE

- ✓ Miniepcidine sono piccoli peptidi che mimano l'attività di epcidina
- ✓ Derivate dalla sequenza AA terminale e modificate per l'attività in vivo
- ✓ Miniepcidine riducono il sovraccarico marziale in modelli animali di emocromatosi HFE e HAMP correlata

Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload Preza GC, Ruchala P, Pinon R, Ramos E, Qiao B, Peralta MA, Sharma S, Waring A, Ganz T, Nemeth E. Journal of Clinical Investigation

Minihepcidins prevent iron overload in a hepcidin-deficient mouse model of severe hemochromatosis Ramos E, Ruchala P, Goodnough JB, Kautz L, Preza GC, Nemeth E, Ganz T. Blood

Grazie per l’attenzione