ROMA TRE
UNIVERSITÀ DEGLI STUDI

Accademia Nazionale dei Lincei

Thalassemias: Past and Future

Paolo Ascenzi …

… and Friends

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De Simone, G., Quattrocchi, A., Mancini, B., di Masi, A., Nervi, C., & Ascenzi, P. (2022) Thalassemias: From gene to therapy. *Molecular Aspects of Medicine*, 84, 101028.

Thalassemias are hereditary disorders characterized by:

- Microcytic and hypochromic anemia

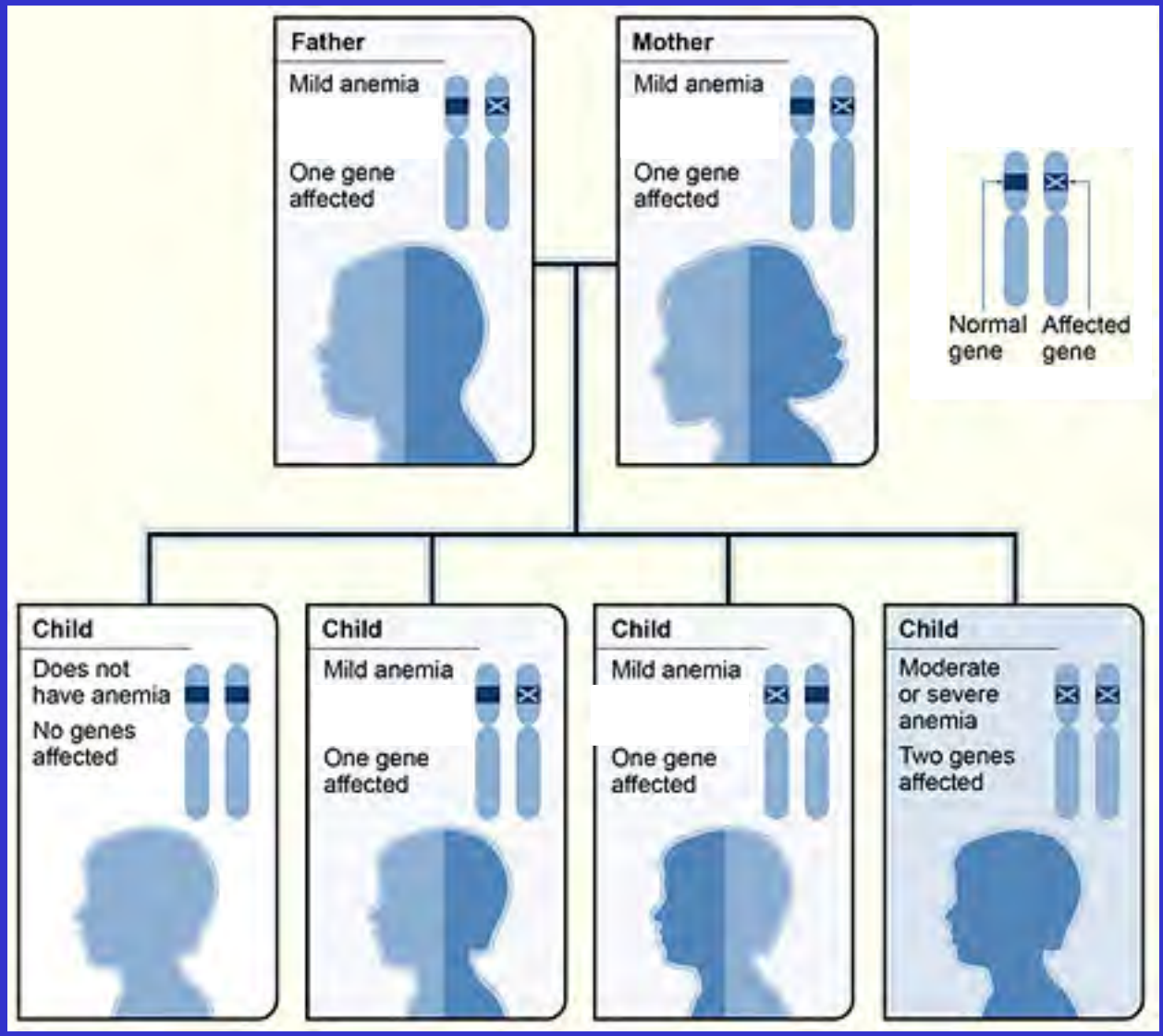
- Hemoglobinopathy due to deficiency of globin chains belonging to either the α- or β-cluster

The presentation and severity of thalassemia depends on the number of defective hemoglobin chains
α, β, γ, δ, δβ, εγδβ

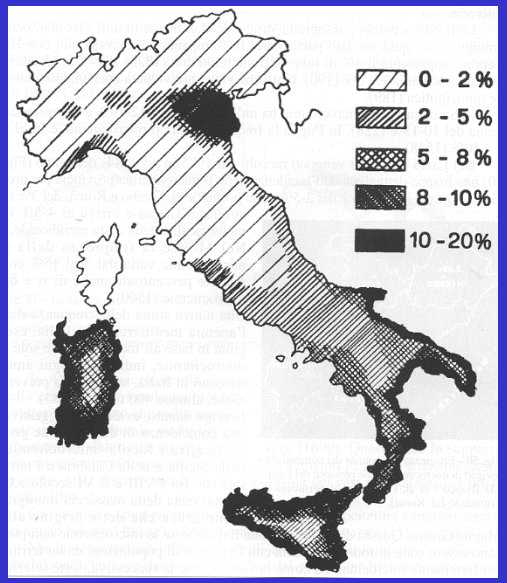
Thalassemia: From Early Milestones to the Present and Future

1889 First report of the homozygous clinical traits of thalassemia
1925 Description of the clinical traits of thalassemia
1925 **I**ncreased osmotic resistance of erythrocytes
1932 The term “Thalassemia” was coined recognizing Mediterranean ethnics (θάλασσα, thàlassa, «sea», and αίμα, àima, «blood»)
1938 Thalassemia is recognized as a genetic disease
1944 First blood transfusion for clinical treatment
1948 Thalassemia is determined to be caused by an abnormal hemoglobin
1964 The thalassemia mutation is found to protect against malaria
1976 Prenatal diagnosis for thalassemia becomes available
1977 Iron chelation improve patient survival
1982 Bone marrow transplantation is first used to cure thalassemia
1985 Pharmacologic HbF induction: use of butyrate and hydroxyurea in clinical trials
2005 Gene therapy clinical trials with improved vectors in patients with severe disorder
2010-2022 Encouraging results from clinical trials of the reactivation of fetal hemoglobin by lentiviral vectors or genome editing

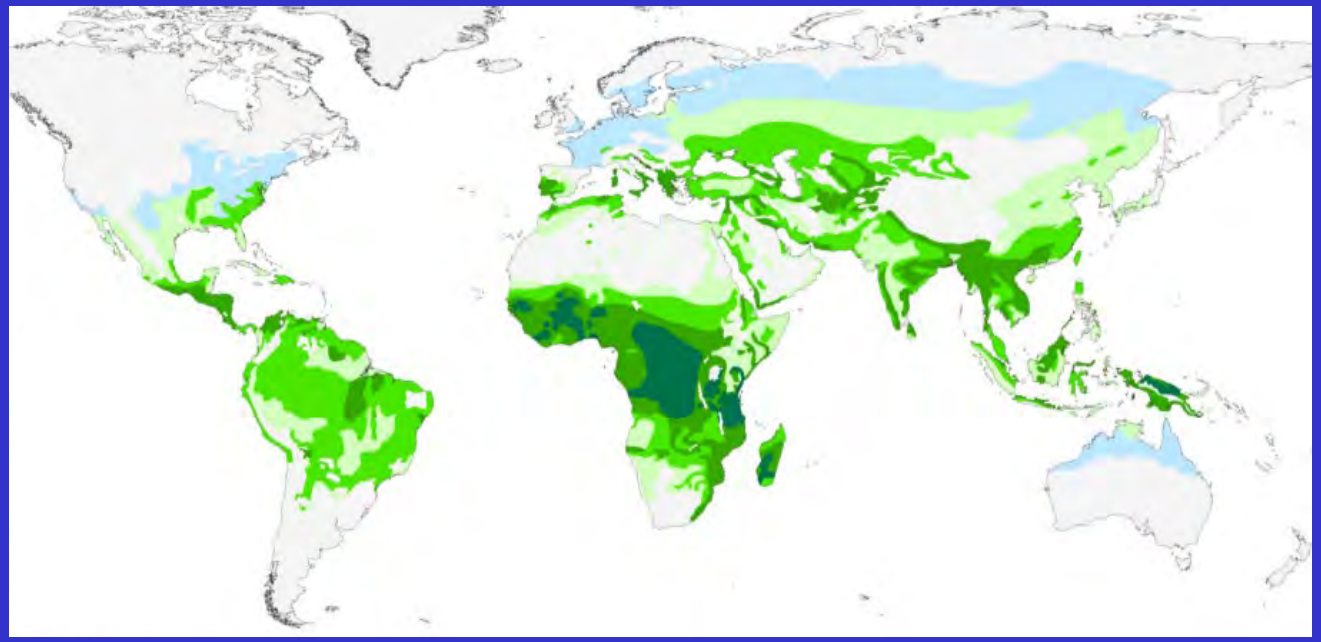
Mendelian inheritance of thalassemia



Geographical distribution



Thalassemia
1.7% of the global population



Malaria
3.6% of the global population

Thalassemia detection in human ancient bones

Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7170–7172, November 1985
Biochemistry

Immunological detection of hemoglobin in bones of ancient Roman times and of Iron and Eneolithic Ages

(heme proteins/paleopathology/skeletal remains)

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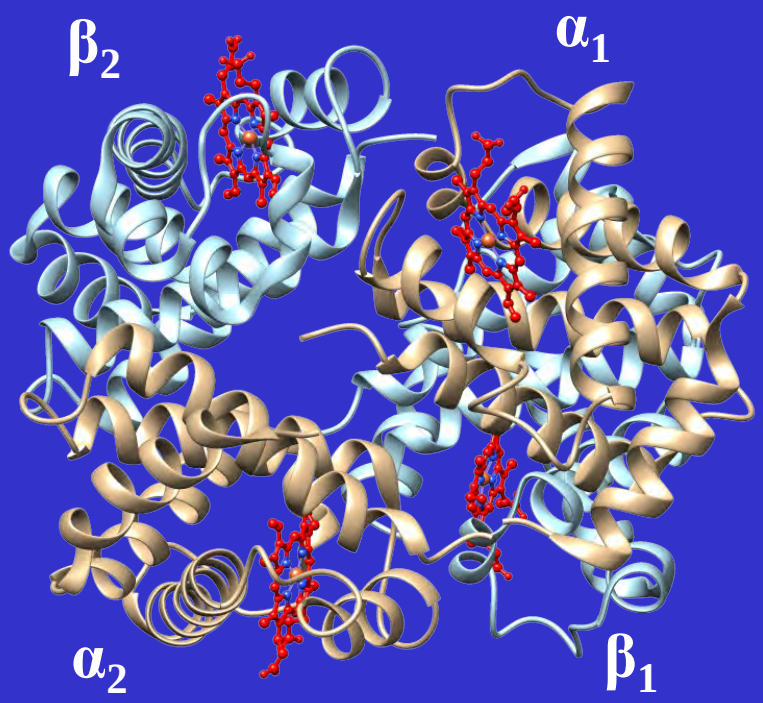
HISTORY OF MEDICINE

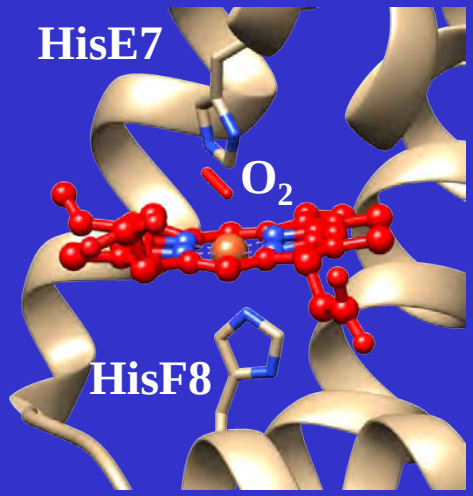
In what ways can human skeletal remains be used to understand health and disease from the past?

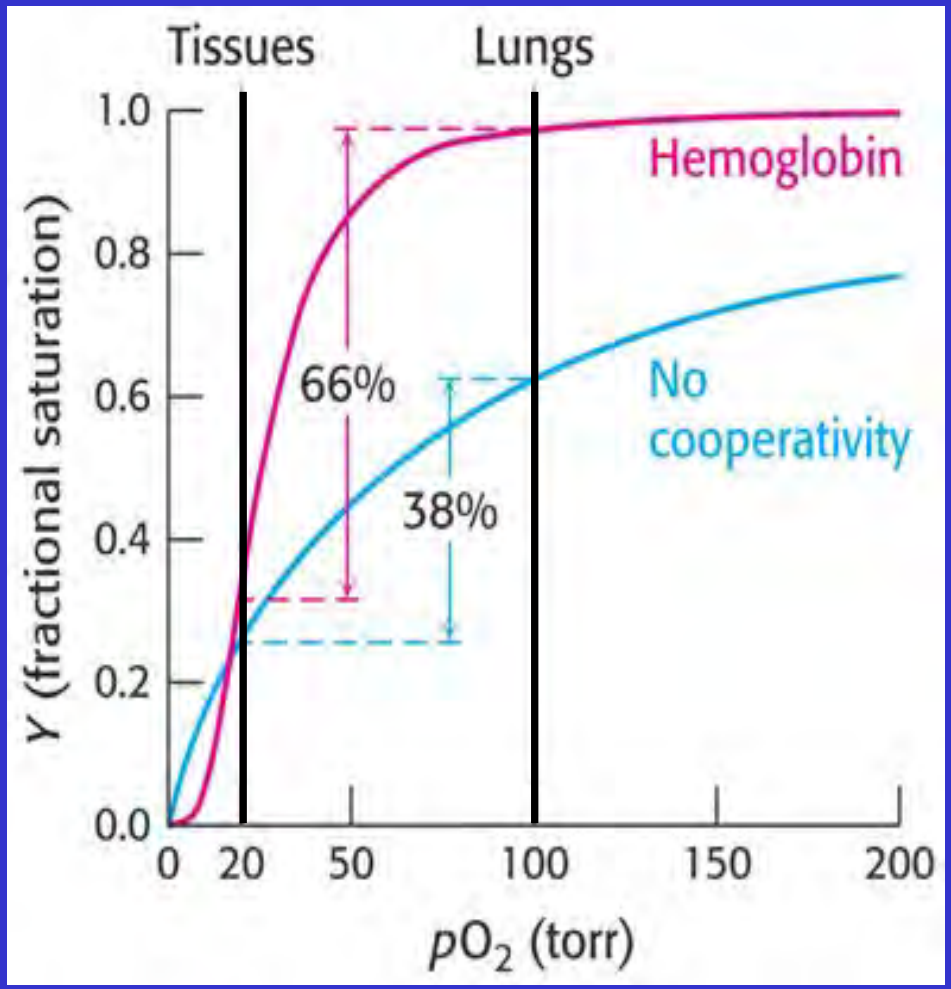
Neil H Metcalfe

Postgrad Med J 2007;**83**:281–284. doi: 10.1136/pgmj.2006.051813

Hemoglobin: the main actor

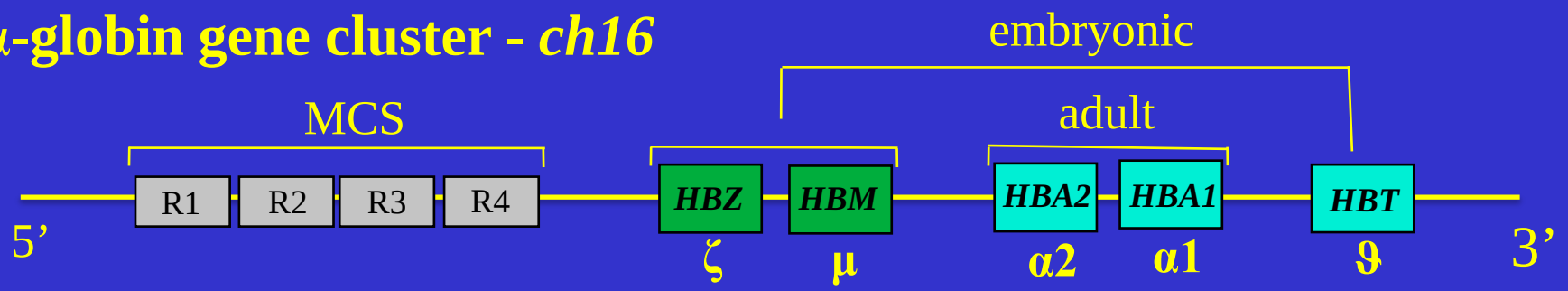




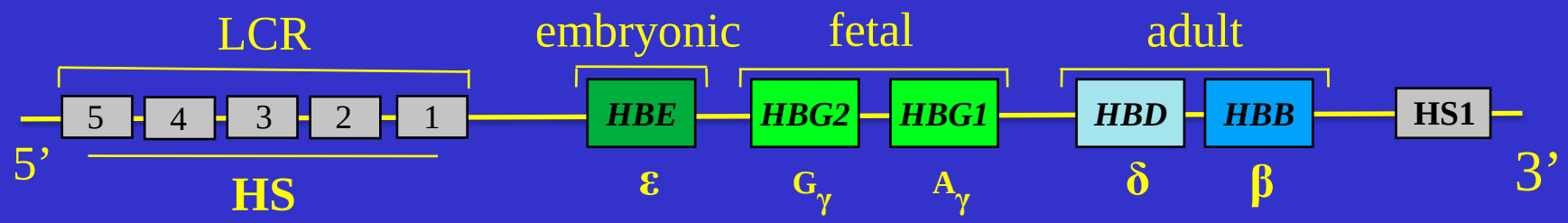


Globin gene clusters

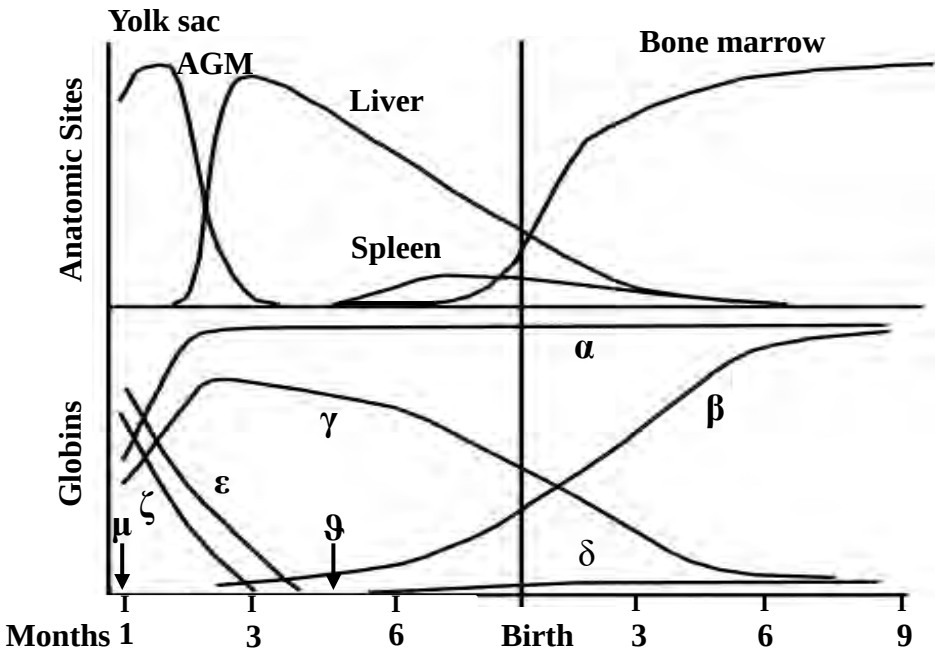
α-globin gene cluster - *ch16*



β-globin gene cluster - *ch11*



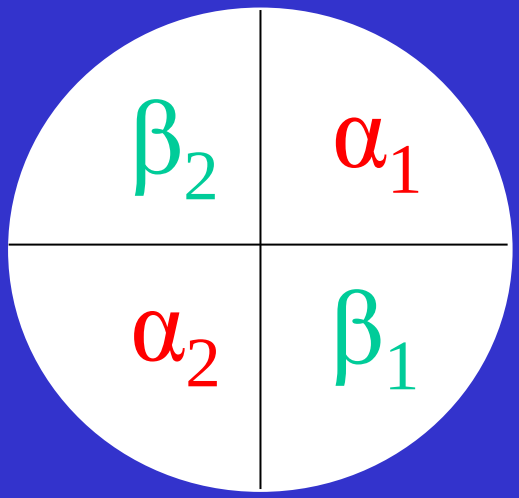
Hemoglobin chains expression during the development of the human hematopoietic system



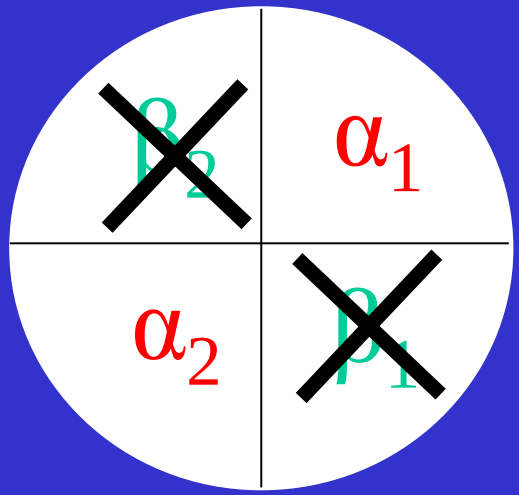
AGM, aorta-gonad-mesonephros

From: Istologia di Monesi, VII Ed., Piccin, Padova, 2018

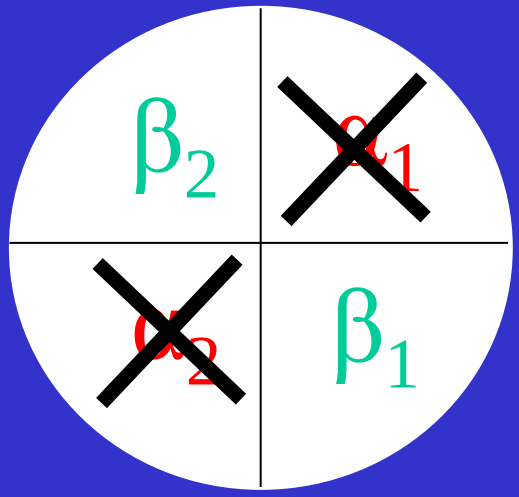
Molecular bases of α- and β-thalassemia



Normal Hb
HbA and HbA2

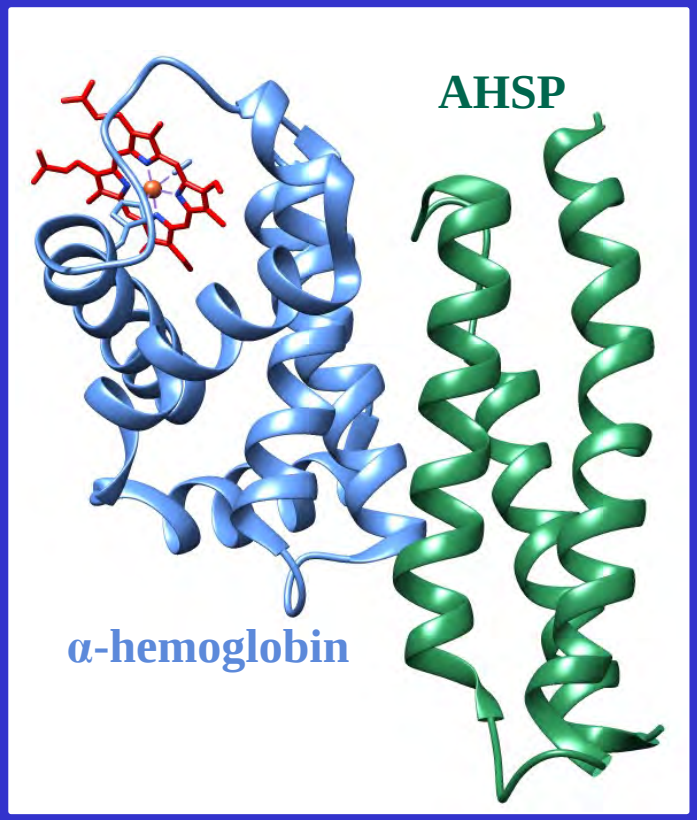


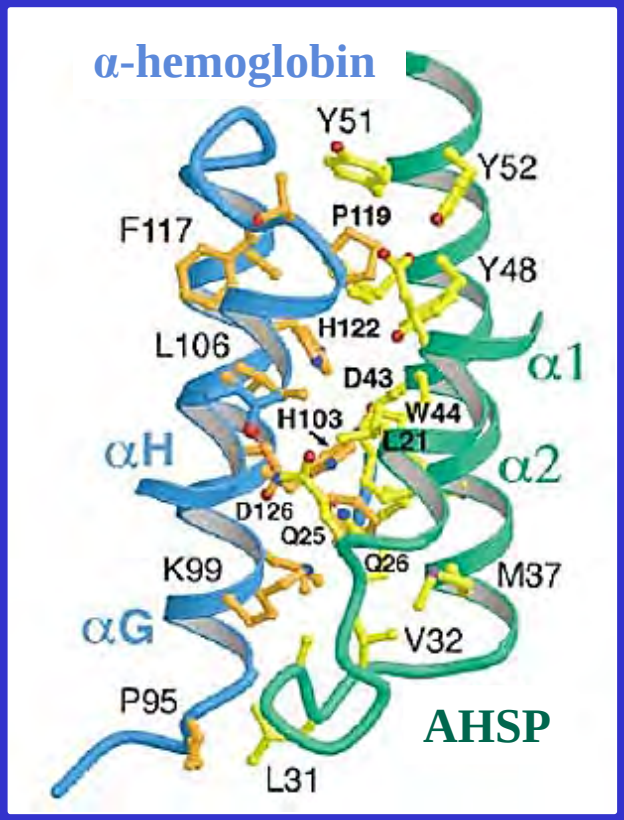
β-Thalassemia

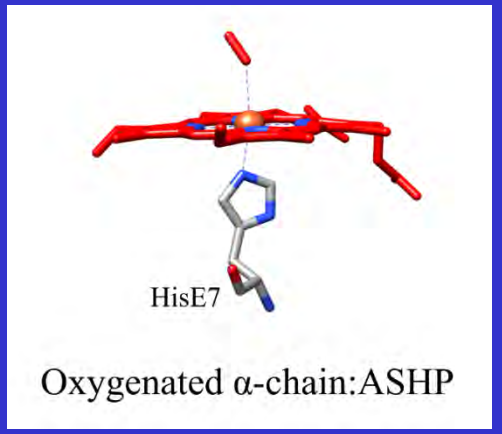


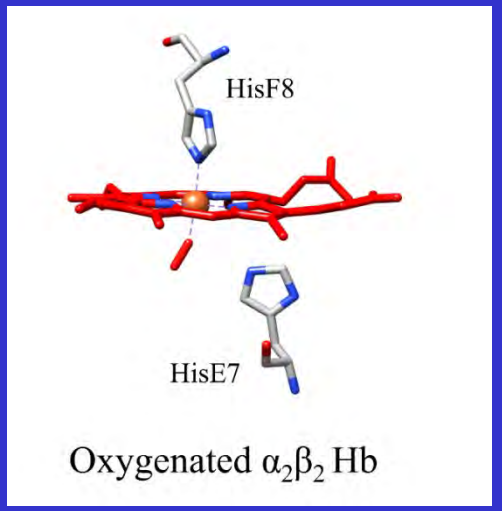
α-Thalassemia

Thalassemic globins belonging to the α-cluster (ζ, μ, ϑ, α)



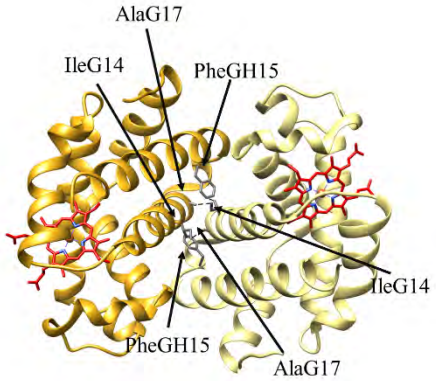




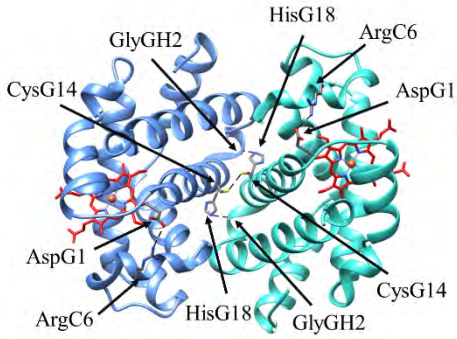


Feng L., et al., (2004) Cell, 119, 629-640.

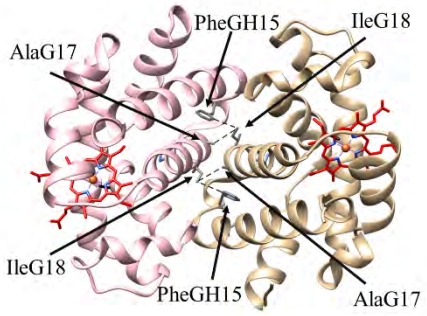
Subunit contacts of thalassemic globins belonging to the β-cluster (ε, γ, δ, β)



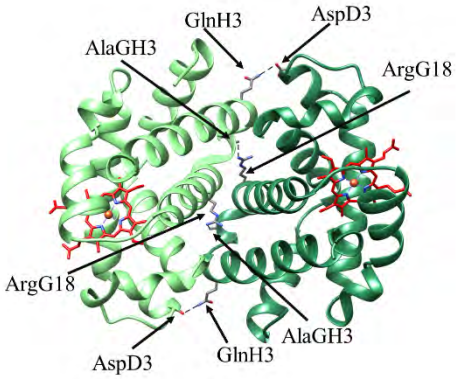
$\varepsilon_1\varepsilon_2$ interface



$\beta_1\beta_2$ interface



$\gamma_1\gamma_2$ interface



$\delta_1\delta_2$ interface

Thalassemic traits

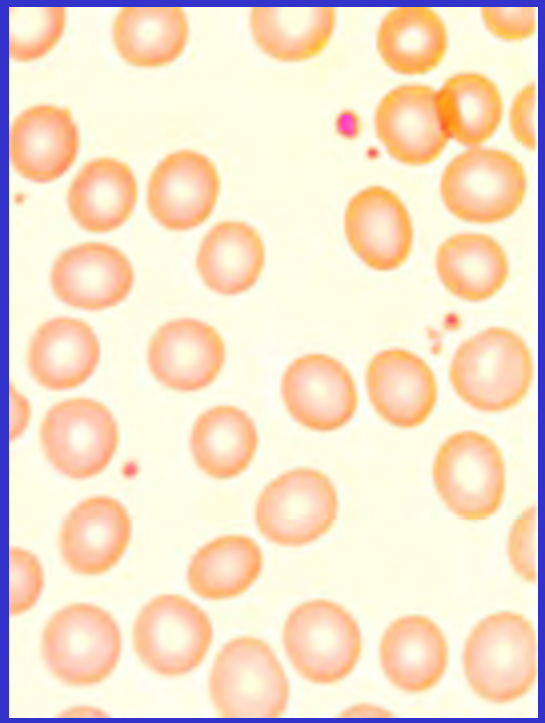
Anemia, beginning early in life
Ineffective erythropoiesis
Extramedullary hematopoiesis
Splenomegaly
Bone deformities
Characteristic *facies* (known as *chipmunk face*)
Red cells with increased resistance to osmotic lysis
Familial incidence

Von Jaksch, R., 1889. Über leukämie und Leucocytose im Kindesalter. Wien Klin. Wchnschr 2, 201

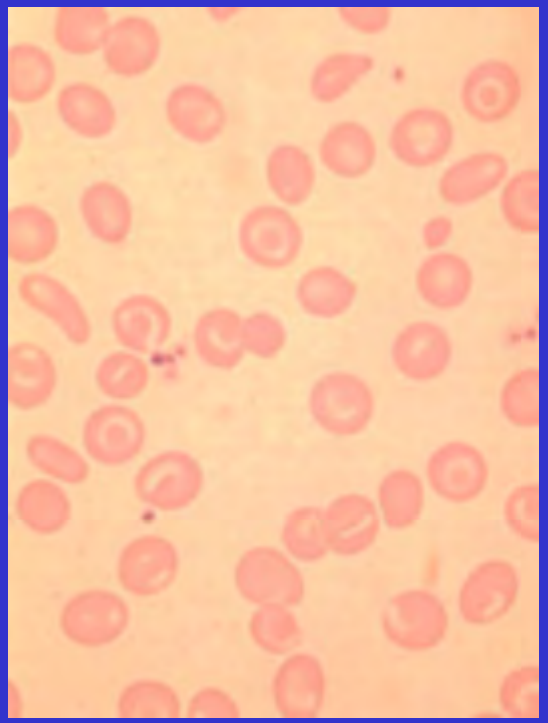
Cooley, T., Lee, P., 1925. Series of cases of splenomegaly in children with anemia and peculiar bone changes. Tr. Am. Pediat. 37, 29–30.

Rietti, F., 1925. Ittero emolitico primitivo. Atti Accad Scient Med Nat Ferrara 2, 14–19.

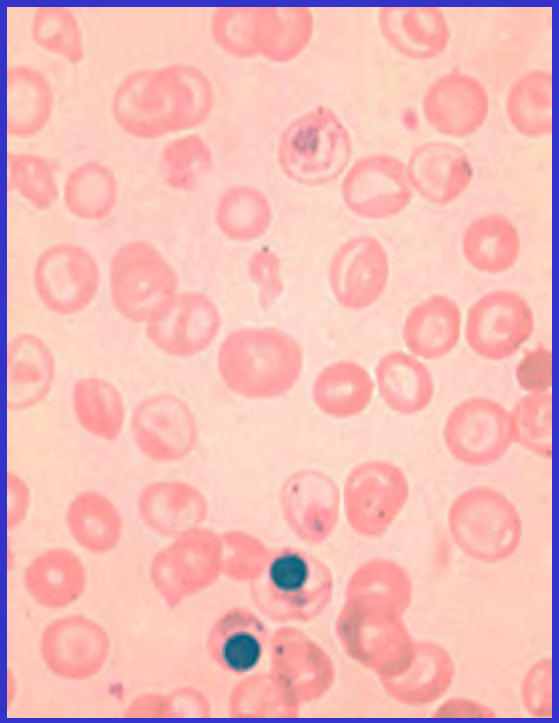
Normal and thalassemic red blood cells (× 1260)



Normal blood



Thalassemia minor

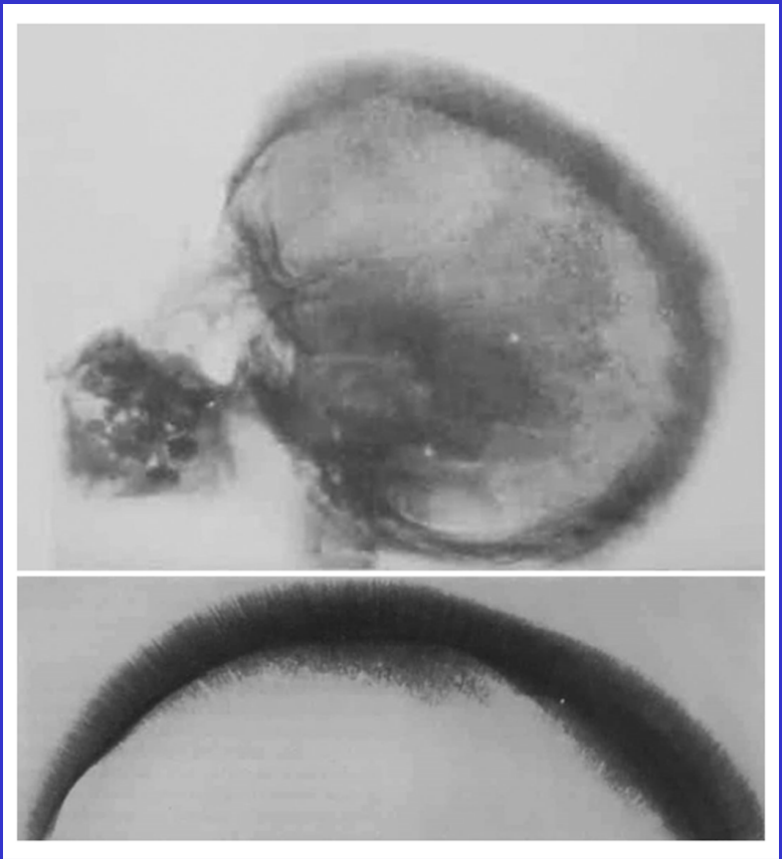


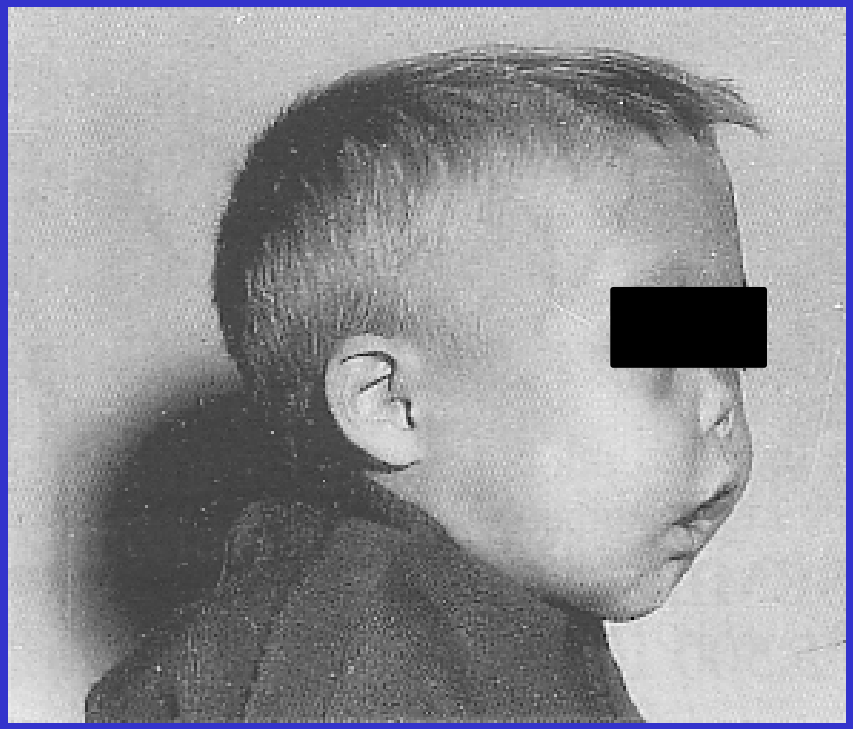
Thalassemia major

Blood smear: anisocytosis and poikilocytosis of red blood cells. Presence of erythroblasts

From: Kapff CT, Jandl JH, *Blood: Atlas and Sourcebook of Hematology*, Little Brown and Co. Boston, 1981.

Skull radiograph of an untransfused children with β-thalassemia major





From: Ascenzi A, Mottura G, *Trattato di Anatomia Patologica per il Medico Pratico*, Vol 1, UTET, Torino, 1971.

Bianco Silvestroni I, *Le Talassemie, Un Problema Medico-sociale: Ieri e Oggi*, Istituto Italiano di Medicina Sociale Editore, Roma, 1998.

Treatment of thalassemia

Blood transfusion and iron chelation

Pharmacological reactivation of human fetal hemoglobin

Hematopoietic stem cell transplantation

Gene therapy

Gene editing

Blood transfusion and iron chelation

Blood transfusion maintains adequate Hb levels and reduces hypoxia and its consequences:

Massive expansion of erythroid precursors in hematopoietic tissues

Bone deformities

Hepatosplenomegaly → splenectomy

The target Hb levels are 9-12 g/dl

The iron chelation therapy prevents hemosiderosis in both transfused and non-transfused patients

The target ferritin levels are < 1000 ng/ml

Iron chelators: deferoxamine, deferiprone, deferasirox

Pharmacological reactivation of human fetal hemoglobin

In β- δ-, δβ-, thalassemia, the excess of α-chains can be neutralized by γ-chains

Drugs raising HbF expression levels

5-azacytidine → demethylating agent
Butyrate → hystone deacetylation inhibitor
Hydroxyurea → ribonucleotide reductase inhibitor
→ soluble guanylate cyclase activator

Hematopoietic stem cell transplantation

Life-time therapy

20 years of thalassemia-free survival is 73%

Production of normal Hb

Defective stem cells are destroyed

Heterologous transplantation

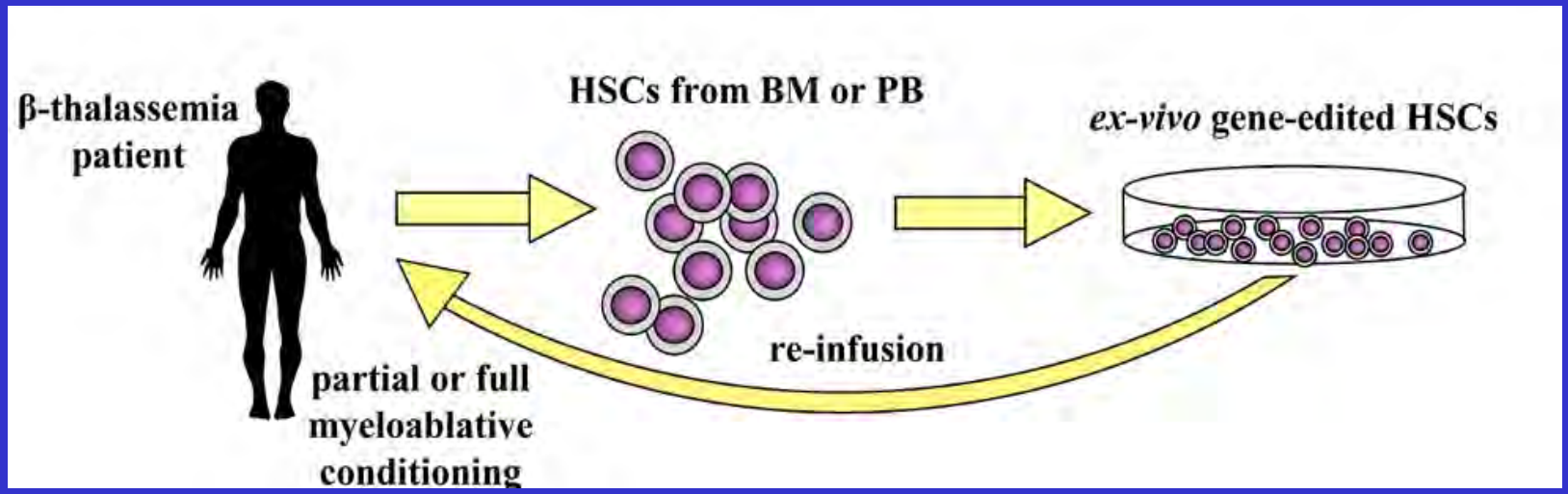
Immunologically matched donor (<20% of the patients)

Immunosuppression

Thomas ED, et al. (1982) Marrow transplantation for thalassaemia. Lancet 31, 227–229.

Lucarelli G, et al. (1987) Marrow transplantation in patients with advanced thalassemia. N Engl J Med. 316, 1050-5.

Gene therapy and genome editing approaches for transfusion-dependent β-thalassemia



Hematopoietic stems cells derived from the patient are genetically modified and re-infused into fully or partially myeloablated patient's bone marrow

Normal differentiation of erythropoietic cells

Life-time therapy

Possible drawbacks: tumor formation, viral toxicity, and germ-line transfer

As a whole and perspectives

Prenatal diagnosis

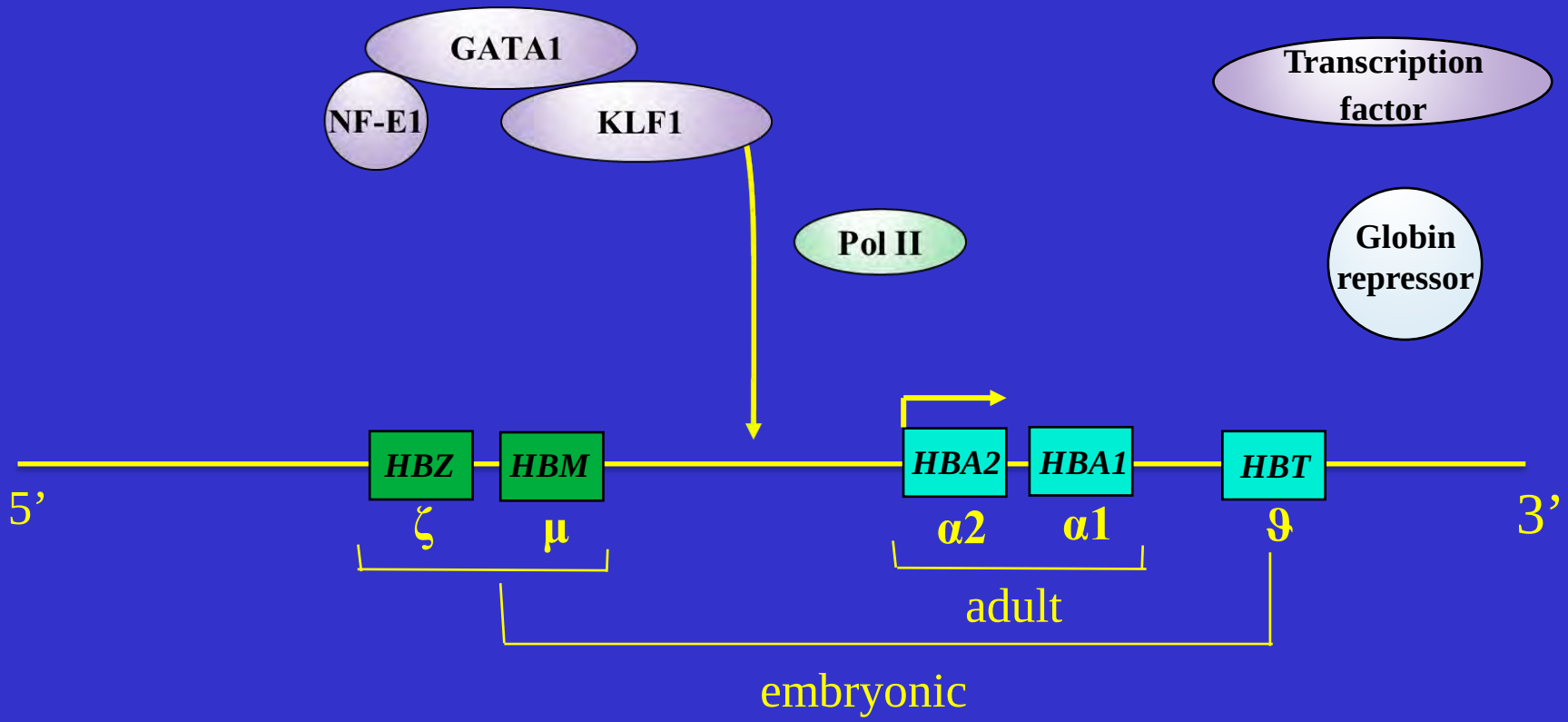
Therapy

Blood transfusion and iron chelation
Bone marrow transplantation
Pharmacologic HbF induction
Gene therapy and genome editing

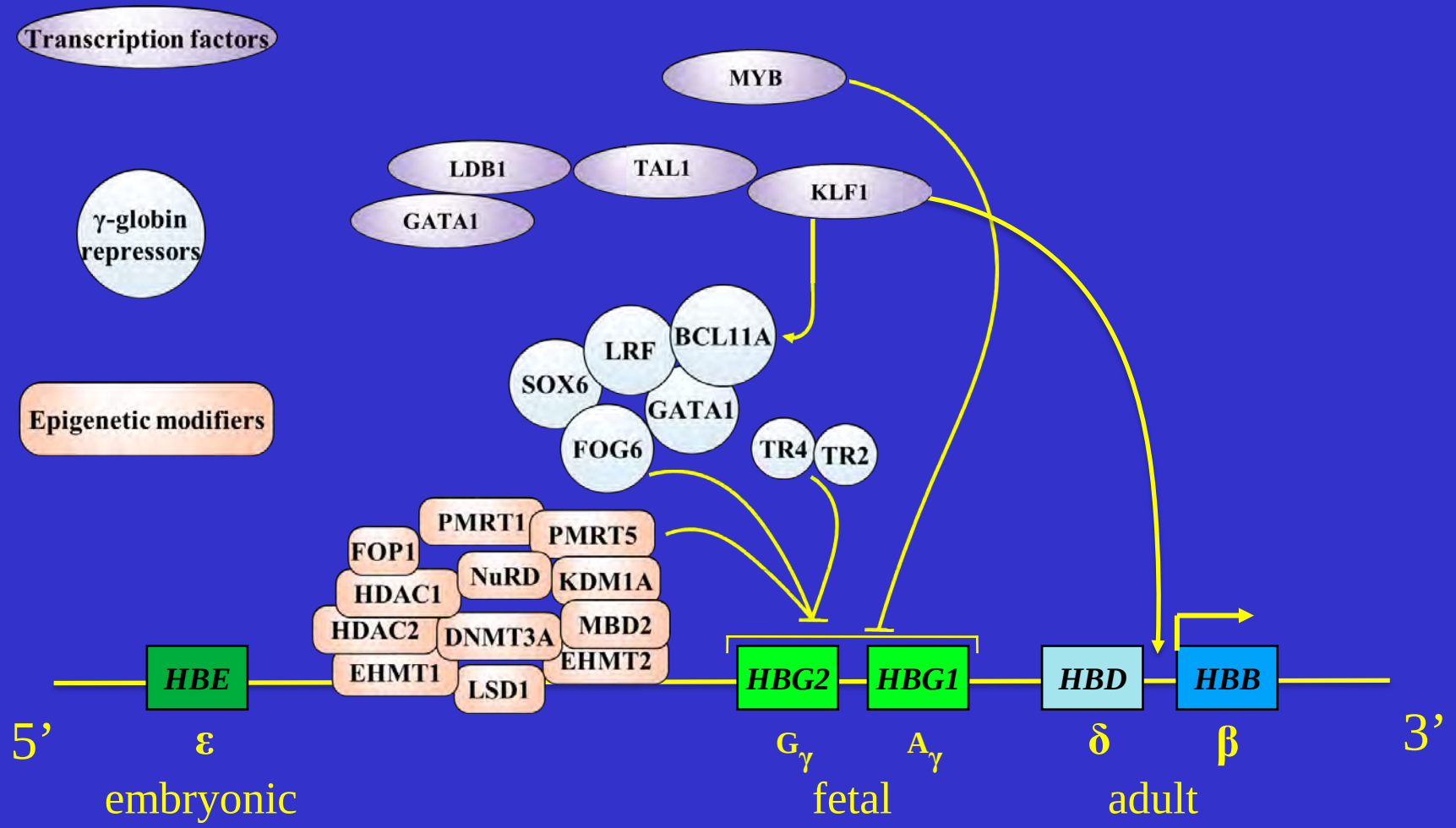
Thalassemia
The Neverending Story

(Michael Ende)

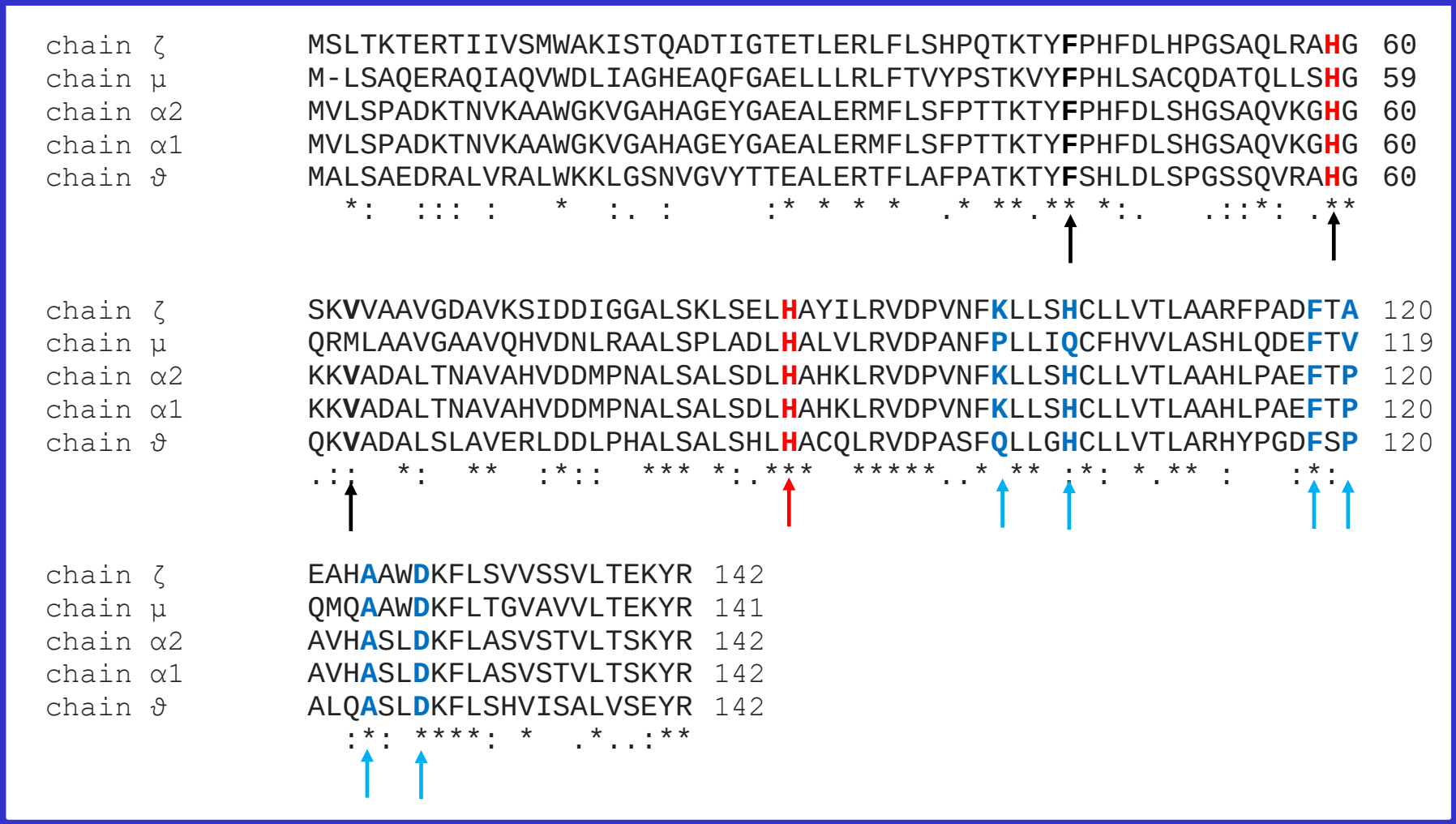
Modulation of the gene cluster α - *ch16*



Modulation of the β-globin gene cluster - *ch11*

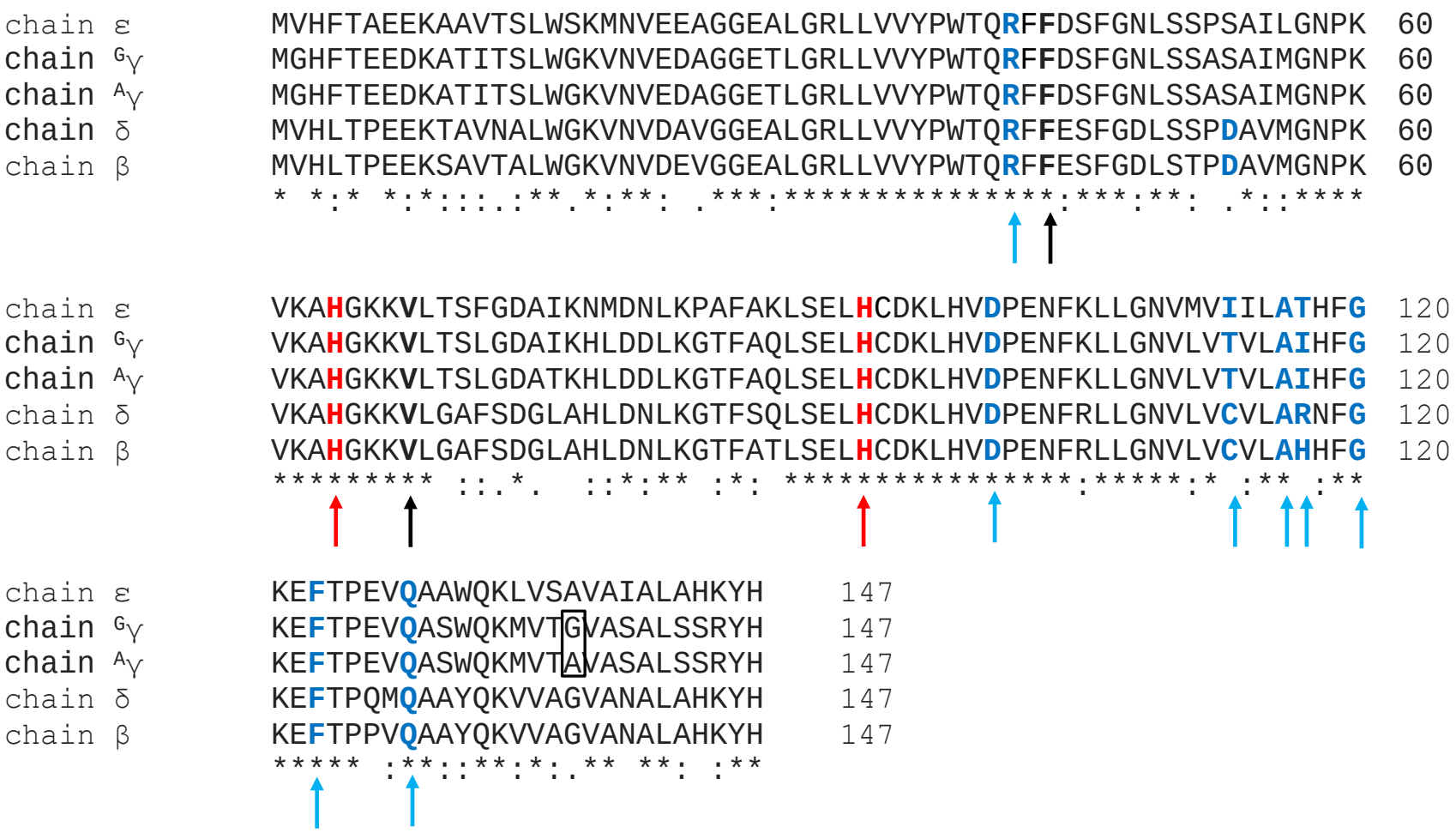


Multiple alignment of human Hb chains belonging to the α-chain cluster

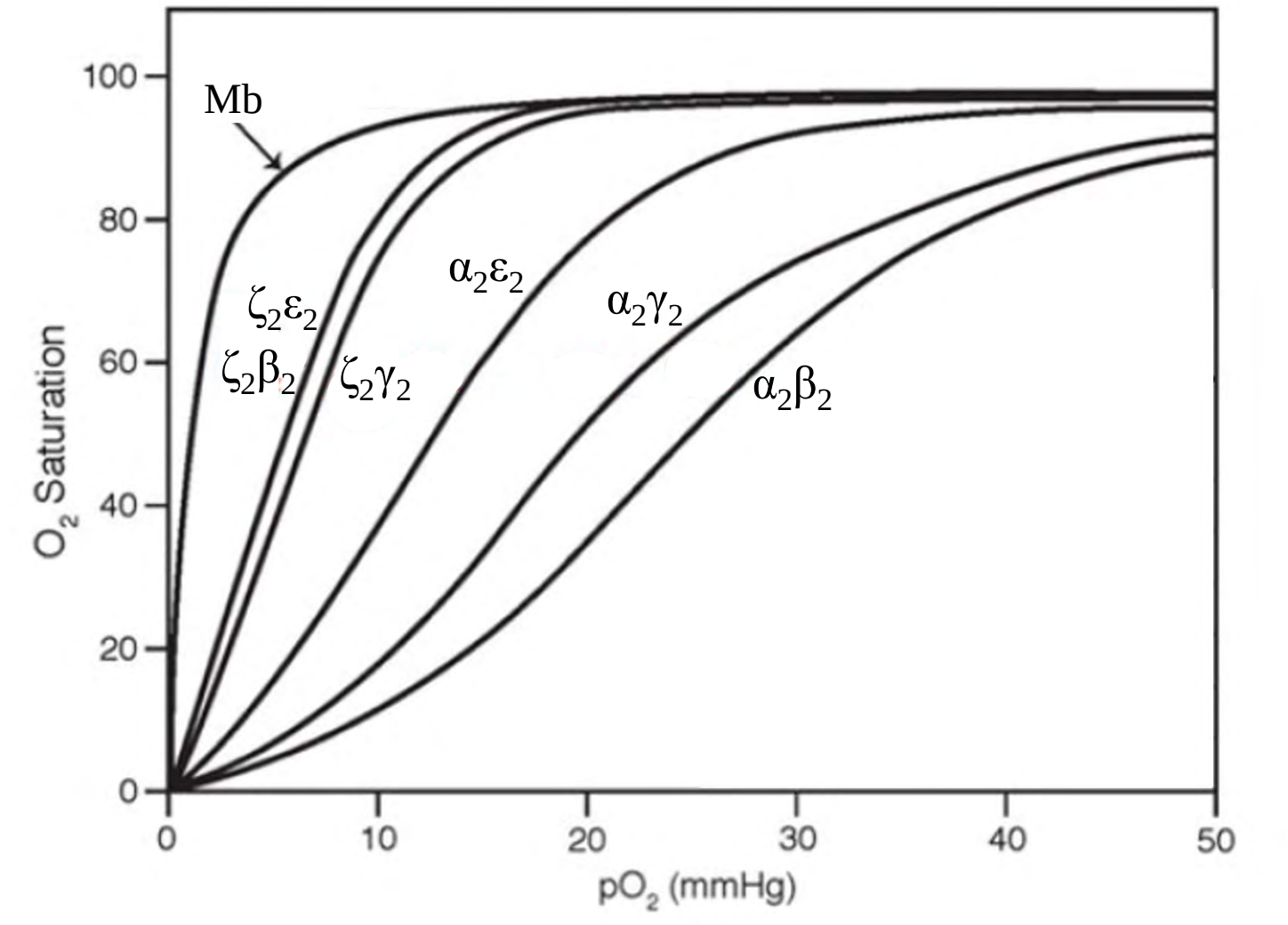


Chains α2 and α1 differ over the 5’ and 3’ untranslated regions and introns

Multiple alignment of human Hb chains belonging to the β-chain cluster



Range of O_2 saturation of normal human Hbs



Manning J.M., et al., Vertebrate and Invertebrate Respiratory Proteins, Lipoproteins and Other Body Fluid Proteins, pp. 275-296, Springer Nature, 2020.

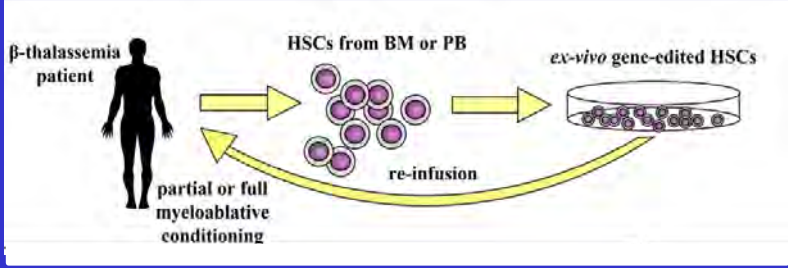
O_2 affinity of human Hbs

Stage of development	Type of Hb		P_{50} value (mmHg)	Hill coefficient
Embryonic Hb (< 12 weeks)	$\zeta_2\varepsilon_2$	Gower I	4	1.9
	$\zeta_2\gamma_2$	Hb Portland	6	2.3
	$\alpha_2\varepsilon_2$	Gower II	12	2.4
Fetal (3-9 months)	$\alpha_2\gamma_2$	HbF	20	2.7
Adult (from birth)	$\alpha_2\delta_2$	HbA2	6.5	2.1
	$\alpha_2\beta_2$	HbA	26	2.8
	β_4		0.5	1
	γ_4	Hb Bart's	0.26	1
	α		0.5	1

Antonini E. and Brunori, M., Hemoglobin and Myoglobin in their Reactions with Ligands, p. 310 North-Holland Publishing Co., Amsterdam, London, 1971.

Manning J.M., et al., Vertebrate and Invertebrate Respiratory Proteins, Lipoproteins and Other Body Fluid Proteins, pp. 275-296, Springer Nature, 2020.

Gene therapy

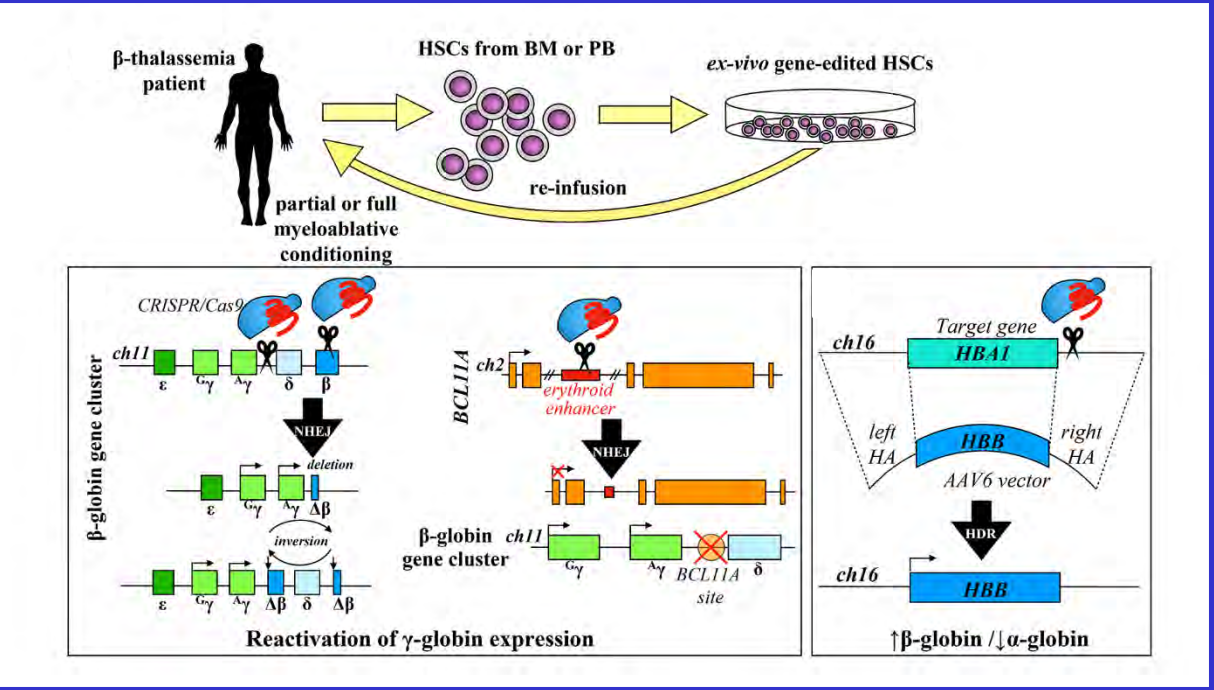


Retroviral vectors for β-thalassemia gene therapy								
Retroviral vector name and type	Sequence elements							Clinical trial
	β-globin			Insulator	β-globin LCR			
	Promoter (bp)	Protein	Intron 2 (bp)		HS2 (bp)	HS3 (bp)	HS4 (bp)	
Mβ6L, γ-retrovirus	-265	WT	374 del	no	423	280	283	N.A.
TNS9, lentivirus	-265	WT	374 del	no	840	1308	1069	Pre-clinical
GLOBE, lentivirus	-265	WT	593 del	no	~1500	~1200	no	Phase I/II
HPV569, lentivirus	-265	βT87Q	372 del	cHS4	646	845	1153	Phase I/II
TNS9.3.55, lentivirus 2014	-615	WT	372 del	no	840	1308	1069	Phase I
TNS9.3.55.A1, lentivirus	-615	WT	~ 370 del	A1	840	1308	1069	N.A.[1]
BB305, lentivirus	-265	βT87Q	372 del	no	646	845	1153	Phase I/II Phase III
LVβ-shα2, lentivirus	-265	βT87Q	374 del + shmiR cassette [q]	no	646	845	1153	N.A.

Lentiviral vector for HbF induction					
Retroviral vector name and type	Sequence elements				Clinical trial
	Expression cassettes		Regulatory sequences		
thEPOR/shmiR BCL11A, lentivirus	ΔEPOR	shmiR cassette	α-globin LCR HS40	ANK	Pre-clinical

Normal differentiation of erythropoietic cells
Gene therapy could lead to tumor formation, viral toxicity, and germ-line transfer

Genome editing



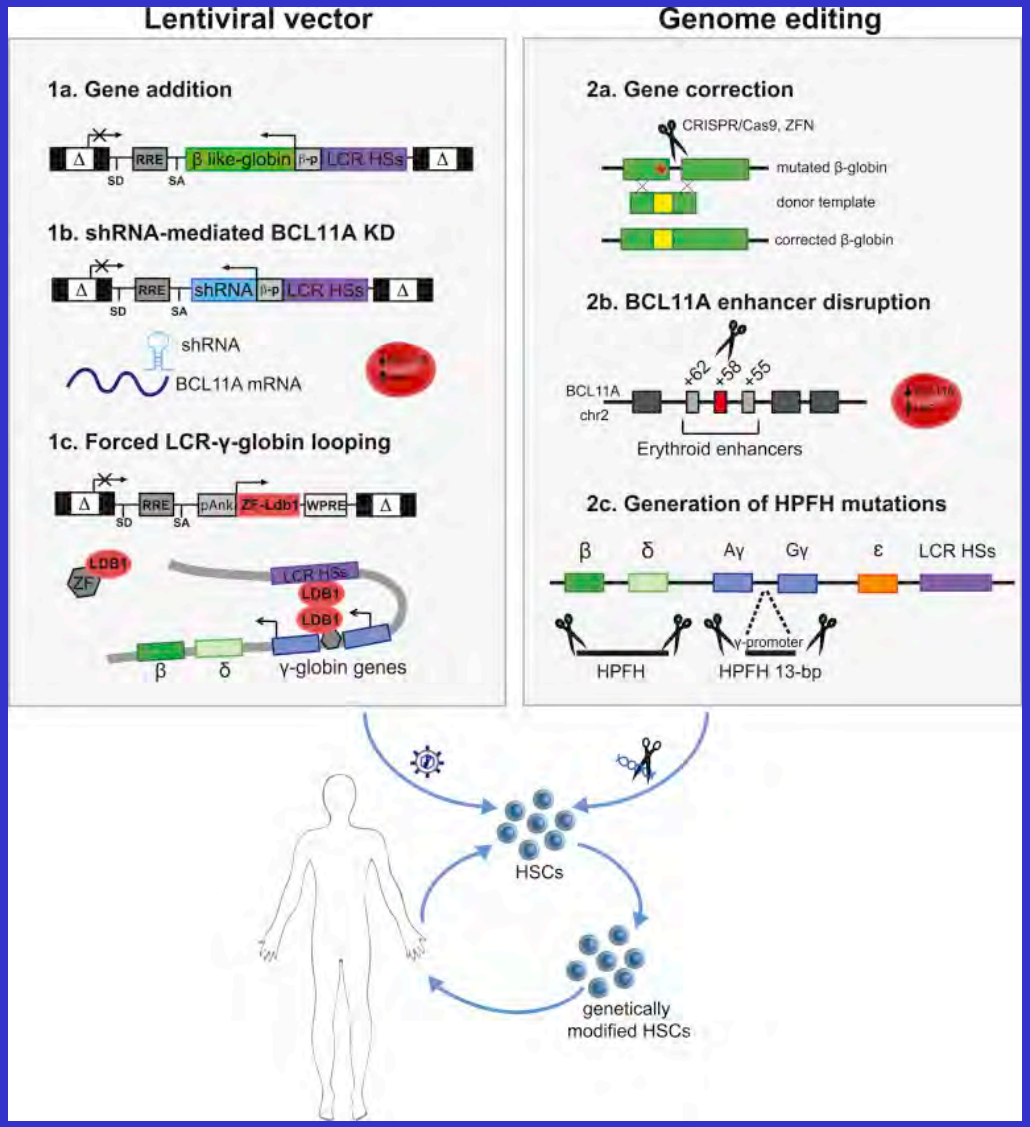
No immunosuppression is required

ife-time therapy

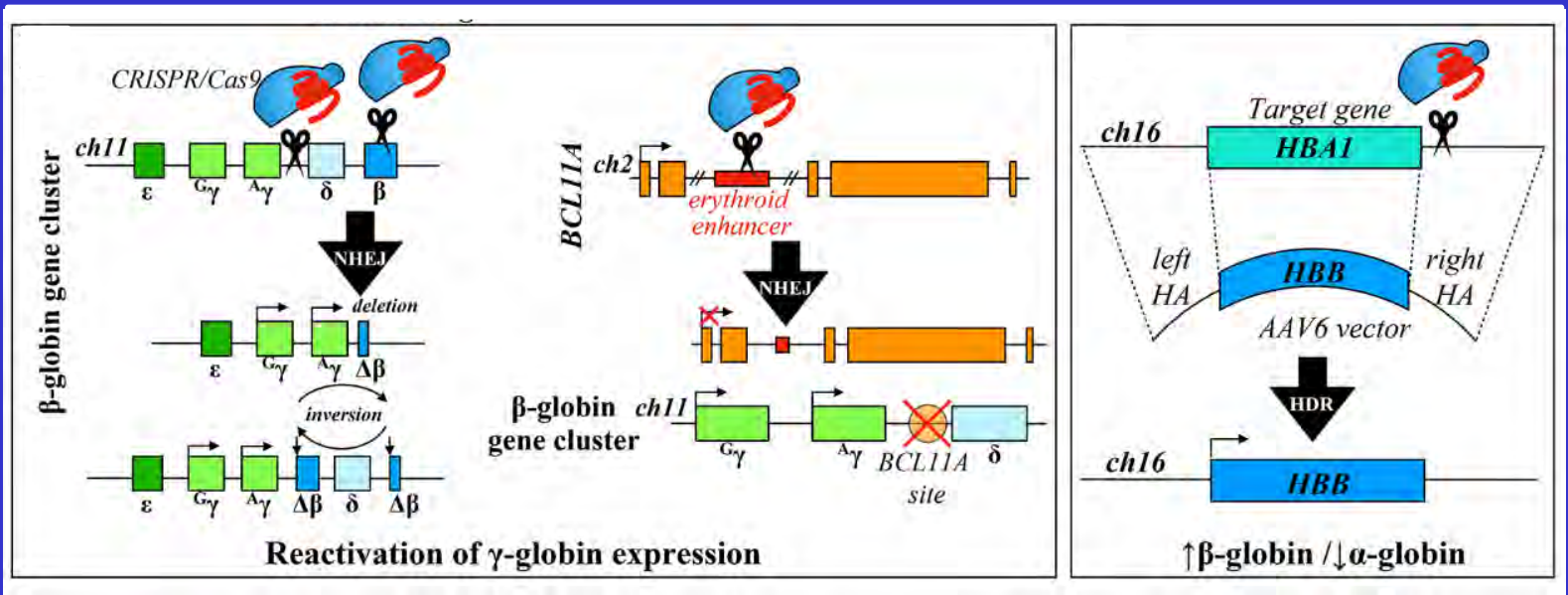
Recipients can produce healthy children

Can show off-target activity

Novel Therapeutic Approaches for β-Hemoglobinopathies

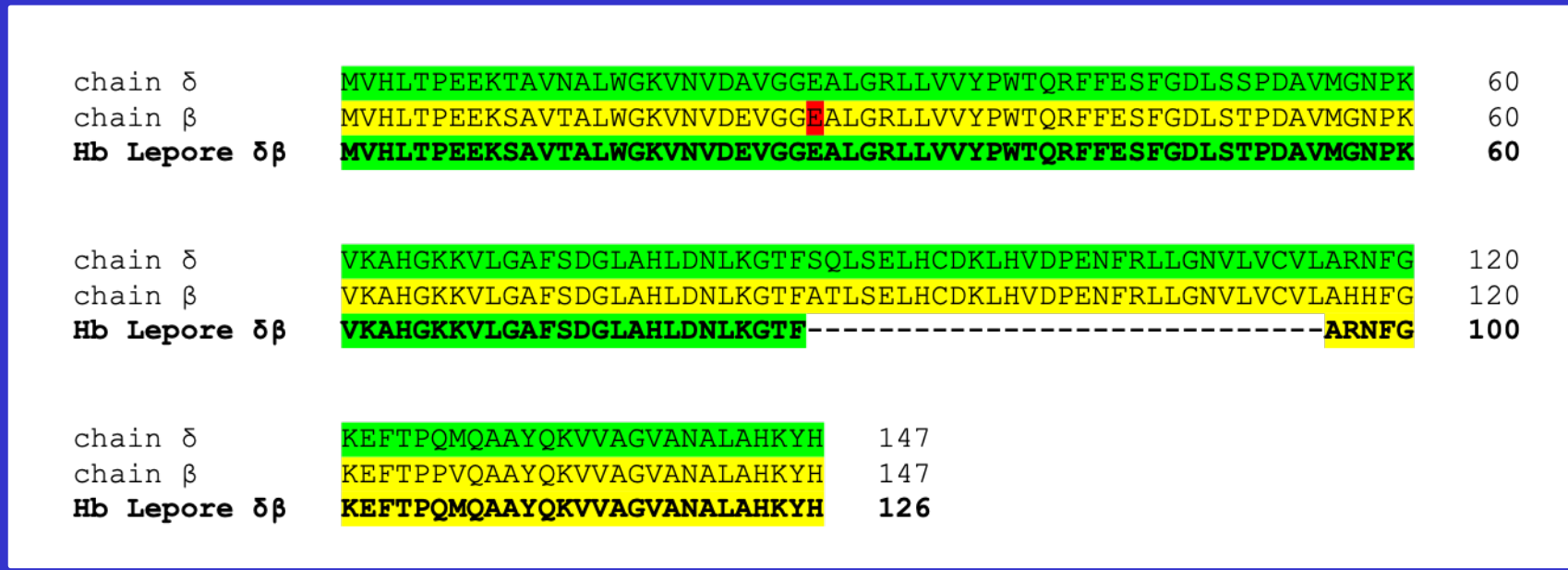


Gene editing



No immunosuppression is required
Life-time therapy
Recipients can produce healthy children
Can show off-target activity

The β-thalassemia paradox: Hb Lepore δβ and HbE (Glu→Lys)



Gerald, P. S., & Diamond, L. K. (1958). A new hereditary hemoglobinopathy (the Lepore trait) and its interaction with thalassemia trait. *Blood*, 13(9), 835–844.

Fucharoen, S., & Weatherall, D. J. (2012). The hemoglobin E thalassemias. *Cold Spring Harbor perspectives in medicine*, *2*(8), a011734.