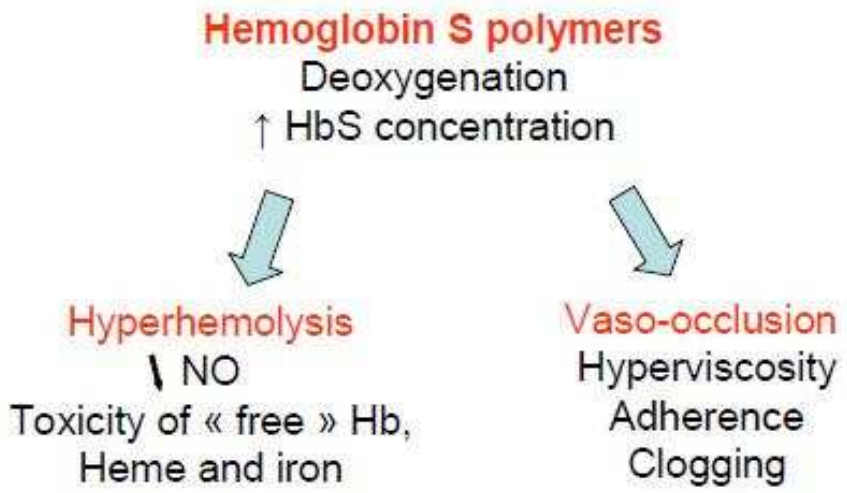
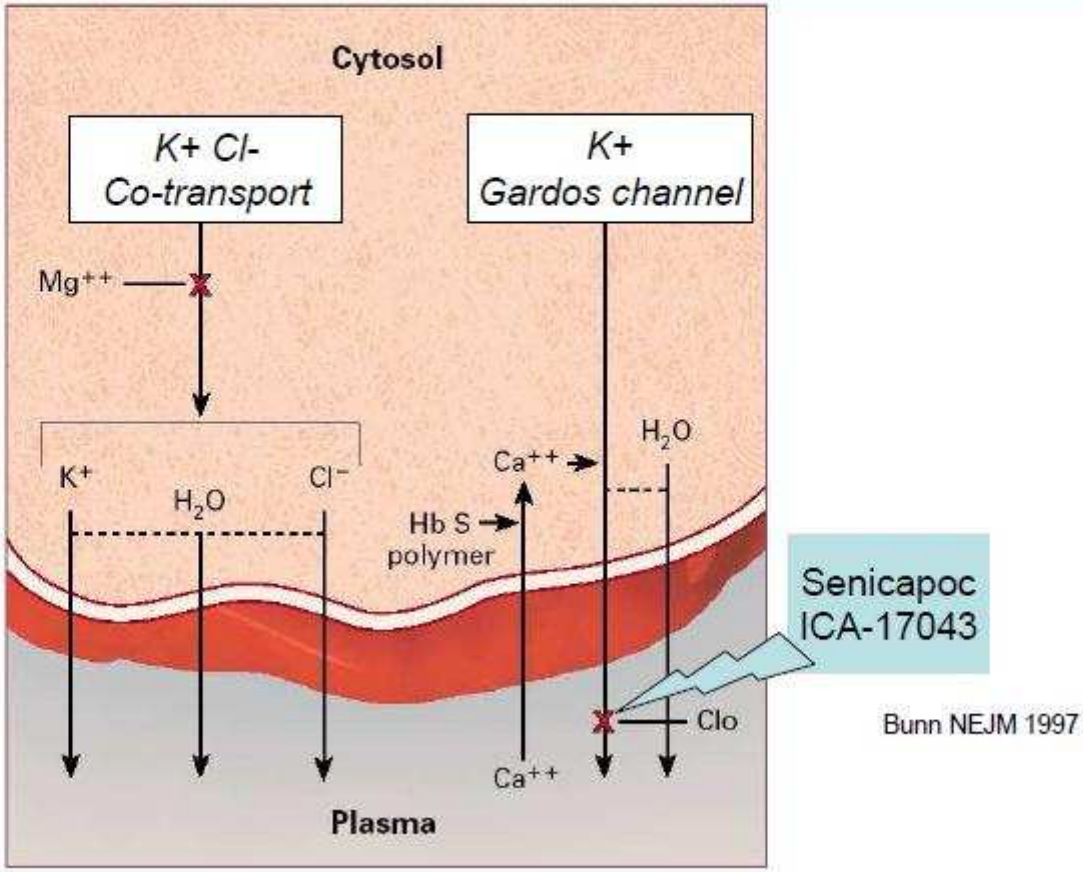
Towards Therapies of Hemoglobin Disorders Targeting Erythroid cells

Yves Beuzard (ESH / EHA / ENERCA) 2010

I Sickle cell disease



Erythrocyte dehydration



Bunn NEJM 1997

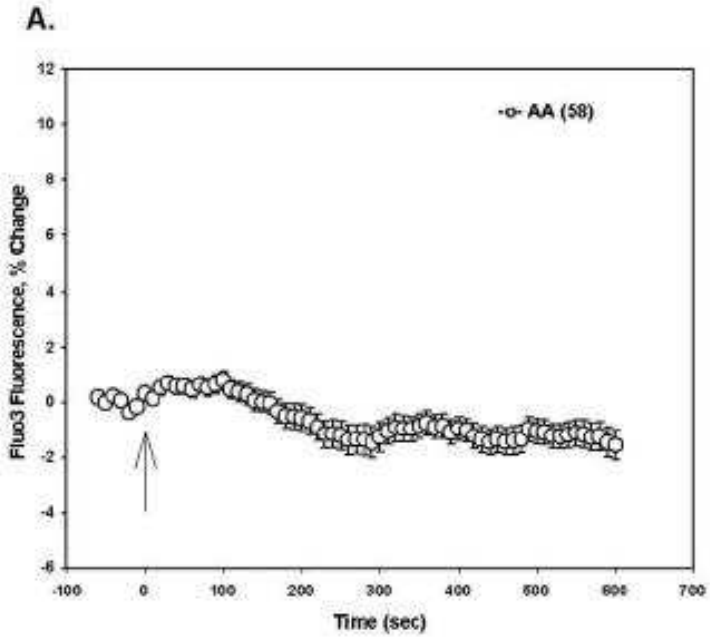
Senicapoc effects

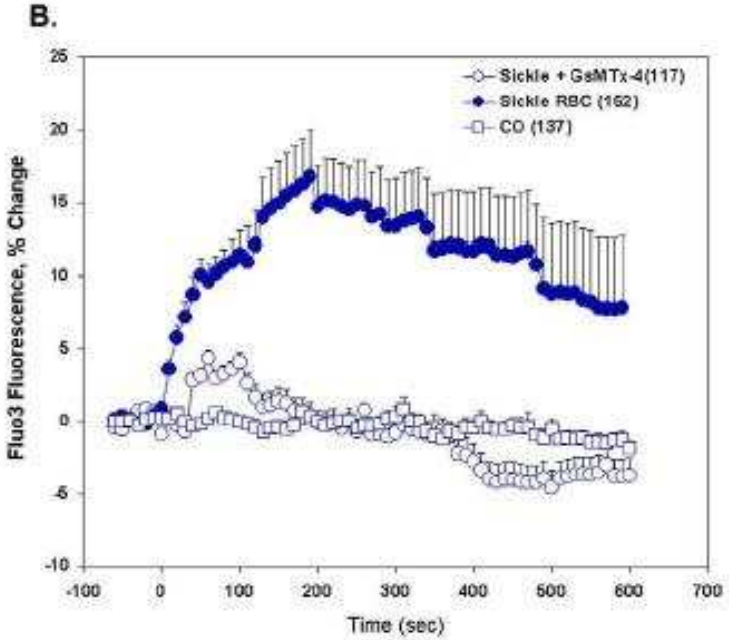
Ataga et al. Blood 2008

- Phase II and phase III study
  - ↓ Hyperhemolysis
  - ↑ Hemoglobin
  - No reduction of sickle cell painful crisis
- New phase III study

Increased [Ca^{2+}] in Sickle Cells Upon Deoxygenation

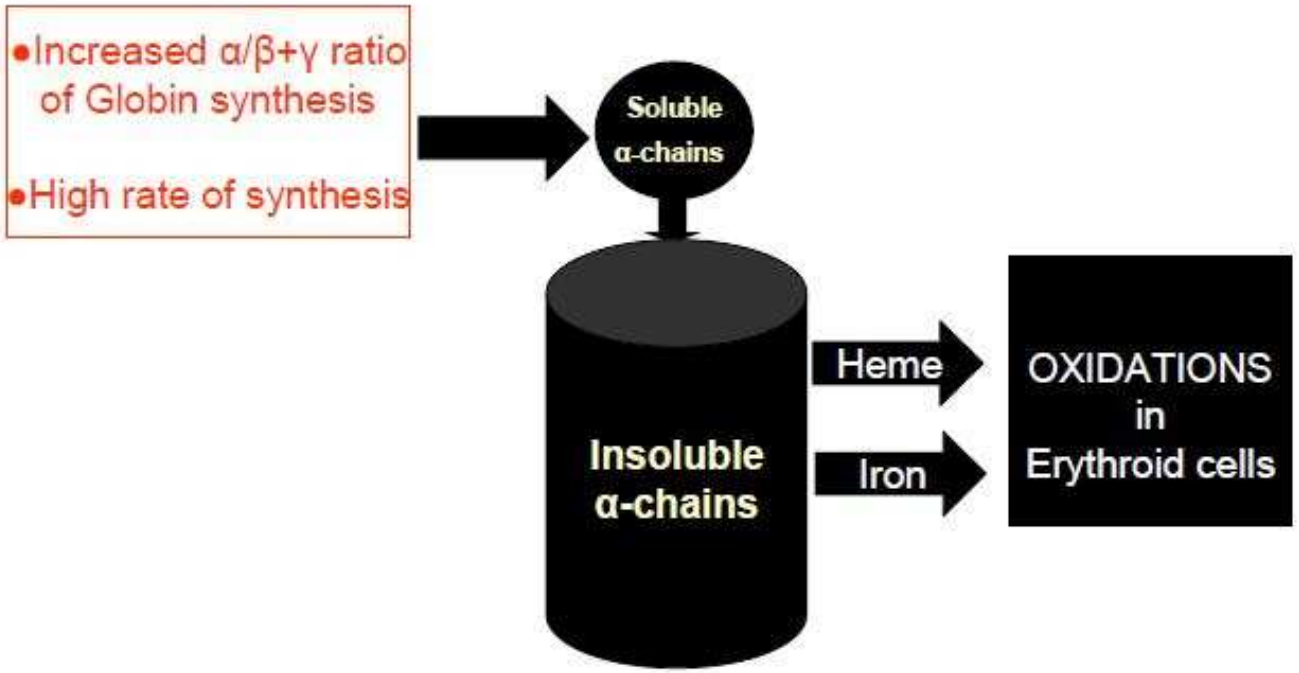
Vandorpe Plos one 2010





GsMTx-4: Grammastola spatulata mechanotoxin-4

II β-Thalassemia



Molecular Factors Modulating β-Thalassemia

- 1) α/β + γ ratio in reticulocytes
  - Human heterozygous β-thal. = 2 (normal = 1)
    : asymptomatic state
  - Human homozygous β-thal. > 2
    : thalassemia major (transfusion dependent)
    : thalassemia intermedia
  - Human dominant mutant = 2
  - Mouse homozygous β-thal ≈ 1. 25
    » Compensation with minor β globin
    » More severe disease

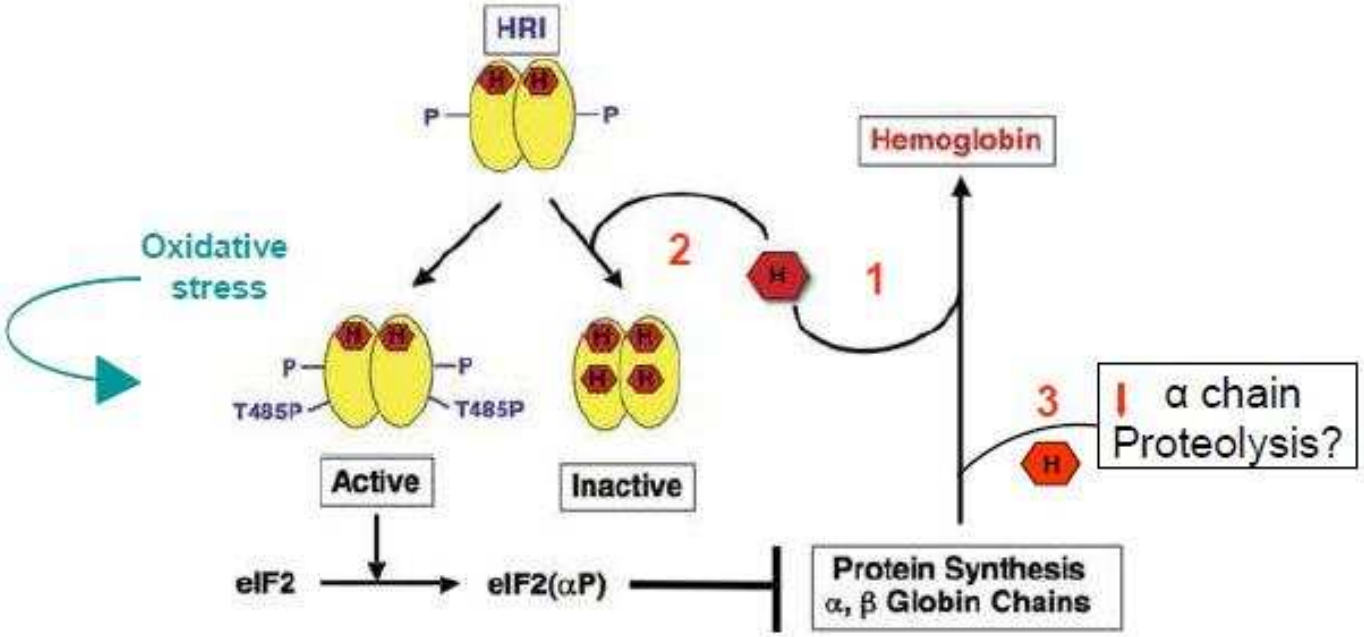
Efficiency of protein synthesis

– Heme regulation of hemoglobin synthesis

Protein synthesis
- ↓ Iron or heme deficiency
- ↓ Oxidative stress
- ↑ Inhibition of heme Regulated Inhibitor (HRI)

Regulation of protein synthesis by Heme

JJ Chen Blood 2007

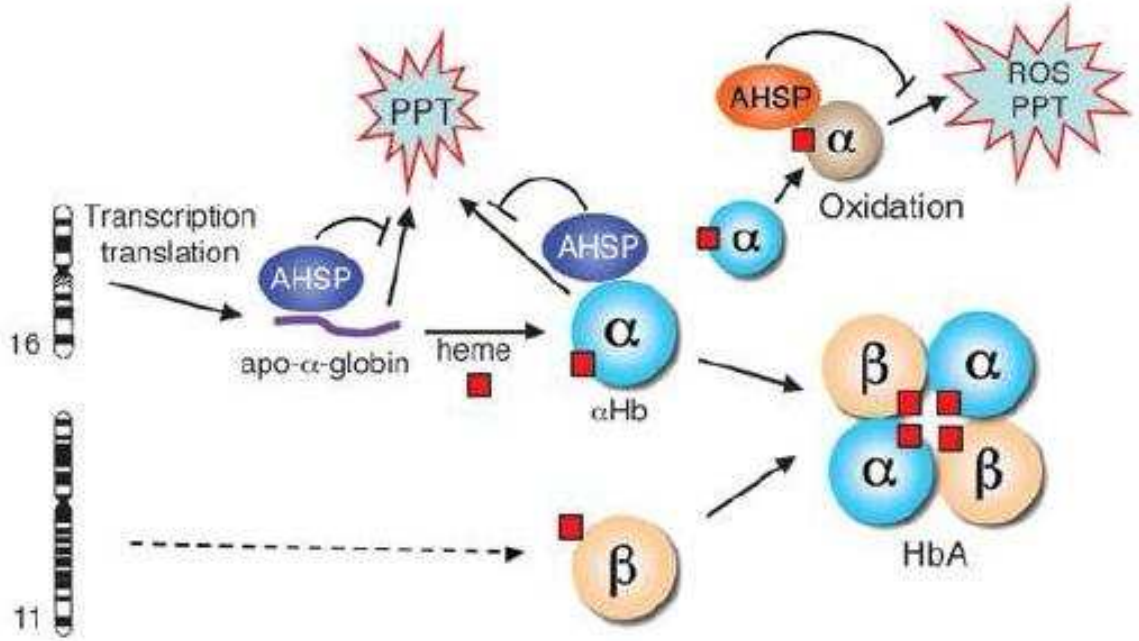


Molecular Factors Modulating β-Thalassemia

- 3) Stability of unpaired globin chains
  - AHSP (**α** **H**emoglobin **S**tabilizing **P**rotein)
- 4) Stability of unpaired α hemoglobin chains
  - AHSP
- 5) Stability of hemoglobins
  - Oxidative damage

Roles of AHSP
α-Hemoglobin Stabilizing Protein

Mollan and Weiss 2009



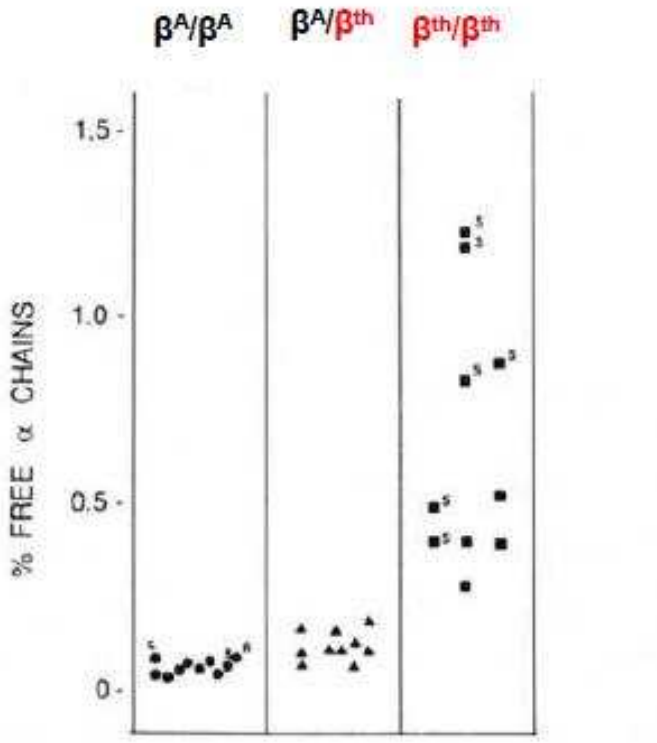
Molecular Factors Modulating β-Thalassemia

- Proteolysis of unpaired α globin and hemoglobin chains
  - ATP dependant
  - ATP independant
- Proteolysis of oxidized (instable) hemoglobin

Pools of α chains

- Soluble pools
  - α-globin
  - α-hemoglobin
- Insoluble pools
  - α-globin
  - α-hemoglobin

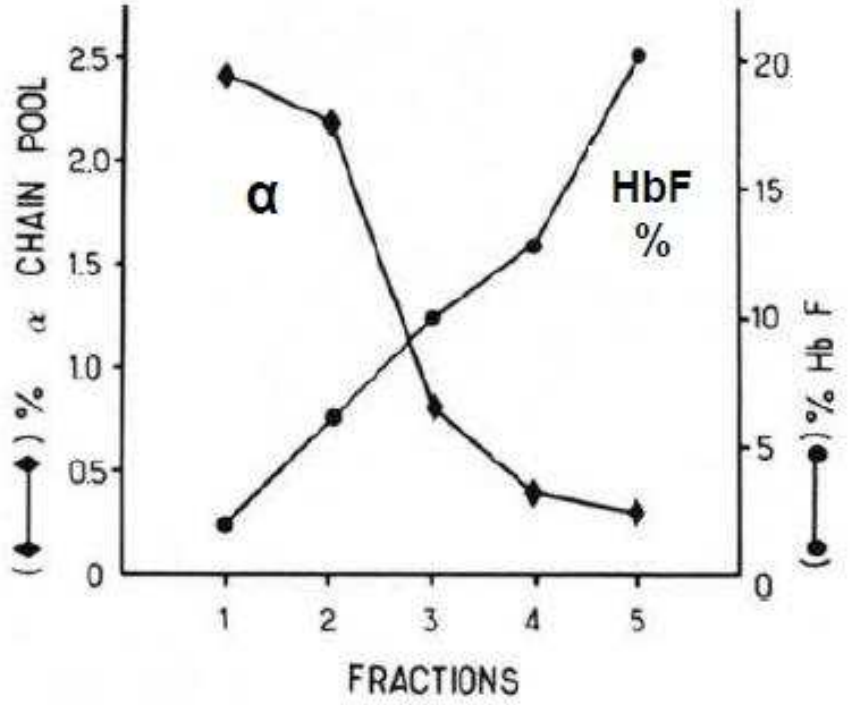
Soluble α Chain Pool in β-thalassemia intermedia



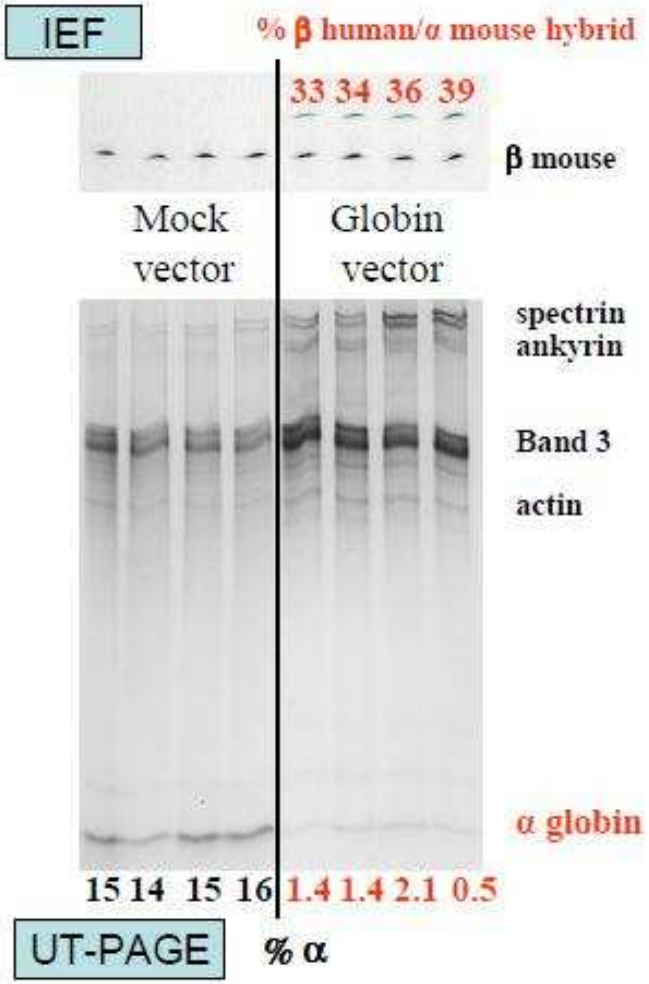
S : splenectomy
R : high reticulocyte counts in controls

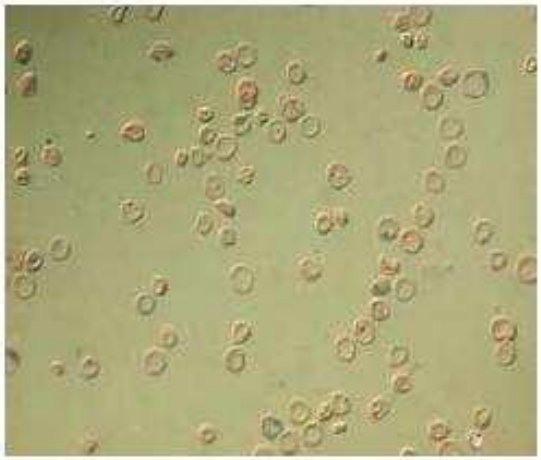
Soluble α Chain Pool in Blood Cells in β thalassemia intermedia

(Cells separated by density gradient)



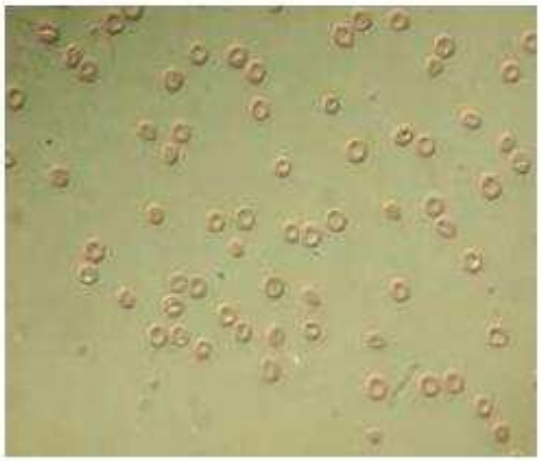
Correction of mouse β-thalassemia (Hbbthal-1/Hbbthal-1) upon upon Lenti-Globin gene transfer





Mock vector

Hb = 9 g/dL



Globin vector

Hb = 13 g/dL

Imren, PNAS 2002

Molecular Factors Modulating β-Thalassemia

Oxidative stress of erythroid cells

- α-Hemoglobin chains
- Free heme
- Free iron

} ROS

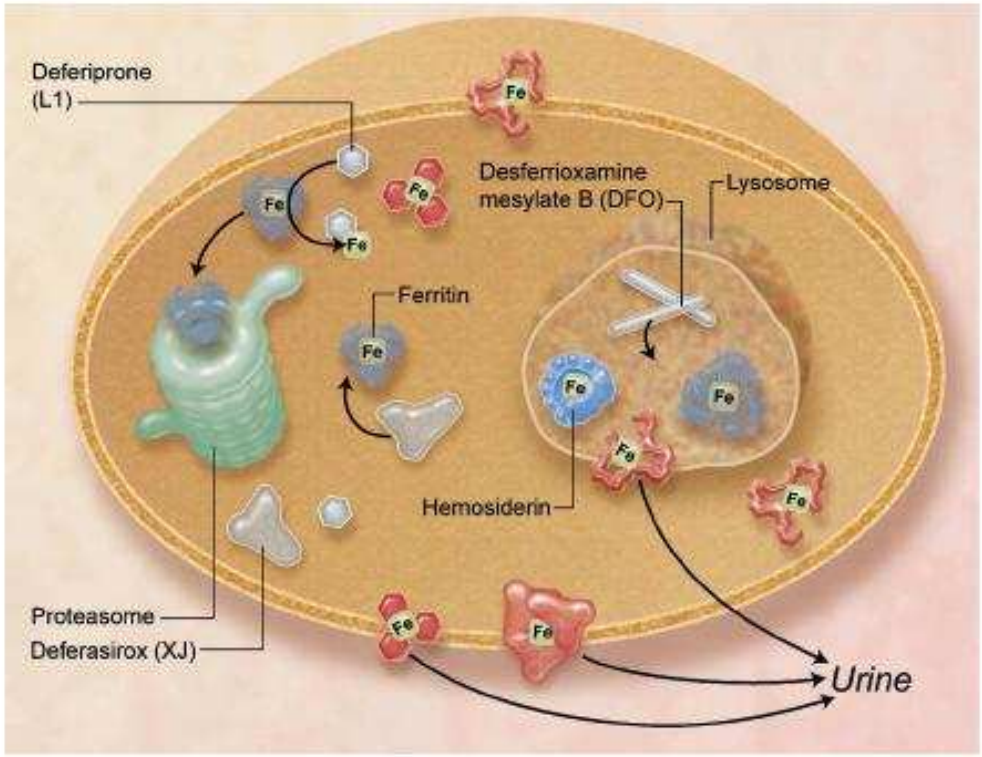
Oxidative damages of erythroid cells

- Proteins (membrane, enzymes, Hb……)
- Lipids
- Transports (KCl cotransport, P-serine….)
- Reducing agents (GSH, Vit. E……)

Cellular Factors Modulating β-Thalassemia

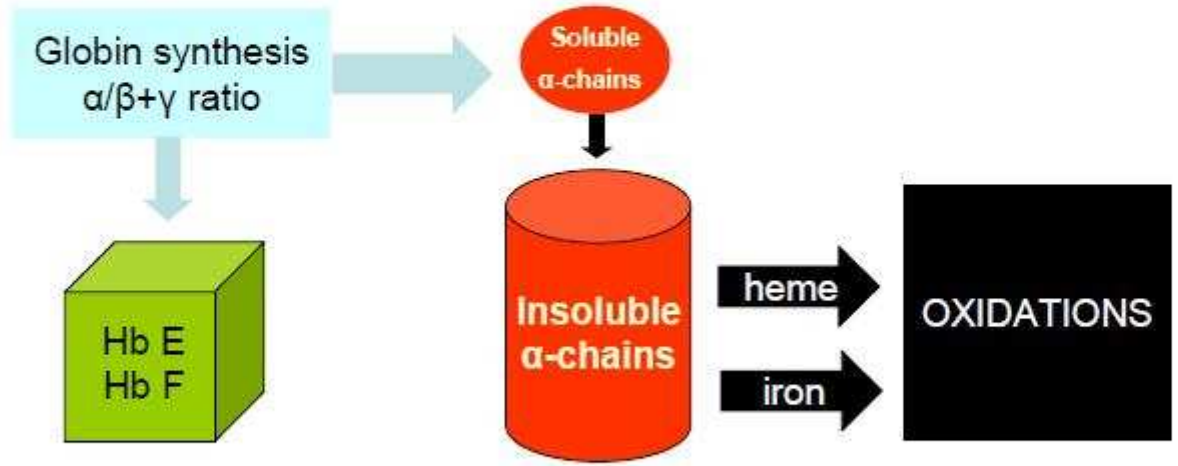
- Ineffective erythropoiesis
  - Proliferation/maturation imbalance
  - Apoptosis of late erythroblasts
  - Inflammation
  - « suboptimal» level of Epo
- Increased cell heterogeneity
  - Erythropoeitic stress
- Hemolysis
  - Extravascular
  - Intravascular

Chelation of intracellular iron



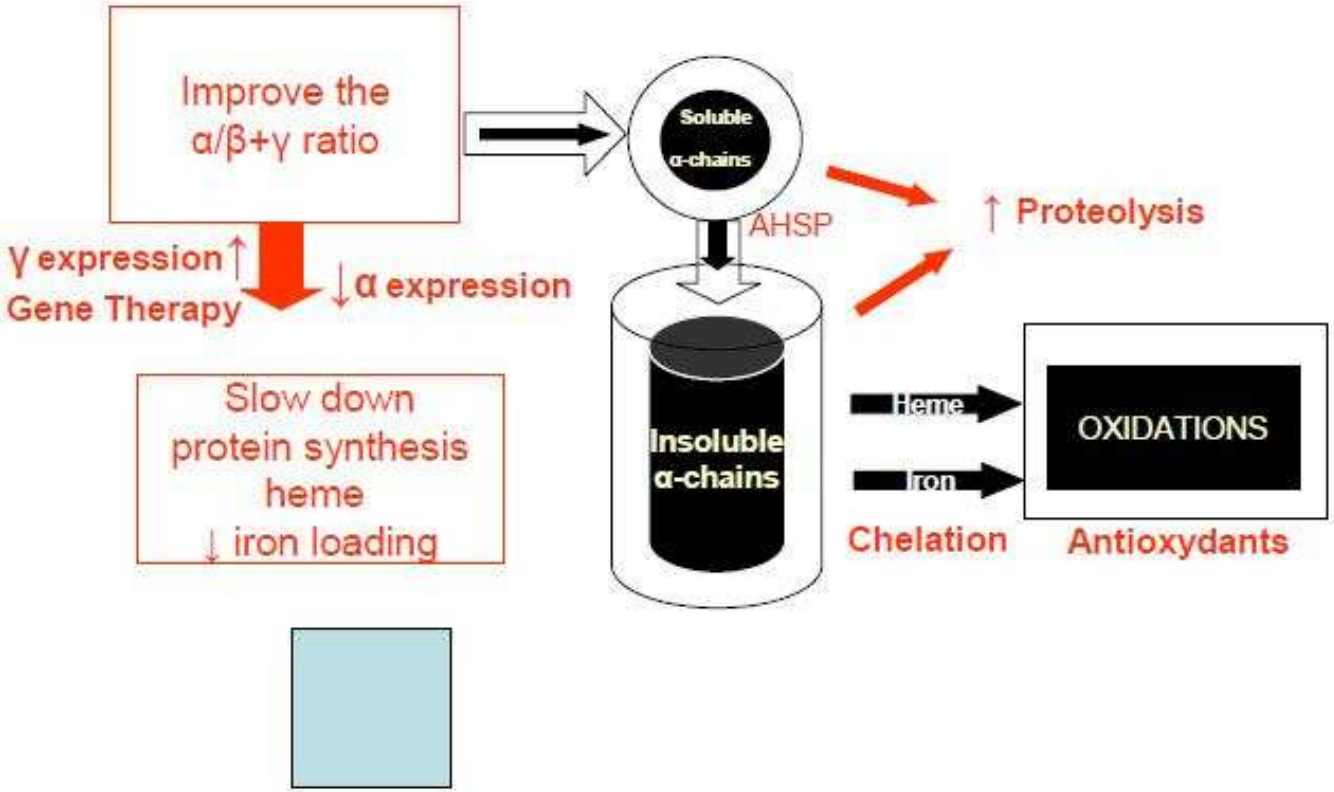
(De Domenico et al. Blood 2009) (Theil Blood 2009)

Conclusion I
Biological assessment of severity



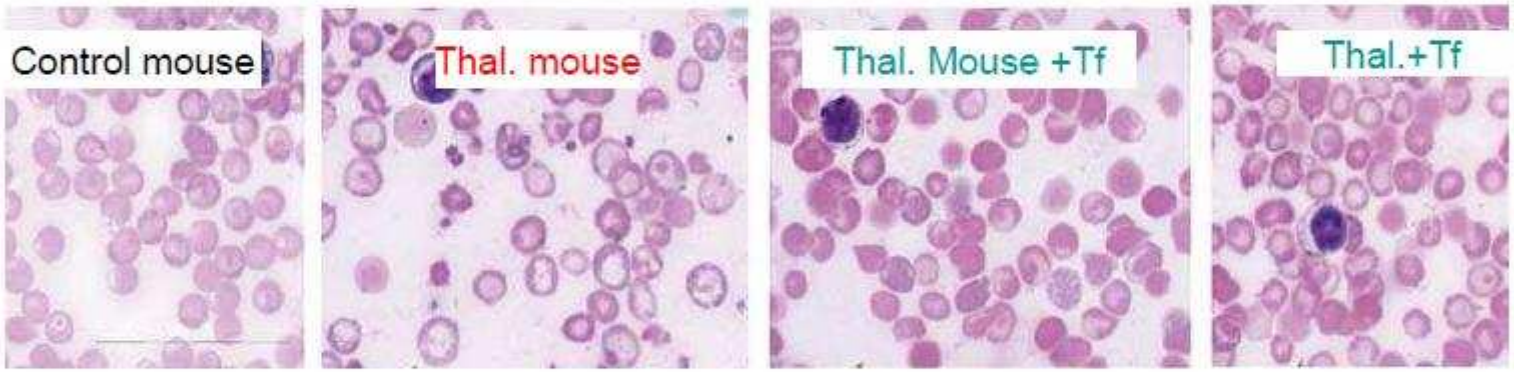
pools of
α chains

Conclusion
Therapeutic targets

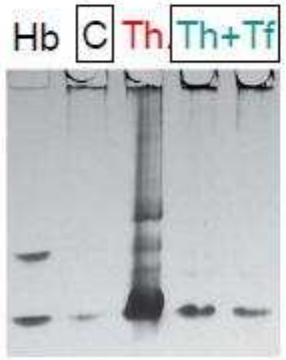


Transferrin (Tf)Therapy in β-thalassemic mice

Huihui Li et al *Nature Med* 2010



α globin precipitation
in red cell ghosts
(UT-PAGE)



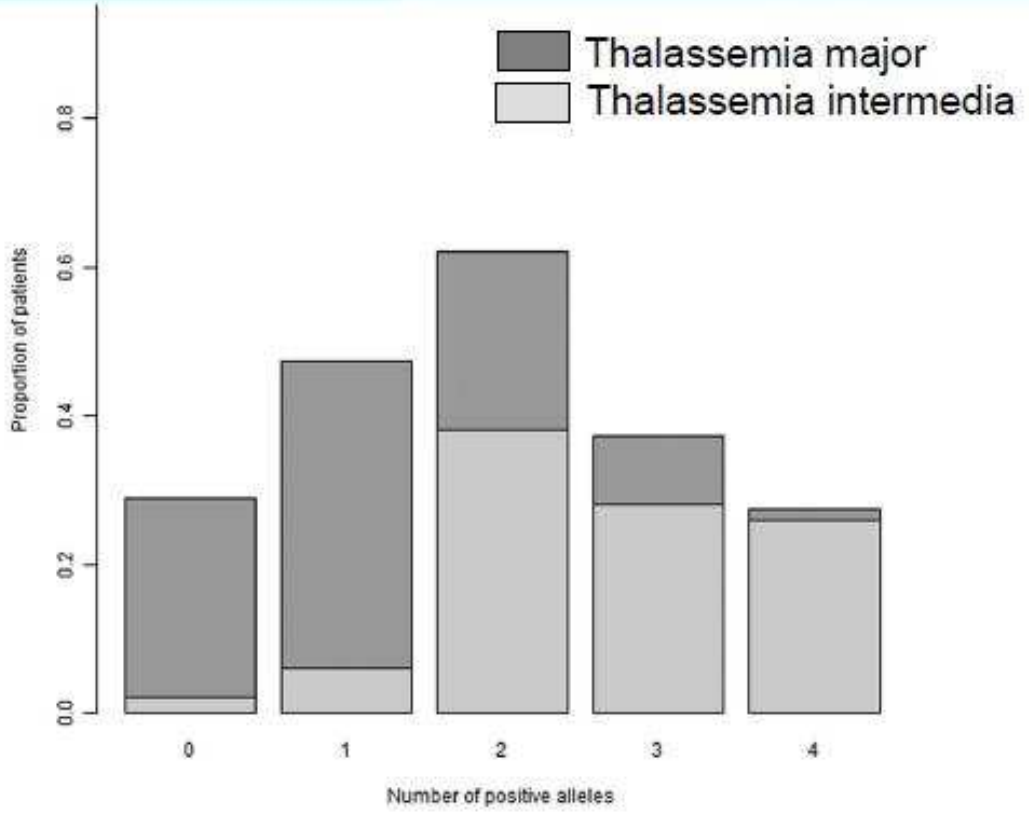
Conclusion II
Therapeutic targets

- Suppression of dyserythropoiesis
  - Jak 2 inhibitor + transfusion
  - HU (HydroxyCarbamide)
  - HU + Epo
- Cell therapy
- Gene therapy

II β-Thalassemia

- I Excess of α chains in erythroid cells
  - 1) Imbalance of globin chains synthesis
    - α/β+γ ratio of synthesis
      - β thalassemic mutation
        - Type : β°, β^{+},β^{E}….
        - Location ($\beta^{dominant}$)
      - α gene dosage
        - <4 : improvement
        - >4 : increased severity
      - Variability of the γ chain expression(HbF):
        - β globin gene cluster haplotypes
        - SNP polymorphisms : BCL11A, HBS1L-MYB,

Number of amaliorating alleles (α thal., BCL11A, HBS1L-MYB) in thalassemia major or intermedia, in Sardinia



Galanello Blood 2009