Innovative therapies for ß-hemoglobinopathies

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Disclosures

Consultant
Isis Pharmaceuticals
Medgenics Pharmaceuticals
Bayer Healthcare Pharmaceuticals
Novartis Pharmaceuticals

Collaboration
Acceleron Pharmaceuticals

Restricted Stock
Merganser Biotech

Research Grants
R01DK095112-NIDDK
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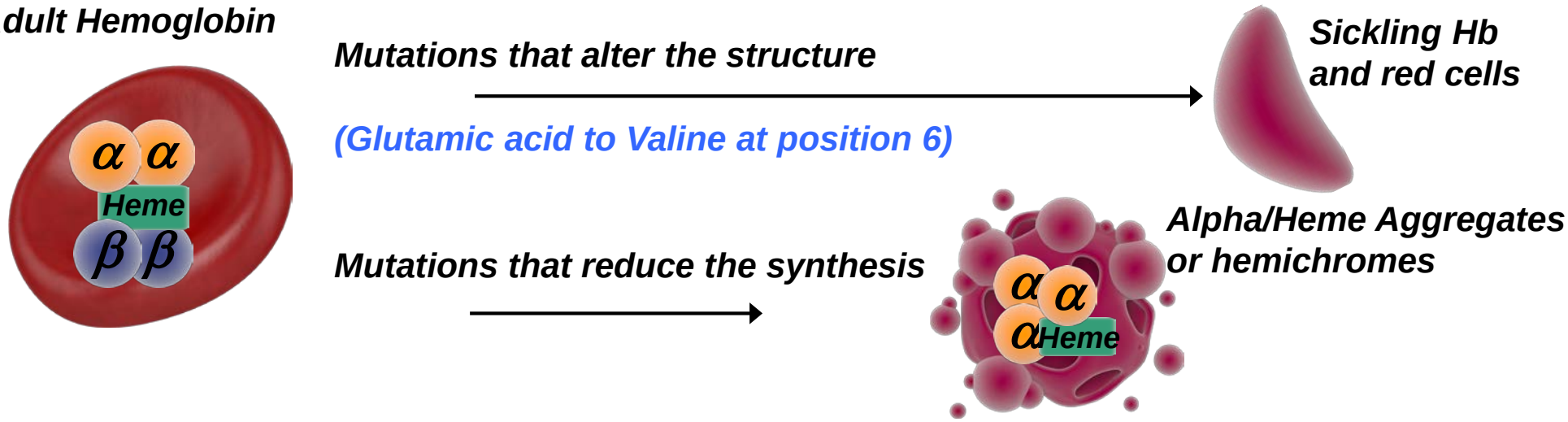
R01DK090554-NIDDK
European Community-FP7-306201
AVLT-Italy

Isis Pharmaceuticals
Merganser Biotech
Bayer Healthcare Pharmaceuticals
Medgenics LLC
Meira GTX

Sickle Cell Anemia and ß-Thalassemia



Consequences of Abnormal ß-Globin Chain Structure

Vaso-Occlusion

Pain

Hemolysis

Anemia

Pulmonary hypertension

Iron overload

Consequences of Reduced ß-Globin Chain Production

Ineffective Erythropoiesis

Anemia

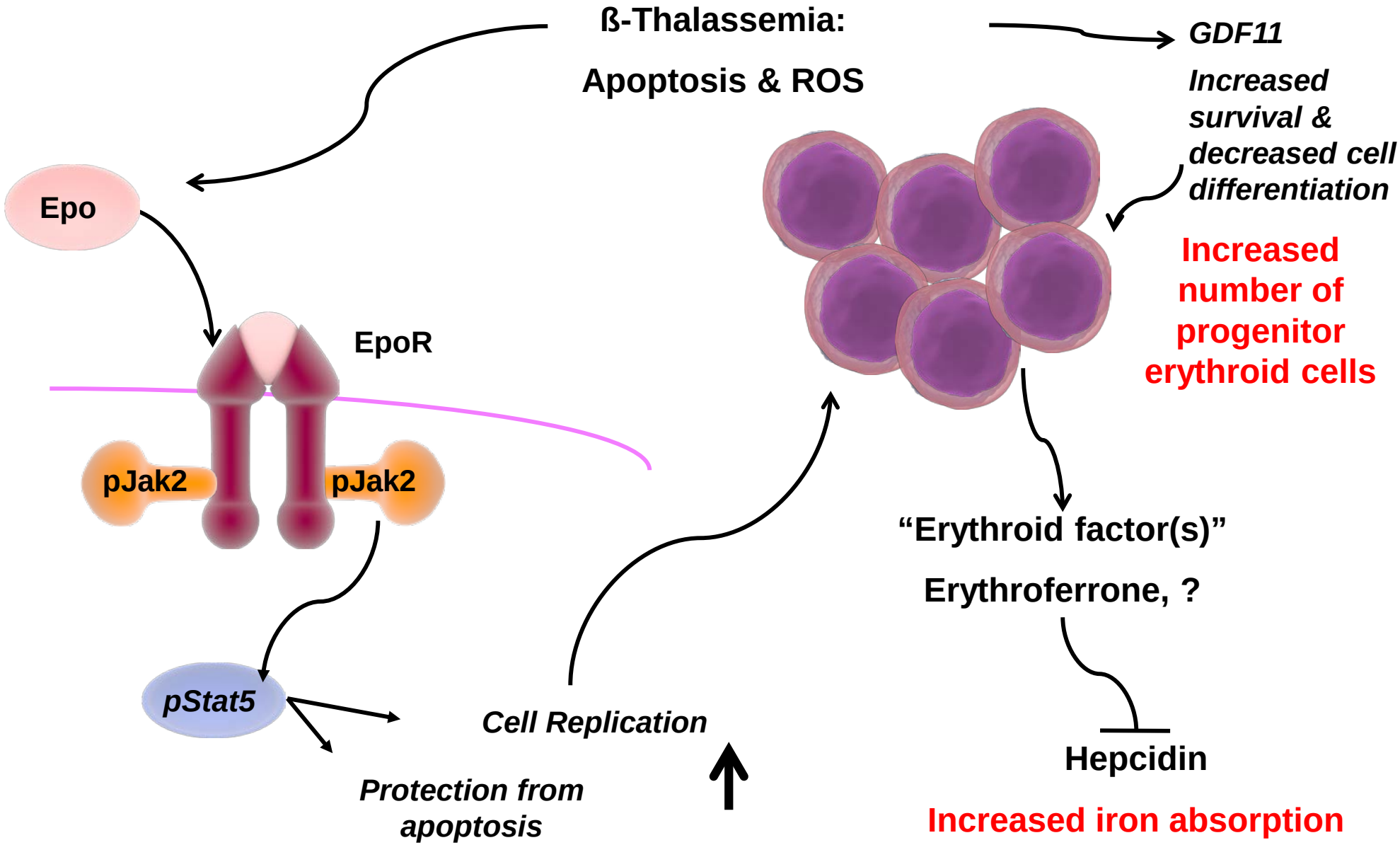
Iron Overload

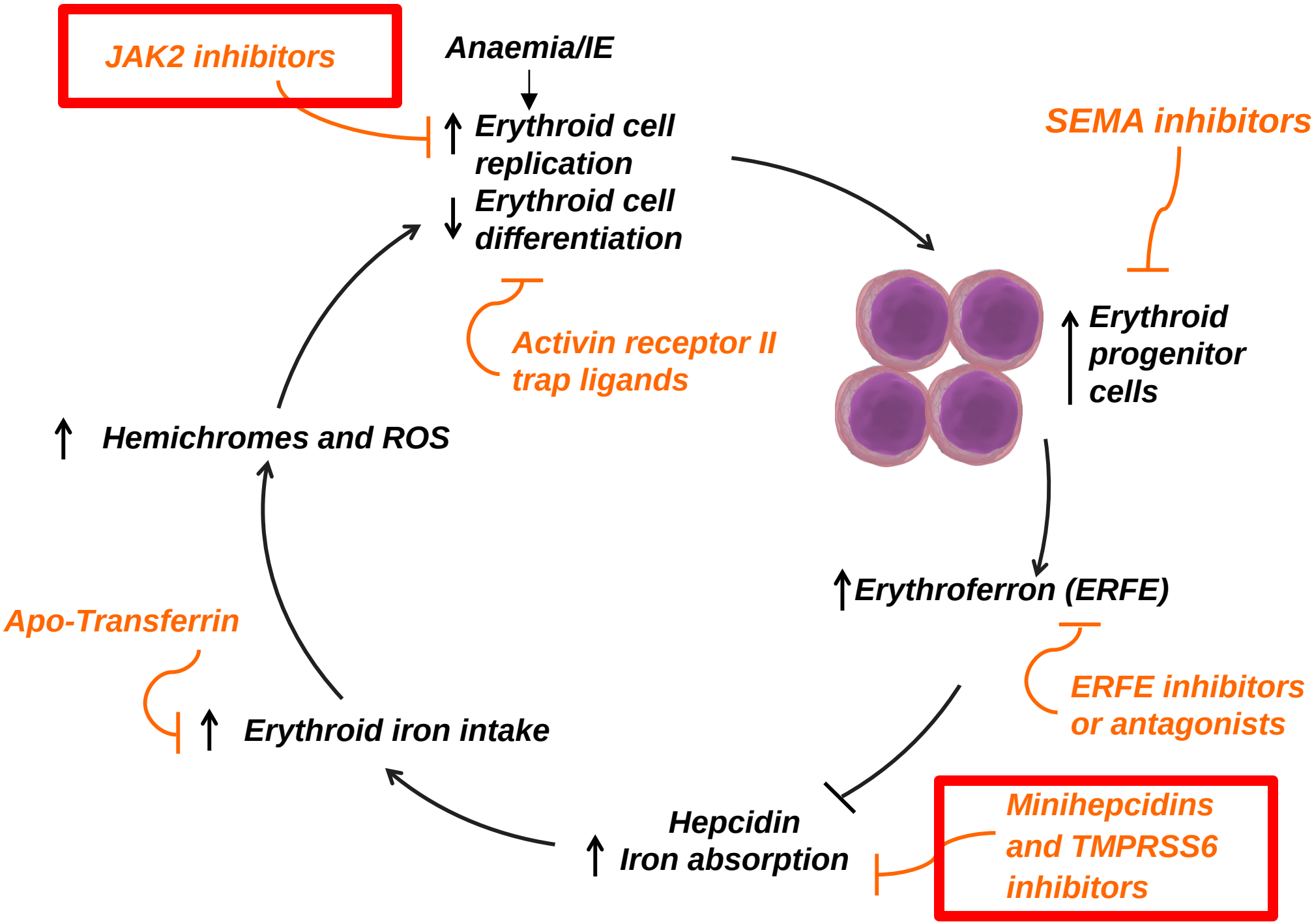
Erythroid Marrow Expansion

Splenomegaly

Thrombosis

Erythropoiesis and iron absorption



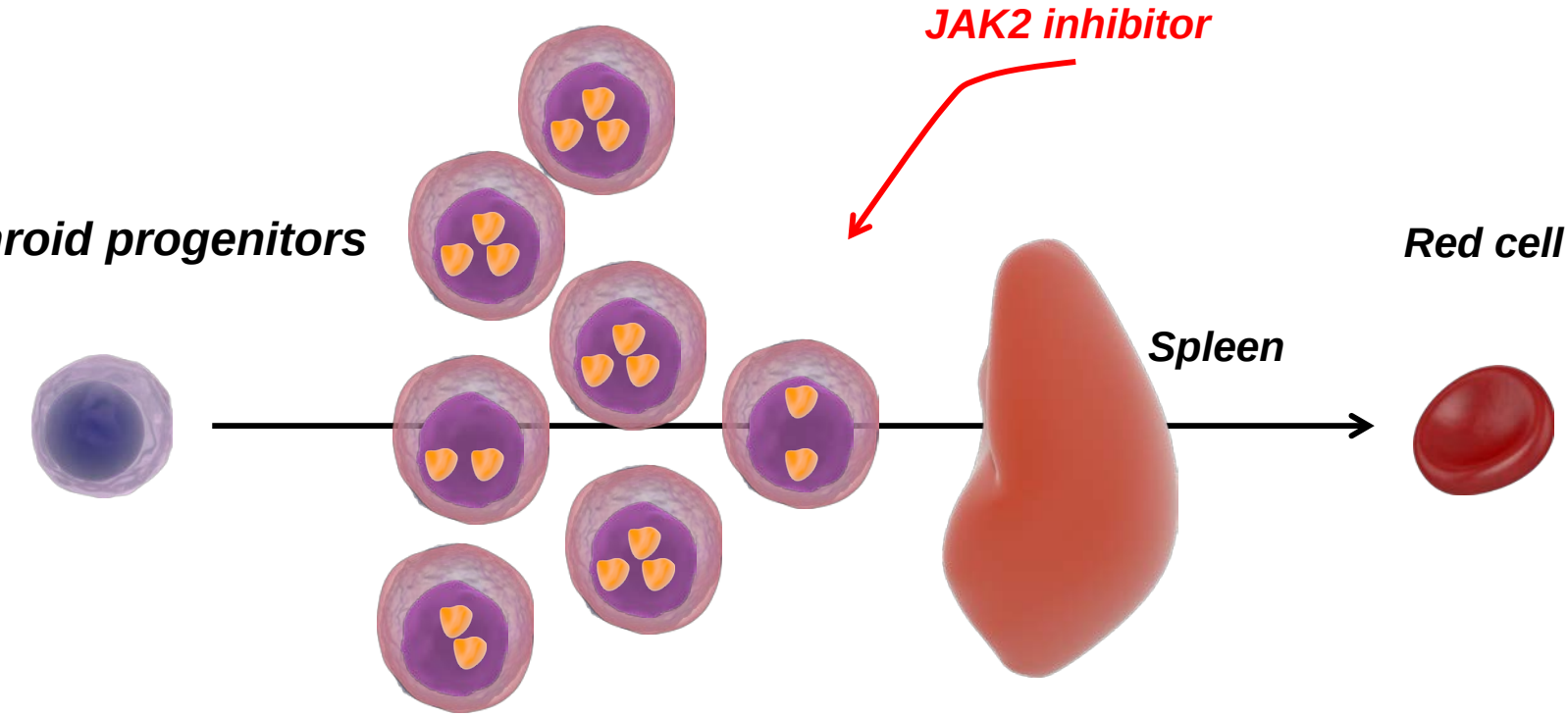

JAK2 inhibitors
Anaemia/IE
↑ Erythroid cell replication
↓ Erythroid cell differentiation
Activin receptor II trap ligands
SEMA inhibitors
↑ Erythroid progenitor cells
↑ Erythroferron (ERFE)
ERFE inhibitors or antagonists
↑ Hepcidin
↑ Iron absorption
Minihepcidins and TMPRSS6 inhibitors
↑ Erythroid iron intake
Apo-Transferrin
↑ Hemichromes and ROS

Potential use of erythroid modulators for the treatment of ß-thalassemia

Potential use of JAK2 inhibitors for the treatment of ß-thalassemia

Potential effect of JAK2 inhibitors on ineffective erythropoiesis

ß-thalassemia → Ineffective erythropoiesis → High EPO levels → pJAK2:

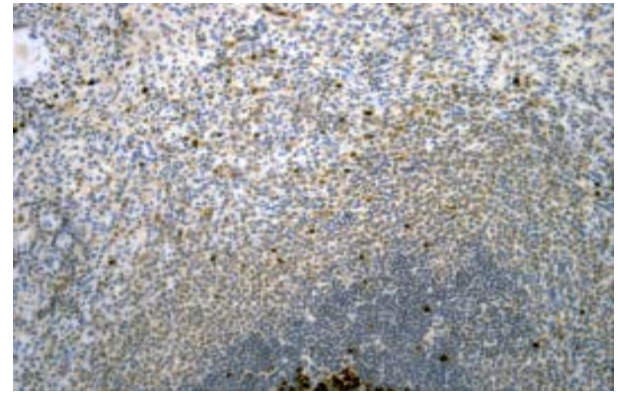
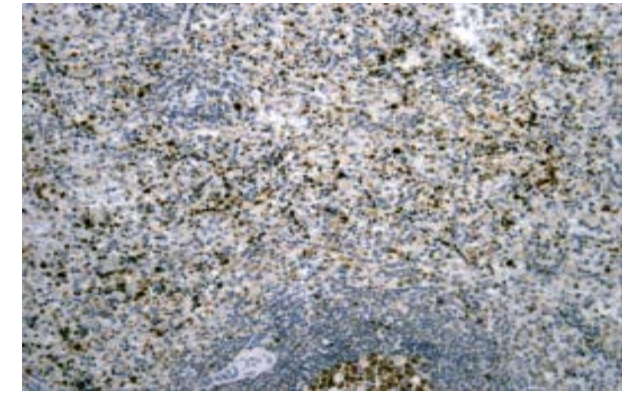
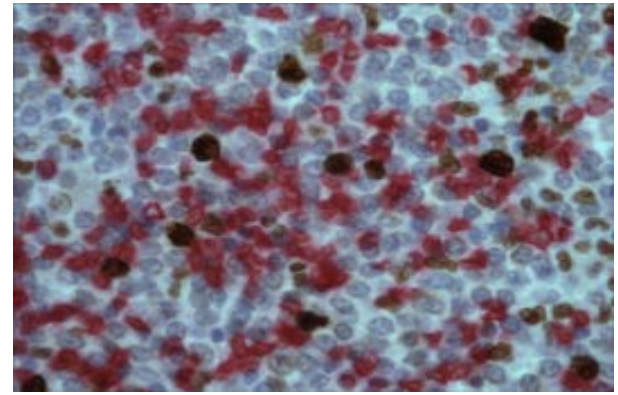
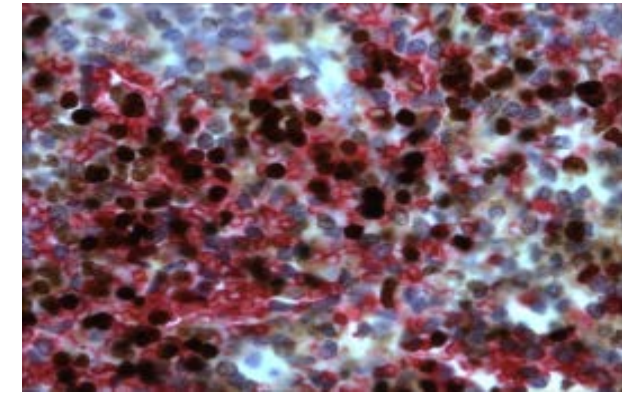


Decreased differentiation of erythroid cells exacerbates ineffective erythropoiesis in β-thalassemia

*Ilaria V. Libani,[1,2] *Ella C. Guy,[1] *Luca Melchiori,[1] *Raffaella Schiro,[1] Pedro Ramos,[1,3] Laura Breda,[1] Thomas Scholzen,[4] Amy Chadburn,[5] YiFang Liu,[5] Margrit Kernbach,[4] Bettina Baron-Lühr,[4] Matteo Porotto,[6] Maria de Sousa,[3] Eliezer A. Rachmilewitz,[7] John D. Hood,[8] M. Domenica Cappellini,[2] Patricia J. Giardina,[1] Robert W. Grady,[1] Johannes Gerdes,[4] and Stefano Rivella[1]

BLOOD, 1 AUGUST 2008 • VOLUME 112, NUMBER 3

Increased erythroid cell proliferation in spleens of human β-thalassaemic specimens

	Normal	*Patient*
Ki67		
Ki67 + glycophorin-C and spectrin		

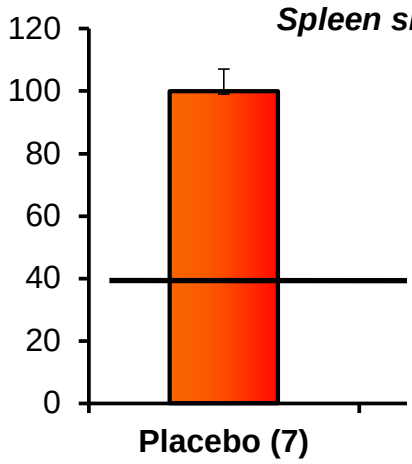
Libani IV, et al. Blood. 2008;112:875-85.

Potential use of Jak2 inhibitors for the treatment of ß-thalassemia

- **In ß-thalassemia anemia is associated with increased EPO levels**
- **This leads to increased activation of the Jak2/Stat5 pathway**
- **One of the main consequences is increased proliferation and expansion of the pool of erythroid progenitors, leading to EMH and exacerbation of IE**

Therefore, administration of Jak2 inhibitors might be helpful to reverse splenomegaly and ameliorate IE

Reduction of splenomegaly following administration of a Jak2 inhibitor in mice affected by β-thalassaemia intermedia is dose-mediated



blood

2008 112: 875-885
Prepublished online May 14, 2008;
doi:10.1182/blood-2007-12-126938

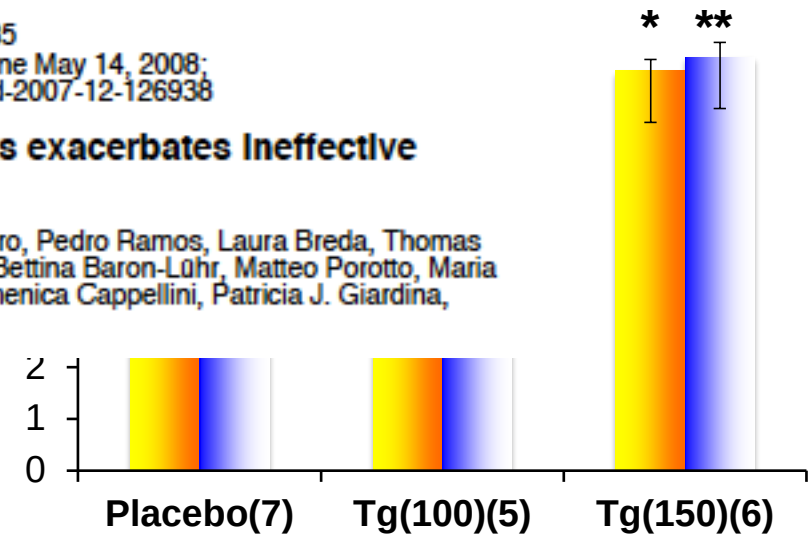
Decreased differentiation of erythroid cells exacerbates ineffective erythropoiesis in β-thalassemia

Ilaria V. Libani, Ella C. Guy, Luca Melchiori, Raffaella Schiro, Pedro Ramos, Laura Breda, Thomas Scholzen, Amy Chadburn, YiFang Liu, Margrit Kernbach, Bettina Baron-Lühr, Matteo Porotto, Maria de Sousa, Eliezer A. Rachmilewitz, John D. Hood, M. Domenica Cappellini, Patricia J. Giardina, Robert W. Grady, Johannes Gerdes and Stefano Rivella

Placebo

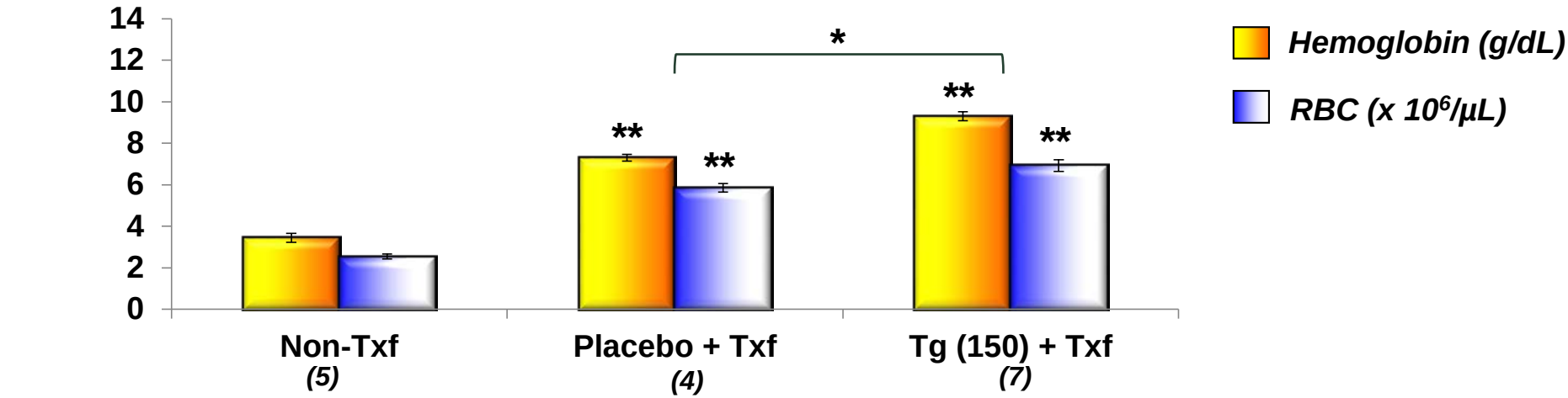
TG 100

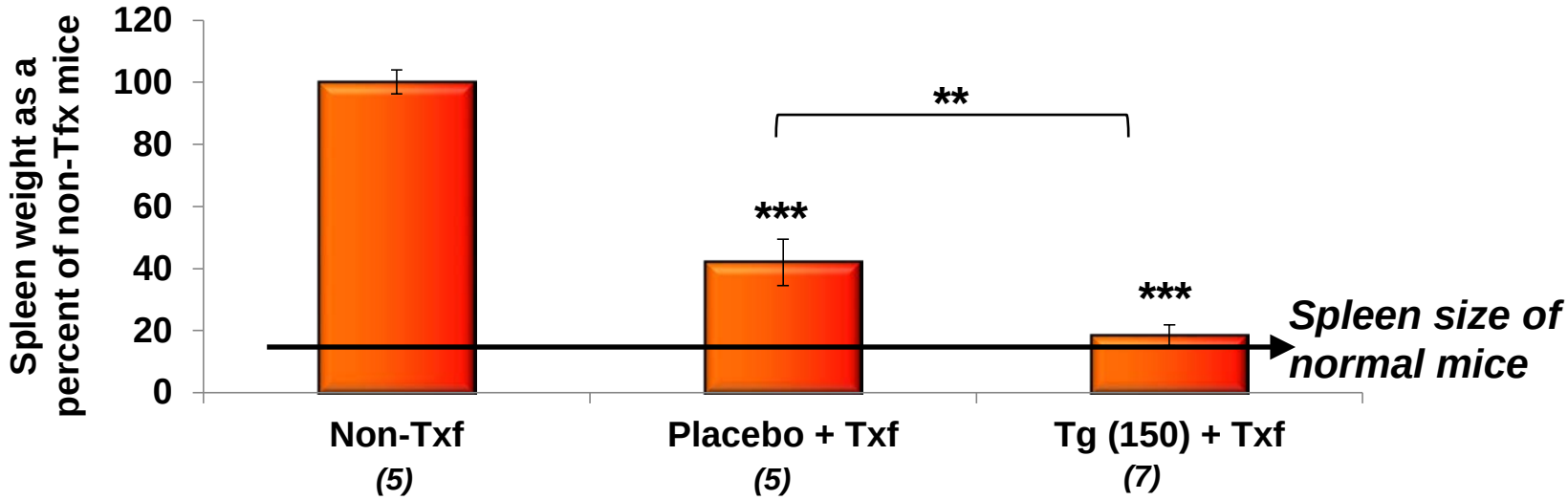
TG 150



Pedro Ramos, Carla Casu

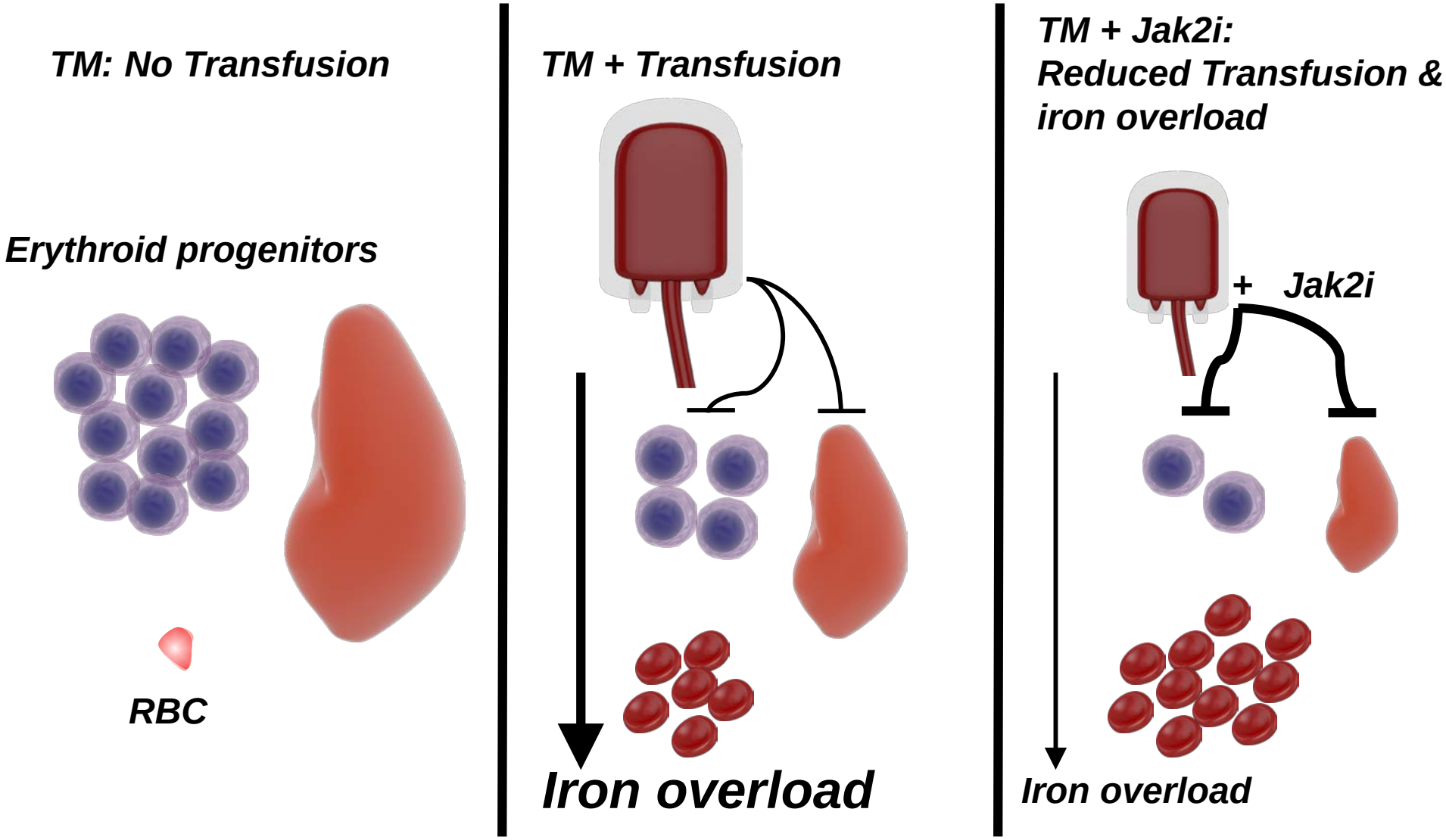
JAK2 inhibitor increases efficacy of transfusion in transfusion-dependent mice affected by β-thalassaemia major





Pedro Ramos, Carla Casu, Luca Melchiori, ASH 2011, San Diego

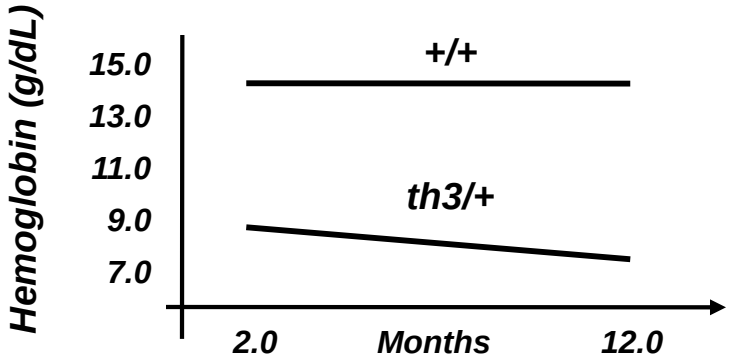
Use of JAK2i in ß-thalassemia major



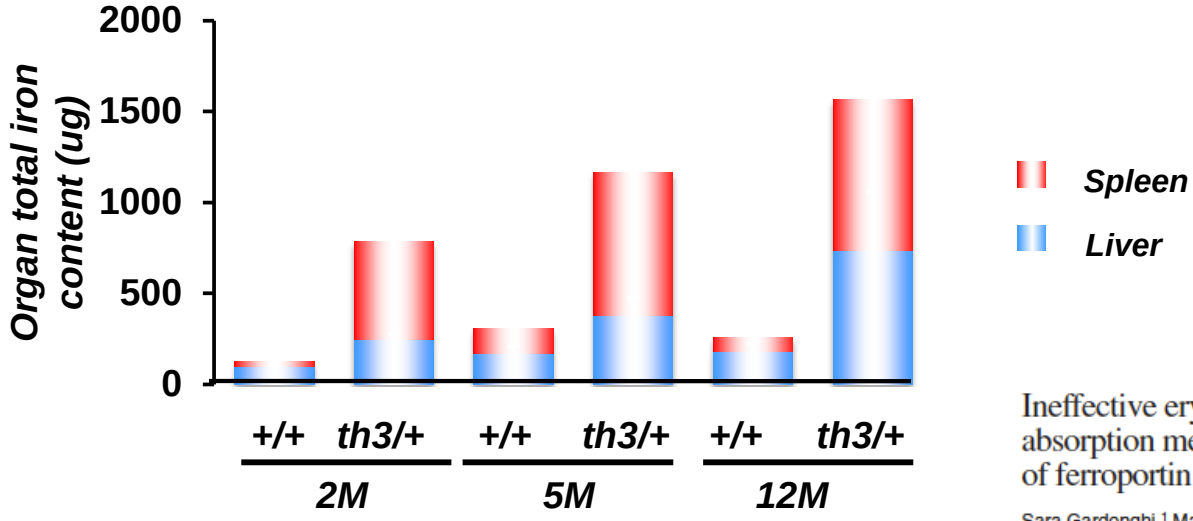
Potential use of Hepcidin agonist or inducers for the treatment of ß-thalassemia

Iron overload and anemia worsen over time in mice affected by ß-thalassemia intermedia

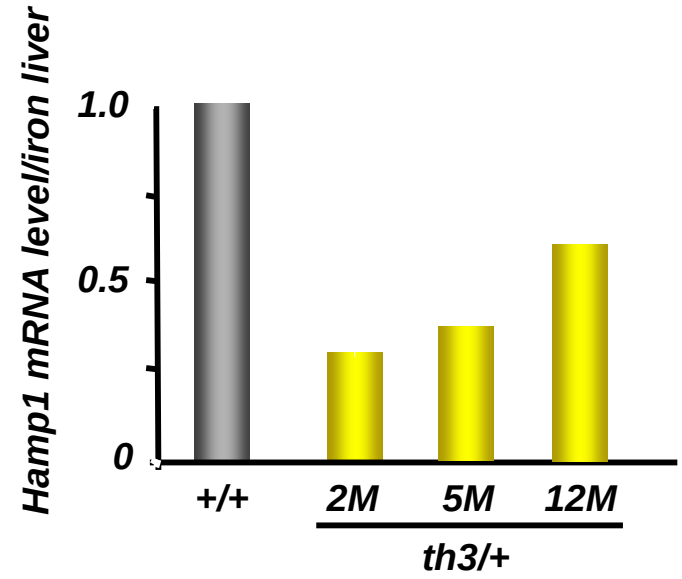
Anemia worsens with time in th3/+ mice



Iron overload increases with time in th3/+ mice



Hepcidin is expressed at low levels in iron overloaded th3/+ mice



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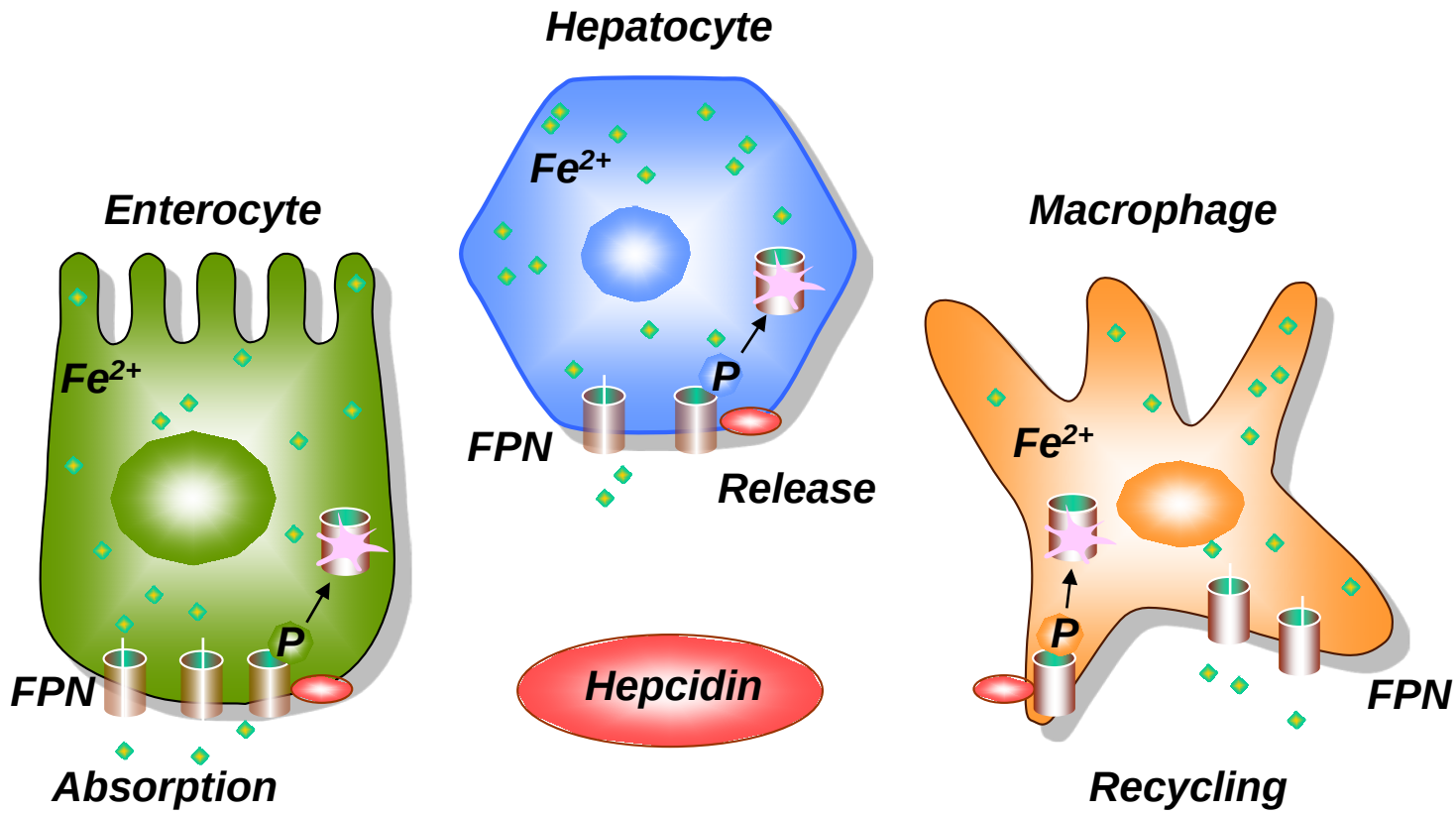
Ineffective erythropoiesis in β-thalassemia is characterized by increased iron absorption mediated by down-regulation of hepcidin and up-regulation of ferroportin

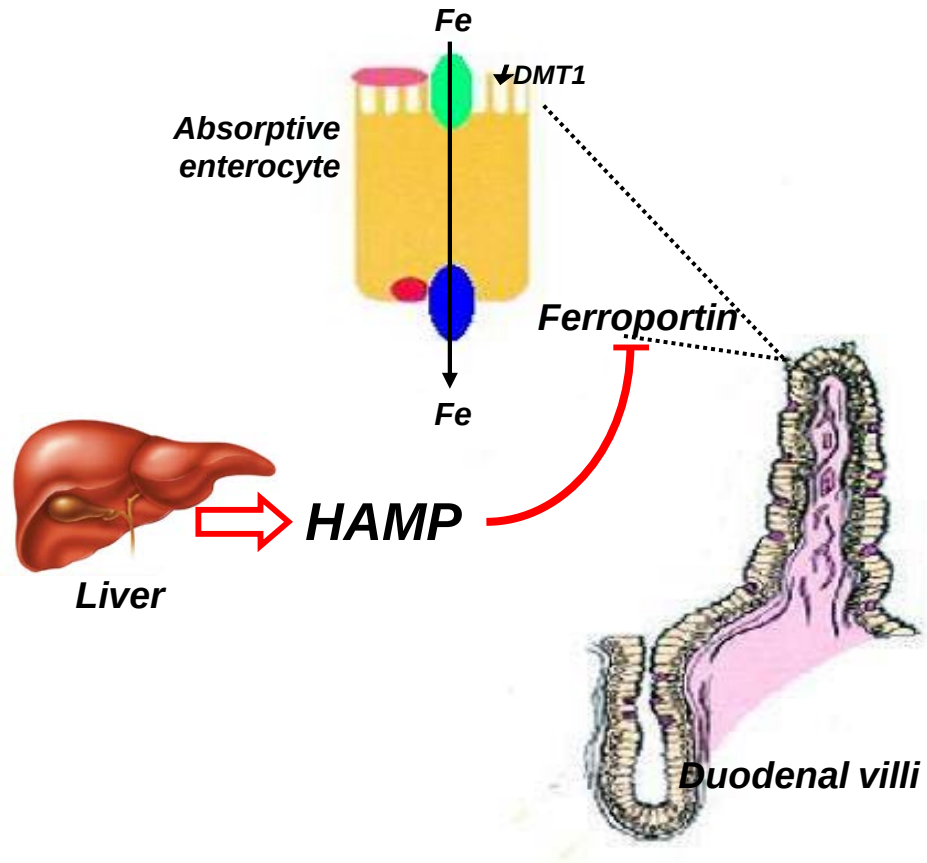
Sara Gardenghi,[1] Maria F. Marongiu,[1] Pedro Ramos,[1,2] Ella Guy,[1] Laura Breda,[1] Amy Chadburn,[3] YiFang Liu,[3] Ninette Amariglio,[4] Gideon Rechavi,[4] Eliezer A. Rachmilewitz,[5] William Breuer,[6] Z. Ioav Cabantchik,[6] Diedra M. Wrighting,[7] Nancy C. Andrews,[7] Maria de Sousa,[1,2] Patricia J. Giardina,[1] Robert W. Grady,[1] and Stefano Rivella[1]

Gardenghi et al. Blood 2007.

Hepcidin, the iron hormone regulator, acts on Ferroportin, the iron exporter



Hepcidin, iron metabolism and erythropoiesis



Hepcidin regulates dietary iron absorption and distribution by triggering the degradation of Fpn1.

Hepcidin is up-regulated by increased iron levels and inflammation.

Hepcidin is down-regulated by erythropoiesis, anemia, and hypoxia.

Hepcidin, a major player in hemochromatosis, iron overload and anemia of chronic disease

- **Hemochromatosis**
- **Secondary Hemochromatosis**

(i.e. ß-thalassemia, MDS)

- **IRIDA**
- **Anemia of Inflammation**
- **Bacterial infections**
- **Cancer**

Hepcidin

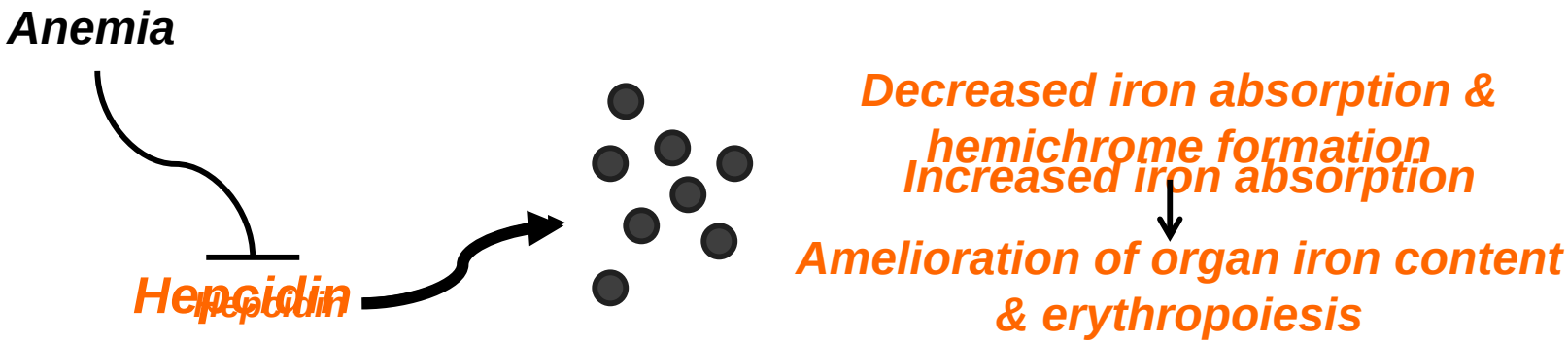
Too little:

- **Increased iron absorption**
- **Increased macrophage iron release**
- **Iron overload**

Too much:

- **Decreased iron absorption**
- **Macrophage iron retention**
- **Anemia**

Hypothesis: Increased levels of Hepcidin in thalassemia intermedia are beneficial to prevent iron overload and ameliorate erythropoiesis



The Journal of Clinical Investigation

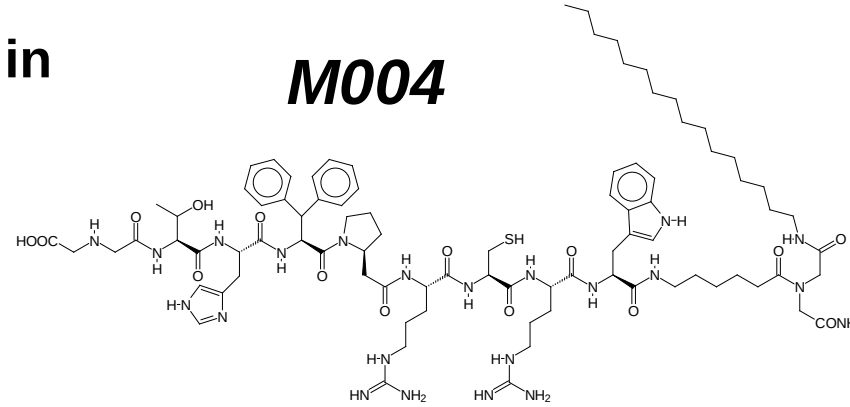
Hepcidin as a therapeutic tool to limit iron overload and improve anemia in β-thalassemic mice

Sara Gardenghi,[1] Pedro Ramos,[1,2] Maria Franca Marongiu,[1] Luca Melchiori,[1] Laura Breda,[1] Ella Guy,[1] Kristen Muirhead,[1] Niva Rao,[1] Cindy N. Roy,[3] Nancy C. Andrews,[4] Elizabeta Nemeth,[5] Antonia Follenzi,[6] Xiuli An,[7] Narla Mohandas,[7] Yelena Ginzburg,[8] Eliezer A. Rachmilewitz,[9] Patricia J. Giardina,[1] Robert W. Grady,[1] and Stefano Rivella[1]

Minihepcidins (MH): background

- **Minihepcidins are short peptide mimetics (9 retro-inverso AA) of hepcidin (25 AA)**
- **Derived from the N-terminal amino acid sequence and modified for in vivo activity**
- **MH are effective in reducing iron overload in animal models of *HFE*- and *HAMP*-related hemochromatosis**

M004



The Journal of Clinical Investigation Volume 121 Number 12 December 2011

Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload

Gloria C. Preza,[1] Piotr Ruchala,[2] Rogelio Pinon,[2] Emilio Ramos,[3] Bo Qiao,[2] Michael A. Peralta,[4] Shantanu Sharma,[5] Alan Waring,[2,6] Tomas Ganz,[1,2] and Elizabeta Nemeth[2]

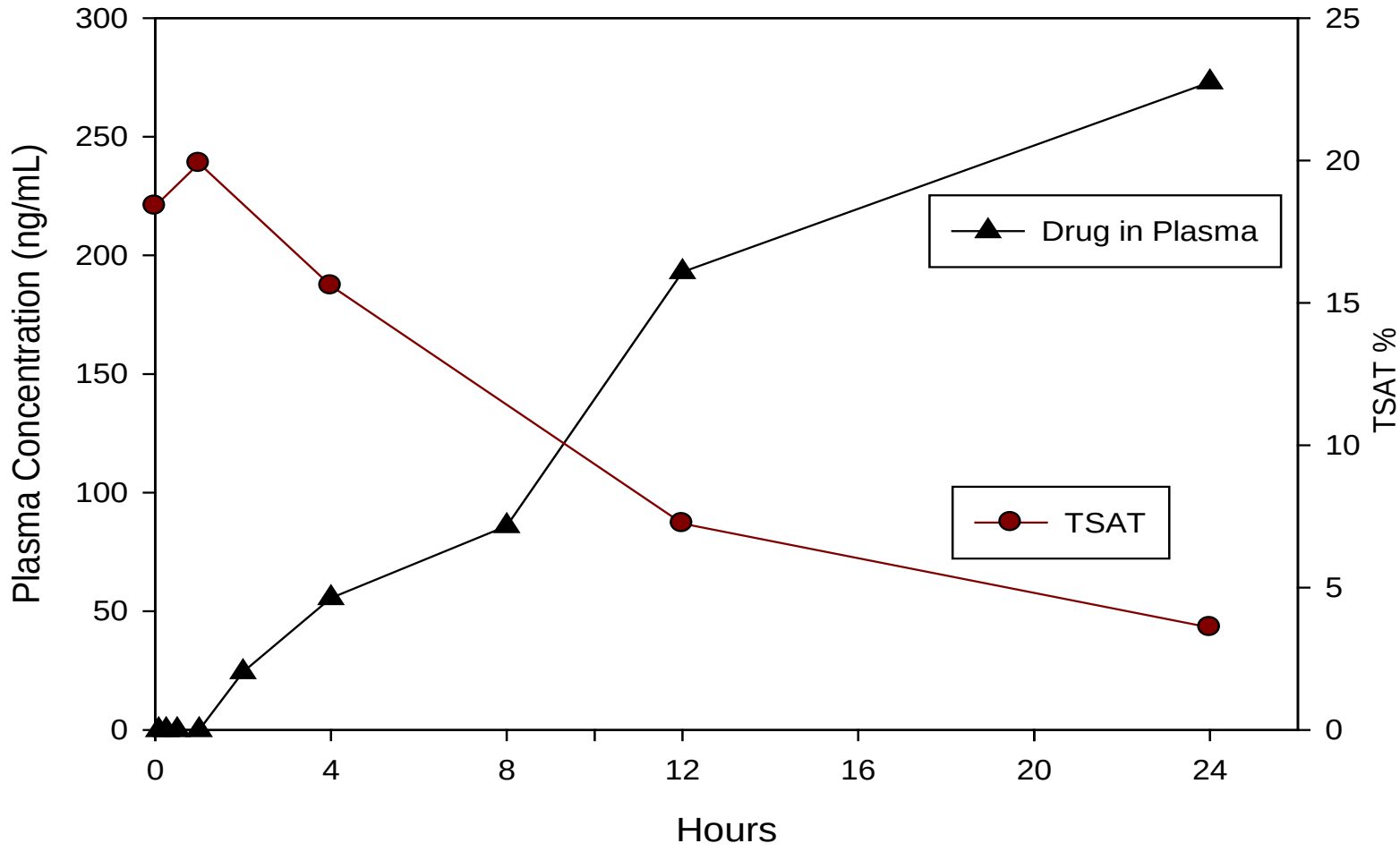
BLOOD, 1 NOVEMBER 2012 • VOLUME 120, NUMBER 18

Minihepcidins prevent iron overload in a hepcidin-deficient mouse model of severe hemochromatosis

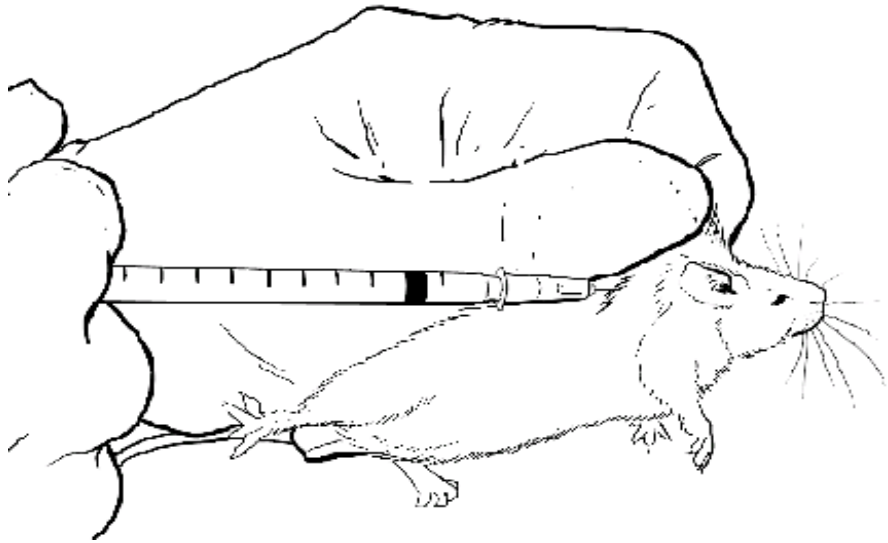
Emilio Ramos,[1] Piotr Ruchala,[2] Julia B. Goodnough,[2] Léon Kautz,[2] Gloria C. Preza,[3] Elizabeta Nemeth,[2] and Tomas Ganz[2,4]

Very high stability of the minihepcidin M004 *in vivo*

Blood levels and effect on transferrin saturation following a single dose of 7.5 mg/kg of M004 in the rat

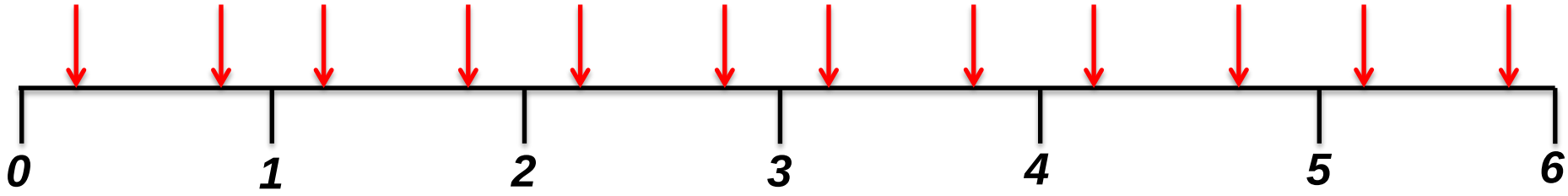


Experimental Protocol

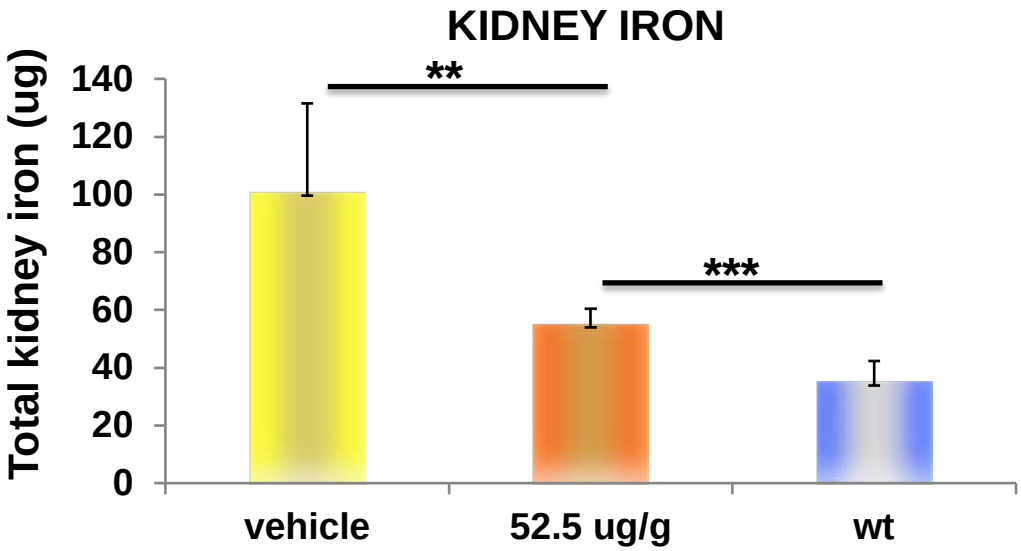


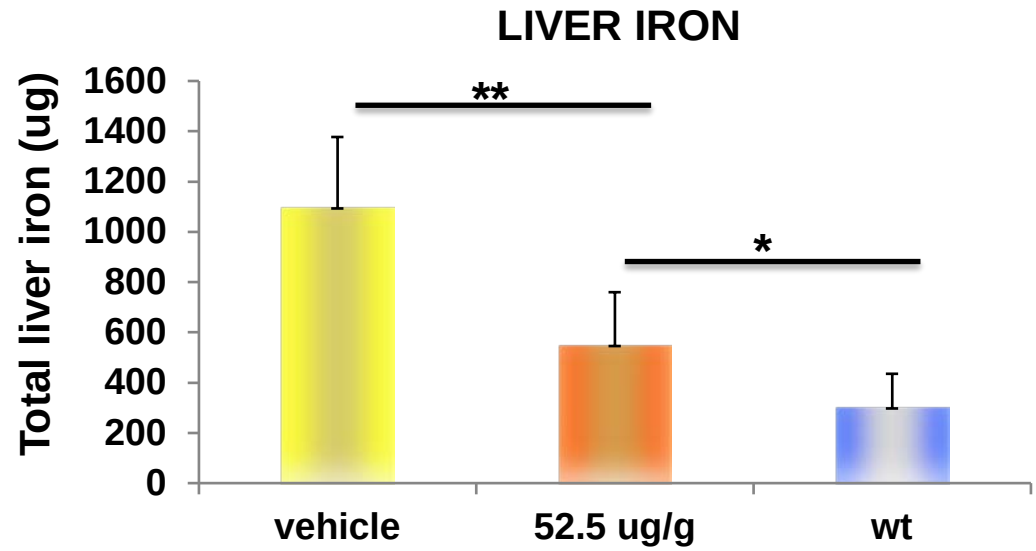
th3/+ animals treated by SC injection with M004 (52.5 µg twice weekly) for six weeks.

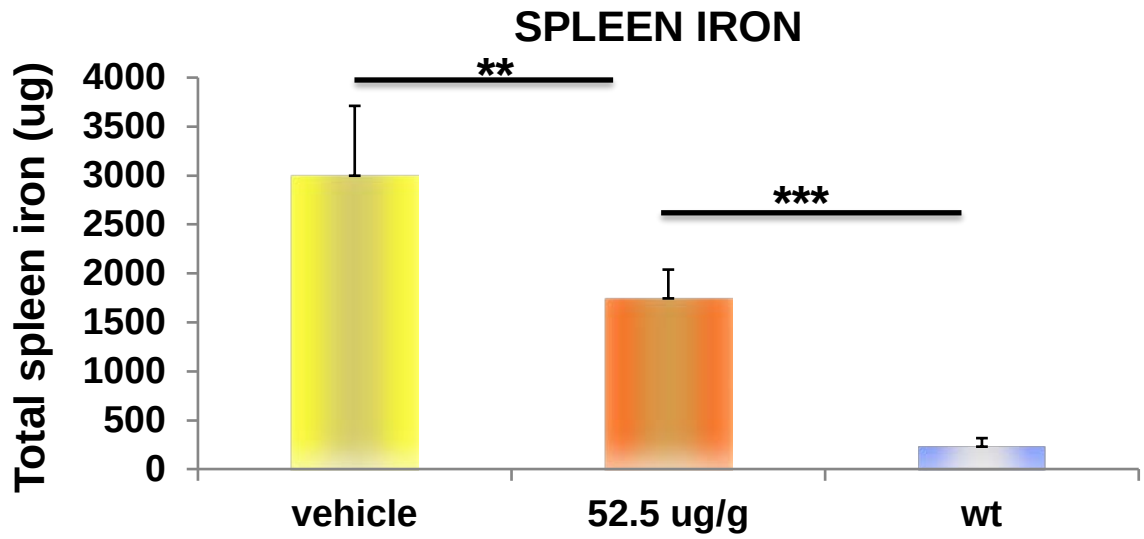
CBC assessment at four and six weeks. Serum and tissue iron assessment at six weeks



Minihepcidin administration significantly reduces liver, spleen and kidney iron concentration in thalassemic mice

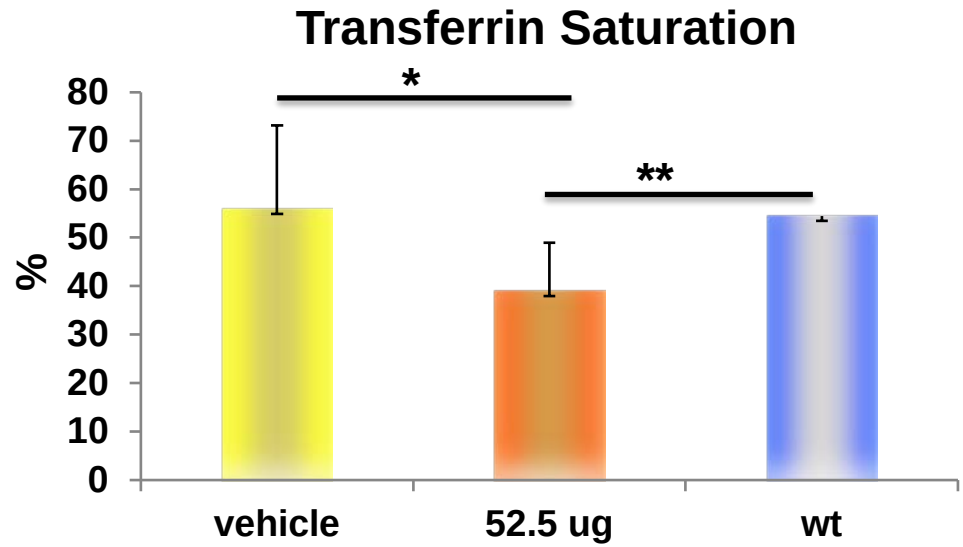


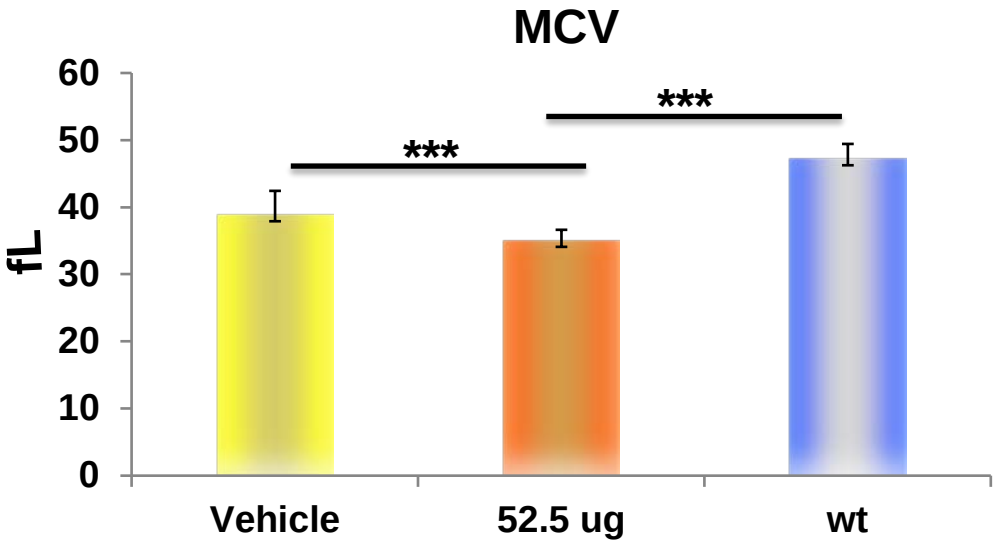


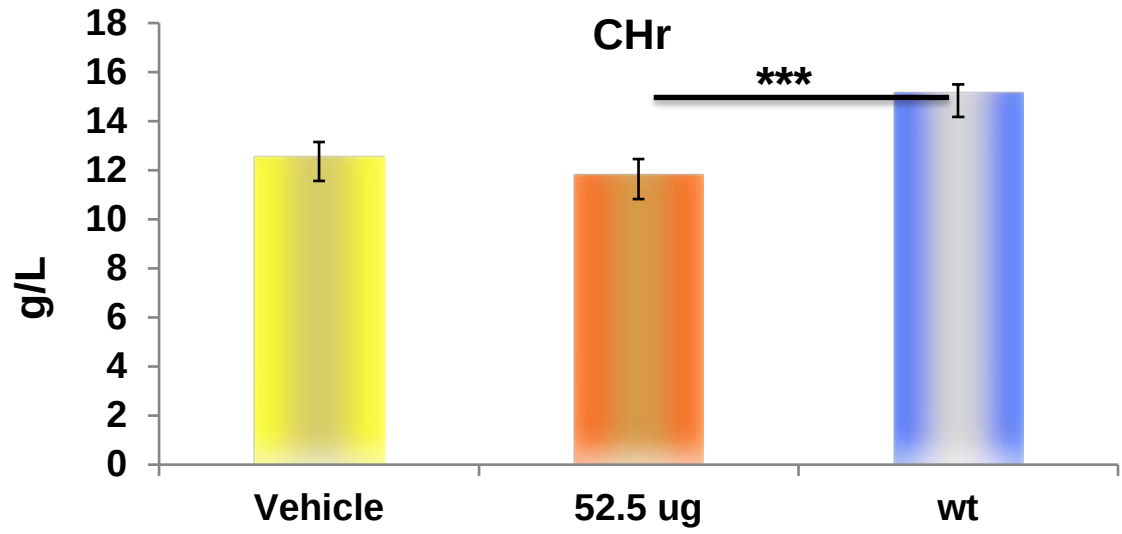


Carla Casu

Minihepcidin administration induces a mild functional iron deficiency







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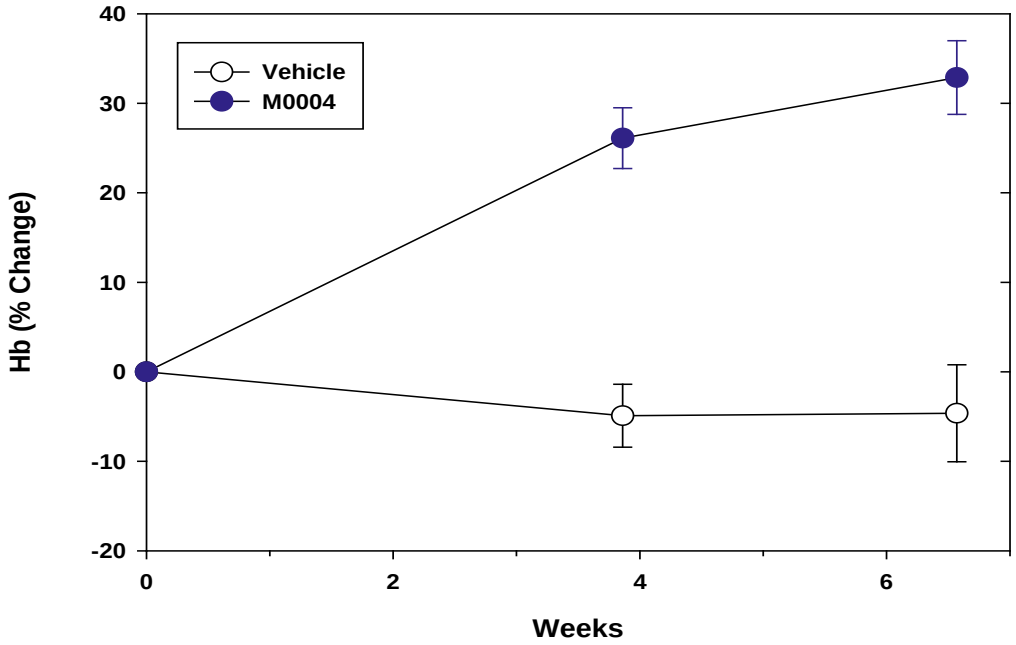
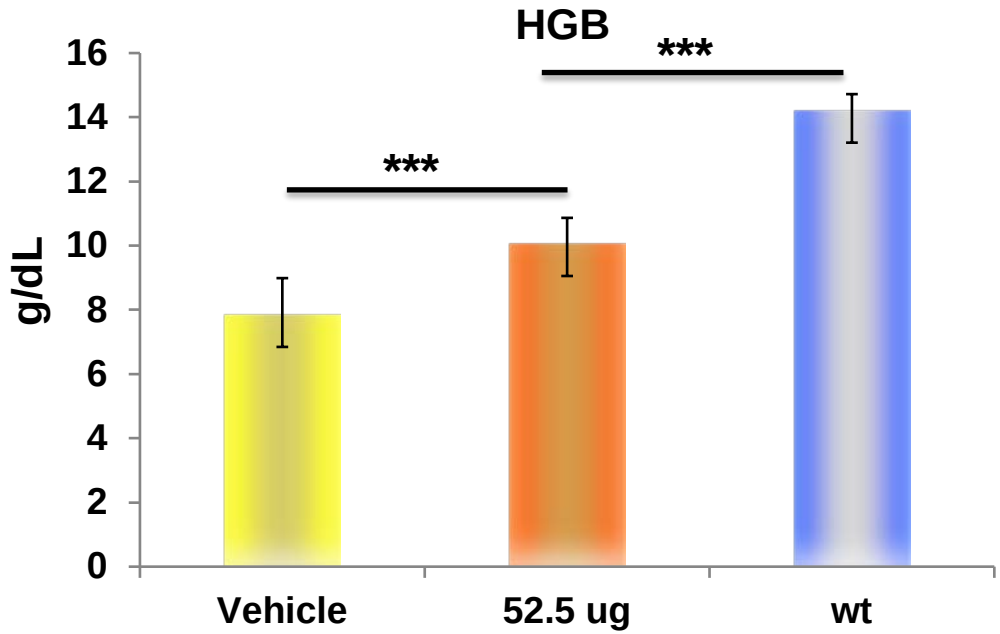
Minihepcidin administration significantly ameliorates hemoglobin levels





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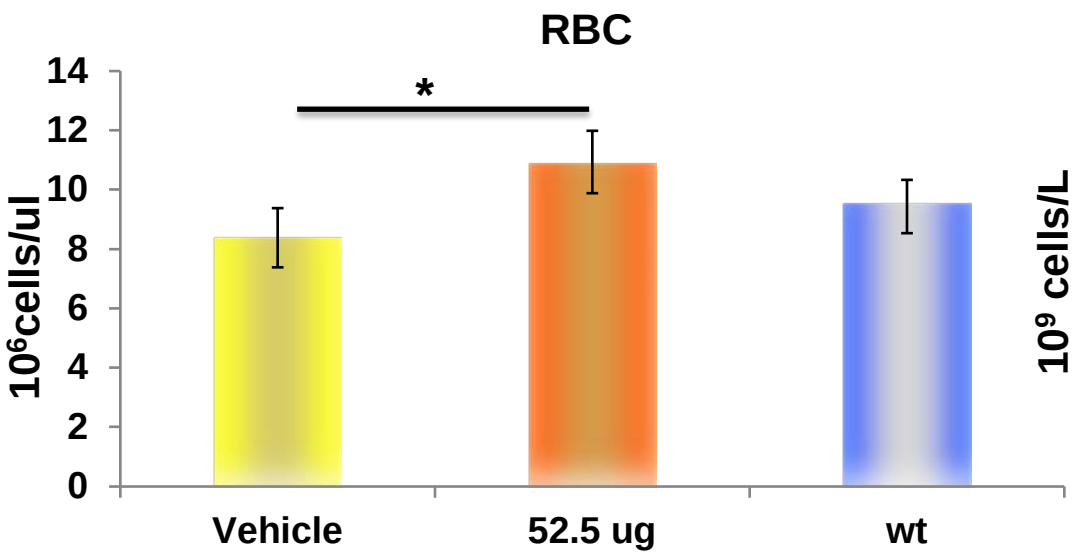
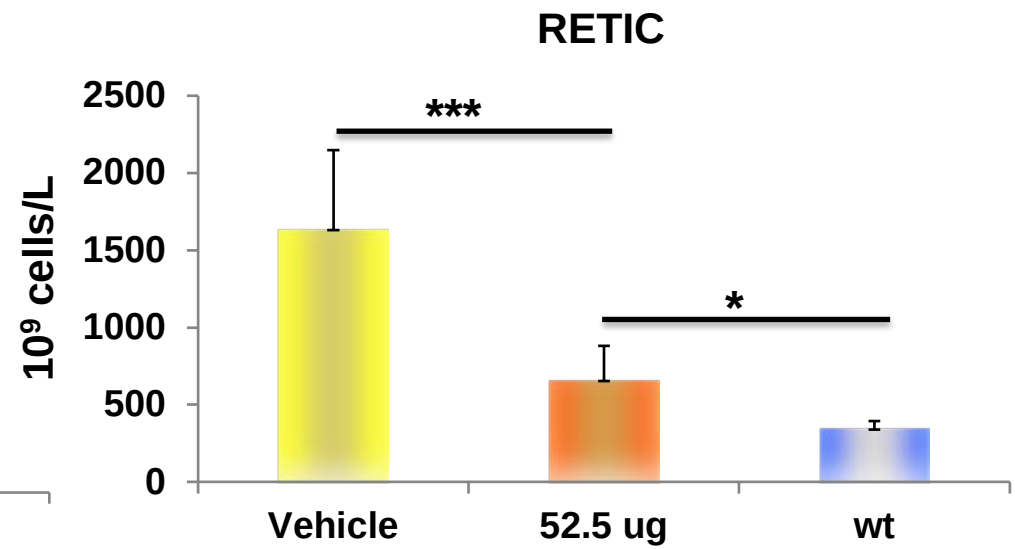
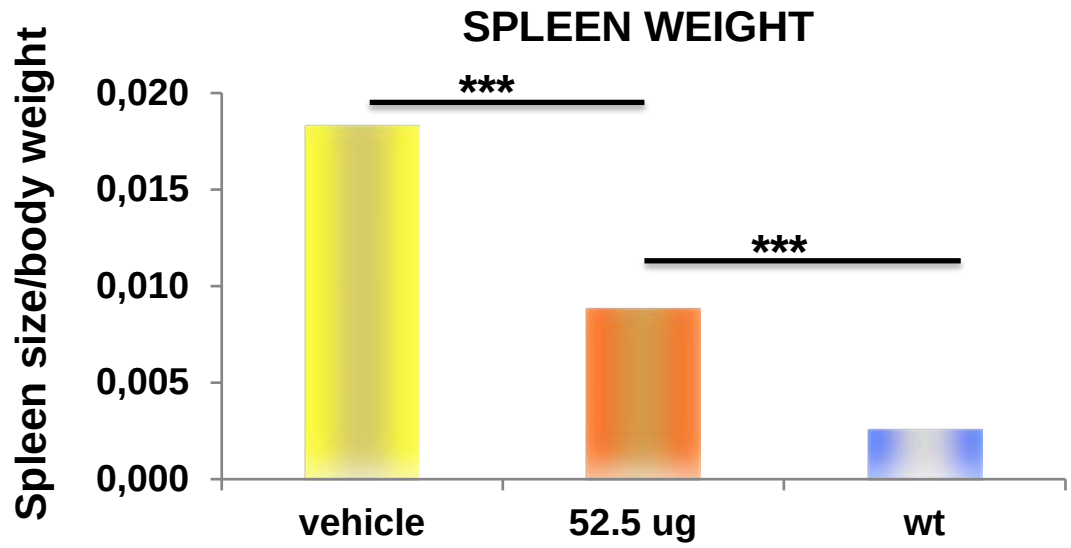
Minihepcidin administration significantly ameliorates erythropoiesis and spleen weight in thalassemic mice







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Administration of minihepcidin is associated with reduced MCH in thalassemic mice

WT + MH		*Th3*/+ + MH	
Hb	↓	*Hb*	↑
RBCs	↓	*RBCs*	↑
Tf sat	↓	*Tf sat*	↓
MCH	↓	*MCH*	↓

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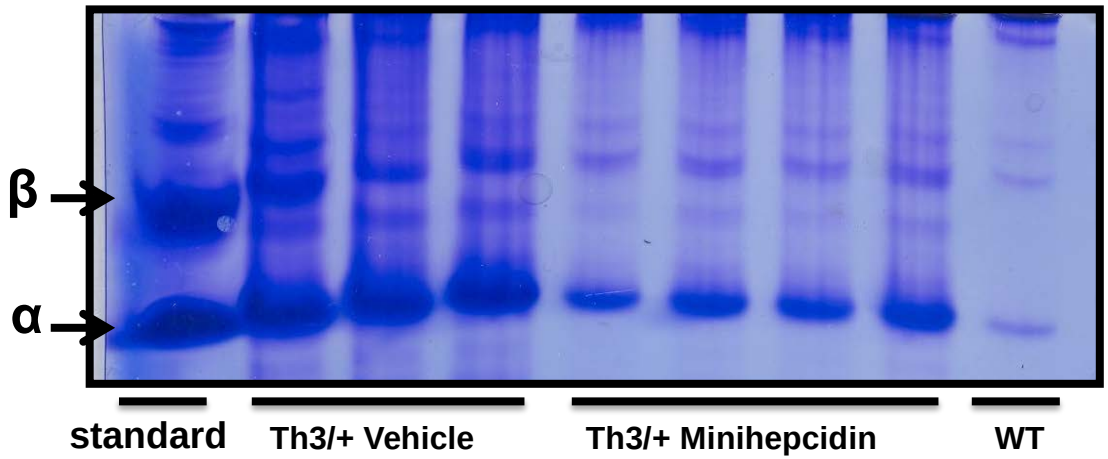
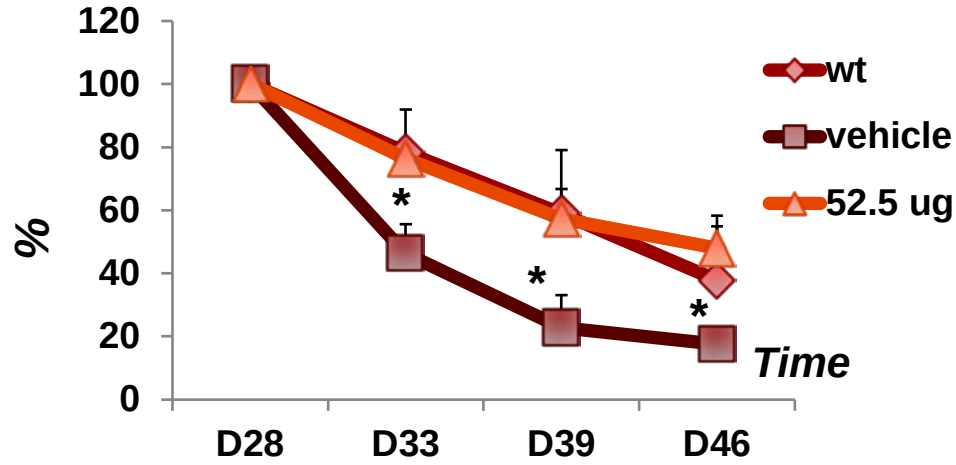
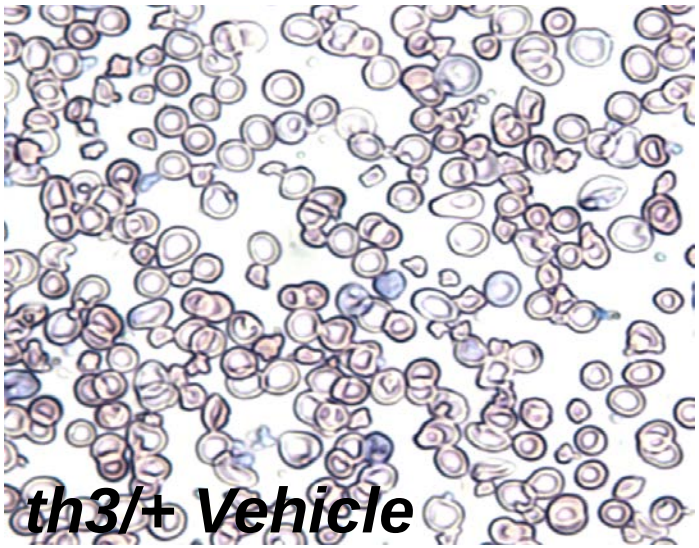
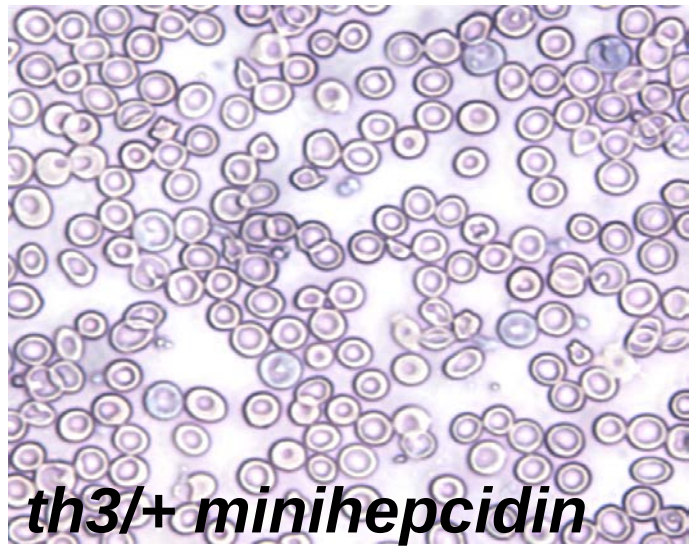
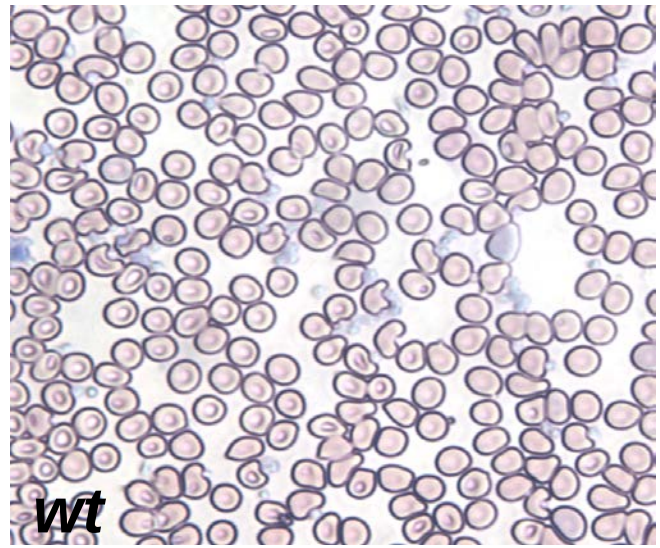
Administration of minihepcidin is associated with reduced erythroid cell damage









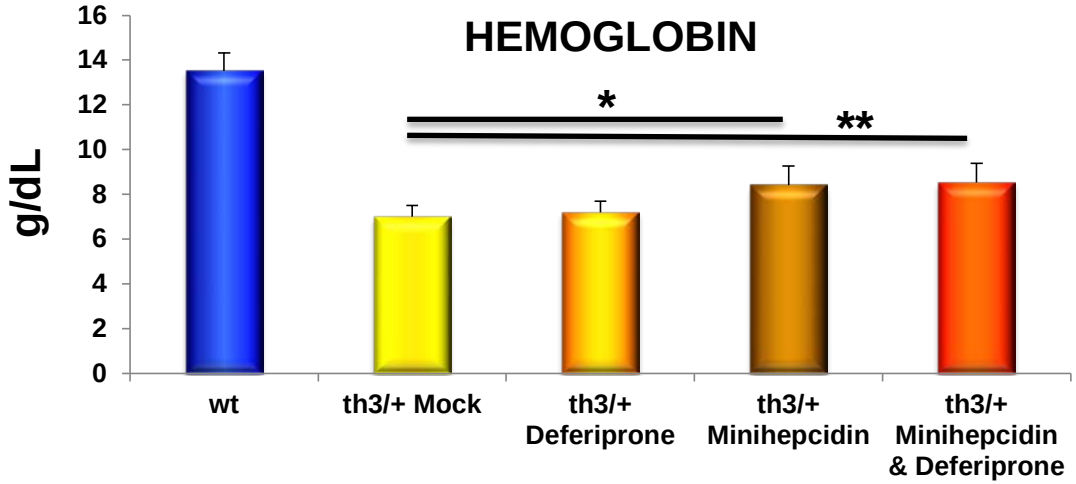


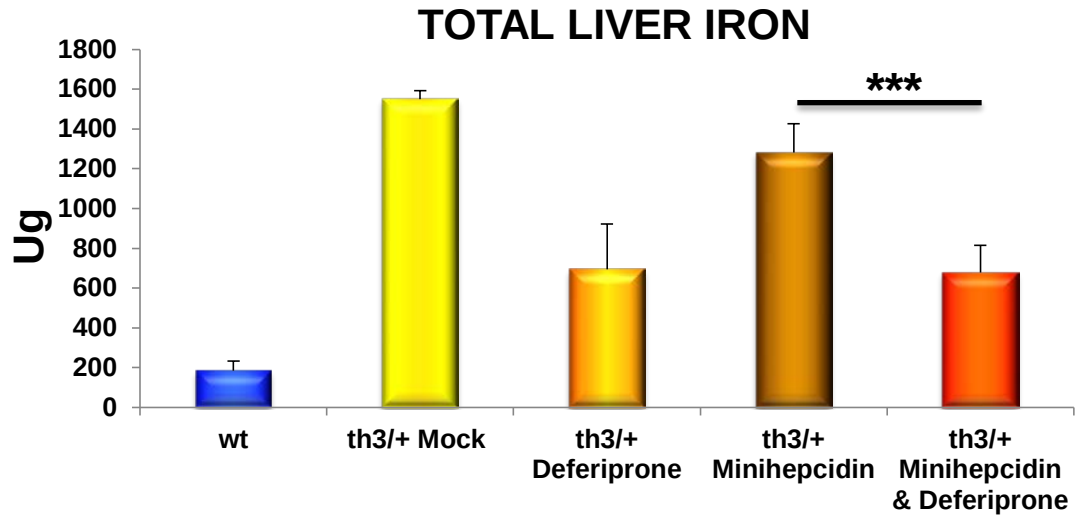
Carla Casu

Combination of MH and iron chelation

Using mice affected by ß-thalassemia intermedia, we evaluated if the simultaneous use of the iron chelator deferiprone (DFP) with MH can combine the positive effects of MH on erythropoiesis with the chelation benefit on organ iron content

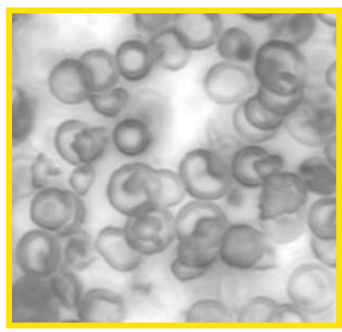
Combination of MH with iron chelation improves organ iron content and erythropoiesis



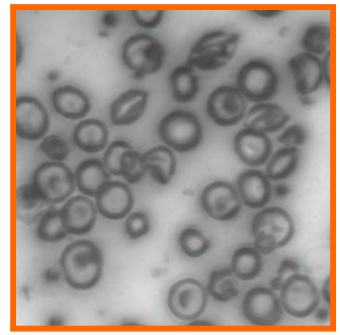


Carla Casu and Paraskevi Rea Oikonomidou

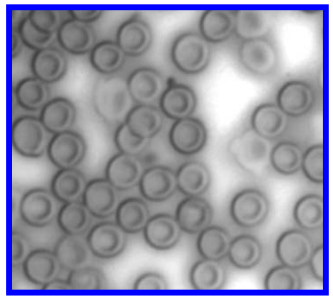
MH, but not Deferiprone, improves RBC morphology and reduces ROS in erythroid cells



Th3/+ Mock



Th3/+ Deferiprone



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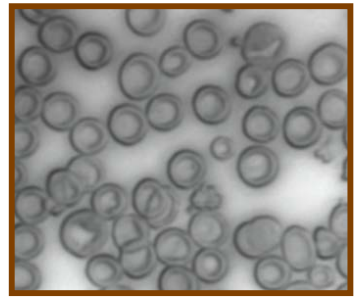
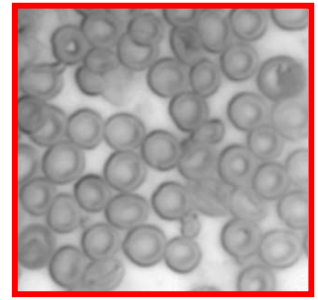
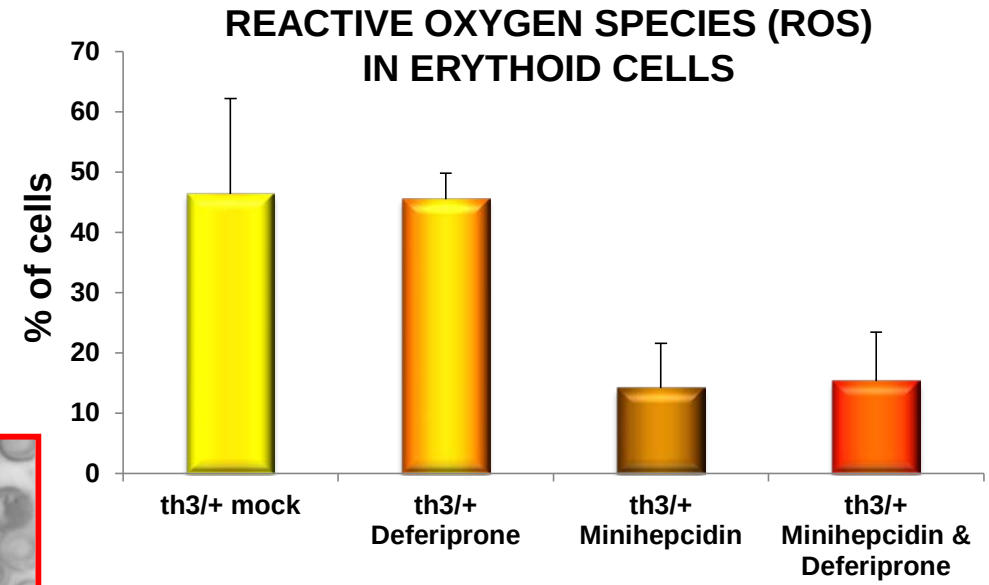


Th3/+ Minihepcidin



Th3/+ Minihepcidin & Deferiprone



Carla Casu and Paraskevi Rea Oikonomidou

Polycythemia vera and ß-thalassemia

Polycythemia vera (PV) is caused by neoplastic proliferation and maturation of erythroid, megakaryocytic and granulocytic elements. In contrast to secondary polycythemias, PV is associated with a low serum level of the hormone erythropoietin (EPO).

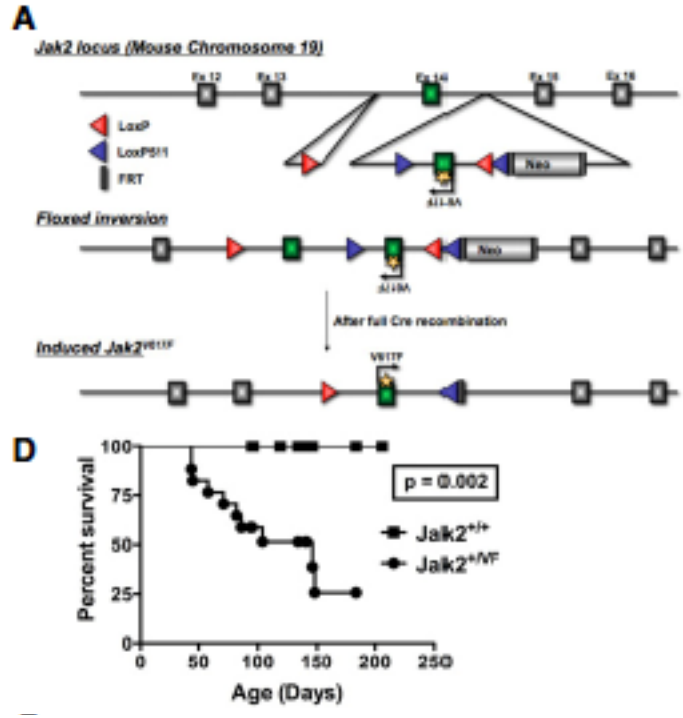
Disorder	*Mutation*	*Main features*	*Main treatment*
Hemoglobinopathies **ß-thalassemia**	**ß-globin gene**	**IE, anemia** ***Increased number of erythroid progenitor cells***	**TX, iron chelation, BMT, Gene therapy (?)**
Myeloproliferative disorder **Polycythemia vera**	**Mostly Jak2^{V617F}**	**Erythrocytosis** ***Increased number of erythroid progenitor cells***	**Phlebotomy, Jak2i**

A mouse model to study Polycythemia vera

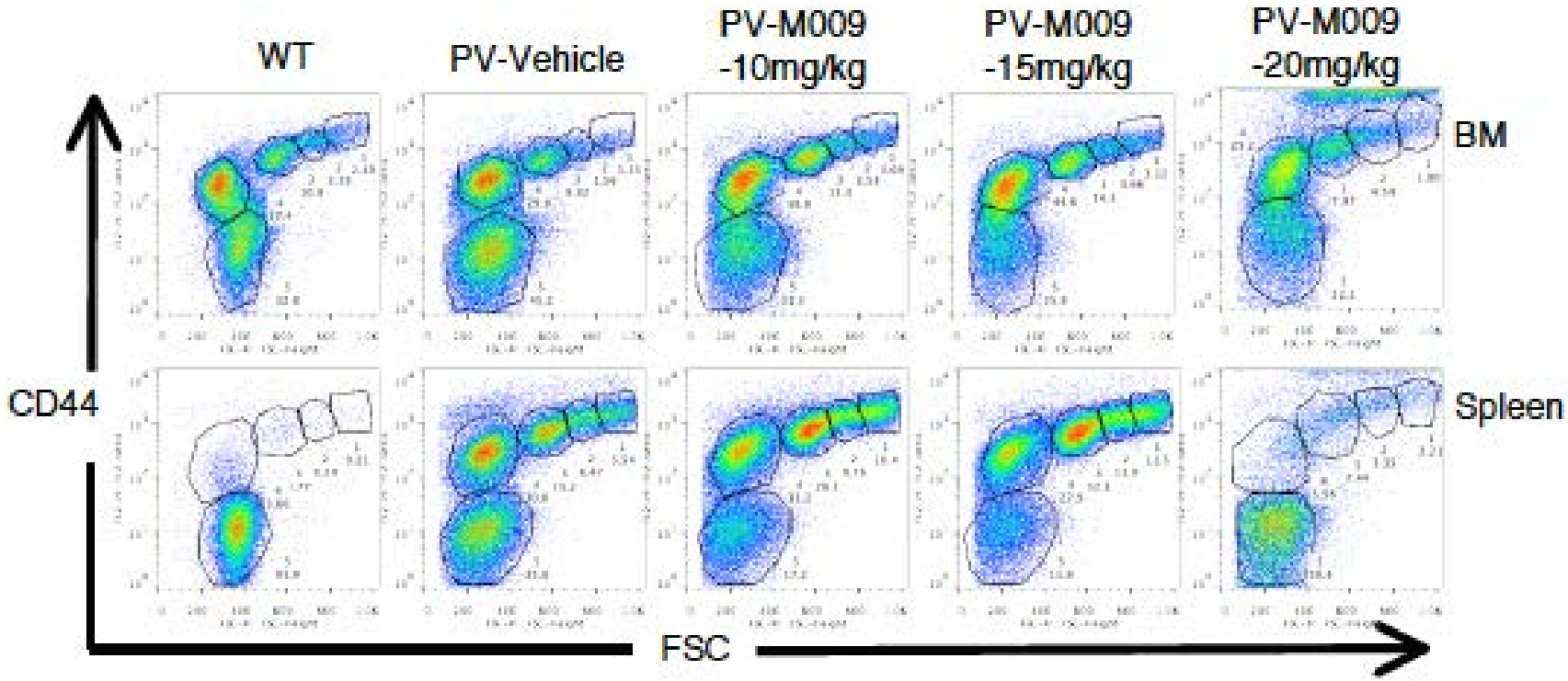
Cancer Cell
Article

Physiological *Jak2V617F* Expression Causes a Lethal Myeloproliferative Neoplasm with Differential Effects on Hematopoietic Stem and Progenitor Cells

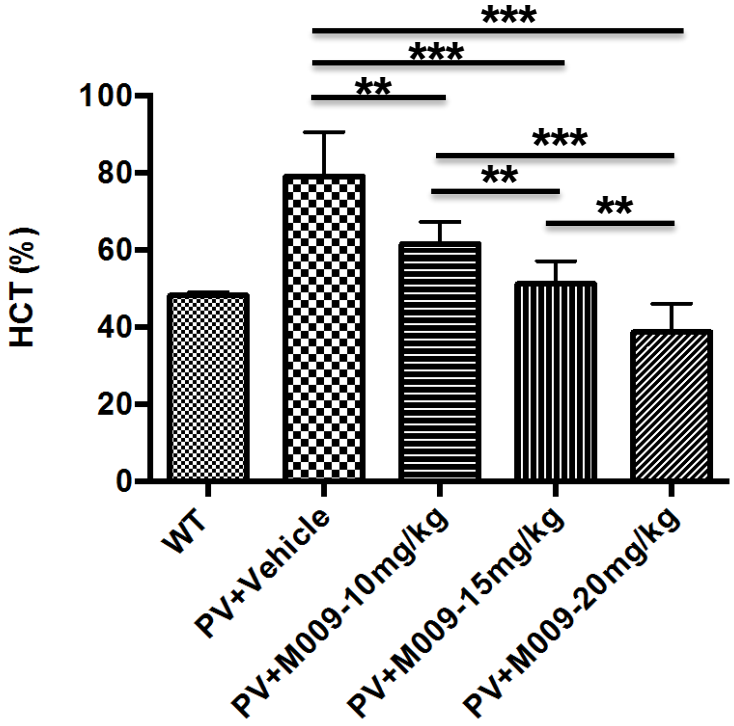
Ann Mullally,[1,2,9] Steven W. Lane,[2,4,9] Brian Ball,[2] Christine Megerdichian,[2] Rachel Okabe,[2] Fatima Al-Shahrour,[2,5] Mahnaz Paktinat,[2] J. Erika Haydu,[2] Elizabeth Housman,[2] Allegra M. Lord,[2] Gerlinde Wernig,[6] Michael G. Kharas,[2] Thomas Mercher,[7] Jeffery L. Kutok,[3] D. Gary Gilliland,[2,8] and Benjamin L. Ebert[1,2,5,*]

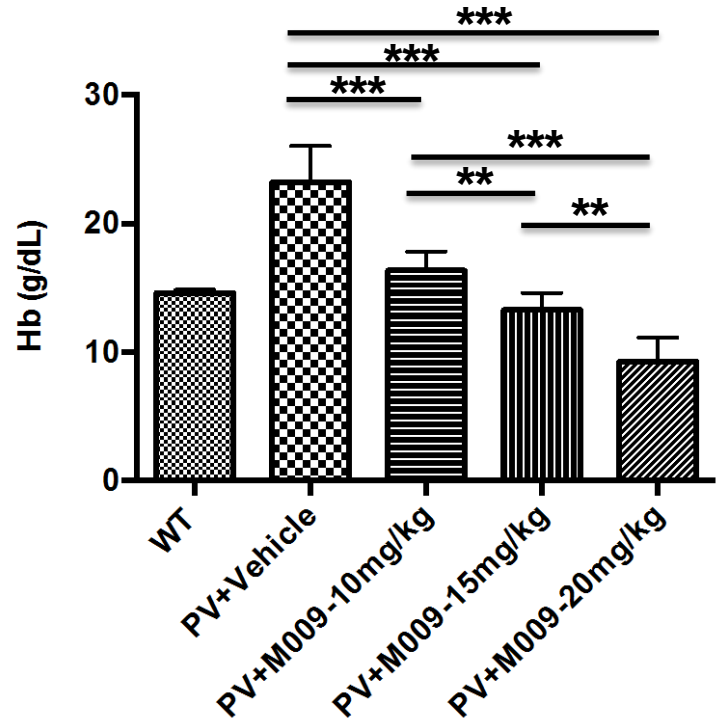


Administration of MH improves erythropoiesis in mice affected by Polycythemia vera

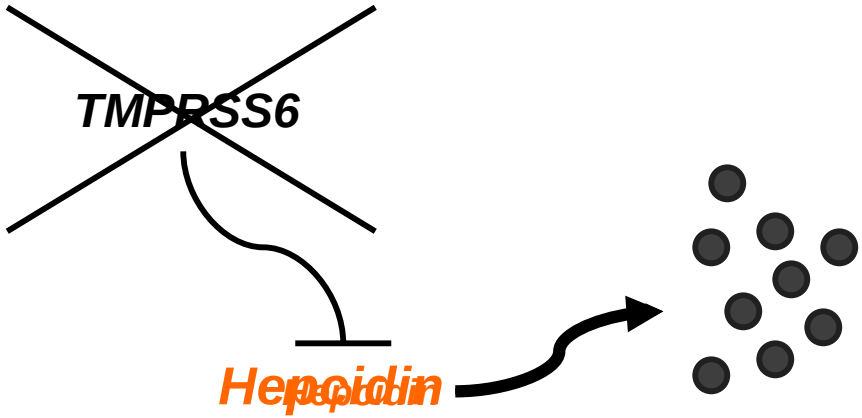


Administration of MH corrects Hb levels and HTC in mice affected by Polycythemia vera





Tmprss6 as a potential target to treat NTDT



Decreased iron absorption and Normal iron absorption recycling

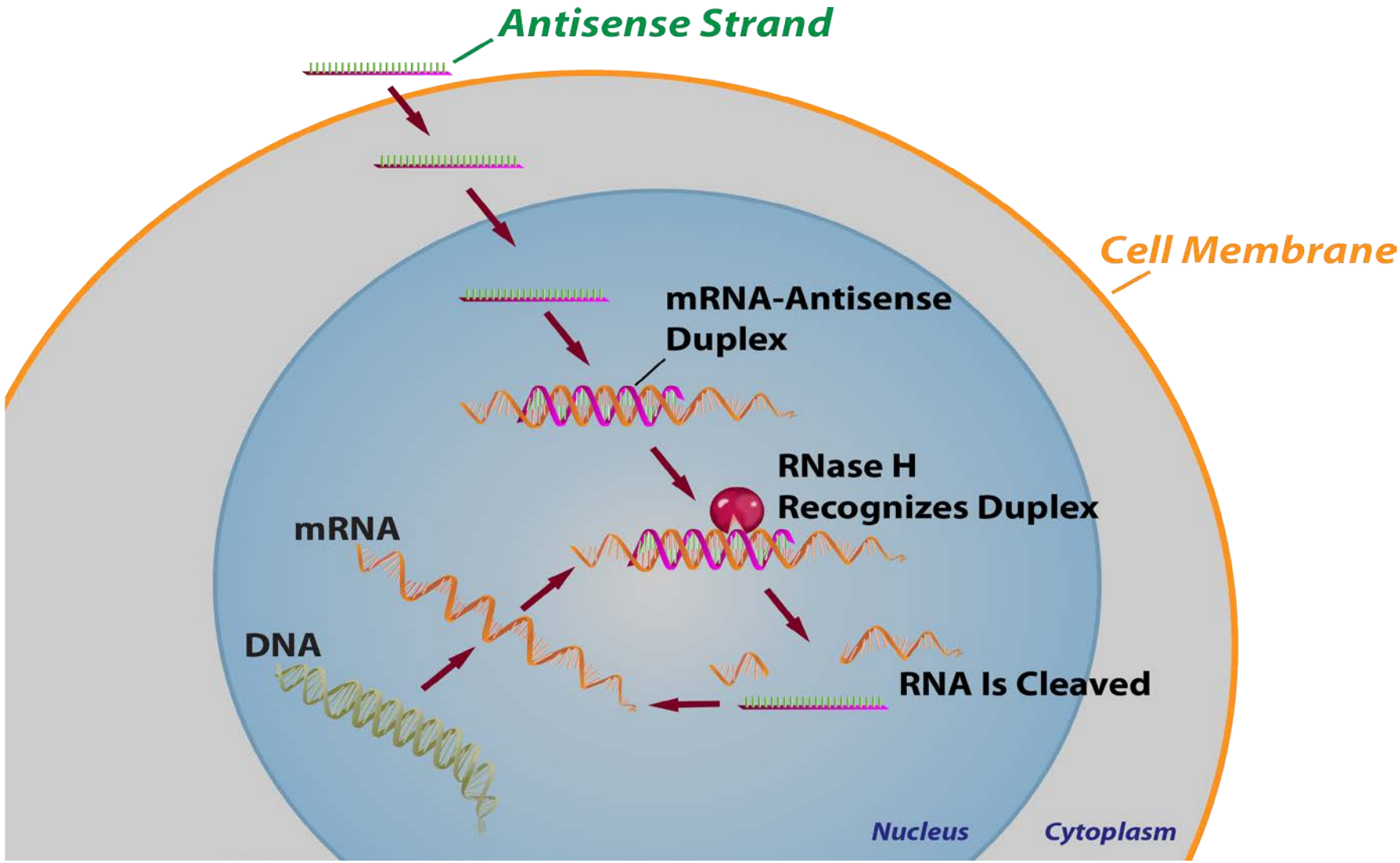
Deletion of *TMPRSS6* attenuates the phenotype in a mouse model of β-thalassemia

Antonella Nai,[1,2] Alessia Pagani,[1,2] Giacomo Mandelli,[3] Maria Rosa Lidonnici,[1,3] Laura Silvestri,[1,2] Giuliana Ferrari,[1,3] and Clara Camaschella[1,2]

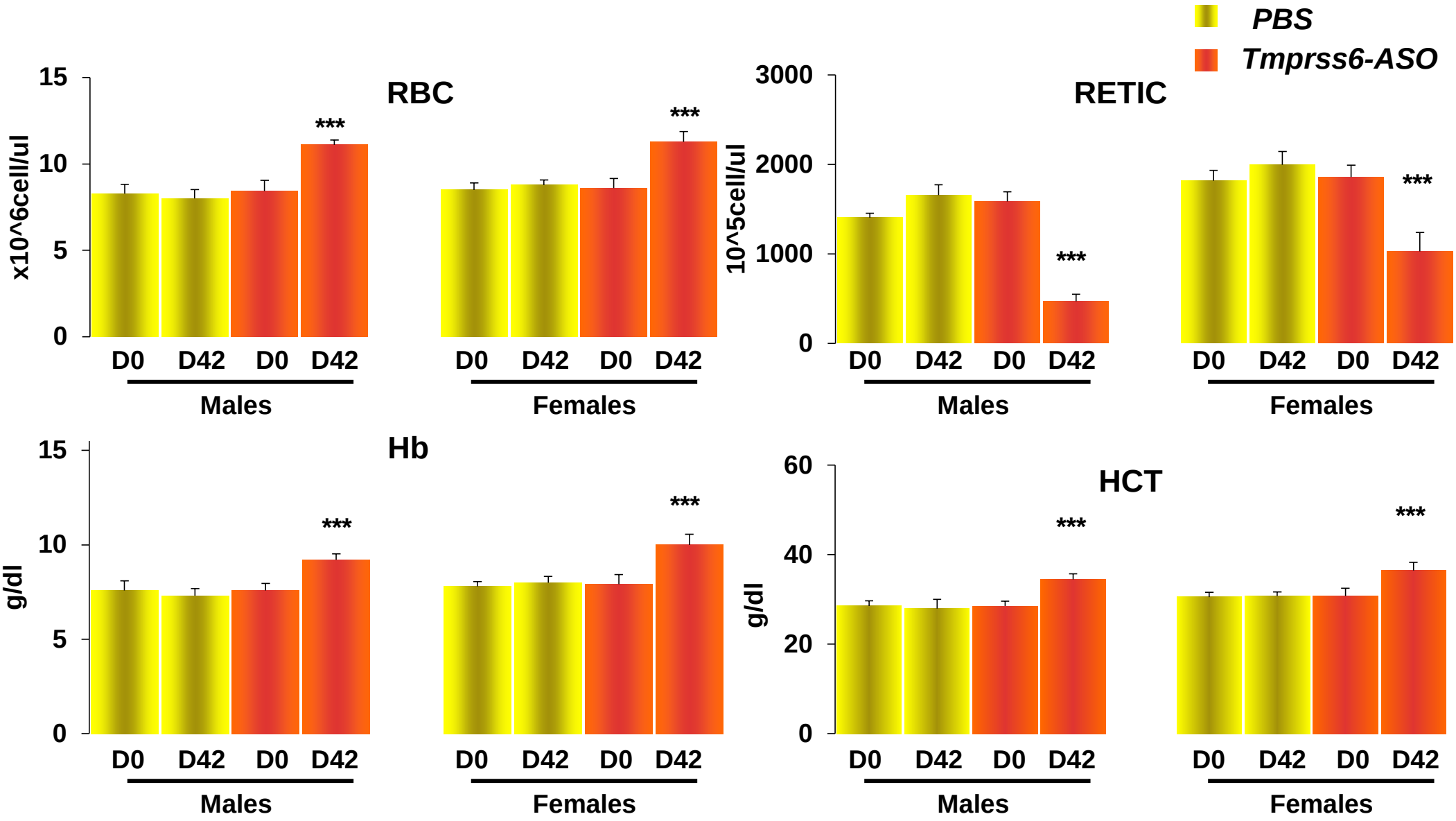
Deletion of *Tmprss6* improves iron overload and erythropoiesis in a mouse model of NTDT

Antisense mechanism of action

RNase H-mediated degradation

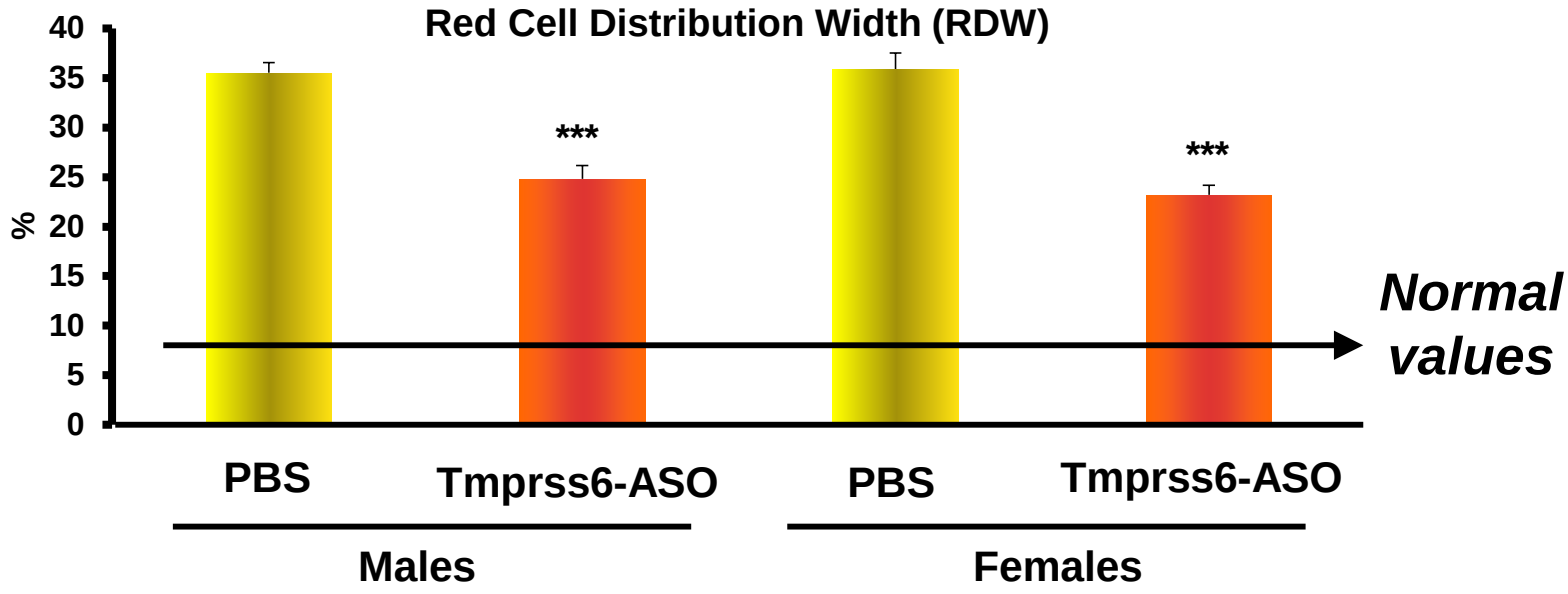


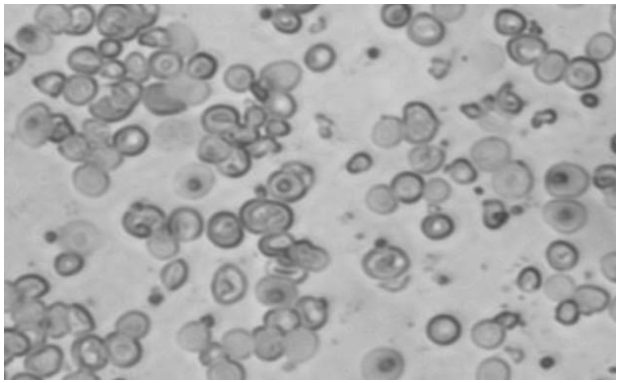
Tmprss6-ASO treatment significantly improves anemia in thalassemic mice (*th3*/+)



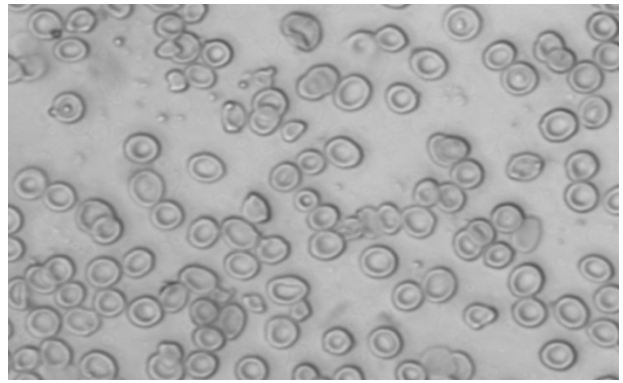
Shuling Guo & Carla Casu

TMPRSS6 ASO treatment improves thalassemic red cell parameters and morphology

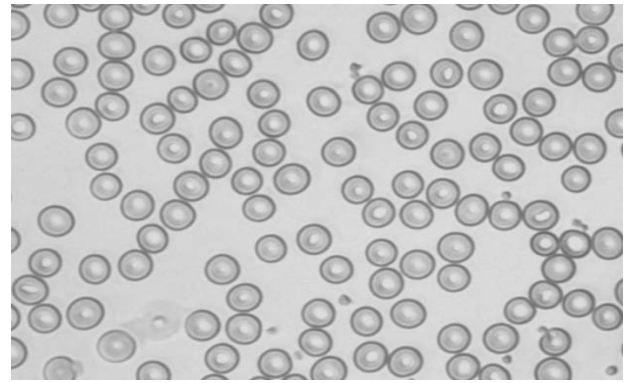




th3/+ PBS



th3/+ Tmprss6-ASO



WT

Carla Casu & Shuling Guo

Use of *Tmprss6* inhibitors improves iron overload in a mouse model of HFE-related hemochromatosis and erythropoiesis and organ iron content in NTDT

The Journal of Clinical Investigation

Reducing TMPRSS6 ameliorates hemochromatosis and β-thalassemia in mice

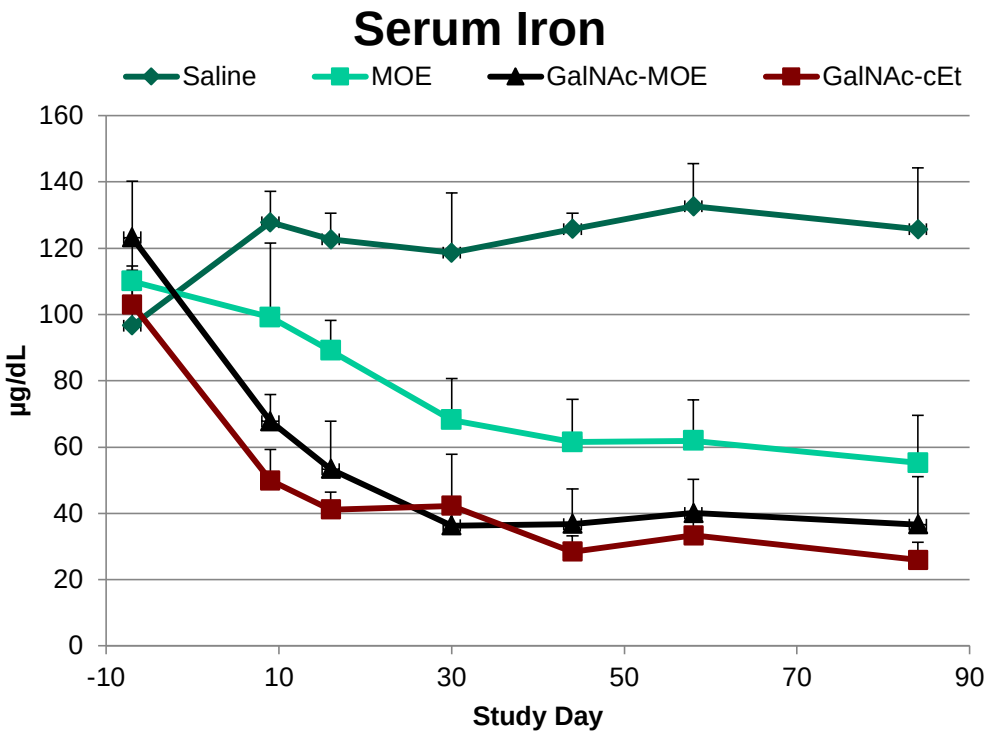
Shuling Guo,[1] Carla Casu,[2] Sara Gardenghi,[2] Sheri Booten,[1] Mariam Aghajan,[1] Raechel Peralta,[1] Andy Watt,[1] Sue Freier,[1] Brett P. Monia,[1] and Stefano Rivella[2,3]

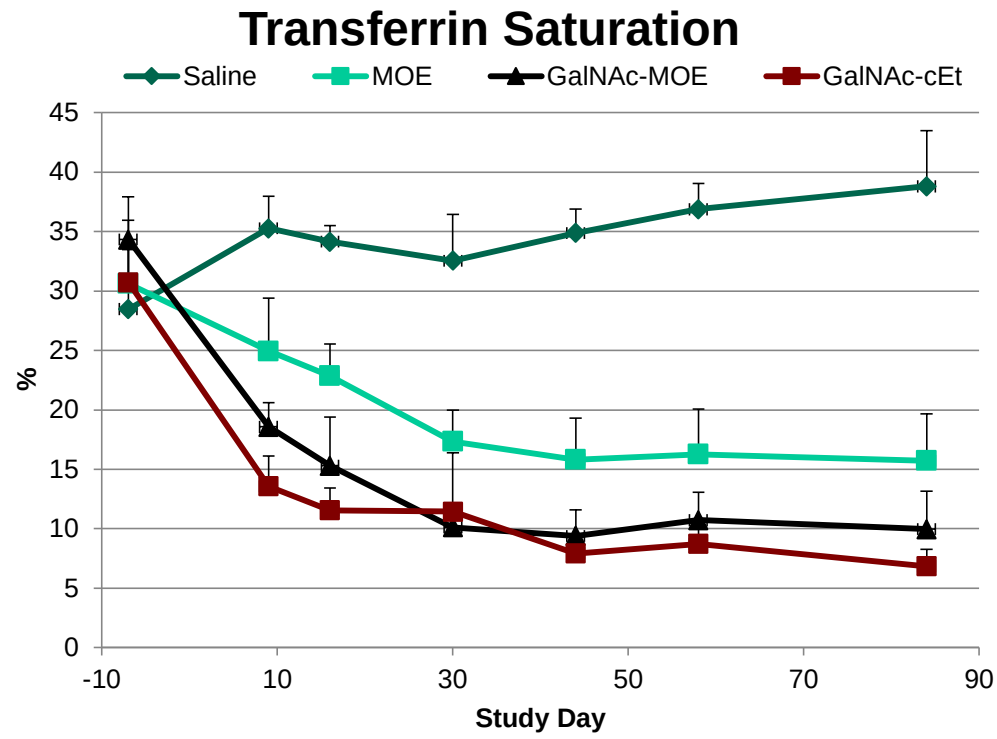
An RNAi therapeutic targeting Tmprss6 decreases iron overload in *Hfe*$^{-/-}$ mice and ameliorates anemia and iron overload in murine β-thalassemia intermedia

Paul J. Schmidt,[1] Iva Toudjarska,[2] Anoop K. Sendamarai,[1] Tim Racie,[2] Stuart Milstein,[2] Brian R. Bettencourt,[2] Julia Hettinger,[2] David Bumcrot,[2] and Mark D. Fleming[1]

TMPRSS6 ASO treatment significantly reduces serum iron and transferrin saturation in non-human primates

- **TMPRSS6-ASO candidates has been selected**
  - **Well tolerated in mice and in monkeys**
  - **Demonstrated excellent activity and pharmacology in both transgenic mice and non-human primates**

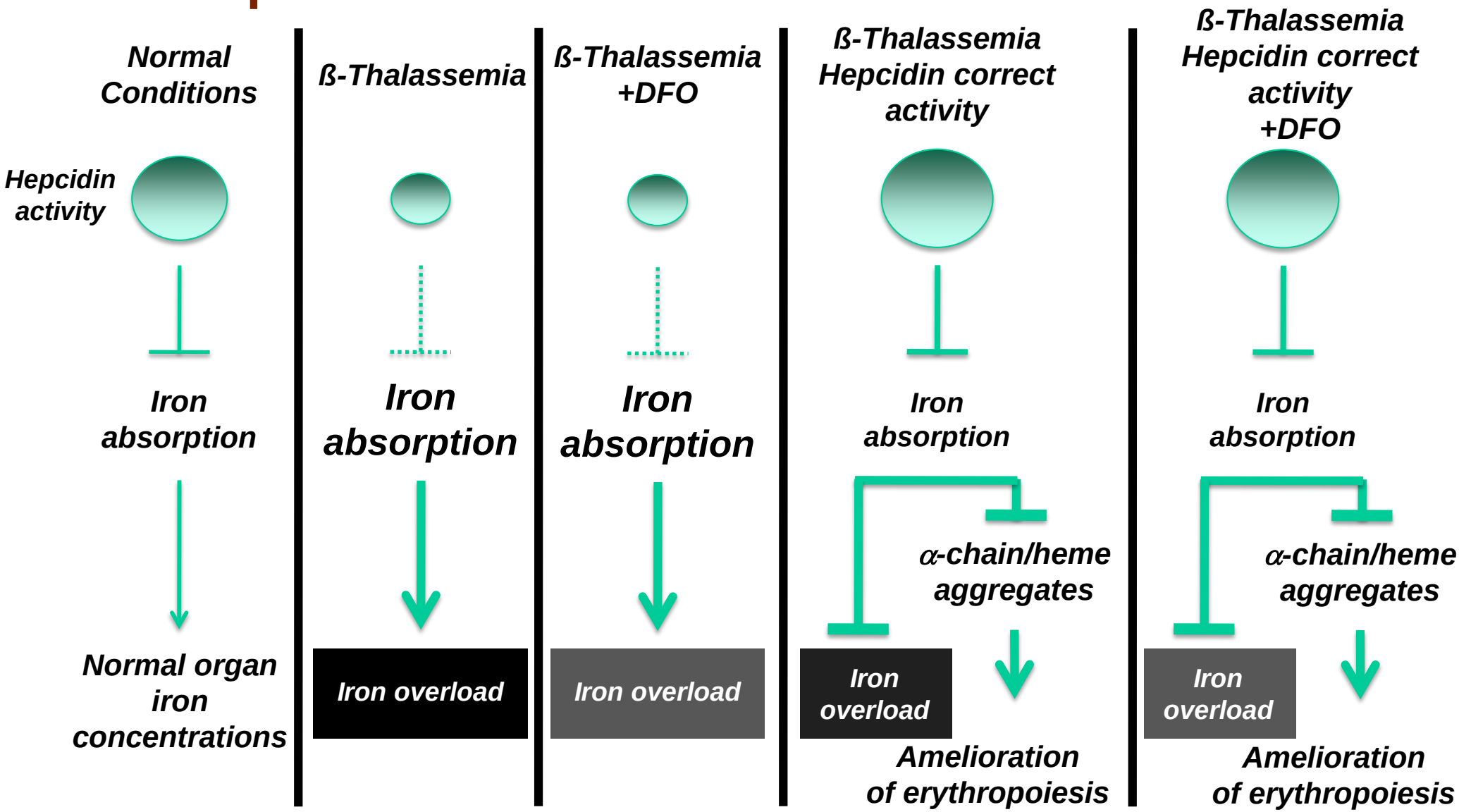




- **Initiation of the Phase 1 clinical trial is expected in 2016**

Shuling Guo, Isis P.

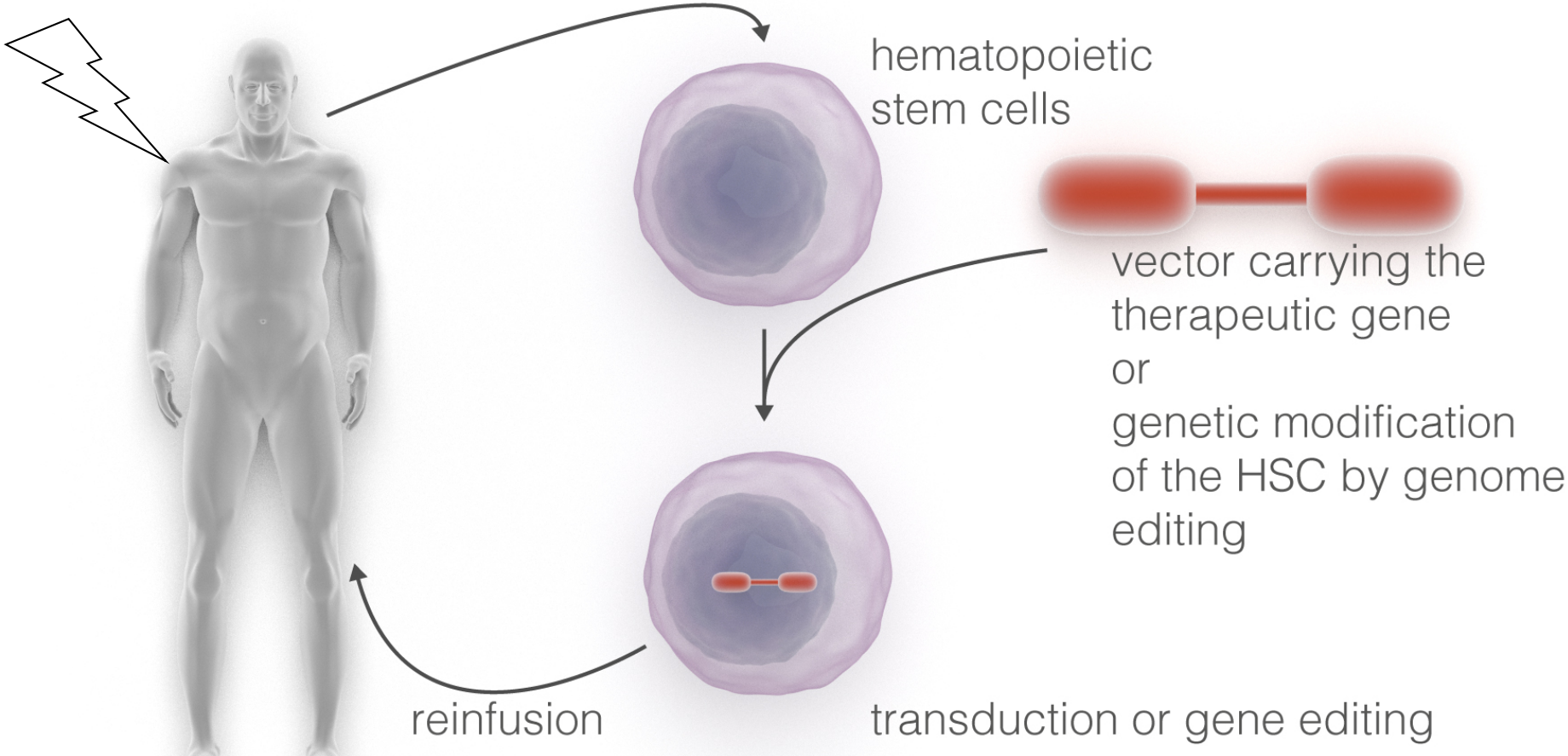
Potential effects of Hepcidin agonists or activators on iron absorption and erythropoiesis in ß-thalassemia in presence or absence of iron chelation



Severe hemoglobinopathies:

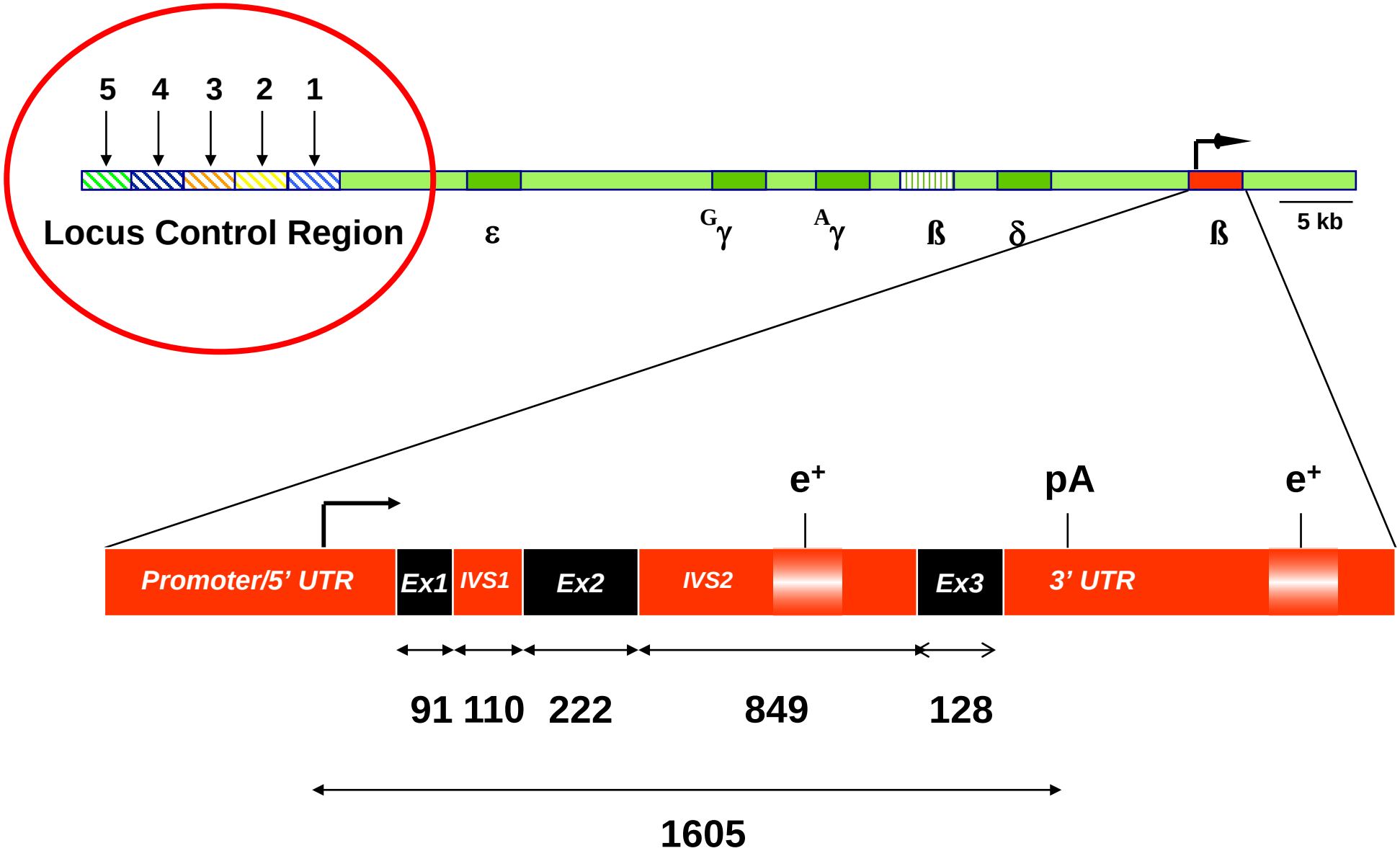
Gene therapy approach

Gene Therapy Schematic Approach

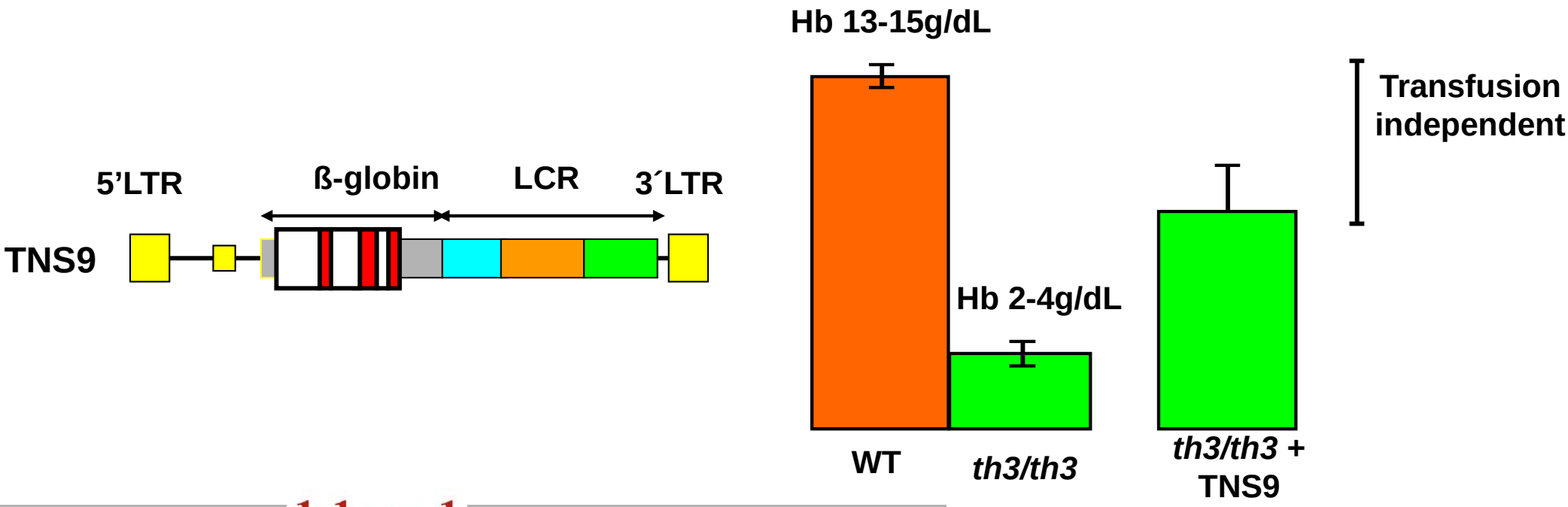


Rivella S; Haematologica 2015

Human ß-globin gene



Therapeutic levels of Hb in mice affected by Cooley's anemia



Plenary paper blood

A novel murine model of Cooley anemia and its rescue by lentiviral-mediated human β-globin gene transfer

Stefano Rivella, Chad May, Amy Chadburn, Isabelle Rivière, and Michel Sadelain

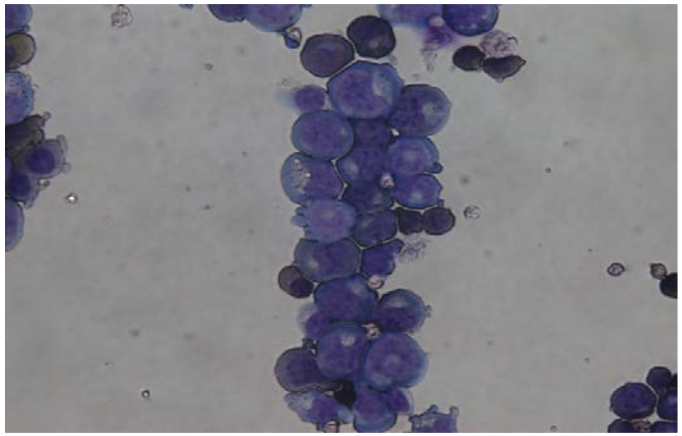
Beta thalassemia patients: ß0/ß0, ß0/ß+ and ß+/ß+

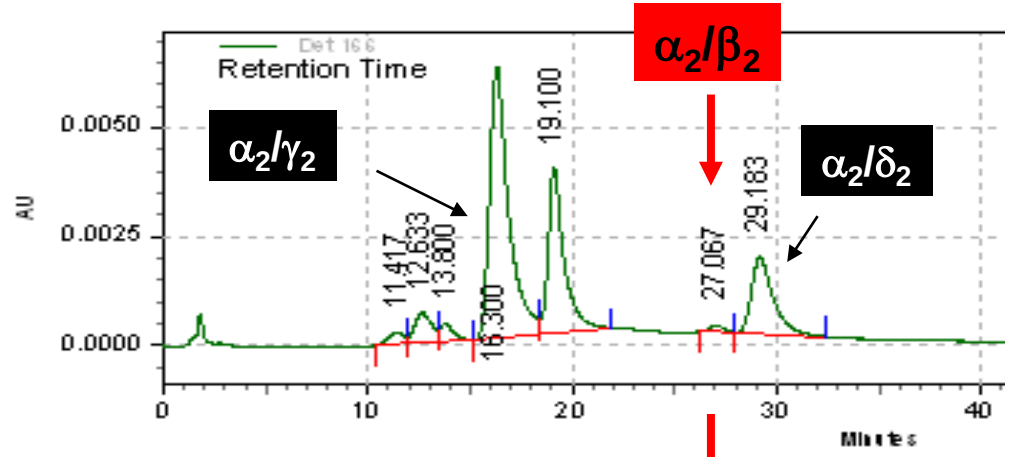
Based on the ß-globin protein synthesis, all mutations can be classified as: ß0, where no ß-globin protein is produced, or ß+, where some, but not sufficient ß-globin chain is made

Therefore, all patients can be classified as ß0/ß0, ß0/ß+ or ß+/ß+, according to all possible combinations of these mutations

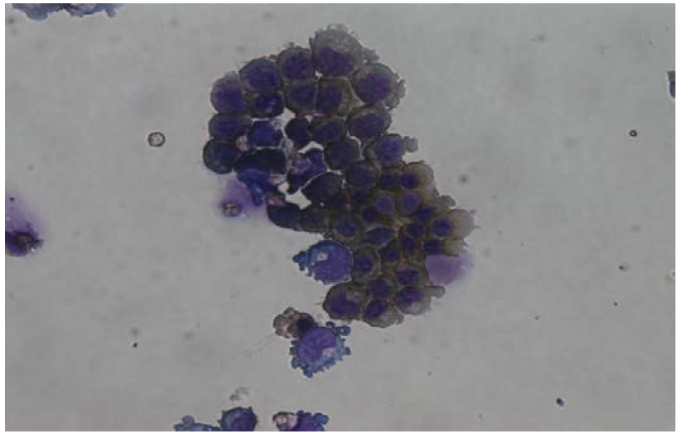
AnkT9W Increases significantly the HbA in ß0/0 specimens

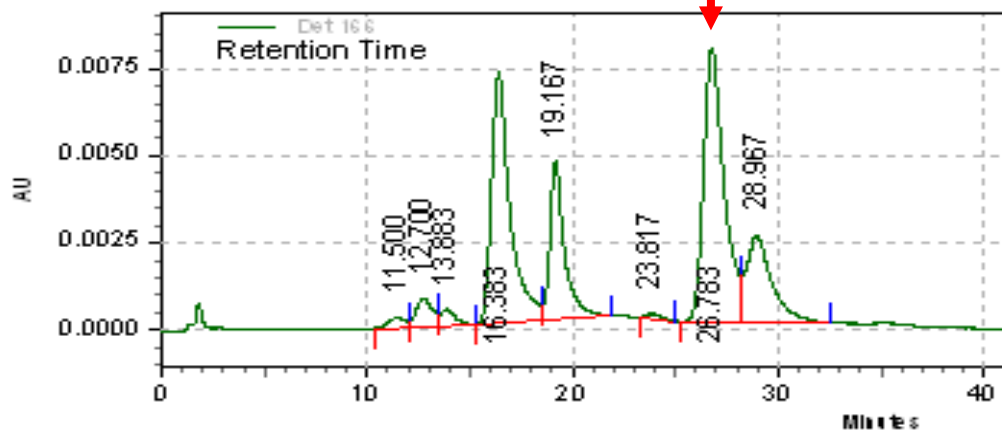
ß⁰/ß⁰ no-vector





ß⁰/ß⁰ + vector

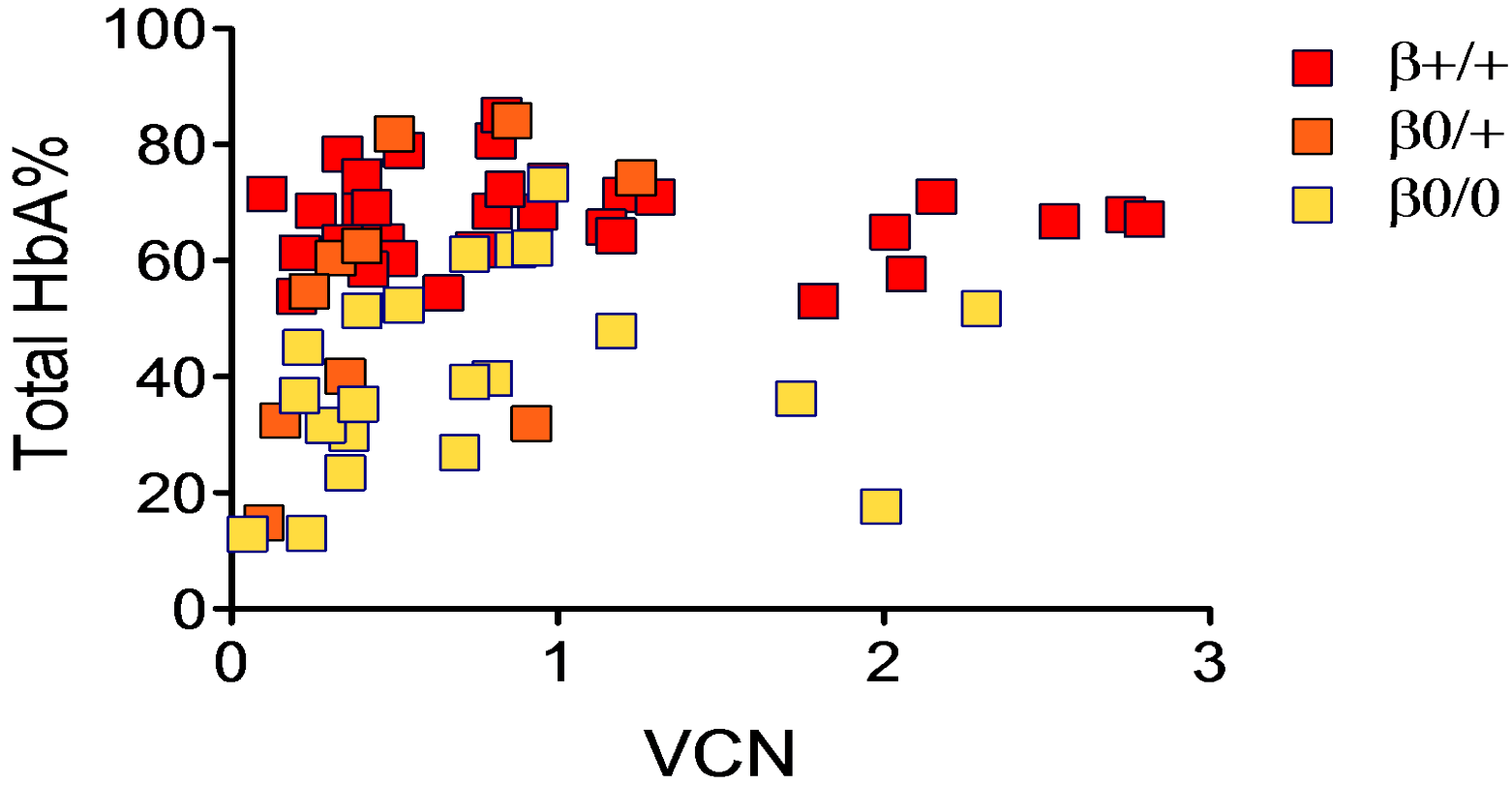




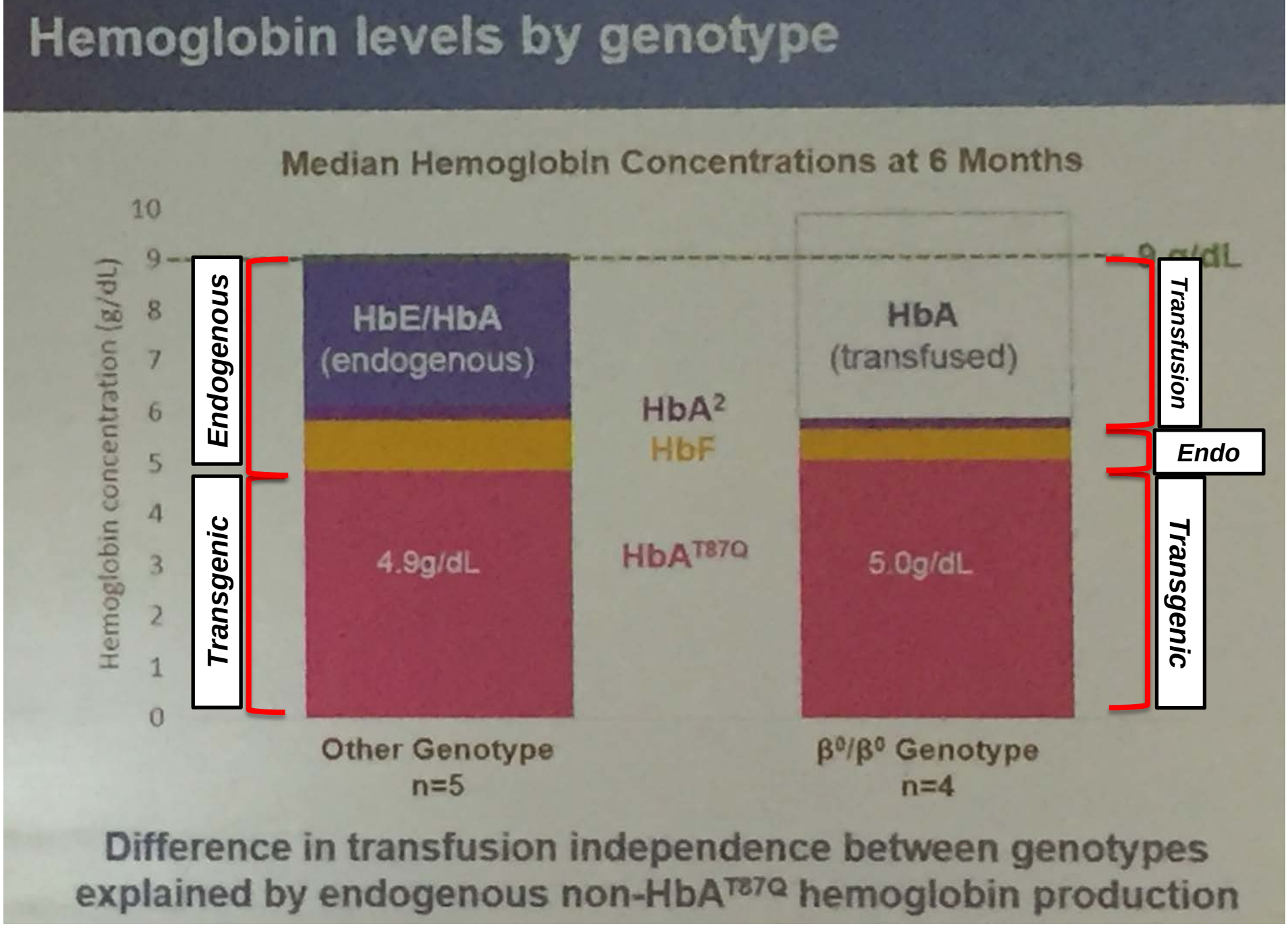
Laura Breda

Patient Genotype Still Matters

Therapeutic Hb synthesis in ß+/+ and ß+/0 cells is reached at lower VCN compared to ß0/0 cells



Breda L, Casu C; PloS One 2012

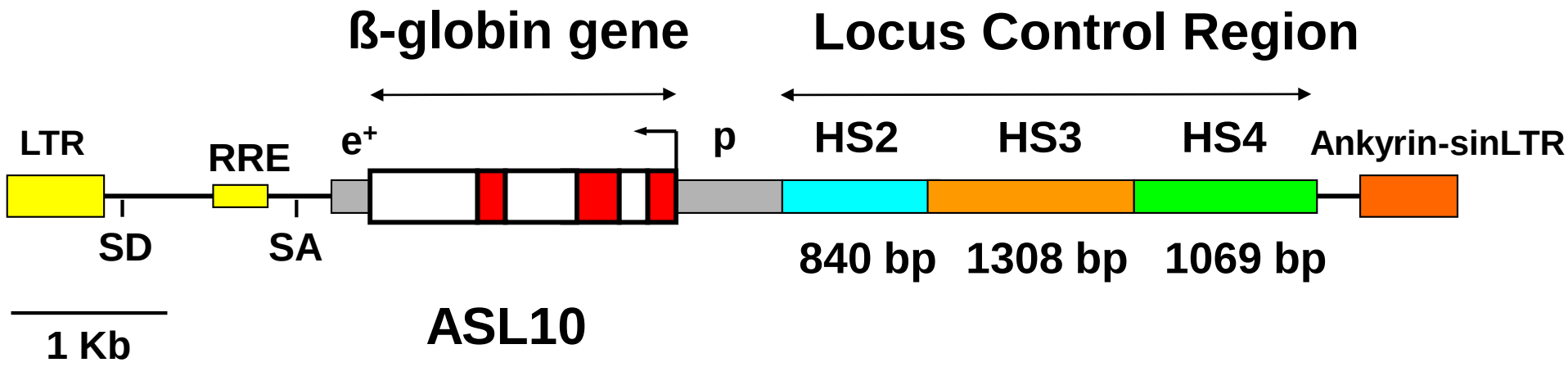


M Walters, ASH 2015, Children's Hospital of Oakland

New vector: ASL10

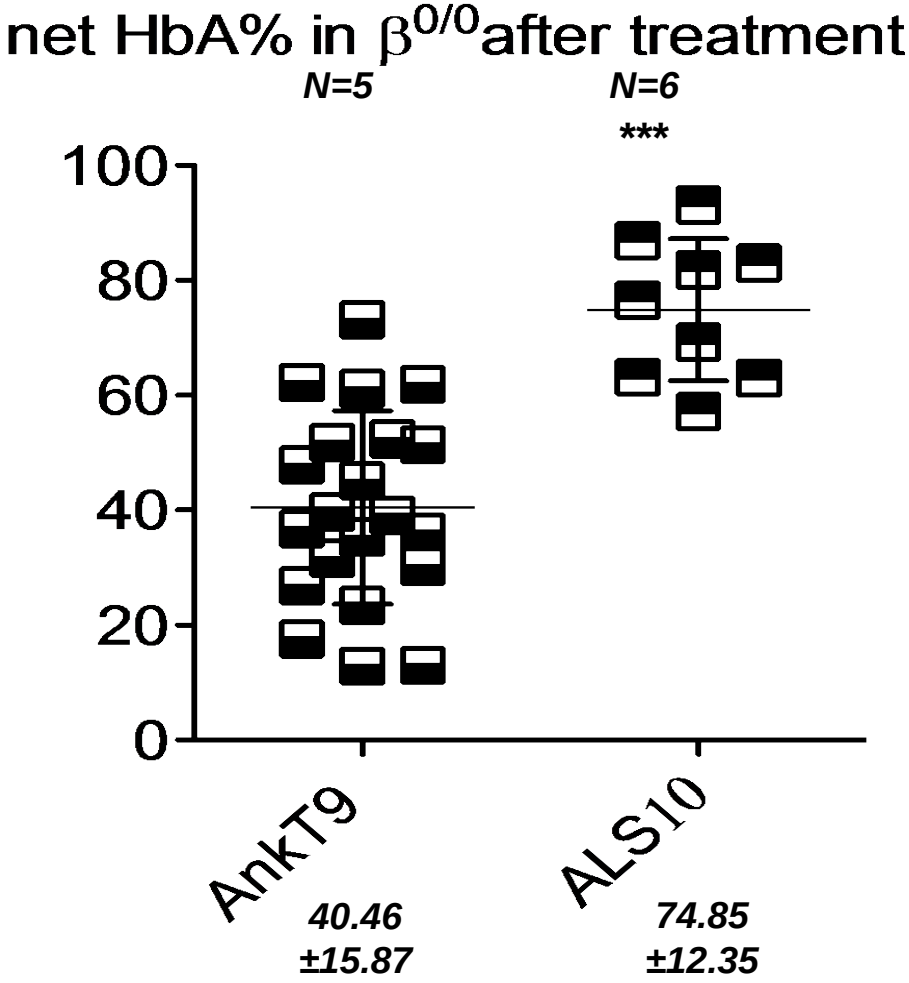
1. Elimination of the WPRE element. Modification of the vector backbone to preserve high titer viral production also in absence of WPRE.

2. Inclusion of the full second ß-globin intron. It contains enhancers and Oct1 binding site that help with LCR looping globin synthesis



Laura Breda, Carla Casu, Alisa Dong

ASL10 performs significantly better in ß0ß0 patient cells compared to AnkT9W

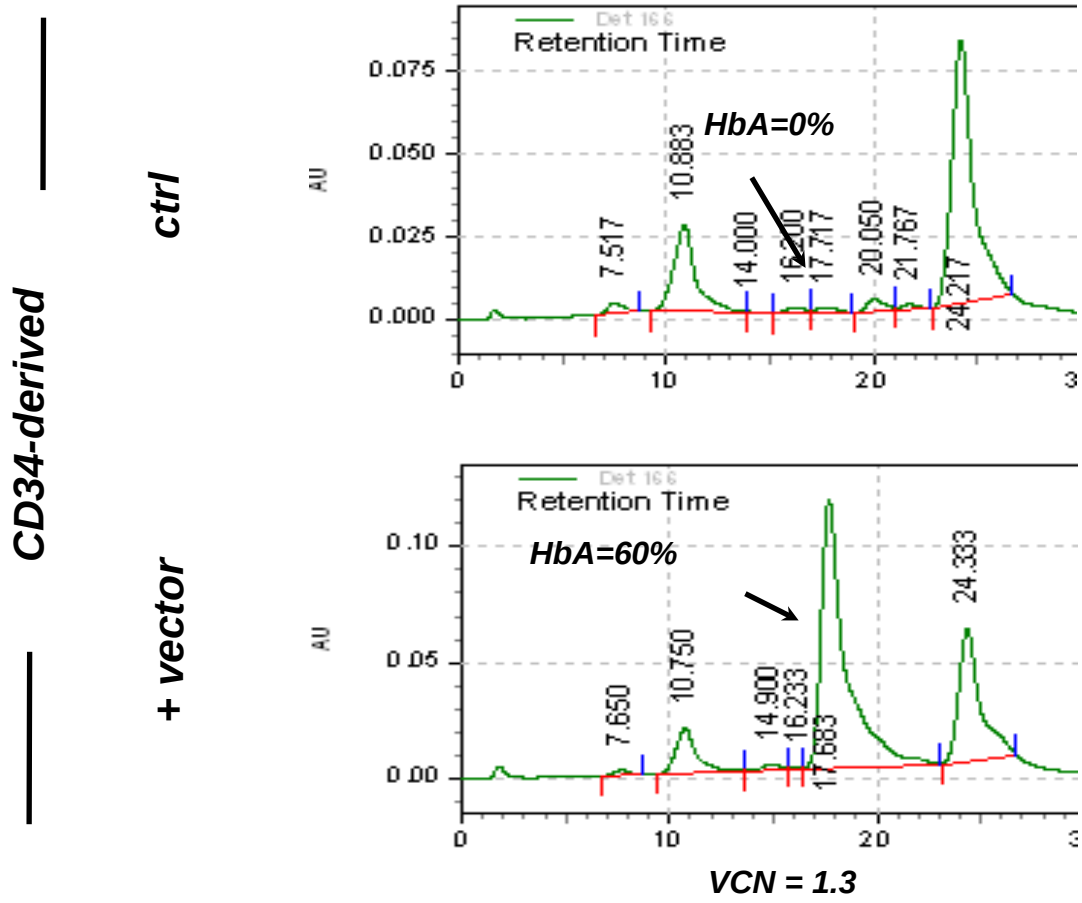


The goals are:

- **Compare ALS10 to vectors already in clinical trial**
- **Generate GMP-quality vector and file and IND application**
- **Clinical trial for ß-thalassemia and SCD**

Alisa Dong and Laura Breda

High level of curative hemoglobin in patient cells from sickle cell patients



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Rivella Lab CHOP