Ten years of Highlights from EHA: Red cells and Iron

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Ten years of Highlights from EHA:

Red cells and Iron

Summary:

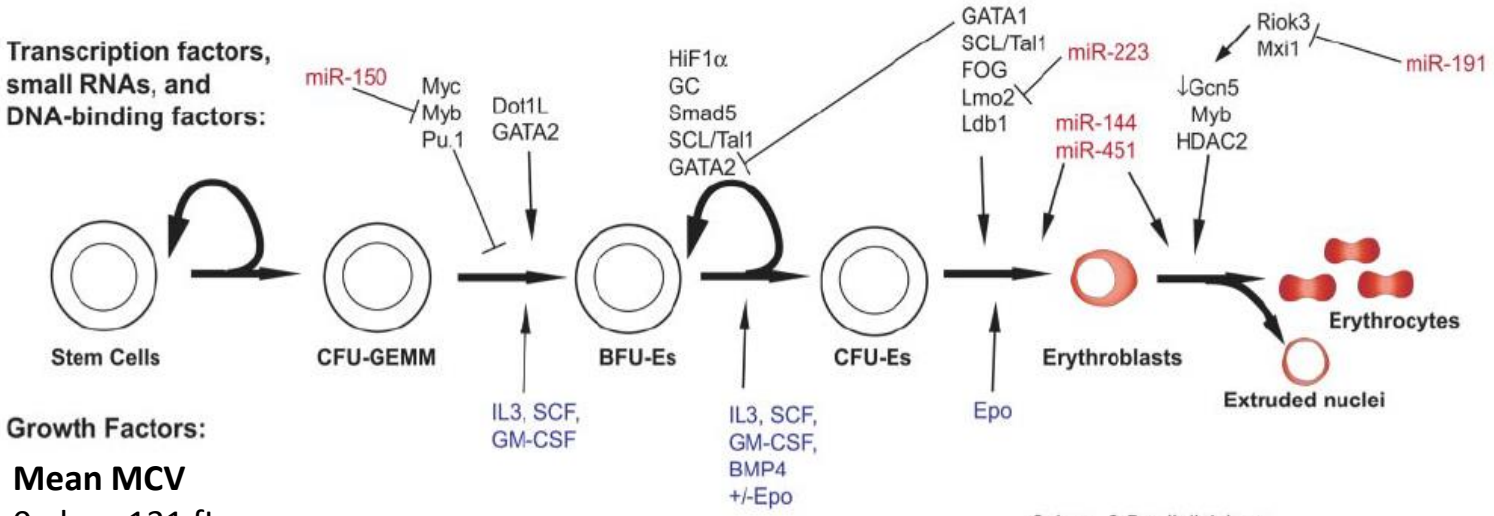
Erythropoiesis : mechanisms

New Diagnostic Approach: new disease-causing genes

New Drugs for inherited anemias

New Treatment Approach: gene therapy and Crisp/Cas9

Molecular Events during Normal Erythropoiesis



Mean MCV

0 day 131 fL

7 day 99 fL

14 day 86 fL

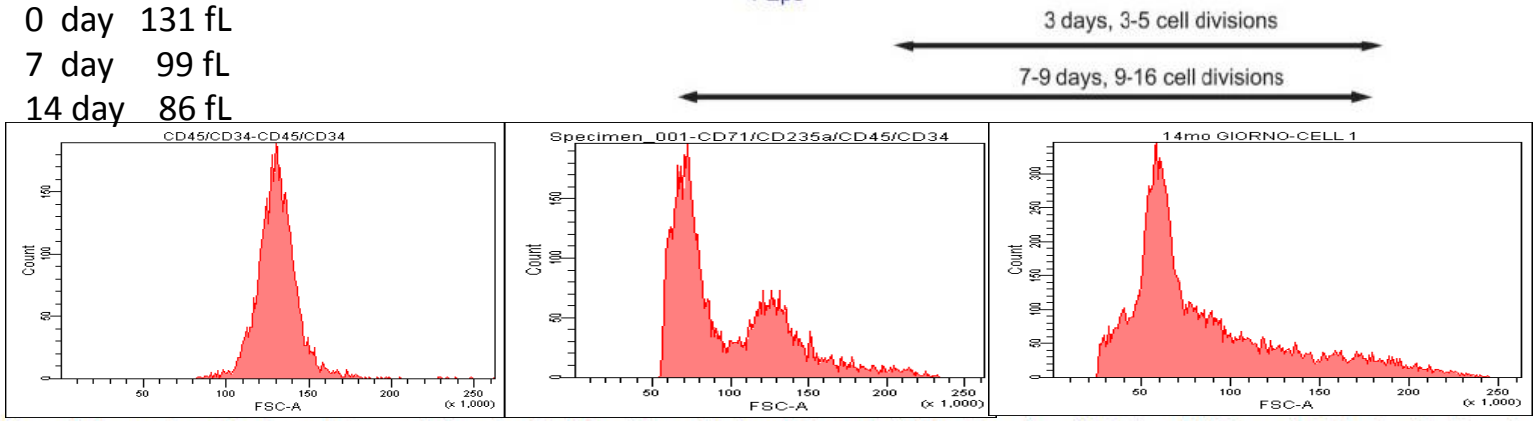
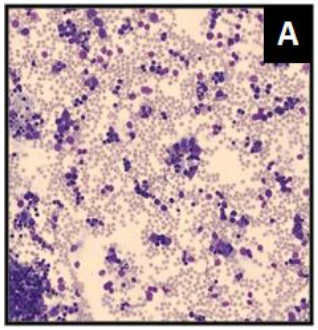
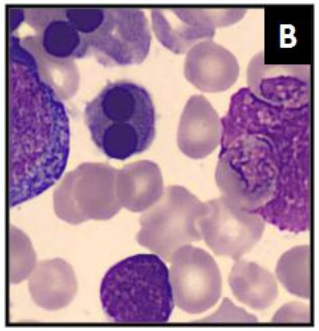


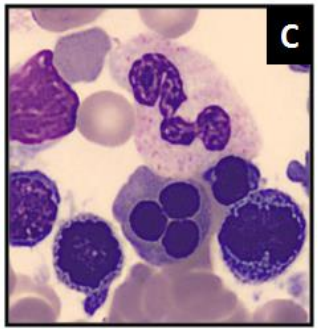
Figure 1. An overview of erythropoiesis: regulation at multiple levels by multiple proteins and miRNAs. Formation of RBCs from HSCs is regulated by signaling through both external factors (blue), such as cytokines and fibronectin, as well as intracellular factors, such as transcription factors (black) and miRNAs (red). Below the differentiation network, a timeline and images are shown for in vitro methylcellulose colony formation of murine BFU-E and CFU-E.

Erythropoiesis block in Inherited Anemias (EIAs)

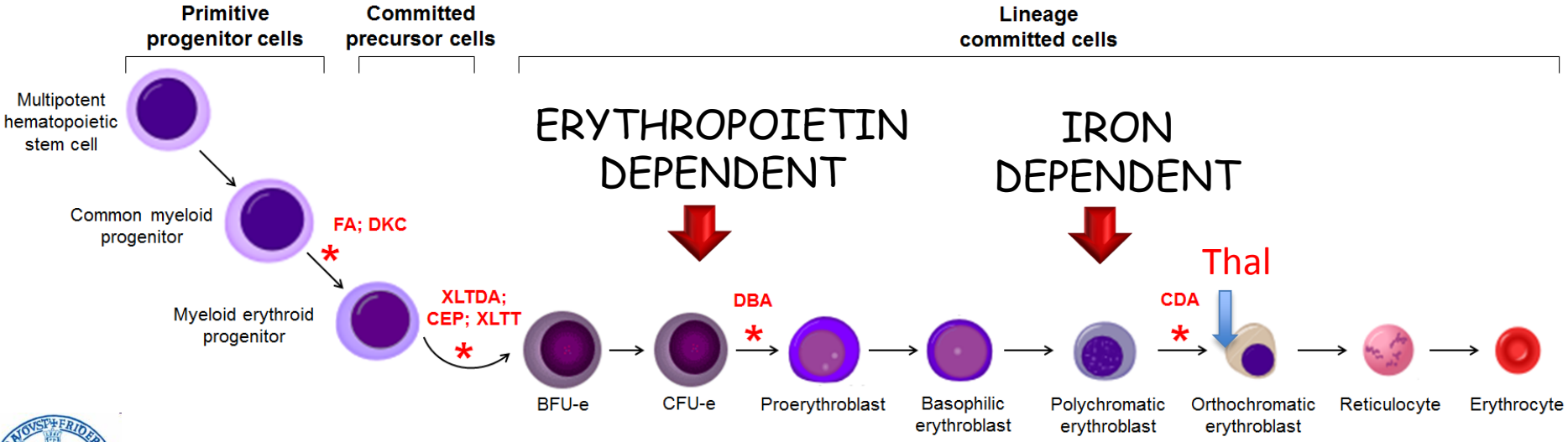
- ✓ **EIAs** are **mendelian** diseases affecting the normal differentiation-proliferation pathway of the erythroid lineage.





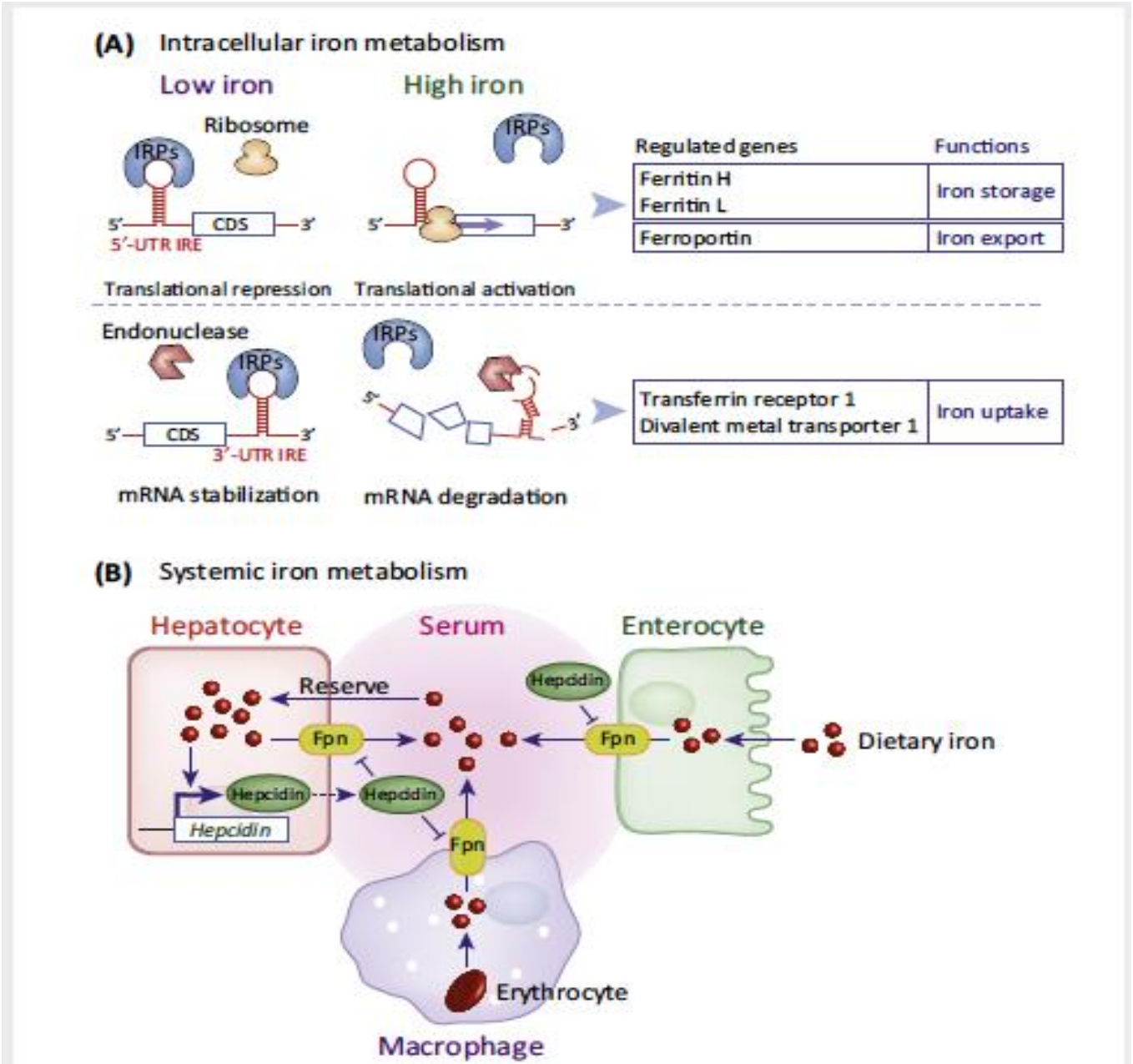


Erythroid hyperplasia with specific morphological alterations involving late erythroblasts

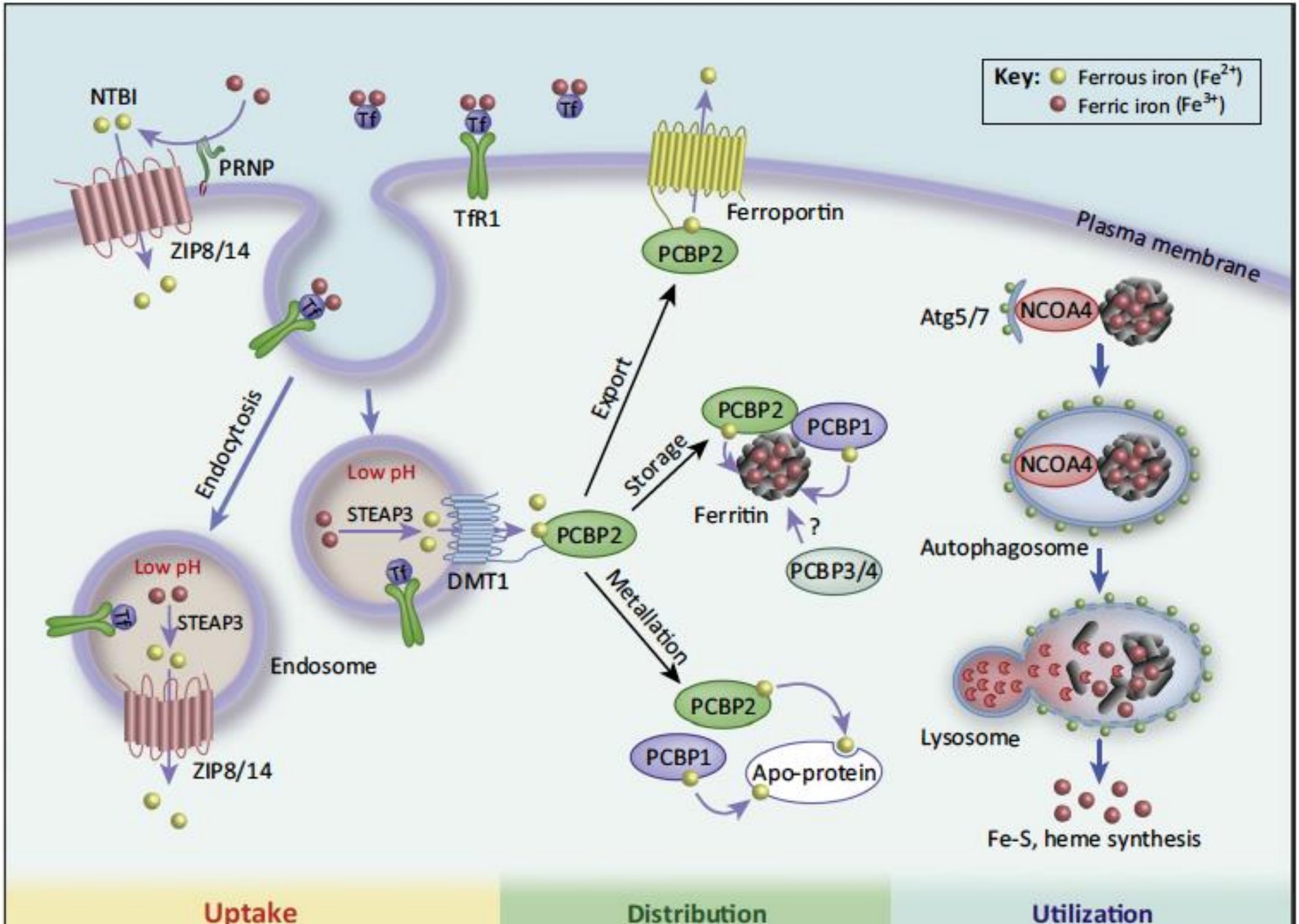


Williams Hematology, Nineth Edition, Chapter 39 by A. Iolascon - McGraw-Hill

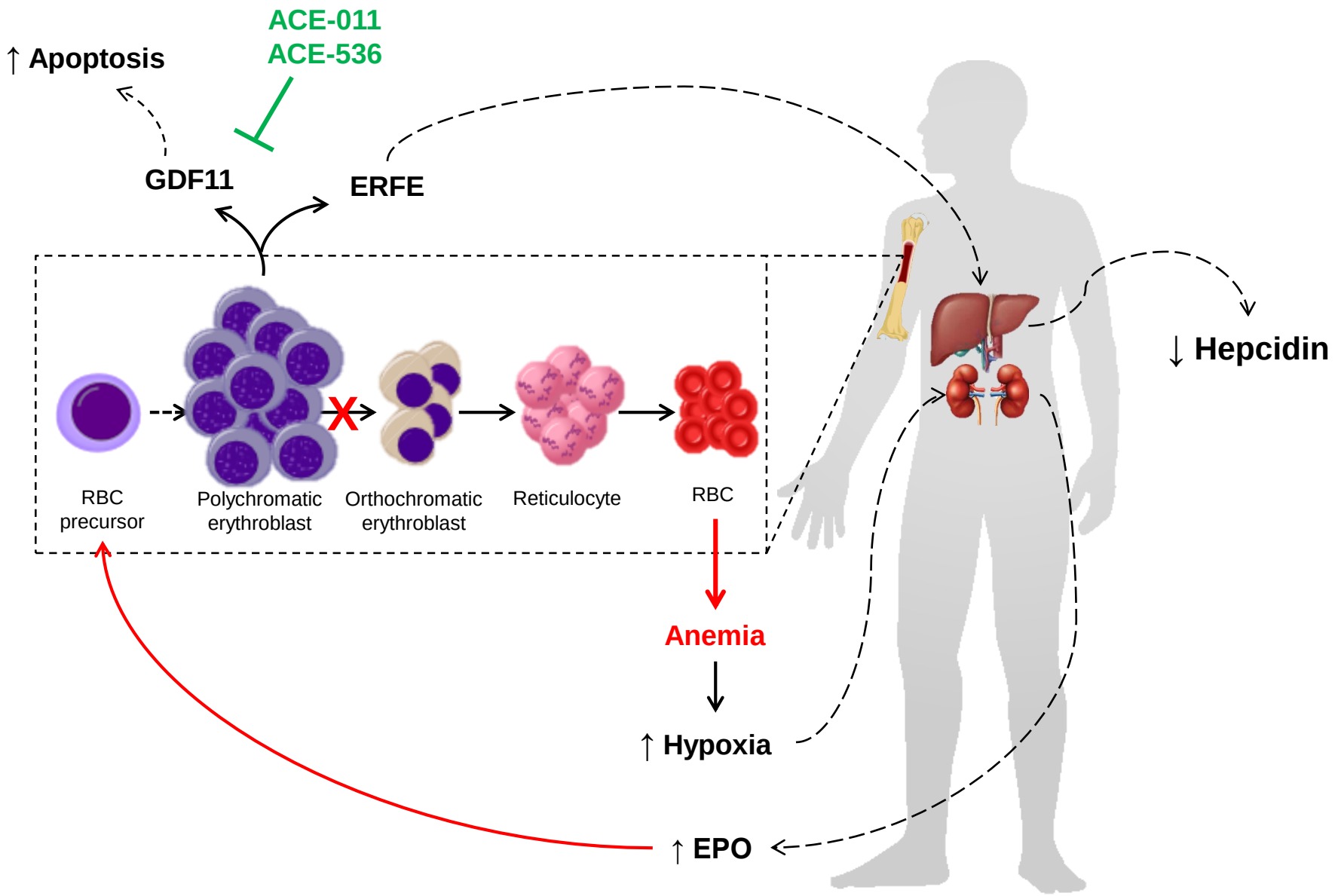
Mechanisms of systemic and intracellular iron homeostasis



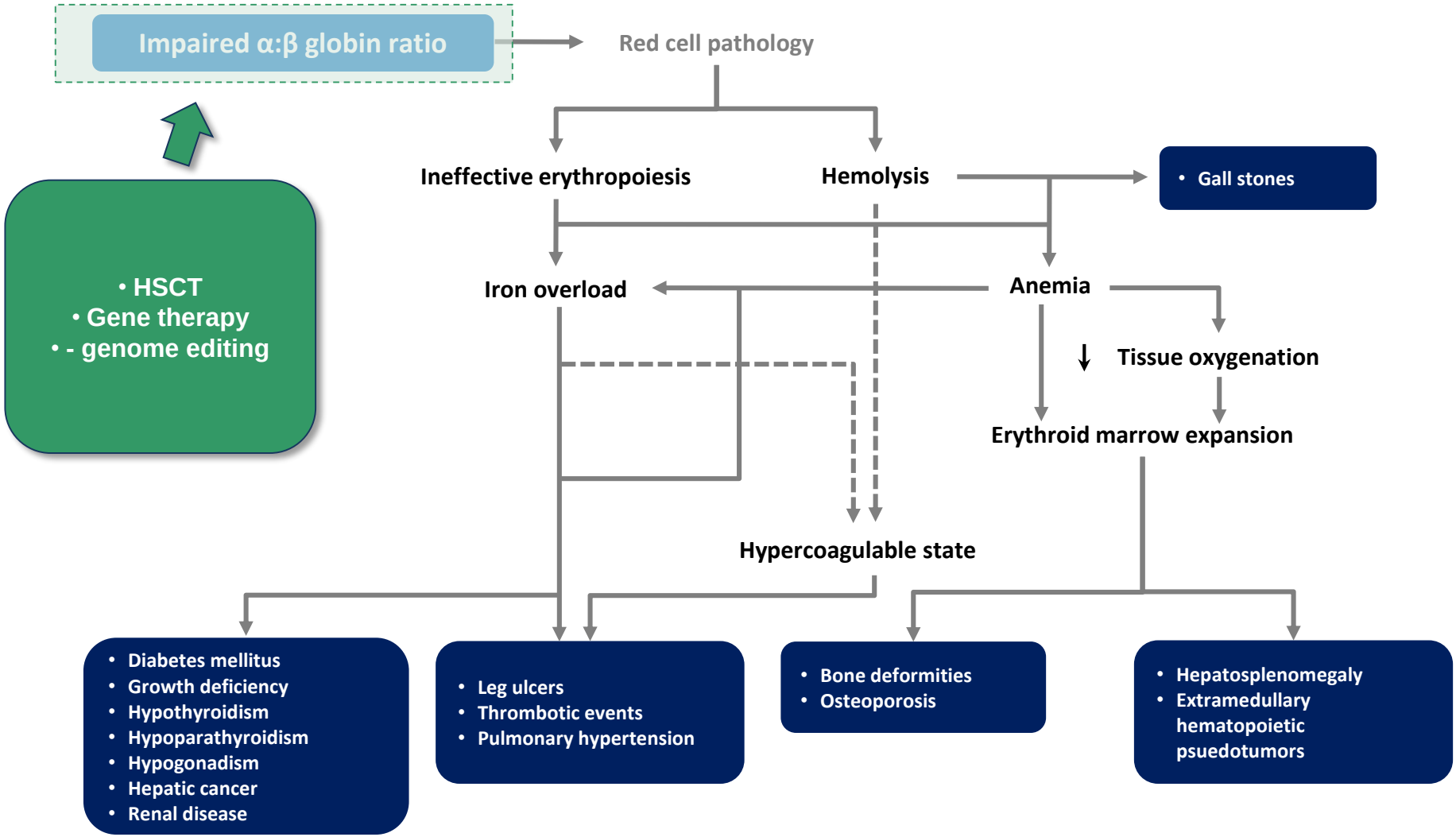
New Mechanisms regulating Iron Intracellular Metabolism



Physiopathology of iron overload

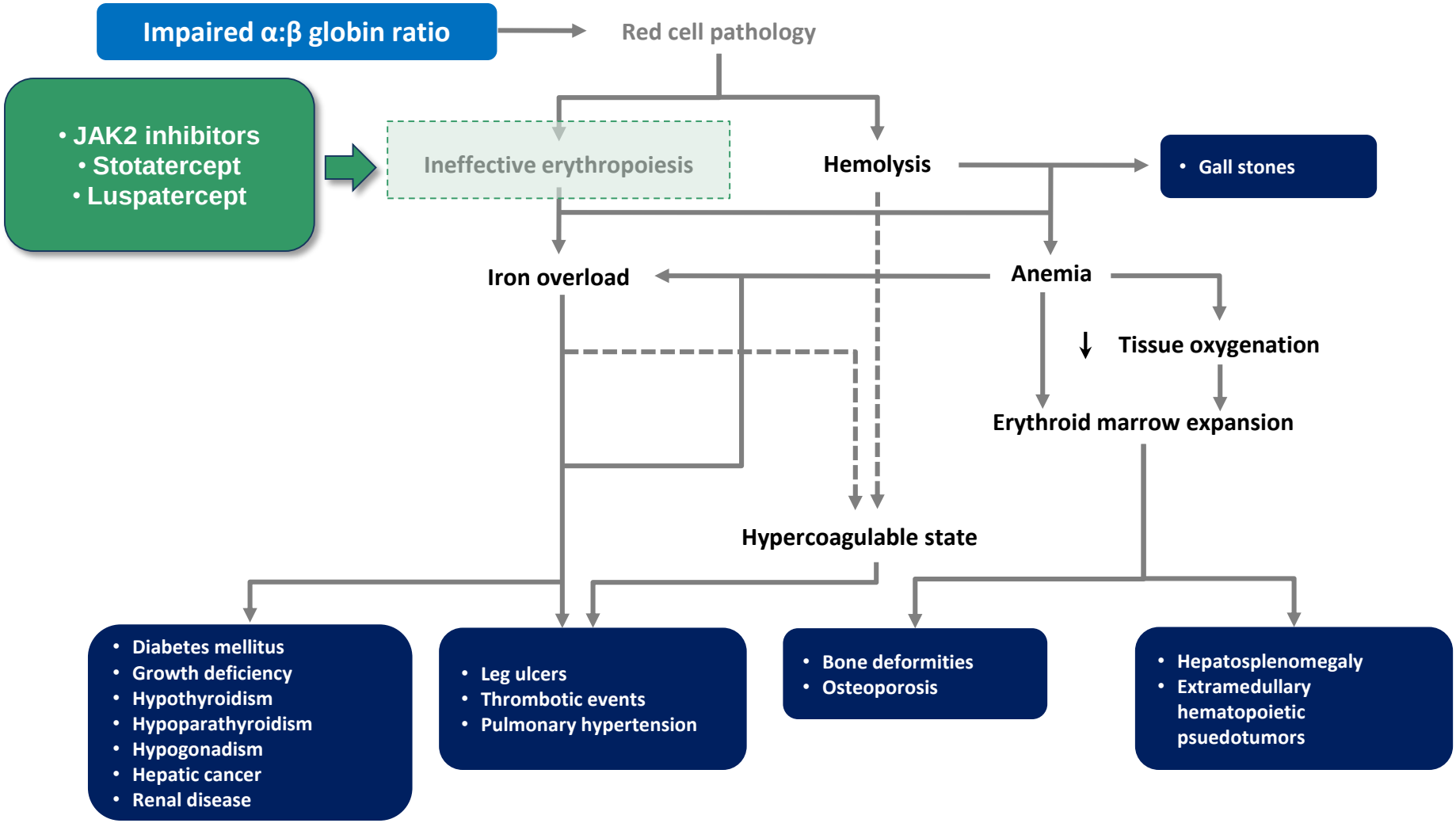


New treatments in thalassemia syndromes: Targeting α/β chain imbalance



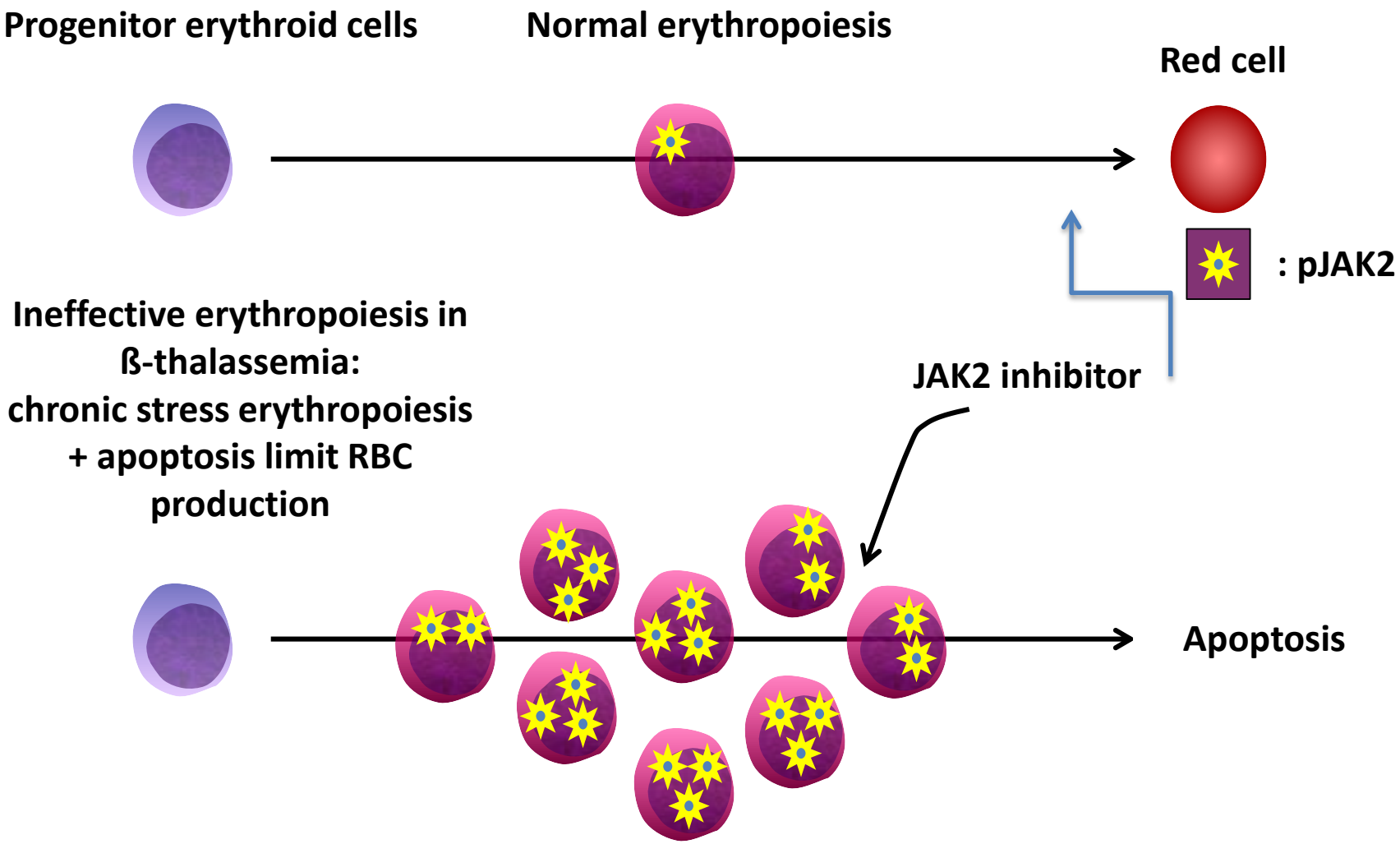
Musallam KM, et al. Haematologica. 2013;98:833-4.

New treatments in thalassemia syndromes: Targeting ineffective erythropoiesis



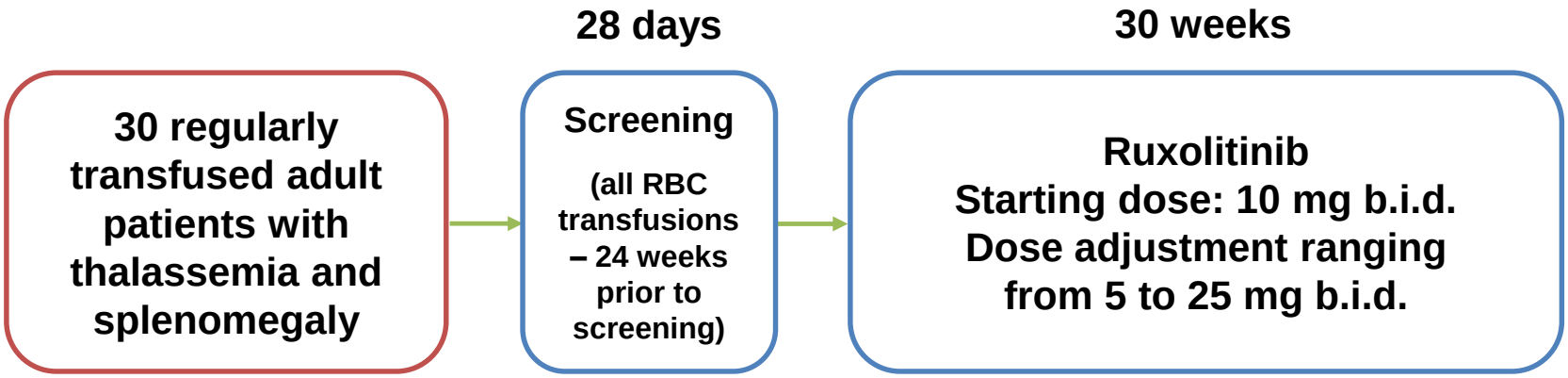
Musallam KM, et al. Haematologica. 2013;98:833-4.

Epo-EpoR-Jak2-STAT5 axis controls red cell production



Melchiori L, et al. Adv Hematol. 2010;2010:938640.

Ruxolitinib: a potent and selective oral JAK1 and JAK2 inhibitor
Phase 2: ruxolitinib in thalassemia

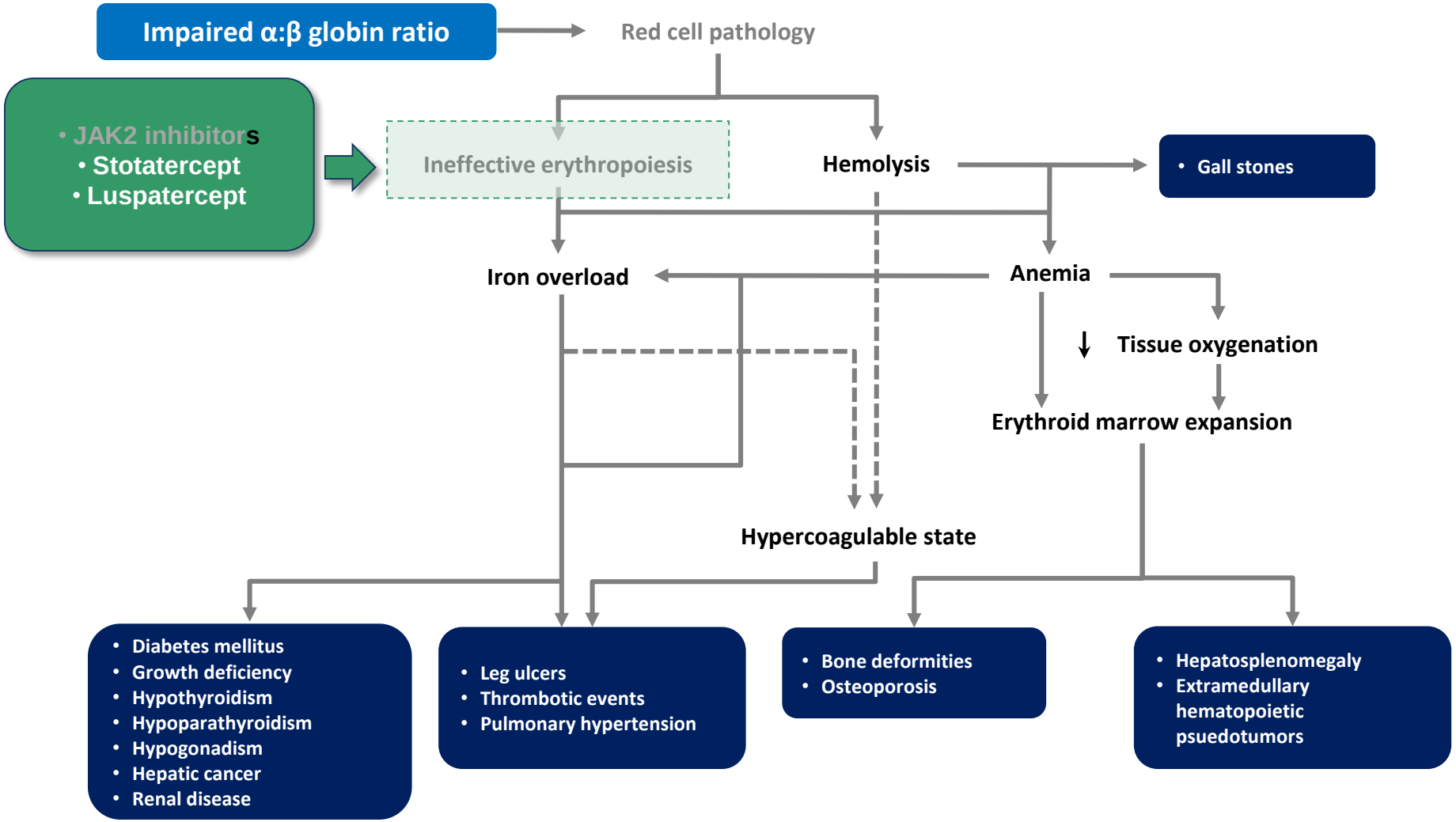


- Primary end-point
  - percent change in RBC transfusion requirement between weeks 6 and 30 compared with baseline
- Secondary end-points
  - change of spleen volume from baseline measured by MRI or CT
  - change of pre-transfusion Hb level from baseline at each post-baseline visit
  - pharmacokinetics
  - safety

CT, computerized tomography; MRI, magnetic resonance imaging.

NCT02049450.

Targeting ineffective erythropoiesis



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

ACE-011 and ACE-536: selective human ActRII receptor ligand TRAP

ACE-011
sotatercept

ActRIIA receptor
(inhibits activin A, B, GDF11)

Fc domain of human IgG_1 antibody

ACE-536
luspatercept

ActRIIB receptor
(only inhibition of GDF11)

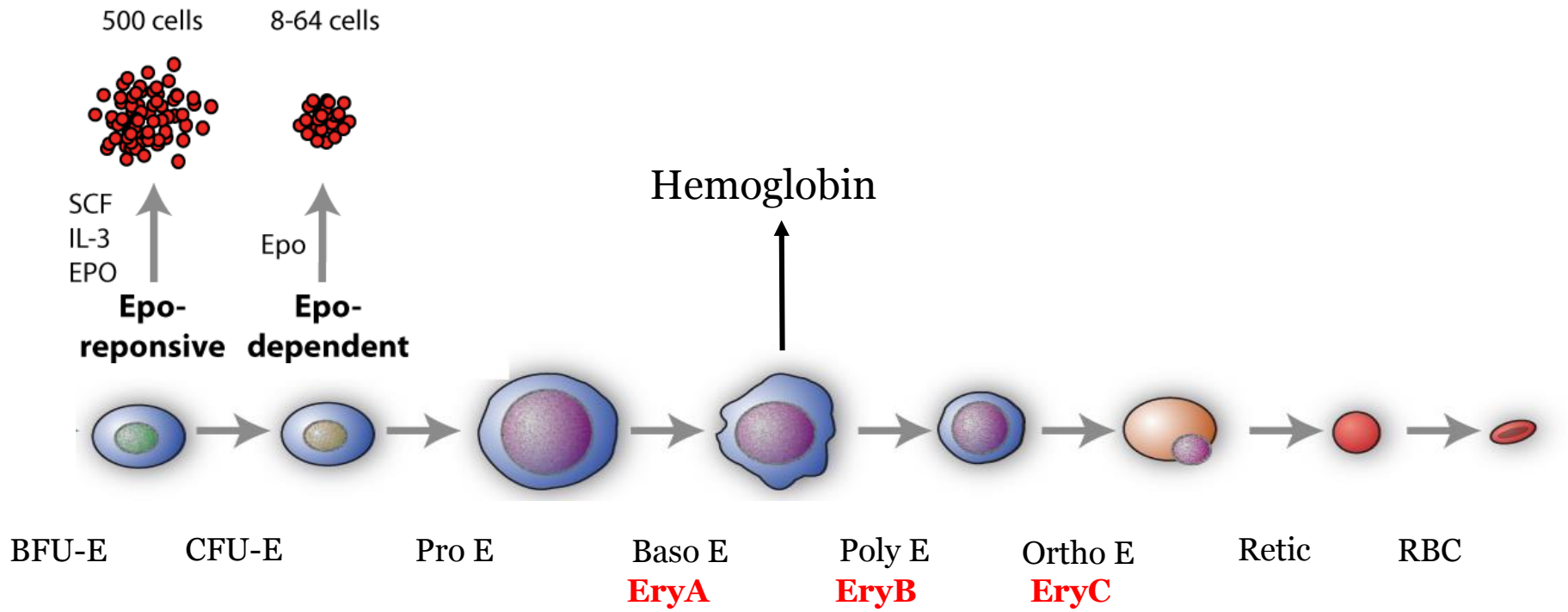
Fc domain of human IgG_1 antibody

- ✓ ACE-011 (SOTATERCEPT) AND ACE-536 (LUSPATERCEPT) ARE HUMAN FUSION RECOMBINANT PROTEINS
- ✓ THEY ACT AS "LIGAND TRAPS" AGAINST TGF-β SUPERFAMILY MEMBER **GDF11**
- ✓ MURINE MODELS OF MDS AND β-THALASSEMIA SHOWED AN AMELIORATION OF HEMATOLOGIC PARAMETERS (RAP-011 and RAP-536)

ActRIIB, activin receptor type IIb.

Suragani R, et al., Nat Med. 2014;20:408-14.

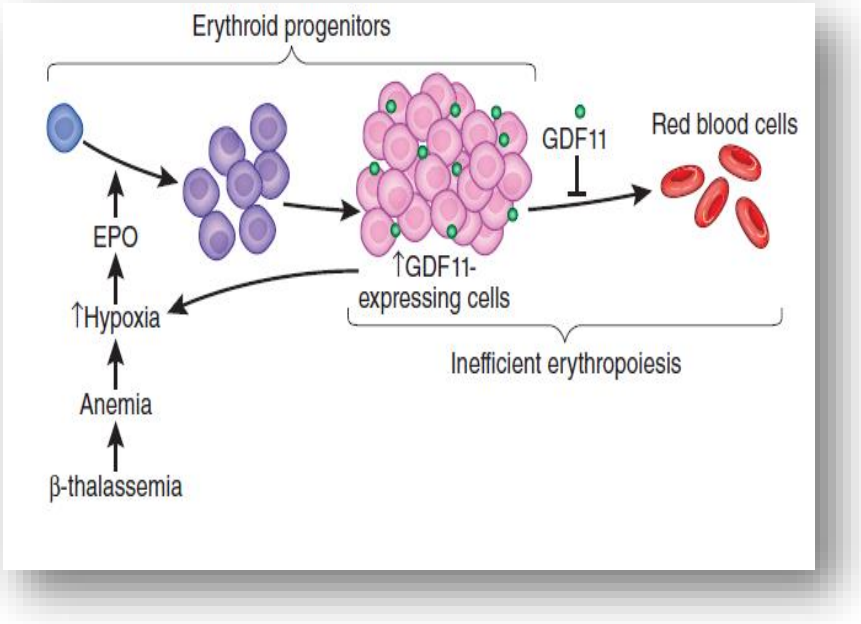
Where Does Sotatercept Impact Erythropoiesis?



How much EPO is required?

14

GDF11 INVOLVEMENT IN INEFFECTIVE ERYTHROPOIESIS

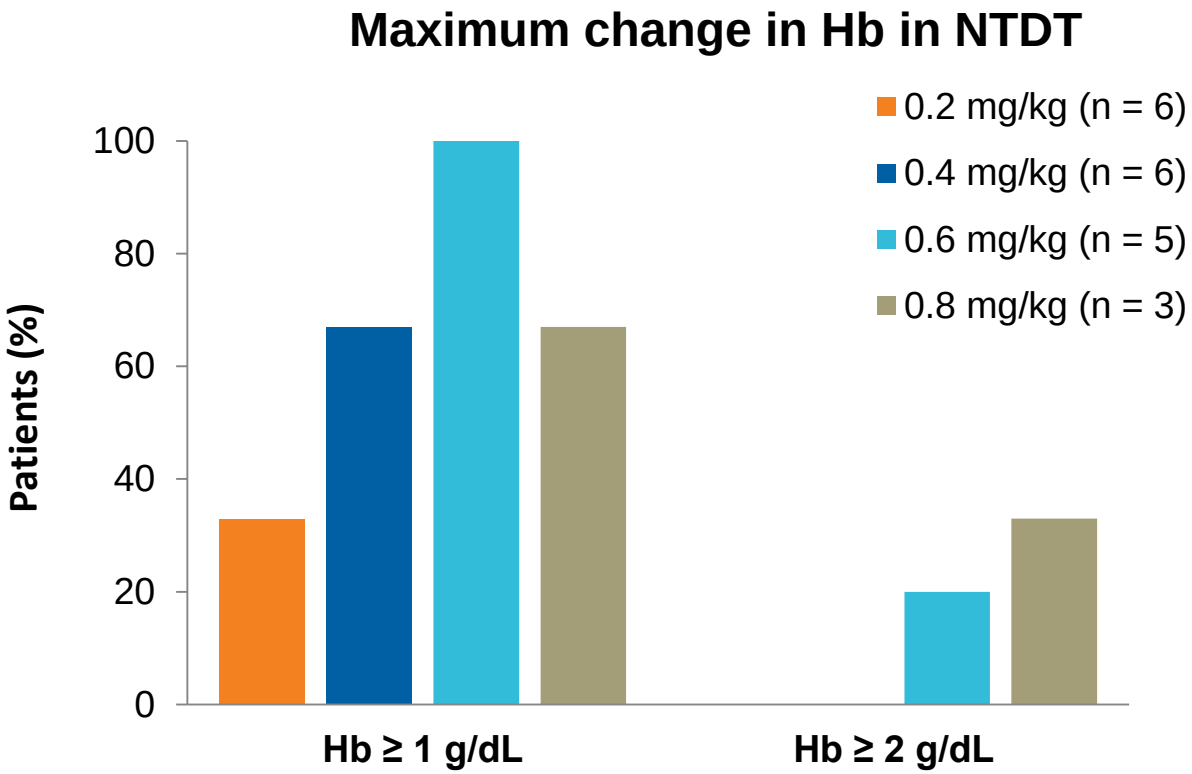


- ✓ DEFECTIVE ERYTHROID DIFFERENTIATION IN β-THALASSEMIA RESULTS IN AN ACCUMULATION OF **GDF11**-EXPRESSING ERYTHROID PROGENITORS

- ✓ **GDF11** MANTAINS THE SURVIVAL OF THESE PROGENITORS AND INHIBITS FURTHER DIFFERENTIATION, AGGRAVATING THE INEFFECTIVE ERYTHROPOIESIS

Robert F Paulson et al. Nature Medicine, 2014

Phase 2 preliminary results: luspatercept* improves anemia in NTDT and TDT

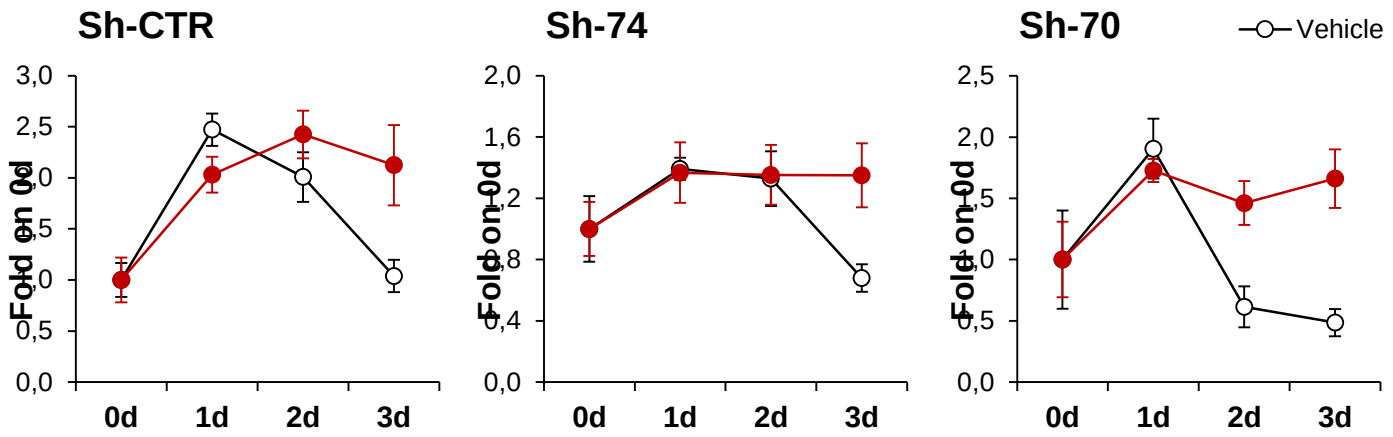


Luspatercept increased Hb levels in NTDT patients, decreased transfusion requirement in TDT patients, and has a favorable safety profile

*s.c. injection once every 3 weeks for up to 5 doses with a 2-month follow-up.
24 patients: 20 NTDT; 4 TDT.

Piga A, et al. EHA 2014. Haematologica. 2014;99 Suppl 1:abstract S664.

EVALUATION OF CYTOTOXICITY AFTER RAP-011 TREATMENT IN K562 sh-*SEC23B* CELLS



- ✓ CYTOTOXICITY ASSAY BY MTT
- ✓ TWO DRUG CONCENTRATIONS (50, 100 µM)
- ✓ TREATMENT WITH RAP-011 50 µM LEADS TO INCREASED SURVIVAL OF BOTH K562 sh-CTR and sh-*SEC23B* cells

Iron Overload in IEs : Case-control study

Table S1. Case-control study

	CDAII cases (*n = 37*)	BT-intermedia cases (*n = 21*)	HS cases (*n = 13*)	Control subjects (*n = 29*)	P[1]	P[2]	P[3]
Age (years)	19.1 ± 3.0 (13.0; 36)	42.1 ± 2.9 (37.0; 21)	21.3 ± 4.8 (15.0; 13)	22.3 ± 1.9 (20.0; 29)	0.39	<0.0001	0.70
Gender (Female/Male)	18 (48.6)/19 (51.4)	10 (47.6)/11 (52.4)	5 (38.5)/8 (61.5)	14 (48.3)/15 (51.7)	0.97	0.94	0.53
Complete blood count							
RBC (10^6/µL)	3.4 ± 0.1 (3.4; 37)	4.5 ± 0.2 (4.4; 21)	4.2 ± 0.1 (4.2; 13)	4.9 ± 0.1 (4.9; 27)	<0.0001	<0.0001	0.0005
Hb (g/dL)	10.0 ± 0.3 (10.0; 37)	9.3 ± 0.2 (9.2; 21)	10.8 ± 0.2 (9.9; 13)	14.3 ± 0.2 (14.2; 27)	<0.0001	0.19	0.23
Ht (%)	29.8 ± 1.0 (29.6; 37)	32.3 ± 0.8 (33.5; 21)	35.2 ± 1.7 (36.9; 12)	43.3 ± 0.6 (42.0; 27)	<0.0001	0.08	0.008
MCV (fL)	88.0 ± 1.4 (87.1; 37)	75.8 ± 3.2 (75.0; 21)	82.3 ± 2.4 (85.3; 13)	87.6 ± 0.9 (87.0; 27)	0.81	0.0002	0.04
MCH (pg)	29.8 ± 0.6 (28.8; 35)	22.2 ± 1.2 (22.5; 21)	28.9 ± 0.6 (29.6; 12)	29.0 ± 0.3 (29.4; 27)	0.31	<0.0001	0.44
MCHC (g/dL)	33.6 ± 0.3 (33.5; 35)	29.0 ± 0.5 (29.5; 21)	35.0 ± 0.7 (34.9; 12)	33.2 ± 0.2 (33.1; 27)	0.26	<0.0001	0.03
PLT (10^3/µL)	417.4 ± 40.9 (357.0; 33)	514.5 ± 70.0 (605.0; 21)	234.5 ± 24.7 (227.0; 13)	245.9 ± 10.6 (246.0; 27)	0.0005	0.11	0.01
RDW (%)	21.0 ± 1.3 (20.7; 27)	-	18.8 ± 1.2 (19.1; 11)	12.4 ± 0.2 (12.5; 27)	<0.0001	-	0.32
Retics abs count (x10^3/µL)	76.3 ± 9.3 (60.0; 36)	44.0 ± 6.7 (51.7; 17)	278.6 ± 57.3 (244.0; 11)	-	-	0.02	<0.0001
BMRI	54.1 ± 6.5 (47.2; 36)	29.1 ± 4.5 (32.2; 17)	208.7 ± 41.6 (196.1; 11)	-	-	0.02	<0.0001
Laboratory data							
Total bilirubin (mg/dL)	3.0 ± 0.5 (2.3; 35)	2.4 ± 0.2 (2.4; 21)	4.3 ± 1.4 (2.1; 13)	0.5 ± 0.1 (0.5; 12)	<0.0001	0.29	0.27
Unconjugated bilirubin (mg/dL)	2.6 ± 0.5 (1.8; 29)	1.8 ± 0.2 (1.7; 21)	4.0 ± 1.4 (1.6; 12)	-	-	0.21	0.24
Serum iron (µg/dL)	159.8 ± 11.5 (163.0; 31)	151.5 ± 13.4 (151.0; 21)	74.7 ± 8.4 (73.0; 11)	71.2 ± 7.9 (59.0; 17)	<0.0001	0.64	0.0001
Ferritin (ng/mL)	275.9 ± 61.0 (148.5; 36)	398.1 ± 67.1 (338.0; 21)	262.0 ± 86.6 (161.0; 10)	-	-	0.20	0.91
Ferritin level/dosage age[§]	29.6 ± 9.7 (13.9; 35)	10.2 ± 2.0 (7.3; 20)	12.9 ± 3.0 (11.6; 10)	-	-	0.14	0.37
Transferrin saturation (%)	71.4 ± 5.5 (70.4; 25)	62.9 ± 5.5 (65.0; 21)	26.0 ± 4.3 (23.6; 9)	-	-	0.28	<0.0001
sTfR (mg/L)	4.1 ± 0.4 (4.0; 17)	9.4 ± 0.6 (9.5; 20)	-	-	-	<0.0001	-

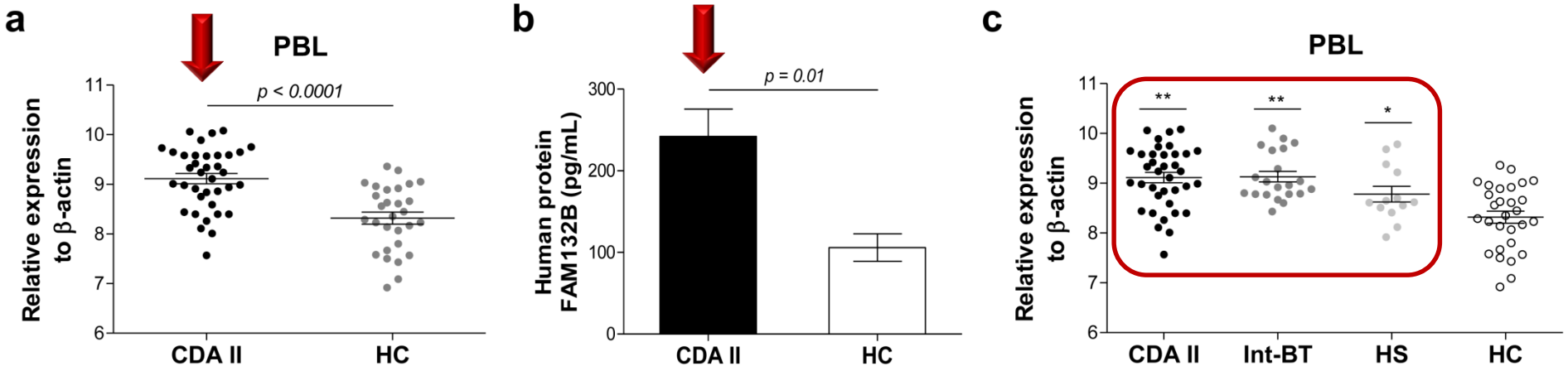
Data are not available for all subjects. For quantitative variables data are presented as average ± SEM (median; n). For qualitative variables data are presented as n (%)/n (%). Student t test for quantitative unpaired data; chi square test for categorical data.

P[1] CDAII cases vs control subjects; P[2] CDAII vs BT-intermedia cases; P[3] CDAII vs HS.

[§] Normalization of ferritin by means of "Ferritin level/dosage age ratio", as described by Iolascon et al, Haematologica 2010; 95(5)

Russo R et al. Blood 2016

Increased levels of ERFE-encoding FAM132B in patients with Congenital Dyserythropoietic Anemia type II



**p<0.0001; *p<0.05 vs HC group

- ✓ CDAII patients exhibit over-expression of ERFE at both gene and protein level
- ✓ When we analyzed ERFE expression in β-thalassemia (BT)-intermedia patients, exhibiting iron overload likewise for CDAII patients, we observed similar results compared to CDAII. Conversely, only a slight increase of ERFE expression was observed in patients with mild well-compensated anemia, such as hereditary spherocytosis (HS)

These data suggested that the marked increased ERFE expression observed in both CDAII and BT-intermedia patients is mainly due to the ineffective erythropoiesis

Russo R et al. Blood 2016

Correlation analysis

Table 1. *FAM132B* expression and clinical correlations in CDAII patients

	Low *FAM132B* (n = 20)	High *FAM132B* (n = 17)	p‡
Age (years)	25.3 ± 4.9 (16.0; 19)	12.1 ± 2.5 (10.0; 17)	0.03
Onset symptoms (years)	7.5 ± 2.5 (5.0; 16)	3.2 ± 1.3 (1.3; 16)	0.14
Gender (Female/Male)	9 (45.0)/11 (55.0)	9 (52.9)/8 (47.1)	0.63
Complete blood count			
RBC (10^6/µL)	3.6 ± 0.2 (3.5; 20)	3.2 ± 0.1 (3.3; 17)	0.05
Hb (g/dL)	10.7 ± 0.5 (10.4; 20)	9.2 ± 0.4 (9.5; 17)	0.02
Ht (%)	31.7 ± 1.4 (30.6; 20)	27.5 ± 1.2 (28.0; 17)	0.03
MCV (fL)	89.7 ± 1.8 (90.2; 20)	86.0 ± 2.2 (84.7; 17)	0.20
MCH (pg)	30.6 ± 0.7 (31.0; 18)	28.9 ± 0.9 (27.9; 17)	0.12
MCHC (g/dL)	33.8 ± 0.4 (33.5; 19)	33.3 ± 0.3 (33.1; 16)	0.32
RDW (%)	19.9 ± 2.5 (18.9; 12)	21.8 ± 1.2 (22.0; 15)	0.48
PLT (10^3/µL)	373.0 ± 41.1 (290.0; 17)	459.2 ± 69.2 (390.0; 17)	0.30
Retics abs count (10^3/µL)	67.4 ± 9.2 (59.2; 20)	87.3 ± 17.5 (79.7; 16)	0.30
Retics (%)	2.0 ± 0.3 (1.5; 20)	2.7 ± 0.6 (2.2; 16)	0.25
Reticulocyte Index	1.3 ± 0.2 (1.2; 20)	1.7 ± 0.3 (1.5; 16)	0.38
Iron balance			
Hepcidin/ferritin	0.04 ± 0.01 (0.02; 16)	0.01 ± 0.003 (0.006; 16)	0.01
Hepcidin (nM)	5.8 ± 1.9 (2.7; 17)	1.0 ± 0.3 (0.6; 16)	0.02
Ferritin (ng/mL)	372.1 ± 107.7 (200.0; 19)	168.5 ± 36.0 (99.8; 17)	0.10
Ferritin level/dosage age§	32.9 ± 17.2 (14.9; 18)	26.1 ± 8.6 (12.7; 17)	0.73
Transferrin saturation (%)	67.7 ± 6.8 (62.5; 19)	81.8 ± 7.8 (86.0; 8)	0.23
Serum iron (µg/dL)	157.8 ± 13.6 (159.5; 18)	162.7 ± 20.4 (172.0; 13)	0.84
sTfR (mg/L)	3.7 ± 0.4 (3.7; 12)	5.1 ± 0.5 (5.7; 8)	0.04
Laboratory data and transfusion regimen			
EPO (mIU/mL)	82.5 ± 19.1 (61.9; 14)	154.3 ± 14.5 (170.1; 13)	0.01
GDF15 (pg/mL)	814.9 ± 251.1 (503.5; 13)	781.9 ± 140.6 (804.0; 9)	0.92
Total bilirubin (mg/dL)	3.7 ± 0.8 (2.5; 19)	2.3 ± 0.3 (2.1; 16)	0.15
Unconjugated bilirubin (mg/dL)	3.1 ± 0.8 (2.2; 17)	1.9 ± 0.3 (1.5; 12)	0.22
Transfusion need (Yes/No)	7 (46.7)/8 (53.3)	10 (58.8)/7 (41.2)	0.49

Data are not available for all patients. For quantitative variables data are presented as average ± SE (median; n). For qualitative variables data are presented as n (%)/n (%)

‡ Student t test for quantitative unpaired data; chi square test for categorical data

§ Normalization of ferritin by means of "Ferritin level/dosage age ratio", as described by Iolascon et al, Haematologica 2010; 95(5)

High-FAM132B patients:

- Reduced Hb
- Reduced Ht
- Increased EPO
- Increased sTfR

- Reduced Hepcidin
- Reduced Hepcidin/ferritin
- Increased Transferrin saturation

However …

The iron balance data do not differ significantly between the two CDA II sub-groups

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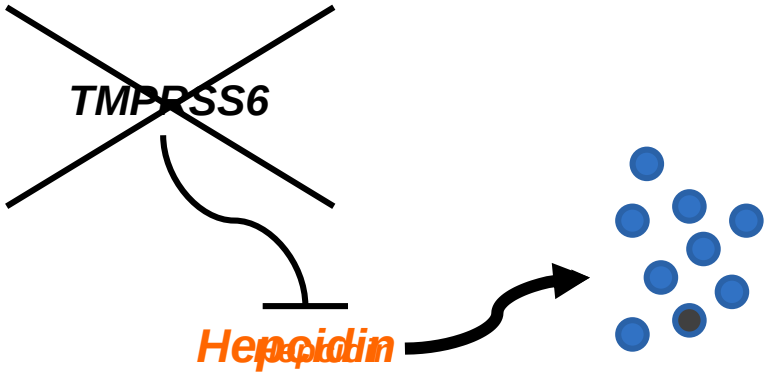
Hypothesis: Increased levels of Hepcidin in thalassemia intermedia are beneficial to prevent iron overload and ameliorate erythropoiesis

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]

Tmprss6 as a potential target to treat NTDT



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

Definitive treatment of ineffective Erythropiesis : Bome Marrow Transplantation

Table 1. Major reported clinical studies that have attempted to improve the outcome of patients with class 3 thalassemia major[5].

	Year	N	Median Age (yrs) / (range)	Proportion in Class 3 (%)	Proportion in Class 3HR (%)	Major defining feature of change in protocol	Treatment related mortality (%)	Graft rejection (%)	EFS	OS (%)
Lucarelli *et al.*[15]*	1996	115	11 (3-16)	100	NA	Bu / Cy based regimen with reduction in Cy total dose from 200 mg/kg to 160 mg/kg	24	35	49	74
Sodani *et al.*[25]	2004	33	11 (5-16)	100	NA	Reduction in Cy dose to ≤160 mg/kg with addition of Flu. Additional therapy from day -45 with immunosuppression with Azt and suppression of erythropoiesis with HU	6	6	85	93
Gaziev *et al.*[19]	2010	71	9 (1.6–27)	57.3	NA	Intravenous Bu, dose adjustments with therapeutic drug monitoring	7	5	87	91
Chiesa *et al.*[26]	2010	53	8 (1-17)	47	NA	Intravenous Bu, dose adjustments with therapeutic drug monitoring	4	15	79	96
Chiesa *et al.*[26]‡	2010	25	NA	100	NA	Intravenous Bu, dose adjustments with therapeutic drug monitoring	4	34	66	96
Bernardo *et al.*[30]	2012	60	7 (1-37)	27^	NA	Treo based conditioning regimen	7	9	84	93
Li *et al.*[29]	2012	82	6 (0.5-15)	NA	NA	Conditioning with age adjusted PK based IV Bu, Cy (110mg/kg), high-dose Flu (200mg/kg), Thio. Additional therapy from day -45 with immunosuppression with Azt and suppression of erythropoiesis with HU	8.5^	4^	88^	91^
Choudhary *et al.*[27]	2013	28	9.6 (2-18)	75	39	Treo based conditioning regimen	21	7	71	79
Anurathapan *et al.*[23]	2013	18	14 (10-18)	100	NA	Conditioning regimen of Flu &IV Bu Pre-conditioning immunosuppression therapy with Flu and Dexa for 1-2 months.	5	0	89	89
Mathews *et al.*[11]	2013	50	11 (2-21)	100	48	Treo based conditioning regimen with PBSC graft in 74%	12	8	79	87
Mathews *et al.*[11]§	2013§	24	12 (3-21)	100	100	Treo based conditioning regimen with PBSC graft in 74%	13	8	78	87
Gaziev *et al.*[24]	2016	37	10 (5-17)	100	NA	As in Sodani *et al.*[25] but with higher dose of Flu (150 mg/kg) and addition of Thio(10 mg/kg)	8	0	92	92

[5]Adapted from Mathews *et al.*[10]; *Only patients <17 years included in this table; ‡Subset of high-risk cases from same paper; ^Includes all adult cases as well (assumed to be Class 3); ^Includes low-risk patients also; §Subset of high-risk cases from same paper. Cy: Cyclophosphamide; Flu: Fludarabine; Dexa: Dexamethasone; Bu: Busulfan; Treo: Treosulfan; Azt: Azathioprine; HU: Hydroxyurea; Thio: Thiotepa. HR: high-risk; EFS: event-free survival; OS: overall survival; NA: not applicable; PK: pharmacokinetics; PBSC: peripheral blood stem cell.

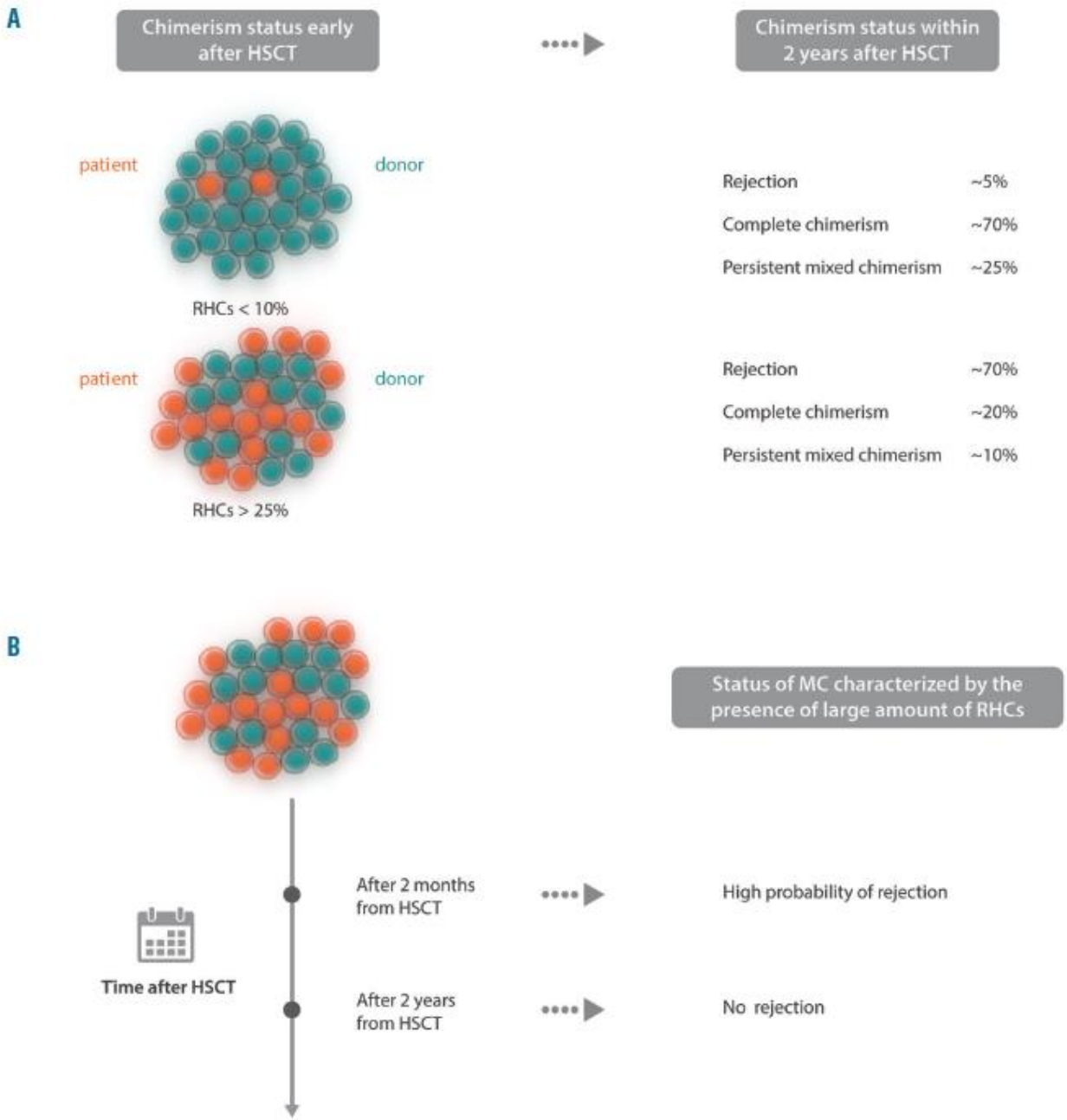


Figure 1. Evolution of chimerism after hematopoietic stem cell transplantation (HSCT). Early mixed chimerism is associated with higher risk of rejection while late chimerism often persists with a stable graft. RHCs: residual host cells. MC: mixed chimerism.

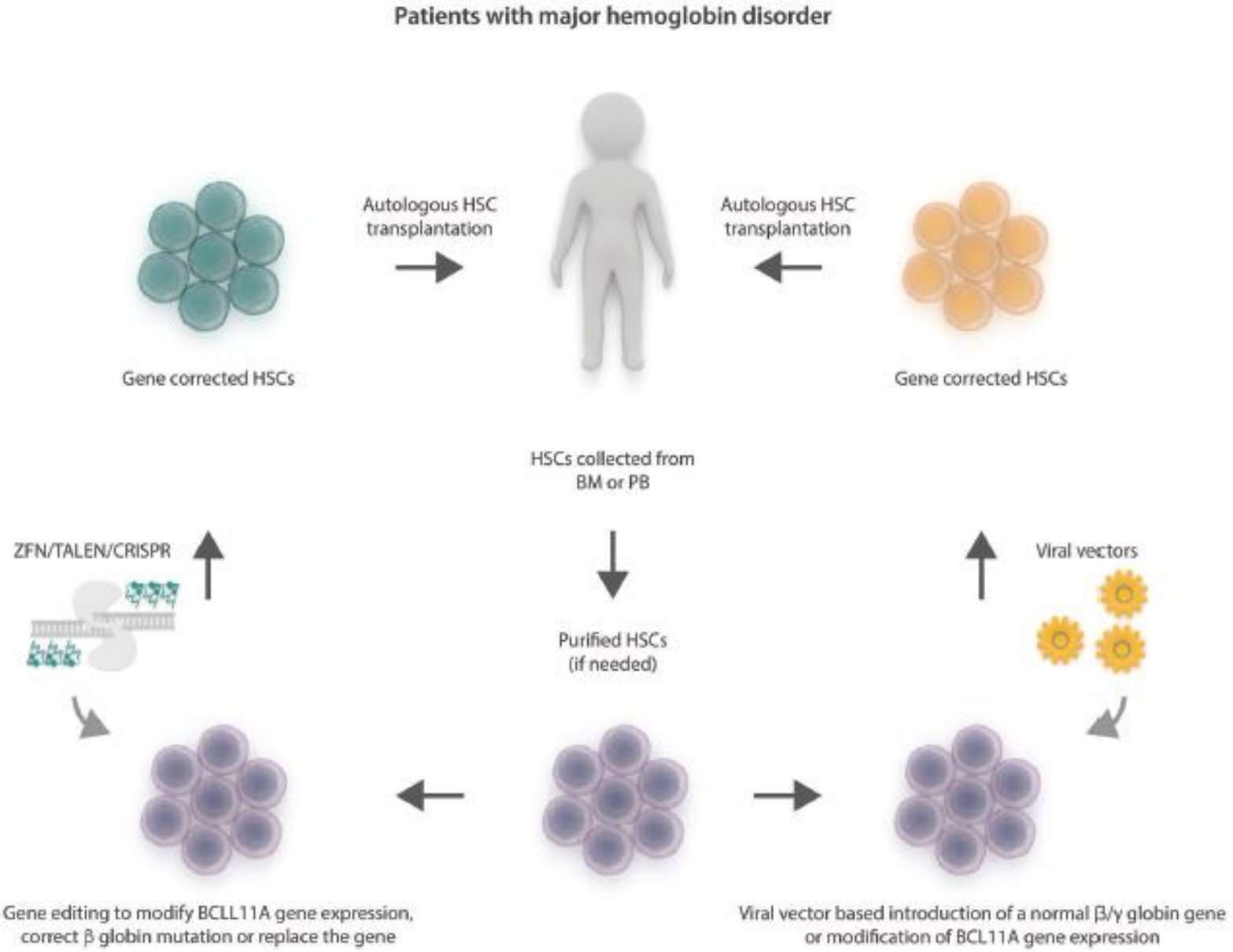


Figure 3. Overview of current approaches to gene therapy for the major hemoglobin disorders. Gene modifications may be through viral vectors or genome editing technologies to achieve the desired therapeutic effect. HSC: Hematopoietic stem cell; BM: Bone marrow; PB: Peripheral blood; ZFN: zinc finger nucleases; TALEN: transcription activator-like effectors with FokI nuclease; CRISPR: clustered regularly interspaced short palindromic repeats.

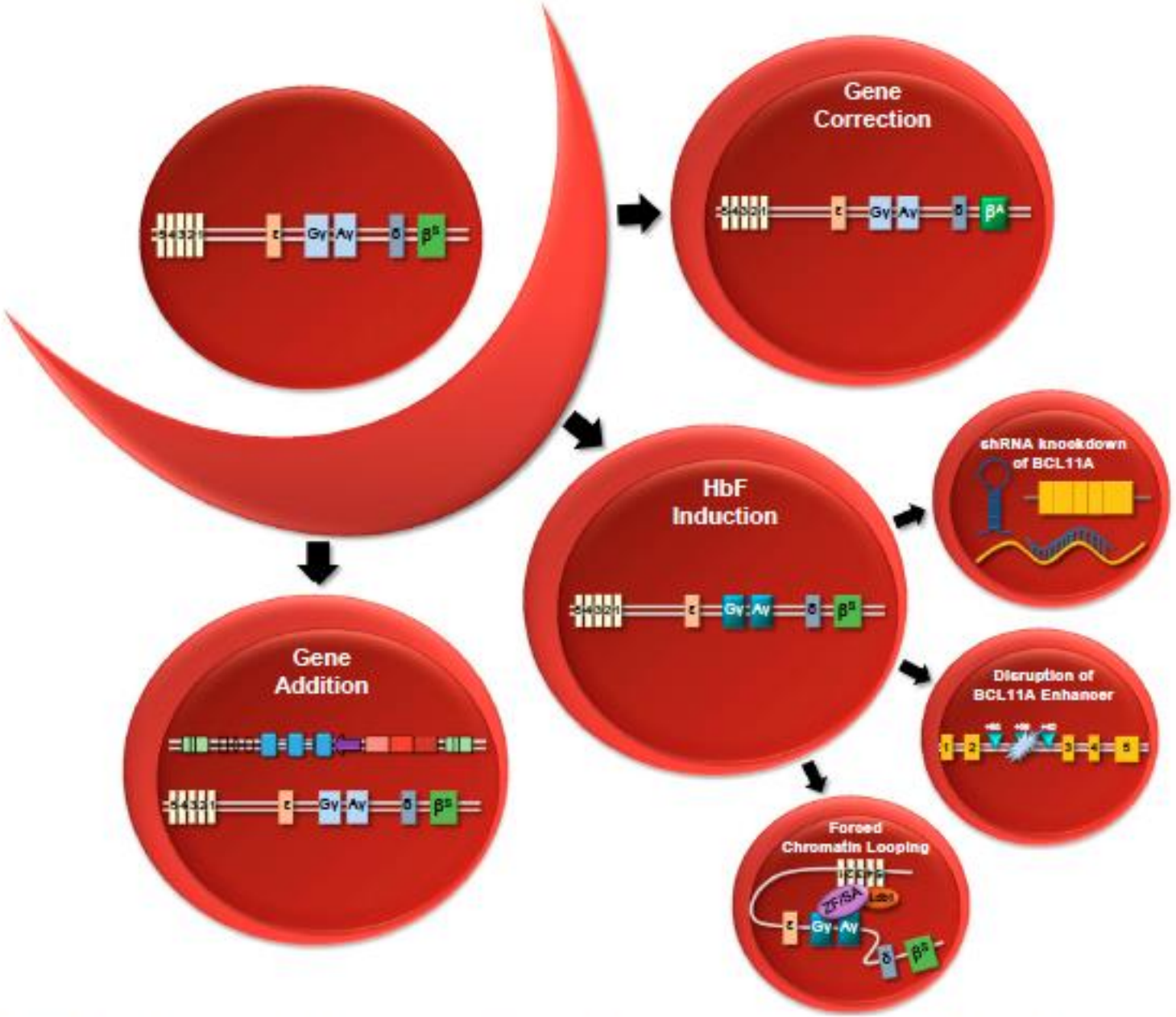
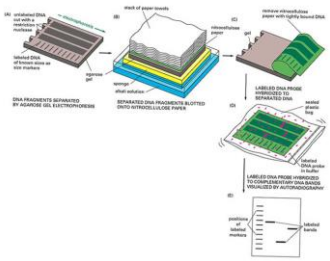


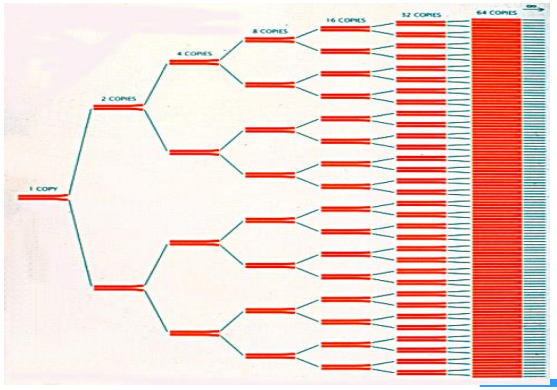
Figure 2. Strategies for gene therapy for SCD: schematic overview of various approaches for correcting the sickle phenotype via gene therapy. Gene correction: targeted genome engineering leads to correction of the sickle mutation such that β^S is repaired as β^A. HbF induction: multiple strategies for induction of γ-globin expression include shRNA-mediated knockdown of BCL11A, targeted disruption of the +58 DNase I HS site in the BCL11A erythroid-specific enhancer, and forced chromatin looping to promote association of the β-globin LCR with the γ-globin genes. Gene addition: integrating lentiviral vector carrying a β-globin, γ-globin, or antisickling β-globin cassette. Ldb1, transcription factor; ZF/SA, zinc-finger self-association domain.

Genetic Era: From DNA structure to Complete Sequence

1953-Discovery of double helix(Watson and Crick)



1975-Southern blotting



1978-First Molecular Diagnosis

1985-Description of PCR technology

1990- Start of Human Genome Project


The New York Times

Genetic Code of Human Life Is Cracked by Scientists

JUSTICES REAFFIRM MIRANDA RULE, 7-2; A PART OF 'CULTURE'

A SHARED SUCCESS

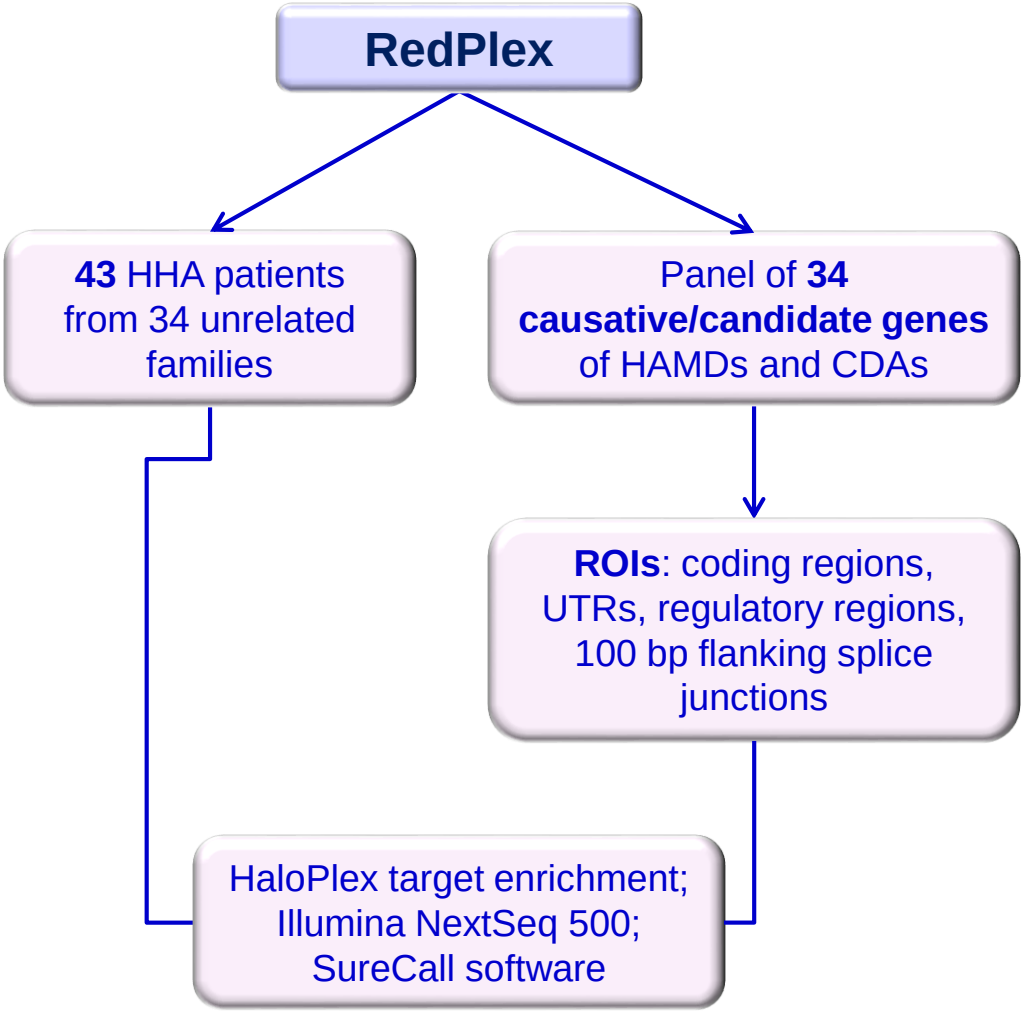
2 Rivals' Announcement Marks New Medical Era, Risks and All

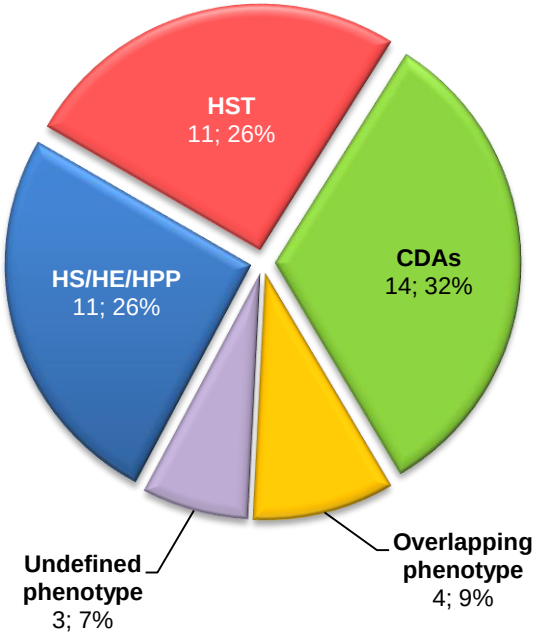
A Pearl and a Hodgepodge: Human DNA

1990-First experiment of Gene Therapy: ADA deficiency correction

2000-First announcement of decodification of entire human genome

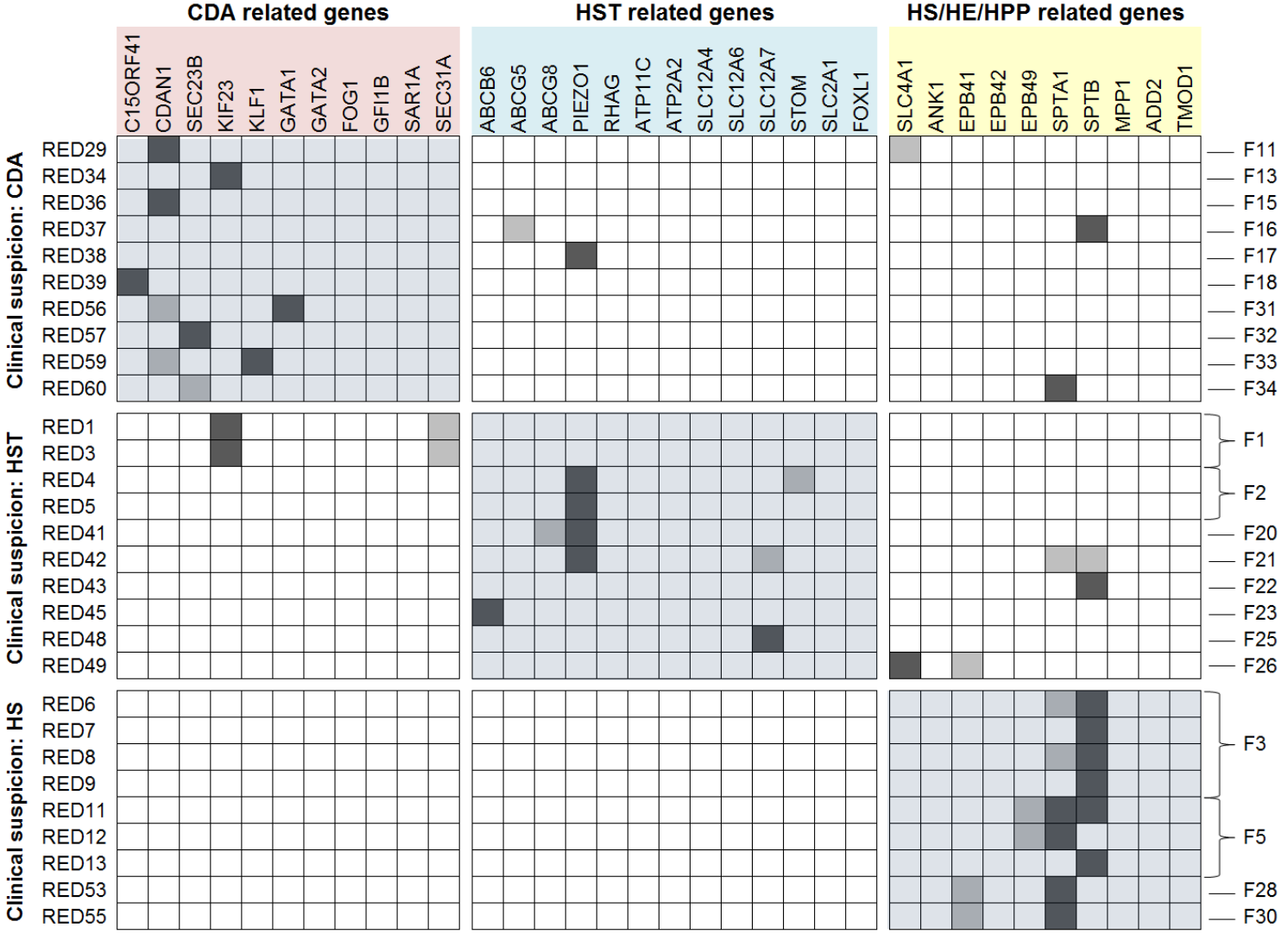
RedPlex: a targeted next generation sequencing-based diagnosis for patients with hereditary hemolytic anemias





- ✓ Target regions (ROIs): 538
- ✓ Total Amplicons: 8874
- ✓ Total Target Bases Analyzable: 239.59 kbp
- ✓ Target Coverage: 99.9 %

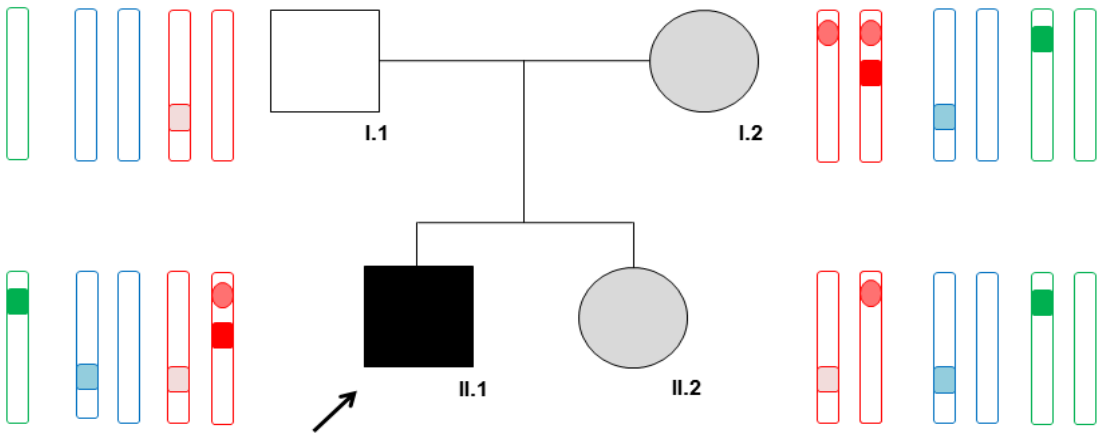
P743. RedPlex: a targeted next generation sequencing-based diagnosis for patients with hereditary hemolytic anemias



- ✓ Dark gray squares highlight prioritized variants
- ✓ Light gray squares indicate clinically related or modifier variants.

RedPlex data study on 29 HHA patients with conclusive diagnosis

Polygenic contribute in monogenic disease



Hereditary Spherocytosis

-*SPTA1* mutations

- *αLELY* variant

Dyserythropoietic phenotype

-*SEC23B* mutation

-*GATA1* modifier variant

SPTA1 LOCUS

WT

c.5029G>A, p.Gly1677Arg

Alpha Lely

c.2319C>A, p.Cys773Ter

SEC23B LOCUS

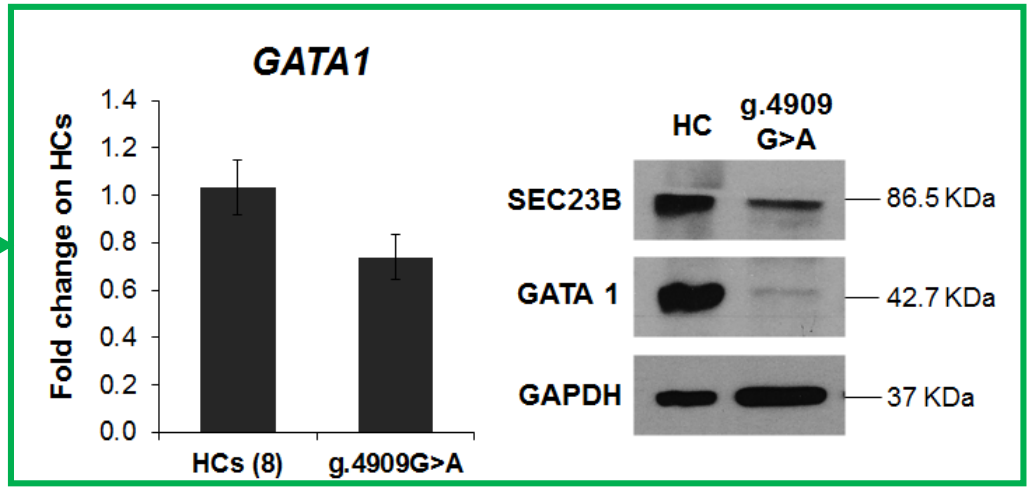
WT

c.1254 T>G, p.Ile418Met

GATA1 LOCUS

WT

g.4909G>A



Russo R. et al, unpublished data 2016

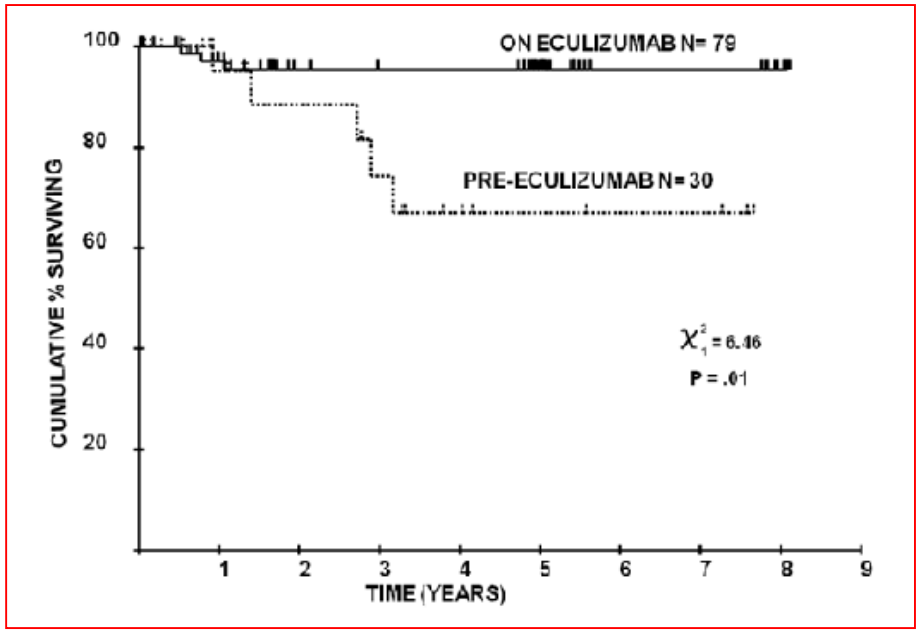
ECULIZUMAB AND PNH: EFFECTS ON SURVIVAL

blood

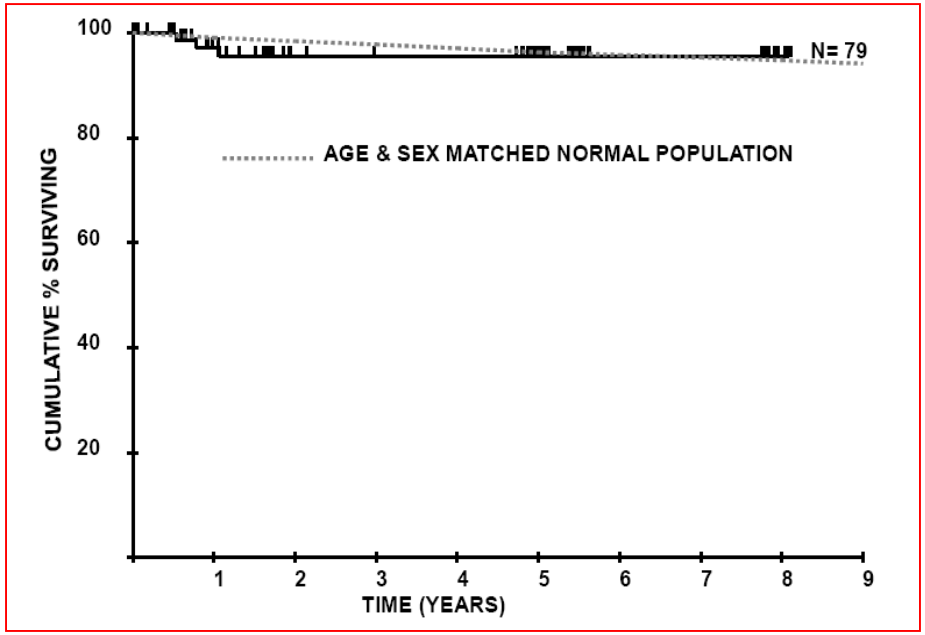
Long term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival

Richard J Kelly, Anita Hill, Louise M Arnold, Gemma L Brooksbank, Stephen J Richards, Matthew Cullen, Lindsay D Mitchell, Dena R Cohen, Walter M Gregory and Peter Hillmen

Untreated vs Ecu-treated PNH



Treated PNH vs normal population



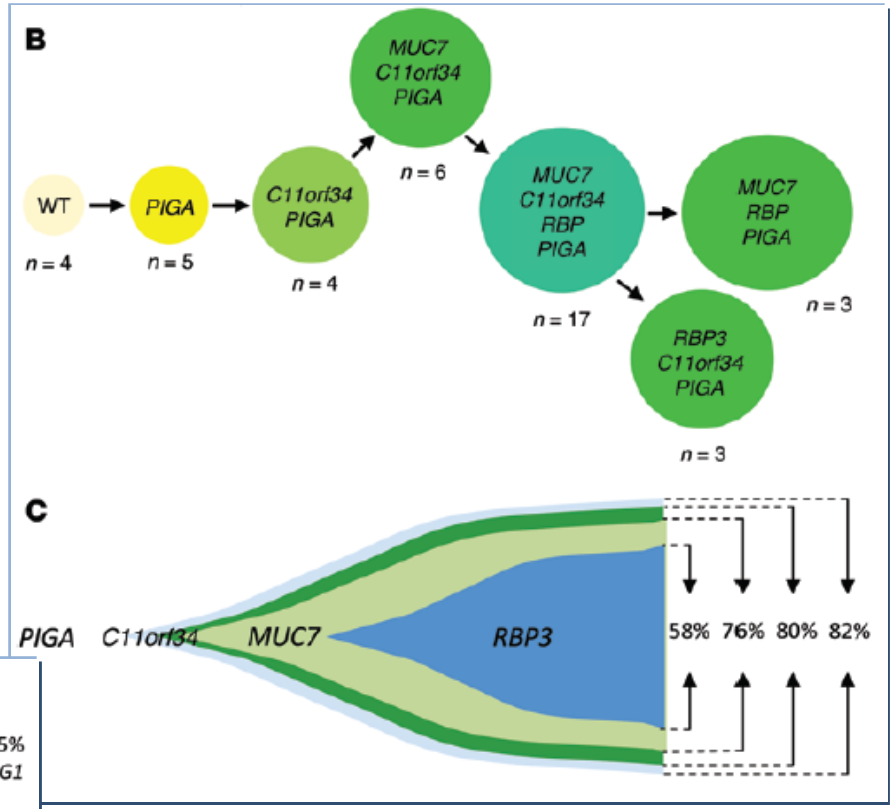
Hill et al, Blood 2011

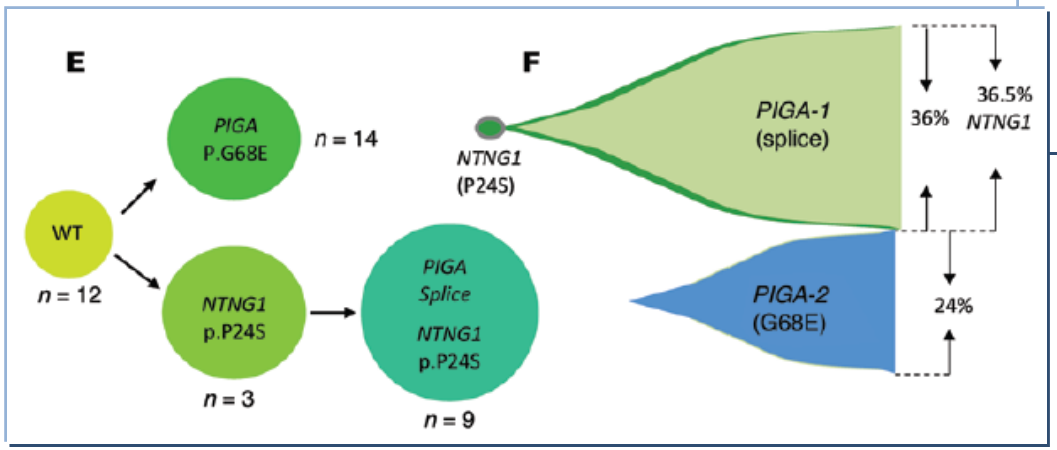
Deep sequencing reveals stepwise mutation acquisition in paroxysmal nocturnal hemoglobinuria

Wenyi Shen,[1,2] Michael J. Clemente,[1] Naoko Hosono,[1] Kenichi Yoshida,[3] Bartlomiej Przychodzen,[1] Tetsuichi Yoshizato,[3] Yuichi Shiraishi,[4] Satoru Miyano,[4,5] Seishi Ogawa,[3] Jaroslaw P. Maciejewski,[1] and Hideki Makishima[1]

Whole exome sequencing and targeted deep sequencing in 60 PNH patients

- ✓ **Additional somatic mutations in 10 out 12 patients studied by WES**
- ✓ **In total, 21 mutations in 21 genes (including some also found in MDS, such as TET2, ASXL1U2AF1, SUZ12, BCOR)**
- ✓ **Clonal architecture re-created by single-colony sequencing**





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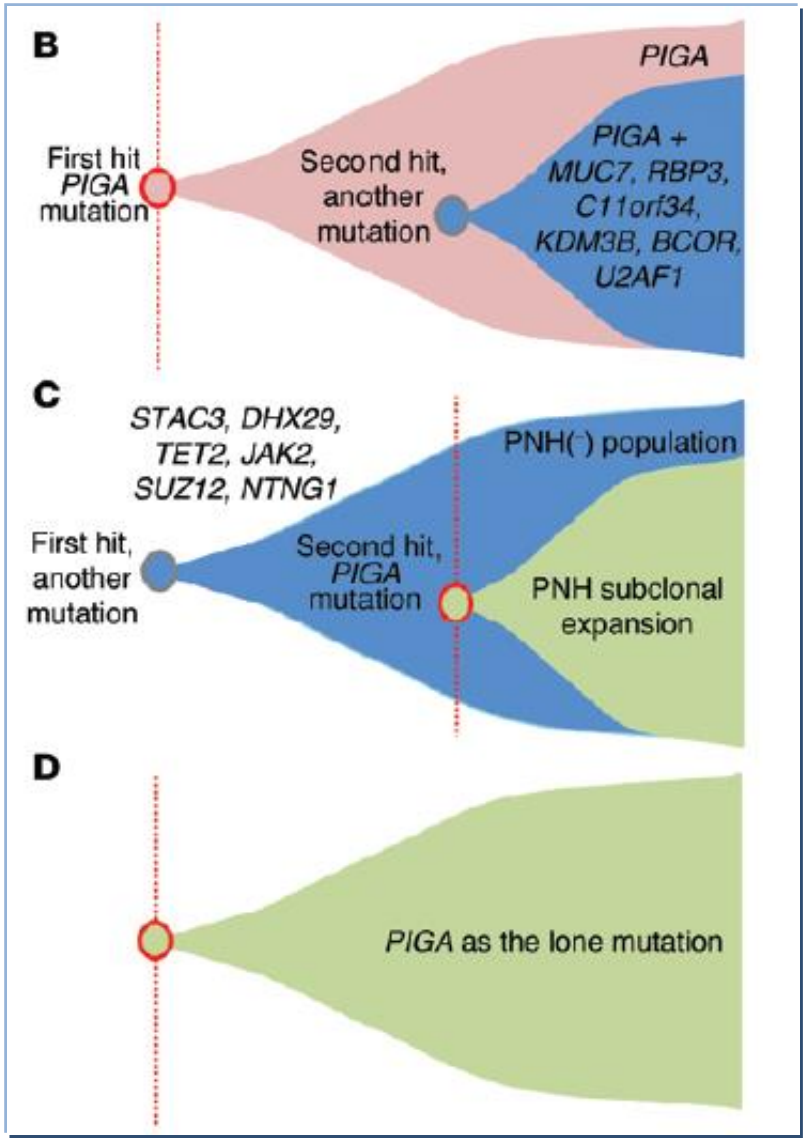

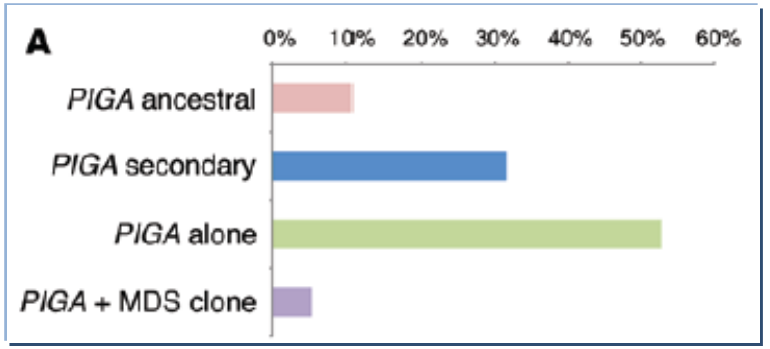

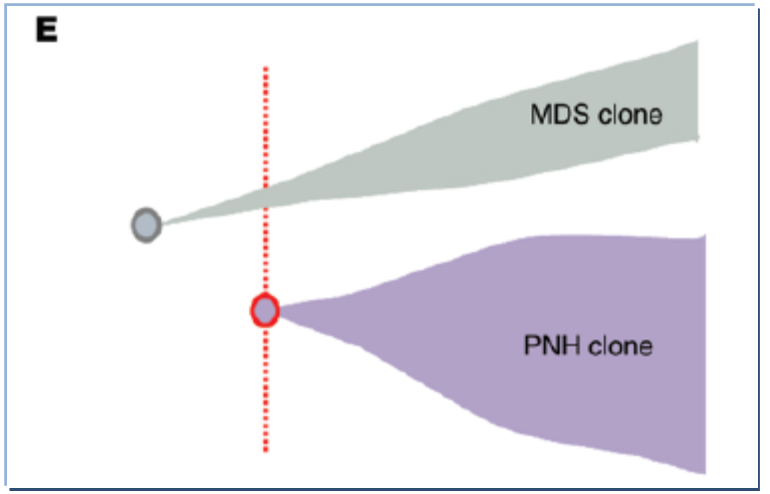

Is PNH a cancer???

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Medical Genetics Unit