9th EDITION
Highlights from EHA

RED BLOOD CELLS AND IRON:
best presentations from
21st EHA Meeting
Copenhagen

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Medical Biotechnology –
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RED BLOOD CELLS AND IRON:

- news on iron metabolism

- new therapeutic approaches for inherited anemias

- new techniques in diagnosis of red cell disorders

Iron metabolism

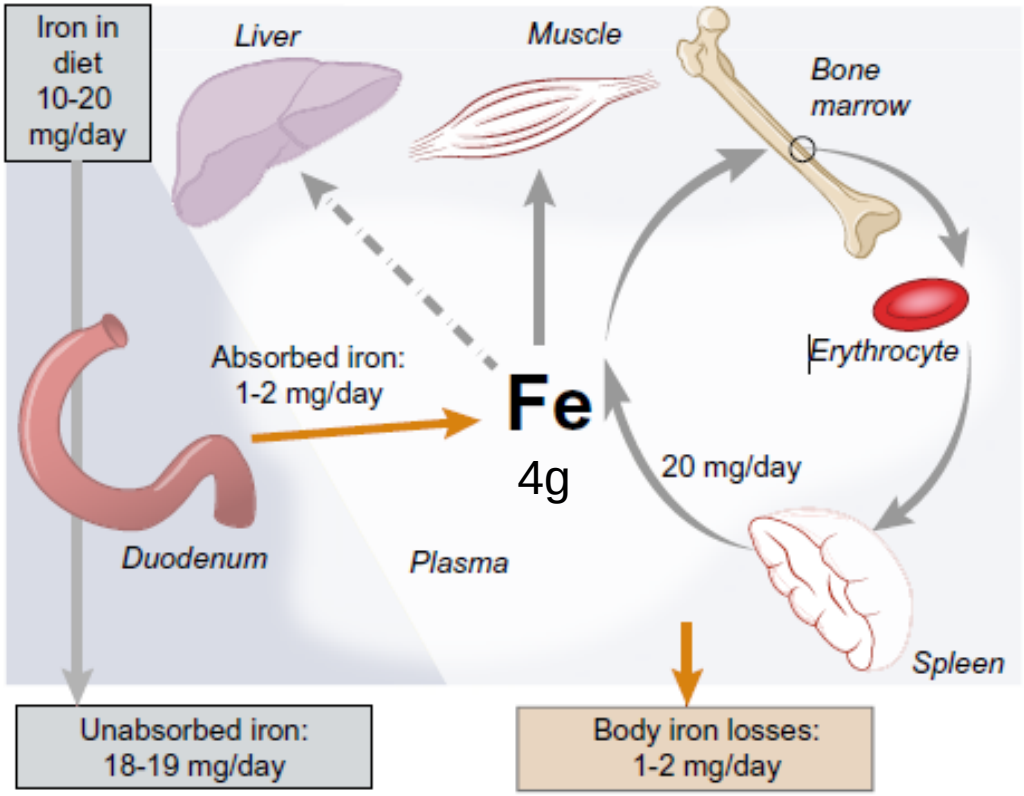
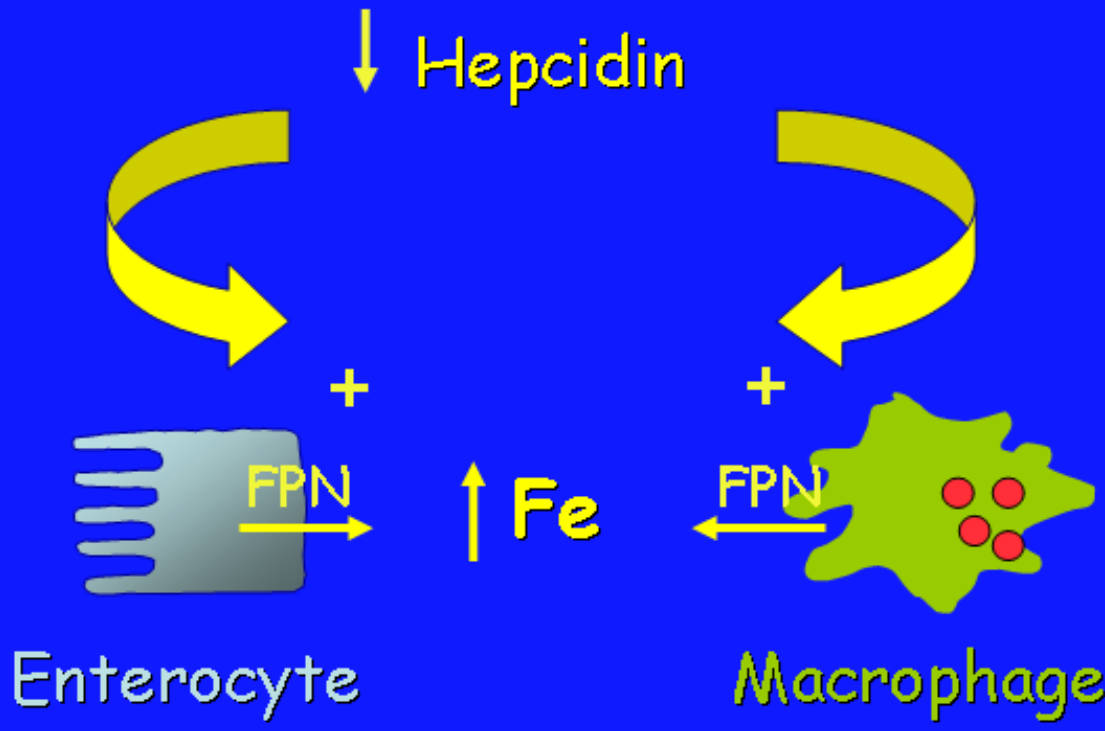


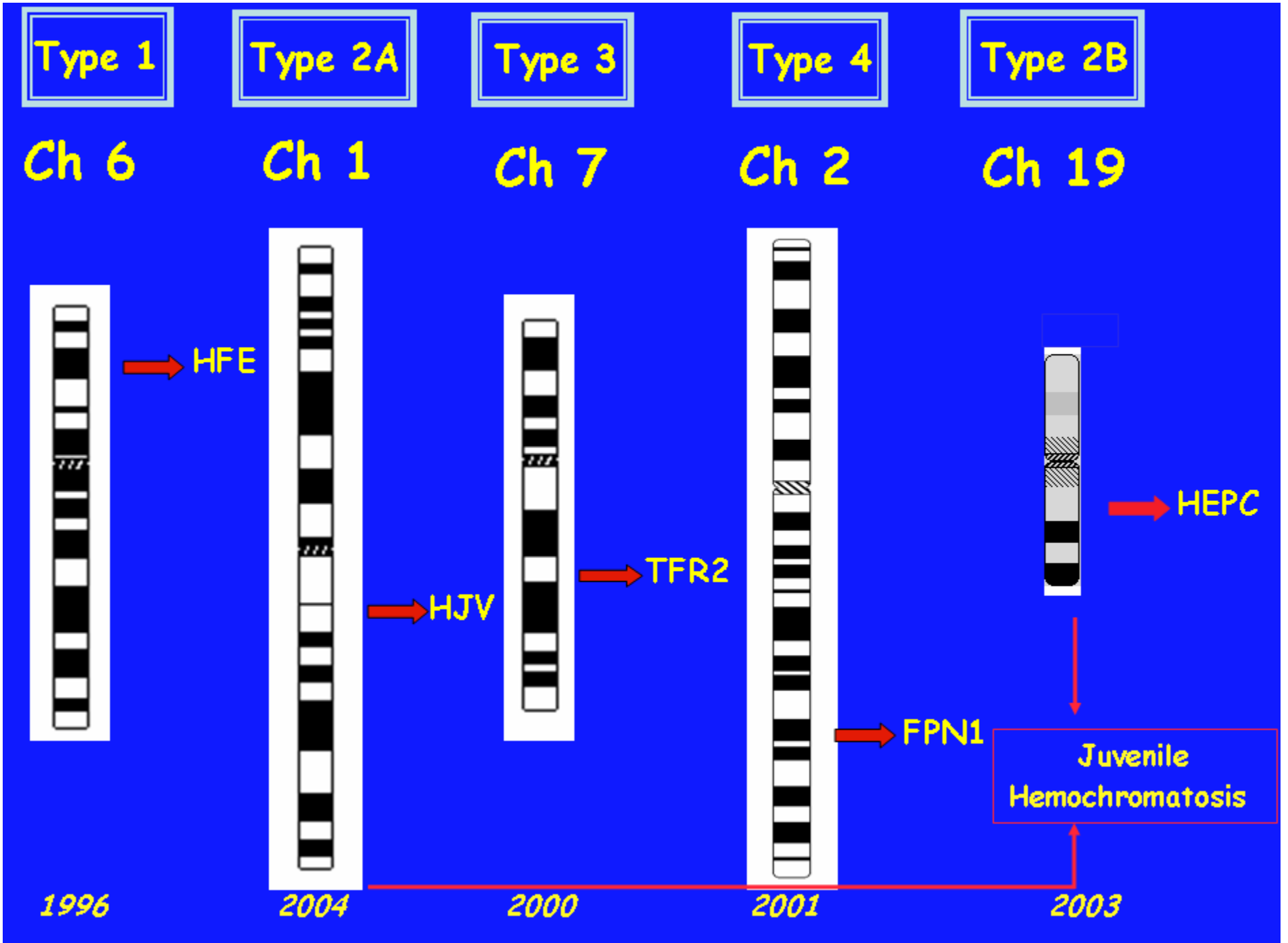
Fig. 1. Iron homeostasis. Plasma iron comes from duodenal absorption and from the spleen (iron recycling following erythrophagocytosis).

- **The total body iron content of an average male adult is about 4 g;**
- **Total iron**
  - **Red cell mass as haemoglobin – 65%-75%**
  - **Muscles as myoglobin – 10%**
  - **Storage as ferritin - 10%**
    - **Bone marrow**
    - **Reticulo-endothelial cells**
    - **Liver (0.5-1 g)**
  - **Other Haem proteins - 5%**
    - **Cytochromes, others**
  - **In Serum - 0.1%**

HEMOCHROMATOSIS MOLECULAR PATHOGENESIS

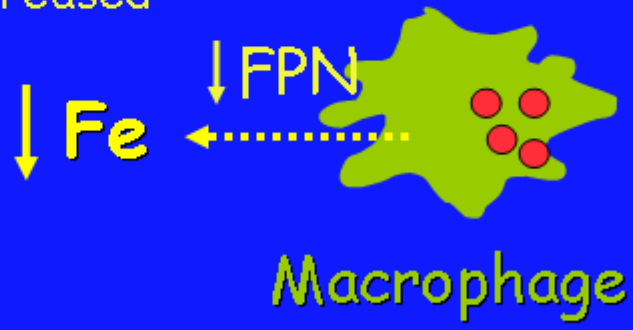
Deregulation of systemic iron homeostasis due to inadequate hepcidin production




Type 1
Type 2A
Type 3
Type 4
Type 2B
Ch 6
Ch 1
Ch 7
Ch 2
Ch 19
HFE
HJV
TFR2
FPN1
HEPC
Juvenile Hemochromatosis
1996
2004
2000
2001
2003

Ferroportin disease (type 4)

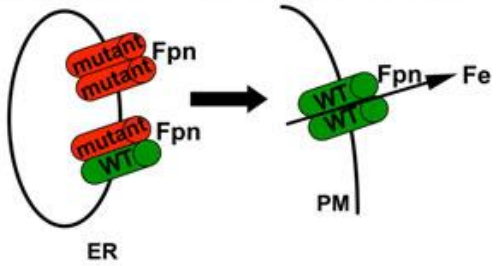
Autosomal dominant
Normal transferrin saturation
High serum ferritin
Iron increased in macrophages
Decreased iron recycling--> marginal anemia
Less clinical complications
Heterozygous mutations of the iron exporter "ferroportin"
Hepcidin levels usually not decreased



(Some cases are similar to classic hemochromatosis)

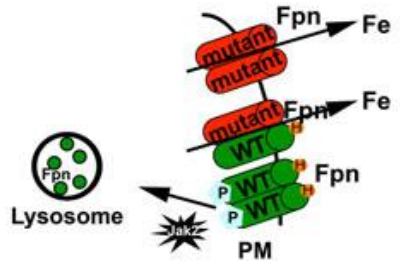
The loss-of-function and gain-of-function dichotomy clarifies how patients with a SLC40A1 mutation can develop variable iron overload diseases

A Fpn mutant does not arrive at the plasma membrane



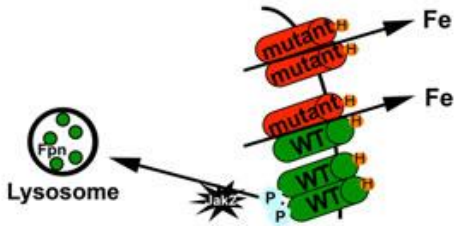
Macrophage iron loading, low to normal transferrin saturation, high serum ferritin

B Fpn mutant does not bind hepcidin



Hepatocyte iron loading, high transferrin saturation, high serum ferritin

C Fpn mutant binds hepcidin but does not get internalized



Hepatocyte iron loading, high transferrin saturation, high serum ferritin

Loss of function (type A)

Mutations affect localization of FPN to the cell membrane and/or iron export function leading to iron sequestration within the cell

Gain of function (type B)

Mutations affect the interaction between hepcidin and FPN, at the binding or signaling step, which normally leads to internalization and degradation of the FPN protein. Iron is thus continually exported from the cells to the plasma, due to increased expression of FPN protein on cell membrane.

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DISSECTING THE CONTRIBUTION OF UNREGULATED MACROPHAGE IRON RECYCLING AND DIETARY IRON UPTAKE IN GENERATING SYSTEMIC IRON OVERLOAD IN HEMOCHROMATOSIS

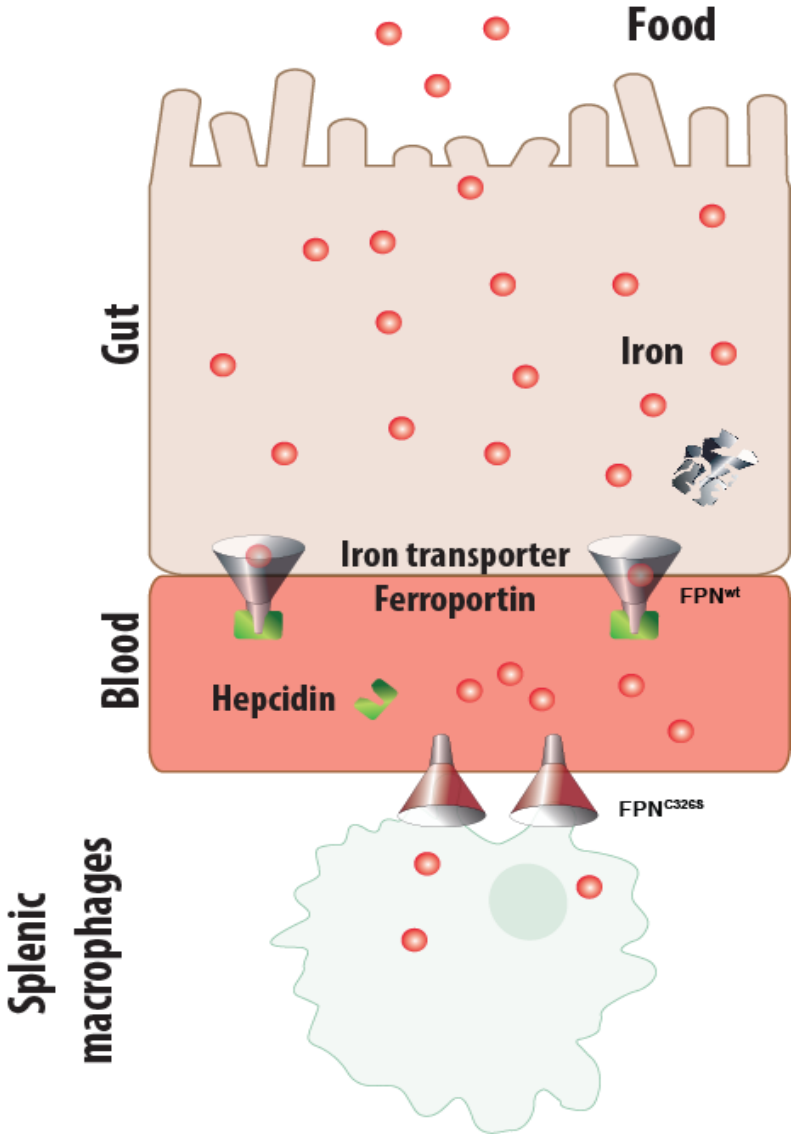
Sandro Altamura et al.

MMPU - Molecular Medicine Partnership Unit

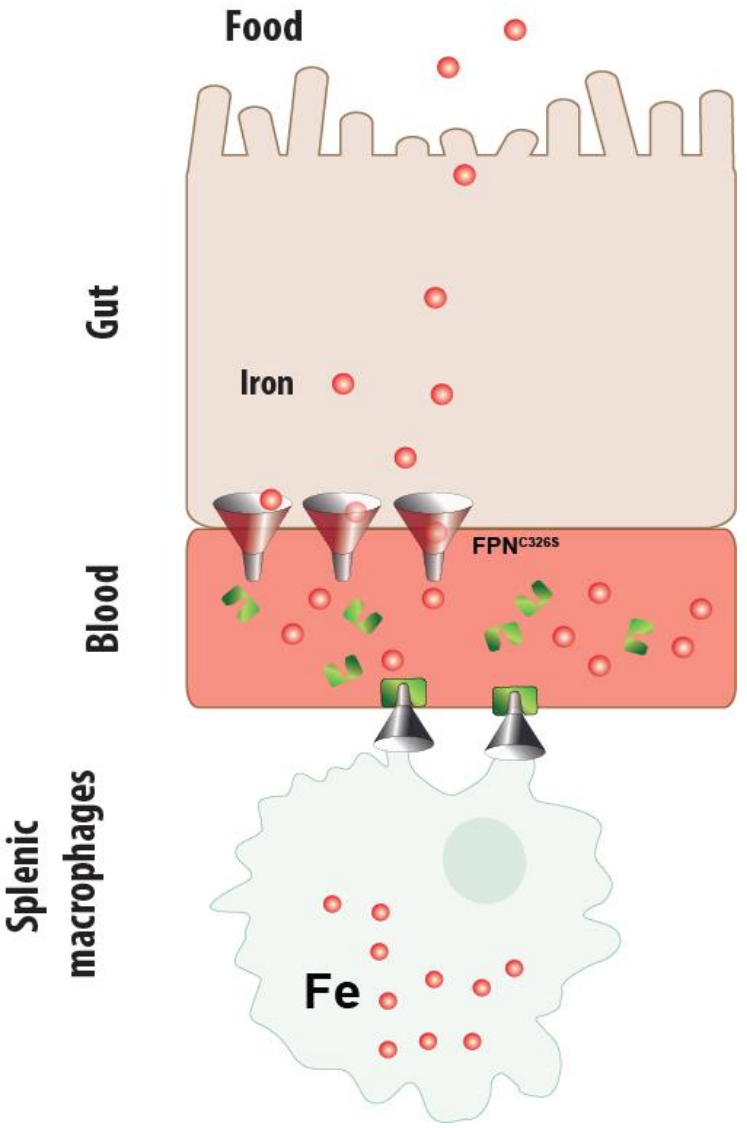
Department of Pediatric Oncology, Hematology, Immunology and Pulmonology; Children's Hospital; Heidelberg University Medical Center

Tissue-specific FpnC326S mice: Hepcidin resistant

Macrophage Specific C326S mice
Fpn-flx X LyzCre



Gut Specific C326S mice
Fpn-flx X VillinCre



THE PYRUVATE KINASE ACTIVATOR AG-348 IMPROVES MURINE β-THALASSEMIC ANEMIA AND CORRECTS INEFFECTIVE ERYTHROPOIESIS

A Matte[1], E. Beneduce[1], A. Siciliano[1], P. A. Kosinski[2], A. Janin[3], C. Lebouef[3], A. Iolascon[4], L. De Falco[4], L. Dang[2], C. Kung[2], L. De Franceschi[1]

[1]Department of Medicine, University of Verona-AOUI Verona, Verona; Italy; [2]Agios Pharmaceuticals, Inc., Cambridge, MA, USA [3]Inserm, U1165, Université Paris 7- Denis Diderot, AP-HP, Hôpital Saint-Louis, F-75010, Paris, France; [4]CEINGE and Dept. of Biochemistry, University of Naples, Naples; Italy

EHA- 8-12 June 2016

β-Thalassemic Syndromes are Worlwide Distributed Hereditary Red Cell Disorder and is a Model of Pathological Erythropoiesis

- **7% of global population is a carrier for severe hemoglobinopathies and β thalassemias are one of the most common inherited red cell disorders**

- **β thalassemias are characterized by absent or reduced synthesis of β globin chains, resulting in accumulation of free α-chain and free pathological heme.**

Weatherall DJ et al. Ann Human Biol 32: 117, 2005; Weatherall DJ Bull WHO 79: 704, 2001; Rund D et al. NEJM 353: 1135, 2005

Potentiation of Endogenous Anti-oxidant Systems as Novel Therapeutic Strategy in β-thalassemia

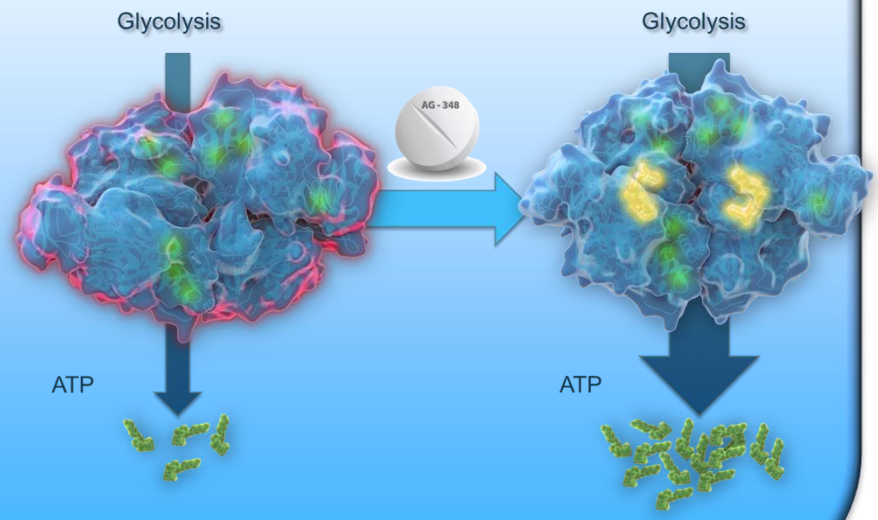
- **In β-thalassemia, exogenous anti-oxidants have been largely studied to limit ROS cytotoxity and ineffective erythropoiesis**

- **Potentiation of endogenous anti-oxidant systems might be a novel interesting therapeutic strategy to face chronic and severe oxidative stress such as in β-thalassemia.**

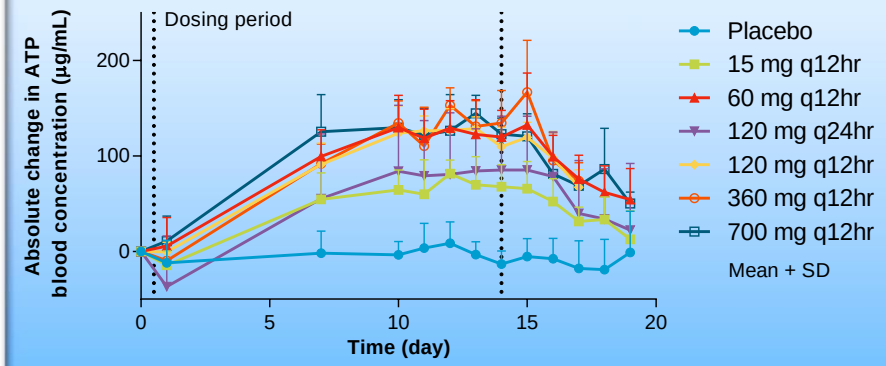
Franco SS et al. Haematologica 99: 267, 2014; Matte A ARS 23: 1284, 2015; Rund D et al. NEJM 353: 1135, 2005; Macari ER et al. Blood 117: 5987, 2011; Schmidt HHHW et al. ARS 23:1130, 2015

AG-348: Allosteric activator of the red cell isoform of pyruvate kinase (PK-R)

AG-348 binds at PK-R enzyme dimer-dimer interface to promote active tetramer

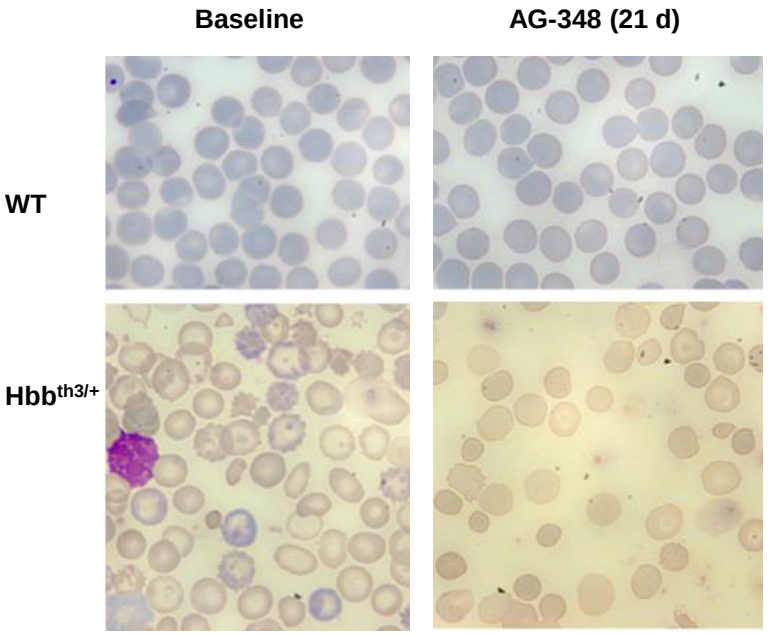


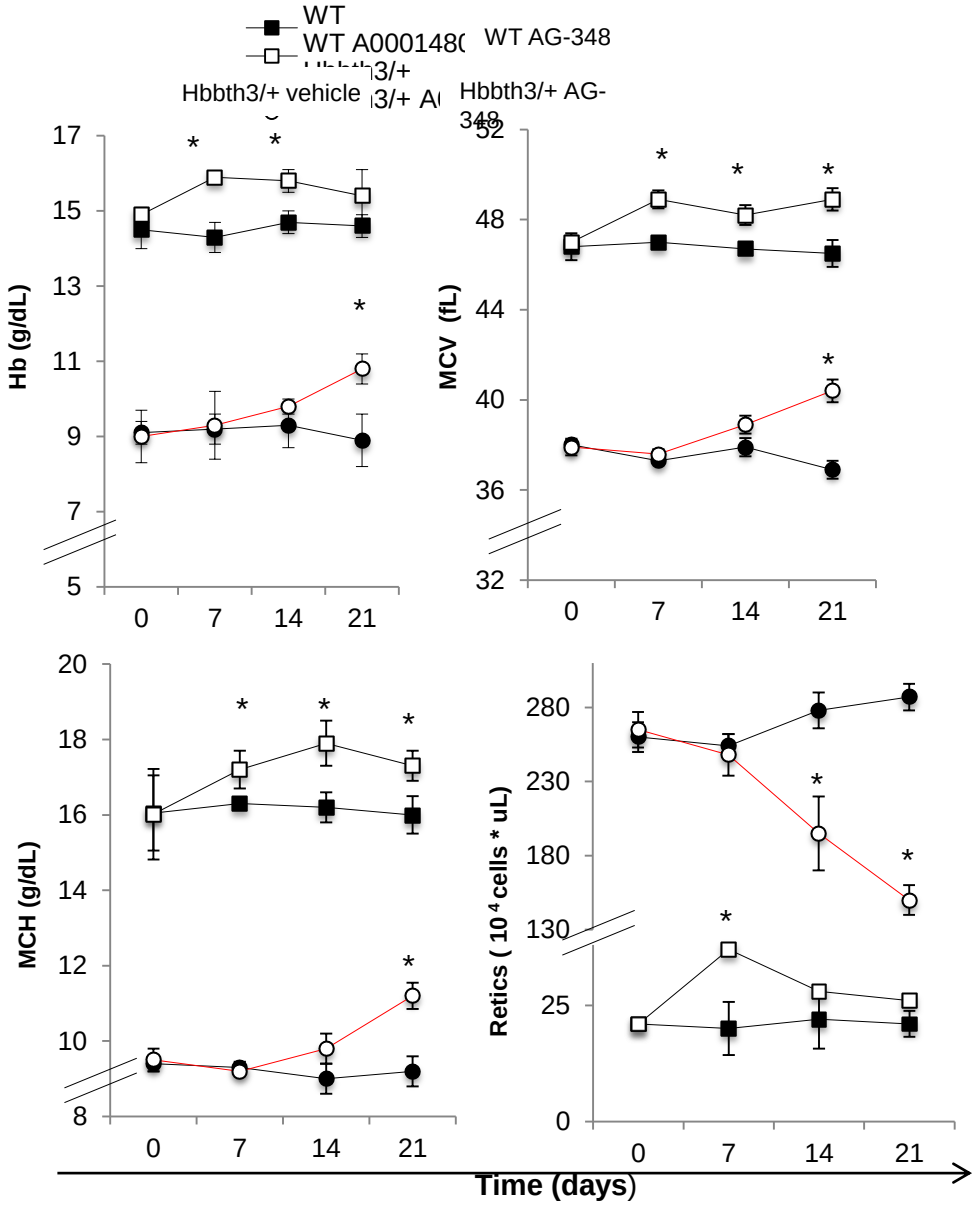
AG-348 increases ATP levels in healthy volunteers (NCT02149966)



AG-348 is currently in Phase 2 testing in patients with pyruvate kinase deficiency (NCT02476916)

AG-348 treatment significantly ameliorates anemia in a mouse model of β-thalassemia





Conclusions

✧ **In β thalassemic mice, AG-348:**

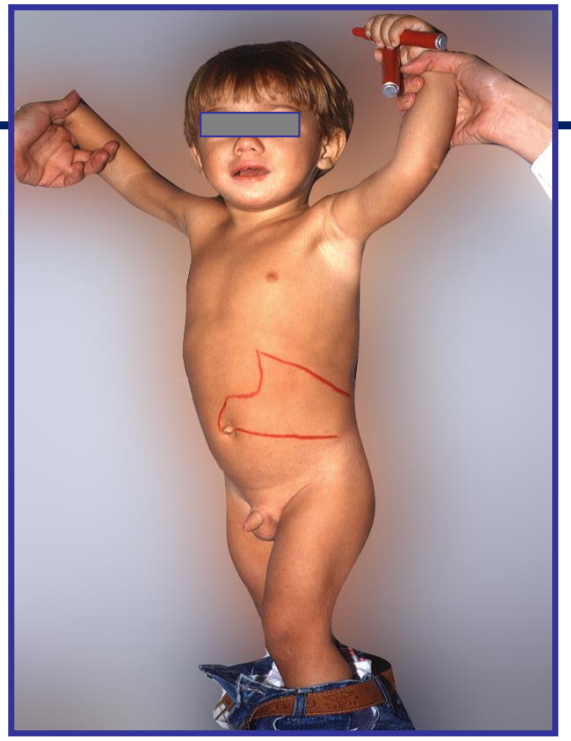
- **Reduces ineffective erythropoiesis, extramedullar erythropoiesis, Erfe expression and ROS levels**
- **Increases Hb levels, reduces reticulocyte count and circulating erythroblasts**
- **Significantly increases RBC survival**
- **Reduces liver iron overload and increases Hamp**

✧ **AG-348 might represent a novel therapeutic approach in clinical management of anemia in β thalassemic syndromes.**

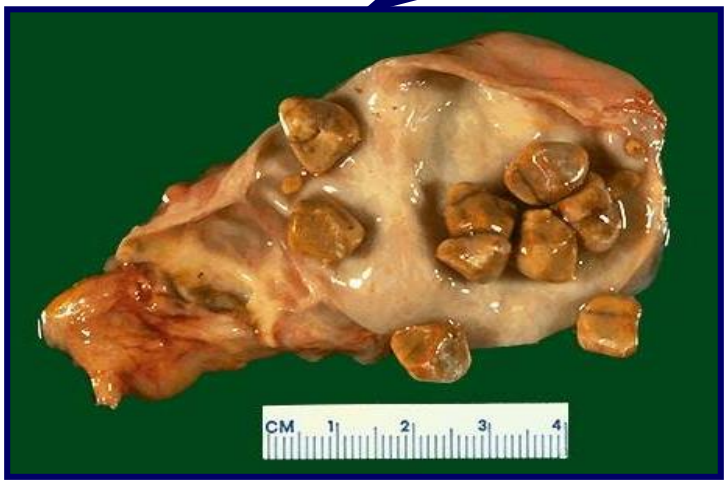
Genetic heterogeneity of Inherited Hemolytic Anemias

Anemia

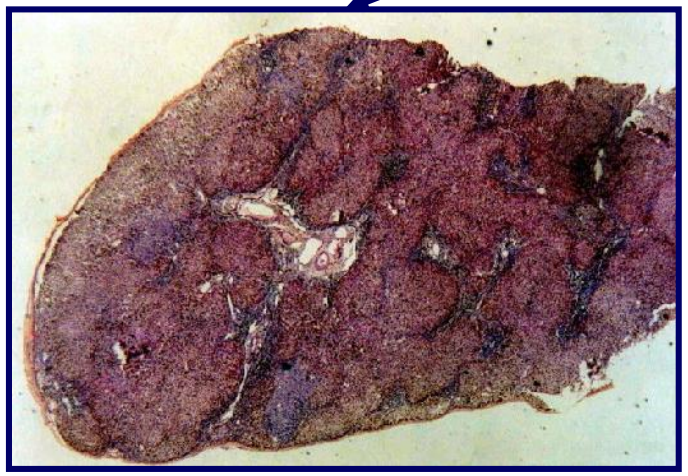
Jaundice



Splenomegaly



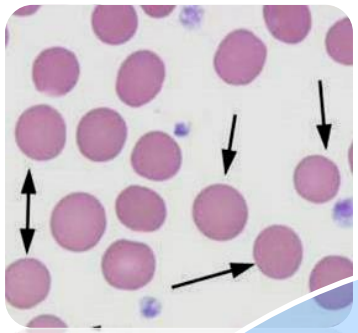
Gallstones



Iron overload

Differential diagnosis

HS



↑ Reticulocyte count

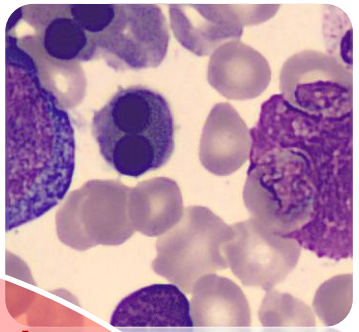
Splenectomy is the standard treatment

Autosomal dominant (75%)

↑ RDW

Normal or slightly increased sTFR

CDA II



Reticulocyte count ↓

Splenectomy slightly increases Hb level

Autosomal recessive

RDW ↑

↑ sTFR

Shared

↑ Unc. Bilirubin

↑ LDH

↓ Haptoglobin

Hepatosplenomegaly

Hemosiderosis

Preliminary study design for DD: tNGS approach

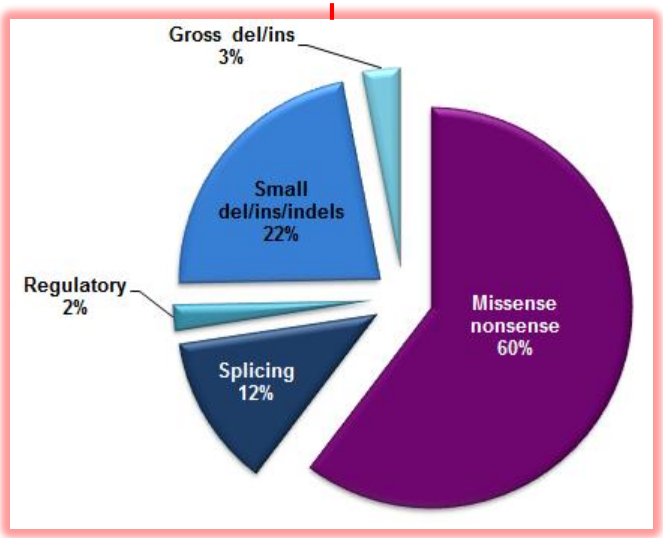
Pilot study

Selection of **15 patients**:
6 with known genotype;
6 with unknown genotype;
1 family

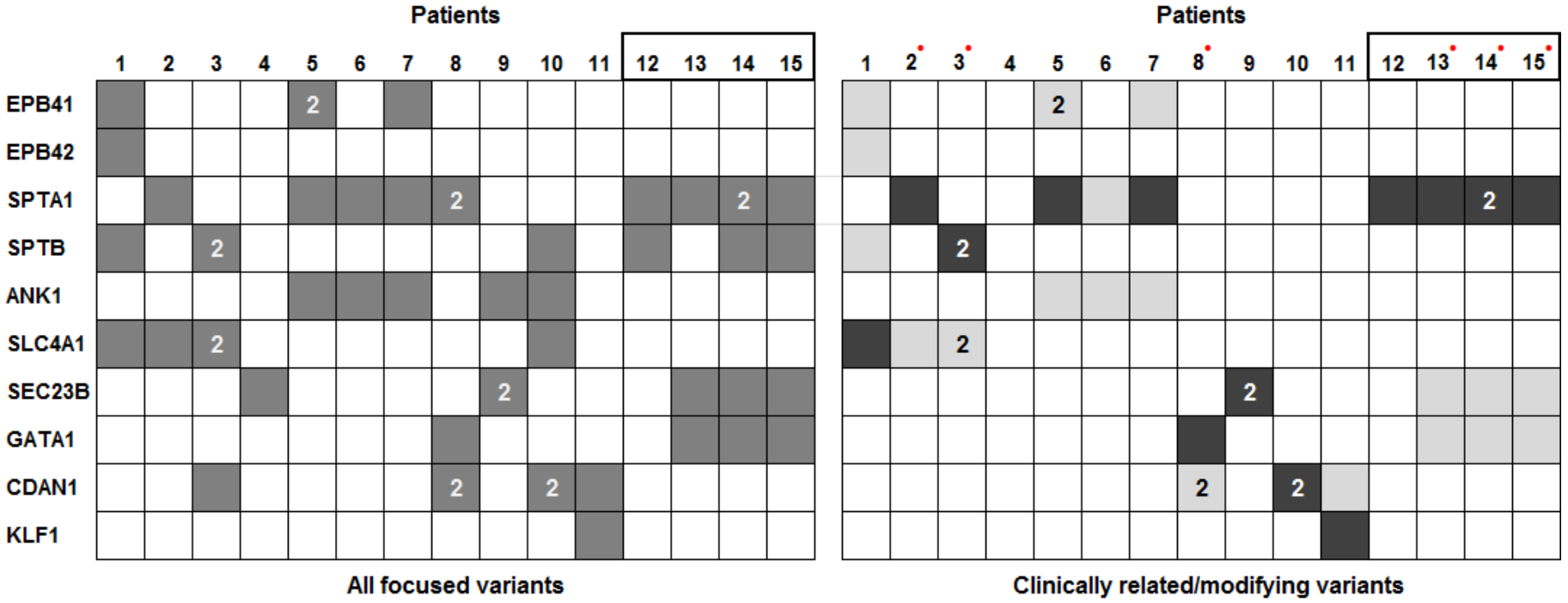
Panel of **10 causative genes** of HS and CDAs
(2012-2013)

Coding regions, UTRs, regulatory regions, **100 bp flanking splice junctions**

Inheritance pattern and validation by Sanger sequencing



Variants in clinical report of targeted-NGS-based diagnosis for HHA patients



Total variants	**62−122**
Off-target gene variants	0−2
Target gene variants	55−105
Intronic and regulatory gene variants	48−92
Coding gene variants	5−13

Variants related to clincal phenotype

Variants modifying clinical phenotype

• SPTA1 α-LELY

Complete pedigree
(12-father; 13-mother; 14-proband; 15-unaf. sister)

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

Roberta Russo[1,2], Immacolata Andolfo[1,2], Francesco Manna[1,2], Antonella Gambale[1,2], Piero Pignataro[1,2], Gianluca De Rosa[2], Achille Iolascon[1,2]

[1] Department of Molecular Medicine and Medical Biotechnology, University of Napoli Federico II, Napoli, Italy;
[2] CEINGE Advanced Biotechnologies, Napoli, Italy

ceinge BIOTECNOLOGIE AVANZATE

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

Gene panel design

SureDesign (https://earray.chem.agilent.com/suredesign/)

Panel of 34 causative or candidate genes of HAMDs and CDAs

Panel of **34 causative/candidate genes** of anemias due to red cell membrane defects and CDAs

Target enrichment and sequencing

ROIs: coding regions, UTRs, regulatory regions, 100 bp flanking splice junctions

Sequencing: Illumina NextSeq 500

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

Bioinformatic analysis

SureCall software

Exclusion of variants with:
- MAF > 0.01
- strand bias > 0.90

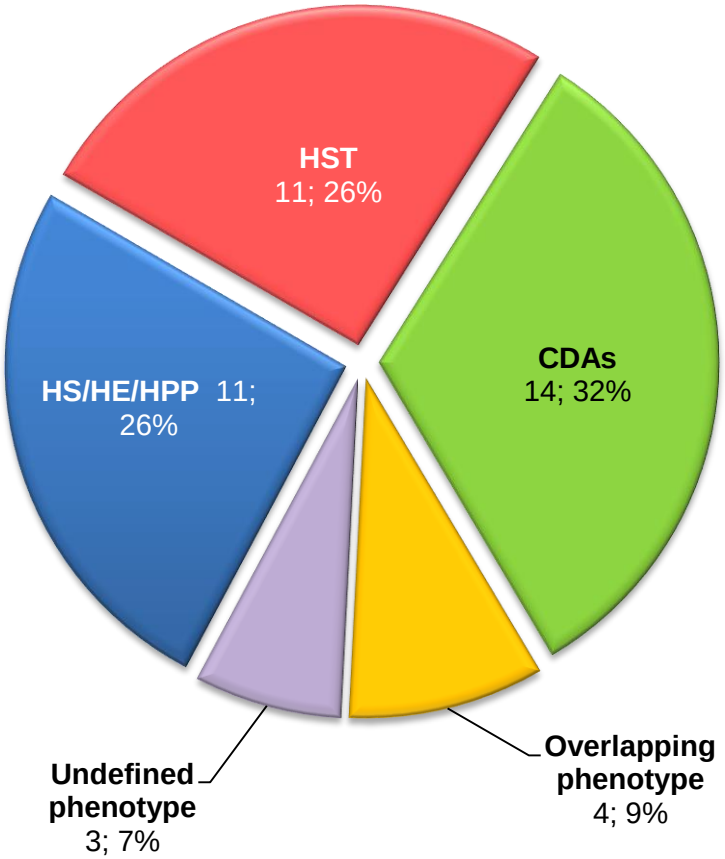
Variant selection

Prioritization by prediction tools: PolyPhen2, SIFT, HSF

Prioritizated variants were confirmed by Sanger sequencing and by the analysis of inheritance pattern

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

Distribution of diagnostic suspicions among **43** patients with hereditary hemolytic anemia (HHA) enrolled in RedPlex study



- ✓ CDAs, Congenital Dyserythropoietic Anemias;
- ✓ HST, Hereditary Stomatocytosis;
- ✓ HS, Hereditary Spherocytosis;
- ✓ HE, Hereditary Elliptocytosis;
- ✓ HPP, Hereditary Pyropoikilocytosis.
- ✓ The term “overlapping phenotype” refers to those patients exhibiting clinical picture shared among different conditions.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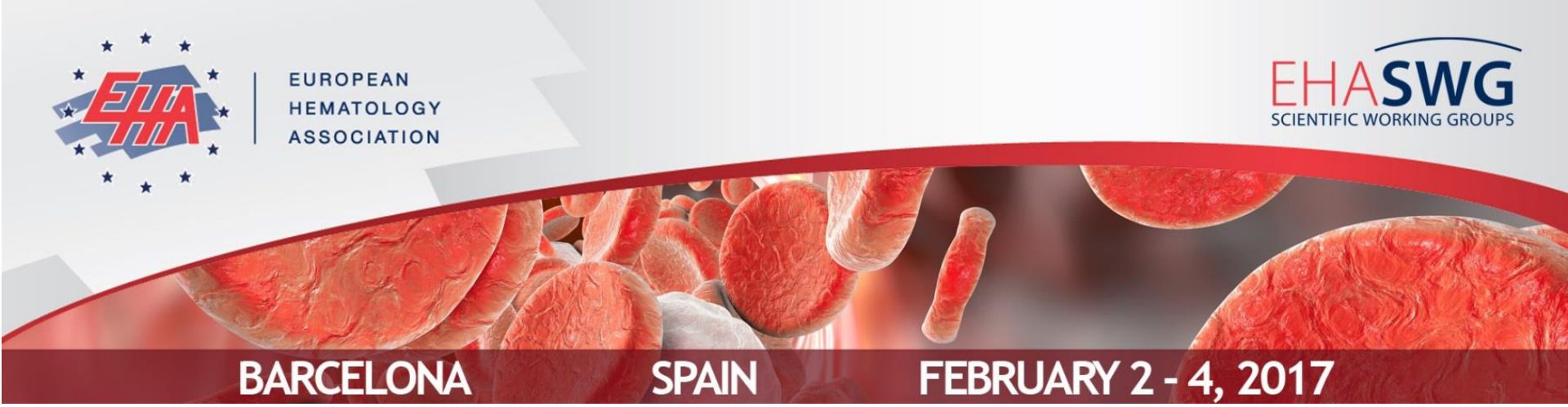
EHA Jean Bernard Lifetime
Achievement Award 2016
has been awarded to
Clara Camaschella
In recognition of her significant contribution to t
understanding of the pathophysiology of inherit
iron metabolism, including hereditary hemochr
genetic iron deficiency and iron-loading anemia

Thanks for your attention



Anemias: Diagnosis and treatment in the Omics Era

Important dates:
Registration: September 15th
Case submission: September 15th

Avenida Palace, Barcelona, Spain
www.ehaweb.org