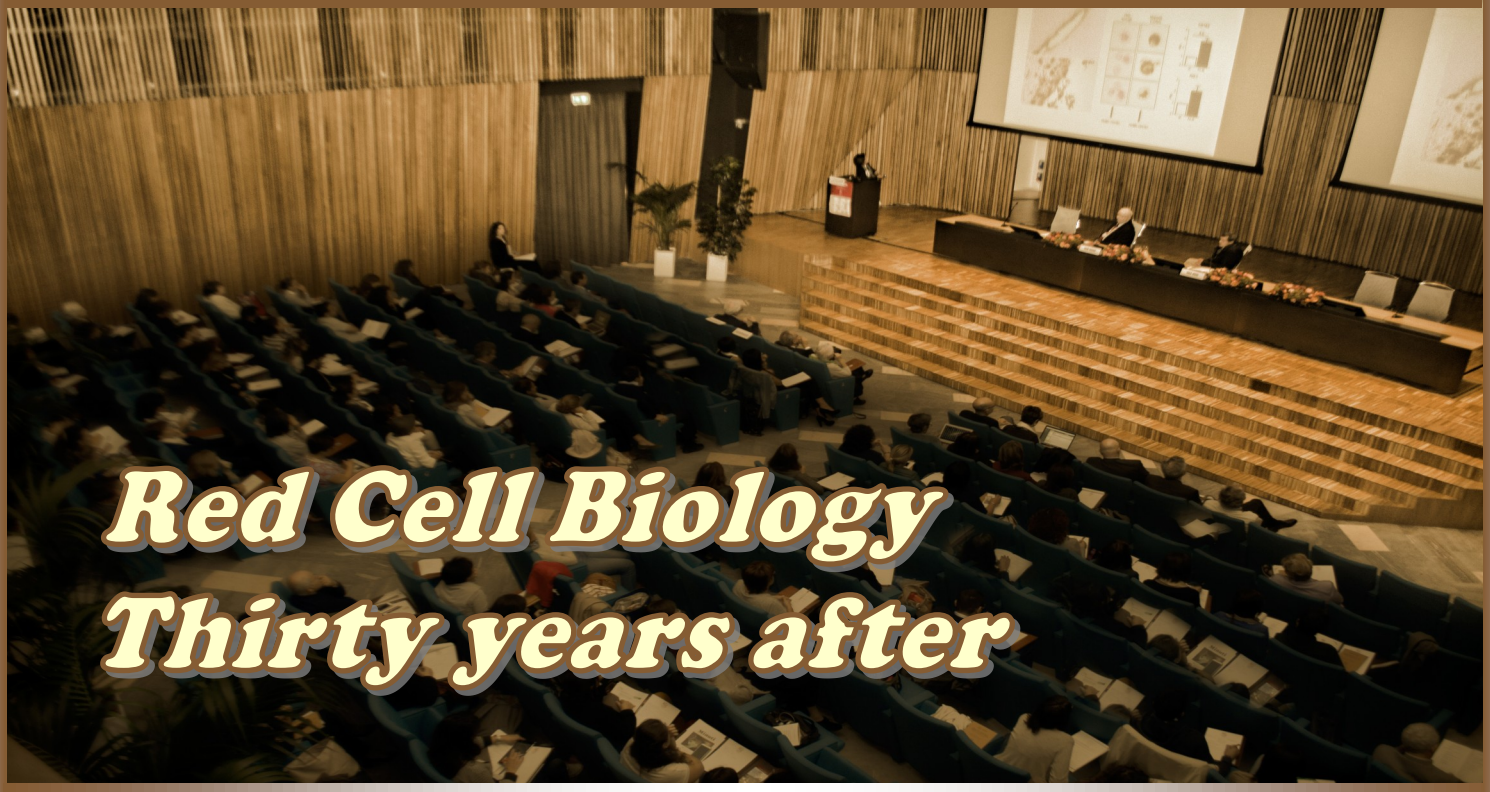


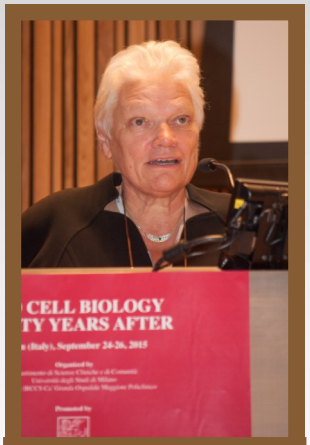
Red Cell Biology
Thirty years after

HIGHLIGHTS

Fondazione
Internazionale
Menarini

Milan (Italy)
September 24 -26, 2015

HIGHLIGHTS



M.D. Cappellini
(Milan, I)

Red Cell Biology: thirty years after!

Professor Cappellini opened the conference mentioning the first "Red Cell Biology" congress held in Florence thirty years earlier and organized by Professor Gemino Fiorelli, a physician and scientist, whose memory remains still alive within the scientific community.

Those meeting had the merit to draw clinicians attention to the findings relating the molecular biology applied to haematology. The actual conference, thirty years later, in Milan University represents a high-level scientific opportunity for international researcher to share the results of their studies that have been carried out on major subjects in this area.

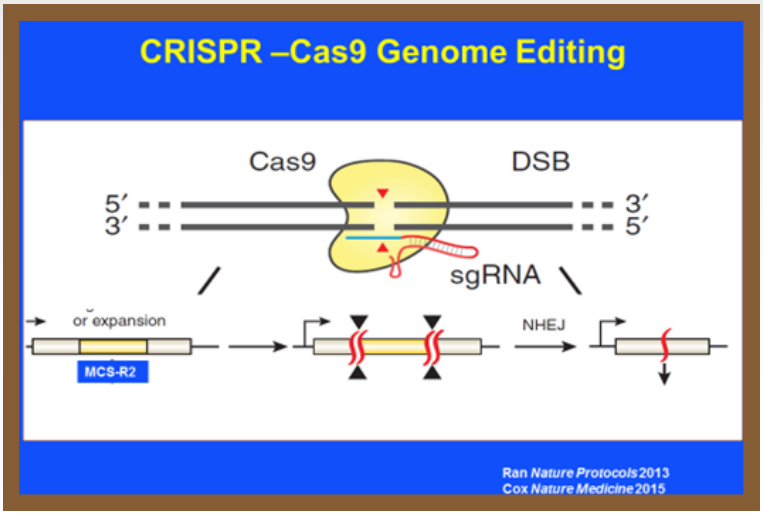
HIGHLIGHTS

D. Higgs
(Oxford, UK)

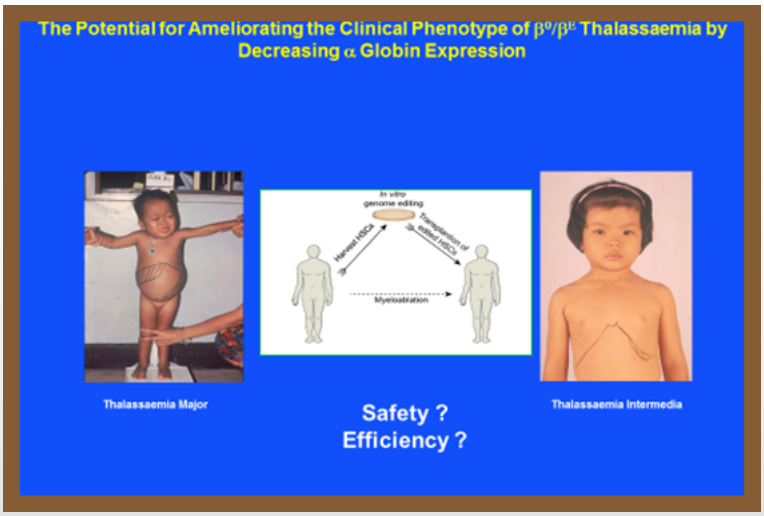
Genome Editing as a Potential Treatment for Thalassaemia

How influent is genome editing on the synthesis of haemoglobin in patients with Thalassaemia?

Professor Higgs answered this question submitting studies performed by his research group. Patients suffering the same genetic damage can show radically different clinical signs. Natural deletion of a single or two α-globin genes improves the prognosis in patients with β-thalassemia.



Then, why could'nt we practically mutate α-genes, that are responsible for α-globin synthesis, to restore globin chain imbalance in patients with b^0/ b^E Thalassaemia?



In his speech, Professor Higgs described how to act to decrease α-globin expression by deleting MCS-R2 enhancer in human chromosome 16.

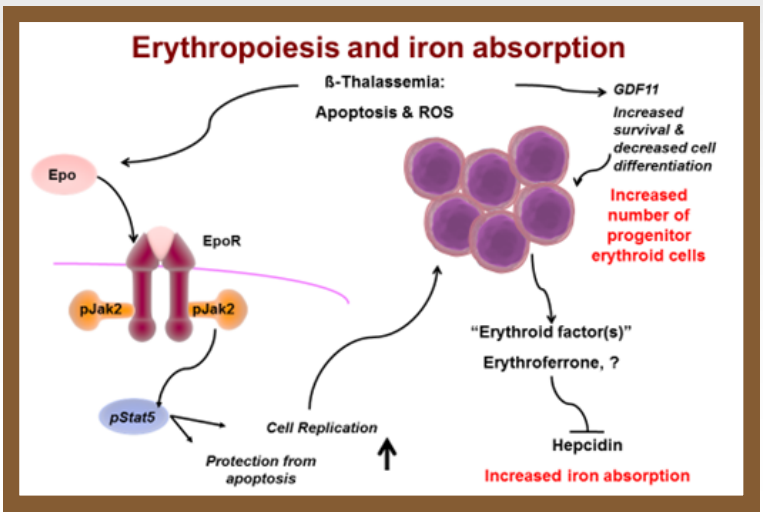
Which is the technique developed by the research group of Professor Higgs?
What are the outcomes on patients with Thalassaemia β?
Can we also use this technique for different types of Thalassaemia other than the β form?

HIGHLIGHTS

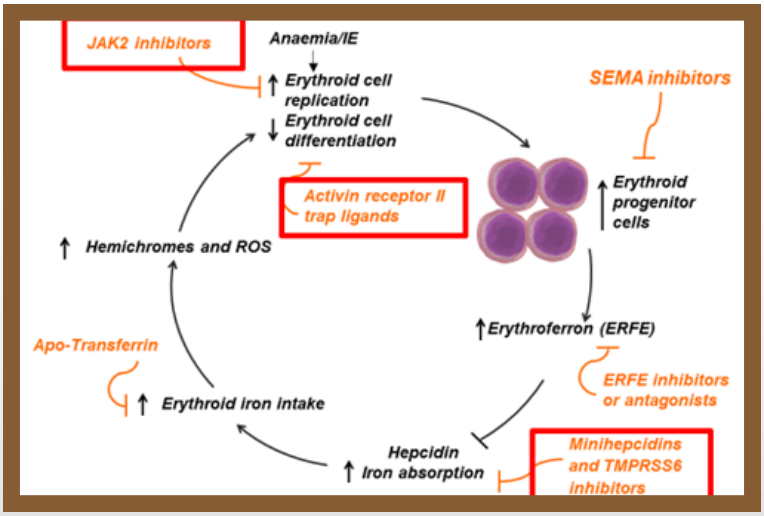
S. Rivella
(New York, USA)

New Therapeutic Approaches to Thalassemia excluding gene therapy

In addition to gene therapy, are there other new treatment options for patients with β-thalassemia?
Professor Rivella focused on this issue submitting research data on the new pharmacological treatments. Regulators of the progenitor erythroid cells including JAK-2 inhibitors represent a new pharmacological class.



Another drug class consists in the inhibitors or antagonists of Hepcidin, an important hormone necessary for the iron hepatic synthesis. Professor Rivella finally submitted data on the Activin receptor-II inhibitors that improve ineffective erythropoiesis by targeting ROS and GDF11 molecules.



What is the mechanism of action of JAK2 inhibitors?
What is the mechanism of action of the antagonists to Hepcidin?
What are the potential effect of the Activin receptor-II inhibitors on the bone metabolism?

HIGHLIGHTS

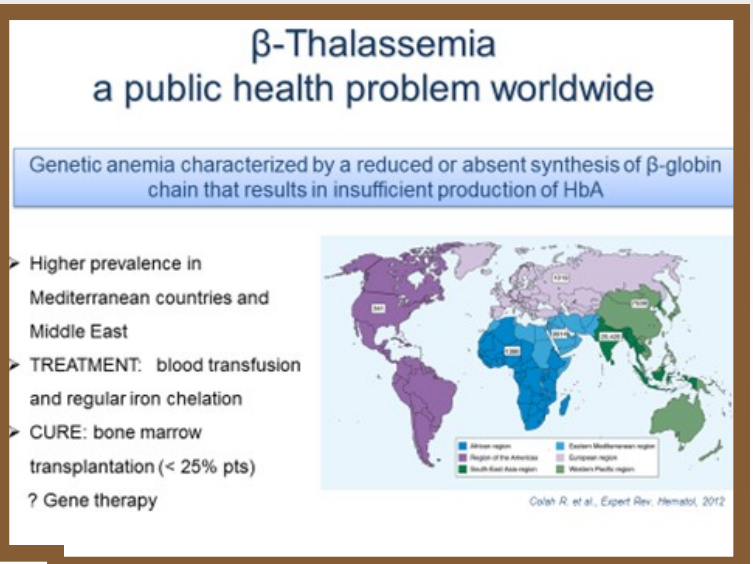
G. Ferrari
(Milan, I)

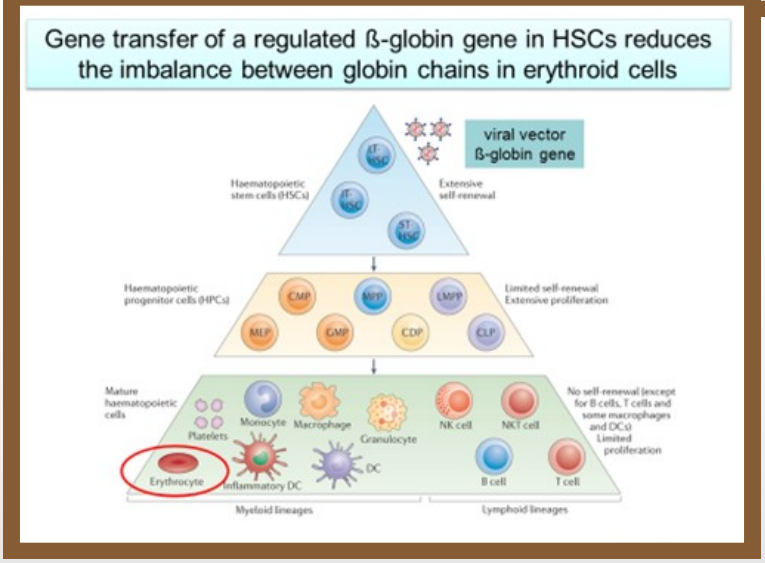
RBC monogenic diseases gene therapy

What are the factors determining a successful gene therapy in treating hematopoietic diseases?

Professor Ferrari introduced her speech with this issue: gene therapy applied to haematopoietic monogenic diseases. The rationale of this therapy is based on the transfer of β gene into somatic cells by retroviral vectors. These types of vectors seem to be the most efficient among those that have been studied.

The other crucial aspect of the gene therapy is the target cells, i.e. the haematopoietic totipotent stem cells. What are the effects of the gene transfer into these cells? In other words, for example, might there be variables in cells related to the source, the age, the environment or the route of administration that could affect the outcome? An answer to this question could come from the current clinical trials.





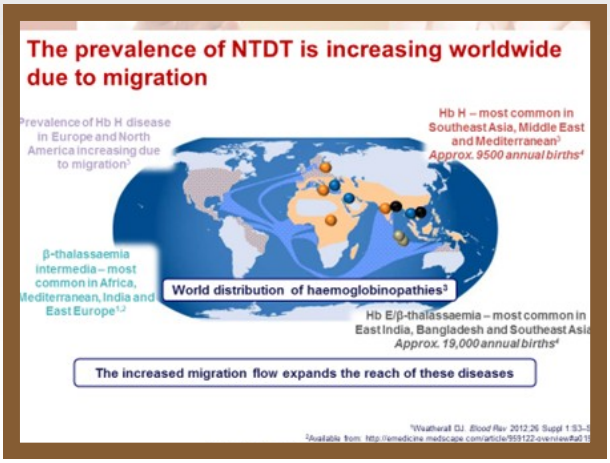
What is the most common characteristic genotype in subjects suffering β-thalassaemia and treated with gene therapy? - - What are the most efficient vectors to transfer β genes into erythropoietic stem cells? - - What are the current clinical trials to evaluate the effect of the variables related to stem cells and used for the outcome of the disease?

HIGHLIGHTS

A. Taher
(Beirut, RL)

Non transfusion dependent Thalassemia

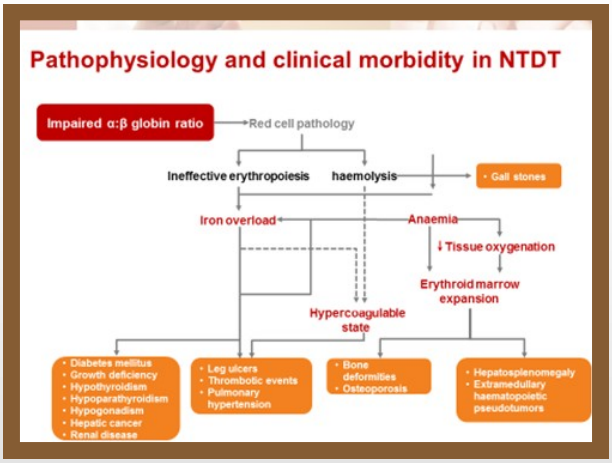
Professor Taher from Beirut spoke about forms of thalassaemia that do not require regular transfusion therapy (NTDT). The prevalence of these disease forms is increasing worldwide due to migration: they are currently present also in North America and Europe, where they were not common until recently.

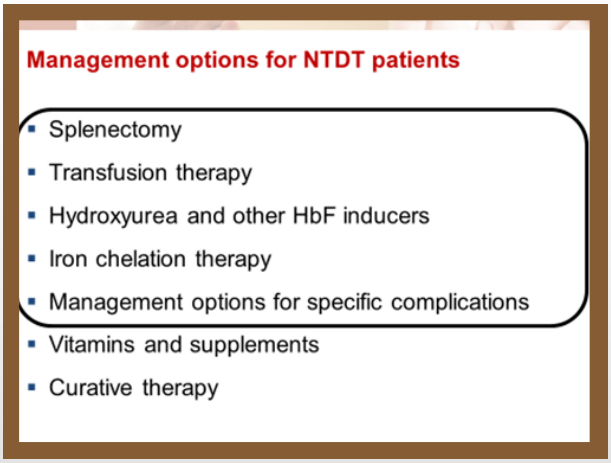


Their pathophysiology is undoubtedly very complex.

A key aspect is that they cause, in their turn, related concomitant diseases in different organs as liver, kidney, thyroid gland and parathyroid gland, bone tissue and vascular tissue. The consequent risk of complications in patients is very high and it increases with advancing age. Professor Taher described some of the major complications causing health damages as leg ulcers, silent cerebral infarctions, vascular events or even neoplastic pathologies.

The level of the quality of life in these patients is rather low and considerably lower than in subjects of same age and free from the disease. How to manage at best this pathology in order to reduce complications and to improve the patient's quality of life?





How prevalent is Thalassaemia in the world? - - What are the main pathophysiological mechanisms causing the disease occurrence? What are the complications? What are the pharmacological and non-pharmacological treatments used to cure Thalassaemia?

Red Cell Biology - Thirty years after
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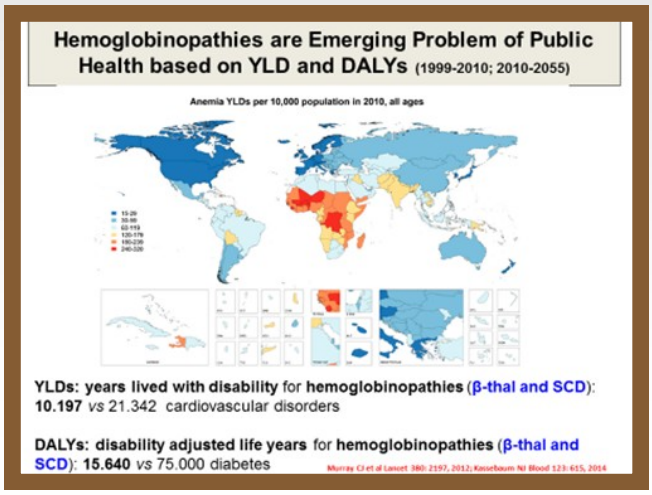
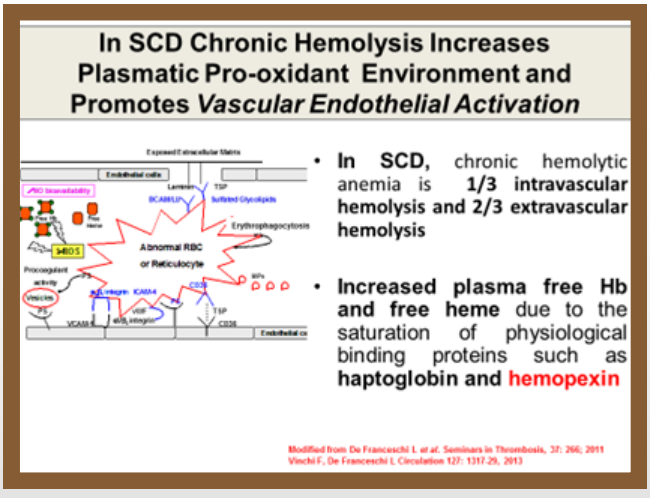
HIGHLIGHTS

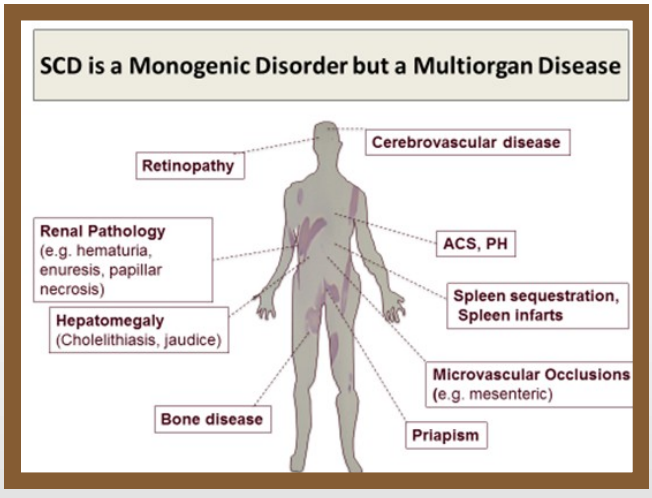
L. De Franceschi
(Verona, I)

Pathophysiology of Sickle Cell Disease

Sickle cell disease is a rising disorder in non-endemic areas and it represents an emerging public health threat due to the associated complications. Professor De Franceschi from Verona tackled this issue starting from the pathophysiology of sickle cell disease and identifying the key role of Erythrocyte Calpain expression. Another central feature seems to be connected to the increase of plasmatic pro-oxidant environments and to the presence of endothelial dysfunction. These are side effects of chronic haemolytic anemia, which is typical for this disease. Endothelial dysfunction is, in turn, responsible for endemic inflammatory vasculopathy. Professor De Franceschi further analysed the pathogenetic mechanisms triggered by these phaenomena. With all this data she intended to explain the severe multi-organ damages that are typical of Sickle Cell disease, which is a monogenic disease responsible for many alterations affecting different organs simultaneously.







What are the main pathophysiological mechanisms of Sickle Cell disease?
How important is Calpain protein? Why endothelial dysfunction appears?
What are the new recent potential pharmacological treatments to cure this pathology?

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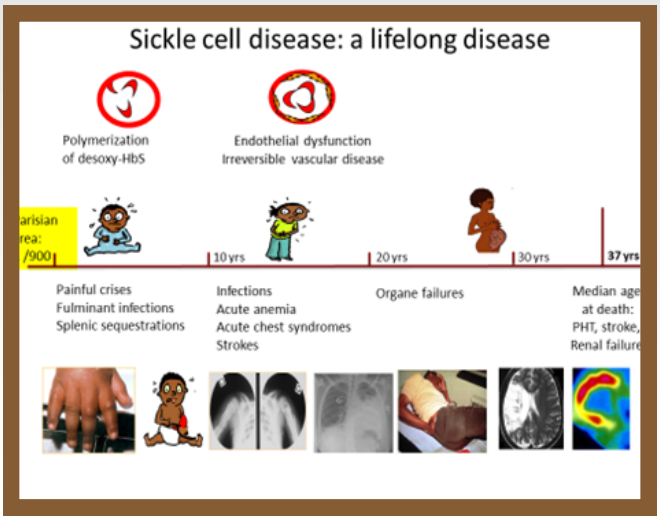
HIGHLIGHTS

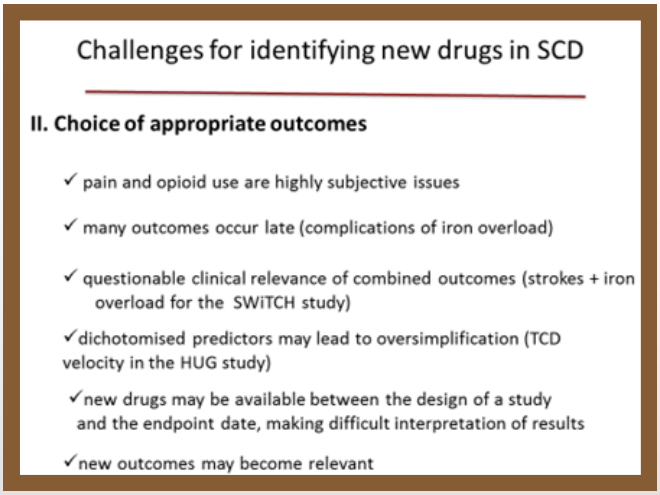
M. De Montalembert
(Paris, F)

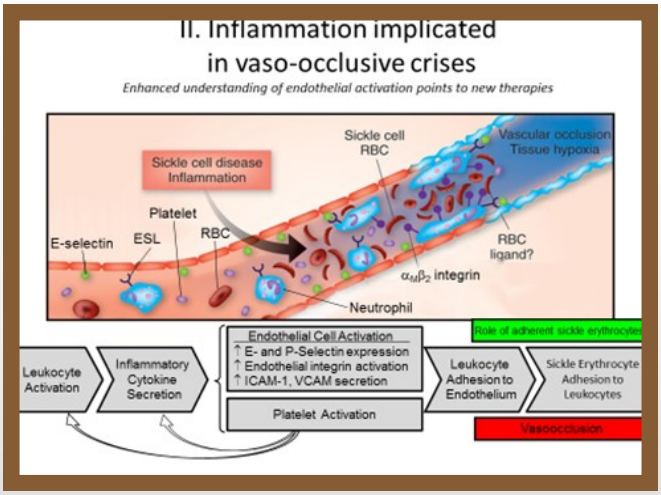
New therapeutic approaches to Sickle Cell Disease

Sickle Cell Disease is a lifelong disease affecting, with specific clinical signs, the entire life of the patients. The average age of death among those patients does not exceed 37 years.



Professor De Montalembert introduced her report emphasising the main problem of this disease in patients: it involves other organs and causes first disability that then results in death. What are the main therapeutic drugs now used for patients with sickle cell disease? Are they effective or have they further serious problems and complications? Professor De Montalembert examined these issues. It is probably essential to identify new drugs acting on patho-physiological mechanisms that involves different organs. This means, on one hand, to identify the priority of the research projects and, on the other hand, to keep in mind the limitations hindering their development. The speaker focused on two key issues: to identify innovative drugs that act selectively on erythrocytes and other drugs that act on the inflammatory states of this disease.





What life expectancy have patients with sickle cell disease?
What are the drugs, currently under study, that may affect the patho-physiological mechanisms of this disease?
How to act on the endothelial dysfunctions that affect these patients?

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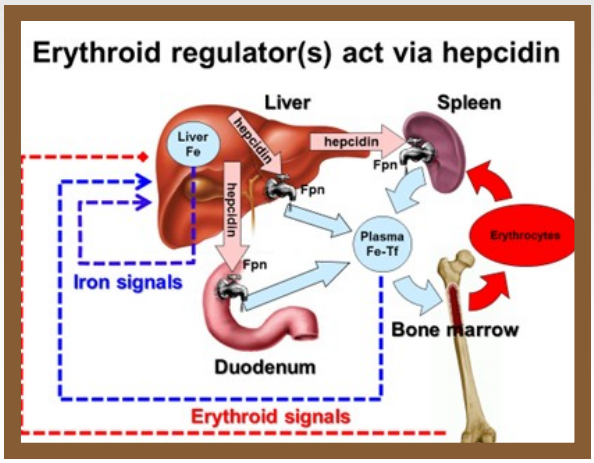
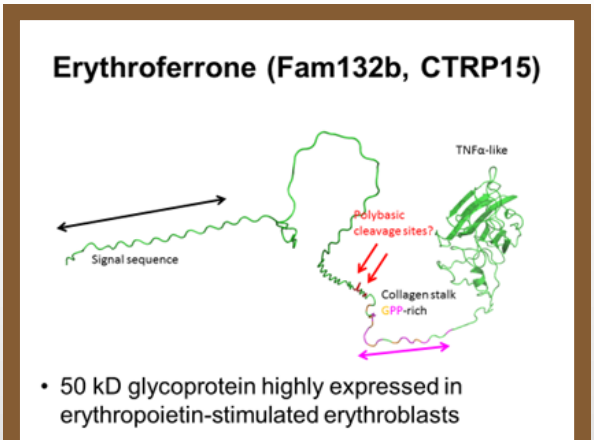
HIGHLIGHTS

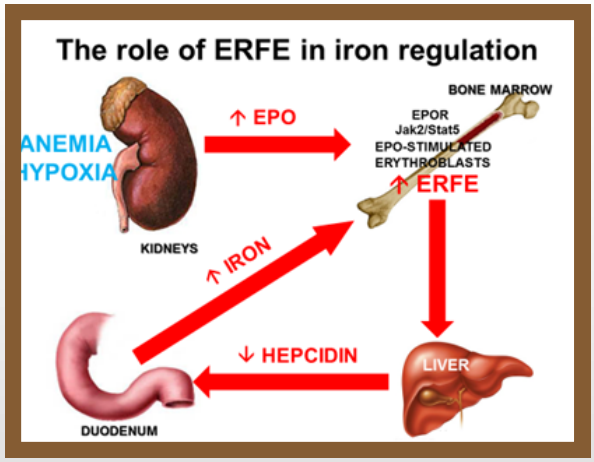
T. Ganz
(Los Angeles, USA)

New insights into iron metabolism

Professor Ganz from Los Angeles submitted very recent data regarding hepcidin, a regulator of iron homeostasis. Hepcidin is a newly discovered hormone that mediates iron homeostasis in the liver and the intestinal iron absorption in the kidney, duodenum and bone marrow. This hormone is regulated by the plasma levels of ferritin and by serum iron. The effect of the stimulation of its synthesis in the liver is a reduced iron absorption in the duodenum and an increase of renal excretion. What are the mechanisms regulating its synthesis? Professor Ganz sought to answer this question that. Is there any erythroid factor regulating hepcidin? Erythropoietin cannot be responsible for it as it is not an erythroid regulator. The erythroid regulator should be in the bone marrow. Based on these considerations, Professor Ganz submitted a list of research data tested in mice and generated by his research group. They detected Erythroferrone (Erfe), a glycoprotein highly expressed particularly in erythropoietin-stimulated erythroblasts in the bone marrow. In the light of these studies on mice, Professor Ganz presented a model that could also be applied to humans but with previous further tests. Special health conditions, as bleeding or infections, determine the expression of erythroferrone synthesis in bone marrow or the secretion of cytokines in the macrophages or the TFR or BMP in the liver. They modulate, in turn, the hepcidin synthesis, the hormone that increase plasma iron levels, acting on its absorption and release from stores.







What are the mechanisms regulating hepcidin expression in liver?
Does Erfe play a role in β-Thalassemia?
Is there any relation between hypoxia effect and hepcidin synthesis?
Should ERFE be applied to humans, could it be successful?

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HIGHLIGHTS

F. Shah
(London, UK)

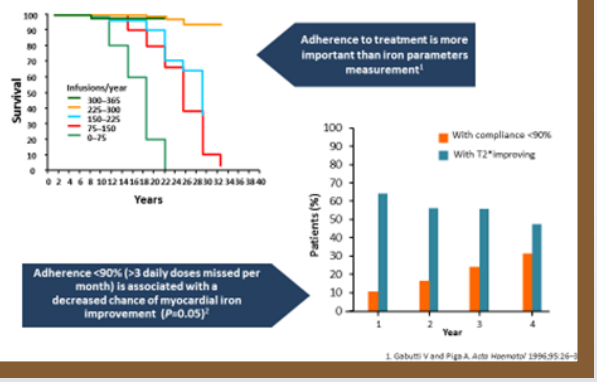
New era of iron chelation

Chelation was born about 30 years ago with the introduction of desferrioxamine to treat patients for acute iron poisoning and with the first clinical trial published in the 70's. Both events had the merit of revealing the fundamental role played by desferrioxamine in reducing the mortality in patients with serum ferritin concentrations and retention in blood. Professor Farrukh from London submitted, with her speech, the latest data on iron chelation therapy. In recent years, the treatment protocols passed from mono to combination therapy with multiple regimens simultaneously. The drugs today in use for chelation are three: desferrioxamine, deferiprone and deferasirox. The novelty are not the drugs, as they were developed during the 80's and 90's, but it regards the form of their combination. Over the years, a great number of data was produced in order to identify the ideal therapy for each type of patient. Today, the treatment protocols provide a threefold drug combination. A key aspect is their synergistic effect. A great index of synergy is noticed in the chelation combinations to remove iron from hepatic cells.

Clinical use of DFO-history

Author	year	finding
Sephton Smith	1962	IM, iron excretion increased but no effect orally
Sephton Smith	1964	Iron excretion increased with age, transfusions and transferrin saturation
Propper	1976	Continuous IV increased excretion more than IM
Propper	1977	SC increased iron excretion more than IM
Pippard	1982	Importance of faecal excretion to iron balance
Marcus	1984	IV high dose continuous
Cohen	1984	IV high dose discontinuous

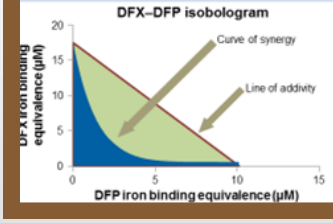
Patient survival and iron removal correlate with adherence to chelation therapy



Synergy with chelator combinations at removal of hepatocellular iron

Chelators	Iron removal (%)	Chelators	Iron removal (%)	Chelators	Iron removal (%)
DFO 10 µM	30.7	DFX 10 µM	25.6	DFP 10 µM	27.5
DFO 10 µM + DFX 5 µM	38.2	DFX 10 µM + DFO 5 µM	36.9	DFP 10 µM + DFO 5µM	38.3
DFO 10 µM + DFP 5 µM	39.3	DFX 10 µM + DFP 5 µM	41.7	DFP 10 µM + DFX 5µM	43.2

All times at 8-hour incubation



Synergy index

Chelator combination	Synergy index (α)
DFX + DFO	0.49
DFP + DFO	0.51
DFP + DFX	0.59

What are the combination treatments currently used?
What are the main discriminant effects to evaluate the drug effectiveness?
How to calculate the synergy index and its real effect?

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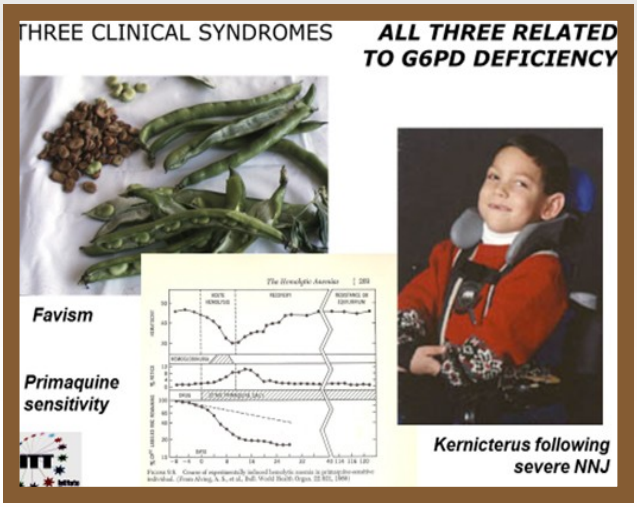
HIGHLIGHTS

L. Luzzatto
(Florence, I)

Glucose 6-Phosphate Dehydrogenase deficiency

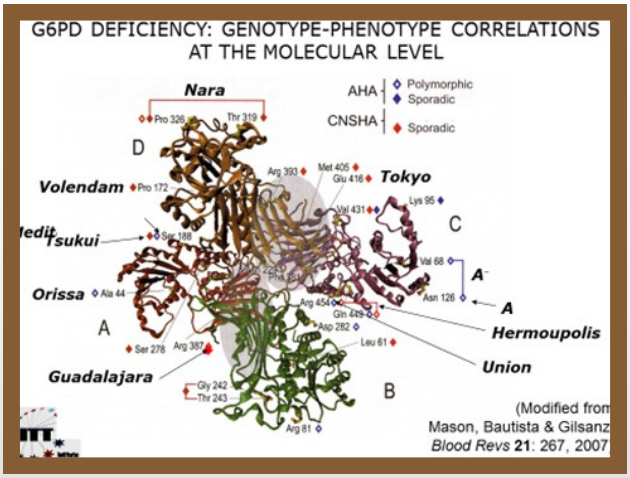
Professor Luzzato highlighted with his speech the main aspects of this enzymatic deficiency causing three syndromes: Favism, Primaquine sensitivity and Kernicterus following severe NNJ. In 1956, Professor Carson conducted the first studies regarding the close correlation between the three syndromes.

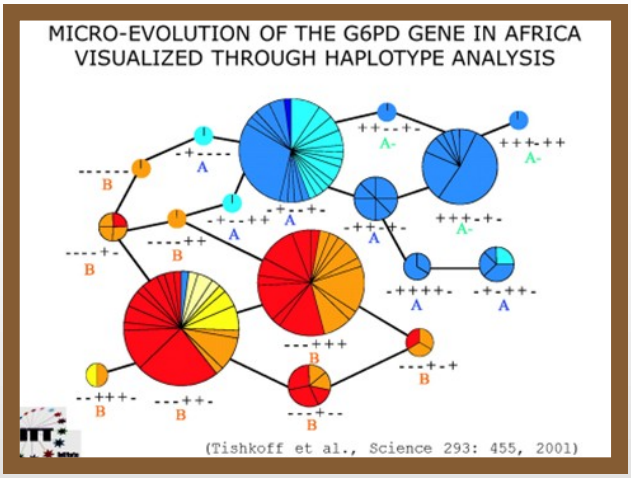


Today, genes have been cloned to encode glucose 6-phosphate dehydrogenase enzyme and we know the consequent specific gene mutations and their correlations with the different molecular phenotypes.

What is the physiological role of this enzyme? The studies conducted in knockout rats for the gene expression regulating G6PD synthesis demonstrate, unequivocally, that the enzymatic deficiency increases oxidative stress and leads embryos to death in case of homozygosity.

Already well known are the drugs that may cause haemolysis in patients with Glucose 6-Phosphate Dehydrogenase deficiency. According to Professor Luzzato it is necessary to develop more representative animal models of this enzyme deficiency. The research community is actively working on this aspect and applies specific protocols for the gene therapy.





What are the gene mutations causing G6PD Glucose 6-Phosphate Dehydrogenase deficiency?
What is the physiology of this enzyme? Do we know everything about it?
What are the most effective animal models to simulate this kind of deficiency?

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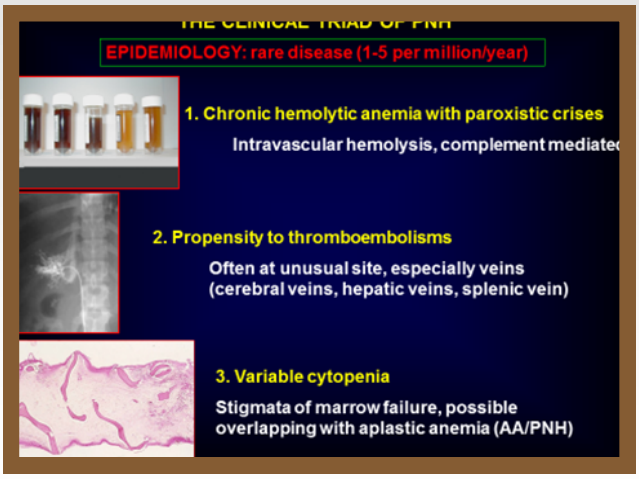
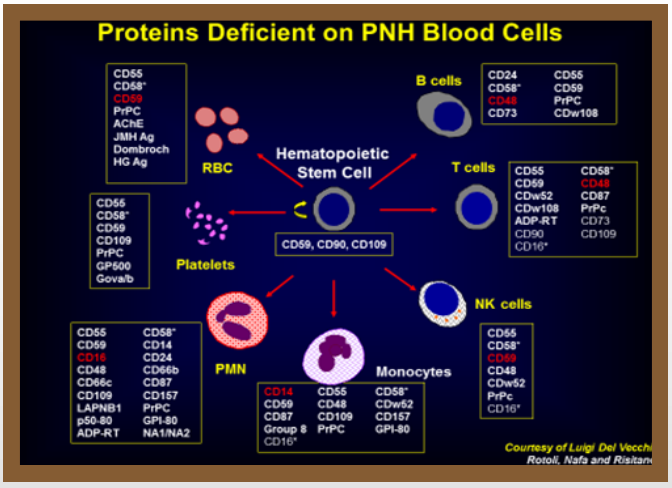
HIGHLIGHTS

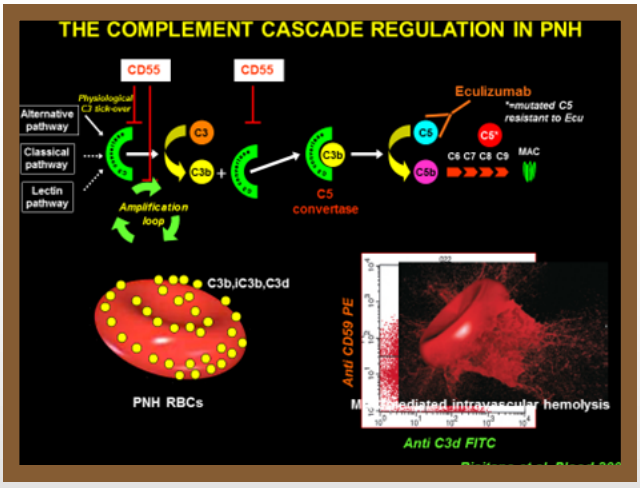
A.M. Risitano
(Naples, I)

Parosysmal nocturnal hemoglobinuria

Starting from the clinical aspects, Professor Risitano from Naples covered this subject extensively trying to define it as complete and comprehensive as possible. He showed a complex framework characterised by a series of aspects that require further clarification. There are, however, some certainties relating, for example, to the genetic of the disease and to the exclusion of its neoplastic nature. The initial characteristic of the disease is the PIG-A gene mutation in totipotent stem cells. From the pathophysiologic point of view, we can define PNH as an immune-mediated disease due to an adaptive immunity mechanism that involves T cells. Most of the patients suffering from this disease present bone marrow failure with frequent pancytopenia and reduced erythroid colony formation. Their average survival rate is about 20 years and they often die for thrombotic disease. Bone marrow transplants is the traditional PNH therapy but patients with hemolytic PNH must be initially treated with Eculizumab. The same treatment is suggested for patients with thrombotic disease.







What are the main patho-physiological theories determining the occurrence of PNH?
Which is the role that the complement plays in disease determinism?
Is the drug treatment with Eculizumab effective for all PNH events?
Are we able to develop new treatments to improve the outcome of this disease?

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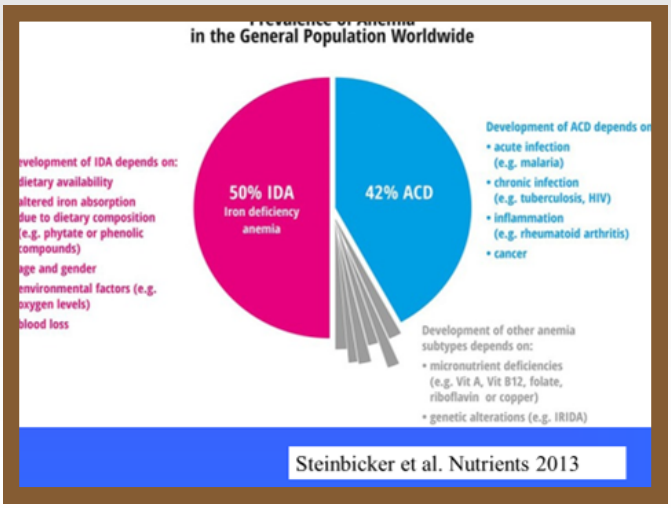
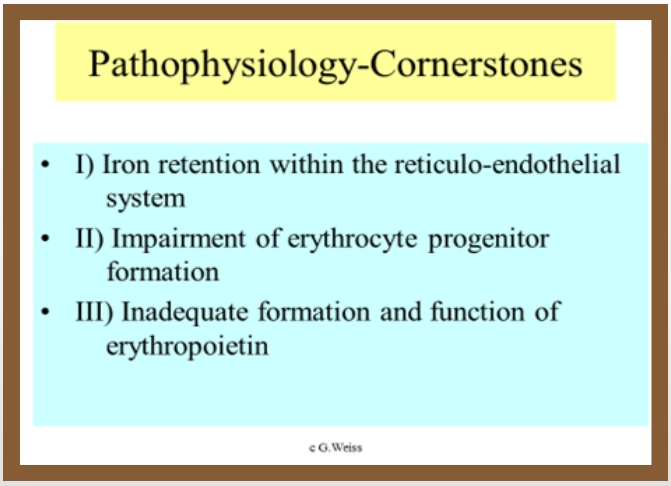
HIGHLIGHTS

G. Weiss
(Innsbruck, A)

Anemia of chronic diseases

In almost 50% of cases Anemia is due to iron deficiency and in the other 50% depends on chronic diseases such as acute infection or immune system deficit or inflammation-based diseases. After this introduction, Professor Weiss described the major pathophysiological mechanisms of Anemia: iron retention within the reticulo-endothelial system, impairment formation of erythrocyte progenitor and inadequate formation and function of erythropoietin. For its pathophysiological mechanisms, Anemia is reported as an immune driven disease. Professor Weiss dealt with the issue of the differential diagnosis between the two types of Anemia: ACD (Anemia of Chronic Disorders) and ACD/IDA (Associated True Iron Deficiency). He emphasised the importance of the therapeutic treatments, which are often diverging. In the last part of the speech, he described the therapeutic protocols and the new therapeutic approaches to cure Anemia of chronic disease.





What are the major pathophysiological mechanisms of Anemia of chronic disease?
What is the role of iron supplements in this form of Anemia?
Which is the correct differential diagnosis? Are there new therapeutic treatments for patients suffering from Anemia due to chronic diseases?

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HIGHLIGHTS

These are just some of the topics addressed during the congress. For further details please consult the website of the Fondazione Internazionale Menarini that contains the full version of the congress talk

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