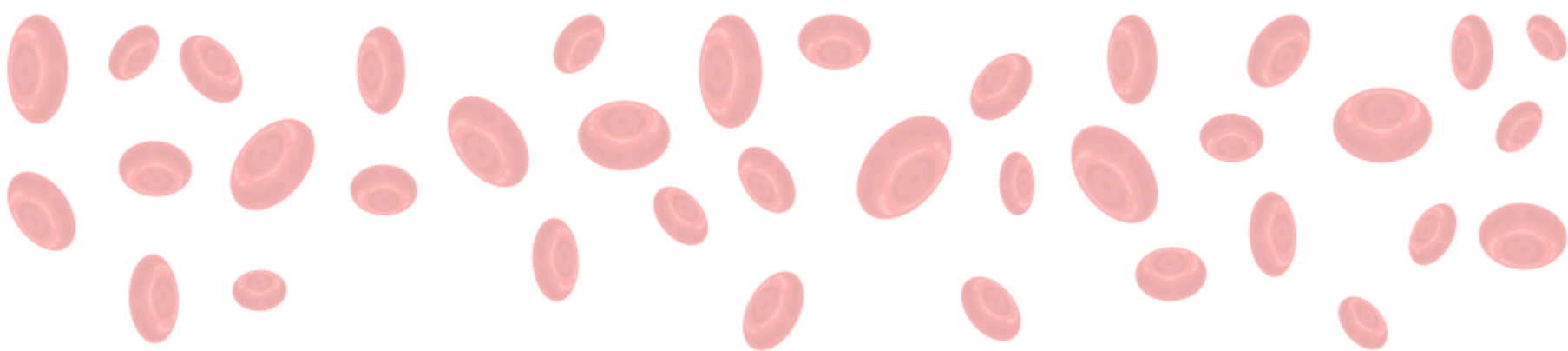




STORY OF RED CELL MEMBRANE DEFECTS AND BEYOND

Achille Iolascon , MD. PHD

Prof. Emeritus of Federico II University of Naples

CEINGE Advanced Biotechnology – Franco Salvatore - Naples



**Globulo
Rosso 2026**

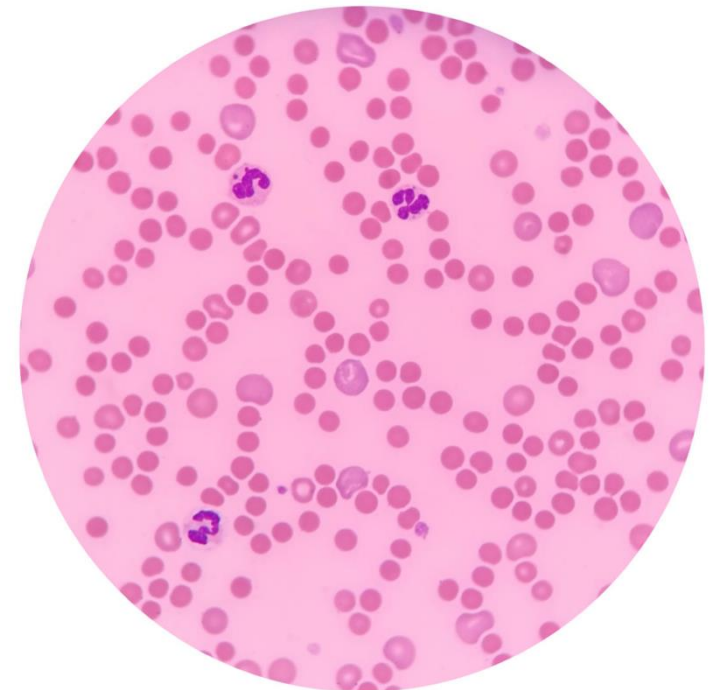
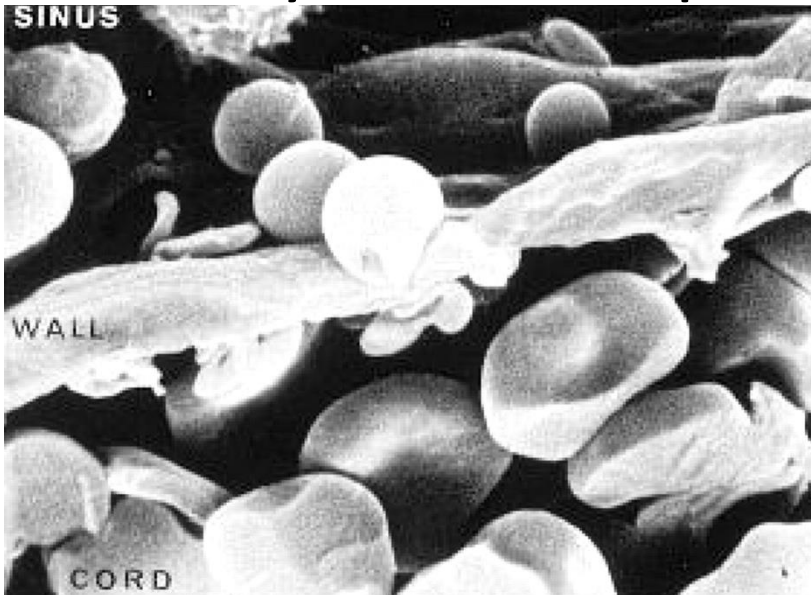
NAPOLI
28-29 SETTEMBRE 2026
Complesso del SS. Marcellino e Festo



- Hereditary spherocytosis is one of the most common inherited hemolytic anemias (1:1500).

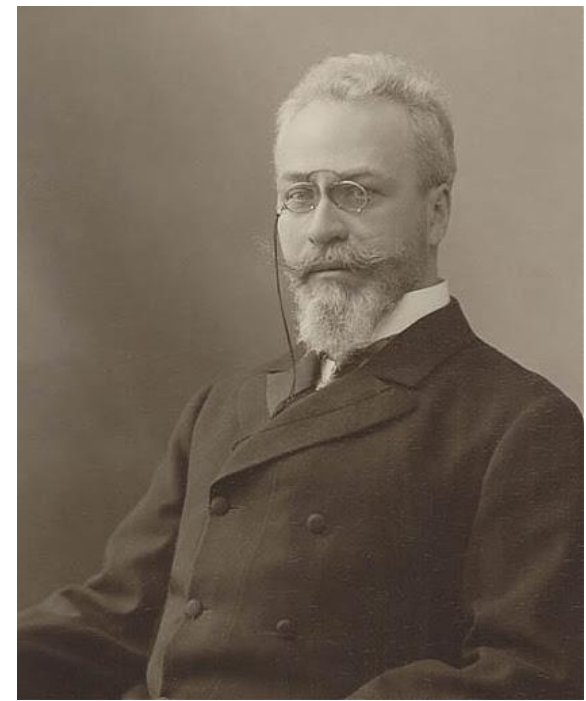


- Erythrocytes lose their biconcave discoid shape and become spherical.
- Spherocytes are less deformable and are destroyed in the spleen



Anatole-Marie-Émile
Chauffard

French physician (1855–1932)
professor in Paris.



Oskar Minkowski

German–Lithuanian internist
and physiologist (1858–1931).
Known for identifying the pancreatic
origin of diabetes mellitus (1889).



Guido Banti

(1852–1914).

Described hemolytic jaundice with splenomegaly and progressive anemia.

Demonstrated that splenectomy could result in complete recovery.

Proposed two forms: hemopoietic and anemopoietic.

Highlighted the spleen's central role in hemolysis.



HS 20th–21st Century Advances

- 1930s–1950s: Recognition that hereditary spherocytosis is distinct from acquired hemolytic anemias.
- 1950s: Identified as a disorder of the red cell membrane rather than hemoglobin.
- 1960s–1970s: Biochemical studies revealed defects in membrane proteins.
 - Spectrin identified as a key cytoskeletal component.
 - Ankyrin discovered as the link between spectrin and band 3.
 - Band 3 recognized as the major anion exchanger of RBCs.
 - Protein 4.2 linked to membrane stability.
- 1980s–1990s: Molecular genetics allowed identification of gene mutations.
 - ANK1, SPTA1, SPTB, SLC4A1, EPB42 mutations characterized.
 - Understanding of autosomal dominant vs recessive forms.
- 2000s–present: Modern diagnostic refinement.
 - EMA-binding test standardized for screening.
 - Flow cytometry improves diagnostic accuracy.
 - Genetic sequencing defines genotype–phenotype correlations.
- Therapeutic evolution:
 - Splenectomy reserved for moderate/severe disease.
 - Partial splenectomy explored to reduce infection risk.
 - Improved management of neonatal jaundice and gallstones.

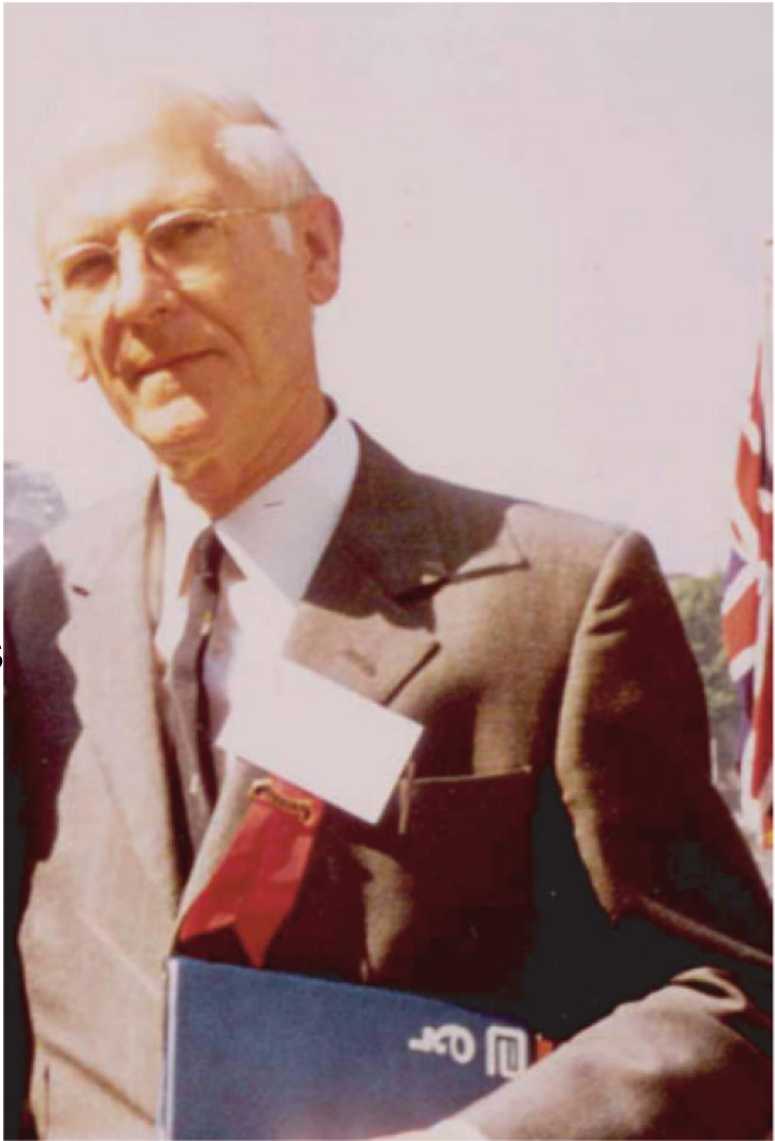


John V. Dacie (UK) – 1940s–1950s

Considered the founder of modern clinical hematology.

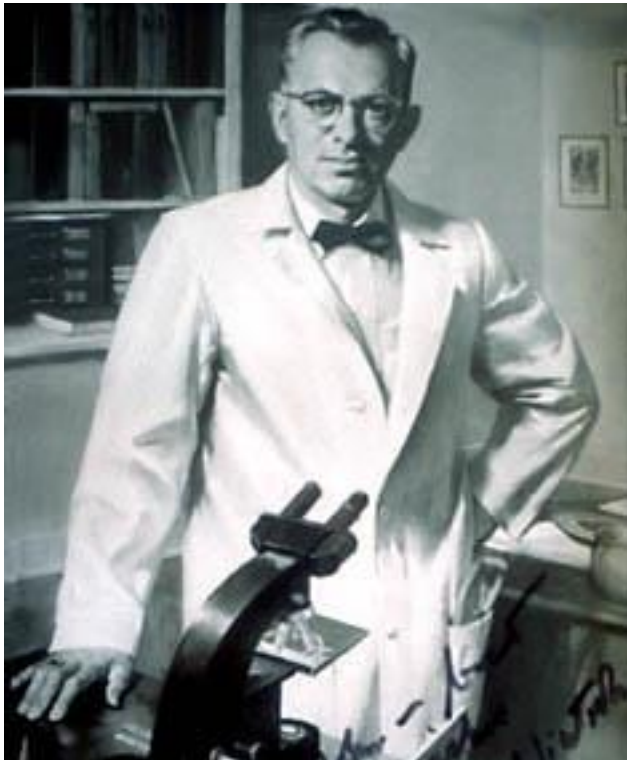
In the first edition of *Practical Haematology* (1950), Dacie systematically described:
the clinical picture of hereditary spherocytosis
its familial pattern
its differences from acquired hemolytic anemias

He showed that HS does not present antibodies against red blood cells, unlike autoimmune forms.



Robin Coombs, Arthur Mourant, Robert Race – 1945

- **They introduced the Coombs test (Direct Antiglobulin Test, DAT).**
- **This test made it possible to distinguish:**
- **spherocytes due to immune causes (DAT+)**
- **spherocytes due to intrinsic red cell defects as in HS (DAT-)**
- **The DAT was the technical breakthrough that allowed the two conditions to be definitively separated.**



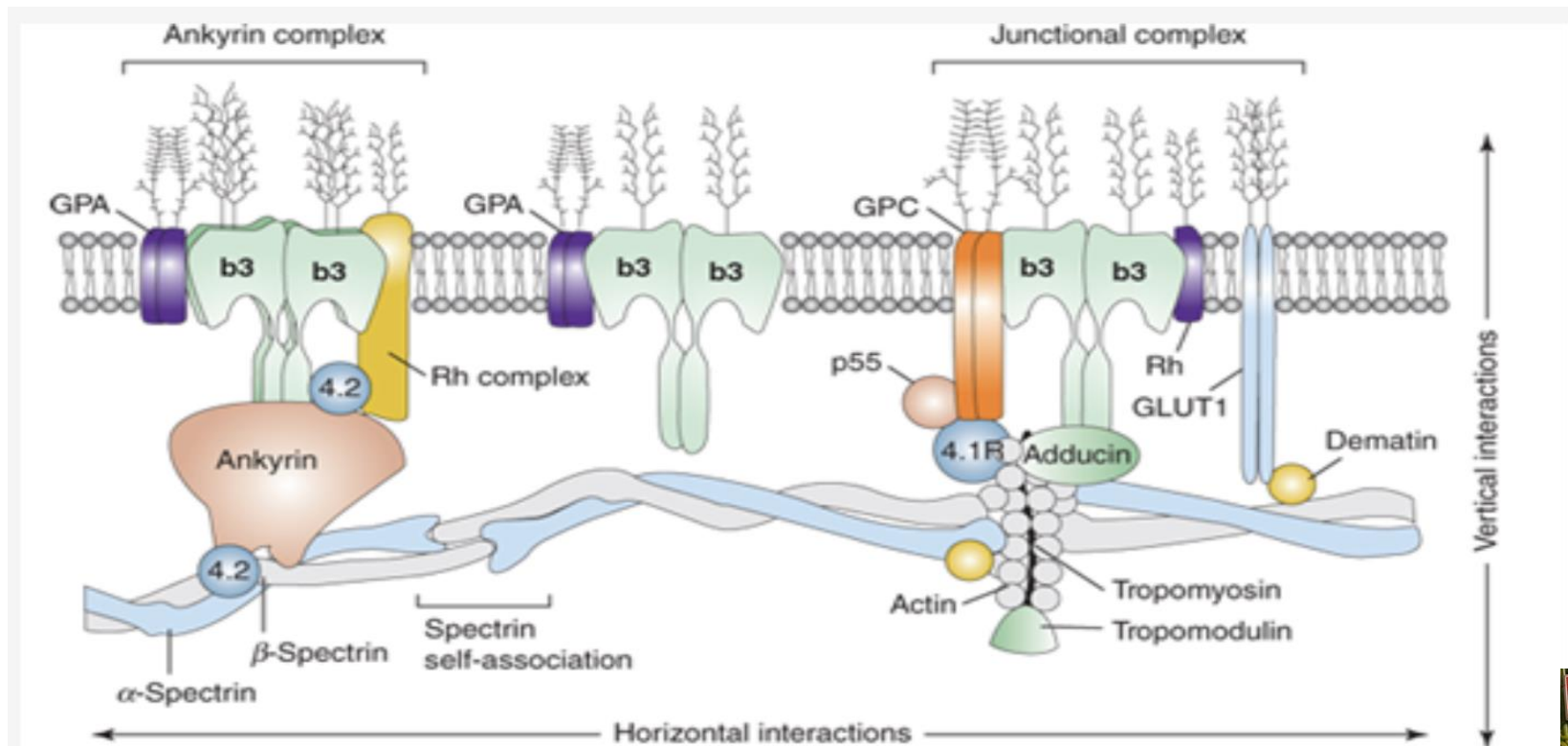
Maxwell Wintrobe – anni 1930–1950

- **Author of *Clinical Hematology* (first ed. 1942).**
- He organized and clarified the classification of hemolytic anemias, recognizing HS as a distinct hereditary entity.

Jirí Palek is one of the fathers of the modern molecular understanding of hereditary spherocytosis, thanks to fundamental contributions in:

- identification of defective proteins
- clarification of membrane pathophysiology
- creation of the genetic framework
- development of membrane analysis techniques
- connection between molecules and clinical phenotype

His work transformed HS from a simple "hereditary spherocytic anemia" to a red cell membrane disease with a defined molecular basis.



Pioneers of Red Cell Membrane Biology



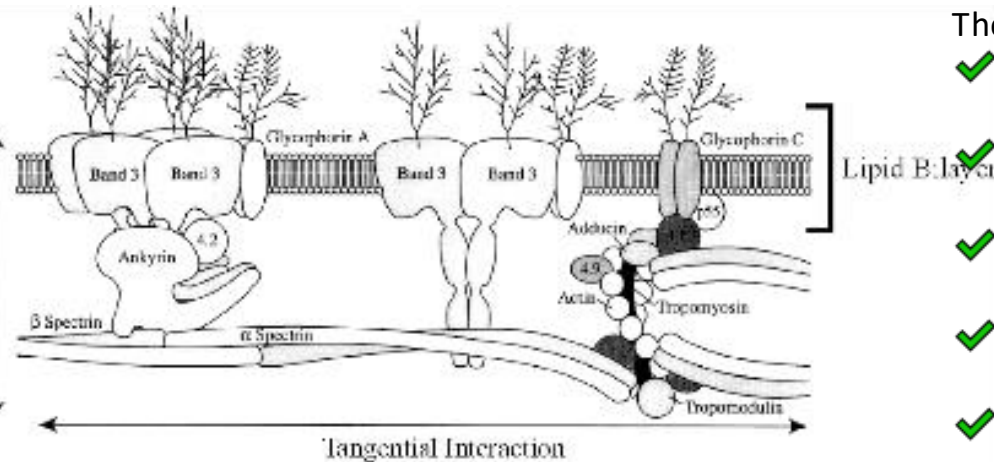
Jiri Palek



Samuel J. Lux



Bernard Forget



They contributed to:

- ✓ defining the architecture of the erythrocyte cytoskeleton
- ✓ linking molecular defects to clinical phenotypes
- ✓ clarifying how spect/ank mutations generate spherocytes
- ✓ forming the modern framework of HS pathophysiology
- ✓ creating the research foundation on which current studies are based

They are considered, pivotal figures in the transition from classical hematology to the molecular biology of spherocytosis.



Peter Agre's Role

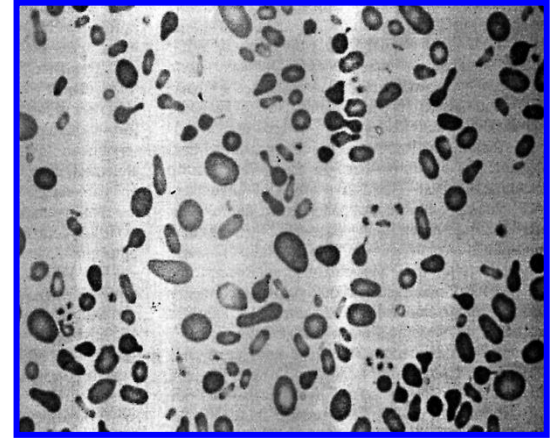
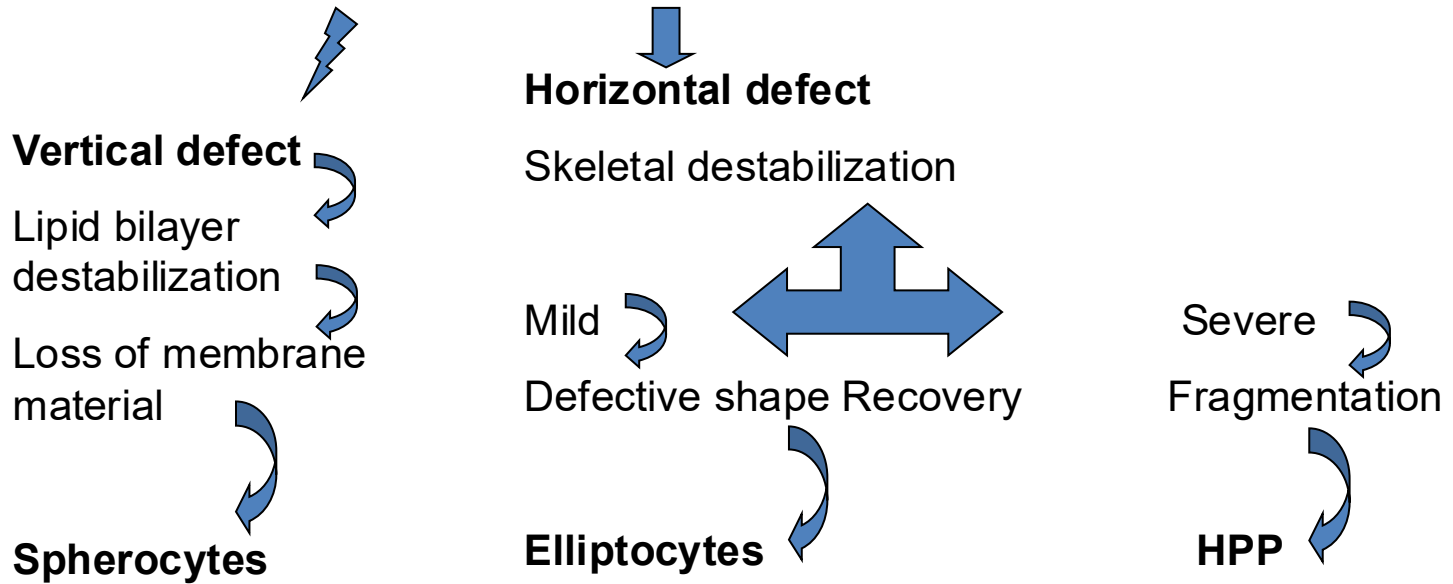
- Key Contributions Related to HS
- Discovery of aquaporin-1 (AQP1) in erythrocyte membrane
- Defined mechanisms of water transport and volume regulation
- Enhanced understanding of RBC membrane architecture
- Advanced biochemical techniques for membrane protein analysis
- Indirectly supported discovery of HS-related membrane defects



SWE-NOBEL CHEMISTRY LAUREATE
10 december 2003



Cytoskeleton and integral membrane proteins



Hereditary Spherocytosis: clinical forms and diagnosis

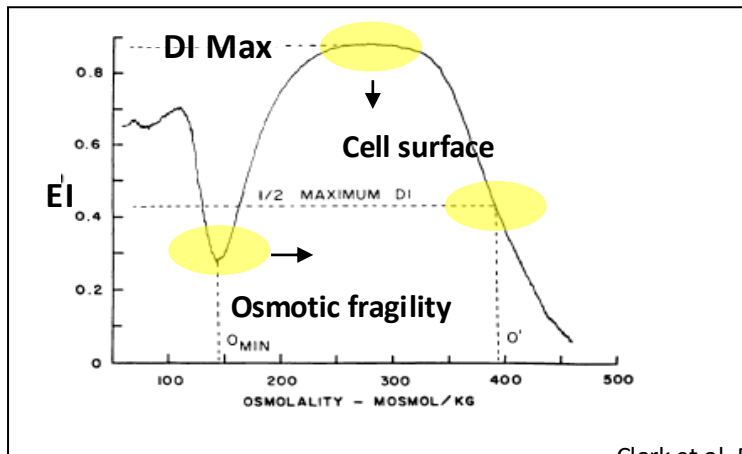
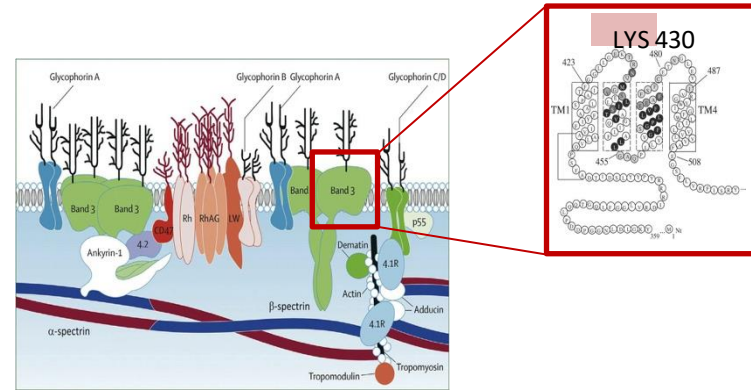
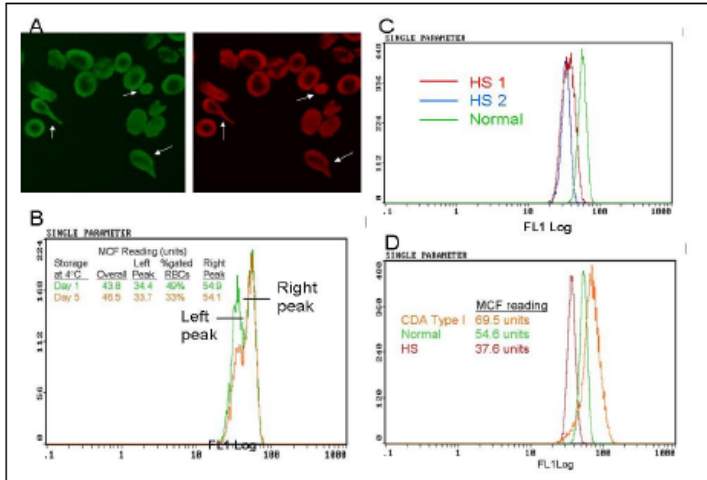
- ❖ **Mild**: enterely compensated hemolysis
- ❖ **Moderate**: anemia with Hb>80g/L
- ❖ **Moderately severe**: anemia with Hb levels ranging between 60-80 g/L
- ❖ **Severe**: transfusion-dependence
 - ❖ Anaemia
 - Increased osmotic fragility
 - especially after incubation
 - ❖ Splenomegaly
 - ❖ Jaundice
 - ❖ Reticulocytosis
 - ❖ Spherocytosis
 - ❖ Elevated MCHC
 - Normal Coombs' test
 - Inheritance
 - Dominant 75%
 - Non Dominant 25%
 - Excellent response to splenectomy



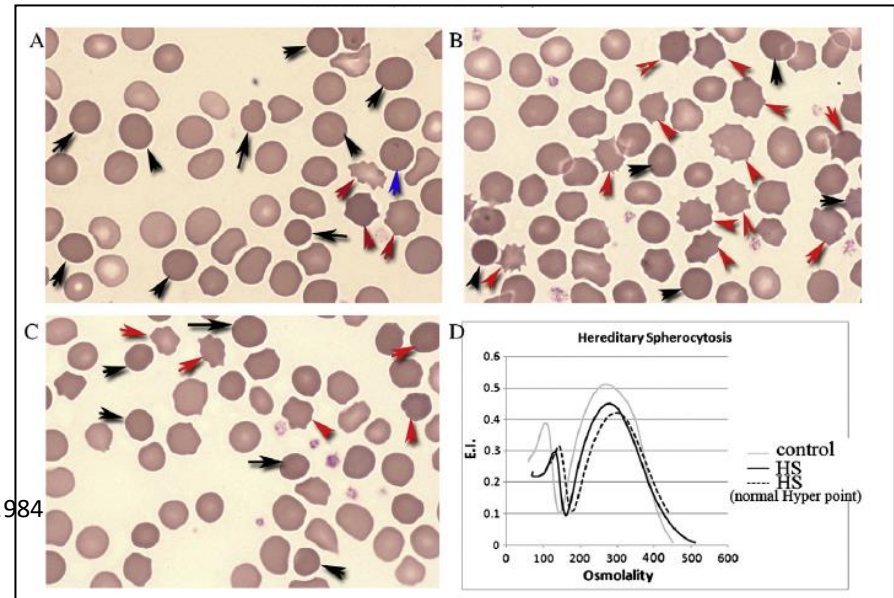


EMA-Test

Fig S5. Confocal images and histograms of EMA-labeled red blood cells
 (A) Confocal images showing both green and red fluorescing EMA-RBCs from a patient. (B) Effect of delay in sample testing on a patient sample giving a dual fluorescence peak. (C) Histograms of HS samples: HS2 gave a broader based peak than HS 1. (D) CDA type I with macrocytosis gave a peak shifted to the right of the normal control.



Clark et al, Blood 1984



Ektacytometry

Biochemical defects in hereditary spherocytosis

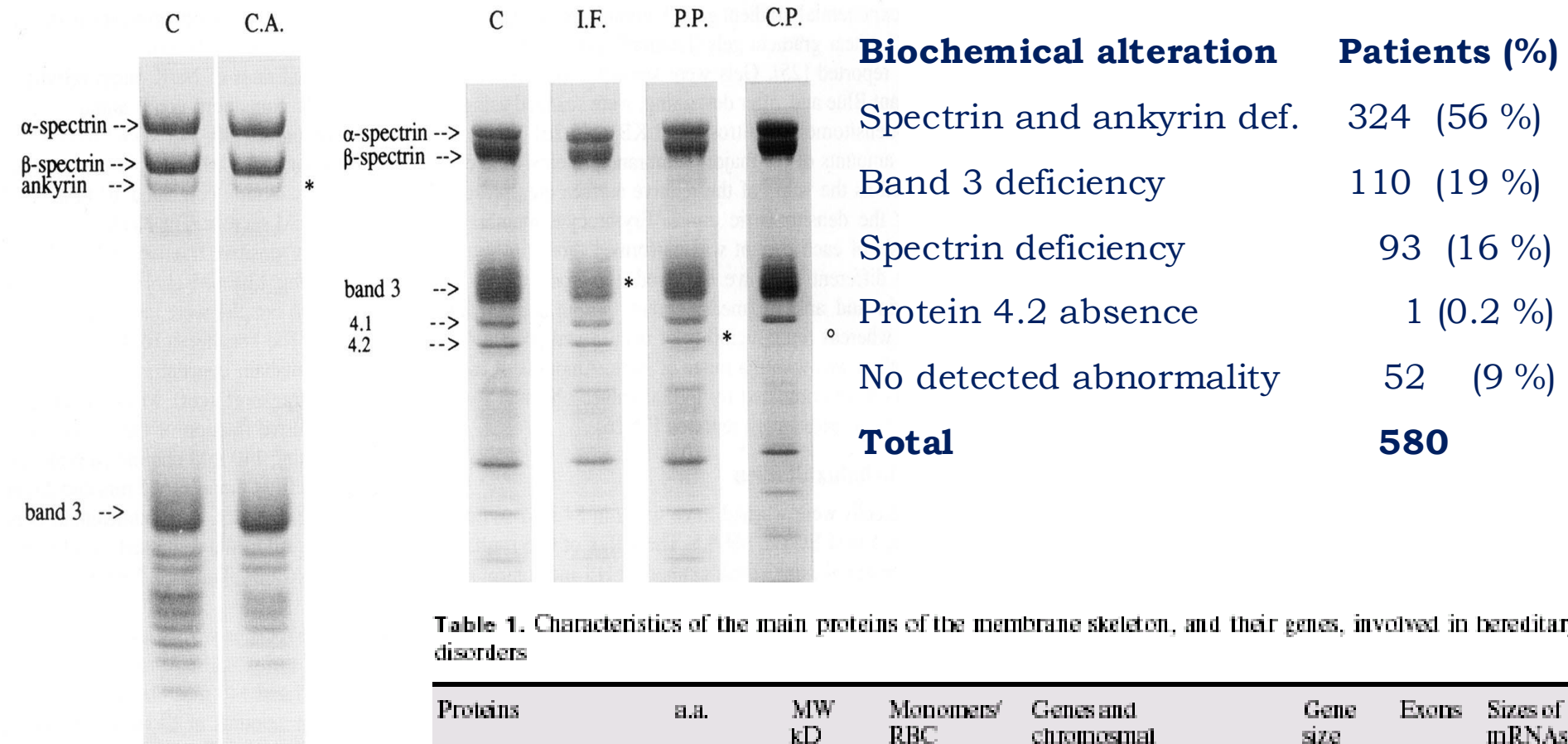


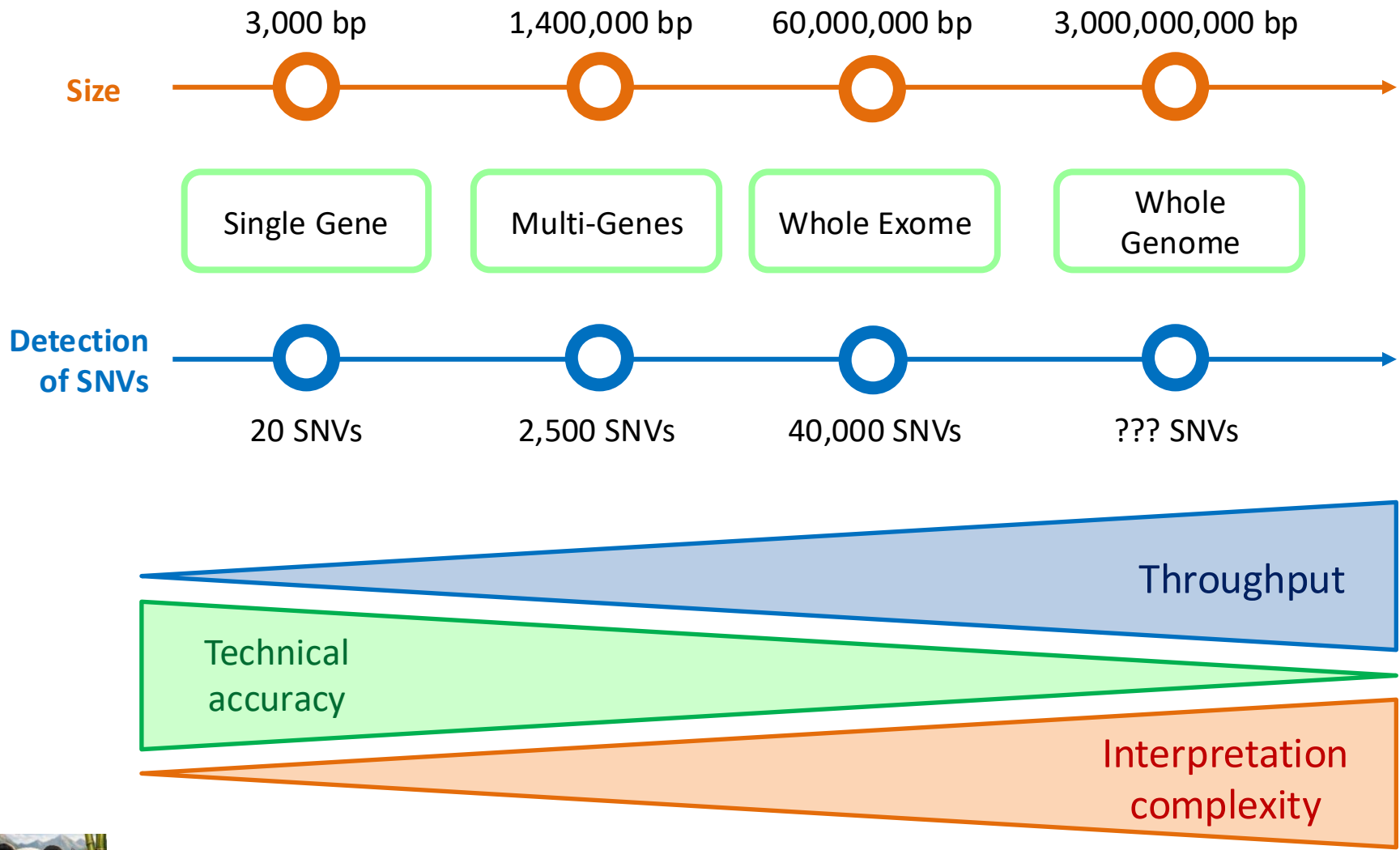
Table 1. Characteristics of the main proteins of the membrane skeleton, and their genes, involved in hereditary disorders

Proteins	a.a.	MW kD	Monomers/ RBC	Genes and chromosomal location	Gene size kb	Exons	Sizes of mRNAs kb
Spectrin α-chain	2,429	281	242,000	<i>SPTA1</i> ; 1q21	80	52	8.0
Spectrin β-chain	2,137	246	242,000	<i>SPTB</i> ; 14q23-q24.1	>100	36	7.0
Ankyrin	1,880	206	124,000	<i>ANK1</i> ; 8p11.2	>120	42	6.8; 7.2
Band 3	911	102	1,200,000	<i>SLC4A1</i> ; 17q21-q22	17	20	4.7
Protein 4.1	588	66	200,000	<i>EPB41</i> ; 1p36.2-p34	>250	>23	5.6
Protein 4.2	691	77	200,000	<i>EPB42</i> ; 15q15	20	13	2.4

a.a. = Amino acid; MW = molecular weight.



The (r)evolution of genetic testing



✓ The molecular data obtained by our t-NGS analysis confirmed the clinical suspicion in **54.2%** of patients (26/48) (**matched phenotype-genotype**)



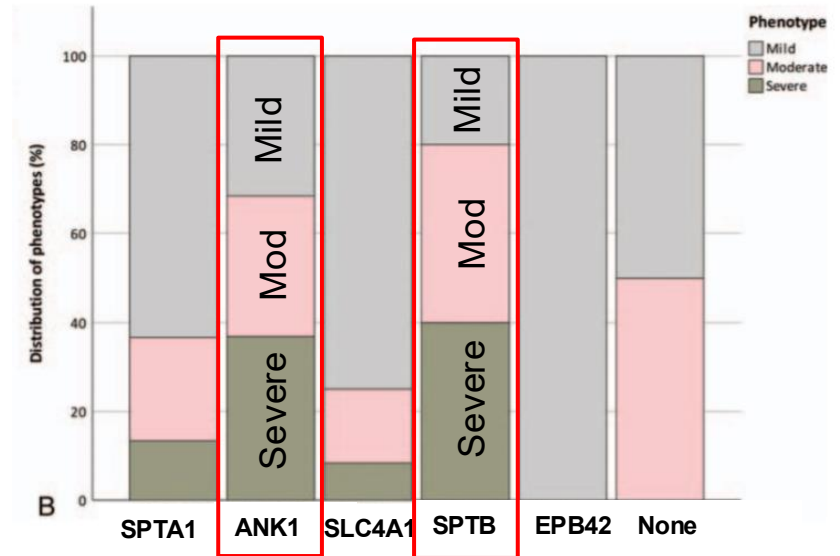
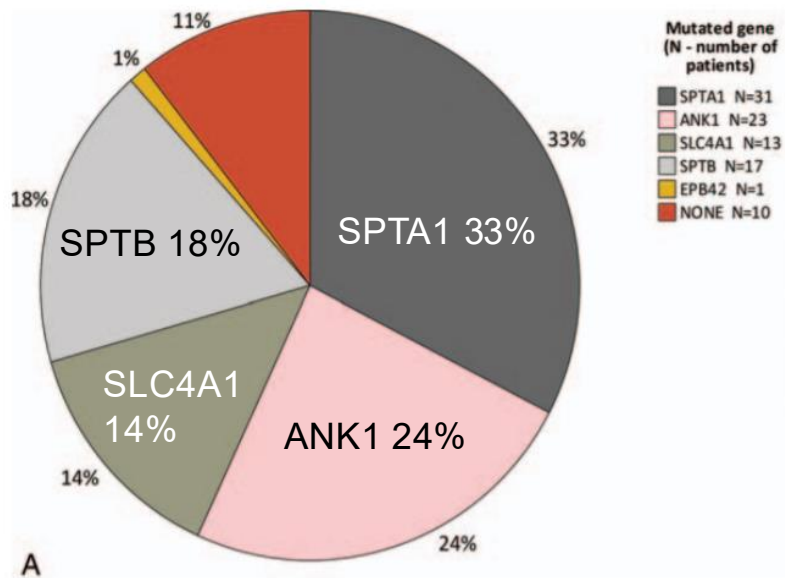
	CDA genes	HST genes	HS/HPP/HE genes	ED genes	Others	Diagnosis
	ALAS2 C15ORF41 CDAN1 GATA1 KIF23 KLF1 PARP4 SEC23B	ABCB6 ABCG5 ABCG8 ATP11C KCNNA PIEZO1 RAG SLC2A1 STOM	SLC4A1 ADD2 ANK1 EPB49 EPB41 EPB42 SPTA1 SPTB TMOD1	AK1 G6PD GCLC GPI GSS HK1 MVK NT5C3A PKG1 PKLR TPP1	CAD GBA LPIN2 SBD5	
Clinical suspicion: CDAI-II-III-IV/XLTA/XLSA	RP0_36 CDAII					CDAIIa
	RP0_38 CDA					DHS1
	RP0_39 CDAII					CDAIIb
	RP0_59 CDAI					CDAIV
	RP1_13 CDAI					PKD
	RP1_16 CDA					DHS1
	RP1_17 CDA					DHS1
	RP1_23 CDAI					PKD
	RP1_41 CDAI					XLSA
	RP1_58 CDAII					PKD
	RP1_59 CDAI-II					PKD
	RP1_60 CDA					HS
	RP1_63 CDA					DHS1
	RP1_67 CDAII					HS
	RP1_71 CDAI					GPI-D
	RP1_72 CDAI					PKD
	RP1_73 CDAI					PKD
	RP1_75 CDA					PKD
	RP1_80 CDA					PKD
Clinical suspicion: DHS1-2/OHS/CHC/FP	RP1_85 CDA					AKD
	RP1_109 CDA					HS
	RP1_112 CDA					EIEE50
	RP0_4 DHS					DHS1
	RP0_5 DHS					DHS1
	RP0_41 DHS					DHS1
	RP0_42 DHS					DHS1
	RP0_43 FP					HS
Clinical suspicion: HS/HE/HPP	RP0_45 DHS					FP
	RP1_4 DHS					DHS1
	RP1_5 DHS					DHS1/CDAII
	RP1_48 STSL					STSL
	RP1_68 DHS					CHC
	RP1_86 DHS					DHS1
	RP0_6 HPP					HPP
	RP0_9 HPP					HPP
	RP0_11 HPP					HPP
	RP0_53 HS					HS
	RP0_55 HS					HS
	RP0_60 HS					HS
	RP1_1 HS					HS
	RP1_49 HS					HS
	RP1_77 HE					SAO
	RP1_78 HE					SAO
	RP1_79 HE					SAO
	RP1_94 HS					HS
	RP1_115 HS					HS
	RP1_116 HS					HS
	RP1_119 HS					HS

- ✓ The multi-gene approach modified the original diagnosis in **45.8%** of patients (**non-matched phenotype-genotype**)
- ✓ 84.6% of patients with matched phenotype-genotype are included in the subgroup of RBC membrane defects
- ✓ **81.8%** of non-matched patients were clinically suspected to suffer from CDA

Russo R, et al. Am J Hematol. 2018

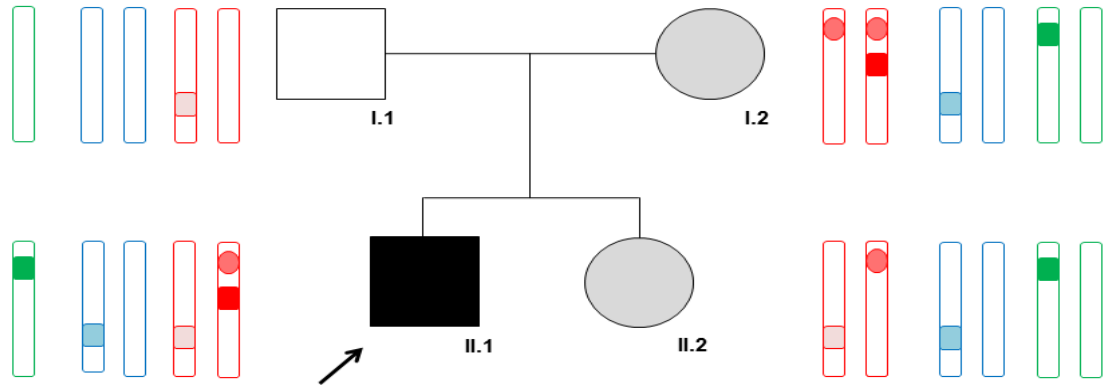


Hereditary Spherocytosis: new genotype-phenotype correlation



- ✓ **SPTA1** mutations were most frequently encountered (33%), primarily in patients with autosomal recessive forms of HS
- ✓ Moderate or severe HS was more frequent in patients with **SPTB** or **ANK1** mutations
- ✓ 30% AR – 70% AD or *denovo* variants

Polygenic contribute in monogenic disease



Hereditary Spherocytosis

- *SPTA1* mutations
- α LELY variant

Dyserythropoietic phenotype

- *SEC23B* mutation
- *GATA1* modifier variant

SPTA1 LOCUS

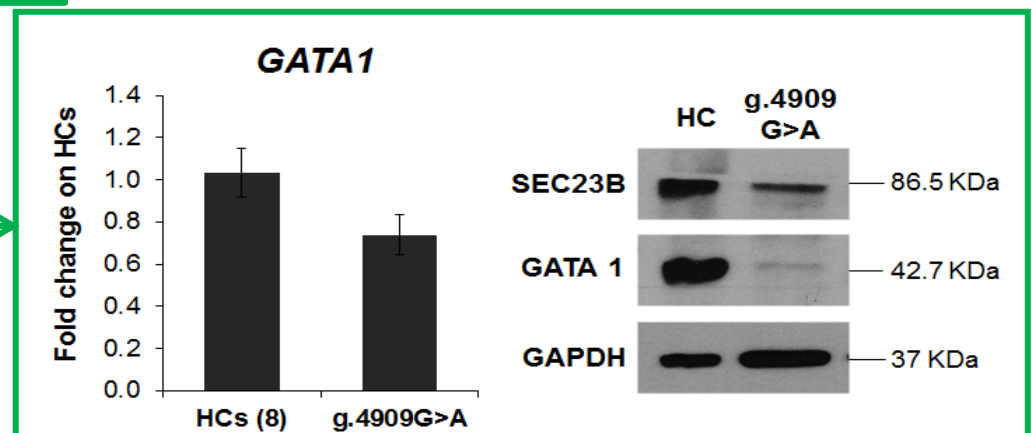
- WT
- c.5029G>A, p.Gly1677Arg
- Alpha Lely
- c.2319C>A, p.Cys773Ter

SEC23B LOCUS

- WT
- c.1254 T>G, p.Ile418Met

GATA1 LOCUS

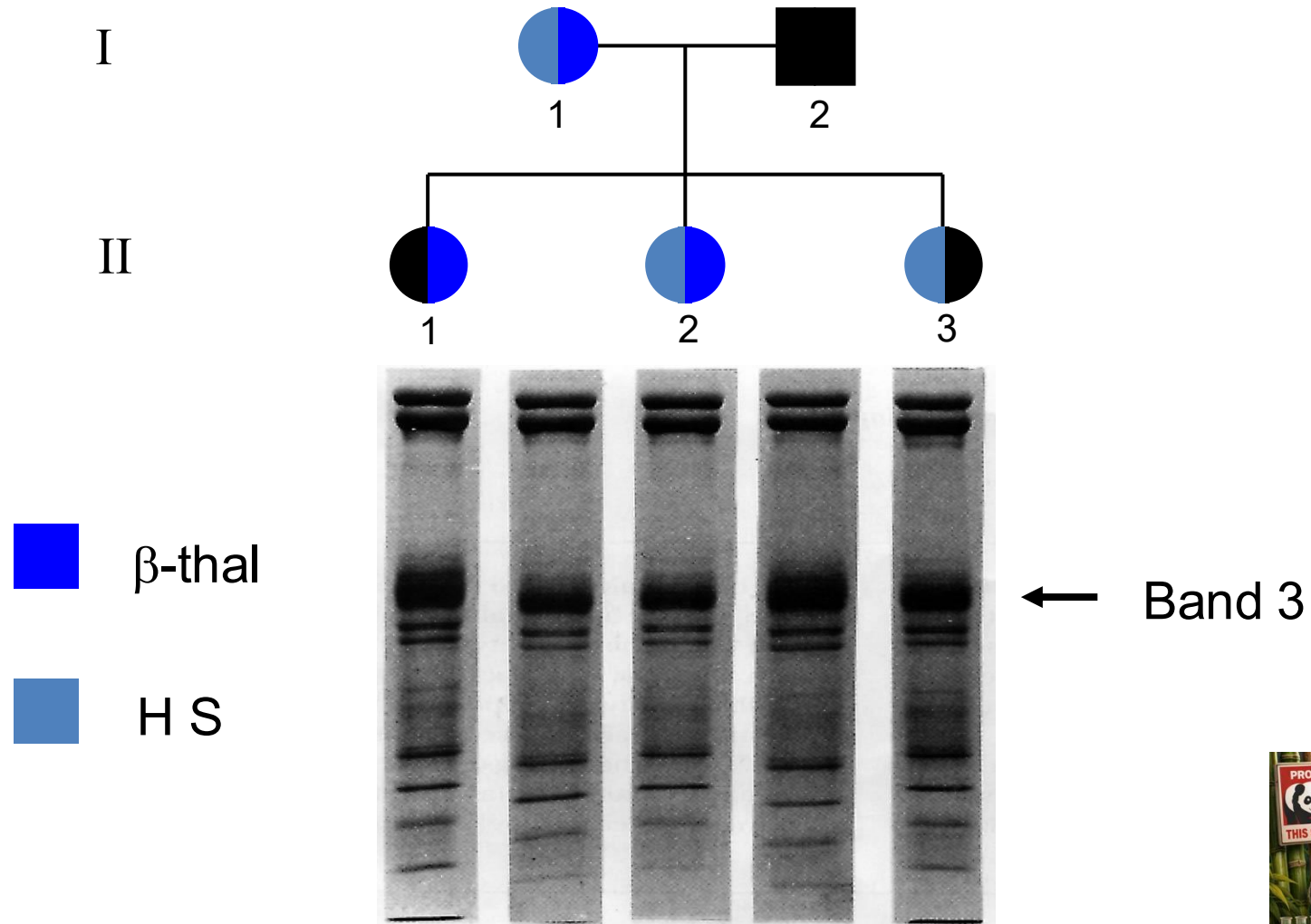
- WT
- g.4909G>A



Russo R. et al, unpublished data 2016



Hereditary Spherocytosis and β -thalassaemia



Therapy :

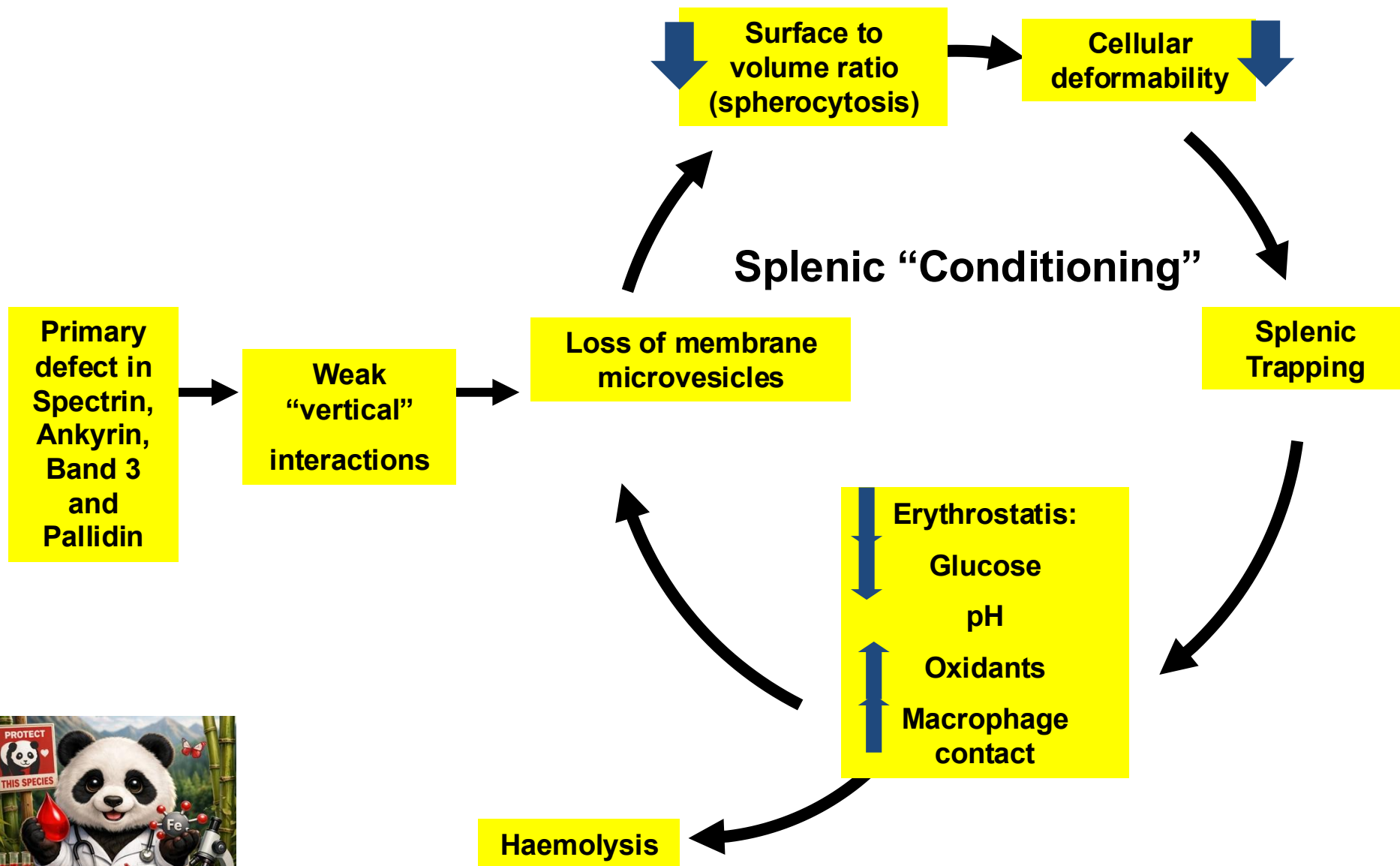
When splenectomy

Transfusion requirements : neonatal vs adult

BMT

Drugs : Mitapivat





GUIDELINE ARTICLE



Recommendations regarding splenectomy in hereditary hemolytic anemias

Achille Iolascon,^{1,2} Immacolata Andolfo,^{1,2} Wilma Barcellini,³ Francesco Corcione,⁴ Loïc Garçon,⁵ Lucia De Franceschi,⁶ Claudio Pignata,⁷ Giovanna Graziadei,⁸ Dagmar Pospisilova,⁹ David C. Rees,¹⁰ Mariane de Montalembert,¹¹ Stefano Rivella,¹² Antonella Gambale,^{1,2} Roberta Russo,^{1,2} Leticia Ribeiro,¹³ Jules-Vives-Corrans,¹⁴ Patricia Aguilar Martinez,¹⁵ Antonis Kattamis,¹⁶ Beatrice Gulbis,¹⁷ Maria Domenica Cappellini,⁸ Irene Roberts¹⁸ and Hannah Tamary¹⁹ on behalf of the Working Study Group on Red Cells and Iron of the EHA

Haematologica 2017
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Table 1. Summary of splenectomy recommendations for hemolytic disorders.

Disease	When splenectomy recommended? *
Hereditary spherocytosis	Patient is transfusion-dependent or suffers severe anemia. Patient has moderate disease: decision based on spleen size and quality of life parameters. No need to perform cholecystectomy.

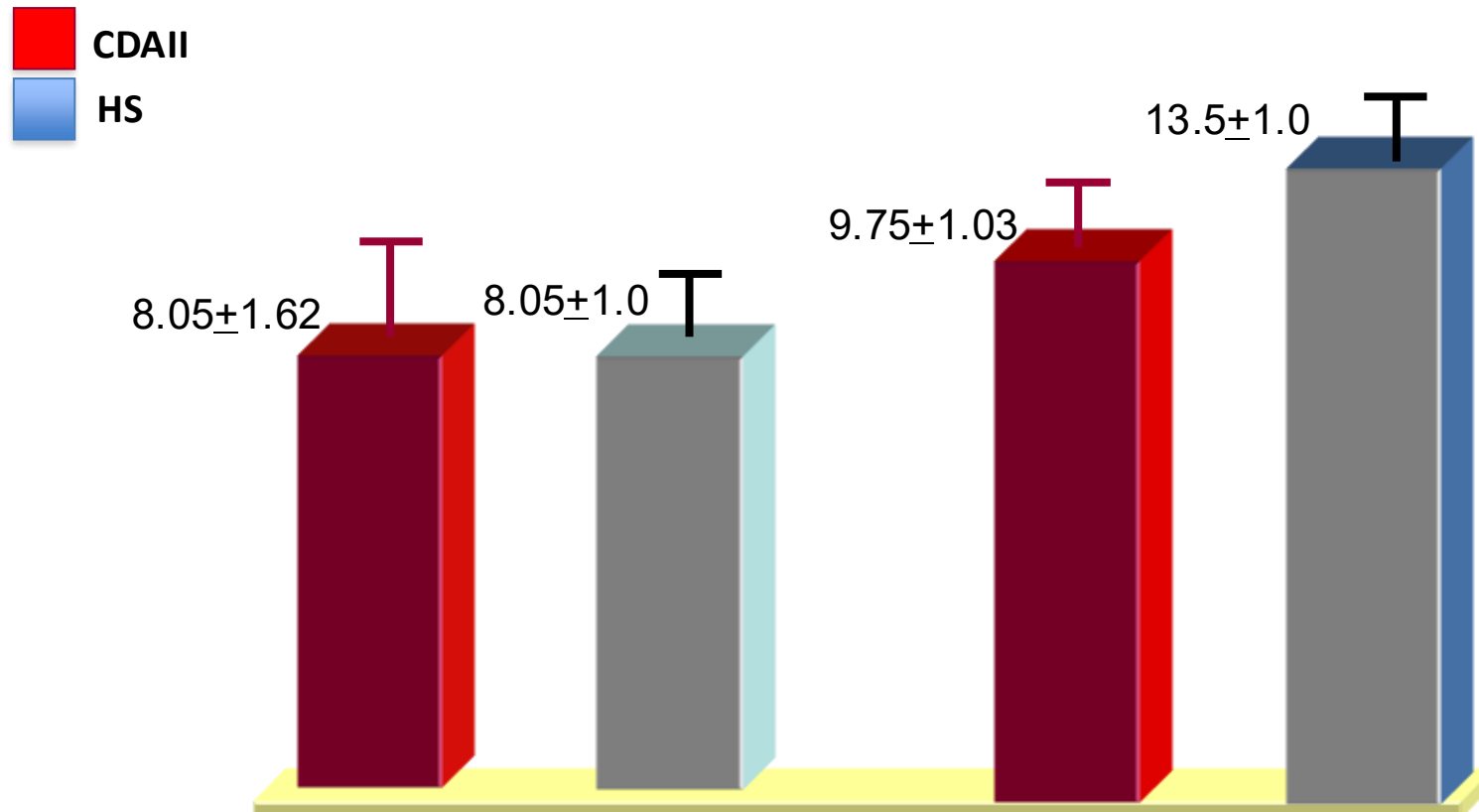
Table 2. Indications for splenectomy in hereditary spherocytosis based on severity of disease*.

Disease severity	Hemoglobin (g/dL)	Reticulocyte count (%)	Bilirubin (μmol/L)	Indication for splenectomy
Severe	< 8	> 10	> 51	Indicated, delay until age >6 years
Moderate	8 - 12	> 6	> 34	Individually tailored based on spleen size and quality of life parameters
Mild	11 - 15	3 - 6	17 - 34	Not indicated

Hemoglobin level and splenectomy



- ✓ Hb level in 19 and 35 patients affected with CDA-II and HS respectively



Splenectomy:

before

after

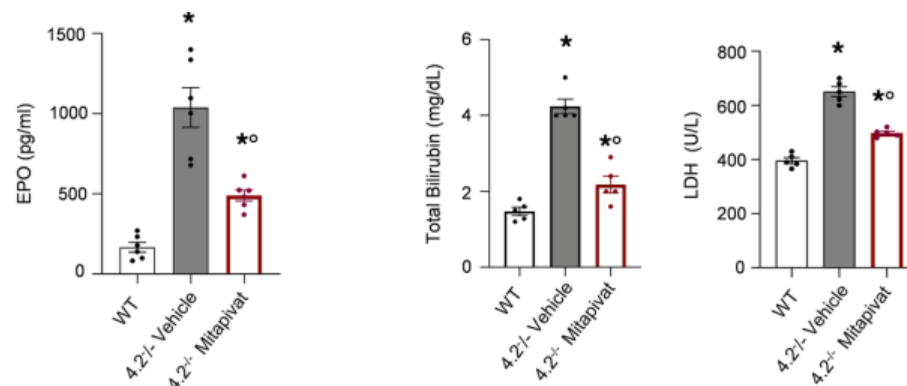
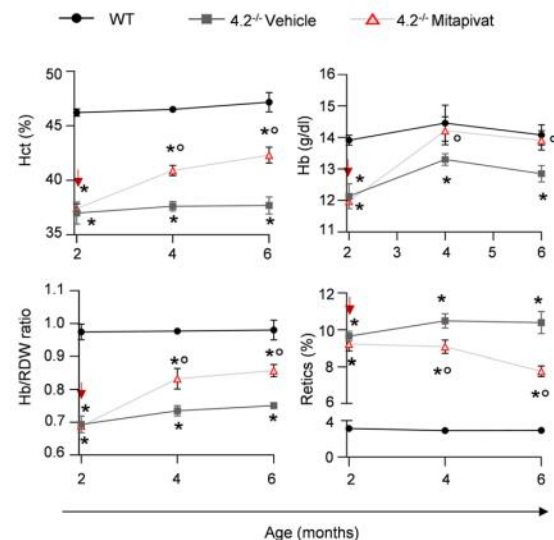
$p < 0.001$



Mitapivat reprograms the RBC metabolome and improves anemia in a mouse model of hereditary spherocytosis

Alessandro Matte,¹ Anand B. Wilson,¹ Federica Gevi,² Enrica Federti,¹ Antonio Recchiuti,³ Giulia Ferri,³ Anna Maria Brunati,⁴ Mario Angelo Pagano,⁴ Roberta Russo,^{5,6} Christophe Leboeuf,^{7,8,9} Anne Janin,^{7,8,9} Anna Maria Timperio,² Achille Iolascon,^{5,6} Elisa Gremese,^{10,11} Lenny Dang,¹² Narla Mohandas,¹³ Carlo Brugnara,^{14,15} and Lucia De Franceschi¹

- In mice genetically lacking protein 4.2 (Epb42), a murine model of HS, we showed increased expression of pyruvate kinase (PK) isoforms in RBCs and abnormalities in the glycolytic pathway
- Mitapivat, a PK activator, metabolically reprogrammed 4.2^{-/-} mouse RBCs with amelioration of glycolysis
- This resulted in improved osmotic fragility, blood count and hemolytic indices
- Mitapivat treatment beneficially affected iron homeostasis
- Splenectomy improves anemia in 4.2^{-/-} mice but mitapivat is non inferior to splenectomy





Poster Abstracts

101.Red Cells and Erythropoiesis, Excluding Iron

Safety and Efficacy of Mitapivat in Adult Patients with Erythrocyte Membranopathies (SATISFY)

Andreas Glenthøj MD PhD¹, Eduard J. Van Beers MD PhD², Richard van Wijk PhD³,
Minke A.E. Rab⁴, Evelyn Groot MSc⁵, Niels G Vejlstrup MD PhD⁶, Selma K Bendtsen MSc⁷,
Nina Toft MD PhD⁷, Jesper Petersen PhD¹, Jens Helby MD PhD¹, Fatiha Chermat⁸,
Pierre Fenaux MD PhD⁸, Kevin H.M. Kuo MD FRCPC, MSc⁹

- This investigator-initiated, **prospective, multicenter phase 2 trial** evaluates the **safety** and **efficacy** of mitapivat in adults with hereditary red blood cell membranopathies, **including hereditary spherocytosis**, and CDA II.
- Approximately **25 genetically confirmed patients with anemia** will be enrolled across centers in Denmark and the Netherlands.
- Participants will receive mitapivat starting at 50 mg twice daily, with possible escalation to 100 mg twice daily after 4 weeks, followed by two 24-week fixed-dose periods.
- The **primary objective** is to assess **safety** through treatment-emergent adverse events, while **secondary endpoints** include **improvement in hemoglobin levels, markers of hemolysis, erythropoiesis, patient-reported outcomes, and spleen size**.
- Exploratory analyses will investigate the effects of pyruvate kinase activation on **red blood cell metabolism and function**, including RBC deformability and membrane stability.

SATISFY Study (NCT05935202):

A phase 2, open-label, multicenter, single-arm trial investigating the safety and efficacy of mitapivat in adults with red blood cell membranopathies, including hereditary spherocytosis.



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Vanessa D'Onofrio
Federica Maria Esposito

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Medical Genetics Unit AOU Federico II

External collaborators

Ospedali Galliera, Genova
University of Verona
Foundation IRCCS Ca' Granda, Milan
CNR-ISASI, Naples
Hacettepe University, Ankara
Boston Children Hospital, USA

Patients and their families



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complex diseases

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