



Le anemie rare: definizione dei nuovi standard terapeutici Le anemie emolitiche ereditarie

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Sistema Socio Sanitario

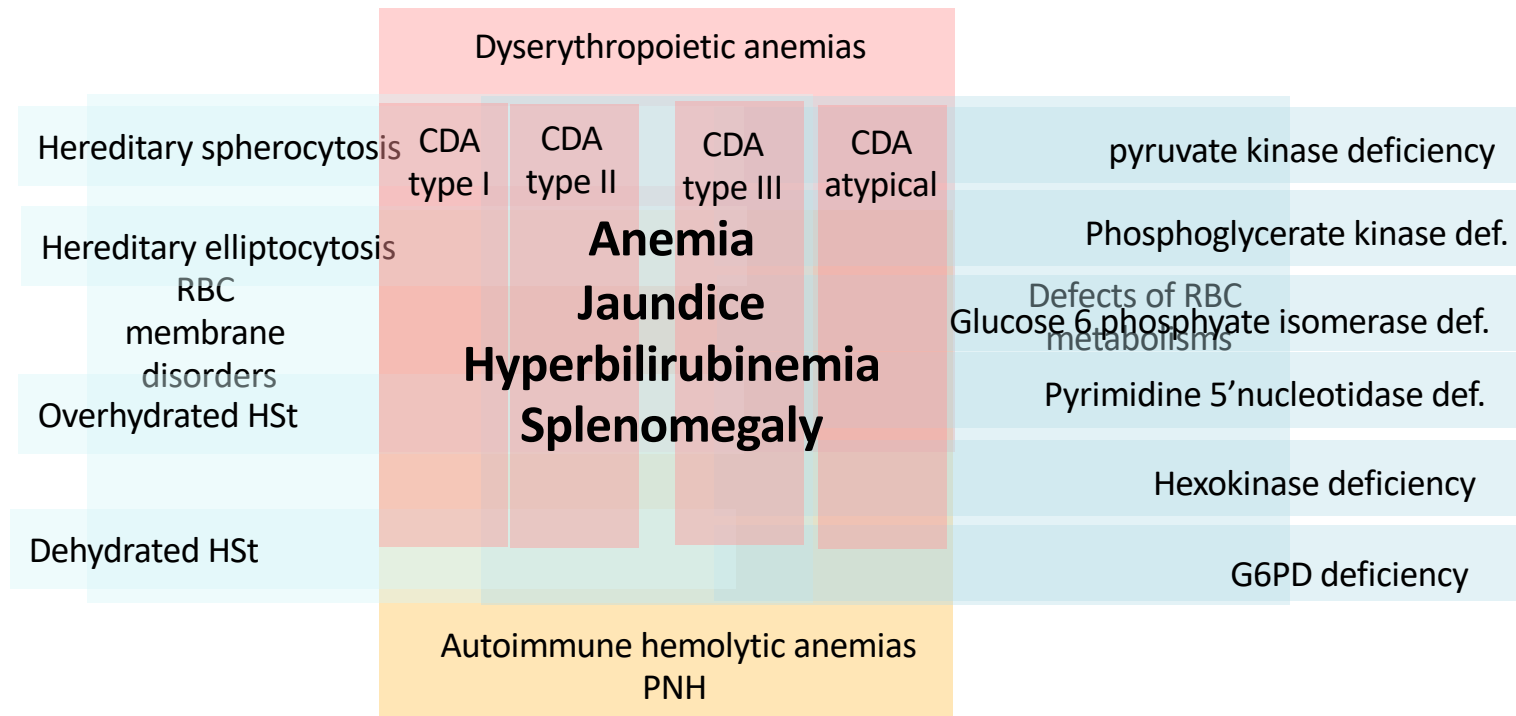


Regione
Lombardia

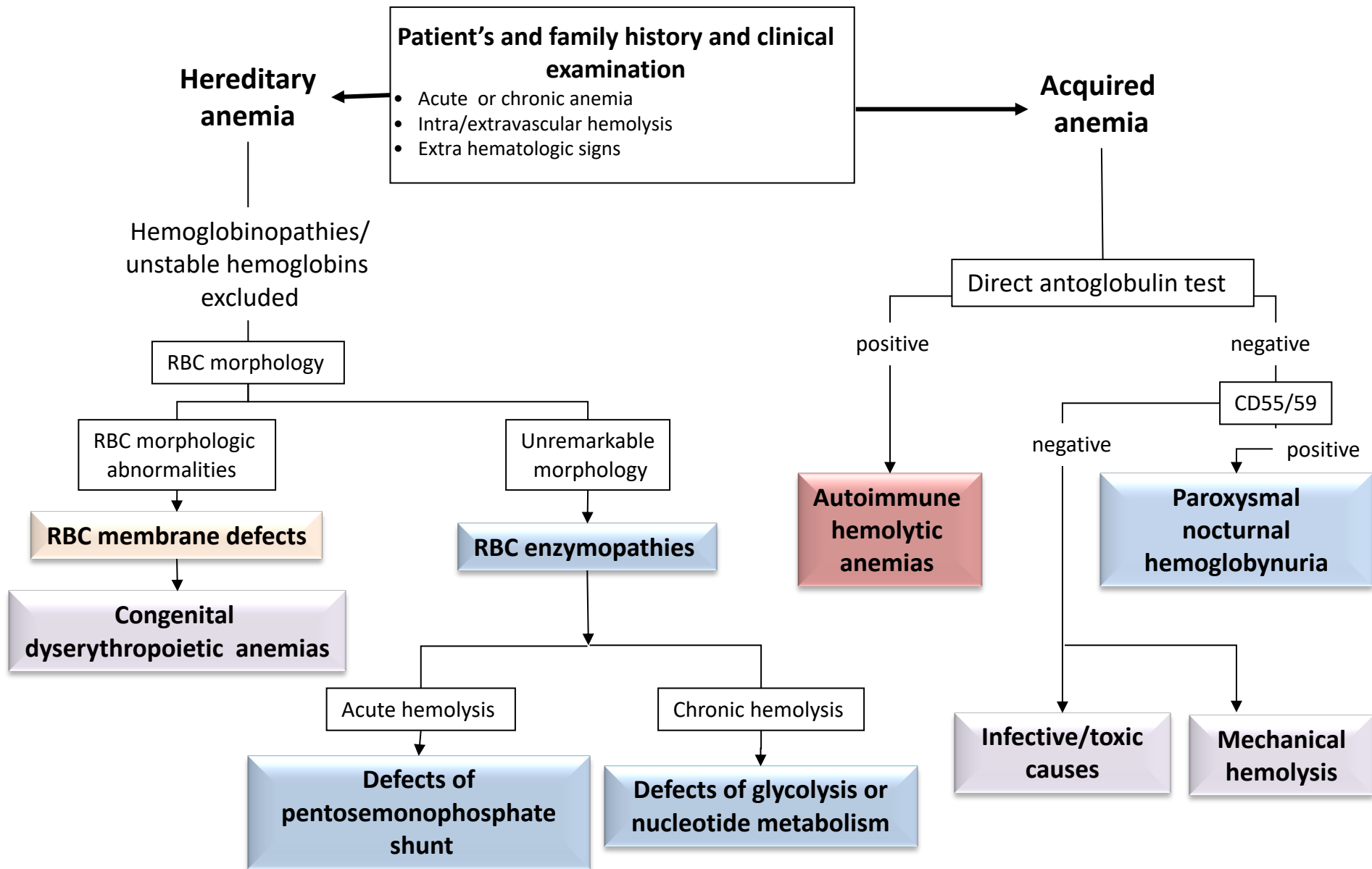
**Primo simposio sulle terapie innovative in Ematologia
Avellino, 30-31 Marzo 2023**

Hemolytic anemias:

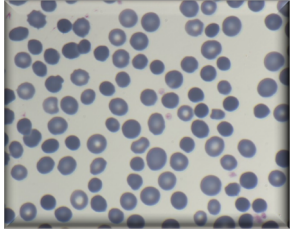
Clinical presentation and differential diagnosis



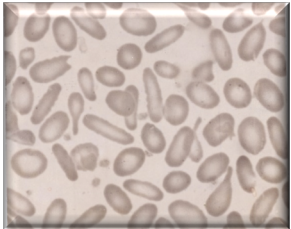
Hemolytic anemias: diagnostic flowchart



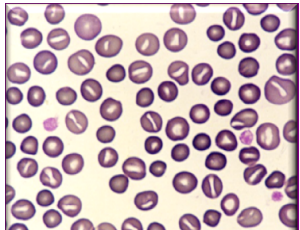
RED CELL MEMBRANE DISORDERS



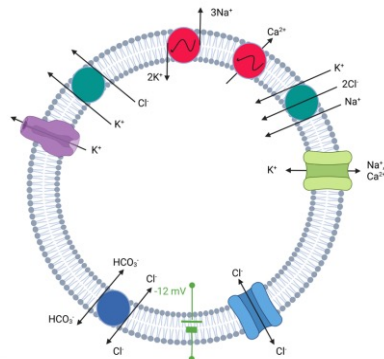
Hereditary Spherocytosis



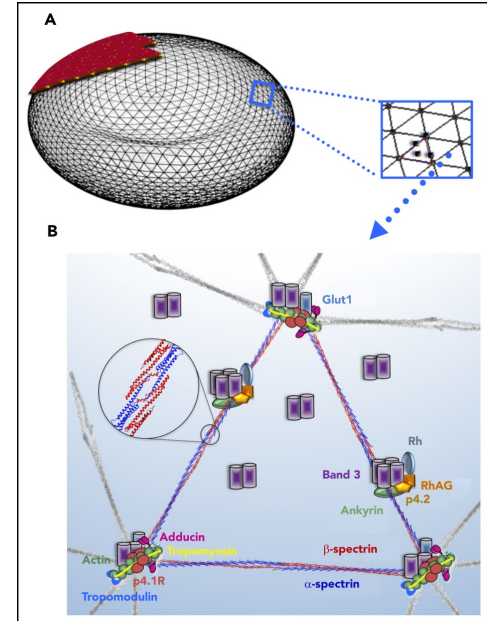
Hereditary Elliptocytosis



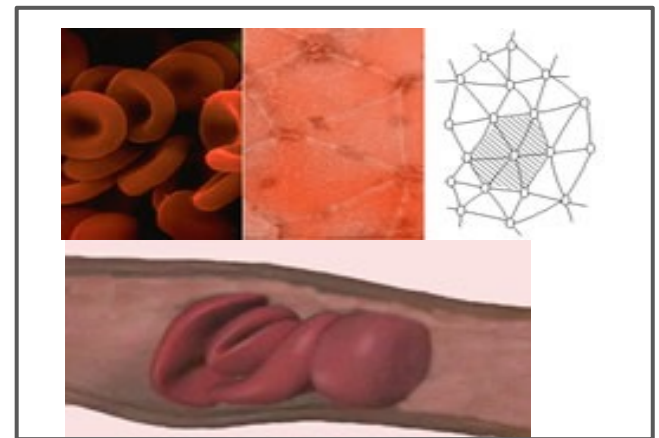
Hereditary Stomatocytosis



Jansen et al, 2021



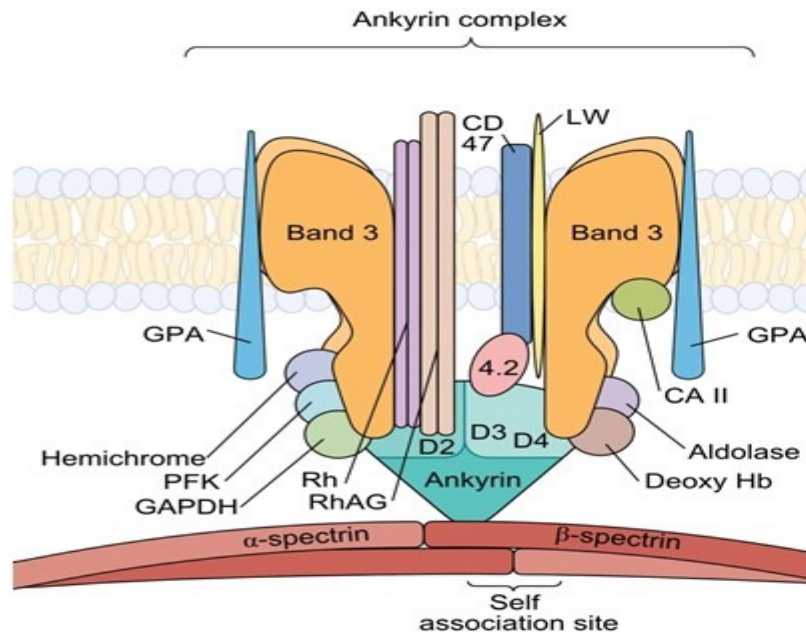
Risinger M, Blood, 2020



RBC disorders: molecular heterogeneity

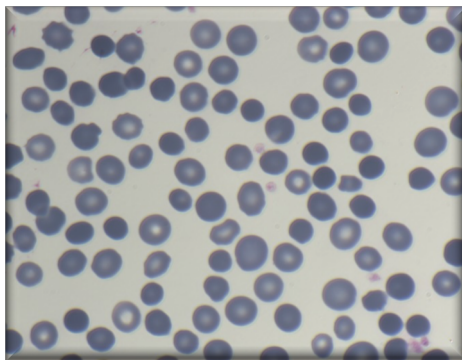
Protein	Gene	Position	Function	Phenotype
a-spectrin	SPTA1	1q23.1	Membrane skeletal network	HS HE/HPP
b-spectrin	SPTB	14q23,3	Membrane skeletal network	HS HE
Ankyrin	ANK1	8p11.21	Vertical interactions	HS
Protein Band 3	SLC4A1	17q21.31	<ul style="list-style-type: none"> •Anion exchange channel •Link to glycolytic enzymes •Vertical interactions 	HS SAO HSt
Protein 4.2	EPB42	15q15.2	Stabilize band3/ankyrin complex	HS
Protein 4.1	EPB41	1p35.3	Stabilize spectrin-ankyrin contact	HE
Glycophorin C	GYPC	2q14.3	Gerbich - blood group	HE
FAM38A	PIEZO1	16q24.3	Mechanosensitive ion channel	HX/ Polycythemia
Gardos channel KCa3.1	KCNN4	19q13.31	Potassium Calcium-Activated Channel	HSt
Rh associated Glycoprotein	RHAG	6p12.3	Rh -blood group	OHSt
GLUT1	SLC2A1	1p34.2	Glucose transporter	CHC
ABC transporter Superfam	ABCB6	2q35	Porphyrin transporter	Fam. PHYK

HEREDITARY SPHEROCYTOSIS

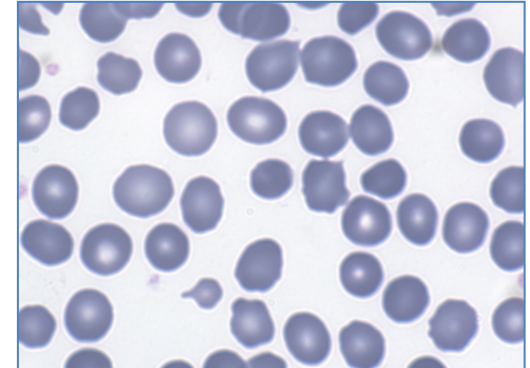
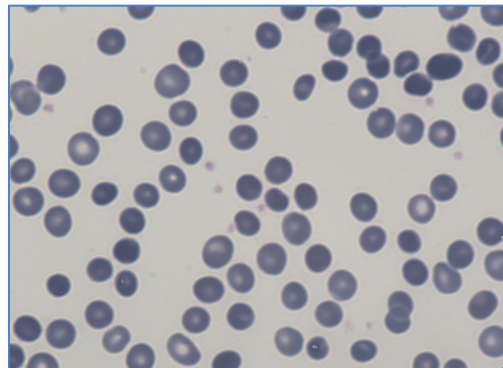


- ✓ Prevalence 1:2000
- ✓ Worldwide distribution
- ✓ Dominant/recessive transmission
- ✓ Variable severity
- ✓ Presence of spherocytes
- ✓ Genes involved SLC4A1, EPB42, SPTA1, ANK1
- ✓ Complete response to splenectomy

AIHA



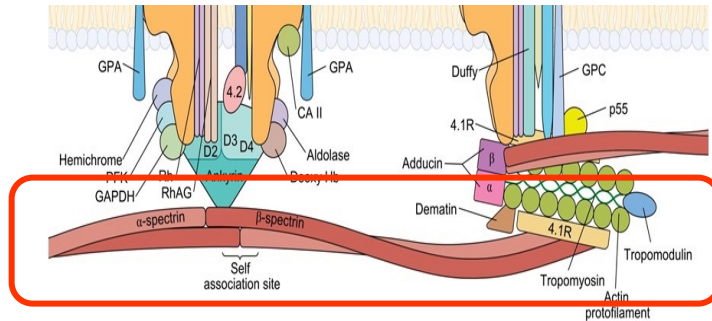
CDA



Demographic features and common complications of 446 patients with hereditary spherocytosis at diagnosis

Characteristics	At diagnosis (N = 446 ^a)
Male/female (N)	239/207
Median age at diagnosis (y, range)	22 (0.1–80)
<18 years old (n = 186)	7 (0.1–17)
≥18 years old (n = 260)	36 (18–80)
Median Follow-up (y, range)	–
Splenomegaly (N, %)	300/374 ^b (80)
Gallstones (N, %)	148/353 ^c (42)
Neonatal jaundice (N, %)	133/446 (30)
Transfused patients (N, %)	130/446 (29)
Cholecystectomy (N, %)	93/446 (21)
Splenectomized (N, %)	72/446 (16)
Exchange transfusion (N, %)	20/446 (4.5)
Aplastic crises (N, %)	21/446 (5)
Infections (N, %)	13/446 (3)
Thromboses (N, %)	4/446 (0.9)

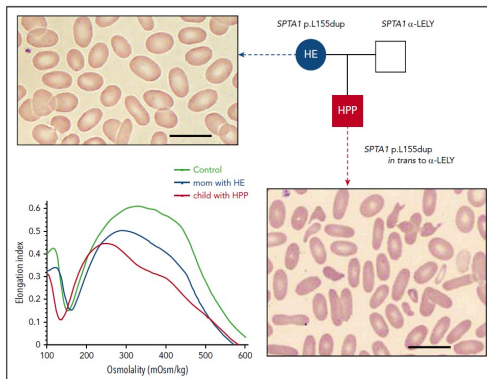
HEREDITARY ELLIPTOCYTOSIS (classical form)



- ✓ Prevalence: 1/1000 -1/4000 up to 1/50 in malaria endemic areas like west and central Africa.
- ✓ Dominant transmission
- ✓ Anemia: Compensated – Mild
- ✓ Genes involved: SPTA1, SPTB; EPB41
- ✓ No therapies required

HEREDITARY PYROPOIKILOCYTOSIS

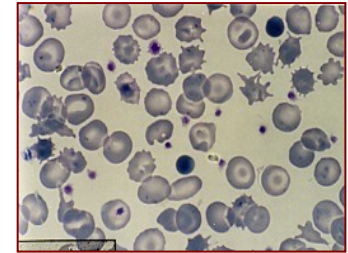
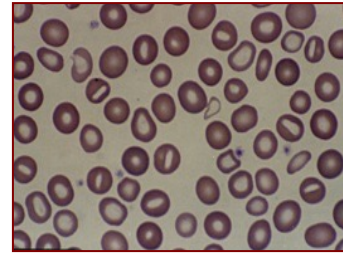
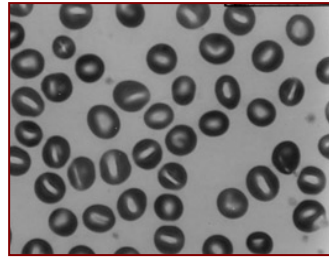
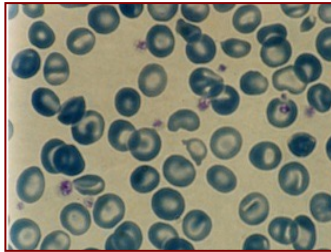
Allele α LELY SNP: c.6531-12C/T, c. 5572C>G Leu1858Val



Risinger M, Blood, 2020

- ✓ Recessive transmission
- ✓ Severe hemolytic anemia – tx dependent
- ✓ Altered morphology, mimicking heat lability
Within a family, HE and HPP may both be present
- ✓ Genes involved : SPTA1, SPTB, EPB41

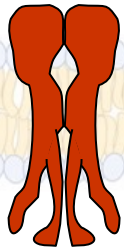
HEREDITARY STOMATOCYTOSIS



Dehydrated
Stomatocytosis



PIEZO1



KCNN4

Overhydrated
Stomatocytosis

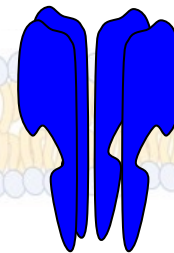


RhAG

Cryohydrocytosis SAO, Sphero-
stomatocytosis

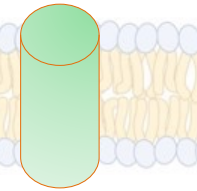


GLUT1



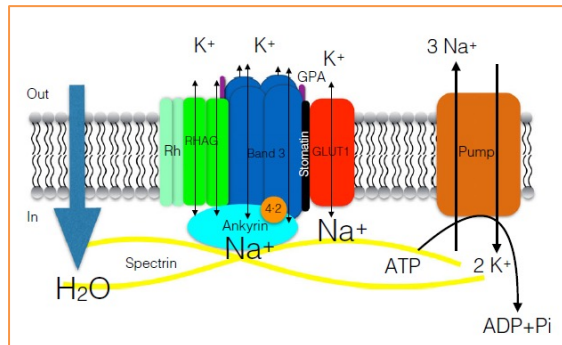
SLC4A1

Familial
pseudoperkalemia

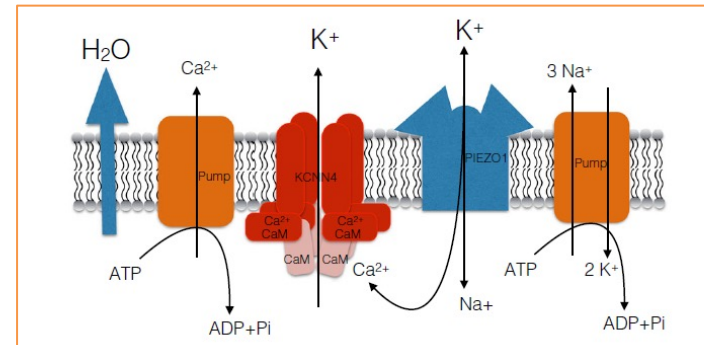


ABCB6

Overhydrated stomatocytosis

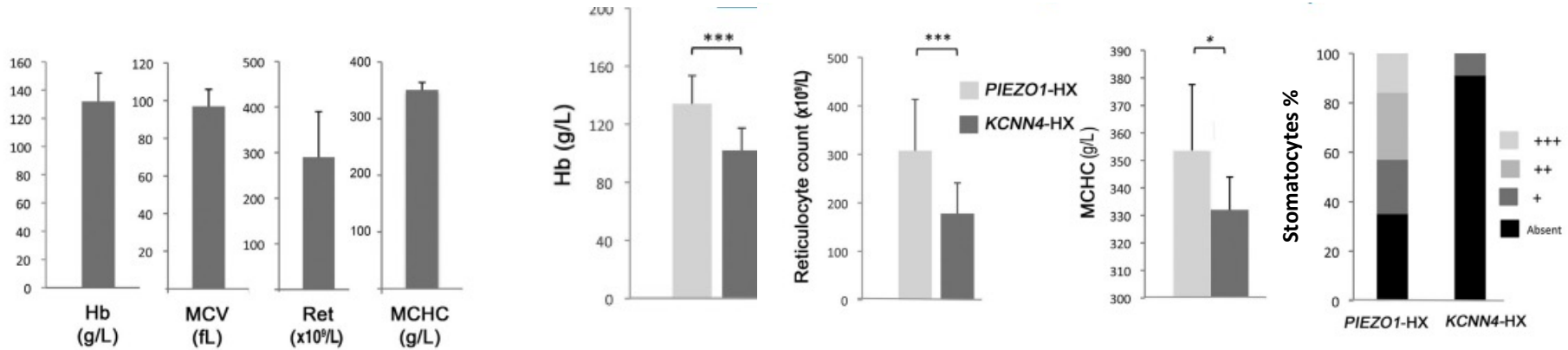


Dehydrated stomatocytosis

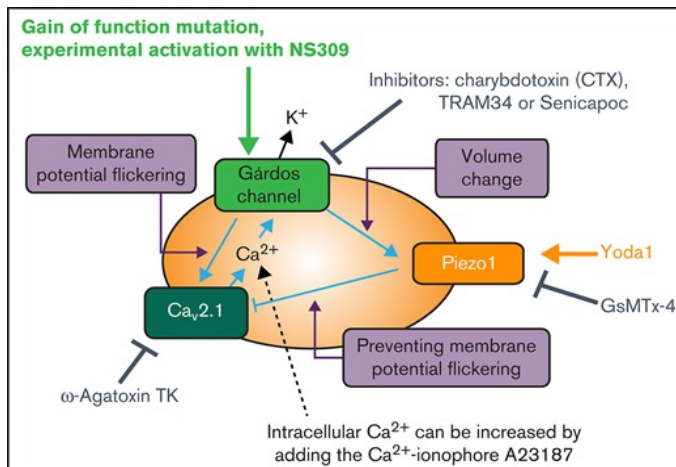


PIEZO1 variants vs KCNN4 Gardos Channelopathy

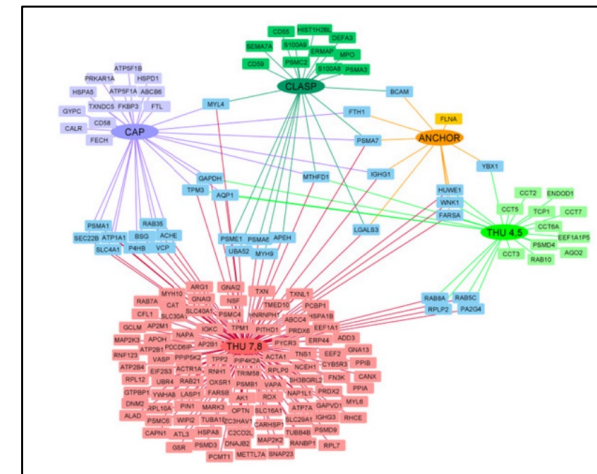
Clinical and biological features in PIEZO1-HX and Gardos channelopathy:
a retrospective series of 126 patients



Picard et al, 2019



Jansen et al, 2021



Andolfo et al, 2023

Diagnostic tools

Osmotic fragility (OF) test (Parpart et al, 1947)	68%
Acidified glycerol lysis test (AGLT) (Zanella et al, 1980)	95%
The Pink test (Vettore & Zanella, 1984)	91%
Hypertonic cryohaemolysis test (Streichman & Gescheidt, 1998)	91%
Eosin-5-maleimide (EMA) binding (King et al, 2000)	93% Se 98% Sp

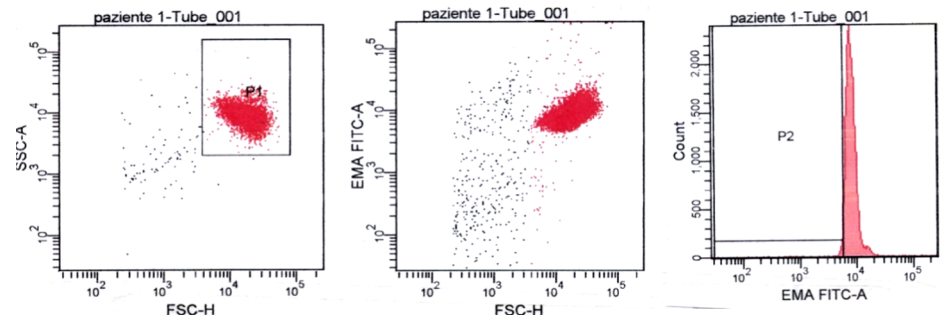
British Journal of Haematology, 2000, **111**, 924–933

Rapid flow cytometric test for the diagnosis of membrane cytoskeleton-associated haemolytic anaemia

MAY-JEAN KING,¹ JUDITH BEHRENS,² CHRIS ROGERS,³ CLARE FLYNN,⁴ DAVID GREENWOOD⁵ AND KEITH CHAMBERS⁶
¹International Blood Group Reference Laboratory, Bristol, ²Department of Haematology, St. Helier Hospital, Carshalton,
³Research and Development Support Unit, Southmead Hospital, Bristol, ⁴Department of Haematology,
St. Mary's Hospital, London, ⁵Department of Haematology, Southmead Hospital, Bristol, and
⁶Department of Haematology, Leicester Royal Infirmary, Leicester, UK

Received 12 June 2000; accepted for publication 13 July 2000

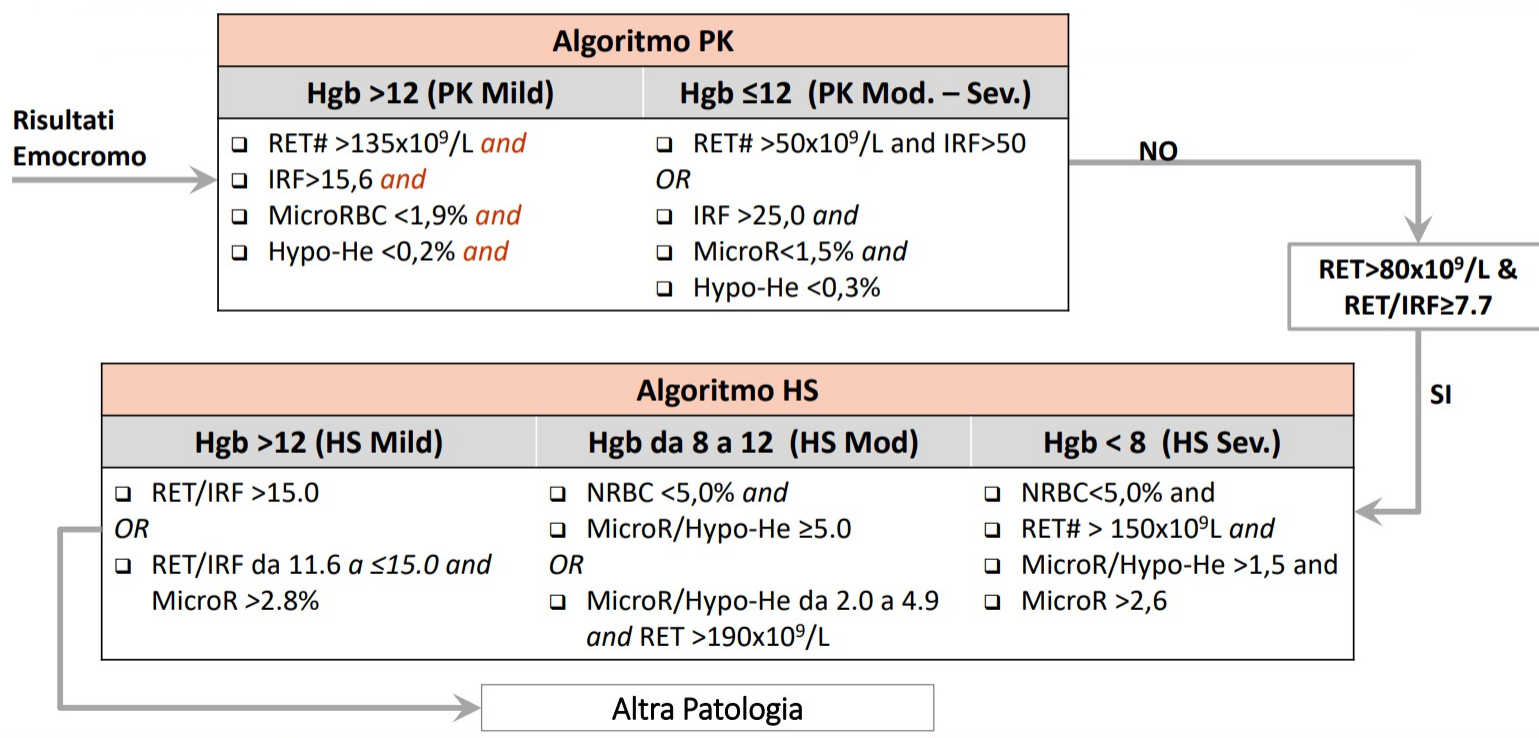
Sensitivity = 92,7%
Specificity = 99,1%.



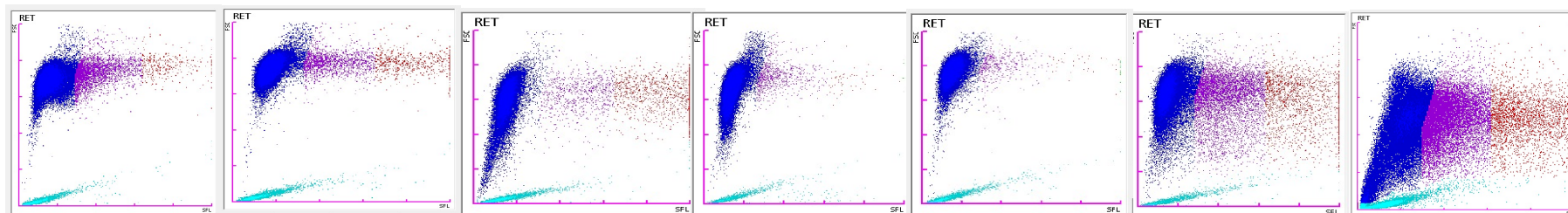
Automated red cell parameters in the prediction of congenital hemolytic anemias

M. Chiron, et al 1999	HS samples MCV < MCV <i>Mean Spherized Corpuscular Volume, assessed during the retics count procedure under hypotonic conditions)</i>	Sensitivity 100% Specificity 93.3%
Danise et al 2001	RDW/HDW ratio significantly greater in CDA II than HS CHDW/CHDW _r ratio significantly lower in CDA II than HS <i>RDW= anisocytosis; HDW= anisochromia; CHDW_r= cell Hb content of reticulocytes</i>	p<0.0002 p<0.0002
Da Costa et al 2001	Reticulocyte volume <100fL HS (except for neonates) Advia H*3 Bayer	
Brosèus, et al 2010	Delta MCV-MSCV >9.6fL Beckman coulter	
Mullier F. et al 2011	Hs screening index: RET ≥80x10 ⁹ /L and RET/IRF >7.7 Ret/IRF; %MicroR; %MicroR%/HypoHe %MicroR: % erythrocytes <60 fL; %Hypo-He: % of erythrocytes Hb<17g/dL (30HS) Sysmex XE-5000	Sensitivity 100% Specificity 99.3%
Persijn L et al 2012	Modification of Mullier algorithm (25 HS) Sysmex XE-5000	Sensitivity 100% Specificity 99%
Lazarova, et al 2014	MRV (mean reticulocyte volume) IRF; Delta MCV-MSCV Beckman Coulter	Sensitivity 100% Specificity 88%
Bobée V et al, 2018	Hs screening index: RET ≥80x10 ⁹ /L and RET/IRF >9.1 Ret/IRF; %MicroR; %MicroR%/HypoHe (47 HS, 17 PKD) Sysmex XE-5000	Sensitivity 100% Specificity 92.1%
Sottiaux JF et al, 2020	Hs screening index: RET ≥80x10 ⁹ /L and RET/IRF >7.7 Ret/IRF; %MicroR; %MicroR%/HypoHe (20 HS) Sysmex- XN	Sensitivity 94,6% Specificity 96,7%

Patologie	MILANO	Literature	TOTALE
HS	56	65	117
PKD	15		15
AEA	50		50
CDA-I, CDA-II	6		6
HSt (PIEZO1 + KCNN4)	9		9
Talassemie, HbS, HbC, HbH	118	4	122
Altre anemie emolitiche	18	29	51
TOTALE	272	94	366



Anche l'occhio vuole la sua parte.....



HS PKD an. lieve PKD an. grave AEA CDAII MDS Thal mayor

PKD

Tot. pazienti	VP	VN	FP	FN	SE	SP	VPN	VPP
366	14	348	3	1	93,3	99,1	99,7	82,3

HS

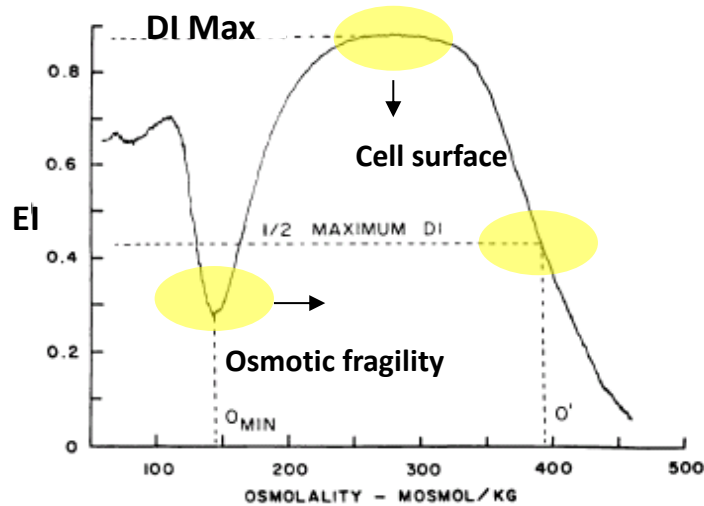
Tot. pazienti	VP	VN	FP	FN	SE	SP	VPN	VPP
366	116	232	17	1	99,1	93,2	99,6	87,2

HS

Algoritmo	VP	VN	FP	FN	SE (%)	SP (%)	VPN (%)	VPP (%)
«Milano»	116	232	17	1	99,1	93,2	99,6	87,2
<u>Mullier</u>	103	227	22	14	88,0	91,2	94,2	82,4
<u>Persijn</u>	99	230	19	18	84,6	92,4	92,7	83,9
<u>Sottiaux</u>	109	224	25	8	93,2	90,0	96,6	81,3
<u>Bobée</u>	97	199	50	20	82,9	79,9	90,9	66,0

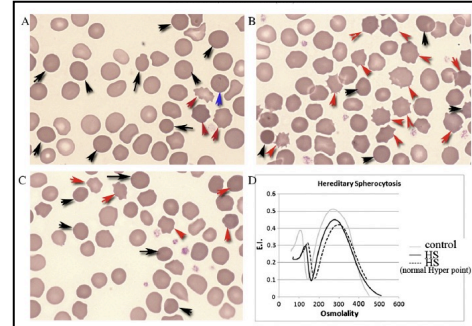


Laser-assisted Optical Rotational Cell Analyzer

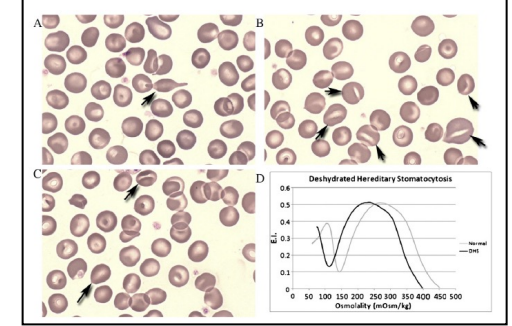


Clark et al, Blood 1984

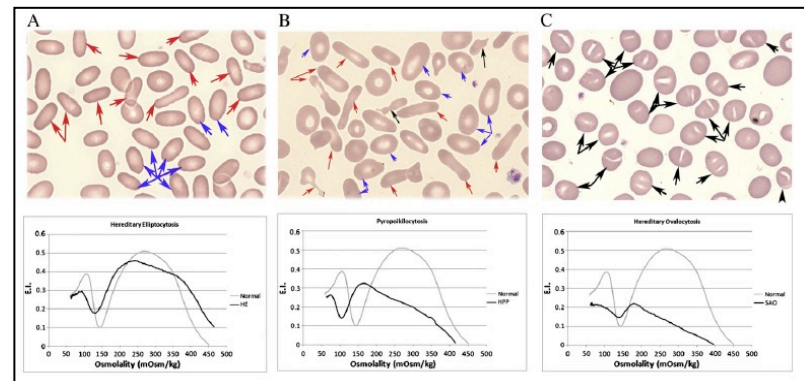
Hereditary Spherocytosis



Dehydrated Stomatocytosis



Hereditary Elliptocytosis



The diagram illustrates the Hexose Monophosphate Shunt pathway, which branches off from the main glycolysis pathway. It is divided into two main sections: EMBDEN-MEYERHOF PATHWAY and GLUTATHIONE METABOLISM.

EMBDEN-MEYERHOF PATHWAY:

- Glucose** is converted to **Glucose-6-phosphate** by the enzyme **HEXOKINASE**, using **ATP** and producing **ADP**.
- Glucose-6-phosphate** is in equilibrium with **Fructose-6-phosphate** via the enzyme **GLUCOSE-6-PHOSPHATE ISOMERASE**.
- Fructose-6-phosphate** is converted to **6-phosphogluconate** by the enzyme **PHOSPHOFRUCTOKINASE**, using **ATP**.
- Glucose-6-phosphate** is also converted to **6-phosphogluconate** by the enzyme **GLUCOSE-6-PHOSPHATE DEHYDROGENASE**, using **NADP** and producing **NADPH**.

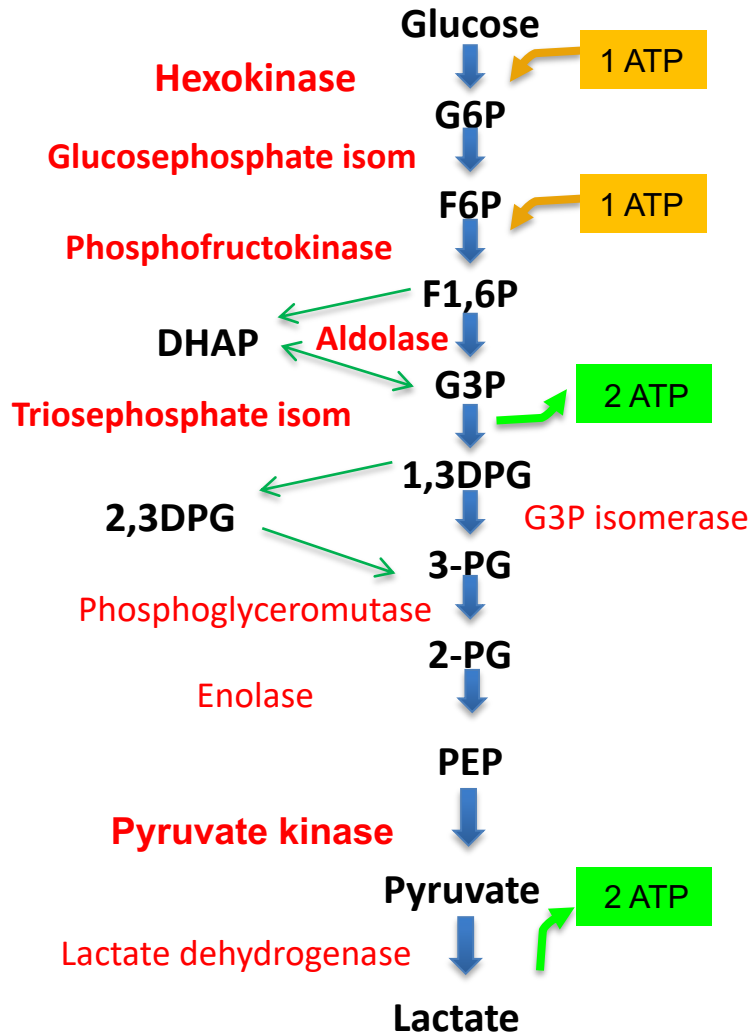
GLUTATHIONE METABOLISM:

- Glutamate + cysteine** are converted to **γ-glutamylcysteine** by the enzyme **γ-GLUTAMYL-CYSTEINE SYNTHETASE**, using **ATP** and producing **ADP**.
- γ-glutamylcysteine** is converted to **GSH** (Glutathione) by the enzyme **GLUTATHIONE SYNTHETASE**, using **ATP/glycine** and producing **ADP**.
- GSH** is converted to **GSSG** (Oxidized Glutathione) by the enzyme **GLUTATHIONE REDUCTASE**, using **NADPH** and producing **NADP**.
- GSSG** is converted back to **GSH** by the enzyme **GLUTATHIONE REDUCTASE**, using **NADPH** and producing **NADP**.
- 6-phosphogluconate** is converted to **6-phosphogluconate** (Note: This appears to be a typo in the original image, likely intended to be 6-phosphogluconate) by the enzyme **GLUTATHIONE REDUCTASE**, using **NADPH** and producing **NADP**.

Erythrocytosis

Hemolytic anemia (acute o chronic)

The Embden-Meyerof pathway



In red blood cell glycolysis is the main source of **Metabolic energy**

- To keep the iron of hemoglobin in the functional form
- To maintain intracellular ions concentration
- To protect from oxydative stress
- To maintain the red cell shape

Enzyme	Gene	Position	N. of cases	Phenotype
Embden-Meyerof pathway				
Hexokinase	<i>HK1</i>	10q22.1	20 cases	CNSHA
Glucosephosphate isomerase	<i>GPI</i>	19q13.11	>50 fam	CNSHA Mental retardation?
Phosphofructokinase	<i>PFK-M</i> <i>PFK-L</i>	12q13.11 21q22.3	~75 cases	Erythrocytosis, minimal hemolysis, Tarui disease, muscle disease
Aldolase	<i>ALDOA</i>	16p11.2	6 cases	CNSHA, mental retardation Dysmorphism
Triosephosphate isomerase	<i>TPI1</i>	12p13	~75 cases	CNSHA, neuromuscular disease, Infections
Phosphoglycerate kinase	<i>PGK1</i>	X13.3	40 cases	CNSHA, neuromuscular disease
Pyruvate kinase	<i>PKLR</i>	1q22	>500 fam	CNSHA



PK deficiency: clinical findings

The PKD Natural History Study

Baseline and retrospective data from patients with PK deficiency (N = 254)

Characteristics		All (N=254)	
		N	
Age at diagnosis (yrs, range)		243	0.4 (0-60.3)
Age at enrollment (yrs, range):	Overall	254	19.0 (0.1-69.9)
	< 18 years old	123	6.4 (0.1-17.7)
	≥ 18 years old	131	36.2(18.0-69.9)
Median number of lifetime transf. (range)		191	18 (1-516)
Splenectomized		150	59% (3.2y)
Transfusion status at Enrollment			
Historically on regular transfusions		79/198	40%
Currently on regular transfusions		23/198	12%
Intermittent transfusions only		56/198	28%
Never transfused		34/198	17%
Historical transfusions with unknown transfusion frequency		6/198	3%
<ul style="list-style-type: none">• Median rise in hemoglobin of 1.6 g/dl, reduction in the transfusion burden in 90% of cases• transfusion dependence persisted despite splenectomy in 10%• Predictors of poor response to splenectomy included: lower pre-splenectomy hemoglobin (p=0.007), higher indirect bilirubin (p=0.005), and Amish descent (p=0.001)			



PK deficiency: Genotype-Phenotype Correlation

	NM/NM, N=29 Median (Range)	M/NM, N=52 Median (Range)	M/M, N=111 Median (Range)	p-value ⁺
Age at diagnosis (years)	0.4 (0-10.9) n=29	0.7 (0-42.3) n=50	1.3 (0-60.3) n=105	0.049
Hemoglobin (g/dl)**	7.9 (6.5-8.9) n=14	8.4 (6.4-12.8) n=21	9.2 (4.3-12.3) n=40	0.003*
Total number of lifetime transfusions	65 (3-991) n=27	25 (1-721) n=38	16 (1-1915) n=81	0.0013*
Maximum ferritin (ng/ml)	1787 (423-13,409) n=22	604 (22-8,220) n=37	573 (31-9,679) n=75	<0.0001*
PK enzyme activity normalized to patient-specific normal range (%)	-41.6 (-152.4-15.2) n=18	-51.9 (-211.1-64.4) n=24	-69.6 (-485.7-117.6) n=60	0.16

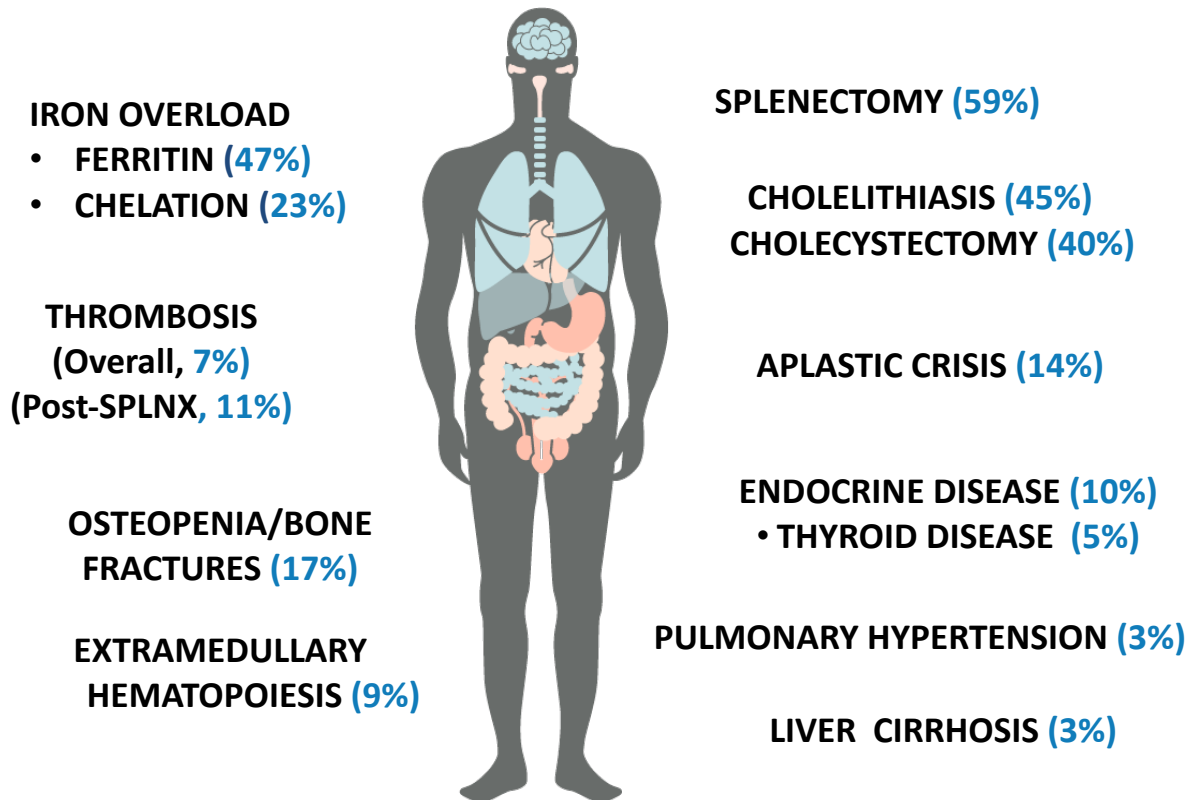
Splenectomy response

	No response (Hb <8 g/dl) n=31	Partial response (Hb 8-<11 g/dl) n=110	Complete response (Hb ≥11 g/dl) n=7	p
Genotype				
M/M	29%	59%	100%	0.0017
M/NM	32%	26%	0%	0.0005
NM/NM	39%	16%	0%	0.5

PK deficiency: complications

The PKD Natural History Study

Baseline and retrospective data from patients with PK deficiency (N = 254)

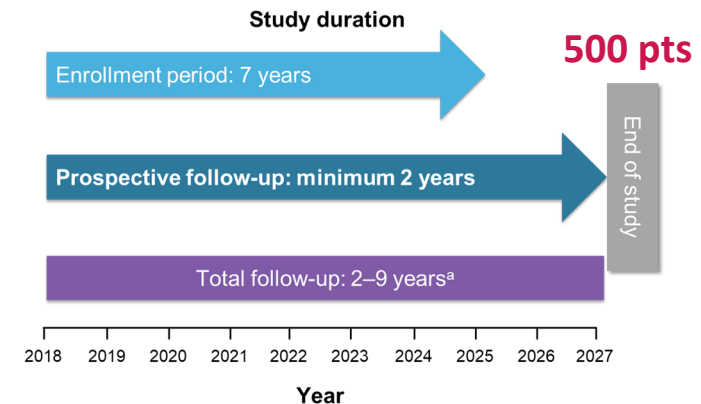


The Peak Registry

An unwavering commitment to patients with PK deficiency

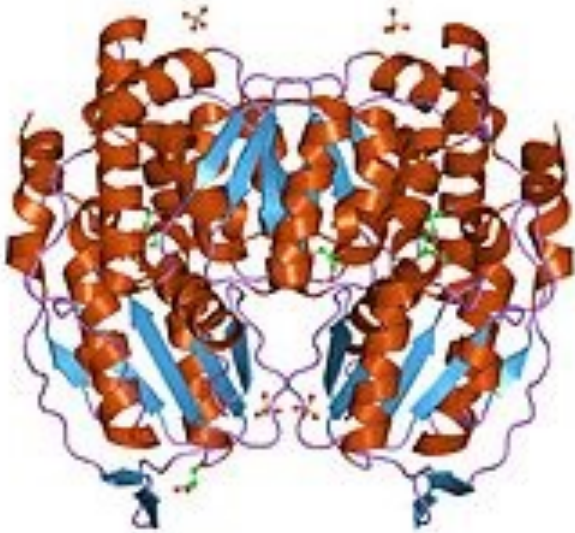
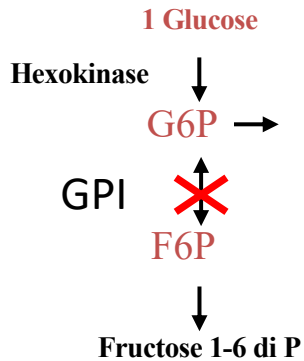
BMJ Open 2023 The Pyruvate Kinase Deficiency Global Longitudinal (Peak) Registry: rationale and study design

Rachael F Grace,¹ Eduard J van Beers,² Joan-Lluis Vives Corrons,³ Bertil Glader,⁴ Andreas Glenhøj,⁵ Hitoshi Kanno,⁶ Kevin H M Kuo,⁷ Carl Lander,⁸ D Mark Layton,⁹ Dagmar Pospíšilová,¹⁰ Vip Viprakasit,¹¹ Junlong Li,¹² Yan Yan,¹² Audra N Boscoe,¹² Chris Bowden,¹² Paola Bianchi¹³



Congress	Citation
ASH, 2022	Grace, R. F., Glenhøj, A., Lander, C., van Beers, E. J., Kanno, H., Vives Corrons, J. L., ... & Glader, B. (2022). Comorbidities and Complications in Pediatric Patients with Pyruvate Kinase Deficiency Enrolled in the Peak Registry. <i>Blood</i> , 140 (Supplement 1), 5316-5318.
ASH, 2022	Glenhøj, A., Grace, R. F., van Beers, E. J., Vives Corrons, J. L., Glader, B., Kuo, K. H., ... & Bianchi, P. (2022). Age of Onset of Complications in Patients with Pyruvate Kinase Deficiency: Analysis from the Peak Registry. <i>Blood</i> , 140 (Supplement 1), 5323-5325.
EHA, 2022	Glenhøj, A., Grace, R. F., van Beers, E. J., Corrons, J. L. V., Glader, B., Kuo, K. H., ... & Bianchi, P. (2022). P1542: Comorbidities and Complications across Genotype in Adult Patients with Pyruvate Kinase Deficiency: Analysis from the Peak Registry. <i>HemaSphere</i> , 6 (Supplement 3), P1542.
ASH, 2021 (Encore: EHA, 2022)	Bianchi, P., Grace, R. F., Corrons, J. L. V., Glader, B., Glenhøj, A., Kanno, H., ... & Van Beers, E. J. (2021). Characterizing Iron Overload By Age in Patients Diagnosed with Pyruvate Kinase Deficiency- Descriptive Analysis from the Peak Registry. <i>Blood</i> , 138 (Supplement 1), 3074.
EHA, 2021 (Encore: SFH, 2021)	Bianchi, P., van Beers, E. J., Vives Corrons, J. L., Glader, B., Glenhøj, A., Kanno, H., ... & Grace, R. F. (2021). Baseline Characteristics by age of a Global Cohort of Patients Diagnosed with Pyruvate Kinase Deficiency-A Descriptive Analysis from the Peak Registry. <i>HemaSphere</i> 5 (Supplement 2), P e566.
ASH, 2020	Grace, R. F., Boscoe, A., Bowden, C., Glader, B., Kanno, H., Layton, D. M., ... & Bianchi, P. (2020). Baseline Characteristics of Patients in Peak: A Global, Longitudinal Registry of Patients with Pyruvate Kinase Deficiency. <i>Blood</i> , 136 (Supplement 1), 39-40.

GPI : dual functional protein



1- homodimer, catalyzes the conversion from G6P to F6P

2- monomer secreted by leucocytes

Neuroleukine : lymphokine,
neurotrophic factor

- Mutants that disrupt the dimerization interface cause loss of catalytic activity: chronic anemia, T195I, T224M, R347C, R347H, T375R, L487F, E495K, I525T, and D539N;

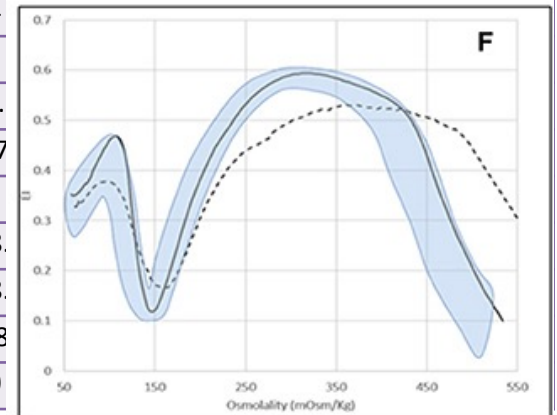
- Mutants that disrupt Monomer Folding are accompanied by neuro disorder: H20P, L339P(exon 1 and 12 mutants)

Kugler et al. 1998

Glucose6-phosphate isomerase deficiency

- ✓ GPI deficiency is the second most common erythro-enzymopathy of glycolysis, after PKD (100 cases).
- ✓ Patients are affected by chronic non-spherocytic hemolytic anemia of variable severity; in rare cases, intellectual disability or neuromuscular symptoms have also been reported.
- ✓ Molecular heterogeneity: about 60 causative mutations identified.
- ✓ **Splenectomy does not always result in the amelioration of anemia** but may be considered in transfusion-dependent patients to reduce transfusion intervals

Pt	Age	Sex	Transfusion	Splenect. (age)	Colecyst. (age)	Hb g/dL pre	Hb g/dL Post splenect.	SFerritin (ng/mL)
1	2	M	Occasional	No	No	6.1-10.2	—	n.a.
2	6	F	Occasional	No	No	6.2-11.6	—	n.a.
3	40	M	Occasional	Yes (9)	No	n.a.	11.5	2356
4	8	F	Occasional	Yes (7)	Yes (7)	9.4		
5	1	M	Occasional	No	No	10		
6	51	F	Occasional	Yes (17)	Yes (18)	n.a.		
7	3	M	Occasional	No	No	11.7		
8	1	M	Regular (4w)	No	No	8.5		
9	18	F	Regular* (4w)	Yes (6)	Yes	5.4-8		
10	23	M	Regular* (4w)	Yes (3)	Yes	2.7-8		
11	18	M	No	No	No	10.8		
12	46	M	No	Yes (45)	No	8.0		



Fermo et al Front Physiol 2019



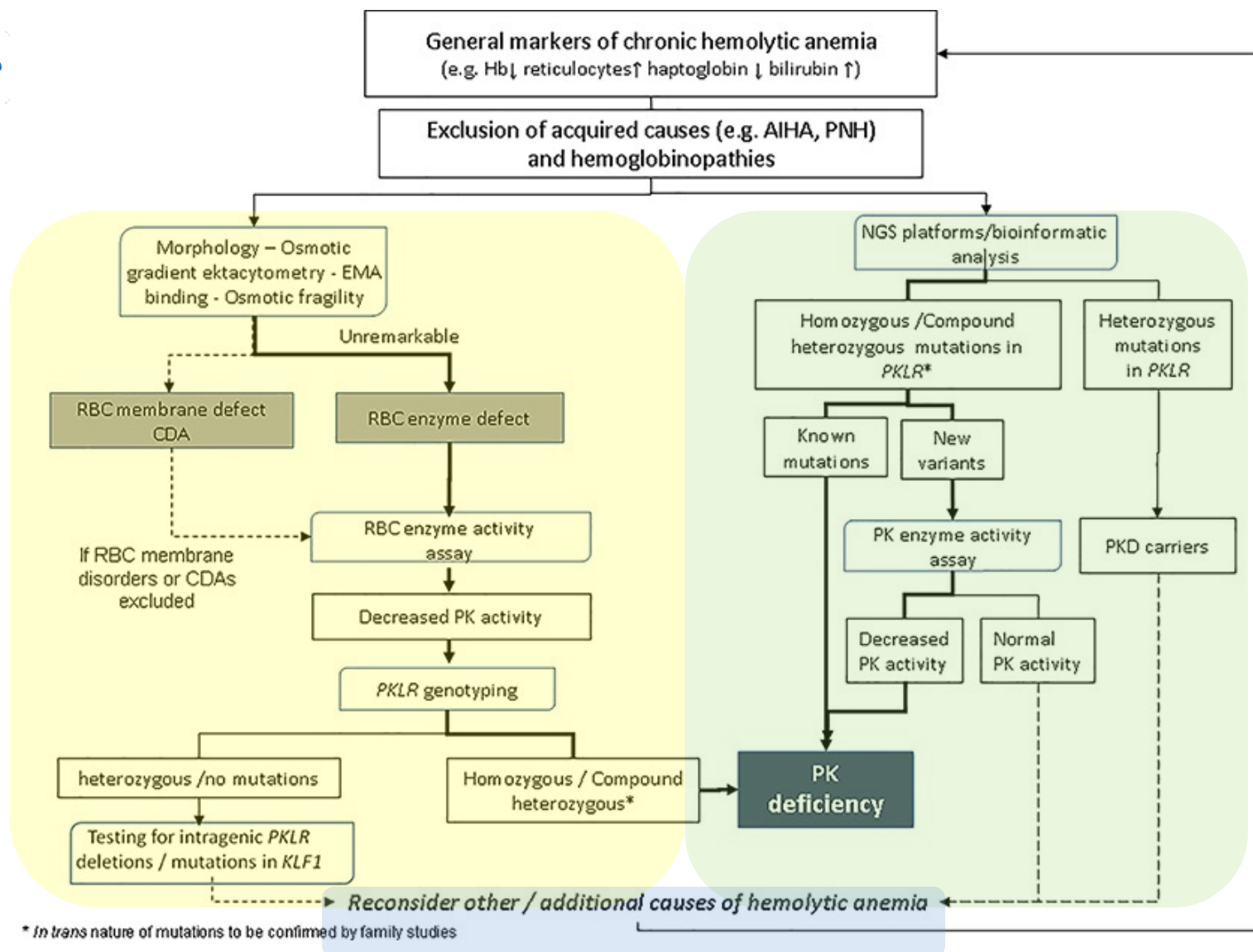
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Sistema Socio Sanitario

Regione
Lombardia

Addressing the diagnostic gaps in pyruvate kinase deficiency:
Consensus recommendations on the diagnosis of pyruvate
kinase deficiency









NGS apport to diagnosis of chronic hemolytic anemias

	N. of genes analysed	N. of cases	Overall sensitivity	Sensitivity in hemolytic patients with no previous diagnosis
Agrawal, et al 2023	28	450 (CHA)		24%
More et al, 2023	5	26 HS	80%	Not studied
Nieto et al, 2022	48	165 (HS)	83%	35%
Fermo, et al. 2021	48	122 (CHA)	74%	35%
Morado et al, 2021	40	99 (CHA)	78%	n.a.
Chonat, et al. 2019	32 (membrane defects)	11 (HS)	100%	Not studied
van Vuren, et al.2019	7 (membrane defects)	95 (HS)	89%	Not studied
Xue, et al. 2019	10 (membrane defects)	10 (HS)	90%	Not studied
Peng, et al, 2018	n.a.	51 (HS)	72%	Not studied
Li, et al., 2018.	217	46 (CHA)	60.9%	n.a.
Russo et al., 2018	34 and 71	74 (CHA)	64.9%	45.8%
Agarwal et al., 2016	28	17 (CHA)	70%	70%
Roy et al., 2016	33	57 (CHA)	38.6%	11%

CHA = Chronic hemolytic anemias, HS = Hereditary spherocytosis, n.a. = not available

The use of next-generation sequencing in the diagnosis of rare inherited anaemias: A Joint BSH/EHA Good Practice Paper*

Noémi B. A. Roy^{1,2}  | Lydie Da Costa³ | Roberta Russo^{4,5}  | Paola Bianchi⁶  |
 Maria del Mar Mañú-Pereira⁷ | Elisa Fermo⁶ | Immacolata Andolfo^{4,5}  | Barnaby Clark¹³ |
 Melanie Proven⁸ | Mayka Sanchez^{9,10} | Richard van Wijk¹¹ | Bert van der Zwaag¹¹ |
 Mark Layton¹² | David Rees¹³  | Achille Iolascon^{4,5}  | British Society for Haematology/
 European Hematology Association

Variant classification (ACMG)

Class 5: Pathogenic	Report if fits phenotype	Further studies <ul style="list-style-type: none"> • Family studies • Functional work: <ul style="list-style-type: none"> • Enzyme levels • EMA dye binding • Ekta • cDNA analysis for splice variants • Patch clamp analysis channelopathies
Class 4: Likely pathogenic	Report if fits phenotype	
Class 3: Variant of uncertain significance (VUS)	Consider further studies	
Class 2: Likely benign	Do not report	
Class 1: Benign	Do not report	

Targeted Next Generation sequencing and diagnosis of congenital hemolytic anemias: a three years experience monocentric study

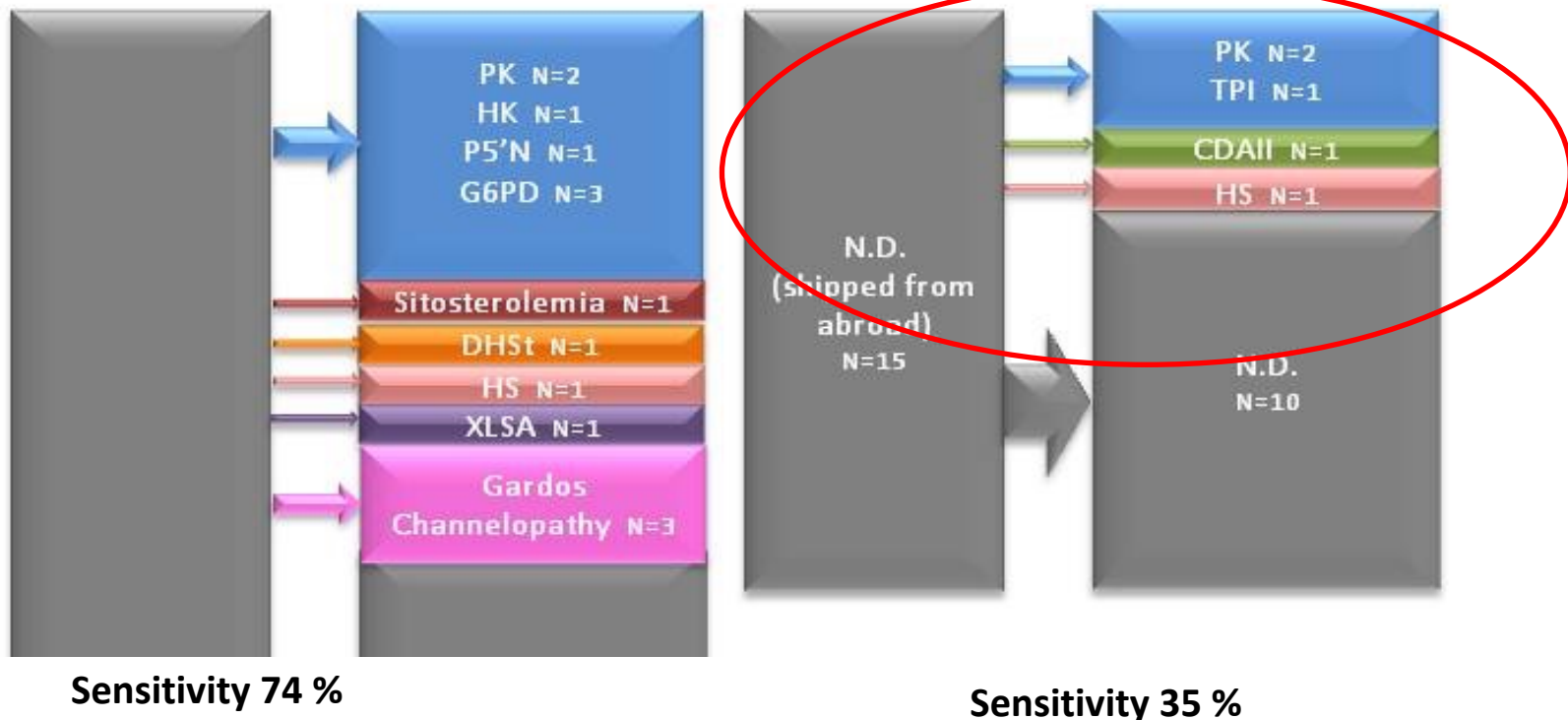
48genes t-NGS platform

2017-2019

122 patients -105 unrelated families

60 patients who reached a diagnosis after first and second level hematologic investigations, to be confirmed at molecular level

62 patients with unexplained chronic haemolytic anemia after extensive hematologic investigations.



T-NGS panel vs laboratory testing

	Laboratory testing	Molecular analysis (NGS)
HS	EMA-binding test Ectacytometry Others	High molecular heterogeneity Consistency with clinical and laboratory features required
HE	Osmotic fragility tests Ectacytometry RBC morphology	High molecular heterogeneity Consistency with clinical and laboratory features required
HSt- <i>PIEZO1</i>	Rbc morphology; Ektacytometry Always requiring molecular testing to confirm diagnosis	Highly polymorphic gene Functional tests mandatory in presence of new variants
HSt-<i>KCNN4</i>	Absence of specific laboratory markers	
RBC enzyme defects	RBC enzyme assay. Always requiring molecular testing to confirm diagnosis	
Familial sitosterolemia	Complete blood count	
Atypical conditions		



Therapy



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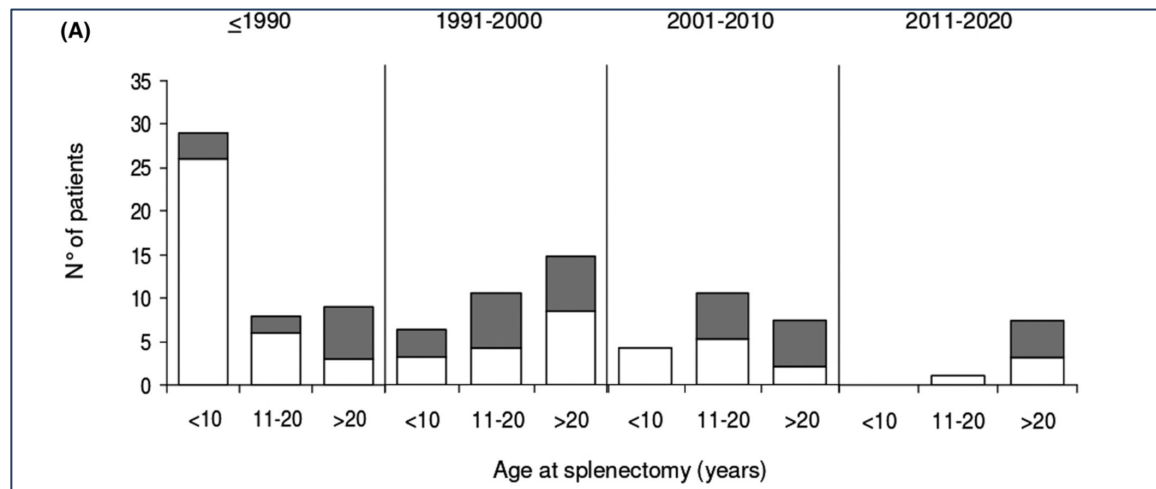
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Changing trends of splenectomy in hereditary spherocytosis

	Before splenectomy	After Splenectomy	P
Hematologic parameters			
Hb (g/dL)	10.4 (7.2-14.7)	15.3 (9.1-18.4)	<0.0001
MCV (fL)	86 (65-105)	87 (74-95)	NS
MCHC (g/dL)	35.6 (29.9-37.5)	353 (30.3-37.4)	NS
RDW (%)	19.2 (11.5-24.7)	13.3 (11.1-22.5)	0.03
Markers of haemolysis			
Reticulocytes (x10 ⁹ /L)	350 (70-648)	157(16-279)	<0.0001
Unconjugated bilirubin (mg/dL)	1.8 (0.2-8.9)	0.6 (0.2-3.2)	0.0003
LDH (U/L)	412 (155-1057)	222 (105-405)	0.01
Iron status parameters			
Iron (µg/dL)	78 (42-224)	100 (26-230)	NS
Transferrin (mg/dL)	260 (174-345)	251 (133-348)	NS
Transferrin saturation (%)	27.5 (14-79)	28 (11 -75)	NS
Serum ferritin (ng/mL)	140 (17-560)	158 (10-1315)	NS



INHERITED ANEMIAS

Management of pyruvate kinase deficiency in children and adults

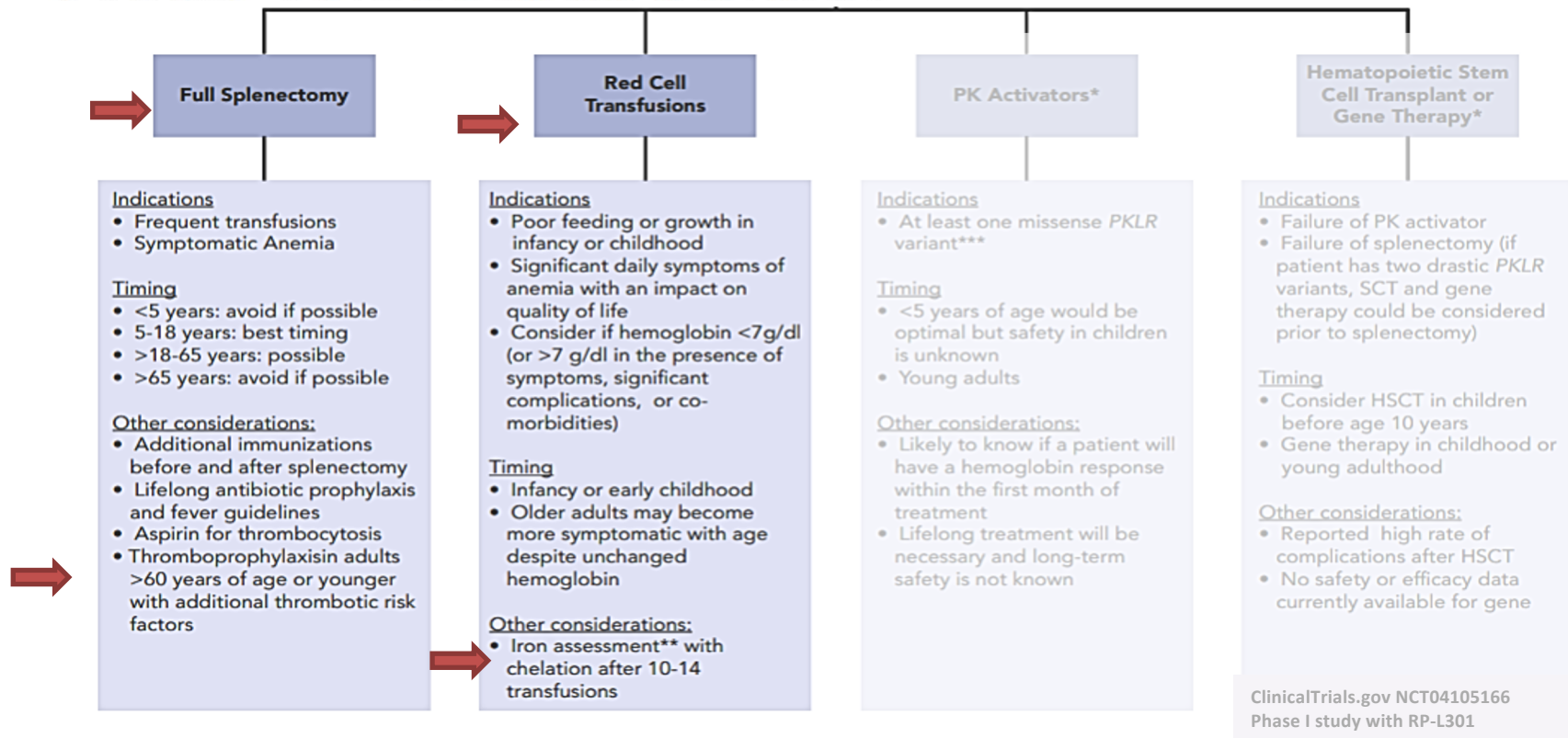
Blood 2020

Rachael F. Grace¹ and Wilma Barcellini²

How we manage patients with pyruvate kinase deficiency

Rachael F. Grace¹, D. Mark Layton,² and Wilma Barcellini,³

1. Department of Pediatrics, University of Colorado, Denver, Colorado, USA; 2. Department of Pediatrics, University of Colorado, Denver, Colorado, USA; 3. Department of Pediatrics, University of Colorado, Denver, Colorado, USA



INHERITED ANEMIAS

Management of pyruvate kinase deficiency in children and adults

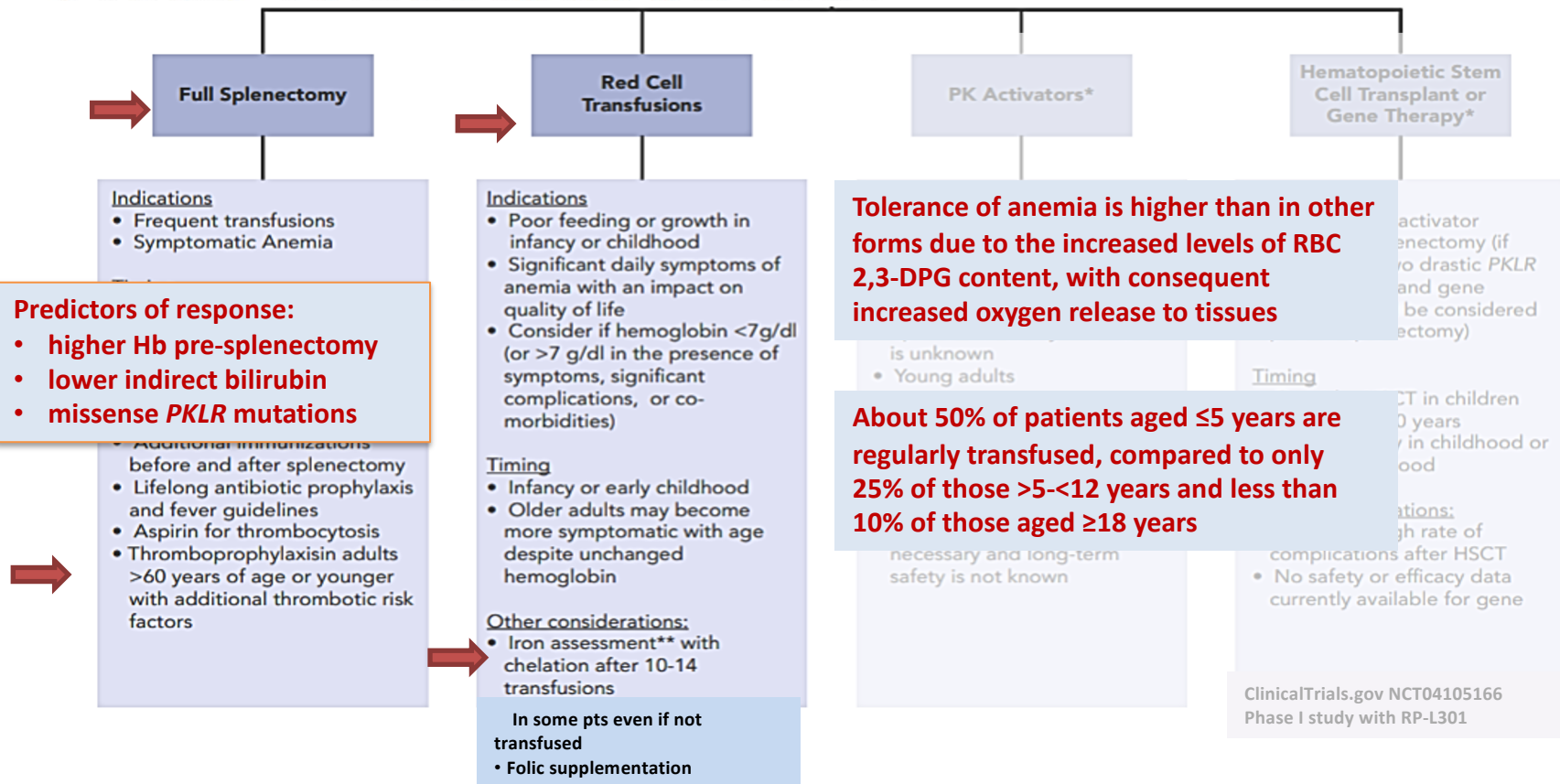
Blood 2020

Rachael F. Grace¹ and Wilma Barcellini²

How we manage patients with pyruvate kinase deficiency

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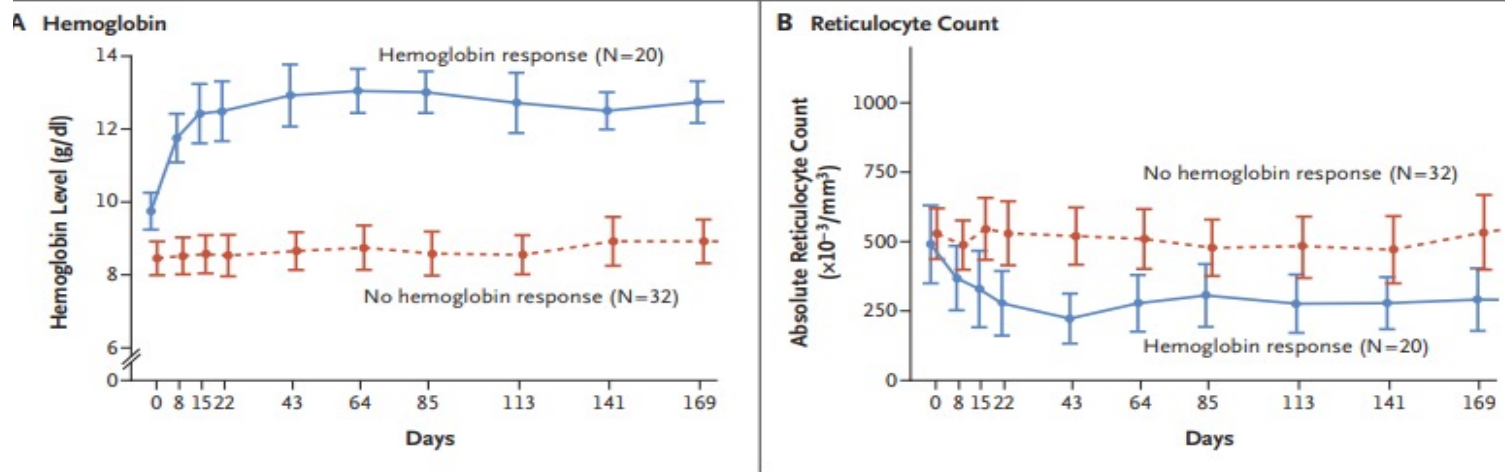
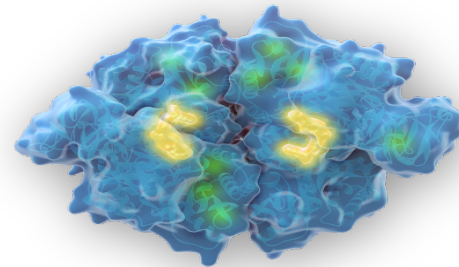
1. Institute of Hematology, University of Verona, Verona, Italy; 2. Department of Hematology, University of Verona, Verona, Italy; 3. Department of Hematology, University of Verona, Verona, Italy



AG-348 Mitapivat in PK deficiency

Safety and Efficacy of Mitapivat in Pyruvate Kinase Deficiency

Rachael F. Grace, M.D., Christian Rose, M.D.,* D. Mark Layton, M.B., B.S., Frédéric Galactéros, M.D., Wilma Barcellini, M.D., D. Holmes Morton, M.D., Eduard J. van Beers, M.D., Hassan Yaish, M.D., Yaddanapudi Ravindranath, M.D., Kevin H.M. Kuo, M.D., Sujit Sheth, M.D., Janet L. Kwiatkowski, M.D., M.S.C.E., Ann J. Barbier, M.D., Ph.D., Susan Bodie, Pharm.D., Bruce Silver, M.D., Lei Hua, Ph.D., Charles Kung, Ph.D., Peter Hawkins, Ph.D., Marie-Hélène Jouvin, M.D., Chris Bowden, M.D., and Bertil Glader, M.D., Ph.D.

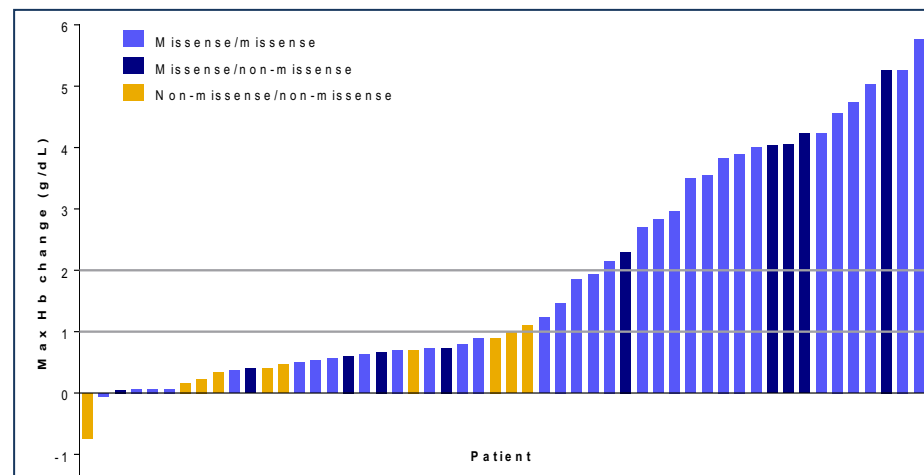
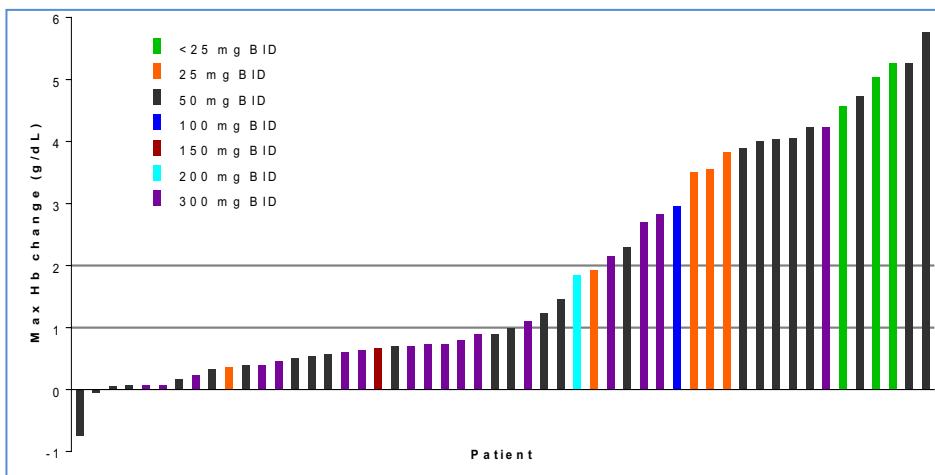


- The administration of mitapivat was associated with a rapid increase in the Hb level in 50% of cases, with a sustained response during the extension phase (median follow-up 29 months)
- Adverse effects were mainly low-grade and transient (headache, hypertriglyceridemia)
- (Funded by Agios Ph; ClinicalTrials.gov number, NCT02476916.)



Mitapivat in PK deficiency

- The mean maximum Hb increase in responders was 3.4 g/dL (range 1.1–5.8 g/dL)
- The median time to hemoglobin response >1.0 g/dl was 10 days (range 8–24 days) and the response was sustained in the majority of patients
- Response occurred through a wide range of doses
- Hb responses were observed only in patients with at least one missense mutation and correlated with baseline *PKLR* protein level.

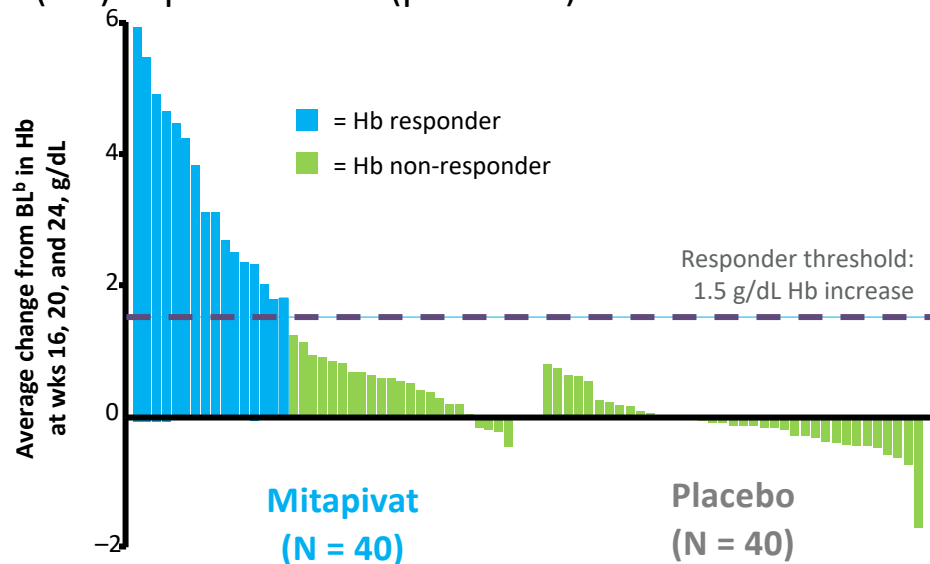


The baseline value is the average of all central assessments within the screening period (42 days prior to Day 1)

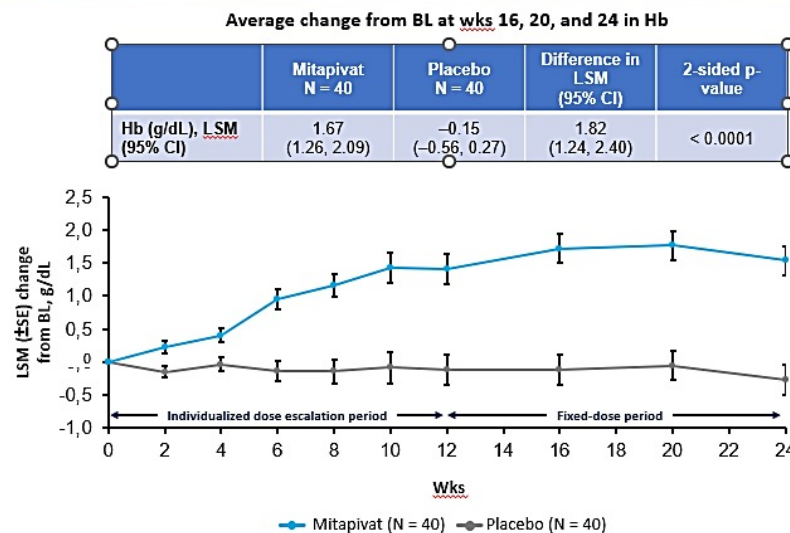
Phase 3, randomized, double-blind, placebo-controlled study that evaluated the efficacy and safety of mitapivat in adult patients with PK deficiency who were not regularly transfused

- Baseline Hb ≤ 10 g/dl
- Dose escalation (5 \rightarrow 20 \rightarrow 50 mg BID)
- Excluded homozygous for R479H mutation or have 2 non-missense mutations, without another missense mutation, in PKLR

16 pts (40%) responded in Mitapivat vs 0 pts (0%) in placebo arm ($p < 0.0001$)



Mitapivat led to early and sustained improvement in Hb and in markers of hemolysis (bilirubin, LDH)



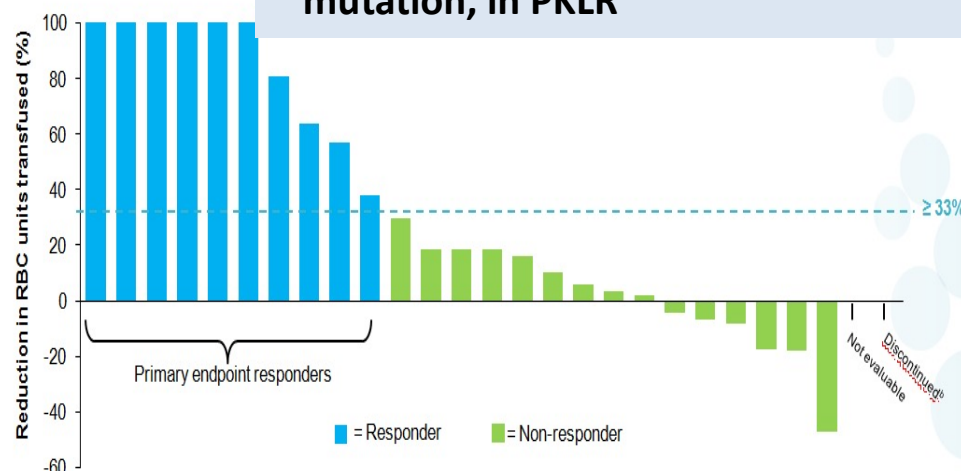


Mitapivat in adult patients with pyruvate kinase deficiency receiving regular transfusions (ACTIVATE-T): a multicentre, open-label, single-arm, phase 3 trial

Andreas Glenthøj, Eduard J van Beers, Hanny Al-Samkari, Vip Viprakasit, Kevin H M Kuo, Frédéric Galactéros, Satheesh Chonat, John Porter, Erin Zagadailov, Rengyi Xu, Abdulafeez Oluyadi, Peter Hawkins, Sarah Gheuens, Vanessa Beynon, Wilma Barcellini, on behalf of the ACTIVATE-T investigators*

- ≥ 6 transfusion episodes in the past 1 y
- Dose escalation (5 \rightarrow 20 \rightarrow 50 mg BID)
- Excluded homozygous for R479H mutation or have 2 non-missense mutations, without another missense mutation, in PKLR

Baseline characteristics	27 patients
Mean age (years)	18–68
Hb (g/dL), mean (SD)	9.2 (0.98)
Ferritin ($\mu\text{g/L}$), mean (SD) ^a	1153.7 (1221.41)
Prior splenectomy, n (%)	21 (77.8)
Prior cholecystectomy, n (%)	23 (85.2)
Prior chelation therapy, n (%)	24 (88.9)
No. RBC transfusion episodes, mean (SD)	9.7 (3.62)
No. RBC units transfused, mean (SD)	16.6 (8.63)



- 37% (10/27) of patients achieved a response with a reduction of transfusions
- 6 patients (22%) became transfusion-free during the fixed-dose period, and 3 patients (11%) achieved normal haemoglobin at least once

Mitapivat demonstrated a significant transfusion burden reduction in patients with PK who were regularly transfused.

Studies correlated with Mitapivat in PKD

Durability of hemoglobin response and reduction in transfusion burden is maintained over time in patients with PKD treated with mitapivat in a long-term extension study

Grace R, et al. EHA, ASH 2022

Long-term treatment with oral mitapivat is associated with normalization of hemoglobin levels in patients with PKD

Barcellini W, et al EHA 2022

Long-term improvements in patients –reported outcomes in patients with PKD treated with Mitapivat

Kuo K, et al ASH 2022

Mitapivat decreases the need for transfusions secondary to poorly tolerated anemia and acute events compared to placebo in patients with PKD who are not regularly transfused

Al-samkari H, et al EHA 2022

Improvements in patient-reported outcomes in mitapivat-treated patients with pyruvate kinase deficiency: a descriptive analysis from the phase 3 Activate trial.

H.M. Kuo K. P1735, EHA 2022

Mitapivat improves ineffective erythropoiesis and reduces iron overload in patients with pyruvate kinase deficiency, J. van Beers E. P1565, EHA 2022

Bone mineral density remains stable in pyruvate kinase deficiency patients receiving long-term treatment with mitapivat , Al-samkari H. P1544, EHA2022

Activate-KidsT/Activate kids: mitapivat in children with pyruvate kinase deficiency who are regularly transfused/who are not regularly transfused. Grace R. P1546 and P1547, EHA 2022: ASH 2022



Mitapivat: a drug for different disorders?

Thalassemia, Sickle cell disease (SCD) and more...

Thalassemia



Kuo et al. Safety and efficacy of mitapivat, an oral pyruvate kinase activator, in adults with non-transfusion dependent α -thalassaemia or β -thalassaemia: an open-label, multicentre phase 2 study. Lancet. 2022

↓ ATP

Sickle cell Anemia



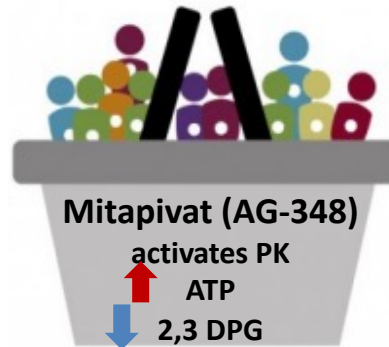
Preliminary results indicate decreased 2,3-DPG, increased ATP, amelioration of anemia and hemolysis in SCD

↑ 2,3 DPG

Hereditary spherocytosis



Matte et al. Mitapivat ameliorates red cell features and decreases anemia in band 4.2^{-/-} mice, a model of hereditary spherocytosis. EHA2022



Pyruvate kinase deficiency

MDS



Acquired Pyruvate Kinase Deficiency In Clonal Myeloid Neoplasms
Al-Samkari, MD, Massachusetts General Hospital clinical trials-gov NCT04902833

Secondary ↓ PK activity



Incomplete *PKLR* genotypes?
Hereditary stomatocytosis?
Other RBC enzyme defects ?



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Hematopoietic Allogeneic Stem Cell Transplantation in PKD



Haematologica. 2018 Feb; 103(2): e82–e86.

doi: [10.3324/haematol.2017.177857](https://doi.org/10.3324/haematol.2017.177857)

PMCID: PMC5792292

PMID: [29242305](https://pubmed.ncbi.nlm.nih.gov/29242305/)

Worldwide study of hematopoietic allogeneic stem cell transplantation in pyruvate kinase deficiency

Stephanie van Straaten,^{1,2,*} Marc Bierings,^{3,*} Paola Bianchi,⁴ Kensuke Akiyoshi,⁵ Hitoshi Kanno,⁶ Isabel Badell Serra,⁷ Jing Chen,⁸ Xiaohang Huang,⁸ Eduard van Beers,⁹ Supachai Ekwattanakit,¹⁰ Tayfun Güngör,¹¹ Wijnanda Adriana Kors,¹² Frans Smiers,¹³ Reinier Raymakers,¹⁴ Lucrecia Yanez,¹⁵ Julian Sevilla,¹⁶ Wouter van Solinge,¹ Jose Carlos Segovia,^{17,18} and Richard van Wijk¹

- 16 cases transplanted between 1996 and 2015 (all European or Asian centers, none in USA)
- Patient's median age at transplantation was 6.5 years, all were transfusion-dependent
- Infectious complications (mostly pneumonia) occurred in 10/16 (62%)
- GvHD grade 4 reported in 6/16 cases (38%)
- The two-year cumulative survival was 74%.
- 5/16 patients (31%) died of transplant-related causes (median survival 13 months, range 2–25 months).

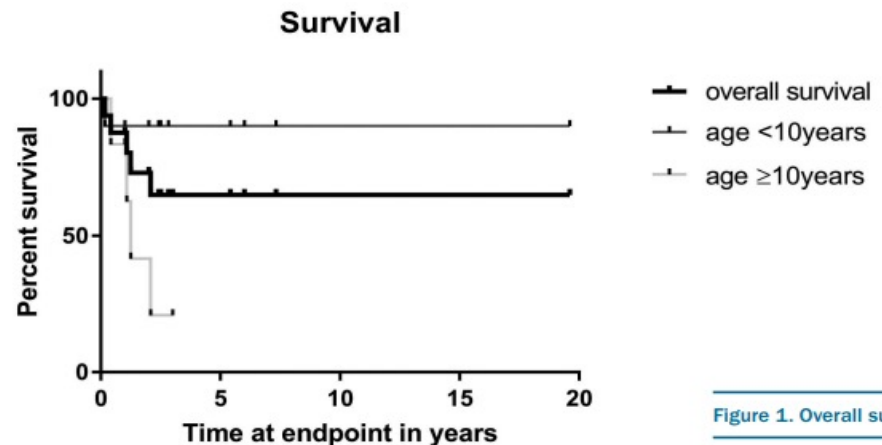


Figure 1. Overall survival, i



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Gene therapy in PKD

Safe and Efficient Gene Therapy for Pyruvate Kinase Deficiency

Maria Garcia-Gomez^{1,2}, Andrea Calabria³, Maria Garcia-Bravo^{1,2}, Fabrizio Benedicenti³, Penelope Kosinski⁴, Sergio López-Manzaneda^{1,2}, Collin Hill⁴, María del Mar Mañu-Pereira⁵, Miguel A Martín^{1,2}, Israel Orman^{1,2}, Joan-Lluis Vives-Corrons⁵, Charles Kung⁴, Axel Schambach⁶, Shengfang Jin⁴, Juan A Bueren^{1,2}, Eugenio Montini³, Susana Navarro^{1,2} and Jose C Segovia^{1,2}

Mol Ther. 2016

- Preclinical gene therapy for PKD, based on a lentiviral vector used to transduce mouse PKD HSCs that were subsequently transplanted into myeloablated PKD mice.
- The procedure normalized erythroid compartment and corrected hematologic phenotype, organ pathology, the glycolytic pathway, with no evidence of genotoxicity

Lentiviral-mediated Gene Therapy for Adults and Children with Severe Pyruvate Kinase Deficiency: Results from an Ongoing Global Phase 1 Study. Shah AJ, et al, ASH 2022

NCT04105166. A Phase 1 clinical trial RP-L301-0119 (NCT04105166) is underway to evaluate lentiviral mediated hematopoietic stem and progenitor cell (HSPC)-targeted gene therapy for adults and children with severe PKD.

Inclusion Criteria: Splenectomized patients with severe PKD (severe and/or transfusion-dependent anemia)

- ✓ **2 adult splenectomized patients** had been enrolled, with an Hb increase from 7.4 to 13.3 g/dL and from 7 to 14.8 g/dL at 18 months, and an improvement in hemolytic markers. No severe adverse events were reported.
- ✓ No red blood cell transfusion requirements following engraftment (transfusion independence).
- ✓ Both patients reported improved quality of life following treatment.

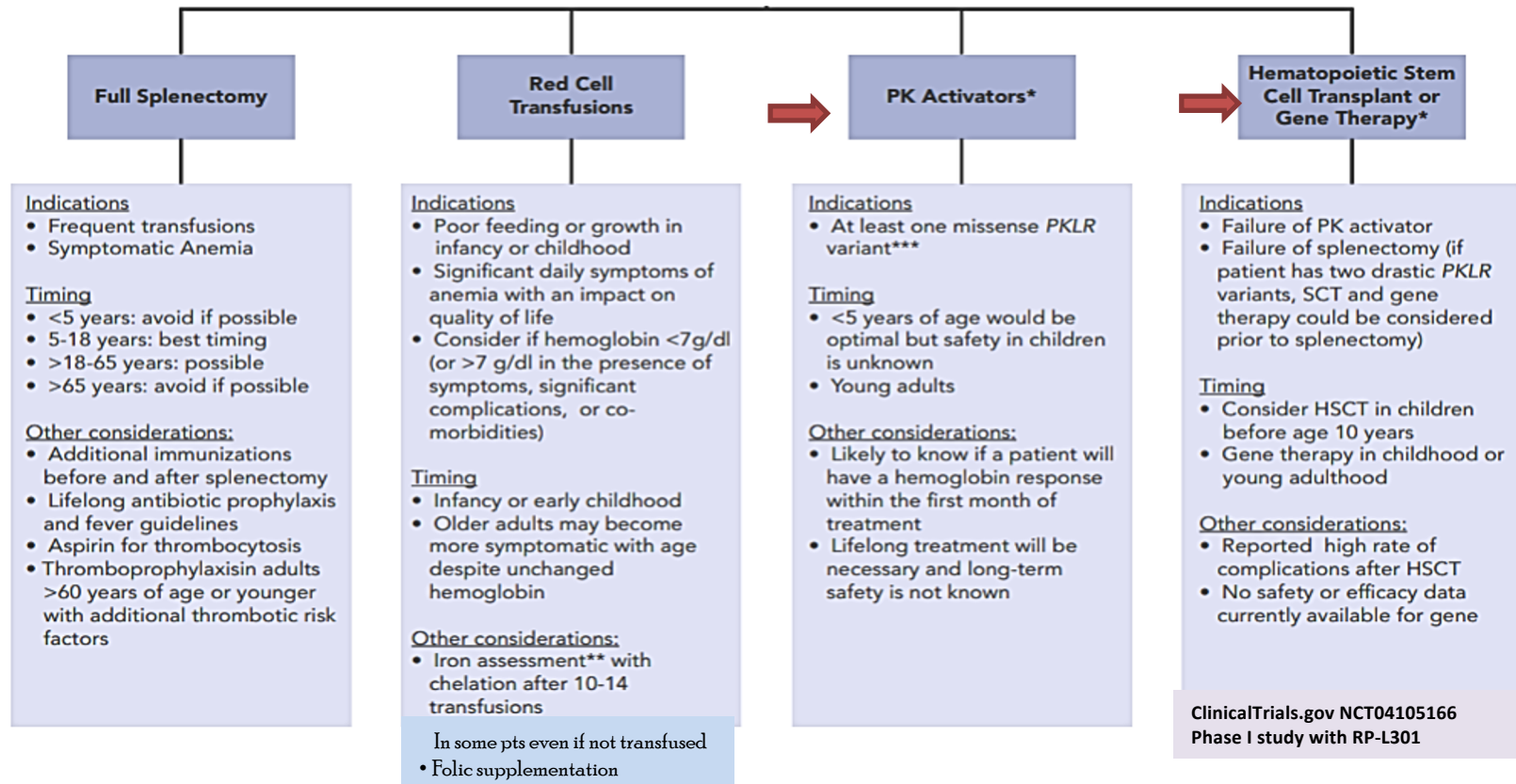


INHERITED ANEMIAS

Management of pyruvate kinase deficiency in children and adults

Blood 2020

Rachael F. Grace¹ and Wilma Barcellini²



Triose phosphate isomerase deficiency

- ✓ TPI deficiency is a multisystemic disorder characterised by:
 - severe hemolytic anemia at birth
 - neurological and cognitive dysfunction, progressive neuromuscular impairment
 - infectious complications

Bone marrow transplantation corrects haemolytic anaemia in a novel ENU mutagenesis mouse model of TPI deficiency.

Conway AJ, Brown FC, Hortle EJ, Burgio G, Foote SJ, Morton CJ, Jane SM, Curtis DJ. *Dis Model Mech.* 2018

Itavastatin and resveratrol increase triosephosphate isomerase protein in a newly identified variant of TPI deficiency.

Van Demark AP, Hrizo SL, Eicher SL, Kowalski J, Myers TD, Pfeifer MR, Riley KN, Koeberl DD, Palladino MJ. *Dis Model Mech.* 2022



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Grazie!



European
Reference
Network

for rare or low prevalence
complex diseases

 Network

Hematological
Diseases (ERN EuroBloodNet)