5 JAHRE
HÄMATOLOGIE HEUTE

Normozytäre Anämien

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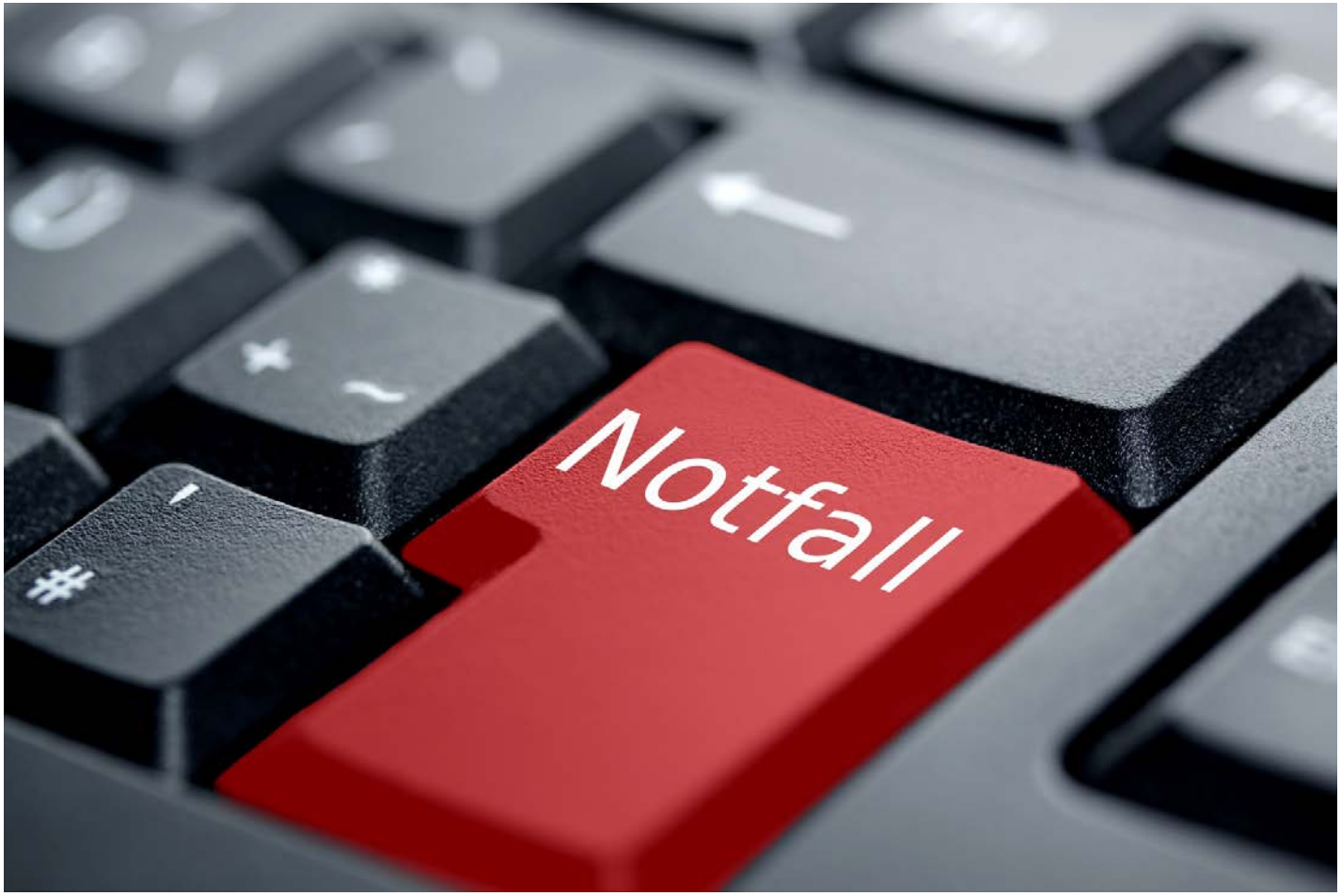
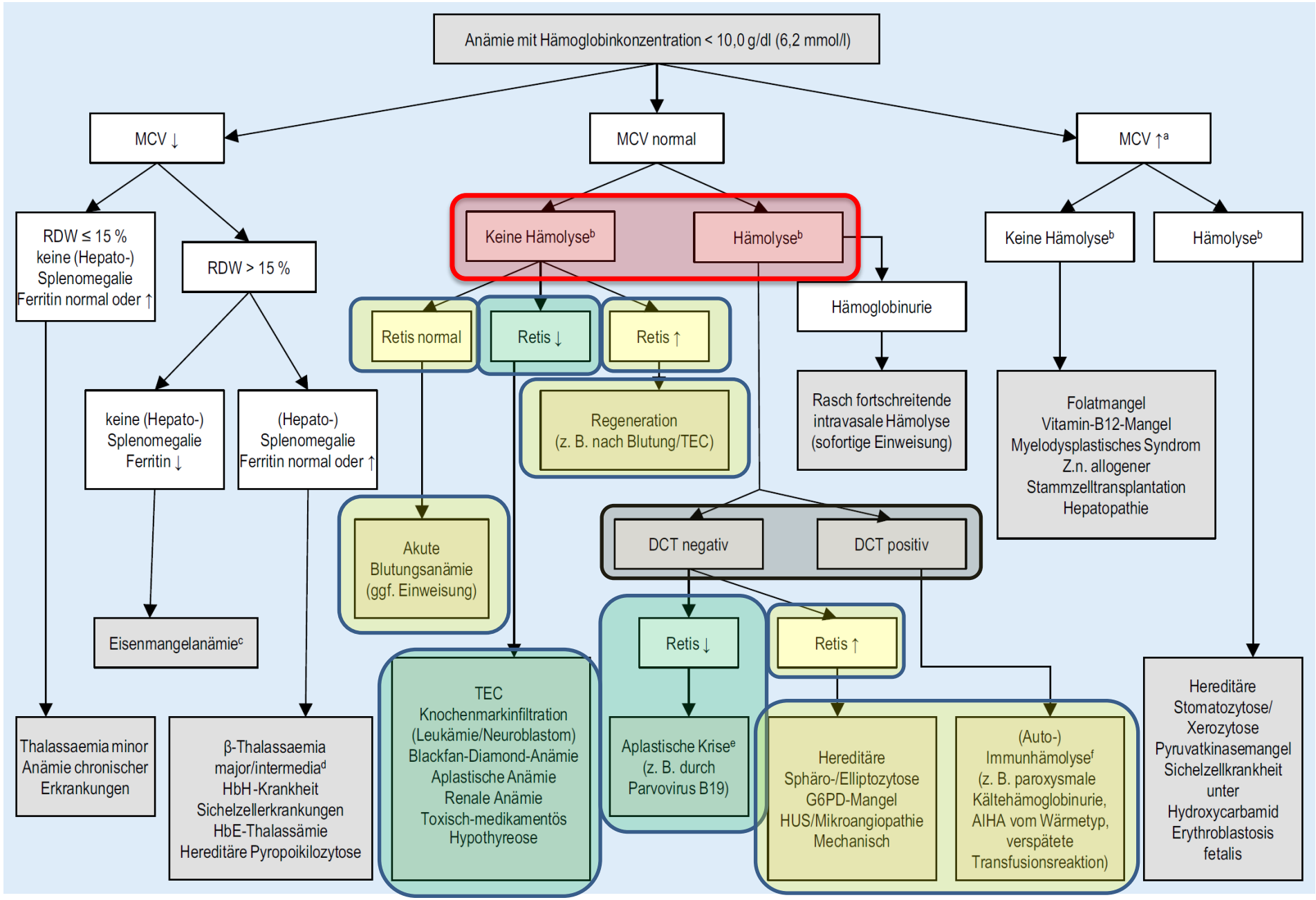
St. Anna Kinderkrebsforschung

Normozytäre Anämien (Hb < 8g/dl) oft




Anämie mit Hämoglobinkonzentration < 10,0 g/dl (6,2 mmol/l)
MCV ↓
MCV normal
MCV ↑[a]
RDW ≤ 15 %
keine (Hepato-)
Splenomegalie
Ferritin normal oder ↑
RDW > 15 %
Keine Hämolyse[b]
Hämolyse[b]
Keine Hämolyse[b]
Hämolyse[b]
Retis normal
Retis ↓
Retis ↑
Hämoglobinurie
keine (Hepato-)
Splenomegalie
Ferritin ↓
(Hepato-)
Splenomegalie
Ferritin normal oder ↑
Regeneration
(z. B. nach Blutung/TEC)
Rasch fortschreitende
intravasale Hämolyse
(sofortige Einweisung)
Folatmangel
Vitamin-B12-Mangel
Myelodysplastisches Syndrom
Z.n. allogener
Stammzelltransplantation
Hepatopathie
Akute
Blutungsanämie
(ggf. Einweisung)
DCT negativ
DCT positiv
Eisenmangelanämie[c]
Retis ↓
Retis ↑
Thalassaemia minor
Anämie chronischer
Erkrankungen
β-Thalassaemia
major/intermedia[d]
HbH-Krankheit
Sichelzellerkrankungen
HbE-Thalassämie
Hereditäre Pyropoikilozytose
TEC
Knochenmarkinfiltration
(Leukämie/Neuroblastom)
Blackfan-Diamond-Anämie
Aplastische Anämie
Renale Anämie
Toxisch-medikamentös
Hypothyreose
Aplastische Krise[e]
(z. B. durch
Parvovirus B19)
Hereditäre
Sphäro-/Elliptozytose
G6PD-Mangel
HUS/Mikroangiopathie
Mechanisch
(Auto-)
Immunhämolyse[f]
(z. B. paroxysmale
Kältehämoglobinurie,
AIHA vom Wärmetyp,
verspätete
Transfusionsreaktion)
Hereditäre
Stomatozytose/
Xerozytose
Pyruvatkinasemangel
Sichelzellkrankheit
unter
Hydroxycarbamid
Erythroblastosis
fetalis

Patient 14 Monate

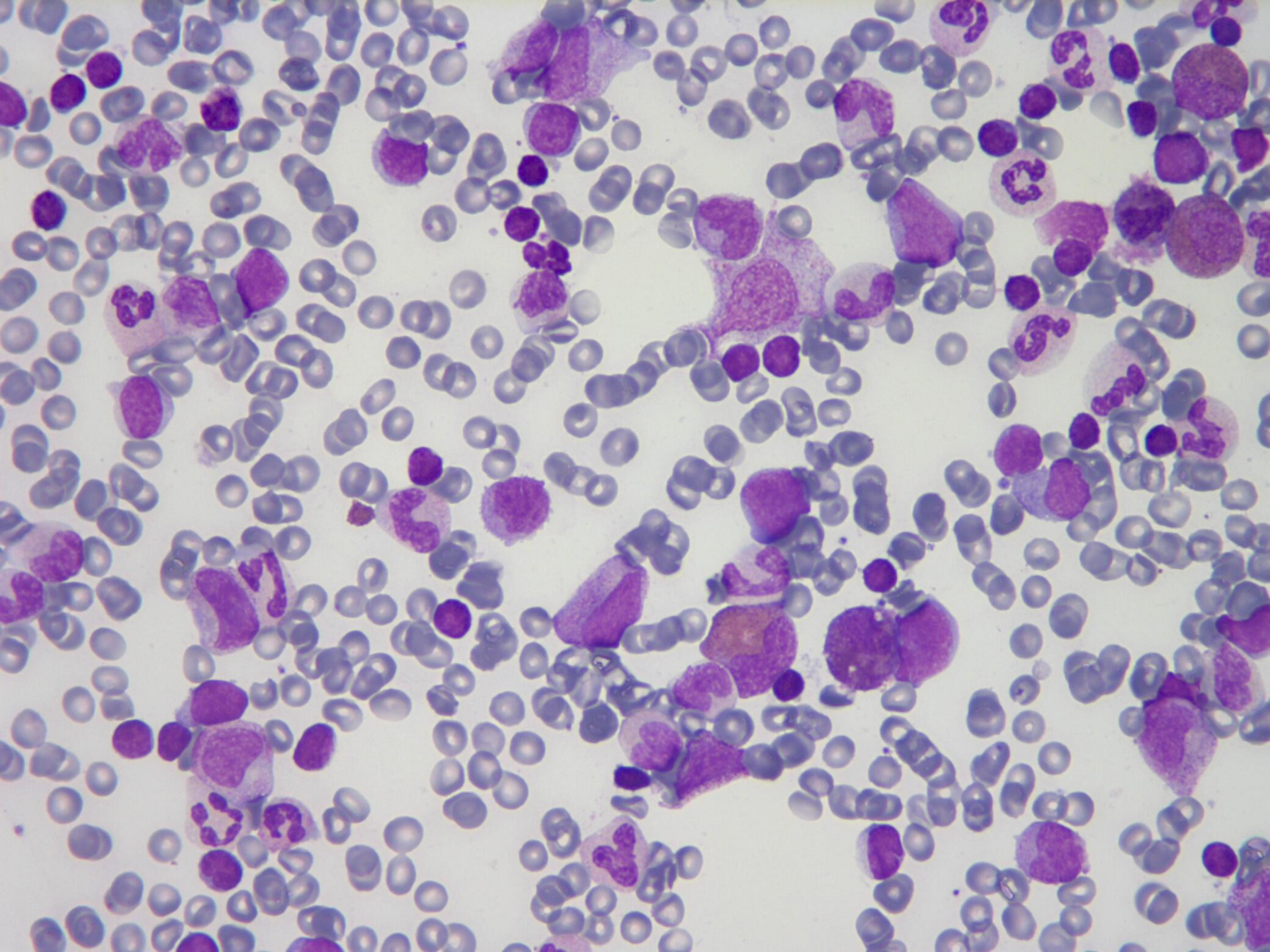
1. Kind; unauff. FA und Geburtsanamnese, normale Entwicklung, bisher keine ernsthaften Erkrankungen

Stationäre Aufnahme wegen auffallender Blässe bei seit 3 Wochen bestehenden afebrilen Infekt der oberen Atemwege

KL: 75,5 cm (10-25%), KG: 8,7 kg (3-10%), Haut: blass, Cor: HA rhythmisch, 1/6 Systolikum, HF 126/min, Pulmo: VA bds., 100% O2-sättigung, Abdomen: H/L n.p.; Lymphknoten: vereinzelt erbsengroße Lymphknoten zervikal; übriger interner Status o.B.

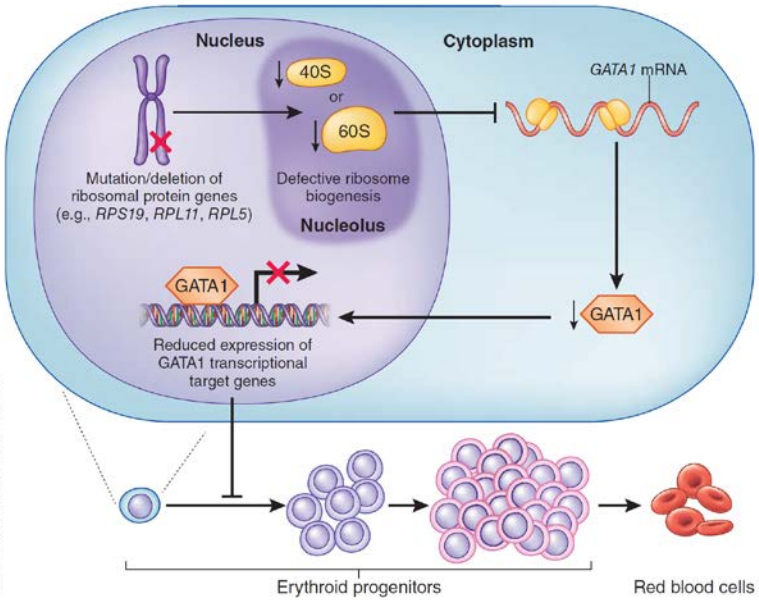
BEFUNDE

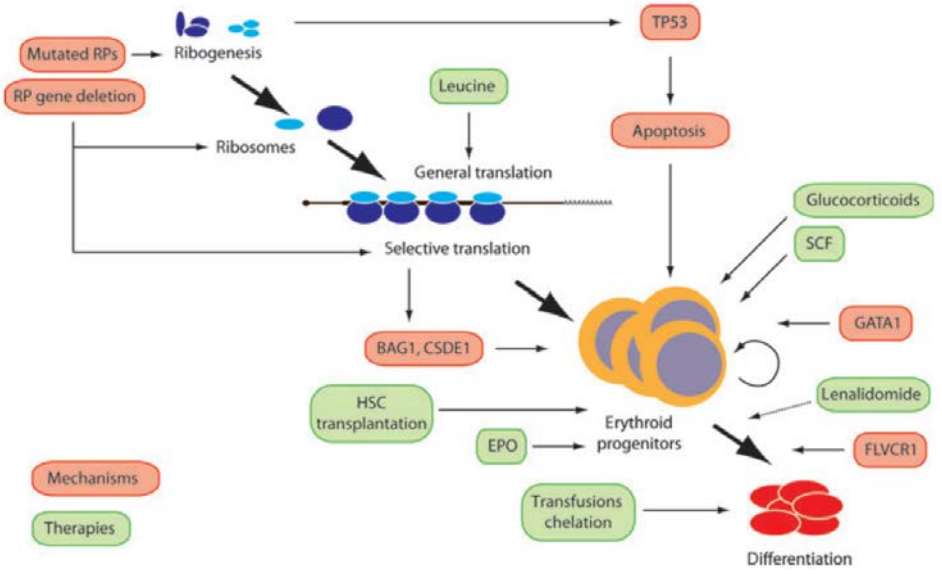
Leukozyten	10.79 G/l	5.00-17.50
Erythrozyten	1.76 T/l	3.50-5.50
Hämoglobin	5.8 g/dl	11.1-14.3
Hämatokrit	17 %	32-43
MCV (mittl.Zellvolumen)	97 fl	73-101
MCH (mittl.Zell-Hb)	33 pg	25-35
MCHC (mittl.Zell-Hb-Konz.)	34 g/dl	26-34
Erythrozytenverteilungsbreite	19.7 %	11.5-14.5
Retikulozyten	4 ‰	2-28
Thrombozyten	343 G/l	150-450
MPV (mittleres Plättchenvol.)	8 fl	7-11
Coombstest direkt	negativ	
Haptoglobin	66 mg/dl	30-200
Kreatinin	0.24 mg/dl	0.10-0.40
Harnsäure	4.2 mg/dl	0.0-6.0
Bilirubin gesamt	0.3 mg/dl	0.2-1.5
LDH	346 U/l	120-300
Ferritin	59 µg/l	7-150



Blackfan Diamond Anämie (DBA)

- 1976 durch Alter et al. definiert als
  - *Moderate to severe macrocytic anemia*
  - *Reticulocytopenia*
  - *Normal bone marrow cellularity with a paucity of erythroid precursors*
  - *Age less than 1 year*





British Journal of Haematology, 2012, **159,** 514–527

There was **no homozygous** variant identified.
The table lists a **heterozygous** variant which is likely causative for the phenotype.

Genetic analysis: Hematology gene panel screen

GENE	CHR	NAME	POS	SNP ID	R	A	IMPACT	AA	Transcript ID	MAF	READS
RPS29	14	RIBOSOMAL PROTEIN S29	50052691	.	C	T	MISSENSE	A47T	ENST00000396020	NA	392

LEGEND

GENE	gene name	**R**	base in reference genome	**ID**	Transcript ID
CHR	chromosome	**A**	alternative base in sample	**MAF**	minor allele frequency
POS	chromosomal position (bases)	**IMPACT**	consequence of variant	**READS**	number of sequencing reads covering the variant
SNP ID	rs number	**AA**	change on amino acid level	**NA**	not annotated

Predictions of variant impact
Polyphen-2: probably damaging
CADD Score: deleterious
SIFT: 35

References
http://genetics.bwh.harvard.edu/pph2/
http://sift.jcvi.org/
http://cadd.gs.washington.edu/

Whole-exome sequencing and functional studies identify *RPS29* as a novel gene mutated in multicase Diamond-Blackfan anemia families

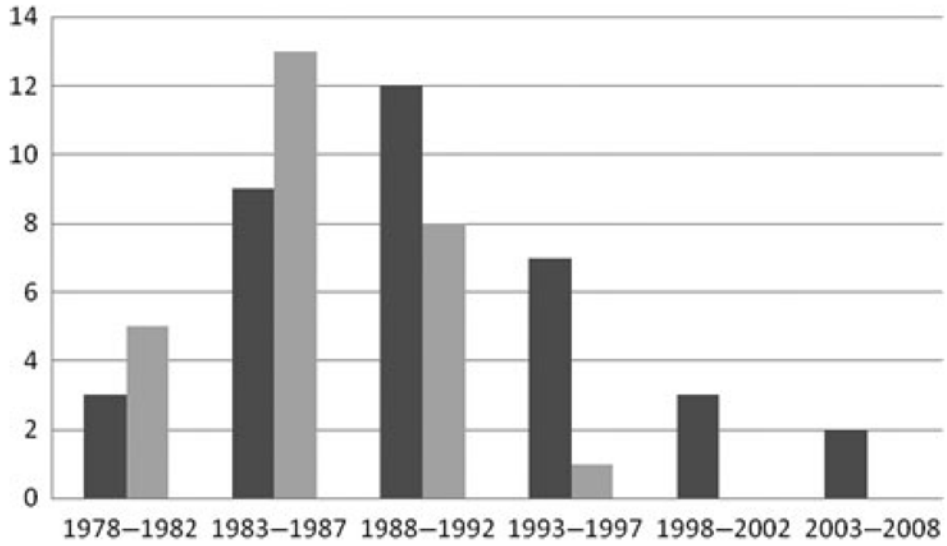
Lisa Mirabello[1], Elizabeth R. Macari[2], Lea Jessop[1], Steven R. Ellis[3], Timothy Myers[1], Neelam Giri[1], Alison M. Taylor[2], Katherine E. McGrath[2], Jessica M. Humphries[2], Bari J. Ballew[1], Meredith Yeager[4], Joseph F. Boland[4], Ji He[4], Belynda D. Hicks[4], Laurie Burdett[4], Blanche P. Alter[1], Leonard Zon[2,5], and Sharon A. Savage[1]

blood®

Diamond-Blackfan anemia is a rare inherited bone marrow failure syndrome (IBMFS). It is characterized by red blood cell (RBC) aplasia and variable congenital anomalies. DBA classically presents with severe anemia in the first year of life and may include craniofacial anomalies such as flat nasal bridge, high arched or cleft palate, and short stature. There is a high incidence of cancer in DBA patients, with particularly high risks of leukemia, myelodysplastic syndrome, colon adenocarcinoma, and osteosarcoma. DBA is inherited in an **autosomal dominant** manner, although disease penetrance is often incomplete and expressivity may be variable, leading to clinical heterogeneity within families. DBA is considered a **ribosomopathy**, a disorder caused by impaired ribosome biogenesis and function, because the majority of DBA patients have a germ-line heterozygous mutation or deletion in a ribosomal protein (RP) gene

Transitorische Erytroblastopenie des Kindesalters (TEC)

- Benigne selbstlimitierende Erkrankung (~1 Monat)
- 1978-2008 ‚Sick Children' (Toronto), N=36
- Medianes Alter bei Diagnose 19 Monate (3 – 50)
- Werte bei Dx (median), Hb 4.4g/dl, MCV 79fl



ACTA PÆDIATRICA
NURTURING THE CHILD

2014 **103**, pp. e288–e294

Patientin 15 Monate

1. Kind in SSW 39+1 SL, GG: 3000 g, Länge: 49 cm, KU: 34 cm, Apgar: 9-10-10, postpartal bei Anämie EK erhalten, V.a. Meningitis Zovirax und Claforan, bis 02/103 Neorecormontherpaie + Fe + Folsäure

Bekannte Thrombopenie seit Geburt >90 G/l keine Blutungsneigung. Seit 2. Lebensmonat bei Pflegeeltern.

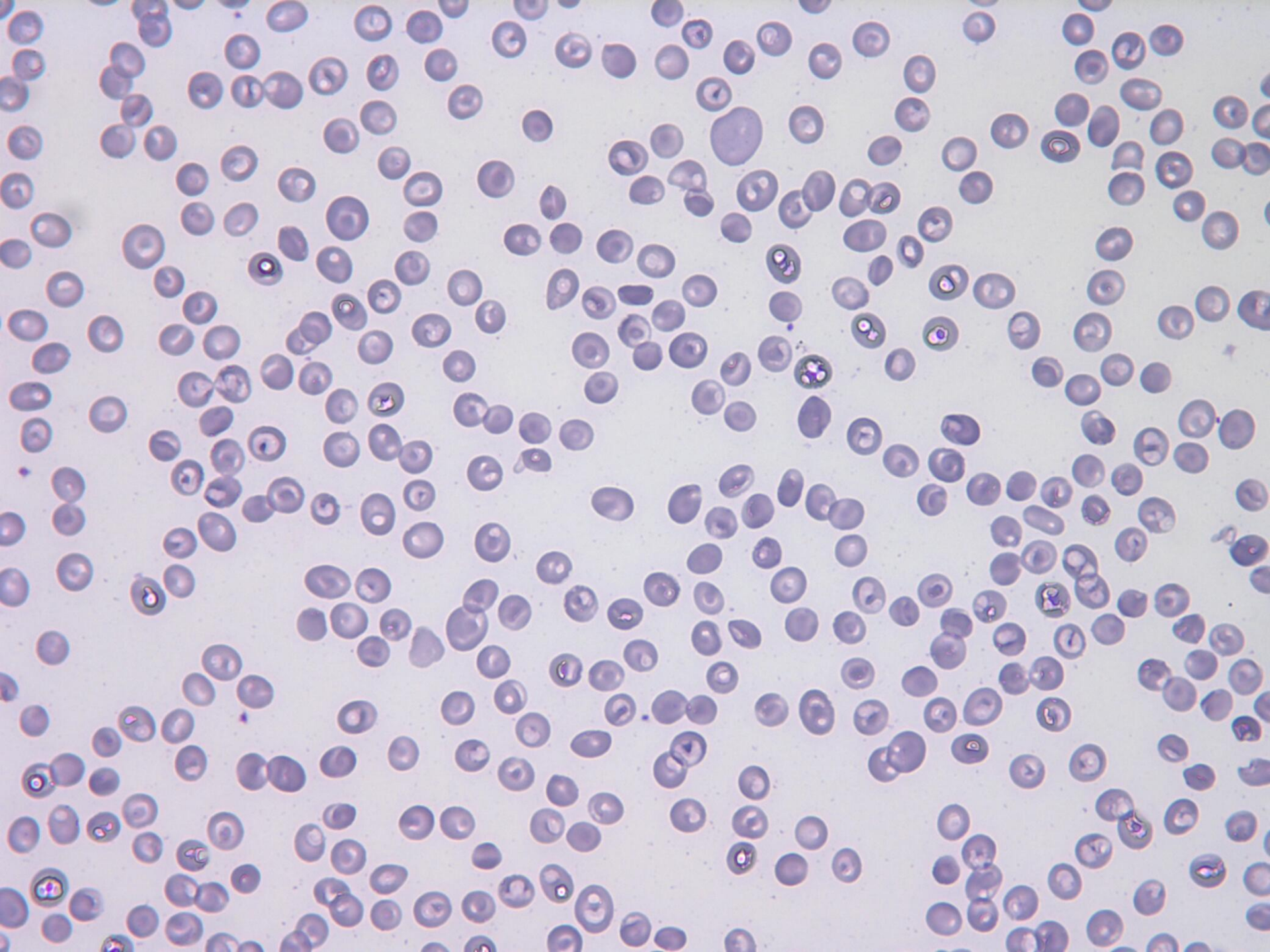
Aufnahme bei Infekt und auffallender Blässe

HÄMATOLOGISCHE BEFUNDE

Leukozyten	9.82 G/l	5.00-17.50
Erythrozyten	**1.77** T/l	3.50-5.50
Hämoglobin	**5.7** g/dl	11.1-14.3
Hämatokrit	**17** %	32-43
MCV (mittl.Zellvolumen)	94 fl	73-101
MCH (mittl.Zell-Hb)	32 pg	25-35
MCHC (mittl.Zell-Hb-Konz.)	34 g/dl	26-34
Erythrozytenverteilungsbreite	*19.8* %	11.5-14.5
Retikulozyten	11 ‰	2-28
Thrombozyten	*97* G/l	150-450
MPV (mittleres Plättchenvol.)	10 fl	7-11

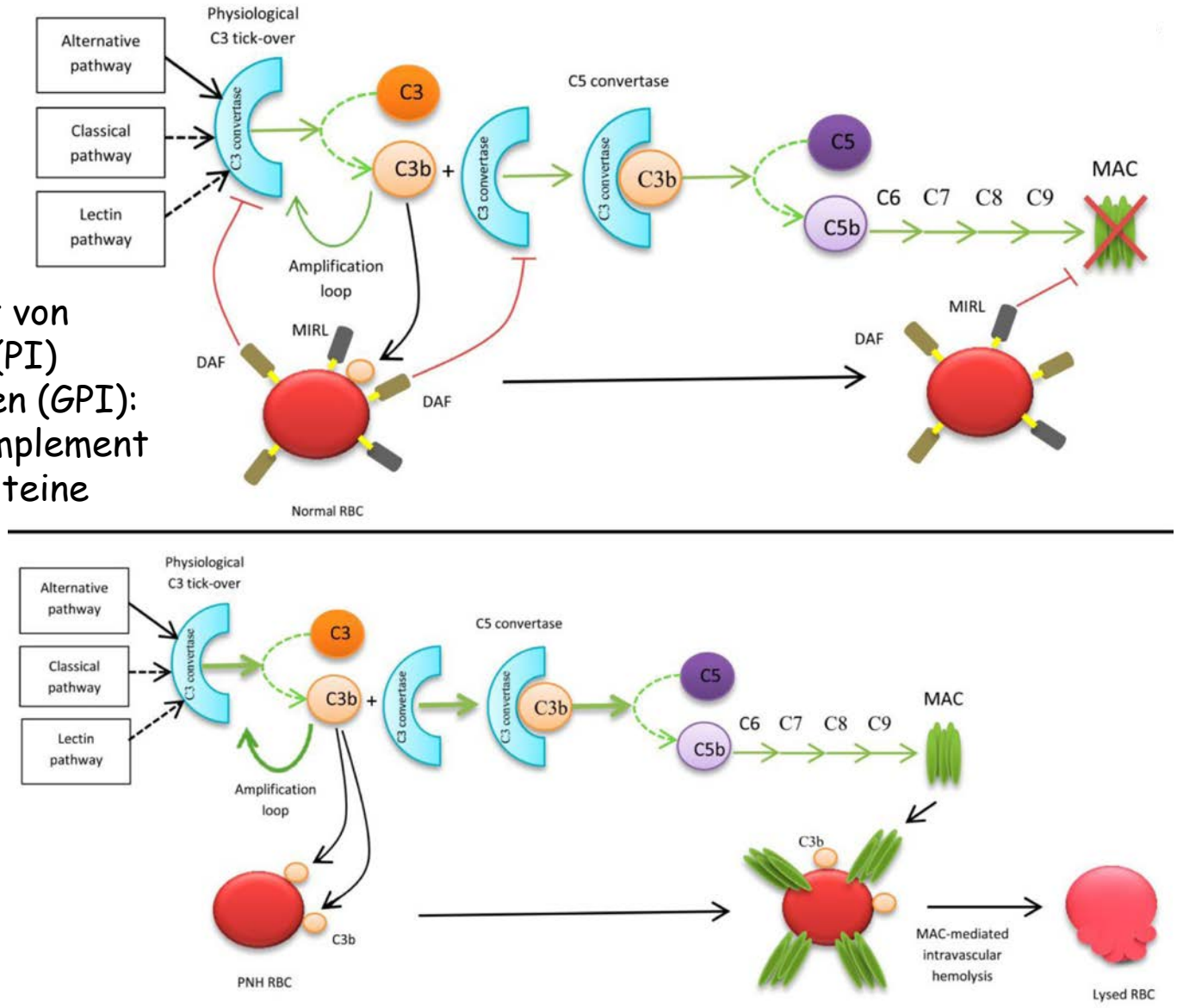
CHEMISCHE BEFUNDE

Harnsäure	2.8 mg/dl	0.0-6.0
Bilirubin gesamt	0.9 mg/dl	0.2-1.5
GOT (ASAT)	23 U/l	0-56
GPT (ALAT)	11 U/l	0-39
Gamma-GT	8 U/l	0-20
LDH	*320* U/l	120-300



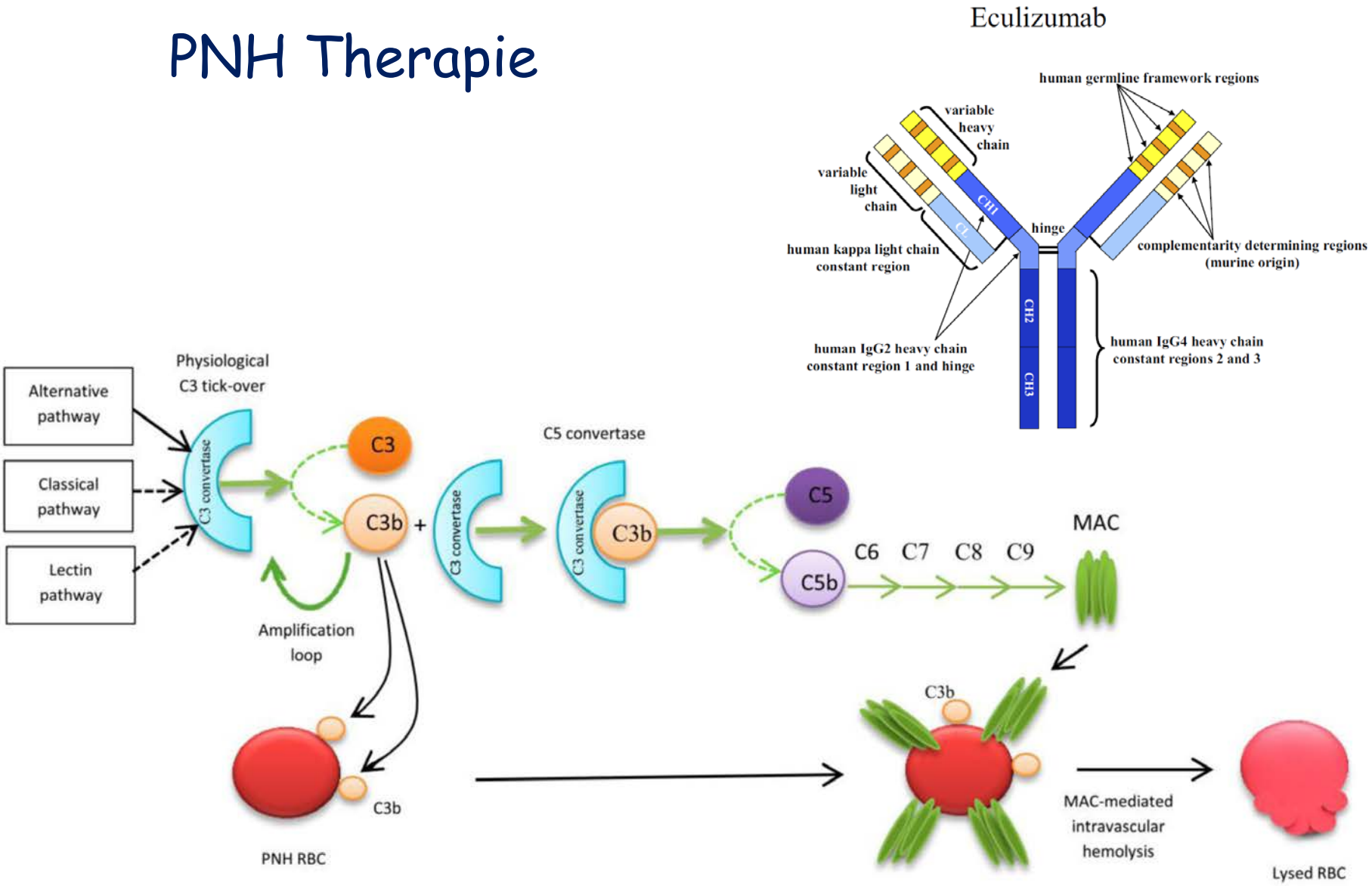
Paroxysmale nächtliche Hämoglobinurie

Partieller Defekt von Phosphoinositol (PI) verankerten Molekülen (GPI): CD55 + CD59 sind Komplement regulatorische Proteine



Journal of Extracellular Vesicles 2014, **3**: 23304

PNH Therapie



Journal of Extracellular Vesicles 2014, **3**: 23304

Blood. 2009;113:6522-6527

PIG-A mutation in a HSC

T cell-mediated auto-immune attack against HSC

Sub-clinical GPI-negative clone

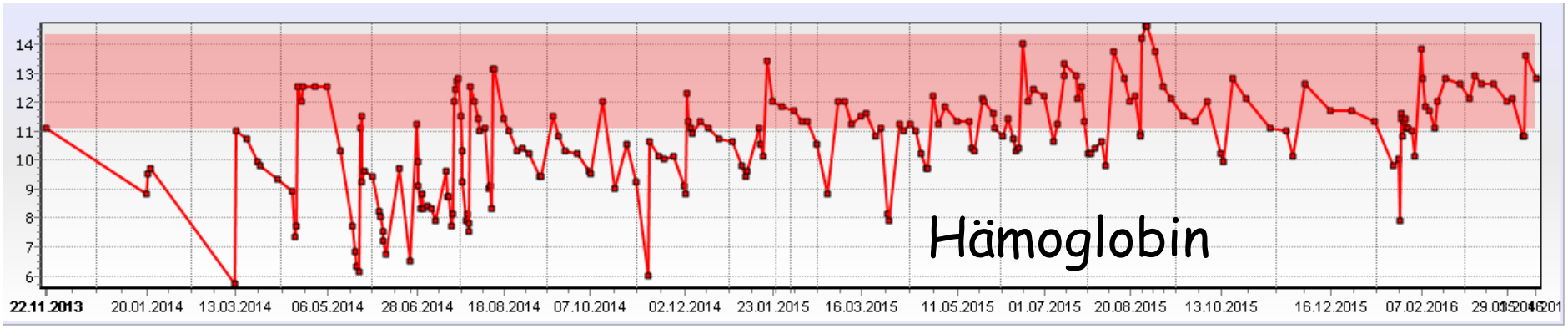
Target: GPI
glycosylphosphatidylinositol (GPI) molecule

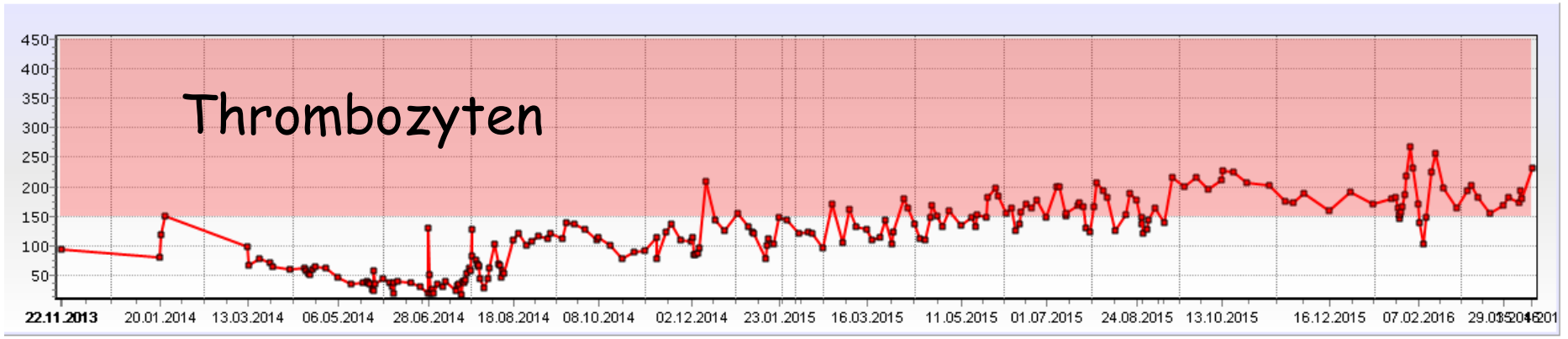
Target: other molecules

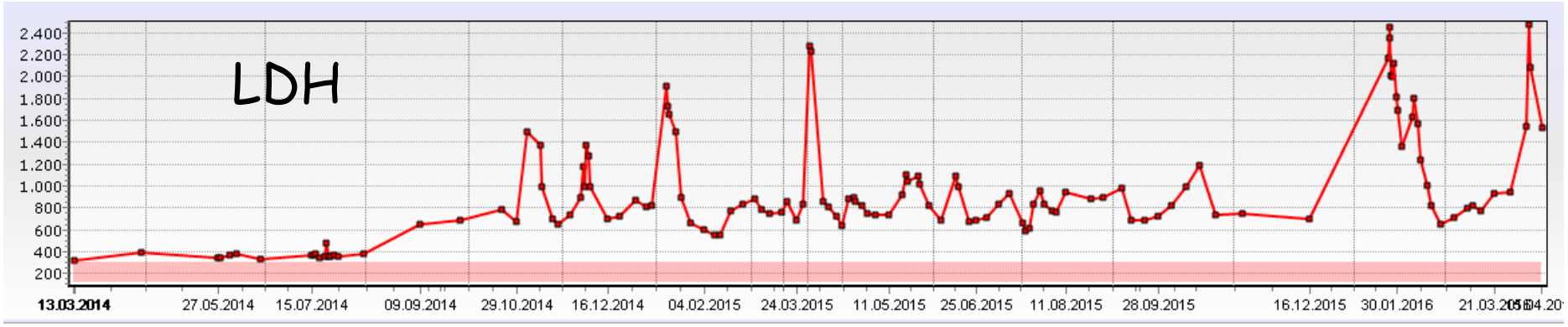
Expansion of GPI-negative clone

APLASTIC ANAEMIA

PNH


Hämoglobin
14
13
12
11
10
9
8
7
6
22.11.2013
20.01.2014
13.03.2014
06.05.2014
28.06.2014
18.08.2014
07.10.2014
02.12.2014
23.01.2015
16.03.2015
11.05.2015
01.07.2015
20.08.2015
13.10.2015
16.12.2015
07.02.2016


Thrombozyten
450
400
350
300
250
200
150
100
50
22.11.2013
20.01.2014
13.03.2014
06.05.2014
28.06.2014
18.08.2014
08.10.2014
02.12.2014
23.01.2015
16.03.2015
11.05.2015
01.07.2015
24.08.2015
13.10.2015
16.12.2015
07.02.2016


LDH
2.400
2.200
2.000
1.800
1.600
1.400
1.200
1.000
800
600
400
200
13.03.2014
27.05.2014
15.07.2014
09.09.2014
29.10.2014
16.12.2014
04.02.2015
24.03.2015
11.05.2015
25.06.2015
11.08.2015
28.09.2015
16.12.2015
30.01.2016

Patientin 7a

Frühgeburt SSW 35+4. Postpartal bei V.a. peripartale Sepsis Thrombopenie des Neugeborenen (Thrombozyten 102.000) Coombs Test und AK – Suchtest negativ.

Thrombozytenwerte 70-100.000, bei Infekten Abfall bis 40.000 Thrombozyten – **chronische ITP**

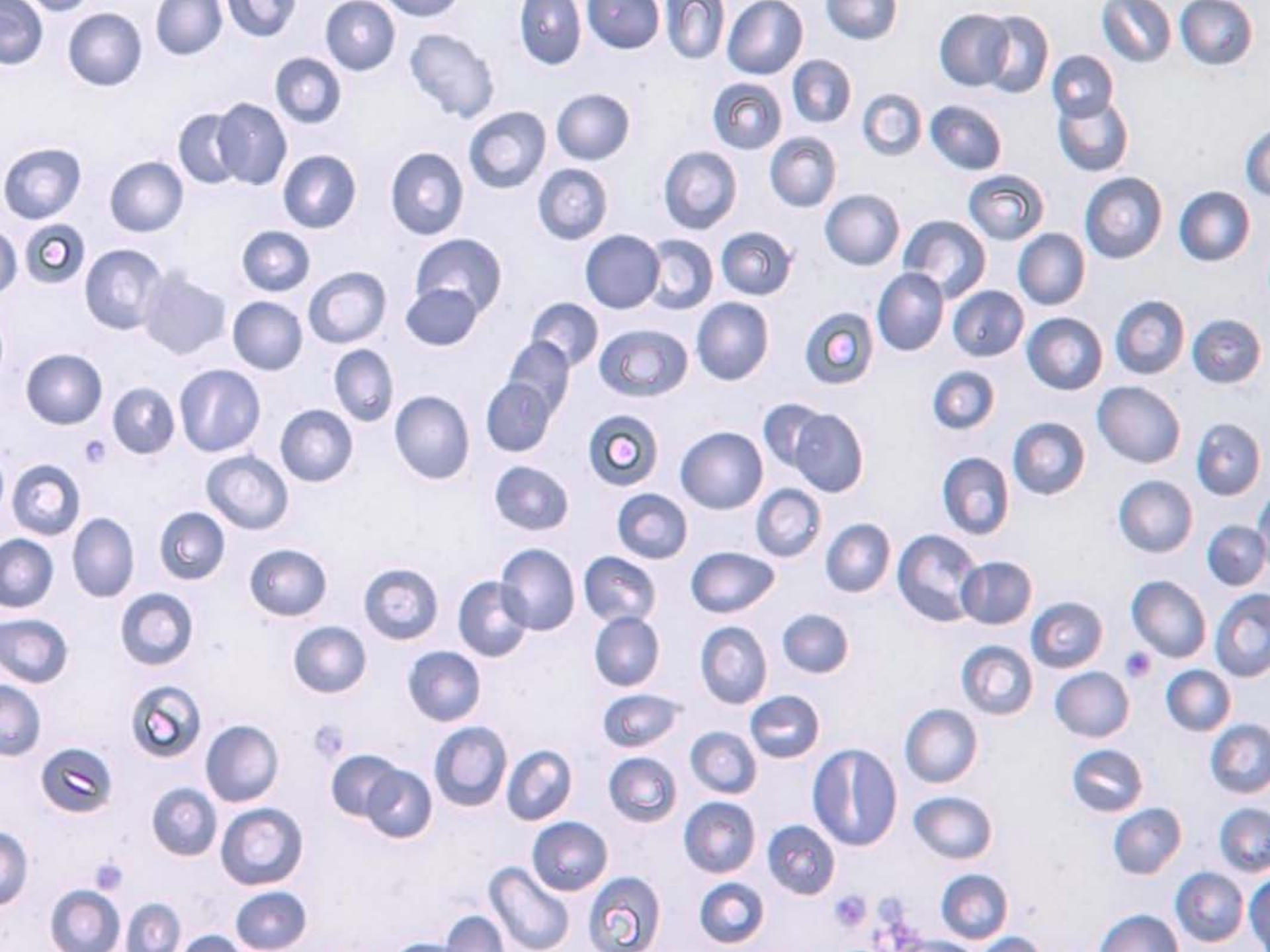
Bis auf eine Pneumonie im 8. Lebensmonat immer ‚gesund'.
Anamnestisch keine schweren Infekte oder Blutungszeichen.

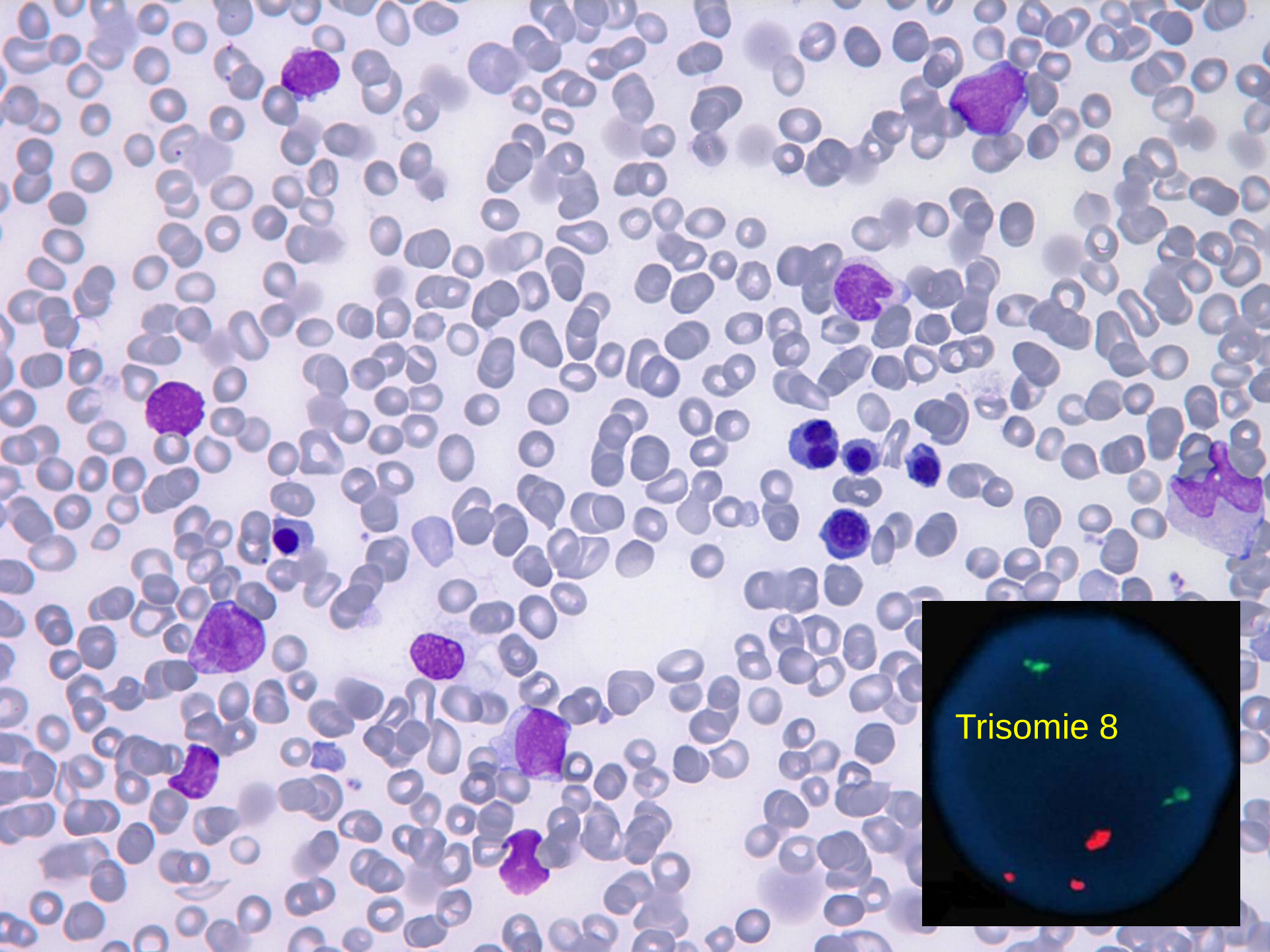
Zuletzt einmalig Epistaxis bei 27.000 Thrombozyten, anschließend Nadir von 19.000 Thrombozyten.

Wechselnde Panzytopenie (geringe Anämie und Leukopenie)

HÄMATOLOGISCHE BEFUNDE

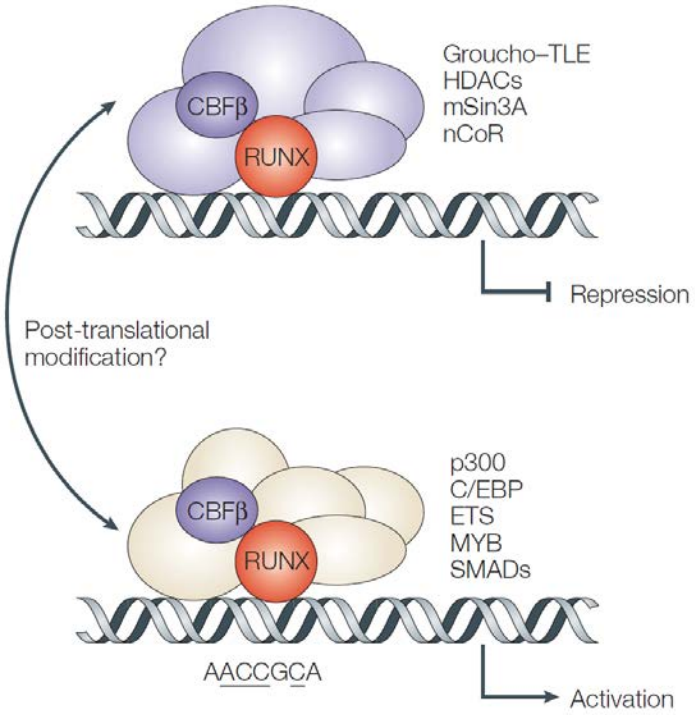
Leukozyten	*3.16* G/l	4.50-13.00
Erythrozyten	*3.56* T/l	4.00-5.20
Hämoglobin	*10.8* g/dl	12.0-16.0
Hämatokrit	34 %	33-45
MCV (mittl.Zellvolumen)	94 fl	78-96
MCH (mittl.Zell-Hb)	30 pg	25-35
MCHC (mittl.Zell-Hb-Konz.)	32 g/dl	30-37
Erythrozytenverteilungsbreite	*21.1* %	11.5-14.5
Retikulozyten	25 ‰	2-28
Thrombozyten	**23** G/l	150-450
MPV (mittleres Plättchenvol.)	11 fl	7-11 fl
Stabförmige Neutrophile	0.03 abs./G/l	0.00-0.68
Neutrophile	1.80 abs./G/l	1.50-8.10
Lymphozyten	*0.92* abs./G/l	1.30-7.00
Monocyten	0.41 abs./G/l	0.15-1.40




Trisomie 8

Myelodysplastisches Syndrom / RAEB

Refractory anemia with excess of blasts
15% Blasten im Knochenmark, Trisomie 8 (80%)



RUNX1-Deletion

Familial platelet disorder (FPD) with a predisposition to acute myeloid leukemia (AML)

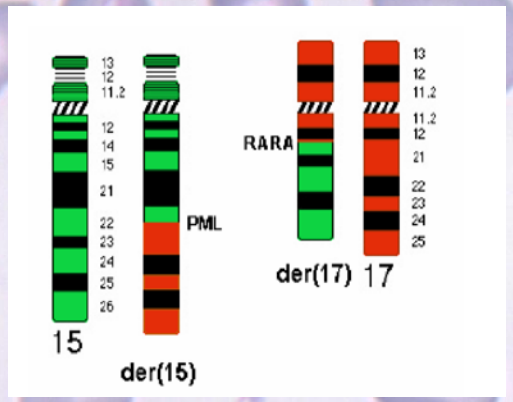
Patientin 9 Jahre

Bisher keine ernsthaften Erkrankungen. Seit einer Woche Epistaxis bei Tonsillitis, Schmerzen im linken Arm – zunehmende Blutungszeichen, V.a. ITP, 1x Ig Gabe

Guter Allgemeinzustand, < 100 Petechien, 5 kleine Hämatome bds. Unterschenkel, Schulter und Ellbogen links bewegungseingeschränkt und Schmerzen bei Bewegung, keine Ln Vergrößerungen, keine Organomegalien, neurologisch unauffällig

BEFUNDE

Leukozyten	12.01 G/l	4.50-13.00
Erythrozyten	2.15 T/l	4.00-5.20
Hämoglobin	6.3 g/dl	12.0-16.0
Hämatokrit	17 %	33-45
MCV	79 fl	78-96
MCH	29 pg	25-35
MCHC	37 g/dl	30-37
RDW	15.4 %	11.5-14.5
Thrombozyten	19 G/l	150-450
MPV	8 fl	7-11
LDH	582 U/l	120-300
Harnsäure	2.6 mg/dl	0.0-6.0
Kreatinin	0.33 mg/dl	0.20-0.70
Blasten	6.49 G/l	
Metamyelocyten	0.24 G/l	
Neutrophile	0.36 G/l	1.50-8.10
Lymphozyten	4.80 G/l	1.30-7.00
Monocyten	0.12 G/l	0.15-1.40



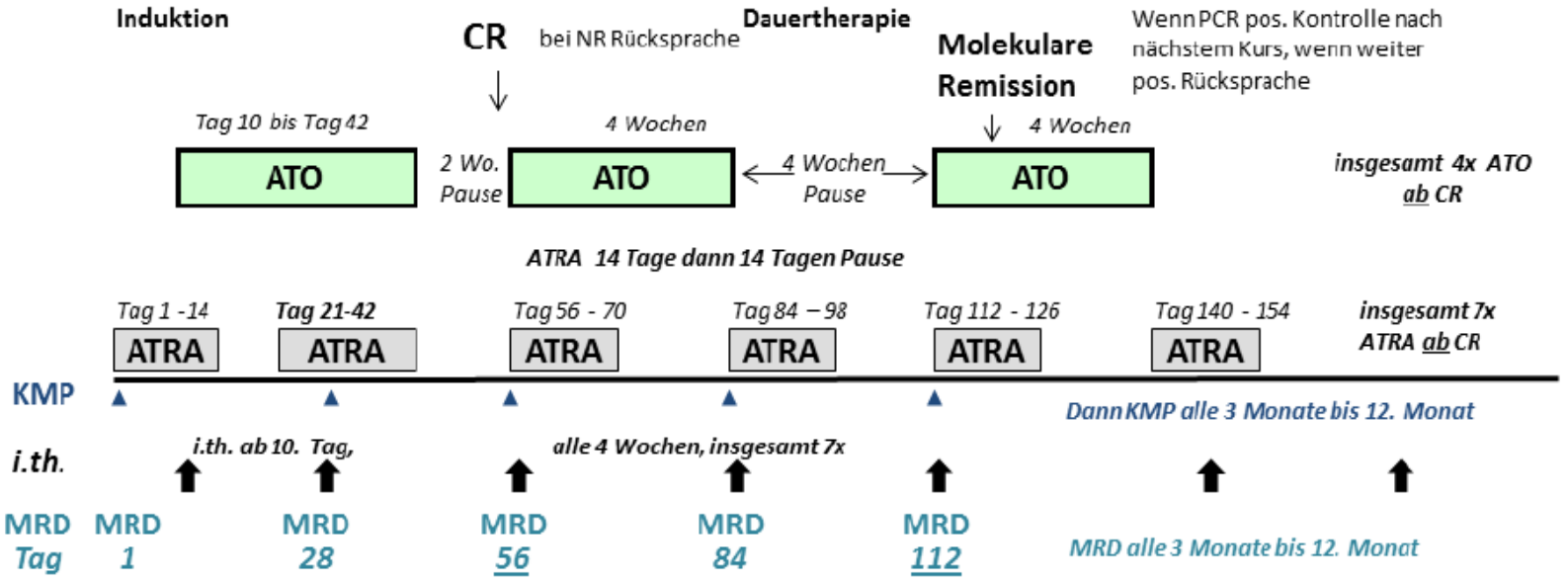
Akute myeloische Leukämie, FAB M3 (APL)

Auer pos., t (15;17), PML/RARa Rearrangement

ZNS-Befall

APL-BFM Empfehlung Standardrisiko

FAB M3 (PML/RARA): ATRA + ATO bei Pat. mit WBC < 10 000/µl



Bei Hinweisen für ein beginnendes ATRA-Syndrom (Kopfschmerzen, Pleura/Perikarderguss, pulmonale Infiltrate, Fieber) sofort Therapie mit Dexamethason (10–15 mg/m²) einleiten

ATRA: Alltrans-Retinol Säure 25mg/m²/Tag oral in 2 geteilten Dosen für 14 Tage, dann 14 Tage Pause, insgesamt 7x ab CR (Ausnahme 2. ATRA Block!)

ATO: Arsentrioxid 0.15 mg/kg/Tag *i.v.* über 1-2 Stunden beginnend am Tag 10 bis zur CR.

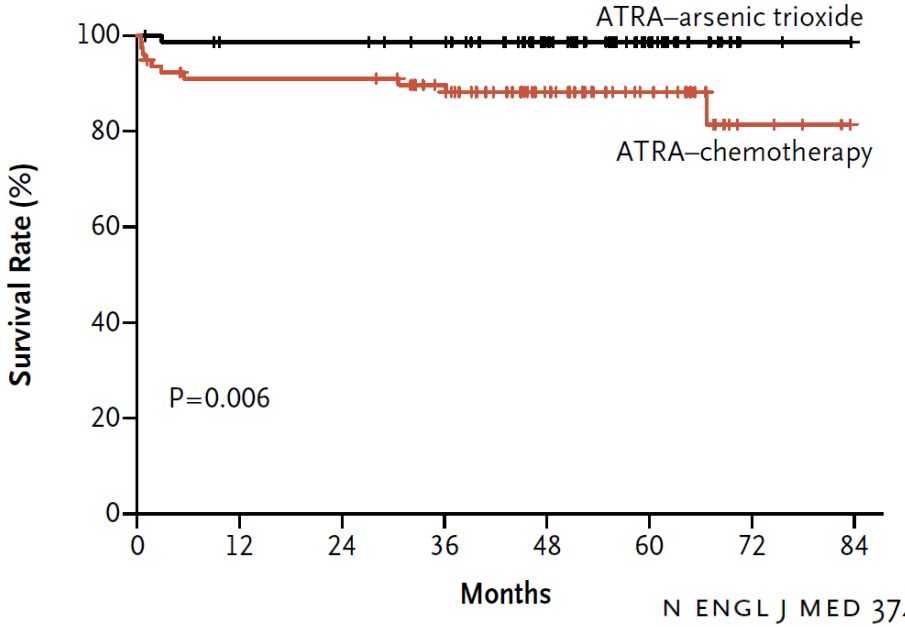
Nach 2 Wochen Pause: Montag – Freitag jeweils 0.15 mg/kg/Tag *i.v.* für 4 Wochen – 4 ATO Zyklen je 4 Wochen – 4 Wochen Pause

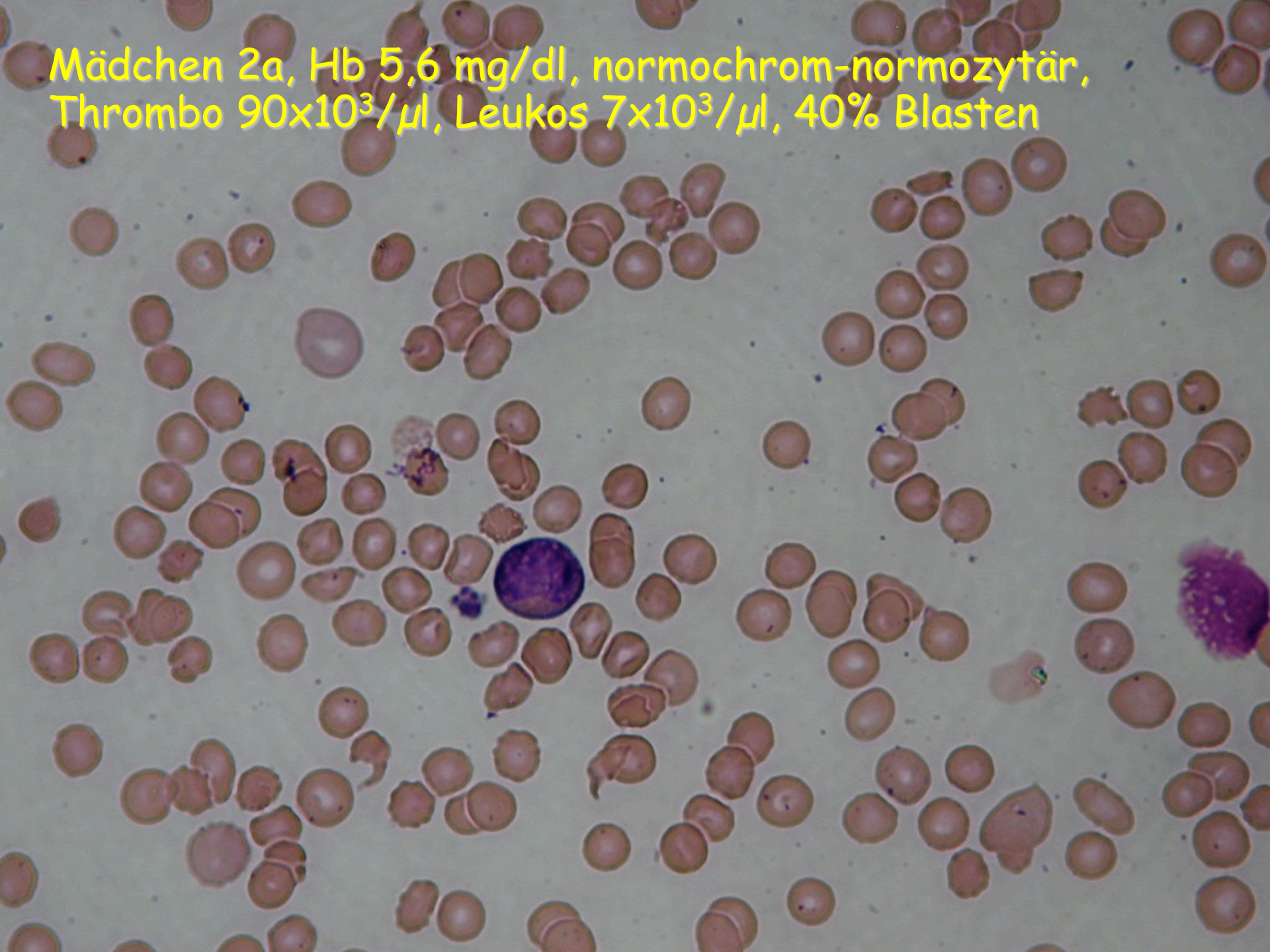
Nebenwirkungen ATO:
Gewichtszunahme, Flüssigkeitsretention, Leukozytose, Verlängerung des QT-Intervalls.

EKG Kontrolle jeweils vor ATO Kurs
Möglich: Periphere Neuropathie, Hyperglykämie, Hautreaktionen.

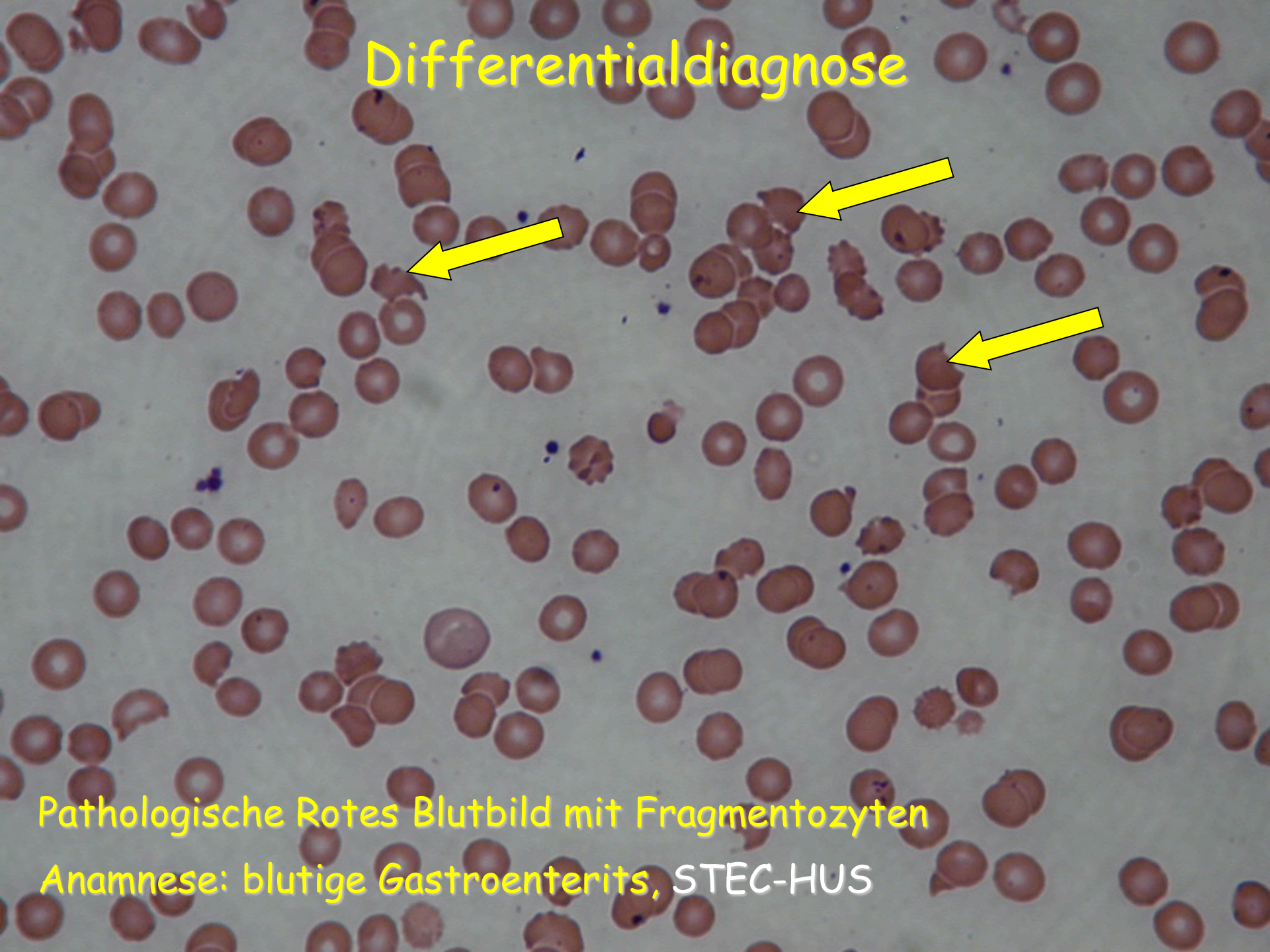
CR	Komplette Remission
MRD:	Minimal residual disease
KMP:	Knochenmarkpunktion
i.th.	Intrathekal Cytarabin altersabhängig


SKISCHULE
Riesneralm


ATRA–arsenic trioxide
ATRA–chemotherapy
Survival Rate (%)
100
80
60
40
20
0
P=0.006
0
12
24
36
48
60
72
84
Months


Mädchen 2a, Hb 5,6 mg/dl, normochrom-normozytär,
Thrombo 90x10³/µl, Leukos 7x10³/µl, 40% Blasten

Differentialdiagnose

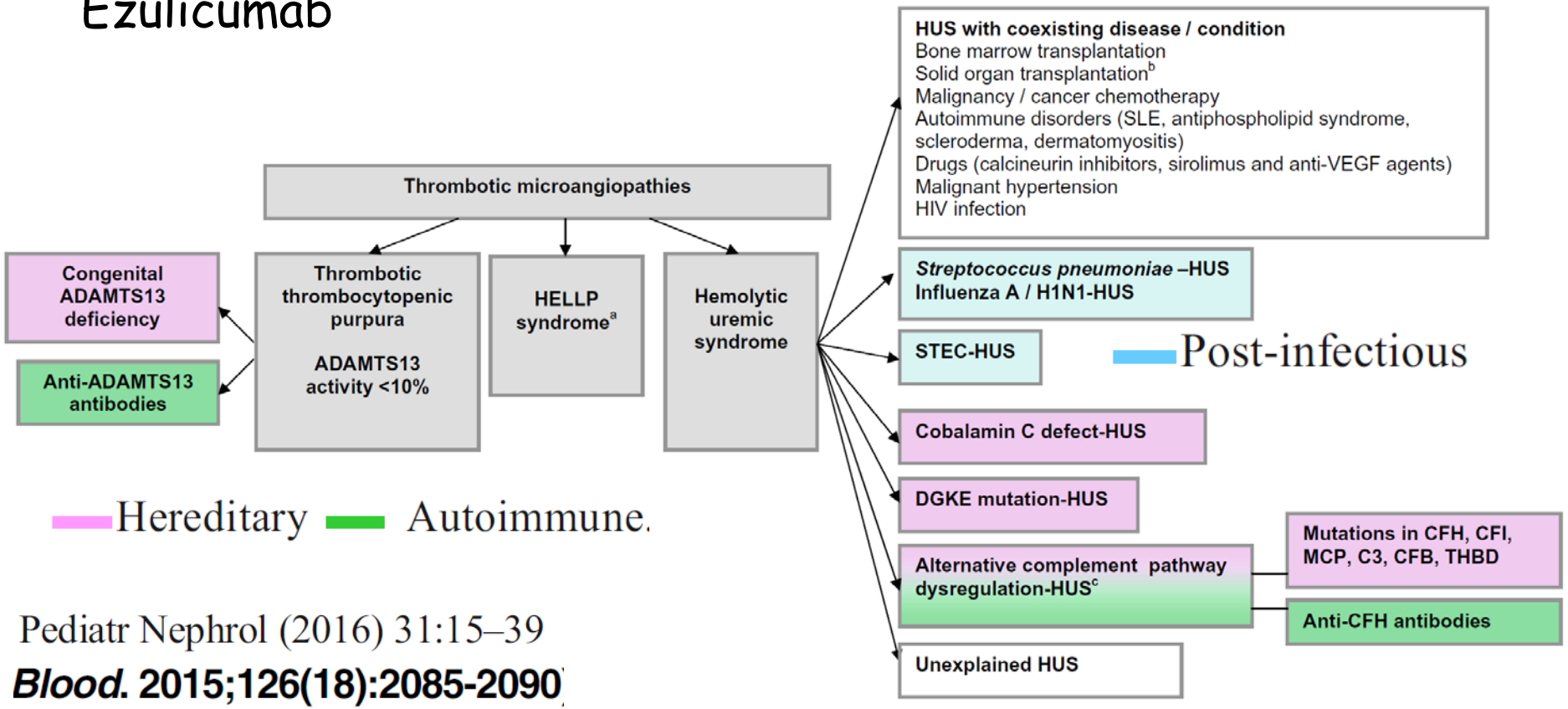


Pathologische Rotes Blutbild mit Fragmentozyten

Anamnese: blutige Gastroenterits, STEC-HUS

Hämolytisch urämisches Syndrom (HUS)

- Typisches HUS (STEC-HUS), Shiga-Toxin von entero-hämorrh. E.coli -> 25% STEC-HUS -> 25% chron. Niereninsuff., 1-5% Mortalität
- Atypisches HUS (aHUS), chron. Rezidivierend, >50% end-stage Nierenversagen, bis 25% Mortalität – gebessert seit Ezulicumab



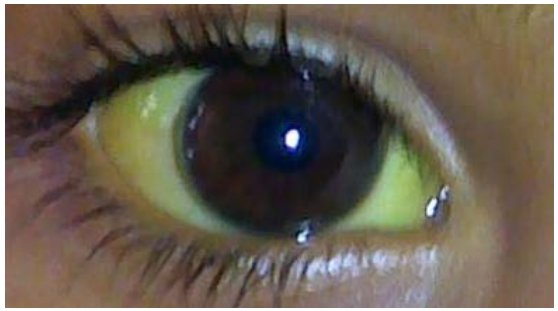
Pediatr Nephrol (2016) 31:15–39

Blood. 2015;126(18):2085-2090

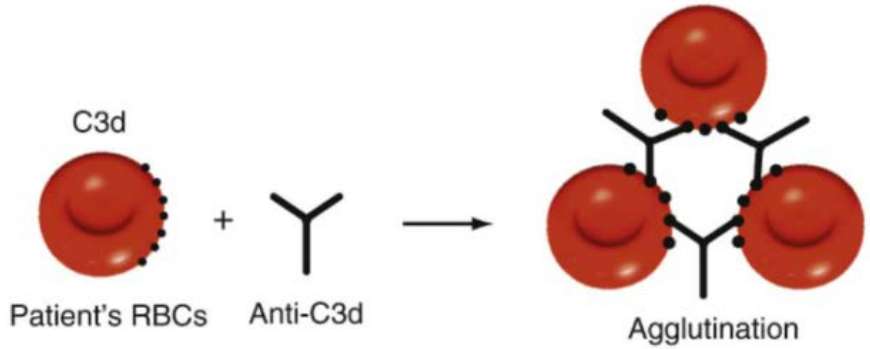
Hämolytische Anämien

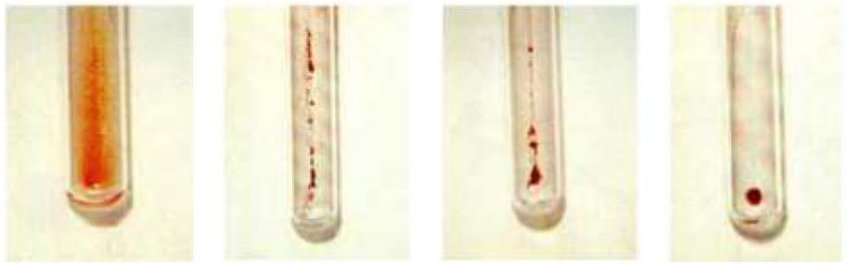
- Hämolyseparameter (indirektes Bili + LDH erhöht, Ahaptoglobinämie)

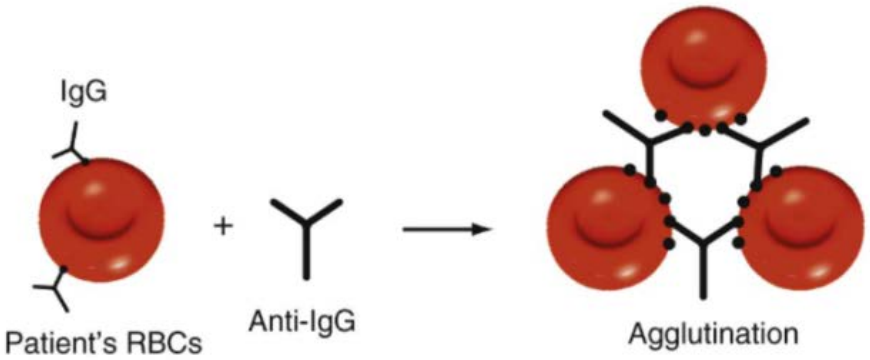
- Urinteststreifen: Hämoglobinurie – sofortige Klinikeinweisung

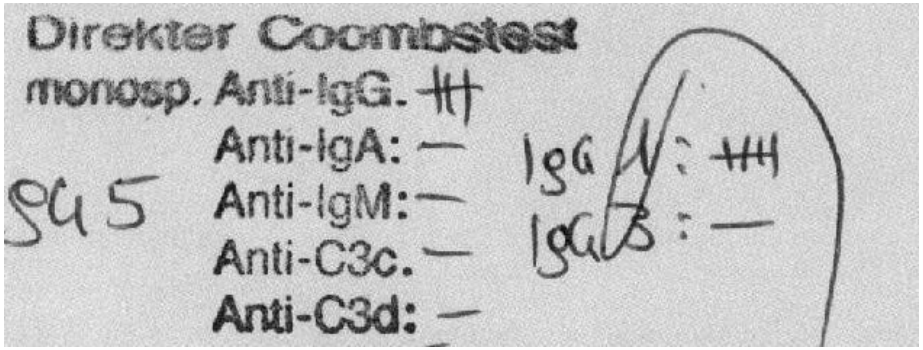


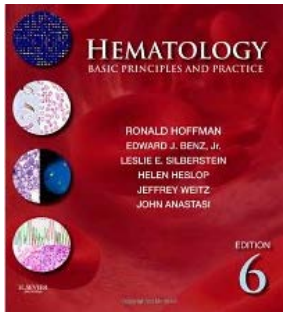
AIHA – Direkter Antiglobulin Test (DAT, Coombs Test)





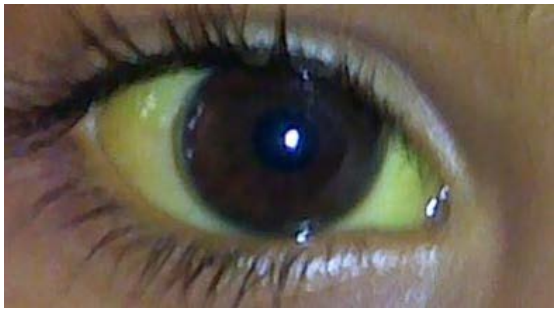






Jäger U, Lechner K. Autoimmune Hemolytic Anemia. Hematology-Basic Principles and Practice.; 6th Edition, 2013, Hoffman R, Benz EJ, Silberstein LE, et al. editors, Elsevier

Patient



- 1a alter Knabe mäßiger AZ
- Anamnestisch seit 2 Tagen Fieber und zunehmender Ikterus, kein Auslandsaufenthalt, lebt seit 4 Monaten bei Pflegeeltern wegen schwerer Vernachlässigung
- klin. Status: Sklerenikterus, Tachykardie (Hf 156/min), Af normal, O_2 Stg. 100%, keine Organomegalie

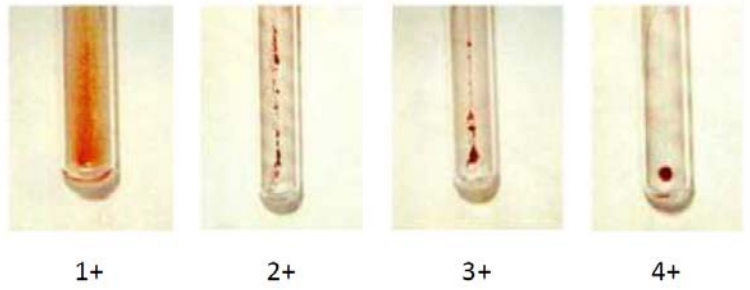
Blutbild

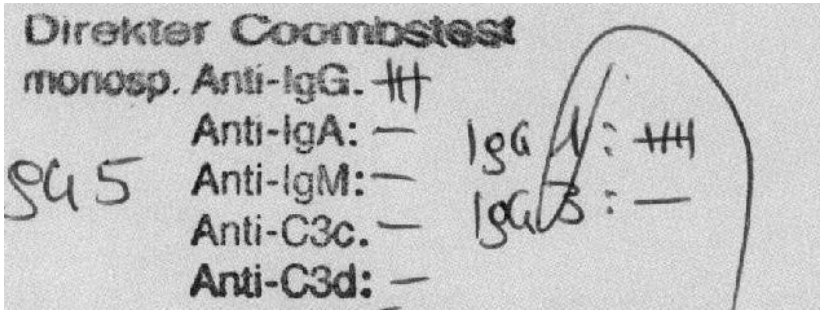
HÄMATOLOGISCHE BEFUNDE

Leukozyten	11.32	G/l
Erythrozyten	1.56	T/l
Hämoglobin	4.5	g/dl
Hämatokrit	12	%
MCV (mittl.Zellvolumen)	77	fl
MCH (mittl.Zell-Hb)	29	pg
MCHC (mittl.Zell-Hb-Konz.)	38	g/dl
Erythrozytenverteilungsbreite	15.3	%
Retikulozyten	44	‰
Thrombozyten	295	G/l
MPV (mittleres Plättchenvol.)	7	fl
Diff. BB: unauffällig		
Normoblasten	3.0	

Labor

Harnsäure	3.1mg/dl
Bilirubin gesamt	***8.8 mg/dl***
Bilirubin direkt	0.55 mg/dl
Bilirubin indirekt	***8.25 mg/dl***
GOT (ASAT)	44 U/l
GPT (ALAT)	23 U/l
Gamma-GT	6 U/l
LDH	***407 U/l***
Creatinkinase	206 U/l
Cholinesterase	7.6 kU/l
Alpha Amylase (Serum)	35 U/l
Haptoglobin	**2 mg/dl**





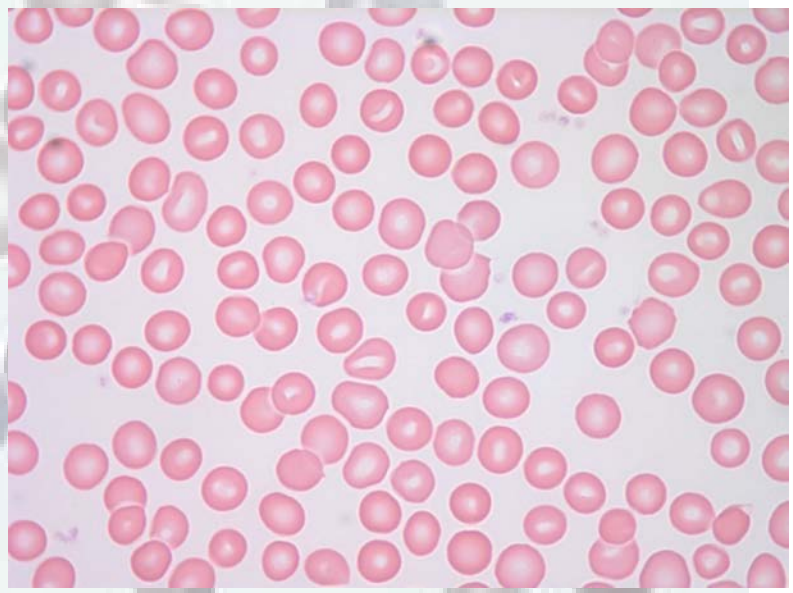
Coombstest direkt ***positiv***

Direkter Coombstest: monospezifische **Anti-IgG +++, Anti-IgG1 +++**, Anti-IgG3 -, Anti-IgA -, Anti-IgM -, Anti-C3c -, Anti-C3d –
Virologie: HSV, Hepatitis, HIV, VZV, CMV, Adeno, etc. NEGATIV

Diagnose: AIHA vom Wärmetyp

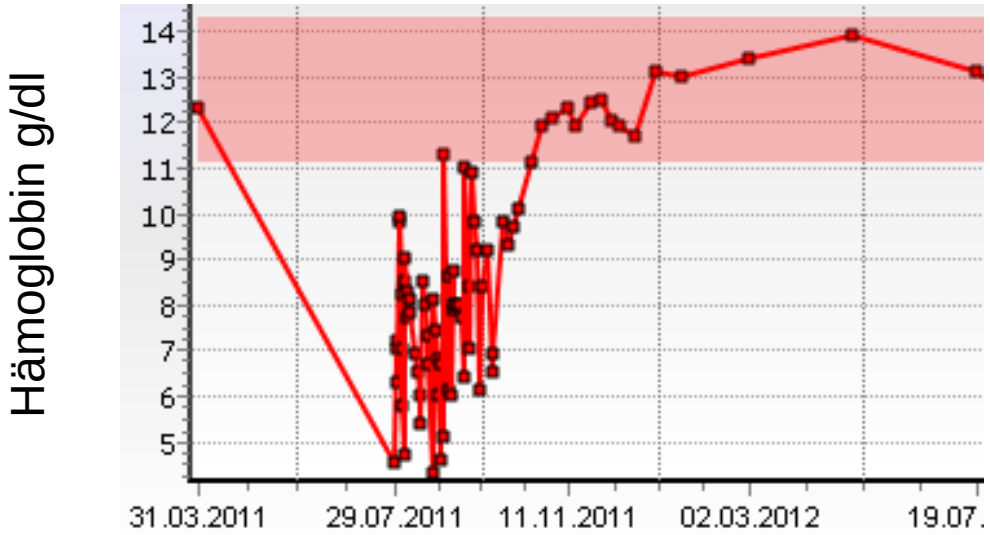
Geldrollenbildung

Mikrosphärozyten



Schweiz Med Forum 2010;10(37):626–633

Blutgruppenserologie Rhesusprotokoll: AB positiv (D+),
Erythrozyten Ak: Pan- und Autoagglutinin (indirekter Coombstest).
Transfusionsempfehlung für Erythrozytenkonzentrate: Blutgruppe AB, Rhesus positiv (D+, K-)
nach Durchführung der biologischen Vorprobe nach Oehlecker.
Direkter Coombstest (polyspezifisch): +++



Steroide 7-9/2011

+ Ig (4x) + Rituximab (9-10/2011) (4x) + EK (4x)

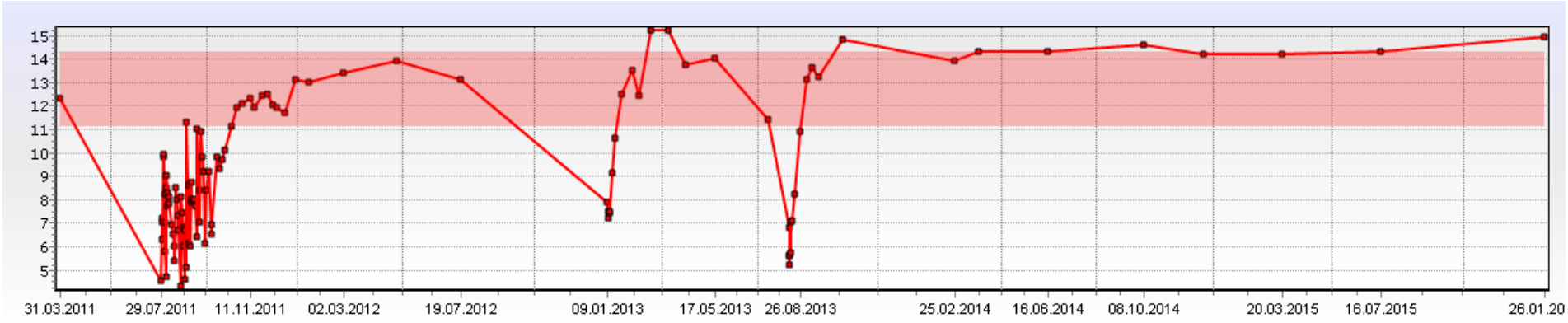
Azathioprim
9/2011 – 1/2012

Verlauf: 2 Rezidive

Therapieende: 1/2012
1. Remission

1.Rezidiv: 01/2013
Steroiddtherapie (1-4/2013)
2. Remission

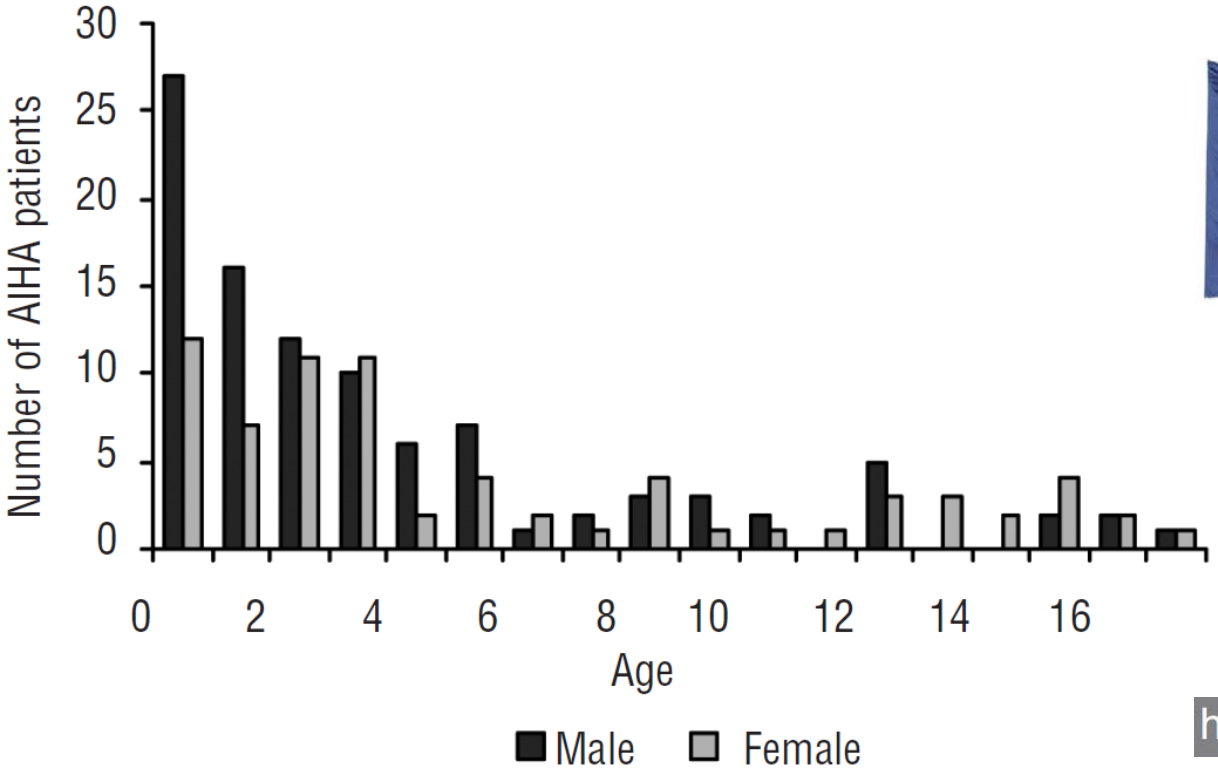
2.Rezidiv: 08/2013
Steroiddtherapie (8–10/2013)
3. Remission



Autoimmun hämolytische Anämie (AIHA)

Seltene Erkrankungen im Kindes- und Jugendalter

Inzidenz AIHAs: 0.2/Mill/Jahr (wahrscheinlich 10-fach höher?)



haematologica | 2011; 96(5)

Autoimmunhämolytische Anämien

PRIMARY AIHA*

- Warm-reactive AIHA
- Paroxysmal cold haemoglobinuria
- Cold agglutinin disease, usually IgM

SECONDARY AIHA†

- Evans' syndrome
- Autoimmune and inflammatory diseases (eg, systemic lupus erythematosus)
- Immunodeficiency (eg, autoimmune lymphoproliferative syndrome)
- Infection (eg, *Mycoplasma pneumoniae* or Epstein-Barr virus)‡
- Malignancy (eg, haematological-leukaemia or lymphoma or solid tumours)§
- Drug-induced§

Vagace JM, *et al*. *Arch Dis Child* 2014;**99**:668–673.

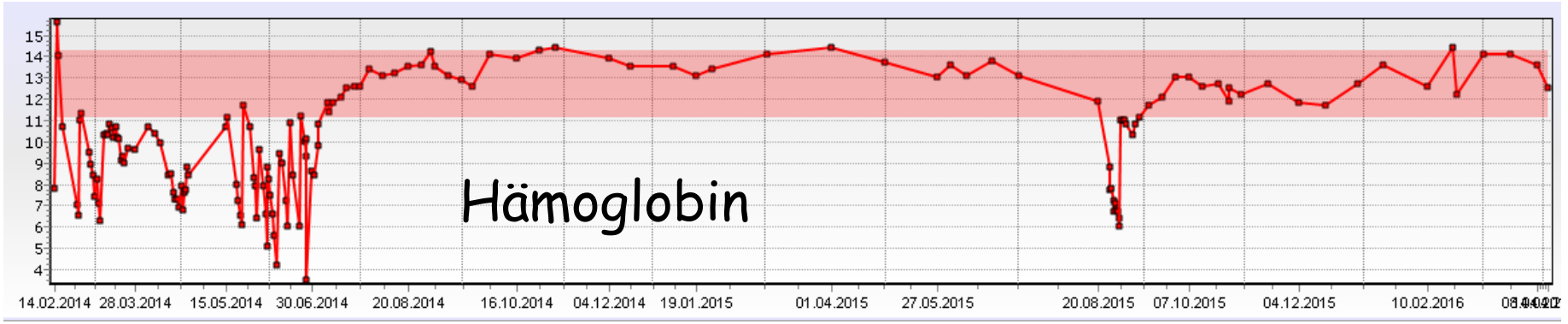
Empfohlene Untersuchungen bei AIHA

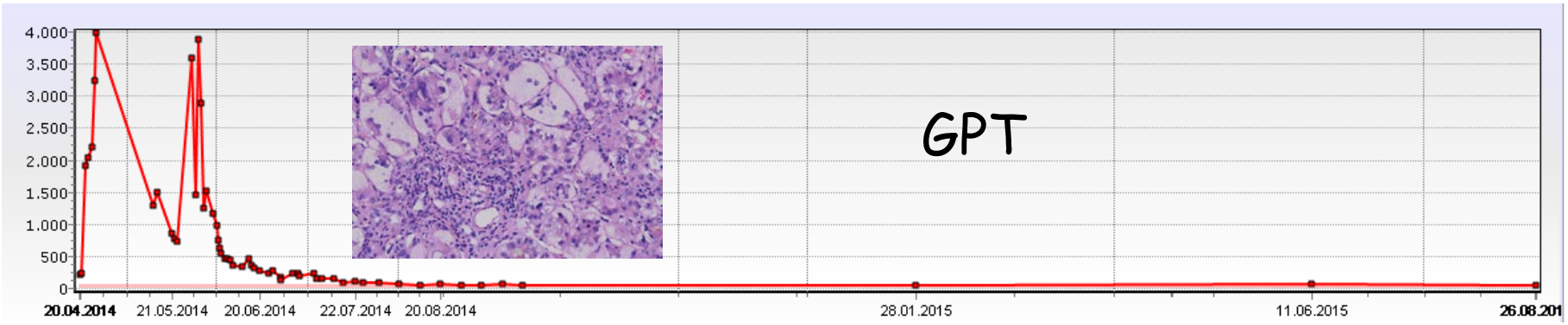
- Complete blood count (including reticulocytes)
- Blood smear.
- Biochemistry: urea, creatine, aspartate aminotransferase, alanine aminotransferase, bilirubin, haptoglobin and LDH.
- Immunoglobulins dosage (IgG, IgA and IgM)
- ANA and anti-DNA if ANA is positive.
- Immunophenotyping of peripheral lymphocytes (should include the quantification of 'double-negative T cells: CD3CD4-CD8-TCRα/β+' if an ALS is suspected)
- Lupus anticoagulant and antiphospholipid antibodies (if antiphospholipid syndrome is suspected or systematically before splenectomy)
- Bone marrow aspiration (if pancytopenia, lymphadenopathy or visceromegaly)
- Chest X-ray and abdominal sonography (if a secondary AIHA is suspected).
- Serological test for Epstein-Barr virus and/or *Mycoplasma pneumoniae* (if cold agglutinin disease has been diagnosed)

Vagace JM, *et al*. *Arch Dis Child* 2014;**99**:668–673.

Autoimmunhämolytische Anämie (AIHA) und Riesenzellhepatitis

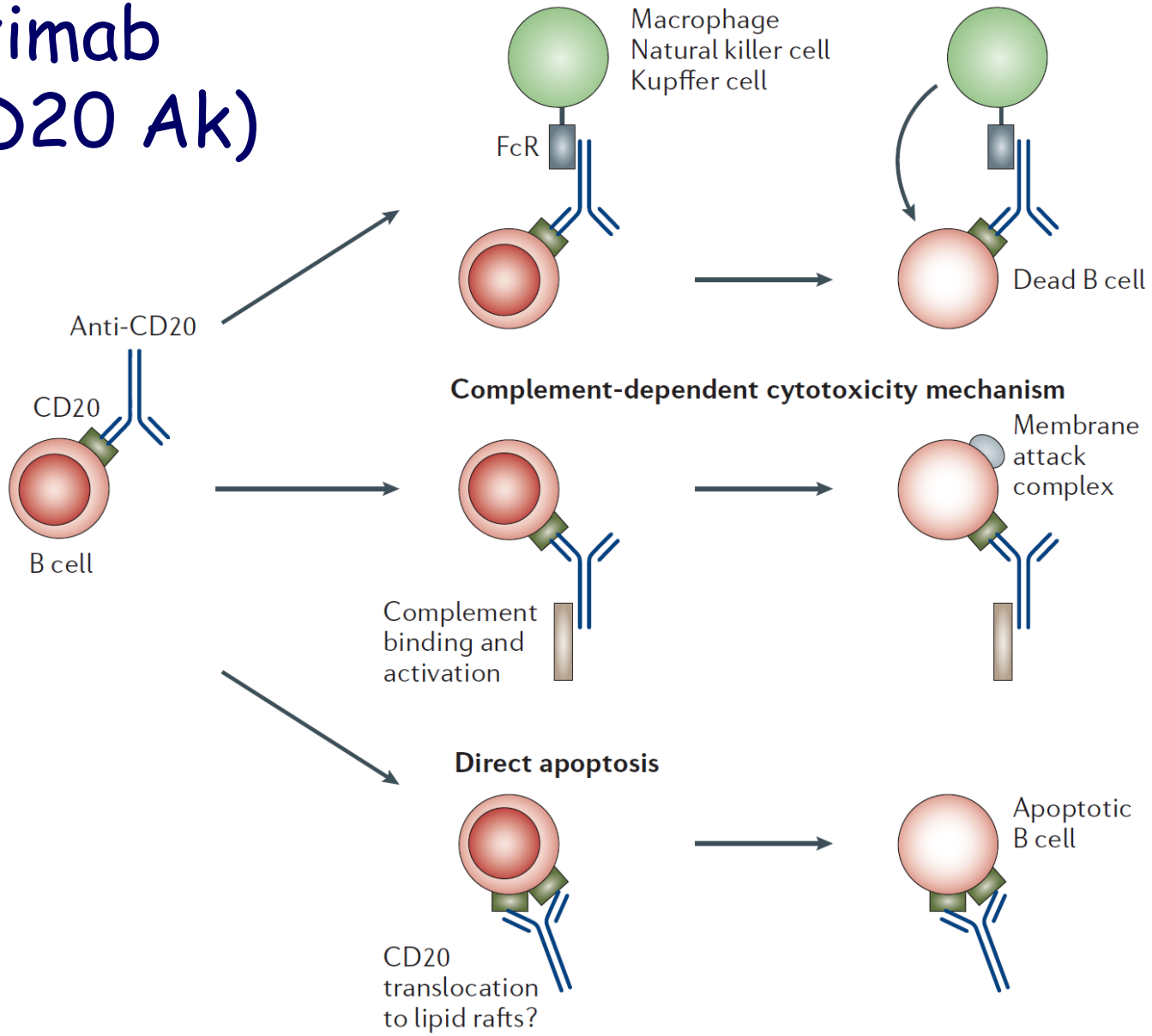
2 Monate altes Mächen, Hb 4g/dl, normochrom-normozytär, DCT pos, IgG++++, Steroid, 6-MP, Rituximab, Blutaustausch





JPGN 2014;58: 669–673

B-Zell Depletion durch Rituximab (Anti-CD20 Ak)



Patientin

- 4a altes Mädchen, dzt. unbeeinträchtigt

- Anamnestisch bei Fieber immer wieder ‚gelbe Skleren' – bei Vater die gleichen Zeichen (keine Diagnose bisher)

- klin. Status: Splenomegalie 4cm unterhalb des Rippenbogens

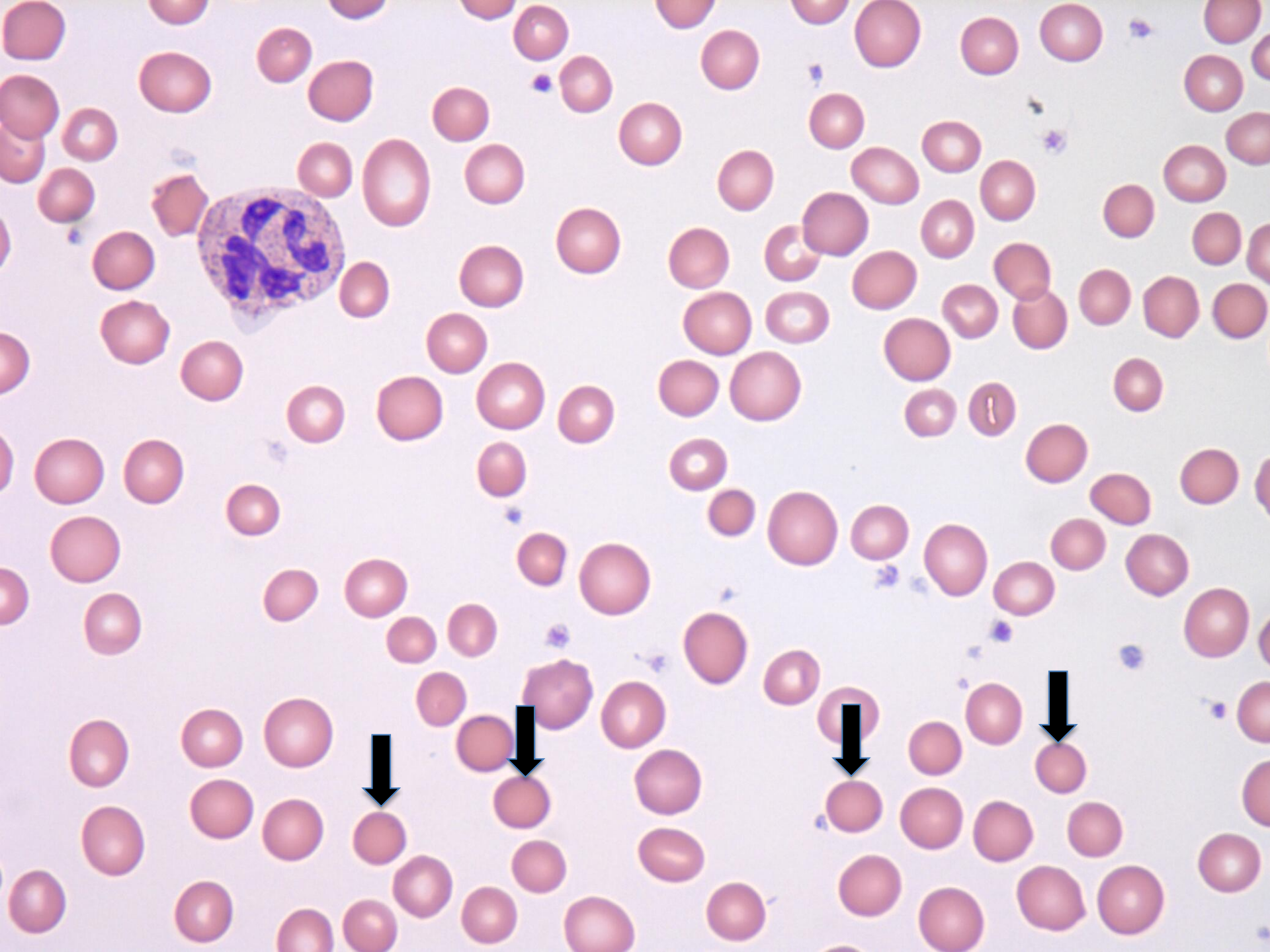
Blutbild

Leukozyten	12.73 G/l	5.00-15.00
Erythrozyten	*3.77* T/l	4.00-5.20
Hämoglobin	*9.6* g/dl	11.1-14.3
Hämatokrit	*27%*	32-43
MCV	*76* fl	75-85
MCH	26 pg	25-35
MCHC	36 g/dl	30-37
RDW	*25.0* %	11.5-14.5
Retikulozyten	92 ‰	2-28
Thrombozyten	642 G/l	150-450

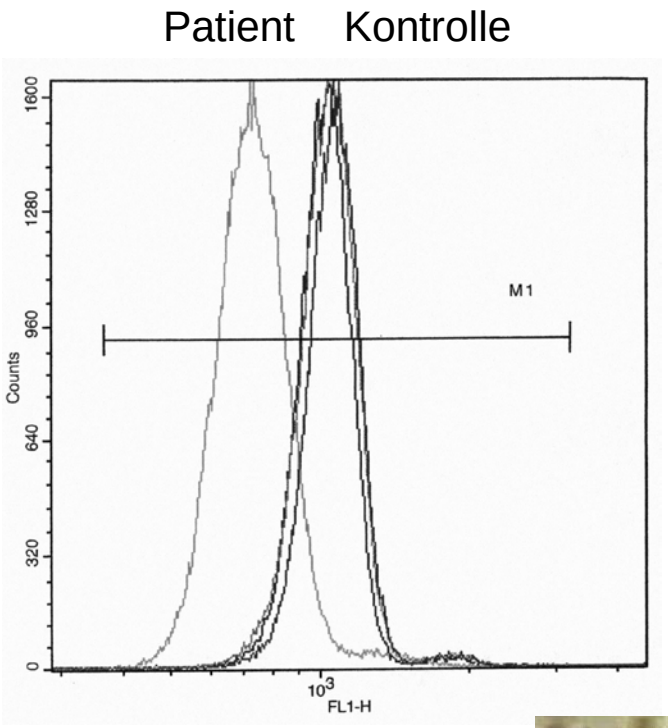
Coombstest direkt	negativ	
Haptoglobin	0 mg/dl	30-200

CHEMISCHE BEFUNDE

Bilirubin total	2,1mg/dl	0.2-1,5
Bilirubin indirekt	1.69 mg/dl	0.00-0.75
LDH	335 U/l	120-300



Bestätigung der Diagnose

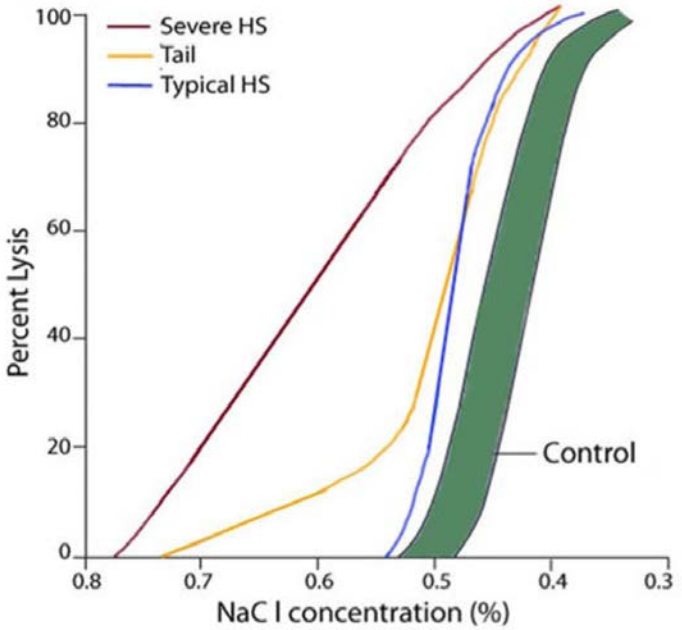


FACS-Analyse (EMA-Test)

Reduzierte Intensität der 5-Eosin-Maleimid Färbung (5-EMA)

Osmotische Resistenz

Reduzierte osmotische Resistenz in HS Zellen

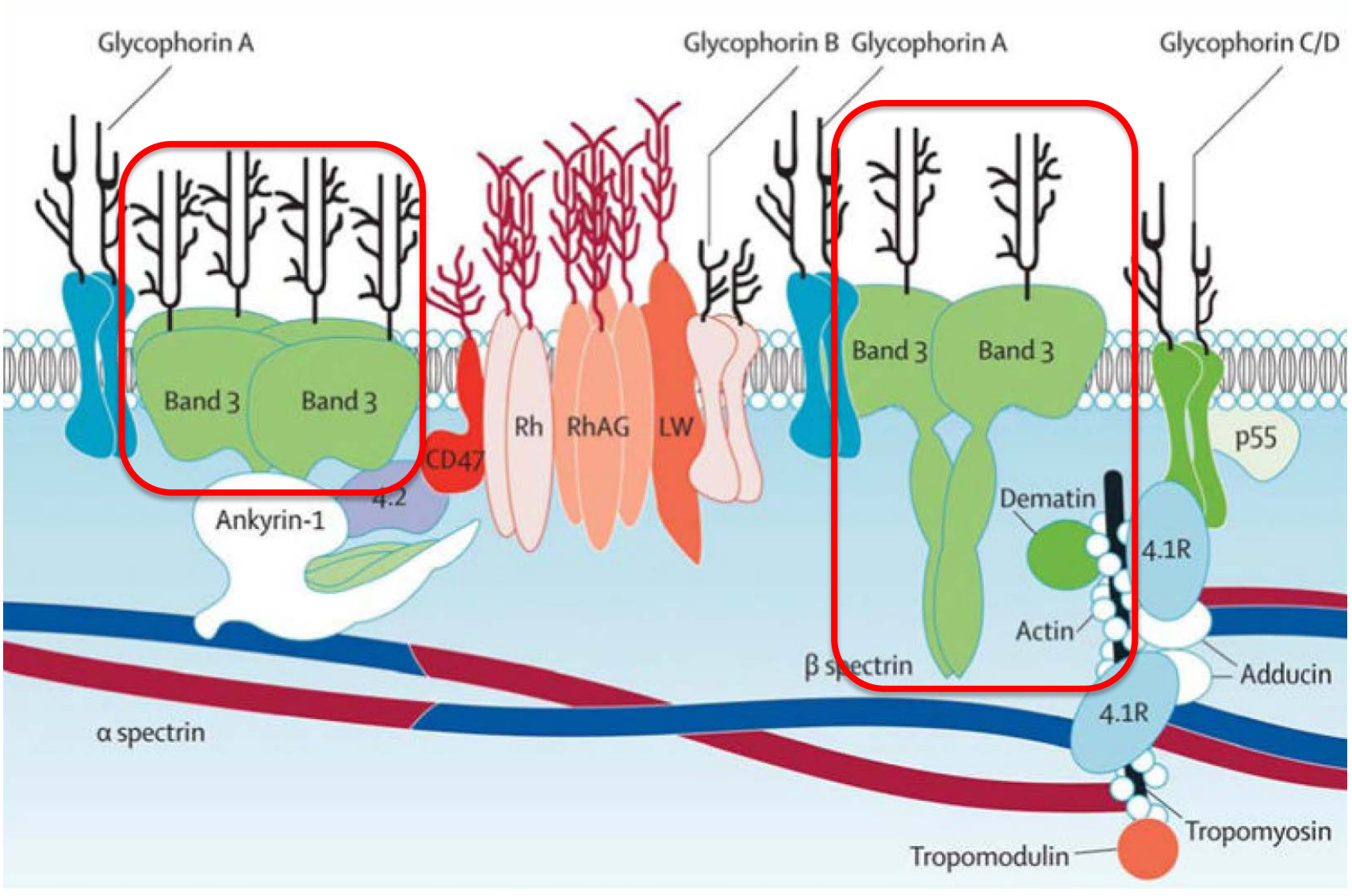


Pediatr Clin North Am. 2013 December ; 60(6): 1349–1362

Schweregrade der Sphärozytose

	Mittelschwere Sphäro-zytose	Schwere Sphärozytose[a]	Sehr schwere Sphärozytose[b]
Anteil an Patienten (%)	60–70	≈ 10	3–4
Hämoglobin			
(g/dl)	8–11	6–8	< 6
(mmol/l)	5–6,8	3,7–5	< 3,7
Retikulozyten (%)	≥ 6	≥ 10 (meist > 15)[c]	≥ 10
Bilirubin			
(mg/dl)	≥ 2	> 2–3	≥ 3
(µmol/l)	≥ 34,2	> 34,2–51,3	≥ 51,3
Sphärozyten u. a. im Blutausstrich	Deutlich vermehrt	Deutlich vermehrt	Mikrosphärozyten und Poikilozyten
Transfusionsbedarf[d]	0–2	≥ 3	Regelmäßig
Indikation zur „near-total splenectomy“	Bei mehreren hämolytischen Krisen (Hb < 8 g/dl, 5 mmol/l; > 2 Transfusionen) oder ausgeprägter Leistungsminderung	Alle Patienten, nicht vor dem 6. Jahr	Alle Patienten, nicht vor dem 6. Jahr

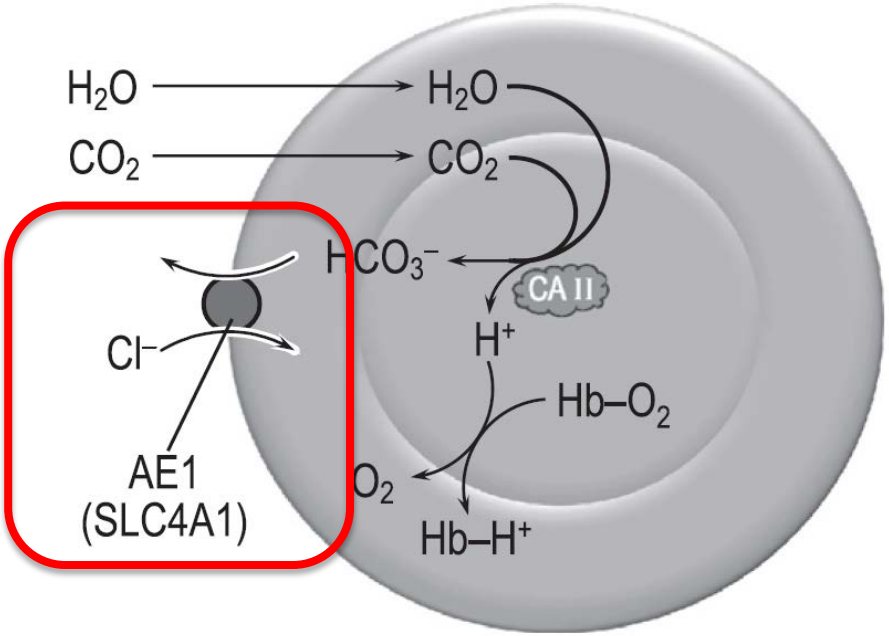
Membranopathien



Pediatr Clin North Am. 2013 December ; 60(6): 1349–1362

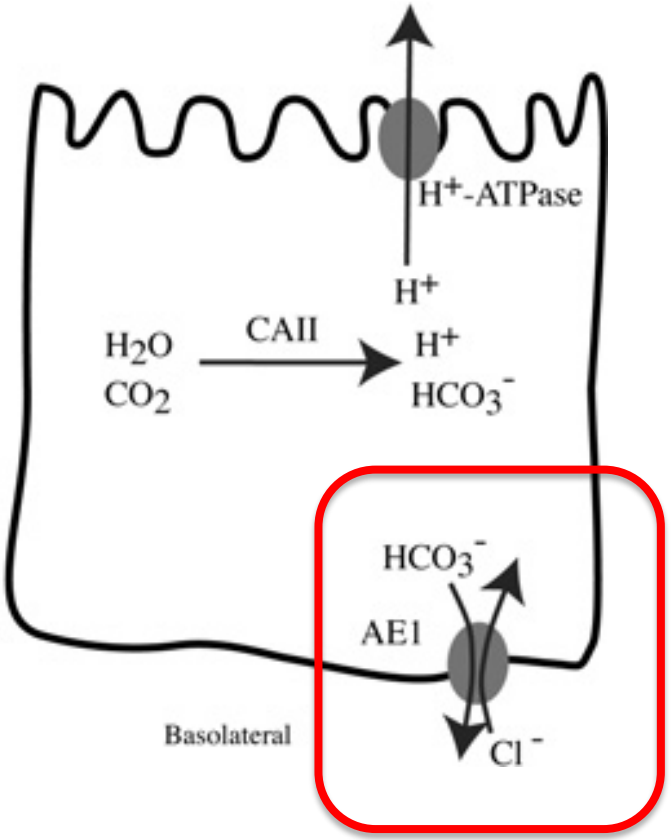
Band 3 oder Solute carrier 4A1 (SLC4A1) oder Anionenaustauscher 1 (AE1)

Erythrocyte

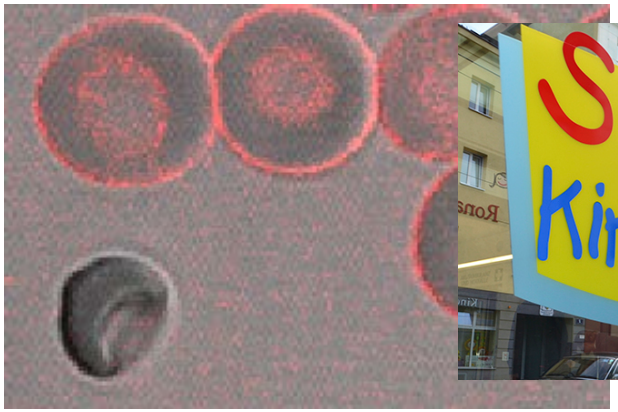


Hereditäre Sphärozytose

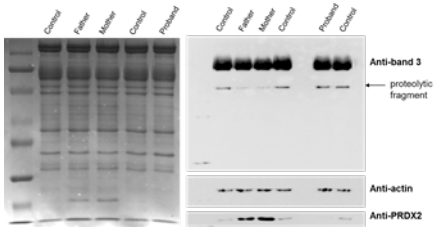
Kidney – collecting duct

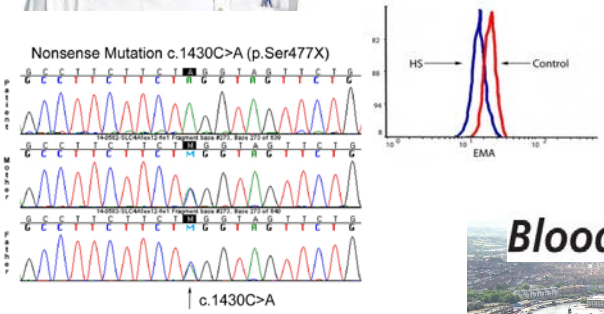


Distale renale tubuläre Azidose (dRTA)



St.Anna
Kinderkrebs
Forschung



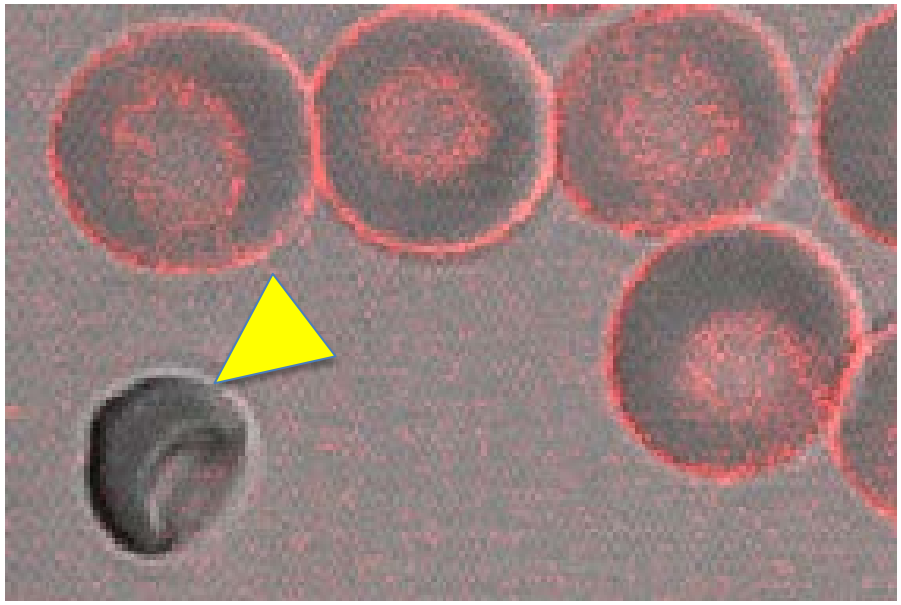


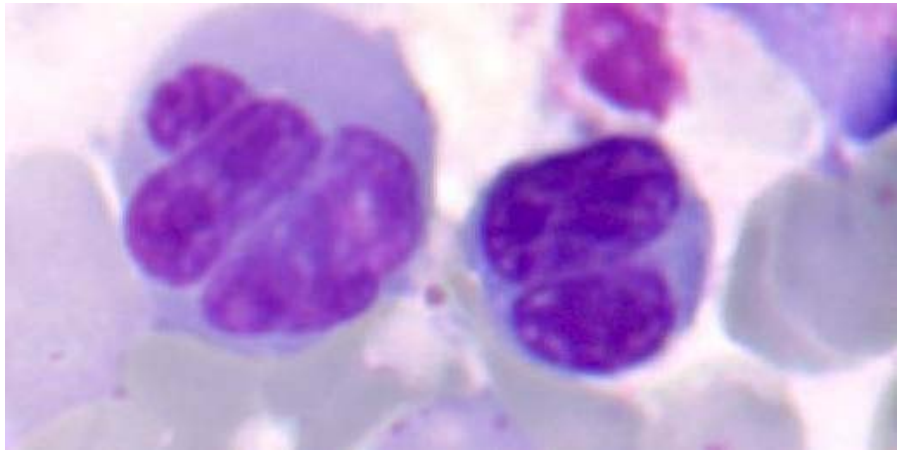
NHS

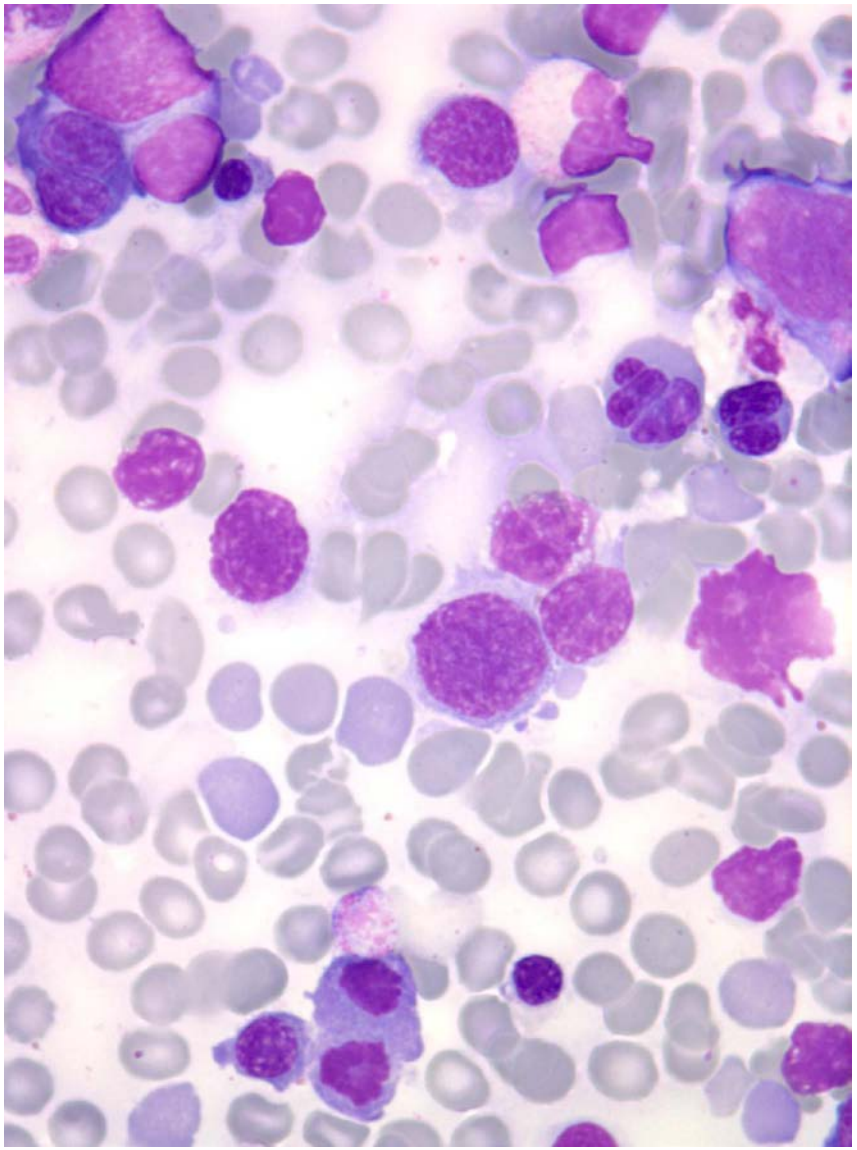
Blood and Transplant

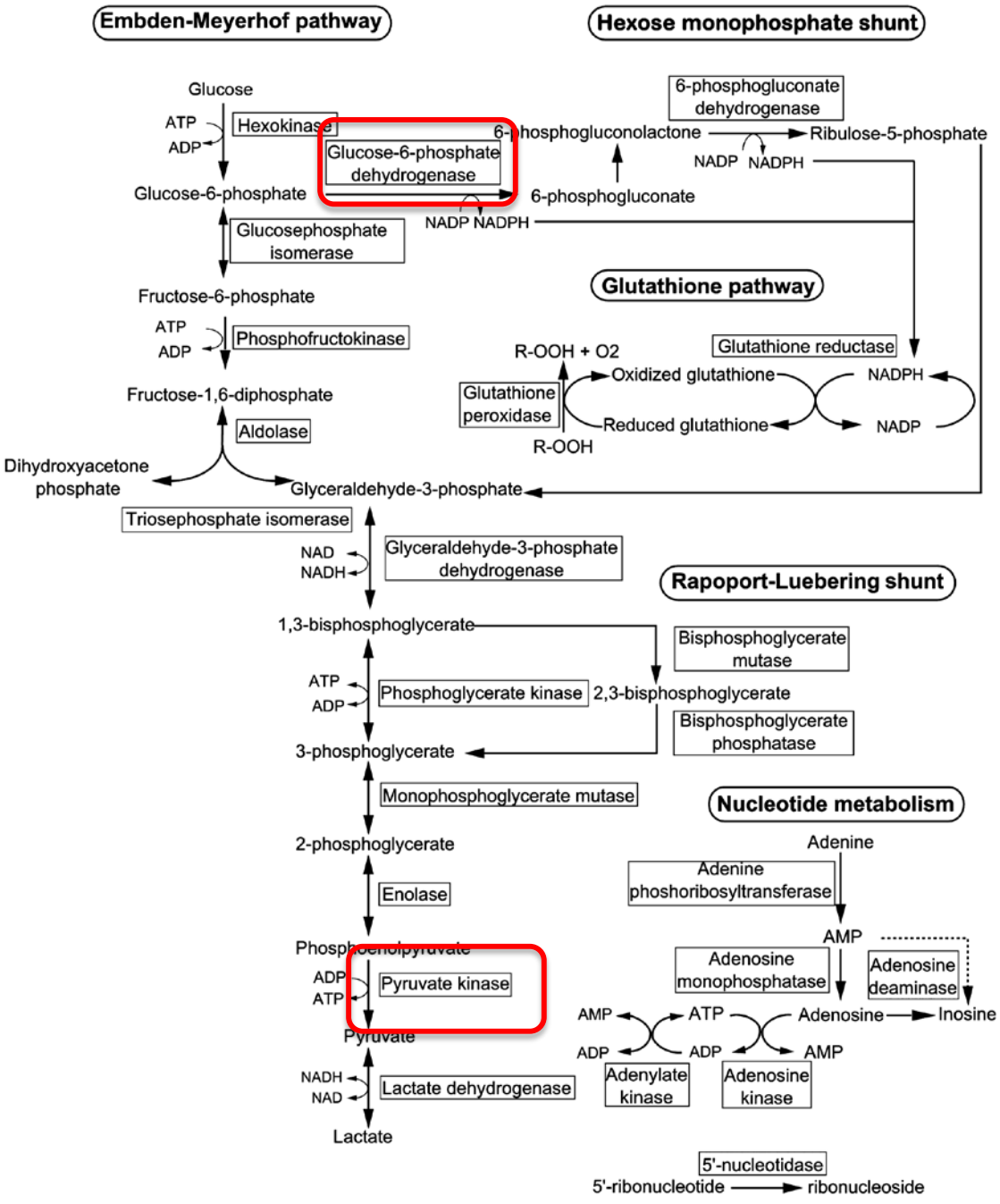
CHUC
CENTRO HOSPITALAR
E UNIVERSITÁRIO
DE COIMBRA

Band 3 null VIENNA







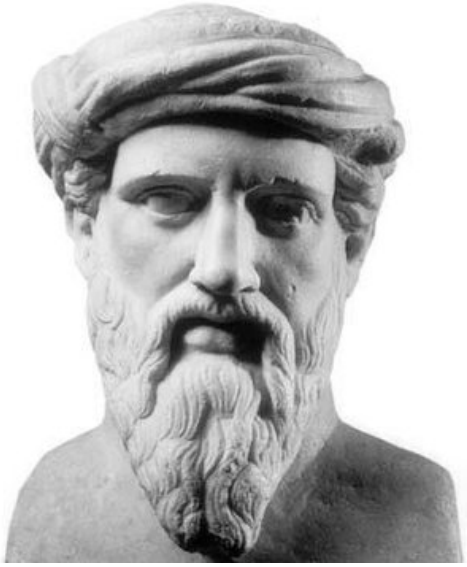


Enzymopathien

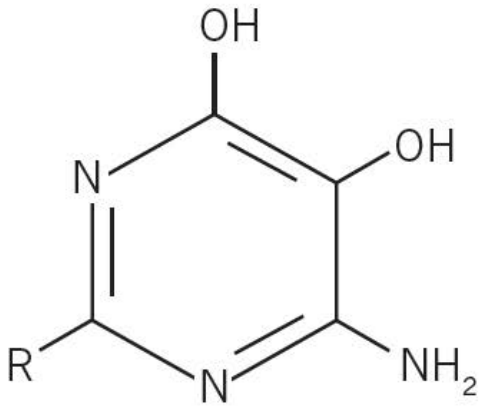
Kongenitale nicht-sphärozytische hämolytische Anämien (CNSHA)

Int. Jnl. Lab. Hem. 2014, **36**, 388–397

Glucose 6-Phosphat Dehydrogenase Mangel

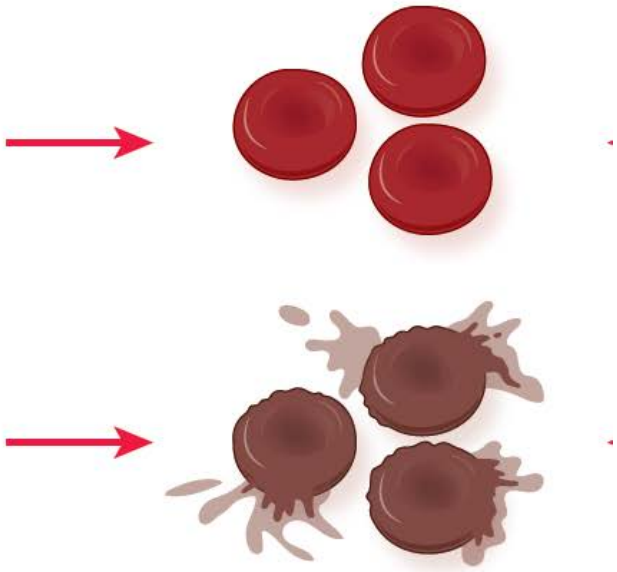


Broad beans



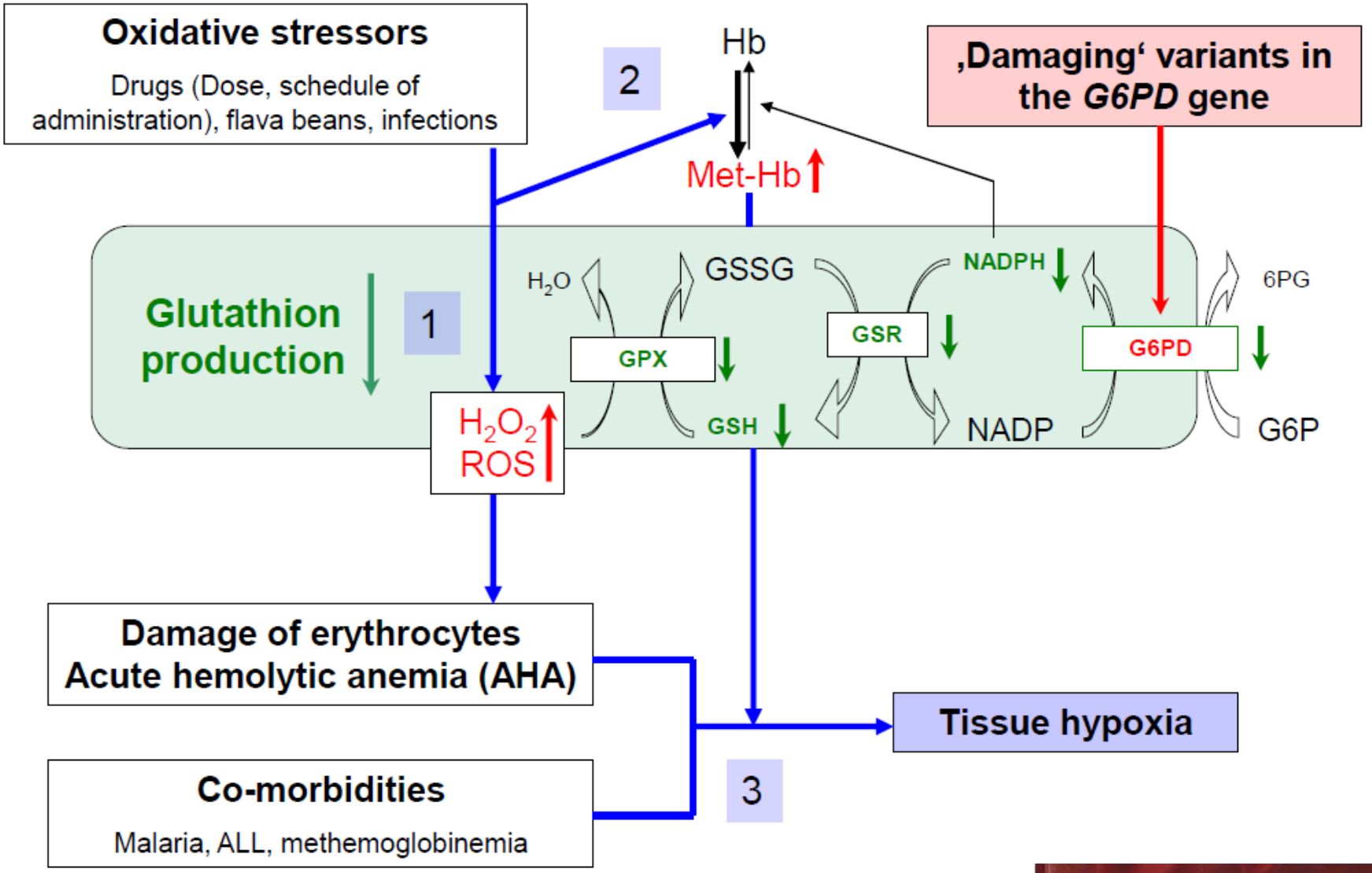
Isouramil/divicine

Normal G6PD
(class IV and V alleles)

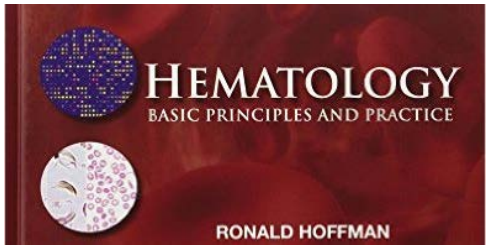


G6PD deficiency
(class I, II and III alleles)

M Relling & WE Evans

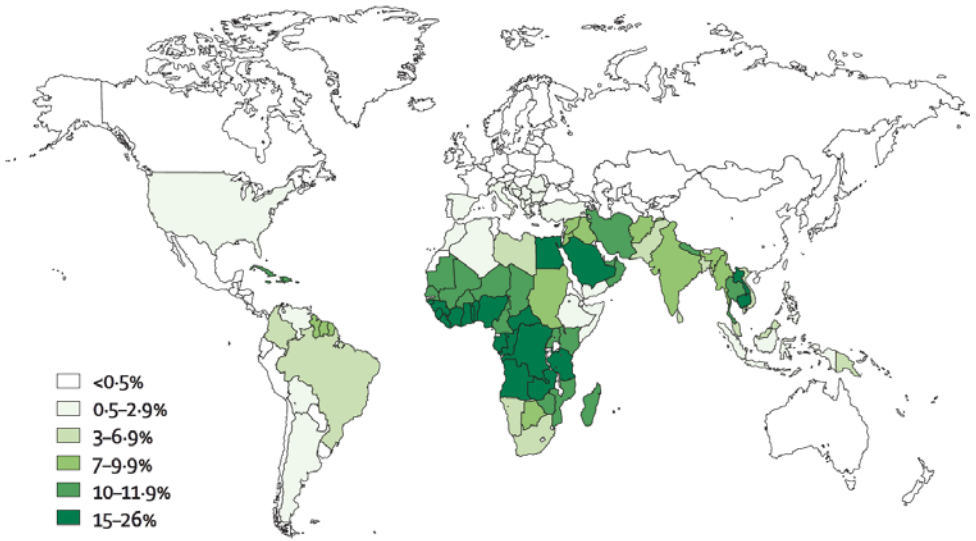


L Kager & WE Evans. Pharmacogenomics in Hematologial Disease. Hematology-Basic Principles and Practice. Eds. Hoffman et al. 7th Edition, 2016, Elsevier *in press*



Glucose 6-Phosphat Dehydrogenase Mangel

- Häufigste Enzymopathie (> 400 Mill.)
- Subtropischer Gürtel/Malariagebiete
- X-linked, schwere Formen fast immer sporadisch, > 140 Mutationen in *G6PD*
- Neonataler Ikterus
- Hämolytische Krisen



Lancet 2008; 371: 64–74

Class I

Severely deficient, associated with chronic non-spherocytic haemolytic anaemia

Class II

Severely deficient (1–10% residual activity), associated with acute haemolytic anaemia

Class III

Moderately deficient (10–60% residual activity)

Class IV

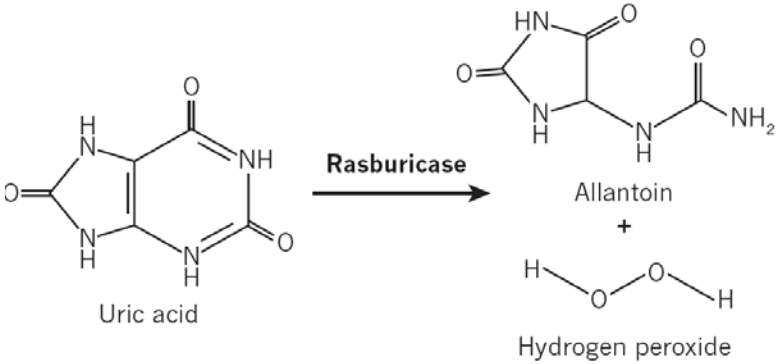
Normal activity (60–150%)

Class V

Increased activity (>150%)

Lancet 2008; 371: 64–74

	Definite association	Possible association	Doubtful association
Antimalarials	Primaquine Pamaquine	Chloroquine	Mepacrine Quinine
Sulfonamides	Sulfanilamide Sulfacetamide Sulfapyridine Sulfamethoxazole	Sulfadimidine Sulfasalazine Glibenclamide	Aldesulfone Sulfadiazine Sulfafurazole
Sulfones	Dapsone	..	..
Nitrofurantoin	Nitrofurantoin	..	..
Antipyretic or analgesic	Acetanilide	Aspirin	Paracetamol Phenacetin
Other drugs	Nalidixic acid Niridazole Methylthionium Phenazopyridine Co-trimoxazole	Ciprofloxacin Chloramphenicol Vitamin K analogues Ascorbic acid Mesalazine	Aminosalicylic acid Doxorubicin Probenecid Dimercaprol
Other chemicals	Naphthalene 2,4,6-trinitrotoluene	Acalypha indica extract	



Lancet 2008; 371: 64–74

PharmGKB
The Pharmacogenetics and Pharmacogenomics Knowledge Base

Ce-M-M-

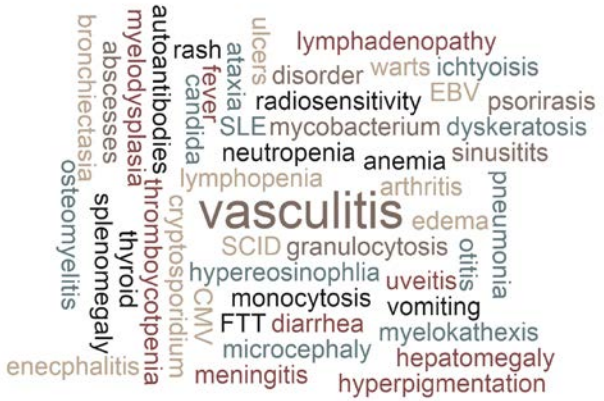
Research Center for Molecular Medicine
of the Austrian Academy of Sciences

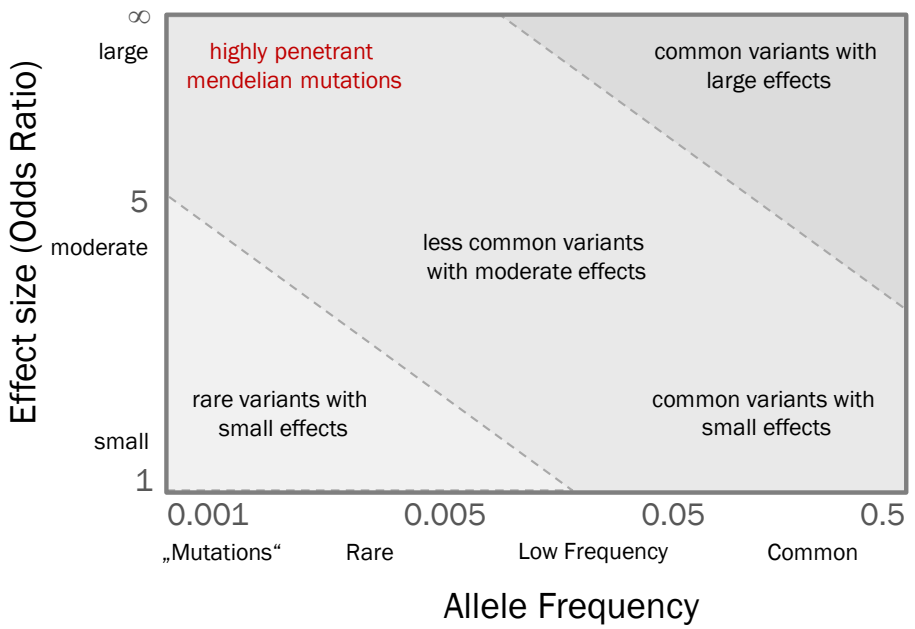
ÖAW
Österreichische Akademie
der Wissenschaften

Targeted Sequencing:
A successful strategy to identify genetic causes of hematological disorders

Lab Kaan Boztug

Linking the phenotype: The role of genetics in disease



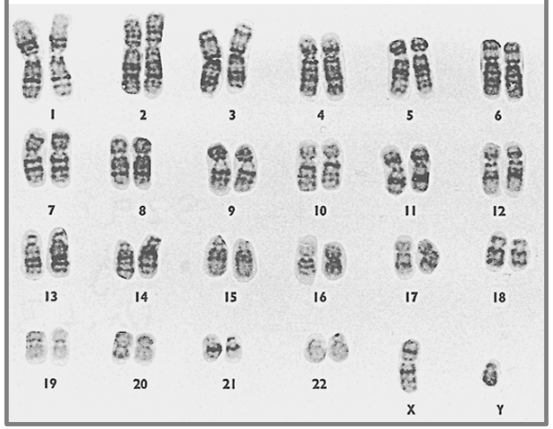


Adapted from Manolio et al. *Nature (2009)*

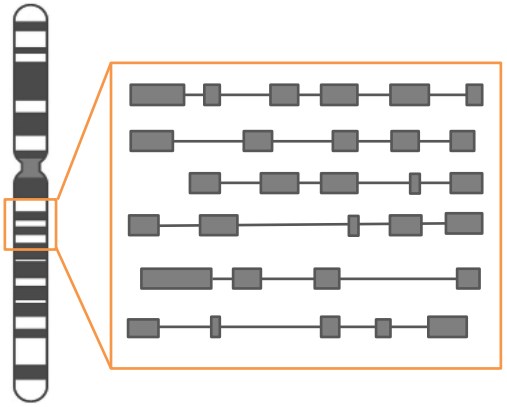
Next Generation Sequencing (NGS) approaches for the identification of rare gene defects

incoming patient material → 1 Genetic screen via targeted next-generation-sequencing (NGS) technology: **Hematology Panel**

Whole Genome Sequencing (WGS)



Exome Sequencing (ES)



Targeted Sequencing

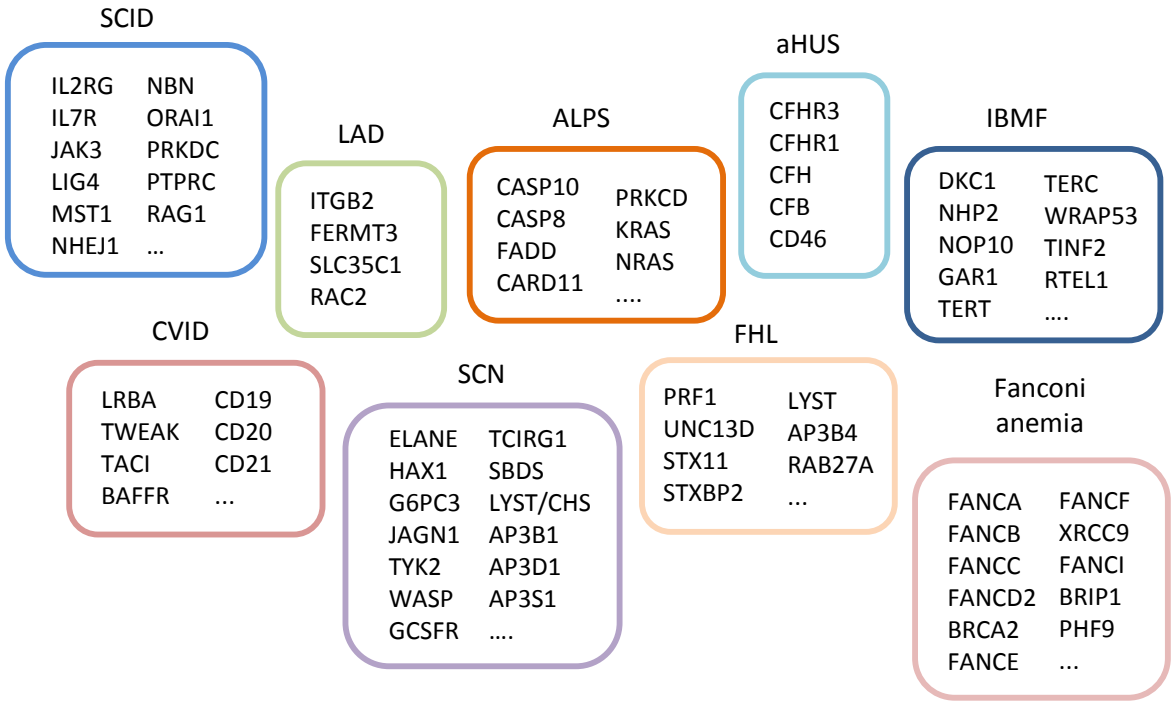
example: SCID Genes	
IL2Rg	*ADA*
JAK3	*PNP*
IL7RA	*DNA ligase IV*
PTPRC	*DCLRE1C*
CD3g	*DNAPkcs*
CD3δ	*AK2*
CD3ε,0	*CD8 alpha chain*
CD247	*ORAI-I*
Coronin-1A	*STIM-1*
CIITA	*NHEJ1*
RFX5	*ZAP70*
RFXAP	*FOXN*
RFXANK	*TBX1*
RAG1	*MST1*
RAG2	*LCK*

Data complexity

Targeted sequencing of hematological relevant genes

Facts about our custom designed PID gene-panel:

- Currently **438** relevant genes targeted which are causative or potentially causative for monogenic hematopoetic disorders
    - all known hematological disease genes > diagnostic
    - several unpublished interesting candidate genes > discovery
- Simultaneous analysis of up to 48 samples (patients) on HiSeq3000 platform

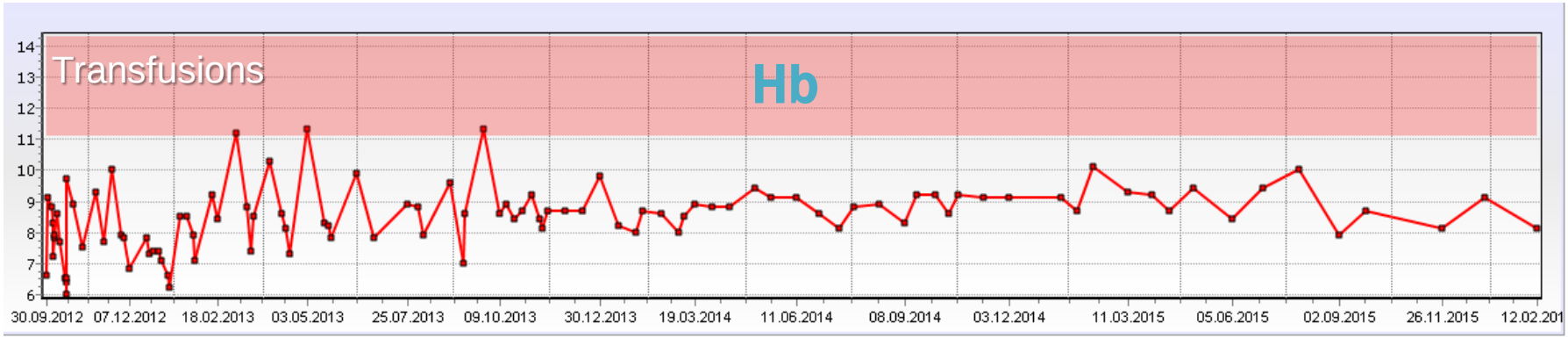


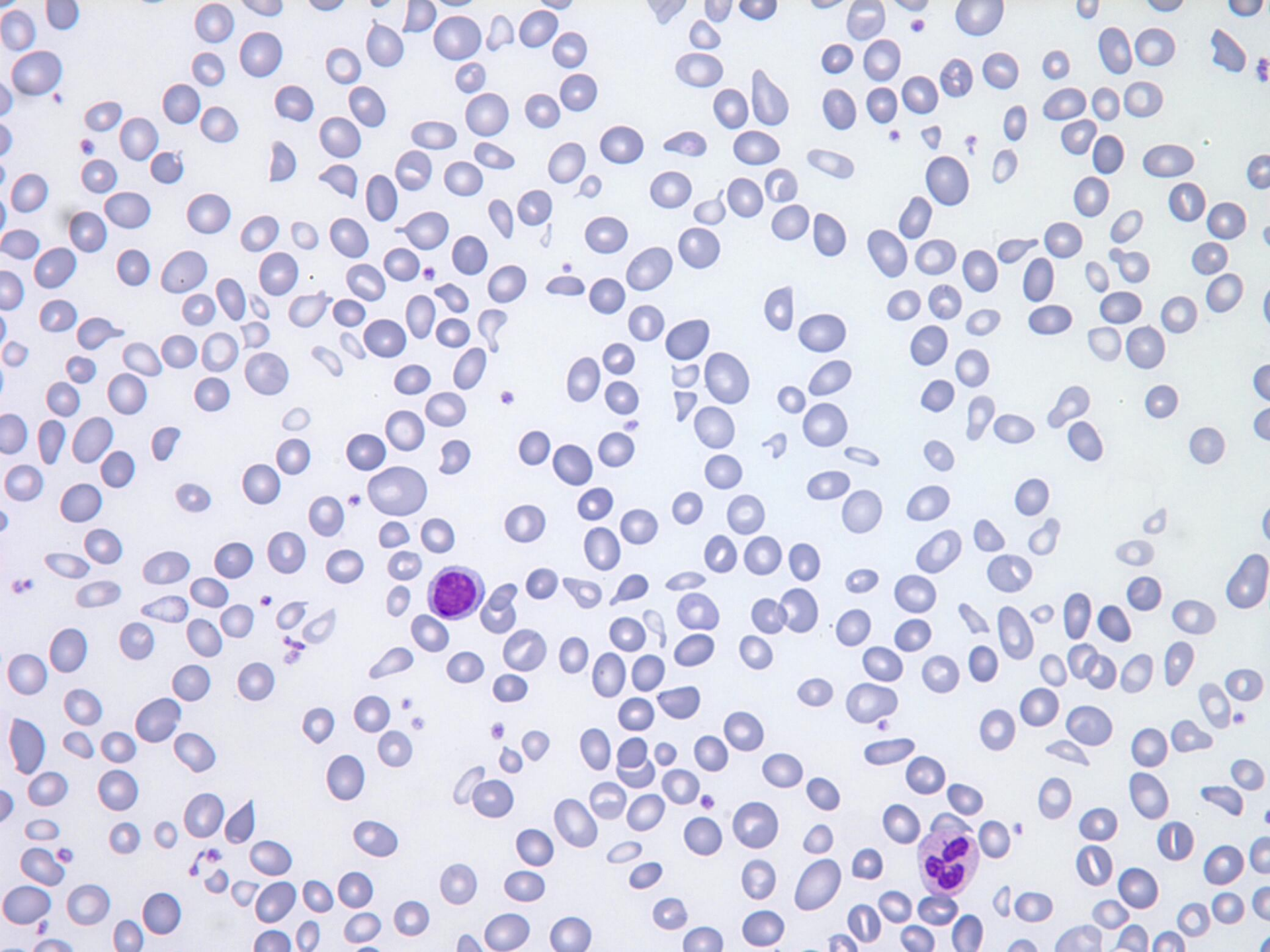
Examples of targeted disease groups

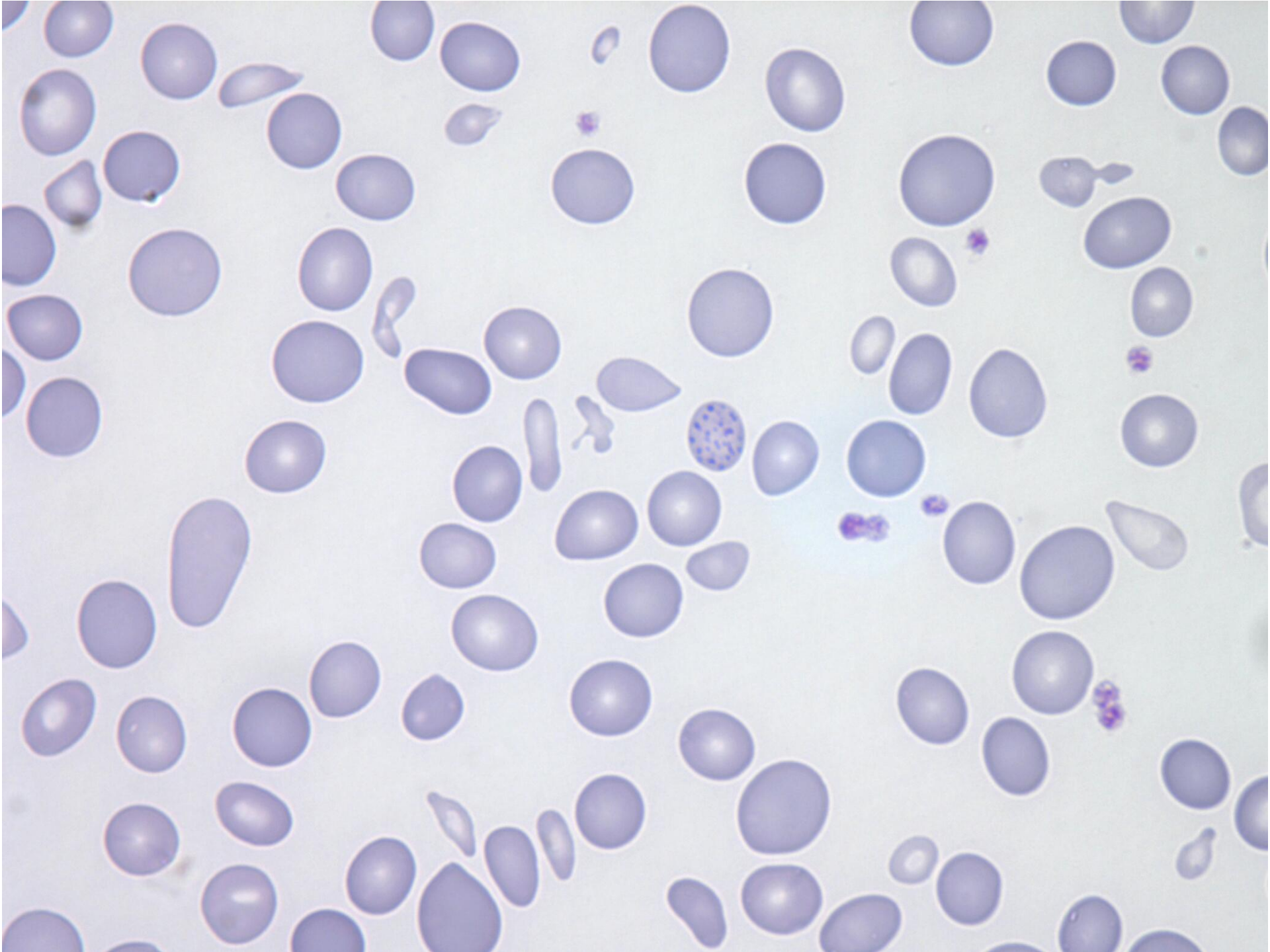
Patient /S.P.

Patient information & clinical data

Patient Code:	
Gender/Age (y):	Male/3.5
Consanguinity:	No
Age at onset:	Birth
Clinical Features/Symptoms:	Initially transfusion dependent, developmental delay
Immunophenotyping:	-
Incoming Diagnosis:	Chronic 'normocytic (upper range)' hemolytic anemia
Therapy & Response:	none
Previous tests/analysis:	EMA (-), Enzymes (-), screening for metabolic diseases (-)
Additional information:	Parents refused BM analysis, FISH (MDS) (-), PNH (-), DEB (-)







There was **no homozygous** variant identified.
The table lists 2 **heterozygous** variants which are likely causative for the phenotype.

Genetic analysis: Hematology gene panel screen

GENE	CHR	POS	SNP ID	R	A	IMPACT	AA	Transcript ID	MAF	READS
CDAN1	15	43022926	rs375339408	G	A	STOP_GAINED	R682*	ENST00000356231	NA	40
CDAN1	15	43016798	.	A	G	MISSENSE	L1192S	ENST00000356231	NA	88

LEGEND

GENE	gene name	**R**	base in reference genome	**ID**	Transcript ID
CHR	chromosome	**A**	alternative base in sample	**MAF**	minor allele frequency
POS	chromosomal position (bases)	**IMPACT**	consequence of variant	**READS**	number of sequencing reads covering the variant
SNP ID	rs number	**AA**	change on amino acid level	**NA**	not annotated

Predictions of variant impact

chr15:43022926 G>A
Polyphen-2: NA
SIFT Score: NA
CADD Score: 38

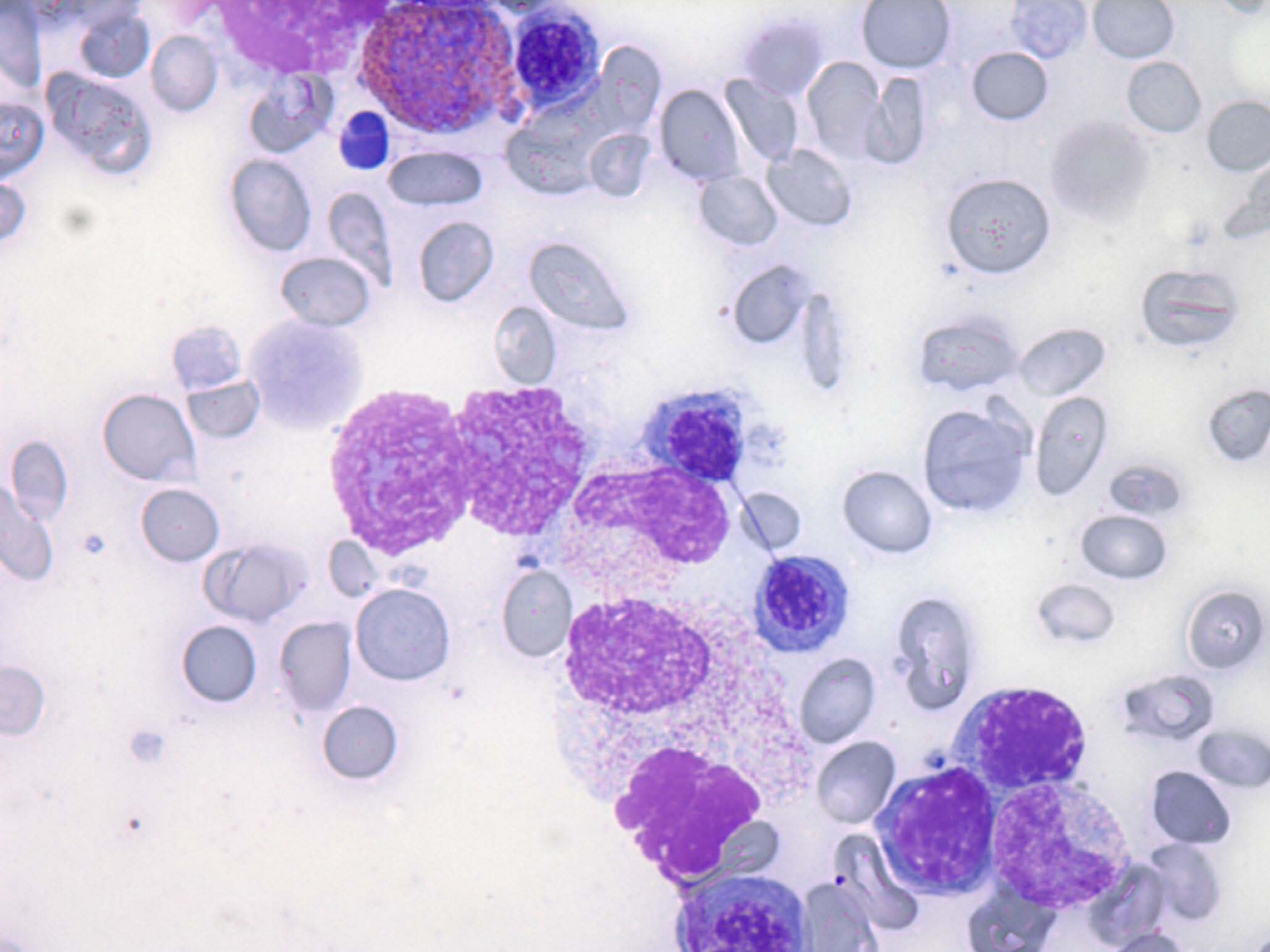
chr15:43016798 A>G
Polyphen-2: probably damaging
SIFT Score: deleterious
CADD Score: 27.3

References
http://www.omim.org/entry/224120
http://genetics.bwh.harvard.edu/pph2/
http://sift.jcvi.org/
http://cadd.gs.washington.edu/

CDAN1 = CODANIN1

Dyserythropoietic anemia, congenital, type Ia (OMIM #224120)

CDA type I is a rare inherited red blood cell disorder characterized by macrocytic anemia, ineffective erythropoiesis, and secondary hemochromatosis. It is occasionally associated with bone abnormalities, especially of the hands and feet (acrodysostosis), nail hypoplasia, and scoliosis. Striking morphologic abnormalities of erythroblasts include the 'Swiss-cheese' abnormality of erythroblasts on electron microscopy. The inheritance is known to be autosomal recessive; the patient comprises compound heterozygosity. – confirmed via Sanger sequencing of patient and parents. The novel missense variant is inherited from the father, the known stop gained variant from the mother.



Zusammenfassung

Neu diagnostizierte normozytär-normochrome Anämie mit Hb < 8g/dl (< 5 mmol/l) ist fast immer ein Notfall

Bei Zeichen einer Hämolyse sofortige weitere Untersuchungen einleiten

Bei zusätzlichen Zytopenien und/oder Vergrößerung von lymphatischen Organen – Onkologie

Migrantion: bedenke hämolytische Krise bei G6PD-Mangel (und Milzsequester bei SDC)

Danksagung

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