- WASp deficiency -
X linked thrombopenia / Wiskott Aldrich syndrome

Coralie Mallebranche, MD, MSc

EuroBloodNET seminar

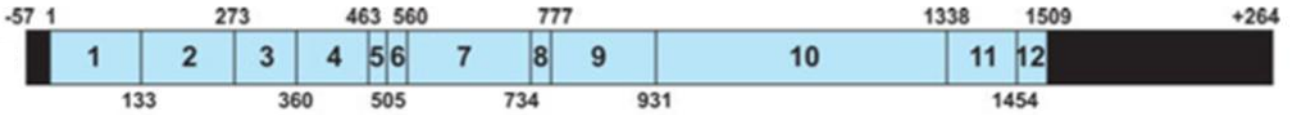
2024, March 20th

- Wiskott Aldrich Syndrome Protein - *Structure & Roles*

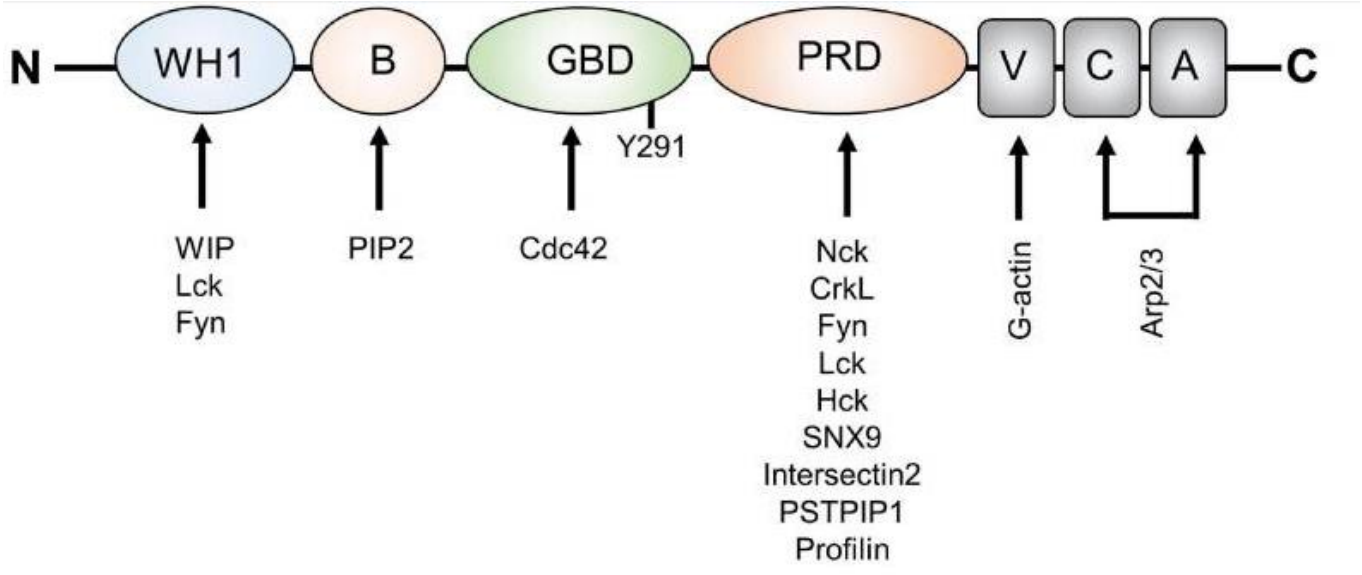
WASp protein

encoded by WAS gene (Xp11.22)

WAS gene

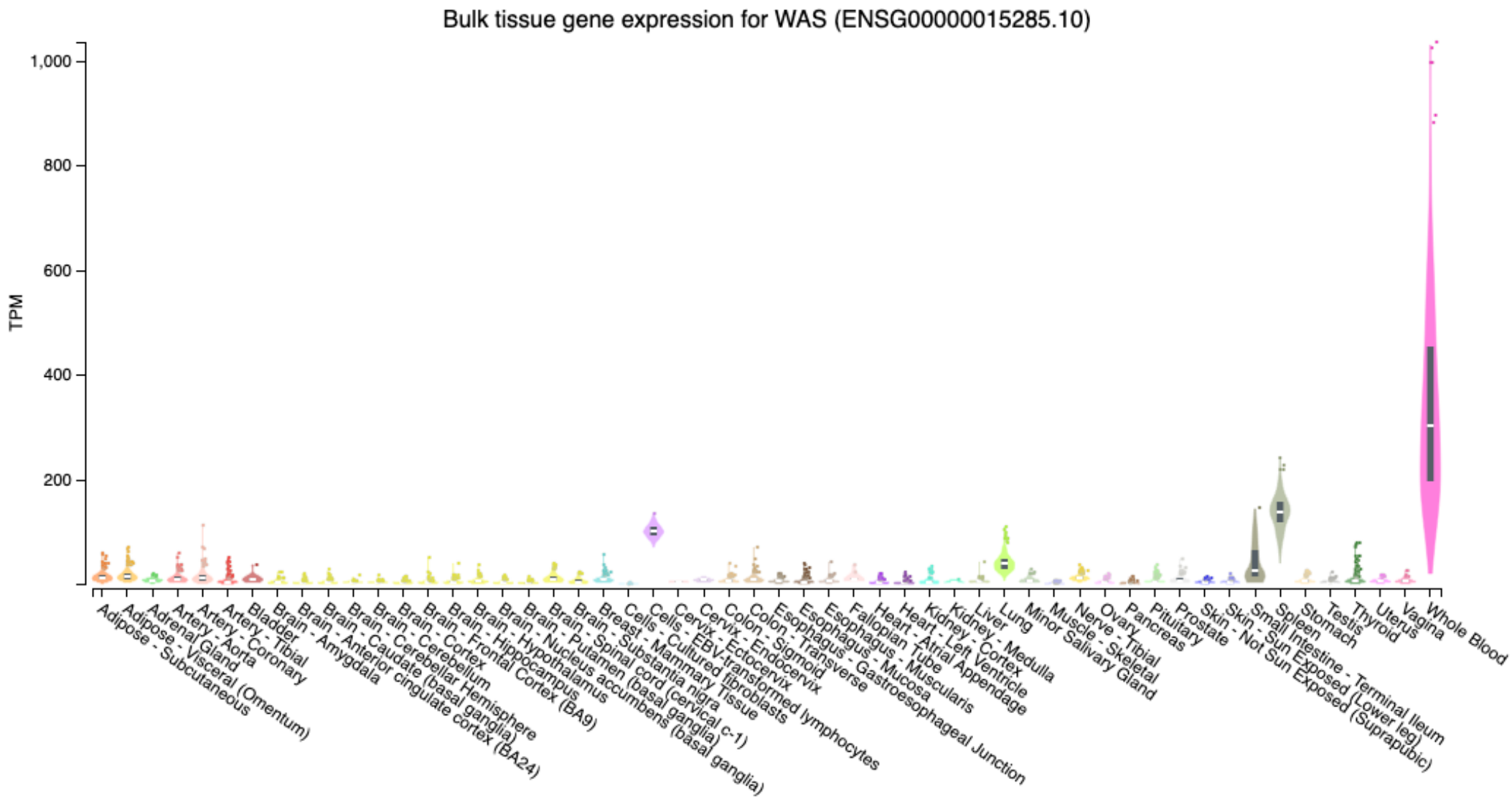


WAS protein



Ngoenkam et al.
Front Cell Dev Bio. 2021

Gene expression (GTEx portal)



Functions of WASp

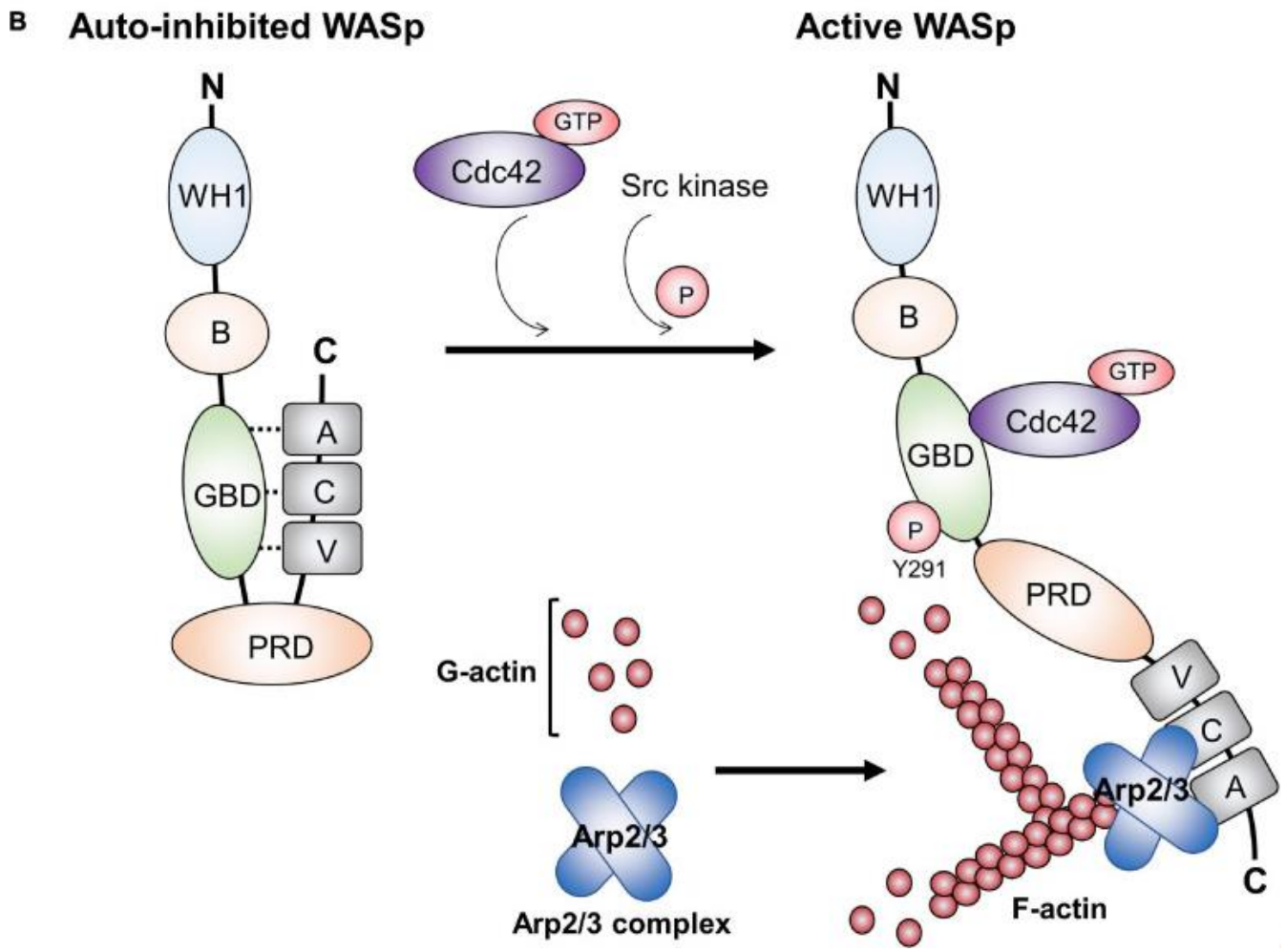
Actine cytoskeleton-independant

- T cell differentiation
- Memory B cell activation, through transcription of B cell co-receptor CD19
- Transcription of inflammatory cytokines
- Transcriptional regulation of myeloid cells

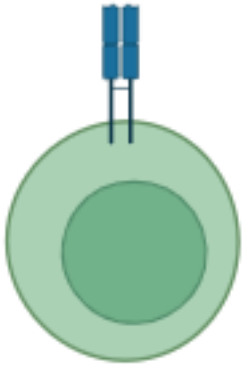
Actine cytoskeleton-dependant +++

Rivers et al.
Eur J Immunol. 2017

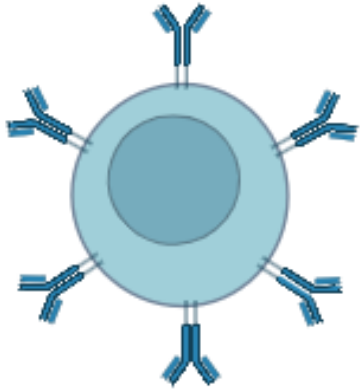
WASp acts as scaffold protein that mediate dynamic changes in the actin cytoskeleton

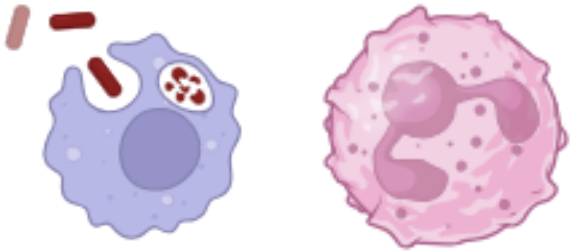


Ngoenkam et al.
Front Cell Dev Bio. 2021

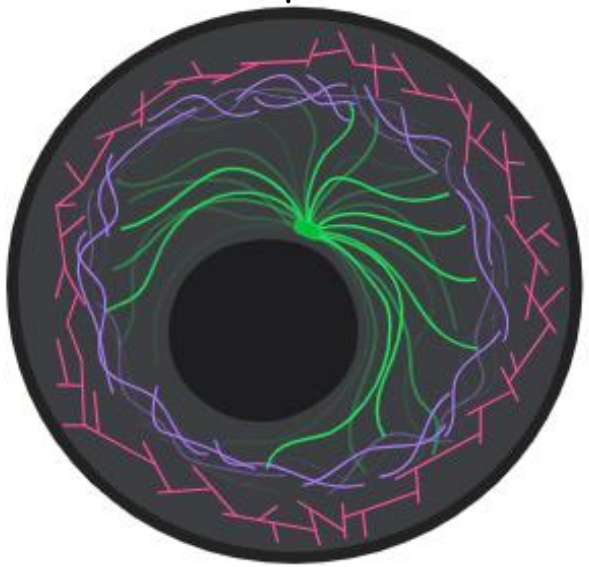


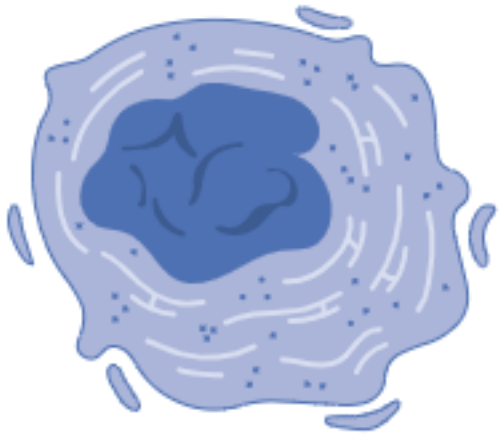
Proliferation
Immune synapse assembling and signaling
Cytokine polarization and release
Migration





Immune synapse assembling and signaling
Cytokine polarization and release
Endothelial adhesion (podosome)
Migration





Platelets formation (premature in BM)
Aggregation
Secretion

Rivers et al. Eur J Immunol. 2017
Nurden et al. JTH. 2011
Sabri et al. Blood. 2006

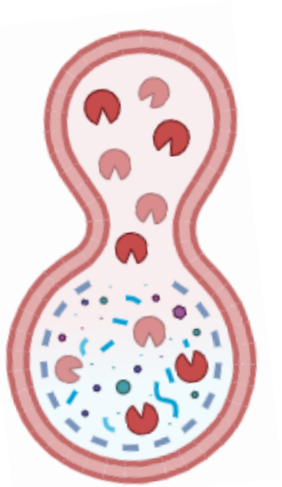
WAS and autoinflammation?

Impaired autophagy

Defective bacterial clearance

Excessive inflammasome activation

Autoinflammatory manifestations?

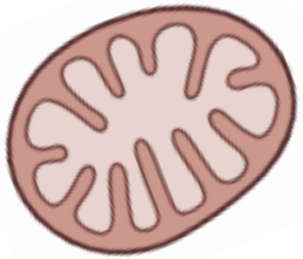


Impaired mitophagy

Impaired maintenance of mitochondrial network integrity

Alteration of mitochondrial respiration

Metabolic dysfunction



Lee et al. Nat Commun 2017

Rivers. eLife. 2020

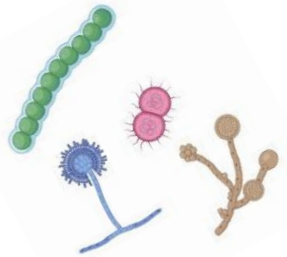
Wiskott Aldrich Syndrome & X-linked thrombocytopenia

Story of the disease

- Wiskott: inherited thrombocytopenia (1937) — Thrombocytopenia / Eczema / Recurrent ear infections
- Dr Robert Aldrich: X-linked thrombocytopenia (1957)
- Description of the immune deficiency associated with the thrombocytopenia (1960)
- Discovery of WAS gene (1994)
- X-linked thrombocytopenia and Wiskott-Aldrich syndrome are diseases with mutations in the WASP gene (1995)

Clinical triad

Recurrent infections



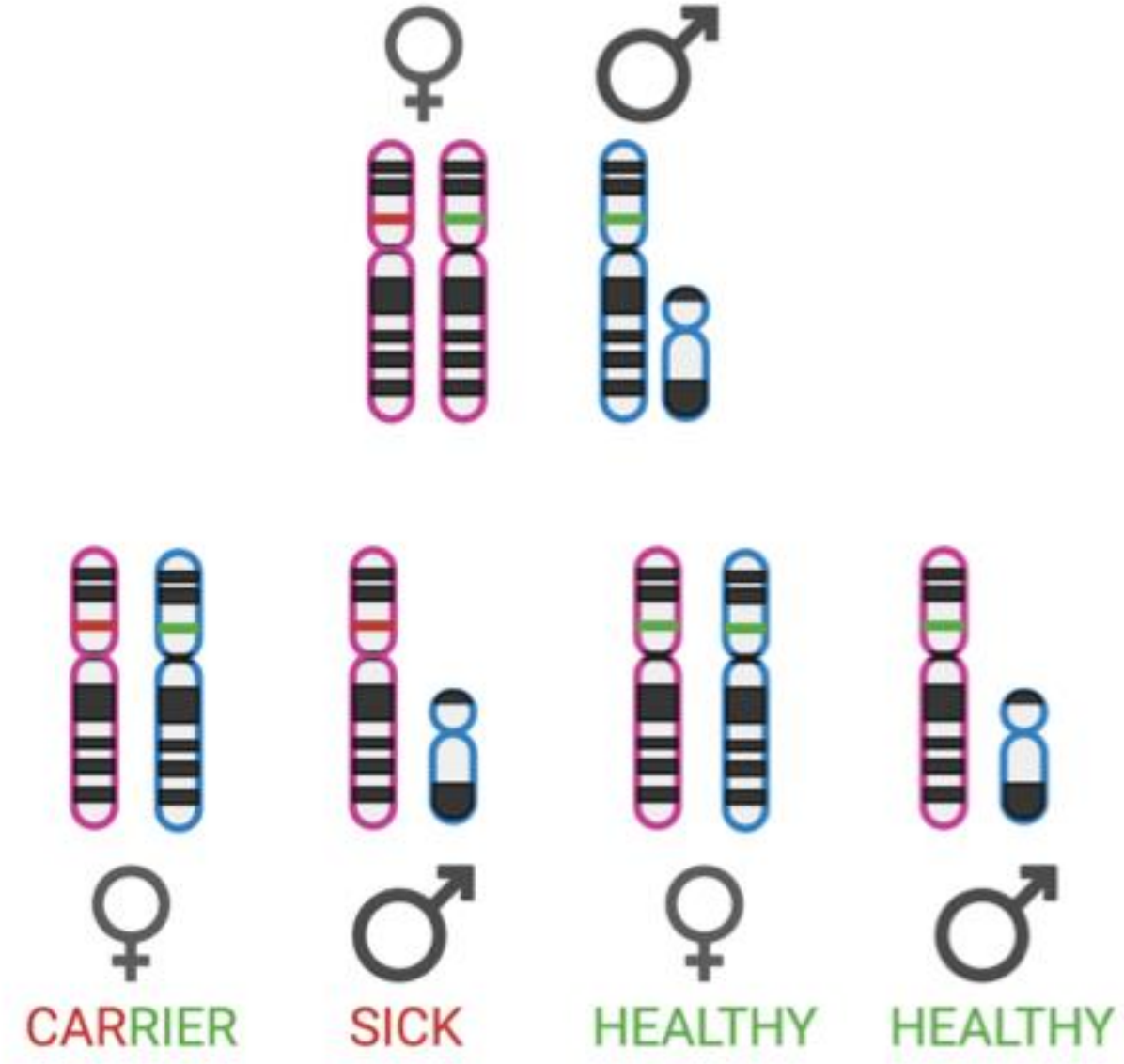
Eczema

Microthrombocytopenia

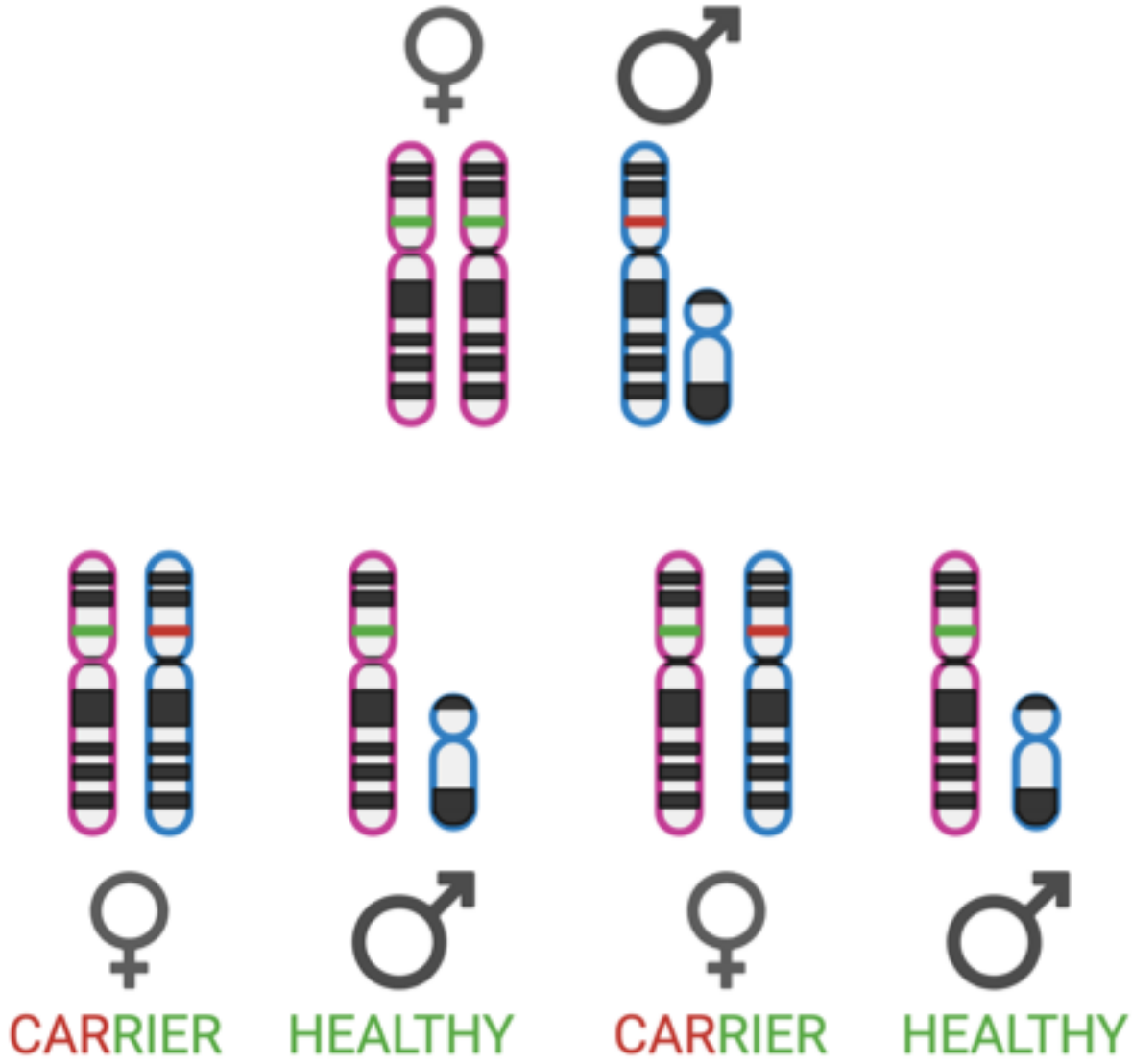
Diagnostic features

- Usually: thrombocytopenia
  - In a boy
  - Presenting during the first year of life
  - Petechiae, bruising, spontaneous/prolonged bleeding
  - Small platelets +++
- Sometimes:
  - Eczema
  - Recurrent infections
- Rare presenting features:
  - Autoimmunity
  - Malignancy

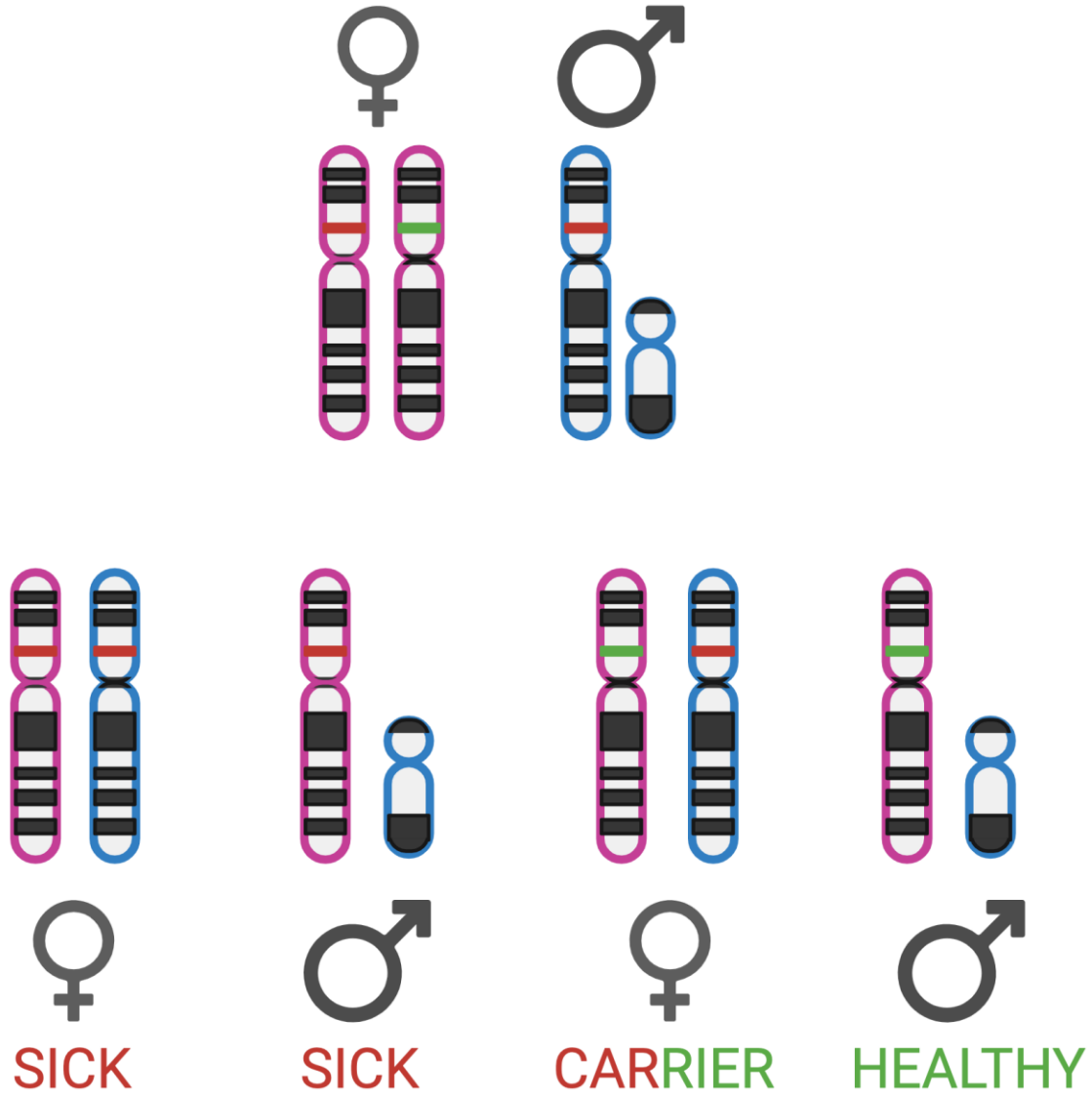
X-linked transmission



X-linked transmission



X-linked transmission

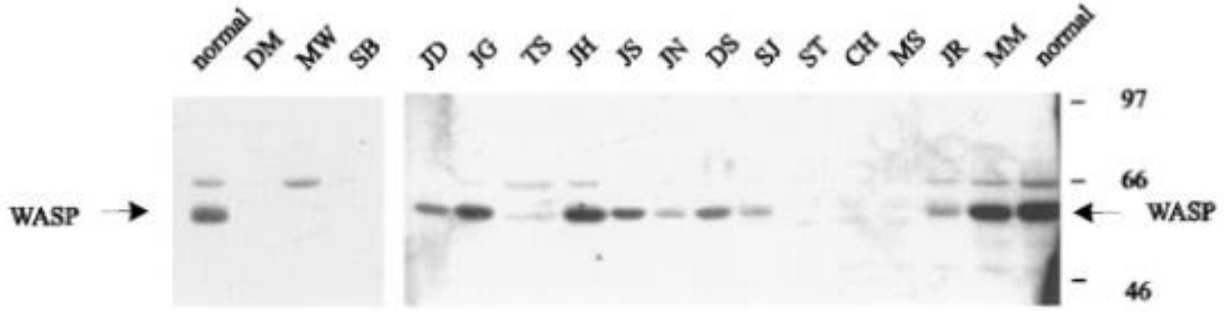


Diagnostic methods

Genetic analysis
612 mutations (Rapid Lite - February 2024)
Nonsense, insertion, deletion, missense, splice site
De novo: 1/3

Western blot / Flow cytometry

Quantification



But

- Normal levels and functionnally impaired WASp
- Absent WASp expression from disturbance of detection

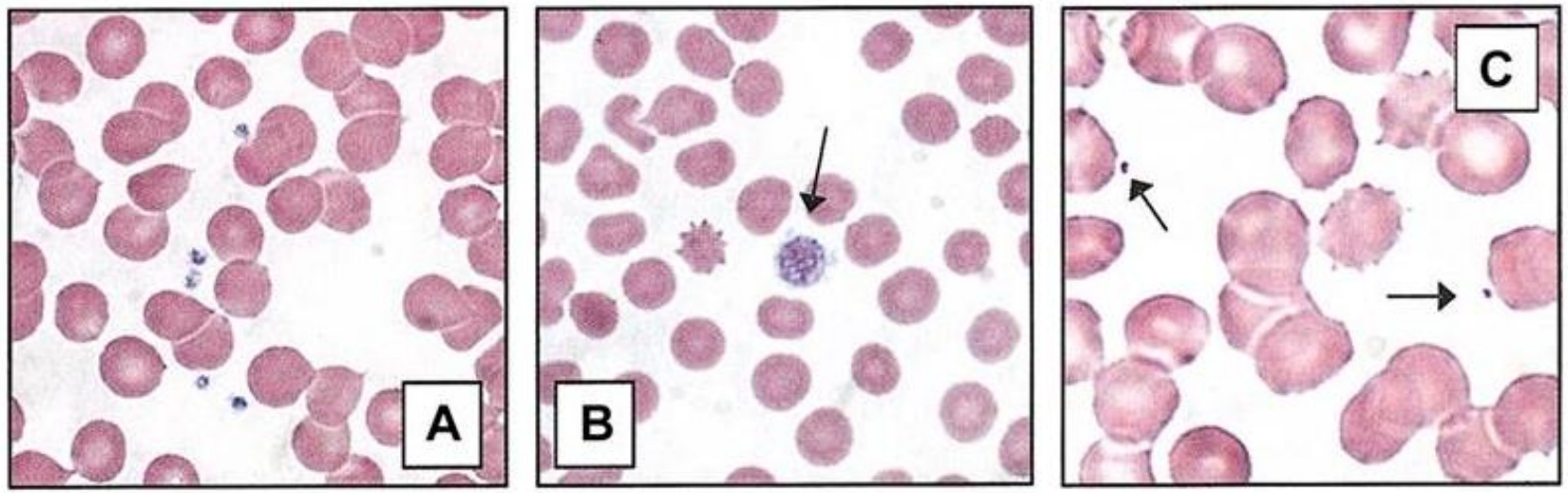
Zhu et al., Blood 1997

Thrombocytopenia

- For all patients from birth
  - Severe (< 20 G/L), moderate (20 – 50 G/L) or mild (> 50 G/L)

- Small platelets (mean volume 5-7 fl)
  - But can be normal

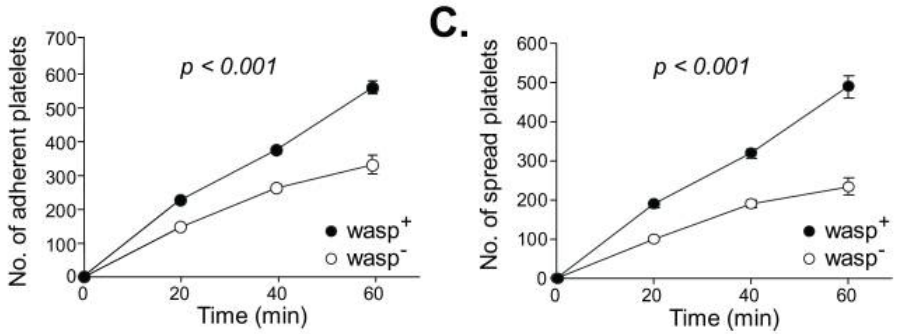
Drachman et al., Blood 2004



Platelet dysfunction?

Shcherbina et al, Br J Haematol. 2010
Shcherbina et al, Br J Haematol. 1999
Semple et al., Br J Haematol. 1997

- Integrin αIIbβ3 outside-in dependent responses
  - Require strong plasma membrane-cortical cytoskeleton interactions
    - Adhesion
    - Spreading
    - Clot retraction and
    - Plug stabilization
  - Deficient in WASp KO platelets



- Responses negatively regulated by stable actin filament linkages between the plasma membrane and the cortical cytoskeleton
  - As phosphatidyl serine expression
  - Enhanced in WASp KO platelets

THROMBOPENIA = normal number of megacaryocytes but abnormal platelet formation (low-number & dysfunction) + early destruction / autoimmunity

Thrombocytopenia

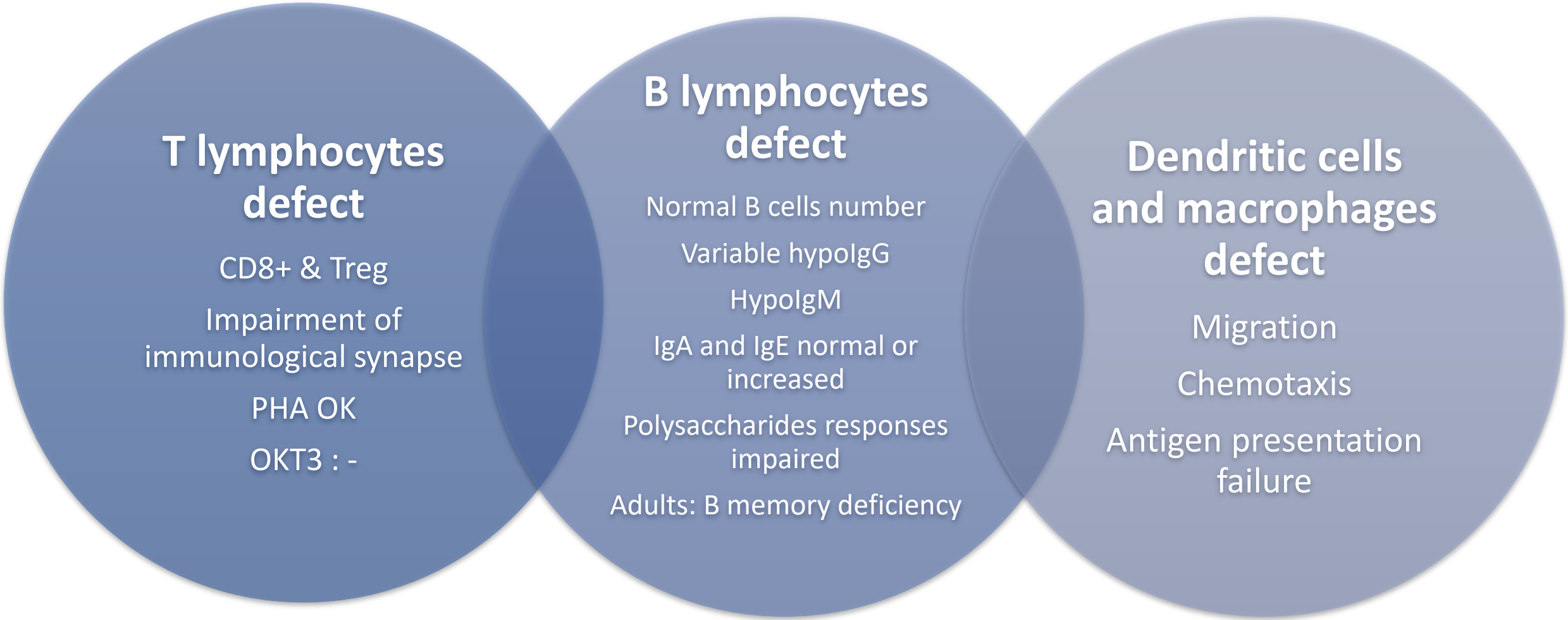
- Variable signs
  - Petechiae
  - Bruising
  - Mucosal bleeding
- Prolonged bleeding
  - Cord
  - Intracerebral
  - Bloody diarrhea

Severe bleeding:
Incidence 10-30 %
6 to 30 % of deaths

Albert et al., Front Pediatr 2019

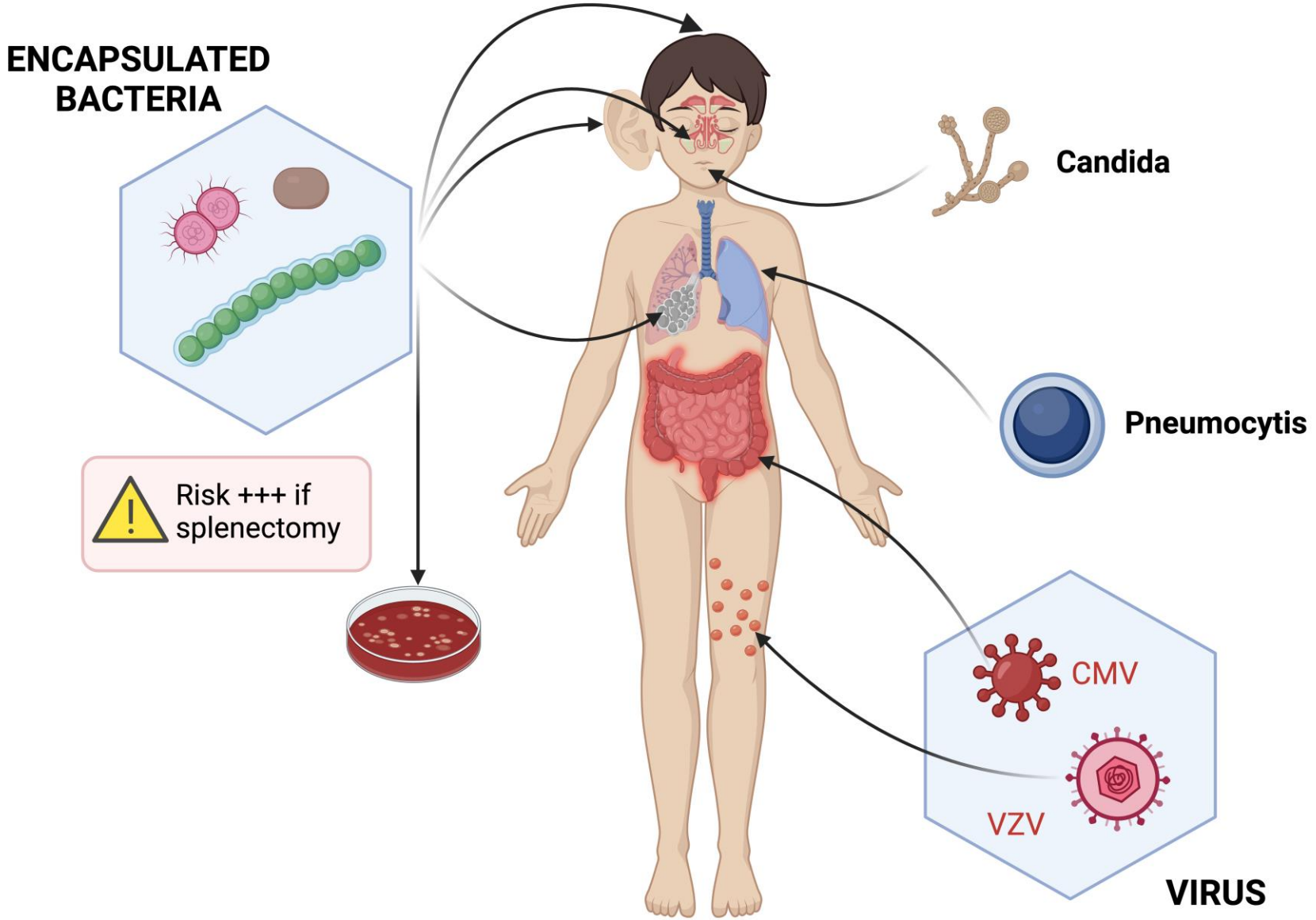
- Severe bleeding: association with autoimmune platelet consumption
- Particular situation: infants with refractory thrombocytopenia

Immunodeficiency



Bosticardo et al. 2009; E Rivers et al 2019

Infections

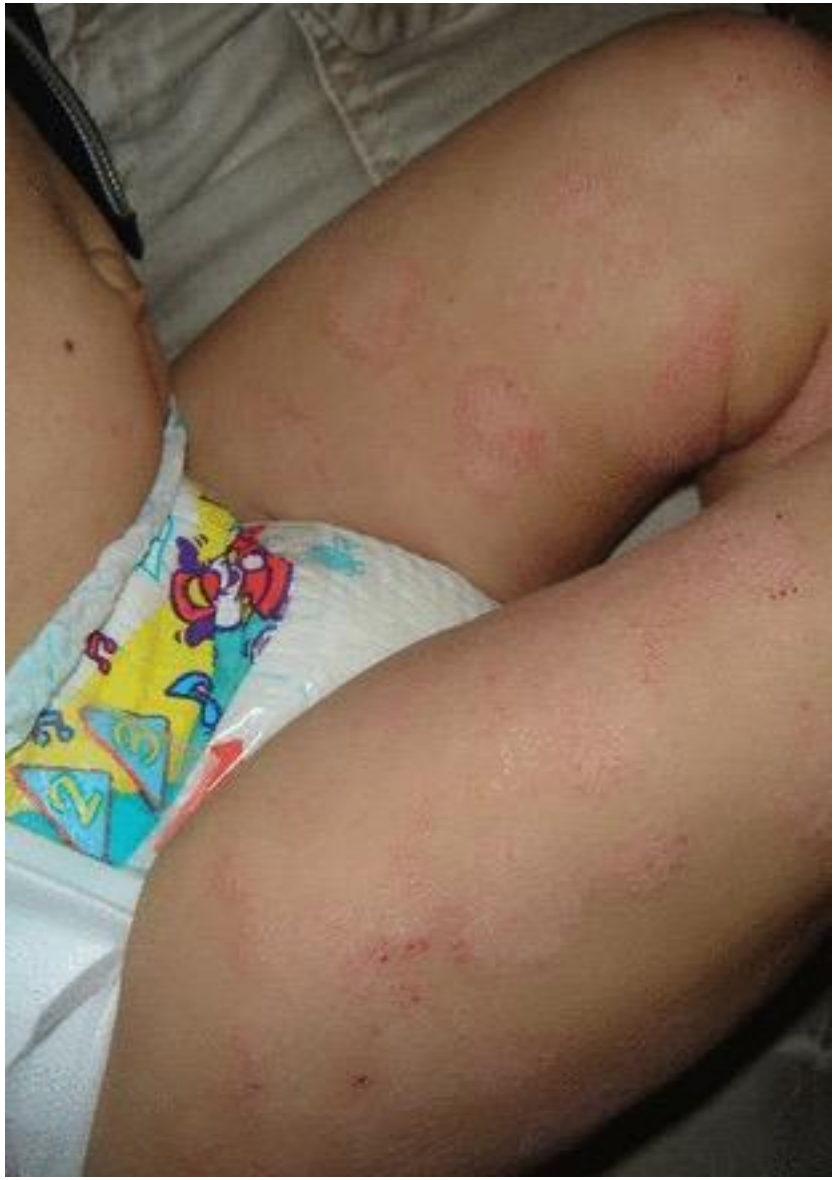


Viral		
CMV	6	17·65
RSV	2	5·88
Unspecified respiratory viruses	2	5·88
EBV	1	2·94
Varicella	1	2·94
HPV	1	2·94
Molloscum	1	2·94
Coxsackie	1	2·94
Adenovirus	0	0·00
Parainfluenza	0	0·00
Fungal		
Fungal pneumonia	3	8·82
Candida	3	8·82
Bacterial		
Chronic otitis media	1	2·94
Recurrent perianal abscesses	1	2·94
Recurrent cellulitis	1	2·94
Atypical mycobacteria	0	0·00
Parasitic		
Cryptosporidium	1	2·94

Elfeky et al, JACI. 2018

Eczema

50 – 80 % of patients
Varying severity
Resembles classical atopic dermatitis

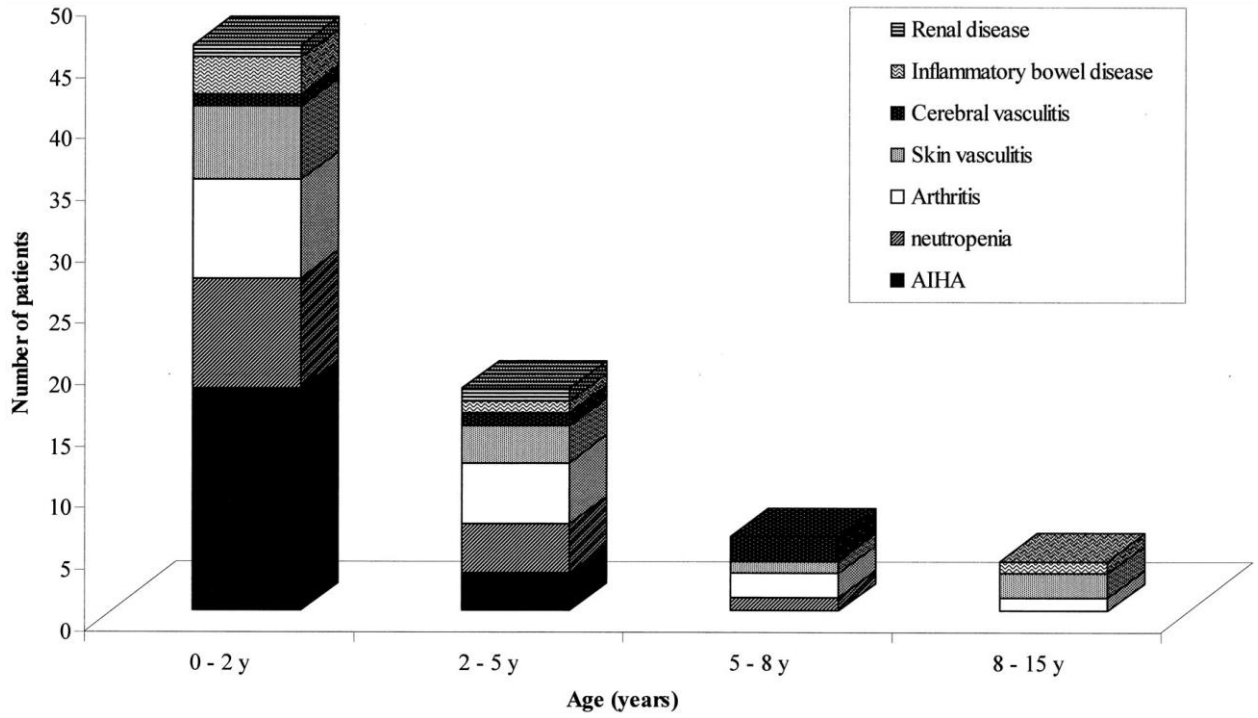


Gomes Loyola Presa et al, 2011

Autoimmunity

- 40 – 72 % of patients
- Autoimmune hemolytic anemia 36 %
  +++
- Neutropenia 25 % / ITP
- Arthritis 29 %
- Inflammatory bowel disease 9 %
- Vasculitis 22 %
  - Skin 7 %
  - Cerebral 3 %
- Renal disease

PATHOPHYSIOLOGY?



Pediatrics. 2003;111(5):e622-e627. doi:10.1542/peds.111.5.e622

Dupuis et al. Ped. 2003
Sullivan et al. J of Ped. 1994
Bosticardo et al. Blood 2009

Renal disease

3 – 19 % ? Around 5 %

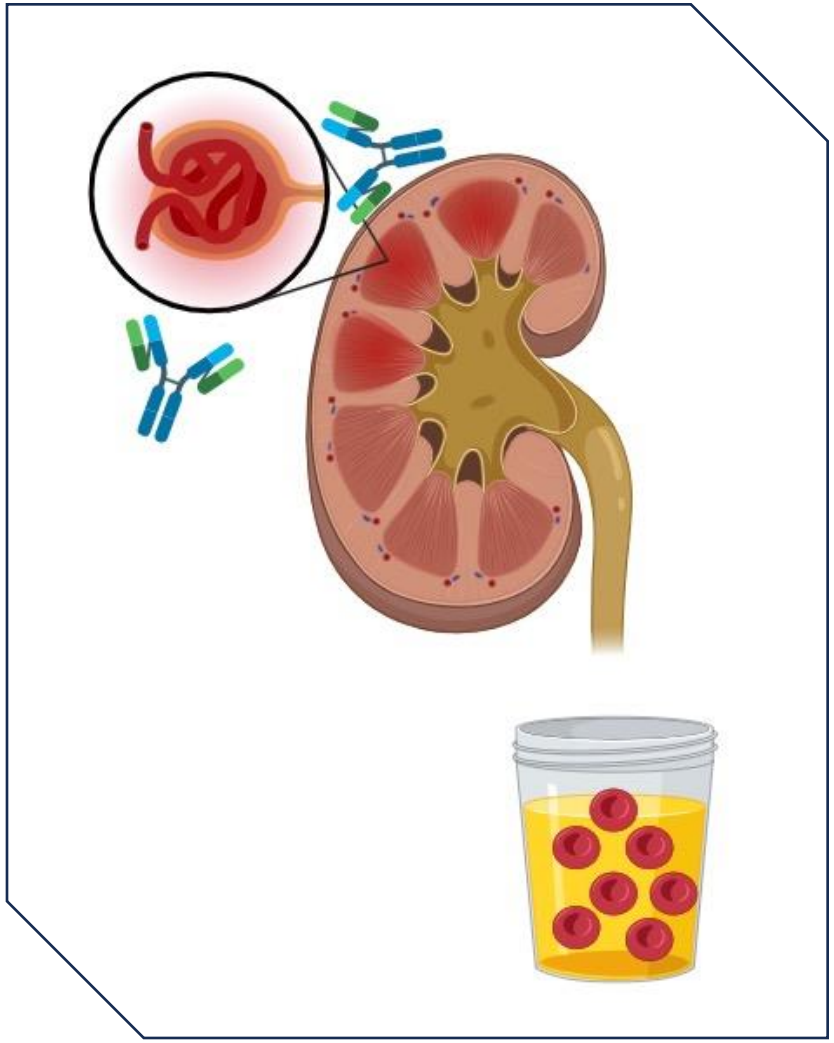
Mostly: IgA nephropathy – Hematuria ++

Auto-immunity? Aberrant glycosylation of IgA

Table 2 Clinical and pathologic findings of nephropathy associated with WAS/XLT

Patient No.	Age	Gender	Clinical diagnosis	Serum IgA level (mg/dl)	Pathologic findings	Reference No.
1	2	Male	WAS	Not described	Interstitial nephritis	15
2	4	Male	WAS	Not described	Chronic proliferative GN with focal crescent formation	15
3	12	Female	WAS variant	Elevated	Immune-complex GN IF: IgA and IgM, C3	33
4	46	Male	WAS	Elevated	MPGN with crescent and mesangial IgA deposit	34
5	33	Male	WAS	Elevated	MPGN IF: negative for IgA	35
6	12	Male	WAS	Elevated	MPGN IF: IgA	36
7	35	Female	WAS carrier	Normal	Diffuse proliferative GN with cellular crescent IF: IgA, fibrinogen, C3	37
8	8	Male	XLT	Elevated	IgAN IF: IgA, C3	38
9	35	Male	XLT	Normal	IgAN IF: IgA, C3	38
10	8	Male	WAS	Elevated	IgAN with FSGS and focal ATIN IF: IgA, C3	39
11	8	Male	XLT	Normal	IgAN IF: IgA, IgG	40
12	21	Male	WAS	Elevated	IgAN	41
13	8	Male	XLT	Not described	IgAN	42
14	16	Male	XLT	Normal	IgAN IF: IgA, C3	Present case

WAS, Wiskott–Aldrich syndrome; XLT, x-linked thrombocytopenia; IgAN, IgA nephropathy; C3, complement3; GN, glomerulonephritis; IF, immunofluorescence; MPGN, membranoproliferative glomerulonephritis; FSGS, focal segmental glomerular sclerosis; ATIN, acute tubulointerstitial nephriti.



Yamaguchi et al. CEN Case Rep. 2022

← Kakio et al. Acta Med Okayama. 2018

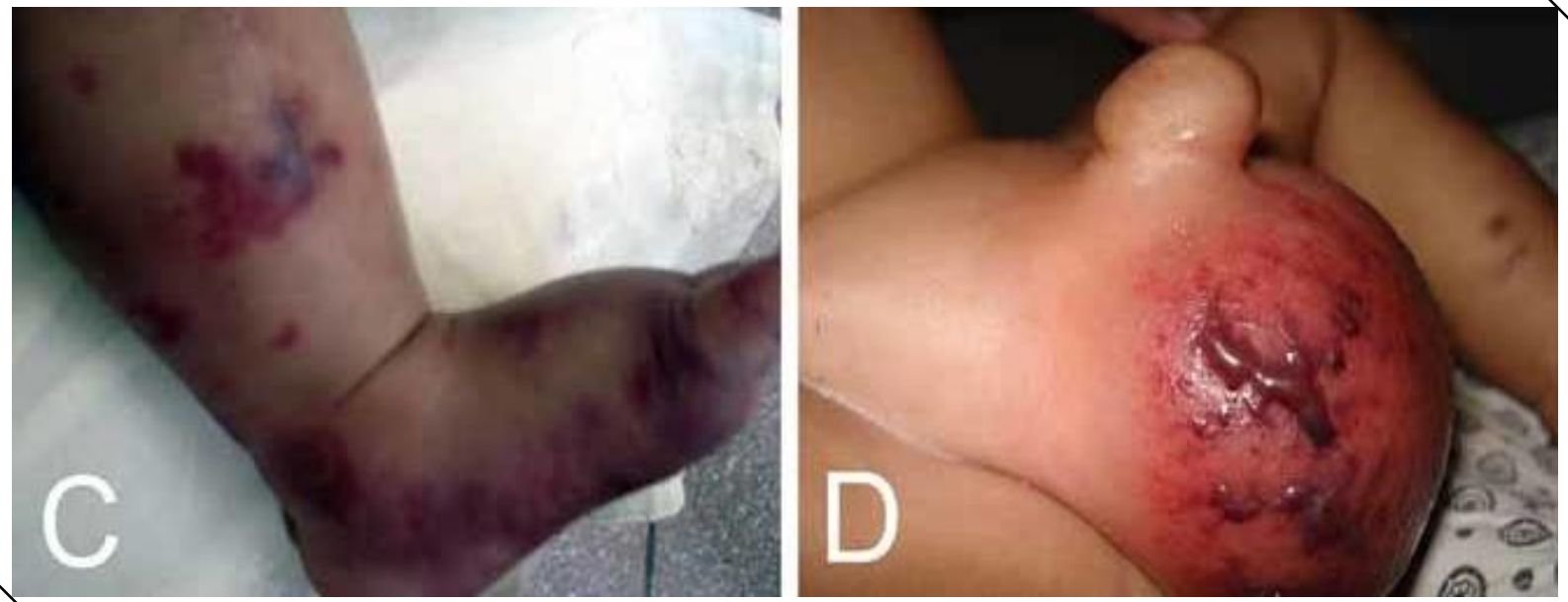
Albert et al. Blood 2010

Dupuis et al. Ped. 2003

Vasculitis

Mostly cutaneous: Leukocytoclastic vasculitis
IgA vasculitis (Henoch-Schönlein purpura)
Aneurysms
Rarely cerebral but severe +++

Linked to high levels of IgA and IgE?
Immunodeposition in the vessel wall leading to necrosis?



Sudhakar et al. Appl Clin Genet. 2021
Chandrakasan et al. PBC. 2011
Dupuis et al. Ped. 2003

Aneurysms

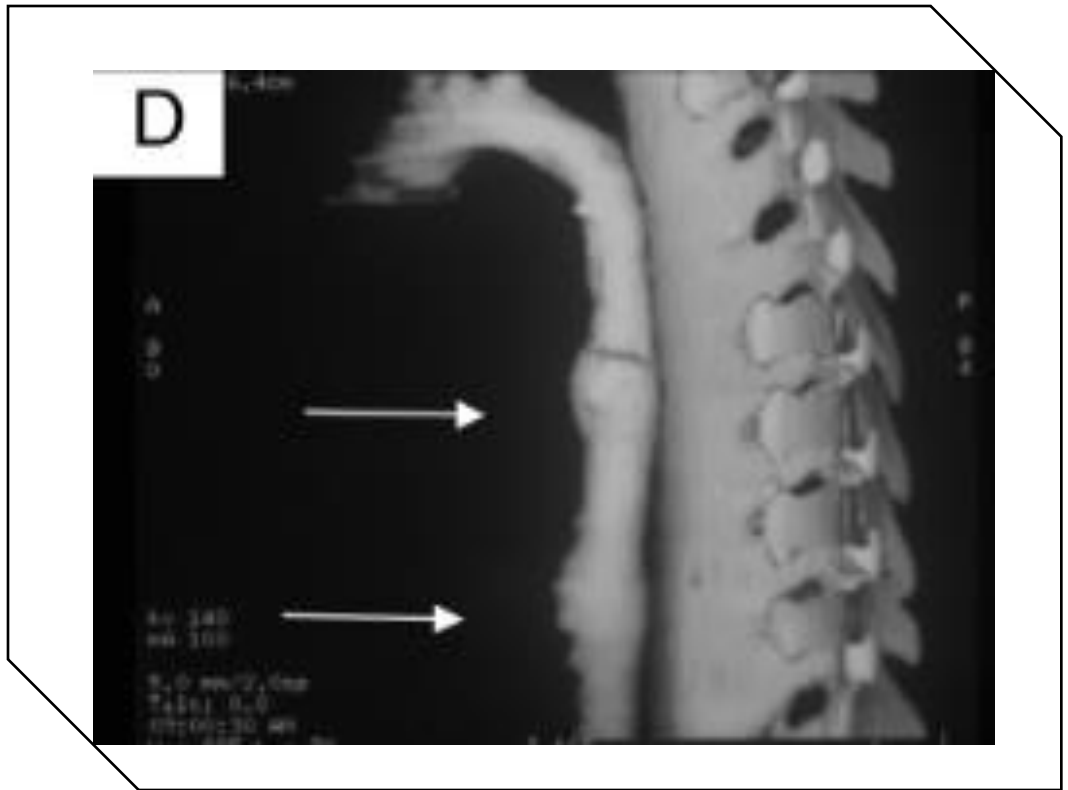
Rare +++

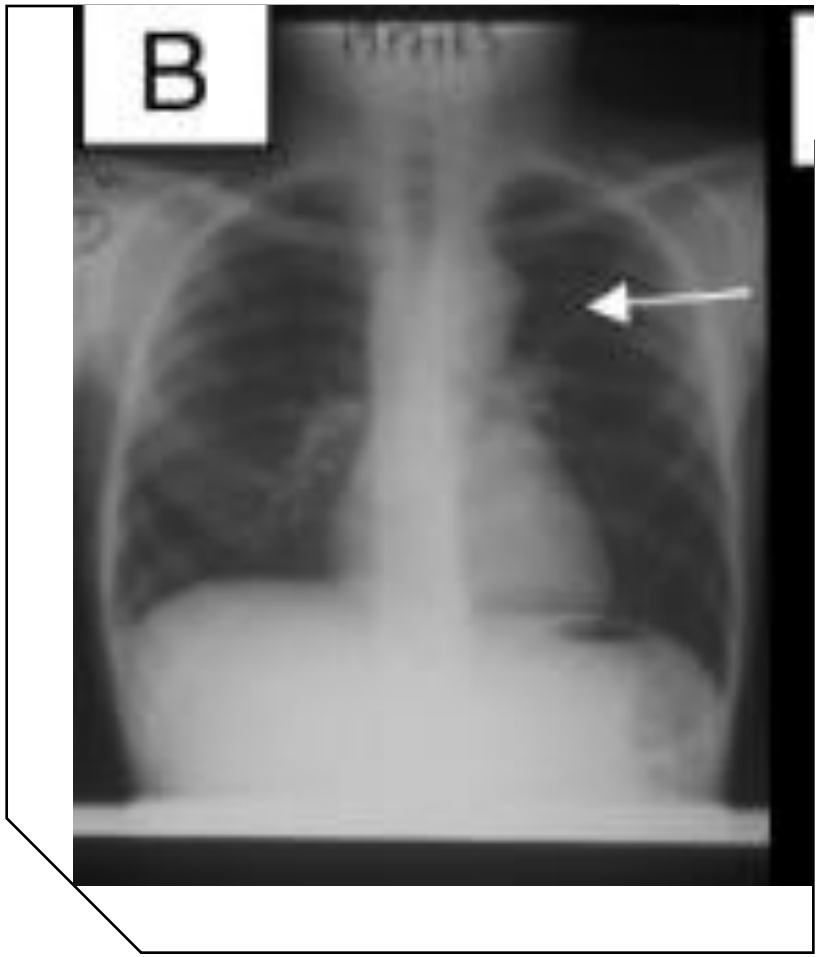
Immune reaction againstIgA/IgE deposit in vessel wall?

Heart, liver, kidney, stomach, coronary, and cerebral

middle and small diameter

Aorta





Pellier et al. Pediatrics.2011

Baratcu et al. JPHO. 2021

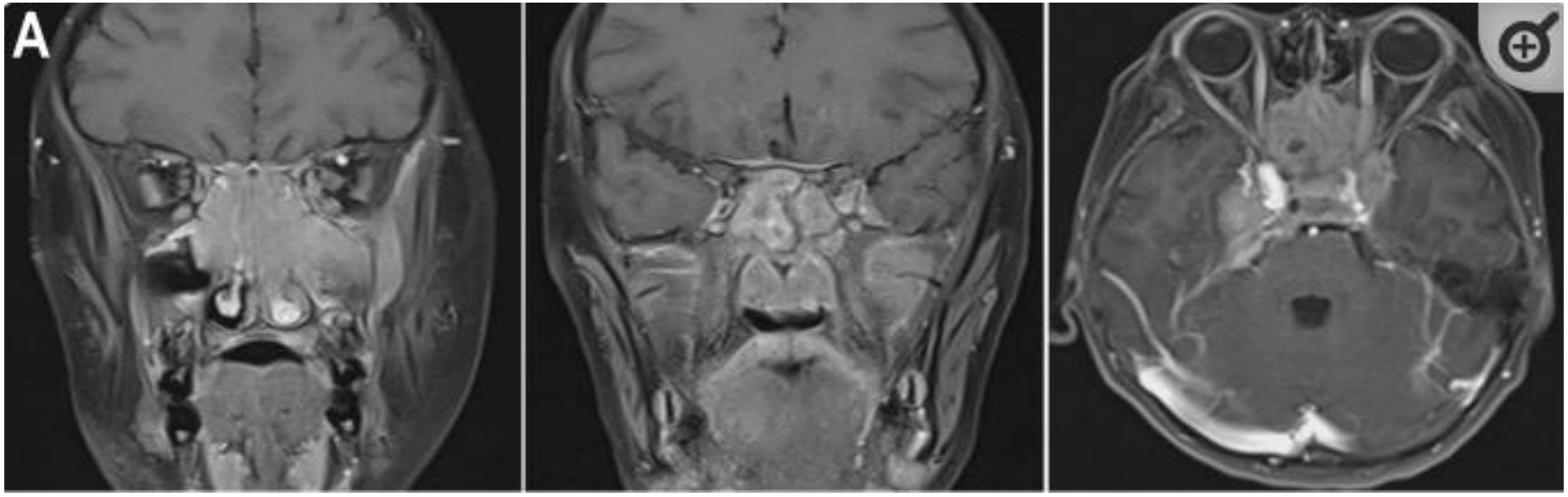
Malignancies

5 – 10 %

Hematological malignancies

- Non Hodgkin lymphoma (B EBV+)
- Leukemia

Poor prognosis than in those with normal WASp



Sun et al. Front Immunol. 2022

Bosticardo et al. Blood. 2009

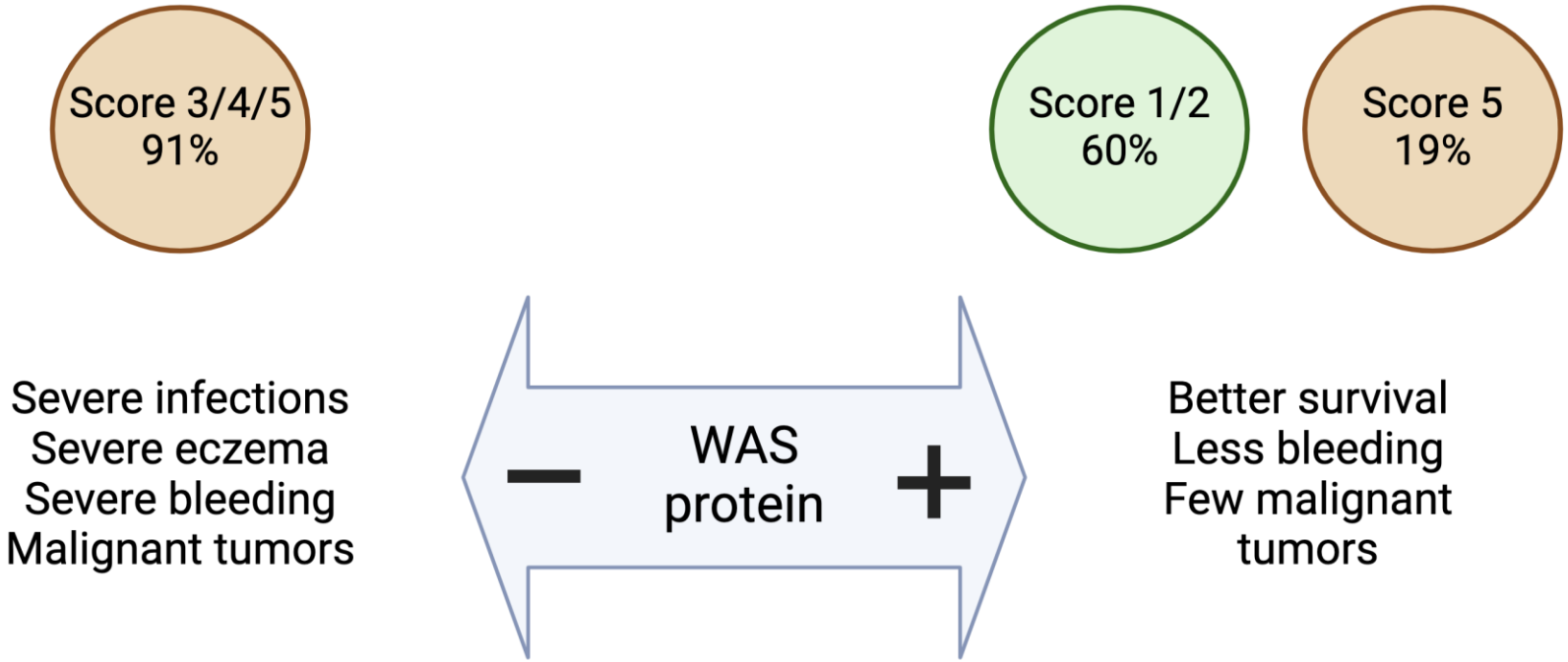
How to assess the severity of WAS/XLT?

Score AT 2 YEARS

Disease phenotype	XLT		Classical WAS phenotype		Severe WAS phenotype	Early-onset severe WAS
Severity score	1	2	3	4	5	EOS
Onset before the age of 2 y	−/+	−/+	−/+	−/+	−	+
Thrombocytopenia	+	+	+	+	+	+
Microthrombocytes	+	+	+	+	+	+
Eczema	−	−/+	+	+/++	+/++	+/++
Immunodeficiency	−	−/+	+	+	+	+
Infections	−	−/+	+	+/++	+/++	+
Autoimmunity and/or vasculitis and/or neoplasia	−	−	−	−	+	+/++
Severe refractory thrombocytopenia	−	−	−	−	−	+*

Mahlaoui et al. Blood 2013 ; Zhu et al. Blood 1995

How to assess the severity of WAS/XLT?



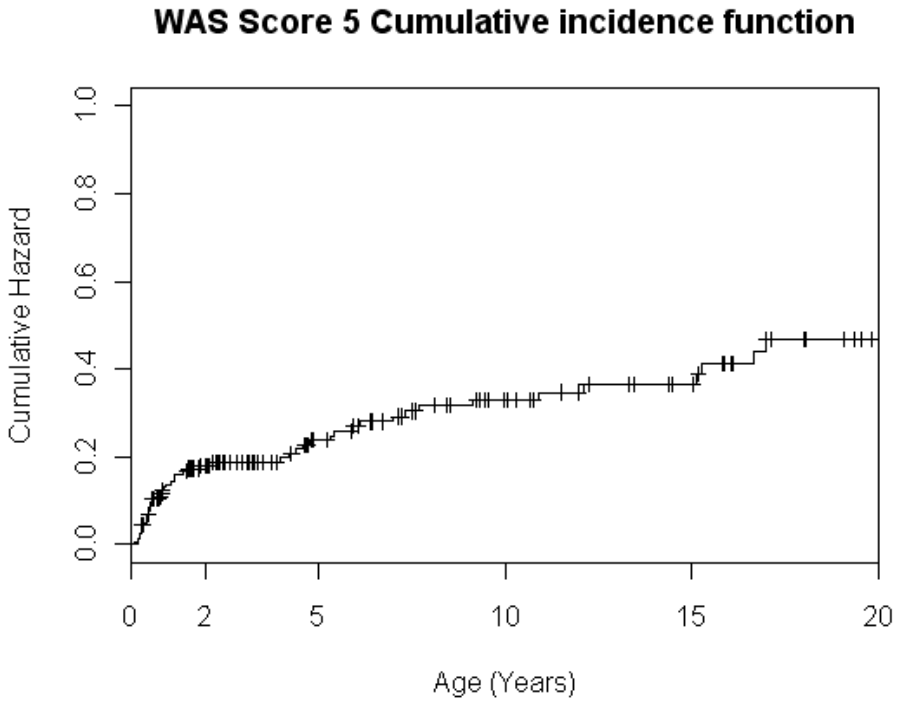
Phenotype correlates with mutation type and protein expression level

Same risk of autoimmunity
22% vs 26%

Based on Imai et al. Blood. 2004

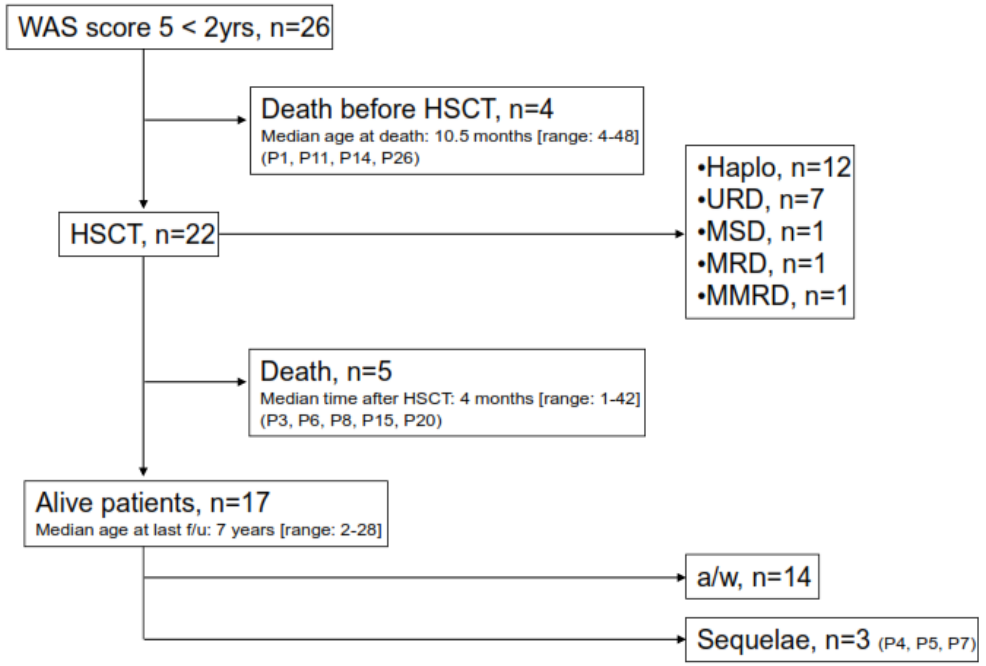
But not everything is predictable…

Some patients have an early onset of life threatening manifestations



Mutations:
10 nonsenses
5 deletions
5 missenses
3 splice site
1 insertion

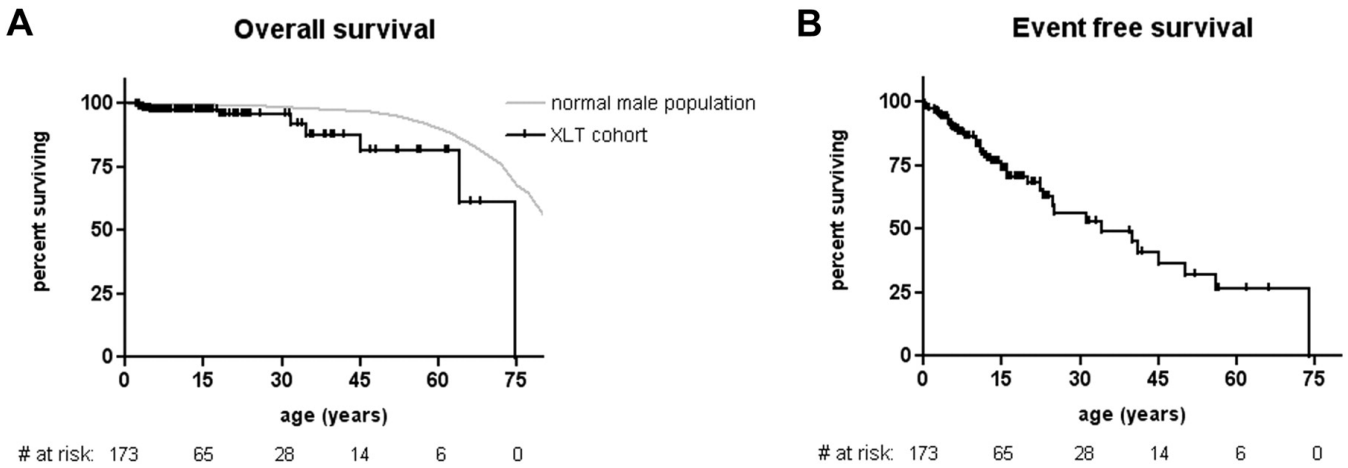
Highly predictive of absence WASp:
19 cases



Mahlaoui et al. Blood 2013

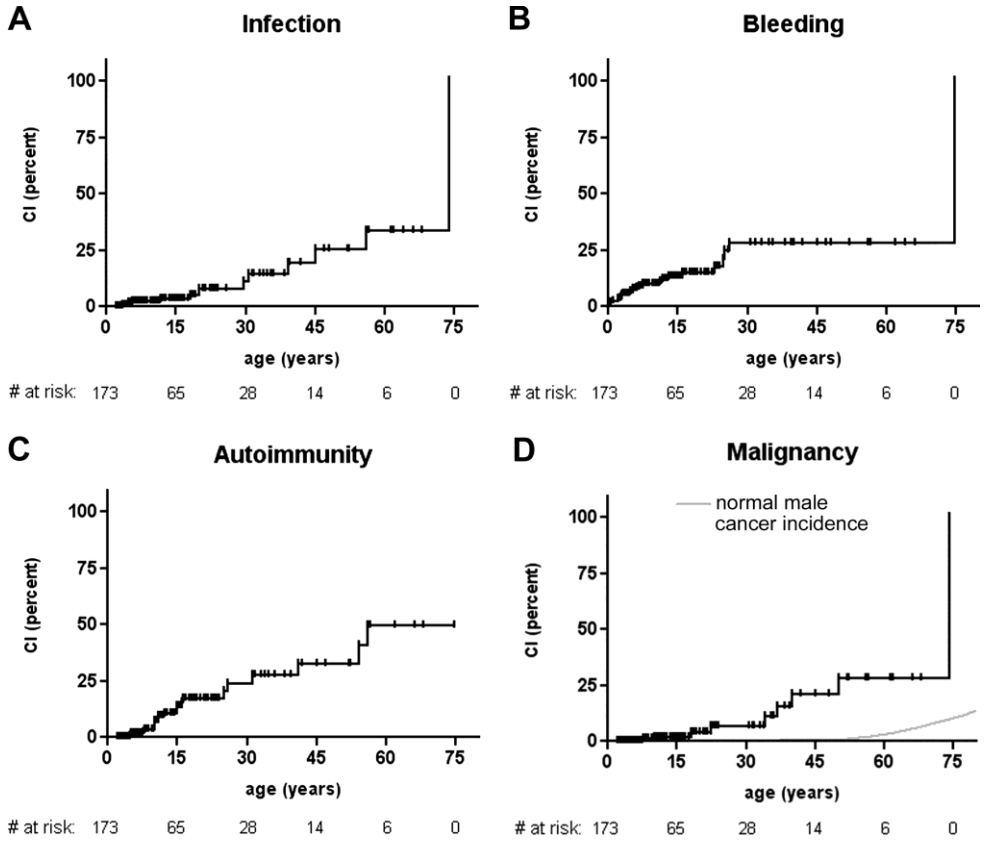
But not everything is predictable…

Some XLT patients do on to develop life-threatening manifestations



OS 15-year 97%
30-year 96%
45-year 81%
60-year 81%

EFS 15-year 74%
30-year 56%
45-year 36%
60-year 27%



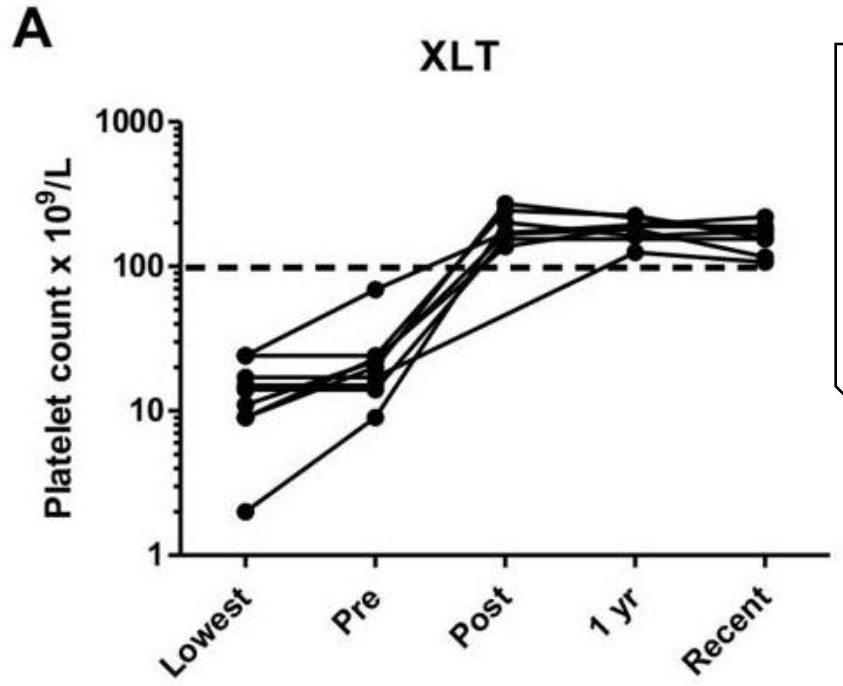
Albert et al. Blood 2010

How to treat WAS/XLT patients ?

Supportive therapy

Thrombocytopenia

- Prevention +++
- NO SYSTEMATIC PLATELET TRANSFUSIONS
- Splenectomy: controversial

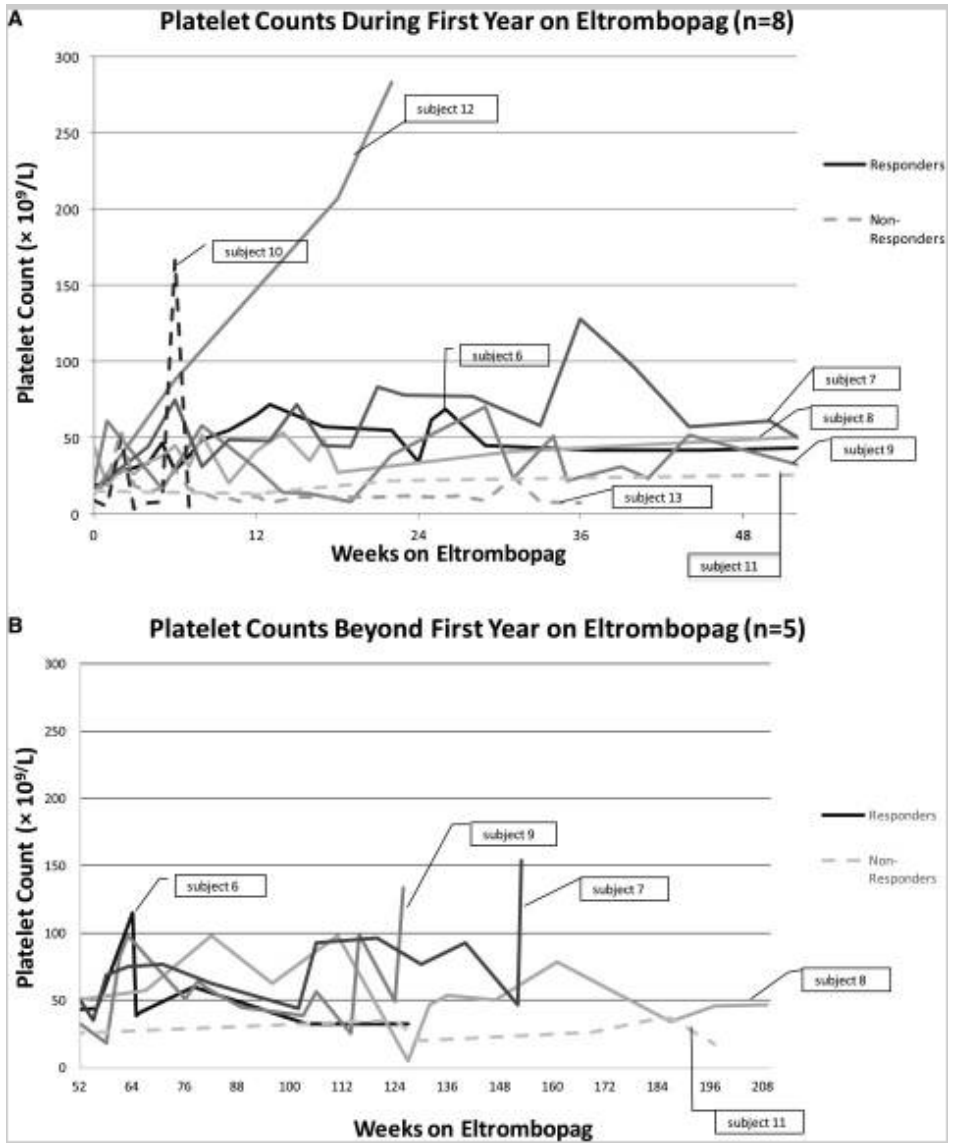


Splenectomy works on platelets but risk of infection +++

Rivers et al. Br J Haematol. 2019
Rivers et al. JACI Pract 2018
Albert et al. Blood. 2010

Thrombocytopenia

- Severe bleeding:
  - Transfusion if needed
- Prolonged nosebleeds:
  - Intravenous tranexamic acid
- Platelets agonist
  - Increases platelet production
  - BUT no improvement in platelet activation
  - Can be used temporarily in specific situations (before surgery…) but not systematically and not on a long-term basis



Zaninetti et al. Haematologica. 2020
Rivers et al. Br J Haematol. 2019
Gerrits et al. Blood. 2015

Thrombocytopenia

- When to suspect an auto-immune platelet consumption?
  - Increase in bruising/petechiae or spontaneous bleeding
  - Acute drop in the platelet count from baseline
  - Failure of platelet transfusions
  - Immunomodulatory treatment:

1st line	Oral prednisolone	Children and adults: 2 mg/kg/day orally for 1–2 weeks then taper gradually, maximum 60 mg/day
or	Methylprednisolone	Children and adults: 4 mg/kg/day intravenously for 4 days then taper gradually, maximum 60 mg/day
1st/2nd line*	Intravenous immunoglobulin	Children and adults: 1 g/kg intravenously as a single dose; consult specialist for guidance on subcutaneous dose *Intravenous immunoglobulin and steroids given together as first line in children
3rd line	Rituximab	375 mg/m^2 weekly for 4 weeks

Rivers et al. Br J Haematol. 2019

Other complications

- Auto-immunity
  - Supportive care and immunosuppression
  - Nephropathy: renal transplantation is sometimes necessary

- Malignancies
  - Standard oncology protocols

BUT : CONSIDER MOVING ON TO DEFINITIVE THERAPY

Rivers et al. Br J Haematol. 2019

How to treat WAS/XLT patients ?

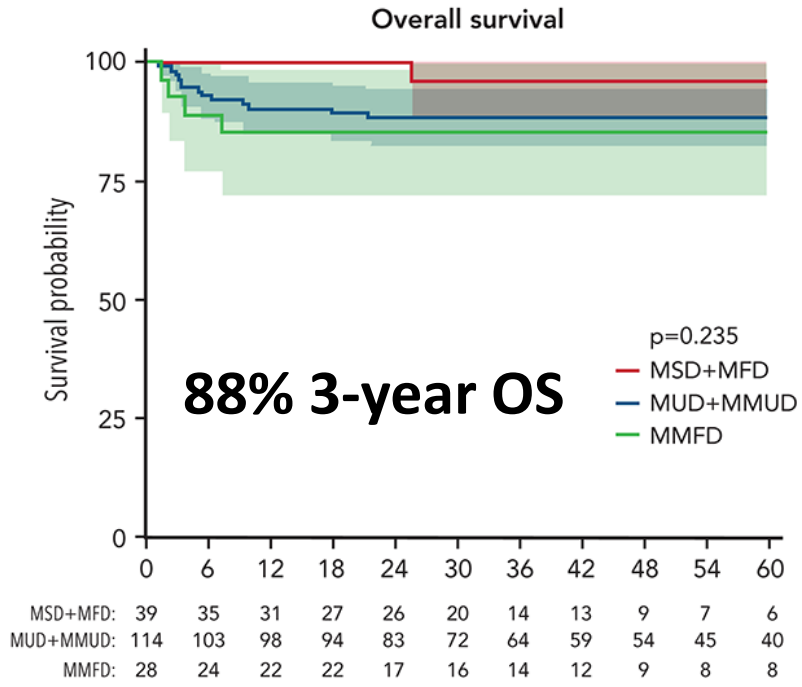
Definitive therapy

Is a definitive therapy indicated?
If so, when?

- DEFINITELY
  - All children with genetic mutation consistent with classical WAS and no WASp
  - Severe WAS or Early-onset severe WAS: as soon as possible
  - As soon as onset of a stade 5 complication
    - Hematological malignancies: as soon as possible after remission
    - Close to kidney transplantation

- MAYBE
  - All patients with impairement of quality of life and donor available

HEMATOPOIETIC STEM CELL TRANSPLANTATION



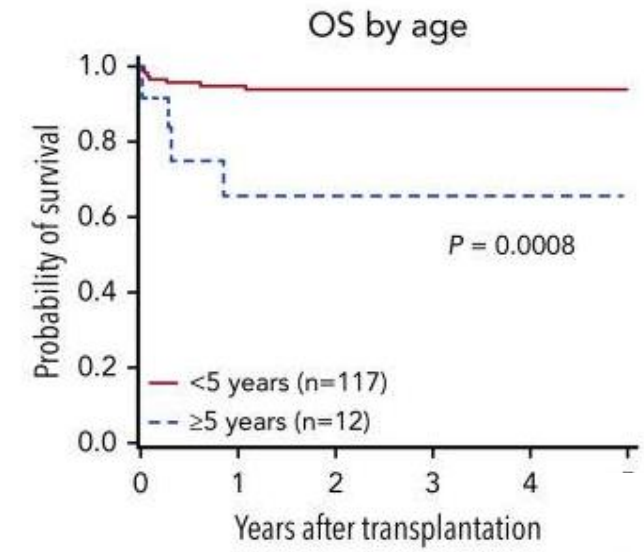
88% 3-year OS

Europe between 2006 and 2017

Albert. Blood 2022

100% OS between 2006 and 2016

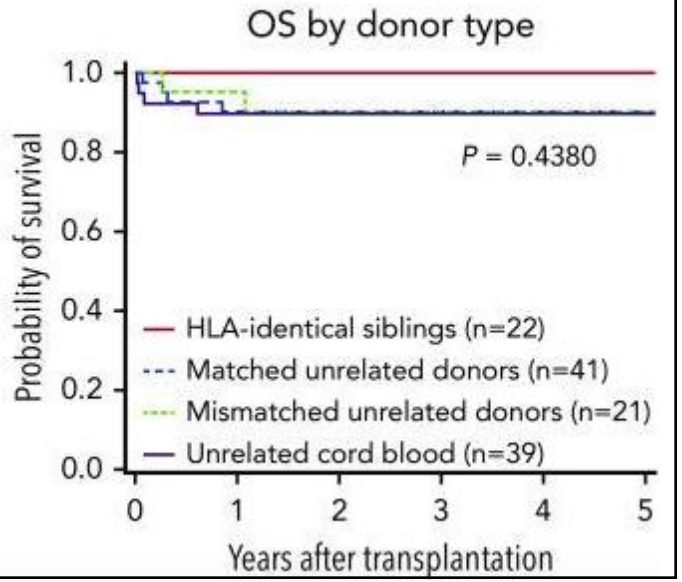
(34 consecutive HSCT) *Elfeky et al. JACI. 2018*



91% 5-year OS

USA between 2005 and 2015

Burroughs. Blood. 2020



HEMATOPOIETIC STEM CELL TRANSPLANTATION

Success with all donor types

Age at transplant

Younger is better?

Before 1 year?

Before 5 year?

Before complications

Conditioning regimen

MAC or RIC?

MAC:

- improved T-cell engraftment
- higher percentage of patients with full donor B-cell chimerism at day 100 and myeloid chimerism at 6 months

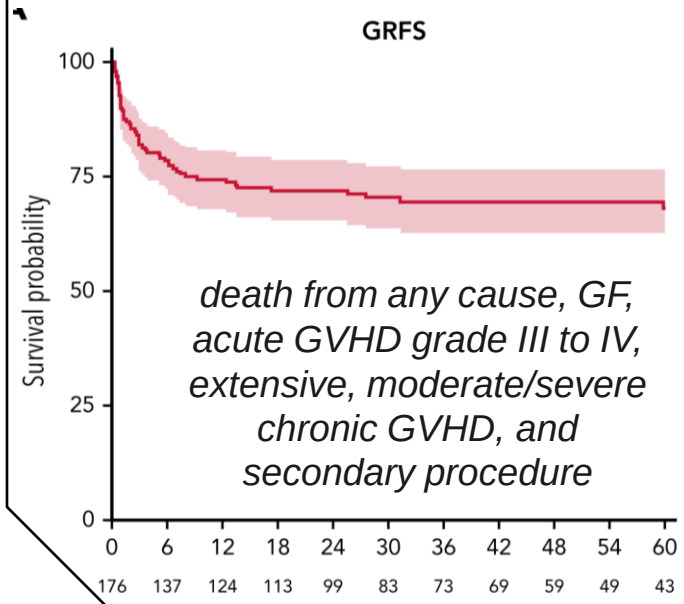
BUT but disease correction is achievable with attenuated conditioning

Albert. Blood 2022

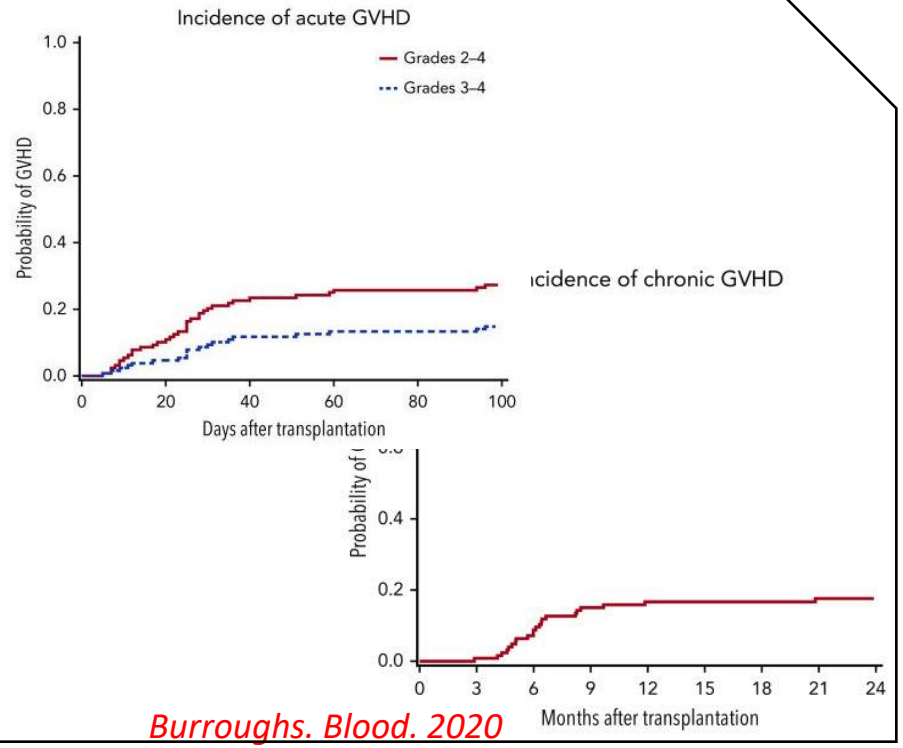
Burroughs. Blood. 2020

HEMATOPOIETIC STEM CELL TRANSPLANTATION

GVHD



Albert. Blood 2022



Burroughs. Blood. 2020

Long term complications

Infertility, secondary malignancies, endocrine effects, and organ dysfunction…

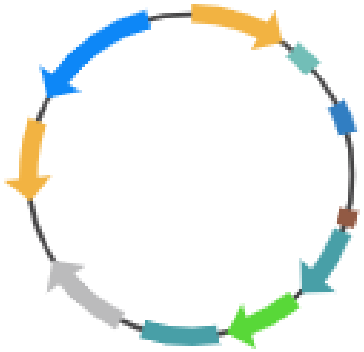
Role of age at transplantation?
Role of conditioning regimen?

Autoimmunity

Resolution of previous autoimmunity
BUT risk of de novo autoimmune disease (14%-15%)

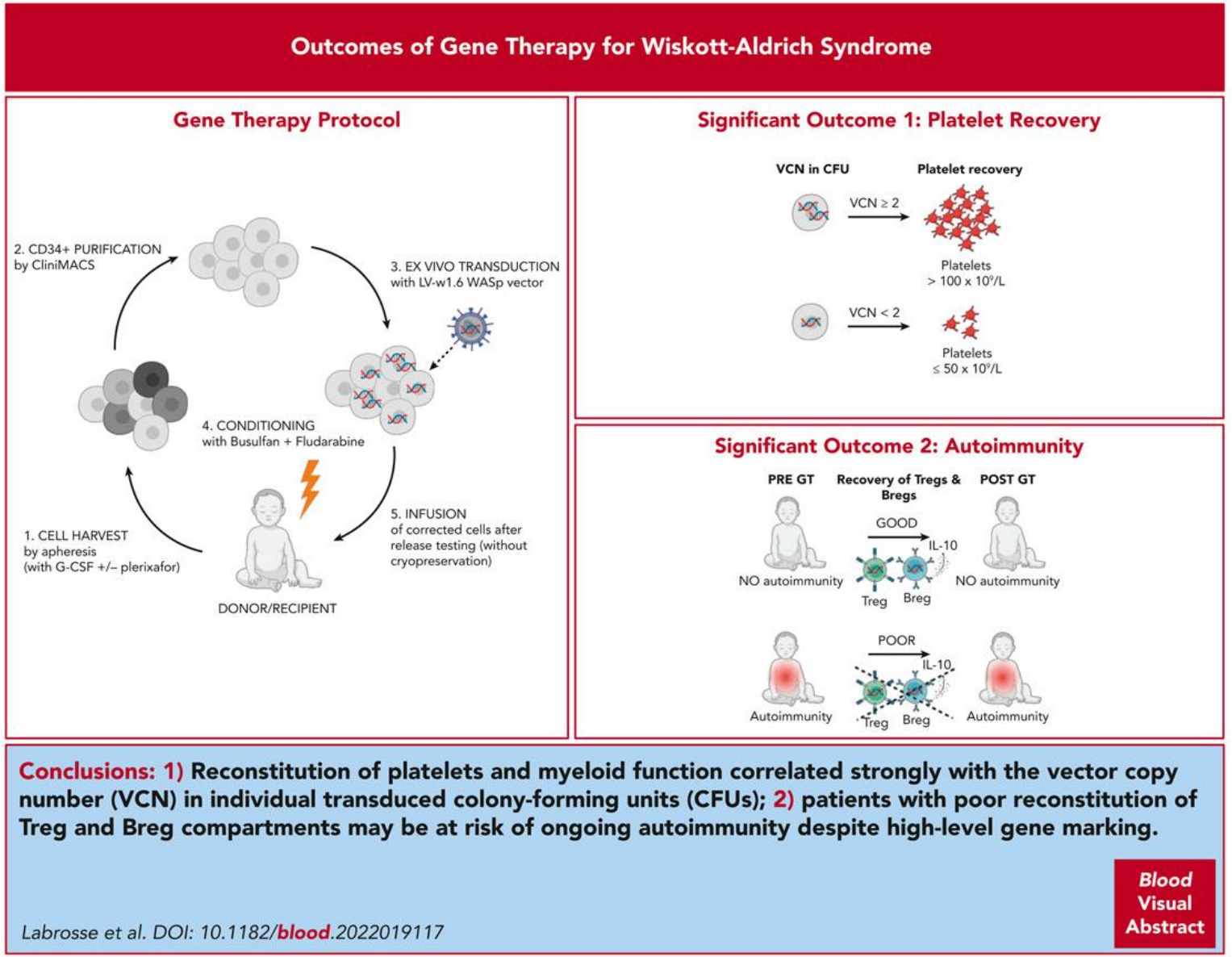
HEMATOPOIETIC STEM CELL GENE THERAPY

Self-inactivating lentiviral vector expressing WASp under the control of a minimal endogenous promoter

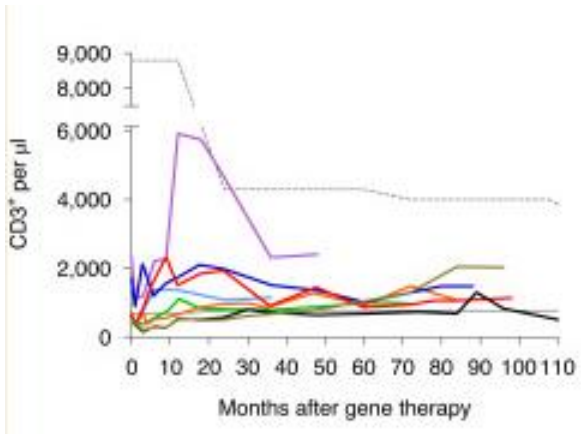


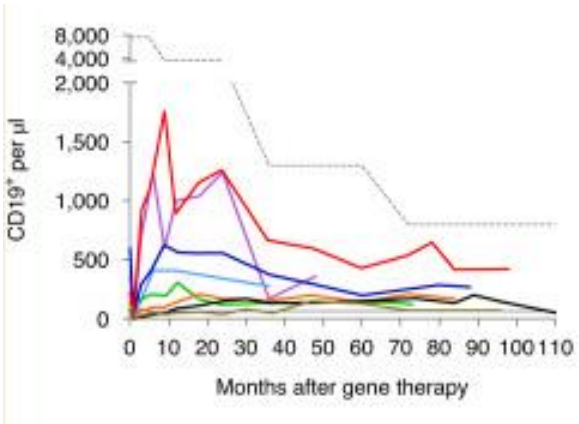
1 death/9 patients
(pre-existing severe viral infection)

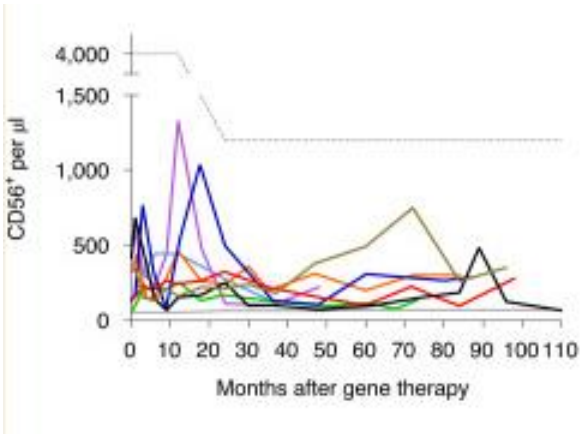
Labrosse et al. Blood. 2023
Magnani et al. Nat Med. 2022
Hacein-Bey Abina. JAMA. 2015

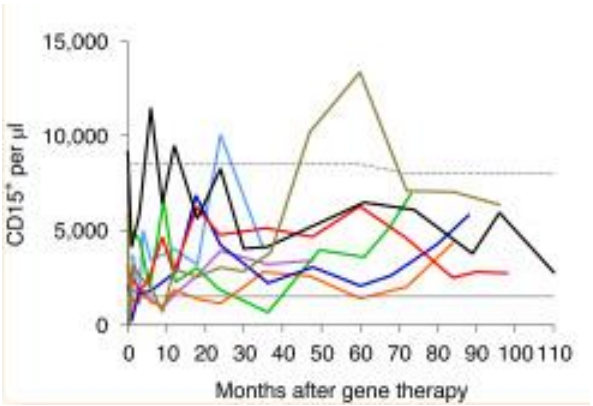


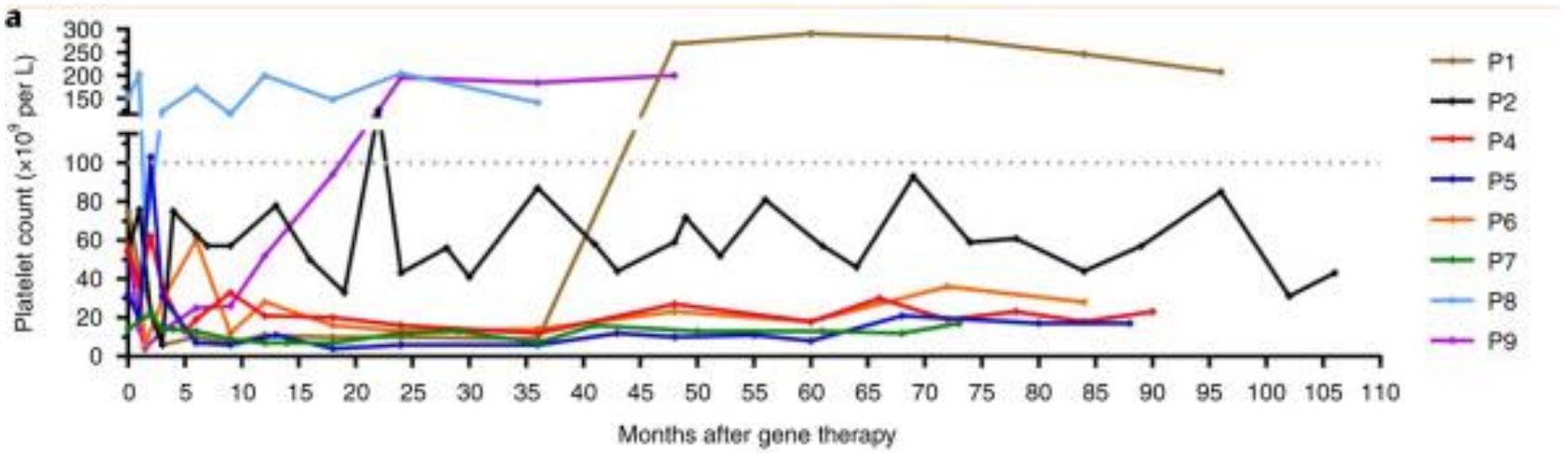
HEMATOPOIETIC STEM CELL GENE THERAPY











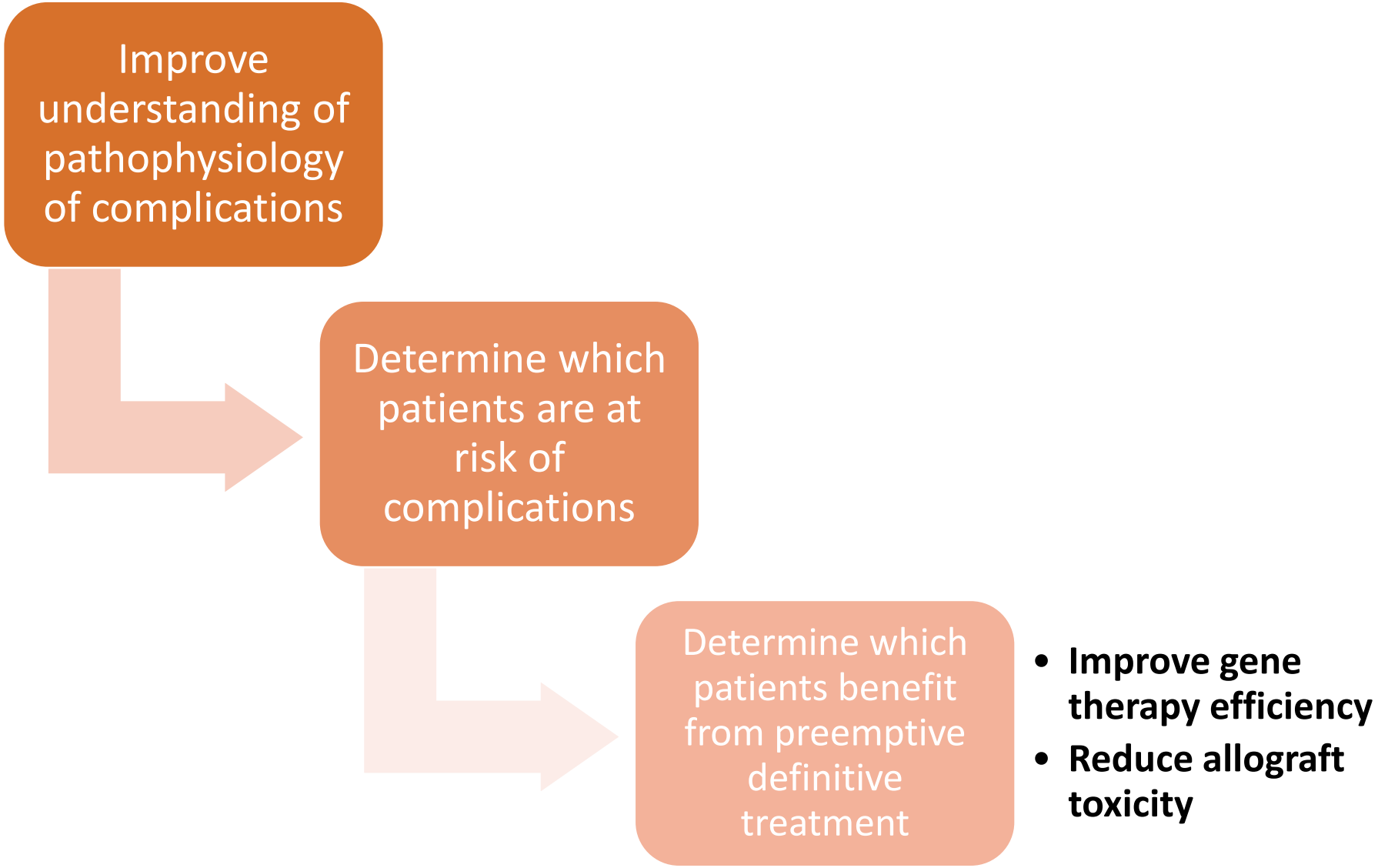
Autoimmunity

No resolution of all pre-transplant autoimmunity

De novo auto-immunity

Magnani et al. Nat Med. 2022

FUTURE CHALLENGES



- **Improve gene therapy efficiency**
- **Reduce allograft toxicity**

European Reference Network

for rare or low prevalence complex diseases

Network Hematological Diseases (ERN EuroBloodNet)