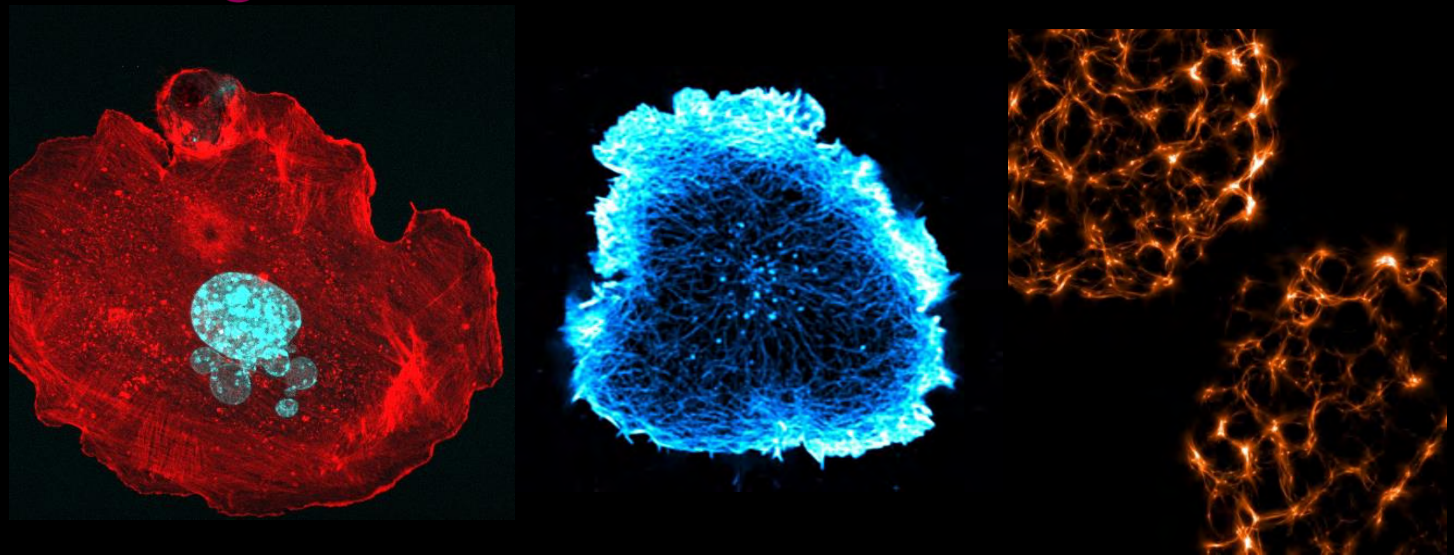
Spectrum of WASp variants: from loss-of-function to gain-of-function

Lisa Westerberg

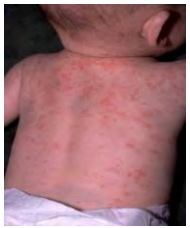
Karolinska Institutet
Dept of Microbiology Tumor and Cell Biology



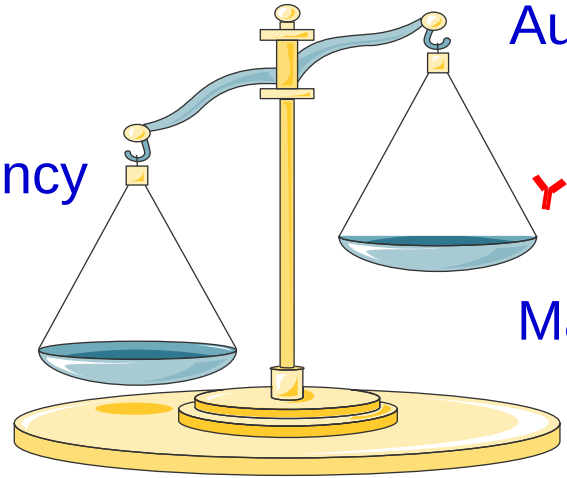
X	No, Nothing to disclose
	Yes, please specify disclosures

Inborn errors of Immunity (IEIs):
Spectrum of immunodeficiency and cancer

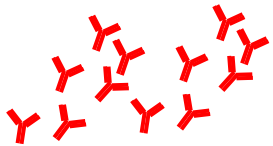
Immunodeficiency



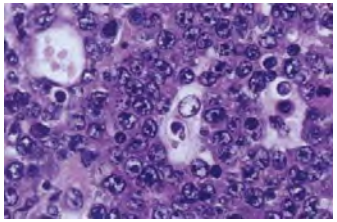
Boy with
Wiskott-Aldrich syndrome

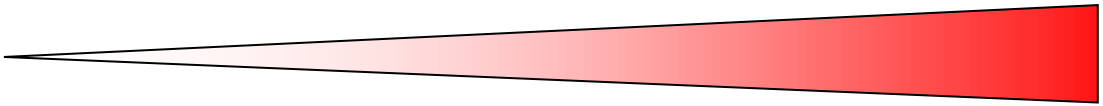


Autoimmunity



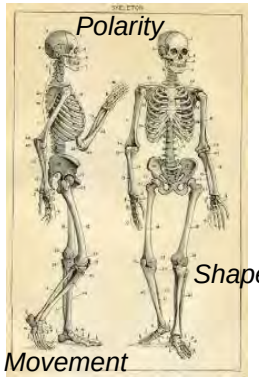
Malignancies

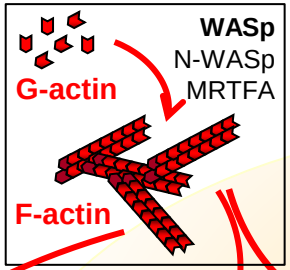




Activity of actin regulators

The immune system is never at rest: The actin cytoskeleton dynamics



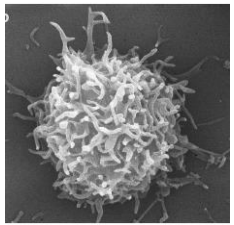


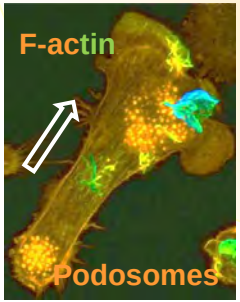
Cell – cell communication

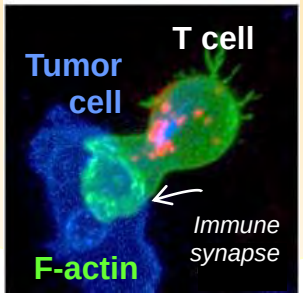
Immunological synapse

Migration

Circulation in blood

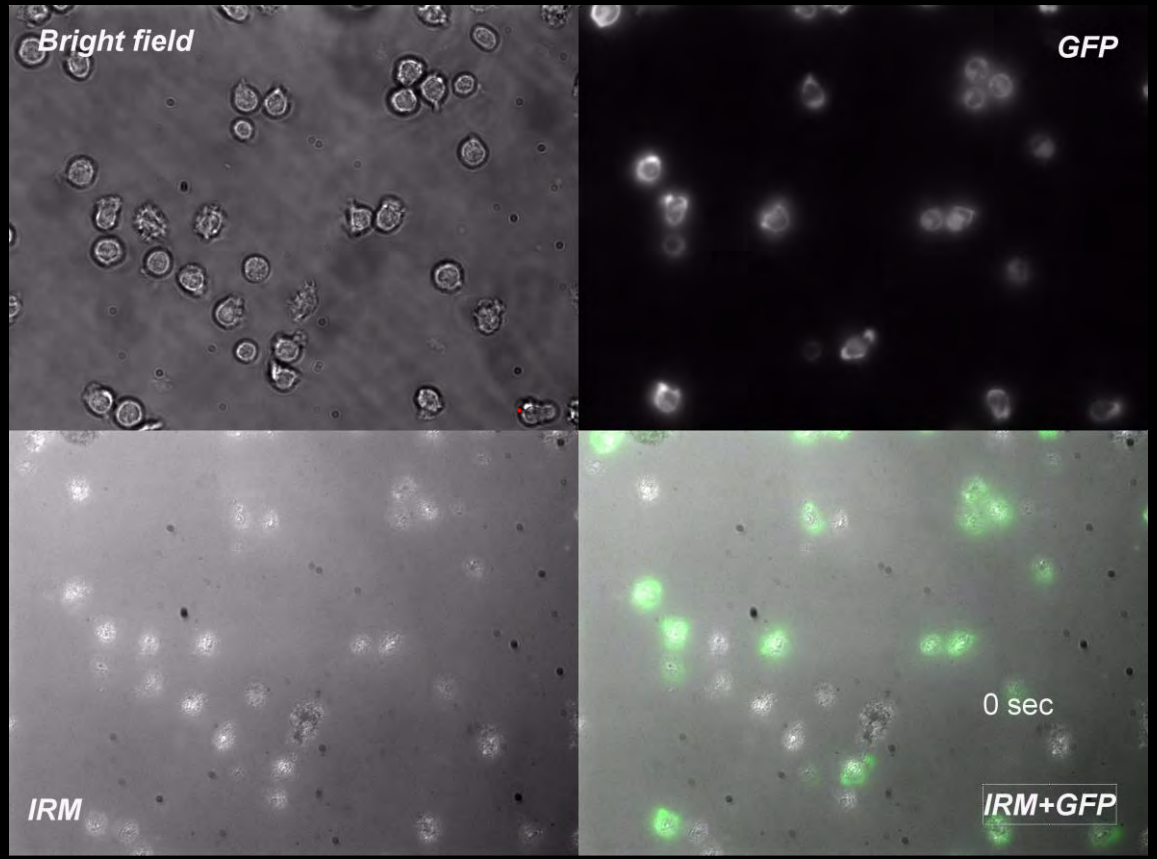






Live cell adhesion of neutrophils on fibrinogen

WASp-L272P(LifeAct) vs WT



”Hematopoietic cells depend on high and fast actin dynamics”

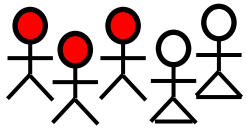
Extra gene copies of actin regulators

Keszei et al, J Clin Invest 2018

Wiskott-Aldrich syndrome and the WASp family of actin regulators

Alfred Wiskott
1937

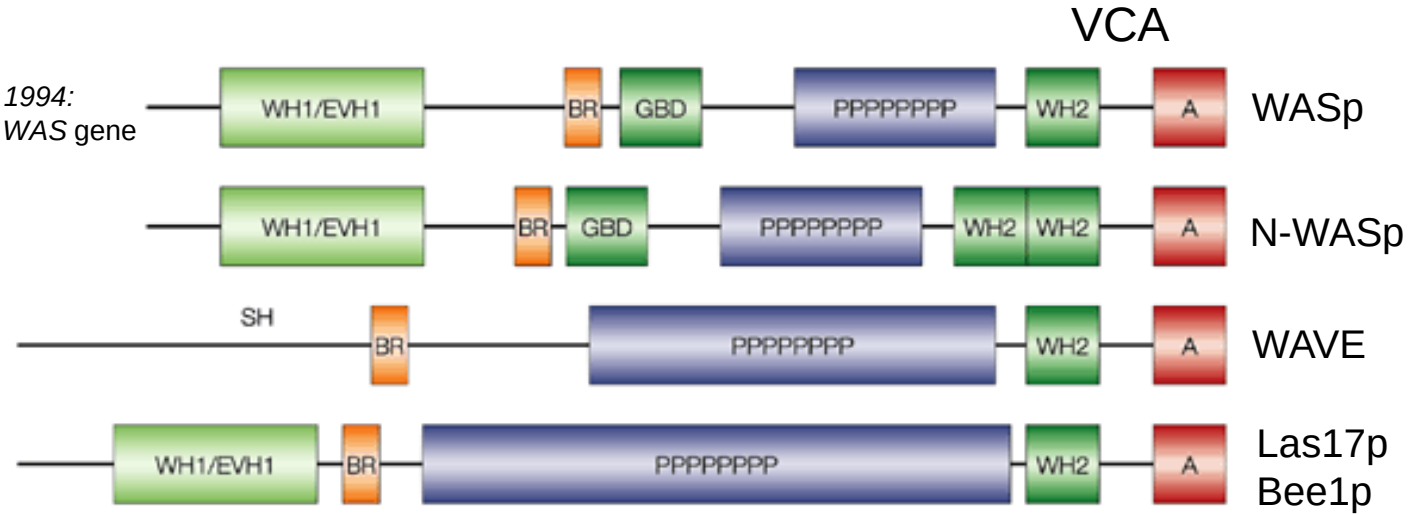
Robert Aldrich
1954



Wiskott-Aldrich syndrome (WAS):
Triad of immunodeficiency,
thrombocytopenia, eczema

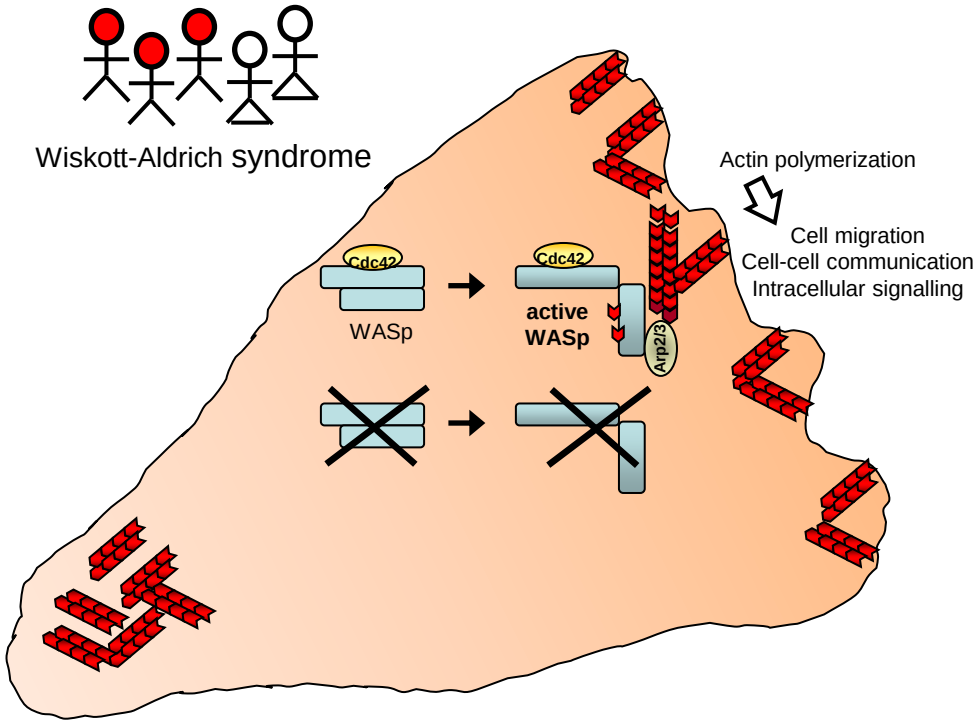
Curative treatment: HSCT or Gene therapy

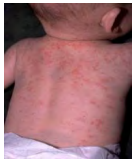
Abo et al, Cell 1994:
Cloning of the *WAS* gene



Thrasher and Burns, Nat Rev Immunol 2010
Saeed, Record, Westerberg, IRCMB 2020

Inborn errors in actin regulation:
Loss-of-function variants of *WAS* cause Wiskott-Aldrich syndrome

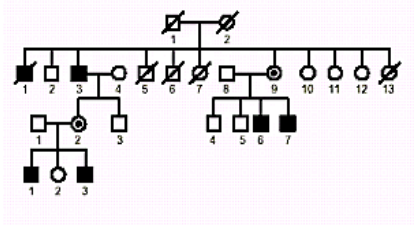




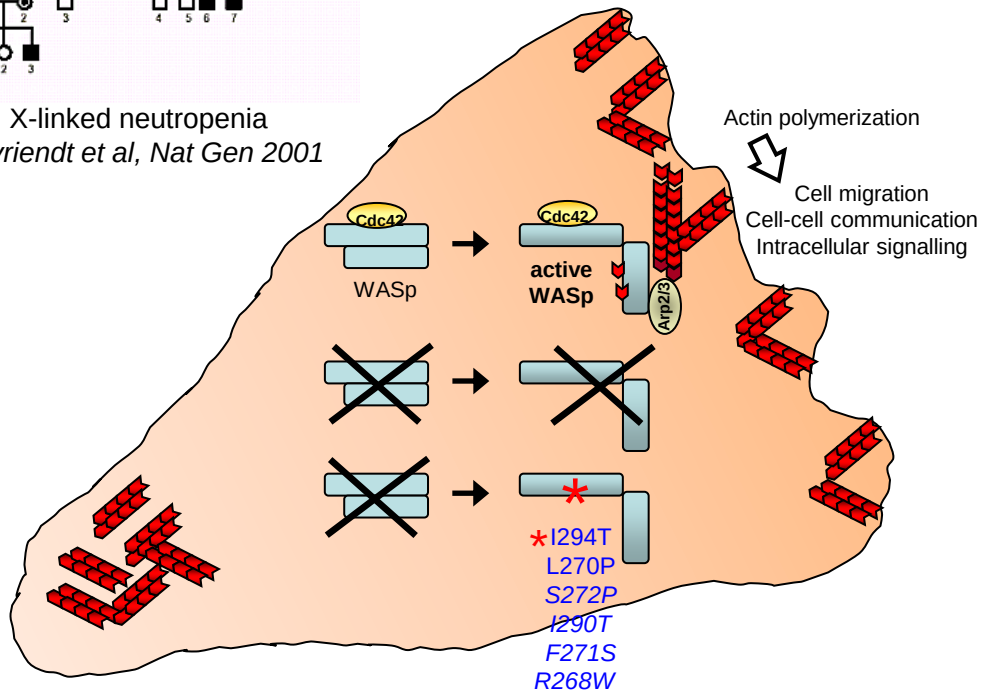
Boy with
Wiskott-Aldrich syndrom

Infections + Autoimmunity
Lymphoma
Eczema

Inborn errors in actin regulation:
Gain-of-function variants of *WAS* cause X-linked neutropenia



X-linked neutropenia
Devriendt et al, Nat Gen 2001

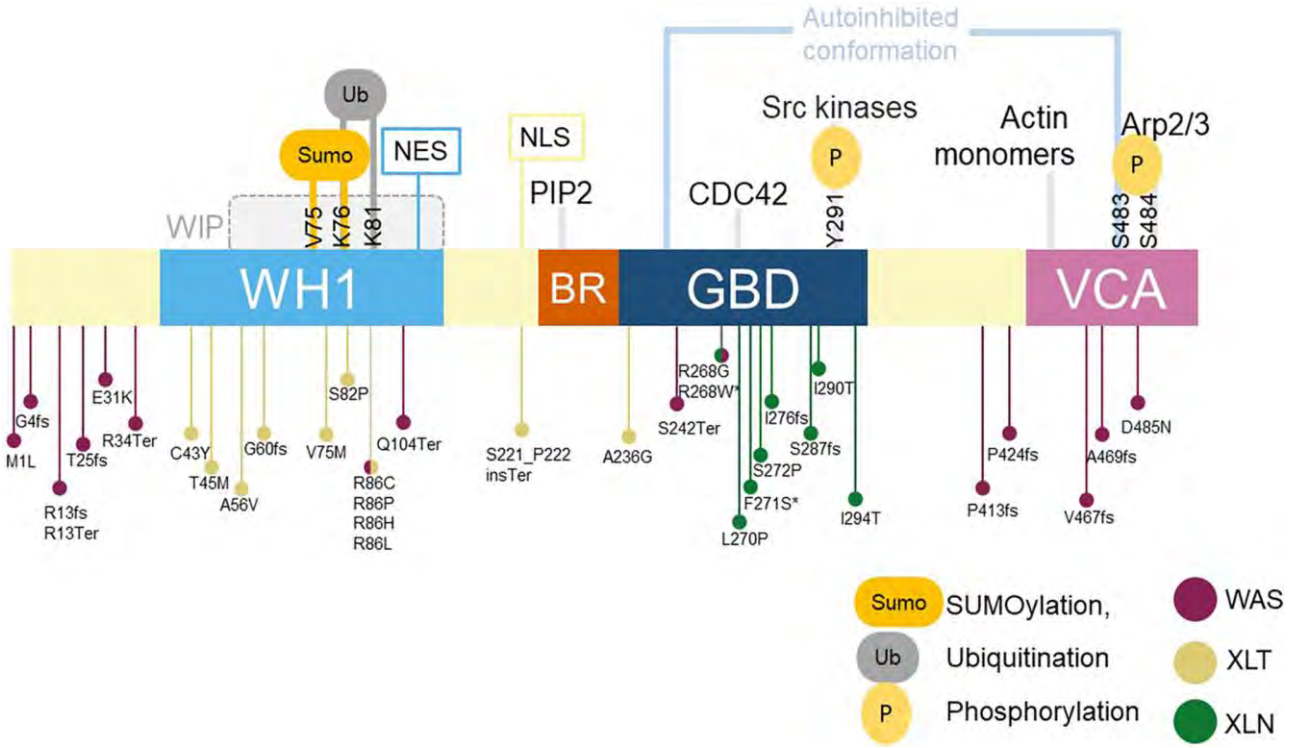


Actin polymerization

Cell migration
Cell-cell communication
Intracellular signalling

Infections + ?

Precision medicine:
>300 variants in the WAS gene lead to inborn error of immunity



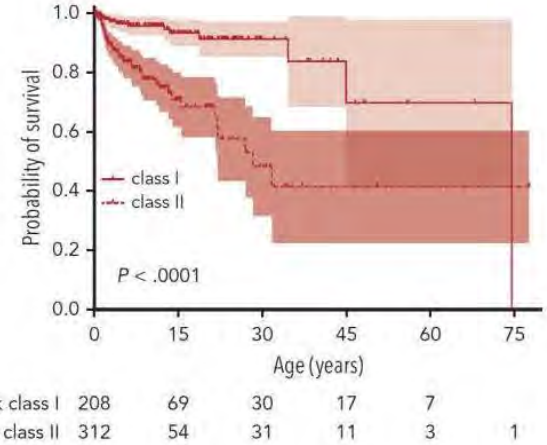
Vieira, Pinho, Westerberg, Ped All Immunol 2023

Wiskott-Aldrich syndrome: disease outcome by genetic variant in >500 patients

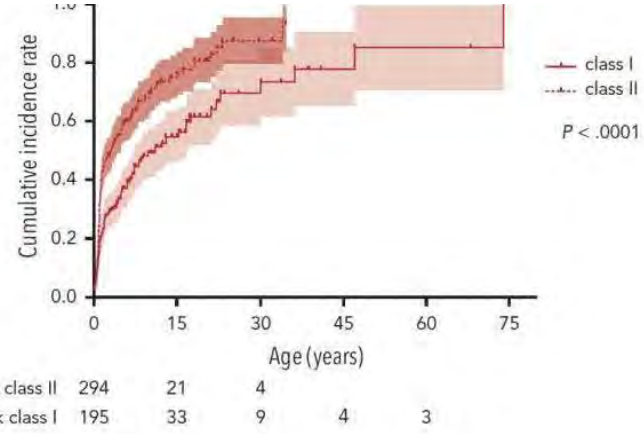
WAS class I: late onset – missense variants in exons 1+2 and c.559+5G>A

WAS class II: early onset – all other variants

Overall survival (OS)
n=520



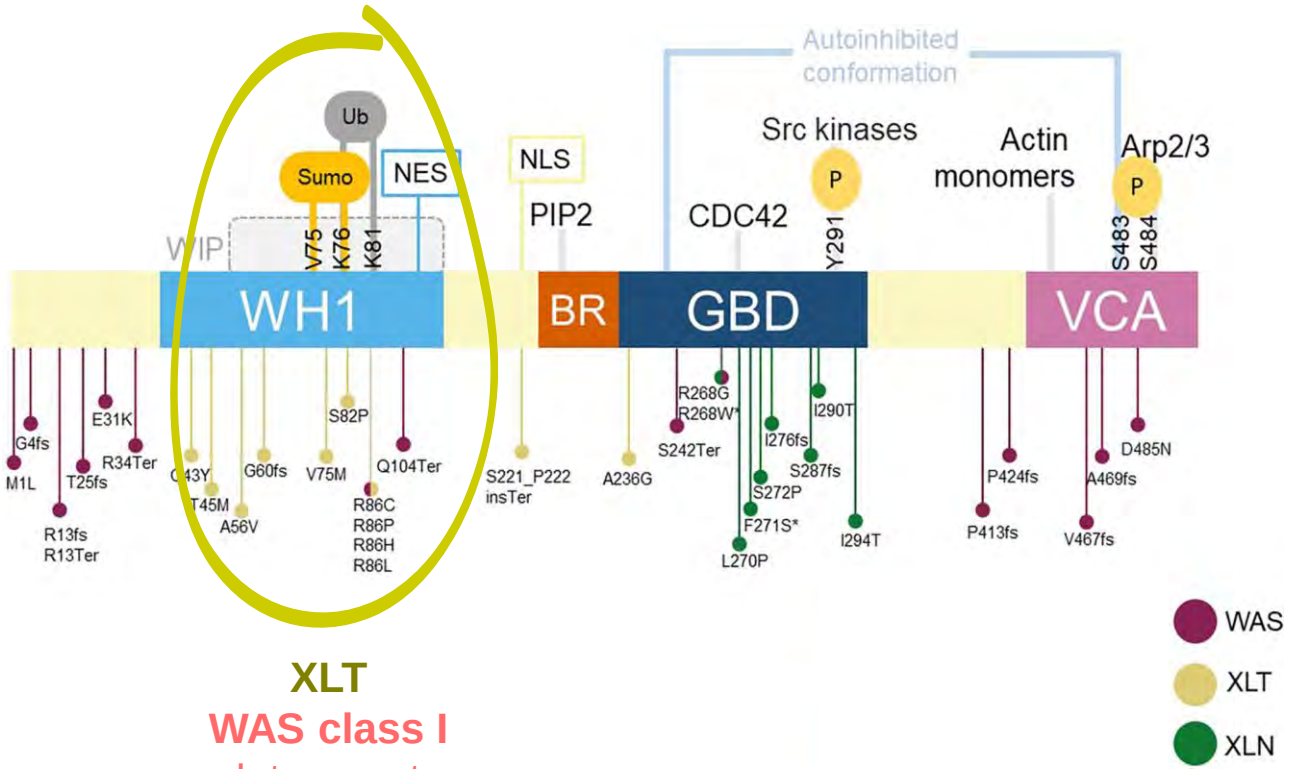
Cumulative incidence of severe disease*
n=489



*Life-threatening bleeding
(Intracranial, gastrointestinal)
Bleeding episode w. blood cell transfusion
Sepsis
Meningitis
Pneumonia w. respitory support
Systemic viral (viremia)
Invasive fungal infection
Autoimmunity
Malignancies

Vallee … Alberts, Wiskott-Aldrich syndrome: a study of 577 patients defines the genotype as a biomarker for disease severity and survival, Blood 2024

Precision medicine:
>300 variants in the WAS gene lead to inborn error of immunity



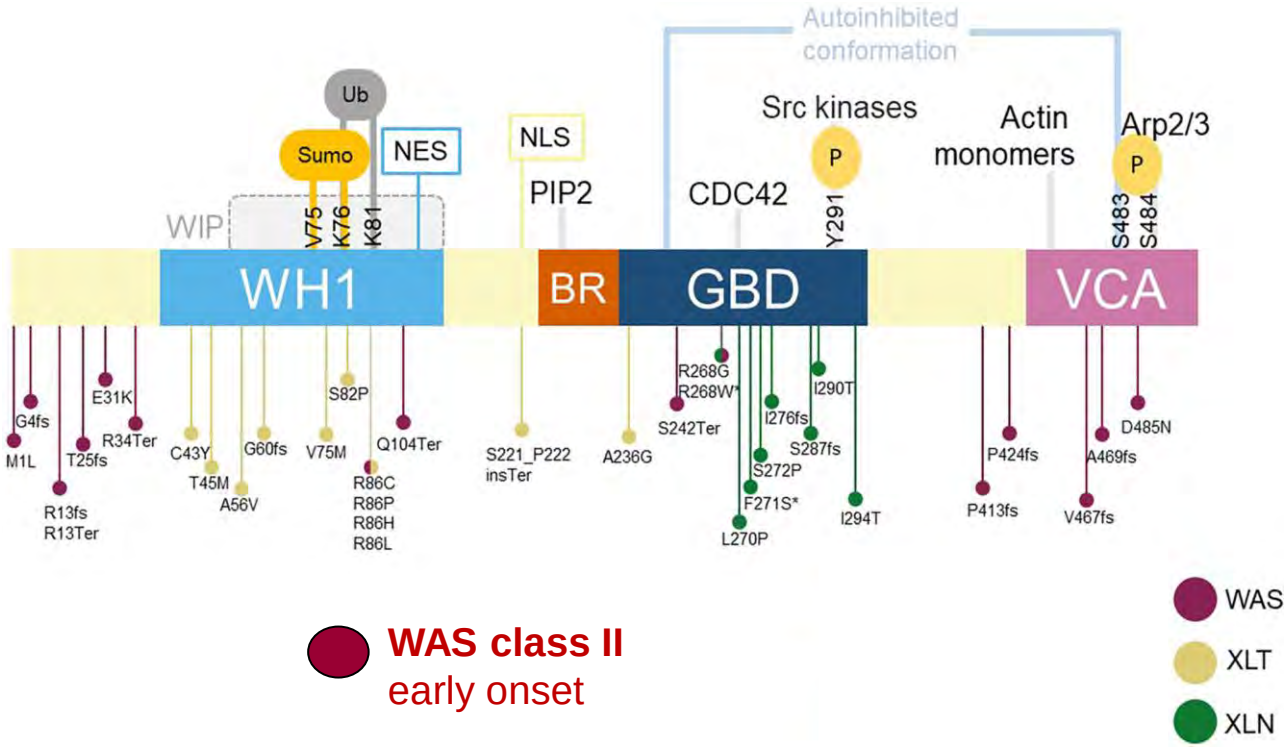
XLT

WAS class I

late onset

Vieira, Pinho, Westerberg, Ped All Immunol 2023

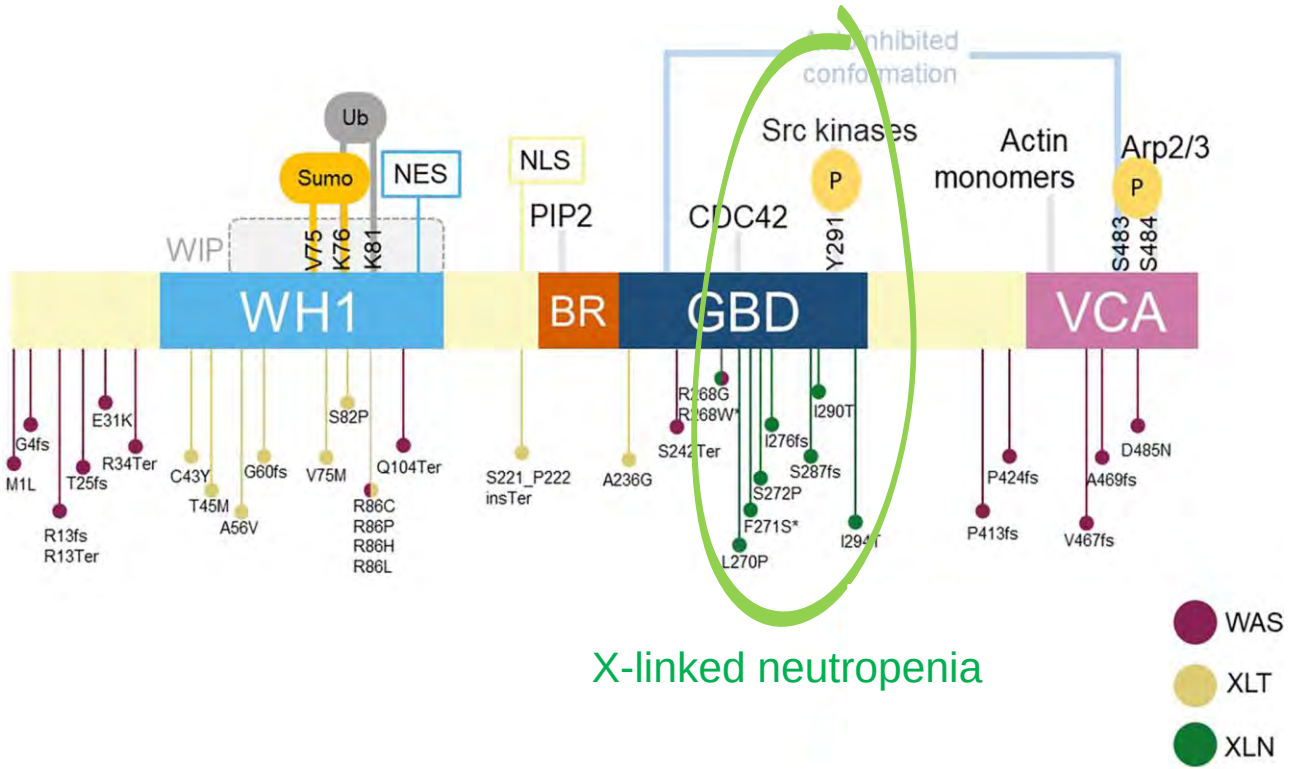
Precision medicine:
>300 variants in the WAS gene lead to inborn error of immunity



WAS class II
early onset

Vieira, Pinho, Westerberg, Ped All Immunol 2023

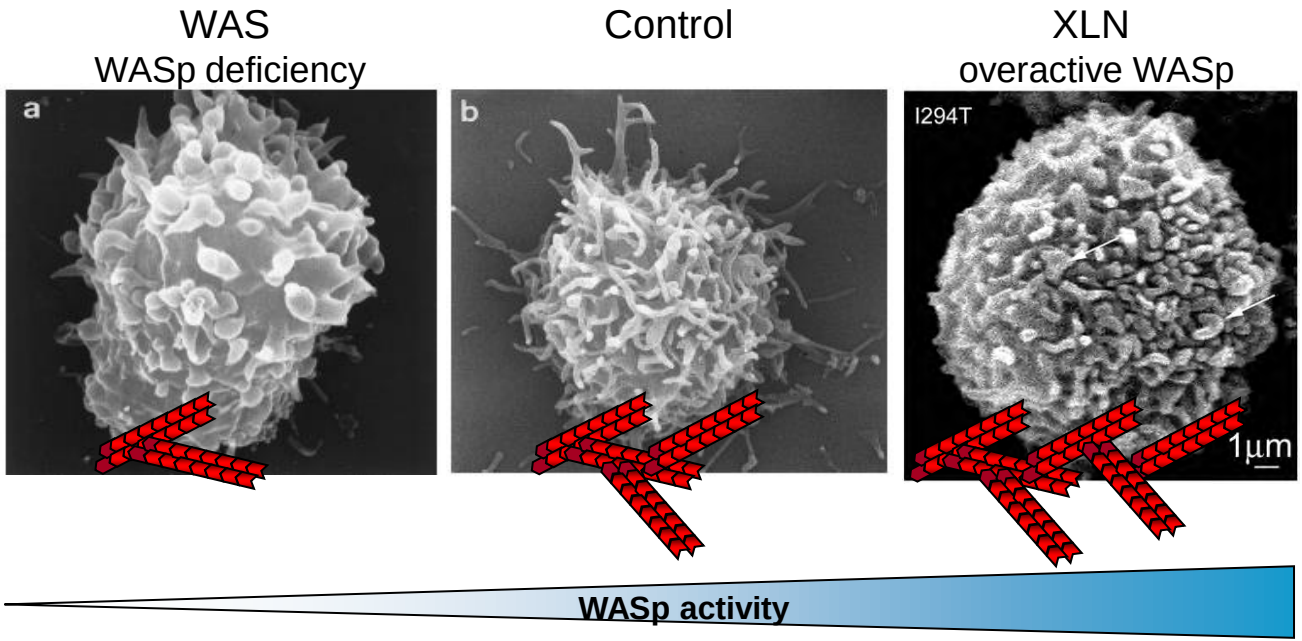
Precision medicine:
>300 variants in the WAS gene lead to inborn error of immunity

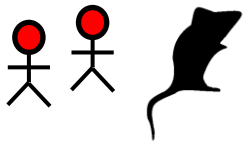


X-linked neutropenia

Vieira, Pinho, Westerberg, Ped All Immunol 2023

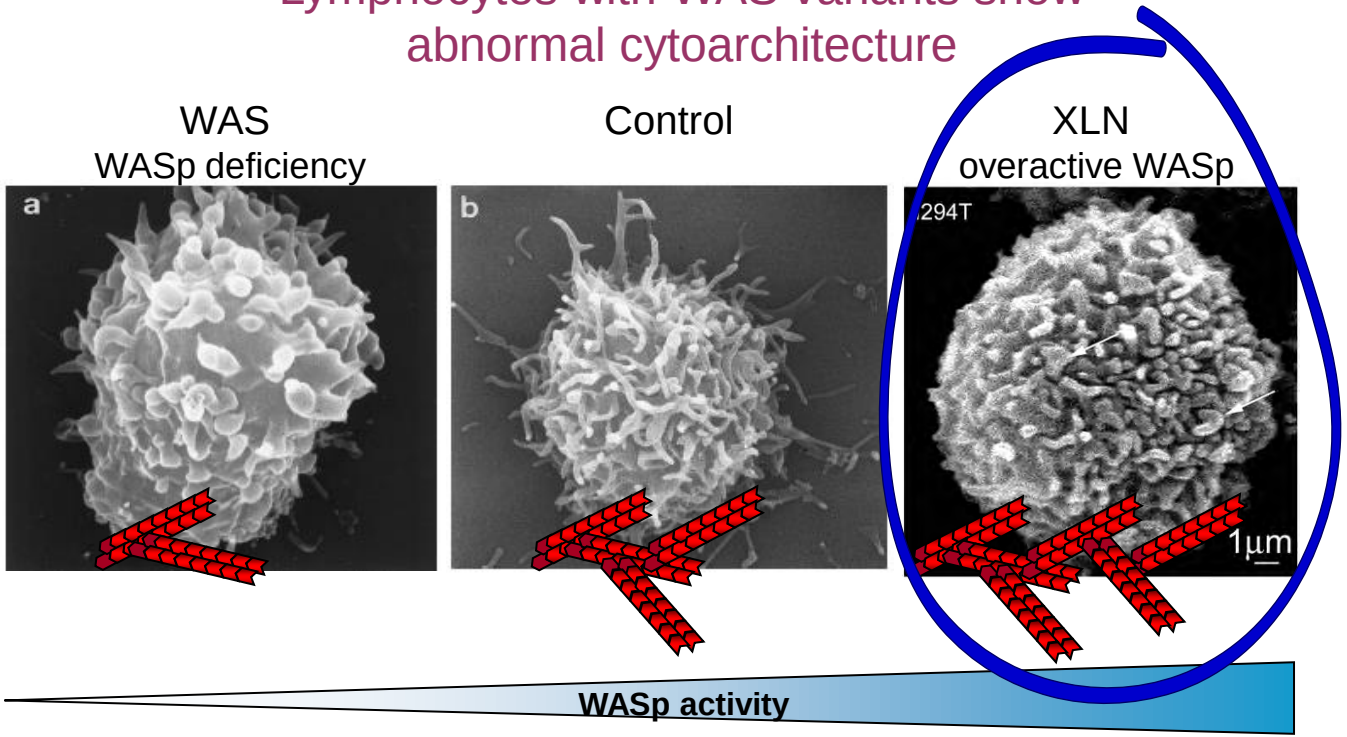
Lymphocytes with WAS variants show abnormal cytoarchitecture





Kenney et al, Blood 1986
Molina et al, Blood 1997
Moulding et al, J Exp Med 2007
Burns et al, Blood 2010
Westerberg et al, Blood 2001, 2005; J Exp Med 2010
Baptista et al, Nature Comms 2016
Kuznetsov et al, Genome Medicine 2017
Keszei et al, J Clin Invest 2018
He et al, JACI 2022

Lymphocytes with WAS variants show abnormal cytoarchitecture



⇨ Very low neutrophils in blood

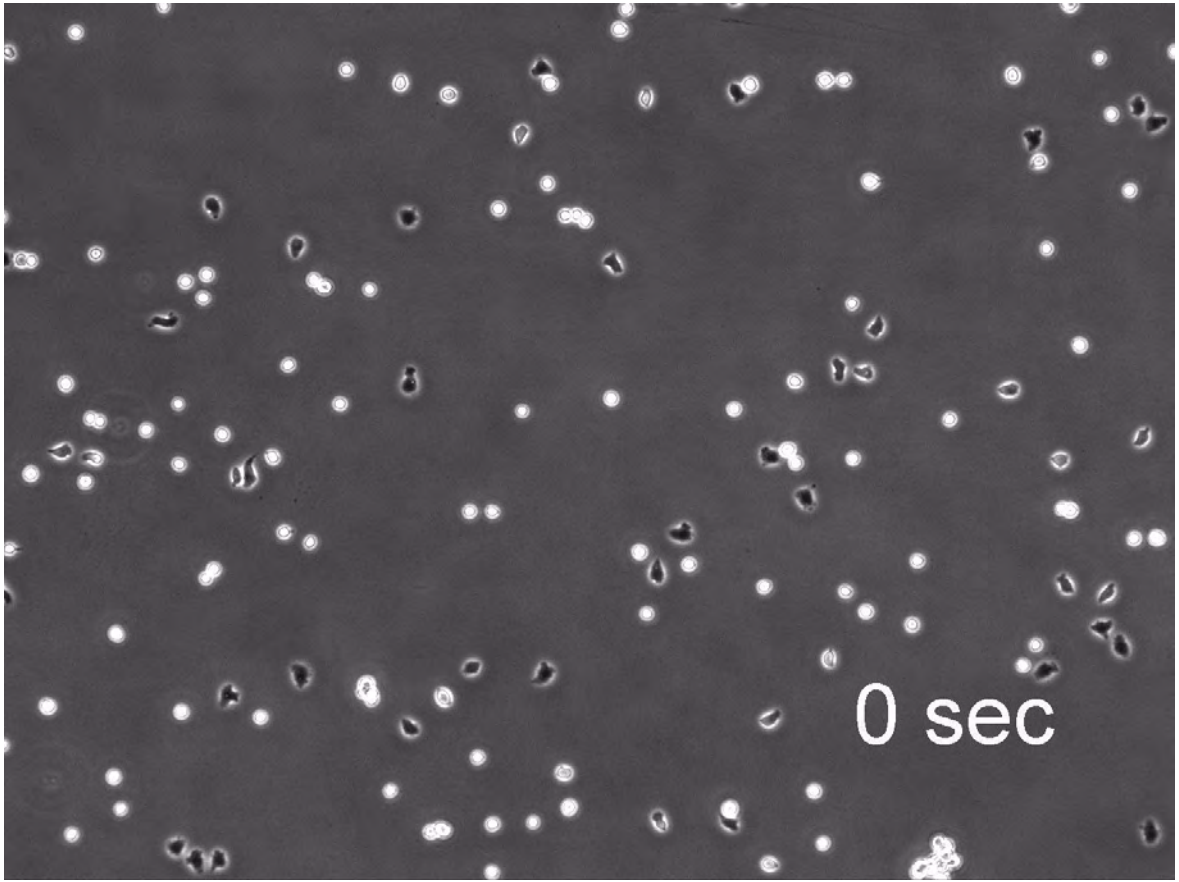
⇨ Relatively mild clinical phenotype

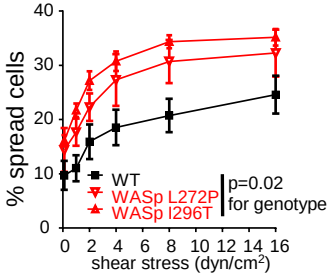
Neutrophils – warriors of the immune system

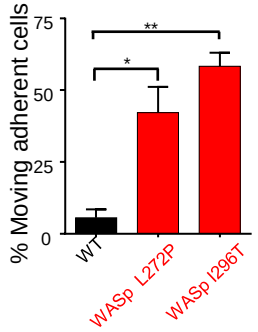
Marton Keszei

Keszei and Westerberg, J Immunol Rep 2014

Mimicking neutrophils transmigration in flow chambers on ICAM1,P-selectin, and CXCL8

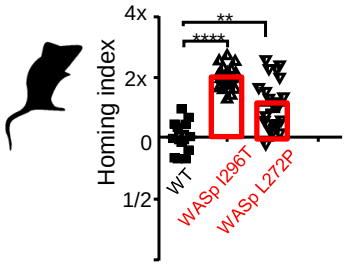




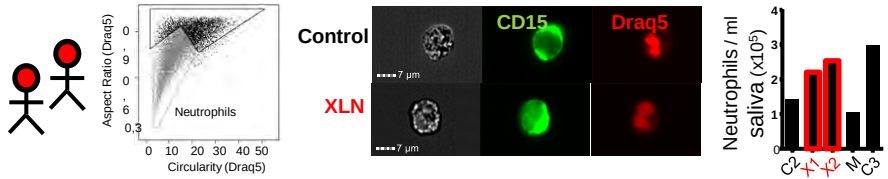


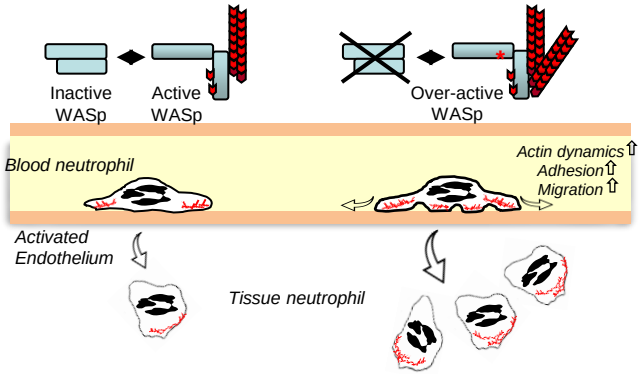
XLN patients with overactive WASp have hyperactive neutrophils in tissues

Sterile Inflamation



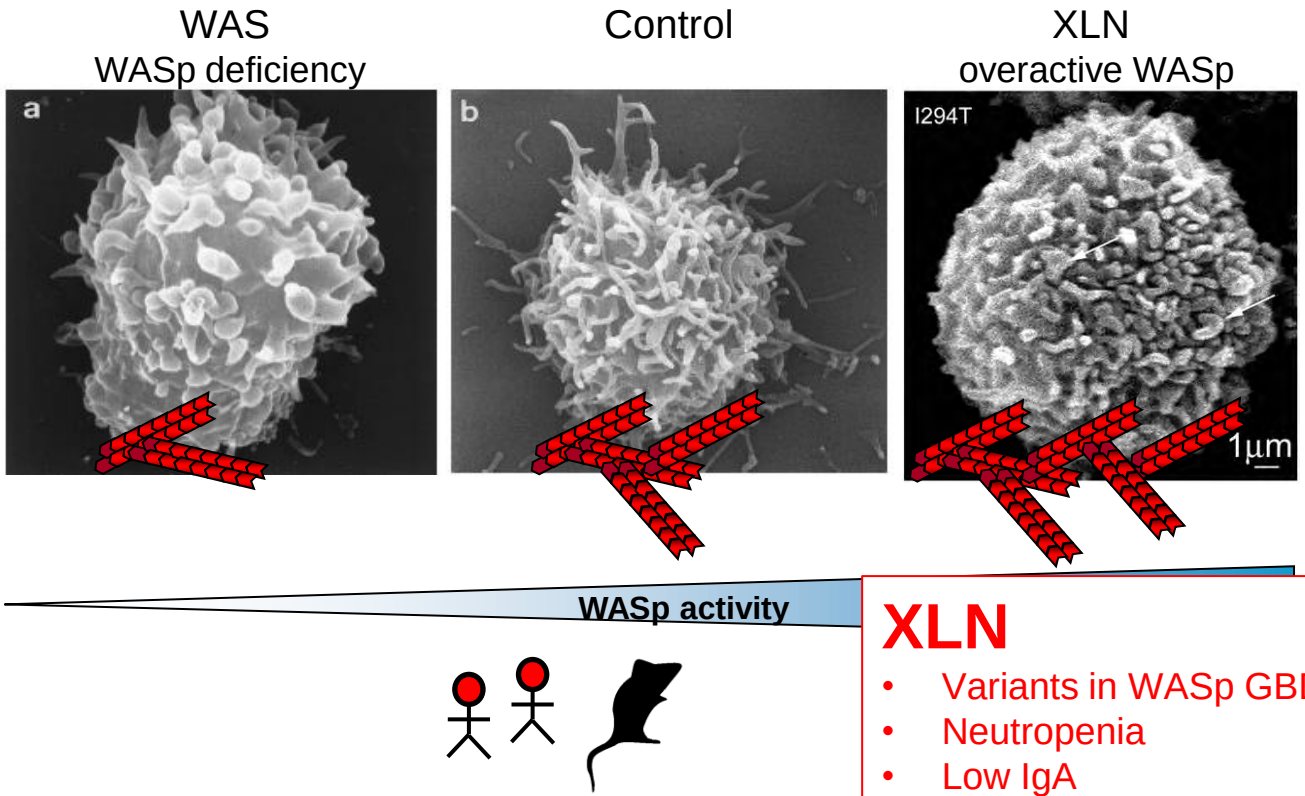
ImageStream analysis





Keszei et al, J Clin Invest 2018

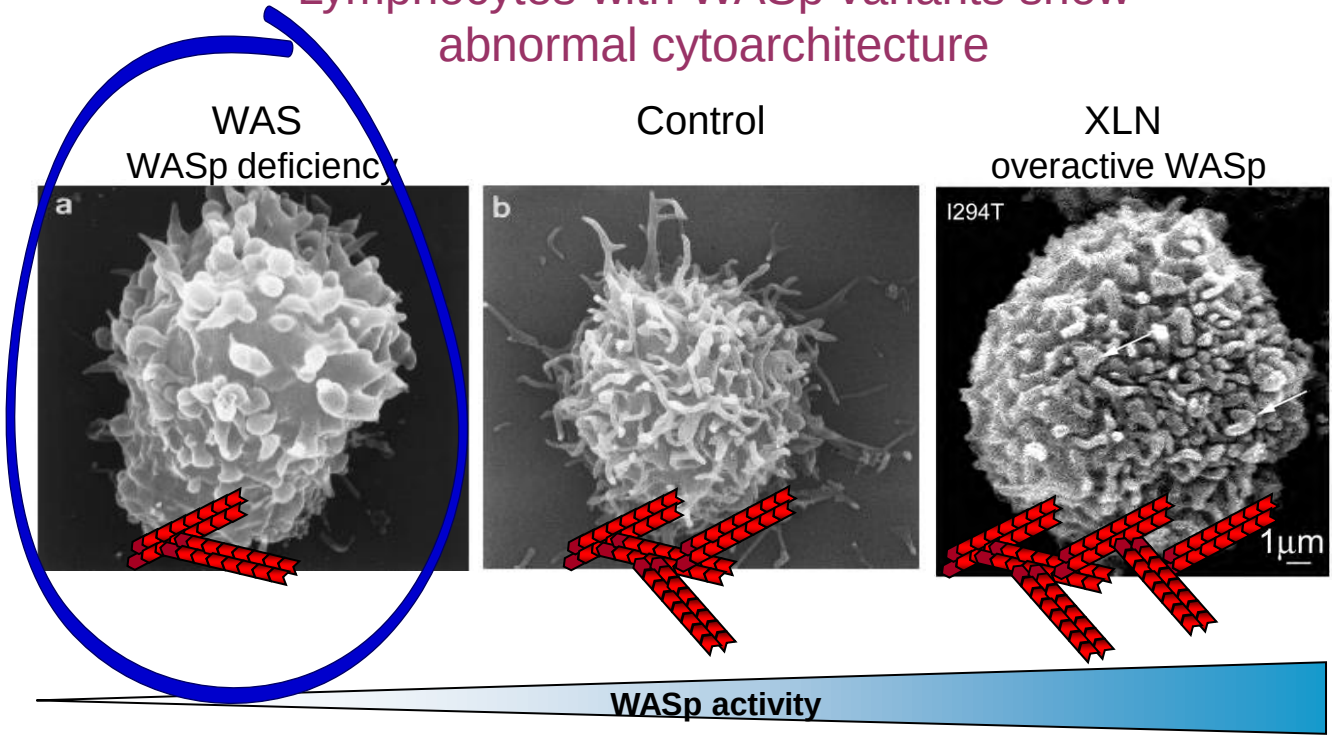
Lymphocytes with WAS variants show abnormal cytoarchitecture



XLN

- Variants in WASp GBD
- Neutropenia
- Low IgA
- Neutrophils in saliva

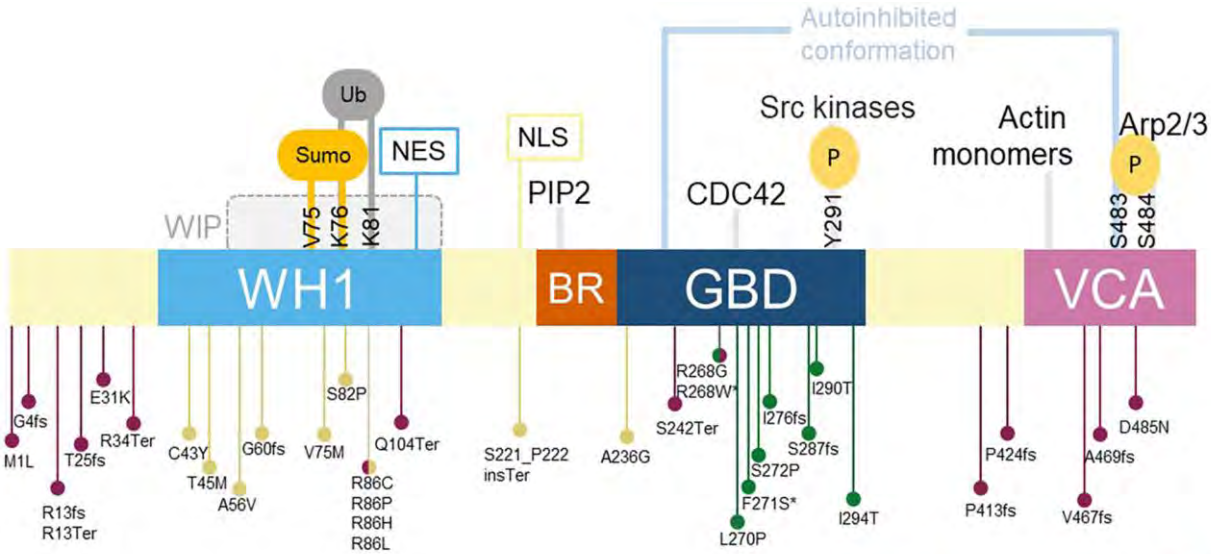
Lymphocytes with WASp variants show abnormal cytoarchitecture



⇨ WAS variant to prediction of clinical outcome?

⇨ New treatment options for WAS?

Precision medicine:
>300 variants in the WAS gene lead to immunodeficiency diseases



Precision medicine:

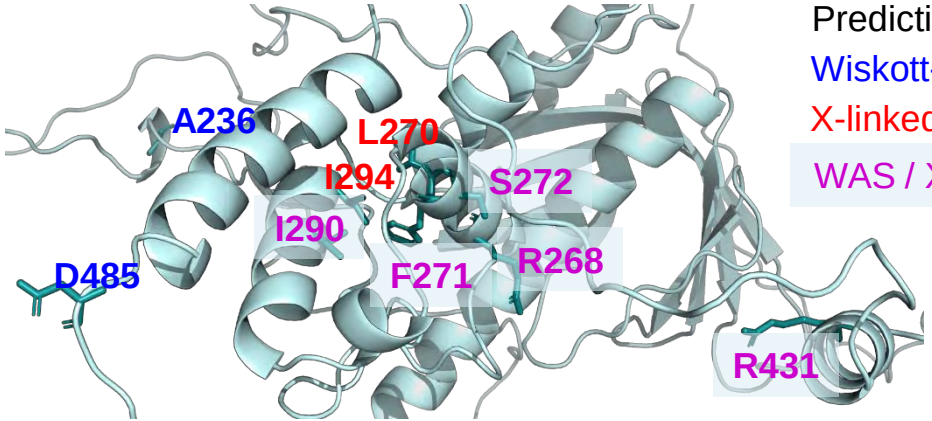
How should we treat this patient?

- Genetic variation analysis
- WASp staining by flow cytometry
- Enough?

Sumo SUMOylation,
Ub Ubiquitination
P Phosphorylation
WAS
XLT
XLN

Vieira, Pinho, Westerberg, Ped All Immunol 2023

Variants in WASp – prediction from clinical phenotype correct?



Alphafold2 structure

Prediction based on clinical data:

Wiskott-Aldrich syndrome

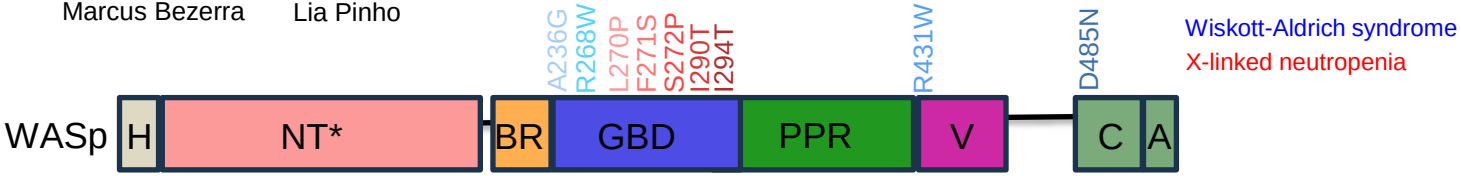
X-linked neutropenia

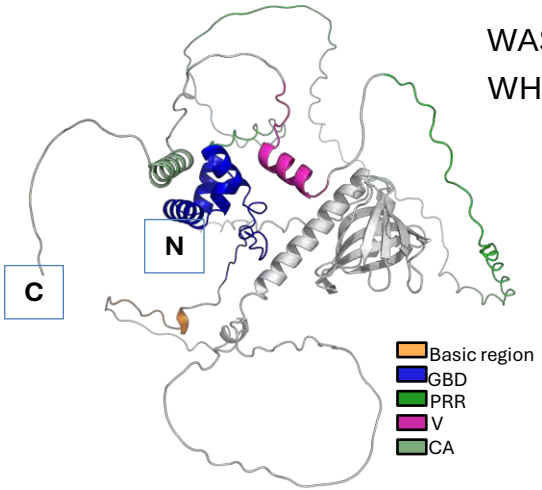
WAS / XLN / not disease causing?

Expression of highly disordered WASp using the spider silk NT* stability tag

Marcus Bezerra

Lia Pinho





Alphafold2 structure

WASp structure is highly disordered:

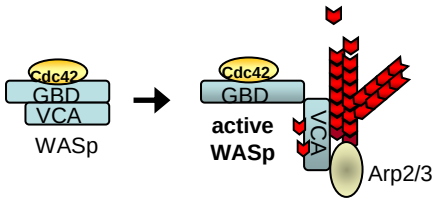
WH1 domain was replaced by a His-tag and a NT*-tag

Leppert et al, Nano Letters 2022

Michael Landreh

Axel Leppert

Methods to study WAS variants

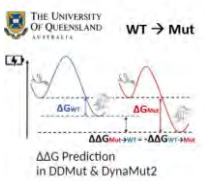


How does WASp variants affect the structure?
- *In silico Modelling*
- *Native Mass Spec*

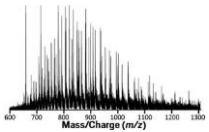
What is the activity of WASp variants?
- *Pyrene actin polymerization assay*
- *Cell based assays*

Structure analysis

In silico



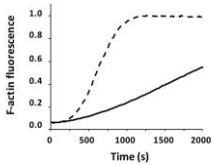
In vitro



Native Mass Spectrometry

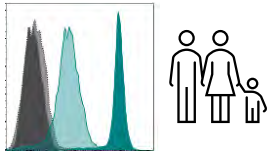
WASp function

Protein analysis



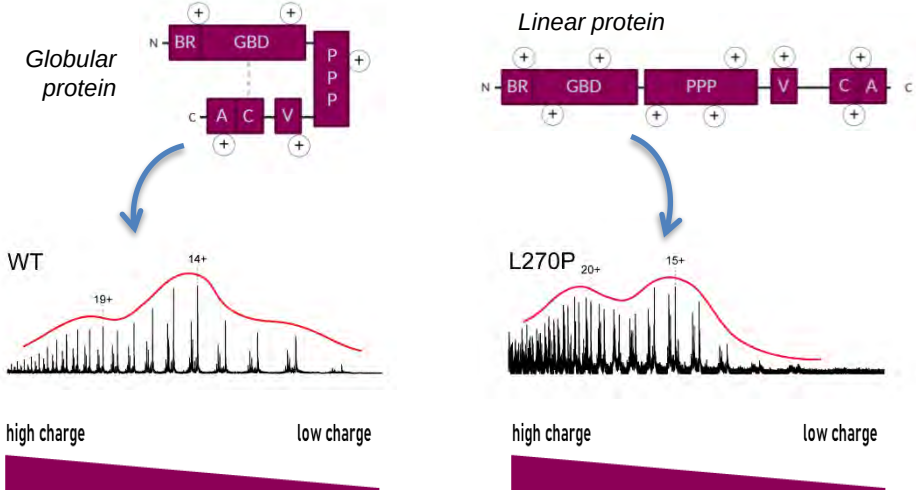
Actin Polymerisation Assay

Cell analysis

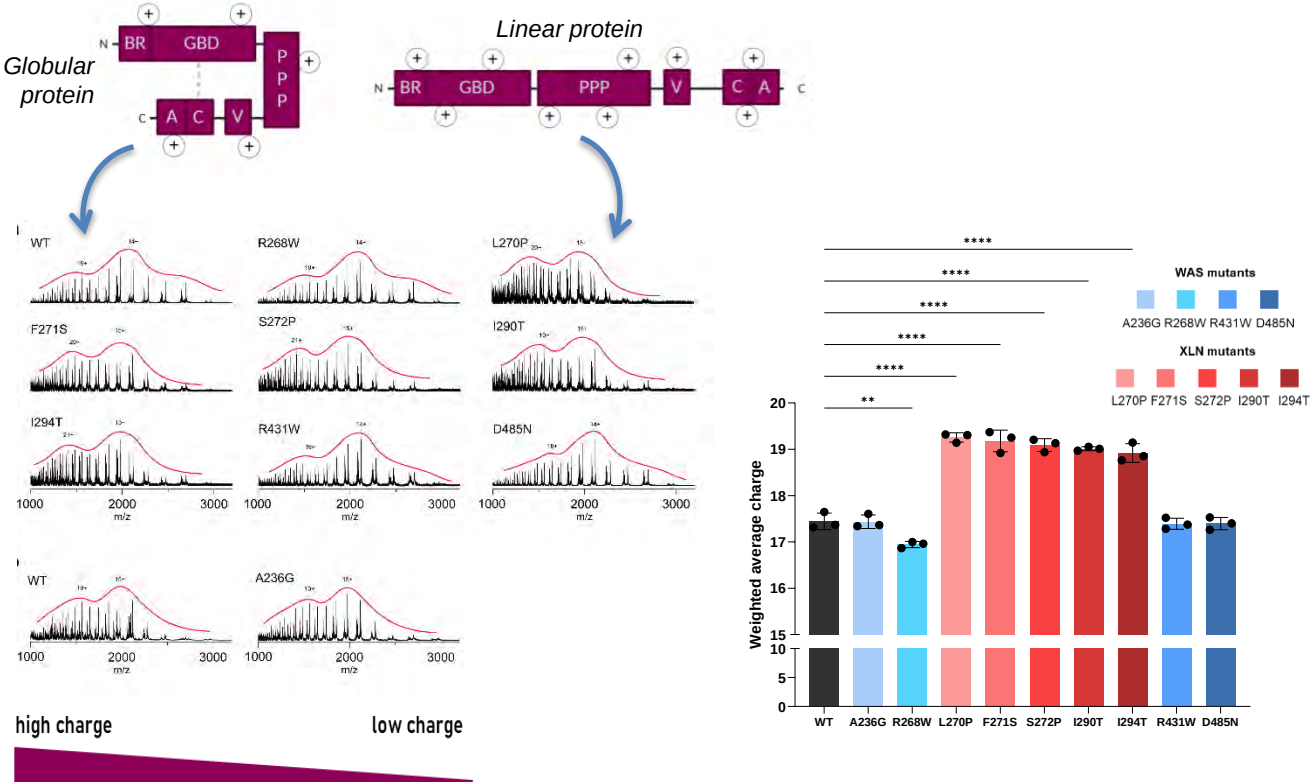


Flow cytometry assays

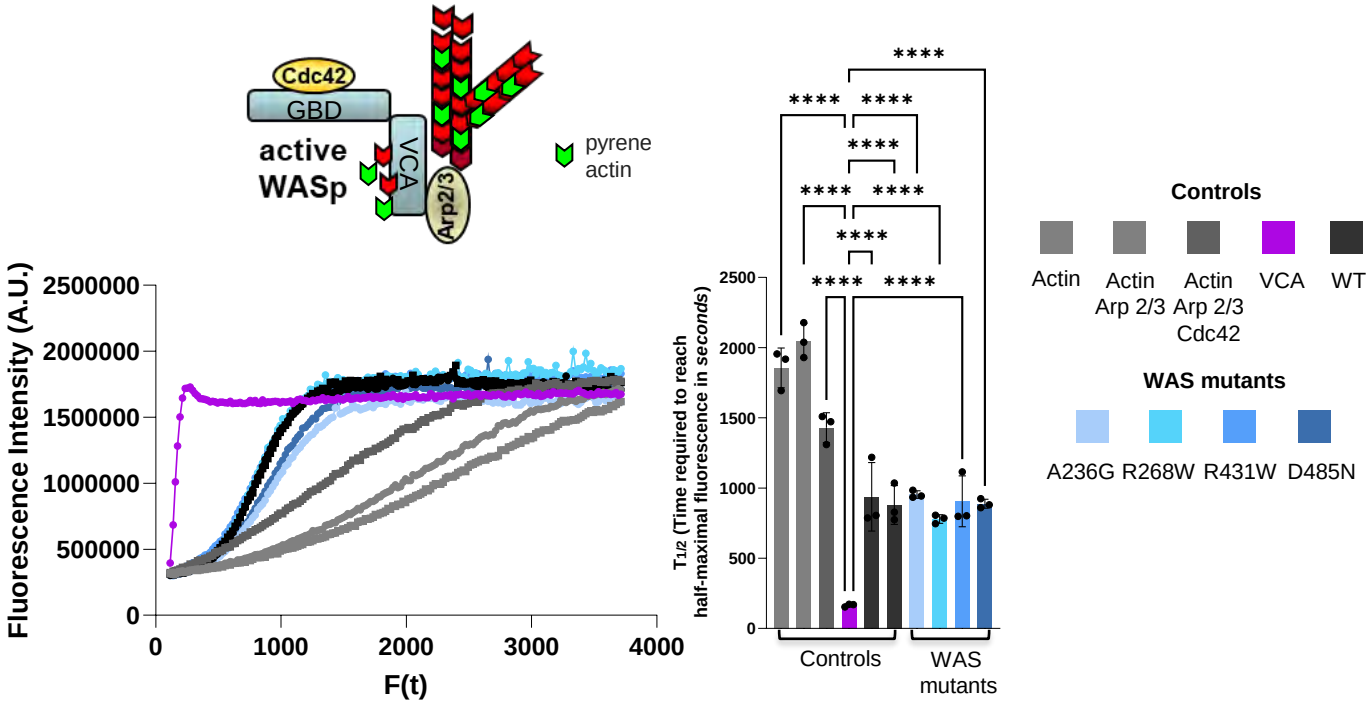
Native mass spectrometry reveals protein folding



XLN variants showed higher weighted average charge state by native mass spectrometry

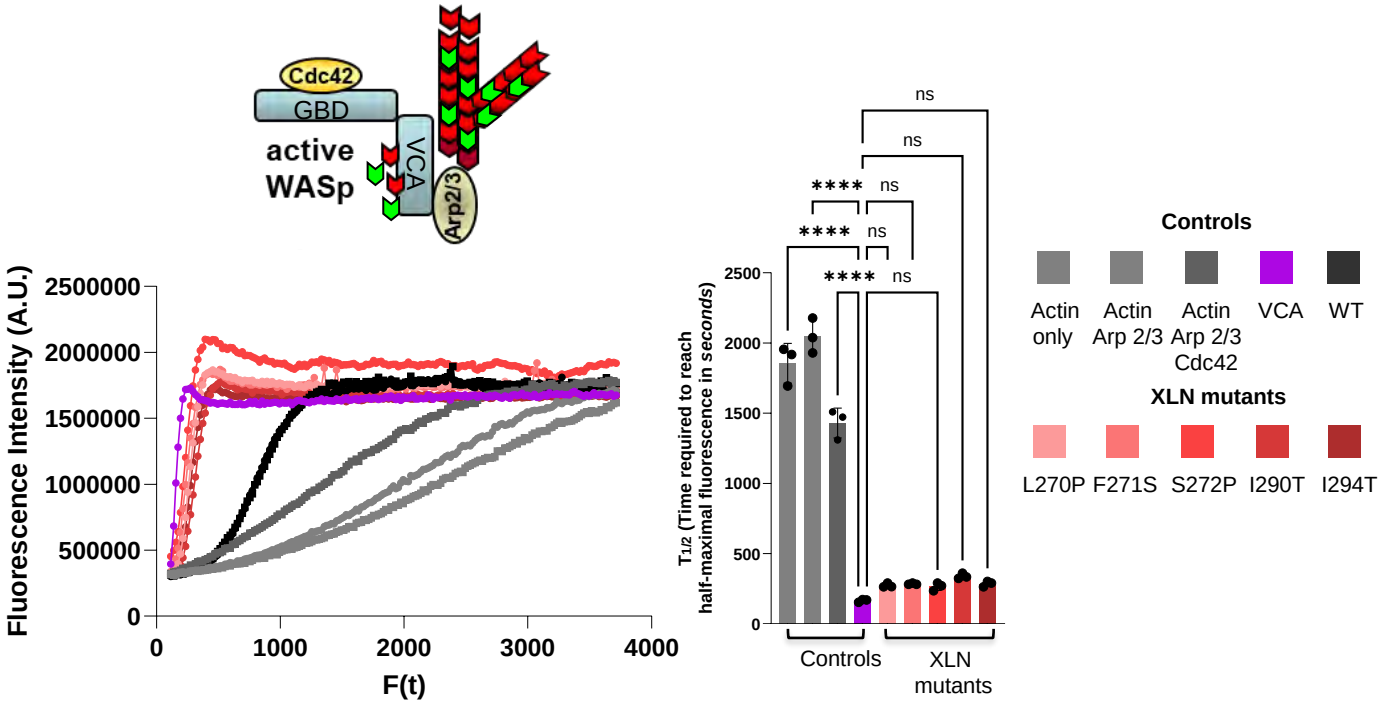


WAS loss-of-function variants show similar actin polymerization to WT-WASp



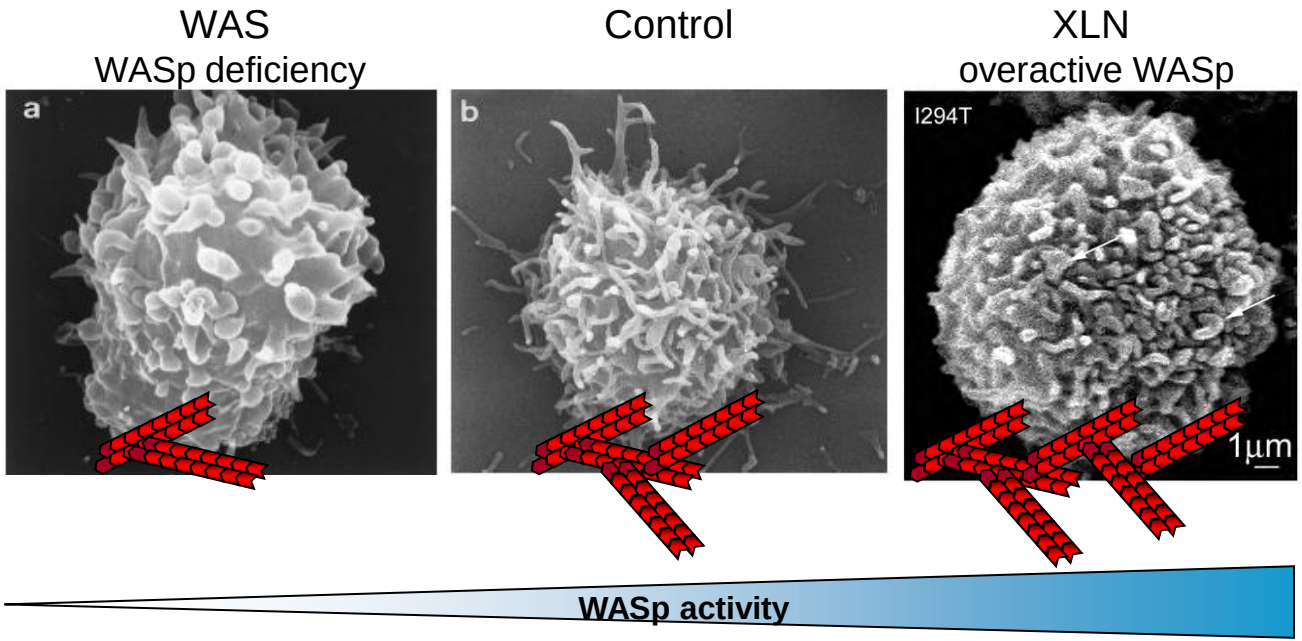
N = 3. Statistical significance calculated using an unpaired, one-way ANOVA and Dunnett's multiple comparisons test.

WAS gain-of-function variants in XLN show similar actin polymerization to the VCA alone

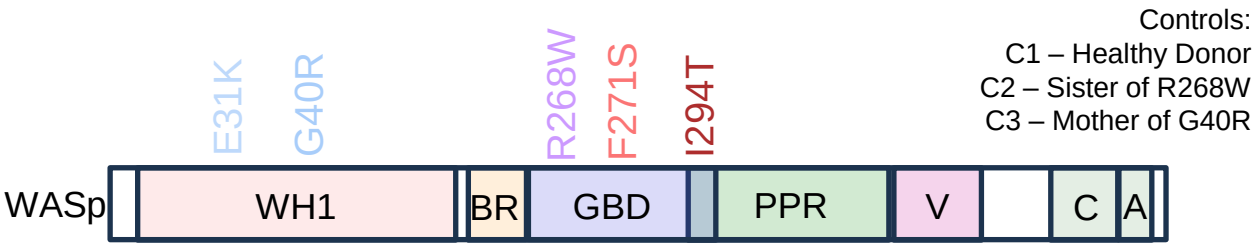


N = 3. Statistical significance calculated using an unpaired, one-way ANOVA and Dunnett's multiple comparisons test.

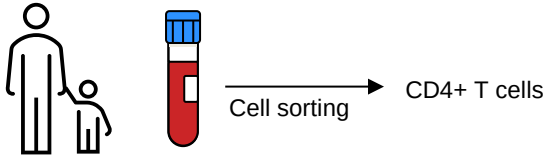
How does patient variants affect T cell functionality?

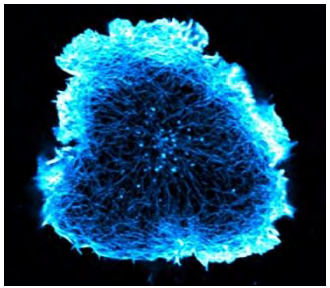


Isolation and culture of CD4 T cells from patient samples to examine T cell functionality

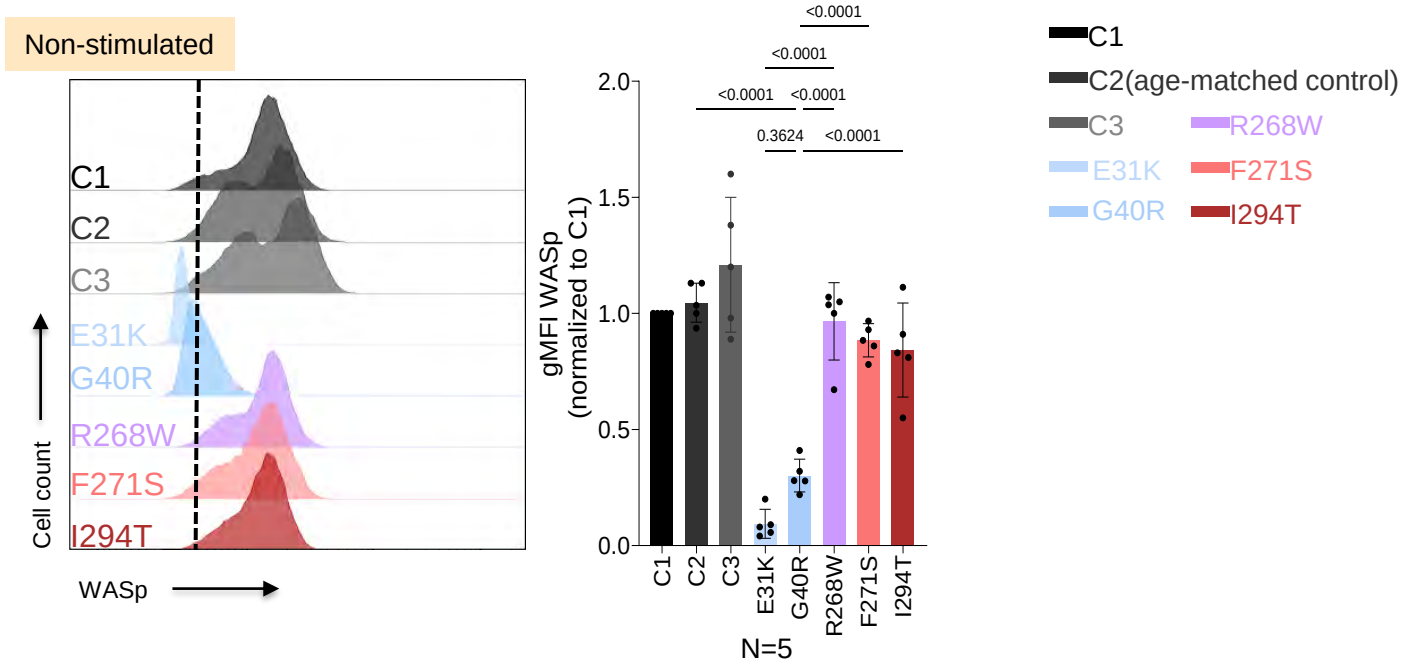


Controls:
C1 – Healthy Donor
C2 – Sister of R268W
C3 – Mother of G40R

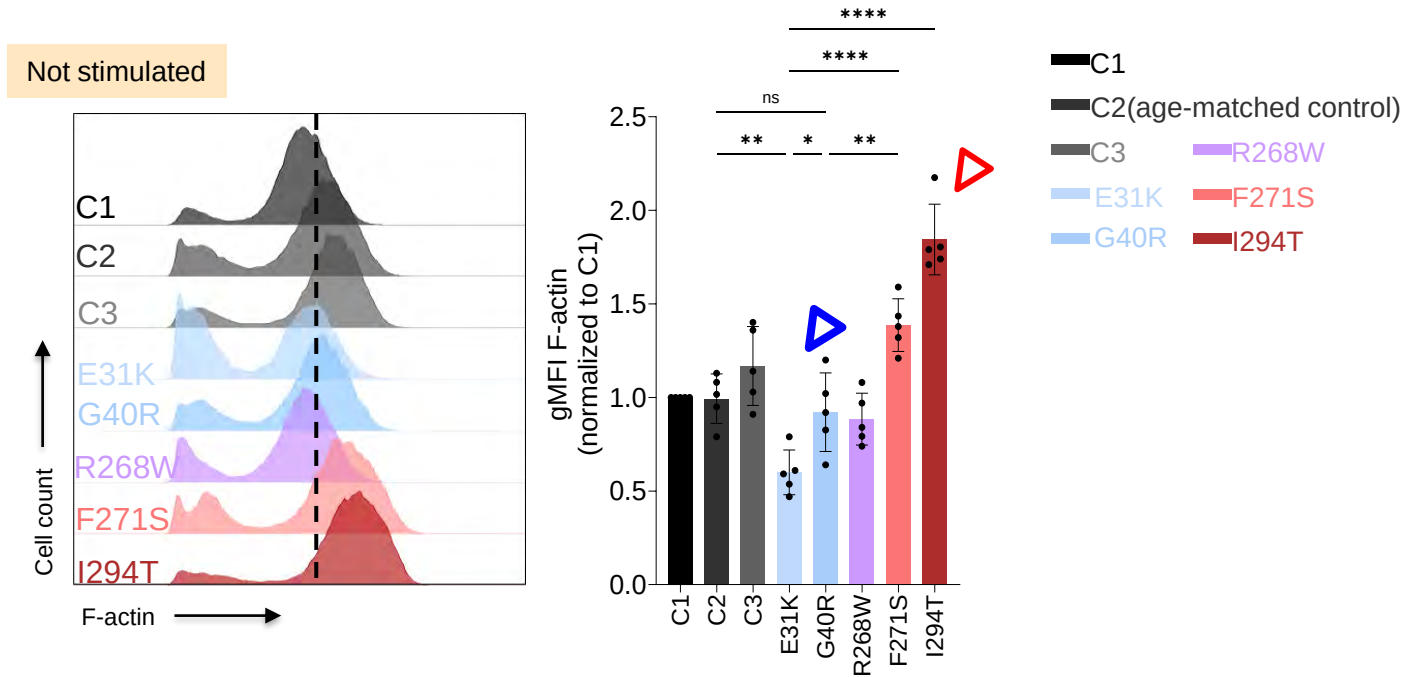




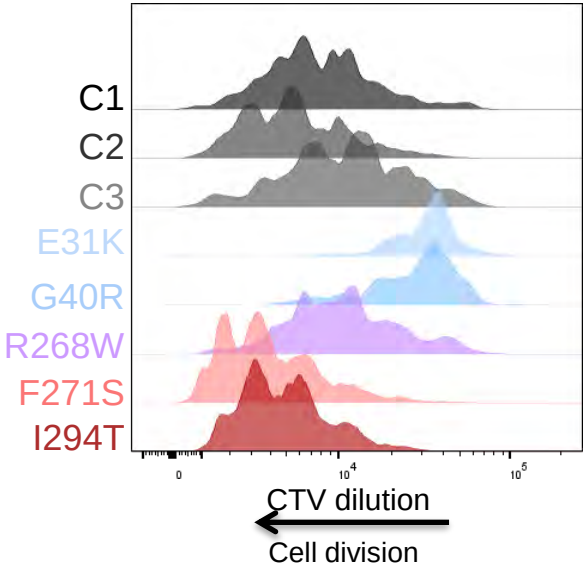
WAS T cells have lower/absent WASp expression

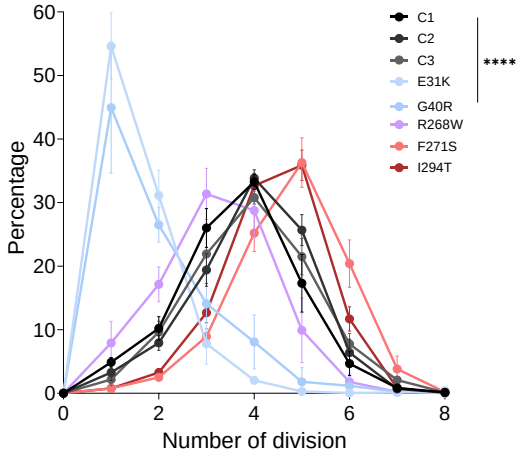


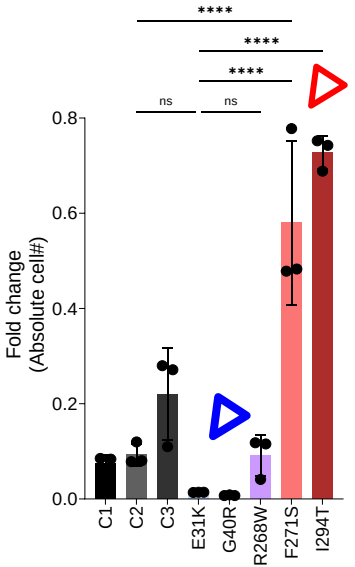
Low WASp expression in WAS-G40R T cells still induce actin polymerization



WAS T cells show poor proliferation upon T cell receptor (anti-CD3) stimulation

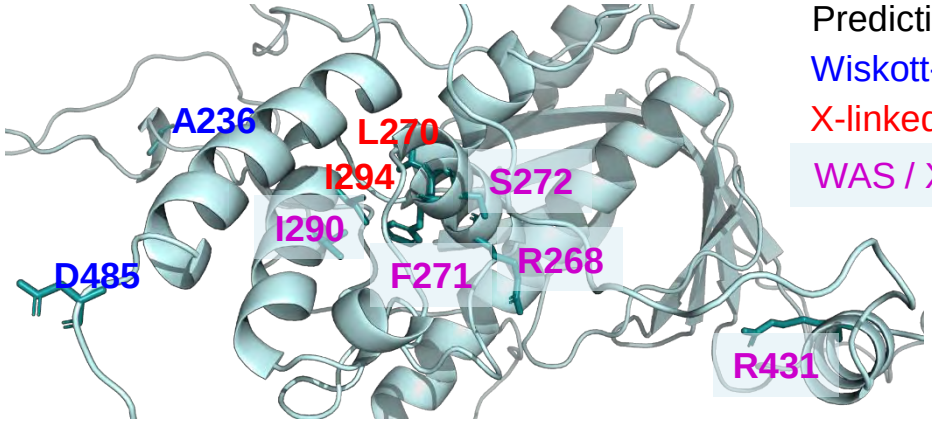






N=3

Variants in WASp – prediction from clinical phenotype correct?



Alphafold2 structure

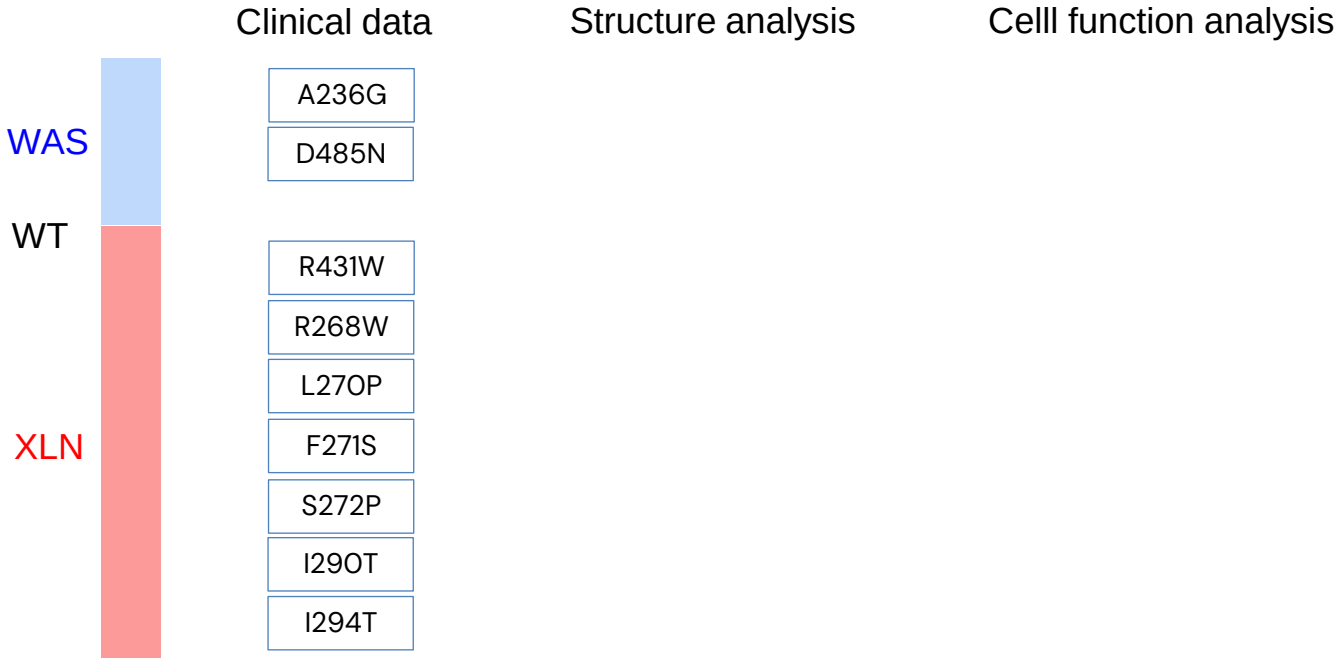
Prediction based on clinical data:

Wiskott-Aldrich syndrome

X-linked neutropenia

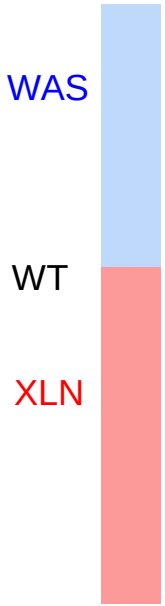
WAS / XLN / not disease causing?

Biochemical and cellular analysis of WAS variants help guiding clinical decisions



Bezerra, Pinho, Leppert et al, In preparation 2024

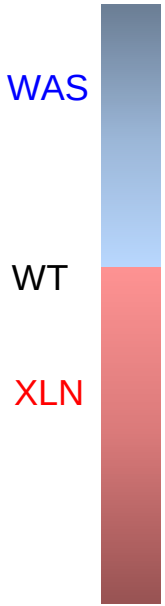
Biochemical and cellular analysis of WAS variants help guiding clinical decisions



Clinical data	Structure analysis	Celll function analysis
A236G	A236G	
D485N	D485N	
	R431W	
	R268W	
L270P	L270P	
F271S	F271S	
S272P	S272P	
I290T	I290T	
I294T	I294T	

Bezerra, Pinho, Leppert et al, In preparation 2024

Biochemical and cellular analysis of WAS variants help guiding clinical decisions



Clinical data	Structure analysis	Celll function analysis
A236G	A236G	E31K
D485N	D485N	G40R
	R431W	
		R268W
L270P	L270P	
F271S		
S272P	S272P	I294T
I290T	I290T	F271S
I294T		

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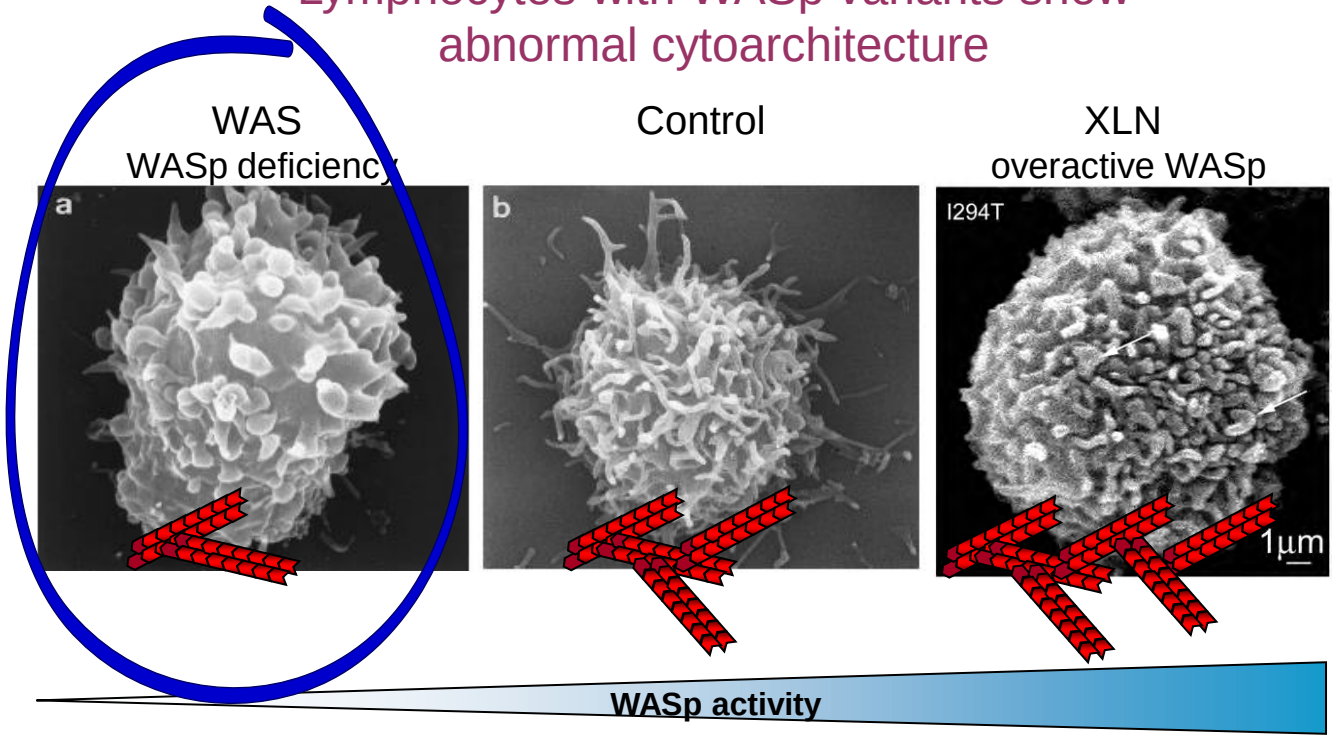
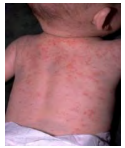
Lymphocytes with WASp variants show abnormal cytoarchitecture



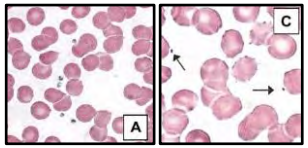
⇨ WAS variant to prediction of outcome?

⇨ New treatment options for WAS?

Thrombocytopenia in WAS patients – difficult to treat

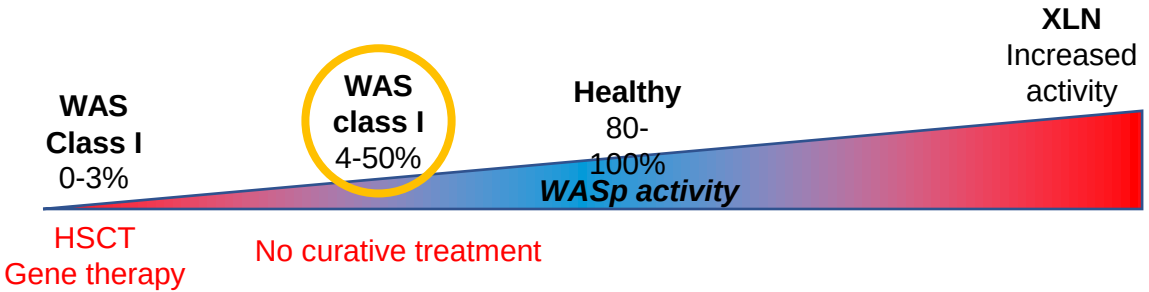


Boy with Wiskott-Aldrich syndrome

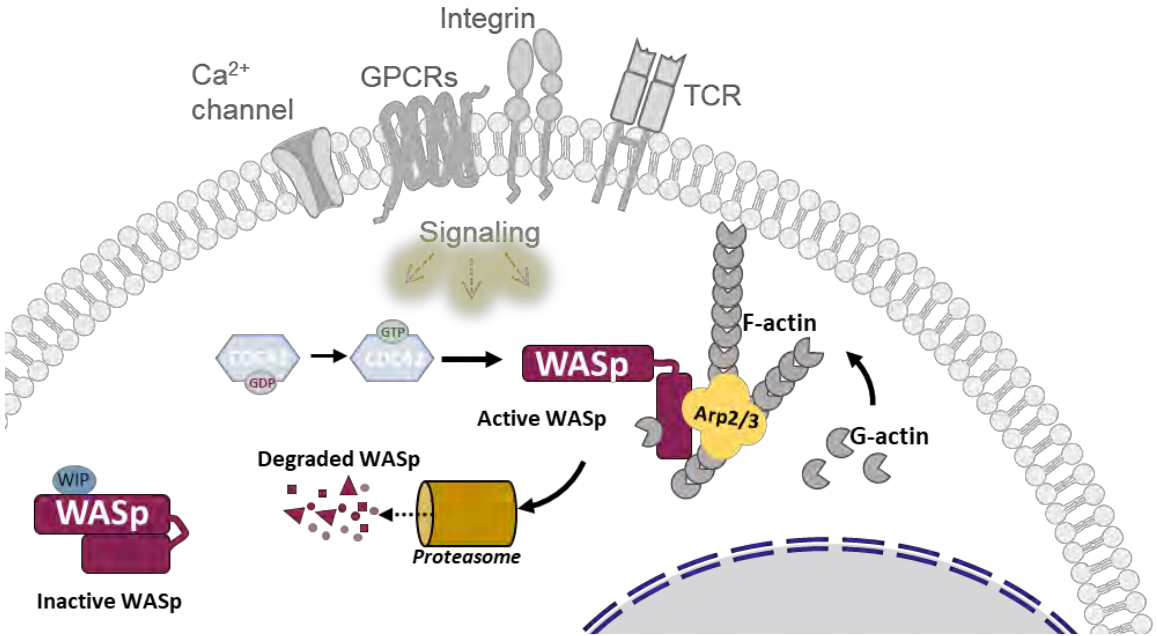


Microthrombocytopenia

- Thrombocytopenia with small platelet size
- Infections, autoimmunity, and malignancies represent severe, potentially fatal complications



WAS variants that de-stabilize **WASp-WIP** interaction induce Thrombocytopenia in WAS/XLT



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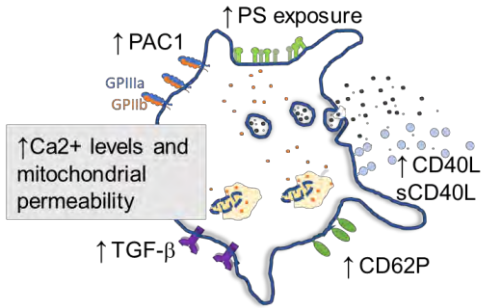
Ramesh N, et al. Proc Natl Acad Sci USA (1997)
Chou HC, et al. Curr Biol (2006)
De la Fuente MA, et al. Proc Natl Acad Sci USA (2007)
Kim AS, et al. Nature (2000)

Thrombocytopenia in WAS – difficult to treat and manage

Impaired Regulation in WAS defective PTLs

Low activation threshold



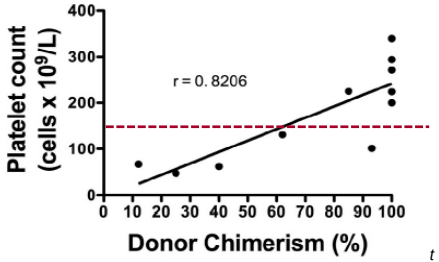
Shcherbina A, et.al., Brit Jour Haematology, 1999
Prislovsky A, et. Al., Exp Haematology, 2008
Sereni L, et.al., J Allergy Clin Immunol, 2018
Obydennyi SI, et.al., Haematologica, 2020
Sereni et al, J Leukoc Biol 2017
Sereni et al, JACI 2018a and 2018b

Platelets size and counts

Patient platelets in different interventions

HSCT

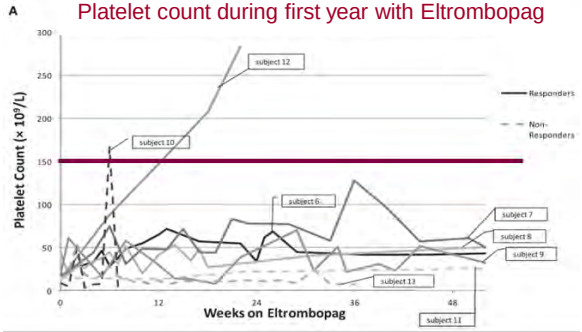
Platelet count vs donor myeloid chimerism



t *(2018)*

Thrombopoietic analogs

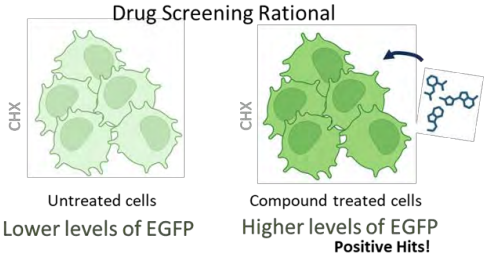
Platelet count during first year with Eltrombopag



Gerrits AJ, et al., *Platelets And Thrombopoiesis* (2015)

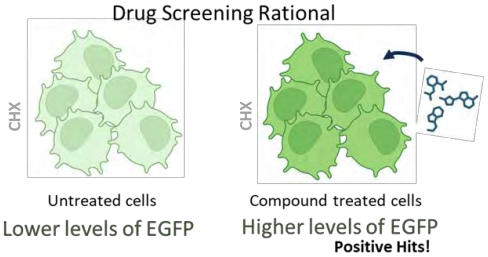
FACS-based drug screening discovers **PTW-08** that prevent WASp degradation

Rhaissa Vieira

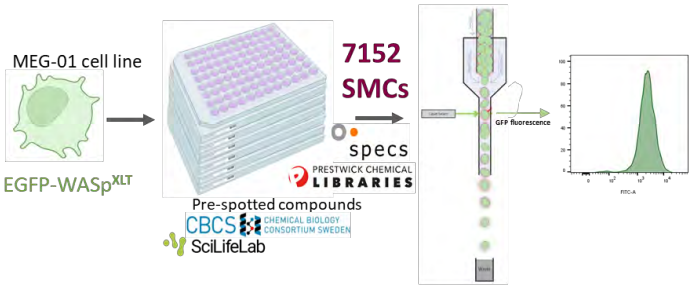


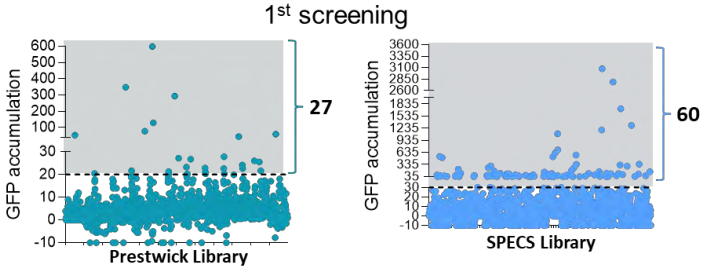
FACS-based drug screening discovers **PTW-08** that prevent WASp degradation

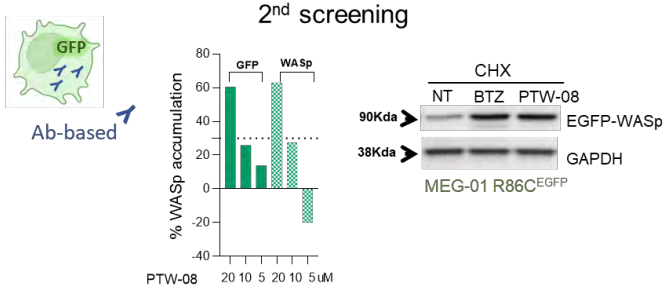
Rhaissa Vieira



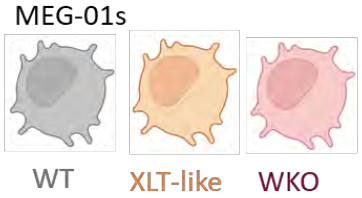
Cell line models and drug-screening summary

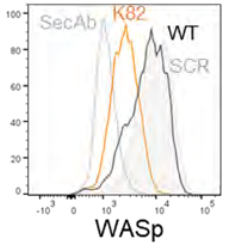


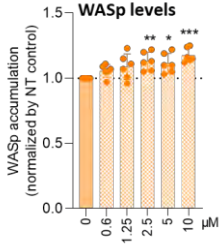


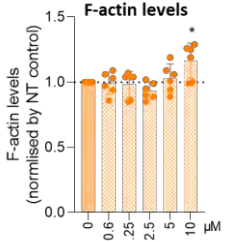


PTW-08 enhances WASp, F-actin, and maturation markers in MEG-01 cell line

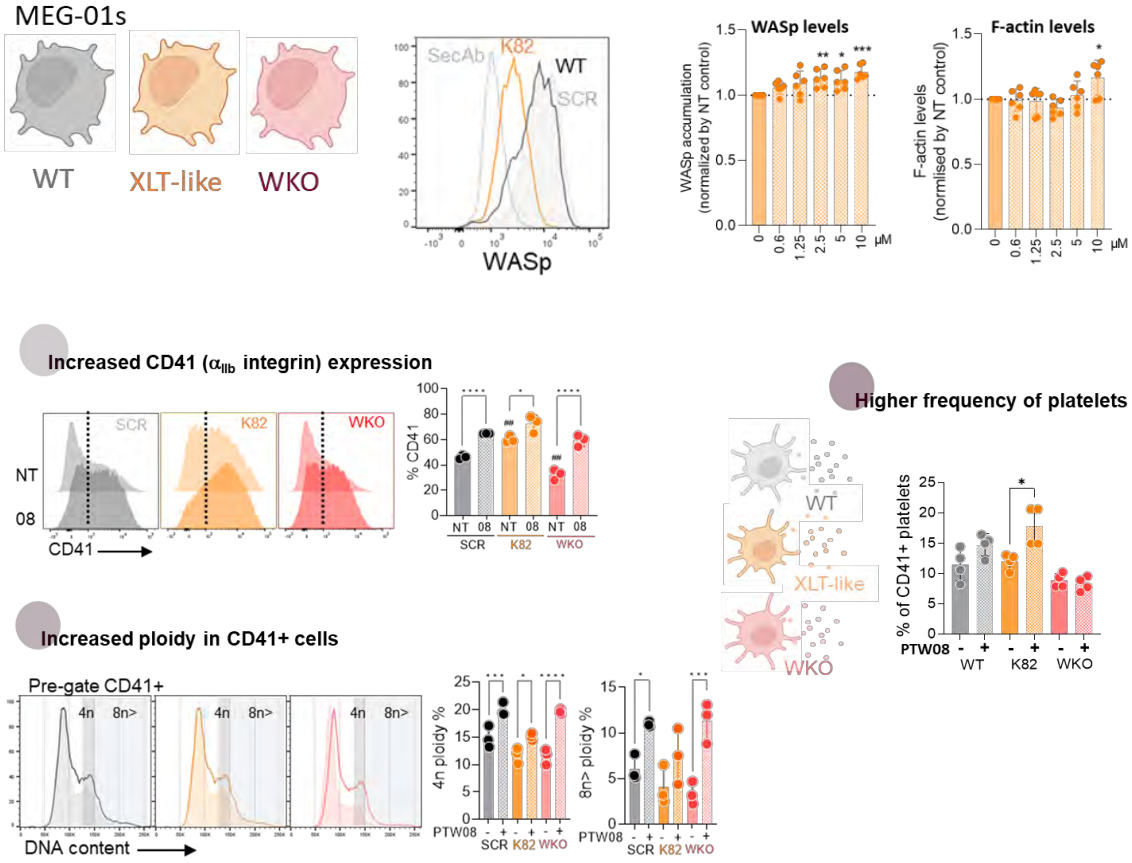




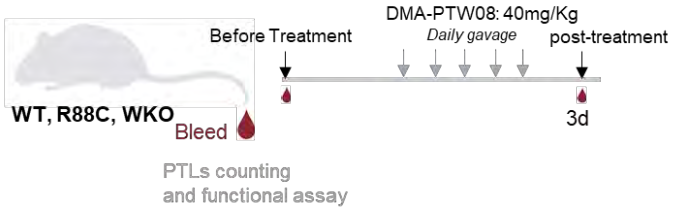


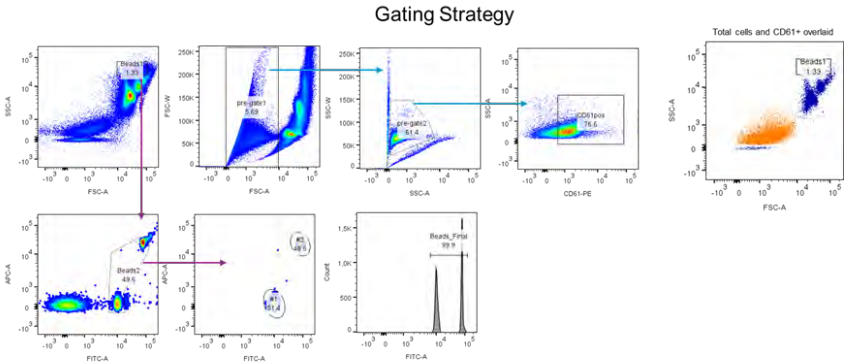


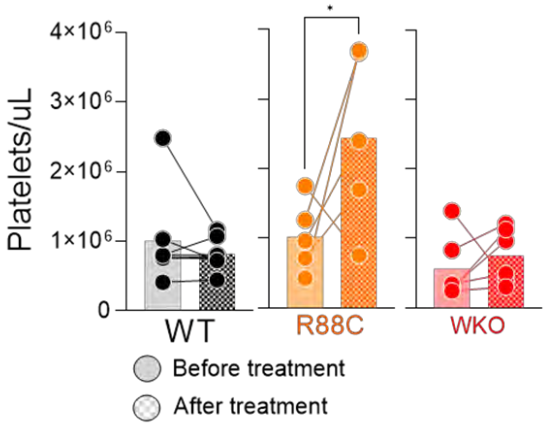
PTW-08 enhances WASp, F-actin, and maturation markers in MEG-01 cell line

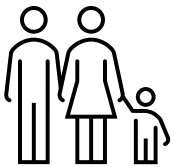


Preliminary: PTW-08 can affect platelet production and function in vivo

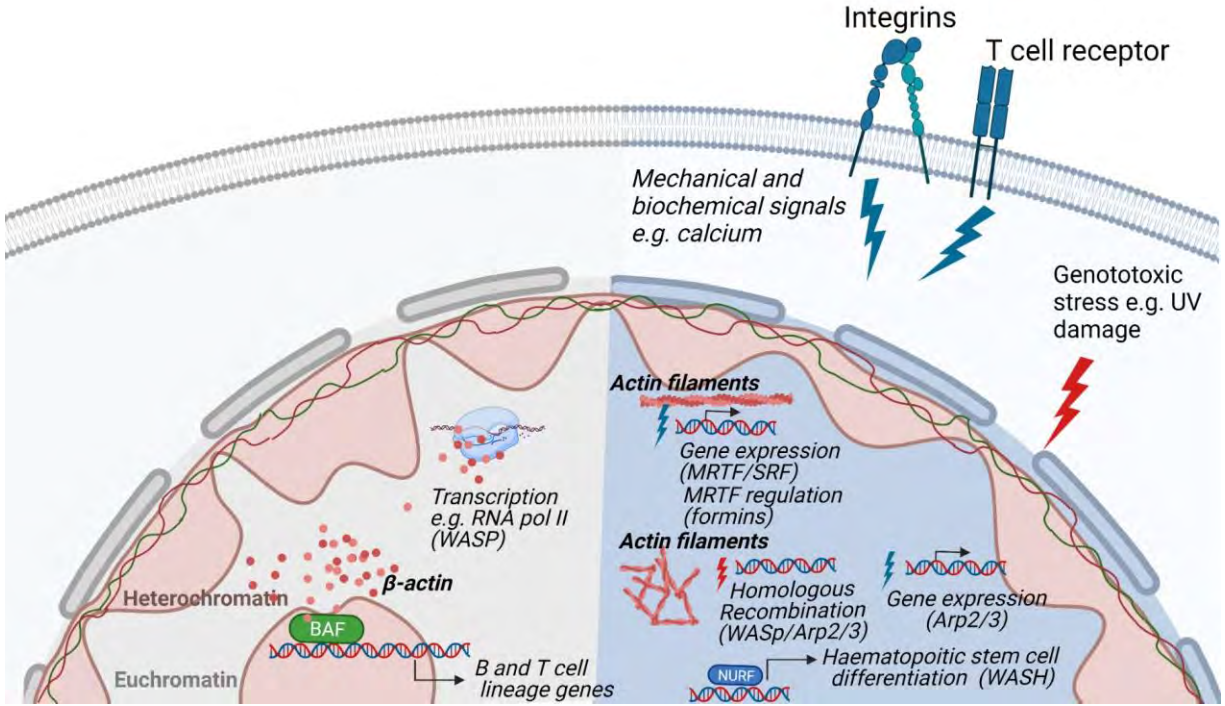








Future perspective of WAS and Actinopathies: Actin regulation in the nucleus



Record, Saeed, Westerberg, Front Dev Cell Biol 2021

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