JOURNÉE ANNUELLE DU CRPP 2025

«Diagnostic différentiel entre le PTI et les thrombopénies constitutionnelles »
Point de vue de la recherche

Frédéric Rieux-Laucat
(frederic.rieux-laucat@inserm.fr)

Scientific Team members

A.Magérus, F.Mazerolles, M. Parlato, M.C.Stolzenberg, O. Pelle, C.Brunaud, L. Giojia, Léa Gaigne, C.Boussard, M.Jeanpierre. F. Zanchetta, Manho Na, O.Debeaupuis, F.Zeco, D.Ho-Nat

Former members

L. Delage, Q.Riller, B.Sorin, E.Crickx, F.Consoni, S Delafontaine.

Clinical Staff

B.Neven, B.Bader-Meunier, P.Quartier, A.Fischer, J.Hadjadj

Primary immune deficiency

Developmental, survival or functional defects of Immune cells

(T effector cells, regulatory T cells, B cells, neutrophils…)

caused by monogenic mutations in immune-related genes

(also called Inborn errors of Immunity, or IEI)

PIDs features:

Severe infections by common pathogens

But also:

Cancers

Auto-immunity/inflammation/allergy

3 main functions of the Immune System:

Clearence of pathogens

Tumor surveillance

Self-control

Monogenic mutations in immune related genes (>500)

can lead to

Primary Immune Deficiency and Dysregulation

(PIDD)

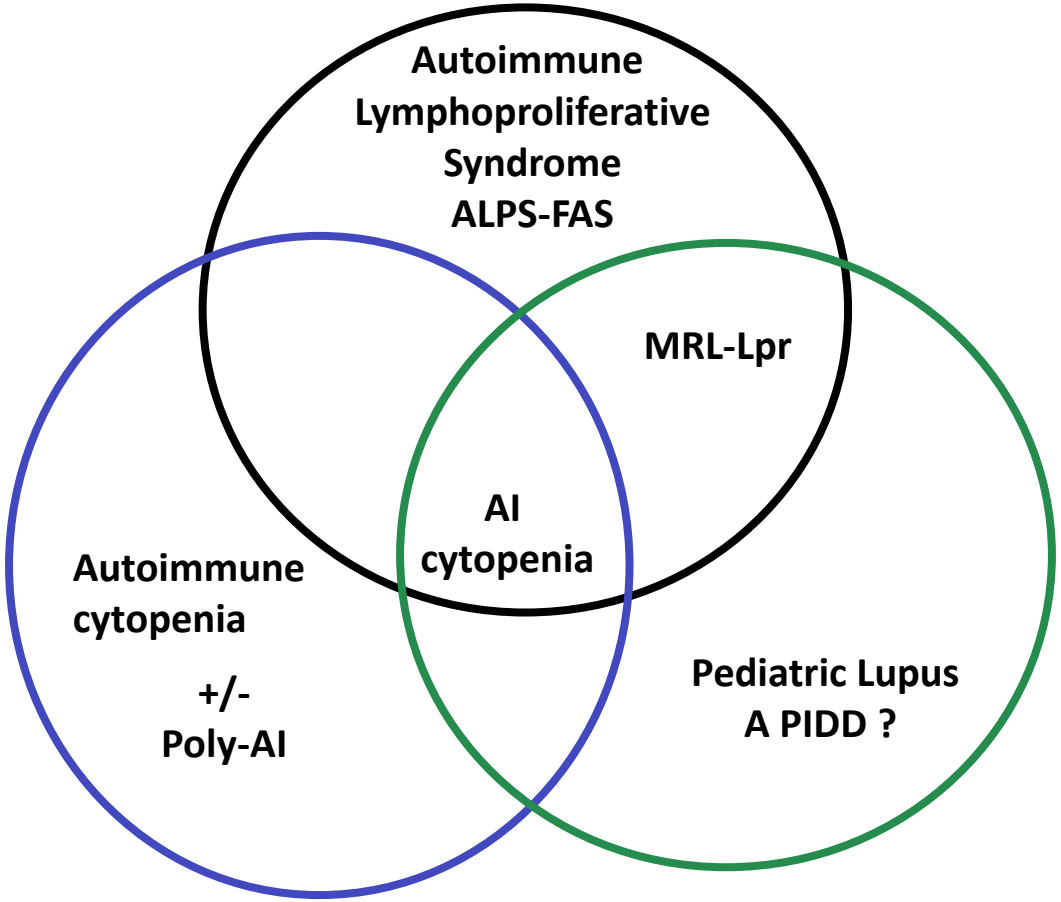
Relative risk (RR) of Autoimmunity in PIDD patients compared to the non-PIDD population

Register of >6000 PIDD cases (CEREDIH reference center: N.Mahlaoui A.Fischer)	Prevalence/ 1×10^3 PIDD pts	Prevalence/ 1×10^3 general population		RR
• Cytopenia	120	1		120
• Autoimmune hemolytic anemia (pediatric age)	25	.03	(France)	830
• Immune thrombocytopenia (adults)	60	1	(France)	60
• Inflammatory Bowel disease (adults)	78	1.8	(France)	43
• Inflammatory Bowel disease (pediatric age)	55	.7		80
• Skin disease	60	6	(France)	10
• Rheumatological disorders	50	8.6		6
• Rheumatoid arthritis (pediatric age)	8	.2	(France)	40

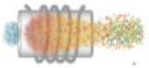
Autoimmune thrombocytopenia can be a presenting feature of PIDD

-> a rationale to search monogenic predisposition

30 years of research in Primary Immune Deficiency and Dysregulation (PIDDs)



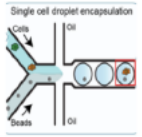
Variants' impact validation



In-depth immunophenotyping (CyTOF)

Cytokine production (Elisa, SIMOA)

Autoantibodies



Single cell RNAseq, ATACseq

Cellular models

Expression of variants in cell lines and primary cells

iPSCs-> differenciation

Functional and molecular assays

Animal models

Phenotype analysis treatments

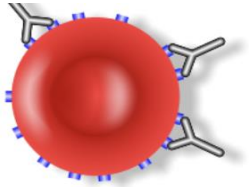
Implementation of multi-Omics analyses

Patients → Patients

Blood Samples → NGS Pipeline

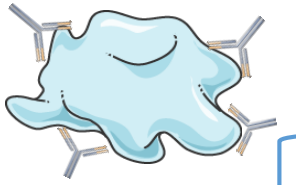
- Known genes → Diagnosis confirmation (Genetic Lab) → Treatments (Clinical dpt)
- unknown genes > Pathophysiological mechanisms → -> Treatments?

Early onset autoimmune cytopenia: all monogenic diseases ?



Autoimmune hemolytic anemia (AIHA)

+



Immune thrombocytopenia (ITP)

Jérome Hadjadj et al., Blood, 2019

Gene (no. of patients)	Mutation type and consequences
Pathogenic mutations, n = 32, 40%	
TNFRSF6 (6)	Heterozygous/LOF
CTLA4 (8)	Heterozygous/LOF
STAT3 (6)	Heterozygous/GOF
PIK3CD (1)	Hetezozygous/GOF
CBL (1)	Heterozygous/LOF
ADAR1 (1)	Heterozygous/LOF
LRBA (4)	Homozygous/LOF
RAG 1 (2)	Compound heterozygous/LOF
TNFRSF6 somatic (1)	Heterozygous/LOF
KRAS somatic (2)	Heterozygous/GOF
Probably pathogenic mutations, n = 20, 25%	
Immune cell receptors	
IFNAR1 (1)	Homozygous/likely LOF
TNFR2 (1)	Heterozygous/likely GOF
TGFBR2 (1)	Heterozygous/likely LOF
Intracellular signaling	
JAK1 (2)	Heterozygous/likely GOF
JAK2 (1)	Heterozygous/likely GOF
PLCG2 (1)	Heterozygous/likely GOF
TRAF3 (1)	Heterozygous/likely GOF
CARD11 (1)	Heterozygous/likely GOF
ARHGEF4 (1)	Heterozygous/likely GOF
PTPN11 (1)	Heterozygous/likely GOF
PARP4 (1)	Compound heterozygous/likely LOF
Apoptosis regulation	
RIPK2 (2)	Heterozygous/likely LOF
APAF1 (1)	Heterozygous/likely GOF
Transcription factors	
IKZF1 (2)	Heterozygous/likely GOF
NFATC1 (2)	Heterozygous/likely GOF
IKZF2 (1)	Heterozygous/likely LOF
No genetic abnormalities, n = 28, 35%	

M+ group 65%

Mainly heterozygous missense mutations

M- group 35%

Key pathways:

Immune Checkpoints
(CTLA-4/LRBA)

JAK/STAT
(JAK1/2gof, SOCS1/PTPN2lof)

Apoptosis
(FASLG/FAS/FADD)

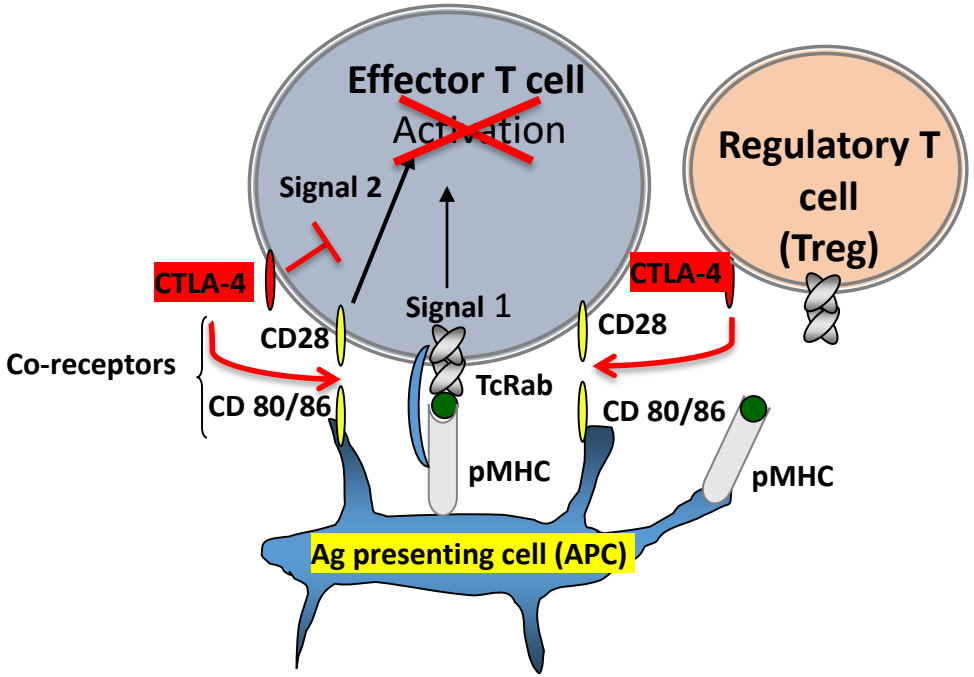
NFkB
(NFkB1/2, RELA, RELB, TNFAIP3)

MTOR
(PIK3CD, R1, …)

Actinopathies
(ARPC1B, WAS, DOCK11, ACTN1…)

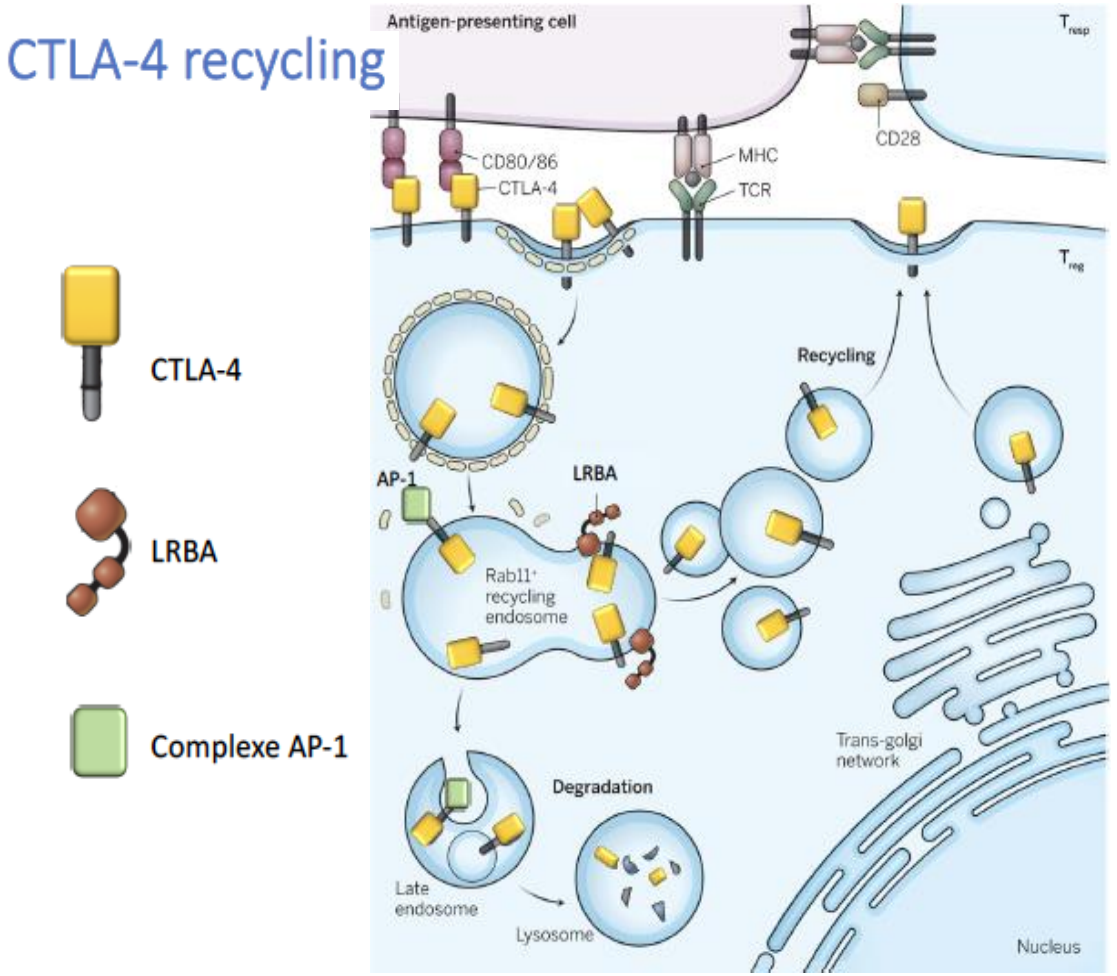
Autoimmune cytopenia associated with Immune checkpoint deficiency

Role of CTLA4 and LRBA in effector and regulatory T cells

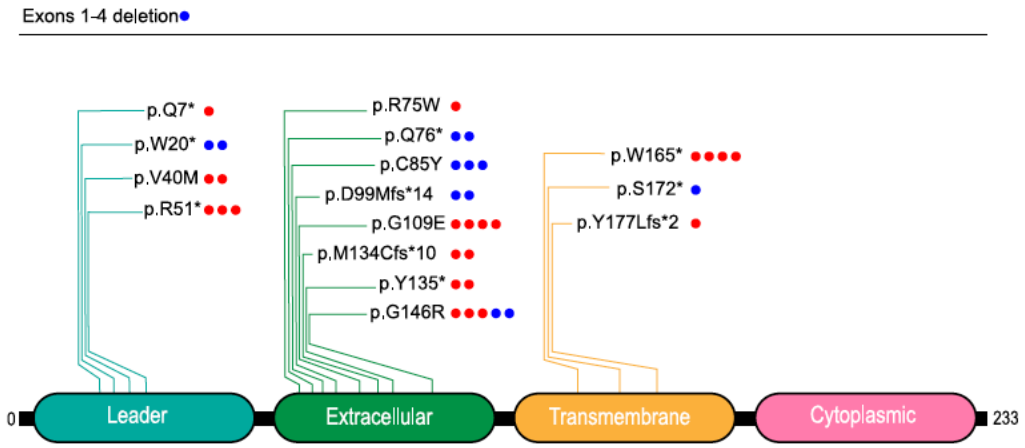


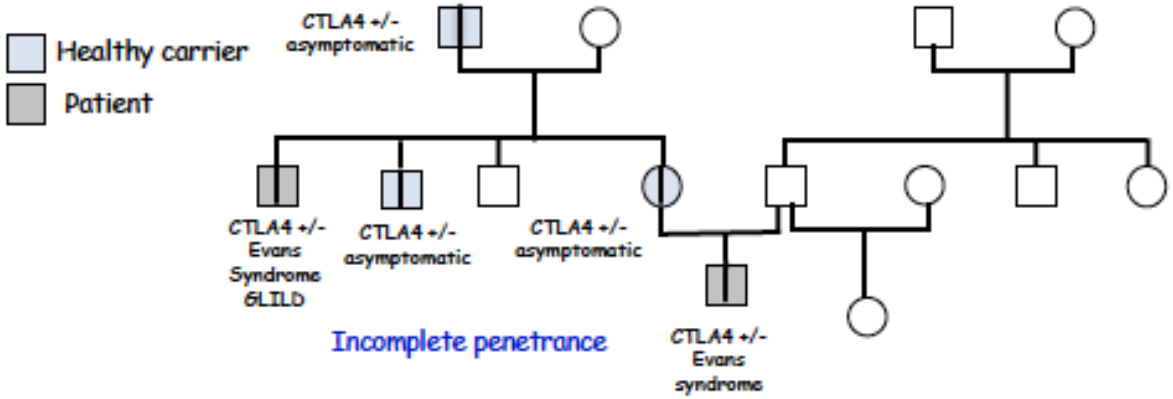
Inducible CTLA4 expression in conventional T cells

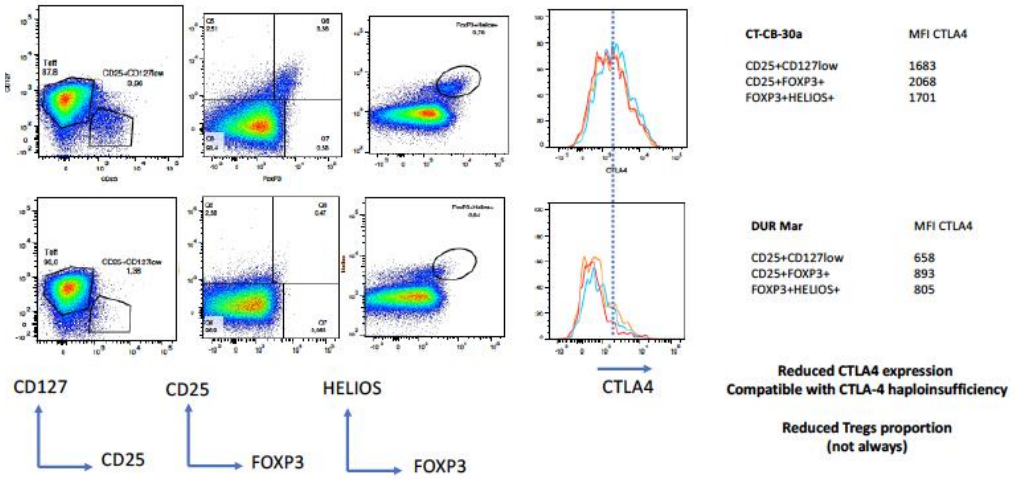
Constitutive CTLA4 expression in Tregs



Autosomal dominant *CTLA-4* mutations with variable clinical penetrance







CT-CB-30a	MFI CTLA4
CD25+CD127low	1683
CD25+FOXP3+	2068
FOXP3+HELIOS+	1701

DUR Mar	MFI CTLA4
CD25+CD127low	658
CD25+FOXP3+	893
FOXP3+HELIOS+	805

Reduced CTLA4 expression
Compatible with CTLA-4 haploinsufficiency

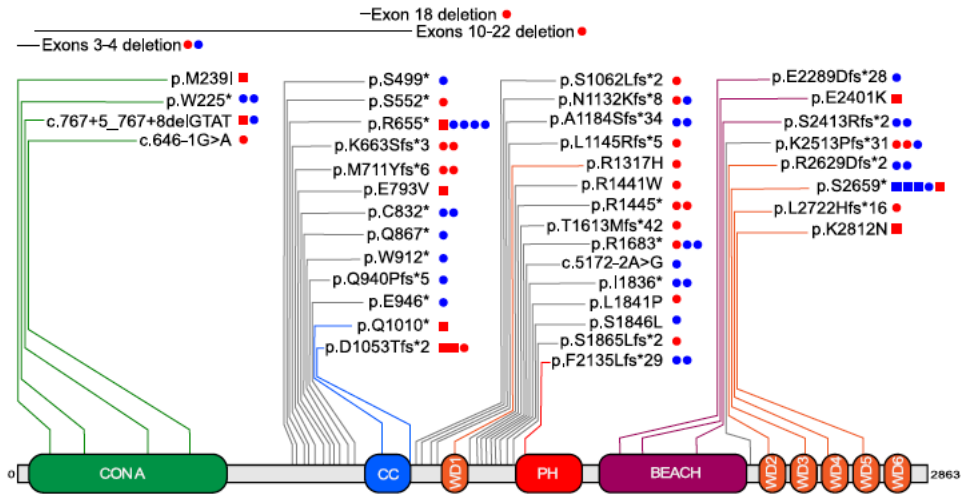
Reduced Tregs proportion
(not always)

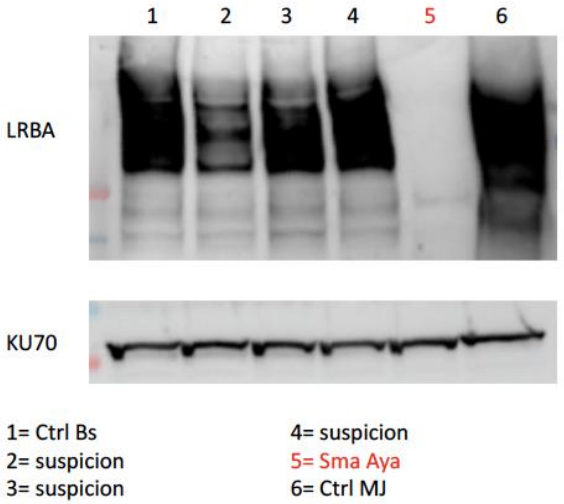
Membrane Staining

intracellular Staining

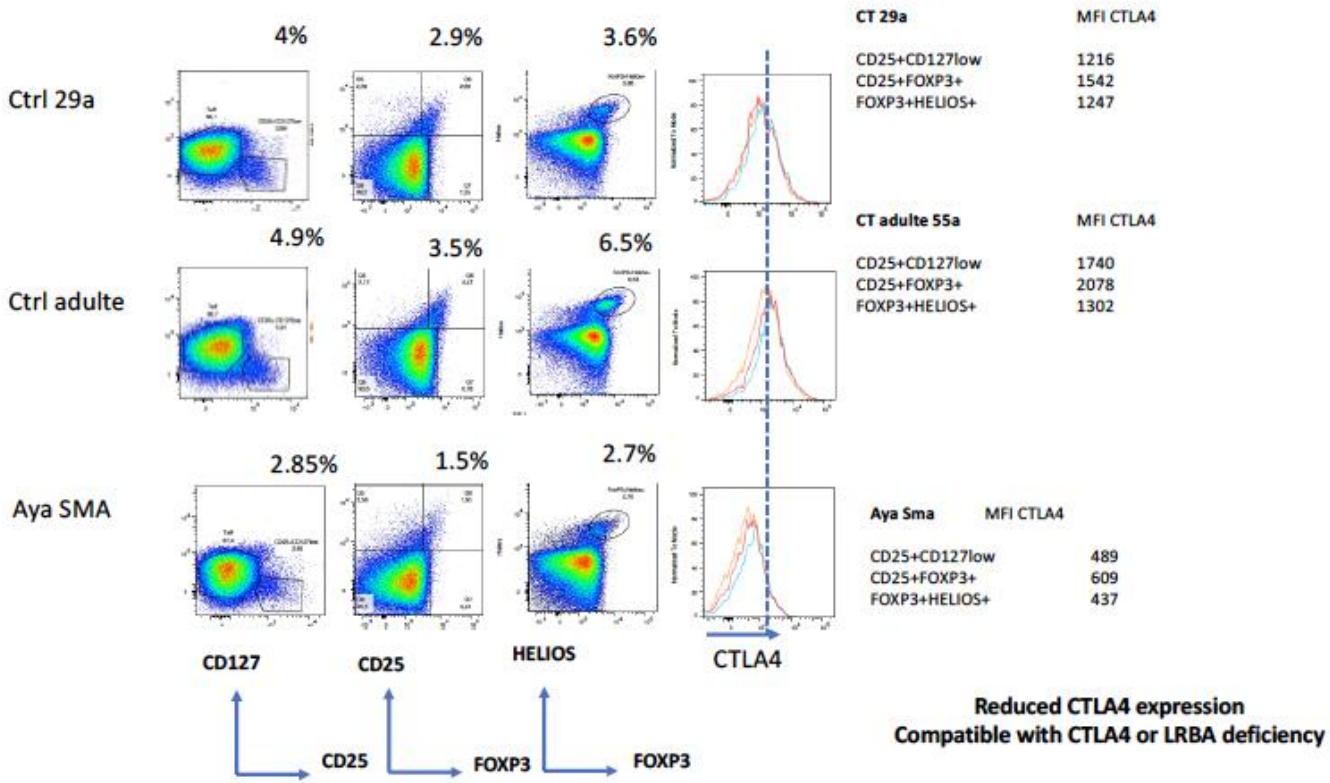
CTLA-4 mutations variable clinical penetrance

Autosomal recessive LRBA mutations: variable clinical manifestations





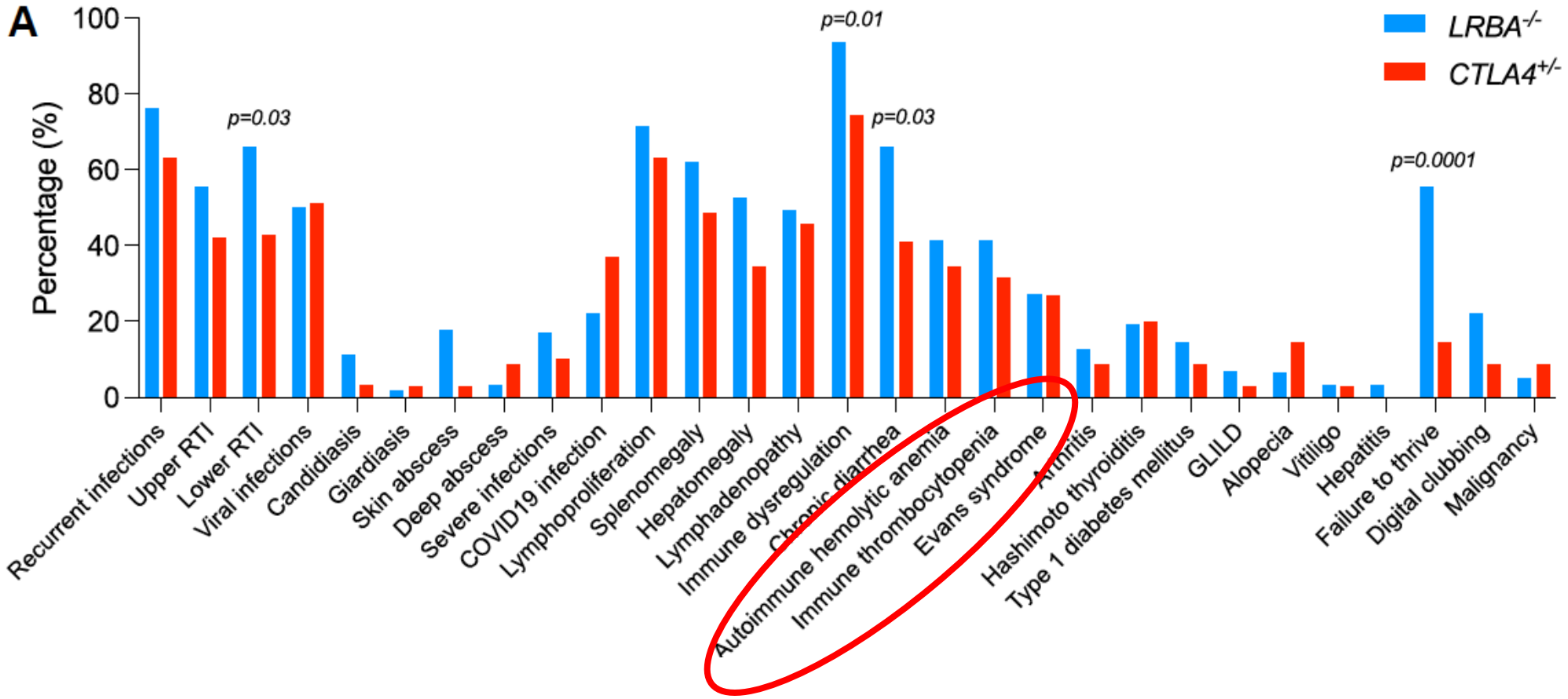
1= Ctrl Bs
2= suspicion
3= suspicion
4= suspicion
5= Sma Aya
6= Ctrl MJ



1-Reduced CTLA-4 expression on Tregs

2-Absent (or profoundly reduced) protein expression on WB (or intracellular staining)

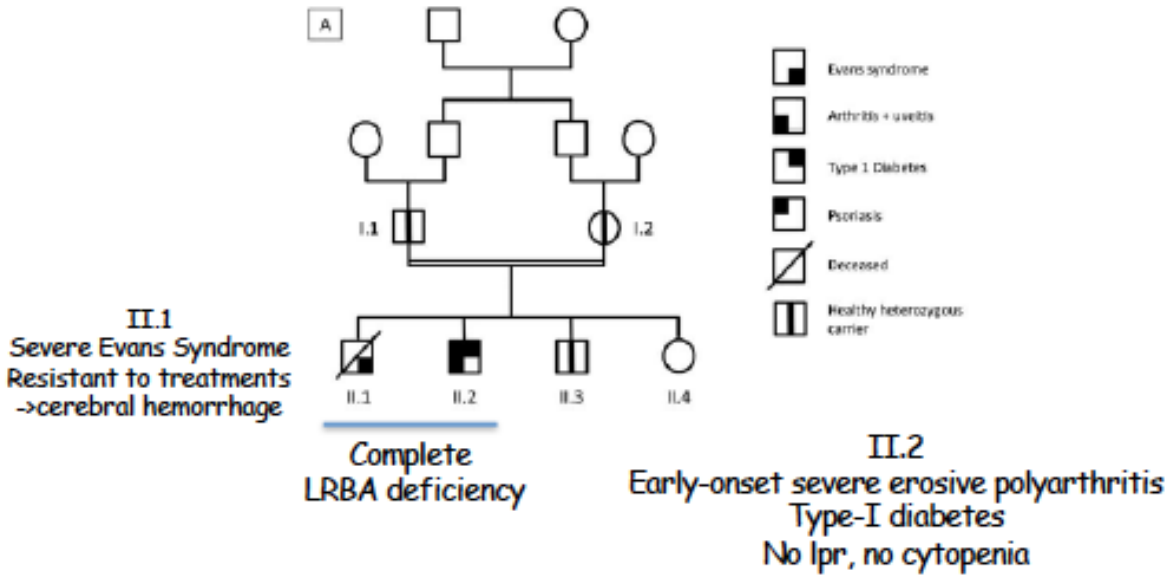
Variable clinical expression in CTLA-4/LRBA deficiencies



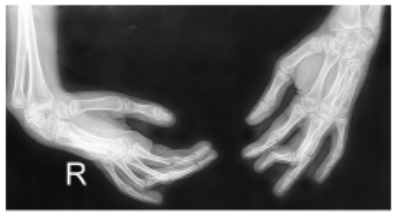
Autoimmune cytopenia in 40-50% of patients with CTLA-4/LRBA deficiencies and could be a presenting clinical feature

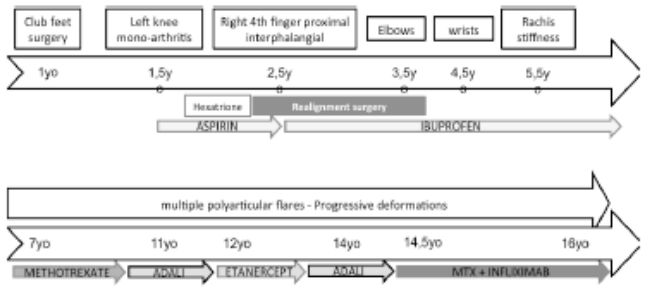
Taghizade et al, JACI 2023

Variable clinical expression autosomal recessive LRBA deficiencies



Patient II.2

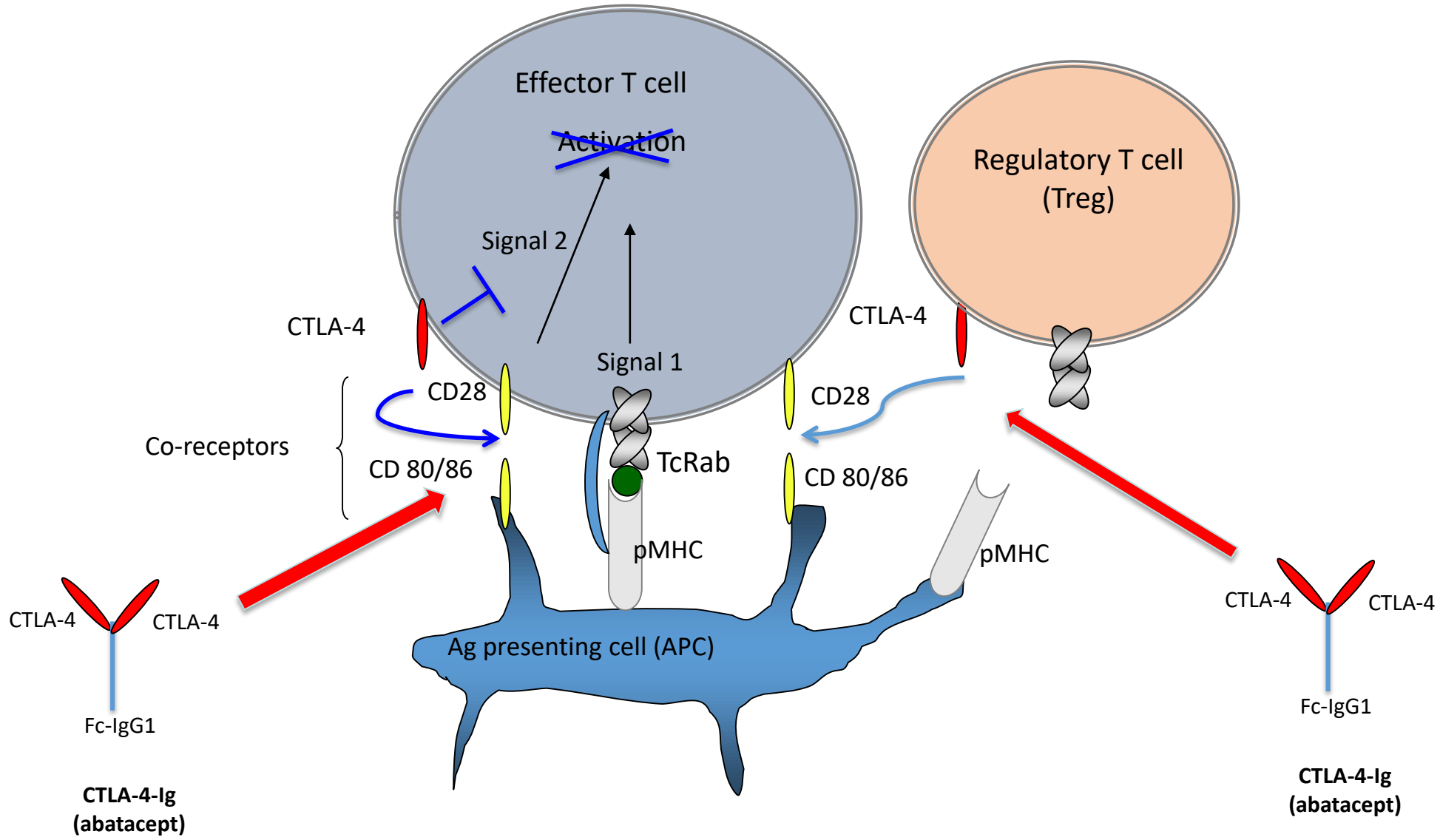




CTLA4 related pathophysiology
➔ Targeted treatment

Levy et al Clin Imm 2016

Role of CTLA4 in effector and regulatory T cells: towards targeted therapeutics

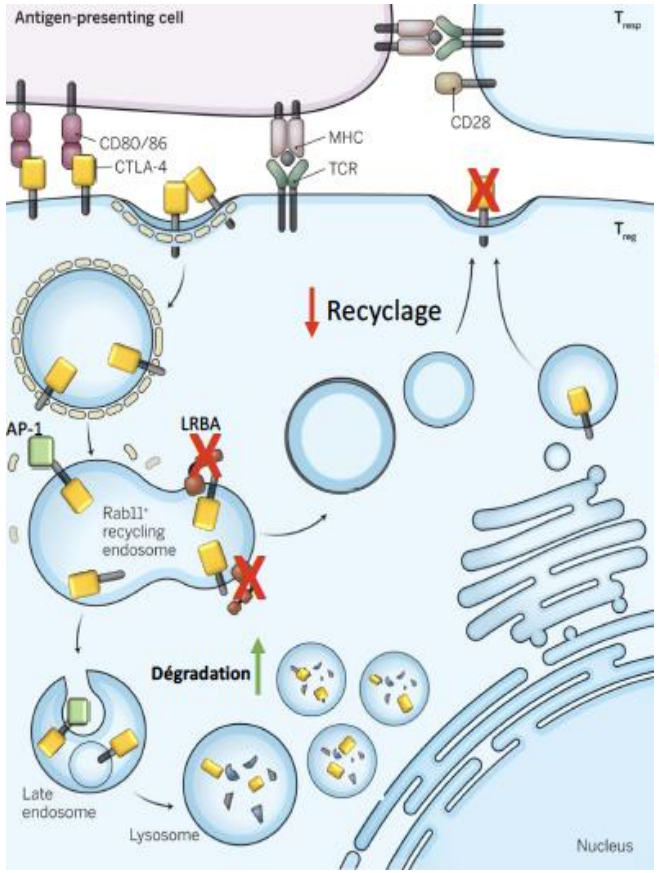


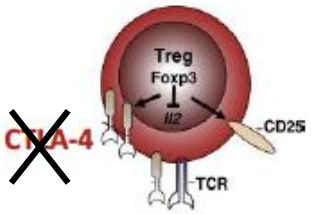
CTLA-4 or LRBA deficiencies are associated with low CTLA-4 expression in conventional T cells and Tregs

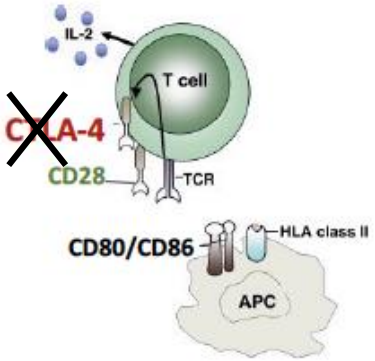
CTLA-4

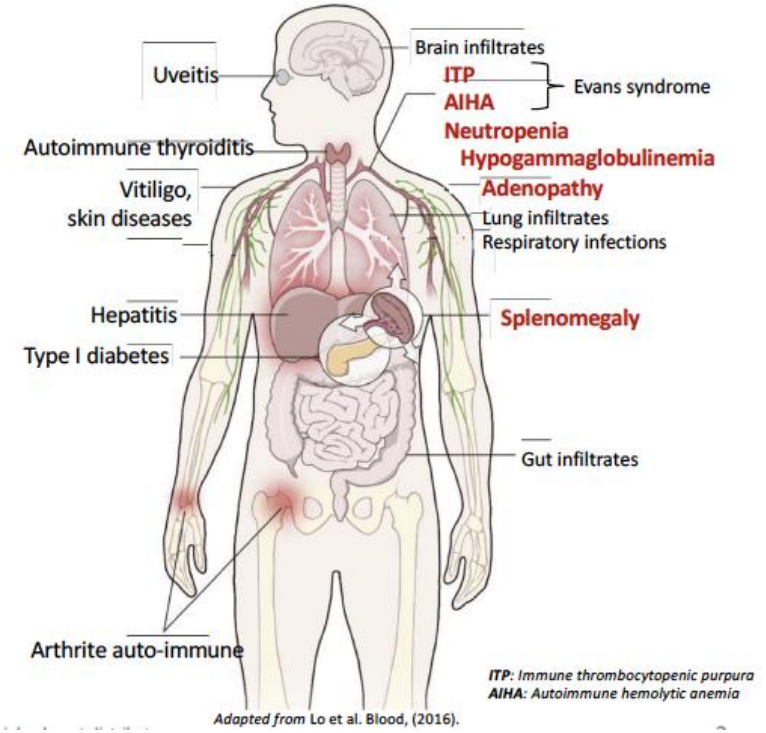
LRBA

Complexe AP-1







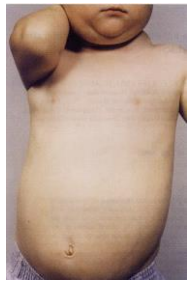


CTLA-4 haplo-insufficiency and LRBA deficiency are genetic phenocopies with low CTLA-4 expression on activated T cells and Tregs leading to various severe early-onset autoimmunity

Autoimmune LymphoProliferative Syndrome with mono-allelic *FAS* mutations: ALPS-FAS

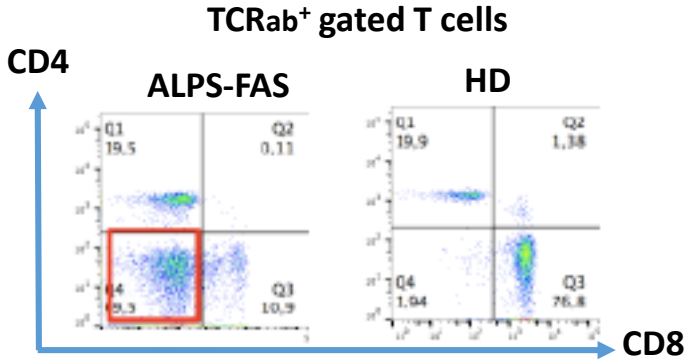
Chronic Non-infectious Non-malignant Lymphoproliferation

- Onset < 5 years (0-18y)
- Splenomegaly (hepatomegaly)
- Adenopathy



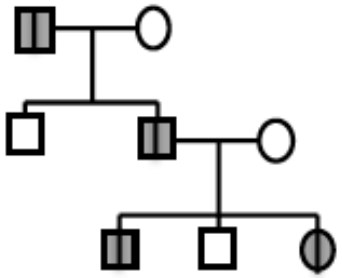
Immunological disorders

- «double negative»T cells (Tcrab CD4 $^{-}$ CD8^{-})
- hyper Ig (G,A)
- Elevated Vit B12, IL10, soluble FASL in sera
- Autoimmune cytopenias (2/3 patients): ITP/AIHA
- Rare organ-specific AI

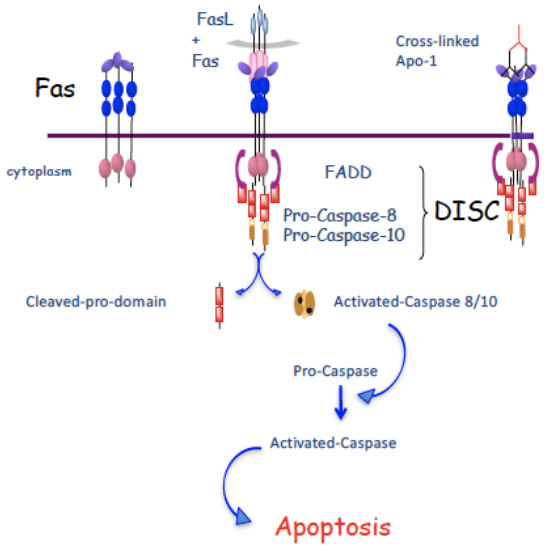


Familial cases

Dominant germline FAS mutations



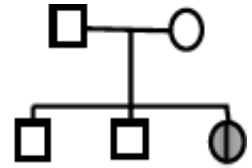
(Rieux-Laucat et al Science 1995)
(Fisher et al Cell 1995)



Impairment of FAS-induced apoptosis

Sporadic cases

Dominant somatic *FAS* mutations

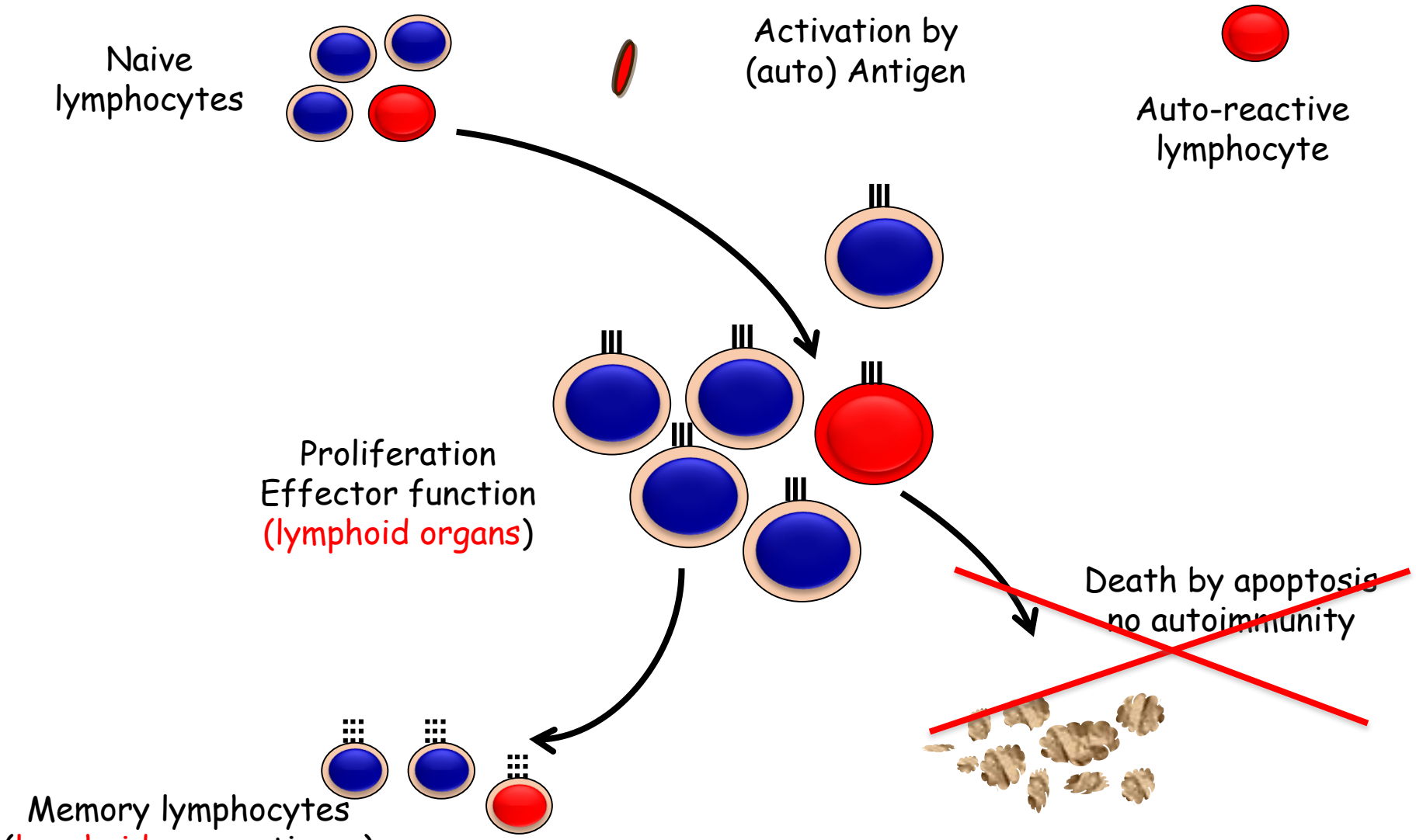


Somatic mutations appear
in hematopoietic precursors

Detected in DNT cells (80-100%)
in T and B cells , monocytes (1-20%)

(Holzelova et al NEJM 2004)

Programmed cell death deficiency: Lymphoproliferation + Auto-immunity



FAS regulates Proliferation in lymphoid organs
But is dispensible in tissues

ALPS-FAS: differential diagnosis

- Découverte d'une thrombopénie à 106 000 puis 71 000 fin octobre 2004 à l'âge de 16 mois devant des lésions cutanées d'allure purpurique aux membres inférieurs. S'y associait une neutropénie autour de 500/mm^3. Le myélogramme était riche en mégacaryocytes, la lignée granuleuse riche, avec un blocage partiel de maturation. Le bilan immunitaire était normal
- Myélogramme/caryotype médullaire/bilan d'hémostase/ échographie abdominale N,
- lymphopénie CD4 avec 850 lymphocytes/mm^3 sans excès de lymphocytes T doubles négatifs.
- Conclusion: **bilan en faveur d'une origine périphérique de la thrombopénie** qui est à 70 000/mm^3 avec 3 500 polynucléaires neutrophiles
- Absence de mutation du gène WAS en 2006 (eczéma et splénomégalie modérée)
- Consultation en octobre 2008: pas de syndrome hémorragique, 100 000 plaquettes, 4 000 polynucléaires neutrophiles.
- l'aspect des plaquettes sur le frottis sanguin n'a jamais montré à priori de particularité évocatrice d'un diagnostic précis, le volume plaquettaire moyen est normal, on peut retenir l'hypothèse d'un PTI chronique bien que l'évolution soit très atypique
- Dernière consultation en janvier 2015 : **débord splénique de 4 cm**, 58 000 plaquettes, 2 000 PN, 1600 lymphocytes, 560 CD4, CD4- CD8- 3 %, PCR EBV négative, FAS ligand 0,6 ng/mL, IL10 0, vitamine B12 891 pg/mL pour une normale entre 180 et 820, test de Coombs négatif : IgG 12,5 g/L, IgA 1,49 g/L, IgM 4,2 g/L, sérologie EBV positive en IgG VCA, EBNA et IgM VCA

-> NGS (research)

-> Homozygous mutation in *NBEAL2*

ALPS-FAS: differential diagnosis

Gray platelet syndrome can mimic autoimmune lymphoproliferative syndrome

Anne Rensing-Ehl, Ulrich Pannicke, Stefanie-Yvonne Zimmermann, Myriam Ricarda Lorenz, Benedicte Neven, Ilka Fuchs, Ulrich Salzer, Carsten Speckmann, Anne Strauss, Eberhard Maaβ, Benedicte Collet, Anselm Enders, Remi Favier, Marie Christine Alessi, Frederic Rieux-Laucat, Barbara Zieger, Klaus Schwarz, Stephan Ehl

Blood. 2015 Oct 15; 126(16): 1967–1969.

IgG g/L* (normal age range, y)	23.41 (5.04-14.74)	10.46 (4.53-9.16)	16.51 (5.04-14.74)	10.6 (6.98-15.84)	13.74 (6.98-15.84)	18.2 (5.04-14.74)
DNT %† (<2.5%)	2.8 (43/μL)	1.7 (24/μL)	2.4 (28/μL)	1.5 (14/μL)	2.3 (35/μL)	5 (57/μL)
Vitamin B_{12},*,‡ mg/dL	3856	>4000	>4000	3758	1843	>2000
sFASL,‡ pg/mL (<200)	759	740	797	549	465	630
IL-10, pg/mL (<20)	6.5	<1	4.7	<1	<1	<1

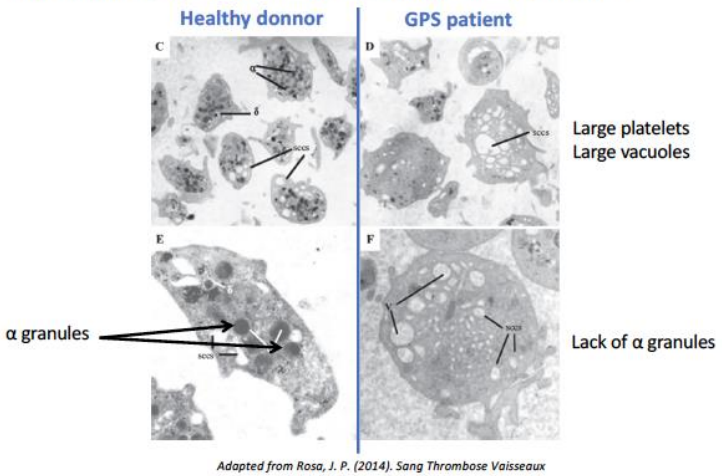
ITP, Splenomegaly, Hyper IgG, Increased soluble FAS-ligand

-> pathophysiology ??

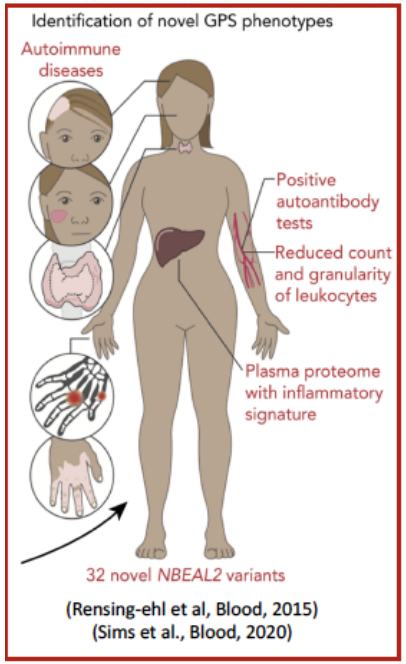
Grey Platelet syndrome: a bleeding disorder with autoimmune features
What are the mechanisms involved in autoimmunity?

Gray platelet syndrome (GPS)

Congenital hemorrhagic syndrome caused by **recessive NBEAL2 mutations**
Abnormal gray platelets and macrothrombopenia
Often splenomegaly, sometimes evolving in progressive myelofibrosis

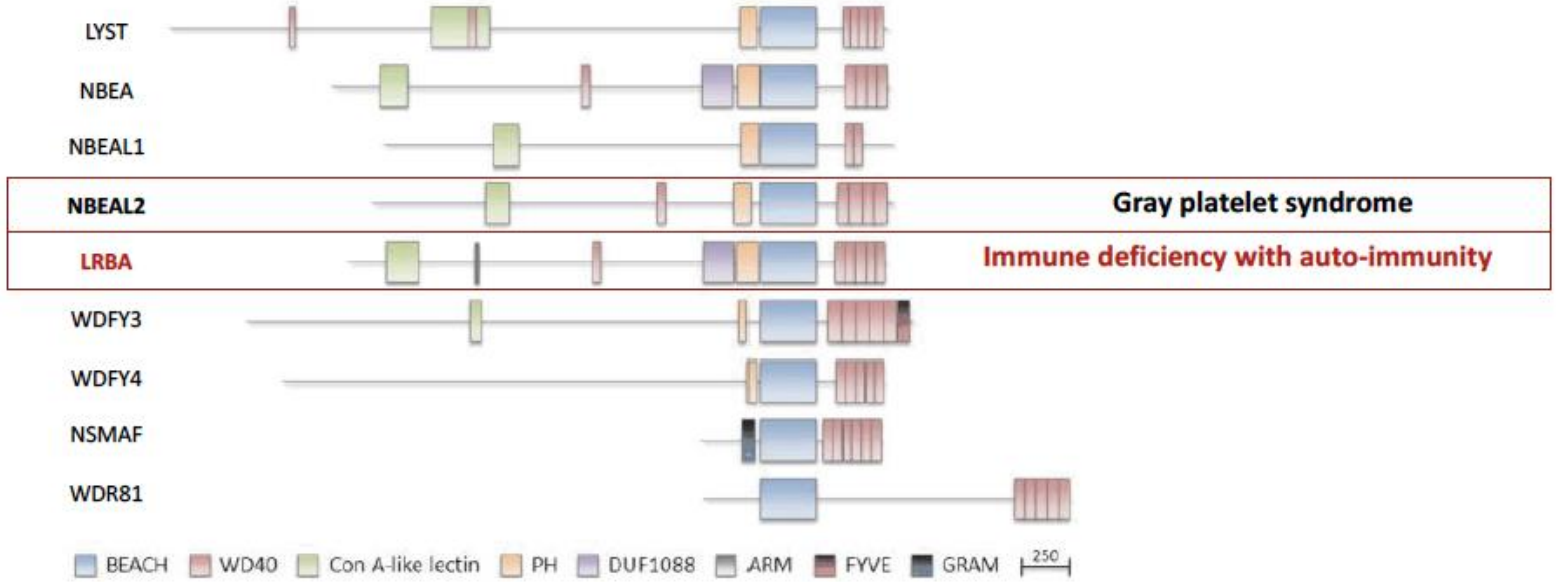


Adapted from Rosa, J. P. (2014). Sang Thrombose Vaisseaux



(Rensing-ehl et al, Blood, 2015)
(Sims et al., Blood, 2020)

BEACH protein family



LRBA is involved in CTLA-4 trafficking through its BEACH domain

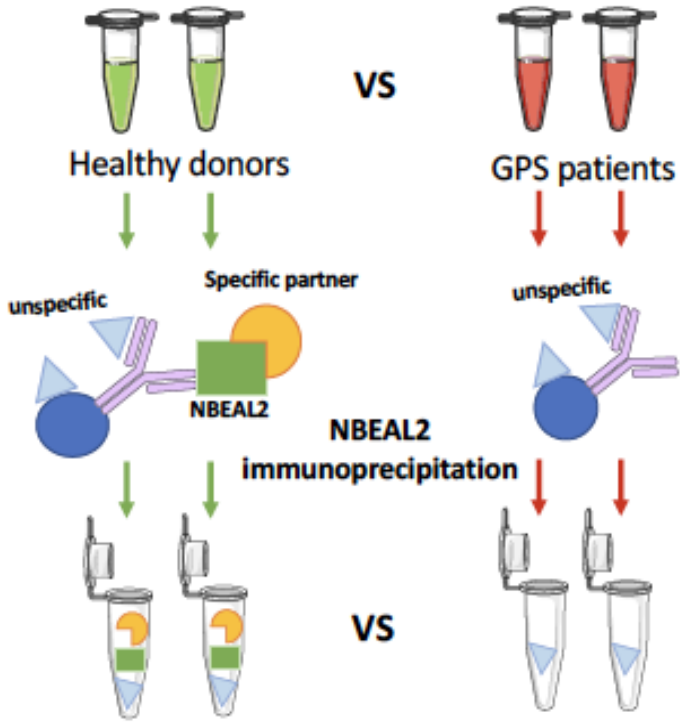
LRBA and NBEAL2 are structurally similar and involved in vesicular trafficking

Adapté de Cullinane et al. Traffic (2013)

Hypothesis:
NBEAL2 is involved in the vesicular trafficking of immune receptors
(Laure Delage, Thèse CIFRE SANOFI)

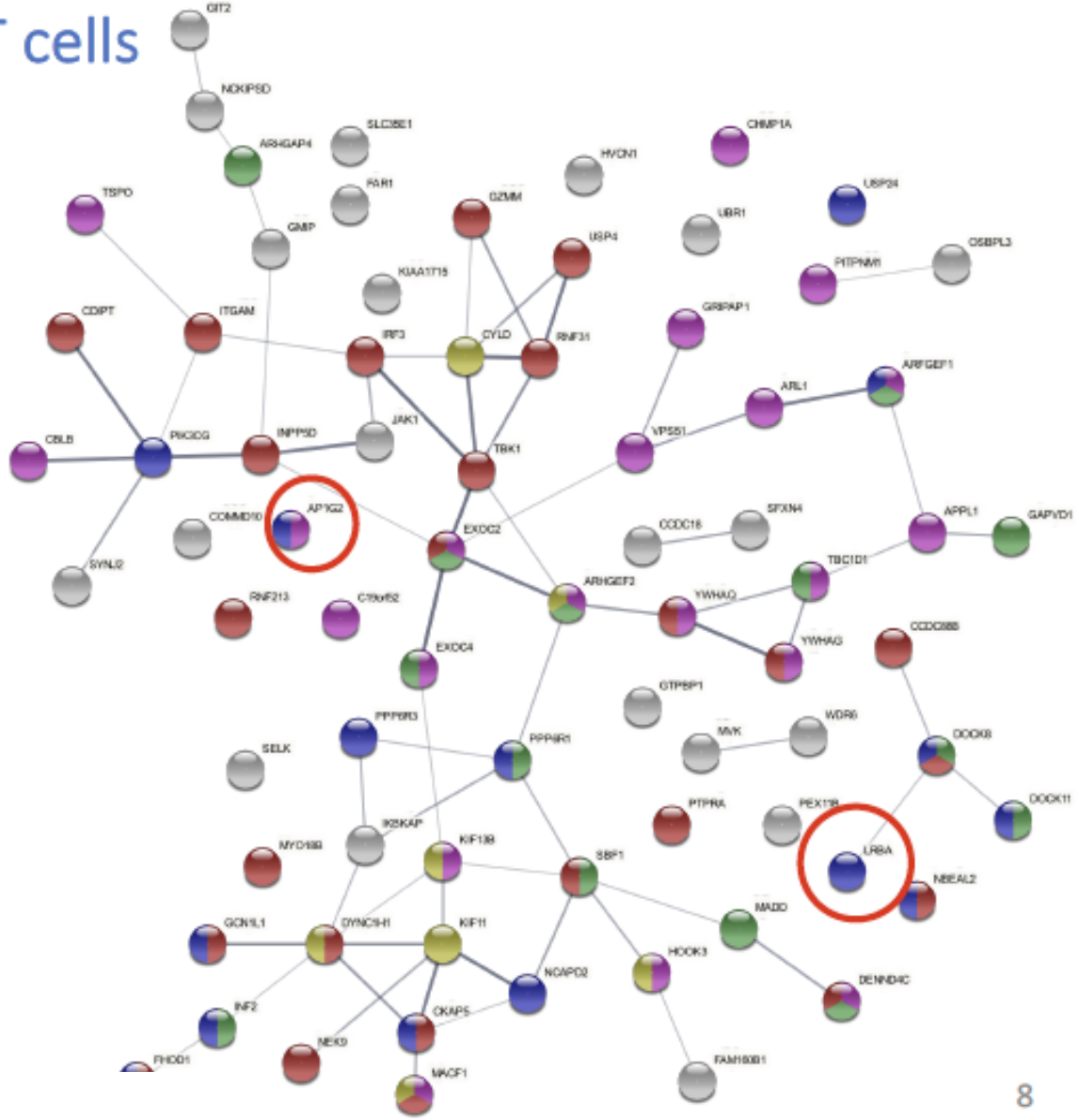
NBEAL2 interacts with LRBA and AP-1: a role in CTLA-4 expression?

NBEAL2 interactome in activated T cells

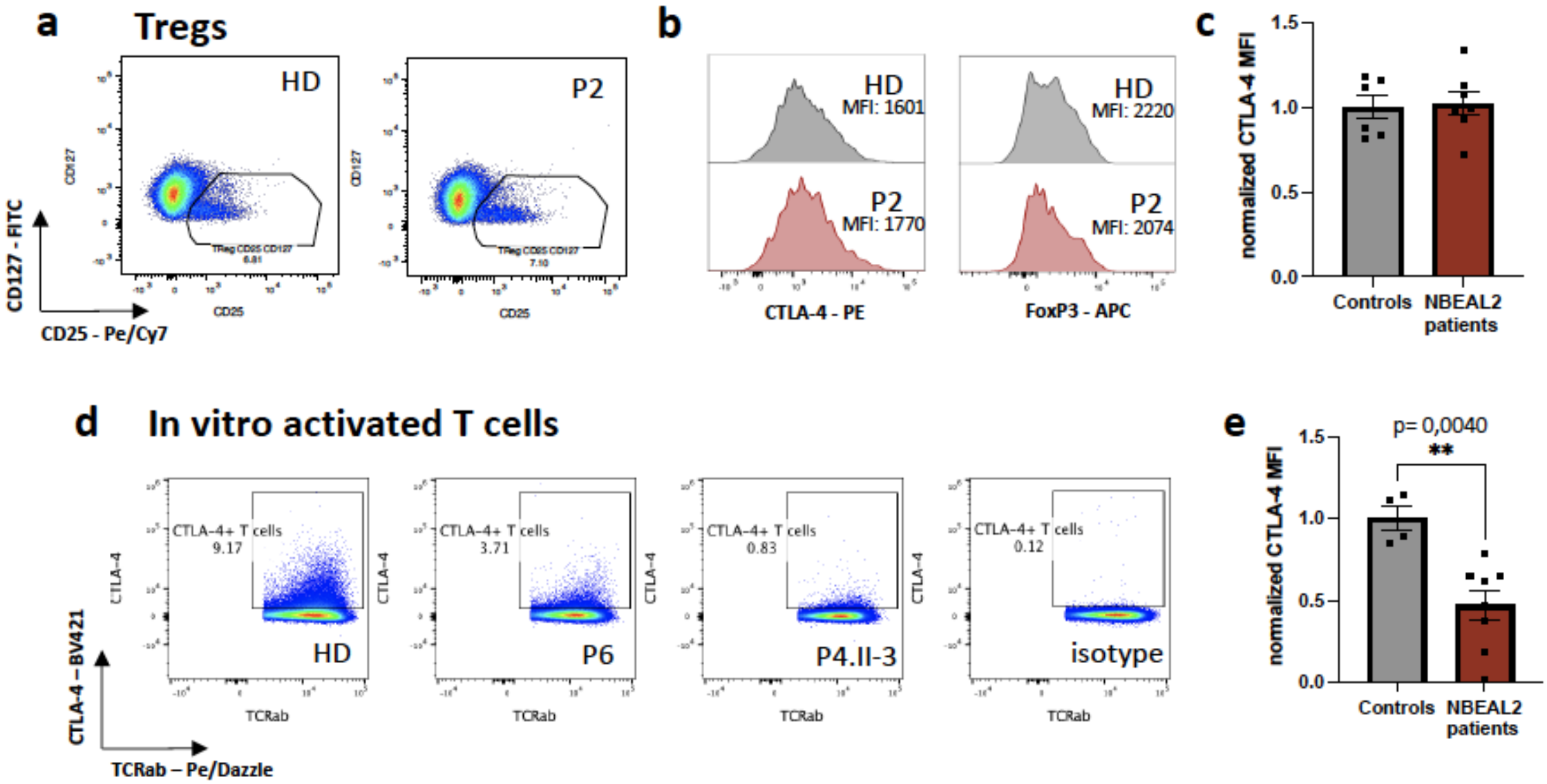


- Immune system (25/76 proteins)
- GTPase binding (14/76 proteins)
- ARM domain containing (15/76 proteins)
- Transport proteins (21/76 proteins)
- Microtubules (7/76 proteins)

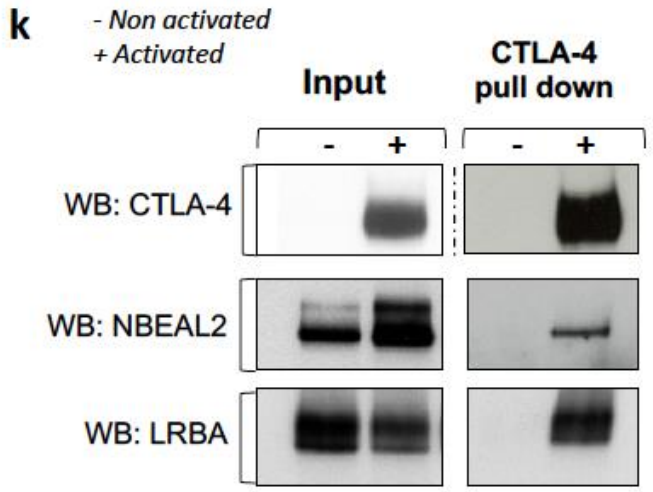
Proteins involved in CTLA-4 trafficking (LRBA, AP1G2)



NBEAL2 deficiency is associated with normal CTLA-4 expression in Tregs
But low CTLA-4 expression in Activated conventional T cells

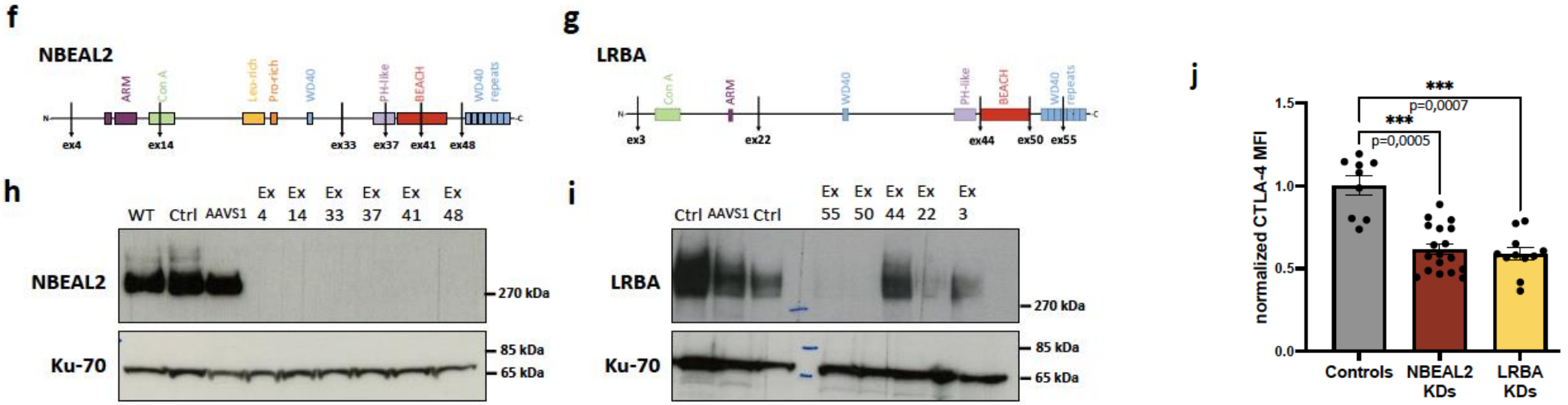


NBEAL2 and LRBA interact with CTLA-4 in primary T cells from healthy controls

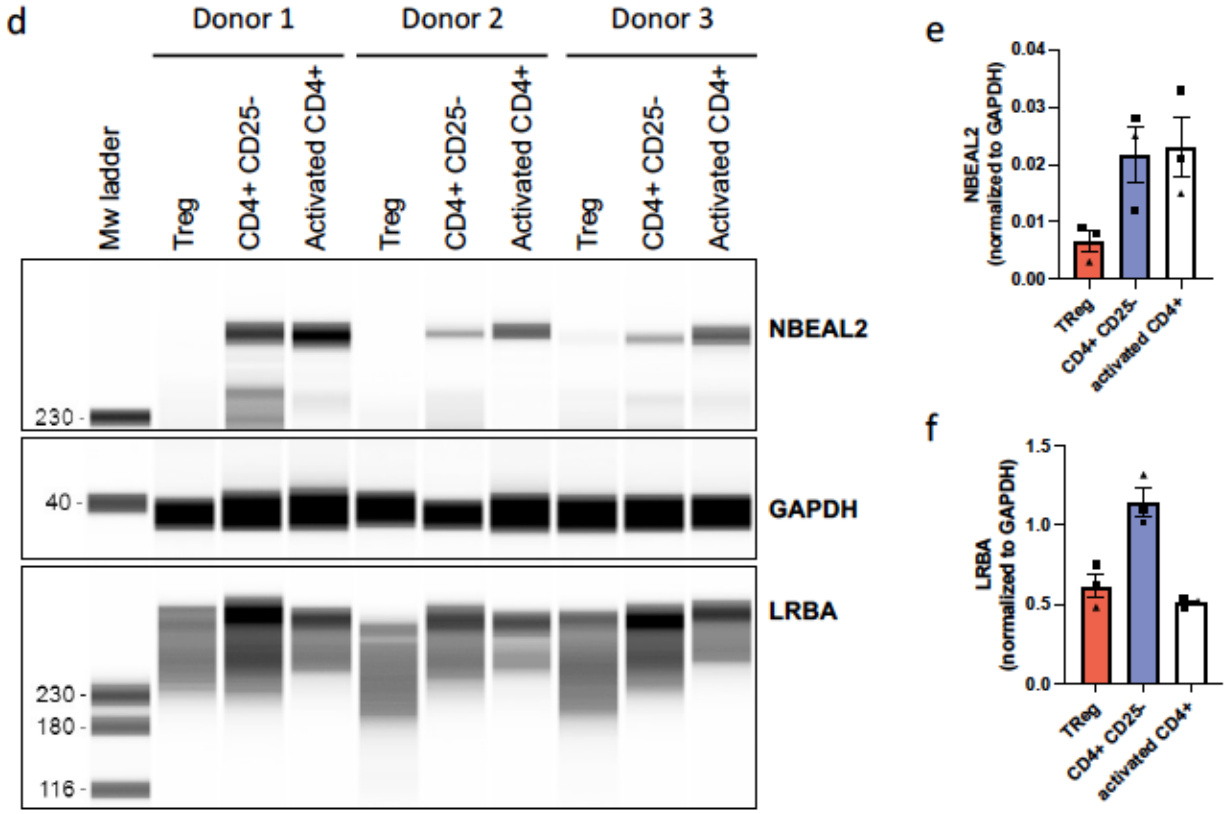


Pull-down of CTLA-4 reveals interaction with LRBA and NBEAL2 in primary activated T cells from healthy controls

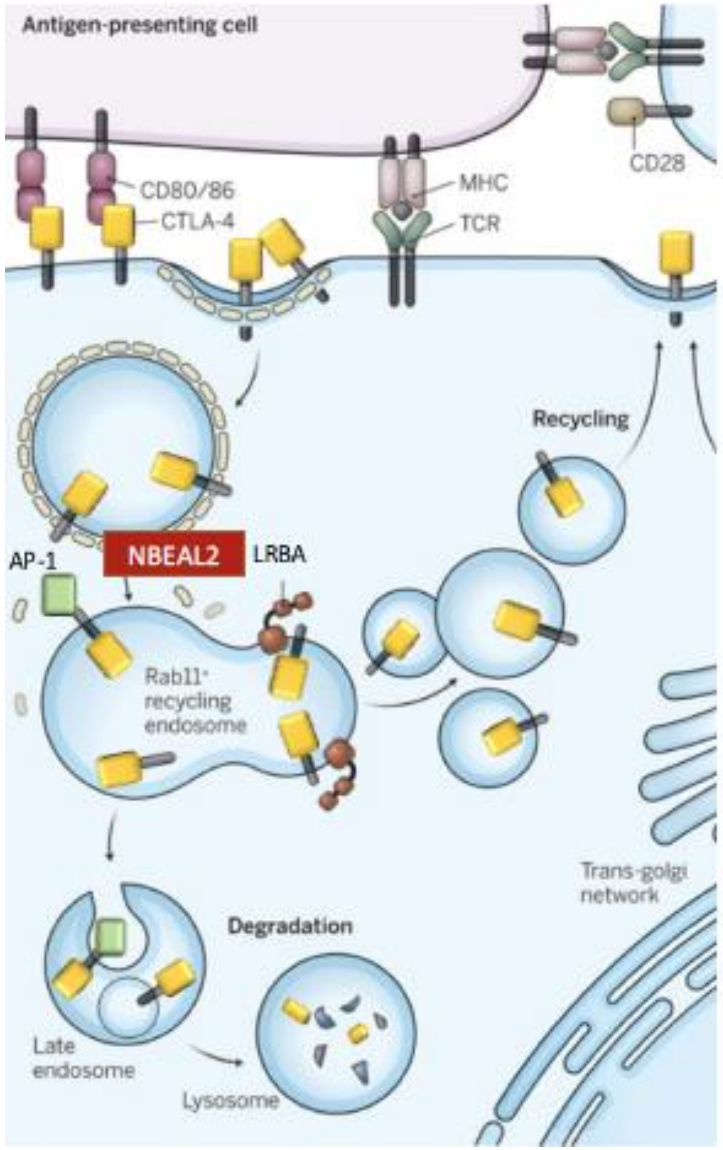
NBEAL2 or LRBA Knock-down by Crispr/Cas9 in primary T cells from healthy controls

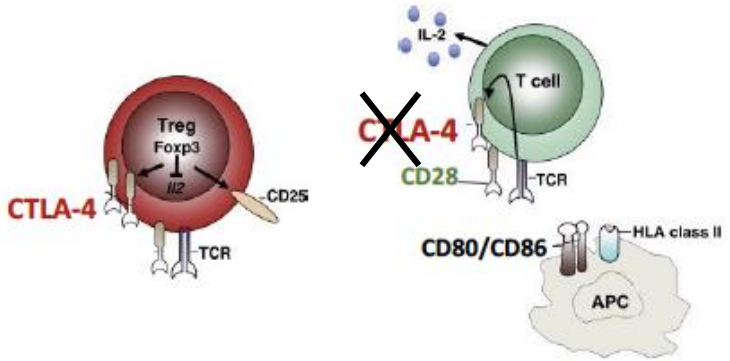


NBEAL2 is expressed in conventional T cells but not in Tregs from healthy controls



NBEAL2 regulates the CTLA-4 expression in conventional activated T cells





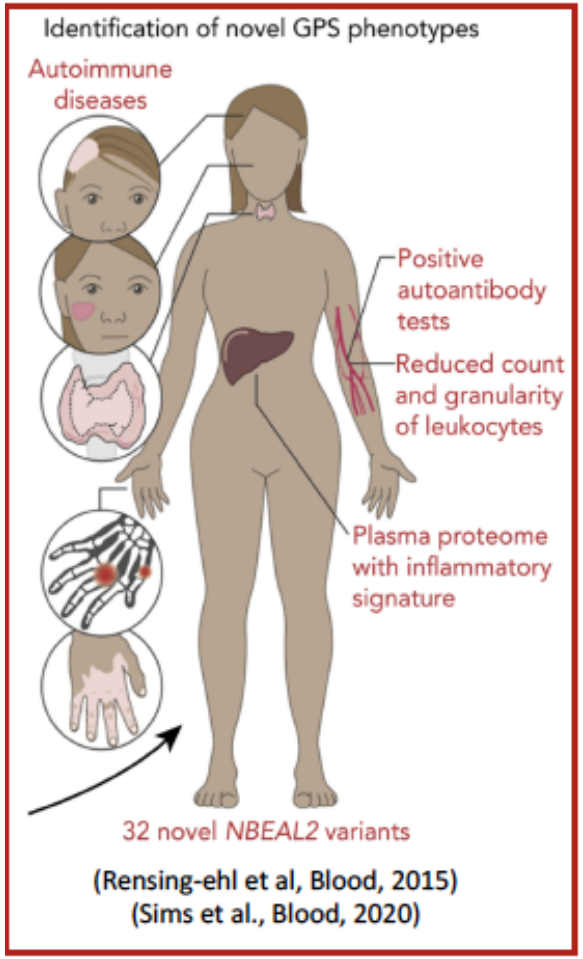
NBEAL2 :

interacts with LRBA

Regulates CTLA-4 expression
on activated conventional T cells
but not on Tregs

Low CTLA-4 expression on activated T cells
Is associated with autoimmunity

-> Abatacept as a potential therapy
To treat autoimmunity in GPS?



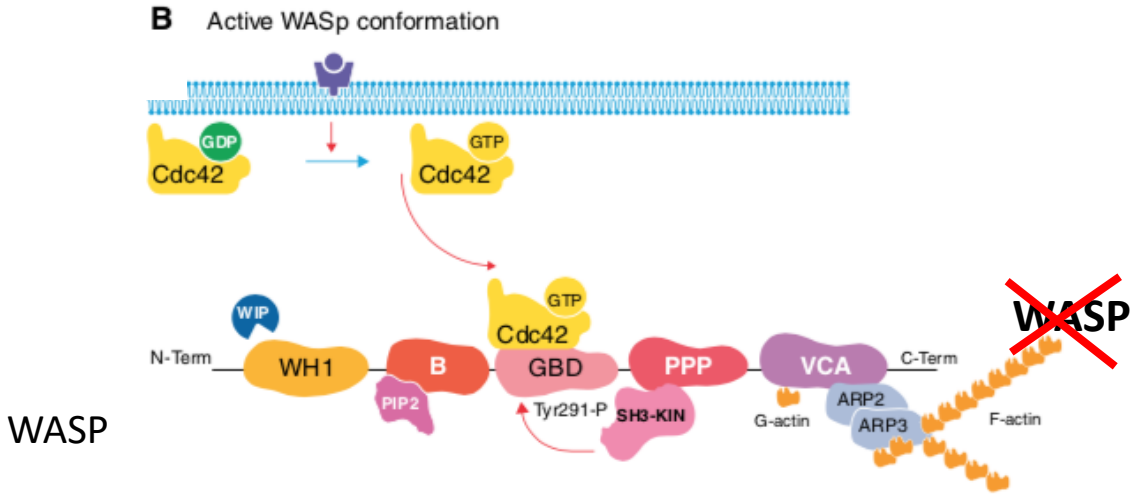
Delage et al Nat.Com 2020

Constitutional Platelet deficiencies: A possible genetic cause of autoimmune cytopenia? Platelets target and culprit in ITP ?

mutations in WAS, NBEAL2, and genes associated with constitutional thrombocytopenia in patients with Autoimmune cytopenia

WAS hemizygote mutations:
-Aldrich syndrome:
X-linked CID, thrombocytopenia with microplatelets, Autoimmunity
-XL thrombocytopenia

WASp KO in mice recapitulates the human disease



WASP

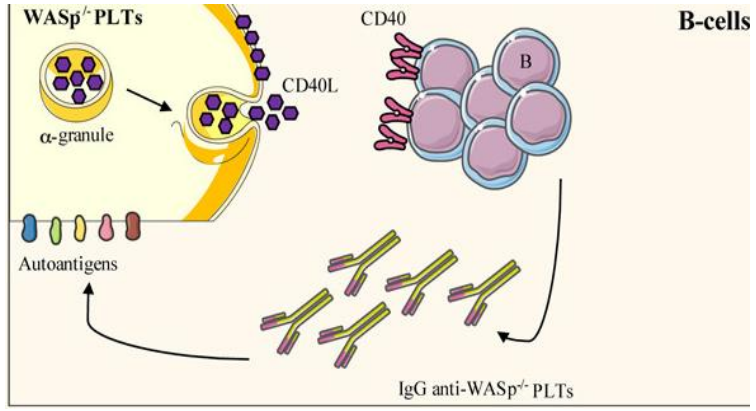
Mice WAS^{KO} in MK

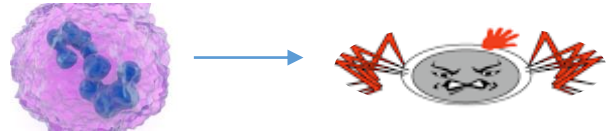
Conditional WASp KO in megakaryocytes:

-> microplatelets

AND

Autoantibodies against platelets





Abnormal platelets production
Can contribute to immune tolerance breackdown

Constitutional Platelet deficiencies: A possible genetic cause of autoimmune cytopenia? Platelets target and culprit in ITP ?

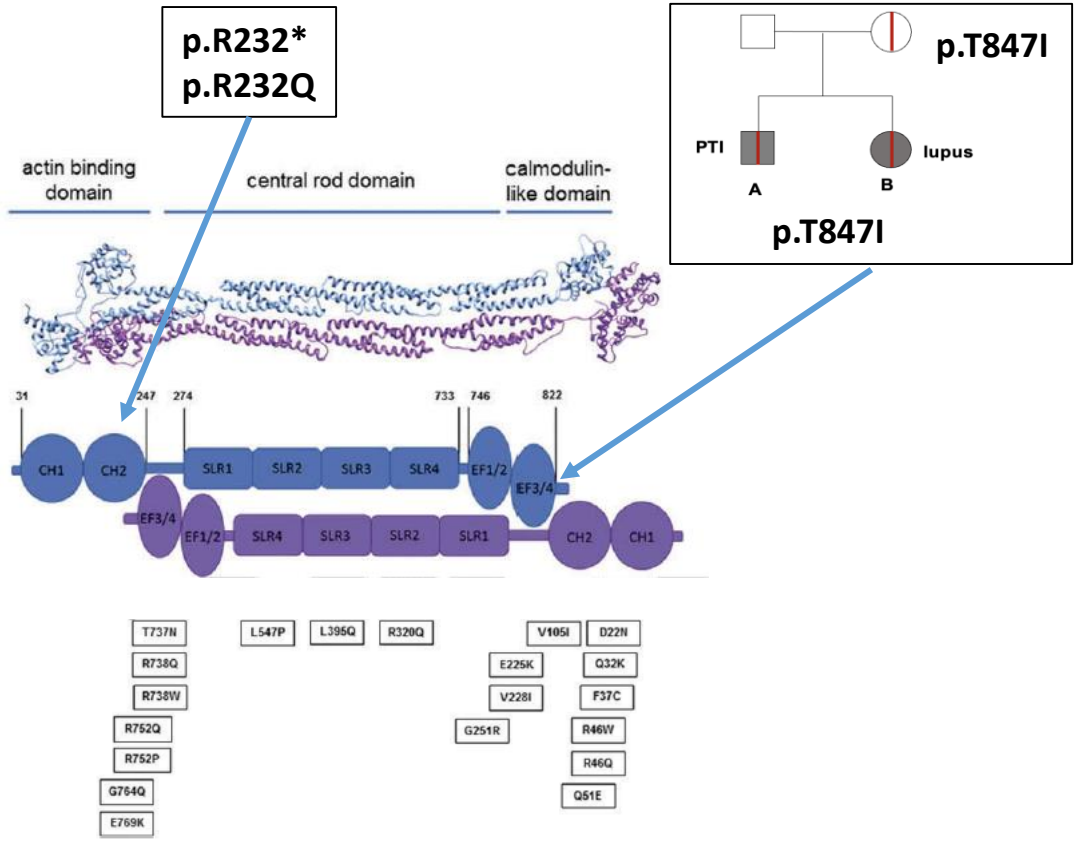
mutations in WAS, NBEAL2, and genes associated with constitutional thrombocytopenia in patients with autoimmune cytopenia

Patients with AI cytopenia
and mutations in genes of constitutional thrombocytopenia

		Gene	protéine	CADD	MAF
Platelet function	P1	TUBB1	p.R297H	28	Nd
	P2	TUBB1	p.E45X	NA	Nd
	P3	ACTN1	p.N462H	28	Nd
	P4	TPM4	p.E75Q	24	Nd
	P5	TRPM7	p.A1254V	23	$4.80x10^{-4}$
	P6	MYH9	p.D1424N	28	Nd
	P7	MYH9	p.S1294N	23	Nd
	P8	MYH9	p.D1627N	24	$5.66x10^{-5}$
	P9	MYH9	p.D1119V	27	Nd
Immune and Platelet function	P10	ANKRD26	p.R858X	38	Nd
	P11	ETV6	p.R369W	34	Nd
	P12	RUNX1	p.M267I	16	$3.93 x10^{-5}$
	P13	JAK2	p.F240V	23	Nd
	P14	PLCG2	p.V431	22	$2,14 x10^{-5}$
	P15	PLCG2	p.D520G	21	$3,21x10^{-5}$

Validation ongoing (F.Zeco)

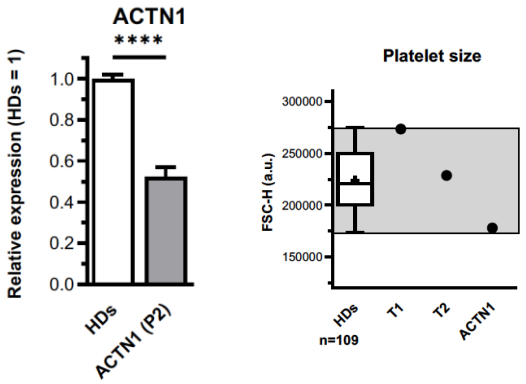
ACTN1 variants



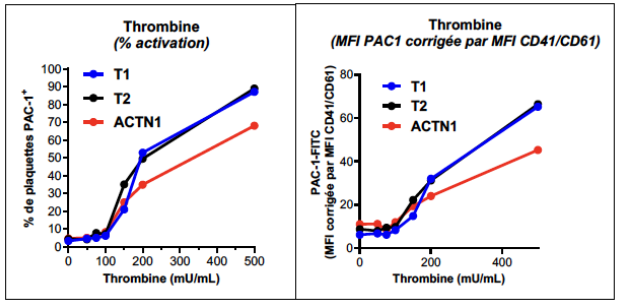
Mutations in constitutional thrombocytopenia

ACTN1 variants: A possible genetic cause of autoimmune cytopenia?

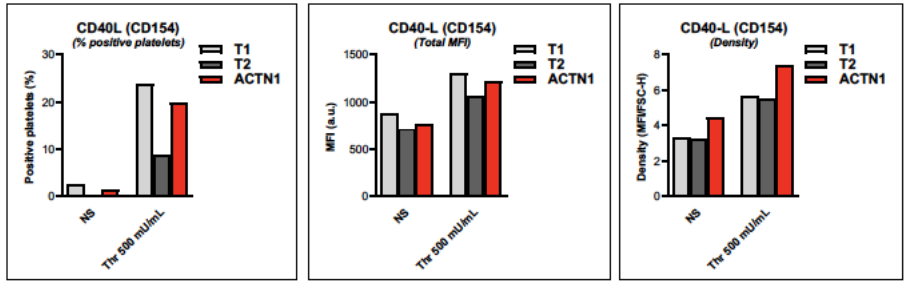
F.Zeco (ongoing PhD study), Coll: F. Adam and A Kauscot (UMRS-1176, KB)



Reduced protein expression and platelets' size

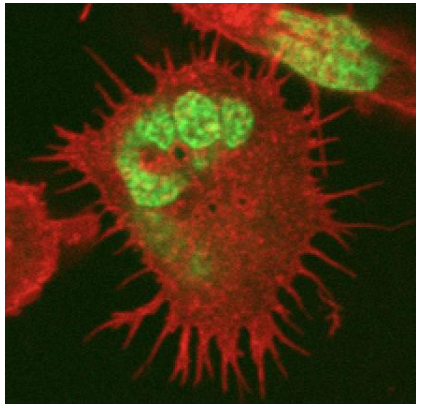


Reduced platelet activation

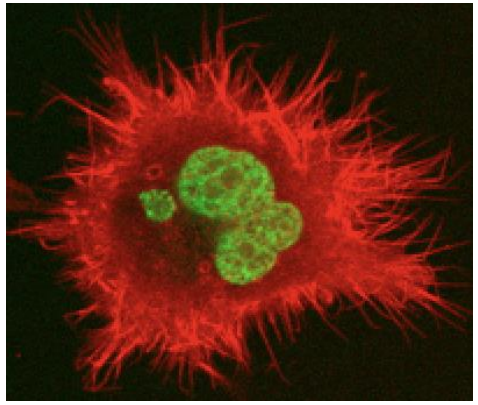


Increased CD40LG density

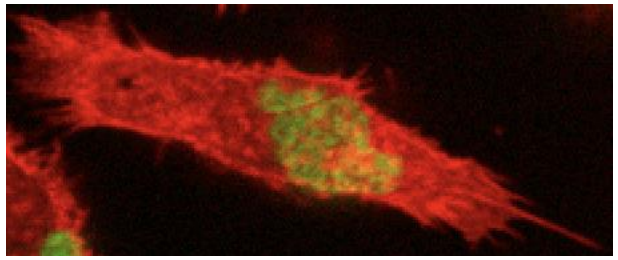
P1



P2



CT

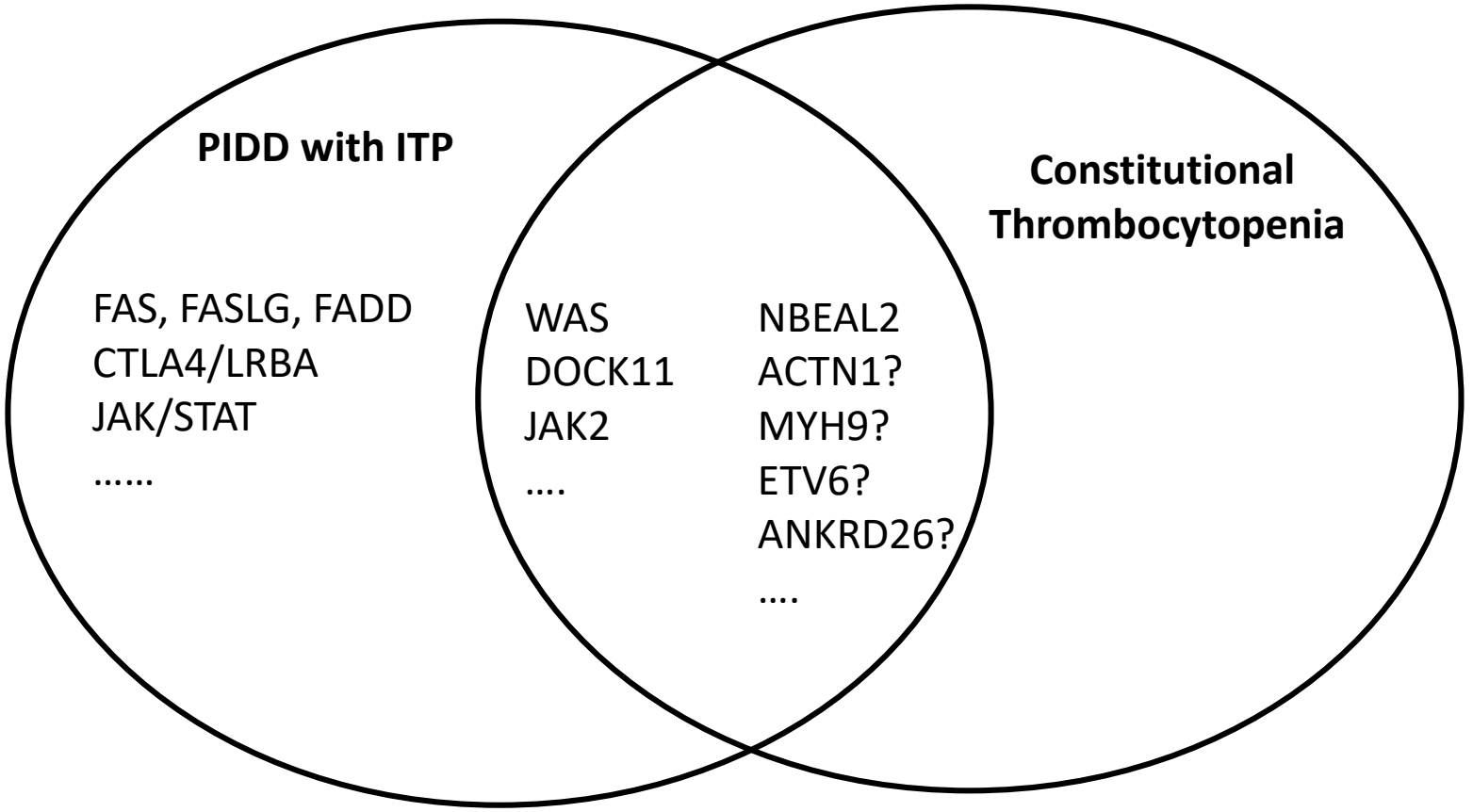


Abnormal morphology in B-EBV cell lines

ACTN1 variants:
Preliminary results on platelets to be confirmed

Role of ACTN1 variants in lymphocytes ?
-> Tregs function (Trogocytosis), T/B cell functions

Constitutional Platelet deficiencies and ITP: Conclusions



Mutations in genes with dual roles in tolerance and platelet biogenesis (WAS, NBEAL2, DOCK11, JAK2…)

Abnormal platelets production/function can contribute to immune tolerance breackdown

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(frederic.rieux-laucat@inserm.fr)