Late-onset enteric viral infection associated with hepatitis (EVAH): a new condition in patients with B-cell deficiencies

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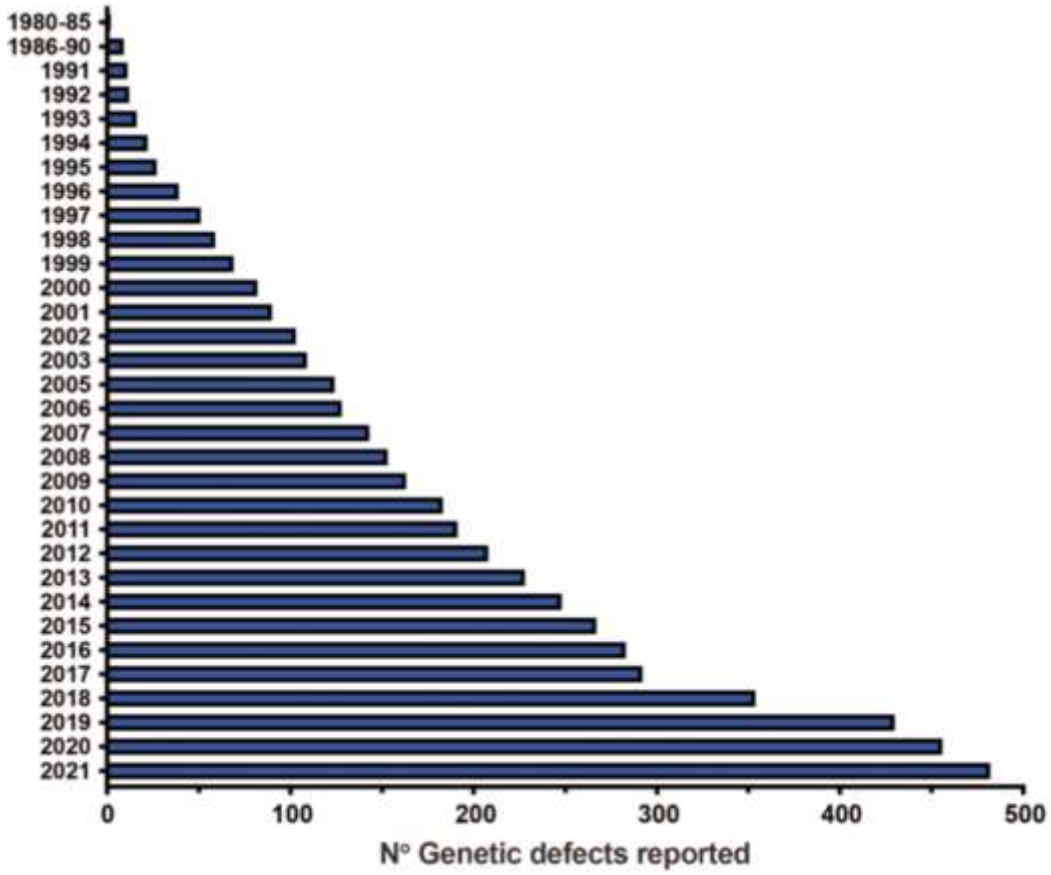
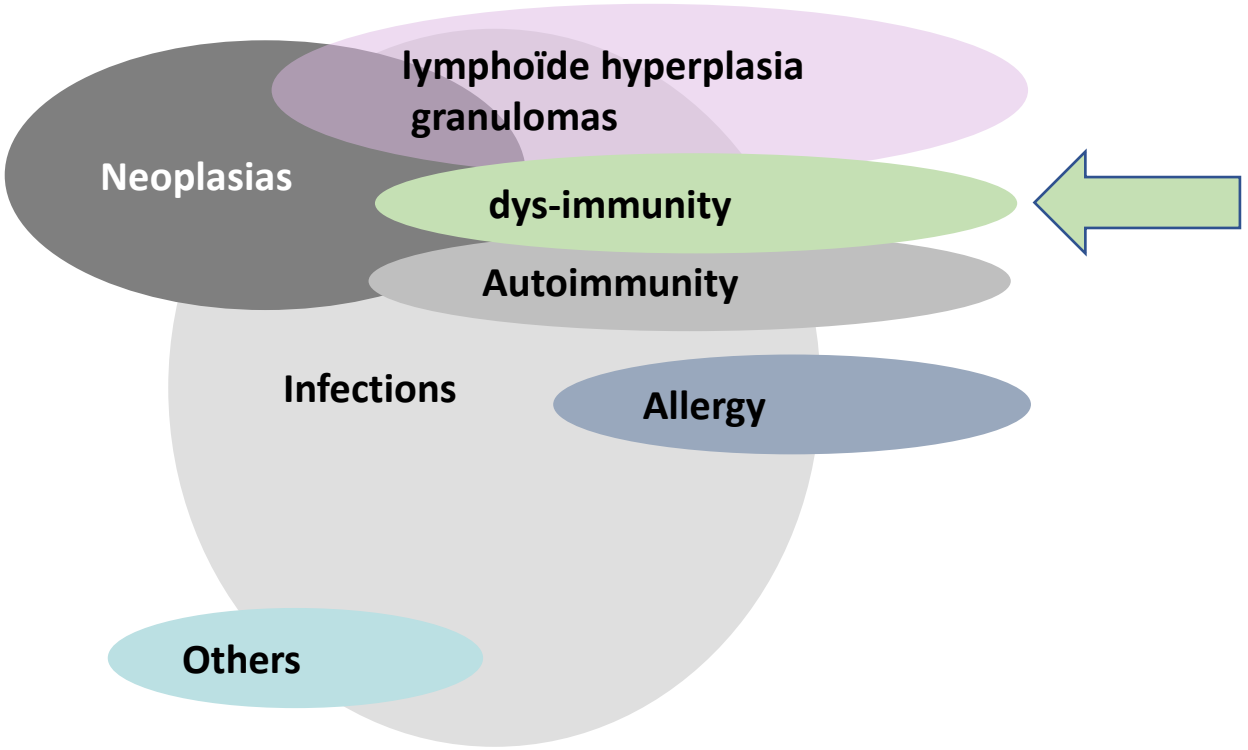
Primary Immune deficiencies (or Inborn Error of Immunity)

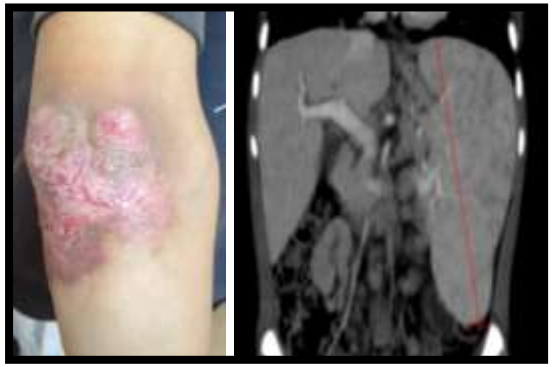




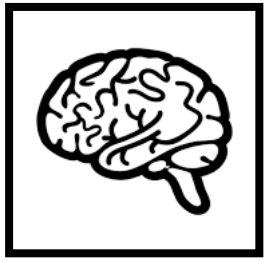
Nunes-Santos et al,, J. Allergy Clin. Immunol, 2022
Pérot et al , Emerging Infectious Diseases journal, 2023

Dys-immune manifestations are frequent

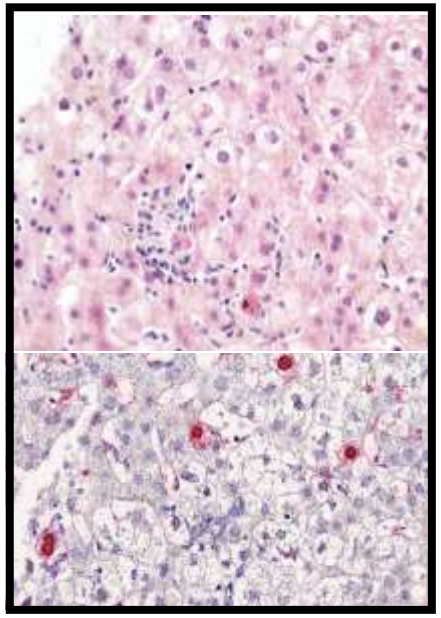
- **Frequent, ill-defined**
- Chronic **benign lymphoproliferation** or **organ specific-inflammation** (skin, liver, lung..)



Vaccine strain of **Rubella**

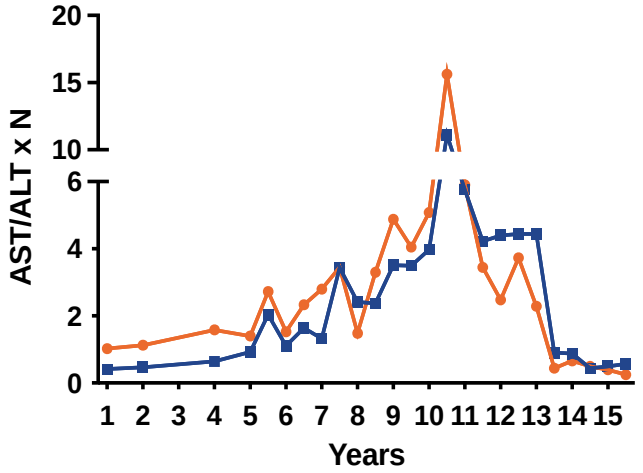


Enterovirus
Avulavirus
Astrovirus..



Circovirus in a transplanted patient

A new story starts here….

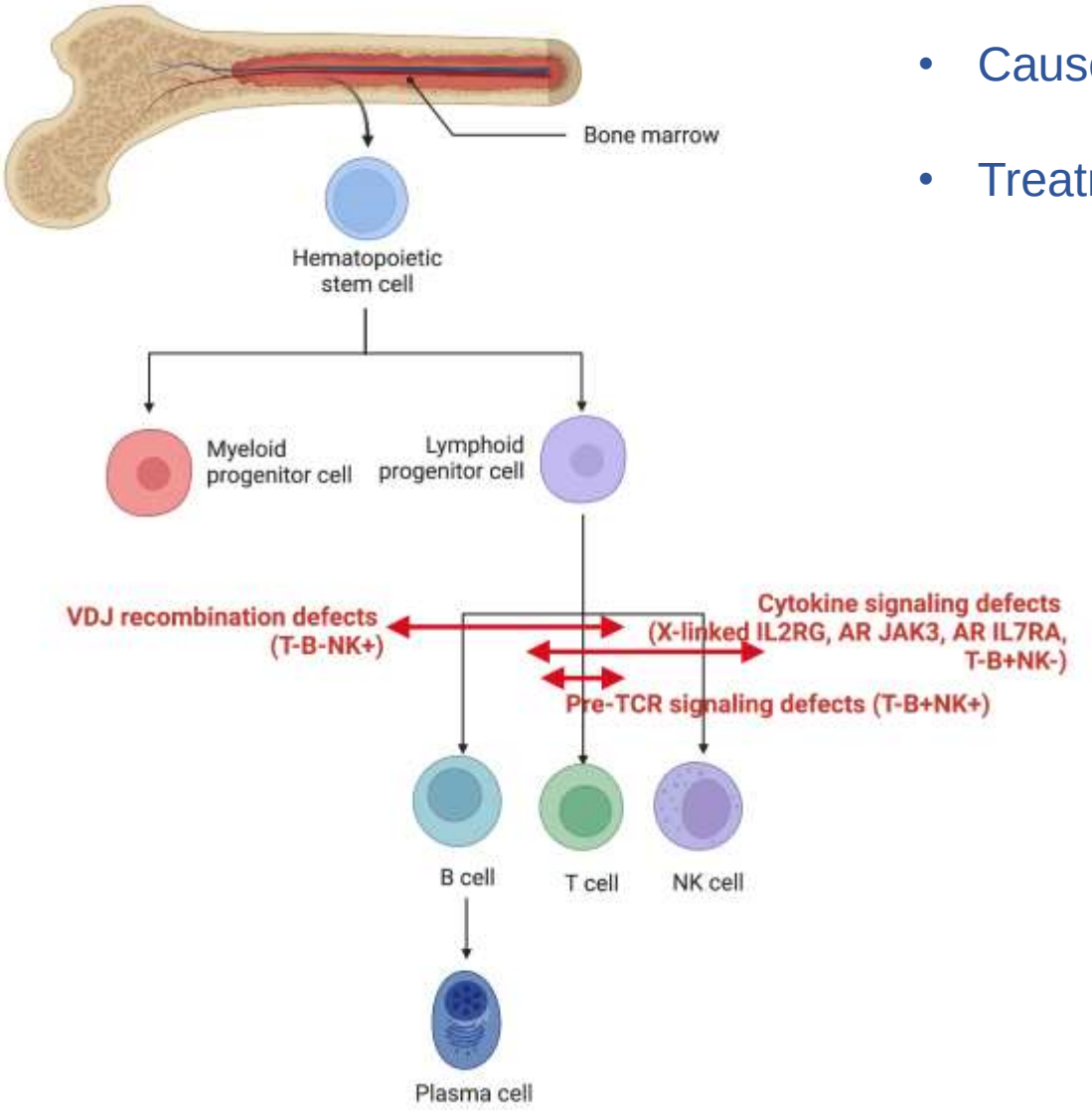


SCID patient: transaminitis
6 years after HSCT

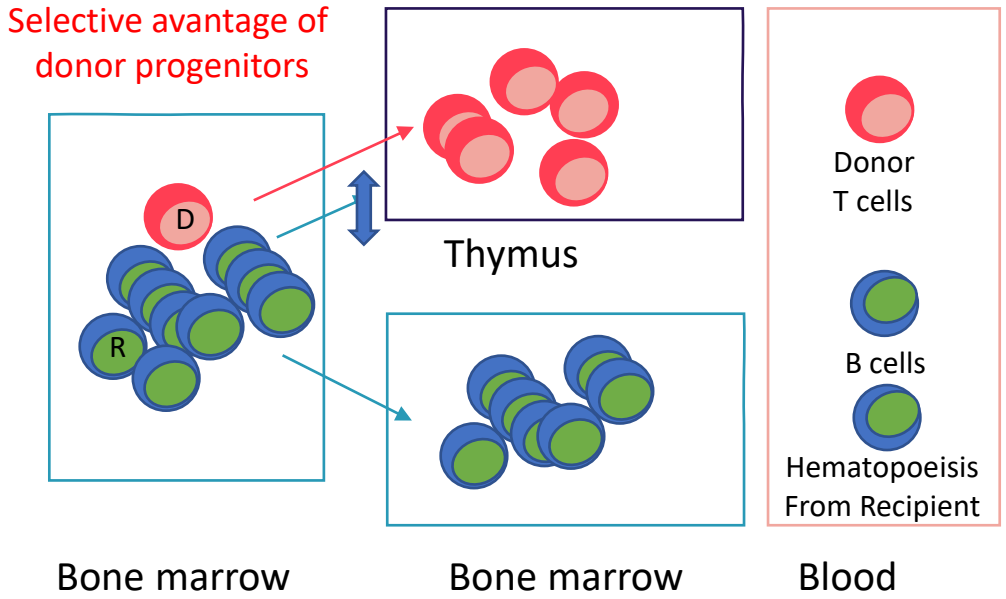
- **mNGS**: new tools: non-supervised detection of pathogens
- Many **applications** in PIDs

Nunes-Santos et al,, J. Allergy Clin. Immunol, 2022
Pérot et al , Emerging Infectious Diseases journal, 2023

Severe combined immune deficiency (SCID)



- Very rare and severe primary immune deficiency
- Caused by an **early arrest in T lymphocyte differenciation**
- Treatment : **Haematopoietic stem-cell transplantation (HSCT)** or **GT**

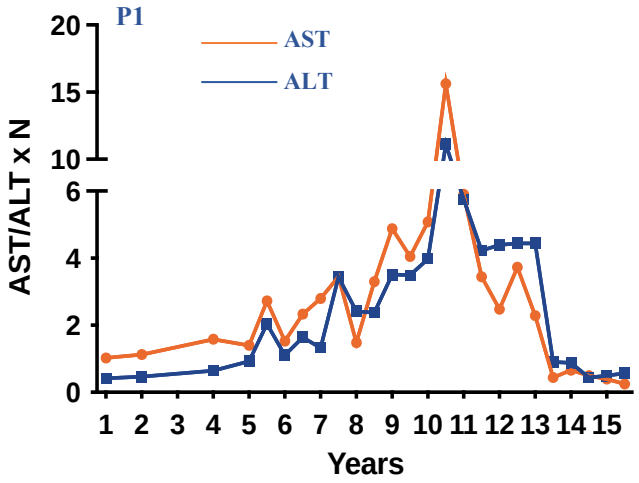


HSCT without myeloablative Chemotherapy is an option but
- persistent B cell deficiency (absent B cells or inefficient)
- decrease thymopoiesis overtime

Persistant hepatitis 6 years after HSCT in a IL2RG SCID

IL2RG deficiency, haploidentical **HSCT without** conditioning regimen

A



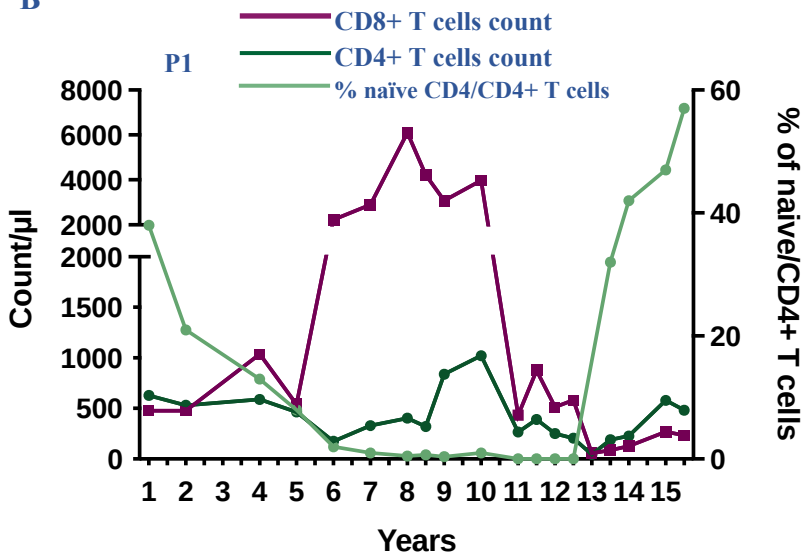
Thrombopenia →

Splenomegaly →

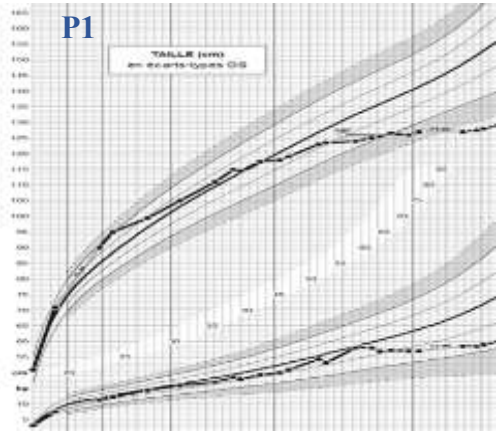
Abominal bloating/diarrhea →

Mild proteinuria →

B



C



→ GVHD, viral (hepatitis A, B, C, E) and autoimmune hepatitis excluded

→ Many treatments tried without efficacy (Steroids, rapamycine, anti-TNF, abatacept, JAKinib….)

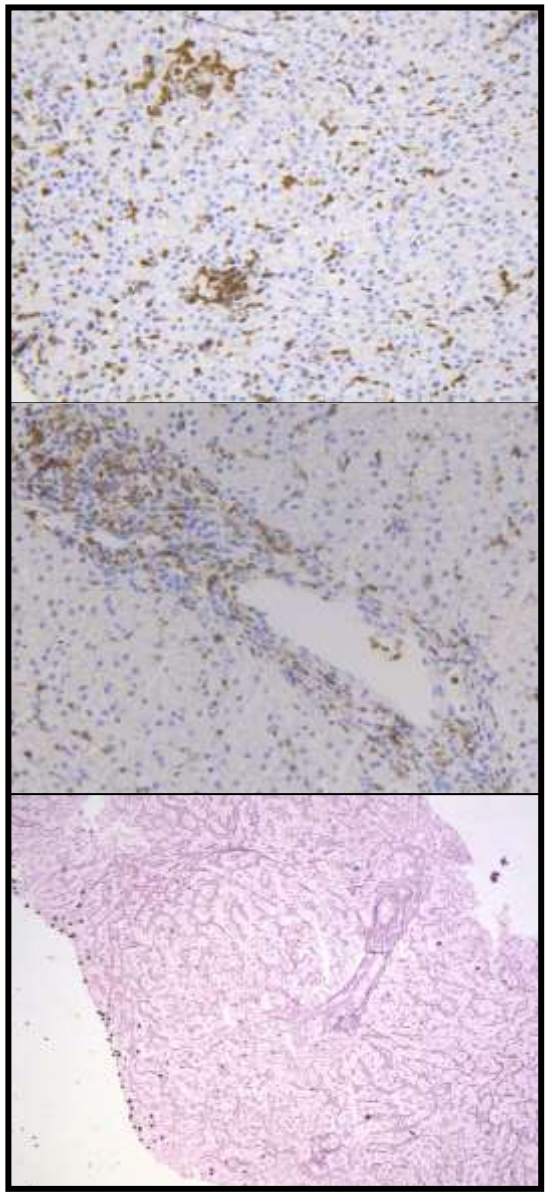
Not an isolated case….

- **12 patients** (IL2RG n= 11, DCLRE1C n= 1)
- Haplo HSCT n= 10, GT n= 1, MSD n=1
- No CR (n= 10) or Bu 8 mg/kg
- **On IgRT** (n= 12)
- **Hepatitis:** median age of 6 y (3-25) (n= 12)
- **Digestive** symptoms (n = 6)
- **Growth failure** (n= 10)
- **Splenomegaly** (n= 4), **nephromegaly** (n= 1)
- Slowly progressive disease, up to **death** in 3

Liver biopsies:

- Inflammatory process
- Lobular/sinusoidal infiltration of CD8+ T cells , granuloma in some cases
- Progressive NRH

(Nodular regenerative hyperplasia)



- **NGS** on **liver** biopsies (8 cases)
  - Aichi virus (n= 3)
  - Norovirus (n= 1)
- Screening of **stools** (9 cases)
  - Norovirus (n= 5)
  - Sapovirus (n= 2)
  - Chronic sheding in all

Enteric viruses found in 9/10 explored patients

- Screening of stools from **healthy transplanted SCID** (no hepatitis) (n= 14): no chronic shedding of enteric viruses

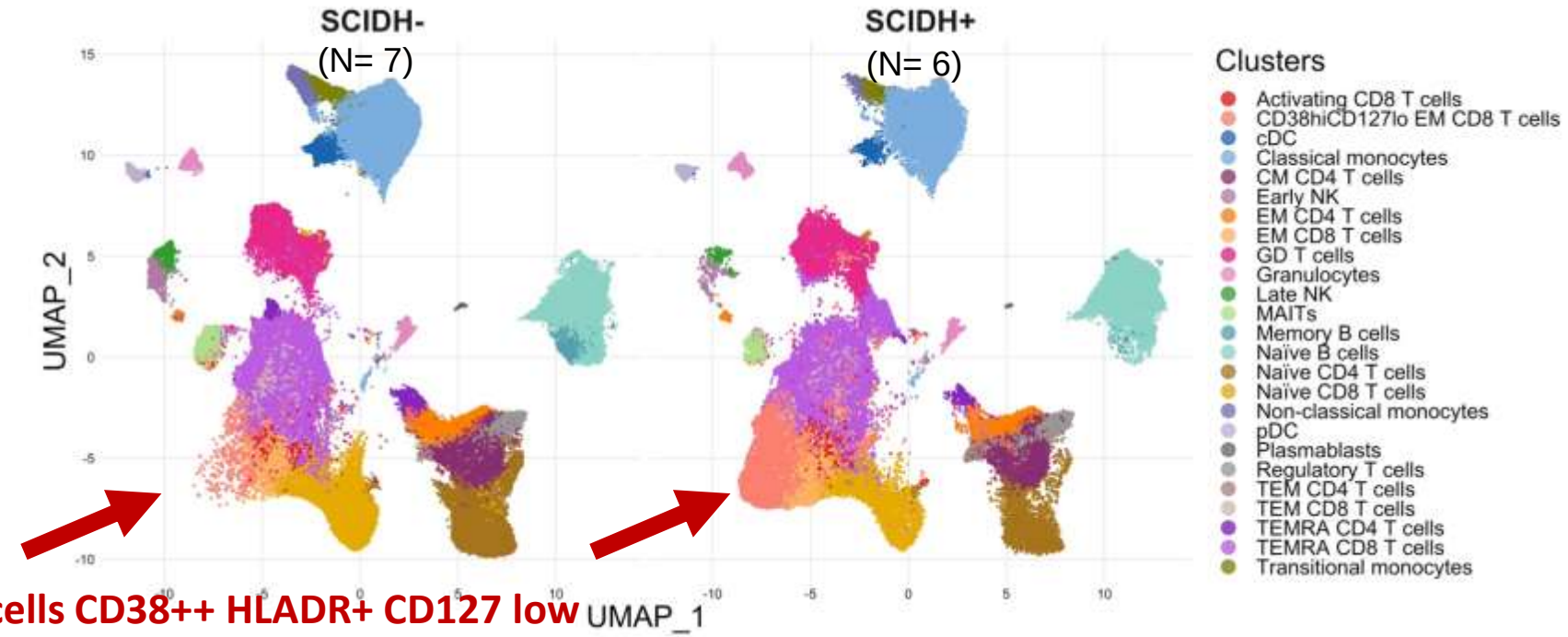
Chronic enteric viral infections in PID

- **Enterovirus and related virus** in agammaglobulinemia: chronic encephalitis

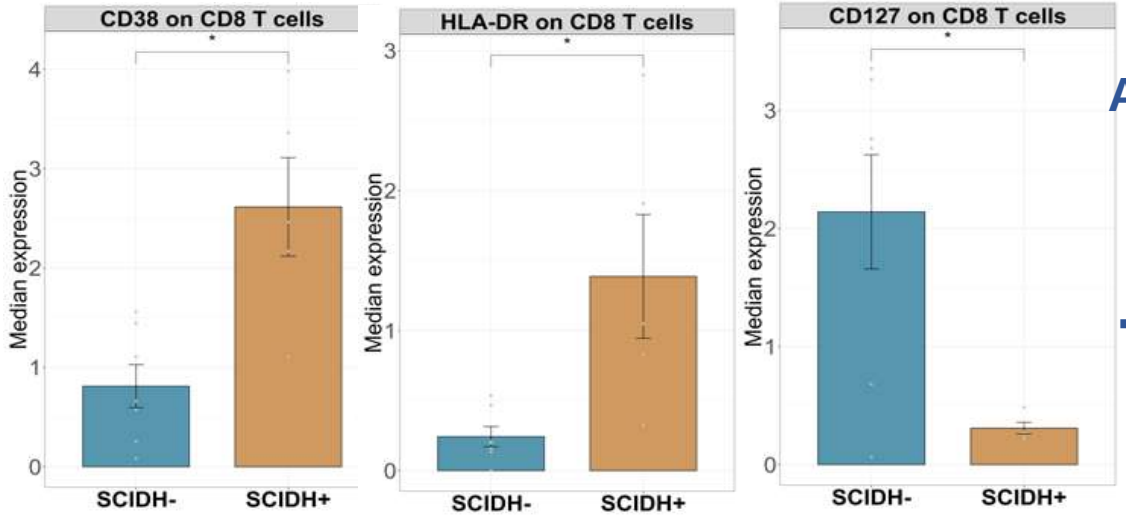
- **Norovirus**: chronic enteropathy in CVID (and XLA) , secondary immunodeficiency (transplantation)

- **Aichi virus (AiV-1)**: small RNA virus, genus *Kobuvirus*, family *Picornaviridae*
  - Immunocompetent: benign gastroenteritis
  - Highly prevalent in the general population
  - In B-cell deficiency: possibly emerging agent in patients with B-cell deficiency
    (*Bucciol et al JoCI 2018)

CyTOF : expansion of an activated CD8+ T cells cluster



EM CD8+ T cells CD38++ HLADR+ CD127 low



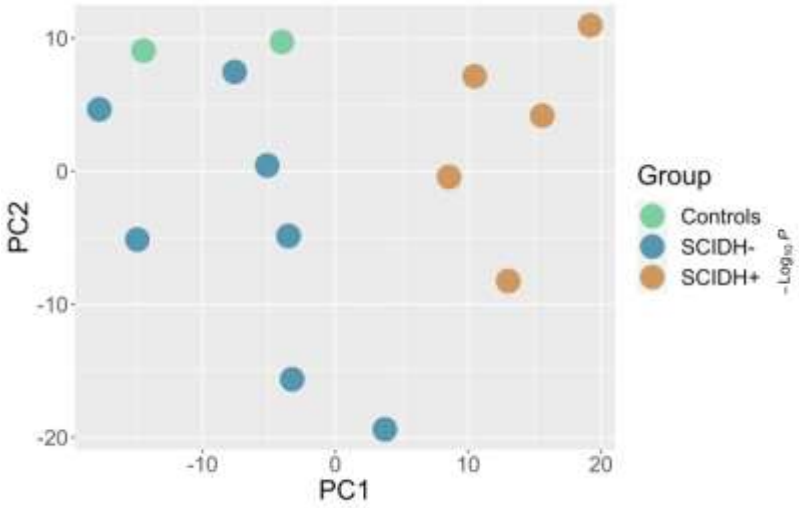
Among all CD8+ T cells, higher expression of CD38, HLA-DR and low expression of CD127

➔ Shift toward an activated phenotype of CD8+ T cells
➔ Low expression of CD127 <u>already reported in chronic viral infection</u> (HIV, HCV)

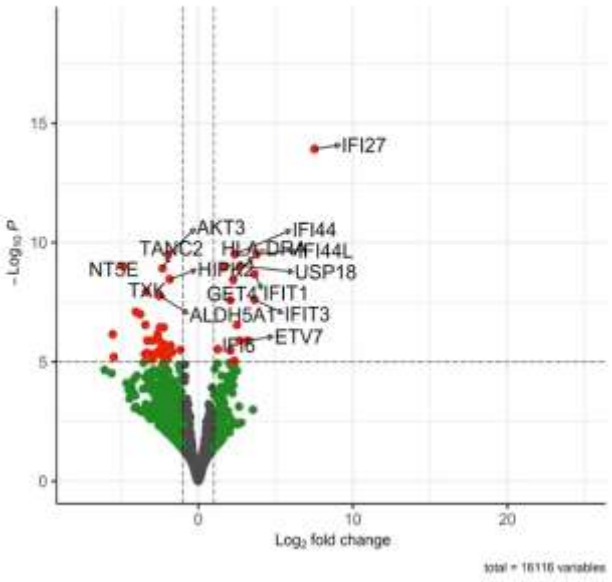
Riller et al, JACI 2023

Single Cell RNA Seq in SCIDH + (n= 6) and SCIDH- (n= 7)

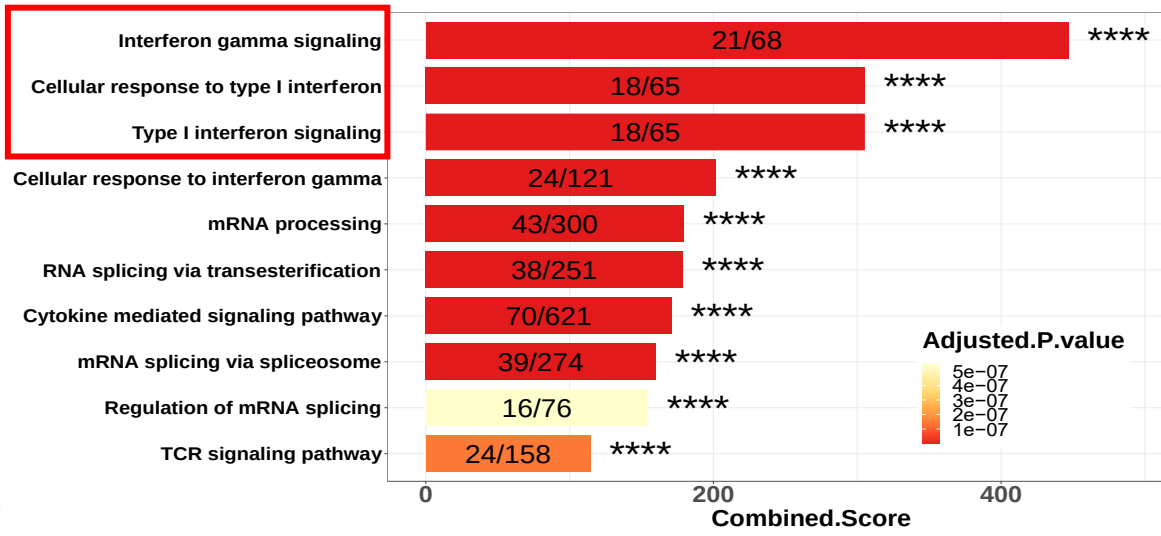
Pseudobulk analysis transcriptome (PCA)



Gene expression of CD8+ T cells highly different between SCIDH+ and SCIDH-



Most of the DEG are ISG



Interferon type I and type II signaling

EVAH as a new entity in IEIs

- **Identification of what we think is a new entity called enteric virus-associated hepatitis (EVAH)**

- **Consequence of defective antibody response against viruses**
  - Other predisposing factors related to quality of T cell reconstitution ?
  - Absence of CR predisposes to B cell defect, decline of thymopoiesis, absence of ILC
  - Absence of Peyer patches as additional factors ?

- **T-cell driven immunopathology (CD8+ T cells)**

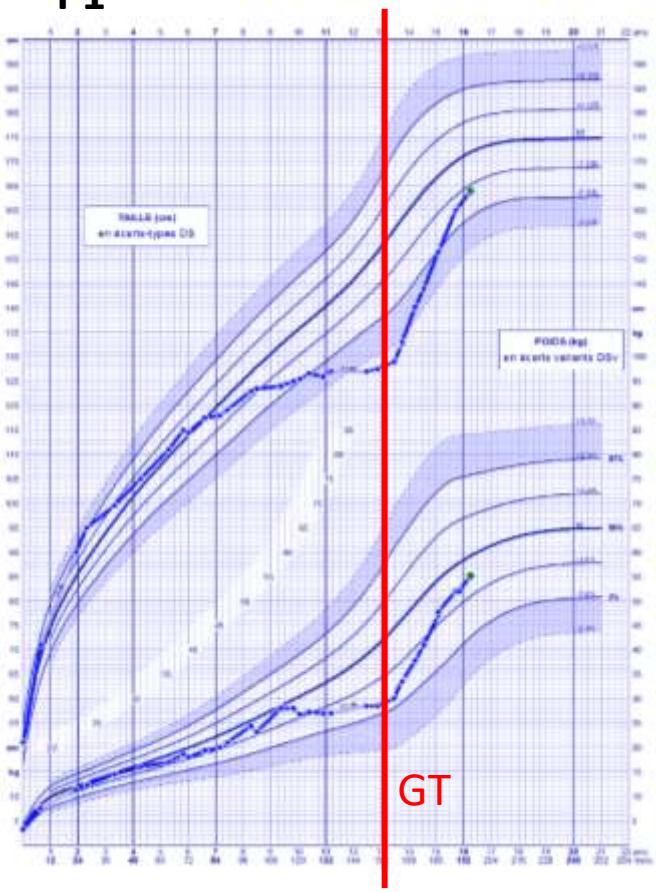
- **Therapeutic consequences (new HSCT or GT, new anti-viral treatment ?)**

Serious condition but Curable, reversible hepatitis

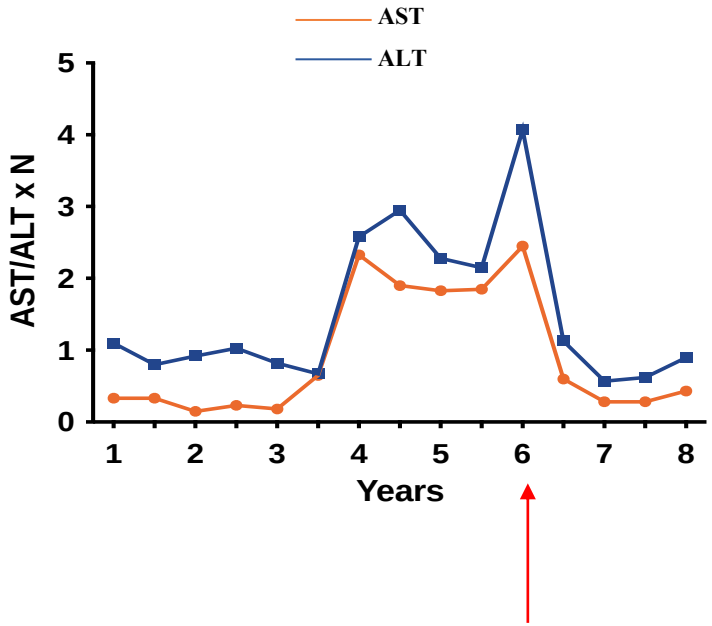
	Treatment	Regimen	Follow-up	Outcome	Liver enzymes
P1	GT*	Low Bu	5y	A.W.- off IRT	Normal
P2	GT*	Low Bu	3y	A.W.- off IRT	Fluctuating
P3	GT*	Low Bu	3y	A.W.- off IRT	Normal
P4	MUD HSCT	Bu 60-65	4y	A.W.- off IRT	Normal
P5°	No treatment			A.W.- off IRT	Spontaneous normalisation
P6	Geno HSCT	TFT	2.5y	A.W. - off IRT	Normal
P7				Progression up to death	
P8	Geno HSCT	No CR	10w	A.W. - off IRT	Normal
P9	None			Progression	Abnormal
P10	Death			Progression up to death	
P11	Death			Progression up to death	
P12	MUD HSCT	Bu 60-65	18m	A.W.- on IVIG	Normal

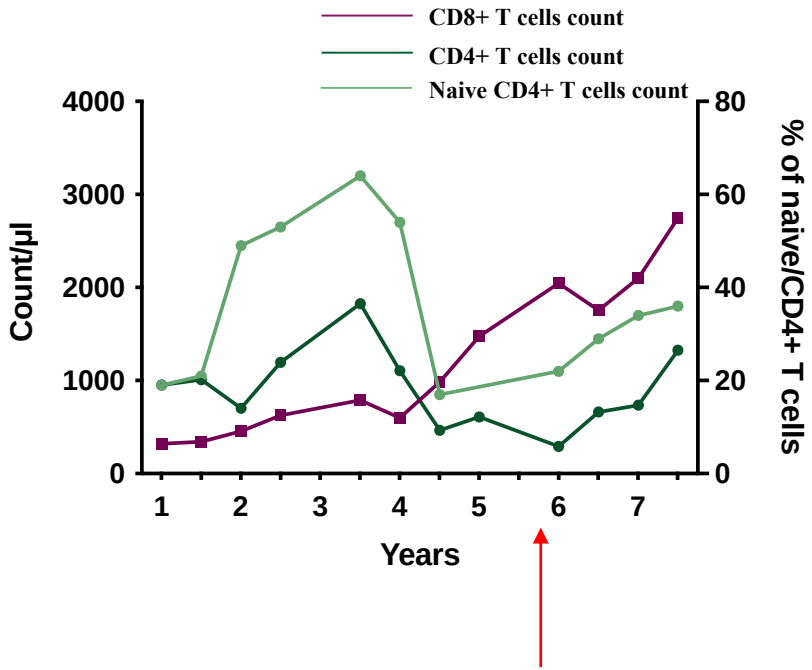
* NIH trial

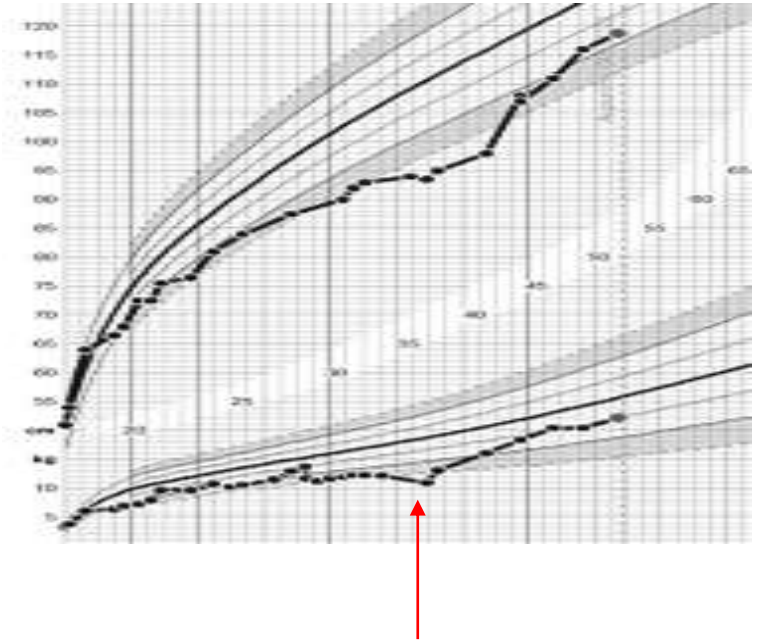
P1



One spontaneous remission







SPONTANEOUS CLEARANCE OF SAPOVIRUS (RED ARROW) ASSOCIATED TO CLINICAL IMPROVEMENT

Hepatopathy in AB deficiency: same entity?

- **5 to 15% of the patients with CVID, 10% in XLA,** sporadic cases in secondary AB deficiency

- chronic **transaminatis** or persistent anicteric **cholestasis**
- Lobular and sinusoidal infiltration, mainly **CD8+ T** cells, **possible granuloma**
- Nodular regenerative hyperplasia **(NRH)** – slowly progressive
- Frequent **splenomegaly and thrombopenia**
- **Chronic enteropathy and NRH** are **frequently associated in CVID**

- Suggestive of an undefined **immune response** in the tissues (autoI or anti-infectious?)
- Reports of recurrence of NRH post liver transplant in CVID
- No pathophysiology= **no specific treatment , poor outcome**

Cohort of patients (transplanted SCID, CID, AB deficiency)

EVAH+ (n= 52):

- chronic transaminitis > 6m or cholestasis > 6 months in the absence of autoantibodies, or classic hepatotropic virus infection
- And/or histologic features (lobular CD8+ T cell infiltration and/or NRH)

EVAH- (n= 32)

- None

- **multiplex PCR** (Norovirus, Rotavirus A, Sapovirus and Astrovirus) **on stools**
- **Simplex PCR (**Entero, Adeno, Aichi Virus, Parechovirus and Astrov MLB) in **stools, urines and plasma**
- **mNGS** in any **biopsies of inflamed organs**

EVAH+ and Virus (+) = 31°/52 (60 %)

- Transplanted SCID	11/16
- CID	14/28
- B-cell defect	3/4
- Acquired B-cell defect	3/4

° chronic Sheding unproved in 5

Aichi Virus n= 9
(in biopsies)

Stools

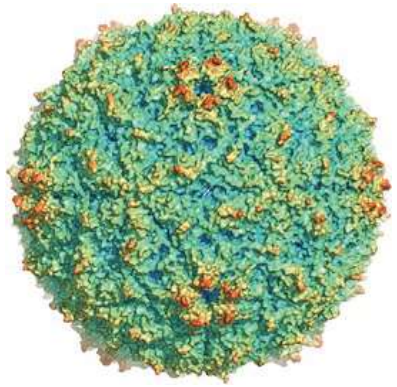
- **Norovirus = 13**
- **Sapovirus= 3**
- **Parechovirus =3**
- **Adenovirus = 2**
- **Kobuvirus= 1**

EVAH- and virus (+) = 2*/32 (6%)

- Transplanted SCID	0/14
- CID	2/9
- B-cell defect	0/7
- Acquired B-cell defect	0/2

* transient sheding

Aichi Virus



- Family of **Picornavirus** (Genus Kobuvirus)
- Small **non enveloped** simple- strained **RNA virus**
- First described in Japan in 1989
- **Mild gastroenteritis** in immuno-competent humans
- **High seroprevalence**: 60% in children of 5y, > 90% in adults

- **9 patients** with Chronic infection (8M/1F)

- **SCID** after HSCT on IRT (n= 3)
- **XLA** (n= 3)
- **CID** (ICF and GINS1)(n= 2)
- **CVID** (n=1)*

- Age at **Onset:** median **9 y** (5 – 40)
- Age at **diagnosis: 16 y** (9- 53)

- Chronic **hepatitis**: **9/9**

- **Renal** abnormalities: **6/9**

(Nephromegaly n= 4, renal insufficiency n= 3)

- **Splenomegaly**: **8/8**
- **Growth** failure: **5/9**
- **Thrombopenia**: **8/9**

*Courtesy Leif Hanitsch, Charité Universitaetsmedizin Berlin, Germany

Conjonctivites purulentes (*Haemophilus influenzae* ++)

Rhinosinusite purulente chronique

Quelques épisodes de surinfection broncho-pulmonaire

AICHI virus + dans les urines

Cytolyse hépatique
1.5-2N

PBH*

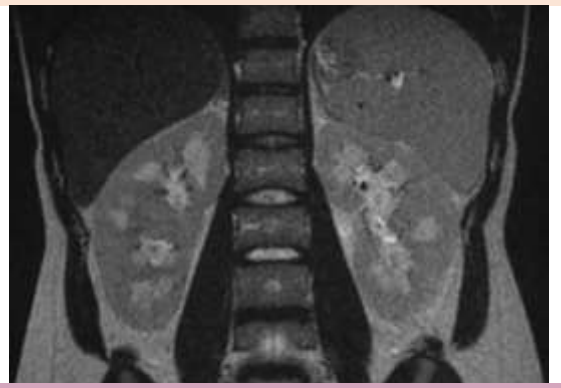
Hépatomégalie + HTP

2001 | 2012-10 ans | 2014 12 ans | 2018 16 ans | 2020 18 ans | 2021

XLA: Diagnostic anténatal-substitution en IG à M3

Altération progressive de la **fonction rénale**
Néphromégalie

DFG 66 DFG 48 DFG 43



*infiltrat intra-sinusoïdal lymphocytaire CD8+ - granulome portal - HNR

Néphromégalie bilatérale - hyperéchogénicité médullaire

P9*

2018 (30 ans)

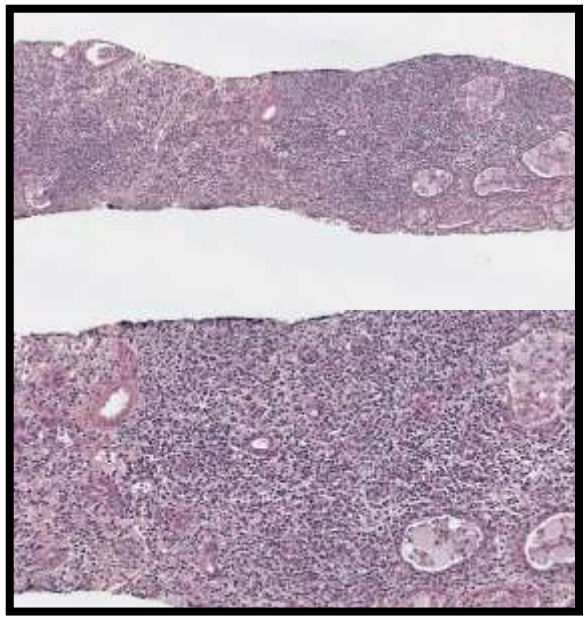
2024 (36 ans)

RAS
Diagnostic tardif a adolescence
Substitution en IG regulière

Gros reins (160 et 165 cm)

Explo hépatique (2021)

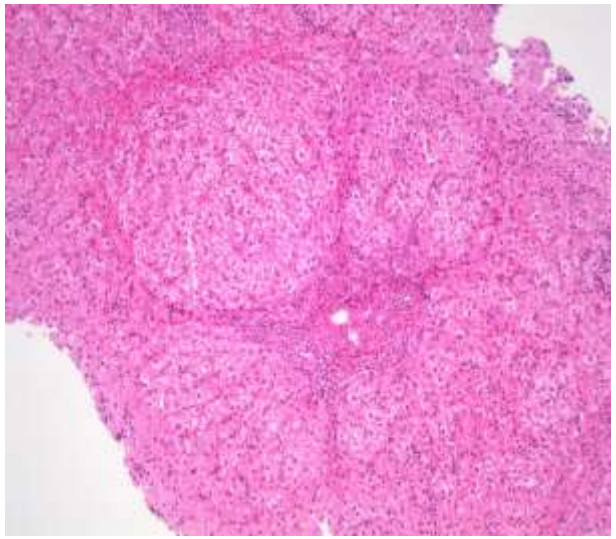
- Fibroscan: normal
- PBH: architecture NLE
- Lympoprolif. Sinusoidale
- Pas d'HNR

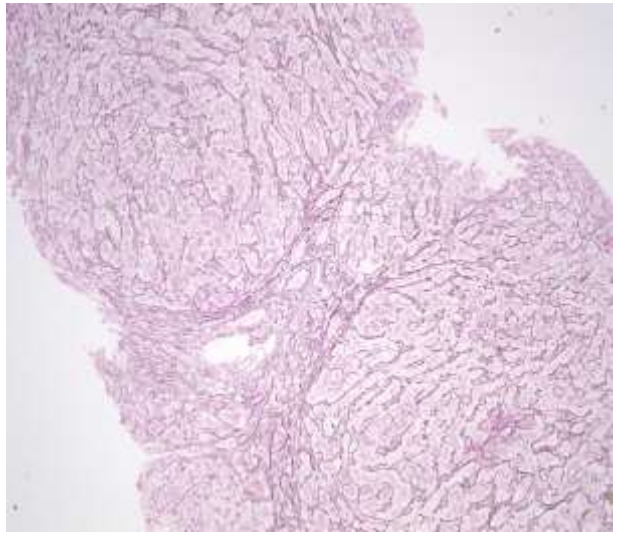


RT-PCR Aichi pos sur la biopsie
Neg plasma et urines

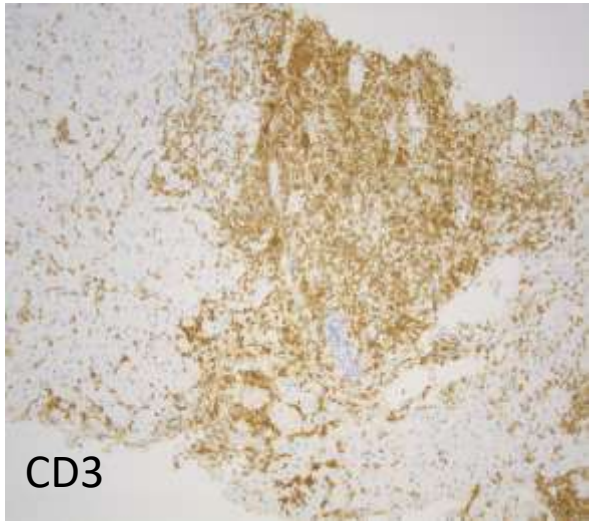
* Khalil EL KAROUI
David BUOD
David BOUTBOUL

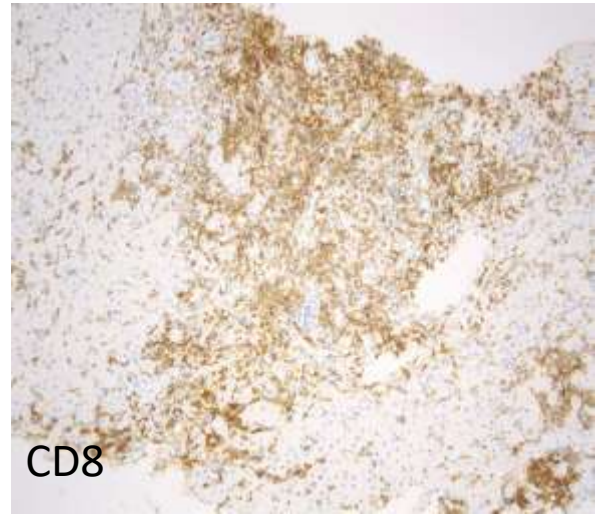
Liver histopathology and diagnostic tools





Nodular Regenerative Hyperpalsia: 8/9





Intense CD3-CD8 lobular and portal infiltration
Oligoclonality

- **Gold standard**: RT-PCR or mNGS on **FROZEN biopsies** of infiltrated organs
- But **low viral loads** (CT range: 32.9 to 42.2)

- RT-PCR **Stools**: only once positive
- RT-PCR on **Plasma and urin :** 50% positive : repeat samples !

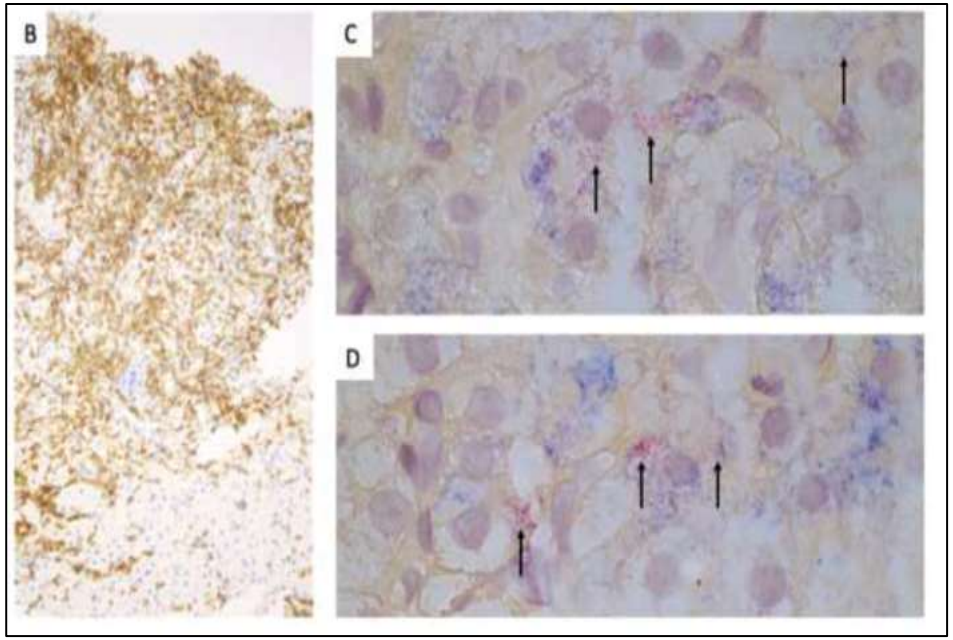
Could Aichi Virus be causative ?

1. the virus is found in affected patients but not in control PIDs

2. Virus is identified in the affected organs
   by *In situ* hybridisation
   (done in 2 patients)

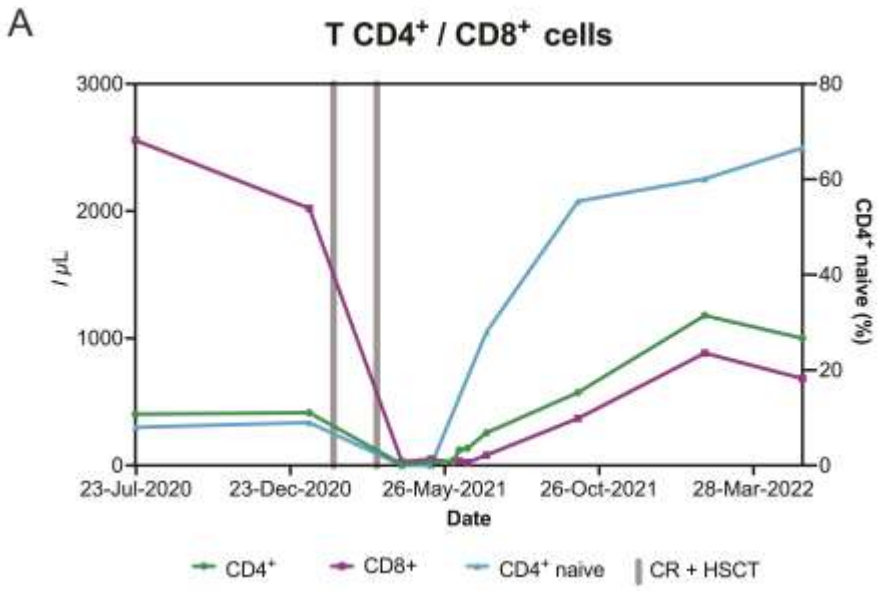
3. Clearance of the virus cure the Disease

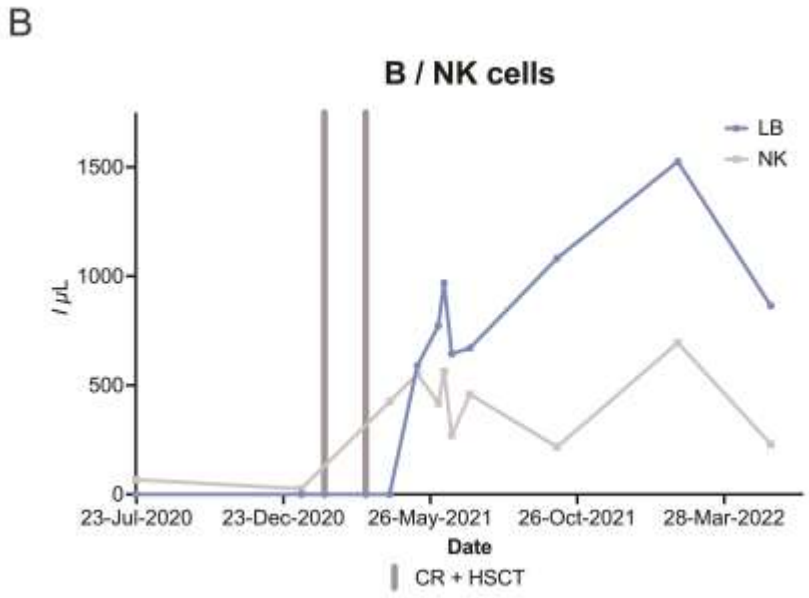
ISH: Aichi signals in infected hepatocytes

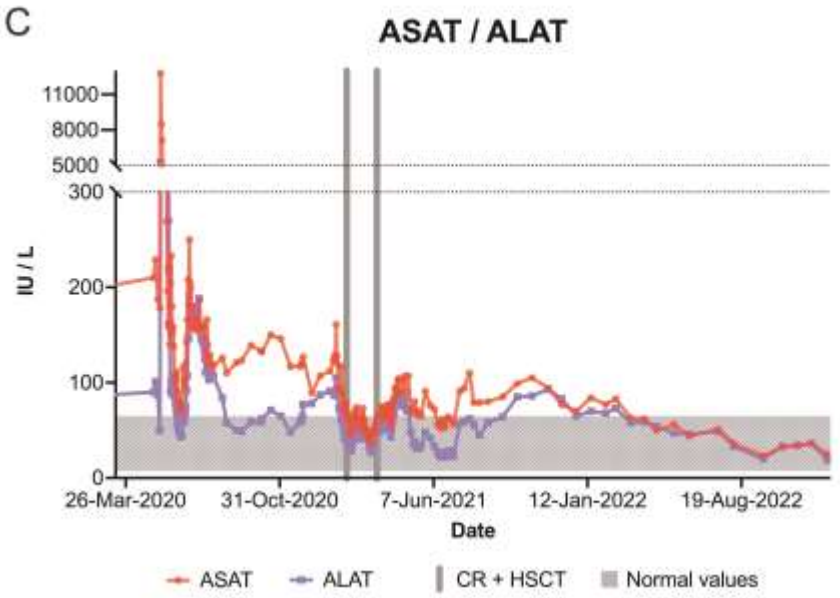


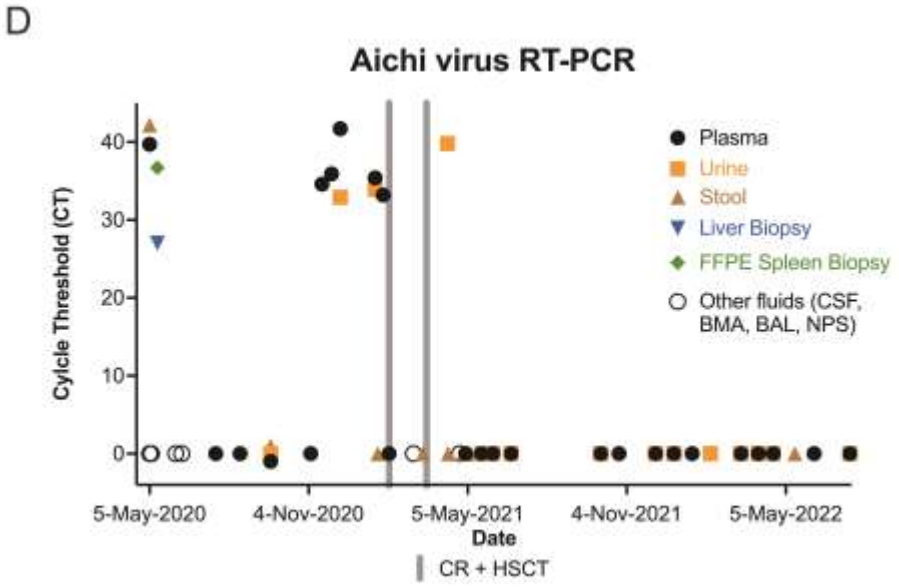
Fourgeaud et al, CID 2023

HSCT(x2) in one XLA patient with Aichi virus infection









HSCT n°1: *Haploid. PBSC*

HSCT n°2: Haploid PBSC

2 years FU

Rapid AiV clearance:

- **26 samples until M15**
- Ø hepatitis from M6

Full donor chimerism

T cell reconstitution from M3

B cell reconstitution:

- IgA/IgM from M2
- **Vaccinations at M8**

AICHI virus multisystemic infection in B-cell deficiency
4 cases in the literature

	Bucciol et al JoCI 2018	Meyts et al JoCI 2022	Meyts et al JoCI 2022	Bekassy et al, JoCI 2022
Disease	XLA	Acquired B cell def	Acquired B cell def	XLA
Age of onset/diag	6 /13	/13	/6	/13
symptoms	Fever, diarrhea, lymphadenopathy epilepsia	HSM	SM	Fever, weight loss, skin rash
Biological findings	Leucopenia, hepatitis, renal failure	hepatitis	hepatitis	Renal failure
Radiologic findings	Nephromegaly	Nephromegaly	Nephromegaly	Nephromegaly
AiV diagnosis	Kidney, liver, spleen, urin, sputum, CSF	Liver, spleen, stools	stools	skin
treatement	HSCT		B cell restauration	HSCT

Conclusions and perspectives

- **EVAH is a new condition** occuring **in B cell deficient patients** (Participation of T cell ID ?)
- characterised **by a T-cell driven immunopathology r**elated **to chronic enteric virus infection**
- **May explain NRH in CVID and** sporadic cases in secondary AB deficiency
- **Aichi Virus**: emerging pathogen in B-cell deficient patients that can cause **tubulointerstitial nephritis** and **nephromegaly**

- **More work has to be done to assess the causality of chronic enteric virus shedding**
  - To characterize epidemiology of enteric viruses among B-cell deficient patients and the link with EVAH
  - to assess causality between viruses detection and pathological findings (including anti-viral CD8+ T cell specificity)
  - To better study the characteristics of the pathological CD8 T cell populations in tissues (gut and liver)

- Understanding pathophysiology of hepatopathy associated to B cell deficiency could pave the way **to new treatments**

Acknowledgements



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- **Muriel Schmutz**
- **Victor Michel**
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