Targeted Next Generation Sequencing as a first line strategy for PID/IEI diagnosis : the biologist's experience

Mathieu Fusaro

Former clinical biologist at « Centre d'étude des déficits immunitaires » (CEDI)

Necker Enfants Malades Hospital (Pr Capucine Picard)

PhD Student at INFINITY (Toulouse)

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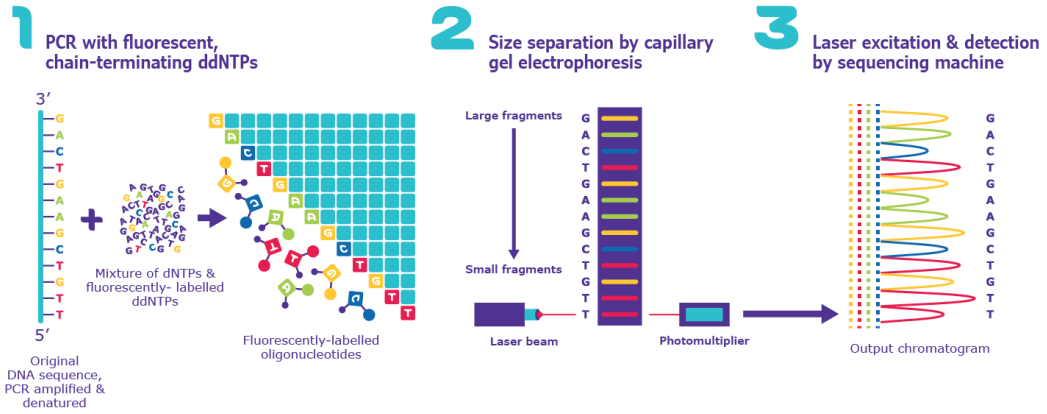
From Sanger to NGS panel for index cases

Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee

Tangye et al. J Clin Immunol. 2020 Jan;40(1):24-64

>450 Inborn Errors of Immunity !
Clinical heterogeneity



Sanger sequencing

Hypothesis-based diagnosis
Cumbersome and expensive for multiple gene testing
No detection of heterozygous CNV

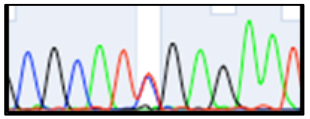
Custom NGS panel was designed in 2015

Capture of exonic regions in 300 genes for which mutations can cause an Inborn Error of Immunity (IEI)

Timeline of genetic strategies in IEI

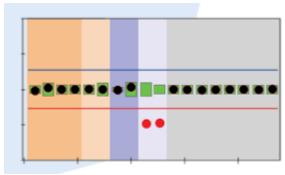
2010 → 2022

Sanger sequencing



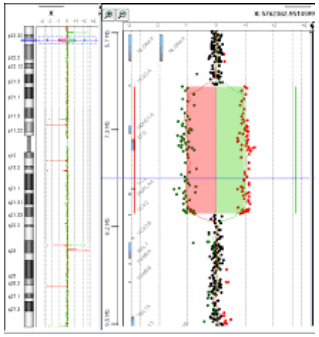
SNV / small indel

MLPA



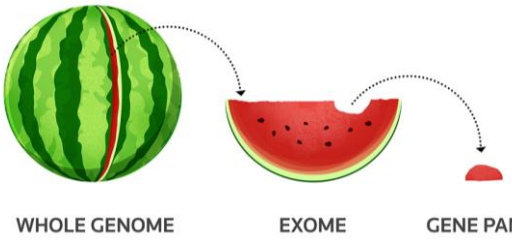
Exon-level CNV

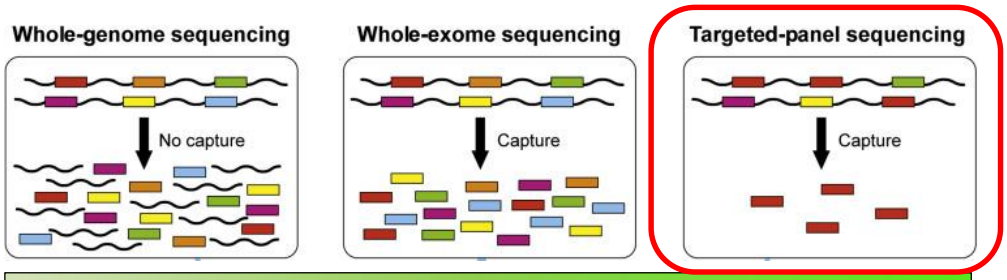
CGH array



Large CNV

Shot-gun sequencing (150bp)





Cost

Complexity of data analysis and storage

SNV – small indels

Mosaïcism (coverage-dependant)

Exon-level CNV

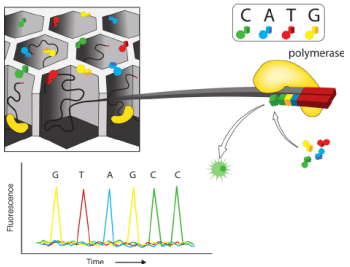
Structurals variations (without exon-CNV)

Repetitive DNA sequence

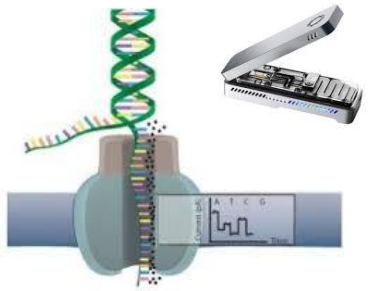
High-homology, pseudogene

Long-read sequencing (10-15kb)

Single-molecule real-time sequencing (SMRT) - Pacbio



Oxford Nanopore Technologies



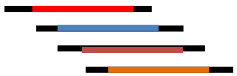
Technical overview

Extraction from whole blood EDTA

Genomic DNA

Enzymatic fragmentation

CAPTURE of regions of interest (custom RNA bait)

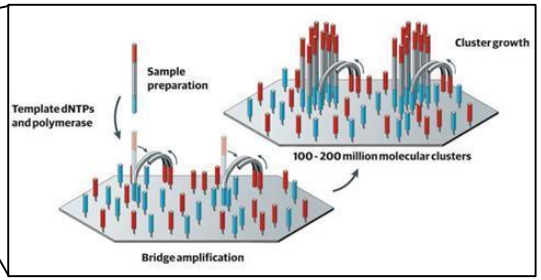


Library

Adaptors, identification sequences

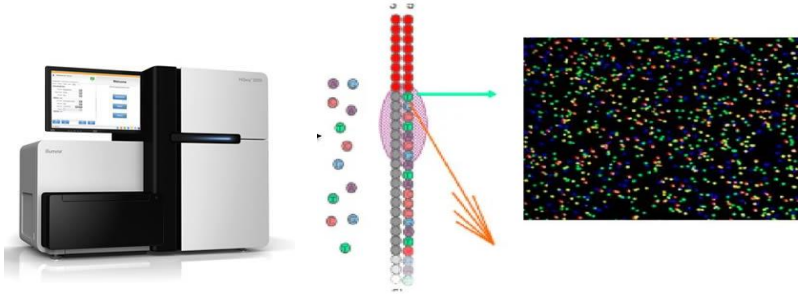
Pool of 8 to 72 patients

Flowcell



Bridge PCR for cluster formation (clonal DNA)

Marion & Nathalie
The pink ladies



Illumina sequencing (Necker Genetic platform)

Bioinformatic analysis

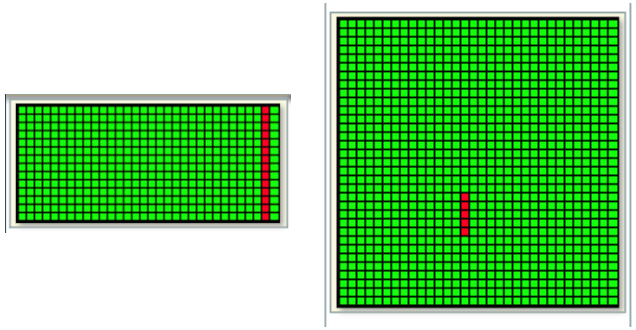
Biological interpretation

Coverage analysis to diagnose Copy Number Variation

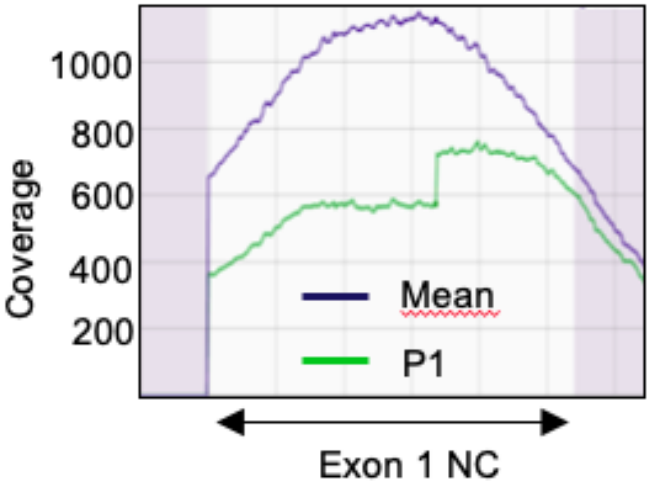
Double normalization of read count (RC) to account for coverage variability between genomic regions and between patients

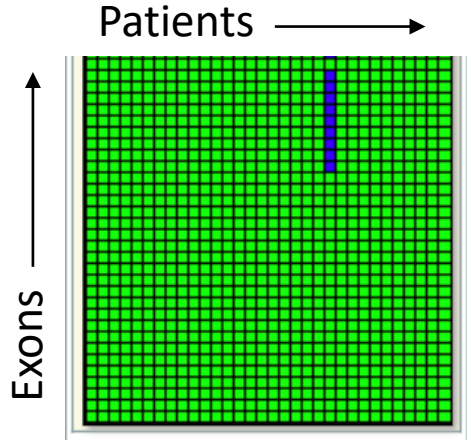
Dup/del score = $\dfrac{(RC^{probe}/RC^{all})_{patient}}{(RC^{probe}/RC^{all})_{mean}} \simeq 1$

RC : read count = coverage



Score < 0,7 => deletion ?





Score > 1,3 => duplication ?

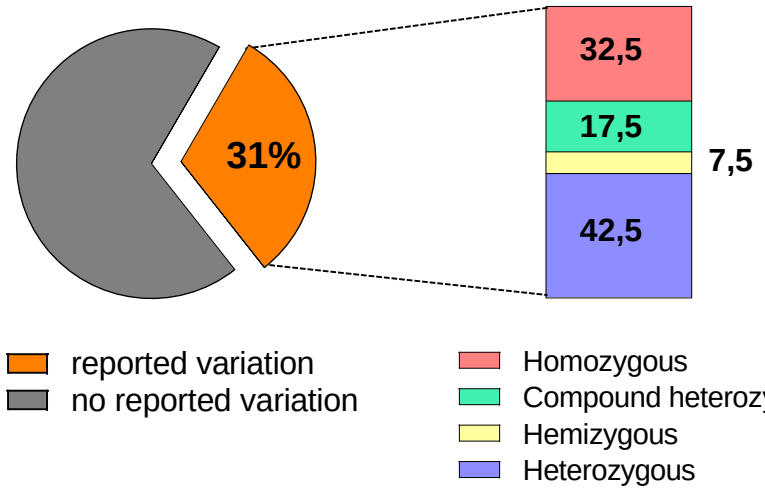
Some statistics on the first 129 PID cohort (2016-2018)

300 genes
4136 regions of interest
1.095 Mbp = 0.034% genome = 3.65% exome

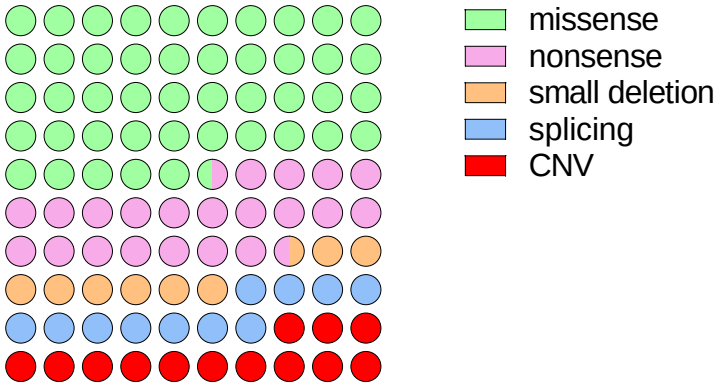
129 patients included in the validation cohort

Mean coverage 539X
>30X : 98.91%

Yield & Transmission



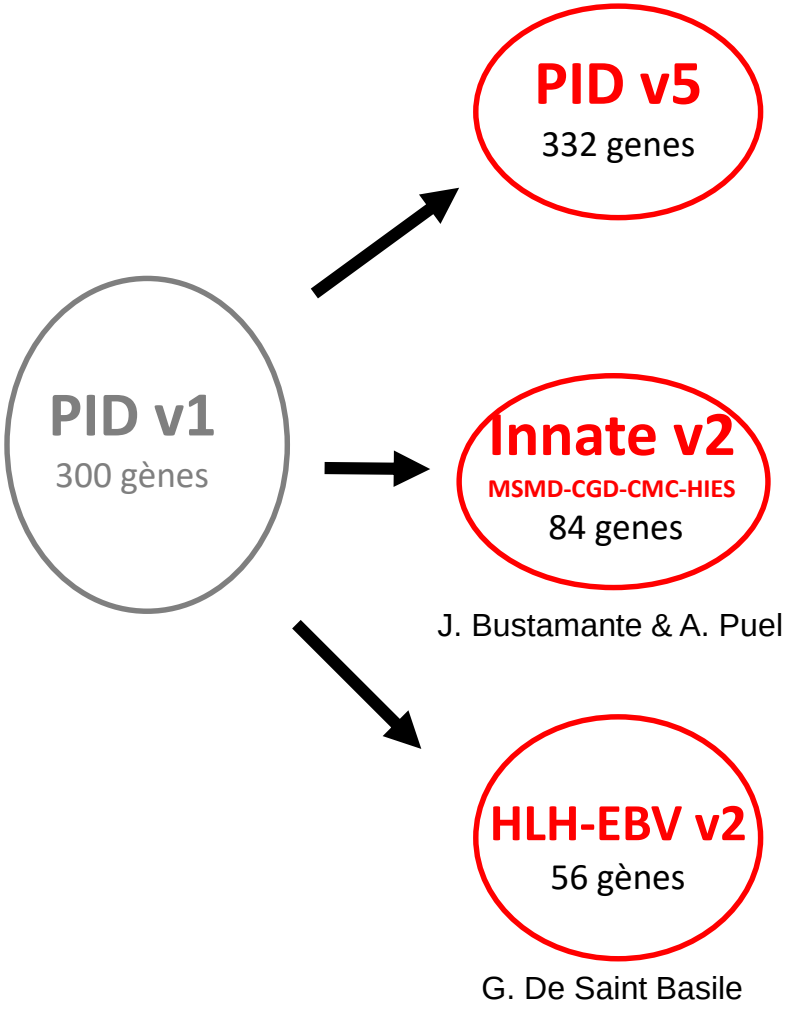
Type of variations

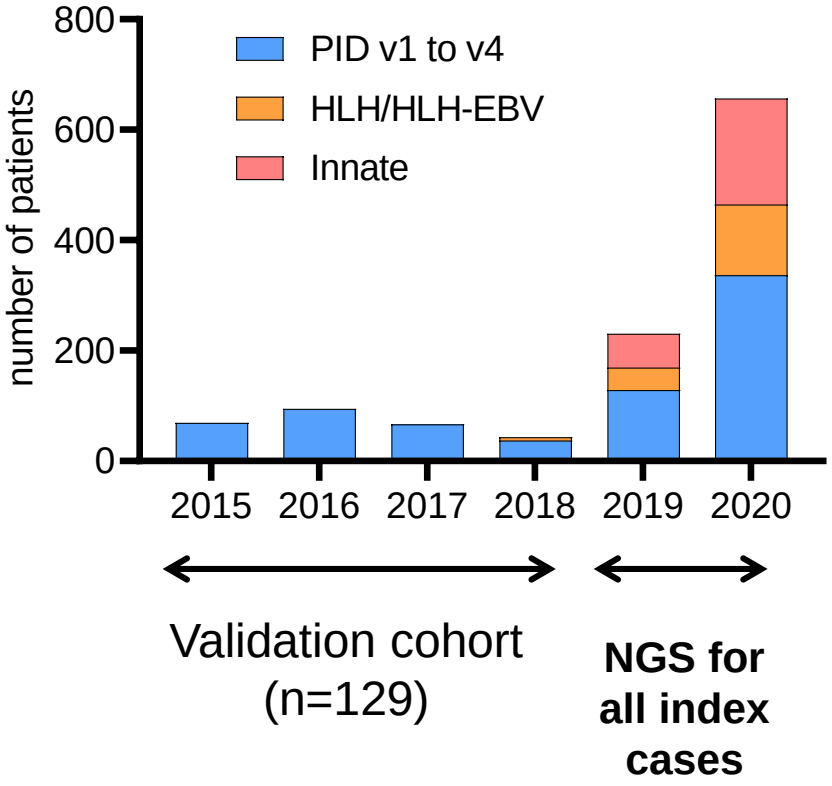


CNV : 13% of the variants
=> increase yield by 4,6%

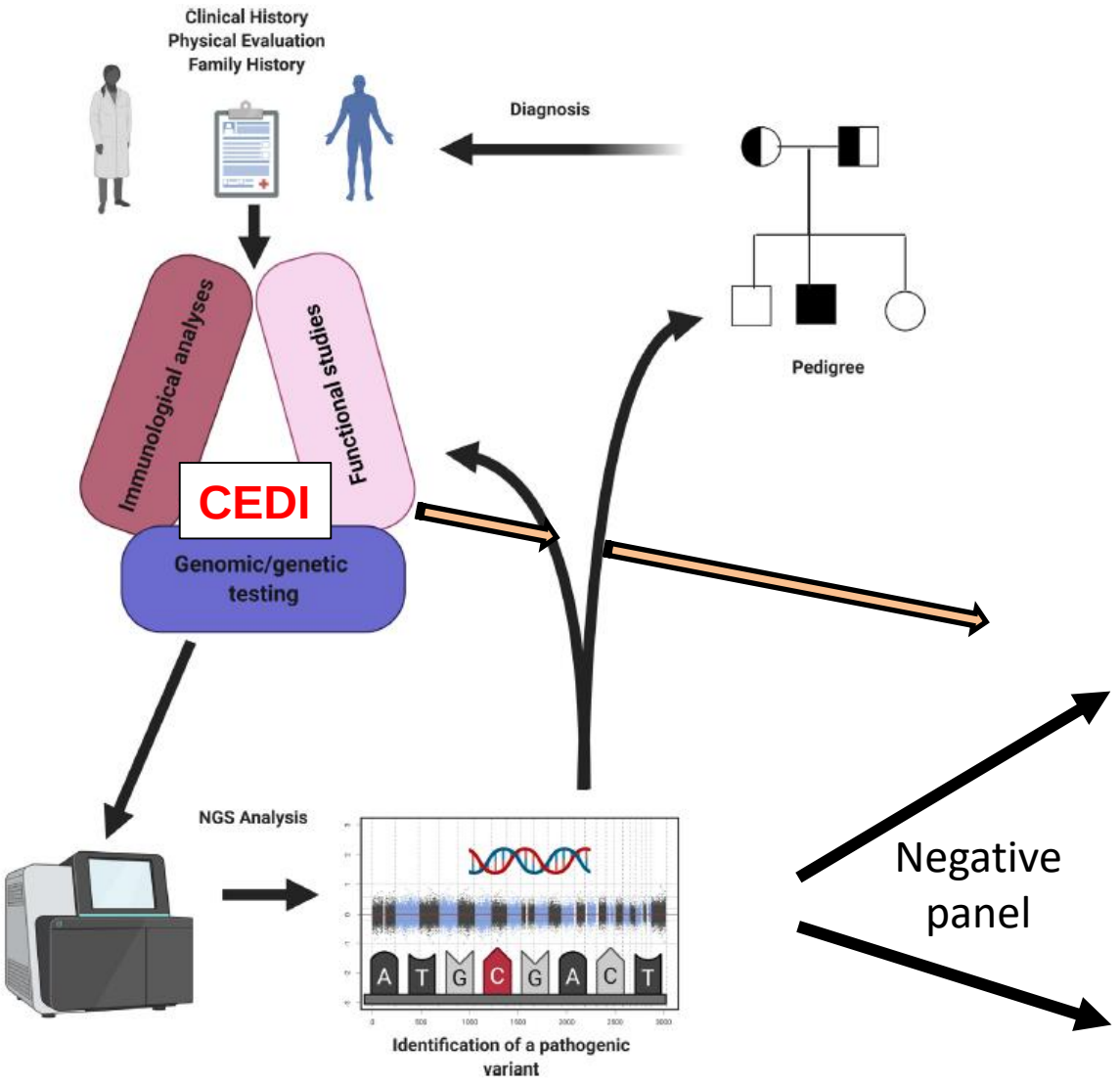
Evolution of the diagnostic strategy

1158 patients studied by tNGS from 2015 to 2020





Integration of NGS panels in the diagnostic workflow



WES

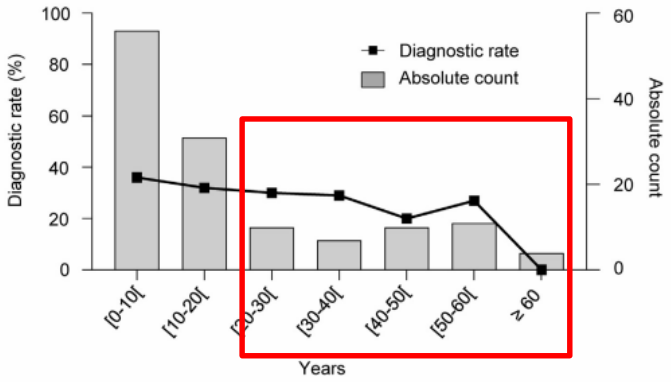
imagine
INSTITUT DES MALADIES GÉNÉTIQUES

WGS

FRANCE MÉDECINE GÉNOMIQUE 2025
aviesan

Adapted from Lee et al, Human Immunology 2021

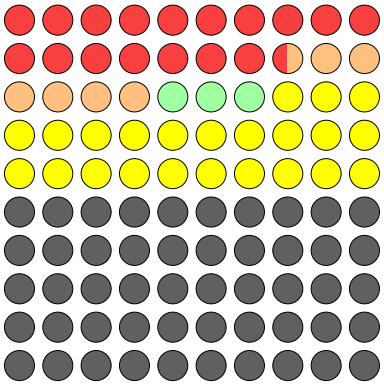
What is the efficiency for adult patients ?



Primary analysis on the PID cohort
Maybe not so bad?

On 46 adult patients, 57% with childhood onset

156 adult patients
Retrospective analysis 2019/2020

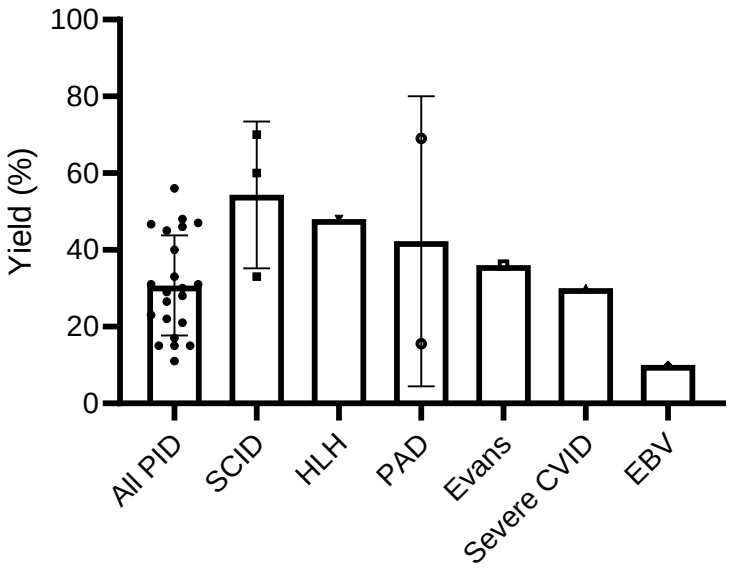


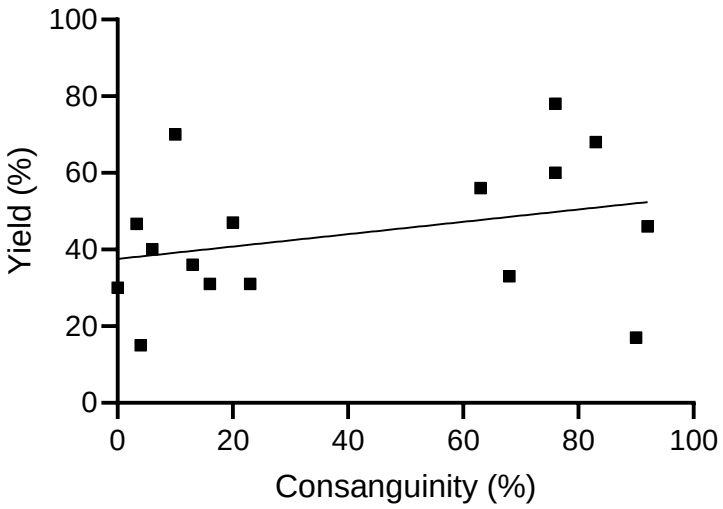
(Likely) Pathogenic **17,5%**
TACI variant **6,5%**
Variant to be further validated
VUS
No variant of interest

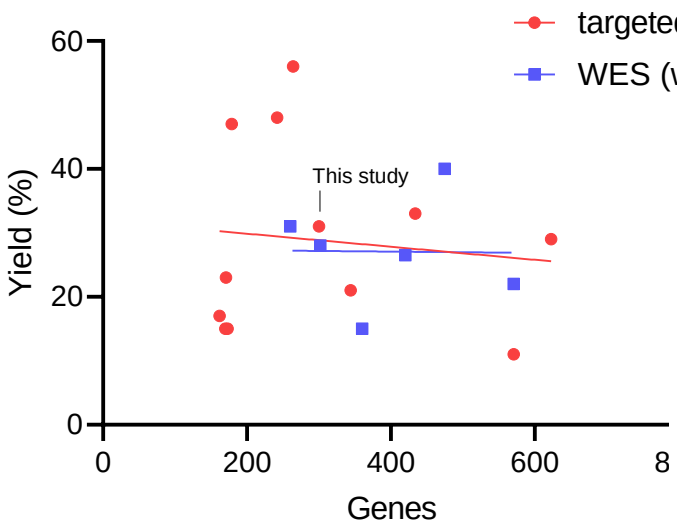
The usual suspects ?

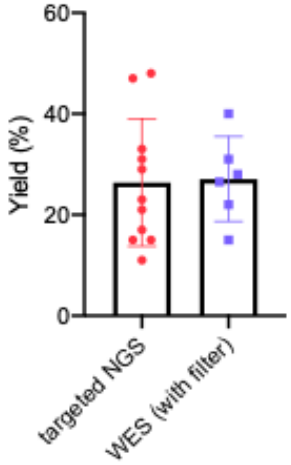
ADA2
BTK
BTK
BTK
CTLA4
CTLA4
CTLA4
CTLA4
ICOS
IKZF1
IKZF1
IRF3
LIG4
NFKB1
NFKB1
NFKB1
NFKB2
NFKB2
NLRC4
RLTPR
RMRP
STAT1
STAT1
STAT3
STAT3
STXBP2
TBX1

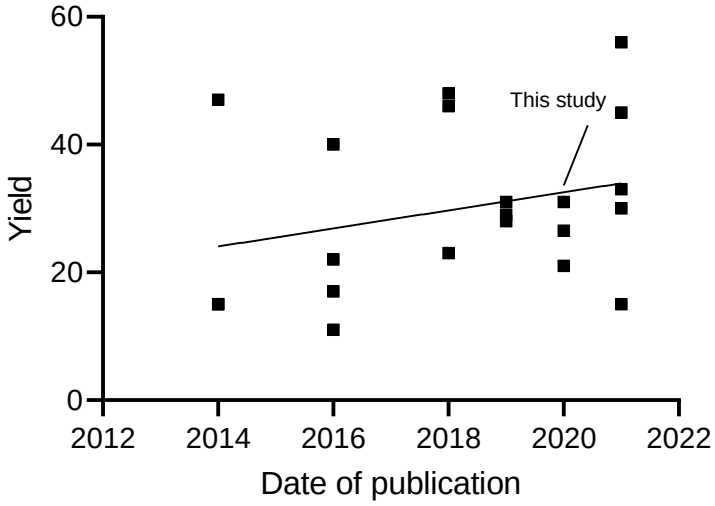
Overview of the litterature (n=30 publications)









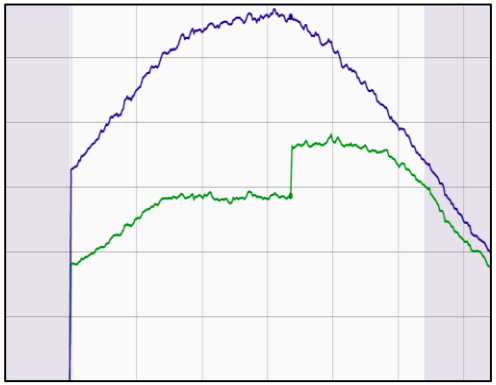


Quick focus on 2 complex cases resolved by tNGS :

1. A copy number variation in a non-coding region leading to haploinsufficiency

2. A patient partially cured before getting sick

A copy number variation in a non-coding region leading to haploinsuffisiency



True deletion of non-coding exon 1 of NFKB1 or false positive?

Patient from A. Maria (Montpellier)

48-years-old man

Since childhood :

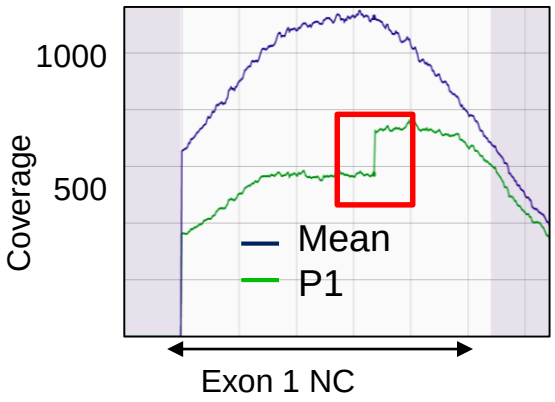
- Sino-pulmonary infections
- Hypogammaglobulinemia (supplemented)

CVID complications : chronic diarrhea with colitis, ITP, NRH with portal hypentension

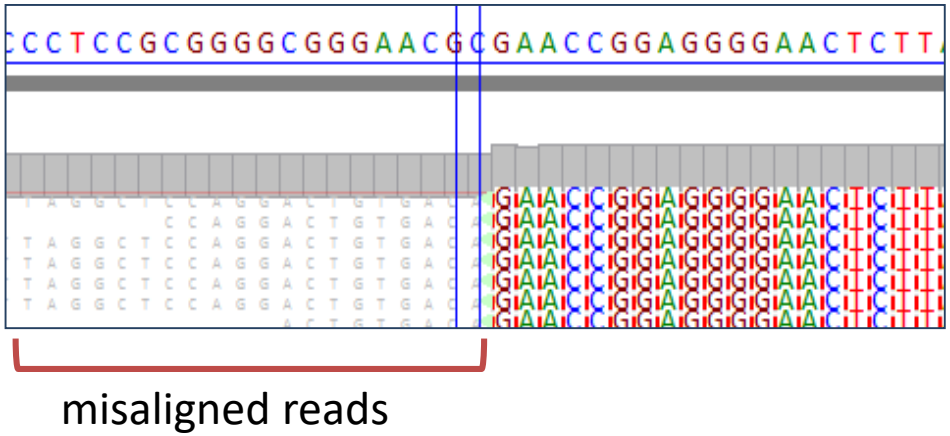
DLBCL EBV+ in 2019

Dup/del score for *NFKB1*

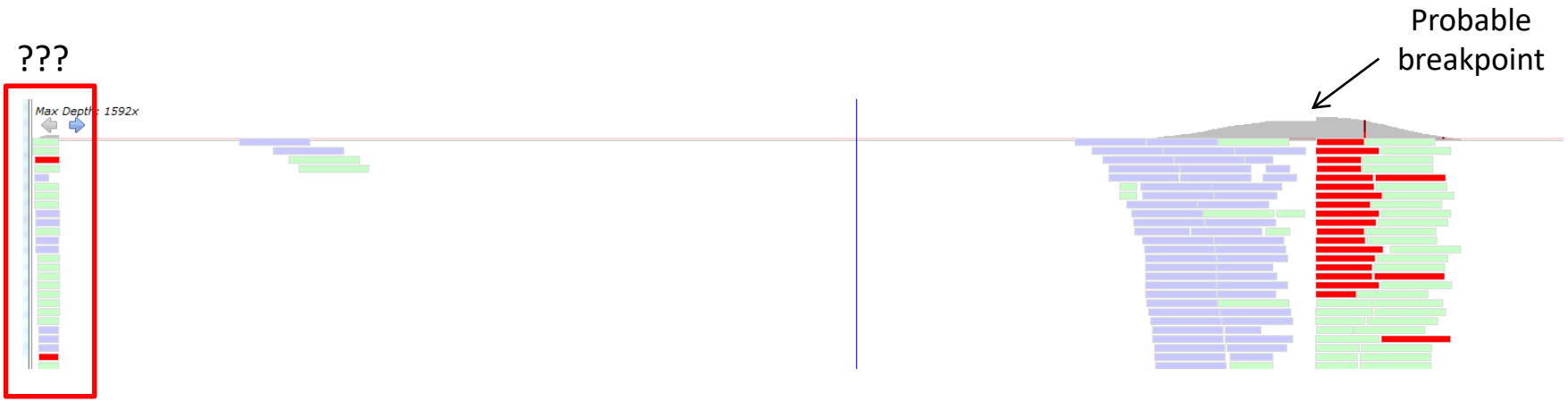
Exon 1NC	0.97	1	0.98	0.62	1.07	1.04	0.97
	1.05	0.93	1.02	0.97	1.03	1.02	0.94
	1.05	0.96	1.06	0.97	1.06	1.03	0.93
	1.05	0.96	1.04	0.94	1.02	0.98	0.93
	1.02	0.9	0.99	1	1.03	1.03	1.02
	1.02	1.06	1.01	0.94	1.03	0.97	0.95
	1.03	1	1.01	1.05	1	1.01	0.96
	0.97	1.02	0.98	0.99	1.04	0.98	1
	1.02	1.05	0.97	0.96	1.06	0.99	1.01



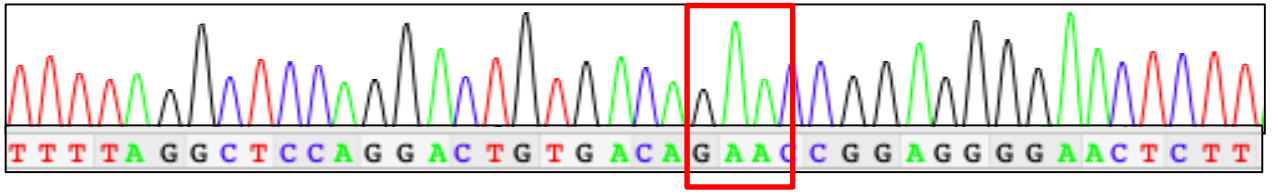
Zoom on coverage drop



Confirmation of the 2,7kb deletion



Breakpoint PCR and sequencing :



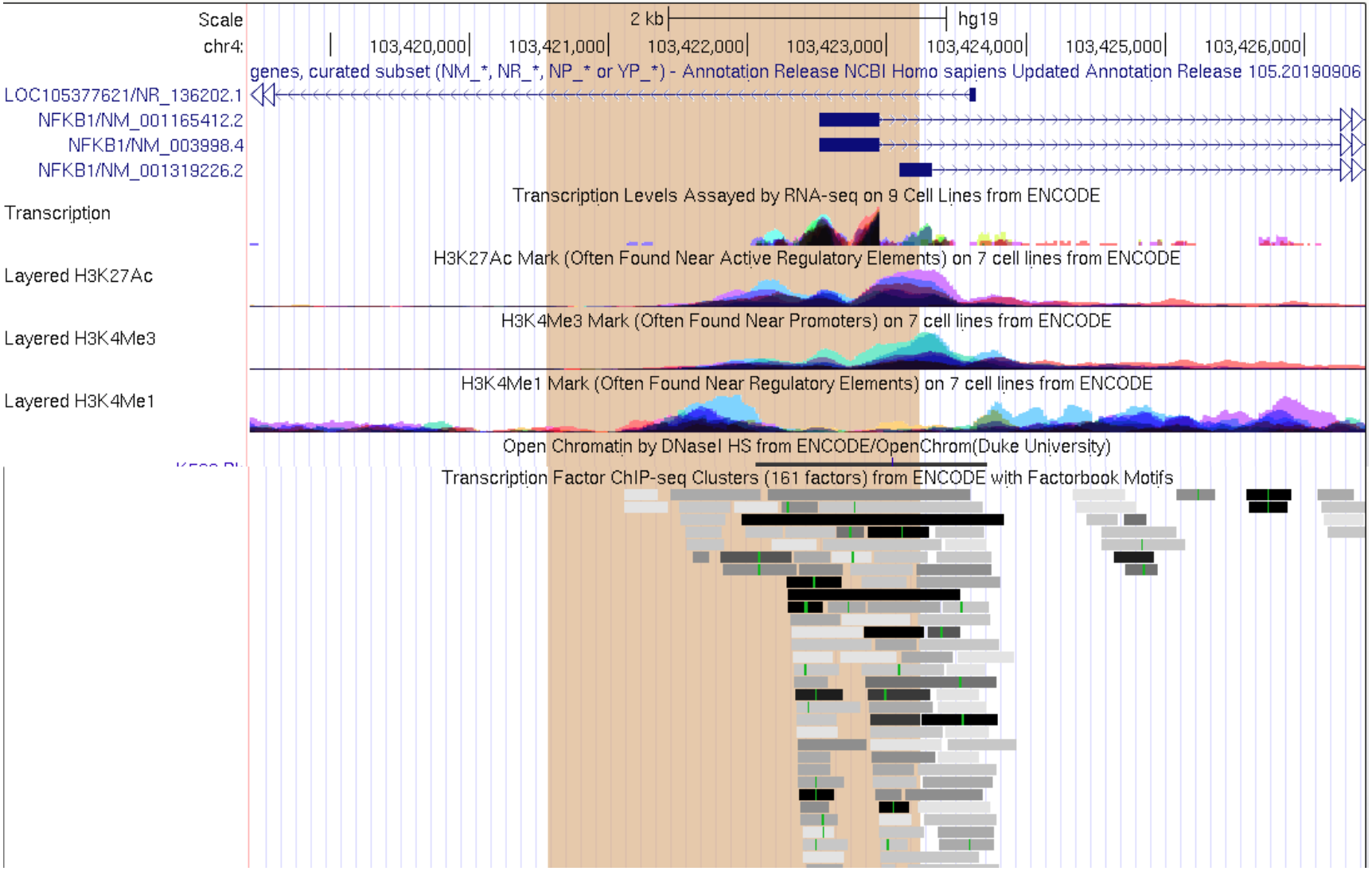
5'UTR TTTTAGGCTCCAGGACTGTGACA**GAA**TAAATTCCCGTTGTAAG

Exon 1NC TCCCTCCGCGGGGCGGGAACGC**GAA**CCGGAGGGGAACTCTT

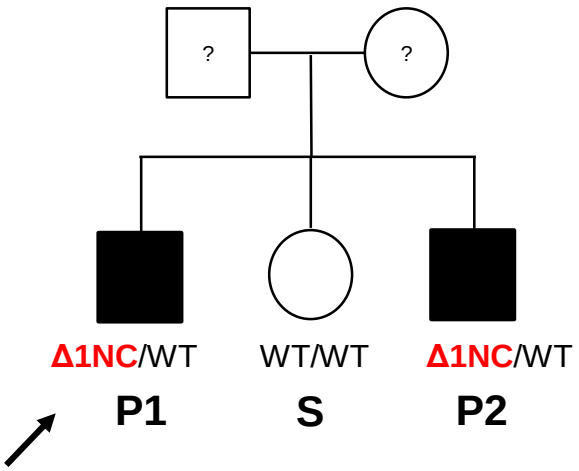
Deletion TTTTAGGCTCCAGGACTGTGACA**GAA**CCGGAGGGGAACTCTT

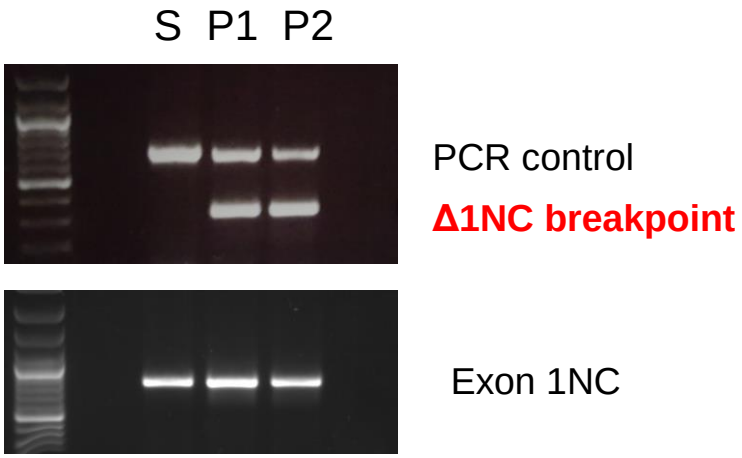
Microhomology junction

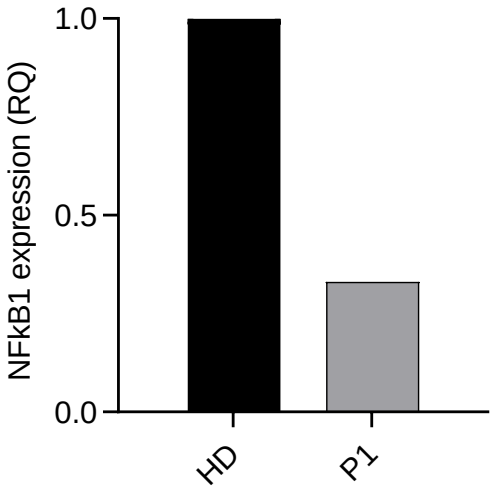
The deletion encompasses promoter and enhancers of *NFKB1*

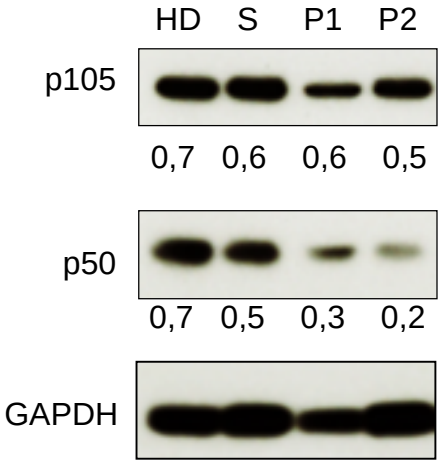


Genetic and functional confirmation



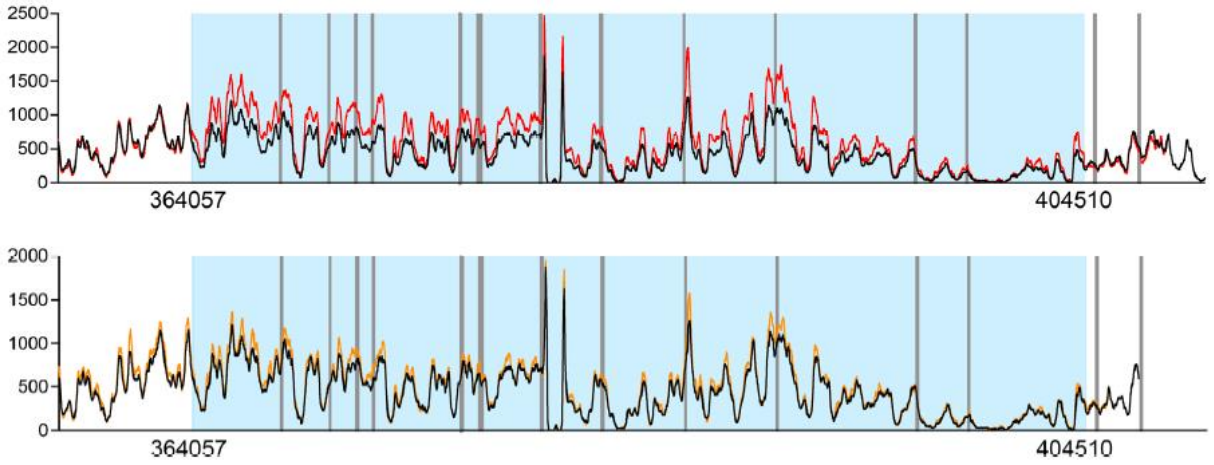






PCR by Marion Heller & qPCR by Laura Barnabei

A patient partially cured before getting sick

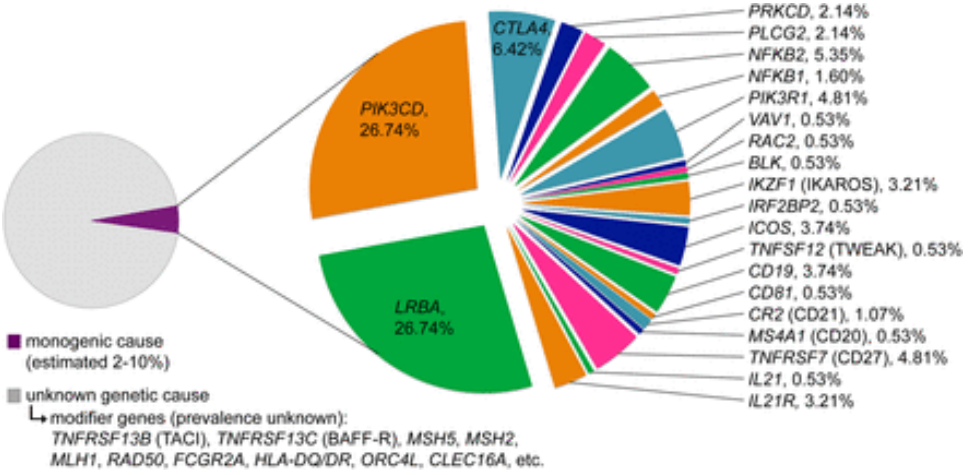


DICK-like phenotype : good candidate for a wide NGS panel ?

Patient from M. Malphettes :
45-years-old man

➢ **Clinical presentation :**
Sino-pulmonary infections since 5y
Moderate/severe psoriasis since 16y
Bilateral bronchiestasie at 37y

➢ **Biological explorations :**
IgGAM : normal
Chronic EBV viremia : low to moderate
Post-vaccinal serology :
- Tetanos : normal
- Pneumococcus : negative



From Bogaert DJA, et al. J Med Genet 2016

Genetic Diagnosis Using Whole Exome Sequencing in Common Variable Immunodeficiency

*Patrick Maffucci[1,21], Charles A. Filion[21], Bertrand Boisson[3,4,5], Yuval Itan[3], Lei Shang[3], Jean-Laurent Casanova[3,4,5,6,7] and Charlotte Cunningham-Rundles[1,2]**

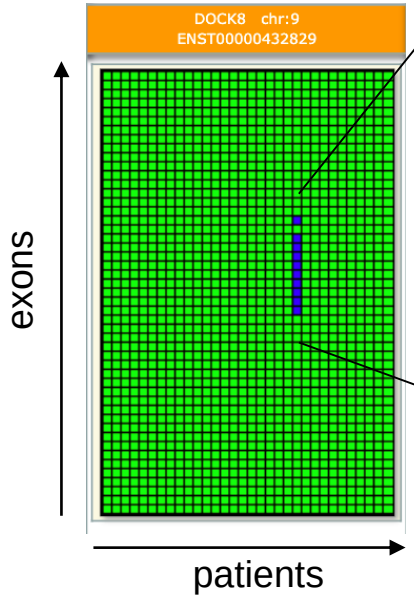
Patients with CVID diagnosis + one of the following criteria :
- Early beginning (<10 yo)
- autoimmunes/inflammatory manifestations
- B cell lymphopenia
- Familial hypogammaglobulinemia

DIAGNOSTIC YIELD = 30%

Two deleterious genetic events in *DOCK8*

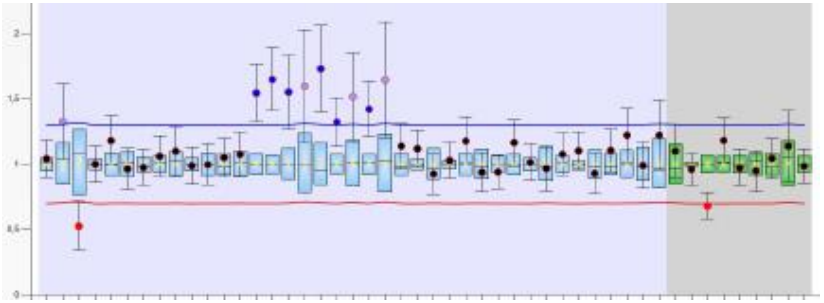
c.5287C>T ; p.(Arg1763*)
exon 41

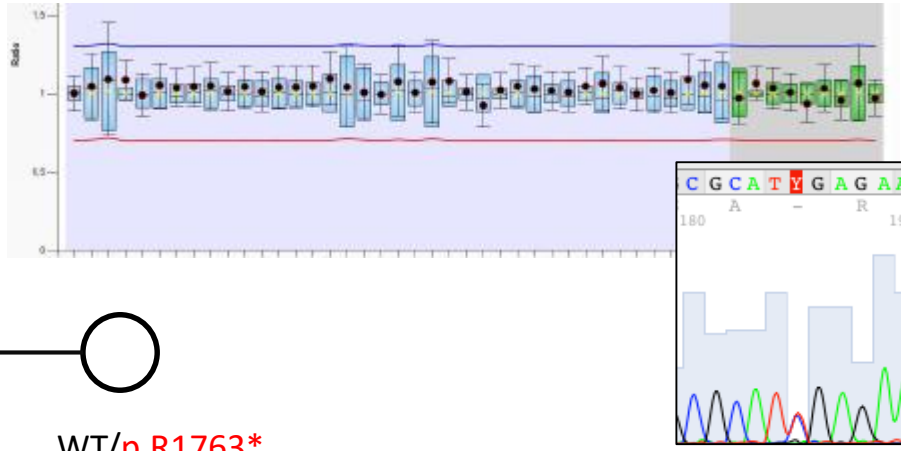
Dup_ex15-26

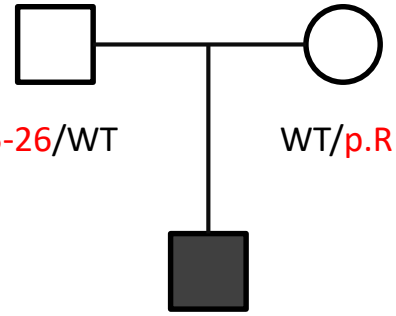


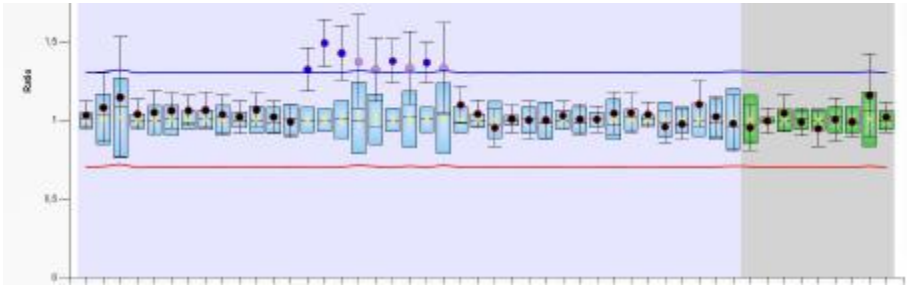
ex13 [338953-339151] primerchr9_338937	1.07 0	1 0	1 0	0.98 0
ex14 [340100-340378] primerchr9_340084	0.99 0	1.01 0	1.06 0	0.99 0
ex15 [367957-368195] primerchr9_367941	1.02 0	1.04 0	0.96 0	1.31 0
ex16 [370165-370363] primerchr9_370149	0.99 0	0.98 0	1.06 0	1.38 2
ex17 [371377-371615] primerchr9_371361	1 0	1.02 0	1.01 0	1.3 0
ex18 [372116-372354] primerchr9_372100	0.96 0	1.1 0	0.98 0	1.39 2
ex19 [376158-376356] primerchr9_376142	1.05 0	0.96 0	1.02 0	1.34 2
ex20 [376954-377232] primerchr9_376938	1.04 0	0.89 0	1 0	1.39 2
ex21 [379713-379991] primerchr9_379697	0.92 0	1.06 0	1.04 0	1.54 2
ex22 [382459-382737] primerchr9_382443	0.95 0	1.01 0	0.95 0	1.43 2
ex23 [386279-386477] primerchr9_386263	0.94 0	0.95 0	1.07 0	1.37 2
ex24 [390419-390617] primerchr9_390403	1.08 0	0.86 0	1.01 0	1.47 2
ex25 [396720-396998] primerchr9_396704	1 0	0.95 0	1.03 0	1.41 2
ex26 [399083-399321] primerchr9_399067	0.98 0	1.03 0	0.91 0	1.35 2
ex27 [404856-405134] primerchr9_404840	1.04 0	1.01 0	1.06 0	1.02 0
ex28 [406880-407118] primerchr9_406864	0.98 0	1.02 0	1.06 0	0.97 0

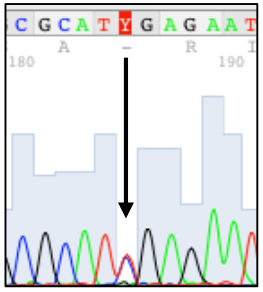
Familial segregation





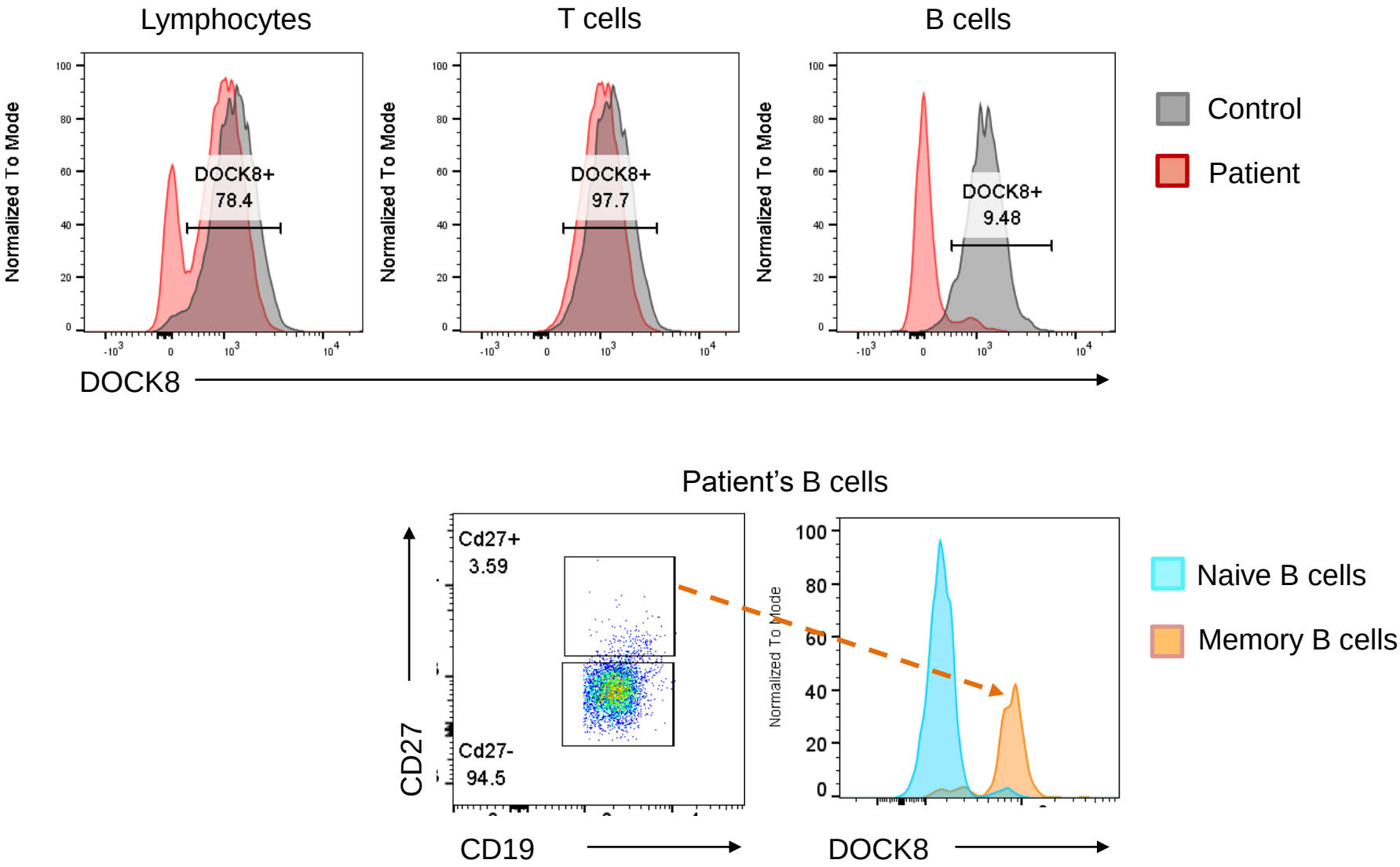






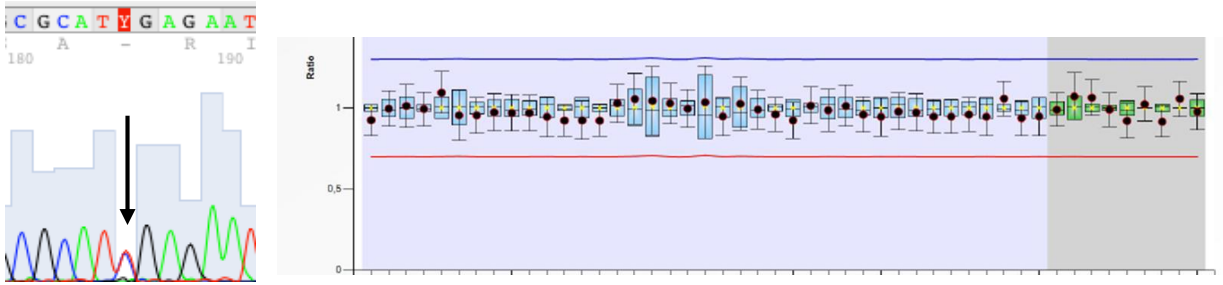
Compound heterozygous

DOCK8 expression by flow cytometry

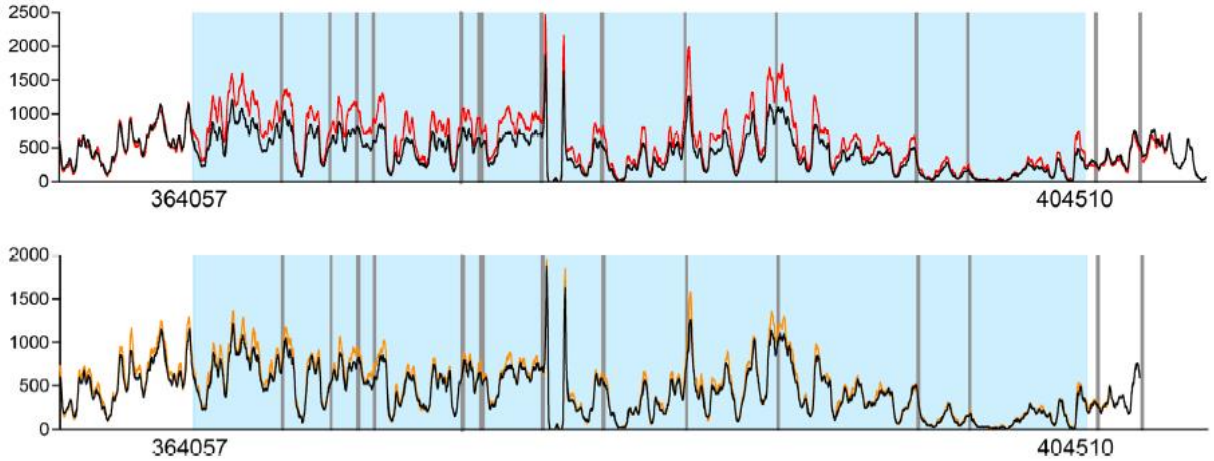


Somatic reversion ?

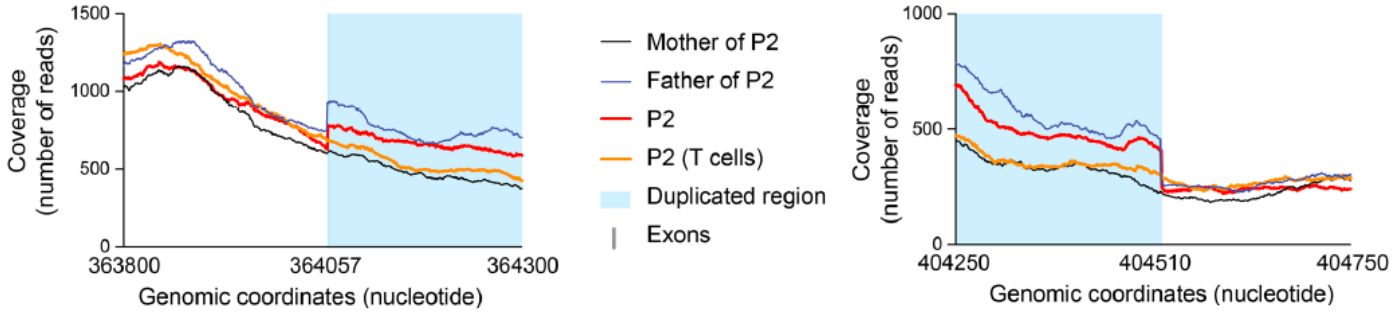
Sanger et MLPA on DNA from T cells (DOCK8+) :



Coverage analysis of *DOCK8* by whole genome sequencing



Discovery of the two breakpoints of the duplication



Whole gene analysis by Christine Bole & Cécile Masson

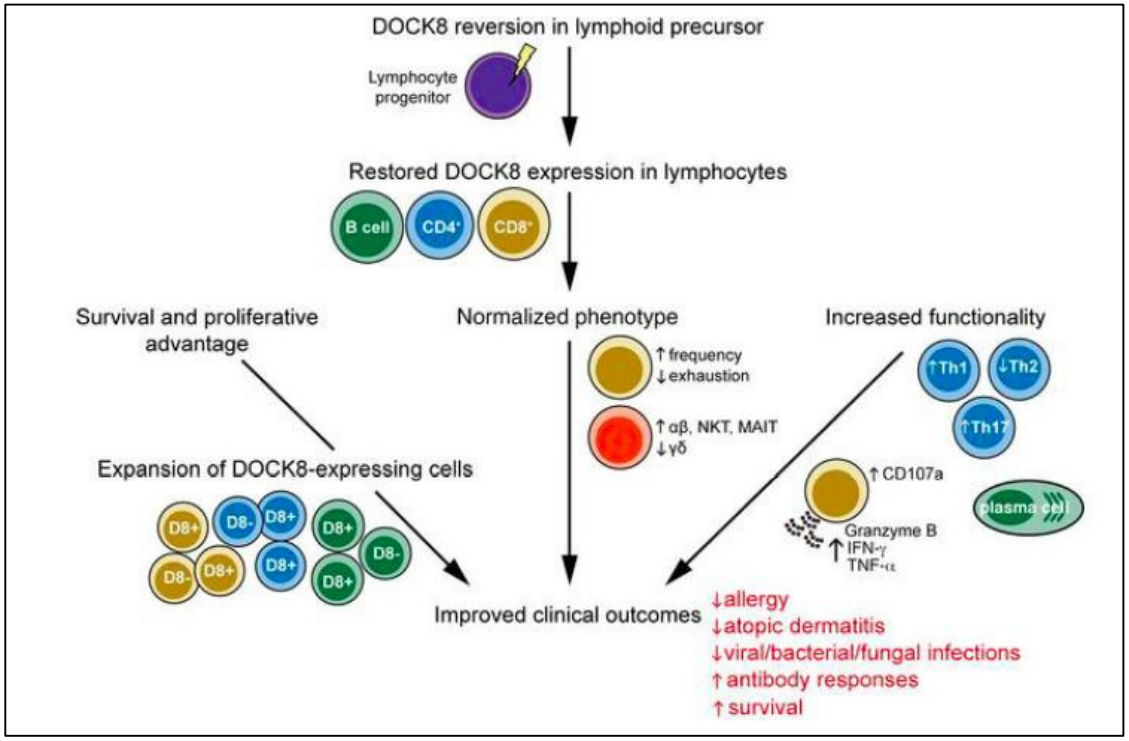
Clinical improvement in 3 DOCK8 revertant patients

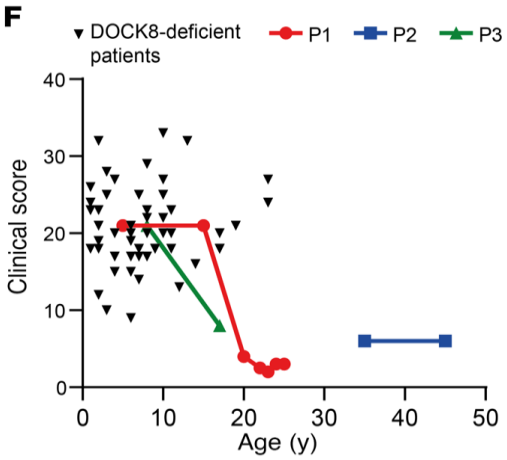
The Journal of Clinical Investigation RESEARCH ARTICLE

Somatic reversion of pathogenic *DOCK8* variants alters lymphocyte differentiation and function to effectively cure DOCK8 deficiency

Bethany A. Pillay,[1,2] Mathieu Fusaro,[3,4,5] Paul E. Gray,[6,7,8] Aaron L. Statham,[1] Leslie Burnett,[1,2,8] Liliana Bezrodnik,[9] Alisa Kane,[1,2,8,10,11,12] Winnie Tong,[8,11] Chrystelle Abdo,[13] Sarah Winter,[3,5,14] Samuel Chevalier,[4] Romain Levy,[3,14,15] Cécile Masson,[3,16] Yohann Schmitt,[3,17,18] Christine Bole,[17] Marion Malphettes,[19] Elizabeth Macintyre,[13] Jean-Pierre De Villartay,[20] John B. Ziegler,[6,7,8] Joanne M. Smart,[21] Jane Peake,[22] Asghar Aghamohammadi,[23] Lennart Hammarström,[24] Hassan Abolhassani,[23,24] Capucine Picard,[3,4,5,14] Alain Fischer,[3,14,25,26] Sylvain Latour,[5] Benedicte Neven,[14,27] Stuart G. Tangye,[1,2,8] and Cindy S. Ma[1,2,8]

Bethany Pillay & Stuart Tangye





CEDI’s team

Aknowledgments

CEDI team (including formers members)

- **Capucine Picard**
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- **Geneviève de Saint Basile**
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Mathieu Fusaro, PharmD, MSc[a,b,c]
Jérémie Rosain, PharmD, MSc[a,b,d]
Virginie Grandin, MSc[b]
Nathalie Lambert, MSc[b]
Sylvain Hanein, PhD[e]
Cécile Fourrage, PhD[e,f,g]
Nicholas Renaud, MSc[e]
Marine Gil, MSc[a]
Samuel Chevalier, AS[b]
Wadih Abou Chahla, MD[h]
Brigitte Bader-Meunier, MD[i]
Vincent Barlogis, MD, PhD[j,k]
Stéphane Blanche, MD[j]
David Boutboul, MD, PhD[l]
Martin Castelle, MD[j]
Thibault Comont, MD[m]
Jean-Sébastien Diana, MD[j]
Claire Fieschi, MD, PhD[k,l]
Lionel Galicier, MD[l]
Olivier Hermine, MD, PhD[a,k,n]
Alain Lefèvre-Utile, MD[j,o]
Marion Malphettes, MD[l]
Etienne Merlin, MD, PhD[p]
Eric Oksenhendler, MD, PhD[k,l,q]
Marlène Pasquet, MD, PhD[r]
Felipe Suarez, MD, PhD[a,k,o]
Isabelle André, PhD[e,s]
Vivien Béziat, PhD[d,e]
Geneviève De Saint Basile, MD, PhD[a,b,t]
Jean-Pierre De Villartay, PhD[e,u]
Sven Kracker, PhD[e,s]
Chantal Lagresle-Peyrou, PhD[e,s]
Sylvain Latour, PhD[c,e]
Frédéric Rieux-Laucat, PhD[e,v]
Nizar Mahlaoui, MD, PhD[j,k]
Christine Bole, PhD[e]
Patrick Nitschke, PhD[e,f]
Elisabeth Hulier-Ammar, PhD[e]
Alain Fischer, MD, PhD[a,i,k,w]
Despina Moshous, MD, PhD[a,i,u]
Bénédicte Neven, MD, PhD[a,i,v]
Alexandre Alcais, MD, PhD[d,e]
Guillaume Vogt, PhD[x,y,z]
Jacinta Bustamante, MD, PhD[a,b,d]
Capucine Picard, MD, PhD[a,b,c,i,k]

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