PNH: stipulations about clonal tribulations

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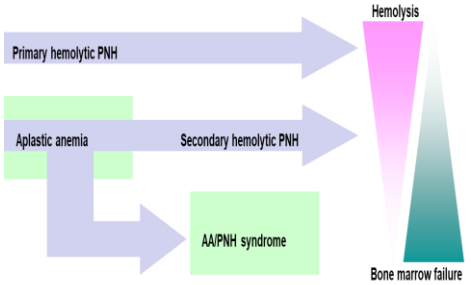
Stubborn PNH

but we know what we do not know



- Is evolution of PNH form of somatic gene rescue in the context of autoimmunity?

  -GPI linked proteins could be the targets but PNH cells do contain proproteins and thus antigens should not be absent?

  -Is antigen present in all cells but PNH cells have secondary immune privilege?

  -Is PNH cell itself immunogenic but how it is spared (i.e. how to explain their expansion)?

- What are the triggers of the auto/immune attack in AA and PNH?
- Mechanisms of secondary immune privilege still unknown?
- What is the mechanistic difference between pPNH and post-AA sPNH?

  -Same genetic predisposition <- different triggers

  -Same triggers -> different genetic predisposition

PNH as immune SGR?

Genetic predisposition

- Immune acquired BMF
- Inherited BMF syndromes
- Aging Hematopoiesis
- Stress hematopoiesis Immunodeficiency Inflammatory stress
- Chemotherapy

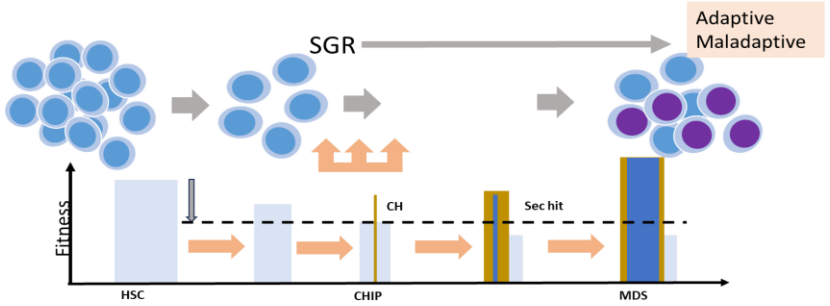
SGR

Immune escape mutations

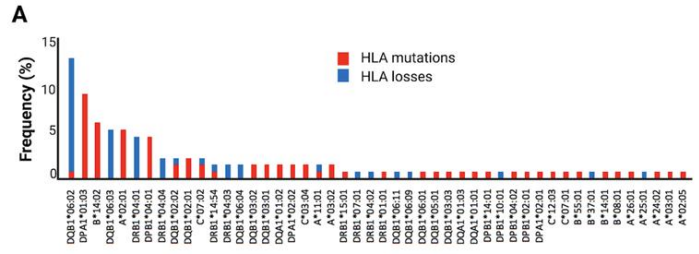
- Clonal recovery
- PNH

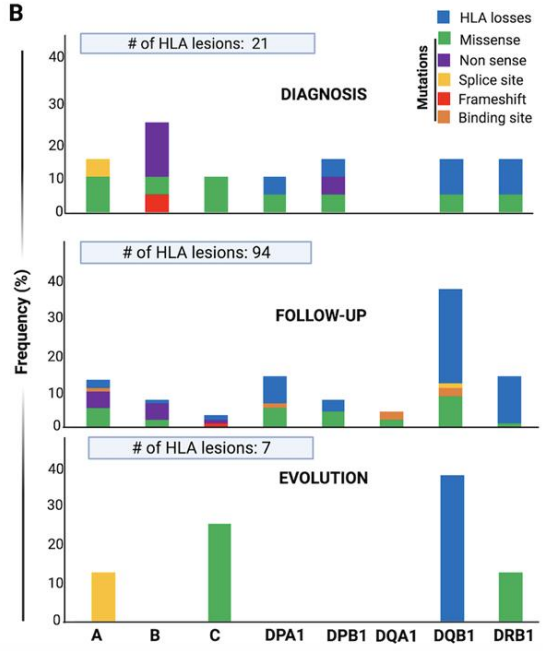
Strong drivers → MDS/AML

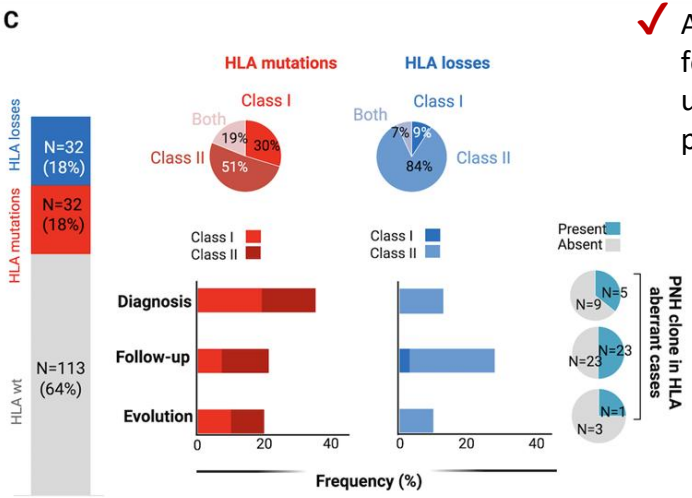
Weak drivers → CHIP

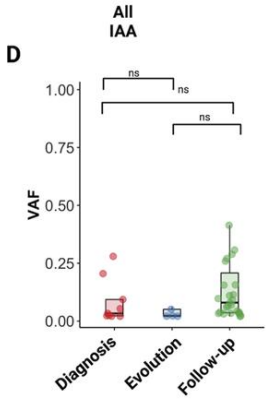


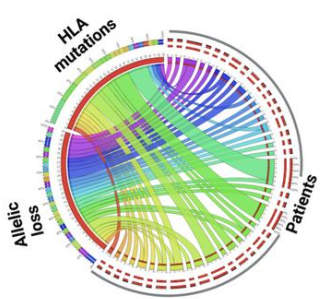
SRG via class I and II HLA mutations











- ✓ At least one HLA aberration was found at diagnosis in 42%, at follow-up in 34% and at the time of progression in 20% of patients.
- ✓ PNH clones were present in almost half of the cases (N=29, 45%) with HLA abnormalities
- ✓ Most mutated class I alleles were A*02:01 and B*14:02, while the most affected class II locus was DQB1
- ✓ hotspot in exon 1 involving class I alleles (particularly B*14:02), (12.5% of all mutant cases) at a median VAF of 9.5% (range: 4.3-20%).
- ✓ No recurrent mutations were identified in class II genes.
- ✓ Allelic losses more frequent in class II gens (DRB1 and DQB1)

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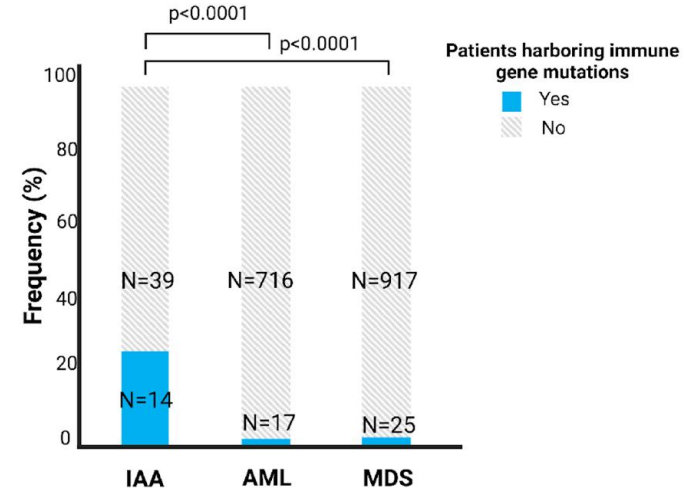
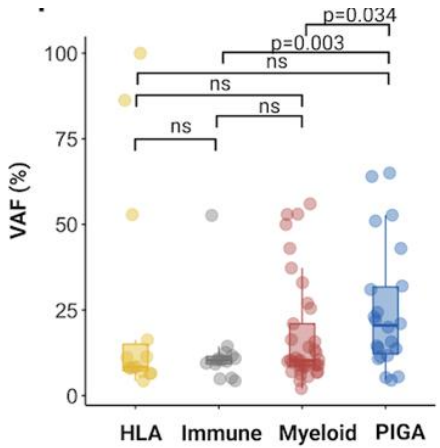
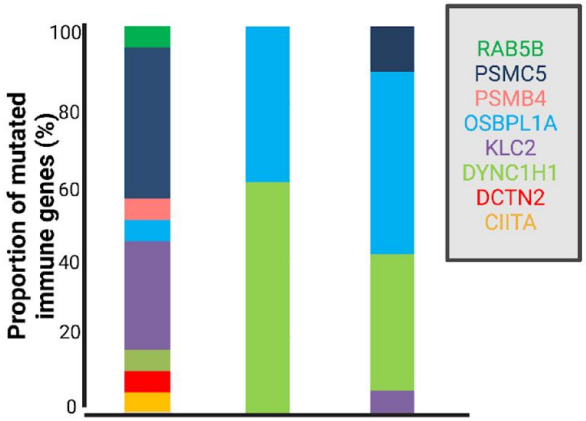
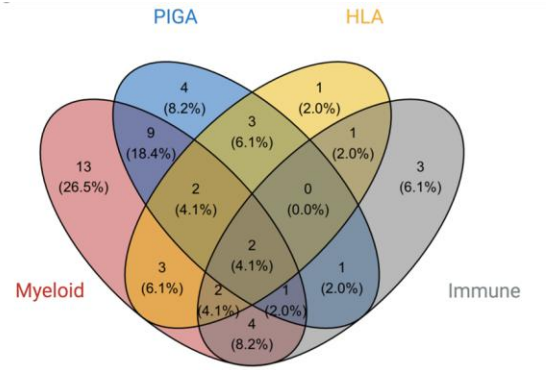
Other somatic hits in presentation and processing









- ✓ **N=53 AA pt WGS (vs 700 AML and >900 MDS)**
- ✓ Genomic analysis of Immune genes involved in antigen presentation and processing machinery other than HLA
- ✓ 26% of the patients, median VAF 10% (lower than PIGA and myeloid drivers), significantly higher frequency than in AML or MDS ($p<0.0001$)
- ✓ Most frequently mutated family of immune genes: proteasome machinery (*PSMC5*) and vesicle transportation (*KLC2*), potentially affecting HLA class I antigen assembly, together with class II HLA transcriptional regulators (i.e., *CIITA)*
- ✓ Somatic hits in immune genes not mutually exclusive with HLA mutations
- ✓ About 26% of patients presented at least one myeloid driver mutation in absence of any PIGA, HLA or immune hit.

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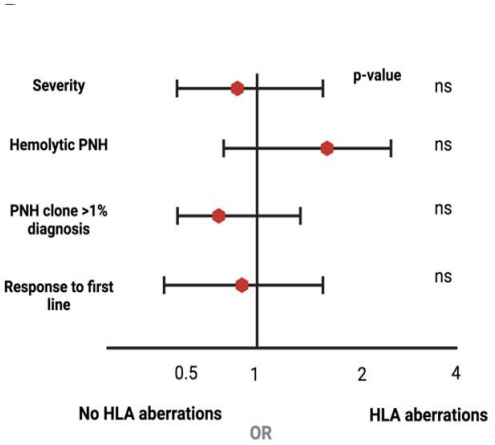
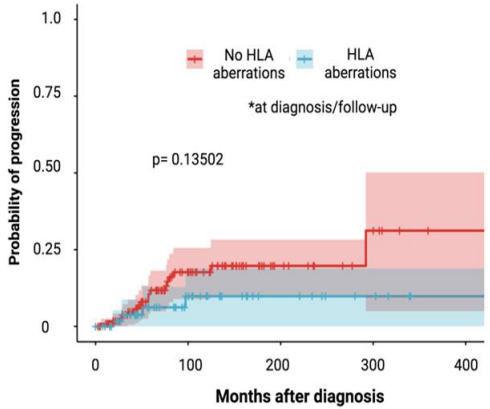
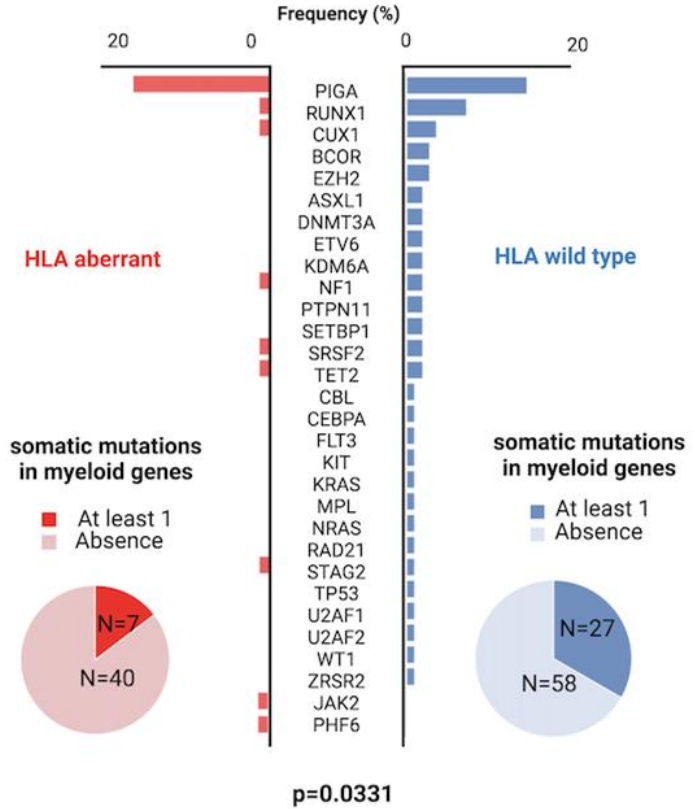
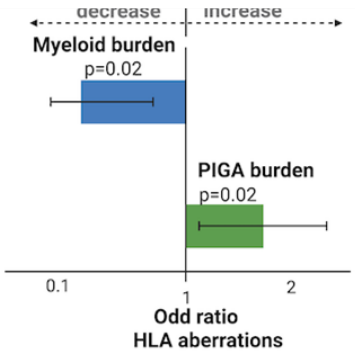
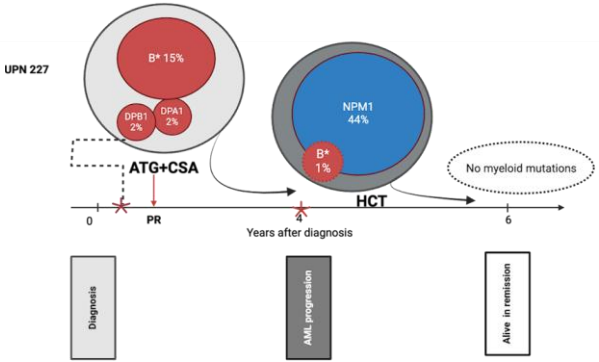
SRG via myeloid driver mutations











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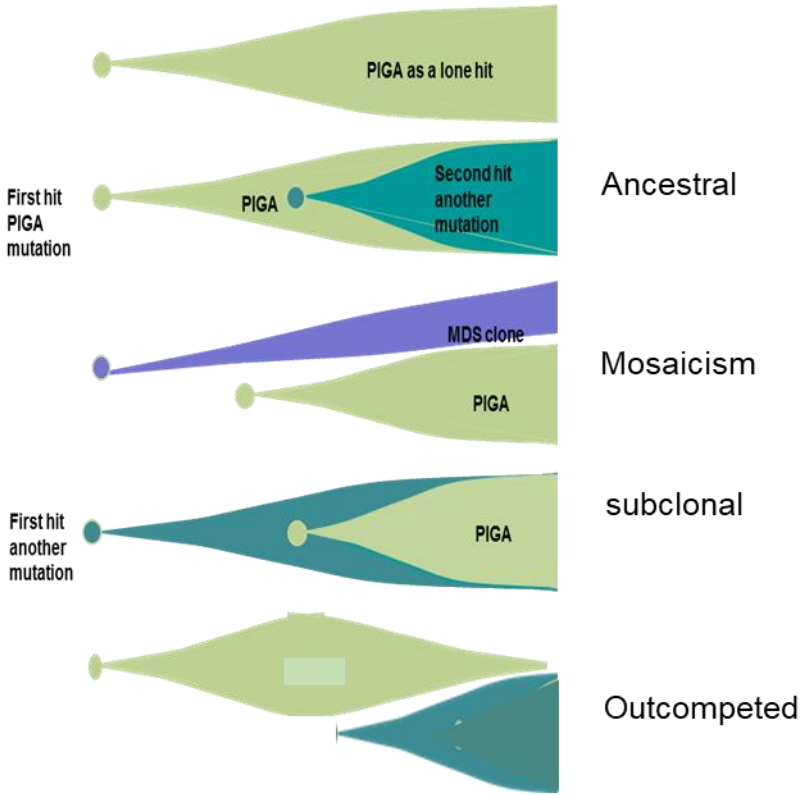
Clonal trajectories in PNH



Caveat:

Clone size in blood may not correspond to the actual disease burden

- PNH/mutant HPSC contribute more to blood production than normal = clone size in blood is underestimate
- Normal HPSC contribute to blood cells production while clonal cells are inhibited = clone size in an overestimate

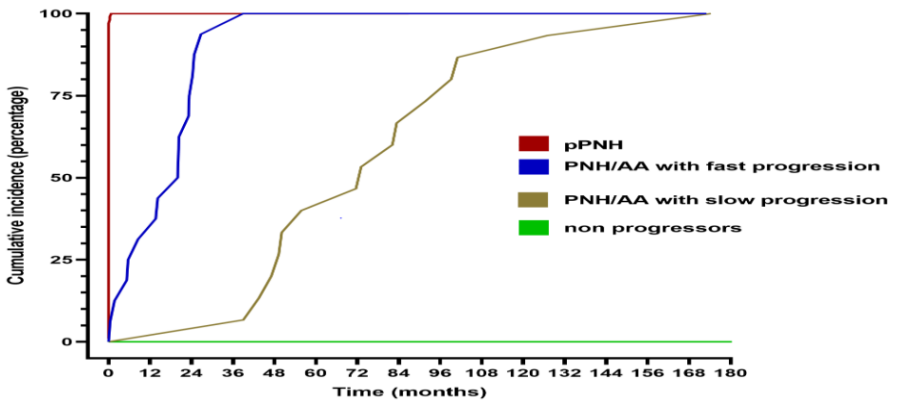
Clonal dynamics: clues to pathogenesis

Evolutions speed may help to identify underpinning of AA/PNH:

- 480 AA, AA/PNH 88 pPNH patients
- pts (F/U60 month = 1533 pty) ‘
- 233/480 PNH clone with 13% sPNH progression at median time of 39 mo.
- 13% progression (31 patients) at median time 39 months (risks: lack of ATG therapy, PIGA mosaicism, >5% PNH, failure to achieve CR)

Criterion: median time to reach an increase by 5,10, 20% in gran clone size: e.g. median time to ↓ of 5% = 23 mo.

1) No PNH clone
2) Slow progressors >23 mo
3) Fast progressors <23 mo
4) pPNH ↓ 0 mo.



Immune privilege

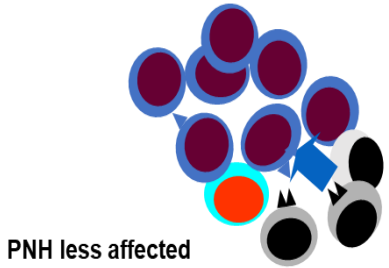
Alternate theory

Strength of immune response

Molecular, pathophysiologic features by group?

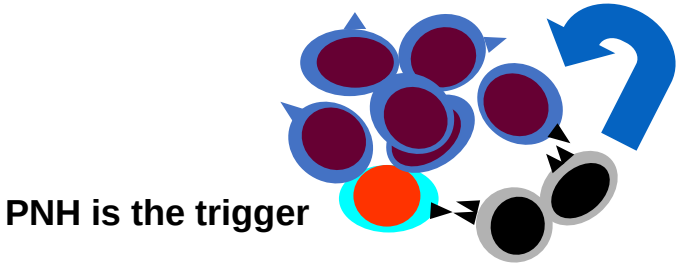
SRG and immune theories of PNH

- Antigens on normal stem cells are the triggers



PNH less affected

Classic theory or secondary privilege theory



Alternative theory

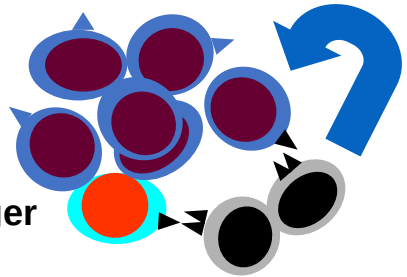
Elimination: AA w/o PNH

AA/PNH syndrome

Primary PNH

Strength of immune response

Alternate theory of PNH

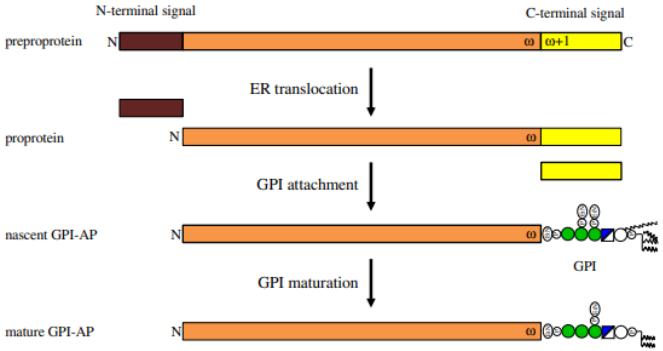


PNH is the trigger

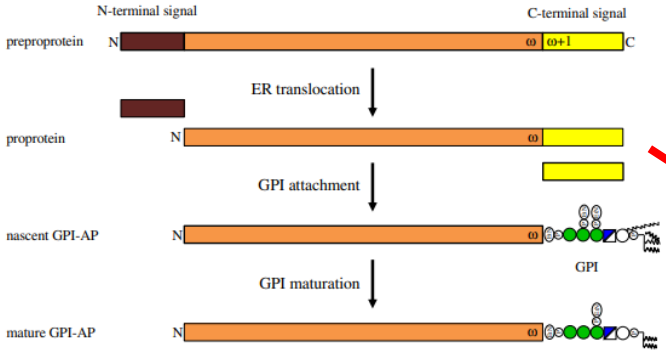
Cross-inhibition of normal stem cells

How PNH cell could be a trigger?

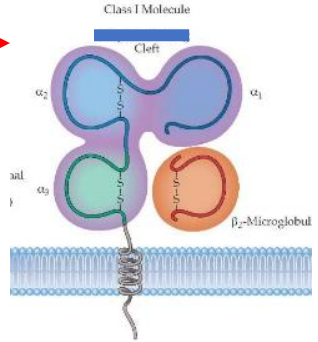
- Analogous to the tumor surveillance reactions
- Altered presentation of antigenic peptides as trigger
- Unprocessed GPI anchored proteins generate neopeptides cross reactive with normal cells.
- Seems incompatible with selective advantage of PNH cells

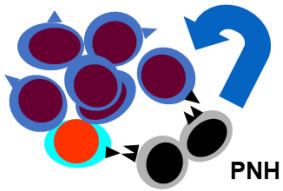


PNH as trigger theory



-14 omega to +1
-1 omega to +14





Antigenic spread

Cross reactive antigenic trigger

Immunopeptidomics

UniProt

GPI- AP Identification N=265

↓

Express in hematopoiesis N=168

↓

Identification of Omega site and capture 14 AA
Omega -13 and -> upstream (c terminus)

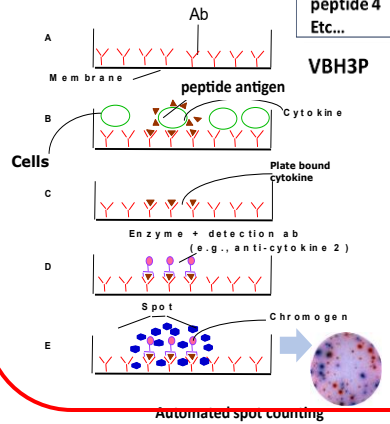
↓

High affinity fit in HLA2 and HLA-B7 top N=20 hits

Elispot

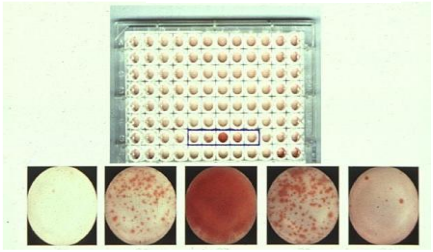
peptide 1
peptide 2
peptide 3
peptide 4
Etc...

VBH3P



Overlapping 9-mer peptides

MSTNPKPQRKTK.....LLALLSCLTV.....
MSTNPKPQR LLALLSCLT
STNPKPQRK LALLSCLTV
TNPKPQRKT
NPKPQRKTK

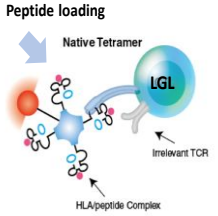


G5 77-85 | G6 78-86 | G7 79-87 | G8 80-88 | G9 81-89

Tetramer flow

peptide 1
peptide 2
peptide 3
peptide 4
Etc...

VBH3P



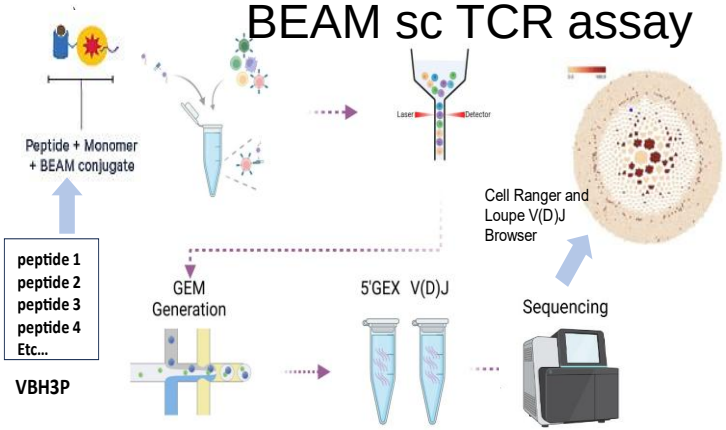
Flow cytometry
CD8, CD3,
VB-family specific

Gate on CD3+CD8+

Gate on VB and Tetramer

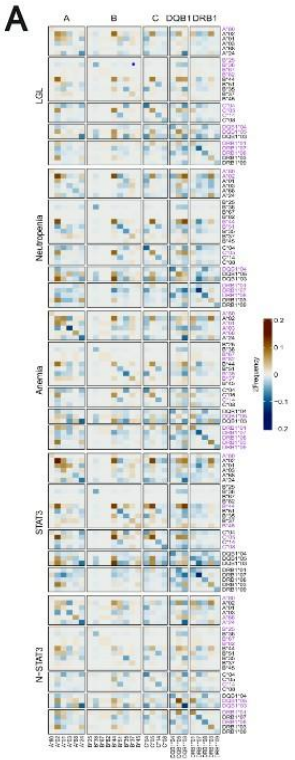
%Tetramer+ cells within VB class+ CD3+/CD8 + fraction
vs
%Tetramer+ cells within VB calls- CD3+/CD8+ VB fraction

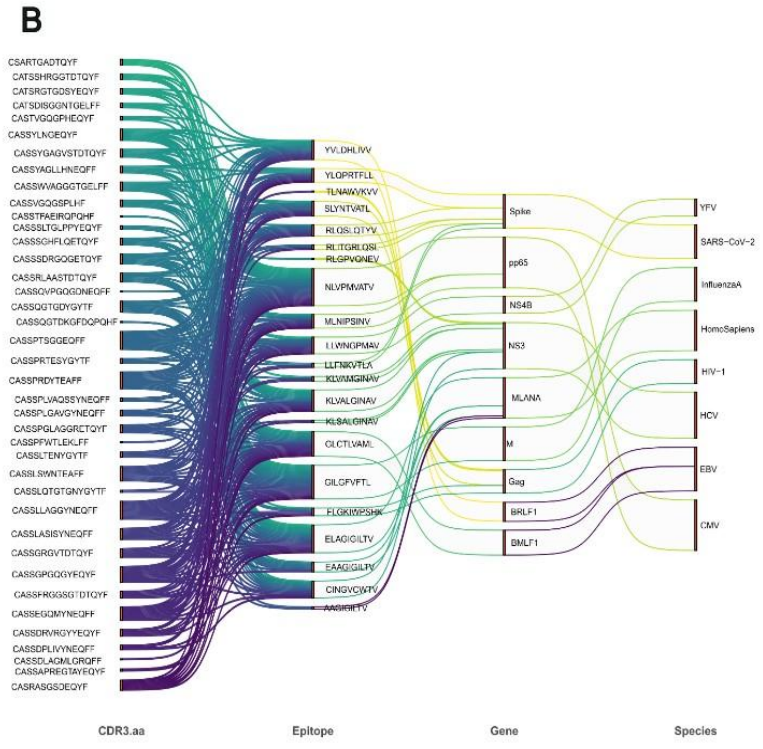
BEAM sc TCR assay



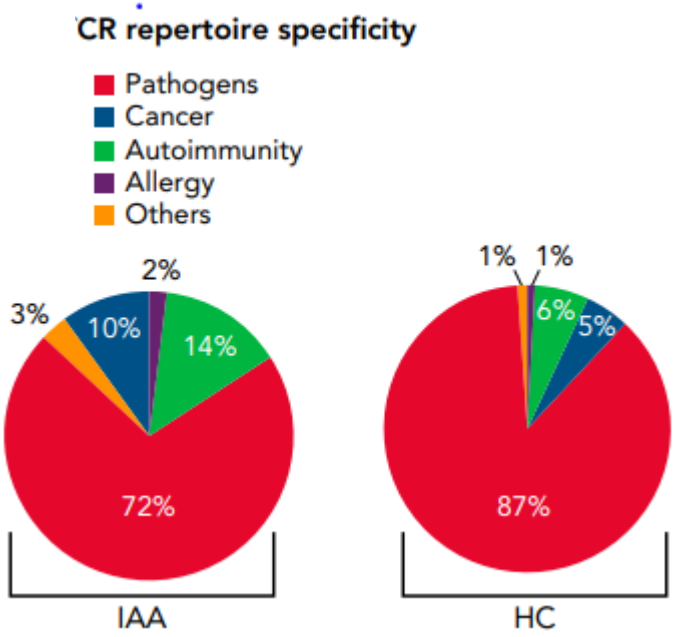
Structure/specificity CDR3 analysis

LGL dominant TCR VB CDR 3 clonotypes specificity pattern

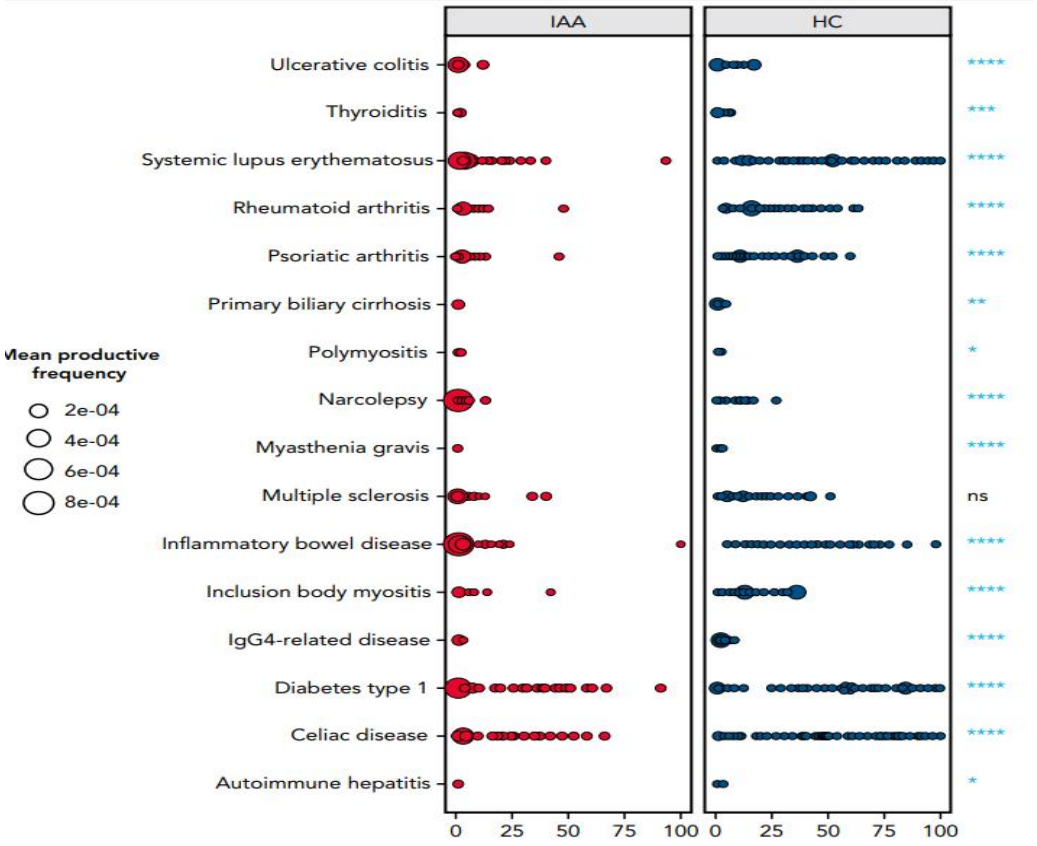


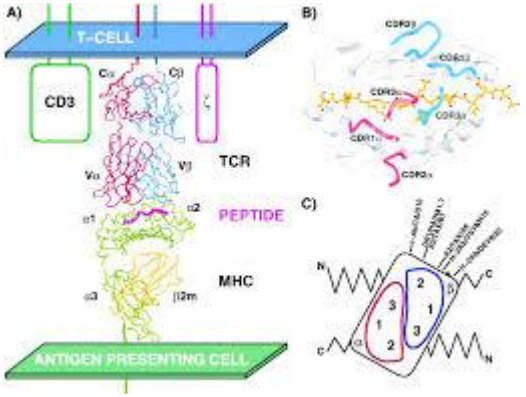


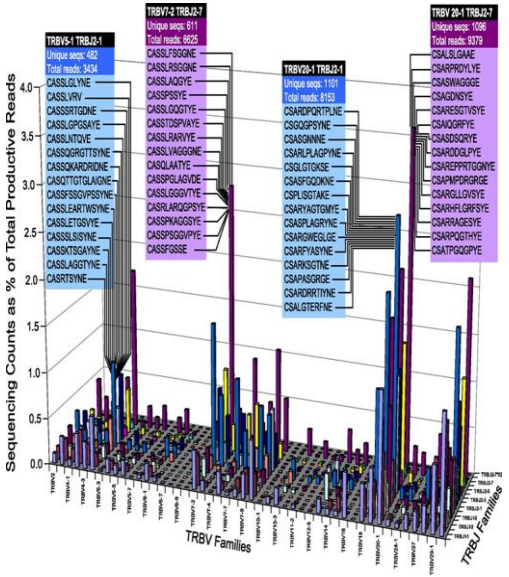
AA specific patter analysis



TCR specificities in AA according to known TCR

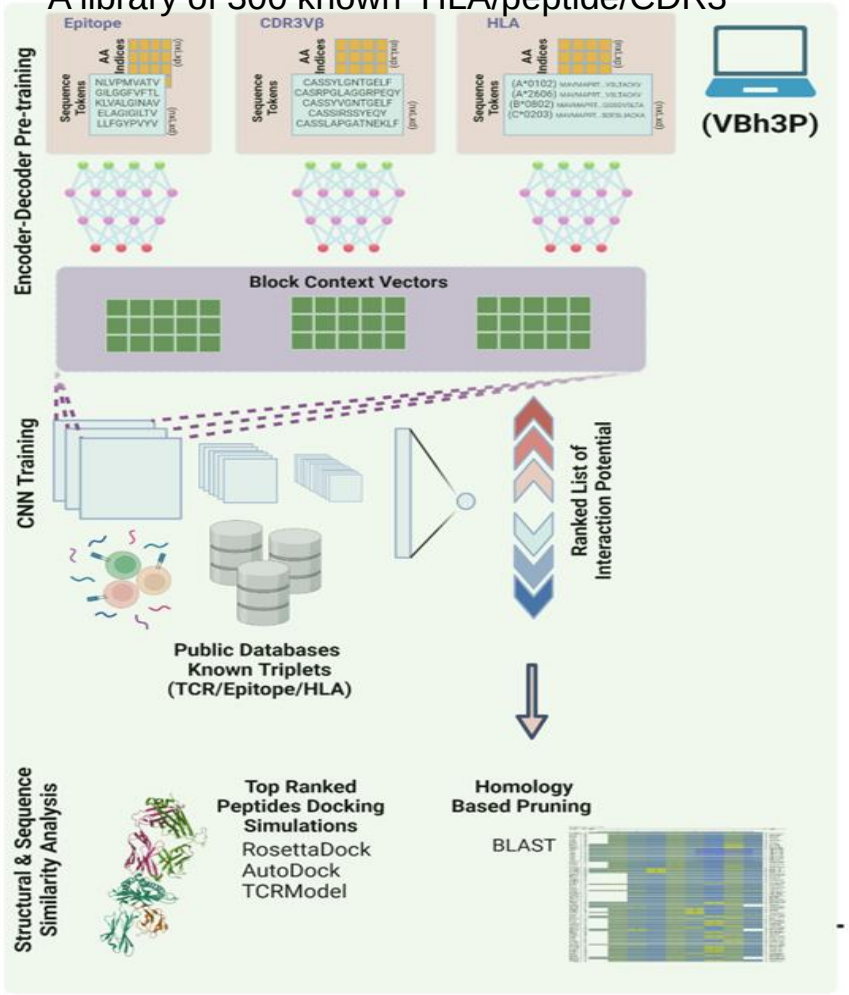


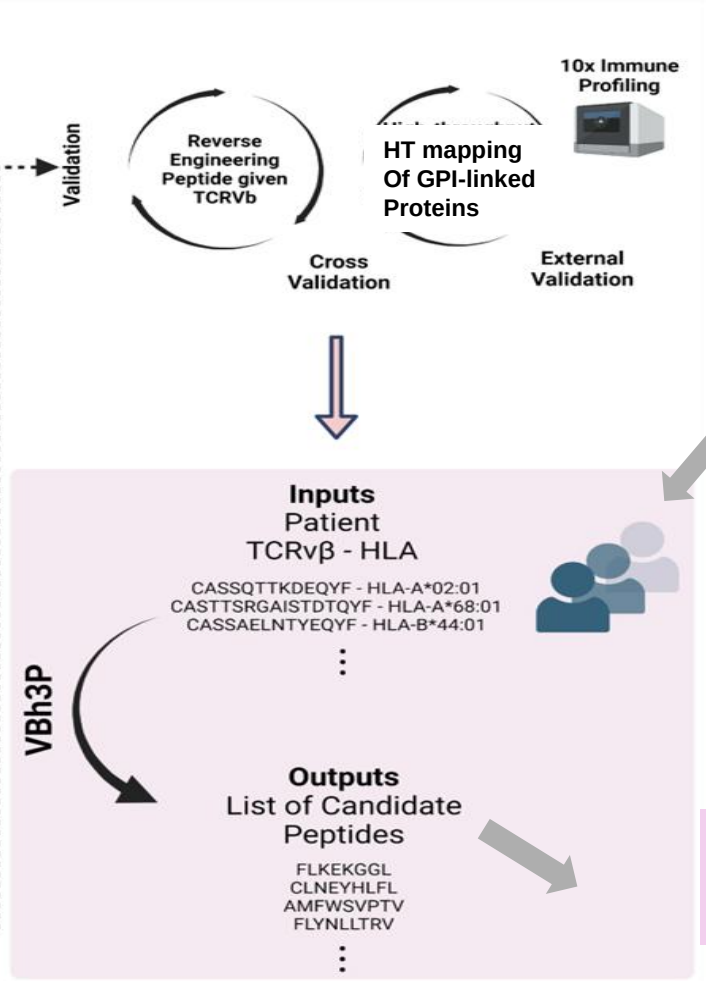




Reverse engineering of the antigenic peptide

A library of 300 known HLA/peptide/CDR3





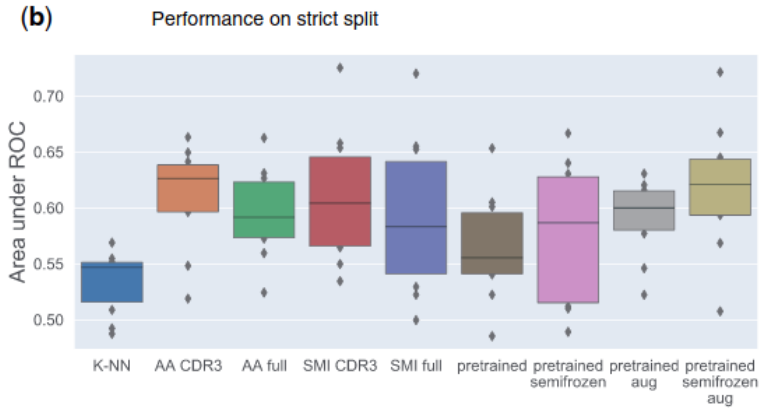
Deep VB CDR NGS in AA/PNH and PNH

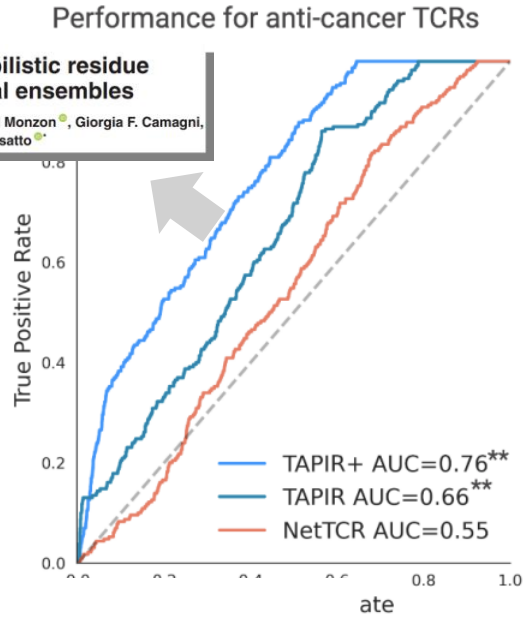
Blast against GPI-proteins or homologues

TCR-HLA peptide characterization

RING 3.0: fast generation of probabilistic residue interaction networks from structural ensembles

Damiano Clementel[1], Alessio Del Conte[1], Alexander Miguel Monzon, Giorgia F. Camagni, Giovanni Minervini, Damiano Piovesan and Silvio C.E. Tosatto*



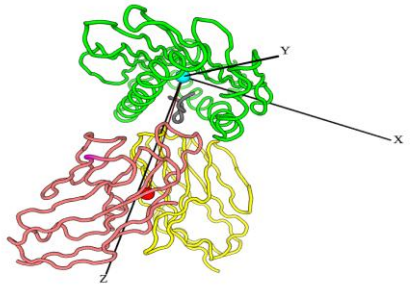


TAPIR: a T-cell receptor language model for predicting rare and novel targets

Authors:
Ethan Fast[1], Manjima Dhar[1], Binbin Chen[1]

TITAN: T-cell receptor specificity prediction with bimodal attention networks

Anna Weber[1,2,*], Jannis Born[1,2] and María Rodríguez Martínez[1,*]



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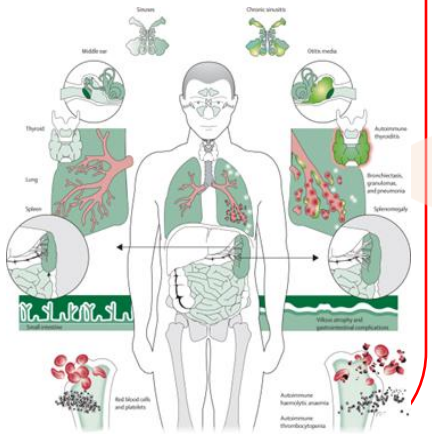
Genetic predisposition theories

Inherited hyperreactivity traits (non Mendelian, low permissive genetic traits + rare inciting event(s)

- Inborn errors of immunity traits -> autoimmune pathologic compensatory response + rare inciting event

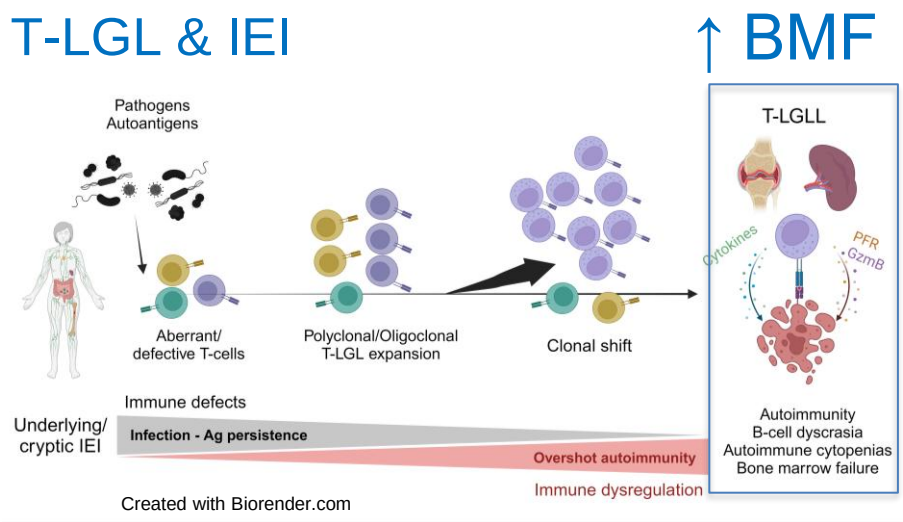
- Classic IEI → SCID → Recessive → Early onset → Severe infections.
- With the advent of NGS → An increasing number of adult-onset IEI has been identified.
- Adult-onset vs. classic IEI:
  - Less-deleterious variants → Dominant traits
  - Monoallelic variants → Recessive diseases
  - +/- Environmental triggers
  - Incomplete penetrance.
  - Variable expressivity.
  - Atypical or delayed manifestations.



Autoimmunity and immunodeficiency can coexist in a paradox fashion.

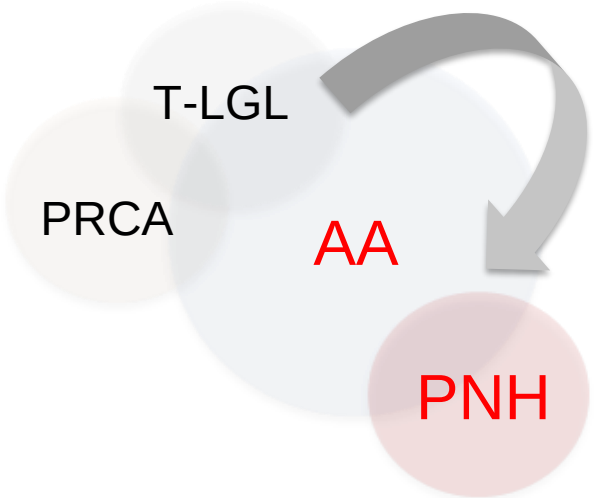
IEI and BMF

- While investigating germline variants implicated in IEI in T-LGL → More frequent in cases with BMF.



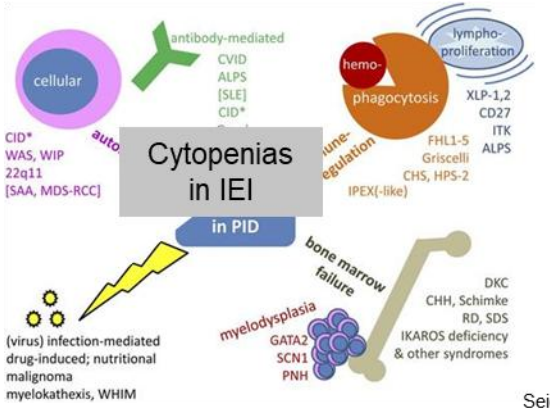
Bravo-Perez et al. ASH23 Abstract#157

- What if similar mechanism operates in AA PNH and AA/PNH.

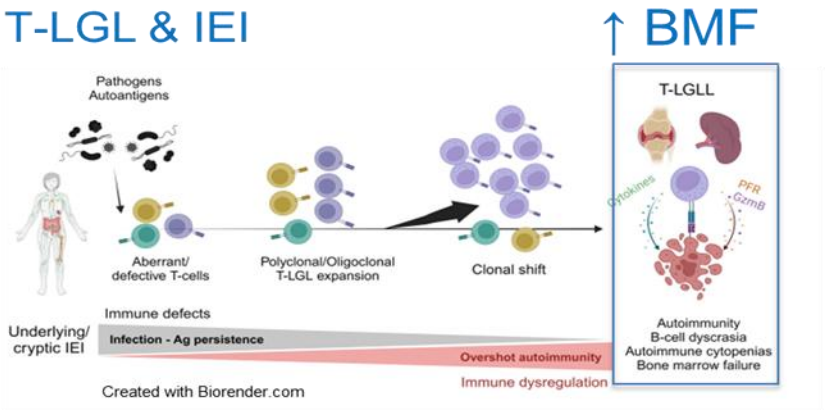


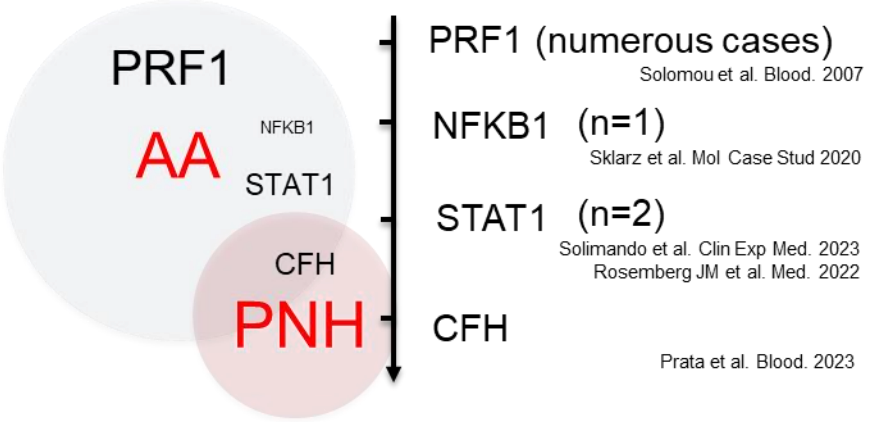
Clinical clues

- Patients with IEI → High frequency of BMF.
- Congenital BMF → IUIS IEI Classification 2022.
- Genes of other IEI → *PRF1* → AA.
- Occasional reports describing AA as the leading manifestation of IEI.
- Complement factor H variants → PNH.
- Systematic, BMF-forward approaches are needed
- While Investigating germline variants implicated in IEI in T-LGL → More frequent in cases with BMF. .

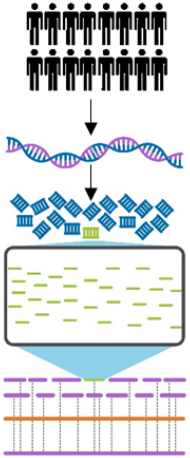


Seidel et al. Blood. 2014
Boushifa et al. J C Immunol. 2022





Analytic pipeline for IEI



163 IEI genes

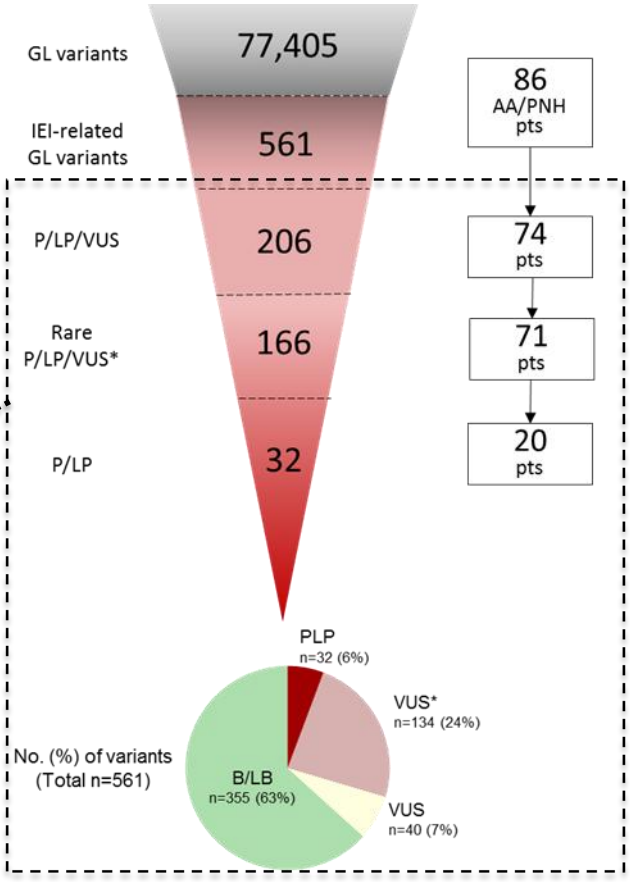
IUIS IEI Classification 2022 (n=454)
Recent publications (n=10)

86 AA/PNH

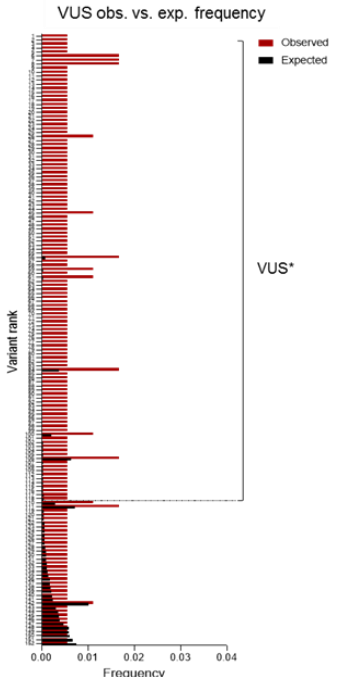
WGS/WES

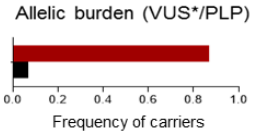
Functionality

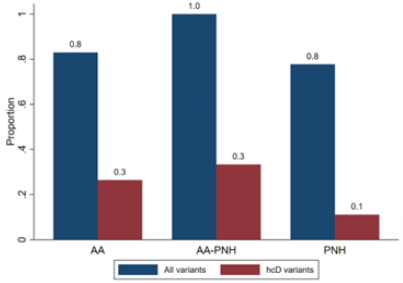
Clinical correlation

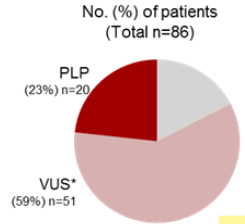


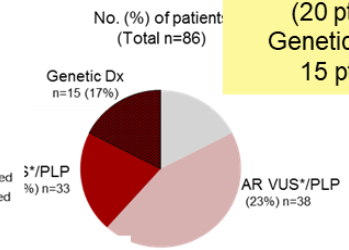
- Combined cellular and humoral immunodeficiency
- Predominantly antibody deficiencies
- Immune dysregulation and autoinflammatory disorders
- Defects in innate immunity
- Congenital phagocytic defects
- Bone marrow failure
- Complement deficiencies



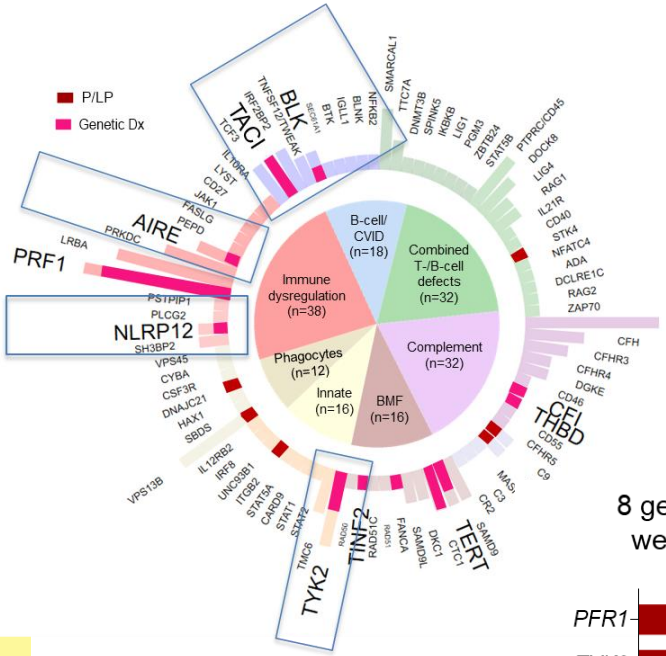




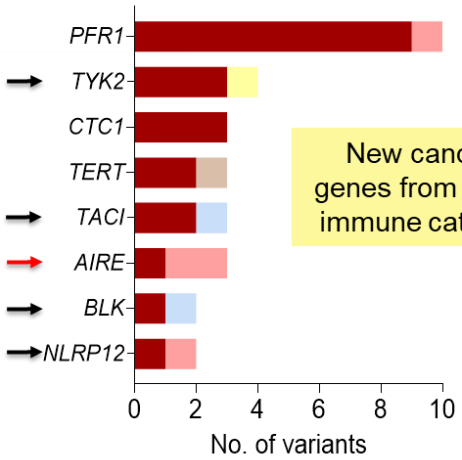




32 P/LP
(20 pts)
Genetic Dx:
15 pts



8 genes with at least 1 PLP
were recurrently mutated



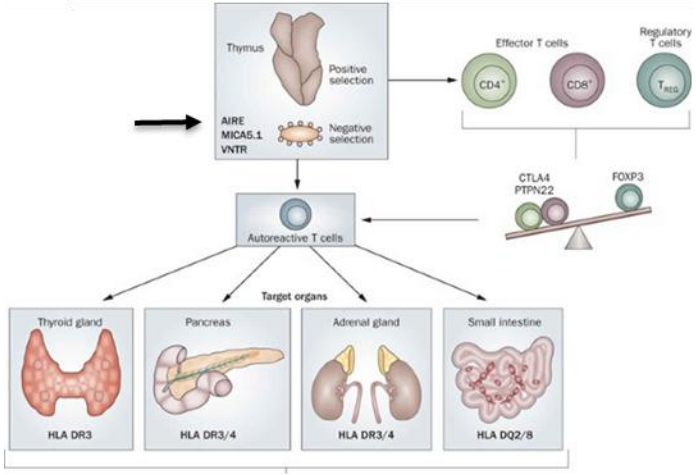
New candidate
genes from different
immune categories

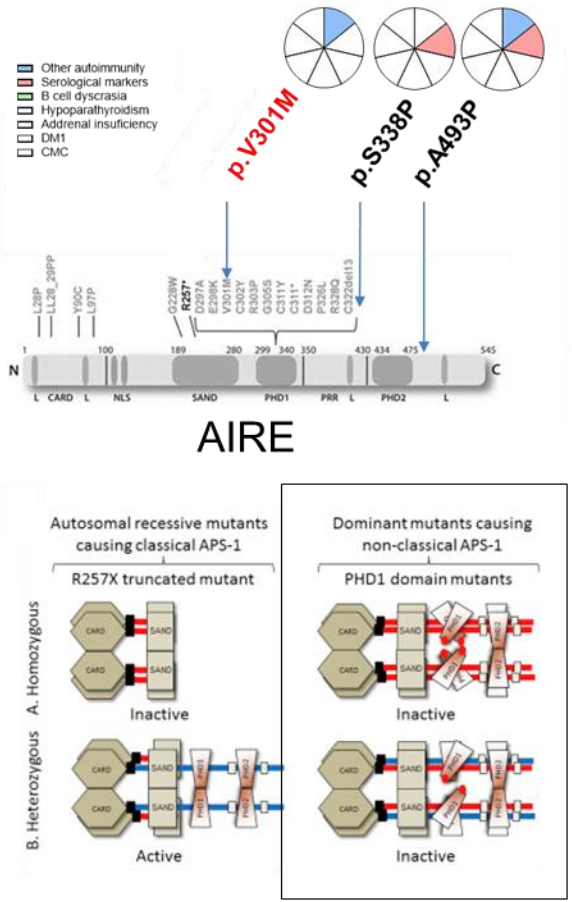
AA/PNH 84% vs. 6%

Recurrent genes

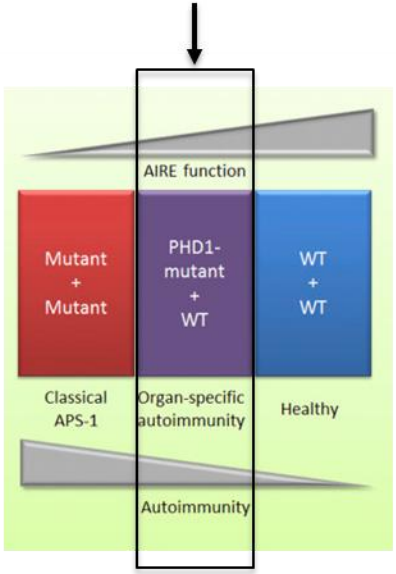
AIRE

- Master regulator of self-tolerance.
- Biallelic mutations → Autoimmune polyendocrine syndrome type 1 (APS-1).

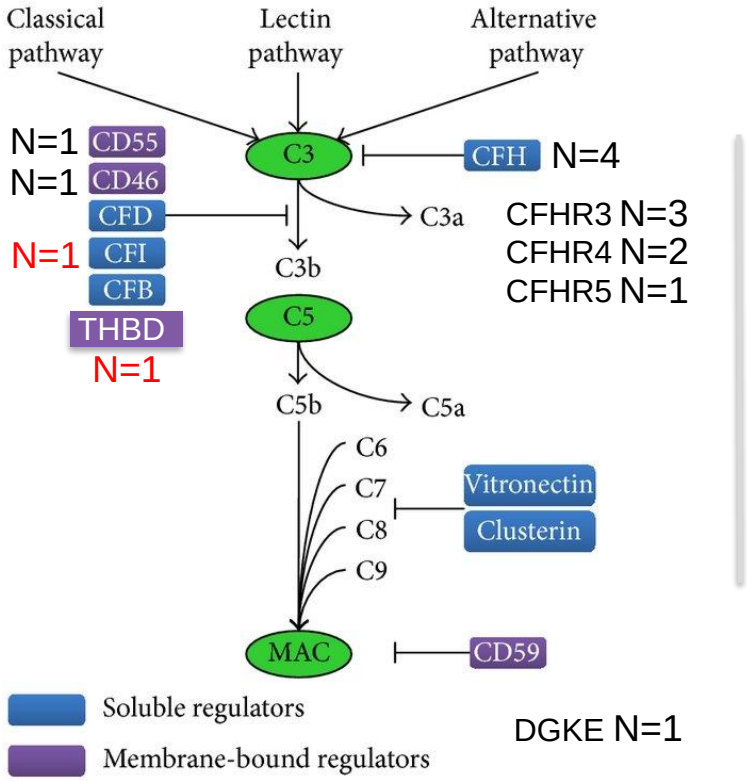




- 3 pts with heterozygous mutations.
- Monoallelic variants can cause an atypical APS-1, adult-onset, organ-specific autoimmunity.
- PHD domains → AIRE complex.
- Dominant negative effect.



Complement positive regulation variants in PNH

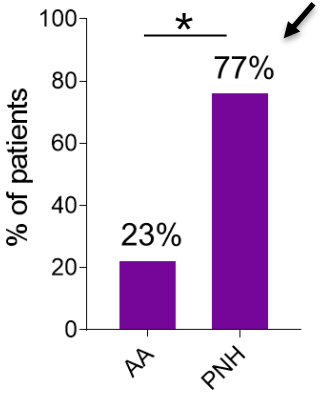


Kawa et al. J Immunol Res. 2014

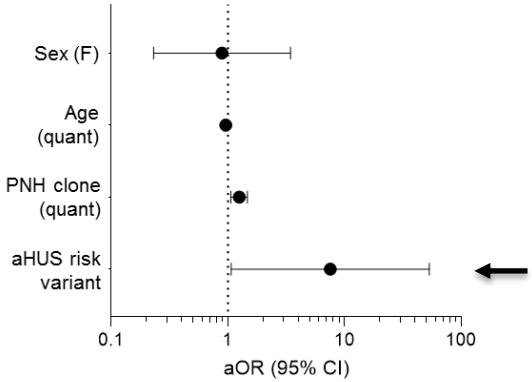
Enriched in PNH

Primary AA → Hemolytic PNH

Disease phenotype

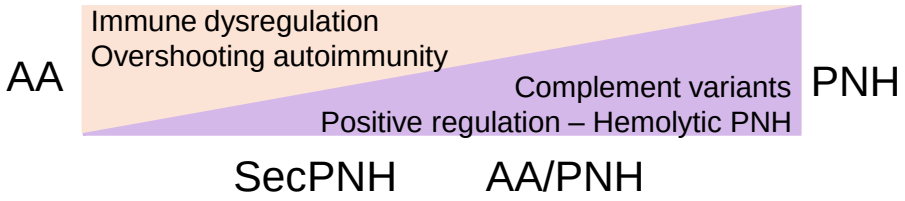


Risk Sec.PNH in primary AA
Multivariable analysis



Conclusions

- The heterogeneity of clinical presentation and clonal dynamic is likely due to the genetic background interaction with somatic gene rescue event of various nature with clonal remission possible.
- Germline variants predisposing to IEI are present in a **significant fraction of cases with AA** .
- The genetic defects found were mostly **heterozygous**, and associated with **dominant and adult-onset traits explaining low penetrance likely in a very specific context**.
- Structural or functional analysis of IEI variants suggests that they may result in **aberrant/defective immune responses**, in which AA may eventually arise.
- **Different immune pathways are asymmetrically distributed**, according to disease phenotype:
- Genetic background may explain disease pathogenesis:
  - In AA predisposition to immune dysregulation,
  - In AA/PNH speed of progression
  - In PNH severity of clinical hemolytic presentation, extravascular hemolysis, thrombotic proclivity.



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Our Laboratory, ASH 2024

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