



université
PARIS-SACLAY



Medicines and Healthcare Technologies Department
UMR 0496é

The localization of T-cell epitopes in biopharmaceuticals : from peptides to gene therapy vectors.

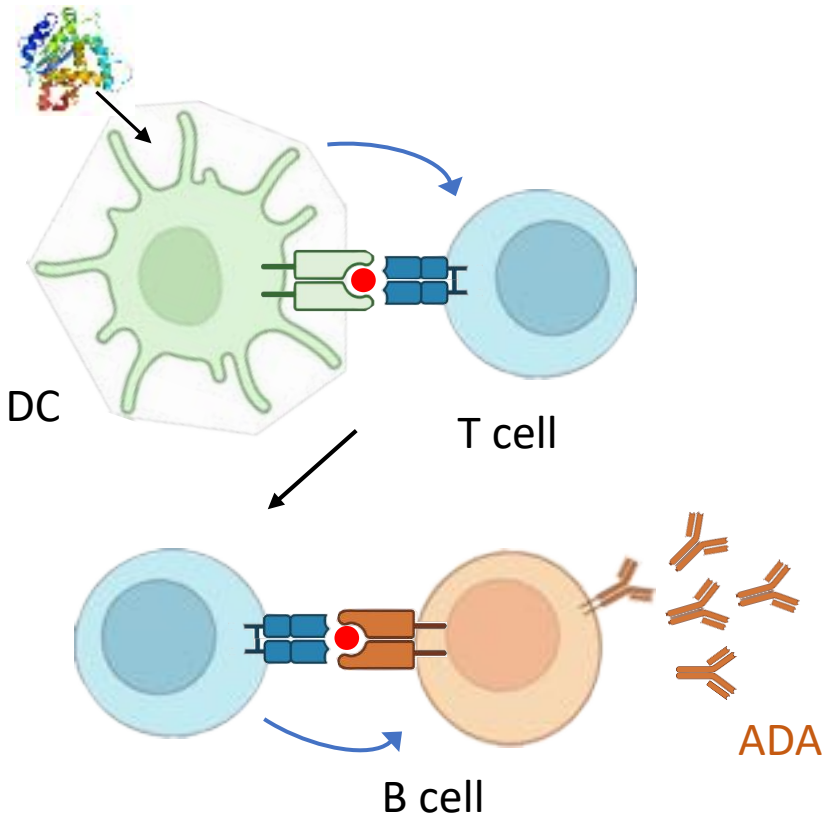
B. Maillere, PhD

Head, laboratory of cellular immunology and biotechnology

Contact: bernard.maillere@cea.fr

T cell response to biopharmaceuticals

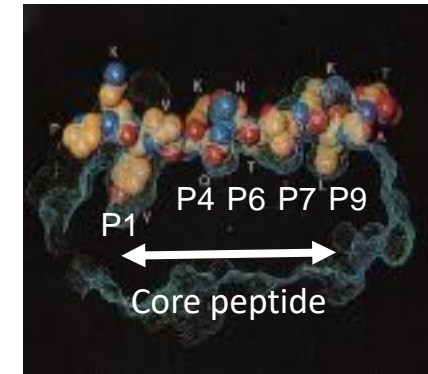
Cellular cooperation



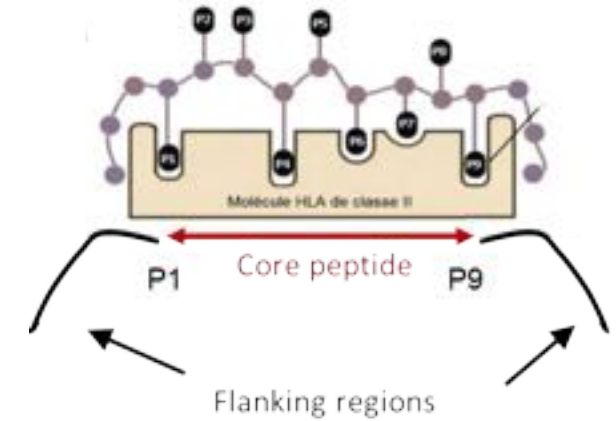
HLA class II molecules



- 2 HLA-DR, 1 HLA-DQ and 1 HLA-DP
- Extended peptide conformation
- Binding site is open at both ends
 - Peptides protrude from the binding site
 - Core + flanking regions
 - Variable size : 13-35 AA
- Five pockets of specificity:
 - P1, P4, P6, P7, P9
 - Dictate the accommodated AA and peptide sequences



Stern et al., 1994

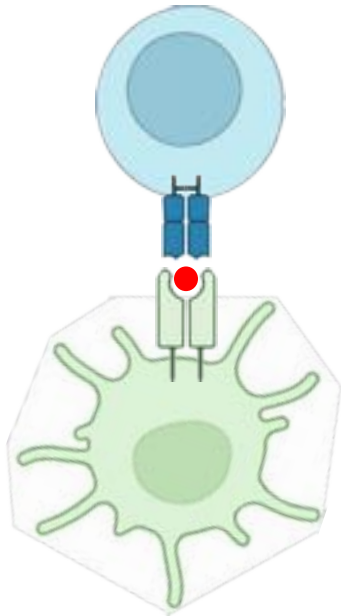


	P1	-	-	P4	-	P6	-	-	P9
DRB1*0101	Y			M		A			L
	F			L		G			
	W								
DRB1*1101	Y			M		R			
	F			L		K			
	W			V					
DRB1*0301	L			D		K			
	F					R			
	I								

Peptide binding motifs :

Rammensee et al., 1995

Properties of T cell epitopes



- **Immunodominance** (Sercarz, 1987)
 - Refers to the contribution in the T cell response to the native antigen (protein, virus ...)
 - ≠ cryptic or sub-dominant epitopes
- **Immunoprevalence** (Oseroff, 2008)
 - Refers to the number of responding donors
 - ≠ promiscuity: number of bound HLA
- **Magnitude (Intensity)**
 - Refers to the activation signal
 - Number of recruited T cells in one donor

T cell epitopes

- Widely differ by their immunoprevalence and magnitude
- Can be ranked in function of their contribution to the T cell response

T cell amplification assay



Healthy donors
Naive T cells

CD4 T cell amplification

IFN- γ EliSpot

• Frequency of specific T cells

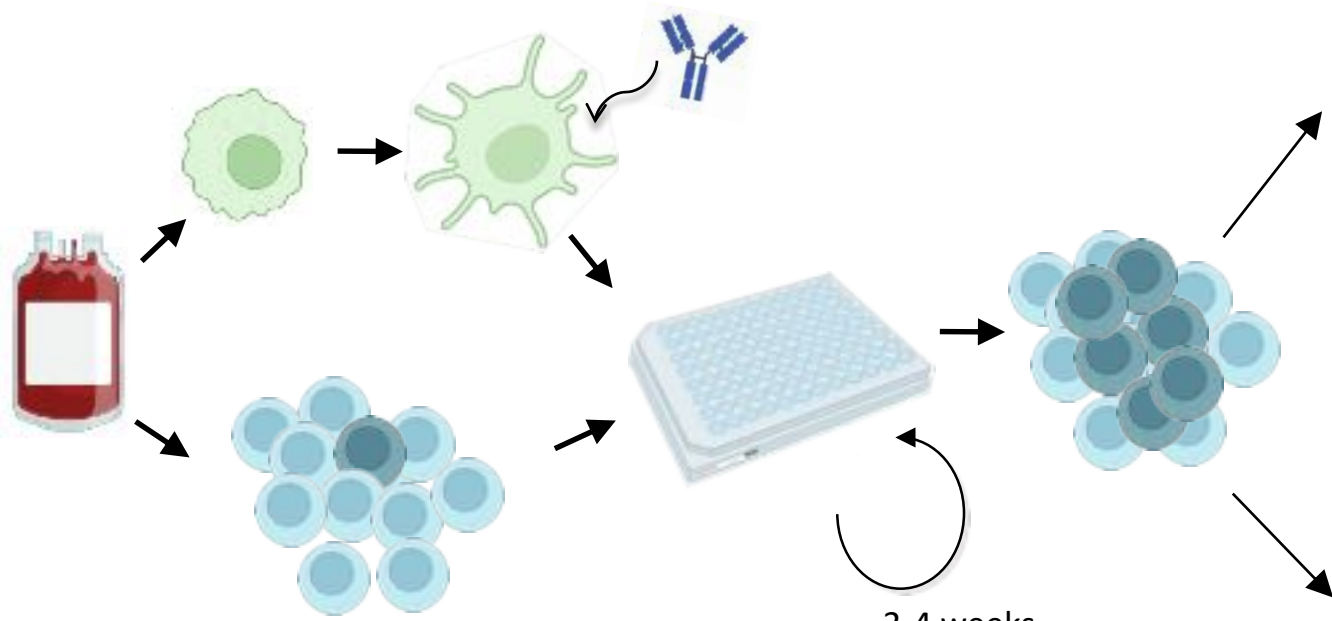


DC



DC
+ Mab

Immunogenicity
risk



Low frequency :
 $1/10^5$ to $1/10^7$

3-4 weeks

• Mapping of T cell epitopes

Overlapping peptides



PBMC



PBMC
+ peptide

Immunogenic
sequences

Journey in the T cell epitope landscape

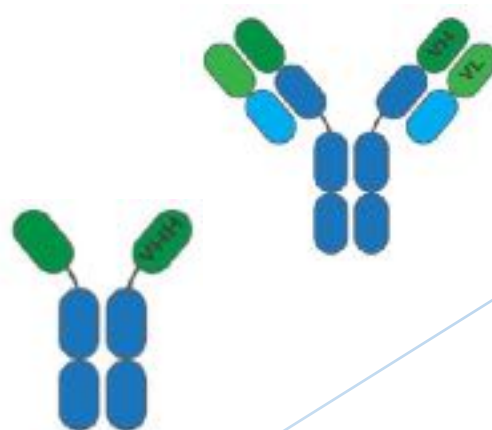
peptide



Animal
toxins



Self proteins

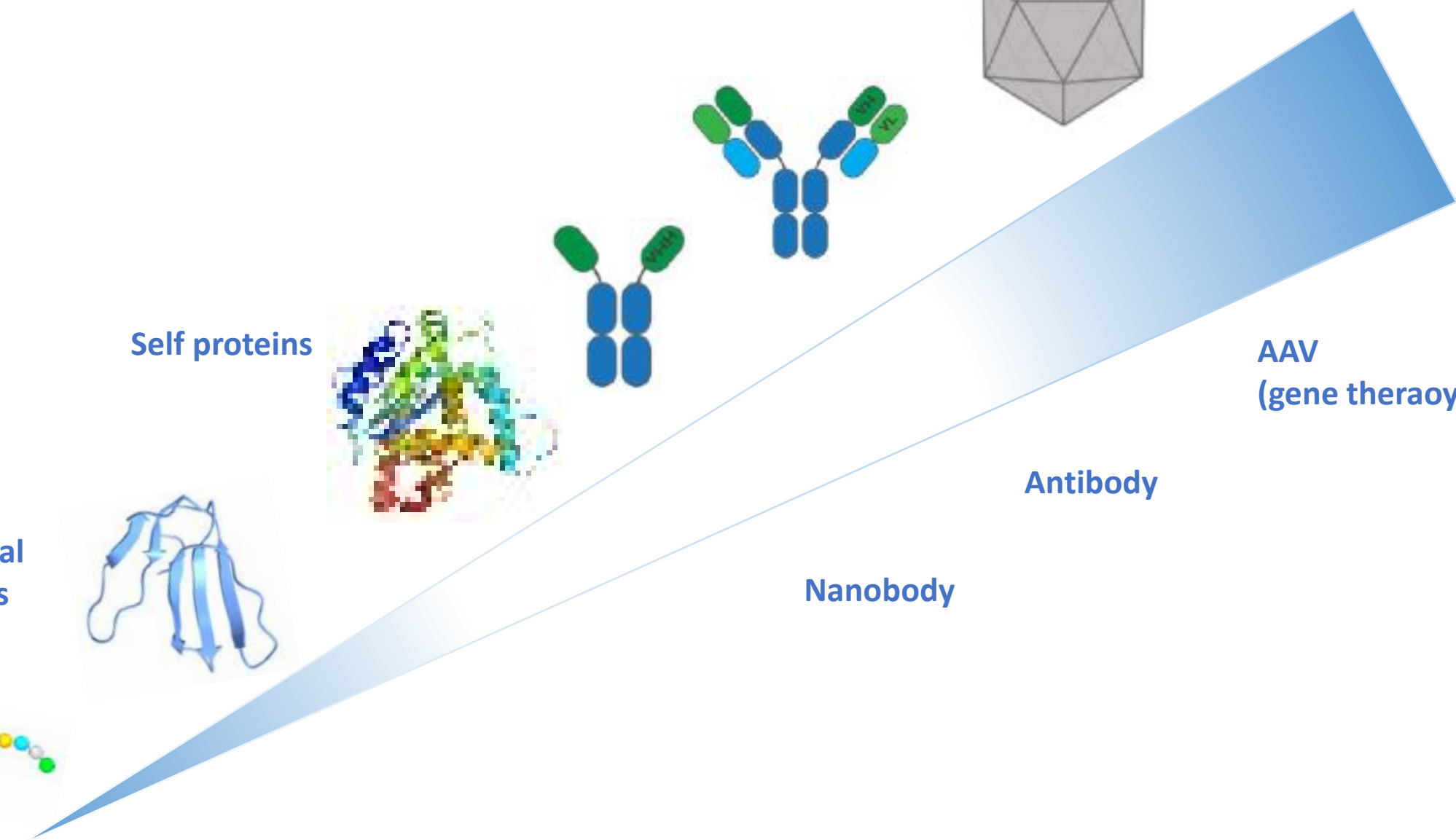


Nanobody



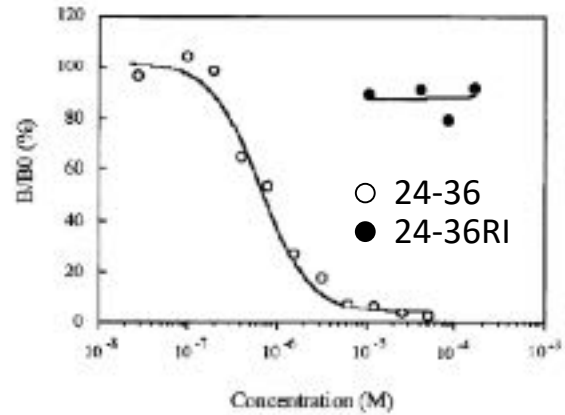
Antibody

AAV
(gene therapy)

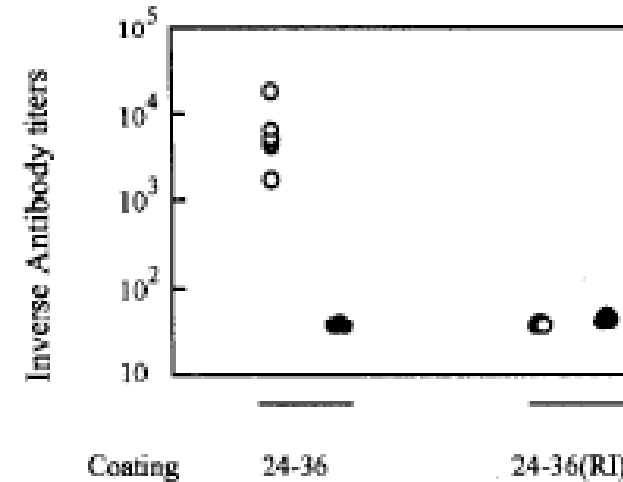


Miminal size for a peptide to be immunogenic

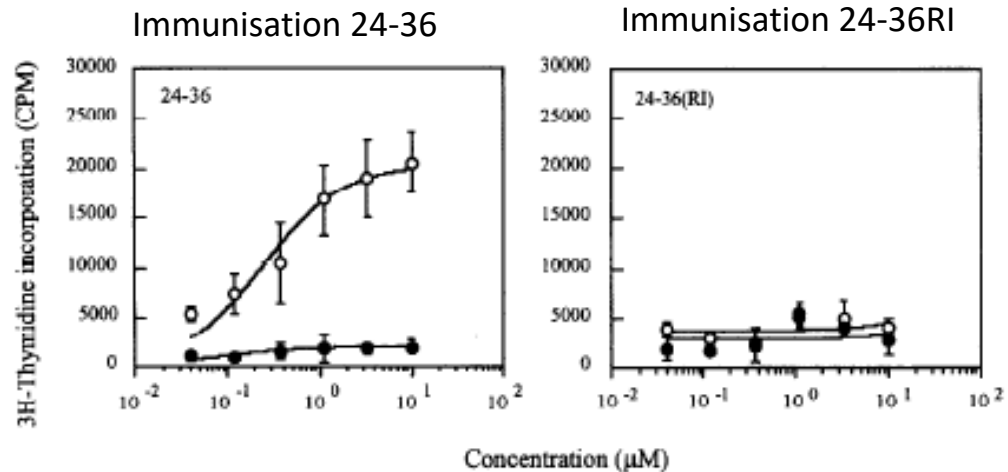
MHC II binding



Antibody response



T cell response



- 12-13 mer peptides can be immunogenic
- Affinity to HLA class II
- Non human origin

low abundant self proteins as biopharmaceuticals

Examples of Immunogenic Proteins					
Protein Category	Protein	Type and Producer Cells	Binding Antibodies	Neutralizing Antibodies	Clinical Consequences
Nonhuman	Insulin	Natural	Yes	Yes	Loss of efficacy uncommon
Human	Glucocerebrosidase	Natural	Yes	Yes	Loss of efficacy
	Factor VIII	Natural	Yes	Yes	Loss of efficacy
	Follicle-stimulating hormone	Natural	No	No	—
Homologous to native protein	IFN- α 2a	rDNA	Yes	Yes	Loss of efficacy
	GM-CSF	rDNA	Yes	Yes	Loss of efficacy
		rDNA	Yes	No	No loss of efficacy
	G-CSF	rDNA	No	No	No loss of efficacy
	IFN- β	rDNA	Yes	Yes	Loss of efficacy
	Epo	rDNA	Yes	Yes	Cross-reacted with endogenous protein and caused adverse effects
	IL-2	rDNA	Yes	Yes	Loss of efficacy
Sequence variants	IFN- β	rDNA	Yes	Yes	Loss of efficacy
	IFN- α Con 1	rDNA	Yes	No	Loss of efficacy not reported

- **EPO and PRCA:** autoimmune disease (Casadevall, 2002 & 2007)
- **IFN- β :** in multiple sclerosis (Peh et al, 2019)
- **IFN- α :** HCV patients (Scagnolari et al 2013)
- **GM-CSF:** colon cancer (Raghammar, Blood, 1994)

- **Spontaneous onset**

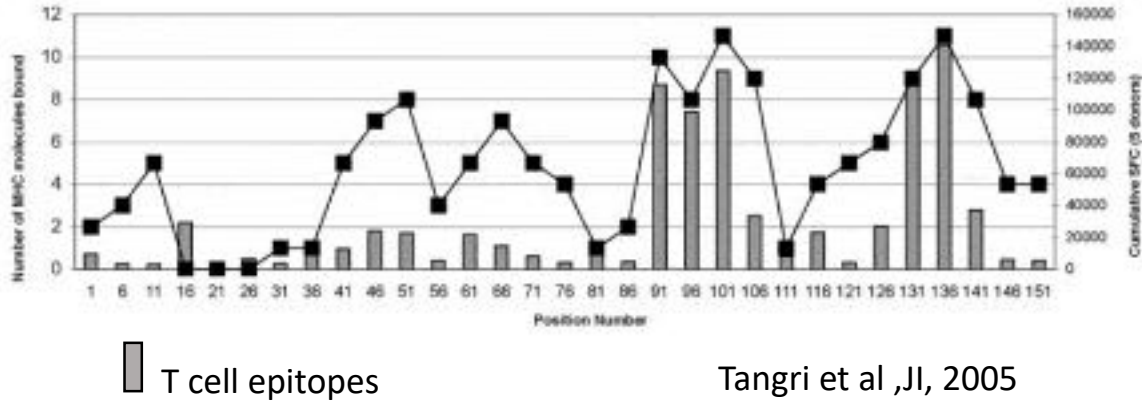
(not linked to protein infusion)

- **IFN- α :** Critical pneumonia Covid-19 (Zhang et al, JEM, 2022)
- **IFN- γ :** Mycobacterial infection (Shih et al, JEM, 2022)
- **GM-CSF:** Pulmonary alveolar proteinosis (Ushida, Blood, 2004)

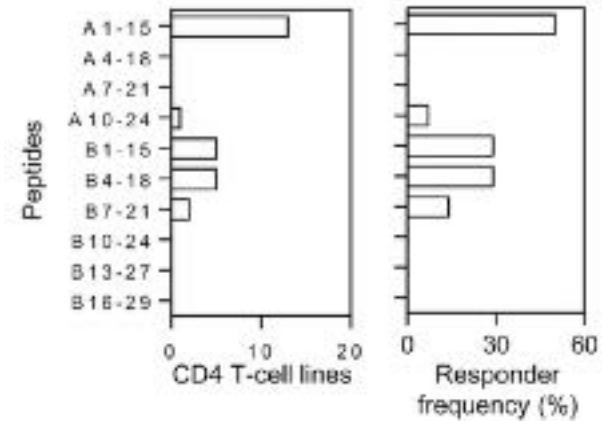
Wardhwa and Thorpe, 2007

T cell epitopes of low abundant biopharmaceuticals

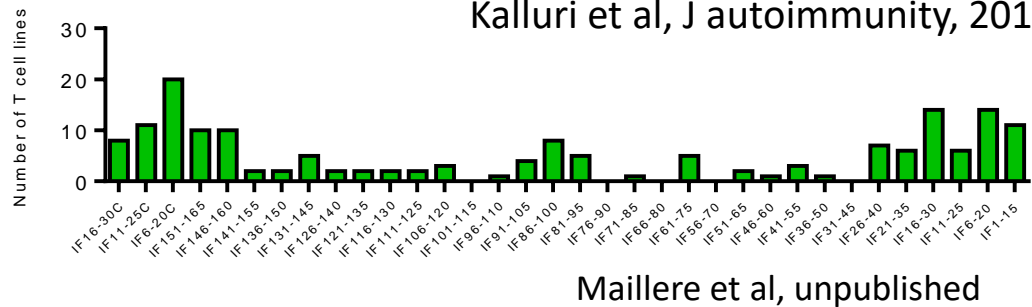
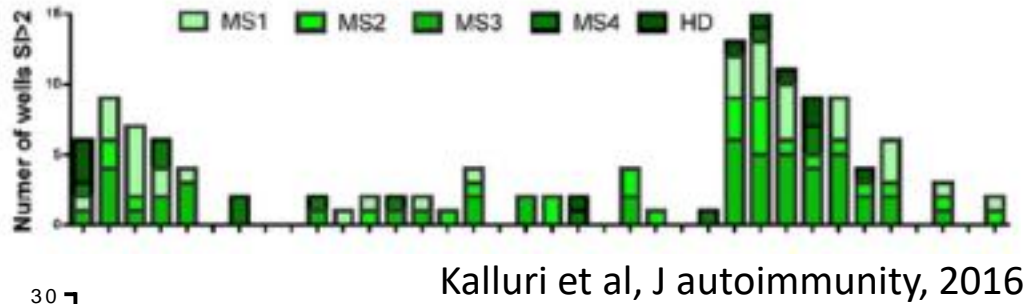
EPO



H2-Relaxin

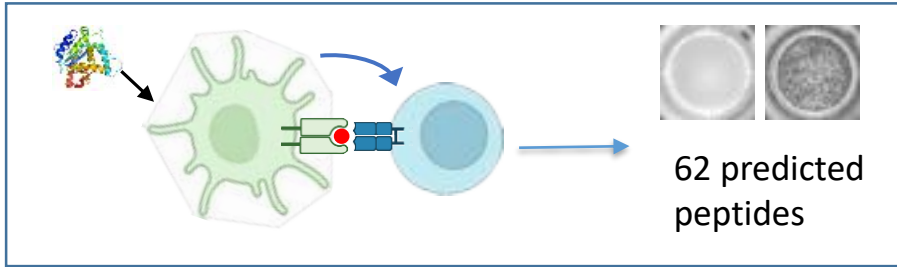


IFN-β

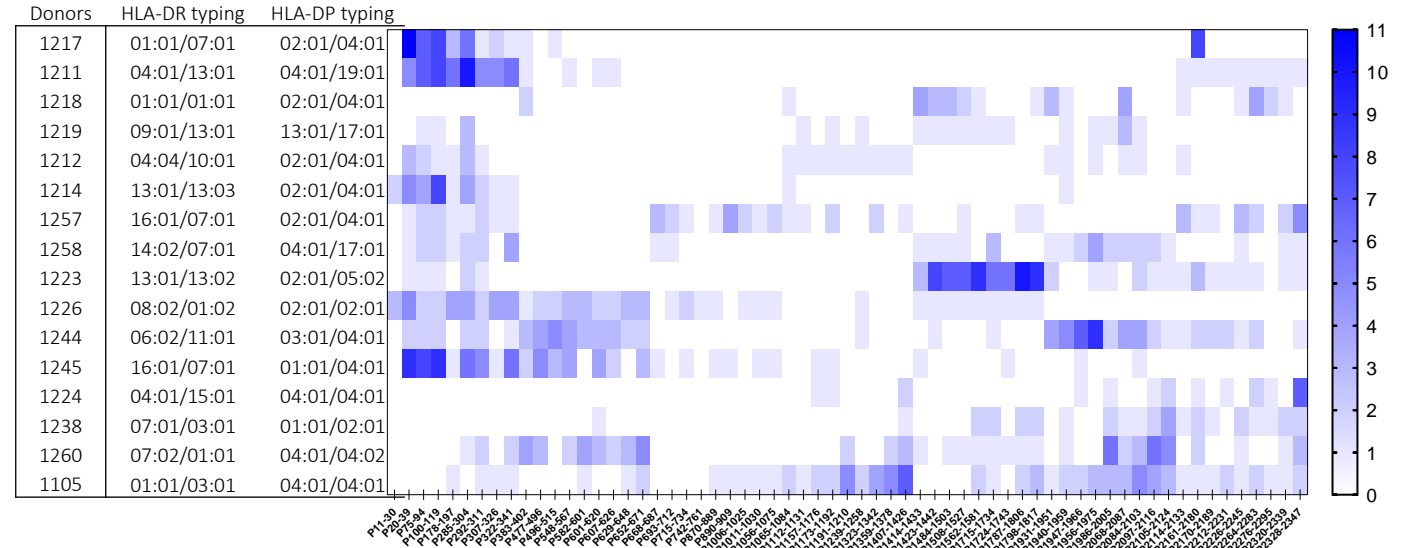


T cell epitopes of recombinant forms of low abundant self proteins are **not mutated**

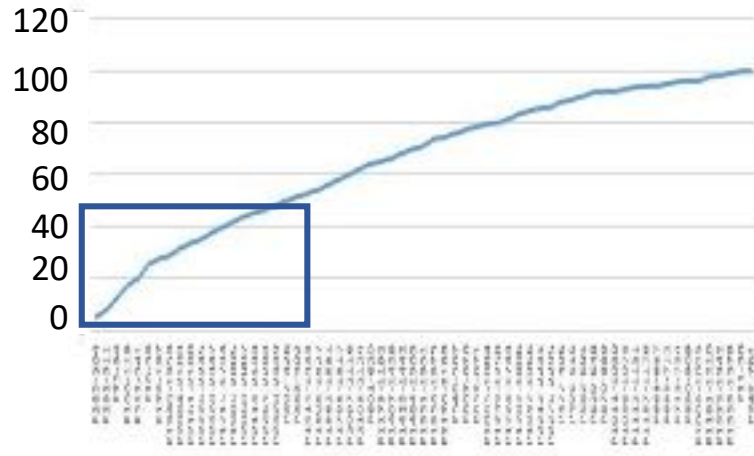
T cell epitopes of FVIII in healthy donors



T cell epitope map



Cumulative % specific T cell lines



872 T cell lines specific for FVIII peptides

18 peptides

- cover 50% of the total T cell response
- Generate a response in all the donors

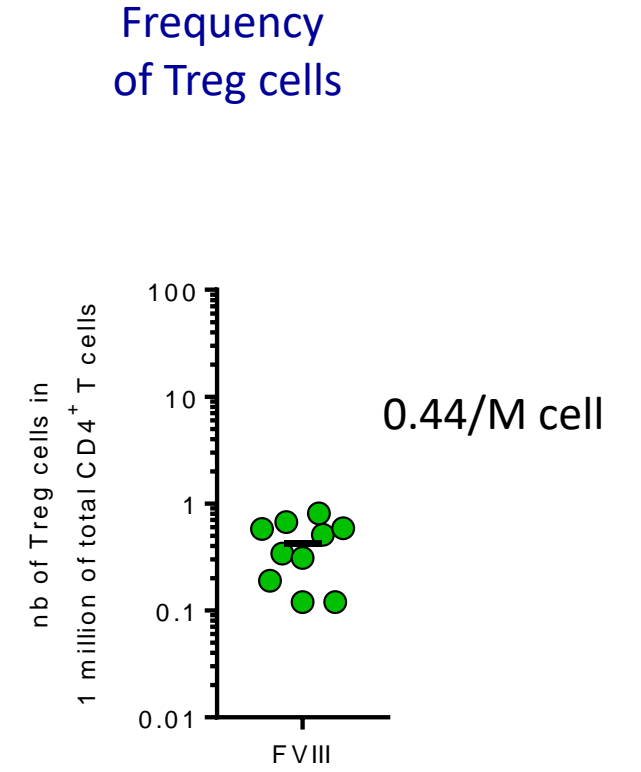
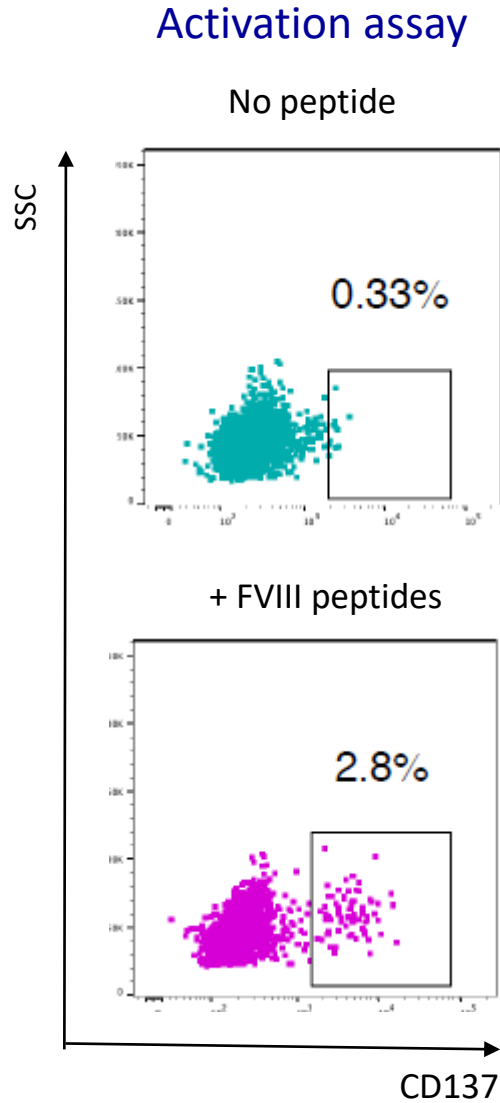
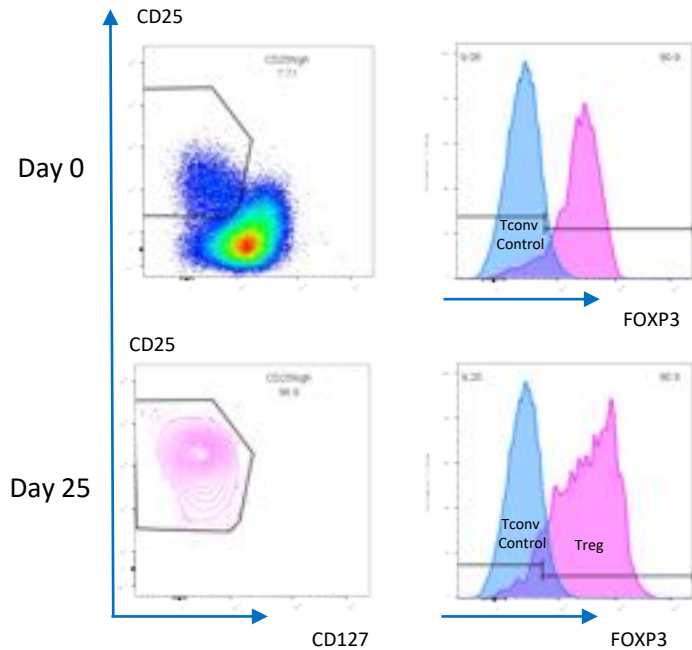
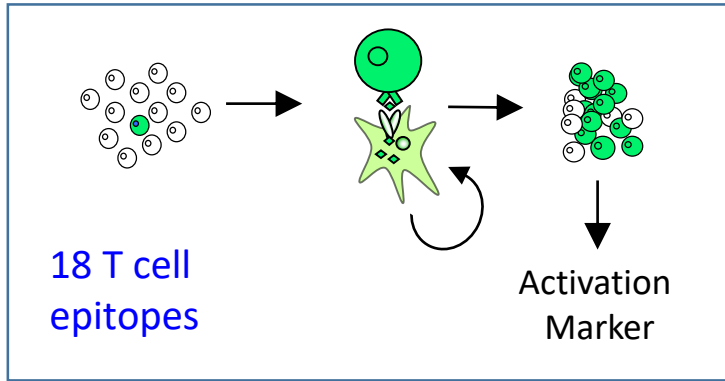
T cell epitopes of FVIII in healthy donors and HA patients

Positions	Sequence	FVIII Domain	T cell response in HA patients
P285-304	VHSIFLEGHTFLVRNHRQAS	A1	/
P292-311	GHTFLVRNHRQASLEISPIT	A1	/
P75-94	DHLFNIAKPRPPWMGLLGPT	A1	/
P100-119	YDTVVITLKNMASHPVSLHA	A1	/
P322-341	LGQFLLFCHISSHQHDGMEA	a1	/
P20-39	ATRRYYLGAVELSWDYMQSD	A1	/
P178-197	LSHVDLVKDLNSGLIGALLV	A1	[1]
P1940-1959	INGYIMDTLPGLVMAQDQRI	A3	/
P2084-2103	KEPFSWIKVDLLAPMIIHGI	C1	[2]
P2161-2180	PPIIARYIRLHPHYSIRST	C1/C2	[3]
P2226-2245	KARLHLQGRSNAWRPQVNNP	C2	[1], [4], [2], [5], [6], [7]
P2328-2347	HPQSWVHQIALRMEVLGCEA	C2	/
P1715-1734	RHYFIAAVERLWDYGMSSSP	A3	/
P1986-2005	KKEEYKMALYNLYPGVFETV	A3	/
P2068-2087	KLARLHYSGSINAWSTKEPF	C1	/
P2114-2133	SLYISQFIIMYSLDGKKWQT	C1	[8], [9], [10], [11]
P2264-2283	TQGVKSLTSMYVKEFLISS	C2	/
P2320-2339	LLTRYLRIHPQSWVHQIALR	C2	[1], [2], [5], [7]

1. Gunasekera D et al. 2023 Front Immunol
2. Ettinger RA et al. 2016 Blood
3. Garnier A et al. 2016 Immunol Cell Biol
4. Ettinger RA et al. 2018 Blood Adv
5. Ettinger RA et al. 2010 Haemophilia
6. Ettinger RA et al. 2009 Blood
7. James EA et al. 2007 J Thromb Haemost
8. Diego VP et al. 2020 J Thromb Haemost
9. Peyron I et al. 2018 Haematologica
10. van Haren SM et al. 2011 Mol Cell Proteomics
11. Jones TD et al. 2005 J Thromb Haemost

HLA-DR and DP restricted T cell epitopes
(inhibition experiments with HLA mAb)

18 FVIII peptides elicit specifically CD4 Foxp3+ Treg cells



Peptide-dependent suppression of allogenic T cells

Immunogenicity of single chain antibodies (nanobodies)



Antibody



Heavy chain
antibody



VHH

- **VHH**

- Initially found in camelidae
- Small size: 12-15 kd
- Single chain
- Flexible: multiple possible combinations

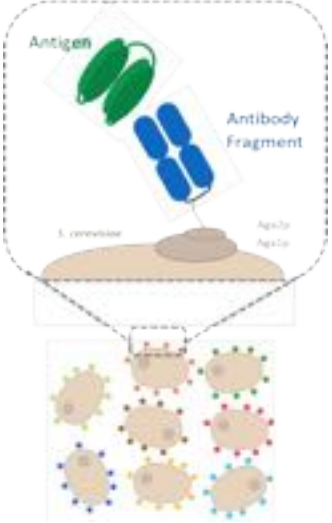
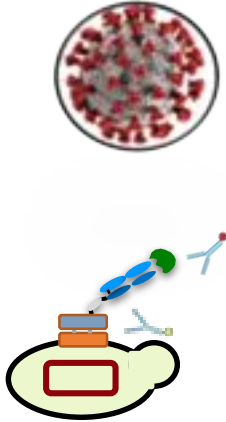
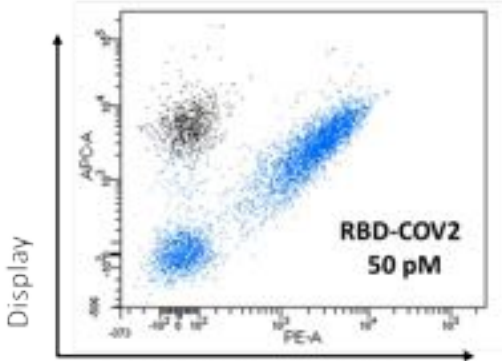
- **Immunogenicity**

- Caplacizumab 2-10%
- envafolimab 36%
- vobarilizumab 11-61%
- sonelokimab 30%
- ozoralizumab 16-44%

Humanisation/germinalisation of the VHH76

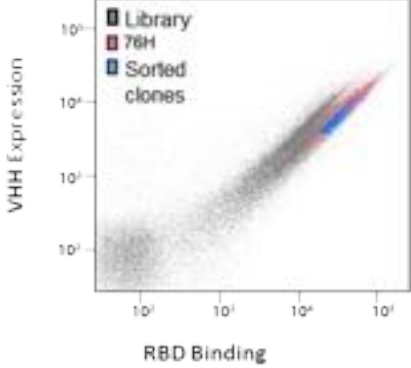
- VHH76:** Laroche et al, Mabs, 2022

Anti-RBD SARS/CoV-2



Library VHH76

- 16 positions
- WT or germline



VHH76 (wt)

VHH 76H

VHH G3



wt

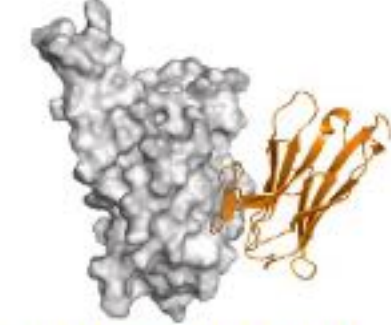
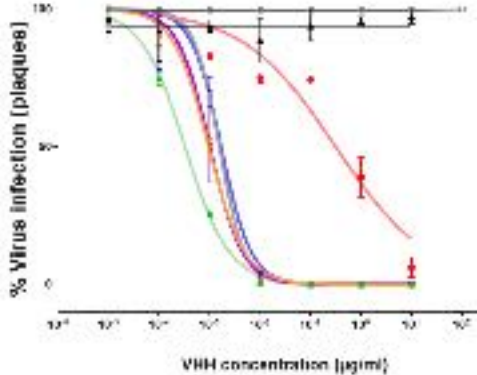
6 mutations

16 mutations

345 pM

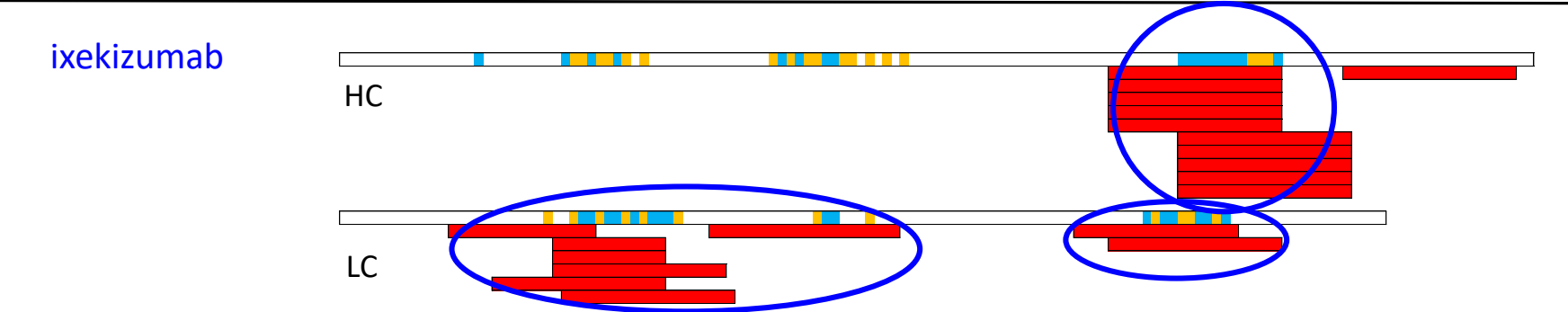
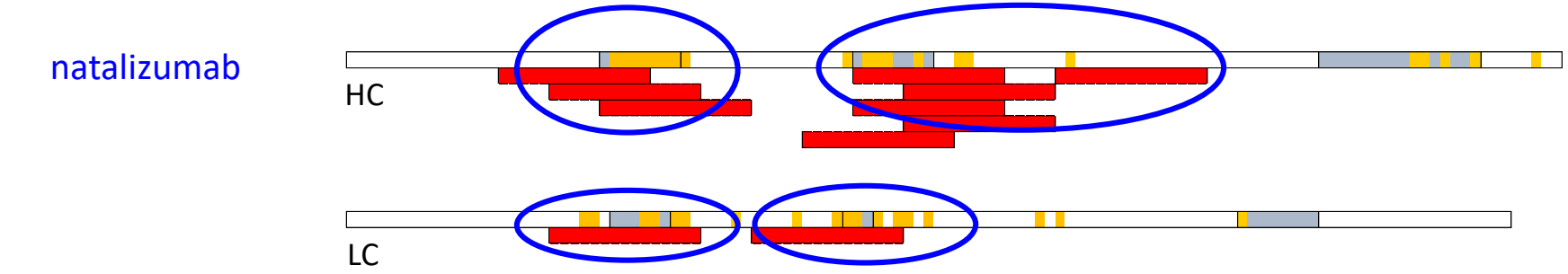
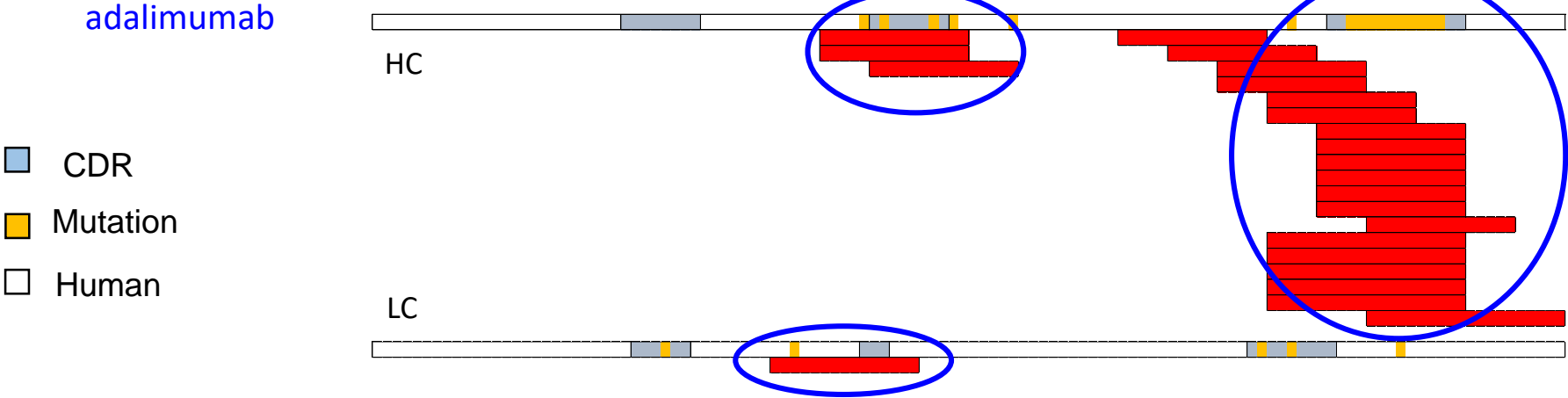
< 100 pM

308 pM



VHH72 mutant 76 (VHH76)

T cell epitopes and mutated regions

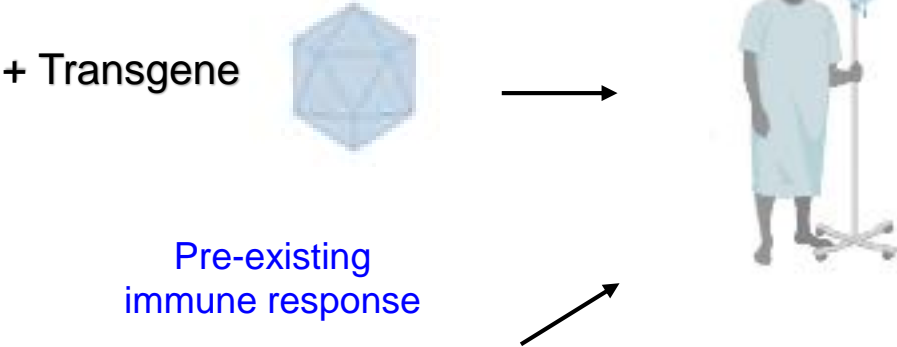


T cell epitopes:
mainly in
mutated regions

Immunogenicity of AAV vectors

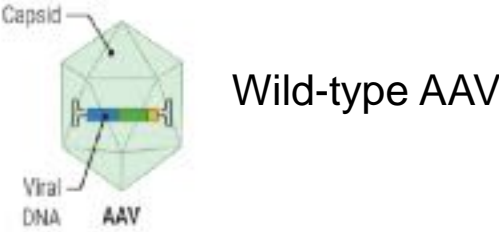
- Adeno-associated virus (AAV) vectors are the leading platform for gene delivery
- Over 200 clinical trials worldwide
- AAV9 : tropism for CNS, muscle, liver, and heart

Recombinant AAV



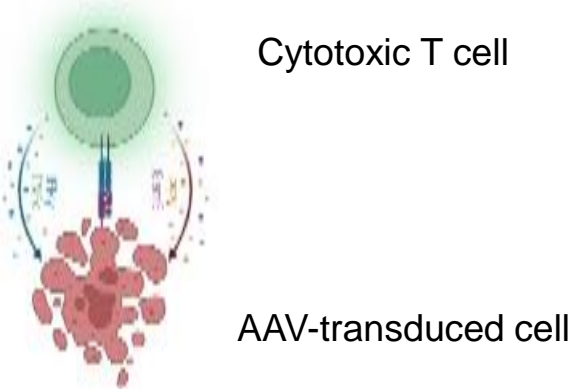
Pre-existing immune response

Natural exposure

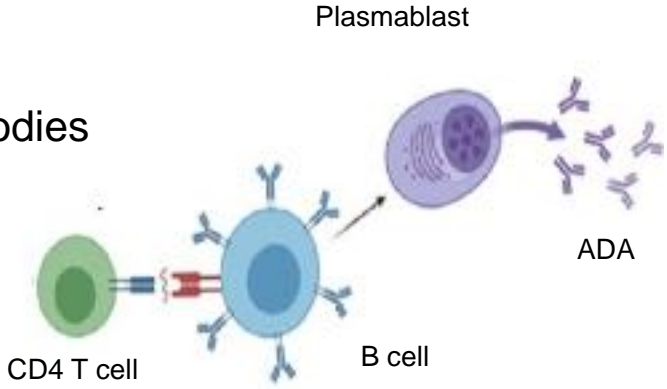


Wild-type AAV

CD8 T cell response

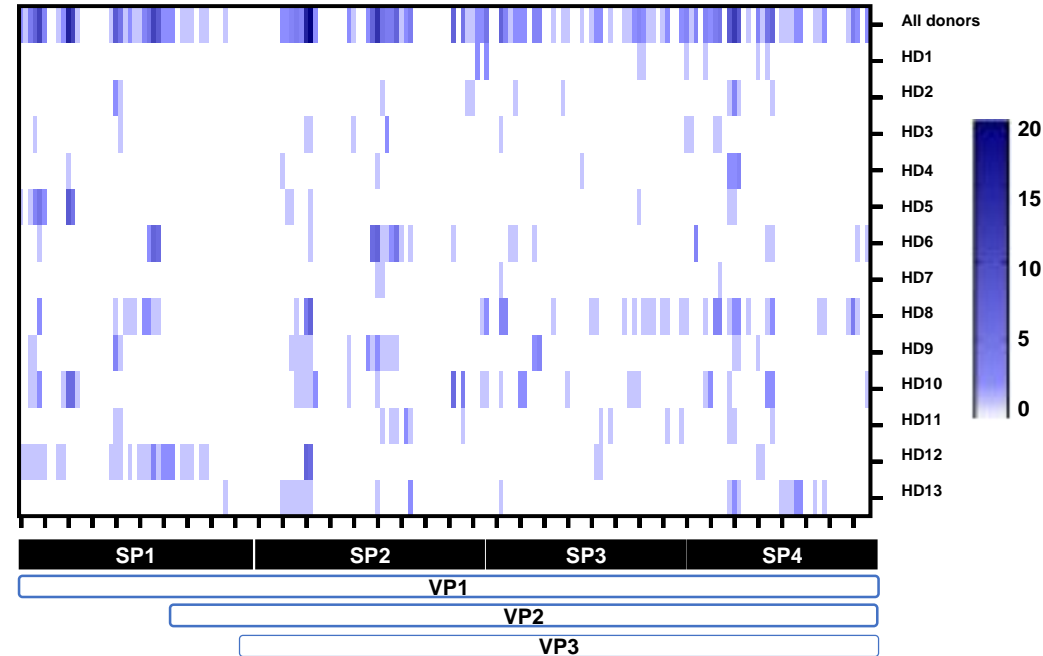
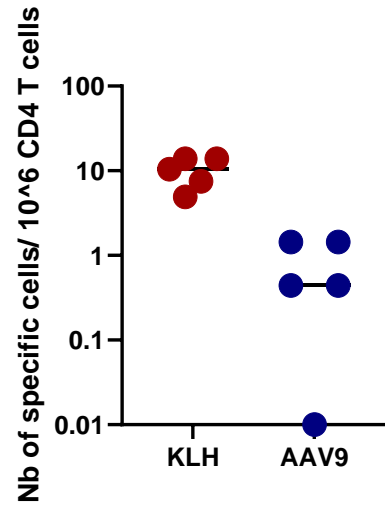


CD4 T cell response
=> Neutralizing antibodies



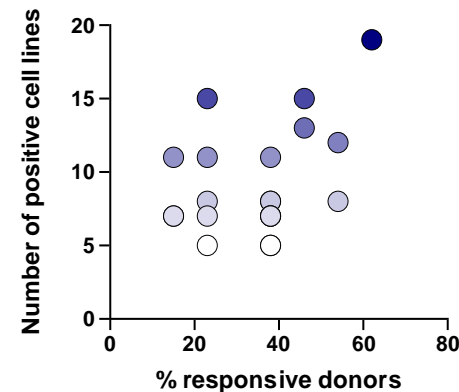
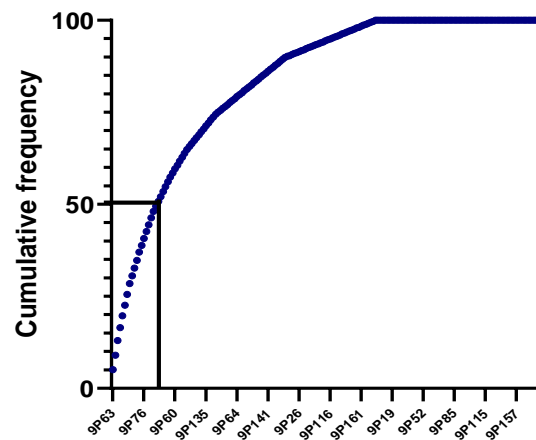
Failure of gene therapy

Specificity of AAV9-specific CD4 T cell



183 peptides VP1
13 healthy donors

374 specific T cell lines

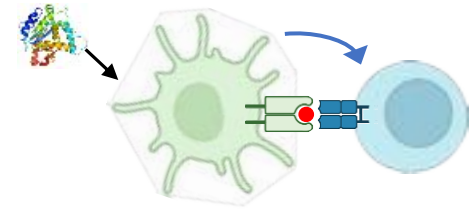


21 peptides:

- 50% total response
- Conserved / non conserved across AAVs

Conclusion

- Using the **sensitive T cell amplification** assay we identified T cell epitopes in multiple biopharmaceuticals : from peptides to AAV vectors
- T cell epitopes widely differ by their **immunoprevalence** and **magnitude**
A **reduced set of peptides** can be sufficient to **cover a large part of the T cell response** (FVIII, AAV)
- T cell epitopes of **recombinant proteins corresponding to low abundant self proteins** are **non mutated**
- T cell epitopes of **therapeutic antibodies** are in **mutated** regions, especially CDRs
- T cell response is detected for the VHH76 and is **diminished by human germinalization**.
T cell epitopes are found in the **CDR3 and in the CDR1 to CDR2 region**
- CD4 T cell eptopes were delineated in AAV gene therapy vectors AAV9 :





université
PARIS-SACLAY

Current team

Evelyne Correia
Stéphane Hua
Rémi Giraudet
Jennifer Mata Orozco
Juan Riveros Velasco
Alicia Roger

Herve Nozach
Steven Dubois
Hugo Dorison
Camille Le Drezen
Eduardo Garcia Calvo
Camille Pitois

Alumni

Sylvain Meunier
Catherine Menier
Moustafa Hamze
Aurélien Azam

Valeria Porcheddu
Batoul Whebi
Adrien Laroche
Benjamin Chalopin
Coline Sivelles
Raphaël Sierocki

Inserm

Marc Pallardy
Isabelle Turbica



<http://www.abirisk.eu/>

Sebastian Spindeldreher
Anette Karle
Niek de Vries
Sabrina Pollastro
Mathieu Allez
Anna Fogdel
Florian Deishammer
Xavier Mariette



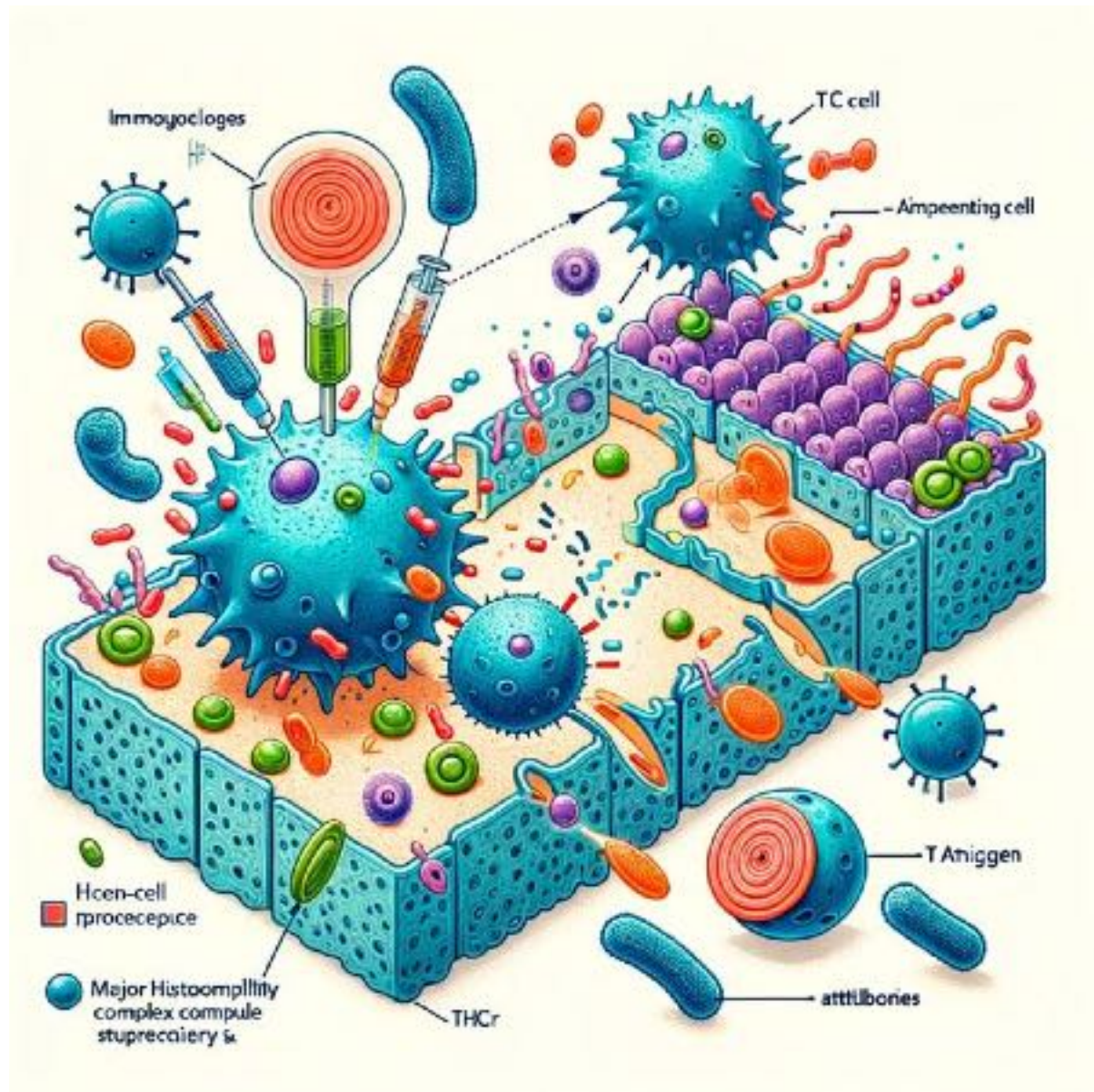
Sebastien Lacroix-Desmazes



Mimoun Azouz
Dean Naisbitt
Adam Lister
Marc Trautwien
Stefan Holtkamp
Guiseppe Ronzitti
Tiziana Labella

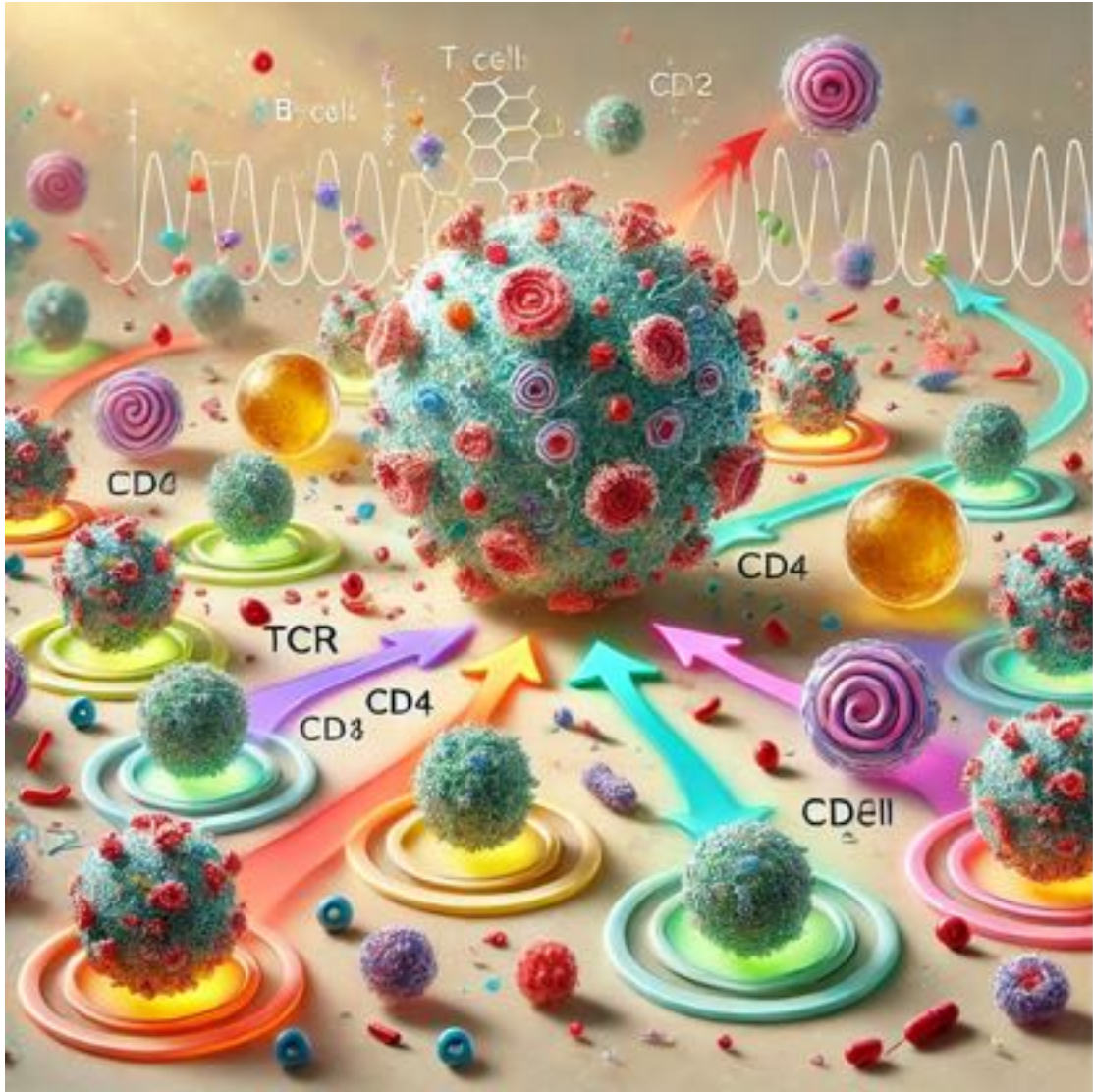


ACCREDITIA



2024

T and B cell cooperation
seen by ChatGPT



2025