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Medicines and Healthcare Technologies Department

## Impact of chemical modifications on T cell response to biopharmaceuticals

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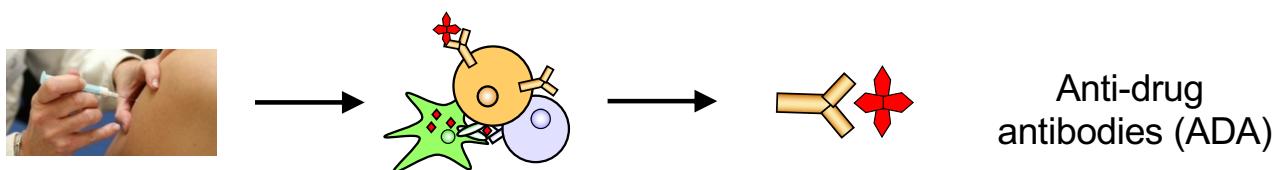
Contact: [bernard.maillere@cea.fr](mailto:bernard.maillere@cea.fr)

**13th Open Scientific EIP Symposium on  
Immunogenicity of Biopharmaceuticals**

Lisbon, Portugal

April 25th – 27th 2022

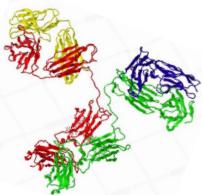
# immunogenicity of therapeutic proteins



- PK alteration
- Therapeutic loss
- Autoimmune or allergic reactions

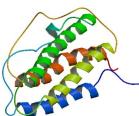
## Chemically modified

Antibodies



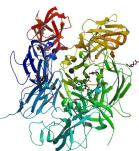
→ Pegylated Fab:  
certolizumab pegol

Cytokines,  
Hormones,  
Growth factors



→ Pegylated: IFN $\beta$ , IFN $\alpha$ , EPO

Replacement  
proteins



→ Pegylated: FVIII, asparaginase

Peptides

→ Non natural amino acids

### Pegylation

- Extension of the half-life
- Reduction of immunogenicity

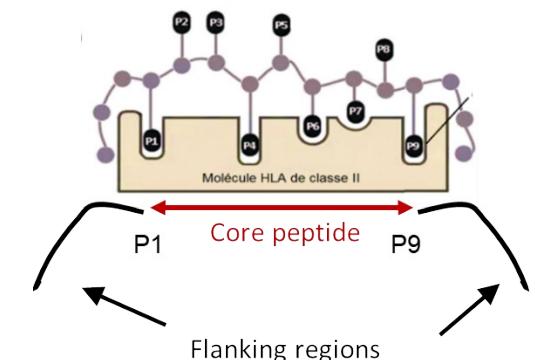
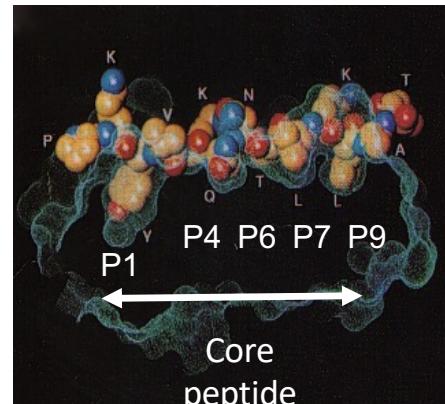
### Non natural amino acids

- Stabilization of conformation
- Reduction of proteolysis

Immunogenicity?  
T cell response?

## Peptide binding to HLA class II molecules

- 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
- Peptide binding motifs :

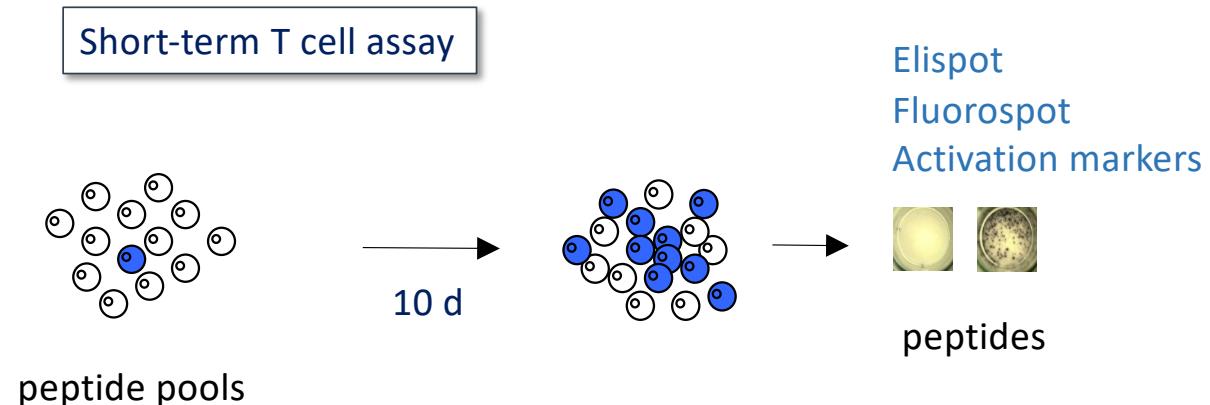
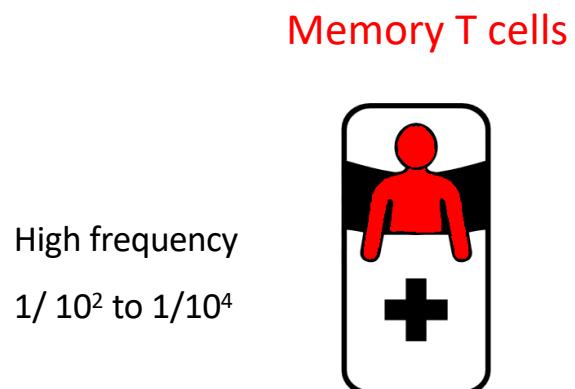
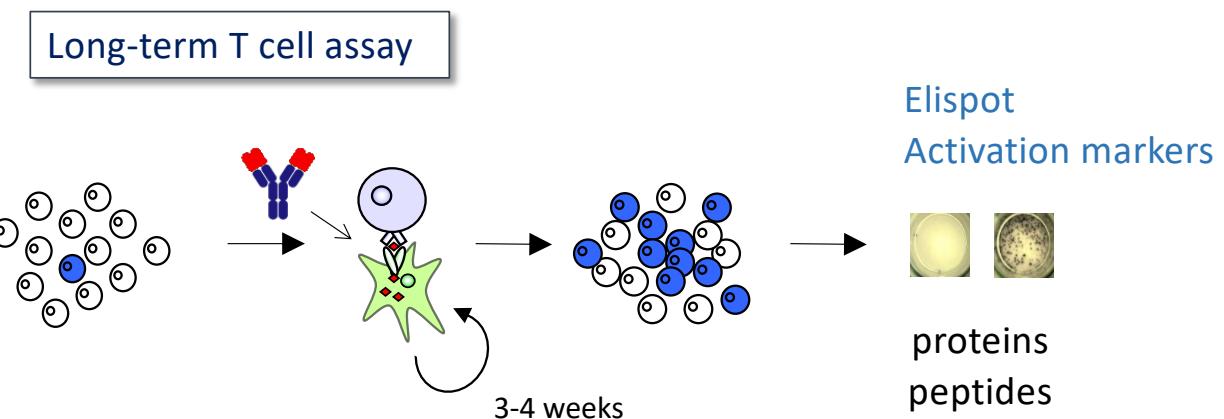
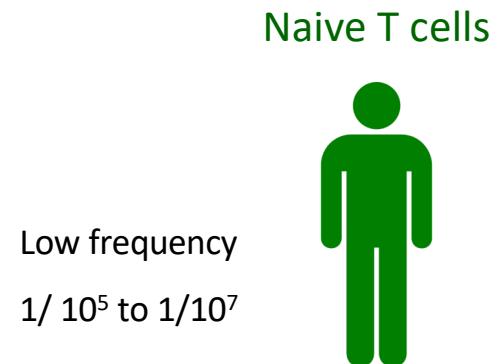


Stern et al., 1994

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

Rammensee et al., 1995

# T cell assays: memory and naive T cells



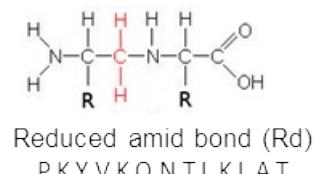
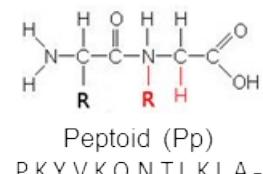
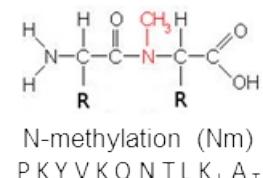
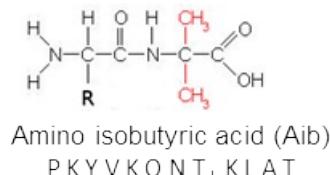
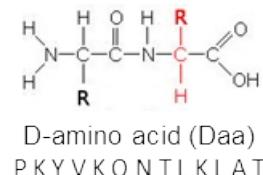
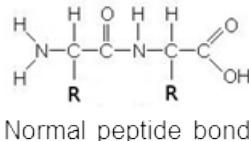
## chemical modifications

- Non natural amino acids
- PEGylation

Immunogenicity? T cell response?

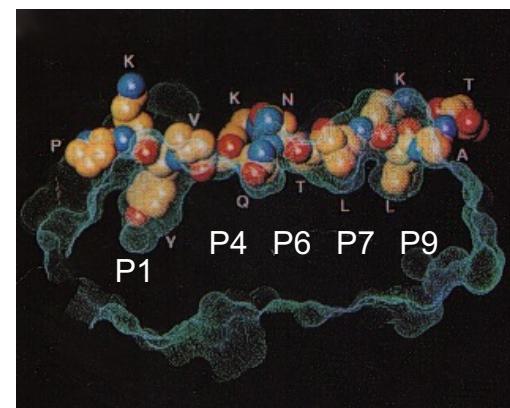
# Introduction of Non-natural Amino Acids Into T-Cell Epitopes to Mitigate Peptide-Specific T-Cell Responses

Aurélien Azam<sup>1,2</sup>, Sergio Mallart<sup>3</sup>, Stephane Illiano<sup>4</sup>, Olivier Duclos<sup>3</sup>, Catherine Prades<sup>1</sup> and Bernard Mailliére<sup>2\*</sup>



## HA 306-318

- Major T cell epitope of Flu HA protein
- Natural/vaccinated immunity to Flu: frequent memory response
- Binding to multiple HLA-DR molecules:  
HLA-DRB1\*0101, 0401, 0701, 1101 & DRB5\*0101

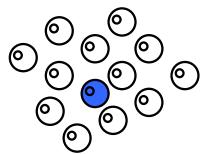


Stern et al., Nature, 1994

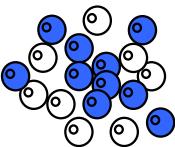
# Antigenicity of HA analogs by HA-specific T cells

Short-term  
T cell assay

Memory response  
To HA peptide

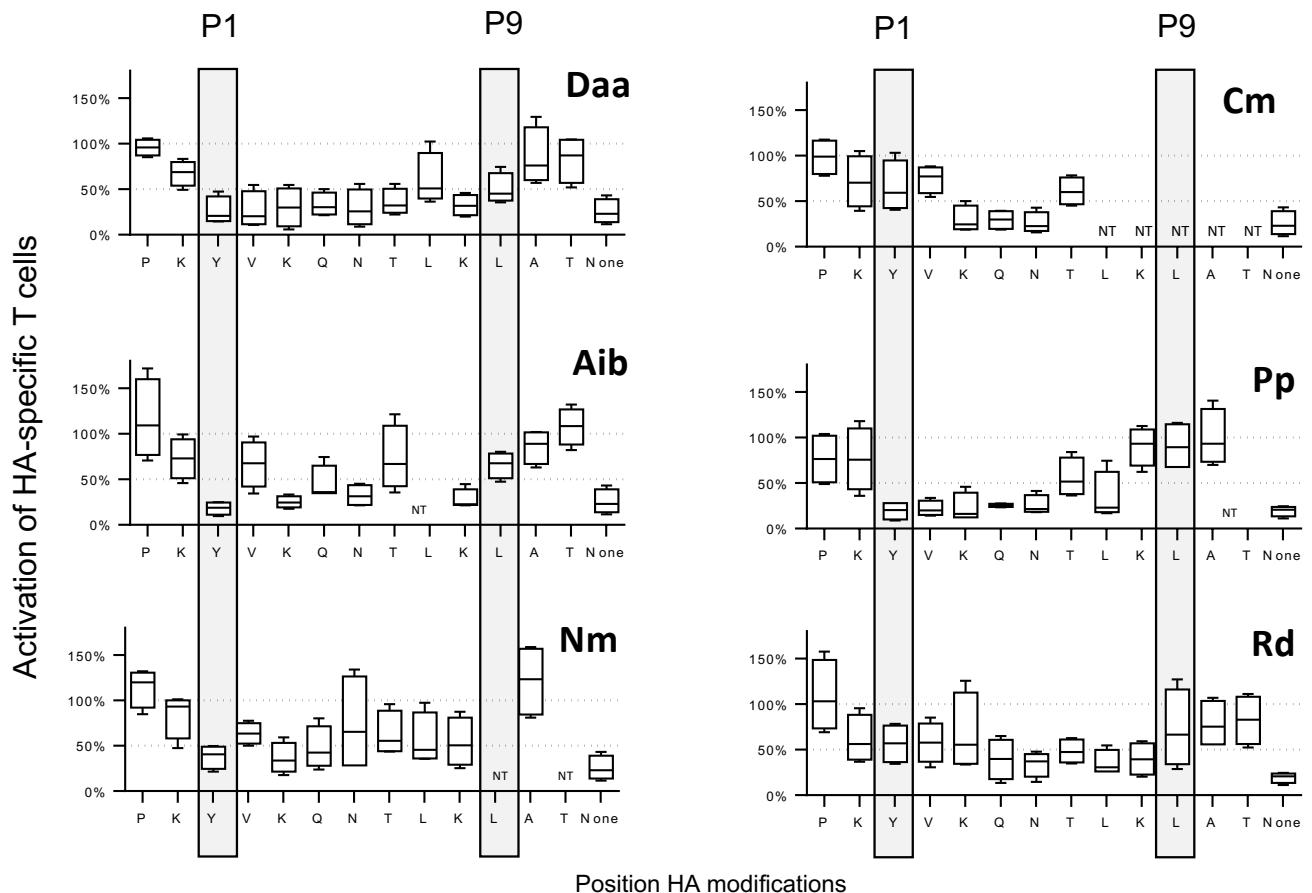


HA Peptide  
(10 d)



Elispot

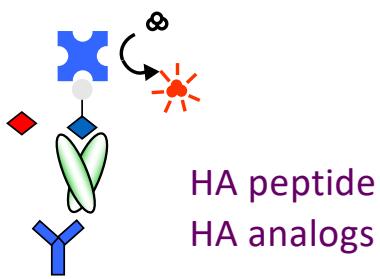
HA analogs



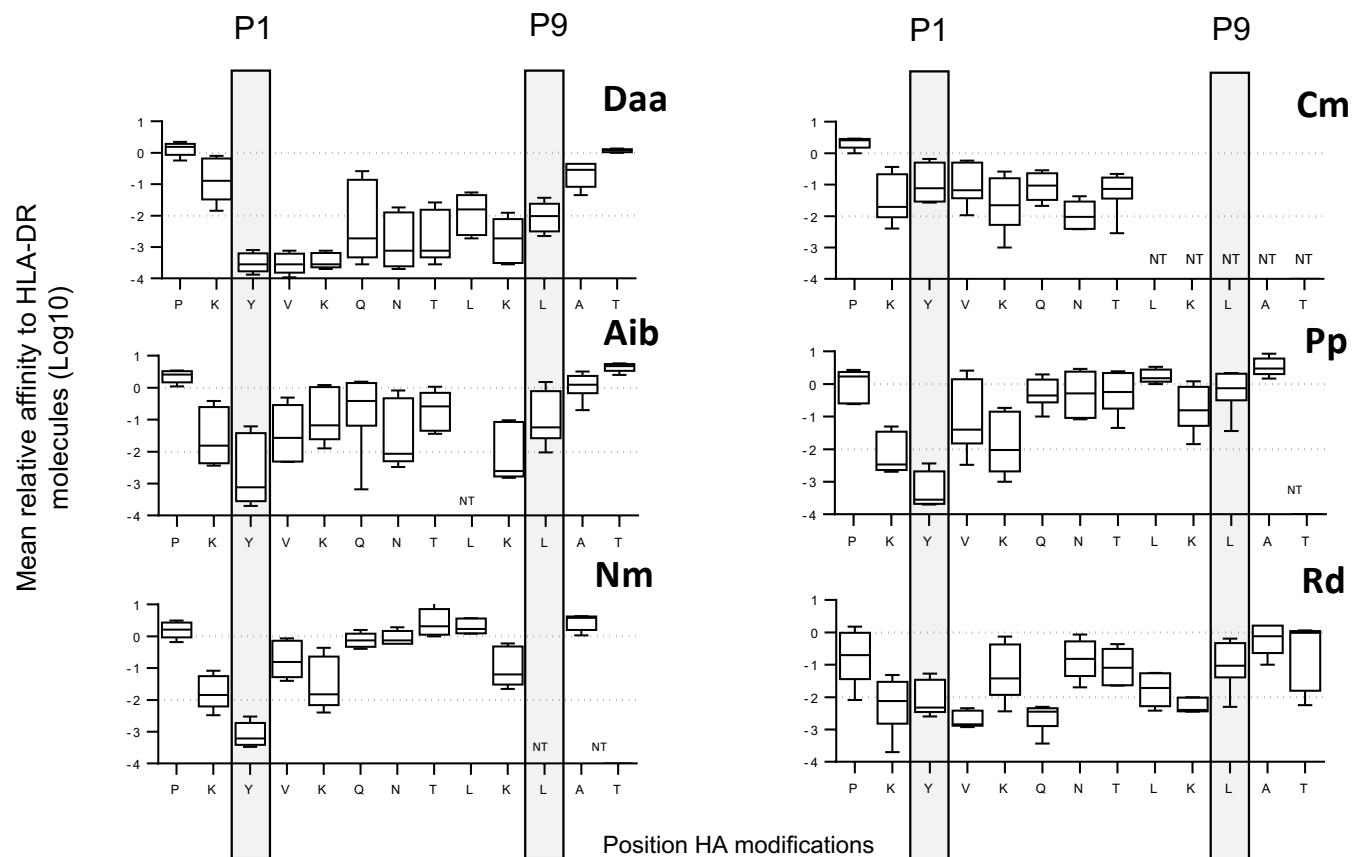
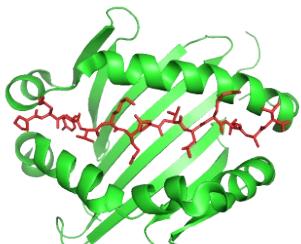
Many positions in the core (P1 to P9)  
diminish T cell activation of HA-specific T-cells

4 donors

# Affinity of HA analogs for HLA-DR molecules

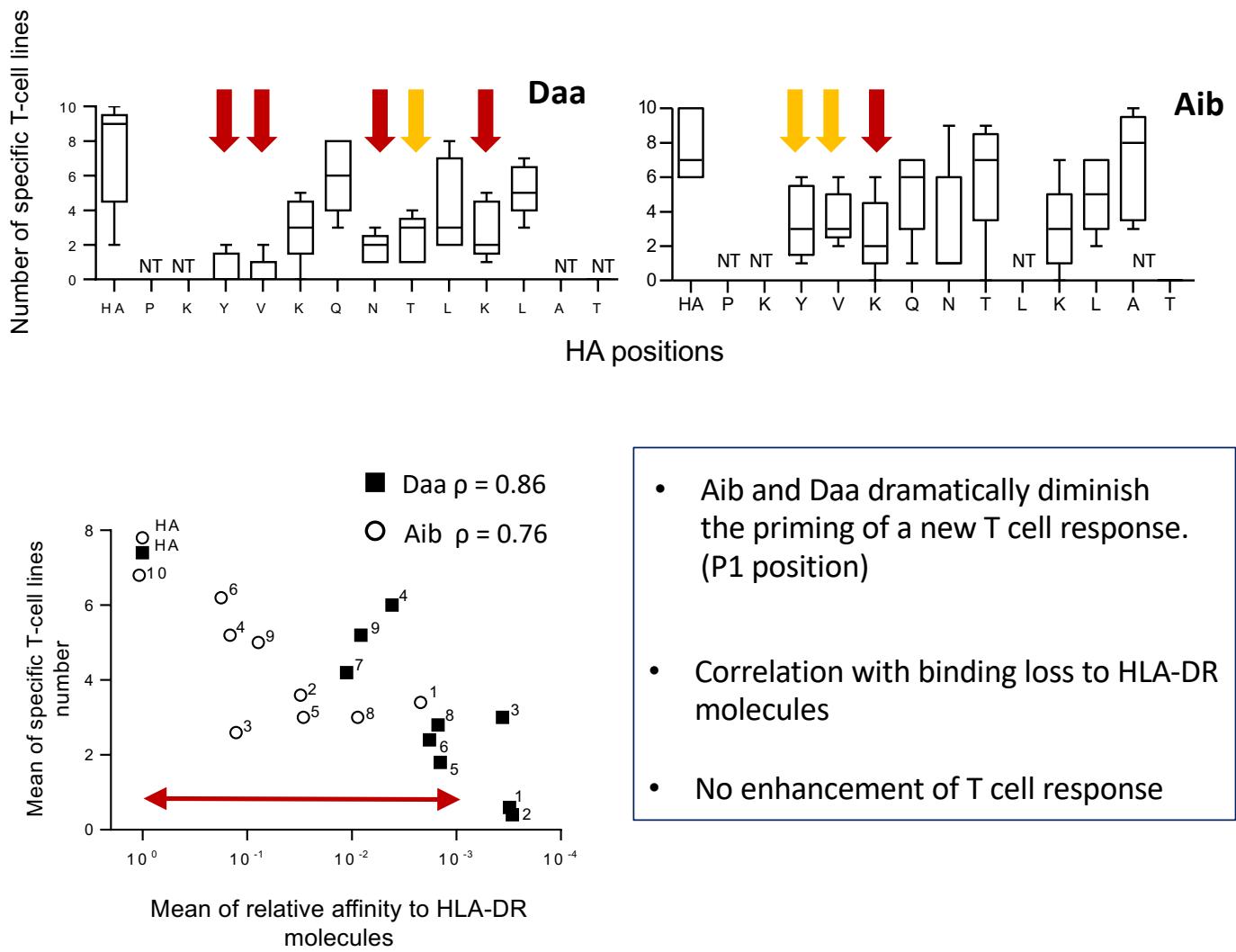
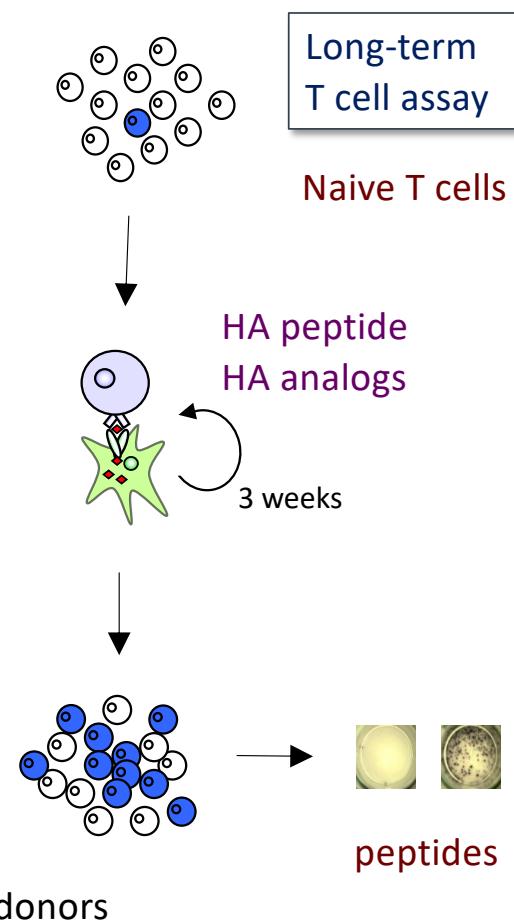


- HLA molecules  
DR1, DR4, DR7,  
DR11, DRB5



- D-AA substitutions in the core lead to strong binding loss (>1000)
- Many substitutions around P1 affect the binding (Aib, Pp, Nm)
- No increase of binding

## T-cell priming capacity of Aib and Daa modified analogs



- Aib and Daa dramatically diminish the priming of a new T cell response. (P1 position)
- Correlation with binding loss to HLA-DR molecules
- No enhancement of T cell response

# D-AA and immunogenicity : an old story

## ROLE OF OPTICAL ISOMERS IN DETERMINING THE ANTIGENICITY OF SYNTHETIC POLYPEPTIDES

By THOMAS J. GILL, III, HANNAH J. GOULD and PROF. PAUL DOTY    Nature , 1963

"The polypeptide composed of D-amino-acids failed to elicit an immune response detectable by the precipitin method in any of the 24 rabbits tested"

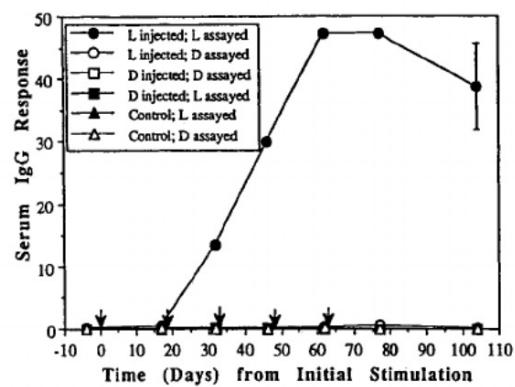
L-AA >> D-AA

## A Comparison of the Immunogenicity of a Pair of Enantiomeric Proteins

D- and L-rubredoxin - Mice

Dintzis et al, Proteins, 1993

L-AA >> D-AA



## Role of Optical Configuration in the Immunogenicity and Specificity of Synthetic Antigens Derived from Multichain Polyproline\*

Jaton et Sela, JBC, 1968

Polymers of Lys, Phe, Glu

L-AA >> D-AA

## Structure-immunogenicity relationship of melittin, its transposed analogues, and D-melittin.

King et al, J immunol, 1994

D- Mellitin - Mice

L-AA >> D-AA

Not cleavable?

# Exploration of Requirements for Peptide Binding to HLA DRB1\*0101 and DRB1\*0401

Hill et al, J Immunol, 1994

Table V. Effect of substitution of D-alanine at each position in simplified peptide on binding to DRB1\*0101 and DRB1\*0401

- D- AA mono-mutations in the core region
- retro inverso

diminish binding to HLA-DR1 and DR4

			IC <sub>50</sub> (nM)	
			DRB1*0101	DRB1*0401
HADP 16.1	RRFAAAAAAAAR	2 ± 2		100 ± 10
D-ala 1	a <sup>a</sup> RFAAAAAAAAAR	2 ± 2		100 ± 10
D-ala 2	RaFAAAAAAAAAR	2 ± 2		320 ± 40
D-phe 3	RRFaaaaAAAAR	400 ± 10	P1	10,000 ± 500
D-ala 4	RRFaAAAAAAAAR	80 ± 5		2,500 ± 100
D-ala 5	RRFaAaAAAAAAAR	20 ± 4		1,400 ± 100
D-ala 6	RRFaaAaAAAAAR	5 ± 2		200 ± 20
D-ala 7	RRFaaaAaAAAAAR	50 ± 5		410 ± 40
D-ala 8	RRFaaaaaAAAAR	25 ± 2		680 ± 50
D-ala 9	RRFaaaaAaAAAAR	40 ± 2		3,500 ± 100
D-ala 10	RRFaaaaAAaAAAAR	100 ± 10		4,700 ± 100
D-ala 11	RRFaaaaAAaAR	20 ± 2	P9	5,100 ± 250
D-ala 12	RRFaaaaAAAaAR	2 ± 1		1,400 ± 100
HADP 41.1	RRAAAAAAAAAR	500 ± 10		20,000 ± 1,000
HADP 18.9	AAYAAAKAAAAAA	1 ± 1		15 ± 2
HADP 18.21	aayaaakaaaaaa	21,000 ± 1,000		>100,000
RI HADP 18.22	aaaaaaakaayaa	9,000 ± 500		31,000 ± 1000
HADP 18.18	AAAAAAKAAAYAA	2,400 ± 500		7,800 ± 500

<sup>a</sup> Lower case letters represent D-amino acids.

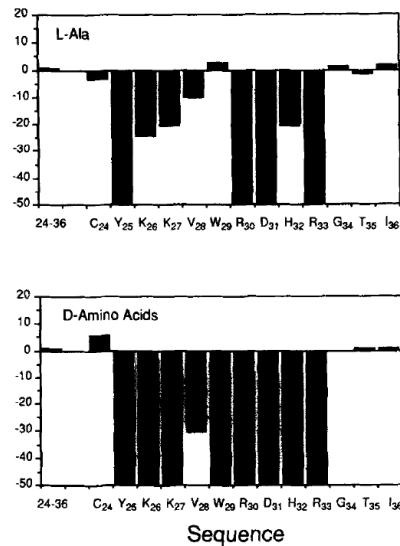
# D-AA and T cell response

## PROBING IMMUNOGENICITY OF A T CELL EPITOPE BY L-ALANINE AND D-AMINO ACID SCANNING

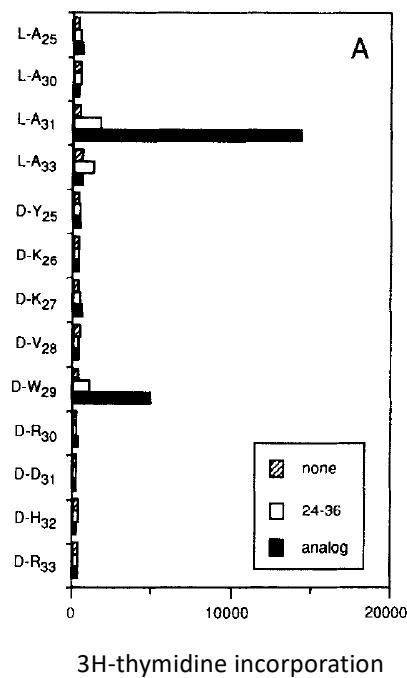
Maillere et al, Mol Immunol, 1995

Snake toxin (*N. nigricollis*)  
peptide 24-36: I-E<sup>d</sup> (BALB/c)

### T Cell hybridoma



### T cell response



### Antibody response

	Antibody titers
24-36	76000 <sup>a</sup>
D-Y <sub>25</sub>	2740
D-K <sub>26</sub>	12960
D-K <sub>27</sub> <sup>b</sup>	25000
D-V <sub>28</sub>	8060
D-W <sub>29</sub> <sup>b</sup>	51000
D-R <sub>30</sub>	11240
D-D <sub>31</sub>	3320
D-H <sub>32</sub>	9620
D-R <sub>33</sub> <sup>b</sup>	30800

D-AA mono-mutations in the core region diminish T and B cell response

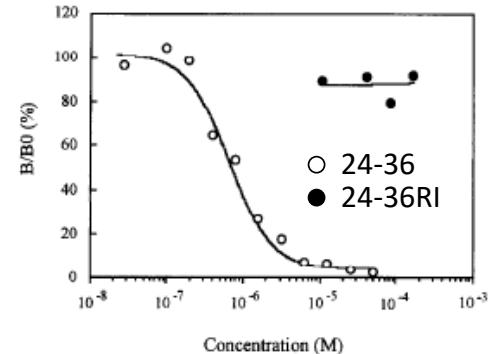
# Immunogenicity of Retro-Inverso peptide

ON THE IMMUNOGENIC PROPERTIES OF RETRO-INVERSO PEPTIDES. TOTAL RETRO-INVERSION OF T-CELL EPITOPE CAUSES A LOSS OF BINDING TO MHC II MOLECULES

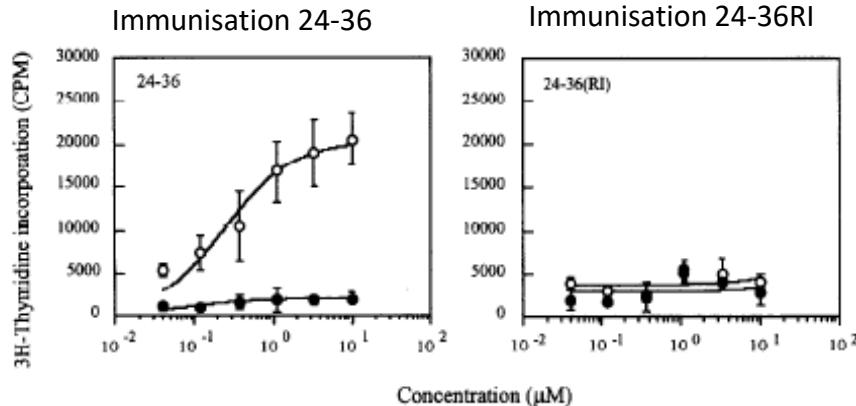
Maillere et al, Mol Immunol, 1997

## MHC II binding

Hydrogen bonds with MHC molecules are crucial



## T cell response

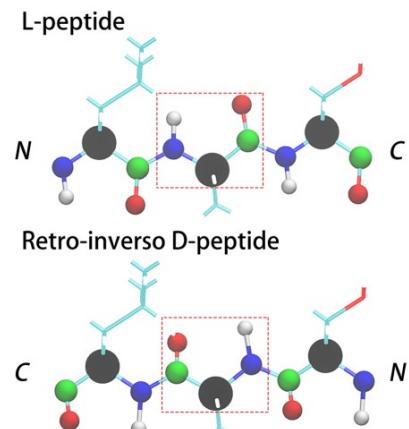


## Retro inverso:

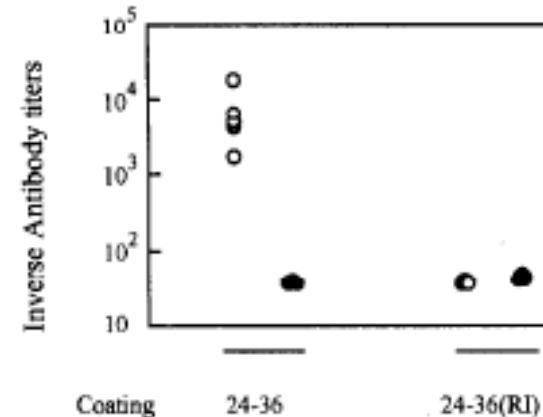
D-AA with a reverse sequence

- Good orientation of the side chains
- Reverse peptidic backbone

peptide 24-36: I-E<sup>d</sup> (BALB/c)



## Antibody response



## Chemical modifications

- Non natural amino acids
- PEGylation

# Pegylation Reduces the Uptake of Certolizumab Pegol by Dendritic Cells and Epitope Presentation to T-Cells

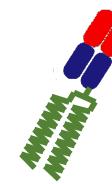
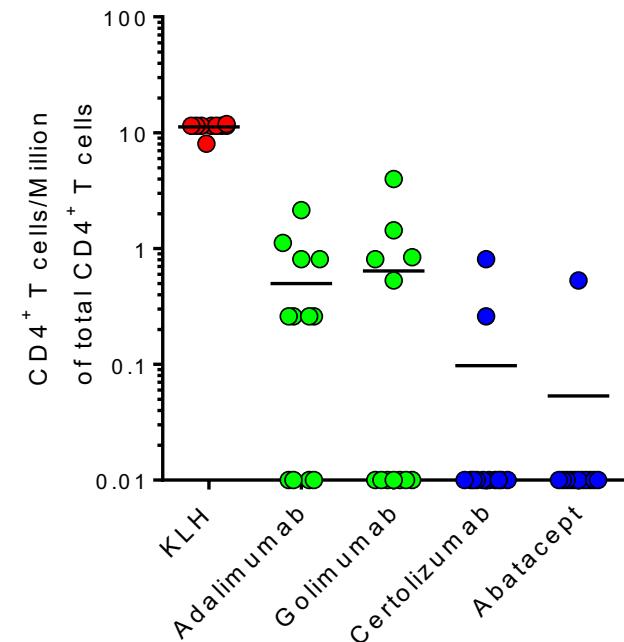
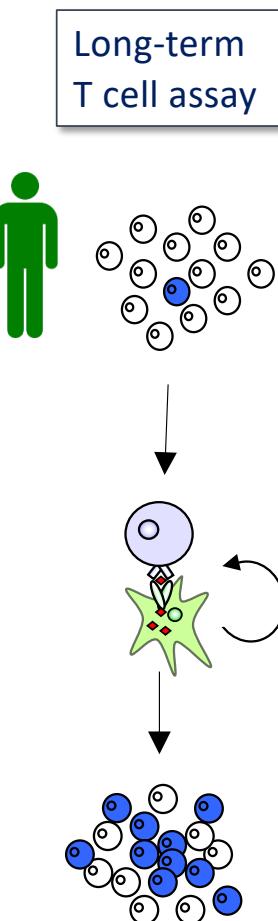
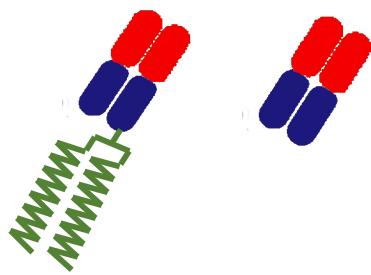


Marie de Bourayne<sup>1</sup>, Sylvain Meunier<sup>1</sup>, Samuel Bitoun<sup>2</sup>, Evelyne Correia<sup>1</sup>, Xavier Mariette<sup>2</sup>, Hervé Nozach<sup>1</sup> and Bernard Maillère<sup>1\*</sup>

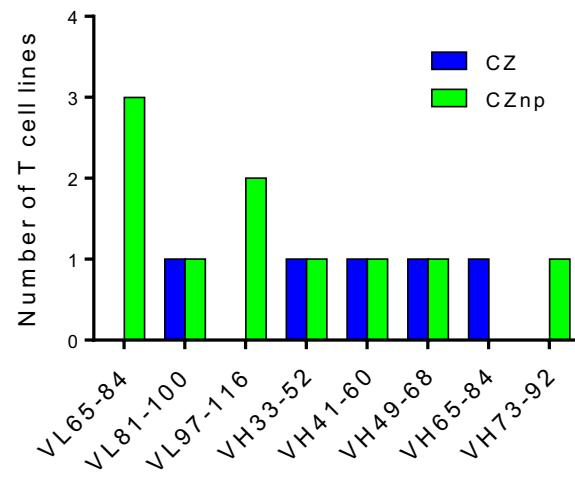
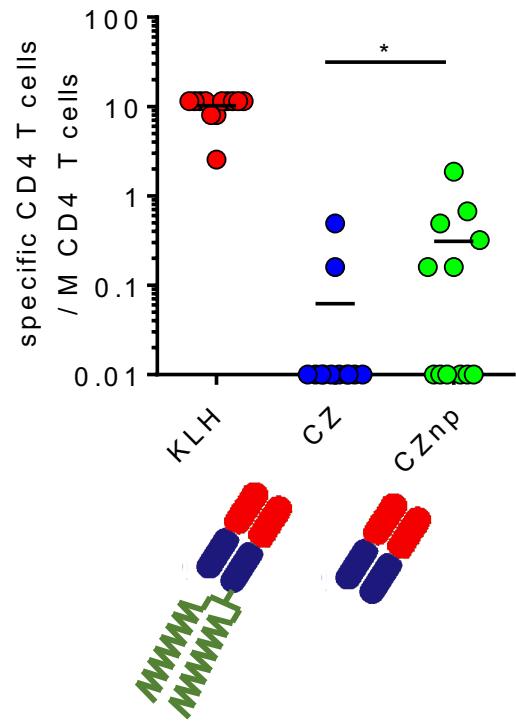
Accepted : February, 4, 2022

## Certolizumab pegol (CTZ):

- Fab of a humanized anti-TNF antibody
- Conjugated to a two branched PEG (40kD)
- PEG increases half-life
- Immunogenicity incidence variable but mainly low titers

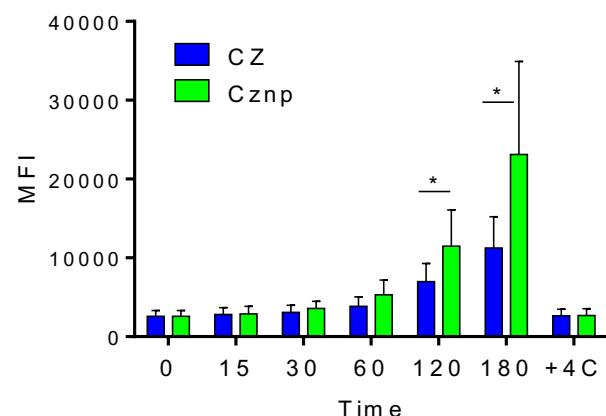
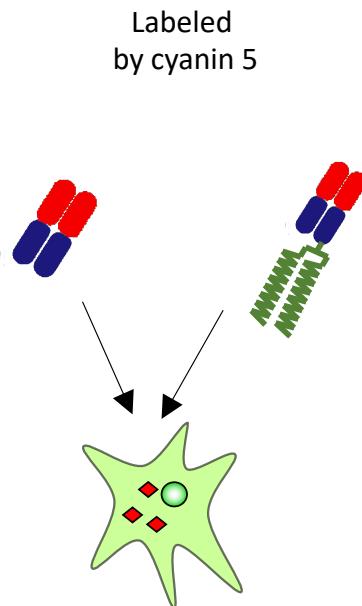


## Pegylation diminishes T cell priming

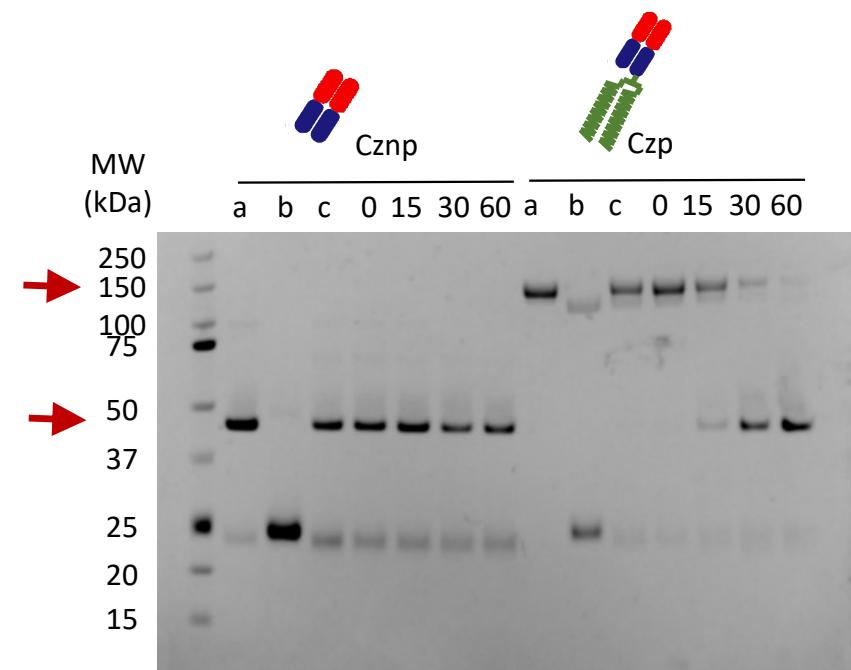


- More T cell precursors with Cznp than CZ
- Many shared T cell epitopes

## Pegylation diminishes the capture by dendritic cells



- Internalization: CZ < CZnp



- PEG does not protect from Cathepsin B degradation

## Take-home messages

- Introduction of **non-natural amino acids** into T-cell epitopes reduces T cell activation and priming (Daa Aib)
- **Pegylation** reduces the uptake of certolizumab pegol by dendritic cells and epitope presentation to T-cells

Foreign = Non immunogenic

# Acknowledgments



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Sebastian Spindeldreher

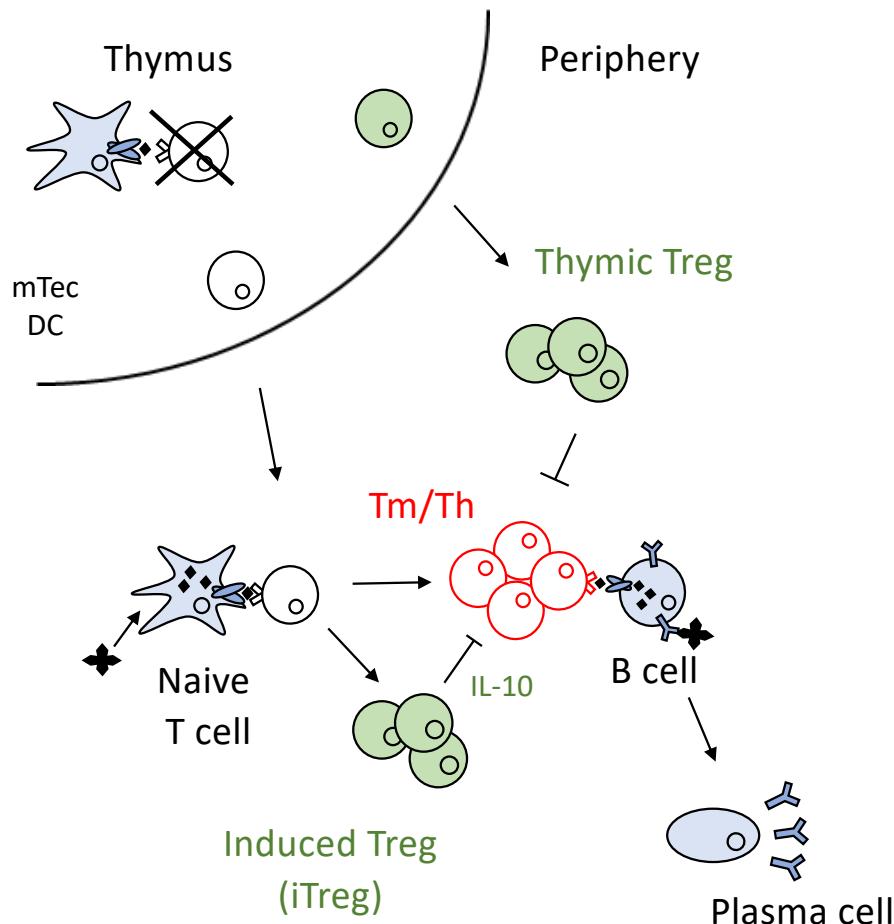


<http://www.abirisk.eu/>

Niek de Vries  
Sabrina Pollastro  
Mathieu Allez  
Anna Fogdel  
Florian Deishammer

And all the WP3 partners

# Regulation of ADA response: evidence of induced Treg



## IL-10-Producing Infliximab-Specific T Cells Regulate the Antidrug T Cell Response in Exposed Patients

Vultaggio, J Immunol, 2017

- : Functional characteristics of IFX-specific T cells, including regulatory cytokines
- Serum IL-10 levels were increased upon drug infusion
- IL-10 mRNA expression was detected in IFX-stimulated PBMCs
- IL-10+ T cell clones suppress IFX-specific T effector cell response through IL-10

