



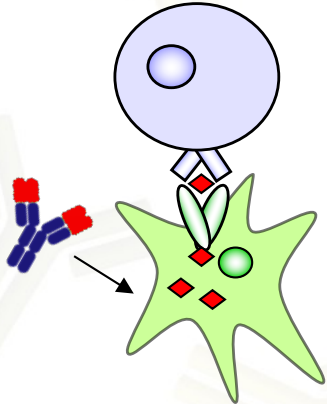
Frederic joliot Institute  
Service of molecular engineering of proteins  
Saclay, France

# Response to T cell epitopes of therapeutic antibodies in healthy donors and in patients

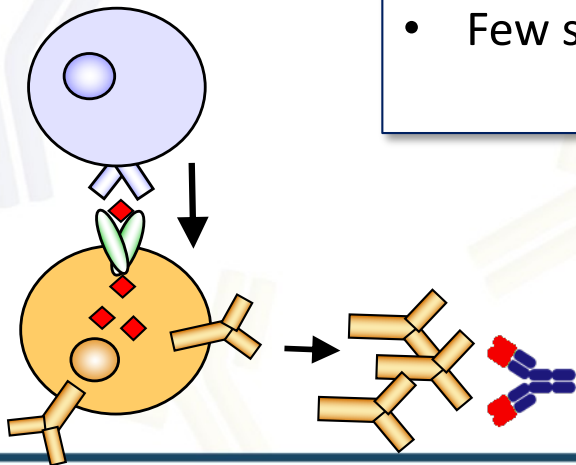
B. Maillere, PhD



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

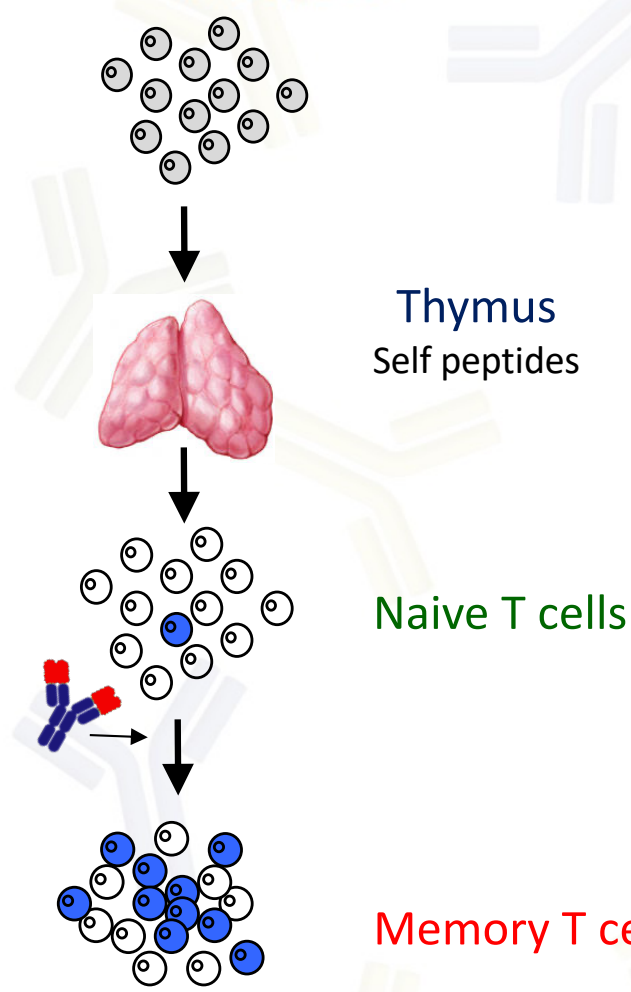


- T cells provide help to B lymphocytes to produce ADA : initiator of the reponse
- Lack of antigen-specific T cells dramatically limits Ab response (nude mice)



- T cell response from healthy donors:  
**perspective of prediction**
- Few studies with patients (E Maggi, B Hemmer)

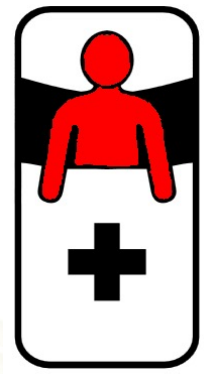
# T cell repertoire: healthy versus patients



Healthy donors



Amplitude and occurrence of T cell response?



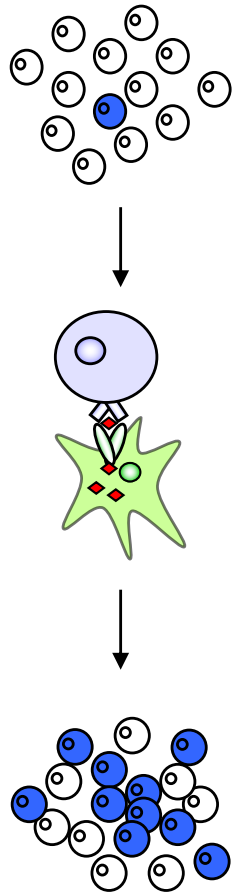
Patients

T cell epitopes?

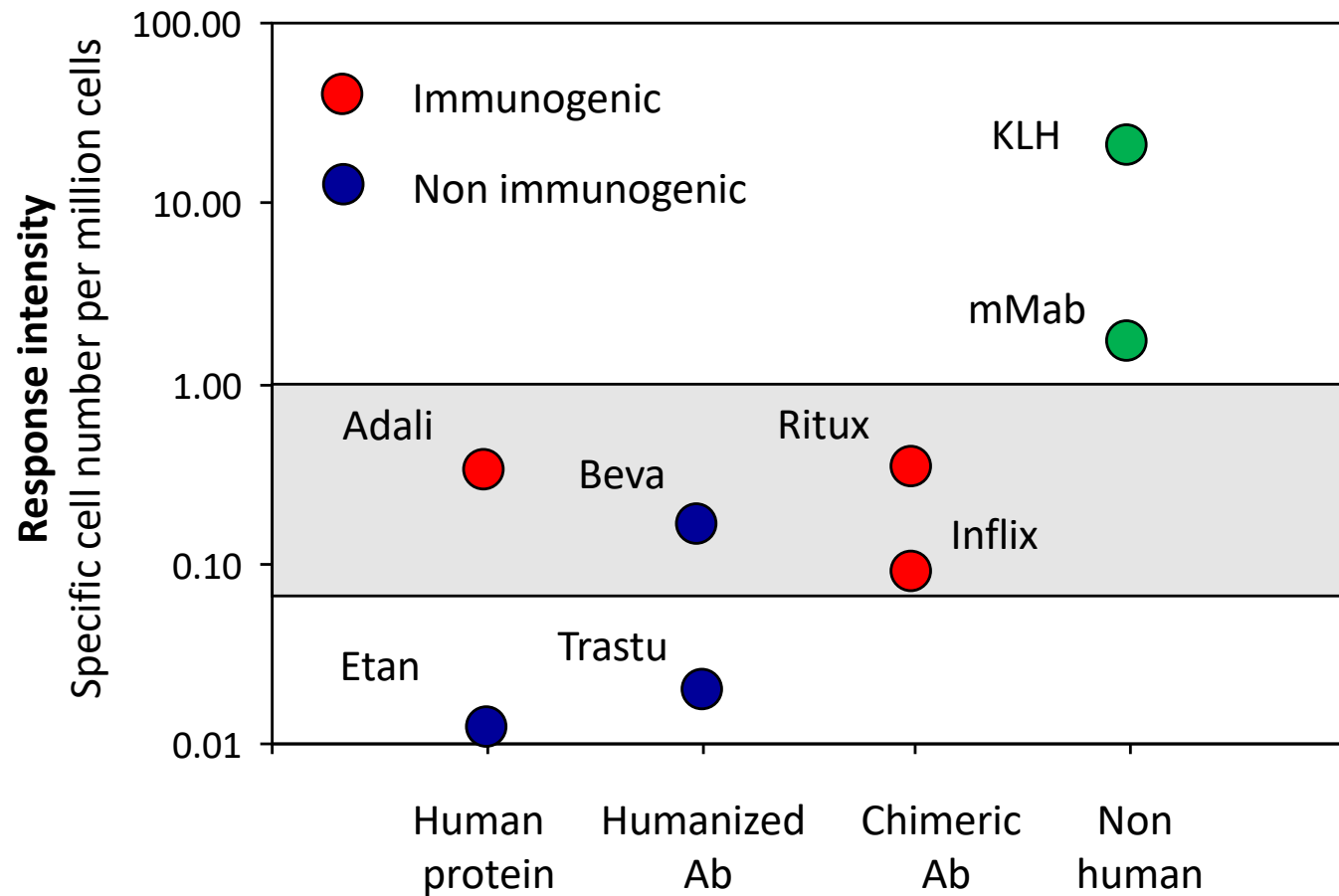
# Quantitative analysis of the CD4 T-cell repertoire specific to therapeutic antibodies in healthy donors

(Maillere, FASEB J, 2011)

Long-term  
T cell assay



Healthy donors



Relationship between size of naive T cell repertoire and immunogenicity

# Humanization of antibodies



Chimeric

- **RITUXIMAB**  
Anti-CD20  
Non-Hodgkin lymphoma: 0.6%  
SLE, RA, Sjogren: 17-50%
- **INFLIXIMAB**  
Anti-TNF $\alpha$   
Crohn, RA, SPA: 30-50%



Humanized

- **NATALIZUMAB:**  
Anti- $\alpha$ 4 integrin  
Multiple sclerosis: 6-21%



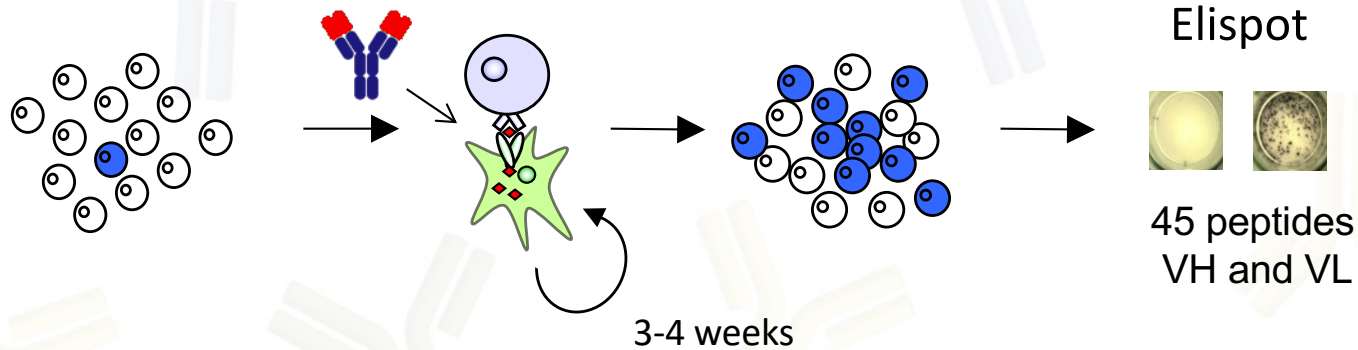
Fully human

- **ADALIMUMAB**  
Anti-TNF $\alpha$   
RA: 30%

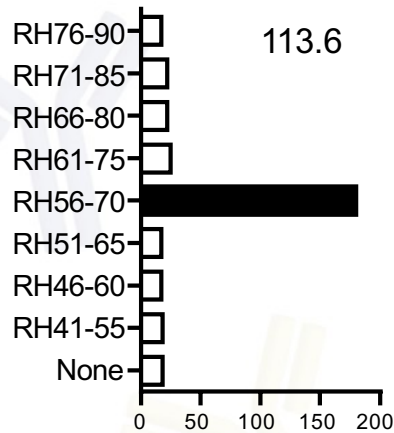
?: ADA rate

# Long-term T cell assays

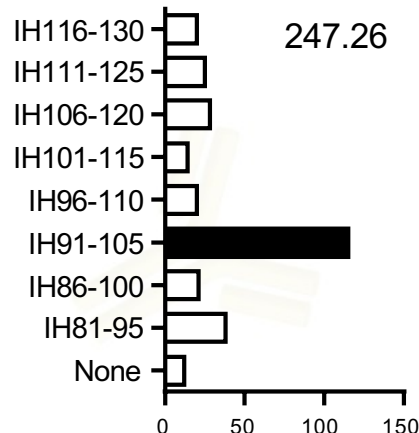
Naive T cells



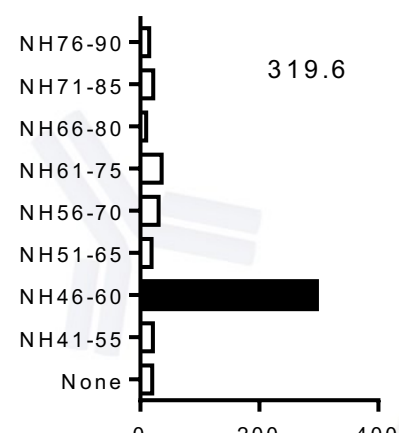
Rituximab



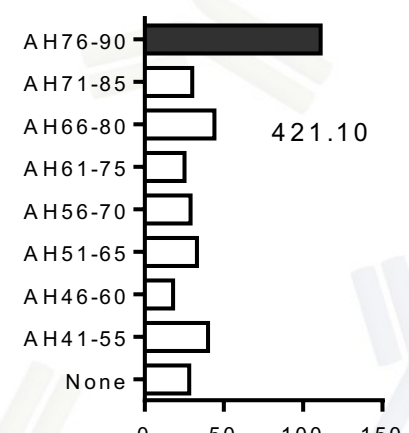
Infliximab



Natalizumab



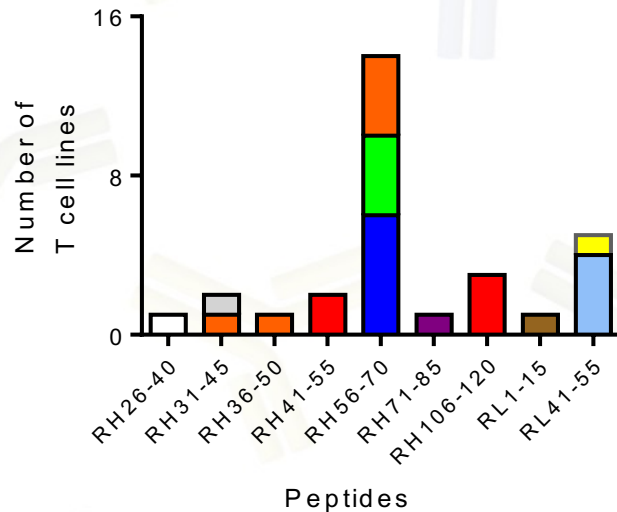
Adalimumab



IFN-γ spots

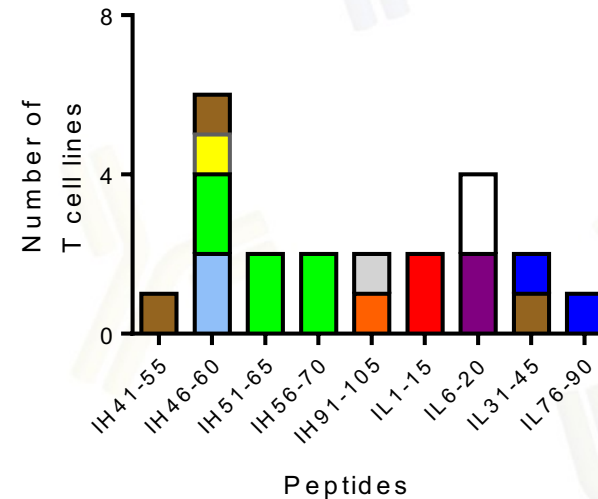
# T cell epitope mapping of Rituximab and Infliximab in healthy donors

## Rituximab



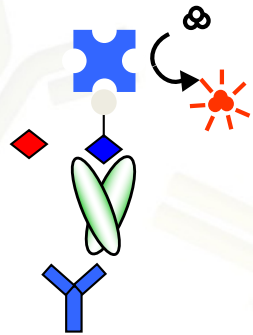
- 11 responders from 15 donors
- 9 epitopes
- VL and VH
- < 3 epitopes/donor
- 3 peptides common to several donors

## Infliximab



- 12 responders from 15 donors
- 9 epitopes
- VL and VH
- < 3 epitopes/donor
- 3 peptides common to several donors

Hamze et al, Frontiers Immunol, 2017



- 11 HLA molecules  
DR1, DR3, DR4, DR7,  
DR9, DR11, DR13,  
DR15, DRB3, DRB4,  
DRB5

Peptides	HLA molecules						HLA haplotypes of responders			
	DR1	DR4	DR7	DR9	DR11	DR15				
RH26-40	216	88	32	177	196	0.4	DR1/DR8			
RH31-45	2	nd	2	60	1.5	122	DR11/DR14	DR1/DR13		
RH36-50	32	156	250	589	378	>1 826	DR11/DR14			
RH41-55	45	>462	3 341	106	463	71	DR13/DR16	DR15/DR15		
RH56-70	>2 404	211	>28 677	12 500	13	>1 826	DR7/DR11	DR11/DR14	DR3/DR11	
RH71-85	4	>462	12 500	816	ND	467	DR3/DR15			
RH106-120	58	>462	40	100	>37 796	11	DR13/DR16			
RL1-15	20	90	10	33	447	117	DR9/DR13			
RL41-55	1	0.1	0.7	2	5	12	DR4/DR7	DR11/DR15		
IH41-55	2 760	10 000	22 727	>4 979	11	238	DR11/DR13			
IH46-60	200	2 424	5.2	657	5	0.4	DR1/DR7	DR4/DR15	DR7/DR11	DR11/DR13
IH51-65	30 000	25 714	29 545	824	1 479	2 000	DR4/DR15			
IH56-70	3 333	1 863	6 818	962	252	>1 335	DR4/DR15			
IH91-105	6	10	9	48	3	13	DR1/DR13	DR11/DR16		
IL1-15	11	>10 526	35	474	7 500	24	DR3/DR7			
IL6-20	17	25 714	45	21	3 260	2	DR7/DR9	DR1/DR13		
IL31-45	183	5	3 015	5 556	362	3	DR1/DR4	DR3/DR4		
IL76-90	20 000	>10 526	>12 205	>4 979	>11 604	27	DR4			

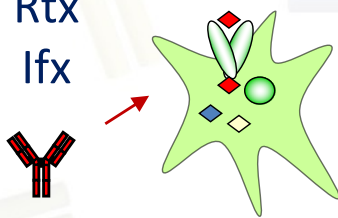
Rituximab

Infliximab

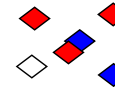
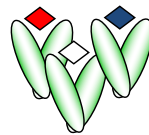
Most of the T cell epitopes exhibit a good affinity for the appropriate HLA molecules.



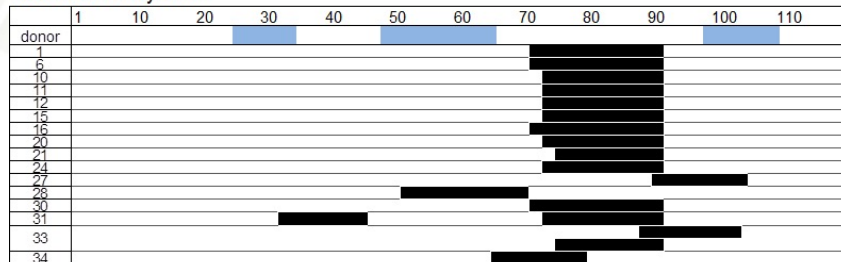
Rtx  
Ifx



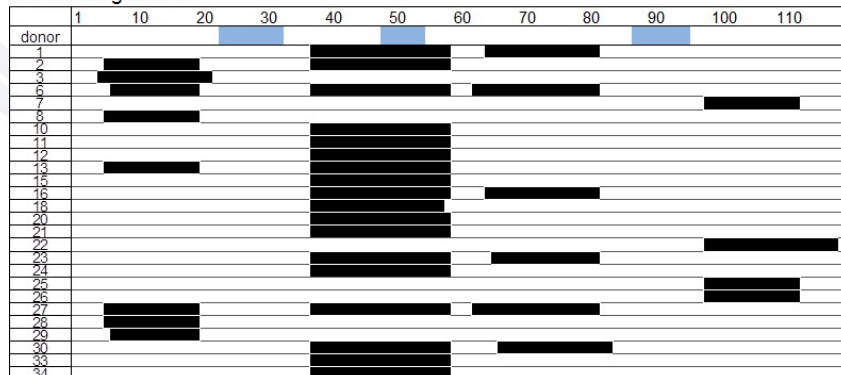
Examples of Ifx and Rtx



rituximab heavy chain



rituximab light chain



Cluster :

multiple length variants with the same HLA-DR binding core

Rituximab:

5 clusters in VH

4 clusters in VL

Infliximab:

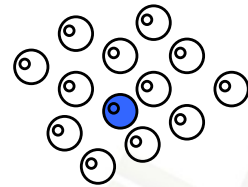
4 clusters in VH

4 clusters in VL

Most of the CD4 T cell epitopes are retrieved from MAPPs

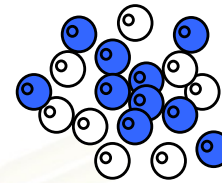
Memory T cells

Short-term T cell assay

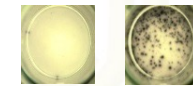


peptide pools

10 d



Elispot

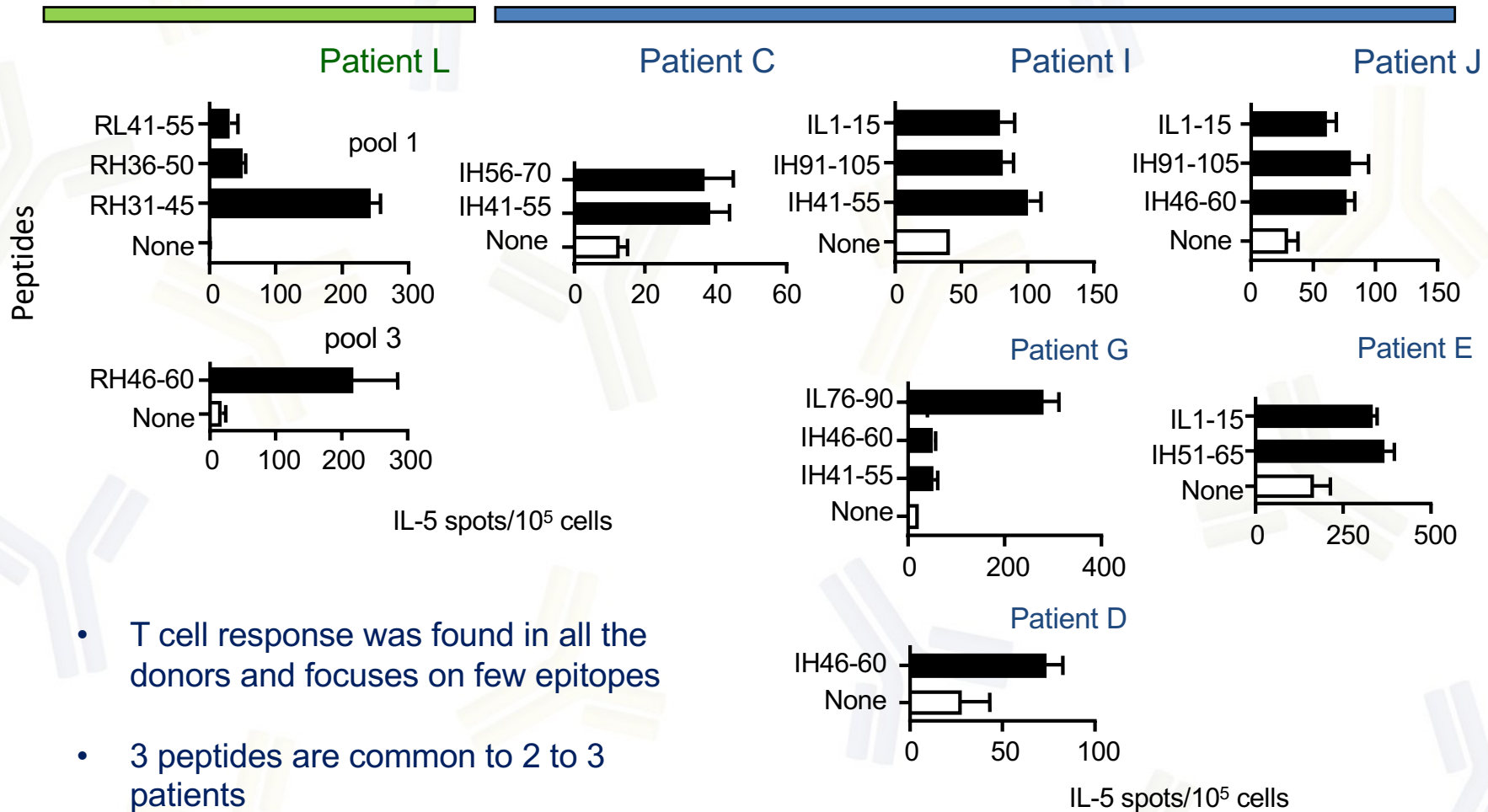


peptides

Patients	Sex	Age	Disease	Treatment	Drug (µg/ml)	ADA (U/ml)
C	M	12	uveitis	lfx	<0.1	63
D	F	57	Crohn.	lfx	0	61
E	F	71	Crohn	lfx	<0.1	>200
G	M	19	Crohn.	lfx	0.13	>200
I	F	23	Crohn.	lfx	0.11	>200
J	M	59	Crohn..	lfx	<0.1	95
L	F	56	r. a.	Rtx	<2	>100

## Rituximab

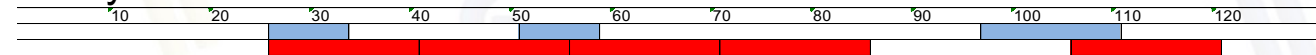
## Infliximab



- T cell response was found in all the donors and focuses on few epitopes
- 3 peptides are common to 2 to 3 patients

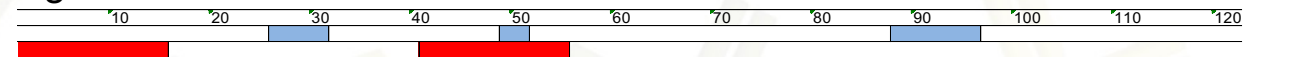
# T cell epitopes: healthy versus ADA+ donors

## Heavy chain



## Rituximab

## Light chain

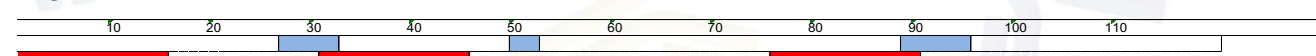


## Heavy chain



## Infliximab

## Light chain

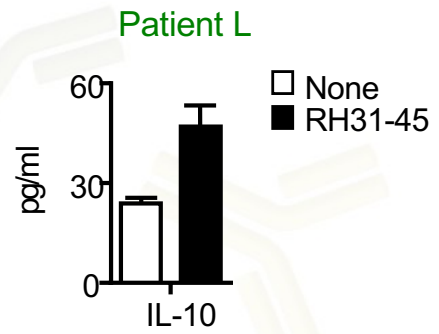


- Healthy
- ADA+ patients
- CDR
- MAPPS

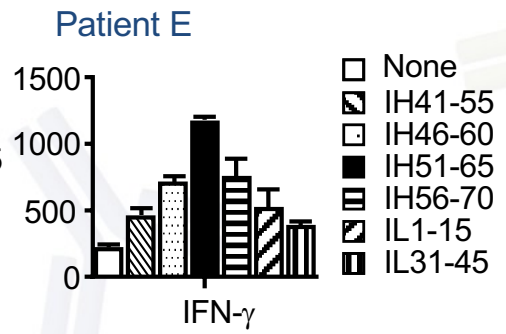
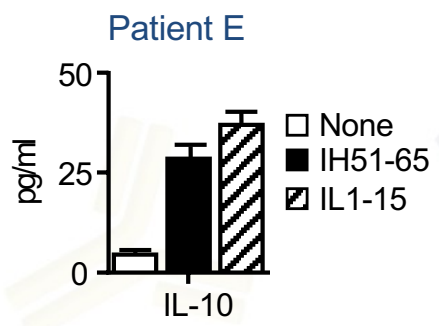
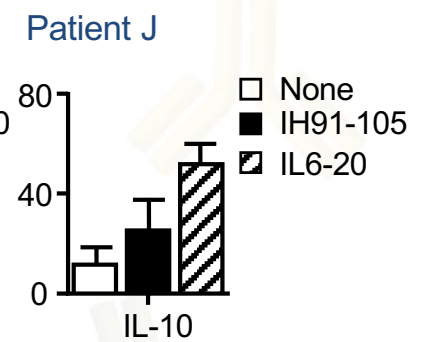
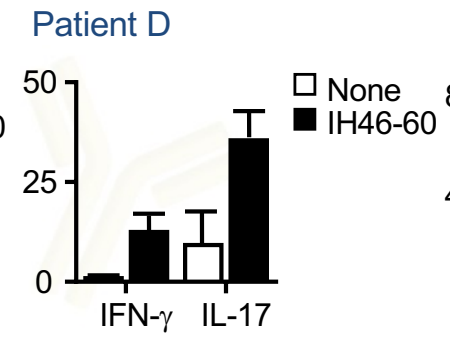
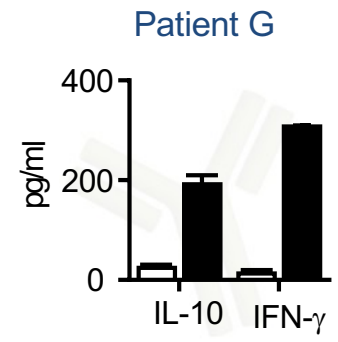
Hamze et al, Frontiers Immunol, 2017

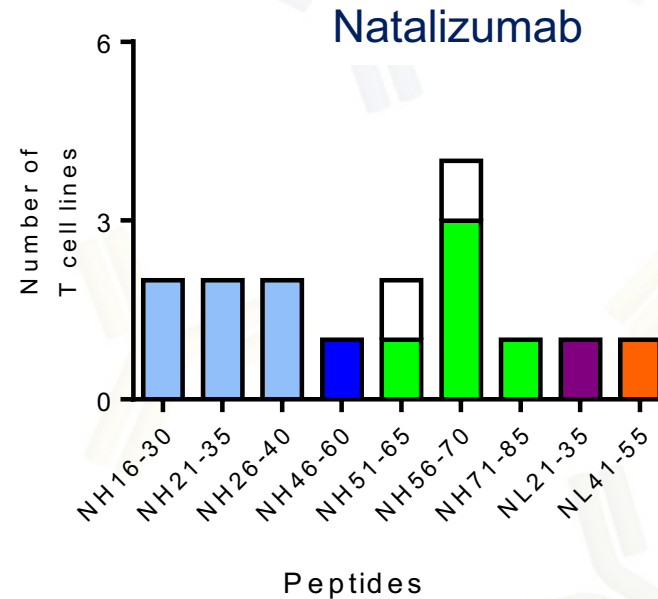
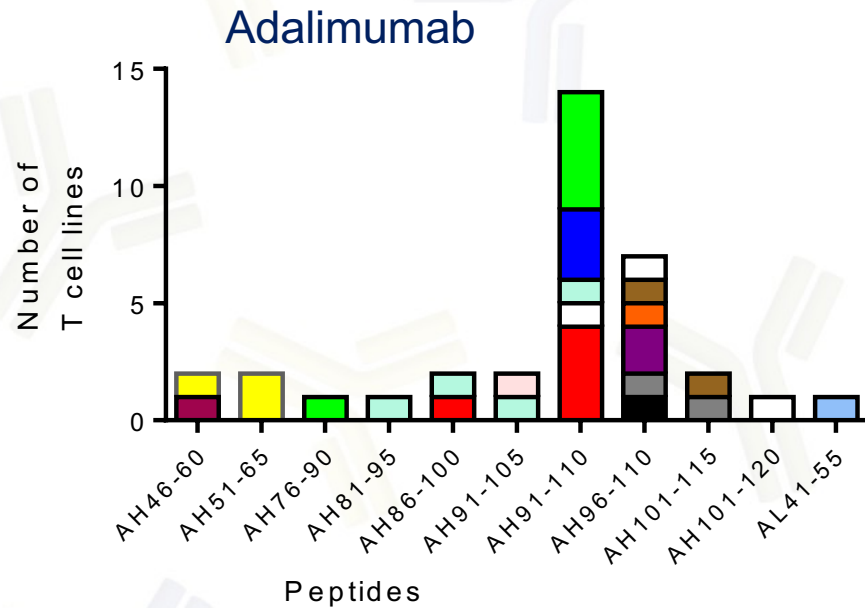
From supernatants of IL5 Elispot assay

## Rituximab



## Infliximab



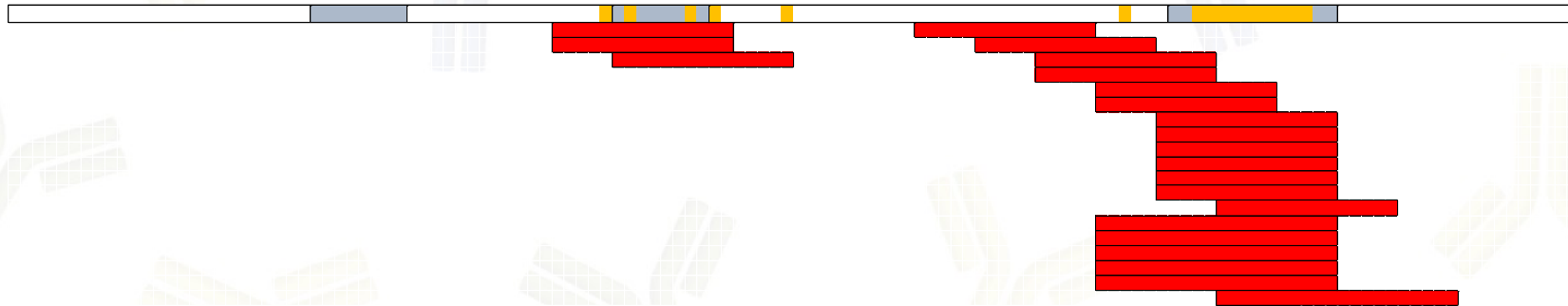


- 14 responders
- 11 epitopes
- Mainly VH
- 91-110 includes 91-105 and 96-110: common to 11 donors

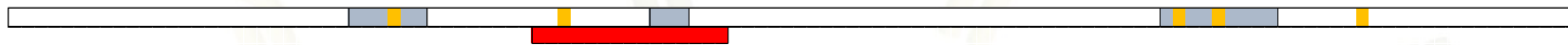
- 6 responders
- 9 epitopes
- VL and VH
- 2 peptides common to 2 donors

## adalimumab

Heavy chain

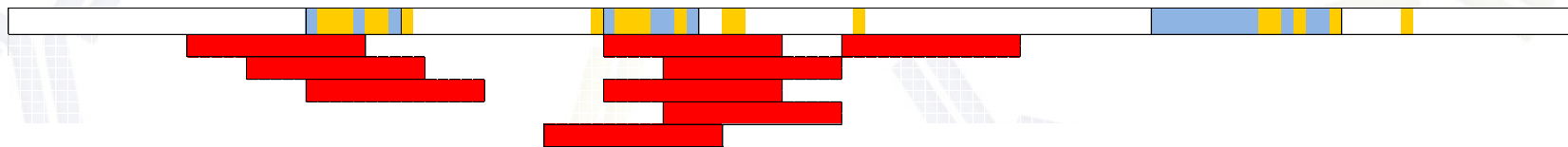


Light chain

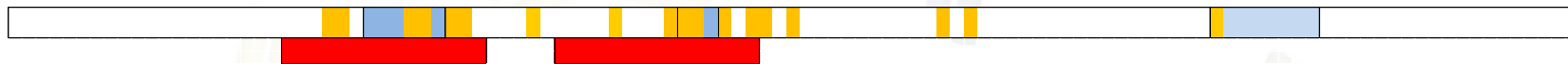


## natalizumab

Heavy chain

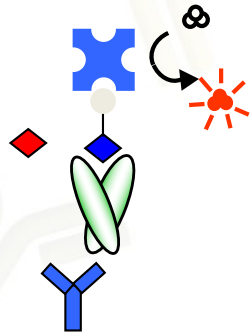


Light chain



■ CDR    ■ mutations

# Binding to HLA-DR molecules of adalimumab and natalizumab peptides

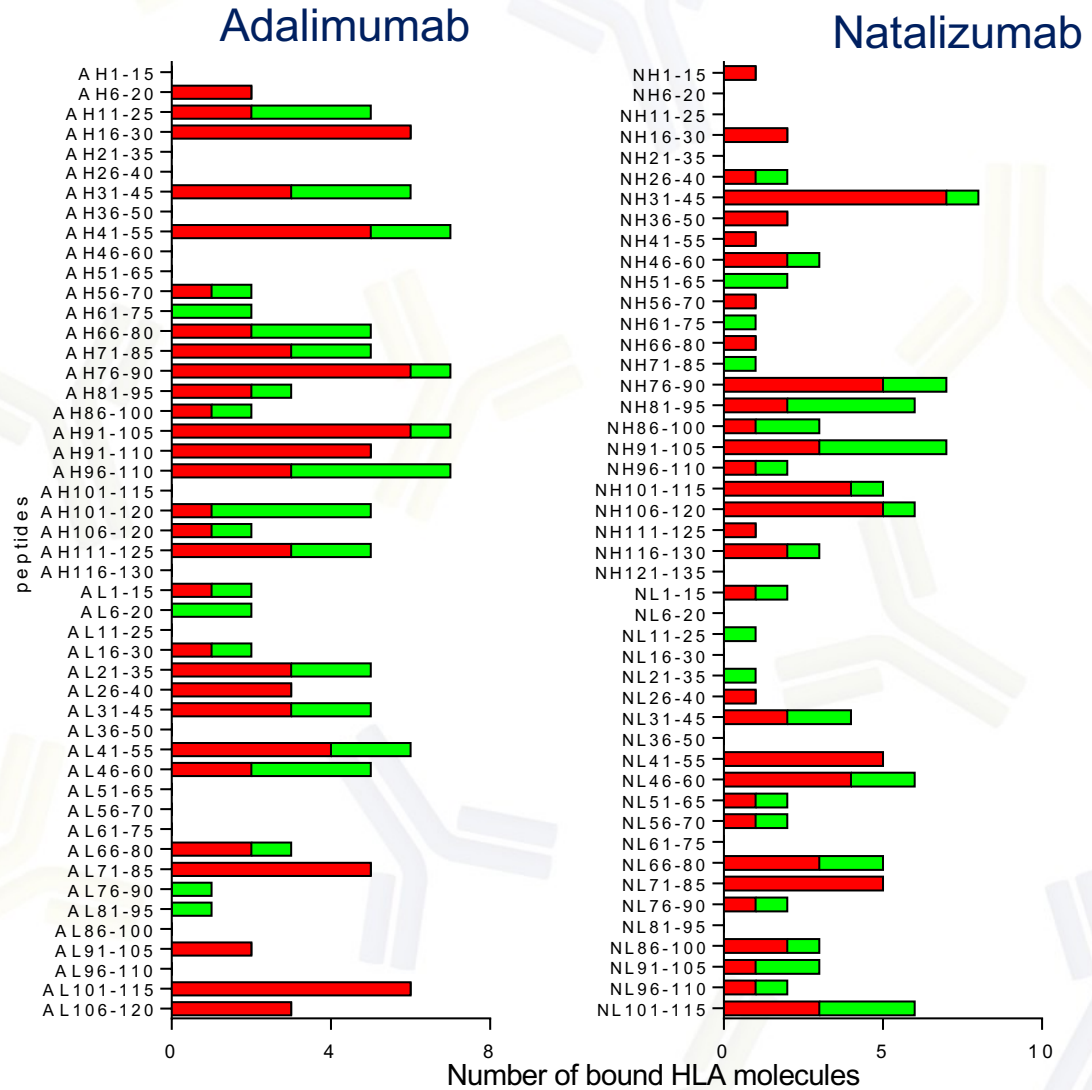


## Adalimumab

8 HLA molecules  
DR1, DR3, DR4, DR7, DR11,  
DR15, DRB4, DRB5

## Natalizumab

10 HLA molecules  
DR1, DR3, DR4, DR7, DR11,  
DR13, DR15, DRB3, DRB4,  
DRB5



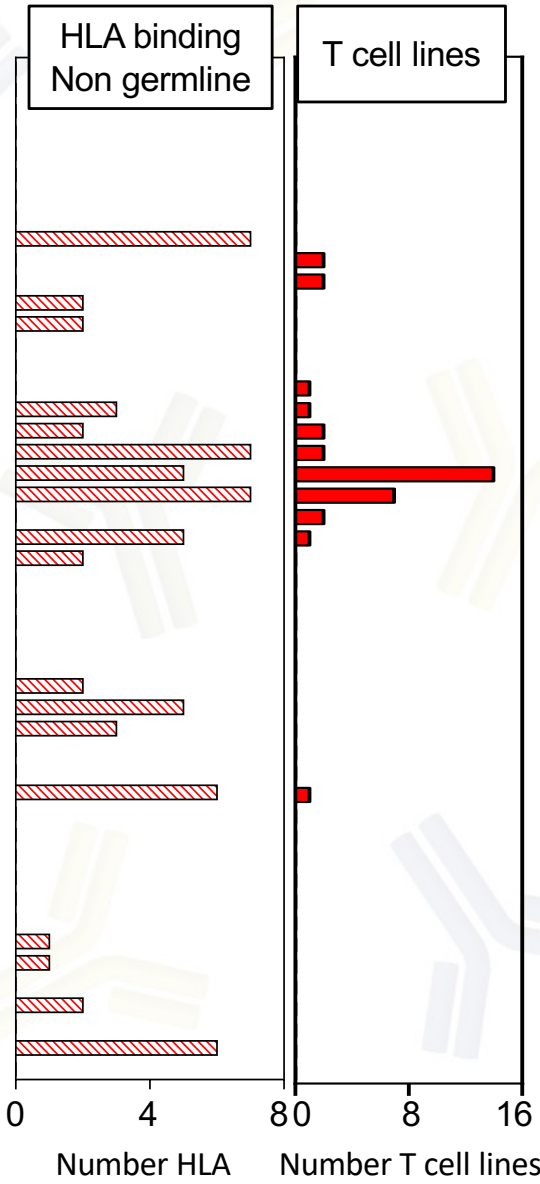




WWW.ABIRISK.EU

# Adalimumab-specific T cell response and HLA binding of non-germline sequences

AH1-15	EVQLVESGGGLVQPG
AH6-20	ESGGGLVQPGRSLRL
AH11-25	LVQPGRSLRLSCAAS
AH16-30	RSLRLSCAASGFTFD
AH21-35	SCAASGFTFDDYAMH
AH26-40	GFTFDDYAMHWVROA
AH31-45	DYAMHWVROAPGKGL
AH36-50	WVRQAPGKGLEWVSA
AH41-55	PGKGLEWVSAITWNS
AH46-60	EWVSAITWNSGHIDY
AH51-65	ITWNSGHIDYADSV
AH56-70	GHIDYADSVGRFTI
AH61-75	ADSVGRFTISRDN
AH66-80	GRFTISRDNKNSLY
AH71-85	SRDNKNSLYLQMNS
AH76-90	KNSLYLQMNSLRAED
AH81-95	LQMNSLRAEDTAVYY
AH86-100	LRAEDTAVYYCAKVS
AH91-105	TAVYYCAKVSYLSTA
AH91-110	TAVYYCAKVSYLSTASSLDY
AH96-110	CAKVSYLSTASSLDY
AH101-115	YLSTASSLDYWQGT
AH101-120	YLSTASSLDYWQGT
AH106-120	SSLDYWQGT
AH111-125	WGQGT
AH116-130	LVTVSSASTKGP
AL1-15	DIQMTQSPSSLSASV
AL6-20	QSPSSLSASVGRVT
AL11-25	LSASVGRVTITCRA
AL16-30	GDRVTITCRASQGIR
AL21-35	ITCRASQGI RNYLAW
AL26-40	SQGI RNYLAWYQOKP
AL31-45	NYLAWYQOKPGKAPK
AL36-50	YQOKPGKAPKLLIYA
AL41-55	GKAPKLLIYAASTLQ
AL46-60	LLIYAASTLQSGVPS
AL51-65	ASTLQSGVPSRFSGS
AL56-70	QSGVPSRFSGSGSGT
AL61-75	RFSGSGSGTDFTLTI
AL66-80	GSGTDFTLTISSLQP
AL71-85	FTLTISSLQPEDVAT
AL76-90	SSLQPEDVATYYCOR
AL81-95	EDVATYYCORYNRAP
AL86-100	YYCORYNRAPYTFGQ
AL91-105	YNRAPYTFGQGTKVE
AL96-110	YTFGQGTKVEIKRTV
AL101-115	GTKVEIKRTVAAPSV
AL106-120	IKRTVAAPSVFI



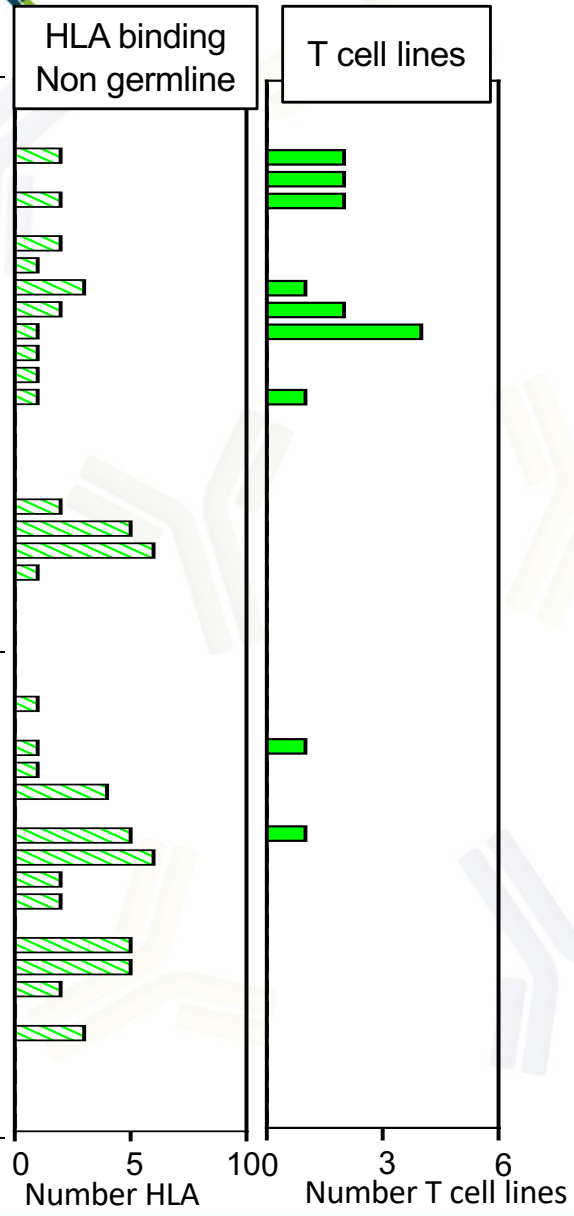
The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° [115303], resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.  
[www.imi.europa.eu](http://www.imi.europa.eu)



# Natalizumab-specific T cell response and HLA binding of non-germline sequences

WWW.ABIRISK.EU

NH1-15	QVQLVQSGAEVKKPG
NH6-20	QSGAEVKKPGASVKV
NH11-25	VKKPGASVKVSKAS
NH16-30	ASVKVSKASGFNFK
NH21-35	SCKASGFNFKDTYIH
NH26-40	GFNFKDTYIHWVRQA
NH31-45	DTYIHWVRQAPGQRL
NH36-50	WVRQAPGQRLEWMGR
NH41-55	PGQRLEWMGRIDPAN
NH46-60	EWMGRIDPANGYTKY
NH51-65	IDPANGYTKYDPKFQ
NH56-70	GYTKYDEKFGQGRVTI
NH61-75	DPKFQGRVTITADTS
NH66-80	GRVTITADTSASTAY
NH71-85	TADTSASTAYMELSS
NH76-90	ASTAYMELSSLRSED
NH81-95	MELSSLRSEDTAVYY
NH86-100	LRSEDTAVYYCAREG
NH91-105	TAVYYCAREGYGNY
NH96-110	CAREGYGNYGVYAM
NH101-115	YVYGVYAMDYWGQ
NH106-120	GVYAMDYWGQGLVT
NH111-125	DYWGQGLVTVSSAS
NH116-130	GTLVTVSSASTKGPS
NH121-135	LTVSSASTKGPSVF
<hr/>	
NL1-15	DIQMTQSPSSLSASV
NL6-20	QSPSSLSASVGDRTV
NL11-25	LSASVGDRTVITCKT
NL16-30	GDRVTITCKTSQDIN
NL21-35	ITCKTSQDINKYMAW
NL26-40	SQDINKYMAWYQQT
NL31-45	KYMAWYQQTTPGKAPR
NL36-50	YQQTTPGKAPRLLIHY
NL41-55	GKAPRLLIHYTSALQ
NL46-60	LLIHYTSALQPGIIPS
NL51-65	TSALQPGIPSRFSGS
NL56-70	PGIPSRFSGSGSGRD
NL61-75	RFSGSGSGRDYFTFI
NL66-80	GSGRDYFTFISSLQP
NL71-85	YFTFISSLQPEDIAT
NL76-90	SSLQPEDIATYYCLO
NL81-95	EDIATYYCLOYDNLW
NL86-100	YYCLOYDNLWTFGQG
NL91-105	YDNLWTFGQGTKVEI
NL96-110	TFGQGTKVEIKRTVA
NL101-115	TKVEIKRTVAAPSVF



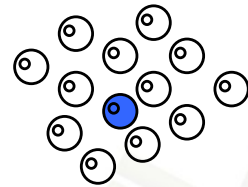
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# T cell response to adalimumab in patients

Memory T cells

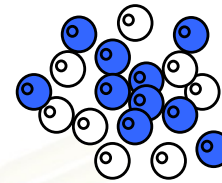


Short-term T cell assay

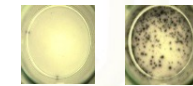


peptide pools

10 d

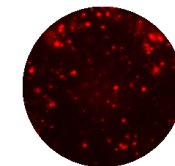
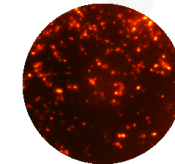
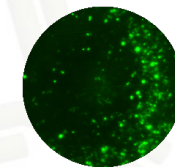


Fluorospot



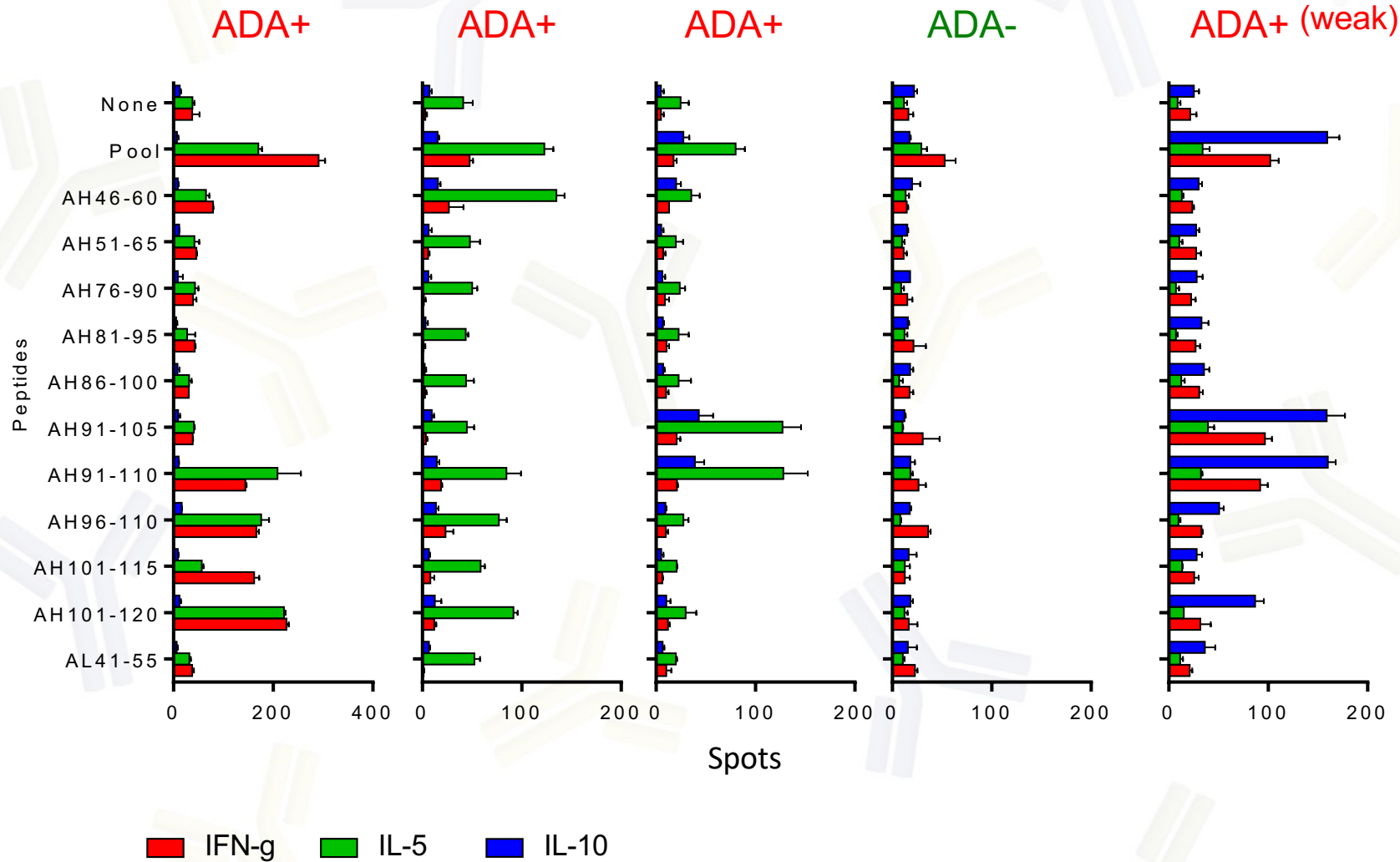
peptides

IFN-g, IL-5, IL-10

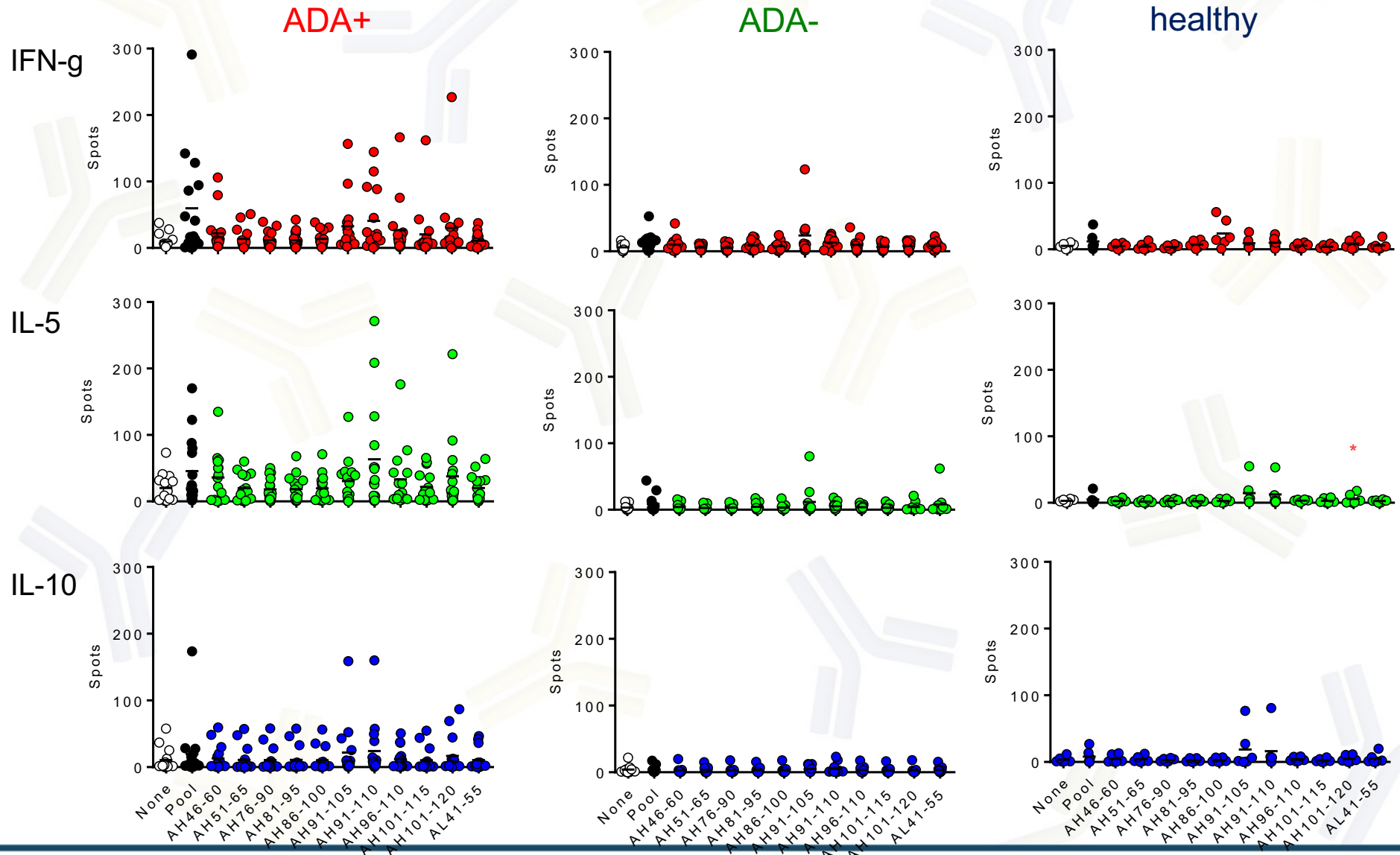


- 15 ADA+ patients
- 11 ADA- patients (>4 months treatment)
- 6 Healthy donors

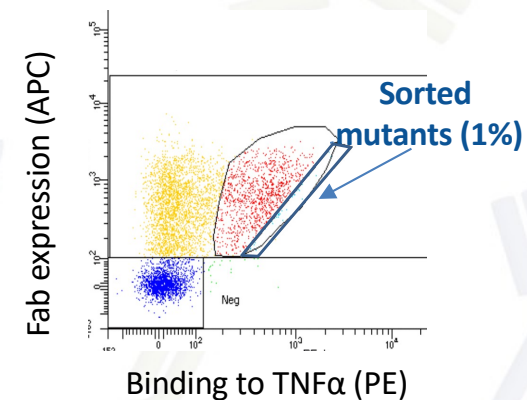
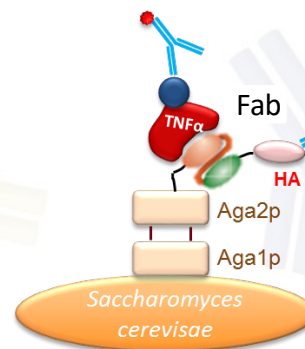
# Individual T cell response to adalimumab



# T cell response to adalimumab in patients



- T cell response to therapeutic antibodies
  - Healthy donors: perspective of immunogenicity prediction
  - ADA+ patients
- T cell epitopes of chimeric antibodies Rtx and lfx
  - Overlap CDR or Fr regions (Chimeric antibodies) of H and L chains
  - Epitopes shared by different donors
- T cell epitopes of human(ized) antibodies Adm and Ntz
  - One major region for Adm shared by multiple donors
  - Lower response for Ntz but multiple T cell epitopes identified
  - Hosted by non-germline sequences
- T cell epitopes identified in healthy donors contribute to T cell responses in patients
  - Diversity of responses including IL-10 response
- Perspectives
  - De-immunized antibodies: T cell epitope removal
  - Immunomonitoring of the ADA response (collaboration Niek de Vries)



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**"It is difficult to make predictions,  
especially about the future"**

**Attributed to Yogi Berra, Groucho Marx, Woody Allen  
, Niels Bohr Enrico Fermi Albert Einstein, Winston  
Churchill, Allan Lamport, Mark Twain George Bernard  
Shaw ....**