

“Eliciting broad anti-HIV neutralizing antibodies by vaccination: lessons learned from natural infection ”

L. Stamatatos

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What factors are linked to the development of cross-reactive NAbs during natural HIV-infection?

Sather, D.N., J. Armann, L. K. Ching, A. Mavrantoni, G. Sellhorn, Z. Caldwell, X. Yu, B. Wood, S. Self, S. Kalams, and L. Stamatatos. 2009. Factors associated with the development of cross-reactive neutralizing antibodies during HIV-1 infection. *J. Virol.* 83; 757-769.

Significant Correlates:

- Time of Infection
- Plasma Viral Load
- Binding Avidity to Env

Covariates	Estimate	Univariate model		Multivariate model	
		95% CI	p-value	95% CI	p-value
Duration of Infection	1.09	(1.05, 1.13)	<0.001	(1.03, 1.12)	0.001
Average Avidity	1.93	(1.63, 2.29)	<0.001	(1.44, 2.06)	<0.001
Average log(VL)	1.99	(1.44, 2.76)	<0.001	(1.24, 2.47)	0.002
Average IgG	2.30	(1.75, 3.01)	<0.001		
Average log(CD4)	0.32	(0.09, 1.16)	0.084		
Average log(CD8)	20.89	(6.60, 66.04)	<0.001		

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Analysis by SCHARP: Steve Self, Blake Wood, Xuesong Yu

How soon following HIV-1 infection can we detect broadly neutralizing antibody responses?

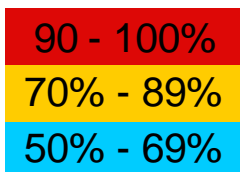
Are they clinically beneficial?

Acute/Early HIV Infection Cohort (Ragon Institute/MGH)

- 17 Subjects, 69 plasma samples
 - Known date of infection / seroconversion
 - Follow-up every 4 months from a few months post infection to up to 7 years post infection
 - Patients are Clade B infected
 - ART-naïve
 - Diverse plasma viral loads, CD4 counts > 200 / ul
-
- Screen all plasma samples against 10 Clade B, 6 Clade C and 4 Clade A isolates. MLV was included as a control for non-Ab-mediated neutralization
 - The neutralization screens were performed initially at a single heat-inactivated plasma dilution of 1:20
 - Over 2600 neutralization assays were performed so far

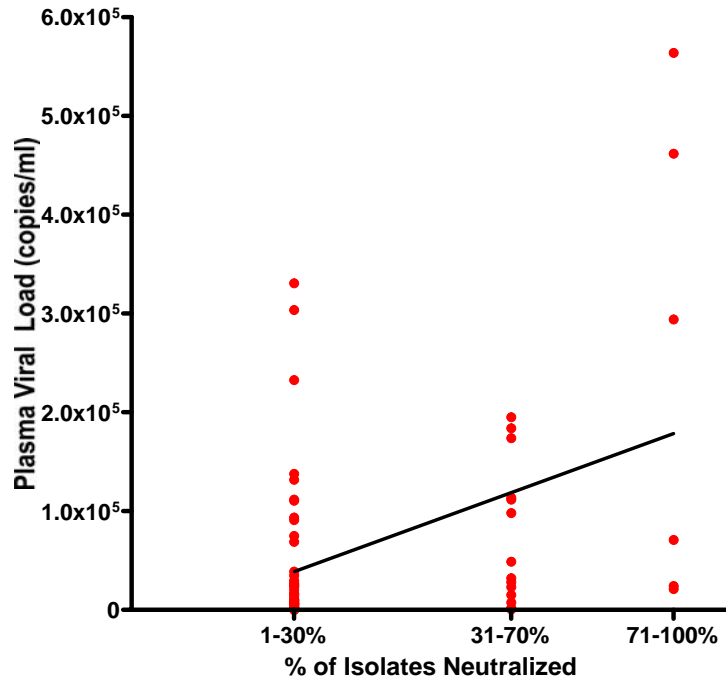
Patient ID	Inf'd for	Clade B									Clade C					Clade A			Breadth					
		SF162.LS	JRFL	YU-2	REJO454 ¹	TRO.11	SC422661 ¹	QH0692.4	CA-AN534	PVO.4	TRJO455 ¹	ZM214M.F	ZM249M.F	ZM109F.P	ZM53M.PB	Du422.1	CAP45.2.C	Q769h5		Q461e2	Q259d2.1	Q168a2		
HC049	<1 yr		(-)	(-)	(-)	(-)																	10%	
	1 yr		(-)	(-)		(-)																		30%
	2.5 yrs																							55%
	3.5 yrs																							85%
	4.5 yrs																							80%
HC053	10 mo		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)													5%	
	1.5 yrs		(-)	(-)		(-)	(-)	(-)	(-)	(-)														20%
	2 yrs			(-)		(-)	(-)	(-)	(-)	(-)														20%
	3 yrs																							50%
	4 yrs																							65%
	5 yrs																							80%
	6 yrs																							60%
	7 yrs																							60%
HC071	5 mo		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	1.5 yrs		(-)	(-)		(-)	(-)	(-)	(-)	(-)														30%
	2.5 yrs			(-)		(-)		(-)	(-)	(-)														45%
	3.5 yrs																							50%
	4 yrs			(-)	(-)		(-)	(-)	(-)	(-)														30%
HC083	1 yr		(-)	(-)		(-)	(-)	(-)	(-)	(-)														15%
HC089	few mo		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	~ 1 yr		(-)	(-)		(-)	(-)	(-)	(-)	(-)														25%
	~ 2 yrs			(-)																				55%
HC093	2 mo		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	1 yr		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	2 yrs		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														20%
	3 yrs			(-)	(-)		(-)	(-)	(-)	(-)														20%
	4 yrs			(-)																				35%
	5 yrs			(-)																				35%
	5.5 yrs			(-)																				25%
HC098	5 mo		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	1 yr		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	2 yrs		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	3 yrs			(-)	(-)	(-)																		20%
	4 yrs		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
HC110	5 mo		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	1 yr		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	2.5 yrs		(-)	(-)																				15%
HC115	10 mo		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	1.5 yrs		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
HC128	2-4 mo		(-)	(-)																				20%
	1 yr			(-)																				65%
	2 yrs																							90%
HC131	8 mo		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	1.5 yrs			(-)																				50%
	2.5 yrs			(-)																				45%
	3 yrs																							75%
HC160	3 mo	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														0%
	1 yr		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
	2 yrs		(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)														5%
3 yrs			(-)																					35%

Legend



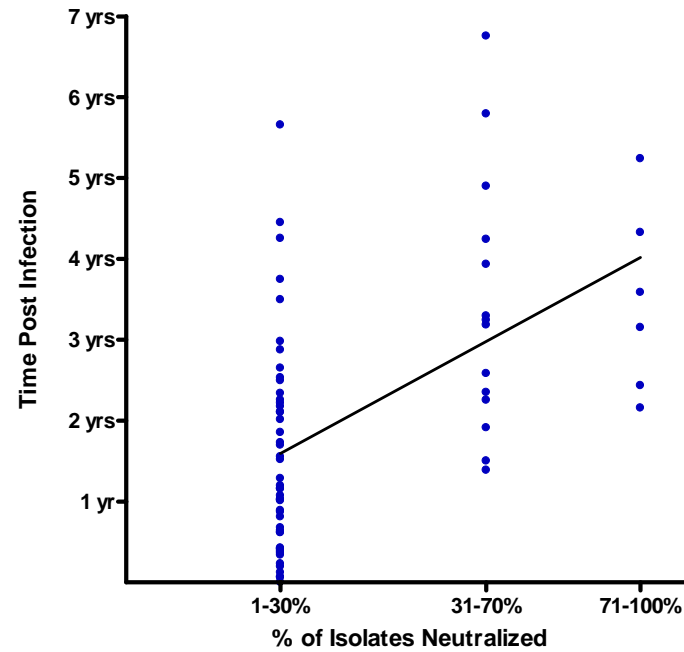
Association between neutralization breadth and plasma viral load, or duration of infection

Plasma Viremia



Spearman R = 0.3615
p value = 0.0026

Duration of Infection

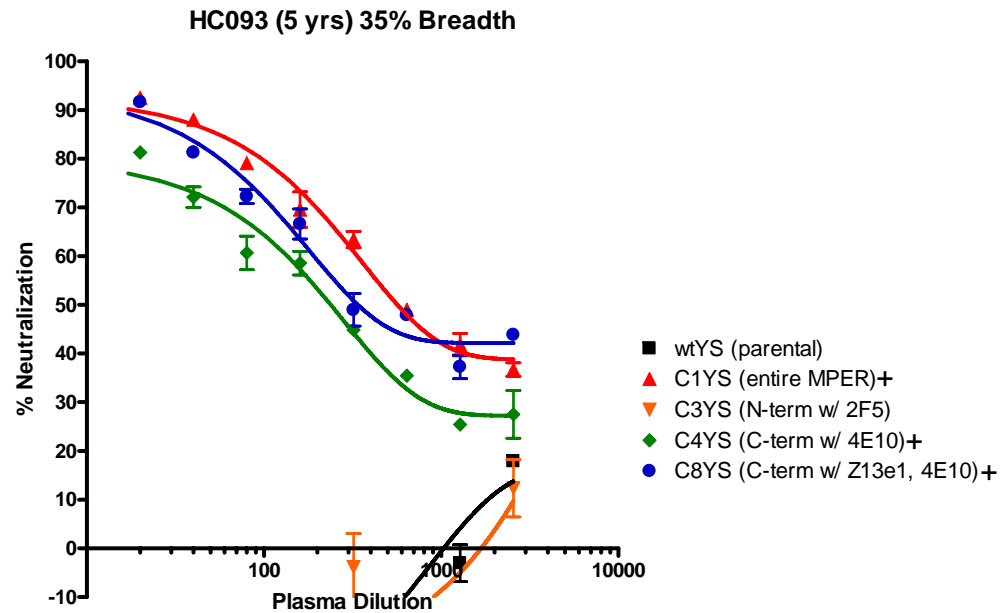
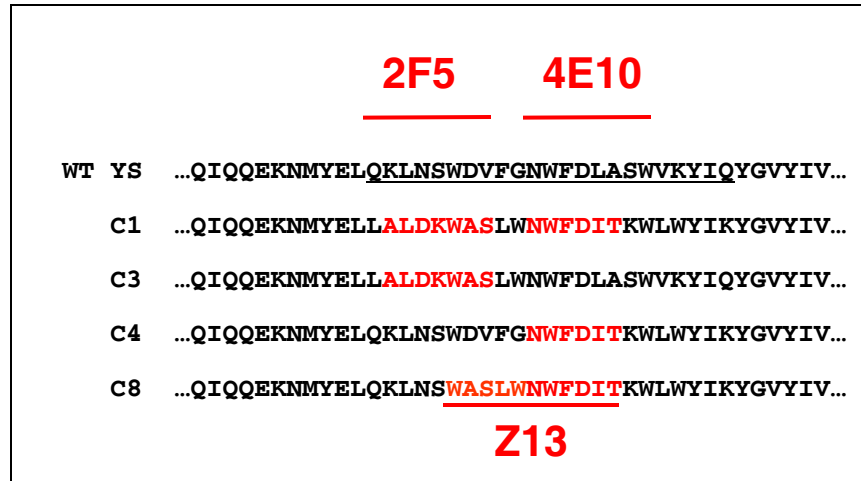


Spearman R = 0.5461
p value < 0.0001

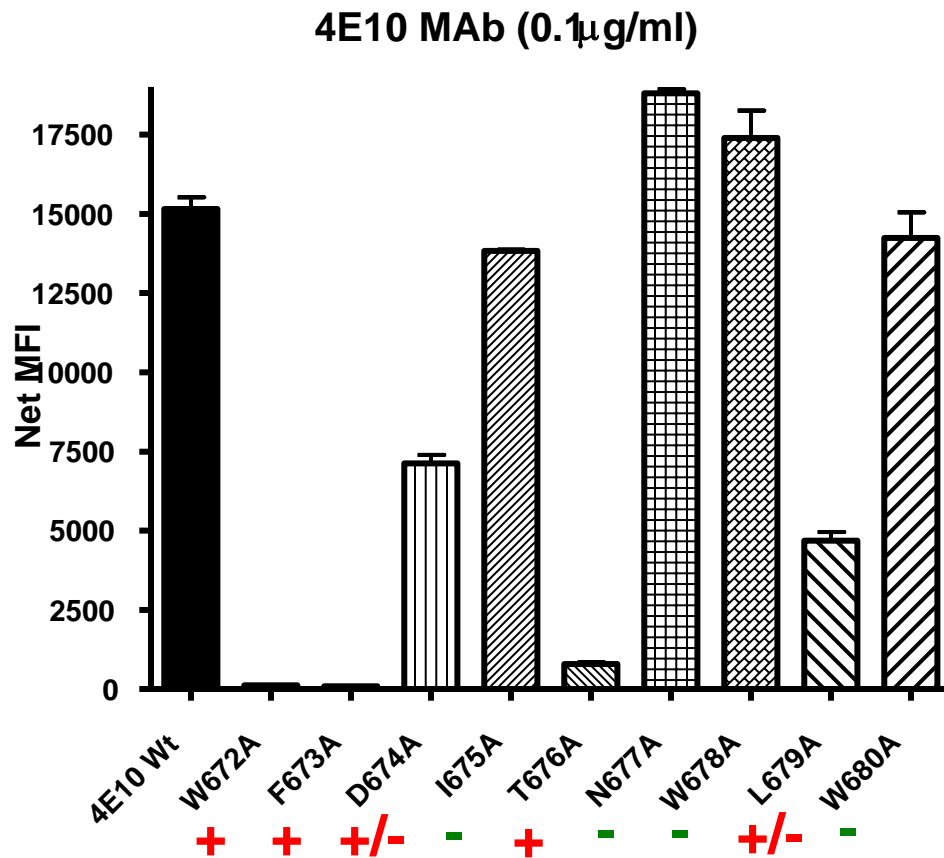
No association between neutralization breadth and initiation of ART

		No Breadth	Breadth.pos	total			
No ART	0	10(77%)	1(25%)	11			
ART	1	3(23%)	3(75%)	6			
	total	13	4	17			
breadth>75% heterologous isolates neutralized at 1:25							
chi-squared test shows that positive breadth is not associated with ART initiation (p.value=0.094)							

Epitope specificities of cross-reactive NABs: the MPER region (Neutralization of HIV-2 / HIV-1 MPER chimeras)



AA recognition pattern of MAb 4E10 (Luminex)



NWFDITNWLWYIRKKK	WT
N A FDITNWLWYIRKKK	W672A
NW A DITNWLWYIRKKK	F673A
NWF A ITNWLWYIRKKK	D674A
NWFD A TNWLWYIRKKK	I675A
NWFDI A NWLWYIRKKK	T676A
NWFDIT A WLWYIRKKK	N677A
NWFDITN A LWYIRKKK	W678A
NWFDITNW A WYIRKKK	L679A
NWFDITNWL A YIRKKK	W680A

AA recognition patterns of anti-4E10 responses in HIV-1+ plasmas

				672	673	674	675	676	677	678	679	680
				W	F	D	I	T	N	W	L	W
MAb 4E10				+	+	+	(--)	+	(--)	(--)	+	(--)
Subject	Years Inf.	Breadth	Pot. Target									
HC049	3.5	85	4E10 like	+	+	+/-	+	+	(--)	(--)	+/-	(--)
CC1782		80	4E10 like	(--)	+/-	+	+/-	+	+/-	+/-	+/-	+/-
HC131	3	75	4E10 like	(--)	+/-	+/-	+/-	(--)	(--)	(--)	+/-	+/-
HC093	5	35	4e10 like	(--)	(--)	+	(--)	+	(--)	+	(--)	+

- The 4E10 epitope is immunogenic in a subset of HIV-1+ subjects
- The specificities of plasma anti-4E10 antibodies differs from that of MAb 4E10

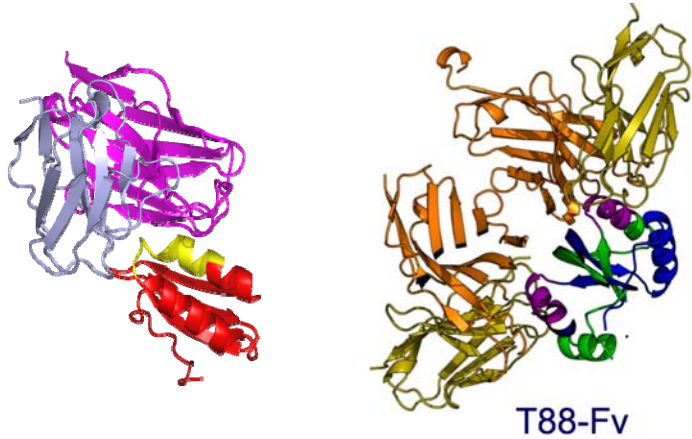
Elicitation of 4E10-like Abs by immunization

The 'epitope scaffold' approach

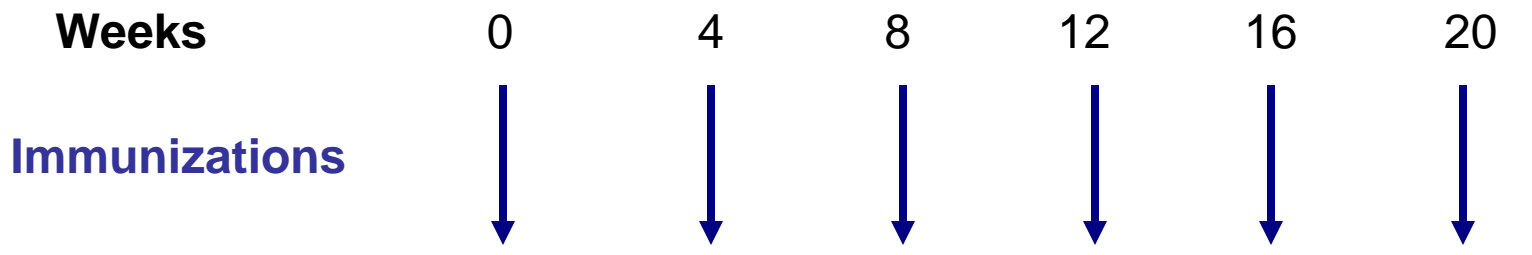
(Bill Schief – Roland Strong)

- *“structural epitope is transplanted, by computational design, to one or more non-HIV scaffold proteins for conformational stabilization”*
- These proteins are validated biophysically, antigenically and crystallographically
- They ‘remove’ anti-4E10 NAbs from the plasma of HIV+ subjects

Immunizations with a 4E10 epitope scaffold (T88)

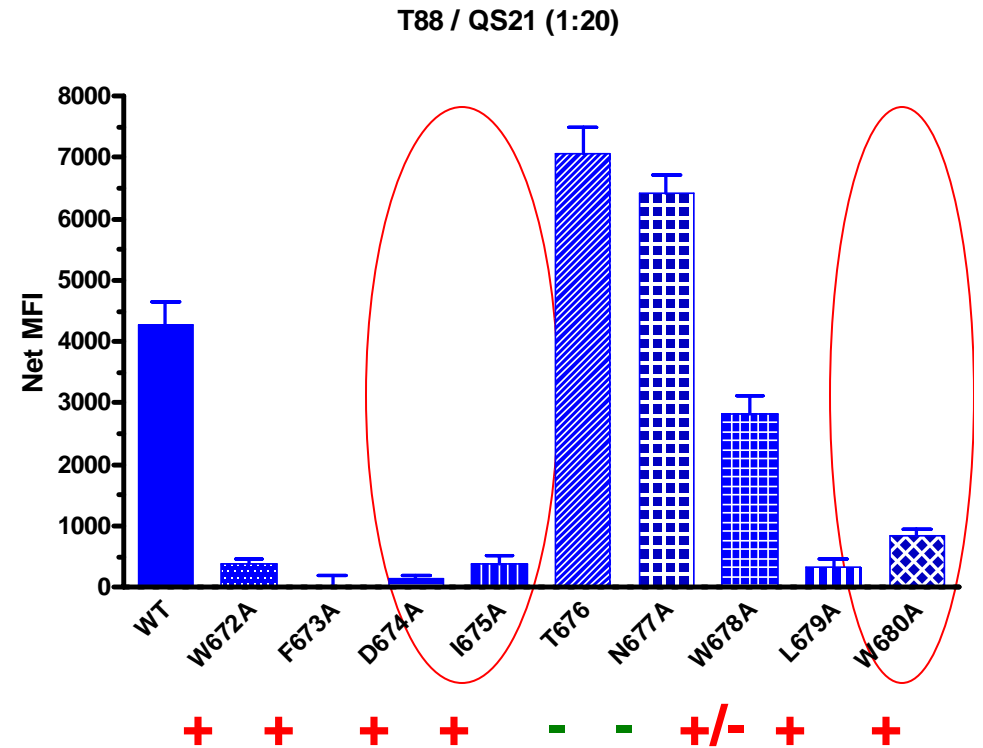
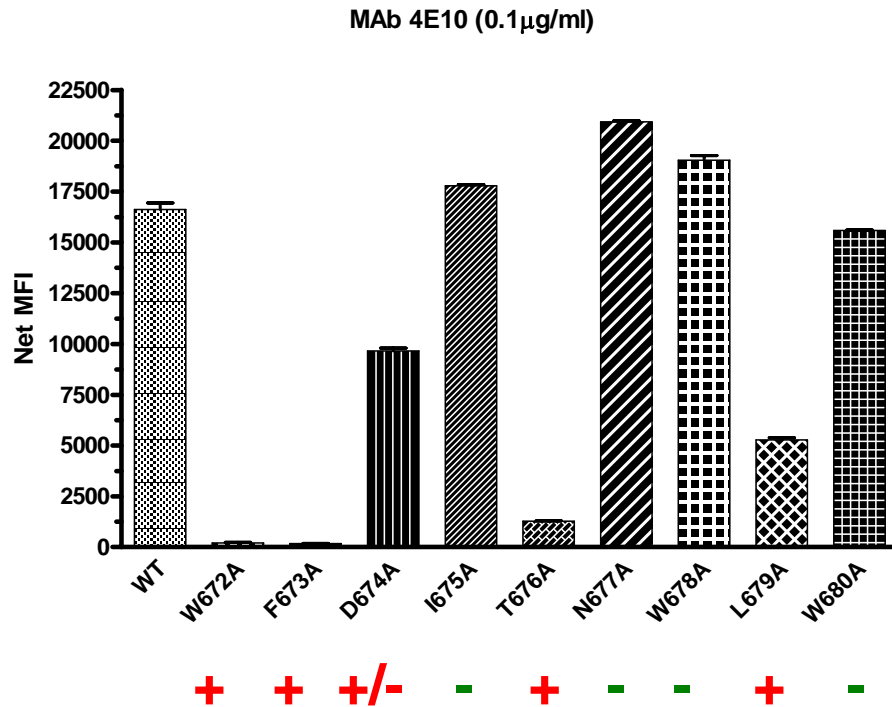


T88 K_D : 5.6nM
NWFDITNWLWYIR



Rabbits; QS21

AA binding patterns of anti-MPER responses in plasma



AA recognition patterns of anti-4E10 responses during infection and during scaffold-immunization

				672	673	674	675	676	677	678	679	680
				W	F	D	I	T	N	W	L	W
MAb 4E10				+	+	+	(--)	+	(--)	(--)	+	(--)
Subject	Years Inf.	Breadth	Pot. Target									
HC049	3.5	85	4E10 like	+	+	+/-	+	+	(--)	(--)	+/-	(--)
HC093	5	35	4e10 like	(--)	(--)	+	(--)	+	(--)	+	(--)	+
HC131	3	75	4E10 like	(--)	+/-	+/-	+/-	(--)	(--)	(--)	+/-	+/-
CC1782		80	4E10 like	(--)	+/-	+	+/-	+	+/-	+/-	+/-	+/-
4E10 Scaffolds				+	+	+	+	(--)	(--)	+/-	+	+

- 4E10 scaffolds elicit Abs against the 4E10 epitope
- They recognize the helical conformation of the epitope
- They do not bind lipids

Summary

~ 20% of HIV+ subjects examined, display 'breadth' 2 – 2.5 years after infection

- Is the 'late' development of breadth due (linked?) to an 'earlier' development of other anti-viral immune responses? (ADCC; CTL)
- Does the development of 'breadth' affect the development of other anti-viral immune responses?

No obvious clinical benefit

Why only a fraction of HIV+ subjects develops breadth during the first ~2 years of infection?

The virus escapes broadly neutralizing antibody responses

- How many pathways of viral-escape from broad neutralizing antibody responses exist?
- Does the number of escape pathways available depend on the epitope specificities of the broadly neutralizing antibody responses?

Abs to the 4E10 epitope are elicited during HIV-1 infection but differ in their AA recognition patterns than MAb 4E10

- There may be more than one way to attack the MPER and the 4E10 epitope in particular
- Implications for immunogen-design (isolate new anti-4E10 MAbs and design immunogens around them rather than MAb 4E10)

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