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

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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 *

		Univariate model		Multivariate model	
Covariates	**Estimate**	**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		

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

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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

		Clade B										Clade C						Clade A				
Patient ID	Inf'ed for	SF162.LS	JRFL	YU-2	REJO4541	TRO.11	SC422661	QH0692.4	CAAN5342	PVO.4	TRJO4551	ZM214M.P	ZM249M.P	ZM109F.P	ZM53M.PB	Du422.1	CAP45.2.0	Q769h5	Q461e2	Q259d2.17	Q168a2	Breadth
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

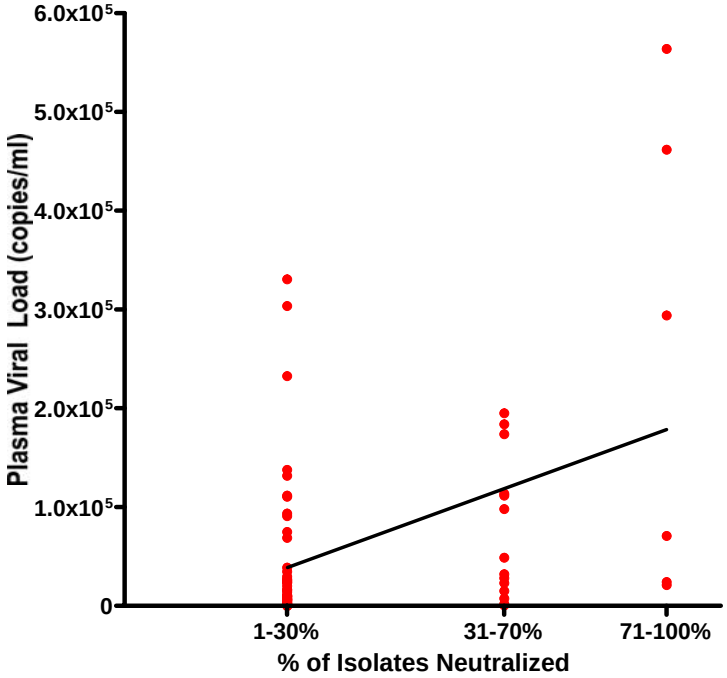
90 - 100%

70% - 89%

50% - 69%

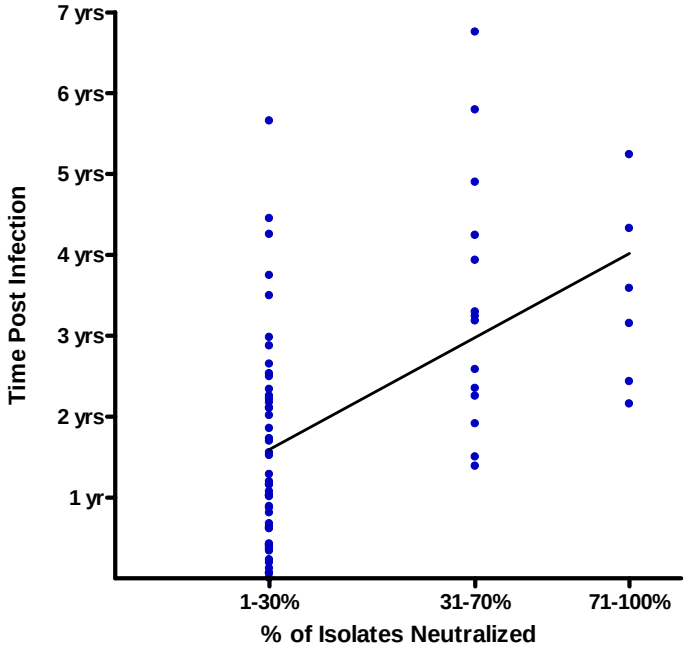
Association between neutralization breadth and plasma viral load, or duration of infection

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

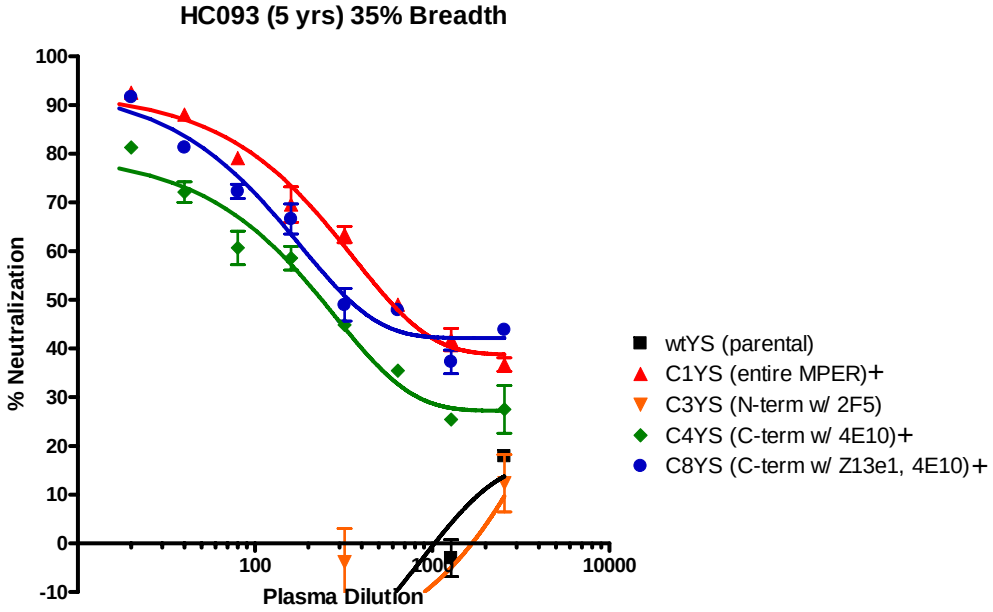
chi-squared test shows that positive breadth is not associated with ART initiation **(p.value=0.094)**

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

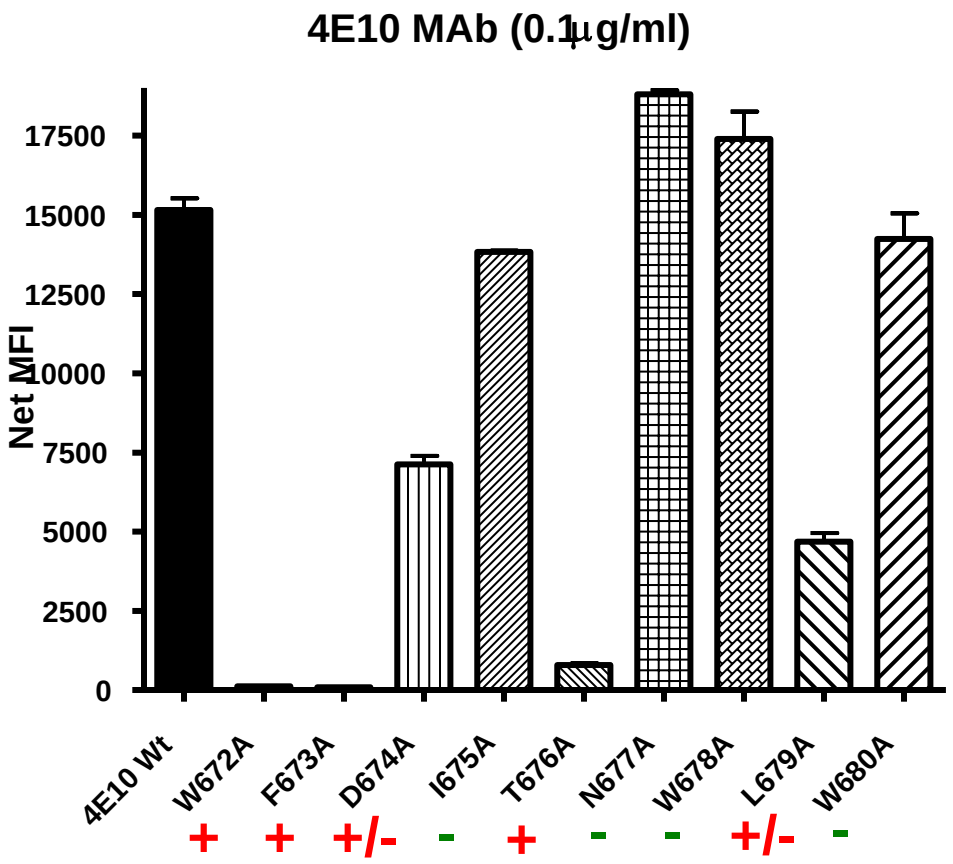
2F5 **4E10**

WT YS	…QIQQEKNMYELQKLNSWDVFGNWFDLASWVKYIQYGVYIV…
C1	…QIQQEKNMYELLALDKWASLWNWFDITKWLWYIKYGVYIV…
C3	…QIQQEKNMYELLALDKWASLWNWFDLASWVKYIQYGVYIV…
C4	…QIQQEKNMYELQKLNSWDVFGNWFDITKWLWYIKYGVYIV…
C8	…QIQQEKNMYELQKLNSWASLWNWFDITKWLWYIKYGVYIV…

Z13



AA recognition pattern of MAb 4E10 (Luminex)



NWFDITNWLWYIRKKK	**WT**
NAFDITNWLWYIRKKK	W672A
NWADITNWLWYIRKKK	F673A
NWFAITNWLWYIRKKK	D674A
NWFDATNWLWYIRKKK	I675A
NWFDIANWLWYIRKKK	T676A
NWFDITAWLWYIRKKK	N677A
NWFDITNALWYIRKKK	W678A
NWFDITNWAWYIRKKK	L679A
NWFDITNWLAYIRKKK	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

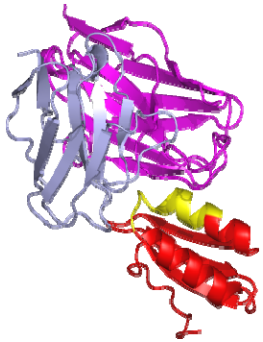
(Bill Schief – Roland Strong)

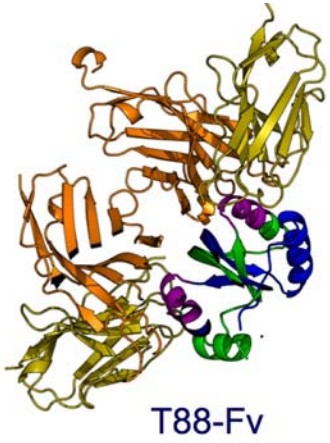
•"*structural epitope is transplanted, by computational design, to one or more non-HIV scaffold proteins for conformational stabilization*"

•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)





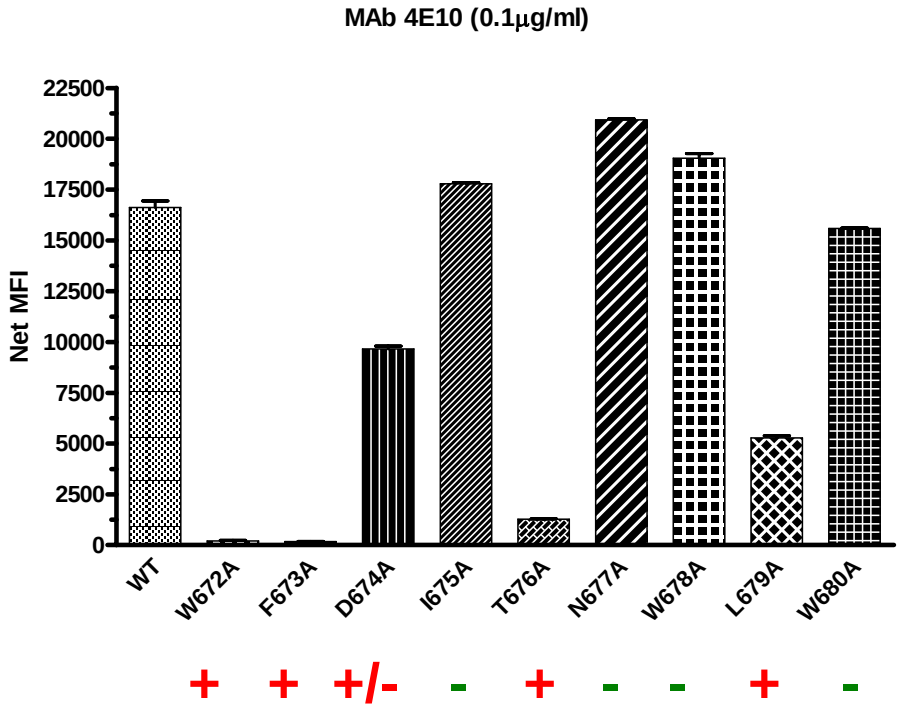
T88 K_D: 5.6nM
NWFDITNWLWYIR

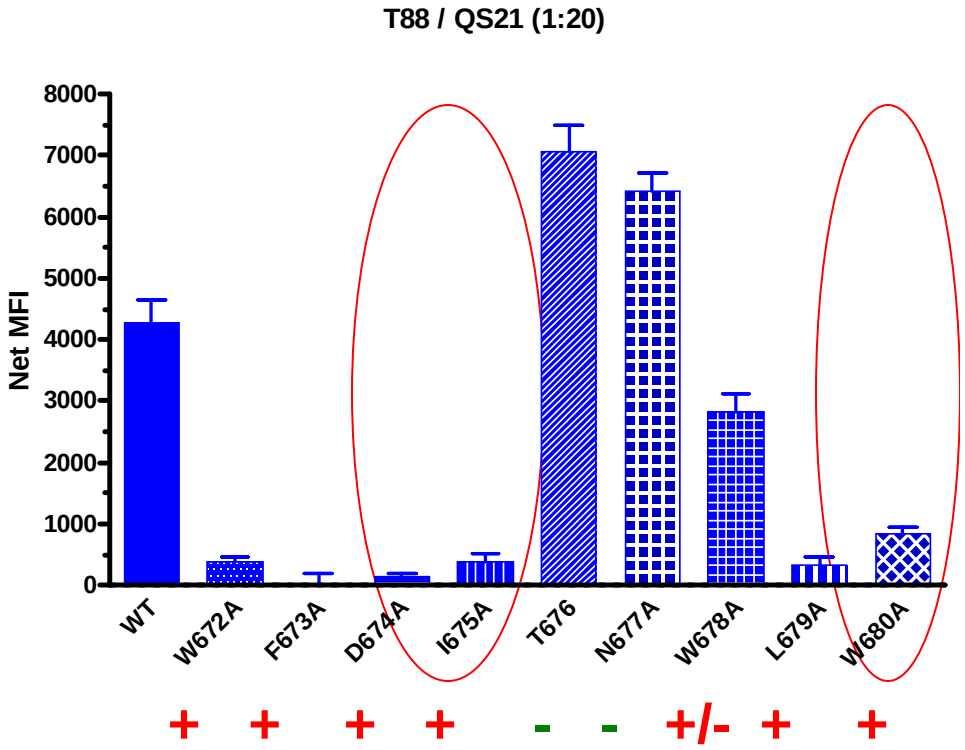
Weeks 0 4 8 12 16 20

Immunizations

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 like**	+	+	+	+	(--)	(--)	+/-	+	+

- 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 breath 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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