Identification of amino acid substitutions associated with neutralization phenotype in the human immunodeficiency virus type-1 subtype C gp120

Kirchherr, J. L., Hamilton, J., Lu, X., Gnanakaran, S., Muldoon, M., Daniels, M., Kasongo, W., Chalwe, V., Mulenga, C., Mwananyanda, L., Musonda, R.M., Yuan, X., Montefiori, D.C., Korber, M.T., Haynes, B.F., & Musonda, R. M. (2011). Identification of amino acid substitutions associated with neutralization phenotype in the human immunodeficiency virus type-1 subtype C gp120. Virology, 409(2), 163-174. DOI: 10.1016/j.virol.2010.09.031

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Outline

- **HIV is a retrovirus with an extremely high rate of mutation that leads to progressive failure of the immune system.**
- The Kirchherr paper studied the role of neutralizing antibodies in attaching to common signature sequences in the HIV virus.
- Collection and analysis of data suggested neutralization to occur within four possible signature sites.
- The V4 region in gp120 was determined to be the most effective area for Nab attachment and virus suppression.
- Future research into the signature sites of the V4 loop is needed to develop a vaccine to induce a greater immune response for the presence of HIV.

HIV is a Highly Diverse Virus Due to Constant Adaptation in Variable Regions

- HIV-1 undergoes rapid genetic mutation.
  - *Env* gene is highly variable.
- HIV is a retrovirus which has 9 subtypes (A-K).
  - Subtype C accounts for 1/2 of HIV-1 infections globally.
- The *Env* gene codes for gp120 and gp41 which are expressed on the surface of the virus.
  - gp120 is an envelope protein which helps the virus avoid detection during the HIV life cycle.
  - V4 region of gp120 on the *env* gene is thought to contribute to patterns of neutralization susceptibility in subtype C viruses (Kirchherr et al. 2010).

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Kirchherr et al. Identified Potential Neutralizing Signature Sequences In The HIV Virus.

- Focus of this paper was to identify what induces Nabs in the HIV virus.
    - Nabs are neutralizing antibodies that are released in response to the HIV-1 infection.
- 15 subjects infected with the subtype C of the HIV virus were studied for neutralization.
    - Neutralization susceptibility was studied in autologous and heterologous plasma.
    - Heterologous neutralization was divided into strong, cross reactive neutralization and weak neutralization.
- The V4 region in the HIV virus was found during analysis of plasma samples to have the greatest neutralization potency.
    - This identification can have further reaching implications for the development of a HIV vaccine.

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Summary of Subjects SGA’s and Functional *Env* Genes

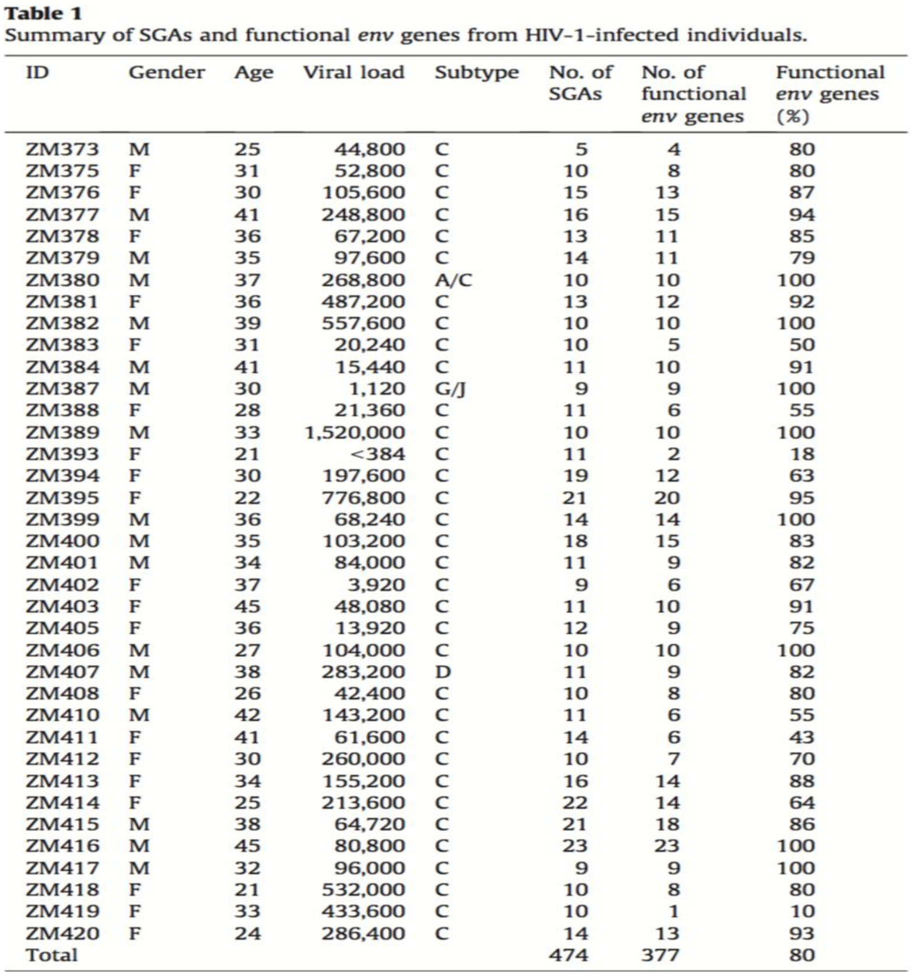


Table 1
Summary of SGAs and functional *env* genes from HIV-1-infected individuals.

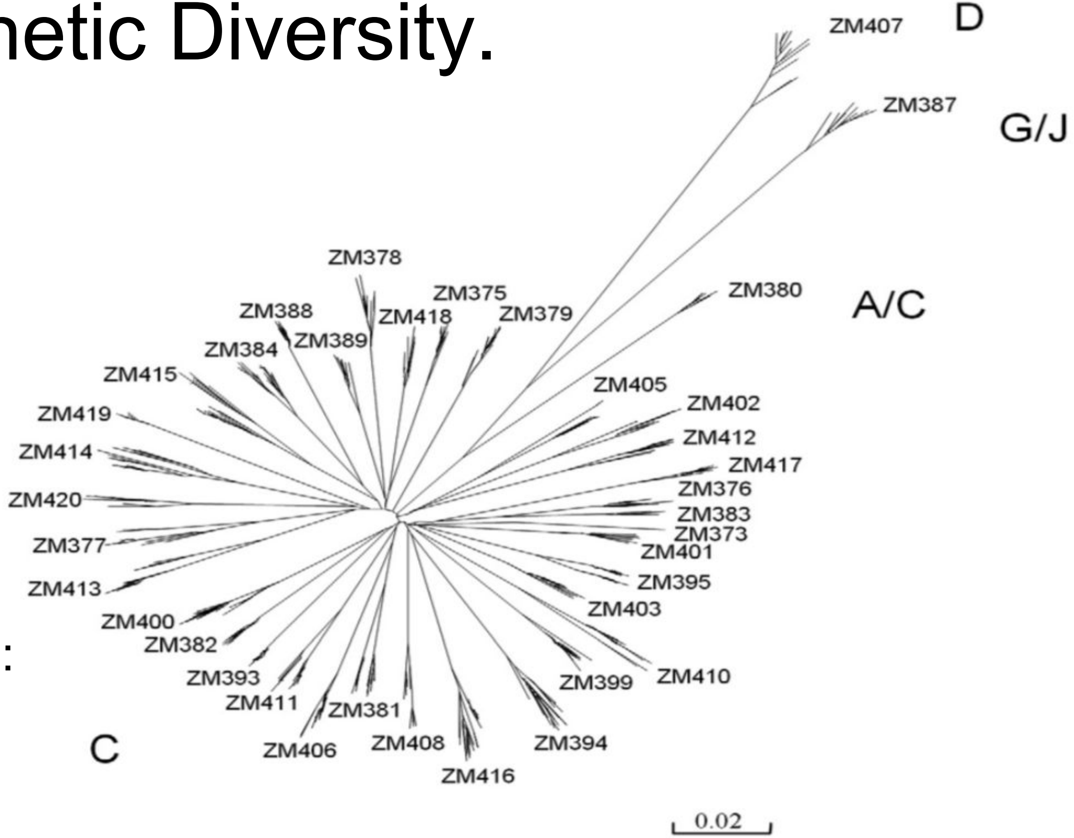
ID	Gender	Age	Viral load	Subtype	No. of SGAs	No. of functional *env* genes	Functional *env* genes (%)
ZM373	M	25	44,800	C	5	4	80
ZM375	F	31	52,800	C	10	8	80
ZM376	F	30	105,600	C	15	13	87
ZM377	M	41	248,800	C	16	15	94
ZM378	F	36	67,200	C	13	11	85
ZM379	M	35	97,600	C	14	11	79
ZM380	M	37	268,800	A/C	10	10	100
ZM381	F	36	487,200	C	13	12	92
ZM382	M	39	557,600	C	10	10	100
ZM383	F	31	20,240	C	10	5	50
ZM384	M	41	15,440	C	11	10	91
ZM387	M	30	1,120	G/J	9	9	100
ZM388	F	28	21,360	C	11	6	55
ZM389	M	33	1,520,000	C	10	10	100
ZM393	F	21	<384	C	11	2	18
ZM394	F	30	197,600	C	19	12	63
ZM395	F	22	776,800	C	21	20	95
ZM399	M	36	68,240	C	14	14	100
ZM400	M	35	103,200	C	18	15	83
ZM401	M	34	84,000	C	11	9	82
ZM402	F	37	3,920	C	9	6	67
ZM403	F	45	48,080	C	11	10	91
ZM405	F	36	13,920	C	12	9	75
ZM406	M	27	104,000	C	10	10	100
ZM407	M	38	283,200	D	11	9	82
ZM408	F	26	42,400	C	10	8	80
ZM410	M	42	143,200	C	11	6	55
ZM411	F	41	61,600	C	14	6	43
ZM412	F	30	260,000	C	10	7	70
ZM413	F	34	155,200	C	16	14	88
ZM414	F	25	213,600	C	22	14	64
ZM415	M	38	64,720	C	21	18	86
ZM416	M	45	80,800	C	23	23	100
ZM417	M	32	96,000	C	9	9	100
ZM418	F	21	532,000	C	10	8	80
ZM419	F	33	433,600	C	10	1	10
ZM420	F	24	286,400	C	14	13	93
Total					474	377	80

- Table1: This table lists each individual subject from which plasma was taken for this experiment.
  - Notable sections include the subtype, number of SGAs and the percent of *Env* genes.

(Kirchherr et al. 2010)

Unrooted Trees Were Formed To Show Genetic Diversity.

- Fig. 1:All subjects within the circle of the unrooted tree represent subtype C subjects.
  - The only outliers are of other subtypes: D, G/J, and A/C



(Kirchherr et al. 2010)

Luciferase Activity Shows Infectivity of HIV-1 Virus

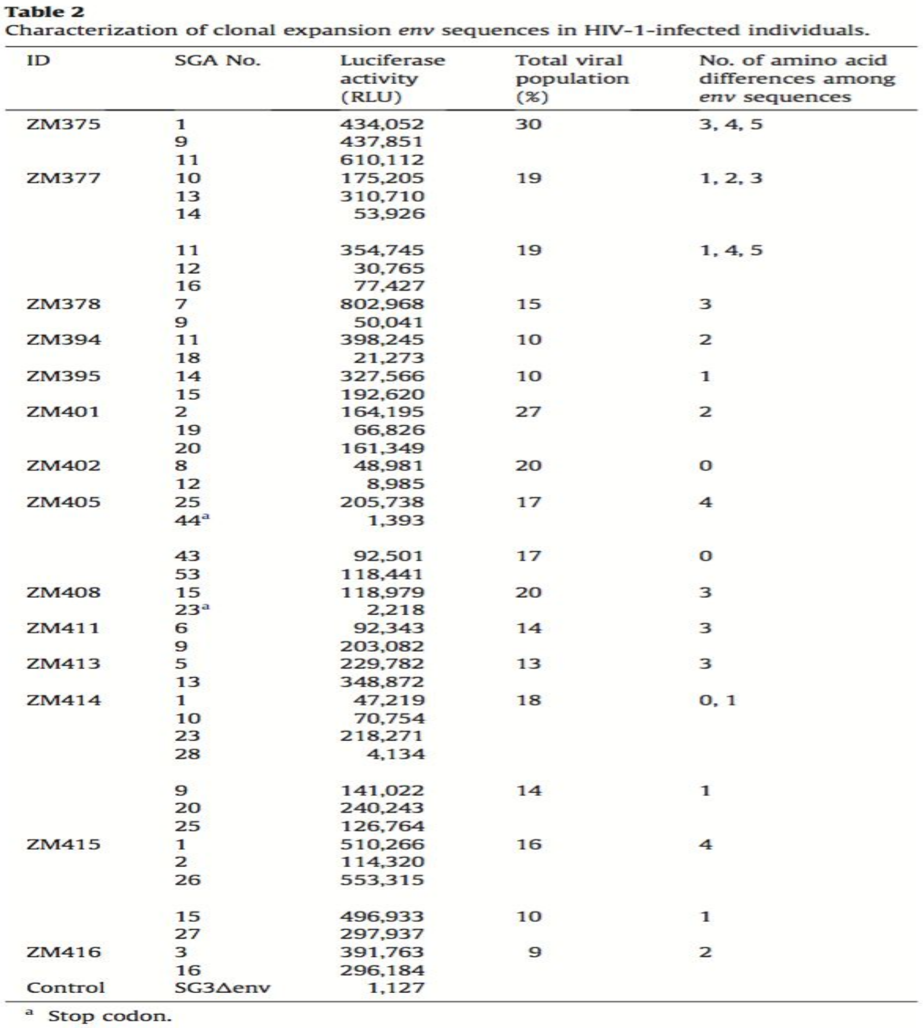


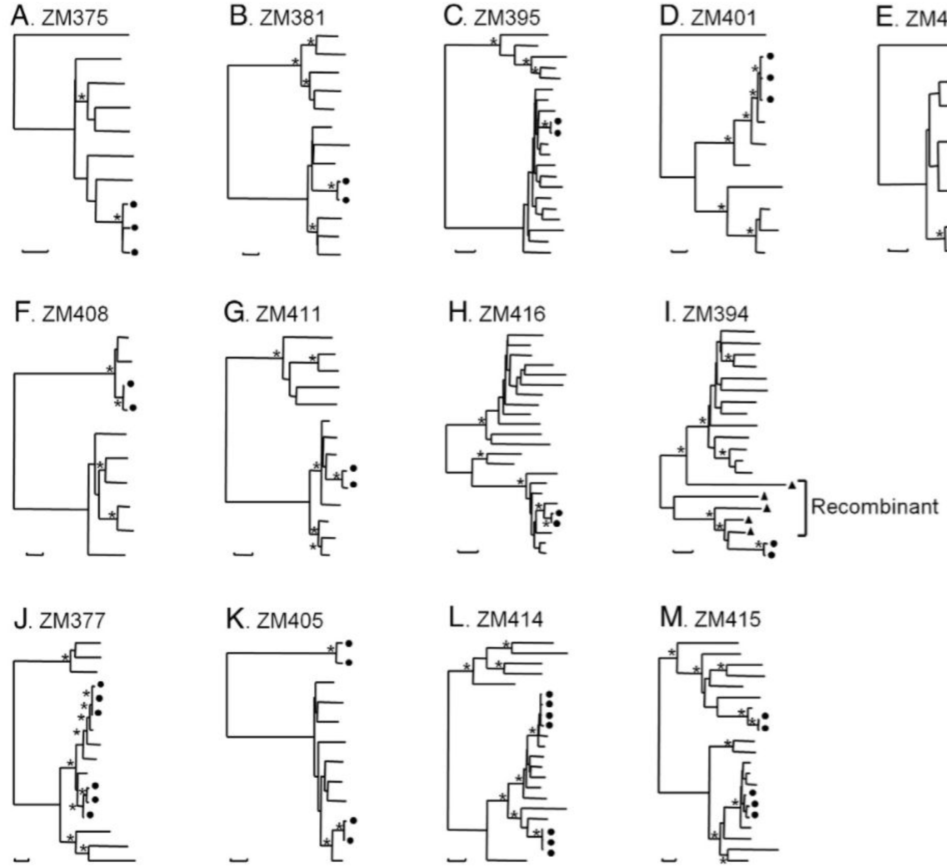
Table 2
Characterization of clonal expansion *env* sequences in HIV-1-infected individuals.

ID	SGA No.	Luciferase activity (RLU)	Total viral population (%)	No. of amino acid differences among *env* sequences
ZM375	1	434,052	30	3, 4, 5
	9	437,851		
	11	610,112		
ZM377	10	175,205	19	1, 2, 3
	13	310,710		
	14	53,926		
	11	354,745	19	1, 4, 5
	12	30,765		
	16	77,427		
ZM378	7	802,968	15	3
	9	50,041		
ZM394	11	398,245	10	2
	18	21,273		
ZM395	14	327,566	10	1
	15	192,620		
ZM401	2	164,195	27	2
	19	66,826		
	20	161,349		
ZM402	8	48,981	20	0
	12	8,985		
ZM405	25	205,738	17	4
	44[a]	1,393		
	43	92,501	17	0
	53	118,441		
ZM408	15	118,979	20	3
	23[a]	2,218		
ZM411	6	92,343	14	3
	9	203,082		
ZM413	5	229,782	13	3
	13	348,872		
ZM414	1	47,219	18	0, 1
	10	70,754		
	23	218,271		
	28	4,134		
	9	141,022	14	1
	20	240,243		
	25	126,764		
ZM415	1	510,266	16	4
	2	114,320		
	26	553,315		
	15	496,933	10	1
	27	297,937		
ZM416	3	391,763	9	2
	16	296,184		
Control	SG3Δenv	1,127		

[a] Stop codon.

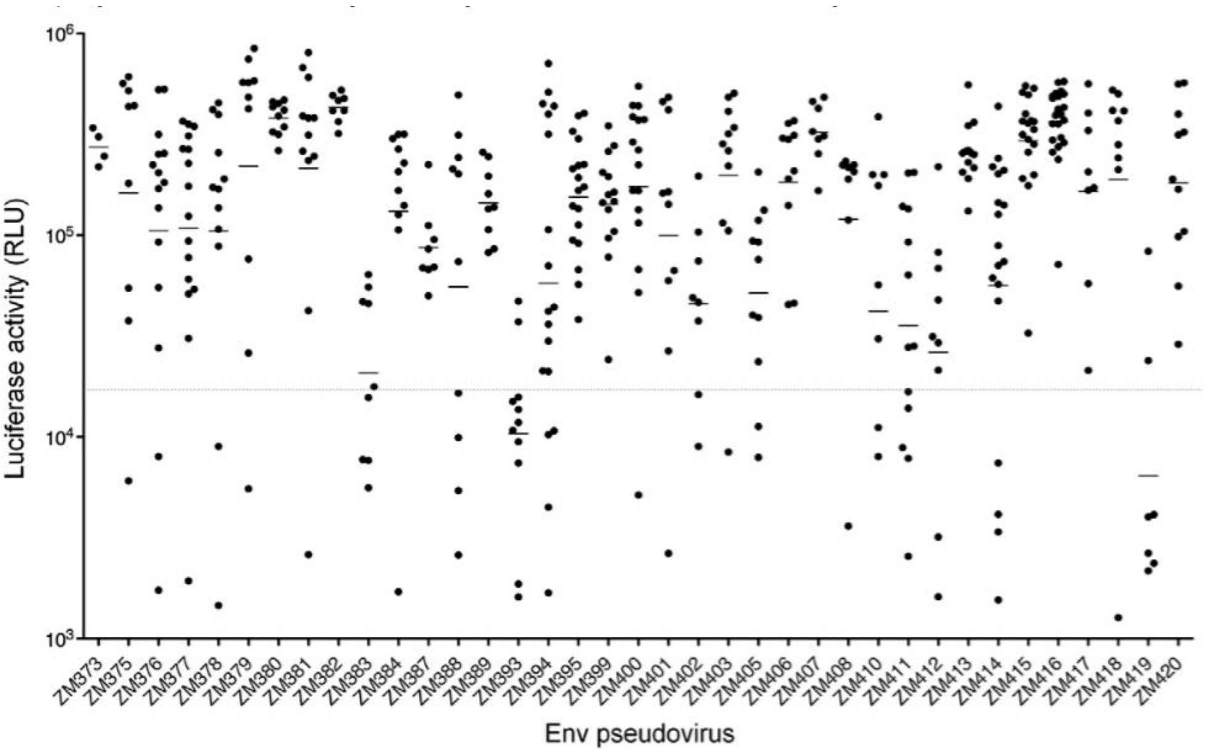
- Table 2: This table contains information that shows the infectivity of the HIV virus.
    - Using PCR, pseudoviruses were formed and then tested in the presence of TZM-bl indicator cells.
    - Luciferase activity indicates the presence of the HIV-1 virus.

(Kirchherr et al. 2010)



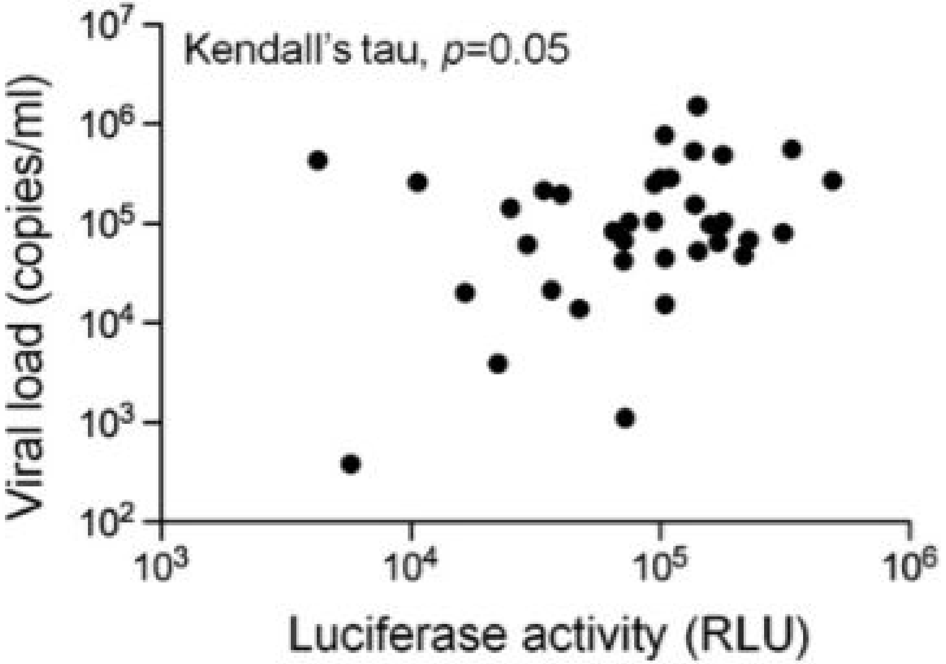
Rooted phylogenetic trees of 13 subjects demonstrating clonal expansion within the *env* gene

(Kirchherr et al. 2010)



Infectivity of 474 *env* pseudoviruses from 37 individuals determined in TZM-bl cells and measured by luciferase activity.

(Kirchherr et al. 2010)

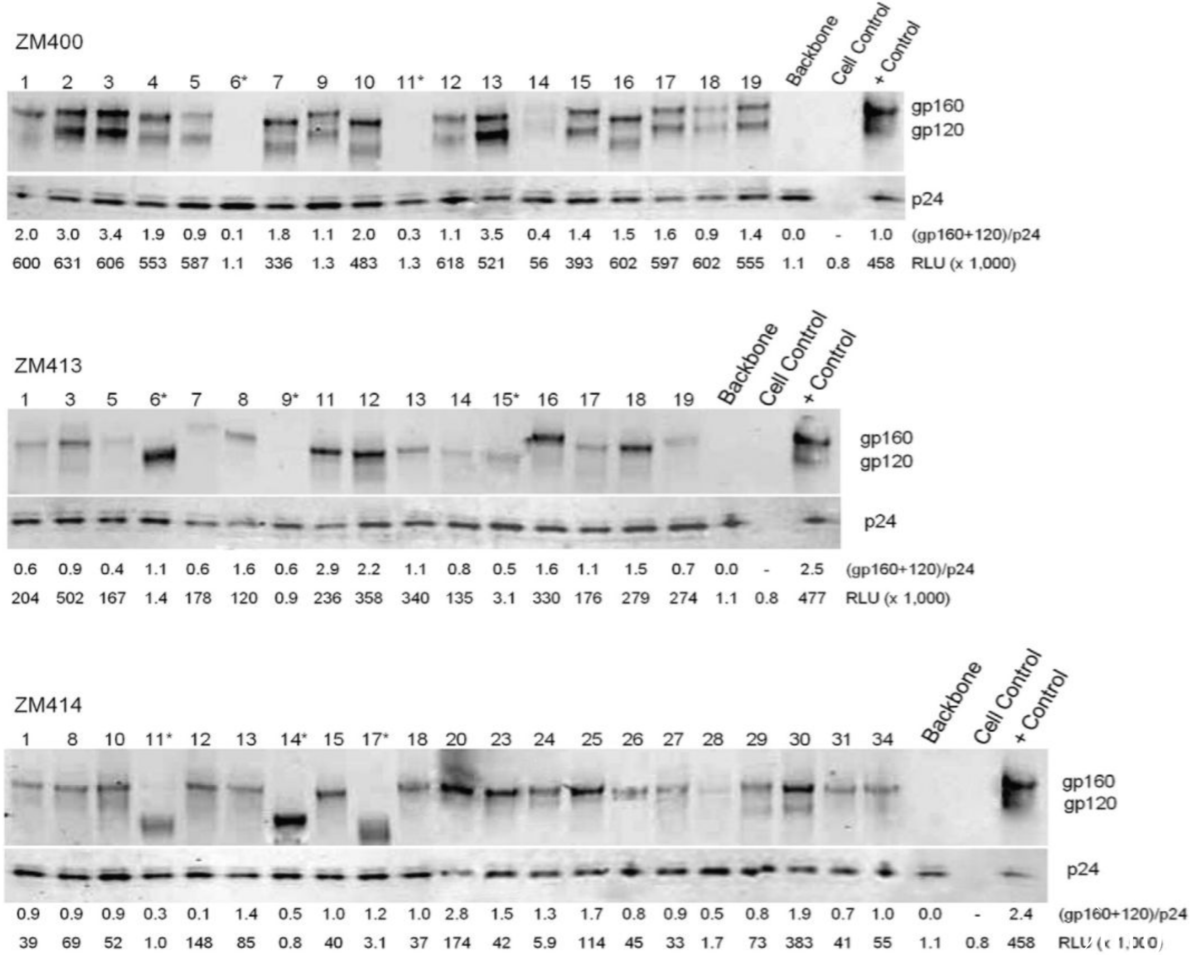


Trend supporting a positive correlation between viral load and pseudovirus infectivity. P-value of 0.05.

(Kirchherr et al. 2010)

Western Blot Analysis Of HIV-1 Proteins In Transfected Cells

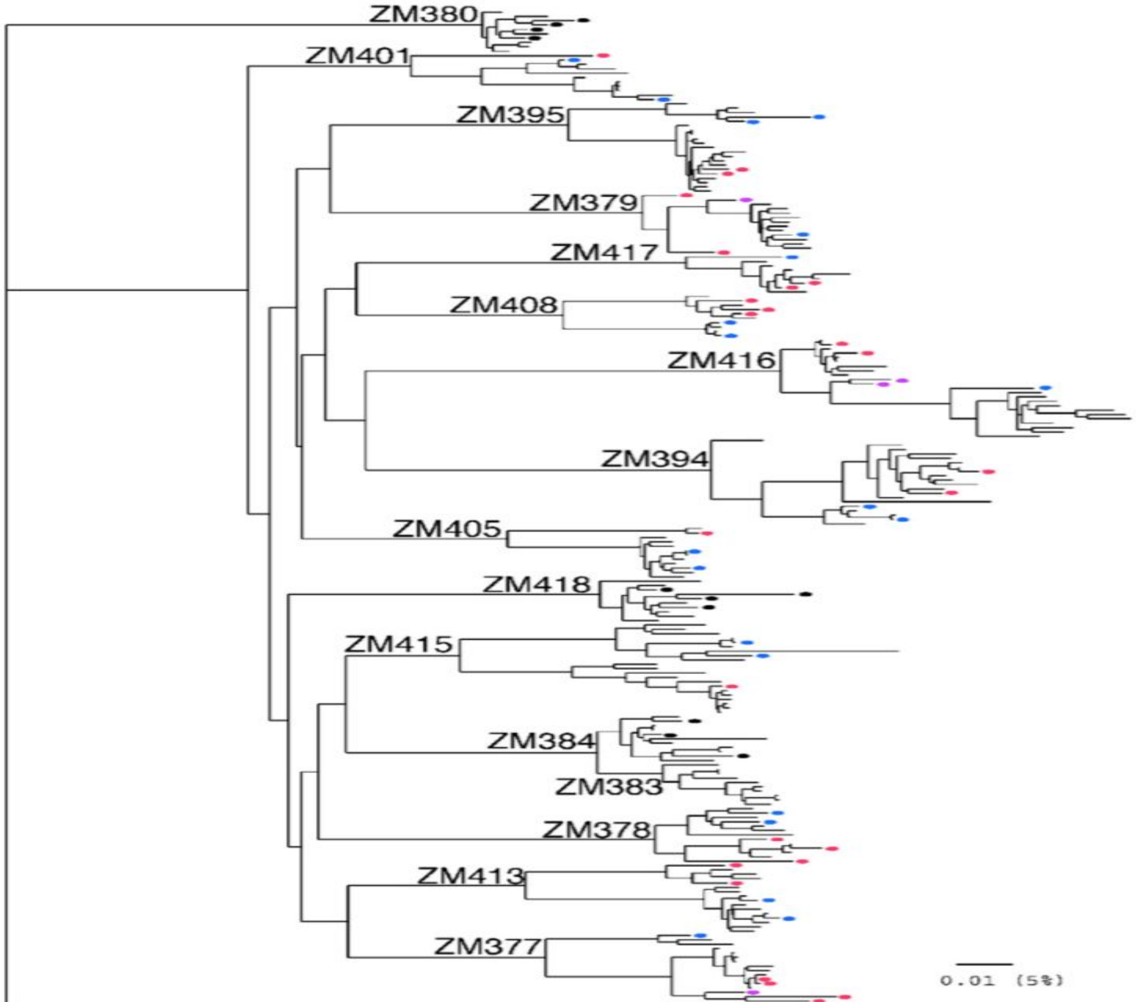
Figure 5:



(Kirchherr et al. 2010)

Phylogenetic tree of *Env* gene sequences

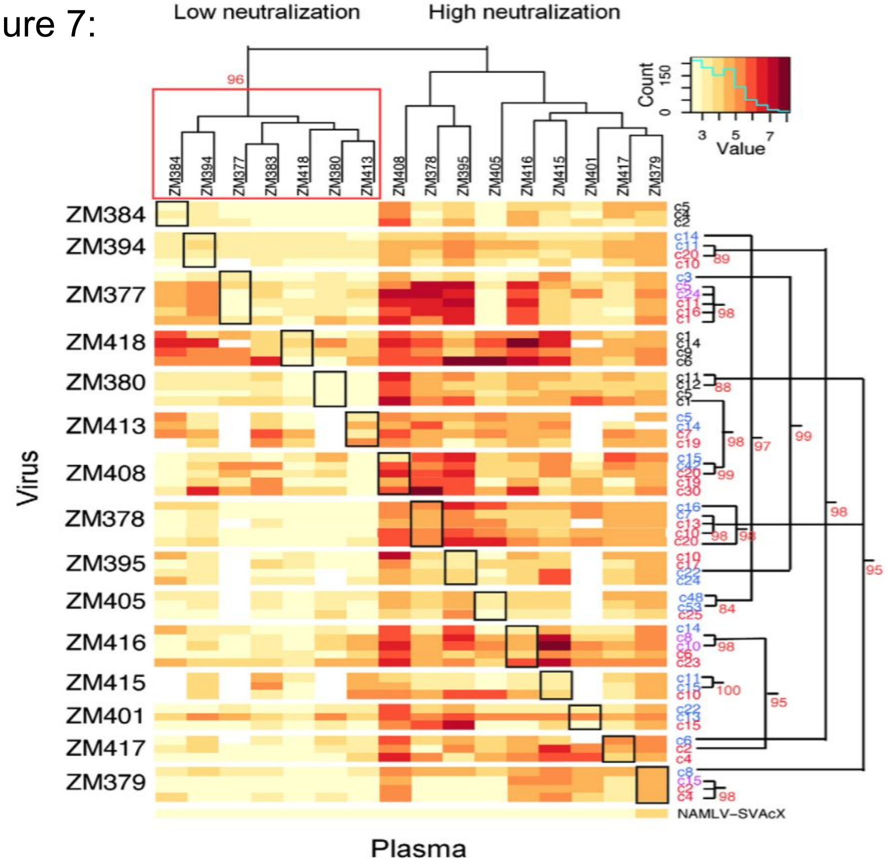
Figure 6:



(Kirchherr et al. 2010)

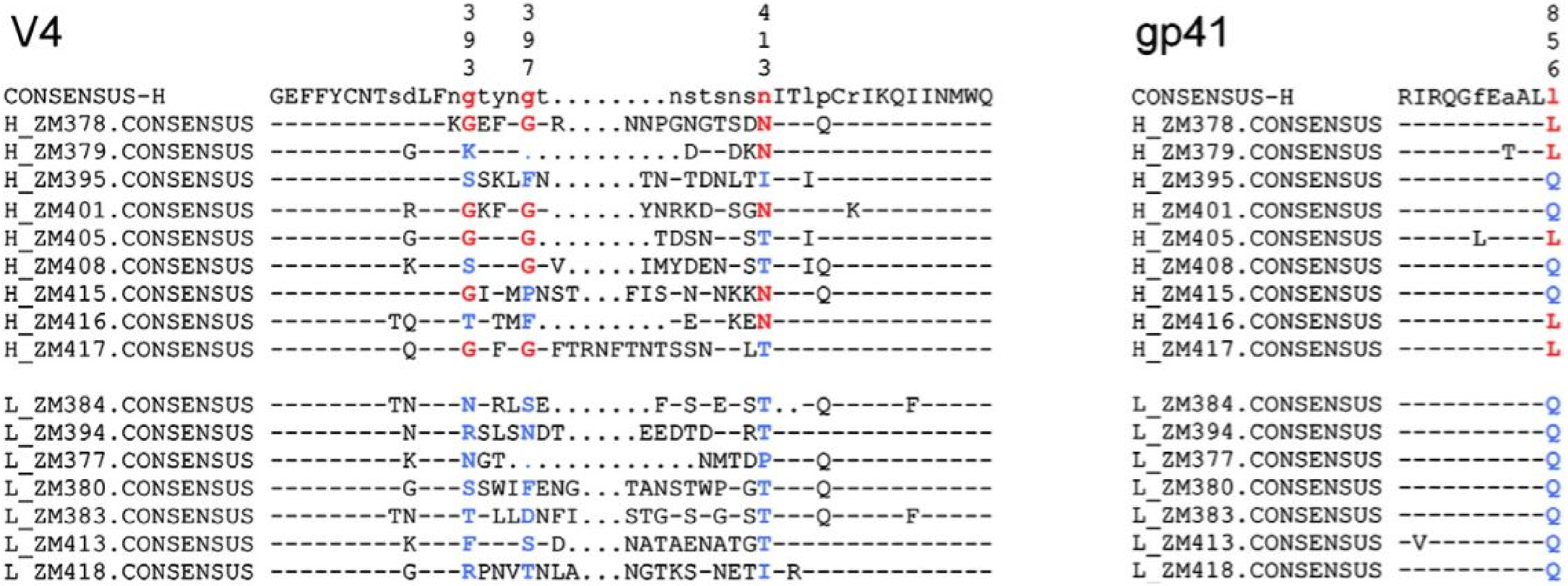
Hierarchical Clustering Of Virus Based On Neutralization Concentrations

Figure 7:



(Kirchherr et al. 2010)

Four Sites for Signature Amino Acid Sequences Were Found; Sites Associated With Potent Neutralization Highlighted



V4

```
                               3   3               4
                               9   9               1
                               3   7               3
CONSENSUS-H       GEFFYCNTsdLFngtyngt........nstsnsnITlpCrIKQIINMWQ
H_ZM378.CONSENSUS ------------KGEF-G-R....NNPGNGTSDN---Q-----------
H_ZM379.CONSENSUS ---------G---K---...........D--DKN---------------
H_ZM395.CONSENSUS -------------SSKLFN......TN-TDNLTI--I------------
H_ZM401.CONSENSUS ---------R---GKF-G-......YNRKD-SGN-----K---------
H_ZM405.CONSENSUS ---------G---G---G........TDSN--ST--I------------
H_ZM408.CONSENSUS ---------K---S---G-V.....IMYDEN-ST--IQ-----------
H_ZM415.CONSENSUS -------------GI-MPNST...FIS-N-NKKN---Q-----------
H_ZM416.CONSENSUS --------TQ---T-TMF.........-E--KEN---------------
H_ZM417.CONSENSUS ---------Q---G-F-G-FTRNFTNTSSN--LT---------------

L_ZM384.CONSENSUS --------TN---N-RLSE.......F-S-E-ST..-Q-----F-----
L_ZM394.CONSENSUS ---------N---RSLSNDT.....EEDTD--RT---------------
L_ZM377.CONSENSUS ---------K---NGT.............NMTDP---Q-----------
L_ZM380.CONSENSUS ---------G---SSWIFENG...TANSTWP-GT---Q-----------
L_ZM383.CONSENSUS --------TN---T-LLDNFI...STG-S-G-ST---Q-----F-----
L_ZM413.CONSENSUS ---------K---F---S-D....NATAENATGT---------------
L_ZM418.CONSENSUS ---------G---RPNVTNLA...NGTKS-NETI-R-------------
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gp41

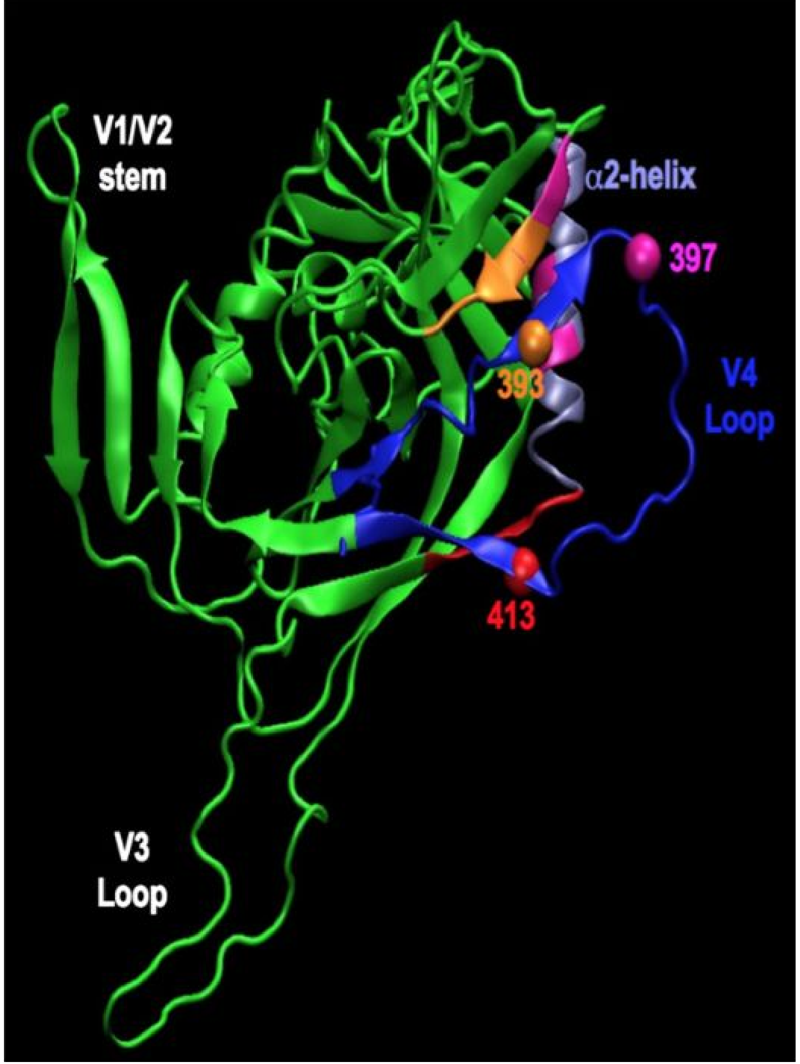
```
                            8
                            5
                            6
CONSENSUS-H       RIRQGfEaALl
H_ZM378.CONSENSUS ----------L
H_ZM379.CONSENSUS -------T--L
H_ZM395.CONSENSUS ----------Q
H_ZM401.CONSENSUS ----------Q
H_ZM405.CONSENSUS -----L----L
H_ZM408.CONSENSUS ----------Q
H_ZM415.CONSENSUS ----------Q
H_ZM416.CONSENSUS ----------L
H_ZM417.CONSENSUS ----------L

L_ZM384.CONSENSUS ----------Q
L_ZM394.CONSENSUS ----------Q
L_ZM377.CONSENSUS ----------Q
L_ZM380.CONSENSUS ----------Q
L_ZM383.CONSENSUS ----------Q
L_ZM413.CONSENSUS -V--------Q
L_ZM418.CONSENSUS ----------Q
```

Fig. 8. The signature amino acid sequences associated with high levels of neutralization activity in plasma. H represents sequences in the group of plasma samples with high neutralizing activity against heterologous viruses, whereas L represents sequences that exhibited weak neutralizing activity. Numbers are used to show corresponding locations in the HXB2 reference strain. Dashes indicate the identical amino acids present in the reference sequence. Periods are used to designate gaps to maintain the alignment. Signature sites associated with potent neutralization are shown in red. Non-signature amino acids in key positions are shown in blue.

(Kirchherr et al. 2010)

CD4-bound Truncated gp120 Structures From B and C Clades Showed That Both Structures Are Similar



(Kirchherr et al. 2010)

Plasmas With High Neutralization Of Heterologous Virus Trended Toward High Neutralization Of Autologous Viruses

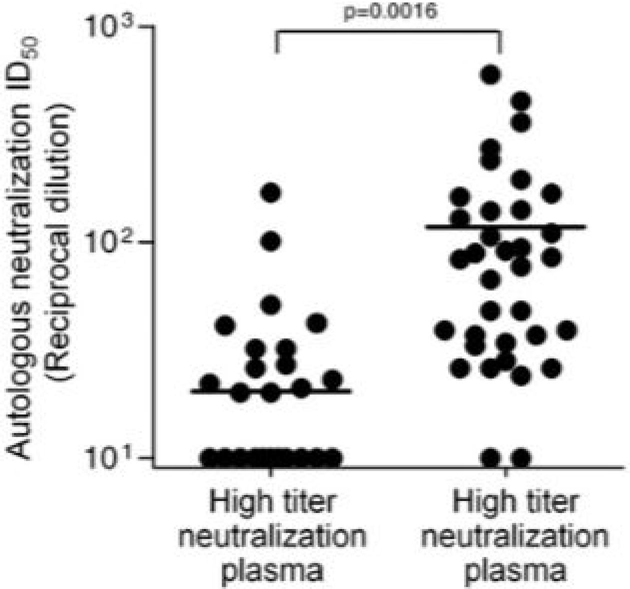


Fig. 10. Comparison of autologous neutralizing activity between low and high heterologous neutralizing plasma samples. Nab titers from low heterologous neutralization plasmas ($n=6$) or high heterologous neutralization plasmas ($n=9$) were compared. Values at *Y*-axis are the reciprocal plasma dilutions at which luciferase activity (RLU) was reduced by 50% relative to virus control wells by autologous plasma.

(Kirchherr et al. 2010)

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- Future research into the signature sites of the V4 loop is needed to develop a vaccine to induce a greater immune response for the presence of HIV.

Analysis of Data Revealed Signature Sequences of Four Amino Acids.

- This paper found variable regions in the gp120 gene were most susceptible to Nabs.
- Four signature sites were observed, three of which were in the V4 region of gp120, and the fourth being in gp41.
- The V4 region consisted of a four amino acid sequence and was the site for Nab binding in autologous and heterologous plasmas.
- Glycine residues in the V4 binding loop play a major role in binding of Nabs.
    - Loss of a gly residue would reduce negative charge in that region, preventing antibody binding.
    - Glycines have no side chains, making them more variable in terms of conforming for attachment.
- Clonal expansion could be an important role in disease proliferation.

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Identification of False Positive Sites Necessary for Future Experimentation

- Previous study by Moore et al. suggests C3V4 region may be responsible for inducing Nabs.
  - Kirchher et al.'s signature sites had amino acids located near this region, suggesting stronger Nab responses.
- The identified sites must each be tested to examine their role in susceptibility to Nabs, since three of them associate with the C3 alpha-2 helical domain, which is thought to contribute to patterns of neutralization susceptibility in subtype C viruses.
- Identification of false positives is necessary before further study
  - One or more of the identified sites is not related to viral susceptibility.
  - Elimination of these sites will allow vaccines to target amino acid sequences in relevant sites and increase the susceptibility of the virus to Nabs.

The Kirchher et al. Paper As A Whole

- The HIV virus has the ability to mutate rapidly, protecting itself against autoimmune response.
- Kirchher et al. studied what aspect of the HIV-1 virus induced response from Nads.
- The gp120 *Env* gene was studied for it’s susceptibility to Nads.
- The V4 region in gp120 contains several signature sequences that have high neutralization potency.
- The results of this study lays the groundwork for further research which can be used to develop a vaccine for the HIV-1 virus.

Acknowledgements

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References

Kirchherr, J. L., Hamilton, J., Lu, X., Gnanakaran, S., Muldoon, M., Daniels, M., Kasongo, W., Chalwe, V., Mulenga, C., Mwananyanda, L., Musonda, R.M., Yuan, X., Montefiori, D.C., Korber, M.T., Haynes, B.F., & Musonda, R. M. (2011). Identification of amino acid substitutions associated with neutralization phenotype in the human immunodeficiency virus type-1 subtype C gp120. Virology, 409(2), 163-174. DOI: 10.1016/j.virol.2010.09.031