A Rare Molecular Signature in HIV-1 Env V1 Associates With bNAb Evolution

Maria C. Hesselman[1], Marius Zeeb[1,2], Peter Rusert[1], Jennifer Mamrosh[3], Samuel Kariuki[4,5], Hugh Murrell[5], Nonhlanhla N. Mkhize[6,7], Kshitij Wagh[3], Penny L. Moore[6,7,8], Carolyn Williamson[5,8], Huldrych F. Günthard[1,2], Roger Kouyos[1,2], Alexandra Trkola[1]

[1]Institute of Medical Virology, University of Zurich, Zurich, Switzerland, [2]Department of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland, [3]Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, USA, [4]Department of Biological Sciences, School of Science, University of Eldoret, Eldoret, Kenya, [5]Institute for Infectious Diseases and Molecular Medicine, Division of Medical Virology, Faculty of Health Sciences, University of Cape Town and National Health Laboratory Service, Cape Town, South Africa, [6]Antibody Immunity Research Unit, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, [7]National Institute for Communicable Disease of the National Health Laboratory Services, Johannesburg, South Africa, [8]Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu Natal, Durban, South Africa

368

A rare twin Cysteine motif in the HIV-1 Env variable loop 1 region is enriched in bNAb inducer strains

Background

- Linking traits of the HIV-1 Envelope glycoprotein (Env) with the development of broadly neutralizing antibodies (bNAb) in natural infection is critical for the development of bNAb vaccines.
- In the Swiss 4.5K screen, we identified 304 bNAb inducers from the Swiss HIV Cohort Study (SHCS) and the Zurich Primary HIV Infection cohort (ZPHI) that form the XbnAb cohort (Rusert *et al. Nat. Med*., 2016).
- The XbnAb cohort provides a unique opportunity to identify common viral determinants of bNAb induction in natural infection among a large group of individuals.

Methods

- A representative Env panel from 36 XbNAb inducers was assessed for neutralization sensitivity.
- V1 Cys were functionally characterized *in vitro* and quantified in diverse HIV-1 cohorts (Swiss4.5K, CAPRISA, AMP) and in the LANL HIV sequence database.

bNAb inducer cohort	Longitudinal HIV cohorts	Public Env sequences	Transmission data set
Swiss 4.5K	SHCS ZPHI CAPRISA[1]	LANL HIV Sequence Database & CATNAP[2]	Antibody mediated protection trials (AMP)[3]

[1]Centre for the AIDS Programme of Research in South Africa, University of KwaZulu Natal, Durban, South Africa.
[2]Yoon *et al*., CATNAP: a tool to compile, analyze and tally neutralizing antibody panels. *Nucleic Acid Res* (2015).
[3]Corey *et al*., Two randomized trials of neutralizing antibodies to prevent HIV-1 acquisition, *NEJM* (2021).

Conclusions

- Extra V1 Cys likely optimize V1 stabilization and epitope shielding during neutralization escape.
- Gains in neutralization resistance and viral fitness with V1 Cys may vary depending on the extent of virus nAb co-evolution.
- bNAb-experienced viruses are likely optimized for both fitness and resistance.
- Recently escaped variants may, despite twin V1 Cys insertions, still have fitness deficits and are rapidly counter-selected when new pressure arises as in the case of VRC01 prophylaxis.

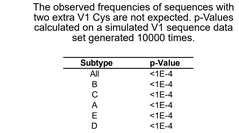
❶ Env of bNAb inducers: Higher neutralization resistance and extra Cys in V1

- XbNAb inducer Envs (N=36) are more neutralization resistant than random chronic Envs.
- XbNAb inducer Envs have longer V1 loops and 6/36 carry extra Cys in the V1 loop.
- Positioning of extra V1 Cys allows the formation of disulfide bridges, suggesting a means of stabilizing the V1 loop.

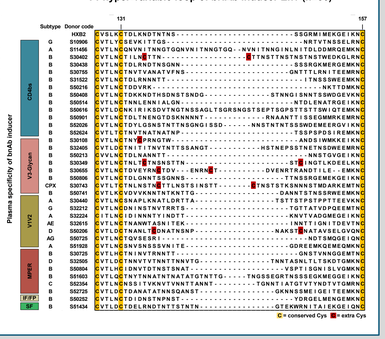
bNAb neutralization

bNAb	Median IC_{50} bnAB inducer Env (n=36)	Median IC_{50} CATNAP-derived chronic Env (n=128)	Wilcoxon p-Value
VRC01	14.48	0.48	<0.001
VRC-PG04	6.68	0.27	<0.001
3BNC117	6.82	0.10	<0.001
N6	0.38	0.07	0.001
PGT128	>25	0.06	0.002
PGDM12	>25	>25	0.135
PGDM21	>25	>25	0.244
PGDM1400	>25	0.19	<0.001
SF12	0.24	0.10	0.536
DH511.11P	0.19	0.49	0.117

V1 loop length
p < 0.0001



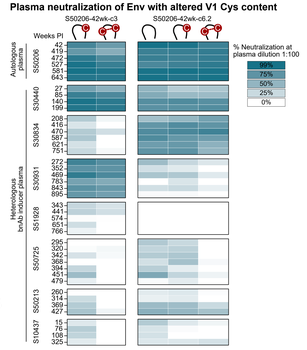
V1 hyper-variable loop of bNAb inducer Env (n=36)



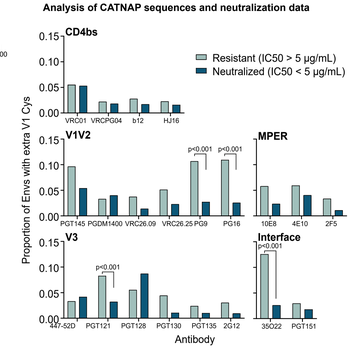
❷ Extra V1 Cys may be involved in immune escape

- *In vitro* molecular evidence from a V1V2 bNAb inducer: Addition of V1 twin Cys decreases, deletion increases plasma neutralization sensitivity.
- Population evidence (CATNAP analysis): Viruses resistant to PG9, PG16, PGT121 and 35O22 have a higher frequency of extra V1 Cys than sensitive viruses.

Plasma neutralization of Env with altered V1 Cys content

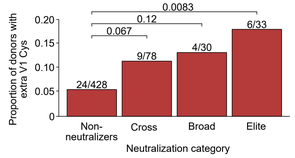


Analysis of CATNAP sequences and neutralization data



❸ Extra V1 Cys are associated with bNAb induction

- Env sequence analysis of Swiss 4.5K participants (N=569): Participants with elite neutralization activity have a higher frequency of extra V1 Cys.
- Logistic regression confirms independent association between elite neutralization and the presence of extra V1 Cys.



Independent contribution of host factors on the presence of extra V1 Cys in Env (Env sequences sampled within one year of the Swiss 4.5K screening time point, n = 569 donors)

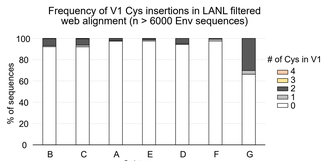
❹ Longitudinal Env evolution insight: Extra V1 Cys are commonly not fixed

- Longitudinal deep sequencing of Env in 100 CAPRISA cohort participants.
- 19/100 participants had at least one sequence with extra V1 Cys at any time point.
- The frequencies of Env with extra Cys fluctuate throughout infection.

❺ Cross-sectional population-wide insight from the Los Alamos HIV sequence database

- The overall frequency of Env with extra V1 Cys is low.
- There is a clear preference for the introduction of two extra V1 Cys.
- The number of Cys in V1 correlates with the length of the V1 hyper-variable region.

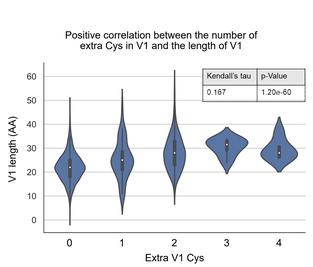
Frequency of V1 Cys insertions in LANL filtered web alignment (n > 6000 Env sequences)



The observed frequencies of sequences with two extra V1 Cys are not expected. p-Values calculated on a simulated V1 sequence data set generated 10000 times.

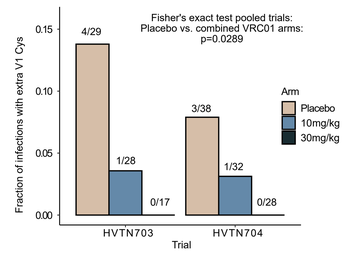
Subtype	p-Value
All	<1E-4
B	<1E-4
C	<1E-4
A	<1E-4
E	<1E-4
D	<1E-4
F	0.06
G	<1E-4

Positive correlation between the number of extra Cys in V1 and the length of V1



❻ AMP trial: Envs with extra V1 Cys were transmitted with higher frequency in the placebo arm.

- Antibody mediated protection (AMP) trials: Analysis of Env sequences from 172 participants (placebo and VRC01 treated) who became infected by the primary 80 week endpoint.
- Nine participants had at least one Env variant with extra V1 Cys.
- The proportion of infections with Env containing extra V1 Cys was higher in the placebo arm than in the combined VRC01 arms.



Acknowledgments

Swiss National Science Foundation

Grant Number: 314730_172790314730B_201266

Swiss National Science Foundation

University of Zurich[UZH]

Contact

Maria Hesselman
hesselman.maria@virology.uzh.ch

