

Mapping Autologous Neutralization Targets in Newly Transmitted Clade C Primary HIV-1 Isolates

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Three desirable properties of humoral vaccine targets are:

1- Sensitive mediators of neutralization

- a- Must be exposed on the surface of native viral particles
- b- Should not be masked by other domains or by glycans
- c- Antibodies bound to these sites should inhibit some function essential for viral entry

2- Highly immunogenic

3- Broadly conserved across many isolates (and clades)

Empirical evidence suggests that for HIV, targets with all three properties may not exist

-Any Envs possessing properties 1 and 2 would be efficiently neutralized by the host, and would not spread throughout the population

Alternate approach

- Characterize targets that are immunogenic and that mediate potent neutralization, but are not widely conserved
- Once such sites are defined, it may be possible to identify additional motifs present in these regions that elicit antibodies that are more cross-reactive and have broader neutralizing activities.
- The breadth of the neutralization response elicited against such targets may be expanded by the use of multivalent immunogens that include multiple allelic forms of these epitopes
- Such targets can be identified by studying autologous neutralizing responses

Recent studies have shown that within a year after infection, patients frequently develop potent neutralizing antibody responses specific for strains present early after infection

Sagar, et al., (2006). Human immunodeficiency virus type 1 V1-V2 envelope loop sequences expand and add glycosylation sites over the course of infection, and these modifications affect antibody neutralization sensitivity. *J Virol* 80, 9586-98.

Li, et al., (2006). Evidence for potent autologous neutralizing antibody titers and compact envelopes in early infection with subtype C human immunodeficiency virus type 1. *J Virol* 80, 5211-8.

Gray, et al, (2007). Neutralizing antibody responses in acute human immunodeficiency virus type 1 subtype C infection. *J Virol* 81, 6187-96.

Little, if anything, is known about the nature of the epitopes that mediate this autologous neutralization

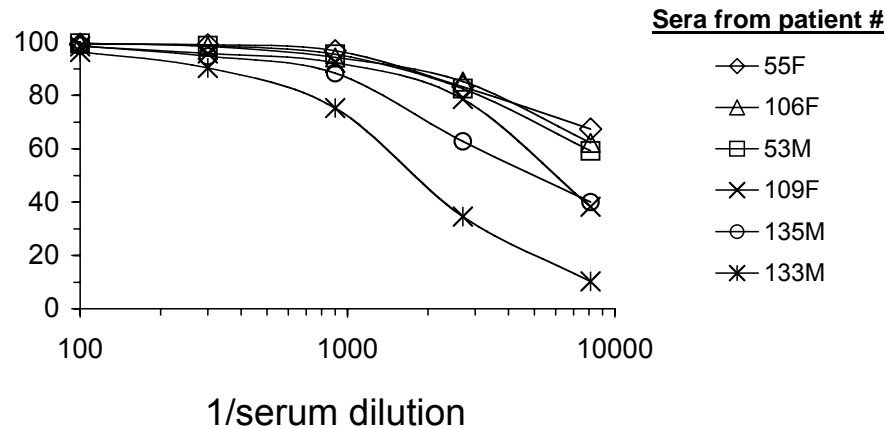
Neutralization of primary clade C HIV-1 isolates by autologous sera

<u>Virus</u>	<u>133 M</u> <u>Month 12</u>	<u>109 F</u> <u>Month 12</u>	<u>106 F</u> <u>Month 24</u>	<u>55 F</u> <u>Month 18</u>	<u>53 M</u> <u>Month 18</u>	<u>135 M</u> <u>Month 25</u>
133 MPB 3.8	3,008	<20	<20	<20	41	<20
109 FPB60	<20	2,801	<20	<20	<20	<20
106 FPB9	<20	<20	271	<20	160	<20
55 FPB3	<20	<20	<20	2,882	276	28
53 MPB21	<20	<20	<20	<20	7,874	<20
135 MPL23a	<20	<20	<20	<20	<20	2,941

Sera from heterosexually infected African subjects were able to neutralize the autologous viruses isolated shortly after infection, but possessed limiting cross-neutralizing activities

Data from Li et al., J. Virol. 80:5211-18, 2006

Neutralization of HIV-1 pseudotyped with SF162 Env with clade B consensus V3 sequence



- The potent neutralization of SF162 by these sera indicates that these sera do possess broadly cross-reactive antibodies against conserved neutralization targets
- The fact that these sera do not cross-neutralize the heterologous primary isolates suggests that the conserved epitopes in those Envs are not accessible, presumably because of effective masking
- The potent neutralization of the autologous viruses by these sera further indicates that the autologous neutralization epitopes are not sensitive to masking

Where are the autologous neutralization targets located?

Analysis of gp120 sequence homology of clade C Env panel

Variable domains

1
 133MPB3.8E MRVVRGMRNRWQWGIWGLG FWMFMICRVM-GDLWVTVVY GVPVWREAKTTLFCASDAKS YEREVHNWATHACVPTDPN PQEIVLGNVTESFNMWKNDM
 106FPB9Env MKVREILRNWRQWGIWGLG FWMMLICNVVGNLWVTVVY GVPVWREAKTTLFCASDAKA YEREVHNWATHACVPTDPS PQEMVLENVTENFNMWKNDM
 53MPB21Env MRVREIPRNYQQWGIWGLG FWMMLICSVV-GNLWVTVVY GVPVWREAKTTLFCASDAKA YEREVHNWATHACVPTDPN PQEMVLENVTENFNMWKNDM
 55FPB4aEnv MRVKGMRNRWQWGIWGLG FWMFLYSGM--GSLWVTVVY GVPVWREAKTTLFCASDAKA YEREVHNWATHACVPTDPN PQEMVLENVTENFNMWKNDM
 135MPL23aE MRVVRGIQRNWQWGIWGLG FWMFMICSGV-GNLWVTVVY GVPVWREAKTTLFCASDAKA YEREVHNWATHACVPTDPN PQELVLGNVTENFNMWKNDM
 109FPB60En MRVKGILRNCCQQWGIWGLG FWMMLICNVV-GNLWVTVVY GVPVWREAKTTLFCASDAKS YEREVHNWATHACVPTDPP PQELVMANVTENFNMWKNDM
 p1531 MRVKGIRKNYQHLWRGGTLL LGMLMICSAV-EKLVWTVVY YDTEVHNWATHACVPTDPN PQEIVLENVTENFNMWKNDM
 Consensus MrVrgi.rNwqgwIwGIlg fwMlmic.vv.gNLWVTVVY GVPVWREAKTTLFCASDAKA Y#rEVHNWATHACVPTDPN PQE.V\$eNVTE#FNMWkN#M

101
 133MPB3.8E VQMHEDIISLWDSLKPCV KLTPLCVTLKCRNANSTGGN VMTTDHVAYNNSMNGEIKNC SFNVTTEIRDKKQNVYALFY RLDVVPVPL--NGN---ST--
 106FPB9Env VQMHEDIISLWDSLKPCV KLTPLCVTLKCVNWNAT--- --SKSNASATNDGSGEMKNC TFNITTEIRDKKRNESALFY KLDIVPLTDNDNN---SG--
 53MPB21Env VQMQEDIISLWDSLKPCV KLTPLCVTLNCSKLN----- -NATDGEMKNC SFNATTELRDKKKQVYALFY KLDIVPLDGRNN---SS--
 55FPB4aEnv VQMHEDIISLWDSLKPCV KLTPLCVTLNCFIT----- -NTTEIKNC TFNMTTELRDIKQGRALFD ILDIVPLEPPNN---SSNY
 135MPL23aE VQMHEDIISLWDSLKPCV KLTPLCVTLCKNATRS----- -NQTTYDNMMDKEIKNC SFNVTTELRDKKKNRMLFY RADIEPLDGNSENESINSESG
 109FPB60En VQMHEDIISLWDSLKPCV KLTPLCVTLNCTSPAA----- -HNSESTRVKHC SFNITTDVDRKQKVNATFY DLDIVPLSSDN---SSNS
 p1531 VQMHEDIISLWDSLKPCV KLTPLCVTLHCTNLKNA--- -TNTKSSNWKEMDRGEIKNC SFKVTTSIRNKMQKEYALFY KLDVVPIDNDNT-----
 Consensus V#QMHEDIISLWDSLKPCV KLTPLCVTL.C.n..... .n...geiKnc sFn.TTe.r#kkq...AlFy .ldIvPl...nn...ss.

V1/V2 domain

201
 133MPB3.8E -EYRLINCNSTSAITQACPKV SFDPIPIHYCAPAGYAILKC NNTKTFSGIGPCHNVSTVQCT HGIKPVVSTQLLNGSLAE EIIIRSENLTNNVKTIIVHL
 106FPB9Env -EYRLINCNSTSAITQACPKV SFDPIPIHYCAPAGYAILKC NNTKTFNGTGPCNVSTVQCT HGIKPVVSTQLLNGSLAEG EIIIRSENLTNDVKTIIVHL
 53MPB21Env -EYRLINCNSTSAITQACPKV SFDPIPIHYCAPAGYAILKC NNTKTFNGTGPCNVSTVQCT HGIKPVISTQLLNGSTAE DIIIRSENLTNNAKTIIVHL
 55FPB4aEnv SEYRLISCNSTSAITQACPKV SFDPIPIHYCAPAGYAILKC NNTKTFNGTGPCNVSTVQCT HGIKPVVSTQLLNGSLAE EIIIRSENLTNNVKTIIVHL
 135MPL23aE DKYRLINCNSTSAITQACPKV TFDPIPIHYCAPAGYAILKC NNTKTFNGIGPCHNVSTVQCT HGIKPVVSTQLLNGSLSE GIIIRSKNLTNDTKTIIVHL
 109FPB60En SLYRLISCNSTSAITQACPKV SFDPIPIHYCAPAGYAILKC NNTKTFSGKGPCNVSTVQCT HGIRPVVSTQLLNGSLAE EIVIRSENLTNDAKTIIVHL
 p1531 -SYKRLINCNSTSAITQACPKV SFEPIPIHYCAPAGYAILKC NNTKTFNGSGPCNVSTVQCT HGIRPVVSTQLLNGSLAEG GVVIRSENLTNDAKTIIVQL
 Consensus .eYrLinCNSTSAITQACPKV sF#PIPIHYCAPAG#AILKC N#KtFng.GPC.NVSTVQCT HGikpv!STQLLNGSLaEe e!IIRSeNLT#N.KTIIVHL

V3, V3' domains

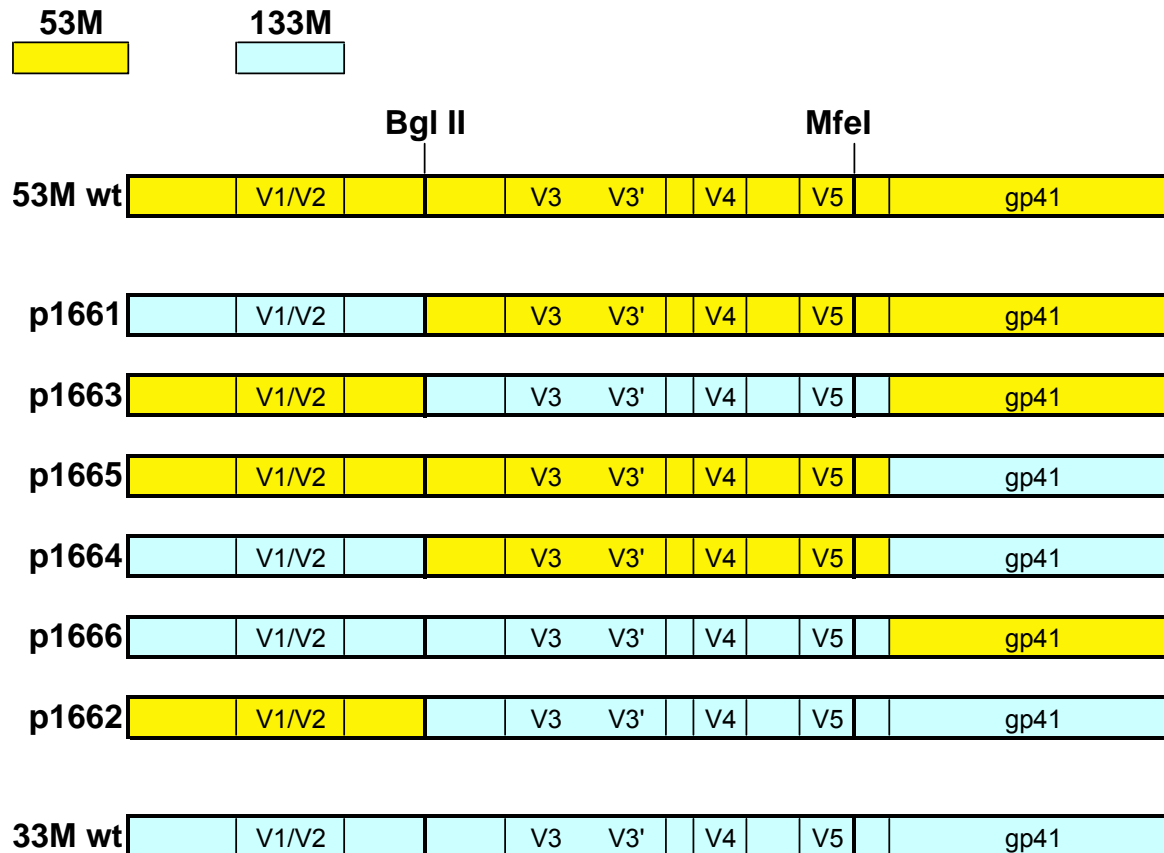
301
 133MPB3.8E NESVEIVCTRPNNNTRKSIR IGPQGFYAT-DIVGDIRQA HCNITRSKWSETISKVKGEL SKHFPNKTIKFAHSSGGDLE ITTHSFNCGGEFFYCNTSNL
 106FPB9Env NESIHITCTRPNNNTRKSIR IGPQGFYATGIIIGDIRQA YCIINSEKWNKALQEVGKKL EHFPPNKTIKFAHSSGGDLE ITTHSFNCRGEFFYCNTSKL
 53MPB21Env NESIEIECTRPNNNTRKSIR IGPQGFYATNIIGDIRQA YCIINKANWNTLHRVSKKL EHFPPNKTIKFAHSSGGDLE ITTHSFNCGGEFFYCNTSSL
 55FPB4aEnv NEPVYIVCTRPNNNTRKSMR IGPQGFYATGDIIGDIRQA HCNISIEKWNTLTKVSKERL KKHFPNKTIKFAHSSGGDLE ITTHSFNCRGEFFYCNTANL
 135MPL23aE NESVAIVCTRPNNNTRKSIR IGPQGFYATGVIIGDIRQA HCNISIEGQWNTLERIKDKL TEYFPDKIKFVHSSGGDLE ITTHSFNCRGEFFYCNTSIL
 109FPB60En NKSVEIECTRPNNNTRKSIR IGPQGFYATGDIIGDIRQA YCINISGEWNETLTKVSEKL KEYF-NKTIKFAHSSGGDLE VTHSFNCRGEFFYCNTSEL
 p1531 KESVEINCTRPNNNTRKSIH IGPGRAFYTTGIIIGDIRQA HCNISIEGKWNTLTKQIVTKL QAQFGNKTIKFAHSSGGDPE IVMHSFNCGGEFFYCNTSL
 Consensus nes!eI.CtrPnNTRKSir IGPgqtF%aTg#!IGDIRQA hCAts.ekWn.tl.!.kl .ehFp#ktI.F. .sGGDLE !tHsFNCRGEFFYCnts.L

V4, V5 domains

401
 133MPB3.8E IS----NSTEGGANSTNIT LPCRICKQIINMWQEVGRAIY TPPIAGNITCKSNITGLLLV RDGGSTRENNNTEIFRPGGG DMRDNWRSELYKVKVVEIKP
 106FPB9Env FNSTYMHNATSARNATNATIT LPCRIRQIINMWQEVGRAMY APPIAGNITCVSNITGLLLV RDGG-NGDTNDTEIFRPGGG DMKNWRSELYKVKVVEIKP
 53MPB21Env KNGTY--NDTDI-YNSTLIT LPCRICKQIINMWQEVGRAMY APPIEGNITCASNITGLLLT RDGGLTNESK--EIFRPGGG DMRDNWRSELYKVKVVEIKP
 55FPB4aEnv NENTF-MNQTDANQTNATIT LPCRICKQIINMWQEVGRAMY APPIENITCNSITGLLIT RDGGENTNDTEIFRPGGG DMRDNWRSELYKVKVVEIKP
 135MPL23aE FN-----TENESSDNIT LPCRICKQFVNMWQEVGRAMY APPIAGNITCNSITGLLLT RDGGLNKNKNGTEIFRPGGG DMRDNWRSELYKVKVVEIRP
 109FPB60En FN-----SNATESNIT LPCRICKQIINMWQEVGRAMY APPIRGEIKCTSNITGLLLT RDGG-NNNNSTEIEIFRPGGG NMRDNWRSELYKVKVVEIKP
 p1531 FNSTW-NNTIGPNNTNGTIT LPCRICKQIINMWQEVGKAMY APPIRGQIRCSSNITGLLLT RDGG-KEISNTTEIFRPGGG DMRDNWRSELYKVKVVEIKP
 Consensus fn.t...n.t...n.t...It LpCRiKQi!nMwQeVgrAmy appI.gNiTc.SnitGllLt RDGG.n...n.teIFRPGGG #Mr#NWRSELYKVKVVEIKP

501
 133MPB3.8E LGIAPTAKRRVVEREKR AVGIGAVFLGFLGAAGSTMGAA SITLTVQARQLLSGIVQQQS NLLRAIEAQQHMLQLTWGFI KQLQTRVLAIERYLKDDQLL
 106FPB9Env LGVAPTEAKRRVVEREKR AVGIGAVLLGFLGAAGSTMGAA SITLTAQARQLVLSGIVQQQS NLLRAIEAQQHLLQLTWGFI KQLQTRVLAIERYLKDDQLL
 53MPB21Env LGIAPTAKRRVVEREKR AVGLGAMFLGFLGAAGSTMGAA SITLTVQARQLLSGIVQQQN NLLRAIEAQQHMLQLTWGFI KQLQARVLAIERYLKDDQLL
 55FPB4aEnv LGIAPTEAKRRVVEREKR AVGIGAVFLGFLGAAGSTMGAA SITLTVQARQLLSGIVQQQS NLLRAIEAQQHMLQLTWGFI KQLQARVLAIERYLKDDQLL
 135MPL23aE LGVAPTEAKRRVVEREKR AVGIGAVFLGFLGAVGSTMGA SITLTAQARQLVLSGIVQQQS NLLRAIEAQQHMLQLTWGFI KQLQARVLAIERYLKDDQLL
 109FPB60En LGIAPTEAKRRVVEREKR AVGIGAVFLGFLGAAGSTMGAA SITLTVQARQLLSGIVQQQS NLLKAIEAQQHLLQLTWGFI KQLQARVLAIERYLKDDQLL
 p1531 LGVAPTEAKRRVVEREKR AVTLGAMFLGFLGAAGSTMGAA SITLTVQARQLLSGIVQQQN NLLRAIEAQQHLLHLTWGFI KQLQARVLAIERYLKDDQLL
 Consensus LG!APTAKRRV#rEKR AVgiGavfLgFlGaAgStMGAA SiTlTvQARQLLSgIvQQQs NLLrAIEAQH\$LqLTWgFI KQLQaRvLaiERyLkDQLL

In order to localize the regions targeted by the autologous neutralizing activity, chimeras were prepared between two of these Envs, 53M and 133M

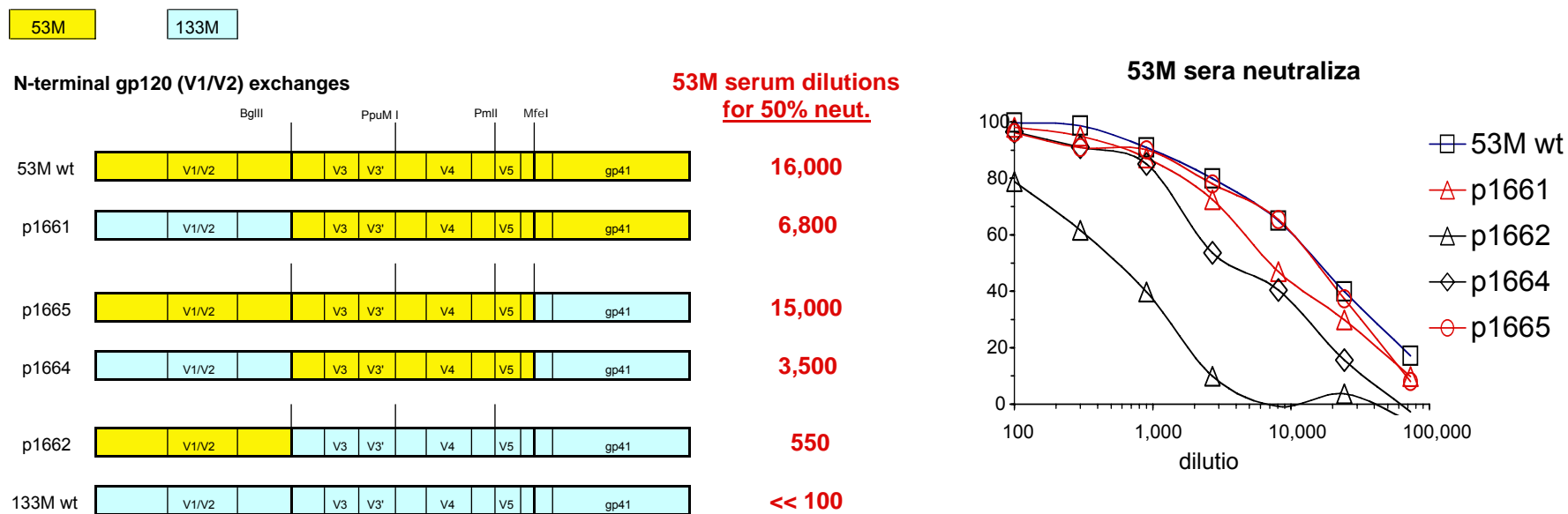


Initial chimeric Envs were constructed using conserved restriction sites that separated the gp120 N-terminus (C1-V1V1-C2), gp120 C-terminus (V3-V3'-V4-C4-V5) and TM domains

All of these chimeric Envs were infectious.

These were used to study autologous targets of 53M serum

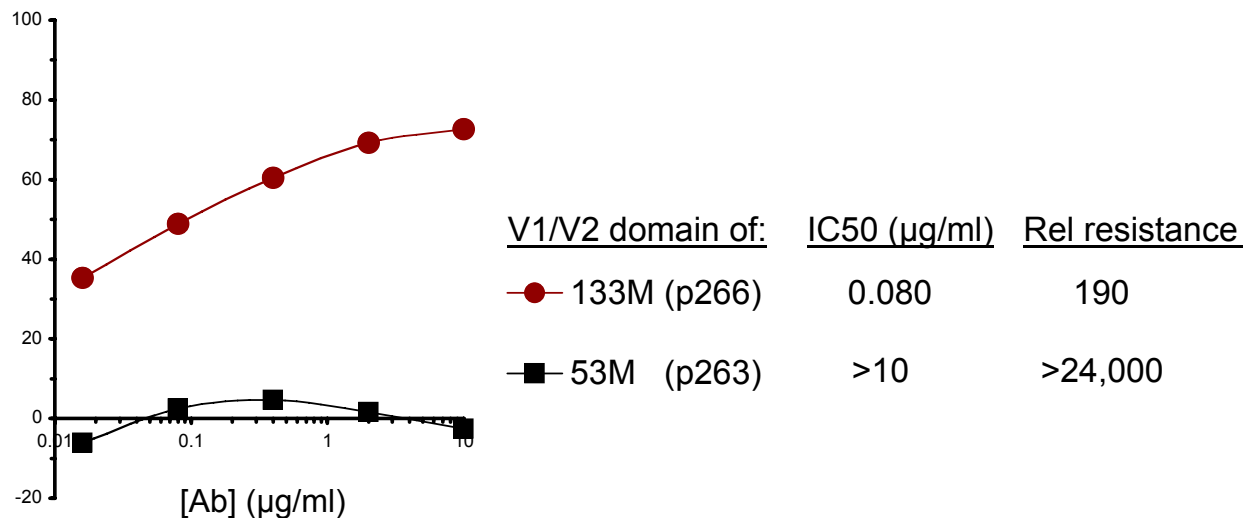
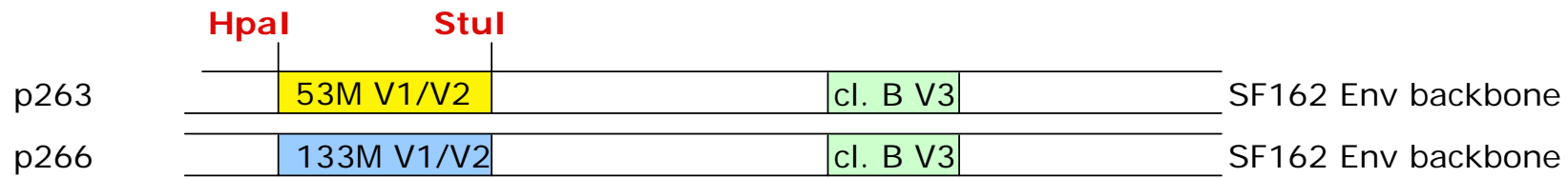
Substituting the N-terminal region of gp120 resulted in significant reduction in the autologous neutralization titer



This is consistent with the presence of potential neutralization targets in the gp120 N-terminal region of 53M (presumably in V1/V2).

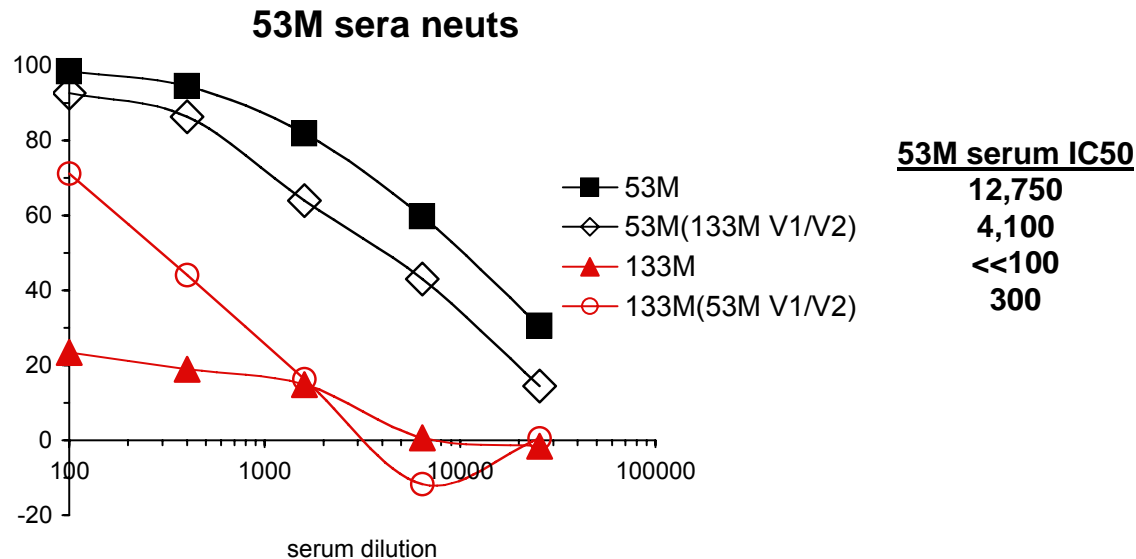
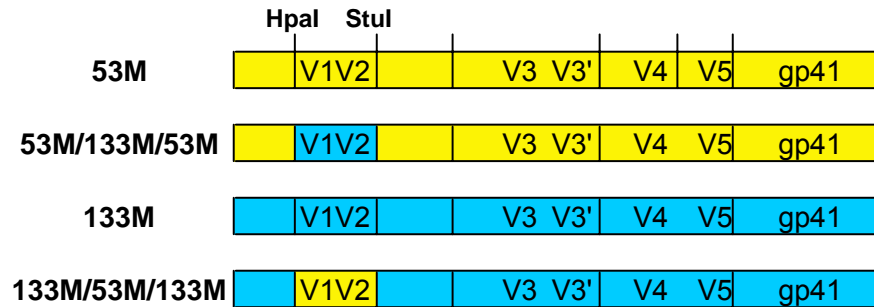
However, might be due to differential masking effects by 53M and 133M V1/V2 regions

The relative masking activities of the 53M and 133M V1/V2 domains was quantitated by exchanging these domains into a standard Env backbone, and examining their sensitivity to neutralization to anti-V3 antibodies



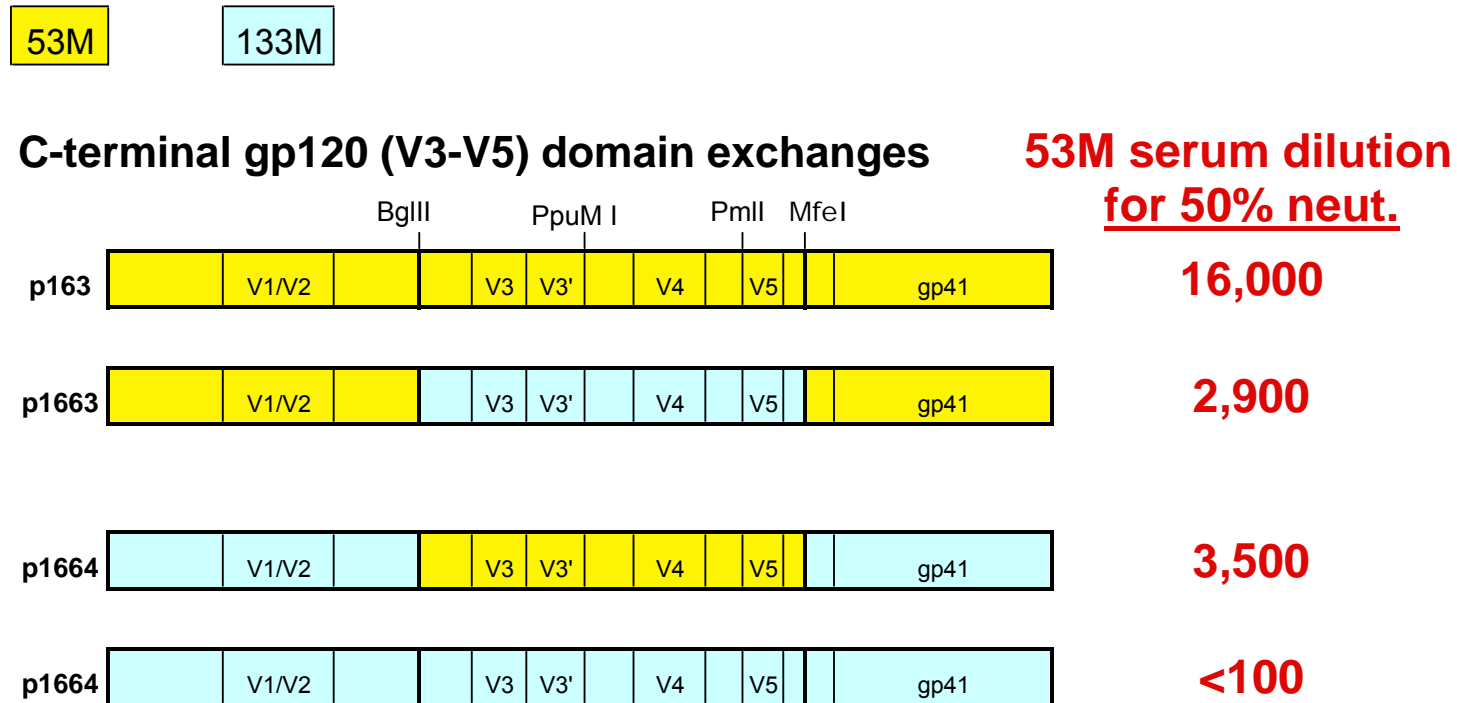
The reduction in neutralizing titer of 53M serum upon replacing the N-terminal gp120 region occurred despite a corresponding reduction in the V1/V2 masking activity

Substituting just the V1/V2 region also reduced the autologous neutralizing titer of 53M serum



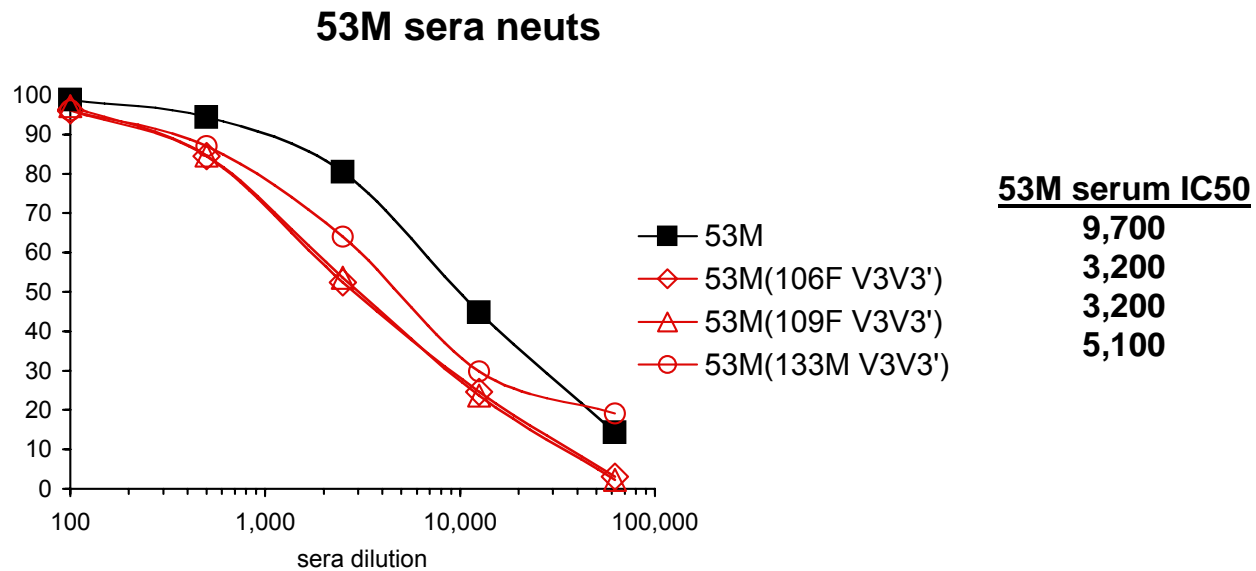
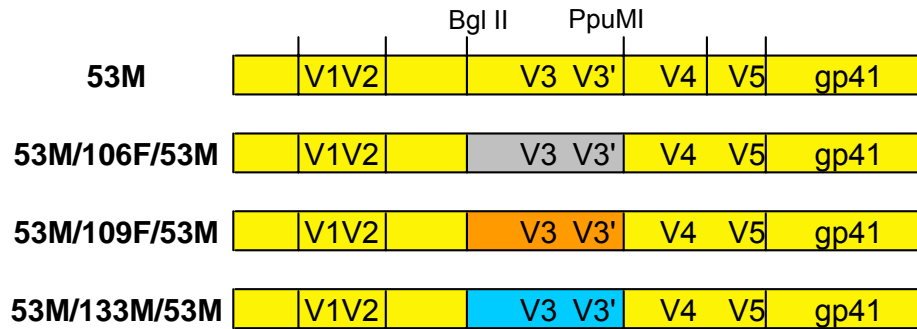
These results suggest the presence of autologous neutralization targets in the V1/V2 domain of 53M Env

Substituting the gp120 C-terminal fragment (including V3-V5) significantly reduced the neutralizing titer of 53M serum

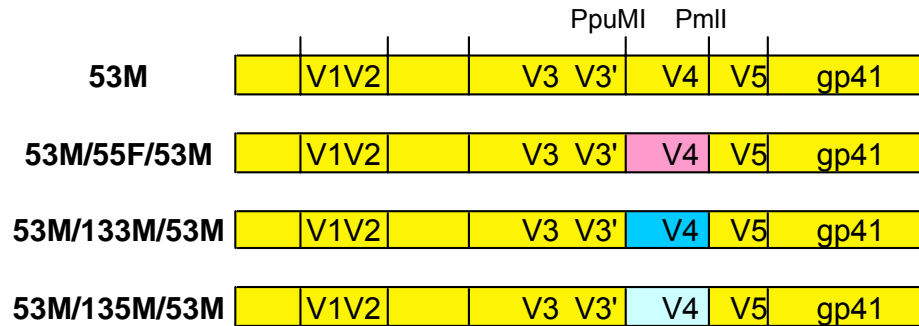


The sites involved were further mapped by exchanging smaller fragments of this region

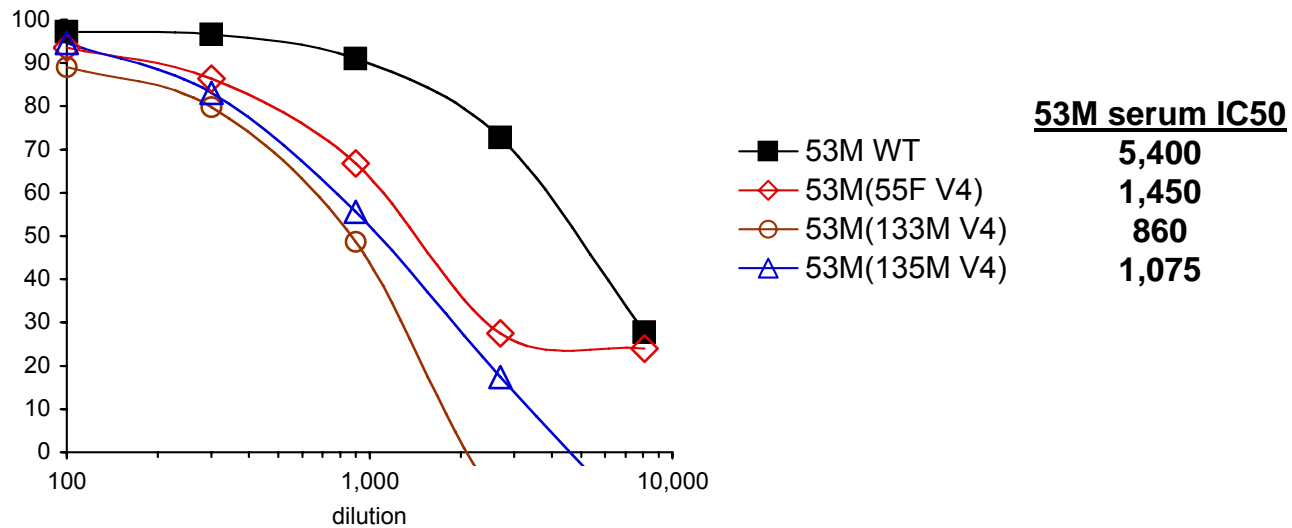
Substituting the V3-V3' region reduced the autologous neutralizing titer of 53M serum



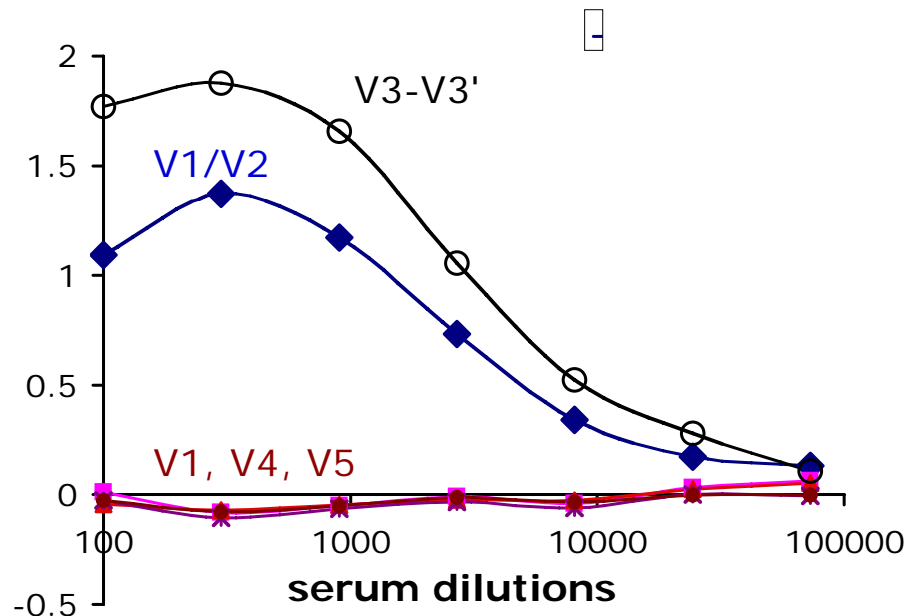
Replacing the V4 region also reduced the autologous neutralizing titer of 53M serum



53M serum neutralizations



Assay of binding activity of 53M serum against soluble fusion proteins expressing isolated variable regions of 53M gp120



53M serum contains binding activity against the autologous V1/V2 and V3-V3' regions, but not against the isolated V1, V4 or V5 regions

This suggests the absence of epitopes localized to the V1, V4 or V5 regions

Mapping autologous neutralization targets in 53M gp41

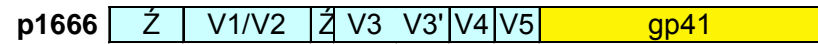
628 C-term heptad repeat region (HR2) | MPER

53M Env	DIW <u>N</u> NTTWMQWDKEV <u>S</u> NYT <u>K</u> TIYK <u>L</u> LEK <u>S</u> Q <u>N</u> Q <u>Q</u> EE <u>N</u> EKDLLALDSWNNLWNWF
133M Env	DIWD <u>N</u> M <u>T</u> WMQWDKEI <u>S</u> NYTNTIYR <u>L</u> LEDSQ <u>N</u> Q <u>Q</u> EKNEKDLLALDSWKNLWNWF
106F Env	DIWD <u>N</u> M <u>T</u> WMQWDKEV <u>S</u> NYTNTIYR <u>L</u> LEDSQ <u>S</u> Q <u>Q</u> EKNEKDLLALDSWKNLWTF
55F Env	DIWD <u>N</u> M <u>T</u> WMEWDREI <u>S</u> NYTNIIF <u>G</u> LL <u>E</u> DSQ <u>N</u> Q <u>Q</u> ERNEKDLLALDKWNNLWNWF
135M Env	EIWN <u>N</u> M <u>T</u> WMQWDKEI <u>S</u> NYTDTIYK <u>L</u> LETSQ <u>S</u> Q <u>Q</u> DKNEKDLLALDSWKNLWNWF
109F Env	EIW <u>G</u> N <u>M</u> <u>T</u> WMQWDKEV <u>S</u> NYT <u>F</u> TIYQ <u>L</u> LEESQ <u>Y</u> Q <u>Q</u> EQNEKELLALNKWNDLWSWF

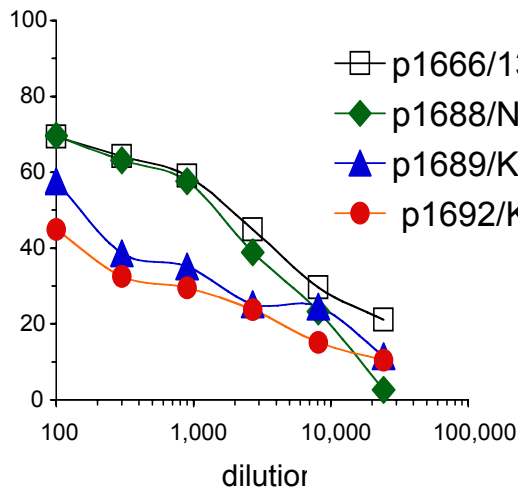
53M Env contains several unique substitutions in the C-terminal heptad repeat region, N-terminal to the MPER region.

To determine the roles of these substitutions and the adjacent glycosylation site in the autologous neutralization sites present in gp41 of this Env, mutations were introduced at these positions in the chimeric p1666 Env.

Mutations in 53M gp41 HR2 region affect autologous neutralization activity



53M sera neutralizat



	616	IC50
p1666 (133M/53M gp41)	<u>N</u> <u>Y</u> <u>T</u> <u>K</u> <u>T</u> <u>I</u> <u>Y</u> <u>K</u> <u>L</u> <u>L</u> <u>E</u> <u>K</u> <u>S</u> <u>Q</u> <u>N</u> <u>Q</u> <u>Q</u> <u>E</u> <u>E</u>	1,800
p1689 (K619N)	---N-----	160
p1692 K619N/K627D)	---N-----D---	<100
p1688 (N616Q)	Q-----	1,500
133M wt	---N---R---D---	<100

All mutations are in p1666 background (133M chimera containing 53M gp41)

53M-specific residues K619 and K627 contribute to autologous neutralization epitopes present in 53M gp41

Removal of the adjacent glycan has no effect on sensitivity to neutralization

This maps a novel neutralization site in the HR2 region

Summary

- These studies suggest that sequences in the V1/V2, V3-V3', V4 and gp41 domains all contribute to the potent autologous neutralizing titer of 53M serum
- Evidence supports the presence of autologous neutralization targets dependent on sequences in the V2 and gp41 domains
- For V3-V3' and V4 it is not clear whether these effects are due to epitopes present in these regions, or to differential masking effects on distal sites
- The nature of the autologous epitopes is unknown- these may be linear, conformational or even quaternary structures

Conclusions

-It is likely that the recognition of multiple neutralization targets contributes to the exceptional autologous neutralizing activity of 53M serum

-Presumably, one or more of these specificities is also responsible for the less potent cross-neutralizing activity of this serum

Conclusions

-Further characterization of these targets, and those that mediate autologous and heterologous neutralizing activities present in other patient sera, may help identify novel regions and epitopes of HIV-1 Env that could be incorporated into new vaccine formulations.

-Identifying more conserved forms of epitopes in these regions, and combining multiple allelic forms into a multivalent formulation, may help overcome the limited cross-reactivities seen for the autologous antibodies directed against these targets.

Collaborators

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Zambia-Emory HIV Research Project

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Susan Allen (Emory)
Joseph Mulenga (Zambia)

This region of HR2 is relatively conserved, and related to the clade C consensus sequence

53MP	E	V	S	N	Y	T	K	T	I	Y	K	L	L	E	K	S	Q	N	Q	Q	E	E	N	E	K	D
133M	E	I	S	N	Y	T	N	T	I	Y	R	L	L	E	D	S	Q	N	Q	Q	E	K	N	E	K	D
106F	E	V	S	N	Y	T	N	T	I	Y	R	L	L	E	D	S	Q	S	Q	Q	E	K	N	E	K	D
55F	E	I	S	N	Y	T	N	I	I	F	G	L	L	E	D	S	Q	N	Q	Q	E	R	N	E	K	D
135MPL23a	E	I	S	N	Y	T	D	T	I	Y	K	L	L	T	E	S	Q	T	Q	Q	D	K	N	E	K	D
109F	E	V	S	N	Y	T	F	T	I	Y	Q	L	L	E	E	S	Q	Y	Q	Q	E	Q	N	E	K	D
CLADE C cons	E	I	S	N	Y	T	D	T	I	Y	R	L	L	E	D	S	Q	N	Q	Q	E	K	N	E	K	D

Do other sequences also contain epitopes in this region?

Would antibodies that recognize such epitopes, including those related to the consensus sequence, also possess neutralizing activities that may be broader than that of those directed against the autologous targets?