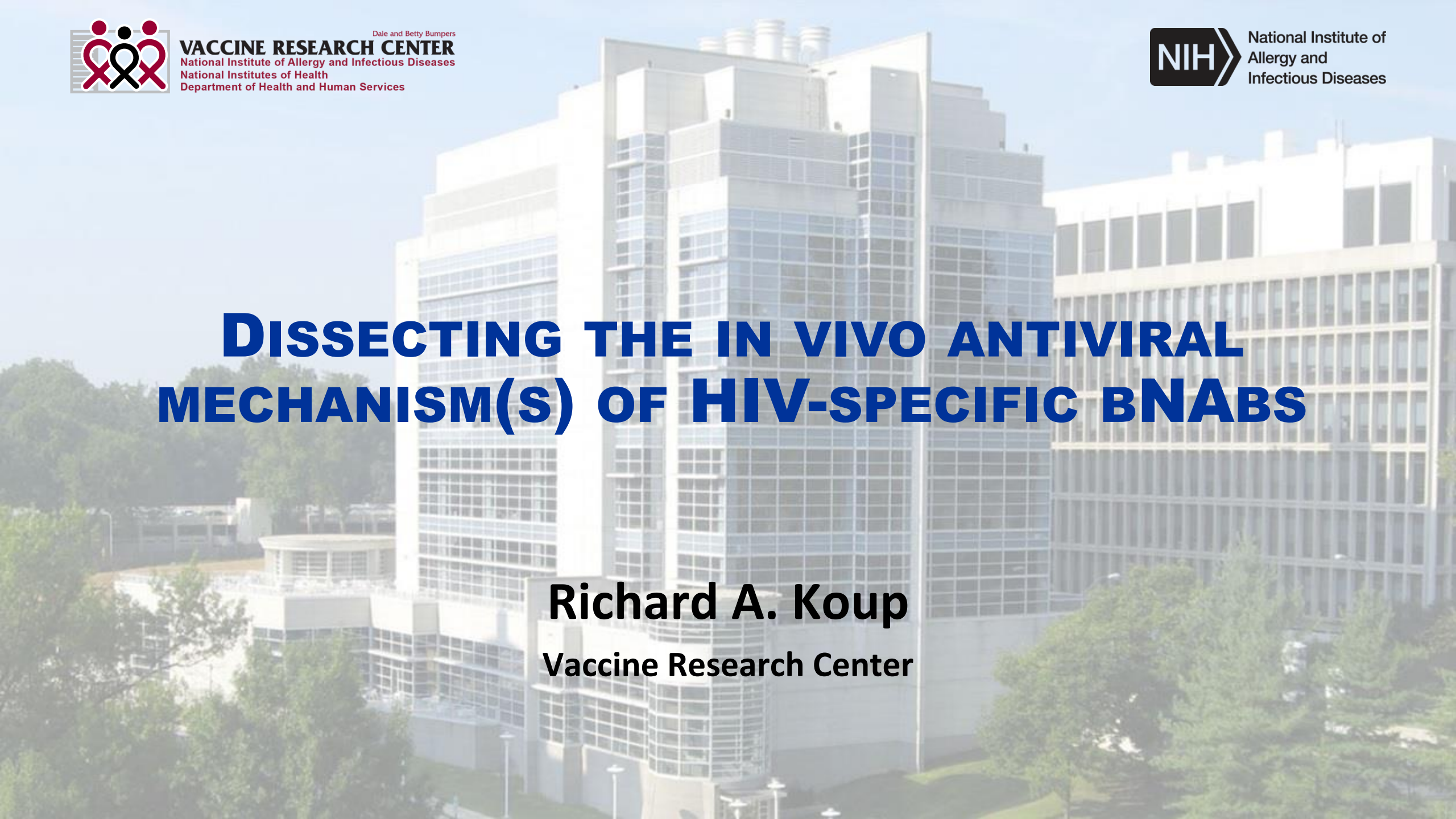


DISSECTING THE IN VIVO ANTIVIRAL MECHANISM(S) OF HIV-SPECIFIC BNABS

Richard A. Koup

Vaccine Research Center

Broadly Neutralizing mAbs in Development

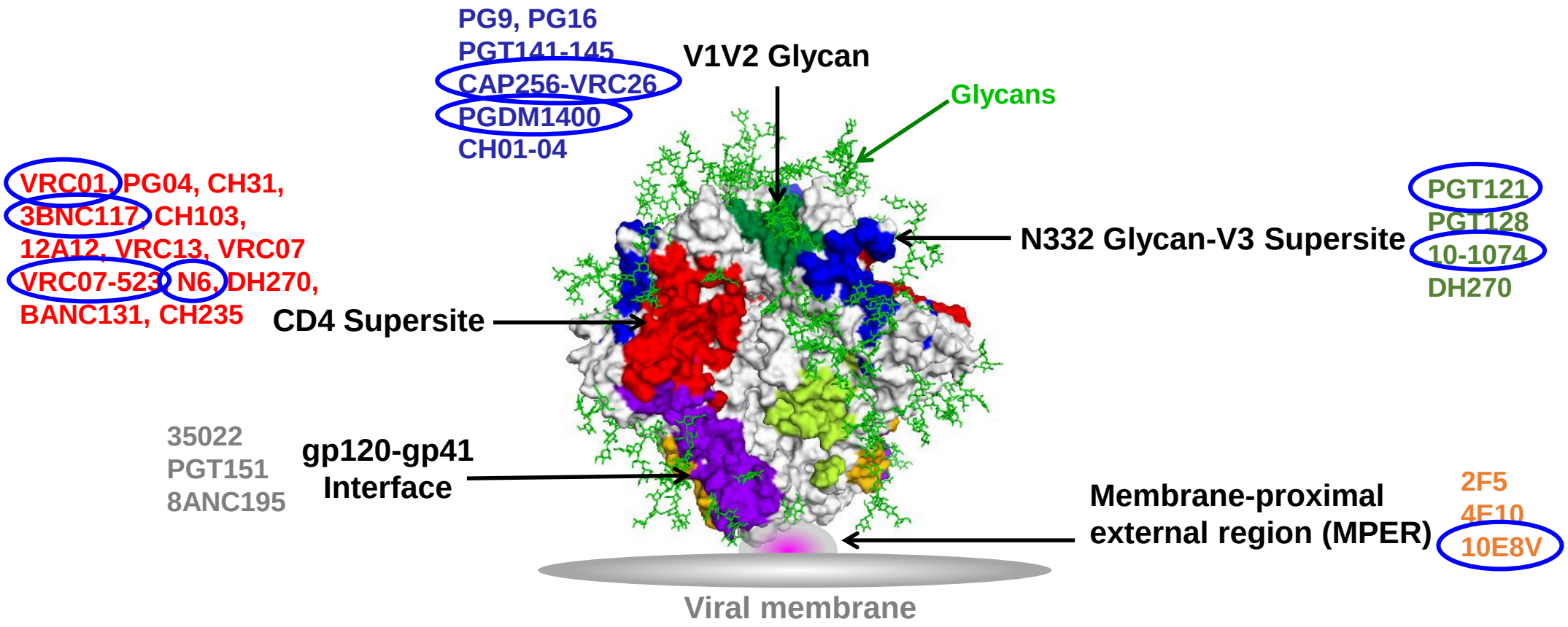
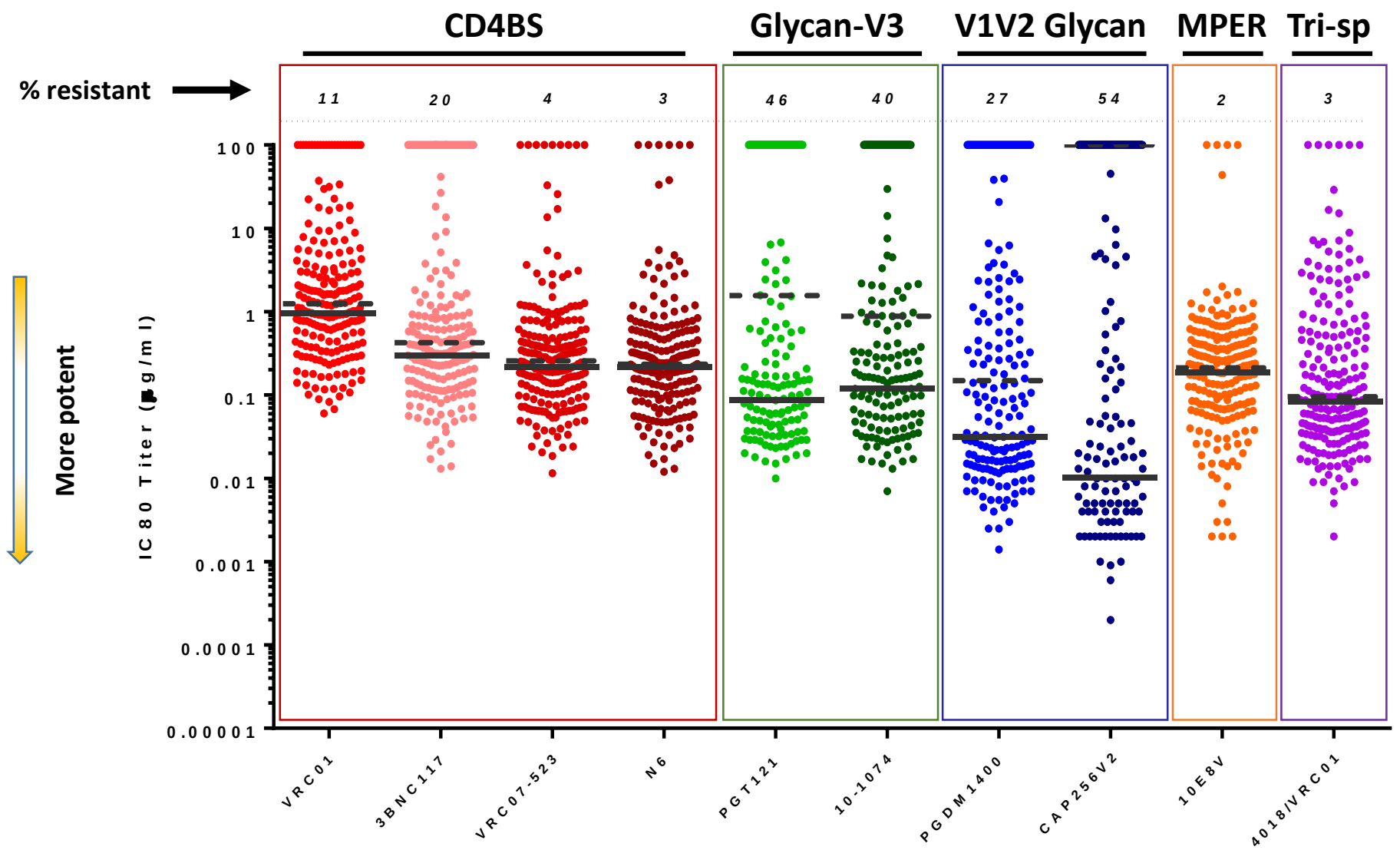


Image by Stewart-Jones, Doria-Rose, Stuckey
Adapted from Stewart-Jones et al Cell 2016 and Pancera et al Nature 2014

Antibodies with Improved Potency/Breadth



Multi-clade virus panel (n=208)

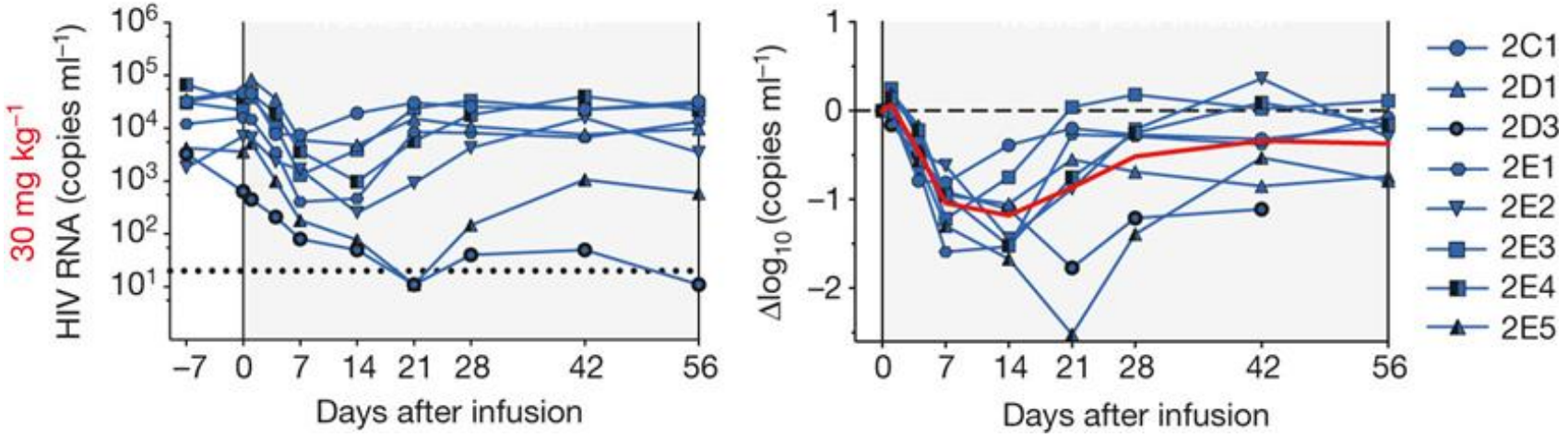
bNAbs have anti-viral activity in vivo

LETTER Nature 522, 487–491 (25 June 2015)

doi:10.1038/nature14411

Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117

Marina Caskey[1]*, Florian Klein[1]*, Julio C. C. Lorenzi[1], Michael S. Seaman[2], Anthony P. West Jr[3], Noreen Buckley[1], Gisela Kremer[4,5], Lilian Nogueira[1], Malte Braunschweig[1,6], Johannes F. Scheid[1], Joshua A. Horwitz[1], Irina Shimeliovich[1], Sivan Ben-Avraham[1], Maggi Witmer-Pack[1], Martin Platten[4,7], Clara Lehmann[4,7], Leah A. Burke[1,8], Thomas Hawthorne[9], Robert J. Gorelick[10], Bruce D. Walker[11], Tibor Keler[9], Roy M. Gulick[8], Gerd Fätkenheuer[4,7], Sarah J. Schlesinger[1] & Michel C. Nussenzweig[1,12]



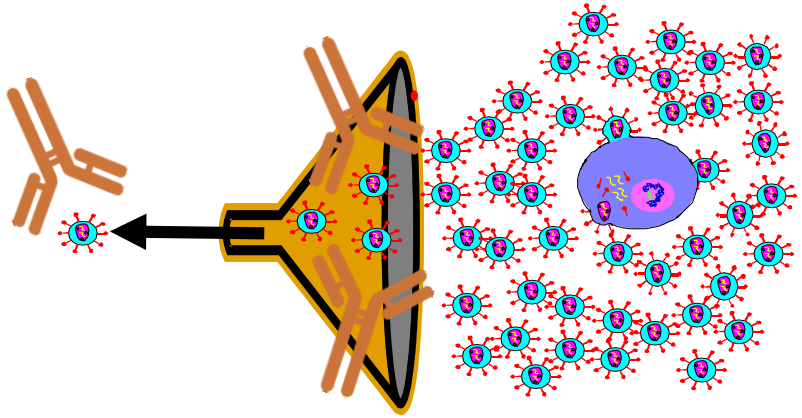
Clinical Use of Antibodies

Prevention and Treatment are Different

Prevention

- Prevent acquisition of infection in high risk individuals

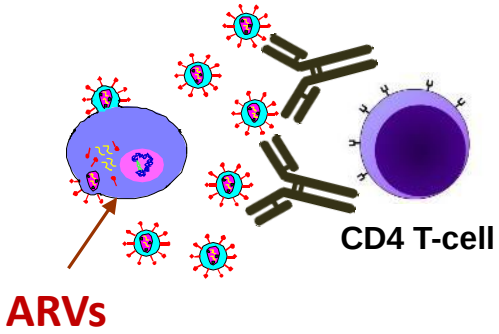
Block
Transmission event

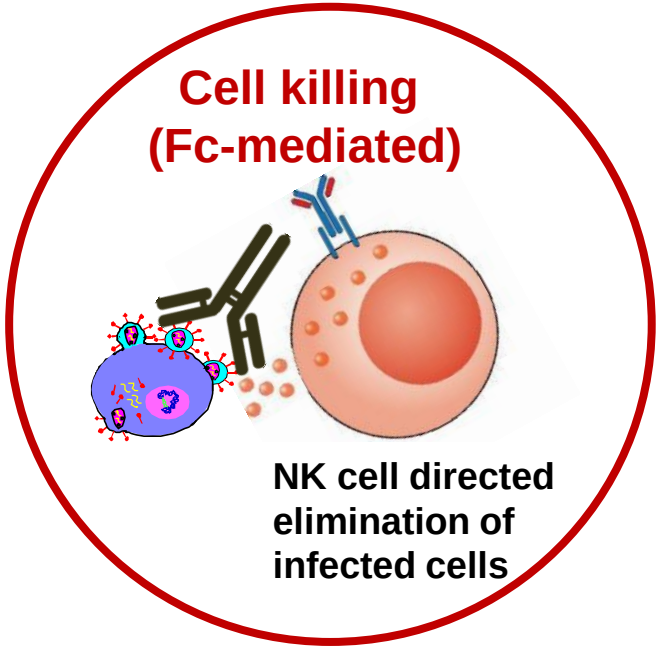


Treatment

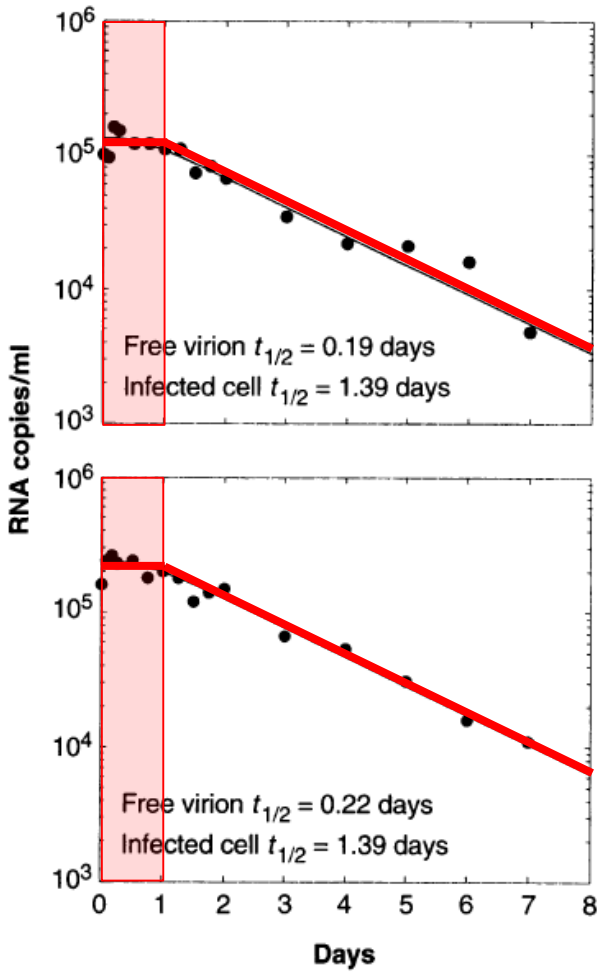
- Kill infected cells; reduce viral reservoir
- Maintain viral suppression induced by ARV

Block viral entry

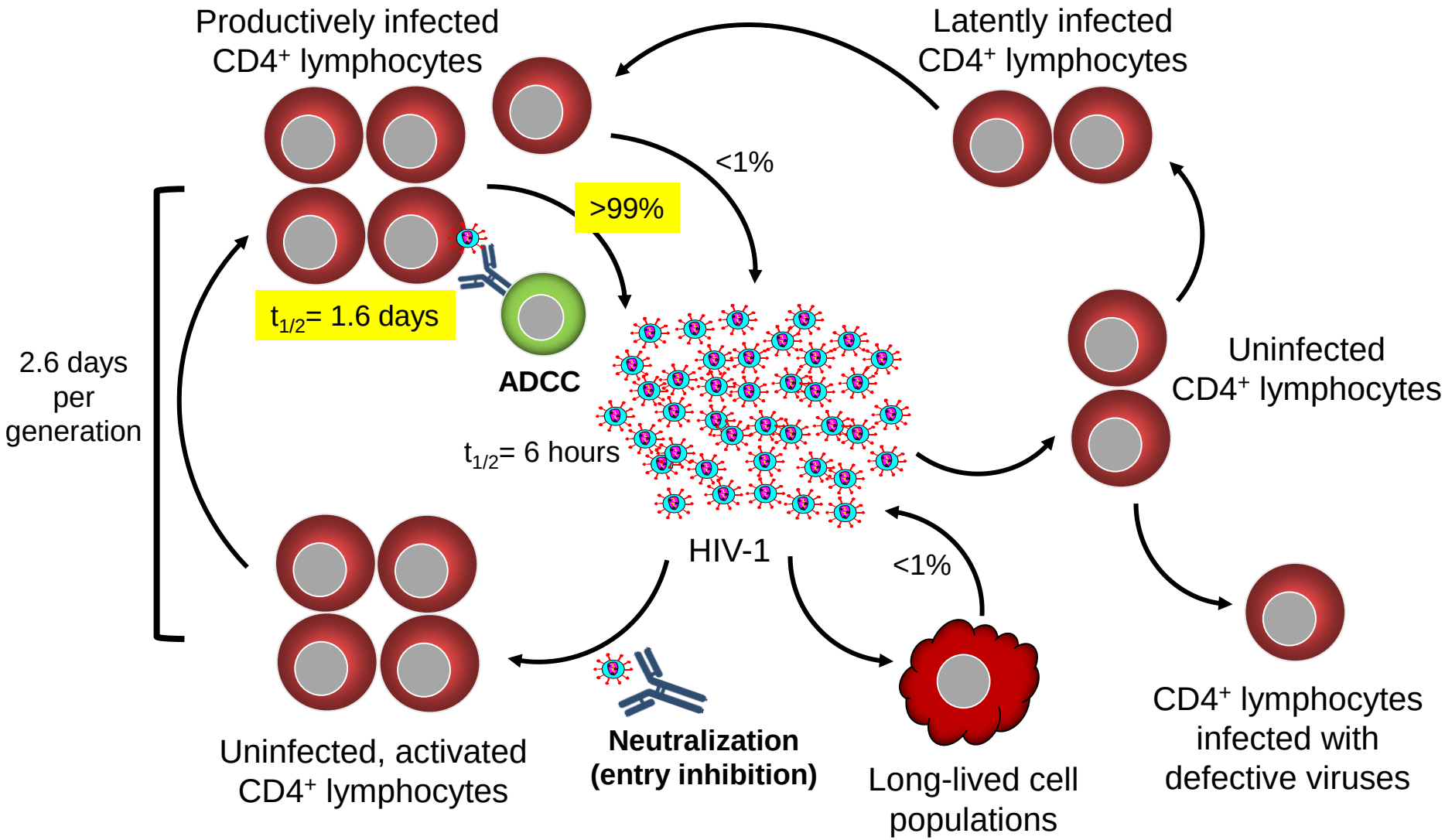




Perelson and Ho, original model

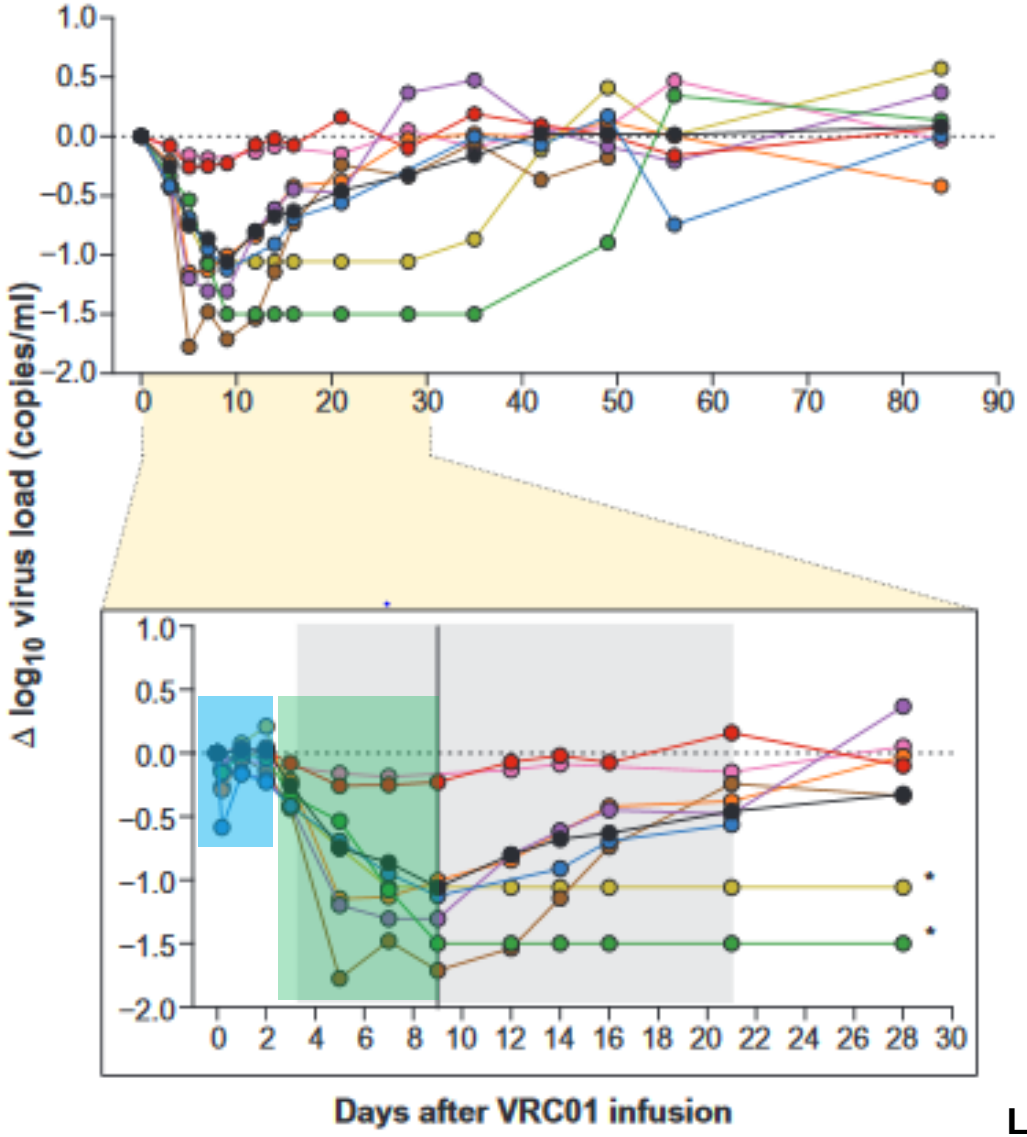


Perelson et al. Science 1996



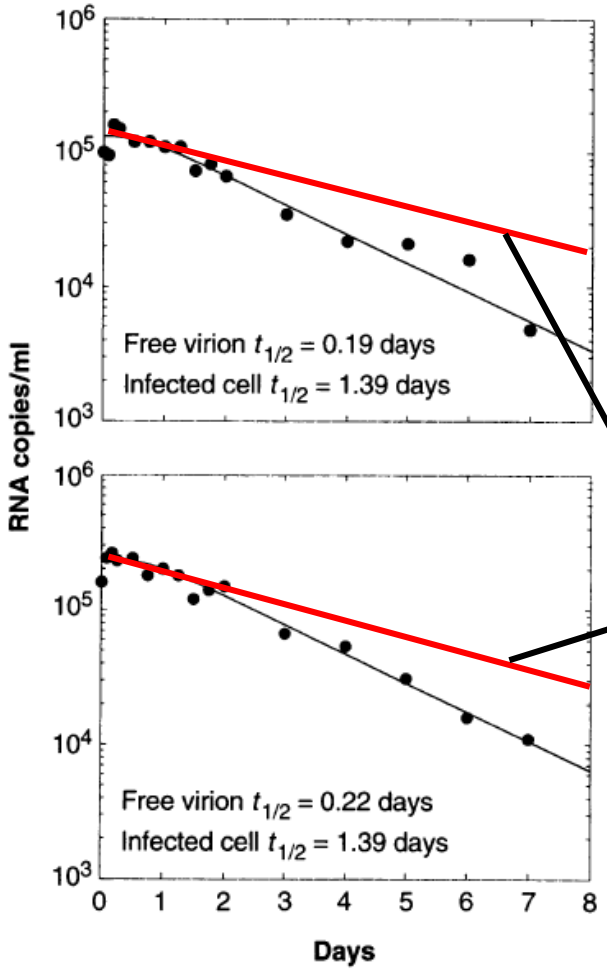
Plasma Virus Decay After mAb Treatment in HIV Infection Suggests Neutralization

VRC01 Phase I Trial
8 HIV-1 infected subjects

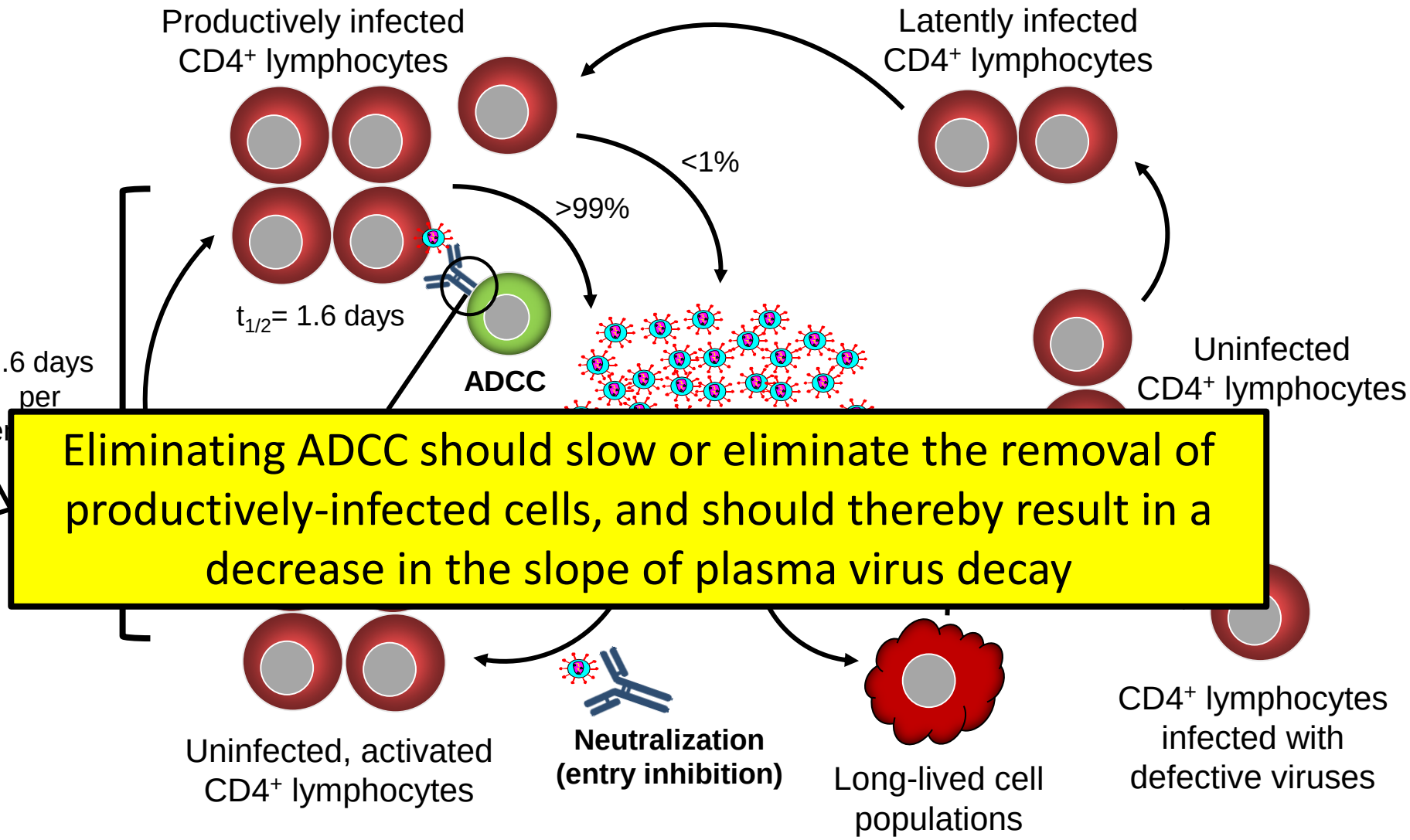


Lynch RM et al. Sci Transl Med 2015

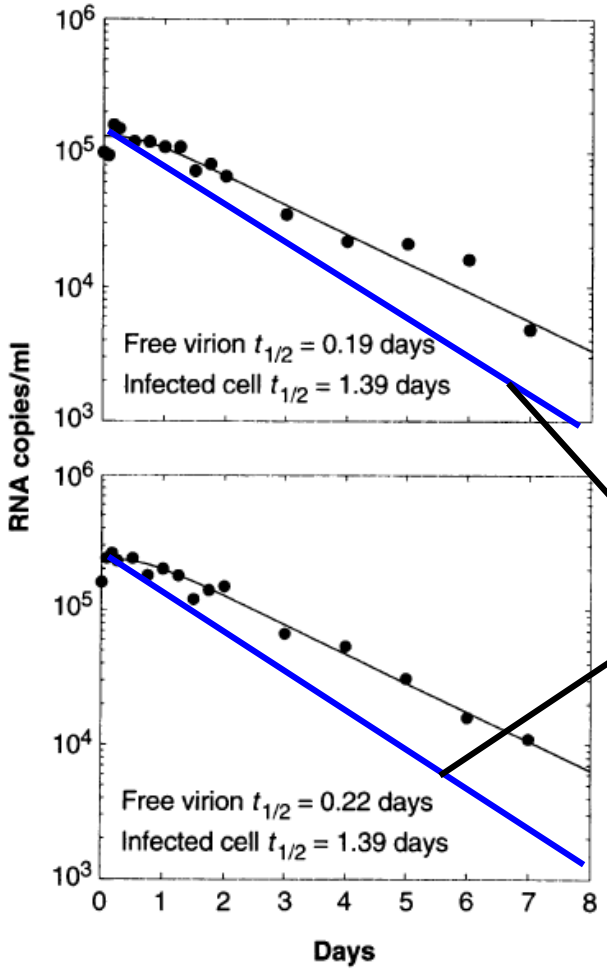
What if we alter ADCC activity?



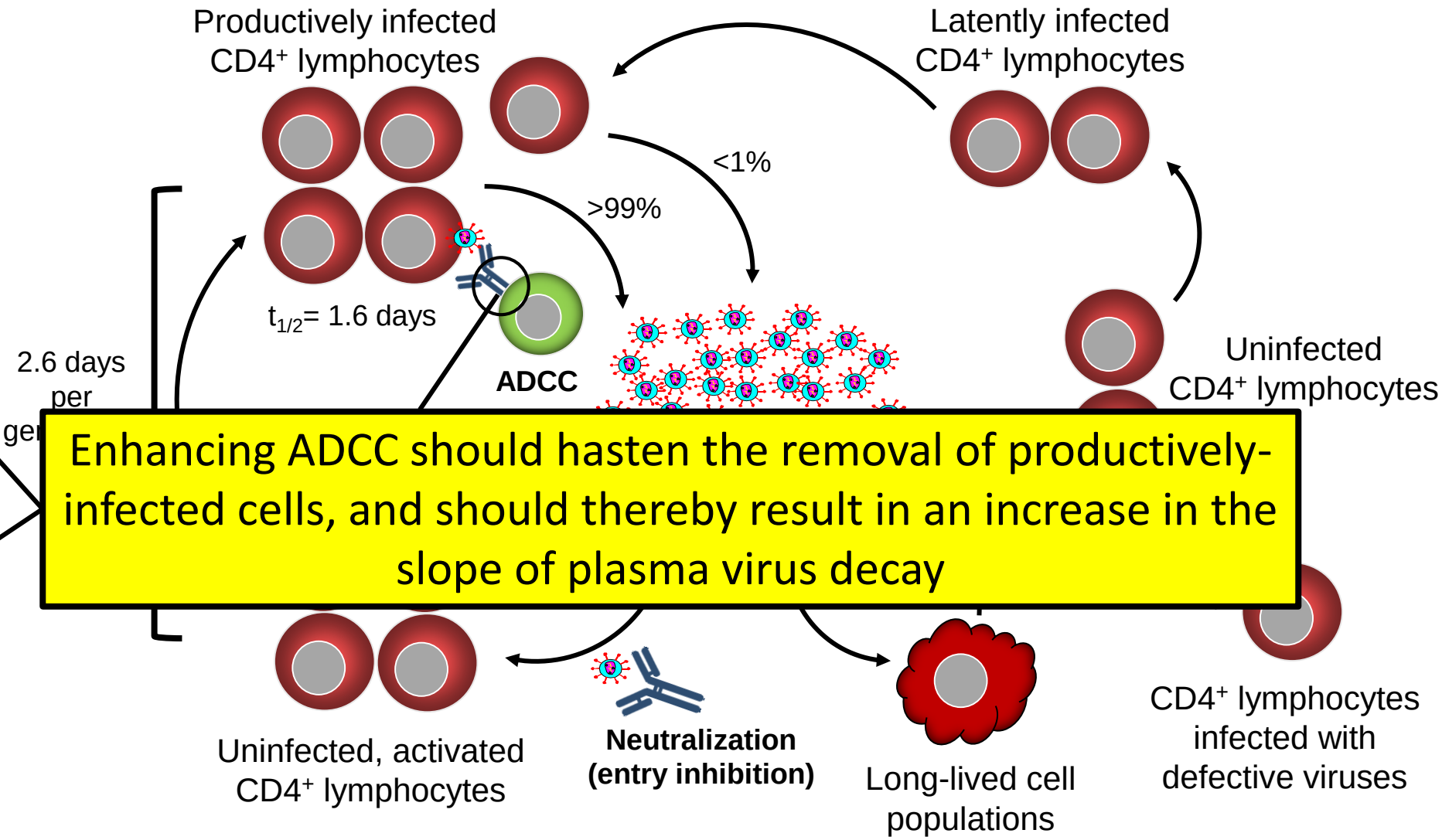
Perelson et al. Science 1996



What if we alter ADCC activity?



Perelson et al. Science 1996



Modulating effector function through FcγR binding

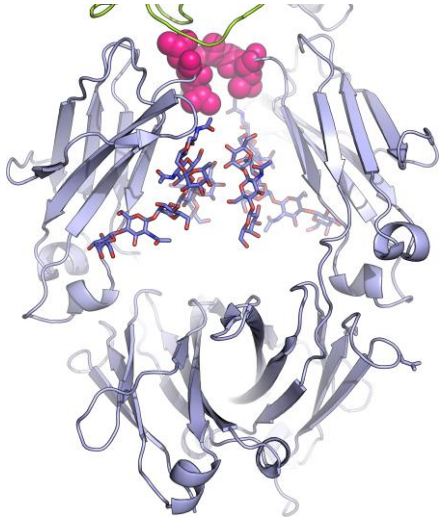
Name	Mutation	Comment	Reference
N297A	N297A	Reduced interactions with all FcγRs	Leabman et al, Mabs (2013) 896-903
LALA	L234A/L235A	Reduced binding to FcγR1a, FcγRIIa, FcγRIIIa and FcγRIIIb	Hessel et al, Nature (2007) 449, 101-104
K322A	K322A	Reduced C1q and C3 binding	Hessel et al, Nature (2007) 449, 101-104
LALA/K322A	L234A/L235A/K322A	Combination of LALA and KA mutations	
FES	L234F/L235E/P331S	Reduced FcγR and C1q binding	Oganesyan et al, Acta Crystallogr D Biol Crystallogr (2008) 64 (700-704)
DEL	S239D/I332E/A330L	Reduced FcγRIIb and enhanced FcγRIIIa binding to improve ADCC	Lazar et al, PNAS (2006) 103, 4005-4010
DEA	S239D/I332E/G236A	Improved FcγRIIa/FcγRIIb binding ratio to improve ADCC	Richards et al, Mol Cancer Ther (2008) 7, 2517-27
FT	H268F/S324T	Increased C1q binding without affecting FcγR	Moore et al, mAbs (2010) 181-189
FTDE	H268F/S324T/S239D/I332E	Increased binding to C1q and FcγR	Moore et al, mAbs (2010) 181-189

Predominantly tested in human IgG – human FcR context
Some mutants tested in human IgG – rhesus FcR context (Ackerman et al)

FcγR Binding Sites in CH2 Domain

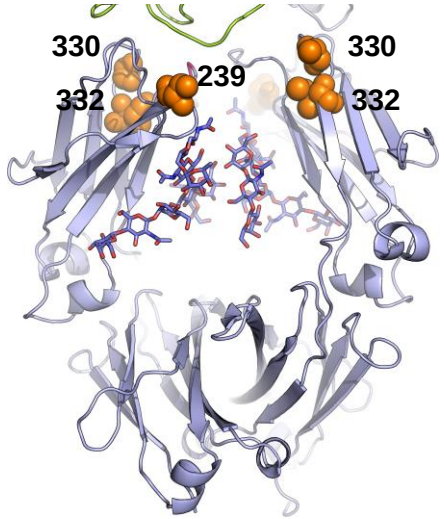
CH2

CH3



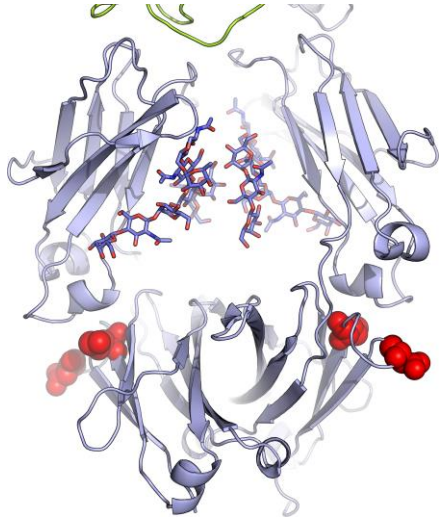
LALA
(L234A/L235A)

OR



DEL
(S239D/A330L/I332E)

+



LS
(M428L/N434S)

Enhanced FcRn binding

Binding in comparison to VRC07-523LS

FcγR ↓

C1q ↓

↑

↓

Modified from Science 293: 1155-1159

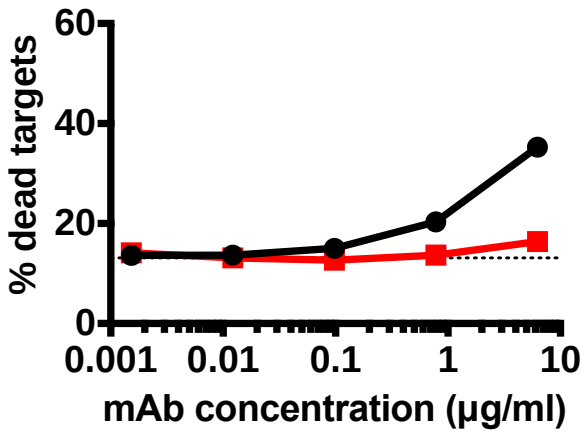
In Vitro Characterization of Fc Mutants

1) Neutralization

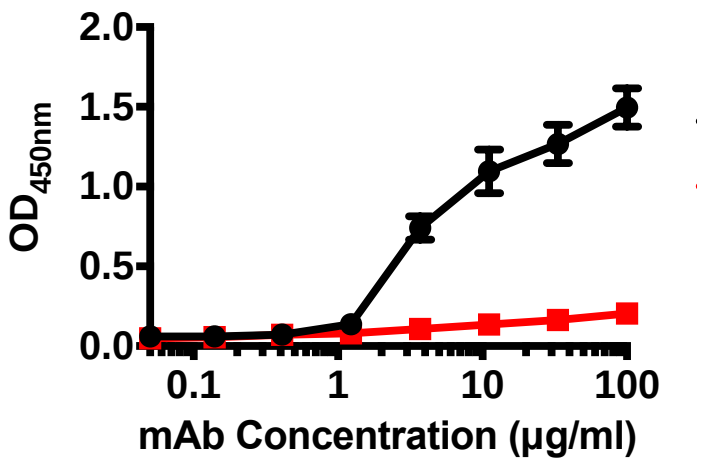
IC80, µg/ml

Virus	VRC07-523-LS	VRC07-523-LS/LALA
Q461.e2.SG3	0.890	0.742
620345.c1.SG3	>50	>50
T250-4.SG3	>50	>50
H086.8.SG3	>50	>50
CNE19.SG3	0.302	0.270
UG024.2.SG3	0.344	0.255
SHIV SF162P3	**1.220**	**1.140**

2) ADCC



3) C1q binding



	Neutralization	ADCC	Complement
VRC07-523LS	⟷	⟷	⟷
VRC07-523LS/LALA	⟷	↓	↓

Not shown: no difference between wt and LS
ADCC assays with NK cells sorted from rhesus macaque blood
Rhesus macaque serum used as source of C1q

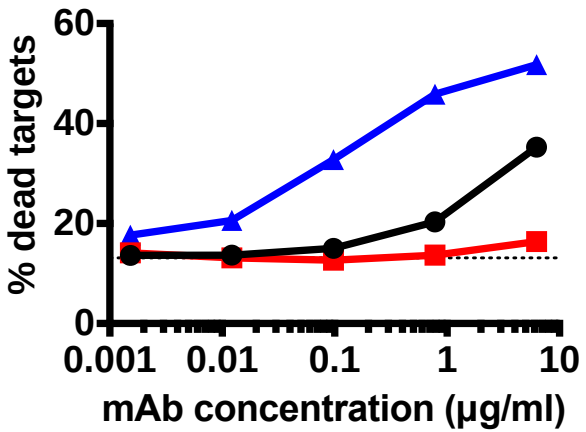
In Vitro Characterization of Fc Mutants

1) Neutralization

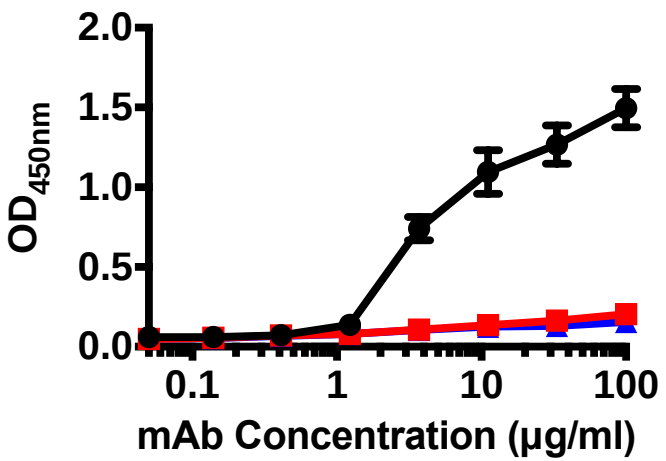
IC80, µg/ml

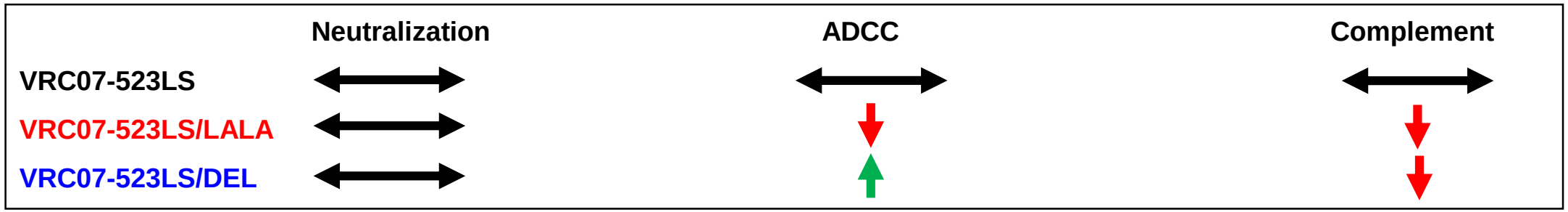
Virus	VRC07-523-LS	VRC07-523-LS/LALA	VRC07-523-LS/DEL
Q461.e2.SG3	0.890	0.742	0.559
620345.c1.SG3	>50	>50	>50
T250-4.SG3	>50	>50	>50
H086.8.SG3	>50	>50	>50
CNE19.SG3	0.302	0.270	0.192
UG024.2.SG3	0.344	0.255	0.207
SHIV SF162P3	**1.220**	**1.140**	**0.811**

2) ADCC



3) C1q binding

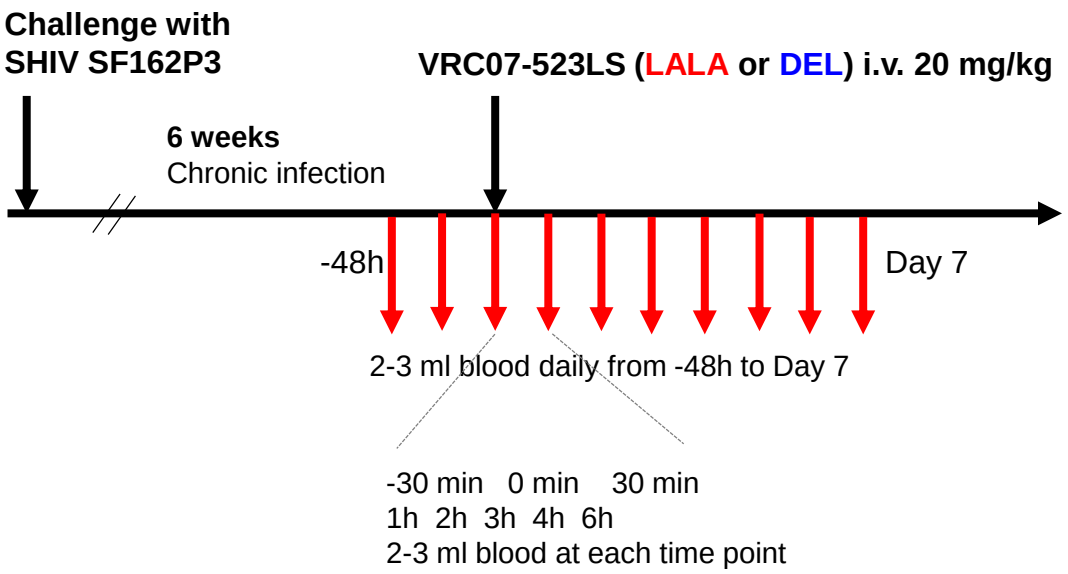


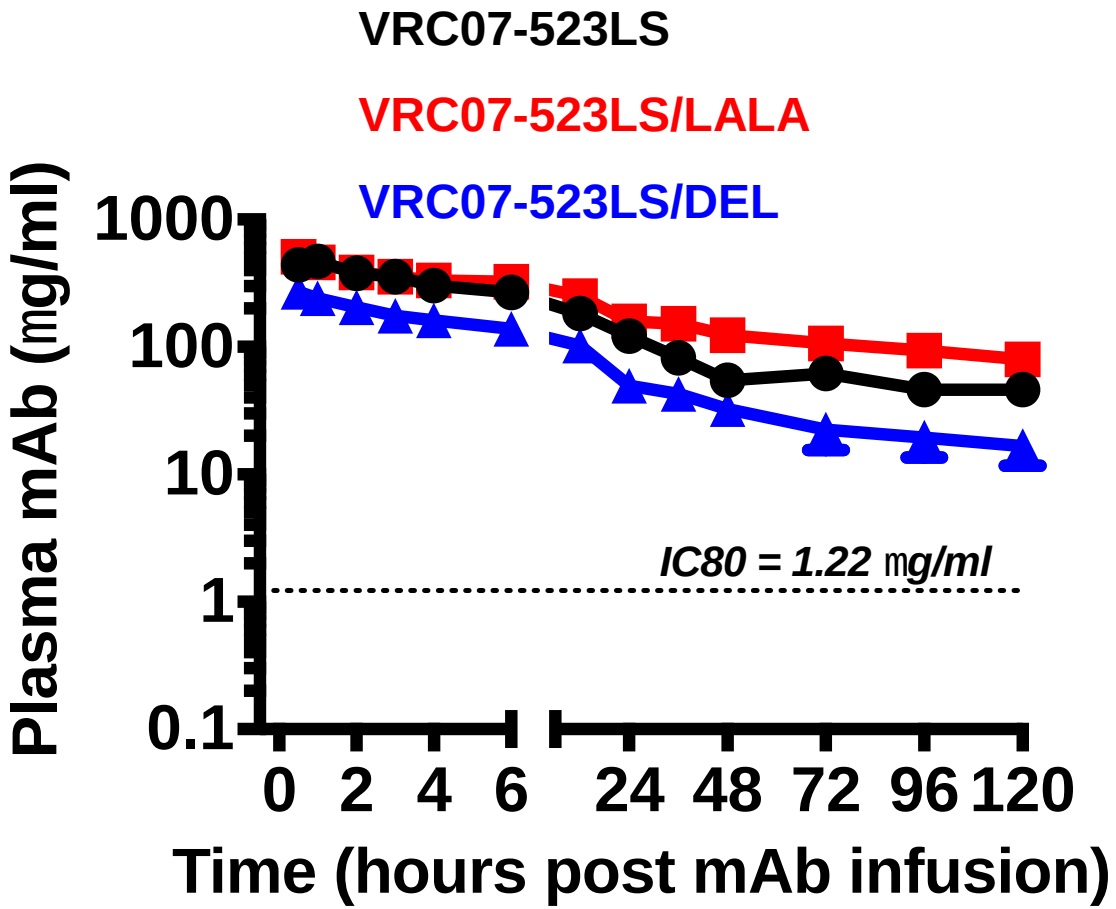


Not shown: no difference between wt and LS
ADCC assays with NK cells sorted from rhesus macaque blood
Rhesus macaque serum used as source of C1q

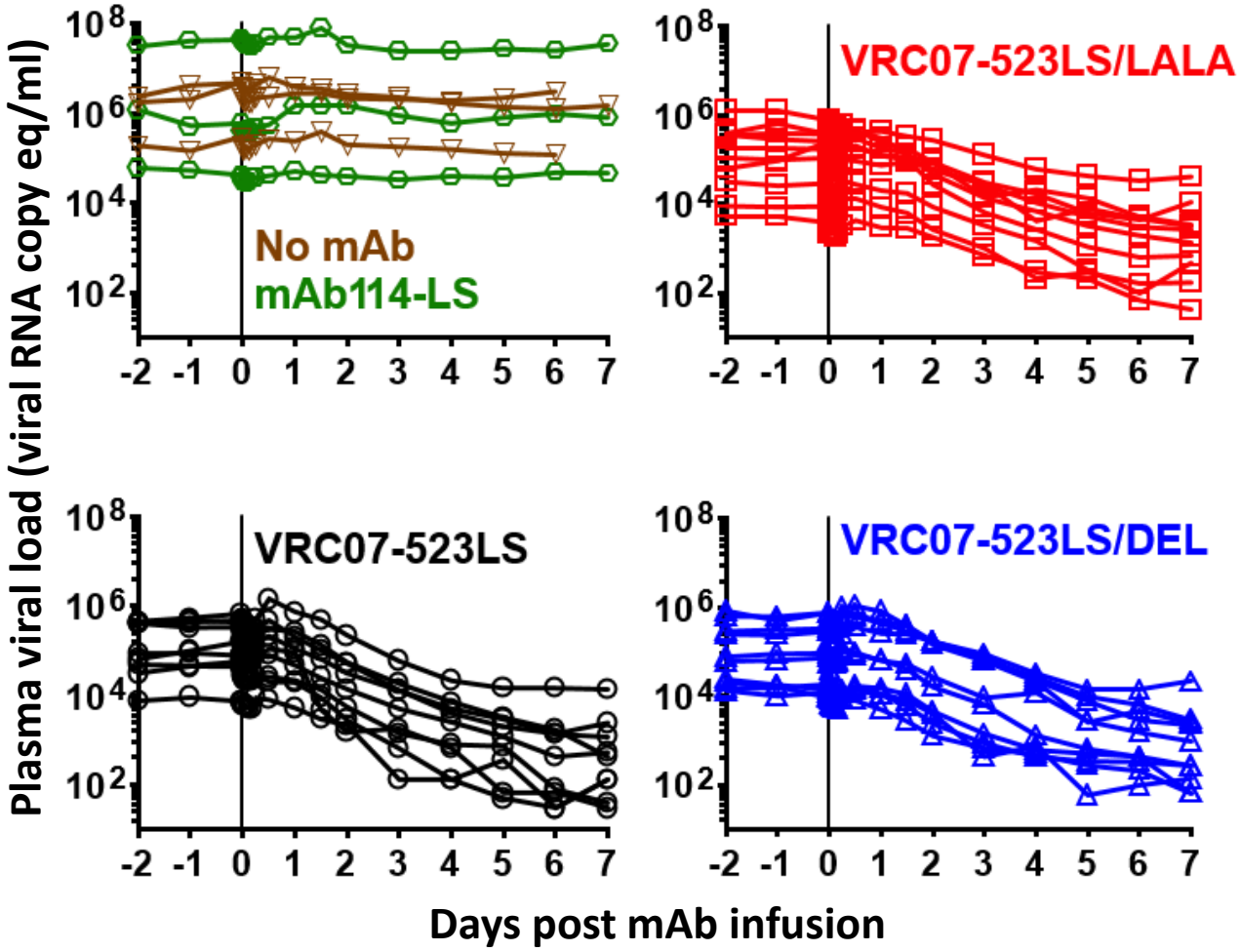
VRC07-523LS Antiviral Study in NHPs

Protocol





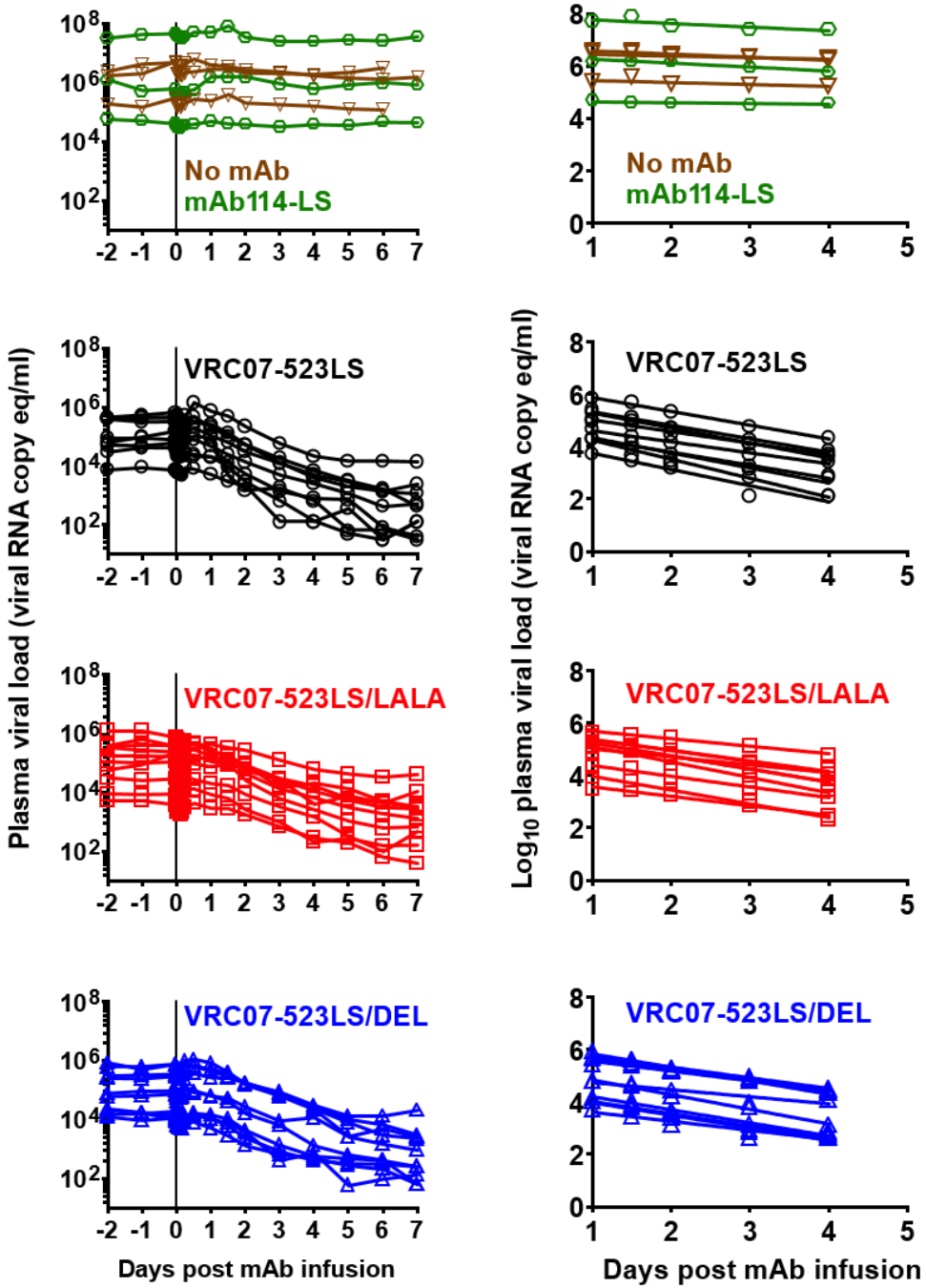
Plasma Virus Loads



No MAb n=3
mAb114-LS (20 mg/kg) n=3
VRC07-523LS (20 mg/kg) n=9
VRC07-523-LS/LALA (20 mg/kg) n=10
VRC07-523-LS/DEL (20 mg/kg) n=10

- Virus loads were stable prior to mAb administration
- All monkeys given VRC07-523xx showed a decline in plasma virus load
- None reached undetectable virus load (good data for calculating kinetics of plasma virus decline)

Plasma Virus Loads



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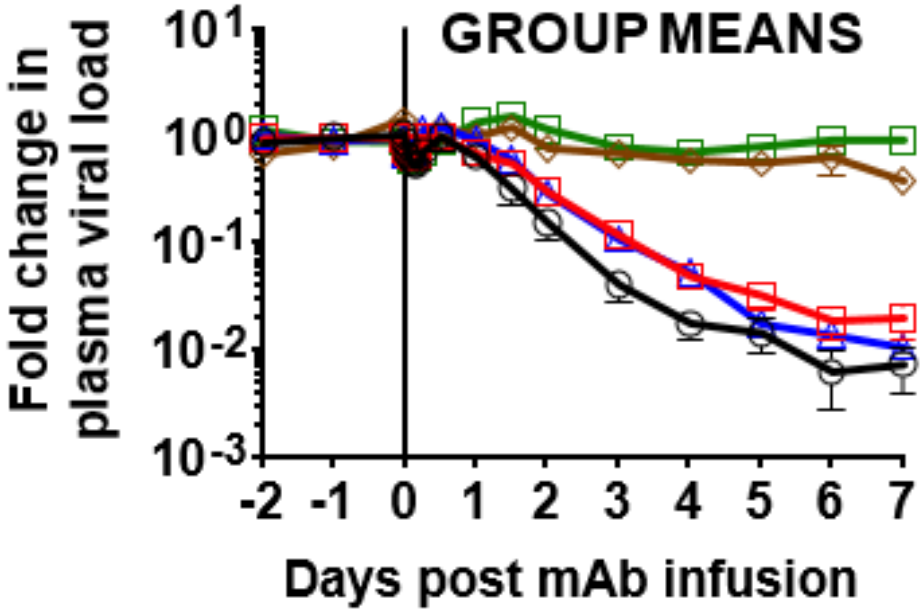
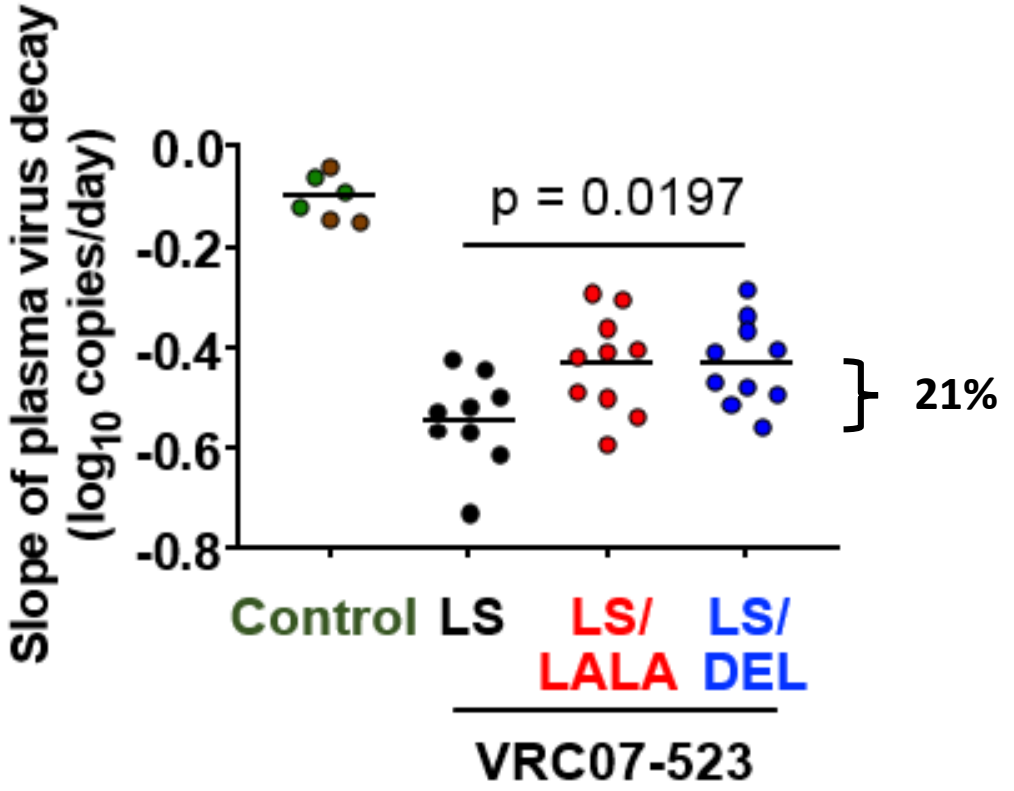
mAb114-LS (20 mg/kg) n=3
VRC07-523LS (20 mg/kg) n=9
VRC07-523-LS/LALA (20 mg/kg) n=10
VRC07-523-LS/DEL (20 mg/kg) n=10

- Virus load decline started to level off after 4 days in some monkeys
- Virus loads varied considerably during the first 6-24 hours in all groups
- Virus load from 1-4 days (5 data points) was used to calculate the slope of plasma virus decline

Normalized Plasma Virus Load Data

Why doesn't increased FcγR binding lead to increased ADCC and a more rapid decline in plasma virus?



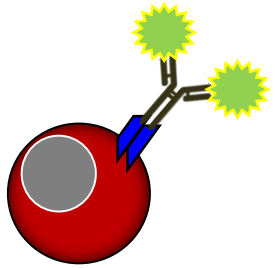


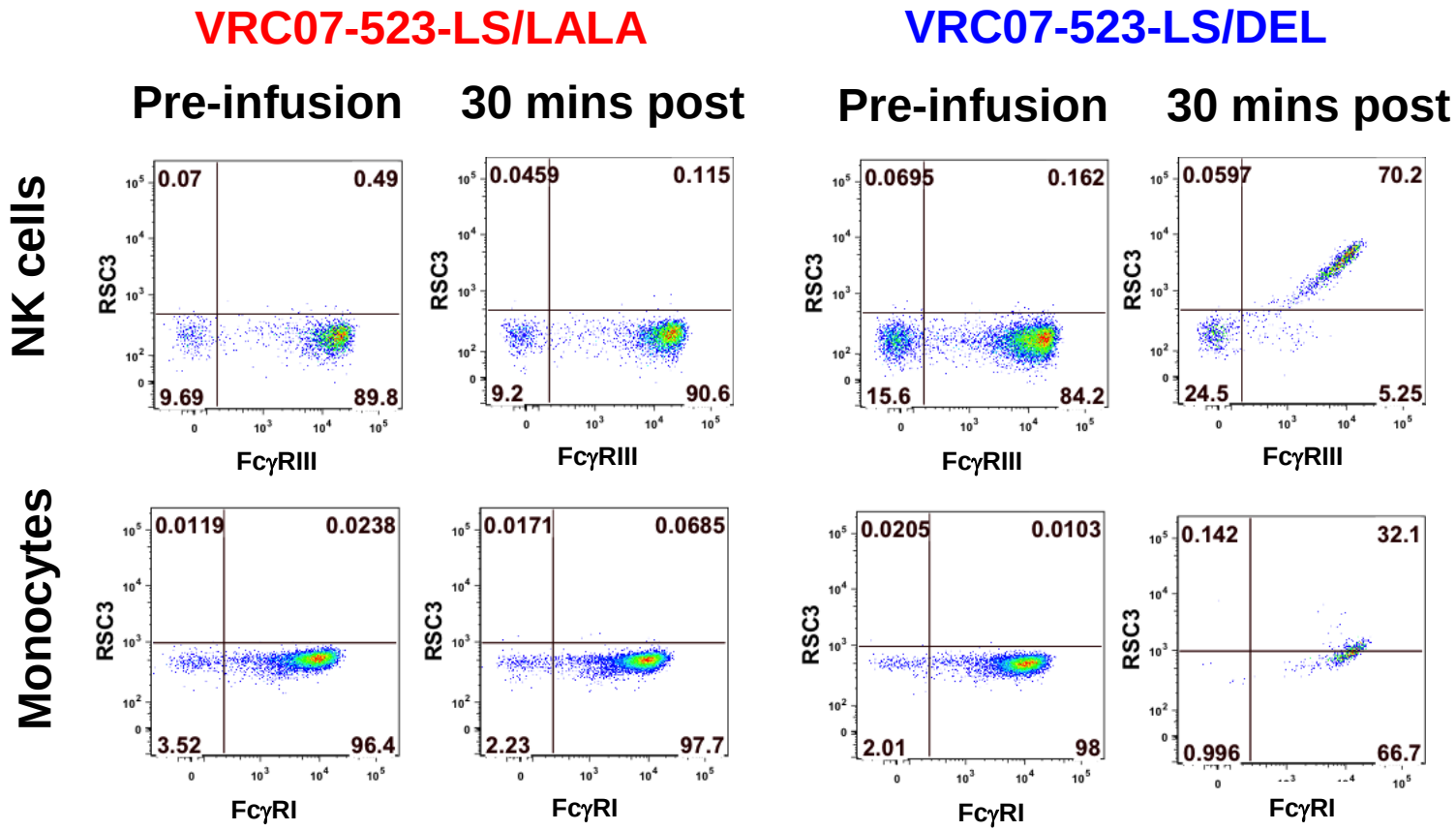
ADCC effector cells are “armed” with antibody after infusion

Fluorescently-labelled RSC3

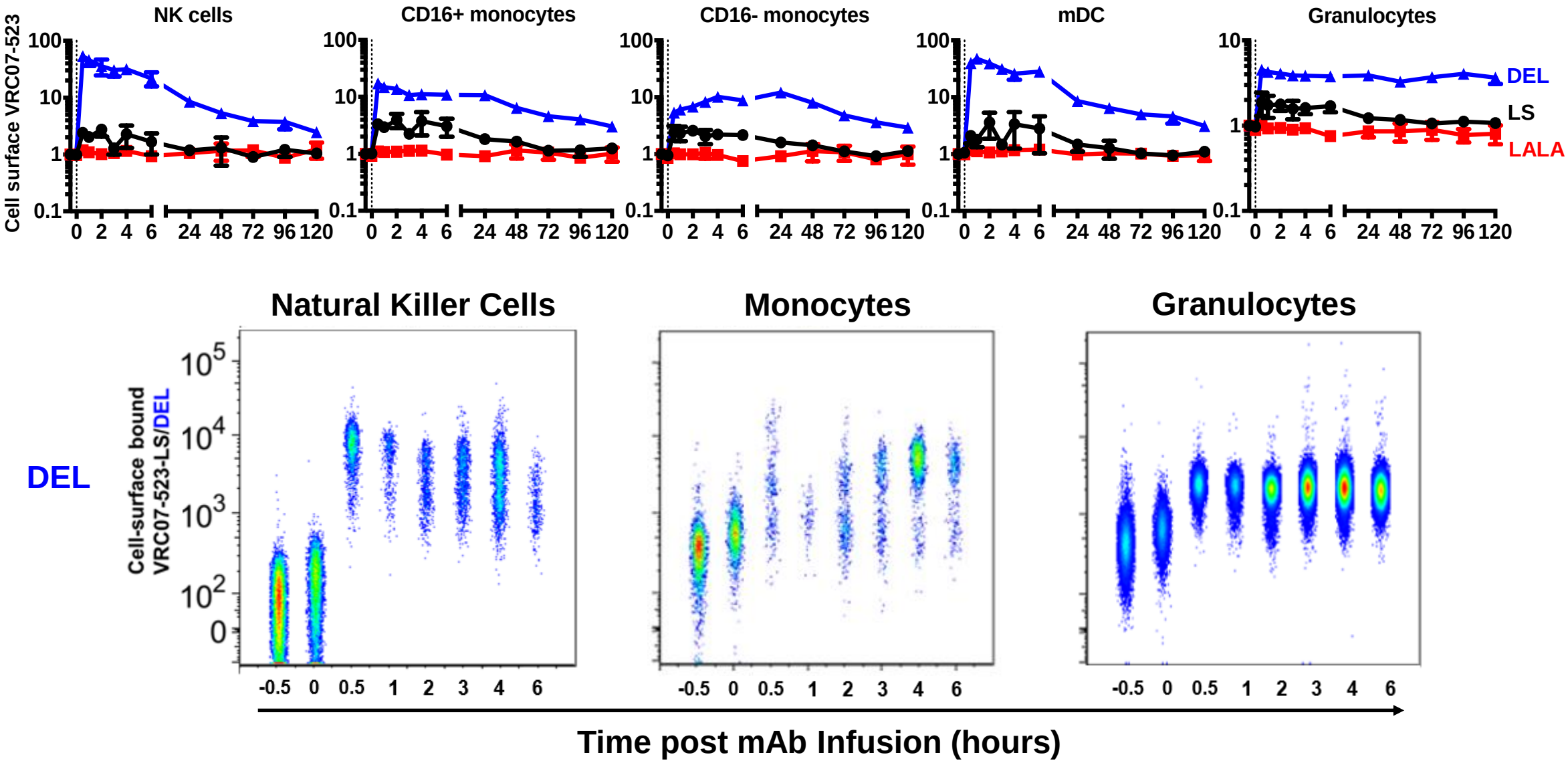
Infused VRC07-523

FcγR





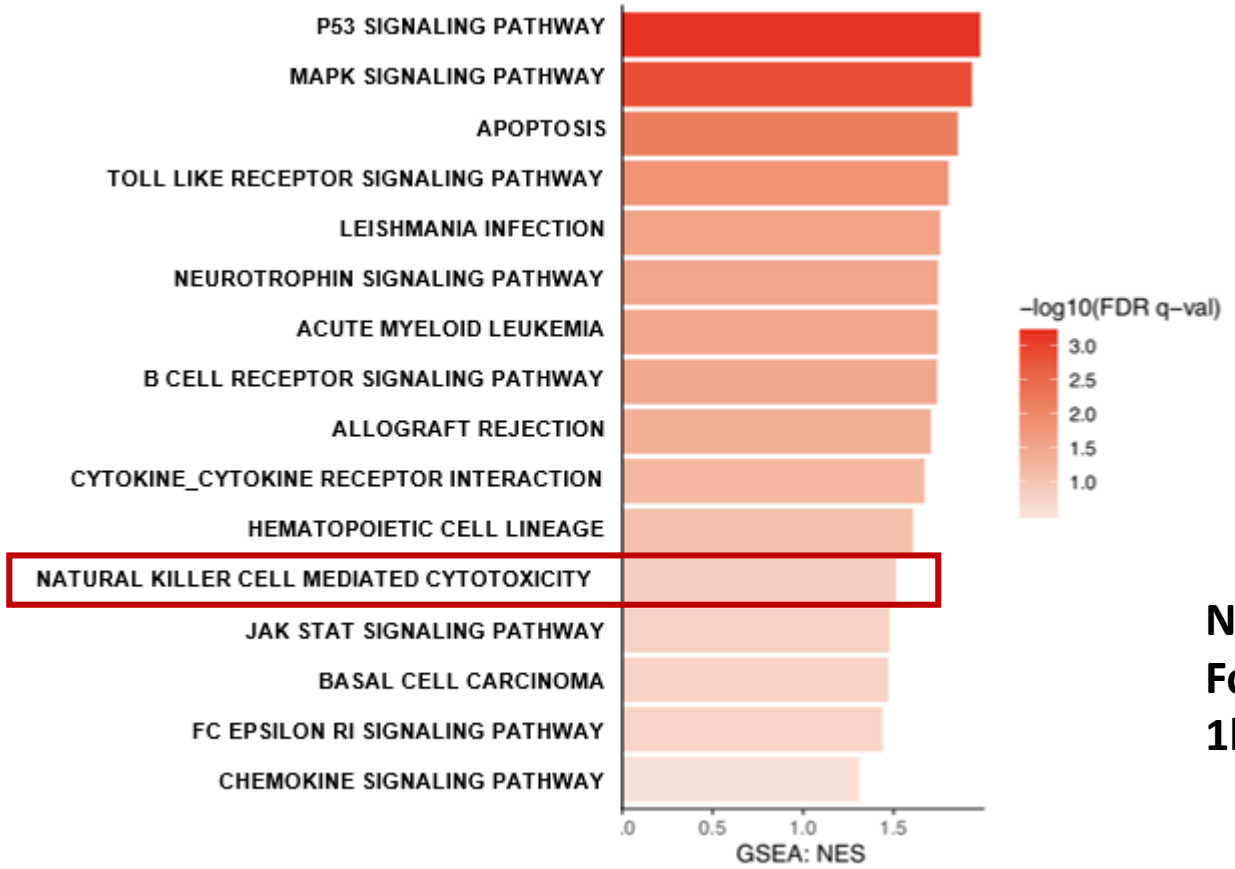
FcγR-expressing cells are pre-armed with DEL mAb

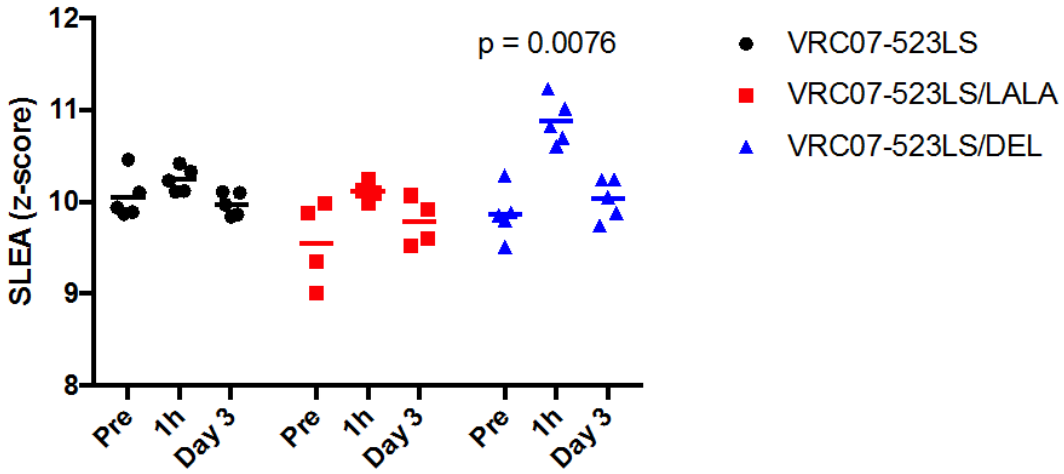


Transcriptomic analysis of NK Cells

NK cells were sorted from monkeys before and 1h and 3d after Ab infusion for RNAseq

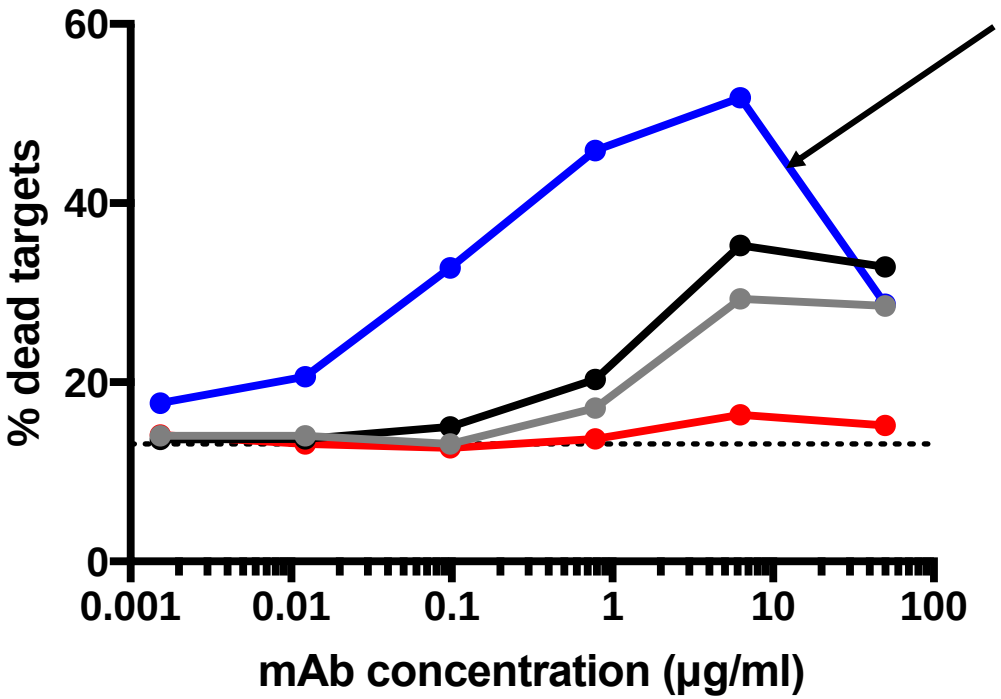
Pathways induced 1 hour after VRC07-523LS/DEL





Network analysis shows TRAIL-mediated apoptosis and FcγRIII-mediated necroptosis are also induced in NK cells 1h after VRC07-523LS/DEL

Prozone Effect



Prozone effect

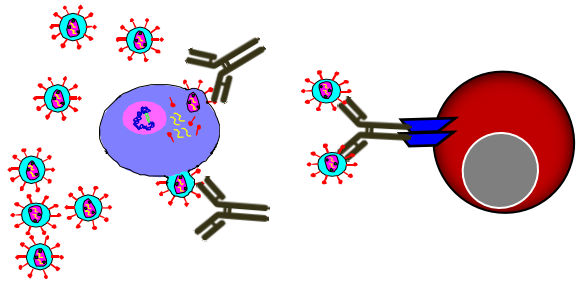
VRC07-523WT

VRC07-523LS

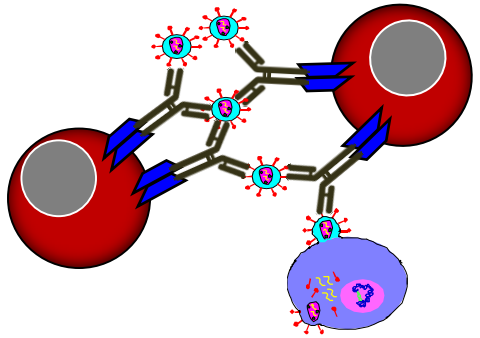
VRC07-523LS/LALA

VRC07-523LS/DEL

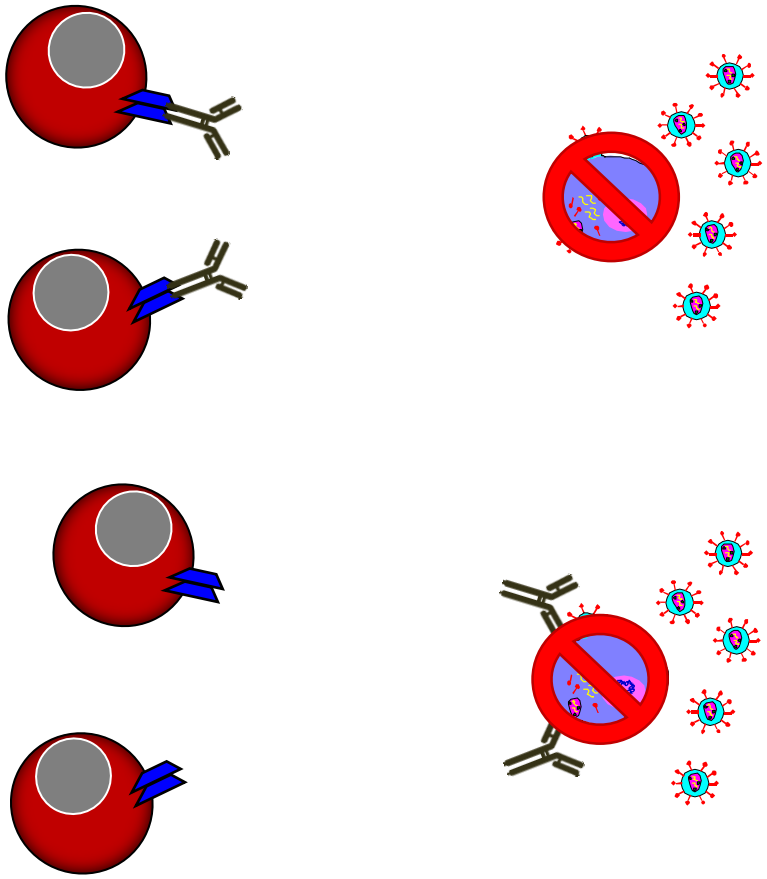
High affinity receptor occupancy



Multivalent binding - anergy

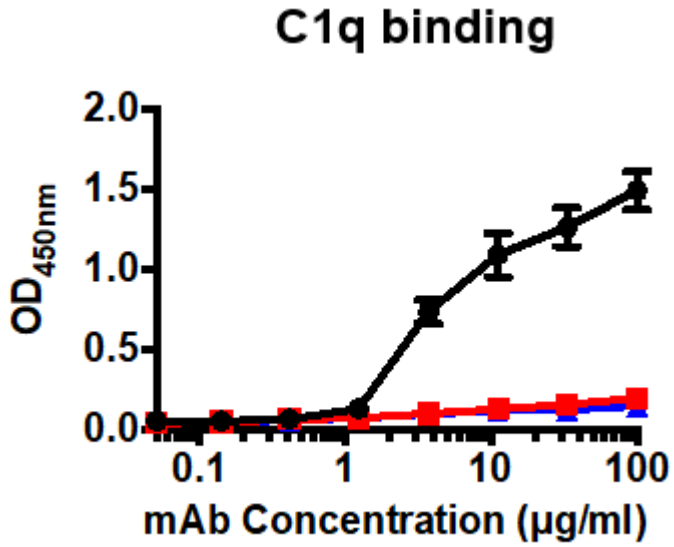


Can this lead to blocking of *in vivo* ADCC?



Maybe ADCC is not the operative effector mechanism

- Antibody-dependent cellular phagocytosis
  - Should have opposite effects of LALA and DEL
- Complement-dependent virolysis



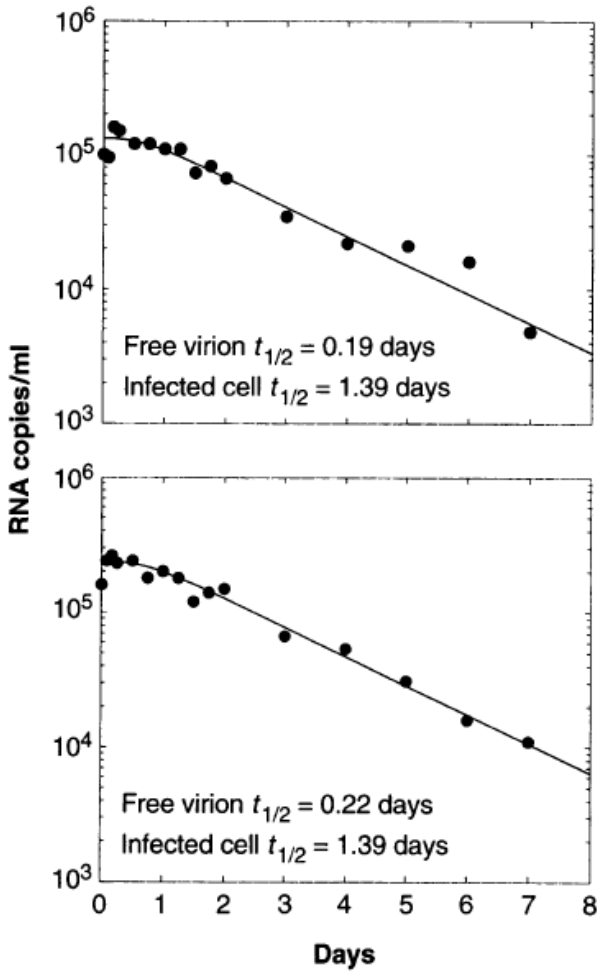
Further work is needed to determine if decreased complement binding results in the decreased antiviral effect of LALA and DEL modified antibodies

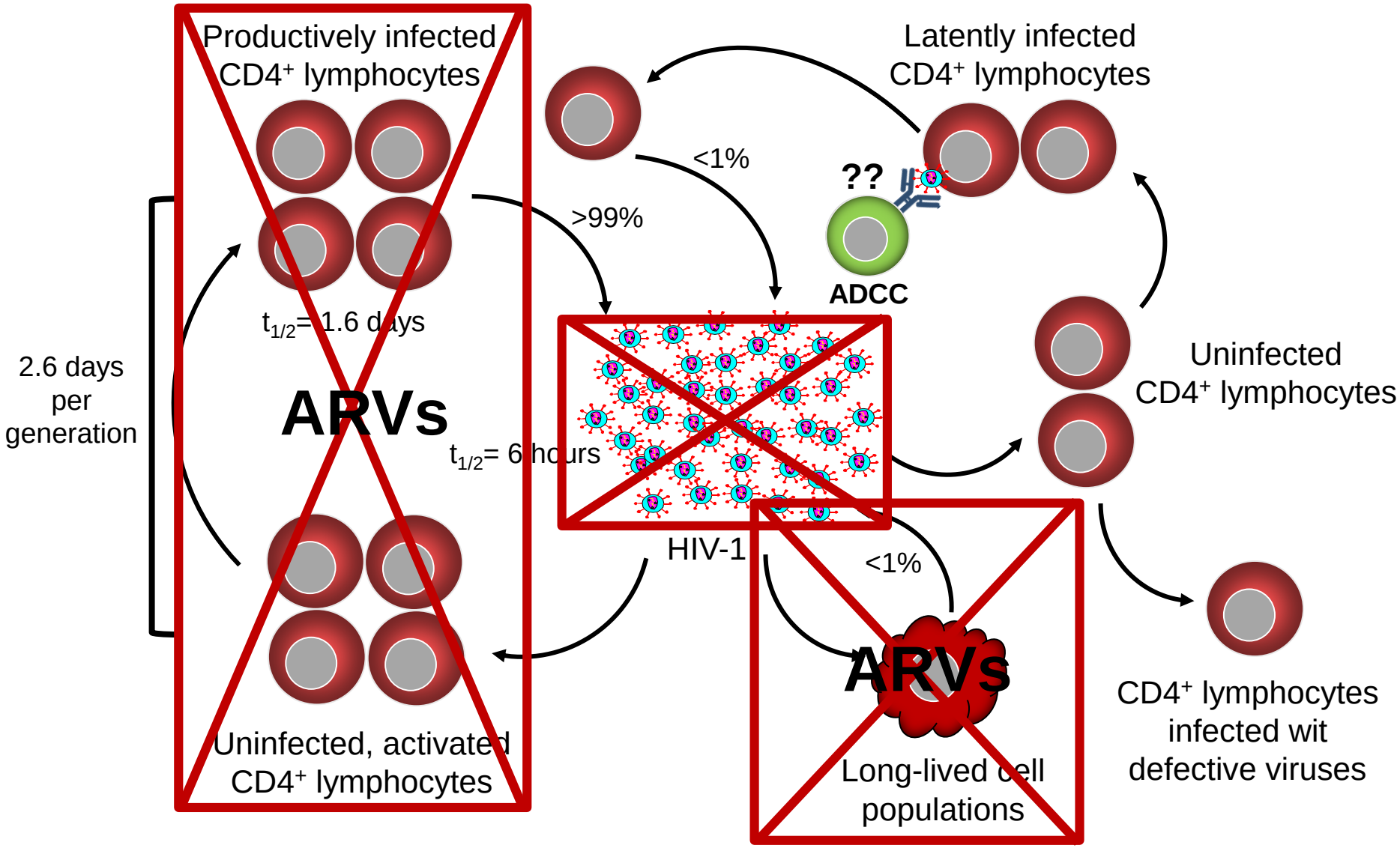
Conclusion

The *in vivo* antiviral effect of VRC07-523 is mediated by a combination of both plasma virus neutralization (entry inhibition) and Fc-dependent functions

- An Fc modification which enhances FcγR affinity had an unexpected effect of decreasing overall *in vivo* anti-viral activity
- Whether this will limit the use of antibodies with similar mutations in cure strategies needs to be determined
- Further work is needed to define the mechanism of the decreased anti-viral effect of DEL modified antibodies *in vivo*

What About the Latent Reservoir?





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