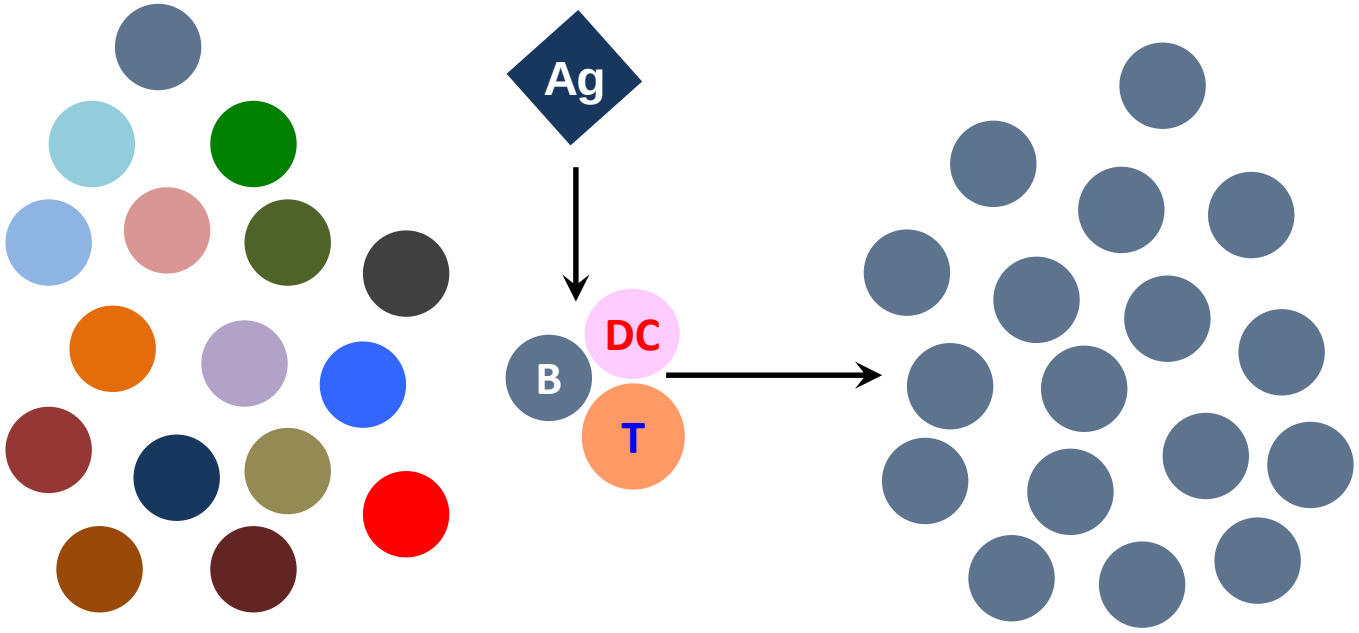
Clonal Selection Theory



Diversity
Specificity
Memory

Burnet, Lederberg, Talmage

Antibody Affinity Maturation:

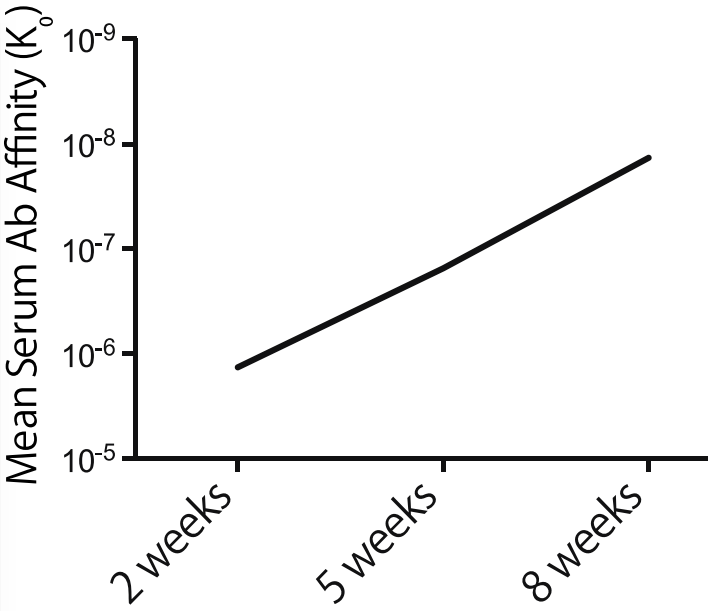
Variations in Affinities of Antibodies during the Immune Response*

HERMAN N. EISEN AND GREGORY W. SISKIND†

From the Department of Microbiology,
Washington University School of Medicine, St. Louis, Missouri

Received February 13, 1964

The affinities of anti-2,4-dinitrophenyl (DNP) antibodies for a variety of 2,4-dinitrobenzenes have been measured by fluorescence quenching and the values obtained have been verified in representative instances by equilibrium dialysis. All populations of anti-DNP molecules examined, whether isolated from pooled sera or from single bleedings of individual rabbits, were heterogeneous in respect to affinity for dinitrobenzenes. The heterogeneity in virtually every instance could be described by the Sips distribution function over a wide range of binding data, and each titration was thus characterized by an average intrinsic association constant (K_o), and by a heterogeneity index (a). Fractional precipitation of antibodies from serum, by addition of limiting amounts of antigen, yielded from the serum of individual rabbits anti-DNP populations that differed as much as 10,000-fold in K_o. Changes in affinity for ε-DNP-lysine were followed by isolating antibodies from sera at various times after a single injection of DNP-bovine-γ-globulin. The K_o increased progressively with time after immunization and the rate of increase was most conspicuous when small quantities of DNP-bovine-γ-globulin were injected initially. The rise in K_o was markedly delayed when large doses of antigen were injected. Antibody populations isolated early after immunization had nearly the same low affinity for both ε-DNP-L-lysine and for 2,4-dinitroaniline, and it is inferred that their specific binding sites are poorly adapted to the dinitroanilino group and insensitive to the norleucine moiety of ε-DNP-lysine. In contrast, antibodies isolated late after immunization bind ε-DNP-lysine strongly, and from their interactions with a variety of dinitrobenzenes it is inferred that their binding sites, on the average, are just about large enough to accommodate ε-DNP-L-lysine. Complex formation was enthalpy driven: ΔH° values ranged from −8 to −20 kcal mole^{-1} for different antibody-ligand pairs, and ΔS° values ranged from −5 to −30 entropy units mole^{-1}. When representative 2,4-dinitrobenzenes were bound by anti-DNP molecules, bathychromic and hypochromic spectral shifts were observed; in the case of antibody-bound 2,4-dinitrotoluene a new absorption peak appeared at 300 mμ. The possibility is raised that charge-transfer contributes to the stability of antibody-dinitrophenyl complexes.

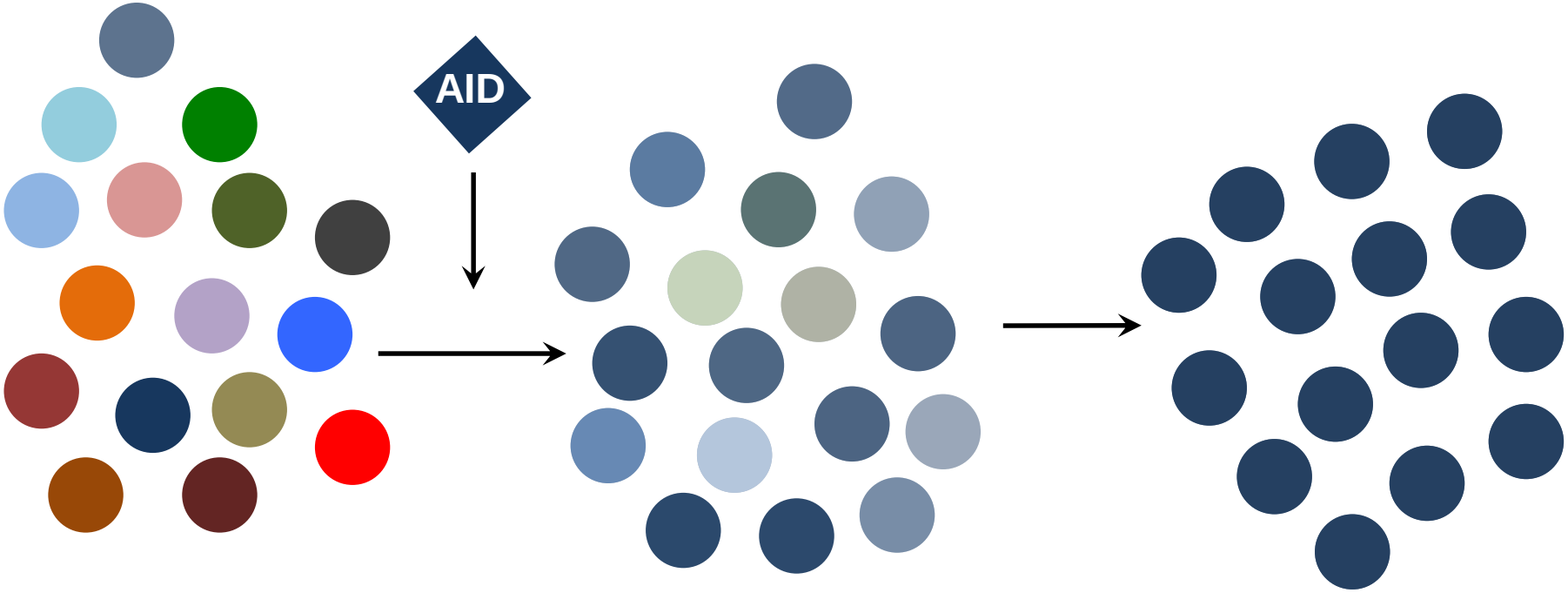


H.N. Eisen & G.W. Siskind, *Biochemistry*, 1964

Cellular basis of affinity maturation

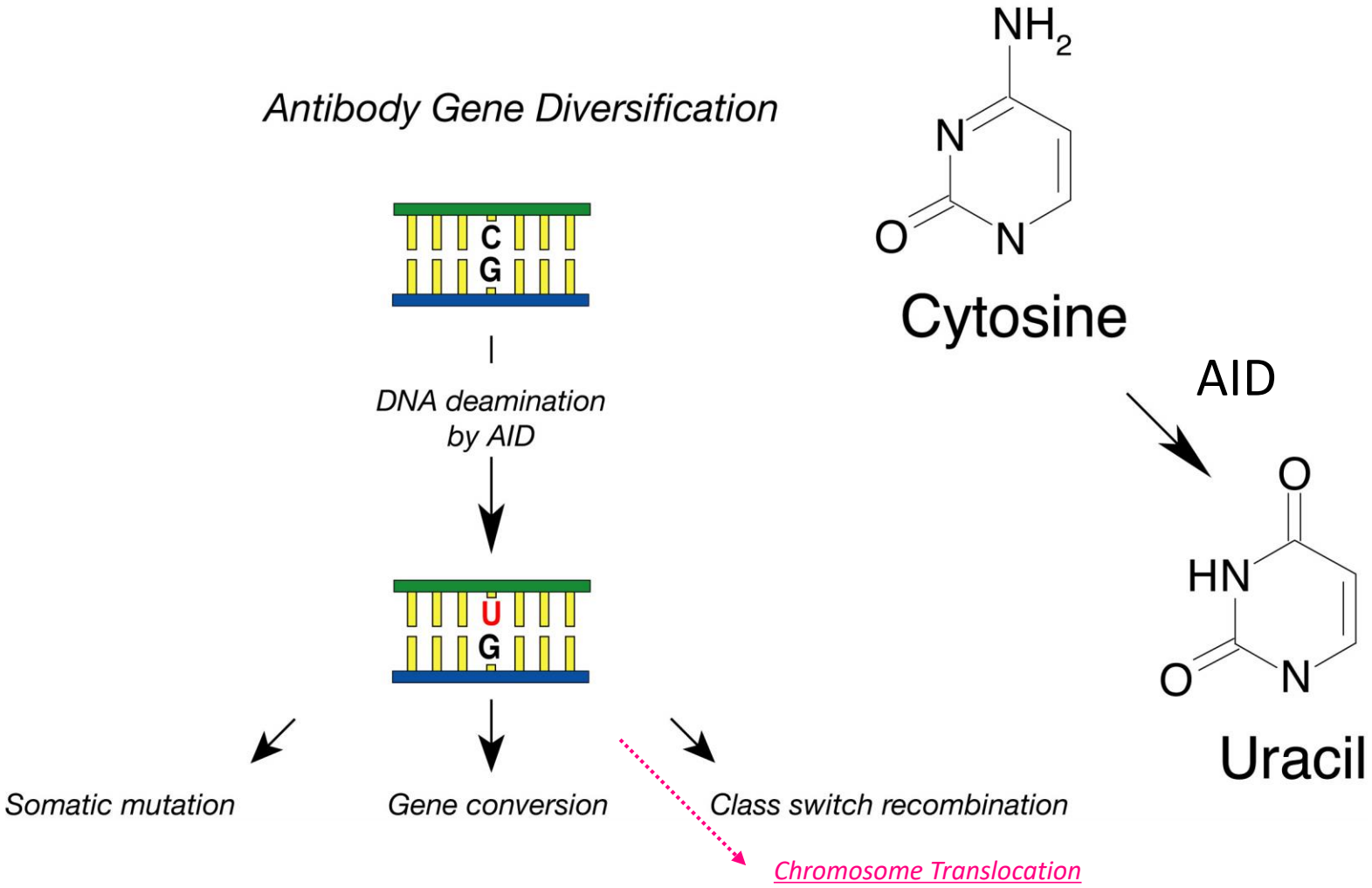
1. Diversification

2. Affinity-based selection



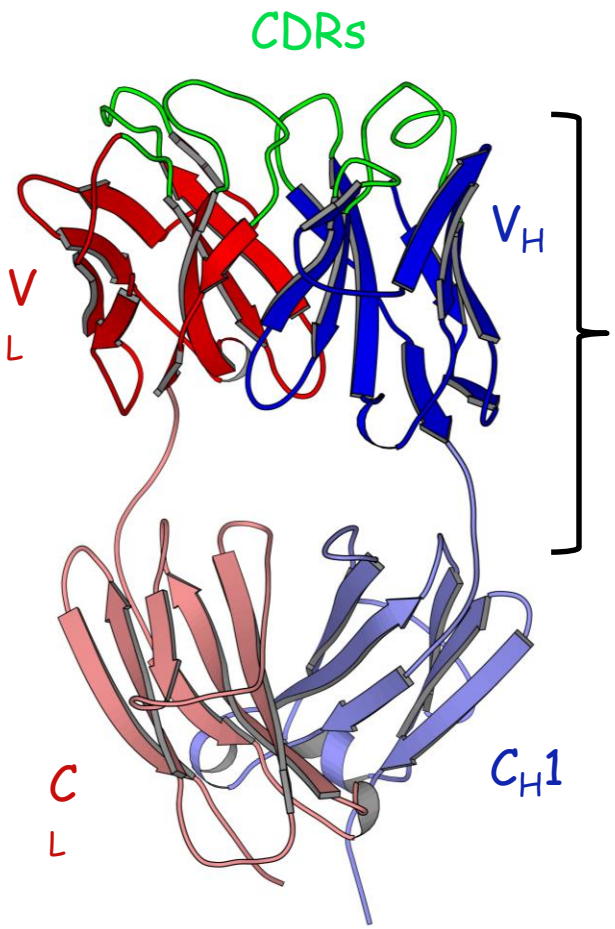
Antibody Affinity

Neuberger Model for Activation Induced Deaminase

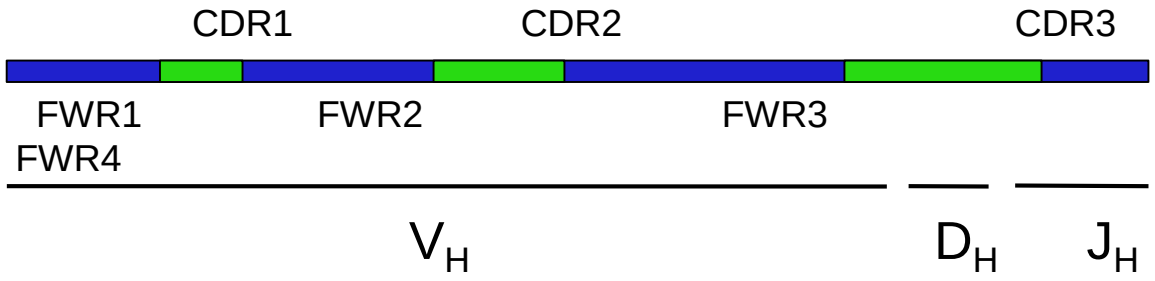


Pavri et al, *Cell* 2010; Basu et al *Cell* 2011; Yamane et al *Nat. Imm.* 2011; Qiang et al *Cell* 2014; Wang et al *PNAS* 2014; Meng et al *Cell* 2014; Robbiani et al *Cell* 2015

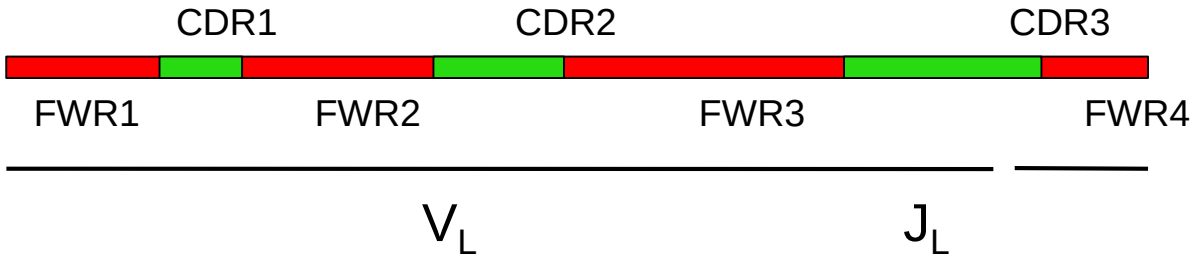
Frameworks and CDRs



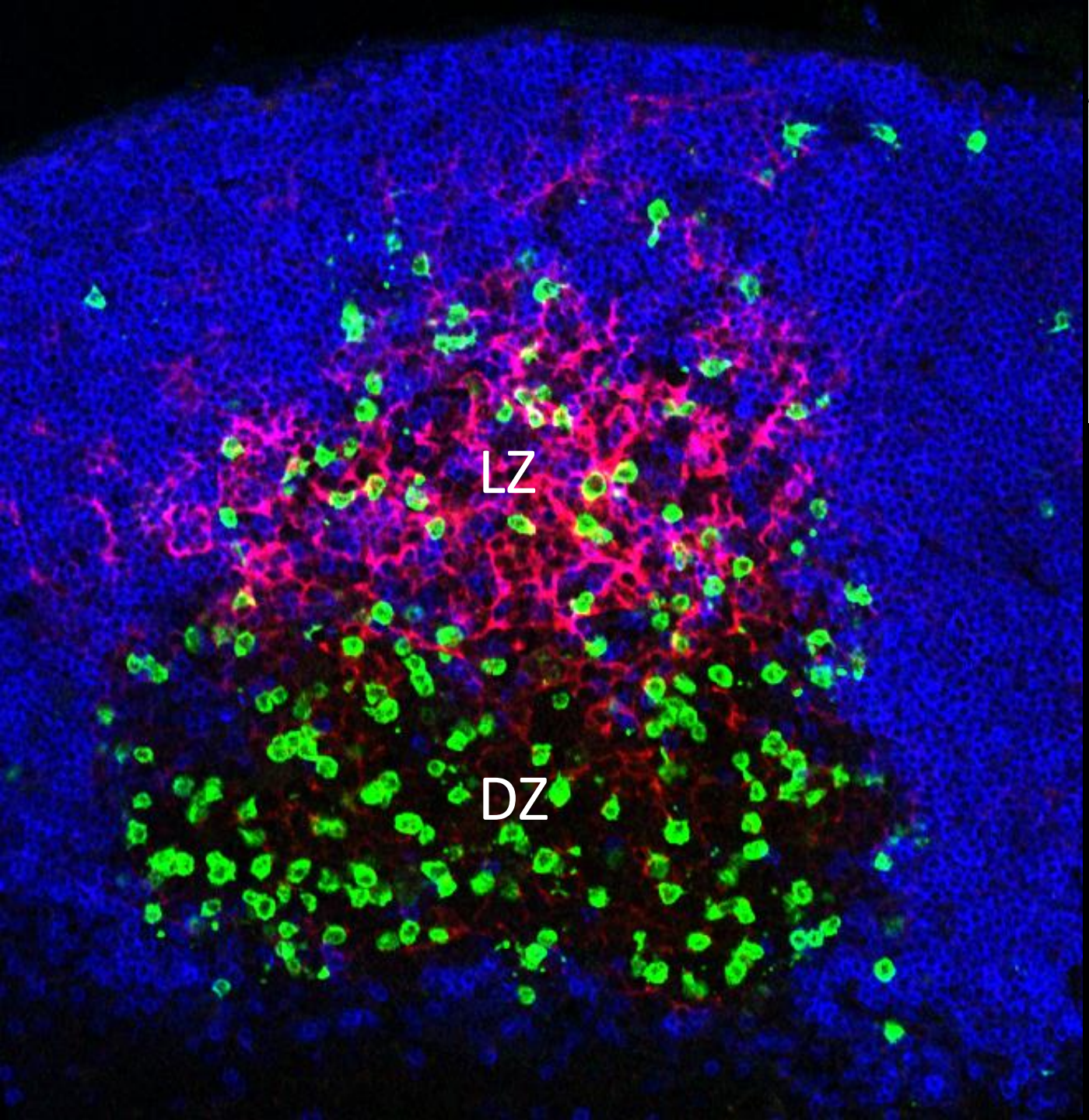
IgH (heavy chain)

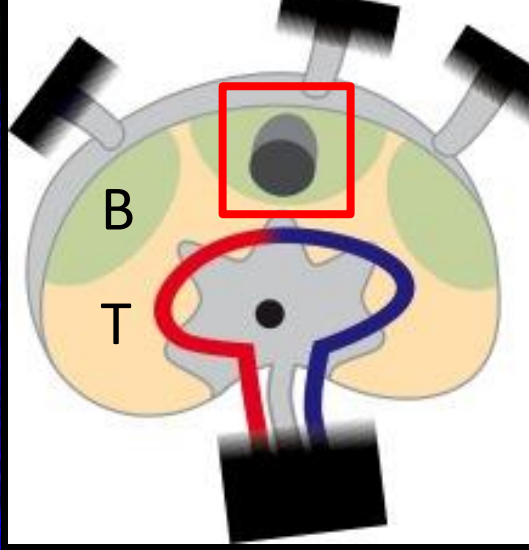


IgL (light chain)



Rogozin, Milstein and Neuberger,
Reynaud and Weill

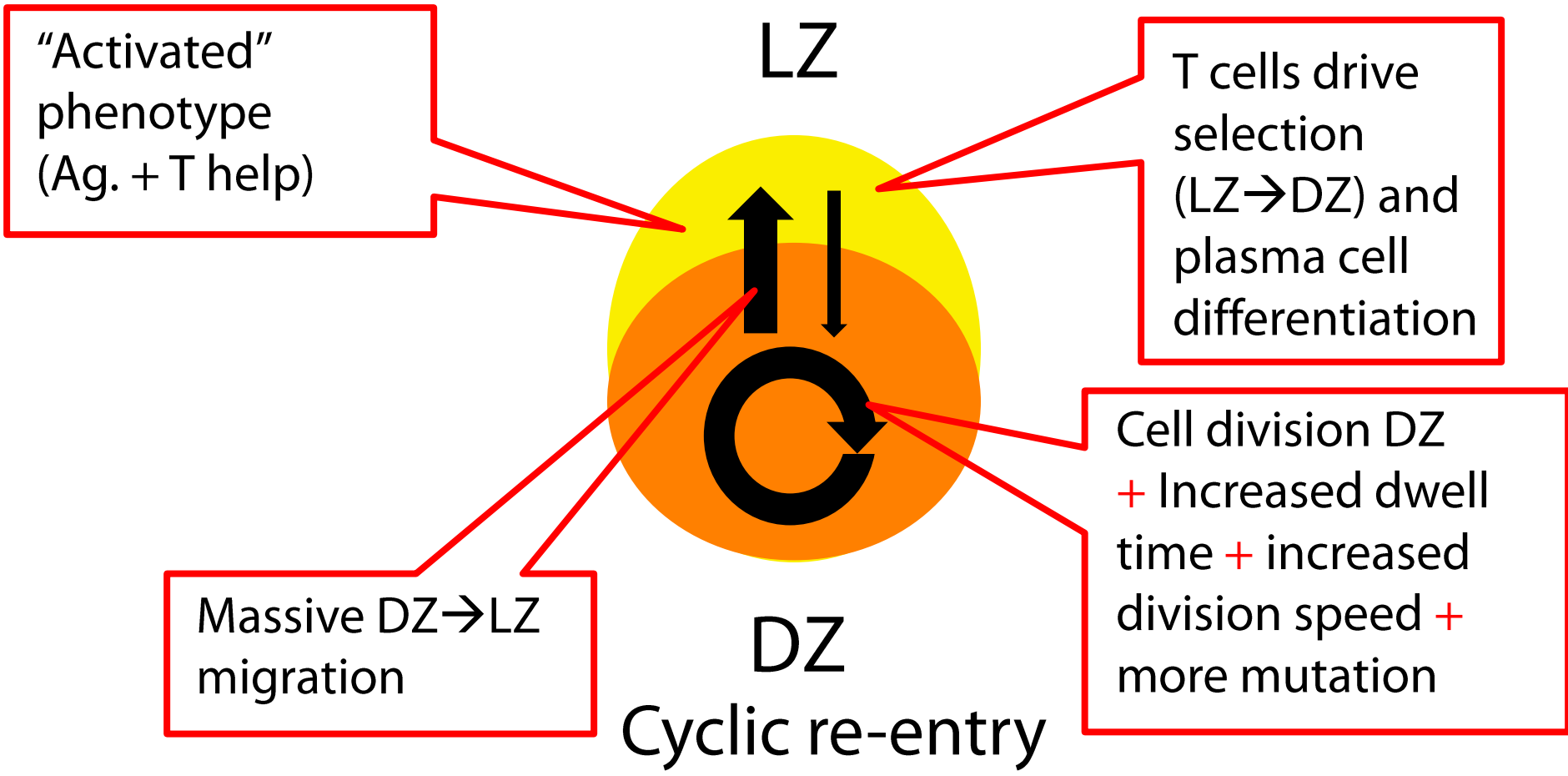




Naïve B cells
GC B cells
FDCs

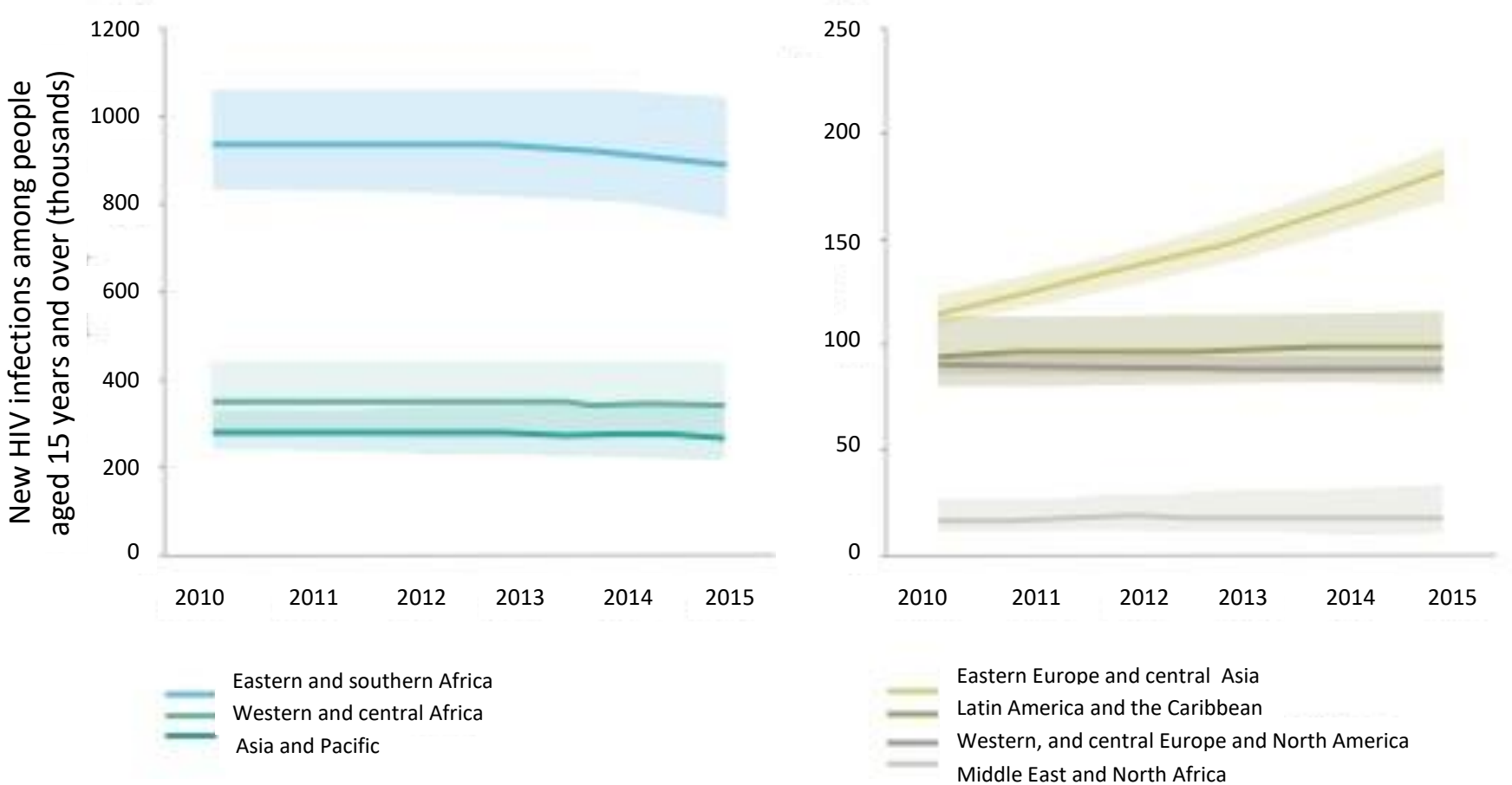
Schwickert et al *Nature* 2008
Victora et al *Cell* 2010

Model: T cells drive B cell selection in GC by directing interzonal migration



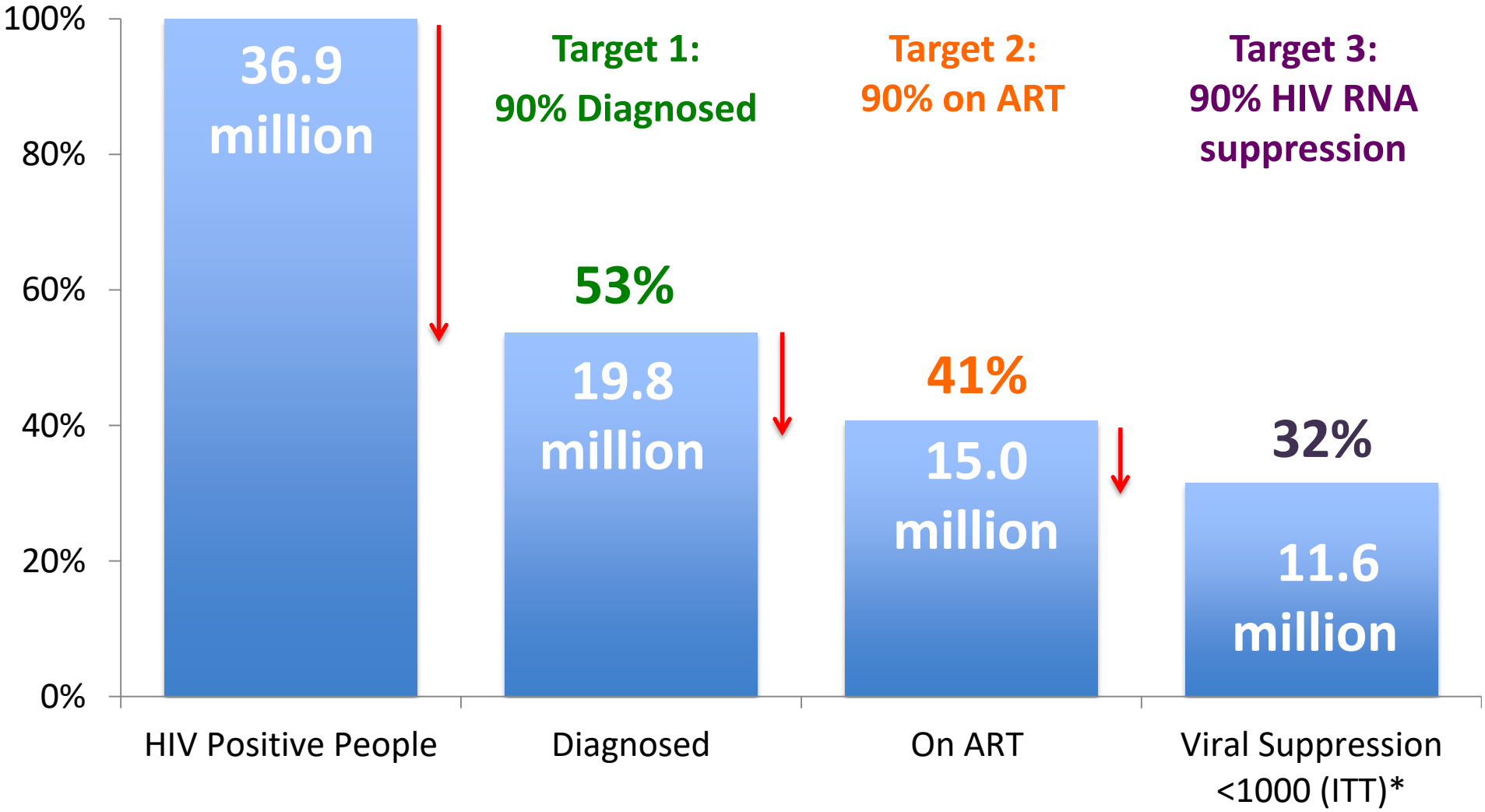
Victora et al, *Cell*, 2010; Shulman et al *Science* 2013
Shulman et al *Science* 2014; Gitlin et al *Nature* 2014
Gitlin et al *Science* 2015; Gitlin et al Immunity 2016

Incidence rates have plateaued



Global AIDS Update (UNAIDS, 2016)

Most HIV-infected people do not have suppressed viral loads

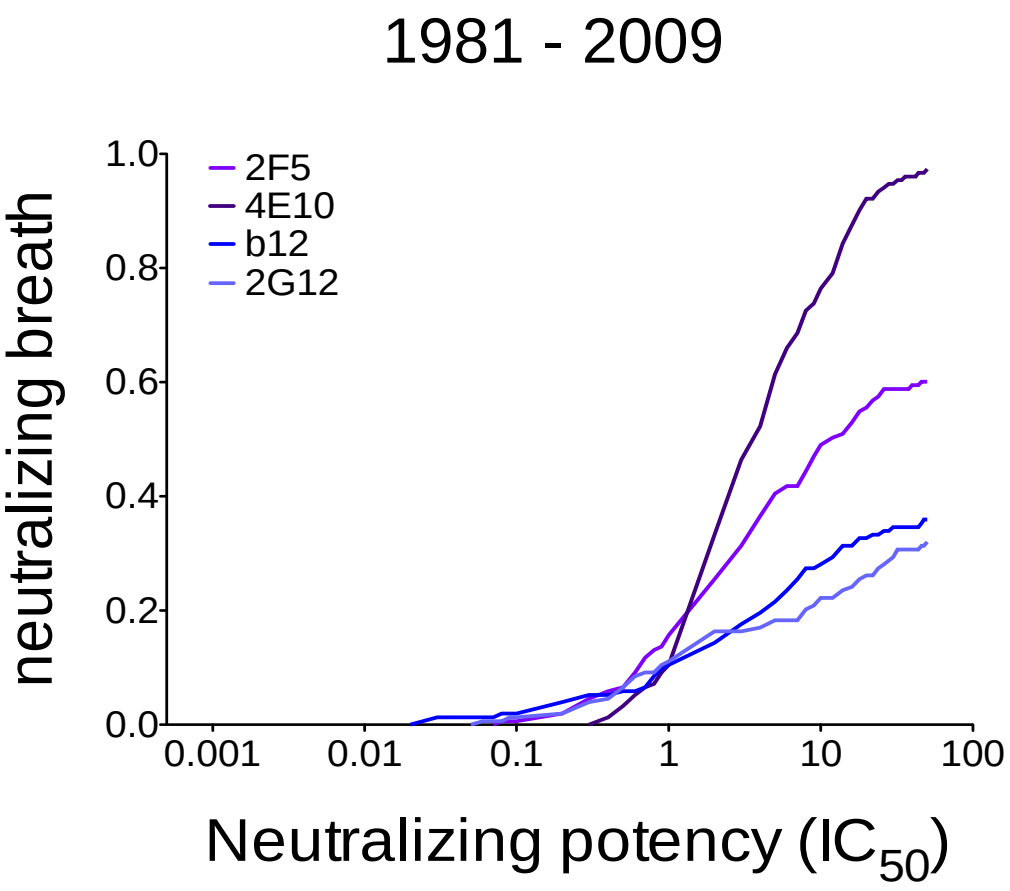


Levi J, IAS 2015 (UNAIDS 2015; McMahon Bull WHO, 2013)

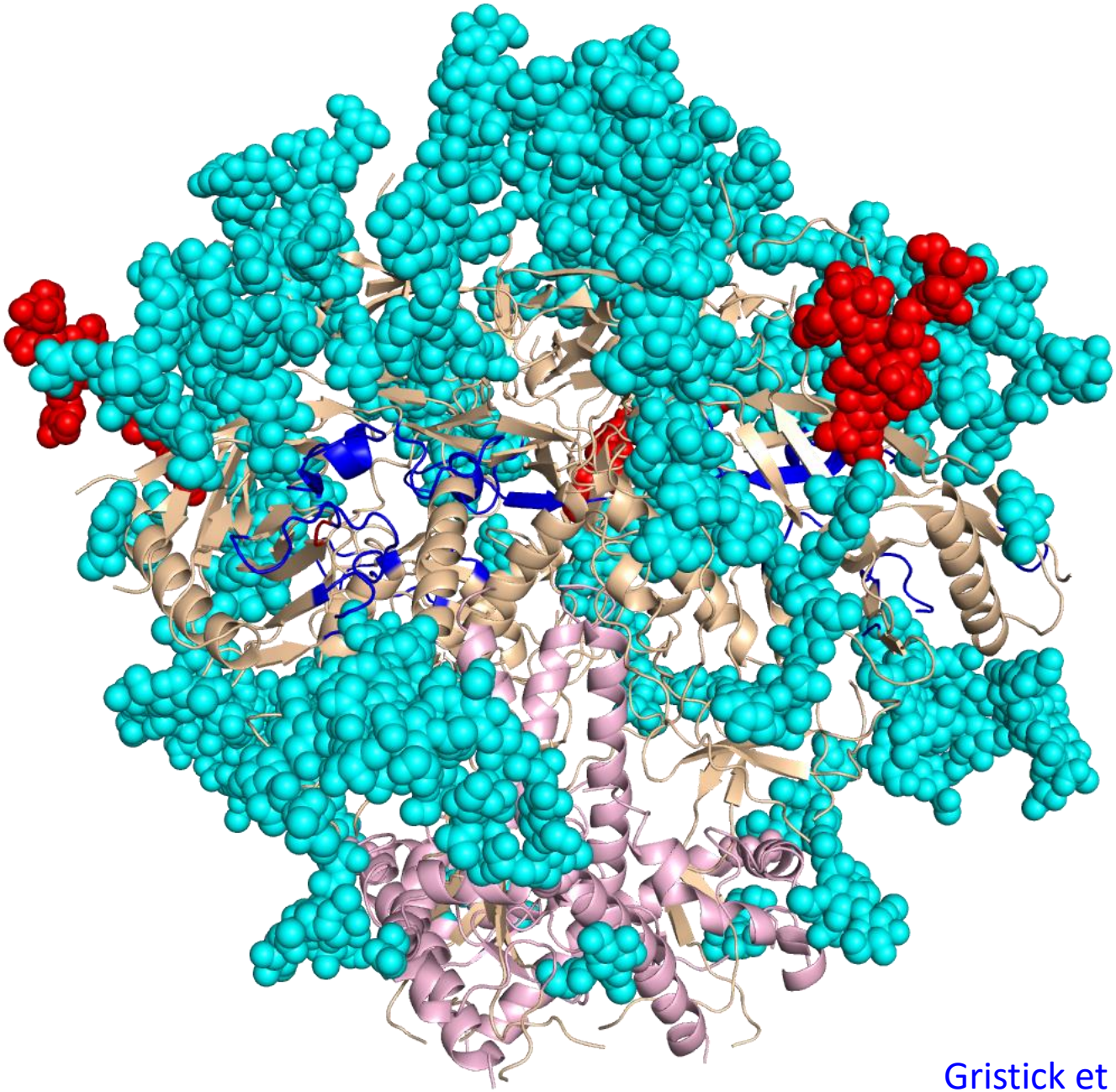
The HIV Problem

- Big global public health problem
- After more than 30 years no vaccine and no cure
- Why?

Broadly neutralizing antibodies against HIV-1

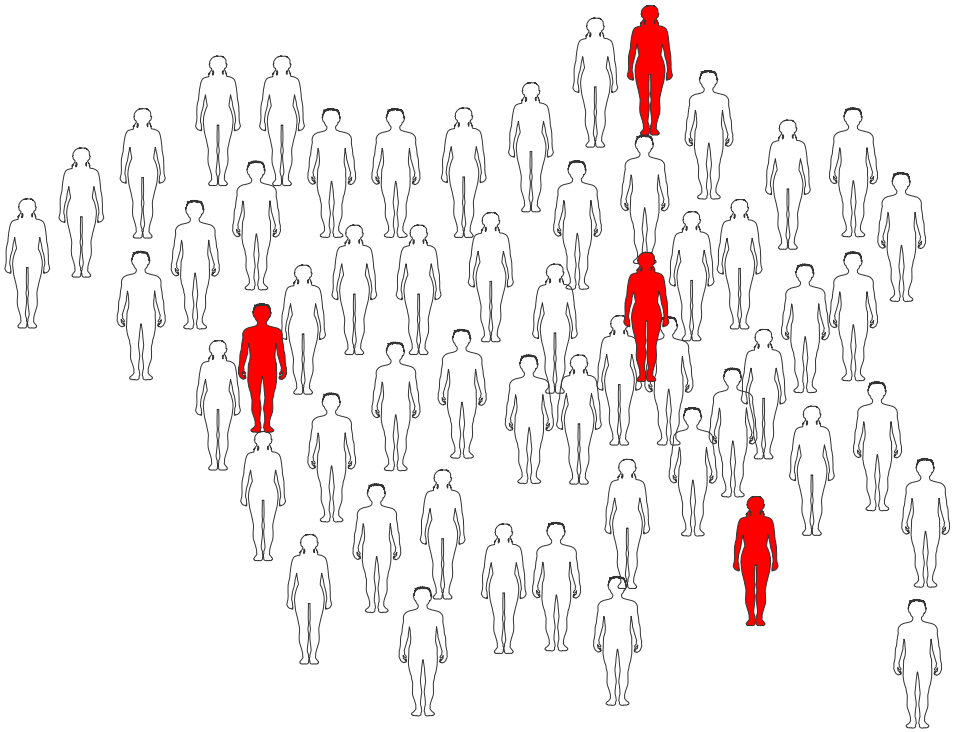


HIV gp160 is shielded by glycans

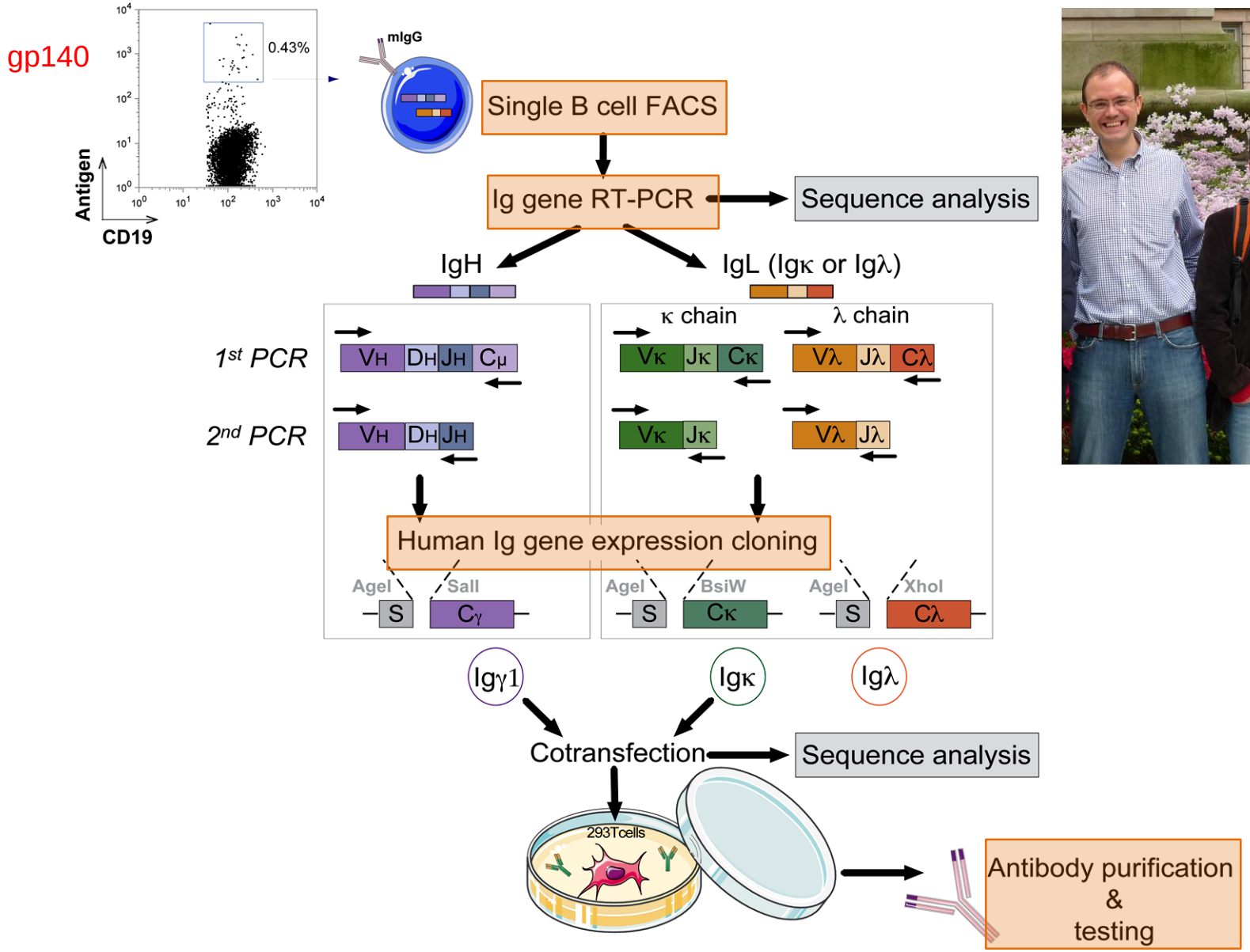


Gristick et al NSMB 2016

5-10% of HIV+ individuals develop broadly neutralizing serum antibodies BUT only after 2-3 years and this has not been reproduced in vaccines

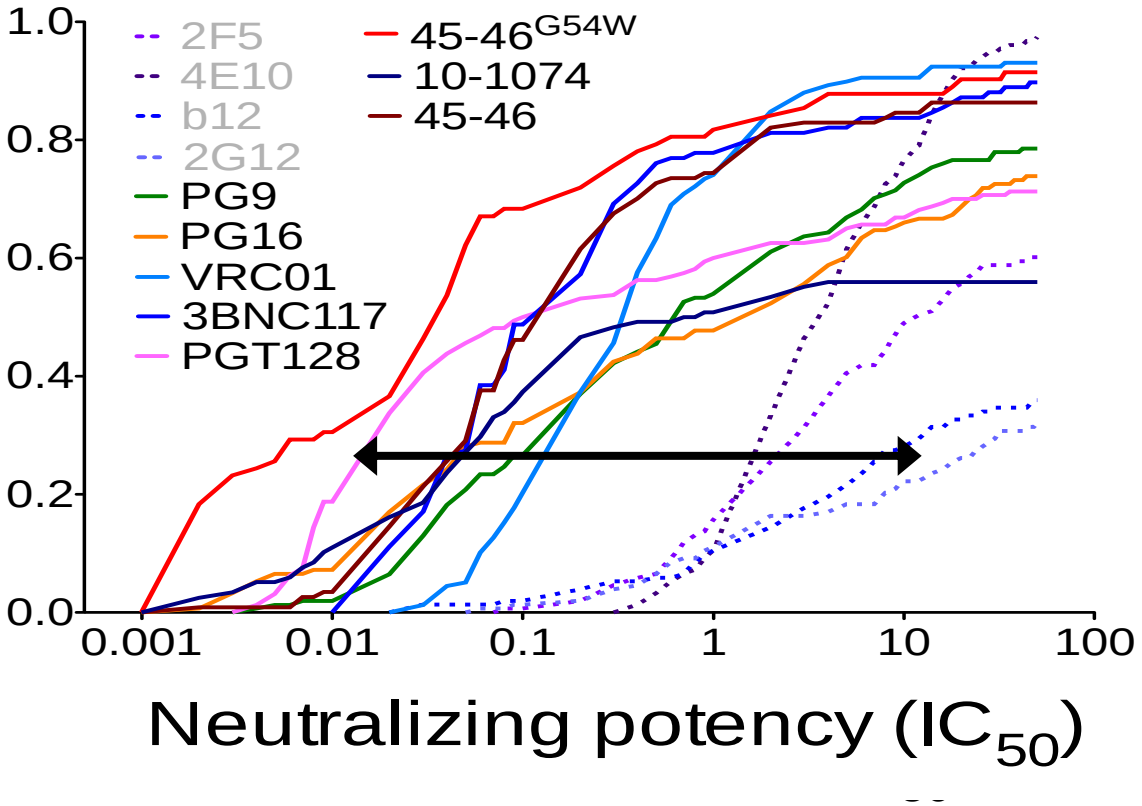


Cloning and Expression of Antibodies from Single Human B cells



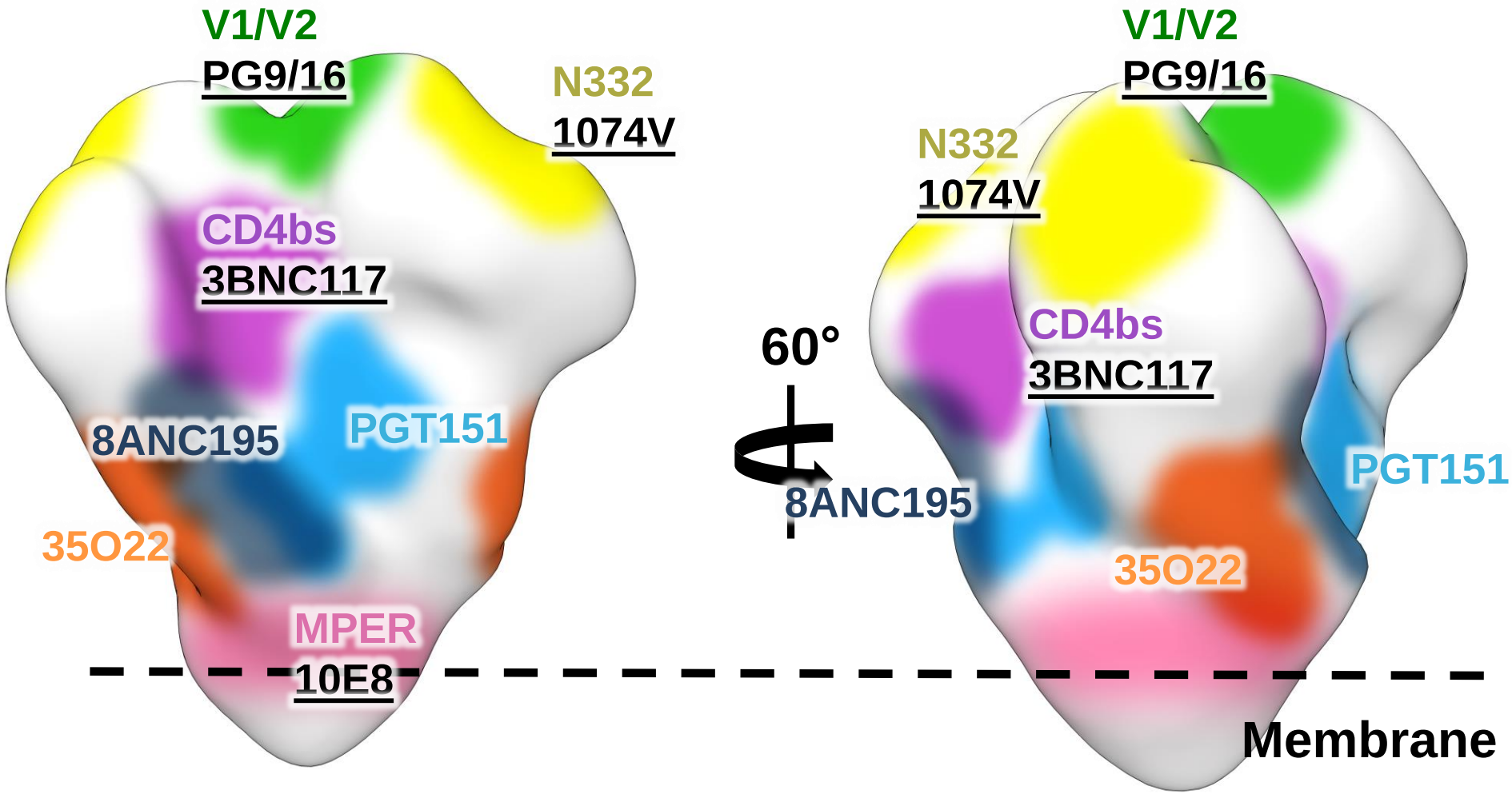
Wardemann H. et al. *Science* 2003 Scheid J. et al. *J Immunol Methods* 2008;
Scheid J. et al. *Nature* 2009; Mouquet H. et al. *Nature* 2010

Broadly neutralizing antibodies against HIV-1



Mouquet et al., *PNAS* 2012; Scheid et al., *Science* 2011; Diskin et al., Science *2011*; Walker et al., Nature *2011*; Wu et al., Science *2010*; Walker et al., Science *2009*

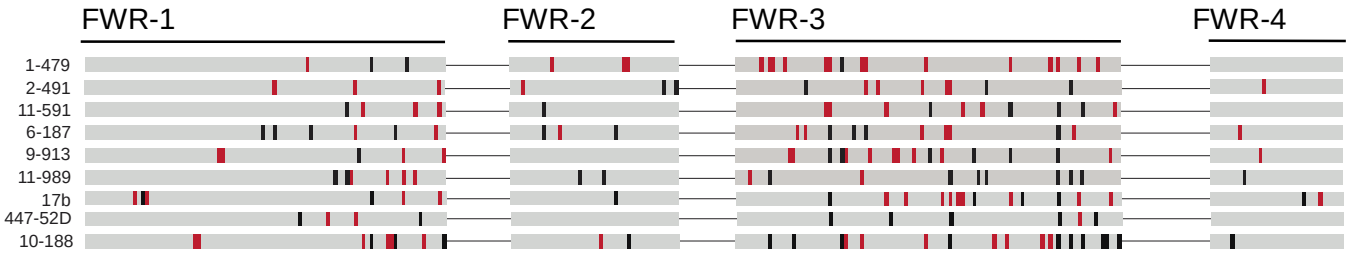
Best in Class Antibodies



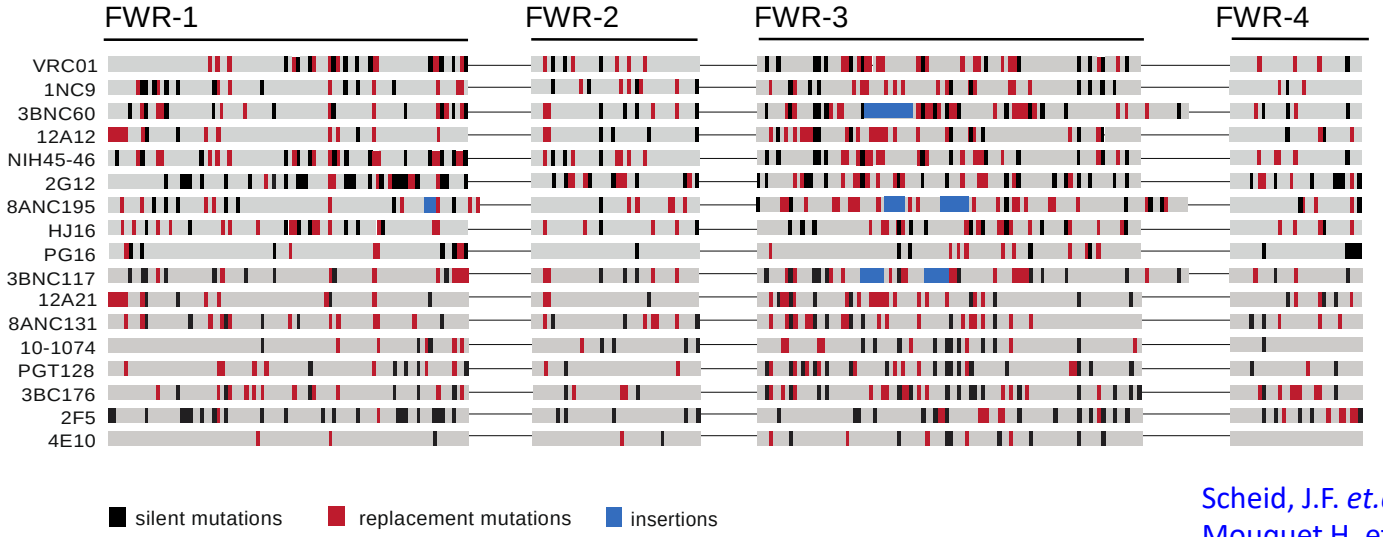
Andrew Ward

Somatic mutations in the framework of HIV-1-neutralizing Abs

Limited neutralizing activity (IgH)

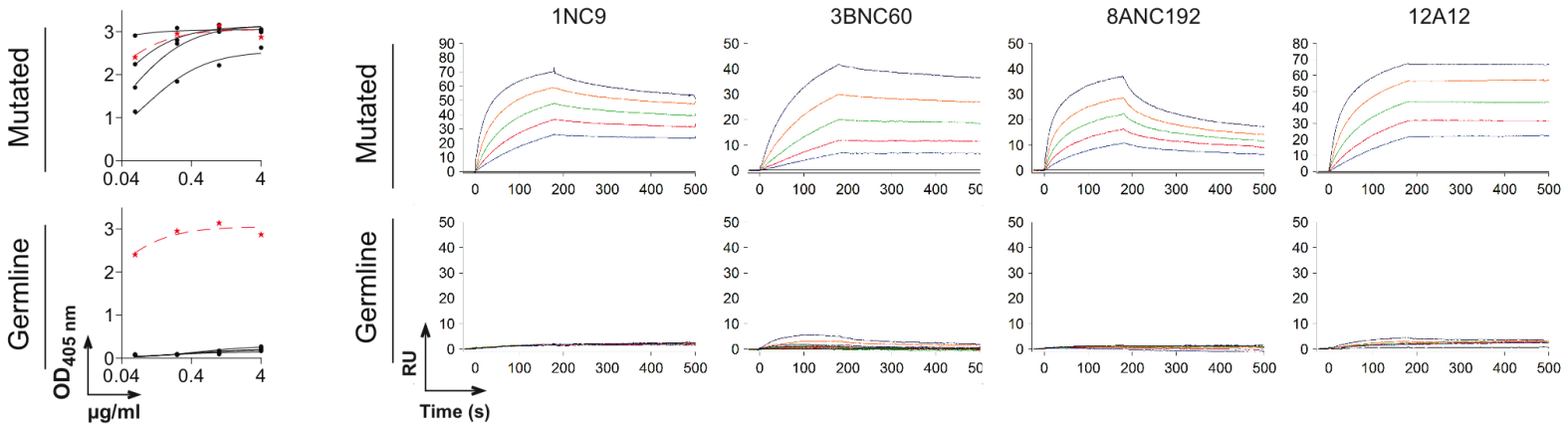


Broadly neutralizing antibodies (IgH)



Scheid, J.F. *et.al*. Nature 2009
Mouquet H. et al Nature 2010
Klein et al *Cell* 2013

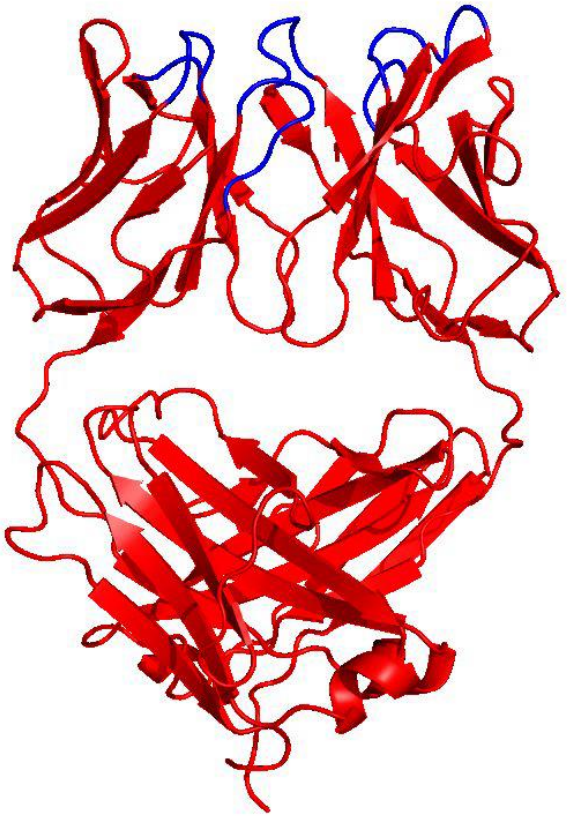
Germline reverted antibodies loose binding and neutralizing activity



IC_{50}	1NC9 (RU08)	3BNC60 (RU01)	8ANC131 (RU10)	12A12 (RU16)	RU08 Germline	RU01 Germline	RU10 Germline	RU16 Germline
MW965.26	>50	<0.04	>50	0.042	>50	>50	>50	>50
BAL.26	1.37	<0.04	0.06	0.017	>50	>50	>50	>50
DJ263.8	>50	<0.04	0.004	0.002	>50	>50	>50	>50
6535.3	>50	0.54	0.20	21.97	>50	>50	>50	>50
RHPA4259	0.09	<0.05	>50	0.086	>50	>50	>50	>50
TRO.11	0.20	<0.05	>50	0.288	>50	>50	>50	>50
PVO.4	0.34	0.09	0.81	0.928	>50	>50	>50	>50
YU2	0.13	<0.05	0.18	0.084	>50	>50	>50	>50

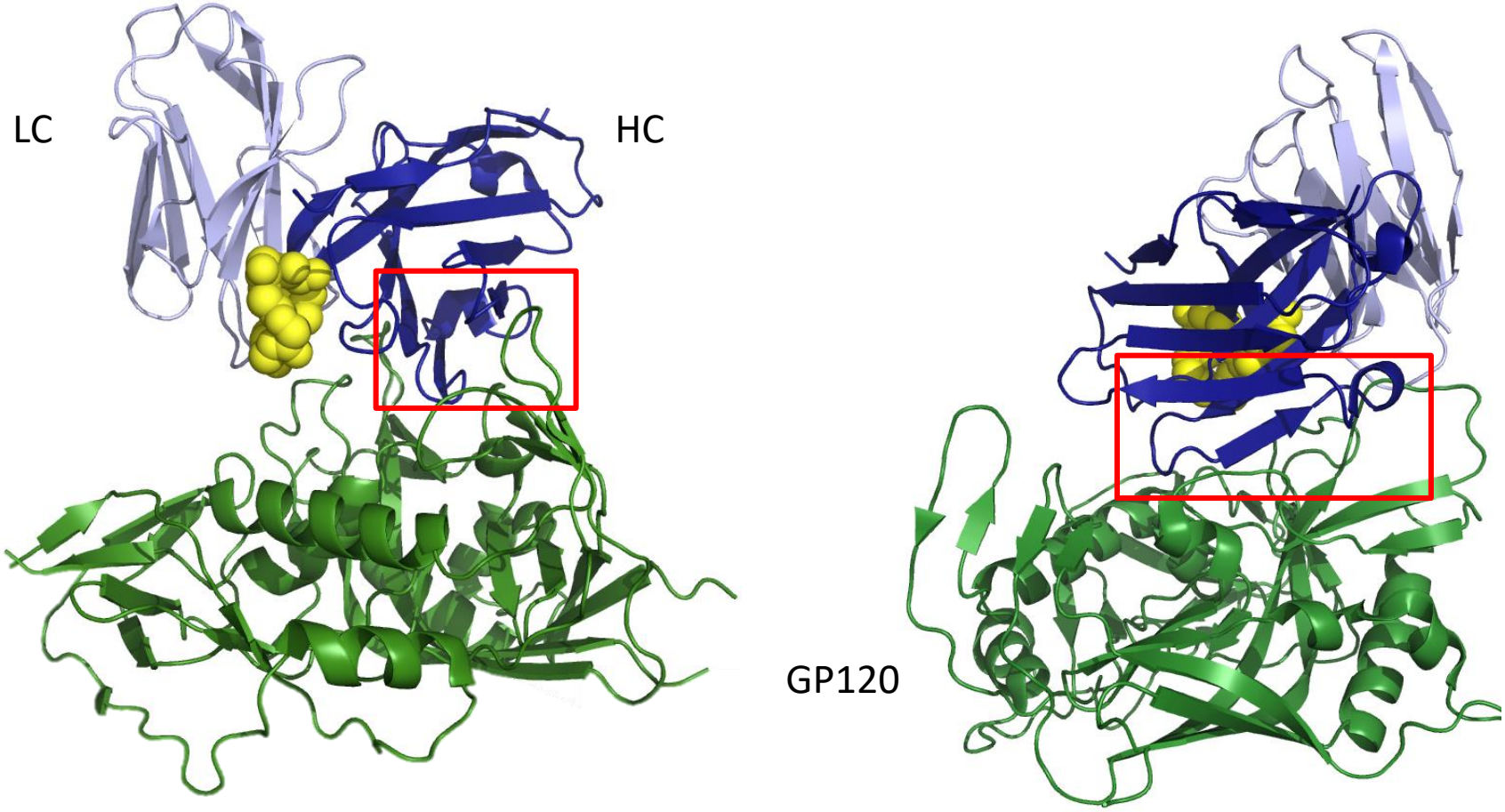
Mouquet. et al *Nature* 2010
Scheid, J. et al *Science* 2011
Klein et al *Cell* 2013

Why so many mutations?



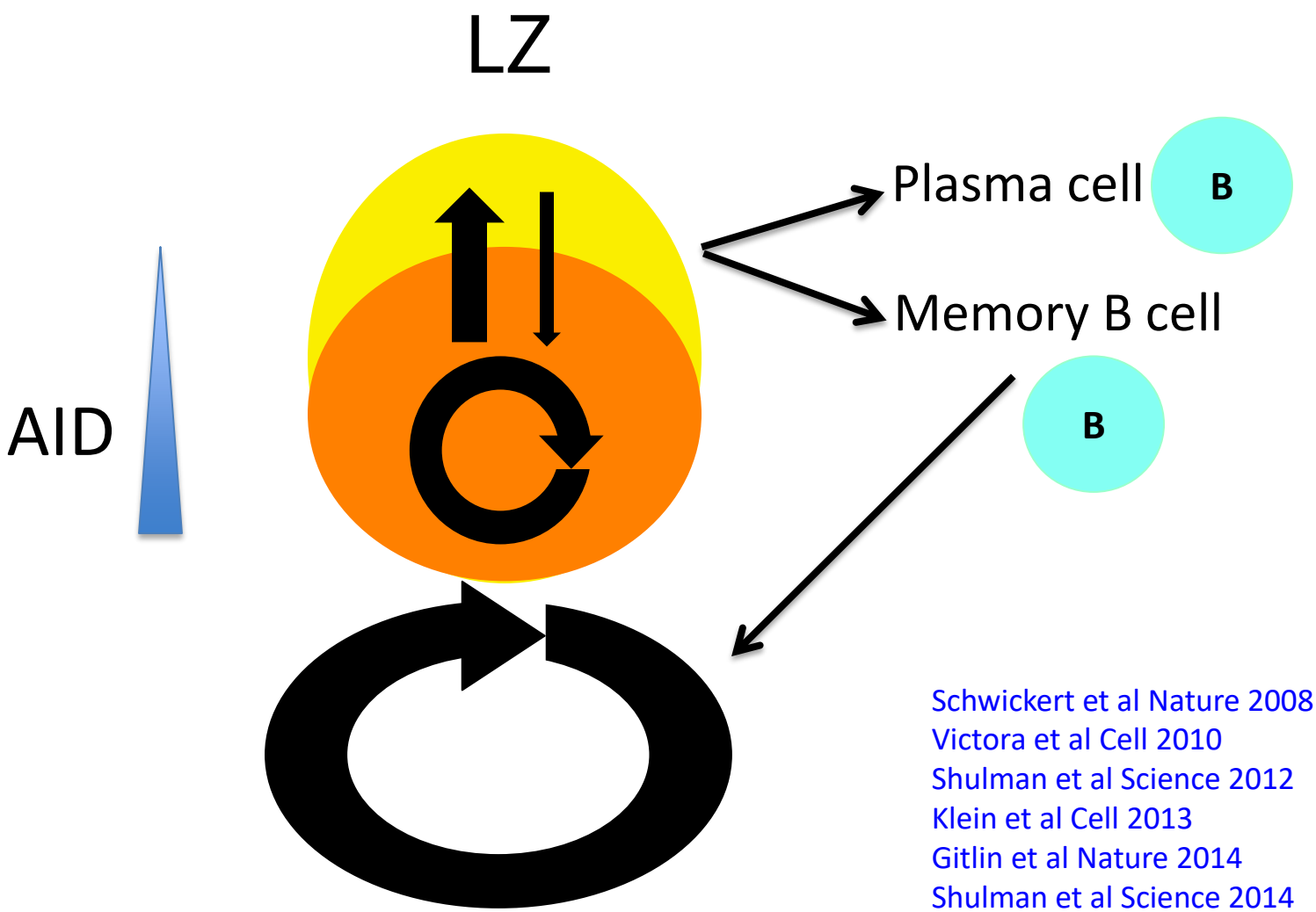
Courtesy of Ron Diskin

Contact by Framework Residues



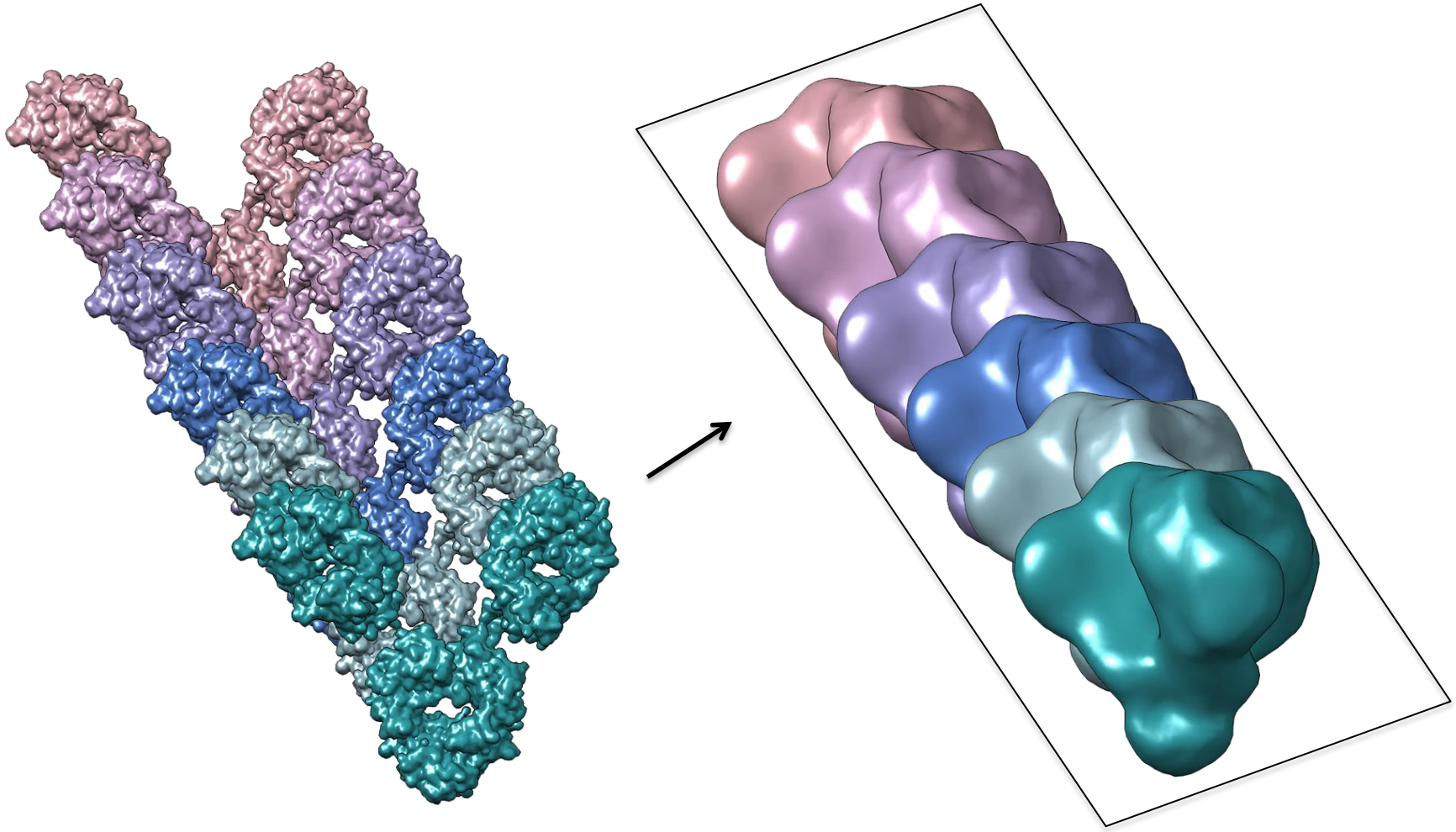
Bill Scheif

Repeated Rounds of Affinity Maturation



Schwickert et al Nature 2008
Victora et al Cell 2010
Shulman et al Science 2012
Klein et al Cell 2013
Gitlin et al Nature 2014
Shulman et al Science 2014

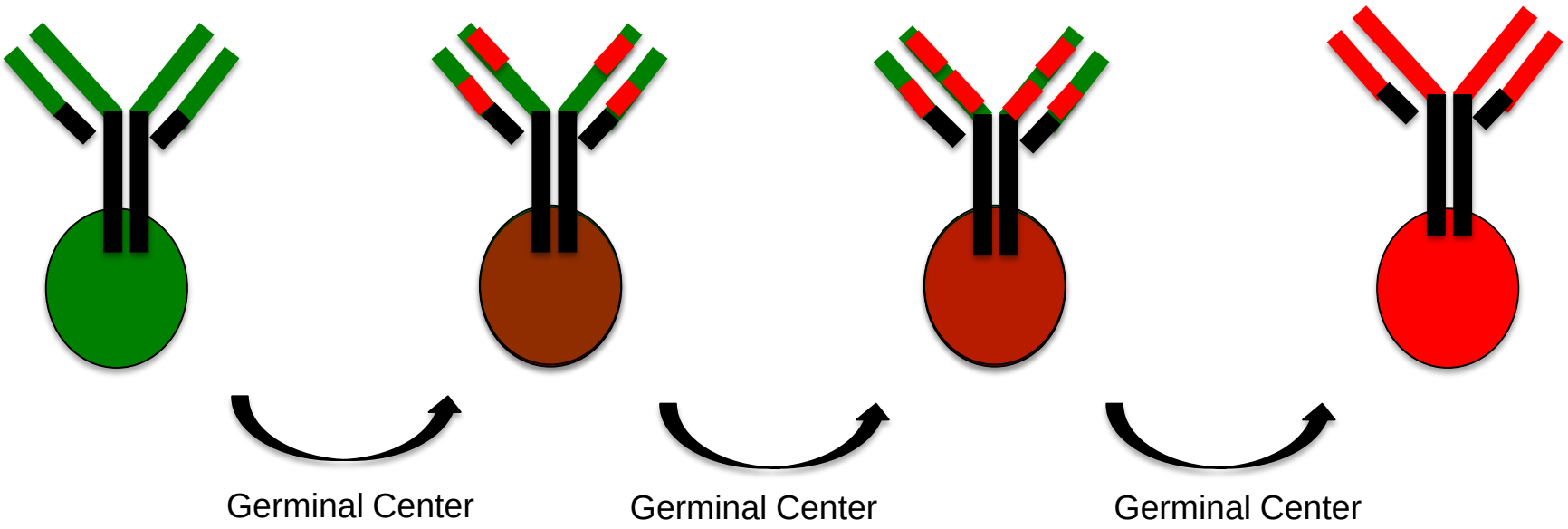
Evolution of a Broadly Neutralizing Antibody



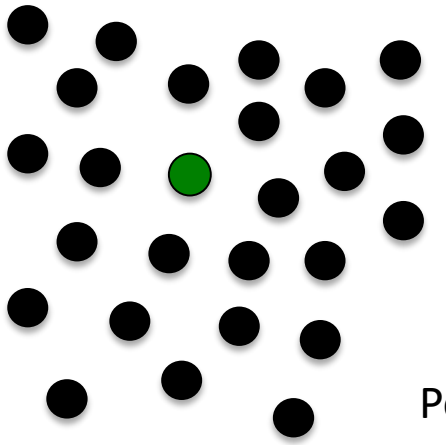
Liao et al *Nature* 2013; Klein et al *Cell* 2013
Moore et al *Nature* 2014

Germ line

Mature bNAbs

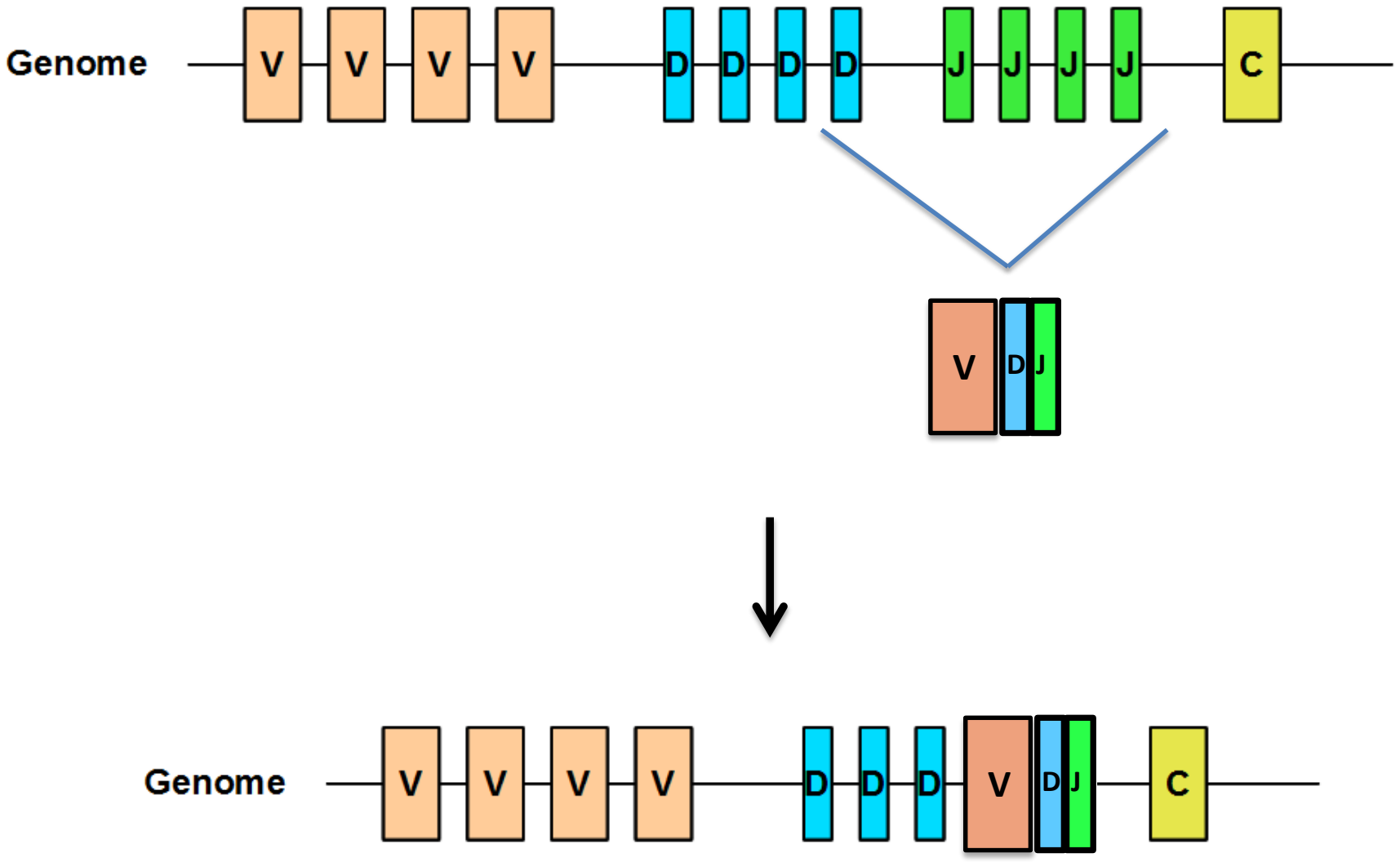


-Germline prediction
-Low precursor frequency
-Competition/Immunodominance
-High Mutation Frequency

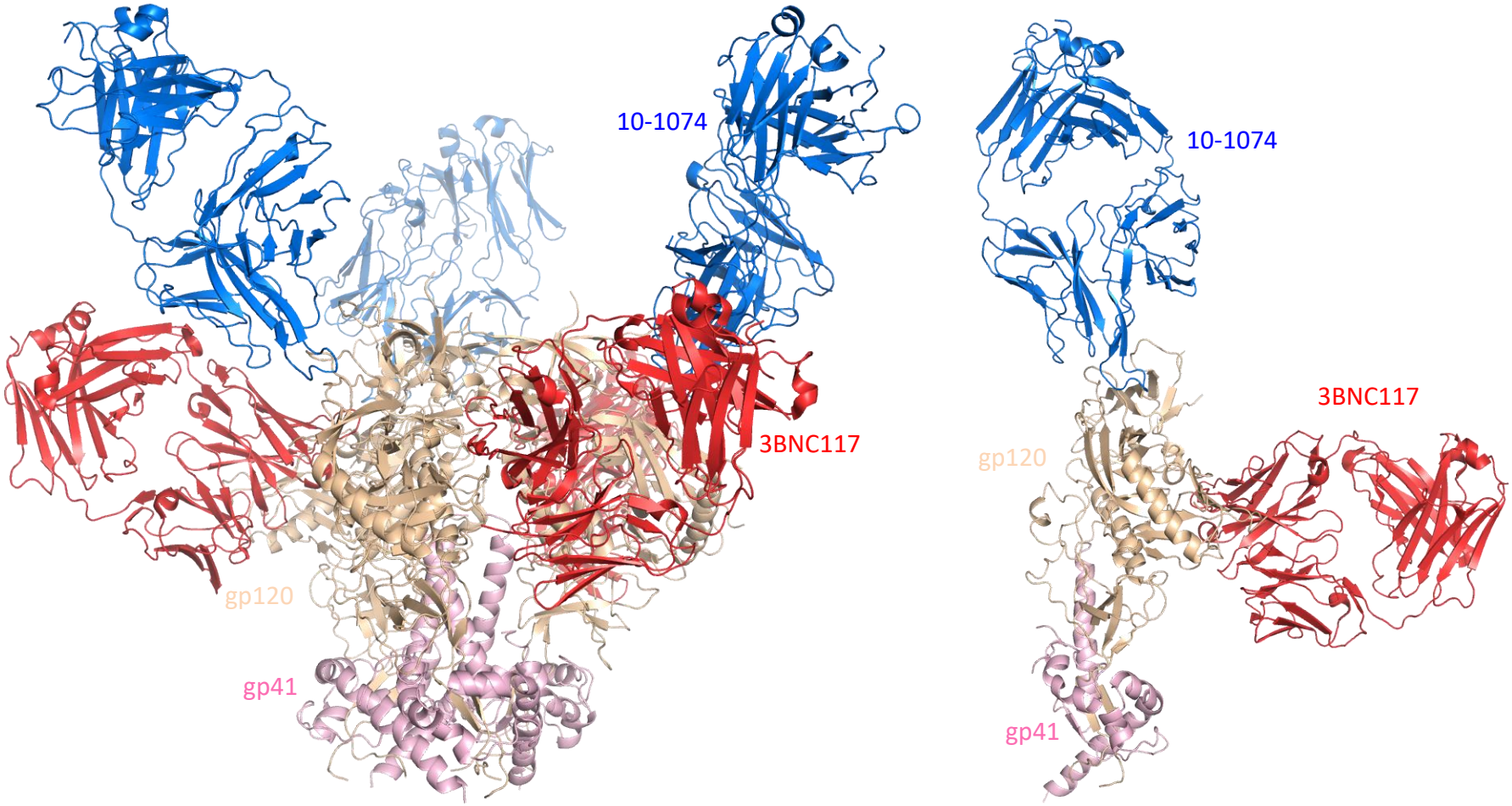


Pool of wt B cells

HIV bNAb KI mice

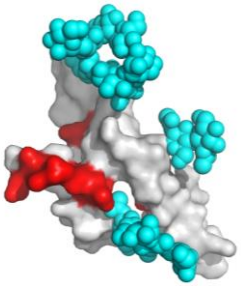


3BNC117 and 10-1074 Target Independent Sites

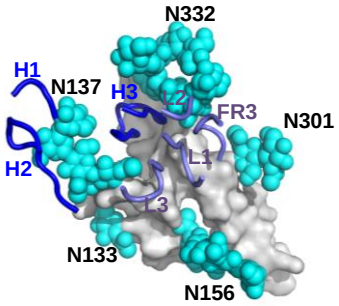


Gristick et al NSMB 2016

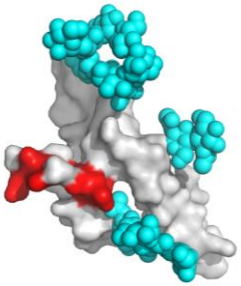
Candidate Antigens



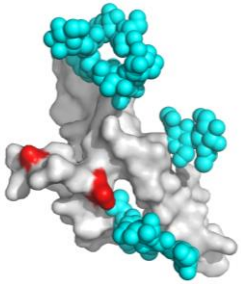
SOSIP-10MUT
Binding to GL_{H3mat}
K_D ~ 1 µM



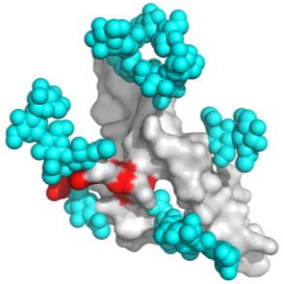
BG505-SOSIP
T332N
No binding to GL



SOSIP-7MUT
Binding to GL_{H3mat}
K_D ~ 50 µM



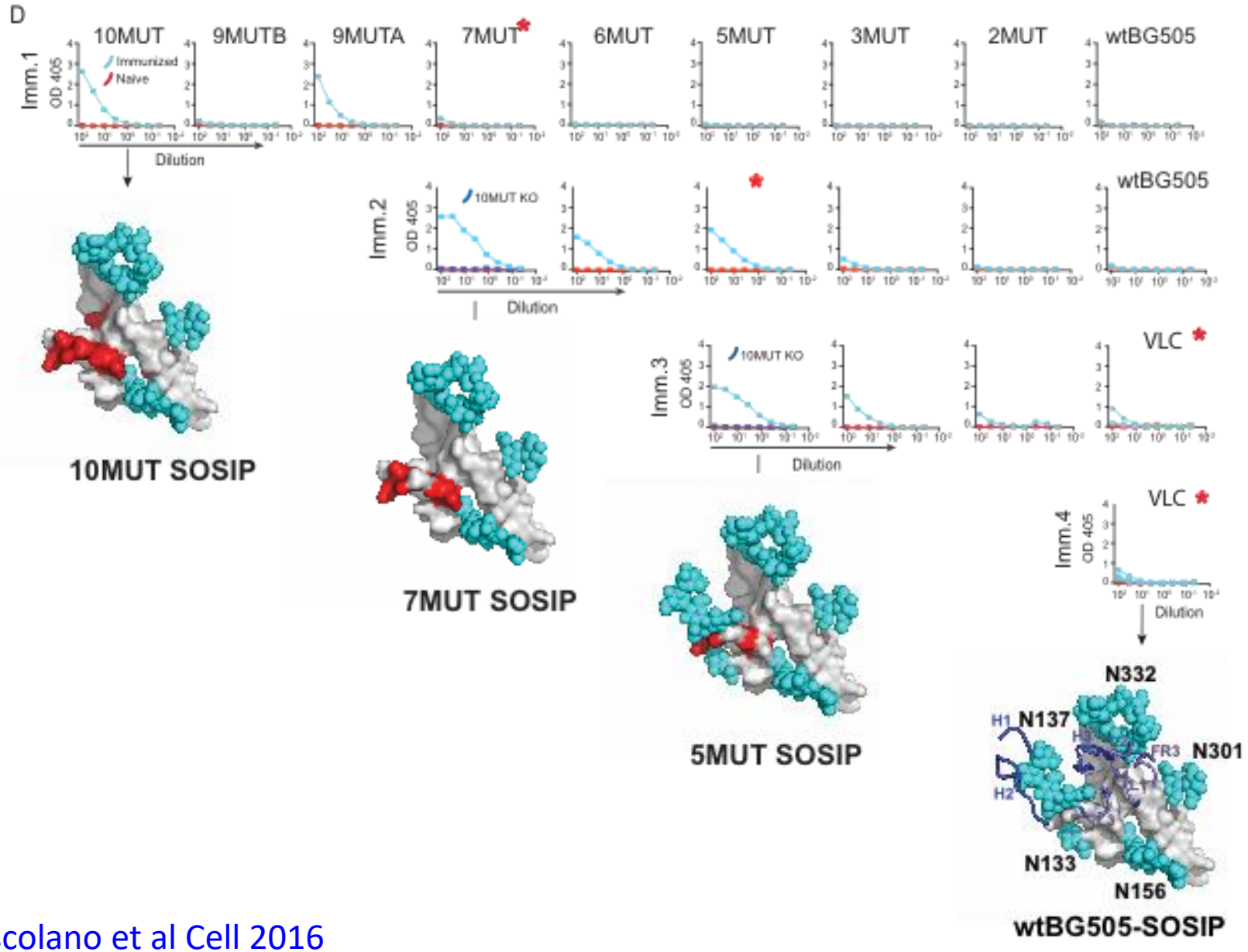
SOSIP-3MUT
Remove V1 glycans N133 and N137
Binding to GL+12



SOSIP-5MUT
Four mutations in V1
Binding to GL+6

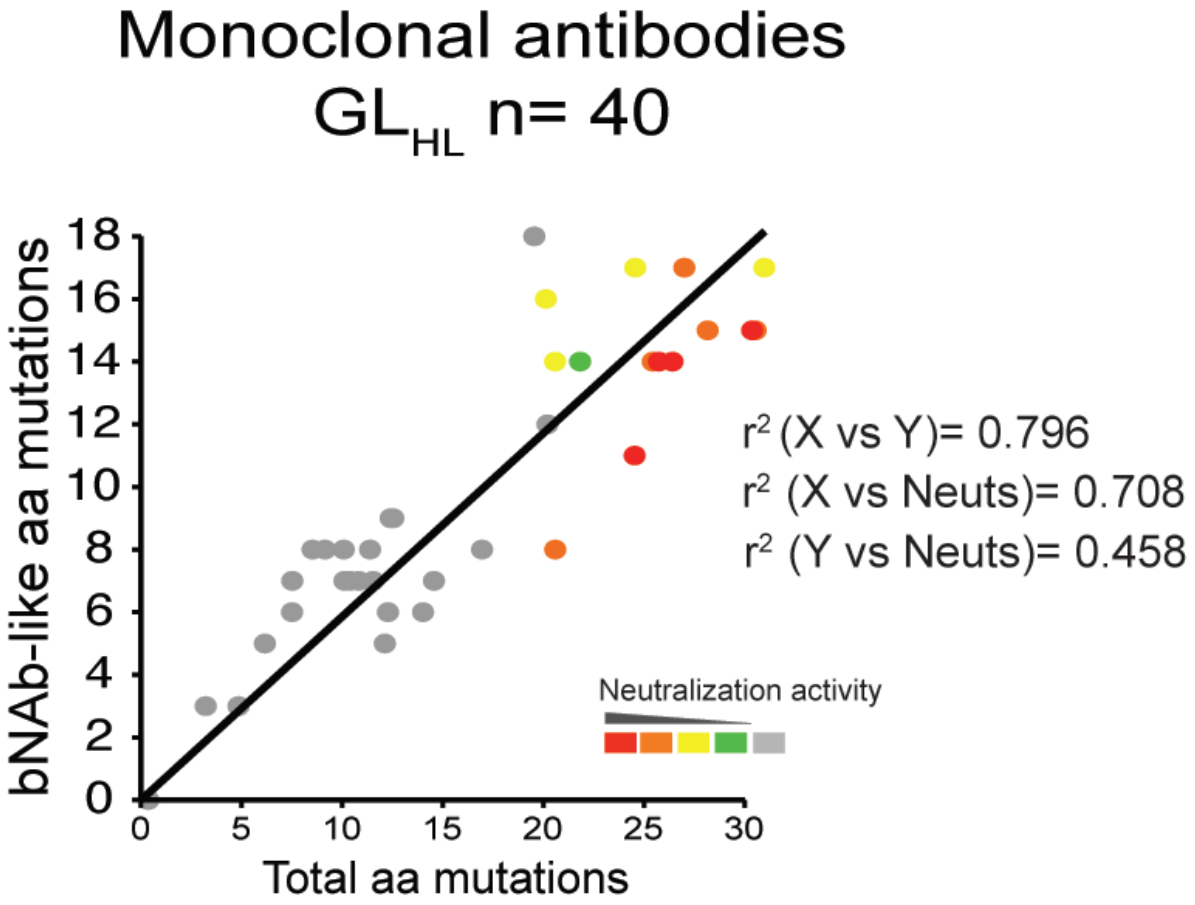
Steichen et al Immunity 2016

ELISA-guided design of sequential protocol



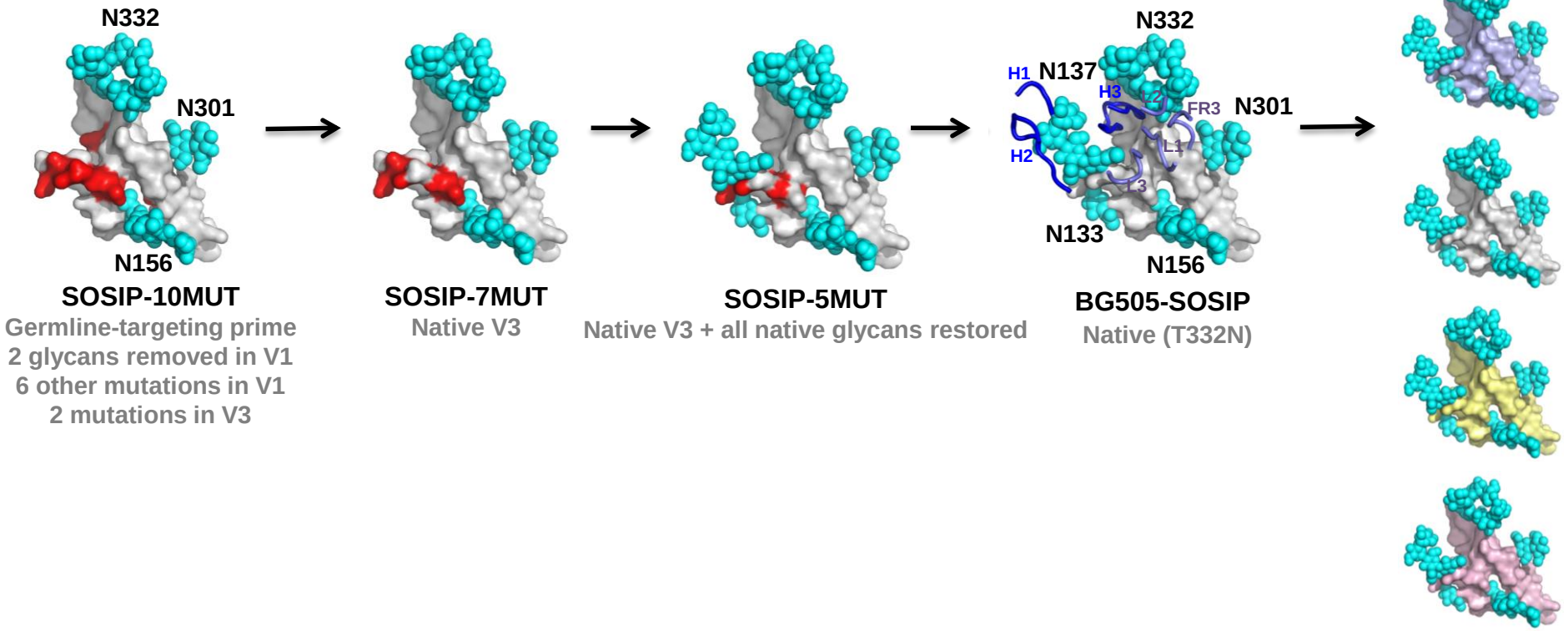
Escolano et al Cell 2016

Elicited antibodies recapitulate PGT121 maturation



Escolano et al Cell 2016

Germline-targeting/boosting strategies to induce bnAbs



Escolano et al Cell 2016

Important Caveats to Consider

- Mice are not humans
- Knock in germline bNAb strongly biases the response
- Precursor frequency is abnormal
- Only a small fraction of the responding cells are germline antigen specific in *Dosenovic et al Cell 2015, Tian et al Cell 2016, Sok et al Science 2016*
- For CD4bs bNAbs N276 remains a key issue
- For glycan patch bANbs we do not have a germline targeting immunogen with sufficiently high affinity

Antibodies are Effective Against HIV

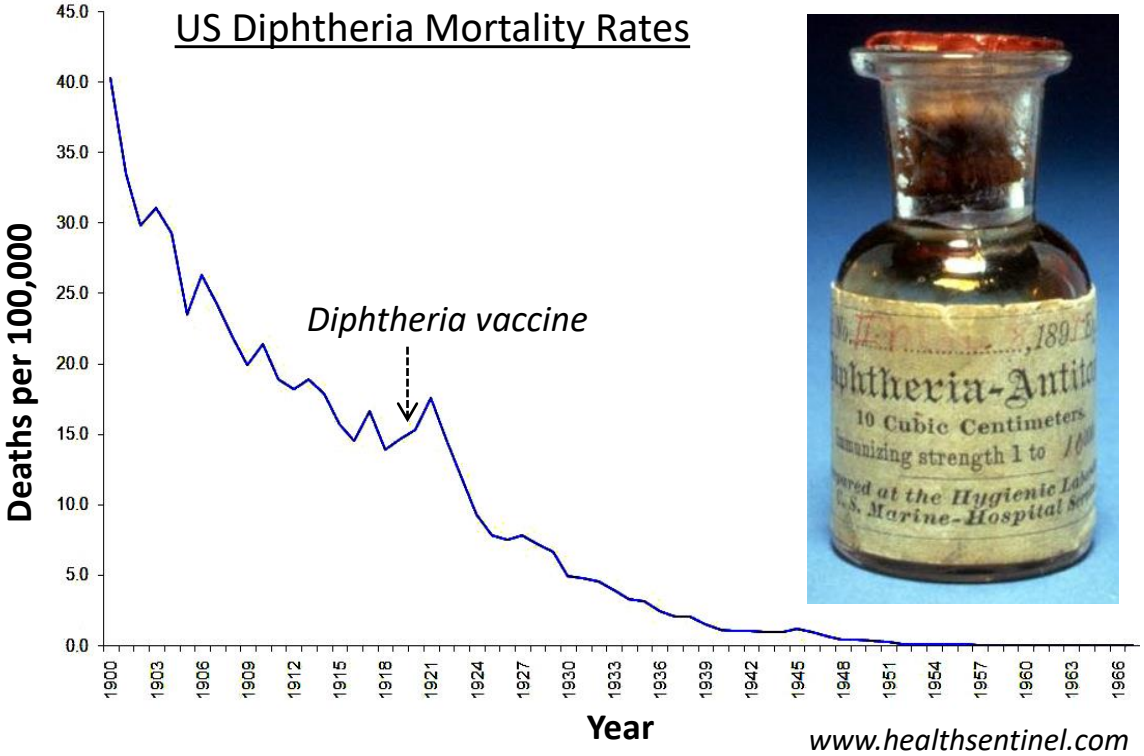
1890-1892 : protective role of Abs


Shibasaburo Kitasato


Emil von Behring

diphtheria and tetanus toxins

1894 : antibody-based therapy against diphtheria in humans

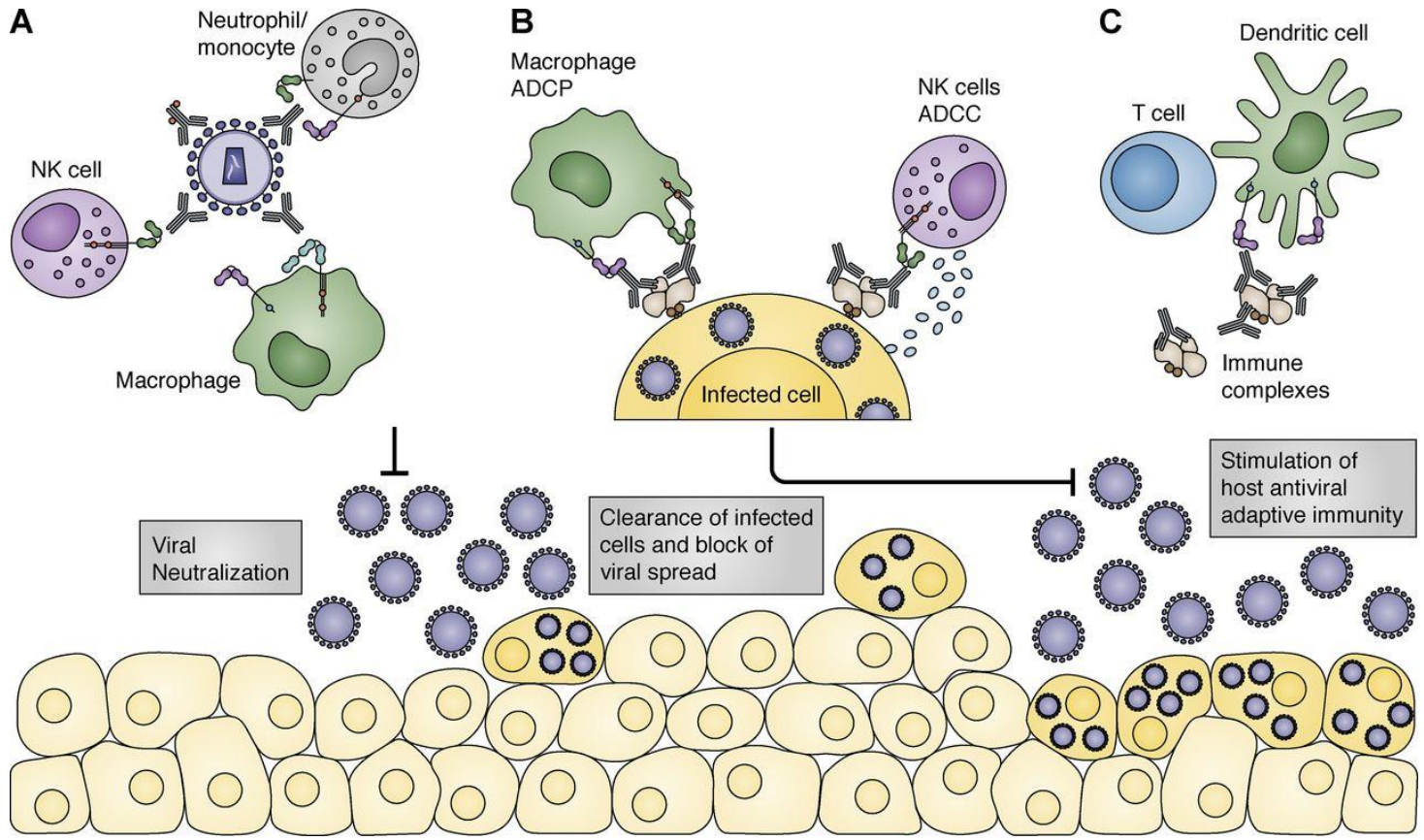


What Roles Might Antibodies have in HIV-1 Prevention or Therapy?

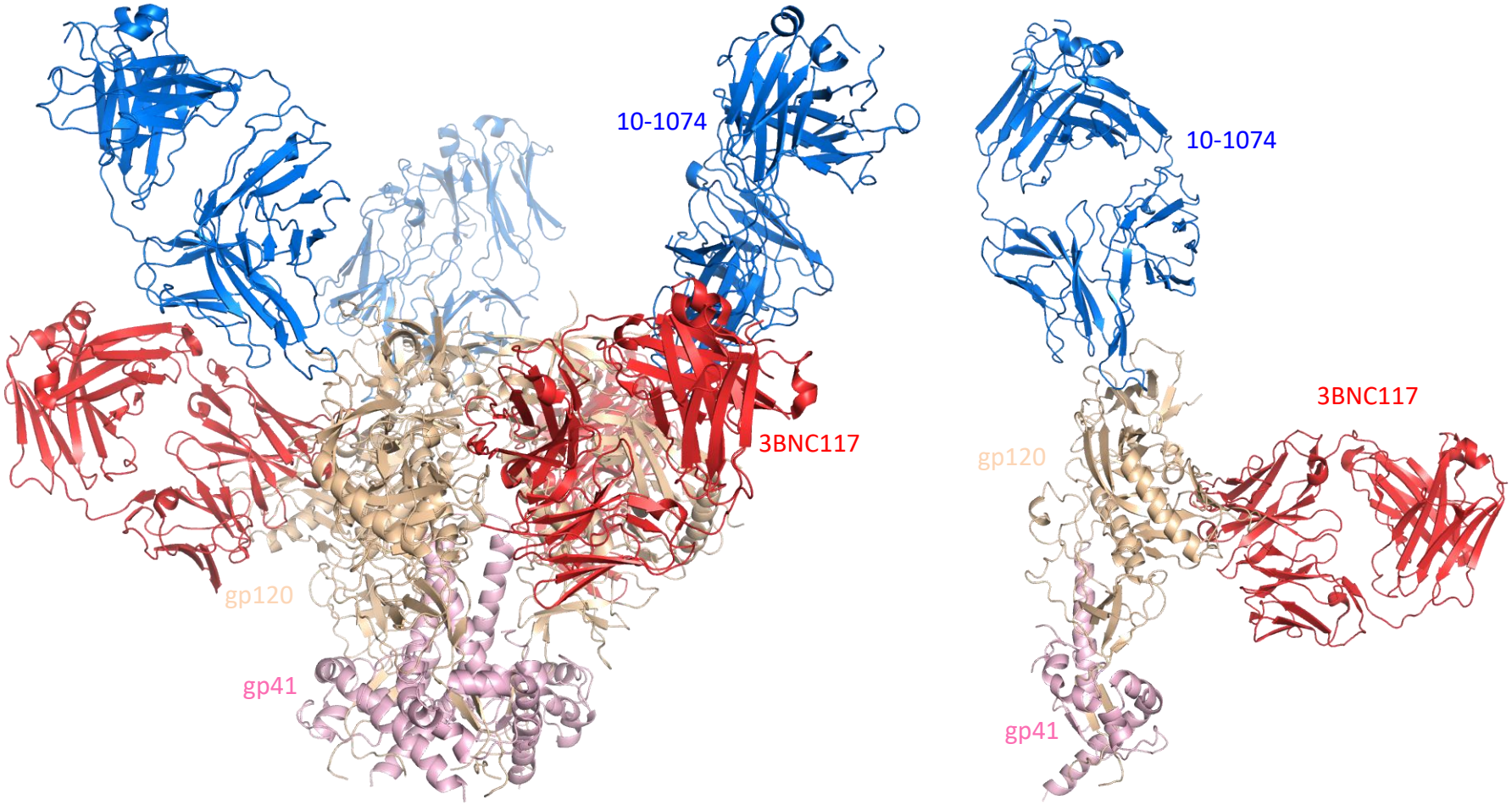
- Passive Protection
- Post-exposure prophylaxis
- Chronic Therapy
- Eradication

Klein et al *Nature* 2012; Horwitz et al *PNAS* 2013; Nishimura et al *Nature* 2013; Barouch et al *Nature* 2013; Nishimura et al *JEM* 2014
Halper-Stormberg et al *Cell* 2014
Bournazos et al *Cell* 2014; Caskey et al *Nature* 2015; Gautam *Nature* 2016

Antibodies Differ from Standard ART in their Potential to Directly Eliminate Virus and HIV-infected Cells and to Create Immune Activating Ab-Ag Complexes



3BNC117 and 10-1074 Target Independent Epitopes



Gristick et al NSMB 2016

What Roles Might Antibodies have in HIV-1 Prevention or Therapy?

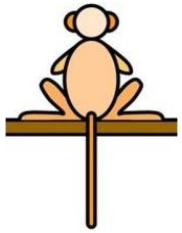
- Passive Protection
- Post-exposure prophylaxis
- Chronic Therapy
- Eradication

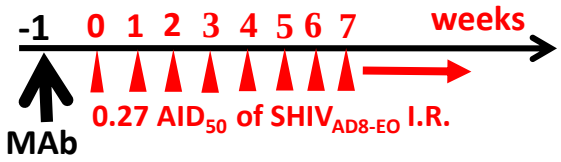
Klein et al *Nature* 2012; Horwitz et al *PNAS* 2013; Nishimura et al *Nature* 2013; Barouch et al *Nature* 2013; Nishimura et al *JEM* 2014
Halper-Stormberg et al *Cell* 2014
Bournazos et al *Cell* 2014; Caskey et al *Nature* 2015; Gautam *Nature* 2016

Study design

Indian Rhesus macaques

Mamu A01, A02, B08 & B17 neg.





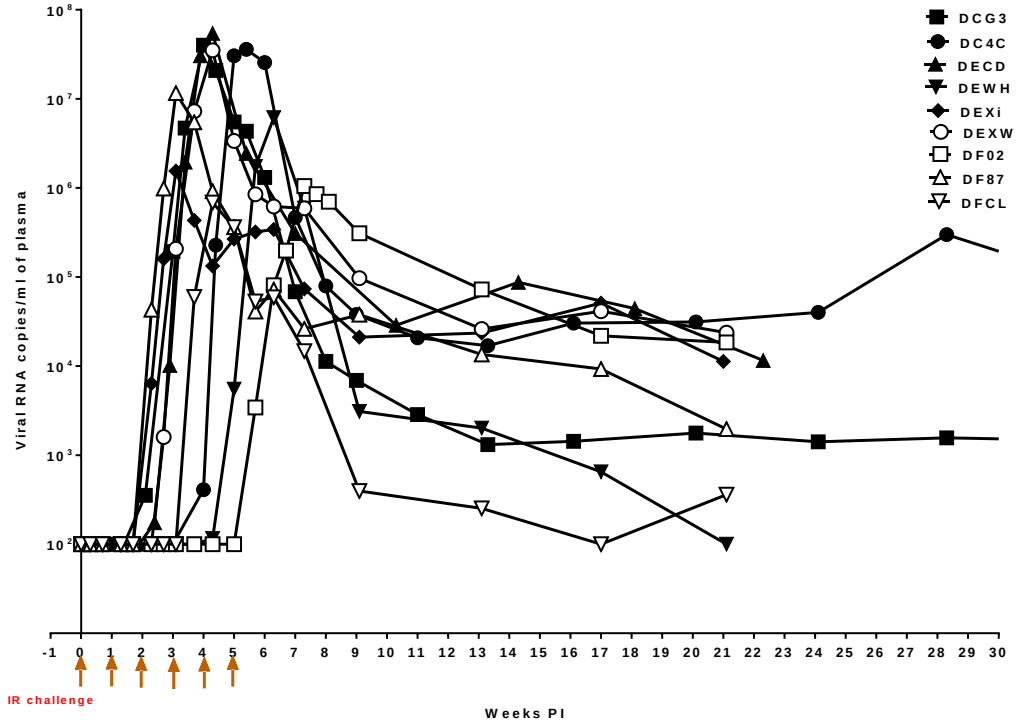
Groups	MAb	Dose/Route	N (no. of animals)
1	None	-	9
2	3BNC117 (CD4 bs)*	20 mg/kg IV	6
3	10-1074 (N332 glycan) *	20 mg/kg IV	6
4	VRC01 (CD4 bs)**	20 mg/kg IV	6
5	VRC01-LS (CD4 bs) **	20 mg/kg IV	6

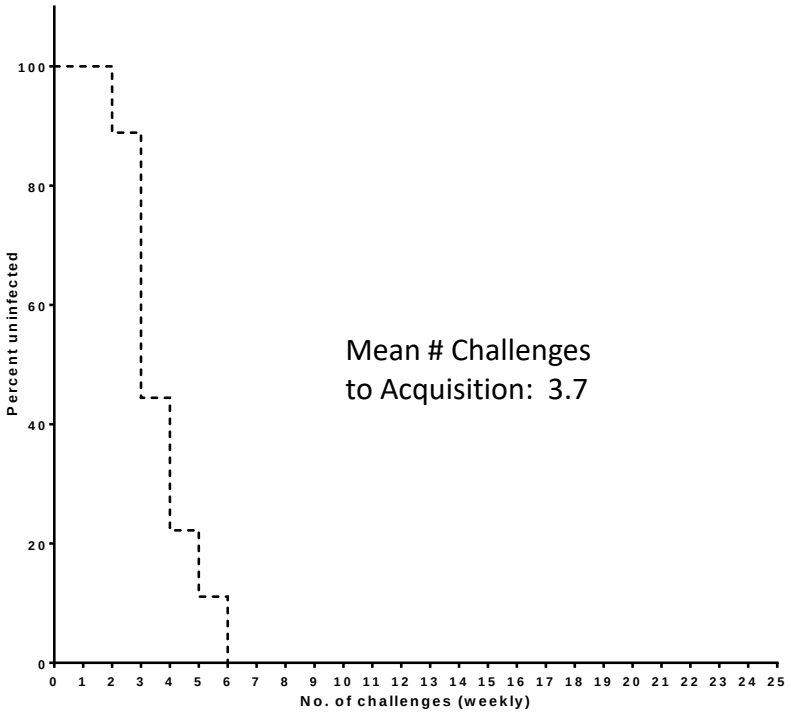
* Michel C. Nussenzweig, Rockefeller University, NY

** John R. Mascola, VRC, NIAID

Gautam et al *Nature* 2016

Six Challenges Were Required to Infect All Nine Untreated Animals





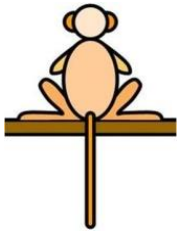
Mean # Challenges
to Acquisition: 3.7

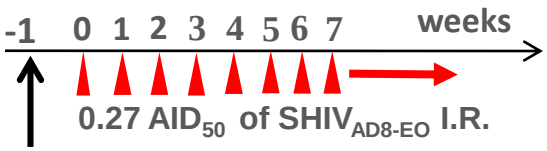
Gautam et al *Nature* 2016

3BNC117 and 10-1074 delay virus acquisition in non-human primates during repeated low-dose rectal challenges

Indian Rhesus macaques

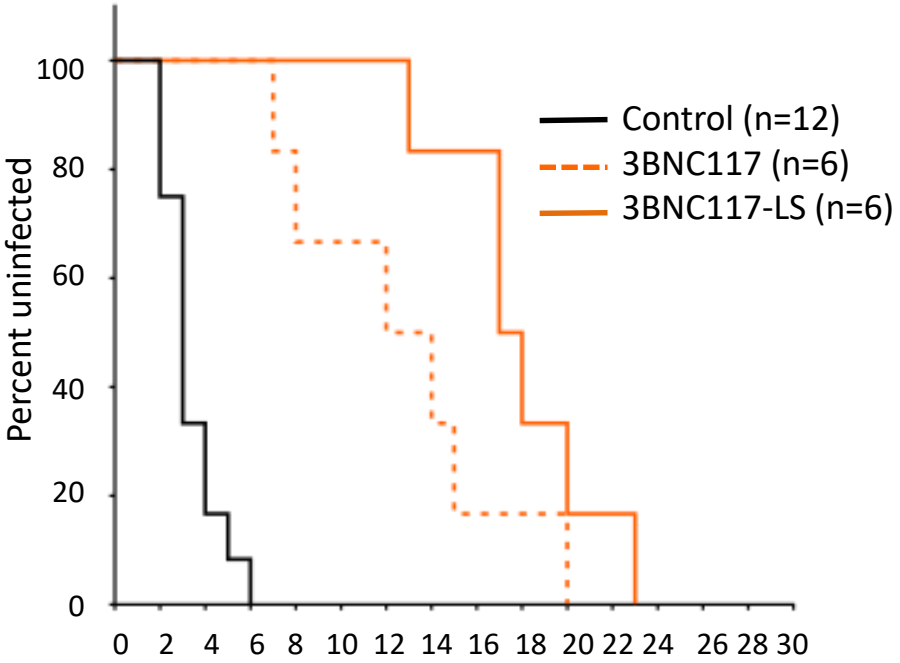
Mamu A01, A02, B08 & B17 neg.



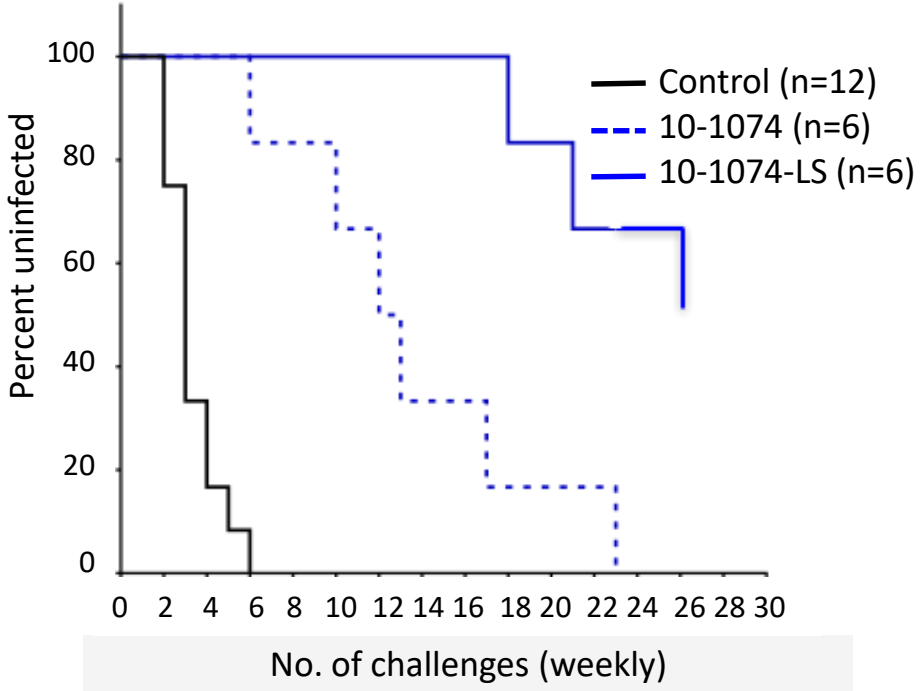


Mab
20 mg/kg, iv

3BNC117-LS



10-1074-LS



Summary

A single injection with native broadly neutralizing antibody protects macaques from infection with SHIV for up to 23 weeks

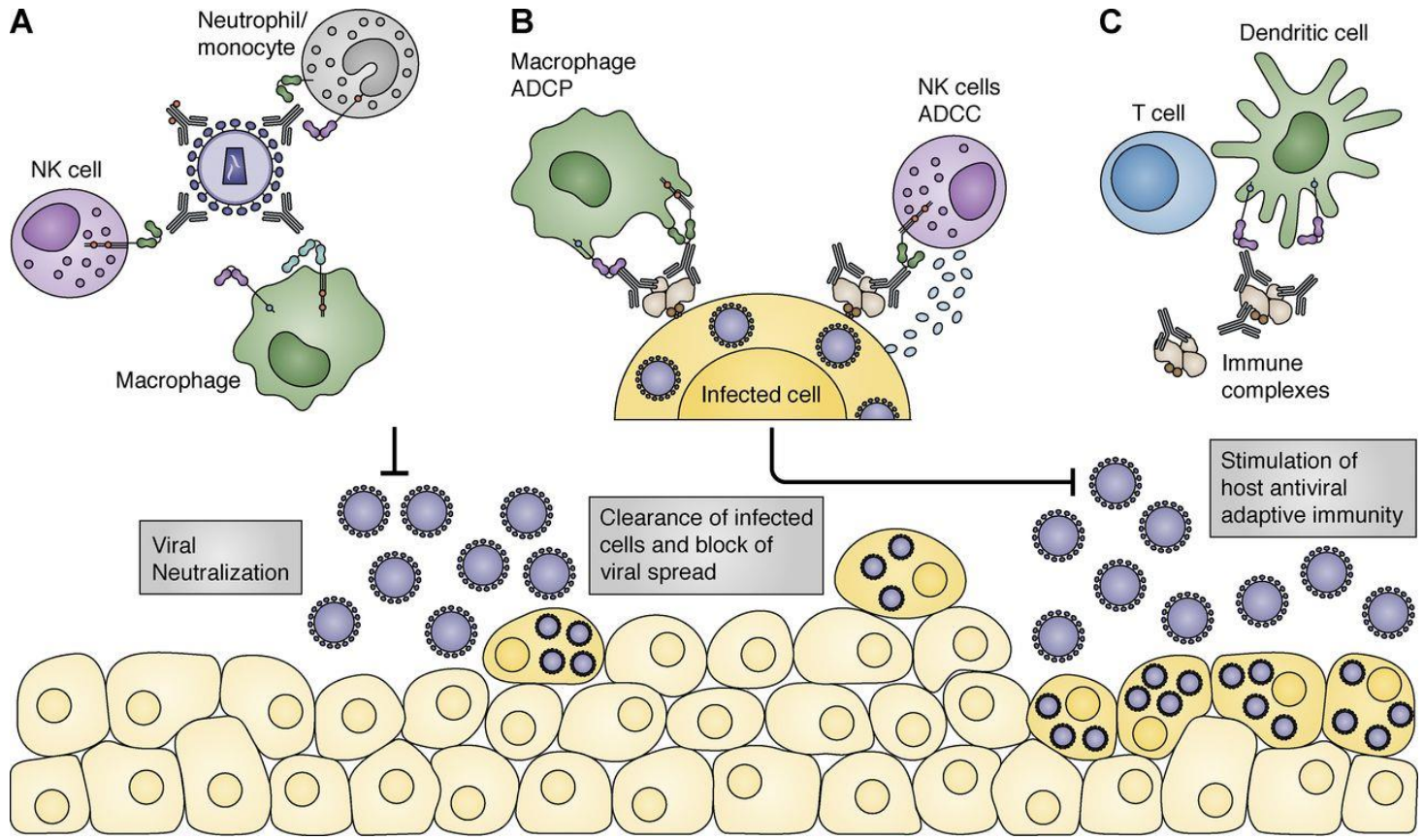
The protective effect is directly related to antibody potency and half-life and is prolonged by mutations that alter FcRn affinity

Protection is achieved at low antibody concentrations ≅ IC_{80}

Human Trials Currently Underway

Gautam et al *Nature* 2016

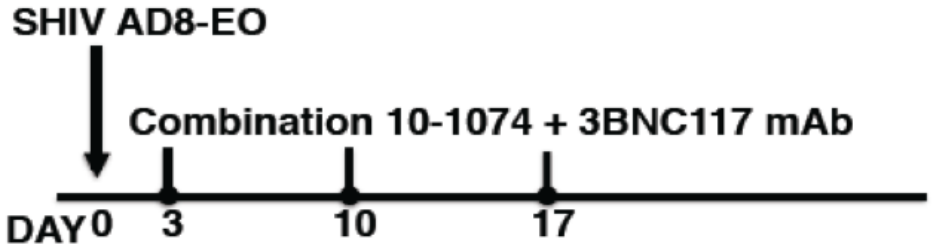
Antibodies Differ from Standard ART in their Potential to Directly Eliminate Virus and HIV-infected Cells and to Create Immune Activating Ab-Ag Complexes



EXPERIMENTAL PLAN

- **Inoculation of rhesus macaques with 1000 $TCID_{50}$ SHIVAD8 by the intrarectal or intravenous route. (Equivalent to 27 AID_{50})**

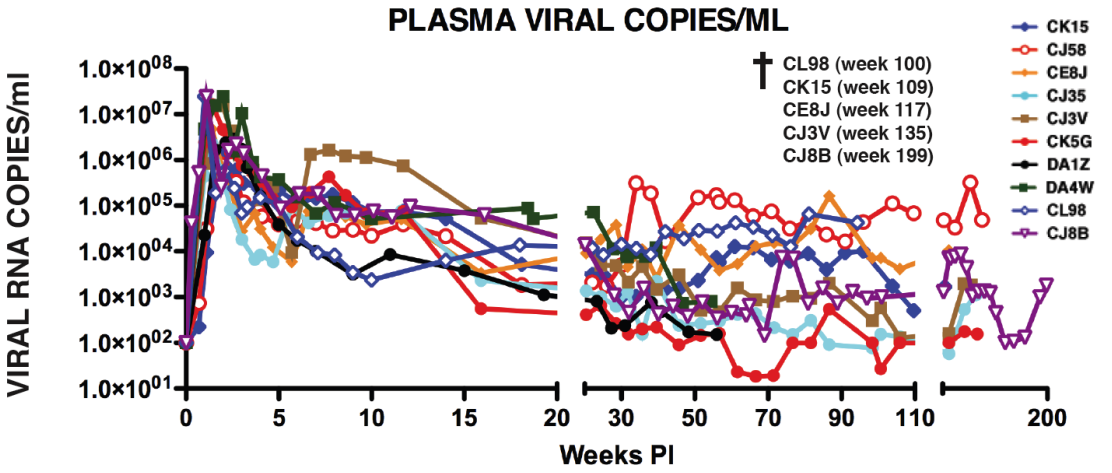
- **Administer a <u>single</u> two week course (3 weekly injections) of combination bNAb treatment (10-1074 mAb + 3BNC117mAb [10mg/kg each]) beginning on <u>day 3</u> post inoculation.**

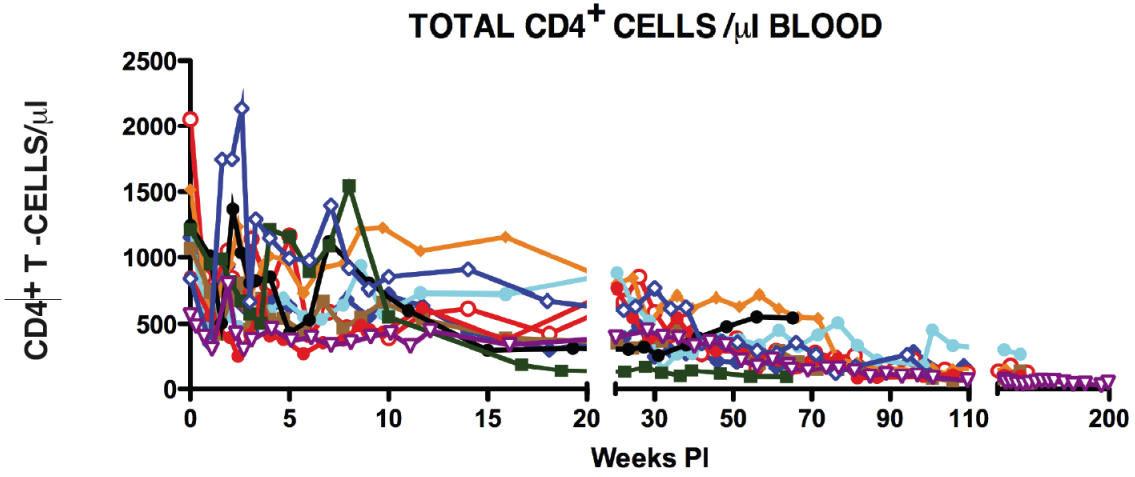


- **Monitor viral loads and $CD4^+$ T cell cell levels.**

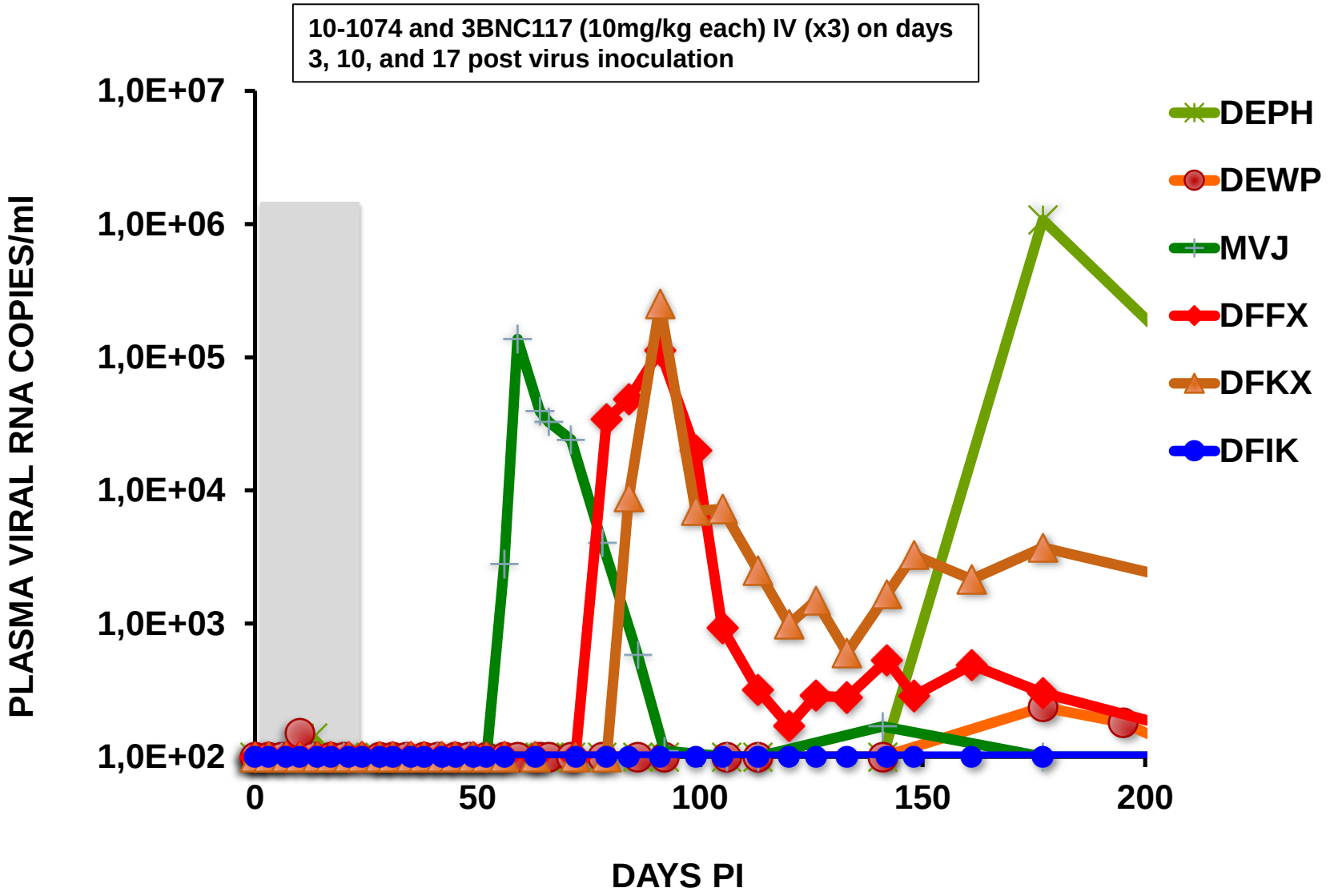
Nishimura et al *Nature* 2017

$SHIV_{AD8}$ INFECTED RHESUS MACAQUES MAINTAIN PLASMA VIREMIA AND EXPERIENCE AN IRREVERSIBLE DECLINE OF $CD4^+$ T CELLS

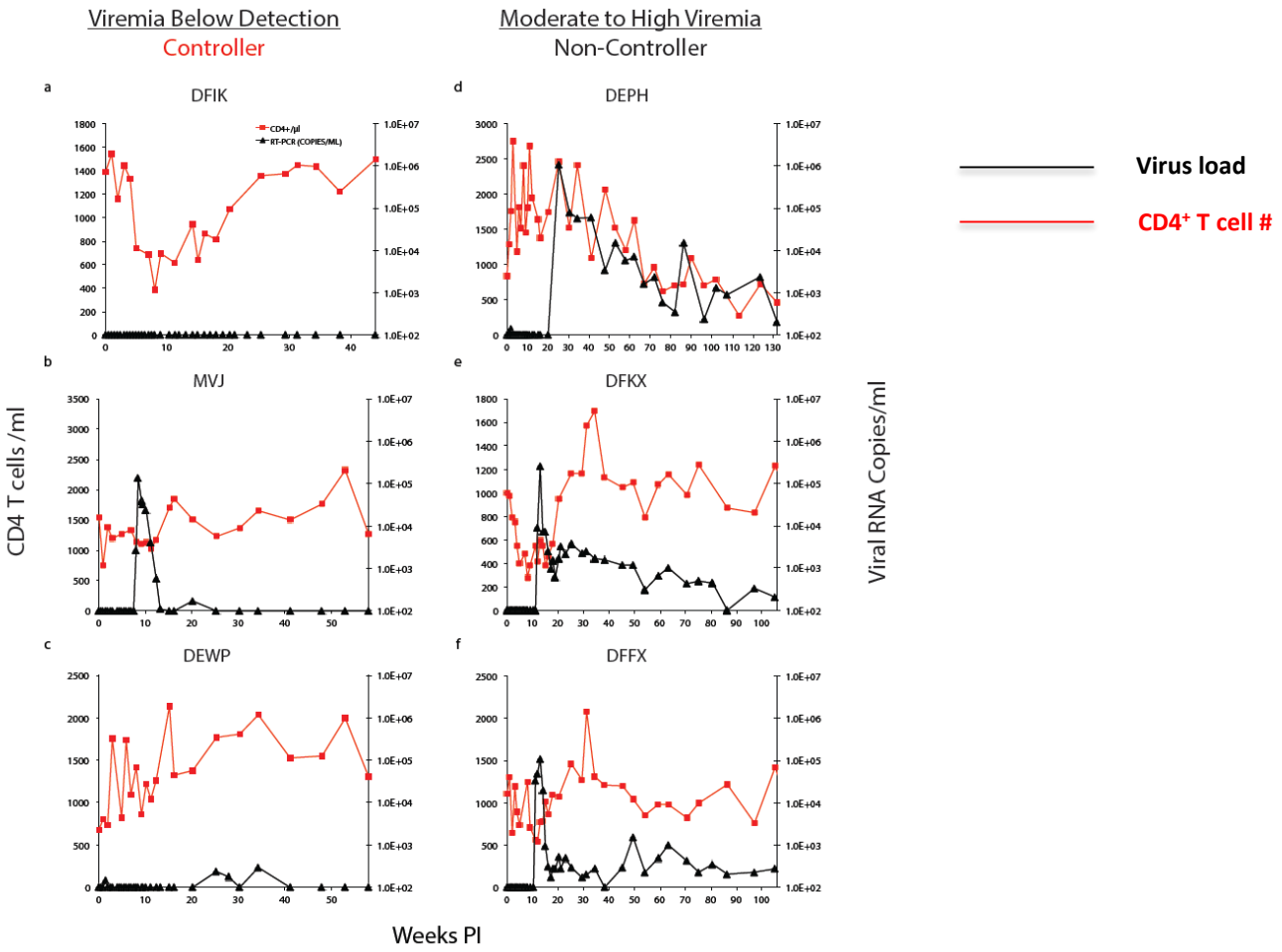




Virus Rebound Occurs 56 to 177 Days Post IR Challenge in bNAb Treated Monkeys

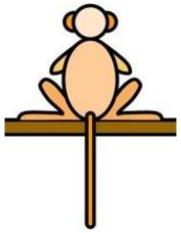


THree PATTERNS OF POST-REBOUND VIREMIA WERE OBSERVED



Indian Rhesus macaques

Mamu A01, A02, B08 & B17 neg.

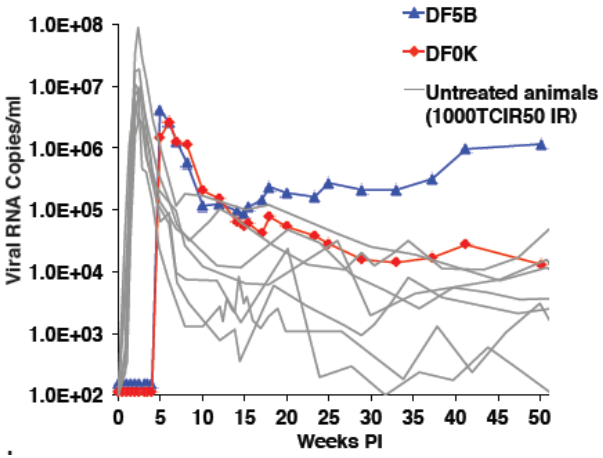


cART

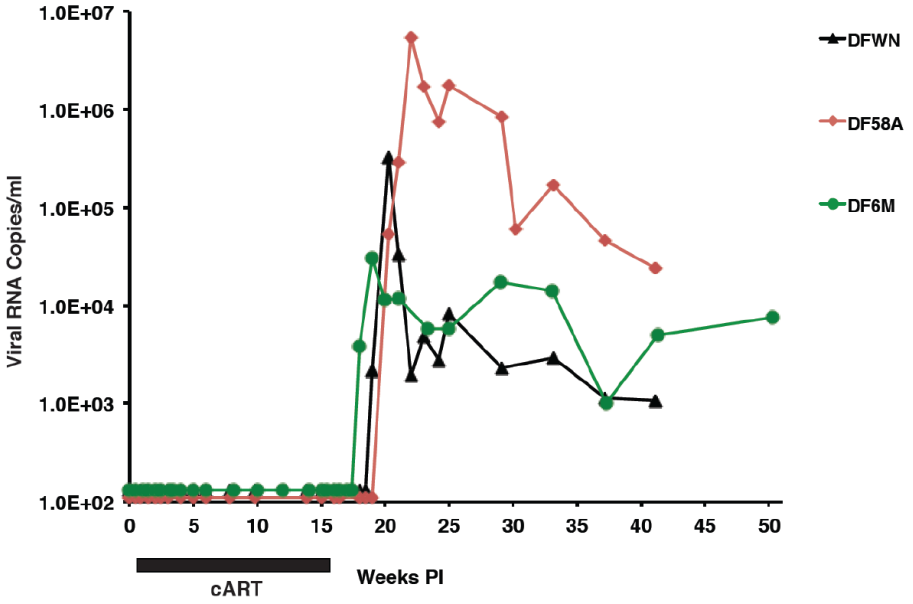
weeks

$SHIV_{AD8-EO}$ 1000 $TCID_{50}$ I.V.

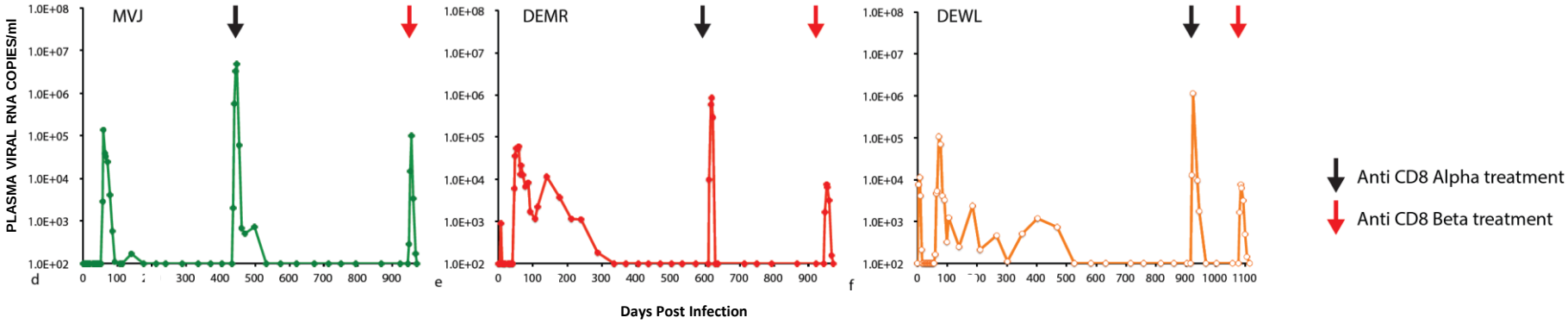
14 days of cART

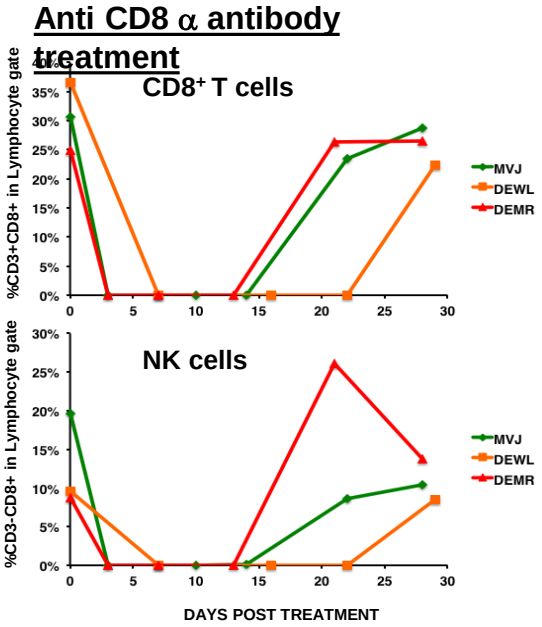


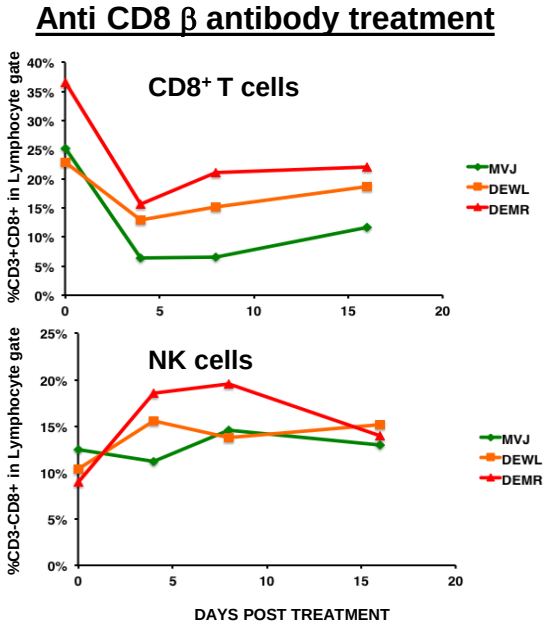
105 days of cART



USE OF ANTI-CD8β TO DEPLETE CD8⁺ T CELLS IN SHVAD8 INFECTED AND bNAb TREATED MONKEYS



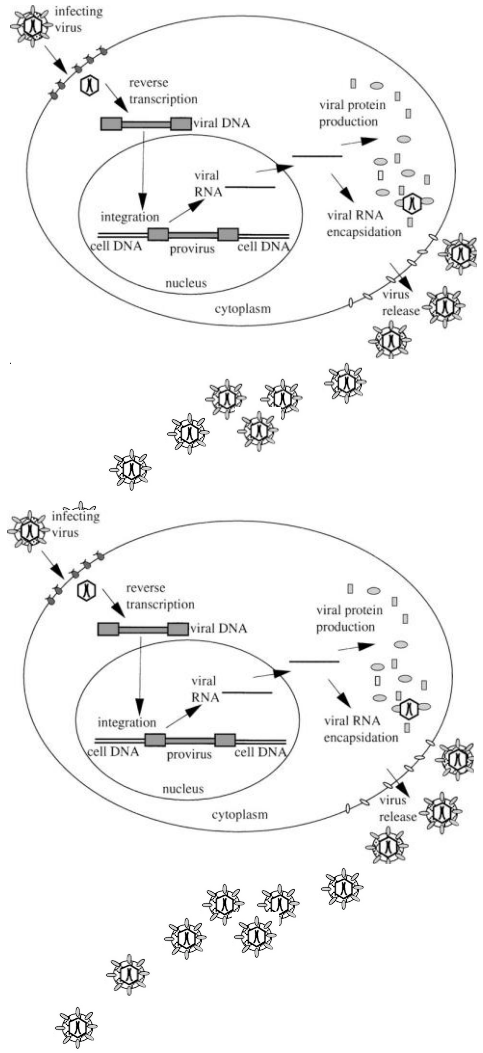




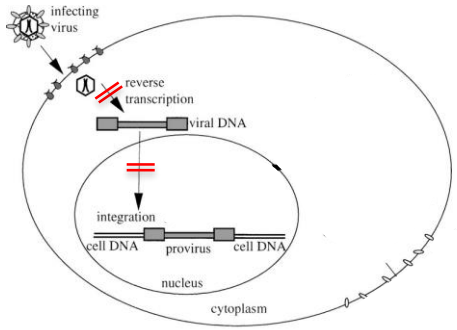
Nishimura et al *Nature* 2017

DIFFERENCES IN cART and bNAb CONTROL OF VIRUS REPLICATION

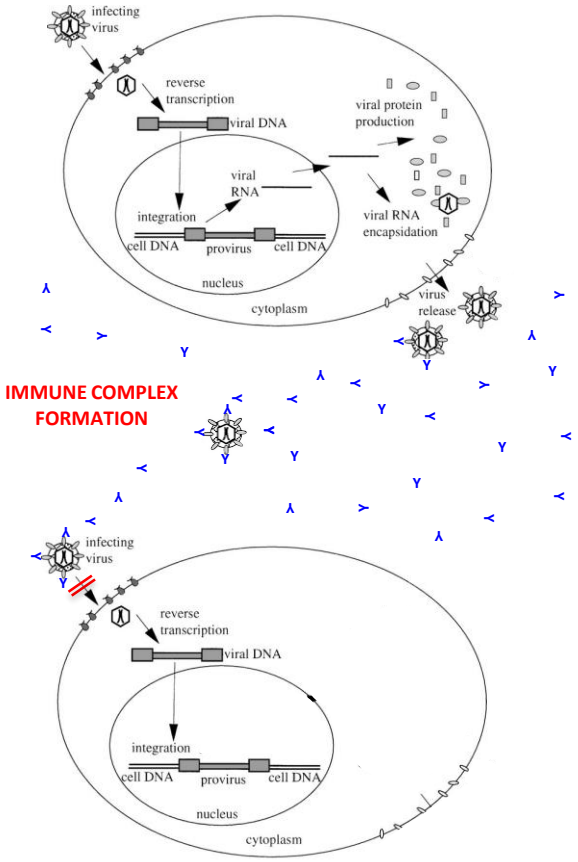
NO TREATMENT



cART THERAPY

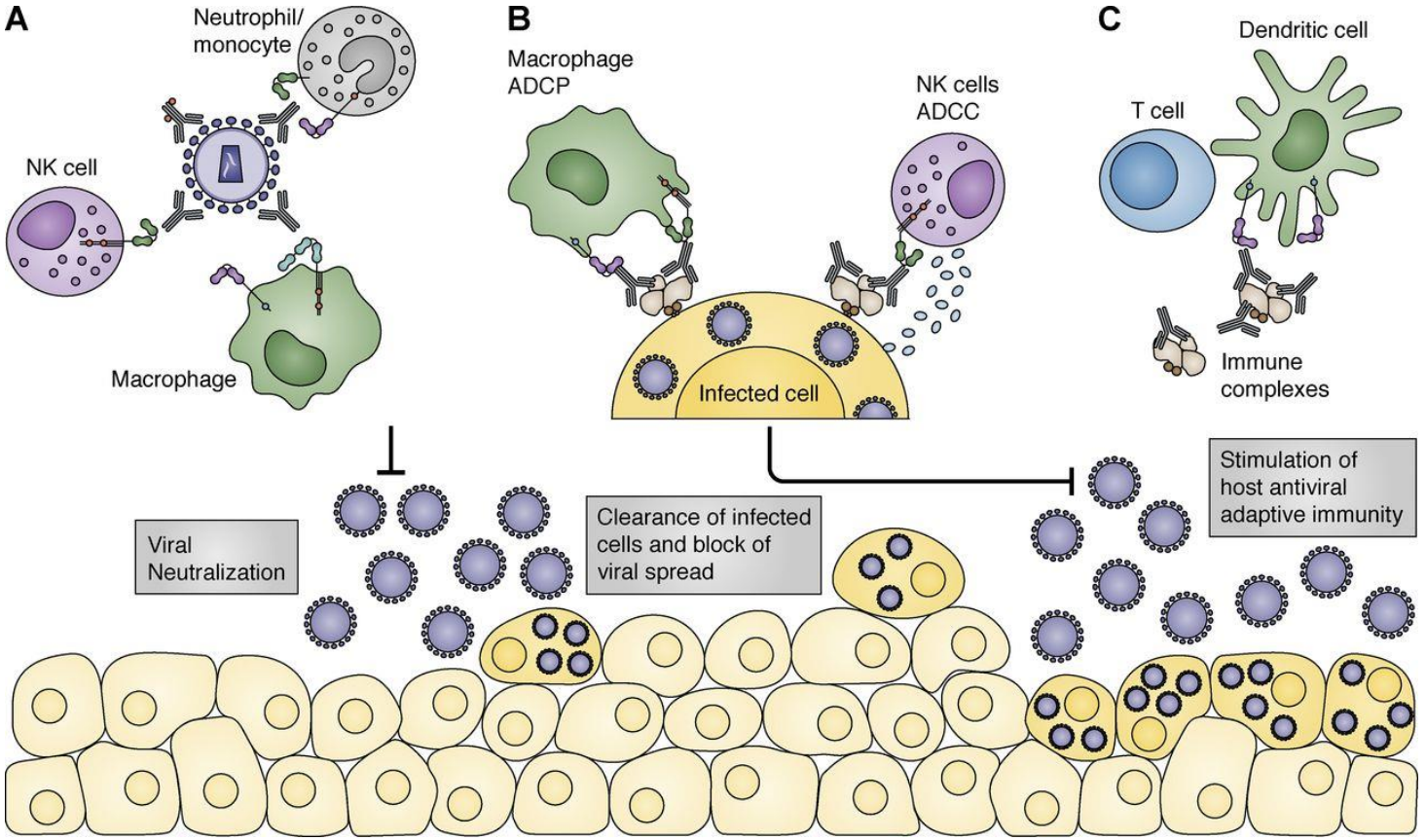


bNAb THERAPY



Nishimura et al *Nature* 2017

Antibodies Differ from Standard ART in their Potential to Directly Eliminate HIV-infected Cells



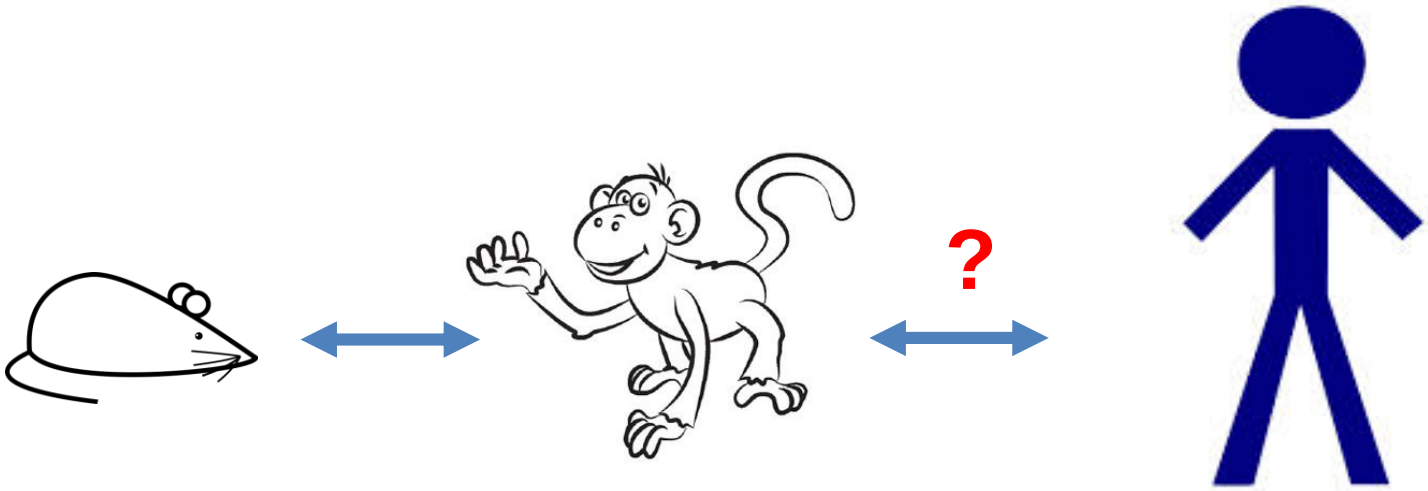
Enhance humoral responses

Schoofs et al., Science 2016

Direct clearance of infected cells

Lu et al, Science 2016

Preclinical Evaluation of bNAb-mediated Therapy



Klein *et al.*, Nature 2012; Horwitz *et al*., PNAS 2013; Shingai *et al*. Nature 2013, Barouch *et al.*, Nature 2013; Halper-Stromberg Cell 2014; Gautam *et al.*, Nature 2016

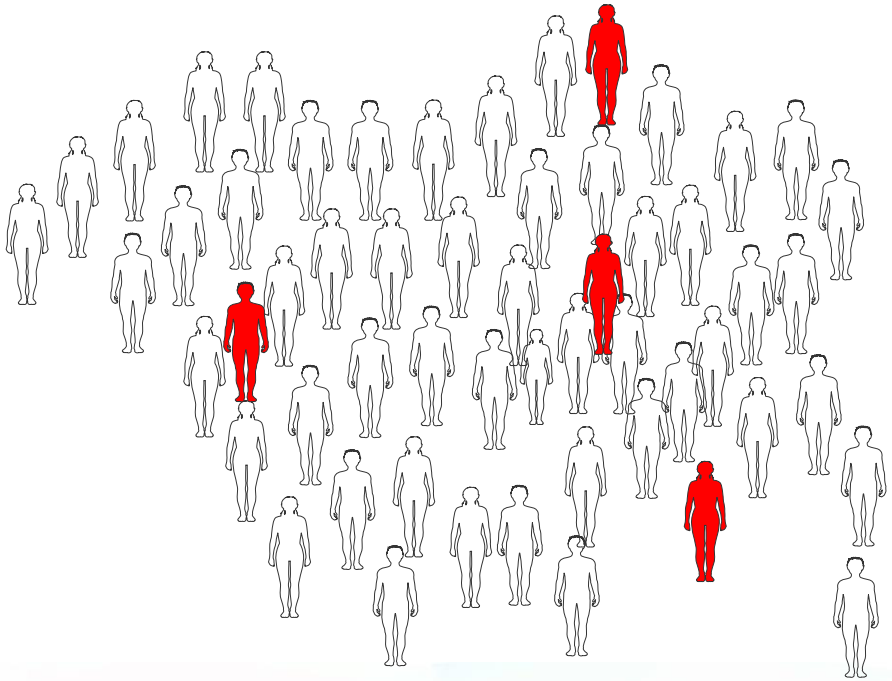
3BNC117 and 10-1074 – Clinical Development

2009	2010	2011	2012	2013
Single Cell Cloning Method		**3BNC117 cloned**	**10-1074 cloned** bNAbs suppress viremia in hu-mice	3BNC117 and 10-1074 suppress viremia and prevent infection in NHP 3BNC117 clinical product

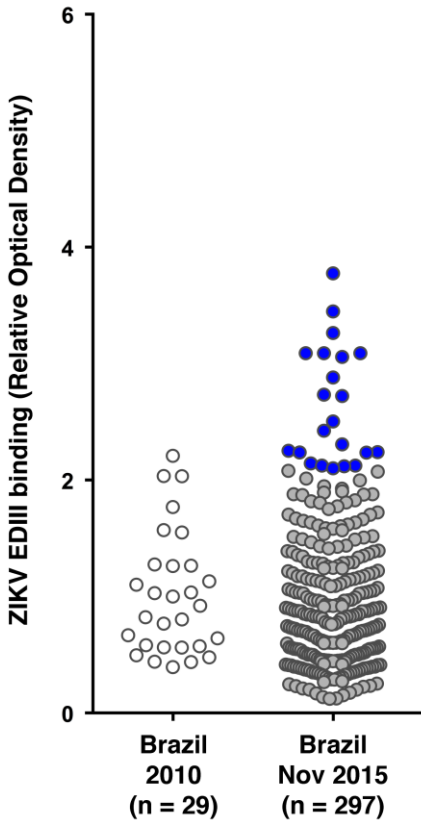
2014	2015	2016	2017	2018
3BNC117 first-in-human study opens 10-1074 clinical product	3BNC117 is safe and suppresses viremia **10-1074 first-in-human study opens.**	3BNC117 delays viral rebound **3BNC117 plus 10-1074 combination studies opens.**	10-1074 is safe and suppresses viremia 3BNC117-LS clinical product. **3BNC117-LS first-in-human study opens.**	10-1074-LS clinical product **10-1074-LS first-in-human study opens.** **3BNC117-LS plus 10-1074-LS combo phase 1 studies open.**

2019	2020	2021	2022
Phase 2b prevention study of 3BNC117-LS plus 10-1074-LS in high-risk individuals opens.		Phase 2b prevention study results are available.	

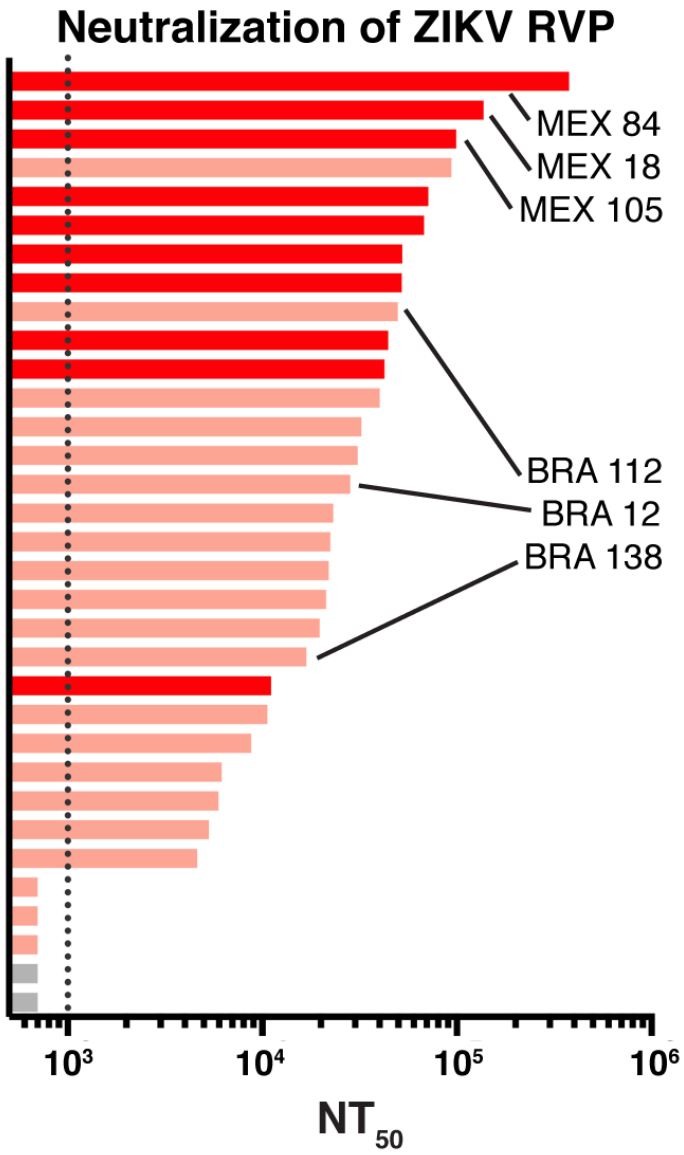
Origin of Zika Virus Neutralizing Antibodies



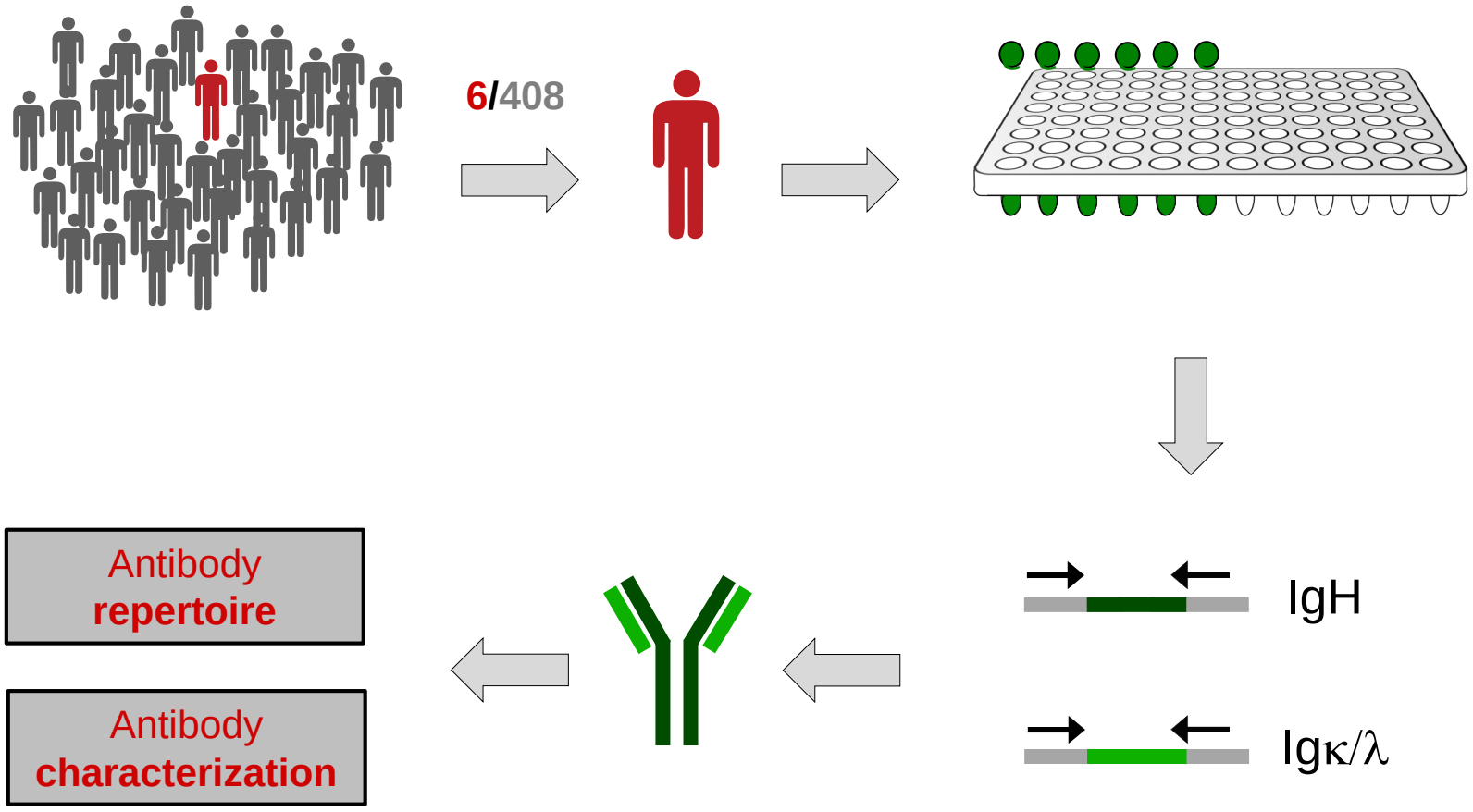
Selection of Donors: Binding to ZIKV EDIII



Selection of Donors: Neutralization

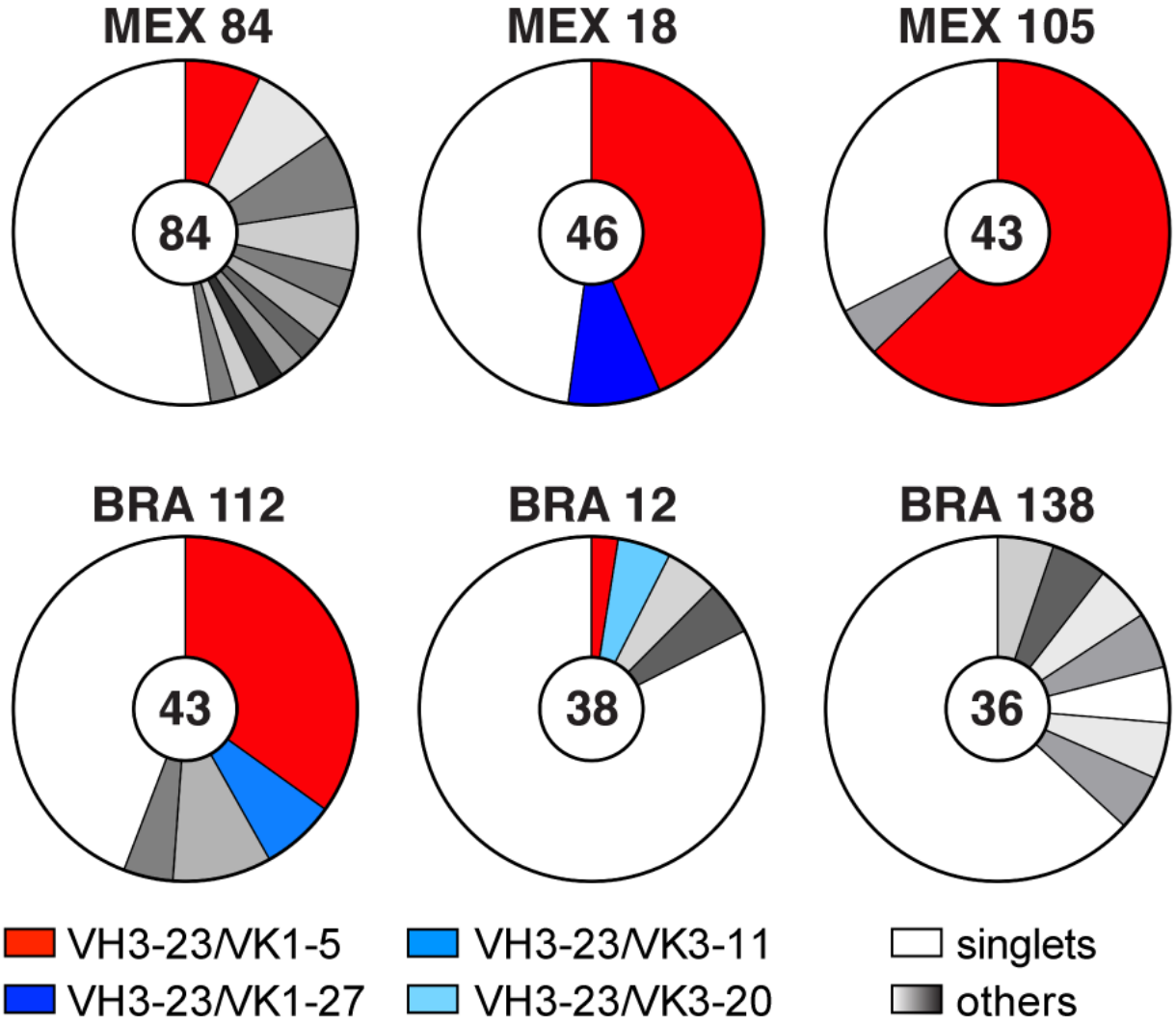


Isolation of ZIKV-specific Antibodies

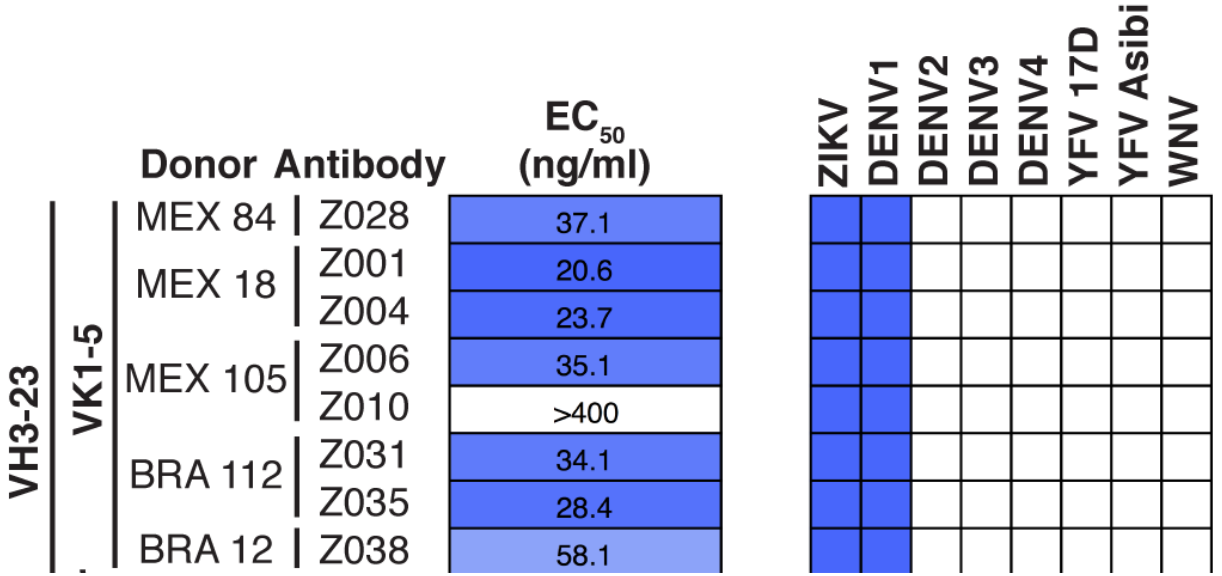


Wardemann et al., Science 2003; Scheid et al., Nature 2009, Scheid et al., Science 2011

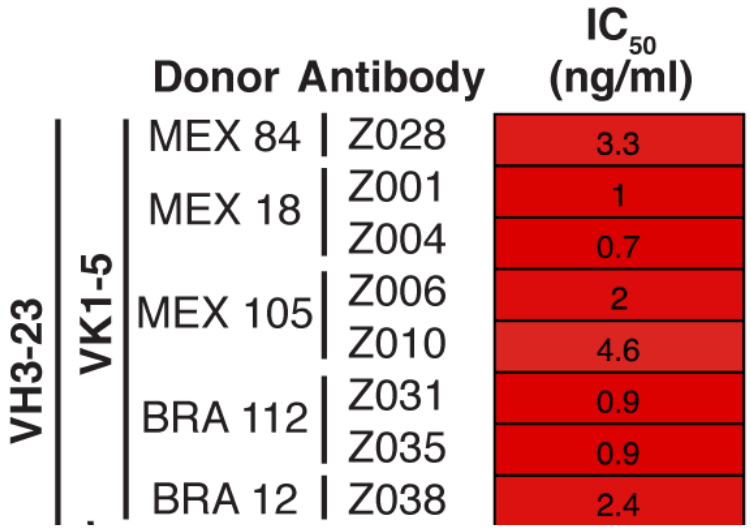
Discovery of Recurrent Antibodies Against the Domain III of the ZIKV Envelope



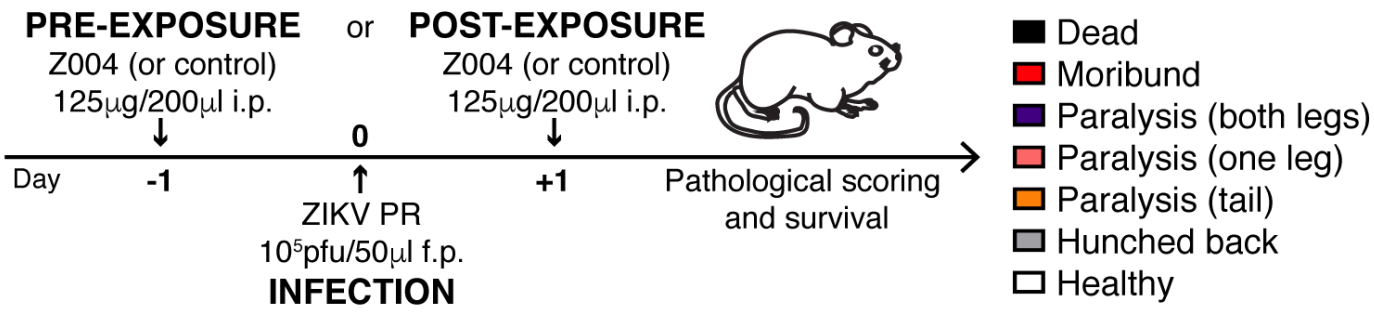
VH3-23/VK1-5 Antibodies Cross-react to DENV1

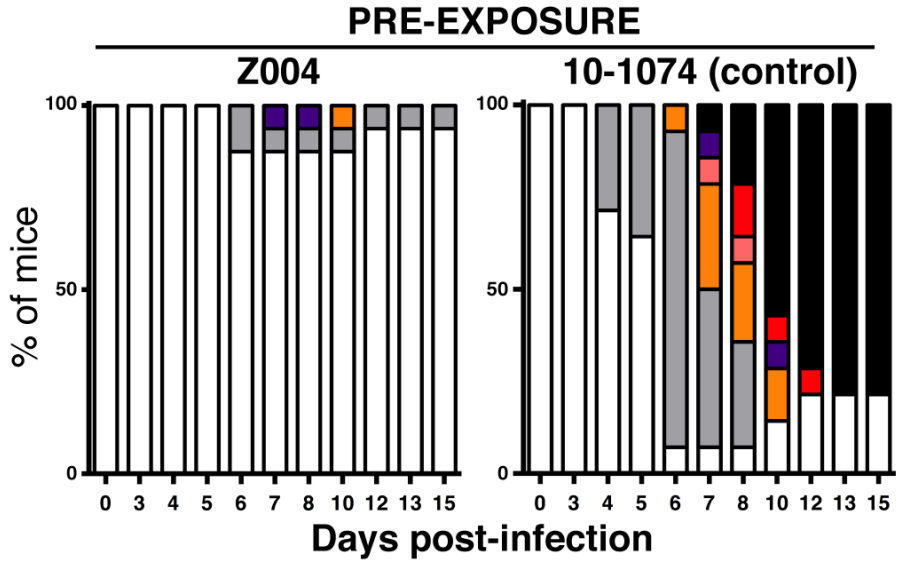


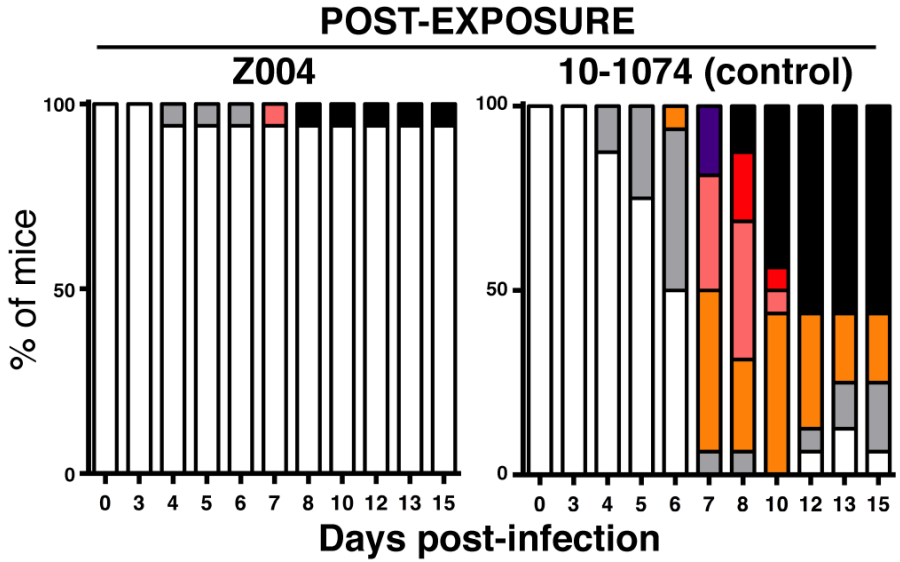
VH3-23/VK1-5 Antibodies Cross-neutralize DENV1



Z004 Neutralizes ZIKV *in vivo*



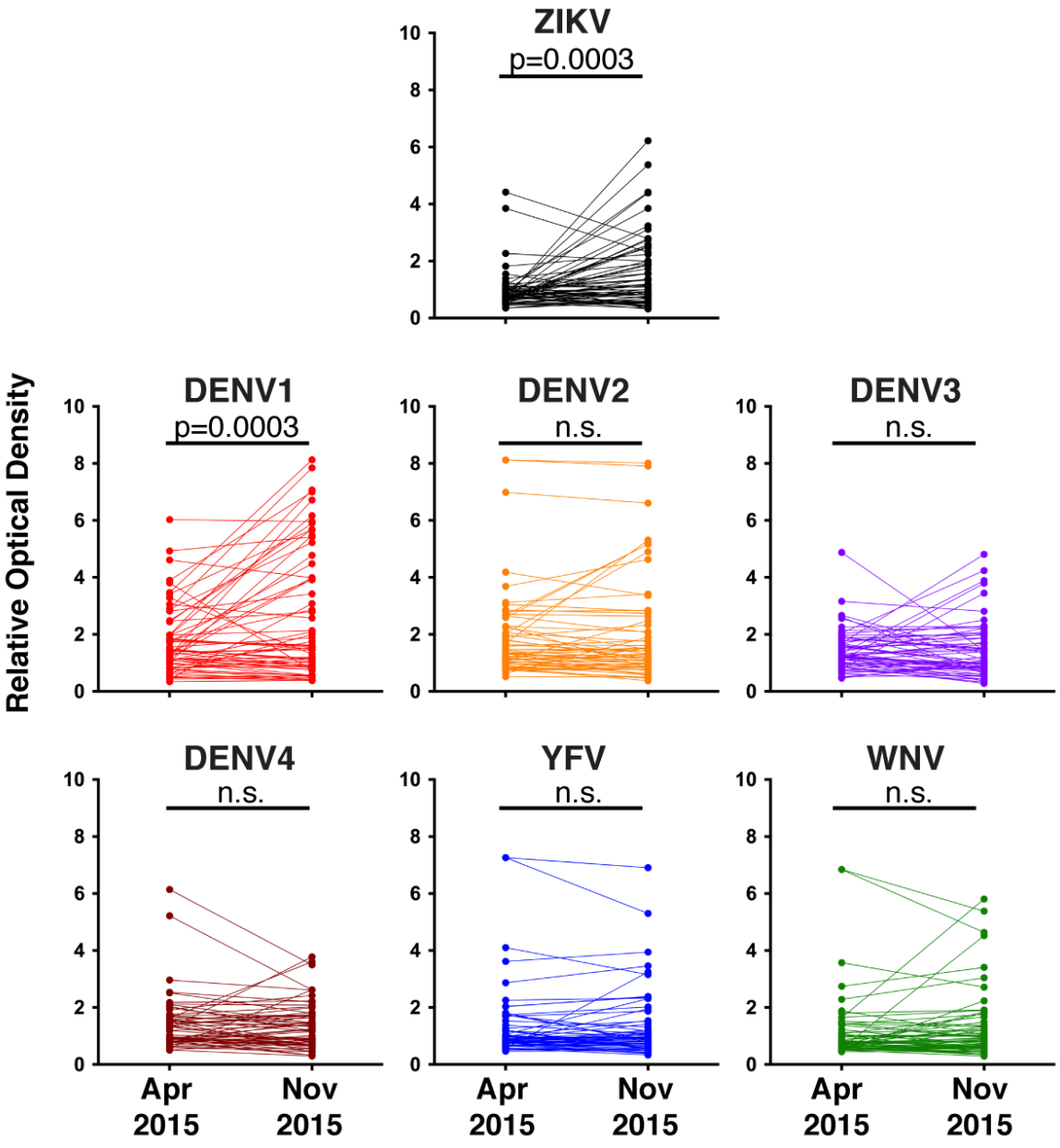




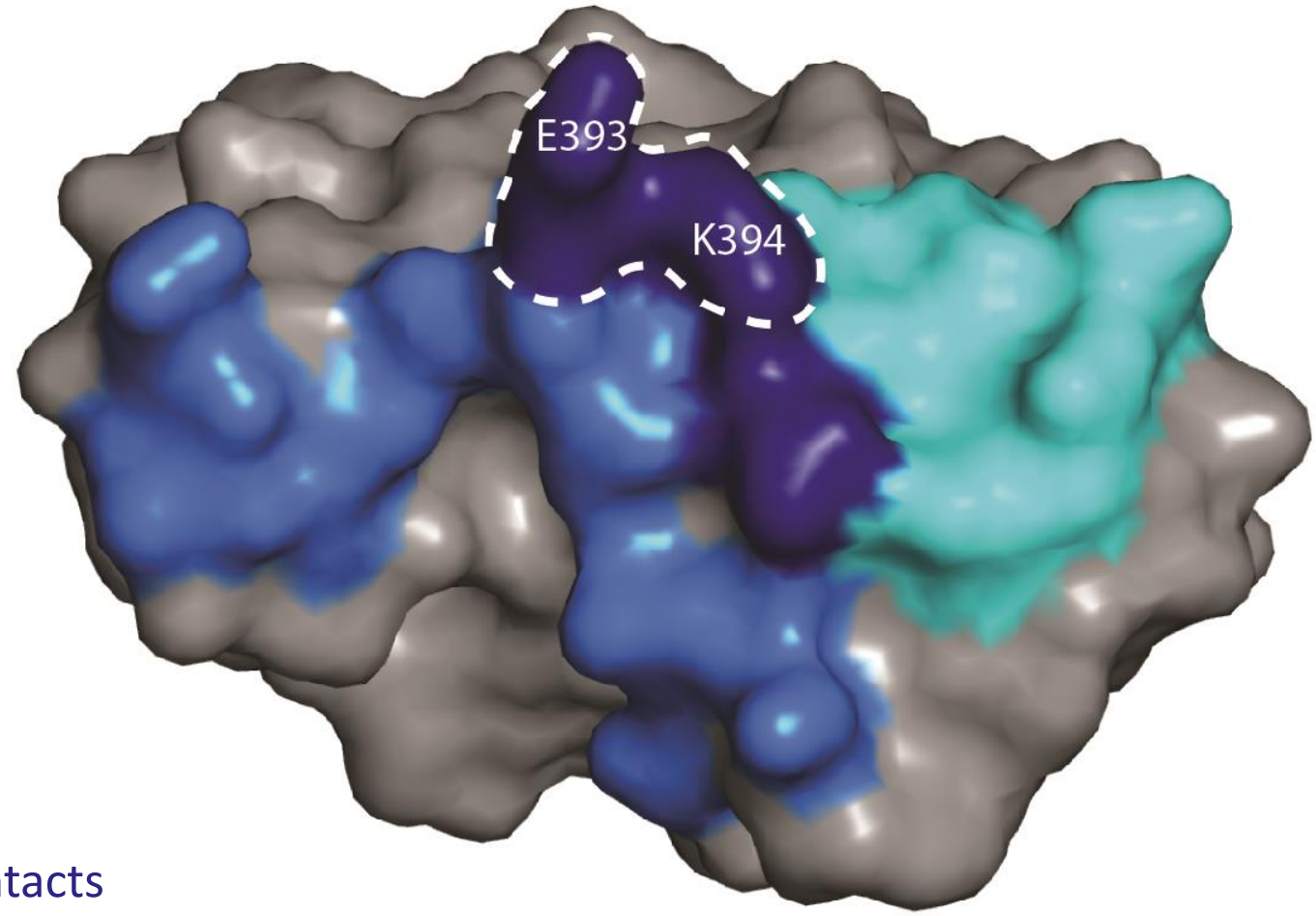
Summary

- VH3-23/VK1-5 antibodies are clonally expanded in 5/6 ZIKV high responders
- VH3-23/VK1-5 antibodies are potent ZIKV neutralizers
- They protect against ZIKV in mice
- VH3-23/VK1-5 antibodies cross-react and neutralize DENG 1
- Antibodies to this region correlate with high neut titers in the population.

History of Flavivirus exposure?



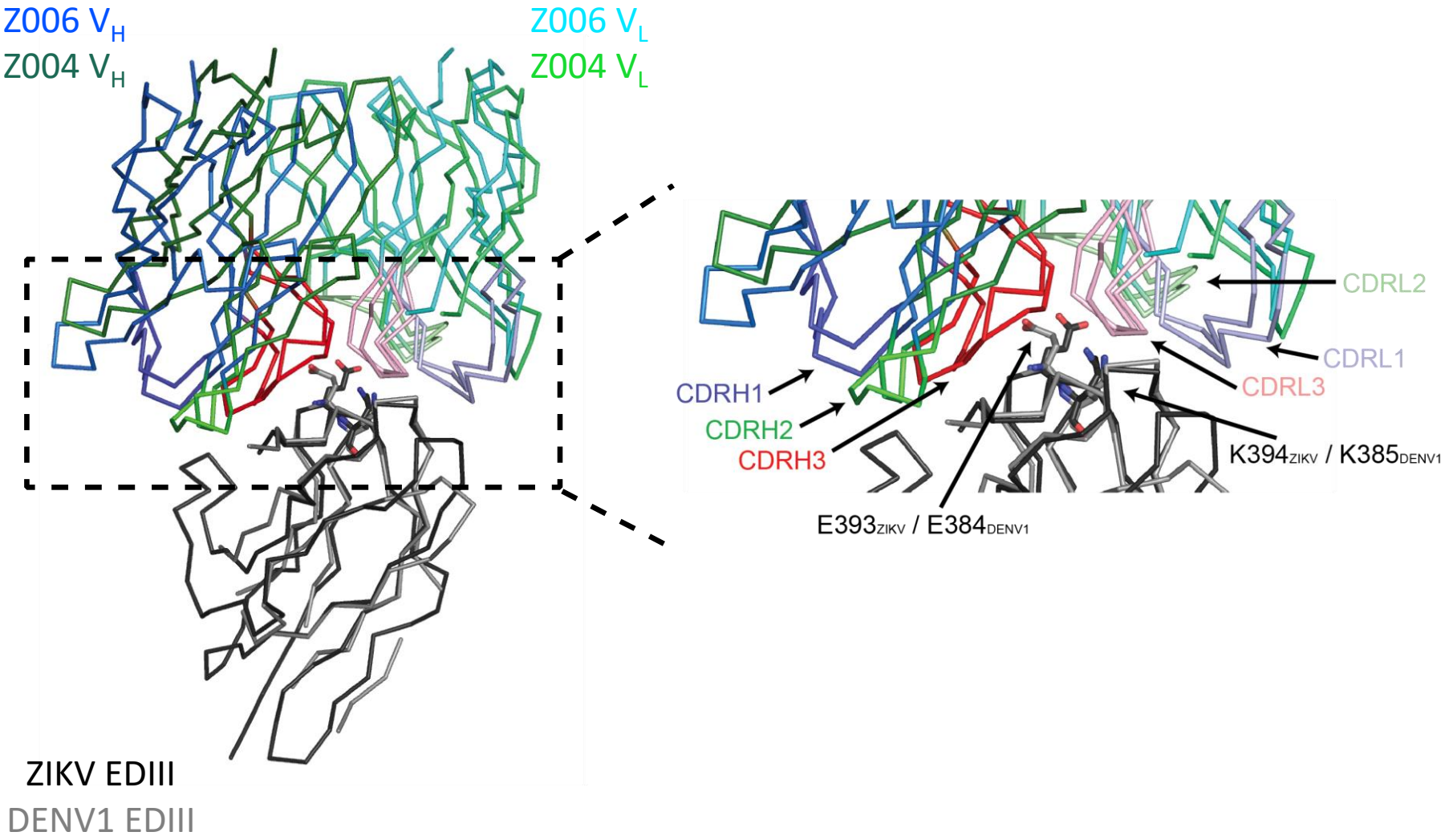
Z006 uses both HC and LC to recognize the ZIKV EDIII Epitope



HC Contacts
LC Contacts
HC & LC Contacts

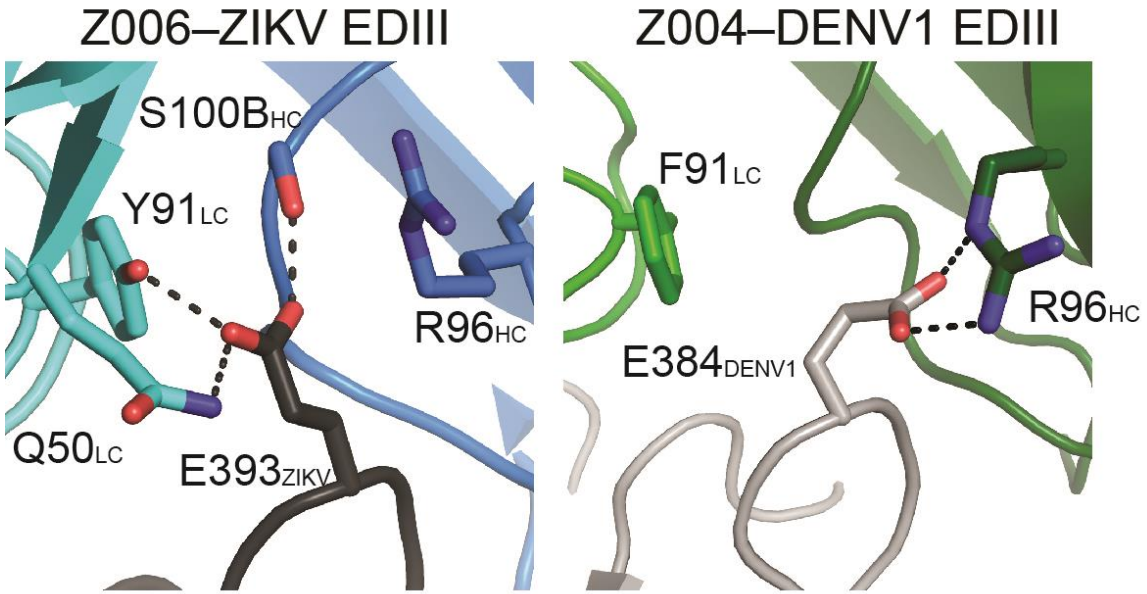
Jennifer Keeffe
Anthony West
Pamela Bjorkman

Structures of V_H3-23/V_K1-5 ZIKV and DENG1 Ab–EDIII Complexes Reveal a Shared Binding Mode

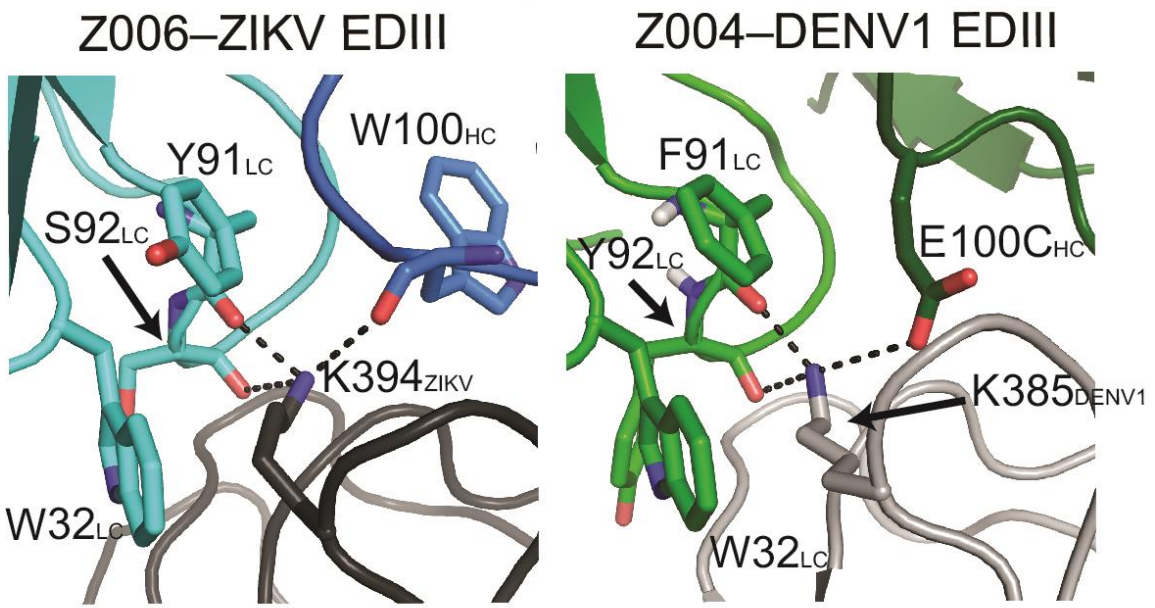


V_H3-23/V_K1-5 ZIKV Fabs contact EDIII E393-K394$_{ZIKV}$/E384-K385$_{DENV1}$

EDIII E393$_{ZIKV}$
EDIII E384$_{DENV1}$

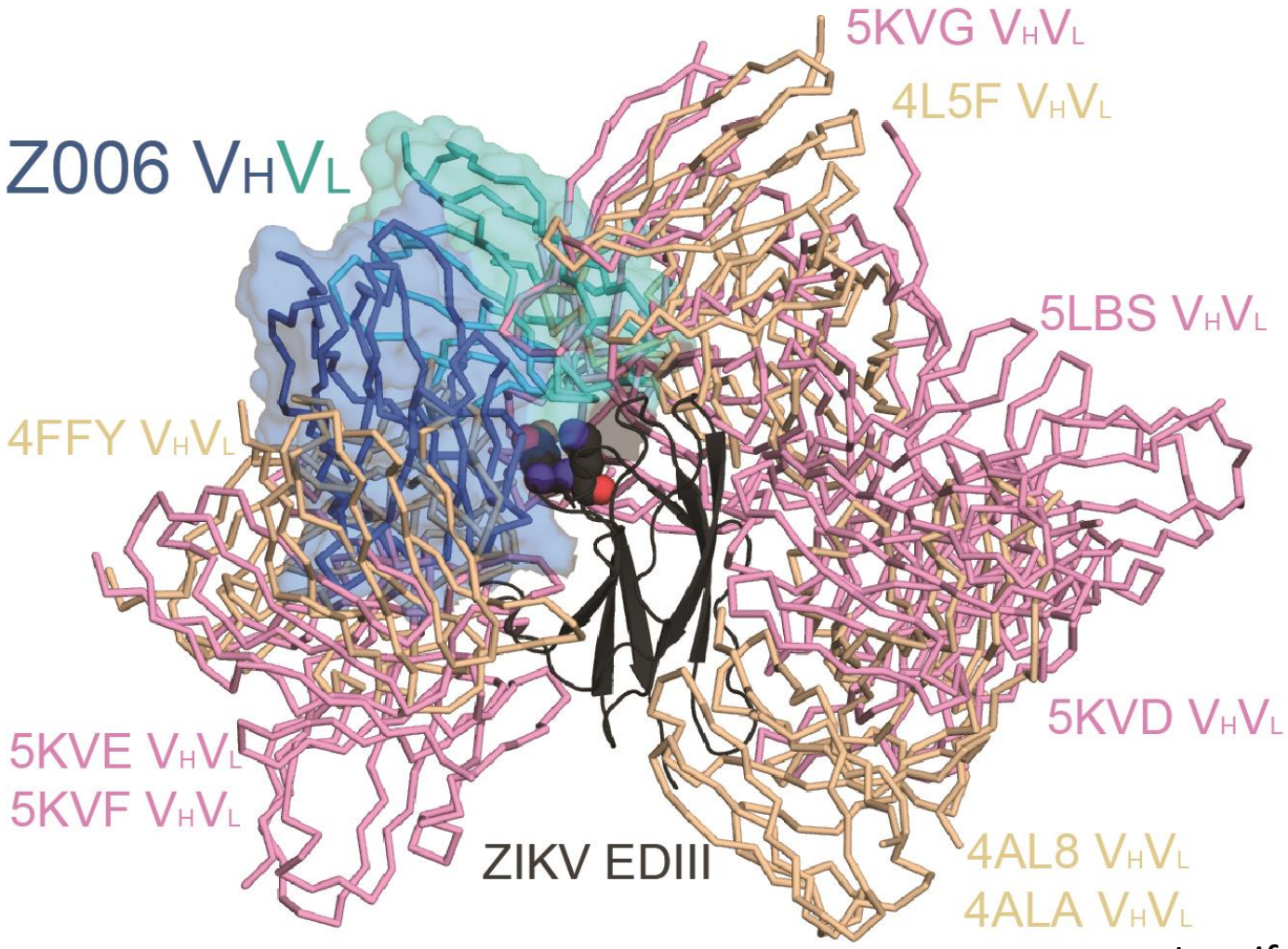


EDIII K394$_{ZIKV}$
EDIII K385$_{DENV1}$



Jennifer Keeffe
Anthony West
Pamela Bjorkman

VH3-23/VK1-5 antibodies recognize a distinct EDIII epitope

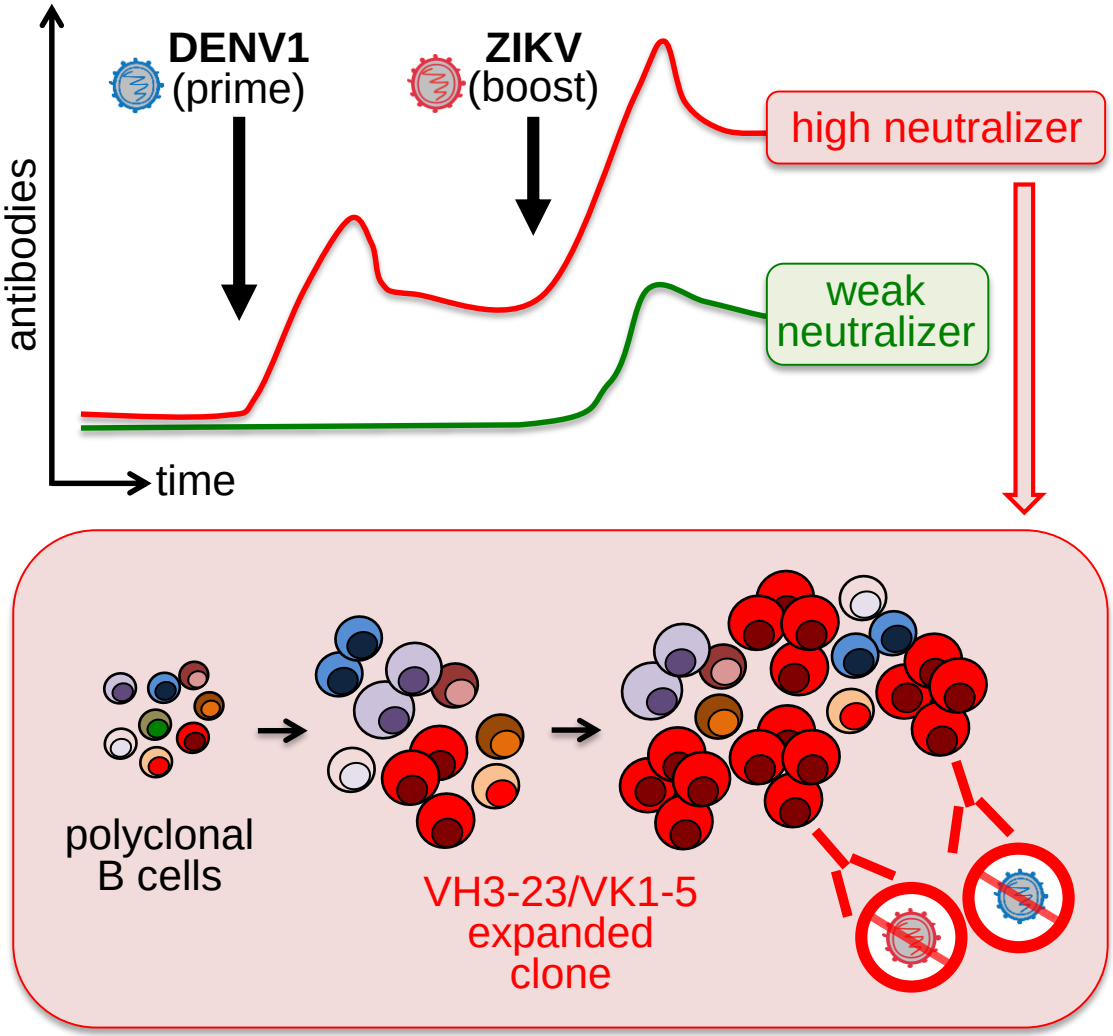


Fab–ZIKV EDIII Complexes

Fab–DENV1 EDIII Complexes

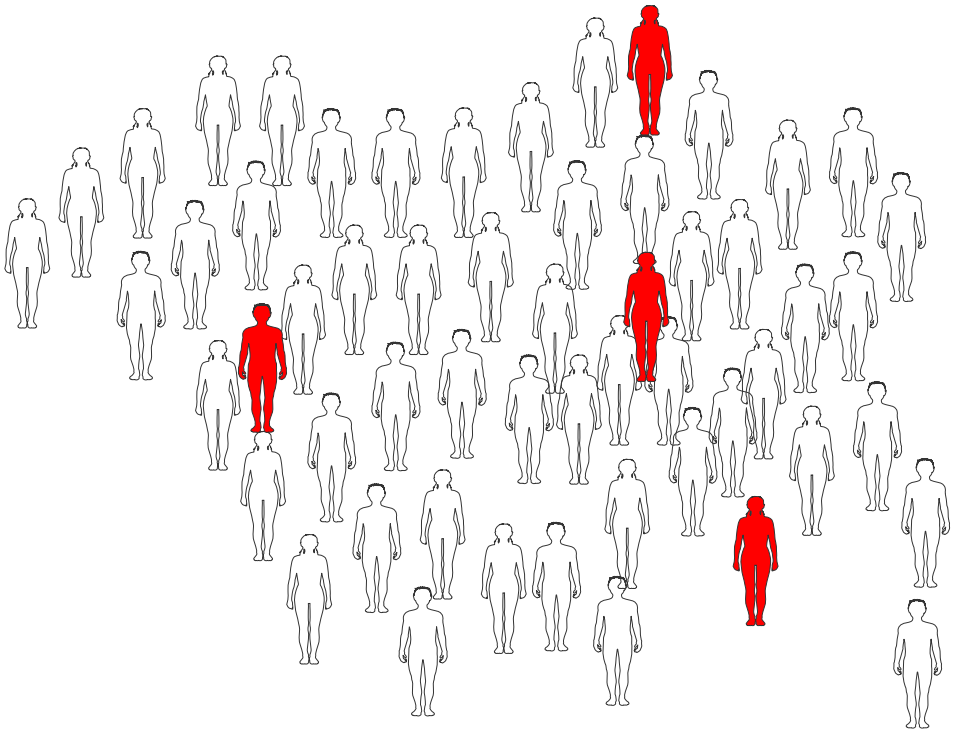
Jennifer Keeffe
Anthony West
Pamela Bjorkman

Prime-Boost Model



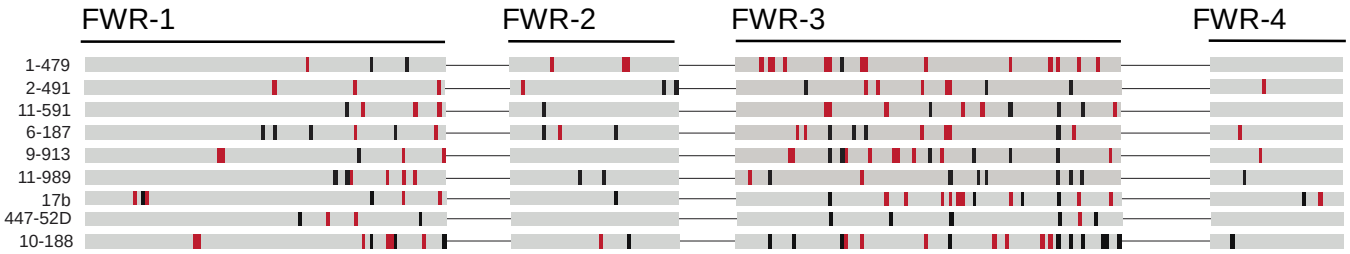
Robbiani et al *Cell* in press

5-10% of HIV+ individuals develop broadly neutralizing serum antibodies BUT only after 2-3 years and this has not been reproduced in vaccines

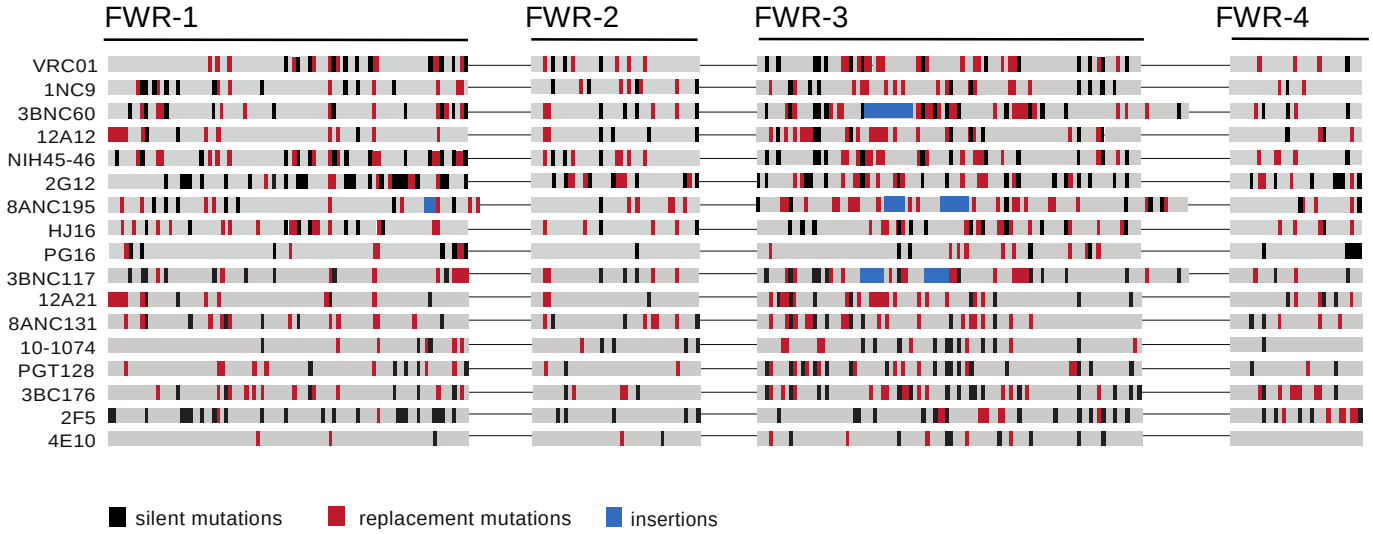


Somatic mutations in the framework of HIV-1-neutralizing Abs

Limited neutralizing activity (IgH)



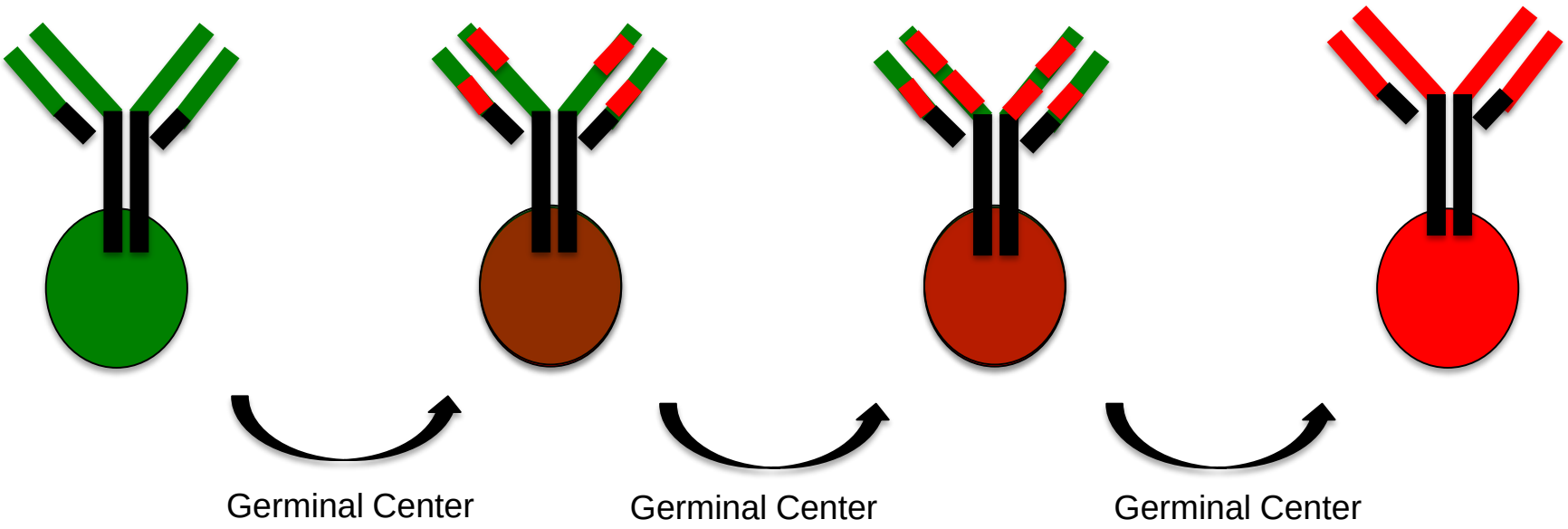
Broadly neutralizing antibodies (IgH)



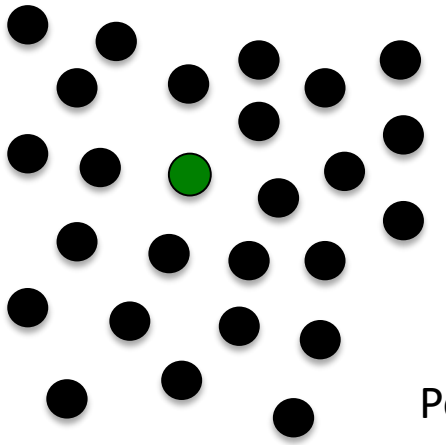
Klein et al *Cell* 2013

Germ line

Mature bNAbs

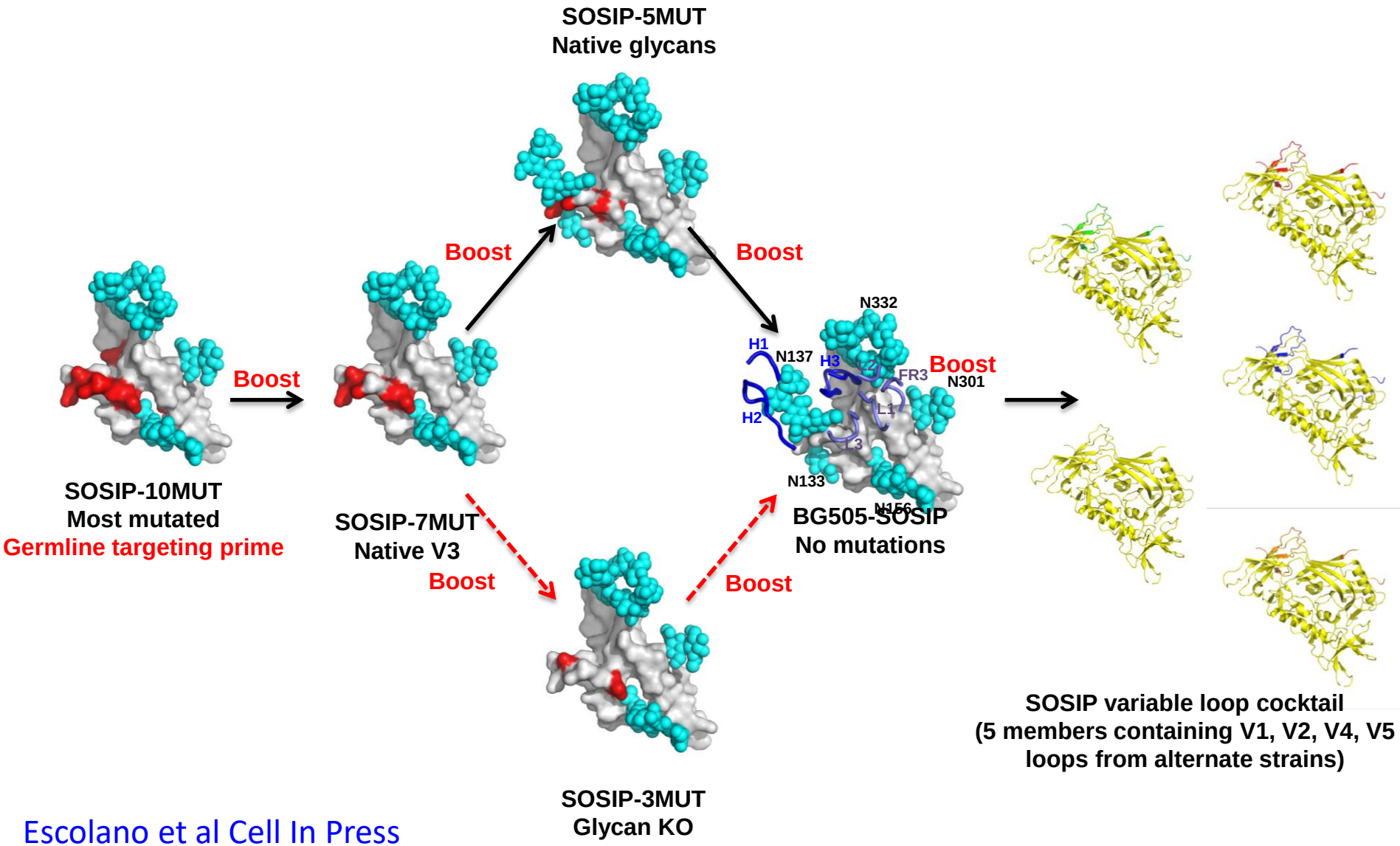


-Germline prediction
-Low precursor frequency
-Competition/Immunodominance
-High Mutation Frequency



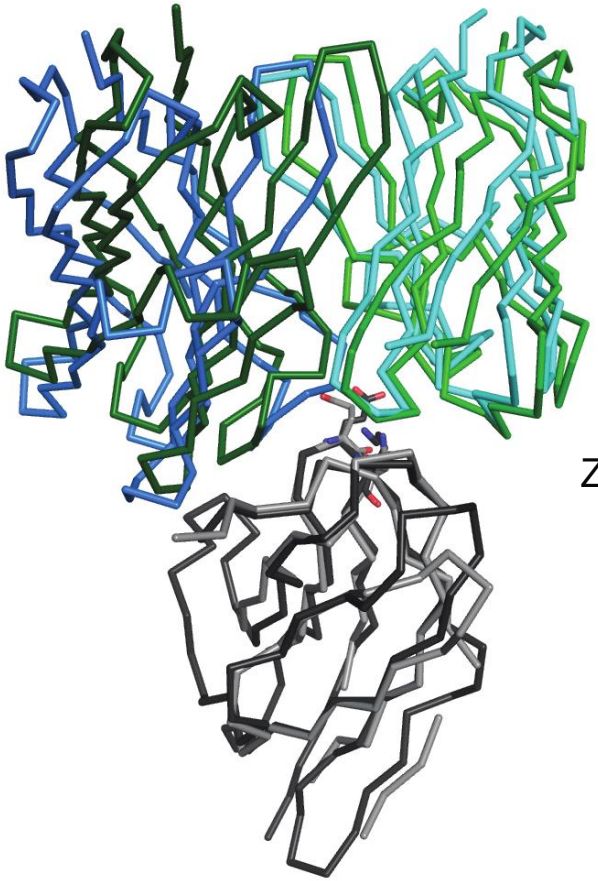
Pool of wt B cells

Germline-targeting strategies to induce 1074/121-like bnAbs



Escolano et al Cell In Press

VH3-23/VK1-5 Recognize the Lateral Ridge

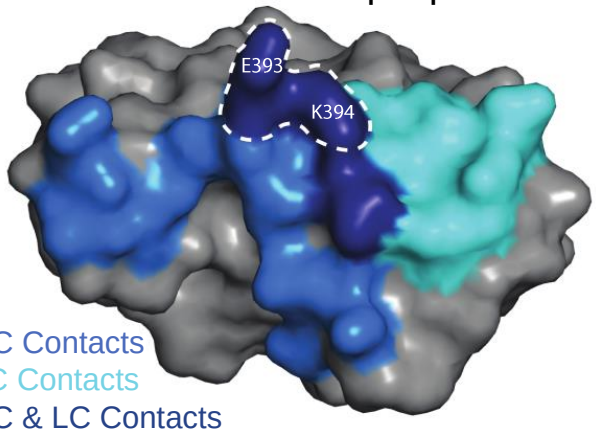


Z006–ZIKV DIII
Z006 HC
Z006 LC
ZIKV DIII

Z004–DENV1 DIII
Z004 HC
Z004 LC
DENV1 DIII

F

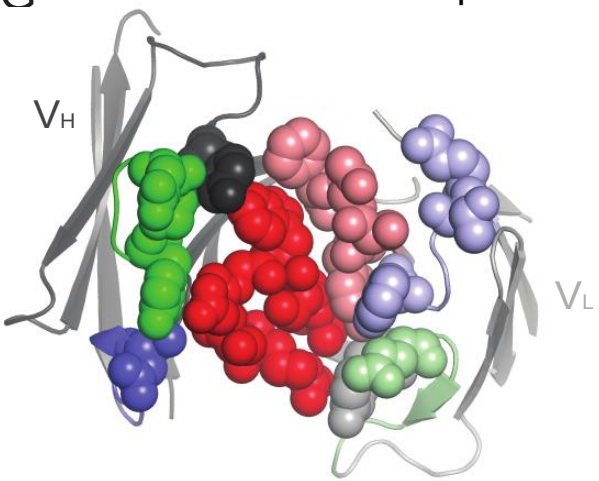
ZIKV DIII Epitope



HC Contacts
LC Contacts
HC & LC Contacts

G

Z006 Fab Paratrope



HC FWR CDRH1 CDRH2 CDRH3
LC FWR CDRL1 CDRL2 CDRL3

Jennifer Keeffe
Anthony West
Pamela Bjorkman

Antibodies are Effective Against HIV

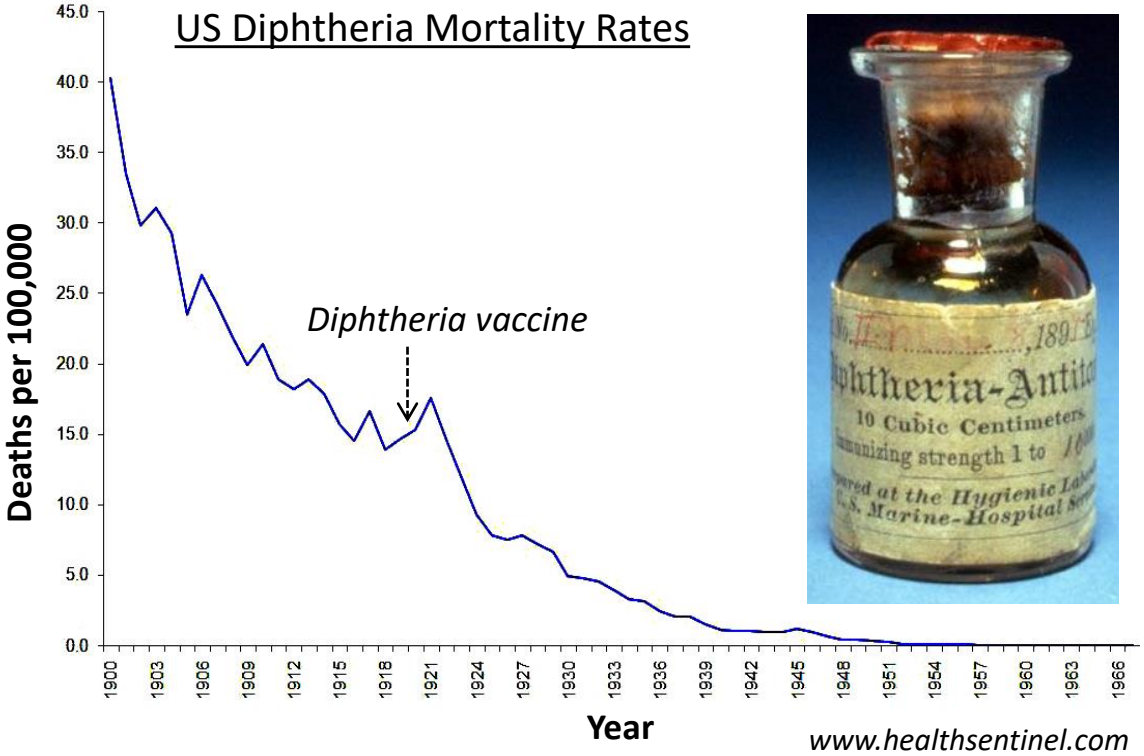
1890-1892 : protective role of Abs


Shibasaburo Kitasato

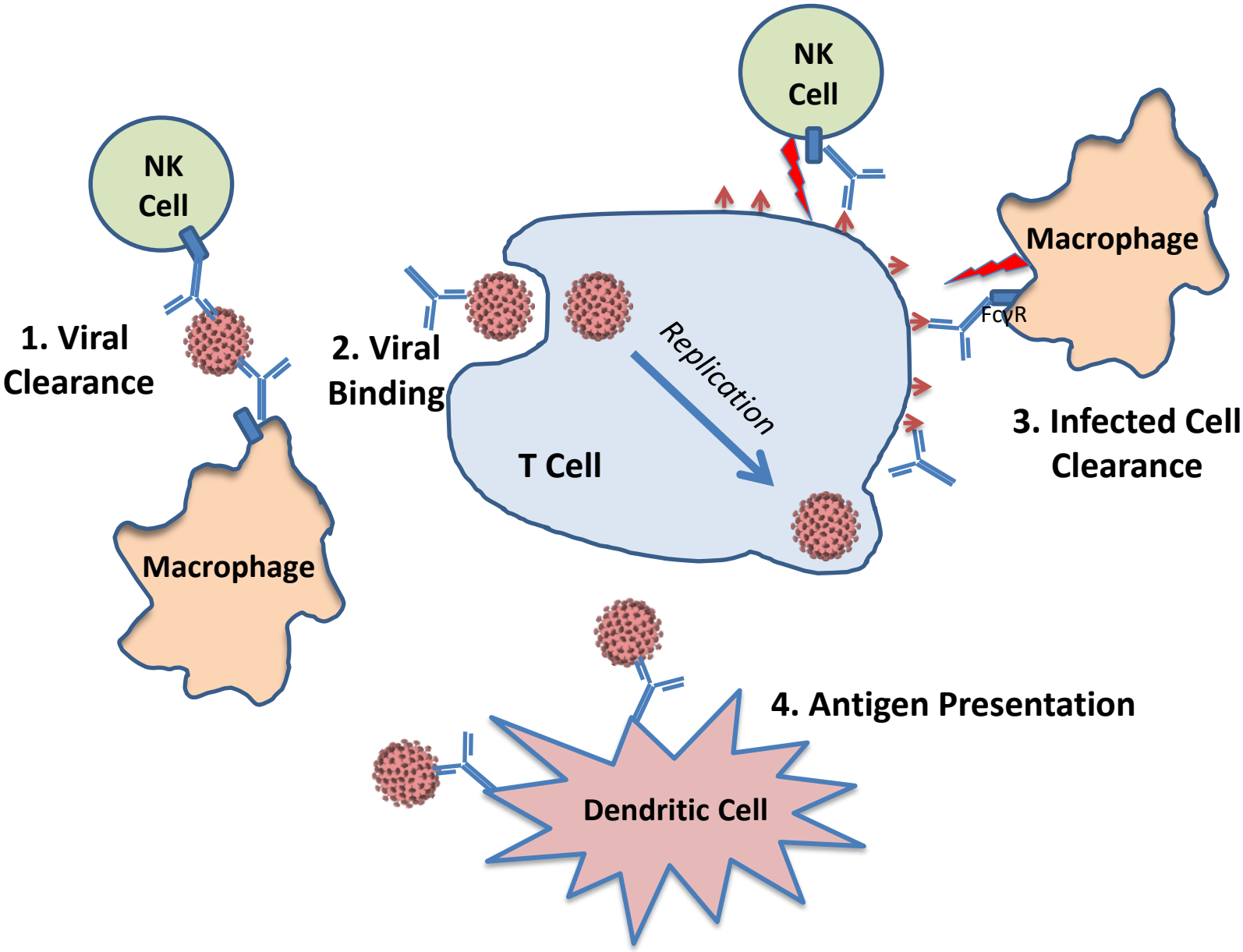

Emil von Behring

diphtheria and tetanus toxins

1894 : antibody-based therapy against diphtheria in humans



Effector Functions



Anti-HIV bNAb trials at the Rockefeller University Hospital

ZIKV Acknowledgments

Study Participants

Rockefeller University

Davide Robbiani
Lion Uhl, Dennis Schaefer-Babajew, Priscilla Olsen, Anna Gazumyan, Lilian Nogueira, Roshni Patel, Tom Eisenreich

Charlie Rice, Peggy MacDonald
Leonia Bozzacco, Stephanie Azzopardi, Mohsan Saeed

Yale University and FIOCRUZ Brazil
Albert Ko & Ricardo Khouri

National Institute for Respiratory Diseases
Gustavo Reyes-Teran & Santiago Avila

Caltech
Pamela Bjorkman
Jennifer Keeffe, Anthony West

Acknowledgements

Study participants

Nussenzweig Laboratory
Johannes Scheid
Marina Caskey
Florian Klein
Pia Dosenovic
Lotta von Boehmer
Amelia Escolano
Ching-Lan Lu
Julio Lorenzi
Malte Braunschweig
Lilian Nogueira
Anna Gazumyan
Joshua Horwitz
Ari Halper-Stromberg
Lillian Cohn

Fred Hutch
Leo Stamatatos
Andy McGuire

NIH
Malcolm Martin
Yoshiaki Nishimura
Rajeev Gautam
Tae-Wook Chun
Richard Koup
Anthony Fauci

Caltech
Pamela Bjorkman
Anthony West
Rachel Galimidi
Harry Gristick

Scripps
Jon Steichen
William Schief
Joseph Jardine
Dan Kulp
Devin Sok
Dennis Burton

University of Cologne
Gerd Faetkenheuer
Gisela Kremer

WCMC
John Moore
Albert Cupo

U. Penn
Beatrice Hahn
Edward F. Kreider

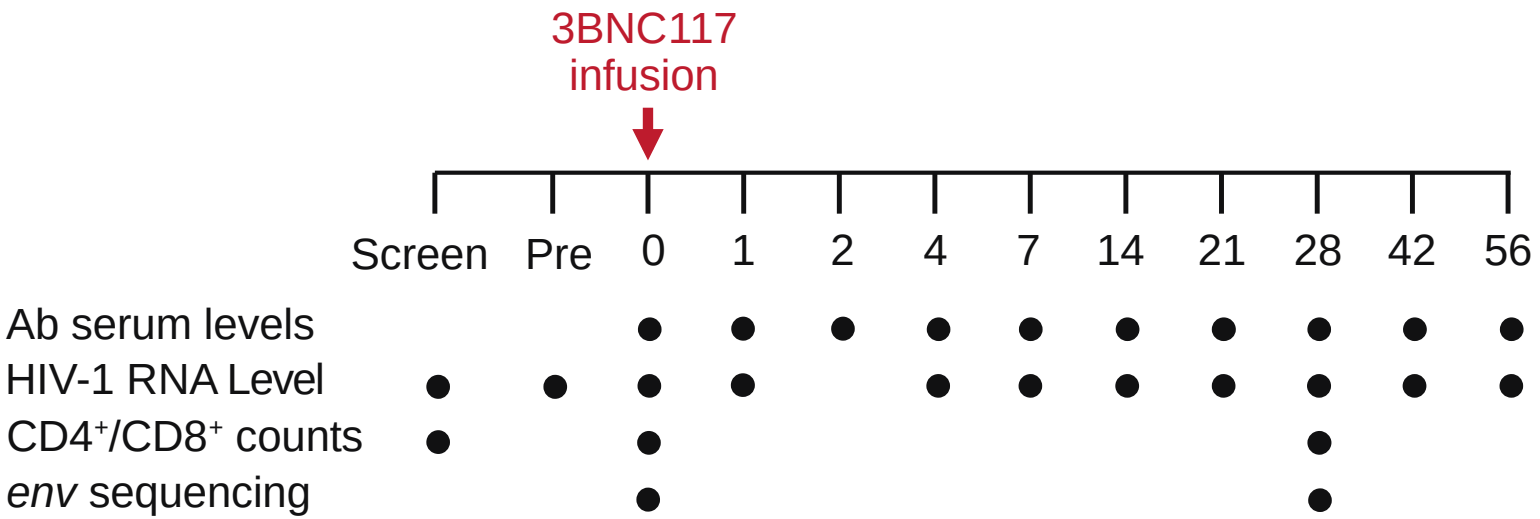
U. Amsterdam
Rogier Sanders

Ragon/MGH and Brigham and Women's Hospital
Lindsey Baden
Bruce Walker

BI-Deaconess/Harvard
Mike Seaman

Support from the Bill and Melinda Gates Foundation, the Rockefeller University, HHMI, NIH

Clinical Investigation of bNAb 3BNC117 (MCA-0835)



Enrollment

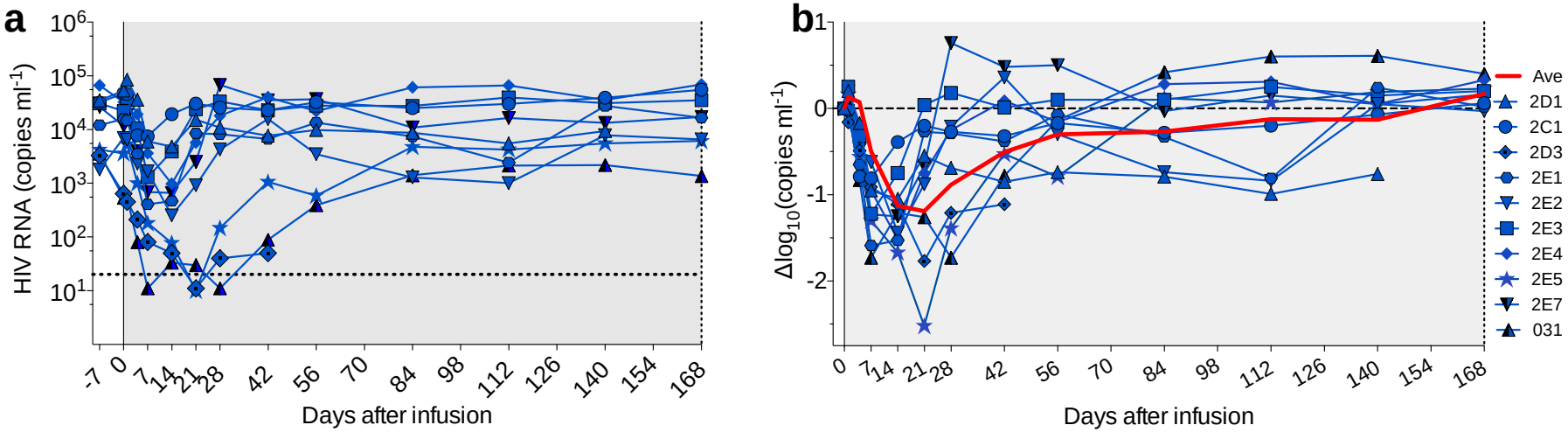
- 49 Subjects
- 29 HIV-1-infected
  - 16 off ART, 13 on ART

Safety/Tolerability

- Well tolerated
- No SAE
- AEs mostly mild (transient fatigue, headache)

Caskey et al *Nature* 2015

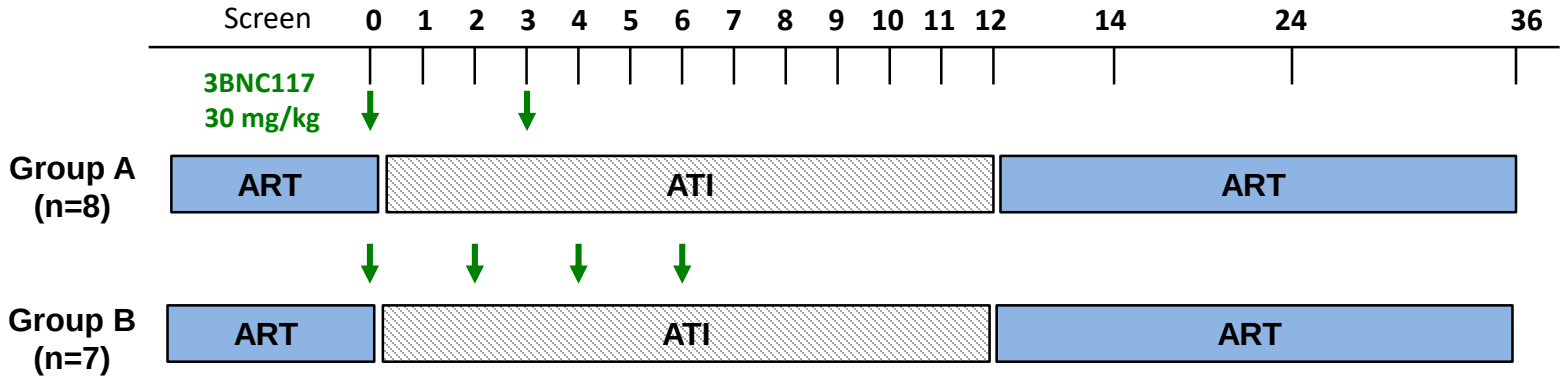
3BNC117 Suppresses Viremia in HIV-infected Individuals



- Viral load decline of 0.8 – 2.5 log copies/ml (**mean 1.48 copies/ml**).
- Plasma viremia was significantly reduced for at least 4 weeks.
- Rebound viruses contained mutations within known antibody contact sites.

Caskey et al., Nature 2015

Can 3BNC117 prevent viral rebound when ART is discontinued?

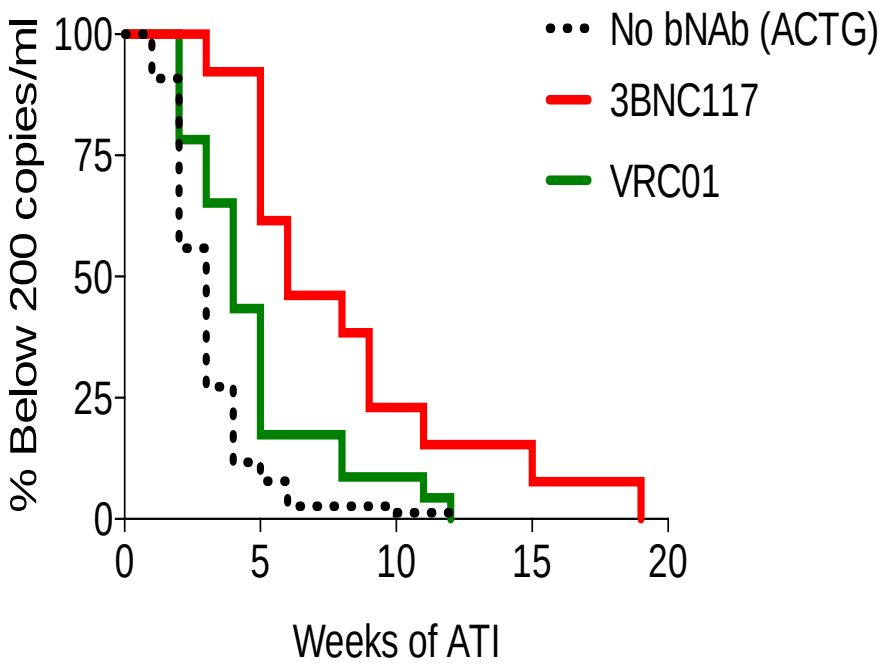


- **Study population**:
- HIV-infected on ART with HIV-1 RNA < 50 copies/ml for at least 12 months
- CD4 count > 500 cells/µl and CD4 nadir > 200 cells/µl
- **Outgrown viruses with 3BNC117 IC_{50} < 2 µg/ml.**

- **ART re-initiation criteria**: HIV-1 RNA ≥ 200 copies/ml OR CD4-T count < 350 cells/µl on two consecutive measurements.

Scheid et al Nature 2016

3BNC117 Delays Viral Rebound during ATI Compared to Historical Controls

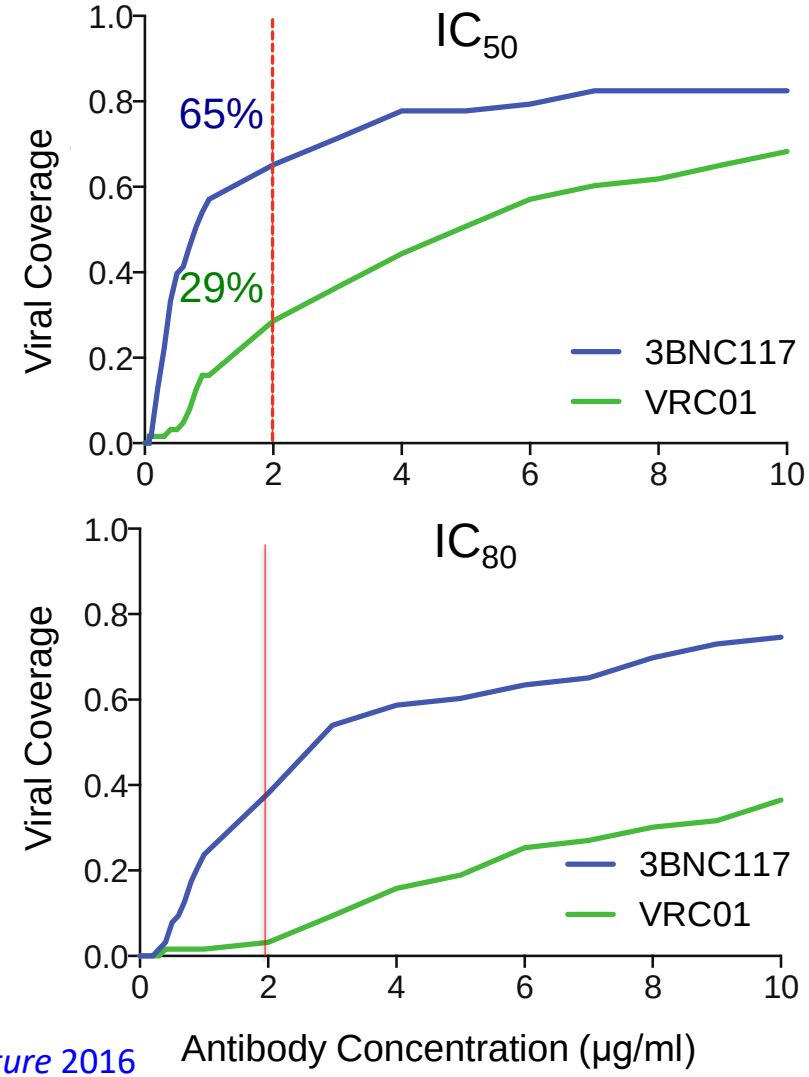


3BNC117 x ACTG: p< 0.0001 VRC01 x ACTG: p< 0.0029

3BNC117 x VRC01: p 0.008

Scheid et al Nature 2016
Bar et al NEJM 2016

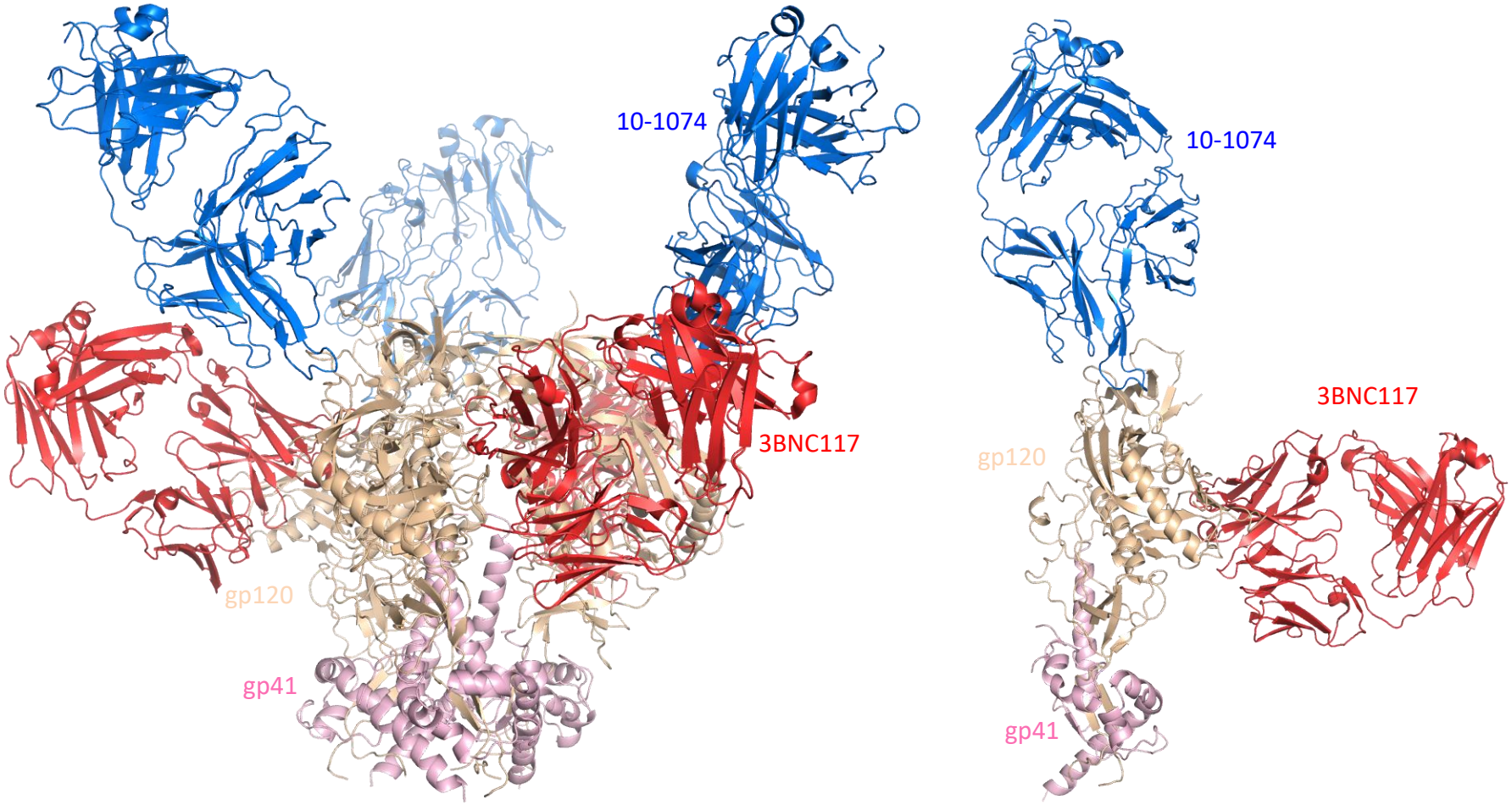
Baseline Sensitivity of Viral Isolates from 88 Individual Viral Outgrowth Cultures



3BNC117 IC_{50} (µg/ml)	n (%)
> 20	10 (11%)
10 - 20	5 (6%)
2.1 – 9.9	16 (18%)
< 2	57 (65%)

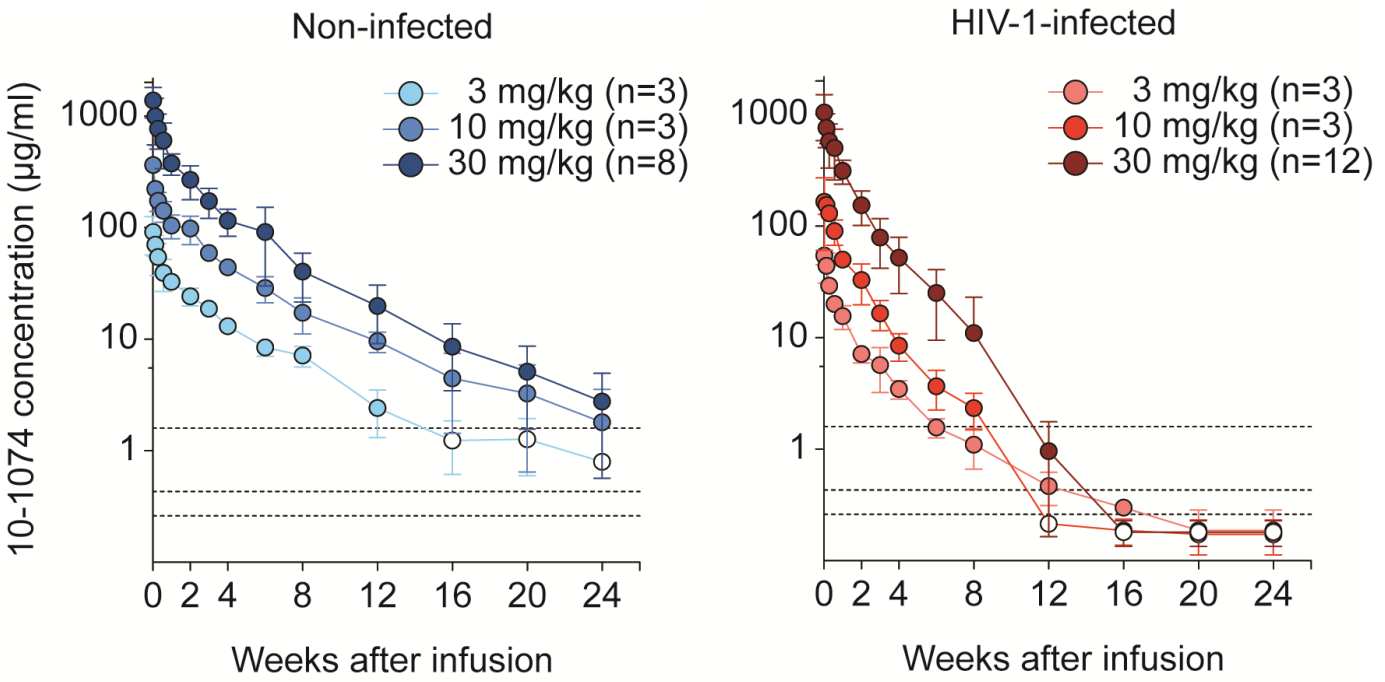
Scheid et al *Nature* 2016

3BNC117 and 10-1074 Target Independent Epitopes



Gristick et al NSMB 2016

10-1074 exhibits preferable PK-characteristics

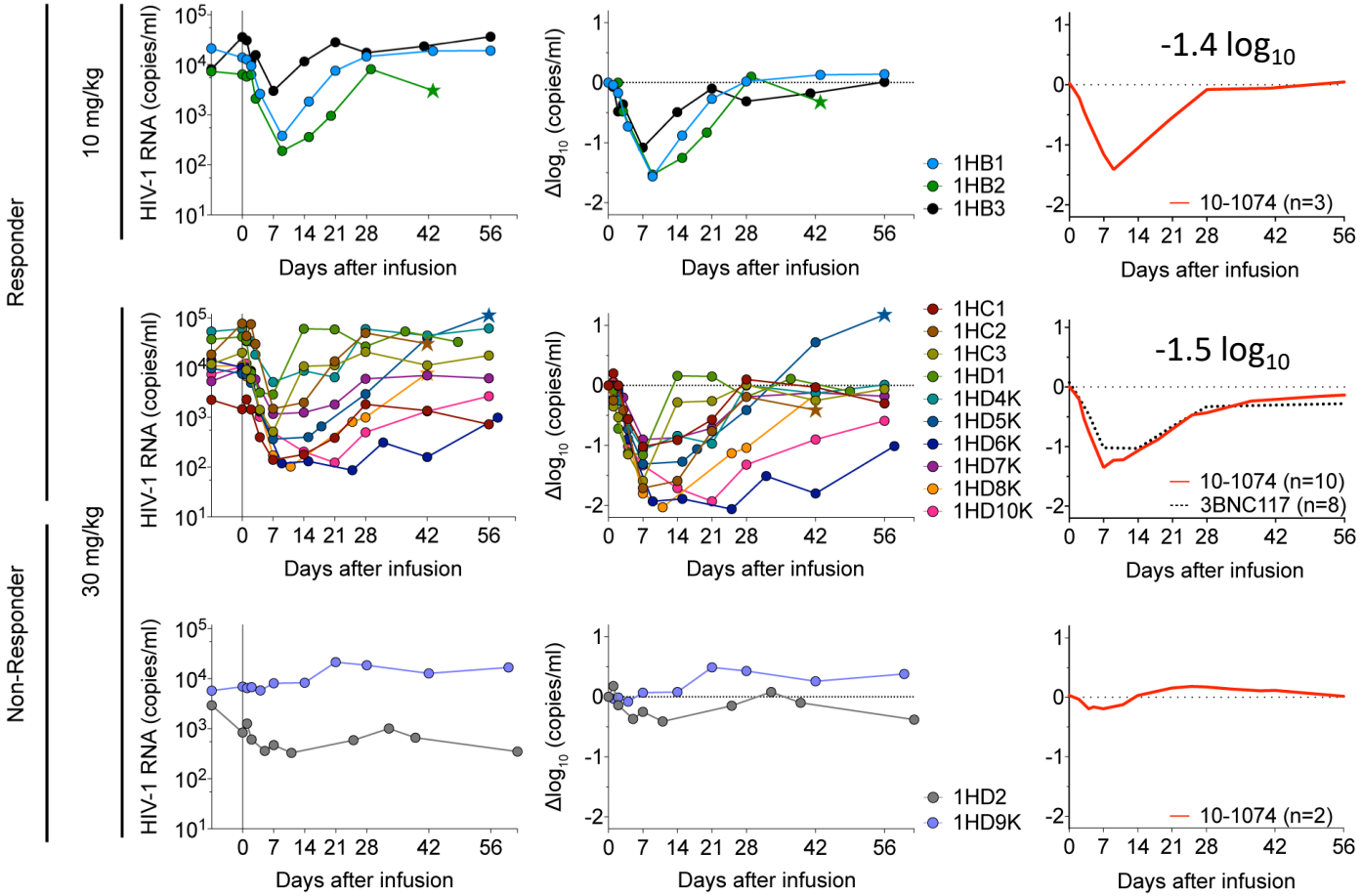


$t_{1/2}$ ~ 24.2 days

$t_{1/2}$ ~ 12.6 days

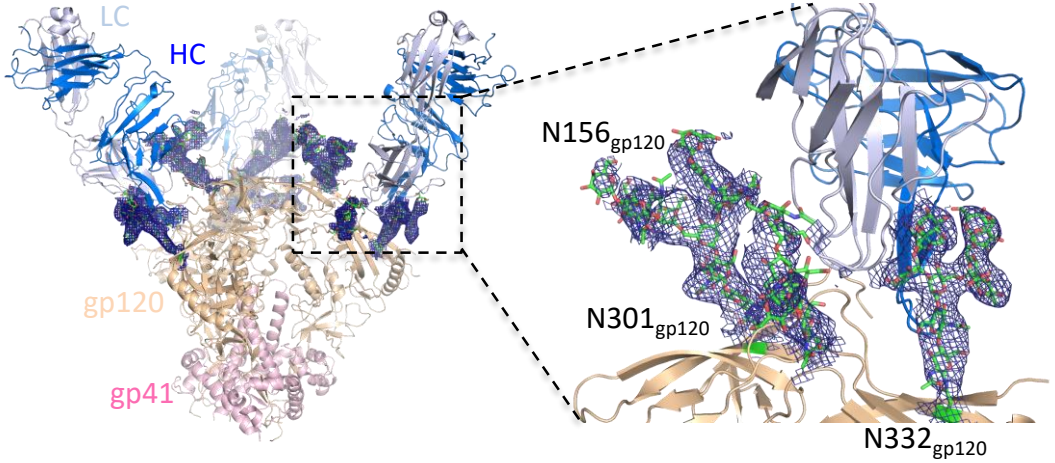
Caskey et al Nature Med 2017

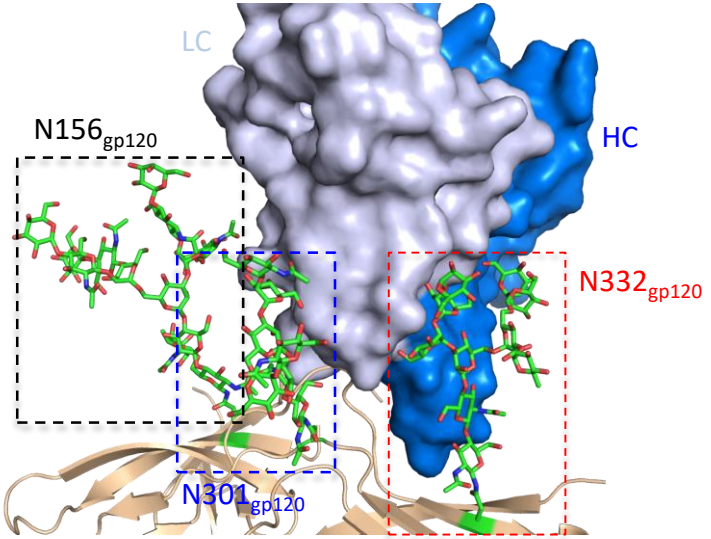
A single infusion of 10-1074 efficiently lowers viremia

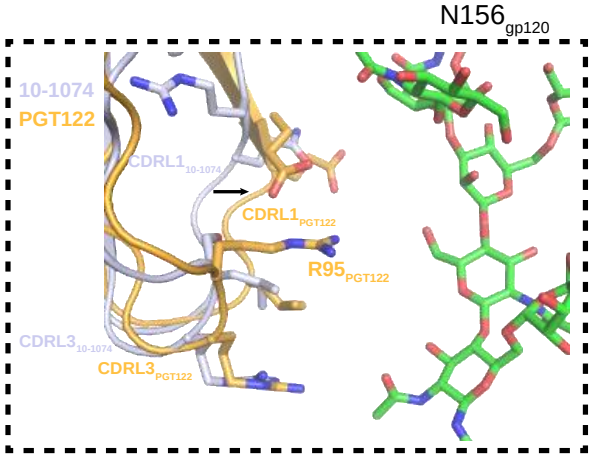


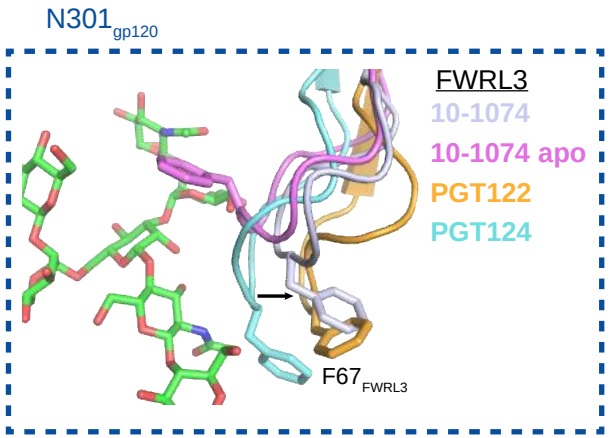
Caskey et al Nature Med 2017

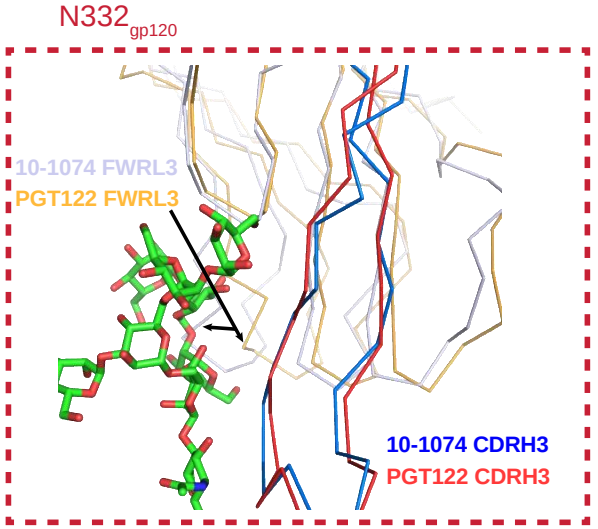
10-1074–BG505 Crystal Structure







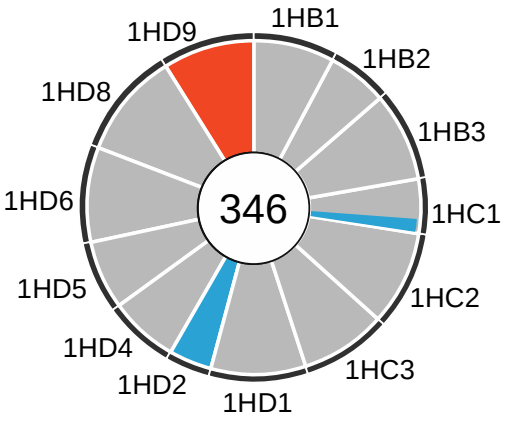


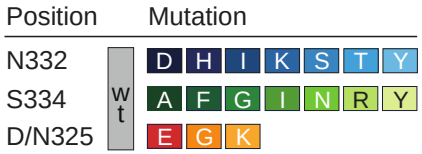


Gristick et al Nat Struct and Mol Bio 2016

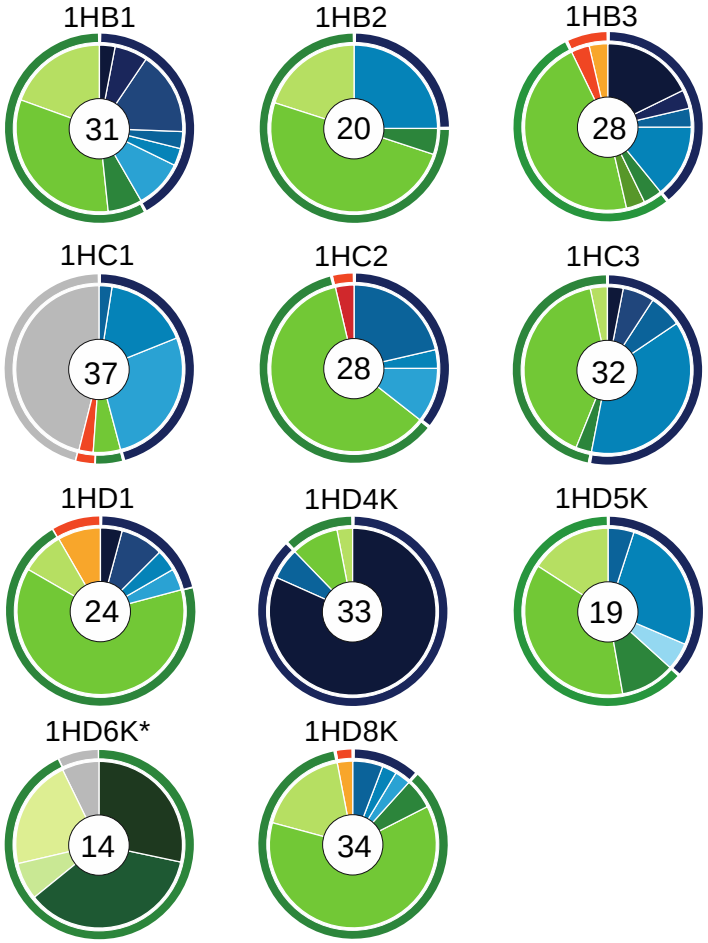
10-1074 selects for recurrent mutations at known contact sites

Day 0

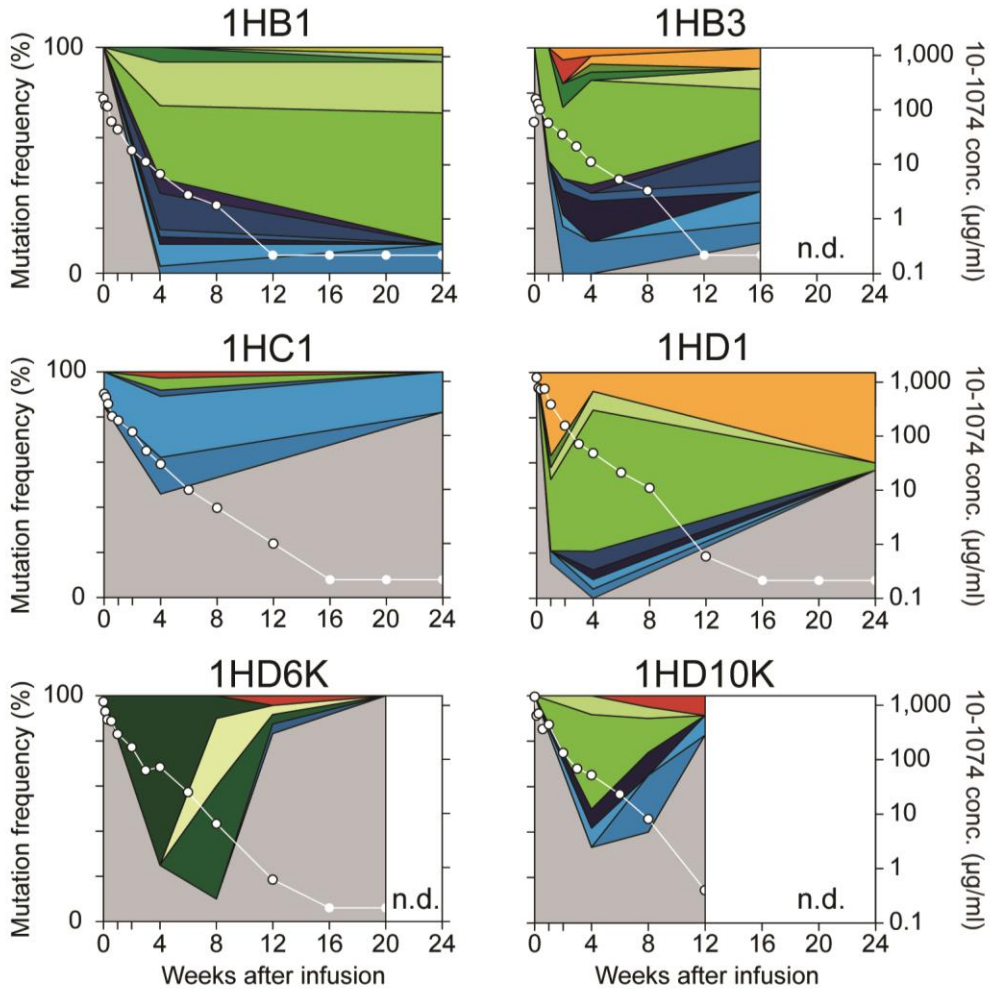




Week 4

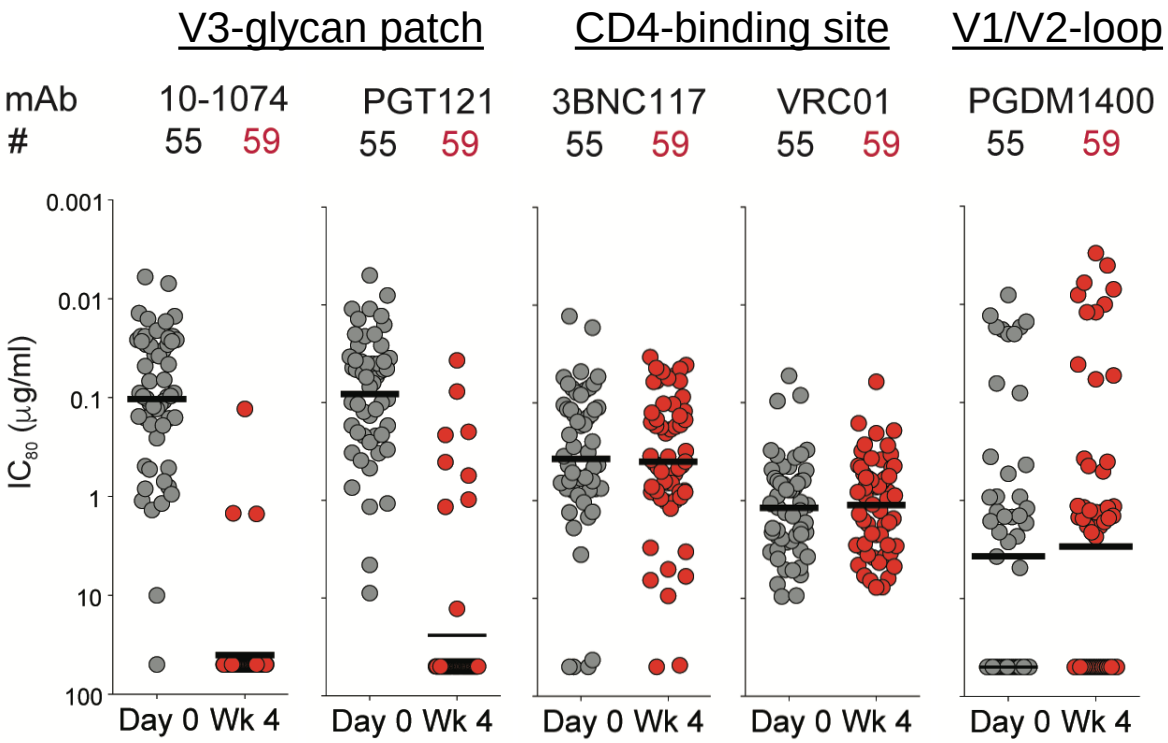


10-1074 escape mutations are lost again over time



Caskey et al Nature Med 2017

10-1074 escape variants remain sensitive to other antibodies



Caskey et al Nature Med 2017

10-1074 Preliminary Observations

32 participants to date

Safety for participants receiving 3, 10, and 30 mg/kg

1. No SAEs
2. Well-tolerated; most reported AEs graded as mild (transient fatigue and headache).

PK

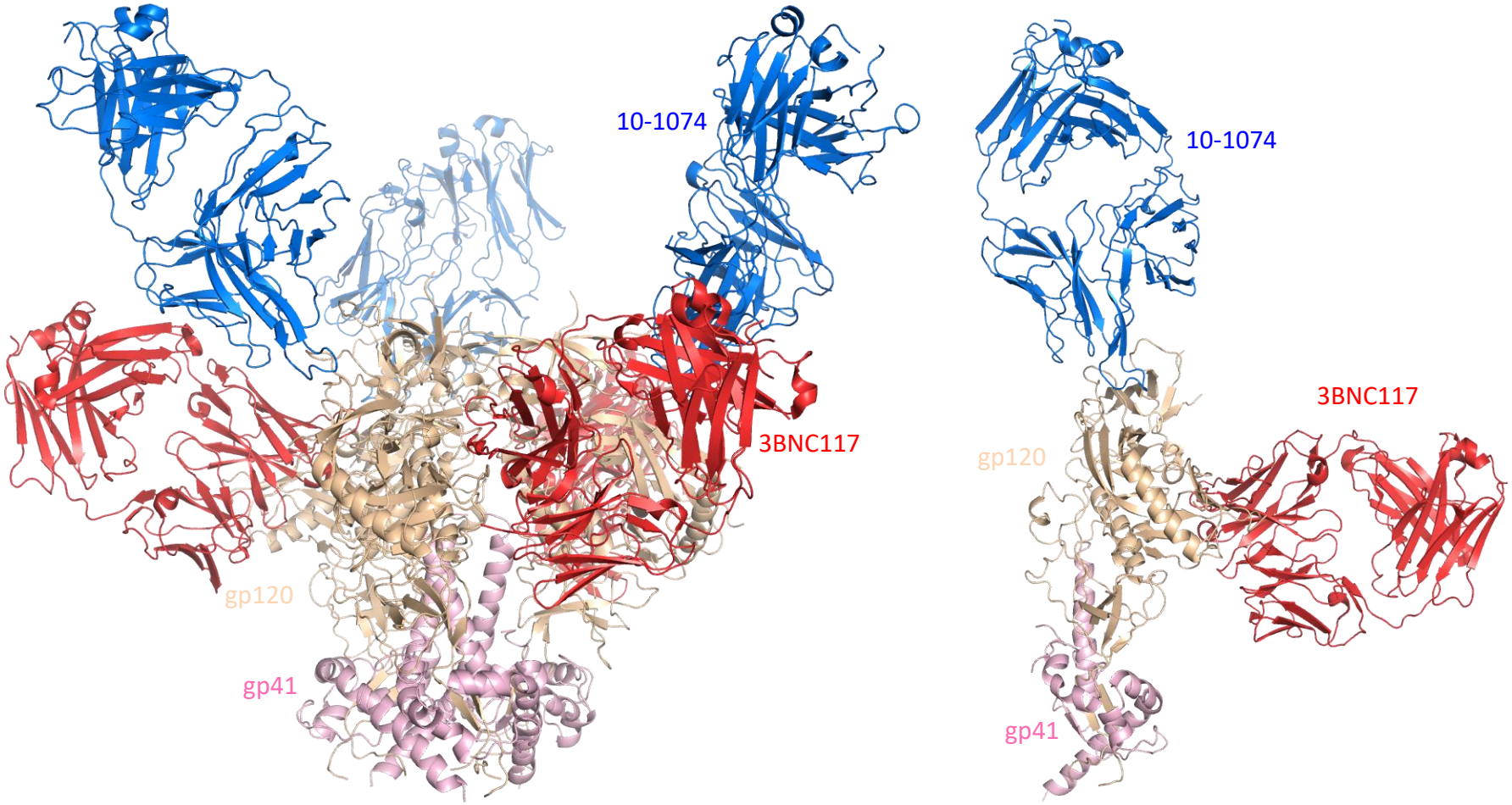
1. Uninfected $T_{1/2}$: 20.3 days
2. Infected $T_{1/2}$: 9.5 days

Virology

1. 30mg/Kg = rapid 1.43_{log} drop in viremia
2. Escape after 4 weeks is uniformly associated with N332 or GDIR mutation
3. Escape mutants remain sensitive to bNAbs targeting other sites

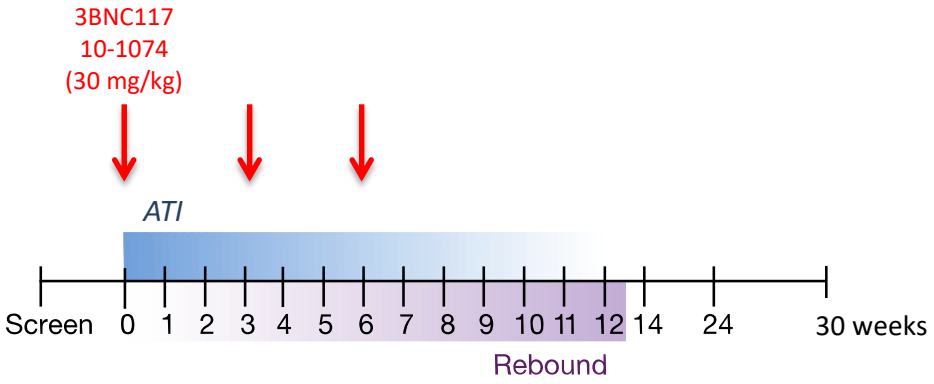
Caskey et al Nature Med 2017

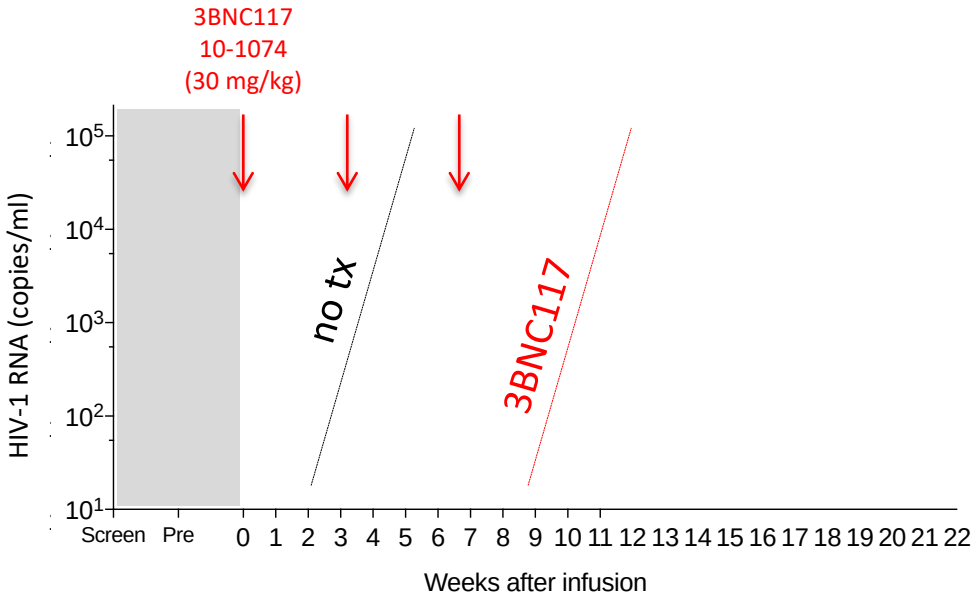
3BNC117 and 10-1074 Target Independent Epitopes

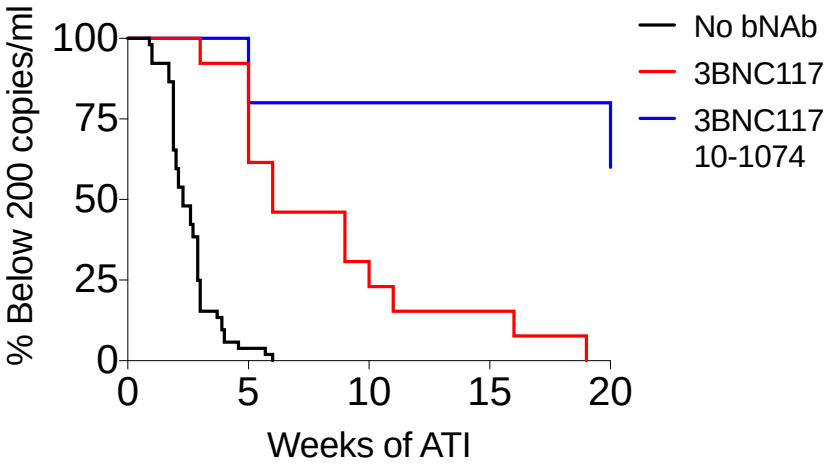


Gristick et al NSMB 2016

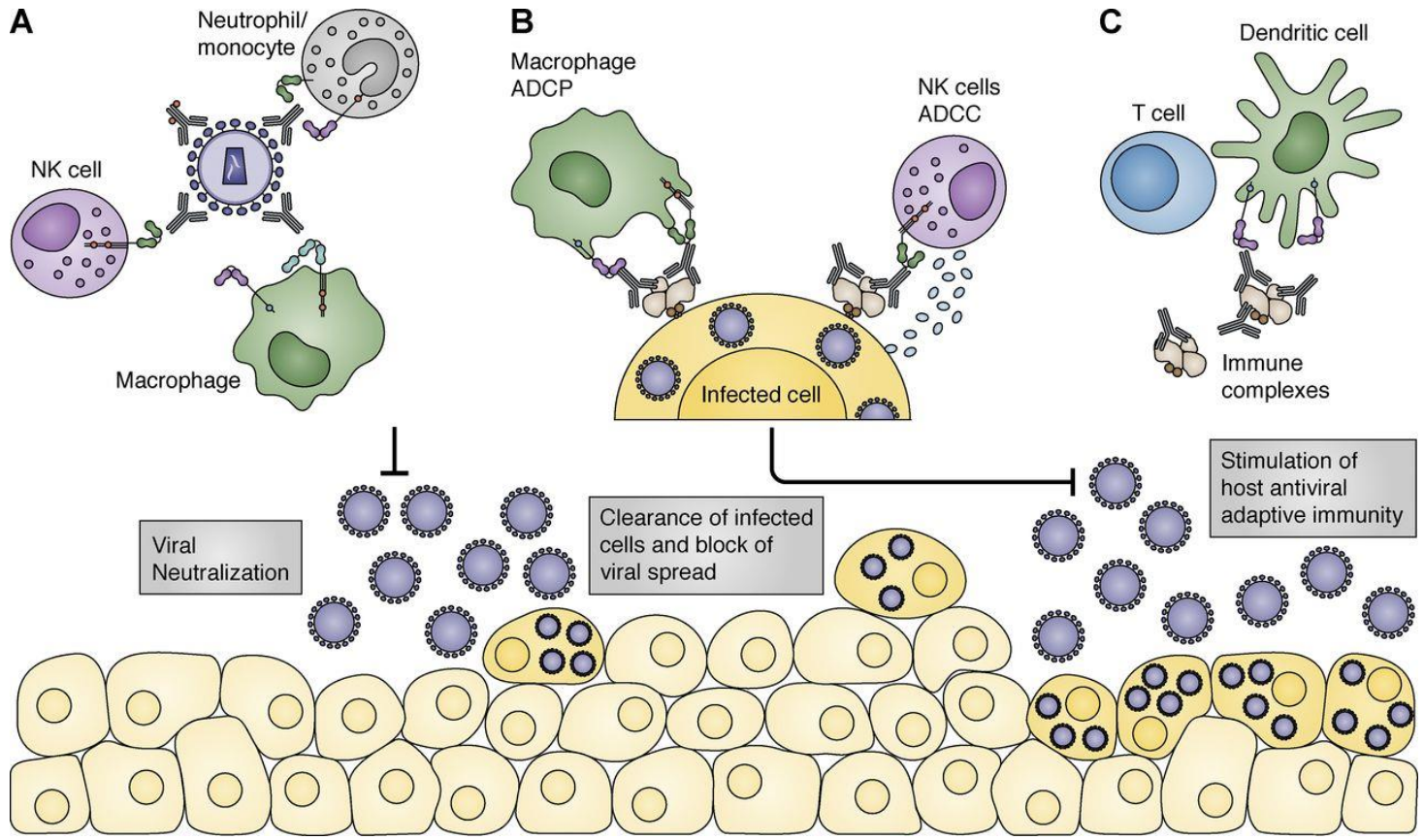
3BNC117 plus 10-1074 delay viral rebound for many weeks



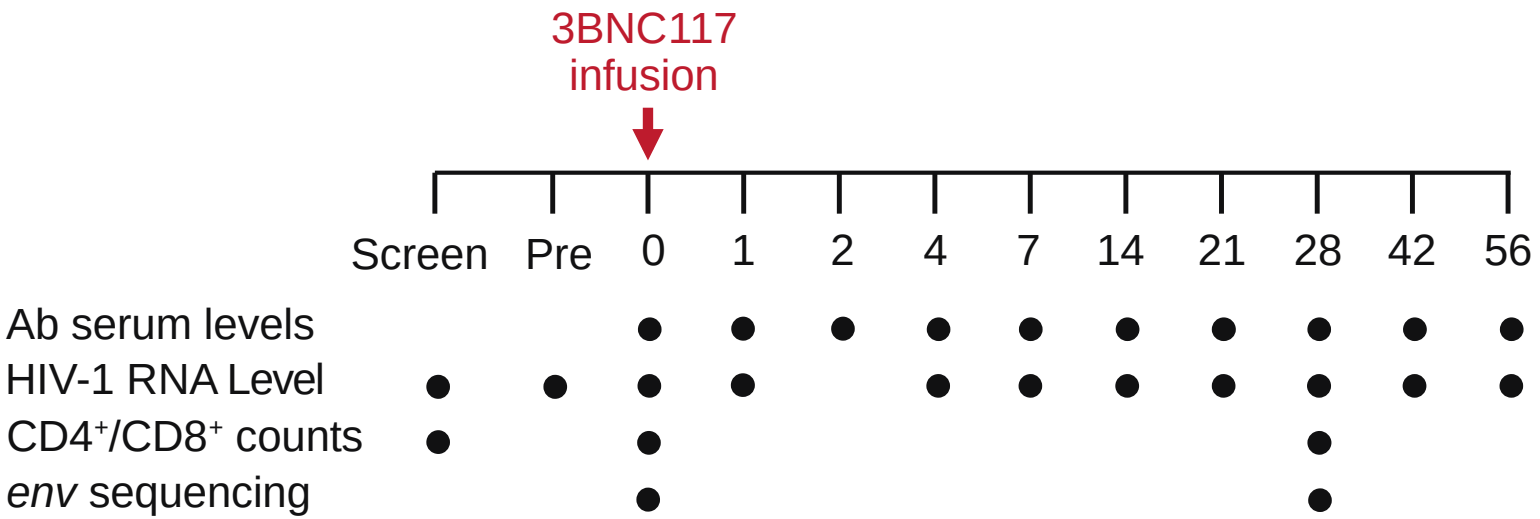




Antibodies Differ from Standard ART in their Potential to Directly Eliminate Virus and HIV-infected Cells and to Create Immune Activating Ab-Ag Complexes



Clinical Investigation of bNAb 3BNC117 (MCA-0835)



Enrollment

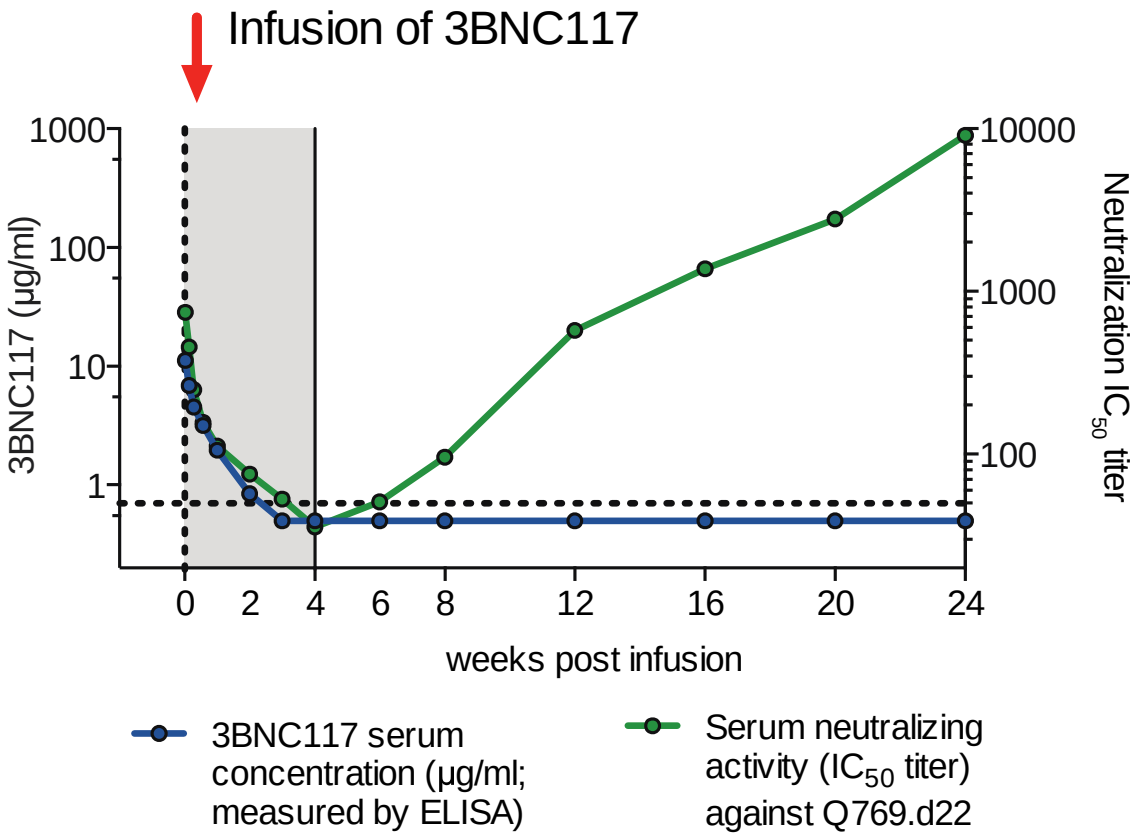
- 49 Subjects
- 29 HIV-1-infected
  - 16 off ART, 13 on ART

Safety/Tolerability

- Well tolerated
- No SAE
- AEs mostly mild (transient fatigue, headache)

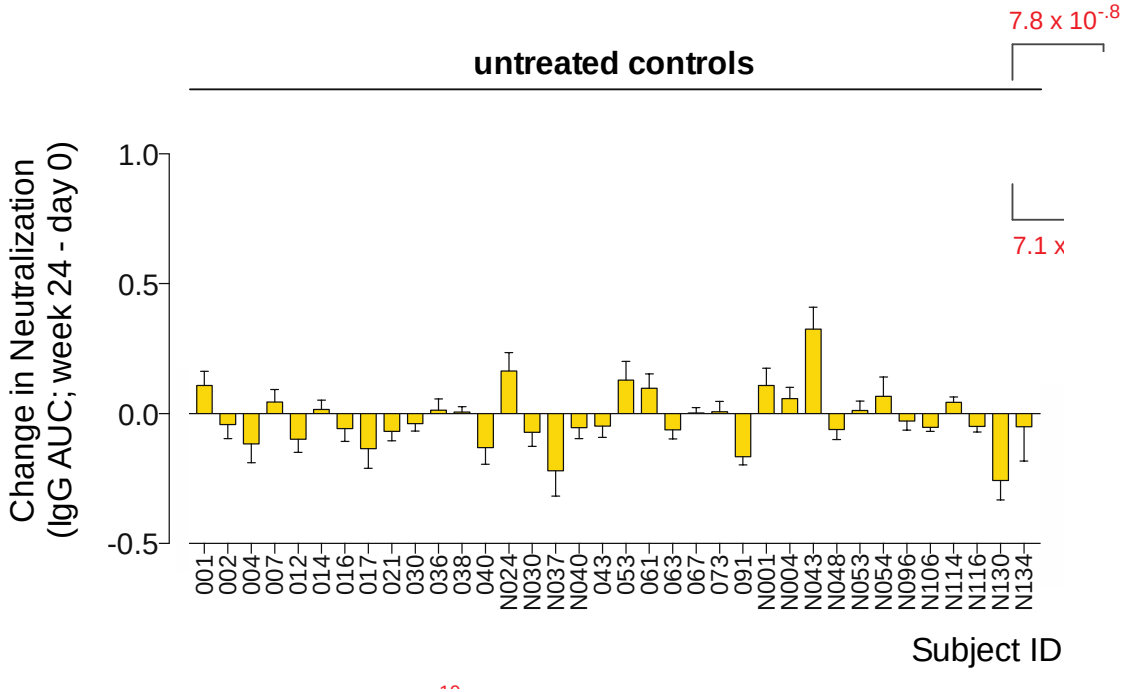
Caskey et al *Nature* 2015

3BNC117 serum concentration and neutralizing activity against Q769.d22 in subject 2A3



Schoofs et al., Science 2016

3BNC117 Enhances Host Humoral Immunity to Heterologous tier 2 HIV-1 viruses



- 3BNC117 infusion 'disturbs' viral quasispecies leading to shifts in viral sequences.
- Both a '**vaccinal effect**' and **responses to newly evolving epitopes** probably contribute to the enhancement of humoral immune responses.

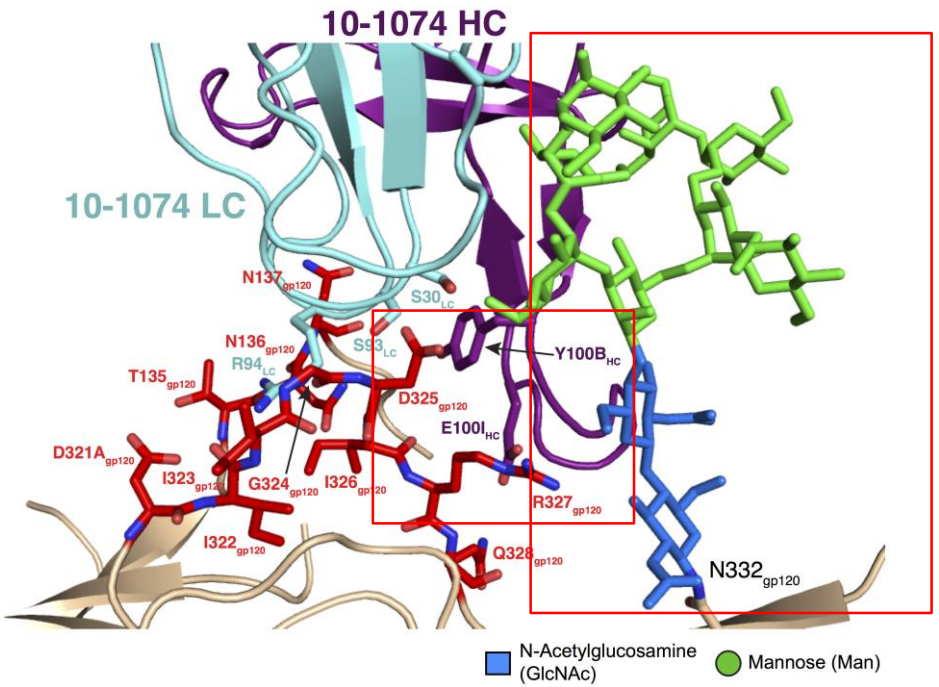
Schoofs et al., Science 2016

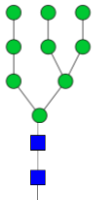
Summary – Clinical Experience with bNAbs

- Generally **safe** in humans at doses up to 40 mg/kg.
- **Half-lives** of about **2.5 weeks**.
- Lead to **significant decline is plasma viremia** (~ 1.5 $\log_{10}$ cp/ml).
- **bNAb monotherapy selects resistant viral strains** (during ATI and in viremic individuals).
- In ART-treated individuals, **3BNC117 significantly delayed viral rebound** compared to historical controls (median 10 wks after 4 doses).
- **3BNC117 engages the host immune system** (through ADCC and increased antigen presentation), and **enhances host humoral responses**.

Caskey et al., Nature 2015; Lynch et al. Science Trans Med 2015; Scheid et al Nature 2016; Bar et al NEJM 2016; Schoofs et al., Science 2016; Lu et al Science 2016; Caskey et al Nature Medicine 2017

10-1074: a glycan-patch antibody highly dependent on N332



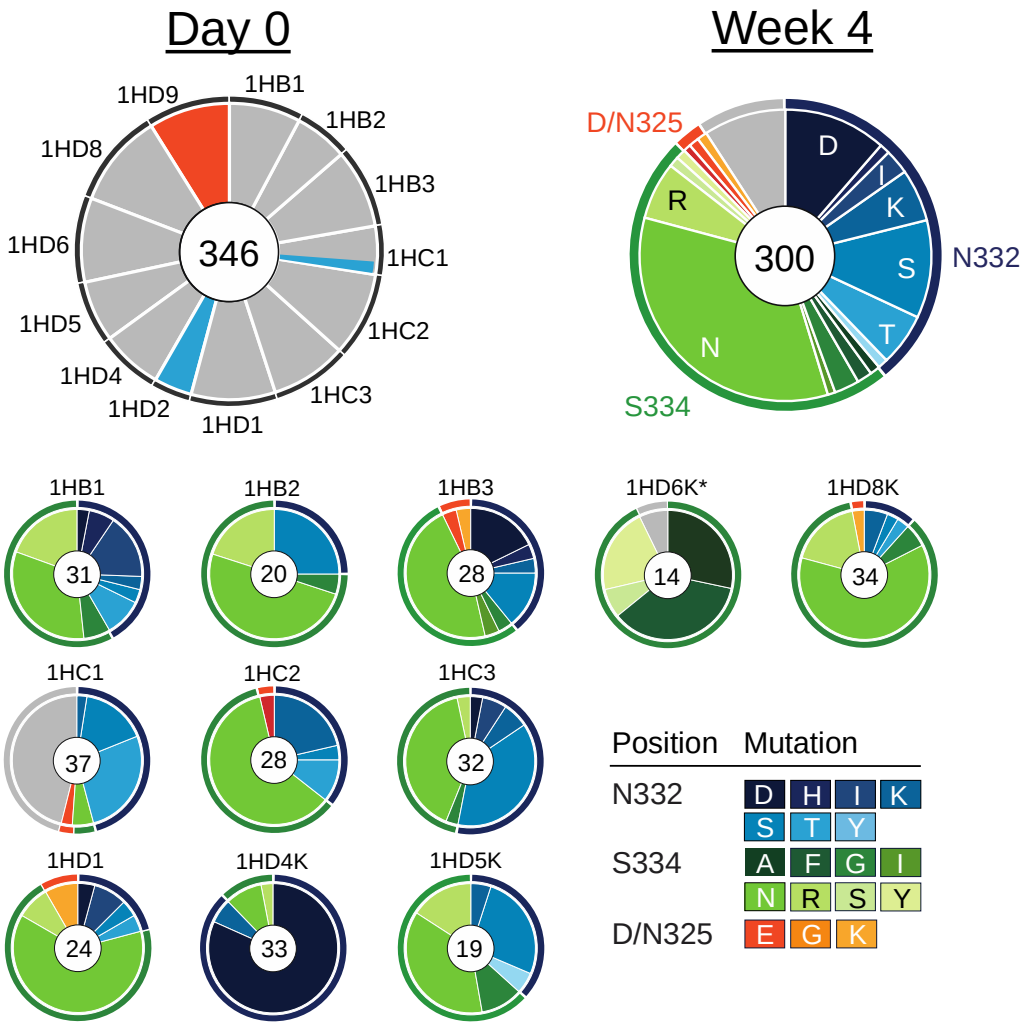


Critical contacts: I G D I R Q A H C N I S

325 327 332 - 334

Gristick *et. al.*, NSMB 2016

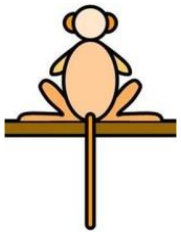
Selection for recurrent mutations at 10-1074 contact sites



Caskey et al Nature Med 2017

Indian Rhesus macaques

Mamu A01, A02, B08 & B17 neg.

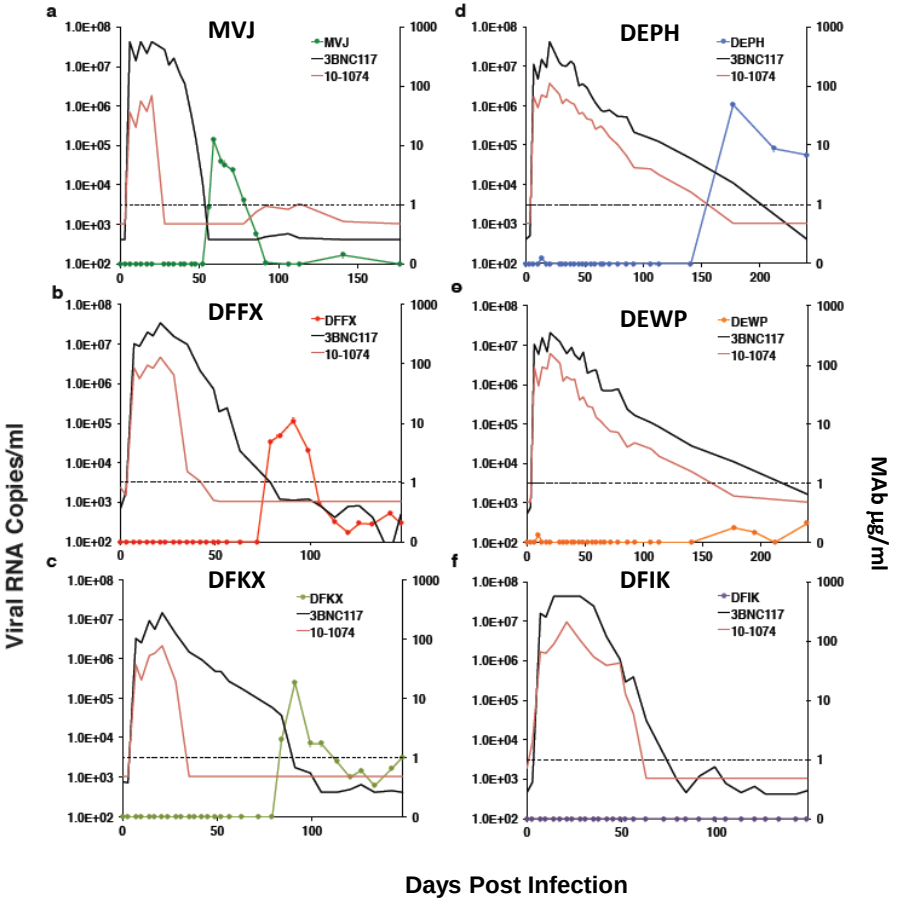


MAb

weeks

$SHIV_{AD8-EO}$ 1000 $TCID_{50}$ I.R.

INTRARECTAL

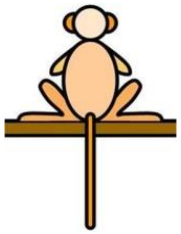


3BNC117

10-1074

Indian Rhesus macaques

Mamu A01, A02, B08 & B17 neg.



MAb

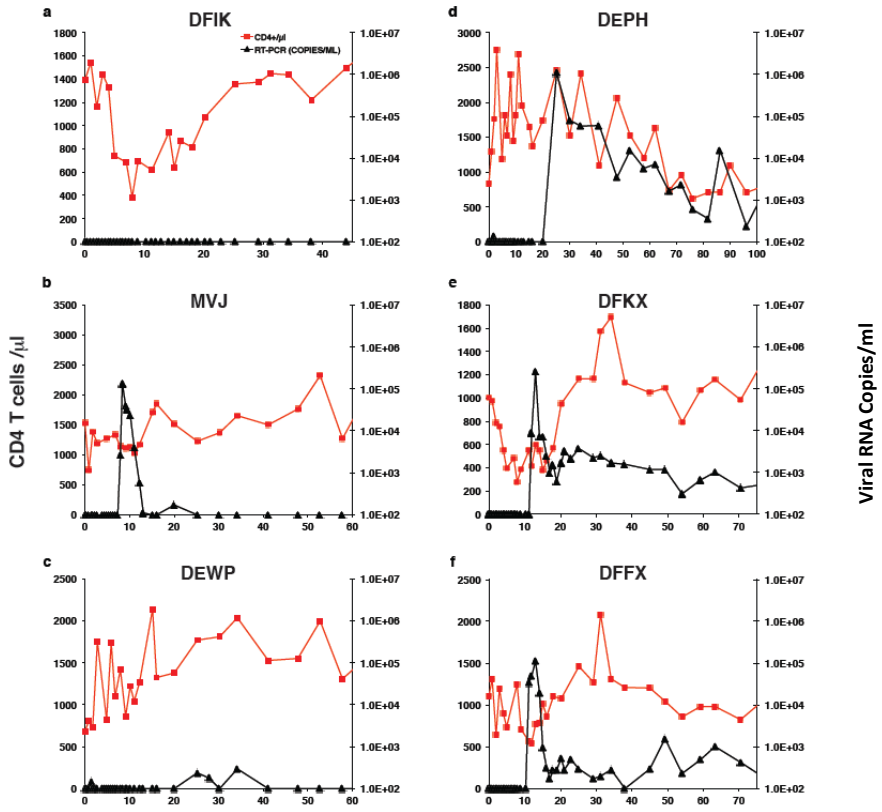
weeks

$SHIV_{AD8-EO}$ 1000 $TCID_{50}$ I.R.

INTRARECTAL

Viremia Below Detection
Controller

Moderate to High Viremia
Non-Controller



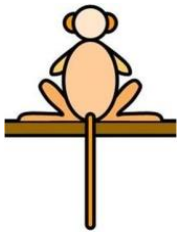
Virus load

$CD4^+$ T cell #

Weeks after infection

Indian Rhesus macaques

Mamu A01, A02, B08 & B17 neg.

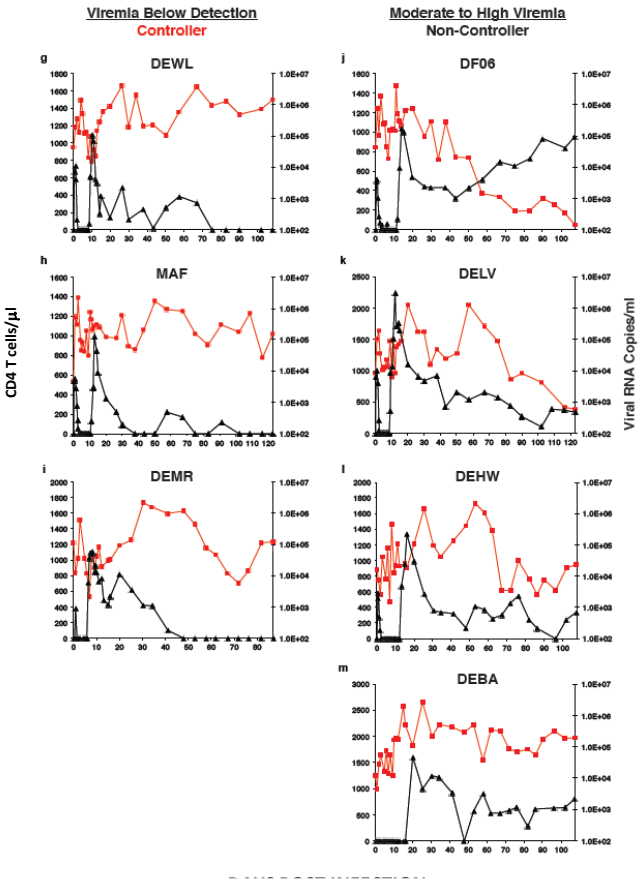


MAb

weeks

$SHIV_{AD8-EO}$ 1000 $TCID_{50}$ I.V.

INTRAVENOUS



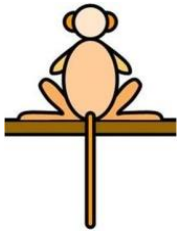
Virus load

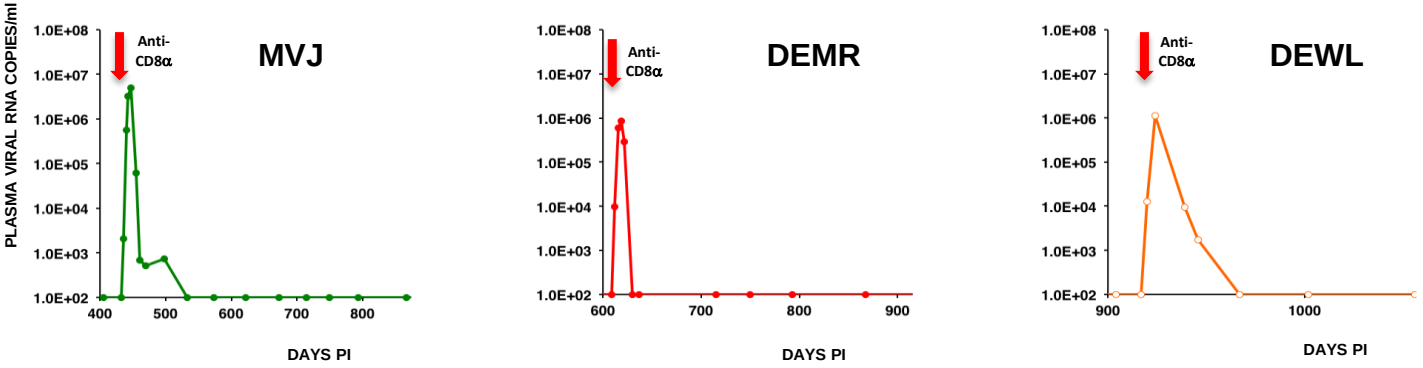
$CD4^+$ T cell #

Weeks after infection

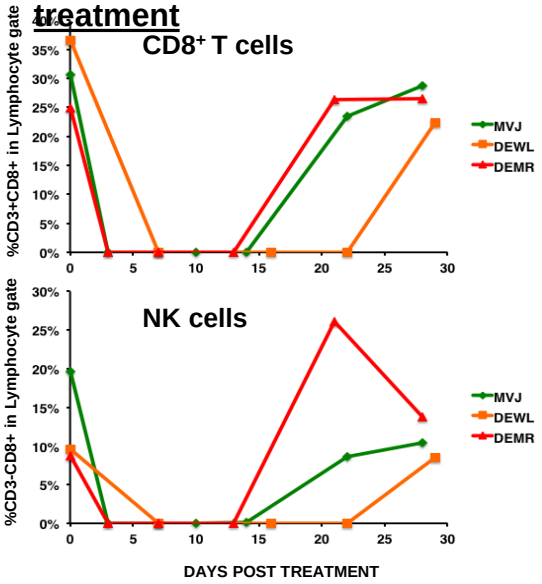
Indian Rhesus macaques

Mamu A01, A02, B08 & B17 neg.



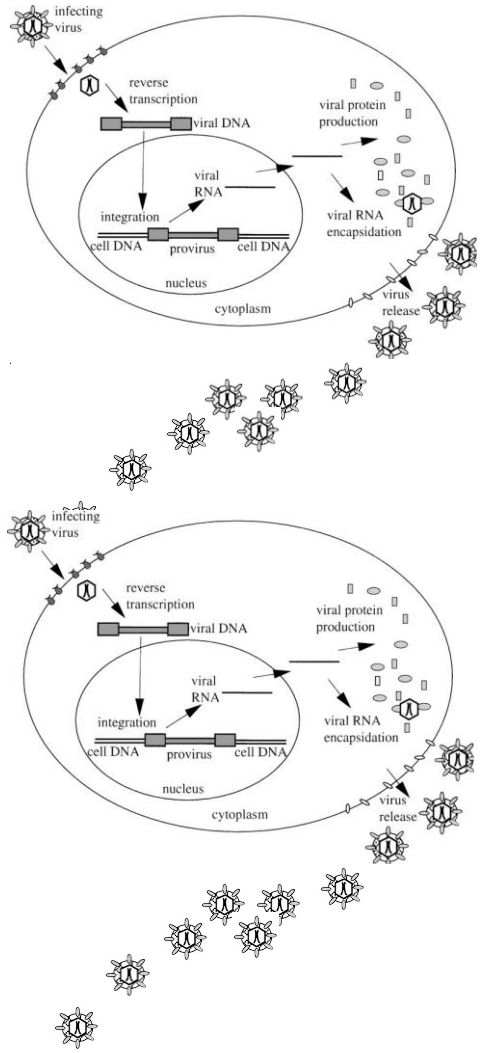


Anti CD8 α antibody treatment

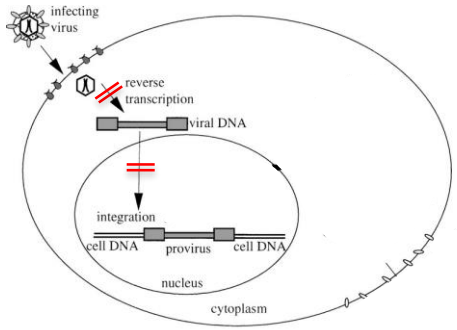


DIFFERENCES IN cART and bNAb CONTROL OF VIRUS REPLICATION

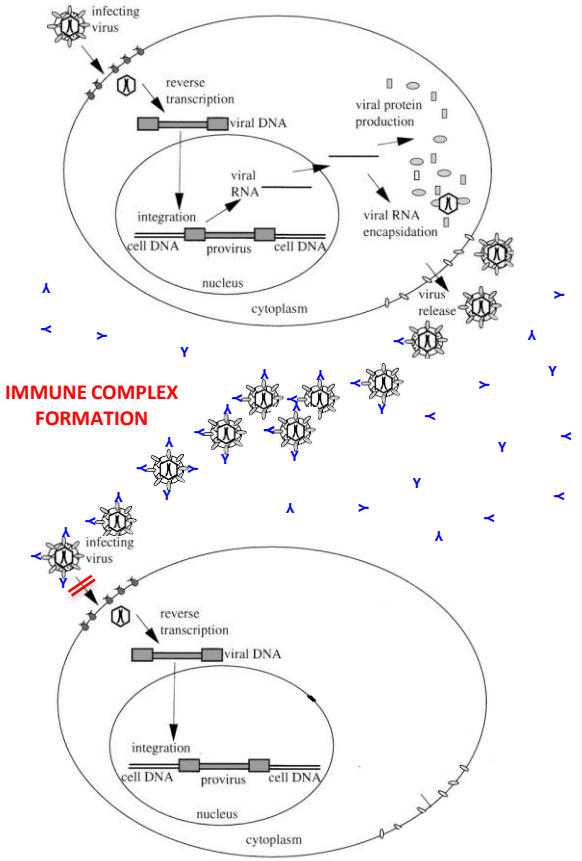
NO TREATMENT



cART THERAPY



bNAb THERAPY



Nishimura et al Nature in Press

SUMMARY

- Elite controller status can be conferred by administering combination bNAbs very early during the acute SHIVAD8/macaque infection.

- bNAb immunotherapy during the acute infection differs from cART by facilitating the emergence of potent immunity able to suppress virus replication.

- Based on the results of the depleting anti-CD8β experiment, $CD8^+$ T cells, not NK, NKT, or γδT cells, presumably induced by immune complexes formed following the administration of bNAbs, mediate long-term control of viremia.

- We are now extending to a more clinically relevant time.

Nishimura et al Nature in Press

Activation of germline B cells

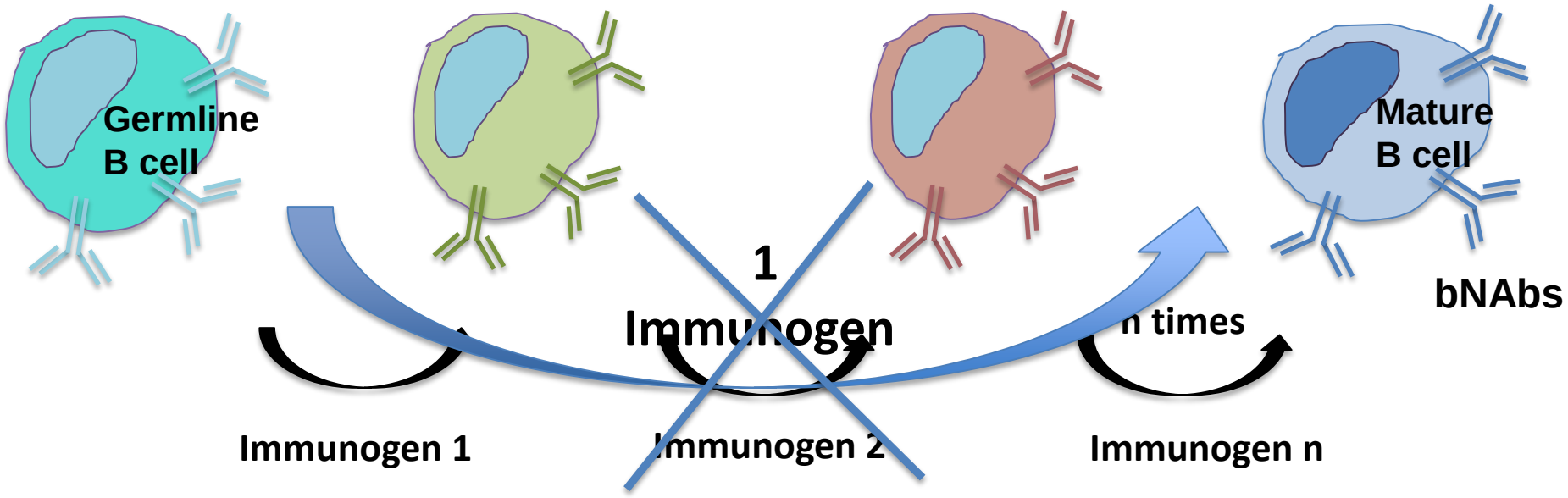
3BNC60/VRC01

GL_{VH} mouse

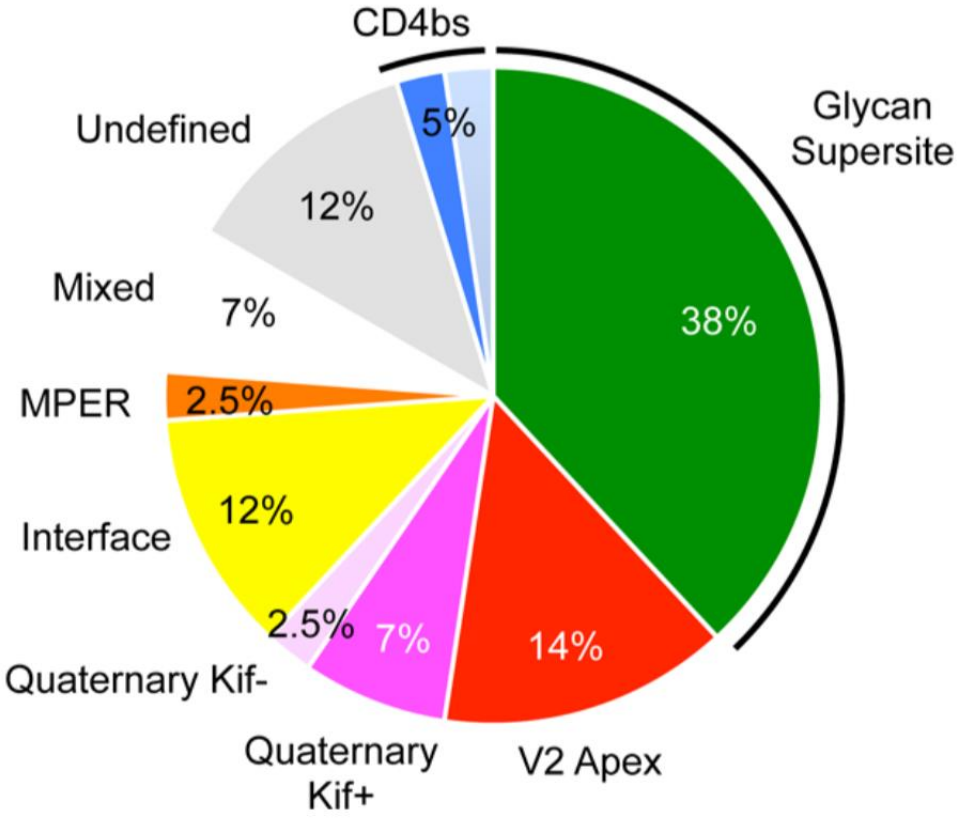
Germline immunogen

B cell activation
CD4bs specific responses
No neutralization
Dosenovic et al, *Cell* 2015
Jardine et al, *Science* 2015

Sequential immunization

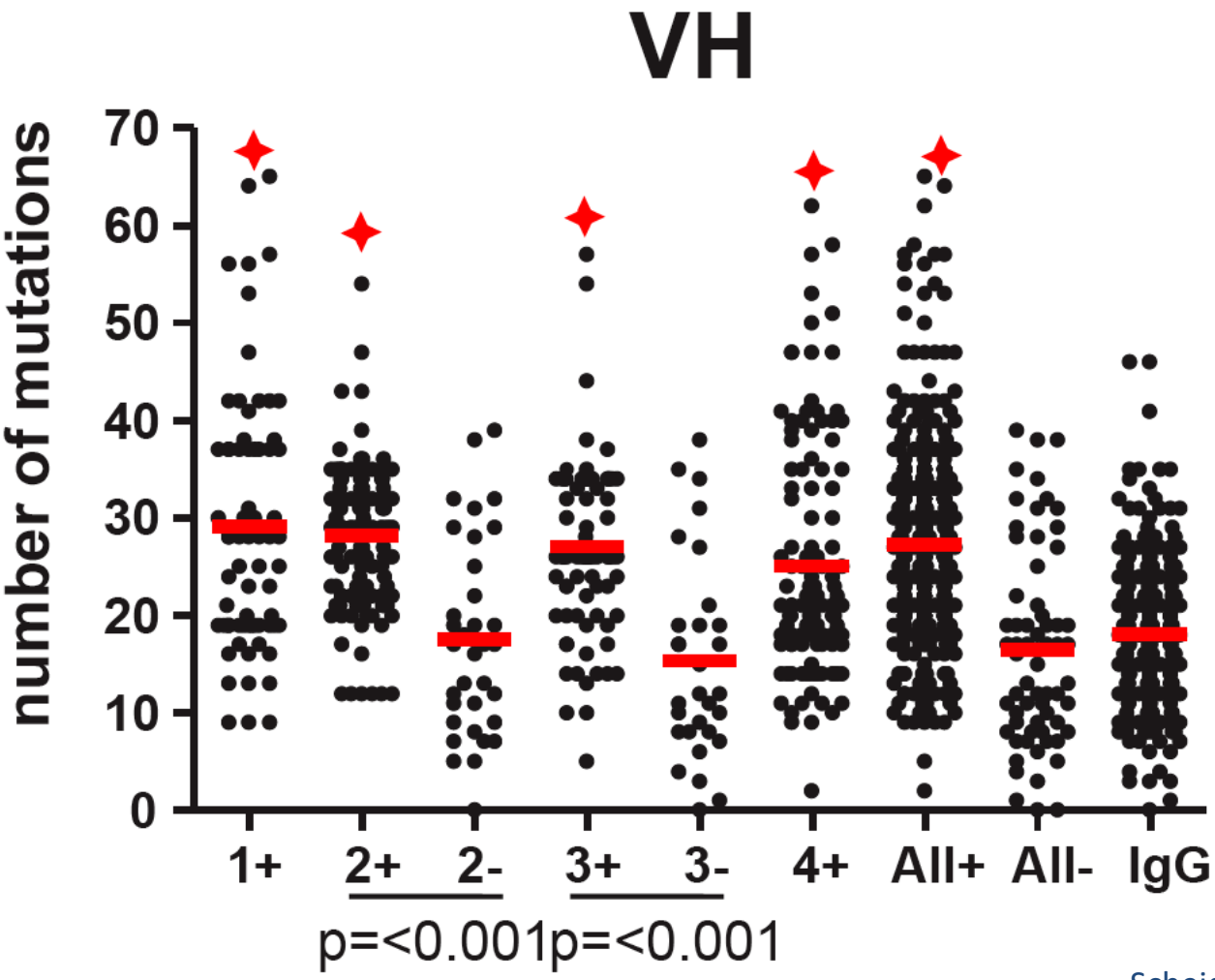


Glycan supersite bnAbs among most common



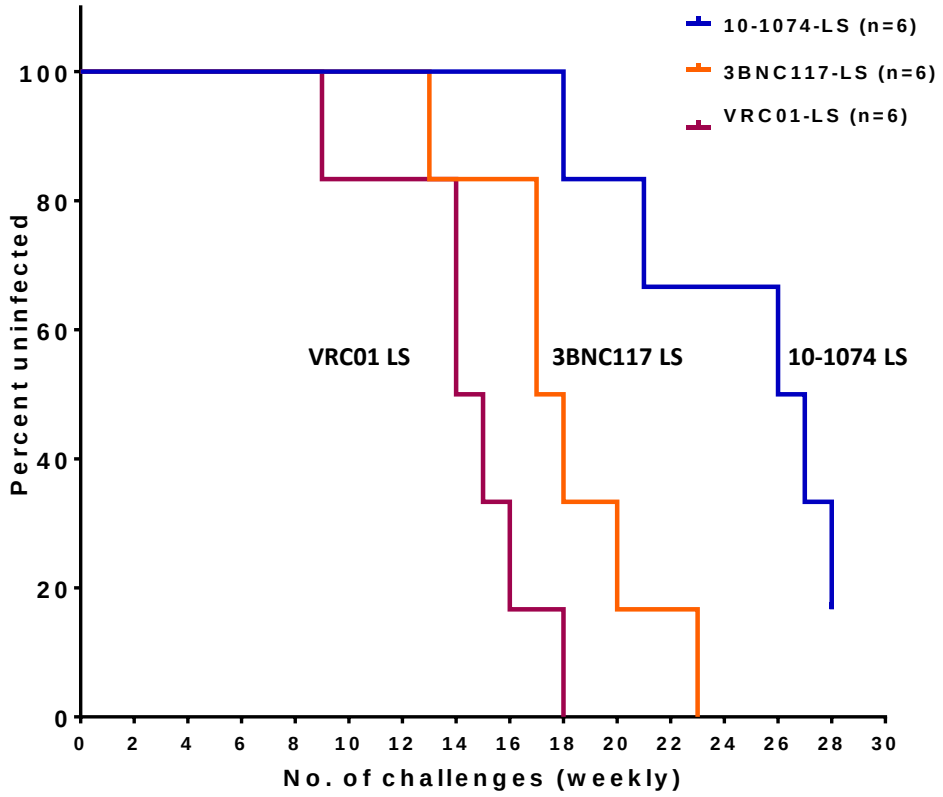
Landais et al Plos Path 2016

Antibodies from gp140+ IgG memory cells are highly mutated



Scheid, J.F. *et.al*. Nature 2009
Mouquet H. et al Nature 2010

THE “LS” MUTATION OF bNAbs SIGNIFICANTLY INCREASES THEIR EFFICACY *in vivo*



10-1074 First-in-Human Study

Study Design: open-label, single infusion

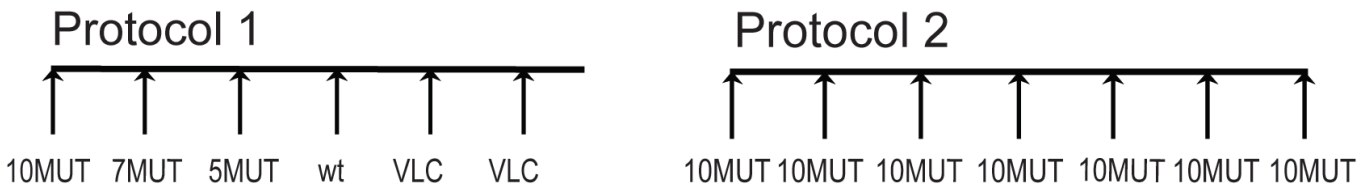
3 dose levels (**3, 10 or 30 mg/kg**)

Enrollment

- 32 participants enrolled
- 18 HIV-1-infected (15 off ART and 3 on ART)
- Baseline HIV-1 VL in participants off ART: 840 – 77,610 cp/ml

Caskey et al Nature Med 2017

Neutralizing monoclonal antibodies elicited by sequential immunization



GL_{HL}

Mouse	Ab	MuLV	R1166	P1981	6535	Q23	Du156	92RW	BG505	IC22	CAAN	JRCSF	T250	HIV001	X2088	91084B7
1	1	ND	ND	0.008	0.036	0.014	0.037	0.015	0.314	0.008	-	0.802	ND	ND	0.819	1.2
1	2	ND	ND	0.004	0.009	0.005	0.011	0.004	0.55	0.003	-	0.334	ND	ND	0.037	0.712
1	3	ND	ND	0.007	0.014	0.016	0.024	0.012	0.598	0.008	-	0.739	ND	ND	0.609	0.862
1	4	ND	ND	0.006	0.015	0.012	0.021	0.013	ND	0.009	-	1.39	ND	ND	0.692	1.34
1	5	ND	ND	0.016	0.026	0.024	0.059	0.022	ND	0.011	-	ND	ND	ND	0.316	ND
7	6	ND	ND	-	-	0.033	-	ND	4.55	1.33	ND	ND	ND	ND	-	-
1	7	ND	ND	0.009	0.043	0.019	0.089	0.007	ND	0.007	0.043	0.47	ND	ND	-	-
7	8	ND	ND	0.126	0.197	0.292	0.239	0.256	ND	0.186	10	ND	ND	ND	-	-
1	9	ND	ND	0.042	0.1	0.068	0.166	0.045	10	0.027	0.243	1.78	ND	ND	-	-
1	10	ND	ND	0.038	0.124	0.058	0.205	0.039	10	0.02	0.267	2.28	ND	ND	-	-
2	11	ND	ND	0.023	-	0.062	0.113	0.183	2.51	0.033	0.449	1.6	ND	ND	-	-
2	12	ND	ND	0.011	-	0.057	0.021	0.387	ND	0.042	10	10	ND	ND	-	-
2	13	ND	ND	0.02	-	0.066	0.182	0.246	ND	0.069	2.5	10	ND	ND	-	-
1	14	ND	ND	0.058	-	10	ND	10	ND	4.85	ND	ND	ND	ND	-	-
1	15	ND	ND	0.02	-	0.272	1.58	0.485	ND	0.096	2.5	ND	ND	ND	-	-

Protocol 2

15	36	ND	ND	ND	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
16	37	ND	ND	ND	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
16	38	ND	ND	ND	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
16	39	ND	ND	ND	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-
15	40	ND	ND	ND	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-

PGT121		ND	ND	0.005	0.005	0.019	0.007	0.002	0.023	0.002	0.031	0.02	0.004	0.041	0.003	0.016

Escolano et al Cell 2016

SUMMARY

- Elite controller status can be conferred by administering combination bNAbs very early during the acute SHIVAD8/macaque infection.

- bNAb immunotherapy during the acute infection differs from cART by facilitating the emergence of potent immunity able to suppress virus replication.

- Based on the results of the depleting anti-CD8β experiment, $CD8^+$ T cells, not NK, NKT, or γδT cells, presumably induced by immune complexes formed following the administration of bNAbs, mediate long-term control of viremia.

- In addition to the 6 Controller macaques, 4 Non-Controller animals maintained their $CD4^+$ T cell counts and suppressed plasma viremia to very low levels for more than 2 years following a single course of bNAb treatment.

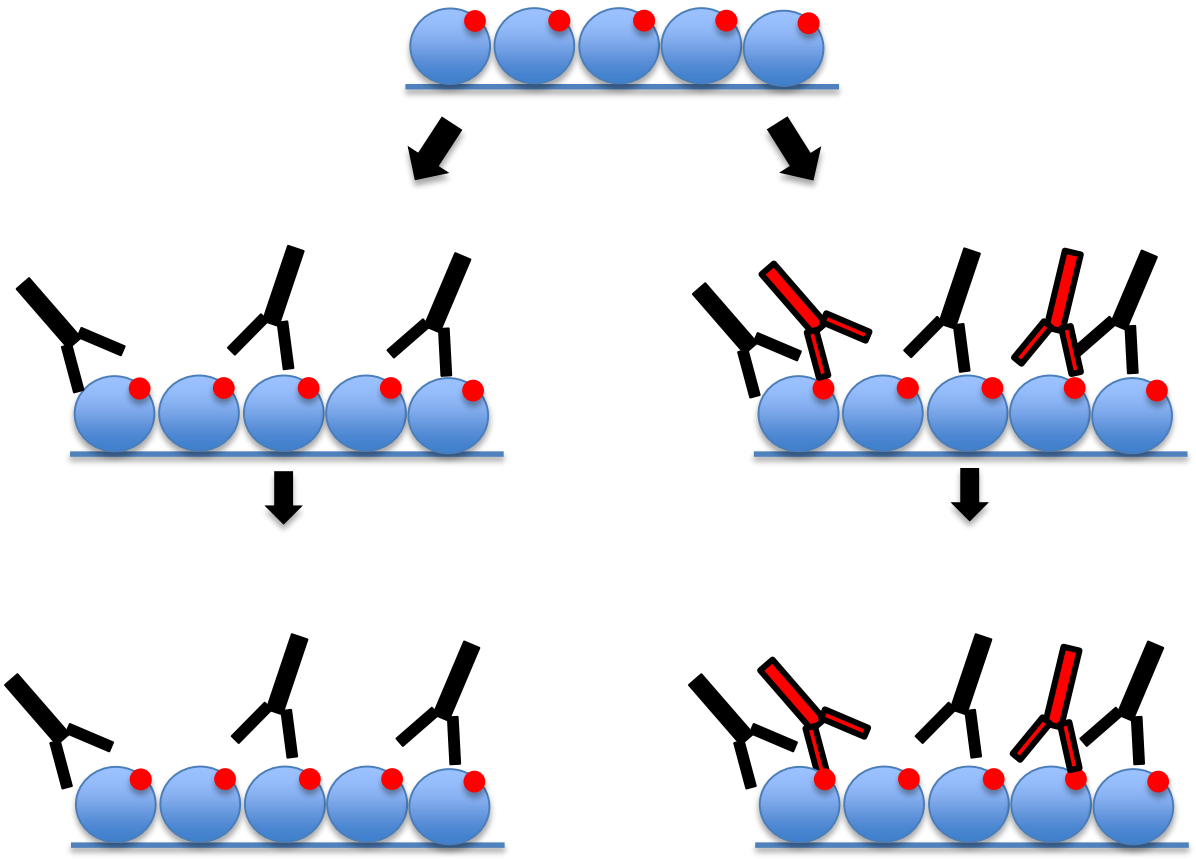
Nishimura et al *Nature* 2017

Discovery of Recurrent Antibodies Against the Domain III of the ZIKV Envelope

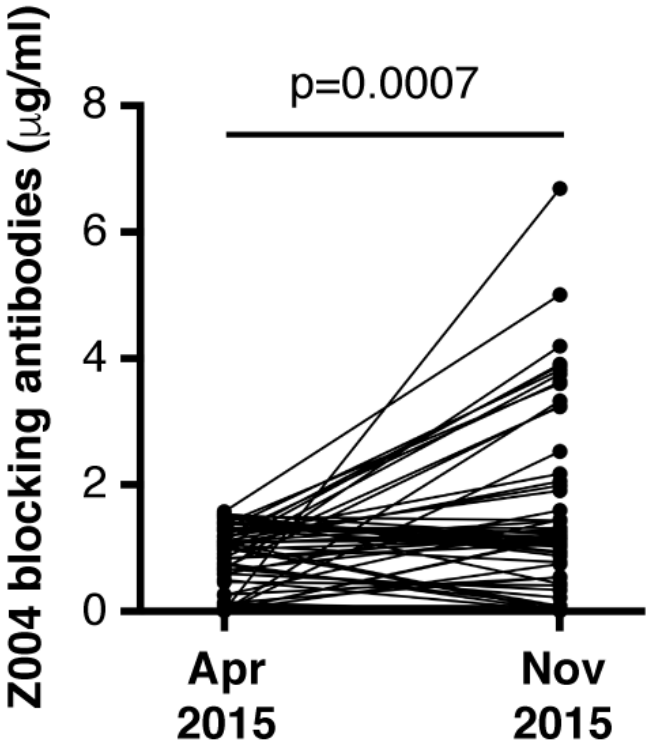
	HEAVY CHAIN			LIGHT CHAIN	
DONOR ID	V GENE	D GENE	J GENE	V GENE	J GENE
MEX 84	IGHV3-23*01	IGHD3-10*01	IGHJ5*01	IGKV1-5*03	IGKJ1*01
MEX 18	IGHV3-23*01	IGHD3-10*01	IGHJ4*02	IGKV1-5*03	IGKJ1*01
MEX 105	IGHV3-23*03	IGHD6-19*01	IGHJ4*02	IGKV1-5*03	IGKJ1*01
BRA 112	IGHV3-23*01	IGHD3-10*01	IGHJ4*02	IGKV1-5*03	IGKJ1*01
BRA 12	IGHV3-23*01	IGHD6-19*01	IGHJ4*02	IGKV1-5*03	IGKJ1*01

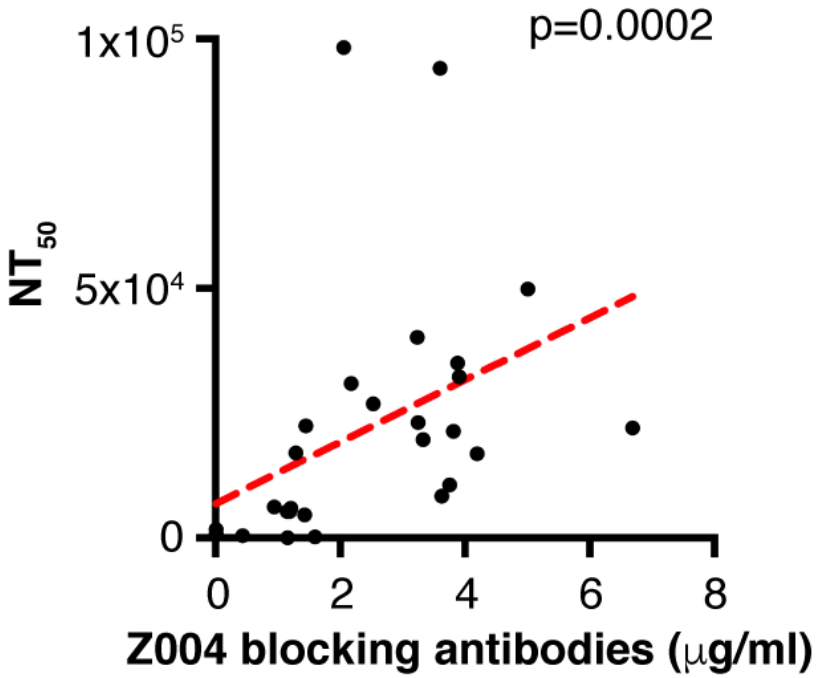
Competition ELISA to Evaluate Antibodies in Serum to the Epitope Recognized by Z004

● = Z004 epitope



Lateral Ridge Antibodies Correlate with ZIKV Neutralization





Z004: Mechanism of Recognition

387

IVIGVGEKKITHHW **ZIKV** (KJ776791)
IVIGVGDKKITHHW **ZIKV African**(MR766)
IVVGAGEKALKLSW **DENV1** (NC_001477)
IIIGVEPGQLKLNW **DENV2** (NC_001474)
IVIGIGDNALKINW **DENV3** (NC_001475)
IVIGVGNSALTLHW **DENV4** (NC_002640)
IIVGRGDSRLTYQW **YFV 17D** (KF769015)
IIVGRGDSRLTYQW **YFV Asibi** (KF769016)
IVVGRGEQQKNHHW **WNV** (NC_009942)