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Measuring *in vitro* Susceptibility to bNAbs

Nicole Doria-Rose, PhD
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October 30, 2023

Measuring *in vitro* Susceptibility to bNAbs

- Most common measurement: **Neutralization Assay**
  - Prevention of target cell infection by free virus
- Neutralization typically measured with **pseudoviruses** on cell lines
  - Other assays: use PBMC-grown virus on cell lines or on PBMC
- Other functions may be measured:
  - Blocking cell-to-cell infection
  - Non-neutralizing functions, *eg* ADCC
  - IgG glycoforms
  - Viral outgrowth

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  - Blocking cell-to-cell infection
  - Non-neutralizing functions, *eg* ADCC
  - IgG glycoforms
  - Viral outgrowth

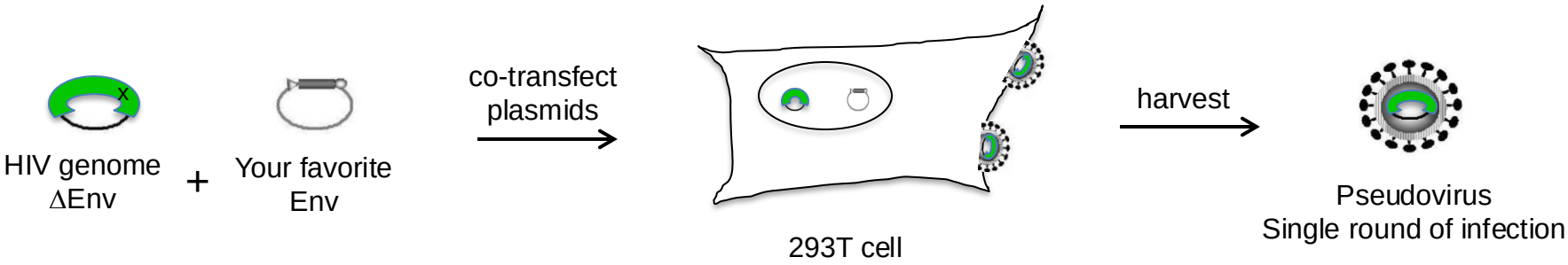
➢ Only neutralization has been shown to correlate with *in vivo* protection

How are the assays done?

What do the numbers mean?

Advantages of Env-pseudoviruses

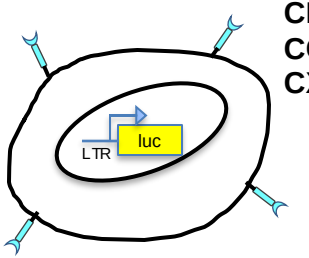
- Single round of infection
- Assay is reproducible
- Quickly produce stocks by co-transfection of standard HIV genome backbone with Env of choice
  - Can have hundreds of Env strains, mutants
  - Do not need to grow large stocks of replicating HIV – do not need BSL3 lab



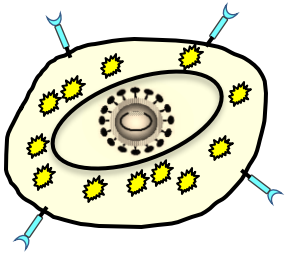
Neutralization Assay

No antibodies
or
Non-neutralizing
antibodies

+



Infection



Add to target cells

Co-incubate 48 hours

Measure infection (luciferase activity)

Neutralization Assay

No antibodies
or
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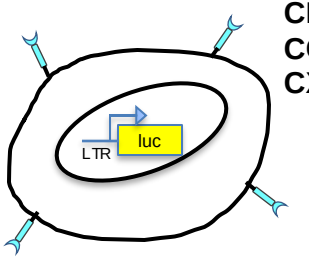
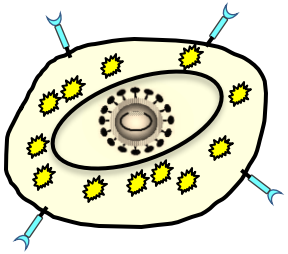
+

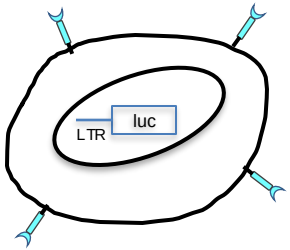


Infection

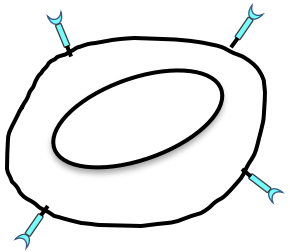


Neutralizing
antibodies

+



No infection



Co-
incubate
1 hour

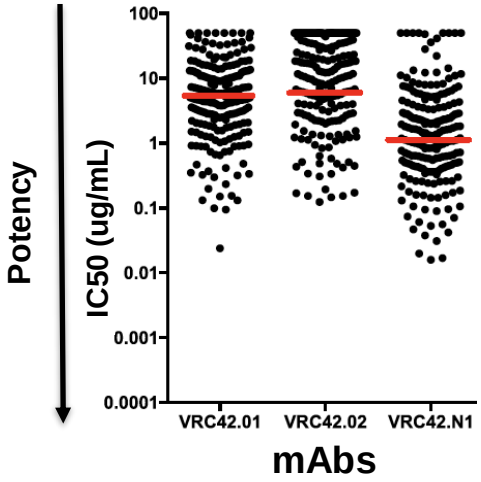
Add to target
cells

Co-
incubate
48 hours

Measure
reduction in
luciferase
activity

Quantifying Neutralization Data

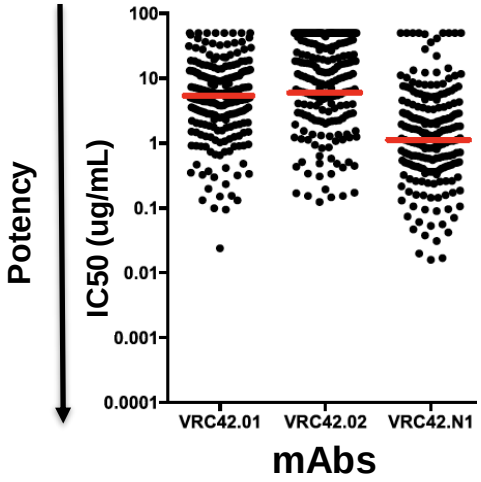
- **IC50** or **IC80**: concentration of antibodies at which 50% or 80% of virus is neutralized
  - ➢ Smaller values = more potent

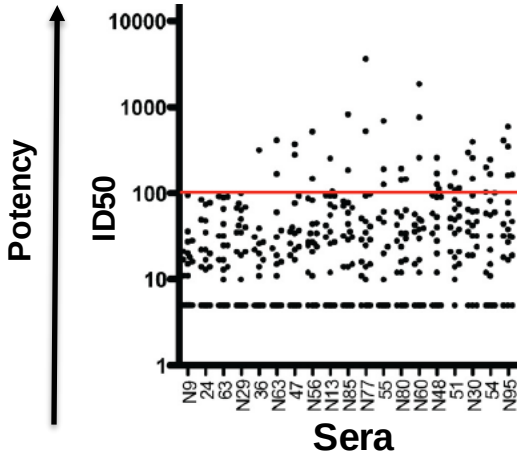


Krebs *et al* 2019 Immunity
Doria-Rose *et al* JVI 2010

Quantifying Neutralization Data

- **IC50** or **IC80**: concentration of antibodies at which 50% or 80% of virus is neutralized
  - ➢ Smaller values = more potent
- **ID50** or **ID80**: reciprocal dilution of serum or plasma at which 50% or 80% of virus is neutralized
  - ➢ Larger values = more potent





Krebs *et al* 2019 Immunity
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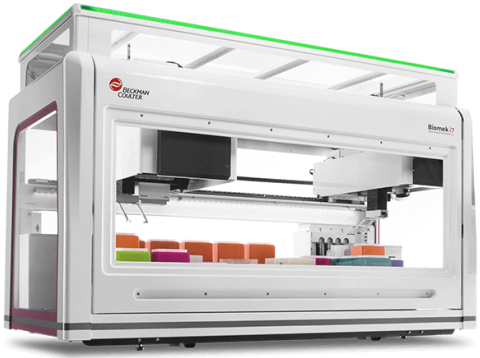
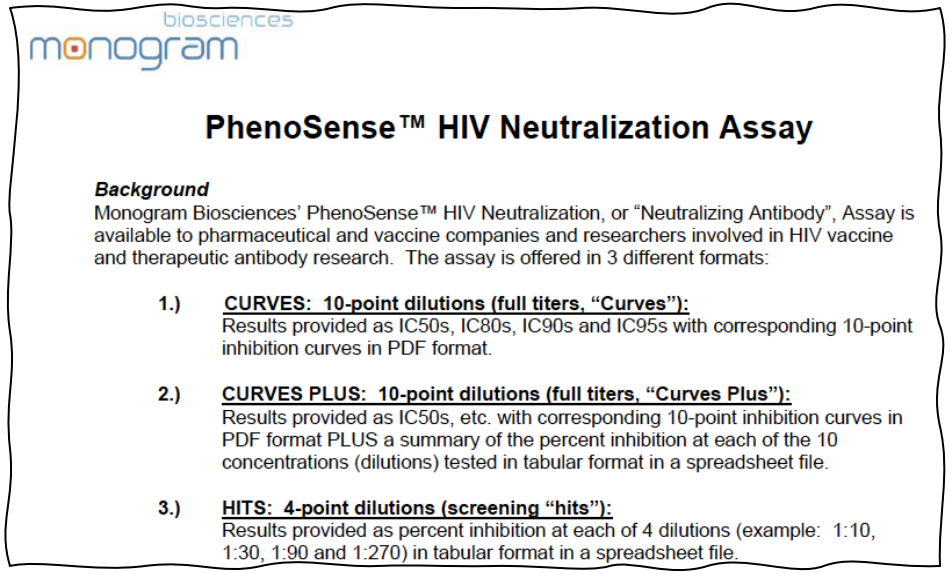
Scale of pseudovirus neutralization assays

- Assays can be done manually or in automated systems
- Small-scale in many research labs
- Large-scale in a few research labs
  - NIH/VRC, IAVI, Duke (Montefiori), Harvard (Seaman)
- Commercially: Monogram



Commercial Testing: Monogram PhenoSense nAb Assay

- Assays for mAbs are validated to CLIA/CAP specifications
- Characterizing novel bNAbs
- Pre-screening Envs from potential participants in antibody therapy trials



biosciences
monogram

PhenoSense™ HIV Neutralization Assay

Background

Monogram Biosciences' PhenoSense™ HIV Neutralization, or "Neutralizing Antibody", Assay is available to pharmaceutical and vaccine companies and researchers involved in HIV vaccine and therapeutic antibody research. The assay is offered in 3 different formats:

1.) **CURVES: 10-point dilutions (full titers, "Curves"):**
Results provided as IC50s, IC80s, IC90s and IC95s with corresponding 10-point inhibition curves in PDF format.

2.) **CURVES PLUS: 10-point dilutions (full titers, "Curves Plus"):**
Results provided as IC50s, etc. with corresponding 10-point inhibition curves in PDF format PLUS a summary of the percent inhibition at each of the 10 concentrations (dilutions) tested in tabular format in a spreadsheet file.

3.) **HITS: 4-point dilutions (screening "hits"):**
Results provided as percent inhibition at each of 4 dilutions (example: 1:10, 1:30, 1:90 and 1:270) in tabular format in a spreadsheet file.

Does *in vitro* neutralization correlate with *in vivo* protection?

NHP: *In vitro* potency predicts *in vivo* protection

- Lower doses of a more potent antibody protect NHP from SHIV challenge

IC50 vs challenge virus:

PGDM1400
0.04 µg/ml

CAP256-VRC26.25-LS
0.003 µg/ml

Julg *et al* 2017 Sci Transl Med

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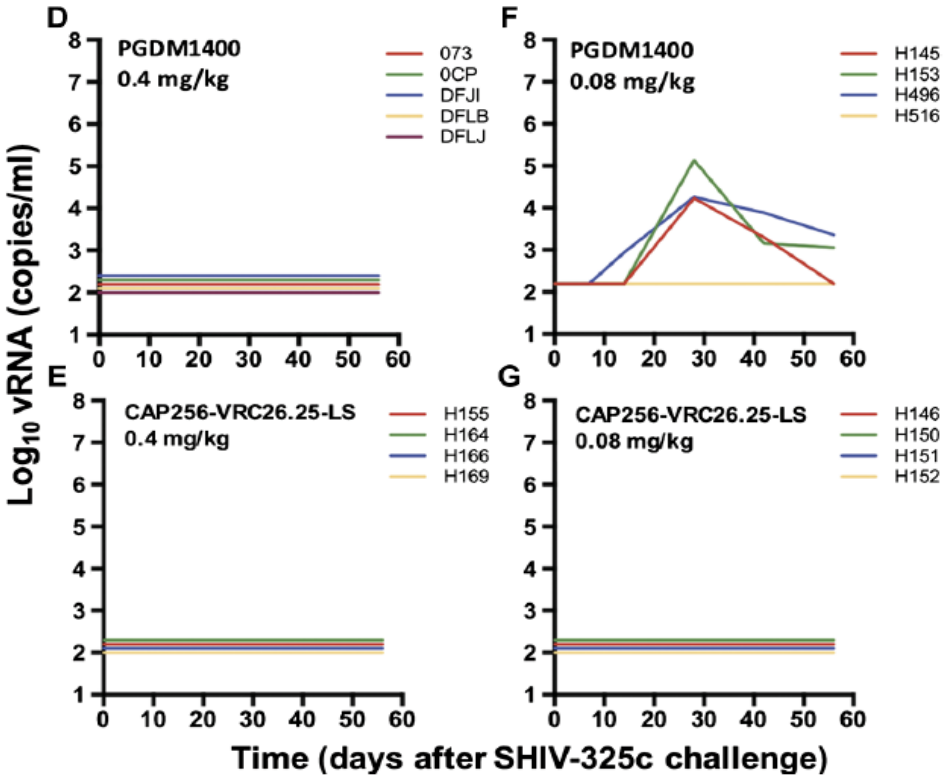
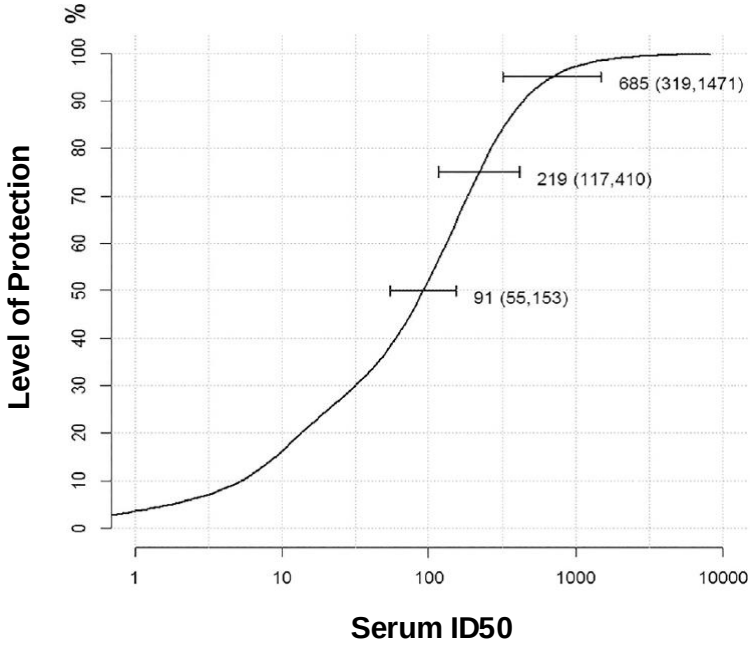


Julg *et al* 2017 Sci Transl Med

NHP: *In vitro* potency predicts *in vivo* protection

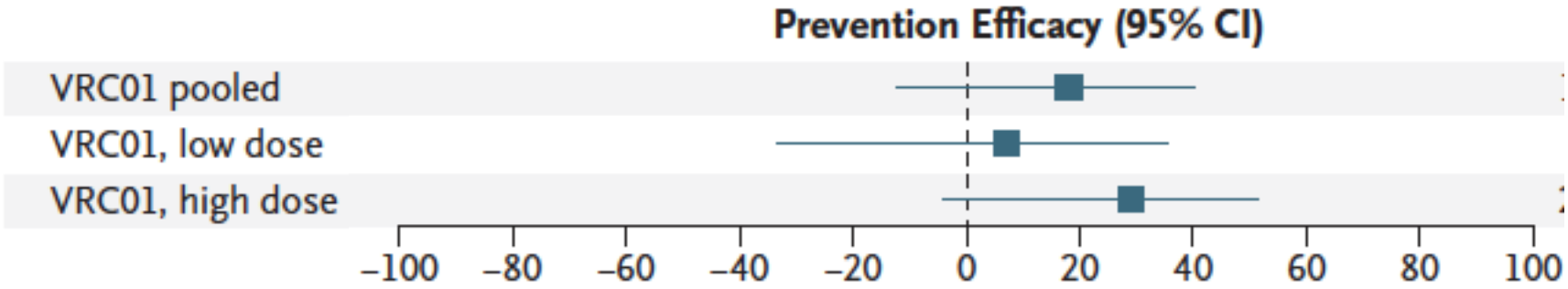
- Lower doses of a more potent antibody protect NHP from SHIV challenge
- Meta-analysis of NHP studies shows correlation of ID50 with protection



Pegu *et al* Cell Host Microbe 2019

Proof-of-concept in Humans: bNAbs for Prevention

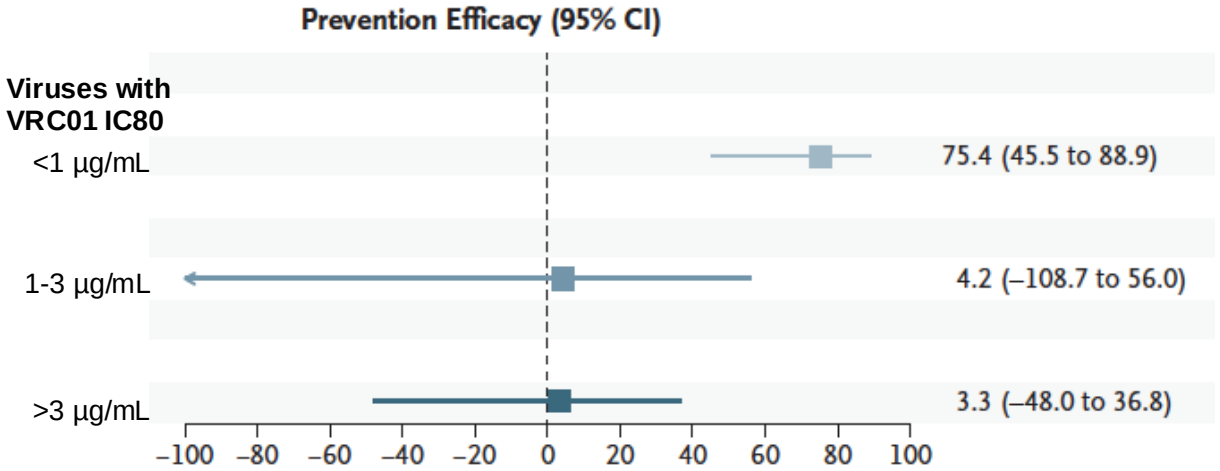
- AMP Clinical Trials: can the bNAb VRC01 prevent HIV infection?
  - HVTN703 (women, Africa) and HVTN 704 (men, Americas), n=4600
  - 10 or 30 mg/kg VRC01 IV, every 8 weeks, *vs* placebo
- No overall preventive effect



Corey *et al* 2021 NEJM

AMP trials: bNAb efficacy against sensitive viruses

- Envs from all infected participants were tested for sensitivity to VRC01 in pseudovirus neutralization assay
- VRC01 prevented infection by very sensitive viruses
  - ➢ Proof of concept that bNAbs can prevent infection
  - ➢ Support for clinical relevance of pseudovirus assay

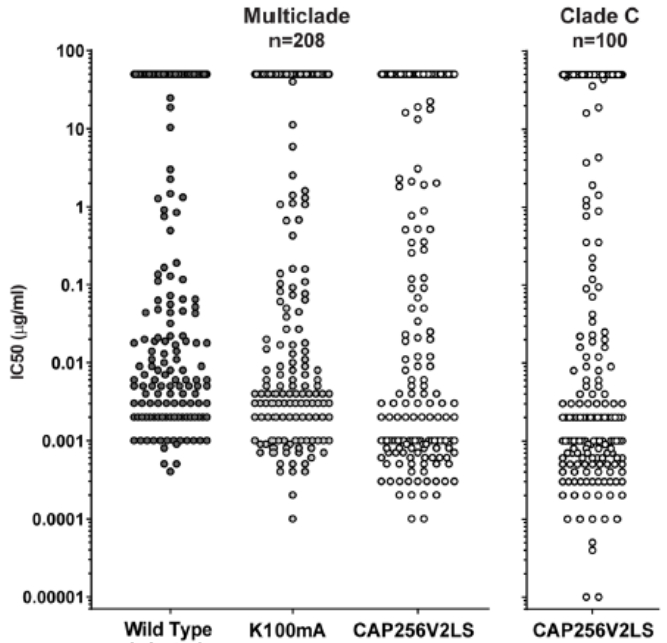


Corey *et al* 2021 NEJM

How can we use neutralization data?

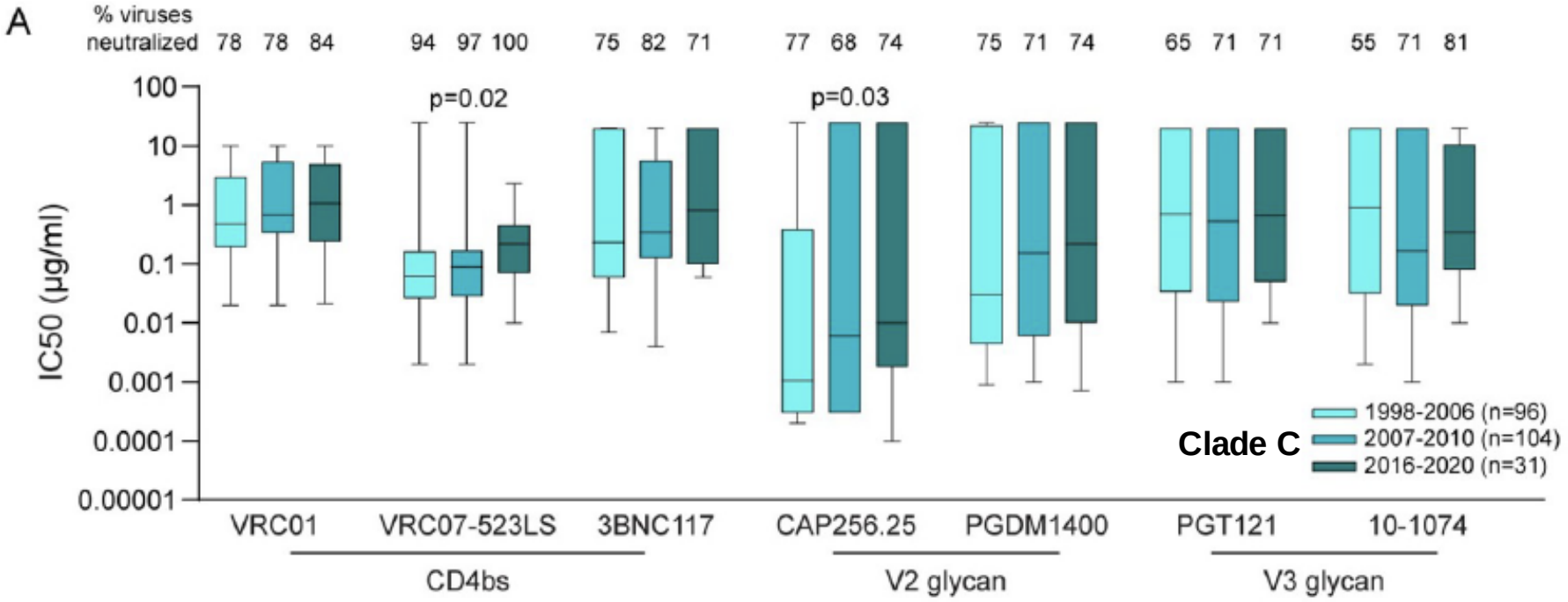
Assessing bNAbs for clinical use

- Measure breadth and potency on large pseudovirus panels:
  - Number of Envs? (often 100-200)
  - Single clade or multi-clade?
  - Local or global?
  - Contemporary?



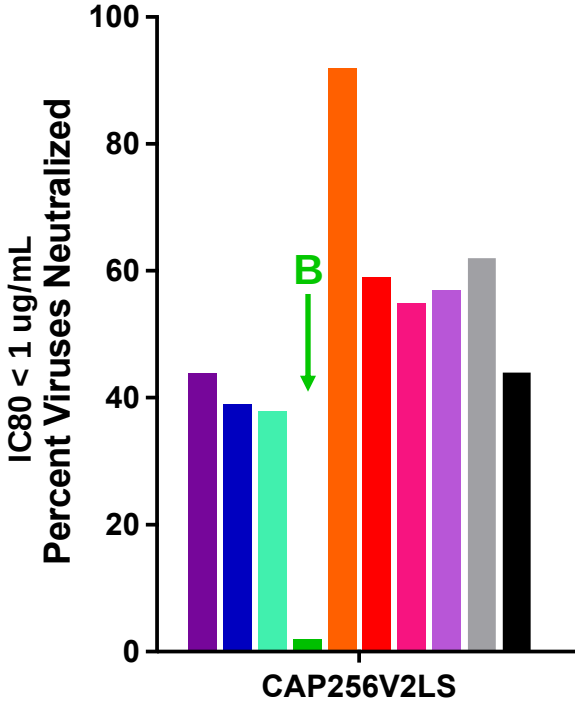
Zhang *et al* Sci Rep 2022

Should we focus on contemporary viruses?



Mkhise *et al* 2023 PLoS Path

Consider Clades and Geography



Clade

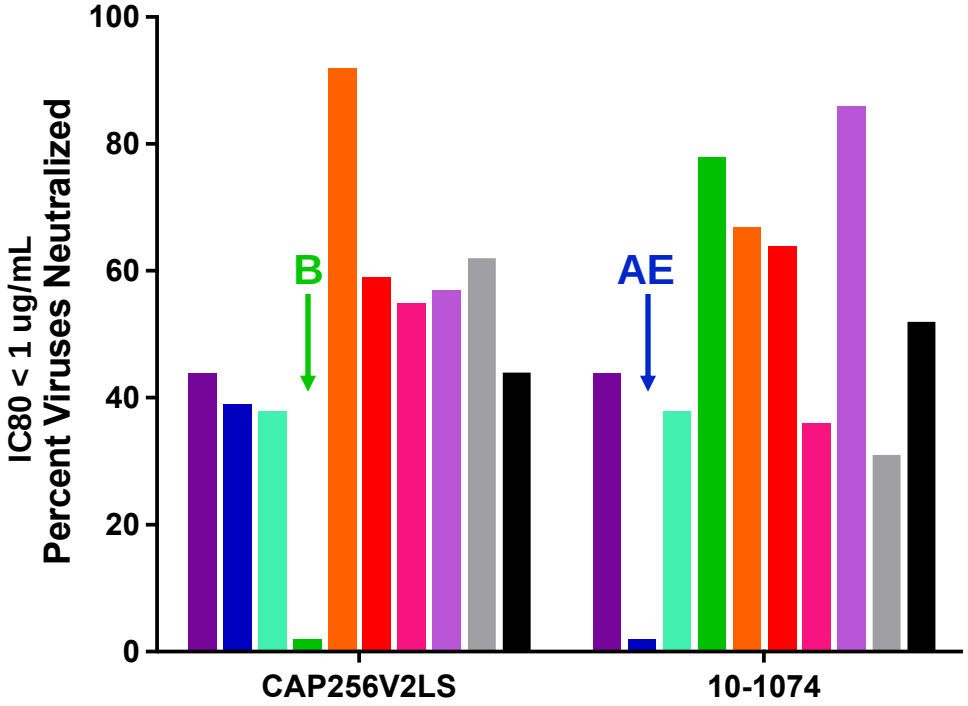
- A
- AE
- AG
- B
- BC
- C
- D
- G
- OTHER
- All Viruses

Other: AC/ACD/AD/CD

n=208

Krisha McKee

Consider Clades and Geography



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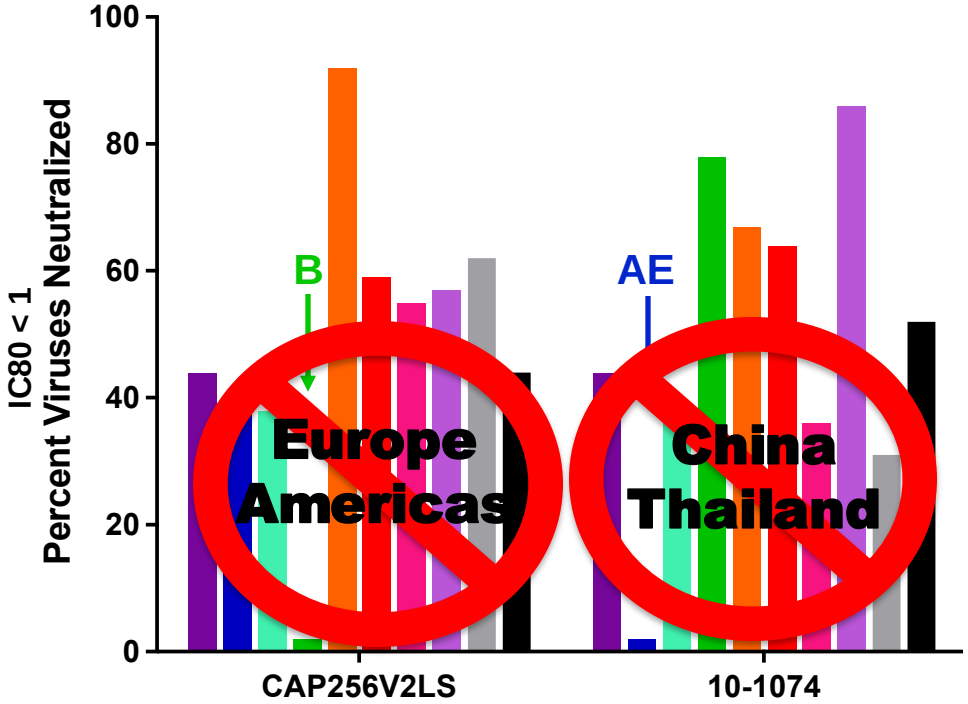
Krisha McKee

Some mAbs Miss Entire Clades: Geographic Restrictions



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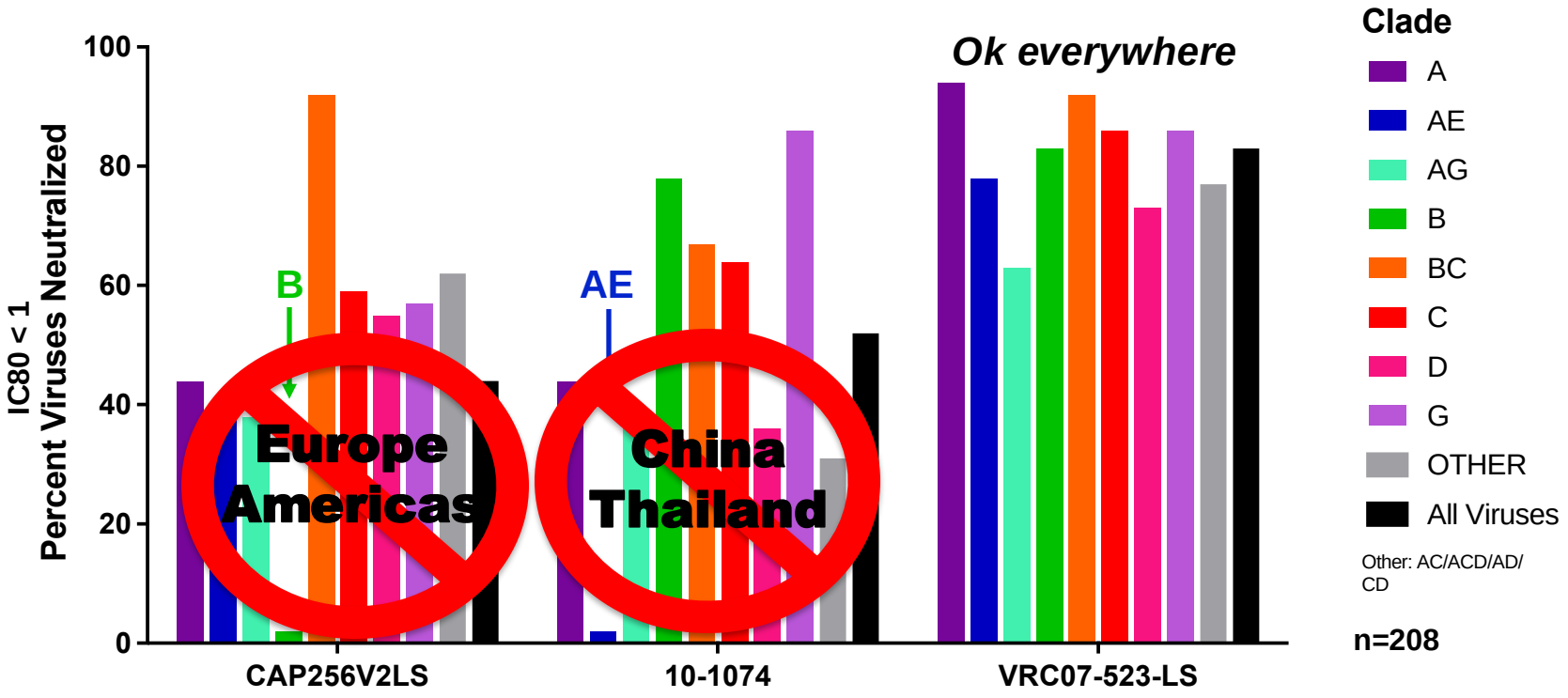
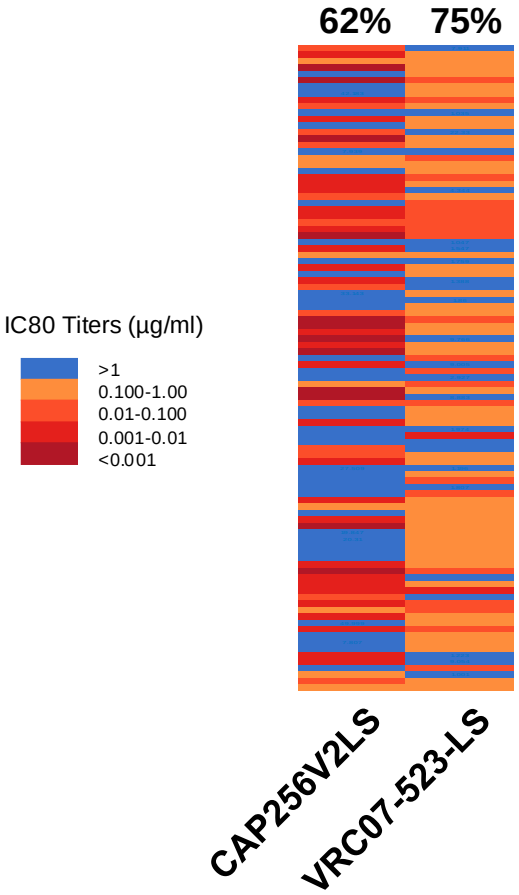
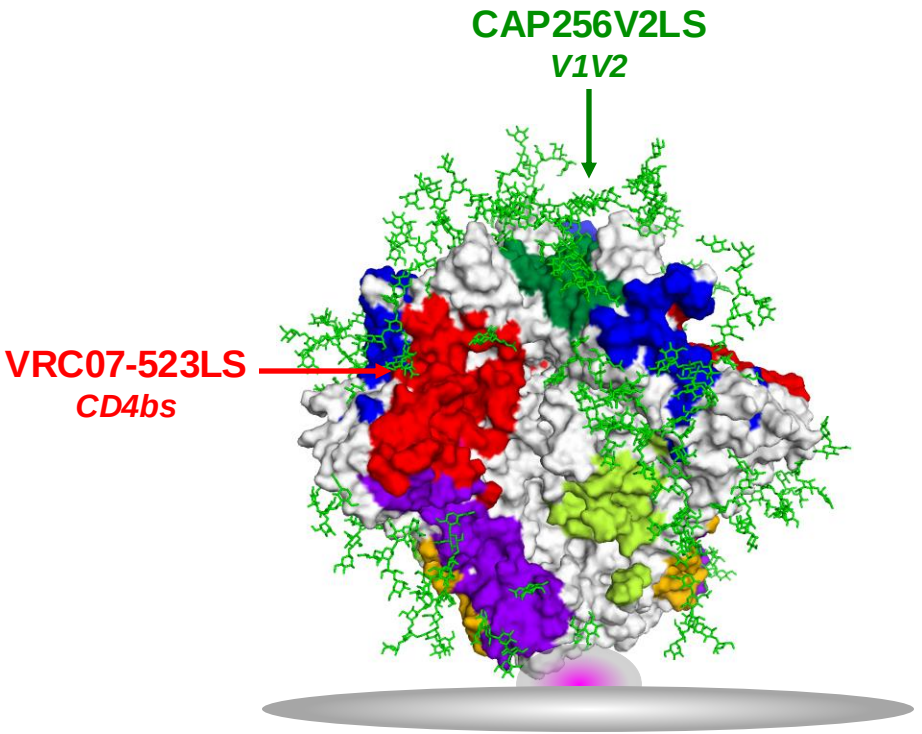


Krisha McKee

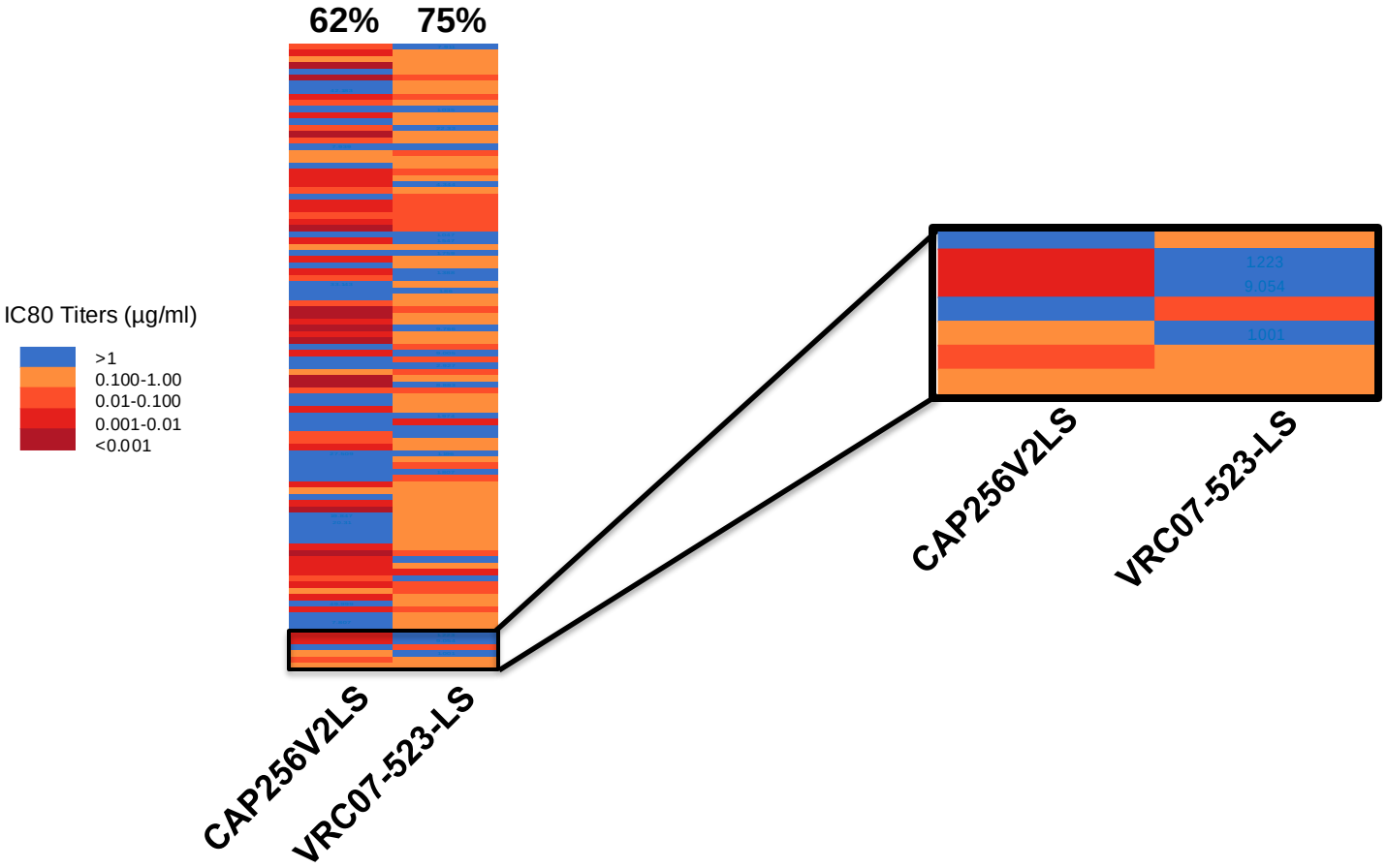
Coverage Complementarity





Krisha McKee
Michael Seaman
Carolyn Williamson

Coverage Complementarity



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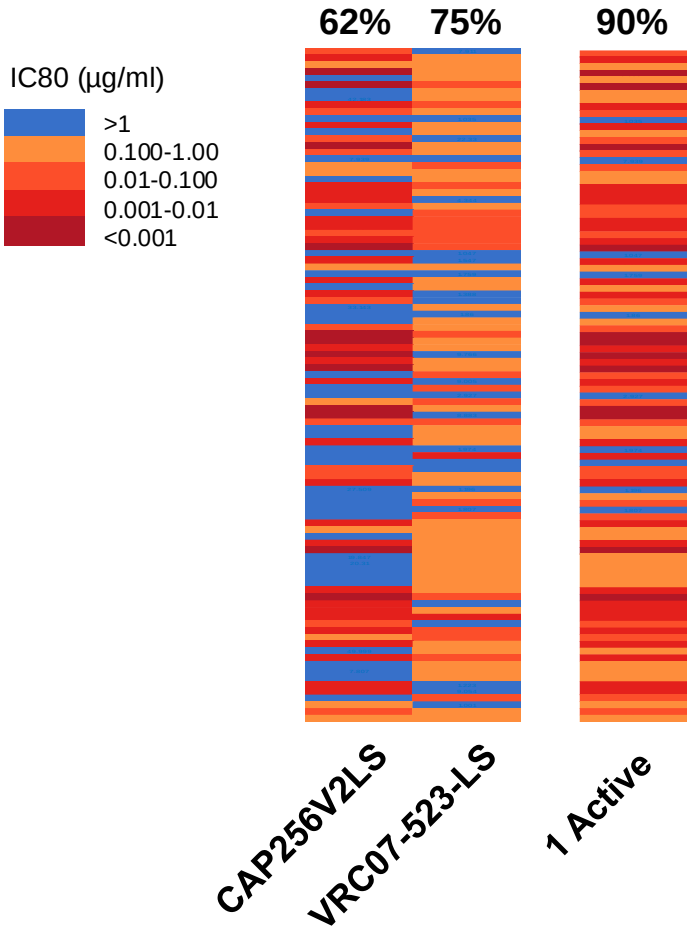


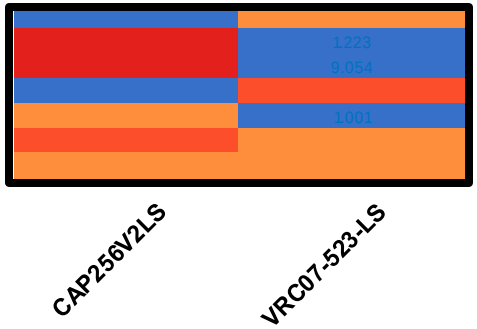
To express activity of combination:

For each virus, count the IC80 of the more potent antibody

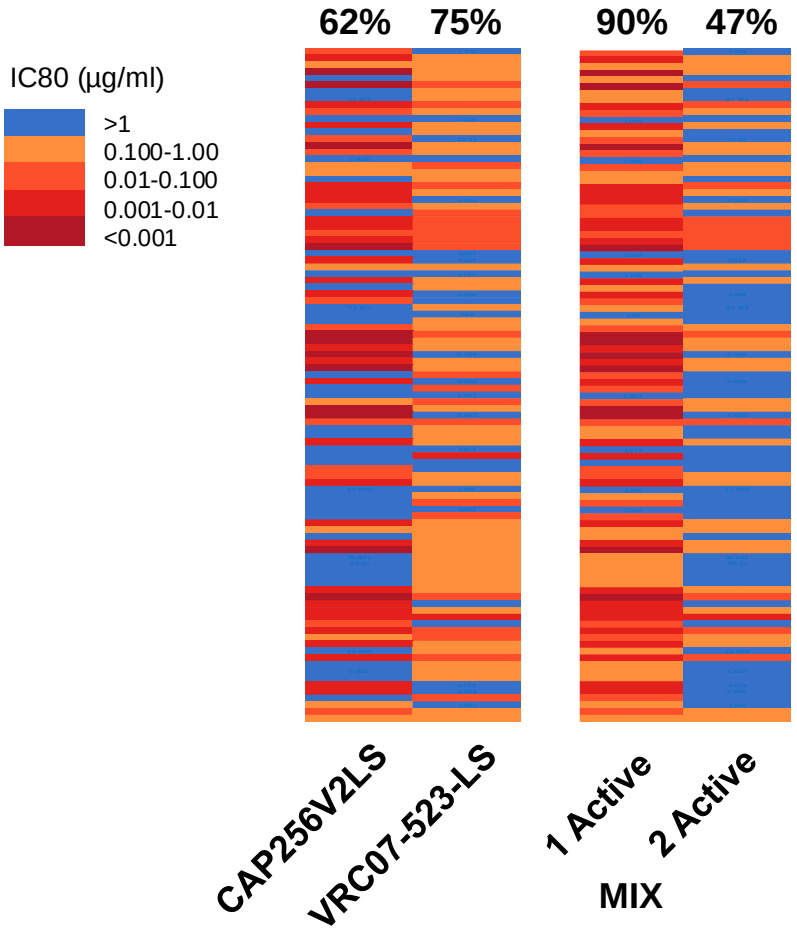
Krisha McKee
Michael Seaman
Carolyn Williamson

Combinations: One mAb active, or more than one?

- We often show coverage assuming at least one mAb is active
  - *ie* count viruses that are sensitive to at least one mAb in the combination
- For some viruses, a combo is effectively monotherapy
  - This is probably acceptable for prevention
  - For treatment, monotherapy is very unlikely to work



Lower Coverage if you Require 2 Active mAbs



To express activity of combination:

<u>1 Active</u>
For each virus, count the IC80 of the more potent antibody

<u>2 Active</u>
For each virus, count the IC80 of the less potent antibody

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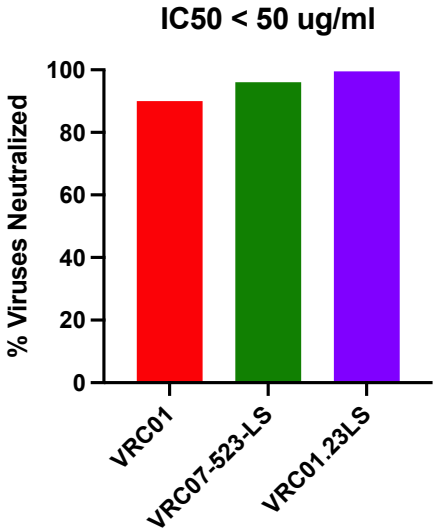
Michael Seaman
Carolyn Williamson

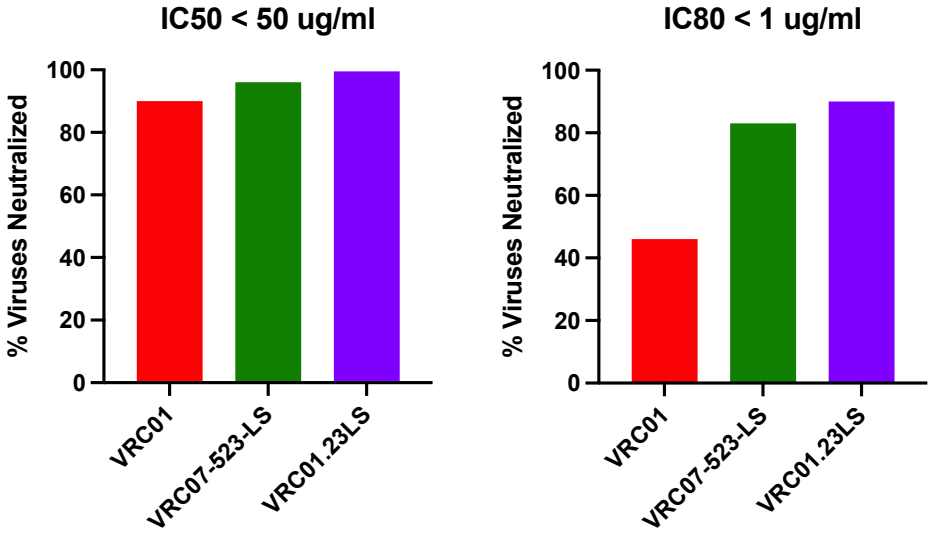
How do we define breadth of coverage?

- Neutralization on large panels of (pseudo)viruses
- Old definition: sensitive if IC50 < 50 µg/ml



How do we define breadth of coverage?

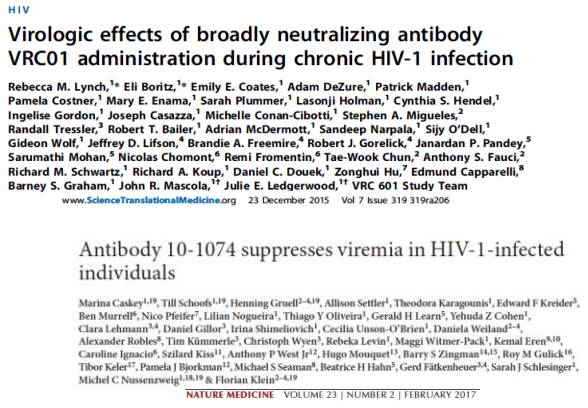
- Neutralization on large panels of (pseudo)viruses
- Old definition: sensitive if IC50 < 50 µg/ml
- Post-AMP: sensitive if IC80 < 1 µg/ml



How can we use neutralization data for treatment trials?

Neutralization assays in therapy trials

- In some bNAb therapy trials (viremic or ATI), subjects were pre-screened for Envs that were sensitive to the study bNAb
  - Clone and test individual Envs
  - Shotgun sequencing, test pseudovirus mix
- Sequencing and sensitivity testing of rebound viruses can help explain loss of bNAb-mediated control



HIV

Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection

Rebecca M. Lynch, Eli Boritz, Emily E. Coates, Adam DeZure, Patrick Madden, Pamela Costner, Mary E. Enama, Sarah Plummer, Lasonji Holman, Cynthia S. Hendel, Ingelise Gordon, Joseph Casazza, Michelle Conan-Cibotti, Stephen A. Migueles, Randall Tressler, Robert T. Bailer, Adrian McDermott, Sandeep Narpala, Sijy O'Dell, Gideon Wolf, Jeffrey D. Lifson, Brandie A. Freemire, Robert J. Gorelick, Janardan P. Pandey, Sarumathi Mohan, Nicolas Chomont, Remi Fromentin, Tae-Wook Chun, Anthony S. Fauci, Richard M. Schwartz, Richard A. Koup, Daniel C. Douek, Zonghui Hu, Edmund Capparelli, Barney S. Graham, John R. Mascola, Julie E. Ledgerwood, VRC 601 Study Team

www.ScienceTranslationalMedicine.org 23 December 2015 Vol 7 Issue 319 319ra206

Antibody 10-1074 suppresses viremia in HIV-1-infected individuals

Marina Caskey, Till Schoofs, Henning Gruell, Allison Settler, Theodora Karagounis, Edward F Kreider, Ben Murrell, Nico Pfeifer, Lilian Nogueira, Thiago Y Oliveira, Gerald H Learn, Yehuda Z Cohen, Clara Lehmann, Daniel Gillor, Irina Shimelovich, Cecilia Unson-O'Brien, Daniela Weiland, Alexander Robles, Tim Kümmerle, Christoph Wyen, Rebeka Levin, Maggi Witmer-Pack, Kemal Eren, Caroline Ignacio, Szilard Kiss, Anthony P West Jr, Hugo Mouquet, Barry S Zingman, Roy M Gulick, Tibor Keler, Pamela J Bjorkman, Michael S Seaman, Beatrice H Hahn, Gerd Fätkenheuer, Sarah J Schlesinger, Michel C Nussenzweig & Florian Klein

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Prolonged viral suppression with anti-HIV-1 antibody therapy

https://doi.org/10.1038/s41586-022-04597-1

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Christian Gaebler, Lilian Nogueira, Elina Stoffel, Thiago Y. Oliveira, Gaëlle Breton, Katrina G. Millard, Martina Turroja, Allison Butler, Victor Ramos, Michael S. Seaman, Jacqueline D. Reeves, Christos J. Petropoulos, Irina Shimelovich, Anna Gazumyan, Caroline S. Jung, Nikolaus Jilg, Johannes F. Scheid, Rajesh Gandhi, Bruce D. Walker, Michael C. Sneller, Anthony Fauci, Tae-Wook Chun, Marina Caskey & Michel C. Nussenzweig

368 | Nature | Vol 606 | 9 June 2022

ARTICLES

nature medicine

OPEN

Safety and antiviral activity of triple combination broadly neutralizing monoclonal antibody therapy against HIV-1: a phase 1 clinical trial

Boris Julg, Kathryn E. Stephenson, Kshitij Wagh, Sabrina C. Tan, Rebecca Zash, Stephen Walsh, Jessica Ansel, Diane Kanjilal, Joseph Nkolola, Victoria E. K. Walker-Sperling, Jasper Ophel, Katherine Yanosick, Erica N. Borducchi, Lori Maxfield, Peter Abbink, Lauren Peter, Nicole L. Yates, Martina S. Wesley, Tom Hassell, Huub C. Gelderblom, Allen deCamp, Bryan T. Mayer, Alicia Sato, Monica W. Gerber, Elena E. Giorgi, Lucio Gama, Richard A. Koup, John R. Mascola, Ana Monczor, Sofia Lupo, Charlotte-Paige Rolle, Roberto Arduino, Edwin DeJesus, Georgia D. Tomaras, Michael S. Seaman, Bette Korber and Dan H. Barouch

THE LANCET HIV

Volume 10, Issue 10, October 2023, Pages e653–e662

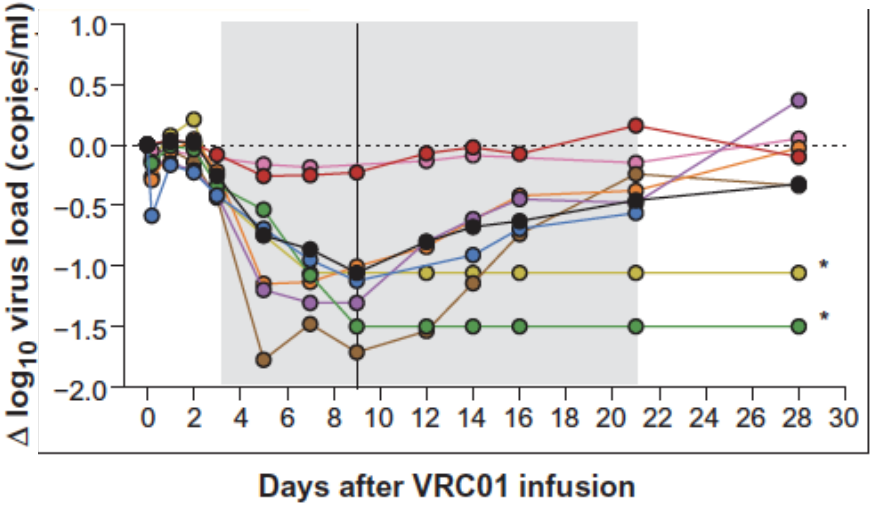
Articles

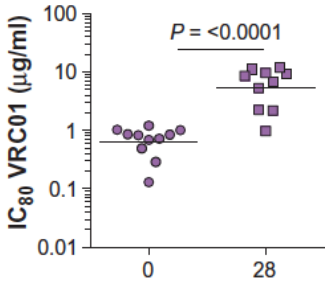
Safety, tolerability, pharmacokinetics, and immunological activity of dual-combinations and triple-combinations of anti-HIV monoclonal antibodies PGT121, PGDM1400, 10-1074, and VRC07-523LS administered intravenously to HIV-uninfected adults: a phase 1 randomised trial

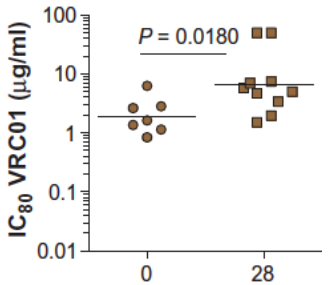
Prof Magdalena E Sobieszczyk MD, Prof Sharon Hannheimer MD, Carmen A Perez MD, Chenchen Yu MS, Theresa Gamble PhD, Deborah A Theodore MD, Wairimu Chege MD, Margaret Yacovone MD, Brett Hanscom PhD, Jack Heptinstall BS, Kelly E Seaton PhD, Lily Zhong MS, Maurine D Miner PhD, Amanda Eaton MBA, Joshua A Weiner PhD, Prof Kenneth Mayer MD, Prof Spyros Kalams MD, Kathryn Stephenson MD, Boris Julg MD, Prof Marina Caskey MD, Prof David Montefiori PhD

Monitoring escape during therapy

- VRC601 trial: Viremic subjects were given one infusion of VRC01
- Some had temporary decrease in viral load
- Envs were sequenced and tested for VRC01 sensitivity
- In some cases, viral rebound was associated with increased resistance to VRC01







Lynch *et al* STM 2015

Conclusions

- Pseudovirus neutralization titers correlate with *in vivo* protection
- The assays are essential for selecting clinical candidates
  - Choice of virus panels, cutoffs may be critical
- The assays can be used clinically in bNAb therapy:
  - Prescreen patients
  - Monitor viral phenotype changes during therapy

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National Institute of Allergy and Infectious Diseases
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Uses of Neutralization Data

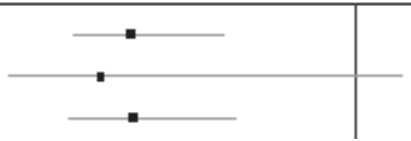
- Discovery, characterization, improvement of bNAbs
  - Breadth
  - Potency
  - Activity against specific clades, local isolates
- Comparing bNAbs to choose clinical products
- Clinical investigation: does neutralization correlate with protection?
- Predicting / monitoring viral escape in treated patients

Pseudovirus neutralization is a correlate of protection for mRNA COVID-19 Vaccine

- Correlates analysis for symptomatic infection in Phase 3 trial of mRNA-1273:
- Binding antibodies and neutralizing antibodies both correlate with protection

C 50% Inhibitory Dilution Pseudovirus Neutralization Titer: Day 57

Group	No. Events	HR (95% CI)
All Vaccine	47	0.42 (0.27, 0.65)
Age ≥ 65	7	0.34 (0.10, 1.12)
Age < 65	40	0.42 (0.26, 0.69)



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PhenoSense nAb Assay: Hx and Applications

- Circa 1996: Development of HIV-1 pseudovirus assays for the measurement of PR/RT inhibitor resistance
- Circa 2001: Adaptation of HIV-1 pseudovirus assays for the measurement of entry inhibitor resistance
- Circa 2001: Adaptation of HIV-1 pseudovirus assays to characterize nAb responses to infection and vaccination
- Circa 2005: Adaptation of HIV-1 pseudovirus assays to determine co-receptor tropism and predict Tx outcome
- Circa 2010: Application of HIV-1 pseudovirus assays for the identification and characterization of novel bnAb
- Circa 2017: Application of HIV-1 pseudovirus assays to evaluate the clinical efficacy of therapeutic bnAb

SBIR-AT-NIAID R43 AI048990 (2001), R44 AI048990 (2003)
SBIR-AT-NIAID R43 AI062522 (2005), R44 AI062522 (2007)

Monogram BIOSCIENCES
LabCorp Specialty Testing Group