# Antibody-based Multipurpose Microbicides

## Antibodies as a Platform Technology for Multipurpose Microbicides:

## Specificity and Versatility

Kevin J Whaley

# Antibodies as Platform Technology for Multipurpose Microbicides

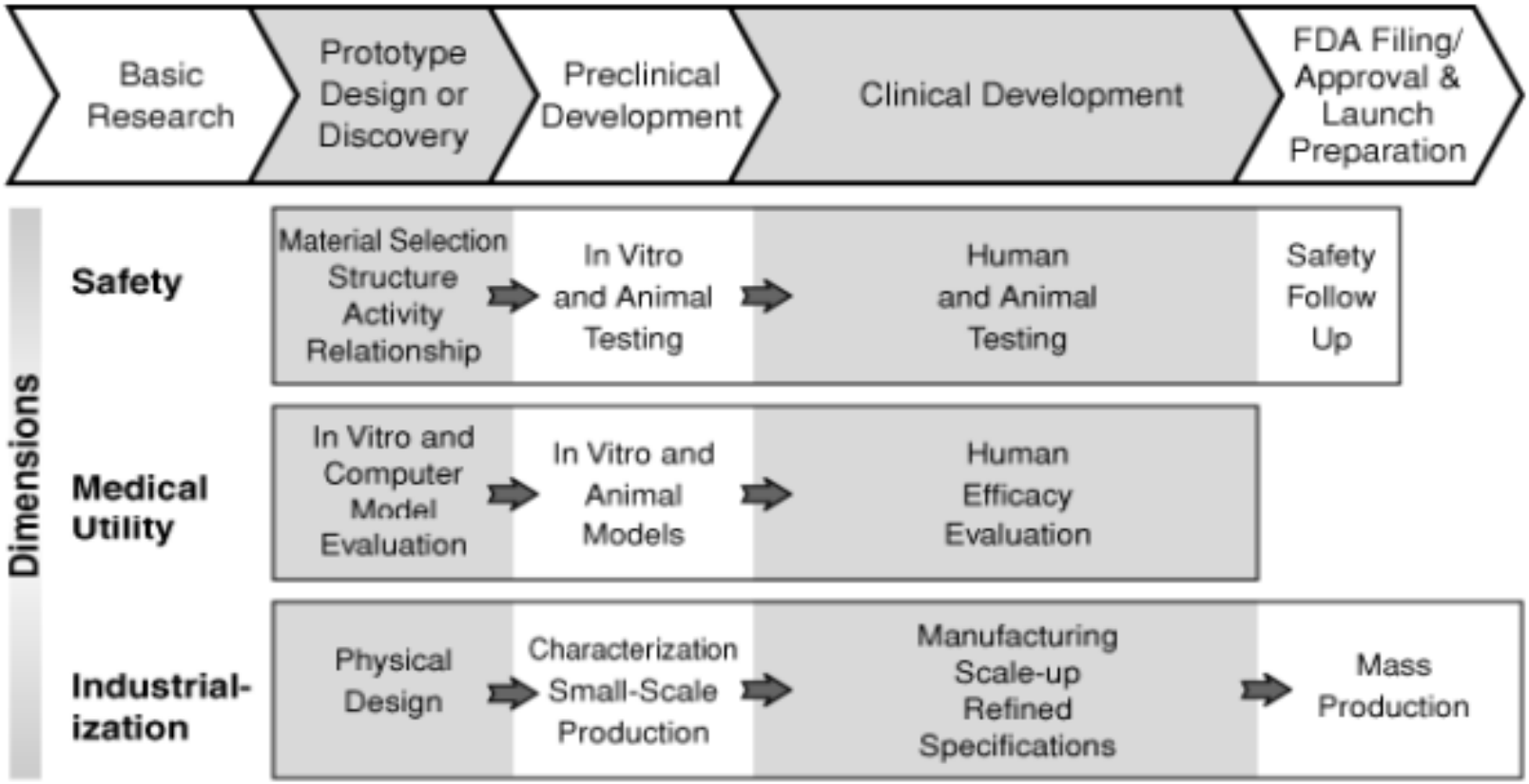
- Commercially available for multiple indications (safety and versatility)
- Multiple mucosal mechanisms (e.g. block binding to receptor, mucus trapping, agglutination/aggregation)
- Antibodies are immune correlates of protection in many vaccines, including the RV144 HIV vaccine trial (efficacy 31.2%, IgG not IgA)

# Versatility of Mucosal Antibodies

| Strategy | Description | How mucosal antibodies could defeat the microbial strategy |
|---|---|---|
| molecular mimicry | microbial antigens similar to host antigens resulting in an absence of an effective immune response (e.g. Treponema pallidum) | use non-inflammatory monoclonals topically against the microbial antigen |
| intracellular or "Trojan Horse" | microbe resides in a host cell thereby evading detection by the immune system (e.g. cell-associated HIV) | use monoclonals against the cell vector |
| induction of ineffective antibodies | microbe exposes non-critical antigens to the immune system, so an ineffective antibody response results (e.g. T. pallidum, Chlamydia trachomatis) | use only monoclonals with proven efficacy |
| soluble antigen competes up antibody | microbe releases soluble antigen which adsorbs antibody (e.g. cell-free HIV) | choose monoclonals against an antigen that is not released; or, use a polyvalent monoclonal to selectively target multivalent antigen on intact microbes |
| protease production | microbe produces an IgA1 protease (e.g. Neisseria gonorrhoeae) | use protease resistant SIgA, engineer out the sensitive site, or include a neutralizing antibody to IgA1 protease |
| antigenic variation | microbe mutates immunodominant antigen (e.g. HIV, N. gonorrhoeae) | use monoclonals against less immunodominant but more conserved antigens |

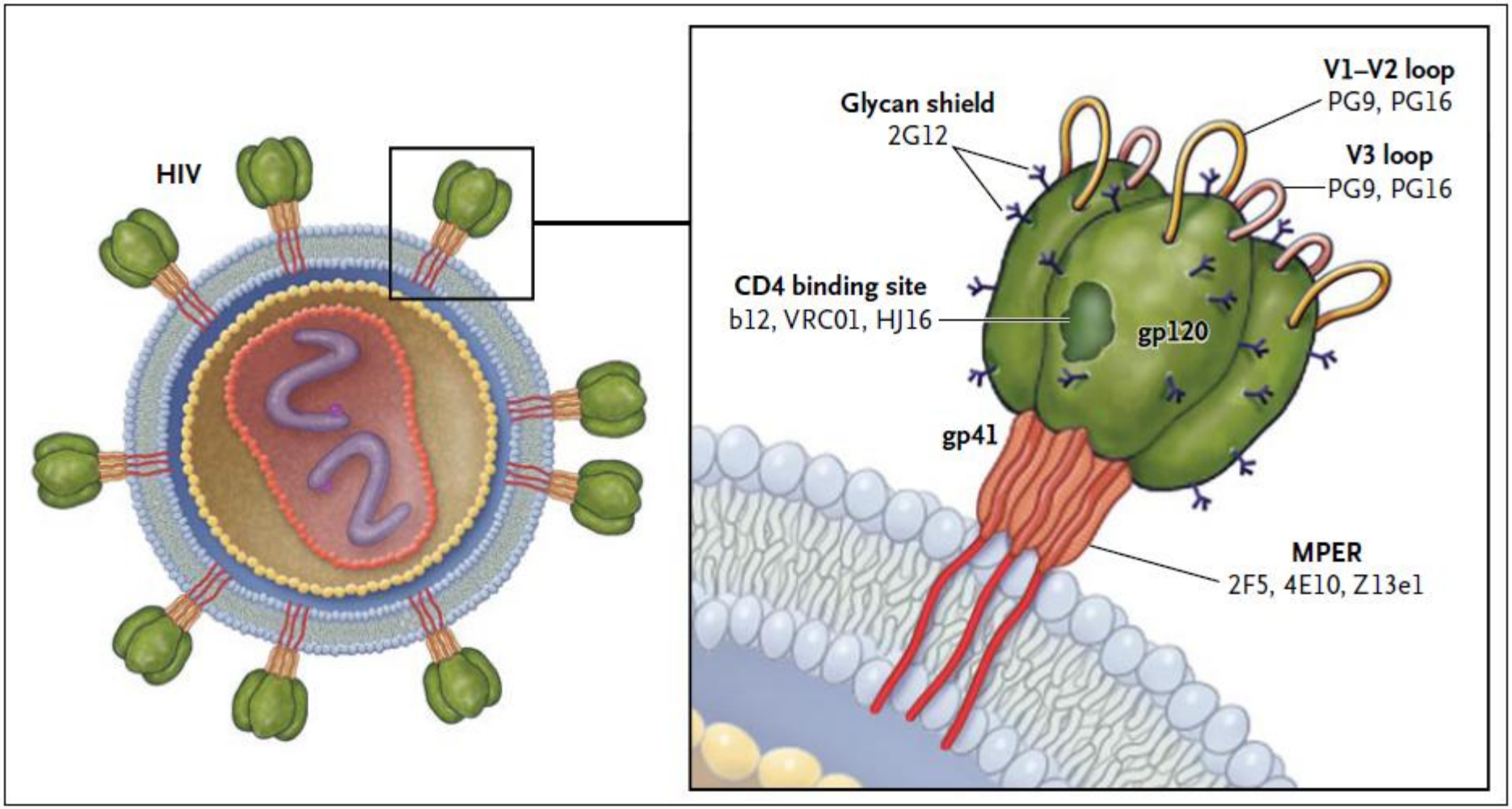
(Adapted from Whaley and Zeitlin, Annales de l'Institut Pasteur 2001)

# Critical Path




(FDA, 2004)

# mAbs to gp120 and gp41




(Koff and Berkley, NEJM 2010)

# HIV Neutralizing mAbs

**A**

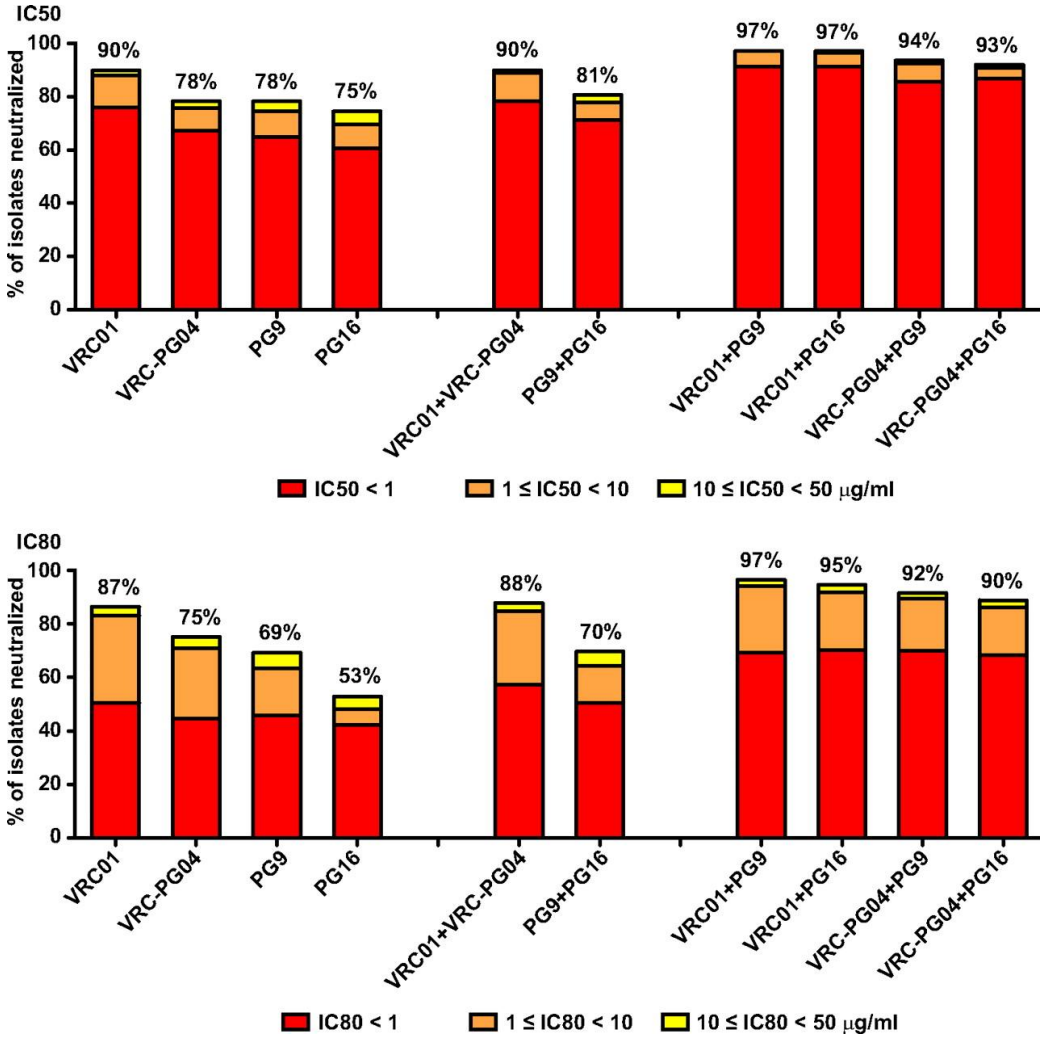
| Clade | No. of viruses | Median $IC_{50}$ (μg/ml) against viruses neutralized with an $IC_{50}$ <50 μg/ml | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | b12 | 2G12 | 2F5 | 4E10 | PG9 | PG16 | PGC14 |
| A | 27 | 6.98 | 17.10 | 5.70 | 6.20 | 0.16 | 0.11 | 41.59 |
| B | 31 | 0.80 | 0.82 | 2.41 | 5.22 | 0.43 | 0.70 | 21.88 |
| C | 27 | 6.46 | 2.93 | 31.51 | 2.97 | 0.22 | 0.25 | 11.97 |
| D | 25 | 1.47 | 7.71 | 3.17 | 4.60 | 0.10 | 0.02 | 38.57 |
| CRF01_AE | 10 | 21.53 | >50 | 0.26 | 0.51 | 0.08 | 0.03 | >50 |
| CRF_AG | 10 | 10.40 | 0.95 | 0.64 | 1.42 | 0.80 | 0.03 | 45.10 |
| G | 15 | 3.07 | 31.03 | 1.24 | 1.44 | 0.29 | 1.21 | >50 |
| F | 15 | >50 | 9.23 | 1.78 | 2.30 | 0.09 | 0.08 | 25.71 |
| Total | 162 | 2.82 | 2.43 | 2.30 | 3.24 | 0.22 | 0.15 | 25.99 |

**B**

| Clade | No. of viruses | Percent viruses neutralized | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | b12 | 2G12 | 2F5 | 4E10 | PG9 | PG16 | PGC14 |
| | | With an $IC_{50}$ <50 μg/ml | | | | | | |
| A | 27 | 30 | 37 | 74 | 96 | 85 | 85 | 11 |
| B | 31 | 58 | 71 | 68 | 97 | 74 | 74 | 29 |
| C | 27 | 33 | 11 | 7 | 96 | 78 | 78 | 19 |
| D | 25 | 48 | 24 | 56 | 96 | 76 | 60 | 8 |
| CRF01_AE | 10 | 30 | 0 | 89 | 100 | 100 | 100 | 0 |
| CRF_AG | 10 | 30 | 50 | 80 | 100 | 80 | 60 | 10 |
| G | 15 | 13 | 20 | 80 | 100 | 87 | 73 | 7 |
| F | 15 | 0 | 21 | 87 | 100 | 67 | 64 | 13 |
| Total | 162 | 35 | 32 | 60 | 98 | 79 | 73 | 15 |
| | | With an $IC_{50}$ <1.0 μg/ml | | | | | | |
| A | 27 | 0 | 4 | 4 | 0 | 70 | 63 | 0 |
| B | 31 | 32 | 39 | 23 | 0 | 45 | 42 | 3 |
| C | 27 | 7 | 0 | 0 | 11 | 56 | 48 | 0 |
| D | 25 | 12 | 8 | 12 | 8 | 48 | 44 | 0 |
| CRF01_AE | 10 | 11 | 0 | 88 | 80 | 70 | 70 | 0 |
| CRF_AG | 10 | 10 | 30 | 60 | 30 | 40 | 50 | 0 |
| G | 15 | 0 | 0 | 27 | 0 | 60 | 33 | 0 |
| F | 15 | 0 | 14 | 13 | 28 | 80 | 79 | 0 |
| Total | 162 | 11 | 12 | 19 | 12 | 57 | 51 | 1 |

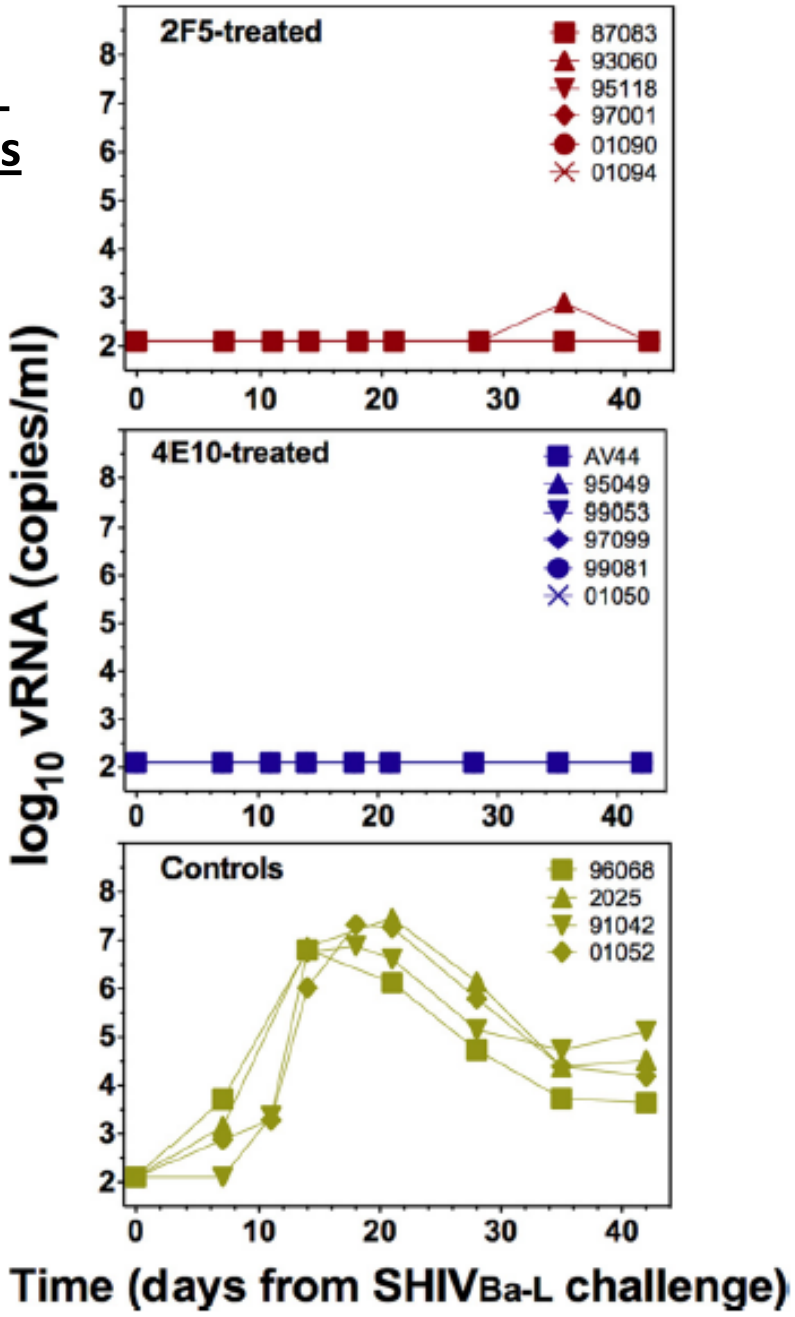
(Walker…Burton, Science, 2009)

**Combining mAbs: Neutralization coverage of a panel of 208 global HIV-1 isolates (190 for VRC-PG04) by MAbs targeting independent epitopes on the Env glycoprotein.**



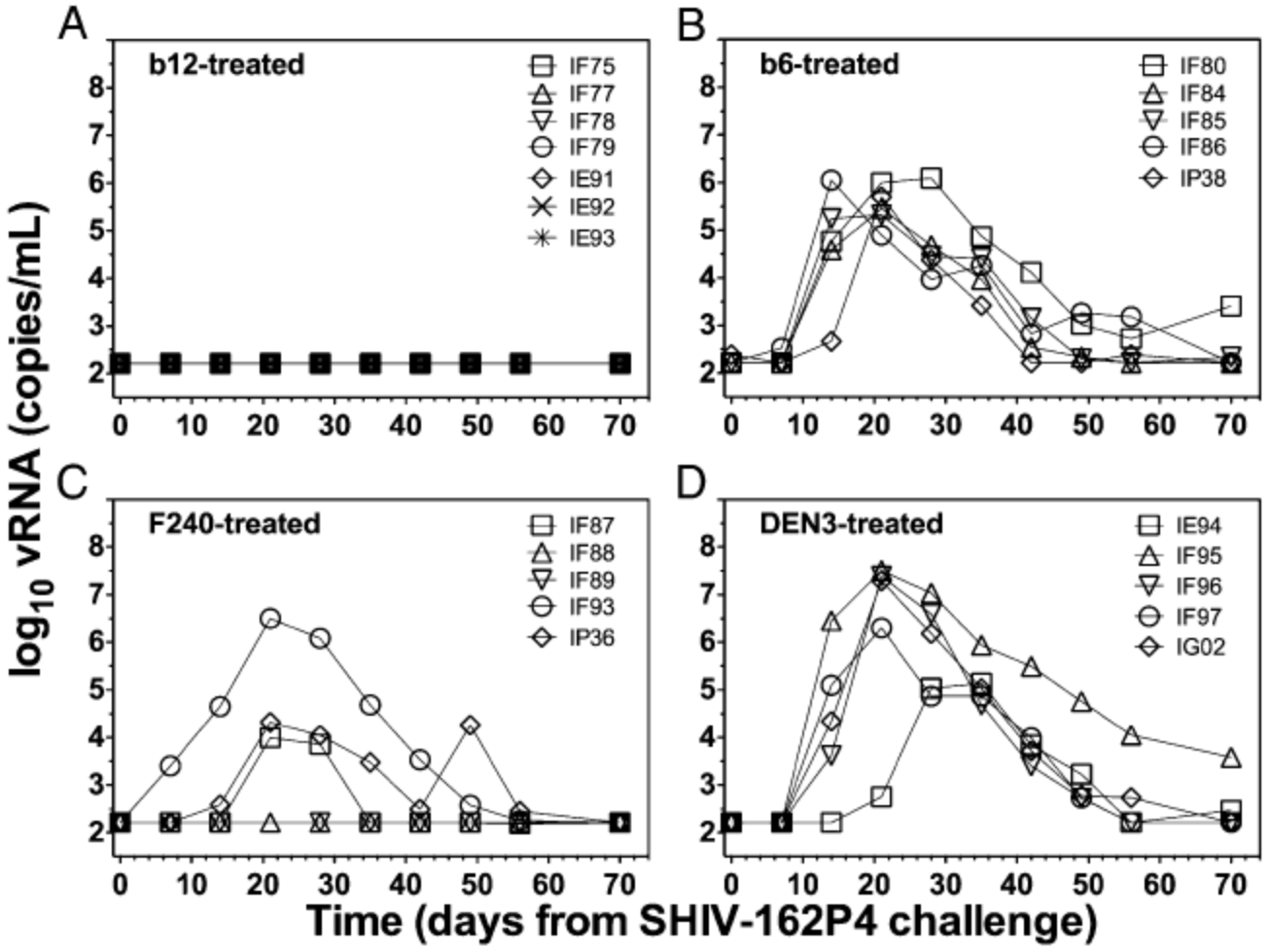

Doria-Rose N A et al. J. Virol. 2012;86:3393-3397

## Preventing Vaginal Transmission in Macaques: 2F5 and 4E10 Mabs




(Hessel…Burton, J Virol. 2010)

# Preventing Vaginal Transmission in Macaques: b12 mAb




Burton…Moore, PNAS 2011

# Preventing Vaginal Transmission in Macaques: TriMab (2F5, 4E10, 2G12) Gel

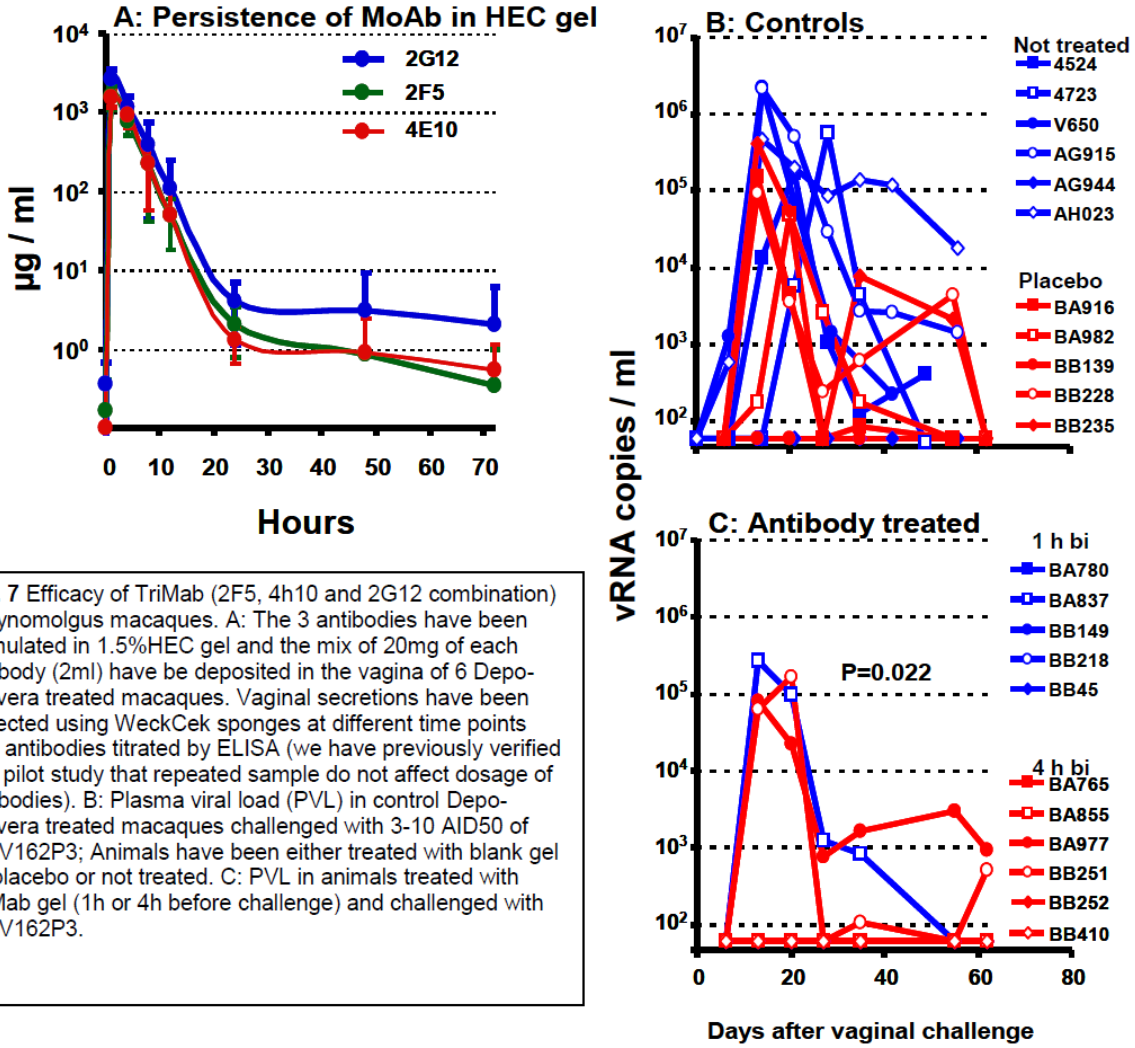



**Fig. 7** Efficacy of TriMab (2F5, 4h10 and 2G12 combination) in cynomolgus macaques. A: The 3 antibodies have been formulated in 1.5%HEC gel and the mix of 20mg of each antibody (2ml) have be deposited in the vagina of 6 Depo-Provera treated macaques. Vaginal secretions have been collected using WeckCek sponges at different time points and antibodies titrated by ELISA (we have previously verified in a pilot study that repeated sample do not affect dosage of antibodies). B: Plasma viral load (PVL) in control Depo-Provera treated macaques challenged with 3-10 AID50 of SHIV162P3; Animals have been either treated with blank gel as placebo or not treated. C: PVL in animals treated with TriMab gel (1h or 4h before challenge) and challenged with SHIV162P3.

(LeGrand, 2010)

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# MABGEL 1
# A phase 1 randomised controlled trial of a triple anti-HIV-1 monoclonal antibody vaginal microbicide

Georgina Morris, Rebecca Wiggins, Sarah Woodhall, Carol Taylor, Brigitta Vcelar, Martin Bland, Charles Lacey;

Centre for Immunology and Infection, Hull York Medical School, & Department of Health Sciences, University of York, & Polymun Scientific, Vienna

# MABGEL Results: Safety Analyses

Mean numbers of AEs assigned as at least possibly related to gel

| Treatment group | Number in group | Mean number of events | Standard deviation |
|---|---|---|---|
| Placebo | 9 | 2.67 | 2.24 |
| Low dose | 9 | 2.11 | 1.69 |
| High dose | 10 | 1.80 | 1.40 |

There were no statistically significant differences in AEs between the groups P = 0.6 (negative binomial regression).

(CROI 2011, courtesy of Morris and Lacey)

# MABGEL Pharmacokinetic results

- There were statistically significant differences between placebo, low dose and high dose group median values at each time point except C2G12 36 hrs post-12$^{th}$ dose
- Clear differences between the high and low dose MAb groups were seen but did not reach statistical significance due to small sample sizes
- MAb levels in serum at low or background levels in all groups, with no statistically significant differences between the arms

(CROI 2011, courtesy of Morris and Lacey)

# First-in-human safety trial of Nicotiana-derived 2G12

(EUDRACT No. 2009-011820-68)

UNIVERSITY OF SURREY

- Study design: A double-blind, placebo-controlled, dose escalation trial in 11 women
- Administration of a single vaginal dose of Nicotiana-derived G12
- Doses: 7, 14 and 28 mg
- Objectives: safety evaluation (clinical safety tests, local reactions, adverse events)
- Evaluate survival of Nicotiana-derivedG12 in vaginal secretions and entry into circulation

Pharma Planta

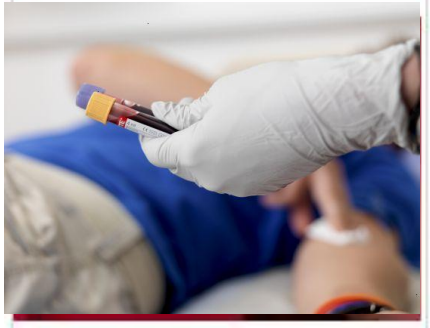
(Courtesy of Julian Ma, 2012)

# 2G12-N: Preliminary results and indications

- All volunteers completed the trial protocol
- No significant changes in clinical tests or clinical inspection
- No clinically significant changes on vaginal examination

**At all doses:**

- Nicotiana-derived G12 is safe and well tolerated
- No clinical changes of concern
- Final report available Q2 2012



(Courtesy of Julian Ma, 2012)

# Summary of Greenhouse Production of Nicotiana-derived 2G12

- Total production time is 6 weeks

  3 weeks for plantlet growth (transgenic 2G12)

  3 weeks for biomass production

  Pesticide free production

- 250Kg Nicotiana tissue/ batch / 250 $m^2$

- Average yield – 10g MAb /batch

- 20 batches / year are possible at this pilot scale

(Courtesy of Julian Ma, 2012)

Pharma Planta

Fraunhofer IME

# Antibody-based Multipurpose Microbicides

**Nicotiana-based Manufacturing**

**A Technology Platform for Multipurpose Microbicides: Speed, Cost, Scale, Versatility**

# SWOT Analysis

Courtesy of Gleba (Icon Genetics), 2012

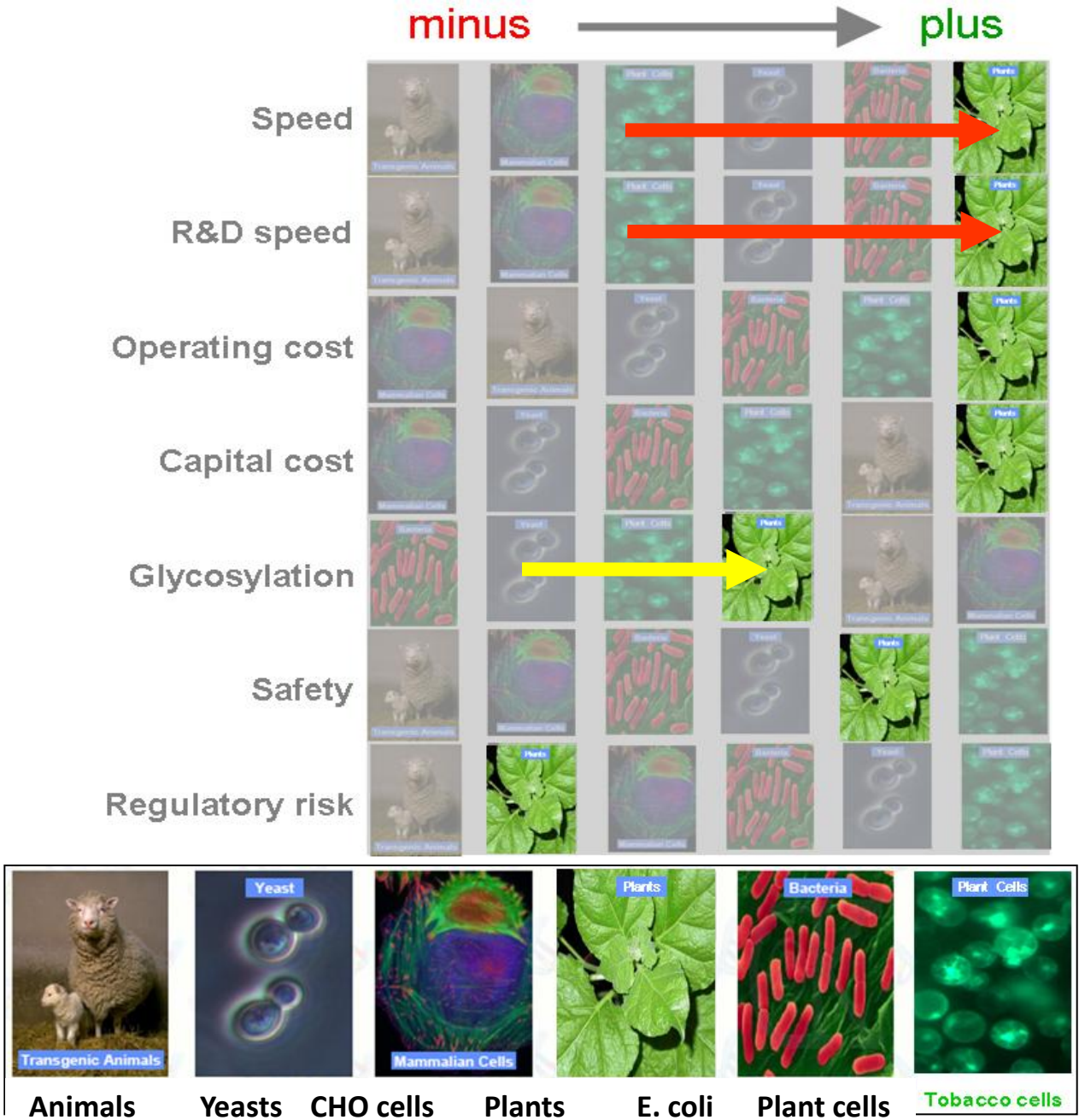
## Transient Plant Production:

### Strengths
- Manufacturing speed
- Fast gene to protein
- Production yield
- Low COGs
- Safety benefits
- Low regulatory risk

### Weaknesses
- No approved product
- No clear guidelines

### Opportunities
- Reduce COGs
- Increase R&D&M speed
- Increase flexibility

### Threats
- Regulatory burden



# IgG glycosylation

*core glycans – plants and mammals:*

*plant glycans:*

*mammalian glycans:*

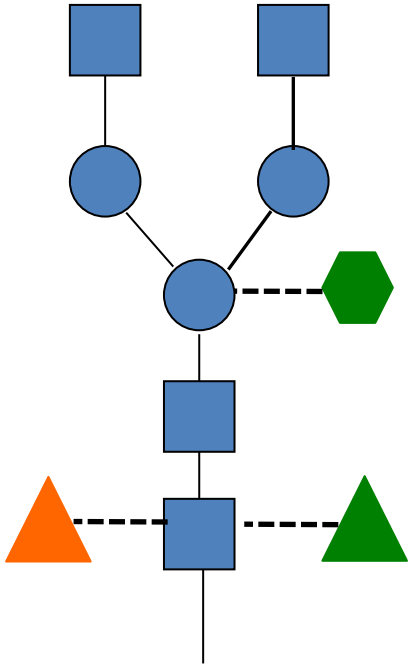
GlcNAc

Mannose

Xylose

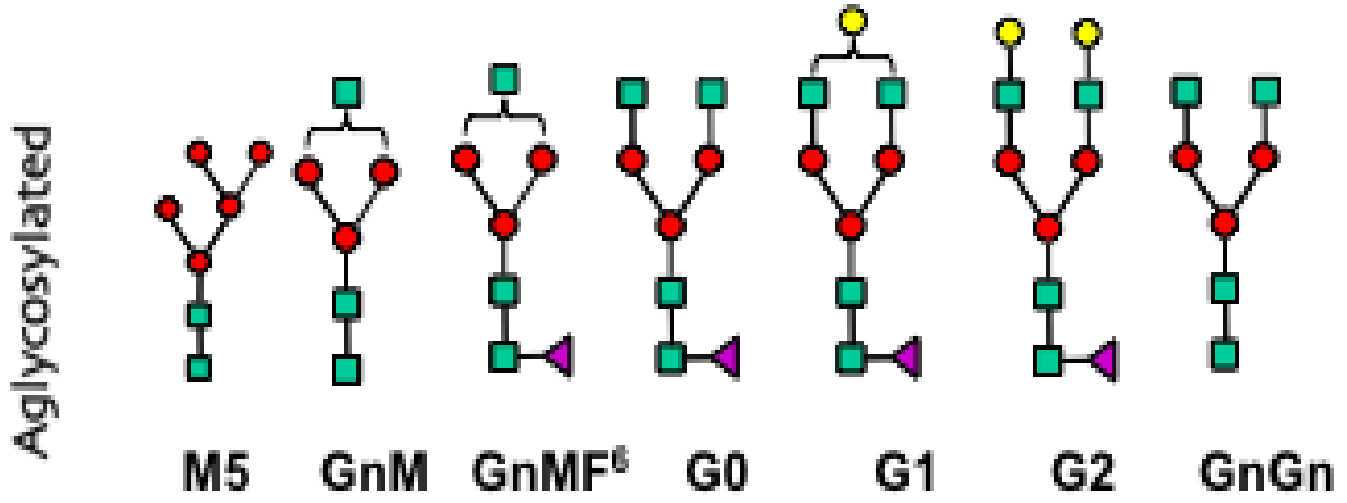
Fucose (α1,3)

Fucose (α1,6)



IgG Asn297

# Glycosylation of h-13F6




| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Rituxan | | | | | 53 | 35 | | 8 |
| h-13F6$_{CHO}$ | | 6 | | 11 | 35 | 27 | 9 | |
| h-13F6$_{\Delta XF}$ | 5 | | 5 | | | | | 90 |
| h-13F6$_{agly}$ | 100 | | | | | | | |

GlcNAc Galactose Mannose core α1,6Fucose

# Antibody IPCP: mapp66 mAbs

- HSV8-N, VRC01-N, 4E10-N
- IgG1, IgG2, IgG3, IgG4, IgA, S-IgA
- GnGn, Aglycosylated, +fucose/xylose, GnGn+galactose, GnGnNaNa

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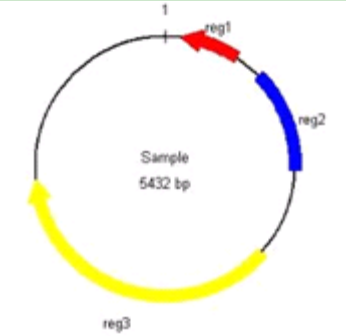
# Mapp66 Upstream




Plasmid Vector

Agrobacterium Strain Development

Infiltration Chamber

Plant AgroInfiltration

- mAbs held in Apoplast

# Large scale manufacturing in *Nicotiana* at KBP

Mapp Biopharmaceutical

KBP
KENTUCKY BIOPROCESSING

- More than 1 acre of indoor controlled growth space

- Recently completed Blue Angel project for DARPA: 10 M doses of H1N1 vaccine under GMP in 1 month

**Study: PAVEG 894, VRC01 produced in N. benthamiana**

Assays: Neutralization in TZM-bl cells

Virus stocks: Derived by transfection in 293T cells.
Stock IDs shown in table.

Report Date: March 8, 2012

| Virus | Tier | Stock ID | Mapp66 VRC01 Lot#12V001 QC#6738-1 | VRC01 (in house control) |
|---|---|---|---|---|
| SF162.LS | 1 | 1825 | 0.34 | 0.43 |
| 6535.3 | 2 | 4439 | 1.76 | 1.59 |
| QH0692.42 | 2 | 2189 | 1.72 | 1.32 |
| PVO.4 | 2 | 845 | 0.38 | 0.44 |
| TRO.11 | 2 | 847 | 0.33 | 0.34 |
| RHPA4259.7 | 2 | 855 | <0.02 | 0.03 |
| AC10.0.29 | 2 | 1798 | 2.35 | 1.82 |
| THRO4156.18 | 2 | 949 | 3.9 | 2.95 |
| REJO4541.67 | 2 | 792 | 0.03 | 0.06 |
| TRJO4551.58 | 2 | 963 | 0.1 | 0.08 |
| WITO4160.33 | 2 | 851 | 0.1 | 0.11 |
| CAAN5342.A2 | 2 | 858 | 3.54 | 2.51 |

(courtesy of David Montefiori, 2012)

**Table 3.** Cost and time comparisons for mAb manufacturing systems

| Manufacturing system | Time to Phase 1 cGMP supply | Cost to Phase 1 cGMP supply |
|---|---|---|
| Mammalian cell culture (CHO, NS0, PER.C6) | 18 months | $5–6 M |
| Transient Nicotiana (magnICON, Geneware) | 6–12 months | $0.5–0.8 M |

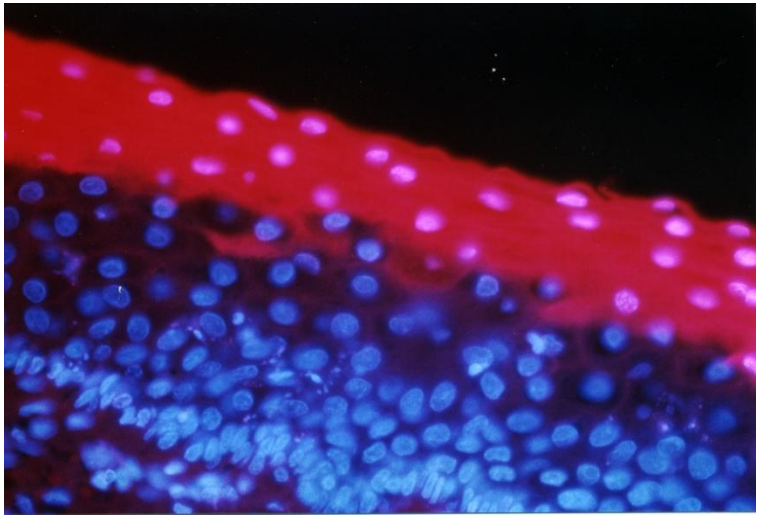
Based upon quotes from Lonza (Slough, UK) and Kentucky BioProcessing (Owensboro, KY).

(Whaley, Hiatt, Zeitlin, Human Vaccines, 2011)

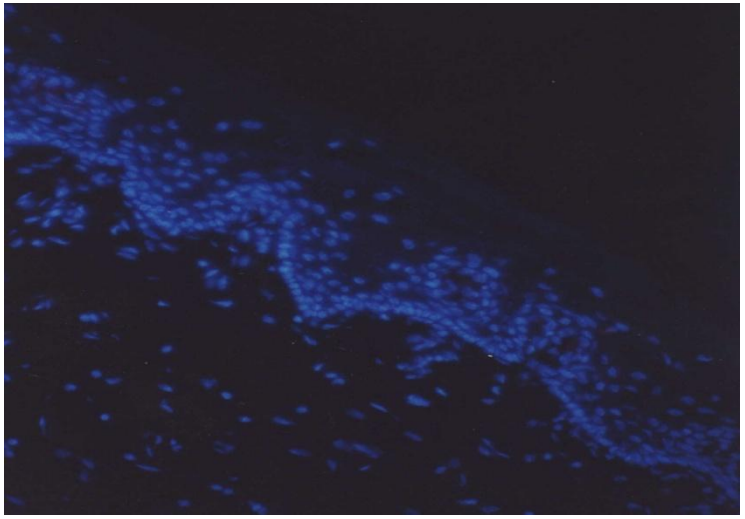
# Antibody-based Multipurpose Microbicides: NIAID funded IPCP

- Purpose: evaluate safety, efficacy, and mechanisms of antibody-based microbicides
- Principal Investigators: Deborah Anderson (BUMC), Thomas Moench (ReProtect), Kevin Whaley (Mapp), Larry Zeitlin (Mapp), Richard Cone (JHU), Sam Lai (UNC), Francois Villinger (Yerkes Primate Center), Thomas Smith (Auritec), Ken Mayer (Fenway), Susan Cu-Uvin (Brown)
- Methods: in vitro, NHP, clinical

# IgG Uptake by Apical Vaginal Epithelial Cells



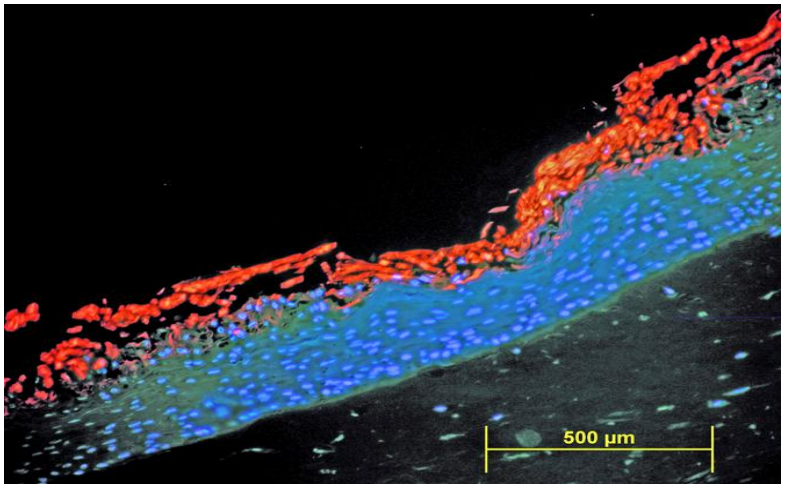
IgG-Cy3



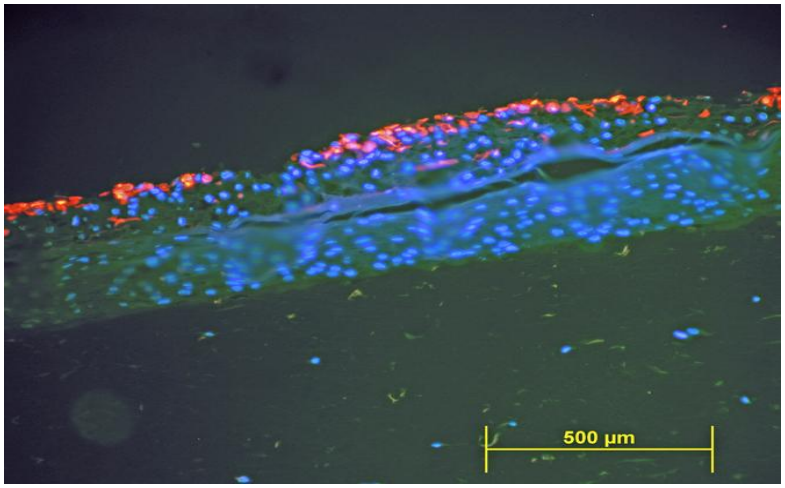
Negative Control

(Courtesy of Deborah Anderson, M2012)

# Retention of HSV-Cy3 mab by vaginal stratum corneum



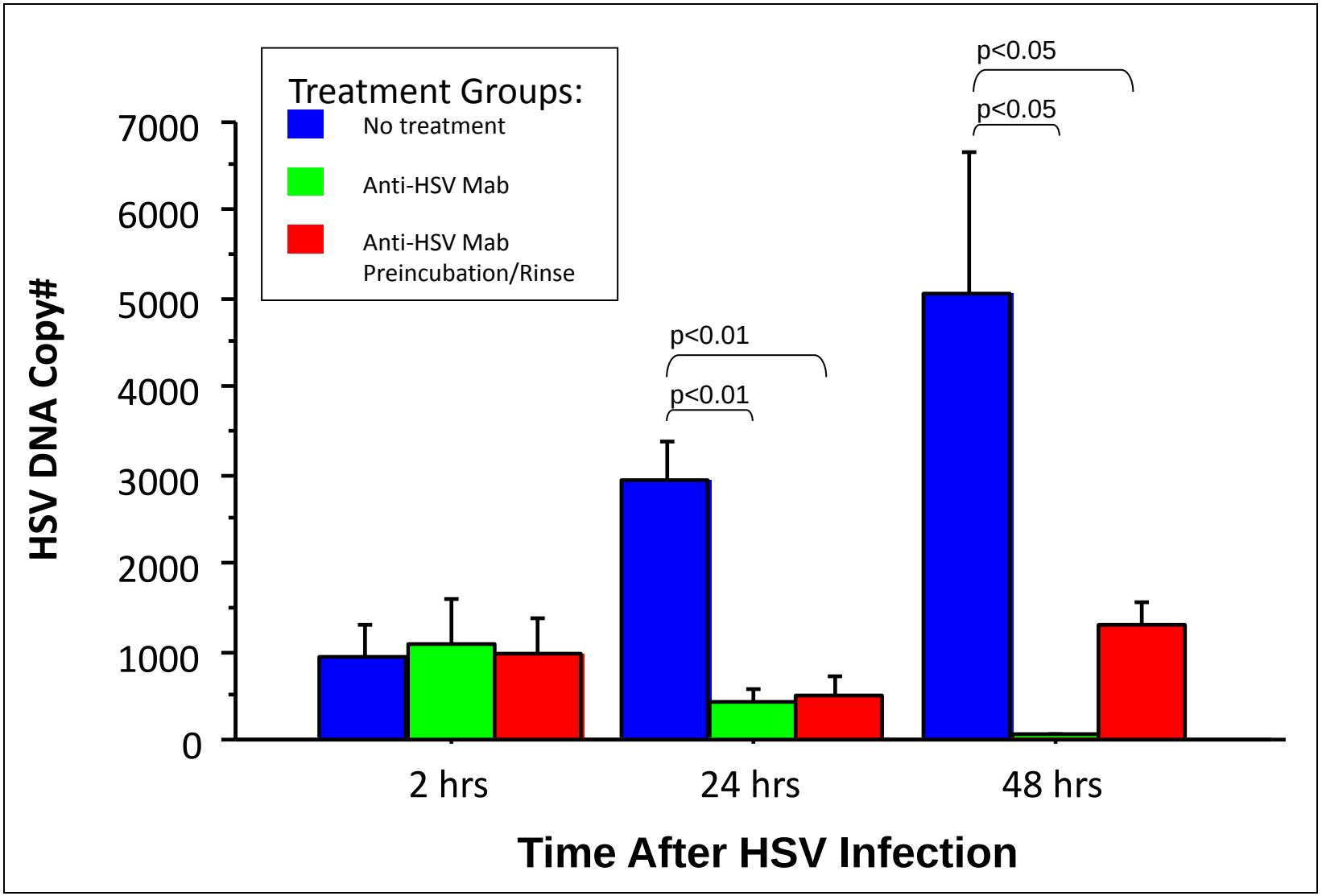

After 1 hour




After 12 hours

(Courtesy of Deborah Anderson, M2012)

# Suppression of HSV-2 Infection *in vitro* with Anti-HSV Mab




(Courtesy of Deborah Anderson, M2012)

# Vaginal Stratum Corneum Battlefront Summary

Pathogens (HIV, HSV-2) can enter the SC

Soluble immunological mediators and leukocytes provide SC immune defense

It may be possible to further fortify SC defense with microbicides (eg. plantibodies)

(Courtesy of Deborah Anderson, M2012)

# Antibodies and Cell-Associated HIV

- Cell-associated vaginal HIV transmission is highly efficient and not prevented by topically applied 1% tenofovir (Swanson and Garcia-Martinez, CROI 2012)

**Table 5.** Antiviral efficacy of m9 in CCR5-dependent cell-to-cell transmission assay

| Compound | $IC_{50}$ (nM) | $IC_{90}$ (nM) |
|---|---|---|
| AMD3100[a] | >10,000 | >10,000 |
| TAK779[b] | 3.0 | 6,420 |
| enfuvirtide | 35 | >1,000 |
| m9 | 0.066 | 2.3 |
| 4E10 | 1.4 | 9.1 |
| 2F5 | 1.5 | 6.4 |

(Zhang…Dimitrov, mAbs 2010)

# Antibody-based Contraception

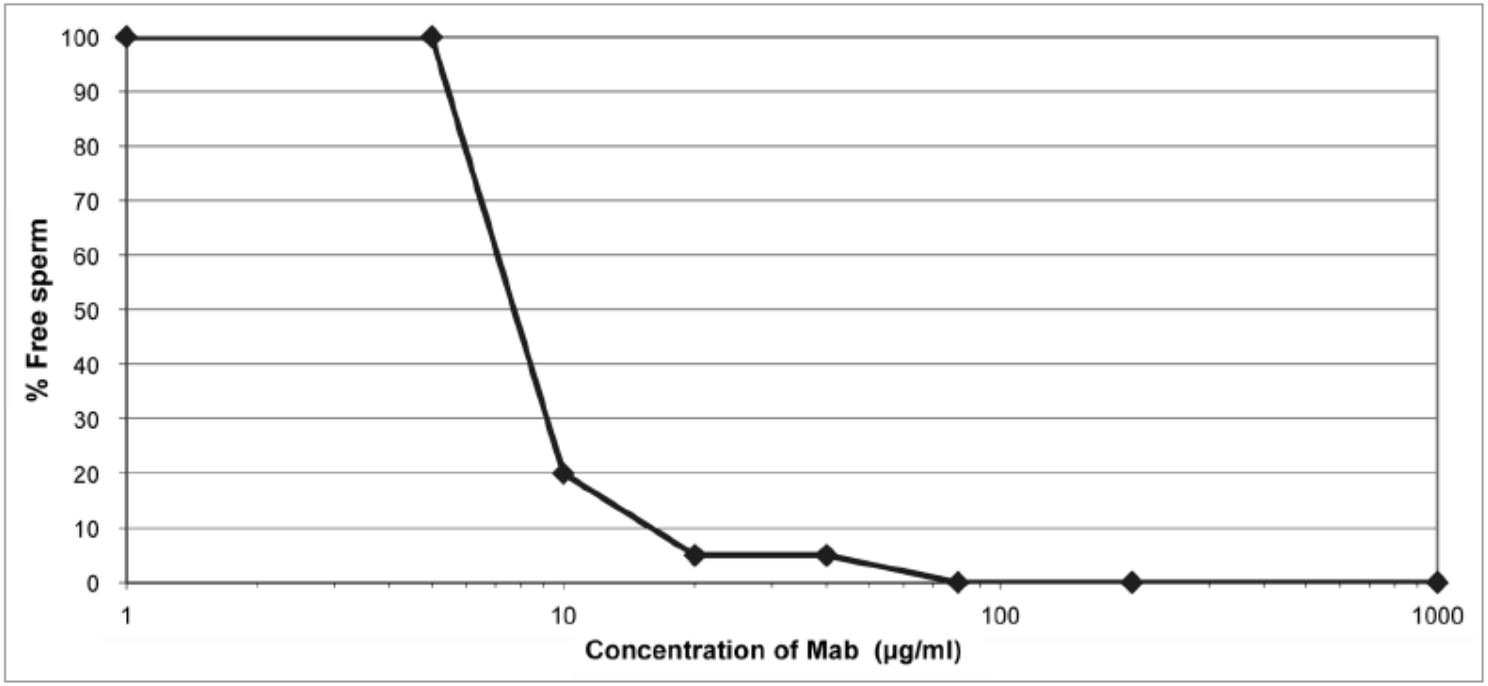



**Figure 2.** Agglutination of human sperm with Mab HC4 produced in *N. benthamiana*. Purified Mab was added to undiluted human semen and observed within 30 seconds via light microscopy.

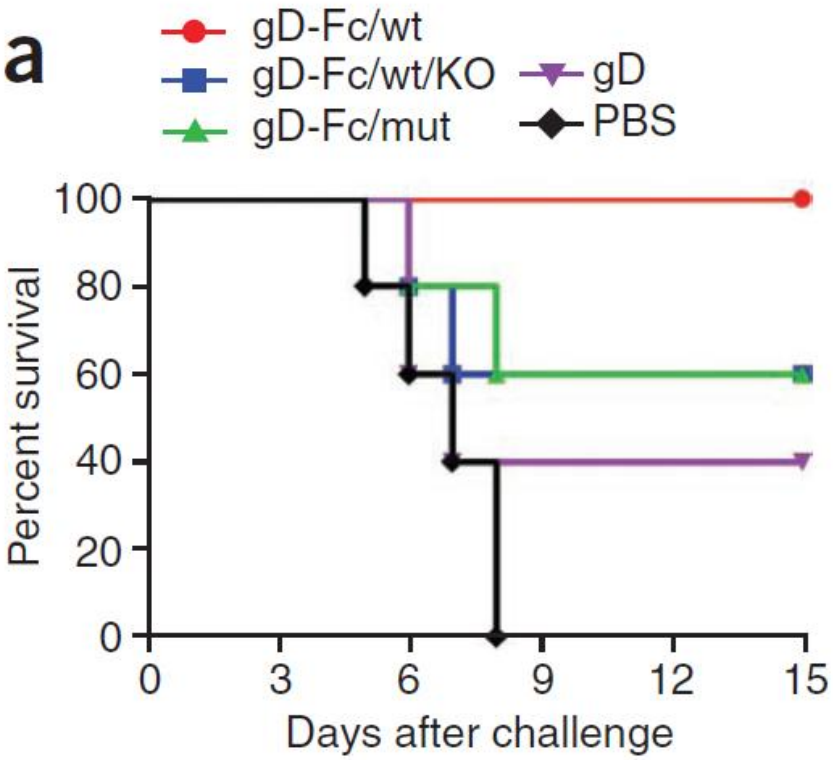
(Whaley, Hiatt, Zeitlin,
Human Vaccines, 2011)

# Anti-inflammatory Antibodies

- HIV upregulates inflammatory cytokines that lead to impairment of mucosal epithelial barrier functions. Antibodies to TNF prevented the loss of barrier function (Nazli et al., PLoS 4-10)
- Mapp is producing TNF and IL-6 mAbs.

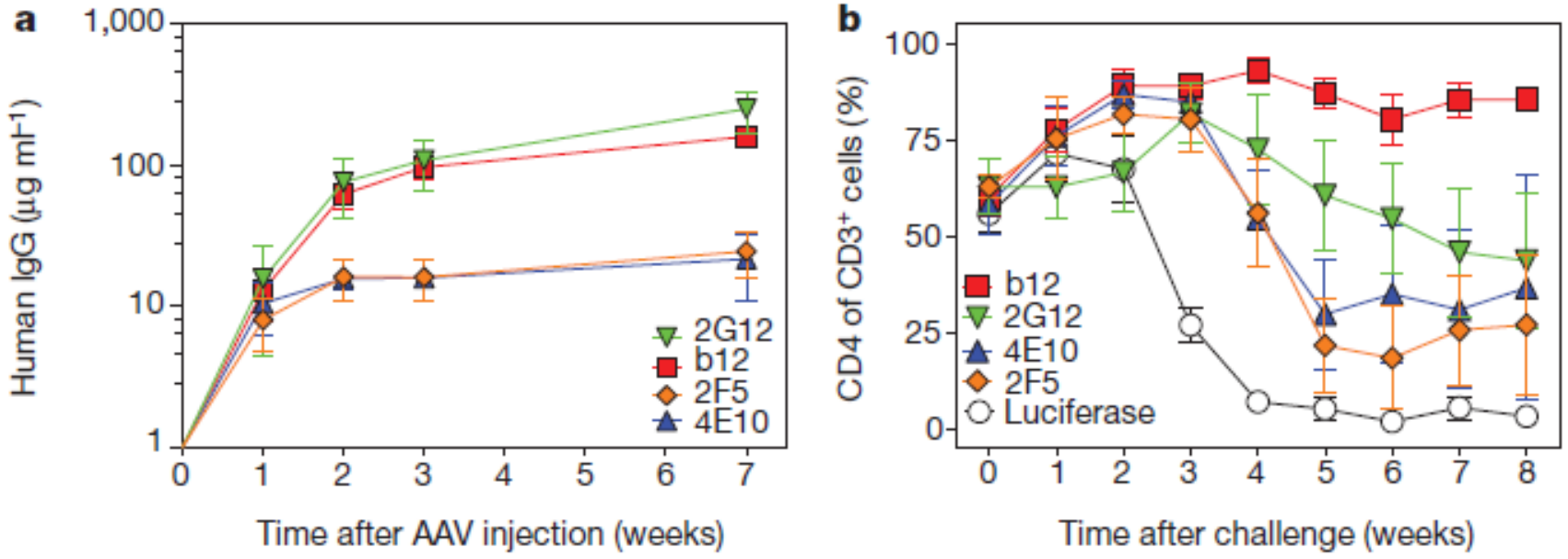
# Anti-Idiotype Vaccines

- FcRn-mediated uptake
- HIV vaccine based on 2F5 anti-Id
- HSV vaccine based on gD-Fc



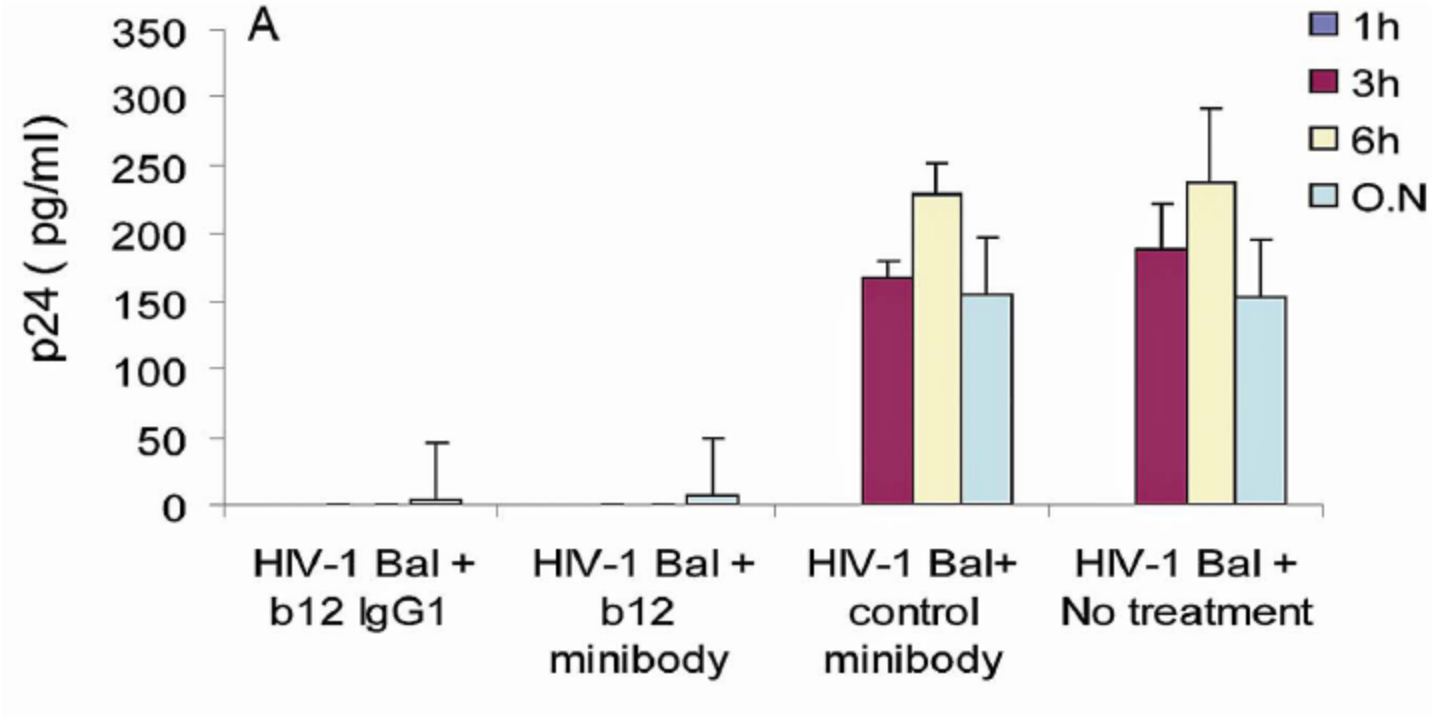

(Ye…Zhu, Nat. Biotech 2011)

# AAV-vectored HIV antibodies and Systemic Protection




(Balazs…Baltimore, Nature 2011)

# AAV-vectored HIV Antibodies and Mucosal Protection




(Abdel-Motal…Anderson… Marasco, PLoS One 2011)

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