Use of monoclonal antibodies in vaginal microbicides

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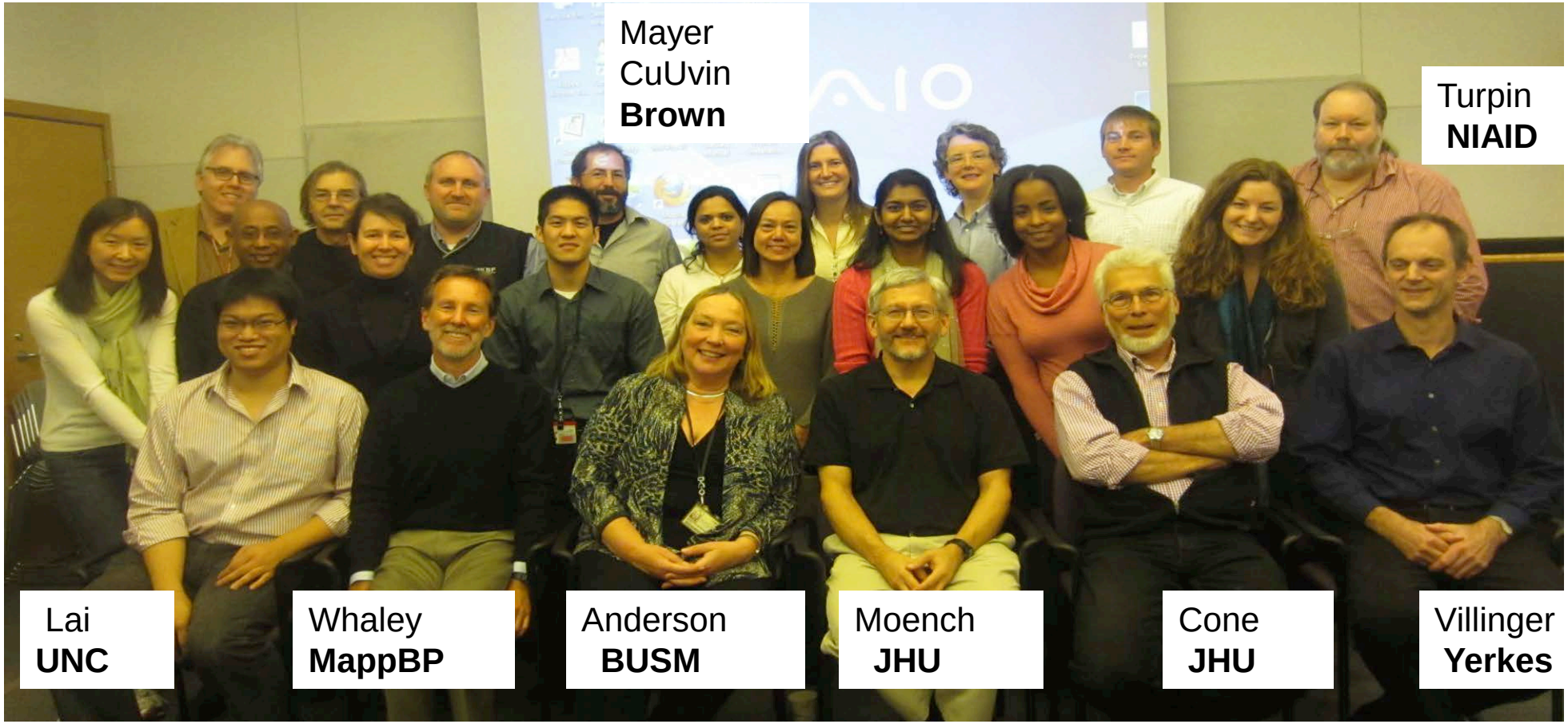
NIH Integrated Preclinical/Clinical Program for HIV Topical Microbicides (IPCP-HTM): Monoclonal Antibody-based Microbicides



Advantages of Monoclonal Antibodies (MAbs) as Vaginal Microbicides

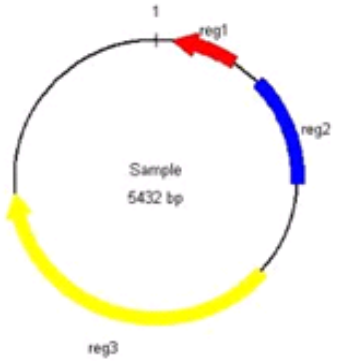
- Antibodies are natural mediators of protection against STIs in the genital tract (safe)

- **Vagina is a poor immune induction site:** Low potential to generate immune response with topical vaginal application (MAbs are effective at low concentrations

- MAb cocktails can be designed to protect against multiple STIs in different risk groups/geographical locations

- Potential use of MAbs in combination with other prevention strategies (ARVs, contraception)

Nicotiana transient transfection system to produce humanized antibodies in plants



Plasmid Vector

Agrobacterium Strain Development

Infiltration Chamber

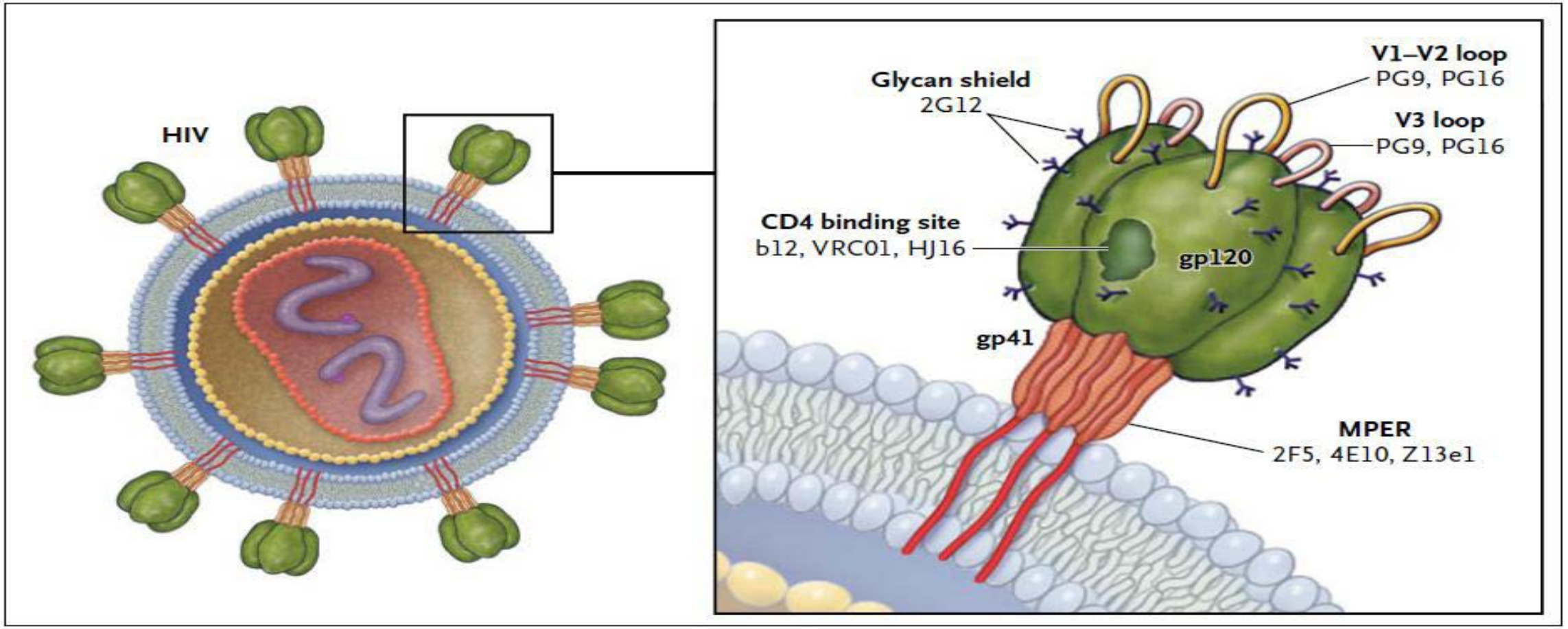
Time, Costs, and Scale of Antibody Manufacturing

- **Mammalian:**
  - Time: several week cycle
  - Costs: Currently $100-$200/g; $10/g is target for purified antibody
  - Scale: 3-(30?)g/L; 20,000L fermenters (three story high) coupled to three story high protein A columns
- **Fungi**
  - Time: 7 day cycle
  - Costs: $10/g is target for purified antibody
  - Scale: 1-3 g/L; 300,000L fermenters
- **Plants (transient)**
  - Time: 7 day cycle
  - Costs: $10/g Target
  - Scale: 0.1-1g/kg biomass; 3,000 kg biomass/acre;
- **Cervicovaginal Antibody Gene Transfer (AAV and DNA plasmid)**
  - Time: Rapid in vivo production with DNA plasmid; days-weeks with AAV
  - Costs: low?
  - Scale: localized production by cervicovaginal tissue

Strategies to Further Lower Cost of Antibody Manufacturing

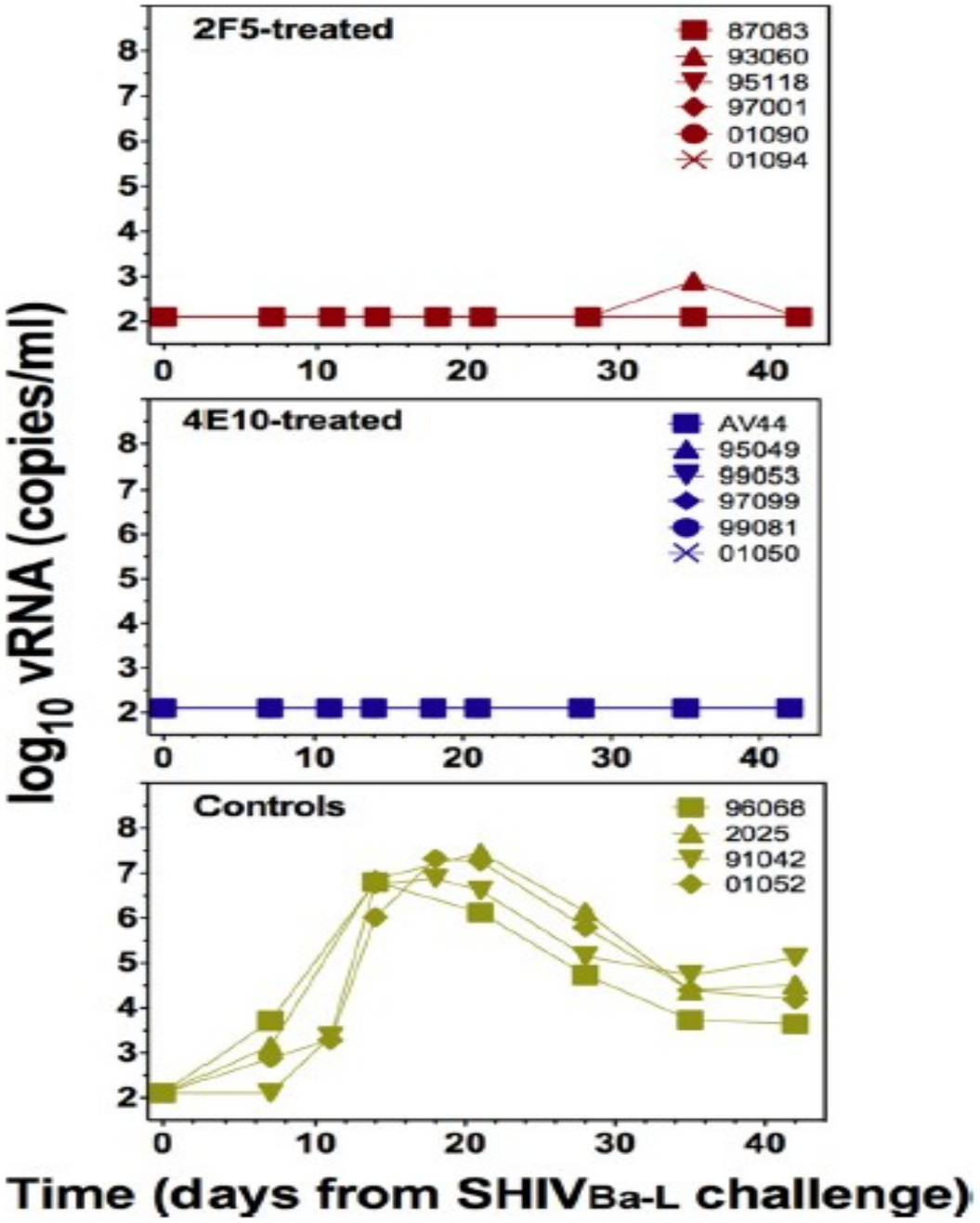
- Improve expression and potency
- Simpler Purification: e.g. flocculation, disposable Protein A, continuous purification
- Extend systemic half-life (2-6 months): YTE, Xtnd Fc mutations
- Local manufacturing of Drug Substance
  - Mammalian: single use bioreactors (SUB)
  - Fungi: large fermenters currently available globally
  - Plants: transgenic, focus on expression and purification
  - Cervicovaginal Antibody Gene Transfer: AAV and DNA plasmids

Potent broadly neutralizing monoclonal antibodies have revolutionized the HIV prevention field



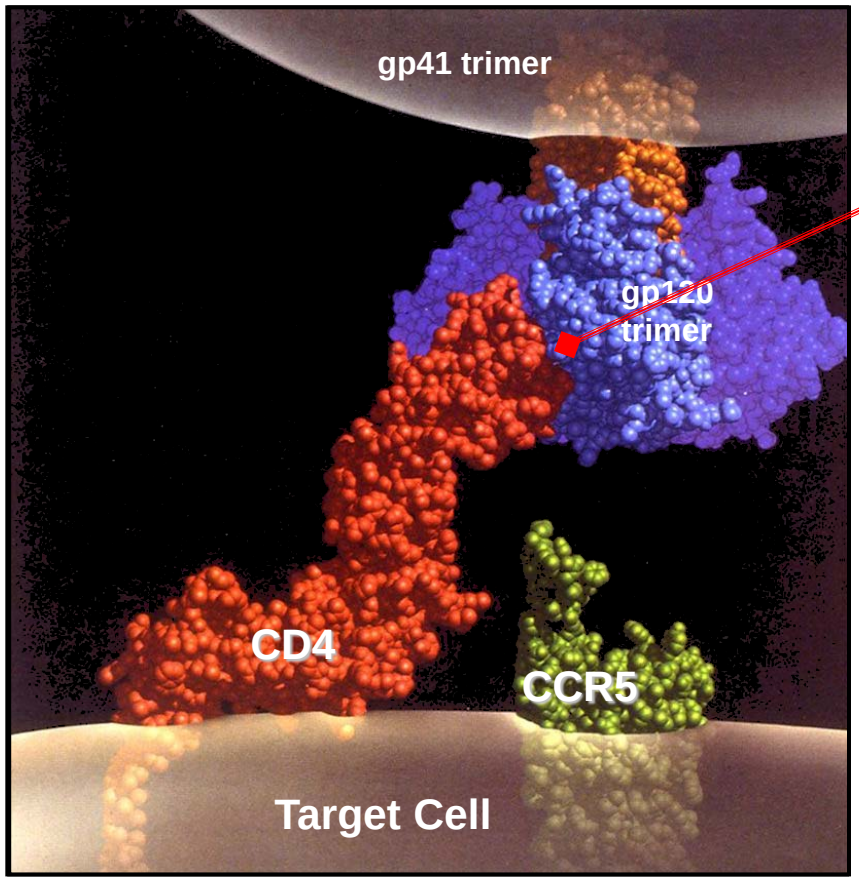
2F5 and 4E10 BnMabs Prevent Vaginal SIV Transmission in Macaques:

(Hessel...Burton, J Virol. 2010)



Mab # 1: VRC01

[blocks attachment of HIV envelope to CD4]

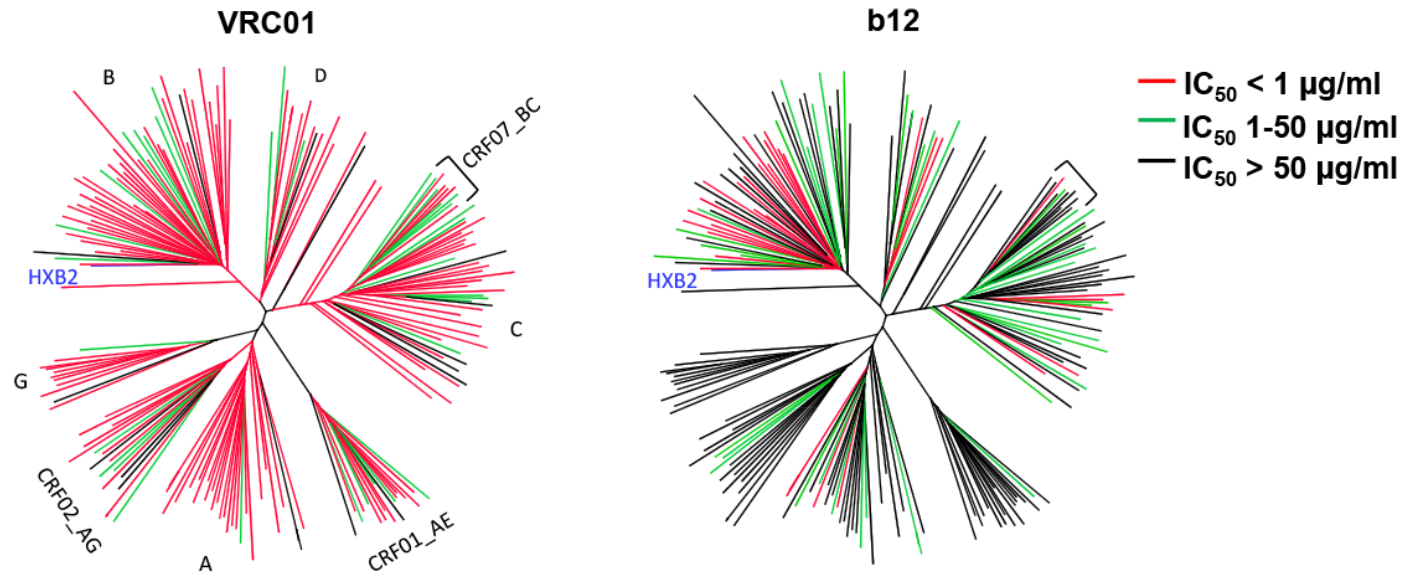


Isolated from individual infected for >15 yrs who controlled VL without ART

Binds to CD4 binding site on gp120 which is functionally conserved: All HIV must bind CD4

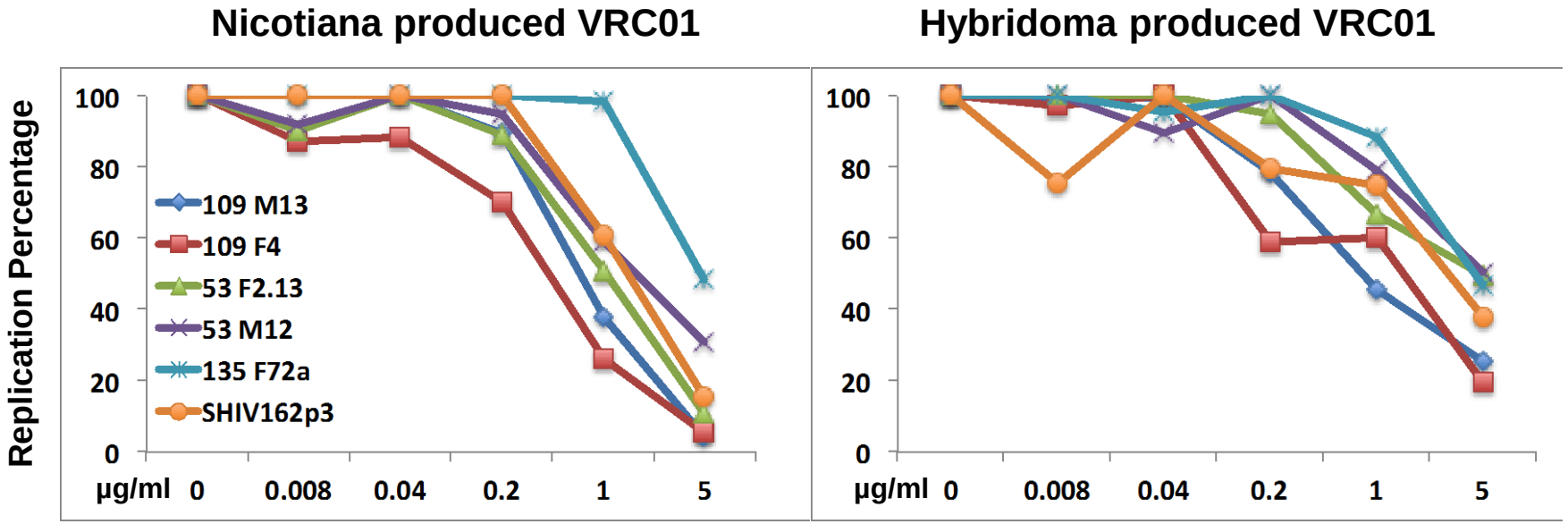
VRC01 neutralize ~90% of diverse viral isolates

VRC01 neutralizes at low concentrations



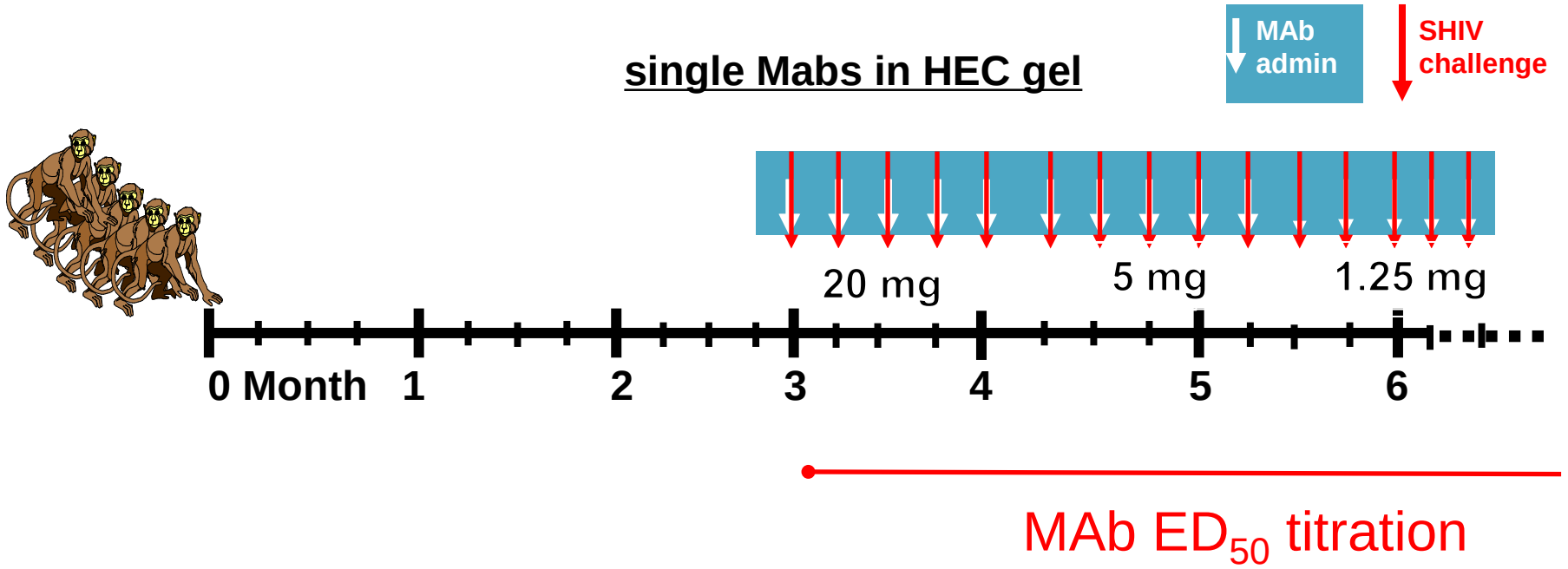
Virus clade	Number of viruses	IC50 < 50 µg/ml		IC50 < 1 µg/ml	
		VRC01	b12	VRC01	b12
A	22	100%	45%	95%	23%
B	49	96%	63%	80%	39%
C	38	87%	47%	66%	13%
D	8	88%	63%	50%	25%
CRF01_AE	18	89%	6%	61%	0%
CRF02_AG	16	81%	19%	56%	0%
G	10	90%	0%	90%	0%
CRF07_BC	11	100%	27%	45%	9%
Other	18	83%	33%	78%	6%
Total	190	91%	41%	72%	17%

Nicotiana and hybridoma produced VRC01: similar neutralization against HIV C and SHIV



	IC50	
	Nicotiana VRC01-N	Hybridoma VRC01
109 M13	0.445	0.442
109 F4	0.296	0.773
53 F2.13	0.465	4.937
53 M12	1.060	>5
135 F72a	4.895	4.612
SHIV162p3	0.972	1.109

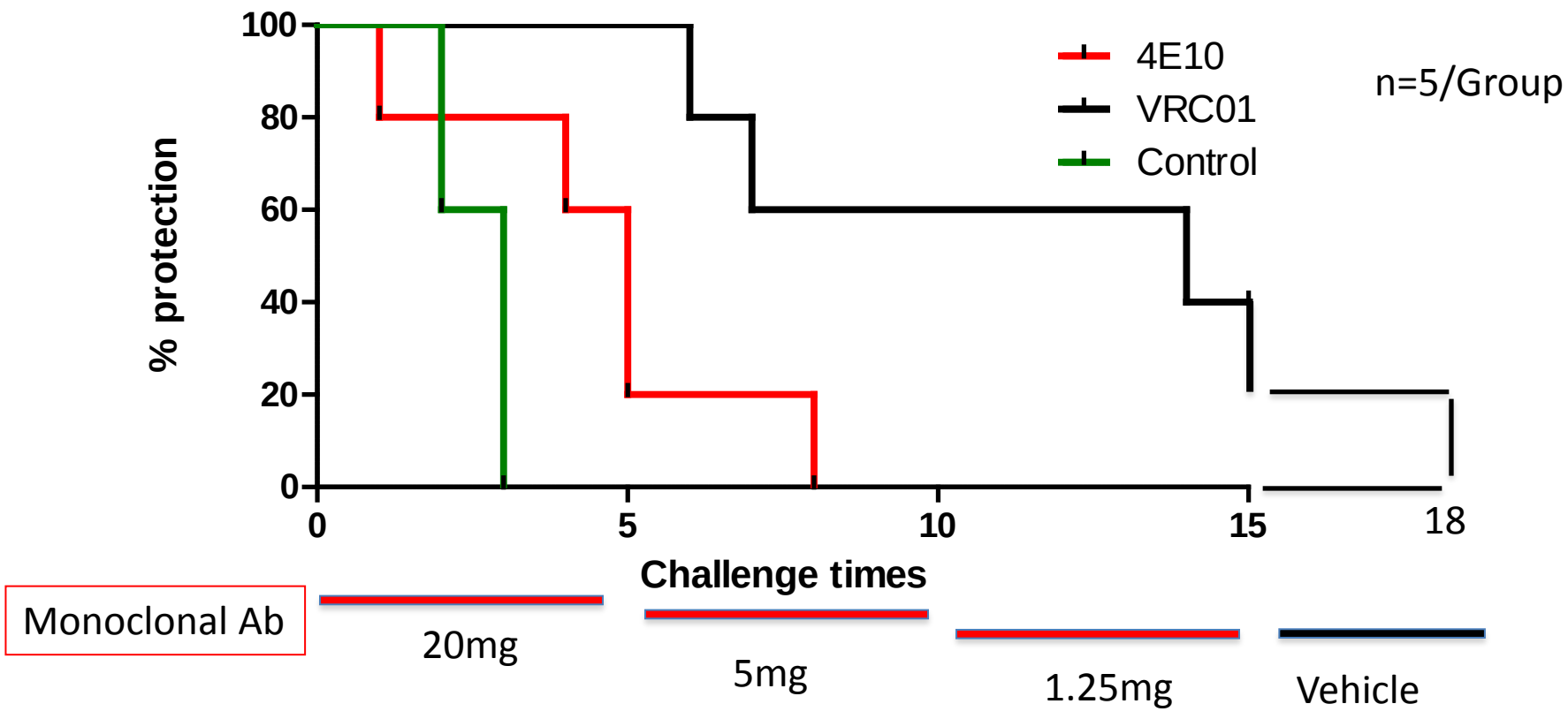
What dose of a single HIV MAb in gel prevents vaginal transmission of a CCR5 tropic SHIV?



Milestones:

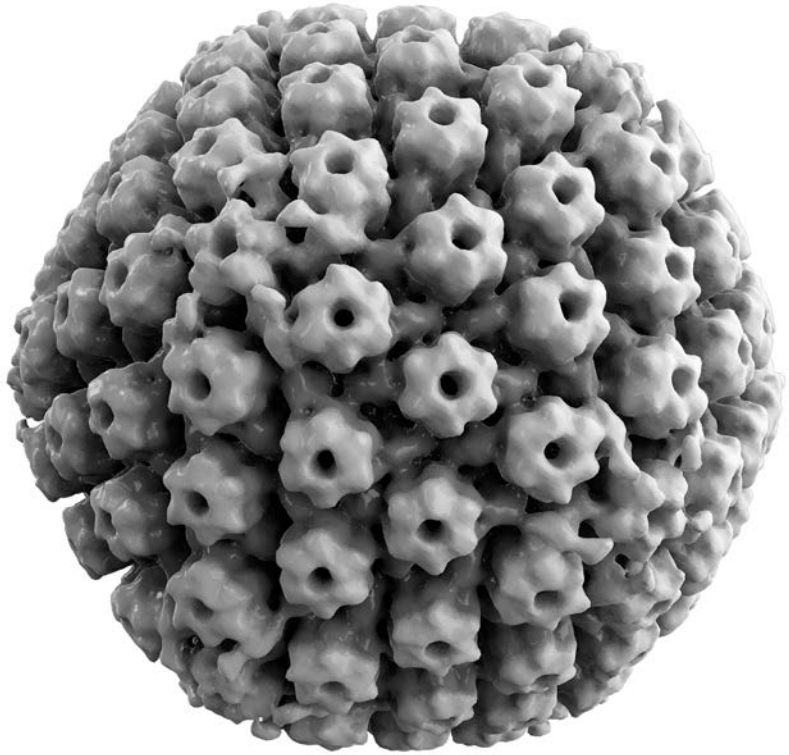
Determine ED_{50} of single MAb.

Microbicide gels with 4E10-N and VRC01-N mAbs protect from SHIV162p3 challenges



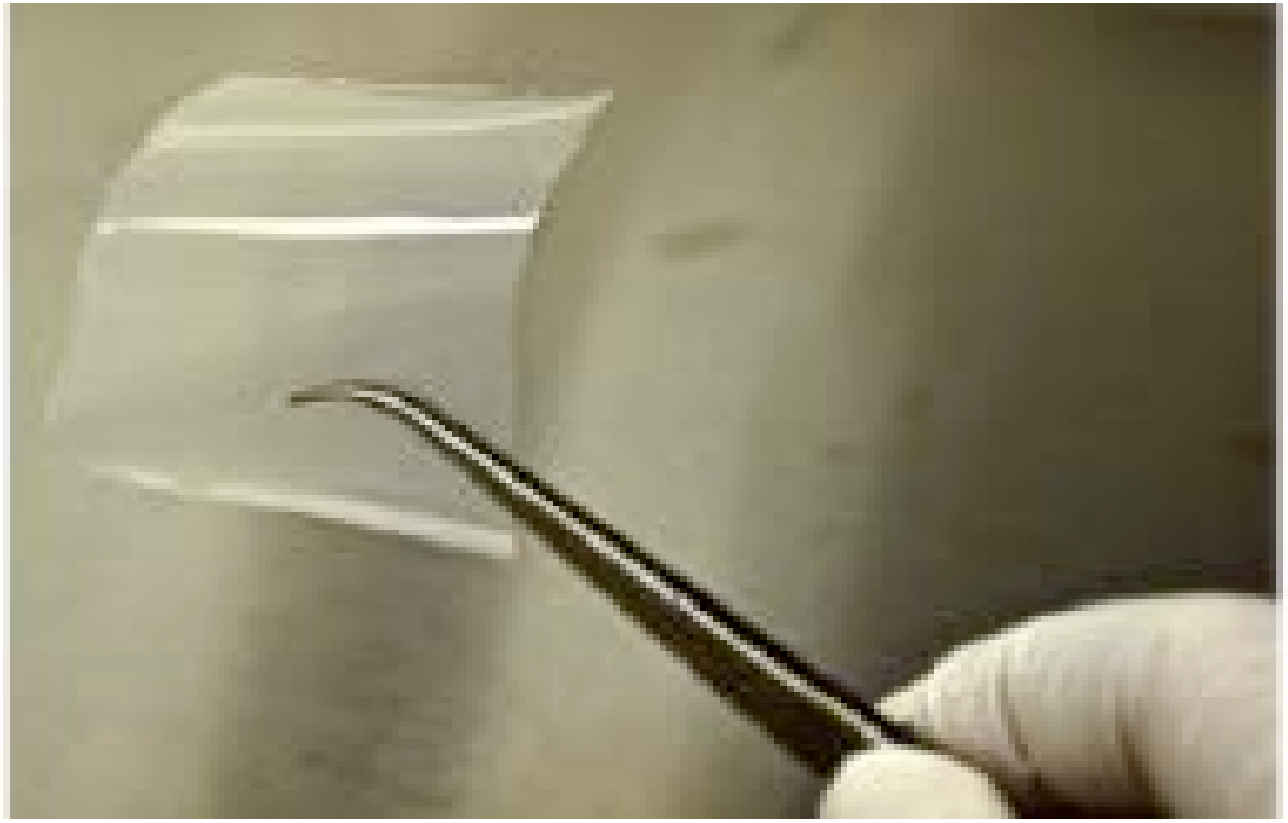
Mab # 2: HSV8 mAb

[binds to glycoprotein D on HSV-1 and -2]



HSV promotes HIV transmission

MB66 Film



Film:

Polyvinyl alcohol 60%
Maltitol 25%
Histidine 0.1%
Polysorbate 20 0.01%
Water 5%

Mabs:

VRC01 10mg
HSV-8 10mg

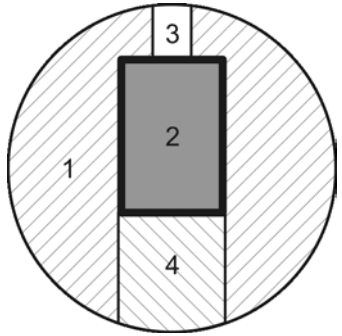
MB66 Film

- Made to clinical grade GMP standards
- Passed stability and toxicology testing in vitro and in vivo
- NHP PK/PD test
- NHP efficacy trial underway
- IND
- Phase 1 clinical trial in women underway
  - Arm 1: 5 women, single dose
  - Arm 2: 15 women, 5 daily doses of MB66 or placebo film

Delivery VRC01-N from intravaginal rings

Macaque-sized pod-IVR

Target: 14 days release in macaque
≥ 28 days release in humans

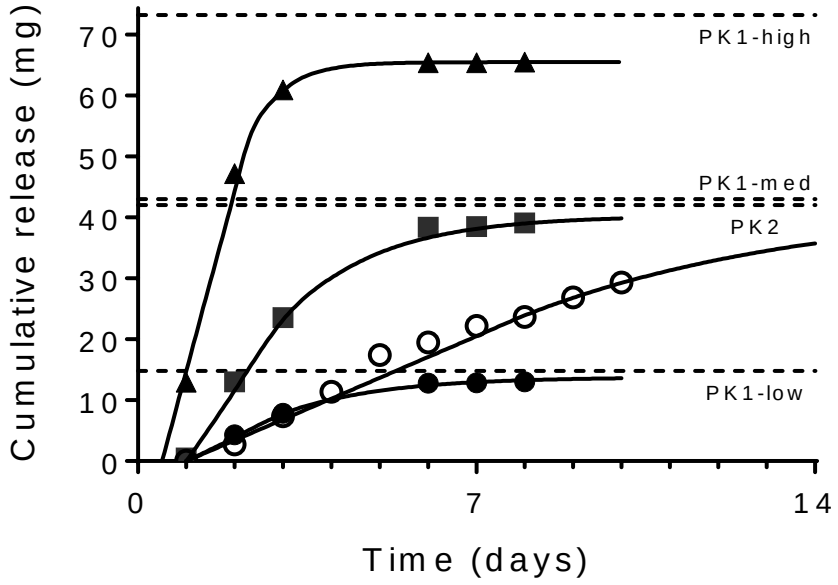


Cross-sectional view through a single pod

1. Silicone ring scaffold
2. Solid VRC01-N formulation core coated with a polylactic acid (PLA) rate controlling membrane (thick black line)
3. Delivery channel to expose a portion of the pod to vaginal fluids and provide the primary release rate control
4. Silicone adhesive backfill to seal pod in IVR

VRC01 IVR Configurations for macaque PK studies

Study-Group	Total VRC01-N Load (mg)	Pods per IVR	*In vitro* release (mg day^{-1})	*In vivo* release estimate[1] (mg day^{-1})
PK1-low	15	2	3.8	1.2
PK1-med	43	6	12	3.5
PK1-high	73	6	30	10
PK2	42	4	3.4	2.3



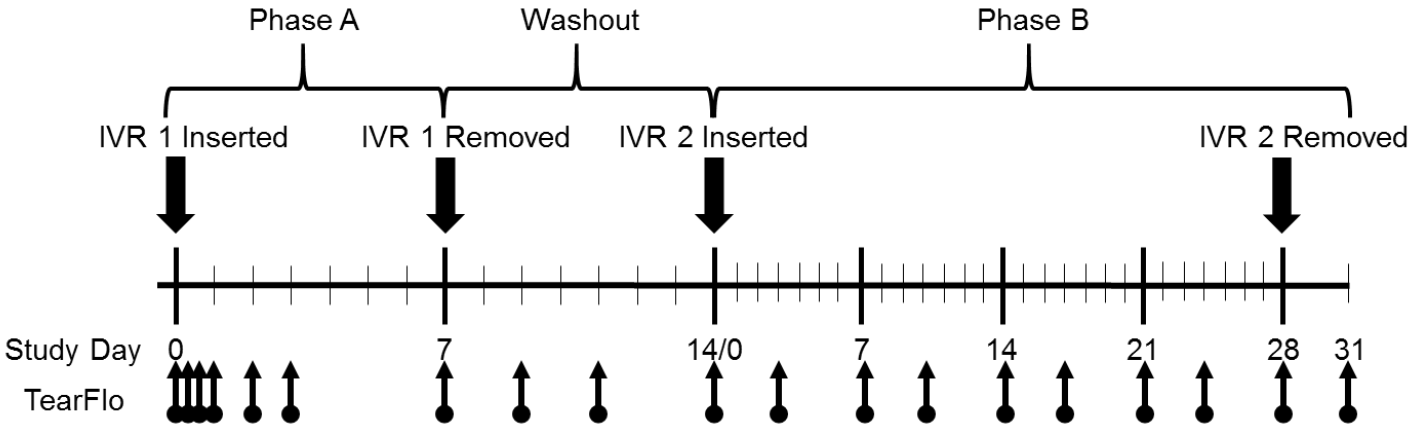
VRC01-N IVR rhesus macaque PK studies

Two-phase PK study design using PK2 IVR configuration

Phase 1: 7 days with intensive early time sampling

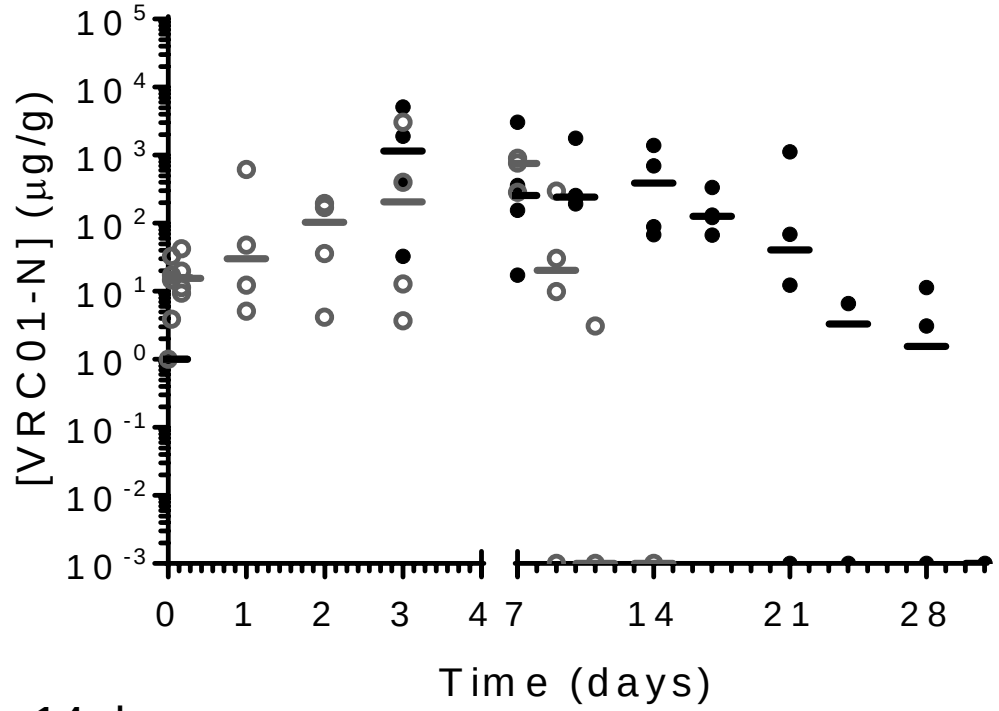
Washout (7 days)

Phase 2: 28 days N = 4 macaques each phase



VRC01 concentration in vaginal fluids

O = 7 day Phase 1 ● = 28 day Phase 2



- Median vaginal fluid levels maintained >10 µg/mL for more than 14 days
- VRC01 structure (SDS PAGE) and binding activity (ELISA) maintained

Highlights from MAb Microbicide Project: Basic research

- Total greenhouse production time = 6 weeks:
  - Pesticide free
  - Average yield – 10g MAb /batch
- VRCO1 MAb-Ns are as effective as VRCO1 huMabs in neutralizing primary HIV isolate and SHIV
- Neutralizing activity not affected by
  - Low pH (2% lactic acid, pH 3.5, 3 hrs)
  - Semen or cervicovaginal secretions
- In vivo and in vitro PK studies indicate MAb retention in the vagina for > 9 hours
  - mAbs are retained in the epithelium, mucus
- VRCO1 MAb-Ns protected macaques from vaginal SHIV infection

Highlights from MAb Microbicide Project: Phase 1 clinical trial

- Large batch of MAb-Ns (VRCO1, anti-HSV-2) have been produced to GMP standards and formulated into film.
  - Stability and toxicology studies complete
  - Primate PK and efficacy studies are underway.
- Phase 1 clinical trial initiated in Q1 2016.
  - First in human mAb-N combination microbicide study.
- Future studies may use vaginal rings to incorporate several antibodies

MB66: Second Generation

Add additional HIV mAbs against cell-free and cell-associated HIV

Candidate Antibodies:

- Free HIV: VRC01 + PGT121
- Cell-associated HIV: HC4

Multipurpose Microbicide

- Anti-sperm: HC4
- Anti-chlamydia

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Clinical Experiment:

Persistence of RhoGAM in human vaginal secretions

- 10 midcycle reproductive-aged women
- 0.8 ml polyclonal human IgG anti-D antibody (RhoGAM, Ortho) was instilled via catheter into the vaginal cavity
- Anti-D activity was titered in vaginal secretions at 1, 24, 48 and 72 hour time points