State of the Art in Microbicide Research

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Vaginal/Cervical Tissues and Natural Barriers to HIV Infection

- Vaginal transmission of HIV during intercourse can occur via vaginal or cervical epithelium
- Women having an intact epithelium without inflammatory changes and a *Lactobacillus*-predominant microflora have a relatively low risk of infection
- Damage to epithelium allows greater access to submucosal T cells, macrophages and dendritic cells
- Microbicides should NOT disrupt innate barriers to infection while decreasing the viral load present in the vagina

What is a Topical Microbicide?

A substance that can be applied topically to prevent transmission of sexually transmitted infections including HIV.

A Tale of Two N-9 Trials

- Cameroon — 1995 - 1998
- UNAIDS — 1996 - 2001

N-9 Film – Effect on HIV Acquisition

	No. of Women	No. of HIV Infections	HIV Incidence 100 p-y	RR (95% CI)
Placebo Film	575	46	6.6	1.0
N-9 Film	595	48	6.7	1.01 (0.68-1.52)

Source: Roddy, NEJM 1998

UNAIDS N-9 Trial - Results Among Women with Regular FU

	No. of HIV infections	HIV incidence 100 p-y	RR (95% CI)
Replens®	33	9.2	1.0
Advantage S®	53	16.4	1.7 (1.1-3.8)

Ref: VanDamme, Lancet 2002: 360:971-7

N-9 Impact on Genital Inflammation

- Classical safety studies required by the FDA did not detect significant safety signals for N-9 during phase 1 and 2 studies except when women used N-9 multiple times daily (Hillier, AIDS, 2005; 39:1-8)
- 3 daily applications of N-9 (150 mg) induced an increase in IL-1, TNF and Il-8, and a decrease in SLPI (Fichorova, J Infect Dis 2001;184:418)

The Change in Microbicide Research Following N-9

- Early concept was that commercially available products could be used to prevent HIV- using approved products for new indications (phase 4 studies)
- In 2000-2002 it became clear that **new** products had to be developed for use as microbicides
- Drug development model: regulatory construct based on phase 1, 2, 3 studies under guidance from FDA

What was the Outcome of the Shift in Microbicide Paradigm?

- Clinical trial sites had to be prepared to conduct studies at standards acceptable to US FDA, rather than epidemiologic standards
- Need for rapid growth in infrastructure at the sites in the developing world: labs, pharmacies, training in regulatory trial conduct
- New drug product development required up-front investment in formulation and scale-up
- HUGE increase in costs for safety testing, clinical trials, drug manufacture and scale-up

Top Priorities After N-9

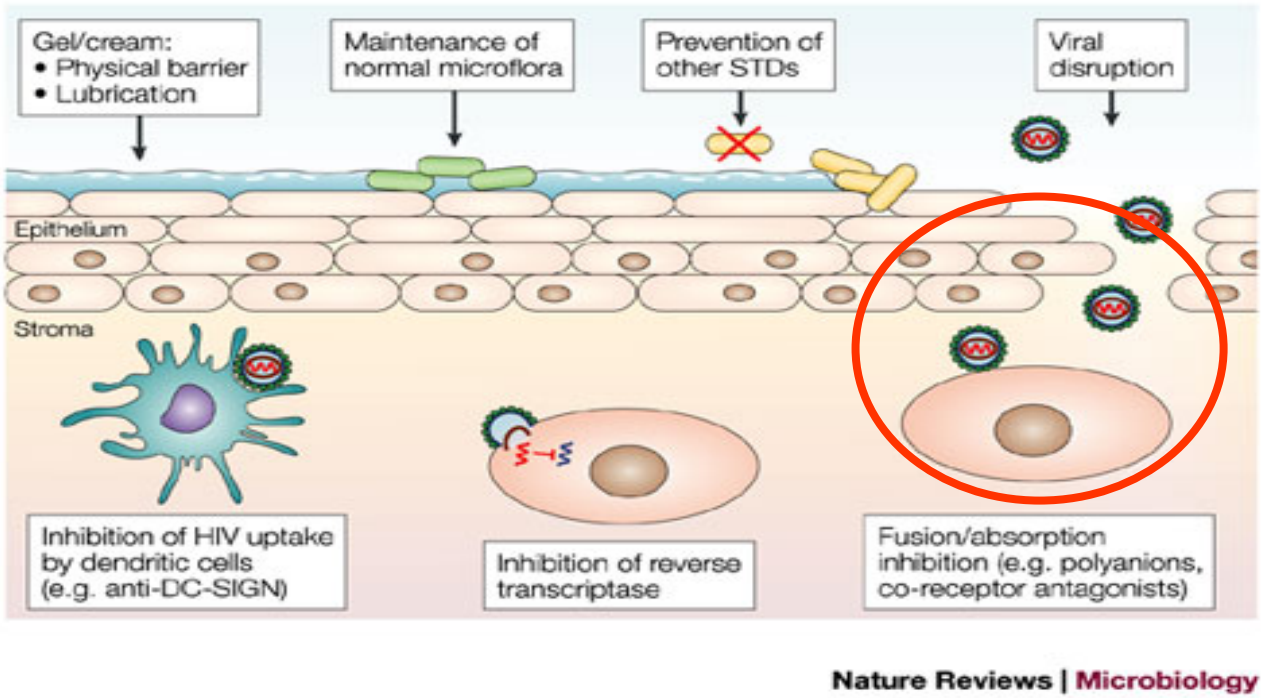
- Engage FDA in defining a regulatory pathway for the evaluation and approval of microbicide products
- Develop clinical trial site infrastructure for regulatory trials
- Get the most promising products into the field to evaluate their effectiveness in large scale trials as soon as possible

Microbicides for Prevention of HIV-1 Transmission

Reduce trauma to mucosa

Maintain vaginal pH

Reduce epithelial inflammation



Shattock RJ and Moore JP. Nature Reviews Microbiology, vol. 1 Oct/2003

What Intervention to Evaluate?

- No clear way yet to identify “best in class” which should move forward
- Real disagreement in the field regarding preference of broad spectrum vs. HIV-specific approach for microbicides
- Important to test different classes of microbicides having different mechanisms of action

Non-trial Issues of Relevance To Topical Microbicides

- Lack of a well-established correlation between in vitro, animal models, and clinical testing
- Insufficient knowledge on vaginal transmission of HIV and other STD pathogens
- Lack of optimal formulations
- Insufficient knowledge on cervico-vaginal and intercourse physiology
- **Insufficient knowledge on of impact of contraceptive hormones, gel products and genital microbes on innate immune defenses in the vagina**

Effectiveness Trials 2006

Sponsors	Product	No. Women Seen	No. Women to Enroll	Complete (Est)
NIAID	BufferGel, PRO 2000	9000	3100	2008
Pop Council	**Carraguard**	12,540	6300	2007
FHI/USAID	Savvy	10,000	4284	2007
FHI/USAID	**Cellulose sulfate**	5000	2160	2007
CONRAD/ USAID	**Cellulose sulfate**	5000	2574	2008
MDP/MRC	**PRO 2000**	20,000	10,000	2008

Carraguard, Cellulose sulfate and PRO 2000 are sulfated or sulfonated polysaccharides

Increasing Concerns Regarding Safety of Broad Spectrum Microbicides in 2007

PRODUCT	OUTCOME
N-9 Gel	Trial stopped, **evidence of harm**
N-9 Film	Trial completed, no evidence of harm or benefit
Savvy (C31G)	Trial stopped due to futility, no evidence of harm
Cellulose Sulfate CONRAD	Trial stopped, trend toward **evidence of harm**
FHI	Trial stopped, no harm

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Focusing on Future Success: The Pros and Cons

- The failure of N-9, cellulose sulfate and Savvy to have any impact on HIV transmission have made some people question whether any microbicide can ever work for prevention of HIV
- The INCREASED risk of HIV in some studies has raised questions about whether any product can be safe enough
- The microbicides having the greatest potency against HIV (ART's) have not yet entered testing
- Products used independent of coitus may not have the same impact on innate immunity
- Products used daily will not require adherence to gel usage just prior to intercourse

Why Would Topical Microbicides Increase Risk of HIV?

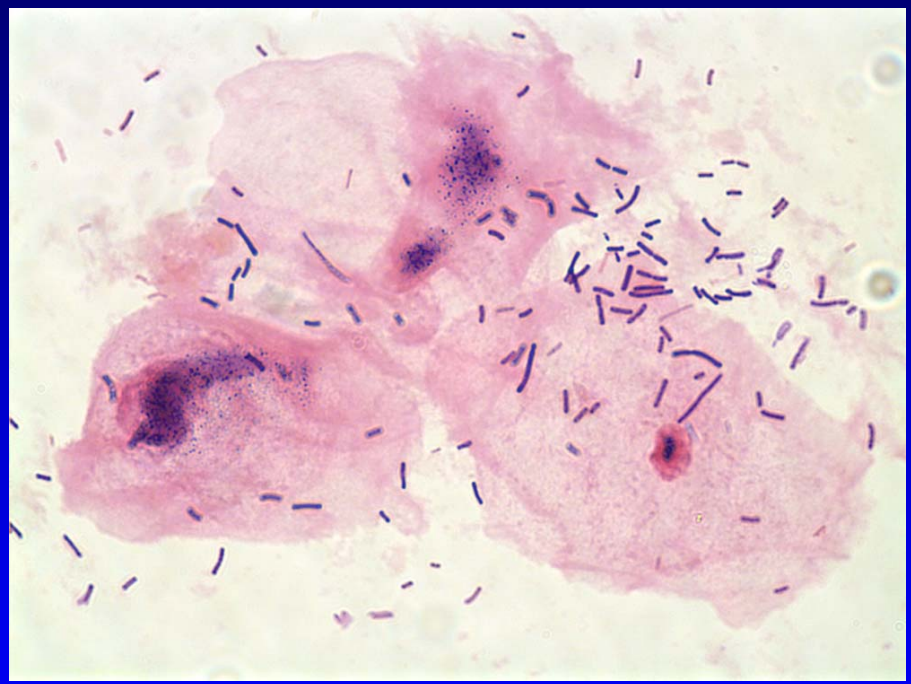
- Increase in local inflammation and recruitment of target cells.
- Disturbances of innate defense factors in the reproductive tract
  - Antiviral activity
  - Antimicrobial peptides
  - SLPI
  - Normal flora
- Exfoliation/disruption of epithelium

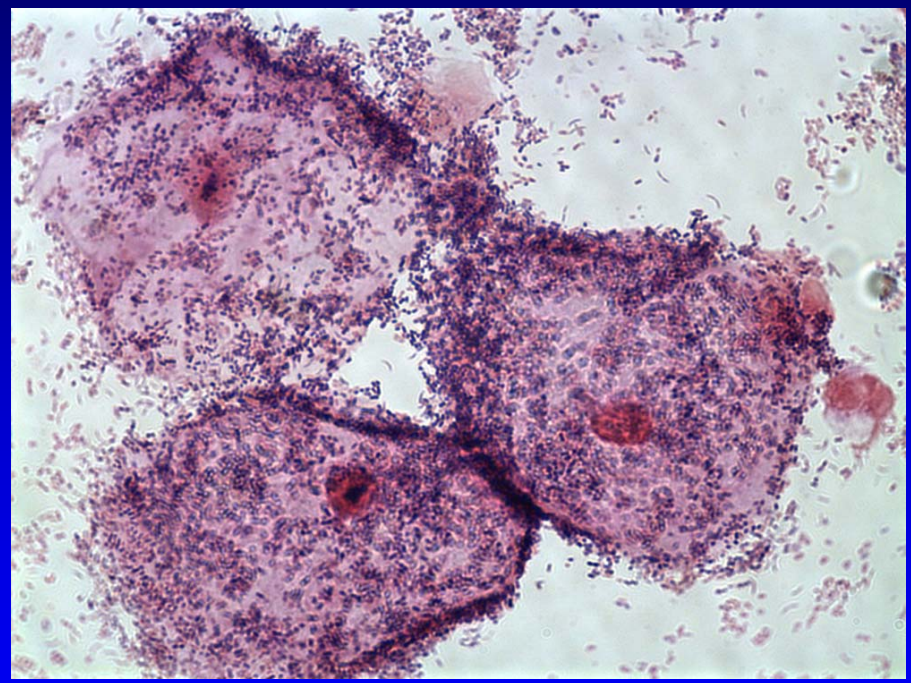
MTN IS EVALUATING THIS VERY CAREFULLY

How Do We Discriminate Between Infection-Related vs. Microbicide-Related Changes in Innate Immune Function of the Reproductive Tract?

Breaks in the Genital Epithelium: Genital Ulcer Disease	vs.	Chemical Epithelial Disruption
Cervical Inflammation: GC, Ct, Trich	vs.	Product-related inflammation
Vaginal inflammation and symptoms: (BV, yeast, Trich) Irritation and Itching	vs.	Vulvar/vaginal irritation due to product use

Why Assess Vaginal Microflora?





1. Because altered flora is associated with acquisition of HIV
2. Because altered flora is linked with changes in cytokines, chemokines, SLPI and other surrogates of safety

Longitudinal Studies Evaluating Vaginal Flora and HIV Acquisition in Women

Author	No Women	Adjusted Risk
Taha, AIDS, 1999	1196 Pregnant women in Malawi	Amsel criteria for BV One 1.5 Two 2.4 Three 3.7 P=0.04
Martin, JID, 1999	Sex workers in Mombasa, Kenya	Absence of vaginal lactobacilli 2.0 (95% CI 1.2-3.5)
Myer, JID, 2005	Non-pregnant women in Cape Town, S. Africa	Nugent >7 OR 2.0 (95% CI; 1.1-3.6)

Vaginal Microbicides: Detecting Toxicities That Increase Pathogen Transmission

- Mouse HSV-2 Vaginal transmission model which
  - Directly tests for toxic effects that increase susceptibility to HSV-2
  - Provides assessment of anti HIV effect
  - Identifies toxic effects that correlate with HSV-2 susceptibility
- Evaluation of N-9, BZK, SDS, components of Savvy, BufferGel, HEC placebo

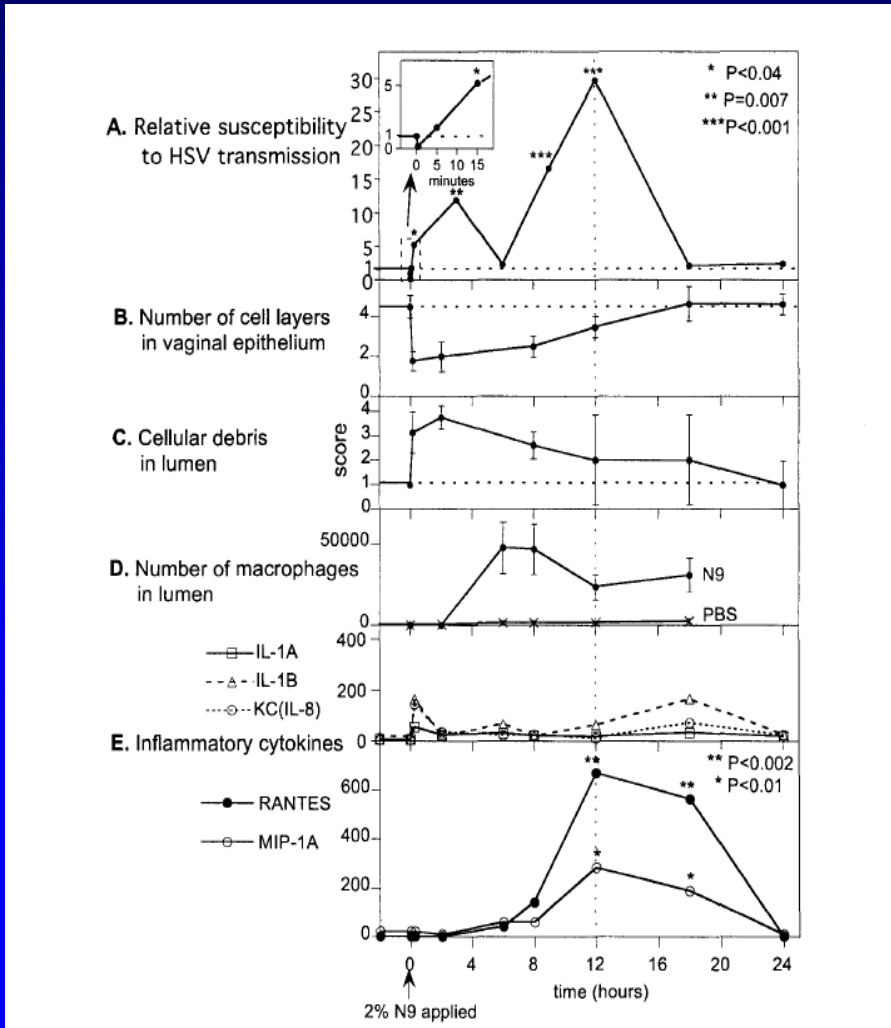
Ref: Cone et al, BMC Infect Dis 2006; 6:90

Vaginal Microbicides: Detecting Toxicities That Increase Pathogen Transmission

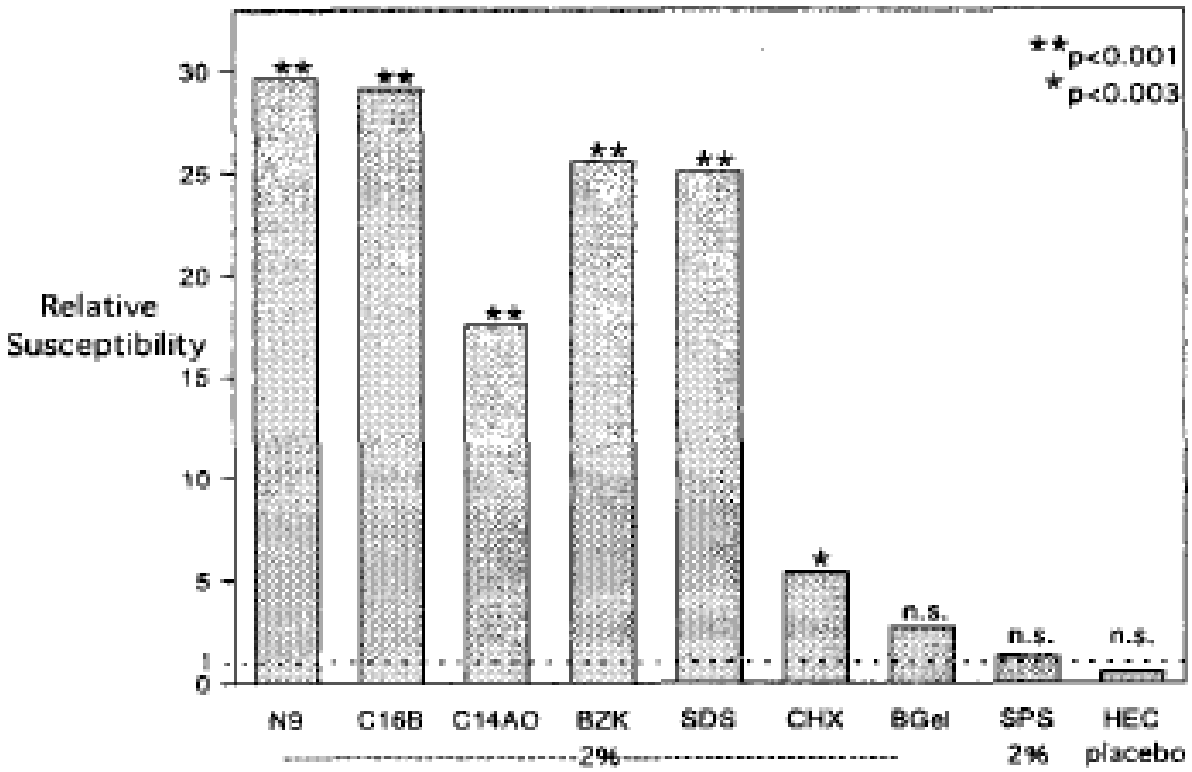
- 20 microliters of product applied to vagina of progesterone-treated mice
- Antiviral activity assessed at 4 hour intervals for up to 24 hours compared to PBS treated controls
- Factors assessed
  - Susceptibility to HSV
  - Number of cell layers
  - Cellular debris
  - Number of receptors
  - Supplementary cytokines

Ref: Cone et al, BMC Infect Dis 2006; 6:90

Vaginal HSV-2 Susceptibility and Toxic Effects vs. Time After a Single Dose of 2% N9



Vaginal HSV-2 Susceptibility 12 Hours After a Single Application of Candidate Microbicides



Ref: Cone et al

Vaginal HSV-2 Susceptibility and Toxic Effects vs. Time After a Single Dose of 2% N9

- N-9, Savvy components, BZK and SDS all **enhance**d HSV-2 susceptibility at 12 hours in mouse model
- Buffer Gel and HEC placebo did not impact HSV-2 susceptibility
- Important targets for safety studies: inflammatory cytokines, exfoliation of columnar epithelium

Ref: Cone et al, BMC Infect Dis 2006; 6:90

Assessing Anti-Viral Activity of Cervicovaginal Fluid Following Microbicide Use

- Assessment of vaginal fluid for anti-HIV and anti-HSV activity prior to and after use of topical microbicide
- HeLa cells or human macrophages inoculated with CVL spiked with replication defective HIV
- Human cervical cells inoculated with CVL and challenged with HSV-2

Ref: Keller, et al. J Infect Dis 2006; 193:27-35

Assessing Anti-Viral Activity of Cervicovaginal Fluid Following Microbicide Use

- Study population:
  - 10 HIV+ women received 2g 0.5% PRO 2000 gel
  - 1 HIV+ women received 2g placebo
- Women with concurrent genital infection excluded
- Cervicovaginal lavage samples obtained 48 hours before and 1 hour after PRO2000 exposure
- Levels of IL-1B, IL-8 and SLP I in CVL measured at each visit.

Ref: Keller, et al. J Infect Dis 2006; 193:27-35

Assessing Anti-Viral Activity of Cervicovaginal Fluid Following Microbicide Use

- Anti HIV activity –
  - 4.0 ± 1.3 log reduction (PRO 2000)
  - 0.2 ± 0.4 log reduction (Placebo)
- Anti HSV activity:
  - 3.0 ± .2 log reduction (PRO 2000)
  - 0.6 ± .2 log reduction (Placebo)
- Marked reduction in IL-1B, IL-8 and SLPI following product exposure, similar for PRO & Placebo

Ref: Keller, et al. J Infect Dis 2006; 193:27-35

Assessing Impact of PRO2000 on Immmune Factors in the Vagina

- 24 healthy nonpregnant women using PRO 2000 DAILY for 14 days
- PRO2000 elicits a decline in immune mediators in the vagina (Beta defensins, immunoglobulins, and IL-1 Ra, a range of cytokines) during product use
- No decline in intrinsic antiviral activity
- Will the temporary decline in some mediators enhance risk?

Ref: Keller, et al, AIDS 2007:21:467-76

Is There Anything We Have Learned that Make us Worry About BufferGel and PRO2000?

- Best models available today show that BufferGel and PRO 2000 do NOT disrupt innate immune function in the vagina
- PRO 2000 has good activity against both HSV and HIV in cervicovaginal fluid
- Our blinded safety data to date do not show worrisome trends

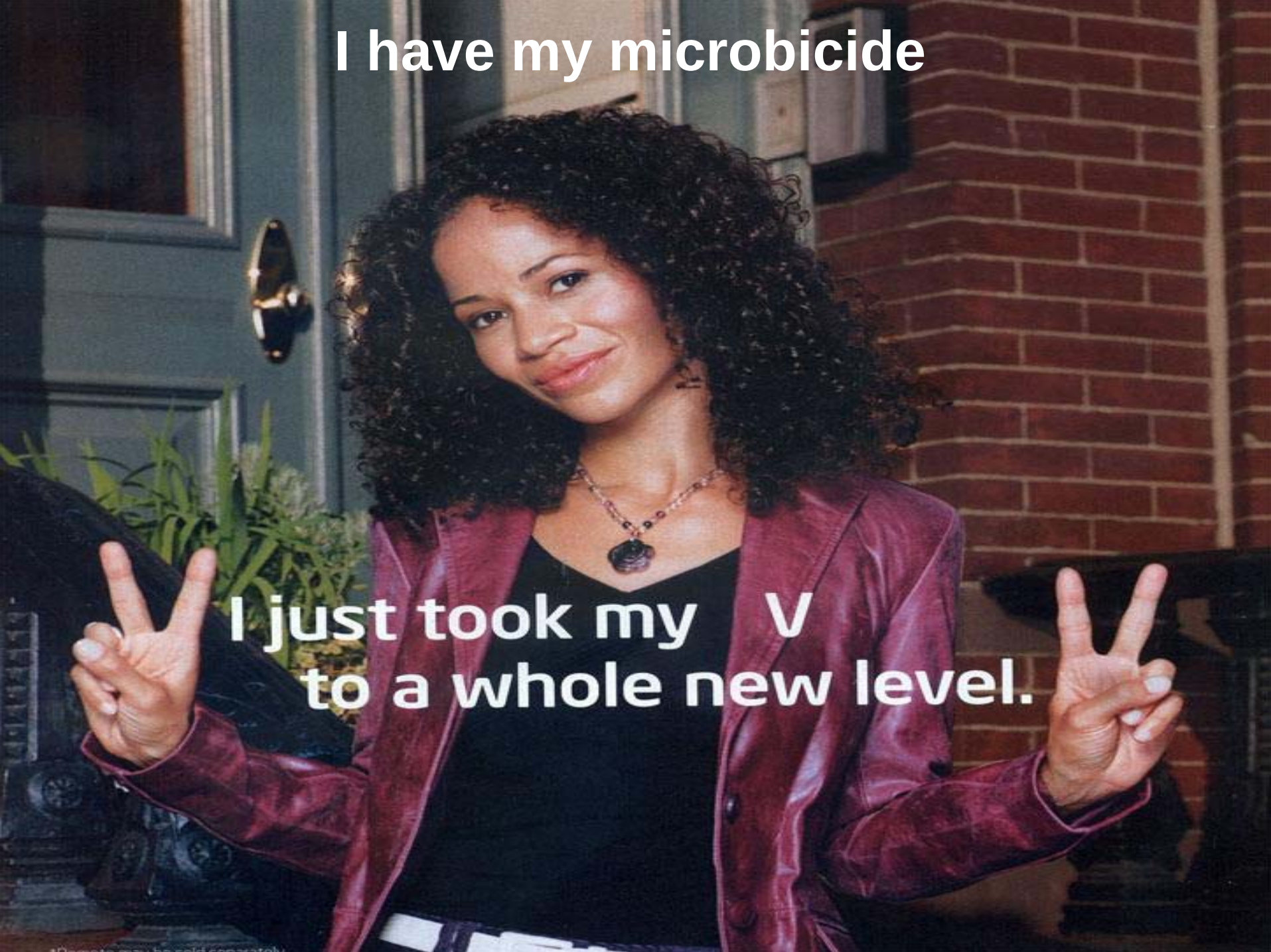
State of the Art in Microbicide Research

- The recent failures in the microbicide field should not discourage us from continuing to press forward with our current studies
- Newer ART based microbicides having high potency against HIV and used independent of coitus have enormous potential

Product Pipeline in 2007*

	Membrane Disruption	Defense Enhancers	Entry Inhibitors	Replication Inhibitors	Other
Pre-Clinical			PSC-Rantes Cyanovirin	MIV-150 TMC-125 UC781 TMC 120 ring PMPA TMC120 gel	Aptamers
Phase 1		Acidform™ Lactobacillus crispatus	VivaGel™ Invisible condom™		
Phase 2/2B		Lactobacillus			Praneem Polyherbal
Phase 3		BufferGel™	Carraguard PRO 2000 (0.5%, 2%)		

*Active planning, ongoing, or recently completed studies


I have my microbicide
I just took my V
to a whole new level.