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Microbicides against HIV and STIs

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Mucosal Infection and Immunity
Section of Infectious Diseases

The New York Times

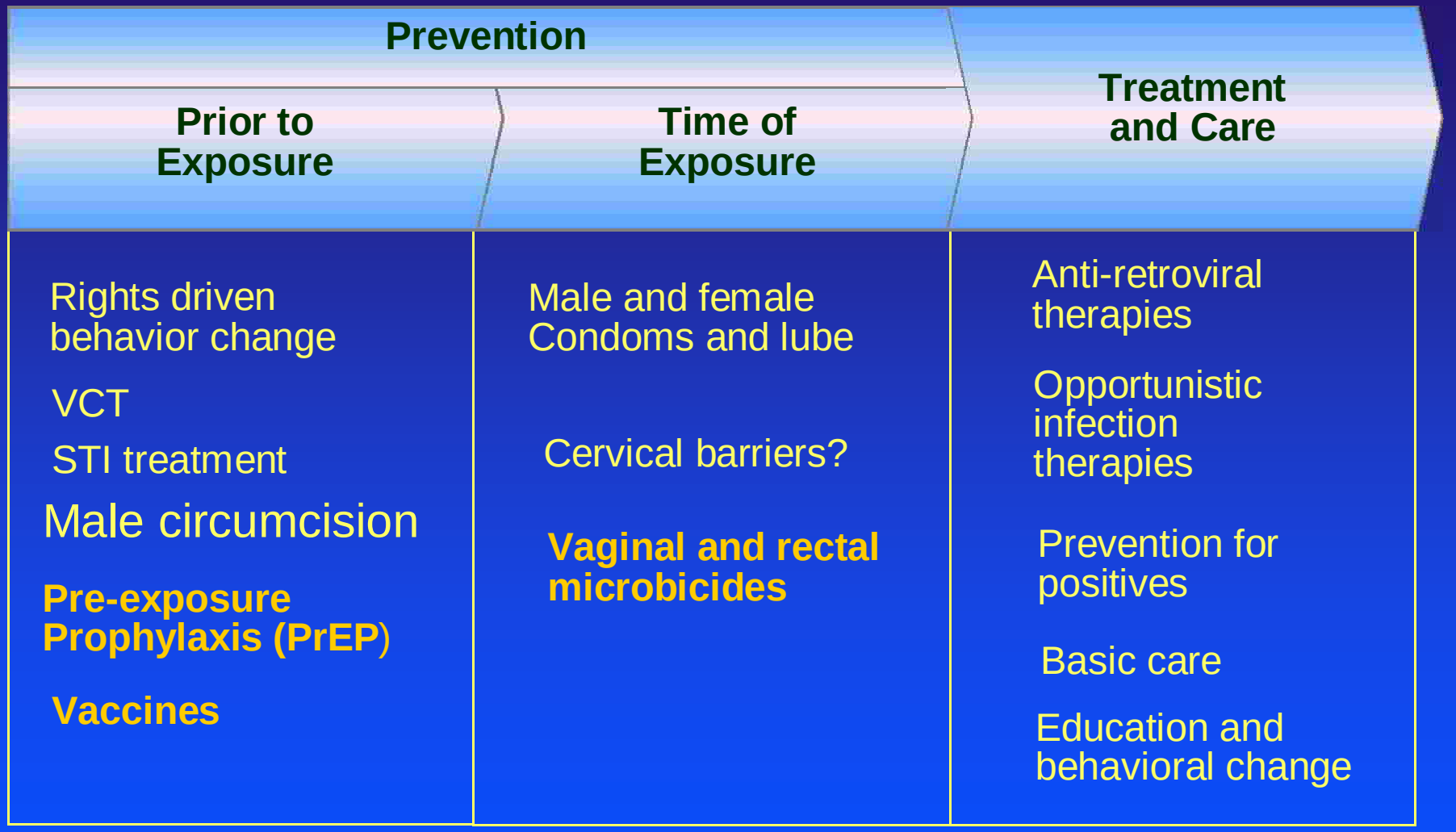
At Front Lines, AIDS War Is Falling Apart

By DONALD G. McNEIL Jr.Published: May 9, 2010

"For every 100 people put on treatment, 250 are newly infected"

"We may **never** be able to provide **universal treatment access** if we are unable to significantly reduce the number of new infections"

Working towards comprehensive approaches to HIV/AIDS



A Microbicide is designed to be

a **safe, effective, acceptable, affordable product** delivered as a single agent or multiple-component strategy in a stably-formulated gel, tablet, film, injectable, and/or device (i.e., ring, diaphragm) to both HIV-negative and, ideally, HIV-positive individuals. Its purpose is **to prevent**, or at least significantly reduce, **acquisition and transmission of HIV** (and possibly other sexually transmitted infections) at the genital vaginal and/or penile) and/or gastrointestinal (colorectal) mucosa.

ALLIANCE FOR MICROBICIDE DEVELOPMENT

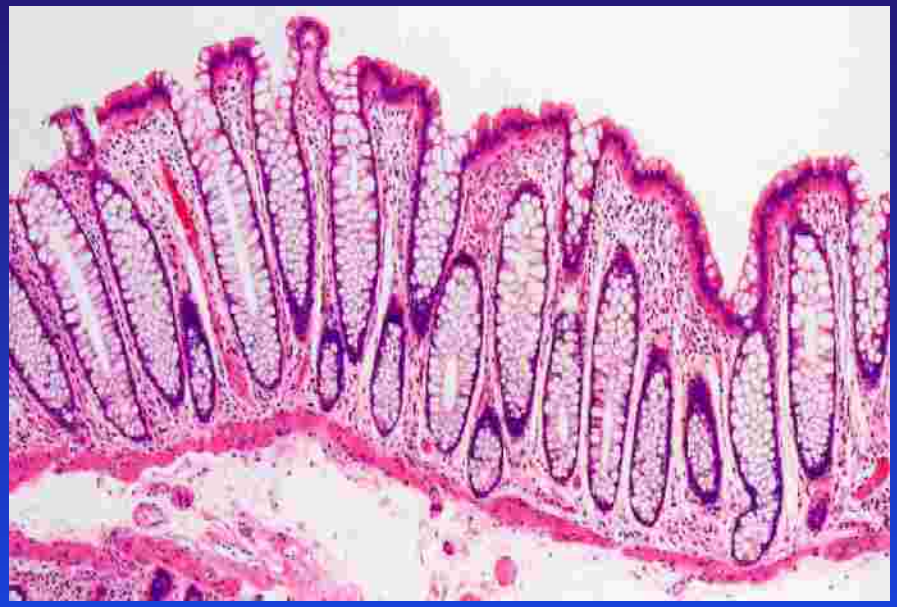
Risk and prevalence of anal sex in MSM and heterosexual women

Behavior	Risk of HIV Infection	Reference
Vaginal Sex	**0.001 – 0.02%** (95% CI: NA)	Kalichman S et al. 2002
Anal Sex	**0.25%** (95% CI: 0.06,0.49)	Vittinghoff E et al. 1999

Group	Prevalence	Reference
MSM	**91%**	PRMS
Women	**8%**	NHSLS, 1992
Women	**20-30%**	Gorbach P et al. 2000
Women	**38.2%**	McBride KR et al. 2010

Vulnerability of colorectal mucosa to HIV-1 infection

Vaginal epithelium



Colorectal epithelium

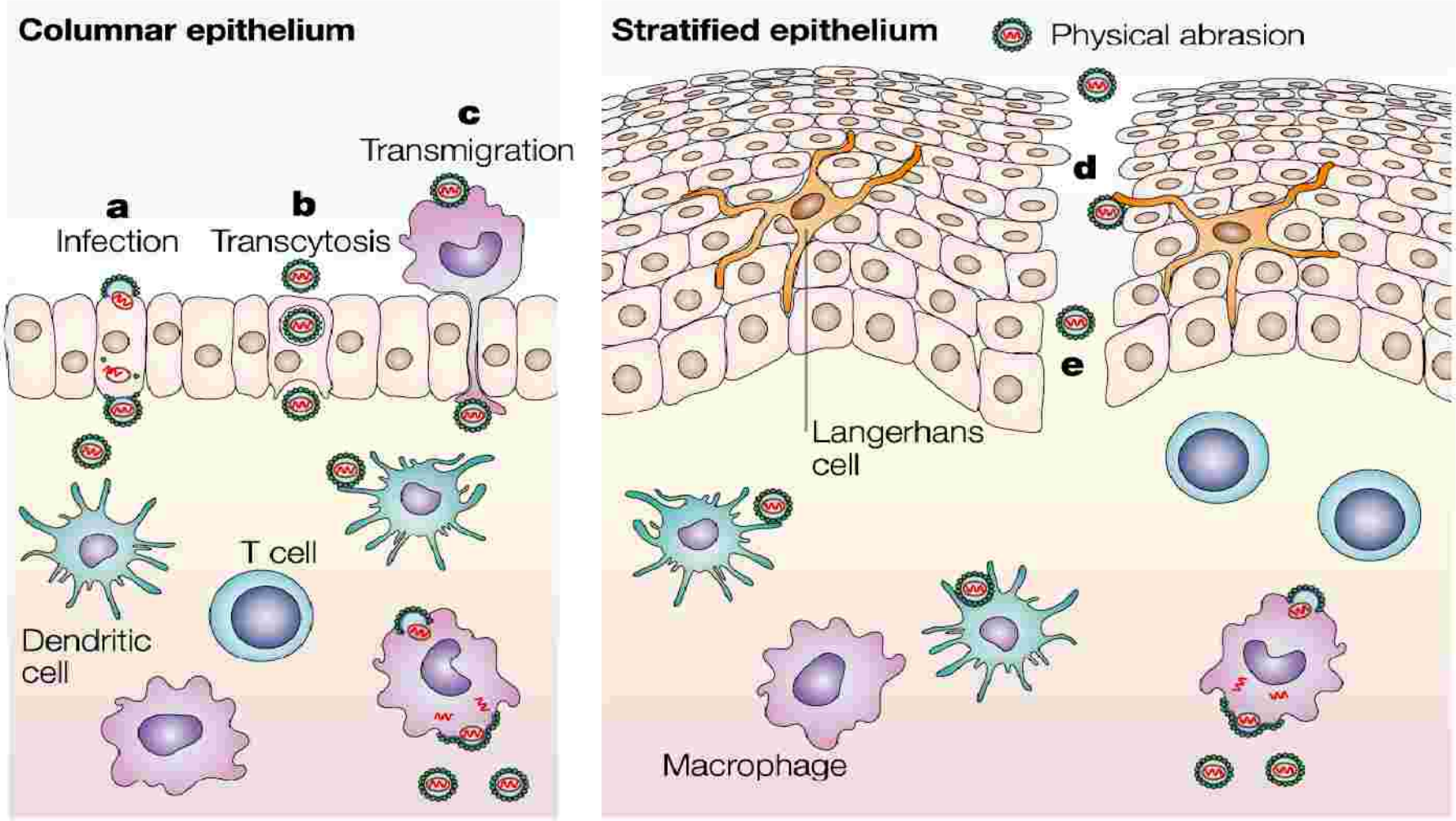
- Single layer of columnar epithelium – easily damaged by RAI
- The intestinal lamina propia contains an abundance of ighly activated target cells for HIV-1 infection

Vaginal and rectal microbicides

Special considerations for the design of microbicides ic for each compartment:

- Some microbicides may be cytotoxic in the rectum and not in the cervicovaginal tract
- Some microbicides may induce “immunological toxicity”
- Dynamics of absorption, local retention and clearance likely to be different in the colorectal tract and in the vaginal compartment

Mucosal transmission



Shattock R and Moore JP (2003) Nat Rev Microbiol

Any microbicide must be

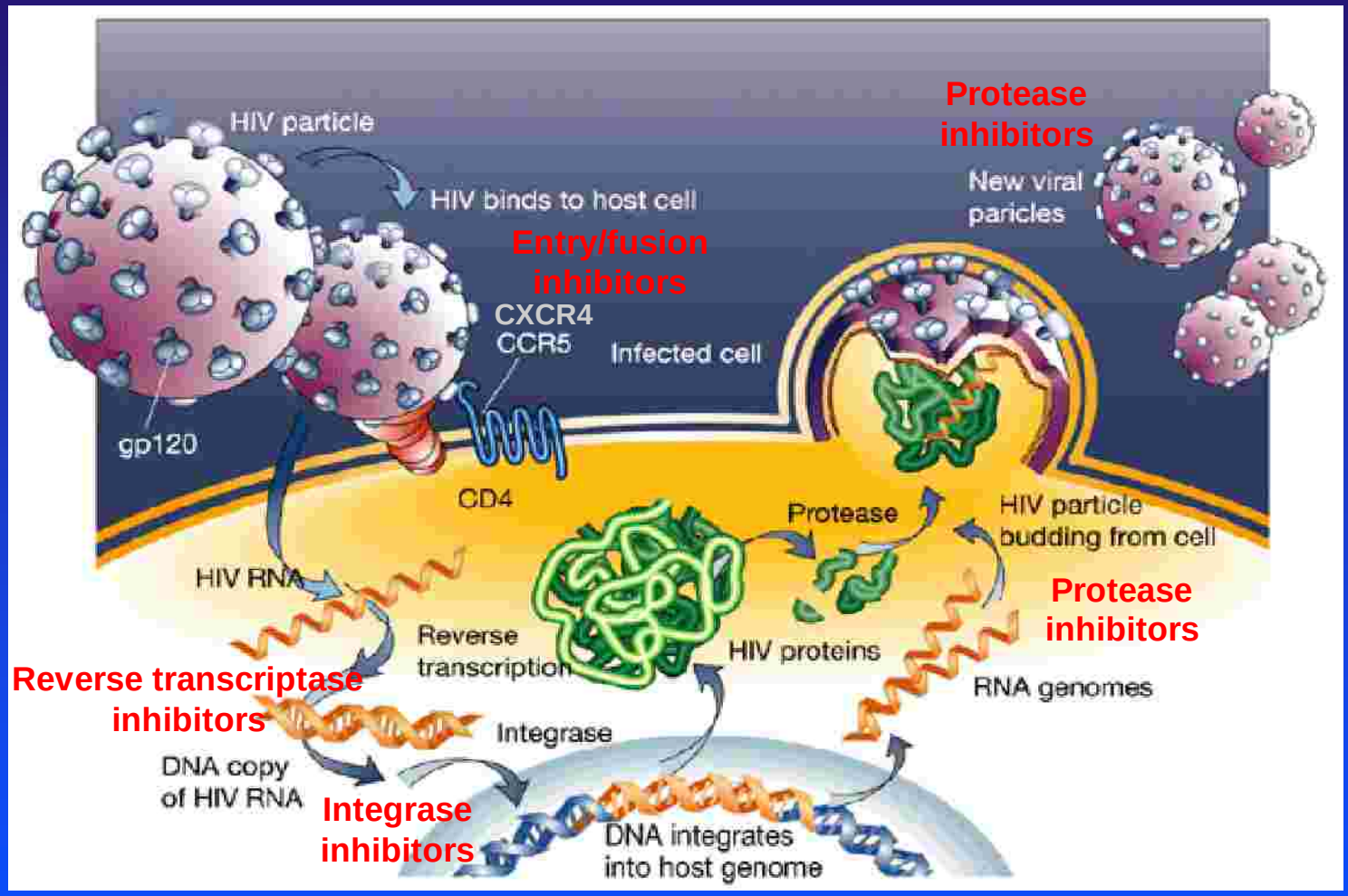
- **Safe** - must have no localized toxicity, including no damage to the vaginal epithelium during sustained, repetitive use, with no localized inflammation.
- **Effective** - must have a significant degree of efficacy in real world situations.
- **Cost effective** - must affordable by those at risk and sustainable by donors
- **User-friendly** - must be compatible with use during sex (consistency, taste, smell etc, must be satisfactory, from both female and le viewpoints).
- **Appropriate drug deliver** - maintenance at the viral portals of entry (GI and genital tracts)
- **High barrier for resistance** - product characteristics should limit hypothetical risks
- **Limited impact on therapy** - product implementation should be closely monitored at a community level

Not all candidate microbicides are equal

Membrane-disruptive agents	pH modifiers	Non-specific entry inhibitors	ARVs	Specific entry inhibitors
N9 C31G Cyclodextrins	Acidform™ Lime Juice Lactobacillus BufferGel™	Cellulose acetate PRO 2000 Carraguard Cellulose Sulfate Polystyrene sulfonate SAMMA K5-N OS	NRTI (TFV) NNRTI Integrase inhibitors Protease inhibitors	CCR antagonist Maraviroc
Specific entry inhibitors	**GMOs**	**Others**	**Combinations**	**Oral PreP**
gp120 binders gp41 binders MAbs Lectins CCR5 & CXCR4 antagonist CD4 blocker (CADA)	Bacteria expressing: CD4, CV-N, gp41i etc	ZFIs siRNA Aptomers Immune modulators	NRTI/NNRTI NNRTI/CCR5 CCR5/gp41/gp120 Microbicide + vaccine	TFV Maraviroc

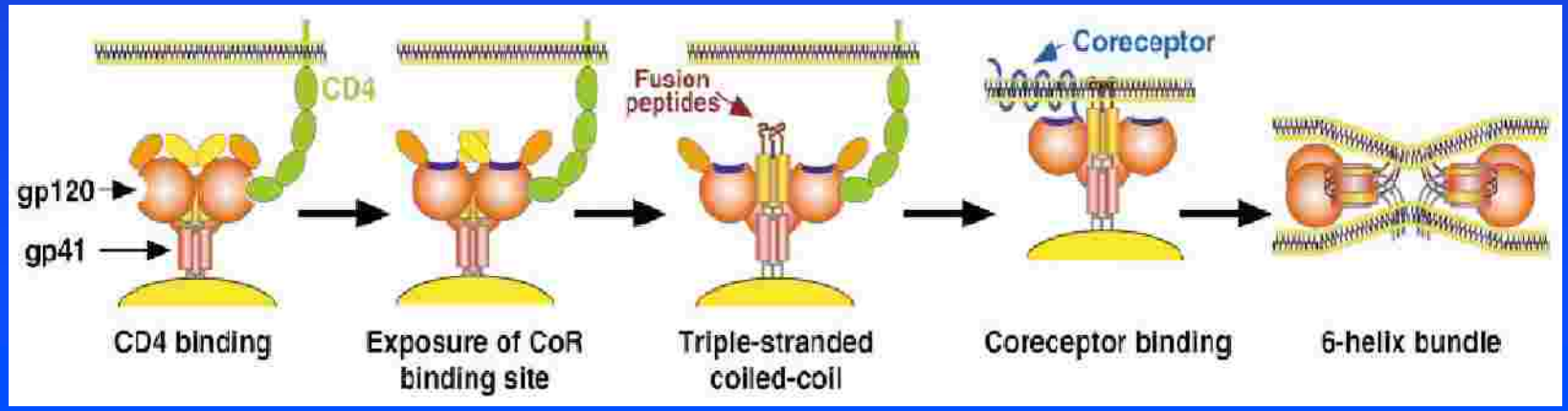
Abandoned Low potency in the clinic preclinical

Antiretroviral targets during the HIV replication cycle



Entry/Fusion inhibitors

- CD4-gp120 binding:
  - Ÿ Blocking the CD4 binding site in gp120: M48-U1, neutralizing antibodies
  - Ÿ Inhibiting conformational change in gp120 induced by CD4 binding: BMS-599793
  - Ÿ Binding lectin-like proteins to mannose moieties on *N*-linked glycans in gp120: cyanovirin-*N*, griffithsin
- Co-receptor (CCR5/CXCR4)-gp120 binding:
  - Ÿ RANTES chemokine analogs: PSC-RANTES, 5P12-RANTES
  - Ÿ Small molecules CCR5 or CXCR4 analogs: CMPD 167, marav c (CCR5) and AMD3100 (CXCR4)
- Six-helix bundle formation:
  - Ÿ Peptides mimicking the C-terminal region (HR2) of gp41: T20 (enfuvirtide), C52L, T1249 and L'644



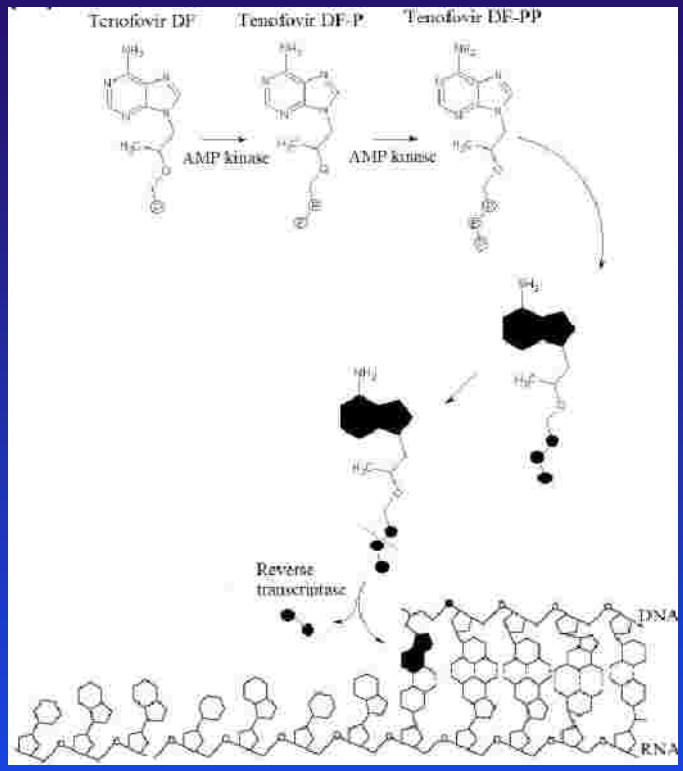
Doms R and Moore JP (2000) J Cell Biol

Reverse transcription inhibitors

- Nucleos(t)ide analog inhibitors (NRTIs):

  metabolically activated by phosphorylation and 5' triphosphates products inhibit the RT by substrate competition. NRTIs lack a 3´-hydroxyl group on the deoxyribose moiety. Hence, once incorporated into the DNA chain, the absence of a 3´-hydroxyl group, blocks further extension of the DNA by RT, and they act as chain terminators

  PMPA (tenofovir), FTC (emtricitabine)

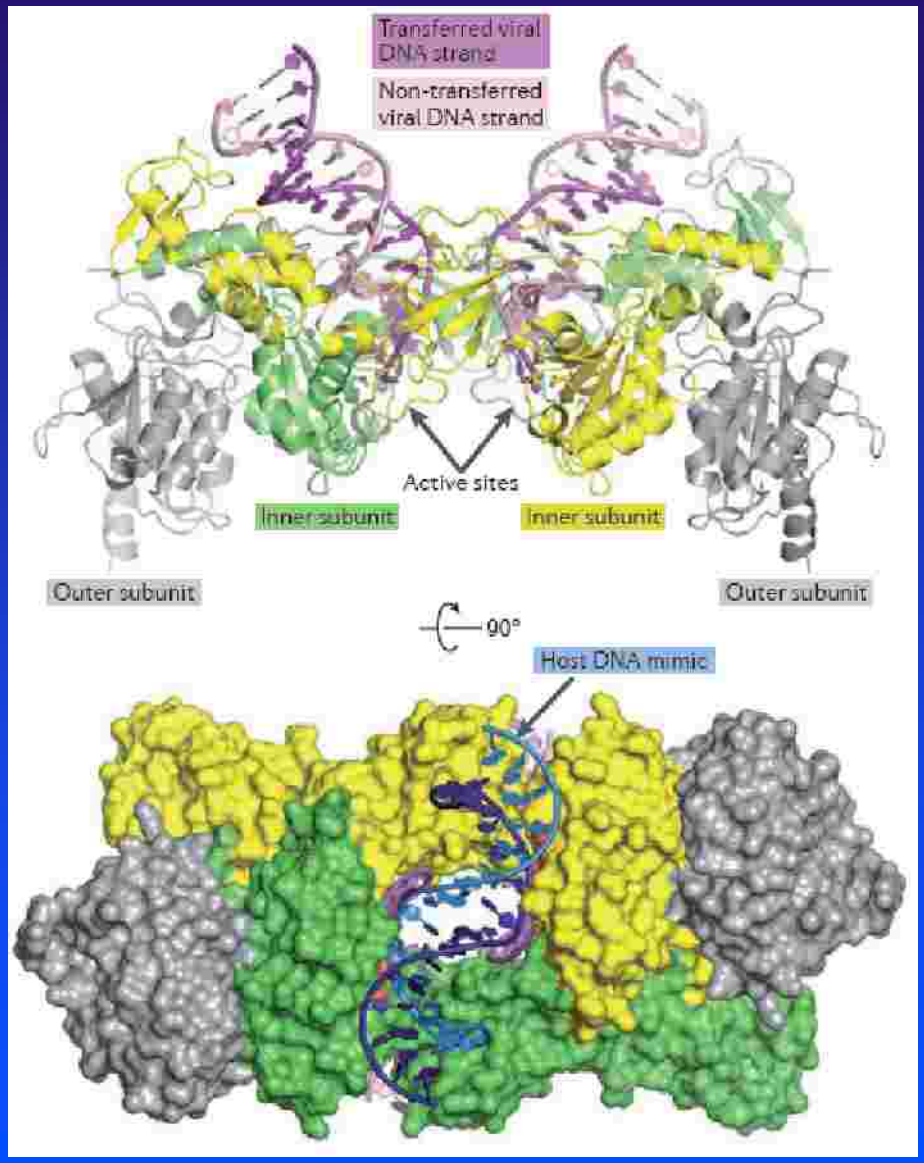


- Nonnucleoside RT inhibitors (NNRTIs):

  group of structurally diverse noncompetitive inhibitors that do not require metabolic activation. They block complementary DNA elongation by binding directly to the enzyme and inducing conformational change of the RT active site, decreasing its affinity for nucleoside binding

  TMC120 (dapivirine), UC781, MIV-150, MIV-170, MC1220, IQP-0528

Integrase inhibitors



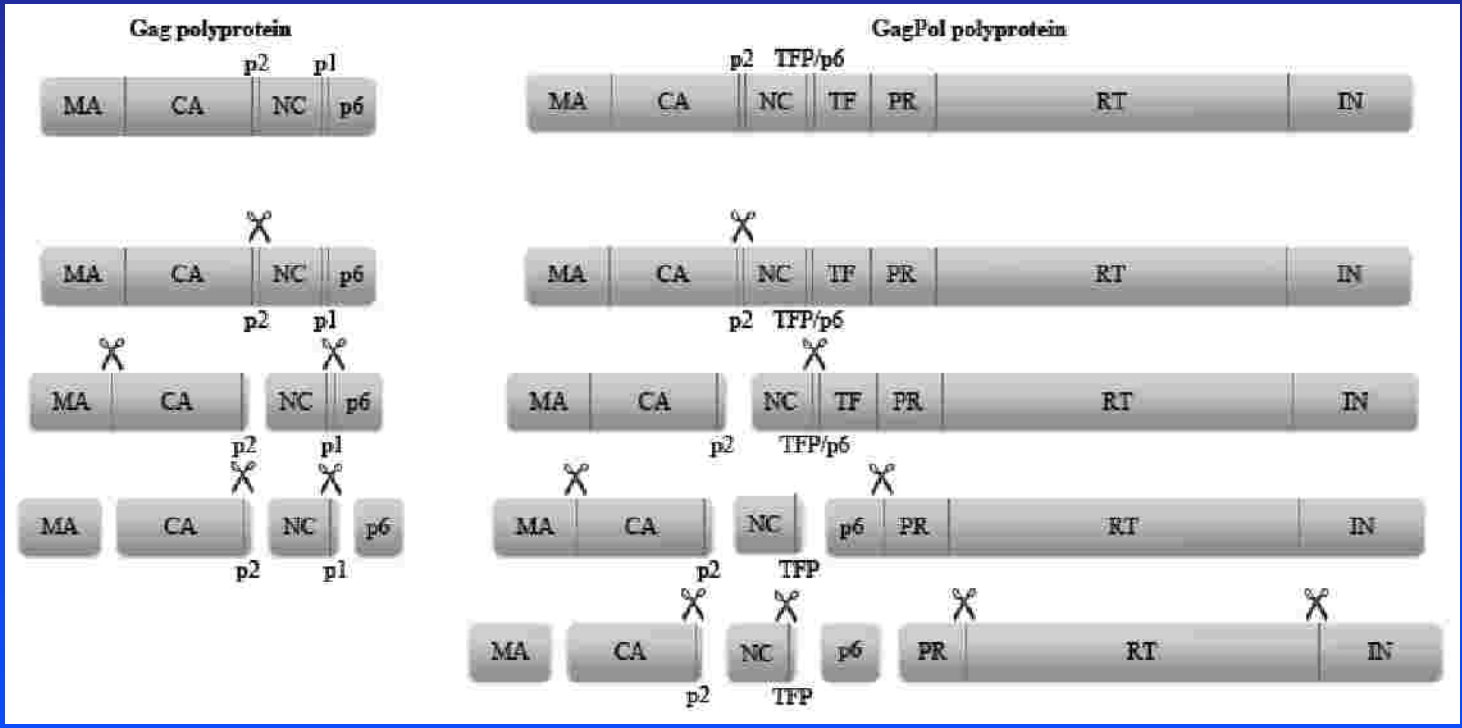
Engelman A and Cherepanov P (2012)

- Viral integrase (IN) possesses two catalytic activities: 3' processing and DNA strand transfer
- A few IN inhibitors have been developed: MK-0518 (raltegravir), elvitegravir, L-870,812
- All these candidate microbicides inhibit DNA strand transfer

Protease inhibitors

- Out of 9 PIs approved for oral used, 4 are candidate m crobicides: saquinavir, darunavir, lopinavir and ritonavir

- They inhibit by binding to the active site of HIV PR



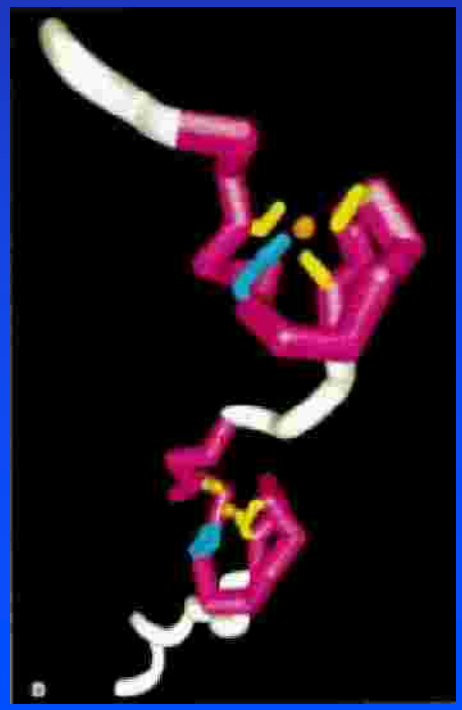
Herrera C and Shattock R (2012)

Zinc-finger inhibitors

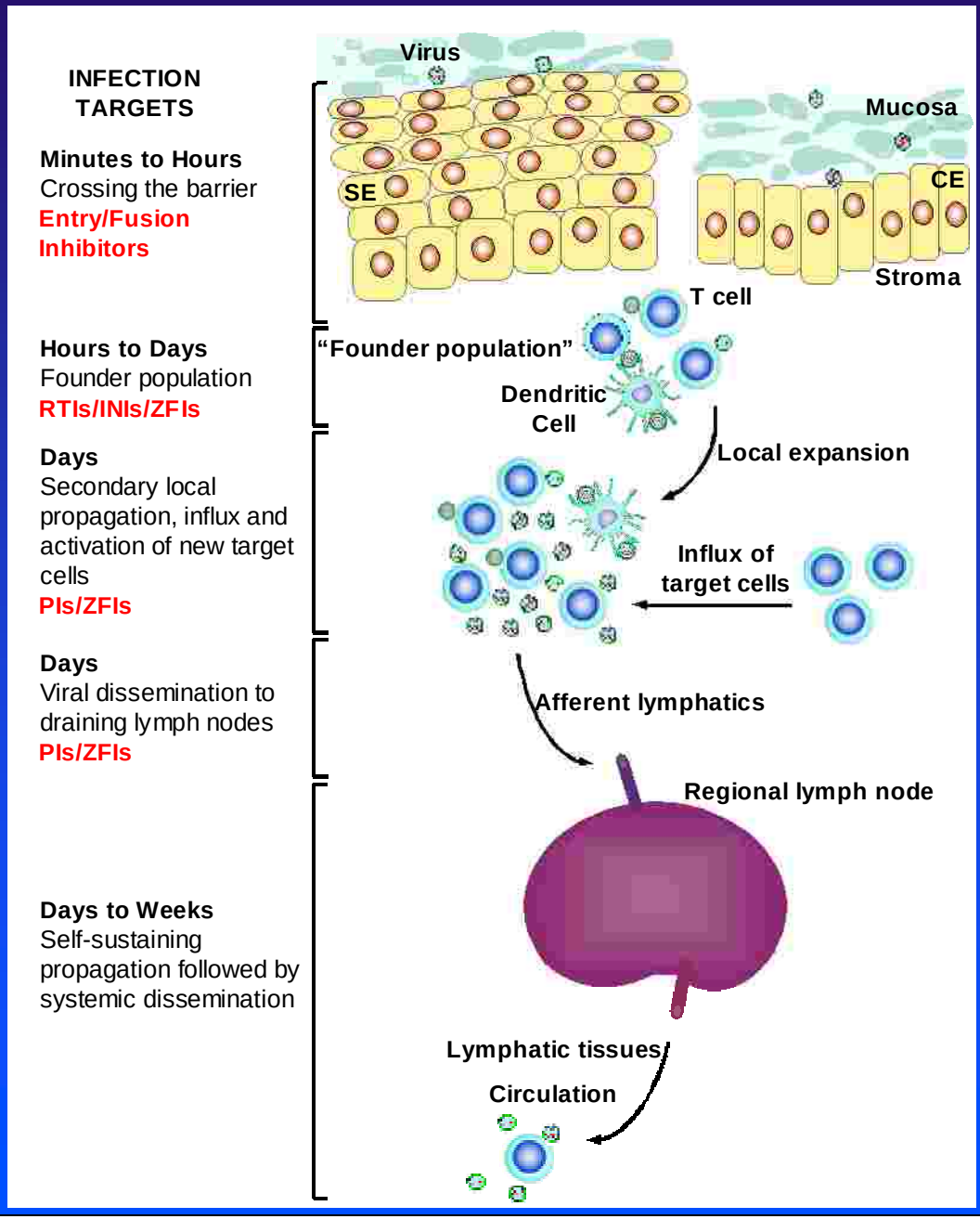
- The nucleocapsid protein (NCp7) has essential functions through out the viral replication cycle (reverse transcription, integration, and dimerization and packaging of the viral genome).

- NCp7 contains two zinc-binding domains forming tight rigid loops

- NCp7 inhibitors (ZFIs) are *S*-acyl-2-mercaptobenzamide thioesters (SAMT): SAMT-8, SAMT-19, SAMT-89 and SAMT-247



Mucosal transmission of HIV-1 and timeline for inhibition



CAPRISA 004: the first partially efficacious microbicide trial

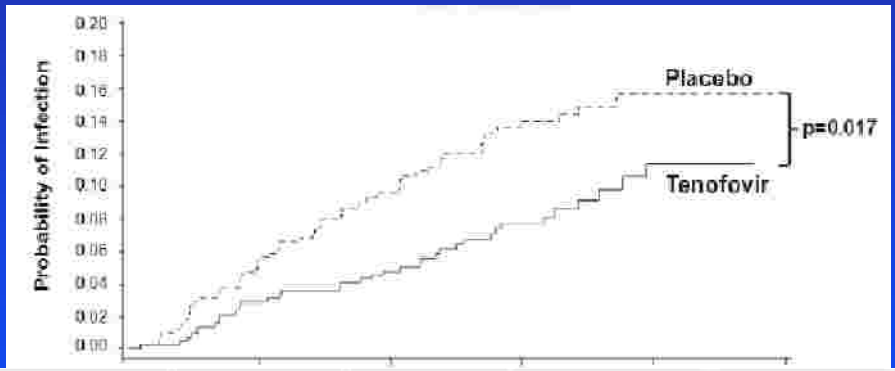
Enrolled Eligible: 889

Tenofovir (PMPA): 445

Placebo: 444

BAT 24 dosing strategy:

- Insert one applicator of gel up to 12 h Before sex
- Insert one applicator of gel as soon as possible withi h After sex
- No more than Two doses in 24 h



Months of follow-up	6	12	18	24	30
Cumulative HIV endpoints	37	65	88	97	98
Cumulative women-years	432	833	1143	1305	1341
HIV incidence rates (Tenofovir vs Placebo)	6.0 vs 11.2	5.2 vs 10.5	5.3 vs 10.2	5.6 vs 10.2	5.6 vs 9.1
Effectiveness (p-value)	**47% (0.064)**	**50% (0.007)**	**47% (0.004)**	**40% (0.013)**	**39% (0.017)**

Karim Q.A. et al. Science 2010

Mucosal environment

- Efficacy of microbicides can be positively influenced factors decreasing infectious dose before crossing mucosae, increasing in ate resistance or preventing establishment/dissemination of initial foci of infection.

- Efficacy could be offset by disturbance of normal flor reduction of innate protective factors or by damage or inflammation of the protective epithelium.

Case-Control Study of seroconvertors and HIV –ve controls from CAPRISA 004	No tenofovir detected (n=38)	
	No cytokine evidence of genital inflammation	Cytokine evidence of genital inflammation
HIV +ve Women	**10**	**7**
HIV –ve Women	**20**	**1**

Cytokine evidence of genital inflammation:
Raised levels of any 3 of IL-1α, IL-1β, TNF-α, IL-8, MIP-1α or MIP-1β

CAPRISA

Adherence needs to be promoted and measured

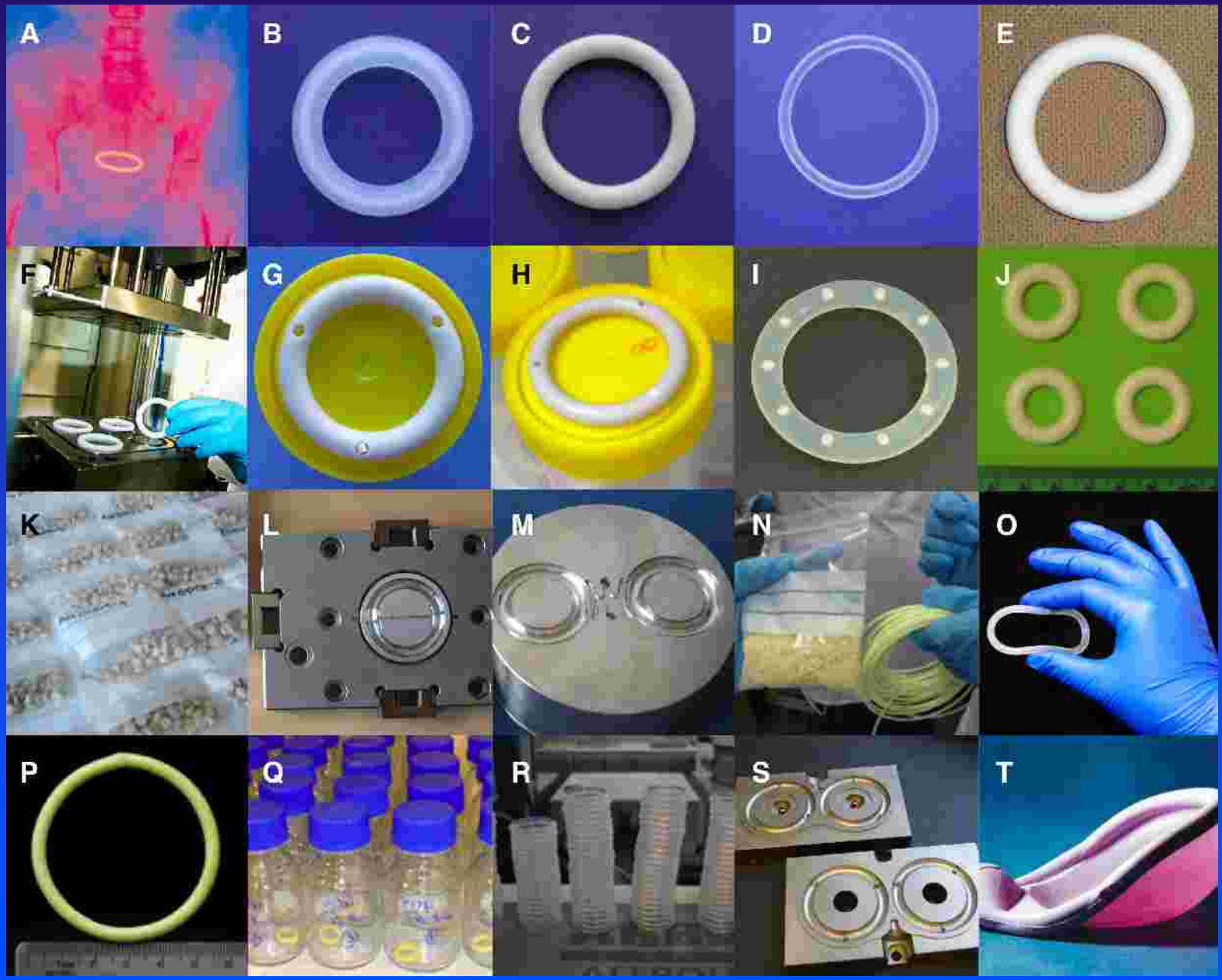
	# HIV	N	HIV incidence		Effect	p-value
			TFV	Placebo		
High adherers (>80% gel adherence)	36	336	4.2	9.3	**54%**	**0.03**
Intermediate adherers (50-80% adherence)	20	181	6.3	10.0	**38%**	0.29
Low adherers (<50% gel adherence)	41	367	6.2	8.6	**28%**	0.30

Karim Q.A. et al. Science 2010

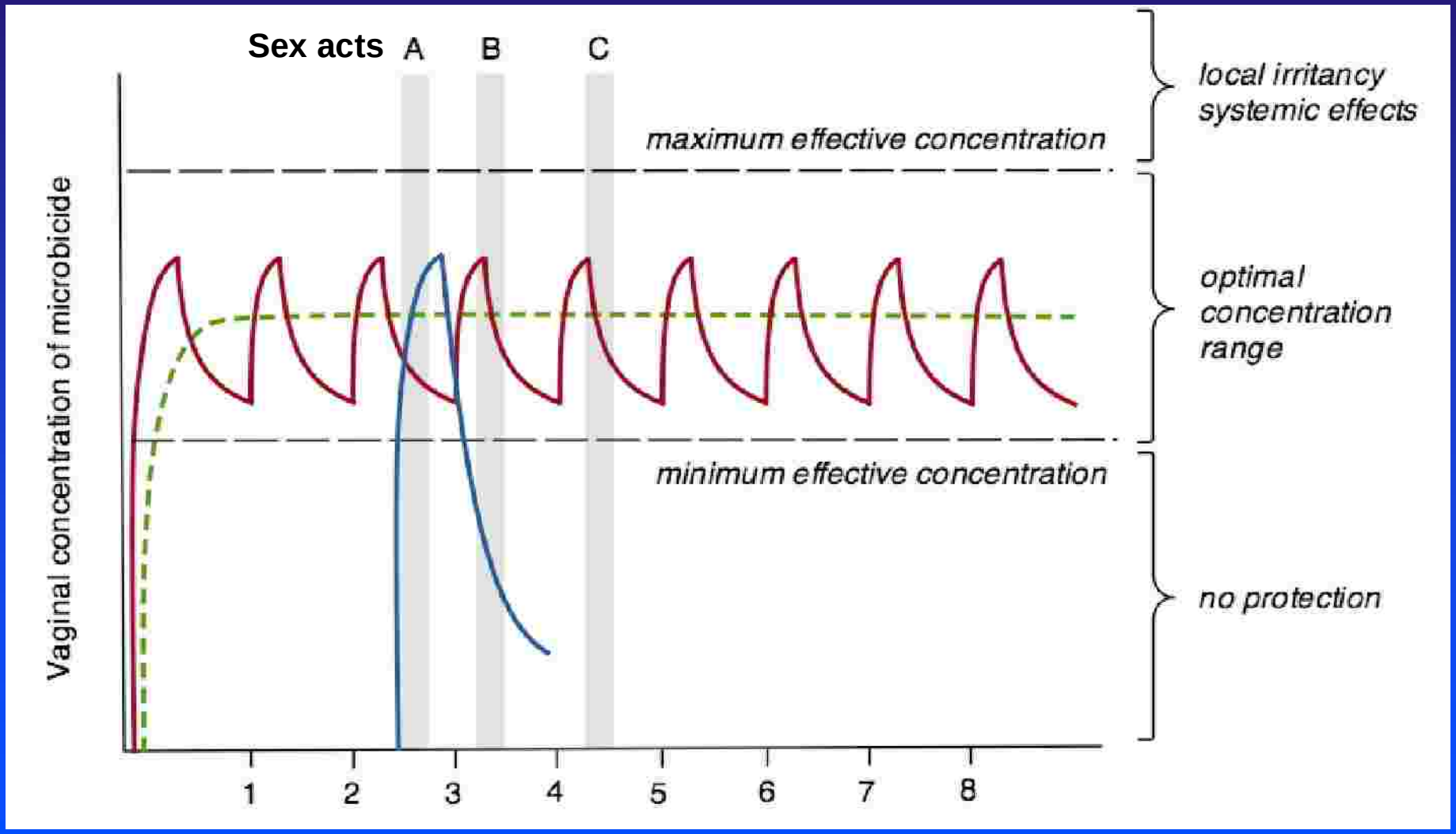
User-friendly - must be compatible with use during sex.

Improve dosing schedule – assess the potential for coital-dependent or independent formulations, and daily/weekly/monthly applications

Prioritization of formulations that maximize adherence



Sustained delivery devices (vaginal rings) offer a pat from coitally-dependent microbicides



Dapivirine rings

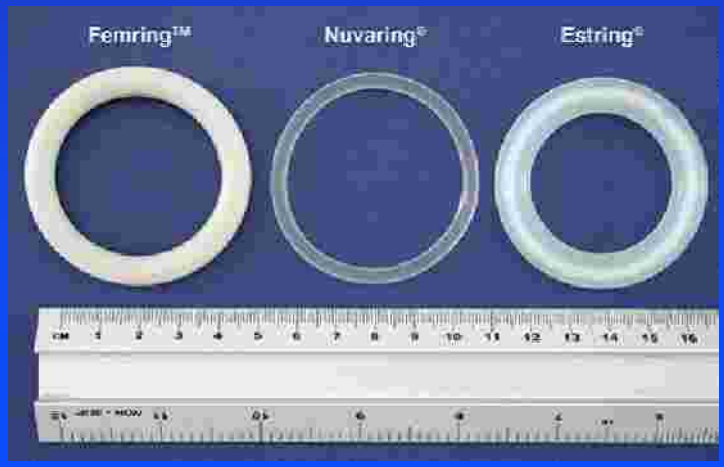
25 mg Res and matrix ring: [dapivirine] in vaginal fluid

Tissue EC_{90}= 3.3 ng/mL

Tissue IC90

Bridging microbicides and contraception: Dual-protection technologies RTI/Progestin ring

- Intravaginal ring made of PU or other polymers
- Long-term controlled release of Prg and RTI
- Prg-only contraceptives are well accepted
- Prg long track-record of safety and efficacy
- Non-contraceptive benefits: reduction of menorrhagia, anemia, endometrial atypia
- UC781 and LNG-loaded rings tested for *in vitro* and *in vivo* (animal) release for at least 1 month



Under development by CONRAD
(Courtesy of Gustavo Doncel)

CONRAD

CAPRISA 004: Impact of tenofovir gel on HSV-2 incidence

	Tenofovir gel n=202*	Placebo gel n=224*
# HSV-2 infections	**29**	**58**
Women-years of follow-up	292.3	287.3
HSV-2 incidence per 100wy (95% CI)	**9.9** (6.6, 14.2)	**20.2** (15.3, 26.1)

**Note: Excludes equivocal HSV-2 results at study exit*

IRR = 0.49 (CI:0.30, 0.78); ***p* = 0.003**

51% protection against HSV-2 by tenofovir gel (CI: 22%-70%)

CAPRISA

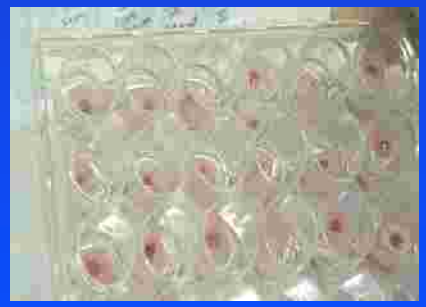
Pre-clinical models to assess candidate microbicides

Phase III clinical trials are expensive (~ $50-$100 million), time consuming and require large numbers of participants.

Activity and safety

- *In vitro* cellular models:
  - Ÿ Cell lines: TZM-bl luciferase reporter cells (2 days), PM-1 $CD4^+$ T cells (7 days), Affinofile cells (with variable levels of CD4 and CCR5)
  - Ÿ Primary cells: peripheral blood mononuclear cells, monocyte-derived macrophages and immature dendritic cells (7 to 14 days)
  - Ÿ Co-cultures with epithelial cells to mimic transepithelial migration of drugs
- *Ex vivo* tissue models:
  - Ÿ Cervical, vaginal, penile and colorectal tissue
  - Ÿ Polarized and non-polarized systems

Explants culture protocol



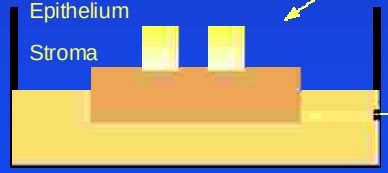
1 h with or without drug

Negative control: tissue only

2 h with or without virus

4 washes in PBS

Colorectal tissue cultured in duplicate



Gelfoam® raft

Samples collected at days 3, 7, 11 and 15

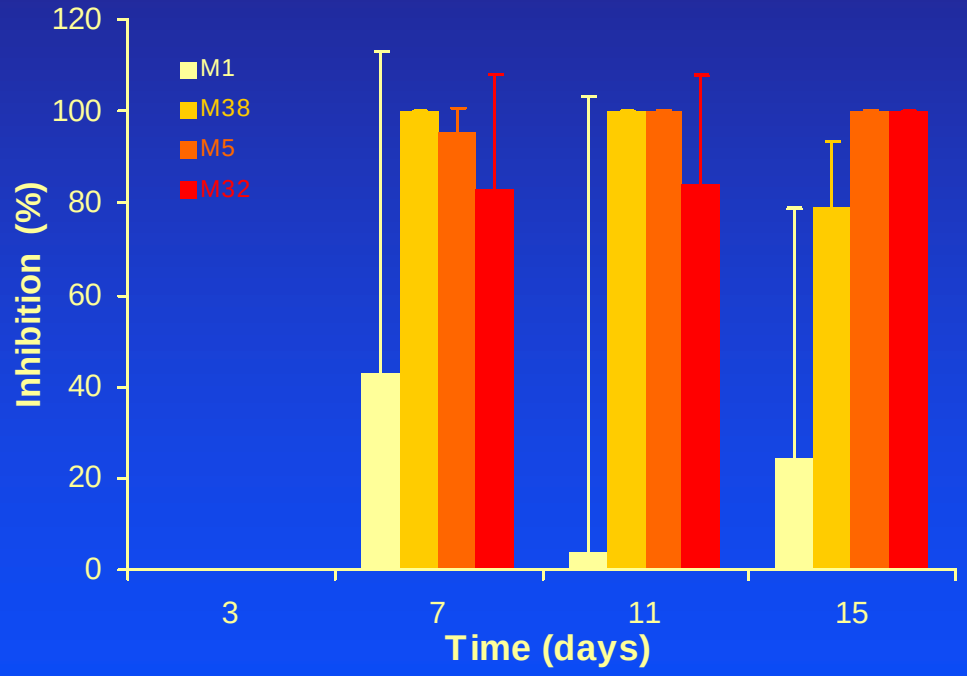
Detection of virus in supernatant (p24 ELISA)

PLoS Medicine | www.plosmedicine.org 1238 August 2008 | Volume 5 | Issue 8 | e157

Prevention of SIV Rectal Transmission and Priming of T Cell Responses in Macaques after Local Pre-exposure Application of Tenofovir Gel

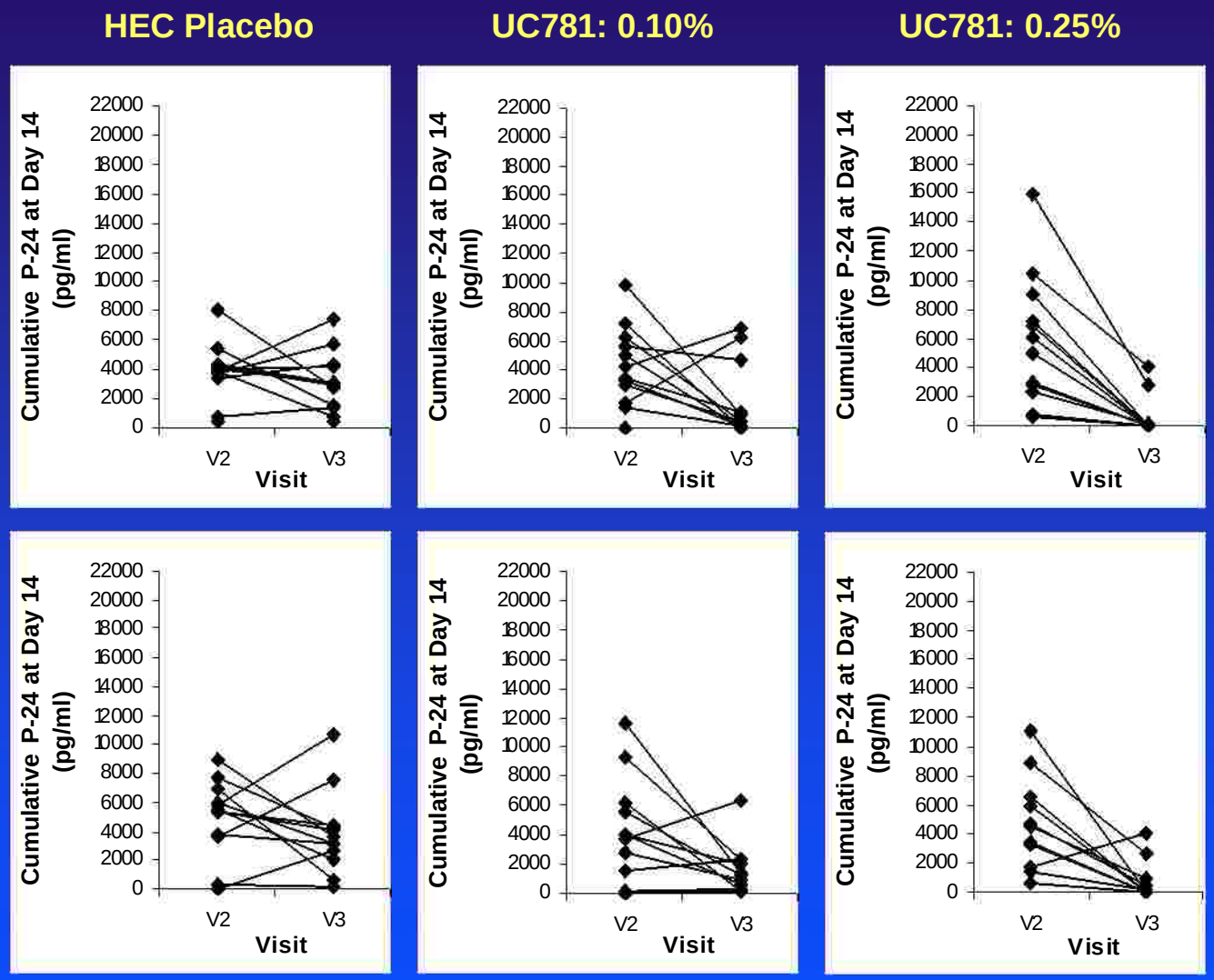
Martin Cranage[1*], Sally Sharpe[2], Carolina Herrera[1], Alethea Cope[1], Mike Dennis[2], Neil Berry[3], Claire Ham[3], Jonathan Heeney[4,5], Naser Rezk[6], Angela Kashuba[6], Peter Anton[7], Ian McGowan[7*], Robin Shattock[1]

- Macaques were dosed *in vivo* with PMPA per rectum 3 h before tissue removal.
- Explants were exposed to virus *ex vivo*.



Ex vivo challenge to assess drug activity *in vivo*

Ex vivo suppression of HIV infection 30' post *In vivo* dosing

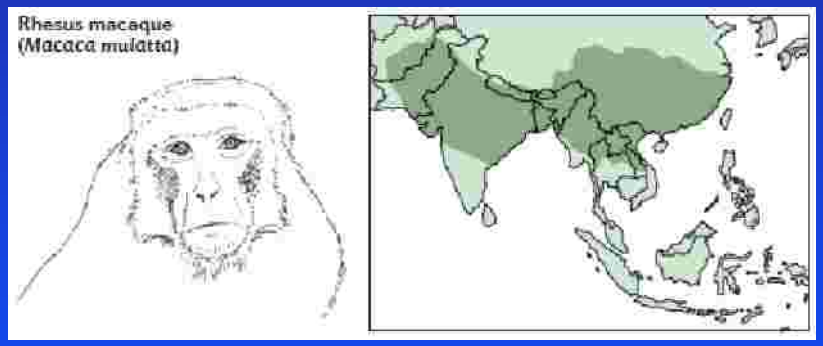


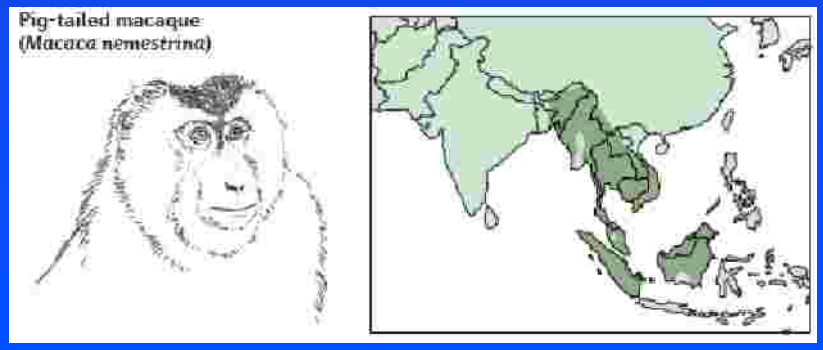
(biopsies from two levels in rectosigmoid; high viral titer for infection used here (10^4 $TCID_{50}$)

Courtesy of Peter Anton

Pre-clinical models to assess candidate microbicides

- *In vivo* animal models:
  - Ÿ Rodents and rabbits used for pre-clinical safety assessment of compounds
  - Ÿ Humanized-mice strains: engineered by transplanting human stem cells to make them susceptible to HIV-1 infection
  - Ÿ Non-human primates: main animal model to study transmissio immunology and pathogenesis of HIV-1 infection as well as efficacy of prevention technologies





Drug distribution and PK/PD in tissue are critical

- Non-human primate studies are now being configured to relate drug distribution (systemic, tissue, topical) to protection (pharmacokinetics (PK)) – *how much drug is needed and where is it need to provide protection?*

- Parallel human studies are being performed to determine drug distribution following application. Hence, providing a bridge between NHPs and human studies

- Studies to determine tissue drug activity are being developed as potential surrogate markers of protection (pharmacodynamics (PD)) – *is the drug active at the site were it should work?*

These are now seen as critical tools for product development

Facing the problem of resistance to microbicides: when and how?

- Risks of resistance for topical ARV microbicides are hypothetical and can only be determined in a clinical trial setting.
- Risks are likely to be different for poorly absorbed products
- ARV resistance is relative, topically applied drugs ma be sufficient to prevent transmission of resistant isolates
- Resistant virus may have reduced fitness for transmission
- In a clinical trial the risk/benefit analysis for the and for study individuals is likely to be low
- Should an ARV approach be successful, this would be in ially implemented as a prescription only product
- Resistance evolution would be monitored with introduction
- Enhanced emphasis would be placed on combination micro ides that reduce the potential for resistance and/or onward transmission

RTI resistance can reduce replication fitness

- Replication of RTI escape mutants in colorectal explants

A17 (K103N, Y181C) NNRTI-resistant

71361-1 (K65R)
8415-2 (K65R, M184V) **NRTI-resistant**

Herrera et al. (2009) AAC

- K65R impairs replication capacity of resistant isolates in plasma from TNF-treated patients: Margot et al (2006) AAC; McColl et al. (2004) J Acq Imm Def Syndr; Miller et al. (2003) Antiviral Therapy; White et al. 2002) AAC

- Clonal viruses containing M230L mutation in RT have diminished replication capacity. Xu et al. (2010) AAC

Could ARV-combinations prevent HIV-1 transmission?

- In conventional HAART regimens, patients receive combinations of three antiretroviral drugs (Fox R. and Gourlay YJ. 2000; Jordan R. et al. 2002; Parpia T. et al. 2001)

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2009, p. 1797–1807 Vol. 53, No. 5
0066-4804/09/$08.00+0 doi:10.1128/AAC.01096-08

Reverse Transcriptase Inhibitors as Potential Colorectal Microbicides[▽]†

Carolina Herrera,[1] Martin Cranage,[1] Ian McGowan,[2] Peter Anton,[3] and Robin J. Shattock[1]*

AIDS 2011, 25:1971–1979

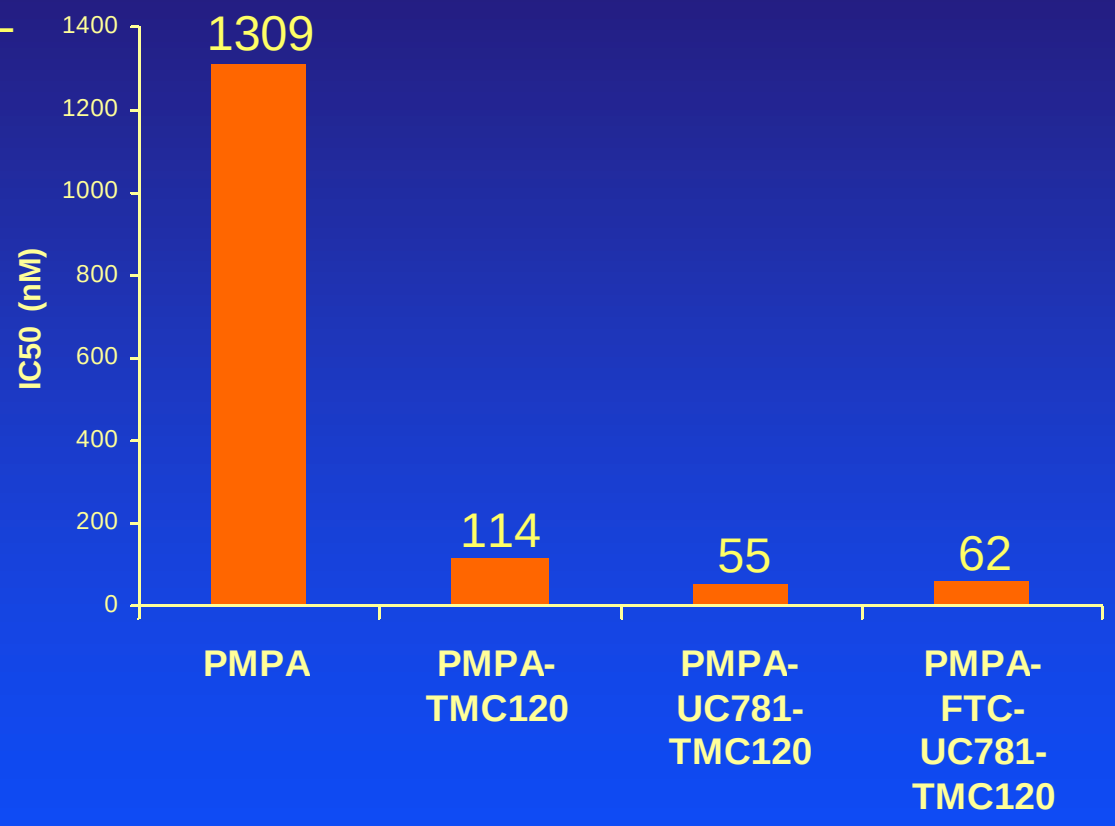
Colorectal microbicide design: triple combinations of reverse transcriptase inhibitors are optimal against HIV-1 in tissue explants

Carolina Herrera[a,*], Martin Cranage[a], Ian McGowan[b], Peter Anton[c] and Robin J. Shattock[a]

Combinations to increase drugs activity

Colorectal explants

HIV-1 BaL



Microbicides based on ARV drug combinations

- New RTIs (MIV170) - RTIs (PMPA, FTC, UC781, TMC120)
- Entry inhibitors (maraviroc, CMP167, 5P12-RANTES, AMD3465, BMS-599793 (DS003), M48U1) - RTIs
- Fusion inhibitors (T20, T1249, L'644 (DS007)) - RTIs
- Protease inhibitors (saquinavir, darunavir) - RTIs
- Zinc fingers inhibitors (SAMT-8, SAMT-19, SAMT-89) - RTIs
- Entry inhibitors - Fusion inhibitors

We might need a combination of prevention strategies

Combining oral PreP with microbicides may deliver better protection

Combining microbicides or oral PreP with partially effective vaccines may provide additional benefit

- Protection during the immunization period
- Reduction of infectious challenge, converting high risk challenge to low risk challenge
- Boosts of local immunity (virus/antigen)
- Broadening of localized immunity through protected exposure to prevalent virus
- While vaccine induces immunity, ARVs may cover intermittent compliance, break through virus and resistance evolution

Concept currently being tested in non-human primates

Prioritizing best-in class

Mechanism of Action	•ARVs - entry, reverse transcriptase, integrase and protease inhibitors
Potency and Selectivity	•High levels of anti-HIV activity and low cellular toxicity/inflammation.
Physical Properties	•Stability under diverse environmental conditions in multiple topical dosage forms
Combination products	•Maximize effectiveness, minimize resistance

Prioritizing best-in class

Stage of Development	•Drugs in late development have higher priority (chemistry, manufacture, and control (CMC) requirements)
Pharmacokinetics/pharmacodynamics (PK/PD)	•Greatly enhances the potential for success in later efficacy studies (NHP/human).
Human Experience	•Drugs already approved for human use have higher priority over those in early development
Adherence	•Multiple dosage forms have priority over complex or expensive forms (sustained release)

Crucial to have multiple products in parallel developm to ensure against development risk

Additional bibliography

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