University of Pittsburgh

MAGEE-WOMENS RESEARCH INSTITUTE

Transforming a Molecule to a New Prevention Product: the Journey Toward an Extended Release Film

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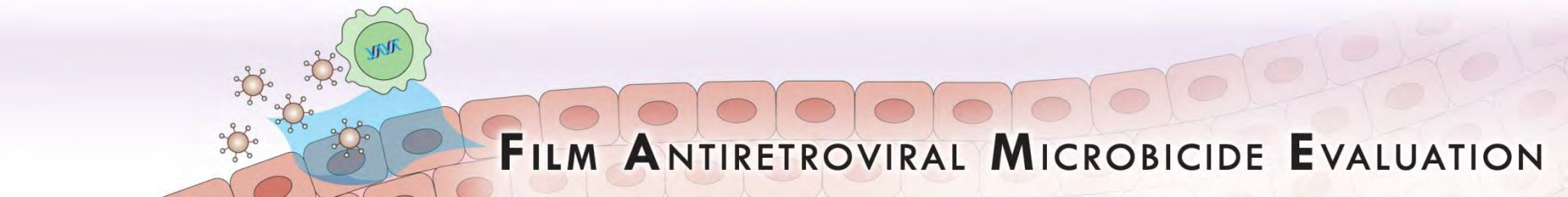
Overview of the Presentation

- Do we need more HIV prevention options for young women?
- Fast dissolving films
  - Can they deliver ARVs to genital tissues?
  - What do films and gels do to the vaginal microenvironment?
  - What does the microbiota do to the drugs?
- Building a film for extended release of ARVs
- Conclusions



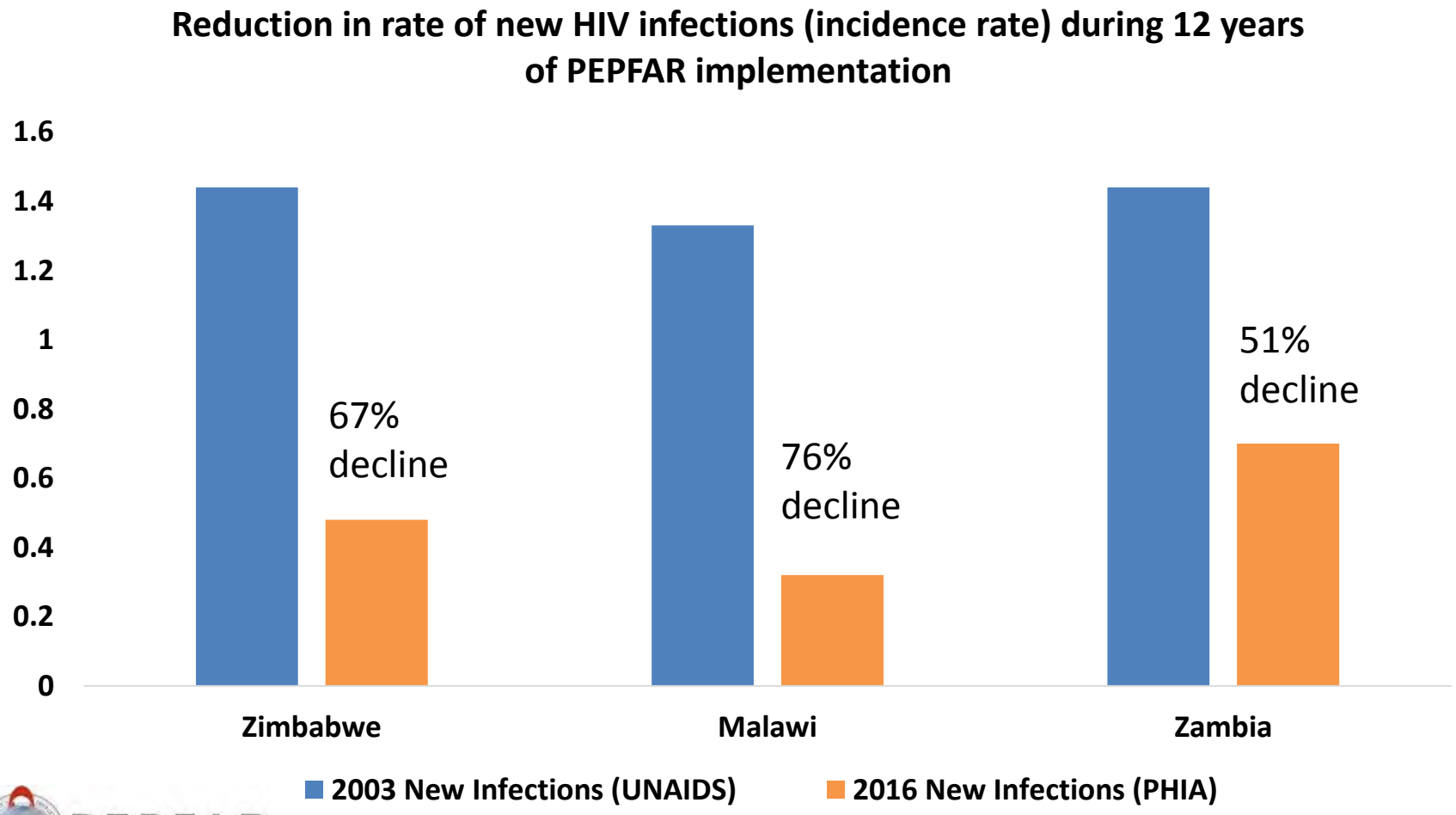
Our world is home to 1.8 billion young people between the ages of 10 and 24, and the youth population is growing fastest in AFRICA nearly 2% every year.

If adolescents were a country they would be the most populous country.

Within this generation are 600 million adolescent girls with specific needs, challenges and aspirations for the future.

PEPFAR
U.S. President's Emergency Plan for AIDS Relief

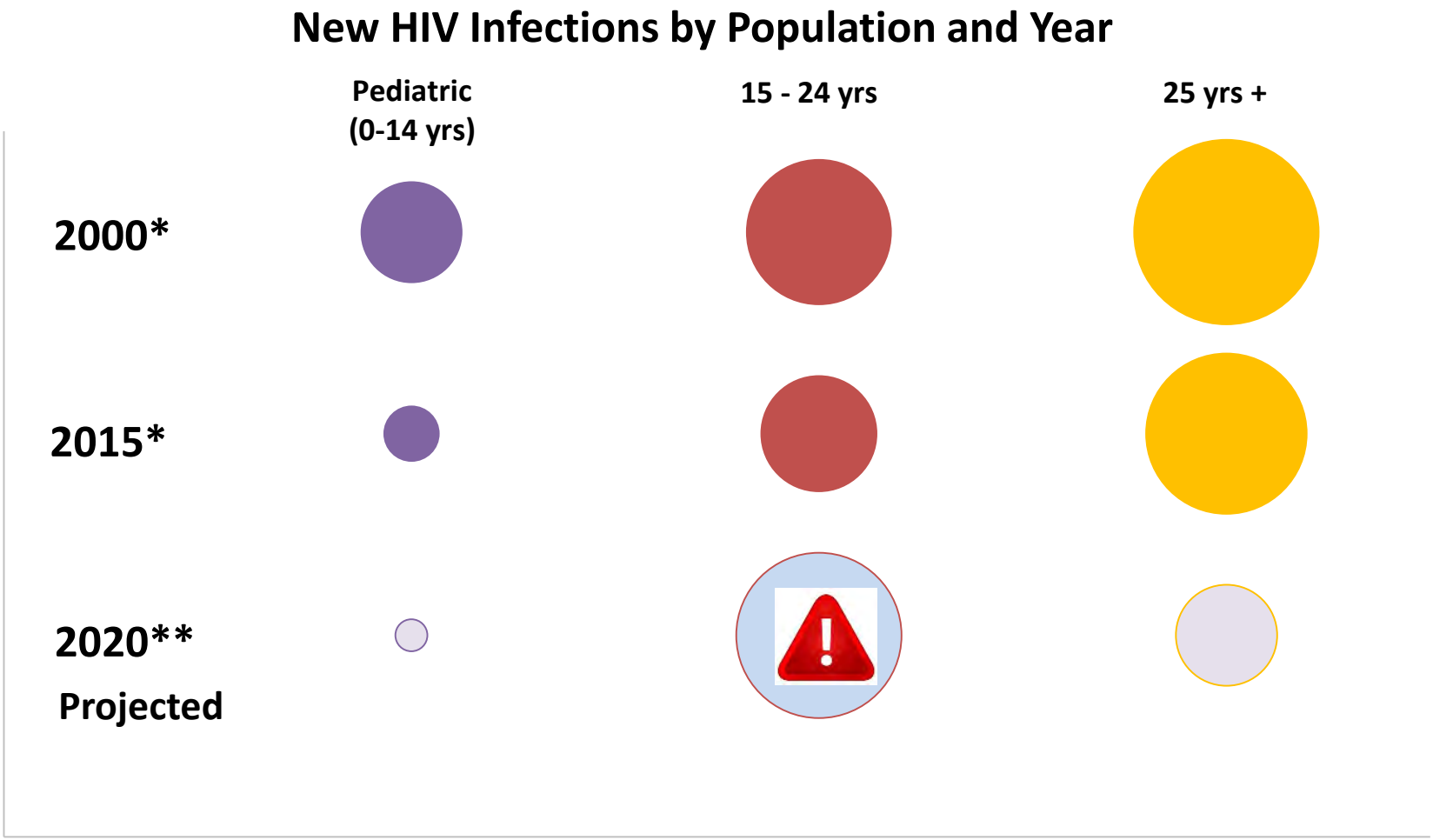
Since the Start of PEPFAR, New HIV Infections Have Declined 51-76%



PEPFAR
U.S. President's Emergency Plan for AIDS Relief

Source: UNAIDS & PHIA IMPACT Studies, 2016

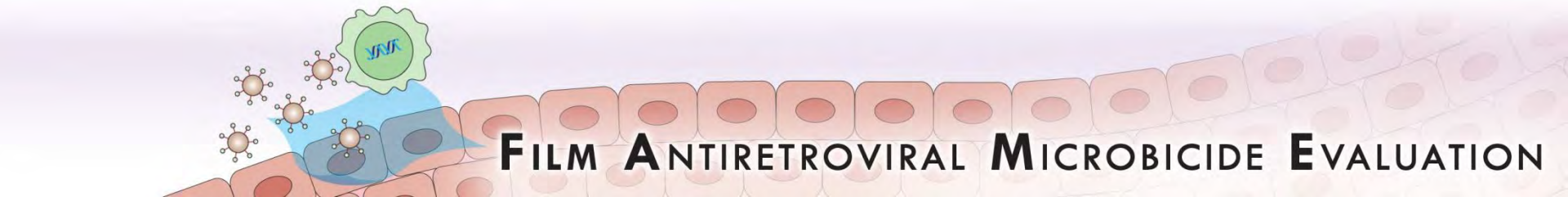
Disproportionate Success in Epidemic Control by Age Group



*Sources: * UNAIDS AIDS info Online Database, 2016; ** 15-24 yrs age group projected based on Africa Development Forum / World Bank 2015, "Africa's Demographic Transition: Dividend or Disaster?"*

Why?

- Increasing population of youth in Africa (youth bulge)
- Rollout of circumcision and treatment as prevention has much less impact on reducing incidence in young women than older women and men

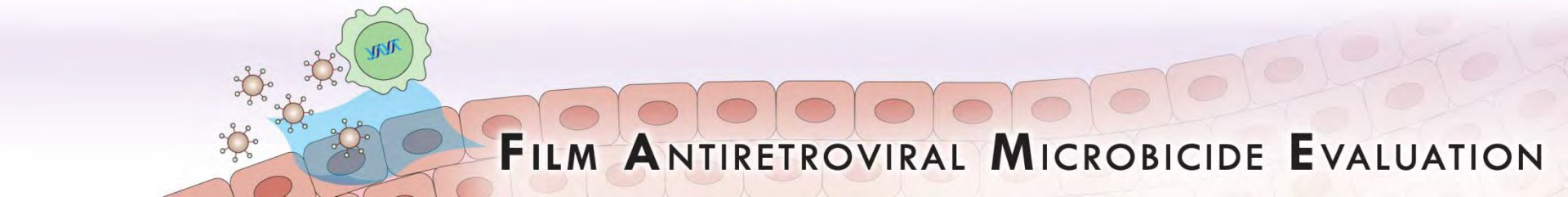


Medicalization of Sex

- To a large extent, HIV medicalized sexuality for many people
  - In HIV endemic areas, to be young and to have sex is be at “high risk”
  - For MSM, to be young and sexually active is to be a “high risk” person
- Some HIV prevention options are nonmedicalized (condoms) while others are very medicalized (oral PrEP)

What Do Young Women Want?

- To be healthy, but not to be reminded every day that they are “at risk” just because they have sex
- Not to be stigmatized by their families and communities for using prevention products
- To be able to access products in less medicalized settings like pharmacies
- To have products that do not require monitoring for toxicity and that look like reproductive health products rather than drugs
- To use single products for family planning and HIV

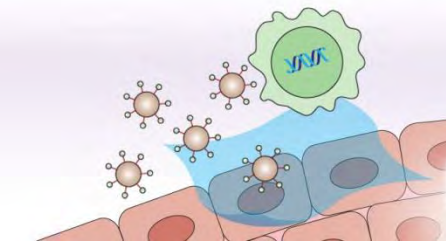


Won't the Tools We Have Be Enough?

- Young people less than 25 years of age have been the least "adherent" to oral treatment and prevention regimens
  - Neither of the studies of oral PrEP conducted in younger women demonstrated PrEP efficacy
  - In iPrEX, MSM <25 years had lower PrEP efficacy than older people
- Injectable ARVs will not require daily adherence but they will require returning for injections every 2 months

“Scorecard” for HIV prevention modalities for women

Modality	Efficacy	Low user burden	Low Cost	Low systemic side effects	Reversibility
Daily oral PrEP					
On demand PrEP					
Injectable Cabotegravir					
PrEP implant					
Vaginal ring					



FILM ANTIRETROVIRAL MICROBICIDE EVALUATION

Promise of multi-purpose technologies (MPTs)

Illustrative challenges (III):

MPT pipeline and investment

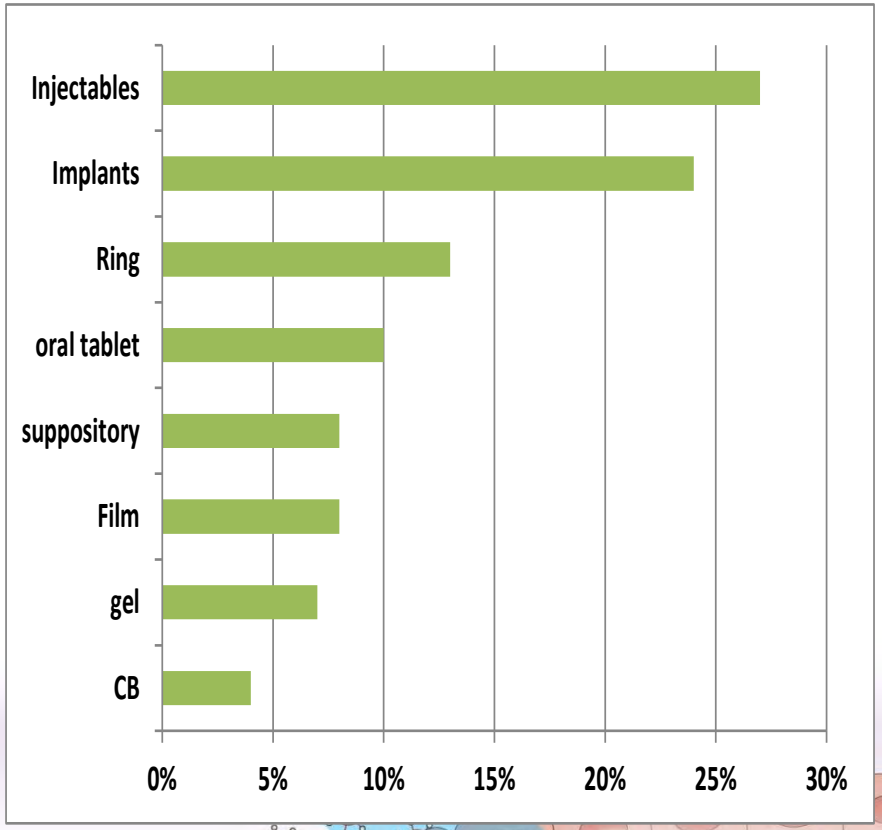
- 4% HIV only
- 2% Pregnancy only

What would women pick if all 4 MPTs were available to them today?

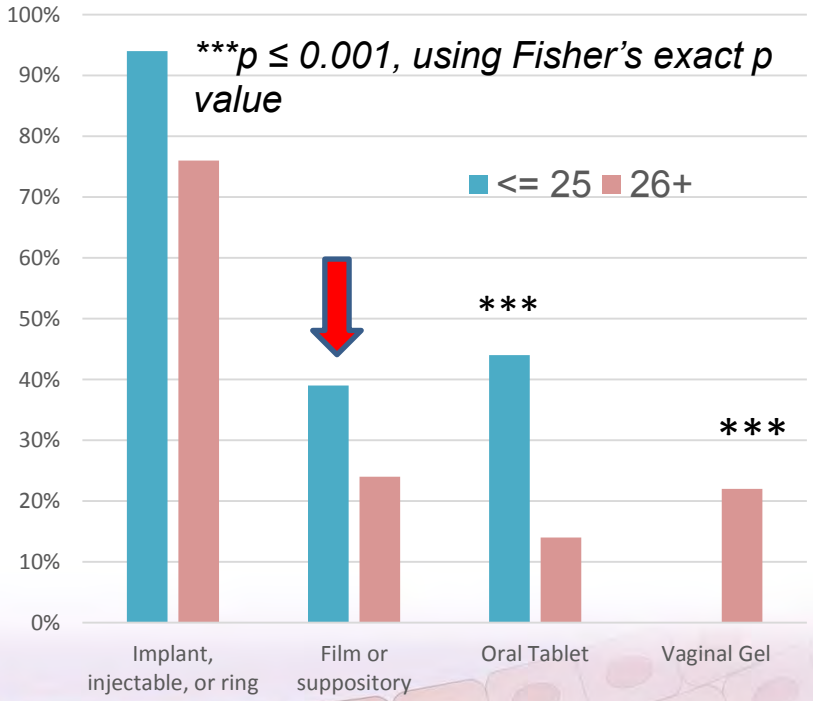
Preferences for prevention products among women in VOICE-D: Luecke, JIAS 2016

Percent of products selected*

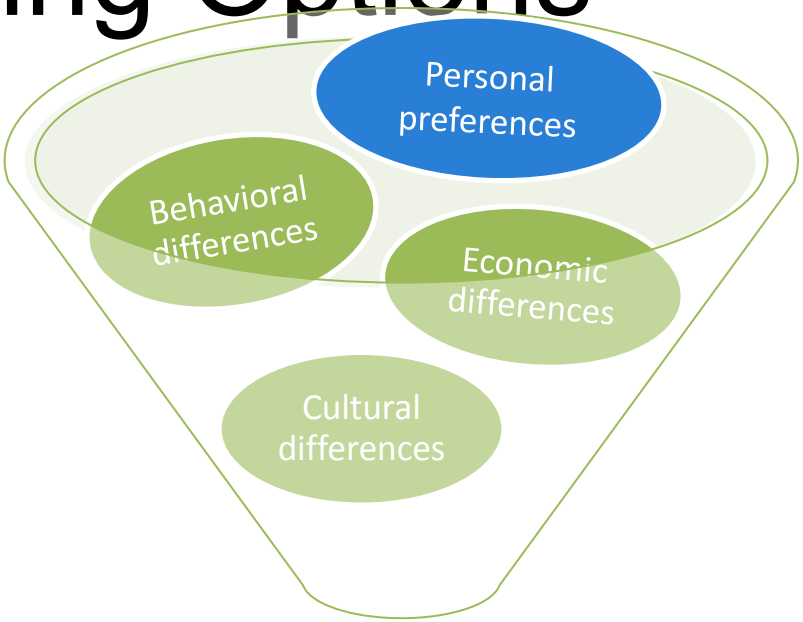
* Multiple selections allowed (median 2, range 0-6)



Young age associated with implant, film or tablet preference. Gel not selected by any young person



Creating Options



Drug Options → Dosage Form Options

Drug Discovery | Preformulation | Formulation | Formulation Assessment | Scale up | First in Human Studies

Co-PIs of FAME

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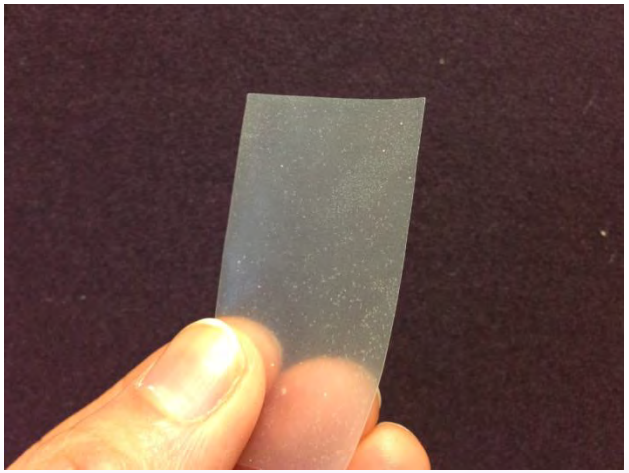
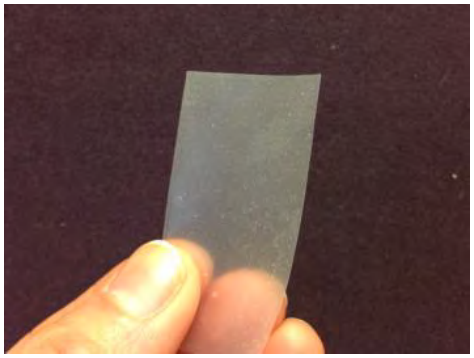
FILM ANTIRETROVIRAL MICROBICIDE EVALUATION

Thin Polymeric Films offer several advantages

- Vaginal films rely on dissolution of drug directly into vaginal fluid, thus decreasing the dilution of endogenous antiviral properties of vaginal fluid
- Because of lower volume, there is reduced sensation of vaginal discharge and less impact on innate protective factors
- For vaginal delivery, films are discreet, portable, inexpensive and easier to store than gel





Development of Coitally Dependent "Quick Dissolve" Thin Films for HIV Prevention

Dapivirine *NNRTI*

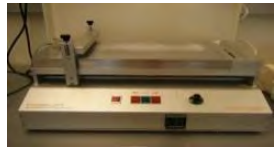
Tenofovir *NtRTI*

Developed at MWRI

IND Submitted to FDA

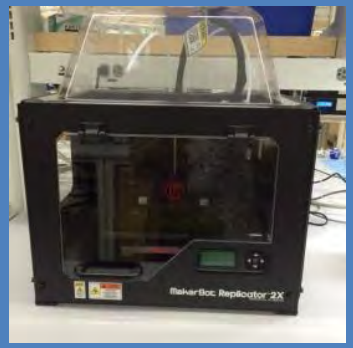
1st in human clinical studies completed

Results:
- Safe
- Acceptable
- Showed *Ex Vivo* Antiviral Activity



Novel Application of Manufacturing Technologies

Hot Melt Extrusion



3D Printing

National Institute of Allergy and Infectious Diseases

Bill & Melinda Gates foundation

International Partnership for Microbicides

C O N R A D
Leaders in Reproductive Health and HIV Prevention

innoteq inc
Innovative High Teq Coated Products

NYU

University of Washington

FILM ANTIRETROVIRAL MICROBICIDE EVALUATION

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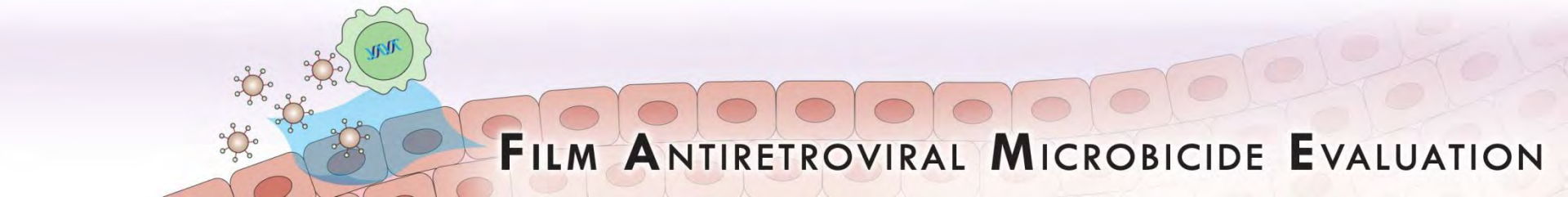
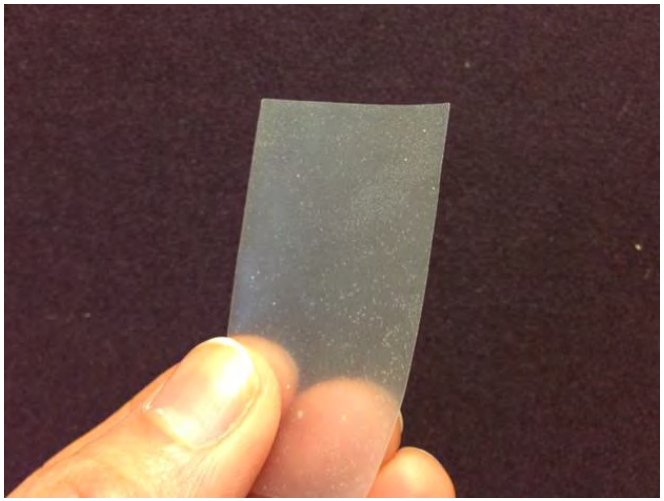
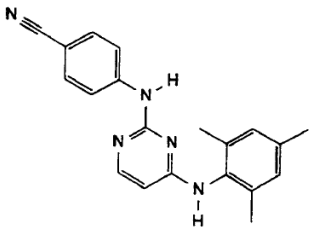
  1. Can they deliver ARVs to genital tissues?
  2. What do films and gels do to the vaginal microenvironment?
  3. What does the microbiota do to the drugs?



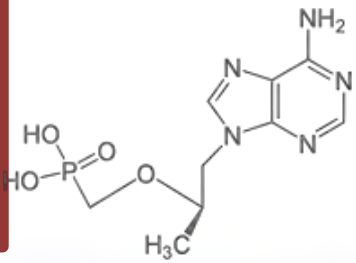
Can Films Deliver ARVs as Well As Gels?

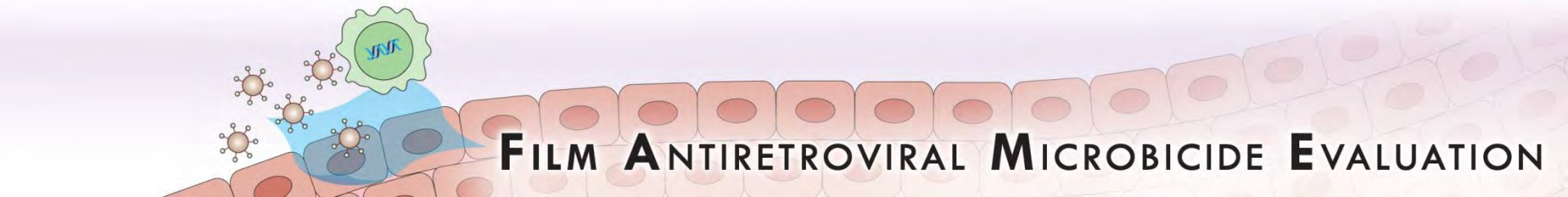


Dapivirine
FAME-01



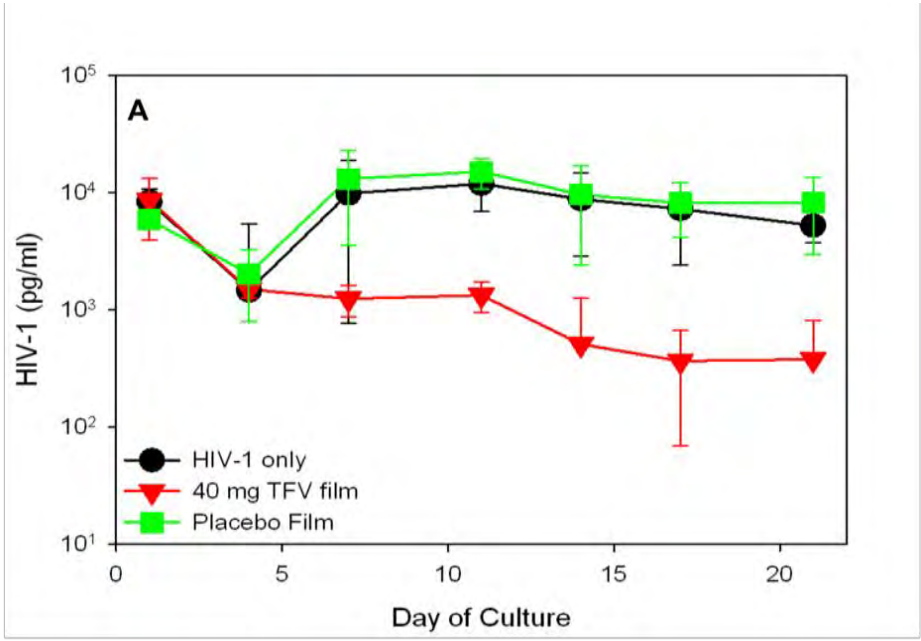
Tenofovir
FAME-02

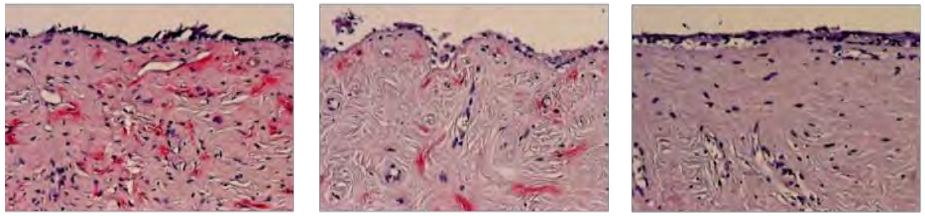




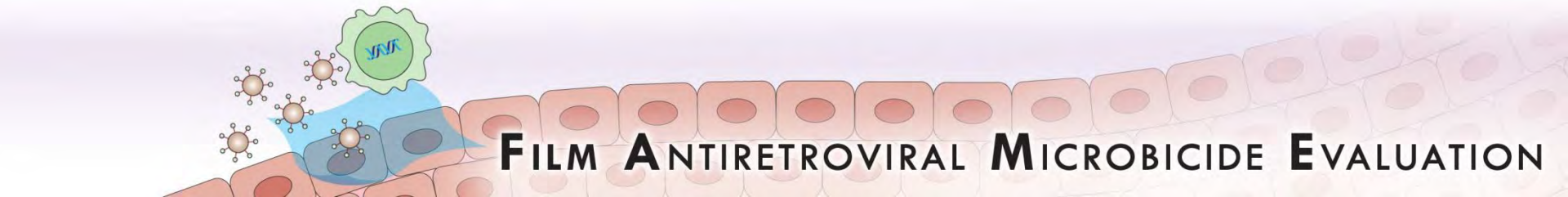
Tenofovir Vaginal Film Development

- Chemical and Physical Characterization
  - Weight, thickness, & appearance
  - Residual Water Content
  - Drug Content Uniformity
  - Dissolution
  - Disintegration
  - Mechanical Strength
- In vitro Safety and Bioactivity
  - *Lactobacillus* compatibility
  - Cell based Toxicity & Bioactivity
  - Ex vivo permeability
  - Ex vivo toxicity
  - Ex vivo HIV challenge study
- R&D Stability Study





Control Placebo Film TFV Film



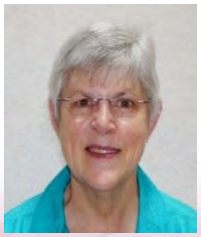
Animal Toxicity Testing

Study Design:

- Animals: New Zealand White Rabbit
- Study duration: 14 days
- Dosing: once daily, 1 mL/Kg of gel test article (2 dosing levels), placebo gel and 4% Nonoxynol-9 gel.

Study Design:

- Animals: Sexually mature female *Macaca nemestrina*
- Design: Two arm (Six macaques) crossover study comparing tenofovir film to placebo film
- Dosing: one 1”x2” film (40 mg tenofovir) once daily for five days one week, followed by four days the next week
- Endpoints: Colposcopy, microflora, vaginal pH, vaginal PMNs



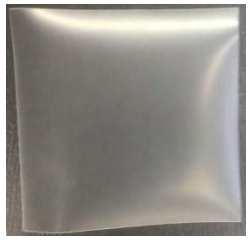
NO SAFETY ISSUES FOUND

Scale up Manufacture

- No significant scale-up issues
- Only small formulation changes required at scale-up

FAME-04 Protocol Summary

- Double blinded, randomized, placebo-controlled phase 1 study
- 75 HIV uninfected women, 18-45 years old
- 7 daily doses
  - Tenofovir gel
  - Placebo gel
  - Tenofovir film (10mg)
  - Tenofovir film (40mg)
  - Placebo film



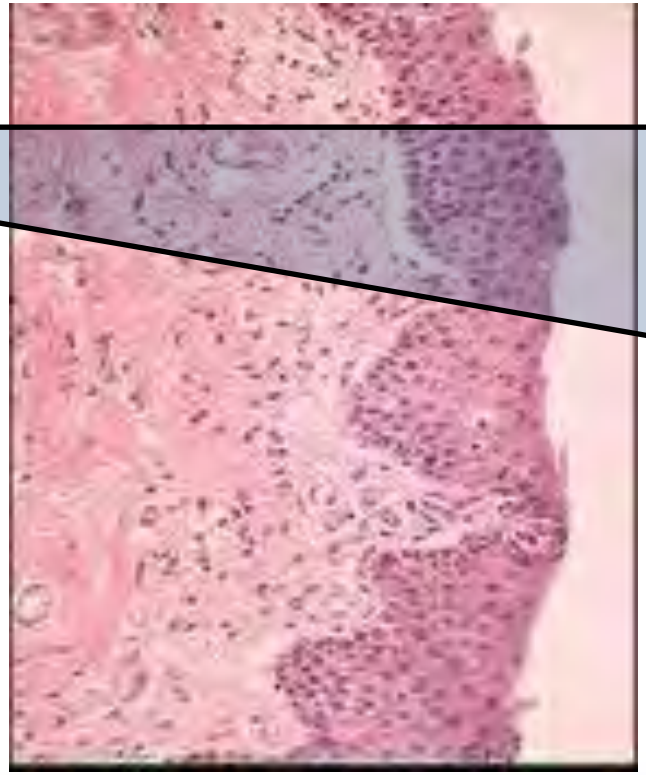
Bunge, et al, CROI 2016 and Hillier et al, CROI 2017

Concentration of Tenofovir

Plasma

Vaginal epithelium

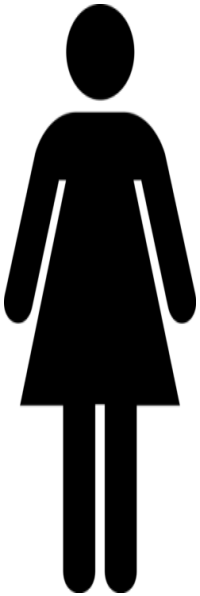
Vaginal fluid



Bunge, et al, CROI 2016 and Hillier et al, CROI 2017

Topical administration of tenofovir

Study Design



Collect:
- vaginal swabs for qPCR for microbiota
- tenofovir applied in clinic

Tenofovir applied daily at home

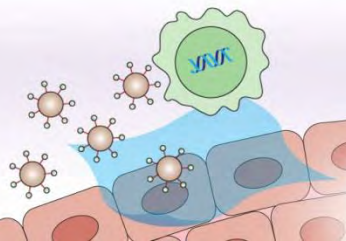
Collect plasma, CVL and vaginal fluid for tenofovir levels

2 hours

Insert 7th dose in clinic

Collect:
- Plasma
- Cervical biopsy

Bunge, et al, CROI 2016 and Hillier et al, CROI 201



FILM ANTIRETROVIRAL MICROBICIDE EVALUATION

Tenofovir Levels: 40 mg in Gel vs Film

Tenofovir (ng/mL)	1% TFV Gel (n=13)	40 mg Film (n=15)	P-value
Plasma TFV trough after 6 doses	**0.86** (0.40, 1.72)	**1.84** (0.46, 2.81)	0.17
Plasma TFV 2 hrs after 7th dose	**2.34** (1.38,4.75)	**2.74** (0.85, 5.31)	0.96
Cervicovaginal lavage 2 hour post-dose	**193 x 10^3** (138 x 10^3, 608 x 10^3)	**181 x 10^3** (114 x 10^3, 320 x 10^3)	0.39

Bunge, et al, CROI 2016 and Hillier et al, CROI 2017

Tenofovir Levels: 40 mg in Gel vs Film

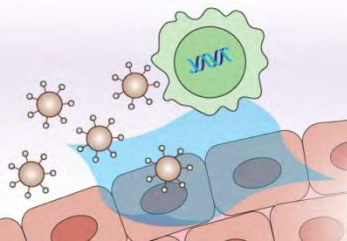
Tenofovir (ng/mg)	**1% TFV Gel (n=13)**	**40 mg Film (n=15)**	**P-value**
Cervicovaginal fluid trough after 6 doses	**532** (311, 622)	**1044** (447, 2171)	0.052
Cervicovaginal fluid 2 hrs after 7th dose	**2850** (2070, 3571)	**8340** (4000, 11,540)	<0.001
Rectal fluid TFV 2 hrs after 7th dose	**33.7** (3.2, 832.2)	**34.0** (15.0, 228.3)	0.75

Bunge, et al, CROI 2016 and Hillier et al, CROI 2017

Tenofovir Diphophosphate Levels: 40 mg in Gel vs Film

TFV-DP levels (fmol/mg)	1% TFV Gel (n=13)	40 mg Film (n=15)	P-value
Cervical tissue 2h post dose 7	**222** (71, 556)	**937** (56, 1457)	0.27
Vaginal tissue 2h post dose 7	**296** (150, 917)	**241** (113, 546)	0.44

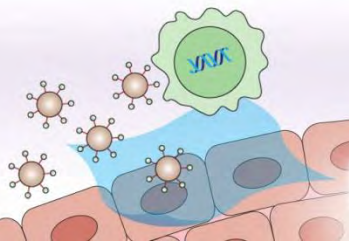
Bunge, et al, CROI 2016 and Hillier et al, CROI 2017



FAME-04

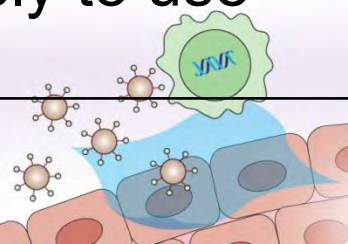
- Films delivered tenofovir as well or better than gel in the following compartments following vaginal administration:
  - Plasma
  - Cervicovaginal lavage
  - Rectal fluid
  - Undiluted cervicovaginal fluid
  - Cervical and vaginal tissues

Bunge, et al, CROI 2016 and Hillier et al, CROI 2017



FAME-04: User preferences

Characteristics	Gel	Film	P-value
Not difficult to insert	94%	51%	0.002
Comfortable	52%	81%	0.25
No Leakage	0%	34%	<0.001
Likely to use	77%	72%	0.14



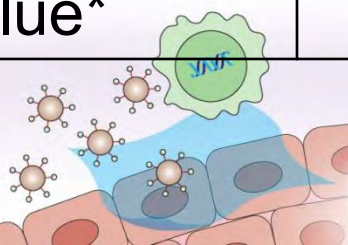
What do films and gels do to the vaginal microenvironment?

- Vaginal microbiota as assessed by qPCR and deep cultivation
- Innate antiviral activity

- Glycomic studies

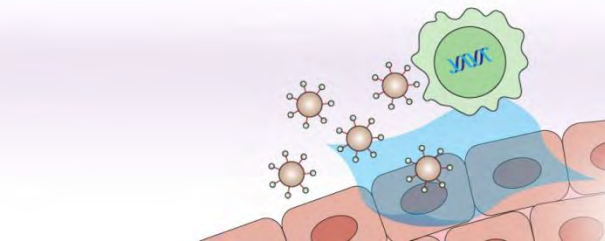
FAME-04: Impact of Gel and Film Polymers on Innate Antiviral Activity

Product	Innate anti-HIV-1 activity (% control)		
	Baseline	2 hours after 7th dose	3 weeks later
Placebo Film (n=14)	70.2 (43.4, 87.4)	85.8 (66.4, 90.8)	69.1 (50.0, 80.8)
P-value*		0.013	0.78
Placebo Gel (n=15)	77.7 (41.9, 89.6)	**34.8 (12.9, 64.3)**	**59.8 (-8.3, 77.3)**
P-value*		**0.003**	**0.036**



Glycomic Studies

- Drugs or formulations that affect the mucus may alter its functions
- Polymers present in the films or gels could plausibly impact innate immunity.
- Glycomic studies compare impacts of products on the glycome using two methods in separate laboratories
  - Impact on mucin proteins and lectins binding in CVL using ELLA assays before and after product use
  - Lectin microarray

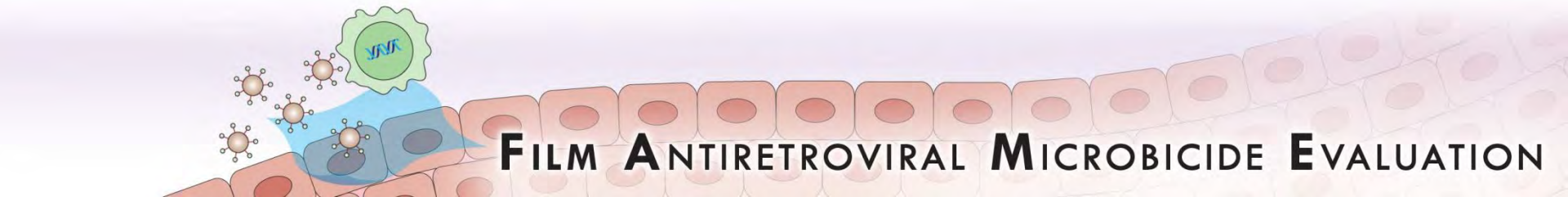


FAME-04 Mucins and Lectin Binding

Butch Moncla, PhD

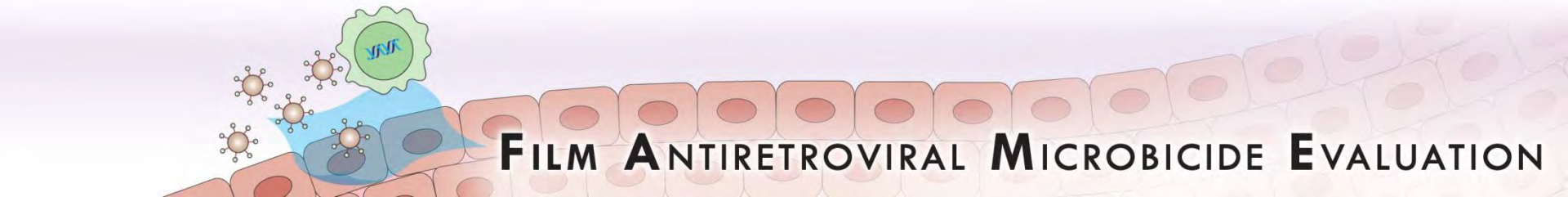
FAME-04 Analyses

- In CVL obtained at the screening visit, 2 hours post product placement and at 4 weeks evaluated the following:
  - Protein content
  - MUCs:
    - Membrane bound mucin proteins: MUC 1 and 16
    - Secreted mucins: MUC 5ac and 7
  - Lectin binding: SNA, GRFT, Jacalin, RCA, ECA



FAME-04: Summary of Mucin and Lectin Binding

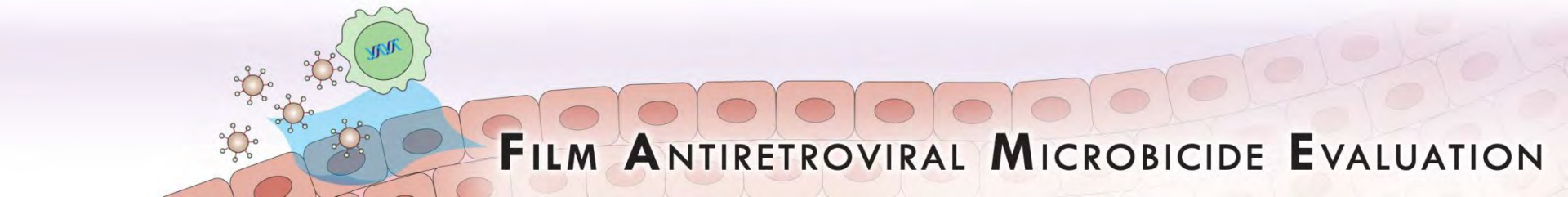
- Film
  - Decreased protein
  - Decreased MUC 1 and 7
  - No effect on MUC 5ac or 16
  - Decreased lectin binding to GRFT and ECA
- Gel
  - Decreased protein
  - Decreased MUC1
  - No effect on MUC5ac, 7 or 16
  - No change in lectin binding



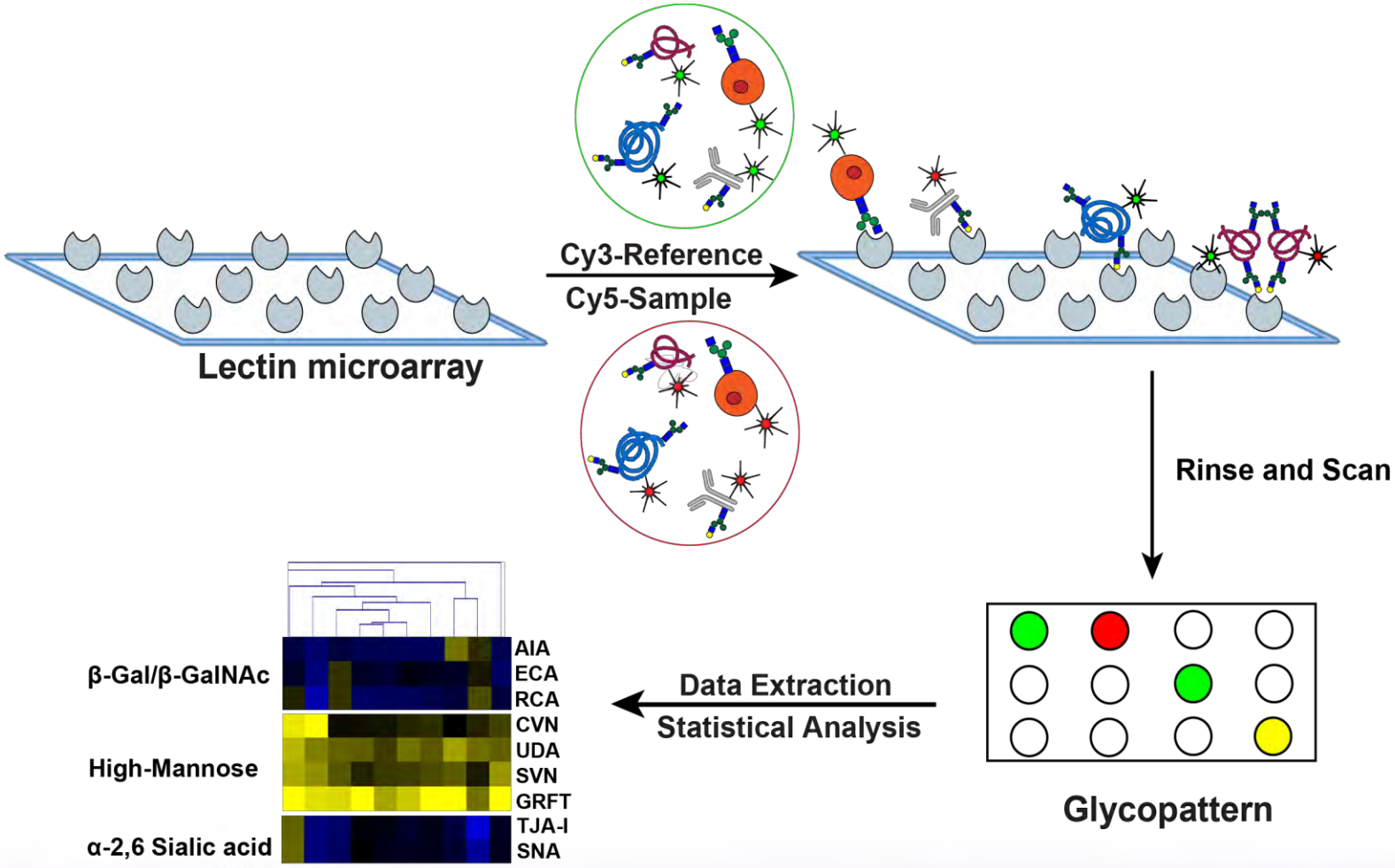
Glycomic Analysis of FAME: Sweet Surprises

Lara K. Mahal

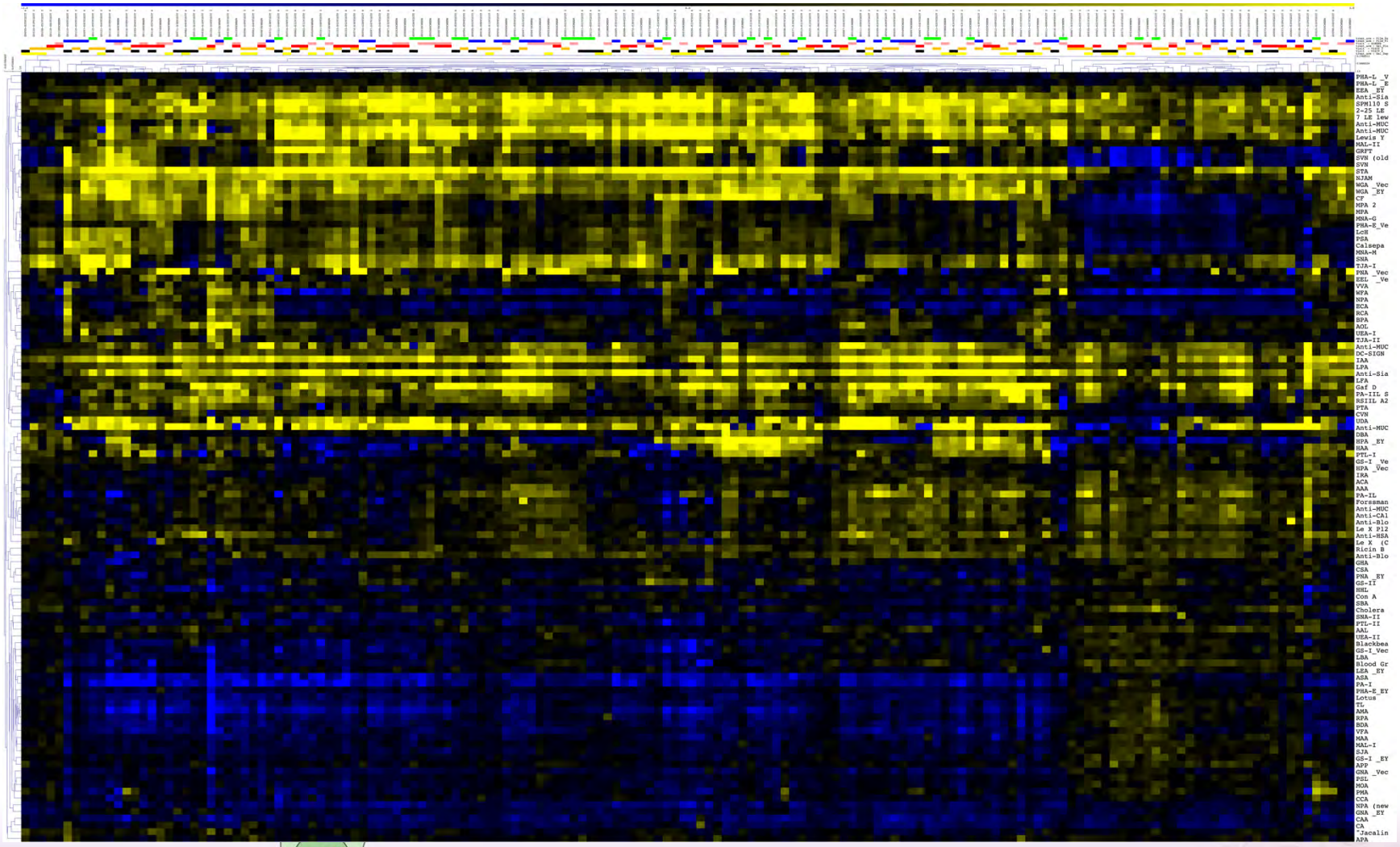
New York University



Lectin Microarrays



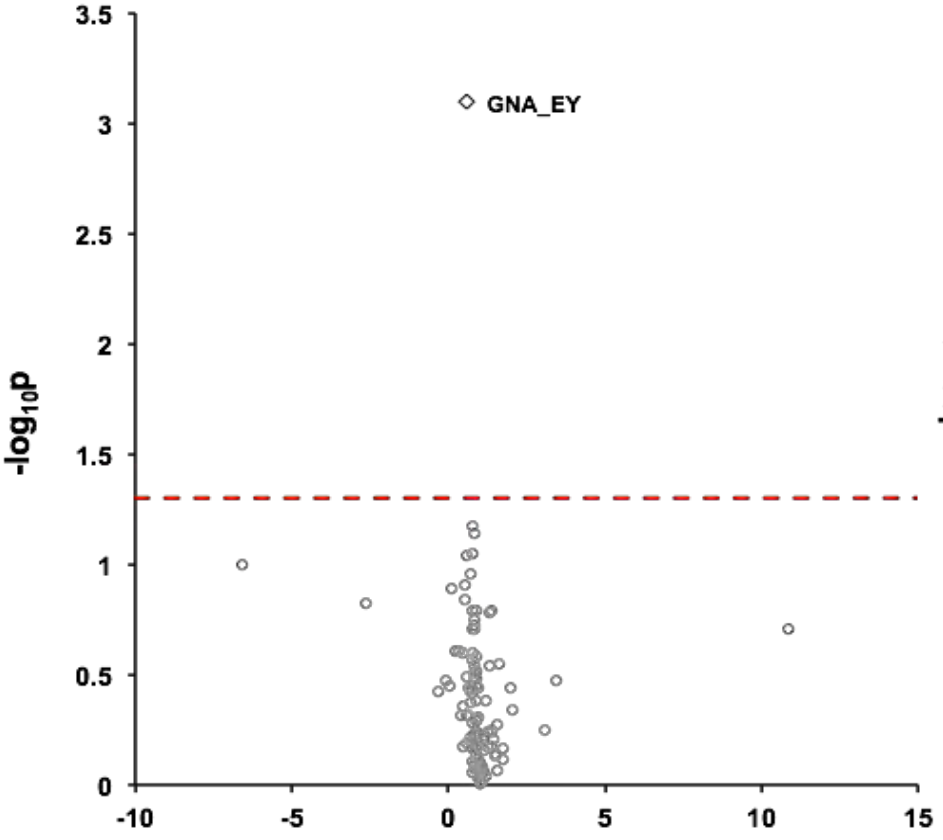
Dual-Color Comparison of FAME

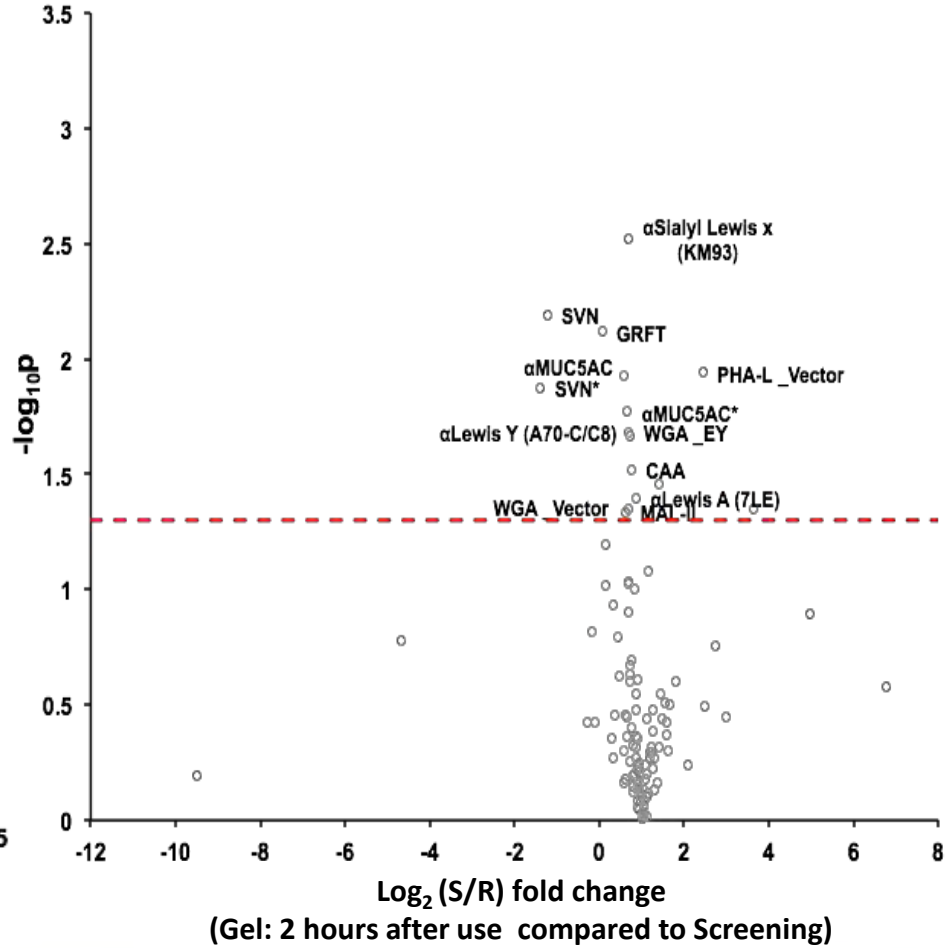


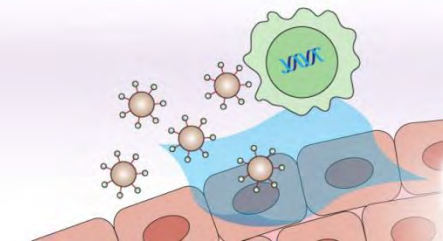
Linlin Wang

FILM ANTIRETROVIRAL MICROBICIDE EVALUATION

Gel but not Film alters the CVL glycome



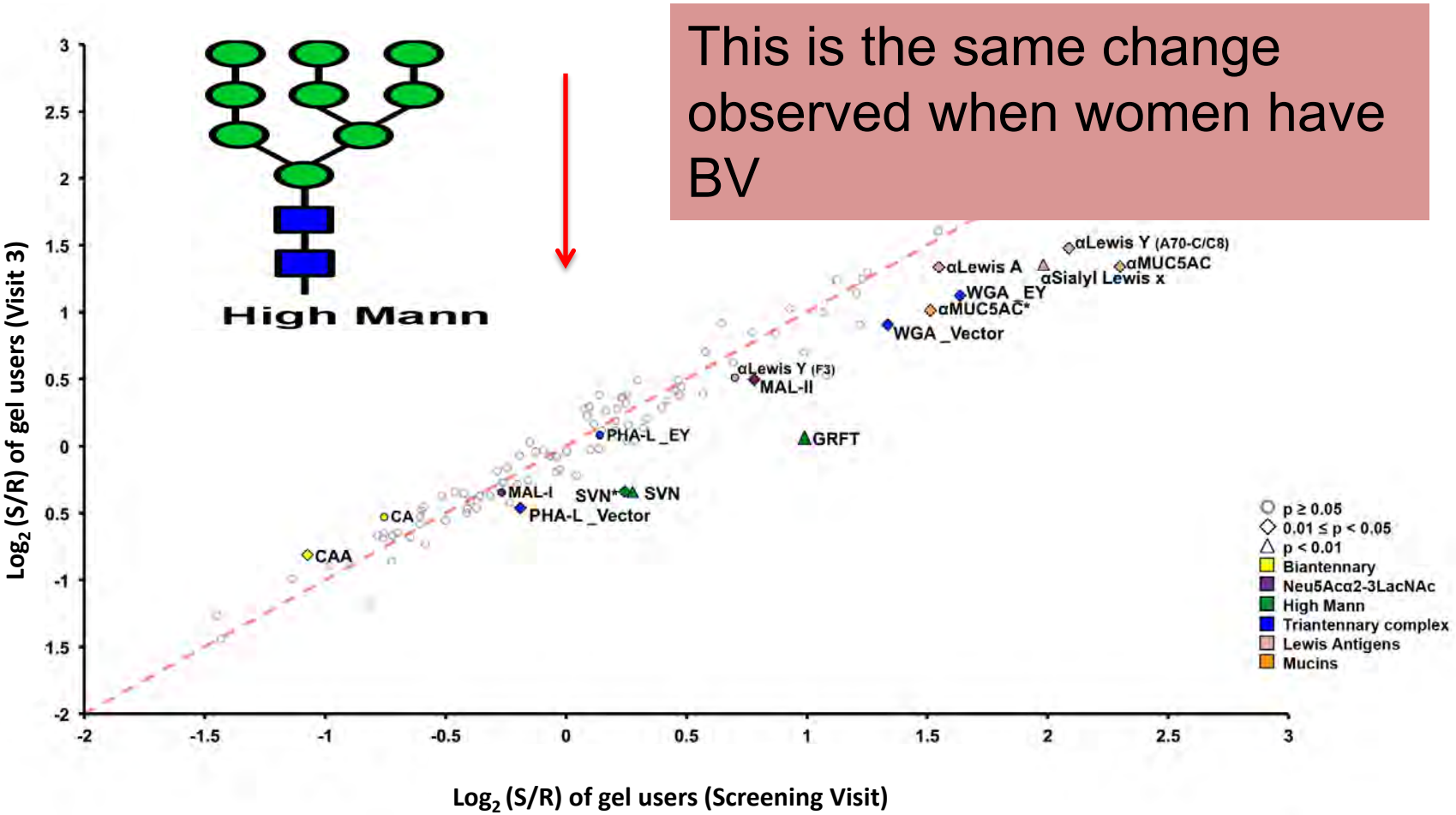


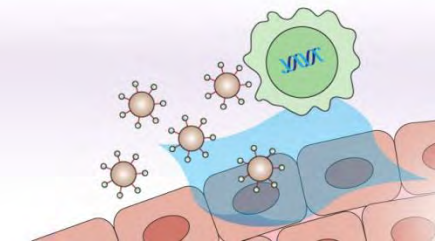


Linlin Wang

FILM ANTIRETROVIRAL MICROBICIDE EVALUATION

Gel alters the CVL glycome

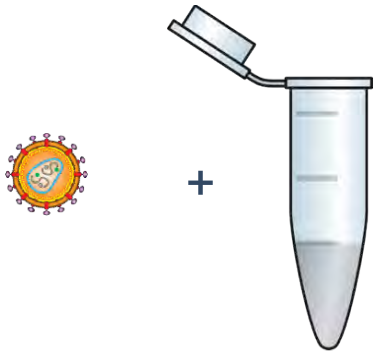




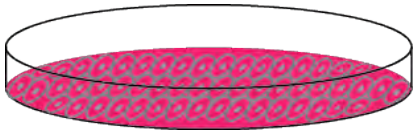
Linlin Wang

FILM ANTIRETROVIRAL MICROBICIDE EVALUATION

Mapping the Glycome onto Innate Immunity: HIV-1 Assay



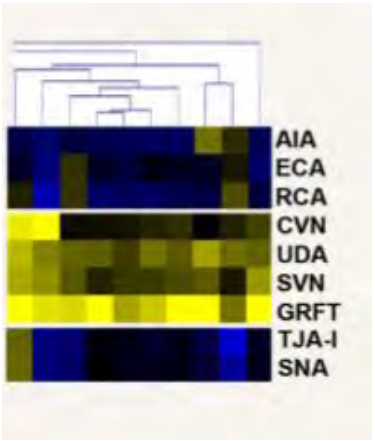
CVL

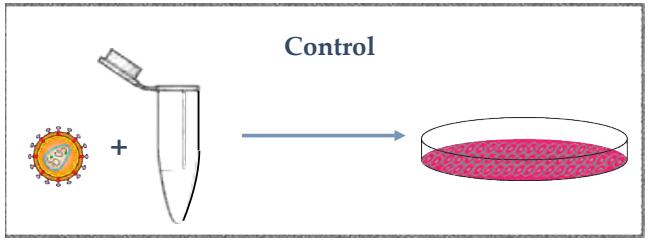


TZM-bl cells

HIV-1 inhibition percentage
Active/ Inactive anti-HIV-1 activities

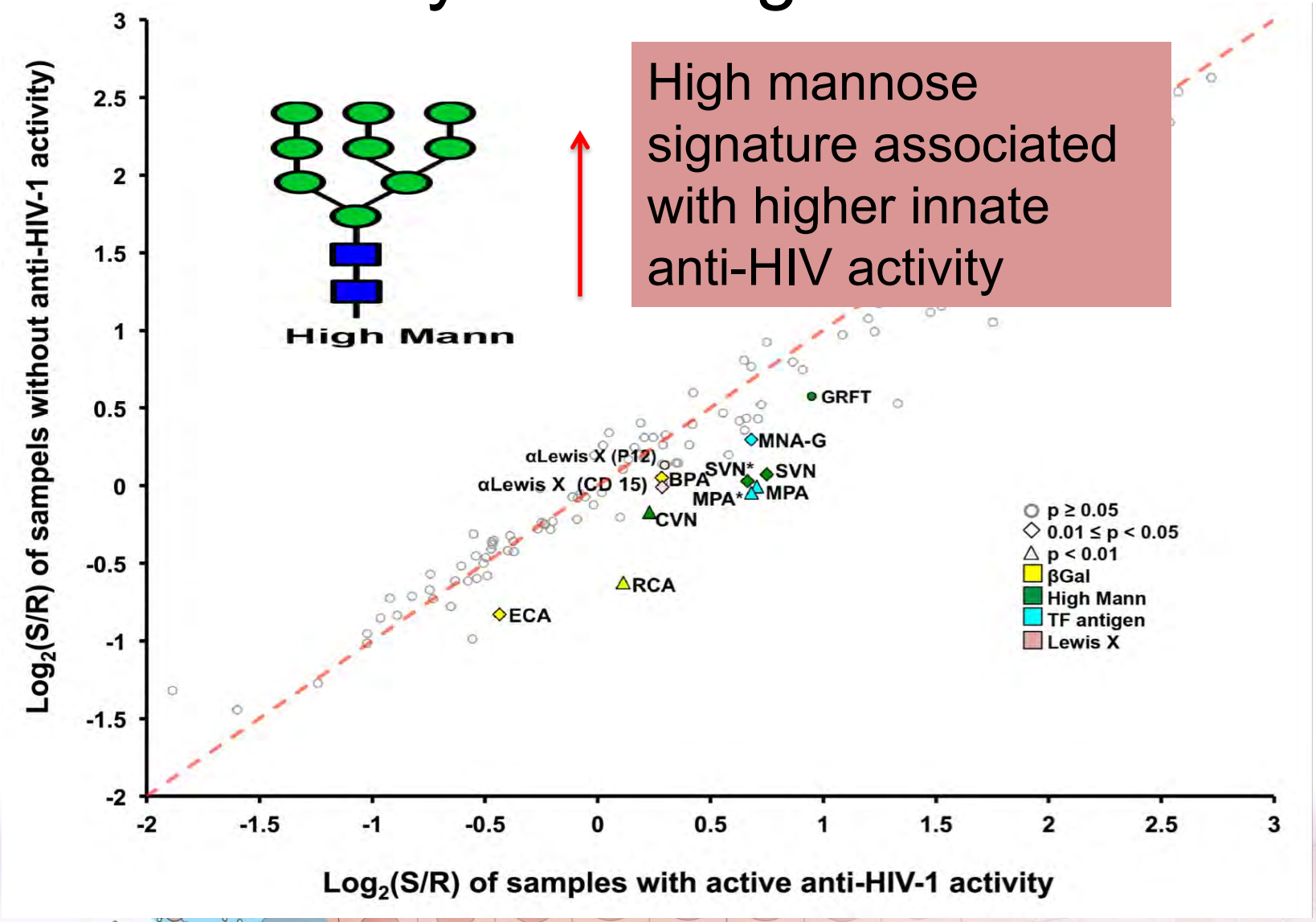
ANOVA





Performed by Dr. Charlene Dezzuti, Magee-Womens Research Institute

Innate Immunity Against HIV-1 has a Glycomic Signature

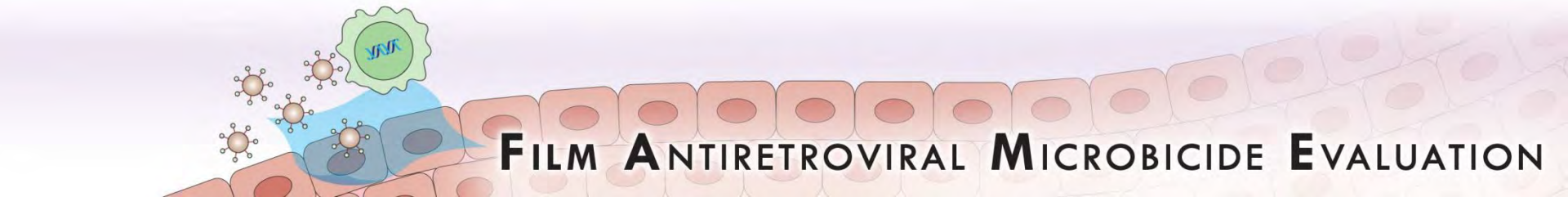


Glycome Conclusions

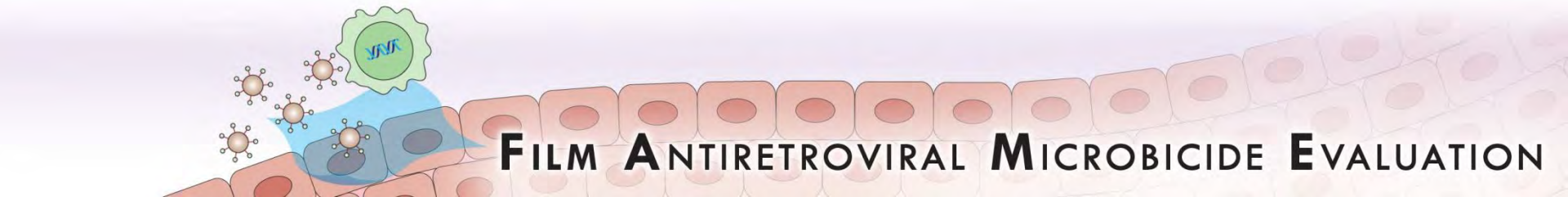
- Drug delivery in gel lowers the level of high mannose glycoproteins, directly impacting innate immunity against HIV-1
- Film does not have this impact.
- The impact of gel on the glycome (an by extension innate anti-viral activity) is not mediated by changes in the microbiome.

Future Work

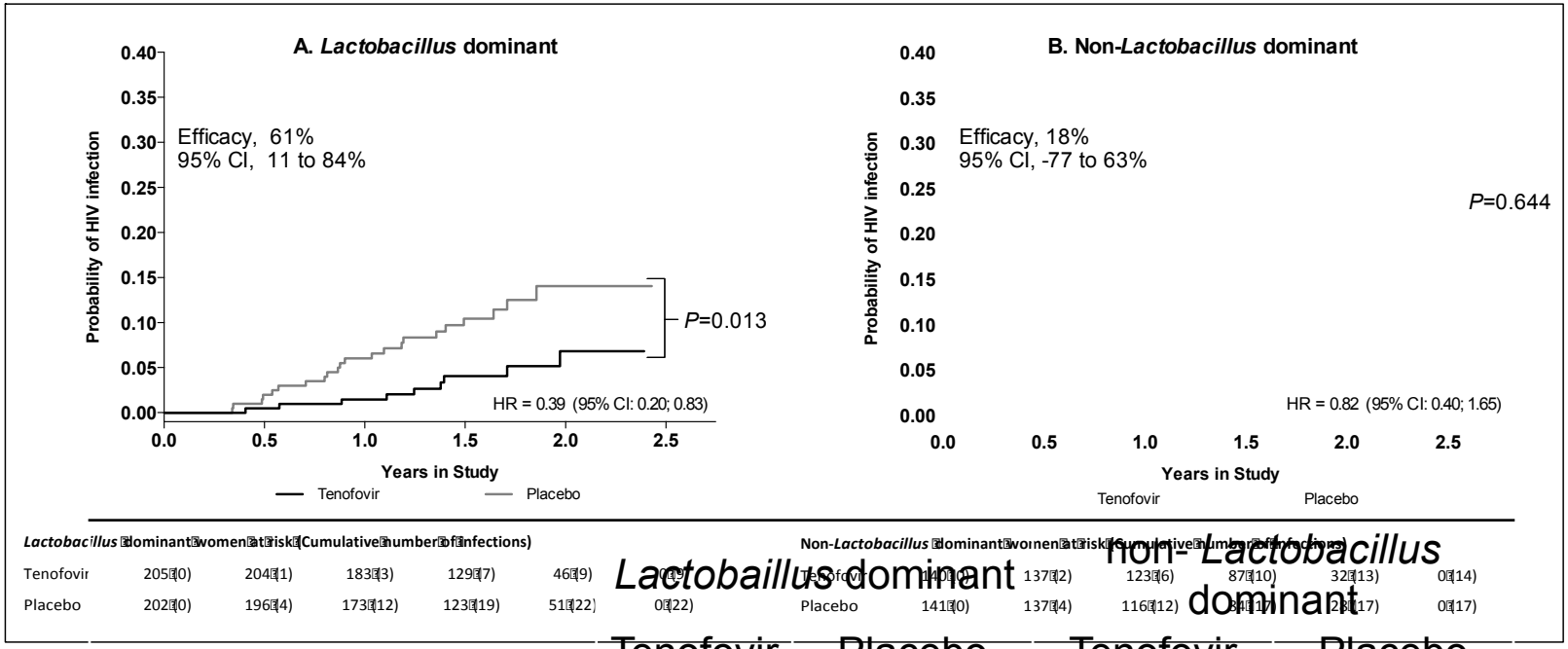
- Identification of high mannose glycoproteins involved in anti-viral activity.



What does the microbiota do to the drugs?



Tenofovir gel effective against HIV with Lactobacillus dominance



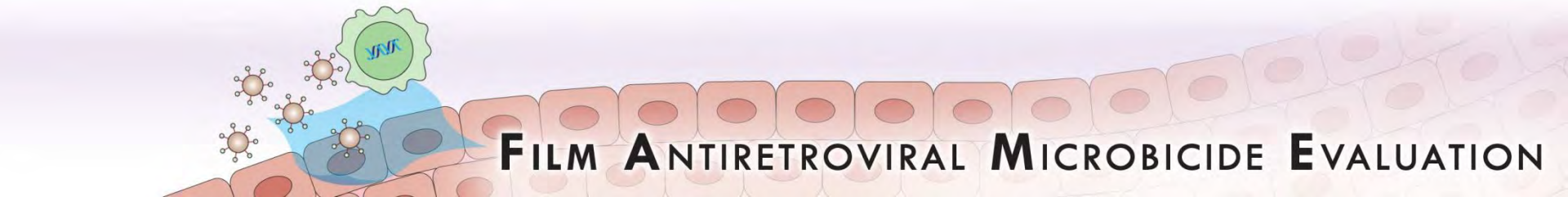
	Tenofovir	Placebo	Tenofovir	Placebo
# HIV-1 infections	9	22	14	17
HIV-1 incidence per 100 person-years	2.7	6.9	6.4	7.8
HIV-1 protection effectiveness 95% CI, *P*-value	**61%** (11, 84), *p*=0.013		**18%** (-77, 63), *p*=0.644	

Klatt/Burgener et al., submitted

FILM ANTIRETROVIRAL MICROBICIDE EVALUATION

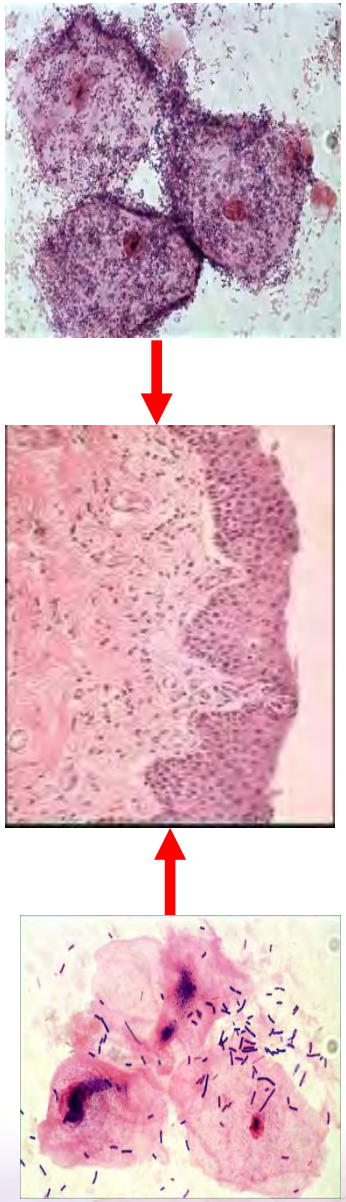
Limitations

- Women having greater numbers of partners and more frequent sexual activity at greater risk of BV and HIV
- Relationship between BV-type microbiota and decreased effectiveness of tenofovir could be attributed to unmeasured differences in behavior



Hypothesis

- Women having microbiota associated with bacterial vaginosis will have decreased levels of tenofovir in genital tissue
- Women having beneficial lactobacilli associated with LB dominant microbiota will have higher levels of tissue tenofovir

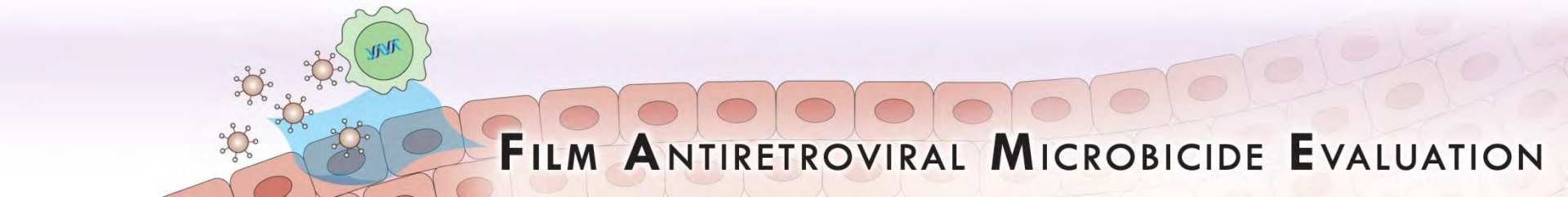


Hillier et al, CROI 2017

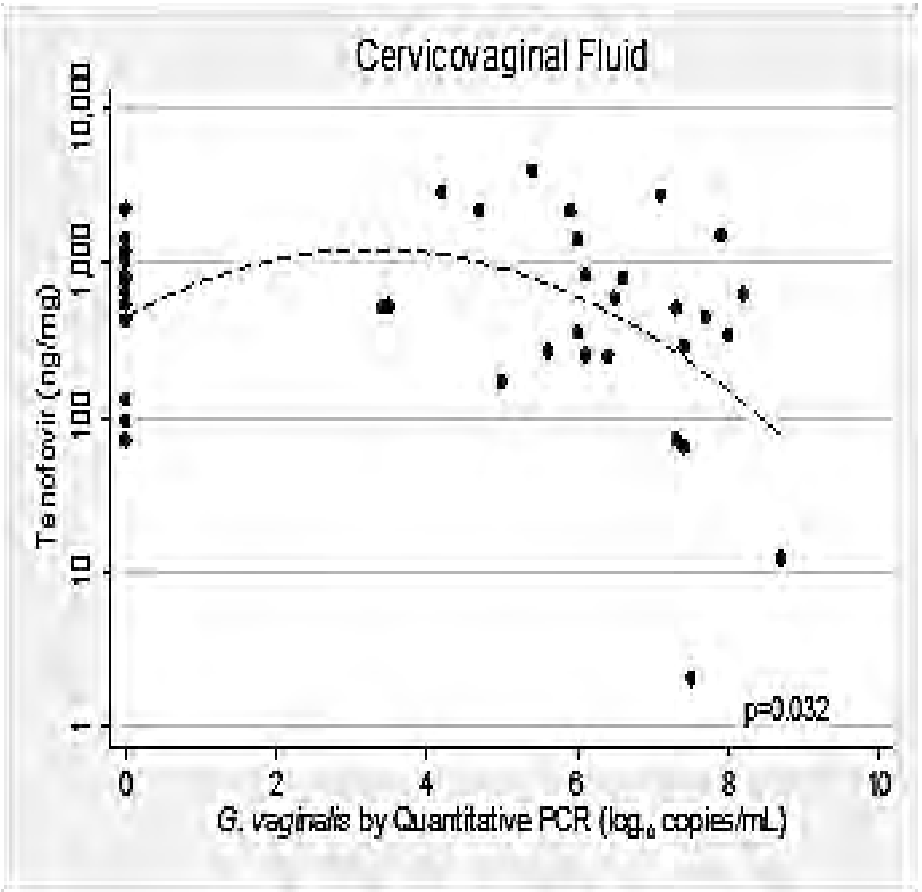
Methods

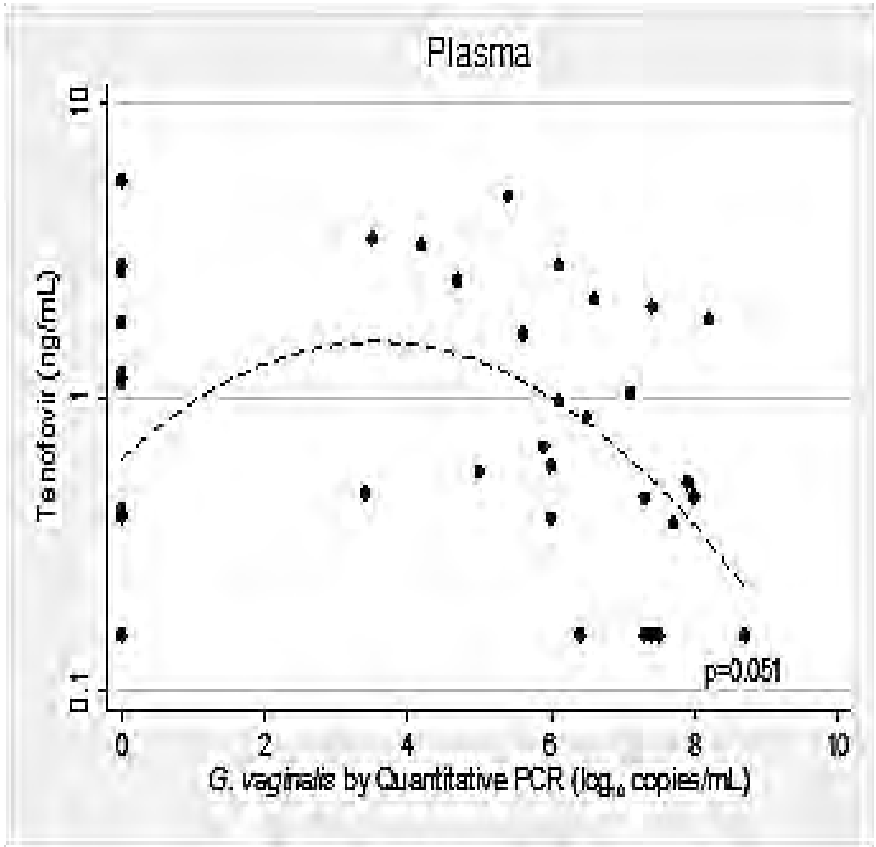
- qPCR performed from vaginal swab samples collected at baseline
  - *Gardnerella vaginalis*
  - *Atopobium vaginae*
  - *Lactobacillus crispatus, L jensenii, L gasseri, L iners*
- Bacterial vaginosis detected using Nugent criteria from a Gram stained vaginal smear collected at baseline
- Statistics:
  - Relationship between vaginal microbiota and TFV concentrations was assessed using linear regression models
  - A quadratic term was included in the models with G. *vaginalis* to improve model fit.
  - Reported P-values are from the global F-test.

Hillier et al, CROI 2017



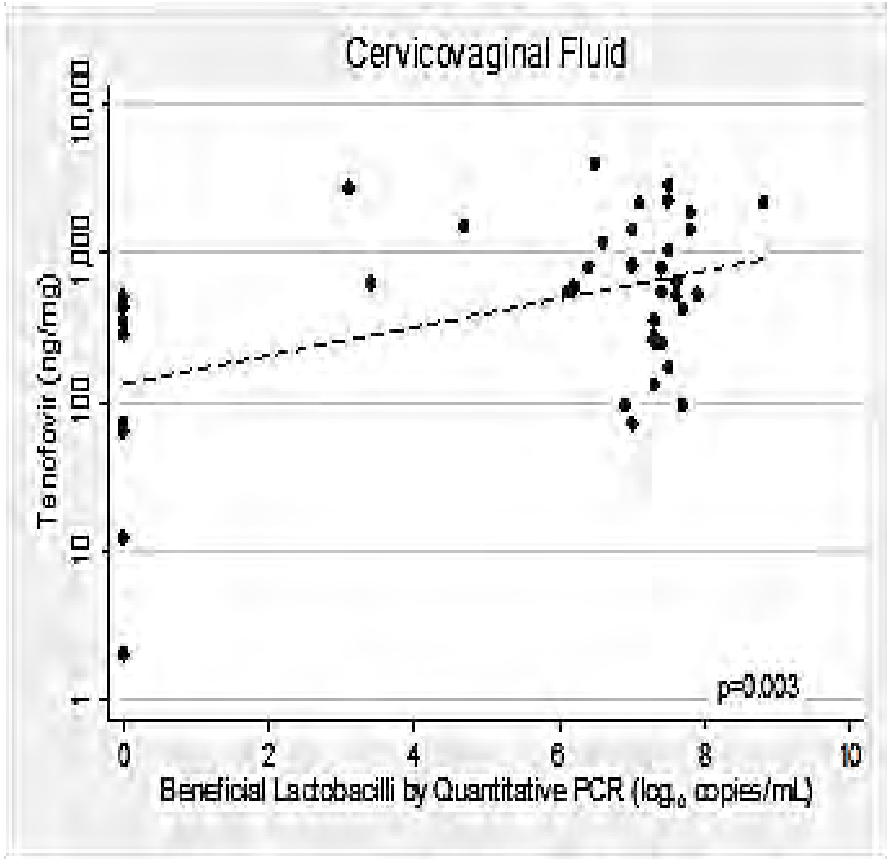
Impact of *G vaginalis* on Tenofovir Levels in Plasma and Vaginal Fluid After 6 Doses

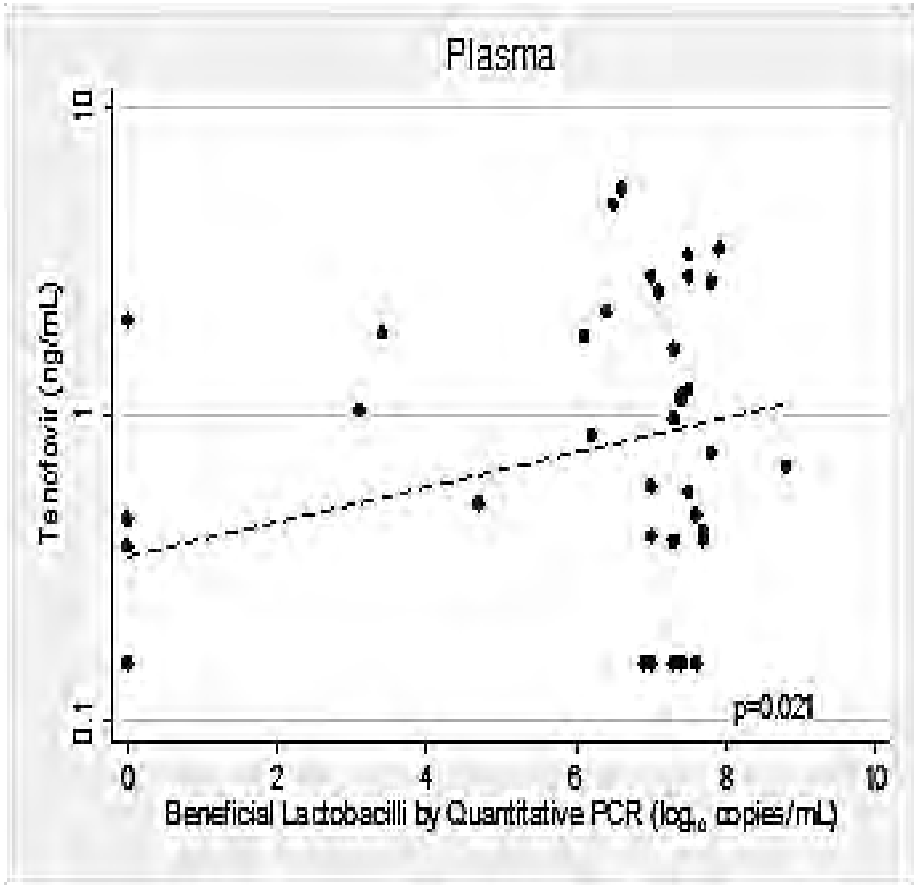




Hillier et al, CROI 2017

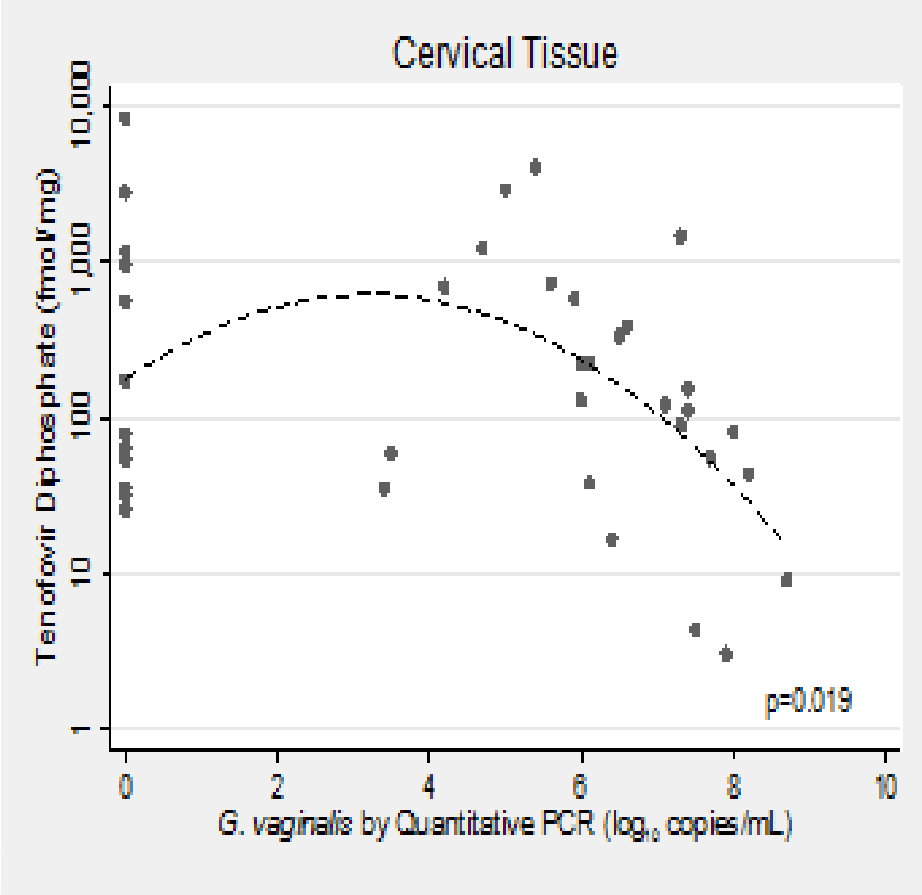
Impact of *L crispatus, L jensenii and L gasseri* on Tenofovir Levels in Plasma and Vaginal Fluid After 6 Doses

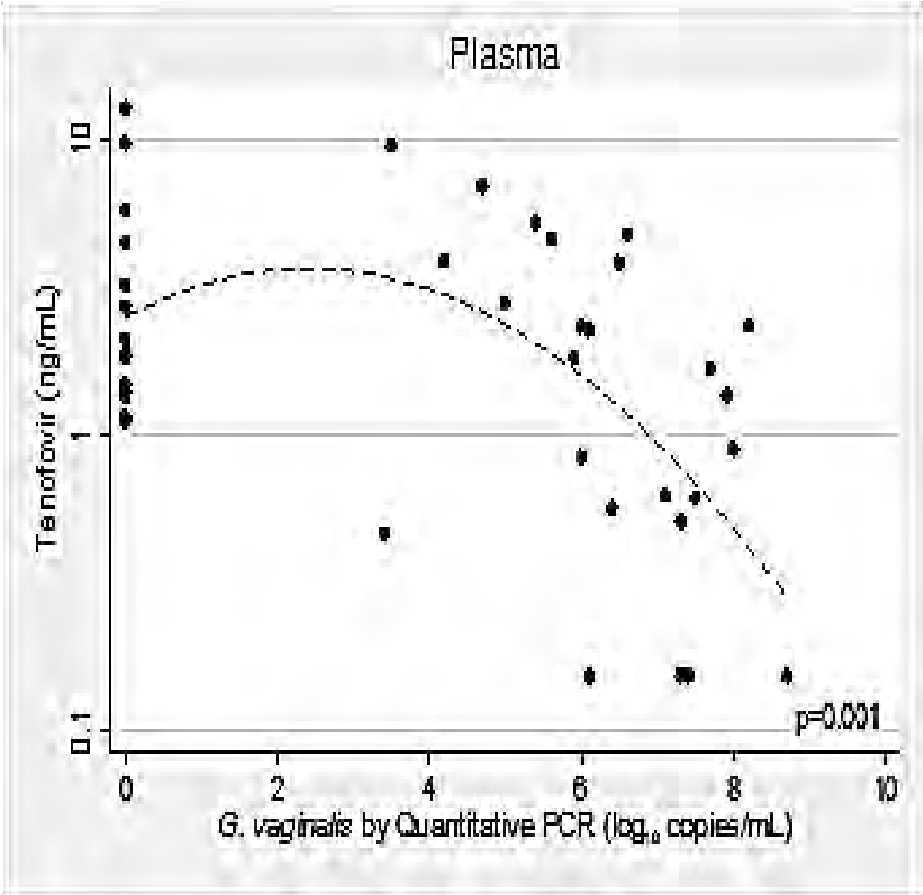




Hillier et al, CROI 2017

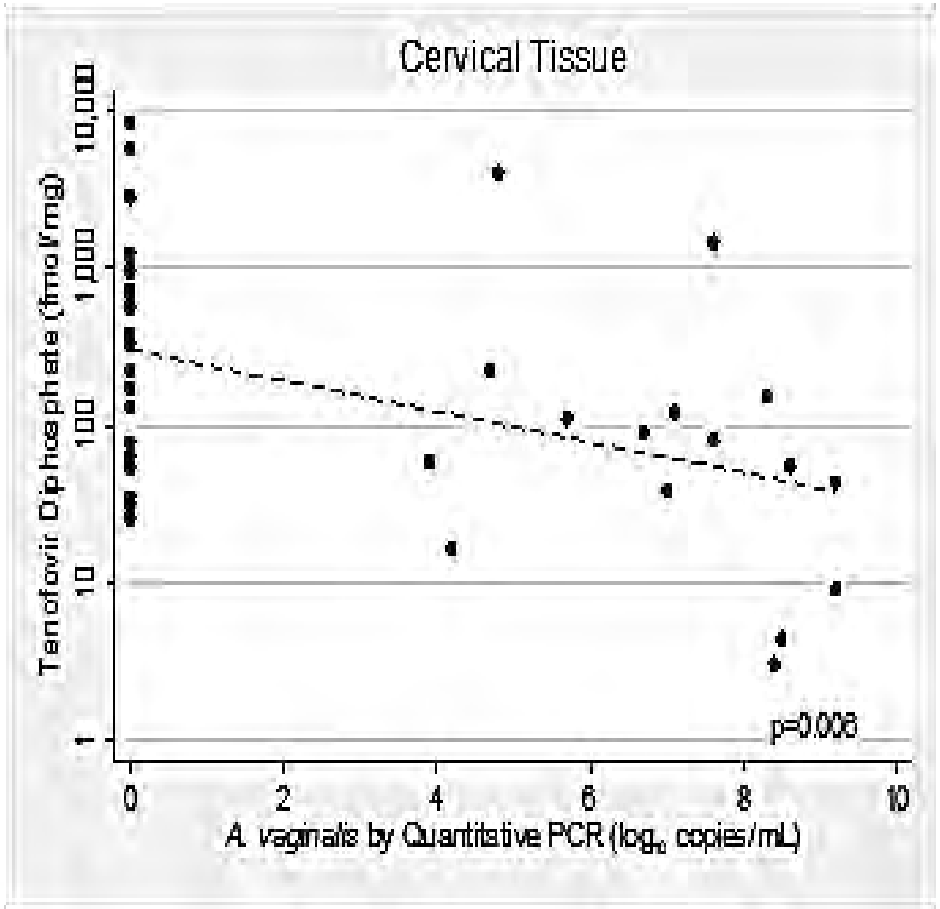
G vaginalis vs Tenofovir diphosphate in Cervical Biopsy Tissue and Tenofovir in Plasma

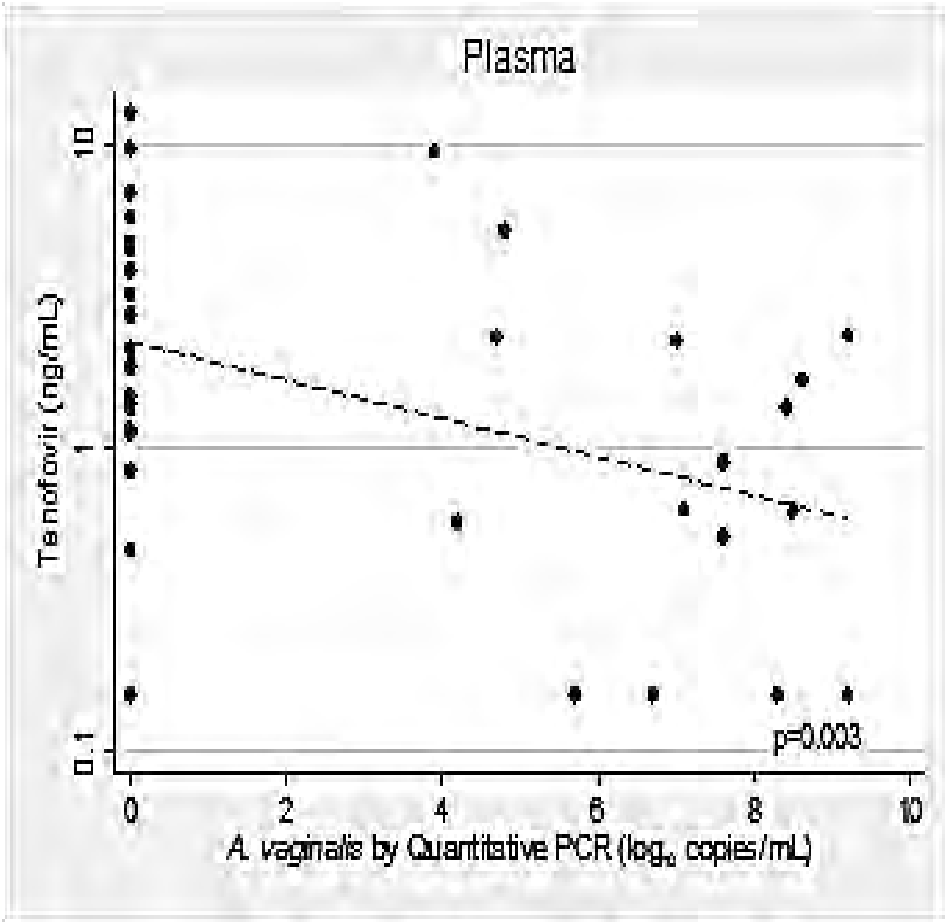




Hillier et al, CROI 2017

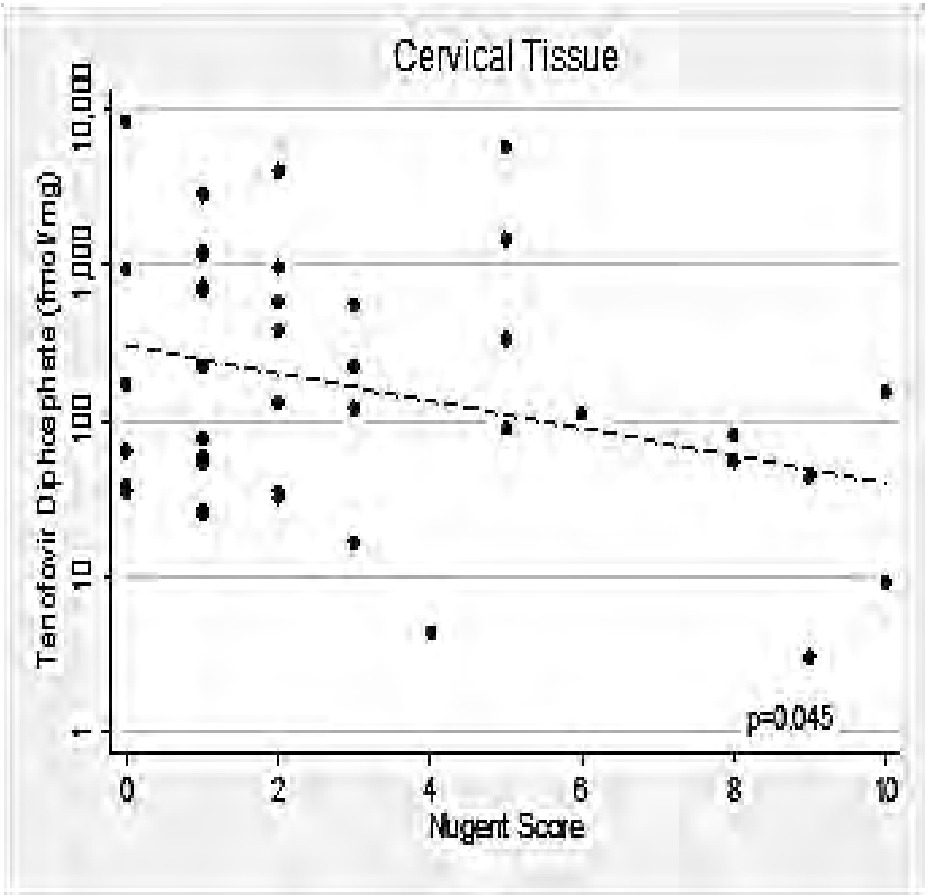
Atopobium vaginae and Tenofovir diphosphate in Cervical Tissue and Tenofovir in Plasma

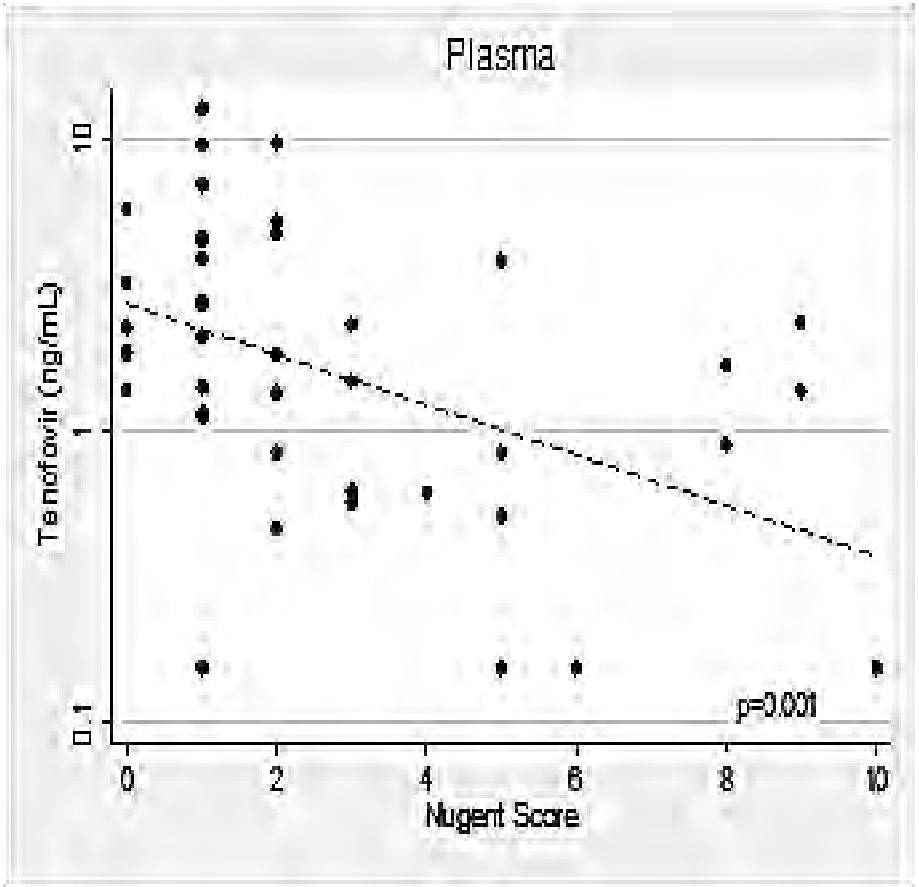




Hillier et al, CROI 2017

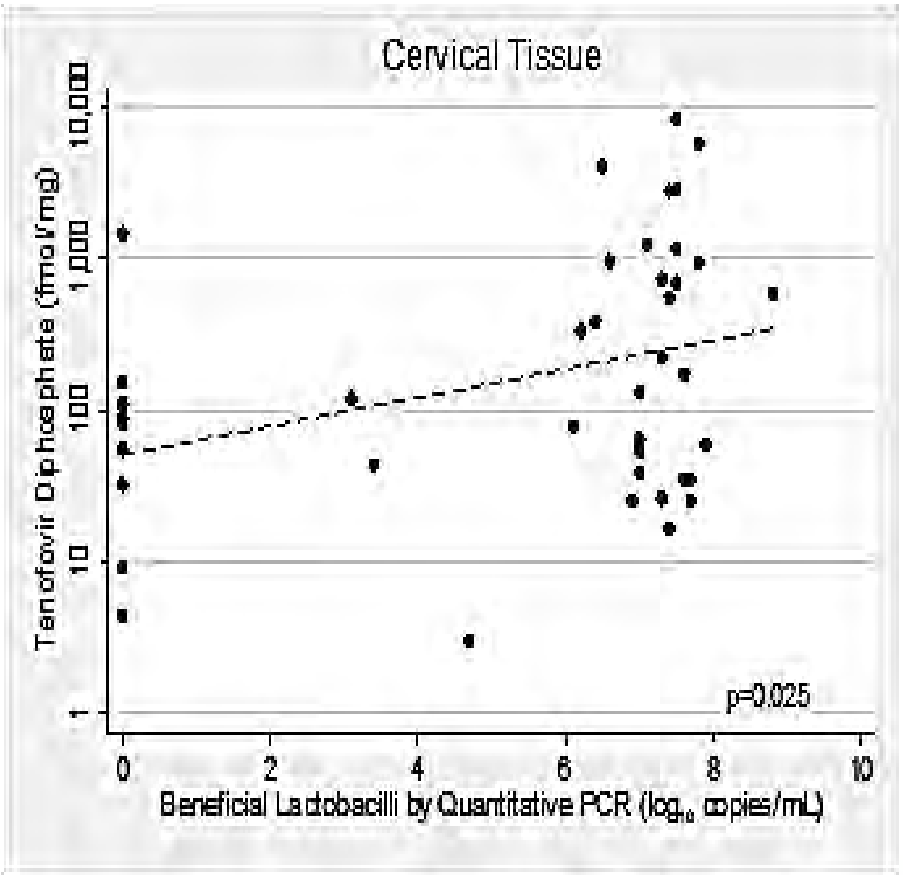
Nugent Score and Tenofovir diphosphate in Cervical Tissue and Tenofovir in Plasma

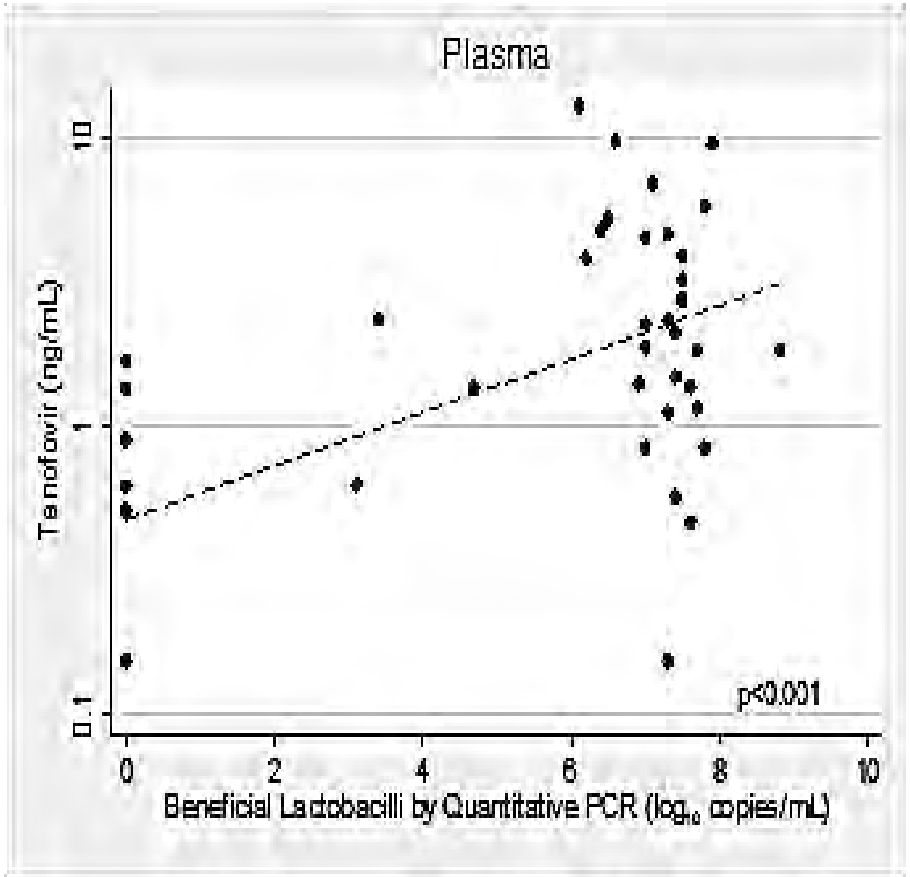


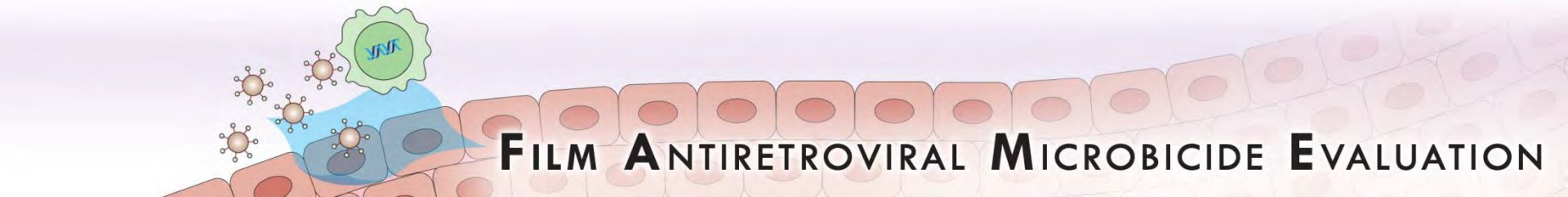


Hillier et al, CROI 2017

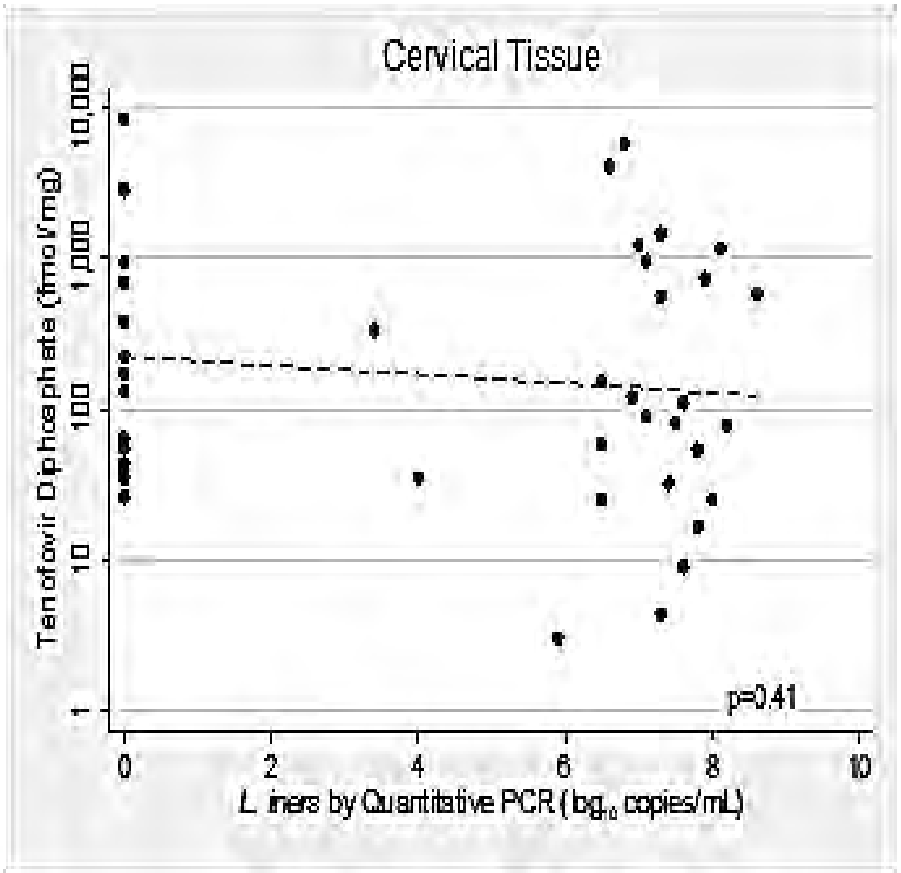
Lactobacillus crispatus, jensenii and gasseri vs Tenofovir diphospate in the Cervix and Tenofovir in the Plasma

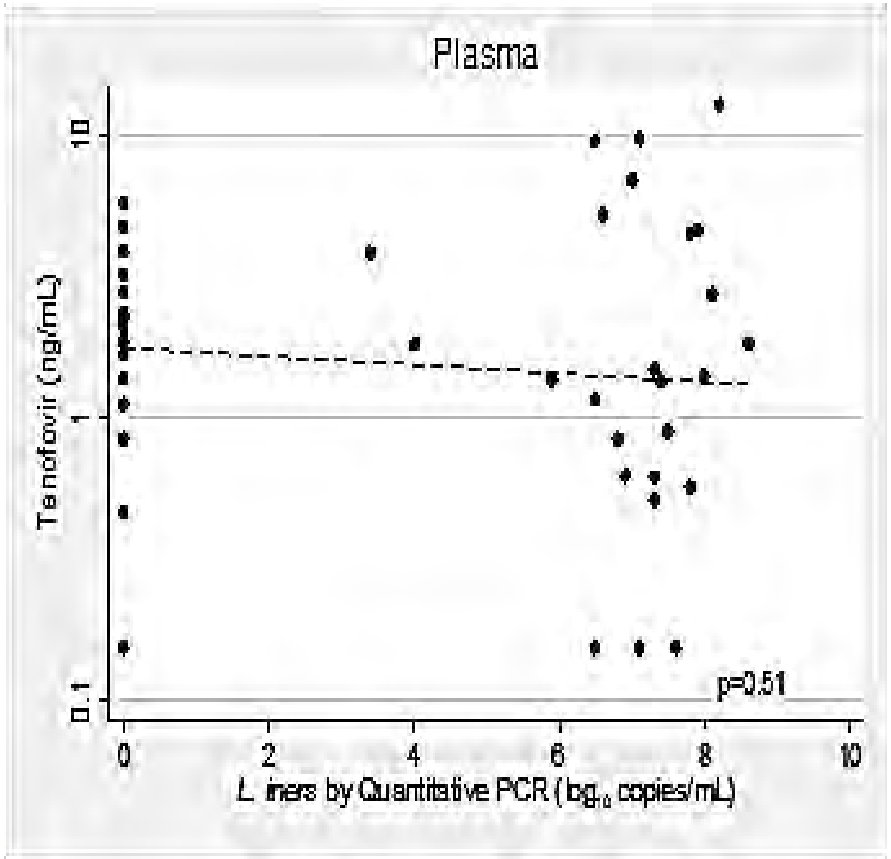






Lactobacillus iners vs Tenofovir diphisphate in the Cervical Tissue and Tenofovir in the Plasma





Summary and Conclusions

- Women having vaginal microbiota associated with bacterial vaginosis have significantly lower levels of tenofovir in plasma and vaginal fluid after 6 daily doses, and less tenofovir in plasma and cervical tissues 2 hours after application of tenofovir to the vagina.

- Higher density of *L crispatus, jensenii and gasseri*, but not *L iners*, was associated with higher systemic and tissue concentrations of tenofovir.

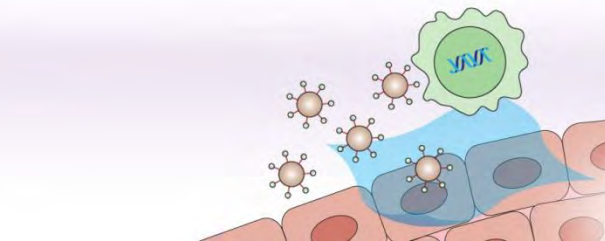
- These data are supportive of the secondary analyses from the CAPRISA study reporting lower effectiveness of tenofovir gel among women having *Lactobacillus*-deficient microbiota

Hillier et al, CROI 2017

So What Did We Learn?

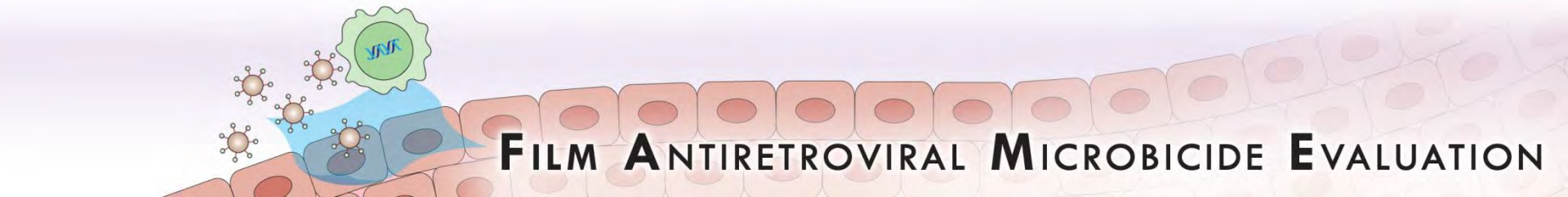
Fast dissolving films

1. Can deliver ARVs to genital tissues as efficiently as gels
2. Neither films nor gels were disruptive to the vaginal microbiota but both caused some changes in mucin expression and glycosylation patterns in the vaginal microenvironment.
   - Gel polymers decreased innate antiviral activity while films did not
   - Lectin micro arrays showed that after gel use, there were changes in the glycome that mapped to anti-HIV activity
3. BV microbiota metabolized tenofovir (but not dapivirine)



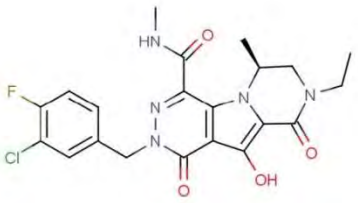
Building a film for extended release of ARVs

- Need a different drug with extended residence in tissues (not dapivrine) and which is not metabolized by vaginal microbiota (not tenofovir)
- Different film polymers that release drug slowly
- Film polymers that do not disrupt innate immunity, vaginal microbiota or glycome

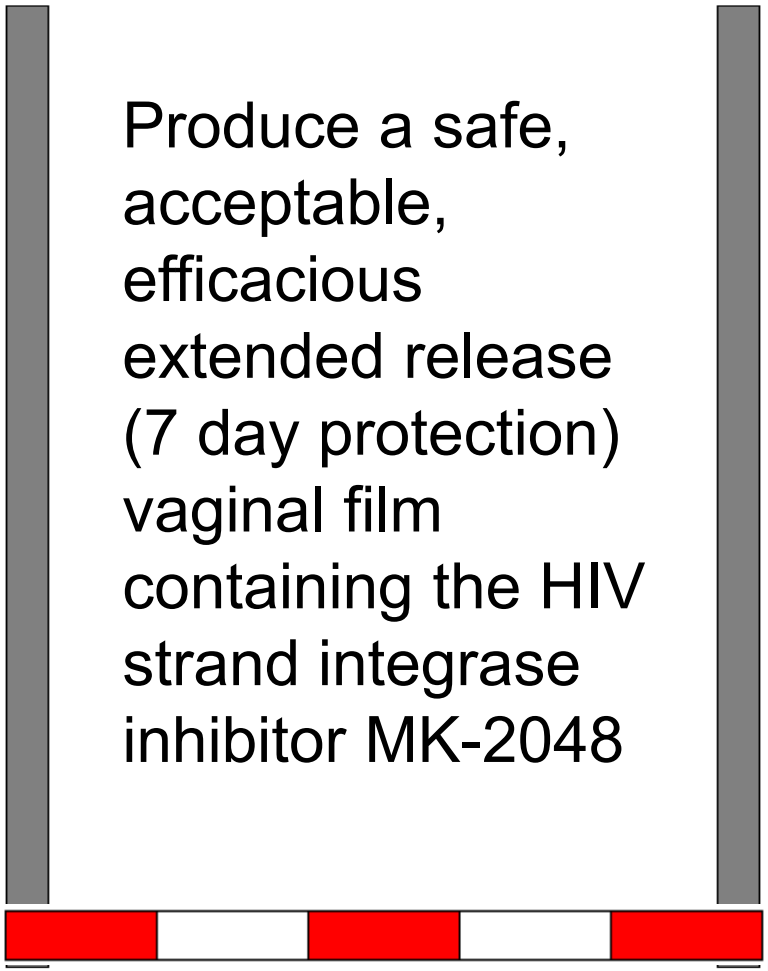


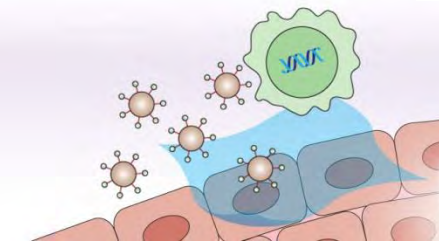
RAISING THE BAR

MK-2048 $C_{21}H_{21}ClFN_5O_4$ MW 461.87



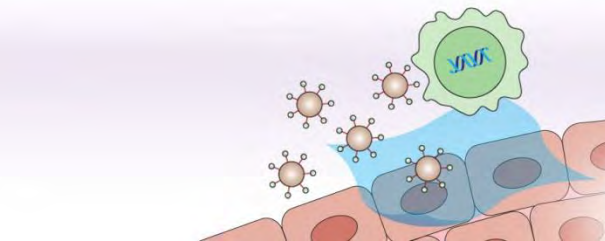
Produce a safe, acceptable, efficacious extended release (7 day protection) vaginal film containing the HIV strand integrase inhibitor MK-2048





Optimization of MK-2048 Film

- Film Composition
  - Base- Create a film base so that regardless of excipient combinations/ratios we can still get a film
    - HPMC E5
    - HEC
    - Plasticizers (PEG 400,Propylene glycol)
  - Extended Release Components
    - HPMC K4M (↑ Molecular Weight)
    - HPC (hydrophobic)
    - Eudragit RS 30D (Extended Release)
  - Size
    - 2x2 cm
  - Drug loading
    - 10 mg/film



Visual Dissolution/Drug Delivery: Placebo & MK-2048 ER Films

Purpose: Document dissolution time for extended release (ER) film platform (placebo) *AND* collect MK-2048 PK data to document drug delivery.

Revised Design

	D0	D3	D7	D10	D14	D17	D21	D24	D27	D30	D34	D36	D41
Visualize Colpo-Photo n=6	√	√	√	√	√	√	√	√	√	√	√	√	√
MK-2048 PK (Vaginal swab) **n=3**	√	√	√	√	√	√	√	√	√	√	√	√	√
Film Dose n=3+3	√		√*		Vag Micro Swab (MWRI)				√				

*Day 0: menstruating; D3 No Film Residual; D7 Apply 2nd Film; D10 Film Present; D14 No Film Residual

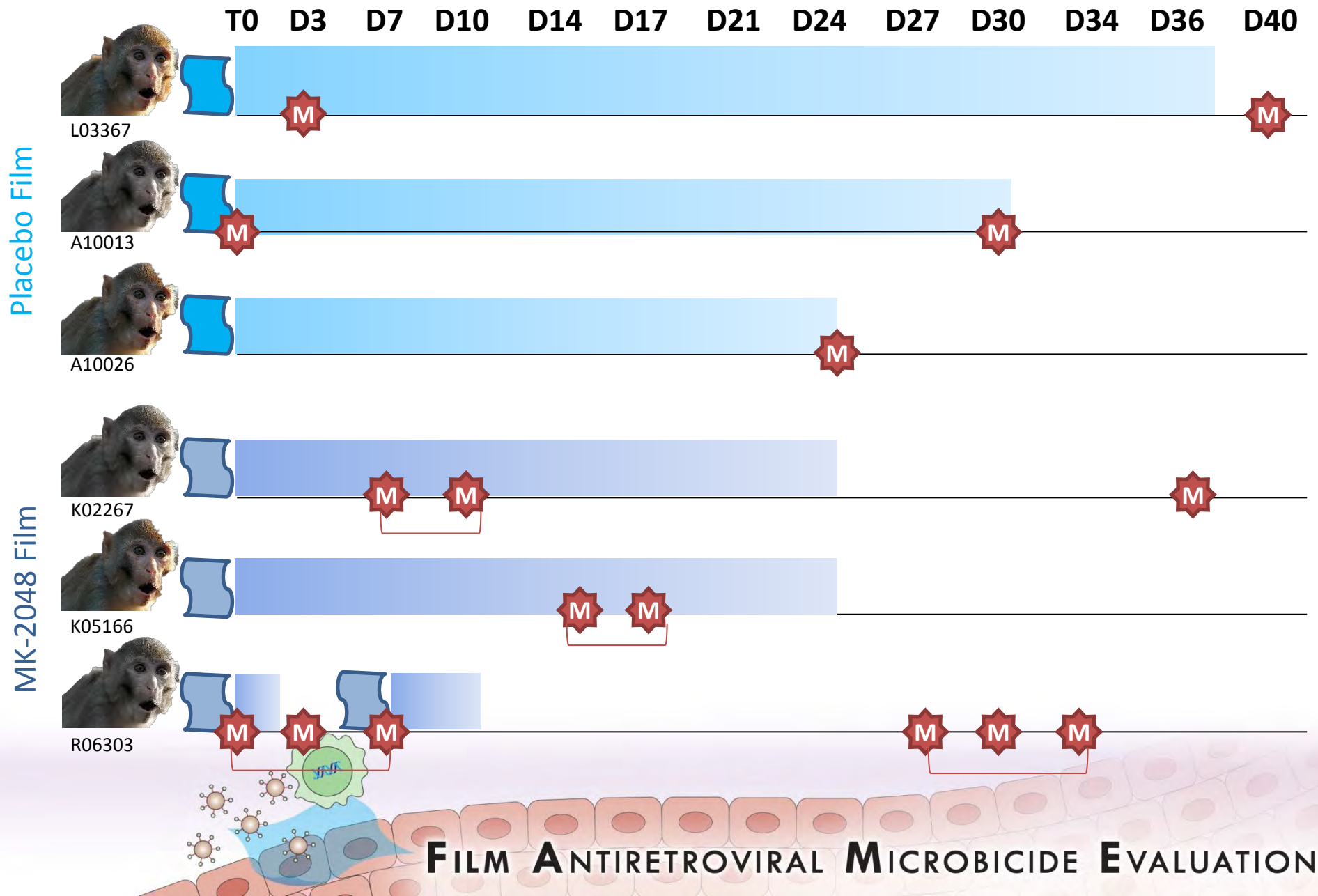
FAME2 Films 1"x1"

Placebo SMG-4-81 A10013. A10026. L03367
MK-2048 SMG-4-79B K02267. K05166, R06303*

F2-2.1

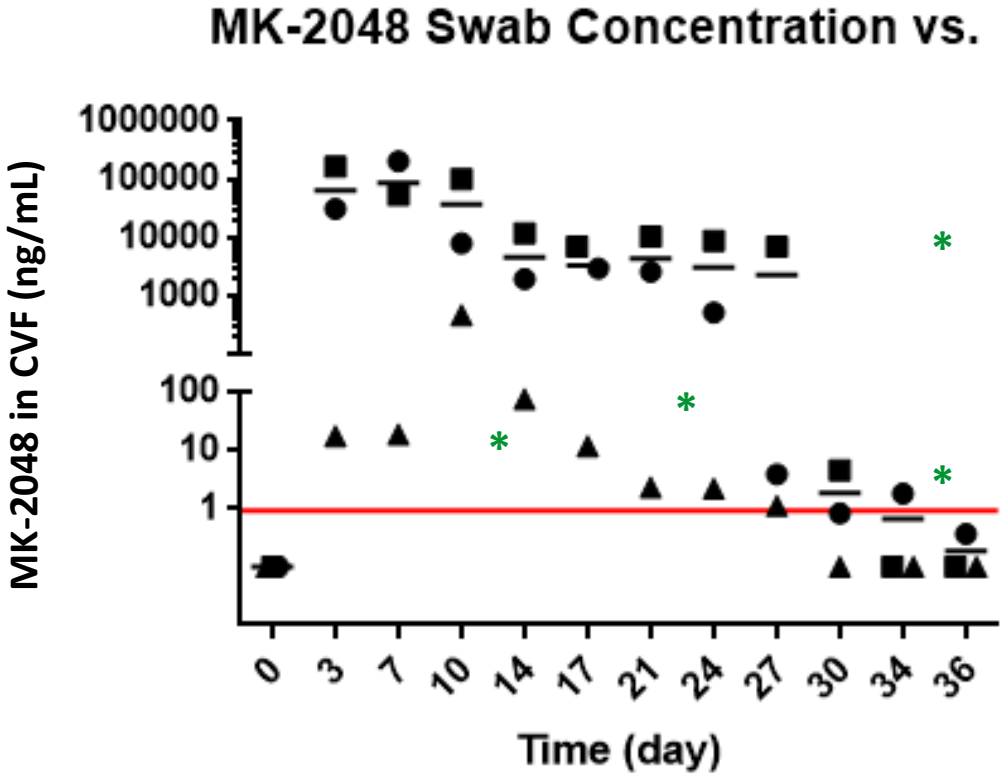
Jan-Mar 2016

NHP Study Results: FILM DISSOLUTION



NHP Study Results

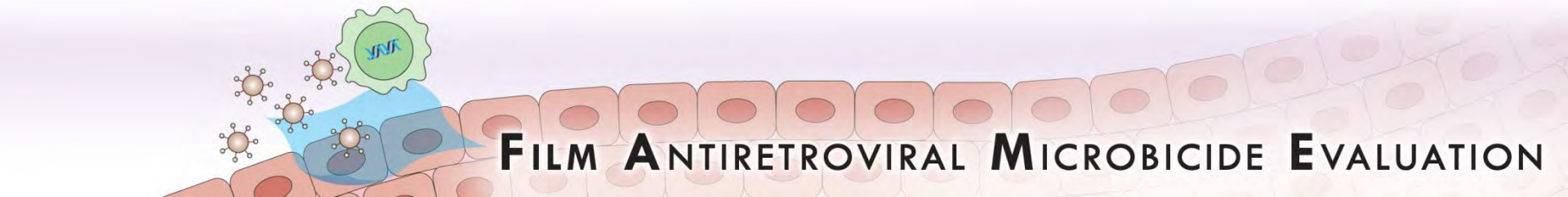
MK-2048 Extended Release Film



	AUC (0-36)d*ng/mL	Css (ng/mL)
▲ R06303	2,079	58
■ K02267	1,257,476	34,930
● K05166	868,866	24,135

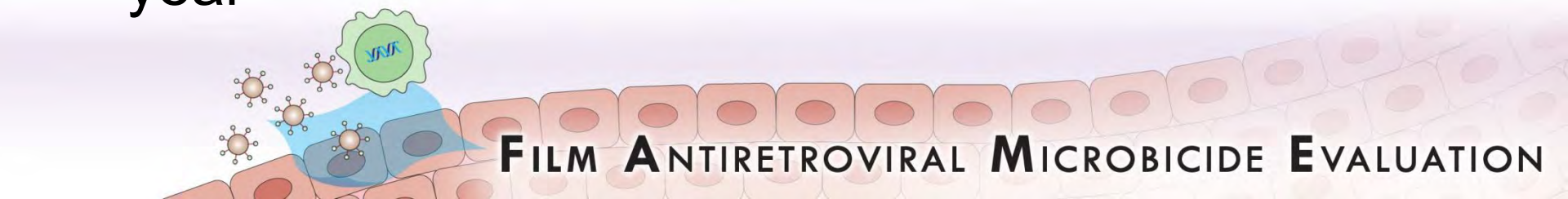
IC_{50}=0.924 ng/mL

*No visual evidence of film



Progress Toward an ER Film

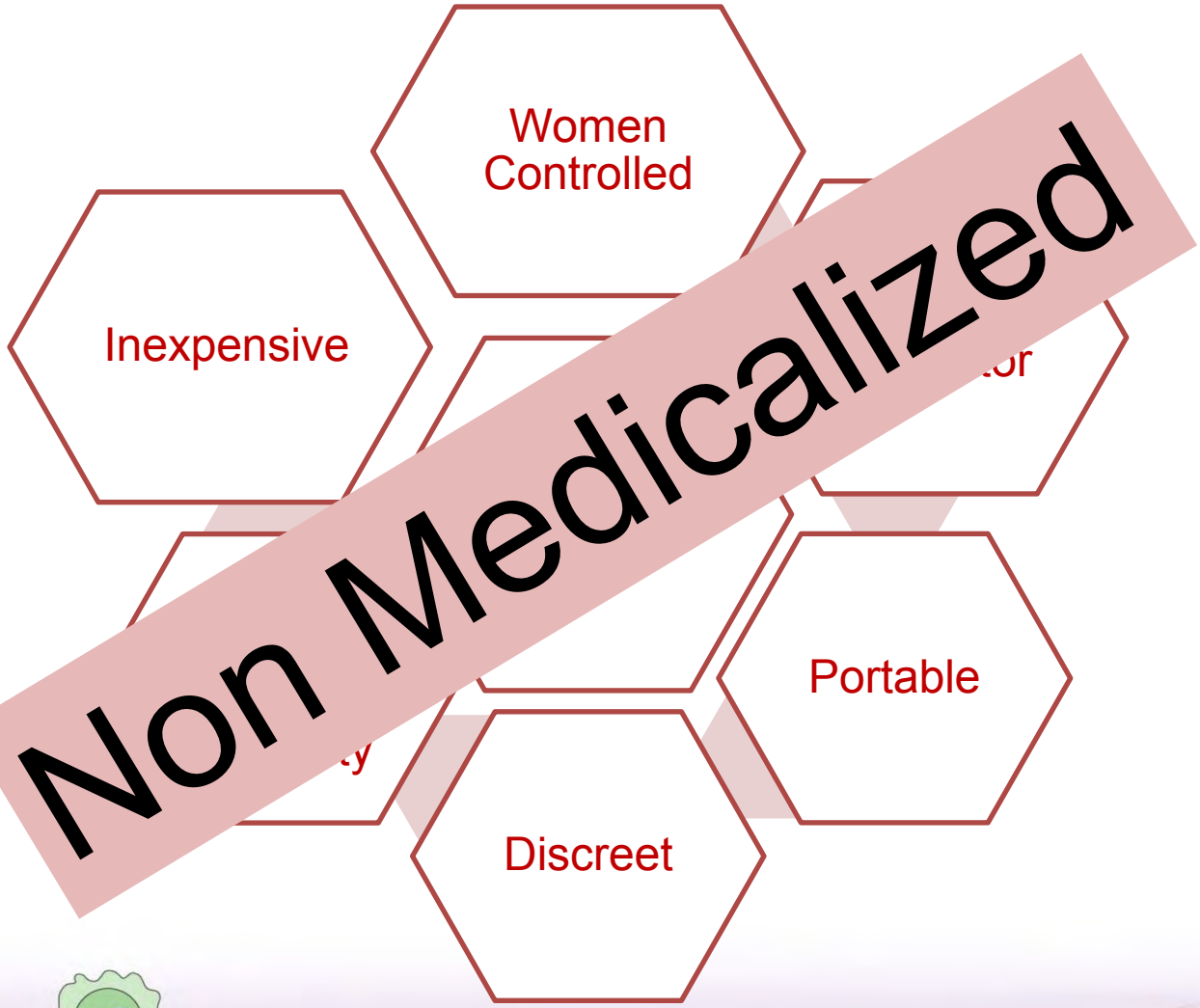
- Proof of principle established in the pig-tailed macaque model that after a single film application drug levels 10,000X the inhibitory dose for 21 days
  - This persisted through menses and sex (data not shown)
  - No safety concerns; no impact on microbiota
- Film polymers used for ER film have been used orally but never vaginally, so doing a safety study of the ER placebo film later this year
- Will do the first in human study of the ER film next year



"Scorecard" for HIV prevention modalities for women

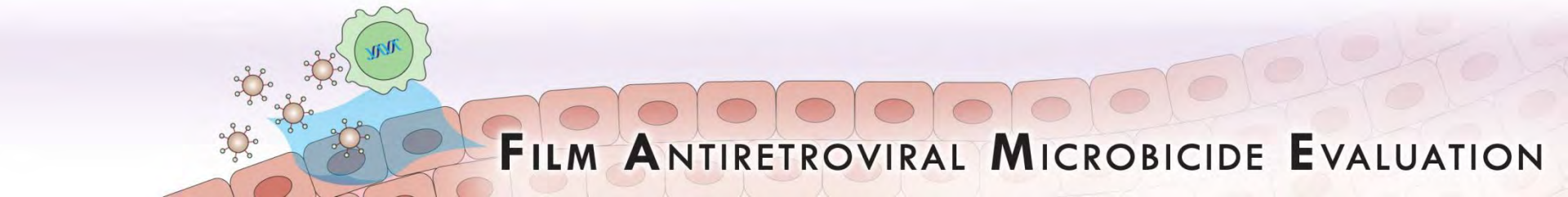
Modality	Efficacy	Low user burden	Low Cost	Low systemic side effects	Reversibility
Daily oral PrEP					
On demand PrEP					
Injectable Cabotegravir					
PrEP implant					
Vaginal ring					
Vaginal film On demand					

Conclusions



Conclusions

- Developing interdisciplinary teams to attack challenges in HIV prevention is super fun!
- We need to focus on end users more as we develop products.
- Films are an attractive dosing option for “on demand” HIV prevention in women



Co-PIs of FAME

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FILM ANTIRETROVIRAL MICROBICIDE EVALUATION

University of Pittsburgh