

Pharmacology Lab Contributions to PrEP Product Development

- *How to turn a sample into drug concentration data*
- *How to use drug data to inform drug development*

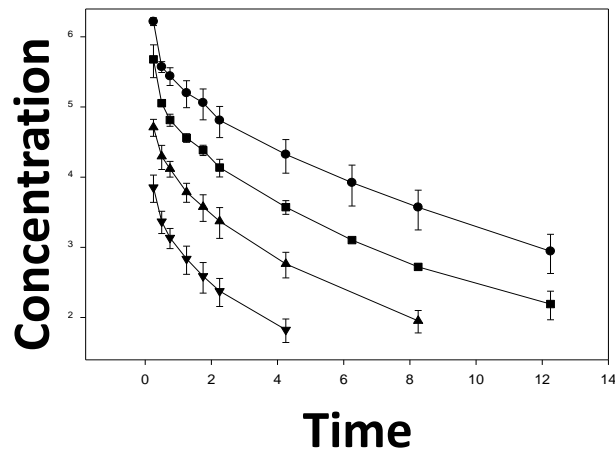
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Craig W. Hendrix, Director, Drug Development Unit
Johns Hopkins University University

LC Pharmacology In Drug Development

- Marriage of Analytical Pharmacology & Clinical Pharmacology
- Applications to all phases of PrEP Drug Development

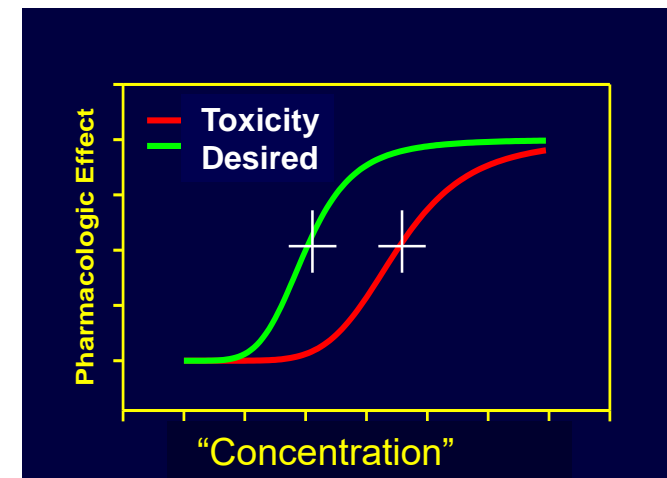
Pharmacokinetics (PK)

- What the body does to the drug
- How to hit the target



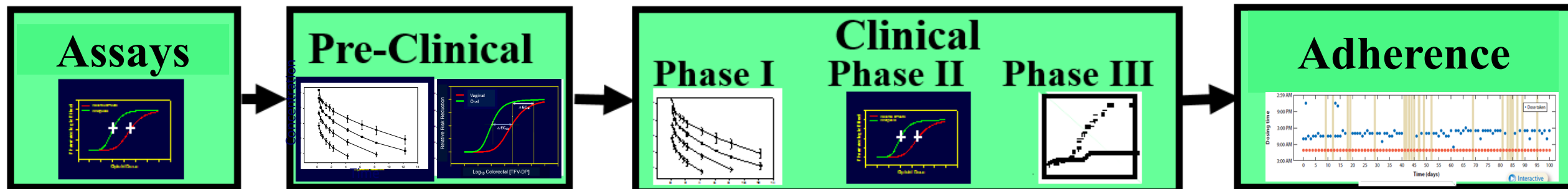
Pharmacodynamics (PD)

- What the drug does to the body & HIV
- Deciding on the target



LC Pharmacology In Drug Development

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Accurate?
Precise?
Reproducible?
Robust?

How much?
How often?

Hit target?

How much?
How often?

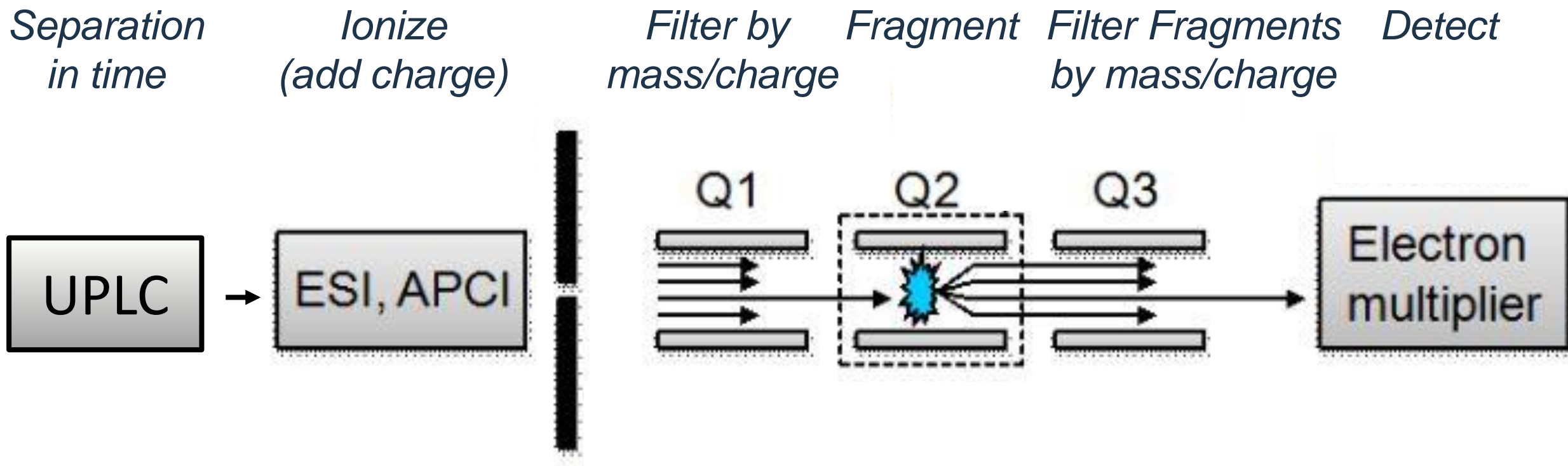
Does it scale?
Hit target?

Does it work?
Target correct?

Drug taken?

Time?
Space?

Mass Spectrometry: Sample to Data



Assay Development & Validation

Test	Acceptance Criteria
Intra-Assay and Inter-Assay Accuracy	Mean of replicates $\leq 15\%$ ($\leq 20\%$ at LLOQ) of nominal conc.
Intra-Assay and Inter-Assay Precision	%CV $\leq 15\%$ ($\leq 20\%$ at LLOQ)
Dilutional linearity	Accuracy: $\leq 15\%$ of nominal conc.; precision $< 15\%$
Partial volume analysis	Accuracy: $\leq 15\%$ of nominal conc.; precision $< 15\%$
Freeze/thaw Stability	
Room temperature Stability	
Extract matrix stability	
Reinjection reproducibility	
Long term Stability	
Solution stability: stock	
Selectivity in blank matrix	IS response $< 5\%$ of IS internal
Matrix Effect Slope Analysis	
Alternate Matrix Evaluation	
Hemolyzed plasma	
Concomitant Medication	Accuracy: $\leq 15\%$ of nominal/QC conc.; precision $< 15\%$ Blank Matrix: response $< 20\%$ of analyte response in LLOQ sample Blank Matrix: IS response $< 5\%$ of IS response in LLOQ sample
Carryover	Blank Matrix: response $< 20\%$ of analyte response in LLOQ sample Blank Matrix: IS response $< 5\%$ of IS response in LLOQ sample
Instrumental Cross-Talk	IS Cross-talk: Analyte response $< 20\%$ analyte response at LLOQ Analyte Cross-talk: IS $< 5\%$ internal standard response

17 Test Categories

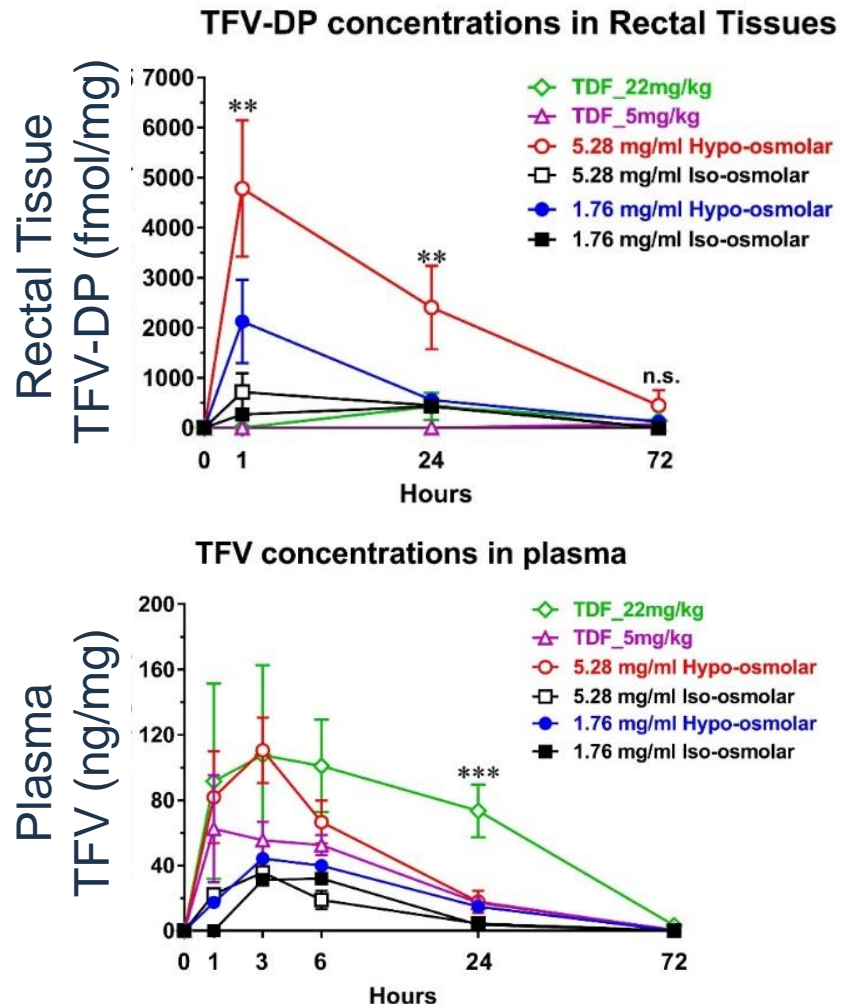
15% Coefficient of Variation for most

CPAL Menu of Assays

Matrix	Collection Device	Analytes
Plasma	K2EDTA	Cabotegravir, CMX157, Dapivirine, Dolutegravir, Efavirenz, Emtricitabine, Ethambutol, Etravirine, Ganciclovir, IQP-0528, Isoniazid, Maraviroc, Medroxyprogesterone acetate, Moxifloxacin, PA-824, Pyrazinamide, Raltegravir, Rifampin, Rifapentine, Desacetyl-rifapentine, Rilpivirine, Tenofovir, Tenofovir alafenamide fumarate, Zidovudine
PBMCs	<div> <p><i>26 analytes (drugs, metabolites)</i></p> <p><i>15 biological matrices/collection devices</i></p> <p><i>79 analyte-matrix pairs</i></p> </div>	
Dried Blood Spots		
Tissue		mbutol, egravir,
Breast Milk		
Cerebrospinal Fluid		
Cervicovaginal Fluid		isoproxil
Cervicovaginal Fluid		
Cervicovaginal Fluid		
Cervicovaginal Lavage	N/A	Dapivirine, Emtricitabine, Maraviroc, Tenofovir, Tenofovir disoproxil fumarate
Rectal Fluid	Weck Cel Sponge	Emtricitabine, Tenofovir
Rectal Fluid	Merocel Sponge	Emtricitabine, Maraviroc, Tenofovir
Rectal Fluid	Dacron Swab	Cabotegravir, Emtricitabine, Dapivirine, Maraviroc, Tenofovir

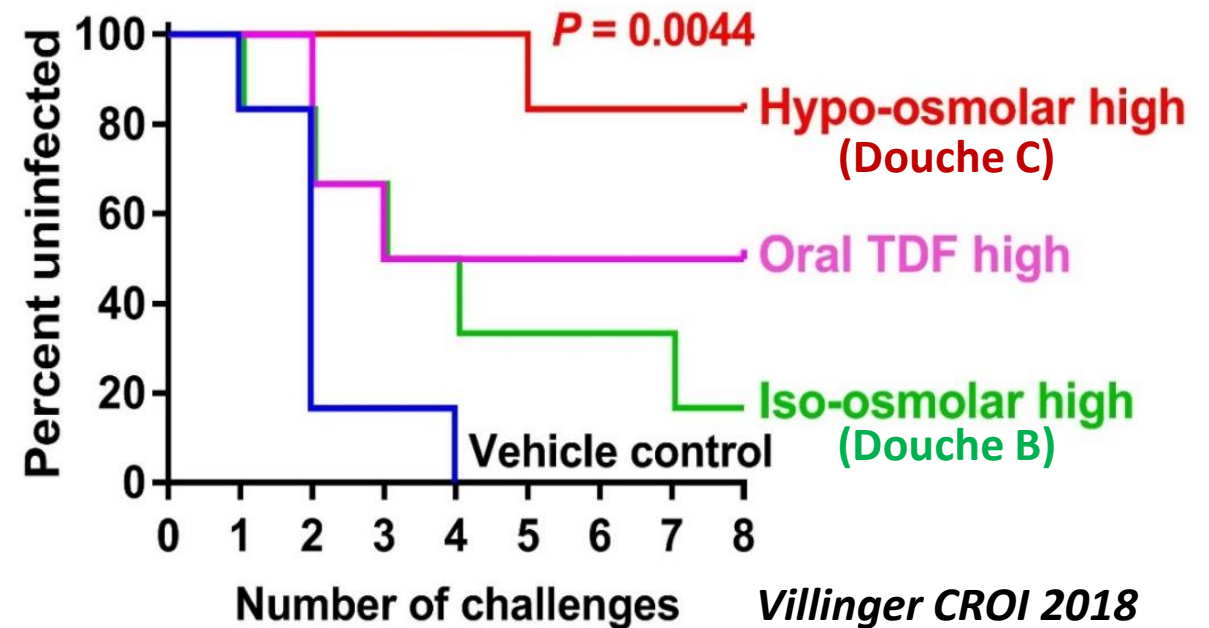
TFV Douche: Macaque SHIV Challenge

Single Dose PK: Oral TDF v. Rectal TFV



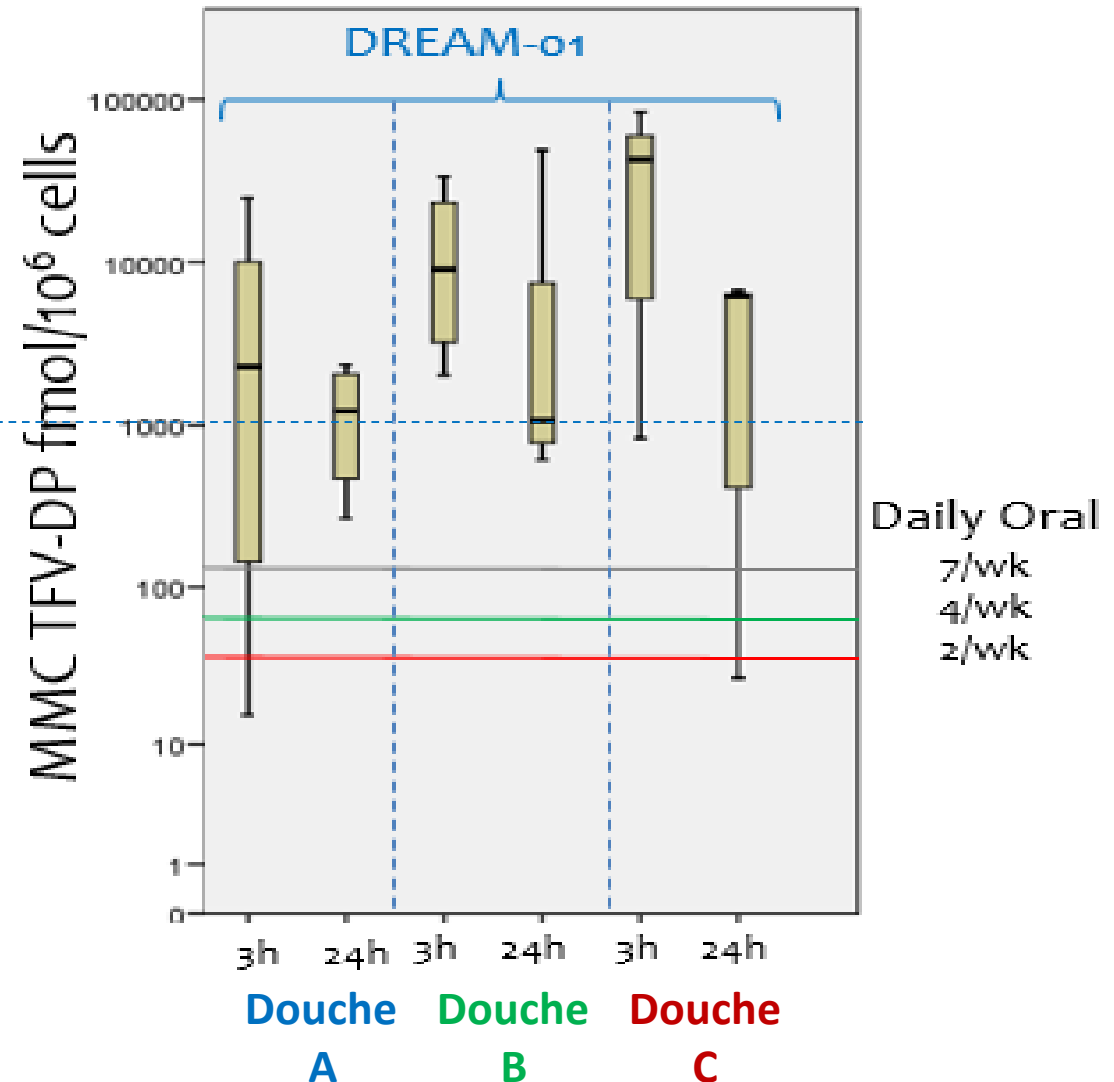
Pharmacodynamics: SHIV Rectal Challenge

- Weekly intrarectal 10^3 TCID₅₀ R5 SHIV
- Weekly plasma viral RNA by qPCR
- “Infected” = 2 vRNA values > 250/mL x 2 wks



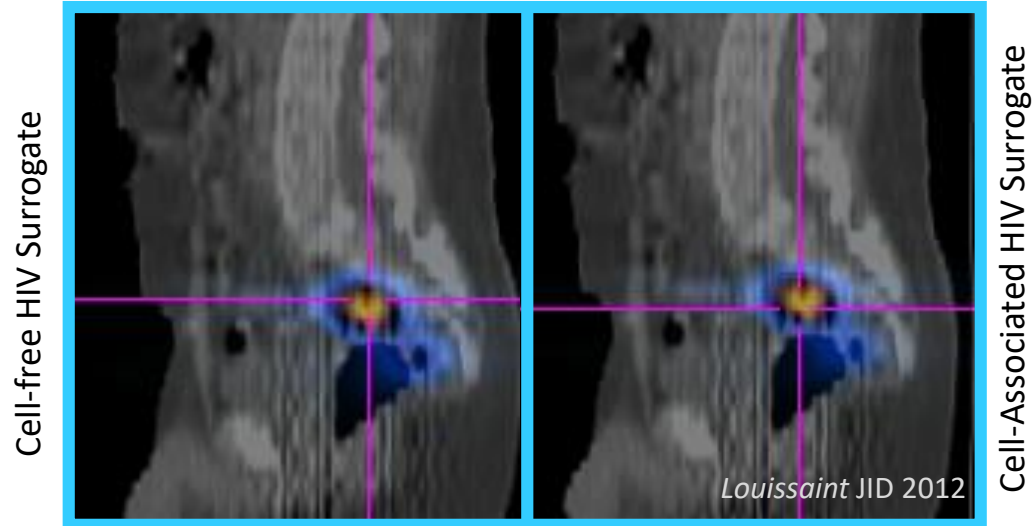
Steady-state oral PK and Challenge Ongoing

Human PK Colon Cell TFV-DP



Colon Luminal HIV & Product Distribution

Define HIV Distribution

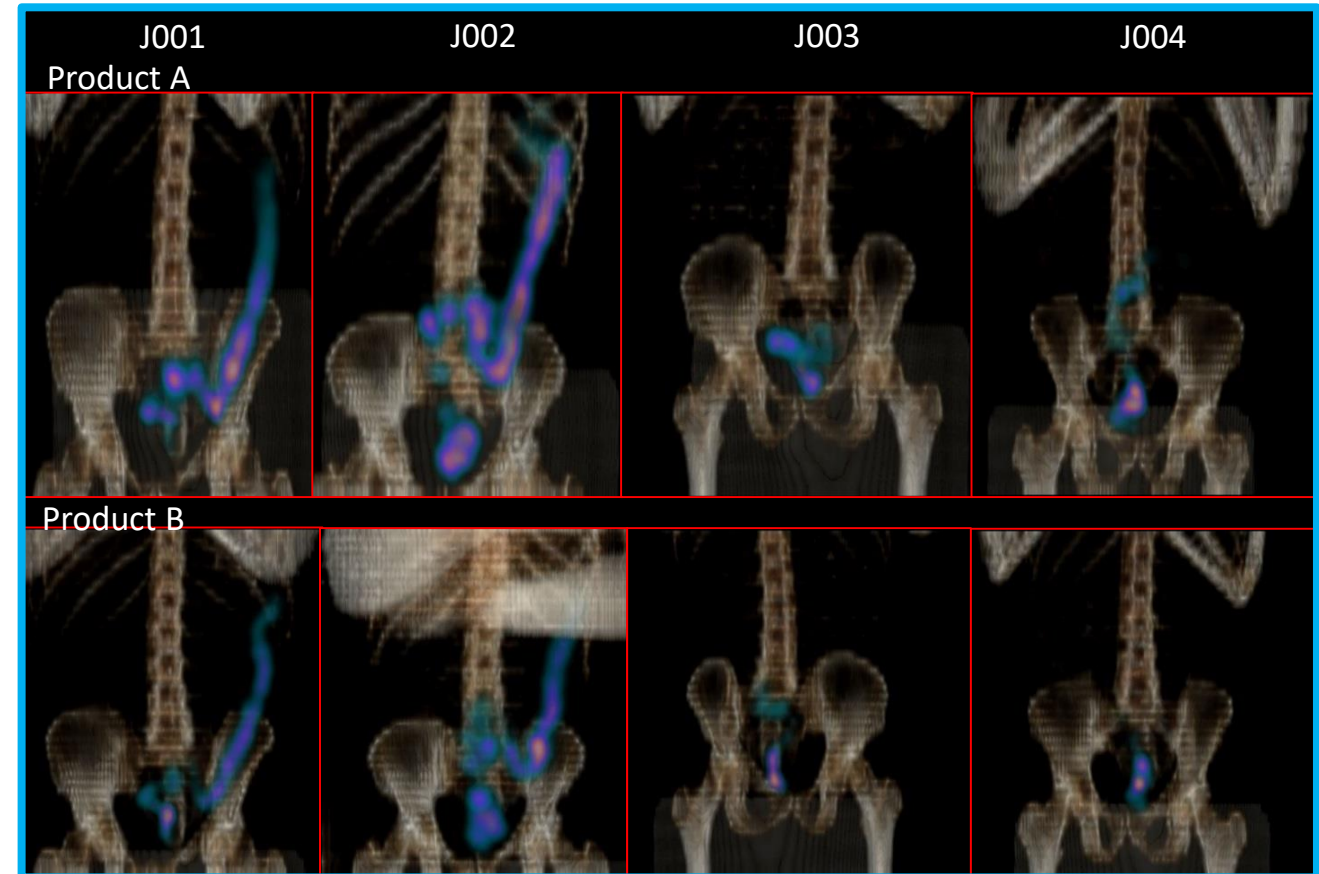


HIV surrogates (SPECT, color) & anatomy (CT, grayscale)

	^{99m} Tc Cell-free	¹¹¹ In Cell-assoc	P value*
	Median (IQR)	Median (IQR)	
1 hr	7 (5, 8)	6 (5, 9)	0.73
4 hr	6 (5, 9)	5 (4, 7)	0.36
8 hr	6 (3, 7)	7 (6, 8)	0.19

*Sigmoidoscope distance adds 4 cm *Weld IAS 2017*

Compare to Product Distribution

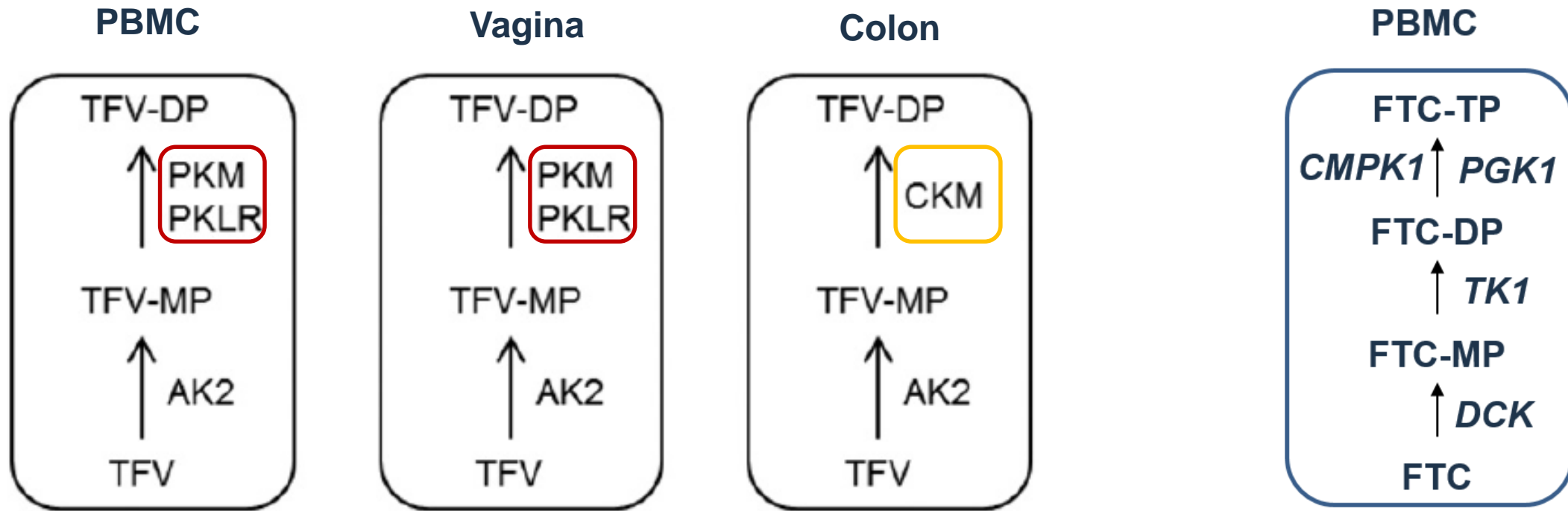


DREAM-01

TFV Activation: Drug Metabolism

Tenofovir Activation

Emtricitabine Activation



Lade et al. EBioMedicine 2015 Jul 9;2(9):1145-52

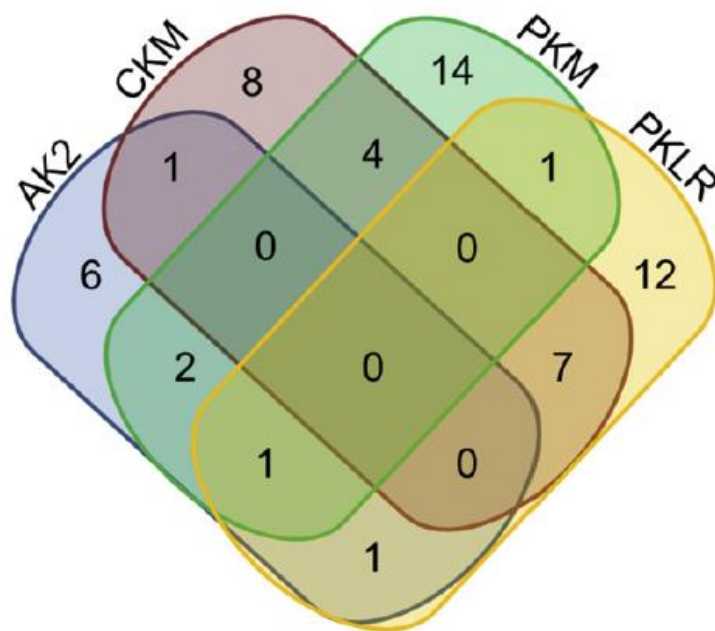
Figuerola. PLoS One. 2018 Apr 11;13(4):e0195764

In vitro PBMC & tissues transfected with siRNA targeting kinases; incubation with substrate

TFV Activation: Pharmacogenetics

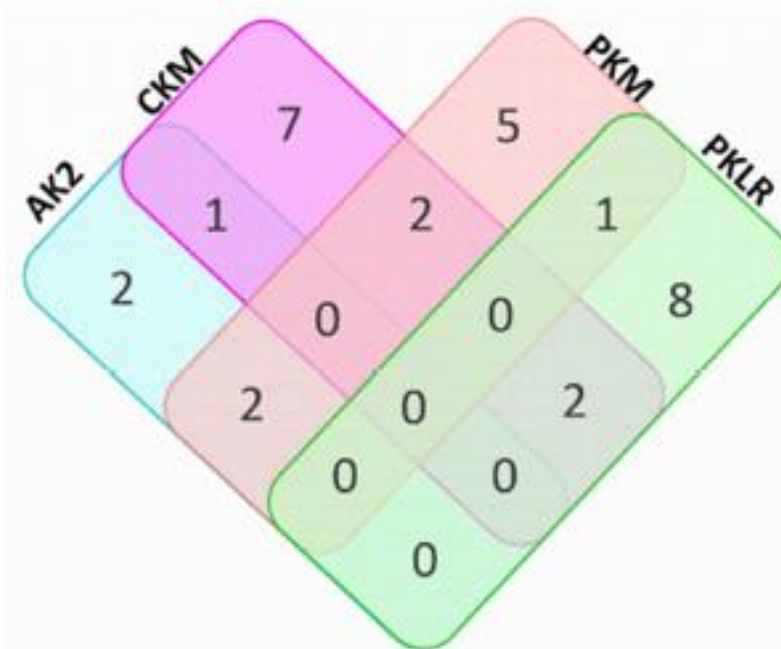
MTN-001

142 ppts typed
6% LOF variations (pred*)



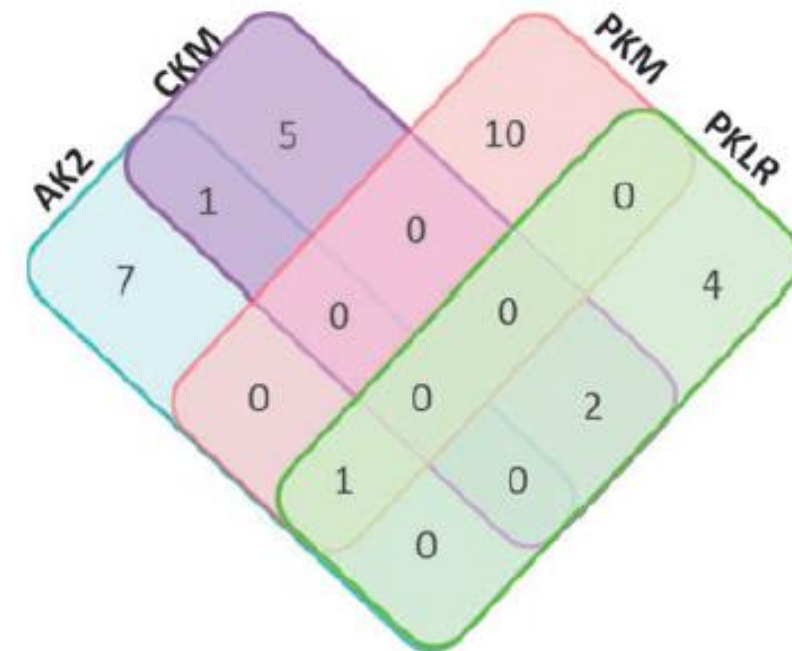
HPTN 067

505 ppts typed
4% LOF variations (pred)



HPTN 069

498 ppts typed
7% LOF variations (pred)



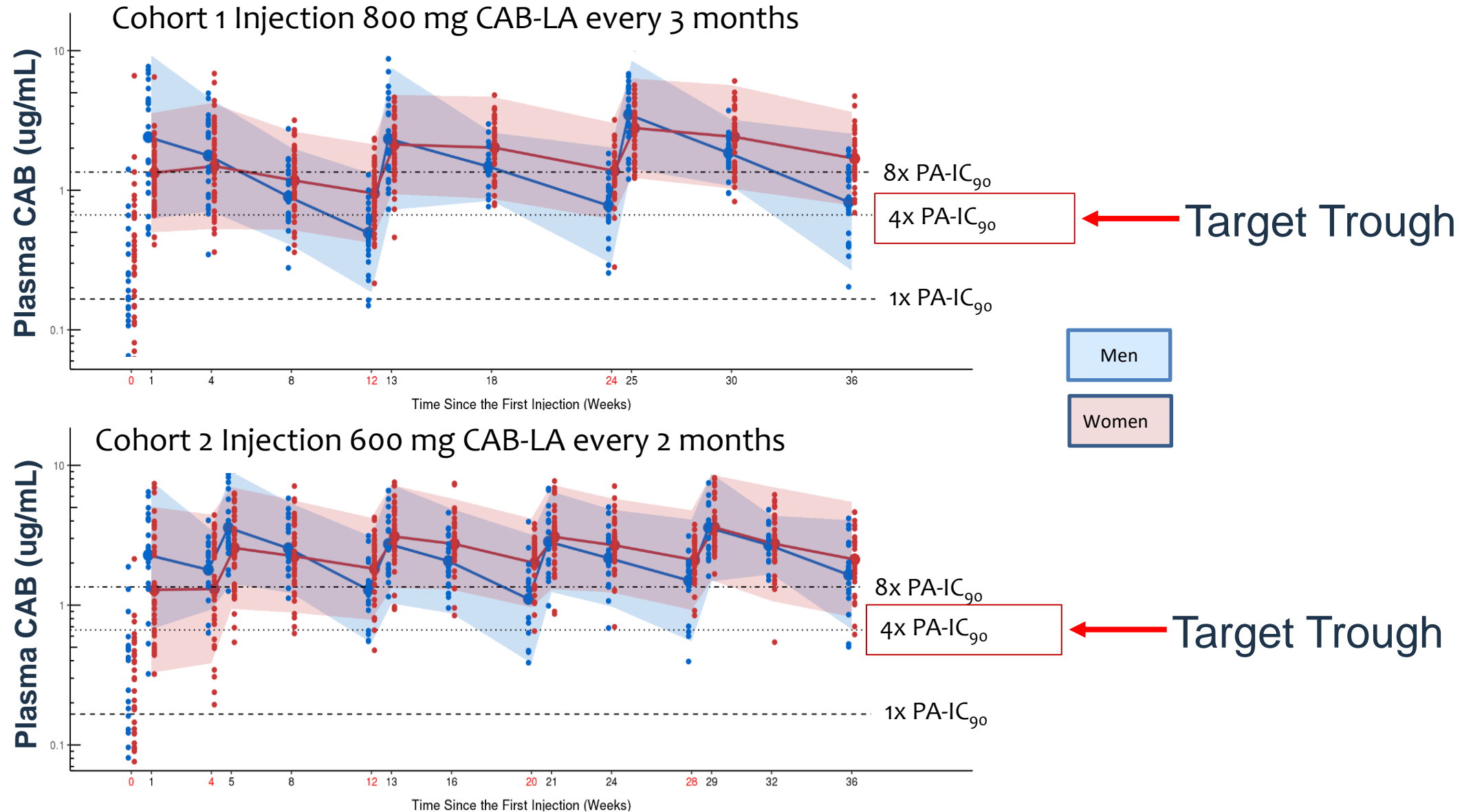
Lade. EBioMedicine 2015 Jul 9;2(9):1145-52

Figuroa. PLoS One. 2018 Apr 11;13(4):e0195764

Figuroa ARHR 2018 May;34(5):421-429

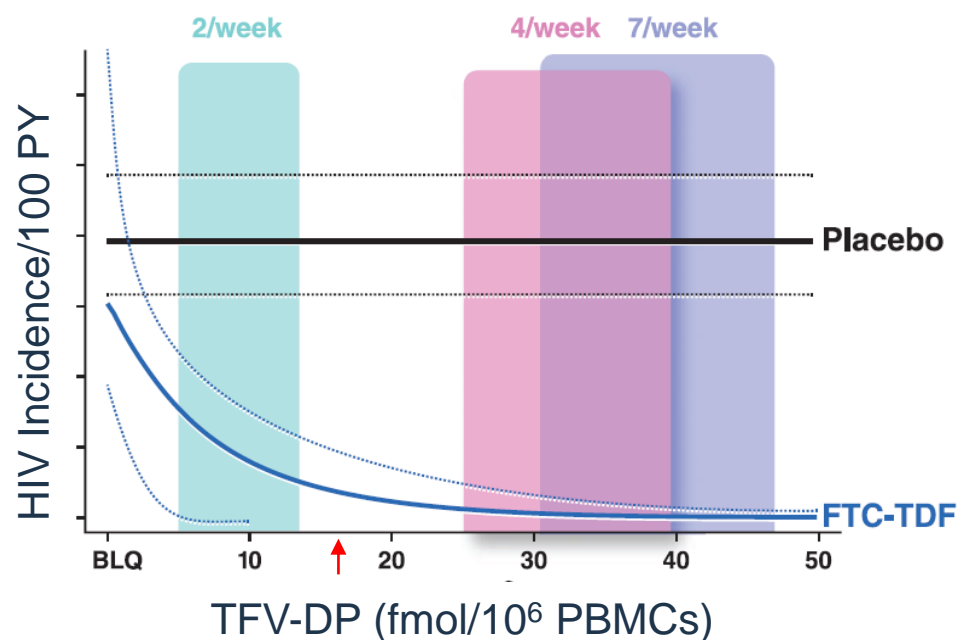
*Next Gen sequencing, loss of function (LOF) predictions based on sequence using SIFT & Polyphen

Phase II HPTN 077 Cabotegravir PK



Phase III Trials: Concentration-Response

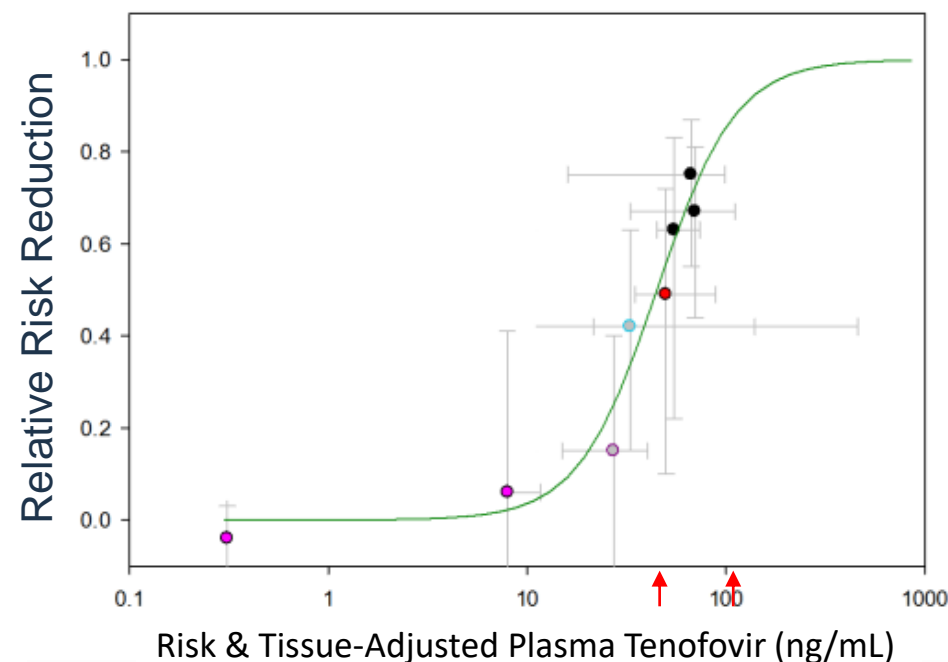
Within Study: iPrEx
Oral Route – Rectal Risk



Controlling for selected covariates
IC₉₀ 16 fmol/10⁶ PBMC

Anderson, *et al.*, Sci Trans Med 2012

Among Studies
All Routes – All Risks

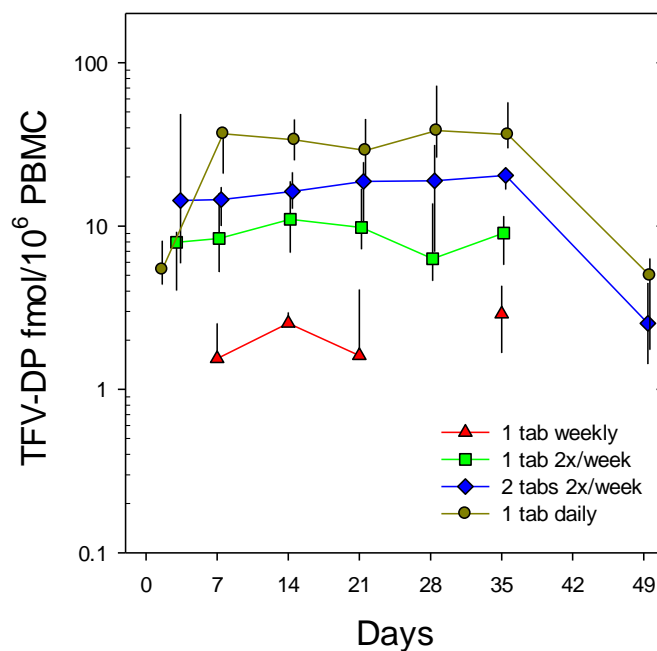


Parameter	Estimate	CV%
E _{max}	0.94	44
EC ₅₀	43	44
EC ₉₀	107	44
Gamma	2.4	56

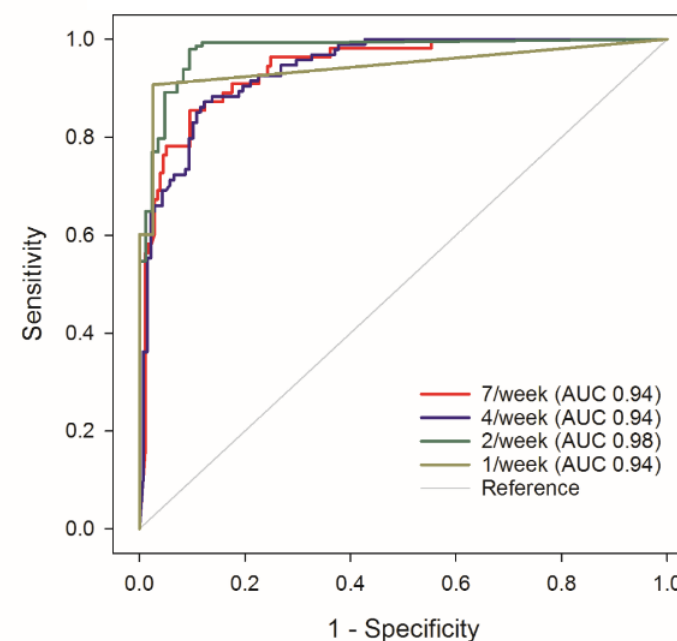
Hendrix, Cell 2013

Phase III Trials: Adherence Assessment

PBMC TFV-DP



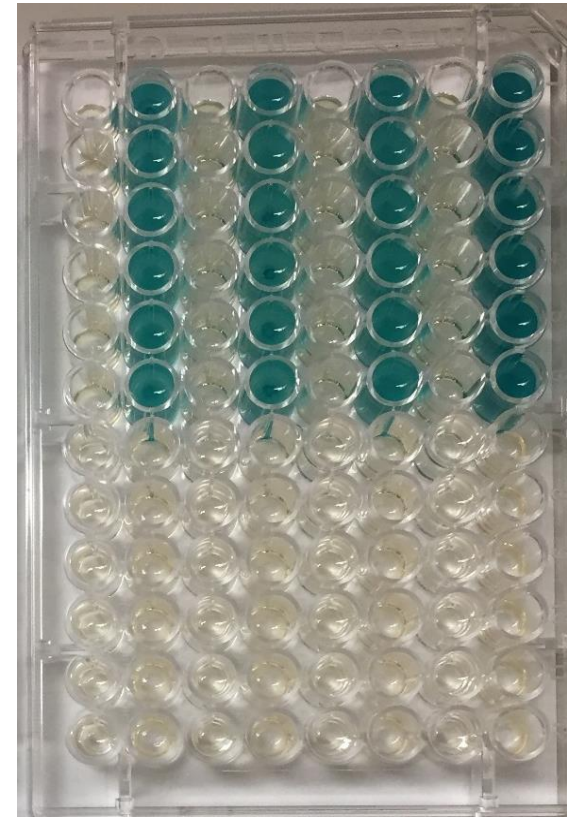
ROC PBMC TFV-DP



Matrix - Analyte	Optimized Cut-off Concentration* ($\geq 90\%$ sensitivity, specificity)			
	7 doses/week	≥ 4 doses/week	≥ 2 doses/week	≥ 1 doses/week
PBMC TFV-DP (fmol/10 ⁶ cells)	16.8 (0.91, 0.82)	9.9 (0.90, 0.80)	5.2 (0.91, 0.93)	1.6 (0.90, 0.97)
PBMC FTC-DP (pmol/10 ⁶ cells)	0.9 (0.91, 0.92)	0.4 (0.90, 0.80)	0.2 (0.91, 1.00)	0.1 (0.90, 1.00)

TFV Enema Tip: Coliform testing

- Gram-negative coliform bacteria in the stool of warm-blooded animals
- Growth enrichment medium w/ color indicator (Coliplate™, Bluewater Biosciences)
- Beta-D-glucosidase produced by coliforms interact with X-gal, yielding a colorimetric product



LC Pharmacology Summary

- Combines expertise in analytical & clinical pharmacology
- Assay development & protocol support to PrEP drug development efforts
- Novel methods developed to deal with special needs of topical PrEP
- Applications:
 - Pre-clinical PK-PD testing to optimize products for clinical study
 - Phase I PK: identify regimen to achieve target concentration & location
 - Phase II Extended Safety: scalability of PK & *ex vivo* PK-PD studies
 - Phase III Efficacy:
 - Concentration-response analysis informs future trials
 - Adherence assessment aids interpretation & may improve PrEP efficacy

ACKNOWLEDGEMENTS

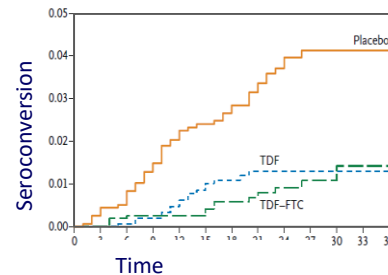
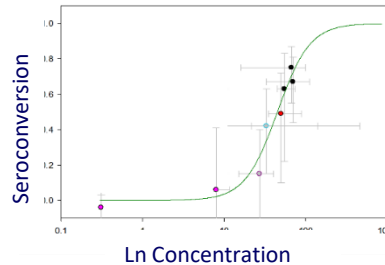
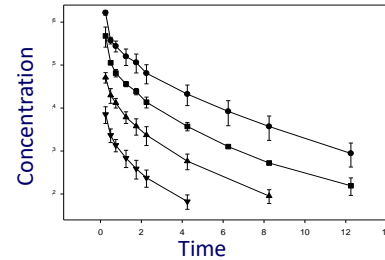
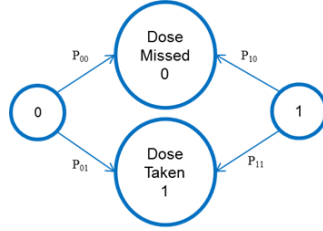
The HIV Prevention Trials Network is funded by the National Institute of Allergy and Infectious Diseases (UM1AI068619, UM1AI068613, UM1AI1068617), with co-funding from the National Institute of Mental Health, and the National Institute on Drug Abuse, all components of the U.S. National Institutes of Health.

LC Pharmacology Network Support

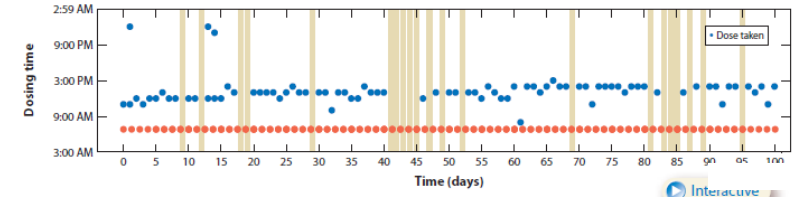
Study	Status	Sample Type	Drug Analytes	Testing Lab Name	Assay Status
HPTN-066	Closed	Plasma	TFV, FTC	JHU CPAL (Marzinke)	completed
		Seminal Plasma	TFV, FTC	JHU CPAL (Marzinke)	completed
		CVF	TFV, FTC	JHU CPAL (Marzinke)	completed
		RF	TFV, FTC	JHU CPAL (Marzinke)	completed
		Tissue	TFV, FTC, TFV-DP, FTC-TP	JHU CPAL (Marzinke)	completed
HPTN-067	Closed	Plasma	TFV, FTC	JHU CPAL (Marzinke)	completed
HPTN-069	Closed	Plasma	TFV, FTC, MVC	JHU CPAL (Marzinke)	completed
		CVF (swab)	TFV, FTC, MVC	JHU CPAL (Marzinke)	completed
		RF (sponge)	TFV, FTC, MVC	JHU CPAL (Marzinke)	completed
		Tissue	TFV, FTC, MVC, TFV-DP	JHU CPAL (Marzinke)	completed
HPTN-073	Closed	Plasma	TFV, FTC	JHU CPAL (Marzinke)	completed
HPTN-076	Closed	Plasma	RPV	JHU CPAL (Marzinke)	completed
		Tissue	RPV	JHU CPAL (Marzinke)	completed
		RF (swab)	RPV	JHU CPAL (Marzinke)	completed
		CVF (swab)	RPV	JHU CPAL (Marzinke)	completed
HPTN-077	Open	Plasma	CBV	JHU CPAL (Marzinke)	in testing phase
HPTN-082	Open	Plasma	TFV, FTC	JHU CPAL (Marzinke)	pending
HPTN-083	Open	Plasma	TFV, FTC, CBV	JHU CPAL (Marzinke)	in testing phase
HPTN 084	Open	Plasma	TFV, FTC, CBV	JHU CPAL (Marzinke)	In testing phase
HPTN-086	In Development	Plasma	CBV	JHU CPAL (Marzinke)	pending

Phase III: Clinical Trial Simulation

- Build models at each stage of development
- Link models across concentration, response, & time
- Compare competing study designs
- Explore “what if?” scenarios to optimize clinical trial design



- Adherence



- Population Pharmacokinetics Model

$$C_e = \frac{D}{V_c} \cdot e^{-k_{et}t}$$

- Pharmacodynamics Model

$$E = \frac{E_{max} \cdot C^\gamma}{EC_{50}^\gamma + C^\gamma}$$

- Disease Progression Model

$$S(t) = S_0 + [E(C) + \alpha] \cdot t$$