

Intracellular Metabolism of Tenofovir Alafenamide in Cervical and Vaginal Epithelial Cells

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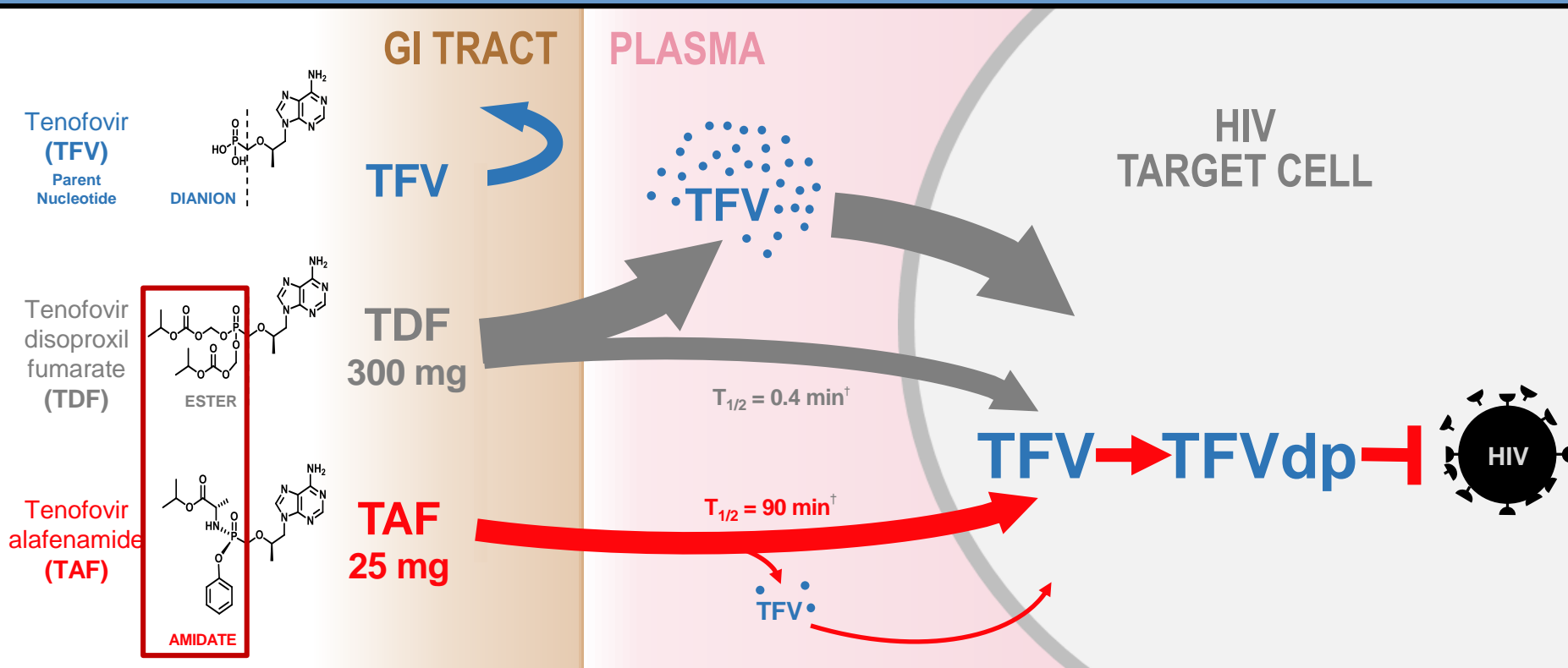
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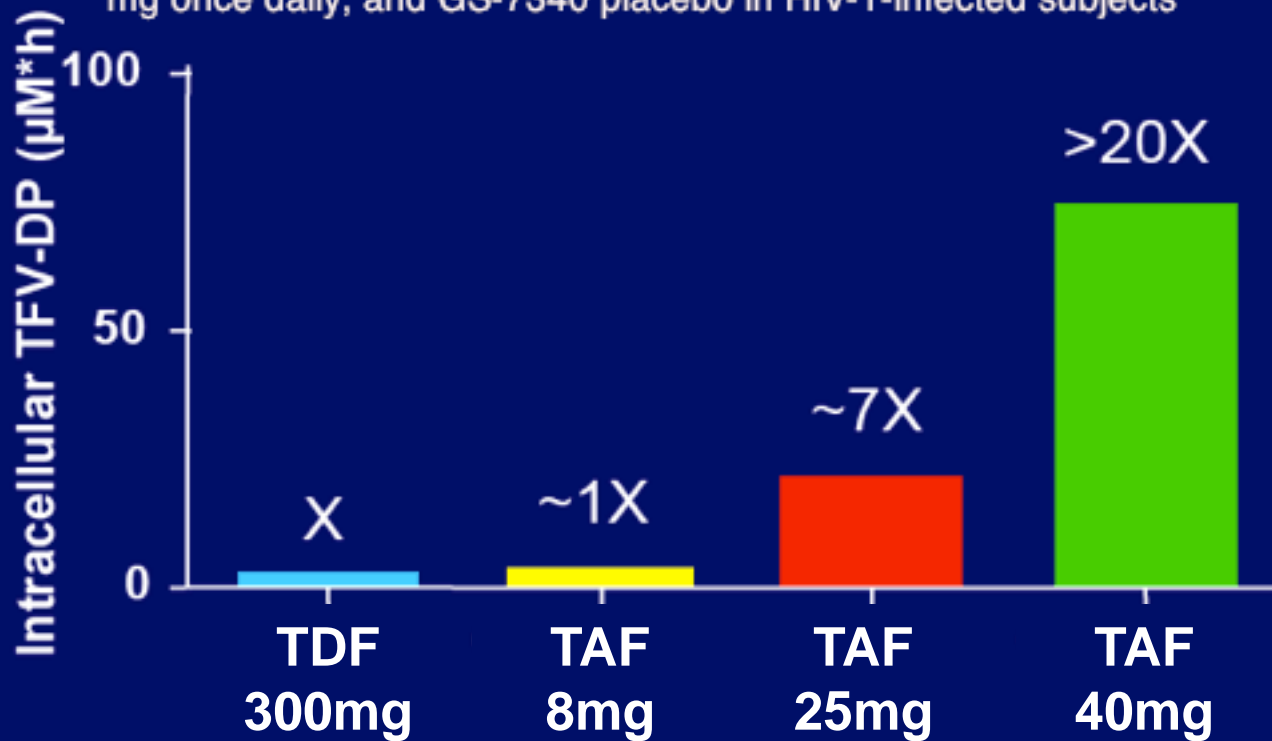
TAF Exhibits Favorable Pharmacology



Adapted from Gupta S. IAS 2015 Oral Abstract #TUAB0103.

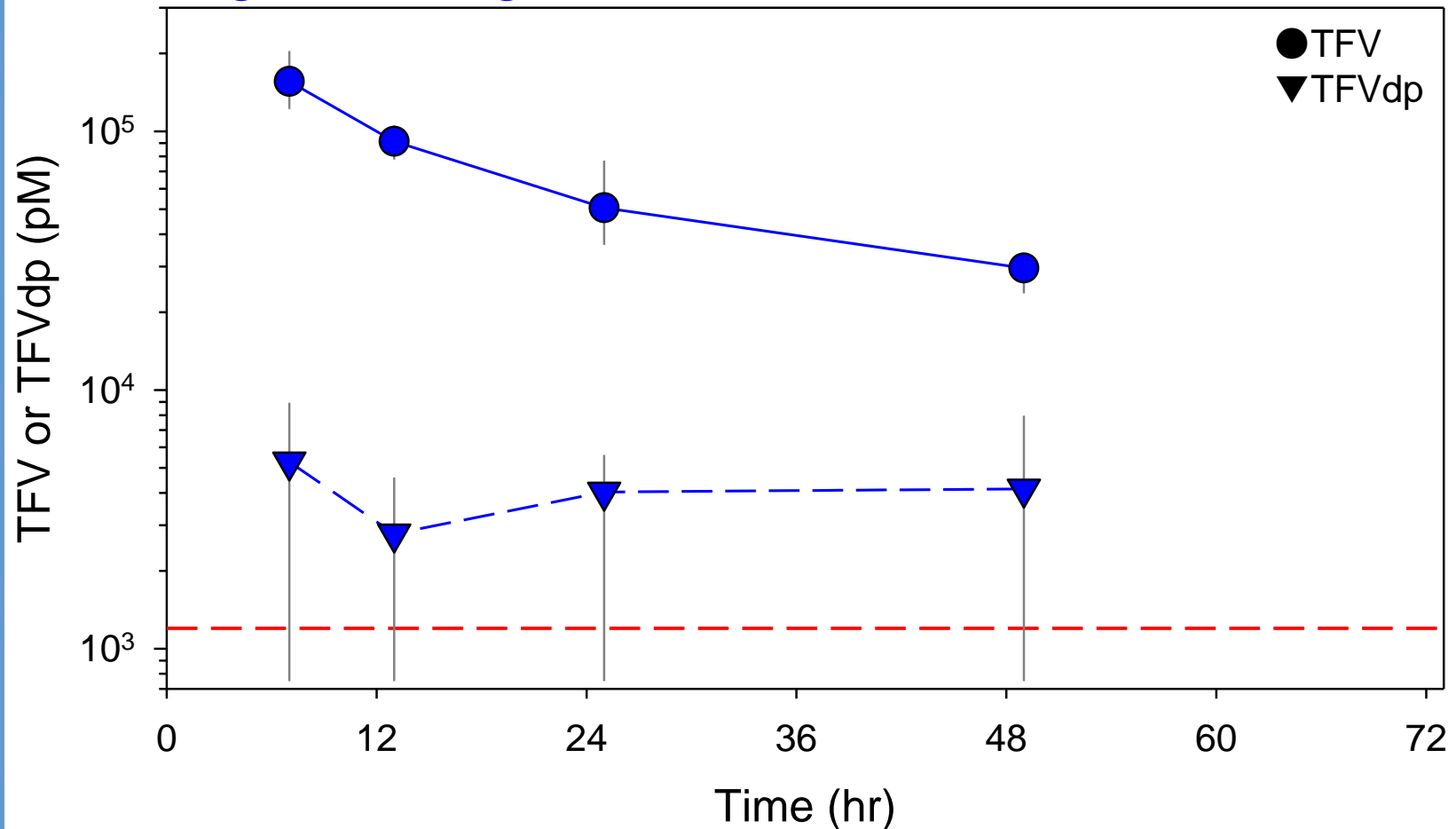
TAF Achieves Higher TFVdp in PBMCs

Methods: 10-day monotherapy study compared GS-7340 8, 25, and 40 mg once daily to TDF 300 mg once daily, and GS-7340 placebo in HIV-1-infected subjects



TFVdp is Lower in FGT Tissues for TAF

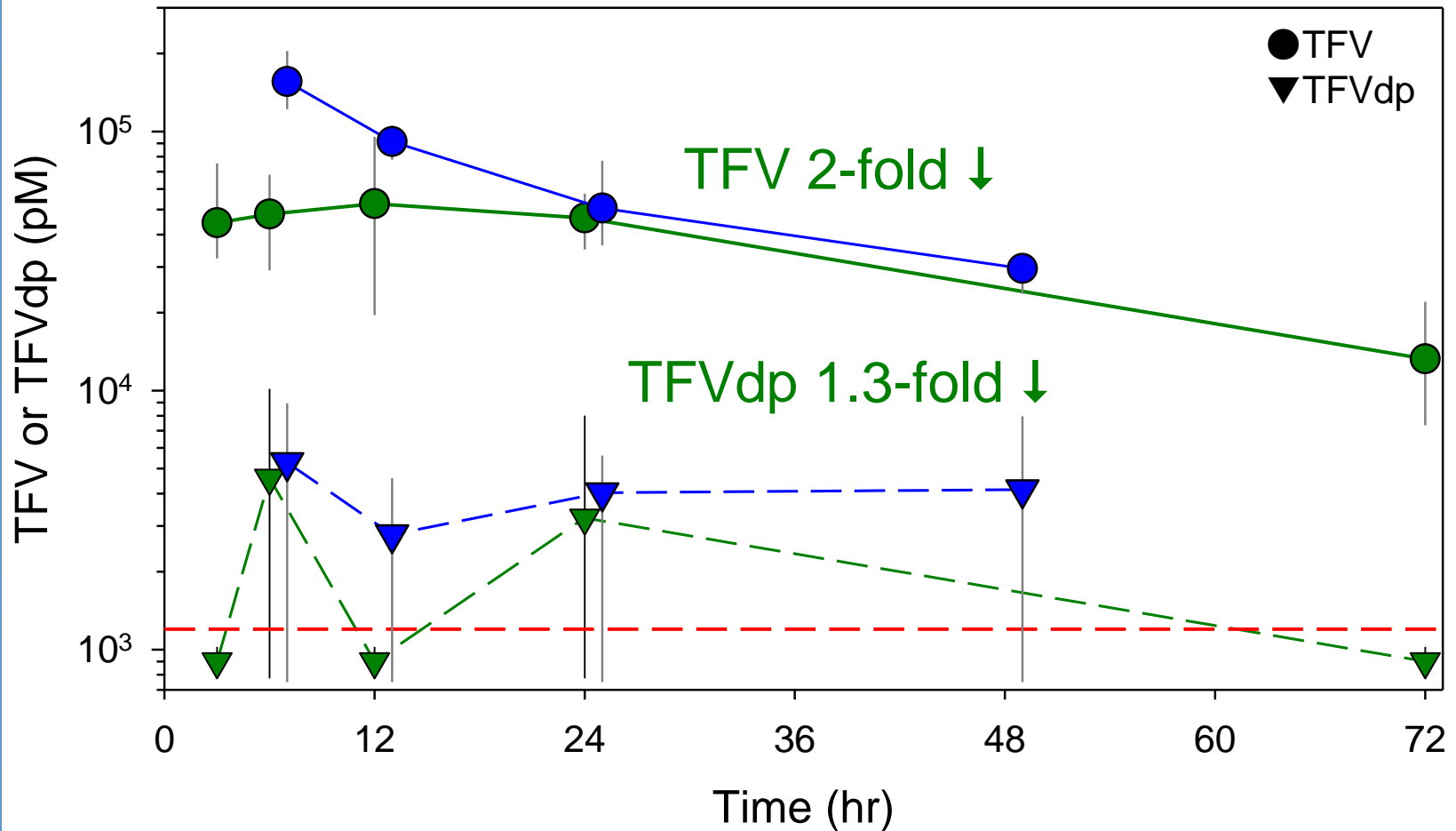
300mg TDF single dose



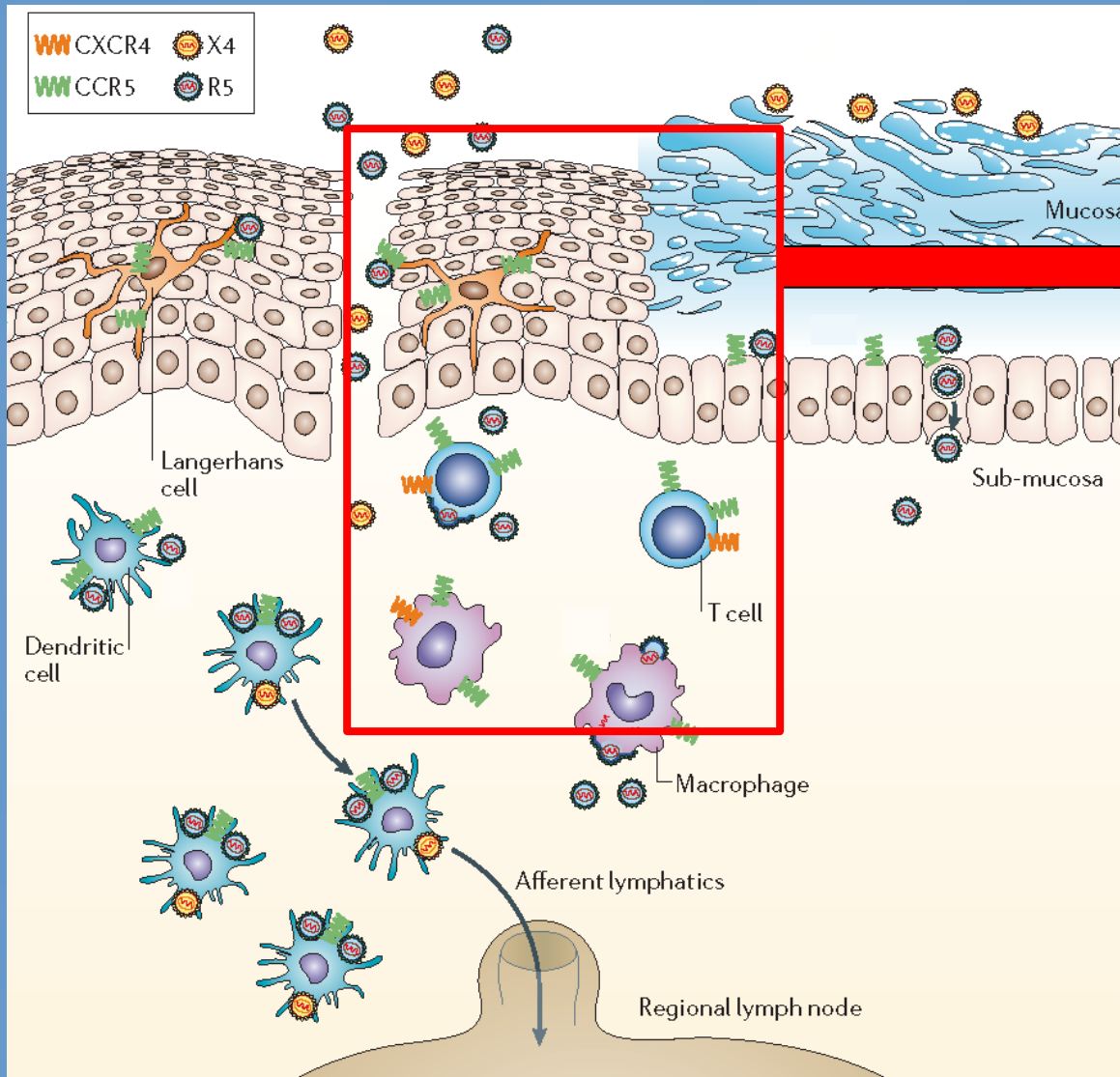
TFVdp is Lower in FGT Tissues for TAF

300mg TDF single dose

25mg TAF single dose

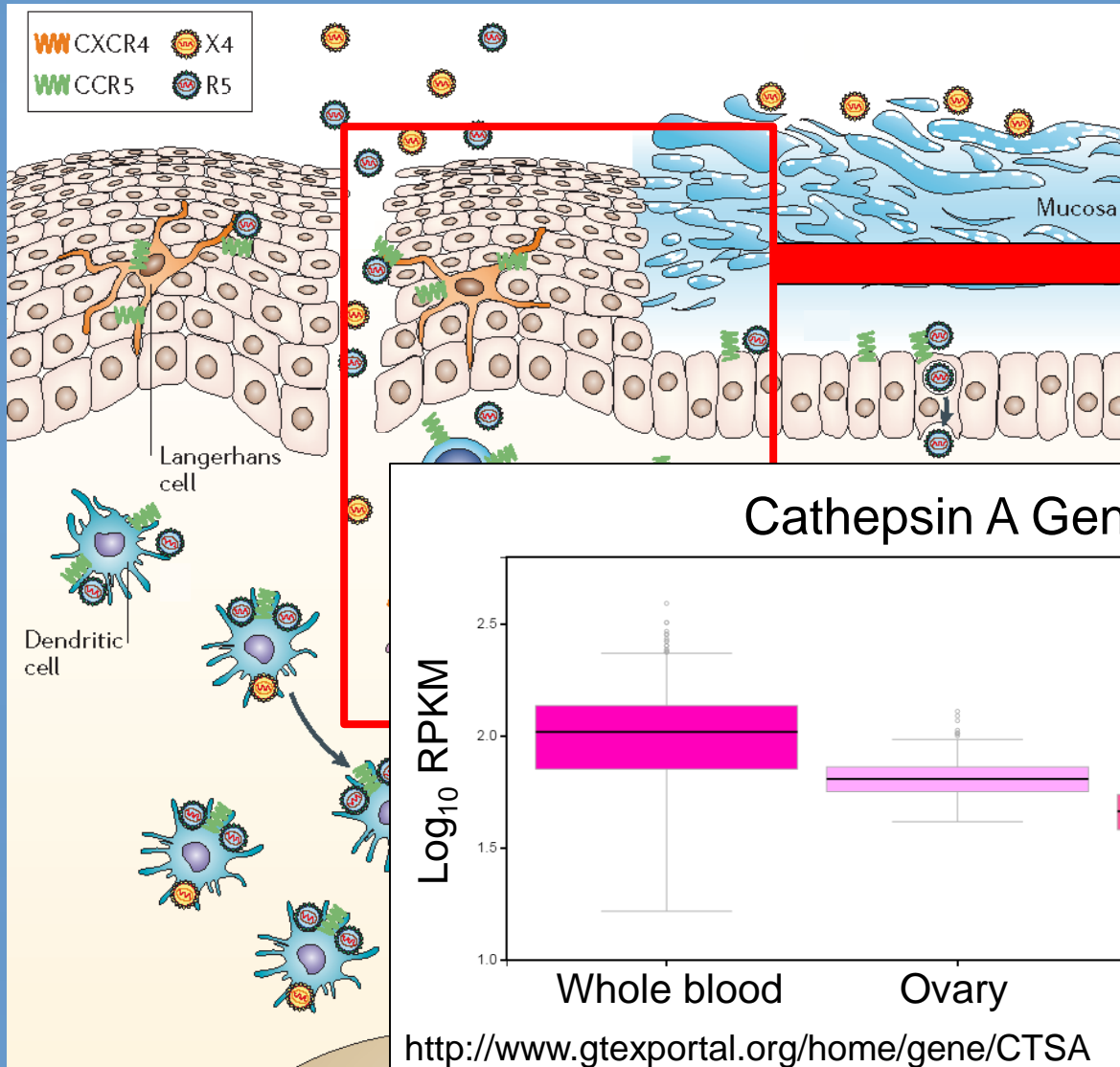


Mucosal Tissue Microanatomy



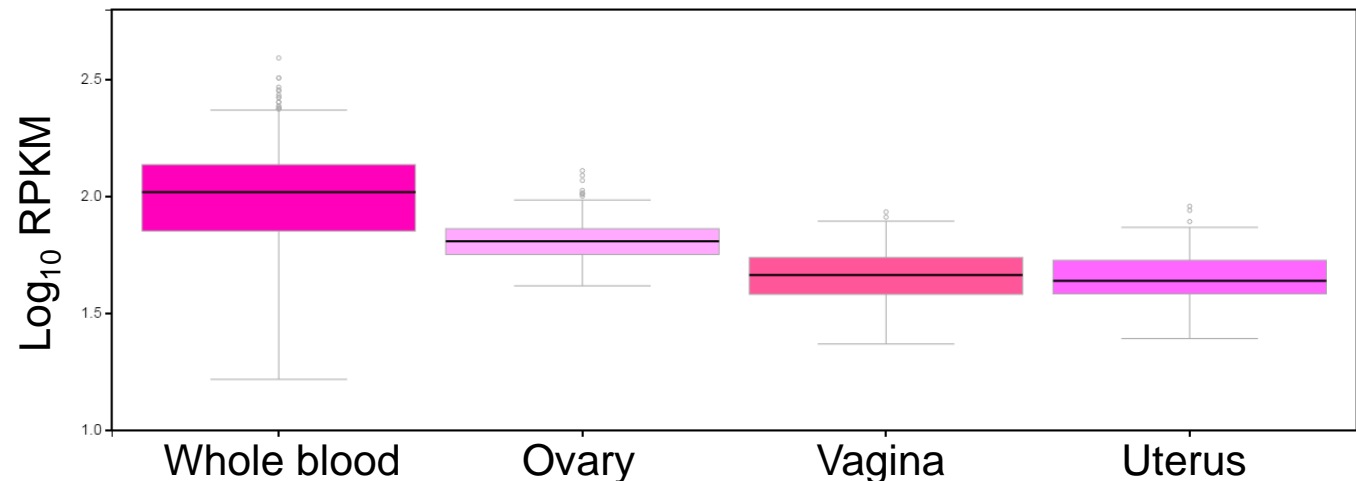
Langerhans Cells
Dendritic Cells
Macrophages
T-Lymphocytes
Epithelial Cells

Mucosal Tissue Microanatomy



Langerhans Cells
Dendritic Cells
Macrophages
T-Lymphocytes
Epithelial Cells

Cathepsin A Gene Expression



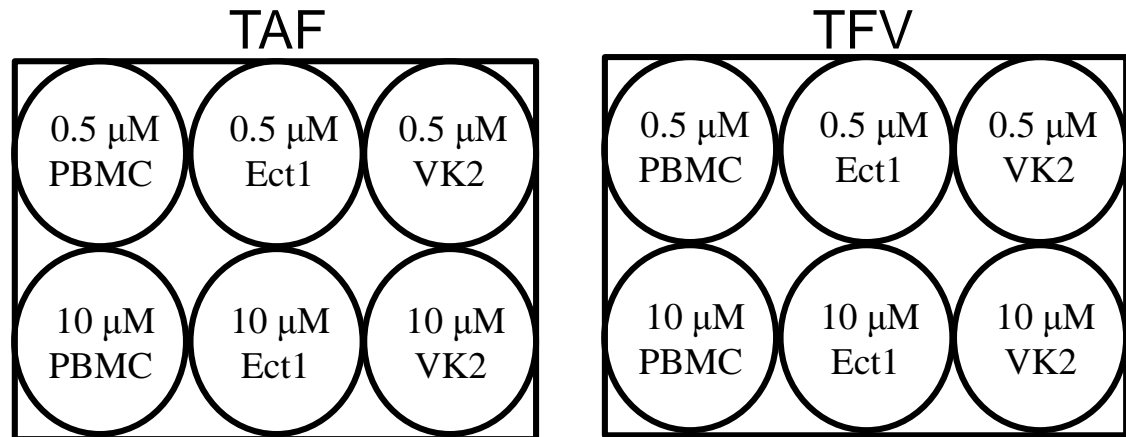
<http://www.gtexportal.org/home/gene/CTSA>

Hypothesis: TFVdp will be
lower in epithelial cells dosed
with TAF vs TFV

In Vitro Approach

Cells: Freshly isolated PBMCs (single healthy volunteer*),
Ect1/E6E7 ectocervical and VK2/E6E7 vaginal cell lines (ATCC®)

Treat with:



Harvest at:



Count on: Muse™ Cell Analyzer

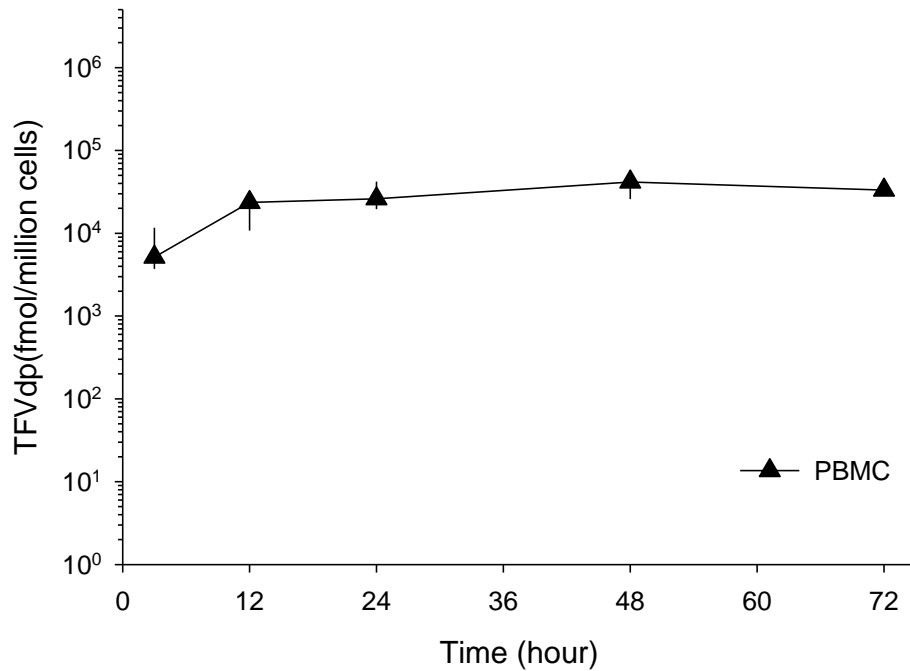
Lyse in: 70:30 methanol:water

Analytical Methods

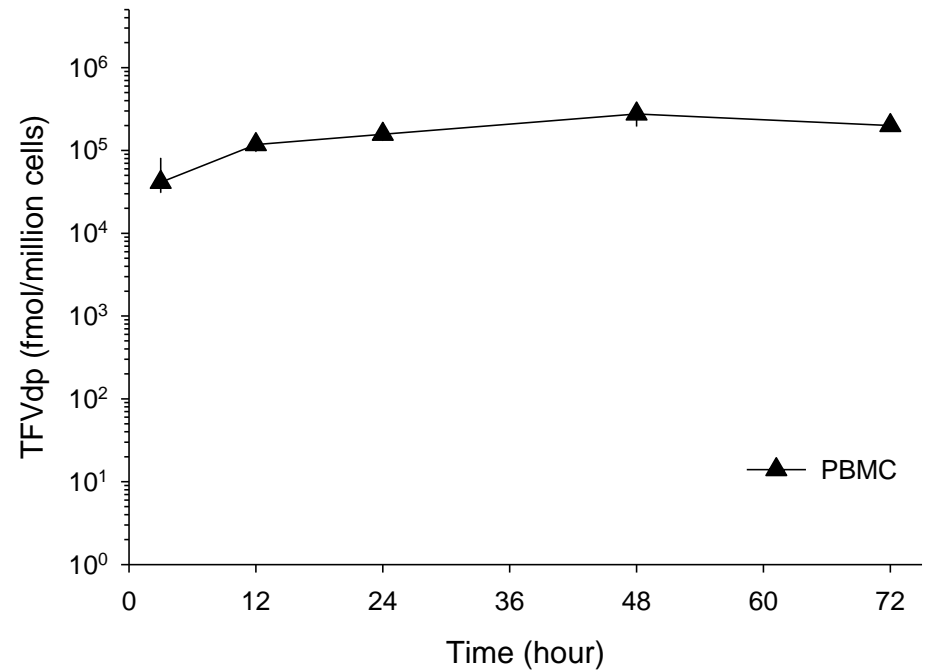
- LC-MS/MS TFVdp quantification: Calibrated range=0.02-500 ng/ml cell lysate
- Statistical Analysis
 - BLQ values imputed at $\frac{1}{2}$ the LLOQ
 - Median area under the concentration time curve (AUC) calculated by linear trapezoidal rule with sparse sampling function in WinNonlin v6.3
 - Pearson's correlation performed for dose-normalized \log_{10} transformed matched samples with SAS v9.3

TFVdp Median (Min, Max) Concentration vs Time: TAF (solid)

0.5 μ M Dosing

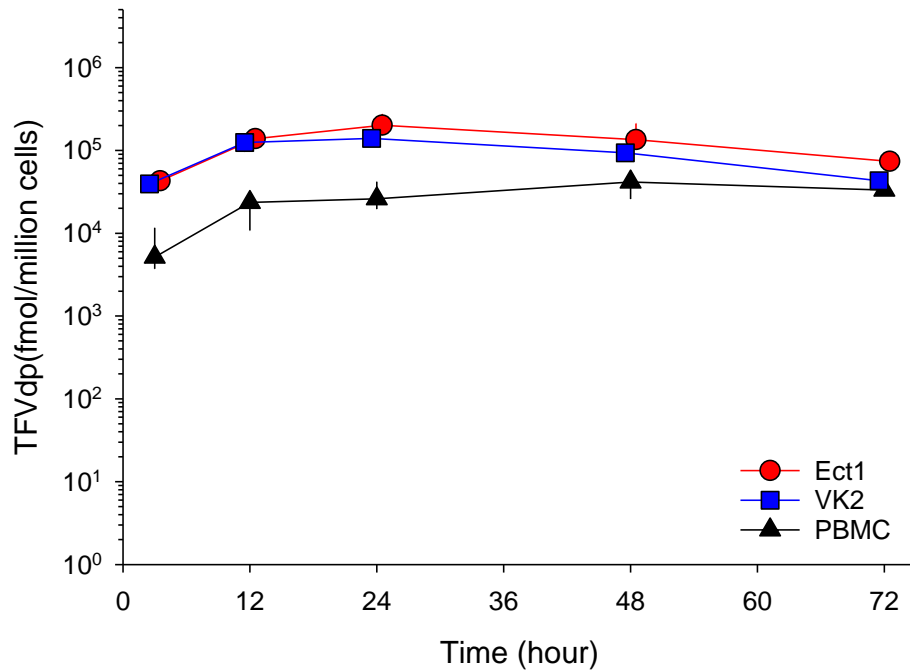


10 μ M Dosing

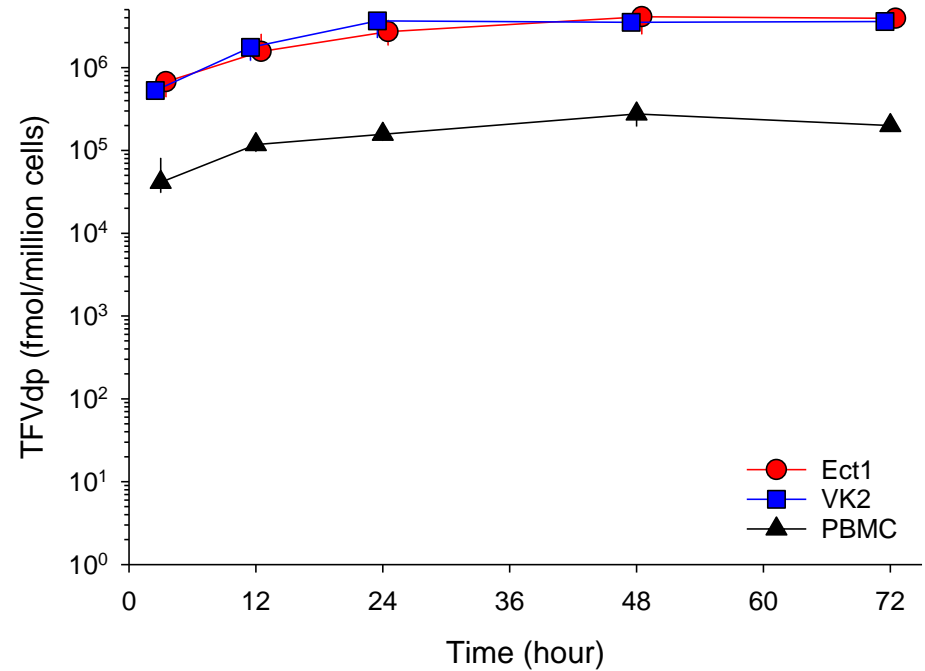


TFVdp Median (Min, Max) Concentration vs Time: TAF (solid)

0.5 μ M Dosing

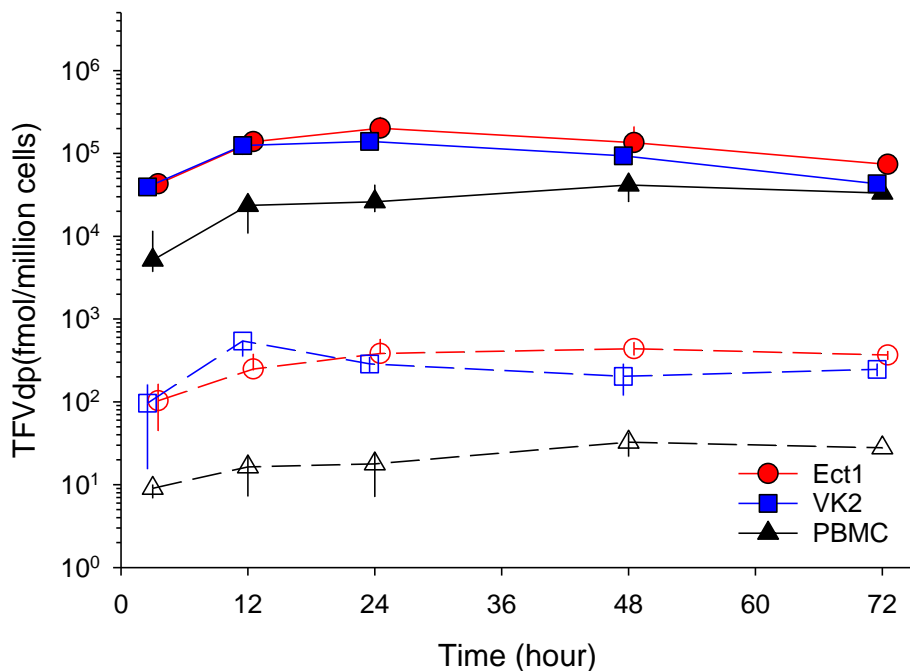


10 μ M Dosing

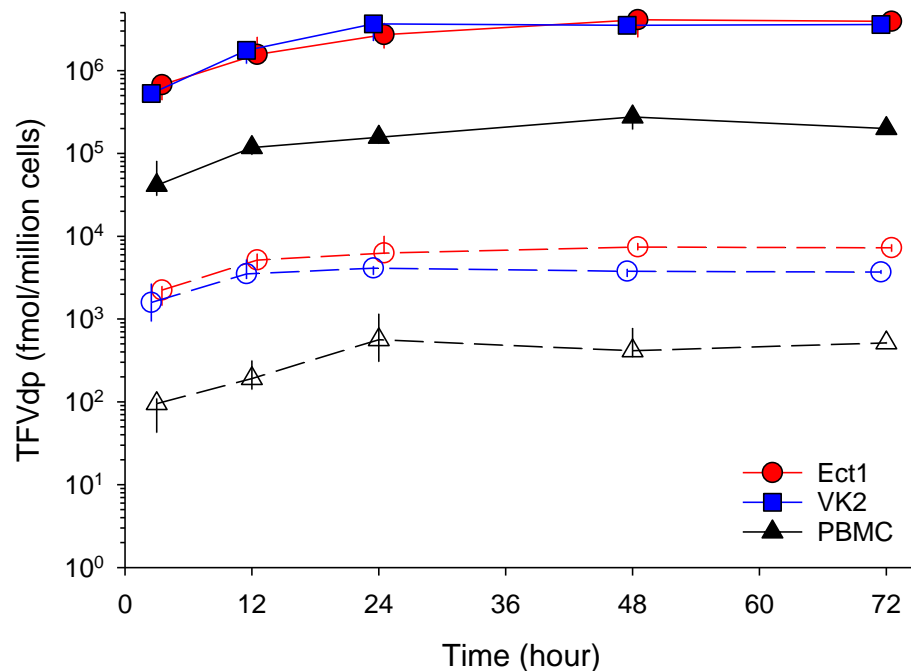


TFVdp Median (Min, Max) Concentration vs Time: TAF (solid) & TFV (dashed)

0.5 μ M Dosing



10 μ M Dosing



TAF AUC_{0-72hr} 192-1305 fold **Higher** compared to TFV

TFVdp AUC_{0-72hr}

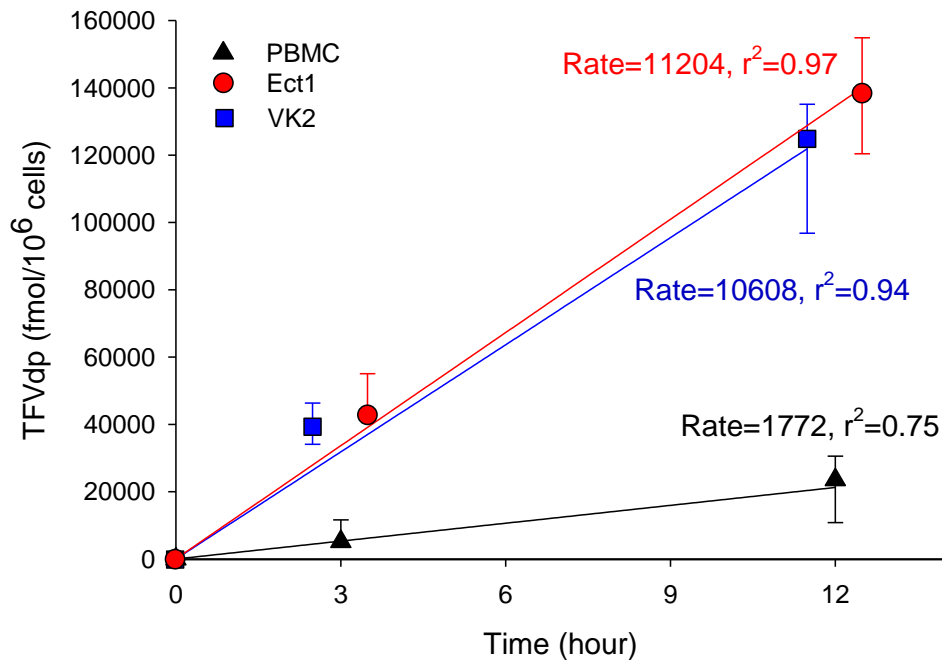
Cell Type	Dose μM	<u>TAF</u>	<u>TFV</u>
		Mean AUC (SE) pmol*hr/million cells	Mean AUC (SE) pmol*hr/million cells
PBMC	0.5	2122 (209)	1.63 (0.173)
	10	12646 (702)	33.5 (5.61)
Ect1	0.5	10037 (989)	26.7 (1.78)
	10	210382 (18701)	469 (28.1)
VK2	0.5	3587 (826)	18.6 (1.60)
	10	60604 (35752)	256 (11.1)

TAF: PBMC 1.7-16.6 fold **Lower** vs Epithelial

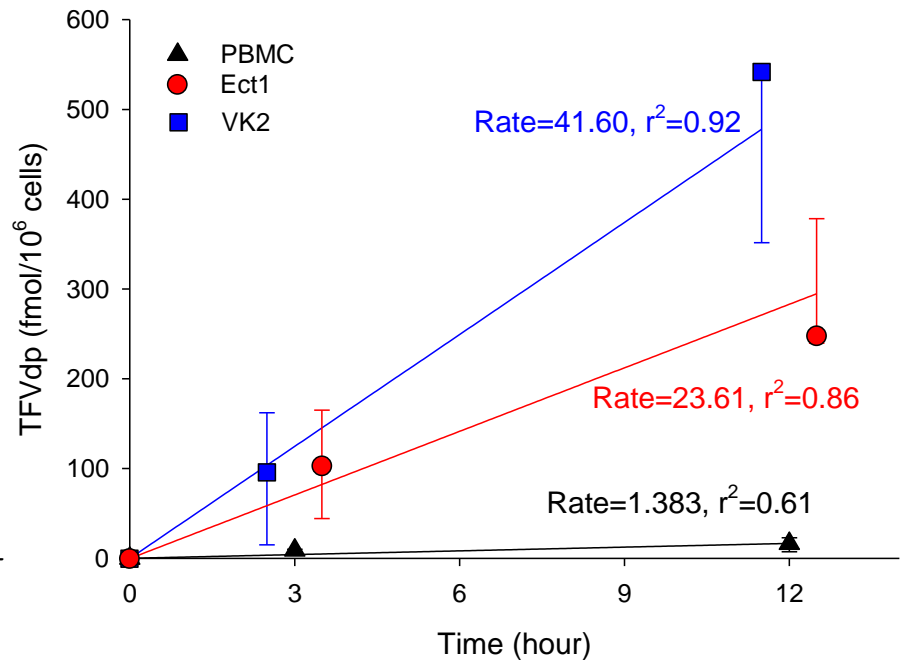
TFV: PBMC 7.6-16.4 fold **Lower** vs Epithelial

TFVdp Rate of Accumulation

0.5 μ M TAF



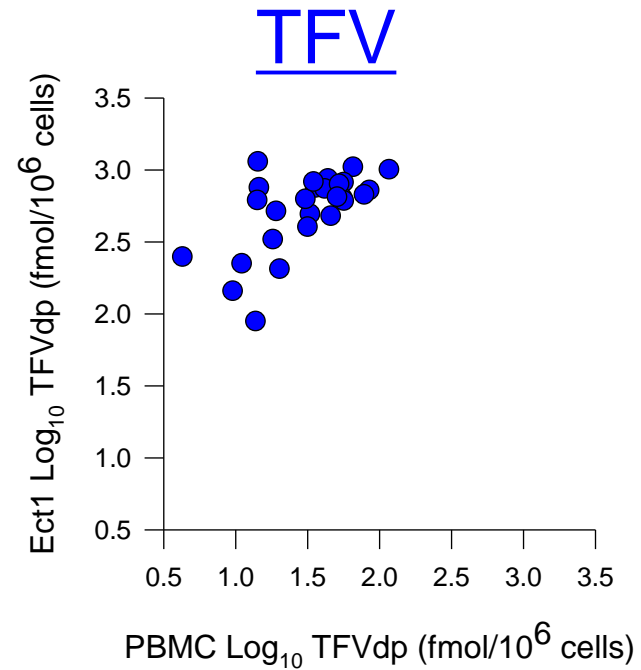
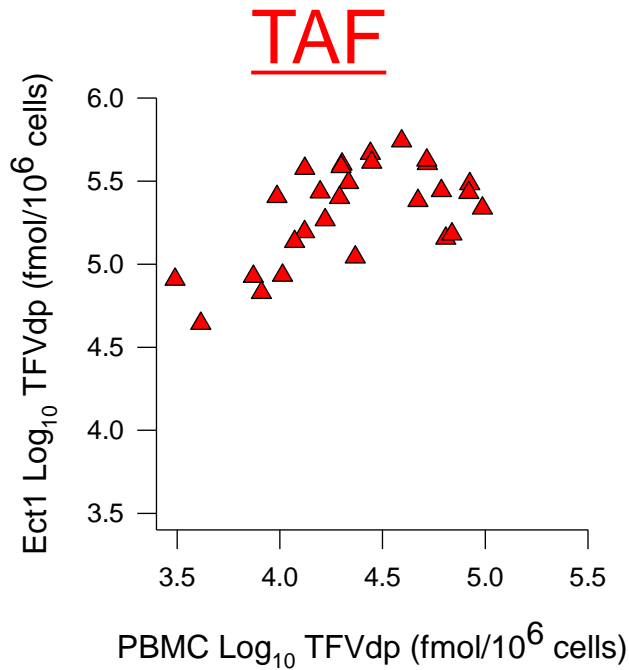
0.5 μ M TFV



Epithelial cells: 6
Times Faster

Epithelial cells: 17-30
Times Faster

TFVdp Correlates Between Cell Types



Pearson's r

	Ect1	VK2	PBMC
Ect1	1	0.71 [†]	0.64*
VK2	0.90 [†]	1	0.45*
PBMC	0.58*	0.42*	1

*p<0.05; †p<0.001

Limitations

- Static exposure over 72 hours
- Immortalized cells exhibit altered phenotypes
 - Ect1 TFVdp ~1.7-fold higher compared to primary ectocervical cells¹

Summary and Conclusions

- TFVdp was HIGHER with matched TAF vs TFV dosing
 - >100-fold \uparrow AUC_{0-72hr} in PBMCs, Ect1 and VK2 cells
- TFVdp was HIGHER in epithelial cells vs PBMCs
 - VK2 \geq 1.7 fold \uparrow AUC_{0-72hr}
 - Ect1 \geq 4.7 fold \uparrow AUC_{0-72hr}
- TFVdp was significantly correlated among cell types
 - Ect1 vs PBMC TFVdp $r^2=0.58$, $p<0.05$
 - VK2 vs PBMCs TFVdp $r^2=0.42$, $p<0.05$
- Presence of epithelial cells within tissue homogenates does not explain previous observations of low TFVdp concentrations in tissue biopsies from women dosed with TAF.