Intracellular Metabolism of Tenofovir Alafenamide in Cervical and Vaginal Epithelial Cells

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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.

Ruane P. CROI 2012 Oral Abstract #103.
TFVdp is Lower in FGT Tissues for TAF

300mg TDF single dose

Garrett K. CROI 2016 Oral Abstract #102LB.
TFVdp is Lower in FGT Tissues for TAF

Garrett K. CROI 2016 Oral Abstract #102LB.
Mucosal Tissue Microanatomy

Langerhans Cells
Dendritic Cells
Macrophages
T-Lymphocytes
Epithelial Cells

Adapted from Margolis L. 2006 Nature Reviews Microbiology.
Mucosal Tissue Microanatomy

Langerhans Cells
Dendritic Cells
Macrophages
T-Lymphocytes
Epithelial Cells

Cathepsin A Gene Expression

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

Adapted from Margolis L. 2006 Nature Reviews Microbiology.
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®)

<table>
<thead>
<tr>
<th>Treat with:</th>
<th>TAF</th>
<th>TFV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 μM PBMC</td>
<td>0.5 μM Ect1</td>
<td>0.5 μM VK2</td>
</tr>
<tr>
<td>10 μM PBMC</td>
<td>10 μM Ect1</td>
<td>10 μM VK2</td>
</tr>
</tbody>
</table>

Harvest at: 3 hr, 12 hr, 24 hr, 48 hr, 72 hr

Count on: Muse™ Cell Analyzer

Lyse in: 70:30 methanol:water

*UNC Center for AIDS Research Sample Collection Protocol IRB 08-0047
Analytical Methods

- LC-MS/MS TFVdp quantification: Calibrated range=0.02-500 ng/ml cell lysate
- Statistical Analysis
  - BLQ values imputed at ½ 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)

0.5μM Dosing

10μM Dosing
TFVdp Median (Min, Max) Concentration vs Time: TAF (solid)

0.5μM Dosing

10μM Dosing
TFVdp Median (Min, Max) Concentration vs Time: TAF (solid) & TFV (dashed)

TAF AUC\textsubscript{0-72hr} 192-1305 fold Higher compared to TFV
TFVdp AUC\(_{0-72hr}\)

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

TAF: PBMC 1.7-16.6 fold Lower vs Epithelial
TFV: PBMC 7.6-16.4 fold Lower vs Epithelial
TFVdp Rate of Accumulation

0.5μM TAF

Epithelial cells: 6 Times Faster

0.5μM TFV

Epithelial cells: 17-30 Times Faster
TFVdp Correlates Between Cell Types

Pearson’s r

<table>
<thead>
<tr>
<th></th>
<th>Ect1</th>
<th>VK2</th>
<th>PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ect1</td>
<td>1</td>
<td>0.90†</td>
<td>0.58*</td>
</tr>
<tr>
<td>VK2</td>
<td>0.90†</td>
<td>1</td>
<td>0.42*</td>
</tr>
<tr>
<td>PBMC</td>
<td>0.58*</td>
<td>0.42*</td>
<td>1</td>
</tr>
</tbody>
</table>

*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 cells

\(^1\text{Shen Z. 2014 PlosOne.}\)
Summary and Conclusions

- TFVdp was HIGHER with matched TAF vs TFV dosing
  - >100-fold ↑ $\text{AUC}_{0-72\text{hr}}$ in PBMCs, Ect1 and VK2 cells
- TFVdp was HIGHER in epithelial cells vs PBMCs
  - VK2 ≥ 1.7 fold ↑ $\text{AUC}_{0-72\text{hr}}$
  - Ect1 ≥ 4.7 fold ↑ $\text{AUC}_{0-72\text{hr}}$
- 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.

This work was supported in part by the Centers for AIDS Research (Grant P30 AI50410) and the John A. and Deborah S. McNeill Jr. Distinguished Professorship