Antiviral Activity of Tenofovir Alafenamide (TAF) against HIV-1 Subtypes and Emergence of K65R

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(See Cox et al - Poster O-07)
Tenofovir Alafenamide (TAF)

TAF:
- novel prodrug of the HIV-1 NRTI tenofovir (TFV)
- requires a lower dose (vs. TDF)
- has greater plasma stability (vs. TDF)
- reduces circulating TFV
- improves the delivery of intracellular TFV
TAF Antiviral Activity

- Panel of HIV-1 and HIV-2 primary isolates tested in PBMCs (~ 3 each).
- TAF is active against all HIV-1 and HIV-2 isolates tested
- TAF mean EC$\text{}_{50} = 3.5$ nM (from 29 HIV-1 isolates tested in PBMCs)

From Callebaut et al AAC, 2015
Clinical Data & Resistance

- TAF has been co-formulated with other ARVs:
  - with FTC, EVG, COBI $\rightarrow$ E/C/F/TAF
  - with FTC, RPV $\rightarrow$ R/F/TAF
  - with FTC $\rightarrow$ F/TAF

- E/C/F/TAF: Clinical Studies conducted worldwide
  - More than 14 subtypes represented in treated population
  - Tx-Naïve: B (86.1%), AE (7.3%), C (1.5%), AG (1.5%), all others (3.6%)

- E/C/F/TAF: Highest efficacy ever seen in treatment naïve population (>$90\%$)
  - Very low resistance observed:
    - 0.8\% by Week 48
    - 1.2\% by Week 96
K65R in HIV-1 Subtype C

Observation:
- Brenner et al., AIDS, 2006
  
  *HIV-1 subtype C rapidly develop K65R resistance to tenofovir in cell culture*

- In vitro TFV dose escalation selections
  - K65R:
    - Subtype B  \(\rightarrow\)  \(~55-75\) weeks
    - Subtype C  \(\rightarrow\)  \(~12\) weeks

- MOA associated with stretch of AAAAA in codons 64-65 in RT

Response:
- Miller et al., AIDS, 2006
  
  *K65R development among subtype C HIV-1-infected patients in tenofovir DF clinical trials*

- In vitro TFV dose escalation selections
  - K65R:
    - Subtype B  \(\rightarrow\)  \(~8-16\) weeks

- HIV-1-infected patients in tenofovir DF
  - Phase 3 Clinical Studies:
    - (Study 903, Study 934)
    - No K65R in patients w/ subtype C
“Updated” Response

- **In vitro**
  - Selection of virus panel: subtypes B and C (64-65-66 in RT)
  - Panel Characterization
  - Dose-escalation Resistance Selection
  - Breakthrough Viral Growth (Resistance Barrier TAF vs. TFV)

- **Analysis of Clinical Studies**
  - Multi-study Database Analysis
## Genotypic & Phenotypic Characteristics of Viral Isolates

<table>
<thead>
<tr>
<th>Isolate ID</th>
<th>Subtype</th>
<th>RT Codons 64 - 65 - 66</th>
<th>Fold Change (EC$_{50}$ FC from Wild-Type Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MT-2 Assay</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TAF</td>
</tr>
<tr>
<td>1</td>
<td>B</td>
<td>AAG AAA AAA</td>
<td>1.1</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>AAG AAA AAA</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>AAG AAG AAA</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>B*</td>
<td>AAA AAG AAA</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>B*</td>
<td>AAA AAG AAA</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>AAA AAG AAG</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>C</td>
<td>AAA AAG AAG</td>
<td>1.1</td>
</tr>
<tr>
<td>9</td>
<td>C</td>
<td>AAA AAG AAG</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>C</td>
<td>AAA AAG AAG</td>
<td>1.0</td>
</tr>
</tbody>
</table>

B*: subtype B with RT codons at positions 64 and 65 that are identical to most prevalent subtype C isolates.
Dose Escalation Resistance Selection (Virus 1 – sub. B)
Dose Escalation Resistance Selection – All isolates

**Virus 1**

**Virus 2**

**Virus 3**

**Virus 4**

**Virus 5**

**Virus 6**

**Virus 7**

**Virus 8**

**Virus 9**

**Virus 10**
Summary of Resistance Selections

- Variability in the time to gain K65R upon repeat with the same isolates
- Trend for subtype C viruses to gain the K65R mutation more rapidly
- Similar trend for the B*viruses (same 64-65 motif as subtype C)
- No consistent difference in time of K65R development between TAF and TFV
Breakthrough Viral Growth

MT-2 cells → HIV-1 infection → 4-6 d → Score CPE

Drug Loading (TAF or TFV) → Cells

Sn → Drug + Media

Repeat every 4-6 days

Up to 4 weeks
Breakthrough Viral Growth – Subtype B

Viral Isolate 1: Subtype B

Drugs used at physiological concentration → 2X less drug → 2X less drug → 2X less drug
Breakthrough Viral Growth – Subtype B (continued)

Viral Isolate 6: Subtype B

Viral Isolate 2: Subtype B
Breakthrough Viral Growth – Subtype B*

Viral Isolate 3: Subtype B*

Viral Isolate 7: Subtype B*
Breakthrough Viral Growth – Subtype C

Viral Isolate 4: Subtype C

Viral Isolate 5: Subtype C
Breakthrough Viral Growth – Subtype C (continued)

**Viral Isolate 9: Subtype C**

- **Setup 1**
- **Setup 2**
- **Setup 3**
- **Setup 4**

**Viral Isolate 10: Subtype C**

- **Setup 1**
- **Setup 2**
- **Setup 3**
- **Setup 4**
### Summary of Breakthrough Viral Growth

<table>
<thead>
<tr>
<th>Isolate ID</th>
<th>Subtype</th>
<th>Codon RT 64-65-66</th>
<th>TAF Phenotypic Fold-Change</th>
<th>Time to Viral Breakthrough (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Setup#1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TAF 400 nM</td>
</tr>
<tr>
<td>M</td>
<td>B</td>
<td>AAG AAA AAA</td>
<td>1.0</td>
<td>&gt;28</td>
</tr>
<tr>
<td>1</td>
<td>B</td>
<td>AAG AAA AAA</td>
<td>1.1</td>
<td>&gt;28</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>AAG AAA AAA</td>
<td>1.3</td>
<td>&gt;28</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>AAG AAG AAA</td>
<td>0.9</td>
<td>&gt;28</td>
</tr>
<tr>
<td>3</td>
<td>B*</td>
<td>AAA AAG AAA</td>
<td>0.9</td>
<td>&gt;28</td>
</tr>
<tr>
<td>7</td>
<td>B*</td>
<td>AAA AAG AAA</td>
<td>1.3</td>
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<td>1.0</td>
<td>&gt;28</td>
</tr>
</tbody>
</table>

- No difference in TFV or TAF antiviral activity between subtype B vs. C viruses
- All WT viruses inhibited by TFV & TAF at pharmacologic concentrations (Setup #1)
- Breakthrough viral growth with TFV, but not TAF, at 2x and 4x lower drug levels (Setups #2-3)
- Breakthrough viral growth with TFV and TAF (8x) (Setup #4)
## Analysis of Clinical Studies

<table>
<thead>
<tr>
<th>Time of Analysis</th>
<th>Subtype</th>
<th>Patients with Data (N)</th>
<th>Virologic Failures (All) n</th>
<th>Virologic Failures (K65R) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>C</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Non-C</td>
<td>540</td>
<td>62</td>
<td>8 (1.5%)</td>
</tr>
<tr>
<td>2016</td>
<td>C</td>
<td>77</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Non-C</td>
<td>5479</td>
<td>245</td>
<td>17 (0.3%)</td>
</tr>
</tbody>
</table>

- **2006**: Analysis of available clinical trial data evaluating 550 patients treated with tenofovir (TDF): K65R not observed in any subtype C patient.

- **2016**: Analysis of available clinical trial data evaluating 5556 patients from 9 clinical studies treated with TDF or TAF: K65R not observed in any subtype C patient and a low incidence (0.3%) was observed in non-subtype C patients.
Conclusion

- As previously reported, subtype C HIV-1 isolates can select K65R more quickly than subtype B viruses \textit{in vitro}.
- This difference appears correlated with codon usage at positions 64-65-66 in RT, and is also found in some subtype B isolates (B*).
- At pharmacologic drug concentrations, both TAF and TFV were able to prevent viral breakthrough all viruses from both subtypes.
- At sub-therapeutic drug concentrations, only TAF maintained antiviral activity against subtype B and C viruses, suggesting that higher intracellular drug concentrations achieved with TAF may result in reduced resistance development among individuals with lower drug adherence regardless of subtype.
- Analyses of patients in clinical studies (n=5556) shows limited virologic failure for patients on TDF- and TAF-containing regimens, with K65R rarely seen overall and not observed in with any subtype C patient (n=77).
Acknowledgements

TAF Teams & Sub-teams

- Stephanie Cox
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- Clinical Virology
- Drug Discovery
- Medicinal Chemistry
- High Throughput Screen.
- Drug Metabolism - PK
- Formulation
- Clinical Pharmacology
- Clinical Operations
- Clinical Research
- Biometrics
- Regulatory Affairs
- Medical Affairs