Entinostat is a histone deacetylase (HDAC) inhibitor selective for class 1 HDACs and activates HIV production from latently infected primary T-cells

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Background

- Two recent clinical trials have shown that the HDACi vorinostat can increase HIV transcription in vivo
  - Archin Nature 2012; Elliot CROI 2013
- Important to identify newer compounds that are more potent, less toxic and perhaps more selective which could move rapidly into clinical trials
- In latently infected cell lines HDAC1, 2 and 3 are major HDACs involved in maintenance of latency
- Inhibitors of Class I but not Class II HDACs increased HIV production from latency in patient derived cells ex vivo
# Histone Deacylases

<table>
<thead>
<tr>
<th>HDAC isoforms</th>
<th>Protein domains</th>
<th>Cellular distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDAC1</td>
<td>482 aa</td>
<td>N</td>
</tr>
<tr>
<td>HDAC2</td>
<td>488 aa</td>
<td>N</td>
</tr>
<tr>
<td>HDAC3</td>
<td>428 aa</td>
<td>N/C</td>
</tr>
<tr>
<td>HDAC8</td>
<td>377 aa</td>
<td>N</td>
</tr>
<tr>
<td>HDAC4</td>
<td>1084 aa</td>
<td>N/C</td>
</tr>
<tr>
<td>HDAC5</td>
<td>1122 aa</td>
<td>N/C</td>
</tr>
<tr>
<td>HDAC7</td>
<td>912 aa</td>
<td>N/C</td>
</tr>
<tr>
<td>HDAC9</td>
<td>1609 aa</td>
<td>N/C</td>
</tr>
<tr>
<td>HDAC6</td>
<td>1215 aa</td>
<td>N/C</td>
</tr>
<tr>
<td>HDAC10</td>
<td>669 aa</td>
<td>N/C</td>
</tr>
<tr>
<td>HDAC11</td>
<td>347 aa</td>
<td>N</td>
</tr>
<tr>
<td>Sirtuins (1-7)</td>
<td>747 aa</td>
<td>N/C Mit.</td>
</tr>
</tbody>
</table>

Marcotullia, BBR Reviews on Cancer, 2011
Aim

To determine the relative potency and toxicity of a panel of HDACi in latently infected primary CD4 T-cells and latently infected cell lines
<table>
<thead>
<tr>
<th>HDACi</th>
<th>Class</th>
<th>Activity in vitro</th>
<th>Activity in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat</td>
<td>Pan</td>
<td>• Cell lines  • Ex-vivo T-cells  • Primary model</td>
<td>Yes (Archin Nature 2012; Elliot CROI 2013)</td>
</tr>
<tr>
<td>Entinostat</td>
<td>Class I</td>
<td>• Cell lines</td>
<td></td>
</tr>
<tr>
<td>MCT-3</td>
<td>Class I</td>
<td>• Cell lines</td>
<td>Research only</td>
</tr>
<tr>
<td>Panobinostat</td>
<td>Pan</td>
<td>• Cell lines  • Ex-vivo T-cells  • Primary model</td>
<td>Ongoing trial</td>
</tr>
</tbody>
</table>
Entinostat

- Class I selective, HDAC1 and HDAC3
  - Khan et al Biochem J 2008; Tatamiya et al AACR meeting 2004

- In 23 Phase I and II trials for conditions including myeloid and lymphocytic leukaemia; non-small cell lung cancer; breast and colorectal cancer
  - clinicaltrials.gov

- Increased histone acetylation and ERK protein translation in tumor tissue

- May suppress T-regulatory cell function
  - Shen et al Nature 2012

- Well tolerated and Ames test negative
Latency models used in this study

- **ACH2 cells**
  - Clonal CD4 T-cell line (derived from CEM cells)
  - Single integrated copy of HIV

- **J-lat 6.3 clone**
  - Clonal CD4 T-cell line (derived from Jurkat cells)
  - Single integrated copy of HIV (full length minus env, nef; + GFP)

- **Primary CD4 T-cell model of chemokine induced latency**
  - Resting CD4 T-cells
  - Random HIV integration sites
Resting CD4 T-cell latency model

- **NL4.3**
- **CCL19**
- **Latency**
- **Viral activation**

**Day**
- -2
- 0
- 4
- +1
- +3
- +7

**HDACi or Mitogen**

Activated PBMC

Saleh, Blood 2007; Cameron, PNAS 2010; Saleh, Retrovirology 2011
Toxicity observed in all cells used

<table>
<thead>
<tr>
<th></th>
<th>ACH2</th>
<th>J-lat6.3</th>
<th>PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat</td>
<td>0.42µM</td>
<td>0.41µM</td>
<td>1.6µM</td>
</tr>
<tr>
<td>Entinostat</td>
<td>0.65µM</td>
<td>0.59µM</td>
<td>1.3µM</td>
</tr>
<tr>
<td>MCT-3</td>
<td>1.58µM</td>
<td>0.93µM</td>
<td>1.7µM</td>
</tr>
<tr>
<td>Panobinostat</td>
<td>0.07µM</td>
<td>0.04µM</td>
<td>0.09µM</td>
</tr>
</tbody>
</table>
All HDACi activated virus production in latently infected cell lines

ACH2

J-lat 6.3

Mean ± SD (n=3)
Entinostat activates HIV production in latently infected primary CD4 T-cells

Overall differences by Kruskal-Wallis
Differences between each HDACi and Vorinostat (paired t-test)  

Mean ± SD (n=4)
No synergism with vorinostat and IL-7

\[ p = 0.07 \]

Mean ± SD (n=3)
No change in HDAC expression with CCL19 treatment
No change in activation markers following HDACi

Mean ± SD (n=4)
Chromatin immunoprecipitation (ChIP) analysis of latently infected T-cells

1. Chromatin Immunoprecipitation
   - Specific Antibody
   - Isotype Control

2. Immunoprecipitation

3. DNA Purification

4. PCR

Mean ± SD (n=3)  * p<0.05
Limitations of study

- Assay was semi-quantitative
- Only in vitro models of latency were tested not patient derived cells
- No assessment of cell death as a result of induction of viral production
- Viral production alone in vitro may not ultimately indicate efficacy in depletion of latently infected cells in vivo
Conclusions

- Variation in toxicity and potency of HDACi between latently infected cell lines and latently infected CCL19-treated CD4 T-cells

- Entinostat, which is selective for Class I HDACi, induced significant viral production in latently infected CCL19-treated CD4 T-cells

- Based on toxicity profile and activity in vitro, entinostat should be considered for future clinical trials
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  - Kirston Barton

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[Logos of Monash University, Burnet Institute, International AIDS Society, and NHMRC]