Elimination of HIV-1 latently infected cells by PKC agonist gnidimacrin alone and in combination with an HDAC inhibitor

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Towards an HIV cure

• Latent HIV reservoir
• “Shock and kill” strategy
• Latency reversing agent (LRA)
  - PKC agonist: prostratin, ingenol esters, bryostatin
  - Histone deacetylase inhibitor (HDACi): SAHA, romidepsin
Daphnane diterpenes from *Stellera chamaejasme*

**Gnidimacrin (GM)**
- PKC agonist
- beta selective

- Dichotomous activity
  - HIV infection
  - latent HIV-1

<table>
<thead>
<tr>
<th></th>
<th>EC$_{90}$ (nM)</th>
<th>IC$_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NL4-3</td>
<td>0.41</td>
<td>2800</td>
</tr>
<tr>
<td>MT4</td>
<td>0.40</td>
<td>4300</td>
</tr>
</tbody>
</table>

GM selectively induced latent HIV-1 activation

**A. P24 level in U1 and ACH-2**

- **B. Cell viability**

### Effect of GM on latent HIV-1 *ex vivo*

<table>
<thead>
<tr>
<th></th>
<th>Viral load</th>
<th>CD4 Counts/µl</th>
<th>ART Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt-1</td>
<td>&lt; 48</td>
<td>419</td>
<td>16</td>
</tr>
<tr>
<td>Pt-2</td>
<td>&lt; 48</td>
<td>679</td>
<td>6</td>
</tr>
<tr>
<td>Pt-3</td>
<td>&lt; 48</td>
<td>592</td>
<td>7</td>
</tr>
<tr>
<td>Pt-4</td>
<td>&lt; 48</td>
<td>693</td>
<td>21</td>
</tr>
<tr>
<td>Pt-5</td>
<td>&lt; 48</td>
<td>835</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Pt-6</td>
<td>&lt; 48</td>
<td>802</td>
<td>15</td>
</tr>
<tr>
<td>Pt-7</td>
<td>&lt; 48</td>
<td>1033</td>
<td>16</td>
</tr>
</tbody>
</table>

PBMCs from patients were provided by DHVI/CAVD/CTVIMC

GM (1nM) reduced proviral DNA and the frequency of latently infected cells

(A) HIV DNA copies/million PBMCs

- **GM**: 87.5, 515, 565
- **SAHA**: 45, 480, 327.5
- **Ctr**: 247.5, 357.5

\[ p = 0.038 \text{ (GM vs Ctr)} \]

(B) IUPM

- **GM**: 0.51, 1.6, 9.6
- **SAHA**: 3.2, 0, 0
- **Ctr**: 8.1, 0, 0

0: Undetectable viral outgrowth; Ctr: DMSO control; IUPM: Infectious units per million PBMCs.

Low dose of GM (20 pM) reduced proviral DNA and the frequency of latent HIV-1 infected cells

Effect of GM on latent HIV-1 in CD8-depleted PBMCs from HIV+ patients

\[ p=0.011 \text{ (GM vs Ctr)} \]

\[ p=0.032 \text{ (GM vs Ctr)} \]

GM=1 nM; SAHA=0.5 µM

Summary of GM studies

• High potency and selectivity

• Consistency in cell and ex vivo models
  - Activates latent HIV-1
  - Eliminates HIV latently infected cells

• Disadvantages
  - Limited source
  - PKC agonist associated side effects
## HDACi TPB and GM/TPB combination

### Activation of Latent HIV-1 in U1 Cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (U1)</th>
<th>CC&lt;sub&gt;50&lt;/sub&gt; (U937)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAHA</td>
<td>1.2 µM</td>
<td>0.78 µM</td>
<td>0.65</td>
</tr>
<tr>
<td>Romidepsin</td>
<td>1.1 nM</td>
<td>0.73 nM</td>
<td>0.66</td>
</tr>
<tr>
<td>TPB</td>
<td>0.93 µM</td>
<td>14 µM</td>
<td>15</td>
</tr>
<tr>
<td>GM</td>
<td>18 pM</td>
<td>&gt;10 nM</td>
<td>&gt;555</td>
</tr>
<tr>
<td>GM+TPB (0.5 µM)</td>
<td>5.6 pM</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

### Thiophenylbenzalmide (TPB) **Isozyme** IC<sub>50</sub> (µM)

- HDAC1: 0.007
- HDAC2: 0.049
- HDAC3: 10
- HDACs 4-8: >10

Elimination of latent HIV-1 infected cells in patient PBMCs by GM/TPB

- GM: 20pM
- GM+TPB: 20pM 1µM
- SAHA: 0.5 µM
- No drug DMSO
**Optimized TPB analog**

- **GM**: 26 pM
- **Ta**: 0.57 µM
- **GM + Ta**
- **DMSO**

Every 3 days

- **HIV P24**
- **Day 18**
- **Proviral DNA**

**SI**

- **TPB**: 15
- **Ta**: 45

**T20**: 1 µg/ml
Elimination of latent HIV-1 infected U1 cells by GM and Ta

Day 18

HIV-1 pol

Beta-2-microglobulin

Day 18

M GM Ta GM+Ta DMSO M GM Ta GM+Ta DMSO M

P24 (pg/ml)

-50 50 150 250 350 450

0 3 6 9 12 15 18

Day

0 3 6 9 12 15 18

0 3 6 9 12 15 18

200bp
TPB suppressed GM-induced IFN-γ in PBMCs

PBMCs (2X10^6/ml) were treated with high- (2,000 pM) or low-dose (20 pM) of GM in the presence of 3 µM or 1 µM TPB (GM-TPB-3; GM-TPB-1) for 24 h. Anti-CD3/CD28 (0.1 µg/ml) were used as a control.
Summary on HADCi/GM combination studies

- Superior selectivity of TPB
- TPB/GM synergy on latent HIV-1 activation/elimination
- TPB suppressed IFN-γ production induced by high dose GM
- Goal achieved by GM/TPB
  1. Reduces the dose of GM required for activity
  2. Mitigates potential side effects of GM
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Thank You