Activating PKC-ε induces HIV expression with improved tolerability

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Background and Methods

- Protein Kinase C (PKC) agonists activate latent HIV, but have limited clinical utility due to toxicity.
- Platelet activation, leading to thrombocytopenia, is a major liability associated with PKC agonists.
- **Hypothesis:** Specifically activating PKC isoforms abundant in T cells, but not in platelets, could improve PKC agonist tolerability.
- **Objective:** Generate small molecule PKC agonists that specifically activate PKC isoforms present in T cells but not in platelets.

**Methods:**
- PKC isoform expression in platelets and T cells was determined by western blot with isoform selective antibodies.
- PKC isoform selectivity of novel PKC agonists was quantified using PKC-GFP translocation in A549 cells.
- Flow cytometry was used to assess T cell and platelet activation in whole blood using CD69 and CD62P, respectively.
- To assess tolerability in vivo, compounds were dosed in rats. T cell activation was assessed by Egr1 induction and platelets were counted.
PKC-ε and η are highly expressed in T cells, but not platelets

- Differential PKC isoform expression in human T cells and platelets by western blotting (n=3 donors)

In CD4 cells from ART-suppressed people living with HIV, PKC agonists induced latent HIV in the presence of an inhibitor of classical PKC isoforms, indicating novel PKC isoforms are sufficient for HIV activation.

Constitutively active PKC-ε or η isoforms induced latent HIV in a Jurkat reporter cell line.
C-233 is more selective for PKC-ε and T cells

PKC-GFP translocations of novel PKC isoforms in A549 cells indicate improved PKC-ε selectivity of C-233 over C-232A.

<table>
<thead>
<tr>
<th>Isoform Translocation</th>
<th>C-232A EC50 (nM)</th>
<th>ε selectivity</th>
<th>C-233 EC50 (nM)</th>
<th>ε selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKC-δ</td>
<td>710</td>
<td>12</td>
<td>47000</td>
<td>196</td>
</tr>
<tr>
<td>PKC-ε</td>
<td>58</td>
<td>1</td>
<td>240</td>
<td>1</td>
</tr>
<tr>
<td>PKC-η</td>
<td>100</td>
<td>2</td>
<td>1100</td>
<td>5</td>
</tr>
<tr>
<td>PKC-θ</td>
<td>180</td>
<td>3</td>
<td>1500</td>
<td>6</td>
</tr>
</tbody>
</table>

ε selectivity: EC50 of each specific isoform/EC50 of ε

C-233 exhibited 3 fold improved potency for T cells relative to platelets in the in vitro whole blood assay that predicted improved tolerability in vivo.

C62P EC50/CD69 EC50 Ratio

1.7 5.1 2.1 2.0 1.8 0.9 1.5

P value: Wilcoxon matched pair T test
C-233 showed improved tolerability in rat studies

- C-233 and C-232A increased Egr1* mRNA to similar levels, consistent with similar T cell activation.
- C-232A induced severe platelet depletion, while C-233 did not.
- 1 of 6 animals treated with C-232A was euthanized, but no deaths occurred with C-233.

* Vemula et.al., Antiviral Res, March 2017
Conclusions

- Platelet activation and depletion is a critical safety liability associated with non-selective PKC agonists and should be carefully monitored in preclinical and clinical studies with PKC agonists.

- Specific targeting of PKC isoforms abundant in T cells but not in platelets, such as PKC-ε, could improve tolerability of PKC agonists that activate HIV.

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