The Latency-Reversing Agent HODHBt Synergizes With IL-15 To Enhance Cytotoxic Function Of HIV-specific CD8+ T-cells

Dennis C. Copertino Jr
Research Specialist
in The Laboratory of Brad Jones
NIAID Early-Stage Investigator Scholarship
Conflicts

• No Conflicts to Report
HODHBt as a Latency Reversing Agent
(3-hydroxy-1,2,3-benzotriazin-4(3H)-one)

- IL-15 holds promise as both a latency-reversing agent and as an NK/CD8+ enhancing agent. In addition, IL-15 is the subject of multiple ongoing clinical trials.
- HODHBt enhances IL-15-mediated reactivation of HIV by increasing STAT5 occupancy of the HIV-LTR.
- While IL-15 drives STAT5 to the nucleus while HODHBt blocks the turnover of STAT5 sequestering STAT5 in its active nuclear form.

Novel insights into mechanism will be presented tomorrow (O.P.5.2 10:30am) by Alberto Bosque

(Bosque et al. Cell Reports, 2017)
Might HODHBt Also Enhance CD8+ T-cell Function?

STATs, including STAT5, play a central role in the control of CD8+ T-cell responses. *(Verdeil, G J et al. Immunol. 2006)*

STATs promote the expression of effector molecules, proliferation, and tissue homing, as well as transcription factors required for CD8 T cell function like T-bet and Eomes. *(Grange, M et al. Cancer Res. 2012; Immunology. 2015; J Immunol. 2013)*

Enhanced STAT5a activation rewires exhausted CD8 T cells during chronic stimulation to acquire a hybrid durable effector like state

Jean-Christophe Beltra¹,²,³, Mohamed S. Abdel-Hakeem¹,²,⁴,⁵, Sasikanth Manne¹,², Zhen Zhang⁶, Hua Huang⁶, Makoto Kurachi⁷, Leon Su⁸, Lora Picton⁶, Yuki Muroyama¹,², Valentina Casella⁹, Yinghui J. Huang¹,², Josephine R. Giles¹,²,³, Divij Mathew¹,², Jonathan Belman¹,², Max Klapholz¹,², Hélène Decaluwe¹⁰, Alexander C. Huang²,³,¹¹,¹², Shelley L. Berger⁶, K. Christopher Garcia⁸,¹³,¹⁴,¹⁵ and E. John Wherry¹,²,³,¹⁶
**Objective**

To improve HIV-specific cytotoxic T-cell responses that could contribute to the reduction of the HIV reservoir.

**Hypothesis**

HODHBt will synergize with IL-15 to enhance both HIV-specific cytotoxic T-cell responses and latency reversing activity. Enhancing both the “kick” and the “kill” required of effective cure strategies.
How Does Treatment With HODHBt + IL-15 Impact HIV-specific CD8+ T Cell Responses *in Vitro*?

### A5321 Study Participants

<table>
<thead>
<tr>
<th>Years on ART</th>
<th>7.2y (4.2-14.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>7 Males, 7 Females</td>
</tr>
<tr>
<td>Race</td>
<td>5 Hispanic, 2 Black, 7 White</td>
</tr>
<tr>
<td>Age</td>
<td>46y (range 31-69)</td>
</tr>
</tbody>
</table>

**Cryopreserved PBMCs**

A5321 on ART 7.2y

**GZMB ELISPOT**
HODHBt Synergizes with IL-15 to Markedly Enhance HIV-specific T-cell Responses

PBMCs isolated from 14 ARV-treated donors were pulsed with different HIV-peptide pools and production of Granzyme B (GZMB) was measured by ELISPOT

**Representative donor** 170,910 cells/well

<table>
<thead>
<tr>
<th></th>
<th>No Peptide</th>
<th>HIV-Gag</th>
<th>HIV-Pol</th>
<th>HIV-Nef</th>
<th>HIV-Env</th>
<th>CMV-pp65</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>0</td>
<td>120</td>
<td>35</td>
<td>13</td>
<td>11</td>
<td>140</td>
</tr>
<tr>
<td>HODHBt</td>
<td>1</td>
<td>125</td>
<td>113</td>
<td>16</td>
<td>6</td>
<td>212</td>
</tr>
<tr>
<td>DMSO</td>
<td>5</td>
<td>97</td>
<td>78</td>
<td>19</td>
<td>6</td>
<td>204</td>
</tr>
<tr>
<td>IL-15</td>
<td>26</td>
<td>103</td>
<td>53</td>
<td>19</td>
<td>2</td>
<td>517</td>
</tr>
<tr>
<td>IL-15+HODHBt</td>
<td>27</td>
<td>310</td>
<td>78</td>
<td>19</td>
<td>124</td>
<td>538</td>
</tr>
<tr>
<td>IL-15+DMSO</td>
<td>13</td>
<td>181</td>
<td>109</td>
<td>96</td>
<td>120</td>
<td>426</td>
</tr>
</tbody>
</table>

PHATotal: 1706, 1680
HODHBt Synergizes with IL-15 to Markedly Enhance HIV-specific T-cell Responses

Relative to IL-15+DMSO, GZMB-releasing responses upon treatment with IL-15+HODHBt were:

- **Gag 4.4-fold** ($p<0.002$), **Pol 2.4-fold** ($p<0.020$), and **Nef 27.8-fold** ($p<0.001$)
What Other Impacts Might HODHBt & IL-15 Have on T-cells?

scRNA-seq. Analysis of PBMCs from PLWH treated with IL-15 versus HODHBt + IL-15
HODHBt + IL-15 Upregulates Antigen-processing and Presentation Pathways and MHC-I in T-cells
Does Treatment With HODHBt + IL-15 Effect Antigen Presentation By CD4+ T Cells?
Treatment with HODHBt + IL-15 Increases Surface MHC-I Expression on CD4^+ T Cells

- 50μM HODHBt + 1ng/ml IL-15
- 100μM HODHBt + 10ng/ml IL-15
- 150μM HODHBt + 25ng/ml IL-15

n=6
Degranulation Assay

CD4+ T-cells isolated from PBMCs treated with DMSO, HODHBt, IL-15, IL-15+HODHBt in ARVs for 4 days

Peptide pulse with Env192 peptide, contains RV9

Wash 4x

CTL Co-Culture for 5h with HIV-RV9 specific CTL 1:1 (E:T)
HODHBt + IL-15 increases Surface MHC-I & enhances functional recognition of targets by HIV specific CTL
We have shown that HODHBt & IL-15 enhanced cytolytic function of HIV specific CD8+ T cells by GZMB ELISPOT. Treatment with HODHBt + IL-15 increases surface MHC-I and antigen presentation by CD4+ T cells.

Are HODHBt + IL-15 sufficient to drive reductions in *ex vivo* HIV reservoirs?
HIV Eradication Assay 2.0 – HIVE 2.0
Assessing Both Latency Reversal & CD8+ Induced Killing of Reservoir

Day 0: Step 1
- PBMCs from PLWH

Step 2: Deplete CD8+ and/or NK
- Magnetic Positive Selection
- Deplete NK Cells
- Deplete CD8+ & NKs
- Complete PBMCs

Day 3: Ultra Sensitive p24
Day 3, 6, 9 Change Media & Flow Cytometry

Day 2: pSTAT5 (Y694) staining performed

Step 3: ex vivo reservoir stimulation
- No NK
- No NK, No CD8
- PBMC
- No CD8
- 1. DMSO
- 2. HODHbt
- 3. IL-15
- 4. HODHbt + IL-15
- 5. HODHbt + IL-15

Enrich for CD4+ cells
Perform IPDA
Treatment with HODHBt + IL-15 Increases Surface Expression of CD69 On Both CD4+ and CD8+ T Cells
Treatment with HODHBt + IL-15 Increases STAT5 Phosphorylation and % of GZMA⁺ Perforin⁺ CTLs

**CD8 Perforin/Granzyme**

- Black: Day 3
- Pink: Day 9

**STAT5 Phosphorylation**

- Green bars represent different treatments:
  - DMSO
  - HODHBt
  - IL-15
  - IL-15 + HODHBt (NO CD8)
  - IL-15 + HODHBt (PBMCs)
IL-15 + HODHBt Was Sufficient to Drive Intact Reservoir Reduction in ARV-Treated Donor

Intact HIV DNA was significantly reduced in both whole PBMCs (p=0.029) and NK-cell depleted conditions (p=0.032), but not in the NK-cell+CD8-depleted condition (p=0.692)
IL-15 + HODHBt Was Not Sufficient to Reduce Reservoir in Two Other ARV-Treated Donors
Conclusions

• IL-15 synergizes with HODHBt to potently induce HIV-specific cytotoxic CD8+ T-cells – revealing strong ex vivo Gzm-B responses

• Together with the known latency reversal activity of HODHBt, this can be sufficient to drive ex vivo HIV reservoir reductions, though this appears to vary by donor –1 out of 3 donors

Future Direction

• Enhancing ‘Kick’ by adding LRAs with other modes of action

• Enhancing ‘Kill’ component by adding agents such as BCL-2 antagonists, or bnAb’s to induce ADCC against infected cells, DARTS or bi-specifics to redirect CD8+ T-cells to kill infected cells, testing combinations of other γc cytokines like IL-2 with IL-15.
Key questions

Can we prepare for ‘next generation’ IL-15 clinical trials by identifying ways to enhance its’ impact on both the ‘kick’ and ‘kill’

Key findings

The compound ‘HODHBt’ synergizes with IL-15 to dramatically enhance HIV-specific cytotoxic T-cell responses in cells from PLWH.

This can be sufficient to reduce HIV reservoirs in cell culture, but not consistently.

Next steps?

Test more ex-vivo reservoirs and add or include combinations of agents which can help enhance the reduction in size of the HIV reservoir.
A special thanks to those who are living with HIV & AIDS around the world! And my sincere gratitude for those who have dedicated their time and samples to the ACTG A5321 study and REACH/other studies as well!

Ask me about the Jones Lab Leukapheresis protocol in NYC. Enrolling HIV+ & HIV- participants.
Those who are living with HIV & AIDS around the world & the ACTG A5321 participants

Massachusetts General Hospital
• Rajesh Gandhi

University of Pittsburgh School of Medicine
• Deborah McMahon
• Bernard Macatangay
• Joshua C. Cyktor
• John W. Mellors

Harvard T.H. Chan School of Public Health
• Ronald Bosch

University of North Carolina at Chapel Hill
• Joseph Eron

Those who are living with HIV & AIDS around the world & the ACTG A5321 participants
Those who are living with HIV & AIDS around the world & the ACTG A5321 participants

Massachusetts General Hospital
- Rajesh Gandhi

University of Pittsburgh School of Medicine
- Deborah McMahon
- Bernard Macatangay
- Joshua C. Cyktor
- John W. Mellors

Harvard T.H. Chan School of Public Health
- Ronald Bosch

University of North Carolina at Chapel Hill
- Joseph Eron

Those who are living with HIV & AIDS around the world & the ACTG A5321 participants
end
Treatment with HODHBt + IL-15 Increases STAT5 Phosphorylation and % of GZMA+ Perforin+ CTLs