



Research Enterprise to Advance a Cure for HIV



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



School of Medicine
& Health Sciences



Weill Cornell
Medicine

Conflicts

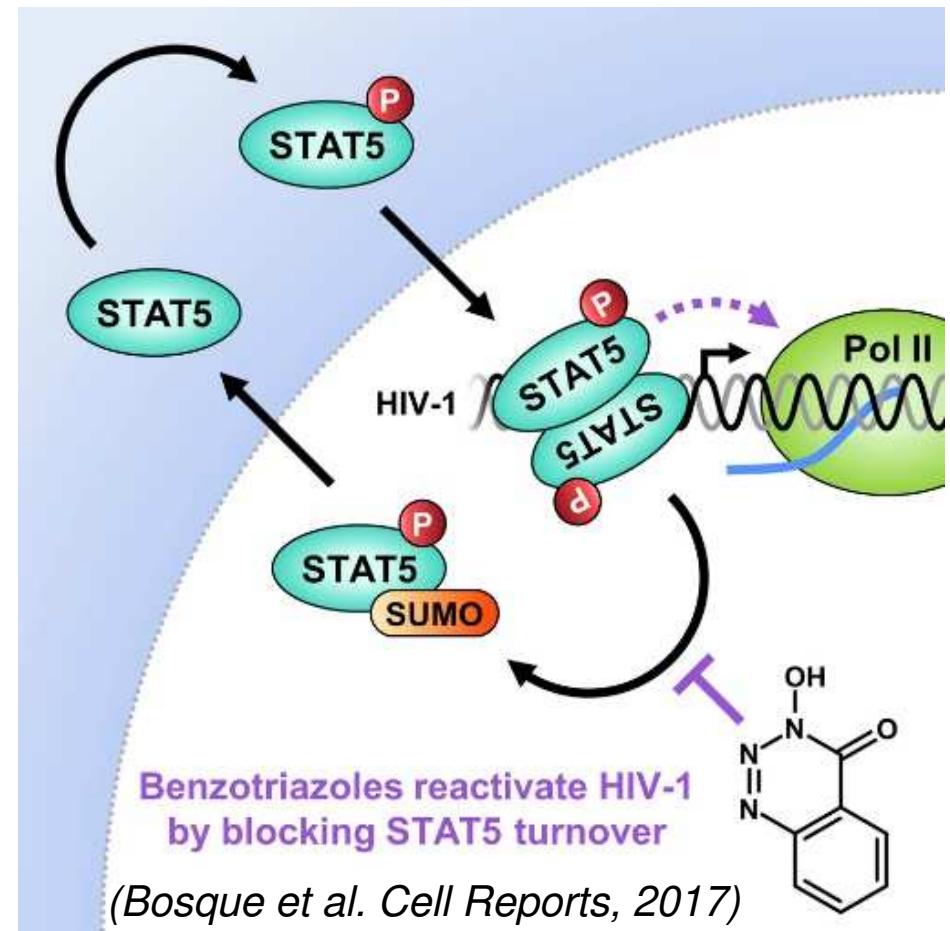
- No Conflicts to Report

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



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*)

In the context of HIV infection, activation in PLWH. (*Krywawych A, Leukoc Biol. 2011*)

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

Jean-Christophe Beltra^{1,2,3}, Mohamed S. Abdel-Hakeem^{1,2,4,5}, Sasikanth Manne^{1,2}, Zhen Zhang⁶, Hua Huang⁶, Makoto Kurachi⁷, Leon Su⁸, Lora Picton⁸, Yuki Muroyama^{1,2}, Valentina Casella⁹, Yinghui J. Huang^{1,2}, Josephine R. Giles^{1,2,3}, Divij Mathew^{1,2}, Jonathan Belman^{1,2}, Max Klapholz^{1,2}, Hélène Decaluwe¹⁰, Alexander C. Huang^{2,3,11,12}, Shelley L. Berger⁶, K. Christopher Garcia^{8,13,14,15} and E. John Wherry^{1,2,3,16}

BioRxiv, 2022

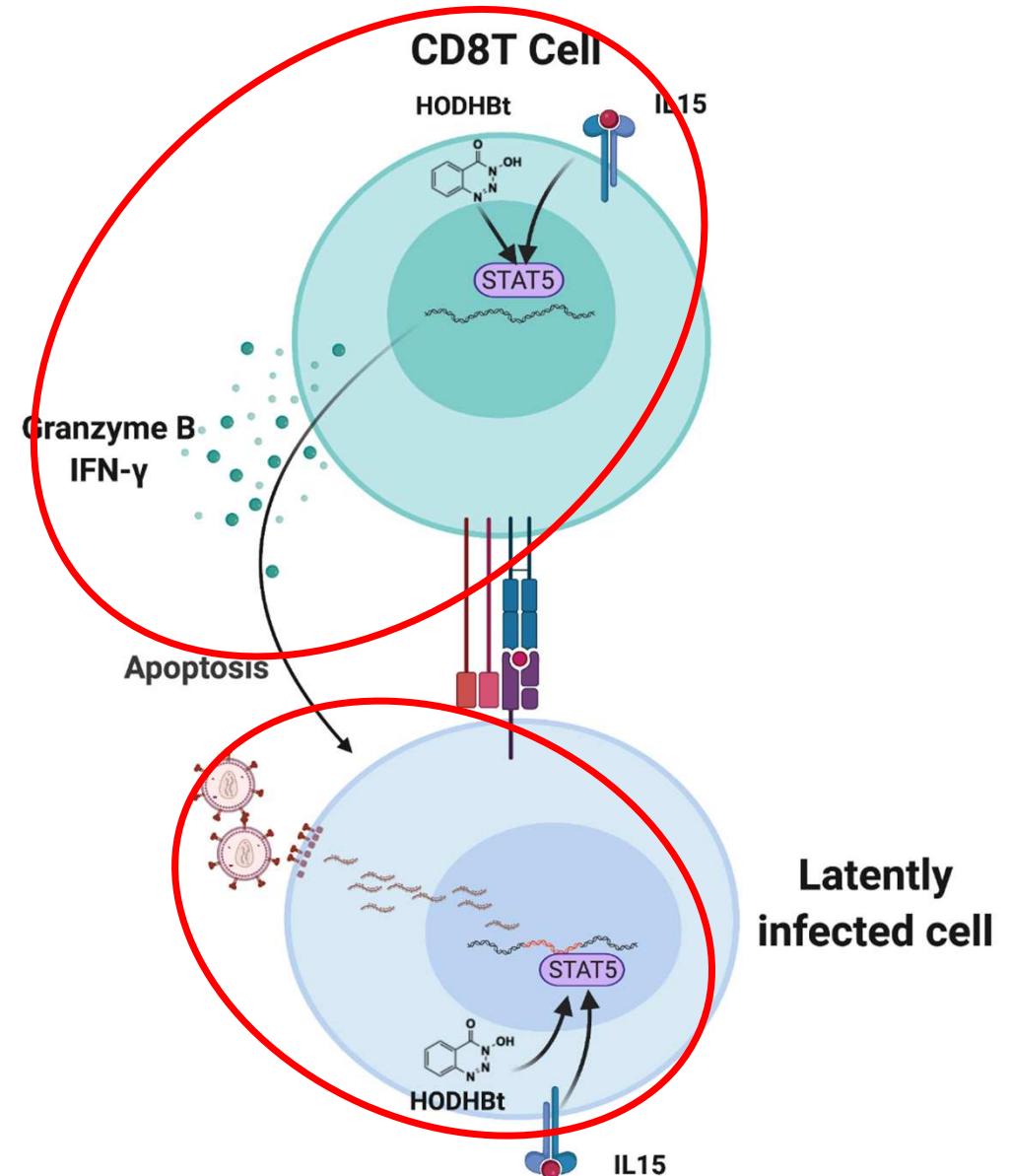
Objective

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	
Years on ART	7.2y (4.2-14.8)
Sex	7 Males, 7 Females
Race	5 Hispanic, 2 Black, 7 White
Age	46y (range 31-69)

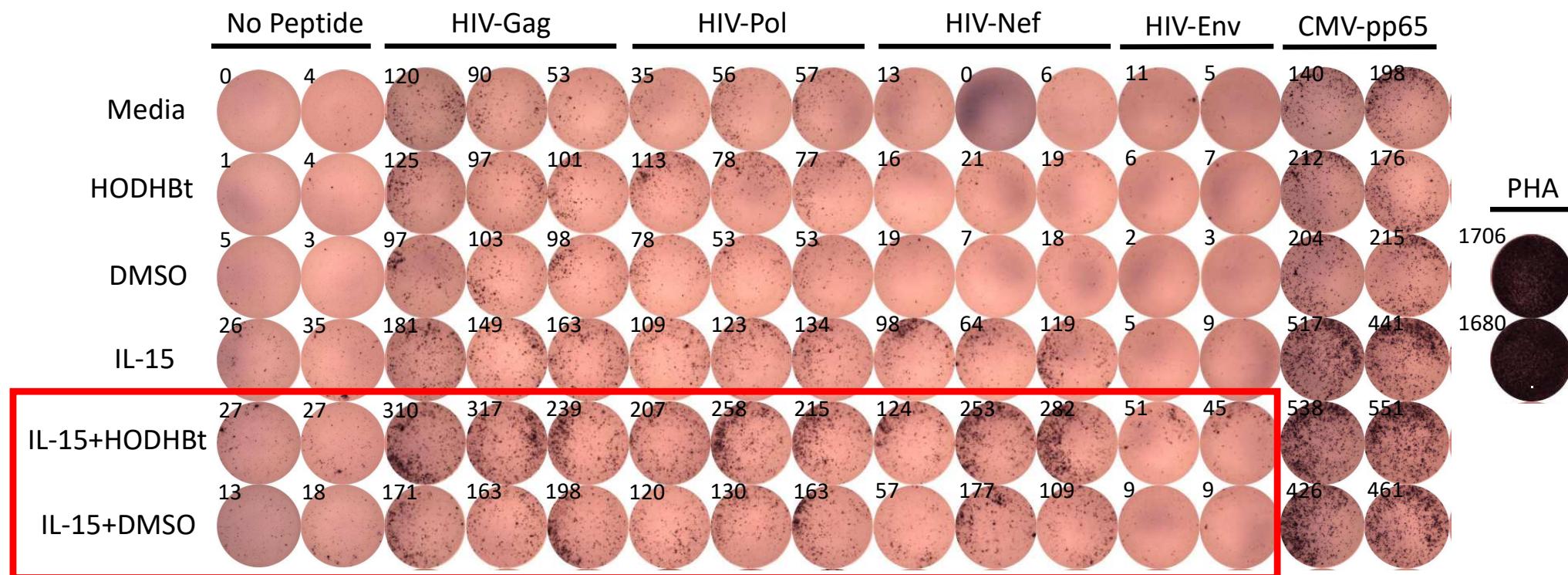


→ Cryopreserved PBMCs → GZMB ELISPOT
A5321 on ART 7.2y

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

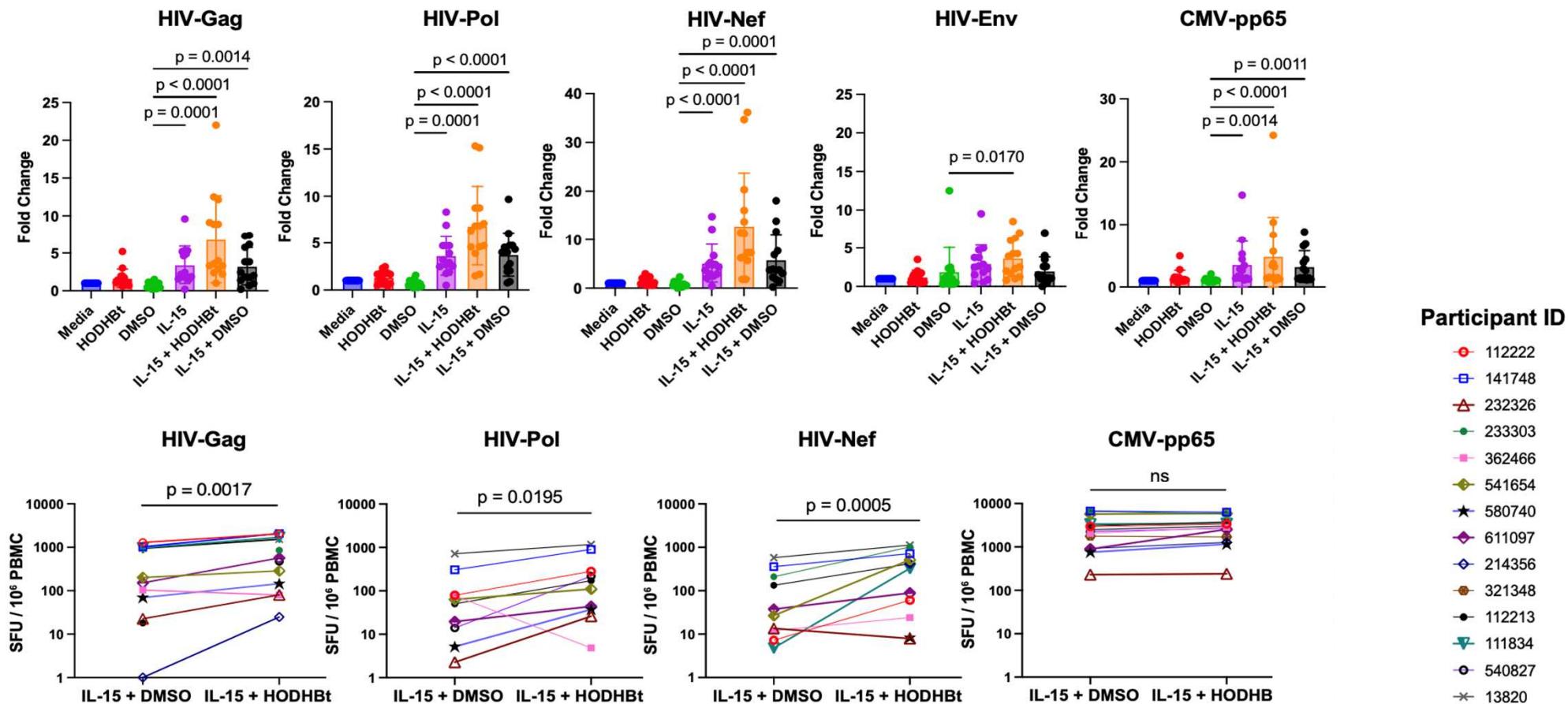
PBMCs isolated from 14 ARV-treated donors were pulsed with different HIV-peptide pools and production of Granzyme B (GZMB) was measured by ELISPOT

Representative donor 170,910 cells/well



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** ($p<0.001$)

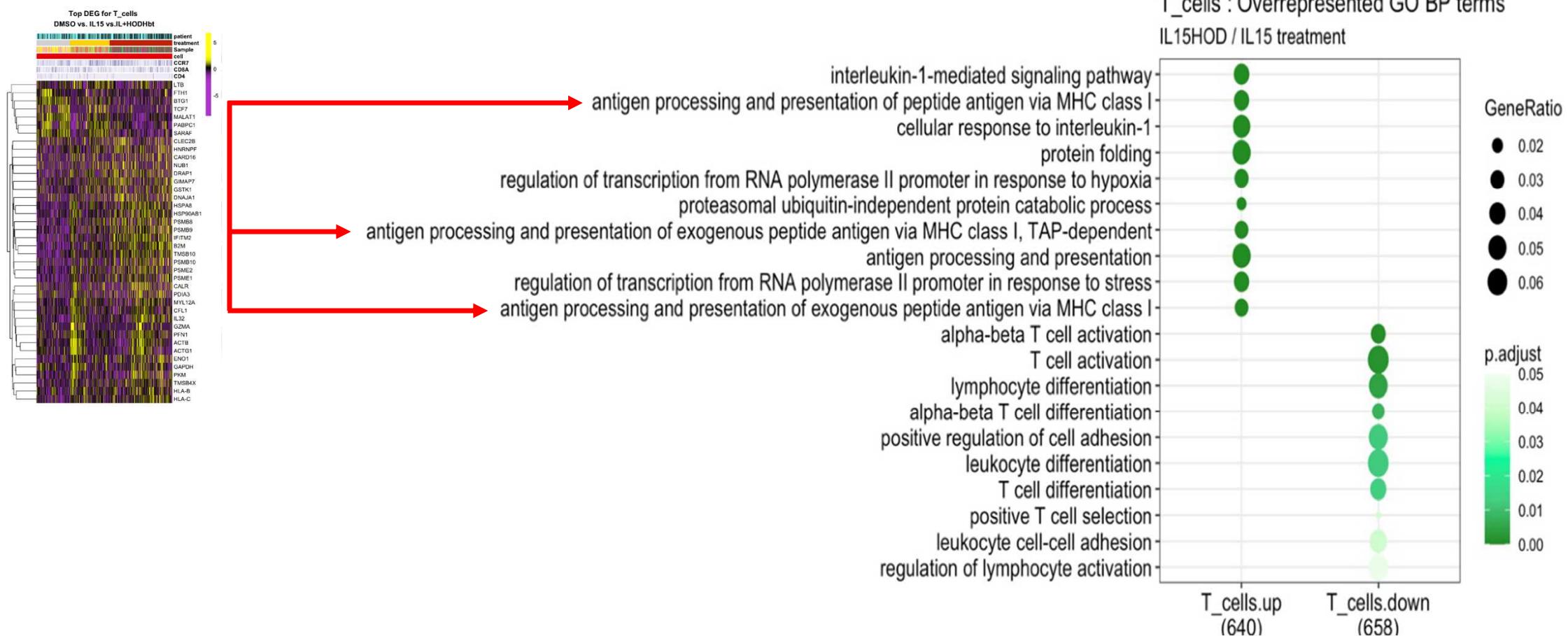


What Other Impacts Might HODHBT & IL-15 Have on T-cells?



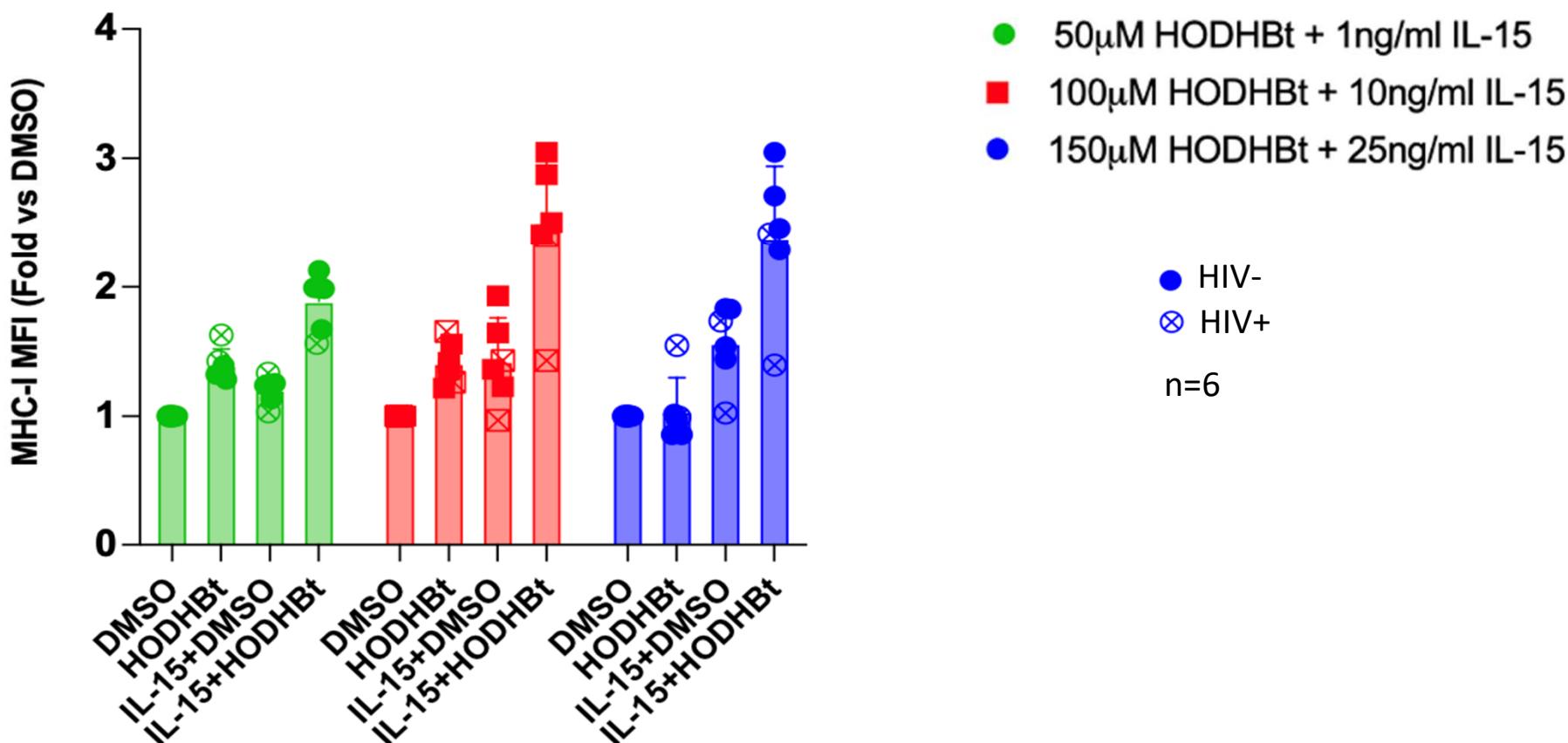
scRNA-seq. Analysis of PBMCs from PLWH
treated with IL-15 versus HODHBT + IL-15

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

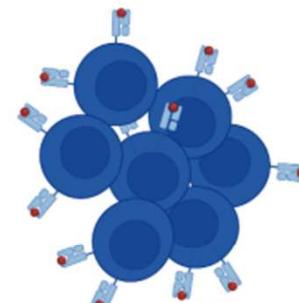


Degranulation Assay

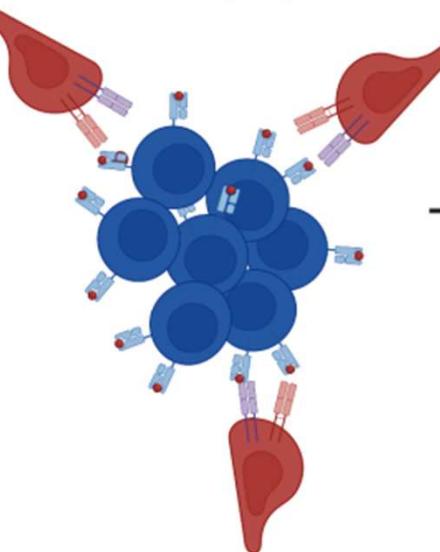
CD4+ T-cells isolated from PBMCs treated with DMSO, HODHBt, IL-15, IL-15+HODHBt in ARVs for 4 days



Peptide pulse with Env192 peptide, contains RV9



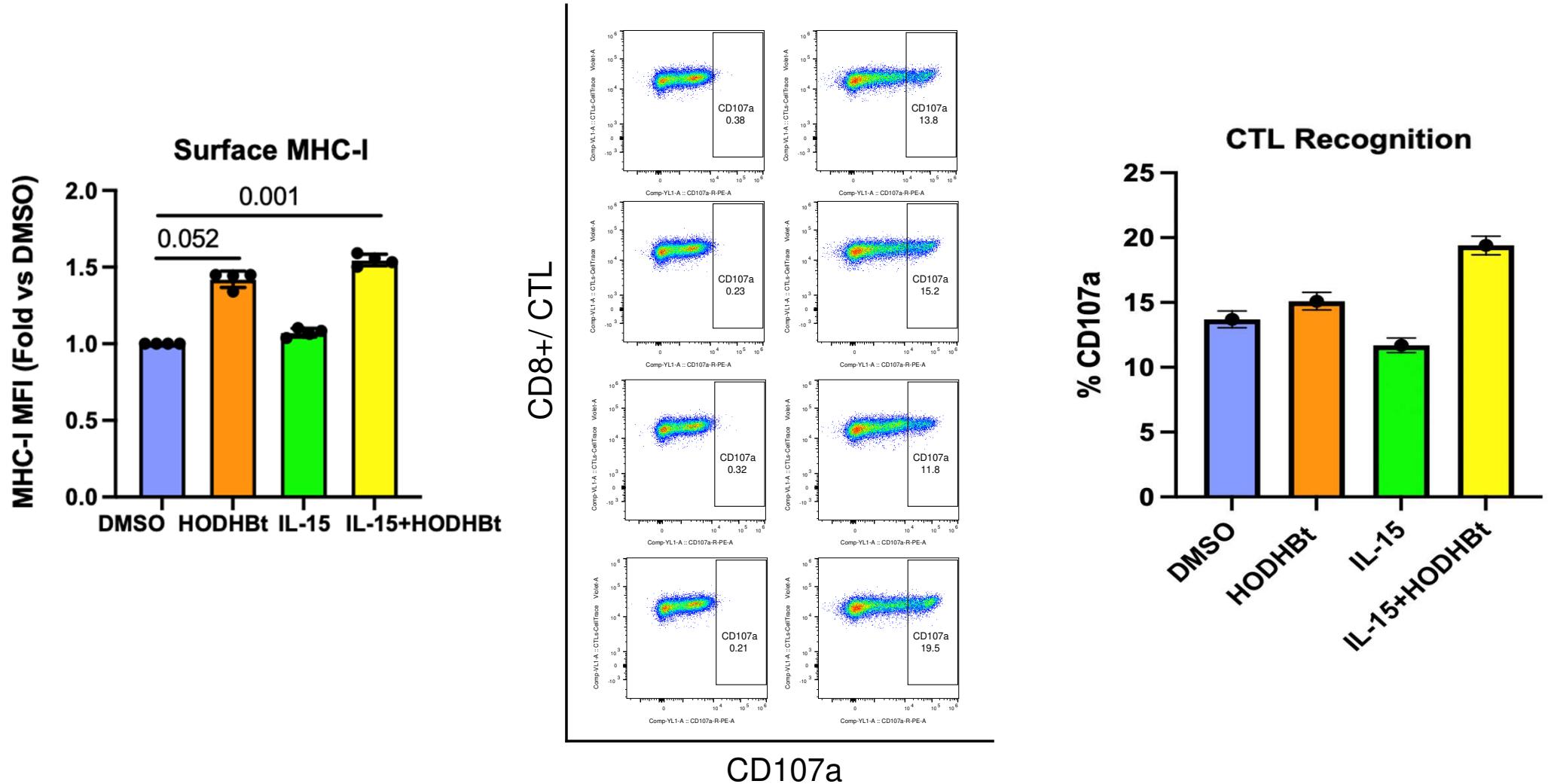
Wash 4x



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



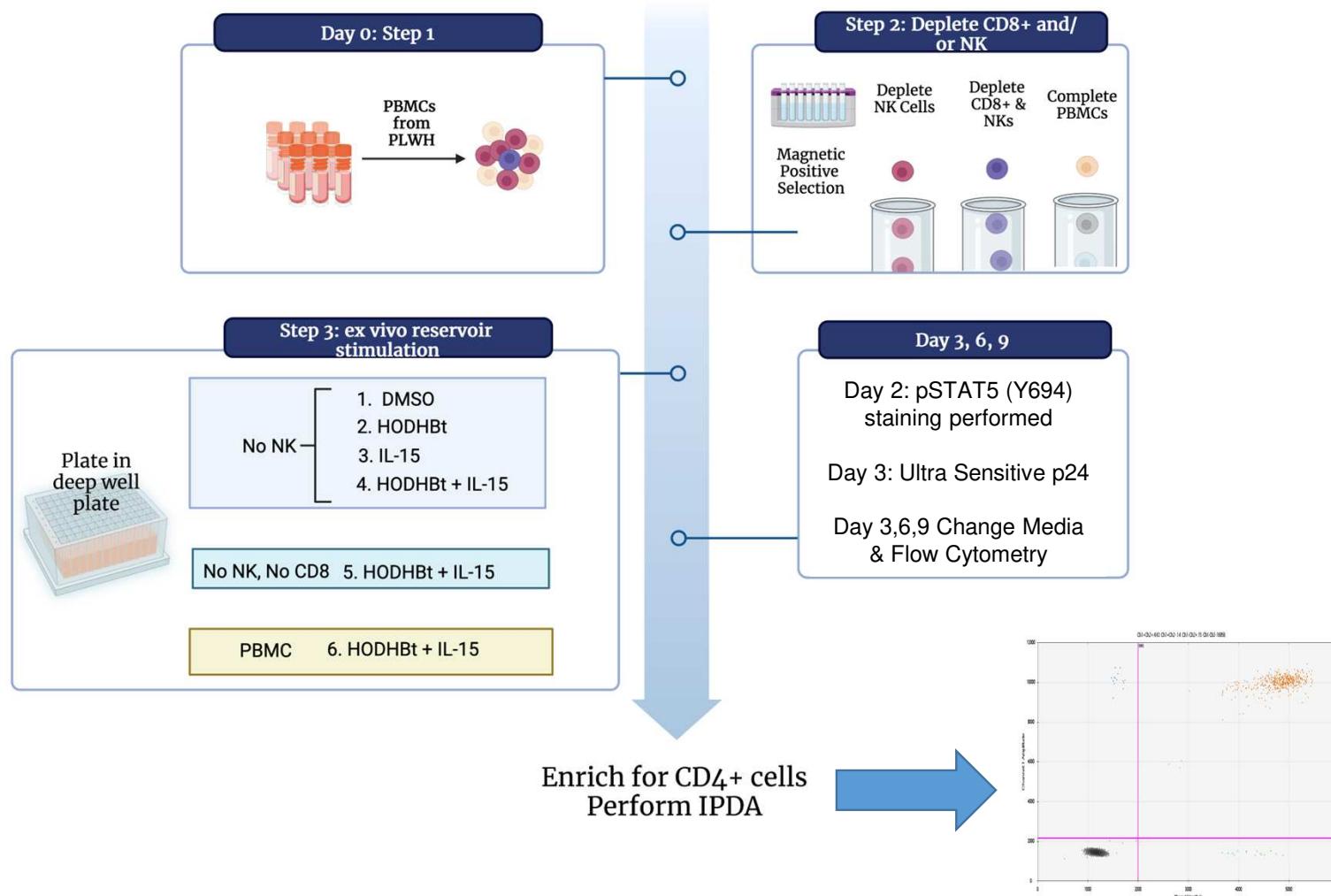
We Have shown that HODHBt & IL-15 Enhanced Cytolytic function of HIV Specific CD8+ T cells by GZMB ELISPOT

Treatment With HODHBt + IL-15 increases surface MHC-I and Antigen Presentation By CD4+ T Cells

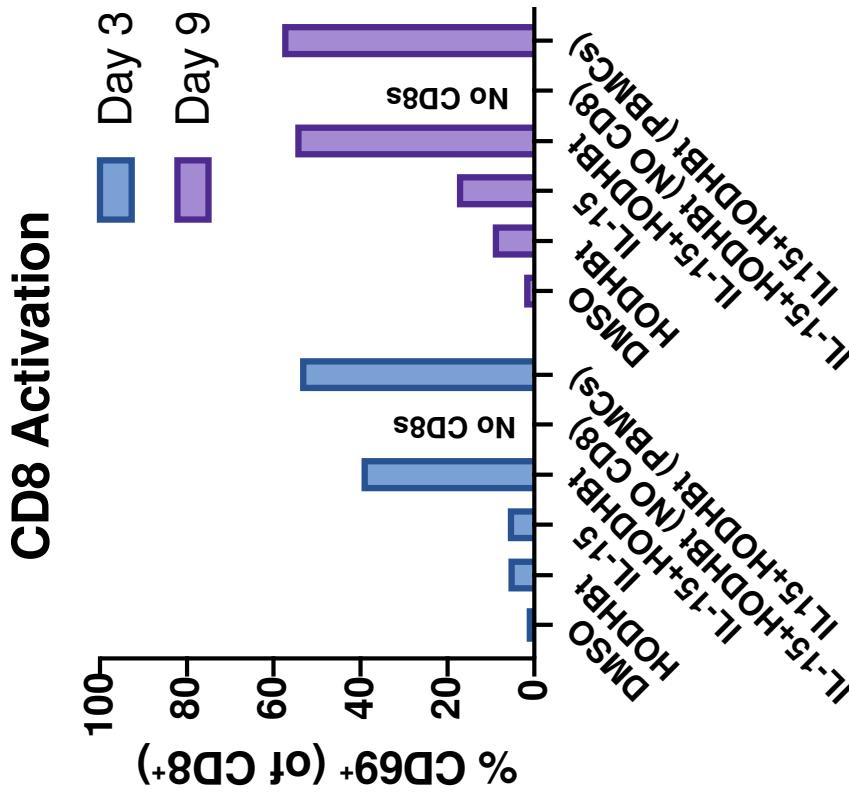
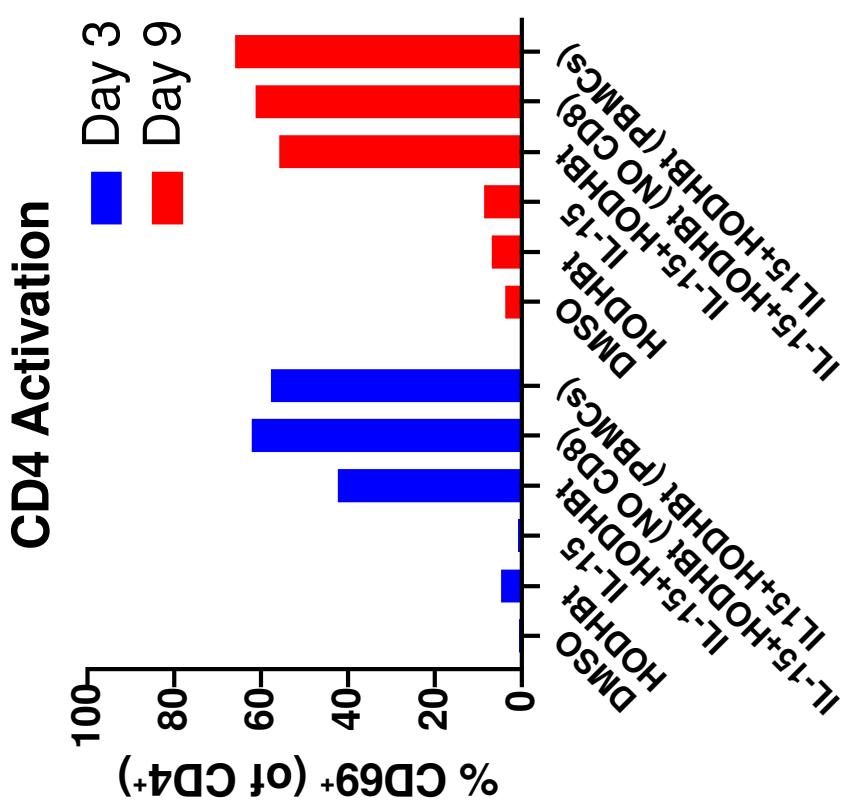
Are HODHBt + IL-15 Sufficient to Drive Reductions in *Ex vivo* HIV Reservoirs

HIV Eradication Assay 2.0 – HIVE 2.0

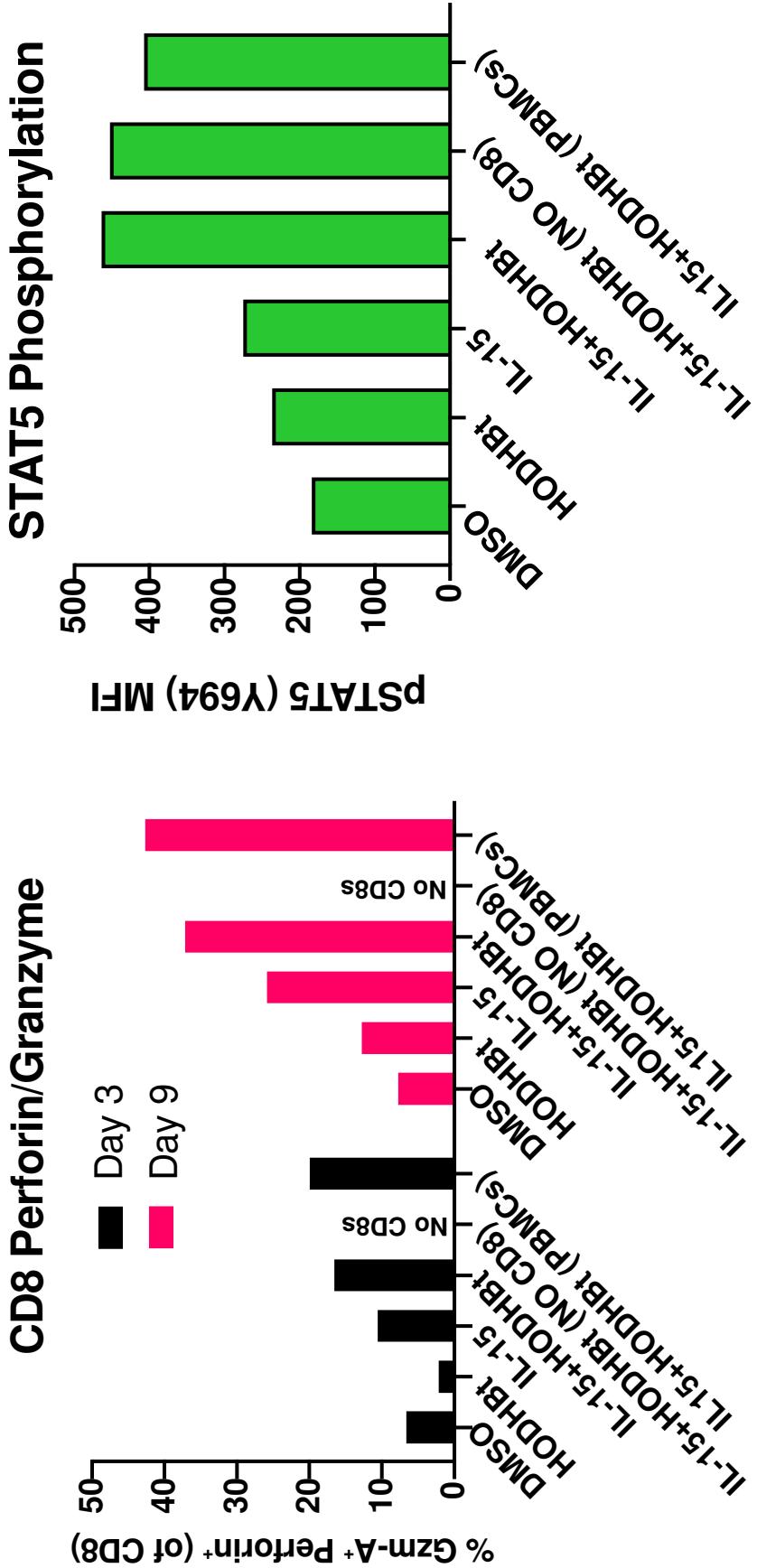
Assessing Both Latency Reversal & CD8+ Induced Killing of Reservoir



Treatment with HODHBT + IL-15 Increases Surface Expression of CD69 On Both CD4+ and CD8+ T Cells

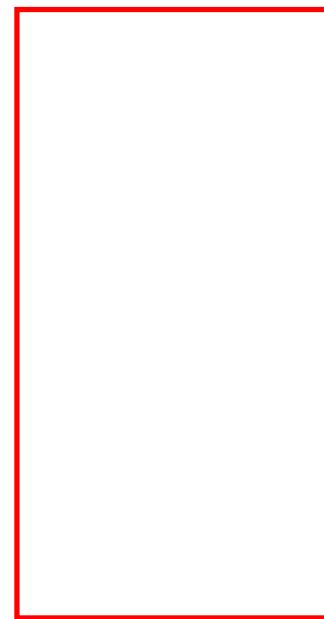
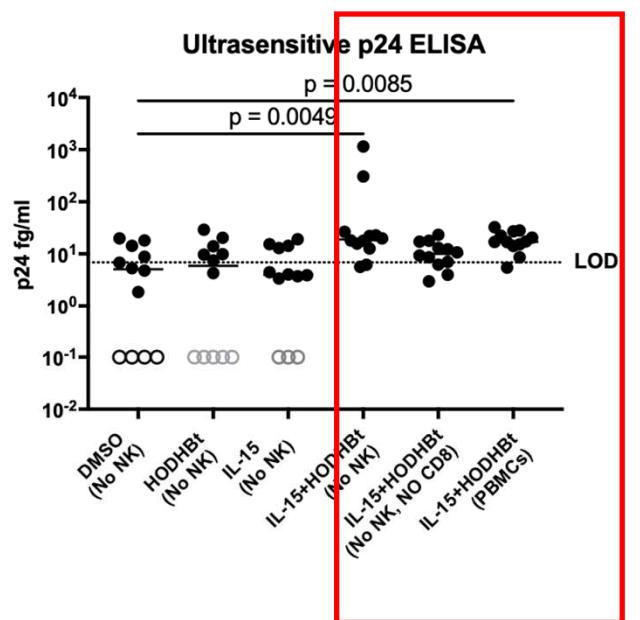


Treatment with HODHBT + IL-15 Increases STAT5 Phosphorylation and % of GZMA+ Perforin+ CTLs

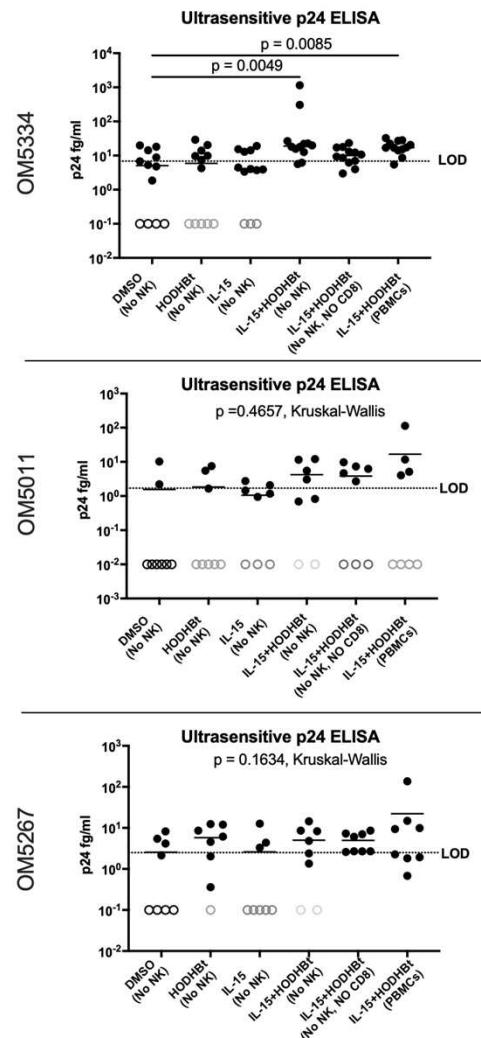


IL-15 + HODHBt Was Sufficient to Drive Intact Reservoir Reduction in ARV-Treated Donor

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 $\gamma\kappa$ cytokines like IL-2 with IL-15.



COMMUNITY SUMMARY

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.





Alberto Bosque



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Callie Levinger



Carissa Holmberg



Natalie Howard

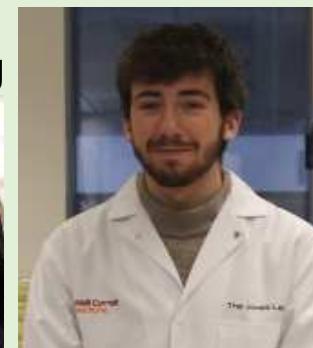


Weill Cornell Medicine

Michael Corley



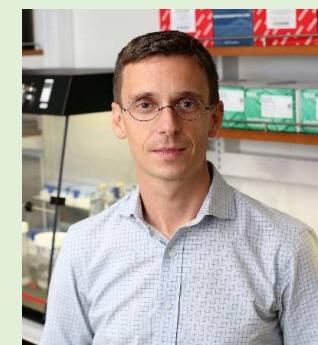
Jared Weiler



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- Paul Zumbo
- Doron Betel
- Andrea Gramatica

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- Bernard Macatangay
- Joshua C. Cykton
- John W. Mellors

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- 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



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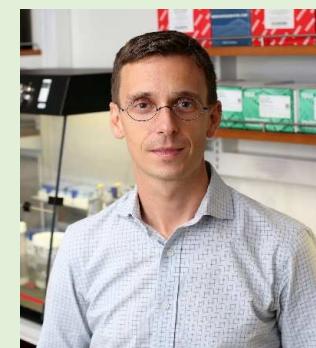
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