Poster 0355

NOD2 and TLR8 Agonists Enhance IL-15 Mediated Activation of HIV Expression

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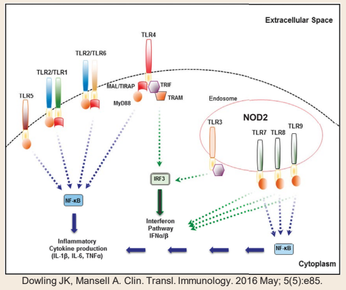
Background

- Antiretroviral therapy (ART) controls HIV viremia but does not eliminate CD4 T cells harboring latent HIV due to minimal expression of HIV antigen.[1,2,4,5]
- Pharmacologic activation of HIV gene expression in latent reservoirs may be required for the elimination of HIV infected CD4 T cells.[2,3]
- Latent CD4 T cells are difficult to eliminate due to minimal expression of HIV antigen.[4,5]
- Activation of Pattern recognition receptors (PRRs) such as toll-like receptors and nucleotide-binding oligomerization domain-like receptors stimulate the immune system and induce T cell activation indirectly through antigen presenting cells (APCs).[6]
- T cell activation of HIV+ CD4 T cells by PRRs can induce latent HIV in various in vitro models.[7]
- IL-15 activates CD4 T cells by engaging IL2Rβγ on the surface of CD4 T cells.

Figure 1: Toll-like receptor signaling in antigen presenting cells.

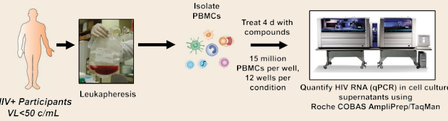


Dowling JK, Mansell A. Clin. Transl. Immunology. 2016 May; 5(5):e85.

- In this study we systematically evaluated PRR agonists for their potential to induce latent HIV and studied the cytokines induced upon PRR agonist treatment of PBMCs from ART suppressed people living with HIV (PLWH).
- We also evaluated the ability of IL-15 to enhance HIV expression.
- To increase the magnitude of HIV expression in latently infected cells we further evaluated combinations of each PRR with IL-15.
- Cytokines released were measured and the CD4 T cell activation profiles of lead PPR+IL-15 combinations were compared.

Methods

Figure 2: HIV expression measurement assay schematic.



- PLWH in this study were on ART with plasma viral load of < 50 copies/ml for > 1 year
- PBMCs obtained through leukapheresis were treated with agonist or DMSO vehicle control in the presence of antiretrovirals (100 nM Efavirenz + 100 nM Elvitegravir).

Methods (cont'd)

Treat PBMCs from suppressed HIV+ people with compound → Day 4 → Collect supernatant and cells for HIV RNA, cytokine production, cell activation markers

- The following parameters were assessed:
  - Supernatant cytokines were assessed 24 h after treatment of PBMCs cytokines using the ProcartaPlex multiplex immunoassay with a custom human 22 analyte panel
  - Supernatant HIV RNA was quantified 4 days after treatment using the Roche COBAS Amplirep/Amplitaq system (12 replicates/condition)
  - CD69, CD25 and Ki67 expression on CD4 T cells was quantified by flow cytometry 4 days after treatment
- Fold HIV activation = $\frac{\text{Mean Agonist vRNA (copies/ml)}}{\text{Mean DMSO vRNA (copies/ml)}}$
- Each point on the graph represents an individual donor.
- Donors were excluded if PMA + Ionomycin induced HIV activation < 5-fold
- Statistical significance was determined using Wilcoxon matched-pairs signed rank test

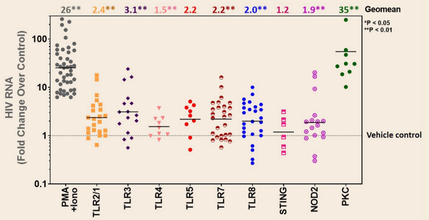
Table 1. Agonists Tested.

PRR	Agonist	Concentration
Toll-like receptor 2/1 (**TLR2/1**)	Pam3CSK4	0.1 µg/ml
Toll-like receptor 3 (**TLR3**)	Poly (I:C) High Molecular Weight	0.1 µg/ml
Toll-like receptor 4 (**TLR4**)	Monophosphoryl lipid A	1 µg/ml
Toll-like receptor 5 (**TLR5**)	Recombinant Flagellin-S. typhimurium	0.5 µg/ml
Toll-like receptor 7 (**TLR7**)	GS-9620	100 nM
Toll-like receptor 8 (**TLR8**)	GS-9688	300 nM
Stimulator of Interferon genes (**STING**)	GSI-066	4 µM
Nucleotide binding oligomerization domain (**NOD-2**)	Romurtide	0.1 µM
Interleukin-15 (**IL-15**)	recombinant human	1 nM
Protein kinase C (**PKC**)	Prostratin	5µM

Concentrations were selected based on relevant literature and dose titrations for cytokine production.

Results

Figure 3. Multiple PRR agonists induce HIV expression.

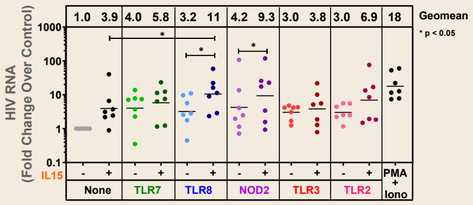


Wilcoxon matched-pairs signed rank test comparing geomean HIV vRNA copies/ml of treatment vs untreated baseline

- Pairwise combinations of each active PRR agonist were assessed and the results showed that HIV induction was comparable to that of the individual PRRs

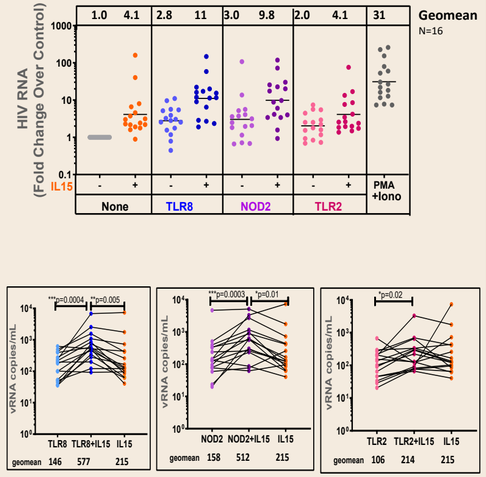
Results (cont'd)

Figure 4. Agonists of TLR8, TLR2, and NOD2 increase HIV expression induced by IL-15.



- HIV expression evaluated as fold change normalized to vehicle control.

Figure 5. IL-15 induces greater HIV expression with TLR8 and NOD2 agonists than TLR2 agonist in an independent set of donors.



- Lines connect results from the same donor

Figure 6. TLR8 agonist drives cytokines induced by the combination of TLR8 + IL-15.

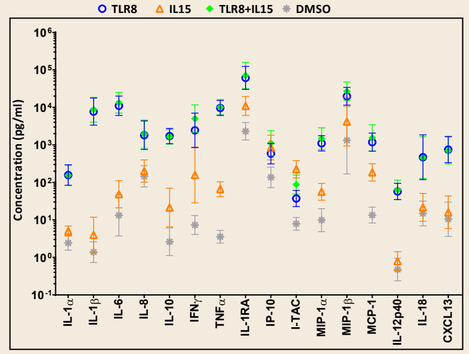
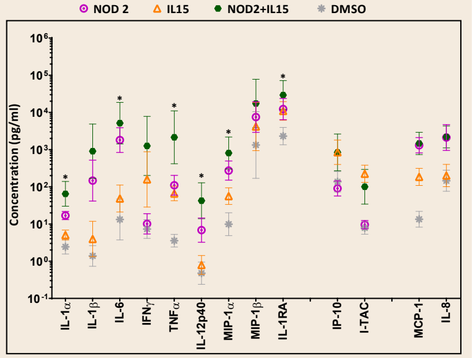
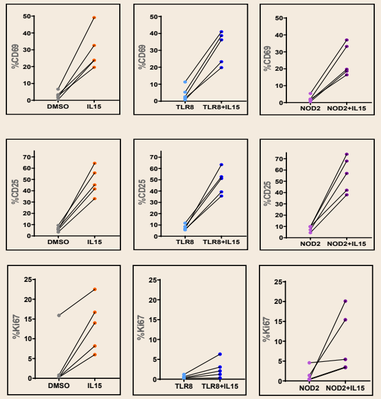


Figure 7. The combination of NOD2 and IL-15 produces higher levels of multiple cytokines than either agonist individually



- Cytokines were assessed from samples activated in figures 5 (n=12; geomean with 95% CI)
- Cytokines induced at least 5 fold are shown
- * p < 0.01 for the combination versus each individual agonist

Figure 8. CD4 T cell activation as measured by CD69, CD25 or Ki67 is primarily driven by IL-15 in TLR8 + IL-15 and NOD2 + IL-15 combinations.



- 5 donors from Figure 5 were assessed for CD4 T cell activation markers
- Lines connect results from the same donor

Conclusions

- Activation of HIV expression by IL-15 can be significantly enhanced by NOD2 and TLR8 agonists.
- Each of these agents has been dosed clinically and should be tested for latency reversal in vivo, ideally in combination with agents targeting the latent reservoir such as broadly neutralizing antibodies.

References: 1. Hernandez-Vargas E, et al. Modeling Kick-Kill Strategies toward HIV Cure. *Frontiers immu* 2017. **2.** Shan L, et al. From reactivation of latent HIV-1 to elimination of the latent reservoir: the presence of multiple barriers to viral eradication. *Bioessays* 2013. 35(6): 544-552. **3.** Deeks S. et al. HIV: Shock and kill. *Nature* 2012. 487:439-440. **4.** Imami N, et al. Multifarious immunotherapeutic approaches to cure HIV-1 infection. *Human Vaccines & Immunotherapeutics* 2015. 11(9): 2287- 2293. **5.** Datta P, et al. HIV-1 latency and Eradication: Past, Present and Future. *Curr HIV Res* 2016. 14(5): 431-441. **6.** Dowling JK, et al. Toll-like receptors: the swiss army knife of immunity and vaccine development. *Clinical & Translational Immunology* 2016. **7.** Novis C, et al. Reactivation of latent HIV-I in central memory CD4+T cells through TLR1/2 stimulation. *Retrovirology* 2013. 10:119.

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