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TLR7 Agonist GS-9620 Activates HIV-1 in PBMCs from HIV-Infected Patients on cART

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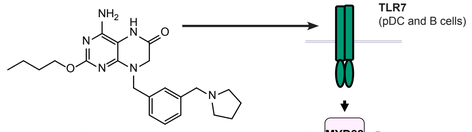
Overview

- Pharmacologic activation of latent HIV reservoirs is considered to be a key part of the strategy towards eradicating HIV-1 infection[1-3].
- GS-9620 is a selective TLR7 agonist currently being evaluated in patients with chronic hepatitis B infection (Phase 2)[4-6].
- In SIV-infected rhesus macaques on suppressive cART, a structurally close analog of GS-9620 induced plasma viremia and reduced viral DNA in PBMCs and tissues (Whitney et al., CROI 2015).
- The present study explores the effect of GS-9620 on HIV activation *ex vivo* in PBMCs from HIV-infected patients on cART.

GS-9620 Background



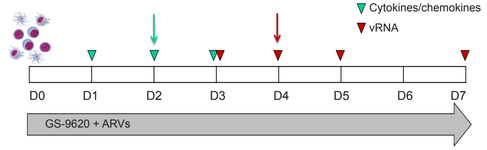
GS-9620

- Potent and selective TLR7 agonist
  - MEC* (TLR7) = 130 nM, MEC (TLR8) > 3 uM
- Orally bioavailable
- Suppressed chronic HBV in animal models[7,8]
- Induced ISGs in absence of systemic IFN-α in HBV-infected patients (Phase I)

*MEC = minimum effective concentration

Methods

- HIV-infected donors on cART (plasma vRNA <50 copies/mL for > 1 yr)
- PBMCs from donors' leukapheresis were treated with DMSO or GS-9620 (100 or 1,000 nM) in the presence of ARVs (EFV + EVG, 100 nM each)
- Cytokines/chemokines in culture supernatants were analyzed using 34-plex Luminex panel for type I IFNs and IFN-inducible cytokines
- HIV-1 RNA in cell-free culture supernatants was quantified by real-time qRT-PCR using the AmpliPrep/COBAS® TaqMan® assay



Results

Figure 1. Activation of HIV by GS-9620 *Ex Vivo* in PBMCs from HIV+ Patients on cART

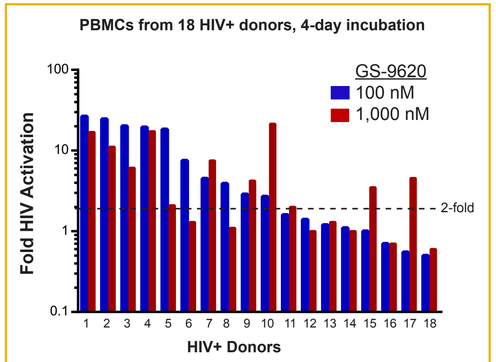


Figure 2. Activation of HIV by GS-9620 *Ex Vivo*

N = 18	GS-9620		
	100 nM	1,000 nM	100 and/or 1,000 nM
N (%) with ≥ 2-fold activation	10 (56)	11 (61)	13 (72)
Fold range if ≥ 2-fold activation	2.7 – 26.6	2.0 – 21.5	2.0 – 26.6
Fold geometric mean if ≥ 2-fold activation	9.3	6.5	9.1

Dose response examples:

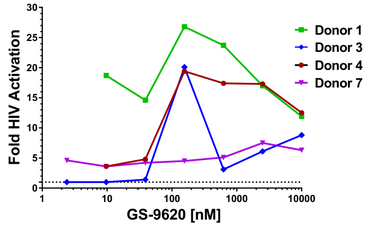
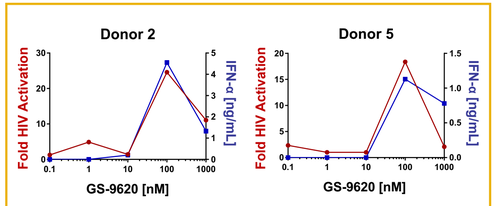


Table 1. Induction of Cytokines/Chemokines by GS-9620 *Ex Vivo*

GS-9620 fold induced vs. DMSO control*	Cytokine
> 100-fold	IFNα, IFNω, IL-1 RA, IL-6, IL-10, IP-10, I-TAC, MIP-1α, MIP-1β, MCP-1
10- to 100-fold	IFNγ, IL-8, IL-29, GROα, IL-1β, TNFα
2- to 10-fold	IL-2, IL-12, IL-15, IL-21, IL-23(p19), RANTES, TRAIL, Granzyme B, SDF-1α
< 2-Fold	BAFF, IFNβ, IL-4, IL-5, IL-12(p70), IL-13, IL-17, IL-18, IL-27, IL-31, sCD40L, TNFS10, MMP-1

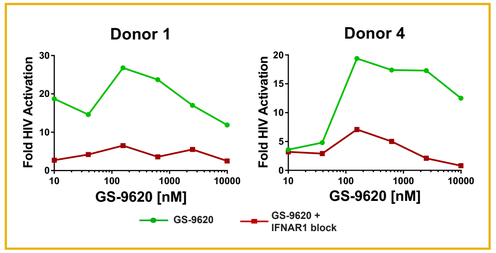
*Peak production of each cytokine/chemokine induced by GS-9620 (1 nM to 10 µM) in Donors 1 – 8

Figure 3. Correlation of GS-9620-Induced IFN-α with HIV Activation



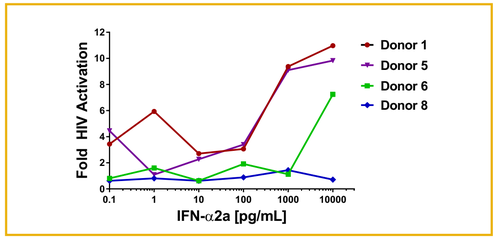
- Examples of PBMCs from 2 HIV+ donors on cART, treated with DMSO or GS-9620
- IFN-α in culture supernatant measured by Luminex on Day 2
- HIV RNA in culture supernatant quantified by qRT-PCR (COBAS) on Day 4

Figure 4. Maximal HIV Activation by GS-9620 Is Dependent on IFN-α/β Receptor Signaling



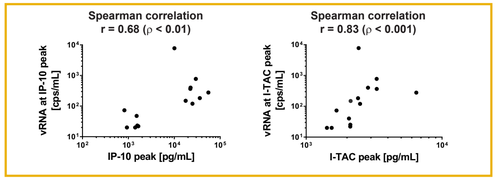
- Examples of PBMCs from HIV+ donors on cART, treated with DMSO or GS-9620 alone or in combination with anti-IFN-α/β receptor mAb (IFNAR1 block)
- HIV RNA in culture supernatant quantified on Day 4
- 85% mean maximal reduction in 4 donors ($p < 0.05$, two-tailed t-test)

Figure 5. Activation of HIV by Recombinant IFN-α *Ex Vivo*



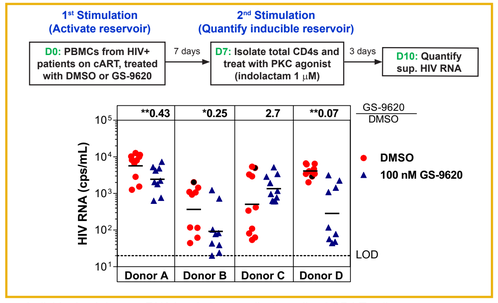
- PBMCs from HIV+ donors on cART (n=4), treated with recombinant IFN-α2a
- HIV RNA in culture supernatant quantified on Day 4

Figure 6. Activation of HIV by GS-9620 Correlated with Peak Production of IP-10 (CXCL10) and I-TAC (CXCL11) Induced by GS-9620



- PBMCs from HIV+ donors on cART (n=14) treated with GS-9620 (1 – 1,000 nM)
- Chemokines and vRNA quantified on Day 2 and Day 4, respectively
- For each donor, vRNA production was plotted as a single point at the GS-9620 concentration that induced peak production of IP-10 or I-TAC. No significant correlation was observed for other cytokines/chemokines measured.

Figure 7. GS-9620 Reduced the HIV Reservoir Re-stimulation by PKC Agonist



- Individual points are vRNA levels from replicate wells of CD4 T cells
- Differences between GS-9620 and DMSO (*p < 0.05, **p < 0.01, two-tailed t-test)

Conclusions

- **GS-9620 activated HIV expression *ex vivo* in PBMCs from HIV-infected patients on cART with prolonged virologic suppression**
  - **GS-9620 (100 - 1,000 nM) induced ≥ 2-fold HIV activation in PBMCs from 13/18 (72%) donors with a geometric mean of 9.1-fold activation.**
  - **Donor-dependent variation in HIV activation may be due to combined "kick" and "kill" effects induced by GS-9620**
- **GS-9620 induced type I IFNs and IFN-inducible cytokines. IFN-α/β receptor signaling was required for maximal activation of HIV by GS-9620**
- **Recombinant IFNα-2a activated HIV in PBMCs from 3/4 donors. IFN-α2a did not activate HIV in CD4 T cells from 2/2 donors (data not shown)**
- **Positive correlation was observed between GS-9620-induced peak levels of IP-10 (CXCL10) and I-TAC (CXCL11) and HIV activation levels. The role of these chemokines in the activation of HIV remains to be established**
- **GS-9620 treatment reduced subsequent response of latent HIV reservoir to PKC agonist-mediated activation. Additional studies are needed to determine if this result is indicative of HIV reservoir reduction**
- **Given these results, and the results of TLR7-induced activation of SIV in cART-suppressed rhesus macaques (Whitney et al, CROI 2015), a clinical study of GS-9620 in HIV-infected patients on cART has been initiated**

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