TLR7 Agonist Treatment of SIV+ Monkeys on ART Can Lead to Complete Viral Remission

Reported by Jules Levin
CROI 2017 Feb 14-16 Seattle, WA

So-Yon Lim1, Christa E. Osuna1, Joseph Hesselgesser2, Alison L. Hill3, Michael D. Miller2, Tomas Cihlar2, Romas Geleziunas2, William A. Lee 2 and James B. Whitney1,4
1Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA; 2 Gilead Sciences, Inc. Foster City, CA 94404; 3Program for Evolutionary Dynamics, Harvard University, Cambridge, MA 02138 USA; 4Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA 02139, USA

CONCLUSIONS

- Consistent with the observed lack of ex-vivo SIV production in both PBMC and LNMC following *in vitro* ConA stimulation (Whiteny et al. CROI2016), two RMs that received either GS-986 (0.1mg/kg) or GS-9620 (0.15mg/kg) maintained undetectable plasma viral load for >1 yr after stopping ART.

- Comparisons of both virologic and immunologic parameters between seven viremic and two remission RMs following TLR7 agonist administration indicate:

  - reduction in cell-associated SIV-DNA from tissue compartments including peripheral blood, lymph node and colorectal mucosa in 67-100 % RMs treated with TLR7 agonists with the most significant decrease in either T_{TM} subset

  - a significant reduction of SIV DNA in T_{CM} from both PBMC and LNMC only in two remission RMs following TLR7 treatment

  - a significant change in peak level of I-TAC (CXCL11) in two remission RMs compared to seven viremic animals during 1-10 doses of TLR7 agonists

  - no significant difference in the peak level of IL-1RA in plasma

  - no significant difference in mRNA levels of ISGs induced following TLR7 agonist treatment

- Longitudinal assessment of two remission RMs following ART stop showed:
  - uniformly negative VOA and VCC results
  - no detectable SIV specific T cell responses measured by IFNγ
  - lack of rebound viremia after in vivo CD8+ T cell depletion
- Adoptive transfer of PBMC and LNMC cells isolated 448 days after ART stop did not induce SIV infection in naïve recipients.
- Administration of GS-986 or GS-9620 to SIV+ ART-suppressed RM is safe, can lower viral set-point after rebound or induce durable long-term remission after ART stop.
- Clinical studies of GS-9620 in ART-treated HIV+ participants are ongoing.

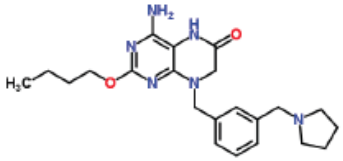
BACKGROUND

- Latent reservoirs of replication competent HIV-1 persist in patients on antiretroviral therapy (ART) and represent the major obstacle to HIV eradication efforts.
- Multiple cure approaches being undertaken, but the focus is on virus reactivation from latency combined with immunomodulation i.e. "shock and kill".
- The identification of pharmaceutical agents capable of safely reversing HIV-1 latency in ART-treated patients is urgently needed.

TLR7

- Expressed in plasmacytoid dendritic cells and B lymphocytes
- Part of the innate immune system linked to adaptive immunity
- TLR7 activation leads to
  - increased antigen presentation
  - enhanced NK and CD8+ T cell activation (KILL)
  - activation of CD4+ T cells

GS-9620 (Vesatolimod)
4-amino-2-butoxy-8-[[3-(pyrrolidin-1-ylmethyl)phenyl]-methyl]-5,7-dihydropteridin-6-one



- Potent, selective and orally deliverable TLR7 agonist
- Anti-viral activity against HBV in animal models and HIV-1 in *in vitro* model

• We previously demonstrated the activity of TLR7 agonists (GS-986 and GS-9620) in SIV-infected ART-suppressed RM to induce :

- activation of immune cells with the greatest change in the effector memory subpopulation of CD4+ and CD8+ T cells and NK cells
- induction of transient plasma viremia
- induction of cytokines/chemokines
- induction of ISGs in the absence of IFN-α
- reduction of viral DNA content in PBMCs, colon and lymphoid tissues

(**Whitney et al. CROI2016**).

Abstract Body:
The persistence of an HIV-1 reservoir in patients on antiretroviral therapy (ART) represents the major obstacle to HIV remission. We have previously reported that oral TLR7 agonists (GS-9620 and GS-986) can induce transient viremia in SIV-infected, ART-treated rhesus monkeys (RMs). After ART release of nine TLR7 agonist-treated RMs, complete remission was noted in two RMs, and two other RMs have exhibited significant control of recrudescent viremia.

After repeated GS-9620 or GS-986 adminsitration and ART stop, we have conducted a long-term follow up of two 'remission' RMs. We also monitored 2 RMs that had rebound viremia after ART stop, as controls. We assessed multiple endpoints including: viral outgrowth (VOA) and viral co-culture (VCC) using lymph node mononuclear cells (LNMC) and PBMC. We assessed SIV-specific T cell responses at multiple time points. We also conducted in vivo CD8+ T cell depletion in remission and viremic control RMs. Finally, we adoptively transferred PBMC and LNMC cells into SIV naïve RMs, from remission RMs prior to TLR7 agonist treatment. In parallel, we also transferred PBMC and LNMC, into SIV naïve RMs, isolated from remisson RMs after GS-9620 or GS-986 treatment and ART stop.

To date, both TLR7 agonist-treated RMs have remained aviremic for more than 1 year after ART cessation. Longitudinal assesment of VOA or VCC in the two remission RMs was uniformly negative, whereas viremic RMs scored consistently postive. Longitudal SIV-specific immune monitoring revealed sustained responses in viremic RMs, but no detectable SIV-specific T cell responses in remission RMs. In vivo CD8+ T cell depletion did not induce rebound viremia in remission RMs, but viremic control RMs exhibited significant increases in SIV RNA levels that later waned as CD8+ T cells recovered. Finally, the adoptive transfer of PBMC and LNMC samples (prior to TLR7 agonist treamtent) induced a persistent SIV infection into naïve RMs. Adoptive transfer of PBMC and LMNC cells from remission RMs did not induce SIV infection in naïve recipients.

Administration of GS-9620 or GS-986 to SIV+ ART-suppressed RM is safe, induces transient

viremia and impacts SIV DNA levels. GS-986 or GS-9620 treatment can delay viral rebound or induce durable long-term remisison after ART cessation in some RMs. These novel findings highlight a possible mechanism of SIV remission after ART cessation and underscore the need for continued investigation of GS-9620 in HIV-1 infected patients on ART.

METHODS

- Indian Rhesus macaques (*Mamu-A*001, B*008, B*17 defined)* were intrarectally (IR) challenged with SIVmac251 (n=11)

- Combination antiretroviral therapy (cART) was initiated day 65 post-infection (TFV, FTC, DTG s.c. q.d.)

- TLR7 agonist treatment was initiated after 400 days of ART suppression.

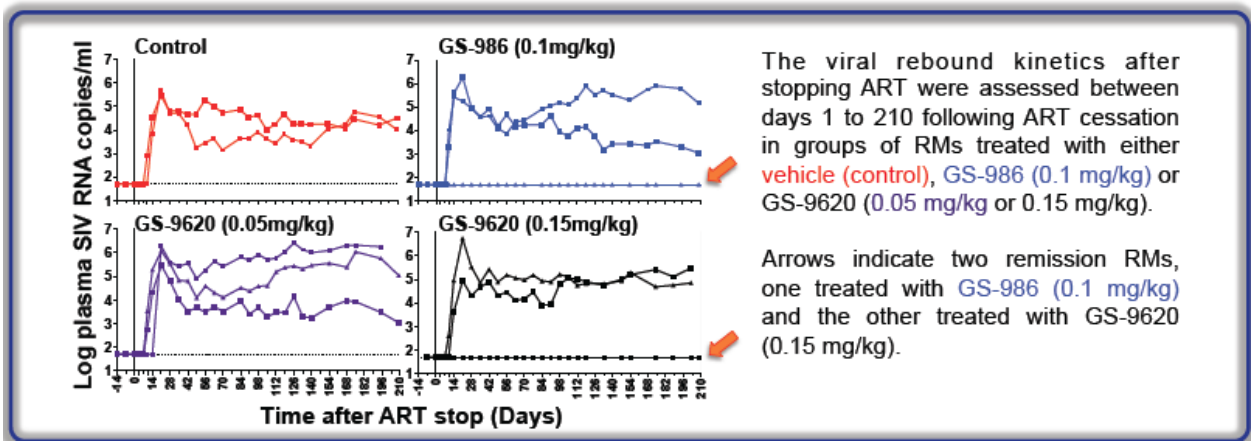
ART stop

Placebo		EOW x 10	3 months	EOW x 9
GS-986	0.1 mg/kg	EOW x 10	3 months	EOW x 9
GS-9620	0.05 mg/kg	EOW x 10	3 months	EOW x 9
GS-9620	0.15 mg/kg	EOW x 10	7 months	

- Endpoints
  - Monitor immune activation and change in plasma viral RNA
  - Perturbation of the reservoir
  - Viral rebound after stopping cART
  - Long-term follow up of remission RMs (n=2)
    - SIV-specific T cell responses
    - Viral outgrowth (VOA) and Viral co-culture (VCC)
    - *In vivo* CD8 depletion
    - Adoptive transfer

RESULTS

Figure 1. SIV plasma RNA rebound kinetics after stopping ART



The viral rebound kinetics after stopping ART were assessed between days 1 to 210 following ART cessation in groups of RMs treated with either vehicle (control), GS-986 (0.1 mg/kg) or GS-9620 (0.05 mg/kg or 0.15 mg/kg).

Arrows indicate two remission RMs, one treated with GS-986 (0.1 mg/kg) and the other treated with GS-9620 (0.15 mg/kg).

Figure 2. Changes in SIV DNA level in memory CD4 T cells isolated from RMs treated with TLR7 agonists

Reduction of SIV DNA level in RMs treated with TLR7 agonists

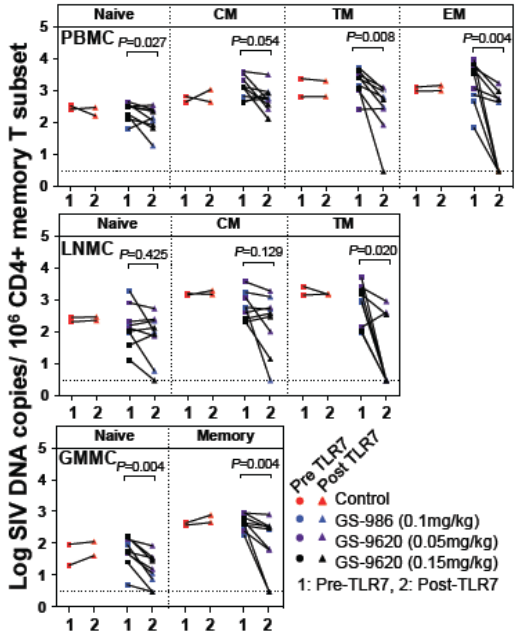


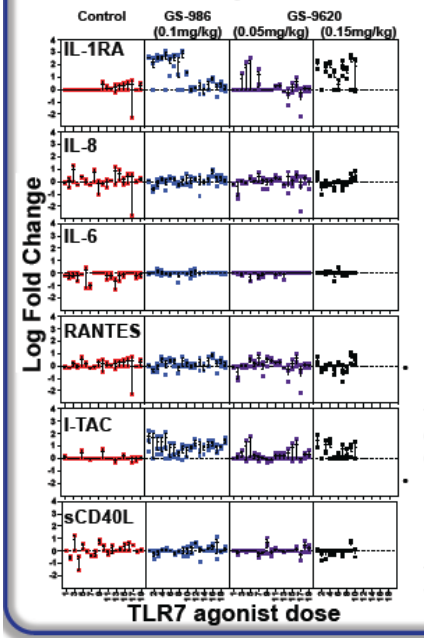
Table 1. Comparison of SIV DNA level between viremic and remission RMs treated with TLR7 agonists

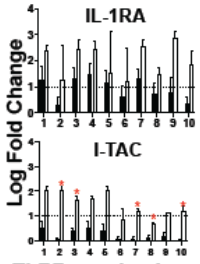
Tissue	Memory Subset	TLR7 agonist treatment	[1]SIV-DNA Viremic (n=7)	[1]SIV-DNA Remission (n=2)	[2]P value
PBMC	T_{CM}	Pre	2.58 ±0.41	2.33 ± 0.20	0.447
		Post	2.16 ±0.36	1.51±0.09	**0.046**
		[3]P value	0.064	**0.034**	
	T_{TM}	Pre	2.31 ±0.45	2.14 ±0.07	0.627
		Post	1.62 ±0.34	0.48 ±0.00	**0.003**
		[3]P value	**0.007**	**0.001**	
	T_{EM}	Pre	1.50 ±0.61	0.80 ±0.45	0.182
		Post	0.95 ±0.61	0.48±0.00	0.333
		[3]P value	0.117	0.420	
LNMC	T_{CM}	Pre	2.12 ±0.41	2.03 ±0.10	0.777
		Post	2.30 ±0.49	0.90 ±0.60	**0.011**
		[3]P value	0.470	0.119	
	T_{TM}	Pre	2.52 ±0.51	2.40 ±0.05	0.214
		Post	1.24±0.92	0.48 ±0.00	0.303
		[3]P value	**0.007**	**0.001**	
GMMC	Memory	Pre	2.36. ±0.40	2.43 ±0.24	0.205
		Post	1.37±0.35	0.48 ±0.00	0.134
		[3]P value	**0.0125**	0.116	

[1]Log SIV-DNA copies/10^6 CD4+ memory T cells are shown.
[2]*P* values calculated for a unpaired t test between viremic and remission RMs.
[3]*P* values calculated for a paired t test between pre and post TLR7 treatment.

Figure 3. *In vivo* induction of cytokines/chemokines and ISGs in RMs following TLR7 administration

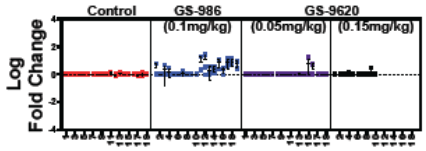
A. Cytokines/chemokines



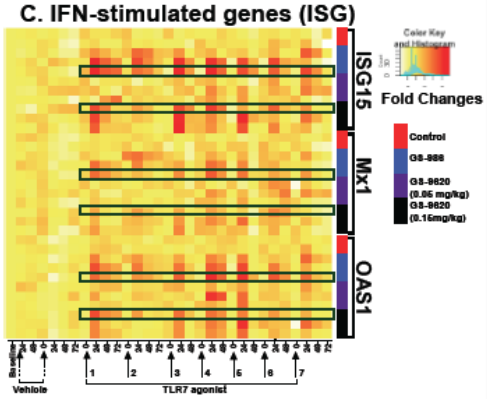


- Significant changes in peak levels of IL-1RA and I-TAC following TLR7 agonist doses were detected after each TLR7 dose.
- Differences in peak level of IL-1RA and I-TAC in two remission animals during 1-10 doses were compared to those levels in seven viremic animals.

B. Induction of IFN-α by TLR7 agonist

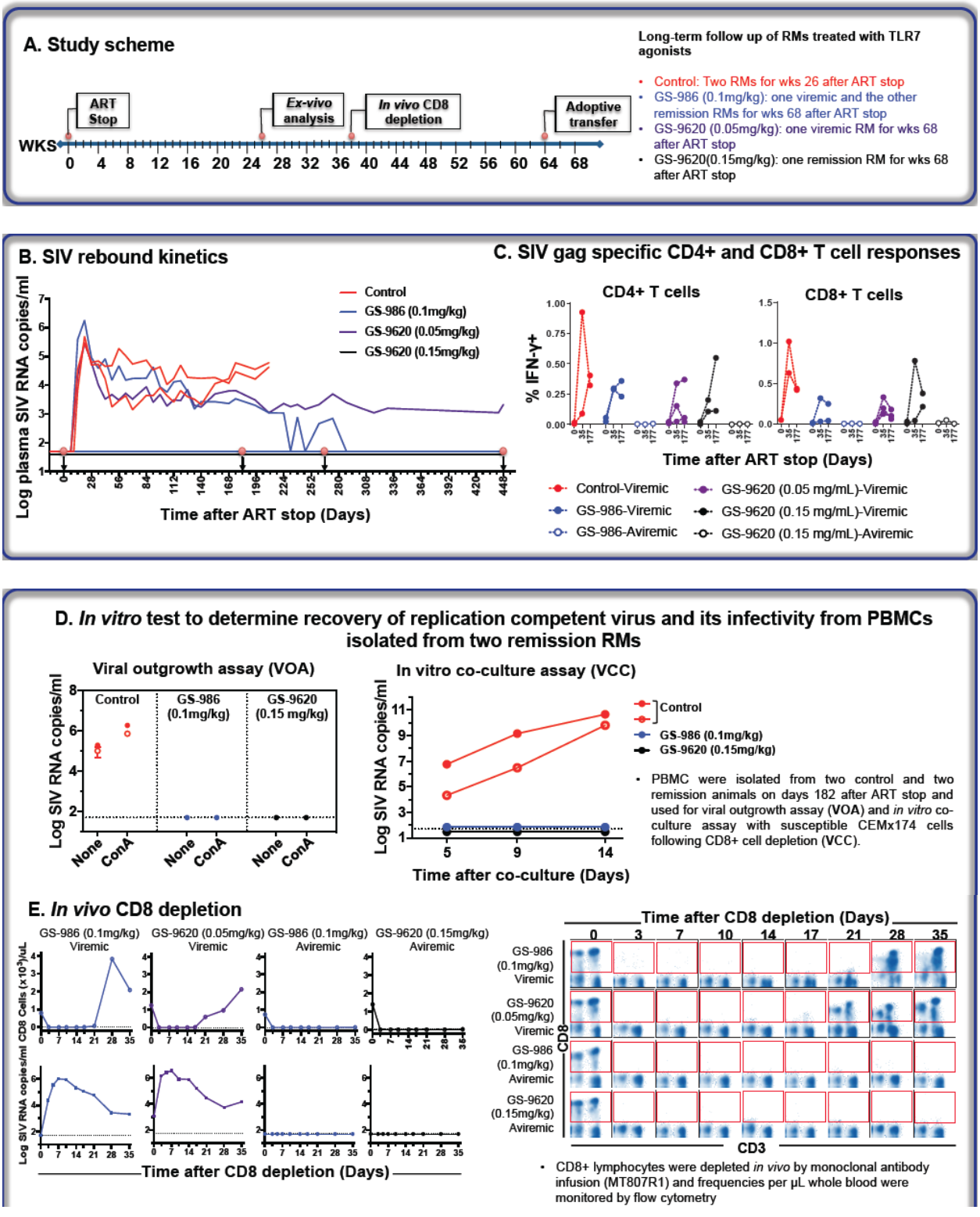


C. IFN-stimulated genes (ISG)

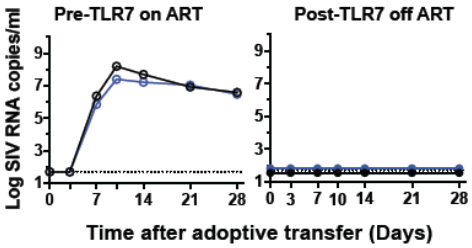


- Induction of ISG15 and OAS1 are highlighted by dark green boxes for two remission RMs treated with GS-986 or GS-9620 (0.15mg/kg)

Figure 4. Long-term follow up of RMs treated with TLR7 agonists after ART stop



F. Adoptive transfer



Pre-TLR7 on ART: GS-986 (0.1mg/kg) Aviremic; GS-9620 (0.15mg/kg) Aviremic

Post-TLR7 off ART: GS-986 (0.1mg/kg) Aviremic; GS-9620 (0.15mg/kg) Aviremic

- Both PBMCs and LNMCs isolated from two remission RMs (GS-986 (0.1mg/kg) Aviremic, GS-9620 (0.15mg/kg) Aviremic) either prior to TLR7 agonist treatment on ART or 448 days after ART stop were infused into naïve monkeys.

ACKNOWLEDGMENTS

This work was supported by Gilead and NIAID grants, AI122942-01, AI091514-01A1 and AI127089-01 to JBW.

Correspondence: jwhitne2@bidmc.harvard.edu