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


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## BACKGROUND

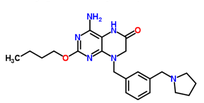
• Latent reservoirs of replication competent HIV-1 persist in patients on antiretroviral therapy (ART) and represent the major obstacle to HIV eradication efforts.

• 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**).

## METHODS

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

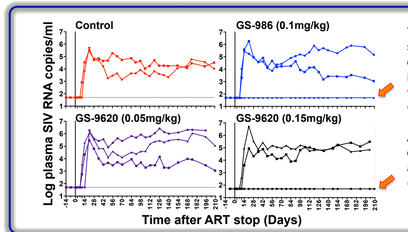
• Combination antiretroviral therapy (cART) was initiated day 65 post-infection (TFV, FTC, DTG s.c. q.d.)

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


• 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

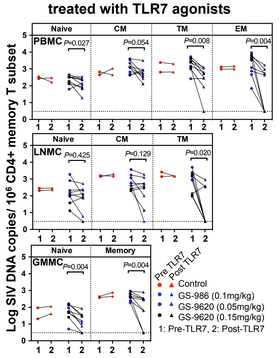
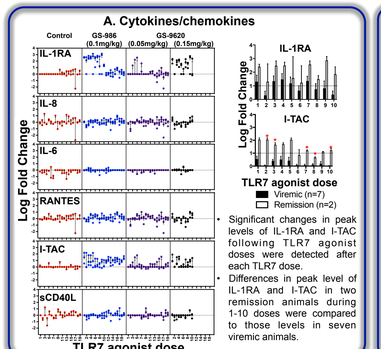



Table 1. Comparison of SIV DNA level between viremic and remission RMs treated with TLR7 agonists

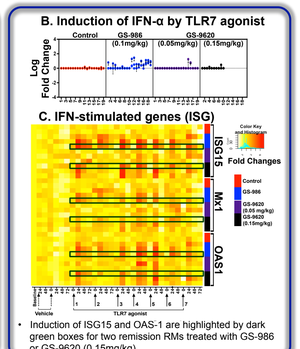
| Tissue | Memory Subset | TLR7 agonist treatment | [1]SIV-DNA Viremic (n=7) | [1]SIV-DNA Remission (n=2) | [2]P value |
|---|---|---|---|---|---|
| PBMC | $T_{CM}$ | Pre | 2.58 ±0.41 | 2.33 ± 0.20 | 0.447 |
| | | Post | 2.16 ±0.36 | 1.51±0.09 | **0.046** |
| | | [3]P value | 0.064 | **0.034** | |
| | $T_{TM}$ | 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**

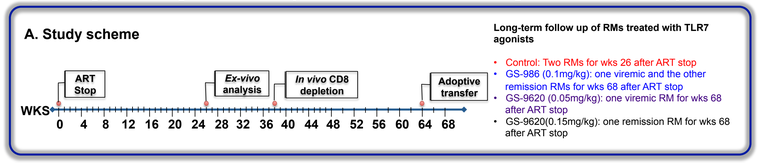



- Significant changes in peak levels of IL-1RA and I-TAC following TLR7 agonist doses were detected after each TLR7 dose.
- Differences in peak level of IL-1RA and I-TAC in two remission animals during 1-10 doses were compared to those levels in seven viremic animals.

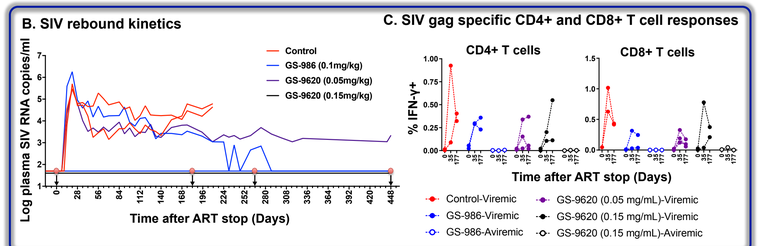



- Induction of ISG15 and OAS-1 are highlighted by dark green boxes for two remission RMs treated with GS-986 or GS-9620 (0.15mg/kg)

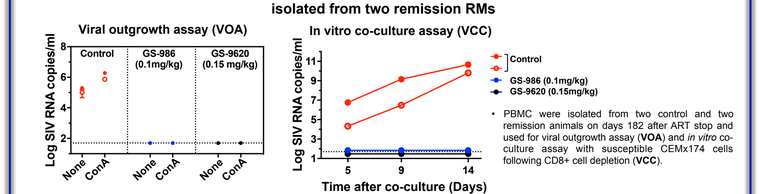
**Figure 4. Long-term follow up of RMs treated with TLR7 agonists after ART stop**




**Long-term follow up of RMs treated with TLR7 agonists**
- Control: Two RMs for wks 26 after ART stop
- GS-986 (0.1mg/kg): one viremic and the other remission RMs for wks 68 after ART stop
- GS-9620 (0.05mg/kg): one viremic RM for wks 68 after ART stop
- GS-9620(0.15mg/kg): one remission RM for wks 68 after ART stop

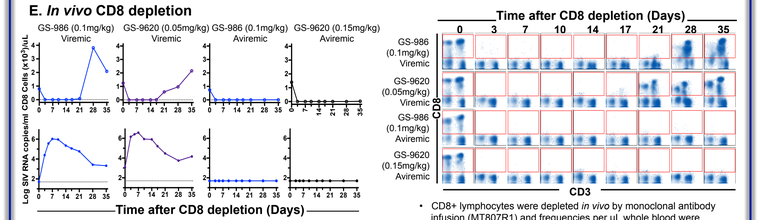



**D. *In vitro* test to determine recovery of replication competent virus and its infectivity from PBMCs isolated from two remission RMs**



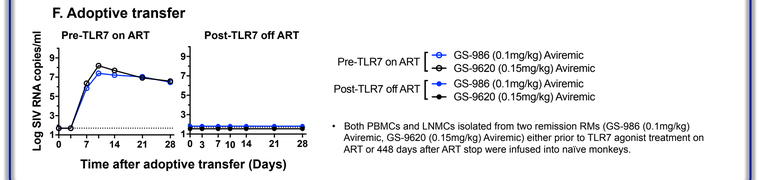

- PBMC were isolated from two control and two remission animals on days 182 after ART stop and used for viral outgrowth assay (**VOA**) and *in vitro* co-culture assay with susceptible CEMx174 cells following CD8+ cell depletion (**VCC**).

**E. *In vivo* CD8 depletion**




- CD8+ lymphocytes were depleted *in vivo* by monoclonal antibody infusion (MT807R1) and frequencies per μL whole blood were monitored by flow cytometry

**F. Adoptive transfer**




- 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.

## 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.

## ACKNOWLEDGMENTS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