#1188: Robust and Persistent SIV Reactivation by N-803 in ART-treated CD8-depleted Macaques

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Abstract

HIV persists indefinitely in antiretroviral therapy (ART)-treated individuals due to a reservoir of latently-infected cells harboring replication-competent virus. Here we used the IL-15 superagonist N-803 as a latency reversing agent (LRA) in ART-suppressed Simian Immunodeficiency Virus (SIV)-infected macaques. N-803 is a complex of an IL-15 superagonist mutant and a dimeric IL-15 receptor αSu/Fc fusion protein. The engineered structure is at least 25-times more biologically potent than IL-15 as it mimics transpresentation and the IgG-Fc component confers better in vivo safety and bio-availability. While N-803 alone did not reactivate virus production, administration after CD8+ lymphocyte depletion induced the most robust and persistent virus reactivation ever observed in humans or macaques, with viremia >60 copies/mL of plasma in 14/14 animals (100%) and 41/56 samples (73.2%) collected one week after each N-803 administration, and viremia >1,000 copies/mL in 6/14 animals (42.9%) and 13/56 (23.2%) of the same samples. Similar results were obtained in ART-treated HIV-infected humanized mice. N-803 deserves further investigation for LRA-based strategies to functionally cure HIV infection.

Rationale

- CD8+ T cells do not decrease the lifespan of cells productively infected with SIV *in vivo*, instead suggesting a non-cytolytic role (Klatt et al., *PLoS Path* 2010)
- CD8+ T cells are not required for the suppression of SIV viremia during ART *in vivo* (Cartwright et al., *Immunity* 2016)
- TCR-activated CD8+ T cells suppress viral replication and LTR-dependent viral transcription (Michelle Zanoni, CROI Abstract #0376)
- CD8+ T cells show a suppression of HIV expression in memory CD4+ T cells during the establishment of infection and help to maintain HIV latency during CD4+ T cell activation (Deanna Kulpa, CROI New Cells on the Block Symposium Speaker)

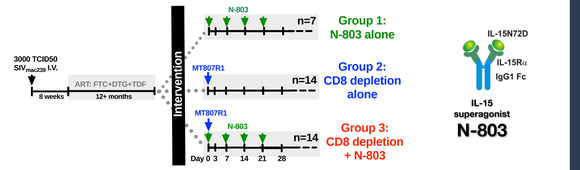
Hypothesis: CD8+ T cells suppress HIV/SIV transcription during HIV/SIV infection. Therefore, effective *in vivo* latency reversal is inhibited by CD8+ T cells. Delivery of N-803, a latency reversing agent, in the absence of CD8+ T cells will permit effective latency reversal.

Methodology

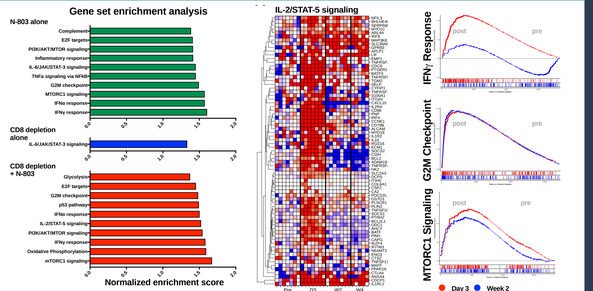
35 rhesus macaques were infected with 3000 TCID50 SIV_{mac239} I.V. and initiated on combination ART 8 weeks later (emtricitabine + tenofovir disoproxil fumarate + dolutegravir). After 1 year of ART, animals were separated into intervention groups. 7 RM received four weekly doses of 100 μg/kg N-803 subQ, 14 received 50 mg/kg of the CD8α-depleting Ab MT-807R1, and 14 RM received both treatments. All animals underwent ART interruption 3 weeks after the last N-803 dose and/or CD8 reconstitution. SIV reactivation was monitored by plasma viremia, cell-associated RNA in peripheral blood and lymph node, and RNAscope analysis in lymph node samples. The size of the viral reservoir was assessed by total cell-associated SIV DNA in the peripheral blood and lymph node. Immunological changes were studied by flow cytometry and RNA sequencing. Diversity of the virus emerging after N-803 and/or CD8 depletion was assessed via single genome amplification.

Additionally, the study was replicated in 23 bone marrow-liver-thymus humanized mice. BLT hu-mice were previously infected with HIVJR-CSF and given ART in chow (FTC+TDF+RAL). N-803 (0.2 mg/kg in PBS) and the control vehicle (PBS) were administered to mice in groups 1 and 3 intravenously in a total volume of 200 microliters. MT807R1 (3 mg/kg in PBS) and the control vehicle (PBS) were also administered intravenously in a total volume of 200 microliters to mice in groups 2 and 3.

Study Design

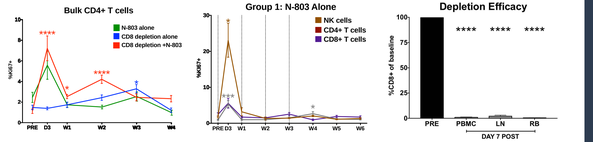


GSEA Reveals CD4+ T cell Activation and Proliferation After N-803



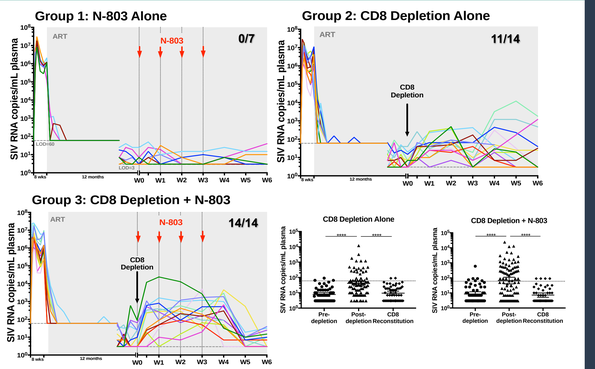
KEY: (left) Sequencing of RNA from CD4+ T cells at day 3 was compared with day 0 for each group. A normalized enrichment score cutoff of greater than 1.35 for upregulated gene sets with a false discovery rate of less than 0.2 was used. **(middle)** N-803 upregulates gene sets related to IL-2/IL-15 signaling in CD4+ T cells at day 3 following N-803 administration alone. **(right)** GSEA of CD4+ T cells from N-803 alone group reveal upregulation of genes relating to antiviral response, proliferation, and T cell signaling.

N-803 Induces Proliferation of Peripheral T cells and NK cells



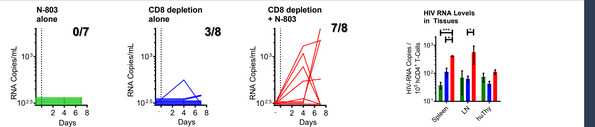
KEY: (left) Comparison of expression of Ki67 on peripheral CD4+ T cells across all groups reveals highest expression in N-803+CD8 depletion group. Statistical analysis using Friedman's test. **(middle)** Comparison of Ki67 expression on CD8+ T cells, CD4+ T cells, and NK cells after N-803 administration alone. Statistical analysis using Kruskal-Wallis test. **(right)** . Depletion efficacy calculated as the %CD8+ T cells as a percentage of the value at pre-depletion baseline and statistics were calculated using Friedman test.

N-803+CD8 Depletion Induces Robust and Persistent Latency Reversal



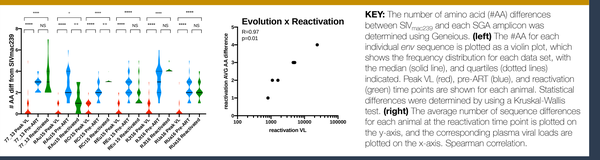
KEY: Changes in viral load throughout infection, ART treatment, and intervention by group. Limit of detections are represented by a horizontal grey dashed line, 60 copies/mL first 11 months, then 3 copies/mL all time points after. Pre-intervention time points and post-intervention time points are at different time scales. Statistical significance of differences in viral loads post-depletion (bottom right) calculated using Kruskal-Wallis test.

N-803+CD8 Depletion Induces Robust HIV Reactivation in BLT hu-Mice



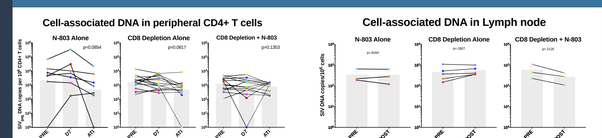
KEY: Changes in viral load pre-intervention, day 4 post-intervention, and at sacrifice (day 7) in BLT hu-mice. Limit of detection for assay is $10^{2.5}$ copies/mL. HIV RNA levels in the tissue (spleen, lymph node, and thymus) were measured upon sacrifice.

Evolution of SIV_{mac239} *env* Sequences Related to Reactivation Viral Load



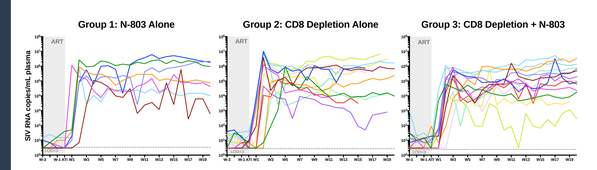
KEY: The number of amino acid (#AA) differences between SIV_{mac239} and each SGA amplicon was determined using Geneious. **(left)** The #AA for each individual *env* sequence is plotted as a violin plot, which shows the frequency distribution for each data set, with the median (solid line), and quartiles (dotted lines) indicated. Peak VL (red), pre-ART (blue), and reactivation (green) time points are shown for each animal. Statistical differences were determined by using a Kruskal-Wallis test. **(right)** The average number of sequence differences for each animal at the reactivation time point is plotted on the y-axis, and the corresponding plasma viral loads are plotted on the x-axis. Spearman correlation.

Interventions do not Decrease Cell-associated SIV DNA Levels



KEY: (left) qPCR was used to measure the number of copies of cell-associated SIV_{gag} DNA in PBMC and the number of copies per 10^6 CD4+ was corrected using CD4+ T cell counts. DNA was extracted from pellets pre-intervention (PRE), day 7 post-intervention (D7), and pre-ART interruption (AT). Friedman test was used to calculate significance. **(right)** RNA was extracted from frozen lymph node pellets and cell-associated SIV DNA measured using qPCR. Statistics were calculated with a Wilcoxon test (paired, non-parametric). The sample averages are indicated by the vertical bar on each graph (±SEM).

Viral Load Rebounds Quickly Following ART Interruption



KEY: Three weeks following either the reconstitution of CD8+ T cells (>100 cells/μL blood) or the last N-803 administration, animals were taken off antiretroviral therapy and viral loads were measured weekly for an average of 6 months before animals were necropsied.

Conclusions

- N-803 administration in CD8-depleted, ART-treated SIV-infected RM induces the most robust and persistent reversal of latency observed to date in humans or nonhuman primates.
- In the absence of a clearance intervention, we did not observe a significant reduction of the reservoir size.
- Combining N-803 and CD8 depletion with an immune-clearing component (i.e. neutralizing antibodies, CD4 mimetics or immunotoxins) may be a powerful shock and kill strategy that profoundly affects reservoir size and stability in ART-treated HIV/SIV infections.

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