No Evidence of Ongoing Viral Replication in SIV-Infected Macaques on cART Initiated in the Chronic Phase of Infection Despite Elevated Residual Plasma Viral Loads

Gregory Del Prete

December 14, 2022

12th EDITION HIV PERSISTENCE DURING THERAPY Reservoirs & Eradication Strategies Workshop DECEMBER 13-16, 2022 www.hiv-persistence.com MIAMI FLORIDA USA

Background

- SIV-infected macaques on cART often have persistent residual PVLs at substantially higher levels than HIV-1 infected people on cART
- Raises questions about relevance of SIV/macaque models for studies of HIV persistence, reservoirs, and cure strategies
- If ongoing viral replication occurs during cART, it may be more easily detected in SIV infected macaques

Study Objectives

- **<u>Question</u>: Is there evidence of ongoing viral replication in SIV infected macaques on cART, where residual PVLs are substantially higher than in HIV infected humans on cART?**

- **<u>Approach</u>: apply methods used to address this question in HIV infected humans on cART:**
  - Treatment intensification in SIV infected animals on cART
  - Assessment of viral sequence evolution and diversity during cART

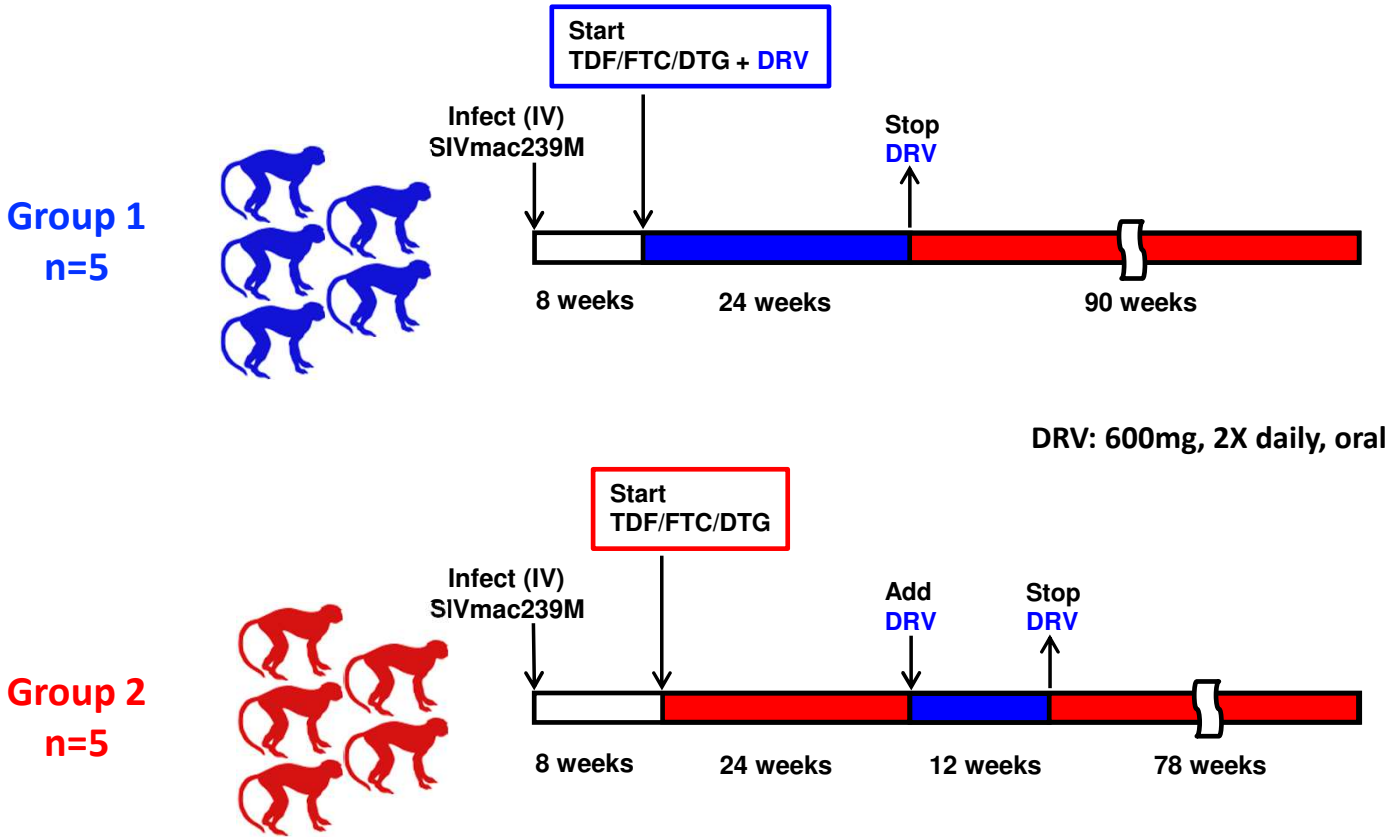
Study Concept

- **SIV infected macaques in chronic phase of infection**

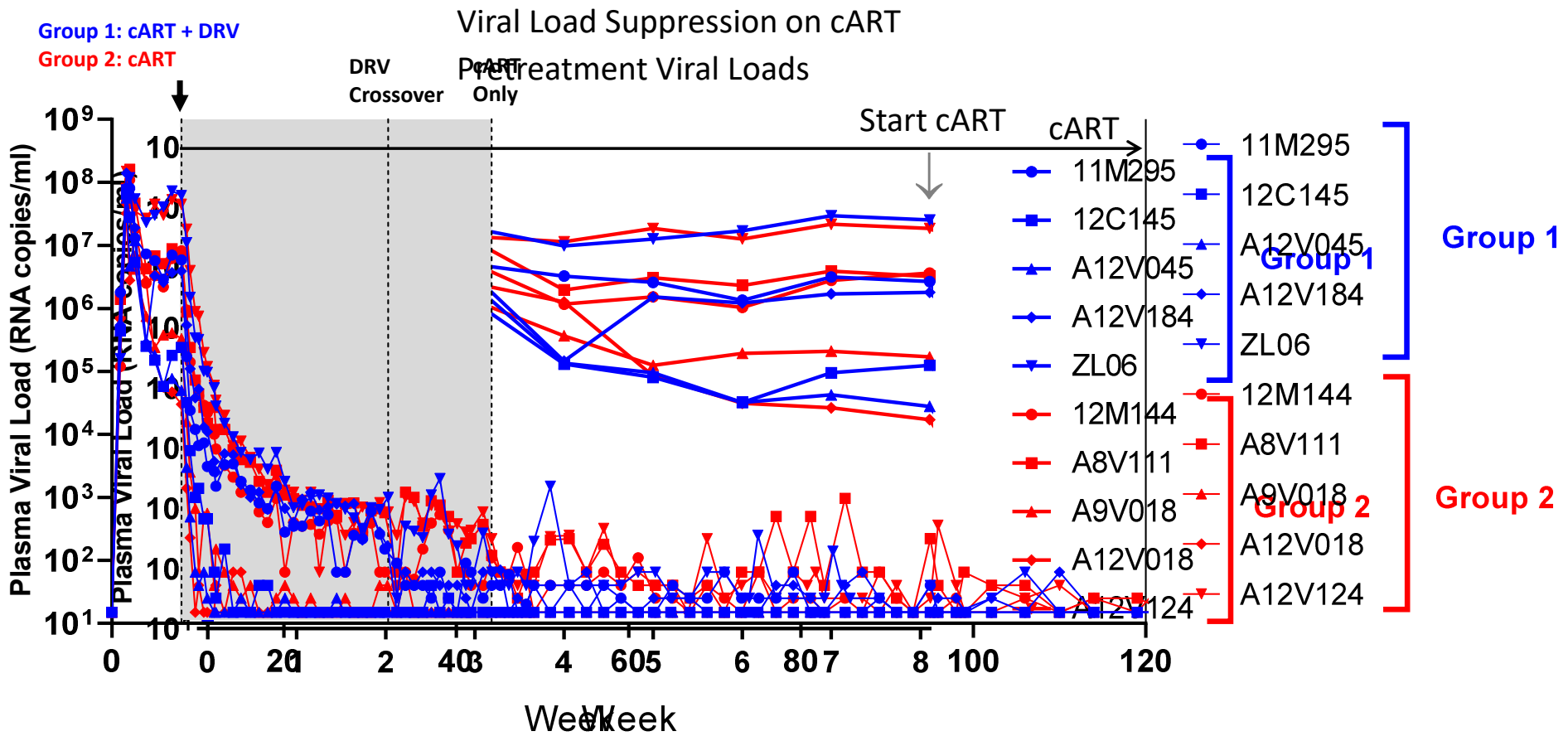
- **Start with benchmark 3-drug cART regimen:**
  - Two RT Inhibitors (NRTI): Tenofovir disoproxil fumarate (TDF), Emtricitabine (FTC)
  - One Integrase Inhibitor (IN-STI): Dolutegravir (DTG)

- **Intensify regimen with 4th drug: protease inhibitor Darunavir (DRV)**
  - Class not included in benchmark regimen; not affected by drug resistance to agents in 3-drug benchmark regimen
  - Active against SIV in rhesus macaques *in vivo* (multi-log viral load suppression, drug resistance emergence)

Study Design

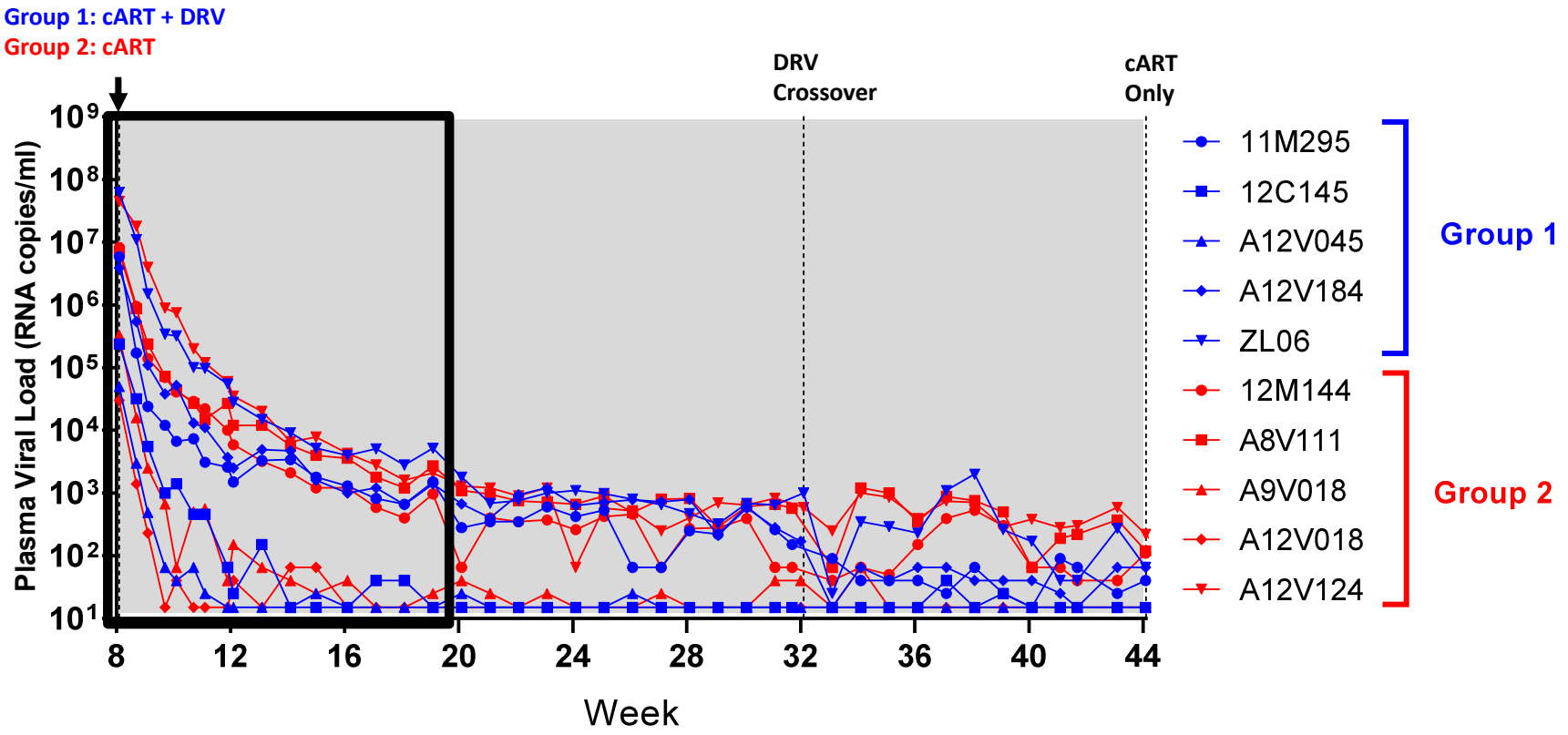


Plasma Viral Loads, Group Assignments

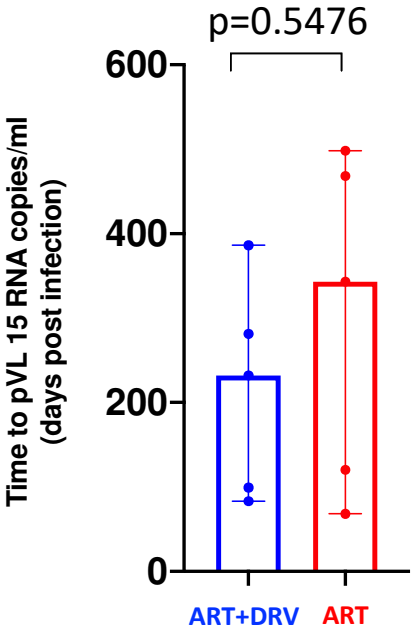


Pretreatment PVL Range: 3 x 10^4 – 6 x 10^7 vRNA copies/ml plasma

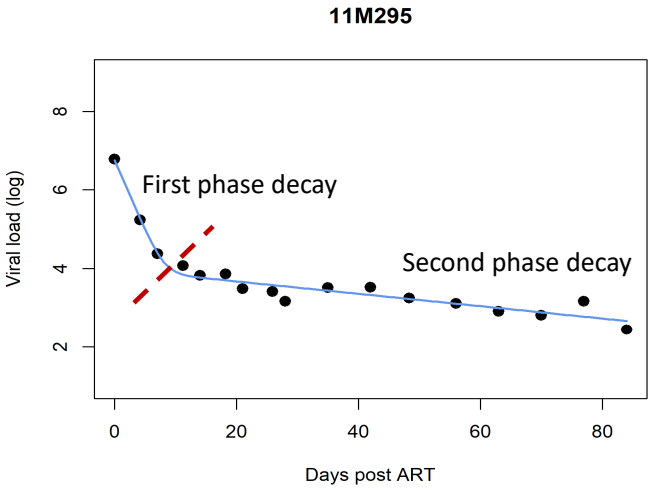
Effect of Intensification on Suppression



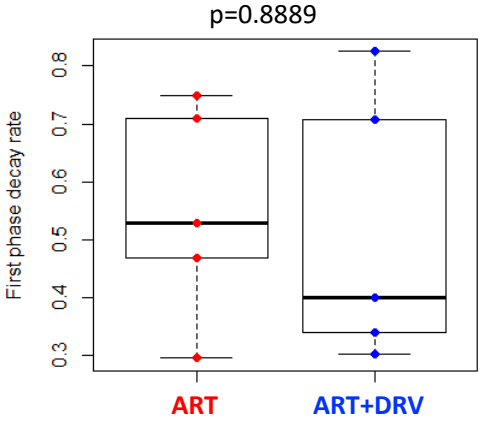
Intensification Does Not Impact Initial PVL Decay Kinetics



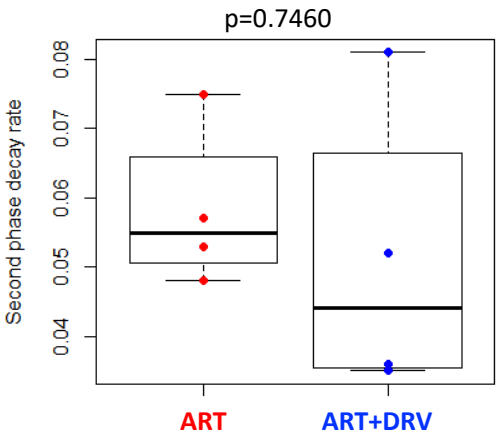
Biphasic initial viral decay in plasma RNA



First phase decay rate

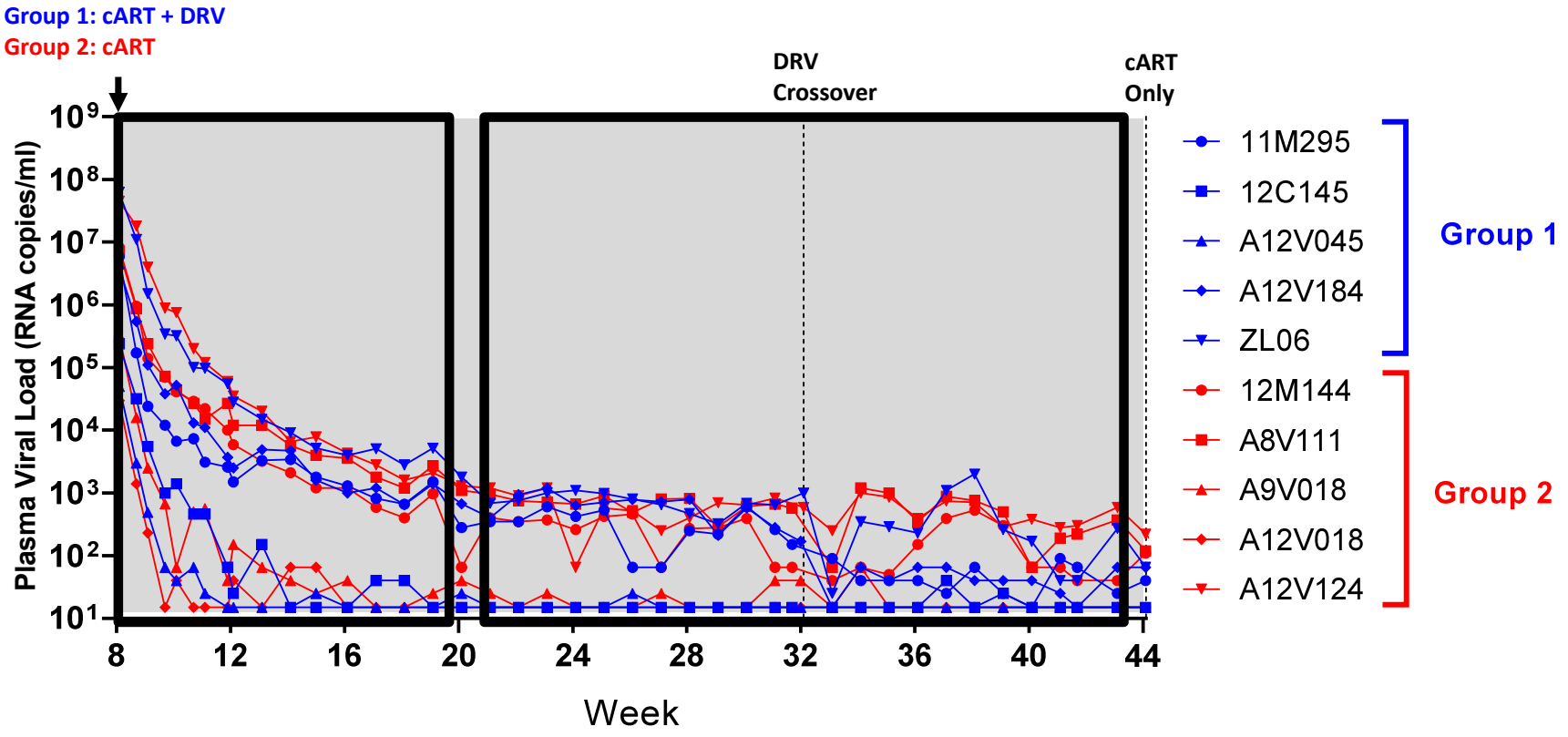


Second phase decay rate



p values- Mann Whitney U test

Effect of Intensification on Suppression

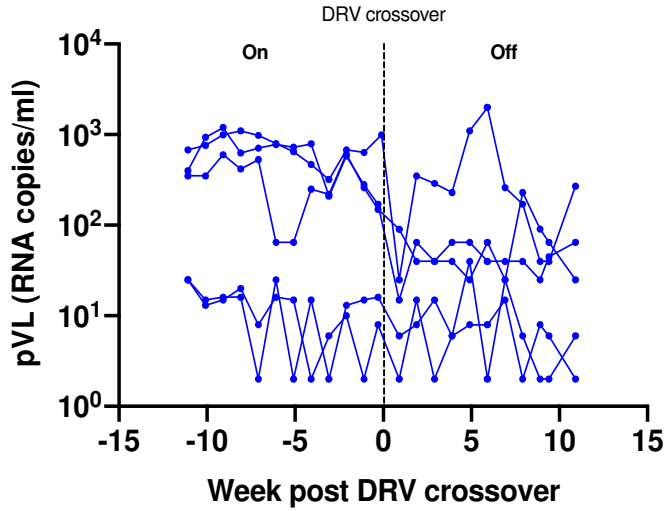


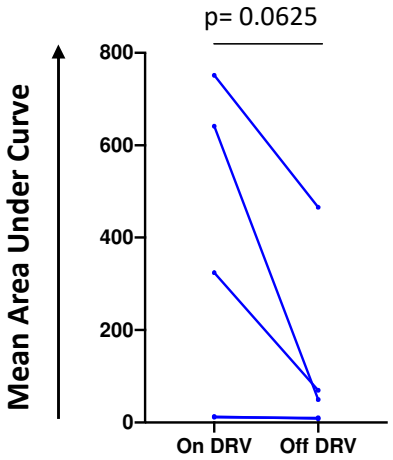
Standard PVL assay threshold = 15 vRNA copies/ml

Ultrasensitive assay (threshold = 2 vRNA copies/ml) for timepoints <15 vRNA copies/ml using standard assay

Intensification Does Not Enhance Magnitude of Suppression

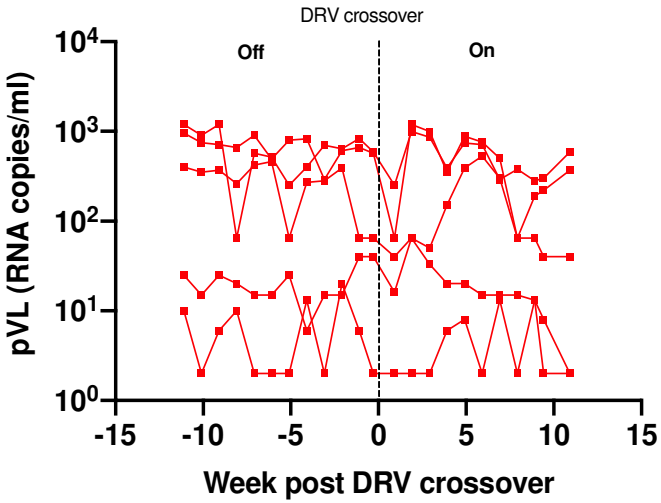
Group 1
cART+DRV
then
cART only

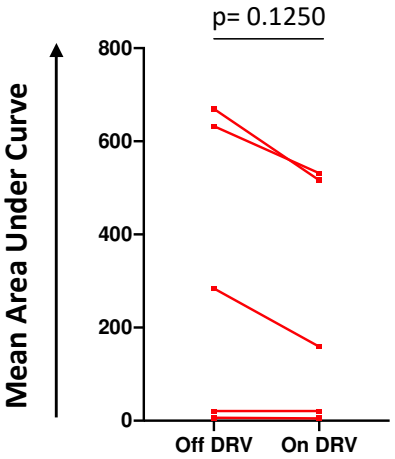




No difference when DRV intensification added vs. removed

Group 2
cART
then
cART+DRV





Viral loads decayed over time regardless of drug intensification or de-intensification.

p values- Wilcoxon matched-pairs signed rank test

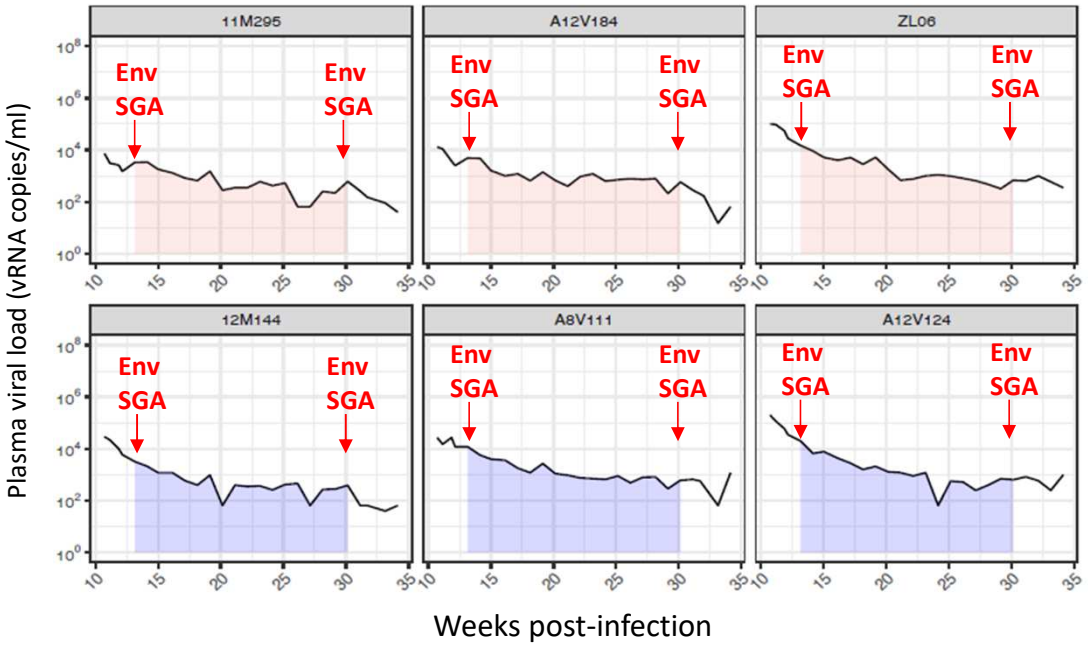
Plasma Viral RNA Sequencing Analyses

- **Look for evidence of viral sequence evolution, indicative of viral replication**

- **Focus on 6 study animals with elevated PVLs on cART**
  - Sufficient vRNA signal for generation of multiple sequences from plasma RNA
  - Env SGA sequencing

Benchmarking viral evolution

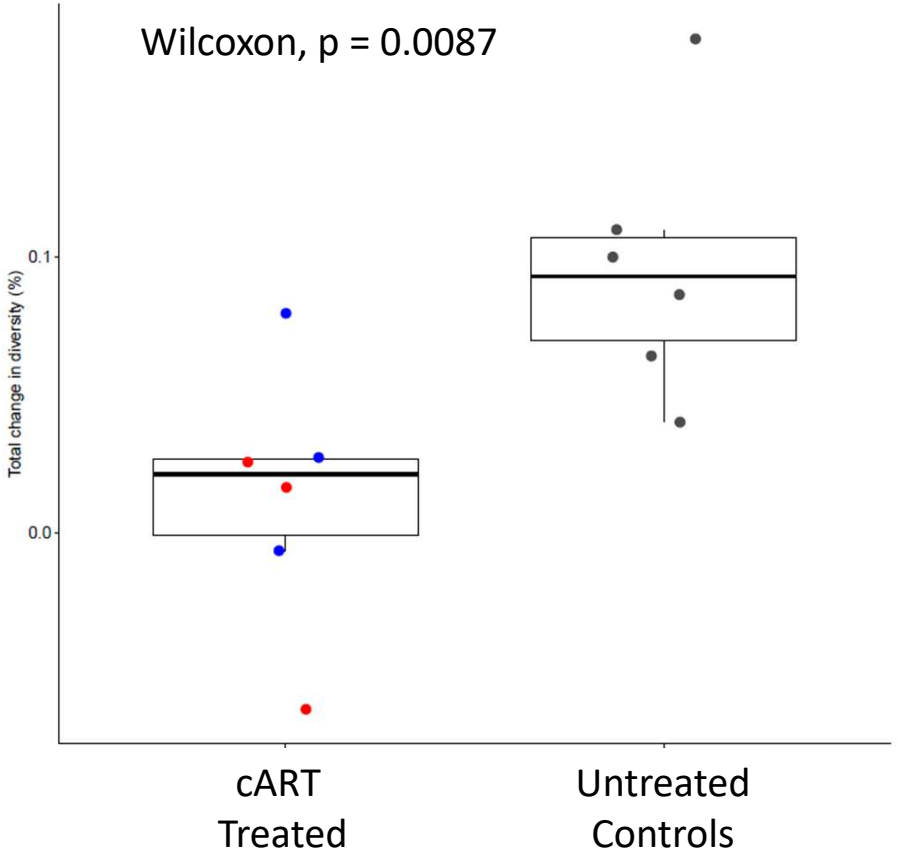
cART Treated Study Animals



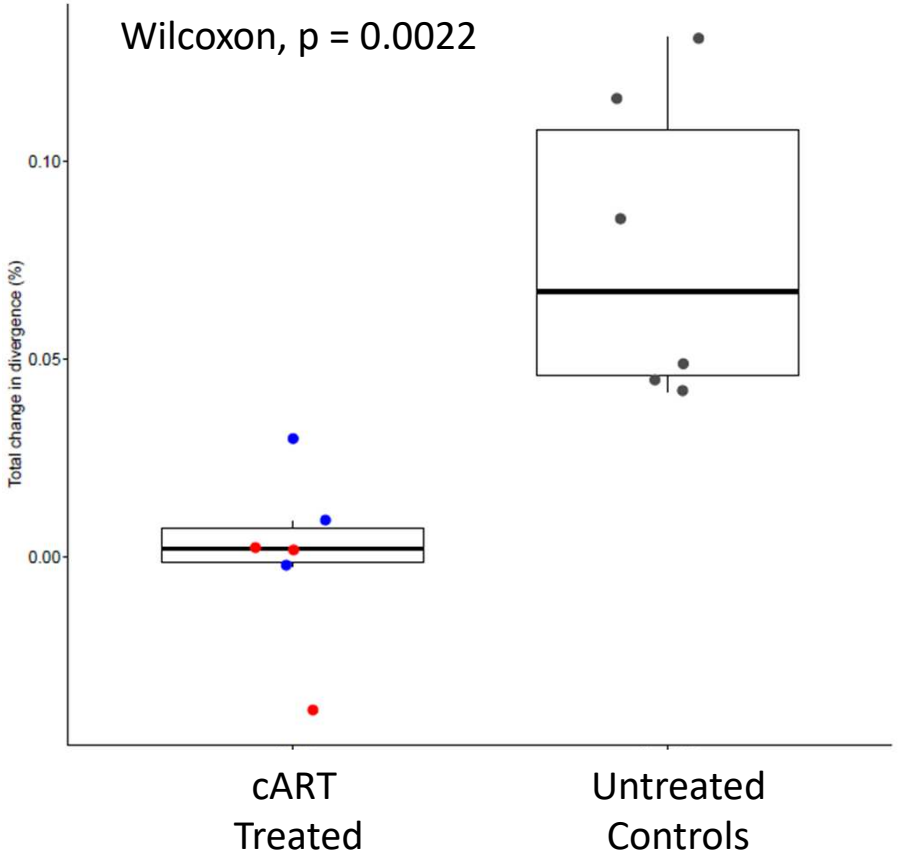
Appropriateness of controls: no significant difference in viral load area-under-curve comparison between cART treated study animals and untreated controls -> comparable total amount of circulating virus

Change in Sequence Diversity and Divergence

Change in Diversity Between Timepoints

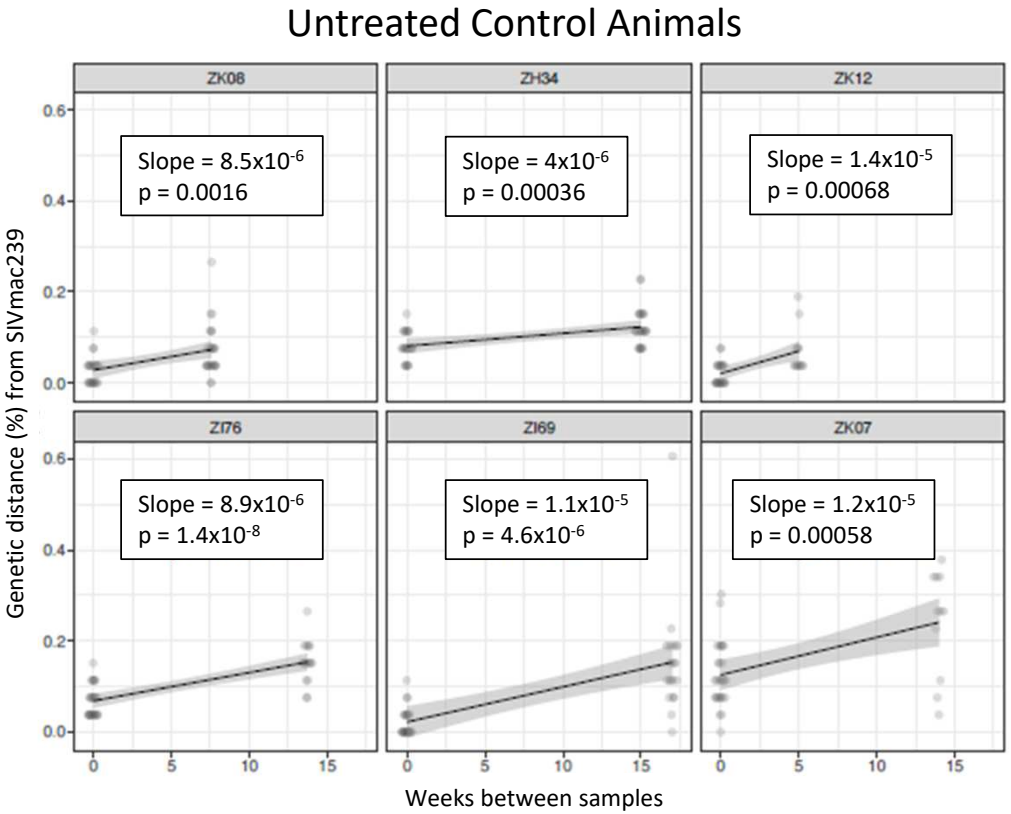


Change in Divergence Between Timepoints



Slope of p-distance over time: Untreated Controls

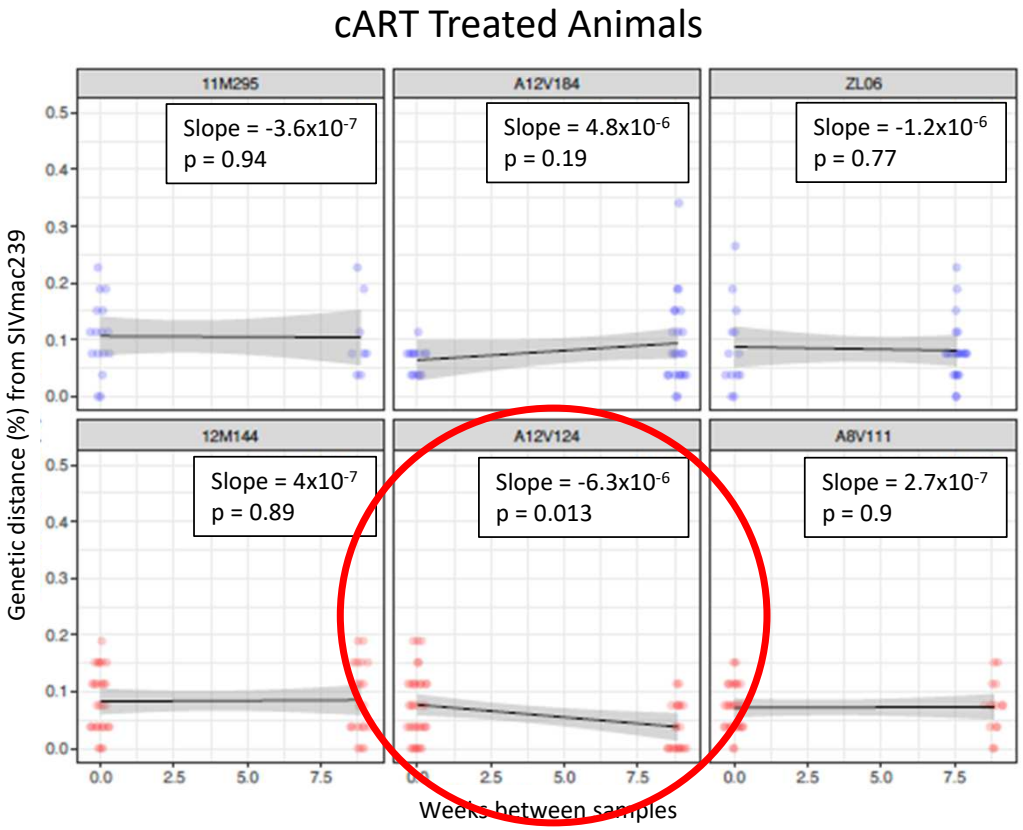
- **P-distance: number of nucleotide substitutions from SIVmac239 genome (inoculum used for all animals)**



- **All untreated animals have a significant, positive p-distance slope**
- **Accumulation of additional mutations between sampling time points**

Slope of p-distance over time: cART Treated Animals

- **P-distance: number of nucleotide substitutions from SIVmac239 genome (inoculum used for all animals)**



- **5/6 animals: slope not significantly different from 0**
- **Animal A12V124: significant *negative* slope, indicating population becoming more ancestral**
- **No accumulation of additional mutations between sampling time points**

Panmixia Test

- **Panmixia = random genetic sampling in subsequent generations, no shift in population structure**
- **Panmixia test: compare populations of SGA sequences obtained from two time points for each animal; p-value for the probability that the populations are the same**
  - Fail panmixia test: multiple possible underlying explanations
  - Pass panmixia test: strong evidence of no viral evolution or replication
- **Untreated control animals: 6 of 6 failed panmixia test**
  - All with $p<0.0001$
- **Animals on cART: 6 of 6 passed panmixia test**
  - Indicates viral sequence population has not changed over time

Conclusions/Community Summary

- No evidence ongoing viral replication in SIV infected rhesus macaques on cART, even in those animals with elevated residual viral loads
- Findings suggest that residual PVL in SIV-infected NHPs on cART is due to virus production from already-infected cells rather than ongoing viral replication
- Results suggest ongoing viral replication is unlikely in HIV-infected people on optimal cART regimens, or that ongoing viral replication will be very difficult to detect in cART-suppressed HIV-infected people using current methods
- Results support relevance of SIV-based NHP models of cART-mediated suppression for studies of viral reservoir and cure strategies

Acknowledgments

AIDS and Cancer Virus Program

Viral Persistence Section
Mukta Nag

Computational Biology Working Group
Taina Immonen
Emek Kose
Andrew Conchas

NHP Research Support Core
Adrienne Swanstrom
Adam Wiles
Rodney Wiles
Colleen Boyenton
Jacob Kiser
Nicholas Zuczek

Quantitative Molecular Diagnostics Core
Jeffrey D. Lifson
Kelli Oswald
Randy Fast
Rebecca Shoemaker
William Bosche
Michael Hull

Viral Evolution Core
Brandon Keele
Christine Fennessey
Agatha Macairan
Laura Newman
Leslie Lipkey

Laboratory Animal Sciences Program
Josh Kramer
Matt Breed
George Muthua
Michelle Metrinko

Data Management Services
Duncan Donohue
Tyler Malys

Frederick National Laboratory for Cancer Research

Funding: NCI contract No. 75N91019D00024/HHSN261201500003I