Viral evolution in a pediatric rhesus macaque model of HIV therapy and rebound

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Abstract

In 2017, approximately 180,000 infants were infected with HIV and 1.8 million children were living with HIV globally. While lifelong combination antiretroviral therapy (ART) can effectively suppress virus replication, ART is not curative due to the establishment of stable latent viral reservoirs immediately after infection. A functional cure able to achieve sustained viral remission will be required to attain an ART-free life. Our study goal was to characterize the kinetics of Simian-Human Immunodeficiency Virus (SHIV) evolution and viral rebound in infant and adult preclinical models of HIV reservoir on ART. In this study, 6 infant and adult rhesus macaques (RMs) were infected with Simian-Human Immunodeficiency Virus (SHIV).C.CH0505.375H.dCT virus via oral and intravenous challenge, respectively. Twelve weeks post infection (wpi), infant and adult RMs were placed on ART for 8 and 12 weeks, respectively. ART was then interrupted and kinetics of viral rebound was measured using qRT-PCR. Viral diversity was measured pre- and post-ART using single genome amplification and sequencing of the HIV env gene. Plasma viral RNA (vRNA) in infants and adults displayed similar kinetics until ART initiation, peaking at 2 wpi. Upon ART initiation, plasma vRNA load was suppressed in infants and adults to undetectable levels within 2-4 weeks. Post-ART, 5/6 infant and 3/6 adult RM rebounded to >150 vRNA copies/ml of plasma within 1-3 weeks. Pre-ART and post-ART, HIV env sequence diversity was greater in adult plasma viruses than in infant plasma viruses, with average pairwise distance values of 0.007 and 0.005, respectively. Post-ART, infant plasma viruses are more closely related to pre-ART viruses than adult plasma viruses, perhaps due to less immune pressure in infants. These findings further delineate the clinically relevant differences in HIV env genetic diversity between infants and adults and emphasize the clear need for highly relevant, preclinical models for the development of pediatric-specific therapeutic and curative strategies to achieve an HIV-free generation.
Introduction & Background

In 2017, an estimated 1.8 million children under the age of 15 were living with HIV and more than 180,000 infants became newly infected with HIV (UNAIDS, 2018). Of these infections, greater than 50% occurred during breastfeeding (UNAIDS, 2018). Thus, the current state of the pediatric HIV epidemic remains a major global public health threat. Despite the widespread availability and success of maternal antiretroviral therapy (ART) in drastically reducing mother-to-child-transmission (MTCT) of HIV to rates as low as 2%, combination ART will not be able to eliminate HIV infections due to limited access to ART in low and middle income countries where the majority of MTCT cases occur, ART-associated infant drug toxicity, development of drug-resistant viral strains, and poor maternal adherence to ART (Lallemant et al., 1994). Adherence to ART is particularly a problem during adolescence, resulting in the development of drug-resistant viral strains (Shaw & Amico, 2016).

Although ART has greatly reduced HIV-related morbidity and mortality through achieving viral suppression, combination ART is not curative due to the establishment of stable latent viral reservoirs within a few hours after infection (Luzuriaga et al., 2015; Persaud et al., 2013). To date, every child who has been removed from ART has eventually demonstrated viral rebound. Notably, in 2014, the absence of detectable viremia following ART cessation was reported in a perinatally HIV-infected infant treated for 18 months starting 30 hours after birth (Persaud et al. 2013). However, virus levels eventually rebounded 28 months after ART interruption (Luzuriaga et al. 2015). This case, referred to in the HIV cure field as the “Mississippi baby,” focused attention on the potential for long-term remission of perinatal HIV infection with very early ART intervention. While rates of in utero and peripartum transmission have greatly declined in recent years due to ART, the greater majority of new pediatric HIV
infections occur postpartum via breastfeeding, and infants that are infected via breastfeeding are frequently diagnosed late and ART initiation is delayed (Luzuriaga & Mofenson, 2016). Despite the notable case of the “Mississippi baby,” the clinical course for pediatric HIV infection even in the most resourced settings requires children to acclimate to lifelong ART. Adherence among infants, children, and adolescents poses particularly unique challenges, such as limited pediatric drug formulations, drug adherence requirements during challenging developmental stages, and potential long-term adverse metabolic consequences (Shaw & Amico, 2016).

The HIV cellular and anatomic latent reservoir is the major barrier to achieving a cure and is the subject of intense scientific investigation. The HIV latent reservoir in ART-treated patients is characterized as a small pool of latently infected memory CD4+ T cells carrying transcriptionally silent, yet replication-competent HIV provirus (Sengupta & Siliciano, 2018). During active infection, the HIV population accumulates genetic diversity with each round of viral replication at a rate of about 1 mutation for every $10^5$ nucleotides copied, largely due to the lack of a proofreading mechanism for the enzyme reverse transcriptase (Figure 1A and 1B) (van Zyl, Bale, & Kearney, 2018). Yet, while on ART, evidence suggests that ongoing replication and associated accumulation of viral diversity comes to a halt (Figure 1B). Reservoir persistence is attributed to clonal proliferation of a subset of infected cells, rather than ongoing viral replication (Figure 1C and 1D). Thus, as represented in the phylogenetic tree, there is more diversity among virus populations pre-ART than there is of near-identical populations on-ART (Figure 1E). However, less is known about the characteristics of viral diversity after treatment interruption during the period of viral rebound. Analytical treatment interruption (ATI) studies are the gold standard approach to characterizing those viruses that emerge during the period of viral rebound after cessation of ART.
It is critical to study the HIV latent reservoir and potential therapeutic interventions in the setting of pediatric HIV infection. However, it is difficult to study the HIV latent reservoir and viral rebound after treatment interruption in children for a number of reasons such as limited sample volume in infants and, more importantly, ethical considerations surrounding cessation of life-saving treatment in children. Thus, there is a clear and urgent need for a highly relevant, pediatric-specific, preclinical experimental model of HIV reservoir and rebound in order to study novel therapies to achieve a functional cure and attain long-term remission. Non-human primate models of Simian-Human Immunodeficiency Virus (SHIV) infection and analytical treatment
interruption are an indispensable tool to study therapeutic and curative strategies without the associated risks of adverse clinical events to participants in clinical trials (Wen, Bar, & Li, 2018).

Study of the influence of ART on HIV genetic diversity through pediatric-specific, preclinical animal models will be critical to the design of vaccines and other curative strategies. To address this gap in research, we have established a non-human primate (NHP) model to study SHIV reservoir and rebound dynamics. In this study, we characterized the kinetics of viral evolution and rebound in an infant and adult rhesus macaque (RM) (Macaca mulatta) model of SHIV infection. In our model, six infant and adult rhesus macaques were infected with a new generation SHIV.C.CH0505.375H.dCT transmitted/founder (T/F) virus, an engineered chimeric virus composed of a Simian Immunodeficiency Virus (SIV) backbone substituted with an HIV-1 envelope (env) gene. This recombinant SHIV challenge virus enables the study of HIV-1 env immunology in an NHP setting.

Infants were infected via oral challenge while adults were infected via intravenous challenge to mimic the distinct modes of infection between infant and adult animals. Twelve weeks post infection (wpi), infant and adult RMs were placed on triple ART for 8 and 12 weeks, respectively. The triple ART regimen included dolutegravir, tenofovir disoproxil fumarate, and emtricitabine. ART was then interrupted and the kinetics of viral rebound was measured using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Viral evolution and diversity were assessed pre and post-ART through single genome amplification and sequencing of the HIV env gene, generation of neighbor-joining phylogenetic trees and highlighter mutation plots, and calculation of average pairwise distances value. Our hypotheses were 1) that after treatment interruption infant plasma virus diversity will be lower than that of adults and 2) that
after treatment interruption infant plasma virus populations will be more closely related to pre-
ART virus populations than that of adults due to a limited selective immune pressure.

Methods

SHIV Construction

The SHIV.C.CH0505.375H.dCT recombinant virus was constructed from a SIVmac766 backbone and the env gene of HIV-1 strain CH0505, a clade C virus isolated from a patient who produced broadly neutralizing antibodies (bNAbs) to the HIV envelope protein (Env) (Figure 2). The construct was further modified via site-directed mutagenesis (S375H) and to enhance CD4 receptor binding and replication in rhesus macaque CD4+ T cells (Figure 2) (Li et al., 2016). The SHIV.C.CH505.375H.dCT challenge stock (generously provided by study collaborator Dr. George M. Shaw, University of Pennsylvania) was prepared by infecting primary activated Indian rhesus macaque CD4+ T cells and 7-14 days later culture supernatants were pooled, as previously described (Li et al., 2016). Virus titers were determined in TZM-bl cells, yielding 6.8x10^6 TCID_50/ml. Previously described in Nelson et al., 2019.

**Figure 2. SHIV construction scheme.** SHIV.C.CH505.dCT was constructed without the carboxy terminal 33 amino acids of HIV-1 gp41 but with the carboxy terminus of SIVmac766 gp41 joined in-frame served as a platform for further SHIV constructions containing vpu–env–gp140 segments of HIV-1 strain CH505. Met-M, Tyr-Y, His-H, Trp-W, and Phe-F were exchanged for the wild-type Ser-S residue at position 375 in Env. SIVmac766 and SHIV sequences are publicly available (GenBank accession nos. KU955514 and KU958484-9) (Li et al., 2016).
**Animal care and sample collection**

At the time of the infection, adult female rhesus macaques ranged from 4 to 10 years of age, and infant rhesus macaques were each 4 weeks of age (Table 1). All macaques were of Indian origin, and from the type D retrovirus-free, SIV-free and STLV-1 free colony of the California National Primate Research Center (CNPRC; Davis, CA). Animals were maintained in accordance with the American Association for Accreditation of Laboratory Animal Care standards and *The Guide for the Care and Use of Laboratory Animals*. For sample collections, animals were sedated with ketamine HCl (Parke-Davis) injected at 10 mg/kg body weight. EDTA-anticoagulated blood was collected via peripheral venipuncture. Plasma was separated from whole blood by centrifugation, and PBMCs were isolated by density gradient centrifugation using Ficoll®-Paque (Sigma) or Lymphocyte Separation Medium (MP Biomedicals). All protocols were reviewed and approved by the University of California at Davis Institutional Animal Care and Use Committee (IACUC) prior to the initiation of the study.

**SHIV challenge of infant and adult monkeys**

In our model, twelve adult RMs between 4 and 10 years of age were challenged intravenously with SHIV.C.CH505 at a dose of $3.4 \times 10^5$ TCID$_{50}$ (Table 1). Six infant RMs were challenged orally beginning at 4 weeks of age. Initially, infants were exposed to SHIV.C.CH505 three times per day for 5 days at a dose of $8.5 \times 10^4$ TCID$_{50}$/ml in an isotonic sucrose solution and bottle-fed, in order to simulate oral acquisition via breastfeeding. After one week, only one infant became infected, and the remaining five infants were challenged three weeks later under sedation at a dose of $6.8 \times 10^5$ TCID$_{50}$/ml until infected. After three weeks one infant remain uninfected and thus was subsequently challenged at an increased dose of $3.4 \times 10^6$ TCID$_{50}$/ml until infected. Methods previously described in Nelson et al., 2019.
Viral RNA load quantification

Plasma RNA load was quantified using a well-established quantitative reverse transcriptase (RT) PCR assay targeting SIVgag RNA. RNA was isolated from plasma samples using the QIAsymphony Virus/Bacteria Midi kit on the QIAsymphony SP automated sample preparation platform (Qiagen, Hilden, Germany). RNA was extracted manually if plasma volumes were limited. Data reported are the number of SIV RNA copy equivalents per ml of plasma, with a limit of detection of 15 viral RNA copies/ml.

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Table 1. Infant and adult SHIV.C.CH505-infected monkey cohort information, number of challenges to infection, and age at infection. F, female; M, male. Grey shaded boxes indicate six animals not included in this cohort of convenience.
ART regimen and analytical treatment interruption

Twelve weeks post infection (wpi), infant and adult RMs were placed on triple ART for 8 and 12 weeks, respectively (Figure 3). The ART regimen was comprised of dolutegravir (DTG), tenofovir disoproxil fumarate (TDF), and emtricitabine (FTC) and was delivered via daily subcutaneous injection. At 20 wpi in infants and 24 wpi in adults, ART was interrupted and the kinetics of viral rebound was measured using qRT-PCR (Figure 3). At 8 weeks post ART interruption animals were necropsied. Blood samples were collected weekly throughout the course of the study.

Figure 3. Timeline of SHIV challenge, ART regimen, and viral rebound post-ART. Red arrows indicate time of infection. Yellow shaded region indicates the period on ART. Green shaded region indicates the period post ART interruption.
Single genome amplification (SGA) of the HIV env gene

Viral RNA was purified from the plasma sample from each animal by the EZ1 Viral RNA Mini Kit 2.0 (Qiagen 955134) and subjected to cDNA synthesis using 1X reaction buffer, 0.5 mM of each deoxynucleoside triphosphate (dNTP), 5 mM DTT, 2 U/mL RNaseOUT, 10 U/mL of SuperScript III reverse transcription mix (Invitrogen), and 0.25 mM antisense primer SHIVEnv.R3out (5’-CTAATTCCTGGTCTGTGGTGAATCCTG-3’). The resulting cDNA was end-point diluted in 96 well plates (Applied Biosystems, Inc.) and PCR amplified using Platinum Taq DNA polymerase High Fidelity (Invitrogen) so that < 30% of reactions were positive in order to maximize the likelihood of amplification from a single genome (Figure 4). A second round of PCR amplification was conducted using 2μl of the first round products as template. SIVmac.F4out (5’-TCATATCTATAATAGACATGGAGACACCC-3’) and SHIVEnvR3out (5’-CTAATTCCTGGTCTGTGGTGAATCCTG-3’) were used as primer pair in the first round of PCR amplification step, followed by a second round with primers SIVmac766.F2in (5’-GGAAATCCTCTCTCAACTATACCGCCCTC-3’) and SIVmac766.R2in (5’-CTATTGCCAATTGTGAATCATTGTTC-3’). PCR was carried out using 1X buffer, 2 mM MgSO4, 0.2 mM of each dNTP, 0.2μM of each primer, and 0.025 U/μl Platinum Taq High Fidelity polymerase (Invitrogen) in a 20μl reaction in Round 1 and in a 50μl reaction in Round 2. Round 1 and Round 2 amplification conditions were 1 cycle of 94°C for 2 minutes, 35 cycles of 94°C for 15 seconds, 50°C for 30 seconds, and 68°C for 3 minutes and 30 seconds, followed by 1 cycle of 68°C for 10 minutes. Round 2 PCR amplicons were visualized by agarose gel electrophoresis, purified by AMPure magnetic bead technology, and sent to Genewiz, Inc for sequencing of the envelope gene using an ABI3730xl genetic analyzer (Applied Biosystems).
Partially overlapping sequences from each amplicon were assembled and edited using Sequencher (Gene Codes, Inc). Sequences with double peaks per base read were discarded. Sequences with one double peak were retained as this most likely represents a Taq polymerase error in an early round of PCR rather than multiple template amplification; such sequence ambiguities were read as the consensus nucleotide. Full-length envelope sequences were aligned and edited using Seaview version 4 (Gouy, Guindon, & Gascuel, 2010). Generation of neighbor-joining phylogenetic trees and calculation of average pairwise distances were performed using MEGA7 (Kumar, Stecher, & Tamura, 2016). Average pairwise distance is an estimate of the number of base substitutions per site from averaging over all sequence pairs within a given group. Highlighter mutations plots were created using a highlighter tool freely available from the Los Alamos National Laboratory HIV Sequence Database:
https://www.hiv.lanl.gov/content/sequence/HIGHLIGHT/highlighter_top.html.
SGA sequences generated for each animal will be deposited in GenBank under accession numbers available at the time of publication.

Figure 4. Single genome amplification (SGA) of the HIV env gene. Viral RNA (vRNA) is extracted from plasma and reverse transcribed into complementary DNA (cDNA). cDNA is then end-point diluted and PCR amplified with a nested primer design to isolate single HIV env gene amplicons.
Results

Plasma viral load kinetics and rebound

Figure 5 depicts viral load kinetics of each animal, with log of viral RNA copies per mL on the y axis and weeks post infection on the x axis. Once infected, both infant and adult animals demonstrated peak viremia at 2 weeks post infection with steady decline thereafter until ART initiation. Additionally, none of the infant or adult animals achieved a set point viral load prior to ART initiation at 12 weeks post infection. Unexpectedly, 2 of the 6 infant animals (46367 in light blue circles and 46359 in light green diamonds) controlled their infection to near undetectable levels (<15 copies/mL) before ART initiation at 12 weeks post infection. Viral load reached undetectable levels for all animals by 4 weeks after ART initiation.

We defined viral rebound in our model as any timepoint after ART interruption at which animal viral load exceeded 150 vRNA copies per mL, or 10 times the limit of detection of the assay. According to these criteria, 5 out of 6 infant and 3 out of 6 adult animals rebounded after treatment interruption. Infant 46352 (in dark blue squares) and adult 39950 (in dark green diamonds) not only correspond to the highest levels of viremia observed at ART initiation, but also demonstrate the fastest time to rebound and highest peak viremia after ART interruption. At 6 weeks post-ART, 30 wpi in adults and 26 wpi in infants, 5 out of 5 adult animals achieved control of rebound viremia, while only 2 out of 5 infant animals achieved similar levels of virologic control at this timepoint. Overall, infant animals displayed less ability to control rebounding virus replication compared to adult animals.
HIV env sequence diversity in infant rhesus macaques

Neighbor-joining phylogenetic trees were generated to visualize evolutionary relationships and sequence diversity of pre-ART infant plasma viruses. In Figure 6, viruses isolated from each animal generally cluster together independently, indicating a lack of sample cross-contamination. Two infants for which we have isolated a representative sample of plasma viruses, 46352 in dark blue and 46367 in turquoise, demonstrate distinct clustering of near identical viruses, suggesting homogeneous sequence diversity. Moreover, we calculated an average pairwise distance value among the infant sequences, which estimates the average number of base substitutions per site over the entire HIV envelope gene. The average pairwise distance for pre-ART infant plasma virus sequences was 0.005, or 0.5% diversity.
In Figure 7, sequences are sorted from top to bottom, one-to-one, in a highlighter mutation plot to match the order of sequences represented in Figure 6. Pre-ART infant plasma virus sequences isolated from each animal generally exhibit a unique pattern of mutation at consistent sites on the HIV env gene within each animal but not between animals. These patterns of mutation appear to be generally homogeneous and are indicative of low sequence diversity. However, this apparent trend of near-identical clustering that suggests low sequence diversity among pre-ART infant plasma virus sequences is not shared among pre-ART adult plasma virus sequences.

**Figure 6.** Neighbor-joining phylogenetic tree of pre-ART infant plasma virus sequences. Each dot is a single virus and each animal is represented with a unique color. Tree is rooted to SHIV CH505 challenges virus in the black circle.
Figure 7. Highlighter plot of pre-ART infant plasma virus sequences. 72 sequences are aligned and compared to the SHIV CH505 challenge virus master sequence positioned at the bottom of the plot. Each colored tick mark is a mutation compared to the master sequence. Each grey shaded region is a deleted region. 2598 nucleotides are in the HIV env gene.
HIV env sequence diversity in adult rhesus macaques

Neighbor-joining phylogenetic trees were generated to visualize evolutionary relationships and sequence diversity of pre-ART adult plasma viruses. In Figure 8, pre-ART adult plasma virus populations isolated from each animal cluster independently, yet demonstrate greater evolutionary distance and markedly less near-identical overlap as compared to pre-ART infant plasma virus sequences in Figure 6. Moreover, the average pairwise distance value for pre-ART adult plasma virus populations was 0.007, or 0.7% diversity. Thus, pre-ART adult plasma virus diversity is 0.2% greater than pre-ART infant plasma virus diversity in this model.

Figure 8. Neighbor-joining phylogenetic tree of pre-ART adult plasma virus sequences. Each dot is a single virus and each animal is represented with a unique color. Tree is rooted to SHIV CH0505 challenges virus in the black circle.
In Figure 9, pre-ART adult plasma virus sequences isolated from each animal generally exhibit a unique pattern of mutation moderately consistent within each animal but not between animals. While there are shared mutations within each animal, similar to the trend seen in pre-ART infant plasma virus sequences, there also appears to be more within animal mutations not attributed to such patterns. The patterns of mutation appear to be more heterogeneous than that of pre-ART infant plasma virus sequences and reflect greater sequence diversity than that of infants as seen in Figure 7.

**Figure 9. Highlighter plot of pre-ART adult plasma virus sequences.** 33 sequences are aligned and compared to the SHIV CH0505 challenge virus master sequence positioned at the bottom of the plot. Each colored tick mark is a mutation compared to the master sequence. Each grey shaded region is a deleted region. 2598 nucleotides are in the HIV *env* gene.
**HIV env sequence diversity in infant and adult rhesus macaques pre- and post-ART**

Neighbor-joining phylogenetic trees were generated for plasma virus sequences isolate from one infant animal (46352) and one adult animal (39950) (Figure 10). These animals are most representative as they had the most single genome amplicon plasma virus sequences available pre- and post-ART among the infant and adult animals. Infant sequences are on the left in squares and adult sequences are on the right in circles. Green represents pre-ART virus, blue represents virus from 2 weeks post-ART, and red represents virus from 8 weeks post-ART. Adult 39950 viral load at 8 weeks post-ART was undetectable and thus there are no sequences for this timepoint. Each tree is rooted to the SHIV CH0505 challenge virus in black.

With respect to the pre-ART plasma virus sequences in both animals, again the infant pre-ART plasma virus sequences are generally near-identical whereas the adult pre-ART plasma virus sequences are not identical and are more heterogeneous. With respect to the post-ART viruses in both animals, a similar trend is exhibited at 2 weeks post-ART, in which the adult post-ART viruses appear to demonstrate more diversity than infant post-ART viruses. Among infant plasma virus sequences isolated from 8 weeks post-ART interruption, greater diversity emerges as viremia is sustained after rebound. Additionally, 2 weeks post-ART infant plasma virus sequences were more distributed among corresponding pre-ART viruses, as compared to 8 weeks post-ART infant plasma virus sequences and that of adults, suggesting that early after rebound, post-ART infant plasma viruses are evolutionarily more closely related to pre-ART viruses. Overall, infant env sequence diversity appears to be more homogeneous as compared to the adult env sequence diversity at both pre- and post-ART timepoints.
Figure 10. Neighbor-joining phylogenetic trees of pre-ART and post-ART infant and adult plasma virus sequences. Each dot is a single virus. Infant (46352) sequences are represented with squares and adult (39950) sequences are represented with circles. Pre-ART sequences are represented in green, 2 weeks post-ART sequences are represented in blue, and 8 weeks post-ART sequences are represented in red. Tree is rooted to SHIV CH0505 challenge virus in the black triangle positioned at the bottom of tree.
Discussion

In this study, we have found evidence to suggest that pre-ART and post-ART, HIV \textit{env} sequence diversity is greater in adult plasma viruses than in infant plasma viruses, and that post-ART, infant plasma viruses are more closely related to corresponding pre-ART viruses. Perhaps these differences may be due to a less robust immune response in the setting of HIV infection early in life and thus less selective pressure on viruses in infant animals as compared to adult animals (Martinez, Permar, & Fouda, 2016). HIV \textit{env} genetic diversity poses major challenges to immune-based therapeutic and curative strategies at all stages of infection. The HIV envelope (Env) protein is the only viral protein expressed on the surface of the virion while circulating in plasma, and thus elicitation of neutralizing antibodies against the HIV envelope is a key target in vaccine development (Cicala, Nawaz, Jelicic, Arthos, & Fauci, 2016). Study of HIV \textit{env} genetic diversity in settings of viral rebound after ART interruption thus is critical to the design of potential therapeutic and curative strategies specific to the pediatric population.

Yet, in the pediatric population, it is challenging to conduct studies of HIV latent reservoir and rebound due to difficulty in obtaining adequate sample volume, ethics of removing children from life-saving therapy, and difficulty in translating findings from adult studies as children are not simply small adults, with respect to differences in immune development and predominant modes of infection (Wen et al., 2018). Thus we have developed a highly relevant, pediatric-specific, preclinical experimental model of HIV therapy and rebound to inform and accelerate the discovery of HIV cure strategies for infected children.

Our study was limited by a number of compounding factors as a cohort of convenience with relatively small sample size in which the infant animals were subjected to 8 weeks of ART while the adult animals were subjected to 12 weeks of ART. Additionally, this study was
completed over a short period of 28 and 32 weeks which limits the potential development of HIV env sequence diversity as compared to clinical studies on the scale of years post infection. During the early phase of infection with one transmitted-founder virus, HIV env diversity increases linearly over time at a rate of approximately 1% per year (Shankarappa et al., 1999). Moreover, low viral load levels and lack of rebound among some animals limited the number of single genome amplicons available for comparison pre- and post-ART.

Our study has not addressed HIV env sequence diversity during the period on antiretroviral therapy or the relationship between pre- and post-ART plasma virus sequences to those on ART. Interestingly, a growing body of evidence suggests that plasma rebound viruses emerging after treatment interruption are recombinants of the circulating latent provirus reservoir (Cohen et al., 2018; Lu et al., 2018; Mendoza et al., 2018; Salantes et al., 2018; Vibholm et al., 2019). These results suggest that emergent plasma rebound viruses after treatment interruption are not dominated by the circulating latent reservoir, but instead represent recombinants of the circulating latent provirus reservoir. These data highlight the importance of analytic treatment interruption studies and a future direction to potentially corroborate these findings in our rhesus macaque model by performing sequencing of the HIV env gene from proviruses found in CD4+ T cells from plasma at timepoints on ART. Characterization of the relationship, or lack thereof, between latent proviruses on ART and the viruses that emerge during rebound may also offer an opportunity to identify predictive markers of viruses that are likely to emerge after treatment interruption. Further investigation into HIV env genetic diversity in pediatric-specific models of HIV latent reservoir, therapy, and rebound will be necessary to inform effective strategies aimed at achieving long-term remission or a functional HIV cure.
References


SHIV.CH505-infected infant and adult rhesus macaques exhibit similar HIV Env-specific antibody kinetics, despite distinct T-follicular helper (Tfh) and germinal center B cell landscapes. bioRxiv 538876; doi: https://doi.org/10.1101/538876


