Time to Viral Rebound After Interruption of Modern Antiretroviral Therapies

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Summary: In A5345, we detected no differences in the timing of viral rebound with modern versus historic ART. Early ART was associated with a significant delay in the time to HIV rebound after ART interruption, lowering the barrier for HIV remission.
ABSTRACT

Background: Development of HIV remission strategies requires precise information on time to HIV rebound after treatment interruption, but there is uncertainty regarding whether modern ART regimens and timing of ART initiation may impact this outcome.

Methods: ACTG A5345 enrolled individuals who initiated ART during chronic or early HIV infection and on suppressive ART for ≥2 years. Participants underwent carefully monitored antiretroviral interruption. ART was restarted upon two successive viral loads ≥1,000 copies/mL. We compared participants of A5345 with participants of 6 historic ACTG treatment interruption studies.

Results: Thirty-three chronic-treated and 12 early-treated participants interrupted ART with evaluable time to viral rebound. Median time to viral rebound ≥1000 HIV RNA copies/mL was 22 days. Acute retroviral rebound syndrome was diagnosed in 9% of chronic-treated and none of early-treated individuals. All participants of the historic studies were on older protease inhibitor-based regimens while 97% of A5345 participants were on integrase inhibitor-based ART. There were no differences in the timing of viral rebound comparing A5345 versus historic studies. In a combined analysis, a higher percentage of early-treated participants remained off ART at post-
treatment interruption week 12 (chronic vs early: 2% vs 9%, P=0.0496). One chronic-tREATED and one early-treated A5345 participant remained off ART for >24 weeks. All participants re-suppressed after ART re-initiation.

Conclusions: Early ART initiation, using either older or newer ART regimens, was associated with a significant delay in the time to HIV rebound after ART interruption, lowering the barrier for HIV remission.

Key words: HIV treatment interruption, viral rebound, posttreatment controller
INTRODUCTION

Antiretroviral HIV therapy (ART) alone cannot eradicate HIV infection and life-long ART is needed to prevent HIV reactivation from long-lived viral reservoirs [1, 2]. However, ART remains inaccessible for many persons with HIV (PWH) while others must manage the complications of prolonged infection and ART use, including stigma, adverse effects, drug-drug interactions, emerging drug resistance, and pill fatigue. Therefore, one of the highest priorities for the HIV field is the search for therapeutic interventions that can eliminate or control the HIV reservoir, leading to long-term ART-free HIV remission [3].

The validation of strategies for ART-free HIV remission will ultimately require demonstration of efficacy through ART interruption studies to confirm a delay in HIV rebound. The results of historic treatment interruption (TI) studies have shown that viral rebound occurs rapidly for most individuals, generally within a few weeks after ART discontinuation [1, 4]. However, many of these TI studies were performed >15 years ago and before the advent of newer ART regimens that are more potent, have improved tolerability and are easier to dose. There is uncertainty as to whether modern ART regimens may achieve improved virologic suppression and potentially delay the time to viral rebound after TI. In addition, there are indications that initiation of ART early after HIV infection will substantially restrict the size of the HIV reservoir [5]. This may delay the timing of HIV rebound and increase the chances of post-treatment HIV control [4, 6, 7], although these findings also need to be evaluated in the setting of modern ART.
AIDS Clinical Trials Group (ACTG) trial A5345 is a prospective TI study to identify biomarker predictors of HIV rebound timing. Participants treated either during chronic or early HIV infection and with virologic suppression were enrolled and underwent TI. We compared the timing of HIV rebound for participants of A5345 with participants on non-NNRTI-based regimens from placebo arms of 6 historic ACTG TI studies. We assessed the timing of HIV rebound in those receiving historic versus modern ART regimens and in those initiating ART during chronic versus early HIV infection. We also evaluated the rate of CD4 cell count decline after stopping ART as this is an important safety consideration for ongoing HIV remission trials that include a TI.

METHODS

Study Design

ACTG study A5345 is a prospective study of factors mediating the timing of HIV rebound after TI. The study enrolled two cohorts of participants: individuals who initiated ART during chronic HIV infection (chronic-treated) or early HIV infection (early-treated). Individuals identified as being treated during chronic infection must have initiated ART >6 months after the estimated date of infection and early-treated participants must have initiated ART during Fiebig stages III-V of acute infection. All participants were between 18 years and 70 years of age, on suppressive ART for ≥2 years with CD4 count ≥500 cells/mm³ and nadir CD4 count ≥200 cells/mm³, and no history of AIDS-defining illness. Participants from historic ACTG TI studies were included if they received no immunologic interventions and were not on a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART regimen.
Procedures

The study involved 4 steps (Supplementary Figure S1). Step 1 includes a lead-in period, such that participants on a non-NNRTI-based regimen were switched to a protease inhibitor (PI) or integrase strand transfer inhibitor (INSTI)-based regimen [8]. All participants who maintained viral suppression during Step 1 were eligible to undergo TI in Step 2. Participants were followed for 48 weeks or until they met the ART restart criteria, whichever occurred first. During the first 8 weeks of the TI, viral loads were monitored twice weekly by the Roche COBAS assay (Quest) and CD4 cell counts every 2 weeks at local CLIA-certified laboratories. Thereafter, viral loads were monitored weekly and CD4 counts every 4 weeks. ART was restarted upon two successive viral loads $\geq 1,000$ copies/mL or based on another of the predefined criteria (Supplementary Table S1). Participants who met the ART reinitiation criteria were restarted on ART (Step 3) and monitored for 24 weeks after viral suppression had been achieved. Those who were found to be posttreatment controllers had the option of extended follow-up off ART (Step 4).

Statistical Analysis

We compared the timing of HIV rebound for participants of A5345 with participants on PI regimens from the placebo arms of 6 historic ACTG TI studies: A371 [9], A5024 [10], A5068 [11], A5170 [12], A5187 [13], and A5197 [14]. Historic ACTG trial participants must not have received an intervention and be on a non-NNRTI-based regimen. The comparison of A5345 and historic TI trial participants was limited to...
viral loads at post-TI weeks 4, 8, and 12 due to limitations in participant follow-up frequency for the historic studies. Fisher’s exact and Wilcoxon tests were used to compare groups.

RESULTS

A total of 48 participants were enrolled from 15 ACTG sites in the US and Thailand and initiated the TI. The analysis population includes 45 of the participants as three individuals restarted ART within 24 weeks without meeting the viral load criteria for ART restart. One individual restarted ART at week 2 due to suspected acute retroviral rebound syndrome (although HIV RNA remained <20 copies/mL) and two participants restarted ART based on personal preference at weeks 4 and 6 (maximum HIV RNAs 815 and 20 copies/mL, respectively, during TI). The A5345 analysis population includes 33 individuals who initiated ART during chronic infection and 12 participants who initiated ART during early infection. Acute retroviral syndrome was diagnosed in 9% of chronic-treated vs none of early-treated participants. Symptoms included grade 1-3 sore throat, lymphadenopathy, fatigue, and/or myalgias, all of which resolved after ART restart.

Viral rebound timing for A5345 participants were compared to historic ACTG TI trial participants (61 chronic-treated and 74 early-treated), all of whom participated in ACTG studies that enrolled participants between 1999 and 2006. A greater proportion of early-treated A5345 participants were Asian compared to the historic control populations; early-treated A5345 participants also had higher pre-ART HIV RNA (P=0.03) and lower nadir CD4 counts (P=0.04). Otherwise, there were no significant differences in the baseline demographics between A5345 participants and
those of historic treatment interruption studies (Table 1). Forty-four of 45 (98%) A5345 participants were on an INSTI-based regimen at TI (Supplementary Table S2). For the historical participants, the most common regimens included amprenavir (51%), nelfinavir (23%) and indinavir (16%) (Supplementary Tables S3-S4).

The median time to viral rebound ≥1000 HIV RNA copies/mL was 22 days for A5345 participants (range 13 to 230 days). When stratified by timing of ART initiation, there was a modest delay in time to HIV rebound for early-treated participants as compared to chronic-treated participants at each viral load threshold (Figure 1a). There was no significant difference in time to viral rebound between A5345 and historic treatment interruption trial participants, when stratified into chronic versus early-treated participants (Figure 1b). In a pooled analysis of A5345 and historic studies, a higher percentage of early-treated participants remained off ART at post-treatment interruption week 12 (chronic vs early: 2% vs 9%, P=0.0496, Supplemental Figure S2). In A5345, one chronic-treated and one early-treated participant remained off ART for >24 weeks, representing 3% of the chronic-treated participants and 8% of early-treated participants (Figure 2). Plasma ARV levels were measured for these two participants at ≥3 time points after treatment interruption and all were undetectable. The pre-ART viral load for the chronic-treated participant was also <1000 HIV RNA copies/mL while for the early-treated participant, it was 1.4 million HIV RNA copies/mL.

There were no significant associations of ART duration or CD4 count with timing of HIV rebound ≥1000 HIV RNA copies/mL. There was a suggestion that higher pre-ART HIV RNA was associated with shorter time to rebound for chronic-treated (Spearman r = -0.37, P=0.09), but not early-treated individuals (r = 0.30, P=0.34). For early-treated participants, there was a correlation between higher nadir
CD4 cell count and time to target detected (but below the limit of quantification) on the viral load assay \( (r = 0.63, P=0.052) \), but not with viral rebound \( \geq 1000 \) HIV RNA copies/mL.

No A5345 participant met the CD4-based ART restart criteria. Despite comparable pre-treatment interruption CD4 counts, A5345 chronic-treated individuals experienced a greater numerical decline in CD4 counts at 2 and 4 weeks post-treatment interruption compared to A5345 early-treated participants (week 2 median CD4 decline for chronic vs early-treated: -86 vs -39 cells/mm\(^3\), \( p=0.19 \), and week 4 CD4 decline: -136 vs -67 cells/mm\(^3\), \( p=0.24 \)), Figure 3). All A5345 participants had successful viral suppression after ART restart. Twenty-four weeks after ART restart, early-treated individuals had a median 20 CD4 cells/mm\(^3\) increase compared to pre-treatment interruption while the CD4 cell count for chronic-treated individuals was median 87 cells/mm\(^3\) lower than baseline. A similar pattern of change was also detected when comparing change in CD4\% over time (Supplemental Figure S3).
DISCUSSION

Much of the HIV TI literature is based off of TI studies performed more than 15-20 years ago. Participants of these studies were on older ART regimens that were less potent, required more frequent dosing and were less tolerable than modern ART regimens. We report precise data on HIV rebound kinetics in 45 participants with virologic suppression on modern ART regimens, including early and chronic-treated individuals, who were enrolled in a closely-monitored prospective TI study with a goal of determining predictors of HIV rebound timing. In A5345, 97% of participants were on an integrase inhibitor before treatment interruption. In comparison, the most common pre-ATI regimens for the historic participants included amprenavir, nelfinavir, or indinavir-based ART. These older ARTs, especially PI-based regimens, have been associated with increased rates of detectable low-level viremia that is concerning for incomplete virologic suppression [15-17]. In addition, it is clear that newer integrase inhibitor-based ART have improved rates of treatment success compared to protease inhibitor or NNRTI-based regimens [18, 19] and treatment intensification with an integrase inhibitor has been reported to alter markers of persistent viral replication in those on a protease inhibitor-based regimen [20]. Despite this, A5345 participants were found to have relatively rapid viral rebound after ART discontinuation that was not significantly different compared to historic control participants. These results are consistent with another recent report of relatively fast viral rebound in chronic-treated individuals on contemporary ART regimens [21] and suggest that while modern ART regimens have made it easier to achieve and sustain virologic suppression in people with HIV, it does not appear to have decreased the barrier to achieving HIV remission.
In the pooled analysis of A5345 and historic participants, as compared with those who initiated ART during chronic HIV infection, individuals who started ART during early infection had a modest delay in timing of viral rebound and a significantly greater likelihood of remaining off ART at week 12. One early-treated and one chronic-treated participant were able to remain off ART for >24 weeks, representing 8% of the early-treated and 3% of the chronic-treated participants. The pre-ART viral load for the chronic-treated participant was noted to be <1000 HIV-1 RNA copies/mL, suggesting that this individual may have been a spontaneous viremic controller. Early initiation of ART has benefits for the health of the patient and to decrease the risk of forward HIV transmission in the community [22, 23]. In addition, early ART initiation restricts the seeding of the HIV reservoir [5, 24] and preserves HIV-specific immune responses [25, 26]. Our results are consistent with these observations and provide additional confirmation that early ART initiation may lower the barrier for HIV remission, making them ideal participants of HIV cure trials. Early-treated individuals had modest CD4 declines during the TI and excellent CD4 count recovery after 24 weeks of virologic suppression post-ART restart, which provide additional reassurance as to the safety of short-term treatment interruption trials in this population. Of note, 50% of the early-treated participants in A5345 were Asian and harbored non-B-subtype HIV, which is a higher percentage than those of historical studies. The role of race and HIV subtype on the timing of HIV rebound is still largely unknown and additional studies are needed in the future.

The success of interventions aimed at achieving HIV remission will ultimately be judged by their ability to show either a significant delay in the timing of HIV rebound or reduction in set point viral load. However, TI studies require lengthier trials and are not without risk. The identification of pre-interruption factors that can
predict the timing or extent of viral rebound may inform the design of the next generation of therapeutics and may speed their evaluation. Our results suggest that lower pre-ART viral load is associated with more delayed viral rebound ≥1000 HIV RNA copies/mL in chronic-treated individuals. This result could reflect a smaller and/or less active HIV reservoir, which have been associated with delayed HIV rebound [4, 27, 28]. Additional studies are underway in A5345 to assess the biomarker predictors of HIV rebound.

While complete reservoir eradication and HIV cure is the ultimate objective, the detection of delayed viral rebound timing through an intervention would represent a tangible sign of progress for strategies towards sustained HIV remission. The results of A5345 demonstrates that while that modern ART is insufficient for lowering the barrier to HIV remission, early ART initiation could be an important component of a comprehensive strategy towards an HIV cure. Additional studies are needed to determine the immune and reservoir mediators of delayed HIV rebound that may act as predictive biomarkers and targets for future interventions.
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Disclosures:

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Potential Conflicts:

JZL reports consulting fees from Abbvie and Jan Biotech, outside the submitted work. Dr Landay consulted for Gilead and Merck, and has received research support from Abbott. RTG reports grants from National Institutes of Health and participation on Scientific Advisory Boards for Gilead and Merck, all outside the submitted work. RWC reports grant NIH AI-106701, paid to their institution, outside the submitted work. BJCM reports research funding (payments made to institution) from Gilead Sciences, outside the submitted work. MCK reports Funding for CTU operations (ACTG CRS): UM1AI069511 from NIAID, during the conduct of the study. DS reports consulting fees from FluxErgy, Bayer, and Arena Pharmaceuticals, outside the
submitted work. SGD reports grant support from NIH, during the conduct of the study; SGD reports a research contract/grant from Gilead and research collaboration from Merck; consulting fees from AbbVie, Eli Lilly, and Immunocore; and serving on Scientific Advisory Board for BryoLogyx and Enochian Biosciences, all outside the submitted work.
REFERENCES

Figure Legends

Figure 1: Viral rebound timing in A5345 and historic treatment interruption trials. 
(A) Percent of participants in A5345 with viral rebound stratified by timing of ART initiation and viral load threshold. (B) Percent of participants with loss of viral suppression based on the 1000 HIV RNA copies/mL threshold in A5345 versus historic control stratified by timing of ART initiation.

Figure 2: A5345 participant viral loads after treatment interruption. (A) Participants who initiated ART during chronic infection and (B) participants who initiated ART during early infection. Open circles are the timepoints when participants reached the ART restart criteria.

Figure 3: Change in CD4 counts at 2 and 4 weeks after treatment interruption (TI) or 24 weeks after ART restart stratified by the timing of ART initiation.
Table 1: Demographics for the A5345 study population and historical controls.

<table>
<thead>
<tr>
<th></th>
<th>A5345 Chronic (N=33)</th>
<th>Historical Chronic (N=61)</th>
<th>A5345 Early (N=12)</th>
<th>Historical Early (N=74)</th>
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<tr>
<td>Age, median (Q1, Q3) years</td>
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<td>43 (40, 49)</td>
<td>38 (34, 47)</td>
<td>37 (28, 42)</td>
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<tr>
<td>Sex, % male</td>
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<td>87%</td>
<td>100%</td>
<td>95%</td>
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<td>Race/ethnicity, %</td>
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<td>3%</td>
<td>8%</td>
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<tr>
<td>Nadir CD4, cells/mm³*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>201-500</td>
<td>82%</td>
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<td>18%</td>
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<td>742</td>
<td>836</td>
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<td>(Q1, Q3) cells/mm³</td>
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<td>(686, 1048)</td>
<td>(654, 892)</td>
<td>(688, 1046)</td>
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<td>Pre-ART Viral Load,</td>
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<tr>
<td>median (Q1, Q3) log₁₀</td>
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<td>(4.8, 7.8)</td>
<td>(4.3, 5.4)</td>
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<tr>
<td>copies/mL*</td>
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*For early-treated participants, nadir CD4 count was lower (p=0.04, Fisher’s exact) and pre-ART HIV-1 RNA was higher (p=0.03, Wilcoxon) for A5345 compared to Historical controls.
Figure Legends

Figure 1: Viral rebound timing in A5345 and historic treatment interruption trials. (A) Percent of participants in A5345 with viral rebound stratified by timing of ART initiation and viral load threshold. (B) Percent of participants with loss of viral suppression based on the 1000 HIV RNA copies/mL threshold in A5345 versus historic control stratified by timing of ART initiation.

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Figure 3: Change in CD4 counts at 2 and 4 weeks after treatment interruption (TI) or 24 weeks after ART restart stratified by the timing of ART initiation.
Figure 3

Change in CD4 count from baseline (median cells/mm³)

-150 -100 -50 0 50

T1 Week 2  T1 Week 4  ART Restart Week 24

Chronic-treated
Early-treated