The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption

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Objectives: Therapies to achieve sustained antiretroviral therapy-free HIV remission will require validation in analytic treatment interruption (ATI) trials. Identifying biomarkers that predict time to viral rebound could accelerate the development of such therapeutics.

Design: A pooled analysis of participants from six AIDS Clinical Trials Group ATI studies to identify predictors of viral rebound.

Methods: Cell-associated DNA (CA-DNA) and CA-RNA were quantified in pre-ATI peripheral blood mononuclear cell samples, and residual plasma viremia was measured using the single-copy assay.

Results: Participants who initiated antiretroviral therapy (ART) during acute/early HIV infection and those on a non-nucleoside reverse transcriptase inhibitor-containing regimen had significantly delayed viral rebound. Participants who initiated ART during acute/early infection had lower levels of pre-ATI CA-RNA (acute/early vs. chronic-treated: median <92 vs. 156 HIV-1 RNA copies/10^6 $CD4^+$ cells, $P < 0.01$). Higher pre-ATI CA-RNA levels were significantly associated with shorter time to viral rebound ($\leq$4 vs. 5–8 vs. >8 weeks: median 182 vs. 107 vs. <92 HIV-1 RNA copies/10^6 $CD4^+$ cells, Kruskal–Wallis $P < 0.01$). The proportion of participants with detectable plasma residual viremia prior to ATI was significantly higher among those with shorter time to viral rebound.

Conclusion: Higher levels of HIV expression while on ART are associated with shorter time to HIV rebound after treatment interruption. Quantification of the active HIV reservoir may provide a biomarker of efficacy for therapies that aim to achieve ART-free HIV remission.

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Keywords: biomarker, expressed reservoir, HIV, monitored antiretroviral pause, treatment interruption, viral rebound

Introduction

Antiretroviral 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 patients and complications of prolonged ART use include side-effects, drug–drug interactions, drug resistance, and pill fatigue. Thus, one of the highest priorities for the HIV field is the search for therapeutic interventions that can

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eliminate or control the HIV reservoirs, leading to long-term ART-free HIV remission [3].

Strategies for ART-free HIV remission will ultimately require validation through analytic treatment interruption (ATI) studies. However, controversy exists as to the risks of ATI studies and their optimal design. In previous ATI studies, ART has generally been stopped for a defined period of time (e.g. 12 weeks or longer) with the viral load set point or $CD4^+$ cell count as the primary outcome metric. A potential risk of this type of study is the exposure of participants to an extended period of viremia, which may lead to immune damage, clinical symptoms [4,5], resistance emergence [6], and increased risk of HIV transmission [7]. More recently, an alternative study design has emerged, termed an intensively monitored antiretroviral pause (IMAP), in which time to viral rebound is the primary outcome. In such studies, participants are monitored intensively after the IMAP and ART is restarted as soon as a viral load threshold is reached [8–10]. Such studies will decrease the duration of viremia during treatment interruption and may thereby decrease risks to participants, especially for individuals whose immune systems are functionally naive to HIV [9,11]. The optimal design of future HIV IMAP studies will require a deeper understanding of the determinants of time to HIV rebound and the extent of $CD4^+$ cell loss.

As it is not feasible to test all promising strategies in ATI or IMAP studies, identifying biomarkers that predict time to HIV rebound would be useful to prioritize promising treatment strategies for further clinical development and to avoid the cost and risk of treatment interruption studies for therapies that are unlikely to result in a delay of viral rebound [10,12]. Quantification of various aspects of the HIV reservoir, including HIV DNA and the expressed HIV reservoir (e.g., as reflected by the levels of cell-associated HIV RNA or residual plasma viremia), are being used to evaluate the efficacy of HIV latency reversing agents in early-phase studies [3,13–16]. Although an analysis of the Short Pulse Anti-Retroviral Therapy at Seroconversion (SPARTAC) trial participants who were treated during acute HIV infection demonstrated that levels of HIV DNA were predictive of time to viral rebound after treatment interruption [17], a more comprehensive analysis of the predictors of viral rebound are needed in participants treated during either acute or chronic HIV infection.

In this study, we performed a pooled analysis of AIDS Clinical Trials Group (ACTG) ATI participants who received no immunologic intervention prior to the ATI. We quantified the distribution of times to HIV rebound and the magnitude of $CD4^+$ cell loss. We also examined the association of several key measures of the HIV reservoir with the timing of viral rebound during ATI. Identifying virologic biomarkers that predict time to viral rebound could accelerate the development of therapies aiming to achieve ART-free HIV remission.

Methods

Study population

Participants in six ACTG ATI studies (ACTG 371 [18], A5024 [19], A5068 [20], A5170 [21], A5187 [22], and A5197 [23]) were included if they were on suppressive ART, received no immunologic interventions (e.g., therapeutic vaccination, interleukin-2), and had HIV-1 RNA less than 50 copies/ml at the time of ATI. For participants of ACTG 371, A5024, A5068, and A5197, viral loads were measured at ATI weeks 1, 2, 3, 4, 6, 8, 10, 12, and 16. A5187 participants had viral loads measured at ATI weeks 2, 4, 6, 8, 10, 12, and 16. A5170 participants had viral load measurements at ATI weeks 4, 8, 12, and 16.

The effect of treatment interruption on participants treated during acute and early infection were evaluated in ACTG 371 [18] and A5187 [22] studies as previously described. The general intention of the A371 inclusion criteria was to enroll participants with acute infections that had occurred within the 4 weeks prior to study entry and early infections that had occurred after 2 weeks to 6 months prior to entry. Participants were treated for 52 weeks prior to the ATI. In A5187, acute HIV infection was defined as having a positive HIV-1 RNA and either a negative or indeterminate western blot; early infection was defined as having a positive enzyme-linked immunosorbent assay (ELISA) or western blot with a nonreactive detuned ELISA and less than 6 months between presumed acute retroviral syndrome and ART initiation. Participants had to be on a stable ART with HIV-1 RNA below 50 copies/ml for at least 6 months.

Viral load and $CD4^+$ cell count measurements

Pre-ATI $CD4^+$ cell count and plasma HIV-1 RNA were defined as the most recent measurements on or before the date of ART discontinuation. Screening $CD4^+$ cell count was defined as the most recent $CD4^+$ cell count prior to the pre-ATI $CD4^+$ cell count. Two commonly used different definitions of viral rebound were assessed: two consecutive HIV-1 RNA levels at least 200 copies/ml or a single HIV-1 RNA level at least 1000 copies/ml.

HIV-1 reservoir quantification

A subset of participants ($N = 124$) had peripheral blood mononuclear cells (PBMCs) and plasma available for HIV reservoir quantification while on ART prior to the ATI. Cell-associated HIV-1 RNA and DNA (CA-RNA and CA-DNA) and plasma residual viremia by a single-copy assay (SCA) were measured [24]. CA-RNA and CA-DNA were isolated from cryopreserved PBMCs using the AllPrep DNA/RNA Mini Kit (Qiagen, Valencia,

California, USA). Cellular integrity for RNA analysis was assessed by the measurement of total extracted RNA and evaluation of the IPO-8 housekeeping gene [25]. CA-RNA and CA-DNA were quantified using a real-time PCR approach with primers/probes targeting conserved regions of HIV LTR/gag as previously described [26]. The CA-RNA assay measures levels of unspliced transcripts, which are late RNA products necessary for the creation of HIV structural proteins and act as the genome for new virions. The unspliced CA-RNA assay is the most commonly used assay in HIV curative studies [3,14,27,28]. HIV reservoir measurements for placebo participants of A5197 were previously published [29]. CA-DNA and CA-RNA levels were normalized by the percentage of $CD4^+$ cells in each sample to determine the HIV DNA and RNA copy number per million $CD4^+$ cells.

Low-level HIV-1 RNA in plasma was measured using the SCA as previously described [24]. The HIV-1 *gag* region was sequenced to assess potential sequence mismatches between the SCA primer/probe and patient sequences that might reduce the efficiency of HIV-1 amplification by the SCA. If any sequence mismatches were identified, SCA was performed on plasma samples with detectable viremia by a commercial assay to confirm concordance of values and expected efficiency of the SCA primer/probes.

Statistical analysis

The analyses of the timing of viral rebound and $CD4^+$ cell decline were performed for all participants at weeks 4, 8, and 12 and stratified by timing of ART initiation and screening $CD4^+$ cell count, which was the $CD4^+$ cell count obtained prior to the pre-ATI time point. The distribution of viral rebound was described using a fourth order polynomial fit. Because A5170 participants had less frequent viral load testing, they were excluded in a subgroup analysis restricted to participants of studies with more frequent measurements. Associations of participant characteristics and HIV reservoir levels with timing of viral rebound were assessed by Wilcoxon rank-sum tests and Fisher's exact tests. Wilcoxon rank-sum test was used to compare the extent of $CD4^+$ cell loss in participants stratified by timing of ART initiation, ART regimen, screening and nadir $CD4^+$ cell counts. Univariate and 2-covariate discrete-time logistic Cox models were also used to evaluate factors associated with the timing of viral rebound. The 2-covariate model was performed as a sensitivity analysis to explore factors that may modify the effect of CA-RNA levels on viral rebound timing while avoiding the risks of overfitting. Evaluation of ART regimen and nadir $CD4^+$ cell count were restricted to participants treated during chronic infection.

Ethics statement

Written informed consent was provided by all study participants for use of stored samples in HIV-related research. This study was approved by the Partners Institutional Review Board.

Results

Participants and study characteristics

A total of 235 participants were included from six ACTG ATI studies. A total of 155 participants initiated ART during chronic infection, 32 during acute infection, and 48 during early HIV-1 infection; Table 1 lists their baseline characteristics. Ninety-one percent of participants were male and 71% were white. The median screening $CD4^+$ cell count was 827 cells/μl and was at or above 800 cells/μl for all patient groups. Participants who were categorized by screening $CD4^+$ cell counts to the

Table 1. Baseline characteristics of participants included in the study.

Characteristic	Total ($N=235$)	Chronic ($N=155$)	Acute ($N=32$)	Early ($N=48$)
Sex, male, *N* (%)	213 (91%)	137 (88%)	31 (97%)	45 (94%)
Age, median years (Q1, Q3)	41 (35, 46)	42 (38, 50)	36 (29, 43)	37 (28, 42)
Race/ethnicity, *N* (%)				
White, non-Hispanic	166 (71%)	106 (68%)	23 (72%)	37 (77%)
Black, non-Hispanic	31 (13%)	25 (16%)	5 (16%)	1 (2%)
Hispanic	32 (14%)	20 (13%)	4 (13%)	8 (17%)
Other	6 (3%)	4 (3%)	0 (0%)	2 (4%)
$CD4^+$ cell count, median cells/μl (Q1, Q3)	827 (698, 1023)	800 (666, 1004)	852 (736, 1050)	827 (687, 1085)
NNRTI-based ART	99 (42%)	94 (61%)	2 (6%)	3 (6%)
IVDU, ever use, N (%)	17 (7%)	15 (10%)	0 (0%)	2 (4%)
Source study, *N* (%)				
A5170	96 (41%)	96 (62%)		
A5197	28 (12%)	28 (18%)		
A5068	19 (8%)	19 (12%)		
A5024	12 (5%)	12 (8%)		
ACTG 371	71 (30%)		28 (88%)	43 (90%)
A5187	9 (4%)		4 (13%)	5 (10%)

ACTG, AIDS Clinical Trials Group; ART, antiretroviral therapy; IVDU, intravenous drug use; NNRTI, non-nucleoside reverse transcriptase inhibitor.

not less than 500 cells/μl category had a median (Q1, Q3) pre-ATI $CD4^+$ cell count of 844 (687, 1050) cells/μl. Those categorized in the lower $CD4^+$ category by screening $CD4^+$ cell count had a median (Q1, Q3) pre-ATI $CD4^+$ cell count of 530 (432, 688) cells/μl. Participants treated during chronic infection had a median 5.1 (3.2, 6.5) years on ART.

Timing of HIV rebound after treatment interruption

Although viral load became detectable by week 4 in the majority of participants, a subset of participants maintained viral suppression for a longer period (Fig. 1a) . The proportion of participants maintaining virologic suppression (viral load < 200 copies/ml) at week 12 was smaller for those who initiated ART during chronic infection as compared with those who initiated ART during acute or early infection (chronic vs. acute vs. early: 3 vs. 9 vs. 15%, $P = 0.01$ Fisher's exact test, Fig. 1b). The proportion of participants who maintained HIV-1 RNA below 1000 HIV-1 RNA copies/ml was also smaller among those who initiated ART during chronic infection, but those comparisons were not statistically significant (Fig. 1c). When stratified by screening $CD4^+$ cell count, those with $CD4^+$ cell count 350–499 cells/μl did not have earlier viral rebound compared with those with $CD4^+$ cell count not less than 500 cells/μl (Fig. S1, http://links.lww.com/QAD/A825).

The timing of ART rebound was also evaluated by ART regimen. This analysis was restricted to those treated during chronic HIV infection because very few participants treated during acute or early infection received a non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing regimen (Table 1). Among participants treated during chronic infection, a significantly higher proportion of those receiving an NNRTI-containing regimen continued to have virologic suppression at week 4 by both viral load thresholds (Figs. 1d and e). After week 4, this difference narrowed such that by weeks 8 and 12, no significant differences were noted between those on NNRTI and non-NNRTI-containing regimens. The majority of participants (83%) discontinued the NNRTI within two days of stopping the remaining antiretroviral medications; only 5% of participants discontinued the NNRTI a week or more before the remaining antiretrovirals.

A subgroup analysis of participants from studies with more frequent HIV-1 RNA measurements was performed that excluded A5170 participants. A total of 139 participants were included in this analysis: 59 individuals treated during chronic infection, 32 treated during acute infection, and 48 treated during early infection. Baseline demographics and $CD4^+$ cell counts were similar between participants in the entire cohort vs. those in this subgroup analysis (data not shown). For participants who initiated ART during chronic infection, the median (Q1, Q3) time to virologic rebound was 3 weeks (2, 5 weeks) using a threshold of at least 200 HIV-1 RNA copies/ml and 4 weeks (3, 5 weeks) using the not less than 1000 HIV-1 RNA copies/ml threshold (Fig. S2, http://links.lww.com/QAD/A825). For participants who initiated ART during acute infection, the median (Q1, Q3) time to virologic rebound was 3 weeks (2, 6 weeks) at both thresholds (Fig. S2, http://links.lww.com/QAD/A825).

$CD4^+$ cell decline after treatment interruption

The median change in $CD4^+$ cell count during the time between treatment interruption and virologic rebound was −55 cells/μl (Q1, Q3: −179 cells/μl, +35 cells/μl) using the viral load threshold of at least 200 copies/ml and −84 cells/μl (Q1, Q3: −229 cells/μl, +12 cells/μl) using the not less than 1000 copies/ml threshold. There were no significant differences in $CD4^+$ cell loss by timing of ART initiation (Fig. 2a and d) or screening $CD4^+$ cell count (Figs. 2b and e). Participants with lower nadir $CD4^+$ cell counts had greater median $CD4^+$ cell loss, but this trend was not statistically significant (Figs. 2c and f).

The extent of CD4 cell loss at the time of viral rebound was compared with $CD4^+$ cell count changes at the end of 12–16 weeks of ATI. $CD4^+$ cell counts were significantly higher at the time of HIV-1 RNA rebound (using either the 200 copies/ml or the 1000 copies/ml threshold) than at the end of 12–16 weeks of ATI (Fig. S3, http://links.lww.com/QAD/A825).

Association between HIV-1 reservoir size and time to viral rebound

A subset of participants ($N = 124$) had PBMC and plasma available for HIV reservoir quantification from time points just before ATI (Table S1, http://links.lww.com/QAD/A825). Participants who initiated ART during acute/early infection ($n = 20$) had lower levels of pre-ATI CA-RNA than those who initiated ART during chronic infection ($n = 104$) (acute/early vs. chronic-treated: median 92 vs. 156 HIV-1 RNA copies/10^6 $CD4^+$ cells, exact Wilcoxon $P < 0.01$). Although pre-ATI levels of CA-DNA were also lower in participants treated during acute/early infection, the difference was not statistically significant (median 250 vs. 518 HIV-1 DNA copies/10^6 $CD4^+$ cells, $P = 0.23$). No significant differences were seen in the proportion of participants with detectable plasma residual viremia pre-ATI between those treated during acute/early vs. chronic infection. There were no significant differences by ART regimen (NNRTI vs. protease inhibitor-based) in pre-ATI CA-RNA, CA-DNA, or the proportion with plasma residual viremia. A modest correlation was detected between levels of pre-ATI CA-RNA and CA-DNA (Spearman $r = 0.18$, $P = 0.04$). Nadir $CD4^+$ cell count was inversely correlated with both CA-DNA and CA-RNA levels (CA-DNA: Spearman $r = -0.26$, $P < 0.01$; CA-RNA: Spearman $r = -0.33$, $P < 0.01$). No significant

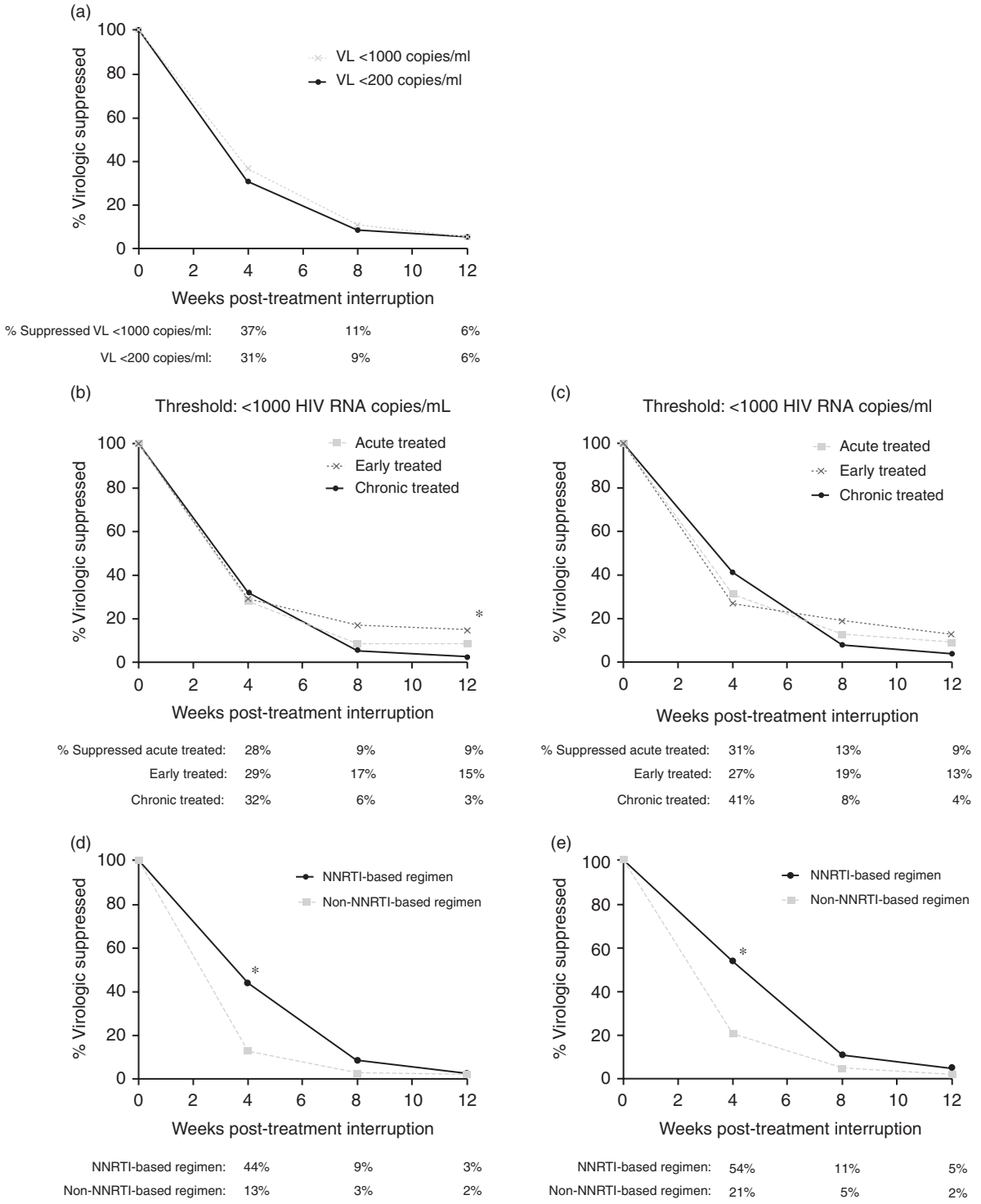

Fig. 1. Virologic suppression after treatment interruption stratified by timing of ART initiation and ART regimen. (a) Cumulative percentage of participants who maintained virologic suppression after treatment interruption based on two viral load (VL) thresholds. (b) Cumulative percentage of participants who remained suppressed stratified by treatment initiated during acute, early, or chronic HIV infection at the 200 HIV RNA copies/ml and (c) 1000 HIV RNA copies/ml viral rebound thresholds. (d) Cumulative percentage of participants who remained suppressed stratified by ART regimen [non-nucleoside reverse transcriptase inhibitor (NNRTI) vs. non-NNRTI] at the 200 HIV RNA copies/ml and (e) 1000 HIV RNA copies/ml thresholds. The 200 HIV RNA copies/ml threshold required a subsequent confirmatory viral load not less than 200 HIV RNA copies/ml. **P* value less than 0.05 by Fisher's exact testing.

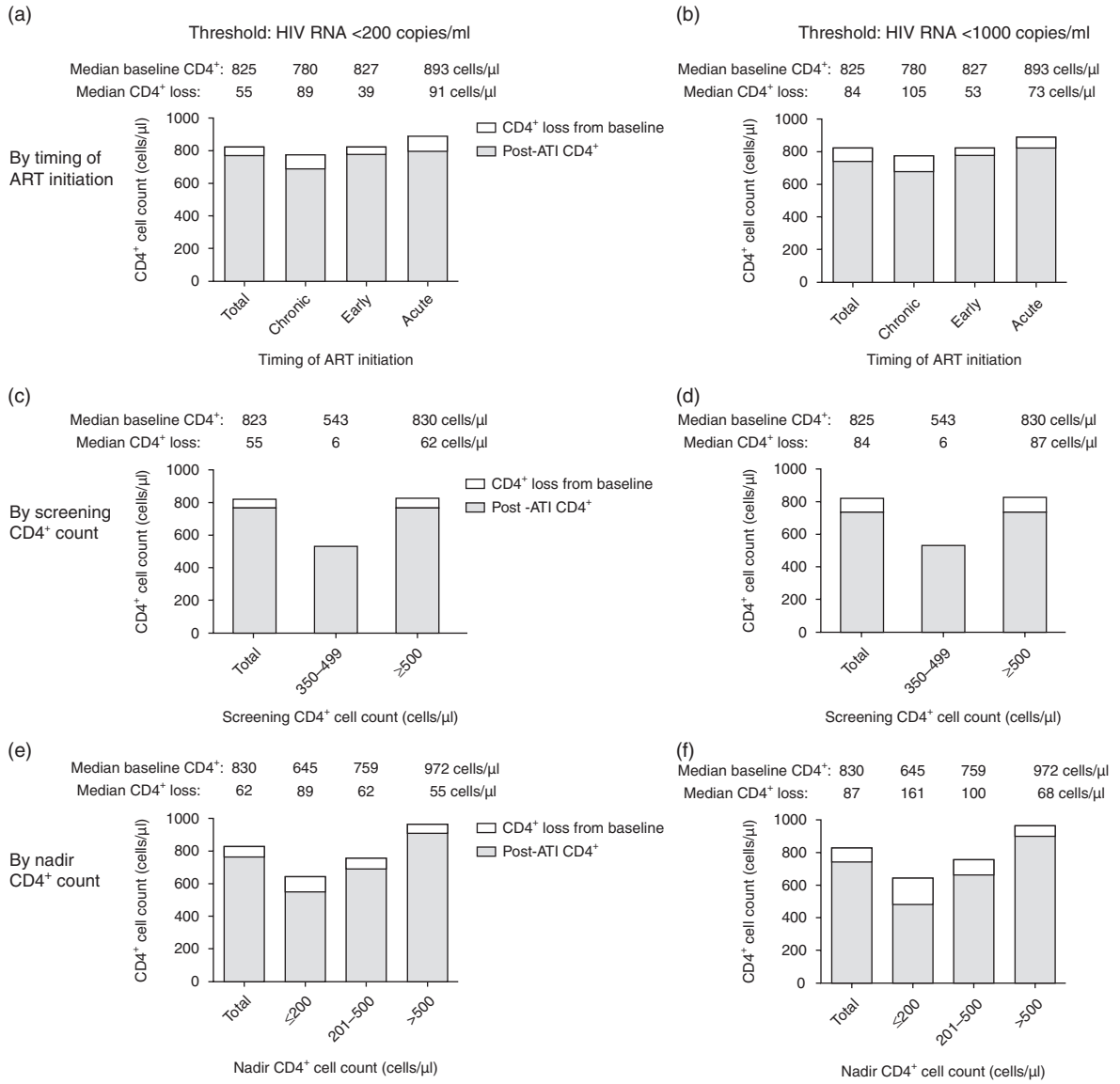

Fig. 2. CD4$^+$ cell loss after treatment interruption. CD4$^+$ cell loss at the time of HIV RNA not less than 200 copies/ml threshold (a) by timing of ART initiation, (b) screening CD4$^+$ cell count, and (c) nadir CD4$^+$ cell count. CD4$^+$ cell loss at the time of HIV RNA not less than 1000 copies/ml threshold (d) by timing of ART initiation, (e) screening CD4$^+$ cell count, and (f) nadir CD4$^+$ cell count.

correlations were detected in the overall population between presence of plasma residual viremia and nadir CD4$^+$ cell count, or levels of CA-DNA and CA-RNA. However, among those who initiated ART during acute/early infection, the presence of plasma residual viremia was significantly associated with higher levels of CA-RNA (Spearman $r = 0.68$, $P = 0.01$).

Higher pre-ATI CA-RNA levels were significantly associated with shorter time to viral rebound to a threshold of either 200 HIV-1 RNA copies/ml [$\leq$4 weeks ($N = 75$) vs. 5–8 weeks ($N = 35$) vs. >8 weeks ($N = 14$): 182 vs. 107 vs. <92 HIV-1 RNA copies/10^6 CD4$^+$ cells, Kruskal–Wallis $P < 0.01$] or 1000 HIV-1 RNA copies/ml (182 vs. 123 vs. <92 HIV-1 RNA copies/10^6 CD4$^+$ cells, $P < 0.01$, Fig. 3a and b). No significant association was seen between CA-DNA levels and timing of viral rebound, regardless of the timing of ART initiation (Figs. 3c and d). The proportion of participants with detectable plasma residual viremia was also significantly higher among those with shorter time to at least 200 HIV-1 RNA copies/ml ($\leq$ 4 vs. 5–8 vs. $\geq$8 weeks: 47 vs. 29 vs. 8%, Fishers exact $P = 0.02$, Figs. 3e and f).

In addition to the HIV reservoir measurements, four other factors were evaluated in the Cox model as predictors of time to viral load of at least 200 HIV RNA copies/ml: timing of ART, sex, nadir CD4$^+$ cell count, and ART regimen. On univariate analysis, factors

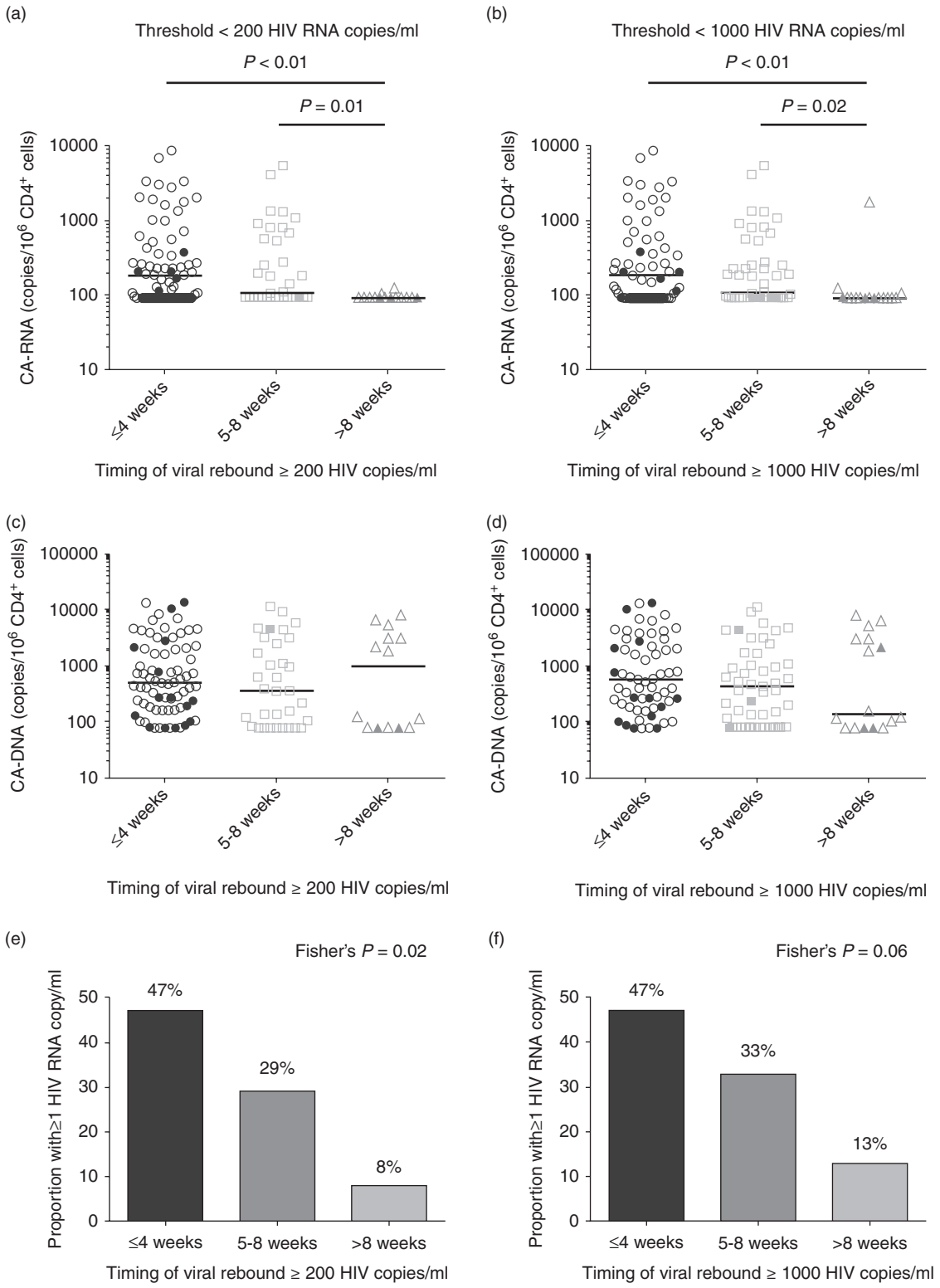

Fig. 3. Association of pre-ATI levels of CA-RNA, CA-DNA, and residual viremia with timing of viral rebound. Levels of pre-ATI CA-RNA categorized by timing of viral rebound to the (a) 200 HIV RNA copies/ml and (b) 1000 HIV RNA copies/ml thresholds. Levels of pre-ATI CA-DNA categorized by timing of viral rebound to the (c) 200 HIV RNA copies/ml and (d) 1000 HIV RNA copies/mL thresholds. (e) Proportion of individuals with pre-ATI residual plasma viremia not less than 1 HIV RNA copy/ml by viral rebound timing at the 200 HIV RNA copy/ml and (f) 1000 HIV RNA copies/ml HIV rebound thresholds. Open symbols represent values from participants treated during chronic infection and closed symbols represent values from participants treated during acute infection. Median values and significance by Wilcoxon rank-sum tests are shown.

significantly associated with earlier timing of viral rebound included CA-RNA [odds ratio (OR) 2.3, $P < 0.01$], presence of plasma residual viremia (OR 3.3, $P < 0.01$), and use of a non-NNRTI-containing regimen (OR 0.41 for NNRTI-containing regimens, $P = 0.02$). The effect of confounding was evaluated by a 2-covariate Cox modeling of CA-RNA with each of the other six variables as predictors of viral rebound timing. The odds ratio for CA-RNA remained stable regardless of the model (Fig. 4). In addition, the presence of plasma residual viremia and ART regimen remained significant when combined in models with CA-RNA levels. The Cox modeling results were generally unchanged at the 1000 HIV-1 RNA copies/ml viral rebound threshold (Table S2, http://links.lww.com/QAD/A825) or when the CA-RNA and CA-DNA values were represented as copies/10^6 PBMCs.

Discussion

In this pooled analysis of ACTG ATI studies, we report an in-depth evaluation of the timing of HIV rebound and $CD4^+$ cell decline, as well as of factors that predict the time of HIV rebound. Although most participants had viral rebound within 4 weeks of stopping ART, a subset maintained ART-free remission for longer periods of time. Participants who initiated ART during acute/early infection and who were on an NNRTI-based regimen were found to have delayed viral rebound. At the time of viral rebound, $CD4^+$ cell loss was relatively modest across ART timing and $CD4^+$ cell count categories. The size of the expressed HIV reservoir was associated with the time to viral rebound after interrupting ART.

Our results show that viral rebound generally occurs quickly after treatment interruption and confirm the rarity of post-treatment controllers, most of whom initiated ART during early infection [30–32]. A more in-depth analysis of participants with sustained post-treatment control within the ACTG ATI studies is needed. One previously published study with more limited participants also compared HIV rebound kinetics between those treated during acute vs. chronic HIV infection and support our findings that those treated during acute infection were more likely to have delayed viral rebound [33]. A second study also found that post-ATI viral loads were lower in those treated during acute infection [34]. Possible explanations for these findings include a more restricted HIV reservoir [35–37] and immune preservation in participants who initiate ART during early infection. In addition, our finding that nadir $CD4^+$ cell count was inversely correlated with levels of both CA-DNA and CA-RNA provide further evidence that $CD4^+$ cell depletion following delayed ART initiation has a sustained impact on HIV reservoir size even years after ART treatment [38].

We found that those who were on an NNRTI-based ART regimen had significantly delayed viral rebound at week 4 compared with those on a non-NNRTI-containing regimen (predominantly protease inhibitor-containing regimen). One possible explanation is the higher risk of incomplete virologic suppression by protease inhibitor-containing regimens seen in some prior studies [39–41], potentially leading to a larger and more active HIV reservoir at baseline [42]. However, we found no difference in pre-ATI HIV reservoir levels between those on an NNRTI- or non-NNRTI-containing regimen, and in the Cox model, NNRTI use was significantly associated with delayed viral rebound even after controlling for pre-ATI CA-RNA levels. Thus, baseline differences in circulating indices of HIV persistence and expression are unlikely to explain

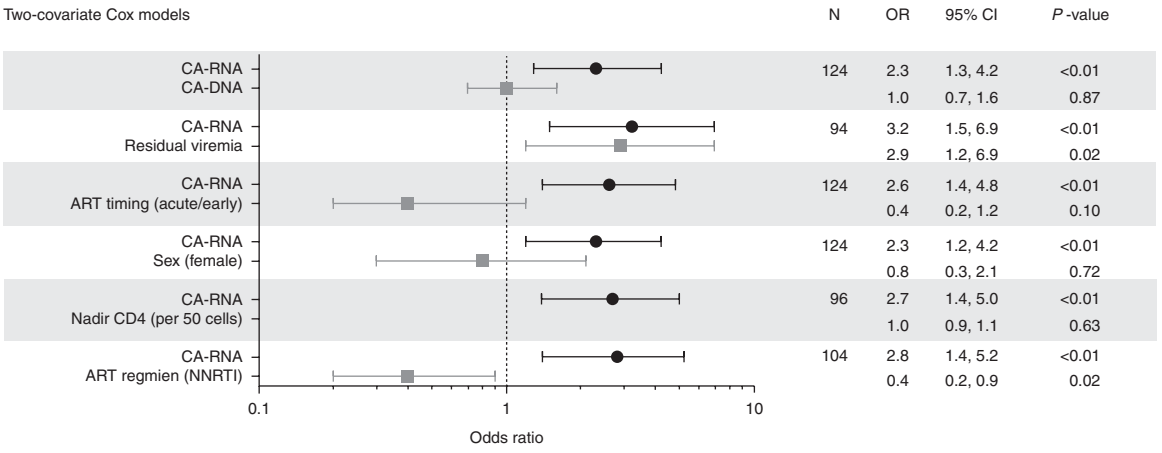

Fig. 4. Two-covariate Cox models of CA-RNA with six other factors as predictors of viral rebound time to not less than 200 HIV-1 RNA copies/ml. CA-RNA and CA-DNA are evaluated as $\log_{10}$ HIV-1 RNA copies/10^6 $CD4^+$ cells and residual viremia as a dichotomous variable (≥1 HIV-1 RNA copies/ml). Cox models with nadir $CD4^+$ cell count and ART regimen were restricted to participants treated during chronic infection. OR, odds ratio; CI, confidence interval.

differences in viral rebound timing by ART regimen. Although differential drug penetration into tissue sites of HIV infection remains a possible explanation [42], our findings are likely due to the significantly longer half-life of the NNRTI class of medications and prolonged NNRTI exposure even after treatment interruption. In a study of participants from ACTG A5095, the estimated half-life of NNRTIs such as efavirenz ranged from 24 to 48 h and drug levels above the 95% inhibitory concentration were estimated to be present for a median of 6–14 days after treatment interruption, depending on the cytochrome P450 genotype [43]. It should also be noted that early differences in viral rebound rates by timing of ART initiation (i.e. acute/early vs. chronic HIV infection) may have been masked by differences in ART regimen use, as a far greater proportion of individuals treated during chronic infection were on an NNRTI-based regimen. The finding that ART regimen is associated with viral rebound kinetics highlights the significance of ART effects in the analysis of ATI studies and the importance of ART standardization for future treatment interruption trials.

HIV treatment interruption studies remain an indispensable method to determine the clinical efficacy of therapeutics aimed at inducing ART-free HIV remission, but concerns remain about participant safety. In this study, we show that using a time-to-viral rebound as the main endpoint results in modest $CD4^+$ cell loss at either the 200 or 1000 HIV RNA copies/ml viral rebound thresholds and across ART timing and $CD4^+$ cell count categories. Participants with lower nadir $CD4^+$ cell counts appeared to have greater $CD4^+$ cell loss, although this difference was not statistically significant. Compared with the traditional 12–16 week ATI approach, we show that using an IMAP study design limited $CD4^+$ cell loss and may further decrease the risks of treatment interruption.

The identification and validation of biomarkers are integral to large-scale drug-development efforts. The discovery of that the plasma HIV-1 RNA level as a biomarker of disease progression led to its use as a surrogate endpoint for ART efficacy trials [44]. This change resulted in significantly shorter and smaller clinical trials and accelerated the pace of new drug development. As the HIV field increasingly focuses on the development of therapeutics to achieve sustained ART-free HIV remission, biomarkers that can predict the timing of viral rebound are needed [10,12]. In this study, we found that the size of the expressed HIV reservoir, as reflected by levels of CA-RNA and residual plasma viremia, were associated with the timing of viral rebound. A recent study of acutely treated participants in the SPARTAC study found that levels of total HIV DNA were predictive of the timing of viral rebound [17]. In our study, the lack of association between CA-DNA and timing of viral rebound could be explained by the accumulation of defective genomes in the HIV DNA reservoir in chronically infected participants [45]. However, an analysis of the french ANRS (National Agency for Research on AIDS) 116 SALTO study did show an association between lower HIV DNA levels and chances of maintaining post-treatment control in participants treated during early chronic infection [32]. Given the breadth of potential virologic, immunologic, and inflammatory biomarkers of HIV rebound kinetics, a systematic prospective evaluation of the optimal set of biomarkers of viral rebound timing is needed. Finally, the validation of predictive biomarkers occurs in stages and confirmation will be needed that changes in any identified biomarker are also associated with changes in clinical outcome.

This study has several limitations. Although chronically infected participants were on ART for a median of 5 years, those treated during acute/early infection were mostly enrolled in ACTG 371 and treated with 52 weeks of ART prior to the ATI. This length of ART is similar to that used in the SPARTAC trial [17], but it is possible that a longer duration of treatment prior to the ATI could further delay the timing of viral rebound. Another limitation is that documented pre-ART viral loads were not available for the majority of participants. In addition, the analysis stratified by screening $CD4^+$ cell counts should be interpreted with some caution given the limited number of participants with $CD4^+$ cell counts 350–499 cells/μl and that the median pre-ATI $CD4^+$ cell count of this group was actually slightly over 500 cells/μl. Finally, the limited number of stored pre-ATI samples available from individuals treated during acute/early infection restricted our ability to perform conclusive subgroup analysis of those participants and precluded the performance of quantitative viral outgrowth assays. Additional studies are also needed on the immunologic and inflammatory predictors of viral rebound.

In this study, we provide a detailed analysis of viral rebound timing and $CD4^+$ decline following treatment interruption, and we identified several factors that predicted the timing of viral rebound. These results will help inform the design and interpretation of future HIV treatment interruption studies. Our results also show that the quantification of the expressed reservoir may provide biomarkers of efficacy for therapies that aim to delay viral rebound, although studies are needed to confirm that interventions that reduce the expressed HIV reservoir will alter viral rebound kinetics. Additional systematic prospective studies are needed to fully assess optimal combination of virologic and immunologic biomarkers predictive of delayed HIV rebound.

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Conflicts of interest

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