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Title: The effect of HIV treatment interruption on subsequent immunologic response

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Abbreviations: ATI, analytical treatment interruption; ART, antiretroviral treatment; HIV, human immunodeficiency virus; IeDEA, International Epidemiology Databases to Evaluate Collaboration; LMM, linear mixed model; PWH, people with HIV

ABSTRACT

CD4 count recovery after antiretroviral treatment (ART) initiation has been thoroughly examined in HIV infection. However, immunologic response after ART restart following care interruption is less well studied. We compare the CD4 trends before disengagement from care and after ART re-initiation.

Data were obtained from the East-Africa International epidemiology Databases to Evaluate AIDS (IeDEA) Collaboration (N= 62,534). CD4 trends before disengagement, during disengagement, and after ART re-initiation were simultaneously estimated through a linear mixed model with two subject-specific knots placed at the time of disengagement and treatment re-initiation. We also estimated CD4 trends conditional on the baseline CD4 value.

10,961 patients returned to care after disengagement from care, with the median (IQR) time of gap in care being 2.7 (2.1, 5.4) months. Our model showed that CD4 increases after ART re-initiation were much slower than those before disengagement. Assuming that disengagement from care occurs 12 months after ART initiation and a three-month treatment gap, CD4 counts at 3 years since ART initiation would be lower by 36.5 cells/μL than those obtained under no disengagement.

Given that poorer CD4 restoration is associated with increased mortality/morbidity, specific interventions targeting at better retention to care are urgently required.

In human immunodeficiency virus (HIV) research, various bio-markers have been used to track disease progression. Among them, the number of CD4-positive lymphocytes is an important predictor of clinical outcomes (1) and it is monitored over time to evaluate the progress of the disease and the state of the immune system (1). In the absence of antiretroviral treatment (ART), following HIV infection, the number of CD4 cells declines over time, though the patient-specific trajectories can vary substantially (2). The introduction of combination ART in 1996 has allowed long-term management of HIV, greatly improving the life expectancy and quality of life of people with HIV (PWH).

CD4 count recovery after ART initiation has been previously thoroughly examined (3–5). Providing treatment adherence, a rapid increase in CD4 counts occurs, followed by a slower increase thereafter. However, a substantial proportion of patients disengages from HIV care (6–8). These patients experience losses in CD4 count, are more likely to experience adverse clinical outcomes, and they can transmit HIV to the community (9). A significant proportion of the patients who have disengaged from care, re-engaged in care in cyclical patterns of patient disengagement from, and re-engagement in care [patient "churn" (10–13)].

Unlike CD4 restoration after the initial ART initiation, the consequences after disengagement from care and, in particular, the CD4 response after ART re-initiation have not been fully investigated. Many studies have examined the impact of planned (14–18) and unplanned (9,19–23) ART interruptions, showing that both are associated with increased mortality, morbidity, and slower CD4 recovery. Fewer studies though have investigated the immunologic consequences of unplanned care interruption, particularly in resource-constrained settings (24). In such settings, HIV care is often combined with nutrition and general support in a holistic approach beyond treatment supplementation. Given that poorer CD4 restoration may lead to increased mortality/morbidity even among those who re-engage in care (22), comparing the potential restoration of CD4 counts after re-initiation of ART with the

CD4 increases after the initial ART initiation (and before disengagement from care) is essential.

In this paper, we focus on PWH who return to care after transient disengagement from care and we compare the CD4 trajectories after re-initiating ART with the corresponding ones before disengagement from care. We hypothesize that the rate of CD4 increase is slower after ART re-initiation compared to that after initial ART initiation as previous studies have suggested (21). To formally examine this hypothesis, we use a unified model for CD4 counts during initial ART initiation, disengagement from care, and ART re-initiation. Modeling these CD4 trajectories is critical in evaluating response to care at the individual level but also in assessing programmatic outcomes at the population level. In the era of universal treatment for PWH ["Treat-All" (25)], the emphasis of current treatment protocols is on monitoring viral suppression, which minimizes onward transmission and is associated with better prognosis at the individual level, rather than on immune reconstitution, leading to longitudinal CD4 data becoming more and more sparse nowadays. From this perspective, the present paper is the last best effort to address the issue of CD4 increase and, more importantly, recovery after ART re-initiation.

METHODS

Study population

Data were obtained from the East-Africa region of the International Epidemiology Databases to Evaluate AIDS (IeDEA) Collaboration (26), a network of HIV care and treatment programs in Kenya, Uganda, and Tanzania which routinely collects clinical/sociodemographic data using an electronic medical record system. Eligible for this study were those who had initiated ART at $\geq$ 18 years of age, had disengaged from and subsequently re-engaged in care, and had at least one observed CD4 count at/after (first) ART initiation from 2001 to 2011. Typically, monthly participants' visits are scheduled with one to three-month ART prescriptions, whereas CD4 counts

are measured biannually. The date of disengagement from care is defined as the midpoint between the date of the last visit before disengagement from care and 2 months after the date of the next scheduled visit. Thus, the working definition of disengagement from care is no clinic visit for 3 months, as 3 months is the maximum time patients could access ART without clinic visits. The study was approved under both the Indiana University Institutional Review Board in the United States and the Institutional Research and Ethics Committee of the Academic Model to Provide Access to Healthcare (AMPATH) in Kenya.

Combined model of CD4 evolution before disengagement from care, during disengagement from care, and after ART re-initiation

CD4 data before and during disengagement from care and after ART re-initiation were modeled by a linear mixed model (LMM) (27) with two subject-specific knots placed at the time of disengagement from care and treatment re-initiation since CD4 trends are drastically affected by these events. As the distribution of CD4 counts is heavily right-skewed, square-root transformation was applied. Let $y_i(t)$ be the observed square-root-transformed CD4 count of the ith patient at $t \geq 0$ years since ART initiation. The knots are incorporated into the model by defining D_i and R_i to be the subject-specific times from ART initiation to care disengagement and treatment re-initiation, respectively. Note that $R_i = \infty$ if the ith patient never restarted ART. The full structure of the model is

$$\begin{aligned} y_i(t) &= (\beta_0 + b_{i0}) + (\beta_1 + b_{i1})t_1 + \beta_2\sqrt{t_1} \\ &+(\beta_3 + b_{i2})t_2 + \beta_4 t_2(D_i - 1.5) \\ &+(\beta_5 + b_{i3})t_3 + \beta_6 t_3(D_i - 1.5) + \beta_7 t_3(R_i - D_i - 0.5) \\ &+\beta_8\sqrt{t_3} + \beta_9\sqrt{t_3}(D_i - 1.5) + \beta_{10}\sqrt{t_3}(R_i - D_i - 0.5) + \epsilon_i(t) \quad (1) \end{aligned}$$

where

$$t_1 = \begin{cases} t, & t \leq D_i \\ D_i, & t > D_i \end{cases}, \quad t_2 = \begin{cases} 0, & t \leq D_i \\ t - D_i, & D_i < t \leq R_i \\ R_i - D_i, & t > R_i \end{cases}, \quad t_3 = \begin{cases} 0, & t \leq R_i \\ t - R_i, & t > R_i \end{cases} \quad (2)$$

$\sqrt{t}$ is included in the model to capture the non-linear evolution of CD4 counts; $\mathbf{b}_i^{\top} = (b_{i0}, b_{i1}, b_{i2}, b_{i3})$ denotes the random effects (b_{i0} random intercept at ART initiation, b_{i1}, b_{i2}, and b_{i3} random slopes before care disengagement, during disengagement, and after ART re-initiation, respectively), which are assumed to follow the multivariate Normal distribution with mean zero and unstructured covariance matrix $\mathbf{D}$, i.e. $N(\mathbf{0}, \mathbf{D})$, and $\epsilon_i(t) \sim N(0, \sigma^2)$ denotes the within-subject error. Thus, correlation in the data is modeled through the random effects, whereas $\epsilon_i(t)$ captures the variability that is unexplained by the model. The population-averaged evolution of CD4 (on the square root scale), $\mu_i(t)$, and the corresponding rate of change before disengagement (i.e. $t < D_i$) are equal to $\mu_i(t) = E\{y_i(t)\} = \beta_0 + \beta_1 t + \beta_2\sqrt{t}$ and $\frac{\partial \mu_i(t)}{\partial t} = \beta_1 + \frac{\beta_2}{2\sqrt{t}}$, respectively. The corresponding CD4 evolution during the period between disengagement from care and ART re-initiation (i.e. $D_i < t \leq R_i$) is equal to $\mu_i(t|D_i) = \beta_0 + \beta_1 D_i + \beta_2\sqrt{D_i} + \beta_3(t - D_i) + \beta_4(t - D_i)(D_i - 1.5)$, which is linear in t, with the corresponding rate of change equal to $\frac{\partial \mu_i(t|D_i)}{\partial t} = \beta_3 + \beta_4(D_i - 1.5)$. Here, times from ART initiation to care disengagement have been centered at $D_i = 1.5$ years, which is close to the corresponding mean time in our application. Note that the rate of CD4 decline during disengagement is allowed to depend on D_i as CD4 decline may be faster at higher CD4 counts (corresponding in general to longer times from treatment initiation to care disengagement), with β_3 denoting the CD4 decline at 1.5 years since ART initiation ($D_i = 1.5$). As CD4 data between disengagement from care and re-engagement in care are unavailable, the model for the CD4 decline is actually informed by the last CD4 measurement before disengagement and all available CD4 measurements from re-engagement in care to ART re-initiation. CD4 evolution after ART re-initiation (i.e. $t > R_i$) is of the form:

$$\begin{aligned}\mu_i(t|D_i, R_i) \quad &= \beta_0 + \beta_1 D_i + \beta_2\sqrt{D_i} \\ &+\beta_3(R_i - D_i) + \beta_4(R_i - D_i)(D_i - 1.5) \\ &+\beta_5(t - R_i) + \beta_6(t - R_i)(D_i - 1.5) + \beta_7(t - R_i)(R_i - D_i - 0.5) \\ &+\beta_8\sqrt{t - R_i} + \beta_9\sqrt{t - R_i}(D_i - 1.5) + \beta_{10}\sqrt{t - R_i}(R_i - D_i - 0.5), \quad (3)\end{aligned}$$

since $t_1 = D_i$ and $t_2 = R_i - D_i$ for $t > R_i$. The corresponding population-averaged slope is $\beta_5 + \beta_6(D_i - 1.5) + \beta_7(R_i - D_i - 0.5) + \frac{\beta_8 + \beta_9(D_i - 1.5) + \beta_{10}(R_i - D_i - 0.5)}{2\sqrt{t - R_i}}$. In the above, we have also centered the time between care disengagement and ART re-initiation $(R_i - D_i)$ at 0.5 years, which approximates the average treatment gap duration in our application. Patients who never restarted ART after re-initiating care contribute to the linear model between disengagement from care and ART restart but do not contribute to the model of CD4 reconstitution after ART restart.

The model, including interaction terms, allows the rate of increase after ART re-initiation to depend on both D_i and R_i, which serves as a proxy of conditioning on CD4 counts at treatment re-initiation. In the main analysis, all available CD4 measurements at/after re-engagement in care were used, ignoring any subsequent disengagement from care. As a sensitivity analysis, though, data are right-censored at the time of the 2nd disengagement.

Comparing CD4 trends before care disengagement and after ART re-initiation

The rate of CD4 increase after ART (re)initiation depends heavily on the baseline CD4 value [i.e., the CD4 count at ART (re)initiation] (4,5). Thus, to compare the CD4 trends before care disengagement with the corresponding ones after ART restart, conditioning on the baseline CD4 value is needed.

In the literature of LMMs, the focus of inference is often on the value $m_i(t) = y_i(t) - \epsilon_i(t)$, which is the individually predicted mean (square root) CD4 count. $m_i(t)$ can be factorized as

$$m_i(t) = \mathbf{x}_i^\top(t)\boldsymbol{\beta} + \mathbf{z}_i^\top(t)\mathbf{b}_i \quad (4)$$

where $\mathbf{x}_i^\top(t)\boldsymbol{\beta}$ denotes the fixed-effect (i.e. population-averaged) part and $\mathbf{z}_i^\top(t)\mathbf{b}_i$ the patient-specific random-effect contribution.

We extended the approach proposed by Harrison and colleagues (28) to compare the CD4 trends before care disengagement and after ART restart conditioning on the baseline CD4 intervals (categories): $[0,50]$, $(50,100]$, $(100,200]$, $(200,250]$, $(250,350]$, $(350,500]$, and $(500,\infty]$ cells/μL. That is, we compare the CD4 evolution before disengagement from care ($t < D_i$) given a baseline CD4 category [e.g. $m_i(0) < \sqrt{50}$] with the corresponding evolution after ART re-initiation conditional on the same CD4 category at treatment re-initiation, e.g.,

$$E\{m_i(t)|m_i(0) < \sqrt{50}\},\ t < D_i \quad (5)$$
$$E\{m_i(t)|m_i(R_i) < \sqrt{50}\},\ t > R_i \quad (6)$$

respectively. It is straightforward to show that, conditional on $\boldsymbol{\beta}$ and $\mathbf{D}$, $\{m_i(t), m_i(0)\}^{\top}$ follows the bivariate Normal distribution with means $\mu_i(t)$ and $\mu_i(0)$ and variances $v_i(t) = \mathbf{z}_i^{\top}(t)\mathbf{D}\mathbf{z}_i(t)$ and $v_i(0) = \mathbf{z}_i^{\top}(0)\mathbf{D}\mathbf{z}_i(0)$, respectively, and covariance between $m_i(t)$ and $m_i(0)$ equal to $\mathbf{z}_i^{\top}(0)\mathbf{D}\mathbf{z}_i(t)$. Such expectations can be calculated using properties of the bivariate Normal distribution (29); the formulas are detailly described in Web Appendix. Note that this is a post-estimation procedure based on the results from the LMM using all relevant data. Calculating corresponding standard errors for (5) and (6) is complicated. To obtain confidence intervals, we relied on a Monte Carlo approach by simulating 1000 random draws from the large-sample distribution of the estimated parameters and repeatedly estimating Equations (5) and (6) for all baseline CD4 strata. 2.5% and 97.5% quantiles of the resulting empirical distribution were used to construct 95% confidence intervals. In addition, at each time point $t < D_i$, we pointwise compared the CD4 trends from ART initiation to care disengagement with the corresponding CD4 trends after ART restart (i.e. at time $t + R_i$), conditional on baseline CD4 category; e.g. for the first baseline CD4 category, we estimated $E\{m_i(t)|m_i(0) < \sqrt{50}\} - E\{m_i(t + R_i)|m_i(R_i) < \sqrt{50}\}$. Confidence intervals were constructed using the above-mentioned Monte Carlo approach.

To further quantify the CD4 loss due to disengagement from care, we also estimated the time from ART re-initiation to reach the CD4 levels at disengagement, $\mu_i(D_i)$,

assuming specific values for D_i and R_i that are representative of our data. That is, we solved the equation $\mu_i(D_i) = \mu_i(R_i + t|D_i, R_i)$ over time t and constructed confidence intervals through the aforementioned Monte Carlo approach.

The appropriateness of the parametric form assumed for the CD4 evolution was assessed by refitting the LMM using a more elaborate structure using natural splines of time with 3 internal knots at 0.5, 1, and 2 years since ART (re)initiation. The fit of the models was evaluated through the AIC criterion.

To investigate the robustness of our findings, an additional sensitivity analysis was performed including only individuals with at least one CD4 count both at/after ART initiation and ART re-initiation. Differences between CD4 increases before care disengagement and after ART restart could be partly attributed to a regression-to-the-mean effect. To investigate the potential regression-to-the-mean effect, we compared the estimated CD4 trajectories of our target population before disengagement from care with the corresponding CD4 trajectories of the whole population initiating ART.

RESULTS

Patient characteristics

In total, 62,534 patients had at least one observed CD4 cell count after ART initiation and initiated ART at ≥ 18 years of age. Of these, 2,647 (4.2%) died while in care, 27,744 (44.4%) were reported to have disengaged from care, and 32,143 (51.4%) were in care by the end of the follow-up time.

Out of 27,744 disengaged patients, 10,961 (39.5%) returned to care, with the latter being the target population of this study. 8,501 (77.6%) had at least one observed CD4 measurement at/after return to care. Descriptive characteristics of the study population, overall, and by availability of CD4 counts after re-engagement in care are provided in Table 1. The median (IQR) time from ART initiation to disengagement from care was 12.5 (4.5, 25.1) months, and from disengagement to re-engagement in

care 2.7 (2.1, 5.4) months. Even though the duration of gap in care was short, a significant loss of CD4 counts was observed, with the median CD4 at disengagement from care reducing from 297 to 248 cells/μL at ART restart. Overall, 93.9% restarted ART after re-engagement in care. Patients who returned to care without observed CD4 measurements had slightly shorter time from ART initiation to disengagement from care, longer time from disengagement from to re-engagement in care, and they were less likely to have re-initiated ART upon return to care compared to those who re-engaged in care with observed CD4 counts (Table 1). After re-engagement in care, 351 (3.2%) died while in care and 5,215 (47.6%) were lost to follow-up at the data-freeze date.

Estimated CD4 trajectories

The parameter estimates from fitting the LMM are provided in Table 2. The model indicates that CD4 counts decline quickly after treatment interruption, with losses tending to become steeper with longer initial ART duration; CD4 increases after ART re-initiation strongly depended on both the time to disengagement (D_i) and the duration between disengagement from care and ART re-initiation ($R_i - D_i$). The estimated CD4 evolution for a typical patient is presented in Figure 1, assuming $D_i = 1.5$ and $R_i = 2$ years. To better evaluate the impact of disengagement from care on long-term immunologic response, we considered further reasonable scenarios regarding D_i and R_i. These are presented in Figure 2. Disengagement, especially when happening soon after ART initiation, is associated with substantially slower CD4 restoration at 3 years since ART initiation. For example, if disengagement from care occurs 12 months after ART initiation and the duration of treatment gap is 3 months, CD4 counts at 3 years since ART initiation would be lower by 36.5 cells/μL than the ones that would have been obtained if there was no disengagement. However, a 3-month treatment gap at 3 months after ART initiation is associated with a corresponding CD4 loss at 3 years since ART initiation of 107.2 cells/μL.

Although CD4 counts increased rapidly both after ART initiation and re-initiation following disengagement from care, there was a trend of slower CD4 cell recovery after ART re-initiation. We compared the estimated CD4 evolution after the initial start of ART and before disengagement from care with the one after ART re-initiation conditionally on being in the same baseline CD4 category. The results are presented in Figure 3. Conditionally on each baseline category, the estimated CD4 increase after initial ART initiation is substantially higher compared to the corresponding one after ART re-initiation. The corresponding results when data are right-censored at the time of the second disengagement are provided in panel (C) of Figure 3. While this analysis leads, "unsurprisingly", to a higher CD4 trajectory, in no way does it increase these to the point of the CD4 curves seen before disengagement. The estimated differences in CD4 counts at each time point along with the corresponding confidence intervals are presented in Web Figure 1; most 95% confidence intervals exclude zero.

Time to reach pre-disengagement CD4 levels

In Figure 4 the predicted times from ART re-initiation to reaching CD4 levels at disengagement from care for several reasonable scenarios involving D_i and R_i are shown. For example, with time from ART initiation to disengagement $D_i = 12$ and treatment gap of $(R_i - D_i) = 3$ months, the estimated time to reach pre-disengagement CD4 levels was 1.8 months (95%CI: 1.5, 2.2 months). Despite this robust CD4 recovery after ART re-initiation, we estimated that, at 1 year after treatment re-initiation, the CD4 count would be lower by 32.3 (95%CI: 26.4, 38.0) cells/μL than the one that would have been achieved if there were no disengagement. For given treatment gap duration $(R_i - D_i)$, the time to reach the CD4 value at disengagement tended to be longer with longer duration of initial ART (D_i) (Figure 4). For example, given treatment gap of $(R_i - D_i) = 3$ months and time from ART initiation to disengagement D_i equal to 18 or 24 months, the estimated times to reach pre-disengagement CD4 levels were equal to 2 months (95%CI: 1.7, 2.4 months) and 2.3 months (95%CI: 1.8, 2.9 months), respectively. As expected, the time to reach CD4

levels at disengagement from care increased with longer periods of treatment gap, for example, when $D_i = 24$ and $R_i - D_i = 12$ months, the time from ART re-initiation to reaching CD4 levels at disengagement was 24.2 (95%CI: 16.6, 35.2.) months.

Sensitivity analyses

The results from the LMM with natural splines of time showed that our initial parameterization using time and square root of time had a better fit to the data compared to the fit based on the natural splines approach. The AIC criterion using the parameterization in Equation (1) was equal to 293,939.6, lower than the AIC when using natural splines (294,658.7).

Results from the sensitivity analysis including individuals with CD4 data both at/after ART initiation and ART re-initiation are provided in Web Figure 2, with identical conclusions to those from Figure 3. The comparison of the estimated CD4 trajectories of our target population with the corresponding ones of the whole population initiating ART is provided in Web Figure 3. By visual inspection, it was apparent that our target population had CD4 profiles only slightly better than those of the whole population initiating ART (26 CD4 cells/μL difference at 3 years since ART initiation).

DISCUSSION

In this paper, we have proposed a unified approach to jointly model (i) the CD4 trends before disengagement, (ii) CD4 decline during disengagement, and (iii) CD4 increase after ART re-initiation of the population who disengaged from and re-engaged in care using a modified linear mixed model with two subject-specific knots placed at the time of disengagement and ART re-initiation. The structure of the model allowed the rate of CD4 decline during disengagement to depend on the time from the initial start of treatment to disengagement from care, as CD4 declines may be faster at higher CD4 counts, which are in general expected with longer times to disengagement.

Furthermore, the rate of CD4 increase after ART re-initiation was assumed to depend on both the time to disengagement and the treatment gap duration, as these two times are closely related to the average CD4 counts at ART re-initiation.

In our study, as in many others, a rapid CD4 increase after ART initiation was observed. Similar to results from studies on treatment interruption (9,14,16,24,30,31), we found a steep decline in CD4 counts during disengagement from care. As expected from past studies (20,21), the evolution of CD4 counts after ART resumption was characterized by a very rapid initial increase, followed by a gradually less steep slope. However, there was a tendency for a slower CD4 recovery compared to that seen after the initial start of ART and before disengagement. These differences became clearer when we conditioned on baseline CD4 levels. This is an important addition to the model, given the large variability in CD4 levels at first ART initiation and ART resumption. Failure to do so muddies the waters when trying to accurately assess the impact of treatment interruption on immune function.

Consistently with the literature (9,20–22,24), we showed that even short periods of disengagement from care are associated with substantially worse long-term immunologic response, especially when disengagement happens soon after ART initiation. This result reflects the slower CD4 increase upon ART resumption rather than the CD4 loss during disengagement since the latter is generally low for short treatment gaps. Moreover, in some cases, the estimated time to reach pre-disengagement CD4 levels was longer than the duration of the gap itself. This is concerning as it implies that the CD4 decline during disengagement may be steeper than the rate of CD4 gain after ART re-initiation, resulting in long periods until losses are reversed. The finding that the time to reach the CD4 levels at disengagement is longer with longer initial ART duration is attributed to the higher CD4 levels at ART re-initiation, in which cases CD4 recovery, at least the initial increase, is slower (4,5,21).

This paper focused on proposing a unified method to model CD4 evolution after ART initiation, during disengagement from care, and after ART re-initiation and on comparing initial and after ART resumption CD4 trends conditional on baseline CD4 levels at ART (re)initiation. However, our results are also of clinical relevance as, despite current guidelines for ART continuity, several PWH have at least temporal treatment interruption in both rich and limited-resource countries (32). Understanding the effects of such treatment interruptions on the future clinical course is very important at both individual and population level as slower CD4 restoration is associated with higher risk of death and AIDS. In particular, the assumption that patients who disengage from care, even only temporarily, have the same immunologic reconstitution upon ART restart as others who remain in care continuously should be revised. Our results also have implications for modeling studies, where an accurate description of CD4 evolution, accounting for non-continuous care, is important. For example, the United Nations (UN) Joint Programme in HIV/AIDS (UNAIDS) produces various projections for the HIV epidemic worldwide using discrete CD4 categories through the Spectrum software (33). Our results may also contribute to current research on analytical treatment interruptions (ATI) for HIV cure (34–36), although we acknowledge that disengagement from care is clinically different from treatment interruption while in care, as in ATI, PWH are closely monitored.

Our study has also some limitations. First, as this study included only individuals who eventually re-engaged in care, our estimate of CD4 decline during disengagement may not be representative of the CD4 decline of the whole population disengaging from care; if the probability of re-engagement is higher when the rate of CD4 decline is steeper, then our model likely over-estimates the rate of CD4 loss. Estimation of CD4 trajectories for the whole population initiating ART would require much more complex technical work such as joint modeling of CD4 levels and competing risks (death while in care and disengagement from care) (37), possibly adjusting for potentially incomplete death ascertainment (38). Second, the slower CD4 recovery after ART re-initiation could be partly attributed to intermittent adherence. However,

we have no reason to believe that the level of compliance would be substantially different between the pre-disengagement and post-re-engagement periods, as our comparison involved the same individuals. In addition, when data were right-censored at the second disengagement, results implied a slightly better CD4 restoration after ART re-initiation compared to that in the main analysis, a finding consistent with the assumption that each treatment interruption has a detrimental effect on CD4 recovery. Regression to the mean could also partly explain the slower CD4 recovery upon ART resumption. However, in our sensitivity analysis, the difference in the estimated CD4 counts between our target population and the whole population initiating ART was very small, not supporting this hypothesis. Third, in our analysis, we included individuals who disengaged from and subsequently re-engaged in care. However, disengaged individuals could have returned to care in a non-IeDEA site. In this work, we have assumed that those who re-engaged in an IeDEA site are representative of the whole population returning to care. Fourth, our estimates may be sensitive to the limited number of available CD4 measurements during ART (re)initiation. Finally, data after re-engagement in care were censored due to death or lost-to-follow-up. As we used a likelihood-based approach modeling all observed data, our results are valid under the assumption that data are missing at random, i.e. the probability of dropping out of the study after return to care depends on the observed CD4 data (39). Whereas this is a reasonable assumption for lost-to-follow-up, death may be missing "not at random", requiring joint modeling of CD4 data and mortality. However, the proportion of deceased individuals post-re-engagement was small to seriously affect our results.

To conclude, this is an important and novel study, becoming increasingly difficult to perform with the decreasing availability of CD4 counts during treatment. We showed that, although CD4 counts increase rapidly both after ART initiation and treatment re-initiation following disengagement, even short periods of disengagement from care and the resulting treatment interruption are associated with slower CD4 cell recovery pointing to the salient advantage of maintaining continuity of care among PWH.

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Table 1. Descriptive characteristics of the patients included in the East-Africa region of the IeDEA Collaboration (from 2001 to 2011) who re-engaged in care according to whether CD4 measurements after re-engagement are available.

Characteristics	Returned with CD4 (*n*=8,501)			Returned without CD4 (*n*=2,460)			Total (*n*=10,961)			*P* value
	No.	%	Median (Q1, Q3)	No.	%	Median (Q1, Q3)	No.	%	Median (Q1, Q3)	
Gender										0.883
Female	5636	66.3		1627	66.1		7263	66.3		
Male	2865	33.7		833	33.9		3698	33.7		
Age at disengagement (yrs)			37.8 (32.0, 44.7)			37.6 (31.2, 44.7)			37.8 (31.9, 44.7)	0.017
CD4 at ART initiation (cells/μL)			139.0 (67.0, 208.1)			138.0 (58.0, 217.8)			139.0 (64.0, 210.0)	0.244
CD4 at disengagement available										<0.001
Yes	1826	21.5		772	31.4		2598	23.7		
CD4 at disengagement (up to 2 months prior to last visit before disengagement) (cells/μL)			293.5 (175.0, 432.8)			314.0 (136.0, 495.2)			297.0 (166.0, 452.8)	0.040
Available CD4 from re-engagement to ART restart[a]										
Yes	3985	36.4		0	0.0		3985	36.4		
CD4 from re-engagement to ART restart[a] (cells/μL)			248.0 (126.0, 412.0)			NA			248.0 (126.0, 412.0)	
Time from ART initiation to disengagement (months)			12.9 (4.9, 25.5)			10.8 (3.4, 23.0)			12.5 (4.5, 25.1)	<0.001
Time from disengagement to re-engagement (months)			2.7 (2.1, 5.0)			3.2 (2.2, 7.1)			2.7 (2.1, 5.4)	<0.001
ART restart										<0.001
Yes	8156	95.9		2131	86.6		10287	93.9		
ART restart at re-engagement in care										0.797
Yes	7003	82.4		2021	82.2		9024	82.3		
Time from disengagement to ART restart (months)[b]	2.9			3.5			3.0			<0.001
Number of available CD4 before disengagement			2.0 (1.0, 4.0)			2.0 (1.0, 3.0)			2.0 (1.0, 4.0)	<0.001
Number of available CD4 post re-engagement			2.0 (1.0, 3.0)			NA			2.0 (1.0, 3.0)	

Abbreviations: ART, combination antiretroviral treatment; NA, not applicable

[a] The first available CD4 count up to 15 days after ART restart was considered.

[b] Values are expressed as median survival

Table 2. Parameter estimates from the LMM including subject-specific knots at the time from ART initiation to disengagement from care (D_i) and ART re-initiation (R_i) fitted to square root CD4 count from the East Africa IeDEA study (from 2001 to 2011) including individuals who disengaged from and subsequently re-engaged in care.

Parameters	Estimate	95% CI	P-value
Intercept $\sqrt{\text{cells}/\mu L}$	12.123	12.015, 12.230	<0.001
t_1[a] $\sqrt{\text{cells}/\mu L}$/year	-1.580	-1.708, -1.451	<0.001
$\sqrt{t_1}$	7.823	7.636, 8.010	<0.001
t_2[b] $\sqrt{\text{cells}/\mu L}$/year	-3.117	-3.348, -2.886	<0.001
$t_2 \times (D_i - 1.5)$	-0.236	-0.435, -0.037	0.020
t_3[c] $\sqrt{\text{cells}/\mu L}$/year	-0.415	-0.583, -0.246	<0.001
$t_3 \times (D_i - 1.5)$	-0.215	-0.358, -0.071	0.003
$t_3 \times (R_i - D_i - 0.5)$	-0.724	-1.059, -0.389	<0.001
$\sqrt{t_3}$	2.511	2.266, 2.756	<0.001
$\sqrt{t_3} \times (D_i - 1.5)$	-0.007	-0.204, 0.191	0.948
$\sqrt{t_3} \times (R_i - D_i - 0.5)$	2.051	1.575, 2.527	<0.001

Abbreviations: ART, combination antiretroviral treatment; D_i, time (years) from ART initiation to disengagement from care; LMM, linear mixed model; R_i, time (years) from ART initiation to ART re-initiation.

[a] $t_1 = \begin{cases} t, & t \le D_i \\ D_i, & t > D_i \end{cases}$, where t denotes time from ART initiation in years.

[b] $t_2 = \begin{cases} 0, & t \le D_i \\ t - D_i, & D_i < t \le R_i \\ R_i - D_i, & t > R_i \end{cases}$

[c] $t_3 = \begin{cases} 0, & t \le R_i \\ t - R_i, & t > R_i \end{cases}$

Figure legends

Figure 1: Estimated CD4 trajectory based on the linear mixed model including subject-specific knots at the time from ART initiation to disengagement from care (D_i) and resumption of treatment (R_i) fitted to data from the East Africa IeDEA study including individuals who disengaged from and subsequently re-engaged in care (from 2001 to 2011). Shown is the estimated CD4 evolution (back-transformed from the square-root scale) assuming that the time from ART initiation to disengagement is $D_i = 1.5$ years and the time gap from disengagement from care to ART re-initiation $R_i - D_i = 6$ months along with the observed CD4 counts (black dots). Also shown is the time it takes to reach the pre-disengagement CD4 levels and the difference of the CD4 levels 6 months after treatment re-initiation with the CD4 levels that would have been observed had disengagement not happened by that time.

Figure 2: Estimated CD4 trajectory based on the linear mixed model including subject-specific knots at the time from ART initiation to disengagement from care (D_i) and ART re-initiation (R_i) fitted to data from the East Africa IeDEA study including individuals who disengaged from and subsequently re-engaged in care (from 2001 to 2011). It is estimated assuming that $D_i = 3$ or $D_i = 12$ months, with the time gap from disengagement to ART re-initiation $(R_i - D_i) = 3$ or $(R_i - D_i) = 6$ months. For comparison, the estimated CD4 evolution assuming no disengagement (solid line) is also presented.

Figure 3: Estimated CD4 evolution since ART initiation (A) and after ART re-initiation (B and C) for individuals who have disengaged from care and subsequently re-engaged in care conditionally on being on the same CD4 category at ART initiation and ART re-initiation. (B): All CD4 data at/after re-engagement in care were used (ignoring any subsequent disengagement from care), and (C) CD4 data have been censored at the time of the second disengagement. Shaded regions correspond to 95% pointwise confidence intervals of the estimated CD4 trajectories. Data from the East Africa IeDEA study were used (from 2001 to 2011).

Figure 4: Time from ART re-initiation to reaching CD4 levels at disengagement from care in relation to time from ART initiation to disengagement from care (D_i) and time from disengagement from care to treatment re-initiation ($R_i - D_i$). Data from the East Africa IeDEA study were used (from 2001 to 2011).