Limiting Cumulative HIV Viremia Copy-Years by Early Treatment Reduces Risk of AIDS and Death

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Background: Viremia copy-years (VCY), a time-updated measure of cumulative HIV exposure, predicts AIDS/death; although its utility in deciding when to start combination antiretroviral therapy (cART) remains unclear. We aimed to assess the impact of initiating versus deferring cART on risk of AIDS/death by levels of VCY both independent of and within CD4 cell count strata ≥500 cells per cubic millimeter.

Methods: Using Concerted Action on Seroconversion to AIDS and Death in Europe (CASCADE) data, we created a series of nested "trials" corresponding to consecutive months for individuals ≥16 years at seroconversion after 1995 who were cART-naive and AIDS-free. Pooling across all trials, time to AIDS/death by CD4, and VCY strata was compared in those initiating vs. deferring cART using Cox models adjusted for: country, sex, risk group, seroconversion year, age, time since last HIV-RNA, and current CD4, VCY, HIV-RNA, and mean number of previous CD4/HIV-RNA measurements/year.

Results: Of 9353 individuals, 5312 (57%) initiated cART and 486 (5%) acquired AIDS/died. Pooling CD4 strata, risk of AIDS/death associated with initiating vs. deferring cART reduced as VCY increased. In patients with high CD4 cell counts, ≥500 cells per cubic millimeter, there was a trend for a greater reduction for those initiating vs. deferring with increasing VCY (P = 0.09), with the largest benefit in the VCY ≥100,000 copy-years/mL group [hazard ratio (95% CI) = 0.41 (0.19 to 0.87)].

Conclusions: For individuals with CD4 ≥500 cells per cubic millimeter, limiting the cumulative HIV burden to <100,000 copy-years/mL through cART may reduce the risk of AIDS/death.

Key Words: viremia copy-years, seroconverters, when to start, cART initiation, CD4 cell count, HIV-RNA

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INTRODUCTION

Although CD4 cell counts are used routinely to monitor adults with HIV infection, viral loads also have an important role in the monitoring and staging of adults with HIV.[1,2] One or 2 values of an individual's viral load are often used to determine combination antiretroviral therapy (cART) failure, their risk of transmitting HIV to others, and to tailor first-line cART regimens.[3–5] However, assessment of an individual's viral load at a single point in time fails to capture cumulative exposure to HIV replication which may have been over a period of 10 years or more. Several investigators have proposed that a measure of cumulative viral burden might provide useful additional information and, in particular, a measurement of viremia copy-years (VCY) has been proposed.[6] VCY is akin to cigarette pack-years when assessing exposure to tobacco; A VCY of 1000 copy-years/mL is the equivalent to an individual having a viral load of 1000 copies per milliliter for an entire year or a viral load of 500 copies per milliliter for 2 years. The measurement of VCY has been shown to predict death and AIDS in both the absence[6] and presence[7,8] of cART, independently of the individual's most recent CD4 count and viral load. This independent association suggests that cumulative HIV burden is associated with an increased risk of development of clinical events through mechanisms other than immunodeficiency.

United States guidelines recommend immediate cART initiation, regardless of CD4 cell count[5,9] due to evidence that exposure to uncontrolled viremia is associated with an increased risk of death, AIDS, and non-AIDS events.[5,10–12] The START (Starting Antiretroviral Treatment Early Improves Outcomes for

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HIV-Infected Individuals) trial has recently reported that waiting to initiate cART until CD4 <350 cells per cubic millimeter increases the likelihood of serious illness or death compared with immediate initiation.[13] VCY serves as a measurement of cumulative exposure to HIV, and so it is important to determine whether VCY contributes to the likelihood of illness and death and whether cART initiation before the accrual of VCY could help optimize clinical and public health HIV outcomes.

Randomized trials are unlikely to be conducted to determine whether accrual of viremia VCY before cART initiation increases mortality because of the difficulty and expertise in enrolling participants soon after seroconversion, and because cART is now recommended in many asymptomatic populations. In addition, there is substantial potential for lead-time bias in analyses using VCY because of variability in the extent of HIV replication an individual will have been exposed to previous enrollment into care. One way to limit this bias is to restrict analyses to participants with serial viral load measurements since a known seroconversion date; such data are available from the Concerted Action on Seroconversion to AIDS and Death in Europe (CASCADE) Collaboration, an international multicenter collaboration of data from persons with well-estimated dates of HIV seroconversion. Previous analyses of CASCADE data have shown a protective effect of initiating cART on AIDS/death at CD4 <500 cells per cubic millimeter [hazard ratio (HR) 0.59 (95% CI: 0.43 to 0.81) and HR 0.75 (0.49–1.14) in CD4 cell strata 200–349 and 350–499, respectively], but no evidence for a reduction in risk at CD4 ≥500 cells per cubic millimeter [HR 1.10 (0.67–1.79)].[14] Here we examine the effect of initiating or deferring cART at different levels of VCY on HIV disease progression. We investigate whether or not individuals with CD4 ≥500 cells per cubic millimeter but high VCY would benefit from starting cART.

METHODS

Study Population

Data from CASCADE in EuroCoord (www.EuroCoord.net) 2013 data update were used for this analysis.[15] Briefly, CASCADE is a cohort collaboration of 29 cohorts of individuals with well-estimated dates of HIV seroconversion from Europe (94%), Australia (2%), Canada (0.5%), and Sub-Saharan Africa (3%). Date of seroconversion is estimated as the midpoint between the last negative and first positive HIV antibody test results with a maximum of 3 years between the test dates (85%), laboratory evidence of acute seroconversion (real-time polymerase chain reaction positivity of incomplete Western blot) (13%), the date of seroconversion illness with a negative and positive test no more than 3 years apart (2%), or by a probability distribution to determine the most likely date of transmission for men with hemophilia infected with HIV after transfusion with clotting factor concentrates (<1% of the sample).

All cohorts contributing to CASCADE received ethical approval from their individual ethics review boards.

Adults (≥16 years old) seroconverting in the cART era (post 1995) were included provided they had at least 1 HIV-RNA measurement between 4 and 12 months after seroconversion. Two Sub-Saharan African cohorts were excluded from this analysis as their CD4 cell count and cART initiation patterns are different from those in industrialized country cohorts.[16]

Study Design

We created a series of sequential nested "trials" corresponding to consecutive months of follow-up beginning 4 months after seroconversion, where each month represents the baseline month for a new trial (Fig. 1). As described previously, this approach allows appropriate adjustment for time-dependent confounding.[17,18] We created new trials with all eligible individuals for each month between January 1996 and May 2013. Individuals were eligible for a trial if they were cART-naive before the baseline month, had a CD4 or HIV-RNA measurement 12 months before the baseline month, and were AIDS-free until the end of the baseline month. Time to AIDS/death was compared in those who initiated cART in each baseline month versus those who deferred, pooling across all trials.

AIDS events in the first year of seroconversion were not considered as disease progression outcomes, but rather as severe seroconversion illness. In addition, invasive candidiasis was not considered an outcome in this analysis as it is typically less severe and associated with longer survival compared with other AIDS-defining conditions.[19–22]

Viremia Measurements

If HIV-RNA could be continuously measured within an individual from seroconversion [with the viral load distribution at any time t called as $V(t)$], then VCY would be calculated as the area under the HIV-RNA curve, or the integral of HIV-RNA from seroconversion to time $t = T$.

$$\mathrm{VCY} = \int_{\mathrm{SC}}^{\mathrm{T}} \mathrm{V}(t)\mathrm{dt}.$$

However, in practice, we do not have continuously measured viral loads, but rather snapshots of HIV-RNA measurements for each individual at irregularly spaced intervals (usually approximately 3 monthly). The best approximation to the integral with the data available can be obtained through use of the trapezoidal rule, which is how we approximated VCY for the remainder of this analysis. At any given time point, J, say, VCY(J) is given by: $\mathrm{VCY(J)} = \sum_{\mathrm{j}=1}^{\mathrm{J}} \frac{t(\mathrm{j}) - t(\mathrm{j}-1) * V(\mathrm{j}) + \mathrm{V}(\mathrm{j}-1)}{2}$. We examined HIV-RNA data for implausible values and identified 3 individuals whose HIV-RNA dropped by factors of 4, 26, and 87 between consecutive measurements and without apparently starting cART. These are far greater drops than would be expected based on the known biological variation of HIV-RNA.[23,24] As all 3 individuals were recorded as having started cART in the following month, we assumed that the date of cART initiation had been incorrectly recorded, and reset the cART start dates for these individuals to 1 month before that recorded.

To estimate HIV exposure equally for all individuals, we removed HIV-RNA measurements taken in the first 3 months of seroconversion, as we were unlikely to capture the well-documented peak in viremia shortly after seroconversion[25] for

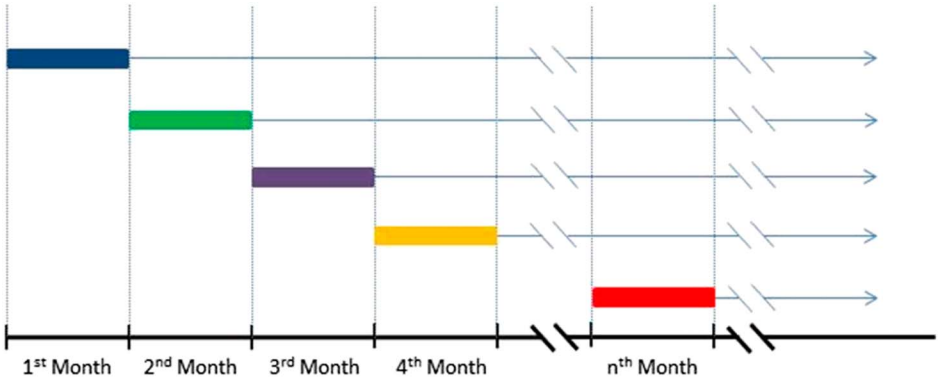

FIGURE 1. A diagram of the "trials" construction. Individuals are assessed for eligibility at the beginning of each month (respective trial baseline). Each eligible individual is classified as having initiated or deferred cART in the baseline month. Time is measured from the beginning of the following month until AIDS, death, or censoring for each eligible individual (excluding any with an outcome during the baseline month). Cox proportional hazards models are used to assess the effect of initiating compared with deferring cART on time to AIDS/death, pooled across all trials.

all individuals. In addition, we assumed that HIV-RNA measurements remained relatively stable over the period 4–12 months (consistent with findings of the viral load stabilizing after the initial peak in viremia), allowing us to make the assumption that an individual's first available HIV-RNA over the period 4–12 months was equal to their HIV-RNA at month 4.

Data Analysis

We describe baseline characteristics between those who initiated or deferred cART during the study period. We estimated the HRs for initiating versus deferring cART by levels of VCY (<10,000 copy-years/mL, ≥10,000–19,999 copy-years/mL, ≥20,000–49,999 copy-years/mL, ≥50,000–99,999 copy-years/mL, ≥100,000 copy-year/mL) pooled across and stratified by CD4 cell count strata (initiate at CD4 <350 cells/mm^3 compared with initiate at higher values, ≥350 cells/mm^3, and initiate at <500 cells/mm^3 compared with initiate at higher values, ≥500 cells/mm^3) using Cox proportional hazards models. We adjusted for trial-independent factors including country of care, sex, HIV transmission risk group, seroconversion year, and trial-dependent factors of current age, time since last HIV-RNA measurement, CD4, VCY, HIV-RNA, and mean number of previous CD4/HIV-RNA measurements per year. Trial-dependent factors were ascertained before the baseline month to ensure they were measured before the decision to initiate or defer cART in the current month. Continuous variables were modeled using restricted cubic splines, all with 3 knots with the exception of current CD4 which was modeled with 5 knots.[26] Most individuals contributed to more than 1 trial, so we used a robust variance estimator to account for within-person correlation. To investigate whether a threshold existed where cART initiation showed the most benefit, we fitted interactions between initiating cART and VCY as a continuous variable with a 3-knot spline.

Furthermore, we investigated whether there was a benefit of incorporating other measures of viremia into the decision about when to initiate cART, namely, current HIV-RNA (most recent measurement), average HIV-RNA (mean of all previous measurements), and maximum HIV-RNA (maximum of all previous measurements). To compare results between all HIV-RNA measurements with VCY, we used the same inclusion criteria for all analyses. We used the Akaike[27] information criteria (AIC), a measure of the relative quality of statistical models which evaluates trade-off between model complexity and goodness of fit, to determine which measure of viremia best fits the data.

RESULTS

Baseline Characteristics

The CASCADE 2013 update contains information on 30,006 individuals, of whom 21,082 seroconverted in the cART era, during or after 1996. Of those, we excluded 916 individuals from African cohorts and 10,813 individuals without at least 1 cART-naive HIV-RNA measurement within 4–12 months of seroconversion, leaving 9353 individuals in the analysis.

Among those seroconverting in the cART era (n = 21,082), men who have sex between men were slightly overrepresented in this analysis compared with those excluded (80% vs. 62%) and those who likely acquired HIV through sex between men and women were slightly underrepresented (9% vs. 29%). Date of seroconversion was later in those included in this analysis [November 2005 (July 2002–August 2008)] than in those excluded [July 2004 (July 2000–March 2008)] explained by availability of routine HIV-RNA measurements within the cohorts. All other baseline characteristics were similar among those included and excluded from this analysis (data not shown).

Of 9353 individuals, 5312 (57%) initiated cART, 326 (3%) acquired AIDS, and 160 (2%) died. Median [interquartile range (IQR) [25th–75th percentile] follow-up was 4.1 (1.8, 7.2) years. Most individuals were men (85%), and modes of HIV transmission included sex between men (71%), sex between men and women (21%), injection drug use (4%), and unknown (4%). Median (IQR) CD4 at cART initiation was 342 (265, 450) cells per cubic millimeter and did not vary by VCY category. Median (IQR) seroconversion age was 33 (27, 40) years between 1996 and 2013. Individuals contributed to a median (IQR) of 21 (13, 36) trials.

Individuals who initiated cART typically had much lower CD4 cell counts and higher HIV-RNA values than those deferring cART. Men were also more likely to defer in the lower viral copy-years strata (Table 1).

Viremia Copy-Years

Pooling across CD4 cell count strata, HRs for the effect of initiating cART compared with deferring on time to AIDS/death significantly decreased as VCY increased (*P*-trend < 0.001). For example, at times when the VCY was in the range 10,000–20,000 copy-years/mL, there was only a modest 9% reduction in the hazard of AIDS/death associated with immediate initiation of cART compared with deferral [HR = 0.91 (95% CI: 0.57 to 1.46)], whereas at times when the VCY was >100,000 copy-years/mL, the estimated reduction in risk of AIDS/death associated with immediate versus deferred initiation was 56% [HR = 0.44 (95% CI: 0.35

TABLE 1. Baseline Characteristic for Individuals Who Initiated or Deferred cART by Levels of VCY

Characteristic	Initiated cART	Deferred cART
VCY < 10,000 copy-years/mL		
"Trial" observations, N	651	50,349
Follow-up, median (IQR) person-years	3.3 (1.4, 6.8)	4.2 (2.1, 7.0)
Male, N (%)	398 (61)	38,869 (77)
Seroconversion year	2005 (2000, 2009)	2004 (2001, 2007)
Seroconversion age	30 (26, 37)	33 (27, 39)
CD4 cell count median (IQR), mm^3	397 (291, 567)	637 (486, 826)
HIV-RNA copies/mL median (IQR) *	3.7 (3.2, 4.1)	3.4 (3.0, 3.8)
VCY median (IQR) *	3.6 (3.2, 3.8)	3.5 (3.0, 3.8)
VCY ≥ 10,000–19,999 copy-years/mL		
Trial observations, N	488	22,825
Follow-up, median (IQR) person-years	3.0 (1.4, 6.0)	4.0 (2.0, 6.6)
Male, N (%)	382 (78)	19,348 (85)
Seroconversion year	2006 (2002, 2009)	2005 (2002, 2007)
Seroconversion age	33 (26, 40)	32 (27, 39)
CD4 cell count median (IQR), mm^3	360 (282, 475)	553 (437, 707)
HIV-RNA copies/mL median (IQR) *	4.3 (3.9, 4.6)	4.1 (3.7, 4.4)
VCY median (IQR) *	4.2 (4.1, 4.2)	4.2 (4.1, 4.2)
VCY ≥ 20,000–49,999 copy-years/mL		
Trial observations, N	1026	39,675
Follow-up, median (IQR) person-years	3.3 (1.4, 6.6)	3.9 (1.9, 6.5)
Male, N (%)	853 (83)	34,804 (88)
Seroconversion year	2006 (2001, 2008)	2004 (2002, 2007)
Seroconversion age	34 (28, 41)	33 (27, 39)
CD4 cell count median (IQR), mm^3	355 (277, 466)	523 (417, 662)
HIV-RNA copies/mL median (IQR) *	4.6 (4.3, 4.9)	4.3 (3.9, 4.6)
VCY median (IQR) *	4.5 (4.4, 4.6)	4.5 (4.4, 4.6)
VCY ≥ 50,000–99,999 copy-years/mL		
Trial observations, N	950	30,925
Follow-up, median (IQR) person-years	3.1 (1.4, 5.7)	3.8 (1.8, 6.2)
Male, N (%)	837 (88)	27,657 (89)
Seroconversion year	2005 (2002, 2008)	2004 (2002, 2006)
Seroconversion age	33 (27, 41)	33 (27, 39)
CD4 cell count median (IQR), mm^3	340 (272, 440)	492 (393, 628)
HIV-RNA copies/mL median (IQR) *	4.8 (4.4, 5.1)	4.5 (4.2, 4.8)
VCY median (IQR) *	4.9 (4.8, 4.9)	4.8 (4.8, 4.9)
VCY ≥ 100,000 copy-years/mL		
Trial observations, N	2102	44,581
Follow-up, median (IQR) person-years	3.5 (1.6, 5.6)	3.9 (1.8, 6.2)
Male, N (%)	1928 (92)	41,702 (94)
Seroconversion year	2005 (2002, 2007)	2004 (2001, 2006)
Seroconversion age	35 (29, 42)	33 (28, 40)
CD4 cell count median (IQR), mm^3	320 (246, 411)	467 (370, 591)
HIV-RNA copies/mL median (IQR) *	5.2 (4.8, 5.5)	4.9 (4.5, 5.2)
VCY median (IQR) *	5.3 (5.2, 5.6)	5.3 (5.1, 5.5)

*Log_{10} copies per milliliter.
IQR, 25th and 75th percentiles.

to 0.55)], Table 2. Among individuals initiating with CD4 ≥350 cells per cubic millimeter, there was a modest trend ($P = 0.11$) toward a greater benefit of immediate initiation of cART (vs. deferral), although the results continued to suggest some benefit of earlier initiation among the group with VCY >100,000 copy-years/mL [HR = 0.68 (95% CI: 0.49 to 0.94)], Table 2. As expected among individuals initiating with CD4 <350 cells per cubic millimeter, immediate initiation was beneficial in all VCY categories (all HR < 1) (see **Table**, Supplemental Digital Content, http://links.lww.com/QAI/A817).

Modeling initiation of cART by VCY as a continuous variable showed the same trends as the categorical analysis, Figure 2. No obvious threshold of copy-years was found; however, pooling CD4 cell count categories, the upper bound of the 95% CI first fell below one when VCY passed 17,343 copies-years/mL, suggesting that among individuals with VCY values above this threshold, immediate initiation of cART may result in a reduction in the risk of AIDS/death. Stratifying by CD4 cell count, in those with CD4 ≥350 cells per cubic millimeter, the upper bound of the 95% CI fell below one when VCY surpassed 52,826 copy-years/mL, again suggesting that among individuals with high CD4 cell counts and VCY values above this threshold, immediate initiation of cART may result in a reduction in the risk of AIDS/death.

Using a CD4 count threshold of 500 cells per cubic millimeter showed similar results. For those with CD4 ≥500 cells per cubic millimeter, the greatest benefit of initiation was seen when VCY >100,000 copy-years/mL [HR = 0.41 (0.19, 0.87), *P*-trend = 0.09], Table 2. Modeling VCY continuously, the upper bound of the 95% CI in those with CD4 ≥500 cells per cubic millimeter fell below one when VCY surpassed 38,152 copy-years/mL. In those with a CD4 count <500 cells per cubic millimeter, there was an overall benefit of treatment initiation in VCY categories >10,000 copy-years/mL (see **Table**, Supplemental Digital Content, http://links.lww.com/QAI/A817).

Other Measures of Viremia

Pooling CD4 strata, the HRs for the effect of initiating cART on time to AIDS/death decreased as most recent HIV-RNA increased (*P*-trend < 0.001) with the largest benefit of initiation seen when current HIV-RNA exceeded 100,000 copies/mL [HR = 0.45 (0.36, 0.57)]. Among individuals with a CD4 count ≥350 cells per cubic millimeter, there was a modest trend (*P*-trend = 0.08) for an increased benefit of immediate initiation (vs. deferral) as the current HIV-RNA increased, with the largest benefit of immediate initiation seen if the current HIV-RNA was >100,000 copies/mL [HR = 0.65 (0.47, 0.89)], Table 2. Stratifying by CD4, there was a benefit of initiating versus deferring for all individuals with CD4 <350 cells per cubic millimeter regardless of current HIV-RNA level, as expected from the VCY analysis. The same trends were seen when modeling VCY and current HIV-RNA continuously, Figure 2, and when considering average and maximum viremia (data not shown).

Using a CD4 threshold of 500 cells per cubic millimeter, similar results were obtained for the average and maximum viremia (data not shown).

Pooling CD4 strata, model fit was best for VCY (minimum AIC, 230115) compared with current (increase in AIC = 238), average (increase in AIC = 124), and maximum HIV-RNA (increase in AIC = 163). Maximum HIV-RNA fits the model best in the CD4 <500 cells per cubic millimeter strata (minimum AIC, 1024345; increase in AIC = 32, 128, 15 for VCY, current, and average HIV-RNA, respectively, for

TABLE 2. The Effect of Initiation Compared With Deferring cART on Time to AIDS/Death by VCY Alone by CD4 Cell Count Strata (≥350, ≥500 Cells/mm^3)

	All Patients			CD4 ≥ 350 Cells/mm^3			CD4 ≥ 500 Cells/mm^3		
	Events, N	HR (95% CI)	*P*, AIC	Events, N	HR (95% CI)	*P*, AIC	Events, N	HR (95% CI)	*P*
VCY, copy-years/mL									
<10,000	198	1.10 (0.74 to 1.63)	0.001*	181	1.04 (0.63 to 1.73)	0.51*	138	0.81 (0.36 to 1.80)	0.56*
10,000–20,000	202	0.91 (0.57 to 1.46)	<0.001†	175	0.79 (0.40 to 1.58)	0.11†	116	0.96 (0.37 to 2.52)	0.09†
20,000–50,000	260	0.69 (0.50 to 0.94)	230,115.30‡	227	0.88 (0.61 to 1.29)	186,803.80‡	166	0.70 (0.37 to 1.31)	113,258.00‡
50,000–100,000	242	0.56 (0.40 to 0.80)		206	0.60 (0.36 to 1.01)		146	0.45 (0.18 to 1.09)	
>100,000	225	0.44 (0.35 to 0.55)		182	0.68 (0.49 to 0.94)		117	0.41 (0.19 to 0.87)	
Current HIV-RNA, copies/mL									
<10,000	180	1.14 (0.81 to 1.61)	0.001*	167	1.37 (0.89 to 2.09)	0.03*	161	0.86 (0.46 to 1.61)§	0.40*
10,000–20,000 ↨	140	0.63 (0.36 to 1.08)	<0.001†	121	0.54 (0.22 to 1.33)	0.08†		—	0.08†
20,000–50,000	211	0.53 (0.37 to 0.76)	230,353.40‡	182	0.55 (0.34 to 0.89)	187,014.80‡	132	0.58 (0.28 to 1.23)	113,454.30‡
50,000–100,000	202	0.62 (0.45 to 0.86)		163	0.80 (0.57 to 1.25)		107	0.61 (0.28 to 1.31)	
>100,000	202	0.45 (0.36 to 0.57)		195	0.65 (0.47 to 0.89)		120	0.38 (0.19 to 0.77)	

*p-heterogeneity (df = 4).
†p-trend (df = 1).
‡AIC, Akaike information criterion.
§By chance, there were no failures among initiators in the CD4 ≥500 cells per cubic millimeter, VCY 10,000–20,000 category, so this category is <20,000 copies per milliliter.

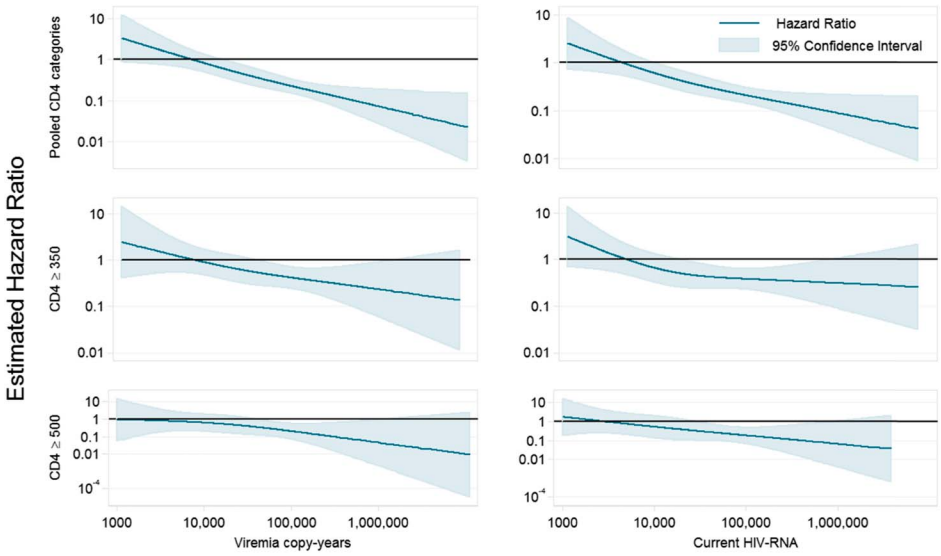

FIGURE 2. The effect of initiating compared with deferring cART on time to AIDS/death by VCY and CD4 cell count modeled continuously with 3 knot splines using the CASCADE data set.

copy-years, current, average, and maximum HIV-RNA, respectively). In the CD4 ≥500 cells per cubic millimeter strata, VCY gave the best model fit (minimum AIC = 113,258.00, increase in AIC = 196, 84, for current, average, and maximum HIV-RNA, respectively).

DISCUSSION

Pooling CD4 cell count strata, there is a benefit of initiating cART as the cumulative and absolute HIV-RNA increases, with benefits observed as the total VCY exceeds approximately 17,500 copy-years/mL. What is of clinical interest, however, is whether there is benefit of immediate cART initiation in individuals with healthy immune systems (CD4 ≥500 cells per cubic millimeter) and high levels of viremia. Among individuals with CD4 ≥500 cells per cubic millimeter, we found a modest benefit of earlier cART initiation for those with high cumulative and absolute HIV-RNA >100,000 copy-years/mL and copies per milliliter, associated with reducing risk of AIDS/death by 59% (13%–81%) and 62% (23%–81%). Our results support the recent evidence from the START trial[28] which found serious illness or death was reduced by 53% among those treated immediately vs. waiting to initiate until CD4 cell count dropped below 350 cells per cubic millimeter.[13]

All measures of viremia showed consistent and similar results with an increased benefit of cART initiation with increasing VCY. Among the pooled and separate CD4 cell count strata, there was not a single viremia measure that consistently showed best model fit using AIC. VCY fits best when pooling CD4 and in the CD4 ≥350 cells per cubic millimeter strata, whereas average viremia fits best in the CD4 <350 cells per cubic millimeter strata. Although VCY incorporates cumulative HIV burden, it requires frequent HIV-RNA measurements from the start of infection, which are not available in most HIV-positive individuals. Even if such measurements are available, cumulative viremia is difficult and time-consuming to calculate. Average and maximum HIV-RNA also require frequent measurements from seroconversion, so too are not relevant for most HIV-positive individuals. Current HIV-RNA, however, is a measure that is easily obtained from all HIV-positive individuals and is therefore of greatest clinical relevance.

Although observational studies are not designed to inform the "when to start" question, we provide evidence that cART initiation is beneficial when CD4 cell counts fall below 350 cells per cubic millimeter, supporting other observational studies.[14,29–31] The START trial has recently reported a modest absolute risk reduction of AIDS, other serious illnesses, and death for cART initiation at CD4 cell counts above 500 cells per cubic millimeter[11] compared with deferring initiation to CD4 below 350 cells per cubic millimeter.[13] Our analysis, using data before guidelines recommending immediate cART initiation, suggests that benefit is likely to be greatest in those with highest viremia burden and adds to the body of evidence which informs clinical guidelines.[32]

We reflected the dynamic process of initiating cART by allowing individuals to contribute information to multiple trials rather than just considering a single point in time. This provided estimates of the average benefit of initiating cART compared with deferring cART at particular levels of CD4 cell counts and cumulative exposure to HIV-RNA. Our estimates can therefore be used to inform trade-offs between initiating treatment at varying points in disease progression compared with the lifelong challenges of initiating therapy, such as adherence and adverse effects.

The availability of HIV-RNA data from HIV seroconversion allowed us to investigate when to start treatment based on a variety of measures of viremia captured during the life course of HIV infection. Of particular importance, there is potential for lead-time bias[33] when measuring cumulative exposure to viremia in sero-*prevalent* cohorts which is essentially eliminated in this sero-*converter* study as we have serial HIV-RNA measurements taken from the date of seroconversion. This is, therefore, the first study, to our knowledge, that has compared the benefit of cART initiation

by these levels of viremia in combination with CD4 cell count. Nevertheless, despite nearly 10,000 seroconverters being included, we were not able to assess the impact of initiating versus deferring within the CD4 strata where decisions on whether cART should be initiated have previously been most controversial (CD4 >350 cells/mm^3).

In addition to AIDS and death, there are several other non–AIDS defining conditions that can affect morbidity and mortality. Increased exposure to viremia has been shown to be associated with cardiovascular disease,[34] multimorbidity,[35] and AIDS and non-AIDS malignancies,[5,36,37] so had these data been available, our estimates could have shown a stronger benefit of cART initiation. CASCADE does not currently collect data on non–AIDS conditions.

Like all observational studies, our estimates rely on the assumption of no unmeasured confounding. We adjusted for some of the most important factors in deciding when to initiate therapy, but it is possible that other unmeasured factors, such as comorbidities or likelihood of adherence, played a role in the initiation of cART in our population. The HRs above one for cART initiation versus deferred treatment, albeit with wide confidence intervals, in the group with low current HIV-RNA suggest we may lack information on some confounders; this could be a particular concern among those with a CD4 count ≥350 cells per cubic millimeter, a group for which not all treatment guidelines recommended initiation of cART during the study period.

It is unlikely that randomized evidence will ever be available on when to initiate cART by these measures of viremia, so applying robust statistical methods to large observational data sets presented here will likely provide the best evidence that will ever be available. Our data suggest that deferring cART in an individual unwilling or unable to start treatment immediately may not impact the risk of AIDS/death provided a healthy CD4 cell count (≥350, 500 cells/mm^3) and low VCY (<50,000 copy-years/mL) are maintained. However, we found consistently that AIDS and death were delayed among those who initiated treatment with CD4 cell counts ≥350 cells per cubic millimeter and VCY >100,000 copy-years/mL.

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