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The impact of age on the prognostic capacity of CD8+ T-cell activation during suppressive antiretroviral therapy

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Abstract

Objective—To assess whether CD8+ T-cell activation predicts risk of AIDS and non-AIDS morbidity during suppressive antiretroviral therapy (ART).

Design—Post-hoc analyses of ART-naïve subjects in prospective ART studies. Subjects with HIV-RNA levels > 200 copies/mL and CD8+ T-cell activation data (%CD38+HLA-DR+) at year-one of ART were selected to determine years 2–5 incidence of AIDS and non-AIDS events.

Methods—We censored data at time of ART interruption or virologic failure. Inverse probability of censoring weighted logistic regression was used to correct for informative censoring.

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A related article, focusing on statistical methods but using the same data, was presented by Judith Lok at ICSA, Boston, June 2012: “Careful selection of surrogate outcomes with application to predictors of clinical events in HIV-positive patients” (invited talk). The submitted article has been presented at CROI, Atlanta, March 2013: “The Prognostic Capacity of CD8+ T Cell Activation During Effective Antiretroviral Therapy (ART) Is Attenuated By Age”.

R.B., C.B., and A.C. were involved in the design and conduct of the ALLRT study. A.L. and M.W. were involved in the conduct of the ALLRT study. J.L. and R.B. carried out the analyses. J.L. drafted a first version of the manuscript. All authors contributed to the final manuscript.

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ACTG A5095: <http://clinicaltrials.gov/ct2/show/NCT00013520>

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Conflicts of interest: AC is a former member of a Data and Safety Monitoring Board for a study sponsored by Merck & Co., has received research support from Schering-Plough (past) and Merck & Co., and is a past stockholder of Abbott, Bristol-Myers-Squibb, Johnson & Johnson, and Pfizer. MW has received honoraria from Gilead Sciences, is on the Speaker’s Bureau for Viiv Healthcare, and received a grant from Pfizer. JL, CB, SD, and RB reported no conflicts of interest.

Results—We included 1025 subjects; 82% were men, median age 38 years, pre-ART CD4 count 255 cells/mm³, and year-one activated CD8+ T-cells 24%. Of these, 752 had 5 years of follow-up; 379 remained on ART and had no confirmed plasma HIV-RNA >200 copies/mL. The overall probability of an AIDS or non-AIDS event in years 2–5 was estimated at 13% (95%-confidence interval [CI] 10–15%), had everyone remained on suppressive ART. Higher year-one activated CD8+ T-cell percentage increased the probability of subsequent events (Odds-Ratio 1.22 per 10% higher [95%-CI 1.04–1.44]); this effect was not significant after adjusting for age. Among those age ≥ 50 years (n=108 at year 1), the probability of an event in years 2–5 was 37% and the effect of CD8+ T-cell activation was more apparent (Odds-Ratio=1.42, p=0.02 unadjusted and adjusted for age).

Conclusions—CD8+ T-cell activation is prognostic of clinical events during suppressive ART although this association is confounded by age. The consequences of HIV-associated immune activation may be more important in those age ≥ 50 years.

Keywords and phrases

Antiretroviral Therapy; HIV/AIDS; CD8+T-cell activation; virologic suppression; loss to follow-up; observational data

Introduction

Despite the many effective treatment options currently available for subjects with HIV infection, AIDS-defining events still cause hospitalizations and deaths, even among subjects on suppressive antiretroviral treatment (ART) [1]. In addition, HIV-infected subjects are at higher risk for serious morbidity and death due to non-opportunistic diseases, such as liver, cardiovascular, renal and malignancies, than the general population [2,3]. To inform the decision on which subjects to institute additional treatments and preventative measures, it is important to identify subjects with poor prognoses despite viral suppression with ART.

Several soluble markers of inflammation and coagulation have been associated with the risk of developing AIDS and non-AIDS morbidity during ART. In the SMART study, which randomized subjects to continuous ART or intermittent CD4+ T-cell count (CD4 count) driven ART, soluble plasma markers of inflammation, monocyte activation, and coagulation (high sensitivity C-reactive protein, interleukin-6, sCD14, and D-dimer) predicted subsequent all-cause mortality; importantly, this effect was also observed in the subset of individuals randomized to continuous ART [4,5]. Similar associations have been observed in FRAM [6], ESPRIT [7], VACS [8], and FIRST studies [9]. However, none of these studies evaluated the prognostic significance of T-cell activation during ART-mediated viral suppression.

T-cell activation has been used as the primary surrogate outcome for several recent randomized controlled trials of immune-based interventions in this setting [10–12], given its superior stability and reproducibility within individuals, but it may reflect distinct pathophysiologic pathways from markers of inflammation and monocyte activation. Most of the larger cohorts with sufficient numbers of events to assess morbidity and mortality have focused on measurements that can be performed with stored plasma. These soluble biomarkers generally reflect activation of the innate immune and coagulation systems. The study of T-cell activation and function requires access to cryopreserved cells and is logistically more challenging. T-cell activation—as defined by co-expression of HLA-DR and CD38—fails to normalize during effective ART [13–15]. The frequency of these cells in untreated HIV disease is a well-established biomarker of disease progression [16,17]. However, the prognostic significance of these cells during suppressive ART has been less well defined. Smurzynski et al ([1]) reported that higher pre-treatment CD8+ T-cell

activation (% CD38/HLA-DR+) was significantly associated with a subsequent AIDS diagnosis, although the effect was attenuated after adjustment for pre-treatment HIV RNA and CD4 count, and the prognostic significance of these markers during early viral suppression was not assessed. Hunt and colleagues found that higher CD8+ T-cell activation during early ART-mediated viral suppression predicted subsequent mortality in a relatively small Ugandan cohort (n=10 deaths, [18]), and in the larger SOCA cohort of North Americans with a history of AIDS (n=64 deaths, [19]), but not in the smaller SCOPE cohort with less advanced disease stage (n=27 deaths, [19]). Hence, the role of T-cell activation as a predictor of disease progression during ART remains unclear.

Modern ART regimens are very effective at indefinitely suppressing HIV replication. Hence the field is now focused on defining the nature of residual disease during suppressive ART. In our current analysis, we assess the impact of CD8+ T-cell activation and CD4 count measured one year after ART initiation on the probability of clinical events in years 2–5 of ART, had everyone maintained ART-mediated viral suppression. Recognizing that even with modern ART, sustained virologic suppression is not achievable for all subjects, we also performed an “on-treatment” analysis using previously published methods [20,21]. Given the associations between aging, inflammation and morbidity in the general population, we also investigated the association between T-cell activation and morbidity/mortality in older individuals.

Methods

Population

We used data from randomized treatment studies including ART-naïve patients (“parent” studies) conducted by the AIDS Clinical Trials Group (ACTG): ACTG protocols 384 [22,23], 388 [24], A5014 [25], and A5095 [26]. We restricted the analysis to the 1036 subjects who were virologically suppressed (HIV RNA < 200 copies/mL) at week 48 or 64 after ART initiation (“year one”), and who had data on CD8+ T-cell activation at that time. All these patients lived in the US. Most (941, 91%) of these subjects also enrolled in the ACTG’s long-term observational study ALLRT [27], which provides standardized long-term follow-up after completion of parent studies. Patients were invited to enroll into the parent studies and ALLRT by their physicians. ALLRT did not provide treatment. At the time of analysis, all participants had potential follow-up of 5 years after ART initiation. We excluded the 11 subjects with an event occurring within 12 weeks prior to the year-one CD8+ T-cell activation measurement, since the level of immune activation could be affected by the previous event.

Evaluations for these studies continued regardless of changes in or discontinuation of ART. Subjects provided written informed consent for participation in these studies. All participating sites received institutional review board approval for the studies.

Outcomes

AIDS-defining events were as defined by the Centers for Disease Control [28] and identified as previously described [1]. Non-AIDS defining events were defined by the methods used in earlier ACTG analyses [29]. The primary outcome was the occurrence of any of the following events in years 2–5 after ART initiation: AIDS-defining event, non-accidental death, hepatic end organ disease (including ascites, esophageal/gastric varices, hepatic encephalopathy), cardiovascular end organ disease (including myocardial infarction, stroke, coronary artery intervention, pulmonary embolism, thrombosis), serious renal disease (including confirmed eGFR < 60 mL/min/1.73 m² using the MDRD equation for eGFR at

least 3 months apart), non-AIDS cancers, hip, spine or wrist fractures, diabetes mellitus, and serious bacterial infections (including pneumonia, bacteremia, meningitis, sepsis).

Loss-to-follow-up

Loss-to-follow-up was defined as the earliest of 6 months after the last HIV RNA measurement, or after 6 months without HIV RNA measurement.

Laboratory measurements

The percentage of CD8+ T-cells co-expressing CD38+/HLA-DR+ (CD8+ T-cell activation) was measured on fresh blood using an ACTG consensus protocol at multiple laboratories. HIV RNA quantification was performed using the Roche Amplicor assay v1.0 or v1.5 (Roche Diagnostic Systems, Branchburg, New Jersey, USA). CD4 cell count and HIV RNA measurements were collected every 8 or 16 weeks.

Statistical analyses

We estimated the probability of experiencing a clinical event in years 2–5 had a subject remained on suppressive ART, dependent on certain prognostic factors. Prognostic factors considered were year-one CD8+ T-cell activation and square root of year-one CD4 count. Additional covariates included in univariate models were age, sex, history of injection drug use (IDU), randomized study, square root of pre-ART CD4 count, pre-ART log₁₀ viral load, year-one naïve CD4 percentage, and the occurrence of an event in year one. Multivariable models included variables that had a p-value less than 0.10 in univariate analyses and year-one CD8+ T-cell activation. Because of current scientific knowledge, we also fit models with pre-ART and year-one CD4 count.

All probabilities and odds ratios (ORs) were estimated for the complete population of 1025 subjects. We censored subjects after they interrupted ART for more than 21 days and at confirmed virologic failure, using a confirmed >200 copies/mL threshold [30]. We then estimated the probability of events with a logistic regression model, using Inverse Probability of Censoring Weighting (IPCW) to address informative censoring [31,32,20]. This approach estimates outcomes in the entire study population, including those subsequently lost-to-follow-up, rather than describing a subgroup selected over time. In practice, the unobserved outcomes of subjects who were lost-to-follow-up/off-suppressive-ART are represented by increasing the weight given to the outcomes of “similar” uncensored subjects.

The weights are obtained from the probability of remaining in follow-up/on suppressive ART at a given time in terms of past observed covariate values (these covariates are the basis for identifying “similar” subjects). We used separate logistic regression models, pooled over the time points, for remaining in follow-up and having ART-induced viral suppression. We divided the data into 28-day periods, with covariates measured at the end of each period [33], carrying values forward until the covariate was measured again.

The variables included in the pooled logistic models for both types of censoring, lost-to-follow-up and off-suppressive-ART, were covariates thought to be predictive of both censoring and clinical events [32]. Based on prior findings [20,34], we included age, sex, history of IDU, pre-ART log₁₀ viral load, year-one CD8+ T-cell activation, square root of CD4 count (time-dependent), viral load 50 (time-dependent), period and period squared, and study stage. Study stage was a categorical variable indicating within-parent-study, end-of-parent-study, or within-ALLRT. If significant, we also included age squared. Confidence intervals were obtained by bootstrap [31], using Efron’s percentile method [35].

In sensitivity analyses, we performed stepwise model selection, starting with the above covariates and also including white race, parent study, and square root of baseline CD4 count, and forcing year-one CD8+ T-cell activation and period in the model. We also investigated sensitivity of our results to excluding subjects with events within 12 weeks before year one: excluding subjects with events within 6 months, and not excluding subjects. Results (not shown) changed minimally. SAS 9.1.3 (SAS Institute Inc., Cary, North Carolina, USA) was used for all analyses.

Results

A total of 1025 subjects were included in our analyses (see Table 1). Among all 1025 subjects, 114 had an event in years 2–5. For the hypothetical scenario had everyone remained on suppressive ART, 379 subjects maintained ART-mediated viral suppression (no confirmed HIV-RNA >200 copies/mL) through year five or until they had a clinical event (uncensored). The 646 censored subjects either stopped drugs (n=216, 33%), had confirmed virologic failure (n=178, 28%), or were off follow-up or did not have viral load measurements for 6 months (n=252, 39%). In subjects censored because of confirmed virologic failure, median time between year-one measurement and confirmed virologic failure was 9 months.

For the multivariable IPCW censoring models, no IDU and a higher current CD4 count were associated with a higher probability of remaining in follow-up for subjects maintaining persistent virus control. The effect of age in this model was nonlinear. Younger age, history of IDU, higher year-one CD8+ T-cell activation, higher current CD4 count, and current HIV RNA above 50 copies/mL were significantly associated with a higher probability of the combined censoring endpoint of virologic failure and ART discontinuation.

Ninety-four of the 379 uncensored subjects had at least one event. Of the first events, 12 were AIDS-defining and 82 non-AIDS-defining. In subjects who were virologically suppressed and had CD8+ T-cell activation data at year one, the overall estimated probability of clinical events in years 2–5 was 13% (95%-CI 10%–15%), had these subjects maintained ART-mediated viral suppression. The risk per year decreased slightly over years 2–5.

Year-one CD8+ T-cell activation was a significant predictor of the probability of an event in years 2–5, with an OR of 1.22 for each 10% higher activated CD8+ T cells (p=0.02, Table 2), had everyone remained on ART and suppressed. This relationship was also seen after adjustment for pre-ART and year-one CD4 counts. However, adjusting for age attenuated the OR of CD8+ T-cell activation to 1.11 (95%-CI 0.94–1.32). Age was a significant predictor of clinical events in all 3 models that included age; in multivariable model C (Table 2), the OR of a clinical event was 2.13 (95%-CI 1.62–2.87, p<0.0001) for each 10-year older age. Neither year-one CD8+ T-cell activation nor year-one CD4 count was significant in the multivariable model that included age. Age positively correlated with year-one CD8+ T-cell activation (p<0.0001), but with a proportion of explained variation of only 0.03. Table 3 summarizes the dependence of clinical events on year-one CD8+ T-cell activation and age.

Inferences were unchanged in the on-treatment analysis, which included follow-up while subjects exhibited virologic failure despite continuing ART (Table 4).

In an exploratory analysis of the effect of the absolute number of activated CD8+ T-cells on the probability of clinical events, for every 100 cells/mm³ higher activated CD8+ T-cells, the OR was 1.16 (95%-CI 1.04–1.31, p=0.01). When adjusted for age, the OR was attenuated to 1.13 (95%-CI 0.97–1.23, p=0.096).

Impact of CD8+ T-cell activation in subjects age ≥ 50 years

In an exploratory analysis we also investigated the effect of CD8+ T-cell activation in the 108 subjects age ≥ 50 years (median age 55, Inter-Quartile-Range (52–59)). We chose the cut-off of 50 as this has been commonly used to define “older” in other HIV cohorts. Of 56 uncensored subjects, 27 had an event. The overall probability of events in subjects ≥ 50 years in years 2–5 had everyone remained on suppressive ART was 37% (95%-CI 24–48%). In subjects ≥ 50 years, the OR of a clinical event was 1.42 (95%-CI 1.07–2.47, $p=0.015$) for each 10% higher CD8+ T-cell activation (Table 5). This relationship remained significant when adjusted for age: OR 1.43 (95%-CI 1.06–2.64, $p=0.018$). While the relationship between CD8+ T-cell activation and clinical events appeared to be stronger in the subgroup of subjects age ≥ 50 years, we did not find significant evidence of interaction by age ($p=0.45$, for age continuous).

Impact of CD8+ T-cell activation on non-AIDS-defining events and death

We next evaluated the risk of non-AIDS-defining events and death, had everyone remained on suppressive ART. For this analysis, of 374 uncensored subjects, 85 had a non-AIDS-defining event or died. Year-one CD8+ T-cell activation significantly predicted subsequent non-AIDS-defining events or death: OR 1.25 (95%-CI 1.06–1.47, $p=0.009$). Adjustment for age attenuated the OR to 1.14 (95%-CI 0.96–1.35). In the model including year-one CD8+ T-cell-activation, year-one square root of CD4 count, and age, the OR for each 10% higher CD8+T-cell activation was 1.18 (95%-CI 0.98–1.41, $p=0.08$), for each 10 years older was 2.25 (95%-CI 1.69–3.11, $p<0.0001$), and for each 3 units higher square root CD4 count was 1.13 (95%-CI 0.96–1.32, $p=0.14$). When including the pre-ART instead of year-one CD4 count, results changed minimally.

Discussion

Even had all individuals maintained optimal virologic response to therapy, an excess risk of non-AIDS morbidity exists in subjects who were virologically suppressed after one year of ART. In addition to low-level HIV replication, this excess risk may be due to multiple non-virologic factors, including traditional risk factors (e.g., substance use), ART toxicity and persistent immune dysfunction/inflammation. Serious non-AIDS-defining events cause much more of this disease burden than AIDS-defining events.

Given the prognostic utility of CD8+ T-cell activation in untreated HIV disease, we analyzed the role of CD8+ T-cell activation in a well-characterized cohort of long-term treated adults enrolled in a clinical trials network. We used IPCW to estimate the risk of disease progression had no one in the cohort experienced confirmed virologic failure or treatment interruptions longer than 21 days, as the vast majority of subjects in modern clinic cohorts appear to now routinely achieve this outcome and this is the group for which immune-based interventions are likely to be used [36].

We found that the frequency of cells expressing CD38 and HLA-DR on CD8+ T-cells (which have long been considered “activated”) measured at year-one of ART-mediated viral suppression predicted an increased risk of subsequent AIDS and non-AIDS events in years 2–5, had everyone remained on suppressive ART. Adjustment for age attenuated the prognostic effect of CD8+ T-cell activation.

These results reach a somewhat different conclusion than the Ugandan study [18] and the SOCA cohort [19]. However, the Ugandan study had many fewer events, and the SOCA cohort had uniformly low pre-ART CD4 counts.

We found a statistically significant association between age and CD8+ T-cell activation in this cohort, which is generally consistent with findings that many markers of inflammation correlate with age in the general population, as well as in HIV-infected individuals suppressed on ART [37,3]. However, the proportion of variation explained by age was only 0.03.

Most studies finding a link between inflammation and disease progression in the general population focused either on the very old [38,39], or on individuals who were at high risk for developing complications (e.g., adults who had acute coronary syndrome [40]). Because it has been used in other HIV cohorts, we defined “older” in this cohort as anyone age 50 years. In this group of individuals who had excess co-morbid conditions and a much higher risk for disease progression, we found a significant effect of CD8+ T-cell activation that remained unchanged even after adjusting for age. While we did not detect statistically significant interaction by age, this subset analysis is consistent with the hypothesis that immune activation is a more important driver of disease risk in older subjects. Based on the geriatrics literature, one might expect inflammation to be more important in the elderly. This may simply be a power issue (older subjects experience more events), but it is also possible that inflammation and immune activation is a more important mediator of disease risk in this population and interventions might be best targeted in this group [29,40]. Many inflammatory markers—including CRP, IL-6 and TNF- α —increase with age [41]. This increase is present even in healthy, older individuals and does not appear to be driven by increased frequency of acute infections. Although it remains unknown if the increased inflammatory burden in the elderly is a cause or consequence of other co-morbid conditions, the consistent observation that inflammation predicts multiple age-associated diseases such as coronary artery disease, cancer, sarcopenia (loss of muscle mass and poor muscle function, typical of older age), frailty and osteoporosis, suggests that chronic inflammation is integral to the aging process, and that the prognostic significance of inflammatory biomarkers might be more apparent in older individuals.

It is also important to note that even in the subset of older subjects, where CD8+ T-cell activation appeared to independently predict morbidity and mortality, the association was relatively modest. Much stronger associations have been observed between markers of inflammation (i.e., IL-6, hsCRP, fibrinogen), monocyte activation (sCD14), and coagulation (D-dimer) and clinical events during treated HIV infection [4–9]. CD8+ T-cell activation may reflect an aspect of pathogenesis that is less closely related to the proximal immunologic events mediating the risk of disease in treated HIV infection than markers of inflammation, monocyte activation, and coagulation. However, more research is needed to better understand the biological mechanisms contributing to CD8+ T-cell activation in well-suppressed individuals on ART, and the connections to cytokines such as IL-6. This may have important implications for identifying targets for novel interventions.

The pre-therapy and on-therapy CD4+ T-cell count has been a consistent predictor of disease progression in many other cohorts. We were surprised to find in our analysis that neither pre-ART CD4 count nor year-one CD4 count were significantly associated with clinical events. This may be related to the fact that most (87%) of the first events after year one were non-AIDS-defining. In addition to renal events, only a small number of non-AIDS defining cancers have been shown to have a relationship with CD4 count. Predicting events after the first year of ART also largely excludes clinical events that may have been related to immune reconstitution inflammatory syndromes (IRIS), which are typically associated with lower pre-ART CD4 counts. Also, the limited effect of a higher CD4 count may be due to our focus on the scenario had everyone remained on (suppressive) ART. For example, [42] did not restrict to HIV RNA suppression, and so the low time-updated CD4 counts in their analysis that were associated with the risk of non-AIDS cancers may have been confounded

with lack of viral suppression. These issues may also play a role in attenuating the predictive value of CD8+ T-cell activation.

A strength of this study is the prospective cohort design, allowing both unadjusted and adjusted analyses. Another strength is the standardized data collection of all the necessary variables. In addition, our analyses have used IPCW to deal with possibly informative censoring, especially important when censoring subjects when they go off (suppressive) ART.

Given our sample size, we could not rule out an age-adjusted OR as high as 1.32 among all subjects. In addition, we have inadequate statistical power to look at the effect of CD8+ T-cell activation on events of a specific type. Another limitation is the fact that T-cell activation was measured in different labs in our study (i.e., measurement error), although an ACTG consensus protocol was used by all labs. Due to the number of laboratories, we did not have sufficient power to further investigate this. In addition, there may have been residual measured or unmeasured confounding which we have not corrected for by IPCW.

To summarize, CD8+ T-cell activation is prognostic for clinical events during effective ART, but this association is largely confounded by age, and is smaller in magnitude than those recently reported for markers of innate immune activation, inflammation, and coagulation. Older HIV-infected individuals are at significantly higher risk of clinical events than younger subjects. The significant and independent association between CD8+T-cell activation and clinical events in subjects age ≥ 50 years suggests that HIV-associated immune activation may be a more important driver of disease in this population, although this hypothesis needs to be confirmed in future studies. Another reasonable explanation is that excess morbidity in older individuals may be causing inflammation even before the clinical event is diagnosed, and that our ability to detect this association is greater in more-affected older adults. However, the latter seems less likely since CD8+ T-cell activation at year 1 had similar prognostic capacity for events occurring after year 2 (see footnotes Tables 2 and 5). The fact that CD8+ T-cell activation appears to be less predictive of clinical events than previously reported inflammatory markers may suggest that interventions specifically targeting innate immune activation and its determinants may hold more promise than interventions specifically designed to decrease CD8+ T-cell activation during treated HIV infection.

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Table 1

Subject characteristics according to year-one CD8+ T-cell activation percentage¹

Covariate	All subjects	CD8+ T-cell activation percentage at year 1	CD8+ T-cell activation percentage at year 1 > 24%
N	1025	519 (51%)	506 (49%)
Age year 1	Median (Q1–Q3)	37 (31–43)	39 (33–45)
Age year 1 at least 50	108 (11%)	34 (7%)	74 (15%)
Sex	Male	82%	83%
History of injection drug use	Yes	10%	8%
Race/ethnicity	White Black Hispanic/Other	50% 35% 15%	44% 26% 30%
Randomized study	ACTG 384 ACTG 388 ACTG A5014 ACTG A5095	62% 1% 4% 34%	45% 1% 5% 49%
Pre-ART log ₁₀ HIV RNA (log ₁₀ copies/mL)	Median (Q1–Q3)	4.83 (4.34–5.43)	4.91 (4.43–5.50)
HIV RNA year 1 (copies/mL)	50 50–200	91% 9%	87% 13%
Pre-ART ² CD4 count (cells/mm ³)	Median (Q1–Q3)	302 (139–446)	198 (67–347)
CD4 count year 1 (cells/mm ³)	Median (Q1–Q3)	491 (319–690)	365 (244–559)
Naïve CD4 percentage year 1 ³	Median (Q1–Q3)	41 (28–52)	34 (22–45)
CD8+ T-cell activation year 1 (cells/mm ³)	Median (Q1–Q3)	111 (69–161)	281 (196–404)
CD8+ T-cell activation percentage year 1	Median (Q1–Q3)	16 (11–20)	35 (29–43)

¹ CD8+ T-cell activation was divided at the median, as percentage 24 versus > 24.

² ART refers to antiretroviral therapy.

³ %CD4+ T-cells co-expressing CD45RA and CD62L.

Table 2

Estimated Odds Ratios (ORs), 95%-CI, and p-value for probability of events¹ in years 2–5 after initiation of antiretroviral treatment (ART), had everyone continued to have viral suppression on ART

	Univariate OR	Adjusted OR for CD8+ T-cell activation year 1 (per 10 units (%)) higher ⁶	Multivariable model A	Multivariable model B	Multivariable model C
CD8+ T-cell activation year 1 (per 10 units (%)) higher ²	1.22 (1.04,1.44) (p=0.02)	--	1.09 (0.91,1.31) (p=0.34)	1.14 (0.96,1.36) (p=0.15)	1.14 (0.95,1.36) (p=0.15)
Age (per 10 years older)	2.17 (1.65,2.92) (p<0.0001)	1.11 (0.94,1.32)	2.04 (1.53,2.80) (p<0.0001)	2.10 (1.59,2.85) (p<0.0001)	2.13 (1.62,2.87) (p<0.0001)
CD4 sqrt ³ year 1 (per 3 units higher) ⁴	1.03 (0.90,1.18) (p=0.68)	1.25 (1.05,1.49)	--	--	1.09 (0.94,1.27) (p=0.24)
Pre-ART CD4 sqrt ³ (per 3 units higher) ⁴	1.06 (0.95,1.18) (p=0.31)	1.26 (1.06,1.49)	--	1.09 (0.97,1.22) (p=0.15)	--
Sex (female, ref male)	1.04 (0.54,1.79) (p=0.92)	--	--	--	--
Injection drug use (ever, ref never)	1.78 (0.65,3.95) (p=0.25)	1.24 (1.06,1.48)	--	--	--
ACTG randomized study 384/388, ref A5014/A5095	0.67 (0.40,1.09) (p=0.10)	1.20 (1.03,1.41)	--	--	--
Pre-ART log ₁₀ RNA (log ₁₀ copies/mL, per unit higher)	0.97 (0.74,1.26) (p=0.81)	--	--	--	--
Naïve CD4 percentage year 1 (per 10 units (%)) higher ⁵	0.81 (0.68,0.95) (p=0.01)	1.15 (0.96–1.37)	0.95 (0.78–1.14) (p=0.54)	--	--
Event in year 1 (yes, ref no)	0.54 (0.00–1.63) (p=0.30)	--	--	--	--

¹ A total of 94 uncensored subjects had events: 12 AIDS-defining events, and 82 non-AIDS-defining events. Non-AIDS-defining events: 33 diabetes mellitus, 15 serious bacterial infections, 11 non-AIDS cancers, 9 cardiovascular end organ disease, 5 non-accidental deaths, 5 thrombosis/embolism, 3 serious renal disease, 1 hepatic end organ disease.

² Sensitivity analysis: for the probability of an event in years 3–5, given no event in year 2: univariate OR 1.27 (1.05–1.55) (p=0.02).

³ Sqrt refers to square root. Reason for sqrt: the difference between CD4=50 and CD4=100 is much more informative than the difference between CD4=600 and CD4=650. Taking a square root accounts for that.

⁴ For example, a CD4 count of 100 versus 49, 294 versus 200, or 643 versus 500 cells/mm³.

⁵ The OR for naïve CD4 percentage (%CD4+ T-cells co-expressing CD45RA and CD62L) at year one was also attenuated by age: age-adjusted OR=0.92 (0.78–1.10), p=0.36.

⁶ These are ORs for CD8+ T-cell activation adjusted for each covariate; for example, the OR for CD8+ T-cell activation adjusted for age is 1.11 (0.94, 1.32).

Table 3

Estimated probabilities of an event in years 2–5 had everyone remained on antiretroviral treatment (ART) and suppressed. Based on logit link for age and year-one CD8+ T-cell activation percentage¹.

Age at year 1	CD8+ T-cell activation percentage at year 1				
	10%	20%	30%	40%	50%
25	3%	4%	4%	5%	5%
30	5%	5%	6%	7%	7%
35	7%	8%	9%	9%	10%
40	10%	11%	12%	13%	14%
45	14%	15%	16%	18%	20%
50	19%	20%	22%	24%	26%
55	25%	27%	29%	31%	34%

¹ We did not include pre-ART or year-one CD4 count, since these effects were not significant and the point estimate indicated higher CD4 was harmful, countering the current scientific knowledge.

Table 4

Estimated Odds Ratios (ORs) and 95%CI, and p-value for probability of events in years 2–5 after initiation of antiretroviral treatment (ART), had everyone remained on ART without treatment interruptions longer than 21 days.

	Univariate OR	Adjusted OR for CD8+ T-cell activation year 1 (per 10 units (%)(higher) ³	Multivariable model A	Multivariable model B	Multivariable model C
CD8+ T-cell activation year 1 (per 10 units (%)(higher)	1.20 (1.04,1.40) (p=0.01)	--	1.08 (0.90,1.27) (p=0.40)	1.13 (0.95,1.32) (p=0.17)	1.16 (0.98,1.36) (p=0.096)
Age (per 10 years older)	1.91 (1.51,2.46) (p<0.0001)	1.12 (0.96,1.30)	1.83 (1.44,2.41) (p<0.0001)	1.87 (1.48,2.44) (p<0.0001)	1.86 (1.47,2.42) (p<0.0001)
CD4 sqrt ¹ yr1 (per 3 units higher) ²	1.05 (0.92,1.19) (p=0.47)	1.24 (1.05,1.45)	--	1.10 (0.95,1.27) (p=0.20)	1.10 (0.95,1.26) (p=0.20)
Pre-ART CD4 sqrt ¹ (per 3 units higher) ²	1.07 (0.97,1.18) (p=0.17)	1.25 (1.07,1.46)	--	--	--
Sex (female, ref male)	1.04 (0.57,1.76) (p=0.91)	--	--	--	--
Injection drug use (ever, ref never)	1.62 (0.69,3.48) (p=0.24)	--	--	--	--
Randomized study 384/388, ref A5014/A5095	0.62 (0.38,0.97) (p=0.03)	1.20 (1.03,1.41)	0.65 (0.39,1.04) (p=0.07)	0.65 (0.39,1.05) (p=0.08)	--
Pre-ART log ₁₀ RNA (log ₁₀ copies/mL, per unit higher)	0.92 (0.72,1.20) (p=0.52)	--	--	--	--
Naïve CD4 percentage year 1 (per 10 units (%)(higher)	0.84 (0.72,0.98) (p=0.03)	1.14 (0.97,1.34)	0.96 (0.80,1.15) (p=0.69)	--	--
Event in year 1 (yes, ref no)	0.76 (0.13,2.15) (p=0.63)	--	--	--	--

¹ Sqrt refers to square root. Reason for sqrt: the difference between CD4=50 and CD4=100 is much more informative than the difference between CD4=600 and CD4=650. Taking a square root accounts for that.

² For example, a CD4 count of 100 versus 49, 294 versus 200, or 643 versus 500 cells/mm³.

³ These are ORs for CD8+ T-cell activation adjusted for each covariate; for example, the OR for CD8+ T-cell activation adjusted for age is 1.12 (0.96,1.30).

Table 5

Estimated Odds Ratios (ORs), 95%CI, and p-values for the probability of events in years 2–5 after initiation of antiretroviral treatment (ART): subjects 50 years and older, had everyone continued to have viral suppression on ART.

	Univariate OR	Adjusted OR CD8+T-cell activation ⁴	Multivariable model
CD8+ T-cell activation year 1 (per 10 units (%) higher) ¹	1.42 (1.07,2.47) (p=0.015)	--	1.51 (1.10,2.91) (p=0.008)
CD4 sqrt ² year 1 (per 3 units higher) ³	1.13 (0.84,1.54) (p=0.43)	1.50 (1.10,2.62)	1.22 (0.89,1.77) (p=0.21)
Age (per 10 years older)	1.14 (0.31,3.55) (p=0.81)	1.43 (1.06,2.64)	0.89 (0.15,3.54) (p=0.90)

¹ Sensitivity analysis: for the probability of an event in years 3–5, given no event in year 2: univariate OR 1.38 (1.01–2.64) (p=0.04).

² Sqrt refers to square root. Reason for sqrt: the difference between CD4=50 and CD4=100 is much more informative than the difference between CD4=600 and CD4=650. Taking a square root accounts for that.

³ For example, a CD4 count of 100 versus 49, 294 versus 200, or 643 versus 500 cells/mm³.

⁴ These are ORs for CD8+ T-cell activation adjusted for each covariate; for example, the OR for CD8+ T-cell activation adjusted for age is 1.43 (1.06,2.64).