Supplementary Online Content

Okulicz JF, Le TD, Agan BK, et al. Influence of the timing of antiretroviral therapy on the potential for normalization of immune status in human immunodeficiency virus 1–infected individuals. *JAMA Intern Med*. Published online November 3, 2014. doi:10.1001/jamainternmed.2014.4010.

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Cohort Description and Statistical Methods

(A) US Military HIV Natural History Study (NHS) cohort description.

The US Military HIV Natural History Study (NHS) is an ongoing, continuous-enrollment, prospective, multicenter, observational cohort study conducted through the Uniformed Services University of the Health Sciences (USU) Infectious Disease Clinical Research Program (IDCRP). The NHS has enrolled more than 5700 Department of Defense (DoD) active duty military service members and beneficiaries since 1986 at 7 Military Treatment Facilities (MTF) throughout the United States. The US military medical system provides comprehensive HIV education, care, and treatment, including the provision of ART and regular visits with clinicians with expertise in HIV medicine at MTFs, at no cost to the patient. Mandatory periodic HIV screening according to DoD policy allows treatment initiation to be considered at an early stage of infection. Eighty-eight percent of the subjects since 1995 have documented seroconversion (ie, a documented negative HIV test preceding a positive HIV test), with a median seroconversion window of approximately 15 months. The median $CD4^+$ count at diagnosis was approximately 500 cells/μL. Active duty personnel are required to visit the MTF at least twice yearly for formal medical evaluation. Following retirement or separation from active duty, all individuals retain health benefits and may continue participation in the cohort study while receiving their primary HIV care either within or outside of the military health care system. Aside from the advantages afforded by the medical system, there are aspects of this cohort that allow for a unique perspective on HIV treatment response. The military population from which these patients are derived consists of highly motivated and disciplined individuals who possess either a minimum of a high school equivalent education (enlisted) or an undergraduate college degree (officers) and maintain rigorous physical standards. As a consequence of periodic random drug screening, the reported rate of injection drug use (IDU) in this population is less than 1%. Thus, many of the factors that typically hinder the clinical response to antiretroviral therapy (ART) in most North American cohorts, such as IDU, homelessness, and unemployment, are minimized or eliminated in the military setting. Additionally, the cohort is racially balanced and geographically diverse reflecting the distribution of individuals with HIV in the United States. In the present study, 5402 NHS participants were evaluated with clinical data from 8/20/1986 to 11/16/2010. The subsets of the NHS participants studied for the 4 primary outcomes are described below.

(B) Study objectives and participants.

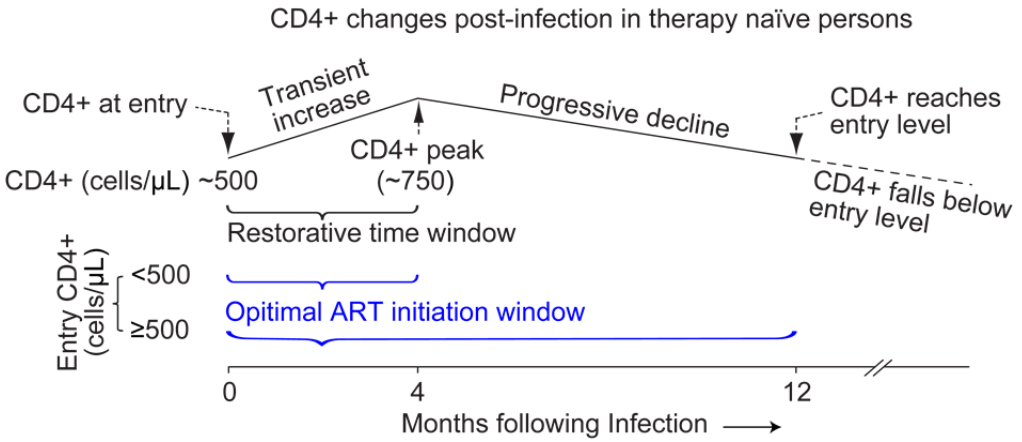
The objective of this study was to investigate the influence of the timing of ART relative to when HIV seroconversion occurred on 4 primary outcomes: normalization of $CD4^+$ T-cell counts ($CD4^+$ normalization), development of AIDS (1987 and 1993 CDC criteria),[1] T-cell activation, and in vivo functional immune responses. We also evaluated the immunologic and clinical (mitigation of AIDS risk) benefits of achieving $CD4^+$ normalization. Normalization of $CD4^+$ T-cell counts was defined as achieving at least 1 $CD4^+$ count of 900 cells/μL or more during VL-suppressive ART. All study participants are from the NHS except for the 13 HIV-uninfected healthy blood donors who were matched by age.

The NHS participants studied for the 4 primary outcomes are described in the main text and illustrated in **Figure 1** and **eFigure 1**. First, $CD4^+$ normalization and AIDS outcomes were examined in 1119 participants with an estimated date of infection (EDS) who met the inclusion/exclusion criteria (**Figure 1)**. Participants who did not achieve viral load (VL) suppression during ART were excluded because the muted $CD4^+$ count recovery in these individuals would have confounded the analyses. Second, immune markers representative of T-cell activation, dysfunction, and responsiveness were assessed in 124 participants (66 with EDS) who were receiving VL-suppressive ART (**eFigure 1)**; the characteristics of these participants are described in **eTable 1**. Third, to determine the effects of the duration of untreated HIV infection on the integrity of functional immune responses in vivo, we evaluated serologic response to hepatitis B virus (HBV) vaccine in 374 participants who received HBV vaccine only after HIV diagnosis (**eFigure 1**); the

characteristics of these participants are shown in **eTables 2 and 3**. A total of 261 of these participants received the HBV vaccine while therapy (ART) naive, whereas 113 received the vaccine during ART.

(C) Conceptual framework and basis for selecting the time intervals and CD4$^+$ thresholds or landmarks used in the present study

The conceptual framework of our study was based on our prior observations made in the San Diego Primary HIV infection cohort[2] wherein we had first defined the CD4$^+$ count trajectory after infection, and then based on this trajectory hypothesized that commencement of ART within specific time windows after infection would be associated with increased normalization of CD4$^+$ counts. This trajectory is depicted in Figure 3A in the main text and is reproduced below.



Normalization of CD4$^+$ T-cell counts. This end point was defined as the attainment of at least 1 CD4$^+$ count that approximated the median CD4$^+$ count identified in HIV-uninfected adults of European or African American descent. A MEDLINE literature review of 26 reports comprising more than 16 000 individuals revealed that the median CD4$^+$ count of the mean CD4$^+$ counts in each of the reported studies was approximately 900 cells/μL[2]. We therefore termed a CD4$^+$ count of 900 cells/μL or above as a *normal* CD4$^+$ T-cell count. In the present study, we confirmed this to be a relevant CD4$^+$ landmark as evaluation of panel CD4$^+$ count data from the (1) US National Health and Nutrition Examination Survey (NHANES; **eTable 5**), and (2) the University of California San Diego HIV Neurobehavioral Research Center cohort (HNRC) (**eTable 6**). We also updated our prior MEDLINE review[2] with an additional reported study that also showed that the median CD4$^+$ count in HIV-uninfected adults was approximately 900 cells/μL.[3] Therefore, consistent with our previous work,[2] we defined normalization of CD4$^+$ counts as attainment of at least 1 CD4$^+$ count of 900 cells/μL or more during VL-suppressive ART.

CD4$^+$ trajectory post infection in therapy-naive persons (see Figure above). Our analysis of the San Diego Primary Infection Cohort showed that the median peripheral blood CD4$^+$ count post infection was approximately 500 cells/μL. Thereafter, CD4$^+$ counts increased spontaneously, peaking to approximately 750 cells/μL at about 4 months since the estimated date of infection (EDI).[2] Thereafter, CD4$^+$ counts declined and approximated entry CD4$^+$ levels approximately 12 months from the EDI. We termed the 4 months since infection as a *restorative time window*, because during this time window there was a trend for CD4$^+$ T-cell counts to rebound spontaneously. Moreover, we observed that initiation of ART within this restorative time window was most beneficial for CD4$^+$ normalization in individuals initiating ART with CD4$^+$ <500 cells/μL. In contrast, in individuals initiating ART with CD4$^+$ ≥500 cells/μL, we observed that the time window post infection within which ART initiation promoted CD4$^+$ normalization could be extended to 12 months since EDI.

Higher versus lower CD4$^+$ counts. In our prior studies, we used the CD4$^+$ count of 500 cells/μL as a threshold for classifying higher versus lower CD4$^+$ counts at ART initiation for 3 reasons: (1) in the primary infection cohort the median CD4$^+$ count at entry was approximately 500 cells/μL and, after a spontaneous rebound in CD4$^+$ counts, levels declined to approximately 500 cells/μL within 1 year post

infection[2]; (2) a survey of 18 495 seroconverting HIV-infected individuals with a median seroconversion window of approximately 280 days (last documented HIV-negative test to first documented HIV-positive test) revealed that the time from EDS to a $CD4^+$ count of approximately 500 cells/μL was approximately 12 months[4], and (3) most current international therapy guidelines[5,6] use the $CD4^+$ count of 500 cells/μL as a threshold for ART initiation.

Earlier versus later ART. Unlike the San Diego primary infection cohort, the NHS is not a primary infection cohort. Since the median seroconversion window was 15 months in the overall NHS and in the participants we evaluated in the present study, we anticipated that there would be few individuals who would be accrued during the first 4 months following infection. Therefore, we elected to use the 12 months from the EDS or entry as a cut-off for classifying earlier versus later ART. This was based on the prior observation in the primary infection cohort that initiation of ART within 12 months of EDI promoted $CD4^+$ normalization, especially in individuals who commenced ART with $CD4^+$ counts of ≥500 cells/μL. Moreover, as noted above, examination of the $CD4^+$ trajectory revealed that participants, on average, had a $CD4^+$ count of approximately 500 cells/μL 1 year post infection.[2] Moreover, in a survey of 18 495 HIV-infected persons who were not receiving ART, the time to reach a $CD4^+$ count of 500 cells/μL was approximately 12 months from the EDS.[4] To maintain uniformity and knowing that the interval between EDS and entry was narrow in the NHS (approximately 10 months), we used a common interval of 12 months from the EDS to starting ART or the study entry to starting ART as a classifier of earlier versus later ART. Study entry was defined as the date when the first $CD4^+$ count was measured after HIV diagnosis and available for analyses.

Since in most instances physicians do not have access to their patients' EDS or proximal (early) seroconversion $CD4^+$ counts and to make the present work more translatable to real-life clinical scenarios, we elected to index the duration of untreated infection (ART timing) from the EDS and study entry. These 2 reference points have distinct implications for clinical care and public health policy if our hypotheses were to be affirmed: (1) the EDS, as it is a defined nodal point of disease initiation and has implications for early point-of-care testing, and (2) study entry into the health care system, as it is a variable nodal point during a patient's disease course and has implications of when to initiate ART so as to maximally mitigate long-term clinical and immunologic sequelae of untreated HIV infection.

(D) Study duration.

Study duration was predefined as 10 years from ART initiation until loss of VL suppression or cessation of ART. This resulted in 3 categories of participants: those with 10 years of follow-up data while on VL-suppressive ART; those who, while on ART, experienced a loss in VL suppression; and finally, those who stopped ART. All of the participants in the present study who stopped ART did so while their VL was suppressed.

Our results were unlikely to be confounded by variable study duration among relevant study groups. Among the 1119 participants we studied for the outcomes of $CD4^+$ normalization and AIDS development, the distribution of these 3 categories of participants defined by their study duration were similar between those initiating ART earlier vs later indexed either by the EDS (P = .18) or by study entry (P = .12) (**eMethods Table 1**, below). In addition, as we show in Table 1 in the main text, the median study duration was similar between participants initiating ART earlier vs later indexed either by EDS (P = .23) or by study entry (P = .59).

eMethods Table 1. Study duration and CD4+ count and VL measurements in participants categorized by earlier versus later ART indexed from EDS or study entry						
	Time from EDS to ART initiation			Time from entry to ART initiation		
	≤12 mo. (n = 292)	>12 mo. (n = 827)	*P* value	≤12 mo. (n = 645)	>12 mo. (n = 474)	*P* value
Groups			.18			.12
10 years on VL-suppressive ART, n (%)	46 (16%)	150 (18%)		107 (17%)	89 (19%)	
Loss of VL suppression before 10 years, n (%)	197 (67%)	572 (69%)		438 (68%)	331 (70%)	
Cessation of ART that suppressed VL before 10 years, n (%)	49 (17%)	105 (13%)		100 (16%)	54 (11%)	
No. of CD4+ measurements per year, median (IQR)	3.26 (2.38-4.99)	3.15 (2.22-4.81)	.30	3.21 (2.38-4.83)	3.09 (2.11-4.86)	.30
No. of VL measurements per year, median (IQR)	3.48 (2.45-5.26)	3.20 (2.18-4.83)	.22	3.36 (2.40-4.99)	3.15 (2.08-5.01)	.23

(E) Viral load (VL) suppression.

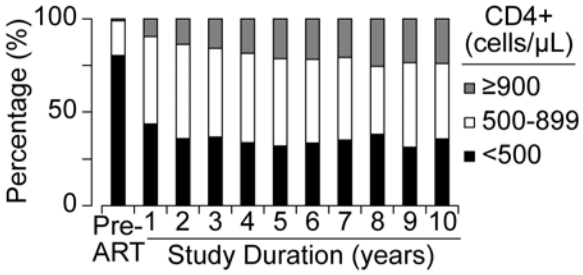
VL suppression was defined as ≥2 consecutive determinations of <400 copies/mL at least 14 days apart. Time to VL suppression was calculated from the date of ART initiation to the first of the 2 consecutive dates at which VL was <400 copies/mL. Our results were unlikely to be confounded by variable measurements of VL among the relevant study groups. The median (IQR) number of VL measurements per year during the study was similar between participants initiating ART earlier vs later indexed by EDS (3.48 [2.45-5.26] vs 3.20 [2.18-4.83], $P = .22$) or by study entry (3.36 [2.40-4.99] vs 3.15 [2.08-5.01], $P = .23$]) (**eMethods Table 1**, above). Moreover, the number of VL measurements per year was not a predictor of the odds of $CD4^+$ normalization (coefficient = −0.002, $P = .50$).

(F) Rate and likelihood of $CD4^+$ normalization.

The rate and likelihood of achieving $CD4^+$ count normalization was determined. While the rate and likelihood are complementary end points, they each have different connotations in HIV disease. For example, a patient may achieve $CD4^+$ normalization during the study period, but may do so much later during follow-up. Thus, in this instance both the odds and rate of normalization can provide important information. Our results were unlikely to be confounded by a variable number of measurements of $CD4^+$ count measurements among relevant study groups. The median (IQR) number of $CD4^+$ measurements per year was similar between participants initiating ART earlier vs later indexed by the EDS (3.26 [2.38-4.99] vs 3.15 [2.22-4.81], $P = .30$) or by study entry (3.21 [2.38-4.83] vs 3.09 [2.11-4.86], $P = .30$). Moreover, the number of $CD4^+$ count measurements per year was not a predictor of the odds of $CD4^+$ normalization (coefficient = −0.002, $P = .38$).

Time to $CD4^+$ normalization was defined as the interval from the date of starting ART to the date of achieving the first $CD4^+$ count of 900 cells/μL or more. To investigate the pattern of $CD4^+$ normalization in the cohort and its relative stability, we analyzed the highest $CD4^+$ count in each year since starting VL-suppressive ART (**eMethods Figure,** below). We stratified the $CD4^+$ counts into 3 strata: <500, 500-899, and ≥900 cells/μL. We found that during the initial 4 years of VL-suppressive ART, the proportion of participants achieving $CD4^+$ normalization increased from approximately 10% to about 20% and it

gradually increased over the subsequent years to approximately 25%. These data suggested that there is durability at the population level in $CD4^+$ recovery during VL-suppressive ART.



(G) AIDS development.

Time to AIDS event was defined as time (years) from ART initiation to the development of the first AIDS event (1987 or 1993 CDC criteria[1]). All participants with an AIDS event (1987 or 1993 criteria) prior to or at ART initiation were excluded from the corresponding analyses.

(H) Covariates.

Demographic as well as all other potential confounding factors that could influence $CD4^+$ normalization and AIDS development were considered in univariate analysis (Table 2, eTable 8, and data not shown). These predefined covariates were age, sex, ethnicity, pre-ART VL, calendar year of ART initiation, ART regimen, duration of VL-suppressive ART indexed from the date of starting ART, and time from ART initiation to VL suppression. The covariates with a significant association were included in the multivariate models.

(I) Logistic regression, Cox proportional hazard modeling, and Kaplan-Meier analysis.

Logistic regression was used to compute the likelihood (odds ratio) of attaining $CD4^+$ normalization during VL-suppressive ART. A Cox proportional hazards model was used to compute the rate ratio (RR) of attaining $CD4^+$ normalization or hazard ratio (HR) of developing the first AIDS event during VL-suppressive ART. All proportionality assumptions were assessed. We also examined the interaction between the $CD4^+$ strata at the time of study entry and pre-ART (higher vs lower) and timing of ART (earlier vs later) on attainment of $CD4^+$ normalization and the development of AIDS. The model with interaction did not fit the data better than a model without interaction. As the interaction was not a significant predictor, it was not included in the model. The Kaplan-Meier method was used to estimate the cumulative probability of achieving $CD4^+$ normalization and developing AIDS while VL was suppressed on ART.

(J) Subgroup analyses.

In subgroup analyses, we determined the conjoint influence of the timing of ART relative to the EDS or study entry as well as $CD4^+$ counts at study entry or ART initiation on recovery of $CD4^+$ counts and AIDS development. All subgroup analyses were predefined and mirrored the statistical plan of our previous study in the San Diego Primary Infection Cohort.[2]

Participants were initially stratified into 4 sets based on $CD4^+$ counts at entry and ART initiation referenced to a $CD4^+$ threshold of 500 cells/μL (**Figure 3B**), and then into 8 subsets based on whether ART was initiated earlier versus later indexed to the EDS (**Figure 3C and D**) or study entry (**eFigure 2**). To parse further the effects of progressively increasing durations of untreated infection on the likelihood of $CD4^+$ normalization, we stratified the 4 $CD4^+$-defined sets shown in Figure 3B into 12 subsets (**Figure 4**; subsets

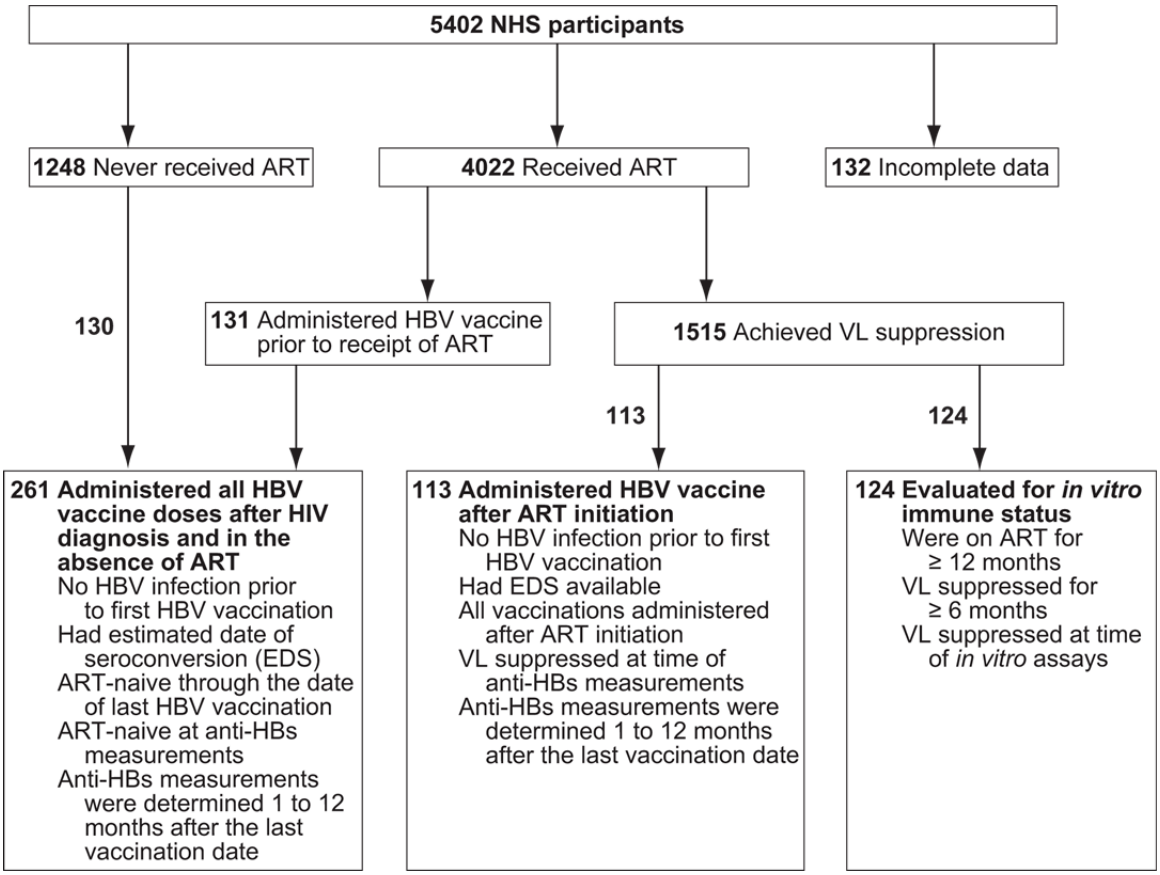
1′ to 12′). Participants were categorized according to whether they initiated ART earlier or later co-indexed to both the EDS and study entry, ie, whether ART was started within 12 months of both EDS and entry (E/E, earlier/earlier), after 12 months from EDS but within 12 months of entry (L/E, later/earlier), and lastly after 12 months from both EDS and entry (L/L, later/later) (**Figure 4**).

Reported *P* values are 2-sided. The models were adjusted for covariates but were not adjusted for multiple comparisons in the prespecified subgroup analyses. The specific statistical methods in each of the figure panels depicted in this study are listed in **eMethods Table 2** below.

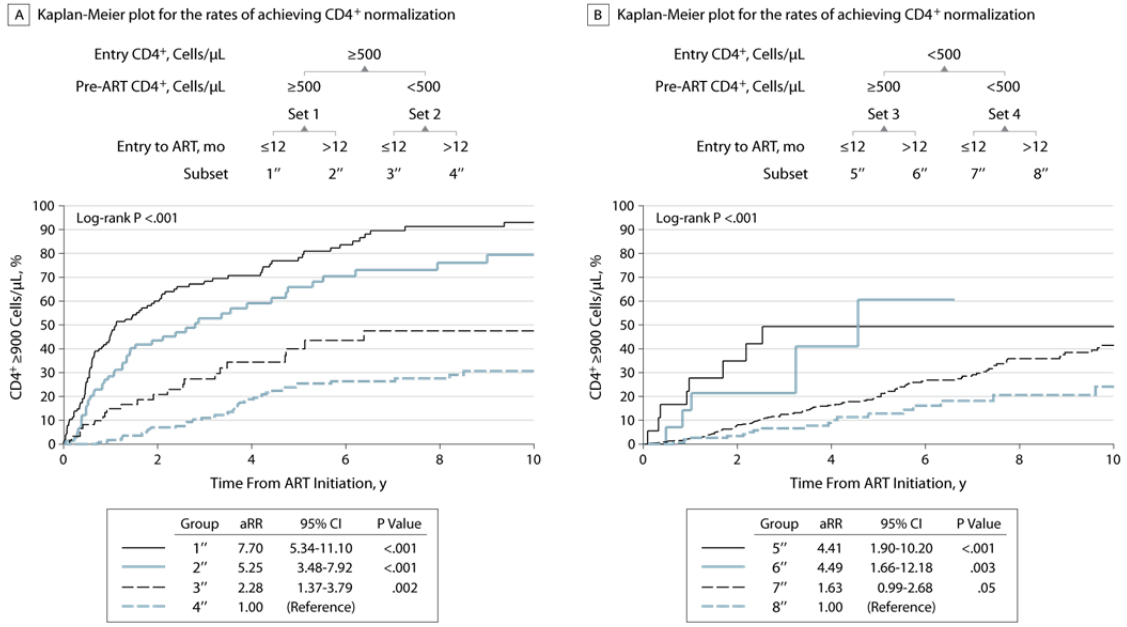
eMethods Table 2. Specific statistical methods used in each of the Figure panels depicted in this study.

Figure	Statistical test used
Figure 2B	Chi-square test.
Figure 2C-E	Mann-Whitney test.
Figure 3	Panel B: median $CD4^+$ counts. Panels C and D: Kaplan-Meier with a log-rank and Cox proportional hazards modeling for computing the rate ratio (RR) and 95% CI with adjustment of covariates.
Figure 4	*$CD4^+$ count change from entry until ART initiation*: Wilcoxon Signed-Ranks test.
	Percent achieving $CD4^+$ normalization: Chi-square test for the indicated groups comprising 3 subsets in each group.
	Odds Ratio of achieving $CD4^+$ normalization: logistic regression for computing the odds ratio and 95% CI with adjustment of covariates.
Figure 5A,B	Kaplan-Meier with a log-rank test and Cox proportional hazards modeling for computing the hazard ratio and 95% CI with adjustment of covariates. Incidence rate ratio (IRR) per 1000 person-years for time to AIDS was also calculated.
Figure 5C	Mann-Whitney test.
Figure 5D, E	Chi-square test.
eFigure 2	Same as Figure 3C and D.

eFigure 1. Sources and Key Characteristics of the NHS Participants in Whom Immune Markers and HBV Vaccine Responses Were Evaluated.



eFigure 2. CD4$^+$ Normalization Rates in NHS Participants According to ART Timing Indexed by Study Entry and CD4$^+$ T-Cell Counts at Entry and ART Initiation. The CD4$^+$-derived sets 1 to 4 shown in main Figure 3B were further stratified into the indicated 8 subsets depending upon whether ART was initiated within or after 12 months of study entry. The rate ratio was adjusted (aRR) for calendar year of ART initiation, ART regimen, duration of VL-suppressive ART indexed from the date of starting ART, and time from ART initiation to VL suppression. Data were obtained from 1119 NHS participants.



eTable 1. Characteristics of the 124 NHS Participants Analyzed for Immune Markers.

Characteristic	Overall	Current $CD4^+$ cells/μL on VL-suppressive ART			
		<500	500-899	≥900	*P* value[a]
Participants, No. (%)	124	38 (30.7)	59 (47.6)	27 (21.7)	–
Male, No. (%)	120 (96.8)	37 (97.4)	56 (94.9)	27 (100.0)	.45
Ethnicity, No. (%)					.56
European American	73 (58.9)	21 (55.3)	34 (58.6)	18 (66.7)	
African American	45 (36.6)	14 (36.8)	22 (37.9)	9 (33.3)	
Age at ART initiation, y	34 (30-40)	34 (30-39)	35 (30-40)	35 (30-41)	.85
Age at experiment, y	38 (32-41)	36 (30-40)	38 (33-41)	38 (33-44)	.39
Study entry $CD4^+$, cells/μL[b]	506 (373-722)	490 (309-622)	488 (358-744)	549 (452-767)	.14
Study entry VL, log_{10} copies/mL	4.25 (3.70-4.74)	4.19 (3.53-4.70)	4.27 (3.66-4.78)	4.31 (3.65-4.77)	.93
Pre-ART $CD4^+$, cells/μL[c]	373 (286-502)	279 (235-394)	384 (302-502)	464 (382-538)	<.001
Pre-ART VL, log_{10} copies/mL	4.57 (3.99-4.96)	4.65 (4.17-4.95)	4.43 (3.79-4.97)	4.52 (4.00-4.94)	.92
Time from EDS to ART initiation, mo[d]	30.0 (13.1-49.2)	36.5 (17.7 -66.4)	23.7 (12.7-42.1)	20.7 (12.2-40.3)	.07
Time from ART to experiment, mo	96.3 (44.0-126.4)	93.5 (34.4-145.9)	93.5 (44.9-121.8)	100.1 (63.6-120.7)	.99

Abbreviations: ART, antiretroviral therapy; EDS, estimated date of seroconversion; IQR, interquartile range; VL, plasma HIV RNA viral load.

Unless otherwise specified, data are expressed as median (IQR).

[a] The *P* values were calculated with the use of Kruskal-Wallis or Chi-square test.

[b] Mann-Whitney test was used for pairwise comparisons of the $CD4^+$ counts at entry between $CD4^+$ strata of 500-899 vs <500 (*P* =.45), ≥900 vs <500 (*P* =.06), and 500-899 vs ≥900 (*P* =.13).

[c] Mann-Whitney test was used for pairwise comparisons of the pre-ART $CD4^+$ counts between $CD4^+$ strata of 500-899 vs <500 (*P* =.009), ≥900 vs <500 (*P* <.001), and 500-899 vs ≥900 (*P* =.01).

[d] Mann-Whitney test was used for pairwise comparisons of the time from EDS to ART initiation between $CD4^+$ strata of 500-899 vs <500 (*P* =.047) or ≥900 vs <500 (*P* =.049), and 500-899 vs ≥900 (*P* =.68).

eTable 2. Characteristics of the 261 NHS Participants Who Received HBV vaccinations While They Were Therapy (ART)–Naive.

Characteristic	Overall	Months from EDS to last HBV Vaccination		*P* value
		≤12	>12	
Participants, No. (%)	261	42 (16.1)	219 (83.9)	
Male, No. (%)	239 (91.6)	40 (95.2)	199 (90.9)	.35
Ethnicity, No. (%)				.71
European American	110 (42.2)	18 (42.9)	92 (42.0)	
African American	109 (41.8)	19 (45.2)	90 (41.1)	
Other	42 (16.0)	5 (11.9)	37 (16.9)	
Age at last vaccination, y	28 (24-32)	24 (22-28)	28 (25-32.1)	<.001
From EDS to last vaccination, mo	21.2 (14.3-38.8)	8.3 (6.4-10.1)	25.0 (17.3-42.5)	<.001
$CD4^+$ at last vaccination, cells/μL	481 (350-606)	491 (400-590)	478 (335-608)	.52
VL at last vaccination, log_{10} copies/mL	3.88 (3.14-4.41)	4.04 (3.33-4.53)	3.85 (3.07-4.40)	.37
No. of participants with anti-HBs ≥10 IU/L, No. (%)	103 (39.5)	24 (57.1)	79 (36.1)	.01

Abbreviations: EDS, estimated date of seroconversion; HBV, hepatitis B virus; IQR, interquartile range; VL, plasma HIV RNA viral load.
Unless otherwise specified, data are expressed as median (IQR).
The *P* values were calculated with the use of the *t* test, Mann-Whitney test, or Chi-square test where appropriate.

eTable 3. Characteristics of the 113 Participants Who Received HBV Vaccinations During VL-Suppressive ART.

Characteristic	Overall	Months from EDS to ART Initiation		*P* value
		≤12	>12	
Participants, No. (%)	113	56 (49.6)	57 (50.4)	
Male, No. (%)	106 (93.8)	53 (94.6)	53 (93.0)	.77
Ethnicity, No. (%)				.08
European American	62 (54.9)	26 (46.4)	36 (63.2)	
African American	41 (36.3)	26 (46.4)	15 (26.3)	
Other	10 (8.8)	4 (7.2)	6 (10.5)	
Age at last vaccination, y	38 (30-43)	35 (28-40)	40 (34-44)	<.001
From ART initiation to last vaccination, mo	48.3 (22.2-96.0)	36.4 (18.9-78.1)	69.9 (31.6-119.7)	.004
$CD4^+$ at last vaccination, cells/μL	637 (523-899)	639 (527-928)	634 (509-872)	.89
No. of participants with anti-HBs ≥10 IU/L, No. (%)	67 (59.3)	38 (67.9)	29 (50.9)	.07

Abbreviations: ART, antiretroviral therapy; EDS, estimated date of seroconversion; HBV, hepatitis B virus; IQR, interquartile range; VL, plasma HIV RNA viral load.
Unless otherwise specified data are expressed as median (IQR).
The *P* values were calculated with the use of *t* test, Mann-Whitney test, or Chi-square test where appropriate.

eTable 4. $CD4^+$ T-Cell Counts in Demonstrated or Presumed HIV-1–Uninfected Individuals of European Descent and African Americans.

Summary Statistics for $CD4^+$ Counts [a]				
	No. of Reports (No. of Individuals)	**Weighted Mean (95% CI)**	**Median (IQR)**	**Range**
$CD4^+$ count				
Whites	16 (11 037)	1011 (1005-1017)	940 (834-1030)	796-1109
Mixed US population	9 (4183)	1016 (1004-1027)	1004 (924-1017)	771-1075
African American	2 (1006)	1077 (1054-1099)	1078 (1055-1100)	1055-1100
Total sample size	27 (16 226)	1015 (1009-1020)	988 (840-1036)	771-1109

Reports from which the summary statistics were derived

Race/ Ethnicity	**Reason for Study**	**Age, Mean (SD, range), y**	**Sex**	**HIV Status**	**No.**	**$CD4^+$ count (cells/μL) Mean (SD or SE; range) or Median (IQR)**	**Ref**
White							
Australian	GN	Mean, 15 (range, 10-37)	52% Female	PN	2538	Mean, 1030 (SD, 270; range, 210-2530)	7
Australian	GN	Mean, 14 (range, 10-22)	48% Female	PN	592	Mean, 1040 (SD, 300; range, 200-2800)	7
UK	GN	Mean, 50 (range, 19-80)	Female	PN	396	Mean, 870 (SD, 330; range, 390-2380)	7
UK/Belgium	RF	(range, 7-17)	22% Female	PN	22	Median, 800 (IQR, 700-1100)	8
UK/Belgium	RF	(range, 18-70)	55% Female	PN	101	Median, 800 (IQ,: 700-1100)	8
Sweden	RF	(range, 20-39)	Combined	PN	75	Mean, 1020 (SE, 39)	9
Sweden	RF	(range, 20- 39)	Male	PN	34	Mean, 929 (SE, 58)	9
Sweden	RF	(range, 20- 39)	Female	PN	41	Mean, 1096 (SE, 52)	9
Sweden	RF	(range, 40-59)	Combined	PN	76	Mean, 834 (SE, 35)	9
Sweden	RF	(range, 40-59)	Male	PN	39	Mean, 718 (SE, 32)	9
Sweden	CT	(range, 40-59)	Female	PN	37	Mean, 956 (SE, 59)	9
Sweden	RF	(range, 60-79)	Combined	PN	68	Mean, 796 (SE, 37)	9
Sweden	RF	(range, 60-79)	Male	PN	36	Mean, 722 (SE, 47)	9

Race/ Ethnicity	Reason for Study	Age, Mean (SD, range), y	Sex	HIV Status	No.	$CD4^+$ count (cells/μL) Mean (SD or SE, range) or Median (IQR)	Ref
Sweden	RF	(range, 60-79)	Female	PN	32	Mean, 880 (SE, 55)	9
Germany	RF	(range, 19-85)	Combined	PN	100	Median, 870 (IQR, 490-1640)	10
Germany	RF		Male	PN	50	Median, 830	10
Germany	RF		Female	PN	50	Median, 930	10
Switzerland	RF	Mean, 50 (range, 24-68)	Combined	PN	70	Median, 691 (IQR, 309-1139)	11
Switzerland	RF	Mean, 49 (range, 23-70)	Male	PN	44	Median, 656 (IQR, 336-1126)	11
Switzerland		Mean, 51 (range, 25 -70)	Female	PN	26	Median, 761 (IQR, 314-1270)	11
Italy	RF	Mean, 37 (range, 18-70)	Combined	PN	946	Mean, 940 (range, 493-1666)	12
Italy	RF	-	Male	PN	532	Mean, 902	12
Italy	RF	-	Female	PN	436	Mean, 989	12
England	RF	(range, 11-79)	Combined	Neg.	600	Mean, 830 (SD, 288; Range, 410-1540)	13
England	RF	Mean, 30 (range, 19-41)	Male	Neg.	50	Mean, 840 (SD, 285)	13
England	RF	Mean, 31 (range, 20-49)	Female	Neg.	50	Mean, 1050 (SD, 377)	13
Belgium	RF	(range, 18-70)	46% Female	Neg.	271		14
UK	RF	-	Combined	Neg.	234	Mean, 831	15
UK	RF	Mean, 31 (range, 19- 67)	Male	Neg.	91	Mean, 754	15
UK	RF	Mean, 28 (range, 17-58)	Female	Neg.	195	Mean, 865	15
UK	RF	Mean, 33 (SD, 6; Range, 23-44)	Male	NP	32	Mean, 954 (SD, 272; range, 460-1430)	15
US	RF	Mean, 38	Male	NP	3467	Mean, 1100 (SD, 400)	16
France	CT	-	-	Neg.	61	Mean, 807 (SD, 378)	17

Race/ Ethnicity	Reason for Study	Age Mean (SD, range), y	Sex	HIV Status	No.	$CD4^+$ count (cells/μL) Mean (SD or SE, range) or Median (IQR)	Ref
France	CT	-	Male	Neg.	16	Mean, 1109 (SD, 399)	17
France	CT	-	-	PN	12	Mean, 844 (SD, 247)	18
Italy	GN	-	Combined	PN	468	-	19
Italy	GN	Mean, 41 (SD, 15)	Male	PN	263	Mean, 903 (SD, 308)	19
Italy	GN	Mean, 40 (SD, 16)	Female	PN	205	Mean, 1018 (SD, 319)	19
Netherlands	RF	-	-	Neg.	1356	Mean, 993 (SD, 319; range; 509-1761)	20
Netherlands	RF	-	-	Neg.	678	Median, 930 (IQR, 490-1750)	21
Netherlands	CT	(range, 18 -64)	~48% Female	PN	59	Median, 908 (IQR, 513-1606)	22
Mixed US							
Baltimore/Los Angeles	RF	(range, 18-60)	-	Neg.	2787	Mean, 1017 (SD, 329)	23
Los Angeles	RF	-	-	Neg.	743		24
US	CT	-	-	PN	19	Mean, 839 (SD, 276)	25
New Mexico	CT	(range, 21-53)	Combined	PN	20	Mean, 1075 (SD, 586)	26
New Mexico	CT	Mean, 76 (range, 67-88)	Combined	PN	25	Mean, 924 (SD, 416)	26
New York	CT	Mean, 39 (SD, 6.7)	-	Neg.	34	Mean, 1013 (SD, 274)	27
US	CT	Median, 25 (IQR, 18-30)	58% Female	Neg.	24	Median, 785 (IQR, 662-860)	28
US	CT	Median, 49 (IQR, 45-66)	54% Female	Neg.	24	Median, 869 (IQR, 658-1111)	28
US	RF	(range, 20-69)	Combined	PN	266	Mean, 1036 (SD, 296; range, 294-1590)	29
US	RF		Male	PN	143		29
USA	RF		Female	PN	123		29
California	CT	Median,38 (IQR, 20-58)	65% Female	PN	49	Mean, 771 (range, 326 -1344)	30
US Air Force	RF	Mean, 49	Male	Neg.	883	Mean, 982 (range, 417- 1841)	31

Race/ Ethnicity	Reason for Study	Age Mean (SD, range), y	Sex	HIV Status	No.	$CD4^+$ count (cells/μL) Mean (SD or SE, range) or Median (IQR)	Ref
South Florida	RF	Mean, 38.1 (range, 21- 67)	67% Female	Neg.	100	Mean, 1003.8 (SD, 304.9; range, 491-2000)	3
African Americans							
US	CT	Median, 32	Female	Neg.	513	Mean, 1055 (SE, 15)	32
US	RF	Mean, 38 (range, 31-45)	Male	PN	493	Mean, 1100 (SD, 400)	16

Abbreviations: CI, confidence interval; CT, control; GN, genetic; IQR, interquartile range; Neg., negative; PN, presumed HIV-negative; UK, United Kingdom; RF, reference.

Mixed USA refers to $CD4^+$ counts data from the USA where the number of individuals who were European American or African American was not specified.

[a]Summary statistics were derived from the $CD4^+$ counts reported for presumed HIV-negative (PN) or documented HIV-1–negative (Neg.) individuals in the studies outlined in the lower half of this table. Reason for study refers to the main purpose of why the study was conducted. Genetics (GN) indicates that the study was conducted to test the genetic basis of inheritance of T-cell counts. Reference (RF) means that the study was conducted to obtain a reference $CD4^+$ value specific for the healthy population studied. Control (CT) denotes that the group studied served as a control for a study in which the association of a process (eg, aging, HIV+) with $CD4^+$ counts were examined. In this instance, only the $CD4^+$ counts from the healthy individuals were used.

eTable 5. $CD4^+$ T-Cell Counts From HIV-1–Uninfected Individuals in the US NHANES.

	1999-2000	2001-2002	2003-2004	2005-2006	1999-2006
No. of subjects[a]	24	10	33	25	92
$CD4^+$ cells/μL					
Mean (SD)	1034.0 (336.89)	834.3 (252.49)	983.9 (396.79)	909.8 (392.16)	960.5 (367.52)
Median (IQR)	994 (787-1298)	861 (587-1073)	922 (666-1198)	870 (588-1082)	904 (686-1126)
Range	273-1640	457-1176	349-1911	412-1859	273-1911

Abbreviations: IQR, interquartile range; NHANES, US National Health and Nutrition Examination Survey.
[a]Data are $CD4^+$ counts (cells/μL) from randomly selected HIV-uninfected individuals represented in the NHANES[33] in the indicated years. Age range is 18-49 years.

eTable 6. Summary of $CD4^+$ T-Cell Counts in Presumed or Confirmed HIV-1–Uninfected Participants.

Source	No. of Individuals	Mean (95% CI)[a]	Median (IQR)	Range
$CD4^+$ counts				
Literature review[b]	16,226	1015 (1009-1020)	988 (840-1036)	771-1109
NHANES[c]	92	961 (885-1036)	904 (686-1126)	273-1911
HNRC[d]	875	968 (947-990)	922 (741-1145)	189-2464

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; HNRC, University of California San Diego HIV Neurobehavioral Research Center[34]; NHANES, US National Health and Nutrition Examination Survey.[33]
[a]Weighted mean and 95% CI calculated from study reports.
[b]Data summarized from eTable 4 from literature review.
[c]Data summarized from eTable 5.
[d]Data of $CD4^+$ T-cell counts from 875 HIV-uninfected participants from HNRC.

eTable 7. Demographic Characteristics of 13 HIV-1–Uninfected Healthy Blood Donors Studied for Immune Markers.	
Variables	
Age, Mean (SD), y	32.3 (6.6)
Male, No. (%)	10 (76.9)
Ethnicity, No. (%)	
European American	5 (38.5)
Hispanic American	7 (53.8)
Asian American	1 (7.7)

eTable 8. Hazard Ratio of Developing AIDS (1987 and 1993 CDC Criteria) During the Study Period.

Model	AIDS (1987 Criteria)		AIDS (1993 Criteria)[a]	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Univariate				
Sex, female vs male	0.77 (0.19-3.15)	.71	1.01 (0.44-2.29)	.99
Ethnicity				
African American vs European American	0.95 (0.56-1.61)	.85	0.99 (0.69-1.43)	.96
Other vs European American	0.39 (0.12-1.27)	.12	0.69 (0.36-1.31)	.26
Age at ART initiation, each increase of 1 y	1.00 (0.96-1.04)	.95	0.99 (0.97-1.02)	.49
Time from EDS to ART initiation, ≤12 vs >12 mo.	0.46 (0.22-0.97)	.04	0.48 (0.30-0.78)	.003
Time from study entry to ART initiation, ≤12 vs >12 mo.	0.46 (0.27-0.78)	.004	0.72 (0.51-1.02)	.06
Conjointly time-indexed categories[b]				
Earlier/ Earlier vs Later/Later	0.14 (0.04-0.45)	.001	0.15 (0.07-0.29)	<.001
Later/Earlier vs Later/Later	0.37 (0.18-0.79)	.01	0.19 (0.10-0.35)	<.001
Earlier/Earlier vs Later/Earlier	0.38 (0.10-1.43)	.15	0.77 (0.32-1.86)	.56
Study entry $CD4^+$, ≥500 vs <500 cells/μL	0.86 (0.51-1.45)	.56	0.67 (0.47-0.96)	.03
Pre-ART $CD4^+$, ≥500 vs <500 cells/μL	0.30 (0.11-0.84)	.02	0.30 (0.16-0.56)	<.001
Pre-ART VL, each increase of 1 $\log_{10}$ copies/mL	1.21 (0.70-2.08)	.50	1.11 (0.77-1.61)	.56
Calendar year of ART initiation, each increase of 1 year	0.82 (0.76-0.88)	<.001	0.80 (0.76-0.84)	<.001
Antiretroviral regimens[c]				
PI-based vs NNRTI-based	2.64 (0.70-10.00)	.15	1.30 (0.54-3.13)	.56
Other vs NNRTI-based	7.13 (2.20-23.09)	.001	6.81 (3.44-13.47)	<.001
Other vs PI-based	2.70 (1.27-5.72)	.01	5.25 (2.82-9.77)	<.001
Length of follow-up from ART initiation, each increase of 1 y	1.16 (1.03-1.31)	.01	1.28 (1.19-1.38)	<.001
Time from ART initiation to VL suppression, each increase of 1 mo	1.02 (1.01-1.02)	<.001	1.02 (1.01-1.02)	<.001
Multivariate				
Earlier/Earlier vs Later/Later	0.41 (0.18-0.93)	.03	0.20 (0.10-0.42)	<.001
Later/Earlier vs Later/Later	0.47 (0.24-0.91)	.02	0.49 (0.29-0.84)	.01

Abbreviations: ART, antiretroviral therapy; EDS, estimated date of seroconversion; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; VL, plasma HIV RNA viral load.

[a] A total of 956 participants were analyzed, as those who had a pre-ART $CD4^+$ <200 cells/μL were excluded from these analyses.

[b] The conjointly time-indexed categories were designated as earlier/earlier, indicating participants who commenced ART ≤12 months from both EDS and entry; later/later, reflecting those initiating ART >12 months from both EDS and entry, and later/earlier, indicating those starting ART >12 months from EDS but ≤12 months from entry.

[c] PI–based was defined as PI therapy without any NNRTI; NNRTI–based was defined as NNRTI therapy without any PI; and the other treatment options were a combination of PI and NNRTI therapy or neither of these drug classes) The rate ratio (RR) and *P* value were calculated with the use of a Cox proportional-hazards model. The multivariate model was adjusted for entry $CD4^+$ counts of ≥500 or <500 cells/μL, pre-ART $CD4^+$ of ≥500 or <500 cells/μL, calendar year of ART initiation, ART regimen, duration of VL-suppressive ART in years, and time from ART initiation to VL suppression.

eTable 9. First Diagnosed AIDS (1987 and 1993 CDC Criteria) Event Among 1119 Participants During the Study Period.

AIDS-Defining Illness	AIDS (1987 Criteria)[a]				AIDS (1993 Criteria)[a,b]			
	Overall	$CD4^+$ Normalization			Overall	$CD4^+$ Normalization		
		≥900	500-899	<500		≥900	500-899	<500
Pneumocystis carinii/jirovecii pneumonia	17 (29.3)	0	7	10	7 (5.5)	0	3	4
Kaposi sarcoma	9 (15.5)	0	5	4	3 (2.3)	0	2	1
Wasting syndrome attributed to HIV	8 (13.8)	1	5	2	3 (2.3)	0	3	0
Non-Hodgkin lymphoma	7 (12.1)	1	2	4	1 (0.8)	1	0	0
AIDS dementia complex	4 (6.9)	1	2	1	2 (1.6)	0	1	1
Candidiasis	3 (5.2)	0	0	3	1 (0.8)	0	0	1
Cryptosporidiosis	3 (5.2)	2	1	0	2 (1.6)	1	1	0
Cytomegalovirus disease	3 (5.2)	1	0	2	2 (1.6)	1	0	1
Cryptococcosis	1 (1.7)	0	1	0	0 (0.0)	0	0	0
Histoplasmosis	1 (1.7)	0	1	0	0 (0.0)	0	0	0
Progressive multifocal leuko-encephalopathy	1 (1.7)	0	0	1	0 (0.0)	0	0	0
Mycobacterium avium intracellulare	1 (1.7)	0	1	0	0 (0.0)	0	0	0
Tuberculosis	0 (0.0)	0	0	0	2 (1.6)	1	1	0
AIDS $CD4^+$ <200 or $\%CD4^+$ <14	0 (0.0)	0	0	0	104 (81.9)	8	55	41
Total	58	6	25	27	127	12	66	49

Abbreviations: ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; VL, plasma HIV RNA viral load. [a] Data shown represents the number of participants (%). [b] A total of 956 participants were analyzed as those who had a pre-ART $CD4^+$ <200 cells/μL were excluded from these analyses.

eTable 10. Likelihood of Achieving HBV Vaccine Response.

Model	Received HBV Vaccinations while being therapy-naïve (n = 261)		Received HBV Vaccination during VL-suppressive ART (n = 113)	
Univariate	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
Sex, female vs male	1.06 (0.44-2.57)	.90	0.91 (0.19-4.27)	.90
Ethnicity				
African American vs European American	1.25 (0.72-2.18)	.43	0.89 (0.40-1.99)	.78
Other vs European American	1.81 (0.91-3.62)	.09	0.63 (0.17-2.41)	.50
Age at entry	1.00 (0.96-1.04)	.91	0.98 (0.93-1.04)	.52
Age at ART initiation, each increase of 1 y	–		0.97 (0.93-1.03)	.31
Age at last HBV vaccination	0.98 (0.94-1.02)	.25	0.98 (0.93-1.02)	.24
Time from study entry to ART initiation, ≤12 vs >12 months	–		1.69 (0.52-5.43)	.28
Time from ART to last HBV vaccination, each increase of 1 mo	–		1.00 (0.99-1.00)	.56
Study entry $CD4^+$, ≥500 vs <500 cells/μL	1.45 (0.87-2.42)	.16	1.31 (0.56-3.04)	.53
Pre-ART $CD4^+$, ≥500 vs <500 cells/μL	–		1.56 (0.62-3.95)	.35
$CD4^+$ at last HBV vaccination, ≥500 vs <500 cells/μL	2.01 (1.21-3.34)	.007	4.32 (1.67-11.19)	.003
Study entry VL, each increase of 1 $\log_{10}$ copies/mL	0.60 (0.44-0.82)	.002	0.91 (0.46-1.80)	.79
Pre-ART VL, each increase of 1 $\log_{10}$ copies/mL	–		1.06 (0.60-1.92)	.82
VL at last HBV vaccination, each increase of 1 $\log_{10}$ copies/mL	0.61 (0.44-0.86)	.004	–	
Calendar year of ART initiation, each increase of 1 y	–		1.05 (0.96-1.14)	.33
Time from EDS to last HBV vaccine administered, ≤12 mo vs >12 mo	2.63 (1.21-4.62)	.01	—	—
Time from EDS to ART initiation, ≤12 mo vs >12 mo	—	—	2.04 (0.95-4.38)	.07
Multivariate				
Model 1: Time from EDS to last HBV vaccine administered, ≤12 mo vs >12 mo	2.75 (1.15-6.59)	.02	—	—
Model 2: Time from EDS to ART initiation, ≤12 mo vs >12 mo	—	—	2.02 (0.91-4.48)	.08

Abbreviations: ART, antiretroviral therapy; EDS, estimated date of seroconversion; HBV, hepatitis B virus; OR, odds ratio; VL, plasma HIV RNA viral load.

Logistic regression modeling was used to estimate the OR. In the multivariate models, the covariates adjusted were as follows:

Model 1: Adjusted for ethnicity, $CD4^+$ (≥500 vs <500 cells/μL) at entry and at last HBV vaccination, VL $\log_{10}$ copies/mL at entry and last HBV vaccination.

Model 2: Adjusted for $CD4^+$ at last HBV vaccination (≥500 vs <500 cells/μL).

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