Relationship of immunologic response to antiretroviral therapy with non-AIDS defining cancer incidence

Elizabeth L. Yanik[a], Sonia Napravnik[a], Stephen R. Cole[a], Chad J. Achenbach[b], Satish Gopal[a], Dirk P. Dittmer[a], Andrew F. Olshan[a], Mari M. Kitahata[c], Michael J. Mugavero[d], Michael Saag[d], Richard D. Moore[e], W. Christopher Mathews[f], Peter Hunt[g] and Joseph J. Eron[a]

Objective: To estimate the association between immunologic response to antiretroviral therapy (ART) and non-AIDS defining cancer (NADC) incidence in HIV-infected patients.

Design: A prospective cohort including patients with at least 1 cell/μl $CD4^+$ cell count and HIV-1 RNA measure after ART initiation between 1996 and 2011 in the Centers for AIDS Research Network of Integrated Clinical Systems, a collaboration of eight HIV clinics at major academic medical centres in the United States.

Methods: Measures of immunologic response were 6-month $CD4^+$ post-ART, latest $CD4^+$ and $CD4^+$ count-years, a cumulative measure of $CD4^+$ lymphopenia. Cox regression with inverse probability-of-exposure weights was used to calculate adjusted hazard ratios of virus-related and virus-unrelated NADC incidence.

Results: Among 9389 patients at ART initiation, median $CD4^+$ cell count was 200 cells/μl [interquartile range (IQR) 60–332)], and median HIV-1 RNA was 4.8 $\log_{10}$copies/ml (IQR 4.3–5.4). Median follow-up was 3.3 years (IQR 1.5–6.5). After 6 months of ART, median $CD4^+$ cell count was 304 cells/μl (IQR 163–469). One hundred and sixty-four NADCs were diagnosed during study follow-up, 65 (40%) considered virus-related. Virus-related NADCs were inversely associated with 6-month $CD4^+$ cell count (hazard ratio per 100 cells/μl increase = 0.71), latest $CD4^+$ cell count (hazard ratio per 100 cells/μl increase = 0.70) and $CD4^+$ cell count-years (hazard ratio per 200 cell-years/μl increase = 0.91) independent of $CD4^+$ cell count at ART initiation, age and HIV-1 RNA response. No associations were found with virus-unrelated NADCs.

Conclusion: Poor $CD4^+$ cell count response was strongly associated with virus-related NADC incidence, suggesting an important role for T-cell mediated immunity in pathogenesis. Lower $CD4^+$ cell count proximal to cancer diagnosis may be a result of subclinical cancer. Intensified cancer screening should be considered for patients on ART with low $CD4^+$ cell counts.

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[a]University of North Carolina, Chapel Hill, North Carolina, [b]Northwestern University, Chicago, Illinois, [c]University of Washington, Seattle, Washington, [d]University of Alabama, Birmingham, Alabama, [e]Johns Hopkins University, Baltimore, Maryland, [f]University of California, San Diego, and [g]University of California, San Francisco, California, USA.

Correspondence to Elizabeth L. Yanik, 9609 Medical Center Dr Rm. 6E-216, Rockville, MD 20850, USA.

Tel: +1 240 276 7186; e-mail: elizabeth.yanik@nih.gov

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Introduction

Among HIV-infected individuals, the burden of non-AIDS-defining cancers (NADCs) is increasing [1], with malignancies such as lung cancer, anal cancer and Hodgkin lymphoma contributing to substantial morbidity and mortality [1–3]. This is largely due to ageing of the HIV population [4,5] and a high prevalence of risk behaviours such as tobacco use, alcohol use and sexual behaviours [6]. However, HIV infection and the resultant immune suppression may also increase cancer risk [7,8]. More severe immunosuppression, as quantified through nadir $CD4^+$ cell count and current $CD4^+$ cell count, is associated with greater incidence of several NADCs [7,9–11]. Effective antiretroviral therapy (ART) would be expected to reduce NADC risk, but prior studies have not found consistent associations between ART use and lower NADC incidence [4,12–14]. These inconsistencies may partly be due to differences in the effectiveness of ART between patients. Immunologic response to ART, as measured by $CD4^+$ cell counts, is likely a major mediator of ART effects on NADC incidence.

Although patterns of cancer incidence differ over time after ART initiation [15,16], the reasons for these patterns are not well understood, including the impact of immunologic ART response. We evaluated the relationship between immunologic ART response and NADC incidence among HIV-infected patients initiating a first ART regimen in the Center for AIDS Research Network of Integrated Clinical Systems (CNICS) between 1996 and 2011.

Materials and methods

Study population

CNICS is a network of eight US HIV clinical cohorts that collects data from HIV-infected patients 18 years of age or older through electronic medical records [17]. CNICS includes detailed information on ART, laboratory measures, demographics and diagnoses, including cancer diagnoses, which have been ascertained and verified through a standardized data collection process [18]. Each CNICS site obtained local institutional review board approval.

We included patients who initiated a first ART regimen, defined as at least three different antiretrovirals, at one of the CNICS sites between 1 January 1996 and 30 August 2011. Among these patients, we included those who had a $CD4^+$ cell count and HIV-1 RNA measure within 12 months prior to ART initiation; were alive for more than 6 months post-ART initiation; and had at least 1 cell/μl $CD4^+$ cell count and HIV-1 RNA measure within the first 6 months post-ART initiation.

Measures of immunologic antiretroviral therapy response

Several measures were used to characterize immunologic ART response on the basis of $CD4^+$ cell counts obtained as a part of routine clinical care. Six-month $CD4^+$ cell count, a measure of early immunologic response, was defined as the latest $CD4^+$ cell count measurement taken within the first 6 months after ART initiation. Latest $CD4^+$ cell count, a time-varying measure of immunologic response, was updated whenever a patient had a new $CD4^+$ cell count result. Finally, $CD4^+$ cell count-years, a time-varying measure of cumulative immunologic response, takes into account both the magnitude and duration of immunologic response using the trapezoidal rule to estimate the area under the curve across multiple $CD4^+$ cell count measurements. Specifically, the accumulation of $CD4^+$ cell count-years is calculated by multiplying the average of two consecutive $CD4^+$ cell counts by the time interval between the two counts and then summing the values across all intervals between counts. Similar methods have been used to calculate cumulative HIV viremia [19,20]. As an example, a patient with a $CD4^+$ cell count of 300 cells/μl for the first year after ART initiation, and a patient with a $CD4^+$ cell count of 200 cells/μl for the first 6 months and a $CD4^+$ cell count of 400 cells/μl for the second 6 months, would both have accumulated 300 cells/μl $CD4^+$ cell count-years 1 year after ART initiation.

All immunologic measures were considered continuously and categorically to identify the most accurate parameterization with relation to NADC incidence. Because immunologic measures proximal to cancer diagnoses may be more likely to be affected by subclinical cancer, analyses were done with 6-month (and 12-month) exposure lags in which immunologic measures were used to predict NADC diagnoses that occurred more than 6 (and 12) months after the immunologic ART response measurement.

Statistical analysis

At-risk time for NADC incidence started after the first 6 months of ART to avoid the inclusion of cancers that developed before ART initiation and to allow time for at least one $CD4^+$ cell count to be obtained after ART initiation. For analyses with 6-month and 12-month exposure lags, at-risk time started at 12 and 18 months, respectively, to allow a minimum of 6 months to assess immunologic measures. Patients remained in follow-up irrespective of ART changes or interruptions until the first of NADC diagnosis, death, loss-to-follow-up (>12 months without a clinic visit), last date of cancer ascertainment for each CNICS site (range: 31 May 2010–31 August 2011) and administrative censoring 10 years after ART initiation.

Cox proportional hazards regression was used to estimate hazard ratios and 95% confidence intervals (95% CIs) as

measures of association and precision, respectively. Immunologic response associations were considered with incidence of all NADCs [21], NADCs known to be related to viral coinfections [human papillomavirus (HPV): anal, squamous cell oral cavity/pharynx, penis, vagina/vulva; Epstein-Barr virus (EBV): Hodgkin lymphoma; hepatitis B virus (HBV) and hepatitis C virus (HCV): liver cancer] [18,22] and virus-unrelated NADCs. Multivariable Cox regression was used to adjust for time-fixed confounders measured at ART initiation, including $CD4^+$ cell count, age, prior antiretroviral use, calendar year, race, sex, transmission risk and CNICS site, with inverse-probability weights used to further adjust for time-varying plasma HIV-1 RNA level.

Inverse-probability weights were applied to account for time-varying confounding introduced by the virologic response occurring before the immunologic response [23–25]. These weights were calculated using linear regression models to estimate the probability density of the observed $CD4^+$ cell counts for each patient with covariates for the HIV-1 RNA measurements from the prior two clinical visits. Application of these weights in the multivariable Cox regression models allows estimation of effects of immunologic response independent of the prior virologic response. In addition, inverse-probability-of-censoring weights were applied to account for differential censoring by prior HIV-1 RNA measurements, and inverse-frequency weights were applied to account for differences in the frequency of obtaining $CD4^+$ cell count tests [24–26]. For each observation, all calculated weights were multiplied together to create a single weight that was used in the regression models (mean total weight = 1.02, SD = 0.79). As 6-month $CD4^+$ cell count was a time-fixed measure not subject to time-varying confounding, total weights for this measure did not incorporate inverse-probability weights to account for time-varying virologic response (mean = 1.00, SD = 0.17), but models were adjusted for the HIV RNA measure prior to the 6-month $CD4^+$ cell count.

All immunologic measures were assessed separately as predictors in bivariable and weighted, multivariable regression models. Associations were also estimated among patients with a $CD4^+$ cell count at ART initiation of less than 200 cells/μl and at least 200 cells/μl to assess effect measure modification. To determine which immunologic measure was the strongest independent predictor, Akaike's information criterions (AICs) were calculated for each multivariable regression model and compared to assess the relative model fit. The lowest AIC represents the model with the best fit to the data indicating the most predictive $CD4^+$ response measure.

Sensitivity analyses were conducted among patients without NADC diagnoses prior to 6 months of ART and among patients with no prior exposure to single or dual ART. All statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Patient characteristics

Of the 25 337 patients enrolled in CNICS at the time of this study, 11 485 initiated a first ART regimen at a CNICS site between 1996 and 2011 and had $CD4^+$ cell count and HIV RNA measures within 12 months prior to ART initiation. Among these patients, 9389 were alive and had obtained at least one HIV RNA level and $CD4^+$ cell count at 6 months after ART initiation.

The 9389 patients included were representative of the entire CNICS population: 20% were women, 43% white and 41% black (Table 1). The median age at ART initiation was 38 years [interquartile range (IQR) 32–45)], and the median calendar year of ART initiation was 2004 (IQR 2000–2007). Most patients initiated a protease inhibitor-based (47%) or nonnucleoside reverse transcriptase inhibitor (NNRTI)-based (43%) ART regimen, whereas a minority initiated a regimen with both a protease

Table 1. Demographic and clinical characteristics of 9389 patients at combination antiretroviral therapy initiation in the CFAR Network of Integrated Clinical Systems, 1996–2011.

Characteristic	*N* (%)
Total	9389
Female sex	1900 (20.2)
Age (years)[a]	38 (32–45)
Race	
White	4032 (43.2)
Black	3814 (40.9)
Hispanic	1065 (11.4)
Other/unknown	478 (5.1)
Injection drug user	1688 (18.0)
MSM	5192 (55.3)
Antiretroviral exposure prior to first ART	2513 (26.8)
ART initiation year[a]	2004 (2000–2007)
ART regimen type	
PI	4415 (47.0)
NNRTI	4025 (42.9)
3 + NRTI	458 (4.9)
NNRTI + PI	351 (3.7)
Other[b]	140 (1.5)
HBV infection prior to ART initiation	1894 (20.1)
HCV infection prior to ART initiation	1562 (16.5)
HIV RNA at ART initiation (log_{10}copies/ml)[a]	4.8 (4.3–5.4)
HIV RNA suppression within 6 months of ART initiation[c]	6009 (64.0)
$CD4^+$ cell count at ART initiation (cells/μl)[a]	200 (60–332)

ART, combination antiretroviral therapy; HBV, hepatitis B virus; HCV, hepatitis C virus; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
[a]Median (IQR) given instead of *N* (%).
[b]Regimens with an integrase inhibitor, fusion inhibitor or entry inhibitor.
[c]HIV RNA suppression defined as achieving <400 copies/ml.

inhibitor and an NNRTI (4%), a triple-nucleoside reverse transcriptase inhibitor regimen (5%) or a regimen including an entry or integrase inhibitor (2%). At first combination ART initiation, 27% of patients had evidence of prior antiretroviral exposure, including single or dual ART use. The median HIV-1 RNA level at ART initiation was 4.8 $\log_{10}$ copies/ml. Within 6 months of ART initiation, 64% of patients were virologically suppressed (<400 copies/ml).

After 6 months of ART, patients were followed for a median of 3.3 years (IQR 1.5–6.5) with a total of 41 538 person-years of follow-up. Over the course of follow-up, 692 deaths occurred and 3156 patients were lost-to-follow-up. Follow-up was censored for 46% of patients at the last date of cancer ascertainment, and 1104 patients were censored at 10 years post-ART initiation.

Immunologic response

The median $CD4^+$ cell count at ART initiation was 200 cells/μl (IQR 60–332). After ART initiation, patients contributed a median of nine $CD4^+$ cell count measurements (IQR 4–17). The median $CD4^+$ cell count was 260 cells/μl (IQR 121–419) during the first 6 months of ART, 324 cells/μl (IQR 182–504) in the second 6 months and 358 cells/μl (IQR 213–539) in months 12–18 of ART. More than 2 years after ART initiation, median $CD4^+$ cell count values only increased slightly (Fig. 1). Patients accumulated a median of 1925 cells*years/μl (IQR 1239–2686) by the 54–60 month time interval after ART initiation equivalent to having an average $CD4^+$ cell count of 385 cells/μl over 5 years (Fig. 1). By 10 years after ART initiation, the median $CD4^+$ cell count-years accumulated was 4572 cells*years/μl (IQR 3144–6152) equivalent to having an average $CD4^+$ cell count of 457 cells/μl over 10 years.

Immunologic response and non-AIDS defining cancer incidence

In total, 164 NADCs were diagnosed at a median of 3.8 years after ART initiation (IQR 2.0–6.8) for a total incidence rate of 395 per 100 000 person-years. Sixty-five NADCs were categorized as virus-related with the most frequent being anal cancer ($N=26$). Ninety-nine NADCs were virus-unrelated with the most frequent being lung cancer ($N=22$) (Table 2).

Higher values for six-month $CD4^+$ cell count, latest $CD4^+$ cell count and $CD4^+$ cell count-years were all associated with lower NADC incidence (Table 3). When examined by type of NADC, all immunologic measures were strongly and inversely associated with virus-related NADCs, even after adjustment for $CD4^+$ cell count at ART initiation, prior HIV-1 RNA measures, age and other demographic and clinical variables. In weighted multivariable regression, a 100 cells/μl increase in a patient's 6-month $CD4^+$ cell count was associated with a 29% lower hazard of virus-related NADC incidence (95% CI 5–47) (Table 3). Similarly, a 100 cells/μl increase in the latest $CD4^+$ cell count was associated with a 30% lower hazard of virus-related NADC incidence (95% CI 7–48). Finally, a 200 cells*years/μl increase in $CD4^+$ cell count-years was associated with a 9% lower hazard of virus-related NADC incidence (95% CI 3–16). The $CD4^+$ cell count-years association with virus-related

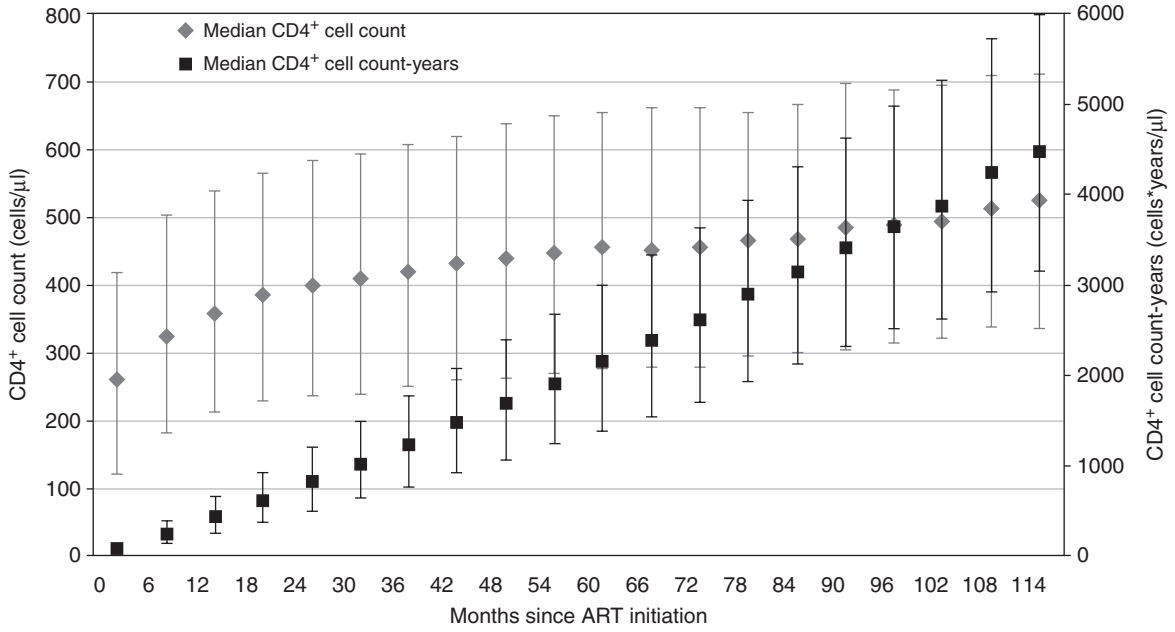

Fig. 1. Distribution of latest $CD4^+$ cell counts and $CD4^+$ cell count-years by 6-month time intervals over the first 10 years after combination antiretroviral therapy initiation. Lines, interquartile range; Symbol, median value.

Table 2. Non-AIDS defining cancer diagnoses occurring more than 6 months after combination antiretroviral therapy initiation in the Center for AIDS Research Network of Integrated Clinical Systems, 1996–2011.

Type of NADC	Number of diagnoses	Median time from ART initiation to cancer diagnosis in years (interquartile range)
Total	164	3.8 (2.0–6.8)
Virus-related	65	3.2 (1.9–5.8)
Squamous cell anal	25	3.8 (2.2–6.3)
Hodgkin lymphoma	16	2.6 (1.9–3.9)
Liver	13	3.7 (1.6–7.2)
Squamous cell oral cavity/pharynx	8	3.3 (1.3–6.3)
Other[a]	3	4.0 (2.5–7.7)
Virus-unrelated	99	3.6 (1.4–6.7)
Lung	21	3.6 (1.0–7.0)
Prostate	17	4.3 (0.8–6.0)
Breast	10	5.2 (3.5–6.5)
Melanoma	9	1.9 (1.4–4.0)
Colorectal	7	2.7 (2.1–6.3)
Other[b]	35	3.0 (1.7–6.7)

ART, combination antiretroviral therapy; NADC, non-AIDS defining cancer. As follow-up started at 6 months after ART initiation, all included cancer diagnoses occurred at least 0.5 years after ART initiation.
[a]Other virus-related cancers include penis, vaginal and vulva.
[b]Other virus-unrelated cancers include bladder, oesophagus, kidney, larynx, leukemia, multiple myeloma, ovary, pancreas, peritoneum, small intestine, soft tissue, testicular, thyroid uterus or nonsquamous cell oral cavity/pharynx.

NADC incidence appeared stronger among patients with a $CD4^+$ cell count at ART initiation of less than 200 cells/μl (adjusted hazard ratio 0.89, 95% CI 0.82–0.98) than among patients with a $CD4^+$ cell count of at least 200 cells/μl (adjusted hazard ratio 0.94, 95% CI 0.81–1.08). When included in these models, $CD4^+$ cell count at ART initiation was inversely, but weakly associated with virus-related NADC incidence (adjusted hazard ratio in model with latest $CD4^+$ cell count 0.90, 95% CI 0.75–1.08).

All measures were assessed with 6-month and 12-month exposure lags. Latest $CD4^+$ cell count was less strongly associated with virus-related NADCs that occurred more than 6 months after the $CD4^+$ cell count measurement (adjusted hazard ratio with 6-month exposure lag 0.85,

Table 3. Associations of measures of immunologic response to combination antiretroviral therapy with non-AIDS defining cancer incidence.

	Bivariable[a]	Weighted, multivariable[b]		
	No lag	No lag	6-month lag	12-month lag
Measures of immunologic ART response		Hazard ratio (95% CI)		
All NADCs		(*N* = 164)	(*N* = 140)	(*N* = 125)
Six-month $CD4^+$ cell count (per 100 cells/μl)	0.90 (0.83–0.97)	0.83 (0.72–0.96)	0.85 (0.73–0.98)	0.82 (0.70–0.97)
Latest $CD4^+$ cell count (per 100 cells/μl)	0.87 (0.81–0.93)	0.90 (0.76–1.07)	0.90 (0.83–0.97)	0.92 (0.84, 1.02)
$CD4^+$ cell count-years (per 200 cells∗years/μl)	0.97 (0.94–1.00)	0.99 (0.95–1.04)	0.97 (0.92–1.01)	0.97 (0.92, 1.02)
Virus-related NADCs[c]		(*N* = 65)	(*N* = 59)	(*N* = 52)
Six-month $CD4^+$ cell count (per 100 cells/μl)	0.75 (0.65–0.87)	0.71 (0.53–0.95)	0.69 (0.51–0.93)	0.69 (0.50–0.96)
Latest $CD4^+$ cell count (per 100 cells/μl)	0.78 (0.70–0.87)	0.70 (0.55–0.89)	0.85 (0.75–0.95)	0.85 (0.74–0.96)
$CD4^+$ cell count-years (per 200 cells∗years/μl)	0.89 (0.84–0.95)	0.92 (0.85–0.99)	0.87 (0.79–0.96)	0.89 (0.80–0.98)
Virus-unrelated NADCs[d]		(*N* = 99)	(*N* = 81)	(*N* = 73)
Six-month $CD4^+$ cell count (per 100 cells/μl)	0.98 (0.89–1.07)	0.90 (0.77–1.04)	0.94 (0.81–1.09)	0.91 (0.77–1.06)
Latest $CD4^+$ cell count (per 100 cells/μl)	0.93 (0.85–1.00)	1.00 (0.86–1.16)	0.93 (0.83–1.03)	0.98 (0.85–1.12)
$CD4^+$ cell count-years (per 200 cells∗years/μl)	1.01 (0.97–1.04)	1.02 (0.98–1.06)	1.00 (0.96–1.05)	1.00 (0.95–1.06)

Each association with an immunologic ART response measure was estimated using a separate regression model.ART, combination antiretroviral therapy; CI, confidence interval; NADC, non-AIDS defining cancer.
[a]Bivariable regression models only included the immunologic ART response measure, without weighting or adjustment for other covariates.
[b]For latest $CD4^+$ cell count and $CD4^+$ cell count-years, weights were applied to account for confounding from HIV RNA measurements from the prior two visits, differential censoring by prior HIV RNA measurements and differential frequency of $CD4^+$ cell count measurements. The mean total weight was 1.02 (SD = 0.79). For 6-month $CD4^+$ cell count, weights were applied to account for differential censoring and differential frequency of $CD4^+$ cell count measurements (mean = 1.00, SD = 0.17). Multivariable analyses additionally adjusted for $CD4^+$ cell count at ART initiation, HIV RNA at ART initiation, prior antiretroviral use, age at ART initiation, year of ART initiation, sex/MSM, IDU, race, CNICS study site.
[c]Virus-related NADCs included squamous cell anal, Hodgkin lymphoma, liver, squamous cell oral cavity/pharynx, penis, vagina and vulva cancer.
[d]Virus-unrelated NADCs included lung, prostate, breast, colorectal, melanoma, kidney, bladder, esophagus, kidney, larynx, leukemia, multiple myeloma, ovary, pancreas, peritoneum, small intestine, soft tissue, testicular, thyroid uterus or nonsquamous cell oral cavity/pharynx.

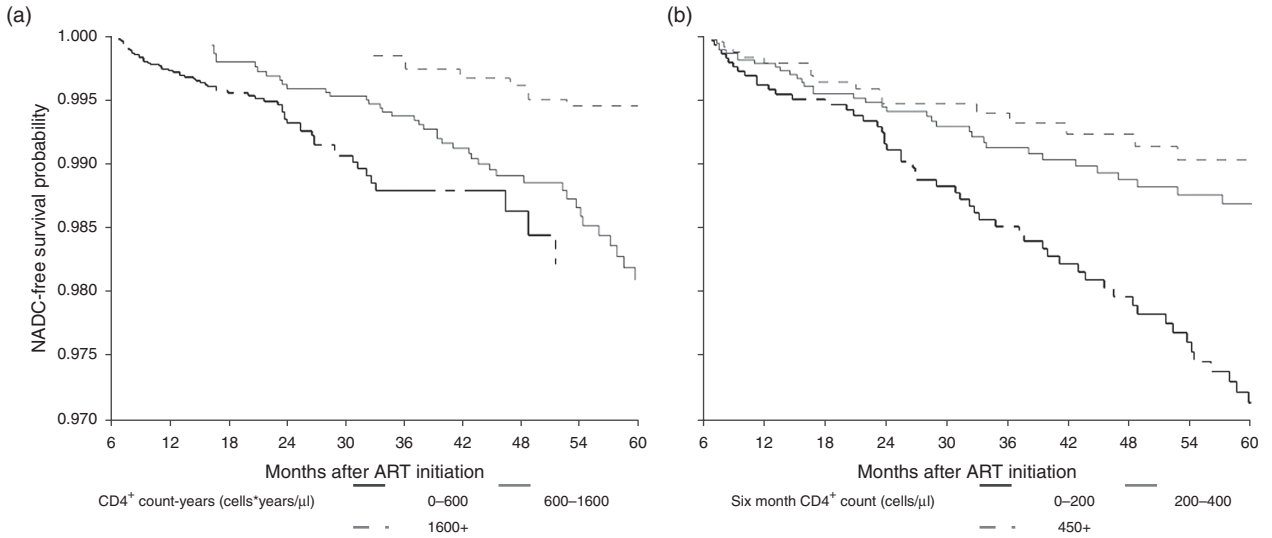

Fig. 2. Time to first virus-related non-AIDS defining cancer diagnosis from 6 months to 5 years after antiretroviral therapy initiation by CD4$^+$ cell count-years category (a) and 6-month CD4$^+$ cell count category (b). Survival probabilities were calculated using the Kaplan–Meier method, with death, loss-to-follow-up and end of cancer ascertainment as censoring events. For CD4$^+$ cell count-years, no patients had accumulated more than 600 CD4$^+$ cell count-years by 6 months post-ART. At the time at which patients accumulated enough CD4$^+$ cell count-years to qualify for a higher CD4$^+$ cell count-years category, they were censored from the lower category and included in the higher CD4$^+$ cell count-year category risk set as late entries. The first patients accumulated more than 600 CD4$^+$ cell count-years at 16 months post-ART (start of solid gray line), and the first patients accumulated more than 1000 CD4$^+$ cell count-years at 33 months (start of dotted gray line).

95% CI 0.75–0.95; Table 3) while other CD4$^+$ cell response measure associations did not change appreciably.

When AICs from separate models for each of the three immunologic response measures were compared, the AIC was lowest for the latest CD4$^+$ cell count model both in bivariable and multivariable analyses (AICs: latest CD4$^+$ cell count = 1070.6, six-month CD4$^+$ cell count = 1075.0, CD4$^+$ cell count-years = 1076.8), indicating that this was the strongest predictor of virus-related NADC incidence. However, after 6-month and 12-month exposure lags were implemented, AICs were lowest for the model using the 6-month post-ART CD4$^+$ cell count (6-month lag AICs: latest CD4$^+$ cell count = 975.8, 6-month CD4$^+$ cell count = 972.6, CD4$^+$ cell count-years = 974.0), indicating that this measure was the strongest predictor of virus-related NADCs occurring at least 6 months after measurement.

When immunologic measures were categorized, associations indicated that virus-related NADC incidence decreased further with each increasing category of improved immunologic response. For example, when compared with the reference category of CD4$^+$ cell count-years of less than 600 cells*years/μl, CD4$^+$ cell count-years between 600 and 1600 cells*years/μl had a hazard ratio of 0.83 (95% CI 0.41–1.65) and CD4$^+$ cell count-years of more than 1600 cells*years/μl had a hazard ratio of 0.30 (95% CI 0.10–0.85) for virus-related NADCs (Fig. 2). Similar findings were observed for latest CD4$^+$ cell count and 6-month CD4$^+$ cell count categories.

No immunologic measures were associated with virus-unrelated cancer incidence in any analyses despite improved precision due to a larger number of cancer events (Table 3).

Similar associations were observed in sensitivity analyses in which only the 6876 antiretroviral-naive patients were included. For instance, among this subgroup, a 100 cells/μl increase in a patient's 6-month CD4$^+$ cell count was associated with a 27% lower hazard of virus-related NADC incidence (95% CI 11–41) in weighted, multivariable analyses. Results were also similar in analyses in which 382 patients were excluded with NADC diagnoses prior to 6 month post-ART initiation. Of these, 382 patients were excluded due to prior NADC diagnoses, 10 had another cancer diagnosis after 6 months of ART.

Discussion

In this study, a greater CD4$^+$ cell count ART response was associated with lower NADC incidence, specifically for NADCs related to viral coinfections (HPV, EBV, HBV or HCV). This association was independent of CD4$^+$ cell count at ART initiation, HIV-1 RNA response, age and

other patient factors, and was consistently observed for measures of early, time-varying and cumulative time-varying immunologic ART response. Inverse associations were specific to virus-related NADCs. No associations were observed for virus-unrelated NADCs. As we did not censor patients with ART interruptions or switches, our measures of immunologic response are influenced by the clinical realities of ART failure or toxicity, consistency of ART drug supply and patient adherence.

$CD4^+$ cell count-years were used as a novel measure to capture the degree and duration of immune response. This may be particularly relevant to virus-related NADCs, as longer periods of immunosuppression may provide greater susceptibility to acquisition, reactivation or persistence of oncogenic viruses [27–33]. Cumulative immunologic ART response was most protective among patients with a $CD4^+$ cell count of less than 200 cells/μl at ART initiation highlighting the importance of prompt initiation of effective ART as a cancer prevention strategy for severely immunosuppressed patients. However, in our analysis, $CD4^+$ cell count-years was not a stronger predictor of NADC incidence than latest $CD4^+$ cell count or 6-month $CD4^+$ cell count, indicating that this more complex measure may not be of clinical importance for NADCs.

Latest $CD4^+$ cell count was the strongest predictor of virus-related NADC incidence. This may reflect immunosuppression and greater oncogenic potential of viral coinfections facilitating the transition to cancer. Conversely, greater $CD4^+$ cell count declines proximal to cancer diagnosis may be a marker of immune dysregulation caused by subclinical cancer. In particular, Hodgkin lymphoma is known to decrease T-cell populations in HIV-infected and HIV-uninfected populations [32,34–36]. Regardless, declines in a patient's most recent $CD4^+$ cell count could be an important indicator to identify HIV-infected individuals for intensified cancer screening strategies.

Although subclinical cancer may alter the latest $CD4^+$ cell count before diagnosis, it is less likely that subclinical cancer is present for $CD4^+$ cell count measurements taken well before cancer diagnosis. When cancer diagnoses were excluded that occurred less than 6 and 12 months after $CD4^+$ cell count measurement, all immunologic measures remained associated, but 6-month post-ART $CD4^+$ cell count became the strongest predictor of virus-related NADC incidence. The strong association of early immunologic response as captured by the 6-month post-ART $CD4^+$ cell count may indicate that the initial early recovery from severe immunosuppression is the most important component of the immunologic ART response for reducing virus-related NADC risk. Potent initial ART regimens and early interventions to maximize ART adherence may be important methods of cancer prevention in the HIV population.

Several prior studies have found similar associations with cancer incidence. Our results parallel those previously found in the ATHENA cohort, in which cumulative exposure to $CD4^+$ cell counts less than 200 cells/μl and latest $CD4^+$ cell count were both associated with infection-related NADC risk [37]. Latest $CD4^+$ cell count has been shown to be associated with virus-related NADC incidence in a number of studies including both ART-treated and ART-naive populations, and these associations have been attributed to the increased risk of oncogenic infection [7,10,37]. However, as mentioned previously, separating immunologic effects of subclinical malignancy from effects of $CD4^+$ lymphopenia resulting from HIV may be difficult.

The interaction between inadequate immune recovery and HPV-associated dysplasia and malignancy may be of particular interest. Low $CD4^+$ cell counts increase the risk of developing cervical cytologic abnormalities [30,31]. However, effective ART has not been convincingly associated with a decreased risk of cervical [38] or anal dysplasia [39]. Multiple factors including competing risks (i.e. prolonged survival on ART allowing greater opportunities for development of dysplasia and cancer) and immunologic experience before and after ART initiation may need to be accounted for when examining this question. Further work should be conducted examining the relationships between immunologic measures, specific oncogenic viruses and precancerous abnormalities.

Our findings are generalizable to other HIV-infected populations in the US given that CNICS is a large multisite clinical cohort from diverse geographic areas. Another strength was our use of comprehensive clinical data on $CD4^+$ cell count and HIV-1 RNA measurements with recently verified cancer diagnoses [18,40]. The use of advanced analytic methods allowed us to accurately estimate cumulative and time-updated immunologic measures and adequately account for confounding due to the close correlation with virologic measures. This study is the first to our knowledge to estimate associations of cumulative immunologic response with NADC incidence and compare them with other immunologic measures in a US HIV-infected population.

A number of limitations should be considered. With the exception of hepatitis B and C, we could not confirm the presence of oncogenic viral infection at the time of virus-related NADC diagnosis, and for certain cancers in this category, tumours may not have had oncogenic viruses present in tissue (e.g. oral pharyngeal squamous cell carcinoma associations with HPV [41]). If a poorer immunologic response increases the risk of viral oncogenesis, then associations would likely be stronger for confirmed virus-associated cancers. Second, we also had limited information on behavioural cancer risk factors, such as tobacco and alcohol use. However, in a

previous CNICS substudy, tobacco and alcohol use were not significantly associated with ART adherence, and thus may be unlikely to greatly confound immunologic response associations with NADC incidence [42]. Finally, information was only available while patients attended a CNICS site. We could not fully capture a patient's immunologic experience prior to ART, though we adjusted for $CD4^+$ cell count at ART initiation. In addition, one-third of patients were lost-to-follow-up and may have experienced different cancer incidence or immune recovery patterns than those observed.

In summary, our findings demonstrate that $CD4^+$ cell count response after ART initiation influences risk for virus-related NADCs. Associations persisted after adjusting for $CD4^+$ cell count at ART initiation, indicating that, beyond the effects of immune status at ART initiation, changes in immune status after ART initiation impact virus-related NADC risk with the influence of early $CD4^+$ cell response being most notable. This highlights the importance of adherence to effective, durable ART to reduce risk of virus-associated cancers in a population at increased risk for repeated episodes of infection with oncogenic viruses. Following ART initiation, if a patient's latest $CD4^+$ cell count is low, this may be an indication to initiate more frequent screening for virus-related NADCs, such as the anal Pap smear for detection of anal dysplasia. This population may also benefit from new screening modalities not currently used in the general U.S. population.

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E.L.Y. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. E.L.Y., S.N., S.R.C., C.J.A., S.G., A.F.O., D.P.D. and J.J.E. conceived the study concept and design. E.L.Y. conducted the data analysis and wrote the initial manuscript. All authors were involved in the interpretation of results and revision of the manuscript and approved the final draft of the manuscript.

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Conflicts of interest

S.N. has received grant support from Pfizer, Bristol-Myers Squibb and Merck; J.J.E is a consultant to Bristol Myers Squibb, GlaxoSmithKline, Merck, ViiV and Janssen, and has received research support (to UNC) from GlaxoSmithKline, Bristol Myers Squibb and Merck; M.J.M. is a consultant for Bristol Myers Squibb, Gilead and Merck; M.S. is a consultant for Bristol Myers Squibb, Merck, Gilead and Janssen. All other authors have no conflicts of interest to declare.

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