CANCER FOLLOWING COMBINATION ANTIRETROVIRAL THERAPY INITIATION: INCIDENCE PATTERNS AND EFFECTS OF ANTIRETROVIRAL RESPONSE

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ABSTRACT

ELIZABETH L. YANIK: Cancer following Combination Antiretroviral Therapy Initiation: Incidence Patterns and Effects of Antiretroviral Response
(Under the direction of Dr. Sonia Napravnik)

Among HIV-infected patients, cancer is a leading cause of morbidity and mortality. While combination antiretroviral therapy (ART) has reduced the incidence of Kaposi sarcoma (KS) and non-Hodgkin lymphomas, the burden of non-AIDS-defining cancers (NADCs) is increasing. However, the patterns and predictors of cancer incidence following ART initiation remain poorly characterized.

The Centers for AIDS Research Network of Integrated Clinical Systems, a collaboration of 8 United States HIV clinical cohorts, was used to evaluate the incidence and timing of cancer among patients initiating first ART, defined as ≥3 antiretrovirals, between 1996 and 2011. Poisson regression was used to estimate incidence rates. Cox regression was used to estimate adjusted hazard ratios to identify patient characteristics at ART initiation associated with subsequent cancer incidence.

Associations between immunologic ART response and NADC incidence were also evaluated among patients with ≥1 CD4 count and HIV RNA measurement by six months after ART initiation. Six month CD4, latest CD4, and CD4 count-years, a cumulative measure of CD4 lymphopenia, were used as measures of immunologic ART response and were considered with 0, 6, and 12-month exposure lags. Inverse probability weights were applied to Cox regression to account for time-varying confounding from the coincident virologic ART response.

Incidence for KS and lymphoma (Hodgkin, non-Hodgkin) were highest in the first six months post-ART start and plateaued thereafter, while incidence for all other cancers combined increased with time from ART initiation. A lower CD4 count at ART initiation was

associated with greater incidence of KS, lymphomas, and human papillomavirus-related cancers. Calendar year of ART initiation was not associated with cancer incidence. All measures of immunologic response were associated with virus-related NADC incidence independent of CD4 count at ART initiation, but none were associated with virus-unrelated NADC incidence. These associations persisted when measures were assessed with 6 and 12-month exposure lags.

Our results underscore recommendations for earlier HIV diagnosis followed by prompt ART initiation. They also highlight the need for cancer prevention and screening efforts throughout the course of HIV care, particularly among patients with poor immunologic response to ART.

DEDICATION

To my mother and my dad, who fostered in me a love of learning from day one, and who have supported and encouraged me in all the paths I have chosen since. They are my example and my inspiration for success in all aspects of life.

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ADC	AIDS-defining cancer
AIDS	acquired immunodeficiency syndrome
ART	combination antiretroviral therapy
CFAR	Center for AIDS Research
CI	confidence interval
CNICS	Center for AIDS Research Network of Integrated Clinical Systems
HIV	human immunodeficiency virus
HR	hazard ratio
IR	incidence rate
IRIS	immune reconstitution inflammatory syndrome
KS	Kaposi sarcoma
NADC	non-AIDS-defining cancers

I. SPECIFIC AIMS

Recent years have seen rising incidence rates of non-AIDS-defining cancers (NADCs) among the HIV population. However, the etiologic pathways through which HIV increases cancer risk and the effects of HIV-specific exposures, such as antiretroviral therapy, on cancer incidence and cancer outcomes are still poorly understood. While it would be intuitive that combination antiretroviral therapy (ART) use would reduce the risk of NADCs through immune reconstitution, studies have shown mixed associations with ART use and NADC incidence, and incidence of some NADCs has increased in the ART era compared to the pre-ART era[1-4]. While these studies have provided a descriptive characterization of NADC incidence in the era of ART, most may not clearly characterize the causal association of modern antiretroviral treatment with malignancies because of latencies between the relevant exposure window and cancer diagnosis, and differences in competing risks between patients from different treatment eras. To better understand the relationship between ART use and cancer incidence the following aims are proposed.

Aim 1: Describe the incidence and timing of cancer diagnoses following combination antiretroviral treatment (ART) initiation.

Hypothesis 1.1: Incidence will be higher within the first year following ART initiation for cancers that may increase due to immune recovery (e.g., Hodgkin's lymphoma) and for cancers detectable through screening tests due to increased medical care.

Hypothesis 1.2: After an initial decrease in incidence following the first year after ART initiation**,** incidence will increase with time after ART initiation due to chronologic aging and cumulative and/or incident carcinogen exposure, including alcohol and tobacco use.

Hypothesis 1.3: The overall incidence of virus-related cancers will be on average greater than the incidence of non-virus-related cancers.

Aim 2: Examine the association of immunologic ART response with the incidence of non-AIDS-defining cancers (NADCs) accounting for time-fixed and time-varying confounders.

Hypothesis 2.1: After controlling for confounders, NADC incidence rates will be lower in patients who achieve immune reconstitution as measured by six month CD4 count, latest CD4 and CD4 count loss-years, a cumulative measure of immunologic response.

Hypothesis 2.2: Immune reconstitution following ART initiation will be more strongly associated with reduced NADC incidence rates when an exposure lag is introduced compared to when immune reconstitution is assessed proximal to NADC diagnosis.

The Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS), an observational clinical cohort collaboration, will be used for these analyses. CNICS has extensive information on antiretroviral treatment, cancer incidence and staging, other diagnoses, and laboratory measurements from eight HIV clinics in the United States. Overall, CNICS includes over 25,000 HIV-infected patients with over 400 incident cancers cases after ART initiation and over 150 incident cases of NADCs occurring in patients six months after ART initiation.

II. BACKGROUND AND SIGNIFICANCE

A. Cancer in the HIV population

From early in the HIV epidemic, cancer has been a prominent source of illness in the HIV population. Initially, this was mostly due to cancers categorized as AIDS-defining (ADCs) which served as markers of advanced HIV progression, specifically Kaposi sarcoma, non-Hodgkin lymphoma, and invasive cervical cancer. With the advent of combination antiretroviral treatment (ART) in 1996, HIV patients are now living longer and facing a new array of non-AIDS-defining illnesses. As incidence and mortality rates from ADCs have declined, non-AIDS-defining cancers (NADCs) now make up a substantial proportion of the cancer burden in HIV populations and have become one of the leading causes of death among HIV-infected individuals in developed countries[1, 5-8]. HIV-infected individuals diagnosed with cancer often present with more advanced disease and have a poorer prognosis than the general population[4, 9-13]. Several studies have demonstrated that HIV-infected individuals are at increased risk of many NADCs when compared to HIV-uninfected individuals, particularly those with known infectious causes[8, 14-16]. In fact, in more recent years relative risks of NADCs such as anal cancer and Hodgkin lymphoma have been shown to be similar to their AIDS-defining counterparts (cervical cancer and non-Hodgkin lymphoma, respectively)[17, 18]. This indicates a need for primary prevention to reduce ADC and NADC incidence as well as identification of groups that may benefit from more frequent screening and faster identification of symptoms. In addition, a better understanding of the etiologic causes for the high incidence in HIV populations may better inform future interventions both in the HIV-infected and general populations.

A number of the most common NADCs in the HIV population have much higher incidence rates than the HIV-uninfected population. While HIV-infected individuals have about 2-3 times the risk of developing any NADC compared to HIV-uninfected individuals, they have 28 times the risk of developing anal cancer, 11 times the risk of developing Hodgkin lymphoma, 3 times the risk of developing lung cancer, 6 times the risk of developing liver cancer, and 4 times the risk of developing skin cancer[15, 19]. The virus-related NADCs have particularly high incidence rates in the HIV population, with the two most common virus-related NADCs being anal cancer (related to human papillomavirus infection) and Hodgkin lymphoma (related to Epstein-Barr virus infection)[14]. While survival rates for both ADCs and NADCs have increased in the ART era, cancer has become a larger contributor to death as infectious causes of death have decreased more dramatically[7, 20-22]. In a recent study of mortality conducted within the Centers for AIDS Research Network of Integrated Clinical Systems, virus-related NADC cases had a 2-year survival rate of 72% while virus-unrelated NADCs had a 2-year survival rate of 51% and ADCs had a 2-year survival rate of 59%[23]. While survival rates among diagnosed NADCs have improved in the ART era, the incidence of NADCs has increased, adding to the burden of disease[24]. The overall rise in incidence of NADCs since the introduction of ART appears to be almost entirely attributable to increased rates of virus-related NADCs[25].

While behavioral differences between the HIV population and the general population are major contributors to the high risk of cancer in HIV-infected individuals these differences do not appear to fully account for the increased risk. For instance in the Multicenter AIDS Cohort Study of gay and bisexual men, anal cancer risk appeared increased in HIV-infected men compared to HIV-uninfected men even after accounting for sexual behaviors[26]. A study of liver cancer found that HIV infection did not predict liver cancer risks independently from the effects of hepatitis C infection and alcohol abuse[27].

However, HIV-infected liver cancer cases have been reported to have a history of alcohol abuse less frequently than their HIV-uninfected cases, and HIV infection may impact cancer risk indirectly through a loss of control of chronic hepatitis infection[24, 28]. Several studies of lung cancer have found that HIV-infected individuals have increased risk compared to HIV-uninfected individuals independent of the effects of smoking behaviors, most notably in patients with low CD4 counts[17, 29-31]. These findings suggest that beyond a higher frequency of risk behaviors that increase NADC incidence in HIV populations, HIV disease may have direct effects on cancer risk.

B. Hypothesized Effects of HIV on Cancer Risk

While a number of biologic mechanisms may account for the higher risk of cancer in the HIV population, immunosuppression clearly may play a role in increasing the risk of infections, the persistence of infections, or the loss of control of latent infections related to oncogenic processes[24, 32-34]. The relationship of immunosuppression with cancer has been clearly established for the two most common ADCs, Kaposi sarcoma and Non-Hodgkin lymphoma, in which the cancers have been linked with human herpes-8 virus and Epstein-Barr virus respectively. Incidence of these two malignancies is closely associated with immunosuppression, and incidence decreases with ART use[1, 3, 35-37]. Immunosuppression may also explain the preponderance of infection-related cancers in the HIV population[14, 38, 39]. This mechanism is further supported by evidence that the majority of cancers with increased incidence in the HIV population have also been found to have increased incidence in populations of transplant recipients, another group of immunosuppressed individuals[40, 41].

While cervical cancer and NADCs have been less strongly linked to immunosuppression, associations persist. Both cervical and anal cancer incidence are

inversely associated with CD4 count and nadir CD4 count[2, 18, 26, 37, 42, 43]. This is further supported by evidence that HIV-related immunosuppression is associated with increased persistence of HPV, increased replication of oncogenic HPV types, and higher incidence of cervical cytological abnormalities[43-46]. Hodgkin lymphoma has shown an inverse association with CD4 count in some populations[47, 48] and a "U"-shaped association in others, in which the highest incidence is seen in those with CD4 counts between 200-250 cells/mm^3 [24, 49]. This could possibly be due to an increase in risk of Hodgkin's lymphoma as a part of immune reconstitution inflammatory syndrome (IRIS) in which diseases may worsen or be 'unmasked' during the immune reconstitution process following ART initiation[24, 50]. IRIS has previously been implicated in cases of Kaposi sarcoma and Non-Hodgkin lymphoma[51]. Liver cancer has also been associated with low CD4 counts, while associations have thus far been inconsistent for lung cancer[3, 29, 32, 52]. Other virus-unrelated cancers have not shown conclusive associations with immune status, though there has been indication of a decreased risk of colorectal cancer with higher CD4 count[3, 17, 53].

In addition to the effects of immunodeficiency, persistent viral replication in patients with HIV may increase inflammation in turn increasing the risk of NADCs through increases in cellular replication and resulting DNA damage[34, 54]. This mechanism may be of particular importance for virus-unrelated cancers with increased risk in HIV populations. Uncontrollable viremia has been shown to predict risk of Kaposi sarcoma and Non-Hodgkin lymphoma independent of CD4 count[17, 32, 36, 55]. While one study has found an association of anal cancer with increased duration of high viremia, this result has not been replicated in other studies[17, 26, 32]. Clear associations with viral replication have not been demonstrated for other NADCs[17, 29, 32]. This may partially be due to limited power to assess cancer-specific effects in most studies with small numbers of cancer cases, particularly if the effects of viral replication are small. For AIDS-related lymphomas,

cumulative viral replication predicts incidence better than latest HIV viral load, but associations of cumulative measures of viremia with other types of cancer have not been assessed[55].

If immunosuppression or viral replication is causally associated with higher cancer incidence we would expect the successful use of antiretroviral therapy in HIV patients to reduce cancer risk, including incidence of ADCs and NADCs, as well as, viral and non-viral mediated cancers.

C. Antiretroviral Use and Cancer

The incidence of Kaposi sarcoma and non-Hodgkin lymphoma have clearly declined since the introduction of ART in 1996, and individual risk for these cancers is greatly reduced with the initiation of ART[32, 56-58]. Despite the intuitive hypothesis that antiretroviral therapy would also decrease the risk of NADCs associated with HIV infection, studies of this association have been inconclusive. Several descriptive studies have compared NADC incidence between the pre-ART era and the combination ART era (generally using 1996 as the beginning of the combination ART era in the United States). The majority of these studies have found either no change or an increase in NADC incidence in the combination ART era, particularly anal cancer and Hodgkin lymphoma[2, 3, 18, 26, 49, 56, 59-61]. Much of this increase is due to the aging of the HIV population, however increases appear to persist after adjustment for age[2, 49, 56, 59, 60]. While these ecologic studies are helpful for descriptive purposes, we should be hesitant to draw any conclusions about causation without individual treatment data or information on other individual level factors. In addition, these studies may not appropriately account for the significant differences in competing risks between the two eras. Specifically, prior to the introduction of effective antiretroviral treatment most HIV-infected patients were dying of

aggressive infectious causes and seldom lived long enough to develop diseases with longer latency periods such as NADCs.

A number of studies have also assessed the association of current ART use or ever vs. never ART use with incidence of NADCs. Most of these studies have also found either no association[3, 26, 29, 32] or positive associations[2, 62] between exposure to ART and NADCs. A study of the association of ART use with liver cancer hinted at a protective association, but estimates of the association were imprecise due to a limited number of cancer outcomes[52]. These studies highlight the importance of screening measures even after successful treatment with ART; however, because oncogenic processes start long before diagnosis, examining ART use at the time of diagnosis may be missing the relevant window of exposure. For virus-related NADCs in particular, the effect of ART may be most prominent at the time of oncogenic viral infection, which likely occurs long before cancer diagnosis. Those infected with oncogenic viruses during an untreated HIV-infected time period likely may not develop diagnosable disease until after entry into care and initiation of ART. NADCs also may be more likely to be diagnosed after ART initiation because of more patient engagement in clinical care after the start of treatment. Finally, categorizing ART use as 'ever vs. never' or 'current vs. not current' may also oversimplify a treatment exposure that may differ dramatically by patient in terms of duration, adherence, and efficacy.

D. Cancer after Antiretroviral Initiation

Regardless of the absence or presence of a causal association between antiretroviral use and cancer risk, the majority of cancer diagnoses in the HIV populations in developed countries now occur in individuals after the initiation of ART[2, 32, 50]. A few studies have described trends in the incidence of specific cancers after ART initiation.

Hodgkin lymphoma was observed to occur at the highest rates within the first three months after ART initiation in a French HIV cohort, with rates similar to those seen in antiretroviral naïve patients after six months[50]. Another study assessed the association of duration of ART exposure with anal cancer incidence, but only 19 cases were included, and while the association appeared to be slightly protective it was not statistically significant[42]. The trends in cancer incidence after ART initiation have not been described for most specific cancer types, and no study has compared incidence trends in this time period between different cancer types. Cancer incidence after ART initiation would be expected to vary over time given the many changes that occur in patients over time after ART initiation including viral suppression, immune reconstitution, changes in adherence, aging, and cumulative exposure to carcinogens. Understanding these cancer risk patterns is an important first step in being able to target screening interventions in HIV patients, and in hypothesizing possible etiologic pathways between ART exposure and cancer incidence.

The specific characteristics of ART such as antiretrovirals included in the regimen and immunologic and virologic response to initiation may be important determinants of cancer risk following ART initiation. However, only a few studies have examined how ART-specific characteristics may affect cancer incidence over time. In a study of a clinical trial comparing antiretroviral treatment interruptions to continuous antiretroviral treatment, ADC incidence was statistically significantly higher in the antiretroviral treatment interruption arm, while NADC incidence did not differ dramatically between the two arms though incidence was slightly higher in the treatment interruption arm[63]. A few laboratory studies have hypothesized effects of particular antiretrovirals with cancer risk[64-66]. However, observational studies have not yet resolved whether the contents of ART meaningfully affect cancer risk following initiation[67, 68]. Only one study has examined the association of virologic and immunologic response after ART initiation with cancer incidence which found infection-related NADC incidence to be associated with greater time

spent with a CD4 count less than 200 cells/mm^3 [69]. Further examination of the relationship between ART response and cancer incidence may help elucidate the mechanisms through which ART use may impact cancer risk.

E. Summary

Cancer continues to be a leading contributor to morbidity and mortality in the HIV population[5, 6, 70]. While the burden of ADCs has decreased over calendar time, the burden of NADCs has increased with the aging HIV population[6]. HIV-infected individuals have higher risk than HIV-uninfected individuals for many NADCs, even after accounting for behavioral and demographic differences. Thus HIV infection may have direct effects on cancer risk. Specifically, both immune suppression and ongoing viral replication have been hypothesized to impact cancer risk[24, 34, 54]. Because of these hypotheses, ART would be expected to reduce NADC incidence, but current studies have shown inconsistent associations between ART use and NADC risk. In fact, the majority of cancer diagnoses now occur in HIV patients after the initiation of ART[2, 32, 50]; however, few studies have described the trends in cancer risk following ART initiation. Additionally, only one study has examined the effects of immunologic and virologic ART response on subsequent NADC incidence.

To address the current gaps in the literature, we plan to better characterize the relationship of ART and ART response with cancer incidence, by using a comprehensive data source and analytic plan that will reduce the limitations present in previous studies.

III. RESEARCH DESIGN AND METHODS

A. Study Population

Data was utilized from the CFAR Network of Integrated Clinical Systems (CNICS) to better understand the relationship between ART initiation, immunologic and virologic ART response, and cancer incidence. CNICS has extensive information on antiretroviral medications, cancer incidence and staging, other medical diagnoses, laboratory measurements, demographics, and medications from eight HIV clinics in the United States. In total, at the time at which data was acquired for this study CNICS included over 25,000 HIV-infected patients with over 300 incident cases of NADCs that occurred in patients after ART initiation[23]. The population was mostly male (81%), predominantly from urban populations, and had a distribution of races representative of the U.S. HIV population[71]: 39% black, 46% white, 11% Hispanic, and 4% other races. CNICS enrollment included patients who initiated clinical care at one of the eight sites after January 1, 1995, and enrollment is continuously ongoing as new patients initiate care at the clinics. In order to be eligible for the study population a patient had to be 18 years of age or older, diagnosed with HIV, and the patient must have attended at least two medical visits within twelve months at one of the eight sites[72]. The eight sites included HIV clinics at Case Western Reserve University; University of Alabama at Birmingham; University of California, San Francisco; University of Washington; University of California, San Diego; Fenway Community Health Center of Harvard University; University of North Carolina, Chapel Hill; and Johns Hopkins University[71, 72].

This study population was advantageous because it provided a large, representative sample of the United States HIV population in care. Information was collected as a part of normal clinical care through patient medical charts and electronic medical records, and so there was very little burden associated with study participation. As a result, large differences between those who participated and those who chose not to participate in the study were unlikely. For example, at the University of North Carolina, Chapel Hill clinic, over 90% of those asked to participate in the study choose to enroll[73]. CNICS was an ideal resource to address our aims as it provided detailed clinical information on a large sample of HIV-infected patients. The CNICS study had also recently verified all cancer diagnoses through a standardized protocol (Table III.1). Because clinical records were available both before and after the date of cancer diagnosis, the CNICS study had the ability to perform rigorous validation procedures through extensive medical record review on every cancer diagnosis occurring while a patient is receiving HIV care at a CNICS clinic.

Multiple measures were undertaken to assure the validity of CNICS data, including data quality protocols regularly conducted at the CNICS data repository located at the University of Washington, Seattle, and checks that were done by our study team to assess accuracy and completeness of the data. At the CNICS data repository, data was regularly integrated using a standardized data mapping system for all eight study sites. Newly integrated data was checked for completeness and logical consistencies at the individual study sites and at the centralized CNICS repository. Automated validity checks were regularly run on the centralized database. For example, HIV viral load measurements must not precede a patient's HIV diagnosis date. Data managers at the repository were regularly in communication with investigators from each study site, and consulted with individual sites to reconcile any inconsistencies or incomplete values in the data[74].

For my study, the population included patients who were HIV-1 seropositive, at least 18 years of age, and who initiated a first ART regimen, defined as the concurrent use of at

least three different antiretrovirals, while in the CNICS cohort between 1996 and 2011. Patients also had to initiate a first ART regimen before the end of cancer ascertainment at their respective CNICS site. The end dates for cancer ascertainment by site were: Case Western Reserve University= 31 May 2010, Fenway Community Health Center= 30 April 2011, Johns Hopkins University= 31 May 2010, University of Alabama= 30 April 2012, University of California-San Diego= 31 May 2010, University of California-San Francisco=31 August 2011, University of North Carolina= 31 May 2011, and University of Washington=30 September 2010. Patients were included who had a cancer diagnosis prior to entry into the CNICS cohort, or who had prior exposure to mono- or dual-antiretroviral therapy. However, sensitivity analyses were conducted in which: 1) only patients with no prior history of a cancer diagnosis were included and 2) only patients who are antiretroviral naïve at ART initiation were included. For cancer-specific analyses, only patients who had a diagnosis of the cancer being studied prior to entry into the CNICS cohort were considered for exclusion.

B. Exposure Measurement

For this study the main exposure of interest was immunologic response to ART. For the assessment of immunologic response, a novel cumulative measure of immunosuppression was developed termed CD4 count-years. This measure was calculated similarly to copy-years viremia, a measure previously developed to capture cumulative viral HIV burden. Copy-years viremia is calculated by estimating the area under the curve of multiple HIV-1 viral RNA measurements over time utilizing the trapezoidal rule[75, 76]. Specifically, two consecutive HIV RNA measurements are averaged and multiplied by the length of time between the two measurements. The resulting values from each interval between measurements are then summed across time. Copy-years viremia has previously been shown to predict the hazard of AIDS or death independently of traditional measures of

HIV viremia such as viral set point, peak viral load, and most recent viral load[75]. Additionally, copy-years viremia following ART initiation in those initiating a first ART regimen has been shown to predict death independently of cross-sectional viral load measures and time-updated CD4 counts[76]. In this study, CD4 count-years was calculated using CD4 count measurements available following ART initiation. As an example, a patient with a CD4 count of 200 cells/mm^3 for the first six months after ART initiation and a CD4 count of 400 cells/mm^3 for the next six months was equivalent to a patient with a CD4 count of 300 cells/mm^3 for one year. While a few studies have previously explored the association of duration of time below 200 CD4 cells/mm^3 with cancer incidence, no published research has yet examined a measure that accounts for both the extent and duration of immunosuppression[32, 69, 77]. The use of CD4 count-years added an innovative component to this research plan. Latest CD4 count, defined as a time-varying measure updated every time a patient received a new CD4 count measurement after ART initiation, and CD4 count at six months following ART initiation, defined as the most recent CD4 count measurement received after ART initiation and before six months post-ART, were also considered as traditional measures of time-varying and short-term immunologic response, respectively.

C. Outcome Measures

The primary outcome of interest for all study aims was cancer diagnosis as documented in clinical records. While patterns and associations with specific cancer types were explored, this study focused on measures of association with groups of common cancers as it was not anticipated that this study would have sufficient power to detect associations with specific cancer types. Cancers were categorized into groups based on prior knowledge of common biologic mechanisms in order to ensure the validity of

inferences drawn from this study and based on prior established categories so as to be comparable with past research (Table III.2). The final cancer groups chosen to highlight in the presentation of results were based on prior knowledge and established categories, as well as similar patterns seen between individual cancer types in the data.

D. Other Measures

A number of other time-fixed and time-varying covariates available in the CNICS database were considered as possible confounders or effect measure modifiers of the associations between baseline patient characteristics, immunologic ART response, and cancer incidence (Table III.3).

E. Statistical Analysis- Aim 1

For the first aim, descriptive analysis of cancer incidence was conducted. Only the first incident cancer following antiretroviral initiation was counted after which the patient was censored from the analysis in order to avoid inclusion of recurrent cancers. Patients were also censored at death, loss-to-follow-up (defined as more than 12 months without a CD4 count or HIV RNA measure), last date of cancer ascertainment by CNICS site (ranging from May 31, 2010-Dec. 31, 2011), and administrative censoring at 10 years after ART initiation. Incidence rates were calculated over all time after ART initiation, in the first six months after ART initiation, and from six months to ten years after ART initiation for each specific cancer, all cancers combined, and various cancer groups as defined in Table III.1. Hazard curves over time since ART initiation were constructed and incidence rates were calculated for six-month and one-year intervals following ART initiation. This characterized the time periods

after ART initiation at which cancer diagnosis was most common, and the timing of NADC incidence was contrasted with the timing of AIDS-defining cancers following ART initiation. Cox proportional hazard regression was used to estimate associations between cancer incidence and patient characteristics at ART initiation, specifically calendar year, CD4 count, HIV viral load, age, and prior exposure to antiretrovirals (mono-therapy, dual-therapy, or an unknown antiretroviral history). The proportional hazards assumption was tested for each patient characteristic by evaluation of the interaction with the log of time since ART initiation. In addition, incidence rate trends were assessed stratified by categories of CD4 count and HIV viral load laboratory values at ART initiation, as well as categories of calendar year of ART initiation, prior antiretroviral exposure, and age at ART initiation.

We anticipated an initial increase in cancer diagnoses due to increased detection with entry into care and ART initiation. Information gained from this aim was used to inform the start of follow-up for Aim 2 in order to avoid this initial detection bias. Additionally, the distributions of all other key covariates were examined in order to guide decisions about categorization or transformation of covariates later in the analysis. For all analyses, if the results of cancer-specific analyses indicated different associations within cancer groups, different possible cancer categorizations were considered. As mentioned earlier, sensitivity analyses were conducted in which patients with a cancer diagnosis or antiretroviral exposure prior to ART initiation were excluded. Results in these subpopulations were compared to those observed in the total population. We performed all analyses in SAS version 9.2[78].

F. Statistical Analysis- Aim 2

For the second aim, survival analysis methods were used to estimate the association of immunologic ART response with NADC incidence. Cox proportional hazards regression

models were used to estimate hazard ratios and 95% confidence intervals. The proportional hazards assumption was evaluated by inclusion of an interaction term with the log of time since ART initiation in the model. Immunologic response as a predictor of NADC incidence was assessed starting from ART initiation. Follow-up for cancer incidence began at six months after ART initiation in order to exclude the excess of cancers initially detected due to increased medical attention following ART initiation, and in order to allow time for measurement of the initial immunologic and virologic response. Patients were followed until the first of NADC diagnosis, death, loss-to-follow-up, last date of cancer ascertainment by CNICS site, or administrative censoring at 10 year after ART initiation.

Potential confounders were considered based on previous knowledge of mechanisms and etiologic pathways for both HIV and cancer. Confounders were assessed in multivariable regression models by evaluating the change-in-estimate and the increase in precision produced from removing each confounder from the model. Covariates that produced a change-in-estimate of greater than or equal to 10% were considered important confounders to be included in the model. An appropriate set of confounders was selected separately for each regression model.

HIV viral load can act as a time-varying confounder affected by prior immunologic response that cannot be accounted for using traditional adjustment methods. As such, marginal structural models that apply inverse probability weights to remove confounding without blocking causal effects were used to disentangle the effects of the immunologic and virologic components of ART response[79-81]. Specifically, linear regression models were used to calculate the probability that a patient would have the CD4 count that was observed based on the HIV RNA measurements from their prior two clinical visits. These probabilities were calculated for each CD4 count observed after ART initiation, and the inverse of these probabilities was used to weight each observation in the final regression model. In order to stabilize these weights, numerators were calculated equal to the probability of the observed

CD4 count measurement given time-fixed covariates, and the same time-fixed covariates were used in the calculation of the probabilities used for the denominator of the weights. Specifically, time-fixed covariates used to stabilize weights were: CD4 count at ART initiation, HIV RNA at ART initiation, injection drug use transmission risk, men who have sex with men transmission risk, prior antiretroviral use, race, calendar year of ART initiation, and age at ART initiation. Inverse-probability-of-censoring weights were calculated to account for differential censoring by time-varying HIV viral load, in which the weights were equal to the inverse of the probability that a patient would be censored given the HIV RNA measurement from their two prior clinical visits. Additionally, inverse-probability-of-frequency weights were applied to account for differences in the frequency of CD4 count measurements in different patient groups[82, 83], in which weights were applied that were equal to the inverse of the probability of receiving a CD4 count measurement within each one week interval following ART initiation. After each of these three set of weights were calculated, total weights were calculated by multiplying together the three weights for each observation. The total weights were applied to the final regression models used to assess the relationship between immunologic response and NADC incidence. The same weights were used for assessment of each measure of immunologic response.

These analyses were also repeated with the use of exposure lags to account for the empirical induction period between immunologic ART response and NADC diagnosis[84]. Exposure lagging was implemented by only including exposures that have occurred a certain time period prior to the time of outcome assessment. In our analysis we explored six-month and twelve-month exposure lags in which only CD4 counts occurring more than six or more than twelve months before cancer ascertainment, respectively, were used as a part of our measures of immunologic ART response. For six month lag analyses, follow-up time started at twelve months after ART initiation to allow time for an initial immunologic response and six months of lag time. Likewise, for twelve month lag analyses, follow-up

time started at eighteen months after ART initiation to allow for an initial immunologic response and twelve months of lag time. Multiple time lengths were explored as these methods can be sensitive to assumptions about the length of the lag time.

This analysis was repeated for cancer groups as previously defined (Table III.1), as well as for specific common NADC diagnoses such as lung cancer and anal cancer. As competing risks, such as death, may likely be associated with ART use, sensitivity analysis was also conducted in which we assessed the impact of observing various patterns of cancer incidence in the absence of death[85].

Table III.1 Incident cancer cases among HIV-infected patients treated for at least six months with ART in the CNICS cohort between 1996 and 2009, from Achenbach et al., 2011.

Cancer Diagnoses	**N (%)**
Kaposi's sarcoma	185 (29)
Non-Hodgkin's Lymphoma non-CNS	114 (18)
Lung	59 (9)
Anal	55 (8)
Hodgkin's Lymphoma	32 (5)
Prostate	27 (4)
Non-Hodgkin's Lymphoma CNS	22 (3)
Liver	22 (3)
Kidney	19 (3)
Melanoma	15 (2)
Breast	11 (2)
Colorectal	11 (2)
Other[a]	78 (12)
Summary Cancer Diagnoses	
Infection-unrelated NADCs	221 (34)
ADCs[b]	321 (49)
Infection-related NADCs[c]	108 (17)

[a]Other cancers: cervical, biliary, bladder/urinary, primary brain, esophagus, oral cavity/pharynx, other head and neck, leukemia, multiple myeloma, other (not specified or unknown origin), ovary, pancreas, penis, peritoneum/retroperitoneum, small intestine, soft tissue, stomach, testicular, thyroid, trachea/pleura, uterus, and vagina/vulva.
[b]ADCs: cervical, Kaposi's sarcoma, and Non-Hodgkin's lymphoma.
[c]Infection-related NADCs: squamous cell anal, squamous cell oral cavity/pharynx, Hodgkin's lymphoma, liver with viral hepatitis, vagina/vulva, and penis.
ART=combination antiretroviral therapy, CNICS= CFAR Network of Integrated Clinical Systems, CNS=central nervous system, NADCs= Non-AIDS-defining cancers, ADCs=AIDS-defining cancers

Table III.2 Categories for grouping cancer diagnosis outcomes

Category Name	Cancers within Category
AIDS-defining cancers	Kaposi sarcoma, non-Hodgkin lymphoma, cervical cancer
Non-AIDS-defining cancers	All cancers not included as AIDS-defining
Virus-related cancers	Kaposi sarcoma, non-Hodgkin lymphoma, cervical cancer, anal cancer, Hodgkin lymphoma, liver cancer in the presence of hepatitis, squamous cell oral cavity/pharynx, vagina/vulva cancer, penis cancer
Virus-unrelated cancers	All cancers not included as virus-related (lung cancer, prostate cancer, colorectal cancer, etc.)
Virus-related non-AIDS-defining cancers	Anal cancer, Hodgkin lymphoma, liver cancer in the presence of hepatitis, squamous cell oral cavity/pharynx, vagina/vulva cancer, penis cancer
Lymphomas	Non-Hodgkin lymphoma and Hodgkin lymphoma
HPV-related cancers	Anal cancer, cervical cancer, squamous cell oral cavity/pharynx cancer, vagina/vulva cancer, penis cancer

Table III.3 Study Variable Descriptions

Variable Name	Description	Type
	Outcome	
Cancer	New cancer diagnosis as indicated by medical records with cancers grouped as stated in Table 2	Binary, Time-to-First-Event
	Primary Exposures	
CD4 count loss-years	Cumulative measure of immunosuppression incorporating the CD4 count loss below 500 cell/mm^3 and duration of exposure starting at ART initiation	Continuous, Time-varying
	Other Exposure Measures	
Latest CD4 count	Most recent measure of CD4 count prior to each time point following ART initiation	Continuous, Time-varying
Six month CD4 count	Most recent CD4 count following ART initiation, but prior to six month after ART initiation	Continuous, Time-fixed
	Covariates	
Baseline CD4 count	Most recent CD4 count prior to ART initiation	Continuous
Baseline Viral Load	Most recent viral load prior to ART initiation	Continuous
Viral load at 24 weeks	HIV viral load nearest to 24 weeks after ART initiation within a window of 12-36 weeks after ART initiation	Continuous, Time-fixed
Most recent viral load	Most recent measure of HIV viral load prior to each time point following ART initiation	Continuous, Time-varying
Sex	Male or Female. Transgendered patients not included in analysis	Binary
Age	Age at ART initiation in years	Continuous
Race	White, Black, Hispanic, or Other	Categorical
IDU	Injection drug user. Identified injection drug use as a HIV transmission risk	Binary
MSM	Men who have sex with men. Man who identified sex with men as a HIV transmission risk	Binary
Calendar Year	Calendar year of ART initiation centered at 1996	Continuous
CNICS site	Indicator of which of the eight CNICS clinical sites patient was attending at the time of ART initiation	Categorical
Type of Antiretroviral Regimen	Categorized by regimen backbone as: non-nucleoside reverse transcriptase inhibitor, protease inhibitor, triple nucleoside reverse	Categorical

	transcriptase inhibitor regimen, or other regimen. Binary variables may also be derived from ART information to indicate the presence or absence of certain antiretrovirals, such as AZT, in the ART regimen.	
BMI	Calculated based on height and weight measurements made nearest to ART initiation date using the formula: [Weight (lb.)*703]/[height(in)^2]	Continuous
AIDS-defining condition	Diagnosis of an AIDS-defining condition (yes vs. no). When AIDS-defining cancers are included as an outcome, this variable will only include non-cancer AIDS-defining conditions.	Binary, Time-varying
Hepatitis B	Categorized as never infected vs. ever infected. Positive laboratory results for Hepatitis B core Antibody, Hepatitis B surface antigen, or Hepatitis B DNA.	Binary, Time-varying
Hepatitis C	Categorized as never infected vs. ever infected. Positive laboratory results for Hepatitis C Antibody or Hepatitis C RNA.	Binary, Time-varying

IV. RESULTS: INCIDENCE AND TIMING OF CANCER FOLLOWING INITIATION OF COMBINATION ANTIRETROVIRAL THERAPY, IN THE UNITED STATES 1996-2011

A. Introduction

While the distribution of cancer types within HIV-infected populations has changed due to advances in treatment and demographic changes, malignancies remain a leading cause of morbidity and mortality[1, 5, 6]. Cancer incidence trends in the HIV population have been thoroughly examined across calendar time[1, 16, 57, 60]. However, incidence rates among HIV patients are not well-described across time relative to initiation of combination antiretroviral therapy (ART), even though most cancers are diagnosed after the initiation of ART[2, 32, 50].

Given the many changes in patient characteristics that occur after ART initiation, including immune reconstitution, HIV replication, aging, and ongoing exposure to carcinogens, cancer incidence over time following ART initiation is expected to be dynamic. Additionally, improvements in access to HIV testing and linkage to care as well as changes in the timing of ART initiation may impact the timing and incidence of cancer diagnoses in treated patients. In a large, diverse U.S. HIV cohort collaboration, the CFAR Network of Integrated Clinical Systems (CNICS), we evaluated trends in cancer incidence rates over time after ART initiation to inform cancer screening and prevention efforts for the HIV population in the ART era. We further identified patient characteristics associated with these trends that may provide insights into the etiology of cancers occurring in HIV-infected populations.

B. Methods

Study Population

This study used data from the CNICS, a collaboration of eight HIV clinic sites that collect information on patients engaged in routine HIV clinical care. CNICS captures extensive and comprehensive electronic medical data, including demographics, medications, co-morbid diagnoses, and laboratory values[72]. CNICS includes over 25,000 patients who have initiated clinical care at one of the eight sites between January 1, 1995 and the present. Within this cohort, cancer diagnoses are verified through a standardized process[23].

For our study, we included all patients who initiated ART with a known start date between Jan. 1, 1996-Aug. 30, 2011, and who had baseline CD4 count and HIV RNA measurements at ART initiation. Baseline CD4 count and HIV RNA measures were values most proximal to, but no more than 12 months before, ART initiation. ART was defined as concurrent initiation of at least three different antiretrovirals. For our primary analyses, we included patients with prior exposure to one or two nucleoside/nucleotide agents (mono- and dual-antiretroviral therapy, respectively) or who had an unknown antiretroviral history prior to initiating ART. We did not analyze person-time prior to ART initiation; however, we did assess effects of prior therapy exposure on our results, and conducted sensitivity analyses in which only patients who were antiretroviral-naive at ART initiation were included.

Patients were followed from ART initiation to the first of the following: cancer diagnosis, death, loss-to-follow-up (defined as 12 months without a clinical visit), or administrative censoring at the last date of cancer ascertainment for each clinic site (range of May 31, 2010-Aug. 31, 2011). Follow-up time was administratively censored at 10 years after ART initiation, at which point <10% of the patients remained under observation. Only

the first cancer diagnosis after ART initiation was included as an event. In our primary analyses we included all patients initiating ART without regard to cancer events prior to ART initiation. To assess the influence of including patients with a previous history of cancer, we performed sensitivity analyses in which patients with cancer diagnoses prior to ART initiation were excluded.

Statistical Analysis

Incidence rates (IRs) were estimated as the total number of cancer diagnoses within the time interval divided by the total number of person-years contributed within the same interval. Overall cancer incidence rates and incidence rates within 6-month and 1-year time intervals following ART initiation were calculated for specific cancers as well as for clinically meaningful pre-defined groups of cancers. Specifically, we assessed incidence rates for cancers using different categorizations including: AIDS-defining cancers (ADCs), non-AIDS-defining cancers (NADCs), lymphomas, HPV-related cancers (including cervical, anal, squamous cell oral cavity/pharynx, vagina/vulva, and penis cancer), virus-related cancers (including all ADCs, all HPV-related cancers, Hodgkin lymphoma, and liver cancer), and virus-unrelated cancers.

Poisson regression was used to contrast incidence rates between time intervals and to estimate trends in incidence rates across time following ART initiation. Baseline clinical characteristics were examined with Cox regression to identify predictors of cancer incidence following ART initiation. Cancers were grouped by common etiology and similar incidence patterns over time when assessing associations with baseline characteristics. Multivariable analyses were conducted with adjustment for all baseline demographic and clinical predictors determined to be associated with cancer incidence based on *a priori* knowledge. Two separate adjustment sets were used: one with CD4 count at ART initiation, and one

with nadir CD4 count any time prior to ART. These two variables were not included simultaneously as they were highly correlated, but were included separately to examine whether choice of CD4 measure affected other estimates differentially, especially for patients with prior antiretroviral exposure. Stratified incidence rates across time were also calculated by CD4 count at ART initiation (≥ or <200 cell/mm^3), age (≥ or <45), and prior exposure to antiretrovirals. Changes in the associations of these predictors with cancer incidence were assessed visually, and through the evaluation of interaction terms with log(time) in regression models.

C. Results

Baseline Characteristics

Of 25,337 patients enrolled in CNICS at the time of this analysis, 11,485 were observed to initiate ART between 1996 and 2011 at a CNICS site with pre-ART CD4 count and HIV RNA levels available. Of these 11,485 patients, 20% were female, 43% were white, 41% black, and 11% Hispanic (Table 4.1). Median age at ART initiation was 38 years (interquartile range [IQR]: 32-45). Twenty-eight percent of patients had exposure to antiretrovirals prior to ART initiation, and the median year of ART initiation was 2004 (IQR: 2000-2007). At ART initiation median CD4 count was 202 cells/mm^3 (IQR: 61-338) and median HIV RNA was 4.8 $\log_{10}$ copies/mL (IQR: 4.3-5.3). Most patients initiated a PI-based regimen (47%), of which 26% included atazanavir/ritonavir and 21% included lopinavir/ritonavir, or a NNRTI-based regimen (42%), of which 90% included efavirenz. In addition, 5% initiated a triple-NRTI regimen, 4% initiated a regimen with a PI and an NNRTI, and 2% initiated with another anchor drug, including integrase inhibitor, fusion inhibitor, and

entry inhibitor regimens. The median length of follow-up after ART initiation was 3.1 years (IQR: 1.4-6.2) with only 9.5% of patients remaining in follow-up at 10 years post-ART.

Cancer Incidence and Timing

During the 46,318 years of follow-up after ART initiation, 457 cancer diagnoses were observed with an IR of 987 cases per 100,000 person-years (95% Confidence Interval [CI]:900-1081) (Table 4.2). The overall incidence of ADCs was similar to that of NADCs (ADC IR: 515 per 100,000 person-years, 95%CI: 454-584; NADC IR: 466 per 100,000 person-years, 95% CI: 408-533). The most common ADC was KS with an IR of 304 per 100,000 person-years (95% CI: 258-358), while the most common NADC was anal cancer with an IR of 69 per 100,000 person-years (95% CI: 49-98). Among women, the most common NADC was breast cancer (IR: 128 per 100,000 person-years; 95%CI: 75-221).

The timing of cancer incidence after ART initiation differed between cancer types. KS had a particularly high incidence within the first 6 months (IR: 1342 cases per 100,000 person-years, 95%CI: 1071, 1683), but incidence decreased dramatically afterwards (IR for second 6 months: 348 per 100,000 person-years, 95% CI: 219-552) and stabilized at a low rate (IR for 6 months-10 years: 164 per 100,000 person-years, 95% CI: 129, 208) (Table 4.2, Figure 4.1). Lymphomas (both Hodgkin and non-Hodgkin) also occurred at a higher incidence in the first 6 months post-ART initiation (IR: 660 per 100,000 person-years, 95% CI: 479-912) compared to 6-12 months after ART initiation (IR: 269 per 100,000 person-years, 95% CI: 160-455), but the absolute change in incidence was smaller than for KS. By contrast, incidence rates of other cancers appeared to progressively increase with time after ART initiation. When combined, all non-KS, non-lymphoma cancers had incidence rates that increased by 7% each year from ART initiation (95% CI: 2%-13%; p=0.009), from 416 to 615 cases per 100,000 person-years in years 1 and 10 post-ART initiation respectively.

Predictors of Cancer Incidence and Time-Modification of Predictors

Several characteristics at the time of ART initiation had distinct relationships with subsequent cancer incidence. Older age increased risk for all cancers except KS in bivariable analyses, and associations appeared the same in multivariable analyses (Table 4.3). Non-lymphoma, HPV-unrelated NADCs were most strongly associated with older age (adjusted HR for 10 year difference in age: 2.33, 95% CI: 1.97-2.74). Associations of older age with cancer risk were consistent throughout time following ART initiation (P for interaction with time>0.05 for all cancers) (Figure 4.2).

CD4 count at ART initiation was the other consistent predictor of cancer incidence. Both in bivariable and multivariable analyses, lower CD4 counts were associated with higher cancer incidence for all cancer groups except the non-lymphoma, HPV-unrelated NADCs. Lower CD4 count was a particularly strong predictor of incidence of KS (Adjusted HR per 100 cells/mm^3 increase: 0.63, 95%CI: 0.54-0.73). The pattern of high KS incidence within the first six months followed by a steep decline in incidence was only seen among those with a CD4 count <200 cells/mm^3 at ART initiation (Figure 4.3, P for interaction of CD4 count with time =0.002). By contrast, those with a CD4 count ≥200 cells/mm^3 had low incidence of KS throughout time after ART initiation (IR: 0.1 per 100 person-years, 95%CI: 0.1-0.2). Low CD4 count was also associated with high incidence of lymphomas and HPV-related cancers, but for these cancers, associations with CD4 count were consistent across time (both P for interaction of CD4 count with time>0.20) (Figure 4.3).

Prior exposure to antiretrovirals was also associated with higher incidence of HPV-related cancer following ART initiation. This association persisted even when nadir CD4 count was included in the model in place of CD4 count at ART initiation. Across all follow-up

time, prior antiretroviral exposure was not associated with incidence of other cancers. However, for lymphomas, patients with prior antiretroviral exposure had lower incidence compared to antiretroviral naïve patients in the first year following ART initiation (Adjusted HR: 0.41, 95%CI: 0.19-0.93), but higher incidence than antiretroviral naïve patients after one year (P for interaction with time <0.05) (Figure 4.4).

Other characteristics examined were weaker predictors of cancer incidence. Higher levels of HIV RNA prior to ART were associated with higher KS incidence, but not with any other cancer after adjusting for CD4 count. No statistically significant associations were found between calendar year of ART initiation and incidence of any cancers in bivariable analysis. After accounting for variables such as age and CD4 count at ART initiation, there was no association between calendar year and any cancer (Table 4.3). The lack of association was robust to parameterization of calendar year as a continuous or categorical variable.

In sensitivity analysis where 489 patients with cancer diagnoses prior to ART initiation were excluded, similar incidence rates and incidence trends were observed and the same predictors of cancer incidence were identified (data not shown). Similarly, our findings were consistent in a subset analysis conducted among 8278 patients who were antiretroviral naïve at ART initiation. In this subset incidence rates and trends were similar to the full study population and factors associated with higher risk were also consistent in both unadjusted and adjusted analyses (data not shown).

D. Discussion

This study of 11,485 patients from a multi-site U.S. clinical cohort of HIV-infected patients followed up to 10 years revealed distinct patterns of cancer incidence following ART initiation. This likely reflects varying etiologic contributions of aging, immunosuppression,

and prior antiretroviral exposure, to the occurrence of specific cancer types. By contrast, cancer incidence did not appear to change over calendar time among ART initiators suggesting that the incremental improvements in ART regimens during the modern ART era have not had dramatic effects on cancer incidence.

The most dramatic change in incidence after ART initiation was seen for KS, which exhibited an incidence higher than all other cancers combined in the first six months and a steep decline thereafter. A similar pattern has been noted in ART initiators in the Swiss HIV Cohort Study[86]. Upon stratification by CD4 count at ART initiation, this incidence pattern was exclusively seen in those initiating with a CD4 count less than 200 cell/mm^3. This observation mirrors results from the HIV/AIDS Cancer Match Study, which demonstrated that severe immunosuppression at AIDS diagnosis was most strongly associated with KS risk in the first six months after AIDS diagnosis, and less strongly thereafter[87]. This finding and prior research demonstrating higher KS incidence among those with lower current CD4 counts[3, 17, 32, 88] are consistent with an early risk driven by more severe immunosuppression. In addition to severe immunosuppression increasing the risk of KS development, these individuals are more likely to experience more rapid immune reconstitution that may unmask previously subclinical KS, a phenomenon referred to as immune reconstitution inflammatory syndrome (IRIS)[51, 89-91].

Lymphomas showed a pattern similar to KS, but with a lower incidence in the first six months and a more gradual decrease in incidence thereafter. A decrease in incidence after six months was seen in both Non-Hodgkin lymphomas and Hodgkin lymphomas. Individuals with no prior antiretroviral exposure and those with lower CD4 counts at ART initiation had higher lymphoma incidence within the first six months. Similar to KS, these patient groups may be more likely to develop lymphoma associated IRIS events after ART initiation. An IRIS effect for lymphoma was hypothesized after a study in France showed a similar incidence pattern when looking at Hodgkin's lymphoma after ART[50]. It is also

possible that early symptoms of subclinical KS or lymphoma may have led to the HIV diagnosis and prompt initiation of ART, with eventual documentation of the definitive cancer diagnosis shortly after starting ART. Regardless, these findings highlight the importance of early HIV diagnosis and timely ART initiation before individuals reach advanced immunosuppression.

All other cancers combined showed an increasing trend over time following ART initiation with a lower incidence within the first six months and a significant trend toward higher incidence over time. This increase is likely a consequence of increasing cancer incidence with advancing age, as noted in the general population. Within this group of cancers that excluded KS and lymphoma, HPV-related cancers showed a strong association with CD4 count at ART initiation and higher incidence in those with prior antiretroviral exposure, which may reflect longer duration of HIV infection. As HIV infection can increase the risk of HPV infection, a lag in time may occur before the increased risk is manifest as increased cancer diagnosis rates in patients with a longer duration of HIV infection[18, 24, 42, 61]. It is also possible that adjustment for nadir CD4, a risk factor for HPV-associated cancers[2, 16, 26, 42], was inadequate if patients with prior antiretroviral exposure experienced their nadir CD4 before entry into a CNICS clinic. We did not observe an association between CD4 count at ART initiation and incidence of cancers that are neither AIDS-defining or associated with viral infections. Others have shown some virus-unrelated NADCs to be associated with the extent of immune suppression[17, 53]. A larger sample size may be needed to observe an association between low CD4 count and virus-unrelated NADCs; however, our data suggest that the relationship is not particularly strong.

Previous studies have described changing trends in cancer incidence within the HIV population as a whole[16, 60, 70]; however, in our population of ART initiators no changes in cancer incidence were observed over calendar time. Our study included more recent calendar years of follow-up than previous studies, and 75% of patients initiated ART in the

year 2000 or later. Trends identified in the larger HIV population are likely indicative of increased uptake of ART, while more recent changes in the potency or durability of first-line ART regimens[92] may have little impact on cancer incidence. This emphasizes the continued need for cancer screening and prevention measures in the HIV population, regardless of continued improvements in ART.

While our sample size was considerable, there were not enough incident cancer cases to conduct separate analyses for all specific cancer types. While we aimed to categorize cancers in etiologically meaningful ways, there may be differing trends for cancers grouped together that were undetectable in our study, particularly within the heterogeneous group of non-lymphoma, non-HPV-related, NADCs. Additionally, even within specific cancer types malignancies may have different etiologic origins. Such is the case with oral cavity/pharynx cancers and non-Hodgkin lymphomas, in which only a proportion are linked to viral co-infection (HPV and EBV, respectively). Despite this limitation, such groupings were necessary in order to discern meaningful trends and associations and can be used to guide more detailed analyses in the future.

This study was notable for the following strengths: a) a large and representative HIV-infected clinical population; b) detailed laboratory and antiretroviral information; and c) well-validated cancer diagnoses. The current study does not account for changes or discontinuations in ART after initiation nor does it account for differences in the immunologic or virologic responses to ART. In a future study we will examine how these changes after ART initiation may impact cancer incidence to expand on these findings and further our understanding of cancer incidence trends over time in this population.

We showed distinct patterns of cancer incidence after ART initiation that differed by cancer type and were modified by several baseline patient characteristics. These findings highlight the importance of cancer screening and cancer prevention efforts throughout the course of HIV clinical care for those cancers for which evidence-based interventions exist.

Currently, robust cancer screening and prevention guidelines have not been established that address the specific needs of HIV-infected individuals. Within the first year after ART initiation, KS and lymphomas are the largest sources of cancer morbidity. After the first year of ART initiation, HPV-associated cancer (particularly anal cancer) and other NADCs become more common. These results suggest that screening for HPV-associated cancers and certain NADCs should be prioritized once patients are on stable ART.

Table IV.1 Demographic and clinical characteristics of 11,485 patients initiating combination antiretroviral therapy in the CFAR Network of Integrated Clinical Systems, 1996-2011

Characteristic	N (%)
Total	11485
Female Sex	2304 (20.1%)
Age[†]	38 (32-45)
Race	
White	4933 (43.0%)
Black	4677 (40.7%)
Hispanic	1273 (11.1%)
Other/Unknown	602 (5.2%)
Injection drug user	2156 (18.8%)
Men who have sex with men	6328 (55.1%)
Antiretroviral exposure prior to first ART	3207 (27.9%)
ART initiation year[†]	2004 (2000-2007)
ART regimen type	
PI	5443 (47.3%)
NNRTI	4835 (42.1%)
3+ NRTI	570 (5.0%)
NNRTI+PI	441 (3.8%)
Other[‡]	196 (1.7%)
CD4 count (cells/mm^3)[†]	202 (61-338)
HIV RNA level (log_{10} copies/mL)[†]	4.8 (4.3-5.3)

[†]Median (IQR) given instead of N (%)
[‡]includes regimens with an integrase inhibitor, fusion inhibitor, or entry inhibitor
ART=combination antiretroviral therapy, PI=protease inhibitor, NNRTI=non-nucleoside reverse transcriptase inhibitor, NRTI=nucleoside reverse transcriptase inhibitor

Table IV.2 Cancer incidence rates within 10 years after combination antiretroviral therapy initiation, CFAR Network of Integrated Clinical Systems 1996-2011

Cancer Type		Incidence rate per 100,000 person-years (95% CI)		
	Total N	0-10 years	0-6 months	6 months-10 years
Overall	457	987 (900-1081)	2405 (2030-2848)	793 (711-884)
ADC	241	515 (454-584)	1881 (1554-2278)	330 (279-390)
KS	143	304 (258-358)	1342 (1071-1683)	164 (129-208)
NHL non-CNS	76	161 (128-201)	357 (230-553)	134 (103-175)
NHL CNS	19	40 (26-63)	160 (84-308)	24 (13-44)
Cervical†	3	30 (10-92)	-	-
NADC	216	466 (408-533)	520 (362-749)	459 (398-530)
Virus-related NADCs	89	192 (156-237)	251 (149-424)	184 (147-231)
Squamous cell anal	32	69 (49-98)	72 (27-191)	69 (47-100)
Hodgkin lymphoma	27	58 (40-85)	144 (72-287)	47 (30-73)
Liver	17	37 (23-59)	18 (3-127)	39 (24-64)
Squamous cell oral cavity/pharynx	9	19 (10-37)	-	-
Other‡	4	9 (3-23)	-	-
Virus-unrelated NADCS	127	274 (230-326)	269 (162-447)	275 (228-331)
Lung	26	56 (38-82)	54 (17-167)	56 (38-85)
Prostate†	20	54 (35-83)	22 (3-159)	58 (37-91)
Breast†	13	128 (75-221)	177 (44-708)	122 (68-221)
Melanoma	10	22 (12-40)	-	-
Colorectal	8	17 (9-35)	-	-
Kidney	5	11 (5-26)	-	-
Other*	45	93 (69-125)	-	-
Lymphomas††	122	259 (217-309)	660 (479-912)	205 (165-253)
HPV-related cancers‡‡	48	104 (78-138)	108 (48-140)	103 (76-140)

†Cervical cancer and breast cancer incidence calculated only among women. Prostate cancer incidence calculated only among men.
‡Other virus-related cancers include penis, vaginal, and vulva
*Other virus-unrelated cancers include bladder, brain, esophagus, larynx, leukemia, multiple myeloma, ovary, pancreas, peritoneum, small intestine, soft tissue, stomach, testicular, uterus, or non-squamous cell oral cavity/pharynx
††Lymphomas included NHL non-CNS, NHL CNS, and Hodgkin lymphoma.
‡‡HPV-related cancers included cervical, squamous cell anal, squamous cell oral cavity/pharynx, penis, vaginal, and vulva cancer
Only overall incidence rates were calculated for cancer types with ≤10 cases.
ADC=AIDS-defining cancer, KS=Kaposi's sarcoma, NHL=non-Hodgkin's lymphoma, CNS=central nervous system, NADC=non-AIDS-defining cancer, HPV=human papillomavirus

Table IV.3 Patient characteristics at combination antiretroviral therapy initiation associated with incidence of first cancer stratified by cancer type, CFAR Network of Integrated Clinical Systems, 1996-2011

	Bivariable	Multivariable[†]
	Hazard Ratio (95% CI)	
Kaposi's Sarcoma		
Age at ART initiation (per 10 year increase)	1.00 (0.84-1.19)	0.99 (0.82-1.21)
Calendar year of starting ART	1.03 (0.99-1.07)	1.01 (0.97-1.07)
Prior mono- or dual-therapy	1.00 (0.70-1.43)	1.34 (0.91-1.99)
CD4 count (per 100 cells/mm^3)	0.64 (0.56-0.73)	0.63 (0.54-0.73)
Viral load (per log_{10} copies/ml)	1.79 (1.42-2.27)	1.31 (1.01-1.70)
Lymphomas		
Age at ART initiation (per 10 year increase)	1.29 (1.07-1.55)	1.30 (1.07-1.58)
Calendar year of starting ART	0.98 (0.93-1.03)	0.97 (0.91-1.02)
Prior mono- or dual-therapy	0.95 (0.64-1.41)	0.86 (0.55-1.33)
CD4 count (per 100 cells/mm^3)	0.81 (0.72-0.91)	0.80 (0.71-0.91)
Viral load (per log_{10} copies/ml)	1.07 (0.85-1.36)	0.85 (0.65-1.11)
HPV-related cancers		
Age at ART initiation (per 10 year increase)	1.33 (0.99-1.79)	1.41 (1.04-1.93)
Calendar year of starting ART	0.96 (0.88-1.04)	1.00 (0.91-1.10)
Prior mono- or dual-therapy	2.33 (1.32-4.13)	2.67 (1.41-5.04)
CD4 count (per 100 cells/mm^3)	0.83 (0.69-0.99)	0.80 (0.65-0.97)
Viral load (per log_{10} copies/ml)	1.13 (0.78-1.65)	1.05 (0.69-1.61)
Non-lymphoma, HPV-unrelated NADCs		
Age at ART initiation (per 10 year increase)	2.34 (2.01-2.74)	2.33 (1.97-2.74)
Calendar year of starting ART	1.01 (0.96-1.06)	1.00 (0.95-1.06)
Prior mono- or dual-therapy	1.19 (0.85-1.69)	1.15 (0.78-1.70)
CD4 count (per 100 cells/mm^3)	0.99 (0.91-1.07)	0.99 (0.89-1.09)
Viral load (per log_{10} copies/ml)	0.95 (0.77-1.18)	0.93 (0.73-1.19)

[†]Multivariable analyses adjusted for other covariates listed as well as CNICS study site, sex/MSM, race, and IDU

ART=combination antiretroviral therapy, MSM=men who have sex with men, IDU=injection drug user, HPV=human papillomavirus, NADC=non-AIDS-defining cancer

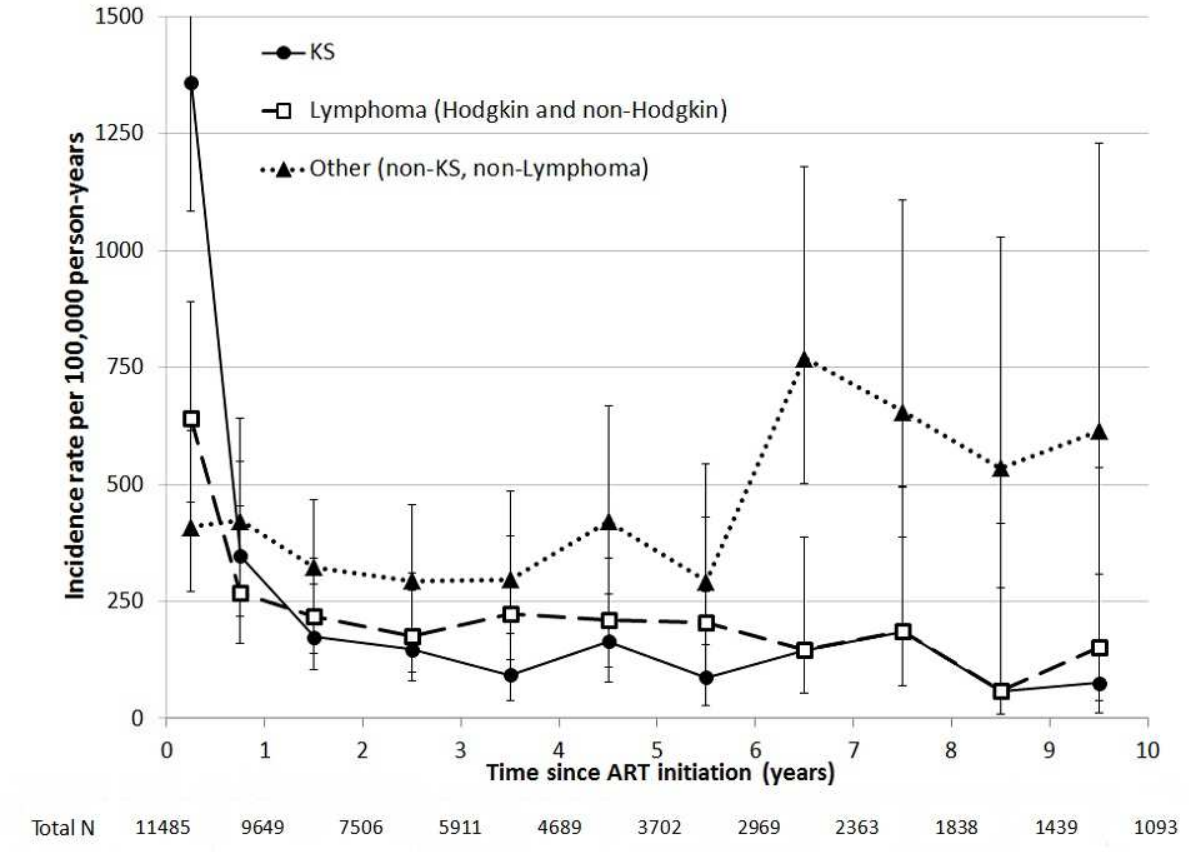

Figure IV.1 Incidence of first cancer across time following initiation of combination antiretroviral therapy (ART), CFAR Network of Integrated Clinical Systems, 1996-2011.

After ART initiation, incidence rates were estimated in the first six months, the second six months, and every year thereafter. The vertical lines extending from each incidence rate estimate represent the 95% confidence interval. Listed below the x-axis are the total numbers of patients remaining in follow-up at the end of each year. Solid line with circles=Kaposi sarcoma incidence; Dashed line with squares=Lymphoma incidence; Dotted line with triangles=Incidence of non-Kaposi sarcoma, non-lymphoma cancers. KS=Kaposi sarcoma, ART=combination antiretroviral therapy.

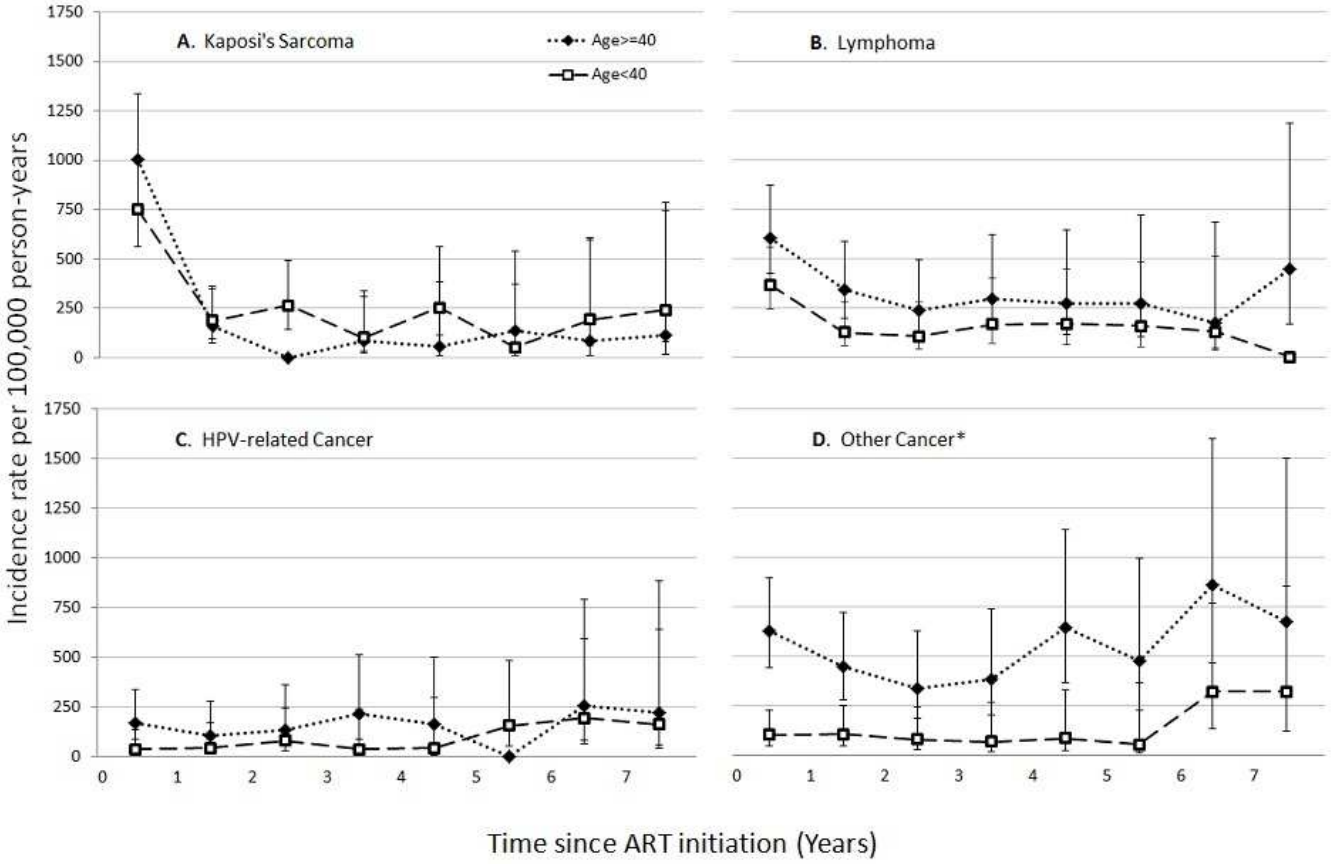

Figure IV.2 Cancer incidence across time following initiation of combination antiretroviral therapy (ART) stratified by age at ART initiation, CFAR Network of Integrated Clinical Systems 1996-2011.

Graphs divided by cancer type: (A) Kaposi sarcoma, (B) Lymphoma, (C) HPV-related cancer, (D) Other cancers. Dotted lines with diamonds=incidence rates among those 40 years of age or older at ART initiation. Dashed lines with squares=incidence rates among those younger than 40 years of age at ART initiation. *Other cancers include lung, liver, prostate, breast, melanoma, colorectal, kidney, bladder, brain, esophagus, larynx, leukemia, multiple myeloma, ovary, pancreas, peritoneum, small intestine, soft tissue, stomach, testicular, uterus, or non-squamous cell oral cavity/pharynx

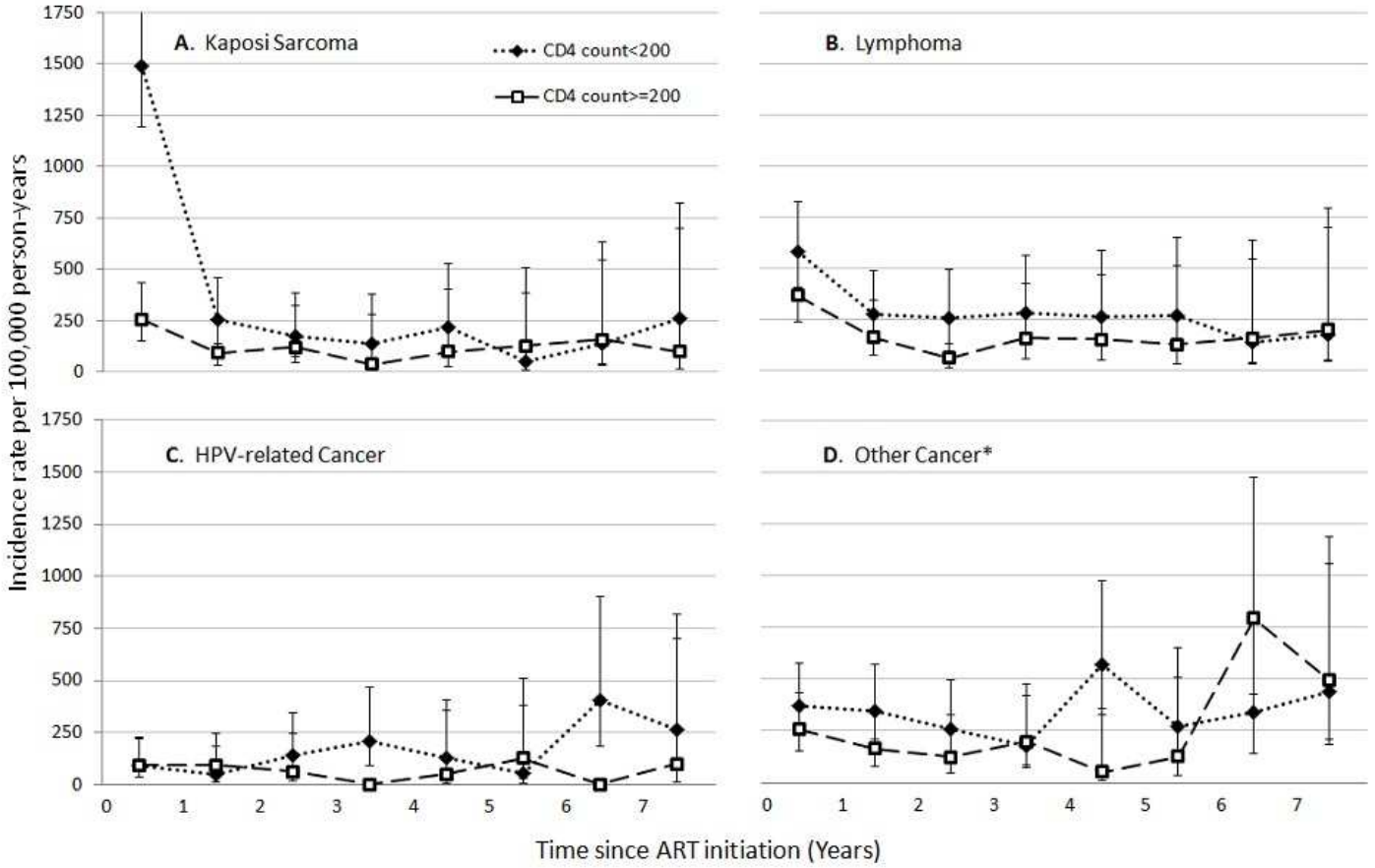

Figure IV.3 Cancer incidence across time following initiation of combination antiretroviral therapy (ART) stratified by CD4 count at ART initiation, CFAR Network of Integrated Clinical Systems 1996-2011.

Graphs divided by cancer type: (A) Kaposi sarcoma, (B) Lymphoma, (C) HPV-related cancer, (D) Other cancers. Dotted lines with diamonds=incidence rates among those with CD4 counts less than 200 cells/mm^3 at ART initiation. Dashed lines with squares=incidence rates among those with CD4 counts greater than or equal to 200 cell/mm^3 at ART initiation. *Other cancers include lung, liver, prostate, breast, melanoma, colorectal, kidney, bladder, brain, esophagus, larynx, leukemia, multiple myeloma, ovary, pancreas, peritoneum, small intestine, soft tissue, stomach, testicular, uterus, or non-squamous cell oral cavity/pharynx

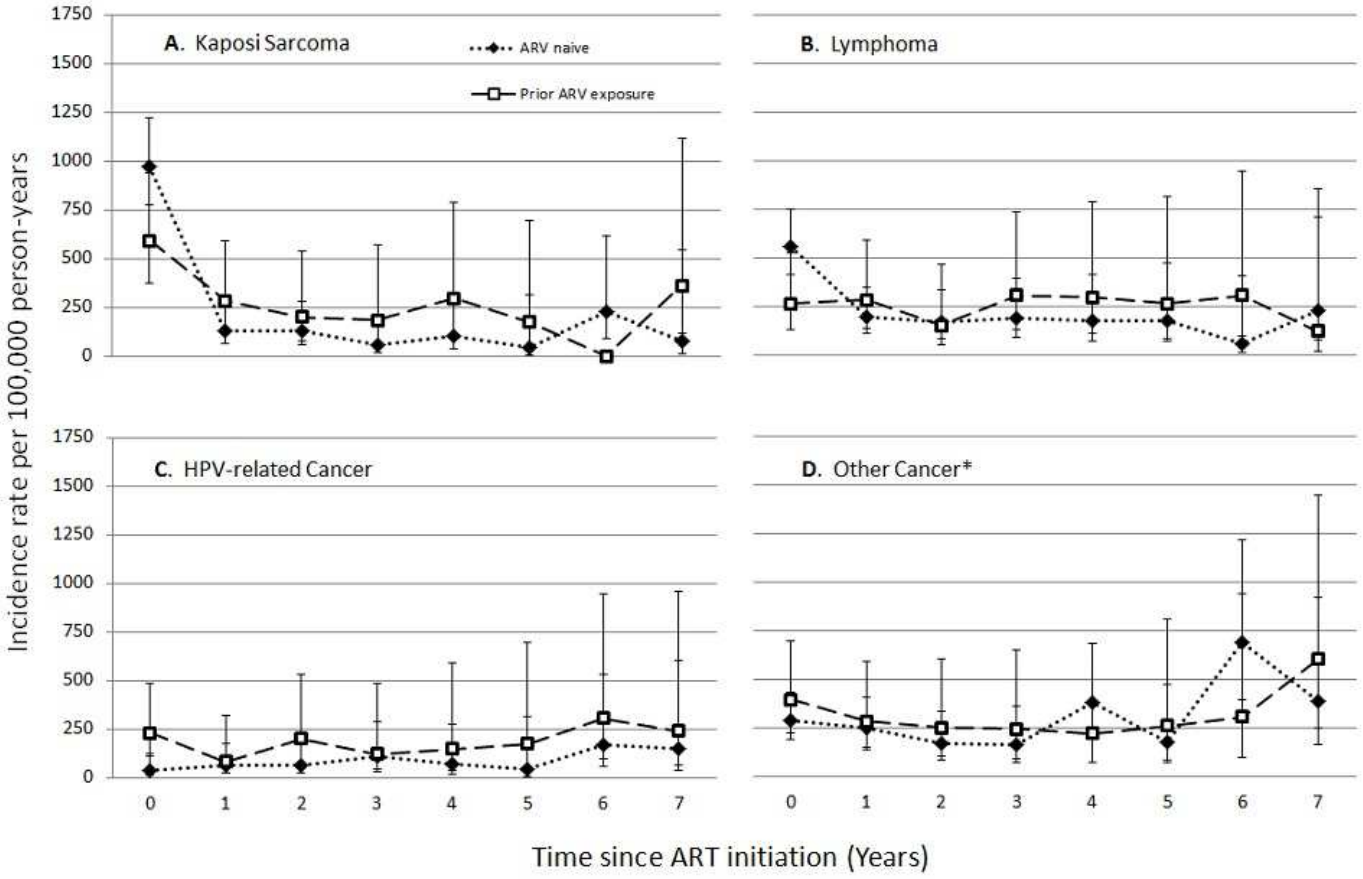

Figure IV.4 Cancer incidence across time following initiation of combination antiretroviral therapy (ART) stratified by antiretroviral history at ART initiation, CFAR Network of Integrated Clinical Systems, 1996-2011.

Graphs divided by cancer type: (A) Kaposi sarcoma, (B) Lymphoma, (C) HPV-related cancer, (D) Other cancers. Dotted lines with diamonds=incidence rates among those who were antiretroviral naïve at ART initiation. Dashed lines with squares=incidence rates among those with prior exposure to mono or dual-therapy or an unknown antiretroviral history at ART initiation. *Other cancers include lung, liver, prostate, breast, melanoma, colorectal, kidney, bladder, brain, esophagus, larynx, leukemia, multiple myeloma, ovary, pancreas, peritoneum, small intestine, soft tissue, stomach, testicular, uterus, or non-squamous cell oral cavity/pharynx

V. RESULTS: RELATIONSHIP OF IMMUNOLOGIC RESPONSE TO ANTIRETROVIRAL THERAPY WITH NON-AIDS-DEFINING CANCER INCIDENCE, CFAR NETWORK OF INTEGRATED CLINICAL SYSTEMS

A. Introduction

Among HIV-infected individuals the burden of non-AIDS-defining cancers (NADCs) is increasing[6], with malignancies such as lung cancer, anal cancer, and Hodgkin lymphoma contributing to substantial morbidity and mortality[6, 7, 59]. This is largely due to the aging of the HIV population[60, 93] as well as a higher prevalence of behavioral cancer risk factors such as tobacco use, alcohol use, and sexual behaviors[24]. However, HIV infection and the resultant immune suppression may also increase cancer risk[17, 94]. More severe immunosuppression, as quantified through measures such as nadir CD4 and current CD4, is associated with greater incidence of several NADCs[17, 35, 52, 53]. We would expect effective antiretroviral therapy (ART) to reduce NADC risk, but prior studies have not found consistent associations between ART use and lower NADC incidence[3, 62, 63, 93]. These inconsistencies may partly be due to differences in the effectiveness of ART between patients. Immunologic response to ART is likely a major mediator of ART effects on NADC incidence.

While patterns of cancer incidence differ over time after ART initiation[50, 95], the reasons for these patterns are not well understood, including the impact of immunologic ART response. We hypothesized that a robust and prolonged immunologic response to ART would correlate with lower NADC incidence, and that this association would be more apparent after a lag time of 6-12 months. We evaluated this hypothesis among HIV-infected

patients initiating a first ART regimen in the Centers for AIDS Research Network of Integrated Clinical Systems (CNICS) between 1996 and 2011.

B. Methods

Study Population

CNICS is a network of eight clinical U.S. HIV cohorts that collects data from HIV-infected patients 18 years of age or older through electronic medical records[72]. CNICS includes detailed information from 1995 to the present on antiretroviral treatment, laboratory measures, demographics, and diagnoses, including cancer diagnoses which have been ascertained and verified through a standardized data collection process[23]. Each CNICS site obtained local institutional review board approval and the current study was approved by the University of North Carolina institutional review board.

We included patients who initiated a first combination ART regimen, defined as ≥3 different antiretrovirals, at one of the eight CNICS sites between Jan. 1, 1996 and Aug. 30, 2011. Among these patients, we included those who: 1) had a CD4 count and HIV RNA measure within 12 months prior to ART initiation; 2) were alive for more than six months after ART initiation; and 3) had at least one CD4 count and one HIV RNA measure within the first six months after ART initiation.

Measures of Immunologic ART Response

Several different measures were used to characterize immunologic ART response based on CD4 cell count values obtained as a part of routine clinical care. Six-month CD4 count was used as a time-fixed measure of early immunologic response and was defined as the latest CD4 measurement taken within the first six months after ART initiation. Latest

CD4 count was used as a time-varying measure of immunologic response and was updated whenever a patient had a new CD4 count result. Finally, CD4 count-years was used as a time-varying measure of cumulative immunologic response. CD4 count-years 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 count measurements. Specifically, the accumulation of CD4 count-years is calculated by multiplying the average of two consecutive CD4 counts by the time interval between the two counts and then summing the values across all intervals between CD4 counts. Similar methods have previously been used to calculate cumulative HIV viremia[75, 76]. As an example, a patient with a CD4 count of 300 for the entire first year after ART initiation, and a patient with a CD4 count of 200 for the first six months and a CD4 count of 400 for the second six months, would both have accumulated 300 CD4 count-years one 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 also 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 measurement of immunologic ART response.

Statistical Analysis

Patient characteristics at ART initiation and patterns of immunologic response following ART initiation were described. At-risk time for NADC incidence started at six months post-ART initiation to avoid the inclusion of cancers that developed before ART initiation and to allow time for at least one CD4 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 still allow a minimum of six months to assess immunologic measures. Patients remained in follow-up irrespective of ART changes or interruptions and were censored at the first of: death, loss-to-follow-up (>12 months without a clinic visit), last date of cancer ascertainment for CNICS site (range: May 31, 2010 until Aug. 31, 2011), and administrative censoring 10 years after ART initiation, when <15% of the study population was still in follow-up.

Cox proportional hazards regression was used to estimate hazard ratios and 95% confidence intervals as measures of the association of immunologic response with NADC incidence. Associations were considered with all NADCs, NADCs known to be related to viral co-infections (HPV, EBV, HBV, and HCV) (anal, Hodgkin lymphoma, liver, squamous cell oral cavity/pharynx, penis, and vagina/vulva cancers)[14, 23], and virus-unrelated NADCs. To evaluate the proportional hazards assumption, product terms with log(time) were assessed. Multivariable Cox regression was used to adjust for time-fixed confounders measured at ART initiation, including CD4 count, age, prior antiretroviral use, calendar year, race, sex, transmission risks, and CNICS site.

Inverse-probability weights were applied to account for time-varying confounding that may be introduced by the close correlation of immunologic response with virologic response, as measured through HIV RNA measurements from the two clinical visits prior to CD4 count measurement[80, 82, 83]. In addition, inverse-probability-of-censoring weights were applied to account for differential censoring by prior HIV RNA measurements, and inverse-frequency weights were applied to account for differences in the frequency of obtaining CD4 count tests that might potentially bias exposure assessment. For each observation, all calculated weights were multiplied together to create a single weight that was used in the regression models. The mean total weight was 1.02 (SD=0.79).

All immunologic measures were assessed separately as predictors in bivariable and weighted, multivariable regression models that accounted for both time-fixed and time-

varying confounding. Associations were also estimated among patients with a CD4 count at ART initiation <200 cells/mm^3 and with a CD4 count at ART initiation ≥200 cells/mm^3 to assess effect measure modification. To determine which immunologic measure was the strongest independent predictor, AICs were calculated for each multivariable regression model and compared to assess the relative model fit.

Sensitivity analyses were conducted among patients without NADC diagnoses prior to six months of ART and among patients who were antiretroviral naïve at ART initiation. All statistical analyses were conducted using SAS version 9.2.

C. Results

Patient Characteristics

Of the 25,337 patients enrolled in CNICS at the time of this study, 11,485 initiated a first combination ART regimen at a CNICS site between 1996 and 2011 and had CD4 count and HIV RNA measures at ART initiation. Among these 11,485 patients, 9,389 were alive and had obtained ≥1 HIV RNA level and CD4 count at six months after ART initiation.

Of the 9,389 patients included, 20% were female, 43% white, and 41% black (Table V.1). The median age at ART initiation was 38 years (IQR: 32-45), and the median calendar year of ART initiation was 2004 (IQR: 2000-2007). Most patients initiated a PI-based (47%) or NNRTI-based (43%) ART regimen, while a minority initiated either a regimen with both a PI and a NNRTI (4%), a triple-NRTI regimen (5%), or a regimen including an entry or integrase inhibitor (2%). At first combination ART initiation, 27% of patients had evidence of prior ART exposure, including single or dual antiretroviral therapy use. The median HIV RNA level at ART initiation was 4.8 $\log_{10}$ copies/ml and by six months post-ART initiation 64% of patients were virologically suppressed (<400 copies/ml).

After six months post-ART initiation, 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 685 deaths occurred, and 2,339 patients were lost-to-follow-up. Follow-up was censored for 54% of patients at the last date of cancer ascertainment. By 10 years after ART initiation, 1,117 patients were still in follow-up.

Immunologic response

The median CD4 count at ART initiation was 200 cells/mm^3 (IQR: 60-332). After ART initiation, patients contributed a median of 9 CD4 count measurements (IQR: 4-17). The median CD4 count was 260 cells/mm^3 (IQR: 121-419) during the first six months after ART initiation, 324 cells/mm^3 (IQR: 182-504) during the second six months and 358 cells/mm^3 (IQR: 213-539) during months 12-18 after ART initiation. From two years post-ART initiation and afterward, the distribution of latest CD4 count values was stable. Figure V.1 demonstrates this over the first five years of ART. Patients accumulated a median of 1925 cells*years/mm^3 (IQR: 1239-2686) by the 54-60 month time interval after ART initiation equivalent to having an average CD4 count of 385 cells/mm^3 over five years (Figure V.1). By ten years after ART initiation, the median CD4 count-years accumulated was 4572 cells*years/mm^3 (IQR: 3144-6152) equivalent to having an average CD4 count of 457 cells/mm^3 per year over ten years.

Immunologic response and NADC incidence

In total, 164 NADCs were diagnosed at a median of 3.8 years after ART initiation (IQR: 2.0-6.8). 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 V.2).

In bivariable analyses, six-month CD4 count, latest CD4 count, and CD4 count-years were all associated with NADC incidence (Table V.3). When examined by type of NADC, all immunologic measures were strongly associated with virus-related NADCs. In weighted multivariable regression a 100 cell/mm^3 increase in a patient's six-month CD4 count was associated with a 29% lower hazard of virus-related NADC incidence (95%CI: 5%-47%) (Table V.3). Similarly, a 100 cell/mm^3 increase in the latest CD4 count was associated with a 30% lower hazard of virus-related NADC incidence (95%CI: 11%-45%). Finally, a 200 cells*years/mm^3 increase in CD4 count-years was associated with an 8% lower hazard of virus-related NADC incidence (95%CI: 1%-15%). The CD4 count-years association with virus-related NADC incidence was stronger among patients with a CD4 count at ART initiation <200 cells/mm^3 (Adjusted HR: 0.89, 95%CI: 0.82-0.98) compared to patients with a CD4 count at ART initiation ≥200 cells/mm^3 (Adjusted HR: 0.94, 95%CI: 0.81-1.08). Associations with other measures were not strongly modified by CD4 count at ART initiation. No immunologic measures were associated with virus-unrelated cancer incidence in bivariable or weighted multivariable analysis, even though precision was better in this group with a larger number of cancer events (Table V.3).

All measures were assessed with 6-month and 12-month exposure lags. Latest CD4 count was less strongly associated with virus-related NADCs that occurred more than six months after the CD4 count measurement (Adjusted HR with 6-month exposure lag: 0.82, 95%CI: 0.73, 0.93; Table V.3). CD4 count-year associations with virus-related NADCs were slightly stronger with increased exposure lag time (Adjusted HR with 6-month exposure lag: 0.88; with 12-month exposure lag: 0.85; Table V.3), while six-month CD4 associations were similar in all analyses. Associations with virus-unrelated NADCs were not identified.

When the AICs from the separate models for each of the three immunologic response measures were compared, the AIC was lowest for the model using latest CD4 count both in bivariable and multivariable analyses (AICs: latest CD4=1070.6, six-month CD4=1075.0, CD4 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 six-month post-ART CD4 count (6-month lag AICs: latest CD4=975.8, six-month CD4=972.6, CD4 count-years=974.0), indicating that this measure was the strongest predictor of virus-related NADCs occurring at least six 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 to the reference category of CD4 count-years less than 600 cells*years/mm^3, CD4 count-years between 600-1600 cells*years/mm^3 had a HR of 0.83 (95%CI: 0.41, 1.65) and CD4 count-years above 1600 cells*years/mm^3 had a HR of 0.30 (95%CI: 0.10, 0.85) for virus-related NADCs (Figure V.2).

Similar associations were observed in sensitivity analyses in which only the 6,876 antiretroviral naïve patients were included, and in which 382 patients with NADC diagnoses prior to six month post-ART initiation were excluded. Of the 382 patients excluded with prior NADC diagnoses, 10 had another cancer diagnosis after six months of ART.

D. Discussion

In this study, a greater immunologic ART response was associated with lower NADC incidence, specifically for NADCs related to viral co-infections (HPV, EBV, HBV, or HCV). This association was independent of CD4 count at ART initiation, age and other patient factors considered, and was consistently observed for measures of early, time-varying, and

cumulative time-varying immunologic response to ART. Inverse associations were specific to virus-related NADCs, while 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 count-years were used as a novel measure to capture both the degree and duration of immune response. Such a measure may be particularly relevant to virus-related NADCs as longer periods of immune suppression may provide greater susceptibility to acquisition, reactivation, or persistence of oncogenic viruses. Importantly, the magnitude of the association of CD4 count-years with virus-related NADC incidence increased with greater exposure lags indicating that the degree of immune suppression close to the time of cancer presentation may be less important than overall duration, likely reflecting oncogenesis which may occur over many years through accumulative precancerous intermediate steps. While these associations were independent of immune status at ART initiation, cumulative immunologic ART response was most protective among patients with a CD4 count less than 200 cells/mm^3 at ART initiation. This relationship highlights the potential importance of effective ART for severely immunosuppressed as a cancer prevention strategy.

Latest CD4 count was the strongest predictor of virus-related NADC incidence. Although this may reflect immunosuppression facilitating a transition from subclinical precancer to overt clinical cancer, greater immune suppression proximal to cancer diagnosis may conversely also be a result of immune dysregulation caused by sub-clinical cancer. This is specifically a concern for Hodgkin lymphoma, which is known to decrease T-cell populations in HIV-uninfected populations[47, 96-98]. While subclinical cancer may have effects on the latest CD4 count before diagnosis, it is less likely to impact CD4 measures less proximal to cancer diagnosis. When cancer diagnoses were excluded that occurred

less than 6 and 12 months after measurement of immunologic response, all immunologic measures remained associated, but six-month CD4 count became the strongest predictor of virus-related NADC incidence. The strong association of early immunologic response as captured by the six-month post-ART CD4 count may indicate that the initial immune reconstitution from the most severe immunosuppression levels is the most important component part of the immunologic ART response for reducing risk of virus-related NADCs. While we only explored exposure lags up to 12 months, it is also possible that associations would be stronger with longer exposure lags.

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 counts below 200 cells/mm^3 and latest CD4 count were both associated with viral coinfection-related NADC risk[69]. Latest CD4 count has been shown to be associated with virus-related cancer incidence in a number of studies including both ART-treated and ART-naïve populations, and these associations have been attributed to the increased risk of oncogenic infection[17, 53, 99]. However, as our results suggest, separating adverse immunologic effects of subclinical malignancy from effects of CD4 lymphopenia resulting solely from HIV may be difficult.

The interaction between inadequate immune recovery and HPV-associated dysplasia and malignancy may be of particular interest. Low CD4 has been found to increase the risk of developing cervical cytologic abnormalities[43, 46]. However, effective ART has not been convincingly associated with decreased risk of cervical[100] or anal dysplasia[101]. Multiple confounding factors including competing risks (i.e. prolonged survival for those on ART which may allow greater opportunities for the development of dysplasia and cancer) and cumulative immunologic experience 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, including the impact of cumulative immunologic history as considered in our study.

Given the large multi-site clinical cohort from which our results were drawn, these findings should be largely generalizable to U.S. HIV patients. In addition, the use of comprehensive clinical data on CD4 count and HIV RNA measurements with recently verified cancer diagnosis[23, 102], and advanced analytic methods allowed us to accurately estimate cumulative and time-updated immunologic measures and adequately account for possible 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 to other immunologic measures in a U.S. HIV-infected population.

A number of limitations of our study should be considered. With the exception of hepatitis, we could not confirm the presence of oncogenic viral infection at the time of virus-related NADC diagnosis, and for some cancers in this category, tumors in individual patients may not be associated with oncogenic viruses (ex. oral pharyngeal squamous cell carcinoma associations with HPV). If a poorer immunologic response increases the risk of viral oncogenesis, then associations found here would likely be stronger for confirmed virus-associated cancers. We also had limited information on behavioral risk factors for cancer, such as tobacco and alcohol use. However, a previous sub-study within CNICS did not demonstrate associations of patient-reported tobacco and alcohol use with ART adherence, and thus these are unlikely to confound associations of immunologic response measures with NADC incidence[103]. Information was only available while patients attended a CNICS site. We could not fully capture a patient's immunologic experience prior to ART initiation, though we did adjust for CD4 count at ART initiation. Additionally, one-fourth of patients were lost-to-follow-up and may have experienced different patterns of cancer incidence than those observed.

In sum, our findings demonstrate that multiple components of the immune response after ART initiation may influence risk for virus-related NADCs. In our study, associations persisted after adjusting for CD4 at ART initiation, indicating that, beyond the effects of immune status at ART initiation, changes in immune status after ART initiation may impact virus-related NADC risk but not that of hitherto non-virus associated NDACs. This highlights the importance of adherence to effective, durable ART which has the demonstrated benefit of reducing cancer risk for infection associated cancers in a population which is at increased risk to experience repeated episodes of infection with oncogenic viruses.

Table V.1 Demographic and clinical characteristics of 9389 patients with six months of follow-up after initiating combination antiretroviral therapy in the CFAR Network of Integrated Clinical Systems, 1996-2011

Characteristic	**N (%)**
Total	9389
Female Sex	1900 (20.2)
Age[†]	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)
Men who have sex with men	5192 (55.3)
Antiretroviral exposure prior to first ART	2513 (26.8)
ART initiation year[†]	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[‡]	140 (1.5)
HIV RNA at ART initiation (log_{10}copies/mL)[†]	4.8 (4.3-5.4)
HIV RNA <400 copies/mL at 6 months post-ART	6016 (64.1)
CD4 count at ART initiation (cells/mm^3) [†]	200 (60-332)
CD4 count at 6 months post-ART (cells/mm^3)[†]	303 (160-466)

[†]Median (IQR) given instead of N (%)

[‡]includes regimens with an integrase inhibitor, fusion inhibitor, or entry inhibitor

ART=combination antiretroviral therapy, PI=protease inhibitor, NNRTI=non-nucleoside reverse transcriptase inhibitor, NRTI=nucleoside reverse transcriptase inhibitor

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

Type of non-AIDS-defining cancer	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[†]	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[‡]	35	3.0 (1.7-6.7)

[†]Other virus-related cancers include penis, vaginal, and vulva.

[‡]Other virus-unrelated cancers include bladder, esophagus, kidney, larynx, leukemia, multiple myeloma, ovary, pancreas, peritoneum, small intestine, soft tissue, testicular, thyroid uterus, or non-squamous cell oral cavity/pharynx

As follow-up started at 6 months after ART initiation, all included cancer diagnoses occurred at least 0.5 years after ART initiation.

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

Measures of immunologic ART response	Bivariable	Weighted, Multivariable[†]		
	No lag	No lag	6-month lag	12-month lag
	Hazard Ratio (95% CI)			
All NADCs	**(N=164)**		**(N=140)**	**(N=125)**
Six month CD4 (per 100 cells/mm^3)	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 (per 100 cells/mm^3)	0.87 (0.81, 0.93)	0.92 (0.77, 1.09)	0.88 (0.81, 0.96)	0.91 (0.84, 1.00)
CD4 count-years (per 200 cells*years/mm^3)	0.97 (0.94, 1.00)	0.98 (0.95, 1.02)	0.97 (0.93, 1.01)	0.95 (0.90, 1.01)
Virus-related NADCs[‡]	**(N=65)**		**(N=59)**	**(N=52)**
Six month CD4 (per 100 cells/mm^3)	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 (per 100 cells/mm^3)	0.78 (0.70, 0.87)	0.70 (0.55, 0.89)	0.82 (0.73, 0.93)	0.81 (0.71, 0.93)
CD4 count-years (per 200 cells*years/mm^3)	0.89 (0.84, 0.95)	0.92 (0.85, 0.99)	0.88 (0.80, 0.98)	0.85 (0.75, 0.97)
Virus-unrelated NADCs*	**(N=99)**		**(N=81)**	**(N=73)**
Six month CD4 (per 100 cells/mm^3)	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 (per 100 cells/mm^3)	0.93 (0.85, 1.00)	1.01 (0.89, 1.14)	0.93 (0.84, 1.03)	0.99 (0.88, 1.11)
CD4 count-years (per 200 cells*years/mm^3)	1.01 (0.97, 1.04)	1.01 (0.97, 1.05)	1.00 (0.96, 1.04)	0.98 (0.93, 1.04)

Each association with an immunologic ART response measure was estimated using a separate regression model.

[†]Total 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 count measurements. Multivariable analyses additionally adjusted for CD4 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.

[‡]Virus-related NADCs included squamous cell anal, Hodgkin lymphoma, liver, squamous cell oral cavity/pharynx, penis, vagina, and vulva cancer.

*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 non-squamous cell oral cavity/pharynx

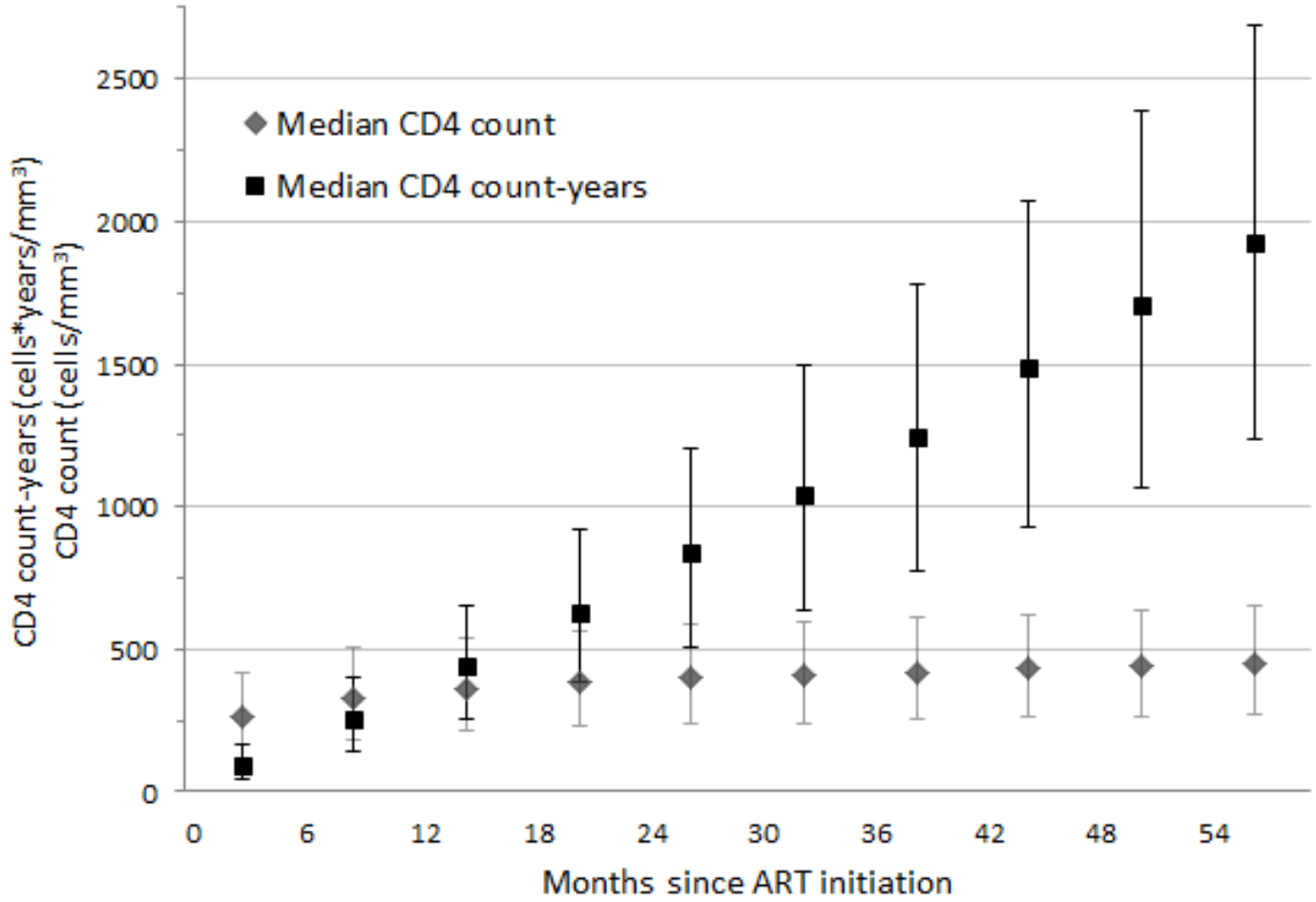

Figure V.1 Distribution of latest CD4 counts and CD4 count-years by six month time intervals following combination antiretroviral therapy (ART) initiation.
Symbol=median value, Lines=interquartile range.

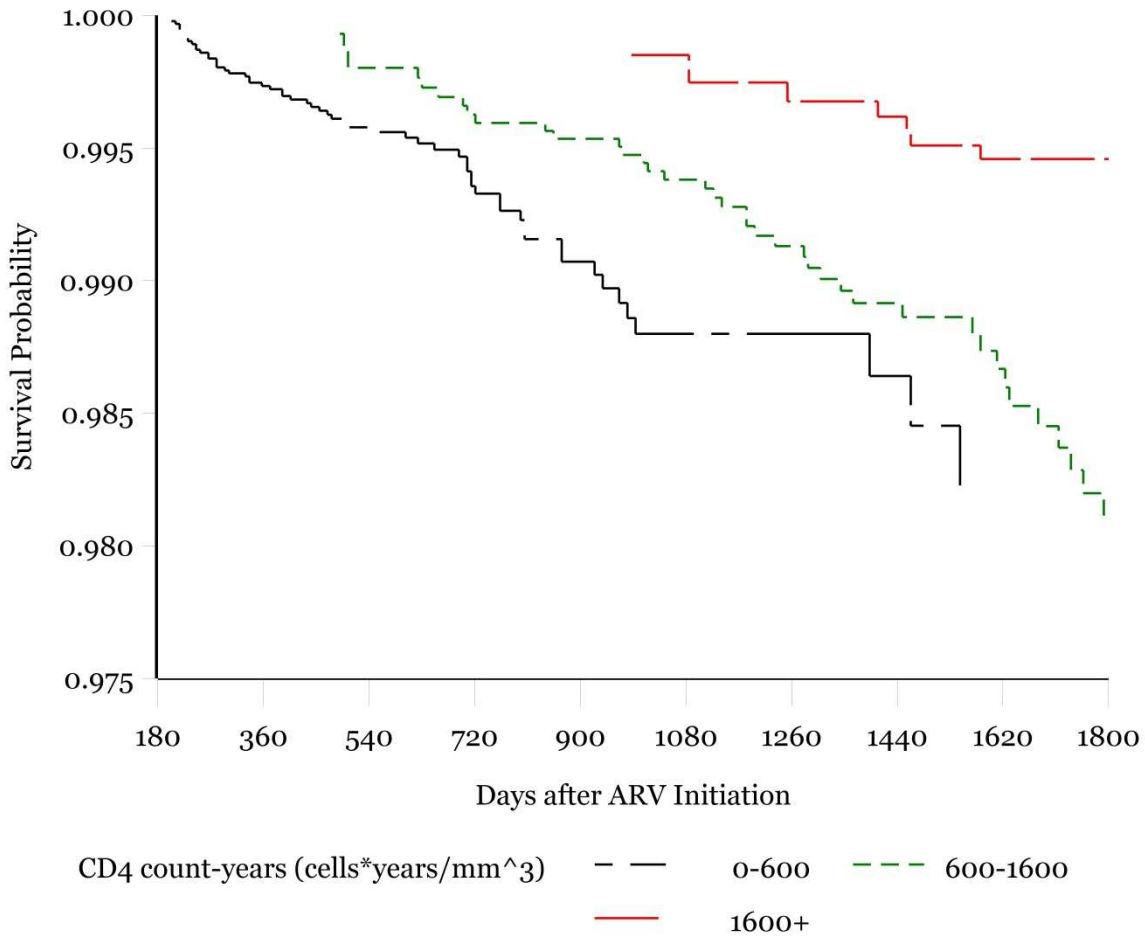

Figure V.2 Time to first virus-related NADC diagnosis by CD4 count-years category.

VI. DISCUSSION

A. Summary of Findings

In this multi-site U.S. observational clinical cohort of 11,485 HIV-infected patients followed up to 10 years a total of 457 cancer diagnoses were observed, 216 of which were non-AIDS-defining. Distinct patterns of cancer incidence following ART initiation were observed for different cancer types. This likely reflects the varying etiologic contributions of aging, immunosuppression, and prior antiretroviral exposure, to the occurrence of specific cancer types, as evidenced by associations of cancer incidence with patient characteristics at ART initiation. Specifically, KS incidence was strongly associated with CD4 count and HIV RNA at ART initiation, lymphoma incidence was associated with CD4 count and age at ART initiation, HPV-related cancer incidence was associated with CD4 count, age, and prior antiretroviral exposure at ART initiation, and other cancers were only associated with age at ART initiation. Incidence did not appear to change over calendar time for any cancer type among ART initiators suggesting that the incremental improvements in ART regimens during the modern ART era have not had dramatic effects on cancer incidence.

Among the 9389 patients in follow-up six months after ART initiation with at least one CD4 count and HIV RNA measurement by that time, a positive immunologic ART response was found to be associated with lower NADC incidence, specifically virus-related NADC incidence. This protective association was independent of CD4 count at ART initiation and was consistently found when assessing measures of short-term immunologic ART response, time-varying response, and cumulative time-varying response. However, all protective

associations were specific to virus-related NADCs, while no associations were found with virus-unrelated NADCs. Among the different immunologic response measures, latest CD4 count was most strongly predictive of virus-related NADC incidence and may be an important indicator for targeting cancer screening. However, immune status close to cancer diagnosis may be affected by subclinical cancer, and thus this may be a case of reverse causation. After 6-month and 12-month exposure lags were implemented, the association between latest CD4 count and virus-related NADC incidence was attenuated and CD4 count at six months post-ART initiation became the strongest predictor of virus-related NADC incidence. Thus a robust early immunologic response may be of particular importance for preventing subsequent virus-related NADCs.

B. Public Health Implications

Elucidating the relationship of antiretroviral use with the incidence of NADCs is clinically important for a number of reasons. The finding that initiation of ART at higher CD4 counts is associated with lower cancer incidence, particularly Kaposi sarcoma, lymphomas, and HPV-related cancers reinforces the benefits of early initiation of ART as has been indicated by previous studies[104, 105]. This also highlights the importance of HIV testing and linkage into care as important steps in ensuring opportunities for early ART initiation. Understanding the effects of antiretroviral treatment response on malignancies can also help in targeting at-risk groups that may benefit from more frequent screening or prevention interventions. Our study indicated that vigilance for Kaposi sarcoma and lymphoma is most important within the first year after ART initiation, and that screening interventions for other cancers may be best targeted after the first year on ART when patients are established on stable ART. We further identified that patients with a poor immunologic response to ART are at higher risk for virus-related NADC incidence, and

thus these patients may benefit the most from prevention interventions and increased screening for those virus-related NADCs for which effective screening tests currently exist (ex. anal pap smear for early detection of anal cancer[106, 107]).

Finally, a better understanding of the relationship between antiretroviral treatment response and cancer incidence may help elucidate the etiologic pathways that increase the risk of cancer among HIV patients. For instance, if better immunologic response reduces cancer incidence, but only after a certain lag time this may indicate that immunodeficiency during early HIV-infection may play an important role in the early stages of cancer even if later immune reconstitution is achieved. Our results showed reduced virus-related NADC incidence with a better immunologic response regardless of lag time, though the strength of associations differed. A poor immunologic response close to cancer diagnosis may be a result of subclinical cancer, but these associations may also be an indicator that immune suppression affects multiple steps in the oncogenic process to varying degrees. Understanding the interaction between the immune system and cancer risk may provide insights about cancer etiology that will also be applicable to the general population. For all of these reasons, examining the relationship between antiretroviral use and cancer helps to further both the HIV and cancer research fields.

C. Strengths

Our study improves upon previous studies of the relationship of ART use with cancer incidence because of a number of strengths. Firstly, we used a large collaboration of eight different HIV observational clinical cohorts from different regions of the United States. As these cohorts collect data through patient medical records, patients have little burden associated with study participation and the cohorts are less vulnerable to volunteer

and non-response bias[72, 108]. Together these characteristics make our findings broadly generalizable to the United States HIV clinical population.

Secondly, CNICS contains detailed information describing antiretroviral use, antiretroviral response, and other relevant variables. Previous studies were often limited in that they only had information available on antiretroviral use at the time of cancer diagnosis, or were only able to place patients into pre-ART or ART eras based on cancer diagnosis dates. The CNICS database is novel in that it contains information on individual antiretroviral history including precise dates of first antiretroviral exposure and dates of first ART regimen as well as information on the types of antiretrovirals contained in each regimen. Laboratory values of HIV viral load and CD4 counts are available whenever taken clinically which allows for estimates of HIV disease progression and immunologic function both prior to and during antiretroviral treatment. As a result, we were able to measure our exposure of interest in a number of ways including novel cumulative measures, such as CD4 count-years. Comparisons between the different measures of immunologic response allow us to better identify the aspects of the immunologic response that are most important for NADC risk.

Lastly, the use of a number of advanced analytic approaches improved upon work previously done to assess these associations. We used inverse probability weights to disentangle the effects of virologic and immunologic ART response, account for differential censoring, and account for differential frequency of laboratory testing. This method has not been commonly used in prior literature examining relationships between ART, immune status, and cancer risk. This potentially resulted in residual confounding in previous studies. We also incorporated exposure lags to account for the etiologic induction period between the effect of treatment response and cancer diagnosis. This method likely better captures causal associations between immunologic response and cancer incidence than estimates obtained with no exposure lag. These approaches combined with a rich data

resource enhance the quality of the inferences that can be made from the results of this study.

D. Limitations

While this research plan will provide new information about the relationship between ART, ART response, and cancer incidence through the use of a comprehensive data source and novel analytic methods, a number of limitations remain. First, substance use information is not fully captured in the CNICS data as it is often unreliably collected in medical records. In addition, the data that is collected on substance use does not include information on the intensity of use. As a result, these variables were not included in any of our analyses. Information on sexual risk behaviors is also not regularly collected beyond the identification of 'Heterosexual Sex' or 'Men who have sex with men' as possible transmission risk factors. While risk factors such as sexual behaviors and substance use may be strongly associated with risk for specific cancers, they have not been found to be associated with virologic and immunologic ART response[103]. However, the potential influence of residual confounding due to these factors must be acknowledged. A subset of individuals within the CNICS cohort have consented to complete full assessments of patient-reported outcomes including questions about transmission risk behaviors and tobacco, alcohol, and drug use. In the future, this subset could be used to inform the validity of measures regularly collected for the larger cohort and evaluate the appropriateness of relying on these measures for analyses in the full cohort.

Another potential weakness of this study population is that information can only be collected on patients for as long as they are attending one of the CNICS study clinics. As a result, limited information is available prior to the initiation of HIV care and baseline values for HIV RNA and CD4 cell count are our best measures of HIV progression prior to ART

initiation. Moving to a different geographic area or deciding to receive HIV care from another facility results in loss-to-follow-up. In addition, because information is collected through medical visits, the frequency of data collected for each participant is dependent upon their frequency of medical appointments and visit attendance. Previous studies of visit attendance and retention into care at the University of North Carolina site, demonstrated that retention and visit attendance may be influenced by such factors as age, AIDS diagnosis, and proximity to the clinic[73, 109]. However, the majority of patients continue to receive care at the clinic for a number of years. Overall, the 6-year probability of retention was 67% (95% CI: 65, 70%), similar to other HIV longitudinal studies[73]. As previously mentioned, inverse probability of censoring weights were applied to account for differential loss-to-follow-up, and inverse probability of frequency weights were applied to account for differences in the frequency of collection of CD4 count measurements.

Our study also did not account for adherence or discontinuations of ART after initiation. As such, exposures such as immunologic response may be driven both by the efficacy of the ART regimen and the adherence to the regimen. While our approach may correctly estimate the causal association of immunologic response measures with NADC incidence, the resulting estimate does not correspond to a particular intervention. In this study, we assume that adherence interventions to improve immunologic response and advances in treatment to improve immunologic response would have equivalent effects on NADC incidence. However, this consistency assumption may be violated, as different interventions could potentially be used to improve a patient's immunologic ART response and the resultant effects on NADC incidence may not be uniform across all interventions[110]. Specific interventions for improving immunologic response should be further evaluated to assess their impact on NADC incidence.

The final limitation of this study is the limited ability to look at specific cancer types, due to the small numbers of cancer cases for individual types of cancer. We did not have

adequate power to detect effects for individual cancer types and thus focused on using larger categories of grouped cancer types as the outcomes of interest. It must be acknowledged that specific cancers within cancer groups may have differing associations with the exposures of interest we considered. In addition, the length of the etiologic induction period can differ widely between cancer types. However, cancer types were categorized in biologically and epidemiologically relevant ways in order to best mitigate this limitation.

E. Future Directions

A number of new studies may be undertaken to further expand upon our results. In addition to the immunologic response associations examined here, we plan to use similar techniques in order to assess associations between virologic response and NADC incidence. Similar to the measures used for immunologic response, we will assess six month viral load, most recent viral load, and viremia copy-years[75]. Preliminary results from these analyses can be found in Appendix C. In this preliminary analysis viremia copy-years are accumulated starting at ART initiation; however, in further planned sensitivity analyses we will limit our patient population to those who have achieved virologic suppression by six months after ART initiation and then measure viremia copy-years starting at six months after ART initiation. Through this method, we can more specifically estimate the effect of increases in viral load after initial successful viral suppression. This may more specifically assess the potential effects of differences in patient adherence to their ART regimens. In addition, exposure lag times and categorical parameterizations of our virologic response measures will be considered such as was done with the analysis of immunologic response.

Another future study of importance will be evaluating the trajectories of CD4 count and viral load measures shortly before cancer diagnosis. This has previously been done in

a study looking specifically at CD4 count trajectories before diagnosis of Hodgkin lymphoma. This study identified statistically significant decreasing trends in CD4 count and identified this as evidence of Hodgkin lymphoma having a deleterious effect on CD4 count. In our study, we hypothesized that this may account for the particularly strong associations between latest CD4 count and virus-related NADC incidence. However, these trends have not been verified in our data, and prior studies have not examined the trajectories of CD4 count or HIV viral load among other types of cancer cases. Using the CNICS data, a case-crossover study could be conducted to compare trajectories during the time immediately before cancer diagnosis (within 6 months or a year of diagnosis) to trajectories during other HIV-infected time periods in the same patient[111, 112]. While such an analysis could not confirm a causal effect of cancer on immunologic or virologic response, it could generate hypotheses that could be further examined in a laboratory setting.

We have not currently evaluated the effects of differences in the contents of ART regimens. Boosted-PIs have been hypothesized to lower cancer risk[113], but these findings should be confirmed and could be evaluated in CNICS or other cohorts with detailed information on antiretroviral medications. Additionally, specific antiretrovirals have been hypothesized to have effects on cancer risk such as the protease inhibitors nelfinavir[64, 65] and ritonavir/saquinavir[66]. Other studies have not shown associations between cancer incidence and antiretroviral class[67, 68], but these associations could be evaluated in further analysis within CNICS.

In addition to evaluating risk factors for cancer incidence, further work should identify clinical characteristics associated with poor outcomes among cancer cases. Some of this work has previously been conducted in CNICS[23], but further studies could better evaluate associations between antiretroviral exposure, time-varying CD4 counts, and time-varying HIV RNA measures with survival and remission following a cancer diagnosis.

F. Conclusions

This study demonstrated that different cancer types have unique patterns of incidence over time after ART initiation. Incidence is predicted by a number of patient characteristics at ART initiation as well as immunologic response to ART. KS and lymphoma had high incidence in the first six months largely driven by poor immune status at ART initiation. Particularly in the case of KS, burden may be reduced substantially if HIV-infected individuals are diagnosed and connected to clinical care before they reach CD4 counts below 200 cells/mm^3. Incidence for other cancers increased over time from ART initiation, indicating the importance of screening and prevention of cancer throughout clinical care. Interventions for particular cancers may be targeted based on the differing distributions of cancer types across time after ART initiation. For instance, screening for NADCs may be best targeted after a year on a stable ART regimen.

Calendar year of ART initiation did not affect the incidence rates of any cancer, after accounting for differences in patient clinical and demographic characteristics over time. This may indicate that improvements in ART regimens and general HIV clinical care have not substantially changed cancer risk. As a result, we can expect that cancer will continue to be a leading cause of morbidity and mortality in the near future and that the establishment of guidelines for cancer prevention and screening in the HIV population will be essential.

While general changes to clinical care may not have had a detectable impact, a better immunologic response to ART is strongly associated with reduced incidence of virus-related NADCs, even after accounting for CD4 count at ART initiation. A robust immunologic response likely reduces the risk of acquisition of oncogenic viruses, as well as potentially reducing the persistence or loss of control of latent oncogenic viruses. Immunologic response within the first six months after ART initiation was the strongest predictor of lower virus-related NADC risk after accounting for a lag in the effect of

immunologic response. This initial response is likely most important because patients are typically recovering from the most severe immunosuppression during this time. This also highlights the importance of adherence to effective ART for the prevention of virus-related NADCs. This coupled with further cancer prevention measures and increased screening can reduce the morbidity and mortality attributable to cancer in the HIV population.

APPENDIX A: SENSITIVITY ANALYSES FOR CHAPTER IV

For chapter IV, sub-analyses were conducted among patients who were antiretroviral naïve at the initiation of their first combination ART regimen. Baseline characteristics are given among patients who were antiretroviral naïve and patients with prior antiretroviral exposure at ART initiation in Table A.1. Associations with cancer incidence among the sub-population of antiretroviral naïve patients are given in Table A.2. The antiretroviral naïve population had a higher proportion of female patients, and Hispanic patients, and patients initiating an NNRTI-based ART regimen. These patients also initiated ART in later years, and had higher CD4 counts and HIV RNA concentrations at ART initiation. However, the associations between patient characteristics and cancer incidence in this subpopulation did not differ noticeably from the results found in the full population including antiretroviral naïve and antiretroviral experience patients.

Table A.1 Demographic and clinical characteristics of 11,485 patients initiating combination antiretroviral therapy stratified by prior antiretroviral experience in the CFAR Network of Integrated Clinical Systems, 1996-2011

Characteristic	N (%)	ARV Naive	ARV experienced
Total	11485	8278	3207
Female Sex	2304 (20.1%)	1579 (19.1%)	725 (6.3%)
Age[†]	38 (32-45)	38 (32-45)	39 (33-45)
Race			
White	4933 (43.0%)	3530 (42.6%)	1403 (43.8%)
Black	4677 (40.7%)	3215 (38.8%)	1462 (45.6%)
Hispanic	1273 (11.1%)	1040 (12.6%)	233 (7.3%)
Other/Unknown	602 (5.2%)	493 (6.0%)	109 (3.4%)
Injection drug user	2156 (18.8%)	1364 (16.5%)	792 (24.7%)
Men who have sex with men	6328 (55.1%)	4677 (56.5%)	1651 (51.5%)
ARV exposure prior to first ART	3207 (27.9%)	-	-
ART initiation year[†]	2004 (2000-2007)	2005 (2001-2008)	2000 (1997-2004)
ART regimen type			
PI	5443 (47.3%)	3496 (42.2%)	1947 (60.7%)
NNRTI	4835 (42.1%)	4053 (49.0%)	786 (24.5%)
3+ NRTI	570 (5.0%)	345 (4.2%)	222 (6.9%)
NNRTI+PI	441 (3.8%)	227 (2.7%)	214 (6.7%)
Other*	196 (1.7%)	157 (1.9%)	38 (1.2%)
CD4 count (cells/mm^3)[†]	202 (61-338)	207 (62-338)	190 (58-339)
HIV viral RNA (log_{10} copies/mL)[†]	4.8 (4.3-5.3)	4.9 (4.4-5.4)	4.6 (4.0-5.2)

[†]Median (IQR) given instead of N (%)

*includes regimens with an integrase inhibitor, fusion inhibitor, or entry inhibitor

ART=combination antiretroviral therapy, PI=protease inhibitor, NNRTI=non-nucleaside reverse transcriptase inhibitor, NRTI=nucleoside reverse transcriptase inhibitor, ARV=antiretroviral

Table A.2 Patient characteristics at ART initiation associated with cancer incidence among antiretroviral naïve patients stratified by cancer group, CFAR Network of Integrated Clinical Systems, 1996-2011

	Bivariable HR (95% CI)	Multivariable* HR (95% CI)	Multivariable 2* HR (95% CI)
Kaposi Sarcoma			
Age at ART initiation (per 10 year increase)	1.06 (0.87-1.30)	1.07 (0.85-1.34)	1.07 (0.85, 1.34)
Calendar year of starting ART	1.03 (0.97-1.08)	1.01 (0.96-1.08)	1.02 (0.96, 1.08)
CD4 count (per 100 cells/mm^3)	0.64 (0.55-0.75)	0.61 (0.51-0.73)	-
Viral load (per log$_{10}$ copies/ml)	1.72 (1.29-2.30)	1.23(0.90-1.70)	1.25 (0.91-1.71)
Nadir CD4 (per 100 cells/mm^3)	0.61 (0.51, 0.73)	-	0.58 (0.47, 0.70)
Lymphomas			
Age at ART initiation (per decade)	1.36 (1.10-1.68)	1.33 (1.07-1.66)	1.33 (1.07-1.67)
Calendar year of starting ART	1.01 (0.95-1.07)	1.01 (0.95-1.08)	1.01 (0.94-1.08)
CD4 count (per 100 cells/mm^3)	0.79 (0.68-0.91)	0.77 (0.66-0.91)	-
Viral load (per log$_{10}$ copies/ml)	1.04 (0.78-1.40)	0.81 (0.59-1.11)	0.83 (0.61-1.14)
Nadir CD4 (per 100 cells/mm^3)	0.78 (0.67-0.92)	-	0.77 (0.65-0.92)
HPV-related cancers			
Age at ART initiation (per decade)	1.52 (1.01-2.28)	1.55 (1.02-2.34)	1.55 (1.03-2.35)
Calendar year of starting ART	0.94 (0.82-1.07)	0.96 (0.84-1.11)	0.96 (0.84-1.11)
CD4 count (per 100 cells/mm^3)	0.82 (0.62-1.07)	0.91 (0.69-1.21)	-
Viral load (per log$_{10}$ copies/ml)	1.97 (1.04-3.72)	1.61 (0.82-3.20)	1.64 (0.83-3.24)
Nadir CD4 (per 100 cells/mm^3)	0.82 (0.61-1.10)	-	0.92 (0.67-1.25)
Non-lymphoma, HPV-unrelated NADCs			
Age at ART initiation (per decade)	2.44 (2.03-2.93)	2.36 (1.94-2.87)	2.36 (1.94-2.87)
Calendar year of starting ART	1.02 (0.95-1.08)	1.00 (0.93-1.07)	1.00 (0.93-1.07)
CD4 count (per 100 cells/mm^3)	0.97 (0.87-1.09)	0.99 (0.88-1.12)	-
Viral load (per log$_{10}$ copies/ml)	0.96 (0.72-1.27)	0.93 (0.68-1.26)	0.91 (0.67-1.24)
Nadir CD4 (per 100 cells/mm^3)	0.95 (0.84, 1.08)	-	0.97 (0.84-1.12)

*Multivariable analyses adjusted for other covariates listed as well as CNICS study site, sex/MSM, race, and IDU. First multivariable model included latest CD4 count at ART initiation, but not nadir CD4 count. Second multivariable model included nadir CD4 count, but not latest CD4 count at ART initiation.

ART=combination antiretroviral therapy, HR=hazard ratio, MSM=men who have sex with men, IDU=injection drug user, HPV=human papillomavirus, NADC=non-AIDS-defining cancer

APPENDIX B: SENSITIVITY ANALYSES FOR CHAPTER V

For chapter V, sub-analyses were conducted among patients who were antiretroviral naïve at the initiation of their first combination ART regimen. Associations between immunologic response measures and NADC incidence among the sub-population of antiretroviral naïve patients are given in Table B.1. Results from combined regression models in this sub-population with all immunologic measures are also given in Table B.2. Results did not differ dramatically from those found in the full population.

Analyses were also conducted in the full population with 6-month and 12-month exposure lags. A subset of these results was given in Chapter V, but the full results are described in Tables B.3-6. The sensitivity of results to patterns of loss-to-follow-up and death were examined in Table B.7 by simulating different scenarios of cancer incidence for the unobserved person-time (time after death or loss-to-follow-up until 10 years after ART initiation). Finally, the trajectories of CD4 count measures in the year prior to a Hodgkin lymphoma diagnosis were examined to qualitatively assess declining CD4 counts as possible evidence of the effects of sub-clinical cancer. CD4 counts did appear to decline in the year prior to cancer diagnosis among Hodgkin lymphoma cases and the trajectories are illustrated in Figure B.1.

Table B.1 Associations of measures of immunologic response to antiretroviral therapy (ART) with non-AIDS-defining cancer incidence among those with no prior antiretroviral exposure

	Bivariable	Multivariable*	
		Weighted	Weighted, Adjusted
Measures of immunologic ART response	**Hazard Ratio (95% CI)**		
All NADCs (N=106)			
Six month CD4 (per 100 cells/mm^3)	0.91 (0.83, 1.00)	0.92 (0.83, 1.02)	0.86 (0.73, 1.02)
Most recent CD4 (per 100 cells/mm^3)	0.84 (0.77, 0.91)	0.98 (0.74, 1.29)	0.89 (0.74, 1.07)
CD4 count-years (per 200 cells*years/mm^3)	0.95 (0.91, 1.00)	0.96 (0.92, 1.00)	0.95 (0.90, 1.00)
Viral-related NADCs (N=38)			
Six month CD4 (per 100 cells/mm^3)	0.73 (0.59, 0.89)	0.72 (0.60, 0.87)	0.85 (0.60, 1.21)
Most recent CD4 (per 100 cells/mm^3)	0.70 (0.60, 0.82)	0.61 (0.47, 0.80)	0.60 (0.43, 0.84)
CD4 count-years (per 200 cells*years/mm^3)	0.85 (0.77, 0.93)	0.85 (0.79, 0.91)	0.89 (0.81, 0.98)
Viral-unrelated NADCs (N=68)			
Six month CD4 (per 100 cells/mm^3)	0.99 (0.89, 1.10)	1.00 (0.90, 1.12)	0.88 (0.73, 1.06)
Most recent CD4 (per 100 cells/mm^3)	0.91 (0.83, 1.01)	1.15 (0.86, 1.53)	0.97 (0.88, 1.08)
CD4 count-years (per 200 cells*years/mm^3)	0.99 (0.95, 1.04)	1.00 (0.96, 1.04)	0.97 (0.92, 1.03)

*Total 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 count measurements. Multivariable analyses additionally adjusted for CD4 at ART initiation, HIV RNA at ART initiation, age at ART initiation, year of ART initiation, sex/MSM, IDU, race, CNICS study site.

Table B.2 Combined model with all immunologic ART response measures with virus-related non-AIDS-defining cancer incidence among those with no prior antiretroviral exposure

	Unadjusted		Adjusted	
Measures of immunologic ART response	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Six month CD4 (per 100 cells/mm^3)	0.91 (0.71, 1.17)	0.4633	1.06 (0.73, 1.53)	0.7754
Most recent CD4 (per 100 cells/mm^3)	0.73 (0.58, 0.92)	0.0072	0.70 (0.55, 0.89)	0.0034
CD4 count-years (per 200 cells*years/mm^3)	1.00 (0.87, 1.14)	0.9906	1.01 (0.88, 1.16)	0.9039

Table B.3 Associations between measures of immunologic response to antiretroviral therapy (ART) with 6-month exposure lags and non-AIDS-defining cancer incidence

	Bivariable	Multivariable*	
		Weighted	Weighted, Adjusted
Measures of immunologic ART response	**Hazard Ratio (95% CI)**		
	All NADCs (N=140)		
Six month CD4 (per 100 cells/mm^3)	0.90 (0.82, 0.97)	0.89 (0.82, 0.97)	0.85 (0.73, 0.98)
Most recent CD4 (per 100 cells/mm^3)	0.89 (0.83, 0.96)	0.90 (0.82, 0.99)	0.88 (0.81, 0.96)
CD4 count-years (per 200 cells*years/mm^3)	0.97 (0.93, 1.00)	0.96 (0.93, 1.00)	0.97 (0.93, 1.01)
	Viral-related NADCs (N=59)		
Six month CD4 (per 100 cells/mm^3)	0.75 (0.64, 0.88)	0.76 (0.66, 0.87)	0.69 (0.51, 0.93)
Most recent CD4 (per 100 cells/mm^3)	0.83 (0.74, 0.93)	0.82 (0.72, 0.94)	0.82 (0.73, 0.93)
CD4 count-years (per 200 cells*years/mm^3)	0.88 (0.82, 0.95)	0.87 (0.80, 0.96)	0.88 (0.80, 0.98)
	Viral-unrelated NADCs (N=81)		
Six month CD4 (per 100 cells/mm^3)	0.98 (0.89, 1.08)	0.98 (0.88, 1.08)	0.94 (0.81, 1.09)
Most recent CD4 (per 100 cells/mm^3)	0.94 (0.86, 1.03)	0.96 (0.86, 1.07)	0.93 (0.84, 1.03)
CD4 count-years (per 200 cells*years/mm^3)	1.00 (0.96, 1.04)	1.00 (0.97, 1.03)	1.00 (0.96, 1.04)

*Total 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 count measurements. Multivariable analyses additionally adjusted for CD4 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.

Table B.4 Combined model with all immunologic ART response measures with virus-related non-AIDS-defining cancer incidence with 6-month exposure lags

	Unadjusted		Adjusted		
Measures of immunologic ART response	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**AIC**
Six month CD4 (per 100 cells/mm^3)	0.83 (0.68, 1.00)	0.0547	0.76 (0.57, 1.02)	0.0704	972.620
Most recent CD4 (per 100 cells/mm^3)	0.98 (0.82, 1.16)	0.7948	0.97 (0.81, 1.16)	0.7285	975.806
CD4 count-years (per 200 cells*years/mm^3)	0.95 (0.86, 1.06)	0.3351	0.95 (0.85, 1.05)	0.3068	973.972

Table B.5 Associations between measures of immunologic response to antiretroviral therapy (ART) with 12-month exposure lags and non-AIDS-defining cancer incidence

	Bivariable	Multivariable*	
		Weighted	Weighted, Adjusted
Measures of immunologic ART response	**Hazard Ratio (95% CI)**		
	All NADCs (N=125)		
Six month CD4 (per 100 cells/mm^3)	0.89 (0.82, 0.97)	0.89 (0.81, 0.98)	0.82 (0.70, 0.97)
Most recent CD4 (per 100 cells/mm^3)	0.90 (0.84, 0.97)	0.92 (0.83, 1.02)	0.91 (0.84, 1.00)
CD4 count-years (per 200 cells*years/mm^3)	0.97 (0.93, 1.00)	0.96 (0.92, 0.99)	0.95 (0.90, 1.01)
	Viral-related NADCs (N=52)		
Six month CD4 (per 100 cells/mm^3)	0.76 (0.64, 0.89)	0.76 (0.65, 0.89)	0.69 (0.50, 0.96)
Most recent CD4 (per 100 cells/mm^3)	0.80 (0.71, 0.91)	0.81 (0.69, 0.95)	0.81 (0.71, 0.93)
CD4 count-years (per 200 cells*years/mm^3)	0.87 (0.80, 0.94)	0.85 (0.76, 0.95)	0.85 (0.75, 0.97)
	Viral-unrelated NADCs (N=73)		
Six month CD4 (per 100 cells/mm^3)	0.97 (0.88, 1.08)	0.97 (0.87, 1.09)	0.91 (0.77, 1.06)
Most recent CD4 (per 100 cells/mm^3)	0.97 (0.88, 1.06)	1.01 (0.90, 1.14)	0.99 (0.88, 1.11)
CD4 count-years (per 200 cells*years/mm^3)	1.00 (0.96, 1.05)	0.99 (0.96, 1.02)	0.98 (0.93, 1.04)

*Total 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 count measurements. Multivariable analyses additionally adjusted for CD4 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.

Table B.6 Combined model with all immunologic ART response measures with virus-related non-AIDS-defining cancer incidence with 12-month exposure lags

	Unadjusted		Adjusted	
Measures of immunologic ART response	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Six month CD4 (per 100 cells/mm^3)	0.85 (0.70, 1.04)	0.1208	0.78 (0.58, 1.06)	0.1082
Most recent CD4 (per 100 cells/mm^3)	0.92 (0.77, 1.10)	0.3751	0.91 (0.75, 1.10)	0.3192
CD4 count-years (per 200 cells*years/mm^3)	0.95 (0.84, 1.07)	0.3688	0.95 (0.84, 1.06)	0.3506

Table B.7 Sensitivity of HR estimates to changes in assumptions about the unobserved incidence rates of virus-related NADCs among patients who died or were lost-to-follow-up in the first 10 years after ART initiation

Simulated virus-related NADC incidence rates (per 100,000 person-years) after censoring for individuals who do not have all follow-up time observed because of loss-to-follow-up or death		**HR (95% CI)**
Six-month CD4<300	Six-month CD4>=300	
Actual data*		0.71 (0.53, 0.95)
191	191	0.75 (0.62, 0.90)
238	238	0.79 (0.66, 0.94)
321	321	0.81 (0.69, 0.95)
128	238	0.91 (0.77, 1.07)
103	470	0.94 (0.81, 1.09)
28	639	1.04 (0.91, 1.19)

*Total virus-related NADC incidence in actual data was 184 cases per 100,000 person-years

Sensitivity analysis conducted for the association between CD4 count at six months after ART initiation and subsequent virus-related NADC incidence.

NADC=non-AIDS-defining cancers, ART=combination antiretroviral therapy, HR=hazard ratio

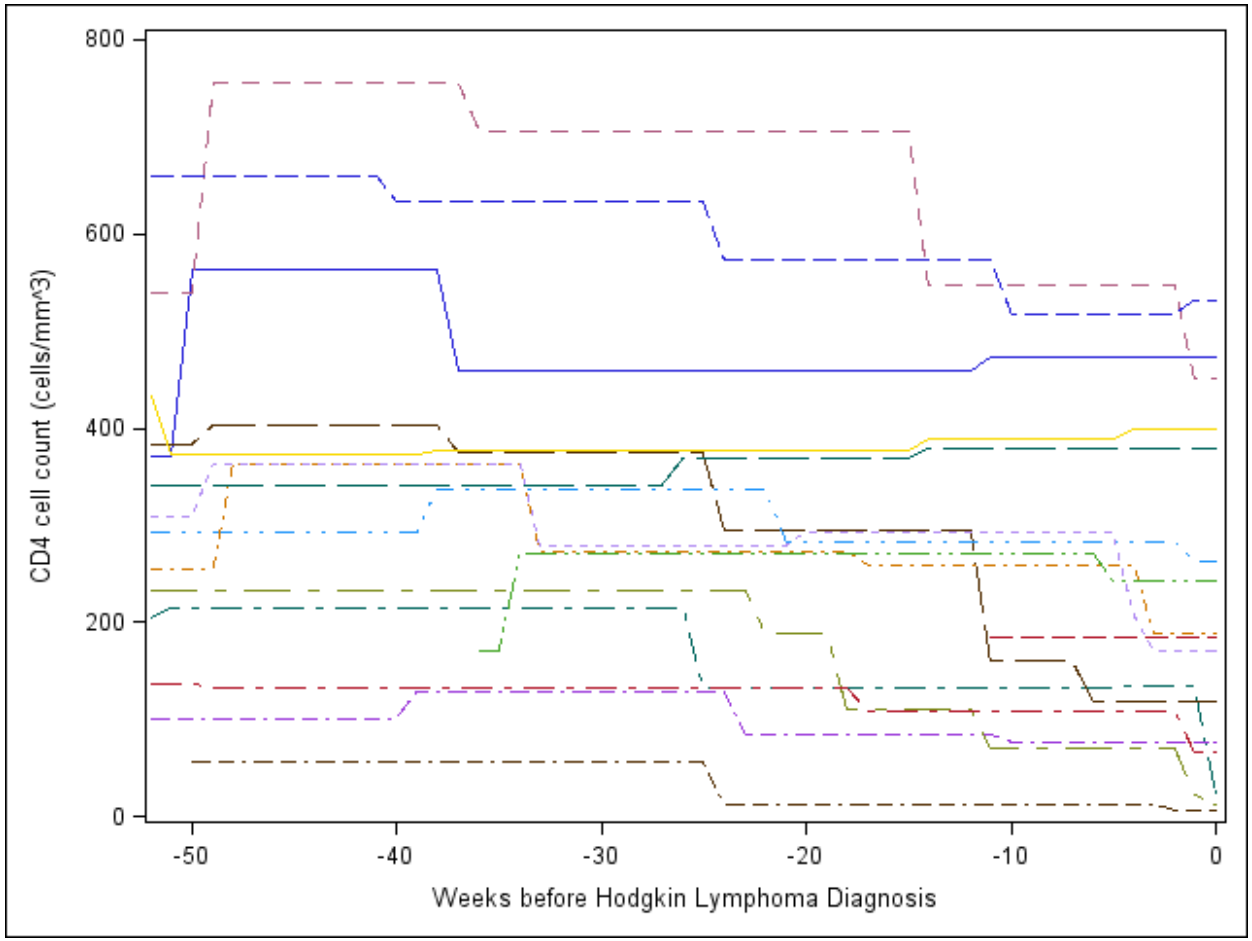

Figure B.1 Trajectory of CD4 counts among Hodgkin lymphoma cases in the year before Hodgkin lymphoma diagnosis

APPENDIX C: PRELIMINARY ANALYSES TO EVALUATE THE ASSOCIATION OF VIROLOGIC ART RESPONSE WITH NADC INCIDENCE

The relationship of virologic ART response with NADC incidence was evaluated in the study population used in Chapter V. HIV RNA at six months, undetectable viral load at six months, latest viral load, and viremia copy-years were all evaluated as measures of virologic ART response. Changes over time in latest viral load measures and viremia copy-years are described in Figure C.1 and Figure C.2. Effect estimates for the association of each measure with total NADC incidence, virus-related NADC incidence and virus-unrelated NADC incidence are given in Table C.1. No statistically significant associations were identified between virologic response and any type of NADC incidence.

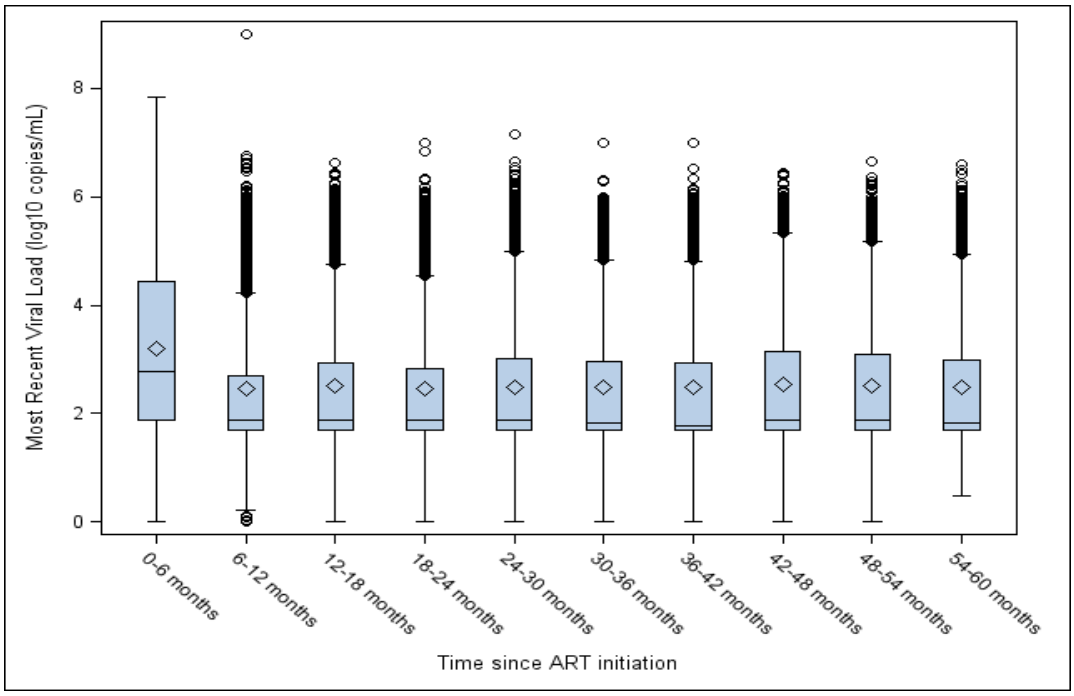

Figure C.1 Distribution of latest viral loads by six month time intervals following combination antiretroviral therapy (ART) initiation.
Box= interquartile range; middle line= median; diamond= mean; “whiskers” extend to 1.5*interquartile range.

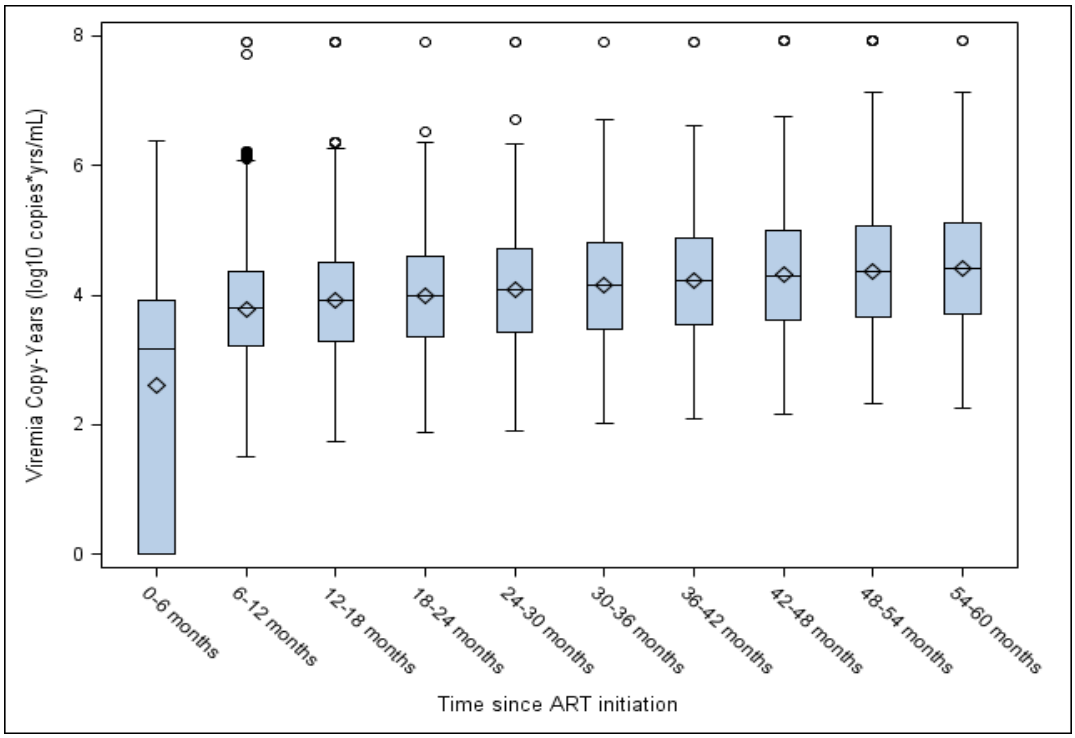

Figure C.2 Distribution of viremia copy-years by six month intervals following combination antiretroviral therapy (ART) initiation.
Box= interquartile range; middle line= median; diamond= mean; “whiskers” extend to 1.5*interquartile range.

Table C.1 Bivariable and multivariable associations of virologic ART response with non-AIDS-defining cancer incidence stratified by cancer group, Center for AIDS Research Network of Integrated Clinical Systems, 1996-2011

	Unadjusted	Adjusted*
Measures of virologic ART Response	**Hazard Ratio (95% CI)**	
All NADCs (164 cases)		
HIV RNA (per $\log_{10}$ copies/ml)	1.07 (0.93, 1.22)	1.05 (0.90, 1.22)
Undetectable HIV RNA (<400 copies/ml)	0.97 (0.69, 1.35)	0.95 (0.67, 1.37)
Latest HIV RNA (per $\log_{10}$ copies/ml)	0.98 (0.86, 1.11)	0.99 (0.86, 1.15)
Viremia copy-years (per $\log_{10}$ copies*year/ml)	1.12 (0.94, 1.33)	1.12 (0.89, 1.41)
Viral-related NADCs (65 cases)		
HIV RNA (per $\log_{10}$ copies/ml)	1.07 (0.86, 1.33)	0.96 (0.76, 1.23)
Undetectable HIV RNA (<400 copies/ml)	0.74 (0.45, 1.23)	0.94 (0.53, 1.65)
Latest HIV RNA (per $\log_{10}$ copies/ml)	1.00 (0.82, 1.22)	1.04 (0.82, 1.34)
Viremia copy-years (per $\log_{10}$ copies*year/ml)	1.18 (0.90, 1.55)	1.11 (0.75, 1.65)
Viral-unrelated NADCs (99 cases)		
HIV RNA (per $\log_{10}$ copies/ml)	1.06 (0.88, 1.27)	1.10 (0.90, 1.33)
Undetectable HIV RNA (<400 copies/ml)	1.18 (0.75, 1.84)	1.07 (0.66, 1.73)
Latest HIV RNA (per $\log_{10}$ copies/ml)	0.96 (0.82, 1.13)	0.95 (0.81, 1.12)
Viremia copy-years (per $\log_{10}$ copies*year/ml)	1.08 (0.86, 1.35)	1.13 (0.86, 1.49)

*Multivariable analyses weighted inverse probability of observed viremia copy-years given the CD4 count measures from prior two visits and CD4 count at ART initiation. Multivariable analyses additionally adjusted for prior antiretroviral, age at ART initiation, year of ART initiation, sex/MSM, IDU, race, CNICS study site

ART=combination antiretroviral therapy, NADC=non-AIDS-defining cancer, CI=confidence interval

REFERENCES

1. Engels EA, Pfeiffer RM, Goedert JJ, Virgo P, McNeel TS, Scoppa SM*, et al.* Trends in cancer risk among people with AIDS in the United States 1980-2002. *AIDS* 2006,**20**:1645-1654.

2. Piketty C, Selinger-Leneman H, Grabar S, Duvivier C, Bonmarchand M, Abramowitz L*, et al.* Marked increase in the incidence of invasive anal cancer among HIV-infected patients despite treatment with combination antiretroviral therapy. *AIDS* 2008,**22**:1203-1211.

3. Clifford GM, Polesel J, Rickenbach M, Dal Maso L, Keiser O, Kofler A*, et al.* Cancer risk in the Swiss HIV Cohort Study: associations with immunodeficiency, smoking, and highly active antiretroviral therapy. *J Natl Cancer Inst* 2005,**97**:425-432.

4. Spina M, Carbone A, Gloghini A, Serraino D, Berretta M, Tirelli U. Hodgkin's disease in patients with HIV infection. *Adv Hematol* 2011,**2011**.

5. Lewden C, Salmon D, Morlat P, Bevilacqua S, Jougla E, Bonnet F*, et al.* Causes of death among human immunodeficiency virus (HIV)-infected adults in the era of potent antiretroviral therapy: emerging role of hepatitis and cancers, persistent role of AIDS. *Int J Epidemiol* 2005,**34**:121-130.

6. Shiels MS, Pfeiffer RM, Gail MH, Hall HI, Li J, Chaturvedi AK*, et al.* Cancer burden in the HIV-infected population in the United States. *J Natl Cancer Inst* 2011,**103**:753-762.

7. Simard EP, Engels EA. Cancer as a cause of death among people with AIDS in the United States. *Clin Infect Dis* 2010,**51**:957-962.

8. Lanoy E, Spano JP, Bonnet F, Guiguet M, Boue F, Cadranel J*, et al.* The spectrum of malignancies in HIV-infected patients in 2006 in France: the ONCOVIH study. *Int J Cancer* 2011,**129**:467-475.

9. Pakkala S, Chen Z, Rimland D, Owonikoko TK, Gunthel C, Brandes JR*, et al.* Human immunodeficiency virus-associated lung cancer in the era of highly active antiretroviral therapy. *Cancer* 2012,**118**:164-172.

10. Spano JP, Costagliola D, Katlama C, Mounier N, Oksenhendler E, Khayat D. AIDS-related malignancies: state of the art and therapeutic challenges. *J Clin Oncol* 2008,**26**:4834-4842.

11. Biggar RJ, Engels EA, Ly S, Kahn A, Schymura MJ, Sackoff J*, et al.* Survival after cancer diagnosis in persons with AIDS. *J Acquir Immune Defic Syndr* 2005,**39**:293-299.

12. Vilchez RA, Finch CJ, Jorgensen JL, Butel JS. The clinical epidemiology of Hodgkin lymphoma in HIV-infected patients in the highly active antiretroviral therapy (HAART) era. *Medicine* 2003,**82**:77-81.

13. Bourcier V, Winnock M, Ait Ahmed M, Sogni P, Pambrun E, Poizot-Martin I*, et al.* Primary liver cancer is more aggressive in HIV-HCV coinfection than in HCV infection. A prospective study (ANRS CO13 Hepavih and CO12 Cirvir). *Clin Res Hepatol Gastroenterol* 2011.

14. Silverberg MJ, Chao C, Leyden WA, Xu L, Tang B, Horberg MA*, et al.* HIV infection and the risk of cancers with and without a known infectious cause. *AIDS* 2009,**23**:2337-2345.

15. Shiels MS, Cole SR, Kirk GD, Poole C. A meta-analysis of the incidence of non-AIDS cancers in HIV-infected individuals. *J Acquir Immune Defic Syndr* 2009,**52**:611-622.

16. Patel P, Hanson DL, Sullivan PS, Novak RM, Moorman AC, Tong TC*, et al.* Incidence of types of cancer among HIV-infected persons compared with the general population in the United States, 1992-2003. *Ann Intern Med* 2008,**148**:728-736.

17. Silverberg MJ, Chao C, Leyden WA, Xu L, Horberg MA, Klein D*, et al.* HIV infection, immunodeficiency, viral replication, and the risk of cancer. *Cancer Epidemiol Biomarkers Prev* 2011,**20**:2551-2559.

18. Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. Risk of human papillomavirus-associated cancers among persons with AIDS. *J Natl Cancer Inst* 2009,**101**:1120-1130.

19. Sissolak G, Sissolak D, Jacobs P. Human immunodeficiency and Hodgkin lymphoma. *Transfus Apher Sci* 2010,**42**:131-139.

20. Mocroft A, Sterne JA, Egger M, May M, Grabar S, Furrer H*, et al.* Variable impact on mortality of AIDS-defining events diagnosed during combination antiretroviral therapy: not all AIDS-defining conditions are created equal. *Clin Infect Dis* 2009,**48**:1138-1151.

21. The Anitretroviral Therapy Cohort Collaboration. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996-2006: collaborative analysis of 13 HIV cohort studies. *Clin Infect Dis* 2010,**50**:1387-1396.

22. Salmon-Ceron D, Rosenthal E, Lewden C, Bouteloup V, May T, Burty C*, et al.* Emerging role of hepatocellular carcinoma among liver-related causes of deaths in HIV-infected patients: The French national Mortalite 2005 study. *J Hepatol* 2009,**50**:736-745.

23. Achenbach CJ, Cole SR, Kitahata MM, Casper C, Willig JH, Mugavero MJ*, et al.* Mortality after cancer diagnosis in HIV-infected individuals treated with antiretroviral therapy. *AIDS* 2011,**25**:691-700.

24. Engels EA. Non-AIDS-defining malignancies in HIV-infected persons: etiologic puzzles, epidemiologic perils, prevention opportunities. *AIDS* 2009,**23**:875-885.

25. Simard EP, Pfeiffer RM, Engels EA. Spectrum of cancer risk late after AIDS onset in the United States. *Arch Intern Med* 2010,**170**:1337-1345.

26. D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD*, et al.* Incidence and epidemiology of anal cancer in the Multicenter AIDS Cohort Study. *J Acquir Immune Defic Syndr* 2008,**48**:491-499.

27. McGinnis KA, Fultz SL, Skanderson M, Conigliaro J, Bryant K, Justice AC. Hepatocellular carcinoma and non-Hodgkin's lymphoma: the roles of HIV, hepatitis C infection, and alcohol abuse. *J Clin Oncol* 2006,**24**:5005-5009.

28. Brau N, Fox RK, Xiao P, Marks K, Naqvi Z, Taylor LE*, et al.* Presentation and outcome of hepatocellular carcinoma in HIV-infected patients: a U.S.-Canadian multicenter study. *J Hepatol* 2007,**47**:527-537.

29. Engels EA, Brock MV, Chen J, Hooker CM, Gillison M, Moore RD. Elevated incidence of lung cancer among HIV-infected individuals. *J Clin Oncol* 2006,**24**:1383-1388.

30. Kirk GD, Merlo C, P OD, Mehta SH, Galai N, Vlahov D*, et al.* HIV infection is associated with an increased risk for lung cancer, independent of smoking. *Clin Infect Dis* 2007,**45**:103-110.

31. Shiels MS, Cole SR, Mehta SH, Kirk GD. Lung cancer incidence and mortality among HIV-infected and HIV-uninfected injection drug users. *J Acquir Immune Defic Syndr* 2010,**55**:510-515.

32. Guiguet M, Boue F, Cadranel J, Lang J, Rosenthal E, Costagliola D*, et al.* Effect of immunodeficiency, HIV viral load, and antiretroviral therapy on the risk of individual malignancies (FHDH-ANRS CO4): a prospective cohort study. *Lancet Oncol.* 2009,**10**:1152-1159.

33. Bedimo RJ, McGinnis KA, Dunlap M, Rodriguez-Barradas MC, Justice AC. Incidence of non-AIDS-defining malignancies in HIV-infected versus noninfected patients in the HAART era: impact of immunosuppression. *J Acquir Immune Defic Syndr* 2009,**52**:203-208.

34. Nguyen ML, Farrell KJ, Gunthel CJ. Non-AIDS-defining malignancies in patients with HIV in the HAART Era. *Curr Infect Dis Rep* 2010,**12**:46-55.

35. Clifford GM, Franceschi S. Cancer risk in HIV-infected persons: influence of CD4(+) count. *Future Oncol* 2009,**5**:669-678.

36. Engels EA, Pfeiffer RM, Landgren O, Moore RD. Immunologic and virologic predictors of AIDS-related non-hodgkin lymphoma in the highly active antiretroviral therapy era. *J Acquir Immune Defic Syndr* 2010,**54**:78-84.

37. Ambinder RF, Bhatia K, Martinez-Maza O, Mitsuyasu R. Cancer biomarkers in HIV patients. *Curr Opin HIV AIDS* 2010,**5**:531-537.

38. Barbaro G, Barbarini G. HIV infection and cancer in the era of highly active antiretroviral therapy. *Oncology Reports* 2007,**17**:1121-1126.

39. Carbone A, Cesarman E, Spina M, Gloghini A, Schulz TF. HIV-associated lymphomas and gamma-herpesviruses. *Blood* 2009,**113**:1213-1224.

40. Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *The Lancet* 2007,**370**:59-67.

41. Engels EA, Pfeiffer RM, Fraumeni JF, Jr., Kasiske BL, Israni AK, Snyder JJ*, et al.* Spectrum of cancer risk among US solid organ transplant recipients. *Journal of the American Medical Association* 2011,**306**:1891-1901.

42. Crum-Cianflone NF, Hullsiek KH, Marconi VC, Ganesan A, Weintrob A, Barthel RV*, et al.* Anal cancers among HIV-infected persons: HAART is not slowing rising incidence. *AIDS* 2010,**24**:535-543.

43. Harris TG, Burk RD, Palefsky JM, Massad LS, Bang JY, Anastos K*, et al.* Incidence of cervical squamous intraepithelial lesions associated with HIV serostatus, CD4 cell counts, and human papillomavirus test results. *JAMA* 2005,**293**:1471-1476.

44. Heard I, Palefsky JM, Kazatchkine MD. The impact of HIV antiviral therapy on human papillomavirus (HPV) infections and HPV-related diseases. *Antiviral Therapy* 2004,**9**:13-22.

45. Palefsky JM, Holly EA, Ralston ML, Jay N. Prevalence and risk factors for human papillomaviruses infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. *The Journal of Infectious Diseases* 1998,**177**:361-367.

46. Massad LS, Ahdieh L, Benning L, Minkoff H, Greenblatt RM, Watts H*, et al.* Evolution of cervical abnormalities among women with HIV-1: evidence from surveillance cytology in the Women's Interagency HIV Study. *J Acquir Immune Defic Syndr* 2011,**27**:432-442.

47. Clifford GM, Rickenbach M, Lise M, Dal Maso L, Battegay M, Bohlius J*, et al.* Hodgkin lymphoma in the Swiss HIV Cohort Study. *Blood* 2009,**113**:5737-5742.

48. Frisch M, Biggar RJ, Engels EA, Goedert JJ, Group A-CMRS. Association of cancer with AIDS-related immunosuppression in adults. *JAMA* 2001,**285**:1736-1745.

49. Biggar RJ, Jaffe ES, Goedert JJ, Chaturvedi A, Pfeiffer R, Engels EA. Hodgkin lymphoma and immunodeficiency in persons with HIV/AIDS. *Blood* 2006,**108**:3786-3791.

50. Lanoy E, Rosenberg PS, Fily F, Lascaux AS, Martinez V, Partisani M*, et al.* HIV-associated Hodgkin lymphoma during the first months on combination antiretroviral therapy. *Blood* 2011,**118**:44-49.

51. Jaffe HW, De Stavola BL, Carpenter LM, Porter K, Cox DR. Immune reconstitution and risk of Kaposi sarcoma and non-Hodgkin lymphoma in HIV-infected adults. *AIDS* 2011,**25**:1395-1403.

52. Clifford GM, Rickenbach M, Polesel J, Dal Maso L, Steffen I, Ledergerber B*, et al.* Influence of HIV-related immunodeficiency on the risk of hepatocellular carcinoma. *AIDS* 2008,**22**:2135-2141.

53. Reekie J, Kosa C, Engsig F, Monforte A, Wiercinska-Drapalo A, Domingo P*, et al.* Relationship between current level of immunodeficiency and non-acquired immunodeficiency syndrome-defining malignancies. *Cancer* 2010,**116**:5306-5315.

54. Engels EA. Inflammation in the development of lung cancer: epidemiological evidence. *Expert Rev Anticancer Ther* 2008,**8**:605-615.

55. Zoufaly A, Stellbrink HJ, Heiden MA, Kollan C, Hoffmann C, van Lunzen J*, et al.* Cumulative HIV viremia during highly active antiretroviral therapy is a strong predictor of AIDS-related lymphoma. *J Infect Dis* 2009,**200**:79-87.

56. International Collaboration on HIV and Cancer. Highly active antiretroviral therapy and incidence of cancer in human immunodeficiency virus-infected adults. *J Natl Cancer Inst* 2000,**92**:1823-1830.

57. Pipkin S, Scheer S, Okeigwe I, Schwarcz S, Harris DH, Hessol NA. The effect of HAART and calendar period on Kaposi's sarcoma and non-Hodgkin lymphoma: results of a match between an AIDS and cancer registry. *AIDS* 2011,**25**:463-471.

58. Shiels MS, Cole SR, Wegner S, Armenian H, Chmiel JS, Ganesan A*, et al.* Effect of HAART on incident cancer and noncancer AIDS events among male HIV seroconverters. *J Acquir Immune Defic Syndr* 2008,**48**:485-490.

59. Simard EP, Pfeiffer RM, Engels EA. Cumulative incidence of cancer among individuals with acquired immunodeficiency syndrome in the United States. *Cancer* 2011,**117**:1089-1096.

60. Franceschi S, Lise M, Clifford GM, Rickenbach M, Levi F, Maspoli M*, et al.* Changing patterns of cancer incidence in the early- and late-HAART periods: the Swiss HIV Cohort Study. *Br J Cancer* 2010,**103**:416-422.

61. Bower M, Powles T, Newsom-Davis T, Thirlwell C, Stebbing J, Mandalia S*, et al.* HIV-associated cancer: has highly active antiretroviral therapy reduced the incidence or improved the outcome? *J Acquir Immune Defic Syndr* 2004,**37**:1563-1565.

62. Powles T, Robinson D, Stebbing J, Shamash J, Nelson M, Gazzard B*, et al.* Highly active antiretroviral therapy and the incidence of non-AIDS-defining cancers in people with HIV infection. *J Clin Oncol* 2009,**27**:884-890.

63. Silverberg MJ, Neuhaus J, Bower M, Gey D, Hatzatkis A, Henry K*, et al.* Risk of cancers during interrupted antiretroviral therapy in the SMART study. *AIDS* 2007,**21**:1957-1963.

64. Shim JS, Rao R, Beebe K, Neckers L, Han I, Nahta R*, et al.* Selective inhibition of HER2-positive breast cancer cells by the HIV protease inhibitor nelfinavir. *J Natl Cancer Inst* 2012,**104**:1576-1590.

65. Bono C, Karlin L, Harel S, Mouly E, Labaume S, Galicier L*, et al.* The human immunodeficiency virus-1 protease inhibitor nelfinavir impairs proteasome activity and inhibits the proliferation of multiple myeloma cells in vitro and in vivo. *Haematologica* 2012,**97**:1101-1109.

66. Barillari G, Iovane A, Bacigalupo I, Palladino C, Bellino S, Leone P*, et al.* Ritonavir or saquinavir impairs the invasion of cervical intraepithelial neoplasia cells via a reduction of MMP expression and activity. *AIDS* 2012,**26**:909-919.

67. Chao C, Leyden WA, Xu L, Horberg MA, Klein D, Towner WJ*, et al.* Exposure to antiretroviral therapy and risk of cancer in HIV-infected persons. *AIDS* 2012,**26**:2223-2231.

68. Krishnan S, Schouten JT, Jacobson DL, Benson CA, Collier AC, Koletar SL*, et al.* Incidence of non-AIDS-defining cancer in antiretroviral treatment-naive subjects after antiretroviral treatment initiation: an ACTG longitudinal linked randomized trials analysis. *Oncology* 2011,**80**:42-49.

69. Kesselring A, Gras L, Smit C, van Twillert G, Verbon A, de Wolf F*, et al.* Immunodeficiency as a risk factor for non-AIDS-defining malignancies in HIV-1-infected patients receiving combination antiretroviral therapy. *Clin Infect Dis* 2011,**52**:1458-1465.

70. Engels EA, Biggar RJ, Hall HI, Cross H, Crutchfield A, Finch JL*, et al.* Cancer risk in people infected with human immunodeficiency virus in the United States. *Int J Cancer* 2008,**123**:187-194.

71. CFAR Network of Integrated Clinical Systems. CNICS: CFAR Network of Integrated Clinical Systems. In. http://www.uab.edu/cnics/2010; 2011.

72. Kitahata MM, Rodriguez B, Haubrich R, Boswell S, Mathews WC, Lederman MM*, et al.* Cohort profile: the Centers for AIDS Research Network of Integrated Clinical Systems. *Int J Epidemiol* 2008,**37**:948-955.

73. Howe CJ, Cole SR, Napravnik S, Eron JJ. Enrollment, retention, and visit attendance in the University of North Carolina Center for AIDS Research HIV clinical cohort, 2001-2007. *AIDS Res Hum Retroviruses* 2010,**26**:875-881.

74. Drozd DR, Lober WB, Kitahata MM, Smith KI, Van Rompaey SE. Developing a relational XML schema for sharing HIV clinical data. In: *AMIA Symposium*; 2005.

75. Cole SR, Napravnik S, Mugavero MJ, Lau B, Eron JJ, Saag MS. Copy-years viremia as a measure of cumulative human immunodeficiency virus burden. *Am J Epidemiol* 2010,**171**:198-205.

76. Mugavero MJ, Napravnik S, Cole SR, Eron JJ, Lau B, Crane HM*, et al.* Viremia copy-years predicts mortality among treatment-naive HIV-infected patients initiating antiretroviral therapy. *Clinical Infectious Diseases* 2011,**53**:927-935.

77. Bower M, Fisher M, Hill T, Reeves I, Walsh J, Orkin C*, et al.* CD4 counts and the risk of systemic non-Hodgkin's lymphoma in individuals with HIV in the UK. *Haematologica* 2009,**94**:875-880.

78. Institute S. SAS version 9.2. In. Cary, NC, USA; 2011.

79. Robins JM, Hernan MA, Brumback B. Marginal structural models and causal inference in epidemiology. *Epidemiology* 2000,**11**:550-560.

80. Cole SR, Hernan MA. Constructing inverse probability weights for marginal structural models. *Am J Epidemiol* 2008,**168**:656-664.

81. Cole SR. Effect of highly active antiretroviral therapy on time to acquired immunodeficiency syndrome or death using marginal structural models. *Am J Epidemiol* 2003,**158**:687-694.

82. Hernan MA, Lanoy E, Costagliola D, Robins JM. Comparison of dynamic treatment regimes via inverse probability weighting. *Basic and Clinical Pharmacology and Toxicology* 2006,**98**:237-242.

83. Hernan MA, McAdams M, McGrath N, Lanoy E, Costagliola D. Observation plans in longitudinal studies with time-varying treatments. *Stat Methods Med Res* 2009,**18**:27-52.

84. Rothman KJ. Induction and latent periods. *Am J Epidemiol* 1981,**114**:253-259.

85. Lau B, Cole SR, Gange SJ. Competing risk regression models for epidemiologic data. *Am J Epidemiol* 2009,**170**:244-256.

86. Franceschi S, Maso LD, Rickenbach M, Polesel J, Hirschel B, Cavassini M*, et al.* Kaposi sarcoma incidence in the Swiss HIV Cohort Study before and after highly active antiretroviral therapy. *British journal of cancer* 2008,**99**:800-804.

87. Biggar RJ, Chaturvedi AK, Goedert JJ, Engels EA. AIDS-related cancer and severity of immunosuppression in persons with AIDS. *J Natl Cancer Inst* 2007,**99**:962-972.

88. Petoumenos K, van Leuwen M, Vajdic C, Woolley I, Chuah J, Templeton D*, et al.* Cancer, immunodeficiency and antiretroviral treatment: results from the Australian HIV Observational Database (AHOD). *HIV Med* 2013,**14**:77-84.

89. Bower M, Nelson M, Young AM, Thirlwell C, Newsom-Davis T, Mandalia S*, et al.* Immune reconstitution inflammatory syndrome associated with Kaposi's sarcoma. *J Clin Oncol* 2005,**23**:5224-5228.

90. Feller L, Anagnostopoulos C, Wood NH, Bouckaert M, Raubenheimer EJ, Lemmer J. Human immunodeficiency virus-associated Kaposi sarcoma as an immune reconstitution inflammatory syndrome: a literature review and case report. *J Periodontol* 2008,**79**:362-368.

91. Achenbach CJ, Harrington RD, Dhanireddy S, Crane HM, Casper C, Kitahata MM. Paradoxical immune reconstitution inflammatory syndrome in HIV-infected patients

treated with combination antiretroviral therapy after AIDS-defining opportunistic infection. *Clin Infect Dis* 2012,**54**:424-433.

92. Willig JH, Abroms S, Westfall AO, Routman J, Adusumilli S, Varshney M*, et al.* Increased regimen durability in the era of once-daily fixed-dose combination antiretroviral therapy. *AIDS* 2008,**22**:1951-1960.

93. Crum-Cianflone N, Hullsiek KH, Marconi V, Weintrob A, Ganesan A, Barthel RV*, et al.* Trends in the incidence of cancers among HIV-infected persons and the impact of antiretroviral therapy: a 20-year cohort study. *AIDS* 2009,**23**:41-50.

94. Dubrow R, Silverberg MJ, Park LS, Crothers K, Justice AC. HIV infection, aging, and immune function: implications for cancer risk and prevention. *Curr Opin Oncol* 2012,**24**:506-516.

95. Yanik E, Napravnik S, Cole SR, Achenbach CJ, Dittmer DP, Olshan A*, et al.* Timing and predictors of cancer incidence following initiation of antiretroviral therapy (ART), CFAR Network of Integrated Clinical Systems 1996-2011. In: *Conference on Retroviruses and Opportunistic Infections*. Atlanta, GA, USA; 2013.

96. Bergmann L, Mitrou PS, Demmer-Dieckmann M, Ruhmann FT, Weidmann E. Impaired T- and B-cell functions in patients with Hodgkin's disease. *Cancer Immunol Immunother* 1987,**25**:59-64.

97. Silivnick DJ, Ellis TM, Nawrocki JF, Fisher RI. The impact of Hodgkin's disease on the immune system. *Semin Oncol* 1990,**17**:673-682.

98. Bohlius J, Schmidlin K, Boue F, Fatkenheuer G, May M, Caro-Murillo AM*, et al.* HIV-1-related Hodgkin lymphoma in the era of combination antiretroviral therapy: incidence and evolution of CD4(+) T-cell lymphocytes. *Blood* 2011,**117**:6100-6108.

99. Vogel M, Friedrich O, Luchters G, Holleczek B, Wasmuth JC, Anadol E*, et al.* Cancer risk in HIV-infected individuals on HAART is largely attributed to oncogenic infections and state of immunocompetence. *Eur J Med Res* 2011,**16**:101-107.

100. Bratcher LF, Sahasrabuddhe VV. The impact of antiretroviral therapy on HPV and cervical intraepithelial neoplasia: current evidence and directions for future research. *Infect Agent Cancer* 2010,**5**:8.

101. Palefsky JM. Antiretroviral therapy and anal cancer: the good, the bad, and the unknown. *Sex Transm Dis* 2012,**39**:501-503.

102. Kitahata MM, Achenbach CJ, Saag CJ. Comment: Age at cancer diagnosis among persons with AIDS. *Ann Intern Med* 2011,**154**:642-643.

103. Kozak MS, Mugavero MJ, Ye J, Aban I, Lawrence ST, Nevin CR*, et al.* Patient reported outcomes in routine care: advancing data capture for HIV cohort research. *Clin Infect Dis* 2012,**54**:141-147.

104. When to Start Consortium. Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies. *Lancet* 2009,**373**:1352-1363.

105. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N*, et al.* Prevention of HIV-1 Infection with Early Antiretroviral Therapy. *New England Journal of Medicine* 2011,**365**:493-505.

106. Lindsey K, DeCristofaro C, James J. Anal Pap smears: Should we be doing them? *J Am Acad Nurse Pract* 2009,**21**:437-443.

107. Goldie SJ, Kuntz KM, Weinstein MC, Freedberg KA, Welton ML, Palefsky JM. The clinical effectiveness and cost-effectiveness of screening for anal squamous intraepithelial lesions in homosexual and bisexual HIV-positive men. *JAMA* 1999,**281**:1822-1829.

108. Lau B, Gange SJ, Moore RD. Interval and clinical cohort studies: epidemiological issues. *AIDS Res Hum Retroviruses* 2007,**23**:769-776.

109. Napravnik S, Eron JJ, Jr., McKaig RG, Heine AD, Menezes P, Quinlivan E. Factors associated with fewer visits for HIV primary care at a tertiary care center in the Southeastern U.S. *AIDS Care* 2006,**18 Suppl 1**:S45-50.

110. Cole SR, Frangakis CE. The consistency statement in causal inference: a definition or an assumption? *Epidemiology* 2009,**20**:3-5.

111. Maclure M. The Case-Crossover Design: A Method for Studying Transient Effects on the Risk of Acute Events. *Am J Epidemiol* 1991,**133**:144-153.

112. Wang S, Linkletter C, Maclure M, Dore D, Mor V, Buka S*, et al.* Future cases as present controls to adjust for exposure trend bias in case-only studies. *Epidemiology* 2011,**22**:568-574.

113. Stebbing J, Bower M. The anti-tumor effects of human immunodeficiency virus protease inhibitors: ready for real time? *Int J Cancer* 2011,**128**:1-2.