

**CD4/CD8 ratio and the risk of Kaposi sarcoma or non-Hodgkin lymphoma in the context of efficiently treated HIV infection: a collaborative analysis of 20 European cohort studies.**

The **(CD4/CD8 ratio and cancer risk)** project Working Group for the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) in EuroCoord”.

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**Summary of the article's main point (max 40 words)**

This study showed that CD4/CD8 ratio and CD8 count, may help clinicians to identify efficiently treated PLHIV with higher risk of developing KS and NHL, still among the most frequent cancers, and that these markers are particularly relevant when  $CD4 \geq 500/mm^3$ .

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## **Abstract**

**BACKGROUND:** A persistently low CD4/CD8 ratio has been reported to inversely correlate with the risk of non-AIDS defining cancer in people living with HIV (PLHIV) efficiently treated by combination antiretroviral therapy (cART). We evaluated the impact of the CD4/CD8 ratio on the risk of Kaposi sarcoma (KS) or non-Hodgkin lymphoma (NHL), still among the most frequent cancers in treated PLHIV.

**METHODS:** PLHIV from the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) were included if they achieved virological control (viral load  $\leq 500$  copies/mL) within nine months following cART and without previous KS/LNH diagnosis. Cox models were used to identify factors associated with KS or NHL risk, in all participants and those with  $CD4 \geq 500/mm^3$  at virological control. We analyzed the CD4/CD8 ratio, CD4 count and CD8 count as time-dependent variables, using spline transformations.

**RESULTS:** We included 56 708 PLHIV, enrolled between 2000 and 2014. At virological control, the median[IQR] CD4 count, CD8 count and CD4/CD8 ratio were 414[296-552]/ $mm^3$ , 936[670-1304]/ $mm^3$ , and 0.43[0.28-0.65] respectively. Overall, 221 KS and 187 NHL were diagnosed, 9[2-37] and 18[7-42] months after virological control. Low CD4/CD8 ratios were associated with KS risk (HR=2.02(95%CI=1.23-3.31)) when comparing CD4/CD8=0.3 to CD4/CD8=1), but not with NHL risk. High CD8 counts were associated with higher NHL risk (HR=3.14(95%CI=1.58-6.22)) when comparing CD8=3000/ $mm^3$  to CD8=1000/ $mm^3$ ). Similar results with increased associations were found in PLHIV with  $CD4 \geq 500/mm^3$  at virological control (HR=3.27(95%CI=1.60-6.56) for KS; HR=5.28(95%CI=2.17-12.83) for NHL).

**CONCLUSION:** Low CD4/CD8 ratios and high CD8 counts despite effective cART, were associated with increased KS/NHL risks respectively, especially when  $CD4 \geq 500/mm^3$ .

## Introduction

Despite the widespread use of effective combination antiretroviral therapy (cART), a higher incidence of AIDS- and non-AIDS-defining events persist in people living with HIV (PLHIV), compared to the general population [1–3]. These appear to be driven by persistent immune activation, including inflammation, despite long-term viral suppression and CD4 restoration thanks to effective cART [4–6].

The CD4/CD8 ratio is considered as a reliable marker of systemic immune activation during successful cART [7,8]. Low CD4/CD8 ratios are associated with higher CD4 and CD8 T-cell activation, exhaustion and senescence, as well as with increased innate immune activation such as monocyte activation, even in individuals with undetectable viremia and  $CD4 \geq 500/mm^3$  [9,10]. Furthermore, in PLHIV receiving efficient cART, the CD4/CD8 ratio inversely correlates with the risk of AIDS-related mortality, and the risk of non-AIDS-defining events, especially non-AIDS-defining cancers [11–14]. However, the impact of the CD4/CD8 ratio on the risk of AIDS-defining cancers, such as Kaposi sarcoma (KS) and non-Hodgkin lymphoma (NHL), has never been studied.

KS and NHL remain among the most frequent cancers in PLHIV receiving cART [15,16]. KS is an angioproliferative malignancy associated with human herpesvirus-8 (HHV-8), whereas NHL can be induced by either Epstein-Barr virus (EBV), HHV-8, or Human T-cell leukemia/lymphoma virus type 1 (HTLV-1), or it may not be viro-induced, depending on subtypes. A previous cohort study conducted in the French Hospital Database on HIV (FHDH-ANRS CO4) found a 35-fold increased KS risk in PLHIV relative to that of the general population, although viremia was suppressed and CD4 counts restored to  $>500/mm^3$  for  $\geq$ two years. For NHL, the risk was 9-fold higher in PLHIV than the general population but dropped to that observed in the general population when

including only PLHIV with suppressed viremia and a stably restored CD4 count [3].

Using data from a large collaboration of European cohort studies of PLHIV (COHERE, Collaboration of Observational HIV Epidemiological Research Europe), we aimed to evaluate the impact of CD4/CD8 ratio restoration on KS or NHL risk in PLHIV on efficient cART, particularly whether the CD4/CD8 ratio provides additional information to the CD4 count, which is the usual immunological predictor for KS and NHL. As initiating cART with high CD4 count is becoming increasingly frequent in the era of early cART recommendations, a secondary analysis was conducted in PLHIV with  $CD4 \geq 500/mm^3$  at virological control [17].

## **Methods**

The COHERE in EuroCoord ([www.cohere.org](http://www.cohere.org)) is a European collaboration of cohorts of people living with HIV (PLHIV) in routine clinical care. A detailed description of COHERE has been previously published [18,19]. For the current project, data were merged in 2015 in COHERE in EuroCoord and 27 European cohorts provided data from 149 028 PLHIV aged  $\geq 16$  years.

## **Inclusion Criteria and Definitions**

HIV-1 infected adults who were  $\geq 16$  years old, initiated cART between 1 January 2000 (when cART became available in all parts of Europe) and 31 December 2014, and achieved virological control within nine months following cART initiation were included. Virological control was defined as the first plasma HIV-1 RNA  $\leq 500$  copies/mL. We used a threshold at 500 copies/mL to account for the heterogeneity of the limit of detection used in methods measuring plasma HIV-1 RNA during the study period and between participating cohorts. At least one CD4/CD8 measurement was required within 6

months after virological control. Cohorts were eligible if CD8 count measurements were reported for  $\geq 70\%$  of blood samples used to measure the CD4 count, to avoid the selection of populations where CD8 measurements would have been performed for medical reason, which could have led to a selection bias. Overall, 20 of the 27 participating cohorts COHERE were selected.

For both the main and secondary analyses, the baseline was defined as the time of the first CD4/CD8 ratio measurement within six months after virological control. In the secondary analysis, the CD4 count of the first ratio measurement had to be  $\geq 500/\text{mm}^3$ . PLHIV with a diagnosis of Kaposi sarcoma (KS) or non-Hodgkin lymphoma (NHL) at baseline or before, were excluded.

### **Statistical analyses**

Continuous variables are expressed as medians and interquartile ranges (IQR), and categorical variables as counts and percentages.

We used separate Cox analyses stratified by cohort, to identify factors associated with KS or NHL risk in the study population. Follow-up was censored at the date of patient's last clinic visit.

Sociodemographic characteristics, baseline characteristics and immune-virological factors were included in univariable analyses. **i)** Sociodemographic characteristics were age at baseline and a composite variable combining sex, HIV exposure risk and geographical origin, consisting of six categories: Men who have sex with men regardless of geographical origin (MSM), intravenous drug users regardless of geographical origin (IDU), Sub-Saharan women, Heterosexual Sub-Saharan men, non-Sub-Saharan women, and Heterosexual non-Sub-Saharan men. **ii)** Baseline characteristics were clinical Center for Disease Control (CDC) stage C, Hepatitis B (HBV)

coinfection (defined by positive HBs antigen), Hepatitis C (HCV) coinfection (defined by positive anti-HCV IgG antibodies), and calendar period of cART introduction (2000-2004; 2005-2009; 2010-2014). **iii)** Immune-virological factors were CD4 count, CD8 count, CD4/CD8 ratio, and virological failure. These factors were considered as time-dependent variables for the analyses. To that end, time was divided into successive six-month periods during the first three years of follow-up, and twelve-month periods thereafter. Indeed, after three years of follow-up, the time between visits was >6 months for 25% of PLHIV. For each period, we used the first value of the CD4/CD8 ratio, corresponding CD4 and CD8 counts, and maximal plasma HIV-1 RNA value. Virological failure was defined as plasma HIV-1 RNA > 500 copies/mL.

We analyzed the CD4/CD8 ratio, CD4 count, and CD8 count as continuous variables, using spline transformations, in order to capture non-linear associations with KS or NHL risk. The knots for the restricted cubic splines were positioned by default, using Harrell recommended percentiles [20,21]. We used tertiles of 0.43, 0.64, 0.91 for the CD4/CD8 ratio, 404, 536, 700/mm<sup>3</sup> for the CD4 count, and 621, 854, 1165/mm<sup>3</sup> for the CD8 count, in the main analysis; For the secondary analysis, tertiles were 0.55, 0.79, 1.10 for the CD4/CD8 ratio, 568, 704, 881/mm<sup>3</sup> for the CD4 count, and 669, 916, 1246/mm<sup>3</sup> for the CD8 count. Other knots based on clinical cut-offs were also tested and gave similar results.

Three models were tested to better understand the impact of immune factors on KS and NHL risk. The first (MV1) was adjusted for the CD4/CD8 ratio, the second (MV2) for both CD4 count and CD8 count, and the third (MV3) for both the CD4/CD8 ratio and CD4 count. All three models were additionally adjusted for variables with a univariable *p* value < 0.20, namely age at baseline, sex, group of HIV exposure risk, origin, calendar period of cART introduction, and virological failure. A *p*-value < 0.05 was considered

significant. Akaike's information criterion (AIC) was used to compare the fit of the three statistical models: the lower the AIC value, the better the fit. A secondary analysis was conducted in PLHIV with  $CD4 \geq 500/mm^3$  at baseline. To test the robustness of our results in PLHIV without virological failure, post-hoc analyses were performed with censored follow-up not only at patient's last clinic visit but also at virological failure. Other post-hoc analyses restricted to patients starting cART from 2005, where the limit of HIV-RNA assay was 50 copies/mL or lower in 99% of measurements, were carried out to evaluate the impact of low viremia ([50-500] copies/mL) on KS/NHL risks.

SAS statistical software version 9.4 (SAS Institute, Inc., Cary, North Carolina, USA) was used for all analyses.

## Results

### Study population

Overall, 56 708 PLHIV from 20 cohorts in 12 countries (France, Austria, Greece, the Netherlands, Germany, Italy, Spain, Denmark, Switzerland, Belgium, Sweden, and United Kingdom) were eligible for the current analyses. **Figure 1** shows the study flowchart. The main reasons for excluding patients were being enrolled in cohorts where CD8 count measurements were recorded in <70% of samples used for CD4 count measurements (n=21 024), having started cART before 2000 (n=36 126), and achieving virological control after 9 months following cART introduction (n=21 127). In addition, 1 600 PLHIV with a KS or NHL diagnosis at baseline or before, were excluded.

Baseline characteristics are shown in **Table 1**. Overall, 27 540 (49%) were MSM, and 14 259 (25%) were women. Most PLHIV were of European origin or from other western countries (including Australia and New Zealand) (n=39 002; 69%), whereas 8 461 (15%) were of Sub-Saharan origin. The median time from cART introduction to



baseline was 2.5 months (IQR=1.2-4.0). At baseline, participants had a median CD4 count of 414/mm<sup>3</sup> (IQR=296-552), a median CD8 count of 936/mm<sup>3</sup> (IQR=670-1304), and a median CD4/CD8 ratio of 0.43 (IQR=0.28-0.65). Only 8% of the study population had a CD4/CD8 ratio  $\geq 1$ , whereas 59% had a very low CD4/CD8 ratio ( $< 0.5$ ) at baseline, and 19 133 (34%) PLHIV had a CD4 count of  $\geq 500$ /mm<sup>3</sup>.

Overall, participants were followed-up for a median of 59 (IQR=30-96) months from baseline, accounting for 307 700 person-years (py). The median number of CD4 count, CD8 count and CD4/CD8 ratio measurements per patient were 15(8-25), 14(7-24) and 14(7-24) respectively. The cumulative incidence of CD4/CD8 ratio restoration  $\geq 1$  from baseline was 28% (95% confidence interval (CI) 27-28) at two years and 45% (95%CI 44-46) at five years. The corresponding cumulative incidence among PLHIV with CD4 $\geq 500$ /mm<sup>3</sup> at baseline, was 46% (95%CI 45-47) and 63% (95%CI 62-64) respectively.

A total of 221 KS and 187 NHL were diagnosed after baseline, corresponding to incidence rates of 72/100 000 py (95%CI 55-89) for KS and 61/100 000 py (95%CI 46-76) for NHL. KS were diagnosed 9 (IQR=2-37) months after baseline, whereas NHL diagnoses occurred 18 (IQR=7-42) months after baseline. KS and NHL occurred in the context of virological failure for 33/221 (15%) and 23/187 (12%) PLHIV, respectively.

The secondary analysis included 19 133 PLHIV-1 with CD4 $\geq 500$ /mm<sup>3</sup> at baseline, of whom 65 presented with KS and 50 with NHL. KS and NHL occurred in the context of virological failure for 14 (21%) and 11 (22%) PLHIV, respectively.

### **Factors associated with the risk of Kaposi sarcoma (Table 2)**

Immune-virological factors associated with KS risk in the three different multivariable models MV1, MV2, and MV3 are presented in **Table 2**. Virological failure

was the strongest factor associated with KS risk in all three models, with a nearly three-fold higher risk.

In MV1, adjusted for CD4/CD8 ratio as the only immune factor, the lower the CD4/CD8 ratio the higher the KS risk (HR from 1.25 for PLHIV-1 with CD4/CD8=0.8, to 2.56 for those with CD4/CD8=0.3, when compared to CD4/CD8=1).

In MV2, which included the CD4 and CD8 counts separately, without the CD4/CD8 ratio, both immune parameters (low CD4 counts and high CD8 counts) were independently associated with KS risk.

MV3 was adjusted for both CD4 count and CD4/CD8 ratio, and confirmed independent associations of both parameters with KS risk: CD4 restoration was associated with decreased KS risk, even for CD4>500/mm<sup>3</sup> [HR=1.57 when CD4=350/mm<sup>3</sup> to HR=0.71 when CD4=650/mm<sup>3</sup>, when comparing to CD4=500/mm<sup>3</sup>]. Furthermore, the CD4/CD8 ratio was gradually associated with KS risk (HR from 1.18 in PLHIV with CD4/CD8=0.8, to 2.02 for those with CD4/CD8=0.3, when comparing to CD4/CD8=1). According to the AIC value, MV3 was the most accurate model (**Table 2**).

Other independent factors associated with higher KS risk were being MSM and being older (**Supplementary Table 1**).

In PLHIV with CD4≥500/mm<sup>3</sup> at baseline, the inverse association between CD4/CD8 ratio and KS risk was stronger than that observed in the entire study population. This is illustrated by **Figure 2A** (overall analysis) and **Figure 2B** (analysis restricted to PLHIV with CD4≥500/mm<sup>3</sup> at baseline) showing the impact of CD4/CD8 ratio on KS risk according to the most accurate model adjusted for both CD4 and CD4/CD8 ratio (MV3).

### **Factors associated with the risk of non-Hodgkin lymphoma (Table 3)**

As observed with KS, virological failure was strongly associated with NHL risk, with a two-fold higher risk in all multivariable models.

Contrary to KS, the CD4/CD8 ratio did not add supplementary predictive information to the CD4 count for NHL risk. However, MV2 showed that NHL risk strongly increased when the absolute CD8 count was very high (HR from 1.61 for CD8=2000/mm<sup>3</sup>, to 3.14 for CD8=3000/mm<sup>3</sup>, compared to CD8=1000/mm<sup>3</sup>). According to the AIC, MV2, adjusted for both CD4 count and CD8 count, was the most accurate model (**Table 3**).

Other independent factors associated with higher NHL risk were being older and male sex (**Supplementary Table 1**).

In PLHIV with CD4≥500/mm<sup>3</sup> at baseline, the positive association between CD8 count and NHL risk was stronger than that observed for the entire study population. This is illustrated by **Figure 3A** (overall analysis) and **Figure 3B** (analysis restricted to PLHIV with CD4≥500/mm<sup>3</sup> at baseline), showing the impact of CD8 count on NHL risk, according to the most accurate model adjusted for both CD4 count and CD8 count (MV2).

### **Post-hoc analyses**

When the follow-up of PLHIV was censored at virological failure, similar associations were found between the CD4/CD8 ratio and KS risk, and between CD8 count and NHL risk (**Supplementary Table 2**). Analyses restricted to PLHIV starting cART from 2005 showed no impact of low viremia ([50-500] copies/mL) on KS/NHL risks (**Supplementary Table 3**).

## **Discussion**

In this large European clinical cohort collaboration, we observed a differential impact of the CD4/CD8 ratio on KS or NHL risk, both AIDS-defining cancers. A low CD4/CD8 ratio was associated with KS risk, even after adjustment for CD4 count and viral load. In contrast, the CD4/CD8 ratio was not associated with NHL risk after adjustment for CD4 count, whereas very high CD8 counts ( $\geq 2000/\text{mm}^3$ ) were strongly associated with NHL risk. As for other HIV-related morbidities virological failure was strongly associated with both KS and NHL risks [3,13].

Secondary analyses conducted in the subgroup of PLHIV with  $\text{CD4} \geq 500/\text{mm}^3$  at baseline showed a stronger association of both the CD4/CD8 ratio for KS risk and the CD8 count for NHL risk.

## **Strength and limitations**

This is the first study to evaluate the impact of the CD4/CD8 ratio on the risk of AIDS-defining cancers. This analysis, based on a large cohort collaboration with a long-term follow-up, allowed the study of rare events separately, in the setting of efficient cART and  $\text{CD4} \geq 500/\text{mm}^3$ . Our findings confirmed the importance of studying cancers separately, since the association between CD4/CD8 ratio and cancer risk differed between the two. Limitations included the absence of information on KS clinical presentation, NHL histological subtypes, and viral serostatus associated with NHL (*i.e.* EBV, HHV-8, HTLV-1), precluding analyses according to these parameters. Evaluating the association between CD4/CD8 ratio and the risk of immune reconstitution inflammatory syndrome was limited since baseline started close to 3 months after cART introduction and most unmasking IRIS events occur within this time frame.

## **Kaposi sarcoma**

Despite large cART-related declines in KS incidence over time, a higher KS risk persists in PLHIV than in the general population, especially for MSM and PLHIV from Sub-Saharan Africa, among whom HHV-8 seroprevalence is elevated [3,15,22,23]. Nevertheless, the clinical context in which KS occurs has considerably changed during the effective cART era. A growing proportion of KS is diagnosed among patients on cART, with higher CD4 counts and/or suppressed viremia, showing different clinical presentations [24].

A previous study of effectively treated PLHIV showed KS to be associated with an increased frequency of T-cells with immunosenescent phenotypes and lower frequencies of naive T-cells [25]. The mechanisms linking immune activation to KS include pro-inflammatory cytokines present in KS microenvironment and involved in the stimulation of angiogenic KS lesions. The KS microenvironment is characterized by inflammatory cellular infiltrates, T-cell infiltrates are low, and associated with very low anti-HHV-8 CD8 T-cell responses in peripheral blood [26,27]. Although the link between immune activation and KS development might be bidirectional, our findings suggest that HIV-related immune activation, reflected by CD4/CD8 ratio, may contribute to KS development in PLHIV on efficient cART.

## **Non-Hodgkin lymphoma**

Efficient cART have reduced the risk of NHL and induced substantial shift in the subtypes of lymphoma observed in PLHIV [24,28]. Higher risks of NHL in PLHIV on cART may be due to persistent low-level HIV replication on cART, resulting in chronic B-cell activation and promoting oncogenic mutations and translocations, leading to

lymphomagenesis [29,30]. Moreover, persistence of an HIV latent reservoir may also influence NHL risk, the HIV proteins promoting B-cell clonogenicity [31].

Here, the CD4/CD8 ratio did not add supplementary predictive information to the CD4 count for NHL risk. Nevertheless, the highest CD8 counts ( $\geq 2000/\text{mm}^3$ ) were associated with elevated NHL risk. Only one nested case-control study has evaluated CD8 counts before NHL diagnosis in PLHIV on cART, in which there was a greater decline in CD8 counts during the last two years preceding NHL diagnosis than that observed in controls ( $-184/\text{mm}^3$  vs  $-19/\text{mm}^3$ ) [32]. Absolute CD8 counts were not reported.

Other studies reported elevated levels of immune activation markers several years before NHL diagnosis, suggesting that immune activation likely plays a key role in NHL development [33,34]. In addition, cytotoxic T-cells, particularly activated CD8 T-cells, are markedly elevated in diffuse Large B-cell Lymphoma, a major subtype among HIV-associated NHL [28]. Furthermore, HHV-8 during Multicentric Castelman disease, and EBV, induce high virus-specific CD8 T-cell responses [35]. Such strong stimulation before cell proliferation may explain the positive association between high CD8 counts and NHL risk.

### **PLHIV with $\text{CD4} \geq 500/\text{mm}^3$**

Early cART recommendations have resulted in changes in the characteristics of the HIV population, with a growing proportion of PLHIV with  $\text{CD4} \geq 500/\text{mm}^3$  [22,24]. CD4/CD8 ratio restoration remains difficult in this population, because of persisting high CD8 counts [36–38]. It has been reported that 25% of PLHIV starting cART with  $\text{CD4} \geq 500/\text{mm}^3$  still had  $\text{CD8} > 1000/\text{mm}^3$  after eight years of suppressive cART [38]. As previously shown, we confirmed that a large proportion (37%) of PLHIV with

CD4 $\geq$ 500/mm<sup>3</sup> at baseline, still had a CD4/CD8 $<$ 1 after five years of suppressed viremia [39].

The association of a low CD4/CD8 ratio with morbidity has already been reported in subjects with CD4 $\geq$ 500/mm<sup>3</sup>, and maintaining a low CD4/CD8 ratio was associated with CD8 T-cell activation [10]. Moreover, a CD8 count above 1000/mm<sup>3</sup> may be a predictor of new AIDS-defining events among PLHIV with CD4 $>$ 500/mm<sup>3</sup> [40]. In our study, the CD4/CD8 ratio and CD8 count appeared to be particularly relevant markers in the context of high CD4 $\geq$ 500/mm<sup>3</sup>, to identify patients with increased risk of developing KS and NHL.

## **Conclusion**

HIV-related morbidities still occur in PLHIV despite suppressed viremia and relative immune restoration. Thus, biomarkers other than the traditional predictive CD4 count may be useful to identify the subset of individuals with a higher risk of morbidities. Previous studies have shown that the CD4/CD8 ratio may help to better monitor immune restoration and identify PLHIV with a higher risk of non-AIDS-defining events, despite efficient cART. Here, we showed that PLHIV with low CD4/CD8 ratios or high CD8 counts, despite efficient cART, had a higher risk of developing KS and NHL respectively, and that these markers, routinely assessed and easily available, may be particularly relevant in PLHIV with CD4 $\geq$ 500/mm<sup>3</sup>. Closer clinical monitoring could be beneficial for PLHIV with persisting low ratios despite efficient cART. However further studies are needed to clarify the impact of the CD4/CD8 ratio on the risk of other HIV-related diseases, to know which specific preventives measures could be implemented.

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## **POTENTIAL CONFLICTS OF INTEREST**

Dr Caby reports personal fees from Gilead France, personal fees from Janssen France, outside the submitted work. Dr Weiss reports personal fees from Merck Sharp Dhome, other from VIV Healthcare, outside the submitted work. Dr Winston reports personal fees from Honoraria and speaker fees, grants from Research Grants, outside the submitted work. Dr Miro reports grants (IDIBAPS during 2017-21) and personal fees from AbbVie, Angelini, Contrafect, Cubist, Genentech, Gilead Sciences, Jansen, Medtronic, MSD, Novartis, Pfizer, and ViiV Healthcare, outside the submitted work. Dr Bonnet reports grants from Janssen, personal fees from ViiV Healthcare, Gilead, MSD, Janssen, outside the submitted work. Dr Reiss reports grants from Gilead Sciences, grants from ViiV Healthcare, grants from Merck & Co, other from Gilead Sciences, other from ViiV Healthcare, other from Merck & Co, other from Teva Pharmaceutical Industries, outside the submitted work. Dr Taylor reports personal fees and other from Gilead, other from ViiV, personal fees and other from Merck, outside the submitted work. Dr MEYER reports grants from ANRS, during the conduct of the study. Dr Boesecke reports personal fees from AbbVie, Gilead, Janssen, MSD, ViiV, grants from NEAT ID, Dt.



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## FIGURE LEGENDS

### Figure 1: Study flow-chart

Abbreviations: HIV-1, Human immunodeficiency Virus type 1; COHERE, Collaboration of Observational HIV Epidemiological Research Europe; cART, combination antiretroviral therapy; KS, Kaposi sarcoma; NHL, non-Hodgkin lymphoma.

### Figure 2: Effect of the CD4/CD8 ratio restoration on KS risk, in the whole study population (A) and in PLHIV with CD4 $\geq$ 500/mm<sup>3</sup> at baseline\* (B).

\*Baseline is the date of the first CD4/CD8 measurement within six months following virological control.

\*\*Plots of computed *HR* from multivariable Cox analysis adjusted for age, sex, group of HIV exposure risk, origin, calendar period of cART introduction, virological failure, CD4 count (MV3). Virological failure, CD4 count and CD4/CD8 ratio were considered as time-dependent variables from baseline. CD4 count, CD4/CD8 ratio were modeled using spline transformations.

Abbreviations: KS, Kaposi sarcoma; PLHIV, people living with Human immunodeficiency virus; *HR*, Hazard Ratio; CI, confidence interval; cART, combination antiretroviral therapy.

### Figure 3: Effect of CD8 counts on NHL risk, in the whole study population (A) and in PLHIV with CD4 $\geq$ 500/mm<sup>3</sup> at baseline\* (B).

\*Baseline is the date of the first CD4/CD8 measurement within six months following virological control.

\*\*Plots of computed *HR* from Multivariable Cox analysis adjusted for age, sex, group of HIV exposure risk, origin, calendar period of cART introduction, virological failure, CD4 count (MV2). Virological failure, CD4 count and CD8 count were considered as time-

dependent variables from baseline. CD4 count and CD8 count were modeled using spline transformations.

**Abbreviations:** NHL, non Hodgkin lymphoma; PLHIV, people living with Human immunodeficiency virus; *HR*, Hazard Ratio; CI, confidence interval; cART, combination antiretroviral therapy.