UNIVERSITA' DEGLI STUDI DI MILANO

Dipartimento di Scienze della Salute

Facoltà di Medicina e Chirurgia

Scuola di Dottorato in Medicina Clinica e Sperimentale

HIV-associated cancers: role of immune parameters and relation with the outcome of chemotherapy

Tutor: Prof.ssa Giulia Carla Marchetti

Supervisor: Prof. Antonella d'Arminio Monforte

Coordinatore del Dottorato: Prof. Emilio Berti

Tesi di Dottorato di Ricerca – XXXI ciclo

Elisa Suardi

Matricola R11388

Anno accademico 2017/2018

Summary

Summary

Background

HIV positive patients present a higher risk of cancer compared to the HIV-negative population. Three tumors, namely Kaposi sarcoma (KS), non-Hodgkin lymphoma (NHL) and invasive cervical cancer (ICC) occurs with particularly high incidence in HIV-positive patients, and have been classified as AIDS-defining malignancies (ADMs).

However, a risk excess is observed for both ADMs and non-AIDS defining malignancies (NADMs).

Co-infection with oncogenic virus occurs as a key pathogenic factor in ADMs (namely, Non-Hodgkin lymphoma (NHL), Kaposi sarcoma (KS), anal and cervical cancer) and in some NADMs (anal cancer, haepatocellular carcinoma (HCC) and Hodking lymphoma (HL). Beside co-infection with oncogenic virus, the pathogenic pathways underlying the development of infection-related and non infection-related cancer in people living with HIV (PLWH) are sustained by a complex network of interactions between various components of the immune system, with cytokines and other pro-inflammatory agents mediating those interactions. However, these mechanisms have not been fully characterized.

Aim

To shed light on this topic, we conducted 3 studies with the following aims:

- Association of T-cells activation markers and CD4+/CD8+ ratio with virus related and non-virus related cancers in HIV-positive patients: High levels of peripheral T-cells immune activation and low CD4+/CD8+ ratio in HIV-positive patients have been associated with comorbidities and increased risk of serious events and deaths, despite effective combination antiretroviral therapy (cART). Thus, we aimed to assess the association of peripheral

immune activation markers (CD8+CD38+ T-lymphocytes) and CD4+/CD8+ ratio with the onset of infection-related and non-infection related cancers in a cohort of HIV-positive patients. We further investigated possible association of CD8+CD38+ T-cells and CD4+/CD8+ ratio with overall survival of HIV-positive patients with virus-related cancer and non virus-related cancer.

- Natural Killer cell populations in HIV associated lymphoma: Natural Killer (NK, CD56+) cells exert anti-cancer and anti-viral actions and their number and function is impaired during HIV infection. In HIV-negative patients with Hodgkin Lymphoma (HL) and diffuse large B-cell lymphoma (DLBCL), NK cell constitute a higher percentage of circulating lymphocytes and are associated with a better outcome. We aimed to evaluate the NK cell population in HIV-associated lymphomas.
- Sex-based differences in factors associated with HPV infection and intraepithelial lesions in a cohort of HIV-positive patients: We aimed to assess the factors associated with Human Papilloma Virus (HPV) infection and with squamous intraepithelial lesions (SILs) in a cohort of HIV-positive patients with focus on gender differences, and possible factors linked with the capability to clear HPV infection, thus influencing the progression of SILs towards cancer.

Materials and methods

For the following studies:

- Association of T-cells activation markers and CD4/CD8 ratio with the onset of virus related and non-virus related cancers in HIV-positive patients;
- Sex-based differences in factors associated with HPV infection and intraepithelial lesions in a cohort of HIV-positive patients;

we enrolled antiretroviral-naive and cART-treated HIV-infected patients in active follow up at the SPID (San Paolo Infectious Disease) Cohort. The SPID cohort was set up in 2006 and includes all HIV positive patients who are attending the outpatients service of Infectious Diseases' Clinic at San Paolo Hospital, Milan, Italy.

According to the study, patients underwent blood exams and gynaecological or surgical visits with colposcopy/anoscopy at baseline (time of first visit); a longitudinal study was performed on an unselected subgroup of patients who repeated the same evaluation after 12-24 months.

Lymphocyte subsets were evaluated by flow cytometric analysis.

For the study on Natural Killer (NK) cells in a cohort of HIV-positive patients, we enrolled patients who attended at the National Centre for HIV malignancies at the Chelsea and Westminster Hospital, London, UK. We included 615 HIV seropositive patients diagnosed with lymphoma between 1988 and 2017. Data on patient characteristics, prognostic factors, treatment and outcomes were extracted from the database.

Lymphocyte subsets were evaluated by flow cytometric analysis.

Comparison of variables between the groups was by Chi-squared test for categorical data and Kruskal-Wallis test for non-parametric continuous variables. The association between CD4+T-cells count and NK cells count was assessed by bivariate analysis. The time interval from lymphoma diagnosis until death, study censoring or loss to follow-up was used to calculate overall survival (OS). Survival curves were plotted according to the method of Kaplan and Meier. The log rank method was used to test for the significance of differences in survival distributions.

Results

Association of T-cells activation markers and e CD4/CD8 ratio with the onset of virus related and non-virus related cancer in HIV-positive patients: we enrolled

140 HIV-positive patients, 115 with virus-related cancer and 25 with non-virus related cancers. Patients with virus-related cancer were younger (p<0.0001), with a shorter time from HIV-diagnosis (p=0.04), more frequently naïve to cART (p=0.009) and with lower CD4+CD8+ ratio (p=0.01) compared to patients with non virus-related cancer. When we analyzed the group of patients on cART, CD4+/CD8+ ratio was comparable in infection-related and non infection-related cancer. Immune activation was similar in the two group.

Neither CD4+/CD8+ ratio nor CD8+CD38+ were predictor of outcome in our cohort.

Natural Killer cell populations in HIV associated lymphoma: we included in the analysis 360 lymphoma cases with full lymphocyte subset analysis at cancer diagnosis.

The percentage of natural killer (NK) cells was significantly higher in patients with Hodgkin lymphoma (HL) (median 9%) than in diffuse large B-cell lymphoma (DLBCL)(6%) and other non-Hodgkin lymphoma (NHL) subtypes (p=0.009). The total NK cell count was significantly higher in patients with undetectable HIV-RNA(p<0.0001) but only weakly correlated with CD4 cell count (PearsonsR^2 =0.11). For 156 patients with DLBCL, the 5-year OS was 64% (95%CI 56-72%) and in univariate analysis neither total NK-cell population (log rank p=0.14) not NK-cell % (log rank p=0.84) were prognostic variables for OS. In multivariate Cox model the only variable associated with OS was the International Prognostic Index (IPI) (log rank p<0.001).

Sex-based differences in factors associated with HPV infection and intraepithelial lesions in a cohort of HIV-positive patients: 472 patients were enrolled.

HPV infection(p<0.001), SIL (p<0.001), HPV persistence (p<0.001) and SIL progression (p=0.018) were all associated with male sex.

Among females, HPV was associated with higher HIV-RNA (p=0.002) and SIL was independently associated with lower CD4+ count (AOR: 0.998; 95%CI: 0.997-1).

In the males group, HPV was associated with homosexual intercourse (AOR: 3.801, 95% CI: 1.82-7.95) while AIDS diagnosis (AOR: 0.387 95% CI:0.176-0.851) and older HIV infection (AOR: 0.996 95% CI:0.992-0.999) were negatively associated with HPV infection; males with SIL were younger (AOR 0.973, 95% CI 0.95-0,997), more frequently men who have sex with men (MSM) (p=0.04) and with higher levels of immune-activation (CD38+CD8+%) (p=0.013) compared to SIL-negative males.

Conclusions

In our cohort, immune activation at the onset of cancer was similar in patients with infection and non infection-related malignancies. Patients with infection-related cancer had lower CD4+/CD8+ ratio, but this difference was no longer significant among patients on cART, supporting the importance of a prompt initiation of cART in restoring immune-deregulation in both virus and non-virus related malignancies.

In this study, CD8+CD38+ and CD4+/CD8+ ratio were not predictive of outcome in HIV-positive patients with cancer.

When we analyzed HIV-positive patients with lymphoma of the National Centre for HIV malignancies at the Chelsea and Westminster Hospital, we found that NK cells deficiency is a key point in the development of non-Hodgkin lymphoma in PLWH and could explain, at least in part, the higher risk for malignancies that persists in the cART era. However, a lower NK cells count was not associated with a worse overall survival of our patients with DLBCL, possibly because of a functional impairment beyond their quantitative depletion. Since in HIV-negative patients immunotherapy targeting NK cells is promising, further studies in people living with HIV (PLWH) may allow comprehending whether NK-cells antitumor function could be restored in these patients, and consequently exert their potential to boost the response to chemotherapy.

Finally, we found that despite an adequate immune recovery, HPV infection and clearance as well as the presence of intraepithelial lesions is largely influenced by risky behaviors and persistent immune activation in males, particularly in recently HIV-infected patients. Among women, on the other hand, the risk of precancerous lesions is linked to immune-suppression as measured by CD4+T-cells count; we suggest that these groups of patients should be considered for more accurate screening and extensive vaccination.

Introduction

Epidemiologic features of oncologic disease in HIV-positive patients

Since the beginning of the HIV pandemic, people living with HIV (PLWH) have been at higher risk of cancer compared to the general population. To date, cancer is responsible of one third of all deaths in HIV-positive patients[1].

Three cancer types, namely Kaposi's sarcoma (KS), non-Hodgkin lymphoma (NHL) and invasive cervical cancer (ICC) have been found to be associated with a particularly high frequency with AIDS, and therefore have classically been referred to as AIDS-defining-malignancies (ADMs) [2].

During 1990-1995, before the introduction of combination antiretroviral therapy (cART), the risk of KS, NHL and ICC in PLW was respectively 2800-fold, 10-fold and 3-fold higher than the risk in the general population[3].

After the advent cART in 1996 the incidence rate of ADMs has decreased, and this trend has been driven mostly by the sharp reduction in the incidence of KS and NHL[4], while the incidence of ICC has not significantly been affected by the advent of cART[5]. However, KS is still one of the most common cancers in Africa and is globally the most common tumor in HIV-infected individuals[6], while NHL still constitute >50% of all ADMs in developed countries[7].

PLWH also show a higher frequency of non-AIDS defining malignancies (NADMs) and with the advent of cART, leading to a prolonged life expectancy in HIV-positive patients, the burden of NADMs has collectively over passed the burden of ADMs[8] [4].

The relative risk of cancer in PLWH is particularly high for cancer with a known or suspected viral origin, with the notable exception of lung cancer and other smoke-related cancer[9].

Indeed, among NADMs the most prevalent is lung cancer, with a risk that is nearly three times higher in the HIV-infected than in the HIV-negative population[10].

A 2.1-fold higher risk of non-melanoma skin cancer and 1.8-fold higher risk of melanoma have been reported in PLWH compared to the general population[11 12 13]. Of note, breast cancer has a similar incidence in HIV-positive and HIV-negative women[14 15]. The incidence of prostatic and colorectal cancer is also similar in HIV-positive compared to HIV-negative individuals[9].

Among non AIDS-defining virus-related cancers, Human Papilloma Virus (HPV)-related anal cancer is particularly frequent in PLWH and specially among men who have sex with men (MSM), who are likely to acquire HPV infection during sexual intercourse, with a 30 to 100-fold higher incidence rate compared to the general population[16 17]. Moreover, the risk for hepatocellular carcinoma (HCC) is 4-fold higher in PLWH than the general population. In a large retrospective cohort study on US veterans, HIV/HCV positive patients showed a higher risk to develop HCC than HIV negative ones[18]. The incidence of HCC has steadily increased among individuals with AIDS in the United States over the past 3 decades, and the relative risk excess for this kind of cancer has remained relatively unchanged over time, including in the cART era.

Further, the risk of Hodgkin lymphoma (HL) has been shown to be 5 to 20-fold higher in PLWH than in the general population, with a similar incidence in the pre- and post-cART era[19].

Risk factors and pathogenetic aspects

Non virus-related cancers

The reasons of the cancer risk excess in PLWH have not been fully clarified. This can be in part explained with the high prevalence of classical cancer risk factors, primarily tobacco use, earlier smoking initiation and increasing proportion of HIV-positive patients aged 50 yrs or older.

A meta-analysis of 113 studies across developed countries, largely in North America and Western Europe, showed that 54% of HIV-infected people were current smokers[20]. A population-based survey of HIV-infected people in care in the United States during 2009 found that 42% were current smokers and another 20% were former smokers[21]. Another study reported the prevalence of smoking among all participants of the Swiss HIV Cohort Study to be 72%, and as high as 96% among injection drug users in the cohort[11].

As regard to the other risk factors for cancer development, a large meta-analysis found hazardous alcohol consumption prevalence not to be higher in PLWH compared to uninfected comparison group[20]. In the same report, the prevalence of obesity and overweight was higher in uninfected patients compared to PLWH.

However, the elevation in cancer incidence appears higher than can be explained by smoking and other classical risk factors alone; immune suppression caused by HIV infection may be determinant, as supported by evidence for an increase in lung cancer risk with advancing time relative to AIDS diagnosis, and among transplant recipients[22]. Inflammation and immune senescence caused by HIV-replication could contribute to cancer risk[23] [2], as well as pro-oncogenic effects of secreted HIV-encoded proteins[23]. Specifically, HIV infection is associated with genetic instability, as suggested by the higher prevalence of microsatellite alteration in HIV-related than in non HIV-related lung cancers. Further, the action of some carcinogen factors may be enhanced in HIV-positive patients, due to the endothelial damage occurring during chronic infection, which could led to a permissive environment for cancer growth[24].

Further, the more rapid immunologic aging in PLWH, which is characterized by a higher prevalence of CD8+CD28- , CD4+CD28- cells, reduction in the number of CD4+CD31+ naïve T-cells, shortened telomeres and increased CD56+CD57+ compared to age-matched HIV-negative patients[25], could lead to an increased risk of cancer.

Virus-related cancers

Non Hodgkin lymphoma

Role of HIV infection: Some of the more common types of systemic NHL occur in the setting of chronic B cell activation, and the effects of chronic B cell activation on lymphomagenesis are numerous, including the accumulation of oncogene mutations and translocations.

HIV infection and consequent immune suppression contributes to oncogenesis through the loss of T-cells control over B-cells function; the subsequent B cells hyperactivation promotes the development of AIDS-NHL through the loss of immunoregulatory control over Epstein-Barr virus (EBV) -infected B cells; further, chronic hyperactivation and pro- inflammatory state promote the proliferation of dysregulated B cells[26].

It has further been proposed that HIV-infected macrophages could drive stimulatory signals that contribute to create a permissive microenvironment for malignant B-cell growth[27].

Role of coinfection with oncogenic virus: alongside the role HIV-infection per se, two gamma herpes viruses, Human Herpesvirus-8 (HHV-8) and EBV, have been shown to play a crucial role in the pathogenesis of a wide range of lymphomas. HHV-8 is involved in the pathogenesis of primary effusion lymphoma (PEL) and large B cells lymphomas arising in patients with multicentric Castleman's disease, whereas

EBV serves as a co-factor in the development of Burkitt lymphoma (BL), Hodgkin lymphoma and diffuse large B-cell lymphoma[28].

Viruses act by directly promoting oncogenesis via the action of viral proteins that regulates cells growth, survival and differentiation.

Further, viral infection and persistence promote a dysregulation in the immune pathways of the host which is crucial in the development of NHL.

Role of Natural Killer cells: Natural Killer cells (NK) are a key component of tumor immune surveillance and play an important role in rituximab-dependent killing of lymphoma[29 30].

NK cells have a high cytotoxic potential and secrete a variety of cytokines and chemokines, such as γ-interferon (IFN- γ), that amplify the recruitment and activation of other participants to the antitumor response.

NK cells are grouped in two different subpopulations, namely CD56dim and CD56bright cells, based on the relative surface density of CD56 antigen[31].

The subset of CD56dim NK have a high, CD16-dependent, cytotoxic activity, while CD56bright NK are potent producers of immuno-regulatory cytokines, and bear low or absent CD16.

A recent study[32] report baseline and long-term analysis of the phenotypic and functional dynamics of peripheral blood NK cell subsets in DLBCL HIV-negative patients, revealing tumor associated as well as therapy dependent alterations of the systemic NK cell compartment.

Role of pro-inflammatory cytokines: a significant associations has been reported between elevated levels of B cell activation biomarkers and subsequent risk of NHL, including numerous cytokines, chemokines and soluble receptors.

Interleukin 6 (IL-6) is a pro-inflammatory cytokine that has been recognized to play a role in promoting tumor growth, and has been found to be elevated in the serum of HIV-positive patients years before the development of NHL[27].

Interleukin 10 (IL-10) is a potent B cell-stimulatory factor and strongly related with NHL risk[33].

A number of groups have evaluated serum biomarkers in an effort to more fully characterize the cytokine signaling underlying the immunological mechanisms involved in the development of AIDS-related NHL, focusing on some cytokines (IL-6), serum free light chains (sFLC), and microRNAs[34]. Some authors evaluated a wide panel of biomarkers, including cytokines and chemokines, in HIV positive patients with NHL compared to HIV-positive lymphoma free control[27]. These studies are mostly retrospective and have been conducted in populations predominantly composed of patients who were not treated with cART prior to the diagnosis of AIDS-NHL[35].

Hodgkin lymphoma

Hodgkin lymphoma (HL) is a B-cell derived malignancies characterized by the presence of malignant Hodgkin/Reed–Sternberg (HRS) cells surrounded by inflammatory infiltrate[36]. Two distinct forms of HL, known as classical Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma, have been recognized and separated on the basis of morphologic, immunophenotypic, and clinical differences[37]. In the general population, classical HL accounts for 95 % of cases, whereas 5 % of the patients are diagnosed with nodular lymphocyte-predominant HL. Conversely, among PLWH the histology of HL tends to be mixed cellularity or lymphocyte-depleted.

Early studies demonstrated a clear causal relationship between Epstein-Barr virus (EBV) infection and HIV-related HL, and is well recognized that HIV-associated HL is linked to EBV in 80 to 100% of cases[38], while only a fraction of HL from the general population is EBV associated.

Among patients with AIDS, the onset of HL seems not be associated to the method of HIV acquisition (homosexual contact, intravenous drug abuse, blood transfusions, heterosexual contact), although the frequency perhaps is higher in people who abuse intravenous drugs[39].

The relation between the development of HL, immune suppression and cART is complex.

Engels et al. found that Hodgkin lymphoma incidence increased substantially following an AIDS diagnosis, but also increased over time in association with the introduction of cART, confirming results from prior studies that described an increasing risk over time subsequent to an AIDS diagnosis, and an elevated risk associated with use of cART[22].

In a previous work, the same group found that as the CD4+ cells count approaches 225-249 cells/mmc the incidence of HL increases, but as the count falls further the incidence declines[40]. These findings suggest that partial immune reconstitution with cART pushes the HIV infected individuals to an intermediate level of immune suppression that causes the highest risk of developing HL. Furthermore, it has been demonstrated that drops in CD4/CD8 ratio in the year prior to HL diagnosis may promote the onset of disease[41].

<u>Kaposi's sarcoma</u>

The etiologic agent of Kaposi's sarcoma (KS) is the Human Herpesvirus-8 (HHV-8), also known as Kaposi's sarcoma Herpes virus (KSHV). KSHV is mainly transmitted via saliva, but the routes of transmission are likely different in different populations. In low KSHV prevalence areas, the virus is predominantly spread among men who have sex with men (MSM), implicating risk factors related to sexual behavior.

Although KSHV transmission by blood transfusion or transplanted organs is documented, based on cost–benefit analyses most countries do not yet routinely screen blood or organ donors for KSHV infection.

The most common cell type in nodular lesions is the spindle-shaped cells (also known as KS cells). The vast majority of these spindle cells express endothelial markers, including CD31, CD34 and Factor VIII, but also markers of lymphatic endothelium, such as vascular endothelial growth factor receptor 3 (VEGFR3), lymphatic vessel endothelial hyaluronan 1 (LYVE1), D2-40 and podoplanin[42]. Ultrastructurally, spindle cells have features of both the lymphatic and vascular endothelium[43].

KSHV latent transcripts, such as latency-associated nuclear antigen (LANA), viral cyclin, viral FLICE/caspase 8 inhibitory proteins (FLIPs) and viral-encoded microRNAs, drive cell proliferation and prevent apoptosis, whereas KSHV lytic proteins, such as viral G protein-coupled receptor, the transmembane glycoprotein K1 and virally encoded cytokines (viral interleukin-6 and viral chemokines) further contribute to the angioproliferative and inflammatory KS lesions through a mechanism called paracrine neoplasia[44].

However, the presence of HHV8 infection alone is not sufficient to explain the insorgence of KS, and a large proportion of patients infected with HHV-8 never develop sarcoma. The most important cofactor for the disease's development is HIV-infection. The incidence of Kaposi's sarcoma is higher among HIV-infected individuals with severe immune suppression, and the prevalence of KS has notably reduced in developed Countries since the introduction of cART[45].

Indeed, patients acquiring HHV-8 infection with pre-existing HIV infection have a significantly higher risk of developing KS. This suggests that an already damaged immune system predisposes to a higher KSHV load, with subsequent KS development. Antiretroviral therapy may reduce the incidence of Kaposi's sarcoma,

as well that of other AIDS-related malignancies, via directly inhibiting tumor development and indirectly by improving the immune surveillance against tumor cells[45].

Moreover, chronic KSHV infection induce secretion of several pro inflammatory cytokines and growth factors, such as IL-6, Oncostatin M52, hepatocyte growth factor (also known as scatter factor)53, fibroblast growth factor 2 and vascular endothelial growth factor 5 (VEGF5), which in turn play a key role in driving the oncogenesis.

HPV-related cancers

Anal cancer, cervical cancer and oropharingeal cancers are strictly related to HPV infection.

Several mechanisms may explain the high prevalence and the greater aggressiveness of HPV-associated diseases in HIV-positive patients. Among these, three elements appear to be of greater importance: direct interaction between the two viruses, impairment of the immune response and chromosomal instability.

The specific cell-mediated immune response plays a fundamental role in the control of HPV infections in both general and in HIV-positive population.

About 80% of sexually active individuals undergo HPV infection during life, but only a few develops a precancerous lesion, probably due to an effective cell-mediated immune response able to control and eradicate the HPV virus.[46]

Similarly, it can be observed that individuals undergoing an iatrogenic immunosuppression secondary to organ transplantation have an increased risk of cervical cancer and vulvar cancer, due to the suppression of the immune response mandatory in this kind of patients.[47]

Little is known about this specific immune response; to date several studies have demonstrated a role of cytotoxic T-cells, specific for E6 and E7 HPV oncoproteins, in the prevention of precancerous lesions.[46]

On the other hand, in general population the immune system response to HPV infection is weak compared to that evoked against a viral systemic infection, such as cytomegalovirus infection, probably due to high compartmentalization of HPV, which is only situated in the epithelial cells of the skin and mucous membranes[48].

Kobayashi et al. have found that in intraepithelial squamous lesions (SILs) lesions of HIV-positive women the concentration of immune cells (CD4 + lymphocytes, macrophages, neutrophils, natural killer) and the expression of interferon-gamma (IFN-γ) are decreased compared to the same lesions in HIV-negative women.[48]Several studies have shown a clear relationship between T-cell infiltration into the site of lesion and disease regression, supporting a role for adaptative T-cell response in lesion regression. The virus specific T-cells of the adaptative response consist of CD4+ and CD8+ populations. CD8+ T-cells have traditionally been thought to play a major role in elimination of viral infection, secreting cytokines like IFN-γ, interleukine-2 (IL-2) and tumor necrosis factor α (TNF-α), displaying cytolytic effects mediated by molecules such as granzyme and perforin, while CD4+ T-cells, which also secrete IFN-γ, mediate killing primarily by engagement with ligands for death receptors such as Fas or TRAIL, resulting in caspase-mediated apoptosis.[49]

HIV infection further weakens immune response against HPV, both through depletion of T-cells and through down-regulation of cytokine compartment.[50]

Beyond HIV-HPV co-infection, there are other factors involved in carcinogenesis: tobacco smoking, high parity, age at first birth, high number of sexual partners, use of oral contraceptives, hormone replacement therapy, dietary deficiency, history of sexually transmitted diseases and socioeconomic status.[51]

Hepatocellular carcinoma

A number of disease entities have been linked to the development of hepatocellular carcinoma (HCC), including primary biliary cirrhosis, hemochromatosis, aflatoxin, and alfa1-antitrypsin deficiency. However, for the vast majority of documented HCC, the primary pathogenetic role is played by Hepatitis C virus (HCV), Hepatitis B virus (HBV) and cirrhosis. Cirrhosis underlies HCC in more than 80% of the cases, and the predominant cause of cirrhosis are HCV and HBV, with a minority of cases sustained by alcohol abuse[52].

HIV, HCV and HBV share common transmission patterns, which explains the frequent co-infection with HIV and one or both hepatotropic virus. Moreover, HIV infection can alterate the natural history of HCV and HBV infection, and co-infected patients show a more rapid progression of viral hepatitis to liver inflammation and cirrhosis[53], even with HIV replication under full control by cART.[54]

Even though HIV itself is not a direct causative agent of HCC, it can fasten the time of progression to fibrosis and cirrhosis through complex mechanisms involving immune suppression and chronic immune activation.

The role of immune status, including CD4+ cells count at the time of cancer diagnosis and nadir CD4+ count, has been studied although not fully clarified.

In the Swiss Cohort[55], the risk of HCC was associated with low CD4+ cell count in the year preceding cancer diagnosis. Conversely, a recent study demonstrated that although cumulative HIV-related immune suppression (nadir CD4+ count, percent of time with undetectable HIV viral load) were not associated with HCC, immune suppression at the time of HCV diagnosis, as measured by CD4+ cell count, was associated with the risk of cancer.[56]

It is likely that, beyond CD4+ cell count, the persistent perturbation of the immune response and the cytokines network play a major role in the pathogenesis of HCC. CD4+ cells depletion may promote hepatic fibrosis through the decreased secretion

of the anti-fibrotic T-helper (Th) 1 cytokine gamma-interferon (IFN-γ). On the other hand, the increased Th2 response is associated with secretion of Th2 cytokines with a recognized pro-fibrotic role (IL-4, IL-5, IL-10, and IL-13)[57].

A study conducted on 52 HBV mono-infected and 54 HIV/HBV co-infected patients demonstrated higher levels of pro-inflammatory markers in co-infected patients, such as Interleukine-6 (IL-6) and soluble CD14 (sCD14), suggesting an enhanced pro-inflammatory response in this group.[58] Further, microbial translocation consequent to CD4+ depletion in the gastrointestinal tract has been linked to liver fibrosis in HIV/HCV co-infection, trough the subsequent chronic inflammation and immune activation[59 60].

Finally, former or active use of drugs and alcohol abuse have to be considered as potential factors involved in the process of liver disease progression, as a large proportion of HIV/HCV positive patients are active/ex intravenous drug users (IDUs) and heavy alcohol consumers[61 62]. Of note, beyond heroine, other substances such as cannabis, cocaine and amphetamine may potentially cause liver damage and worsening of fibrosis[54 63 64].

Aim

Co-infection with oncogenic virus occurs as a key pathogenic factor in AIDS-defining malignancies (ADMs) (namely, Non-Hodgkin lymphoma (NHL), Kaposi sarcoma (KS), anal and cervical cancer) and in some non-AIDS defining malignancies (NADMs) (anal cancer, haepatocellular carcinoma (HCC) and Hodgkin lymphoma (HL).

Beside co-infection with oncogenic virus, the pathogenic pathways underlying the development of infection-related and non infection-related cancer in people living with HIV (PLWH) are sustained by a complex network of interactions between various components of the immune system, with cytokines and other pro-inflammatory agents mediating those interactions. However, these mechanisms have not been fully characterized.

CD4+/CD8+ ratio is correlated with markers of immune activation and immune-senescence, such as lower levels of peripheral naïve T-cells and increased proportion of terminally-differentiated T-lymphocytes. The normalization of CD4+/CD8+ ratio above 1 is slow even during suppressive combination antiretroviral therapy (cART), mainly due to the persistent expansion of the CD8 T-cells compartment[65], and reflect persistent immune activation in PLWH[66]. A recent study conducted in the Swiss cohort demonstrated that the risk to develop Hodgkin lymphoma, a non-AIDS-related but Epstein-Barr virus (EBV)-triggered cancer, was higher in patients with very low CD4+/CD8+ ratio (<0.25). On the other hand, the expression of the immune activation marker CD38 on CD8T-lymphocytes has been linked with HIV infection progression and immune senescence, even in the contest of suppressive cART. It's role in cancer pathogenesis in PLWH has been poorly studied.

Moreover, In the context of HIV-associated lymphoma, there is considerable interest in the role of natural killer (NK, CD56+) cells, as they may contribute to the host response to anticancer therapy[32].

Finally, we focused on the demographic and immunological factors that promote HPV persistence and carcinogenesis in HIV positive patients, as anal and cervical cancers are highly prevalent amongst PLWH.

In this context, the aims of this study were:

- to assess the association of peripheral immune activation markers (CD8+CD38+ T-lymphocytes) and CD4/CD8 ratio with the onset of infection-related and non-infection related cancers in a cohort of HIV-positive patients, and possible association of these markers with overall survival.
- To evaluate the natural killer (NK) cells population in a cohort of HIV positive patients with diagnosis of lymphoma and possible association with the overall survival.
- To evaluate possible factors associated with Human Papilloma virus (HPV) infection at anal and cervical site and with the presence of precancerous lesions (squamous intraepithelial lesions-SILs) in a cohort of HIV-positive patients with focus on gender differences, and possible factors linked with the capability to clear HPV infection, thus influencing the progression of SILs towards cancer.

We thus performed the following studies:

- Association of T-cells activation markers and CD4/CD8 ratio with virus related and non-virus related cancers in HIV-positive patients.
- Natural Killer cell populations in HIV associated lymphoma.
- Sex-based differences in factors associated with HPV infection and intraepithelial lesions in a cohort of HIV-positive patients.

Materials and methods

For the following studies:

- Association of T-cells activation markers and CD4/CD8 ratio with virus related and non-virus related cancers in HIV-positive patients;
- Sex-based differences in factors associated with HPV infection and intraepithelial lesions in a cohort of HIV-positive patients;

we enrolled antiretroviral-naive and cART-treated HIV-infected patients in active follow up at the SPID (San Paolo Infectious Disease) Cohort. The SPID cohort was set up in 2006 and includes all HIV positive patients who are attending the outpatients service of Infectious Diseases' Clinic at San Paolo Hospital, Milan, Italy. To date, 1127 patients are included, 1069 with active follow up, with a median age of 45 years; 795 (74%) are males and 1008 (94%) are on cART.

According to the study they were enrolled in, consecutively visited HIV-positive female and male patients signed an informed consent for anal/genital cancer screening from October 2002 to February 2016. Inclusion criteria were: to be HIV positive, older than 18 years, without history of HPV vaccine, asymptomatic for genital distress or discharge. Exclusion criteria were: refusal to sign the informed consent. Both therapy-naive patients and subjects receiving cART were included.

Lymphocyte subsets were evaluated by flow cytometric analysis, using 50 µl ethylenediaminetetraacetic acid (EDTA) peripheral blood incubated for 30 min at 4°C with fluorochrome-labeled monoclonal antibodies (mAbs) (CD4+, CD8+, CD38+, CD8+/CD38+). After incubation, erythrocyte lysis and fixation of marked cells were performed using the Immuno-Prep EPICS kit and Q-prep Work Station (Coulter Electronics, Hialeah, FL, USA). The CD4+/CD8+ ratio was calculated as a ratio of the absolute CD4+ and CD8+ cell counts.

For the study:

- Natural Killer cell populations in HIV associated lymphoma

we enrolled HIV-positive patients of the National Centre for HIV malignancies, Chelsea and Westminster Hospital, London (UK). At the Centre, the routine data on all individuals who attend are prospectively collected, including 615 HIV seropositive patients diagnosed with lymphoma between 1988 and 2017. Data on patient characteristics, prognostic factors, treatment and outcomes were extracted from the database.

For the immune subset analysis, total lymphocyte and subset analysis was performed using whole blood stained with murine antihuman monoclonal antibodies to CD4 (T helper cells), CD8 (a cytotoxic T-cell marker), CD19 (B cells), and CD16/56 (natural killer cells) (TetraOne; Beckman Coulter, High Wycombe, United Kingdom) and were evaluated on an Epics XL-MCL (Beckman Coulter) multiparametric 4-color flow cytometer.

Results

Association of T-cells activation markers and CD4/CD8 ratio with virus-related and non virus-related cancers in a cohort of HIV positive patients.

Introduction

Co-infection with oncogenic virus occurs as a key pathogenic factor in AIDS-defining malignancies (ADMs), namely Non-Hodgkin lymphoma (NHL), Kaposi sarcoma (KS) and cervical cancer, and in some non AIDS-defining malignancies (NADMs), such as anal cancer, haepatocellular carcinoma (HCC) and Hodgkin lymphoma (HL). However, beside co-infection with oncogenic virus, the pathogenic pathways underlying the development of infection-related and non infection-related cancer in people living with HIV (PLWH) are sustained by a complex network of interactions between various components of the immune system, with cytokines and other pro-inflammatory agents mediating those interactions, which have been only partially characterized.

Thus, beyond immune suppression as measured by CD4+ cells count, the identification of markers of perturbation of the immune-subsets and of chronic inflammation has been advocated to predict the risk of cancer in PLWH.

CD4+/CD8+ ratio is correlated with markers of immune activation and immune-senescence, such as lower levels of peripheral naïve T-cells and increased proportion of terminally-differentiated T-lymphocytes. The normalization of CD4+/CD8+ ratio above 1 is slow even during suppressive combination antiretroviral therapy (cART), mainly due to the persistent expansion of the CD8 T-cells compartment[65], and reflect persistent immune activation in PLWH[66].

Failure in reaching a normal CD4/CD8 ratio has been associated with the risk of non-AIDS defining events globally, and especially with the risk of non-AIDS defining cancers in PLWH[66]. On the other hand, the expression of the immune activation marker CD38 on CD8T-lymphocytes has been linked with HIV infection progression and immune senescence, even in the contest of suppressive cART, and contributes to the pathogenesis of both AIDS and non-AIDS associated diseases[67].

Thus, we aimed to assess the association of CD4+/CD8+ ratio and CD8+CD38+ with the onset of virus related and non virus-related cancers in a cohort of HIV-positive patients, and the possible influence of these markers on mortality in our cohort.

Patients and methods

Study design and study population

The SPID (San Paolo Hospital Infectious Disease) cohort was set up in 2006 and includes all HIV positive patients who are attending the outpatients service of Infectious Diseases' Clinic at San Paolo Hospital, Milan, Italy. To date, 1127 patients are included, 1069 with active follow up, with a median age of 45 years; 795 (74%) are males and 1008 (94%) are on cART.

We included in the present study all the consecutively visited HIV-positive patients who received a diagnosis of infection-related and non-infection related cancers from January 2000 to August 2018. Both therapy-naive patients and subjects receiving cART at the time of cancer diagnosis were included.

Prospectively collected clinico-pathologic and treatment data on all individuals included where analyzed.

Non-Hodgkin Lymphoma (NHL), Hodgkin Lymphoma (HL), Kaposi's sarcoma (KS), HPV-related cancers (cervical cancer, anal cancer, laryngeal cancer), hepatocellular carcinoma (HCC) were classified as infection-related cancers. Lung cancer, breast

cancer, prostate cancer, colorectal cancer, thyroid cancers were classified as non infection-related cancers.

T- lymphocyte subsets

Lymphocyte subsets were evaluated by flow cytometric analysis, using 50 µl ethylenediaminetetraacetic acid (EDTA) peripheral blood incubated for 30 min at 4°C with fluorochrome-labeled monoclonal antibodies (mAbs) (CD4+, CD8+, CD38+, CD8+/CD38+). After incubation, erythrocyte lysis and fixation of marked cells were performed using the Immuno-Prep EPICS kit and Q-prep Work Station (Coulter Electronics, Hialeah, FL, USA). The CD4+/CD8+ ratio was calculated as a ratio of the absolute CD4+ and CD8+ cell counts.

Statistical analysis:

Data were presented as absolute numbers, percentages and median, interquartile range (IQR) for categorical and quantitative variables, respectively. Comparisons between patients with virus-related cancer versus non virus-related cancer were assessed by Chi-square test for nominal variables and non parametric Mann-Whitney test for continuous variables. The same analysis were conducted to explore factors associated with virus-related and non virus-related cancers in the group of patients on cART at the time of cancer diagnosis.

The time interval from cancer diagnosis until death, study censoring or loss to follow-up was used to calculate overall survival. Survival curves were plotted according to the method of Kaplan and Meier[68]. The log rank method was used to test for the significance of differences in survival distributions.

Analyses were conducted using SPSS (SPSS, version 21.0).

Results:

140 patients had a cancer diagnosis between 1/01/2000 and 27/06/2018, 115 (82%) virus related and 25 (18%) non virus-related. 114/140 (81%) patients were males and the median age of the entire study population at the time of cancer diagnosis was 47 years (IQR: 39-52). 37/140 patients (26%) had a diagnosis of AIDS-related disease preceding or concomitant to the diagnosis of cancer and 77/140 (55%) were on cART at the time of cancer diagnosis.

The clinico-pathological characteristics of study population by virus-related and non virus related cancers are shown in table 1.

Patients with virus-related cancers were younger compared with patients with non virus-related cancers (44 yrs, 36-51 vs. 56 yrs 48-61 p<0.001), more frequently males (99/115, 86% vs. 15/25, 60% p=0.002) and MSMs (62/115, 53% vs. 7/25, 28% p=0.003). Moreover, virus-related cancer patients were more recently diagnosed with HIV infection compared to non virus-related cancers patients (median, IQR: 48 months, 0.25-158 vs. 144 months, 26-239 p=0.04) and less frequently on cART at the time of cancer diagnosis (57 pts, 51% on cART vs. 20 pts, 80% on cART p=0.007).

Accordingly, CD4+ cells count (median, IQR: 219 cells/mmc, 112-481 vs. 400 cells/mmc, 305-563 p=0.004) was lower in patients with virus related-cancer.

The CD4+/CD8+ ratio was <1 in both the groups of patients, with significantly lower values in the group of virus-related cancer compared to non virus-related cancer (median, IQR: 0.33 0.14-0.60 vs. 0.54, 0.34-0.73 p= 0.01).

Nadir CD4+ (p=0.97) and CD8+CD38+ as cells count (p=0.99) and percentage (p=0.08) were comparable in the two groups of patients .

Given the higher percentage of patients on antiretrovirals among individuals with virus-related cancer, we conducted the same comparisons in the group of patients on cART (77/140, 55%) at the time of cancer diagnosis (Table 2).

Patients on cART with infection related and non infection-related cancer were comparable for length of HIV infection (median, IQR: 138 months, 29-229 vs. 163

months, 51-256 respectively, p=0.38), percentage of patients on virological suppression (33 pts, 58% vs. 15 pts, 75% respectively, p=0.203), nadir CD4+ (median, IQR: 118 cells/mmc, 49-225 vs. 128 cells/mmc, 84-209 p=0.93) and prevalence of previous or concomitant AIDS disease (18/57 patients, 31% vs.7/20 35% p=0,51). However, patients with virus-related cancer had been exposed to cART for a shorter length of time at the time of cancer diagnosis (median, IQR: 59 months, 19-186) compared to patients with non virus-cancer (median, IQR: 180 months, 81-239, p=0.01).

Lower CD4+cell count (median, IQR: 280 cells/mmc, 152-556 vs. 414 cells/mmc, 305-608 p=0.025) and younger age (median, IQR: 48 yrs, 40-52 vs. 57 yrs, 51-65 p<0.001) were associated with virus-related cancer; nevertheless, among cART treated patients, the CD4+/CD8+ ratio was <1 in both groups without differences between infection-related and non infection-related cancer (median, IQR: 0.47, 0.25-0.71 in virus-related cancer vs. 0.59, 0.38-0.83 in non infection-related cancer, p=0.21) .

Survival data

96/140 patients were treated with curative intent with systemic chemotherapy and/or surgery according to cancer type. 38/140 patients were treated with cART alone as they had a diagnosis of T0 stage KS. 6/140 patients received best supportive care.

39/140 (28%) patients died during follow up, 28/39 (72%) with virus related cancer and 13/39 (33%) with non virus related cancer.

For 23/39 patients the cause of death was progressive disease, 3 patients died for intercurrent AIDS-defining disease (namely, neurotoxoplasmosis, micobacteriosis and IRIS KS), 3 patients for chemoteherapy related-cause. 10 patients died for unrelated causes in remission.

After a median follow up of 177 months (95% CI: 147-207), the 5 yrs probability of death for the whole population was 28% (95% CI: 19-35%).

When we compared the Kaplan-Meier survival curves of patients with infection and non infection-related cancer, we found that the 5 yrs probability of death was 22% (95% CI: 13-29) for infection-related cancers vs. 56% (95% CI: 34-78) for non-infection related cancer (log rank p=0.004).

The influence of low CD4+/CD8+ ratio (CD4+/CD8+ <4) and CD8+CD38+ percentage on mortality was evaluated using a Cox proportional hazards model. Neither CD4+/CD8+<4 (p=0.75) nor CD8+/CD38+ percentage (p=0.79) were prognostic variables for overall survival.

Discussion

Among PLWH, different type of malignancies with and without a known viral-trigger display different strength of association with immune suppression as measured by CD4+, and the risk excess for cancer observed in PLWH persist after cART introduction, which is now anticipated to restore the CD4+ cell count. Moreover, among non-AIDS related malignancies, a number of cancer types do have a recognized viral-triggered origin, and could present in the contest of more dysfunctional immune subset compared to non-virus related cancer.

In this study we aimed to assess the association of CD4+/CD8+ ratio and CD8+CD38+, both markers of immune deregulation, with the onset of virus related and non virus-related cancers.

We found that patients with a cancer with known viral-origin were younger and less frequently on antiretrovirals at the time of cancer diagnosis compared with patients

with non-infection related cancer. Accordingly, they showed deeper levels of immune suppression as measured by CD4+ cell count. This may be mostly ascribable to the inclusion into viral-related cancers of KS and NHL, which are strongly related to advanced immune suppression; on the other hand, other virus-related cancer, namely HL and HCC, clearly have a weaker relationship with immune suppression[69]. Due to the low number of cases, we were not able to explore the effect of CD4+ T-cell count on specific types of cancers.

Peripheral levels of CD8+CD38+ were comparable in the two groups, with no difference between virus and non-virus related cancer. This is surprising, because higher levels of CD8+CD38+ are observed in the contest of several acute and chronic infections[70], and tend to decline when viral replication is suppressed. It could be hypothesized that the high burden of antigenic stimulation in infection-related cancer during HIV infection can lead to CD8 T-lymphocyte cell dysfunction or exhaustion, as has been demonstrated in the contest of primary HIV-infection.[71]

In both the groups, the CD4+/CD8+ ratio was below 1 at the time of cancer diagnosis, but the median CD4+/CD8+ ratio was significantly lower in virus-related cancer compared to non virus-related cancer. However, when we analyzed patients on cART at the time of cancer diagnosis, the ratio was <1 for both virus-related and non virus-related cancer without significant differences between the two groups. In our cohort, patients with virus-related cancer had been on antiretrovirals for a significantly shorter time compared to non virus-related cancer. These data support the hypothesis that cART, even though for short time of exposure, can partially revert, though not completely restore, the CD4+/CD8+ ratio, even in the contest of infection-related malignancies.

Our finding that malignancies with a known viral origin had a lower 5 yrs risk of death compared to non-infection-relate cancer may be attributable to the presence,

among the former group, of KS and cervical cancer, which are burdened by low mortality; alternatively, the higher 5-yrs probability of death among non virus-related cancer may be linked to older age, and further stratified analysis are necessary to clarify this difference.

Immune-activation has been widely studied as a risk factor for several non-communicable disease in PLWH, including cancer[72], and has been indicated as a predictor of mortality in HIV-positive patients with history of AIDS. However, in this study, we did not find an association between immune-activation or CD4+/CD8+ ratio and mortality. It is possible that levels of immune activation in our cohort reflect the presence of persistent inflammatory stimuli, that drive mortality through parallel pathways.

Indeed, the role of CD4+/CD8+ ratio in predicting AIDS and non-AIDS related morbidity and mortality has not been fully clarified.

A large study involving Infectious Disease centers in Europe and North America failed to demonstrate an association between CD4+/CD8+ and all-cause mortality in a HIV population with high CD4+ counts and suppressed viral load[73]. Both low CD4+/CD8+ ratio and high CD8+ count were associated with AIDS mortality in this population, suggesting that both CD4+/CD8+ ratio and CD8 count could account for some excess AIDS mortality in a HIV population that was otherwise healthy. A recent study showed that the CD4+/CD8+ ratio did not add predictive information on morbidity to the CD4+ cells count, except for non-AIDS cancers[74]. It is likely that associations may only be with specific causes of death, that we were unable to look at in this study. Indeed, a major limitation of this study is small sample size, and larger cohort analysis would allow to better interpret survival data.

In conclusion, we found that immune-activation at the onset of cancer was similar in patients with infection and non infection-related malignancies.

Patients with infection-related cancer had lower CD4+/CD8+ ratio, but this difference was no longer significant among patients on cART, supporting the importance of a prompt initiation of cART in contrasting the immune-deregulation in both virus and non-virus related malignancies.

In this study, CD8+CD38+ and CD4+/CD8+ ratio were not predictive of outcome in HIV-positive patients with cancer.

Natural Killer cell populations in HIV associated lymphoma

Introduction

Cancer is a leading cause of morbidity and mortality in people living with HIV (PLWH),particularly since the advent of combination antiretroviral therapy (cART). After Kaposi sarcoma, non-Hodgkin lymphoma (NHL) is the most common malignancy in PLWH[75]. The pathogenic pathways underlying the development of lymphoma in PLWH suggest a complex network of interactions between components of the immune system, with cytokines and other pro-inflammatory agents mediating many of these interactions. In this context there is considerable interest in the role of natural killer (NK) cells as they may contribute to the host response to anticancer therapy[32].

NK cells are characterized by a CD3-/CD56+ phenotype and include two subpopulation defined on the basis of CD56 and CD16 expression. The majority of circulating NK cells (>90%) are $CD56^{dim}$, express CD16, and exert cytotoxic activity against tumor cells. The minority are $CD56^{bright}$ NK cells with low or absent CD16 expression and are generally confined to lymph nodes.$CD56^{bright}$ NK cells have limited cytotoxic activity and predominantly exhibit immune-regulatory functions through cytokines production upon stimulation[76 77] .

In HIV-negative patients, NK cells constitute a higher percentage of circulating lymphocytes and are associated with a better outcome in both Hodgkin lymphoma and diffuse large B-cell lymphoma (DLBCL)[78]. On the other hand, NK cell number and function are impaired during chronic HIV infection[79]. Furthermore, low CD4+ T-cells

count has been linked with depletion of the NK cells compartment in patients with AIDS-related lymphoma, suggesting a poor prognosis[80]

In this study we aim to evaluate the NK cell compartment in a large cohort of PLWH with NHL, and its possible association with the outcome of anticancer treatment in DLCBCL.

Methods

Patient selection and data collection

At the National Centre for HIV malignancies at the Chelsea and Westminster Hospital we prospectively collect routine data on all individuals who attend, including 615 HIV seropositive patients diagnosed with lymphoma between 1988 and 2017. Data on patient characteristics, prognostic factors, treatment and outcomes were extracted from the database.

Immune subsets

Total lymphocyte and subset analysis was performed using whole blood stained with murine antihuman monoclonal antibodies to CD4 (T helper cells), CD8 (a cytotoxic T-cell marker), CD19 (B cells), and CD16/56 (natural killer cells) (TetraOne; Beckman Coulter, High Wycombe, United Kingdom) and were evaluated on an Epics XL-MCL (Beckman Coulter) multiparametric 4-color flow cytometer.

Statistical analysis

Comparison of variables between the groups was by Chi-squared test for categorical data and Kruskal-Wallis test for non-parametric continuous variables; all p values are two-sided. The association between CD4+T-cells count and NK cells count was assessed by bivariate analysis. The time interval from lymphoma diagnosis until death, study censoring or loss to follow-up was used to calculate overall survival.

Survival curves were plotted according to the method of Kaplan and Meier[68]. The log rank method was used to test for the significance of differences in survival distributions[81].

The study complied with local regulations and was approved by the institutional review boards of Chelsea and Westminster Hospital. Patient details were anonymized and therefore patient consent was not required.

Results

Full lymphocyte subset analysis at lymphoma diagnosis was available for 361 patients (271 high grade B-cell NHL and 90 HL). Among the 271 patients with NHL, 157 (58%) had DLBCL, 62 (23%) Burkitt lymphoma, 18 (6%) primary effusion lymphoma, 17 (6%) plasmablastic lymphoma and 17 (6%) primary cerebral lymphoma (PCL). For these 271 patients (229 (84%) were male and the mean age at NHL diagnosis was 43 years (range 19-75). The median duration of HIV infection at NHL diagnosis was 31 months (range 1-295), 57 (21%) had a prior AIDS diagnosis and 153 (56%) were established on cART, of whom 60% had an undetectable plasma HIV viral load. The clinicopathological features and International Prognostic Index (IPI) scores for the 157 patients with DLBCL are shown in table 3.

Lymphocyte subsets at baseline

The median CD56+ cells count was below the reference threshold (normal range: 90-600 cells/mm^3) irrespective of histology, without significant differences between the histological groups; however, the NK cells as a percentage of total lymphocyte counts was significantly higher in patients with HL (median 9%) than in DLBCL (median 6%) and other NHL subtypes (p=0.009).

As expected, CD4+ T-cells count and percentage were low in all histology groups, with the lowest valuesin patients with PCL (median79 cells /mm^3) compared to

other groups (p=0.01). Similarly, CD19 B-cell counts and percentages were lower in HL (median 58 cells/mm^3) than in NHL histological groups. See Table 4.

For the whole group of 361 patients, CD56+ cell count was positively associated with CD4+ T-cell count although thecorrelation coefficient between the variables was very weak (R^2=0.11, Figure 2A). On the other hand, plasma HIV viral suppression (recorded as below the level of detection in assay used at the time of analysis) was strongly associated with higher CD56+ cell count and percentage at the time of lymphoma diagnosis (p<0.0001) (Figure 2B).

Survival data and prognostic factors analysis

Of the 157 patients with DLBCL, 149 were treated with curative intent with combination chemotherapy; 83 R-CHOP (rituximb, cyclophosphamide, doxorubicin, vincristine and prednisolone), 63 CDE (cyclophosphamide, doxorubicin and etoposide), 2 R-CODOX-M/IVAC (rituximab, cyclophosphamide, doxorubicin, vincristine, methotrexate / ifosfamide, etoposide, cytarabine). The median follow-up for these 149 patients is 8.5 years (maximum 18.5) and the 5 year overall survival is 66% (95%CI: 58-74%)(See Figure 2). See table 3 for clinicopathological details of these 149 patients. Thirty-one patients have died of NHL (15 refractory and 16 relapsed lymphoma), 10 died of chemotherapy related causes and 9 died in remission.

The IPI group score was strongly associated with overall survival for 149 patients with DLBCL who were treated with curative intent (Log rank proportional hazards p<0.0001). The influence of NK cell population on overall survival was evaluated using a Cox proportional hazards model stratified for IPI. Neither total NK cell population (p=0.62) nor NK cells as a percentage of lymphocytes (p=0.76) were prognostic variables for overall survival. Similarly, CD4 (p=0.73), CD4% (p=0.37), CD8

(p=0.86), CD19 (p=0.99) and CD19% (p=0.10) did not influence overall survival. However, CD8% was significantly associated with overall survival(p=0.034) in the stratified model although without stratification for IPI, CD8% did not predict survival (p=0.17).

Discussion:

NK cells are large granular lymphocytes that express neither surface immunoglobulins nor T-cell receptors. They are an important mediator of innate immune responses recognizing and destroying "stressed" cells including viral infected cells and tumour cells. These cells are recognized by NK cells because they lack self-molecules including MHC class I. Following NK cell activation by this recognition, perforin and granzymes are released resulting in the death of target cells by apoptosis. Thus NK cells have an important role in the innate defense against virus infected cells and cancer cells. Previous studies have demonstrated reduced numbers of NK cells in PLWH[79] and this could contribute to the survival of virus infected cells including Herpesvirus infected lymphoid cells that are the origin of most HIV associated NHL.

In this cohort study we found that the median NK cell count at lymphoma diagnosis was below the reference threshold and that the NK cell count expressed as a cells as a percentage of total lymphocyte counts was significantly lower in patients with AIDS defining lymphomas than in HL (p=0.009). As expected, CD4+ T-cells count and percentage were low in all histology groups, with the lowest values in patients with PCL (median 79 cells /mm^3) but highest in HL (median 271 cells/mm^3). Whilst overall CD56+ cell counts were positively associated with CD4+ T-cell counts, the correlation coefficient between the variables was very weak (R^2=0.11). These findings support the hypothesis that loss of CD56+ cells could contribute to lymphoma development in PLWH, especially the AIDS defining non-Hodgkin lymphomas. Of course this

analysis does not include a control group of PLWH who do not have lymphomas and it is not possible to correct for other confounding variables.

A low NK cell count at diagnosis has been shown to be associated with adverse outcome in a trail that enrolled 140 HIV negative patients with poor risk DLBCL who were treated with induction anthracycline based chemotherapy followed by autologous stem cell transplantation. Indeed circulating NK cell count was inversely associated with response rate and event-free survival in multivariate analysis independent of IPI[78]. In contrast, in this study, we found no correlation between total NK cell count (or NK cells as a percentage of lymphocytes) and overall survival in 149 patient with HIV associated DLBCL. There are several possible explanations for the different findings in PLWH. Firstly, the median NK cell count at lymphoma diagnosis was 104 cells/mm3 in the HIV negative cohort but only 63 in our patients. Secondly, only 37 (26%) HIV negative patients received rituximab as part of their treatment whilst in our cohort 86 (58%) received rituximab. This is important because antibody-dependent cellular cytotoxicity is largely mediated by NK cells because cells oposonised with antibodies can be recognised by FcϒRIII (CD16) receptors expressed on NK cells, leading to NK cell activation and target cell apoptosis(10). A further limitation, in both studies, is lack of functional assessment of NK cell activity, specifically the expression of activating and inhibitory receptors whose balance determines the anticancer activity of NK cells[82].

Indeed the precise role of NK cells in contributing to the efficacy of therapeutic antibodies has not been fully clarified. However, strategies that enhances the ADCC of NK cells and the capability of monoclonal antibody to boost NK-related citoxicity have been explored in HIV-negative patients[83] [84] [85]. Specifically, the use of immunemodulatory antibodies has shown to be promising[86] [87] .

A recent study[85] compared UL16-binding protein (ULBP) 2 and B7 homolog 6 (B7-H6), two ligands of the NK activating receptors NK2GD (natural killer group 2 member D) and NKp30, in terms of capability to boost the NK ADCC. ULBP2 and B7H6 were fused with a single chain fragment variable of the CD20 antibody 7D8 and both the immune ligands (ULBP2:7D8 and B7H6:7D8) promoted *in vitro* the ADCC induced by the monoclonal antibodies rituximab and daratumumab synergistically; this indicates that the co-engagement of FcϒRIII and either NK2GD and NKp30 enhances NK citotoxity, and could promote the efficacy of therapeutic antibodies.

A further approach is to quantitatively increase the effector cells involved in ADCC. This has been obtained by stimulating human peripheral mononuclear cells with interleukin 2 (IL-2) to generate lymphokine-activated killer (LAK) cells, that contain NK cells[88] [89] [90]. Recently, in a clinical trial including 12 patients with follicular lymphoma[51], the increased expansion of NK cells within the LAK pool has been shown to correlate with cytotoxicity against CD20-positive tumor cells and with rituximab-related ADCC in vivo. LAKs were administered in those patients together with rituximab as a maintenance therapy after 6 cycle of R-CHOP, and this is important with a view to attempt to eradicate CD20-positive cells and to prevent tumor relapse.

Our study indicates that NK cells deficiency is a key point in the development of non-Hodgkin lymphoma in PLWH and could explain, at least in part, the higher risk for malignancies that persists in the cART era.

However, a lower NK cells count was not associated with a worse overall survival of our patients with DLBCL, possibly because of a functional impairment beyond their quantitative depletion.

The function of NK cells in the setting of HIV-associated lymphoma may indeed have different features from those observed in the HIV-negative population. Since in HIV-negative patients immunotherapy targeting NK cells is promising, further studies in PLWH may allow comprehending whether NK-cells antitumor function could be restored in these patients, and consequently exert their potential to boost the response to chemotherapy.

Sex-based differences in factors associated with HPV infection and intraepithelial lesions in a cohort of HIV-positive patients

Introduction

Human Papilloma Virus (HPV) infection is the most common sexually transmitted disease worldwide and its persistence is associated with several types of cancers[91 92]. Despite the advent of combination antiretroviral therapy (cART), HIV-positive patients present a several fold greater incidence of cervical cancer[93] and 30 to 100-fold higher incidence rate of anal cancer compared to the HIV-negative population[16 94 95]. To date, three vaccines (bivalent, quadrivalent and nonavalent) have been licensed for the prevention of infection by the most diffused high-risk (HR) and low-risk (LR) HPV genotypes and showed also high efficacy in preventing cervical and anal intraepithelial lesions [96 5].

The effect of HIV on the natural history of HPV infection is still a matter of debate, since

many elements affect a full characterization of the factors that promote HPV persistence and carcinogenesis in HIV positive patients: the host and virus-related variations in the immune capability to clear the infection, the different susceptibilities to infection by anatomic site, the influence of risky behaviors[97 98]. Further, the role of cART on HPV infection and on intraepithelial lesions is still unclear. Some studies demonstrated an increased incidence in anal cancer and no significant change in the overall incidence of cervical cancer among HIV-positive

individuals between 1992 and 2003, while others reported that cART significantly decreased the risk of cervical cancer but not anal cancer.[99 100 101]Indeed, few studies assessing the natural history of HPV infection include men and women from the same cohort or setting, since the vast majority of reports derives from cohorts composed by HIV positive men who have sex with men (MSM) or women only, or assessed the natural history of HPV among heterosexual couples[100 102].

It has been hypothesized that HPV infection could persist independently from CD4+ T-cells count in female population; thus, HPV natural history might be mostly linked to behavioral factors and traditional risk factors, such as infection with high risk HPV genotype (HR-HPV genotype) or co-infection with multiple HPV genotypes.

Palefsky et al. demonstrated that higher CD4+ T-cells count in men who have sex with men does not necessary protect against intraepithelial anal lesion development[103 17], while other authors found a protective effect of prolonged cART (same regimen received for 4 years or more) against grade 2 and 3 anal intraepithelial neoplasia (AIN 2-3), after adjusting for nadir CD4+ T-cells count[104].

The aim of our study was to assess the prevalence and the factors associated with HPV infection and with intraepithelial lesions at anal and cervical site in a cohort of HIV-positive men and women asymptomatic for HPV cared at the same clinical center. We further investigated possible factors linked with the capability to clear HPV infection, thus influencing the progression of intraepithelial lesions towards cancer.

Patients and methods

Study design and study population

The SPID (San Paolo Hospital Infectious Disease) cohort was set up in 2006 and includes all HIV positive patients who are attending the outpatients service of Infectious Diseases' Clinic at San Paolo Hospital, Milan, Italy. To date, 1127 patients

are included, 1069 with active follow up, with a median age of 45 years; 795 (74%) are males and 1008 (94%) are on cART.

We included in the present study all the consecutively visited HIV-positive female and male patients who signed an informed consent for anal/genital cancer screening from October 2002 to February 2016. Patients were diagnosed and treated according to national guidelines and agreements. All data analysed were collected as part of routine diagnosis and treatment. Further, every patient signs a consent form for collection of body samples (blood, biopsies) and clinical data at the first clinical visit at our centre. The form has been approved by the ethics committee of our centre.

Inclusion criteria were: to be HIV positive, older than 18 years, without history of HPV vaccine, asymptomatic for genital distress or discharge. Exclusion criteria were: refusal to sign the informed consent. Both therapy-naive patients and subjects receiving cART were included.

Each patient underwent blood exams at baseline. At the same time point (time of first visit), women underwent a gynaecological evaluation with colposcopy and cervical swab. Men underwent a surgical visit with anoscopy and anal swab. A longitudinal study was performed on an unselected subgroup of patients who repeated the same evaluation after 12-24 months.

Blood exams were performed to analyse CD4+ T-cell count, HIV viral load and peripheral T-lymphocytes immune phenotypes (CD38+CD8+). During gynaecological and surgical examinations cervical and anal specimens were collected for cytological assessment and detection of HPV infection through HPV-DNA genotyping.

Cervical/anal cytology and HPV detection

Anal and cervical samples were collected using a Dacron swab to perform a pap smear. The cytologic smears were evaluated by expert cyto-pathologists belonging to the Pathology Department of San Paolo Hospital, and classified according to the

2001 Bethesda System terminology as negative, atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), high-grade intraepithelial lesion (HSIL), or squamous cell carcinoma[105].ASC-US was considered as a form of LSIL and, in the statistical analysis, patients belonging to the two classes were grouped together.

Specimens from the cervical and anal mucosae were also collected in liquid-based cytology medium (PreservCytologic) for HPV-DNA assessment. HPV-DNA was detected with PCR using both the L1 consensus primers MY09/MY11 and the E6/E7 consensus primers PU-1M/PU-2R. Viral genotyping was performed using a direct sequencing kit (BD Terminator Kit v 1.1, Applied Biosystems, Life Technologies) on an automated capillary electrophoresis sequencer (ABI3130, Applied Biosystems, Life Technologies). The oncogenic risk of the different viral genotypes was defined according to the epidemiologic classification by Muñozet al.[12]

T-Lymphocyte Immunophenotype

Lymphocyte subsets were evaluated by flow cytometric analysis, using 50 µl ethylenediaminetetraacetic acid (EDTA) peripheral blood incubated for 30 min at 4°C with fluorochrome-labeled monoclonal antibodies (mAbs) (CD4, CD8, CD38, CD127). After incubation, erythrocyte lysis and fixation of marked cells were performed using the Immuno-Prep EPICS kit and Q-prep Work Station (Coulter Electronics, Hialeah, FL, USA).

Clinical variables

At baseline demographic (sex, age and sexual behaviour), clinical (HPV infection at anal/cervical site and HPV genotype), immunological (total number and percentage of CD4+, CD8+, CD38+CD8+ T-cell) and HIV-related characteristics (time from HIV

diagnosis, CDC classification, ongoing cART regimen and duration, current HIV viral load, nadir and current CD4+ T-cells) were recorded.

According to ano-genital HPV infection, patients were defined as HPV-positive (HPV+) or HPV-negative (HPV-).

In the longitudinal study, patients with HPV persistence were defined as the subjects HPV-positive at baseline (T0) who remained HPV-positive at follow up (T1) and were compared to patients who cleared HPV infection (HPV-positive at T0 who become HPV-negative at T1). Histological progression was defined as evolution to a higher grade of dysplasia or development of cancer, while histological regression was defined as reversion to a lower grade of dysplasia or normalization of anal/ cervical cytology.

Statistical analysis

Data were presented as absolute numbers, percentages and median, interquartile range (IQR) for categorical and quantitative variables, respectively. Comparisons between HPV-positive versus HPV-negative patients and between patients with SIL versus patients with negative cytology, according to gender, were assessed by Chi-square test for nominal variables and non parametric Mann-Whitney test for continuous variables. Multivariate logistic regression models were performed to analyse factors independently associated with HPV infection and presence of SIL, according to gender. To explore eventual associations between cART exposure and HPV infection or SIL, we performed the same analyses in the group of cART-treated patients.

Analyses were conducted using SPSS (SPSS, version 21.0).

Results

A total of 472 patients were consecutively enrolled, 327 (69%) men and 145 (31%) women. Cytology and HPV-DNA were available at baseline for all the patients. Main characteristics of the study population according to sex are shown in Table 5.

Males and females were comparable for median age, current CD4+ T cells count at the time of enrollment and percentage of patients with AIDS-defining events; females were most frequently HIV-infected through heterosexual contacts (96/145, 66%) and IDU (36/145, 25%), while males where mostly MSMs (254/327, 78%). Not surprisingly, syphilis was more frequent in the males group and HCV co-infection amongst females reflecting a higher percentage of IDUs.

Women showed a more advanced HIV-infection as they presented a longer duration of HIV infection and a lower nadir of CD4+ T-cells compared to men.

Women were also more frequently on cART, with a longer duration of median cART exposure and more frequently on a PI-based regimen as compared to men.

Prevalence of HPV infection at baseline

The global prevalence of HPV infection in our cohort was 74% (350/472 patients).71/145 (49%) women and 279/327 (85%) males were HPV positive at baseline ($p<0.001$).

42 out of the 71 (59%) HPV positive women and 223 out of 279 (80%) HPV positive men harbored high-risk (HR) genotypes ($p=0.001$).The most represented HR HPV genotypes were HPV 16 (93/265 pts, 35%), HPV 58 (70/265 pts, 26%), HPV 31 (50/265 pts, 19%) and HPV 18 (46/265 pts, 7%); the distribution of HR and low-risk (LR) HPV genotypes is shown in Figure 4.

22/145 (31%) women and 141/279 (50%) men displayed multiple genotypes infection ($p=0.008$).

To investigate the possible role of cART on HPV infection, we compared patients on cART (353/472, 75%) with and without HPV infection. Interestingly, HPV positive

patients showed shorter exposure to antiretroviral therapy compared to the HPV-negative ones (median, IQR: 36, 10 – 108 months vs 57, 26-130months; p=0.01).
68/353 patients (20%) showed detectable plasma viral load (HIV-RNA >50 cp/ml). HPV infection was associated with incomplete viral control (HPV+ patients with HIV-RNA>50 cp/ml: 56, 22% vs. HPV- patients with HIV-RNA> 50 cp/ml:12, 11% p=0.03). In the multivariate logistic regression model, after adjusting for nadir CD4+, duration of HIV infection, being MSM and duration of cART, detectable HIV-RNA was confirmed as independent predictor of HPV infection in cART-treated patients (AOR 2.68 vs undetectable patients, 95% CI 1.04-6.88,p=0.04).

Factors associated with HPV infection according to gender

By examining the study population separately according to gender (Table 6a),we observed that among females HPV infection was associated with higher HIV-RNA levels whereas there was no correlation with cART status (HPV positive pts on cART 67/71, 94% vs HPV negative pts on cART 66/74, 89% p=0.26).
Among males (Table 6a), HPV+ patients were more frequently MSM and showed a shorter time since HIV diagnosis, less AIDS events and higher nadir and current CD4+, in comparison to HPV- subjects. HPV+ males were also less frequently on cART with a higher proportion of detectable HIV-RNA. In the multivariate analysis, MSM (AOR: 3.8 95%CI 1.818-7.946, p=0.0001), lower probability of AIDS(AOR: 0.4 95% CI:0.176-0.851 p=0.02), and shorter time since HIV diagnosis (AOR: 0.1 95% CI:0.992-0.999 p=0.03) were associated with HPV, adjusting for age, CD4+ T cells nadir, current CD4+ count, current HIV-RNA and presence of cART.

Cervical and anal cytology at baseline

224/472 patients (47%) presented normal cervical/anal cytology at baseline; 218(46%) ASCUS/LSIL and 30 (6%) HSIL.

As expected, the presence of ACSUS/LSIL/HSIL was associated with HPV infection (SIL: HPV positive patients 221/248, 89% vs HPV negative patients 27/248, 11%; p=0.0001), infection with HR-HPV (SIL: HR-HPV 175/248, 72% vs LR-HPV 46/248, 19% vs HPV negative 27/248 (9%); p=0.0001) and co-infection by multiple HPV genotype (SIL: multiple genotypes 126/248, 50% vs single genotype 97/248, 39% vs HPV negative 27/248 (9%);p=0.0001). Women were more frequently free from intraepithelial lesions (99/145, 68% vs 125/327, 38%, p<0.001) compared to men (Table 1).

Factors associated with SIL according to gender

Among women (Table 6b), lower CD4+ count and higher HIV-RNA were associated with SIL. We found no differences in age, risk factors, time since HIV diagnosis, AIDS events, CD4+ nadir and cART between SIL-positive and SIL-negative females. In the multivariate model, lower CD4+ T-cells count was confirmed as an independent predictor of intraepithelial lesions (AOR: 0.1; 95%CI: 0.1-1, p=0.05) adjusting for current HIV-RNA.

In the males group (Table 6b) patients with SIL were younger, more frequently MSM, with a shorter duration of HIV infection and a higher nadir CD4+ T-cells count compared to patients with a normal cytology. Further, males with intraepithelial lesions were less likely to be cART-exposed compared to SIL-negative ones, and they showed higher Log_{10} HIV-RNA; the proportion of activated CD38+CD8+ T-cells was also higher in SIL-positive patients. When we entered the variables in the multivariate model, only younger age was confirmed as a predictor of precancerous lesion in males (AOR 0.1, 95% CI 0.9-0,1 p=0.003 every year more) adjusting for MSM, time since HIV diagnosis, cART, logVL, CD4+ nadir, CD8+CD38+.

We then analyzed males on cART and found that men presenting with SIL were cART-exposed for a shorter time (p<0.001) compared to men with a normal cytology. In the multivariate model, shorter duration of cART (AOR 0.1, 95%CI 0.979-

0.994 every month more) was an independent predictor of SIL, adjusting for nadir CD4+ count, current HIV-RNA and MSM.

Factors associated with HPV clearance

155/472 patients underwent at least a second gynecological/surgical evaluation with cervical/anal sampling, after a median of 19 (IQR 13-30) months. A total of 30 patients (19%) cleared HPV: 16 women (52%) and 14 males (11%)($p<0.001$) (clearance group),whereas 125 (81%) patients showed persistence of HPV infection (persistence group)(Table 7).

Patients in the persistence group harbored more frequently HR-HPV genotypes ($p<0.001$) and were more frequently co-infected with multiple HPV genotypes ($p=0.001$) compared with patients in the clearance group. In the univariate analysis, female sex, longer time since HIV diagnosis and being on cART with less frequently detectable HIV-RNA and lower levels of CD8+/CD38+% T-cells were associated with the capability to clear HPV infection.

Among males, HPV clearance was associated with longer exposure to cART (Median, IQR: 73 months, 31-177 vs 31 months, 11-19 $p=0.04$) and lower levels of immune activation (CD38+CD8+% median, IQR; 1%, 1-2 vs 3%, 1-6 $p=0.01$).Unlike males, in women we observed that a shorter cART exposure (clearance: 35, 6-109 vs persistence: 117, 37-178; $p=0.03$) was associated with HPV clearance.

Factors associated with the progression of dysplastic lesions

190/472 patient had a cytological evaluation at follow up visit. 112 patients (77%) showed SIL persistence with a stable grade of dysplasia, or a stable negative cytology, 46 (23%) showed a regression to a lower grade of dysplasia and 32 (16%) a progression towards a higher grade of dysplasia (Figure 5).

Male sex was associated with changes in the cytological status, both in terms of progression and regression of dysplasia, while females were more likely to maintain a stable cytology: in comparison with no modification of cytology, men more commonly than females displayed progression of SIL (males: 29/32 pts, 90% vs females: 3/32 pts, 9% p=0.01) and were also characterized by higher prevalence of regression to lower grade of dysplasia, with a trend towards statistical significance (males: 38/46 pts, 83% vs females: 8/46 pts, 17% p=0.07). Patients with progression of dysplasia were more frequently MSMs (23/32, 72%) compared to patients with stable cytology (62/122, 55% p=0.038) and had a more recent HIV diagnosis (40 months (19-115) vs 67 months (20-180) p=0.037).

We further analyzed factors associated with changes in cytological status in the males group and we found that males who showed a regression to a lower grade dysplasia presented lower levels of CD8+ T-cell activation at baseline compared with patients with cytological stability (CD38+CD8+%, Median, IQR: 2% 1-4 vs 3% 1-7 p=0.04). Males who progressed towards higher grade dysplasia showed a trend to higher nadir CD4+ T-cells count compared to patients who remained stable (progression: 369 cells/mmc, 271-537 vs no modification: 325 cells/mmc, 202-427,p=0.05).

Discussion

In our study cohort, we describe prevalence rates of HPV infection at cervical/anal site that overpass those reported for HIV-negative women and men, underlying the increased risk to acquire HPV and to develop precancerous lesions and potentially invasive cancer in people living with HIV[99 106 107 108]. Our data, together with well consolidated epidemiological evidences, indicates the need for a strong public health campaign aimed at widening the HPV vaccination coverage in this population.

We also found a significantly higher prevalence of HPV infection in men compared to women, and detection of HR-HPV and multiple genotypes infection were also more frequent in males.

We might explain such differences both with the higher frequency of risk-taking behavior among MSM[109] [110] [111] (i.e. condom-less sex, use of recreational drugs) possibly resulting in the exposure to multiple HPV genotypes, and with the characteristics of the rectal mucosa per se, undergoing inflammation for sexual practices.

Previous reports underlined the association of low CD4+ T-cell counts and increased HIV loads with the incidence of cervical HPV infections, as well as with the risk of progression of cervical SIL.[112] [113] [114]Among women of our cohort, higher plasma HIV-RNA was an independent predictor of HPV infection, whereas none of the other HIV-related or demographic factors yielded significant association. Therefore, uncontrolled HIV infection itself seems to lead to an increased susceptibility to HPV infection, probably by altering local immunity and tight junctions function. It could also be hypothesized that high HIV viral load while on antiretroviral therapy, underlying lower adherence, could be also linked to risky sexual behavior and ultimately to higher probability of getting infected by HPV.

Previous studies conducted in HIV-positive males demonstrated an association between impaired immunity as measured by current and nadir CD4+ T-cells count; conversely, other authors found a positive association between higher CD4+T-cells levels and detection of HPV at anal site[115]. We showed that HPV-positive males were more recently HIV-infected, presented less AIDS events and higher current and nadir CD4+ versus HPV-uninfected patients. As expected, they also showed a shorter duration of cART exposure.

These findings suggest that men, and particularly MSM, are highly prone to HPV infection despite a recently acquired HIV-infection and a preserved immune CD4+

count, identifying behavioral attitudes as more relevant in the risk of HPV acquisition than CD4+ depletion in HIV+ men.

As males were more frequently HPV-infected compared to women, they also showed a higher prevalence of precancerous lesions at enrollment, and the presence of dysplasia was independently associated with younger age, which is in line with previous reports[114].

Moreover, in our cohort, higher T-cell activation rather than current CD4+ count appeared to directly influence the presence of SIL in males. The higher prevalence of intraepithelial lesions in patients with an activated lymphocytes profile suggests that HIV-driven immune de-regulation, by leading to an inadequate protection against the oncogenic action of HPV, could promote the development of precancerous lesions despite inadequate CD4+ recovery.

Further, we found that men with SIL were less likely to be cART exposed compared with men with a normal cytology, and among those on cART the duration of treatment-exposure was shorter; these data point towards a protective role of a prompt introduction of cART to prevent precancerous lesion, which is consistent with the capability of cART to control, even though not completely, the peripheral immune-activation[116 117].

We further evidenced that, besides favoring HPV infection, history of homosexual intercourses was also a risk factor for precancerous lesions, underlying the importance of screening for behavioral factors.

Among women, in contrast, the presence of SIL was associated with higher Log_{10} HIV-RNA, lower CD4+ T-cells levels and lower CD8+ T-cells percentage; lower CD4+ T-cells count was further confirmed as an independent predictor of intraepithelial lesions, providing evidence that HPV-mediated oncogenic modifications in women are largely driven by immune suppression.

In the longitudinal analysis, we showed that men presented significantly lower rate of HPV clearance compared with women, being more likely to maintain a persistent HPV infection. In the female group, a shorter duration of antiretroviral therapy was the only factor associated with the capability to clear the HPV infection, but our data might be inconclusive possibly due to the small numbers of patients. On the other hand, higher levels of peripheral immune activation markers were associated with both HPV persistence and SIL progression among men, whereas those men who had been able to clear HPV presented longer exposure to cART compared with those who did not clear the virus. Taken together, these data confirm the importance of immune activation in promoting HPV persistence and carcinogenesis and the role of cART in improving HPV clearance.

Our study has several limitations. A major one is that we can't rule out possible confounding due to the difference in epithelial cells type at the cervical and anal sample site[102]; studies assessing HPV detection and clearance by multi-sites sampling are needed to better clarify the natural history of the virus in different tissue settings.

Further limitations are represented by the small number of women in the longitudinal cohort and the absence of HIV-related or immunological correlates with the outcome of HPV clearance and cytological modifications overtime.

However, data directly comparing the natural history of HPV in men and women are scarce, and in our cohort we confirm the high prevalence of HPV infection observed in people living with HIV, especially in males and MSM.

We found that, despite an adequate immune recovery, HPV infection and clearance as well as the presence of intraepithelial lesions is largely influenced by risky behaviors and persistent immune activation in males, particularly in recently HIV-infected patients. Among women, on the other hand, the risk of precancerous

lesions is linked to immune-suppression as measured by CD4+T-cells count; we suggest that these groups of patients should be considered for more accurate screening.

We think that further longitudinal, long-term follow up studies are required to better investigate the role of cART in the natural history of HPV infection and in the progression of cytological abnormalities. Further, HPV vaccination has to be strongly recommended to all HIV positive men and women, independently form the age.

Figures and Tables

Table 1. Characteristics of study population at the time of cancer diagnosis.

Characteristics of study population	Virus-related cancers (115)	Non virus-related cancers (25)	P value
Male sex°	99 (86)	15 (60)	0.002
Age(years) *	44 (36-51)	56 (48-61)	**<0.001**
Homosexuals ° **Heterosexuals** **IDUs**	62 (53) 25 (22) 28 (24)	7 (28) 14 (56) 4 (16)	**0.003**
AIDS diseases °	81 (73%)	18 (72%)	0.55
Months since HIV diagnosis *	48 (0.25-158)	144 (26-239)	0.04
HCVAb positive °	40 (35)	10 (40)	0.66
cART°	57 (51)	20 (80)	**0.007**
Log10 HIV-RNA (cp/ml)*	3.43 (1.62-4.84)	1.69 (1.59-4.8)	0.128
Nadir CD4+ (cells/mmc)*	118 (49-265)	135 (71-210)	0.97
CD4+ (cells/mmc)*	219 (112-481)	400 (305-563)	**0.004**
CD8+ (cells/mmc)*	818 (563-1162)	861 (569-1310)	0.54
CD4+/CD8+ ratio*	0.33 (0.14-0.60)	0.54 (0.34-0.73)	**0.01**
CD8+CD38+ (cells/mmc)*	137 (76-315)	95 (45-849)	0.99
CD8+CD38+ (%)*	6 (3-17)	4 (1.5-6)	0.088

*Data are presented as the median (IQR). °Data are presented as absolute numbers (%). P values for comparison between virus-related and non-virus related cancer

Abbreviations: IDUs: injection drug users; cART: combination antiretroviral therapy

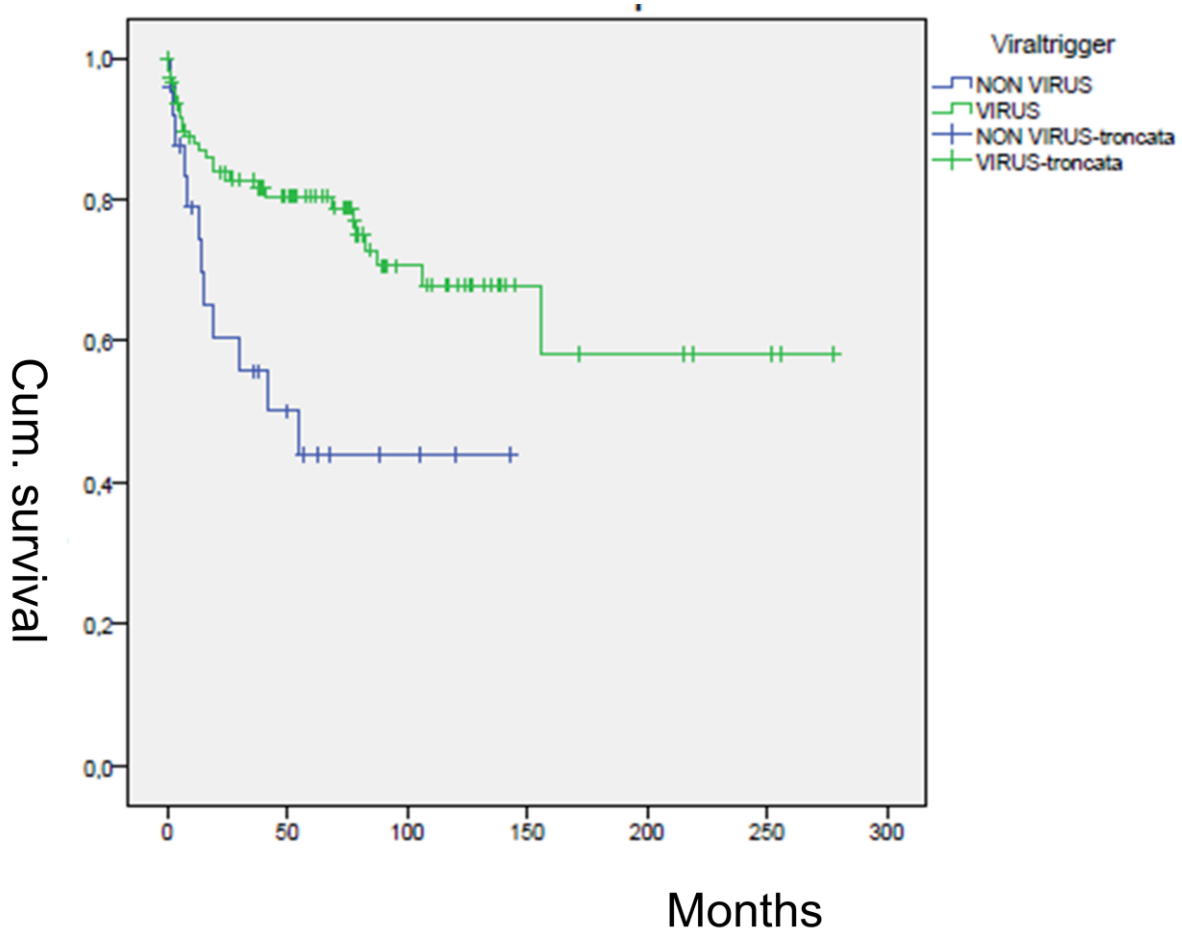
Table 1. Factors associated with virus-related and non virus-related cancers in cART-treated patients

Characteristics of population (N=77)	Virus-related cancers (n=57)	Non virus-related cancers (n=20)	P value
Male sex°	45 (79)	12 (60)	0.09
Age(years) *	48 (40-52)	57 (51-65)	**<0.001**
Homosexuals ° **Heterosexuals** **IDUs**	21 13 23	6 10 4	**0.06**
AIDS diseases °	18 (31)	7 (35)	0.51
Months since HIV diagnosis *	138 (29-229)	163 (51-256)	0.38
HCVAb positive °	32	7	0.19
HIV-RNA <40 cp/ml	33 (58)	15 (75)	0.21
Months on cART*	59 (19-186)	180 (81-239)	**0.01**
Nadir CD4+ (cells/mmc)*	118 (49-225)	128 (84-209)	0.93
CD4+ (cells/mmc)*	280 (152-556)	414 (305-608)	**0.025**
CD8+ (cells/mmc)*	756 (326-1146)	861 (569-1310)	0.28
CD4+/CD8+ ratio*	0.47 (0.25-0.71)	0.59 (0.38-0.83)	0.21
CD8+CD38+ (Cells/mmc)*	88 (49-137)	86 (39-606)	0.73
CD8+CD38* (%)*	4 (2-6)	2.5 (1-6)	0.24

*Data are presented as the median (IQR). °Data are presented as absolute numbers (%). P values for comparison between virus-related and non-virus related cancer in patients on cART.

Abbreviations: IDUs: injection drug users; cART: combination antiretroviral therapy.

Figure 1. Kaplan Meier overall survival curve for patients with virus-related and non-virus related cancer



(Logrank p=0.004)

Table 3. Clinicopathological features of 157 patients with HIV associated DLBCL

Male gender°	131/157 (83%)
Mean age at DLBCL diagnosis (range)*	45 years (19-75)
Prior AIDS defining illness°	31/157 (20%)
On cART at DLBCL diagnosis°	97/157 (62%)
Plasma HIV VL undetectable on cART at DLBCL diagnosis°	56/97 (58%)
Stage°	I: 19/157 (12%) II:12/157 (8%) III: 11/157 (7%) IV: 115/157 (73%)
ECOG PS°	0: 11/156 (7%) 1: 67/156 (43%) 2: 28/156 (18%) 3: 23/156 (15%) 4: 27/156 (17%)
IPI°	Age>60: 16/157 (10%) PS>1: 79/156 (51%) Raised LDH: 101/155 (65%) Stage>2: 126/157 (80%) >1 extranodal site: 70/155 (45%)
IPI group	L: 38/155 (25%) LI: 35/155 (23%) HI: 38/155 (25%) H: 44/155 (28%)

*Data are presented as the median (IQR). °Data are presented as absolute numbers (%).
Abbreviations : DLBCL: diffuse large B-cell lymphoma; cART: combination antiretroviral therapy; ECOG PS: Eastern Cooperative Oncology Group (ECOG) performance status; IPI: International Prognostic Index

Table 4. Immunological parameters at lymphoma diagnosis by histology

	All (n=361)	NHL (n=271)	DLBCL (n=157)	BL (n=62)	PBL (n=17)	PEL (n=18)	PCL (n=17)	HD (n=90)	Kruskal-Wallis
CD56+ (IQR) cells/mm³	64 (92)	64 (89)	63 (80)	69 (76)	69 (125)	58 (89)	24 (74)	74 (117)	p=0.10
CD56+ % (IQR)	6.0 % (8.0)	6.0% (8.0)	6.3% (7.7)	5.0(8.0)	5.0% (5.2)	3.2% (6.0)	5.5% (6.2)	9.0% (8.0)	p=0.0092
CD4+ (IQR) cells/mm³	216 (282)	115 (253)	153 (289)	221 (231)	253 (253)	216 (300)	79 (331)	271 (317)	p=0.010
CD4+ % (IQR)	17% (16)	15% (15)	15% (16)	13% (8.0)	20% (16)	14% (12)	12% (20)	24% (15)	p<0.0001
CD8+ (IQR) cells/mm³	566 (613)	591 (642)	560 (526)	854 (1039)	837 (1000)	822 (902)	406 (608)	515 (546)	p=0.0006
CD8+ % (IQR)	55% (21)	57% (22)	51% (21)	58% (20)	52% (20)	58% (24)	54% (24)	51% (19)	p=0.166
CD19+ (IQR) cells/mm³	103 (132)	115 (145)	93 (127)	158 (216)	225 (142)	121 (97)	113 (151)	58 (93)	p<0.0001
CD19+ % (IQR)	8% (10)	9.7% (12)	8.5% (8.7)	11% (12)	18% (13)	9.0% (12)	14% (13)	5.5% (6.0)	p<0.0001

Data are presented as median and interquartile ranges (IQR).

Abbreviations: DLBCL= diffuse large B-cell lymphoma; BL= Burkitt lymphoma; PBL=plasmablastic lymphoma; PEL= primary effusion lymphoma; PL= primary cerebral lymphoma

Figure 2. CD56+ count at lymphoma diagnosis for all hisologies

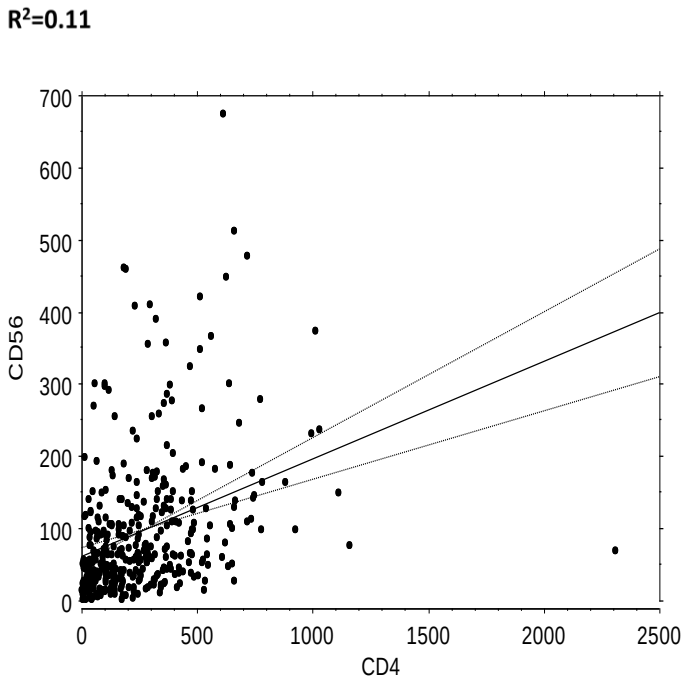


Figure 2A. Bivariate scattergram of CD4+ and CD56+ at lymphoma diagnosis for all histologies (n=360) (Bartlett's test of sphericity p<0.0001).

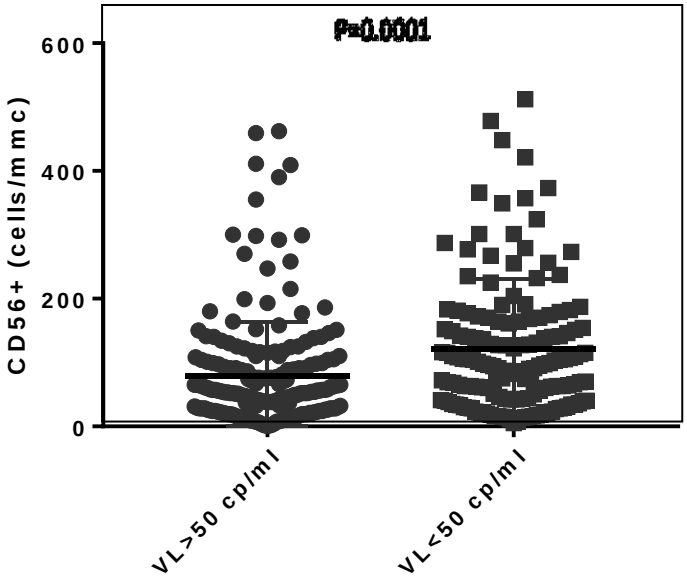


Figure 2B. CD56+ count at lymphoma diagnosis for all histologies (n=360) by HIV virological status.

Figure 3. Kaplan Meier overall survival curve for 149 patients with HIV associated DLBCL treated with combination chemotherapy with curative intent

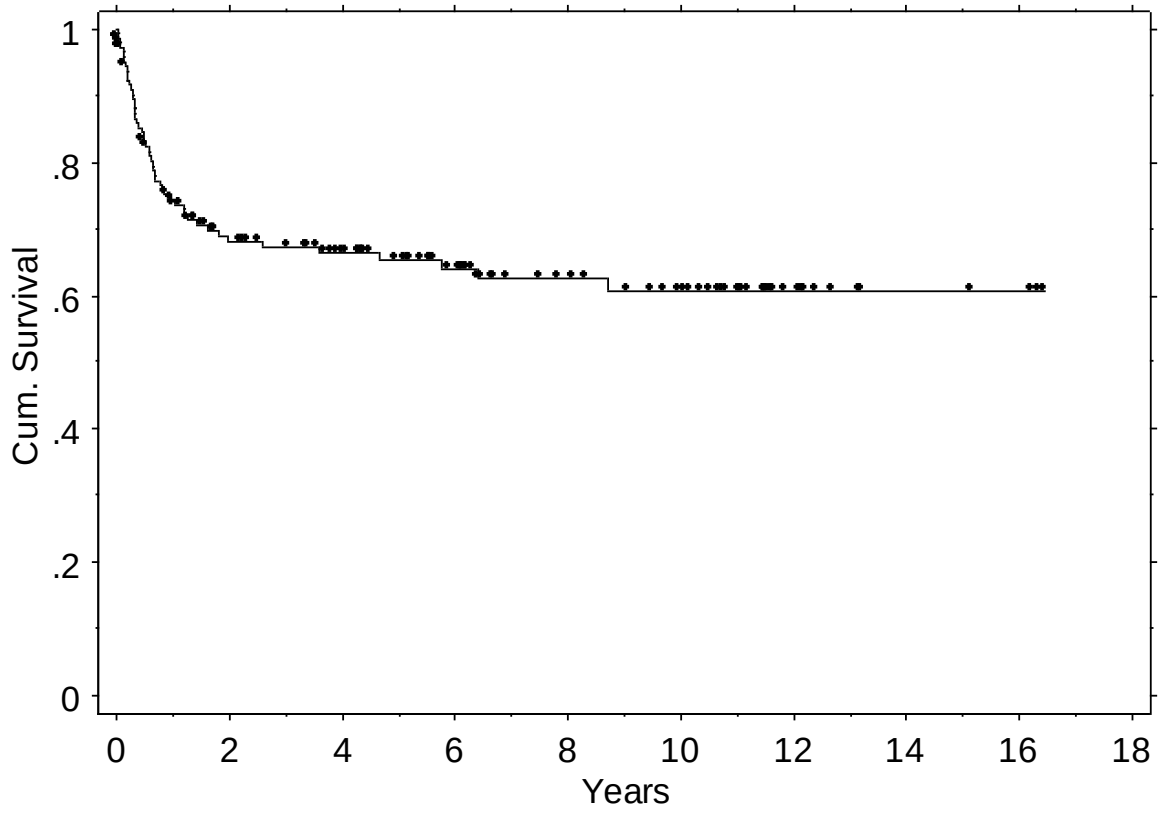


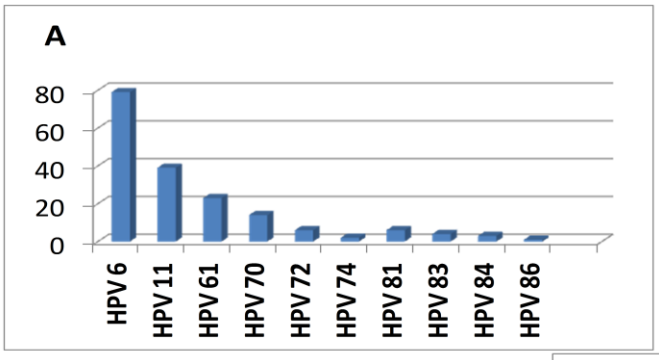
Table 5. DLBCL treated with curative chemotherapy (n=149)

Male gender°	123/149 (83%)
Mean age at DLBCL diagnosis (range)*	45 years (19-75)
Prior AIDS defining illness°	27/149 (18%)
On cART at DLBCL diagnosis°	91/149 (61%)
Plasma HIV VL undetectable on cART at DLBCL diagnosis°	53/91 (58%)
Stage°	I: 18/149 (12%) II: 12/149 (8%) III: 11/149 (7%) IV: 108/149 (72%)
ECOG PS°	0: 10/149 (7%) 1: 66/149 (44%) 2: 27/149 (18%) 3: 22/149 (15%) 4: 24/149 (16%)
IPI°	Age>60: 15/149 (10%) PS>1: 74/149 (50%) Raised LDH: 97/149 (65%) Stage>2: 119/149 (80%) >1 extranodal site: 66/149 (44%)
IPI group°	L: 37/149 (25%) LI: 34/149 (23%) HI: 37/149 (25%) H: 41/149 (28%)
Chemotherapy°	R-CHOP: 84/149 (56%) CDE: 63/149 (42%) R-CODOX-M/IVAC: 2/149 (2%)
Relapsed°	29/149 (19%)
Dead°	50/149 (34%)
Survival*	2 year OS: 70% (95%CI: 62-78%) 5 year OS: 66% (95%CI: 58-74%) 10 year OS: (95%CI: 52-71%)

*Data are presented as the median (IQR). °Data are presented as absolute numbers (%).

Legend: DLBCL: diffuse large B-cell lymphoma; cART: combination antiretroviral therapy; ECOG PS: Eastern Cooperative Oncology Group (ECOG) performance status; IPI: International Prognostic Index

Figure 4. Prevalence of different HPV genotypes (n=350/472, 74%)



LR-HPV: 85/350 (25%)

A: Prevalence of Low-risk HPV genotypes **(LR-HPV)**

B: Prevalence of High-risk genotypes **(HR-HPV)**

The most frequent HPV genotypes are listed on the abscissa. The percentage indicates the prevalence of each genotype within the total LR- HPV genotype (A) and HR-HPV genotypes (B) in the HPV-positive population.

HR-HPV: 265/350 (75%)

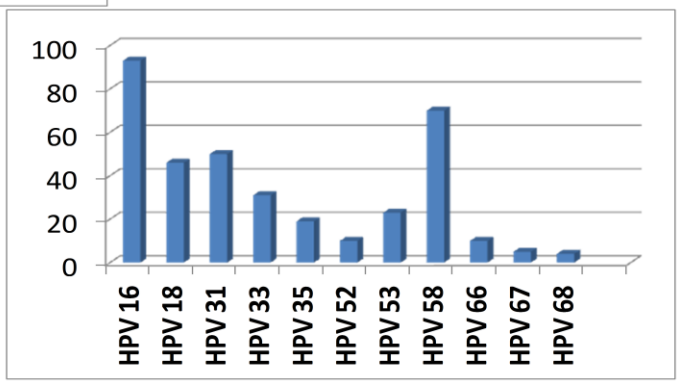


Table 5. Characteristics of the study population according to gender

Baseline parametres	Population (n=472)	Males (n=327)	Females (n=145)	p
Age (years) *	41 (34-47)	40 (33-46)	43 (39-48)	0.066
Homosexuals°	254 (54%)	254 (77.7%)	-	**0.0001**
Heterosexuals	144 (30%)	48 (14.7%)	96 (66.2%)	
IDUs	58 (12%)	22 (6.7%)	36 (24.8%)	
Other/not known	16 (4%)	3 (0.9%)	13 (9%)	
Months since HIV diagnosis*	49 (14-144)	34 (8-100)	144 (48-222)	**0.0001**
AIDS diseases°	84 (18%)	54 (17%)	30 (23%)	0.113
HCV Ab positive°	60 (13%)	32 (10%)	28 (19%)	**0.016**
HCV Ab negative	403 (85%)	289 (88%)	114 (79%)	
Not known	9 (2%)	6 (2%)	3 (2%)	
cART °	353 (75%)	220 (67%)	133 (92%)	**0.0001**
PI–based cART°	129 (36%)	62 (28%)	67 (50%)	**0.0001**
NNRTI-based cART	170 (48%)	113 (51.6%)	57 (43%)	
INSTI-based cART	53 (15%)	44 (20%)	9 (7%)	
Others	1 (1%)	1 (0.4%)	0	
Months on cART *	43.5 (14-110)	32 (8.25-90.7)	72 (31.5-134.2)	**0.01**
Nadir CD4+ T cells (cells/mmc)*	285 (164-432)	322 (191-480)	203 (105-288)	**0.0001**
CD4+ T cells (cells/mmc)*	504 (368-660)	512 (394-664)	477 (337-659)	0.364
CD4+% *	27 (21-32)	27 (21-32)	31 (20-38)	0.753
CD8+% *	48 (40-57)	48 (40-56)	47 (38-59)	0.312
CD127+CD4+% *	15 (10-21)	15 (10-20)	17 (12-23)	0.421
CD127+CD8+% *	19 (12-27)	20 (12-28)	17 (12-25)	0.215
CD38+CD8+% *	3 (1-7)	3 (1-7)	2 (1-5)	0.067
Log_{10} HIV-RNA (cp/mL)*	1.73 (1.59-3.33)	1.77 (1.59-3.9)	1.59 (1.59-1.79)	**0.0001**
HIV-RNA > 50 cp/mL°	177 (37%)	146 (45%)	31 (21%)	**0.0001**
HPV negative °	122 (26%)	48 (15%)	74 (51%)	**0.0001**
HPV positive	350 (74%)	279 (85%)	71 (49%)	
HPV HR genotypes	265 (56%)	223 (68%)	42 (29%)	
HPV LR genotypes	82 (18%)	55 (16.8%)	27 (19%)	
Not known genotype	3 (1%)	1 (0.2%)	2 (1%)	
Multiple HPV genotypes °	163 (46%)	141 (50%)	22 (31%)	**0.008**
Negative cytology °	224 (47.4%)	125 (38%)	99 (68.3%)	**0.0001**
LSIL	218 (46.2%)	179 (55%)	39 (26.9%)	
HSIL	30 (6.4%)	23 (7%)	7 (5%)	

*Data are presented as the median (IQR). °Data are presented as absolute numbers (%). P values for comparison between males and females.

Abbreviations: IDUs: injection drug users; cART: combination antiretroviral therapy; PI: Protease Inhibitor; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor; INSTI: Integrase Strand Transfer Inhibitors; HPV: Human Papilloma virus; HPV HR genotypes: Human Papilloma Virus High-risk genotypes; HPV LR genotypes: Human Papilloma Virus Low-risk genotypes; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade intraepithelial lesion.

Table 6. Factors associated with HPV infection (a) and with SIL (b) in females and males

Table 6a. Factors associated with HPV infection			
Females (N 145)			
Characteristics	**HPV negative (N 74)**	**HPV positive (N 71)**	**p**
Log_{10} HIV-RNA (cp/mL)*	1.59 (1.59-1.75)	1.77 (1.59-2.03)	**0.002**
HIV-RNA >50 cp/mL°	12 (16%)	19 (27%)	0.146
SIL °	9 (12%)	36 (51%)	**0.0001**
Males (N 327)			
Characteristics	**HPV negative (N 48)**	**HPV positive (N 279)**	**p**
Months since HIV diagnosis *	73 (18-144)	31 (7-85)	**0.002**
Homosexuals ° **Heterosexuals** **IDUs** **Other/notknown**	24 (50%) 9 (19%) 13 (27%) 2 (4%)	230(82%) 39 (14%) 9 (3%) 1 (0.3%)	**0.0001**
AIDS diseases °	17 (35%)	37 (13%)	**0.0001**
HCV Ab positive ° **HCV Ab negative** **Not known**	10 (21%) 36 (75%) 2 (4%)	22 (7.8%) 253 (90%) 4 (1%)	**0.007**
HBsAg positive ° **HBsAg negative** **Not known**	17 (35%) 30 (63%) 1 (2%)	53 (19%) 221 (79%) 5 (2%)	**0.036**
cART°	38 (79%)	182 (65%)	**0.057**
Months on cART*	47 (23-132)	29 (7-84)	**0.039**
Log_{10} HIV-RNA (cp/mL)*	1.59 (1.59-2.52)	1.77 (1.59-4.09)	**0.006**
HIV-RNA >50 cp/mL°	14 (29%)	132 (47%)	**0.024**
Nadir CD4+ (cell/mmc)*	214 (117-3158)	335 (200-484)	**0.004**
CD4+ (cell/mmc) *	444 (358-581)	519 (402-681)	**0.026**
HPV HR genotypes ° **HPV LR genotypes** **Not known genotype**	- -	223 (79.9%) 55 (19.7%) 1 (0.4%)	
Multiple HPV genotypes °	-	141 (50%)	
SIL	14 (29%)	188 (67%)	**0.0001**

Table 6b. Factors associated with SIL			
Females (N 145)			
Characteristics	**SIL negative (N 99)**	**SIL positive (N 46)**	**p**
Log_{10} HIV-RNA (cp/mL)*	1.59 (1.59-3.17)	1.77 (1.59-1.94)	**0.024**
HIV-RNA >50 cp/mL°	19 (19%)	12 (26%)	0.488
CD4+ (cell/mmc) *	525 (368-687)	420 (305-598)	**0.033**
CD4+% *	34 (25-40)	21 (14-28)	**0.033**
CD8+% *	40 (36-56)	51 (49-63)	**0.017**
HPV negative ° **HR HPV genotypes** **LR HPV genotypes**	64 (66%) 19 (20%) 14 (14%)	10 (22%) 23 (50%) 13 (28%)	**0.0001**
Males (N 327)			
Characteristics	**SIL negative (N 125)**	**SIL positive (N 202)**	**p**
Age (years) *	42 (34-47)	39 (33-45)	**0.026**
Months since HIV diagnosis *	46(11-123)	31 (7-85)	**0.035**
Homosexuals ° **Heterosexuals** **IDUs** **Other/notknown**	85 (68%) 25 (20%) 12 (10%) 3 (2%)	167 (83%) 23 (11%) 10 (5%) 2 (1%)	**0.044**
HBsAg positive ° **HBsAg negative** **Not known**	34 (27%) 91 (73%) 0	36 (18%) 160 (79%) 6 (3%)	**0.026**

cART °	92 (74%)	128 (64%)	**0.055**
Months on cART *	44 (22-121)	23 (5-62)	**0.0001**
Log_{10} HIV-RNA (cp/mL)*	1.59 (1.59-3.17)	2 (1.59-4.08)	**0.031**
HIV-RNA >50 cp/mL°	45 (36%)	101 (50%)	**0.009**
Nadir CD4+ (cell/mmc)*	279 (154-449)	342 (213-492)	**0.039**
CD127+CD8+% *	18 (10-25)	22 (14-30)	**0.0001**
CD38+CD8+% *	2 (1-7)	3 (2-9)	**0.013**
HPV negative ° **HR genotypes** **LR genotypes**	35 (28%) 68 (54%) 22 (18%)	14 (69%) 155 (77%) 33 (16%)	**0.0001**
Multiple HPV genotypes °	29/90 (32%)	112/188 (59%)	**0.0001**

*Data are presented as the median (IQR). Mann Whitney test. °Data are presented as absolute numbers (%). Chi-square test. P values for comparison between HPV negative and HPV positive and between SIL negative and SIL negative patients.

Abbreviations: SIL: squamous intraepithelial lesion; IDUs: injection drug users; cART: combination antiretroviral therapy; HPV: Human Papilloma Virus; HPV HR genotypes: Human Papilloma Virus High-risk genotypes; HPV LR genotypes: Human Papilloma Virus Low-risk genotypes

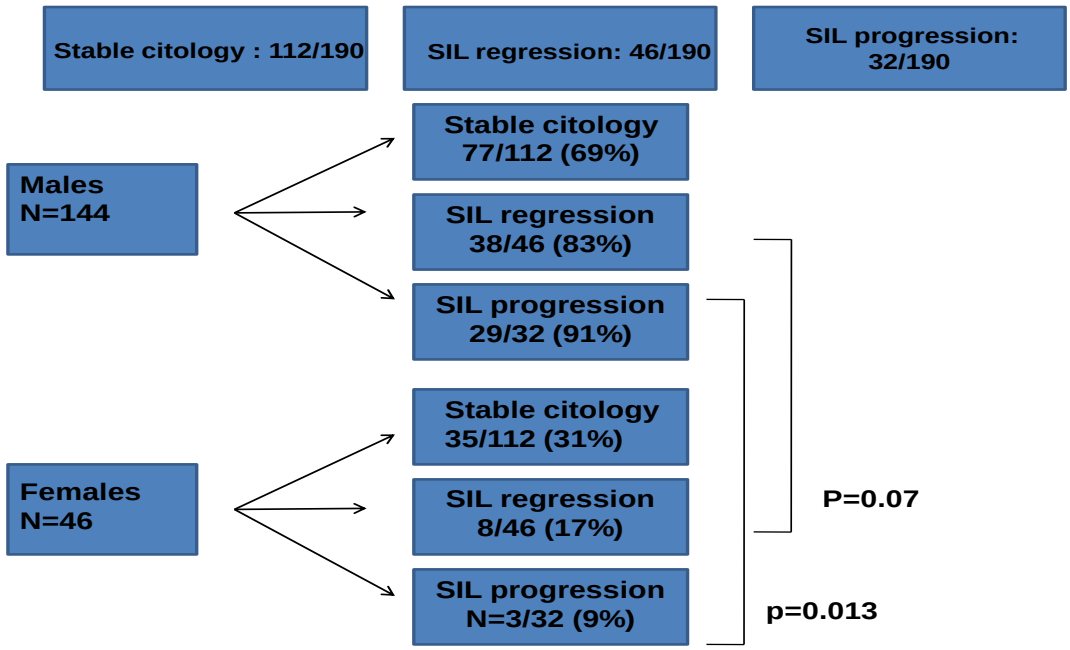
Table 7. Factors associated with clearance of HPV infection

Characteristics	HPV persistence (n 125)	HPV Clearance (n 30)	p
Age, years*	41 (34-46)	41 (38-49)	0.155
Male°	110 (88%)	14 (47%)	**0.0001**
Months since HIV diagnosis*	41 (18-142)	113 (42-186)	**0.007**
Homosexual epidemiology° Heterosexual epidemiology° IDUs° Other/not known°	95 (76%) 21 (16.8%) 8 (6.4%) 1 (0.8%)	10 (33%) 14 (47%) 6 (20%) 0	**0.0001**
Previous AIDS diseases°	10 (8%)	5 (20%)	0.117
HCV positive°	11 (8%)	5 (17%)	0.203
HCV negative°	114 (91%)	25 (83%)	
HCV unknown°	0	0	
HBSAg pos°	28 (22.4%)	9 (30%)	0.615
HBsAg neg°	96 (76.8%)	21(70%)	
HBV unknown°	1 (0.8%)	0	
On cART°	85 (68%)	26 (90%)	**0.019**
cART duration*	57 (26-130)	56 (22-111)	0.495
Log10 cp/mL HIV RNA*	1.77 (1.59-4.25)	1.77 (1.59-1.77)	**0.052**
CD4+ T cell nadir, cell/mmc*	322 (180-448)	270 (143-394)	0.25
CD4+ T cells count, cell/mmc*	516 (378-714)	448 (337-560)	0.162
CD4+ T cells %*	28 (21-34)	32 (23-34)	0.181
CD8+%*	45 (39-56)	45 (38-53)	0.464
CD127+CD4+%*	16 (10-22)	17 (11-23)	0.246
CD127+CD8+%*	15 (10-23)	14 (10-21)	0.741
CD38+CD8+%*	3 (1-6)	1 (1-3)	**0.003**
HR HPV genotypes°	105 (84%)	15 (50%)	**0.0001**
LR HPV°	20 (16%)	15 (50%)	
Multiple HPV genotypes°	72 (57%)	12g (41%)	**0.001**
SIL°	87 (70%)	12 (40%)	**0.002**

*Data are presented as the median (IQR). Mann Whitney test. °Data are presented as absolute numbers (%). Chi-square test. P values for comparison between patients who cleared the HPV infection and patients with HPV-infection persistence.

Abbreviations: IDUs: injection drug users; cART: combination antiretroviral therapy; HPV: Human Papilloma Virus; HPV HR genotypes: Human Papilloma Virus High-risk genotypes; HPV LR genotypes: Human Papilloma Virus Low-risk genotypes; SIL: squamous intraepithelial lesion

Figure 5. Cytological evaluation at follow up (N=190)



Bibliography

1. Bonnet F, Burty C, Lewden C, et al. Changes in cancer mortality among HIV-infected patients: the Mortalité 2005 Survey. *Clin Infect Dis*. 2009;48(5):633-639. http://www.ncbi.nlm.nih.gov/pubmed/19202627. Accessed July 30, 2018.
2. Borges AH, Dubrow R, Silverberg MJ. Factors contributing to risk for cancer among HIV-infected individuals, and evidence that earlier combination antiretroviral therapy will alter this risk. *Curr Opin HIV AIDS*. 2014;9(1):34-40. doi:10.1097/COH.0000000000000025.
3. Shiels MS, Engels EA. Evolving epidemiology of HIV-associated malignancies. *Curr Opin HIV AIDS*. 2017;12(1):6-11. doi:10.1097/COH.0000000000000327.
4. Dubrow R, Silverberg MJ, Park LS, Crothers K, Justice AC. HIV infection, aging, and immune function. *Curr Opin Oncol*. 2012;24(5):506-516. doi:10.1097/CCO.0b013e328355e131.
5. Kojic EM, Conley L, Bush T, et al. Prevalence and Incidence of Anal and Cervical High-Risk Human Papillomavirus (HPV) Types covered by Current HPV Vaccines among HIV-Infected Women in the Study to Understand the Natural History of HIV/AIDS in the Era of Effective Therapy (The SUN Study). *J Infect Dis*. February 2018. doi:10.1093/infdis/jiy087.
6. Maskew M, Fox MP, van Cutsem G, et al. Treatment response and mortality among patients starting antiretroviral therapy with and without Kaposi sarcoma: a cohort study. Schulz TF, ed. *PLoS One*. 2013;8(6):e64392. doi:10.1371/journal.pone.0064392.
7. Arvey A, Ojesina AI, Pedamallu CS, et al. The tumor virus landscape of AIDS-related lymphomas. *Blood*. 2015;125(20):e14-22. doi:10.1182/blood-2014-11-599951.
8. Shiels MS, Pfeiffer RM, Gail MH, et al. Cancer Burden in the HIV-Infected Population in the United States. *JNCI J Natl Cancer Inst*. 2011;103(9):753-762. doi:10.1093/jnci/djr076.
9. 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. *Lancet (London, England)*. 2007;370(9581):59-67. doi:10.1016/S0140-6736(07)61050-2.
10. Winstone TA, Man SFP, Hull M, Montaner JS, Sin DD. Epidemic of lung cancer in patients

with HIV infection. *Chest*. 2013;143(2):305-314. doi:10.1378/chest.12-1699.

11. 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(5):611-622. doi:10.1097/QAI.0b013e3181b327ca.
12. Muñoz N, Bosch FX, de Sanjosé S, et al. Epidemiologic Classification of Human Papillomavirus Types Associated with Cervical Cancer. *N Engl J Med*. 2003;348(6):518-527. doi:10.1056/NEJMoa021641.
13. Silverberg MJ, Leyden W, Warton EM, et al. HIV infection status, immunodeficiency, and the incidence of non-melanoma skin cancer. *J Natl Cancer Inst*. 2013;105(5):350-360. doi:10.1093/jnci/djs529.
14. Goedert JJ, Schairer C, McNeel TS, et al. Risk of breast, ovary and uterine corpus cancers among 85 268 women with AIDS. *Br J Cancer*. 2006;95(5):642-648. doi:10.1038/sj.bjc.6603282.
15. Spano J-P, Lanoy E, Mounier N, Katlama C, Costagliola D, Heard I. Breast cancer among HIV infected individuals from the ONCOVIH study, in France: therapeutic implications. *Eur J Cancer*. 2012;48(18):3335-3341. doi:10.1016/j.ejca.2012.05.019.
16. Park IU, Palefsky JM. Evaluation and management of anal intraepithelial neoplasia in HIV-negative and HIV-positive men who have sex with men. *Curr Infect Dis Rep*. 2010;12(2):126-133. doi:10.1007/s11908-010-0090-7.
17. Palefsky JM, Holly EA, Efirdc JT, et al. Anal intraepithelial neoplasia in the highly active antiretroviral therapy era among HIV-positive men who have sex with men. *AIDS*. 2005;19(13):1407-1414. http://www.ncbi.nlm.nih.gov/pubmed/16103772. Accessed January 15, 2018.
18. 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(31):5005-5009. doi:10.1200/JCO.2006.05.7984.
19. Seaberg EC, Wiley D, Martínez-Maza O, et al. Cancer incidence in the multicenter aids cohort study before and during the HAART era. *Cancer*. 2010;116(23):5507-5516. doi:10.1002/cncr.25530.
20. Park LS, Hernández-Ramírez RU, Silverberg MJ, Crothers K, Dubrow R. Prevalence of non-HIV cancer risk factors in persons living with HIV/AIDS: a meta-analysis. *AIDS*. 2016;30(2):273-291. doi:10.1097/QAD.0000000000000922.

21. Altekruse SF, Shiels MS, Modur SP, et al. Cancer burden attributable to cigarette smoking among HIV-infected people in North America. *AIDS*. 2018;32(4):513-521. doi:10.1097/QAD.0000000000001721.
22. Engels EA, Biggar RJ, Hall HI, et al. Cancer risk in people infected with human immunodeficiency virus in the United States. *Int J cancer*. 2008;123(1):187-194. doi:10.1002/ijc.23487.
23. Borges ÁH, Silverberg MJ, Wentworth D, et al. Predicting risk of cancer during HIV infection: the role of inflammatory and coagulation biomarkers. *AIDS*. 2013;27(9):1433-1441. doi:10.1097/QAD.0b013e32835f6b0c.
24. Mitsuyasu RT. Non-AIDS-defining cancers. *Top Antivir Med*. 22(3):660-665. http://www.ncbi.nlm.nih.gov/pubmed/25101532. Accessed July 31, 2018.
25. Rickabaugh TM, Kilpatrick RD, Hultin LE, et al. The Dual Impact of HIV-1 Infection and Aging on Naïve CD4+ T-Cells: Additive and Distinct Patterns of Impairment. Unutmaz D, ed. *PLoS One*. 2011;6(1):e16459. doi:10.1371/journal.pone.0016459.
26. Pantanowitz L, Carbone A, Dolcetti R. Microenvironment and HIV-related lymphomagenesis. *Semin Cancer Biol*. 2015;34:52-57. doi:10.1016/j.semcancer.2015.06.002.
27. Nolen BM, Breen EC, Bream JH, et al. Circulating Mediators of Inflammation and Immune Activation in AIDS-Related Non-Hodgkin Lymphoma. Unutmaz D, ed. *PLoS One*. 2014;9(6):e99144. doi:10.1371/journal.pone.0099144.
28. Tumwine LK, Orem J, Kerchan P, Byarugaba W, Pileri SA. EBV, HHV8 and HIV in B cell non Hodgkin lymphoma in Kampala, Uganda. *Infect Agent Cancer*. 2010;5(1):12. doi:10.1186/1750-9378-5-12.
29. Tarazona R, Casado JG, Delarosa O, et al. Selective depletion of CD56dim NK cell subsets and maintenance of CD56bright NK cells in treatment-naive HIV-1-seropositive individuals. *J Clin Immunol*. 2002;22(3):176-183. doi:10.1023/A:1015476114409.
30. Subleski JJ, Jiang Q, Weiss JM, Wiltrout RH. The split personality of NKT cells in malignancy, autoimmune and allergic disorders. *Immunotherapy*. 2011;3(10):1167-1184. doi:10.2217/imt.11.117.
31. Moretta L. Dissecting CD56dim human NK cells. *Blood*. 2010;116(19):3689-3691. doi:10.1182/blood-2010-09-303057.
32. Cox MC, Battella S, La Scaleia R, et al. Tumor-associated and immunochemotherapydependent long-term alterations of the peripheral blood NK cell

compartment in DLBCL patients. *Oncoimmunology*. 2015;4(3):1-12. doi:10.4161/2162402X.2014.990773.

33. Vendrame E, Hussain SK, Breen EC, et al. Serum levels of cytokines and biomarkers for inflammation and immune activation, and HIV-associated non-Hodgkin B-cell lymphoma risk. *Cancer Epidemiol Biomarkers Prev*. 2014;23(2):343-349. doi:10.1158/1055-9965.EPI-13-0714.
34. Thapa DR, Hussain SK, Tran W-C, et al. Serum microRNAs in HIV-infected individuals as pre-diagnosis biomarkers for AIDS-related non-Hodgkin lymphomas (AIDS-NHL). *JAIDS J Acquir Immune Defic Syndr*. 2014;66(2):1. doi:10.1097/QAI.0000000000000146.
35. Hussain SK, Hessol NA, Levine AM, et al. Serum biomarkers of immune activation and subsequent risk of non-hodgkin B-cell lymphoma among HIV-infected women. *Cancer Epidemiol Biomarkers Prev*. 2013;22(11):2084-2093. doi:10.1158/1055-9965.EPI-13-0614.
36. Ul-Haq I, Dalla Pria A, Suardi E, et al. Blood Epstein–Barr virus DNA does not predict outcome in advanced HIV-associated Hodgkin lymphoma. *Med Oncol*. 2018;35(4):53. doi:10.1007/s12032-018-1099-2.
37. Vockerodt M, Cader FZ, Shannon-Lowe C, Murray P. Epstein-Barr virus and the origin of Hodgkin lymphoma. *Chin J Cancer*. 2014;33(12):591-597. doi:10.5732/cjc.014.10193.
38. Guidoboni M, Ponzoni M, Caggiari L, et al. Latent membrane protein 1 deletion mutants accumulate in reed-sternberg cells of human immunodeficiency virus-related Hodgkin's lymphoma. *J Virol*. 2005;79(4):2643-2649. doi:10.1128/JVI.79.4.2643-2649.2005.
39. Thompson LDR, Fisher SI, Chu WS, Nelson A, Abbondanzo SL. HIV-associated Hodgkin lymphoma: a clinicopathologic and immunophenotypic study of 45 cases. *Am J Clin Pathol*. 2004;121(5):727-738. doi:10.1309/PNVQ-0PQG-XHVY-6L7G.
40. Biggar RJ, Jaffe ES, Goedert JJ, Chaturvedi A, Pfeiffer R, Engels EA. Hodgkin lymphoma and immunodeficiency in persons with HIV/AIDS. *Blood*. 2006;108(12):3786-3791. doi:10.1182/blood-2006-05-024109.
41. Clifford GM, Rickenbach M, Lise M, et al. Hodgkin lymphoma in the Swiss HIV Cohort Study. *Blood*. 2009;113(23):5737-5742. doi:10.1182/blood-2009-02-204172.
42. Jussila L, Valtola R, Partanen TA, et al. Lymphatic endothelium and Kaposi's sarcoma spindle cells detected by antibodies against the vascular endothelial growth factor receptor-3. *Cancer Res*. 1998;58(8):1599-1604. http://www.ncbi.nlm.nih.gov/pubmed/9563467. Accessed September 5, 2018.

43. Orenstein JM. Ultrastructure of Kaposi Sarcoma. *Ultrastruct Pathol*. 2008;32(5):211-220. doi:10.1080/01913120802343871.
44. Mesri EA, Cesarman E, Boshoff C. Kaposi's sarcoma and its associated herpesvirus. *Nat Rev Cancer*. 2010;10(10):707-719. doi:10.1038/nrc2888.
45. Stolka K, Ndom P, Hemingway-Foday J, et al. Risk factors for Kaposi's sarcoma among HIV-positive individuals in a case control study in Cameroon. *Cancer Epidemiol*. 2014;38(2):137-143. doi:10.1016/j.canep.2014.02.006.
46. Nakagawa M, Stites DP, Palefsky JM, Kneass Z, Moscicki AB. CD4-positive and CD8-positive cytotoxic T lymphocytes contribute to human papillomavirus type 16 E6 and E7 responses. *Clin Diagn Lab Immunol*. 1999;6(4):494-498. http://www.ncbi.nlm.nih.gov/pubmed/10391849. Accessed September 5, 2018.
47. De Vuyst H, Lillo F, Broutet N, Smith JS. HIV, human papillomavirus, and cervical neoplasia and cancer in the era of highly active antiretroviral therapy. *Eur J Cancer Prev*. 2008;17(6):545-554. doi:10.1097/CEJ.0b013e3282f75ea1.
48. Kobayashi A, Greenblatt RM, Anastos K, et al. Functional attributes of mucosal immunity in cervical intraepithelial neoplasia and effects of HIV infection. *Cancer Res*. 2004;64(18):6766-6774. doi:10.1158/0008-5472.CAN-04-1091.
49. Hibma MH. The Immune Response to Papillomavirus During Infection Persistence and Regression. *Open Virol J*. 2012;6(1):241-248. doi:10.2174/1874357901206010241.
50. Woodworth CD, McMullin E, Iglesias M, Plowman GD. Interleukin 1 alpha and tumor necrosis factor alpha stimulate autocrine amphiregulin expression and proliferation of human papillomavirus-immortalized and carcinoma-derived cervical epithelial cells. *Proc Natl Acad Sci U S A*. 1995;92(7):2840-2844. http://www.ncbi.nlm.nih.gov/pubmed/7708734. Accessed September 5, 2018.
51. García-Muñoz R, López-Díaz-de-Cerio A, Feliu J, et al. Follicular lymphoma: in vitro effects of combining lymphokine-activated killer (LAK) cell-induced cytotoxicity and rituximab- and obinutuzumab-dependent cellular cytotoxicity (ADCC) activity. *Immunol Res*. 2016;64(2):548-557. doi:10.1007/s12026-015-8747-9.
52. Aguayo A, Patt YZ. Liver cancer. *Clin Liver Dis*. 2001;5(2):479-507. http://www.ncbi.nlm.nih.gov/pubmed/11385973. Accessed September 10, 2018.
53. Engels EA, Frisch M, Lubin JH, Gail MH, Biggar RJ, Goedert JJ. Prevalence of hepatitis C virus infection and risk for hepatocellular carcinoma and non-Hodgkin lymphoma in AIDS. *J Acquir*

Immune Defic Syndr. 2002;31(5):536-541. http://www.ncbi.nlm.nih.gov/pubmed/12473843. Accessed September 11, 2018.

54. Mastroianni CM, Lichtner M, Mascia C, Zuccalà P, Vullo V. Molecular mechanisms of liver fibrosis in HIV/HCV coinfection. *Int J Mol Sci*. 2014;15(6):9184-9208. doi:10.3390/ijms15069184.
55. Clifford GM, Rickenbach M, Polesel J, et al. Influence of HIV-related immunodeficiency on the risk of hepatocellular carcinoma. *AIDS*. 2008;22(16):2135-2141. doi:10.1097/QAD.0b013e32831103ad.
56. Kramer JR, Kowalkowski MA, Duan Z, Chiao EY. The Effect of HIV Viral Control on the Incidence of Hepatocellular Carcinoma in Veterans With Hepatitis C and HIV Coinfection. *JAIDS J Acquir Immune Defic Syndr*. 2015;68(4):456-462. doi:10.1097/QAI.0000000000000494.
57. Mehal WZ, Friedman SL. The Role of Inflammation and Immunity in the Pathogenesis of Liver Fibrosis. In: *Liver Immunology*. Totowa, NJ: Humana Press; 2007:111-121. doi:10.1007/978-1-59745-518-3_10.
58. Crane M, Avihingsanon A, Rajasuriar R, et al. Lipopolysaccharide, immune activation, and liver abnormalities in HIV/hepatitis B virus (HBV)-coinfected individuals receiving HBV-active combination antiretroviral therapy. *J Infect Dis*. 2014;210(5):745-751. doi:10.1093/infdis/jiu119.
59. Balagopal A, Philp FH, Astemborski J, et al. Human Immunodeficiency Virus-Related Microbial Translocation and Progression of Hepatitis C. *Gastroenterology*. 2008;135(1):226-233. doi:10.1053/j.gastro.2008.03.022.
60. García-Álvarez M, Berenguer J, Guzman-Fulgencio M, et al. Bacterial DNA Translocation and Liver Disease Severity Among HIV-Infected Patients With Chronic Hepatitis C. *JAIDS J Acquir Immune Defic Syndr*. 2012;61(5):552-556. doi:10.1097/QAI.0b013e31826ea109.
61. Hernandez MD, Sherman KE. HIV/hepatitis C coinfection natural history and disease progression. *Curr Opin HIV AIDS*. 2011;6(6):478-482. doi:10.1097/COH.0b013e32834bd365.
62. Justice AC, Lasky E, McGinnis KA, et al. Medical Disease and Alcohol Use Among Veterans With Human Immunodeficiency Infection. *Med Care*. 2006;44(Suppl 2):S52-S60. doi:10.1097/01.mlr.0000228003.08925.8c.
63. Hézode C, Roudot-Thoraval F, Nguyen S, et al. Daily cannabis smoking as a risk factor for progression of fibrosis in chronic hepatitis C. *Hepatology*. 2005;42(1):63-71.

doi:10.1002/hep.20733.

64. Maurer HH. Chemistry, Pharmacology, and Metabolism of Emerging Drugs of Abuse. *Ther Drug Monit*. 2010;32(5):544-549. doi:10.1097/FTD.0b013e3181eea318.

65. Helleberg M, Kronborg G, Ullum H, Ryder LP, Obel N, Gerstoft J. Course and Clinical Significance of CD8 $^{+}$ T-Cell Counts in a Large Cohort of HIV-Infected Individuals. *J Infect Dis*. 2015;211(11):1726-1734. doi:10.1093/infdis/jiu669.

66. Serrano-Villar S, Sainz T, Lee SA, et al. HIV-Infected Individuals with Low CD4/CD8 Ratio despite Effective Antiretroviral Therapy Exhibit Altered T Cell Subsets, Heightened CD8+ T Cell Activation, and Increased Risk of Non-AIDS Morbidity and Mortality. Silvestri G, ed. *PLoS Pathog*. 2014;10(5):e1004078. doi:10.1371/journal.ppat.1004078.

67. Medrano LM, Garcia-Broncano P, Berenguer J, et al. Elevated liver stiffness is linked to increased biomarkers of inflammation and immune activation in HIV/hepatitis C virus-coinfected patients. *AIDS*. 2018;32(9):1095-1105. doi:10.1097/QAD.0000000000001787.

68. Kaplan EL, Meier P. Nonparametric Estimation from Incomplete Observations. *J Am Stat Assoc*. 1958;53(282):457. doi:10.2307/2281868.

69. Engels EA, Biggar RJ, Hall HI, et al. Cancer risk in people infected with human immunodeficiency virus in the United States. *Int J Cancer*. 2008;123(1):187-194. doi:10.1002/ijc.23487.

70. Cao W. Parallel decline of CD8+CD38+ lymphocytes and viremia in treated hepatitis B patients. *World J Gastroenterol*. 2011;17(17):2191. doi:10.3748/wjg.v17.i17.2191.

71. Hoffmann M, Pantazis N, Martin GE, et al. Exhaustion of Activated CD8 T Cells Predicts Disease Progression in Primary HIV-1 Infection. Swanstrom R, ed. *PLOS Pathog*. 2016;12(7):e1005661. doi:10.1371/journal.ppat.1005661.

72. Brites-Alves C, Luz E, Netto EM, et al. Immune Activation, Proinflammatory Cytokines, and Conventional Risks for Cardiovascular Disease in HIV Patients: A Case-Control Study in Bahia, Brazil. *Front Immunol*. 2018;9:1469. doi:10.3389/fimmu.2018.01469.

73. Trickey A, May MT, Schommers P, et al. CD4:CD8 Ratio and CD8 Count as Prognostic Markers for Mortality in Human Immunodeficiency Virus-Infected Patients on Antiretroviral Therapy: The Antiretroviral Therapy Cohort Collaboration (ART-CC). *Clin Infect Dis*. 2017;65(6):959-966. doi:10.1093/cid/cix466.

74. Noelie Hema M, Ferry T, Dupon M, et al. Low CD4/CD8 Ratio Is Associated with Non AIDS-Defining Cancers in Patients on Antiretroviral Therapy: ANRS CO8 (Aproco/ Copilote)

Prospective Cohort Study. 2016. doi:10.1371/journal.pone.0161594.

75. Bower M, Palfreeman A, Alfa-Wali M, et al. British HIV Association guidelines for HIV-associated malignancies 2014. *HIV Med*. 2014;15:1-92. doi:10.1111/hiv.12136.

76. Leal FE, Premeaux TA, Abdel-Mohsen M, Ndhlovu LC. Role of natural killer cells in HIV-associated malignancies. *Front Immunol*. 2017;8(MAR). doi:10.3389/fimmu.2017.00315.

77. Cheng M, Chen Y, Xiao W, Sun R, Tian Z. NK cell-based immunotherapy for malignant diseases. *Cell Mol Immunol*. 2013;10(3):230-252. doi:10.1038/cmi.2013.10.

78. Plonquet A, Haioun C, Jais JP, et al. Peripheral blood natural killer cell count is associated with clinical outcome in patients with aaIPI 2-3 diffuse large B-cell lymphoma. *Ann Oncol*. 2007;18(7):1209-1215. doi:10.1093/annonc/mdm110.

79. Meier U, Owen R, Taylor E. Shared alterations in NK cell frequency, phenotype, and function in chronic human immunodeficiency virus and hepatitis C virus infections. *J …*. 2005;79(19):12365-12374. doi:10.1128/JVI.79.19.12365.

80. Mercier-Bataille D, Sanchez C, Baier C, et al. Expression of activating receptors on natural killer cells from AIDS-related lymphoma patients. *AIDS Res Ther*. 2014;11(1):38. doi:10.1186/1742-6405-11-38.

81. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. *Br J Cancer*. 1977;35(1):1-39. http://www.ncbi.nlm.nih.gov/pubmed/831755. Accessed September 26, 2018.

82. Smyth MJ, Hayakawa Y, Takeda K, Yagita H. New aspects of natural-killer-cell surveillance and therapy of cancer. *Nat Rev Cancer*. 2002;2(11):850-861. doi:10.1038/nrc928.

83. Kohrt HE, Houot R, Marabelle A, et al. Combination strategies to enhance antitumor ADCC. *Immunotherapy*. 2012;4(5):511-527. doi:10.2217/imt.12.38.

84. Desjarlais JR, Lazar GA, Zhukovsky EA, Chu SY. Optimizing engagement of the immune system by anti-tumor antibodies: an engineer's perspective. *Drug Discov Today*. 2007;12(21-22):898-910. doi:10.1016/j.drudis.2007.08.009.

85. Kellner C, Günther A, Humpe A, et al. Enhancing natural killer cell-mediated lysis of lymphoma cells by combining therapeutic antibodies with CD20-specific immunoligands engaging NKG2D or NKp30. *Oncoimmunology*. 2016;5(1):1-12. doi:10.1080/2162402X.2015.1058459.

86. Kohrt HE, Houot R, Goldstein MJ, et al. CD137 stimulation enhances the antilymphoma activity of anti-CD20 antibodies. *Blood*. 2011;117(8):2423-2432. doi:10.1182/blood-2010-

08-301945.

87. Binyamin L, Alpaugh RK, Hughes TL, Lutz CT, Campbell KS, Weiner LM. Blocking NK cell inhibitory self-recognition promotes antibody-dependent cellular cytotoxicity in a model of anti-lymphoma therapy. *J Immunol*. 2008;180(9):6392-6401. http://www.ncbi.nlm.nih.gov/pubmed/18424763. Accessed September 26, 2018.
88. Murphy WJ, Parham P, Miller JS. NK Cells—From Bench to Clinic. *Biol Blood Marrow Transplant*. 2012;18(1):S2-S7. doi:10.1016/j.bbmt.2011.10.033.
89. Weber JS, Yang JC, Topalian SL, Schwartzentruber DJ, White DE, Rosenberg SA. The use of interleukin-2 and lymphokine-activated killer cells for the treatment of patients with non-Hodgkin's lymphoma. *J Clin Oncol*. 1992;10(1):33-40. doi:10.1200/JCO.1992.10.1.33.
90. Berdeja JG, Hess A, Lucas DM, et al. Systemic interleukin-2 and adoptive transfer of lymphokine-activated killer cells improves antibody-dependent cellular cytotoxicity in patients with relapsed B-cell lymphoma treated with rituximab. *Clin Cancer Res*. 2007;13(8):2392-2399. doi:10.1158/1078-0432.CCR-06-1860.
91. Abbas A, Yang G, Fakih M. Management of anal cancer in 2010. Part 2: current treatment standards and future directions. *Oncology (Williston Park)*. 2010;24(5):417-424. http://www.ncbi.nlm.nih.gov/pubmed/20480740. Accessed January 11, 2018.
92. Smyczek P, Singh AE, Romanowski B. Anal intraepithelial neoplasia: review and recommendations for screening and management. *Int J STD AIDS*. 2013;24(11):843-851. doi:10.1177/0956462413481527.
93. Abraham AG, D'Souza G, Jing Y, et al. Invasive cervical cancer risk among HIV-infected women: a North American multicohort collaboration prospective study. *J Acquir Immune Defic Syndr*. 2013;62(4):405-413. doi:10.1097/QAI.0b013e31828177d7.
94. Schim van der Loeff MF, Mooij SH, Richel O, de Vries HJC, Prins JM. HPV and anal cancer in HIV-infected individuals: a review. *Curr HIV/AIDS Rep*. 2014;11(3):250-262. doi:10.1007/s11904-014-0224-x.
95. Sammarco ML, Ucciferri C, Tamburro M, Falasca K, Ripabelli G, Vecchiet J. High prevalence of human papillomavirus type 58 in HIV infected men who have sex with men: A preliminary report in Central Italy. *J Med Virol*. 2016;88(5):911-914. doi:10.1002/jmv.24406.
96. Lin C, Franceschi S, Clifford GM. Human papillomavirus types from infection to cancer in the anus, according to sex and HIV status: a systematic review and meta-analysis. *Lancet Infect Dis*. 2018;18(2):198-206. doi:10.1016/S1473-3099(17)30653-9.

97. Reusser NM, Downing C, Guidry J, Tyring SK. HPV Carcinomas in Immunocompromised Patients. *J Clin Med*. 2015;4(2):260-281. doi:10.3390/jcm4020260.

98. Mooij SH, Van Santen DK, Geskus RB, et al. The effect of HIV infection on anal and penile human papillomavirus incidence and clearance. *AIDS*. 2015;30(1):1. doi:10.1097/QAD.0000000000000909.

99. Patel P, Hanson DL, Sullivan PS, 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(10):728-736. http://www.ncbi.nlm.nih.gov/pubmed/18490686. Accessed January 11, 2018.

100. Blitz S, Baxter J, Raboud J, et al. Evaluation of HIV and highly active antiretroviral therapy on the natural history of human papillomavirus infection and cervical cytopathologic findings in HIV-positive and high-risk HIV-negative women. *J Infect Dis*. 2013;208(3):454-462. doi:10.1093/infdis/jit181.

101. Chaturvedi AK, Madeleine MM, Biggar RJ, Engels EA. Risk of human papillomavirus-associated cancers among persons with AIDS. *J Natl Cancer Inst*. 2009;101(16):1120-1130. doi:10.1093/jnci/djp205.

102. Mbulawa ZZA, Marais DJ, Johnson LF, Coetzee D, Williamson A-L. Impact of Human Immunodeficiency Virus on the Natural History of Human Papillomavirus Genital Infection in South African Men and Women. *J Infect Dis*. 2012;206(1):15-27. doi:10.1093/infdis/jis299.

103. Piketty C, Darragh TM, Heard I, et al. High Prevalence of Anal Squamous Intraepithelial Lesions in HIV-Positive Men Despite the Use of Highly Active Antiretroviral Therapy. *Sex Transm Dis*. 2004;31(2):96-99. doi:10.1097/01.OLQ.0000109515.75864.2B.

104. De Pokomandy A, Rouleau D, Ghattas G, et al. HAART and progression to high-grade anal intraepithelial neoplasia in men who have sex with men and are infected with HIV. *Clin Infect Dis*. 2011;52(9):1174-1181. doi:10.1093/cid/cir064.

105. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA*. 2002;287(16):2114-2119. http://www.ncbi.nlm.nih.gov/pubmed/11966386. Accessed January 15, 2018.

106. Ren X, Ke W, Zheng H, et al. Human Papillomavirus Positivity in the Anal Canal in HIV-Infected and HIV-Uninfected Men Who Have Anal Sex with Men in Guangzhou, China: Implication for Anal Exams and Early Vaccination. *Biomed Res Int*. 2017;2017:2641259.

doi:10.1155/2017/2641259.

107. Giuliano AR, Lu B, Nielson CM, et al. Age-Specific Prevalence, Incidence, and Duration of Human Papillomavirus Infections in a Cohort of 290 US Men. *J Infect Dis*. 2008;198(6):827-835. doi:10.1086/591095.

108. Choudhury SA, Choudhury NA, Humphrey AD, et al. Higher Prevalence of Human Papillomavirus-Related Cervical Precancerous Abnormalities in HIV-Infected Compared to HIV-Uninfected Women. *J Natl Med Assoc*. 2016;108(1):19-23. doi:10.1016/j.jnma.2015.12.003.

109. Mayer KH, Bush T, Henry K, et al. Ongoing sexually transmitted disease acquisition and risk-taking behavior among US HIV-infected patients in primary care: implications for prevention interventions. *Sex Transm Dis*. 2012;39(1):1-7. doi:10.1097/OLQ.0b013e31823b1922.

110. Cingolani A, Zona S, Girardi E, et al. Incidence and factors associated with the risk of sexually transmitted diseases in HIV-infected people seen for care in Italy: data from the Icona Foundation cohort. *HIV Med*. 2015;16(7):412-420. doi:10.1111/hiv.12226.

111. Mayer KH, O'Cleirigh C, Skeer M, et al. Which HIV-infected men who have sex with men in care are engaging in risky sex and acquiring sexually transmitted infections: findings from a Boston community health centre. *Sex Transm Infect*. 2010;86(1):66-70. doi:10.1136/sti.2009.036608.

112. Firnhaber C, Van Le H, Pettifor A, et al. Association between cervical dysplasia and human papillomavirus in HIV seropositive women from Johannesburg South Africa. *Cancer Causes Control*. 2010;21(3):433-443. doi:10.1007/s10552-009-9475-z.

113. Levi JE, Fernandes S, Tateno AF, et al. Presence of multiple human papillomavirus types in cervical samples from HIV-infected women. *Gynecol Oncol*. 2004;92(1):225-231. http://www.ncbi.nlm.nih.gov/pubmed/14751163. Accessed January 16, 2018.

114. Mbulawa ZZA, Marais DJ, Johnson LF, Boulle A, Coetzee D, Williamson AL. Influence of human immunodeficiency virus and CD4 count on the prevalence of human papillomavirus in heterosexual couples. *J Gen Virol*. 2010;91(12):3023-3031. doi:10.1099/vir.0.020669-0.

115. Aar F Van, Mooij SH, Van Der Sande MAB, et al. Anal and penile high-risk human papillomavirus prevalence in HIV-negative and HIV-infected MSM. *Aids*. 2013;27(18):2921-2931. doi:10.1097/01.aids.0000432541.67409.3c.

116. Leng CY, Low HC, Chua LL, et al. Human papillomavirus 16 (HPV16) and HPV52 E6-specific immunity in HIV-infected adults on combination antiretroviral therapy. *HIV Med*.

2017;18(5):321-331. doi:10.1111/hiv.12432.

117. Cadogan M, Dalgleish AG. Chapter 9 HIV Induced AIDS and Related Cancers: Chronic Immune Activation and Future Therapeutic Strategies. *Adv Cancer Res*. 2008;101:349-395. doi:10.1016/S0065-230X(08)00409-0.