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Long-term nitrite inhalant exposure and cancer risk in MSM

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Objectives: Nitrite inhalants (poppers) are commonly used recreational drugs among MSM and were previously associated with elevated rates of high-risk sexual behavior, HIV and human herpesvirus type 8 (HHV-8) seroconversion, and transient immunosuppressive effects in experimental models. Whether long-term popper use is associated with cancer risk among MSM in the HAART era is unclear.

Design: Prospective cohort study of cancer risk in 3223 HIV-infected and uninfected MSM in the Multicenter AIDS Cohort Study from 1996–2010.

Methods: Poisson regression models were used to examine the association between heavy popper use (defined as daily or weekly use for at least 1 year) and risk of individual cancers or composite category of virus-associated cancers.

Results: Among all participants, heavy popper use was not associated with increased risk of any individual cancers. Among HIV-uninfected men aged 50–70, heavy popper use was associated with increased risk of virus-associated cancer with causes linked to human papillomavirus, HHV-8, and Epstein–Barr virus in models adjusted for demographics, number of sexual partners, immunological parameters ($CD4^+$ cell counts or $CD4^+/CD8^+$ ratios), and hepatitis B and C viruses [incidence rate ratio (IRR), 95% confidence interval (CI) 3.24, 1.05–9.96], or sexually transmitted infections (IRR 3.03, 95% CI, 1.01–9.09), as was cumulative use over a 5-year period (IRR 1.012, 95% CI 1.003–1.021; $P = 0.007$). There was no significant association between heavy popper use and virus-associated cancer in HIV-infected men.

Conclusions: Long-term heavy popper use is associated with elevated risk of some virus-associated cancers with causes related to human papillomavirus, HHV-8, and Epstein–Barr virus infections in older HIV-uninfected MSM independent of sexual behavior and immunological parameters.

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Introduction

The rising incidence of non-AIDS-defining malignancies (non-ADMs) among HIV-infected individuals in the HAART era is not fully explained by improvement in HAART regimens or traditional risk factors such as immunodeficiency, aging, and smoking [1–3]. Identifying modifiable behavioral factors affecting cancer risk among persons living with HIV or AIDS (PLWHA) could help to explain this rise in cancer burden and guide the development of prevention strategies targeting these factors. The number of HIV-infected people over the age

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of 50 is increasing, and this population is at higher risk for many types of cancer compared with the general population, particularly those with causes related to infection by oncogenic viruses such as human papillomavirus (HPV), human herpesvirus type 8 (HHV-8), Epstein–Barr virus (EBV), and hepatitis B and C viruses (HBV/HCV) [4–6]. However, risk estimates for non-ADMs vary considerably among HIV-infected cohorts [4], reflecting differences in demographics, lifestyle, ascertainment, competing risks, and other factors.

MSM are overrepresented among PLWHA, yet they continue to be disadvantaged by health disparities. Cancer burden among HIV-infected MSM is frequently examined in cohorts of mixed sexual orientation and sex [7,8]. A limitation of these studies is that some risk factors more specific to MSM may be overlooked. HIV-infected MSM have a greater burden of virus-associated non-ADMs in the HAART era compared with the general population, particularly those caused by viruses that are sexually transmitted such as HPV and HHV-8 [9]. These virus-associated cancers often have a worse prognosis among HIV-infected compared with uninfected populations [10,11] and remain a leading cause of mortality among PLWHA. Studies focused on MSM are needed to improve the understanding of health disparities related to cancer risk, screening, and outcomes.

Recreational drug use is common among MSM who engage in high-risk sexual behavior [12–14]. One of the most commonly used recreational drugs is nitrite inhalants (amyl nitrite, butyl nitrite, isobutyl nitrite; commonly referred to as 'poppers') [15,16]. Previous studies have reported significant associations between popper use and higher rates of high-risk sexual behavior and more frequent acquisition of HIV [12,14,15,17–19]. Despite their potential for harmful effects at high doses [15,16,20–22], popper use remains prevalent among MSM around the world, ranging from 20–40% among MSM cohorts in the United States, Canada, United Kingdom, and China [12,14,19,23–26]. Unlike stimulants and other recreational drugs that correlate with high-risk behavior among MSM, poppers have transient immunosuppressive effects in animal models, particularly at high doses with frequent exposure [21,27–29]. Poppers gained notoriety early in the AIDS epidemic as a potential cause of AIDS and Kaposi sarcoma [16,30], but these notions were later debunked [31,32]. Thereafter, poppers were associated with increased risk of Kaposi sarcoma in some pre-HAART cohorts [33–35], although other studies did not find this association [23,36]. In subsequent studies, popper use was associated with risk of HHV-8 seroconversion or anal cancer precursor lesions among HIV-uninfected MSM [37–39]. Together, these findings raise the possibility that long-term popper use may be associated with increased risk of some virus-associated cancers because of associated high-risk behavior, transient immunosuppressive effects, or a combination of both factors. Here, we investigated the association between heavy popper use and cancer risk in a prospective cohort study of HIV-infected and uninfected MSM with shared demographic and behavioral traits as an internal comparison.

Methods

Study cohort

The study is a nested prospective study in the Multicenter AIDS Cohort Study (MACS), an ongoing prospective study of MSM. The MACS was established in 1984, enrolling 6972 HIV-infected and HIV-uninfected MSM over three recruitment waves [1984–85 ($n = 4957$), 1987–91 ($n = 665$), and 2001–03 ($n = 1350$)] across four study sites (Los Angeles, Chicago, Baltimore, and Pittsburgh). Behavioral, clinical, and laboratory data were collected at semiannual visits as described [40,41]. Eligible participants were 3223 HIV-infected and HIV-uninfected men over the age of 18 with at least one study visit during 1996–2010. Study participants who seroconverted after 1996 were excluded ($n = 143$). Institutional Review Boards at each study site approved the research and all participants provided written informed consent.

Data collection and risk factor classification

The MACS public data set release 23 (p23) was translated to a local SQL database and used for the analyses. Nitrite inhalant (popper) use was the primary exposure of interest. Self-reported popper use, recorded at semiannual visits, was used to characterize longitudinal patterns of use. Study participants reporting daily or weekly popper use for at least 1 year of study enrollment were classified as heavy users; light use (monthly or less) or no use comprised the control group. Cumulative exposure was defined as a continuous variable (mean days of use per year over first 5 years following enrollment). Polydrug use was defined by at least weekly amphetamines, cocaine, crack, or heroin use for at least 1 year during follow-up. The number of male sexual partners and sexually transmitted infections (syphilis and genital warts) was summarized over the first 3 visits following enrollment to avoid bias resulting from longer follow-up. Early HAART (1996–2000) and late HAART (2001–2010) eras were treated as time-varying covariates. Antiretroviral therapy use, mean viral load, and mean $CD4^+$ or $CD8^+$ cell counts and $CD4^+/CD8^+$ ratios were summarized as time-varying covariates using data from the last two visits because recent values are more likely to impact cancer risk.

Cancer outcomes

A total of 327 fatal and nonfatal incident cancers occurring during the study period were classified using International Classification of Diseases for Oncology, third edition (ICD-O-3) codes. Incident cancers

(excluding basal cell carcinoma and benign cancers) were ascertained continuously during follow-up using cancer registry linkage data, available medical records and death certificates, and self-reported cancer diagnoses [40]. Cancers were classified into 10 categories based on body sites and histology [40]. A subset of analyses employed a composite category, 'virus-associated cancers', comprised of diagnoses associated with infection by the oncogenic viruses EBV [non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma], HHV-8 (Kaposi sarcoma), and HPV (anal cancer). Liver cancer and squamous cell carcinoma of the skin, which are associated with HBV/HCV or certain oncogenic subtypes of HPV, respectively, were not included in this composite category because of low HBV/HCV prevalence and confounding by ultraviolet exposure, respectively. ICD-O-3 site codes for anal cancers were 21.0–21.8 and 19.9–20.9 based on a prior study in the MACS cohort [41]. ICD-O-3 site codes for oropharyngeal squamous cell carcinoma were 2.9, 4.9, and 10.2. AIDS-defining malignancies were Kaposi sarcoma and NHL, whereas other cancers were non-ADMs.

Statistical analysis

Incidence rates per 100 000 person-years were calculated by dividing the total number of incident cancers by cumulative years of follow-up. Follow-up was defined by last visit, death, or first instance of the incident cancer. Study participants with multiple cancer diagnoses were evaluated for each cancer individually [40] and those with multiple reports of the same type of cancer at different body sites were counted only for the first instance. A diagnosis of the incident cancer before 1996 was left censored. Poisson regression models adjusted for age, race, and calendar period (early vs. late HAART era) were used to examine the association of risk factors with the 10 most common cancers. In models stratified by HIV-infection status, interaction between heavy popper use and age was tested for significance; interaction between heavy popper use and HIV-infection status was also tested in unstratified models. Analyses restricted to HIV-infected study participants were adjusted for age, race, heavy popper use, calendar period, and time-varying $CD4^+$ cell count (<200 vs. ≥ 200 cells/μl) and plasma viral load (>400 vs. $\leq$ 400 HIV RNA copies/ml). Categorical and cumulative exposure models were stratified by HIV-infection status and age, adjusted for race, calendar period, and number of sexual partners, and sequentially adjusted for immunological parameters ($CD4^+$ cell counts or $CD4^+/CD8^+$ ratios), HBV/HCV, sexually transmitted infections (syphilis or genital warts), smoking, and polydrug use. Study participants with missing values for time-varying $CD4^+$ cell count or viral load (approximately 10% of HIV-infected study participants) were excluded from adjusted analyses. Analyses of skin cancers were restricted to whites ($n = 2108$) because of limited outcomes in non-whites. Lung cancer incidence was evaluated only in study participants who reported smoking on at least one study visit ($n = 2426$). Statistical analysis was performed in R version 3.2.1 (R project for Statistical Computing, Vienna, Austria).

Results

Cohort characteristics of groups by HIV infection and popper use

We identified a cohort of 3223 men (HIV-uninfected, $n = 1660$; and HIV-infected, $n = 1563$) enrolled from 1996–2010, contributing 28 849 person-years with median follow-up of 11 (IQR 7–14) and 8 (IQR 5–14) person-years, respectively (Table 1). Heavy popper users (20.3 and 33.0% of HIV-uninfected and HIV-infected study participants, respectively) were slightly older at entry, with more accrued follow-up, sexual partners, and sexually transmitted infections (syphilis and genital warts) and less non-whites and HBV/HCV coinfected study participants compared with controls of the same HIV status. Heavy popper users reported significant exposure over time, though longitudinal patterns of use varied between individual study participants (Supplemental Digital Content 1, http://links.lww.com/QAD/B60). Heavy popper users reported more daily marijuana use (20.3 vs. 10.9% and 23.4 vs. 16.0% for HIV-uninfected and HIV-infected popper users vs. controls, respectively) and weekly or more amphetamine use (2.9 vs. 1.5% and 8.4 vs. 2.8% for HIV-uninfected and HIV-infected popper users vs. controls, respectively). Heavy popper users and respective control groups had similar time-varying $CD4^+$ and $CD8^+$ cell counts, $CD4^+/CD8^+$ ratios, percentage with viral load more than 400 copies/ml (at or above the limit of detection of the available test at some earlier visits), and antiretroviral therapy use (Table 1) and similar mean $CD4^+$ cell counts across calendar years 1996–2010 (Supplemental Digital Content 2, http://links.lww.com/QAD/B60). The number of enrolled study participants increased in 2002–2003 because of the last recruitment wave, which focused on enrolling more racial/ethnic minority men.

Crude incidence rates of virus-associated and nonvirus-associated cancers

A total of 327 incident cancers were identified among 296 participants: 269 with a single cancer diagnosis, 24 with two cancer diagnoses, and three participants with three or more cancer diagnoses. The analysis of cancer risk focused on categories with at least 10 incident cancers. Crude incidence rates of NHL, squamous cell carcinoma of the skin, prostate cancer, and other cancers were higher in heavy popper users than controls among both HIV-infected and uninfected study participants (Table 2). The crude incidence rate of anal cancer was higher among heavy popper users only in HIV-uninfected study

Table 1. Demographic and clinical characteristics of groups by HIV status and popper use.

	HIV-negative			HIV-positive[a]		
	All ($n=1660$)	None or light popper use ($n=1322$)	Heavy popper use ($n=338$)	All ($n=1563$)	None or light popper use ($n=1175$)	Heavy popper use ($n=388$)
Cumulative person-years						
Median (IQR)	11 (7–14)	8 (7–14)	14 (8–14)	8 (5–14)	8 (4–13)	11 (5–14)
Age at entry visit						
Median (IQR)	43 (38–49)	43 (37–49)	45 (40–51)	41 (36–46)	40 (36–45)	43 (38–48)
Race/ethnicity *n* (%)						
White	1207 (72.7)	902 (68.2)	305 (90.2)	914 (58.5)	588 (50)	326 (84)
Black/African-American	312 (18.8)	294 (22.2)	18 (5.3)	438 (28)	406 (34.6)	32 (8.2)
Other	141 (8.5)	126 (9.5)	15 (4.4)	211 (13.5)	181 (15.4)	30 (7.7)
Smoking[b] *n* (%)						
No	1195 (72)	960 (72.6)	235 (69.5)	987 (63.1)	736 (62.6)	251 (64.7)
Yes	465 (28)	362 (27.4)	103 (30.5)	576 (36.9)	439 (37.4)	137 (35.3)
Sexual partners at first three visits[c]						
≥10 partners	894 (53.9)	639 (48.3)	255 (75.4)	790 (50.5)	497 (42.3)	293 (75.5)
Sexually transmitted infections[c]						
Syphilis	189 (11.4)	134 (10.1)	55 (16.3)	427 (27.3)	313 (26.6)	114 (29.4)
Genital warts	359 (21.6)	249 (18.9)	110 (32.5)	495 (31.7)	339 (28.9)	156 (40.2)
Hepatitis C infection[d] *n* (%)	103 (6.2)	95 (7.2)	8 (2.4)	193 (12.3)	167 (14.2)	26 (6.7)
Hepatitis B infection[d] *n* (%)	71 (4.3)	54 (4.1)	17 (5)	145 (9.3)	111 (9.4)	34 (8.8)
$CD4^+$ cell count (cells/μl)[f] *n* (%)						
<200		3 (0.2)	0 (0)		175 (15.9)	62 (17.8)
200–349		13 (1.0)	2 (0.6)		183 (16.6)	51 (14.6)
350–499		49 (3.7)	14 (4.1)		215 (19.5)	77 (22.1)
≥500		1256 (95.1)	322 (95.3)		531 (48.1)	158 (45.4)
$CD4^+$ + nadir <200 (cells/μl)[d] *n* (%)		13 (0.9)	13 (3.8)		520 (44.2)	202 (52)
$CD8^+$ cell count median (IQR)[e]		493 (356–675)	543.5 (397–697)		823 (565–1097)	788 (561–1143)
$CD8^+$ cell count <300 (cells/μl)[e]		208 (15.7)	37 (10.9)		49 (4.4)	24 (6.9)
$CD4^+/CD8^+$ ratio <0.5[e]		7 (0.5)	0 (0)		474 (42.9)	151 (43.9)
$CD4^+/CD8^+$ ratio <1[e]		101 (7.6)	33 (9.8)		911 (82.5)	293 (84.2)
Viral load >400 copies/ml[e] *n* (%)					356 (33.3)	94 (27.8)
ART use[e] *n* (%)					891 (76.7)	322 (84.1)
AIDS diagnosis[f]					282 (24)	139 (35.8)

ART, antiretroviral therapy; IQR, interquartile range.
[a]Seroconversion 1996 or earlier.
[b]Smoking half pack per day or more for at least 1 year within study.
[c]Evaluated over first three visits following enrollment.
[d]Any time following enrollment to study endpoint.
[e]Time-updated values (based on mean value at last two visits if available). Group percentages calculated for study participants with data.
[f]At baseline visit.

participants, whereas the crude incidence rate of melanoma was higher among heavy popper users only in HIV-infected study participants. Overall, a trend of increased rates of virus-related cancer was observed among heavy popper users compared with respective control groups. As expected, we observed increased rates of Kaposi sarcoma, NHL, anal cancer, Hodgkin lymphoma, liver cancer, and lung cancer among HIV-infected compared with HIV-uninfected study participants.

Heavy popper use and risk of individual cancers

Heavy popper use was associated with significantly elevated risk of NHL (IRR, 1.98; 95% CI, 1.1–3.57) and marginally elevated risk of squamous cell carcinoma of the skin (IRR, 1.54; 95% CI, 0.9–2.63) only in unadjusted models (Table 3). Heavy popper use was not associated with risk of any incident cancers in adjusted models. By contrast, HIV-infection was associated with increased risk of Kaposi sarcoma (IRR, 29.66; 95% CI, 7.07–124.44), NHL (IRR, 5.63; 95% CI, 2.73–11.59), anal cancer (IRR, 6.79; 95% CI, 2.75–16.78), squamous cell skin cancer (IRR, 1.91; 95% CI, 1.11–3.3), and liver cancer (IRR, 4.21; 95% CI, 1.03–17.28) and showed marginally significant association with risk of lung cancer in adjusted analysis (IRR, 2.89; 95% CI, 0.94–8.92); the association between HIV infection status and risk of prostate cancer or melanoma was null. All cancers except Kaposi sarcoma and melanoma had significantly higher incidence rates in study participants aged 50–60 and older than 60 compared with those aged less than 50.

Heavy popper use and risk of virus-associated cancer in HIV-infected study participants

Next, we evaluated the most prevalent virus-associated cancers in analyses of only HIV-infected study participants (Table 4). Heavy popper use showed borderline association with NHL (IRR, 1.91; 95% CI, 0.98–3.73) only in unadjusted analysis; the association was non-significant in models adjusted for age, race, $CD4^+$ cell

Table 2. Incidence rates of cancers by HIV status and popper use.

	HIV-negative						HIV-positive					
	All		None or light popper use		Heavy popper use		All		None or light popper use		Heavy popper use	
	No.	IR	No.	IR	No.	IR	No.	IR	No.	IR	No.	IR
AIDS-defining malignancies												
Kaposi sarcoma	2	12.2 (1.5–44.2)	1	8 (0.2–44.8)	1	25.7 (0.6–143.2)	39	299.7 (213.1–409.7)	28	301.4 (200.2–435.5)	11	295.6 (147.6–528.9)
Non-Hodgkin lymphoma	10	61.3 (29.4–112.8)	7	56.4 (22.7–116.2)	3	77 (15.9–224.9)	36	266.8 (186.9–369.4)	20	208.7 (127.5–322.3)	16	409.6 (234.1–665.2)
Non-AIDS-defining malignancies												
Adenocarcinoma of colon	3	18.4 (3.8–53.7)	1	8.1 (0.2–44.9)	2	51.3 (6.2–185.3)	3	22.1 (4.6–64.5)	2	20.8 (2.5–75)	1	25.3 (0.6–140.8)
Anal cancers	6	36.8 (13.5–80)	3	24.1 (5–70.6)	3	77 (15.9–225.1)	26	207.3 (137.7–299.6)	20	219.3 (135.7–335.2)	6	178 (71.6–366.8)
Hodgkin lymphoma	2	12.2 (1.5–44.2)	2	16.1 (1.9–58.1)	0		4	29.5 (8–75.5)	3	31.2 (6.4–91.3)	1	25.2 (0.6–140.6)
Lung[a]	5	42.6 (13.8–99.5)	3	34.1 (7–99.7)	2	68 (8.2–245.7)	9	87.7 (40.1–166.4)	7	95.1 (38.2–195.9)	2	68.8 (8.3–248.7)
Prostate	32	197.7 (135.2–279.1)	23	186.3 (118.1–279.6)	9	234.3 (107.1–444.8)	19	140.3 (84.5–219.1)	11	114.8 (57.3–205.4)	8	202 (87.2–398.1)
Squamous cell carcinoma, skin[b]	28	217 (144.2–313.6)	16	170.1 (97.2–276.2)	12	343.2 (177.4–599.6)	27	312.9 (206.2–455.3)	16	303.3 (173.4–492.5)	11	328.1 (163.8–587)
Melanoma[b]	18	139.4 (82.6–220.4)	14	149.1 (81.5–250.1)	4	113.7 (31–291)	7	79.9 (32.1–164.7)	2	37.4 (4.5–135)	5	146.7 (47.6–342.4)
Liver	3	18.4 (3.8–53.7)	3	24.1 (5–70.5)	0		7	51.4 (20.7–106)	6	62.2 (22.8–135.4)	1	25.2 (0.6–140.5)
Other cancers[c]	17	104.2 (60.7–166.9)	11	88.7 (44.3–158.8)	6	153.5 (56.3–334.1)	23	169.3 (107.3–254)	14	145.2 (79.4–243.6)	9	228.2 (104.4–433.2)

Number of incident cancers and IR represented as incidence rate per 100 000 person-years. Number of study participants in each group is same as shown in Table 1. IR, incidence rates.
[a]Calculated using study participants that reported smoking during any time following enrollment, $n = 2426$.
[b]No incident cancers were observed in non-white study participants; analysis restricted to white study participants.
[c]Denotes composite category of less frequently occurring cancers: esophageal, stomach, and pancreatic adenocarcinoma ($n = 7$), bladder cancer ($n = 6$), and 20 other types of cancer, all observed three times or less.

Table 3. Univariate and multivariate analysis of risk factors associated with common cancers.

	Virus-associated cancers										Non-virus-associated cancers					
	Kaposi sarcoma		Non-Hodgkin lymphoma		Anal cancer		Squamous cell carcinoma, skin		Liver cancer		Lung cancer		Prostate cancer		Melanoma	
Covariates	IRR (95% CI)	*P*	IRR (95% CI)	*P*	IRR (95% CI)	*P*	IRR (95% CI)	*P*	IRR (95% CI)	*P*	IRR (95% CI)	*P*	IRR (95% CI)	*P*	IRR (95% CI)	*P*
Univariate models																
HIV serostatus																
HIV-positive	24.48 (5.91, 101.38)	<0.001	4.35 (2.16, 8.77)	<0.001	5.64 (2.34, 13.62)	<0.001	1.44 (0.85, 2.45)	0.174	2.8 (0.72, 10.83)	0.136	2.06 (0.69, 6.14)	0.196	0.71 (0.4, 1.25)	0.237	0.57 (0.24, 1.37)	0.212
HIV-negative	Reference		Reference		Reference		Reference		Reference		Reference		Reference		Reference	
Popper use																
Heavy popper use	1.18 (0.6, 2.32)	0.627	1.98 (1.1, 3.57)	0.022	1.17 (0.56, 2.45)	0.674	1.54 (0.9, 2.63)	0.113	0.31 (0.04, 2.46)	0.269	1.11 (0.35, 3.53)	0.865	1.41 (0.79, 2.52)	0.252	1.2 (0.53, 2.71)	0.665
Light or none	Reference		Reference		Reference		Reference		Reference		Reference		Reference		Reference	
Age (years)																
50–60	0.65 (0.31, 1.34)	0.241	1.54 (0.81, 2.93)	0.191	2.21 (1.01, 4.8)	0.046	3.49 (1.56, 7.85)	0.002	3.23 (0.59, 17.62)	0.176	5.69 (1.18, 27.4)	0.03	5.13 (2.05, 12.85)	<0.001	1.42 (0.55, 3.68)	0.471
>60	0.99 (0.41, 2.4)	0.975	1.99 (0.9, 4.4)	0.089	3.05 (1.23, 7.59)	0.016	9.39 (4.23, 20.81)	<0.001	8.34 (1.53, 45.53)	0.014	10.32 (2, 53.2)	0.005	18.81 (7.74, 45.69)	<0.001	2.99 (1.12, 7.96)	0.029
<50	Reference		Reference		Reference		Reference		Reference		Reference		Reference		Reference	
HAART era[a]																
Early HAART	2.7 (1.46, 4.98)	<0.001	3.33 (1.86, 5.96)	<0.001	0.34 (0.12, 0.97)	0.043	0.3 (0.14, 0.66)	0.003	0.29 (0.04, 2.25)	0.233	0.44 (0.1, 1.98)	0.286	0.41 (0.18, 0.9)	0.026	0.51 (0.19, 1.36)	0.180
Late HAART	Reference		Reference		Reference		Reference		Reference		Reference		Reference		Reference	
Multivariate model																
HIV-positive	29.66 (7.07, 124.44)	<0.001	5.63 (2.73, 11.59)	<0.001	6.79 (2.75, 16.78)	<0.001	1.91 (1.11, 3.3)	0.02	4.21 (1.03, 17.28)	0.046	2.89 (0.94, 8.92)	0.065	0.96 (0.53, 1.75)	0.904	0.63 (0.26, 1.55)	0.314
Heavy popper use	0.84 (0.42, 1.67)	0.614	1.38 (0.75, 2.53)	0.299	1 (0.46, 2.16)	0.994	1.3 (0.76, 2.25)	0.34	0.25 (0.03, 2.02)	0.193	0.86 (0.26, 2.85)	0.803	1.3 (0.71, 2.38)	0.389	1.25 (0.55, 2.85)	0.600
Age (years)																
50–60	1.04 (0.49, 2.21)	0.924	2.39 (1.22, 4.66)	0.011	2.52 (1.14, 5.61)	0.023	3.17 (1.39, 7.23)	0.006	4.17 (0.75, 23.33)	0.104	6.51 (1.32, 32.17)	0.022	5.56 (2.19, 14.12)	<0.001	1.22 (0.46, 3.22)	0.694
>60	2.7 (1.06, 6.85)	0.037	4.76 (2.05, 11.08)	<0.001	4.77 (1.83, 12.47)	0.001	8.96 (3.9, 20.58)	<0.001	14.71 (2.46, 87.89)	0.003	14.3 (2.56, 80.04)	0.002	21.3 (8.4, 54.03)	<0.001	2.32 (0.83, 6.5)	0.110
Early HAART era	3.17 (1.66, 6.08)	<0.001	4.5 (2.43, 8.35)	<0.001	0.49 (0.17, 1.44)	0.194	0.48 (0.21, 1.09)	0.08	0.54 (0.07, 4.45)	0.567	0.73 (0.16, 3.38)	0.689	0.72 (0.32, 1.61)	0.421	0.6 (0.25, 1.44)	0.254

CI, confidence interval; IRR, incidence rate ratio.
Multivariable models were adjusted for age, race, HIV status, popper use, and HAART era.
[a]Early HAART era (1996–2000), late HAART era (2001–2010).

Table 4. Univariate and multivariate analysis of risk factors associated with virus-associated cancers in HIV-positive study participants.

	Kaposi sarcoma		Non-Hodgkin lymphoma		Anal cancer		Squamous cell carcinoma, skin	
Covariates	IRR (95% CI)	*P*	IRR (95% CI)	*P*	IRR (95% CI)	*P*	IRR (95% CI)	*P*
Univariate models								
Popper use								
Heavy popper use	1.24 (0.6, 2.55)	0.554	1.91 (0.98, 3.73)	0.058	0.84 (0.36, 1.97)	0.685	1.07 (0.48, 2.39)	0.863
Light or none	Reference		Reference		Reference		Reference	
Age (years)								
50–60	0.96 (0.46, 2.03)	0.921	1.62 (0.79, 3.34)	0.189	2.31 (1.02, 5.22)	0.045	3.52 (1.22, 10.14)	0.02
>60	0.81 (0.19, 3.44)	0.775	2.7 (1, 7.33)	0.051	4.14 (1.44, 11.92)	0.008	11.2 (3.75, 33.41)	<0.001
<50	Reference		Reference		Reference		Reference	
Viral load (copies/ml)								
>400	11.35 (5.3, 24.32)	<0.001	19.41 (8.06, 46.76)	<0.001	1.88 (0.85, 4.15)	0.12	4.16 (1.89, 9.16)	<0.001
≤400	Reference		Reference		Reference		Reference	
$CD4^+$ cell count (cells/μl)								
<200	8.27 (4.2, 16.28)	<0.001	17.72 (8.93, 35.17)	<0.001	3.5 (1.49, 8.24)	0.004	3.89 (1.55, 9.75)	0.004
≥200	Reference		Reference		Reference		Reference	
HAART era[a]								
Early HAART	3.33 (1.7, 6.52)	<0.001	4.94 (2.49, 9.81)	<0.001	0.48 (0.17, 1.39)	0.177	0.81 (0.34, 1.93)	0.629
Late HAART	Reference		Reference		Reference		Reference	
Multivariate model								
Heavy popper use	1.11 (0.52, 2.37)	0.78	1.38 (0.69, 2.78)	0.366	0.74 (0.3, 1.82)	0.515	0.97 (0.43, 2.17)	0.935
Age (years)								
50–60	1.55 (0.72, 3.38)	0.265	2.9 (1.37, 6.12)	0.005	2.54 (1.09, 5.92)	0.031	4.63 (1.55, 13.82)	0.006
>60	1.95 (0.44, 8.62)	0.38	9.16 (3.23, 26.03)	<0.001	4.87 (1.6, 14.78)	0.005	16.87 (5.31, 53.58)	<0.001
Viral load >400 (copies/ml)	8.3 (3.54, 19.46)	<0.001	11.92 (4.46, 31.88)	<0.001	1.49 (0.57, 3.88)	0.416	4.63 (1.9, 11.27)	0.001
$CD4^+$ <200 cells/μl)	2.83 (1.33, 5.99)	0.007	5.31 (2.46, 11.46)	<0.001	3.75 (1.36, 10.36)	0.011	2.32 (0.82, 6.51)	0.111
Early HAART era	2.46 (1.18, 5.14)	0.017	3.57 (1.67, 7.6)	0.001	0.53 (0.18, 1.6)	0.263	1.05 (0.41, 2.69)	0.921

CI, confidence interval; IRR, incidence rate ratio.
Multivariable models were adjusted for age, race, HIV status, popper use, CD4+ cell counts (cells/μl), viral load (copies/ml), and HAART era.
[a]Early HAART era (1996–2000), late HAART era (2001–2010).

count, viral load, and HAART era. Heavy popper use showed no significant association with risk of Kaposi sarcoma, anal cancer, or squamous cell carcinoma of the skin. By contrast, low $CD4^+$ cell count (<200 cells/μl) was associated with increased risk of Kaposi sarcoma (IRR, 2.83; 95% CI, 1.33–5.99), NHL (IRR, 5.31; 95% CI, 2.46–11.46), and anal cancer (IRR, 3.75; 95% CI, 1.36–10.36), whereas viral load (>400 copies/ml) was associated with increased risk of Kaposi sarcoma (IRR, 8.3; 95% CI, 3.54–9.46), NHL (IRR, 11.92; 95% CI, 4.46–31.88), and squamous cell carcinoma of the skin (IRR, 4.63; 95% CI, 1.9–11.27). Older age was associated with risk of NHL, anal cancer, and squamous cell skin cancer, but not Kaposi sarcoma. These analyses restricted to HIV-infected study participants showed no significant associations of popper use with virus-associated cancers in adjusted models.

Heavy popper use and risk of virus-associated cancer in HIV-uninfected study participants

Next, we evaluated the association between heavy popper use and risk of virus-associated cancers in HIV-uninfected study participants. Given low numbers of individual virus-associated cancers (Table 2), statistical power was not sufficient to investigate individual cancers. Therefore, we used a composite category of virus-associated cancer comprised Kaposi sarcoma, NHL, Hodgkin lymphoma, and anal cancer. The interaction between older age and heavy popper use was significant in adjusted models evaluating the composite of virus-associated cancer in HIV-uninfected study participants ($P = 0.041$). Furthermore, the burden of virus-associated cancer as a proportion of all incident cancer was higher in heavy popper users vs. controls among older HIV-uninfected study participants (43.75 vs. 18.75% incident cancers for heavy users vs. controls aged 56–70, respectively; $P = 0.066$, χ^2 test). This association in older HIV-uninfected study participants was further evaluated in Poisson regression models with sequential adjustment for $CD4^+$ cell counts, HBV/HCV, sexually transmitted infections, smoking, and polydrug use. Heavy popper use was associated with increased risk of the composite of virus-associated cancers among HIV-uninfected study participants aged 50–70 years in models adjusted for

Table 5. Univariate and multivariate analysis of risk factors associated with virus-associated cancer[a] in older HIV-uninfected study participants aged 50–70.

	Categorical exposure models		Cumulative exposure models	
Covariates	IRR (95% CI)	*P*	IRR (95% CI)	*P*
Univariate models				
Popper use[b]	2.30 (0.83, 6.34)	0.108	1.011 (1.002, 1.020)	0.014
≥10 sexual partners[c,d]	0.71 (0.26, 1.96)	0.507	—	
HBV/HCV infection[e]	2.33 (0.66, 8.25)	0.191	—	
Sexually transmitted infections[d]				
Syphilis	1.25 (0.35, 4.43)	0.731	—	
Genital warts	0.99 (0.31, 3.10)	0.980	—	
Smoking [f,g]	1.07 (0.24, 4.70)	0.931	—	
Polydrug use[h]	0.97 (0.13, 7.38)	0.976	—	
$CD4^+$ cell count[g] <500 cells/μl	2.93 (0.66, 13.11)	0.159	—	
$CD4^+/CD8^+$ ratio[g] <1	2.20 (0.61, 7.87)	0.227	—	
Multivariate models				
Model 1				
Popper use	2.98 (1.00, 8.84)	0.049	1.012 (1.003, 1.021)	0.008
≥10 sexual partners	0.64 (0.22, 1.89)	0.420	0.824 (0.287, 2.363)	0.719
Model 1 + $CD4^+$				
Popper use	3.04 (1.01, 9.12)	0.047	1.012 (1.003, 1.021)	0.007
Model 1 + $CD4^+$ + HBV/HCV				
Popper use	3.24 (1.05, 9.96)	0.041	1.013 (1.004, 1.022)	0.004
Model 1 + $CD4^+$ + syphilis				
Popper use	3.03 (1.01, 9.09)	0.048	1.012 (1.003, 1.021)	0.007
Model 1 + $CD4^+$ + genital warts				
Popper use	3.02 (1.00, 9.12)	0.050	1.012 (1.003, 1.021)	0.009
Model 1 + $CD4^+$ + smoking				
Popper use	3.04 (1.01, 9.13)	0.048	1.012 (1.003, 1.022)	0.009
Model 1 + $CD4^+$ + polydrug use				
Popper use	3.04 (1.02, 9.13)	0.047	1.012 (1.003, 1.021)	0.007

CI, confidence interval; IRR, incidence rate ratio.
Multivariable models were adjusted for race. HBV, hepatitis B virus; HCV, hepatitis C virus.
[a]Virus-associated cancer is a composite category of cancers with causes linked to EBV (NHL, $n = 8$), HPV (anal cancer, $n = 8$), and HHV-8 (Kaposi sarcoma, $n = 2$). One study participant with prior thyroid cancer diagnosis was censored from analysis.
[b]Categorical models compared heavy vs. light or no use. Cumulative exposure models evaluated a continuous variable (mean days of use per year over first 5 years following enrollment).
[c]vs. less than 10 sexual partners.
[d]Evaluated over first three visits following enrollment.
[e]Any time following enrollment to study endpoint.
[f]Smoking half a pack or more on average during follow-up.
[h]Polydrug use included at least weekly amphetamines, cocaine, crack, or heroin use for at least 1 year during follow-up.
[g]Time-updated values.

demographics, number of sexual partners, and $CD4^+$ cell counts (IRR, 3.04; 95% CI, 1.01–9.12); further adjustment for HBV/HCV, sexually transmitted infections, smoking, and polydrug use did not attenuate this association (Table 5). Models adjusted for $CD4^+/CD8^+$ ratios in place of $CD4^+$ cell counts gave similar results (IRRs 2.98–3.13; 95% CIs, 1.02–9.58, 0.99–8.9, and 0.99–9.03 for models with further adjustments for HBV/HCV, syphilis, and genital warts, respectively). The association of cumulative poppers exposure with virus-associated cancer was evaluated in adjusted models using a continuous variable. Increasing cumulative exposure was significantly associated with increased risk of virus-associated cancer among older HIV-uninfected study participants (IRRs 1.012–1.013, 95% CI 1.003–1.022, per day of use per year over the first 5 years following enrollment; Table 5). Sensitivity analyses excluding last recruitment wave individuals ($n = 324$) did not attenuate these associations (IRRs 3.83 and 1.013, 95% CIs 1.84–12.53 and 1.004–1.022 for categorical and cumulative exposure models, respectively, adjusted as in Model 1+CD4). In contrast to HIV-uninfected men, we found no significant association between heavy popper use and risk of virus-associated cancer in older HIV-infected study participants in categorical and cumulative exposure models (Supplemental Digital Content 3, http://links.lww.com/QAD/B61).

Discussion

In this prospective study of HIV-infected and uninfected MSM in the HAART era, the association between heavy popper use and risk of any individual cancer was null. However, heavy popper use was independently associated with increased risk of virus-associated cancers with causes linked to HPV, HHV-8, and EBV among older HIV-uninfected men aged 50–70 in categorical and cumulative exposure models adjusted for number of sexual partners, $CD4^+$ cell counts or $CD4^+/CD8^+$ ratios, HBV/HCV, sexually transmitted infections, smoking, and polydrug use. Frequent popper use was associated with high-risk sexual behavior, as previously described [12,14,15,17–19], thereby increasing probability of exposure to HPV and HHV-8. However, we did not detect a significant association between number of sexual partners or sexually transmitted infections and risk of virus-associated cancer in HIV-uninfected study participants. Previous studies demonstrated transient immunosuppressive effects of poppers, which may increase efficiency of transmission and replication of some oncogenic viruses, especially when popper exposure occurs at high doses [16,21,27,28,42]. Given these findings, it remains unclear whether the excess risk of virus-associated cancer associated with popper use is related to more sexually transmitted viral infections, transient immunosuppression, or other mechanisms.

A previous study reported increased risk of anal cancer precursor lesions in MSM with recent popper use at any level [37]. Here, we provide further evidence that popper use, in particular daily or weekly use for one year or longer, is associated with risk of virus-related cancer in HIV-uninfected MSM and this risk becomes more significant over the age of 50. The early cohort enrolled in 1984–85 had more older men with heavy popper use, but sensitivity analyses excluding the last cohort enrolled in 2001–2003 did not attenuate the association of popper use with risk of virus-related cancer in older HIV-uninfected men. Thus, our main findings are influenced by age and HIV serostatus, but not confounded by calendar period.

Contrary to expectations, we did not find an association between heavy popper use and risk of virus-associated cancer among HIV-infected study participants. The most likely explanation for this lack of effect of poppers in HIV-infected study participants is the stronger effect of HIV-related immunodeficiency on risk of virus-associated cancer compared with any effects of popper use. Furthermore, HHV-8 and high-risk HPV seroprevalence is likely to be higher among HIV-infected compared with HIV-uninfected study participants, irrespective of popper use. HIV infection was associated with increased risk of two HPV-associated cancers, anal cancer and squamous cell carcinoma of the skin. Ultraviolet radiation exposure is the major risk factor for squamous cell carcinoma of the skin in the general population, but emerging evidence implicates oncogenic HPV subtypes as an etiologic agent or cofactor [43,44]. While low time-updated $CD4^+$ cell count was associated with risk of anal cancer in HIV-infected individuals, consistent with [45,46], time-updated viral load (> 400 copies/ml) was associated with risk of squamous cell carcinoma of the skin. The association of recent viral load with squamous cell carcinoma of the skin contrasts with findings of an earlier study [47], but is consistent with Silverberg *et al.* [48].

Several studies reported reduced risk of prostate cancer among HIV-infected men compared with age-matched controls [49,50]. In contrast, we found similar incidence rates of prostate cancer in HIV-infected men vs. controls with similar demographic and behavioral traits. These conflicting results for prostate cancer incidence rates by HIV status may be explained by: lower prevalence of AIDS in our study compared with earlier studies; HAART era study period and adjustments for early vs. late HAART era in our study, which reduces competing risks related to immunodeficiency; and differences in screening rates and ascertainment bias. Similar to findings in the general population, prostate cancer risk had high racial disparity, with two to three-fold higher risk among African-Americans [50].

Limitations of this study are similar to other cohort studies of this scale [40,41] in that statistical power was limited by

the number of cases. We did not have a sufficient number of individual virus-associated cancers to evaluate their associations with popper use in HIV-uninfected study participants, so it remains possible that an association could have been missed. Ascertainment bias may be another factor contributing to imprecise estimates of cancer risk. Heavy popper users tend to have more years of education and may also have more engagement with medical care, whereas HIV-negative MSM may have less ascertainment of anal cancer compared with HIV-positive MSM. Polydrug use, which is more frequent among heavy popper users, may also contribute to imprecise estimates of cancer risk. However, adjustment for polydrug use did not attenuate the association of popper use with risk of virus-associated cancer in HIV-uninfected men. A previous study reported increased risk of anal cancer precursor lesions with recent popper use at any level [37]; we detected higher crude incidence rates for anal cancer in HIV-uninfected men with heavy popper use vs. controls, but no significant association in adjusted analyses. The low number of cases and under-ascertainment of anal cancers may contribute to these discrepant findings. Last, we lacked direct evidence supporting the viral origin of virus-related cancers, some NHL subtypes are not virus-related, and HHV-8 or EBV virological markers were not available in the MACS public data analyzed herein. Despite these limitations, we identified several significant associations in univariate and multivariate analyses that warrant further investigation in larger cohorts.

In conclusion, we evaluated the association between long-term heavy popper use and cancer risk in a longitudinal cohort of MSM using a nested prospective study design that integrated clinical, laboratory, and behavioral data. Although heavy popper use was not associated with increased risk of any individual cancers among all men, daily or weekly popper use for at least 1 year and increasing cumulative exposure were associated with elevated overall risk of virus-associated cancers with causes linked to HPV, HHV-8, and EBV in HIV-uninfected MSM aged 50–70 years. These findings suggest that prevention strategies targeting popper use may reduce the burden of these virus-associated cancers in MSM.

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Conflicts of interest

There are no conflicts of interest.

References

1. Deeken JF, Tjen ALA, Rudek MA, Okuliar C, Young M, Little RF, Dezube BJ. **The rising challenge of non-AIDS-defining cancers in HIV-infected patients.** *Clin Infect Dis* 2012; **55**:1228–1235.
2. Rubinstein PG, Aboulafia DM, Zloza A. **Malignancies in HIV/AIDS: from epidemiology to therapeutic challenges.** *AIDS* 2014; **28**:453–465.
3. Yanik EL, Katki HA, Engels EA. **Cancer Risk among the HIV-infected elderly in the United States.** *AIDS* 2016; **30**:1663–1668.
4. Dubrow R, Silverberg MJ, Park LS, Crothers K, Justice AC. **HIV infection, aging, and immune function: implications for cancer risk and prevention.** *Curr Opin Oncol* 2012; **24**:506–516.
5. Robbins HA, Pfeiffer RM, Shiels MS, Li J, Hall HI, Engels EA. **Excess cancers among HIV-infected people in the United States.** *J Natl Cancer Inst* 2015; **107**:.
6. Silverberg MJ, Chao C, Leyden WA, Xu L, Tang B, Horberg MA, *et al*. **HIV infection and the risk of cancers with and without a known infectious cause.** *AIDS* 2009; **23**:2337–2345.
7. Guiguet M, Boue F, Cadranel J, Lang JM, Rosenthal E, Costagliola D, Clinical Epidemiology Group of the FHDH-ANRS CO4 cohort. **Effect of immunodeficiency, HIV viral load, and antiretroviral therapy on the risk of individual malignancies (FHDH-ANRS CO4): a prospective cohort study.** *Lancet Oncol* 2009; **10**:1152–1159.
8. Simard EP, Pfeiffer RM, Engels EA. **Cumulative incidence of cancer among individuals with acquired immunodeficiency syndrome in the United States.** *Cancer* 2011; **117**:1089–1096.
9. Boehmer U, Cooley TP, Clark MA. **Cancer and men who have sex with men: a systematic review.** *Lancet Oncol* 2012; **13**:e545–e553.
10. Coghill AE, Shiels MS, Suneja G, Engels EA. **Elevated cancer-specific mortality among HIV-infected patients in the United States.** *J Clin Oncol* 2015; **33**:2376–2383.
11. Rabkin CS. **AIDS and cancer in the era of highly active antiretroviral therapy (HAART).** *Eur J Cancer* 2001; **37**:1316–1319.
12. Colfax G, Coates TJ, Husnik MJ, Huang Y, Buchbinder S, Koblin B, *et al*., EXPLORE Study Team. **Longitudinal patterns of methamphetamine, popper (amyl nitrite), and cocaine use and high-risk sexual behavior among a cohort of san francisco men who have sex with men.** *J Urban Health* 2005; **82**:i62–i70.
13. Colfax G, Guzman R. **Club drugs and HIV infection: a review.** *Clin Infect Dis* 2006; **42**:1463–1469.
14. Daskalopoulou M, Rodger A, Phillips AN, Sherr L, Speakman A, Collins S, *et al*. **Recreational drug use, polydrug use, and sexual behaviour in HIV-diagnosed men who have sex with men in the UK: results from the cross-sectional ASTRA study.** *Lancet HIV* 2014; **1**:e22–e31.
15. Romanelli F, Smith KM, Thornton AC, Pomeroy C. **Poppers: epidemiology and clinical management of inhaled nitrite abuse.** *Pharmacotherapy* 2004; **24**:69–78.
16. Haverkos HW, Kopstein AN, Wilson H, Drotman P. **Nitrite inhalants: history, epidemiology, and possible links to AIDS.** *Environ Health Perspect* 1994; **102**:858–861.
17. Plankey MW, Ostrow DG, Stall R, Cox C, Li X, Peck JA, Jacobson LP. **The relationship between methamphetamine and popper use and risk of HIV seroconversion in the multicenter AIDS cohort study.** *J Acquir Immune Defic Syndr* 2007; **45**:85–92.
18. Ostrow DG, Plankey MW, Cox C, Li X, Shoptaw S, Jacobson LP, *et al*. **Specific sex drug combinations contribute to the majority of recent HIV seroconversions among MSM in the MACS.** *J Acquir Immune Defic Syndr* 2009; **51**:349–355.
19. Buchbinder SP, Vittinghoff E, Heagerty PJ, Celum CL, Seage GR 3rd, Judson FN, *et al*. **Sexual risk, nitrite inhalant use, and lack of circumcision associated with HIV seroconversion in men who have sex with men in the United States.** *J Acquir Immune Defic Syndr* 2005; **39**:82–89.
20. Soderberg LS, Barnett JB. **Possible mechanisms of immunotoxicity following in vivo exposure to the inhalant, isobutyl nitrite.** *Adv Exp Med Biol* 1995; **373**:189–192.
21. Guo GL, Rose D, Flick JT, Barnett JB, Soderberg LS. **Acute exposure to the abused inhalant, isobutyl nitrite, reduced T cell responsiveness and spleen cellularity.** *Toxicol Lett* 2000; **116**:151–158.
22. Soderberg LS. **Increased tumor growth in mice exposed to inhaled isobutyl nitrite.** *Toxicol Lett* 1999; **104**:35–41.
23. Chao C, Jacobson LP, Jenkins FJ, Tashkin D, Martinez-Maza O, Roth MD, *et al*. **Recreational drug use and risk of Kaposi's sarcoma in HIV- and HHV-8-coinfected homosexual men.** *AIDS Res Hum Retroviruses* 2009; **25**:149–156.
24. Li D, Yang X, Zhang Z, Qi X, Ruan Y, Jia Y, *et al*. **Nitrite inhalants use and HIV infection among men who have sex with men in China.** *Biomed Res Int* 2014; **2014**:365261.
25. Lampinen TM, Mattheis K, Chan K, Hogg RS. **Nitrite inhalant use among young gay and bisexual men in Vancouver during a period of increasing HIV incidence.** *BMC Public Health* 2007; **7**:35.
26. Shoptaw S, Stall R, Bordon J, Kao U, Cox C, Li X, *et al*. **Cumulative exposure to stimulants and immune function outcomes among HIV-positive and HIV-negative men in the Multicenter AIDS Cohort Study.** *Int J STD AIDS* 2012; **23**:576–580.
27. Soderberg LS, Barnett JB. **Exposure to the abused inhalant, isobutyl nitrite, compromises both antibody and cell-mediated immunity.** *Adv Exp Med Biol* 1993; **335**:265–268.

28. Soderberg LS. **Immunomodulation by nitrite inhalants may predispose abusers to AIDS and Kaposi's sarcoma.** *J Neuroimmunol* 1998; **83**:157–161.
29. Newell GR, Adams SC, Mansell PW, Hersh EM. **Toxicity, immunosuppressive effects and carcinogenic potential of volatile nitrites: possible relationship to Kaposi's sarcoma.** *Pharmacotherapy* 1984; **4**:284–291.
30. Haverkos HW. **Nitrite inhalant abuse and AIDS-related Kaposi's sarcoma.** *J Acquir Immune Defic Syndr* 1990; **3 (Suppl 1)**:S47–S50.
31. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. **Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma.** *Science* 1994; **266**:1865–1869.
32. Schechter MT, Craib KJ, Gelmon KA, Montaner JS, Le TN, O'Shaughnessy MV. **HIV-1 and the aetiology of AIDS.** *Lancet* 1993; **341**:658–659.
33. Haverkos HW, Drotman DP. **Measuring inhalant nitrite exposure in gay men: implications for elucidating the etiology of AIDS-related Kaposi's sarcoma.** *Genetica* 1995; **95**:157–164.
34. Archibald CP, Schechter MT, Craib KJ, Le TN, Douglas B, Willoughby B, *et al*. **Risk factors for Kaposi's sarcoma in the Vancouver Lymphadenopathy-AIDS Study.** *J Acquir Immune Defic Syndr* 1990; **3 (Suppl 1)**:S18–S23.
35. Armenian HK, Hoover DR, Rubb S, Metz S, Kaslow R, Visscher B, *et al*. **Composite risk score for Kaposi's sarcoma based on a case-control and longitudinal study in the Multicenter AIDS Cohort Study (MACS) population.** *Am J Epidemiol* 1993; **138**:256–265.
36. Lifson AR, Darrow WW, Hessol NA, O'Malley PM, Barnhart JL, Jaffe HW, *et al*. Rutherford GW. **Kaposi's sarcoma in a cohort of homosexual and bisexual men. Epidemiology and analysis for cofactors.** *Am J Epidemiol* 1990; **131**:221–231.
37. Chin-Hong PV, Vittinghoff E, Cranston RD, Browne L, Buchbinder S, Colfax G, *et al*. **Age-related prevalence of anal cancer precursors in homosexual men: the EXPLORE study.** *J Natl Cancer Inst* 2005; **97**:896–905.
38. Casper C, Wald A, Pauk J, Tabet SR, Corey L, Celum CL. **Correlates of prevalent and incident Kaposi's sarcoma-associated herpesvirus infection in men who have sex with men.** *J Infect Dis* 2002; **185**:990–993.
39. Pauk J, Huang ML, Brodie SJ, Wald A, Koelle DM, Schacker T, *et al*. **Mucosal shedding of human herpesvirus 8 in men.** *N Engl J Med* 2000; **343**:1369–1377.
40. Seaberg EC, Wiley D, Martinez-Maza O, Chmiel JS, Kingsley L, Tang Y, *et al*., Multicenter AIDS Cohort Study (MACS). **Cancer incidence in the multicenter AIDS Cohort Study before and during the HAART era: 1984 to 2007.** *Cancer* 2010; **116**:5507–5516.
41. D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, *et al*. Jacobson LP. **Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study.** *J Acquir Immune Defic Syndr* 2008; **48**:491–499.
42. Dax EM, Adler WH, Nagel JE, Lange WR, Jaffe JH. **Amyl nitrite alters human in vitro immune function.** *Immunopharmacol Immunotoxicol* 1991; **13**:577–587.
43. Connolly K, Manders P, Earls P, Epstein RJ. **Papillomavirus-associated squamous skin cancers following transplant immunosuppression: one notch closer to control.** *Cancer Treat Rev* 2014; **40**:205–214.
44. Harwood CA, McGregor JM, Proby CM, Breuer J. **Human papillomavirus and the development of non-melanoma skin cancer.** *J Clin Pathol* 1999; **52**:249–253.
45. Silverberg MJ, Neuhaus J, Bower M, Gey D, Hatzakis A, Henry K, *et al*. **Risk of cancers during interrupted antiretroviral therapy in the SMART study.** *AIDS* 2007; **21**:1957–1963.
46. Engels EA, Biggar RJ, Hall HI, Cross H, Crutchfield A, Finch JL, *et al*. **Cancer risk in people infected with human immunodeficiency virus in the United States.** *Int J Cancer* 2008; **123**:187–194.
47. Crum-Cianflone N, Hullsiek KH, Satter E, Marconi V, Weintrob A, Ganesan A, *et al*. **Cutaneous malignancies among HIV-infected persons.** *Arch Intern Med* 2009; **169**:1130–1138.
48. Silverberg MJ, Leyden W, Warton EM, Quesenberry CP Jr, Engels EA, Asgari MM. **HIV infection status, immunodeficiency, and the incidence of non-melanoma skin cancer.** *J Natl Cancer Inst* 2013; **105**:350–360.
49. Silverberg MJ, Chao C, Leyden WA, Xu L, Horberg MA, Klein D, *et al*. **HIV infection, immunodeficiency, viral replication, and the risk of cancer.** *Cancer Epidemiol Biomarkers Prev* 2011; **20**:2551–2559.
50. Marcus JL, Chao CR, Leyden WA, Xu L, Klein DB, Horberg MA, *et al*. **Prostate cancer incidence and prostate-specific antigen testing among HIV-positive and HIV-negative men.** *J Acquir Immune Defic Syndr* 2014; **66**:495–502.