Published in final edited form as:

AIDS. 2016 June 19; 30(10): 1629–1637. doi:10.1097/QAD.0000000000001053.

Is Nelfinavir Exposure Associated with Cancer Incidence in HIV-positive individuals?

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Abstract

Objective—Nelfinavir exhibits potent anti-cancer properties against a range of tumours. However, in 2006/07, nelfinavir supplies were accidentally contaminated with a carcinogen. This analysis investigated the association between nelfinavir use and cancer risk in HIV-positive persons.

Design—Observational cohort study.

Methods—D:A:D study data was analyzed using Poisson regression models to examine associations between cancer incidence and cumulative nelfinavir exposure, current nelfinavir exposure, and exposure to nelfinavir between 1/Jul/2006–30/Jun/2007.

Results—A total of 42,006 individuals (50% white, 73% male) contributed 303,005 person-years of follow-up between 1/Jan/2004–1/Feb/2014. At study enrolment, median age was 40 (interquartile range [IQR] 33–46) years and 8,305 individuals had a history of nelfinavir use (median duration 1.7 [IQR 0.7–3.4] years). During follow-up, nelfinavir was used by 2,476 individuals for a median of 1.7 (IQR 0.7–3.8) years; 1,063 were exposed to nelfinavir between 1/Jul/2006–30/Jun/2007. Overall, 2,279 cancers were diagnosed at a rate of 0.75 (95% confidence interval [95% CI] 0.72–0.78) per 100 person-years. Neither greater cumulative exposure to

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Conflicts of Interest:

The authors declare no conflicts of interest.

Table, Supplemental Digital Content 1.doc

Table, Supplemental Digital Content 2.doc

nelfinavir (adjusted risk ratio [aRR] 0.93 for every additional 5 years, 95%CI 0.82–1.06, $p=0.26$) nor current use of nelfinavir (aRR 0.98 vs. other protease inhibitor use, 95%CI 0.68–1.41, $p=0.92$) were associated with cancer risk. The adjusted risk of cancer for participants exposed to nelfinavir between 1/Jul/2006–30/Jun/2007 compared to those receiving other treatment over this period was 1.07 (95%CI 0.78–1.46, $p=0.68$).

Conclusions—Nelfinavir use was not associated with a lower cancer incidence than other protease inhibitor regimens. As of Feb/2014, exposure to the 2006/07 contamination of nelfinavir does not appear to be associated with increased cancer incidence.

Keywords

HIV; Nelfinavir; Cancer; Ethyl mesylate; Kaposi's sarcoma

Introduction

Protease inhibitors (PIs) were designed specifically to inhibit HIV protease, yet serendipitously have been found to have broad antineoplastic and antiviral activity.^[1,2] In particular, nelfinavir exhibits anti-cancer properties (e.g. inhibition of Akt signalling, induction of cancer cell autophagy and apoptosis, proteasome inhibition) against a range of cancers including myeloma, breast cancer, ovarian cancer, liposarcoma, melanoma, non-small cell lung cancer, diffuse large B-cell lymphoma, glioma, hepatocellular carcinoma, and prostate cancer.^[3–15] It has also been shown to suppress Kaposi's sarcoma-associated herpesvirus replication *in vitro*.^[16] Since the approval of nelfinavir as an antiretroviral in 1997, more efficacious alternatives with fewer adverse effects have taken over the role of PIs in antiretroviral therapy (ART). However, clinical trials are currently being undertaken to investigate whether nelfinavir could be repositioned as an anti-cancer agent.^[17–26]

All ART, by increasing CD4 cell count, substantially reduces the risk of AIDS- and non-AIDS-defining cancer in HIV-positive individuals.^[27–29] Portsmouth *et al* (2003) reported that the incidence of Kaposi's sarcoma in the HIV-positive population decreased from 30/1000 person-years before 1995 (the pre-ART era) to 0.03/1000 person-years in 2001 and that non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART had a similar protective effect to PI-based ART.^[29] However, the relative efficacy of nelfinavir-based ART compared to other ART in preventing cancer has only been clinically evaluated in one small study of HIV-positive individuals which found no difference between regimens.^[30] The potentially protective effect of nelfinavir against Kaposi's sarcoma and other specific cancers has not been assessed in any HIV cohort.

In June 2007, Viracept® (nelfinavir mesylate) was subject to an international recall due to a manufacturing fault that led to the product containing high levels of ethyl mesylate, a genotoxic substance that has been associated with increased rates of lung, kidney, brain, liver, breast and uterine cancer in animal models.^[31] The contamination arose from Roche's manufacturing plant in Switzerland. All nelfinavir users living outside of the USA, Canada and Japan (where nelfinavir supplies were not manufactured by Roche) at the time were considered to be at risk of exposure. In the worst case scenario, up to 25,000 people may have consumed contaminated nelfinavir between late 2006 and early 2007. The most

severely contaminated batches were distributed between March 2007 and June 2007 and individuals may have taken highly contaminated nelfinavir for up to three months.^[32–34] Following a review of toxicology studies,^[31,35–45] the European Medicines Agency concluded that the contamination did not increase the risk of developing cancer and that further follow-up of affected individuals was not required.^[33] Nevertheless, long-term clinical data supporting this decision have not been reported.

The objective of this analysis was to evaluate the potential of nelfinavir as a cancer preventative in HIV-positive persons enrolled in a very large cohort study, and to assess the rates of cancer in individuals exposed to nelfinavir between late 2006 and early 2007.

Methods

The Data collection on Adverse events of Anti-HIV Drugs (D:A:D) Study has been described in detail elsewhere.^[46–49] Briefly, it is a prospective study formed by the collaboration of eleven cohorts in Europe, Australia, and the United States. All participating cohorts in D:A:D followed local national guidelines/regulations regarding patient consent and/or ethical review. Information on all AIDS events, including all new AIDS-defining cancers (Kaposi's sarcoma, non-Hodgkin's lymphoma, and cervical cancer), has been provided prospectively on an annual basis since the start of the study in 1999. Since 2008, information has also been collected on non-AIDS-defining cancers (other than basal or squamous cell skin cancer, pre-cancers, and relapses). All participating cohorts have been collecting information prospectively on non-AIDS-defining cancers from 2008 or earlier; information was also collected retrospectively on events occurring between 1 January 2004 and 31 January 2008. Detailed information on each cancer is collected on a specific case report form. All reported events are validated centrally at the D:A:D coordinating center with a proportion of events selected for discussion with an external consultant oncologist. All events are regularly monitored for accuracy, and random monitoring is performed at participating centers to ensure complete ascertainment of events.

D:A:D participants were followed from the latest of 1 January 2004 or D:A:D study entry (baseline), until the earliest of a first incident cancer diagnosis, 1 February 2014, death, or six months after the last visit. As in previous analyses of the data set, each individual's follow-up was split into a series of consecutive one month periods and his/her age and clinical status (including ART use) at the start of each period was established. At the time of study entry, individuals may have already been exposed to ART, with exposure then accruing over follow-up.

Endpoints

We performed analyses on all cancer, AIDS-defining cancer, non-AIDS-defining cancer, and non-infection-related cancer diagnoses. Non-infection-related cancers were considered those not normally associated with an infectious agent. These included multiple myeloma, leukemia, melanoma, and cancers of the lung, brain, kidney, testes, breast, colon, rectum, prostate, bladder, bone, body of uterus, lip, pancreas, gall bladder, oesophagus, or connective tissue. Some cancer codes in the D:A:D database cannot be clearly identified as non-infection-related (for example, head and neck cancer) and were therefore not included in this

list. This was because the aim of investigating non-infection-related cancers was to assess the anti-cancer potential of nelfinavir where restoration of immune function was less protective. Several of the more frequently occurring cancer types were also considered separately (non-Hodgkin's lymphoma, Kaposi's sarcoma, lung cancer, anal cancer). In each analysis, follow-up of participants diagnosed with a cancer other than the one of interest was right censored at the time of cancer diagnosis to avoid any bias that might be introduced through more frequent subsequent monitoring for cancer.

Statistical analysis

Poisson regression models were used to assess associations between the incidence of cancer and: 1) cumulative exposure to nelfinavir; 2) current exposure to nelfinavir; and 3) exposure to nelfinavir between 1 July 2006 and 30 June 2007 (defined as the risk period for exposure to contaminated nelfinavir). A patient's cumulative exposure to therapy at the start of each one month follow-up period was calculated (including the duration of treatment before D:A:D enrollment and during follow-up) and the result used to assign the patient-month (and any endpoint events that occurred during that month) to the appropriate exposure category. Patients were considered to be currently using a regimen when it was their prescribed ART at the beginning of a patient-month. The effect of nelfinavir exposure between 1 July 2006 and 30 June 2007 and cancer incidence was only evaluated amongst individuals who had contributed some follow-up time on ART over that period so as to limit the influence of calendar year and different ART use on our results. We were not able to specifically identify individuals that were exposed to contaminated nelfinavir.

Treatment covariates were time-updated, so an individual's treatment status could change over time; reported adjusted rate ratio (aRR) estimates for cumulative ART exposure were scaled to reflect the impact of each additional five years of ART exposure on the outcome thereby enhancing their clinical relevance. Each model compared exposure to ART regimens (categorized as: nelfinavir-based-ART; non-nelfinavir, PI-based ART; NNRTI-based ART; other ART; and no ART), either as cumulative exposure or current exposure. Models were additionally adjusted for gender, mode of HIV acquisition, cancer diagnosis prior to baseline (all as fixed covariates), age (as a continuous, time-updated covariate), hepatitis B surface antigen status and hepatitis C antibody status (as time-updated covariates). All analyses were performed using SAS[®], version 9.3 (SAS Institute Inc, Cary, NC).

Sensitivity analyses

Because of the potential role of CD4 count and HIV viral load as confounders for initiation of ART and factors on the causal pathway between the initiation of ART and cancer development, our primary analyses did not include adjustment for these variables. Interpretation of rate ratios is impeded by the selection bias introduced in such analyses.^[50] Nevertheless, sensitivity analyses considered whether our conclusions regarding cumulative and current nelfinavir use were modified by adjusting for the latest (time updated) CD4 count and HIV RNA level. We also investigated the effect of adjusting for calendar year.

Results

A total of 42,006 participants (from the nine D:A:D cohorts that provide data on both AIDS- and non-AIDS-defining cancers) were included, accounting for 303,005 person-years of follow-up. Participant characteristics are described in Table 1. At baseline, 8,305 individuals had a history of nelfinavir exposure and the median (interquartile range [IQR]) duration of past exposure was 1.7 (0.7–3.4) years. Over the course of follow-up, 2,476 individuals contributed a median of 1.7 (IQR 0.7–3.8) years of nelfinavir exposure. Loss-to-follow-up rates were between 2–3% for each calendar year included in the analysis.

Overall, 2,279 cancers were diagnosed at a rate of 0.75 (95% confidence interval [CI] 0.72–0.78) per 100 person-years; 810 were AIDS-defining (0.27 [0.25–0.29]), 1469 were non-AIDS-defining (0.48 [0.46–0.51]), 763 were non-infection-related (0.25 [0.23–0.27]), 377 were non-Hodgkin's lymphoma (0.12 [0.11–0.14]), 375 were Kaposi's sarcoma (0.12 [0.11–0.14]), 252 were lung cancer (0.08 [0.07–0.09]), and 162 were anal cancer (0.05 [0.05–0.06]).

Association between cancer incidence and cumulative nelfinavir exposure

The incidences of each type of cancer stratified by cumulative exposure to different types of ART are shown in Table, Supplemental Digital Content 1. Table 2 shows the adjusted associations between increasing cumulative exposure to ART (per additional five years) and the eight cancer categories/cancers analyzed. Greater cumulative exposure to nelfinavir was not associated with a significant change in overall cancer incidence but was associated with an elevated risk of non-AIDS-defining cancer (aRR 1.19 per additional five years, 95% CI 1.03–1.37, $p=0.02$). All regimens, including nelfinavir-based ART, were associated with a significant reduction in AIDS-defining cancer, non-Hodgkin's lymphoma, and Kaposi's sarcoma (see Table 2). While increased exposure to non-nelfinavir, PI-ART was associated with a higher risk of anal cancer (aRR 1.40 per additional five years, 95% CI 1.16–1.71, $p<0.01$), no such association was found for nelfinavir-based ART. Rates of non-infection-related cancer and lung cancer were unchanged by greater exposure to any category of ART.

Association between cancer incidence and current nelfinavir exposure

The incidences of each type of cancer stratified by current exposure to different types of ART are shown in Table, Supplemental Digital Content 2. The adjusted associations between current ART exposure and cancer incidences are shown in Table 3. Rates of cancer were non-significantly different between individuals receiving nelfinavir-based treatment and other PI-based ART. The incidences of non-infection-related cancer and lung cancer were not associated with current ART regimen. However, rates of all cancer, AIDS-defining cancer, non-AIDS-defining cancer, non-Hodgkin's lymphoma, Kaposi's sarcoma, and anal cancer were lower in individuals using NNRTI-based ART compared with those using non-nelfinavir, PI-ART (see Table 3). Not using ART was associated with a significantly higher risk of all cancer, AIDS-defining cancer, non-Hodgkin's lymphoma, and Kaposi's sarcoma, and a lower risk of non-AIDS-defining cancer and anal cancer (see Table 3).

Association between cancer incidence and nelfinavir exposure between 1st July 2006 and 30th June 2007

Overall, 32,346 persons contributed at least one day of follow-up on ART between 1 July 2006 and 30 June 2007; of these, 7,743 had received nelfinavir at some point over follow-up. For the 1,063 individuals who were exposed to nelfinavir between 1 July 2006 and 30 June 2007, total follow-up time was 8,050 person-years. For the remaining 23,267 individuals under follow-up over the same period but who were using non-nelfinavir-based ART between 1 July 2006 and 30 June 2007, total follow-up time was 167,788 person-years. Overall, 1,061 cancers occurred (228 AIDS-defining, 833 non-AIDS-defining, 426 non-infection-related). Figure 1 shows the incidences of all, AIDS-defining, non-AIDS-defining, and non-infection-related cancers were similar amongst individuals exposed to nelfinavir in the risk period and other nelfinavir users. These comparisons remained similar when adjusted for important demographic and lifestyle variables and hepatitis status (as described in the Methods section; Table 4).

Sensitivity analyses

Our conclusions regarding the association between nelfinavir exposure and cancer incidence were unchanged in cumulative and current exposure models that additionally adjusted for the latest CD4 cell count and HIV RNA level, or for calendar year (results not shown).

Discussion

Despite strong evidence that nelfinavir exhibits potent anticancer properties,^[3–16] the only association we found between nelfinavir exposure (both increasing cumulative exposure and current exposure versus no ART) and reduced cancer incidence occurred with AIDS-defining cancers and is most likely explained by ART-induced immune reconstitution.^[51–55] We did not observe a specific cancer protective effect of nelfinavir use over and above that seen for other PIs. Additionally, our results indicate that the 2006/07 carcinogenic contamination of nelfinavir was not associated with an elevated rate of cancer amongst those most likely to have been exposed.

In an analysis of 2,499 HIV-positive people enrolled in a US cohort, a similar relationship between nelfinavir use and cancer incidence was found.^[30] While the authors suggested that their results may have been related to the need for a larger cohort to detect a specific protective effect of nelfinavir, our findings show that this is probably not the case. It cannot be ruled out, however, that a study of longer follow-up duration could reveal an association between nelfinavir use and the incidence of slow-progressing cancers. An alternative explanation might be that nelfinavir at doses used for HIV suppression are insufficient for cancer prevention. Interestingly, the maximum tolerated dose of nelfinavir was not determined during initial development. Clinical trials evaluating the efficacy of high dose nelfinavir in cancer treatment will soon provide further insight into optimal oncological dosing.^[17, 19, 25]

PIs induce apoptosis and inhibit nuclear factor κ B in Kaposi's sarcoma cell lines, ^[56] and also exhibit a host of anti-cancer properties in other cancer types.^[2] Despite the fact that

only nelfinavir displays inhibitory activity against Kaposi's sarcoma-associated herpesvirus, [16] we found that nelfinavir use had a similar effect on Kaposi's sarcoma incidence to other PI-based ART. Importantly, insufficient dosing is unlikely to explain this result as nelfinavir is active against Kaposi's sarcoma-associated herpesvirus at *in vitro* concentrations similar to those achieved in plasma with dosing used to treat HIV.[16]

Consistent with an earlier D:A:D analysis,[27] we found cumulative and current exposure to NNRTI-based ART was associated with a lower risk of both AIDS and non-AIDS-defining cancers compared with PI-based ART. Further discussion of these findings can be found in the original paper although the mechanism behind this observation remains unclear and does not appear to be associated with different rates of immune recovery with NNRTIs and PIs. [27] Adding to this dialog, we found cumulative exposure to nelfinavir-based ART appears to partially drive the positive association between PI-exposure and non-AIDS-defining cancer. However, the difference in cancer risk between NNRTI- and PI-based ART did not extend to non-infection-related cancers. Interestingly, the risk of both non-AIDS-defining cancer and anal cancer was lower in patients not currently using ART when compared with patients using a non-nelfinavir, PI-based regimen. The effect size of these associations were similar to those seen with NNRTI-based ART which suggests the increased risk of non-AIDS-defining cancer with PI-based ART compared to NNRTI-based ART may be related to an increased rate of cancer with PI exposure rather than a lower rate of cancer with NNRTI use.

The global recall of nelfinavir in June 2007 prompted a series of studies to investigate the extent of DNA damage induced by high doses of ethyl mesylate and the likely impact on cancer occurrence in humans. Animal studies were undertaken to establish a threshold dose beyond which DNA damage begins to accumulate.[36,37,57] This was estimated at 25 mg/kg/day. Following extrapolation of these data to humans, it was shown that even at an exposure 30- to 370-fold higher than that ingested by nelfinavir users (estimated at 0.055 mg/kg/day in the worst case scenario), the chromosomal damage incurred would still be effectively managed by cellular DNA repair mechanisms.[40-42] The European Medicines Agency was satisfied by this body of work and concluded that further follow-up of affected individuals was not required.[33] Although we were unable to specifically identify individuals exposed to contaminated nelfinavir in our analysis, our results provide the first clinical evidence supporting the abovementioned toxicology studies. Since the adjusted rate ratios reported are very close to one, it seems unlikely these results would be altered by a larger study. However, it cannot be discounted that a study with a maximum follow-up time greater than 7.5 years after contaminated nelfinavir exposure might reveal different results to ours.

As most cancer diagnoses result in hospitalization, information on which is generally passed back to D:A:D cohorts, we do not believe the cancer rates reported here are underestimated. Nevertheless, there were several limitations to this study that have not been mentioned above. Numerous factors may affect cancer risk, yet, to avoid overfitting our models, we only adjusted for age, gender, mode of HIV acquisition, hepatitis B surface antigen status, hepatitis C antibody status and history of cancer. The possibility that other potential confounding factors influenced our analysis cannot be ruled out. We were also hindered by

low event numbers for many cancer types. This prevented analysis of a number of specific cancers and meant we could not look at any individual cancer types in our analysis of persons exposed to nelfinavir between 1 July 2006 and 30 June 2007.

Nelfinavir use was not associated with reduced cancer incidence compared to other PI-based ART regimens. As of February 2014, exposure to the 2006/07 contamination of nelfinavir does not appear to be associated with increased cancer incidence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Support:

The Data on Adverse Events of Anti-HIV Drugs (D:A:D) study was supported by the Highly Active Antiretroviral Therapy Oversight Committee (HAARTOC), a collaborative committee with representation from academic institutions, the European Medicines Agency, the United States Food and Drug Administration, the patient community, and pharmaceutical companies with licensed anti-HIV drugs in the European Union: AbbVie, Bristol-Myers Squibb, Gilead Sciences Inc., ViiV Healthcare, Merck & Co Inc. and Janssen Pharmaceuticals. Supported also by a grant [grant number DNRF126] from the Danish National Research Foundation (CHIP & PERSIMUNE); by a grant from the Dutch Ministry of Health, Welfare and Sport (ATHENA); by a grant from the Agence nationale de recherches sur le sida et les hépatites virales [ANRS, Action Coordonnée no.7, Cohortes] to the Aquitaine Cohort; The Australian HIV Observational Database (AHOD) is funded as part of the Asia Pacific HIV Observational Database, a program of The Foundation for AIDS Research, amfAR, and is supported in part by a grant from the U.S. National Institutes of Health's National Institute of Allergy and Infectious Diseases (NIAID) [grant number U01-AI069907] and by unconditional grants from Merck Sharp & Dohme; Gilead Sciences; Bristol-Myers Squibb; Boehringer Ingelheim Roche; Pfizer; GlaxoSmithKline; Janssen Pharmaceuticals. The Kirby Institute is funded by The Australian Government Department of Health and Ageing, and is affiliated with the Faculty of Medicine, UNSW Australia; by grants from the Fondo de Investigación Sanitaria [grant number FIS 99/0887] and Fundación para la Investigación y la Prevención del SIDA en España [grant number FIPSE 3171/00], to the Barcelona Antiretroviral Surveillance Study (BASS); by the National Institute of Allergy and Infectious Diseases, National Institutes of Health [grants number 5U01AI042170-10, 5U01AI046362-03], to the Terry Bein Community Programs for Clinical Research on AIDS (CPCRA); by grants from the BIOMED 1 [grant number CT94-1637] and BIOMED 2 [grant number CT97-2713] programs and the 5th framework program [grant number QLK2-2000-00773], the 6th Framework (LSHP-CT-2006-018632), and the 7th Framework (FP7/2007-2013, EuroCoord n° 260694) programmes of the European Commission and unrestricted grants by Janssen R&D, Merck and Co. Inc., Pfizer Inc., GlaxoSmithKline LLC, (the participation of centres from Switzerland is supported by The Swiss National Science Foundation (Grant 108787)) to the EuroSIDA study; by unrestricted educational grants of AbbVie, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Pfizer, Janssen Pharmaceuticals to the Italian Cohort Naive to Antiretrovirals (The ICONA Foundation); and by a grant from the Swiss National Science Foundation (grant #148522) to the Swiss HIV Cohort Study (SHCS).

DCB conceived the idea and wrote the first draft of the manuscript. CS performed the statistical analysis. CS, AG, LR, FB, PR, AdAM, OK, AP, MB, GF and JDL provided critical input at all stages of the project. ML supervised the overall conduct of this project and provided critical inputs on the analysis and manuscript. The writing committee would like to acknowledge the D:A:D study participants and the D:A:D study Steering Committee (see Appendix 1).

References

1. Chow WA, Jiang C, Guan M. Anti-HIV drugs for cancer therapeutics: back to the future? *Lancet Oncol.* 2009; 10:61–71. [PubMed: 19111246]
2. Gantt S, Casper C, Ambinder RF. Insights into the broad cellular effects of nelfinavir and the HIV protease inhibitors supporting their role in cancer treatment and prevention. *Curr Opin Oncol.* 2013; 25:495–502. [PubMed: 23872785]
3. Gills JJ, Lopiccolo J, Tsurutani J, Shoemaker RH, Best CJ, Abu-Asab MS, et al. Nelfinavir, A lead HIV protease inhibitor, is a broad-spectrum, anticancer agent that induces endoplasmic reticulum

stress, autophagy, and apoptosis in vitro and in vivo. *Clin Cancer Res.* 2007; 13:5183–5194. [PubMed: 17785575]

4. Kraus M, Bader J, Overkleeft H, Driessen C. Nelfinavir augments proteasome inhibition by bortezomib in myeloma cells and overcomes bortezomib and carfilzomib resistance. *Blood Cancer J.* 2013; 3:e103. [PubMed: 23454896]
5. Shim JS, Rao R, Beebe K, Neckers L, Han I, Nahta R, et al. Selective inhibition of HER2-positive breast cancer cells by the HIV protease inhibitor nelfinavir. *J Natl Cancer Inst.* 2012; 104:1576–1590. [PubMed: 23042933]
6. Bono C, Karlin L, Harel S, Mouly E, Labaume S, Galicier L, et al. The human immunodeficiency virus-1 protease inhibitor nelfinavir impairs proteasome activity and inhibits the proliferation of multiple myeloma cells in vitro and in vivo. *Haematologica.* 2012; 97:1101–1109. [PubMed: 22271897]
7. Bruning A, Burger P, Vogel M, Rahmeh M, Gingelmaiers A, Friese K, et al. Nelfinavir induces the unfolded protein response in ovarian cancer cells, resulting in ER vacuolization, cell cycle retardation and apoptosis. *Cancer Biol Ther.* 2009; 8:226–232. [PubMed: 19106637]
8. Guan M, Fousek K, Jiang C, Guo S, Synold T, Xi B, et al. Nelfinavir induces liposarcoma apoptosis through inhibition of regulated intramembrane proteolysis of SREBP-1 and ATF6. *Clin Cancer Res.* 2011; 17:1796–1806. [PubMed: 21355074]
9. Gupta AK, Li B, Cerniglia GJ, Ahmed MS, Hahn SM, Maity A. The HIV protease inhibitor nelfinavir downregulates Akt phosphorylation by inhibiting proteasomal activity and inducing the unfolded protein response. *Neoplasia.* 2007; 9:271–278. [PubMed: 17460771]
10. Jiang W, Mikochik PJ, Ra JH, Lei H, Flaherty KT, Winkler JD, et al. HIV protease inhibitor nelfinavir inhibits growth of human melanoma cells by induction of cell cycle arrest. *Cancer Res.* 2007; 67:1221–1227. [PubMed: 17283158]
11. Kawabata S, Gills JJ, Mercado-Matos JR, Lopiccolo J, Wilson W 3rd, Hollander MC, et al. Synergistic effects of nelfinavir and bortezomib on proteotoxic death of NSCLC and multiple myeloma cells. *Cell Death Dis.* 2012; 3:e353. [PubMed: 22825471]
12. Petrich AM, Leshchenko V, Kuo PY, Xia B, Thirukonda VK, Ulahannan N, et al. Akt inhibitors MK-2206 and nelfinavir overcome mTOR inhibitor resistance in diffuse large B-cell lymphoma. *Clin Cancer Res.* 2012; 18:2534–2544. [PubMed: 22338016]
13. Pyrkó P, Kardosh A, Wang W, Xiong W, Schonthal AH, Chen TC. HIV-1 protease inhibitors nelfinavir and atazanavir induce malignant glioma death by triggering endoplasmic reticulum stress. *Cancer Res.* 2007; 67:10920–10928. [PubMed: 18006837]
14. Sun L, Niu L, Zhu X, Hao J, Wang P, Wang H. Antitumour effects of a protease inhibitor, nelfinavir, in hepatocellular carcinoma cancer cells. *J Chemother.* 2012; 24:161–166. [PubMed: 22759761]
15. Yang Y, Ikezoe T, Takeuchi T, Adachi Y, Ohtsuki Y, Takeuchi S, et al. HIV-1 protease inhibitor induces growth arrest and apoptosis of human prostate cancer LNCaP cells in vitro and in vivo in conjunction with blockade of androgen receptor STAT3 and AKT signaling. *Cancer Sci.* 2005; 96:425–433. [PubMed: 16053514]
16. Gantt S, Carlsson J, Ikoma M, Gachelet E, Gray M, Geballe AP, et al. The HIV protease inhibitor nelfinavir inhibits Kaposi's sarcoma-associated herpesvirus replication in vitro. *Antimicrob Agents Chemother.* 2011; 55:2696–2703. [PubMed: 21402841]
17. [[accessed: 15 May 2015]] Nelfinavir for the Treatment of Gammaherpesvirus-Related Tumors (NCT02080416). Available from: https://clinicaltrials.gov/ct2/show/NCT02080416?term=%22nelfinavir%22+and+%22cancer%22&recr=Open&no_unk=Y&rank=2
18. [[accessed: 15 May 2015]] Systemic Therapy and Chemoradiation in Advanced Localised Pancreatic Cancer-- 2 (SCALOP-2) (NCT02024009). Available from: https://clinicaltrials.gov/ct2/show/NCT02024009?term=%22nelfinavir%22+and+%22cancer%22&recr=Open&no_unk=Y&rank=3
19. [[accessed: 15 May 2015]] Nelfinavir and Lenalidomide/Dexamethasone in Progressive Multiple Myeloma (NCT01555281). Available from: https://clinicaltrials.gov/ct2/show/NCT01555281?term=%22nelfinavir%22+and+%22cancer%22&recr=Open&no_unk=Y&rank=4

20. [[accessed: 15 May 2015]] A Phase II Single-arm Intervention Trial of Nelfinavir in Patients With Grade 2/3 or 3 Cervical Intraepithelial Neoplasia (NCT01925378). Available from: https://clinicaltrials.gov/ct2/show/NCT01925378?term=%22nelfinavir%22+and+%22cancer%22&recr=Open&no_unk=Y&rank=5
21. [[accessed: 15 May 2015]] A Study of Nelfinavir Added to Cisplatin Chemotherapy Concurrent With Pelvic Radiation for Locally Advanced Cervical Cancer (II-IVA) (NCT02363829). Available from: https://clinicaltrials.gov/ct2/show/NCT02363829?term=%22nelfinavir%22+and+%22cancer%22&recr=Open&no_unk=Y&rank=8
22. [[accessed: 15 May 2015]] A Phase II Trial of a Protease Inhibitor, Nelfinavir (NFV), Given With Definitive, Concurrent Chemoradiotherapy (CTRT) in Patients With Locally-Advanced, Human Papilloma Virus (HPV) Negative, Squamous Cell Carcinoma Larynx (NCT02207439). Available from: https://clinicaltrials.gov/ct2/show/NCT02207439?term=%22nelfinavir%22+and+%22cancer%22&recr=Open&no_unk=Y&rank=6
23. Brunner TB, Geiger M, Grabenbauer GG, Lang-Welzenbach M, Mantoni TS, Cavallaro A, et al. Phase I trial of the human immunodeficiency virus protease inhibitor nelfinavir and chemoradiation for locally advanced pancreatic cancer. *J Clin Oncol*. 2008; 26:2699–2706. [PubMed: 18509182]
24. Dennis PA, Blumenthal G, Ballas M, Gardner E, Kawabata S, LoPiccolo J, et al. A phase I study of nelfinavir, an FDA approved HIV protease inhibitor, in adults with refractory solid tumors. *J Clin Oncol (Meeting Abstracts)*. 2009:2583.
25. Pan J, Mott M, Xi B, Hepner E, Guan M, Fousek K, et al. Phase I study of nelfinavir in liposarcoma. *Cancer Chemother Pharmacol*. 2012; 70:791–799. [PubMed: 22983015]
26. Rengan R, Mick R, Pryma D, Rosen MA, Lin LL, Maity AM, et al. A phase I trial of the HIV protease inhibitor nelfinavir with concurrent chemoradiotherapy for unresectable stage IIIA/IIIB non-small cell lung cancer: a report of toxicities and clinical response. *J Thorac Oncol*. 2012; 7:709–715. [PubMed: 22425919]
27. Bruyand M, Ryom L, Shepherd L, Fatkenheuer G, Grulich A, Reiss P, et al. Cancer risk and use of protease inhibitor or nonnucleoside reverse transcriptase inhibitor-based combination antiretroviral therapy: the D: A: D study. *J Acquir Immune Defic Syndr*. 2015; 68:568–577. [PubMed: 25763785]
28. Grabar S, Abraham B, Mahamat A, Del Giudice P, Rosenthal E, Costagliola D. Differential impact of combination antiretroviral therapy in preventing Kaposi's sarcoma with and without visceral involvement. *J Clin Oncol*. 2006; 24:3408–3414. [PubMed: 16849755]
29. Portsmouth S, Stebbing J, Gill J, Mandalia S, Bower M, Nelson M, et al. A comparison of regimens based on non-nucleoside reverse transcriptase inhibitors or protease inhibitors in preventing Kaposi's sarcoma. *AIDS*. 2003; 17:F17–F22. [PubMed: 12853764]
30. Crum-Cianflone NF, Hullsiek KH, Marconi V, Weintrob A, Ganesan A, Barthel RV, et al. The impact of nelfinavir exposure on cancer development among a large cohort of HIV-infected patients. *J Acquir Immune Defic Syndr*. 2009; 51:305–309. [PubMed: 19412116]
31. Gocke E, Burgin H, Muller L, Pfister T. Literature review on the genotoxicity, reproductive toxicity, and carcinogenicity of ethyl methanesulfonate. *Toxicol Lett*. 2009; 190:254–265. [PubMed: 19857796]
32. Lutz WK. The Viracept (nelfinavir)--ethyl methanesulfonate case: a threshold risk assessment for human exposure to a genotoxic drug contamination? *Toxicol Lett*. 2009; 190:239–242. [PubMed: 19695319]
33. European Medicines Agency. Questions and answers on the follow-up to the contamination of Viracept (nelfinavir) with ethyl mesilate. London: 2008 Jul 24.
34. Pozniak A, Muller L, Salgo M, Jones JK, Larson P, Tweats D. Elevated ethyl methanesulfonate (EMS) in nelfinavir mesylate (Viracept, Roche): overview. *AIDS Res Ther*. 2009; 6:18. [PubMed: 19660105]
35. Gerber C, Toelle HG. What happened: the chemistry side of the incident with EMS contamination in Viracept tablets. *Toxicol Lett*. 2009; 190:248–253. [PubMed: 19857795]
36. Pfister T, Eichinger-Chapelon A. General 4-week toxicity study with EMS in the rat. *Toxicol Lett*. 2009; 190:271–285. [PubMed: 19442710]

37. Gocke E, Ballantyne M, Whitwell J, Muller L. MNT and MutaMouse studies to define the in vivo dose response relations of the genotoxicity of EMS and ENU. *Toxicol Lett.* 2009; 190:286–297. [PubMed: 19446969]
38. Gocke E, Muller L, Pfister T. EMS in Viracept--initial ('traditional') assessment of risk to patients based on linear dose response relations. *Toxicol Lett.* 2009; 190:266–270. [PubMed: 19439165]
39. Gocke E, Wall M. In vivo genotoxicity of EMS: statistical assessment of the dose response curves. *Toxicol Lett.* 2009; 190:298–302. [PubMed: 19857797]
40. Lave T, Birnbock H, Gotschi A, Ramp T, Pahler A. In vivo and in vitro characterization of ethyl methanesulfonate pharmacokinetics in animals and in human. *Toxicol Lett.* 2009; 190:303–309. [PubMed: 19695315]
41. Lave T, Paehler A, Grimm HP, Gocke E, Muller L. Modelling of patient EMS exposure: translating pharmacokinetics of EMS in vitro and in animals into patients. *Toxicol Lett.* 2009; 190:310–316. [PubMed: 19695316]
42. Muller L, Gocke E, Lave T, Pfister T. Ethyl methanesulfonate toxicity in Viracept--a comprehensive human risk assessment based on threshold data for genotoxicity. *Toxicol Lett.* 2009; 190:317–329. [PubMed: 19443141]
43. Muller L, Gocke E. Considerations regarding a permitted daily exposure calculation for ethyl methanesulfonate. *Toxicol Lett.* 2009; 190:330–332. [PubMed: 19857798]
44. Muller L, Singer T. EMS in Viracept--the course of events in 2007 and 2008 from the non-clinical safety point of view. *Toxicol Lett.* 2009; 190:243–247. [PubMed: 19857794]
45. Walker VE, Casciano DA, Tweats DJ. The Viracept-EMS case: impact and outlook. *Toxicol Lett.* 2009; 190:333–339. [PubMed: 19866516]
46. Friis-Moller N, Sabin CA, Weber R, d'Arminio Monforte A, El-Sadr WM, Reiss P, et al. Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med.* 2003; 349:1993–2003. [PubMed: 14627784]
47. Friis-Moller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio Monforte A, et al. Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study. *AIDS.* 2003; 17:1179–1193. [PubMed: 12819520]
48. Friis-Moller N, Reiss P, Sabin CA, Weber R, Monforte A, El-Sadr W, et al. Class of antiretroviral drugs and the risk of myocardial infarction. *N Engl J Med.* 2007; 356:1723–1735. [PubMed: 17460226]
49. Worm SW, Bower M, Reiss P, Bonnet F, Law M, Fatkenheuer G, et al. Non-AIDS defining cancers in the D:A:D Study--time trends and predictors of survival: a cohort study. *BMC Infect Dis.* 2013; 13:471. [PubMed: 24106926]
50. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. *Epidemiology.* 2004; 15:615–625. [PubMed: 15308962]
51. Chao C, Leyden WA, Xu L, Horberg MA, Klein D, Towner WJ, et al. Exposure to antiretroviral therapy and risk of cancer in HIV-infected persons. *AIDS.* 2012; 26:2223–2231. [PubMed: 22951631]
52. Crum-Cianflone N, Hullsiek KH, Marconi V, Weintrob A, Ganesan A, Barthel RV, et al. Trends in the incidence of cancers among HIV-infected persons and the impact of antiretroviral therapy: a 20-year cohort study. *AIDS.* 2009; 23:41–50. [PubMed: 19050385]
53. Guiguet M, Boue F, Cadranet J, Lang JM, Rosenthal E, Costagliola D, et al. Effect of immunodeficiency, HIV viral load, and antiretroviral therapy on the risk of individual malignancies (FHDH-ANRS CO4): a prospective cohort study. *Lancet Oncol.* 2009; 10:1152–1159. [PubMed: 19818686]
54. Polesel J, Clifford GM, Rickenbach M, Dal Maso L, Battegay M, Bouchardy C, et al. Non-Hodgkin lymphoma incidence in the Swiss HIV Cohort Study before and after highly active antiretroviral therapy. *AIDS.* 2008; 22:301–306. [PubMed: 18097233]
55. Shiels MS, Cole SR, Wegner S, Armenian H, Chmiel JS, Ganesan A, et al. Effect of HAART on incident cancer and noncancer AIDS events among male HIV seroconverters. *J Acquir Immune Defic Syndr.* 2008; 48:485–490. [PubMed: 18614916]

56. Pati S, Pelser CB, Dufraine J, Bryant JL, Reitz MS Jr, Weichold FF. Antitumorigenic effects of HIV protease inhibitor ritonavir: inhibition of Kaposi sarcoma. *Blood*. 2002; 99:3771–3779. [PubMed: 11986235]
57. Gocke E, Muller L. In vivo studies in the mouse to define a threshold for the genotoxicity of EMS and ENU. *Mutat Res*. 2009; 678:101–107. [PubMed: 19376265]

Figure 1.

Incidence (per 100 person-years) of cancers amongst participants that used nelfinavir between 1 July 2006 and 30 June 2007 compared to other nelfinavir users*

*Error bars represent 95% confidence interval. 1 July 2006 to 30 June 2007 was defined as the risk period for exposure to contaminated nelfinavir.

Table 1

Participant baseline characteristics

Number of participants		42006	(100.0%)
Gender	Male	30716	(73.1%)
Age (years)	Median (IQR)	40	(33, 46)
Cohort	Swiss HIV Cohort, Switzerland	7091	(16.9%)
	ATHENA, The Netherlands	12084	(28.8%)
	Nice Cohort, France	1505	(3.6%)
	Aquitaine, France	3072	(7.3%)
	BASS, Spain	597	(1.4%)
	EuroSIDA, Europe	10836	(25.8%)
	AHOD, Australia	750	(1.8%)
	ICONA, Italy	3467	(8.3%)
	Brussels St. Pierre, Belgium	2604	(6.2%)
	Mode of HIV acquisition	MSM	18404 (43.8%)
Ethnic group	IDU	6007	(14.3%)
	Heterosexual	14948	(35.6%)
	Other/unknown	2647	(6.3%)
	White	20977	(49.9%)
	Black African	2958	(7.0%)
Year of entry	Other	848	(2.0%)
	Unknown	17223	(41.0%)
	2004	27198	(64.8%)
	2005	3046	(7.3%)
	2006	3868	(9.2%)
	2007	2571	(6.1%)
	2008	4602	(11.0%)
Smoking status	2009	721	(1.7%)
	Current smoker	17020	(40.9%)
	Ex-smoker	7359	(17.7%)
	Never smoker	11530	(27.7%)
CD4 count (cells/mm ³) (n=39956)	Unknown	5727	(13.8%)
HIV RNA (log ₁₀ copies/ml) (n=39001)	Median (IQR)	434	(282, 620)
Hepatitis C antibody	Median (IQR)	2.3	(1.7, 4.3)
	Negative	27705	(66.0%)
	Positive	7899	(18.8%)
Hepatitis B surface antigen	Unknown	6402	(15.2%)
	Negative	28950	(68.9%)
	Positive – active	1956	(4.7%)
	Positive - inactive	5905	(14.1%)
	Unknown	5195	(12.4%)
Previous cancer		2384	(5.7%)

Number of participants	42006	(100.0%)
Previous AIDS diagnosis	9901	(23.6%)

MSM=men who have sex with men; IDU=intravenous drug user; IQR=interquartile range

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Table 2
Association between cancer and cumulative exposure (per additional five years) to different antiretroviral therapy regimens*

	All		AIDS-defining		Non-AIDS-defining		Non-infection-related	
	aRR (95%CI)	p-value	aRR (95%CI)	p-value	aRR (95%CI)	p-value	aRR (95%CI)	p-value
Nelfinavir-ART	0.93 (0.82, 1.06)	0.26	0.59 (0.45, 0.77)	<0.01	1.19 (1.03, 1.37)	0.02	1.17 (0.96, 1.43)	0.12
Non-nelfinavir, PI-ART	0.82 (0.78, 0.87)	<0.01	0.47 (0.41, 0.54)	<0.01	1.05 (0.99, 1.13)	0.12	0.98 (0.89, 1.07)	0.63
NNRTI-ART	0.68 (0.63, 0.73)	<0.01	0.27 (0.23, 0.33)	<0.01	0.97 (0.89, 1.05)	0.43	0.97 (0.86, 1.08)	0.54
Other ART	0.92 (0.85, 1.00)	0.05	0.65 (0.55, 0.77)	<0.01	1.09 (0.99, 1.19)	0.08	1.03 (0.90, 1.17)	0.68
	Non-Hodgkin's		Kaposi's sarcoma		Lung		Anal	
Nelfinavir-ART	0.68 (0.48, 0.96)	0.03	0.46 (0.29, 0.75)	<0.01	0.93 (0.63, 1.36)	0.70	1.10 (0.67, 1.80)	0.71
Non-nelfinavir, PI-ART	0.53 (0.45, 0.63)	<0.01	0.36 (0.29, 0.45)	<0.01	1.02 (0.87, 1.19)	0.84	1.40 (1.16, 1.71)	<0.01
NNRTI-ART	0.29 (0.22, 0.37)	<0.01	0.36 (0.29, 0.45)	<0.01	0.90 (0.74, 1.10)	0.31	1.10 (0.84, 1.42)	0.50
Other ART	0.59 (0.46, 0.76)	<0.01	0.21 (0.16, 0.29)	<0.01	1.03 (0.83, 1.29)	0.78	1.25 (0.94, 1.67)	0.13

* Adjusted for age, gender, mode of HIV acquisition, hepatitis B surface antigen/hepatitis C antibody status and history of a previous cancer.

ART= antiretroviral therapy; PI=protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; aRR=adjusted rate ratio; CI=confidence interval.

Table 3
Association between cancer and current exposure to different antiretroviral therapy regimens*

	All			AIDS-defining			Non-AIDS-defining			Non-infection-related		
	aRR (95%CI)	p-value	aRR (95%CI)	aRR (95%CI)	p-value	aRR (95%CI)	aRR (95%CI)	p-value	aRR (95%CI)	aRR (95%CI)	p-value	
Nelfinavir-ART	0.98 (0.68, 1.41)	0.92	1.28 (0.72, 2.28)	0.40	0.86 (0.54, 1.37)	0.52	0.73 (0.35, 1.55)	0.41				
Non-nelfinavir, PI-ART	1	-	1	-	1	-	1	-	1	-	-	
NNRTI-ART	0.81 (0.73, 0.89)	<0.01	0.73 (0.61, 0.88)	<0.01	0.85 (0.76, 0.95)	<0.01	0.93 (0.79, 1.09)	0.38				
Other ART	0.85 (0.71, 1.02)	0.07	0.78 (0.56, 1.10)	0.16	0.88 (0.71, 1.08)	0.21	0.97 (0.73, 1.29)	0.84				
No ART	1.39 (1.23, 1.56)	<0.01	2.35 (1.97, 2.80)	<0.01	0.78 (0.65, 0.93)	<0.01	1.01 (0.80, 1.28)	0.92				
Non-Hodgkin's												
Kaposi's sarcoma												
	aRR (95%CI)	p-value	aRR (95%CI)	aRR (95%CI)	p-value	aRR (95%CI)	aRR (95%CI)	p-value	aRR (95%CI)	aRR (95%CI)	p-value	
Nelfinavir-ART	1.64 (0.81, 3.35)	0.17	0.60 (0.15, 2.44)	0.48	0.90 (0.28, 2.83)	0.85	0.79 (0.19, 3.23)	0.75				
Non-nelfinavir, PI-ART	1	-	1	-	1	-	1	-	1	-	-	
NNRTI-ART	0.76 (0.59, 0.98)	0.04	0.73 (0.55, 0.97)	0.03	0.87 (0.65, 1.15)	0.33	0.66 (0.47, 0.94)	0.02				
Other ART	0.76 (0.47, 1.23)	0.27	0.63 (0.35, 1.15)	0.13	1.01 (0.63, 1.63)	0.97	0.58 (0.28, 1.19)	0.14				
No ART	1.97 (1.47, 2.49)	<0.01	3.12 (2.42, 4.02)	<0.01	0.98 (0.65, 1.48)	0.92	0.58 (0.33, 1.00)	0.05				
Lung												
Anal												

* Adjusted for age, gender, mode of HIV acquisition, hepatitis B surface antigen/hepatitis C antibody status and history of a previous cancer.

ART= antiretroviral therapy; PI=protease inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; aRR=adjusted rate ratio; CI=confidence interval.

Table 4

Association between cancer and nelfinavir exposure between 1 July 2006 and 30 June 2007 *

	AIDS-defining			Non-AIDS-defining			Non-infection-related		
	aRR (95%CI)	p-value	aRR (95%CI)	p-value	aRR (95%CI)	p-value	aRR (95%CI)	p-value	p-value
Exposed to nelfinavir in risk period	1.07 (0.78, 1.46)	0.68	0.58 (0.25, 1.31)	0.19	1.23 (0.88, 1.72)	0.23	1.25 (0.78, 2.00)	0.35	
Exposed to nelfinavir outside risk period	1.07 (0.74, 1.21)	0.31	1.02 (0.77, 1.36)	0.87	1.08 (0.94, 1.25)	0.27	1.07 (0.87, 1.30)	0.53	

* Adjusted for age, gender, mode of HIV acquisition, hepatitis B surface antigen/hepatitis C antibody status and history of previous cancer. 1 July 2006 to 30 June 2007 was defined as the risk period for exposure to contaminated nelfinavir. Reference group for each treatment category is all other ART exposure.

aRR=adjusted rate ratio; CI=confidence interval.