JAIDS Journal of Acquired Immune Deficiency Syndromes Publish Ahead of Print
DOI: 10.1097/QAI.0000000000000662

The effect of cumulating exposure to abacavir on the risk of cardiovascular disease events in patients from the Swiss HIV Cohort Study

Jim Young, PhD,[a]* Yongling Xiao, PhD,[b,c] Erica E.M. Moodie, PhD,[c] Michal Abrahamowicz, PhD,[c]

Marina B. Klein, MD MSc,[d] Enos Bernasconi, MD MSc,[e] Patrick Schmid, MD,[f]

Alexandra Calmy, MD PhD,[g] Matthias Cavassini, MD,[h] Alexia Cusini, MD,[i]

Rainer Weber, MD,[j] Heiner C. Bucher, MD MPH,[a,k] and the Swiss HIV Cohort Study

[a] Basel Institute for Clinical Epidemiology and Biostatistics, University Hospital Basel, Basel, Switzerland

[b] Analysis Group Inc, Montreal, Canada

[c] Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Canada

[d] Department of Medicine, Royal Victoria Hospital, McGill University Health Centre, Montreal, Canada

[e] Division of Infectious Diseases, Regional Hospital of Lugano, Lugano, Switzerland

[f] Division of Infectious Diseases and Hospital Epidemiology, Cantonal Hospital St. Gallen, St Gallen, Switzerland

[g] Division of Infectious Diseases, University Hospital Geneva, Geneva, Switzerland

[h] Division of Infectious Diseases, University Hospital Lausanne, Lausanne, Switzerland

[i] Department of Infectious Diseases, Bern University Hospital and University of Bern, Bern, Switzerland

[j] Division of Infectious Diseases and Hospital Epidemiology, University Hospital and University of Zürich, Zurich, Switzerland

[k] Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland

* Corresponding author

Jim Young, Basel Institute for Clinical Epidemiology and Biostatistics, University Hospital Basel, CH-4031 Basel, Switzerland.

Tel: +41 61 265 3100; fax: +41 61 265 3109; e-mail: james.young@usb.ch

Running head: Abacavir and cardiovascular disease

Counts: abstract 250, manuscript 3680; tables 2; figures 3.

Meetings

1. 17th International Workshop on HIV Observational Databases, 11-13 April 2013, Cavtat, Croatia [Oral, closed meeting].

2. 7th IAS Conference on HIV Pathogenesis, Treatment and Prevention, 30 June-3 July 2013, Kuala Lumpur, Malaysia [Poster, Abstract MOPE070]. Abstract available at http://pag.ias2013.org/Abstracts.aspx?AID=1149.

3. 29th International Society for Pharmacoepidemiology Conference, August 25 – August 28 2013, Montreal, Canada [[Oral, Abstract 1; oral, Abstract 396]. Abstracts published in *Pharmacoepidemiology and D rug Safety*, 22(S1):193-194 and available at: http://onlinelibrary.wiley.com/doi/10.1002/pds.3512/pdf.

Conflicts of interest and sources of funding

This work was funded by the Canadian Institutes of Health Research (grant number 81275) and Canada's Natural Sciences and Engineering Research Council (grant number 331271) and as project SHCS 658 within the framework of the Swiss HIV Cohort Study, which is supported by the Swiss National Science Foundation (grant number 33CS30 134277). The Basel Institute for Clinical Epidemiology and Biostatistics is supported by grants from Santésuisse and from the Gottfried and Julia Bangerter-Rhyner-Foundation. EEMM is supported by a Chercheur boursier career award from the Fonds de recherche du Québec – Santé (FRQS); MA is a James McGill Professor at McGill University. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

MBK has served on advisory boards for ViiV Healthcare and Gilead Sciences and received travel grants from ViiV Healthcare. RW has received travel grants from Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, and Pfizer; his institution has received unrestricted educational grants from GlaxoSmithKline and ViiV Healthcare. HCB has received travel grants, honoraria and unrestricted research grants from Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Pfizer and ViiV Healthcare. All other authors have no conflicts.

Abstract

Background: Patients with HIV exposed to the antiretroviral drug abacavir may have an increased risk of cardiovascular disease (CVD). There is concern that this association arises because of a channelling bias. Even if exposure is a risk, it is not clear how that risk changes as exposure cumulates.

Methods: We assess the effect of exposure to abacavir on the risk of CVD events in the Swiss HIV Cohort Study. We use a new marginal structural Cox model to estimate the effect of abacavir as a flexible function of past exposures while accounting for risk factors that potentially lie on a causal pathway between exposure to abacavir and CVD.

Results: 11,856 patients were followed for a median of 6.6 years; 365 patients had a CVD event (4.6 events per 1000 patient years). In a conventional Cox model, recent – but not cumulative – exposure to abacavir increased the risk of a CVD event. In the new marginal structural Cox model, continued exposure to abacavir during the past four years increased the risk of a CVD event (hazard ratio 2.06, 95% confidence interval 1.43-2.98). The estimated function for the effect of past exposures suggests that exposure during the past 6 to 36 months caused the greatest increase in risk.

Conclusions: Abacavir increases the risk of a CVD event: the effect of exposure is not immediate, rather the risk increases as exposure cumulates over the past few years. This gradual increase in risk is not consistent with a rapidly acting mechanism, such as acute inflammation.

Keywords: HIV, antiretroviral therapy, reverse transcriptase inhibitors, adverse effects, marginal structural models

1. Introduction

In 2008, an analysis by the D:A:D collaboration of observational cohorts showed that recent exposure to the antiretroviral drug abacavir was associated with an increased risk of cardiovascular disease (CVD) events.[1] Subsequent meta-analyses of randomised controlled trials failed to find evidence of this association.[2-5] The D:A:D emphasised that stopping smoking would do more to reduce the risk of a heart attack than stopping abacavir and noted that the absolute risk of such events was low.[6] Nevertheless, their results caused an unprecedented change in prescribing behaviour.[7]

Neither cumulative nor past exposure to abacavir seemed to increase the risk of these events [1] and the D:A:D collaboration observed that while current use was a risk, this risk appeared to reverse shortly after the use of abacavir ceased.[8] These factors led the collaboration to suggest that a rapidly acting mechanism, such as vascular inflammation, could be responsible for the increase in risk. However subsequent biomarker studies proved inconclusive [9-12] and analyses of other observational cohorts led to inconsistent results.[9,10,13,14]

There is also lingering concern that any association between abacavir and CVD could be an artefact of either 'channelling bias' or the failure to adjust for potential confounders such as renal function or injection drug use.[9] Indeed patients at higher risk of CVD were more likely to receive abacavir [1,15] (a 'channelling bias' [16] or 'confounding by indication' [17]). The D:A:D did not adjust for time varying risk factors such as blood lipid levels and blood pressure because, if they lie on a causal pathway between exposure to abacavir and CVD, adjusting for them could 'adjust away' the effect of interest.[18] This situation necessitates more complex methods of analysis; marginal structural modelling [19] in particular has been recommended.[18,20,21]

In most analyses, it is not known how the effect of exposure to a drug cumulates over time. Assuming a simple relationship between exposure and outcome can erode the power to detect a relationship and give a misleading picture of how best to minimise the risk of an adverse event.[22] We assess the effect of exposure to abacavir on the risk of CVD events in the Swiss HIV Cohort Study (SHSC). First we reproduce the D:A:D's analysis using SHCS data; then we consider the likely results had they used more complex statistical methods. We fit a new marginal structural model [23] to estimate the effect of abacavir as a flexible function of past exposures while accounting for risk factors that potentially lie on a causal pathway between exposure to abacavir and CVD.

2. Methods

2.1. Patients

The SHCS is a prospective cohort with continuing enrolment of HIV-infected adults.[24] Since 1 April 2000, a cardiovascular risk assessment has been part of follow up visits scheduled every six months at one of seven outpatient clinics or at the office of a collaborating physician. At each assessment, blood pressure, smoking status, weight and body fat loss or gain are recorded and a blood sample taken to measure blood lipids. In this study, we include all patients with at least one cardiovascular risk assessment.

2.2. Outcome, covariate and exposure definitions

We consider a composite outcome to maximise the number of suitable disease events. As in the D:A:D study,[1] we define a CVD event as the first occurrence of either a myocardial infarction, an invasive cardiovascular procedure or a cardiovascular related death. Each myocardial infarction or invasive cardiovascular procedure was documented in a checking chart;[25] since 2005, each death has been documented using a CoDe cause of death form.[26,27]

As in the D:A:D study, each patient's follow-up is divided into consecutive one-month periods in our analyses. To reproduce the D:A:D's analysis, we adjust for the same covariates in our conventional multivariate models. Hence these models have time-fixed covariates for demographic characteristics (age, sex, likely transmission through injection drug use, Caucasian ethnicity) and CVD risk factors (family history of coronary heart disease, previous CVD event); and time-varying covariates for CVD risk factors (smoking status and body mass index, updated each follow up visit), calendar year, and cumulative exposure to 15 other antiretroviral drugs (with a separate covariate for each drug updated each month).

Time varying covariates identified by the D:A:D as potentially on a causal pathway between exposure to abacavir and CVD are not included in our conventional multivariate models but are accounted for in our marginal structural Cox models. These covariates are represented by separate indicators for hypertension, dyslipidaemia and diabetes (and in a sensitivity analysis, an indicator for chronic kidney disease); indicators for three Framingham risk score categories;[28] and continuous measures of CD4 cell count and log 10 HIV RNA (viral load).

When estimating the effect of abacavir as a flexible function of past exposures, exposure is represented by an indicator variable with value one if the patient was taking abacavir on the first day of the month. Other estimates of the effect of abacavir use exposure indicators and duration of exposure as at the first day of the month derived from the exact dates the patient started and stopped taking abacavir.

2.3. Statistical Analyses

We analyse time to a first CVD event using various forms of the Cox proportional hazard model. For each patient, follow up begins at their first CVD risk assessment. A patient with no CVD event during follow up is censored at a death unrelated to CVD, six months after their last CVD assessment if lost to follow up or at the end of the study (30 September 2012), whichever comes first. As in the D:A:D's analyses, we assume that censoring is uninformative.

2.4. Conventional modelling

We fit three conventional Cox models; all adjust for the same covariates but the history of exposure to abacavir is represented in different ways. The first model reproduces an analysis reported by the D:A:D, with two time varying exposure variables: one for the total duration of past use (cumulative use), the other an indicator of any exposure within the previous six months (recent use). The other two conventional models use exposure variables suggested by the results of our cumulative exposure modelling. These results suggest that current exposure to abacavir might be protective and that exposure during the past 6 to 36 months causes the greatest increase in the risk of a CVD event. Hence the second model has three exposure variables: cumulative use as before, but with recent use partitioned into two indicators – use in the current month and use in the previous one to six months. The third model has exposure to abacavir represented by three variables – current use, use in the previous one to six months and the total duration of use over the past 7 to 36 months.

2.5. Marginal structural modelling

We also fit the models described above as marginal structural Cox models using stabilised inverse probability of treatment weights (Section 1, Supplemental Digital Content, http://links.lww.com/QAI/A680). This process requires eight different logistic regression models in order to estimate the probability that in a given month a patient either starts treatment with

abacavir (if abacavir-naive) or continues treatment with abacavir (if already exposed) given the most recent values of confounding variables. The process also allows relationships between confounding variables and treatment to change after February 2008 because prescribing behaviour changed after the D:A:D's results were published.[7,29]

2.6. Cumulative exposure modelling

We fit a new marginal structural model that estimates the effect of abacavir as a flexible function of past exposure while using the same inverse probability of treatment weights as above.[23] Exposure to abacavir is defined as a weighted sum of use in each past month, with (exposure) weights found by estimating a cubic spline for the relative importance of exposure at different times in the past. We assume that exposure more than four years ago would have no effect on the current risk of a CVD event. We consider nine alternative weight functions (Section 2, Supplementary Digital Content, http://links.lww.com/QAI/A680): these differ in their degree of flexibility and in whether weights are forced to take zero value at both the beginning and end of the four year interval, or just at the end of four years, or can take values other than zero at all times. A zero weight at the beginning of the four year interval implies there is a lag between exposure and its effect on the current risk of an event. Having selected the best fitting weight function, we estimate a hazard ratio comparing two different treatment strategies – always exposed to abacavir over the entire four years versus never exposed over this period.

2.7. Additional analyses

We re-fit our weighted models with a time varying indicator of chronic kidney disease added to the covariates used to calculate inverse probability of treatment weights (Section 3, Supplemental Digital Content, http://links.lww.com/QAI/A680). This sensitivity analysis requires a truncated data set, limited to follow up after January 2002 when routine serum creatinine measurement began in the SHCS.[31] We define chronic kidney disease as an estimated glomerular filtration rate (calculated using CKD-EPI equation [32]) below 60 ml/min/1.73m^2.

In two unplanned sensitivity analyses, we re-fit models for abacavir after excluding patients infected with HIV through injection drug use and after excluding patients exposed to abacavir before their first cardiovascular risk assessment (Section 4, Supplemental Digital Content, http://links.lww.com/QAI/A680). The second of these analyses avoids a bias that would arise if

existing uses of abacavir were in a sense 'survivors' at low risk of CVD,[33]and its population of abacavir naive patients corresponds to the 'full population' used in a recent analysis by the NA-ACCORD.[34]

We also carry out a set of analyses for two other antiretroviral drugs from the same drug class: didanosine and tenofovir (Sections 5 and 6, Supplemental Digital Content, http://links.lww.com/QAI/A680). The D:A:D collaboration found that recent exposure to didanosine was also associated with an increased risk of CVD events.[1] Didanosine and abacavir are both guanosine analogues and hence might plausibly have similar effects. On the other hand tenofovir was not associated with an increased risk of CVD events, even though subject to the same channelling biases as abacavir.[35]

3. Results

3.1. Patients

As at October 2012, 11,924 patients in the SHCS had at least one cardiovascular risk assessment and 11,856 patients provided follow up with all covariates available. These 11,856 patients have been followed for a total of 80,004 patient years with a median follow up of 6.6 years (interquartile range, IQR, 2.8 to 11.6). Of these patients, 1549 were exposed to abacavir before assessments began, for a median duration of 0.7 years (IQR 0.2 to 1.4). During follow up, 4052 patients were exposed to abacavir, for a median duration of 3.4 years (IQR 1.3 to 6.0) and of these, 2297 stopped taking abacavir during follow up and 821 re-started again. During follow up, 365 patients had a CVD event (3.0%): of these, 195 had been exposed to abacavir (53%), for a median duration of 3.4 years (IQR 1.0 to 5.9). Half of the CVD events included a myocardial infarction (Table 1). Of the 11,491 patients without a CVD event, 4312 had been exposed to abacavir (38%), for a median duration of 3.3 years (IQR 1.0 to 6.0). Patients who had a CVD event were older and more likely to be men, currently smoking, with a previous CVD event or a family history of such events (Table 1). They were also more likely to have diabetes, chronic kidney disease, hypertension, dyslipidaemia or lipodystrophy and had higher Framingham risk scores than those without an event.

3.2. Conventional and marginal structural modelling

In our first conventional model (Table 2), the risk of a CVD event increased with recent exposure to abacavir (hazard ratio, HR, 1.50, 95% confidence interval (CI) 1.12 to 2.00) with weaker evidence of

an increase with greater cumulative exposure (HR 1.04, 95% CI 0.99 to 1.10, per year). These estimates are close to the equivalent estimates reported by the D:A:D (HR 1.63, 95% CI 1.30 to 2.04, for recent exposure and HR 1.03, 95% CI 0.96 to 1.10, per year for cumulative exposure [1]).

The other two conventional models use exposure variables suggested by the results of our cumulative exposure modelling (Table 2, footnotes). The first of these two models suggests that recent exposure in the past zero to six months can be partitioned into current exposure and recent exposure in the previous one to six months. In this second model, current exposure has a protective effect (HR 0.36, 95% CI 0.23 to 0.55) while recent exposure increases the risk of a CVD event (HR 3.69, 95% CI 2.36 to 5.75) such that the mixing of current and recent exposure in the first model understates the risk posed by the latter. The third model suggests that cumulative exposure during the past seven months to three years (HR 1.25, 95% CI 1.04 to 1.51, per year) does indeed increase the risk of a CVD event, as predicted by our cumulative exposure modelling.

Re-fitting these three models as marginal structural models led to very similar estimates (Table 2).

3.3. Cumulative exposure modelling

Of the nine exposure weight functions, the best fitting weight function had a single knot and weights of zero at both the beginning and end of the four year interval (Figure 1, left). This function implies that exposure to abacavir did not immediately increase the current risk of a CVD event; rather this risk reflects cumulating exposure to abacavir over the past 6sto 36 months. Of note, weight functions where the effect of current exposure could have a weight other than zero had negative weights for the earliest months of the interval suggesting that current exposure might have a protective effect (Section 2, Supplemental Digital Content, http://links.lww.com/QAI/A680). The total effect of always being exposed to abacavir, during the entire four year period, versus never being exposed was HR 2.06, 95% CI 1.43 to 2.98 (Figure 1, right). Cumulative exposure modelling without inverse probability weights gave a similar estimate of this total effect (HR 2.10, 95% CI 1.58 to 2.78; Figure 1, right).

3.4. Additional analyses

Estimates of the effect of abacavir were not attenuated when an indicator for chronic kidney disease was added to the covariates used to calculate inverse probability of treatment weights. Estimates of the effect of abacavir were not attenuated in unplanned analyses of patients not infected through

injection drug use and of abacavir naive patients. Cumulative exposure modelling suggested exposure to didanosine had early harmful and then later protective effects (Figure 2) while exposure to tenofovir had if anything a protective rather than a harmful effect (Figure 3). Results for these additional analyses are summarised in Sections 3 to 6 of the Supplemental Digital Content(http://links.lww.com/QAI/A680).

4. Discussion

Our results suggest that the risk of a CVD event increases as past exposure to abacavir cumulates, but only for a limited period. Exposure during the past 6 to 36 months causes the greatest increase in risk; both current exposure and exposure more than three years ago cause little additional increase in risk. Acute inflammation has been suggested as an explanation for the increase in CVD risk with exposure to abacavir, because the risk seemed associated with recent and not past exposure.[1,8] Our results suggest other explanations should be sought because the increase in risk is not immediate and it cumulates so that past exposure within the last three years still influences current risk.

Note that the relative risks presented in Table 2 should not be interpreted too literally. The models in this table illustrate how different partitions of time – into current, recent or cumulative use – can lead to different clinical conclusions. Our estimated weight function (Figure 1, left) does not require this arbitrary partitioning and is therefore a more reliable basis for drawing clinical conclusions. Having estimated this weight function, a contrast between any two treatment histories can be generated and we show one contrast of obvious interest – the effect of always being exposed to abacavir, over a four year period, versus never being exposed (Figure 1, right).

With our data, we were able to reproduce estimates reported by the D:A:D despite the changes in prescribing behaviour brought about by the publication of their results. While the SHCS contributes data to the D:A:D, only 45% of our 365 events occurred prior to February 2007 and might therefore have been included in their original analysis. The results of our cumulative exposure modelling explain seemingly inconsistent results from earlier studies. If the harmful effects of exposure cumulate but only for a finite period, and yet patients are exposed to abacavir for much longer, cumulative exposure per year will appear weakly harmful at best.[13,35] Exposure to abacavir more than six months earlier may well appear harmful, although studies may lack the power to really confirm or rule out such an effect.[1,36] Recent use should appear harmful, as it has in many studies,[1,14,35,36] but

may underestimate the short term risk if current use is included in the definition of recent use, because current use appears protective.[13]

An early protective effect could arise because abacavir, as part of an effective therapy, reduces viral replication, a risk factor for CVD events,[37,38] or because of a 'reverse causation bias' [39] if patients at high risk of a CVD event were taken off abacavir after only a short exposure but then went on to have such an event. Our modelling suggests that after the D:A:D's results were published, patients with a previous CVD event or a high Framingham risk score were taken off abacavir (Section 1, Supplemental Digital Content, http://links.lww.com/QAI/A680). But of the 53 high risk patients who stopped taking abacavir after February 2008, only two went on to have a CVD event and both had at least five year's exposure to abacavir.

This change in prescribing behaviour was considered prudent.[9] However for patients that smoke, giving up smoking leads to a greater reduction in CVD risk than avoiding exposure to abacavir.[6,40] For many patients , the increase in relative risk with exposure to abacavir will be acceptable, if other risk factors for CVD are absent,[41] given the low rate of CVD events – 4.6 per 1000 patient-years in these data – and that alternatives such as tenofovir also have side-effects.[31,42] The question of whether – and how – abacavir increases the risk of cardiovascular disease is still important. The recently approved co-formulation of dolutegravir, a new integrase inhibitor, with abacavir and lamivudine provides a one pill once a day regimen that is likely to prove popular with patients.[43,44] Integrase inhibitors are well-tolerated antiretrovirals because they do not interfere with normal cellular processes [45] and are therefore considered suitable for patients at risk of cardiovascular disease.[46]

Strengths of this study include that this is an analysis of data from a single cohort. This avoids the additional variation that arises when contributing cohorts in a multi-cohort collaboration use different methods to collect and measure data. Our confidence intervals for estimates of effect sizes are of a similar width to those reported in the D:A:D's original study,[1] yet in our data we have only half the number of CVD events (365 versus 693 events). We use modelling that does not require strong assumptions about the relationship between exposure and outcome. As a consequence, in our results we see a relationship between exposure to abacavir and the risk of CVD events that is both plausible – in that risk lags exposure and does not cumulate indefinitely – and explains seemingly inconsistent results from earlier studies. Finally, unlike other observational studies, our analyses also account for covariates potentially on a causal pathway between exposure to abacavir and CVD; this reduces the residual confounding that would otherwise arise when those exposed to abacavir are at

greater risk of CVD than the unexposed.[18] Note that estimates in Table 2 with and without marginal structural modelling are similar, vindicating those who maintained that such modelling would not have altered the conclusions of their analyses.[1,29,35] However, marginal structural modelling was important in our analysis of tenofovir (Figure 3).

We note the following study limitations. As in the D:A:D study,[1] not all patients were abacavir-naïve at the start of follow up, with 13% of patients pre-exposed. Those pre-exposed to abacavir had a higher prevalence of dyslipidemia and of moderate or high Framingham risk scores (data not shown) but our modelling of continued use of abacavir took such factors into account. A causal interpretation of our results is only possible if there is no unmeasured confounding.[19] We did not adjust for time dependent injection drug use because routine recording of this only began in July 2008. Note however that sensitivity analyses of abacavir naive patients and of patients not infected through injection drug use gave similar results to the main analysis. We did not have sufficient events to warrant cumulative exposure modelling of the risk of myocardial infarction alone (see Table 1).

The implication of these results is that a rapidly acting mechanism, such as acute inflammation,[1,8] may not be responsible for the increased risk of CVD with exposure to abacavir. A possible early protective effect and a later cumulative harmful effect suggest more gradual processes. One possibility for a cumulative harmful effect is mitochondrial toxicity,[47] as abacavir may interact with cytidine analogues lamivudine and emtricitabine.[48] The heart, with its high metabolic demand, is rich in mitochondria and is susceptible to mitochondrial damage, especially as it ages.[49] Several mechanisms could be involved: equivalent modelling of the risk of CVD with exposure to didanosine suggests that the two drugs may affect CVD in different ways. Our results for didanosine suggest an unexpected dual effect – a rapid early harmful effect followed by a later protective effect (Figure 2). This might explain why other studies show that recent exposure to didanosine is harmful but that cumulative exposure has no net effect [1,35] or even a protective effect.[36] In the updated D:A:D analysis, plots showing the rate of myocardial infarction with cumulative exposure are consistent with what we report here – with abacavir, the rate increases and then levels off after two to three years of exposure; with didanosine, the rate seems to peak after about one to two years of exposure and may then decline.[35]

Our results suggest a number of directions for future research. First one could reconsider more gradual processes that might give rise to an increasing risk of CVD with cumulating exposure to abacavir. Second one could look for evidence of a protective effect with current exposure to abacavir in data collected before the D:A:D's results prompted clinicians to take high risk patients off abacavir.

Third one could consider whether the harmful effects of abacavir and didanosine might involve substantially different processes. While our analyses suggest that exposure to abacavir increases the risk of CVD, they also suggest that acute processes are unlikely to be the cause.

5. Acknowledgements

This work was funded by the Canadian Institutes of Health Research (grant number 81275) and Canada's Natural Sciences and Engineering Research Council (grant number 331271) and as project SHCS 658 within the framework of the Swiss HIV Cohort Study, which is supported by the Swiss National Science Foundation (grant number 33CS30 134277). The Basel Institute for Clinical Epidemiology and Biostatistics is supported by grants from Santésuisse and from the Gottfried and Julia Bangerter-Rhyner-Foundation. EEMM is supported by a Chercheur boursier career award from the Fonds de recherche du Québec – Santé (FRQS); MA is a James McGill Professor at McGill University. The funders had no role in study design, data collection and analysis, the preparation of the manuscript or the decision to publish.

The members of the Swiss HIV Cohort Study are: Aubert V, Barth J, Battegay M, Bernasconi E, Böni J, Bucher HC, Burton-Jeangros C, Calmy A, Cavassini M, Egger M, Elzi L, Fehr J, Fellay J, Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (President of the SHCS), Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hösli I, Kahlert C, Kaiser L, Keiser O, Klimkait T, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Metzner K, Müller N, Nadal D, Pantaleo G, Rauch A (Chairman of the Scientific Board), Regenass S, Rickenbach M (Head of Data Center), Rudin C (Chairman of the Mother & Child Substudy), Schmid P, Schultze D, Schöni-Affolter F, Schüpbach J, Speck R, Taffé P, Tarr P, Telenti A, Trkola A, Vernazza P, Weber R, Yerly S.

6. References

1. Sabin CA, Worm SW, Weber R, et al. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multi-cohort collaboration. *Lancet*. 2008;371:1417-1426.

2. Brothers CH, Hernandez JE, Cutrell AG, et al. Risk of myocardial infarction and abacavir therapy: no increased risk across 52 GlaxoSmithKline-sponsored clinical trials in adult subjects. *J Acquir Immune Defic Syndr*. 2009;51:20-28.

3. Ribaudo HJ, Benson CA, Zheng Y, et al. No risk of myocardial infarction associated with initial antiretroviral treatment containing abacavir: short and long-term results from ACTG A5001/ALLRT. *Clin Infect Dis*. 2011;52:929-940.

4. Cruciani M, Zanichelli V, Serpelloni G, et al. Abacavir use and cardiovascular disease events: a meta-analysis of published and unpublished data. *AIDS*. 2011;25:1993-2004.

5. Ding X, Andraca-Carrera E, Cooper C, et al. No association of abacavir use with myocardial infarction: findings of an FDA meta-analysis. *J Acquir Immune Defic Syndr*. 2012;61:441-447.

6. Lundgren J. Position statement by the D:A:D steering committee. Available at: http://www.cphiv.dk/Ongoing-Studies/DAD/Position-Statement.

7. Antoniou T, Gillis J, Loutfy MR, et al. Impact of the Data Collection on Adverse Events of Anti-HIV Drugs Cohort Study on abacavir prescription among treatment-naive, HIV-infected patients in Canada. *J Int Assoc Provid AIDS Care*. 2014;13:153-159.

8. Sabin CA, Worm S, Phillips AN, et al. Abacavir and increased risk of myocardial infarction - reply. *Lancet*. 2008;372:804-805.

9. Behrens GM, Reiss P. Abacavir and cardiovascular risk. *Curr Opin Infect Dis*. 2010;23:9-14.

10. Triant VA. HIV infection and coronary heart disease: an intersection of epidemics. *J Infect Dis*. 2012;205 Suppl 3:S355-S361.

11. Hattab S, Guihot A, Guiguet M, et al. Comparative impact of antiretroviral drugs on markers of inflammation and immune activation during the first two years of effective therapy for HIV-1 infection: an observational study. *BMC Infect Dis*. 2014;14:122.

12. Wohl DA, Arnoczy G, Fichtenbaum CJ, et al. Comparison of cardiovascular disease risk markers in HIV-infected patients receiving abacavir and tenofovir: the nucleoside inflammation, coagulation and endothelial function (NICE) study. *Antivir Ther*. 2014;19:141-147.

13. Bedimo RJ, Westfall AO, Drechsler H, et al. Abacavir use and risk of acute myocardial infarction and cerebrovascular events in the highly active antiretroviral therapy era. *Clin Infect Dis*. 2011;53:84-91.

14. Choi AI, Vittinghoff E, Deeks SG, et al. Cardiovascular risks associated with abacavir and tenofovir exposure in HIV-infected persons. *AIDS*. 2011;25:1289-1298.

15. Obel N, Farkas DK, Kronborg G, et al. Abacavir and risk of myocardial infarction in HIV-infected patients on highly active antiretroviral therapy: a population-based nationwide cohort study. *HIV Med*. 2010;11:130-136.

16. Petri H, Urquhart J. Channeling bias in the interpretation of drug effects. *Stat Med*. 1991;10:577-581.

17. Psaty BM, Koepsell TD, Lin D, et al. Assessment and control for confounding by indication in observational studies. *J Am Geriatr Soc*. 1999;47:749-754.

18. Triant VA, Josephson F, Rochester CG, et al. Adverse outcome analyses of observational data: assessing cardiovascular risk in HIV disease. *Clin Infect Dis*. 2012;54:408-413.

19. Hernan MA, Brumback B, Robins JM. Marginal structural models to estimate the causal effect of zidovudine on the survival of HIV-positive men. *Epidemiology*. 2000;11:561-570.

20. Aberg JA, Ribaudo H. Cardiac risk: not so simple. *J Infect Dis*. 2010;201:315-317.

21. Bavinger C, Bendavid E, Niehaus K, et al. Risk of cardiovascular disease from antiretroviral therapy for HIV: a systematic review. *PLoS One*. 2013;8:e59551.

22. Abrahamowicz M, Beauchamp ME, Sylvestre MP. Comparison of alternative models for linking drug exposure with adverse effects. *Stat Med*. 2012;31:1014-1030.

23. Xiao Y, Abrahamowicz M, Moodie EEM, et al. Flexible marginal structural models for estimating the cumulative effect of a time-dependent treatment on the hazard: reassessing the cardiovascular risk of didanosine treatment in the Swiss HIV Cohort. *J Am Stat Assoc*. 2014;109:455-464.

24. Schoeni-Affolter F, Ledergerber B, Rickenbach M, et al. Cohort profile: the Swiss HIV Cohort Study. *Int J Epidemiol*. 2010;39:1179-1189.

25. Swiss HIV Cohort Study. Checking charts. Available at: http://www.shcs.ch/64-checking-charts.

26. Swiss HIV Cohort Study. Cause of death form (CoDe). Available at: http://www.shcs.ch/userfiles/file/documents/CODE.pdf.

27. Kowalska JD, Friis-Moller N, Kirk O, et al. The Coding Causes of Death in HIV (CoDe) Project: initial results and evaluation of methodology. *Epidemiology*. 2011;22:516-523.

28. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA*. 2001;285:2486-2497.

29. Sabin C, Reiss P, Ryom L, et al. Is there continued evidence for an association between abacavir and myocardial infarction risk? Available at: http://www.croiconference.org/sites/default/files/abstracts/747LB.pdf.

30. Volinsky CT, Raftery AE. Bayesian information criterion for censored survival models. *Biometrics*. 2000;56:256-262.

31. Young J, Schafer J, Fux CA, et al. Renal function in patients with HIV starting therapy with tenofovir and either efavirenz, lopinavir or atazanavir. *AIDS*. 2012;26:567-575.

32. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604-612.

33. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. *Am J Epidemiol*. 2003;158:915-920.

34. Pallella FJ, Althoff KN, Moore RD, et al. Abacavir use and risk for myocardial infarction in the NA-ACCORD. Available at: http://www.croiconference.org/sites/default/files/posters-2015/749LB.pdf.

35. Worm SW, Sabin C, Weber R, et al. Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D:A:D) study. *J Infect Dis*. 2010;201:318-330.

36. Lang S, Mary-Krause M, Cotte L, et al. Impact of individual antiretroviral drugs on the risk of myocardial infarction in human immunodeficiency virus-infected patients: a case-control study nested within the French Hospital Database on HIV ANRS cohort CO4. *Arch Intern Med*. 2010;170:1228-1238.

37. Lundgren JD, Babiker A, El-Sadr W, et al. Inferior clinical outcome of the CD4+ cell count-guided antiretroviral treatment interruption strategy in the SMART study: role of CD4+ Cell counts and HIV RNA levels during follow-up. *J Infect Dis*. 2008;197:1145-1155.

38. Calmy A, Gayet-Ageron A, Montecucco F, et al. HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial. *AIDS*. 2009;23:929-939.

39. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. *Epidemiology*. 2004;15:615-625.

40. Friis-Moller N, Thiebaut R, Reiss P, et al. Predicting the risk of cardiovascular disease in HIV-infected patients: the data collection on adverse effects of anti-HIV drugs study. *Eur J Cardiovasc Prev Rehabil*. 2010;17:491-501.

41. Kowalska JD, Kirk O, Mocroft A, et al. Implementing the number needed to harm in clinical practice: risk of myocardial infarction in HIV-1-infected patients treated with abacavir. *HIV Med*. 2010;11:200-208.

42. Ryom L, Mocroft A, Kirk O, et al. Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. *J Infect Dis*. 2013;207:1359-1369.

43. Waters LJ, Barber TJ. Dolutegravir for treatment of HIV: SPRING forwards? *Lancet*. 2013;381:705-706.

44. Karmon SL, Markowitz M. Next-generation integrase inhibitors : where to after raltegravir? *Drugs*. 2013;73:213-228.

45. Pommier Y, Johnson AA, Marchand C. Integrase inhibitors to treat HIV/AIDS. *Nat Rev Drug Discov*. 2005;4:236-248.

46. Lennox JL. The use of HIV-1 integrase inhibitors in antiretroviral naive patients. *Curr Opin HIV AIDS*. 2012;7:409-414.

47. Koczor CA, Lewis W. Nucleoside reverse transcriptase inhibitor toxicity and mitochondrial DNA. *Expert Opin Drug Metab Toxicol*. 2010;6:1493-1504.

48. Venhoff N, Setzer B, Melkaoui K, et al. Mitochondrial toxicity of tenofovir, emtricitabine and abacavir alone and in combination with additional nucleoside reverse transcriptase inhibitors. *Antivir Ther*. 2007;12:1075-1085.

49. Chaudhary KR, El Sikhry H, Seubert JM. Mitochondria and the aging heart. *J Geriatr Cardiol*. 2011;8:159-167.

50. Sylvestre MP, Abrahamowicz M. Flexible modeling of the cumulative effects of time-dependent exposures on the hazard. *Stat Med*. 2009;28:3437-3453.

Figure 1. The effect of exposure to abacavir on the risk of cardiovascular disease events: the estimated weight function (left) and the estimated total cumulative effect (as a hazard ratio) of always being treated with abacavir over the past 48 months versus never being treated with abacavir (right). Exposure more than four years ago was assumed to have no effect on current risk. Functions are shown for cumulative exposure modelling with both marginal structural (solid curve) and conventional (dashed curve) Cox models.[23,50] Of the nine alternative weight functions considered, the best fitting weight function had a single knot and (exposure) weights of zero at both the beginning and end of the four year interval.

Figure 2. The effect of exposure to didanosine on the risk of cardiovascular disease events: the estimated weight function (left) and the estimated total cumulative effect (as a hazard ratio) of always being treated with didanosine over the past 30 months versus never being treated with didanosine (right). Exposure more than 30 months ago was assumed to have no effect on current risk. Functions are shown for cumulative exposure modelling with both marginal structural (solid curve) and conventional (dashed curve) Cox models.[23,50] Of the nine alternative weight functions considered, the best fitting weight function had a single knot and (exposure) weights of zero at both the beginning and end of the 30 month interval.

Figure 3. The effect of exposure to tenofovir on the risk of cardiovascular disease events: the estimated weight function (left) and the estimated total cumulative effect (as a hazard ratio) of always being treated with tenofovir over the past 48 months versus never being treated with tenofovir (right). Exposure more than four years ago was assumed to have no effect on current risk. Functions are shown for cumulative exposure modelling with both marginal structural (solid curve) and conventional (dashed curve) Cox models.[23,50] Of the nine alternative weight functions considered, the best fitting weight function had a single knot and (exposure) weights of zero at both the beginning and end of the four year interval.

Table 1: Characteristics of patients at the time of their first cardiovascular disease event or if no such event, at their last follow up visit – median or proportion**.**

Characteristic	Patients with a cardiovascular disease event				Patients without a cardiovascular disease event
	All	Exposed to antiretroviral therapy [a]			
		Exposed to abacavir	Exposed to didanosine	Exposed to tenofovir	
General					
Number of patients	365	195	151	148	11491
Male	0.83	0.83	0.81	0.83	0.70
Age (years)	45	44	46	44	35
CD4 cell count (cells/µL)	470	500	460	460	510
Suppressed viral load [b]	0.76	0.80	0.75	0.82	0.76
Body mass index > 26 kg/m^2	0.25	0.23	0.22	0.26	0.26
Current smoker	0.61	0.58	0.59	0.61	0.47
Ex-smoker	0.21	0.22	0.22	0.25	0.21
Infected through injection drug use	0.22	0.22	0.24	0.26	0.19
Cardiovascular disease					
Previous event [c]	0.14	0.12	0.13	0.12	0.01
Family history [d]	0.19	0.16	0.20	0.18	0.11
Diabetes mellitus [e]	0.15	0.15	0.15	0.17	0.06
Chronic kidney disease [f]	0.21	0.17	0.21	0.11	0.09

Hypertension					
Use of anti-hypertensive medication	0.43	0.43	0.36	0.45	0.18
Arterial hypertension [g]	0.59	0.61	0.54	0.64	0.34
Lipid levels					
Total cholesterol (mmol/L)	5.4	5.5	5.6	5.4	4.9
HDL cholesterol (mmol/L)	1.1	1.1	1.1	1.1	1.2
Use of lipid lowering medication	0.32	0.32	0.35	0.33	0.10
Dyslipidemia [h]	0.65	0.63	0.68	0.62	0.36
Lipodystrophy [i]	0.45	0.46	0.54	0.48	0.27
Framingham risk score [j]					
Low (<10%)	0.40	0.41	0.42	0.40	0.76
Moderate (10-20%)	0.45	0.43	0.42	0.47	0.21
High (>20%)	0.15	0.17	0.15	0.14	0.03
Cardiovascular disease event [k]					
Myocardial infarction	0.51	0.56	0.57	0.47	NA
Invasive cardiovascular procedure [l]	0.72	0.72	0.72	0.76	NA
Cardiovascular death	0.12	0.10	0.14	0.08	NA

Abbreviations: NA, not applicable.

[a] Patients could be exposed to none of these three drugs or to one or more.

[b] HIV RNA undetectable or below 50 copies / mL.

[c] Cardiovascular disease event before the patients first cardiovascular risk assessment.

[d] Myocardial infarction or stroke before the age of 50 in any first degree relative.

[e] Clinical diagnosis, or casual plasma glucose >11.1 mmol/L, or on anti-diabetic medication or insulin.

[f] Estimated glomerular filtration rate (calculated using CKD-EPI equation [32]) < 60 ml/min/1.73m^2

[g] Systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg, or on anti-hypertensive medication.

[h] Total cholesterol > 6.2 mmol/L, HDL cholesterol < 0.9 mmol/L, or on lipid-lowering medication.

[i] Patient and clinician report either body fat loss or body fat gain.

[j] Estimated risk of cardiovascular disease in the next 10 years.

[k] More than one event can occur during the same month.

[l] Coronary angioplasty/stenting, coronary artery by-pass grafting, carotid endarterectomy, procedures on other arteries.

Table 2.The relative risk of a cardiovascular disease event for patients exposed to abacavir.

Exposure parameters	Conventional model [a]		Marginal structural model [b]	
	Hazard ratio	(95% CI)	Hazard ratio	(95% CI)
Model 1 [c]				
Cumulative exposure (per year)	1.04	(0.99, 1.10)	1.04	(0.97, 1.10)
Recent exposure within past 0 to 6 months	1.50	(1.12, 2.00)	1.63	(1.14, 2.32)
Model 2 [d]				
Cumulative exposure (per year)	1.05	(1.00, 1.10)	1.05	(0.98, 1.11)
Recent exposure within past 1 to 6 months	3.69	(2.36, 5.75)	4.61	(2.59, 8.23)
Current exposure	0.36	(0.23, 0.55)	0.28	(0.15, 0.50)
Model 3 [e]				
Cumulative exposure within the past 7 to 36 months (per year)	1.25	(1.04, 1.51)	1.22	(0.98, 1.52)
Recent exposure within past 1 to 6 months	3.20	(1.97, 5.19)	4.06	(2.24, 7.34)
Current exposure	0.35	(0.22, 0.54)	0.27	(0.15, 0.50)

[a] Models adjusted for age, sex, likely transmission through injection drug use, Caucasian ethnicity, family history of coronary heart disease, previous CVD event, smoking status, body mass index, calendar year, and cumulative exposure to 15 other antiretroviral drugs.

[b] Models fit using inverse probability weights, with weights found using 8 different logistic regression

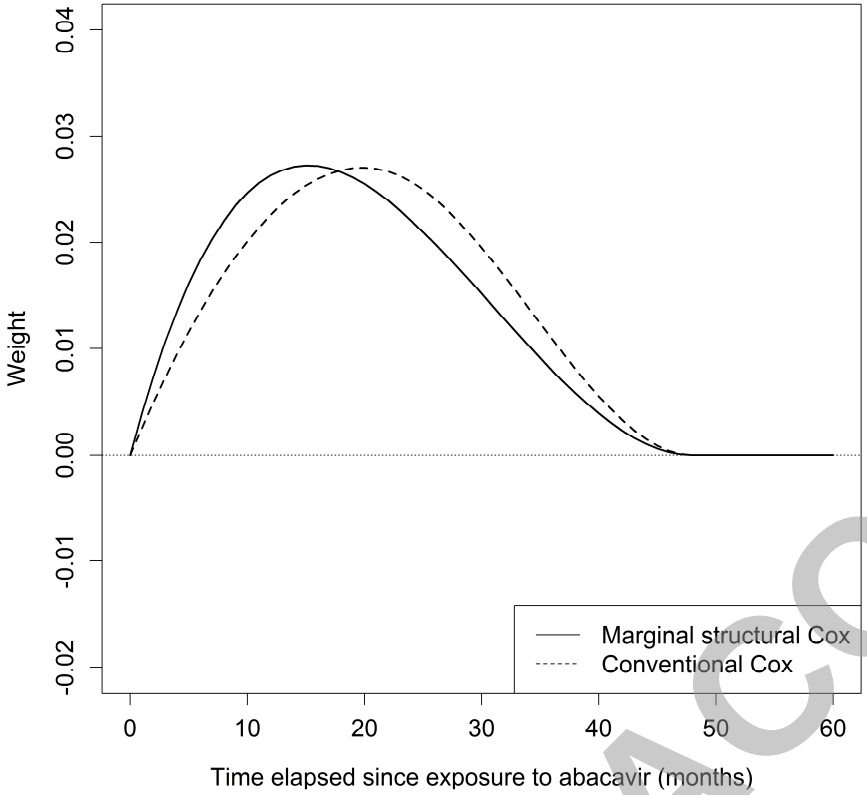
models. The covariates in these models included those used in the conventional models plus indicators for hypertension, dyslipidaemia, diabetes, Framingham risk score categories and continuous measures of CD4 cell count and log 10 HIV RNA.

[c] Model 1 reproduced an analysis reported by the D:A:D - their estimates were HR 1.03, 95% CI 0.96 to 1.10, per year for cumulative exposure and HR 1.63, 95% CI 1.30 to 2.04, for recent exposure [1].

[d] Model 2 was suggested by cumulative exposure modelling – weight functions where the effect of current exposure could have a weight other than zero had negative weights for the earliest months of the four year interval suggesting that current exposure had a protective effect.

[e] Model 3 was suggested by cumulative exposure modelling – the best fitting weight function (Figure 1, left) suggested that cumulating exposure to abacavir over the past 6 to 36 months causes the greatest increase in the risk of a CVD event.

Figure 1. The effect of exposure to abacavir on the risk of cardiovascular disease events: the estimated weight function (left) and the estimated total cumulative effect (as a hazard ratio) of always being treated with abacavir over the past 48 months versus never being treated with abacavir (right). Exposure more than four years ago was assumed to have no effect on current risk. Functions are shown for cumulative exposure modelling with both marginal structural (solid curve) and conventional (dashed curve) Cox models.[23,50] Of the nine alternative weight functions considered, the best fitting weight function had a single knot and (exposure) weights of zero at both the beginning and end of the four year interval.



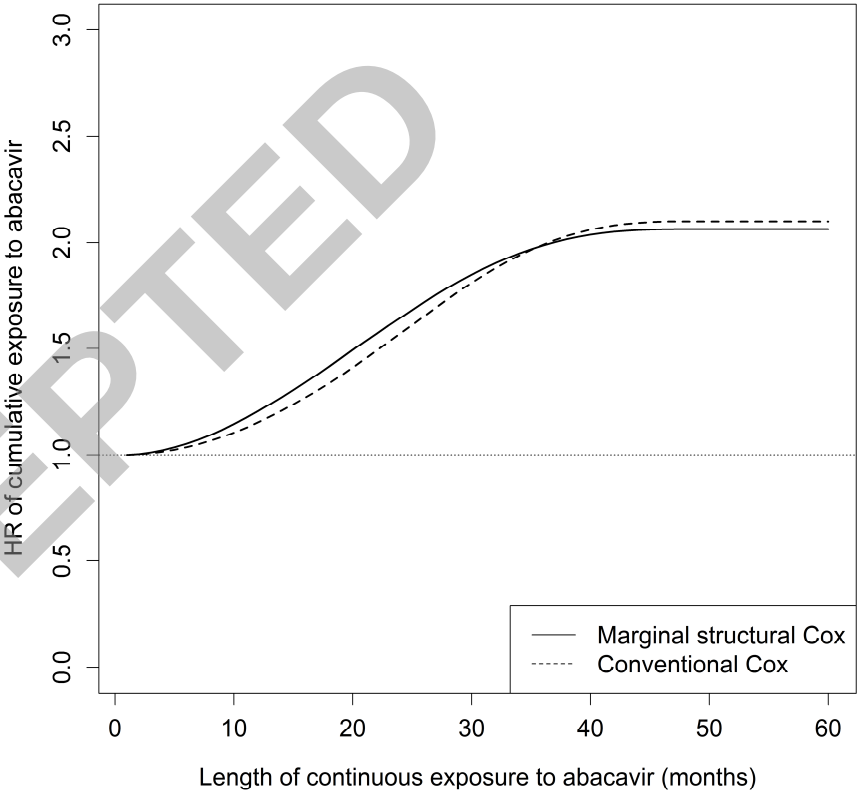
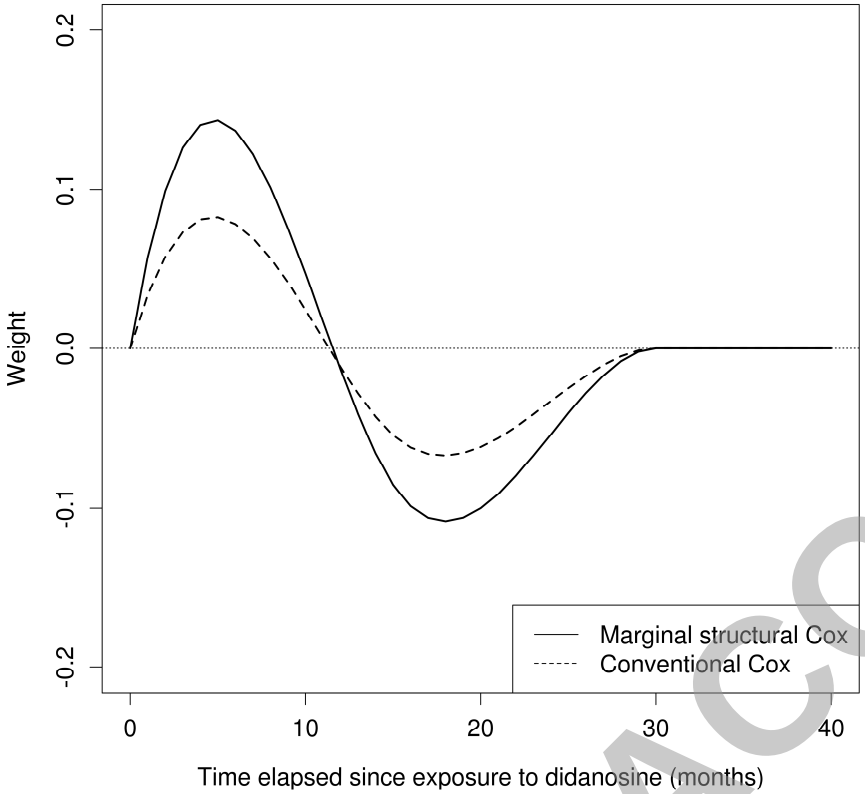


Figure 2. The effect of exposure to didanosine on the risk of cardiovascular disease events: the estimated weight function (left) and the estimated total cumulative effect (as a hazard ratio) of always being treated with didanosine over the past 30 months versus never being treated with didanosine (right). Exposure more than 30 months ago was assumed to have no effect on current risk. Functions are shown for cumulative exposure modelling with both marginal structural (solid curve) and conventional (dashed curve) Cox models.[23,50] Of the nine alternative weight functions considered, the best fitting weight function had a single knot and (exposure) weights of zero at both the beginning and end of the 30 month interval.



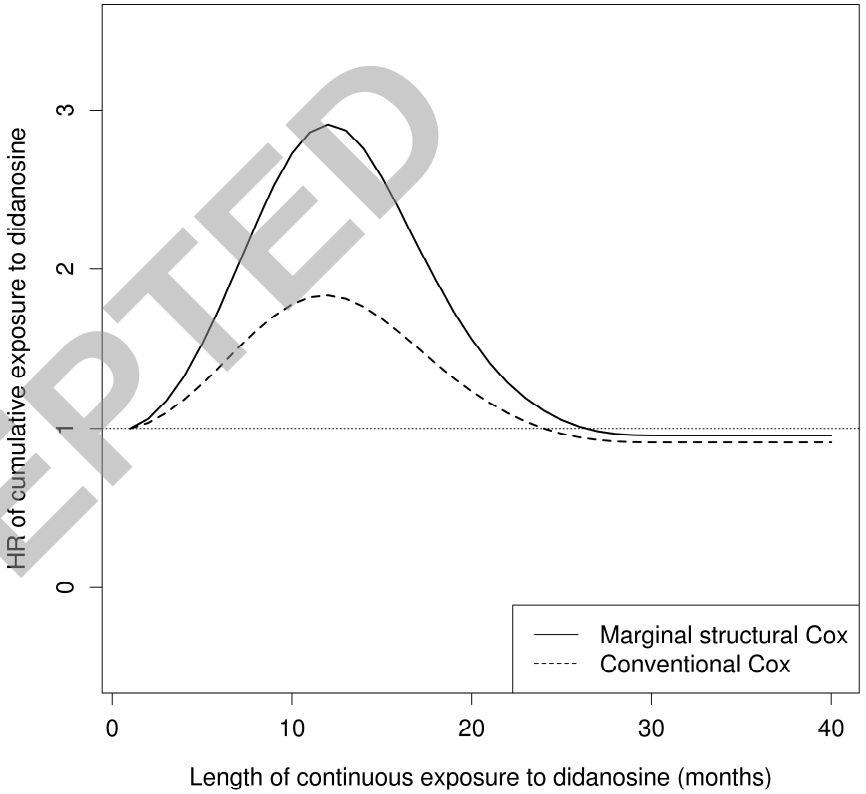


Figure 3. The effect of exposure to tenofovir on the risk of cardiovascular disease events: the estimated weight function (left) and the estimated total cumulative effect (as a hazard ratio) of always being treated with tenofovir over the past 48 months versus never being treated with tenofovir (right). Exposure more than four years ago was assumed to have no effect on current risk. Functions are shown for cumulative exposure modelling with both marginal structural (solid curve) and conventional (dashed curve) Cox models.[23,50] Of the nine alternative weight functions considered, the best fitting weight function had a single knot and (exposure) weights of zero at both the beginning and end of the four year interval.