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

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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.\textsuperscript{1} Subsequent meta-analyses of randomised controlled trials failed to find evidence of this association.\textsuperscript{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.\textsuperscript{6} Nevertheless, their results caused an unprecedented change in prescribing behaviour.\textsuperscript{7}

Neither cumulative nor past exposure to abacavir seemed to increase the risk of these events \textsuperscript{7} 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.\textsuperscript{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 \textsuperscript{9-12} and analyses of other observational cohorts led to inconsistent results.\textsuperscript{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.\textsuperscript{9} Indeed patients at higher risk of CVD were more likely to receive abacavir \textsuperscript{1,15} (a ‘channelling bias’ \textsuperscript{16} or ‘confounding by indication’ \textsuperscript{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.\textsuperscript{18} This situation necessitates more complex methods of analysis; marginal structural modelling \textsuperscript{19} in particular has been recommended.\textsuperscript{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.\textsuperscript{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 \textsuperscript{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. 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, 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; since 2005, each death has been documented using a CoDe cause of death form.

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; 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{31} We define chronic kidney disease as an estimated glomerular filtration rate (calculated using CKD-EPI equation\textsuperscript{31}) below 60 ml/min/1.73m\textsuperscript{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,\textsuperscript{33} and its population of abacavir naive patients corresponds to the ‘full population’ used in a recent analysis by the NA-ACCORD.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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).

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 6 to 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{1,36} Recent use should appear harmful, as it has in many studies,\textsuperscript{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.\textsuperscript{13}

An early protective effect could arise because abacavir, as part of an effective therapy, reduces viral replication, a risk factor for CVD events,\textsuperscript{37,38} or because of a ‘reverse causation bias’\textsuperscript{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.\textsuperscript{9} However for patients that smoke, giving up smoking leads to a greater reduction in CVD risk than avoiding exposure to abacavir.\textsuperscript{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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{43,44} Integrase inhibitors are well-tolerated antiretrovirals because they do not interfere with normal cellular processes\textsuperscript{45} and are therefore considered suitable for patients at risk of cardiovascular disease.\textsuperscript{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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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,\textsuperscript{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.\textsuperscript{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 naïve 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,\textsuperscript{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,\textsuperscript{47} as abacavir may interact with cytidine analogues lamivudine and emtricitabine.\textsuperscript{48} The heart, with its high metabolic demand, is rich in mitochondria and is susceptible to mitochondrial damage, especially as it ages.\textsuperscript{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 \textsuperscript{1,35} or even a protective effect.\textsuperscript{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.\textsuperscript{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

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6. References


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. 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. 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. 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.

<table>
<thead>
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<th>Characteristic</th>
<th>Patients with a cardiovascular disease event</th>
<th>Patients without a cardiovascular disease event</th>
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<tbody>
<tr>
<td></td>
<td>All Exposed to antiretroviral therapy</td>
<td>Exposed to abacavir Exposed to didanosine Exposed to tenofovir</td>
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<tr>
<td>General</td>
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<td></td>
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<tr>
<td>Number of patients</td>
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<td>Age (years)</td>
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<td>Suppressed viral load</td>
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<td>Body mass index &gt; 26 kg/m²</td>
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<tr>
<td>Use of anti-hypertensive medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>0.59</td>
<td>0.61</td>
</tr>
<tr>
<td>Lipid levels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.4</td>
<td>5.5</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Use of lipid lowering medication</td>
<td>0.32</td>
<td>0.32</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.65</td>
<td>0.63</td>
</tr>
<tr>
<td>Lipodystrophy</td>
<td>0.45</td>
<td>0.46</td>
</tr>
<tr>
<td>Framingham risk score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (&lt;10%)</td>
<td>0.40</td>
<td>0.41</td>
</tr>
<tr>
<td>Moderate (10-20%)</td>
<td>0.45</td>
<td>0.43</td>
</tr>
<tr>
<td>High (&gt;20%)</td>
<td>0.15</td>
<td>0.17</td>
</tr>
<tr>
<td>Cardiovascular disease event</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>0.51</td>
<td>0.56</td>
</tr>
<tr>
<td>Invasive cardiovascular procedure</td>
<td>0.72</td>
<td>0.72</td>
</tr>
<tr>
<td>Cardiovascular death</td>
<td>0.12</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not applicable.
Patients could be exposed to none of these three drugs or to one or more.

HIV RNA undetectable or below 50 copies / mL.

Cardiovascular disease event before the patient’s first cardiovascular risk assessment.

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

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

Estimated glomerular filtration rate (calculated using CKD-EPI equation $^{32}$) < 60 ml/min/1.73m$^2$

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

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

Patient and clinician report either body fat loss or body fat gain.

Estimated risk of cardiovascular disease in the next 10 years.

More than one event can occur during the same month.

Coronary angioplasty/stenting, coronary artery by-pass grafting, carotid endarterectomy, procedures on other arteries.
<table>
<thead>
<tr>
<th>Exposure parameters</th>
<th>Conventional model&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Marginal structural model&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio (95% CI)</td>
<td>Hazard ratio (95% CI)</td>
</tr>
<tr>
<td>Model 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative exposure (per year)</td>
<td>1.04 (0.99, 1.10)</td>
<td>1.04 (0.97, 1.10)</td>
</tr>
<tr>
<td>Recent exposure within past 0 to 6 months</td>
<td>1.50 (1.12, 2.00)</td>
<td>1.63 (1.14, 2.32)</td>
</tr>
<tr>
<td>Model 2&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative exposure (per year)</td>
<td>1.05 (1.00, 1.10)</td>
<td>1.05 (0.98, 1.11)</td>
</tr>
<tr>
<td>Recent exposure within past 1 to 6 months</td>
<td>3.69 (2.36, 5.75)</td>
<td>4.61 (2.59, 8.23)</td>
</tr>
<tr>
<td>Current exposure</td>
<td>0.36 (0.23, 0.55)</td>
<td>0.28 (0.15, 0.50)</td>
</tr>
<tr>
<td>Model 3&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative exposure within the past 7 to 36 months (per year)</td>
<td>1.25 (1.04, 1.51)</td>
<td>1.22 (0.98, 1.52)</td>
</tr>
<tr>
<td>Recent exposure within past 1 to 6 months</td>
<td>3.20 (1.97, 5.19)</td>
<td>4.06 (2.24, 7.34)</td>
</tr>
<tr>
<td>Current exposure</td>
<td>0.35 (0.22, 0.54)</td>
<td>0.27 (0.15, 0.50)</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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.

<sup>b</sup> Models fit using inverse probability weights, with weights found using 8 different logistic regression
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.

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. 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.

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. 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.
Time elapsed since exposure to abacavir (months)

Weight

Marginal structural Cox
Conventional Cox

Length of continuous exposure to abacavir (months)

HR of cumulative exposure to abacavir

Marginal structural Cox
Conventional Cox
**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.\textsuperscript{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. 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.