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Cardiovascular Disease & Use of Contemporary Protease Inhibitors: The D:A:D Study

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Abstract

Background

While older HIV protease inhibitors (PIs) have been associated with excess cardiovascular disease (CVD) risk it is unclear whether this also applies to more contemporary PIs.

Methods

We followed D:A:D cohort participants from 2009 to the earliest of CVD (centrally validated myocardial infarction, stroke, sudden cardiac death or invasive cardiovascular procedures), last visit plus six months or 2016. Poisson regression models assessed associations between CVD and use of the contemporary PIs atazanavir/ritonavir and darunavir/ritonavir.

Findings

During 6.96 (IQR 6·28-7·08) years median follow-up 1,157 persons developed CVD (Incidence rate 5·34/1000 PYFU [95%CI 5·03-5·65]). The CVD incidence increased gradually from 4·91 [4·59-5·23]/1000 PYFU in individuals unexposed to darunavir/ritonavir to 13·67 [8·51-18·82]/1000 PYFU in those exposed >6 years, while atazanavir/ritonavir changes were less pronounced (5·03 [4·69-5·37] to 6·68 [5·02-8·35] /1000 PYFU). After adjustment, keeping factors on the potential causal pathway from PI/ritonavir use to CVD fixed at baseline, darunavir/ritonavir , but not atazanavir/ritonavir, use was associated with excess CVD risk (IRR 1·59 [1·33-1·91] and 1·03 [0·90-1·18]/5 years, respectively). The association remained after adjustment for time-updated factors on the potential causal pathway; myocardial infarction and stroke separately; bilirubin; and stratifying for using darunavir/ritonavir as the first ever PI/ritonavir, with a non-nucleoside reverse transcriptase inhibitor, with prior virological failure and at high CVD risks.

Interpretation

Cumulative darunavir/ritonavir, but not atazanavir/ritonavir, use was associated with gradually increasing CVD risks. Causal inference is limited by the observational study nature, but the findings call for investigations into possible mechanisms.

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Manuscript Text

Introduction

Advances in the antiretroviral treatment for controlling HIV have led to significantly lower levels of drug related adverse events [1]. Meanwhile recent data suggest that the cardiovascular disease (CVD) risk is increasing amongst people living with HIV due to increased frequency and severity of CVD risk factors such as hypertension, diabetes, dyslipidemia and chronic kidney disease (CKD)[2]. With an aging HIV-positive population at increasing CVD risk, it is therefore becoming increasingly important to tailor antiretroviral treatment to fit the individual risk profile [3]. Earlier studies, including prior analyses from the Data collection on Adverse effects of antiretroviral Drugs (D:A:D) study, have demonstrated associations between longer cumulative use of older HIV protease inhibitors (PIs) indinavir and ritonavir boosted lopinavir and CVD [4-7]. The mechanism of this association is believed to be, at least partly, mediated by dyslipidemia, and drugs within the PI drug class are known to be associated with different metabolic profiles [8].

Presently, the two most frequently used PIs are ritonavir boosted darunavir and atazanavir [1, 9]. Darunavir/ritonavir and atazanavir/ritonavir also remain part of the recommended first line treatment combinations for treatment-naïve adult HIV-positive persons in a number of guidelines including the European AIDS Clinical Society (EACS), the British HIV Association (BHIVA) and the US Department of Health and Human Services (DHHS) guidelines [1, 9, 10].

It is unknown if use of more contemporary PIs in the current modern antiretroviral treatment era pose a similar CVD risk as the older PIs. In a prior D:A:D analysis, atazanavir/ritonavir was not found to be associated with excess CVD risk, however the follow-up time in that analyses was relatively short [11].

Methods

Study population

The D:A:D study is a prospective cohort collaboration established in 1999 following more than 49,000 HIV-1-positive persons from 11 cohorts in Europe, the United States and Australia; details have been published previously [4]. The primary study aim is investigation of associations between antiretroviral treatment and serious non-AIDS events. Data on centrally validated clinical events include myocardial infarction (MI), sudden cardiac death, stroke, invasive cardiovascular procedures (ICP) and fatal cases is collected in real-time during routine clinical care, regularly monitored and reviewed by external experts. Data on socio-demographic factors, cardiovascular risk factors and treatment, laboratory biomarkers, antiretroviral treatment and HIV-variables including HIV viral load (VL), CD4 counts, AIDS events and viral hepatitis is collected electronically at enrolment and at every six months thereafter.

Endpoint definition

CVD was defined as a composite endpoint including MI, stroke, sudden cardiac death and ICP: coronary bypass, coronary angioplasty and carotid endarterectomy. All events are reported using study specific designated event forms, and centrally adjudicated blind to the individual's use of antiretroviral treatment using standardised algorithms including the WHO MONICA Study for MIs and sudden cardiac death (www.chip.dk/Studies/DAD/Study-Documents)[12, 13].

Statistical analyses

We included data from D:A:D study participants under follow-up from January 1st 2009 (reflecting the wider darunavir/ritonavir licensing in Europe and use of more contemporary antiretrovirals) until the earliest of incident CVD, last visit plus six months or February 1st 2016. We calculated the incidence rates of CVD per 1000 person years of follow-up (PYFU) and stratified by cumulative (per 5 year) exposure to atazanavir/ritonavir and darunavir/ritonavir. We used Poisson regression models to assess associations between incident CVD and use of atazanavir/ritonavir and darunavir/ritonavir after adjustment for potential confounders. Due to concerns about adjustment for factors on the potential causal pathway between use of darunavir/ritonavir and atazanavir/ritonavir and CVD our primary model was *a priori* designed to adjust for such factors at baseline only. Factors considered to potentially lie on the causal pathway included body mass index (BMI), dyslipidaemia (total cholesterol >6.2 mmol/L, high-density lipoprotein cholesterol <0.9 mmol/L, triglyceride >2.3 mmol/L, or use of lipid-lowering treatment), CD4 count, diabetes (confirmed on an event form or use of antidiabetics) and CKD (confirmed, $\geq$3 months apart, eGFR $\leq$ 60 mL/min/1.73m^2). Other factors included in the primary model were gender, race, age, prior CVD, enrolment cohort, baseline date, HIV risk acquisition and nadir CD4 count (all time-fixed), cumulative use of lopinavir/ritonavir and indinavir, recent use of abacavir, VL, prior AIDS, family history of CVD, smoking, hypertension (blood pressure >140/>90 or use of antihypertensives) and viral hepatitis B and C status (all time-updated).

We calculated cumulative exposure to antiretroviral drugs as in previous D:A:D analyses [4, 12]. Before drug initiation both cumulative exposure time and time since stopping the drug was zero. When stopping the drug the cumulative time of exposure stopped increasing, and the time off the drug started accumulating. If the person restarted the drug, time since stopping was set back to zero and cumulative exposure started increasing from the point it had previously stopped.

In a sensitivity analysis we allowed the factors on the potential causal pathway between darunavir/ritonavir and atazanavir/ritonavir use and CVD to change during follow-up by adjusting for these as time-updated variables. Prior analyses have suggested that increased levels of bilirubin may have a cardio-protective effect[14]. As atazanavir/ritonavir is associated with hyperbilirubinemia in additional models we investigated the impact of adjusting for time-updated bilirubin levels (fitted as a continuous and categorical variable). In further sensitivity analyses we investigated the effect of excluding those never exposed to

atazanavir/ritonavir or darunavir/ritonavir respectively, separating the CVD composite endpoint to investigate if the association differed for coronary (MI) or cerebrovascular events (stroke) and to exclude everyone with prior history of CVD. To assess the specificity of any potential PI/ritonavir association with CVD in a separate analysis we also examined the association between cumulative use of efavirenz, another frequently used third antiretroviral agent, and CVD.

To assess the possibility of confounding by indication we also tested the primary model associations for interactions with the D:A:D CVD risk score (very high (>10%) vs low (<1%) five-year estimated CVD risk) and the impact of virological failure (defined as VL >50 copies/mL >6 months after starting/changing treatment), using darunavir/ritonavir as the first ever PI-containing regimen, using antiplatelet drugs and a later baseline date[3].

All statistical analyses were carried out using SAS version 9·3 (Cary, NC, USA).

No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

During a median follow-up of 6·96 years (interquartile range, IQR, 6·28-7·08) 1,157 of 35,711 persons (3·24%) experienced a CVD event with an incidence rate (IR) of 5·34/1000 persons years of follow-up (PYFU) [95% confidence interval, CI, 5·03-5·65], figure 1.

At baseline, median age was 44 years (IQR 38-51) and median CD4 count 501 cells/mm^3 (IQR 360-689), 73·61% were male, 47·84% white, 85·38% antiretroviral treatment experienced of whom 76·42% were virologically suppressed, table 1. With regards to traditional CVD risk factors 40·18% had dyslipidemia, 39·24% were current smokers, 9·72% had hypertension, 5·05% diabetes and 1·26% prior CVD. Those with incident CVD were generally older, had lower median CD4 counts and a higher proportion were at high 5-years estimated CVD- and CKD risks as compared to those without CVD.

At baseline 18·44% and 3·97% were ever exposed to atazanavir/ritonavir and darunavir/ritonavir respectively. At time of last visit 26·62% and 22·27% were ever exposed to atazanavir/ritonavir and darunavir/ritonavir respectively. At time of CVD the median exposure to atazanavir/ritonavir, among those ever exposed, was 3·07 (IQR 1·21-5·33) and for darunavir/ritonavir 2·56 (IQR 1·21-4·01) years. There were no individuals on darunavir/ritonavir monotherapy included in the analysis.

The crude CVD IR increased gradually from 4·91/1000 PYFU [4·59-5·23] in individuals never exposed to darunavir/ritonavir to 13·67/1000 PYFU [8·51-18·82] in individuals exposed >6 years. The

atazanavir/ritonavir IR also increased, although more slowly, from 5·03/1000 PYFU [4·69-5·37] in individuals never exposed to atazanavir/ritonavir to 6·68/1000 PYFU [5·02-8·35] in individuals exposed >6 years, figure 2. In univariate analysis both cumulative use of atazanavir/ritonavir (incidence rate ratio, IRR, 1·25/5 years [1·10-1·43]) and darunavir/ritonavir (1·93/5 years [1·63-2·28]) was associated with significantly increased CVD rates, figure 3 and appendix 1 page 22. After adjustment for potential confounders in our primary model, where factors on the potential causal pathway from PI/ritonavir use to CVD were fixed at baseline, only the darunavir/ritonavir association remained statistically significantly associated with CVD (adjusted IRR, aIRR, 1·59/5 years [1·33-1·91]). The number needed to treat to harm (NNTH) for darunavir/ritonavir stratified by the underlying estimated five-year CVD risk were 533 [314-706]/5 years and 15 [13-17]/5 years for those at low vs very high CVD risk, figure 4.

Additional adjustment using time-updated values for the factors potentially on the causal pathway to CVD did not impact the overall association (darunavir/ritonavir 1·53/5 years [1·28-1·84]), suggesting that the association between darunavir/ritonavir and CVD is not mediated by any of these factors, and most notably not by dyslipidaemia, figure 3. Approximately 90% of those with CVD had $\geq$1 cholesterol measurement with a median of 1·81 (IQR 0·98-2·44) measurements annually.

Adjustment for time-updated bilirubin levels, regardless of whether this was fitted categorically or as a continuous variable, did not impact on the associations with CVD (atazanavir/ritonavir 1·05/5 years [0·89-1·23] and darunavir/ritonavir 1·60/5 years [1·31-1·96]), figure 3. Likewise we found no evidence of an interaction between atazanavir/ritonavir or darunavir/ritonavir and bilirubin on CVD rates (atazanavir/ritonavir p= 0·68 and darunavir/ritonavir p=0·21 for interaction).

Figure 2 suggests an overall lower rate of CVD in those never exposed to atazanavir/ritonavir or darunavir/ritonavir, which could increase the associations observed between the two drugs and CVD. These lower rates did, however, not explain the associations, as the darunavir/ritonavir association remained similar (1·42/ 5 years [1·08-1·87]) in an intention to treat analysis excluding person-years of follow-up in those without exposure to darunavir/ritonavir, while the atazanavir/ritonavir association declined below 1 (0·93/5 years [0·75-1·15]).

Excluding individuals with any prior CVD also showed consistent results for darunavir/ritonavir and CVD 1·59/5 years [1·31-1·92].

Separating the composite CVD endpoint into stroke and MI reduced the overall power of the analysis with 477 MIs (IR 2·18 [1·98-2·30]) and 395 strokes (1·80 [1·62-1·98]), but with entirely consistent results (darunavir/ritonavir and MI 1·51/5 years [1·13-2·02] and darunavir/ritonavir and stroke 1·49/5 years [1·08-2·07]). Excluding individuals with any prior CVD further reduced power with 432 MIs (IR 2·00 [1·81-2·18]**)**

and 379 strokes (IR 1·75 [1·57-1·93]), but also showed consistent results between darunavir/ritonavir and MI (1·53/5 years [1·13-2·07]) and a slightly weaker association with stroke (1·37/5 years [0·97-1·94]).

A 'dip' in the CVD IR appears after 3-4 years of atazanavir/ritonavir and 4-5 years of darunavir/ritonavir use. A number of exploratory analyses investigated possible explanations including the impact of individual participating cohort and calendar year, but found no clear explanation for these lower rates. Censoring follow up times before the dips (3 years for atazanavir/ritonavir and 4 years for darunavir/ritonavir), the observations strengthened for both drugs (atazanavir/ritonavir 1·38/5 years [0·80-2·39] and darunavir/ritonavir 2·26/5 years [1·54-3·32]), but remained statically non-significant for atazanavir/ritonavir. Therefore, this variation over time may reflect a chance finding. By including all data, including the dip, we are underestimating the strength of the association between use of atazanavir/ritonavir and darunavir/ritonavir and CVD.

As the D:A:D study does not collect data on drug doses, as a proxy for using darunavir/ritonavir 600/100 mg bid vs 800/100 mg qd we assessed if associations differed depending on having experienced virological failure or not. The association remained similar amongst those with (1·67/5 years [1·39-2·01]) and without prior virological failure (2·02/5 years [1·00-4·01]), and further there was no observed difference between darunavir/ritonavir being used as the first ever PI/r containing regimen or not (p=0·29 for interaction). Associations were also unaffected after stratification for whether darunavir/ritonavir was used in combination with non-nucleoside reverse transcriptase inhibitor or not (p=0·43). Additionally there was no evidence of an interaction between the darunavir/ritonavir and CVD association in those at high vs low estimated 5 year D:A:D CVD risk (p=0·12), and in those with vs without prior CVD (p=0·51). Finally, adjustment for (ever) use of antiplatelets including aspirin, which is predominantly used in individuals with prior CVD or high estimated CVD risk did not impact the association (atazanavir/ritonavir 1·03/5 years [0·90-1·17]) and darunavir/ritonavir 1·52/5 years [1·26-1·82]).

In a final analysis we examined the use of another frequently used third antiretroviral agent, efavirenz, with 42·98% ever exposed at baseline and 49·44% at time of CVD, without any evidence of an association with CVD (0·93/5 years [0·86-1·02]).

Discussion

In this large heterogeneous cohort of contemporary treated HIV-positive persons, cumulative use of darunavir/ritonavir, but not atazanavir/ritonavir, was independently associated with a small, but gradually increasing risk of centrally adjudicated CVD events. For individuals at high CVD risk i.e. with an absolute five-year CVD risk of 10%, using darunavir/ritonavir for five years would increase the absolute CVD risk to almost 16%. Expressed differently, if 15 persons at very high CVD risk were exposed to darunavir/ritonavir for five years, one may develop CVD (NNTH= 15) attributable to darunavir/ritonavir. As the NNTH varies

depending on the underlying absolute CVD risk (from 15 to 533 going from very high to low five-year risk), caution with darunavir/ritonavir use is particularly warranted in those at high CVD risk.

Cautious interpretation of our findings is indeed warranted due to the observational nature of the study and the risks of unmeasured confounding. However, as darunavir/ritonavir is currently recommended as part of a first line treatment regimens in several guidelines, and use of darunavir/ritonavir is increasing in recent years, our findings creates impetus for other studies to investigate possible mechanisms[1, 9] . We also encourage other large studies to undertake analyses of darunavir/ritonavir and CVD to investigate reproducibility of our findings. There is currently not enough follow-up time in the D:A:D study to investigate if discontinuation of darunavir/ritonavir use will lead to reductions in the observed increased CVD incidence. Such analyses will however be paramount for further building on the evidence of potential causality. Meanwhile, given the large size, relatively long follow-up, heterogeneous nature, rigorously defined CVD endpoints, strength of the darunavir/ritonavir CVD association, biological gradient and specificity of the finding this safety signal should lead to thoughtful considerations for whether other more CVD friendly drugs are available as part of individual care.

The strength of the observed darunavir/ritonavir association is of a similar size as we have previously observed in the years 1999-2005 for the older PIs indinavir (1·47/5 years) and lopinavir/ritonavir (1·54/5 years), but in contrast the darunavir/ritonavir association does not seem to be modified by dyslipidemia, or any of the other factors hypothesized to potentially lie on the causal pathway to CVD[4, 12]. Furthermore, our data do not suggest this lack of an effect modification is due to lack of lipid measurements. Previous studies collectively suggest a lipid profile of darunavir/ritonavir that is similar or slightly superior to that of atazanavir/ritonavir in treatment-experienced individuals, but superior to that of lopinavir/ritonavir [15-17]. Several studies have suggested that certain PIs may contribute to the CVD pathogenesis via changes in lipid pathways, also via other lipid pathways than those directly measurable by more simple definitions of dyslipidemia [6, 18-20]. Another possibility is that the mechanism include entirely different atherosclerotic pathways that are not captured by more traditional CVD risk factors [21] similar to what has been documented for abacavir and CVD[22, 23]. One study has, as such, suggested that PIs generally increases levels of oxidative stress more than other antiretrovirals which may in turn contribute to development of CVD [24].

The differentiated associations between cumulative use of darunavir/ritonavir and atazanavir/ritonavir and CVD confirm our earlier analyses of atazanavir/ritonavir and CVD, and do not suggest a uniform PI-drug class or ritonavir-specific effect on CVD risk. A small study has however suggested higher intracellular doses of ritonavir in persons on darunavir as opposed to persons on atazanavir, which may increase risk of adverse effects including CVD [25]. To ensure comparability we included only individuals on boosted atazanavir and darunavir in this analysis, however a prior D:A:D subanalysis found consistent results when including unboosted atazanavir [11]. Another recent study found that cumulative use of darunavir/ritonavir,

but of no other PI was associated with markers of subclinical coronary atherosclerosis, but also longer HIV duration, viral replication and lower CD4 count [26]. The significant univariate association between atazanavir/ritonavir and CVD seemed to be explained primarily by increasing age in those on atazanavir/ritonavir [11]. We found no evidence to support the hypothesis that the lack of an association between atazanavir/ritonavir and CVD was explained by a protective effect of atazanavir/ritonavir related increased bilirubin levels. It is possible that it is not an atazanavir/ritonavir associated increased bilirubin level per se, but rather that other PIs than atazanavir/ritonavir do not cause bilirubin changes that mediates the differential CVD association. Additional insight into the mechanism of the apparent lack of association between ATV/r and CVD and the role of bilirubin is however necessary, as more indirect bilirubin-related cardio-protective pathways may be involved. Conversely, the lack of an atazanavir/ritonavir and CVD association may also reflect that atazanavir/ritonavir does not activate proatherogenic pathways the same way other PIs do.

In terms of alternative commonly used third antiretroviral agents, we found no evidence of an association between CVD and cumulative efavirenz use, whilst there is still not adequate long follow-up data on integrase inhibitors to reliably undertake such safety analyses.

None of the original trials [15, 16, 27] investigating darunavir/ritonavir efficacy and safety reported, or were able to report, increased CVD rates. It is possible that this lack of data is related to the high risk of type II errors in these trials due to the general short follow-up time, limited size, focus on laboratory endpoints, and for the majority inclusion of individuals with a relatively low CVD risk. Further, these studies were not designed specifically or powered to investigate longer-term adverse effects. The European darunavir/ritonavir product information does however declare angina pectoris an uncommon event (up to 1/100), and acute MI a rare event (up to 1/1000) associated with darunavir/ritonavir use, while such safety data is not included in the US package insert[28, 29].

After the initial investigations in D:A:D, Janssen has also evaluated safety data on darunavir/ritonavir exposure and CVD in a recent poster presentation[30]. The investigators did not find supportive evidence for an association between darunavir/ritonavir and CVD, but the study had significantly less power compared to the data presented here with 5,721 primarily treatment naïve persons (vs 35,711 persons in D:A:D with substantial treatment experience), 66 CVD events (vs 1,1157 in D:A:D) and a median follow-up of 1.8 years (vs 6·96 years in D:A:D).

It is unlikely that the observed darunavir/ritonavir association with CVD is explained by confounding by indication. Darunavir/ritonavir was, due to its high genetic barrier, initially used predominantly as part of salvage therapy. By limiting follow-up to after 2009 in this analysis we aimed to reflect a broader use of darunavir/ritonavir. In addition our data showed no difference when stratifying analyses according to

whether darunavir/ritonavir was used as first ever PI regimen or not, and a robust association was found in both those with and without a history of virological failure. Also our data did not suggest an interaction with recent abacavir use which has consistently been associated with CVD in D:A:D. Finally, we accounted for common factors known to influence the choice of a specific ART regime and for factors related to CVD risks.

Limitations

The study has important limitations to acknowledge. Due to the observational nature of the study we are unable to exclude the possibility of unmeasured confounding, and it is therefore not possible to draw definitive conclusions on causal inference of our findings. As the study does not collect data on drug doses, we are further unable to directly assess if using darunavir/ritonavir 600/100 mg bid vs. 800/100 mg qd differ in the association with CVD, although associations were not significantly different when comparing use in those with virological failure (where a 600/100 mg bid dosage is recommended) versus those without (800/100 mg qd recommended). The ODIN trial showed a better lipid profile when using darunavir/ritonavir qd as compared to bid, but in our analysis additional adjustment for time-updated dyslipidaemia did not change associations [(27)]. While darunavir/ritonavir is always boosted by a second agent (ritonavir or cobicistat) atazanavir may be used unboosted, however we only have very limited data on unboosted atazanavir after 2009 and are unable to study the CVD risk from using atazanavir unboosted. We have too limited follow-up data on cobicistat to enable analysis using an alternative PI boosting agent than ritonavir to investigate if the observed associations differ.

Conclusions

In this large heterogeneous cohort of HIV-positive persons with a median follow-up time of seven years, cumulative use of darunavir/ritonavir, but not atazanavir/ritonavir, was independently associated with a gradually increasing risk of centrally adjudicated CVD. Our findings remained consistent in a high number of sensitivity analysis, and call for a cautioned approach to darunavir/ritonavir use in particular in individuals at high CVD risk. The darunavir/ritonavir association with CVD was, in contrast to findings with older PIs, not modified by dyslipidemia, and calls for further investigations of possible mechanisms.

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Role of authors

L. Ryom had full access to all the data in the study and takes responsibility for the integrity of the data and analysis. L. Ryom, A. Mocroft and J. Lundgren proposed and developed the research question, and A. Mocroft performed the statistical analyses. W. El-Sadr, P. Reiss, O. Kirk, M. Law, A. Phillips, R. Weber, E. Fontas, A. d' Arminio Monforte, S. de Wit, F. Dabis, C.I. Hatleberg and C. Sabin contributed with ideas around study design and interpretation of data. L. Ryom wrote the first draft of the manuscript. All authors have seen and contributed to the final version of the manuscript.

Conflicts of Interests

L. Ryom, J. Lundgren, E. Fontas, C.I. Hatleberg, W. EL-Sadr, F. Dabis and S. De Wit have reported no conflicts of interest. P. Reiss has served as a scientific advisor to Bristol-Myers Squibb, Gilead Sciences, Grupo Ferrer, GlaxoSmithKline, Janssen Pharmaceuticals, Merck, Inc and ViiV Healthcare. He has served on data and safety monitoring boards and endpoint adjudication committees for Janssen Pharmaceuticals and his institution has received honoraria for speaking engagements at scientific conferences from Bristol-Myers Squibb, Gilead Sciences, Inc and GlaxoSmithKline. He has received research support from Gilead Sciences, ViiV Healthcare, Merck, Inc, Janssen Pharmaceuticals, Bristol-Myers Squibb, Abbott, and Boehringer Ingelheim. O. Kirk had prior/present board membership at ViiV Healthcare, Gilead Sciences and Merck, received payment for lectures and/or for development of educational presentations from Abbott, Gilead Sciences and Tibotec and had travel/accommodations/meeting expenses paid by Abbott, BMS, Gilead Sciences, Merck and ViiV Healthcare. M. Law has received research grants from Boehringer Ingelheim, Bristol Myer Squibb, Gilead Sciences, GlaxoSmithKline, Janssen Pharmaceuticals, Merck, Pfizer and Hoffman-LaRoche. A d'Arminio Monforte has past board membership at Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceuticals and Merck. A. Phillips received personal fees from Gilead Sciences, Abbvie, GlaxoSmithKline Vaccines and grants from Bristol-Myers Squibb. R.W. received travel grants from Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Merck Sharp & Dome, Pfizer, Roche, TRB Chemedica, and Tibotec, the clinic has received unrestricted educational grants from GlaxoSmithKline, ViiV, and Gilead Sciences. C. Sabin received personal fees from Gilead Sciences, Bristol-Myers Squibb, Janssen Pharmaceuticals, Abbott Pharmaceuticals, and ViiV Healthcare. A. Mocroft has received consultancy fees/honoraria/speaker fees from Bristol-Myers Squibb, Pfizer, Merck, Boehringer Ingelheim, and Gilead Sciences.

Ethical approval

All participating cohorts followed local national guidelines/regulations regarding patient consent and/or ethical review. In particular, of the countries represented by the participating cohorts, only Switzerland and Australia require specific ethical approval for D:A:D in addition to that required for their national cohorts (Swiss HIV Cohort Study and AHOD), France, Italy, and Belgium do not require specific ethical approval over-and-above that required for the individual cohorts (Nice/Aquitaine, Brussels St. Pierre and IcoNA, respectively), and the Netherlands do not require any specific ethical approval as data is provided as part of HIV care (ATHENA). For the EuroSIDA study (which includes the data from the BASS and Swedish cohorts), which contains participants from across many European countries, each participating site has a contractual obligation to ensure that data collection and sharing is done in accordance with national legislation; each site principal investigator either maintains appropriate documentation from an ethical committee (if required by law) or has a documented written statement to say that this is not required.

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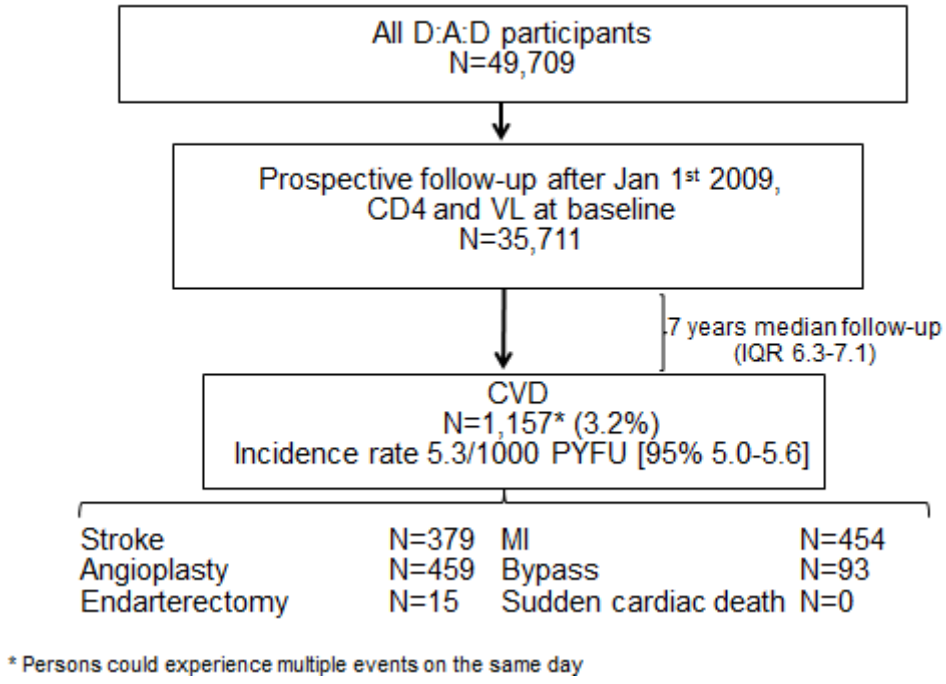
Figure 1 Inclusion of D:A:D Participants into the Analysis



* Persons could experience multiple events on the same day

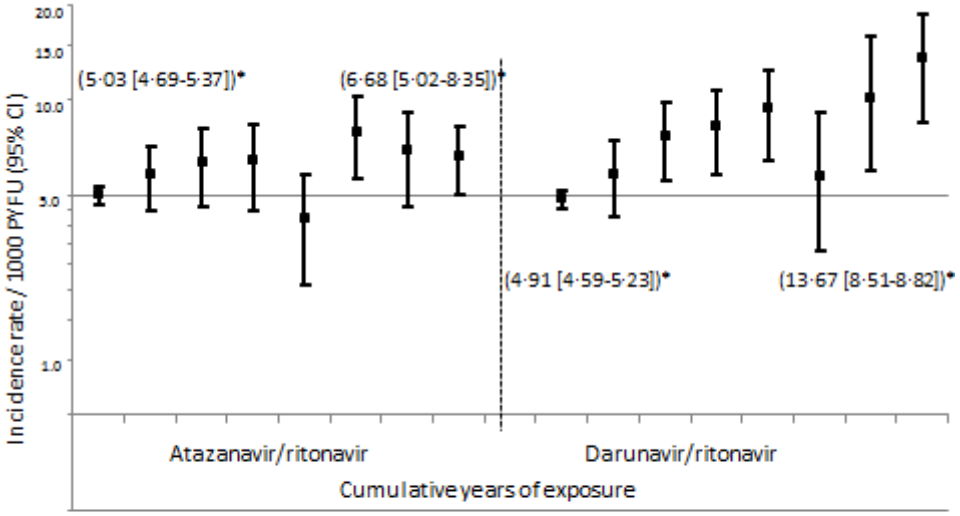
Table 1 Characteristics at baseline for all included and those experiencing a CVD event

		All		CVD	
		N	%	N	%
All		35711	100·00	1157	3·20
Gender	Male	26288	73·61	1022	88·33
Ethnicity	White	17085	47·84	579	50·04
	African	2379	6·66	32	2·77
	Other	727	2·04	17	1·47
	Unknown	15520	43·46	529	45·72
HIV	MSM	16447	46·06	647	55·92
exposure	IDU	4484	12·56	124	10·72
group	Heterosexual	12605	35·30	317	27·40
	Other	2175	6·09	69	5·96
Hepatitis B[1]	Negative	32564	91·19	1052	90·92
	Positive	1439	4·03	51	4·41
	Unknown	1708	4·78	54	4·67
Hepatitis C[2]	Negative	26792	75·02	890	76·92
status	Positive	6864	19·22	199	17·20
	Unknown	2055	5·75	68	5·88
ARV naïve	Yes	4944	13·84	67	5·88
Ever cART	Yes	30490	85·38	1084	93·69
Ever atazanavir/ritonavir	yes	6586	18·44	280	24·20
Ever darunavir/ritonavir	yes	1419	3·97	102	8·82
Ever efavirenz	yes	15350	42·98	572	49·44

VL <400 copies/mL	Yes	27290	76·42	999	86·34
Smoking status	Current	14014	39·24	550	47·54
	Previous	8299	23·24	296	25·58
	Never	9391	26·30	204	17·63
	Unknown	4007	11·22	107	9·25
Prior AIDS	Yes	9799	27·44	421	36·39
Prior CVD	Yes	451	1·26	97	8·38
Diabetes[3]	Yes	1805	5·05	163	14·09
Dyslipidemia[4]	Yes	14347	40·18	657	56·78
Hypertension[5]	Yes	3471	9·72	220	19·01
D:A:D CKD Risk score[6]	≤-1	8577	27·56	91	8·72
	0-4	10597	34·05	268	25·77
	≥5	11952	38·40	685	65·61
D:A:D CVD risk score[6]	1%	8463	25·47	46	4·19
	1-5%	18787	56·54	504	45·94
	5-10%	4226	12·72	316	28·81
	>10%	1753	5·28	231	21·06
		Median	IQR	Median	IQR
Age (years)		44	38-51	52	46-60
CD4 (cells/mm^3)		501	360-689	357	500-713
Nadir CD4 (cells/mm^3)		210	100-322	165	67-272

1. HBV positive: positive HBV surface antigen, HBV e antigen, or HBV DNA 2. anti-HCV positive and HCV-RNA positive/unknown 3. Confirmed in a case report form or use of antidiabetics 4. total cholesterol >6.2 mmol/L, high-density lipoprotein cholesterol <0.9 mmol/L, triglyceride >2.3 mmol/L, or lipid-lowering treatment 5. Blood pressure >150/>100 or use of antihypertensive treatment 6. Estimated as a five-years risk

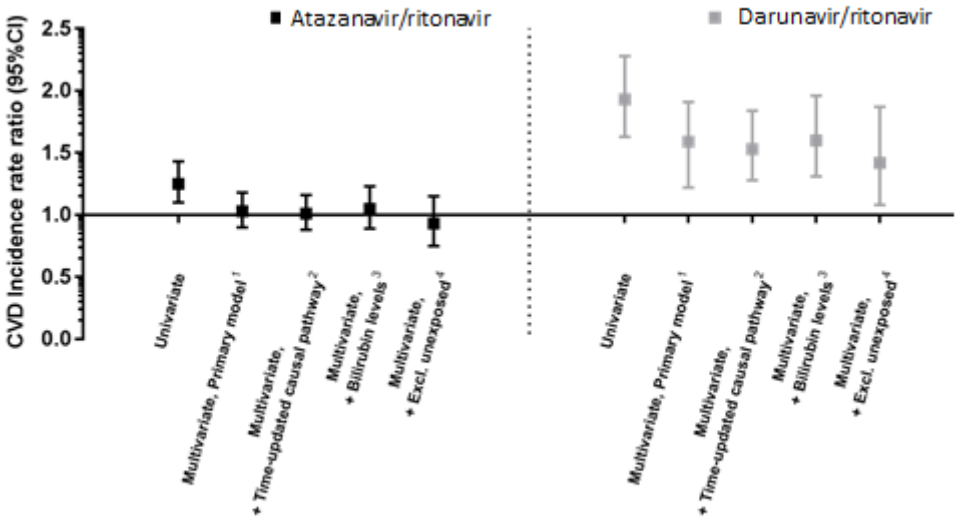
Figure 2 Crude Incidence Rates of CVD per 1000 Persons years of Follow-up Stratified by Cumulative Use of Atazanavir/ritonavir and Darunavir/ritonavir



	0	0-1	1-2	2-3	3-4	4-5	5-6	>6	0	0-1	1-2	2-3	3-4	4-5	5-6	>6
Events	824	75	49	41	26	46	34	62	909	52	51	44	39	17	18	27
PYFU	163785	12886	7631	6369	6144	5757	4898	9278	185246	8845	6591	5285	4100	2940	1768	1975

*Numbers in parenthesis refer to 0 and >6 years exposure for atazanavir/ritonavir and darunavir /ritonavir respectively

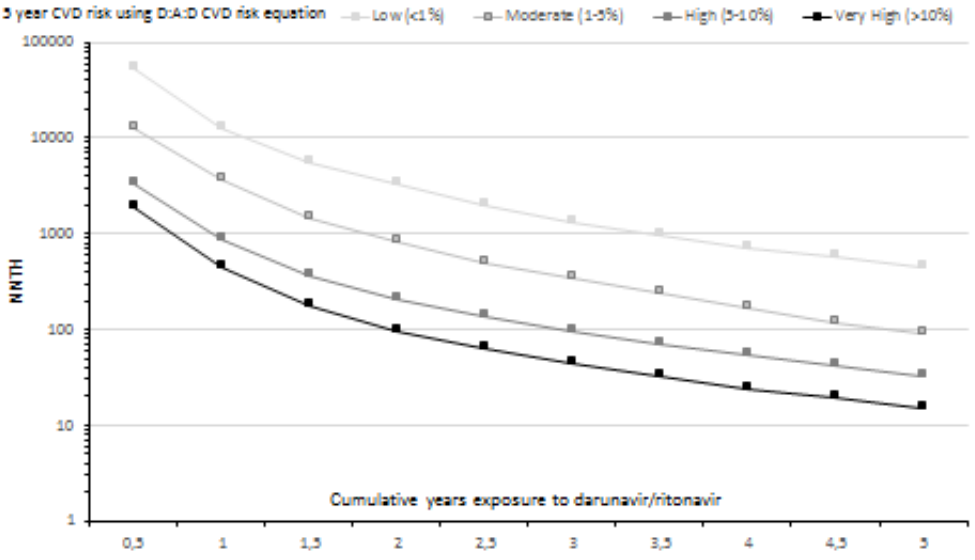
Figure 3 Association Between Cumulative Atazanavir/ritonavir and Darunavir/ritonavir Use and CVD



1. The primary multivariate model was adjusted for cumulative exposure to darunavir/ritonavir, atazanavir/ritonavir, lopinavir/ritonavir, indinavir, on abacavir, prior AIDS, HIV viral load, hepatitis B and C status, family history of CVD, hypertension, smoking, (all as time-updated covariates), gender, age, race, enrollment cohort, HIV risk of acquisition, CD4, CD4 nadir, prior CVD, BMI, diabetes, dyslipidemia (incl. lipid-lowering drugs), CKD and date of baseline (all as fixed covariates at baseline).
Subsequent multivariate models were additionally adjusted for: 2. Variables on the potential causal pathway (BMI, dyslipidaemia, CD4 count, diabetes and CKD) from atazanavir/ritonavir or darunavir/ritonavir use to CVD were all time-updated, 3.Time-updated bilirubin levels and 4. Excluding person-years of follow up with no exposure to atazanavir/ritonavir and darunavir/ritonavir respectively.

Figure 4 Darunavir/ritonavir Numbers Needed To Treat To Harm (NNTH)

Stratified By The D:A:D CVD Five-Year Risk Score



	Low risk	Moderate risk	High risk	Very high
NNTH* /5 years use of darunavir/ritonavir	533 [95%CI 314-706]	91 [95%CI 81-102]	33 [95%CI 30-38]	15 [95%CI 13-17]

*NNTH makes the assumption that the association between darunavir/ritonavir use and CVD is causal. The D:A:D study is observational and confounding by indication cannot be excluded and therefore the study cannot conclude the relationship between darunavir/ritonavir & causal

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