



Cardiovascular disease and use of contemporary protease inhibitors: the D:A:D international prospective multicohort study

Lene Ryom, Jens D Lundgren, Wafaa El-Sadr, Peter Reiss, Ole Kirk, Matthew Law, Andrew Phillips, Rainer Weber, Eric Fontas, Antonella d' Arminio Monforte, Stéphane De Wit, Francois Dabis, Camilla I Hatleberg, Caroline Sabin, Amanda Mocroft, on behalf of the D:A:D study group*

Summary

Background Although earlier protease inhibitors have been associated with increased risk of cardiovascular disease, whether this increased risk also applies to more contemporary protease inhibitors is unknown. We aimed to assess whether cumulative use of ritonavir-boosted atazanavir and ritonavir-boosted darunavir were associated with increased incidence of cardiovascular disease in people living with HIV.

Methods The prospective Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study consists of people living with HIV-1 from 11 cohorts in Australia, Europe, and the USA. Participants were monitored from Jan 1, 2009, until the earliest of a cardiovascular event, 6 months after the last visit, or until Feb 1, 2016. The outcome of interest was the incidence of cardiovascular disease in adults (aged ≥ 16 years) living with HIV who were being treated with contemporary treatments. We defined cardiovascular disease as centrally validated myocardial infarction, stroke, sudden cardiac death, or use of invasive cardiovascular procedures, including coronary bypass, coronary angioplasty, and carotid endarterectomy. We used Poisson regression models to assess the associations between cardiovascular disease and the contemporary protease inhibitors atazanavir and darunavir (both boosted with ritonavir).

Findings 49 709 participants were enrolled in the original cohort from 1999 onwards; 35 711 (71.8%) participants with available data on CD4 cell count and viral load at the 2009 baseline were included in the current analysis, and 13 998 (28.2%) participants had insufficient follow-up data after 2009. During a median 6.96 years of follow-up (IQR 6.28–7.08), 1157 people developed cardiovascular disease (incidence rate 5.34 events per 1000 person-years; 95% CI 5.03–5.65). The incidence rate of cardiovascular disease progressively increased from 4.91 events per 1000 person-years (4.59–5.23) in individuals unexposed to ritonavir-boosted darunavir to 13.67 events per 1000 person-years (8.51–18.82) in those exposed to the drug for more than 6 years. The changes associated with ritonavir-boosted atazanavir were less pronounced, showing an incidence rate of 5.03 cardiovascular events per 1000 person-years (4.69–5.37) in unexposed individuals to 6.68 events per 1000 person-years (5.02–8.35) in participants exposed for more than 6 years. After adjustment, keeping factors on the potential causal pathway from boosted protease inhibitor use to cardiovascular disease fixed at baseline, ritonavir-boosted darunavir use was associated with increased risk of cardiovascular disease (incidence rate ratio 1.59; 95% CI 1.33–1.91 per 5 years additional use), but use of ritonavir-boosted atazanavir was not (1.03; 0.90–1.18). This association remained after adjustment for time-updated factors on the potential causal pathway; myocardial infarction and stroke separately; plasma bilirubin concentration; and after stratification by use of ritonavir-boosted darunavir as the first ever protease inhibitor, used in combination with a non-nucleoside reverse transcriptase inhibitor, by previous virological failure, and by those at high risk of cardiovascular disease.

Interpretation Cumulative use of ritonavir-boosted darunavir, but not of ritonavir-boosted atazanavir, is associated with progressively increasing risk of cardiovascular disease. Causal inference is limited by the observational nature of the D:A:D study. Our findings should prompt investigation into the possible underlying mechanisms of this finding.

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Introduction

Improvements to antiretroviral treatment, which is used to control HIV, have substantially reduced the incidence of drug-related adverse events.¹ However, a 2014 study² suggested that the risk of cardiovascular disease is increasing among people living with HIV because of

increased frequency and severity of cardiovascular disease risk factors such as hypertension, diabetes, dyslipidaemia, and chronic kidney disease. With an ageing population of people living with HIV, at growing risk of cardiovascular disease, antiretroviral treatment tailored to fit individuals' risk profiles are increasingly

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See [Comment](#) page e267

*D:A:D study group members are listed in the appendix

Department of Infectious Diseases, Centre of Excellence for Health, Immunity and Infections, Section 2100, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark (L Ryom PhD, Prof J D Lundgren DMSc, O Kirk DMSc, C I Hatleberg MD); ICAP at Columbia University, New York, NY, USA

(Prof W El-Sadr MPH); Harlem Hospital, New York, NY, USA (Prof W El-Sadr); Academic Medical Centre, Division of Infectious Diseases and Department of Global Health, University of Amsterdam, Amsterdam, Netherlands (Prof P Reiss PhD); HIV Monitoring Foundation, Amsterdam, Netherlands (Prof P Reiss PhD); The Kirby Institute, University of New South Wales, Sydney, NSW, Australia

(Prof M Law PhD); Research Department of Infection and Population Health, University College London, London, UK (Prof A Phillips PhD, Prof C Sabin PhD,

Prof A Mocroft PhD); Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

(Prof R Weber DMSc); Public Health Department, Centre Hospitalier Universitaire (CHU) de Nice, Nice, France (E Fontas PhD); Department of Health Sciences, Infectious and Tropical Diseases Clinic, Azienda Ospedaliera, Milan, Italy (Prof A d'Arminio Monforte PhD); Department of

Infectious Diseases, CHU Saint-Pierre, Brussels, Belgium (S De Wit PhD); and CHU de Bordeaux and INSERM U897, Bordeaux University, Bordeaux, France (Prof F Dabis PhD)

Correspondence to: Dr Lene Ryom, Department of Infectious Diseases, Centre of Excellence for Health, Immunity and Infections, Section 2100, Rigshospitalet, University of Copenhagen, 2100 Copenhagen, Denmark lene.ryom.nielsen@regionh.dk

See Online for appendix

Research in context

Evidence before this study

Several first-generation protease inhibitors used in HIV treatment have been associated with an increased risk of cardiovascular disease. We used PubMed to identify all clinical trials and observational studies that reported associations between protease inhibitors and cardiovascular disease endpoints. The search terms that we used were “myocardial infarction”, “MI”, “stroke”, “cerebrovascular”, “cardiovascular disease”, “CVD”, with “AND HIV” or “AND antiretroviral treatment”, “protease inhibitors”, “indinavir”, “lopinavir”, “saquinavir”, “atazanavir”, and “darunavir”. We also searched for “adverse effects” with “AND protease inhibitors”, “indinavir”, “lopinavir”, “saquinavir”, “atazanavir”, and “darunavir”. The search included studies from the beginning of time until Aug 6–8, 2016, with no language restrictions. Evidence from observational and mechanistic studies suggest that an underlying mechanism between longer cumulative use of indinavir and ritonavir-boosted lopinavir and cardiovascular disease risk could, at least partly, be mediated by dyslipidaemia, and drugs within the protease inhibitor drug class have different metabolic profiles. Whether use of more contemporary protease inhibitors such as ritonavir-boosted darunavir and atazanavir are also associated with an increased risk of cardiovascular disease is unknown.

Added value of this study

After a median follow-up of 7 years, 1157 of 35 711 study participants developed a centrally validated cardiovascular disease event. After adjustment for potential confounders, ritonavir-boosted darunavir was associated with a 59% increased risk of cardiovascular disease per 5 years of additional use, whereas use of ritonavir-boosted atazanavir was not associated with cardiovascular disease. Several sensitivity analyses

confirmed the robustness of this association, including adjustment for several factors on the potentially causal pathway between protease inhibitor use and cardiovascular disease. These analyses also separated cardiovascular disease into myocardial infarction and stroke; adjusted for plasma bilirubin concentration, which may be cardioprotective and is associated with ritonavir-boosted atazanavir use; and stratified by participants for whom ritonavir-boosted darunavir use was their first ever protease inhibitor, by use of this drug in combination with a non-nucleoside reverse transcriptase inhibitor, by history of virological failure, and by participants at high risk of cardiovascular disease.

Implications of all the available evidence

With an ageing population of people living with HIV at increasing risk of cardiovascular disease, tailoring of antiretroviral treatment to fit the individuals' risk profiles is increasingly important. Ritonavir-boosted darunavir is currently recommended as part of first-line treatment regimens in several international guidelines. In this prospective multicohort study with rigorously defined cardiovascular disease endpoints and relatively long follow-up, we identified a progressively increasing risk of cardiovascular disease with longer ritonavir-boosted darunavir use. Although the strength of the association between ritonavir-boosted darunavir and cardiovascular disease is similar to that reported for the first-generation protease inhibitors, the ritonavir-boosted darunavir association does not seem to be moderated by dyslipidaemia. These findings should prompt evaluation of whether other antiretrovirals that do not impart a risk of cardiovascular disease are available as part of individual care, particularly for people with HIV who are at high risk of cardiovascular disease.

needed.³ Earlier studies,^{4–7} including previous analyses from the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study, have shown longer cumulative use of the older protease inhibitors indinavir and ritonavir-boosted lopinavir is associated with cardiovascular disease. The mechanism of this association is believed to be at least partly mediated by dyslipidaemia, and different protease inhibitors are associated with different metabolic profiles.⁸

The two most commonly used protease inhibitors presently are darunavir and atazanavir, both boosted with ritonavir.^{1,9} Ritonavir-boosted darunavir and atazanavir are part of the recommended first-line treatment combinations for treatment-naive adults with HIV in several guidelines, including guidelines by the European AIDS Clinical Society, the British HIV Association, and the US Department of Health and Human Services.^{1,9,10}

Whether contemporary protease inhibitors, such as darunavir and atazanavir, pose a similar risk of cardiovascular disease as previous protease inhibitors is unknown. A previous analysis¹¹ of the D:A:D cohort showed no association between ritonavir-boosted

atazanavir and increased risk of cardiovascular disease; however, the follow-up duration of that analysis was relatively short.

We therefore aimed to assess whether cumulative use of ritonavir-boosted atazanavir and ritonavir-boosted darunavir was associated with increased incidence of cardiovascular disease in people living with HIV in the D:A:D study.

Methods

Study design and participants

The D:A:D study is a prospective cohort collaboration that was established in 1999. This study monitored more than 49 000 adults (aged ≥ 16 years) with an HIV-1 infection from 11 cohorts in Australia, Europe, and the USA who were receiving routine clinical care; details of the D:A:D cohort have been published previously.⁴

All participating cohorts followed local or national guidelines or regulations regarding patient consent and ethical review. Of the countries with participating cohorts, only Australia and Switzerland require specific ethical approval for the entire D:A:D cohort in addition to

that required for their national cohorts (the Australian HIV Observational Database and the Swiss HIV Cohort Study); France (Nice and Aquitaine cohorts), Italy (ICONA cohort), and Belgium (Brussels Saint-Pierre cohort) do not require specific ethical approval more than that required for the individual cohorts, and the Netherlands (AIDS Therapy in the Netherlands project) does not require any specific ethical approval, because data are provided as part of HIV care. For the EuroSIDA study, which includes the data from the Barcelona Antiretroviral Surveillance Study and Swedish cohorts, among participants from many European countries, each participating site has a contractual obligation to ensure that data collection and sharing are done in accordance with national legislation; each site's 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.

Procedures

The primary aim of the D:A:D study is to investigate the associations between antiretroviral treatment and serious non-AIDS adverse events. We report data on clinical events that we centrally validated, including myocardial infarctions, sudden cardiac death, strokes, use of invasive cardiovascular procedures, and deaths. These data were collected in real time during routine clinical care and were regularly monitored and reviewed by external experts. Data on sociodemographic factors, cardiovascular risk factors and treatment, laboratory biomarkers, current antiretroviral treatment, and HIV-associated variables, including HIV viral load, CD4 cell count, AIDS-associated events, and presence of viral hepatitis were collected electronically at enrolment and every 6 months thereafter. All cardiovascular events were reported by use of study-specific designated event forms and were centrally validated at the D:A:D coordinating centre in Copenhagen by the study coordinators, who were masked to the antiretroviral treatment drugs used. Validation was based on the use of standardised algorithms, such as in the WHO MONICA Study^{12,13} of myocardial infarctions and sudden cardiac death, as described in the study protocol.

Outcomes

Cardiovascular disease was assessed as a composite endpoint that included myocardial infarctions, strokes, sudden cardiac deaths, and invasive cardiovascular procedures (coronary bypass, coronary angioplasty, and carotid endarterectomy).

Statistical analysis

For our analysis, we included D:A:D study participants with available follow-up data from a baseline on Jan 1, 2009 (reflecting the wider ritonavir-boosted darunavir licensing in Europe and use of more contemporary antiretrovirals), until the earliest of a

cardiovascular disease event, the last visit plus 6 months, or Feb 1, 2016. We calculated the incidence rates of cardiovascular disease per 1000 person-years of follow-up and stratified by cumulative exposure to ritonavir-boosted atazanavir or darunavir as a risk over 5 years. Incidence rate ratio (IRR) is therefore expressed per 5 years throughout. We used Poisson regression models to assess the associations between incident cardiovascular disease and use of ritonavir-boosted atazanavir or darunavir after adjustment for potential confounders. Because of concerns about adjustment for factors on the potential causal pathway between use of ritonavir-boosted darunavir and atazanavir and cardiovascular disease, our primary model was a priori designed to adjust for such factors at baseline only. Factors that might lie on the causal pathway included body-mass index, dyslipidaemia (defined as 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 cell count, diabetes (confirmed on an event form or by use of antidiabetics), and chronic kidney disease (defined as two measurements of estimated glomerular filtration rate ≤ 60 mL/min per 1.73 m², made ≥ 3 months apart). Other factors included in the primary model were sex, race, age, previous cardiovascular disease, enrolment cohort (ie, time of enrolment into the D:A:D study) and corresponding baseline date, HIV risk group (ie, men who have sex with men, intravenous drug users, heterosexual), nadir CD4 cell count (all fitted as time-fixed variables), cumulative use of ritonavir-boosted lopinavir and indinavir, recent use of abacavir (ie, within the last 6 months), viral load, previous AIDS diagnosis, family history of cardiovascular disease, smoking, hypertension (blood pressure higher than 140/90 mm Hg or use of antihypertensives), and viral hepatitis B and C status (all fitted as time-updated variables).

We calculated cumulative exposure to antiretroviral drugs as in previous D:A:D analyses.^{4,12} Before starting a drug, cumulative exposure time and time since stopping the drug were 0. When stopping the drug, the cumulative time of exposure remained constant, and the time since stopping the drug began to increase. If the person restarted the drug, time since stopping was set back to 0 and cumulative exposure started increasing from the point at which it had previously stopped.

In a sensitivity analysis, we allowed the factors on the potential causal pathway between ritonavir-boosted darunavir and ritonavir-boosted atazanavir use and cardiovascular disease to change during follow-up by adjusting for these as time-updated variables. Previous analyses¹⁴ have suggested that increased concentrations of plasma bilirubin might have a cardioprotective effect. Because ritonavir-boosted atazanavir is associated with hyperbilirubinaemia in other models, we investigated the effects of adjusting for time-updated bilirubin concentrations (fitted as a continuous and categorical variable). In further sensitivity analyses, we investigated the effect of

For the protocol and related documents see www.chip.dk/studies/DAD/study-documents

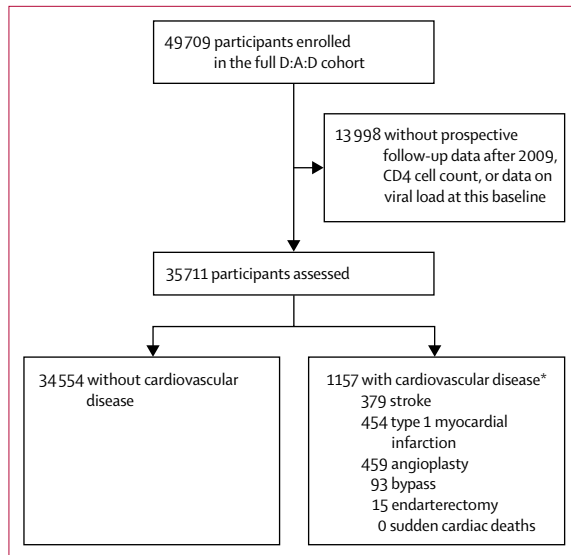


Figure 1: Study profile for this analysis

*Participants could have several events on the same day.

excluding participants who were never exposed to ritonavir-boosted atazanavir or ritonavir-boosted darunavir, separating the cardiovascular disease composite endpoint to investigate whether the association differed between coronary (myocardial infarction) and cerebrovascular events (stroke), and to exclude everyone with a history of cardiovascular disease. In a separate analysis, we examined the association between cumulative use of efavirenz, another frequently used third antiretroviral agent, and cardiovascular disease to assess the specificity of any potential association of boosted protease inhibitor with cardiovascular disease.

To assess the possibility of confounding by indication, we tested the primary model associations for interactions with the D:A:D cardiovascular disease risk score (very high [$>10\%$] vs low [$<1\%$] 5 year estimated risk of cardiovascular disease) and the effects of virological failure (defined as a viral load of >50 copies per mL more than 6 months after starting or changing treatment), use of ritonavir-boosted darunavir as the first ever protease inhibitor-containing regimen, use of antiplatelet drugs, and a later baseline date (ie, after Jan 1, 2009).³ All statistical analyses were done with SAS version 9.3 (Cary, NC, USA).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. 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

The full D:A:D cohorts consisted of 49 709 participants, of whom 35 711 (71.8%) had prospective follow-up data after Jan 1, 2009, including data on CD4 cell count and

viral load. 13 998 (28.2%) participants did not have prospective follow-up data after 2009, CD4 cell count data, or data on viral load at the 2009 baseline, and so were not included in the current analysis (figure 1). During a median follow-up of 6.96 years (IQR 6.28–7.08), 1157 (3.2%) of 35 711 participants had a cardiovascular disease event, at an incidence rate of 5.34 events per 1000 person-years of follow-up (95% CI 5.03–5.65).

At baseline, median age was 44 years (IQR 38–51), median CD4 count was 501 cells per μL (360–689), 73.6% of participants were male, 47.8% were white, 85.4% had ever received combination antiretroviral treatment, and 76.4% were virologically suppressed (table). With regard to traditional cardiovascular risk factors, 40.2% of participants had dyslipidaemia, 39.2% were current smokers, 9.7% had hypertension, 5.1% had diabetes, and 1.3% had previous cardiovascular disease. Participants with incident cardiovascular disease were generally older and had lower median CD4 cell counts, and a higher proportion of these participants had a high 5 year risk of cardiovascular disease (risk score $>10\%$) and chronic kidney disease (risk score >5) compared with participants who did not have a cardiovascular disease event. At baseline, 18.4% of participants were ever exposed to ritonavir-boosted atazanavir and 4.0% to ritonavir-boosted darunavir. At the time of last visit, 26.6% participants were ever exposed to ritonavir-boosted atazanavir, and 22.3% to ritonavir-boosted darunavir. At the time of a cardiovascular disease event, median exposure to ritonavir-boosted atazanavir among those ever exposed was 3.07 years (IQR 1.21–5.33); median exposure to ritonavir-boosted darunavir was 2.56 years (1.21–4.01). None of the individuals included in this analysis received ritonavir-boosted darunavir monotherapy.

The crude cardiovascular disease incidence rate progressively increased from 4.91 events per 1000 person-years (95% CI 4.59–5.23) in individuals never exposed to ritonavir-boosted darunavir to 13.67 events per 1000 person-years (8.51–18.82) in individuals exposed for more than 6 years. For ritonavir-boosted atazanavir, the incidence rate also increased, although less, from 5.03 events per 1000 person-years (4.69–5.37) in individuals never exposed to 6.68 events per 1000 person-years (5.02–8.35) in individuals exposed for more than 6 years (figure 2). In univariate analyses, cumulative use of ritonavir-boosted atazanavir (IRR 1.25 per 5 years; 95% CI 1.10–1.43) and ritonavir-boosted darunavir (1.93 per 5 years; 1.63–2.28) was associated with significantly increased rates of cardiovascular disease (figure 3; appendix p 22).

After adjustment for potential confounders in our primary model, where factors on the potential causal pathway from ritonavir-boosted protease inhibitor use to cardiovascular disease were fixed at baseline, only the darunavir association remained significantly associated with cardiovascular disease (adjusted IRR 1.59 per 5 years; 95% CI 1.33–1.91); the adjusted IRR for atazanavir

	All	With cardiovascular disease
Total participants	35 711	1157 (3.2%)
Sex		
Male	26 288 (73.6%)	1022 (88.3%)
Female	9423 (26.4%)	135 (11.7%)
Ethnicity		
White	17 085 (47.8%)	579 (50.0%)
African	2379 (6.7%)	32 (2.8%)
Other	727 (2.0%)	17 (1.5%)
Unknown	15 520 (43.4%)	529 (45.7%)
HIV exposure group		
MSM	16 447 (46.1%)	647 (55.9%)
IDU	4484 (12.6%)	124 (10.7%)
Heterosexual	12 605 (35.3%)	317 (27.4%)
Other	2175 (6.1%)	69 (6.0%)
Hepatitis B status		
Negative	32 564 (91.2%)	1052 (90.9%)
Positive*	1439 (4.0%)	51 (4.4%)
Unknown	1708 (4.8%)	54 (4.7%)
Hepatitis C status		
Negative	26 792 (75.0%)	890 (76.9%)
Positive†	6864 (19.2%)	199 (17.2%)
Unknown	2055 (5.8%)	68 (5.9%)
ART history		
ART-naive	4944 (13.8%)	67 (5.9%)
Ever combination antiretroviral therapy	30 490 (85.4%)	1084 (93.7%)
Ever ritonavir-boosted atazanavir	6586 (18.4%)	280 (24.2%)
Ever ritonavir-boosted darunavir	1419 (4.0%)	102 (8.8%)
Ever efavirenz	15 350 (43.0%)	572 (49.4%)
Viral load under 400 copies per mL	27 290 (76.4%)	999 (86.3%)
Smoking status		
Current smoker	14 014 (39.2%)	550 (47.5%)
Previous smoker	8299 (23.2%)	296 (25.6%)
Never smoked	9391 (26.3%)	204 (17.6%)
Unknown	4007 (11.2%)	107 (9.3%)

(Table continues in next column)

	All	With cardiovascular disease
(Continued from previous column)		
Previous AIDS event	9799 (27.4%)	421 (36.4%)
Previous cardiovascular disease	451 (1.3%)	97 (8.4%)
Participants with diabetes	1805 (5.1%)	163 (14.1%)
Participants with dyslipidaemia	14 347 (40.2%)	657 (56.8%)
Hypertension	3471 (9.7%)	220 (19.0%)
Chronic kidney disease risk score‡		
≤1	8577 (27.6%)	91 (8.7%)
0–4	10 597 (34.1%)	268 (25.8%)
≥5	11 952 (38.4%)	685 (65.6%)
Cardiovascular disease risk score‡		
1%	8463 (25.5%)	46 (4.2%)
1–5%	18 787 (56.5%)	504 (45.9%)
5–10%	4226 (12.7%)	316 (28.8%)
More than 10%	1753 (5.3%)	231 (21.1%)
Age (years)	44 (38–51)	52 (46–60)
CD4 count (cells per µL)	501 (360–689)	357 (500–713)
CD4 count nadir (cells per µL)	210 (100–322)	165 (67–272)

Data are n (%) or median (IQR). MSM=men who have sex with men. IDU=intravenous drug users. ART=antiretroviral therapy. *Positive for hepatitis B DNA, hepatitis B surface antigen, or hepatitis B e antigen. †Positive for anti-hepatitis C virus or hepatitis C virus RNA. ‡5 year risk in individuals who could be assessed.

Table: Baseline characteristics of all participants, and of participants who had a cardiovascular disease event

(1.03 per 5 years; 0.90–1.18) was not significant. The number needed to treat to harm for ritonavir-boosted darunavir, stratified by the underlying estimated 5 year cardiovascular disease risk, was 533 people (314–706) for those at low risk of cardiovascular disease and 15 people (13–17) for those at very high risk (figure 4).

Additional adjustment with time-updated values for the factors that could potentially be on the causal pathway to cardiovascular disease did not affect the overall association (ritonavir-boosted darunavir adjusted IRR 1.53 per 5 years; 95% CI 1.28–1.84), suggesting that the association between ritonavir-boosted darunavir and cardiovascular disease is not mediated by any of these factors, and, most notably, not by dyslipidaemia

(figure 3). About 90% of participants with cardiovascular disease had at least one cholesterol measurement, with a median of 1.81 measurements (IQR 0.98–2.44) annually. Adjustment for time-updated plasma bilirubin concentrations, regardless of whether this was fitted categorically or as a continuous variable, did not affect the associations between cumulative use of either ritonavir-boosted protease inhibitor and cardiovascular disease: the adjusted IRR was 1.05 (95% CI 0.89–1.23) per 5 years for ritonavir-boosted atazanavir and 1.60 (1.31–1.96) per 5 years for ritonavir-boosted darunavir (figure 3). Similarly, we found no evidence of an interaction between ritonavir-boosted atazanavir ($p=0.68$) or darunavir ($p=0.28$) and plasma bilirubin concentration on rates of cardiovascular disease.

The incidence of cardiovascular disease in participants who were never exposed to ritonavir-boosted atazanavir or darunavir seems lower than in those who were exposed (figure 2), which could increase the associations observed between the two drugs and cardiovascular disease. However, these lower rates did not explain the associations because the ritonavir-boosted darunavir association remained similar (adjusted IRR 1.42 per 5 years; 95% CI 1.08–1.87) in an intention-to-treat analysis that excluded follow-up in participants not exposed to ritonavir-boosted darunavir or ritonavir-boosted atazanavir, whereas the

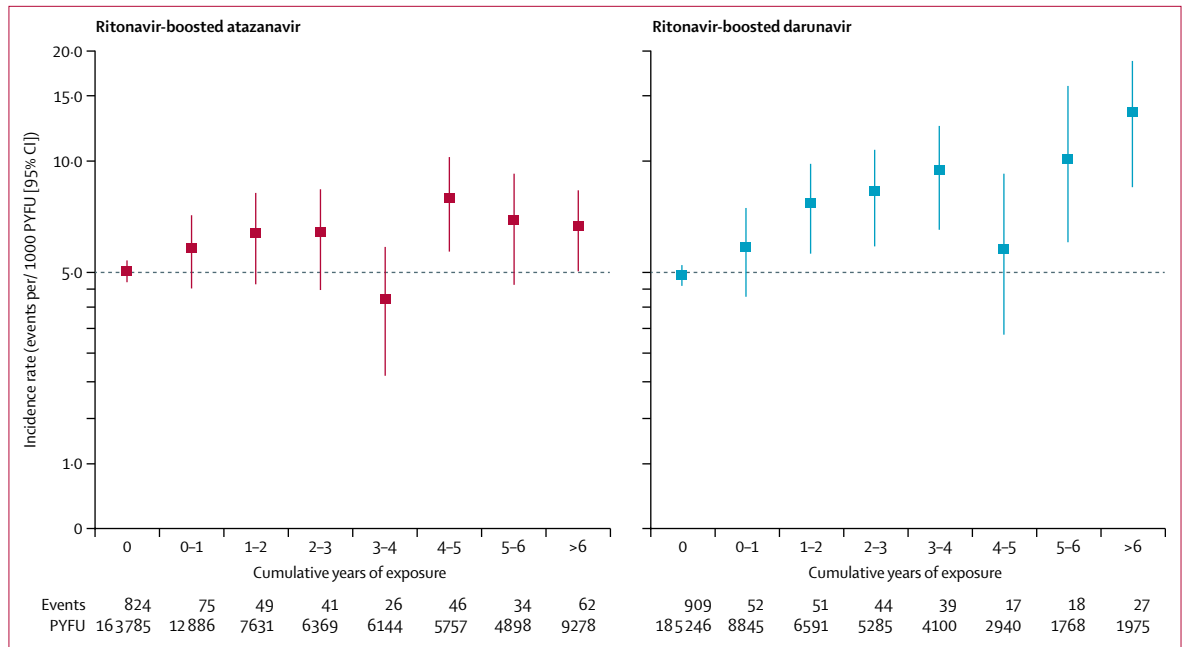


Figure 2: Unadjusted incidence rates of cardiovascular disease per 1000 PYFU, stratified by cumulative use of ritonavir-boosted atazanavir and ritonavir-boosted darunavir
PYFU=person-years of follow-up.

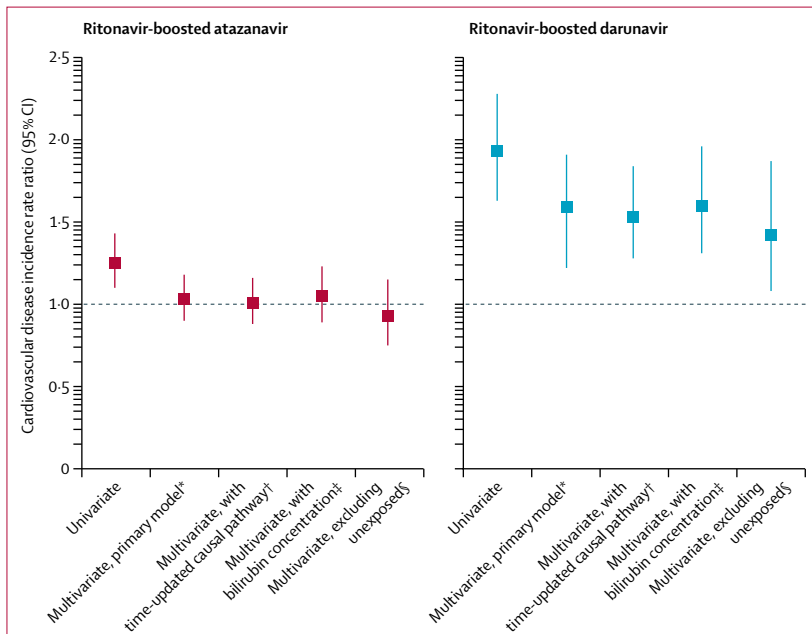


Figure 3: Association between cumulative ritonavir-boosted atazanavir and ritonavir-boosted darunavir use and cardiovascular disease

*Adjusted for cumulative exposure to ritonavir-boosted darunavir, ritonavir-boosted atazanavir, ritonavir-boosted lopinavir and indinavir, recent abacavir treatment, previous AIDS, HIV viral load, hepatitis B and C status, family history of cardiovascular disease, hypertension, smoking (all as time-updated covariates), sex, age, race, enrolment cohort, risk of HIV acquisition, CD4 and CD4 nadir cell counts, previous cardiovascular disease, body-mass index, diabetes, dyslipidaemia (including use of lipid-lowering drugs), chronic kidney disease, and date of baseline (all as fixed covariates at baseline). †Also adjusted for variables on the potential causal pathway (body-mass index, dyslipidaemia, CD4 count, diabetes, and chronic kidney disease) from ritonavir-boosted darunavir or ritonavir-boosted atazanavir to cardiovascular disease, as time-updated covariates. ‡Also adjusted for time-updated bilirubin concentration. §Excluded person-years of follow-up with no exposure to ritonavir-boosted darunavir and ritonavir-boosted atazanavir.

ritonavir-boosted atazanavir association was less than 1.00 (0.93 per 5 years; 0.75–1.15). Exclusion of individuals with any previous cardiovascular disease did not affect the association between ritonavir-boosted darunavir and cardiovascular disease (1.59 per 5 years; 1.31–1.92).

When we separated the composite cardiovascular disease endpoint, we recorded 477 myocardial infarctions (incidence rate 2.18 events per 1000 person-years; 1.98–2.30) and 395 strokes (1.80 events per 1000 person-years; 95% CI 1.62–1.98). Even with reduced power of the analysis, use of ritonavir-boosted darunavir remained significantly associated with increased risk of myocardial infarction (adjusted IRR 1.51 per 5 years; 95% CI 1.13–2.02) and stroke (1.49 per 5 years; 1.08–2.07) when investigated separately. Exclusion of individuals with any previous cardiovascular disease further reduced the power of the analysis: 432 myocardial infarctions (2.00 events per 1000 person-years; 95% CI 1.81–2.18) and 379 strokes (1.75 events per 1000 person-years; 1.57–1.93). However, the association between ritonavir-boosted darunavir and myocardial infarction remained similar (adjusted IRR 1.53 per 5 years; 95% CI 1.13–2.07) but slightly weakened for stroke (1.37 per 5 years; 0.97–1.94).

A brief decrease (that was subsequently reversed) in cardiovascular disease incidence occurred after 3–4 years of ritonavir-boosted atazanavir and 4–5 years of ritonavir-boosted darunavir use. Several exploratory analyses investigated possible explanations, including the effects of individual participating cohorts and calendar year, but

found no clear explanation for the lower rates of cardiovascular disease at these timepoints than would be expected. If follow-up times before the decreases (3 years for ritonavir-boosted atazanavir and 4 years for ritonavir-boosted darunavir) were censored, the associations statistically strengthened for both drugs (ritonavir-boosted atazanavir 1.38 per 5 years [95% CI 0.80–2.39] and ritonavir-boosted darunavir 2.26 per 5 years [1.54–3.32]), but remained non-significant for ritonavir-boosted atazanavir. Therefore, this variation over time might reflect a chance finding. By including all data, including the brief decreases, we are underestimating the strength of the association between use of ritonavir-boosted atazanavir and ritonavir-boosted darunavir and cardiovascular disease.

Since the D:A:D study does not collect data on drug doses, as a proxy for using 600 mg darunavir and 100 mg ritonavir twice a day versus 800 and 100 mg once a day, we assessed whether the association between use of these drugs and cardiovascular disease differed with virological failure. The association between ritonavir-boosted darunavir use and cardiovascular disease was similar among participants with previous virological failure (adjusted IRR 1.67 per 5 years; 95% CI 1.39–2.01) and those without (2.02 per 5 years; 1.00–4.01); furthermore, there was no observed difference between ritonavir-boosted darunavir being used as the first ever regimen containing a ritonavir-boosted protease inhibitor or not ($p=0.29$ for interaction). This association was also unaffected after stratification by whether ritonavir-boosted darunavir was used in combination with a non-nucleoside reverse transcriptase inhibitors or not ($p=0.43$). Additionally, there was no evidence of an interaction between ritonavir-boosted darunavir use and cardiovascular disease in participants at high versus low estimated 5 year risk with the D:A:D cardiovascular risk score ($p=0.12$), and in those with and without previous cardiovascular disease ($p=0.51$). Finally, adjustment for ever-use of antiplatelet drugs, including aspirin, which is predominantly used in individuals with previous cardiovascular disease or high estimated risk of cardiovascular disease did not affect the association: ritonavir-boosted atazanavir adjusted IRR 1.03 per 5 years (95% CI 0.90–1.17) and ritonavir-boosted darunavir 1.52 per 5 years (1.26–1.82).

In a final analysis, we examined the use of another frequently used third antiretroviral drug, efavirenz; 43.0% of participants had been exposed at baseline and 49.4% had been exposed at the time of a cardiovascular disease event, without any evidence of an association between efavirenz and cardiovascular disease (adjusted IRR 0.93 per 5 years; 95% CI 0.86–1.02).

Discussion

In this large heterogeneous cohort of people with HIV, cumulative use of ritonavir-boosted darunavir, but not ritonavir-boosted atazanavir, was independently associated with a small, but progressively increasing risk of centrally

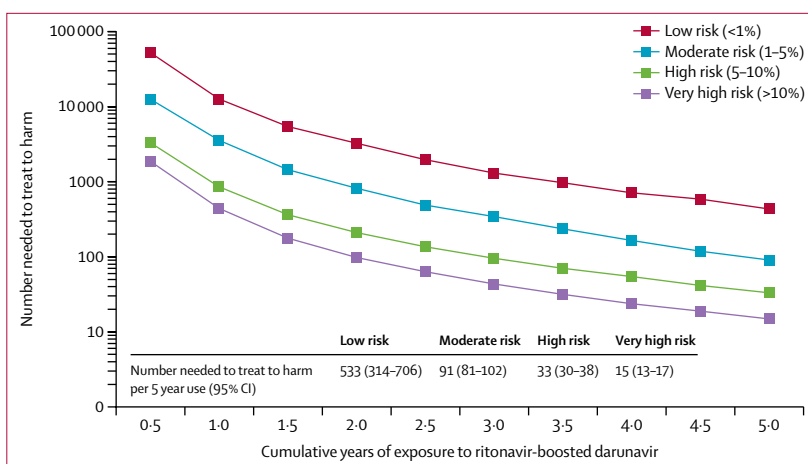


Figure 4: Number needed to treat to harm after cumulative ritonavir-boosted darunavir use, stratified by D:A:D cardiovascular disease 5 year risk score

validated cardiovascular disease events. For individuals at high risk of cardiovascular disease (ie, with an absolute 5 year cardiovascular disease risk of 10%), use of ritonavir-boosted darunavir for 5 years would increase the absolute cardiovascular disease risk to almost 16%. Expressed differently, if 15 people at very high risk of cardiovascular disease were exposed to ritonavir-boosted darunavir for 5 years, one might develop cardiovascular disease that was attributable to ritonavir-boosted darunavir (number needed to treat to harm equals 15 people). Because the number needed to treat to harm varies depending on the underlying absolute cardiovascular disease risk (from 15 at very high 5 year risk to 533 at low 5 year risk), caution with ritonavir-boosted darunavir use is particularly warranted in people at high risk of cardiovascular disease.

Cautious interpretation of our findings is warranted because of the observational nature of the study and the risks of unmeasured confounding. However, because ritonavir-boosted darunavir use is recommended as part of a first-line treatment regimen in several guidelines, and its use has increased, our findings create an impetus for other studies to investigate possible mechanisms by which the increased risk of cardiovascular events might occur.¹⁹ We also encourage other large studies to do analyses of the association between ritonavir-boosted darunavir and cardiovascular disease to investigate the reproducibility of our findings. Follow-up time in the D:A:D study is insufficient to investigate whether discontinuation of ritonavir-boosted darunavir use will lead to reductions in the observed increased incidence of cardiovascular disease. Such analyses will, however, be paramount to build on the evidence of potential causality. Meanwhile, given the large size of the study, relatively long follow-up, diversity of participants, rigorously defined cardiovascular disease endpoints, strength of the association between ritonavir-boosted darunavir use and cardiovascular disease, biological gradient, and specificity of the findings, these new data

about possible issues with safety should prompt careful consideration of whether an alternative drug without any known risk of cardiovascular disease is available as part of individual care.

The strength of the observed association of ritonavir-boosted darunavir with cardiovascular disease is of a similar magnitude as we observed in 1999–2005 for the older protease inhibitors indinavir (adjusted IRR 1.47 per 5 years) and ritonavir-boosted lopinavir (1.54 per 5 years); by contrast, the association of cardiovascular disease with ritonavir-boosted darunavir use does not seem to be modified by dyslipidaemia or any of the other factors hypothesised to potentially lie on the causal pathway to cardiovascular disease.^{4,12} Furthermore, our data do not suggest that the observed absence of an effect modification by dyslipidaemia is because of an inadequate number of lipid measurements. Previous studies^{15–17} suggest a lipid profile for ritonavir-boosted darunavir treatment similar or slightly superior to that of ritonavir-boosted atazanavir in treatment-experienced individuals, but superior to that of ritonavir-boosted lopinavir. Several studies^{6,18–20} have suggested that certain protease inhibitors might contribute to cardiovascular disease pathogenesis via changes in lipid pathways and via other lipid pathways than those directly measurable by more simple definitions of dyslipidaemia. Another possibility is that the mechanism underlying cardiovascular disease development includes entirely different atherosclerotic pathways not captured by analyses of more traditional cardiovascular disease risk factors,²¹ similar to what has been documented for abacavir and cardiovascular disease.^{22,23} One study²⁴ has suggested that protease inhibitors increase oxidative stress more than other antiretrovirals, which could subsequently contribute to development of cardiovascular disease.

The different findings between cumulative use of ritonavir-boosted darunavir and ritonavir-boosted atazanavir and cardiovascular disease confirm our earlier analyses¹¹ that showed no association between ritonavir-boosted atazanavir and cardiovascular disease, and do not suggest a uniform protease inhibitor drug class or ritonavir-specific effect on risk of cardiovascular disease. A small study²⁵ has, however, shown higher intracellular doses of ritonavir in people receiving darunavir than people receiving atazanavir, which might increase risk of adverse effects, including cardiovascular disease. To ensure comparability, we included only individuals who were using ritonavir-boosted atazanavir and darunavir in this analysis; however, a previous D:A:D subanalysis¹¹ found similar results when including unboosted atazanavir. Another study²⁶ in 2016 found that cumulative use of ritonavir-boosted darunavir, but of no other protease inhibitor, was associated with markers of subclinical coronary atherosclerosis, but also longer time with an HIV diagnosis, ongoing viral replication, and lower CD4 cell count. The significant association between ritonavir-boosted atazanavir and cardiovascular disease in the univariate analysis seemed to be explained

primarily by the increasing age of participants receiving ritonavir-boosted atazanavir.¹¹ We found no evidence to support the hypothesis that the absence of an association between ritonavir-boosted atazanavir and cardiovascular disease was explained by the protective effect of an increased plasma bilirubin concentration related to ritonavir-boosted atazanavir treatment. It is possible that it is not the increased plasma bilirubin concentration that is related to ritonavir-boosted atazanavir use directly, but instead that other protease inhibitors do not cause changes in plasma bilirubin concentration, which mediates the difference in associations with cardiovascular disease. However, additional insight into the reason for the apparent absence of association between ritonavir-boosted atazanavir and cardiovascular disease and the role of bilirubin is necessary, because more indirect bilirubin-related cardioprotective pathways could be involved. Conversely, the absence of an association between ritonavir-boosted atazanavir and cardiovascular disease could also mean that ritonavir-boosted atazanavir does not activate proatherogenic pathways in the way that other protease inhibitors do.

In terms of alternative, commonly used third antiretroviral drugs, we found no evidence of an association between cardiovascular disease and cumulative efavirenz use, and there are still inadequate long follow-up data on integrase inhibitors to reliably do such safety analyses.

None of the original trials^{15,16,27} that investigated the efficacy and safety of ritonavir-boosted darunavir reported, or could report, increased cardiovascular disease rates. This data shortage might be related to the high risk of type II errors in these trials because of the general short follow-up time, limited size, focus on laboratory endpoints, and (in most) inclusion of individuals with low cardiovascular disease risk. Furthermore, these studies were not designed specifically or powered to investigate longer-term adverse effects. The European ritonavir-boosted darunavir product information does, however, declare angina pectoris an uncommon event (up to one per 100 person-years of follow-up), and acute myocardial infarction to be a rare event (up to one per 1000 person-years of follow-up) associated with ritonavir-boosted darunavir use, but such safety data are not included in the US package insert.^{28,29}

After initial investigations in the D:A:D study, Janssen also evaluated safety data on ritonavir-boosted darunavir exposure and cardiovascular disease in a 2017 poster presentation.^{30,31} The investigators did not find evidence to support an association between ritonavir-boosted darunavir and cardiovascular disease, but the study had substantially less power compared with our data. The study by Janssen analysed 5721 people living with HIV, who were predominantly naive to treatment (*vs* 35711 people in our study, who had a substantial treatment history) and 66 cardiovascular disease events (*vs* 1157 events in our study), with a median follow-up of 1.8 years (*vs* 7.0 years in our study).

It is unlikely that the observed association between ritonavir-boosted darunavir and cardiovascular disease is explained by confounding by indication. Ritonavir-boosted darunavir was, because of its high genetic barrier, initially used predominantly as part of salvage therapy. By limiting follow-up in this analysis to after 2009, we aimed to reflect a broader use of ritonavir-boosted darunavir. Additionally, our data showed no difference when stratifying analyses by use of ritonavir-boosted darunavir as first-ever protease inhibitor regimen or not, and a robust association was found in participants with and without a history of virological failure. Also, our data did not suggest an interaction of cardiovascular events with recent abacavir use, which has consistently been associated with cardiovascular disease in the D:A:D study. Finally, we accounted for common factors known to affect the choice of a specific antiretroviral regimen and for factors related to cardiovascular risks.

Our study has important limitations to acknowledge. Because of the observational nature of the study, we are unable to exclude the possibility of unmeasured confounding, and we cannot draw definitive conclusions on causal inference of our findings. Because the study does not collect data on drug doses, we are also unable to directly assess whether 600 mg darunavir with 100 mg ritonavir twice a day versus 800 mg with 100 mg once a day differ in their associations with cardiovascular disease, although associations were not significantly different when comparing use in participants with virological failure (where 600 mg with 100 mg twice a day is recommended) versus those without (800 mg with 100 mg once a day recommended). The ODIN trial²⁷ showed a better lipid profile with ritonavir-boosted darunavir once a day compared with twice a day, but, in our analysis, additional adjustment for time-updated dyslipidaemia did not change associations. Although darunavir is always boosted by a second agent (ritonavir or cobicistat), atazanavir can be used unboosted; however, we have only very limited data on unboosted atazanavir after 2009 and are unable to study the cardiovascular disease risk associated with its use. We have inadequate follow-up data on cobicistat to enable analysis with an alternative protease inhibitor boosting agent than ritonavir to investigate whether the observed associations differ.

In this large heterogeneous cohort of people living with HIV with a median follow-up time of 7 years, cumulative use of ritonavir-boosted darunavir, but not ritonavir-boosted atazanavir, was independently associated with a progressively increasing risk of centrally validated cardiovascular disease events. Our findings remained similar across several sensitivity analyses, and call for a cautious use of ritonavir-boosted darunavir in individuals at high risk of cardiovascular disease. The association between ritonavir-boosted darunavir use and cardiovascular disease was, in contrast to findings with older protease inhibitors, not modified by adjusting for dyslipidaemia,

and calls for further investigations of possible mechanisms of this association.

Contributors

LR had full access to all data in the study and takes responsibility for the integrity of the data and analysis. LR, JDL, and AM proposed and developed the research question, and AM did the statistical analyses. WE-S, PR, OK, ML, AP, RW, EF, Ad'AM, SDW, FD, CIH, and CS contributed to the study design and interpretation of data. LR wrote the first draft of the manuscript. All authors have seen and contributed to the final version of the manuscript.

Declaration of interests

PR has served as a scientific adviser to Bristol-Myers Squibb, Gilead Sciences, Grupo Ferrer Internacional, GlaxoSmithKline, Janssen Pharmaceutica, Merck, and ViiV Healthcare; has served on data and safety monitoring boards and endpoint adjudication committees for Janssen Pharmaceutica; reports honoraria to his institution for speaking engagements at scientific conferences from Bristol-Myers Squibb, Gilead Sciences, and GlaxoSmithKline; and reports research support from Gilead Sciences, ViiV Healthcare, Merck, Janssen Pharmaceutica, Bristol-Myers Squibb, Abbott Laboratories, and Boehringer Ingelheim. OK reports previous and current board membership at ViiV Healthcare, Gilead Sciences, and Merck; reports payment for lectures, or for development of educational presentations, or both, from Abbott Laboratories, Gilead Sciences and Janssen Therapeutics; and reports travel, accommodation, or meeting expenses paid by Abbott Laboratories, Bristol-Myers Squibb, Gilead Sciences, Merck, and ViiV Healthcare. ML reports research grants from Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Janssen Pharmaceutica, Merck, Pfizer, and Roche. AP reports personal fees from Gilead Sciences, AbbVie, GlaxoSmithKline Vaccines and grants from Bristol-Myers Squibb. RW reports travel grants from Abbott Laboratories, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, GlaxoSmithKline, Merck, Pfizer, Roche, TRB Chemedica, and Janssen Therapeutics; and reports unrestricted educational grants from GlaxoSmithKline, ViiV, and Gilead Sciences to his institution. Ad'AM reports previous board membership at AbbVie, Bristol-Myers Squibb, Gilead Sciences, Janssen Pharmaceutica, and Merck. CS reports personal fees from Gilead Sciences, Bristol-Myers Squibb, Janssen Pharmaceutica, Abbott Laboratories, and ViiV Healthcare. AM reports consultancy fees, honoraria, or speaker fees from Bristol-Myers Squibb, Pfizer, Merck, Boehringer Ingelheim, and Gilead Sciences. LR, JDL, WE-S, EF, SDW, FD, and CIH declare no competing interests.

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