Association between Integrase Strand Transfer Inhibitors and Cardiovascular Disease in People Living with HIV: A multicentered Prospective Study from the RESPOND Cohort Consortium

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Abstract

Summary

Background Although associations between older antiretroviral drug classes and cardiovascular disease (CVD) in people living with HIV (PLWH) are well described, data regarding a possible association with integrase strand transfer inhibitors (INSTIs) are limited. Our aim was to investigate if exposure to INSTIs was associated with an increased incidence of CVD.

Methods

RESPOND is a prospectively multicentered collaboration between 17 pre-existing European and Australian cohorts, that follows > 32 000 adult PLWH in clinical care after Jan 1, 2012.

Included participants were required to have CD4 cell counts and HIV viral load measured in the 12 months before or within 3 months after baseline. (latest of cohort enrollment or Jan 1, 2012); these were subsequently followed to the earliest of the first CVD event (myocardial infarction, stroke, or invasive cardiovascular procedure), last follow-up, or Dec 31, 2019.

Multivariable negative binomial regression was used to assess associations between CVD and INSTI-exposure.

Findings Out of 29,340 PLWH, 47·7% were exposed to an INSTI. During 160,252 person-years of follow-up (PYFU), 748 individuals experienced a CVD event (incidence rate, IR, 4·67/1000 PYFU [95% confidence interval, 4·34–5·01]). The crude CVD IR increased from 4·19/1000 PYFU [3·83–4·57] in those with no INSTI-exposure to 8·46 [6·58–10·71] at >0-6 months exposure and decreased after 24 months of exposure, to levels similar to individuals never-exposed to INSTIs. Compared to those never-exposed, the risk of CVD was elevated within the first 24 months of INSTI-exposure (>0–6 months adjusted incidence rate ratio:1·85 [1·44–2·39], 6–12 months: 1·19 [0·84–1·68], 12–24 months of exposure: 1·46 [1·13–1·88], p <0·01) and thereafter fell to levels similar to those never-exposed. Results were consistent across a range of sensitivity analyses, and according to age, estimated 5-year D:A:D CVD risk score and calendar year before or after 2014 ($p_{interaction}$ >0.25, for all)

Interpretation Although the potential for unmeasured confounding and channelling bias cannot fully be excluded, INSTIs initiation was associated with an early onset, excess incidence of CVD in the first two years of exposure, after accounting for known CVD risk factors and across a wide range of sensitivity analyses. These early findings call for analyses in other large studies, and the potential underlying mechanisms explored further.

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Research in context:

Evidence before this study

We searched Pubmed for observational studies and clinical trials, using the Mesh-terms “cardiovascular disease, “myocardial infarction” OR “cerebrovascular disorder” OR “Stroke” OR “cardiovascular Procedures” with AND “Antiretroviral Therapy, Highly Active” OR ”Anti-Retroviral Agents” OR ”HIV integrase inhibitors” OR ”raltegravir” OR ”elvitegravir” OR ”dolutegravir” OR ”bictegravir,” or AND ”HIV,” in the period from index start to Aug 1, 2021, without any date or language restrictions.

Associations between the risk of cardiovascular disease and the use of older antiretroviral drugs are well described. The risk has been described as a gradual increase with longer cumulative exposures, for the boosted protease inhibitors indinavir, lopinavir, and darunavir, and as a rapid and maintained risk increase, reversible upon discontinuation, for the nucleotide-reverse-transcriptase-inhibitor, abacavir. However, investigations of a potential association between the use of the newer integrase inhibitor drug-class — which are recommended as first-line treatment in most guidelines — and cardiovascular disease are still scarce. Nonetheless, such studies are warranted, as an increasing number of studies suggest that integrase inhibitors are linked to weight gain and associated conditions such as metabolic syndrome, which could, in turn, lead to cardiovascular disease. A recent retrospective, US-based, found no such association, although not assessing exposure time and excluding clinical events within the first 90 days.

On the other hand, an older analysis spanning the period 2003 – 2015 from the US Veterans Affairs cohort found that myocardial infarctions and strokes were less likely with atazanavir treatment than integrase inhibitor treatment. However, the analysis was not dedicated to examining a potential relationship between integrase inhibitors and cardiovascular disease. Moreover, the integrase inhibitor group was relatively small, with only a limited number of second-generation Integrase inhibitors included.

Whether a relationship between cumulative exposure to integrase inhibitors and cardiovascular disease exists when examined in well-powered studies with firmly defined end-points, including centrally adjudicated events, is unknown

Added value of this study

During 6.16 years median follow-up time and 160,252 person-years, 748 of the 29,340 individuals included experienced a rigorously defined cardiovascular event. When comparing individuals never-exposed to an integrase inhibitor to individuals exposed for >0–6 months, >6–12 months, >12–24 months, >24–36 months, and >36 months, we found that the relative risk of CVD increased almost two-fold in the first six months of exposure, after adjustment for potential confounders. The association remained until 24 months of exposure

— albeit at a lower relative risk than in the initial six months. The association was similar across a wide range of sensitivity analyses that tested the robustness of the findings; included analyses that excluded individuals with prior CVD and analyses that excluded invasive cardiovascular procedures for the composite cardiovascular end-point. Exploratory models adjusting for factors on the potential causal pathway to CVD, such as BMI, lipids, glucose, blood pressure, and CD4 count, as time-updated variables, did not lower the relative risk, suggesting that the association was not mediated through classic cardiovascular risk factors. The association was similar for individuals above or below 50 years of age and individuals at low or high 5-year estimated cardiovascular risk, respectively. In addition, the risk did not vary by immune/virologic status or by the period before or after availability of second-generation integrase inhibitors

Implications of all the available evidence

With cardiovascular disease remaining a common cause of morbidity and mortality among people living with HIV, it is paramount that treatment given to suppress HIV does not add to the cardiovascular risk profile. Therefore, insights into CVD risk factors, including the potential role of individual antiretroviral agents, remain crucial.

In this large, multi-national cohort study using meticulously defined cardiovascular end-points, we observed an almost two-fold increased risk of cardiovascular disease, after accounting for other known risk factors, within the first six months of exposure to integrase inhibitors when compared to individuals never-exposed to an integrase inhibitor. The risk remained elevated until two years of exposure, although the risk was higher in the initial six months. We did not find any evidence suggesting that known cardiovascular risk factors mediated the increased risk or that the strength of the association depended on underlying estimated cardiovascular risk.

Our results call for investigations in other large studies and further exploration of potential underlying mechanisms.

Introduction

With modern combination antiretroviral therapy (ART), the life expectancy for people living with HIV (PLWH) has approached that of the HIV-negative population.[1] Yet, as the population ages, non-AIDS comorbidities such as cardiovascular disease (CVD) and risk factors hereof are seen with increasing frequency.[2] Therefore, continuous assessments of modern antiretroviral drugs are needed to tailor ART regimens to fit individual needs, taking the complex interactions between ART, comorbidities, lifestyle factors, and non-ART medication into consideration.[3,4]

A safety signal linking ART-exposure to incident myocardial infarctions (MIs) first appeared in a 2003 publication from The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study.[5] Follow-up studies published in 2007 and 2010 from the same group could, in part, attribute this to a 47% and 54% increase in the relative risk of MIs per 5 years of exposure to the older protease inhibitors (PI), indinavir, and ritonavir-boosted lopinavir, respectively.[6,7] An additional analysis from the D:A:D study further suggested that recent exposure to the nucleoside-reverse transcriptase inhibitor, abacavir (ABC), increased the relative risk of MIs by 90%.[8] Subsequently, both these findings were reproduced in other independent studies,[9] although not consistent across all studies.[10] Whereas a pro-atherosclerotic lipid profile is now generally considered to underlie the association for older PIs[11], a platelet hyperactivity mechanism has been suggested as the link between ABC and CVD.[12] Both cases illustrate that the process from initially observing a potential safety signal to establishing a plausible causal mechanism evolves over time and requires the involvement of many different types of studies. The recent report of a 59% increase in CVD risk per 5 years exposure to the ritonavir-boosted darunavir,[13] not explained by dyslipidemia, serves as an example of the initiation of one such process — underlining the continued need for large-scale pharmacovigilance research of potential adverse effects of antiretroviral drugs. To date, no studies have reported an association between CVD and the use of non-nucleotide reverse transcriptase inhibitors (NNRTIs).[7,13]

Due to their potent suppression of HIV viremia, rapid immune reconstitution, and high genetic barrier to resistance,[15–18] unboosted integrase strand transfer inhibitors (INSTIs) are recommended as first-line treatment in North American and European guidelines.[3,4] Although INSTIs are generally well-tolerated,[15–17,19] recent studies have suggested a possible association between INSTI use, weight gain, and metabolic syndrome;[20–22] factors that in turn could lead to CVD. However, only limited data exist on a potential association between rarely occurring CVD events and INSTI-exposure.[23,24] Therefore, data from large-scale, prospective, observational collaborations with extended follow-up and rigorously defined clinical end-points are warranted.

In this study, we investigated if exposure to INSTIs was associated with an increased incidence of CVD within the RESPOND cohort consortium.

Methods

Study design and participants

A detailed consortium profile for REPSOND has been published elsewhere.[25] In brief RESPOND was formed in 2017, dedicated to the study of HIV and other infectious diseases, as a prospectively multicentered collaboration between 17 pre-existing European and Australian cohorts. RESPOND participants are required to more than 18 years of age, INSTI naïve prior to Jan 1, 2012, and to have a CD4 cell counts and HIV viral load measured in the 12 months before or within 3 months after baseline (see statistical analysis below),

Ethical considerations

Participants consent to share data with RESPOND according to local requirements. Enrolled participants are pseudonymised by assigning a unique identifier by the participating cohort before data transfer. According to national or local requirements, all cohorts have the approval to share data with RESPOND. Data are stored on secure servers at the RESPOND coordinating centre in Copenhagen, in accordance with current legislation and under approval by The Danish Data Protection Agency (approval number 2012-58-0004, j.nr.: RH-2018-15, 26/1/2018), under the EU General Data Protection Regulation (2016/679).

Procedures

All included individuals had data retrospectively collected for at least five years prior to their enrollment into RESPOND — a complete history of ART and clinical events was requested for all individuals. In addition, prospective data have been collected annually since 2017. The systematic data collection includes demographics (e.g., sex, age, region of origin), viral hepatitis co-infection, and HIV-specific information (e.g., HIV viral load [VL], CD4 cell counts, AIDS), detailed information on ART including start/stop dates, and reasons for discontinuation. Further, non-ART medications, biochemical measures (e.g., lipids, creatinine, glucose, and Hb1Ac, cardiovascular risk factors (prior CVD, smoking, body-mass-index [BMI], hypertension, renal function, and diabetes mellitus [hereafter referred to as diabetes]), and incident clinical events (including CVD, cancers, liver- and renal failure) are also collected.

Outcomes

We assessed CVD using a composite endpoint consisting of fatal and non-fatal MIs, strokes, and invasive cardiovascular procedures (ICPs: coronary angioplasty/stenting, coronary bypass surgery, and carotid endarterectomy). CVD events occurring within 12 months of the last clinical visit before RESPOND enrolment and thereafter were reported using designated case report forms. Subsequently, the case report forms were centrally validated by a trained medical doctor, using standardised algorithms based on WHO's MONICA study.[26] CVD events occurring before this point were collected but not centrally validated.

Statistical analysis

We followed INSTI naïve individuals, from the latest of cohort enrolment or Jan 1, 2012 (baseline) to the earliest of the first CVD event, last follow-up visit, or Dec 31, 2019 (administrative censoring date). We allowed CVD events prior to baseline, but only included incident events of a different subtype after baseline (e.g. if the person had experienced a MI before baseline we would not include a subsequent MI during follow-up, whereas we would count a stroke). We did not count ICPs performed within 72 hours of an MI.

Logistic regression, adjusted for calendar time, was used to assess whether or not individuals at a higher estimated 5-year D:A:D CVD risk[27] preferentially started an INSTI, compared to other contemporary third-drug antiretrovirals within the period. We used the 5-year risk estimate rather than the 10-year risk estimate, as the median follow-up in the population did not exceed ten years.

ART exposure was calculated based on the D:A:D study methodology described elsewhere.[5] In brief, follow-up from each participant was divided into a series of consecutive one-month periods, adding each month on a drug, the person's cumulative exposure for that specific drug. If treatment stopped, the exposure count remained static with no addition to the cumulative exposure of that drug. However, should the specific treatment be reinitiated, time would be added to the cumulative exposure. We repeated this process for each ARV that an individual had received. Finally, we added drug exposure prior to the baseline to the cumulative exposur**e.**

In these first analyses, we assessed INSTI-exposure as a class exposure consisting of raltegravir (RAL), cobicistat-boosted elvitegravir (EVG/c), dolutegravir (DTG), and bictegravir (BIC), as the analytical power at the time of the analysis was insufficient to assess exposure to individual INSTIs. Based on exploratory analyses determining whether a potential relationship between CVD incidence and INSTI-exposure was linear or not, we analysed INSTI exposure as a categorical variable, with categories of 0 months (unexposed), >0 – 6, >6 – 12, >12 – 24, >24 – 36, and >36 months of exposure; the 0-exposure group refers to those who were never exposed to an INSTI, at any time, and per definition, includes both ART experienced and ART naïve individuals. As RESPOND has complete ART history and precise dates of CVD events, we were able to determine INSTI exposure prior to CVD events, for those exposed to INSTI.

CVD incidence rates (IR) were calculated per 1000 person-years of follow-up (PYFU), stratified by duration of INSTI-exposure.

Binomial regression models using generalised estimating equations and robust standard errors were used to examine a potential association. A priori, the model was adjusted for sex, ethnicity, region, HIV acquisition risk, age, body mass index (BMI), CD4 cell count, CD4 nadir, hypertension, dyslipidemia, diabetes, prior AIDS-defining conditions, prior CVD, and chronic kidney disease (CKD) all fitted at baseline. We included smoking and antiretroviral drugs previously associated with CVD (cumulative exposure to indinavir, ritonavir-

boosted lopinavir, boosted darunavir, didanosine, and recent ABC exposure [current or within six months]) in the model as time-updated variables. An unknown category accounted for missing categorical data in the model. Due to collinearity with cumulative ART exposure, we did not include calendar time or treatment experience in the model. Definitions and variable fitting are shown in the legend of Table 1 and Figure 2 and 3, respectively. To investigate the potential overfitting of the model, we performed a sensitivity analysis, adjusted only for the estimated 5-year D:A:D CVD risk score.[27]

We used exploratory analyses to assess the effect of fitting factors on the potential causal pathway from INSTI-exposure to CVD (CD4 cell count, BMI, hypertension, diabetes, CKD, and dyslipidemia) as time-updated variables to evaluate if this would attenuate a potential signal and indicate a mediator effect. Subsequently, we added time-updated platelet counts to the model, assessing a potential platelet-dependent mechanism, such as blood-clotting.

To test the primary model's robustness, we further performed analyses with models that excluded ICPs from the composite CVD endpoint or excluded individuals with any CVD before baseline. Other sensitivity analyses only included centrally validated CVD events or individuals who switched/initiated a new ART regimen after Jan 1, 2012.

We also examined if the CVD incidence and association with INSTI-exposure varied depending on the estimated 5-year CVD risk score, sex, or age (<50 years and ≥50 years) by testing the relevant interactions. In addition, we also examined potential variation due to differences in the availability of first and second-generation INSTIs, by testing a potential interaction with calendar-year before or after Jan 1, 2014.

We used Stata/SE 15·0 (StataCorp LLC) for all performed analyses. All p-values are two-sided, with a p-value <0.05 defined as statistically significant.

Role of funding source

As per RESPOND governance (https://chip.dk/Portals/0/files/RESPOND/Study%20documents/RESPOND%20governance%20and%20procedures_v6_2020SEP30.pdf?ver=2020-10-20-163958-080), funders of the study were also academic collaborators, and employees/associates could be included as co-authors if they met the ICJME criteria. However, funding bodies (incl. employees/associates hereof), were not in a position to veto study design, data collection, data analysis, data interpretation, and/or writing of the manuscript.

RESULTS

Among 32,487 eligible individuals within RESPOND, 3147 participants were excluded, leaving 29,340 (90·3%) INSTI-naïve individuals for inclusion, as shown in the inclusion flowchart, Figure 1, which also notes specific reasons for the exclusion. There were some differences in baseline characteristics between those included and excluded. Compared to those included, a larger proportion of excluded participants were ART-naïve (1678/3147 [53·3%] vs. 7172/29,340 [24·4 %]), and a lower proportion had one or more comorbidities (1343/3147 [42·7%] vs 20,913/29,340 [71.3%]).

Of the 29,340 included individuals, 47·7% were exposed to one or more INSTIs during follow-up (8647 individuals to DTG, 3344 to EVG/c, 3296 to RAL, and 840 to BIC). The majority were white, males of Western European origin , with men who have sex with men as the predominant risk category (table 1).

During a median follow-up of 6·16 years (interquartile range, IQR: 3·87 – 7·52; 160,252 person-years of FU, PYFU), 748 individuals experienced a CVD event (299 MIs, 228 strokes, and 221 ICPs); crude incidence rate (IR) 4·67/1000 PYFU (95% confidence interval, 95% CI, 4·34 – 5·01). Traditional CVD risk factors, such as current smoking, hypertension, dyslipidemia, CKD, and diabetes, were more prevalent at baseline for those who developed a CVD event during follow-up ($p<0·001$ for all; Table 1). Further, individuals who experienced a CVD event were older than those who did not, and their 5-year estimated risk of CVD at baseline was consequently higher ($p<0·001$). Additional details on prior ART usage among those with and without incident CVD and those exposed and unexposed to INSTIs are provided in Supplementary tables 1 and 2.

Compared to those at low estimated 5-year risk of CVD, the odds of starting an INSTI showed an upward going linear trend, being significantly higher for those with moderate, high , and very high risk of CVD ($p<0.001$ for all; Figure 2). Further, the results were consistent but slightly more pronounced when only assessing ART-experienced individuals.

The crude CVD IR increased from 4·19/1000 PYFU (95% CI, 3·83 – 4.57) in those with no INSTI-exposure to a peak IR of 8·46/1000 PYFU (6·58 – 10·71) at >0–6 months of INSTI-exposure, and then gradually weakened, returning to rates similar to no INSTI-exposure, after >12–24 months INSTI-exposure, figure 3A. After adjusting for potential CVD confounders, the CVD IR ratio (aIRR) remained significantly higher at >0-6 months of INSTI-exposure when compared to those never exposed (aIRR 1·85 [95% CI, 1·44 – 2·39]; figure 3B). The aIRR remained elevated at >6 – 12 months of exposure (1·19 [0·84 – 1·68]) and >12 – 24 months of exposure (1·46 [1·13 – 1·88]), although the associations were weaker than within the first six months. After 24 months of exposure, aIRRs decreased to levels comparable to those with no INSTI-exposure (0·89 [0·62 – 1·29] and 0·96 [0·69 – 1·33] at >24–36 and >36 months, respectively)

Fitting CD4 cell count, BMI, hypertension, diabetes, CKD, and dyslipidemia as time-updated variables yielded results consistent with the primary analysis, as was the case when adding time-updated platelet counts to the model, Table 2. In addition, all performed sensitivity analyses were consistent with the primary analysis, Table 2.

Although we did not have the statistical power to perform adjusted analyses, crude IR for MIs and strokes was consistent with the primary analysis (numbers not shown). Moreover, as only 15% of total strokes caused by cerebral hemorrhages, we could not meaningful separate ischemic and hemorrhagic strokes.

We found no evidence suggesting that the association between INSTI-exposure and CVD differed according to baseline CVD risk score or age group ($p_{interaction}$ = 0·27 for both), indicating that the association was similar in both younger and older individuals and individuals at high and low estimated CVD risk. Likewise, the association was similar before and after Jan 1, 2014 ($p_{interaction}$ = 0·63), and for men and women ($p_{interaction}$ = 0·28).

Moreover, while we did not have adequate statistical power to stratify individuals based on treatment experience at baseline, we tested the interaction between INSTI-exposure and treatment experience at baseline defined as ART-naïves, ART-experienced with a VL ≥200 copies/mL, or ART-experienced with a VL <200 copies/mL, in a subsequent analysis. However, we found no evidence that the association differed between the groups ($p_{interaction}$ = 0·18).

To further investigate the impact of immunologic and virologic status on the CVD risk, we conducted an exploratory post hoc analysis focused on the first six months after starting an INSTI; stratifying individuals according to good, poor, or intermediate immunologic and virologic markers[28] at the time of INSTI initiation (good: CD4 count ≥500 cells/μL and VL <200 copies/mL, poor: CD4 count ≤350 cells/μL and VL >200 copies/mL intermediate: all other combinations respectively). However, we did not find any difference in the association between the groups (p = 0·20).

Discussion

To our knowledge, this is the first assessment of a potential association between INSTI-exposure and the incidence of CVD, which applies data derived from a large and multi-national cohort of PLWH seen in routine clinical care, with prospectively collected data and rigorously defined and centrally adjudicated end-points. After accounting for CVD risk factors, we observed that INSTI use was associated with an almost two-fold greater CVD incidence in the first six months of exposure compared to no INSTI-exposure. Although the association was relatively weaker after the initial six months, it persisted until 24 months of exposure, after which the incidence decreased to levels comparable to that of no INSTI-exposure. Findings were consistent across a wide range of sensitivity analyses, with no evidence suggesting that the association between INSTI-exposure and CVD incidence differed according to underlying estimated CVD risk strata, age group, sex, calendar time, or immune/virologic status.

Randomised clinical trials (RCTs) and observational studies, including the RESPOND cohort itself, have suggested an association between INSTI use and increase in BMI,[20–22] especially within the first 12 months of initiating ART, and potentially also with metabolic syndrome.[22] Therefore, as higher BMI is associated with CVD, it could be hypothesised that INSTI-exposure might increase CVD risk over time. Conversely, we found a rapid increase in CVD incidence after INSTI initiation, which was no longer present beyond 24 months of exposure — a pattern of association different from that previously described for cumulative exposure to certain PIs[7,8,13] and recent exposure to ABC.[8,9] Nevertheless, the strength of the association, with an estimated relative risk increase of 85% within the first six months, and 46% between 12 to 24 months, were similar in magnitude to previous reports for both cumulative exposure boosted PIs and recent ABC exposure. If the association indeed turns out to be causal, it could imply that CVD develops quickly after INSTI initiation in individuals with a distinct underlying vulnerability. However, it is possible that unmeasured confounding may have played a part in our findings.

The increased likelihood of starting an INSTI in persons with a higher estimated 5-year CVD risk indicates at least some degree of confounding by indication, with individuals at risk of CVD preferentially starting an INSTI-based regimen. However, it is important to note that the association found between INSTI use and incident CVD remained after adjusting for CVD risk profiles, including ABC and other antiretroviral drugs previously associated with CVD. Further, the association was also observed for individuals with a low estimated 5-year CVD risk, suggesting that the findings cannot alone be explained by confounding by indication. Nevertheless, the lack of such an interaction warrants a cautious interpretation with the test's limited statistical power.

RESPOND's observational nature does not allow us to establish causality of the found association. However, we examined possible mediator effects in exploratory analyses, adjusting for any effects of time-updated BMI, hypertension, diabetes, dyslipidemia, and CKD. These adjustments showed no attenuation in CVD risk; therefore, none of these are likely to mediate it, consistent with these factors leading to CVD via slow developing atherosclerosis and would not account for the rapid increase in CVD rates as seen here. In addition, prior findings from RESPOND analyses examining incident dyslipidemia[29] and hypertension[30] have not found an increase in these events within a period that precedes or matches the increased CVD risk incidence seen here, although, the time of the event was not the main focus of these analyses Nevertheless, here we focused on the potential relationship between CVD and INSTIs more broadly, not restricting the population, and understanding the potential effects of INSTI-related weight gain is of increasing clinical interest. Therefore, future RESPOND studies will investigate potential associations between CVD risk factors and CVD in greater detail for the population experiencing weight gain related to INSTIs.

Overall, the lack of an attenuated effect after adjusting for BMI and other known CVD-risk factors suggests one of two possible explanations: either that we have not captured the CVD risk factors through which INSTIs act to increase CVD adequately, or that the association is in fact not causal, and explained by unmeasured CVD risk factors in the INSTI-exposed population. A third possible explanation for our findings is that INSTIs can increase CVD risk via a different mechanism unrelated to known CVD risk factors. Such an effect could be similar to the drug-induced platelet hyperreactivity, suggested as the mechanisms linking ABC to CVD,[12] or the antibody-mediated clot formation and thrombocytopenia seen in vaccine-induced immune thrombotic thrombocytopenia.[31] However, introducing time-update platelet count into our model did not affect the relative risk, although we cannot adequately address thrombocyte function and other potential pathways in this study. We encourage further examinations of the possible underlying mechanism for the association observed here in mechanistic studies.

INSTI treatment can cause a rapid increase in CD4 cell count in individuals initiating treatment with a low CD4 cell count. Therefore, increased occurrence of the immune-reconstitution-inflammatory syndrome, or a similar phenomenon, with immunological changes that could mimic symptoms of CVD or even cause type II, non-atherosclerotic, MIs could also be suspected to underlie our findings. However, it is important to underline that RESPOND's clinical event definitions exclude all suspected type II MIs and stroke cases due to other causes such as opportunistic infections and cancers. Moreover, in addition to the low number of ART-naïve individuals included here, there was no apparent difference seen in the first six months when stratifying individuals by CD4 cell count and VL at the time of INSTI initiation and the risk was similar for ART naïve, and ART experienced individuals. In addition, adding time-updated CD4 cell count to our model did not influence the CVD risk in any substantial way. Therefore, immune-reconstitution-inflammatory syndrome or

a related condition as an explanation seems unlikely — even though we did not assess CD4/CD8 ratios in these analyses, as it is not available for all participants at present.

Focusing exclusively on those on INSTIs, using 0-6 months as a reference, lower CVD rates after 24 months might be suggested. However, such an interpretation is not without caveats. If confounding by indication explains the initial 0-6 months peak, a comparison with this group would be biased towards lower rates. Moreover, to confirm a decrease >24 compared to 0-6 would require substantially longer follow-up to also rule out an increase in CVD with long term exposure beyond 3 years.

Contrary to our findings here, no RCTs assessing INSTIs have reported a short-term increase in CVD incidence.[15–18] Nevertheless, it is worth noting that while RCTs are essential to determine ART efficacy and safety, they do generally not have the large sample size or duration of follow-up needed to uncover rarely occurring events such as CVD. Although investigations of CVD occurrence with INSTI-exposure are still scarce, a recently published US-based analysis showed no association between INSTI use and CVD.[24] Nevertheless, the analysis had a retrospective design, did not assess CVD incidence stratified by exposure time, and excluded CVD events occurring in the first three months of INSTI initiation. Therefore, an immediate effect may have been overlooked and further diluted by not accounting for events shortly after INSTI initiation. In addition, an older analysis from the US Veteran's Affairs cohort, assessing potential cardioprotective effects of atazanavir, reported hazard ratios of MI and stroke that were lower for atazanavir than for INSTI, in line with our findings. Even so, the study period of the analysis spanned from 2003-2015, and the INSTI group was relatively small, including only a limited number of individuals treated with second-generation INSTIs.[23]

There are several limitations of our analysis to address. Firstly, as this is an observational study, we cannot exclude the potential for residual confounders or channelling bias as discussed above. We have applied the same methodology developed and used in D:A:D pharmacovigilance analyses adjusting for a number of potential confounders and performed numerous consistent sensitivity analyses, interpreting results cautiously and conservatively.[5-8] Nevertheless, propensity score matching could have been considered an alternative to traditional regression analyses, even though such methods also have their limitations.

Secondly, we did not have adequate analytical power to restrict the analyses to only include ART-naïve individuals or provide reliable estimates for individual INSTIs use at present. Therefore, we assessed all INSTIs collectively as a class for a combined population of ART-naïve and ART-experienced individuals; explored further, in post-hoc power calculation, we found less than 50% power to detect a 1.8-fold increase in the incidence of CVD in the first 6 months of exposure to the most frequently used INSTI in RESPOND, DTG, versus those not exposed to DTG.

We acknowledge there may be within-class differences in CVD risk among INSTIs, as shown for PIs,[13] and differences in CVD risk assessments. However, reporting on potential class effects follows that of earlier

studies, including D:A:D, and allows for timely reporting of a potential safety signal of currently used ART, allowing for investigation in other studies and examination of potential mechanisms.[6–8] Assessments of CVD incidence with cumulative use of individual INSTIs, stratified by ART experience, will be a focus area for RESPOND going forward as follow-up time within the cohort increases.

Finally, as RESPOND only includes individuals naïve to INSTIs before 2012, we could not directly examine the relationship between CVD and use of NNRTIs or PI/b within the same analysis, as very few individuals within the cohort were naïve to these two drug classes by Jan 1 2012, and thus the statistical power was insufficient.

In summary, while we cannot exclude possible channelling bias and residual confounding, we found that after accounting for CVD risk factors — including the use of ABC — INSTI-exposure was associated with an almost two-fold higher CVD risk in the first six months after INSTI initiation. The increased risk persisted up to 24 months of use, albeit with lower risk. The association was similar in individuals with high and low estimated CVD risk and across a wide range of sensitivity analyses. These early findings call for analyses in other large studies, and the potential underlying mechanisms explored further..

Author contribution:

BN, LG, AM, LR, and JDL proposed and developed the research question,

BN wrote the first draft of the manuscript.

LG conducted the statistical analyses.

JDL, JMM, KG-P, GW, CS, SDW, FW, LP, APM, CM, AC, CP, AM, JJV, AS, AVA, AC, LB-M, HFG, FR, RZ, HG, LDR, CN, MvdV, MM and CMU contributed to the study design, interpretation of data and revision of the manuscript.

BN, LG, AM, and LR have verified the underlying data

All authors have seen and contributed to the final version of the manuscript.

Potential conflicts of interest

BN, LR, LDR, LBM no conflicts of interest

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Hospital Bonn, University Hospital Cologne participates The International Cohort Consortium of Infectious Disease (RESPOND)

The full RESPOND study group can be found at https://www.chip.dk/Research/Studies/RESPOND/Study-group and is listed in the Appendix.

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Data sharing statements:

The RESPOND Scientific Steering Committee (SSC) encourages the submission of concepts for research projects. Online research concepts (please see https://chip.dk/Portals/0/files/RESPOND/RESPOND%20governance%20and%20procedures_v6_2019SEP30.pdf?ver=2019-10-02-144419-230) should be submitted to the RESPOND secretariat (respond.rigshospitalet@regionh.dk). The secretariat will direct the proposal to the relevant Scientific Interest Group, where the proposal will initially be discussed for scientific relevance before being submitted to the SSC for review.

Once submitted to the SSC, the research concept's scientific relevance, relevance to RESPOND's ongoing scientific agenda, design, statistical power, feasibility, and overlap with already approved projects will be evaluated. Upon completion of the review, feedback will be provided to the proposer(s). In some circumstances, a revision of the concept may be requested. If the concept is approved for implementation, a writing group will be established consisting of the proposers (up to 3 persons who were centrally involved in developing the concept), representatives from RESPOND cohorts, and representatives from the Statistical Department and Coordinating Center. All persons involved in the process of reviewing these research concepts are bound by confidentiality.

All data within RESPOND from individual cohorts are de-identified. The present RESPOND data structure and a list of all collected variables and their definition can be found in the latest version of "Standard Operating Procedure for data transfer in RESPOND, EuroSIDA, MISTRAL, and CARE," of the publicly available at https://chip.dk/Research/Studies/RESPOND/Study-documents.

For any inquiries regarding data-sharing, please contact the RESPOND secretariat (respond.rigshospitalet@regionh.dk) and Dorthe Raben, Director of Research Coordination (Dorthe.raben@regionh.dk

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Table 1: Baseline demographics and clinical characteristics, overall and stratified by cardiovascular event

		Overall, n = 29340		**CVD Event,** n = 748		**No CVD Event,** n = 28592	
		n	(%)	n	(%)	n	(%)
Sex	Male	21818	(74·4)	655	(87·6)	21163	(74·0)
	Female	7478	(25·5)	93	(12·4)	7385	(25·8)
Ethnicity	White	20419	(69·6)	611	(81·7)	19808	(69·3)
	Black	2983	(10·2)	20	(2·7)	2963	(10·4)
	Other	1267	(4·3)	15	(2·0)	1252	(4·4)
Geographical region	West Europe	12810	(43·7)	443	(59·2)	12367	(43·3)
	South Europe and Argentina	6626	(22·6)	140	(18·7)	6486	(22·7)
	North Europe and Australia	7069	(24·1)	129	(17·2)	6940	(24·3)
	East Europe	2832	(9·7)	36	(4·8)	2796	(9·8)
Risk of HIV acquisition	Men sex with men	13229	(45·1)	362	(48·4)	12867	(45·0)
	Intraveneous drug use	3993	(13·6)	117	(15·6)	3876	(13·6)
	Heterosexual sex	10253	(34·9)	216	(28·9)	10037	(35·1)
	Other	654	(2·2)	15	(2·0)	639	(2·2)
CD4 cell nadir (cells/µL)	<200	11925	(40·6)	398	(53·2)	11527	(40·3)
	200-350	8757	(29·8)	202	(27·0)	8555	(29·9)
	350-500	4325	(14·7)	74	(9·9)	4251	(14·9)
	>500	4333	(14·8)	74	(9·9)	4259	(14·9)
Prior AIDS	Yes	5785	(19·7)	221	(29·5)	5564	(19·5)
ART treatment status	ART-naïve	7172	(24·4)	58	(7·8)	7114	(24·9)
	ART-experienced, VL <200 cp/mL	19951	(68·0)	647	(86·5)	19304	(67·5)
	ART-experienced, VL ≥200 cp/mL	2217	(7·6)	43	(5·7)	2174	(7·6)
BMI (kg/m^2)	<18·5	873	(3·0)	18	(2·4)	855	(3·0)
	18·5 - <25	11321	(38·6)	335	(44·8)	10986	(38·4)
	25 - <30	1547	(5·3)	51	(6·8)	1496	(5·2)
	>30	5159	(17·6)	162	(21·7)	4997	(17·5)
Smoking status	Never	8207	(28·0)	191	(25·5)	8016	(28·0)
	Current	8196	(27·9)	305	(40·8)	7891	(27·6)
	Previous	2261	(7·7)	90	(12·0)	2171	(7·6)
Hypertension[±]		5683	(19·4)	330	(44·1)	5353	(18·7)
Diabetes[≠]		1170	(4·0)	99	(13·2)	1071	(3·7)
Dyslipidaemia[┼]		17984	(61·3)	633	(84·6)	17351	(60·7)
Prior CKD[⊤]		541	(1·8)	44	(5·9)	497	(1·7)
Prior CVD[⊥]		666	(2·3)	94	(12·6)	572	(2·0)
		Median	IQR	Median	IQR	Median	IQR
Age (years)		44·3	(36·2-51·3)	53·4	(47·5-61·5)	44·0	(36·0-51·0)
CD4 (cells/µL)		524·0	(357·0-715·0)	554·0	(388·5-752·0)	523·0	(355·8-714·0)
Platelets (cells/nL)		200	(134-248)	213	(165-260)	200	(133-248)

Percentage of overall unknowns: ethnicity: 15·9, Risk of HIV acquisition: 4·1, Prior AIDS: 5·4, BMI: 35·6, smoking status: 36·4, hypertension: 17·5, diabetes: 20·8, CKD: 9·8, and CVD: 9·8

p<0·001 for all comparisons5

Abbreviations: HIV: human immunodeficiency virus, AIDS: Acquired Immune Deficiency Syndrome, µL: microliter, nL: nanoliter ART: antiretroviral therapy, VL: (HIV) viral load, cp: copies, BMI: body mass index, CKD: Chronic kidney disease, CVD: Cardiovascular disease, IQR: Interquartile range

[±]Hypertension: Blood pressure systolic >140 mm Hg, diastolic >90 mm Hg or use of antihypertensive drugs

[≠]Diabetes: random blood glucose > 11·1 mmol/L, HbA1c > 48 mmol/mol, use of antidiabetic drugs or a noted diagnosis of diabetes

[┼]Dyslipidimia: Total cholesterol >6·2 mmol/L, high-density lipoprotein (HDL)cholesterol <0·9 mmol/L, triglyceride >2·3 mmol/L, or use of lipid-lowering treatment)

[⊤]Prior CKD: ≥2 estimated glomerular filtration rate (eGFR) measure <60 mL/min/1·73m^2

[⊥]Prior CVD: MI, stroke, and ICPs, (coronary angioplasty/stenting, coronary bypass surgery, and carotid endarterectomy)

Table 2: Adjusted incidence rate ratio by cumulative exposure to INSTIs, compared to no INSTI-exposure in exploratory- and sensitivity analyses.

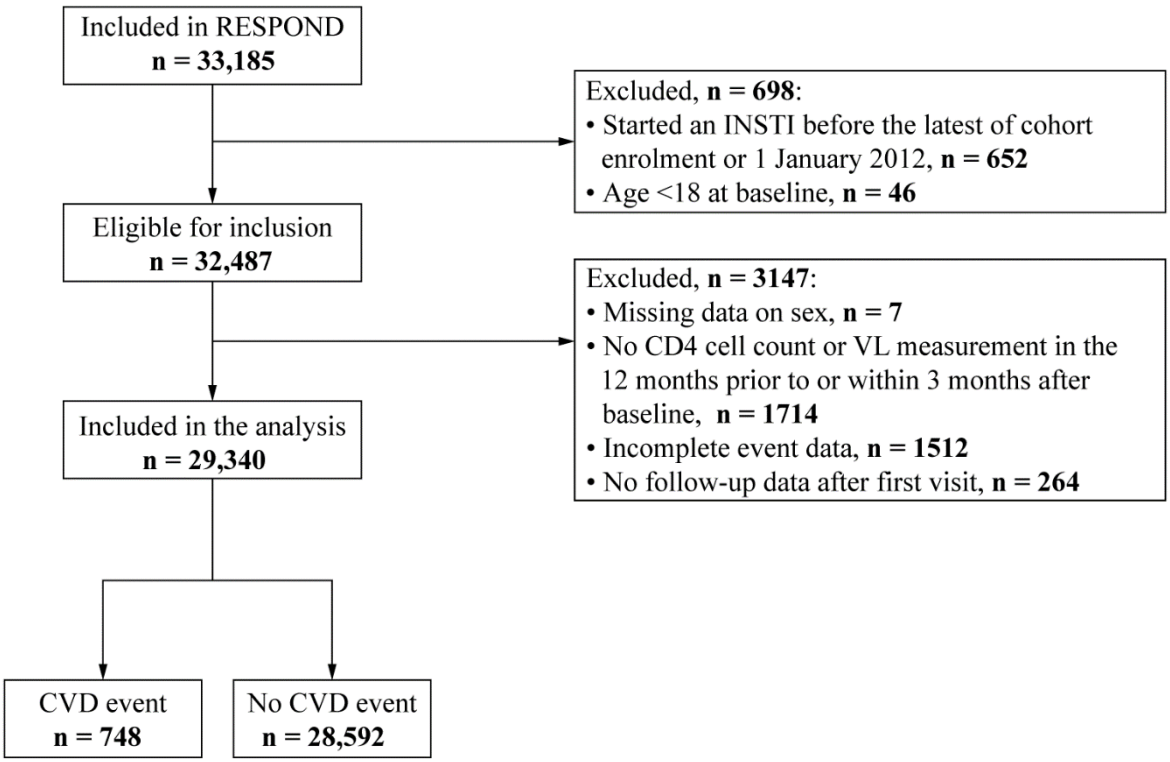
		Cumulative INSTI-exposure												
		0 months (reference)		>0 – 6 months		>6 – 12 months		>12 – 24 months		>24 – 36 months		>36 months		Global p-values
	n include	aIRR (95% CI)	Events (PYFU)	aIRR (95% CI)	Events (PYFU)	aIRR (95% CI)	Events (PYFU)	aIRR (95% CI)	Events (PYFU)	aIRR (95% CI)	Events (PYFU)	aIRR (95% CI)	Events (PYFU)	
Primary model †	29,340	1 (ref)	506 (120714)	1·85 (1·44 – 2·39)	69 (8154)	1·19 (0·84 – 1·68)	34 (6489)	1·46 (1·13 – 1·88)	69 (10327)	0·89 (0·62 – 1·29)	31 (7287)	0·96 (0·69 – 1·33)	39 (7938)	<0·0001
Model with time-updated factors on the potential causal pathway *	29,340	1 (ref)	506 (120714)	1·92 (1·47 – 2·52)	69 (8154)	1·09 (0·74 – 1·61)	34 (6489)	1·27 (0·95 – 1·70)	69 (10327)	0·81 (0·54 – 1·22)	31 (7287)	0·87 (0·61 – 1·26)	39 (7938)	<0·0001
Model with time-updated factors on the potential causal pathway + platelets ⌂	29,340	1 (ref)	506 (120714)	1·93 (1·47 – 2·52)	69 (8154)	1·09 (0·74 – 1·61)	34 (6489)	1·27 (0·95 – 1·70)	69 (10327)	0·82 (0·54 – 1·23)	31 (7287)	0·88 (0·61 – 1·27)	39 (7938)	<0·0001
Model only adjusted for D:A:D 5-year CVD risk score ¤	29,340	1 (ref)	506 (120714)	2·07 (1·61 – 2·66)	69 (8154)	1·29 (0·91 – 1·83)	34 (6489)	1·61 (1·25 – 2·07)	69 (10327)	1·00 (0·70 – 1·45)	31 (7287)	1·11 (0·80 – 1·53)	39 (7938)	<0·0001
Excluding individuals with prior CVD at baseline #	28,674	1 (ref)	445 (118141)	1·83 (1·39 – 2·41)	60 (7976)	1·12 (0·77 – 1·63)	29 (6366)	1·36 (1·03 – 1·80)	58 (10111)	0·86 (0·58 – 1·28)	27 (7141)	0·97 (0·69 – 1·38)	35 (7731)	0·0002
Excluding ICPs from the composite CVD outcome ɵ	29,340	1 (ref)	353 (120714)	1·77 (1·30 – 2·41)	47 (8154)	1·13 (0·74 – 1·73)	23 (6489)	1·55 (1·15 – 2·08)	52 (10327)	0·73 (0·45 – 1·17)	18 (7287)	0·93 (0·63 – 1·38)	27 (7938)	0·0003
Including only individuals who started/shifted regimen after Jan 1, 2012 ×	20,782	1 (ref)	118 34081	1·76 (1·31 – 2·37)	73 8609	1·18 (0·82 – 1·71)	38 6863	1·41 (1·05 – 1·89)	74 10922	0·98 (0·68 – 1·43)	37 7730	1·03 (0·72 – 1·46)	44 8412	0·0023
Including only centrally adjudicated CVD events ±	21,188	1 (ref)	145 (40886)	1·37 (0·89 – 2·12)	26 (4121)	1·30 (0·82 – 2·06)	22 (3744)	1·33 (0·93 – 1·90)	48 (7149)	0·93 (0·61 – 1·42)	27 (6006)	0·88 (0·59 – 1·31)	34 (7533)	0·22

†**primary model, adjusted for** age (per 10 years older), sex (male, female), ethnicity (Black, White, other), region (West Europe, South Europe and Argentina, North Europe and Australia, East Europe), BMI (kg/m; <18·5, 18·5-<25, 25-<30 and >30), HIV acquisition risk (MSM, heterosesual contact IDU, CD4 cell count (per 100 cell/μL higher), hypertension (yes/no), diabetes (yes/no), prior AIDS (yes/no), prior CVD (yes/no), prior CKD (yes/no), dyslipidaemia (yes/no), all fixed at baseline. In additon smoking and antiretroviral drugs previously associated with CVD (cumulative exposure to indinavir, ritonavir-boosted lopinavir, boosted darunavir, didanosine, and recent ABC exposure [current or within six months]) were included in the model as time-updated variables.

* As primary model, with BMI, hypertension, diabetes, dyslipidemia, CKD, and CD4 cell count fitted as time-updated variables instead of at baseline
⌂ As primary model, with BMI, hypertension, diabetes, dyslipidemia, CKD, and CD4 cell count fitted as time-updated variables instead of at baseline + time-updated platelet count
¤ Model adjusted only for D:A:D 5-year CVD risk score at baseline
As primary model, excluding individuals with prior CVD at baseline
ɵ As primary model, excluding ICPs from the composite CVD outcome
× As primary model, including only individuals who started/shifted regimen after the RESPOND baseline, Jan 1, 2012
± As primary model , including only centrally validated CVD events. However, as the median time of CVD event was before the validation period, the model included a substantially lower number of events (302 vs 748) and had limited statistical power.

Abbreviations: aIRR: adjusted Incidence rate ratio, 95% CI: 95 % confidence interval, PYFU: person years of follow-up, BMI: Body mass index, CVD: cardiovascular disease, CKD: chronic kidney disease, INSTI: integrase strand transfer inhibitor

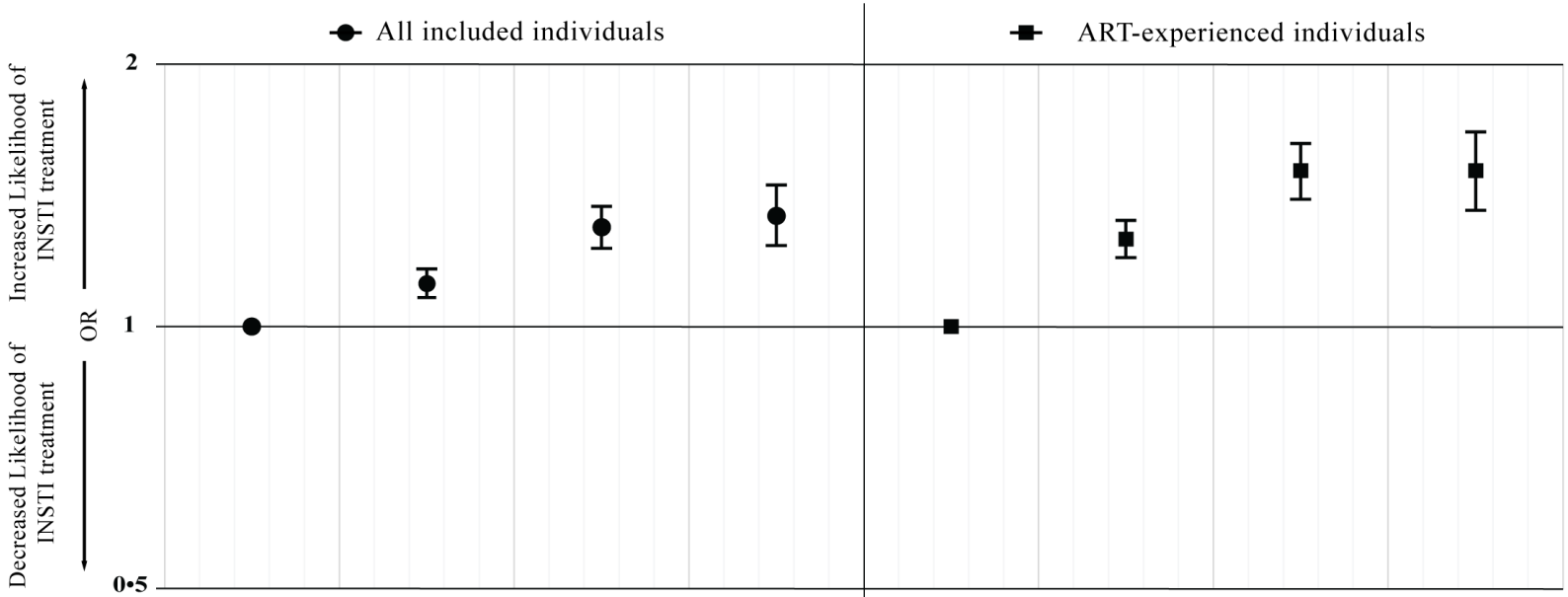
Figure 1: Flowchart depicting the participant inclusion process



Abbreviations. CVD: Cardiovascular disease, INSTI: integrase strand transfer inhibitor, VL: viral load

Note that more that one reason for exclusion may apply

Figure 2: Calendar time adjusted odds of starting an INSTI by D:A:D estimated 5-year CVD risk score category

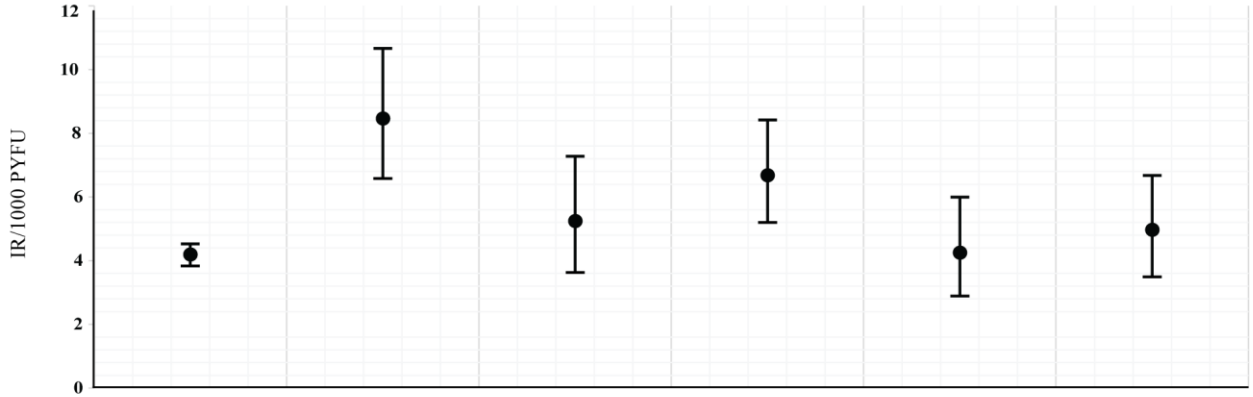


Estimated D:A:D 5-year CVD risk	< 1%	≥1 – <5%	≥5 – <10%	≥10%	< 1%	≥1 – <5%	≥5 – <10%	≥10%
Adjusted odds ratio [95% CI]	1	1·12 [1·08–1·17]	1·30 [1·23–1·38]	1·34 [1·24–1·46]	1	1·26 [1·20–1·33]	1·51 [1·40–1·63]	1·51 [1·36–1·68]
P-value	(ref)	<0·001	<0·001	<0·001	(ref)	<0·001	<0·001	<0·001

Abbreviations: CVD: cardiovascular disease, INSTI: integrase strand transfer inhibitor, D:A:D: Data Collection on Adverse events of Anti-HIV Drugs, ART: antiretroviral therapy, 95% CI: 95% confidence interval

Figure 3: A: Crude IR of CVD/1000 PYFU by cumulative exposure to INSTIs. B: Adjusted incidence rate ration (IRR) by cumulative exposure to INSTIs, compared to no INSTI-exposure

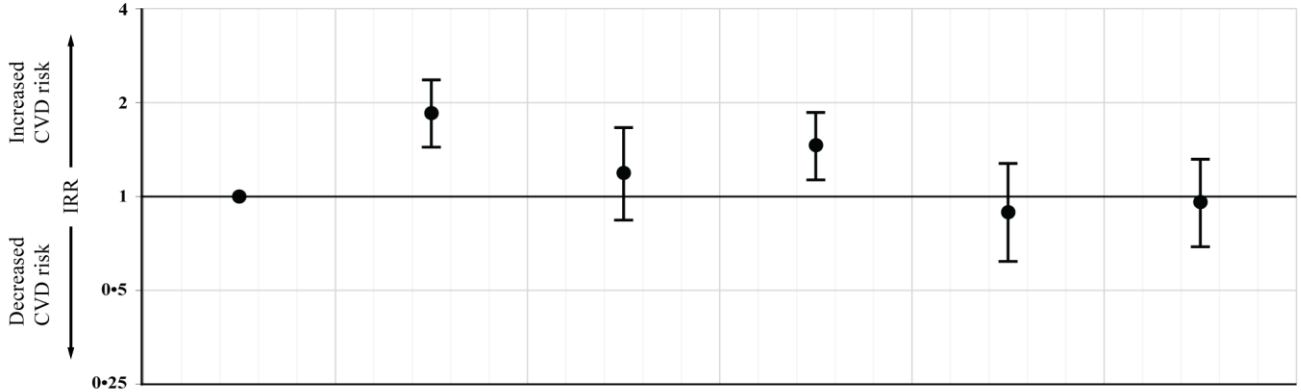
Figure A



	0 months	0 – 6 months	6 – 12 months	12 – 24 months	24 – 36 months	>36 months
Persons at risk (n)	29340	13602	12103	10894	8376	5733
PYFU	120714	8154	6489	10327	7287	7938
Events (n)	506	69	34	69	31	39
IR [95% Confidence interval]	4·19 [3·83–4·57]	8·46 [6·58–10·71]	5·24 [3·63–7·32]	6·68 [5·20–8·46]	4·25 [2·89–6·04]	4·91 [3·49–6·72]

Figure B

	0 months	0 – 6 months	6 – 12 months	12 – 24 months	24 – 36 months	>36 months
IRR [95% Confidence interval]	reference	1·85 [1·44–2·39]	1·19 [0·84–1·68]	1·46 [1·13–1·88]	0·89 [0·62–1·29]	0·96 [0·69–1·33]



Multivariable model adjusted for: age (per 10 years older), sex (male, female), ethnicity (Black, White, other), region (West Europe, South Europe and Argentina, North Europe and Australia, East Europe), BMI (kg/m; <18·5, 18·5-<25, 25-<30 and >30), HIV acquisition risk (MSM, heteroseksual contact IDU, CD4 cell count (per 100 cell/µL higher), hypertension (yes/no), diabetes (yes/no), prior AIDS (yes/no), prior CVD (yes/no), prior CKD (yes/no), dyslipidaemia (yes/no), all fixed at baseline. In addition Smoking and antiretroviral drugs previously associated with CVD (cumulative exposure to indinavir, ritonavir-boosted lopinavir, boosted darunavir, didanosine, and recent ABC exposure [current or within six months]) were included in the model as time-updated variables.

Abbreviations: MSM: Men who have sex with men, IDU: intravenous drug use, BMI: Body mass index, CVD: cardiovascular disease, CKD: chronic kidney disease, INSTI: integrase strand transfer inhibitor, PYFU: person years of follow-up, IR: incidence rate, IRR: incidence rate ratio.

Supplementary Table 1: Prior exposure as numbers, percentage and median cumulative exposure to specific drugs within different ART classes among individuals that did experience a CVD event during follow-up, stratified by INSTI exposed or not INSTI exposed during follow-u

		INSTI exposed	Not INSTI exposed
ARVs	exposure	n = 242	n = 506
PIs (IDV,LPV/r, DRV/b)	n	159	311
	%	65.7 %	61.5%
	Median cumulative exposure, months (IQR)	56 (22-114)	58 (23-95)
NRTIs (ABC, DDI)	n	180	318
	%	74.4%	62.8%
	Median cumulative exposure, months (IQR)	72 (29-127)	79 (30-127)
NNRTIs (EFV)	n	103	245
	%	42.6%	48.4%
	Median cumulative exposure, months (IQR)	49 (17-112)	53 (17-115)

Abbreviations: PIs: protease inhibitors, ARVs: antiretroviral drugs, IDV: indinavir, LPV/r: ritonavir boosted lopinavir, DRV/b: cobicistat or ritonavir boosted darunavir, NRTI: nucleos(t)ide reverse transcriptase inhibitors, ABC: abacavir, DDI: Didanosine, NNRTI: non-nucleotide reverse transcriptase inhibitors, EFV: efavirenz, IQR: interquatile range, CVD: cardiovascular disease, INSTI: integrase inhibitor

Supplementary Table 2: Prior exposure as numbers, percentage and median cumulative exposure to specific drugs within different ART classes among individuals that did not experience a CVD event during follow-up, stratified by INSTI exposed or not INSTI exposed during follow-up

		INSTI exposed	Not INSTI exposed
ARVs	exposure	n = 13360	n =15232
PIs (IDV,LPV/r, DRV/b)	n	6944	7507
	%	52.0%	49.3%
	Median cumulative exposure, months (IQR)	49 (19-96)	51 (20-96)
NRTIs (ABC, DDI)	n	7830	6202
	%	58.6%	40.7%
	Median cumulative exposure, months (IQR)	53 (26-111)	64 (26-111)
NNRTIs (EFV)	n	4637	6657
	%	34.7%	43.7%
	Median cumulative exposure, months (IQR)	56 (16-111)	60 (19-115)

Abbreviations: PIs: protease inhibitors, ARVs: antiretroviral drugs, IDV: indinavir, LPV/r: ritonavir boosted lopinavir, DRV/b: cobicistat or ritonavir boosted darunavir, NRTI: nucleos(t)ide reverse transcriptase inhibitors, ABC: abacavir, DDI: Didanosine, NNRTI: non-nucleotide reverse transcriptase inhibitors, EFV: efavirenz, IQR: interquatile range, CVD: cardiovascular disease, INSTI: integrase inhibitor