"Cardiovascular risks associated with protease inhibitors for the treatment of HIV"

Abstract

Introduction: Cumulative use of some first-generation protease inhibitors has been associated with higher rates of dyslipidemia and increased risk of cardiovascular disease. The protease inhibitors most commonly in use are atazanavir and darunavir, which have fewer detrimental lipid effects and greater tolerability. This paper aims to review the evidence of a potential association of these contemporary protease inhibitors with the risk of ischemic CVD and atherosclerotic markers.

Areas covered: We searched for publications of randomized trials and observational studies on *PubMed* from 1st January 2000 onwards, using search terms including: protease inhibitors; darunavir; atazanavir; cardiovascular disease; cardiovascular events; dyslipidemia; mortality; carotid intima media thickness; arterial elasticity; arterial stiffness and drug discontinuation. Ongoing studies registered on *clinicaltrials.gov* as well as conference abstracts from major HIV conferences from 2015-2020 were also searched.

Expert opinion: Atazanavir and darunavir are no longer part of first-line HIV treatment, but continue to be recommended as alternative first line, second- and third-line regimens, as part of two drug regimens, and darunavir is used as salvage therapy. Although these drugs will likely remain in use globally for several years to come, baseline CVD risk should be considered when considering their use, especially as the population with HIV ages.

Article highlights box:

- History and biological properties of PIs
- Association of contemporary PIs with surrogate markers for atherosclerotic CVD
- Contemporary PIs and association with clinical CVD
- PI boosters and risk of CVD
- Possible biological mechanisms for contemporary PIs and association with CVD
- Clinical approach to the use of contemporary PIs

Keywords:

Atazanavir, darunavir, HIV, cardiovascular disease, atherosclerotic markers, boosters, clinical management

1. Introduction

Protease inhibitors (PIs) have been a major component of combination antiretroviral therapy (cART) regimens since their first introduction in 1995. Together with nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and integrase strand transfer inhibitors (INSTIs), these four drug classes have resulted in a significant reduction in HIV mortality and morbidity in people living with HIV (PLWH) [1], who typically achieve a reduction in viral load and increase in CD4 count soon after treatment initiation. With optimal maintenance of cART, morbidity and mortality risk is significantly reduced, resulting in a life expectancy of PLWH that now, in some studies, approaches that of the general population [2,3].

As AIDS-related morbidity and mortality have decreased, the life expectancy of PLWH has increased and, with that, the research focus is increasingly directed towards the prevention and management of non-communicable comorbid conditions. In particular, an increased risk of ischemic cardiovascular disease (CVD), (including myocardial infarctions (MIs), strokes and invasive cardiovascular procedures), has been reported [4-6], likely resulting from multiple factors, including traditional CVD risk factors (e.g. smoking, dyslipidemia, hypertension, diabetes and chronic kidney disease) [4][7-9]), HIV-related factors (e.g. ongoing inflammation, immune activation, microbial translocation) [10,11]) and the pro-atherogenic effects of some antiretroviral drugs (ARVs), including certain PIs and abacavir (ABC) [12-15].

Of the first generation PIs, cumulative use of indinavir (IDV), (fos)amprenavir ((f)APV), and ritonavir (RTV) have been associated with higher rates of dyslipidemia and an increased risk of CVD in primarily observational studies [6][13-19]; whereas similar associations with nelfinavir (NFV) or saquinavir (SQV) have not been observed [15,16][18]. Higher rates of dyslipidemia and an increased risk of CVD were also observed following the introduction of lopinavir (LPV), generally used in combination with pharmacologically boosted doses of RTV (LPV/r) [6][15][18]. In randomized controlled trials (RCTs), two newer PIs - i) atazanavir (ATV), used both in an unboosted form (ATV) and boosted (ATV/b) with either RTV (ATV/r) or cobicistat (ATV/c) and ii) darunavir, which is always pharmacologically-boosted (DRV/b) with either RTV (DRV/r) or cobicistat /DRV/c) - appeared to have fewer detrimental lipid effects and greater tolerability

than LPV/r and other first generation PIs [20,21].

Among PLWH with access to CVD care, the absolute age-adjusted risk of CVD mortality has decreased over the past decade [1][22]. However, CVD mortality rates remain higher in PLWH compared to HIV-negative individuals at all ages [22], and the proportionate CVD mortality has increased as deaths from AIDS-related causes have declined [22,23]. As the population of PLWH ages, the incidence of CVD is expected to increase, and as PLWH also have higher rates of comorbid conditions at any given age compared to their HIV-negative counterparts [24], this can contribute to poorer survival. Modelling studies suggest that by 2030, nearly 30% of PLWH will have >3 comorbidities [25], although greater awareness of co-morbidity management and antiretroviral options may counterbalance this increased age-related risk.

While several reviews and commentaries have discussed the CVD risk associated with the use of (predominantly older) PIs [6][26-28], this paper aims to provide a more comprehensive review on the latest evidence to describe the potential risks of ischemic CVD among those receiving the two PIs most often used in current practice (ATV and DRV) as well as the impact of these drugs on surrogate markers of atherosclerotic disease. Possible biological mechanisms and suggested approaches for clinical practice will also be reviewed. Discussion of non-ischemic CVD (i.e. heart failure, arrythmias and peripheral artery disease), use of other ARVs and other CVD risk factors which may be prevalent in PLWH are beyond the scope of this review.

Methods

We searched for publications of RCTs and observational studies on PubMed. Papers were included if they were in English and were published from 1st January 2000 onwards, as ATV and DRV were licensed in 2003 and 2005, respectively. We used the following search terms based on the aim of the review to describe contemporary PIs, surrogate atherosclerotic markers and CVD: *protease inhibitors; darunavir; atazanavir; cardiovascular disease; dyslipidemia; mortality; carotid intima media thickness; carotid intima media thickness AND atazanavir; carotid intima media thickness AND darunavir; arterial elasticity AND atazanavir; arterial elasticity AND darunavir; arterial stiffness AND atazanavir; arterial stiffness AND darunavir; discontinuation of atazanavir; discontinuation of darunavir; atazanavir AND cardiovascular events; darunavir AND cardiovascular events*. Cardiovascular disease was used as a broad search term to include ischemic MIs and strokes. We also searched studies registered on *clinicaltrials.gov* as well as conference abstracts from four major HIV conferences (Conference on Retroviruses and Opportunistic infections; HIV Glasgow; European Clinical AIDS Society (EACS); the International AIDS Society) from 2015-2020. We only searched conference abstracts for the past five years as it was anticipated that any presentations pre-dating this would by now be published and hence identified through the publication search.

3. Protease inhibitors; history and drug biological properties

PIs act by inhibiting the HIV protease, thus forming a cleavage of the HIV precursor polyprotein required for formation of viral proteins necessary for the effectivity of HIV virions which then inhibits viral replication [29]. In 1995-96, SQV, IDV and RTV were the first US Food and Drug Administration approved PIs to be introduced to care in combination with two NRTIs. These combinations permitted the maintenance of viral suppression whilst simultaneously providing a higher genetic barrier against drug resistance. Although PIs offered substantial benefits over NRTI-based mono- or dual-regimens, the approval of LPV in 2000, with its improved efficacy and tolerability compared to older PIs, particularly when boosted with RTV (LPV/r) [30], heralded a new era of PI therapy. This was soon followed by the licensing of ATV and DRV.

As many PIs have a short half-life this would require frequent dosing and a high pill burden if not pharmacologically boosted, most are now used as part of regimens that also include low boosting doses of either RTV or cobicistat (introduced in 2014). RTV is rarely used as a PI in its own right given the toxicities associated with full doses.

Following the introduction of the INSTIs, including raltegravir (RAL), dolutegravir (DTG) and bictegravir) and newer effective NNRTIs (rilpivirine and doravirine), cART regimens have changed substantially and PI use is now declining. INSTIs are now generally preferred to PIs due to their reduced potential for drug-drug interactions and improved tolerability [31-33], however, boosted PIs are still an important part of alternative first line, second -and third line regimens and are used as part of two drug regimens [31-33]. Partly as a result, and partly due to the superior toxicity profiles and efficacy of DRV/r and ATV/r [20,21], LPV/r is now rarely used in most resource-rich settings.

4. Results

Use of contemporary PIs and association with CVD risks

PLWH with CVD have been reported to be younger at the time of their primary CVD event than HIV-negative individuals; to have less classic symptomatology [5][34] and a reduced number of coronary artery lesions but the same degree of multivessel disease [35];a higher degree of non-calcified plaques and vulnerable plaques typically associated with acute coronary syndromes [36][37][38]; and different stroke mechanisms with a predisposition to cerebral artery remodeling and impaired cerebral vasoreactivity [39,40], which may all suggest a different underlying pathogenesis of atherosclerosis. The higher prevalence of subclinical atherosclerosis in PLWHIV, which has been associated with higher risk of CVD [11][41,42], is demonstrated through surrogate markers such as carotid intima media thickness (CIMT), arterial stiffness or elasticity, the degree of atherosclerotic plaques or inflammatory markers. As it remains unclear whether these factors translate directly into an increased risk of clinical CVD outcomes, we will consider this body of evidence separately.

4.1 Association of contemporary PIs with surrogate markers for atherosclerotic CVD

CIMT has been reported to be a more sensitive marker of subclinical atherosclerosis than arterial stiffness [43] and is more strongly associated with traditional CVD risk factors than with markers of inflammation or immunosuppression [43,44]. PLWH exposed to mostly older PIs have been shown to have a higher degree of CIMT compared to those exposed to NNRTI-based cART, a finding not fully explained by the more favorable lipid profile in the NNRTI-treated individuals [45]. More recent studies have linked exposure to DRV/r to increased CIMT: among 119 ART-naïve individuals in the PREVALEAT II cohort, the 39 individuals treated with DRV/r demonstrated a significantly higher risk of developing CIMT over 12 months than the 31 individuals treated with EFV [46]. In contrast, a substudy from the ATADAR RCT of 33 individuals found neither ATV/r or DRV/r to be associated with CIMT progression [43], with ATV exposure (boosted or unboosted) appearing to result in a decrease in CIMT. This finding has also been observed by others [47]. In an RCT of tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) used in combination with either RAL, ATV/r or DRV/r, slower CIMT progression was seen in those treated with ATV/r than in those treated with DRV/r, an effect that was partly mediated by hyperbilirubinaemia [48]. A follow-up study demonstrated that

high- and low-density lipoprotein (HDL, LDL) changes were not significantly associated with CIMT progression in any treatment group [49]. However, despite some reported associations with contemporary PI use, findings are inconsistent, and the studies include a relatively small number of participants and limited follow-up time (Table 1).

PI associations with several other CVD surrogate markers have been evaluated. For example, in the START study, there was no improvement in arterial stiffness/elasticity when comparing individuals receiving immediate or deferred cART [50] including contemporary PIs. The study was however limited in the ability to study individual PIs and by a small sample size. Similar to the general population, measurement of early plaque deposition via the coronary artery calcium score obtained by chest computer tomography angiography (CTA) as a proxy for atherosclerosis has been shown to be of benefit in PLWH [51], and may be a more a stronger predictor of CVD risk than CIMT alone [52]. In men participating in the Multicenter AIDS Cohort Study, cumulative DRV/r exposure was associated with an increased presence of calcified plaque and increased extent of coronary artery calcium and total plaque scores, but not with the extent of calcified plaque in fully-adjusted models, and associations with increased presence of coronary stenosis and the extent of mixed plaque were of borderline statistical significance [53]. In the Swiss HIV Cohort Study, ATV use was associated with any type of plaque, independent of other CVD risk factors [54].

Markers of inflammation and coagulation activation, such as D-dimer, C-reactive protein (CRP), interleukin-6 (IL-6), and macrophage activation markers CD14- and CD163 have been associated with increased CVD risk and the presence of subclinical atherosclerosis in several studies of PLWH [44][55]. One study compared the impact of DRV/r vs ATV/r on soluble markers of inflammation, including IL-6, among 85 and 73 PLWH receiving either DRV/r or ATV/r, respectively, finding no significant association with the use of either PI [56]. In the PREVALEAT II cohort [46], those receiving ATV/r and DRV/r experienced declines in D-dimer and CRP, and although these findings could indicate a beneficial impact of these drugs on the inflammatory cascade, they did not explain the greater observed impact on CIMT of DRV/r compared to EFV [46]. Finally, despite some differences in inflammatory markers (such as CRP, D-dimer, IL-6, CD14 and CD163) and immune activation between 234 individuals receiving either RAL, ATV/r or DRV/r in the ACTG 6250 substudy, there was no consistent evidence that

any treatment-related changes in these markers differed between those receiving RAL- or PI-based regimens [57]. Hence, findings supporting an association between the use of contemporary PIs and inflammatory markers are so far limited.

4.2 Dyslipidemia

Dyslipidemia is an important individual CVD risk factor in both PLWHIV and HIV-negative individuals, and a common adverse effect of PI treatment. The CVD risk associated with older PIs was observed to be at least partly mediated by dyslipidemia [18]. Studies on dyslipidemia are summarized in Table 2. The CASTLE and ARTEMIS studies evaluated changes in lipid profiles between those treated with ATV/r or DRV/r, respectively, and compared these to changes seen in individuals treated with LPV/r. ATV/r use was associated with significantly smaller changes in total cholesterol (TC), LDL and triglycerides than LPV/r [20]. DRV/r was also associated with smaller increases in TC and triglycerides than LPV/r, although changes in HDL and LDL levels were similar between the two regimens [21]. The ATADAR Study evaluated changes in TC and tolerability between cART-naïve individuals randomized to either ATV/r or DRV/r in combination with TDF/FTC and observed no significant differences between the two regimens, although there was a trend towards a better TC/HDL ratio in those receiving ATV/r [58]. Overall, the findings of this study supported those of the CASTLE and ARTEMIS studies with DRV/r having a smaller effect on cholesterol than LPV/r when compared to ATV/r.

Substudies of the ATADAR Study further evaluated differences in body composition between those receiving DRV/r and ATV/r, with ATV/r being associated with higher triglyceride levels and more total- and subcutaneous fat than DRV/r. Conversely, there was no difference between the drugs in terms of cholesterol fractions [59]. Another substudy demonstrated improvements in the LDL sub-fraction phenotype at 48 weeks in those treated with DRV/r, with no improvement seen in those receiving ATV/r; this was associated with a smaller increase in triglycerides in those receiving DRV/r [60]. Hence, whilst differences in lipid profiles between ATV/r vs DRV/r have been reported, these are generally small.

Comparing ATV/r and DRV/r to INSTIs, the NEAT 022 RCT investigated the effect of an immediate vs deferred switch (after 48 weeks) from primarily RTV-boosted ATV or DRV to DTG in virologically-suppressed older individuals (>50 years) with a Framingham risk score ≥10%. TC

and other lipid fractions (except HDL) improved regardless of the PI used at baseline, the switch was well-tolerated and virological efficacy was maintained [61]. A similar beneficial lipid profile in those treated with DTG vs DRV/r was reported in the FLAMINGO trial [62]. Furthermore, in a study of 1797 individuals randomized to ATV/r, DRV/r or RAL, comparable increases in TC and LDL were observed between the two PIs, but these increases were greater than those seen in the RAL group; in contrast, incidence of metabolic syndrome increased to the same degree in all three groups [63]. Others have reported no difference in lipidomic profiles between individuals on INSTI- vs DRV/r-based cART, although studies are limited by their cross-sectional design and small sample size [64]. Whilst greater increases in some lipid fractions have been reported after initiation of these PIs than after initiation of INSTIs, these increases are generally smaller and less clinically relevant than increases associated with first generation PIs. Current NRTIs to be combined with boosted PIs are most commonly TDF or tenofovir alafenamide (TAF) and FTC. The EMERALD Study investigated the effect of an immediate vs deferred switch (after 52 weeks) from primarily RTV or Cobi-boosted DRV and FTC/TDF to DRV/c+/FTC/TAF in virologically suppressed treatment experienced individuals. Only small increases in the TC:HDL ratio and fasting lipid levels were observed in both the immediate and deferred switch arm at week 96, and only a small portion of individuals were started on lipid lowering therapy in those randomized to DRV/c+/FTC/TAF [65].

4.3 Interpretation of findings

The utility of surrogate markers and clinical relevance for CVD risk prediction in PLWH remains uncertain. Many studies are limited by small sample size and limited follow up time, and the association between CIMT, other surrogate markers and CVD risk has been shown to be inconsistent across studies due to different methodologies [52][55]. The addition of surrogate measures of atherosclerosis, such as CIMT, does not improve the prognostic value of CVD risk scores [66]; the measurement of plaque burden by CTA is also not readily implemented in all settings for routine screening and no inflammatory marker has yet proven consistently useful in routine CVD screening. Further, the somewhat inconsistent findings relating to such markers and lipids suggests that if there is an association between use of contemporary PIs and CVD it is less likely to be substantially mediated via any of these established pathways. Finally, the

literature is still limited concerning the impact of switching from PIs to INSTIs and from TDF to TAF in boosted PI regimens on other surrogate markers than lipid levels.

4.4 Contemporary PIs and association with clinical CVD

Relatively few studies have evaluated the association of contemporary PIs with clinical CVD, details are summarized in Table 3. The multinational D:A:D (Data collection on adverse events of Anti-HIV drugs) cohort study, using data from 35,711 participants followed for a median of 7.0 years (including 454 type 1 Mis, 379 strokes and 567 invasive CVD procedures), reported that the CVD event rate increased from 4,9 events /1000 person-years in individuals unexposed to DRV/r to 13,7 events /1000 person-years in those exposed for >6 years [67]. In multivariable analyses, DRV/r was associated with increased risk of CVD (incidence rate ratio 1,6 [95% CI 1·3–1·9] /5 years additional use) [67]. The size of the association was similar to that reported for the older PIs, and, in contrast to the latter, was unchanged after adjustment for lipid changes. ATV and ATV/r were not associated with CVD risk, consistent with earlier analyses [68], and associations were similar for stroke and MI when analyzed separately. All CVD events were prospectively collected, monitored and centrally validated against validated algorithms (https://www.chip.dk/Portals/0/files/Study%20documents/DAD_MOOP_revised2013.pdf)[67]. Other studies re-investigating this observation have not to date observed any such association between DRV use and increased CVD risk; a nested case-control study within the French Hospitals Database on HIV (including prospectively reported 408 ischemic MIs; 109 MIs and 288 controls on ATV and 41 MIs and 107 controls on DRV) assessed by a cardiologist and validated on the basis of the American College of Cardiology (ACC)/European Society of Cardiology definition, showed no increased risk of MI associated with exposure to boosted DRV or ATV [69]; In an Italian post-hoc analysis of the TMC114-HIV4042 observational study on the efficacy and tolerability of DRV/r-based regimens, DRV use was not associated with increased CVD risk, although the study was limited by the relatively small sample size (875 DRV/r treated individuals, 1566 person-years) and number of events (23 CVD events not restricted to ischemic CVD, with event reports being validated using the ACC/American Heart Association atherosclerotic cardiovascular disease events definition and the Framingham general CVD definition)[70]; In a Brazilian cohort of 2960 cART-treated PLWH (109 CVD

events/deaths (including both ischemic and non-ischemic CVD events), validated using hospital records and discharge diagnoses coded according to the International Classification of Diseases, Tenth Revision (ICD-10) code and "Coding of Death in HIV" (CoDe)), each additional year of exposure to LPV/r, ATV/r and DRV/r were associated with a reduction in the incidence of CVD of 19, 40 and 35% after adjustment, respectively [71]; A retrospective US study of 516 and 504 individuals on ATV and DRV, respectively, with a mean follow-up of 6.6 years, found that the time to first ischemic MI or stroke (identified through chart examination of diagnosis codes and clinical notes and further validated by review of cardiac catheterization or brain imaging reports) was longer in those on ATV than in those on DRV. After adjustment, those on ATV also had a significantly lower risk of a new CVD event and a longer survival [72]. Finally, two reports investigated the association of DRV with CVD risk in several RCTs; the first was an evaluation of 19 Janssen-sponsored phase 2–4 studies (5713 individuals followed for a median of 2 years, 115 ischemic CVD events, validated based on Medical Dictionary for Regulatory Activities preferred terms) which found no evidence of CVD risk associated with the use of DRV/r [73]. The observed CVD incidence rate (95% CI) /1000 person-years in the overall pooled population (any DRV dose) was 6.2 (2.9–11.9), with the cumulative CVD event rates for those exposed to DRV at any dose declining over time. The second was a meta-analysis including 22 RCTs, which reported a low CVD event rate overall, although findings were limited by short follow-up and a very small number of CVD events [74].

Differences in study findings may be explained by differences in study design. Compared to the D:A:D Study, most RCTs had a shorter follow up-time (48-96 weeks) and fewer CVD events [73][74], this was also true for some of the observational studies [69-71]. Together, this may result in an increased risk of type II errors or false negative results. Furthermore, the definition of CVD events varies across the studies particularly regarding the exclusion [67][69][72,73] or inclusion [70,71] of non-ischemic CVD events. Finally, whereas the D:A:D Study and others used validation of CVD events based on standardized algorithms and CVD definitions [67][69,70], others have used chart examinations and diagnosis codes/medical terms to identify CVD events [71-73]. Hence, the heterogeneity in CVD events included in the studies and the variety of validation methods may also explain why the signal observed by the D:A:D Study have not, as of yet, been re-produced in other studies.

In contrast to DRV/r, ATV/r has not been associated with an increased CVD risk in several studies [67,68][72][75,76] . ATV can cause hyperbilirubinemia, and is associated with a decreased risk of CVD events in the general population [77]. Similarly, in PLWH, mild hyperbilirubinaemia has been shown to slow the progression of atherosclerosis [48][78], as well as being inversely related to CVD risk [79]. Unconjugated bilirubin acts as an antioxidant suppressing lipid oxidation and has anti-inflammatory, complement inhibitory, and possibly anti-thrombotic and lipid-lowering properties. In the prospective Centers for AIDS Research Network of Integrated Clinical Systems cohort, hyperbilirubinemia was not associated with a lower risk of ischemic MIs, but was associated with a lower risk of a composite CVD endpoint (including 392 ischemic MIs and 160 strokes) after stratification for ATV use [80]. Hyperbilirubinaemia was also independently associated with the decreased CVD risk seen in those receiving ATV in a retrospective study [72]. Bilirubin appeared to only partly mediate the association between ATV use and CVD risk in adjusted analyses, raising the possibility that there may be a, as of yet unidentified, cardio-protective mechanism of ATV unrelated to bilirubin. Alternatively, this may also indicate that associations could be confounded by Gilberts syndrome, a genetic disorder where bilirubin levels are consistently high [81], given the relatively high prevalence of this condition in the general population where affected individuals are known to develop substantially higher bilirubin levels on ATV [82]. The D:A:D Study did not observe an association between ATV use and CVD risk mediated by hyperbilirubinaemia, nor was there evidence to support an interaction between DRV/r or ATV/r with bilirubin levels [67]. It is also possible that bilirubin levels may be cardio-protective via other pathways or that ATV/r does not impact on proatherogenic pathways in the same manner as other PIs. Thus, whilst the evidence appears to consistently point to ATV *not* being associated with increased CVD risk, a potential cardio-protective effect of ATV-induced hyperbilirubinemia remains uncertain.

Whereas some studies have demonstrated lower CVD and all-cause mortality in ATV-treated individuals compared to those treated with DRV [72][76], no studies have, to date, investigated whether the potential increased CVD risk associated with DRV/r directly translates into increased CVD mortality.

4.5 Boosters and risk of CVD

ATV and DRV are metabolized in the liver by the cytochrome P450 (CYP) enzyme CYP3A, which results in insufficient plasma concentrations to suppress viral replication. As RTV and cobicistat are able to inhibit these enzymes, co-formulation of ATV and DRV with a sub-therapeutic dose of RTV or cobicistat results in pharmaco-enhancement. RTV, which is itself associated with lipid increases, may accumulate in cells to a higher degree in individuals treated with DRV than in those treated with ATV [83]. Thus, RTV boosting might have an additional effect on the increased CVD risk observed for DRV. However, a large RCT found no evidence of a difference in RTV plasma concentrations between those receiving ATV/r, DRV/r and RAL over a 48-week period [63] and previous studies have similarly failed to demonstrate a difference in CVD risk between those receiving unboosted vs boosted ATV [68]. However, cobicistat appears to have a reduced lipidemic potential compared to RTV. A significant decrease in triglyceride levels was observed in a retrospective study including 173 PLWH on DRV/r monotherapy who switched to DRV/c monotherapy, whereas total-, HDL-, and LDL-cholesterol levels remained stable [84]. Another retrospective observational study of 299 individuals receiving mono-dual or triple ART regimens including DRV/r, reported significant reductions in total, -LDL and HDL cholesterol and triglycerides for individuals with dyslipidemia at baseline when switched to DRV/c [85]. Newer reports have also observed reductions in LDL and triglyceride levels in PLWH switching from ATV/r to ATV/c [86]. Studies comparing the risk of CVD events in those receiving contemporary PIs boosted with either RTV or cobicistat have not yet been conducted, but these results may suggest that the increased CVD risk associated with DRV/r might be partly reduced by changing the booster.

4.6 Interpretation of findings

Recently, several studies have analyzed associations between use of newer PIs and CVD to investigate if these have similar risks to those observed for the older PIs. These studies have primarily been of an observational design with longer follow-up than is usually seen in RCTs [26]. The D:A:D analysis remains the largest study to assess the association between exposure to DRV/r and CVD risk [67], using a robust statistical analysis based on validated clinical endpoints and reporting a clinically important dose-response relationship. These findings

support an association is cumulative in nature with risk depending on underlying CVD risk; studies of individuals at lower CVD risk may therefore fail to detect such an association, particularly if follow-up is short. The magnitude of the association reported in the D:A:D Study was of clinical relevance, with a low number needed to harm (NNTH) for those at high CVD risk (stratified by the underlying estimated D:A:D CVD 5 year risk score) of 15 people (95% CI 13-17) vs. 533 people [95% CI 314–706] for those at low CVD risk [67]. Several smaller observational studies have not, however, observed such an association, although the possibility of type II error due to limited follow-up time (particularly among individuals on DRV and ATV) and CVD events in those on the drugs cannot be excluded.

Considering the severity of the reported safety signal in the D:A:D Study, and the inconsistent findings from smaller studies, other adequately-powered observational studies are needed to investigate the reproducibility of this finding. However, as the use of boosted DRV is declining these studies may not be feasible, and if the association should be causal, it is still a good sign that it is not very common and highly depending on underlying CVD risk. Observational studies also have important limitations; they are unable to demonstrate causality and future studies will be limited by channeling bias as DRV/r is now rarely recommended for use in those at high underlying CVD risk [31]. Despite this, large prospective observational studies can play an important role in understanding the long-term effects of ARV exposure and in monitoring CVD risk over time. Moving forward, it is unlikely that any adequately powered RCT will evaluate the CVD risk associated with contemporary PIs; by their nature, RCTs are generally of short duration, i.e. rarely designed to investigate effects over longer time periods, have a limited sample size and a focus on laboratory rather than hard clinical CVD endpoints. Furthermore, the typical inclusion of those at lower CVD risk may also limit the ability of RCTs to determine the true risk associated with each drug. As such, observational studies, with their limitations, may provide the only evidence-base for associations with CVD in the future.

5. Possible Biological mechanisms of PIs and risk of CVD

Possible biological mechanisms are summarized in Figure 1; the main biological pathways through which cumulative use of the older PIs (primarily IDV and LPV) were believed to

increase the risk of CVD included changes in lipid levels and other negative metabolic impacts such as increased risk of metabolic syndrome/fat abnormalities and insulin resistance/diabetes [6][13-21][30][87,88]. However, the impact on CVD risk of some PIs does not appear to be fully explained by the impact of the drugs on these factors.

It has been proposed that PIs trigger reactive oxygen species production that promotes cell death, impaired mitochondrial function and dysregulation of the ubiquitin–proteasome system (UPS), directly and indirectly driving the process of metabolic complications related to increased risk of CVD [88]. Furthermore, a cellular condition referred to as endoplasmic reticulum (ER) stress, involving the accumulation of unfolded or misfolded proteins, is involved in the pathogenesis of CVD [89], and ER stress is buffered by the adaptive reaction of the unfolded protein response (UPR). *In vitro* and *in vivo* animal studies on PIs (including APV, ATV, RTV, IDV) have linked the ER stress -and subsequent UPR response induced by these PIs to dysregulation of lipid metabolism with accumulation of intracellular free cholesterol/lipids, foam cell formation and cell apoptosis in metabolically important macrophages [90], where cell apoptosis may lead to a preservation of harmful macrophage phenotypes [91]. LPV/r may also induce ER stress and impair autophagy activity (another intracellular protein degradation system) in adipocytes [92]. Furthermore, via activation of the UPR, ATV, LPV, IDV and RTV have been shown to impact the increased release of inflammatory cytokines by macrophages (including tumor necrosis factor-alpha, IL-6), which are highly presented in atherosclerotic plaques and in addition to accumulation of lipids play an important role in the atherosclerotic process [93,94].

Other mechanisms have also been studied; Accumulation of cholesteryl esters and foam cell formation inside macrophages have been linked to RTV induction of the inflammatory mediator CD36 [95,96]; In one *in vitro* study, LPV/r and ATV/r were observed to activate the adipose renin angiotensin system which may play a role in the development of hypertension and CVD events [97]. Finally, some of the original PIs have been linked to endothelial dysfunction [98], a well-established response to CVD risk factors preceding the development of atherosclerosis involving diminished production and availability of nitric oxide and/or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors. DRV/r, ATV/r and LPV/r have been shown to induce endothelial cell dysfunction, reactive

oxygen species production, inflammation and senescence *in vitro,* with little or no effect induced by DRV/r, a moderate effect by ATV/r but a stronger effect by LPV/r [99]. Others have observed that although treatment with DRV/r, ATV/r or NNRTI-based regimens resulted in reductions in levels of CVD-associated biomarkers, markers of endothelial dysfunction and monocyte activation remained elevated [100].

Studies on possible biological mechanisms for increased CVD risk are primarily based on older PIs and LPV/r, and the complexity of these mechanisms suggests that some PIs, to a different extent, may be involved in several processes contributing or predisposing to atherosclerotic disease, including changes in lipid pathways not necessarily captured through normal measurements of dyslipidemia. These mechanisms may be of relevance to contemporary PIs although it appears that the PI effect is not an overall class effect.

6. Clinical approach

The aging population of PLWH calls for particular caution and an increased focus on comorbidity risk, including CVD and related conditions that often cluster together (e.g. hypertension, dyslipidemia, diabetes, obesity and ischemic heart disease). Whilst most older PIs have now been phased out, evidence from several observational studies suggests a cumulative CVD risk of these drugs which may persist after they are discontinued [6][13-18], and historic use of these drugs should therefore be taken into account when assessing CVD risk [101], in addition to the potential additive effect posed by contemporary PIs.

Given the potential severity of an increased CVD risk in those exposed to DRV/r for longer periods of time, it is currently recommended that exposure to DRV/r is considered in the context of other CVD risk factors that might be present, as the relative impact of a drug-related increase in risk would be greater for those already at elevated CVD risk [28][31]. It remains unknown whether discontinuation of DRV/r will lead to a reduction of this risk [67].

Since the contemporary PIs have different adverse risk profiles, it is important to take individual baseline CVD risk profiles into consideration in clinical practice applying systematic screening and management, as recommended in the current EACS guidelines [31]. This includes initial estimation of CVD risk through available risk scores, the identification of CVD risk factors and modification of these where possible, and the appropriate initiation of primary

or secondary prophylaxis. The D:A:D CVD risk score is currently the only HIV-specific CVD risk score available for PLWH which takes cART exposure into account [101]. cART modification is currently recommended if the estimated 10-year CVD risk of the individual exceeds 10% [31], and lipid thresholds have also been amended in recent guidelines to align with those used in the general population [31,32]. Thus, statins may be a viable option for individuals on contemporary PIs where switches to alternative regimens may not be possible, and although careful evaluation must be made of potential drug-drug interactions with PIs as well as other ARV drugs [102], the use of statins is still suboptimal in PLWH [103].

7. Conclusion

We have summarized the evidence to support an association between the two main contemporary PIs, ATV and DRV, using a comprehensive approach including both the risk of subclinical and manifest ischemic CVD. The evidence of any association between ATV and DRV with subclinical CVD is overall inconsistent. The initial cumulative safety signal between DRV/r and CVD reported in the large D:A:D Study was of a size similar to that seen for first generation PIs, but this association has not been confirmed in other smaller studies. In contrast, the accumulated evidence does not suggest an association between ATV/r use and CVD risk. As these PIs are likely to continue to be in use in coming years, it is important to consider their potential impact on CVD risk, particularly among those already at high risk, identified through available CVD risk scores. As CVD is one of the most prevalent comorbidities causing morbidity and mortality in PLWH , any signals of a potential drug-associated increase in the risk of this require further exploration in appropriately designed and powered studies.

8. Expert opinion

Although ATV and DRV are no longer part of first-line cART regimens in ART-naïve individuals and thus are being phased out in many parts of the world due to other available efficient alternatives with better toxicity profiles and fewer drug-drug interactions, they continue to be recommended as alternative first line, second-and third line regimens [31-33]. Due to the high genetic barrier for resistance, DRV/b also remains a treatment option for individuals with poor adherence and as salvage therapy for those experiencing virological failure [31-33][104,105]. Furthermore, weight gains have been reported in individuals receiving INSTIs and/or TAF [106]. PIs may therefore be once again seen as an attractive option for those at low CVD risk in whom the potential lipid benefits of INSTIs may be partly or fully outweighed by the risk of weight gain.

Controlling viral replication with fewer drugs and identifying simplified suppressive ART strategies consisting of two-drug regimens has become a key issue in the long-term management of PLWH for reasons including cumulative toxicity, drug-drug interactions and polypharmacy. Although not recommended as first line options, in selected groups with sustained virological suppression, and in treatment-experienced individuals in whom NRTI and or NNRTI use is not possible, dual therapy with contemporary PIs may be a viable option and is increasingly in use [31-33]; DRV/r+lamivudine (3TC) has demonstrated non-inferior therapeutic efficacy and similar tolerability at 48 weeks compared with triple therapy with DRV/r, FTC or ABC and TDF [107], where a significant increase in the TC:HDL cholesterol ratio was not observed. Furthermore, a meta-analysis of seven RCTs in 1624 patients [108], showed that rates of virological suppression at 48 weeks in both treatment-naïve and -experienced individuals on either PI/r+3TC or PI/r+TDF were non-inferior to those seen with triple therapy with significantly fewer discontinuations for adverse events. Dual combinations of DTG with unboosted ATV is another intriguing NRTI-sparing option that would also avoid pharmacological boosting and where a significant decrease in TC, TG and fasting glucose were also observed in study participants of older age and at high CVD risk 109. Recent findings suggest that two-drug regimens including contemporary PIs (most commonly RAL+ATV/b) may offer a potential option with no increased impact on risk of CVD events110.

For these reasons, and as a considerate portion of PLWH remain on PI therapy [111], contemporary PIs are likely to remain in use globally for several years to come and remain pertinent to study; Studies on two-drug regimens with boosted PIs combined with INSTIs or 3TC are still limited to date and further research is needed on the longer term impact on atherosclerotic surrogate markers and risk of clinical CVD events; the reversibility potential of the adverse effects of boosted PIs including the CVD association once the boosted PIs are discontinued remain unanswered, and the literature is still limited concerning the impact of switching from PIs to INSTIs and from TDF to TAF in boosted PI regimens on other surrogate markers than lipid levels as well as risk of clinical CVD events.

In light of the current evidence on CVD risk discussed in this review, baseline CVD risk should be considered when considering PI use, and as the population of PLWH ages, it is essential that we remain vigilant regarding CVD risk not only in relation to existing drugs but also for those that are newly developed in the future.

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Table 1. Recent studies on ATV and DRV and association with CIMT

	Type of study	Outcome	Number of participants	Follow up time	Association with increased CIMT		No association with increased CIMT	
Study					**Atazanavir**	**Darunavir**	**Atazanavir**	**Darunavir**
Maggi et al, Atherosclerosis 2017 [46]	Longitudinal cohort study	Progression to CIMT after ART initiation (ATV/r, DRV/r or EFV based regimens)	119 ATV/r: 49 DRV/r: 39 (EFV: 31)	12 months		OR 6.5 (95% CI 1.2,36.6) relative to EFV based regimen	OR 2.1 (95% CI 0.4,11.8) relative to EFV based regimen	
Gonsalez-Gordon, HIV Med 2018 [43]	Substudy within the ATADAR Study (RCT)	Progression to CIMT after ART initiation comparing ATV/r vs.DRV/r in combination with TDF/FTC	33 ATV/r: 16 DRV/r: 17	12 months				DRV/r treated had faster progression than those on ATV/r; median (IQR) changes of 117 (-2, 143) vs. -6 (-58,89) µm, resp. (P = 0.0917).
Saint-Martin et al, AIDS 2015 [47]	Comparative prospective cohort	CIMT at baseline, 6, 12, and 18 months in ATV-treated vs. non-ATV-treated individuals	229; 33 ATV treated and 99 without ATV exposure	18 months			CIMT significantly decreased at 12 (0.636 mm vs. 0.676; P=0.05) and 18 months (0.611mm vs. 0.675, P=0.018)	
Stein et al AIDS 2015 [48]	RCT	Progression to CIMT after ART initiation (ATV, DRV or RAL)	334 -	144 weeks	IR 8.2, 95% confidence interval (5.6, 10.8) mm/year]	IR 12.9 (10.3, 15.5) mm/year		

RCT: Randomized controlled trial; CIMT: carotid intima media thickness; ART: antiretroviral therapy; ATV: atazanavir:ATV/r: atazanavir/ritonavir; DRV: darunavir; DRV/r: darunavir/ritonavir; RAL: raltegravir; EFV: efavirenz; TDF: tenofovir disoproxil fumarate; FTC: emtricitabine; IR: incidence ratio; OR: odds ratio; IQR: interquartile range; mm: millimeter; µm: micrometer CI: confidence interval

Table 2. Studies on ATV and DRV and lipid changes

	Type of study	Outcome	Number of participants	Follow up time	Lipids (TC, HDL, LDL, TG)
Study					**ATV and DRV**
Martinez et al, HIV Med 2014 [58]	RCT, ATADAR Study	Change in lipid levels ATV/r vs. DRV/r	178 ATV/r: 90 DRV/r: 88	96 weeks	Greater decrease of TC:HDL ratio with ATV/r vs. DRV:/r estimated difference (95% CI) −1.02;(−2.35 to +0.13; P = 0.07). No significant difference in TC and similar changes TG
Martinez et al, CID 2015 [59]	RCT, ATADAR Substudy	Body fat composition and lipid changes ATV/r vs. DRV/r	178 ATV/r: 78 DRV/r: 80	96 weeks	TG: Mean change (standard deviation): ATV/r +38.89 (71.74 mg/dL) vs. DRV/r +15.65 (69.53) p=.0567 No significant changes in TC, LDL and HDL Body fat, limb fat and subcutaneous abdominal adipose tissue increased more in the ATV/r arm than in the DRV/r arm. The changes in the ATV/r arm were associated with higher insulin resistance
Saumoy et al, J Antimicrob Therapy 2015 [60]	RCT, ATADAR Substudy	Standard lipid parameters and LDL subfraction phenotypes ATV/r vs. DRV/r	86 ATV/r: 45 DRV/r: 41	48 weeks	TC and HDL increased significantly in both arms LDL increased significantly only in those on DRV, TG increased significantly only in those on ATV The apolipoprotein A-I/apolipoprotein B ratio increased only in the ATV/r arm, the LDL subfraction phenotype improved in the DRV/r arm and worsened in the TAV/r arm (related to the greater increase in TG in the ATV /r arm).
Gatell et al, CID 2019 [61]	RCT, NEAT022 Study	Efficacy and safety of immediate vs. deferred switch from PI/r incl. ATV/r, DRV/r, LPV/r or other PI/r + NRTI backbone to DTG- based regime	415 Immediate switch to DTG+2 NRTIs: 205 Deferred switch: 210	96 weeks	TC and other lipid fractions (except HDL) significantly improved in both groups overall regardless of baseline PI/r strata. No change in proportions on statins. Trend toward a reduction in the CVD risk score at 5 years after 48 weeks in both groups based on Framingham and D:A:D Study equations
Molina et al, Lancet HIV 2015 [62]	RCT, Flamingo trial	Efficacy and safety of DTG vs. DRV/r+ TDF/FTC or ABC/3TC	488 DTG: 242 DRV/r: 242	96 weeks	Mean increase in fasting LDL was significantly higher in the DRV/r group than in the DTG (adjusted mean difference –0·33 mmol/L, 95% CI (–0·45 to –0·21) Greater increases in TC and TG from baseline in DRV/r group Mean TC:HDL ratio increased in both groups More post-baseline laboratory abnormalities occurred in the DRV/r group for TC (46 vs. 18%) and LDL (36 vs.16%), and LDL abnormalities were significantly higher in the DRV/r group (22 vs. 7%, p<0·0001)

Ofotkun et al, CID 2014 [63]	RCT, ACTG 5257	Metabolic effects for ATV/r, DRV/r and RAL	1797 ATV/r: 602 DRV/r: 595 RAL: 600	96 weeks	Comparable increases TC, TG, LDL for ATV/DRV; each PI had greater increases relative to RAL (all P ≤ .001 at week 96). Modest increases in HDL in all arms
Mena et al, Nature Scientific reports, 2019 [64]	Cross sectional study	Difference in lipidomic profile between DRV and INSTI based regimen	62 DRV/r: 25 INSTI: 37	-	Higher concentrations of TC, LDL and TG, and lower HDL in DRV/r -treated vs. INSTI-treated group, but the difference was not statistically significant The lipidomic profile including other lipid parameters (ceramides, phosphatidylinositols, diacylglycerols, phosphatidylethanolamines, triacylglycerols and lysophosphatidylethanolamines) did not differ between DRV/r and INSTI-based regimens

RCT: Randomized controlled trial; PI: protease inhibitor; PI/r: protease inhibitor/ritonavir; ATV: atazanavir: ATV/r: atazanavir/ritonavir; DRV: darunavir; DRV/r: darunavir/ritonavir;LPV: lopinavir; LPV/r: lopinavir/ritonavir; RAL: raltegravir; DTG: dolutegravir; INSTI: integrase strand inhibitor; NRTI: nucleoside reverse trancriptase inhibitor; TDF: tenofovir disoproxil fumarate; FTC: emtricitabine; ABC: abacavir; 3TC: lamivudine TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides; D:A:D: Data collection on Adverse events of anti-HIV drugs; CI: confidence interval

Table 3. Studies on association between atazanavir and darunavir and CVD risk

	Type of study	Number of participants and CVD events	Outcome	Study period, Person years of follow up or median follow-up time Exposure time to ARV/DRV	Central validation of clinical event	Association to increased CVD risk		No association with increased CVD risk	
Study						**ATV**	**DRV**	**ATV**	**DRV**
Ryom et.al, D:A:D Study, HIV Lancet 2018 [66]	Prospective observational cohort	35.711 1157 CVD events MIs: 454 Stroke: 379 ICPs: 567 Sudden cardiac death: 0	Ischemic CVD event risk with cumulative use of DRV/r and ATV/r	2009-2016 Median follow-up of 6·96 years At the time of CVD event, median exposure to ATV/r: 3·07 years (IQR 1·21–5·33); Median exposure to DRV/r: 2·56 years (1·21–4·01)	Yes		Adjusted IR 1.59; 95% CI (1.33–1.91) per 5 years additional use	Adjusted IR 1.03; 95% CI (0.90–1·18) Per 5 years additional use	
Costagliola et al, ANRS-CO4 (FHDH), CID 2020 [68]	Nested case-control study within prospective observational cohort	1658 408 MIs/1250 controls ATV exposed:109/288 DRV-exposed:41/107	Risk of ischemic MI with cumulative use of DRV and ATV	2006-2012 At the time of MI event, median exposure to ATV: 1.9 years (10th–90th percentile: 0.1–4.7) Median exposure to DRV: 1.0 year (10th–90th percentile: 0.2–2.5)	Yes			Adjusted OR 1.54; 95% CI, (0.87–2.73) per 5 years of exposure	Adjusted OR 0.51; 95% CI, (0.11–2.32) Per 5 years of DRV exposure
Antinori et al Drug Design, Development and Therapy 2019 [69]	Post-hoc analysis of the TMC114-HIV4042 observational study	875 23 CVD events (MI, cardiac death, stroke, heart failure, coronary insufficiency,	Incidence of CVD adverse events and predictors for CVD risk	12-42 months 1,566 PYRS DRV exposed prior to study: 642 DRV exposed from start of study: 233	Yes				Adjusted HR (95%CI) 1.02 95% CI (0.90, 1.15)

		angina, transient ischemic attack, and peripheral artery disease)							
Diaz et al BMC 2017 [70]	Prospective cohort study	2960 109 CVD events (Heart/vascular disease, ischemic heart disease, stroke, venous thrombosis and pulmonal embolism, CVD-related death)	Assessment of predictors for CVD morbidity and mortality including cART	2000-2010 16,140 PYRS Median follow up: 4.68 years [IQR), 2.34–9.09]), 62 % had PI exposure (9973 PYRS) PI/r-exposed unspecified: 78 ATV/r-exposed: 13 DRV/r-exposed: 6	No			Adjusted IR 0.60, 95% CI (0.45–0.81) per additional year of exposure	Adjusted IR 0.65, CI 95% (0.45–0.95) per additional year of exposure
Li et al, J Am Heart Ass 2020 [71]	Retrospective cohort study	1020 57 CVD events Ischemic MI: Stroke: New CVD:78 (coronary artery disease, peripheral vascular disease, carotid artery stenosis, and transient ischemic attack)	ATV exposure compared to DRV exposure and 1) Primary outcome: Ischemic MI or stroke 2) Secondary outcome: 1+ new CVD and all-cause mortality	2003-2019 Median follow up: 6.6±3.4 years ATV-exposed: 516 DRV-exposed: 504	No			1) Crude IR 4.65 vs.13.55; IR ratio, 0.34; 95% CI (0.20–0.60; P<0.001). Adjusted HR, 0.38; 95% CI (0.21–0.71) Significantly longer time to first MI or stroke vs. DRV (log-rank P<0.001) 2) Adjusted HR, 0.53; 95% CI (0.33–0.86) Significantly longer time to all-cause death (log-rank P<0.001)	

Opsomer et al Drugs in R&D (2018) [72]	Review of 19 clinical trials (Janssen), postmarketing pharmacovigilance databases (Janssen global safety database) and epidemiological data (3 US administrative claims databases)	5713 115 CVD events (Cerebrovascular disease, broad ischemic heart disease and MIs, ICPs)	Evaluation of CVD risk associated with DRV/r exposure	2006-2016 18,643 PYRS Median treatment duration 1.9 years;(IQR 0.94–2.75); range 0–6.1 years	No				Overall CVD event rate, per 1000 PYRS: 6.15, 95% CI,(2.91–11.89). Cumulative CVD event rates for DRV/r users (any dose) generally declined over time. CVD IRs did not increase with exposure to DRV/r with increasing yearly intervals, and there were no CVD events in exposure intervals of > 3 years
Antinori et al, Scientific reports Nature 2018 [73]	Metaanalysis of 22 RCTs	6924 10 CVD events Non-specified CVD events: 7 MIs: 2 Stroke:1	Evaluation of efficacy, safety (adverse and serious adverse events) and tolerability of DRV/r-based regimens	48-96 weeks	No				The proportion of CVD events in the DRV/r-treated individuals was 0.18% (9/4992). For DRV/r, the IR was 1.44 per 1000 person-years

D:A:D: Data collection on adverse events of Anti-HIV drugs; FHDH ANRS CO4: French hospital database on HIV; RCT: randomized controlled trial; CVD: cardiovascular disease; MI: myocardial infarction; ICP: invasive cardiovascular procedure; ATV: atazanavir: ATV/r: atazanavir/ritonavir; DRV: darunavir; DRV/r: darunavir/ritonavir; CI: confidence interval; IR: incidence rate, IRR: incidence rate ratio; OR: odds ratio; HR: hazard ratio; IQR: interquartile range; PYRS: person years

Figure: Possible biological mechanisms for the association of PIs with risk of CVD

Figure legends

CVD: cardiovascular disease; UPS: Ubiquitin–proteasome system; UPR: Unfolded protein response**;** NO: Nitric oxide; TC: total cholesterol; LDL: low density lipoprotein; TG: triglycerides;

Clockwise from right;
1.Dyslipidemia: Elevated levels of TC, LDL, TG; **2. Altered glucose metabolism/insulin resistance/diabetes**;

3.Activation of adipose renin angiotensin system: LPV/r and ATV/r may activate the adipose renin angiotensin system in adipocytes and hereby induce adipocyte dysfunction including lipid loss, insulin resistance, oxidative stress and inflammation leading to hypertension and increased risk of CVD:

4. Endothelium reticulum stress: APV, ATV, RTV, IDV can induce ER stress and cause accumulation of intracellular free cholesterol and lipids and activation of the UPR in metabolically important macrophages. LPV/r induced ER stress and impairment of autophagy activity are involved in dysregulation of lipid metabolism in adipocytes; **5.Endothelial dysfunction:** Preceding the development of atherosclerosis involving diminished production and availability of NO and/or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors. DRV/r, ATV/r and LPV/r have been shown to induce endothelial cell dysfunction; **6.Elevated inflammatory markers**: PIs can impact increased release of inflammatory cytokines (including TNF-alpha and IL-6) by regulating the intracellular translocation of RNA binding protein proteins in macrophages, resulting in foam cell formation and mediation of several processes in atherosclerotic disease; **7. Triggering of reactive oxygen species:** PIs (e.g LPV/r) can trigger production that promotes cell death, impaired mitochondrial function and dysregulation of the UPS, where attenuation of the UPS may lead to transcriptional changes that contribute to a perturbed lipid metabolism, fueling a pro-atherogenic milieu as well as altering ion channels and interfering with electric signals in the myocardium; **8. Induction of CD36:** Upregulation of accumulation of cholesteryl esters inside macrophages by RTV induction of CD36 has also been shown to result in foam cell formation and consequently atherosclerotic plaques, not captured by normal measurements of dyslipidemia; **9.Body fat abnormalities:** Lipodystrophia typically associated with 1st generation PIs including peripheral lipoatrophy, fat accumulation within the abdomen and over the cervical vertebrae, hyperlipidemia, and insulin resistance