# Antiretroviral molecules and cardiovascular diseases

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**SUMMARY**

Antiretroviral therapy has effectively tackled HIV replication and prevented the development of AIDS-related complications in the majority of HIV-positive patients. This pharmacological approach has dramatically increased the life expectancy of HIV-positive subjects transforming HIV infection into a chronic disease. Notwithstanding this major improvement in HIV disease management, several HIV-positive patients show an earlier and significant onset of aging-related chronic conditions such as cardiovascular disease, osteoporosis, diabetes and neoplasias with respect to uninfected individuals. In particular, cardiovascular diseases are associated with both HIV infection and antiretroviral treatment, and represent major clinical complications in HIV-positive patients. Here, we discuss the interaction between antiretroviral therapy and cardiovascular system in HIV-positive patients focusing on the antiretroviral-related mechanisms involved in cardiovascular alterations.

*KEY WORDS:* HIV, Cardiovascular diseases, cART.

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## INTRODUCTION

Combined antiretroviral therapy (cART) mainly consists of the association of at least three antiretroviral drugs (DHSS 2009, Antinori *et al.* 2012). These drugs target critical steps in the viral replication cycle and are divided into fives classes of antiretrovirals: the nucleoside/nucleotide reverse transcriptase inhibitors (N(n)RTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors (IIs), and entry inhibitors (EIs) specifically fusion and CCR5 inhibitors (Figure 1). N(n)RTIs generally constitute the backbone of antiretroviral treatment and current treatment guidelines recommend an initial regimen consisting of two N(n)RTIs with either one PI, one NNRTI or one integrase inhibitor (raltegravir). EIs such as enfuvirtide and maraviroc were approved more recently and their use, previously limited to cART-experienced patients demonstrating therapeutic failure, now extends to specific antiretroviral combinations (DHSS 2009, Ghosh *et al.* 2011, Antinori *et al.* 2012). The advent of cART has changed the evolution of HIV disease: the incidence of deaths in the HIV-infected population has dropped sharply compared with the pre-cART era (Blanco *et al.* 2010) and life-expectancy has increased to more than 35 years from the time of diagnosis (Lohse *et al.* 2007). Although these results led to a major improvement in HIV treatment and management, cART has been associated with the impairment of organs and tissues such as heart, kidney, bone and fat tissue (Borderi *et al.* 2009, Gandhi *et al.* 2012, Maggi *et al.* 2012, Triant 2012). In particular, cardiovascular diseases are important complications both in cART-treated and naïve HIV-positive patients. The incidence of atherosclerosis, myocardial infarction

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(MI), pulmonary hypertension, peripheral and cerebrovascular lesions, endocarditis and pericardial diseases (Holmberg *et al.* 2002, Currier *et al.* 2003, Dubé *et al.* 2003, Friis-Moller *et al.* 2003, Mary-Krause *et al.* 2003, Grinspoon & Carr 2005, Ho & Hsue 2009, Hakeem *et al.* 2010) is increased in HIV-positive subjects with respect to uninfected individuals and cardiovascular diseases represent a major cause of death in HIV-positive individuals (Mocroft *et al.* 2010, Smith *et al.* 2010). Some epidemiological and clinical studies undertaken in both the pre-cART and post-cART eras, have demonstrated that HIV infection is significantly associated with atherosclerosis and cardiovascular events and may be considered an independent risk factor for MI (Currier *et al.* 2003, Triant *et al.* 2007). Although cART effectively tackles HIV disease development, selected antiretrovirals have been associated with an enhancing role in atherosclerosis induction and cardiovascular damage. Hence, HIV infection per se and cART play a major role in the increase in cardiovascular events especially in patients with long-standing exposure to classical risk factors. This report analyses the mechanisms involved in cART-dependent induction of atherosclerosis and cardiovascular injuries in HIV-positive patients.

*Clinical studies on the correlation between antiretroviral and cardiovascular diseases*

The relationship between cART and cardiovascular diseases was firstly analysed in 2003 in a large retrospective study on 36766 HIV-positive patients (Bozzette *et al.* 2003). No significant increase in cardiovascular disease was found in HIV-positive subjects treated with cART for 40 months, suggesting that a short treatment did not induce cardiovascular disease but may tackle the HIV-related cardiovascular impairment.

Subsequent reports have analysed HIV-positive

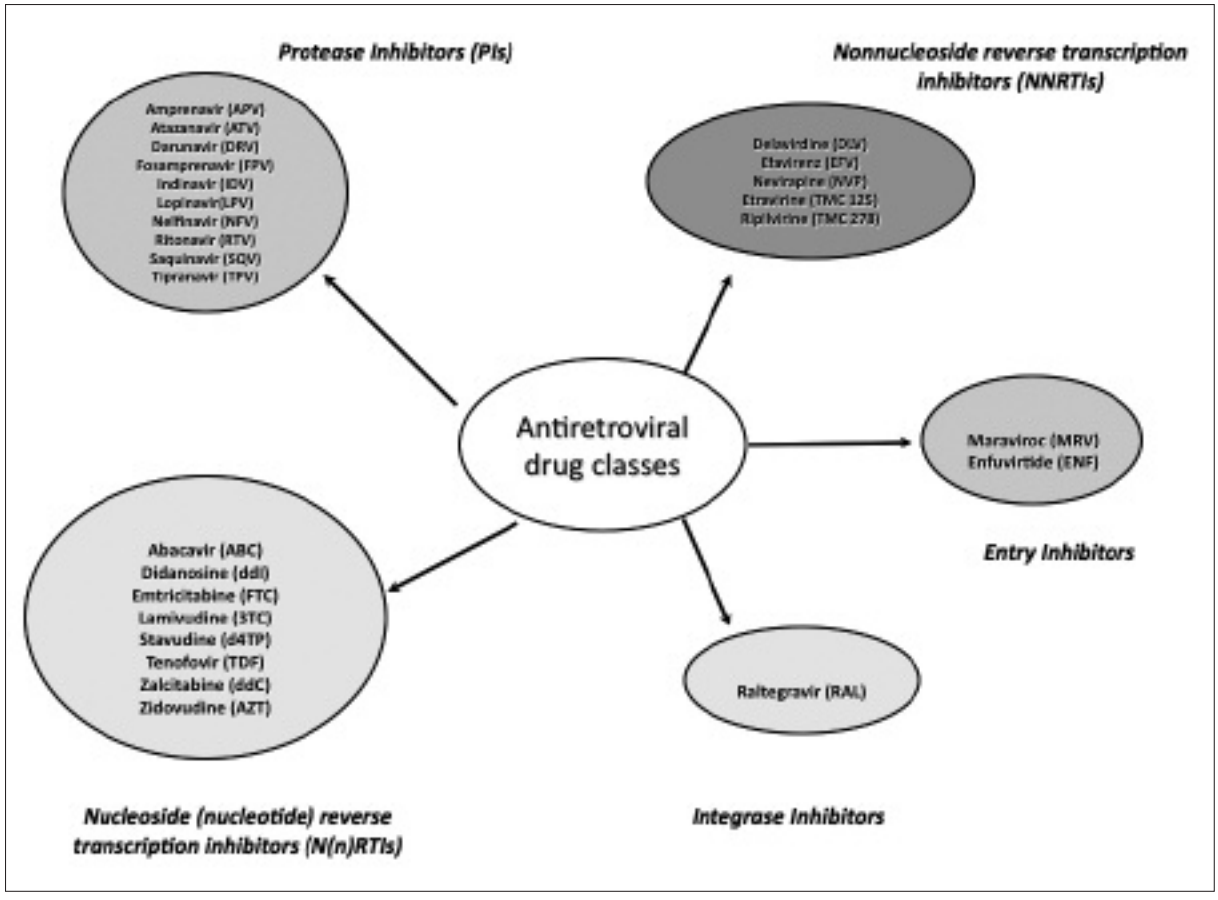


FIGURE 1 - *Antiretroviral classes.*

individuals treated by cART for a longer period describing an increase in cardiovascular disease in cART-treated patients particularly when PIs were used (Mary-Krause *et al.* 2003, Farrugia *et al.* 2009). The DAD study (Sabin *et al.* 2008), performed on 23.437 participants, demonstrated that MI increased in direct correlation with the longer period of PI-containing antiretroviral treatment up to six-to-seven years of exposure (relative risk 1.16, 95% CI 1.09 to 1.23, per year of exposure p<0.0001). Two further studies confirmed that PI treatment induced a significant risk of MI (Holmberg *et al.* 2002, Barbaro *et al.* 2003). Interestingly, the analyses on the impact of specific antiretroviral drug class demonstrated that PIs, but not NNRTIs, were related to an increased annual relative risk of MI. An analysis of French hospital databases on 34976 HIV-positive individuals showed that PI-based cART treatment longer than 18 months determined a significant risk of MI compared with HIV patients treated with PIs for less than 18 months (Mary-Krause *et al.* 2003). The DAD study evaluated the correlation between N(n)RTIs and cardiac disease risk. Recent use of abacavir (ABC) and didanosine (but not another three NRTIs tested: zidovudine, stavudine and lamivudine), was associated with MI risk, but the risk reverted to baseline six months after the cessation of ABC and didanosine-containing therapy (Sabin *et al.* 2008, Farrugia *et al.* 2009). In the SMART study, 2752 HIV-positive patients were treated with ABC, didanosine or an alternative N(n)RTI to determine the risk for MI or other major cardiovascular diseases such as stroke, coronary arterial disease and death from cardiovascular accidents (Lundgren *et al.* 2008). Interestingly, only ABC treatment showed a significant increase in cardiovascular disease compared to other NRTIs (Lang *et al.* 2010) and in the presence of intermittent therapy dynamics (Lundgren *et al.* 2008, Lang *et al.* 2010). The association between ABC therapy and cardiovascular risk was confirmed by other four studies such as FHDB (74958 patients), STEAL (357 patients), QPHID (7053 patients) and a recent report by Choi and coworkers (10931 patients) comparing different antiretroviral regimens with or without ABC (Lang *et al.* 2010, Martin *et al.* 2010, Choi *et al.* 2011, Durand *et al.* 2011). In contrast, a recent meta-analysis of randomised clinical trials did not support the hypothesis that ABC-containing cART regimens carry a greater risk of MI or major cardiovascular events relative to comparative cART (Cruciani *et al.* 2011). Furthermore, the ACTG A5001/ALLRT study analysed data from 5056 individuals initiating randomised antiretroviral treatment in AIDS Clinical Trials Group studies with 1704 started on ABC therapy (Ribaudo *et al.* 2011) showing no risk of MI associated with initial antiretroviral treatment containing ABC. This lack of association was also described in a retrospective observational study VACCR (19424 patients) where cumulative use of ABC was compared to tenofovir (TDF) (Bedimo *et al.* 2011). These controversial results preclude a determination of the true impact of ABC on cardiovascular risk suggesting a cautious use of this compound in HIV-positive patients with classical cardiovascular risk factors. Analysis of NNRTIs, integrase or entry inhibitor drugs did not show a significant increase in cardiovascular risk.

*Role of protease inhibitors (PIs) in cardiovascular impairment*

PIs tackle HIV-1 replication cycle inhibiting the cleavage of viral polypeptides into mature and functional proteins. Current members of PI antiretroviral class include ritonavir (RTV), atazanavir (ATV), amprenavir (APV), indinavir (IDV), nelfinavir (NFV), darunavir (DRV), saquinavir (SQV), fosamprenavir (FPV), tipranavir (TPV), and lopinavir (LPV) (De Clerq 2009). It is noteworthy that RTV has a strong inhibitory activity on the cytochrome P450 isoenzyme 3A4 (CYP3A4) and CYP2D6 and is often therapeutically associated, at low doses, with another PI in cART regimens determining a clinically significant increase in serum levels of other PIs (Worm *et al.* 2010). This pharmacokinetic enhancement of some PIs with low-dose RTV provides a reduction in pill burden and dosing frequency. As reported above, clinical and epidemiological studies show that long-term treatment with PIs has been associated with an increased risk of premature atherosclerosis and MI even though the specific involvement of each PI in MI risk has not been elucidated. Recently, the DAD study provided some data on the relationship between MI risk and treatment with some PIs such as NFV, SQV, IDV or LPV/RTV. The relative risk for MI is increased with IDV (RR=1.12, 95%

CI=1.07–1.18) and LPV/RTV (RR=1.13, 95% CI=1.05–1.21) whereas no significant differences were observed with SQV or NFV (Worm *et al.* 2010). PIs could exert these negative effects on the cardiovascular system, by acting on the enhancement and induction of some classical risk factors and altering vascular functional and structural homeostasis (Figure 2). PIs are associated with a significantly higher incidence of alterations in lipid metabolism and glucose regulation that are classically involved in the development of atherosclerosis (Crowe *et al.* 2010, Brown & Glesby 2011). The Swiss cohort study detected a dyslipidemia in PI-treated patients: hypercholesterolemia and hypertriglyceridemia were about two-fold more common among patients treated with a cocktail associated with PIs with respect to those without PIs (Periard *et al.* 1999). Hypercholesterolemia (>240 mg/dL) and hypertriglyceridemia (>500 mg/dL) were detected in 60% and 75% of PI-treated HIV individuals respectively. In particular, PIs are associated with a cholesterol increase in VLDL and IDL lipoprotein whereas the increase in LDL-C and HDL-C is variable. Hypertriglyceridemia is a very common lipid abnormality in PI-treated patients and it is detectable in all lipoprotein fractions coupled with an increase in apo-lipoprotein B-100. In particular, full doses of RTV determines hypertriglyceridemia causing triglyceridaemia levels exceeding 1 g/dl (Ho & Hsue 2009) in some cases. It is noteworthy that RTV is currently used at lower concentrations to boost the other PIs administrated and then its action on triglyceride levels is scaled down (Dubé & Cadden 2011). An analysis of the correlation between specific PI and hypertriglyceridemia showed (Dubé & Cadden 2011) the greater negative effect of some associations such as TPV/RTV, LPV/RTV and FPV/RTV (Eron Jr *et al.* 2006, Hicks *et al.* 2006, Mills *et al.* 2009). Intermediate effects are detectable with SQV/RTV (Kurowski *et al.* 2002, Fontas *et al.* 2004) IDV/RTV (Dragsted *et al.* 2003) and NFV (administrated without RTV) (Murphy *et al.* 2003, Dubé *et al.* 2005) whereas minor effects were seen with newer PIs such as ATV (ad-

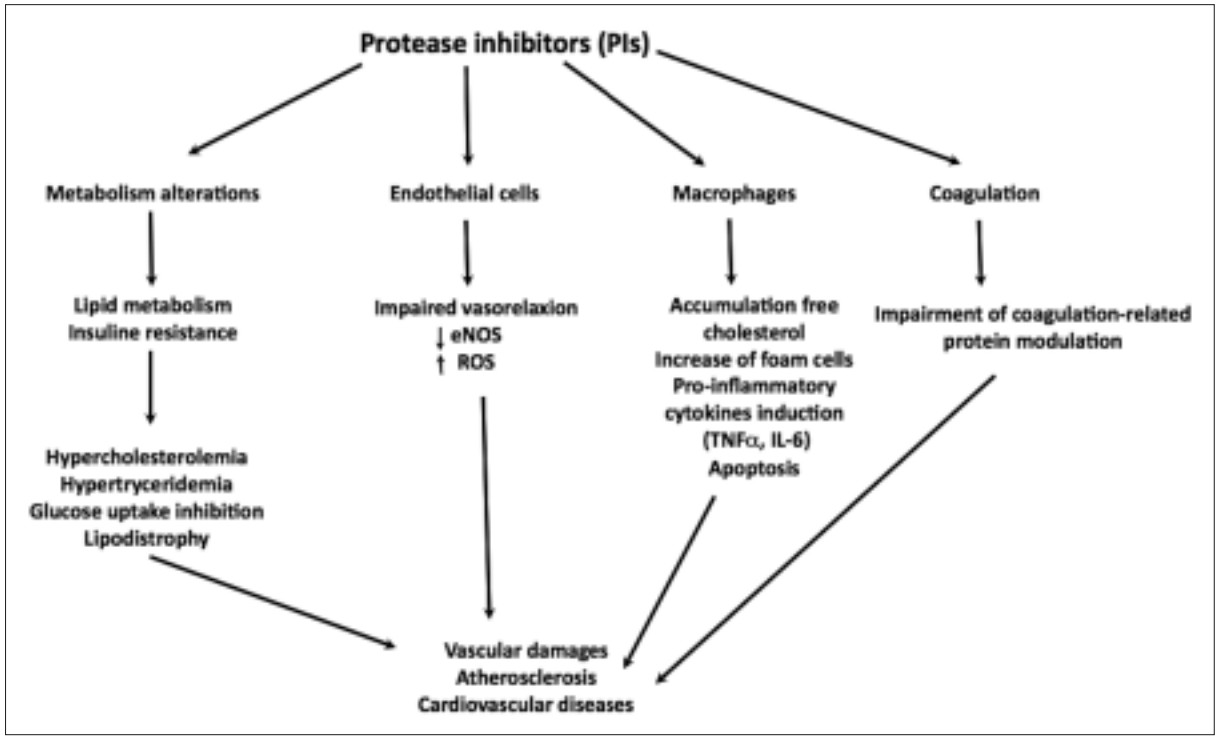


FIGURE 2 - *PIs effects on the cardiovascular system. PIs showed a complex interaction namely with the metabolic and cellular targets involved in atherosclerosis development. As reported in the text, these PI-related mechanisms are demonstrated only for some PIs with varying pathological effects.*

ministered with RTV at a low dose) (Murphy *et al.* 2003, Noor *et al.* 2004, Dubé *et al.* 2008) and DRV/RTV (Mills *et al.* 2009) (Figure 3). PI-associated dyslipidemia does not represent a real antiretroviral class effect because the lipid abnormalities are related to the use of specific PI drugs and genetic susceptibility (Tarr *et al.* 2010). In vitro experiments have disclosed some of the mechanisms involved in PI-related dyslipidemia induction. In cultured human and rat hepatoma cells and primary hepatocytes achieved from transgenic mice, RTV and SQV inhibit proteasomal degradation of nascent apolipoprotein B, the major protein component of triglyceride and cholesterol-rich plasma lipoproteins (Liang *et al.* 2001). This suggests that PI-inhibition of pre-secretory ApoB degradation at the proteasome might increase the assembly and secretion of very low density (VLDL) and low-density lipoproteins (LDL). In addition, LDL clearance is reduced because of down-regulation of LDL-R and LDL-R-related protein (LRP) mRNA and protein level in the presence of NFV whereas APV, IDV, RTV and SQV did not affect these proteins. NFV exerts its effect by reducing levels of active SREBP1 in the nucleus. LDL-R and LRP are two factors involved in lipoprotein catabolism and vessel wall integrity by down-regulating levels of active nuclear SREBP-1 (Tran *et al.* 2003). SREBP-1 is highly expressed in adipocytes and in liver cells increasing lipogenesis and lipid accumulation through the modulation of PPARγ activation (Carr *et al.* 1999, Sudano *et al.* 2006). IDV and NFV but not APV impaired adipose cell differentiation altering to different extent protein expression of SREBP-1, CAAAT/enhancer binding protein alpha (C/EBPα) and fatty acid synthase (FAS) and the distribution of laminin A/C and B (Caron *et al.* 2003). Interestingly, a study performed on HIV-infected patients treated by cART with PIs showed that PI treatment is associated with a significant increase in VLDL- and IDL-apoB concentrations when compared to non-PI-treated patients (Petit *et al.* 2003). Studies performed in vitro using C3H10T1/2 stem cells, a cell model currently used for the analysis on adipocyte differentiation and lipid metabolism, proved that in these cells NFV, SQV, and RTV reduce triglyceride accumulation, lipogenesis, and expression of the adipose markers, aP2 and LPL with a decrease of oil red O-staining of cytoplasmic fat droplets whereas APV and IDV showed low effects. Moreover, NFV, SQV and RTV increased acute lipolysis in adipocytes suggesting that some PIs may block adipogenesis and stimulate fat catabolism (Lenhard *et al.* 2000a). It is noteworthy that an additional report indicated that NFV, SQV and RTV stimulated triglyceride synthesis whereas APV and IDV had no effect in the HepG2 cell model. This confirms the different impact of specific PIs on lipid regulation also depending on the cell model employed (Lenhard *et al.* 2000b). Lipodystrophy is charac-

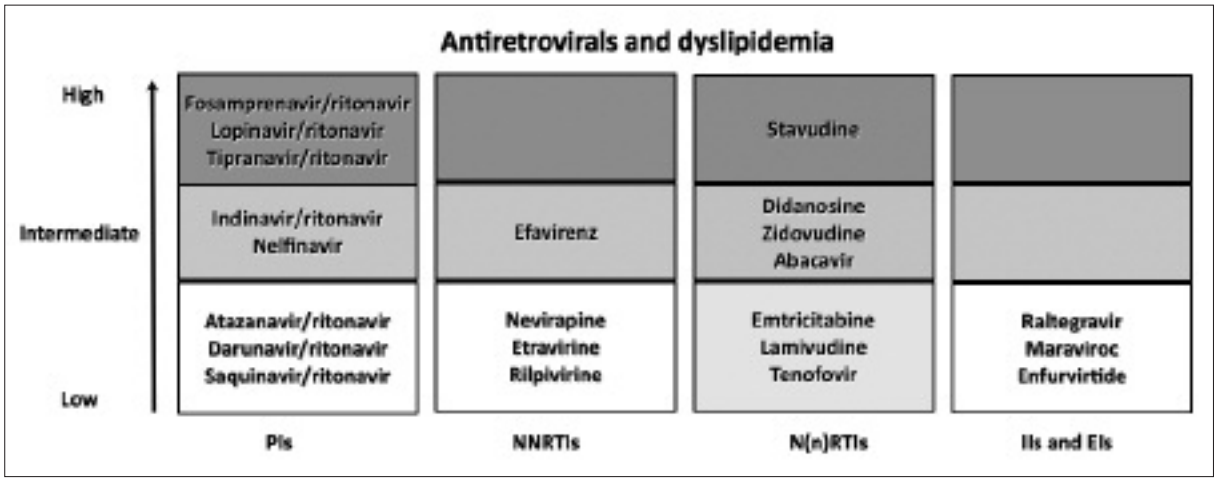


FIGURE 3 - *Antiretroviral drugs and dysipidemia. The relationship between antiretrovirals and the degree of dyslipidemia has already been described (Blanco et al. 2010, Dubé & Cadden 2011). The antiretrovirals were divided into different classes and the relative strength in the association to dyslipidemia is indicated in each drug class.*

terized by an impairment of fat body distribution with uniform subcutaneous and peripheral fat loss and a relative preservation or increase in visceral fat and is an independent cardiovascular risk factor (Masia *et al.* 2010). HIV-positive patients may show relative central adiposity and fat accumulation in the dorso-cervical region and neck (Carr & Cooper 2000, Mooser & Carr 2001, Grinspoon & Carr 2005). Although dyslipidemia and impaired glucose tolerance are not detected in every patient with fat redistribution, fat redistribution has been associated with a cluster of metabolic abnormalities, such as insulin resistance, loss of glucose tolerance, hypertriglyceridemia, and low serum levels of HDL-C (Mooser & Carr 2001, Sekiya *et al.* 2008). The analysis of depot-specific adipocyte cell lines has shown that subcutaneous adipocytes are more susceptible to the PI effects than visceral adipocytes (Kovsan *et al.* 2009, Caron-Debarle *et al.* 2010). Furthermore, an analysis of human subcutaneous adipose tissue (SAT) explants revealed that some PIs increase FFA, IL-6 and TNFα production by activating the NFkB pathway eliciting a paracrine loop between adipocytes and macrophages not seen in visceral adipose tissue (VAT) (Grinspoon & Carr 2005). These results indicate that SAT is more sensitive to the adverse effects of some PIs than VAT. PIs induce lipodystrophy especially when associated with stavudine and didanosine, two NRTIs (Grinspoon & Carr 2005). In addition to impairment of lipid metabolism, PIs are related to dysfunction of glucose regulation. In particular, insulin resistance may result from the direct effects of PIs and HIV infection and changes in fat distribution (Grinspoon 2003, Van Wijk *et al.* 2011). The prevalence of insulin resistance in the cART-treated population is not known, but cross-sectional studies estimate that up to 30-40% of patients are insulin resistant whereas the prevalence of impaired glucose tolerance and diabetes mellitus is below 10-15% (Behrens *et al.* 1999, Carr *et al.* 1999, Calza *et al.* 2008). Treatment with IDV and RTV In HIV-negative adults disclosed a down-regulation of insulin sensitivity one month after the start of treatment (Noor *et al.* 2002, Lee *et al.* 2004, Noor *et al.* 2004). There is also evidence that IDV, APV, NFV and RTV directly inhibit the uptake of glucose in insulin sensitive tissues such as fat and skeletal muscle by selectively inhibiting the glucose transporter 4 (Glut-4) without interfering with insulin post-receptor signalling (Murata *et al.* 2000, Behrens *et al.* 2002). Lipodystrophy and insulin resistance are also linked to adipocytokines activity (Brinkman *et al.* 1999, Bastard *et al.* 2002). Lipodystrophy is also characterised by PI-related impairment of differentiation of pre-adipocytes to adipocytes resulting in decreased synthesis of adiponectin and leptin with a down-regulation of matrix metalloproteinase expression (Weyer *et al.* 2001, Bourlier *et al.* 2005, Tsiodras *et al.* 2010). Adiponectin is lower in HIV patients with lipodystrophy and visceral fat accumulation than controls (Weyer *et al.* 2001, Tsiodras *et al.* 2010), and the reduction of adiponectin levels is correlated with insulin resistance and dyslipidemia (Weyer *et al.* 2001, Tong *et al.* 2003). In addition, a study on 131 HIV-positive subjects demonstrated that insulin sensitivity is correlated with serum content of adiponectin and the adiponectin/leptin ratio (Vigouroux *et al.* 2003). These data were confirmed in a subsequent cross-sectional study where adiponectin levels were positively correlated with insulin sensitivity (Kosminski *et al.* 2003).

In addition to dyslipidemia, PIs exhibit adverse effects on different cells involved in the development of atherosclerosis such as endothelial cells and macrophages. Impaired homeostasis in the endothelial layer is involved in the development of atherosclerosis. Since impaired vasorelaxation in response to pharmacological agents is considered as an indicator of early endothelial impairment and vascular dysfunctions (Freiman *et al.* 1986, Benzuly *et al.* 1994, Wang *et al.* 2007), the effects of PIs on endothelium-dependent vasorelaxation were investigated by measuring flow-mediated vasodilation (FMD) of the brachial artery in PI-treated HIV individuals. HIV subjects receiving PIs showed a significant reduction of FMD with respect to HIV individuals treated without PIs (Stein *et al.* 2001). These results were substantially confirmed in IDV-treated healthy volunteers, who showed a clear decrease of FMD after one month of treatment (Shankar & Dubé 2004). Moreover, FMD was impaired by a mean 3,6% in HIV-positive children treated with PIs compared with 1,8% in the group of HIV-positive children treated in the absence of PIs (Charakida *et al.* 2005).

Clinical plasma concentrations of specific PIs

were tested in an animal model represented by pig carotid and coronary arteries. In these experimental conditions, RTV perfusion showed that endothelium-dependent vasorelaxation was down-modulated in PI-treated porcine carotid arteries with respect to untreated controls (Conklin *et al.* 2004). RTV, APV and SQV determined a significant reduction in endothelium-dependent vasorelaxation even in porcine coronary arteries (Fu *et al.* 2005) confirming FMD instrumental data (Stein *et al.* 2001, Shankar & Dubé 2004, Charakida *et al.* 2005). These endothelial dysfunctions have mainly been related to the PIs effects on nitric oxide synthase (NOS) and reduction/oxidation (ROS) systems. Nitric oxide (NO) plays a pivotal role in maintaining vascular tone preventing platelet and leukocyte adhesion to the endothelial layer. NO causes relaxation of vascular smooth muscle and regulates endothelium-dependent vasorelaxation. In mammalian tissues, NO is catalysed by NOS family enzymes including inducible(iNOS), endothelial (eNOS) and neural NOS (nNOS). In endothelial cells, NO is constitutively synthetized from L-arginine by eNOS and several reports have demonstrated that a dysfunction of synthesis release and activity of NO is correlated to vascular damage. In particular, eNOS inhibition induces a reduction of endothelium-dependent vasorelaxation and an accelerated development of atherosclerosis (Kawashima 2004, Shimokawa *et al.* 2010). RTV, APV, IDV, NFV and SQV elicit a significant reduction (40-50%) of eNOS messenger RNA (mRNA) and protein levels in cultured human coronary artery endothelial cells with respect to untreated controls. NO release was analysed in porcine coronary arteries by measuring the accumulation of the NO degradation products nitrite and nitrate. Nitrite in the supernatant of cultured arteries was significantly reduced by 33.5% for RTV and by 28.7% for APV compared with untreated arteries whereas SQV, IDV, and NFV decreased nitrite levels but to lesser extent (Fu *et al.* 2005, Wang *et al.* 2007). Reactive oxygen species (ROS) are pivotal chemical mediators that can alter lipids, nucleic acids and proteins determining oxidative stress (Simionescu 2009). They have major pathophysiologic consequences in the vascular system because an enhanced ROS formation could elicit the attenuation of endothelium-dependent dilation by NO, resulting in impaired organ perfusion, endothelial layer homeostasis, cell damage, vessel structure lesion and systemic hypertension (Heistad *et al.* 2009). NFV and SQV specifically elicited ROS production in vascular smooth muscle cells and co-treatment PIs plus N-acetyl-cysteine or the anti-oxidant enzyme catalase significantly decreased ROS induction (Rudich *et al.* 2005, Wang *et al.* 2007). Further reports confirmed this PI activity, IDV or NFV, combined with zidovudine and efavirenz, up-regulate ROS synthesis in human aortic endothelial cells and superoxide anion content is significantly increased in endothelial and smooth muscle cell of porcine coronary arteries when treated by RTV or APV (Mondal *et al.* 2004, Chai *et al.* 2005a, Chai *et al.* 2005b). The mechanisms involved in the induction of ROS have to be yet elucidated but RTV significantly increased nicotinamide adenine dinucleotide phosphate (NADPH)-stimulated superoxide production in porcine carotid arteries (Zhong *et al.* 2002). By contrast, RTV and IDV treatment in endothelial cells elicited endothelial permeability and a mitochondrial DNA damage inducing mitochondrial dysfunction that may contribute to oxidative stress (Zhong *et al.* 2002, Fiala *et al.* 2004, Chen *et al.* 2005) because mitochondria are one of the major sources of ROS production (Zinkevich *et al.* 2011). It is noteworthy that PI treatment induces mitochondrial alterations such as reduced cellular respiration and ATP production, decreased mitochondrial membrane potential and mitochondrial DNA damage. Beside these adverse effects on endothelial biology, PIs also affect macrophages that are the most important cell type in atherosclerotic lesions and play a pivotal role in the genesis and development of atherosclerotic damage. Studies performed on THP-1 cells and human PBMCs demonstrated that RTV up-regulates CD36 expression and accumulation of cholesteryl esters suggesting an impairment in cholesterol regulation with a subsequent accumulation of sterol in macrophages and their transformation in the typical foam cells (Dressman *et al.* 2003). Interestingly, a recent report on RTV effects in mouse peritoneal macrophages indicated that RTV treatment could stimulate foam cell formation through PKC activation (Xiang *et al.* 2011). A more extensive study on PIs activity in macrophages has shown that therapeutic concentrations of RTV, IDV and ATV cause an accumulation of intracellular free cho-

lesterol up-regulating CD36 and LDL-R expression. This increase in intracellular free cholesterol is associated with a depletion of endoplasmatic reticulum stress with activation of an unfolded protein response that can determine a complex gene modulation with caspase-12 and apoptosis activation in macrophages (Zhou *et al.* 2005). In addition, all PIs except APV can elicit the up-regulation of important pro-inflammatory cytokines such as TNFα and IL-6 through the positive modulation of RNA binding protein HuR (Zhou *et al.* 2007). This suggests a positive effect of PIs in the enhancement of chronic inflammation in atherosclerotic arteries. These data on PIs/macrophages interactions indicate that some PIs elicit a pro-atherogenic activity due to cholesterol modulation impairment, pro-inflammatory cytokine and apoptosis induction. These effects may enhance and promote the development of atherosclerosis but further studies are needed to fully understand the PI-mediated effects on macrophage biology.

Some PIs are also related to the onset of thrombotic events (George *et al.* 1999, Sullivan *et al.* 2000, Majluf-Cruz *et al.* 2004, Lijfering *et al.* 2007, Bibas *et al.* 2011). IDV and SQV were associated with an increased risk of VTE in an HIV-positive population (George *et al.* 1999, Jacobson *et al.* 2004, Shen *et al.* 2004), but these data were not confirmed (Crum-Cianflone *et al.* 2008). The mechanisms of this effect, remain unsettled even though PIs are thought to interfere with hepatic metabolism, specifically cytochrome P450 metabolism and the regulation of thrombotic proteins. In addition, lipodystrophy is related to an increased risk of developing an abnormal coagulation profile, such as increased fibrinogen, D-dimer, PAI-1, or protein S deficiency (Lyonne *et al.* 2008) with a possible impairment of anticoagulant regulation.

*Role of N(n)RTIs in cardiovascular impairment*

N(n)RTIs represent the most important antiretroviral class in the assessment of initial cART, and zidovudine was the first drug approved for the treatment of HIV. The antiretroviral molecules currently classified in this class are: zidovudine (AZT), stavudine (d4TP), zalcitabine (ddC), didanosine (ddI), abacavir (ABC), lamivudine (3TC), emtricitabine (FTC) and tenofovir (TDF). It is noteworthy that TDF is included in this class even though it is a nucleotide reverse transcription inhibitor. As described above, the increased MI risk induced by N(n)RTIs was detected when ABC or ddI was used within the preceding six months, whereas other N(n)RTIs did not seem to be involved in cardiovascular impairment. ABC effects on the cardiovascular system is a matter of debate because several trials displayed controversial results as described above. Studies have been performed on N(n)RTI modulation of lipid metabolism and cell lineages involved in atherosclerosis and cardiovascular diseases. N(n)RTI-containing treatment showed a prevalence of hypertriglyceridemia and hypercholesterolemia of 23% and 10% respectively (Tsiodras *et al.* 2000). Treatment with d4TP determines an increase in plasma lipid levels whereas TDF has no significant effects on lipid content but may have a slightly lowering effect on triglyceride and cholesterol levels (Lee *et al.* 2004, Gallant *et al.* 2004). An analysis of the specific N(n)RTI relationship with dyslipidemia (Figure 3) suggested that d4TP shows higher effects on dyslipidemia. AZT, ddI and ABC exhibit intermediate effects whereas FTC, 3TC and TDF had lower effects (Dubé & Cadden 2011). ABC may be classified as N(n)NRTI with intermediate effect because some trials have indicated a more important effect of ABC on dyslipidemia than TDF (Moyle *et al.* 2006, Martinez *et al.* 2009, Sax *et al.* 2011). It is noteworthy that only thymidine NRTIs (d4TP and AZT) are linked to lipoatrophy and lipodystrophy development (Nolan *et al.* 2003, Hammond *et al.* 2007, Villaroy *et al.* 2010). These two NRTIs cause mitochondrial toxicity, in part by inhibiting the mtDNA polymerase gamma (Pinti *et al.* 2006, Cote 2007), and determine fat hypertrophy in visceral depots (Galli *et al.* 2002, Miller *et al.* 2003, Caron *et al.* 2008, Boothby *et al.* 2009, Flint *et al.* 2009, Haubrich *et al.* 2009, Hsue *et al.* 2009, Van Voderen *et al.* 2009, Stankov *et al.* 2010). AZT and d4TP decrease mitochondrial DNA content in human adipocytes maintaining respiratory chain activity whereas they increase oxidative stress, MCP-1, CRP and IL-6 also down-regulating adiponectin and leptin (Lewis *et al.* 2003, Caron *et al.* 2008, Flint *et al.* 2009, Hsue *et al.* 2009, Stankov *et al.* 2010). The mitochondrial toxicity is due to competitive inhibition of these NRTIs with normal nucleotides for mitochondrial DNA polymerase gamma that

regulates mitochondrial DNA replication and repair. This competition elicits a mitochondrial damage with a progressive mitochondrial depletion that can determine cell toxicity (Lewis *et al.* 2003). Despite their association with pro-atherogenic processes such as lipodystrophy and dyslipidemia, the DAD study (Sabin *et al.* 2008) showed that d4TP and AZT did not elicit an increased risk of MI suggesting a complex scenario whose mechanisms have yet to be assessed.

N(n)RTIs have also been investigated to establish whether these drugs could affect the endothelial layer and coagulation. Some observations showed an ABC-related dysfunction of endothelial and platelet homeostasis (Hsue *et al.* 2009). ABC treatment induces a greater dysfunction of the endothelium documented by lower artery flow mediated dilatation in a cohort of long-term ABC-treated HIV patients with respect to ABC-untreated controls (Hsue *et al.* 2009). In addition, ABC represents a guanosine analogue, that can be involved in the competitive inhibition of soluble guanylyl-cyclase, a negative regulator of platelet function, increasing platelet reactivity (Gresele *et al.* 2012). It is noteworthy that an analysis of platelet activation in HIV-positive patients treated for six to 12 months with ABC or TDF showed a significant increase in platelet activation markers (sPsel, sPLA2 and sGPV) in the plasma of ABC-treated patients but not in TDF-treated patients. Moreover, ABC-treated patients showed enhanced ex vivo platelet aggregation (Gresele *et al.* 2012). The increased platelet reactivity was also confirmed by another two studies demonstrating that ABC induced a higher platelet reactivity after exposure to a large array of agonists (Satchell *et al.* 2010, Satchell *et al.* 2011). Some studies have also reported that ABC treatment could determine an increase in inflammatory biomarkers such as IL-6 and CRP (27% and 16% respectively) in comparison with HIV-positive patients treated with other NRTIs. This may suggest that the activation of vascular inflammation is implicated in the genesis of the controversial ABC-related increased risk of cardiovascular disease (Caron *et al.* 2003, Lundgren *et al.* 2008). Interestingly, a two-year prospective study performed in 67 HIV-infected patients on inflammatory biomarkers suggested a possible association between TDF-based therapies and progression of subclinical atherosclerosis progression but additional analyses on a larger cohort are needed (Aragones *et al.* 2012).

Studies performed on AZT in mice have shown that after 35 days of treatment the murine aortas exhibited a 34% reduction of maximum endothelium-dependent relaxation compared with the controls and a five-fold decrease in acetylcholine sensitivity probably due to ROS production (Sutliff *et al.* 2002, Jiang *et al.* 2006, Kline *et al.* 2008). In vitro AZT induced changes in the proliferation and survival of endothelial cells probably related to an impairment of mitochondrial homeostasis (Herbert *et al.* 2004, Jiang *et al.* 2007). These observations indicate that some N(n)RTIs can determine an alteration of lipid metabolism/distribution and an impairment of the endothelial layer but, at the moment, the impact of these drugs on atherosclerosis and cardiovascular injury has to be yet addressed. Further basic and clinical studies must be performed to determine whether these molecules have pathologic effects on the cardiovascular system.

*Role of NNRTIs in cardiovascular impairment*

NNRTIs are non-competitive inhibitors of viral reverse transcriptase. Nevirapine (NVP), delavirdine (DLV), efavirenz (EFV) and the recent etravirine (TMC 125) and rilpivirine (TMC 278) are the currently approved therapeutic molecules belonging to this antiretroviral class.

Clinical studies where NNRTI-based cART regimens were compared to PI-based therapeutic combination, showed that NNRTIs were not associated with an increased risk of premature atherosclerosis (De Saint Martin *et al.* 2006) and MI (Friis-Moller *et al.* 2007). The DAD study showed that the risk of MI did not change significantly under NNRTI-based therapy. When adjusted for other MI factors, NNRTI treatment was associated with a stable risk (RR=1.00, 95% CI=0.93–1.09, P=0.92) (Van Leth *et al.* 2004a). Interestingly, NNRTIs did not induce an atherogenic lipid profile(Figure 3) in HIV patients (Murphy & Smith 2002, Young *et al.* 2005). EFV and NVP increase HDL cholesterol levels, and, to a lesser extent, LDL cholesterol and non-HDL cholesterol without significantly changing the resulting total/HDL cholesterol ratios (Van Leth *et al.* 2004b, Dubé & Cadden 2011). EFV determines a mild increase in triglycerides and LDL cholesterol when compared to NVP (Fichtenbaum

2010). In the 2NN study, 1267 subjects were randomised to EFV or NVP (Fichtenbaum 2010) and HDL-C increased 42.5% and 32.7% in the NVP and EFV groups, respectively, whereas triglycerides and LDL-C also increased 26.9% and 35.4% and 31.1% and 40.1% in the NVP and EFV groups, respectively. The higher increase in HDL-C has been attribuited to a counterbalance to the other lipid changes lowering the overall risk of MI events (El-Hadri *et al.* 2004). Interestingly, although initial studies failed to implicate EFV in lipodystrophy, the ACTG study found that EFV may be associated with lipoatrophy more frequently than LPV/RTV when combined with d4TP or AZT treatment (Haubrich *et al.* 2009, Caron-Debarle *et al.* 2010). In agreement, EFV down-regulates SREBP-1c expression and exerts an anti-adipogenic effects in cultured adipose cells (El-Hadri *et al.* 2004, Rodriguez *et al.* 2005, Esposito *et al.* 2009, Giralt *et al.* 2011). On the other hand, NVP does not influence hMADS adipose cell differentiation (Vernochet *et al.* 2005) or even induces brown adipocyte differentiation (Rodriguez *et al.* 2005). The analysis of lipid abnormalities and changes in lipid levels in the DUET-1 and DUET-2 trials showed that etravirine did not determine an altered lipid profile (Girard *et al.* 2012). The ECHO and THRIVE trials studied the efficacy and safety of ripilvirine versus efavirenz at 48 weeks of treatment in HIV naive patients. Interestingly, changes in HDL-cholesterol and pro-atherogenic lipid profiles were less consistent with ripilvirine than with EFV, but the total cholesterol/HDL-cholesterol ratio was similar between ripilvirine and EFV-treated groups (Cohen *et al.* 2012). These observations suggest that switching to a cART regimen with NNRTIs may reduce cardiovascular effects in HIV patients with a high risk of atherosclerosis and cardiovascular impairment.

*Role of integrase inhibitor in cardiovascular impairment*

The integrase inhibitor raltegravir is the only integrase inhibitor approved for therapeutical use. Raltegravir inhibits viral integrase activity and then blocks the proviral integration process of proviral DNA into host DNA. Raltegravir does not show negative effects on the cardiovascular system and lipid metabolism. In the STARTMRK study, raltegravir combined with TDF/FTC in treatment-naive patients, induced viral suppression equivalent to EFV combined with TDF/FTC through 156 weeks of therapy but raltegravir was associated with fewer drug-related adverse events and smaller elevations in lipid levels (Rockstroh *et al.* 2011). The SWITCHMRK 1 and 2 multicentre randomised controlled trials compared patients switching raltegravir for LPV/RTV with respect to patients continuing with LPV/RTV in HIV-infected patients with stable viral suppression by LPV/RTV-based combination therapy. Raltegravir-treated patients showed a decrease in serum lipid concentrations with respect to individuals in whom continuation of LPV/RTV was assured (Eron *et al.* 2010). In vitro analysis using 3T3-L1 cells, has demonstrated that raltegravir did not significantly affect adipogenesis or lipid metabolism indicating no interference in lipid regulation (Minami *et al.* 2011, Perez-Matute 2011). Although further studies are required to improve the knowledge of raltegravir activity on the cardiovascular system, raltegravir is currently considered a molecule with no relevant toxicity on this target. In addition, while several PIs and NNRTIs are involved in CYP450 or other transporter system inhibition, with a higher risk of clinically significant drug interactions, raltegravir is not involved in the CYP450 system, and may be a useful option to employ to minimise interactions with other drug classes (Tseng *et al.* 2012).

*Role of entry inhibitors in cardiovascular impairment*

The CCR5 receptor blocker maraviroc, and HIV fusion inhibitor enfuvirtide are the two only molecules acting on viral entry approved for HIV disease therapy. Maraviroc did not show significant activity on the cardiovascular system. The MERIT study showed that maraviroc was not associated with increased levels of total cholesterol, LDL-C or triglycerides (MacInnes *et al.* 2011) thus showing an absence of activity in lipid metabolism and regulation. Moreover, maraviroc is a CCR5 binding antagonist and some studies have indicated a supporting role for CCR5 and its ligands CCL3, CCL4 and CCL5 in the initiation and progression of atherosclerosis. In particular, CCR5 might likely be critical for recruiting monocytes in the development of atherosclerotic plaques. Furthermore, the CCR5 deletion poly-

morphism CCR5delta32 has been associated with a reduced risk of cardiovascular disease and both CCR5 antagonism and gene deletion decrease atherosclerosis in mouse models suggesting that the CCR5 antagonist maraviroc might show cardiovascular-protective effects (Jones *et al.* 2011). Although few studies were performed on enfuvirtide-related effects, the drug does not seem to exhibit cardiovascular negative effects. A recent study analysed the effects of enfuvirtide on lipid profiles at 48 weeks of treatment comparing enfuvirtide plus an optimised background regimen versus optimized background alone (control group) in treatment-experienced patients. In this study, total cholesterol, LDL, VLDL, HDL and triglyceride plasma levels did not show any significant variation (Cooper *et al.* 2011).

## CONCLUSIONS

Combined ART has provided a dramatic change in HIV disease evolution preventing the development of AIDS in the majority of cases with an increase in life expectancy. In spite of this improvement in the control of HIV replication, some antiretrovirals are correlated with an higher risk of cardiovascular diseases because of relative toxicity and interference with lipid metabolism and cell lineages involved in vessel structure homeostasis. These negative effects have suggested the use of cART switch to reduce the risk of cardiovascular diseases especially in HIV-positive patients showing additional independent risks of cardiovascular diseases (Negredo *et al.* 2006). The rationale underlying the switch in therapy is related to the awareness that not all antiretroviral drugs have the same toxicity especially on lipidic metabolism, insulin resistance, diabetes etc. Interestingly, some clinical trials have suggested specific changes in cART regimen association to minimise these collateral negative events such as, for example, the substitution of PI/RTV with an NNRTI or PI with reduced metabolic impact (ATZ, SQV or DRV) or raltegravir, and the substitution of d4T, ZDV or ABC with TDF (Martin *et al.* 2004, Moyle *et al.* 2006, Madruga *et al.* 2007, Molina *et al.* 2008, Eron *et al.* 2010). Future research on the antiretroviral mechanisms involved in cardiovascular impairment will be pivotal to tailor a cART with negligible cardiovascular and metabolic effects to improve the management of HIV-positive patients.

### Conflicts of interests

All Authors declared no conflict of interests.

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