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Calmy, Alexandra

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Université de Genève,
Département de médecine interne
Professeur H.Bounameaux

Optimizing HIV drug therapy

Alexandra Calmy,
MD

Thèse présentée pour
l'habilitation au titre de privat
docent de l'université de
Genève

ABSTRACT

The spectrum of drugs used in HIV-infected patients has dramatically changed since triple antiretroviral combinations were introduced, albeit at the expense of some severe adverse events, in 1996. Long term complications of antiretroviral drug exposure, such as HIV lipodystrophy, as well as organ-specific disease of heart and bone are, therefore, a critical issue when designing antiretroviral regimens. Because it is difficult to predict the occurrence of lipodystrophy, and because there is no therapeutic agents able to combat lipodystrophy once established, avoidance of thymidine nucleoside analogues remains the most useful strategy to prevent and treat lipoatrophy; although this approach can worsen dyslipidaemia. Decreasing thymidine analogue use as well as the availability of new drugs and new drug classes led to a reduced likelihood of lipodystrophy development. Metabolic syndrome can and should be assessed as it predicts type 2 diabetes as well as cardiovascular events in HIV-infected individuals. Ongoing HIV replication is a risk factor for serious non-AIDS events, including cardiovascular disease. Therefore, HIV RNA suppression is imperative in all patients on antiretroviral therapy. Finally, HIV-infected adults on antiretroviral therapy, particularly in those receiving a boosted protease inhibitor, have a high prevalence of low bone mineral density. The estimation of fracture risk with the WHO FRAXTM tool deserves further validation in HIV-infected adults.

In conclusion, prevention, detection and treatment of various non-infectious comorbidities has become essential for HIV-infected individuals exposed to a life-long antiretroviral therapy and goes beyond the sole management of the lipodystrophy syndrome.

PREFACE

I would like to express my gratitude to Professor Bernard Hirschel, with whom I have the pleasure to work since more than 10 years, and who always trusted me, encouraged my choices and gave me a space to develop my own projects within the HIV unit. I certainly wouldn't be presenting my PD thesis today without his continuous support. He particularly allowed to me to pursue a sustained activity in the associative world (Médecins Sans Frontières) in relationship with HIV/AIDS global issues – which collaboration certainly helped to shape my understanding on HIV/AIDS as a global, chronic, political as well as deadly disease.

I am grateful to the service of Infectious Diseases in Geneva University Hospital, and particularly to Professor Daniel Lew who, despite several differences in our vision, knew how to make me go ahead.

I had the pleasure to work several years in internal medicine ward (Professors A-F Junod and A Perrier) and there is no doubt that the direction I took in HIV medicine was closely related to the interest in internal medicine they transmitted to me.

I did a two years training in clinical research at St Vincent's Hospital; I benefited from a very close supervision from Professor Andrew Carr, who was my supervisor for my PhD thesis. I would like to express him my full gratitude.

I wish to thank all co-authors of the presented published or submitted papers who all gave their written permission to use this published material as part of the present PhD thesis. None of the work could have been done without their commitment and patience for which I am very grateful.

All papers and figures have been reproduced with the permission of journals Editors whom I would like to thank warmly.

Finally, I hope that I haven't discourage my three daughters to undertake medical studies. They went through difficult times and bravely supported watching television while the computer was busy...Fabrice, my partner, was a key supporter as well. I wish to dedicate this PD thesis to them all.

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ABBREVIATIONS

3TC	lamivudine*
ABC	abacavir
AE	adverse event
AZT	zidovudine
BMD	bone mineral density
cART	combined antiretroviral therapy
CVD	cardiovascular disease
d4T	stavudine
D:A:D	Data Collection of Adverse events of Anti-HIV Drugs
DM	diabetes mellitus
EFV	efavirenz
FTC	emtricitabine
HAART	highly active antiretroviral therapy
IRIS	Immune reconstitution inflammatory syndrome
LPV/r	ritonavir-boosted lopinavir
MI	myocardial infarction
MS	metabolic syndrome
NRTI	nucleoside analogue reverse transcriptase inhibitor
NNRTI	non-nucleoside analogue reverse transcriptase inhibitor
NVP	nevirapine
PI	protease inhibitor
PNP	polyneuropathy
RCT	randomized clinical trial
SAT	subcutaneous adipose tissue
SMART	Strategies for Management of Antiretroviral Therapy trial (clinical trial.gov number: NCT00027352)
STACCATO	Swiss-Thai-Australia Treatment Interruption Trial (clinical trial.gov number: NCT00113126)
TAHOD	Treat Asia HIV Observational Database
TDF	tenofovir
TNF- α	tumour necrosis factor-alpha
VAT	visceral adipose tissue

**Drug names are used in full in the text, and abbreviated forms are used in the tables and figures.*

1. AN INTRODUCTION TO DRUG TOXICITIES

The spectrum of drugs used in HIV-infected patients has dramatically changed since triple antiretroviral combinations were introduced, albeit at the expense of some severe adverse events, in 1996. Twenty-four antiretroviral drugs have been approved since 1986. The efficacy of combination antiretroviral therapy (cART, also referred as highly active antiretroviral therapy [HAART]) for compliant cART-naïve patients over 1 to 2 years now approaches 90% and this percentage may be difficult to improve. Drug safety is likely to be the most important factor to distinguish one antiretroviral regimen from another.

cART generally comprises three drugs including two nucleoside analogue reverse transcriptase inhibitors (NRTIs) and either one protease inhibitor (PI) or one non-nucleoside analogue reverse transcriptase inhibitor (NNRTI) (Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents, Department of Health and Human Services, USA, 2009).

There is a distinction between the terms ‘toxicity’ and ‘adverse event’. An adverse event is an unwanted event occurring when a patient is on antiretroviral therapy, but the drug causality remains to be proven; an adverse event is likely to be multifactorial. Cardiovascular events and osteopenia are examples of cART-related adverse events. Toxicity is an unwanted event that is related to an identifiable drug; the offended drug can be substituted by another drug with a different safety profile. Lactic acidosis is an example of drug class toxicity.

Anti HIV drug names and their common or serious adverse events are summarized in table 1.

Table 1

Licensed anti-HIV drugs: common and serious toxicities

Class	Drug (abbreviation)	Common side effects	Serious side effects
Nucleoside analogue reverse transcriptase inhibitor (NRTI) <i>Many NRTI toxicities are due to mitochondrial toxicity</i>	Abacavir (ABC)	Well tolerated	Hypersensitivity reactions. Cardiovascular events in patients with known risks factors.
	Didanosine (ddl)	Peripheral neuropathy, nausea	Lactic acidosis Pancreatitis
	Emtricitabine (FTC)	Well tolerated	Rare cases of lactic acidosis / hepatitis / anaemia in combination with other NRTIs
	Lamivudine (3TC)	Well tolerated	Same toxicity profile as emtricitabine
	Stavudine (d4T)	Peripheral neuropathy, lipoatrophy	Lactic acidosis pancreatitis
	Tenofovir (TDF)	Renal tubular dysfunction, Reduced bone mineral density	Renal insufficiency, Fanconi syndrome
	Zidovudine (AZT)	Anaemia, nausea, headache, asthenia	Bone marrow suppression, lactic acidosis
	Abacavir + 3TC	<i>Fixed dose combinations have the side effects of each drug component.</i>	
	AZT + 3TC		
	Tenofovir + FTC		
	AZT + 3TC + Abacavir		
Non-nucleoside analogue reverse transcriptase inhibitor (NNRTI)	Efavirenz (EFV)	Neuropsychiatric side effects	Teratogenicity in non human primates Hepatitis
	Etravirine	Rash, nausea	Hepatitis especially in hepatitis B and C co-infected patients. Rare cases of Stevens-Johnson syndrome.
	Nevirapine (NVP)	Hepatitis, hypersensitivity	Stevens-Johnson syndrome, Toxic epidermal necrolysis Hepatitis
Protease Inhibitor (PI)	Atazanavir	Indirect hyperbilirubinaemia,	PR interval prolongation

Class	Drug (abbreviation)	Common side effects	Serious side effects
<i>Gastro intestinal toxicity is common in this drug class</i> <i>Hyperlipidaemia is seen with all PIs except unboosted atazanavir</i> <i>All ritonavir-boosted PIs can cause bleeding episodes in haemophiliacs</i>		nephrolithiasis	
	Darunavir*	Skin rash, headache	Hepatitis, Hypersensitivity
	Fosamprenavir*	Skin rash	
	Indinavir*	Nephrolithiasis, hyperglycemia, indirect hyperbilirubinaemia, metallic taste, alopecia	
	Lopinavir + ritonavir (LPV/r)	Hypertriglyceridaemia, hyperglycaemia	
	Nelfinavir	Diarrhoea	
	Ritonavir	Hyperglyceamia	
	Saquinavir*		
	Tipranavir*	Hepatitis, skin rash	Liver failure, intracranial haemorrhage
Integrase inhibitor	Raltegravir	Nausea, diarrhea, headache, fever, dizziness	myositis
CCR5 blocker	Maraviroc	Abdominal pain, upper respiratory infection, cough, rash, orthostatic hypotension	Hepatotoxicity, cardiovascular events in at risk patients
Fusion inhibitor	Enfuvirtide	Local injection site reactions, pneumonia	Hypersensitivity

* recommended for use with low dose ritonavir as a booster.

Indeed, adverse events are the leading cause of cART discontinuation in patients receiving their first cART regimen, both in developed (**A Carr et al**, AIDS 2009) and in resource-limited settings (**A Calmy et al**, AIDS 2006). Although life-threatening toxicities are rare (Table 1), side effects may negatively affect a patient's ability to be adherent – potentially leading to a poorer outcome. Lipodystrophy is a good example of the complex relationship between the occurrence of an adverse event and adherence. Although it has been observed that greater adherence is associated with a greater risk of lipodystrophy (**G Guaraldi et al**, HIV Clin Trial 2003), the strength of this relationship diminishes over time – suggesting that, over time,

patients with a more severe lipodystrophy are more likely to become nonadherent and to develop drug resistance (**A Ammassari et al**, J Acquir Immune Defic Syndr 2002).

International treatment recommendations have delayed the start of potent cART mainly because of long term toxicities in patients on long term exposure to antiretroviral drugs (**SM Hammer et al**, JAMA 2008). Moreover, the SMART trial, the largest HIV clinical trial ever conducted (5472 participants in 33 countries), demonstrated that people randomized to an intermittent therapy had an increased risk of HIV disease progression and death (**W El Sadr et al**, New Engl J Med 2007). Moreover, it also showed patients receiving episodic therapy experienced major complications such as cardiovascular, kidney or liver diseases. These results completely changed the paradigm that drug toxicity was a direct cause for all sorts of end organ diseases. Cardiovascular events, for example, occur in patients after drug cessation, leading to the hypothesis that HIV RNA replication as such could be a cardiovascular risk factor.

As complications do not resolve by interrupting treatment, and as life-long treatment is likely to be the rule for the next few years, understanding and preventing drug toxicity are important to ensure successful, long-term management of HIV-infected patients. Long term complications deserve special attention as most new antiretroviral drugs are marketed before there is a large clinical experience in all patient populations. Furthermore, most data come from randomized trial that rarely last longer than 48 weeks.

I aimed at showing how various toxicities associated with cART can be assessed, prevented and managed. I focused toxicities occurring in resource-rich settings where there is no limitation of drug formulary.

2. MOVING FROM LIPODYSTROPHY TO NON INFECTIOUS END ORGAN DISEASES

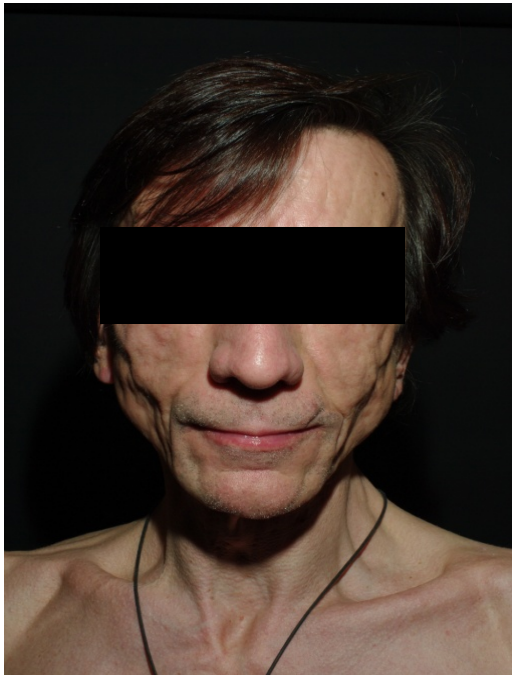
2.1 HIV Lipodystrophy

2.1.1 Background

HIV lipodystrophy is characterized by peripheral, subcutaneous fat loss in the face, arms, legs, and buttocks (referred to as lipoatrophy) and relative, central fat accumulation in the neck, breasts, and abdomen (referred to as lipohypertrophy) (**A Carr et al**, Lancet 1999). These body shape changes are sometimes referred as the fat redistribution syndrome, or as HIV lipodystrophy syndrome. Lipodystrophy is associated with several atherogenic metabolic abnormalities, including low plasma levels of high-density (HDL) cholesterol, hypertriglyceridaemia, insulin resistance, and, less commonly, hyperglycemia.

The prevalence of lipodystrophy is high (ranging from 10% to 30% of patients after one or two years of therapy, and up to 70% in some cross-sectional series) and depend on the type of drug used (**A Carr et al**, AIDS 1998, **PW Mallon et al**, AIDS 2003). We however showed that both lipohypertrophy and lipoatrophy are diminishing problems in patients commencing contemporary antiretroviral regimens ([Appendix, A Nguyen, A Calmy et al, Lipodystrophy and weight changes: Data from the Swiss HIV Cohort Study, 2000 – 2006; HIV Med 2008; 17:142-150, publication # 4](#)), with a prevalence rate estimated at around 10% at 2 years in patients initiating a 'preferred' regimen in 2008 (**RH Haubrich et al**, AIDS 2009).

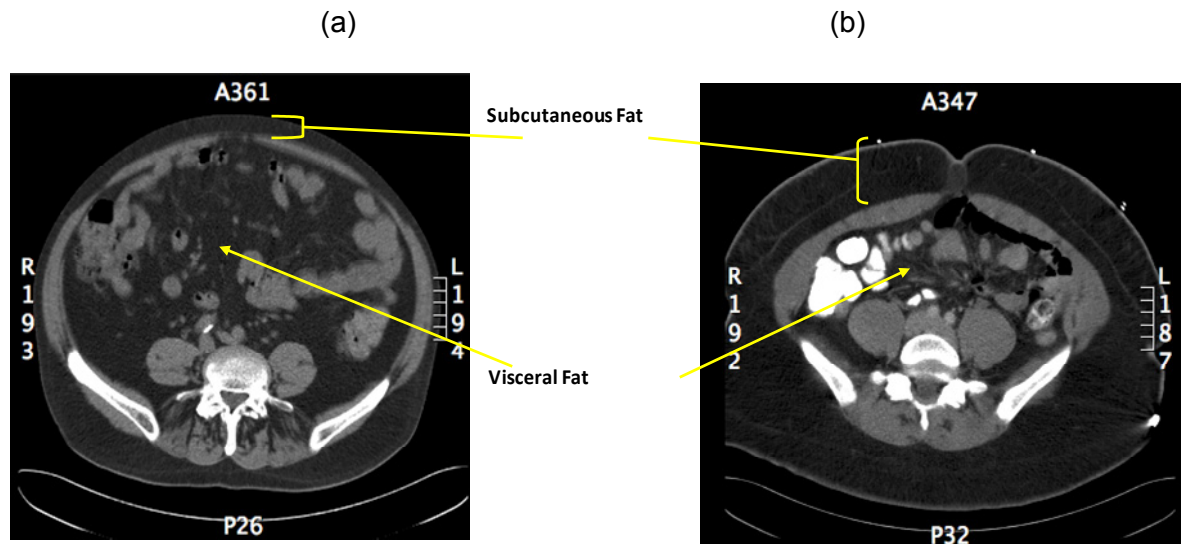
Figure 1 Severe facial lipoatrophy in a man.



How to diagnose lipodystrophy

The diagnosis of lipodystrophy is challenging. The definition of lipodystrophy remains subjective; most longitudinal studies rely on subjective patient or physician report for diagnosis. The need for objective measures was obvious very early after the first descriptions of lipodystrophy. Dual-energy X-Ray absorptiometry (DXA) measures body composition including the percentages and absolute amount of total, limb and truncal fat. Single-slice computed tomography (CT) at the level of the fourth or fifth lumbar vertebra assesses the areas of visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and their ratio. Ultrasound has been considered a simple and cheap diagnostic tool to assess facial thickness, but its reliability is controversial (**Ascher B et al**, Dermatol Surg 2006, **R Gulizia et al**, AIDS 2006). MRI is a more sensitive tool for quantifying fat than CT. MRI was shown in a pilot study to accurately quantify fat changes in multiple anatomic areas (dorso-cervical lipoma, face fat wasting, or visceral fat accumulation) (**M Bickel et al**, HIV Med 2007). Figure 2 shows how CT shows evidence of fat accumulation and subcutaneous fat loss in an HIV-infected patient with lipodystrophy.

Figure 2 Computed tomography imaging of visceral fat accumulation and loss of subcutaneous fat (a) and with obesity (b) (L5 level)



Carr and colleagues developed an objective case definition with the aim of providing a objective, sensitive, specific, and broadly applicable case definition of HIV lipodystrophy, particularly for regulatory and research purposes (**A Carr et al**, Lancet 2003). In models derived from a subset of randomly selected cases and controls, the case definition models that incorporated only clinical, or only clinical and metabolic, variables had lower sensitivity and specificity than the model that also included body composition data measured by imaging. However, as DXA and CT are not routinely performed (and certainly not reimbursed in most countries) for this indication, lipodystrophy is mostly diagnosed subjectively.

As the prevalence of body shape changes varies depending on the definition applied, cohort comparisons, analysis of risk factors or evaluation of therapeutic interventions are challenging (**VM Carter et al**, HIV Med 2001).

Table 2 summarises the clinical, anthropometric and laboratory characteristics of HIV lipodystrophy

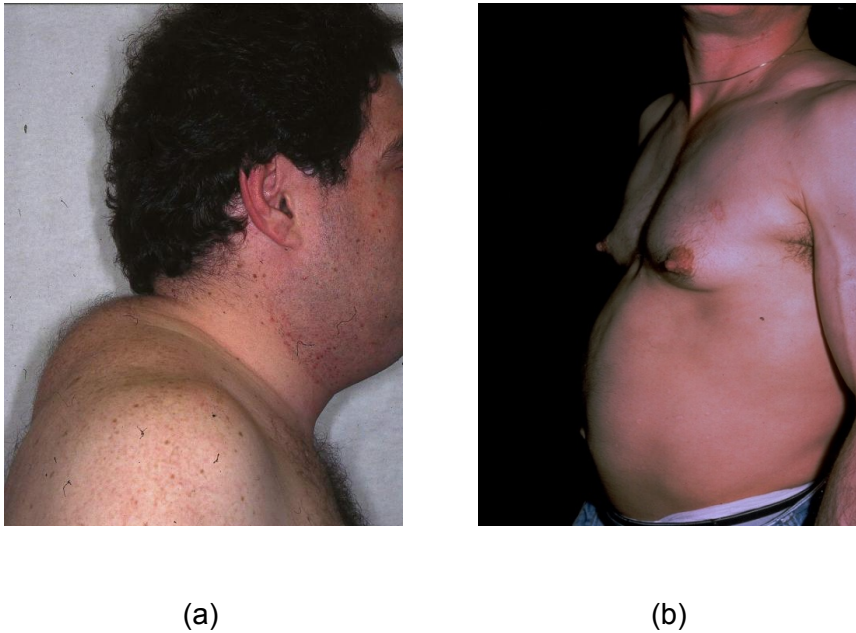
Table 2 Features of HIV lipodystrophy

Features	Lipoatrophy	Lipohypertrophy (fat accumulation)
Clinical	• Facial atrophy • Leg, arm and buttock atrophy. • Prominent veins.	• Trunk • Dorso-cervical spine • Breast • Lipomata
Body composition	• Total fat reduced • Limb fat reduced	• Intra-abdominal fat
Laboratory		• Waist : hip ratio increased • VAT : SAT ratio increased • Hypertriglyceridaemia • HDL cholesterol decreased • Hyperlactataemia • Insulin resistance
Association with antiretroviral drugs	Certain: Stavudine > zidovudine >> other nucleoside inhibitors	Controversy: Protease inhibitors

* VAT: visceral abdominal tissue, **SAT: subcutaneous abdominal tissue, *** Low-density lipoprotein, ****HDL: High density lipoprotein

Examples of fat accumulation (buffalo hump and visceral fat accumulation) will be presented in figure 3.

Figure 3 Buffalo hump (a) and visceral fat accumulation (b) in a man



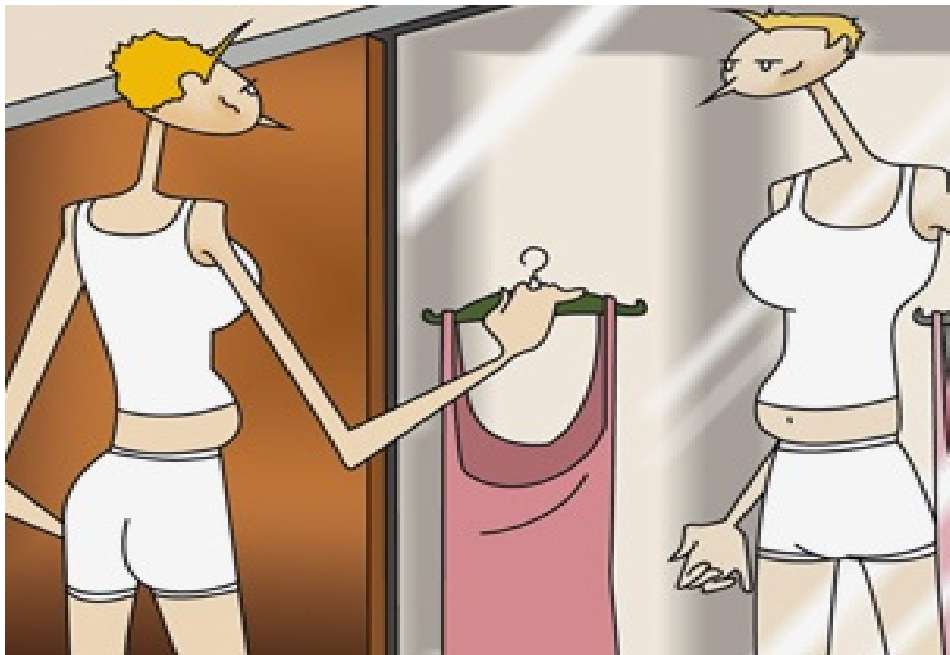
In summary, lipodystrophy definition ideally include DXA and CT (**A Carr et al**, Lancet 2003); while invaluable for small-scale studies, they are not practical for routine use in patients because of cost, exposition to radiation (CT Scan) or machine availability (DXA Scan). Instead, larger cohorts and epidemiological studies most often rely only on the subjective impressions of patients and/or physicians.

Lipodystrophy is not only an esthetic problem

Lipodystrophy and fat accumulation are not trivial: they can stigmatize patients and may reduce treatment adherence (**A Ammassari et al**, J Acquir Immune Defic Syndr. 2002). Clinical experience suggests that lipodystrophy can have an influence on health-related quality of life, producing erosion of self-esteem, anxiety, negative mood and decreasing the social functioning of patients experiencing this condition. Unfortunately, similarly to the absence of consensus on the best definition of lipodystrophy, there is no agreement on the best method for measuring its consequences. Most studies revealed an association between the presence of a lipodystrophy syndrome and either depression (**R Burgoyne et al**, Qual

Life Res. 2005), anxiety and social distress (**J Blanch et al**, Clin Infect Dis. 2004) or suboptimal adherence to cART (**JK Kemppainen et al**, AIDS Care 2006).

Figure 4 The discrepancy between the former and the new self image (*reproduced from Geneva University Hospital “Pulsations” journal, with permission*)



Patients with HIV lipodystrophy also demonstrate an increased utilization of healthcare services with associated increased healthcare costs as compared to HIV-infected patients without lipodystrophy. The magnitude of these increases remains to be adequately determined (**JS Huang et al**, AIDS Res Ther 2008).

The obesity and associated lipid and glucose disturbances of HIV lipodystrophy resemble the metabolic syndrome (MS), which is associated with an increases risk for cardiovascular events and diabetes in the general population (**National Cholesterol Education Program Third Report [NCEP]**, Circulation 2002). Although there are still controversies on the prevalence of MS in HIV-infected patients receiving cART, HIV-infected adults with MS have been found to be at increased risk for subclinical atherosclerosis as measured by the internal carotid intima-media thickness (**A Mangili et al**, Clin Infect Dis 2007).

Pathogenesis

Causes of lipodystrophy are not completely elucidated. NRTI-associated mitochondrial toxicity is increasingly implicated in lipodystrophy (**W Lewis et al**, *Nature Rev Drug Discov* 2003). Mitochondrial toxicity induced by thymidine analogues leads to fat cell loss, dysfunction or both, and hence lipodystrophy, even if it may not be the only mechanism involved in the development of this disorder. Mitochondrial DNA polymerase- γ is inhibited by some NRTIs (the most potent inhibitor of polymerase- γ is zalcitabine, followed by stavudine and didanosine) and thus causes depletion of mtDNA encoded enzymes resulting in mitochondrial dysfunction (**TN Kakuda et al**, *Clin Ther* 2000). Interestingly, NRTIs have been shown to negatively influence mRNA expression predating any measurable reduction of cellular mtDNA content (**PW Mallon et al**, *J Infect Dis* 2005). This finding indicated that NRTIs induce an early downregulation of mitochondrial transcription, that could affect mtDNA replication and critical mitochondrial functions, independent from an inhibitory activity on polymerase- γ . If this hypothesis is true, then other component able to compete in the mitochondrial cycle with NRTIs might prevent mitochondrial damage. Uridine is a pyrimidine precursor and so might replenish intracellular pyrimidine pools; in vitro, uridine abrogates mitochondrial toxicities of nucleoside reverse transcriptase inhibitor in adipocyte cell culture (**UA Walker et al**, *AIDS* 2004). We tested this hypothesis in a randomized trial, comparing patients with uridine, pravastatin, or both drugs to treat moderate to severe lipodystrophy (**Appendix, A Calmy, M Bloch, et al. No significant effect of uridine or pravastatin for HIV lipodystrophy in men who have ceased thymidine nucleoside analogue therapy: a randomized trial. HIV Med 2009, in press, publication # 8**).

Along with mtDNA depletion, abnormal adipocyte differentiation has been reported in fat tissue biopsies from patients on cART. Adverse effects of protease inhibitors on adipocyte differentiation have been described in cultured cells, probably acting at the step of sterol regulatory element-binding proteins (SREBPs) maturation and nuclear localization (**M Caron et al**, *Diabetes* 2001).

Few in vitro data propose some explanation as to how protease inhibitors could lead to visceral fat accumulation. In a 3T3-L1 preadipocyte model, **TA Nguyen et al** (AIDS 2000) reported that ritonavir enhances adipocyte differentiation in culture. Also, lopinavir/r can promote adipocyte growth in vitro (**K El Hadri et al**, J Biol Chem 2004).

It is unclear whether fat hypertrophy and fat atrophy in patients receiving cART represent one or more than one phenomenon. Risk factors for fat atrophy and hypertrophy are not identical (**A Calmy et al**, HIV Med 2008) suggesting a pathogenesis that is at least partially independent, with practical consequences: if fat accumulation is a redirection of fat normally stored in peripheral adipocytes to central depots, then the best treatment of fat accumulation would be the treatment of fat atrophy. If, however, fat accumulation is an effect of aging, of recovery from HIV-wasting, or a separate direct effect of cART, then the two conditions need to be managed separately. Cross-sectional studies tend to show the lack of association between central lipohypertrophy and lipoatrophy (**P Bachetti et al**, J Acquir Immune Defic Syndr. 2005) - and similarly improvement in fat atrophy after NRTI cessation has not been associated with visceral fat loss (**MA Boyd et al**, J Infect Dis 2006), suggesting that the second hypothesis is more probable.

2.1.2 Risks factors for lipodystrophy

Cohort studies have revealed several potential risk factors for lipoatrophy including older age, low CD4+ lymphocyte count, and high plasma HIV viral load (**KA Liechtenstein et al**, AIDS 2001, **E Martinez et al**, Lancet 2001, **JR Bogner et al**, J Acquir Immune Defic Syndr 2001, **M Saves et al**, Clin Infect Dis 2002). Reports from observational studies on the role of sex have been contradictory (**E Martinez et al**, Lancet 2001, **E Bernasconi et al**, J Acquir Immune Defic Syndr 2002, **JR Bogner et al**, J Acquir Immune Defic 2001, **M Saves et al**, Clin Infect Dis 2002, **M Galli et al**, Arch Intern Med. 2002). Some authors have found that non-white individuals were at lower risk of developing lipoatrophy when exposed to

antiretroviral therapy (**D Nolan et al**, J HIV Ther 2001, **KA Lichtenstein et al**, J Acquir Immune Defic Syndr 2003).

However, in prospective studies lipoatrophy is most closely related to the use of particular, namely thymidine NRTIs (tNRTIs) and first generation protease inhibitors (**PW Mallon et al**, AIDS 2003, **SH Lowe et al**, HIV Clin Trials 2008, **JE Gallant et al**, JAMA 2004, ACTG384, **Van Vonderen et al**, PLoS One 2009). One trial also observed significant gain in visceral fat with zidovudine-based cART, whereas controls receiving an NRTI-sparing regimen of nevirapine and lopinavir/r did not. It is possible that the incidence of lipodystrophy will decrease with newer drugs that induce less dyslipidaemia and glucose intolerance. Indeed the use of abacavir or tenofovir-based backbone regimens together with efavirenz is associated with a very low rate of lipoatrophy after 3 years of therapy (**D Podzamczek et al**, J Acquir Immune Defic Syndr 2007), which reflect the dominant effect of tNRTI treatment choice in the subsequent development of lipoatrophy.

Recent data also suggest that the choice between a ritonavir-boosted protease inhibitor and an NNRTI may also be important. Efavirenz has not traditionally been linked with the development of lipodystrophy (**JA Perez-Molina et al**, J Antimicrob Chemother 2008). One randomized, prospective study revealed greater limb fat loss with nelfinavir treatment, compared with efavirenz treatment over 144 weeks (**MP Dubé et al**, J Acquir Immune Defic Syndr 2007). A substudy of another trial of subjects randomized to receive either efavirenz or unboosted atazanavir with a zidovudine-lamivudine backbone showed similar effects of efavirenz and atazanavir on total and regional fat (**JG Jemsek et al**, Clin Infect Dis 2006). With this in mind, results from the ACTG 5142 study are more surprising: 32% of patients receiving two NRTIs plus efavirenz developed DXA-defined lipoatrophy, compared to 18% of patients receiving 2NRTIs plus lopinavir/r, and 8% for patients only efavirenz and lopinavir/r (**RH Haubrich et al**, AIDS 2009). It is not known if this greater incidence of fat loss in patients who received efavirenz represents lipoatrophy from efavirenz or adipocyte growth

from boosted lopinavir. Supporting the latter possibility, another study found that ritonavir-boosted atazanavir was associated with less lipodystrophy over 2 years relative to those receiving unboosted atazanavir (**GA McComsey et al**, Clin Infect Dis 2009). Low dose ritonavir may, therefore, stimulate adipocyte growth as has been found *in vitro* (**A Nguyen et al**, AIDS 2000). The 8% rate for tenofovir-lamivudine was similar to that of the lopinavir/r-efavirenz group, suggesting that tenofovir-lamivudine causes little if any lipodystrophy. Increasing limb fat mass (without an increase in visceral fat or insulin resistance) has also been observed in other recent studies that evaluated boosted fosamprenavir (**CB Hicks et al**, AIDS Res Hum Retroviruses 2009) or boosted tipranavir with tenofovir and lamivudine (**A Carr et al**, AIDS 2008).

2.1.3 Metabolic abnormalities in the lipodystrophy syndrome

Lipodystrophy is associated with atherogenic lipid abnormalities, low levels of HDL cholesterol, insulin resistance, and, less commonly, hyperglycaemia. Early after the introduction of cART, lipid disturbances affected nearly 60% of patients on treatment (**G Behrens et al**, AIDS 1999). The Data Collection of Adverse Events of Anti-HIV Drugs (D:A:D study) demonstrated that the prevalence of total cholesterol plasma concentration above 6.2 mmol/L was 27% for PI recipients, 23% for NNRTI recipients, and 10% for patients on a triple NRTI regimen, as compared with 8% for untreated patients (**N Friis-Møller et al**, AIDS 2003).

Prior to the cART era, glucose intolerance and diabetes were uncommon in HIV-infected adults. In the early cART era, rates of glucose metabolism disorders (including type 2 diabetes and glucose intolerance) as high as 25% were reported in PI recipients (mainly indinavir) with lipodystrophy and dyslipidaemia (**A Carr et al**, Lancet 1999). More recent data have shown a 10% prevalence of type 2 diabetes after 4 years of follow-up, as compared with a 3% risk for HIV-uninfected adults (**TT Brown et al**, Arch Intern Med 2005). After

adjusting for body mass index and age, HIV-infected adults had a four-fold greater risk of diabetes than HIV-uninfected adults.

Most PI, with the exception of atazanavir when not ritonavir-boosted (**MA Noor et al**, AIDS 2006, **GA McComsey et al**, Clin Infect Dis 2009, **M Sension et al**, J Acquir Immune Defic Syndr. 2009), are associated with an elevation of total cholesterol, triglycerides and LDL cholesterol, an atherogenic association. Interestingly, HDL cholesterol levels increased by 20 to 30% with ART including two NRTIs and either a NNRTI or a ritonavir-boosted PI (**MA Johnson et al**, J Acquir Immune Defic Syndr 2006, **M van der Valk et al**, AIDS 2001). This increase suggests that any deleterious effect of PIs on HDL cholesterol levels (as seen in HIV-uninfected volunteers) is less important than the effect of cART to increase HDL cholesterol by suppressing HIV replication.

The NNRTI nevirapine has a more favorable impact on lipids than most protease inhibitors. A comparison of indinavir and nevirapine found a greater increase in HDL cholesterol levels in patients receiving nevirapine as compared with patients on PIs (**M Van der Valk et al**, AIDS 2001). This finding was confirmed later on by a large study comparing two NNRTIs, efavirenz and nevirapine (**F Van Leth et al**, Lancet 2004). Nevirapine was consistently associated with a tendency of increased level of HDL-C (**F Van Leth et al**, PLoS Med 2004, **M Van der Valk et al**, AIDS 2001). Efavirenz however has shown a lipid pattern very similar to that observed the PI lopinavir/r (**RH Haubrich et al**, AIDS 2009).

The contribution of the stavudine to lipid abnormalities was suggested early in the cART era (**PN Kumar et al**, HIV Med 2006). Later, a large prospective, randomized study compared two different backbones: stavudine or tenofovir, in association with efavirenz and lamivudine (**Gallant JE et al**, JAMA 2004). After 48 weeks of follow-up, patients receiving stavudine were more likely to have hypertriglyceridaemia than patients receiving tenofovir. Increased tNRTI exposure in the D:A:D cohort was a risk factor, remaining significant even after adjustment for lipodystrophy (**S de Wit et al**, Diabetes Care 2008). The strongest association in this study was use of stavudine, but zidovudine was also a risk factor; PI

exposure was not a risk. The metabolic profile of new drugs is incompletely explored as yet; studies in highly experienced patients with etravirine have shown that elevations in total cholesterol and LDL-C as well as initiation of lipid lowering therapy were more common in etravirine-treated subjects compared with those in the placebo arm (**A Lazzarin et al**, Lancet 2007, **JV Madruga et al**, Lancet 2007).

Table 3 Summary of lipid and glycaemic abnormalities of individual antiretroviral drugs

Agents	Lipid changes	Impact on glucose metabolism
<u>NRTIs</u>		
Abacavir	No change	No change
Didanosine	↑TC, ↑TG	Unclear
Emtricitabine Lamivudine	Lipid and glycaemic abnormalities of lamivudine are difficult to distinguish from those of the other antiretrovirals with which it is combined. Emtricitabine and lamivudine have the same safety profile.	
Tenofovir	No change	No change
Stavudine	↑TC, ↑↑TG	Insulin resistance acutely and secondary to lipoatrophy
Zidovudine	↑TC, ↑TG	Insulin resistance acutely and secondary to lipoatrophy
<u>NNRTIs</u>		
Efavirenz	↑ TC, TG	No change in insulin sensitivity
Etravirine	↑ TC	?
Nevirapine	↑ TC, TG (less than EFV)	No change in insulin sensitivity
<u>PIs</u>		
Ampranavir	↑ TC, TG	No change
Atazanavir	No or minimal effect	No change
Darunavir/r	↑ TC, (TG?)	No data
Indinavir	↑ TC, TG	↑↑ insulin resistance
Lopinavir/r	↑ TC, ↑↑ TG	↑ insulin resistance acutely, but not with chronic cART*
Ritonavir	↑ TC, ↑↑ TG	↑ insulin resistance acutely

Saquinavir	No or minimal effect	No change
Tipranavir	↑ TC, TG	No change
<u>CCR5 inhibitor</u>		
Maraviroc	No change**	No data
<u>Integrase inhibitor</u>		
Raltegravir	No change***	No change
<u>Entry inhibitor</u>		
Enfuvirtide	No change****	No change

Abbreviations; TC: total cholesterol; HDL: high-density lipoprotein, LDL: low-density lipoprotein; TG: triglycerides

*in healthy volunteers (**MA Noor et al**, AIDS 2006); data not supported in HIV-infected individuals **

From MERIT substudy, **E de Jesus et al**, CROI 2008 *** From SWITCHMRK 1 and 2, **JJ Eron et al**, CROI 2009 **** From TORO trial, **A Lazzarin et al**, New Engl J Med 2003

Classical risk factors (abdominal obesity, physical inactivity, increasing age) also contribute to the overall risk of dyslipidaemia. In a 10-year, prospective assessment of lipid levels, the Multicenter AIDS Cohort Study analyzed 50 men who acquired HIV infection. Lipid levels decreased after seroconversion, but three years after cART initiation TC and LDL-C increased significantly, whereas HDL-C level remained lower than before HIV infection. The increase in serum TC was observed in patients with a longer follow-up, suggesting that the aging population could also participate to this increase in cholesterol levels (**SA Riddler et al**, JAMA 2003).

Genotypic variations in apolipoprotein C-III also can affect the triglyceride response to cART (**M Amedo et al**, Pharmacogenet Genomic 2007; **PE Tarr et al**, Antiviral Ther, 2007).

Risk factors for diabetes include increasing age, obesity, lipoatrophy and fat accumulation, a family history of diabetes, Metabolic Syndrome and hepatitis C infection (**C Yoon et al**, Acquir Immune Defic Syndr. 2004; **MK Jain et al**, HIV Med 2007).

2.1.4 Management of lipoatrophy

2.1.4.1 Switching strategies

Switching from tNRTI therapy to abacavir, tenofovir or no NRTI for 1 or 2 years resulted in statistically significant but only modest improvements in limb fat mass (around 0.4 kg over one year) with most patients continuing to have lipoatrophy (**A Martin et al**, AIDS 2004; **M John et al**, J Acquir Immune Defic Syndr 2003, **GJ Moyle et al**, AIDS 2006). Even with ongoing increases at the same rate, lipoatrophy might take well over 5 years to resolve for many patients without additional intervention. Therefore, other interventions are warranted.

2.1.4.2 Antiretroviral cessation

Complete cART cessation has not been extensively studied. A non randomized study (**MJ Kim et al**, Antivir Ther 2007) evaluated gene expression in subcutaneous abdominal adipose tissue of 40 patients before and 6 months after the complete interruption of cART. Although no data on body composition were available, the authors did not observe any difference in subjectively reported lipoatrophy. The morphology of adipose tissue, however, was modified with less inflammation, fewer macrophages and lipogranulomata. The authors also reported an increase in mitochondrial DNA content in these patients.



The SMART trial, which randomized patients with CD4+ lymphocyte counts >350 cells/mm³ to intermittent, CD4-guided ART (stop ART >350 and start <250 cells/mm³) (drug conservation) or continuous ART (viral suppression) (**El Sadr et al**, New Engl J Med 2006), provided useful information on how treatment cessation may affect body composition on

patients with moderate immunosuppression. In a substudy, 142 and 133 patients allocated to the drug conservation and viral suppression arms, respectively, had metabolic and DXA assessment. In this mostly male (19% female), ART-experienced population, 39% had had provider-reported lipoatrophy; at 12 months, limb fat was modestly improved in the drug conservation arm when compared to the viral suppression arm (mean changes in limb fat

percent: +0.2% and -0.2%, respectively $p = 0.03$) (**F Visnegarwala et al**, HIV Clin Trials 2007). No new data on treatment cessation effect on body composition can be expected; results from the main SMART trial do not suggest that this is a viable strategy in the long term with current medications.

2.1.4.3 Medical interventions

Several medical interventions have been evaluated to treat lipodystrophy in randomized trials: thiazolinediones, uridine and pravastatin for lipoatrophy; and metformin, growth hormone and growth hormone analogues for visceral fat accumulation. Thiazolinediones are agonists of the peroxisome proliferator-activated receptor *gamma* (PPAR- γ) receptor and are insulin-sensitizing drugs used for the treatment of type 2 diabetes mellitus. The PPAR- γ receptor is strongly expressed in adipocytes and in particular subcutaneous adipocytes. PPAR- γ agonists stimulate the differentiation and growth of adipocytes and promote storage of circulating lipid. Thiazolinediones stimulate adipogenesis via effects on PPAR- γ , and troglitazone (no longer marketed because of severe liver side effects) increased subcutaneous fat in patients with congenital lipodystrophy (**E Arioglu et al**, Ann Intern Med 2000). Rosiglitazone, from the same family, was not found to improve lipoatrophy in two randomized studies over 24 weeks (**J Sutinen et al**, Antiviral Ther 2003) or 48 weeks (**A Carr et al**, Lancet 2004). In contrast, Hadigan (**C Hadigan et al**, Ann Intern Med, 2004) and Bejjani (**D El Bejjani et al**, CROI 2009) reported significant increase of leg fat with rosiglitazone, and one study found a significant increase with pioglitazone (**L Slama et al**, Antivir Ther. 2008). Overall, the data suggest a greater response in patients no longer receiving a tNRTI, which can down regulate PPAR γ expression in adipose tissue of HIV-uninfected adults (**PW Mallon et al**, J Infect Dis 2005); therefore, a thiazolinedione is unlikely to be a substitute for tNRTI cessation. Pioglitazone may be preferred as it does not appear to have the same adverse lipid effects (**A Calmy et al**, AIDS 2003).

Uridine is a pyrimidine precursor that is widely distributed in healthy adults. It is synthesized in the body and involved in a number of biochemical reactions, such as UMP and UDP formation, and protein glycosylation. It has been hypothesized that supplying uridine as an

exogenous source of pyrimidine precursors could attenuate NRTI toxicity. *In vitro*, uridine abrogates the mitochondrial toxicity to adipocytes and hepatocytes of stavudine and zidovudine, but not of didanosine (UA Walker et al, Antiviral Ther 2006). Uridine (36g [1 sachet] tid for 10 consecutive days every month for 12 weeks) was assessed in lipoatrophic, HIV-infected adults receiving a tNRTI. There was a significant and substantial increase in limb fat with uridine relative to placebo (between-group difference 0.92 kg; $p < 0.005$). No toxicity was observed, including for HIV viral load, except for bitter taste that lead to early withdrawal in one patient (J Sutinen et al, Antiviral Ther 2007). A second study of 16 lipoatrophic adults receiving stavudine found the same dose of uridine over 32 weeks was safe and associated with subjective improvement in lipoatrophy and a non-significant increase in adipose mitochondrial DNA content (G McComsey et al, Eur J Clin Nutr 2008). The question remains as to whether uridine may have any positive effect in patients no longer receiving a tNRTI. We tested the hypothesis that uridine would increase limb fat in patients not receiving a tNRTI in the randomized trial presented in Appendix 1 (Appendix, A Calmy, M Bloch, et al. No significant effect of uridine or pravastatin for HIV lipoatrophy in men who have ceased thymidine nucleoside analogue therapy: a randomized trial. HIV Med 2009, in press, publication # 8).

Another potential new candidate for treatment of fat atrophy is pravastatin. Pravastatin is a HMG-CoA reductase inhibitor ('statin'). Pravastatin produces its lipid-lowering effects in two ways. First, as a consequence of its reversible inhibition of HMG-CoA reductase activity, it effects modest reductions in intracellular pools of cholesterol. This results in an increase in the number of LDL receptors on cell surfaces and enhanced receptor-mediated catabolism and clearance of circulating LDL. Pravastatin also inhibits LDL production by inhibiting hepatic synthesis of VLDL, the LDL precursor. Mallon and al. conducted a 12-week, placebo-controlled study of pravastatin 40 mg nocte in HIV-infected male PI recipients with fasting total cholesterol greater than 6.5 mmol/l, almost all of whom had previously ceased stavudine and zidovudine for lipoatrophy. They found that pravastatin increased subcutaneous fat, both limb fat on DXA and SAT on CT (PW Mallon et al, AIDS 2006). This

positive effect was unlikely to represent a false-positive outcome as subcutaneous fat mass increased in two regions using two different imaging techniques. The mechanism of this effect, however, is unknown; in particular, there was no inverse correlation between increase in subcutaneous fat mass and reduction in total cholesterol with pravastatin.

We have attached in the appendix 1 the results of our randomized trial in which both uridine and pravastatin were assessed for their effects on lipoatrophy ([Appendix, A Calmy, M Bloch et al, No significant effect of uridine or pravastatin for HIV lipoatrophy in men who have ceased thymidine nucleoside analogue therapy: a randomized trial, HIV Med, in press, publication # 8](#)).

2.1.4.4 Surgery

In the absence of proven therapy for lipoatrophy, surgical correction, particularly in the face, remains an option, although expensive. Various reconstructive procedures have been used to improve facial lipoatrophy. Bioabsorbable fillers such as polylactic acid have been successful ([DL Carey et al, J Acquir Immune Defic Syndr. 2007](#), [DL Carey and al, HIV Med 2009](#)), but need be repeated over time. Permanent fillers are durable, but may be difficult or impossible to remove if complications occur ([DH Jones et al, Dermatol Surg 2004](#)). In many countries, surgical repair of lipoatrophy is not reimbursed.

Figure 5 Effect of facial cosmetic surgery in a lipoatrophic patient



2.1.5 Management of lipohypertrophy

Lifestyle interventions including a low lipid and caloric-restrictive diet and aerobic exercise are usually recommended to patients with lipodystrophy. The impact of these interventions on lipoatrophy and fat accumulation are not well known and controversial; one might reasonably hypothesize that both a restrictive diet and aerobic exercise could reduce peripheral fat as well as reduce central fat. A randomized trial on lipoatrophic adults with metabolic syndrome showed that an intensive intervention 3 days per weeks for 6 months may reduce central fat accumulation, but may also aggravate peripheral fat atrophy (**KV Fitch et al**, AIDS 2006). An older study, however, showed that sedentarity was a risk factor for both lipoatrophy and lipodystrophy (**P Domingo et al**, Antiviral Ther 2003).

Given the role of insulin resistance in the HIV lipodystrophy, researchers have evaluated the role of insulin-sensitizing drugs as treatment options. Metformin reduced visceral fat and insulin resistance among HIV-infected adults, but also reduced subcutaneous fat (**T St Marc et al**, AIDS 1999; **C Hadigan et al**, JAMA 2000). A 16-week randomized trial found that metformin (up to 1000 mg twice daily) improved insulin sensitivity without significant change in body composition over 16 weeks (**K Mulligan et al**, AIDS 2007). Unfortunately, side

effects were prevalent, with nearly half the recipients requiring dose modification or permanent discontinuation of metformin.

Growth hormone and growth hormone analogues have also been tested to treat visceral fat accumulation. Although high-dose growth hormone significantly improved abdominal adiposity, it had significant, dose-dependent, side effects. Visceral fat was also reduced by 15.2% in lipodystrophic adults randomized to receive the growth hormone-releasing hormone analogue, tesamorelin (2 mg daily for 26 weeks) (J Falutz et al, New Engl J Med 2007). Tesamorelin appeared to be much better tolerated than growth hormone and did not substantially reduce subcutaneous fat mass, but is not yet approved for this indication and is not available commercially.

Table 3 provides a summary of assessed interventions for both lipoatrophy and lipohypertrophy

Table 4 Interventions for HIV lipoatrophy and central fat accumulation

<i>Intervention</i>	<i>Lipoatrophy</i>	<i>Central fat accumulation</i>	<i>Comments</i>
Diet and aerobic exercise	Worsens	Improves	• Reduce systolic blood pressure and weight in adults with metabolic syndrome, but do not alter lipid levels • Diet must not affect meals necessary for absorption of antiretroviral therapy
Thymidine nucleoside analogue switch	Improves (about 0.4kg limb fat at 12 months)	No change	• High risk of virological failure without substitution • No effect on lipids or insulin resistance demonstrated • No excess risk of virological failure with abacavir or tenofovir substitution
Protease inhibitor switch	No change	Reduction in one study	• Inception studies show more lipoatrophy with nelfinavir and less lipoatrophy with ritonavir-boosted lopinavir/r, both relative to efavirenz

Intervention	Lipoatrophy	Central fat accumulation	Comments
Uridine	Improved (+0.7kg limb fat at 12 weeks)	Increased	• Hyperlipidaemia improves • Variable effects on insulin resistance • No change in HDL cholesterol • HDL cholesterol decreased • Unlicensed “dietary” supplement • Expensive – about US\$300 per month
Statin	Improved with pravastatin (+0.5kg limb fat at 12 weeks)	No change	• Total and LDL cholesterol fall by about 25% • No change in insulin resistance or triglycerides • Pravastatin preferred as cholesterol-lowering agent, as has no significant cytochrome P450-mediated interaction with antiretroviral therapy • Effects of other statins on lipoatrophy are unknown
Thiazolinediones	Possibly improves with pioglitazone	No change	• Improves insulin resistance • Rosiglitazone increased triglycerides and LDL cholesterol • Reduces liver fat
Metformin	Worsens	Slight improvement	• Improves insulin resistance, blood pressure and possibly hypertriglyceridaemia
Growth hormone	Worsens	Improves	• Overall reduction in VAT:SAT ratio • No effect on triglyceride levels • Improves total and LDL cholesterol • Transient deterioration in insulin resistance • Risks of fluid retention, arthralgias • Maintenance therapy required to sustain effect on

<i>Intervention</i>	<i>Lipoatrophy</i>	<i>Central fat accumulation</i>	<i>Comments</i>
Tesamorelin (Growth hormone-releasing hormone analogue)	Worsens	Improves (reduced VAT by 20%)	intra-abdominal fat • Overall reduction in VAT:SAT ratio • Minimal safety risk with achievement of physiologic GH levels • Modest increase in HDL cholesterol • Need for maintenance therapy not known

2.2 Cardiovascular diseases: is HAART compatible with HEART?

2.2.1 Background

Cardiovascular disease is the number one cause of mortality in the general population. There is an increased rate of coronary heart disease (CHD) in HIV-infected individuals. CHD has become the fourth cause of morbidity and mortality in this population (**C Lewden et al**, J Acquir Immune Defic Syndr 2008). Most data come from retrospective analysis of cohorts (**M Mary-Krause et al**, AIDS 2003), administrative databases (**SA Bozette et al**, N Engl J Med 2003; **D Klein et al**, J Acquir Immun Defic Syndr 2002; **VA Triant et al**, J Clin Endocrinol Metab 2007; **JS Currier et al**, J Acquir Immun Defic 2003) or prospective HIV cohorts (**N Friis-Møller et al**, New Engl J Med 2003; **N Friis-Møller et al**, N Engl J Med 2007).

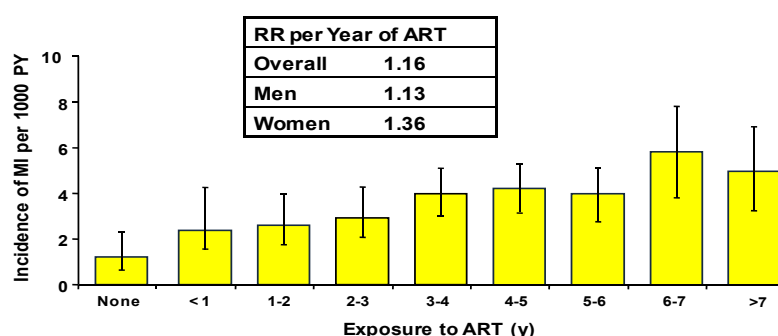
Table 5 Rate of myocardial infarction in cohort studies

<i>Studies</i>	<i>Number of patients/number of events</i>	<i>Rate of MI among HIV+ patients / 1000 yr of follow-up</i>	<i>Endpoint</i>	<i>PI effect detected (yes/no)</i>
N Friis-Møller, 2007	23,437/345	3.6	MI	Yes
M Mary-Krause, 2003	34,976/66	-	MI	Yes
SA Bozette, 2003	36,766/1207	8.1	CVD	No
J Currier, 2003	28,513/1360	4.1	CVD	Not stated (?)
D Klein, 2002	5,000/162	4.3	CVD	Yes
VA Triant, 2009	13,851/189	11.3	CVD	Not stated (?)

The strongest epidemiological evidence relating the risk of cardiovascular disease with

the duration of combination cART comes from the Data Collection on Adverse Events of Anti-HIV Drug (D:A:D) study. The D:A:D study is the largest, prospective, multinational, observational study of cardiovascular disease in HIV infection and was specifically designed to capture a signal for an increase risk of myocardial infarction. Eleven cohorts worldwide are participating, with a total current enrolment of more than 35,000 patients from 188 clinics in 21 countries in Europe, USA and Australia; 345 (0.9%) developed their first myocardial infarction (MI) during follow-up, of which 28% were fatal; overall incidence of MI was 3.5 per 1000 person-years. The incidence of myocardial infarction increased with longer exposure to cART (RR [relative risk] 1.17, $p < 0.0001$] per year of exposure (Figure 6). This increased risk with cART exposure duration was similar across gender and age.

Figure 6 Incidence of MI per year of exposure to ART



** Slide adapted and provided by M Battegay (reproduced with permission)*

It is important to state that in all of the above studies that classical risk factors were also strong predictors of myocardial infarction. For example, in the D:A:D study, increased age (per year older) was associated with a 6% increased risk, male sex with a 110% increase, diabetes with a 90% increase, smoking with a 290% increase and hypertension with a 80% increase risk of MI. The size of each of these risks is very comparable to what is known for HIV non-infected individuals (**J Currier et al**, Circulation 2008).

The combined contribution of classical risk factors, most of them modifiable, is probably greater than for cART. Overall, for patients with little or no cardiovascular risk factors, the absolute risk of developing a myocardial infarction, although increasing with cART exposure, remains relatively low.

The SMART study was designed to assess the hypothesis that intermittent cART would be associated with a lower rate of cardiovascular events, which had largely been associated with the deleterious metabolic effects of cART. The study was stopped earlier than expected when an interim analysis found that, paradoxically, intermittent, CD4+ lymphocyte count-guided cART was associated with a 50% greater risk of cardiovascular events relative to continuous cART (although the incidence of symptomatic myocardial infarction did not differ between groups). These results have raised several questions relative to the understanding of this unexpected phenomenon.

2.2.2 Pathogenesis

Both HIV and the use of cART have an impact on the heart and vasculature in HIV-infected adults. HIV can directly infect myocytes, although replication rate is low (**W Lewis et al**, Prog Cardiovasc Dis 2000). This may result in left ventricular dysfunction and dilated cardiomyopathy, a disease that was well recognized before the cART era. HIV can also directly infect vascular smooth muscle and endothelial cells and cause endothelial dysfunction through several mechanisms (**M Dubé et al**, Circulation 2008). HIV Tat protein, in synergy with TNF- α , can bind to endothelial integrin and induce endothelial proliferation, expression of intercellular adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin), so increasing the migration of monocytes into endothelium. Also, persistent inflammation and immune activation can increase oxidative stress and contribute to endothelial dysfunction. Recently, another hypothesis that could at least partly explain SMART findings was suggested; the expression of HIV nef, a protein that enhances HIV

replication and infectivity, inhibits ABCA1-dependent cholesterol efflux from macrophages and thus preventing apoA-I lipidation; this could be a contributing factor to the low HDL cholesterol levels of untreated HIV infection (**Z Mujawar et al**, PLoS Biol 2006).

2.2.3 cART-related risks factors in HIV-infected individuals

Cardiovascular complications of HIV infection such as cardiomyopathy or pericarditis have been reduced by cART. Many drugs used in cART are, however, associated with the development of metabolic complications such as dyslipidaemia or impaired glucose metabolism, which are well known risk factors for ischaemic, cardiovascular disease.

2.2.3.1 Use of protease inhibitors

The increased risk for cardiovascular events demonstrated in D:A:D with cART is primarily driven by exposure to protease inhibitors (RR 1.16, 95% confidence interval, 1.10 to 1.23) (**N Friis-Møller et al**, New Engl J Med 2007). The association with PI exposure is only partially explained by lipid levels, suggesting that protease inhibitors may induce ischemic heart disease through at least one other mechanism. The association between the use of PIs and increased cardiovascular risk was not consistently found in other studies; a retrospective analysis of cardiovascular and cerebrovascular disease among 36,766 patients who received care for HIV infection at Veterans Affairs facilities in the United States between January 1993 and June 2001 found that newer therapies for HIV were associated with significant mortality benefits, and these benefits were not diminished by any increase in the rate of cardiovascular or cerebrovascular events or related mortality (**N Bozette et al**, N Engl J Med 2003). The endpoints in this study, however, were not validated.

2.2.3.2 Use of nucleoside analogues

The role of other drug classes was recently revised in another D:A:D analysis. Surprisingly, the D:A:D investigators found that neither zidovudine nor stavudine, both of which cause dyslipidaemia and insulin resistance, was associated with myocardial infarction or stroke.

However, recent and ongoing abacavir or didanosine therapy was each independently associated with an increased risk of MI, by 90% and 46%, respectively. The 90% excess risk associated with abacavir appears high compared to the 16% annual excess risk associated with protease inhibitor therapy. Additional analysis confirmed that use of tenofovir was not associated with an increased risk of myocardial infarction. The original findings of an increased risk of cardiovascular events when using abacavir were recently confirmed by other groups. A case-control study nested within the French Hospital Database compared the 289 HIV-infected patients in the database who experienced a confirmed first myocardial infarction between January 2000 and December 2006 with 884 controls of the same sex and similar age (**S Lang et al**, CROI 2009). Patients with recent abacavir exposure had twice the risk of a myocardial infarction than unexposed individuals (OR 1.97), but only if abacavir had been initiated in the previous 12 months.

Randomized studies including abacavir were less consistent in showing an increased risk of MI when exposed to abacavir. The STEAL study, for example, reported the possibility of a higher number of heart problems among patients using abacavir and lamivudine compared with emtricitabine and tenofovir (**Martin A et al**, Clin Infect Dis 2009); the AIDS Clinical Trials Group ALLRT study (ACTG A5001) included 3205 patients who initiated first-line cART in five randomized trials; among them, 781 commenced a regimen containing abacavir. Sixty-three severe, cardiovascular events and 27 myocardial infarctions were reported. In this study of cART-naïve patients, no significant association between recent abacavir use and myocardial infarction was observed (**C Benson et al**, CROI 2009). Abacavir is not known to have any other long-term toxicity, so the mechanism by which abacavir might cause CVD is unknown. A possible explanation might be platelet hyperreactivity (**C Satchell et al**, CROI 2009) to various clot-stimulating agents (adenosine diphosphate, epinephrine, and collagen). To assess whether abacavir was associated with impaired endothelial function, Hsue and colleagues measured endothelial function by measuring flow-mediated dilation of the brachial artery in 61 antiretroviral-treated patients (49% with abacavir) who had undetectable

plasma HIV RNA levels (**P Hsue et al**, AIDS 2009). They found that, even after adjustment for traditional and HIV-specific risk factors, current abacavir use was independently associated with lower endothelial function.

2.2.3.3 Risk factors related to lifestyle

Patients infected with HIV are more likely to have a lifestyle associated with an increased cardiac risk, in particular a high prevalence of smoking. Reports suggest that 50-70% of individuals living with HIV are current smokers compared with 21.6% in the general population in the United States (**R Niaura et al**, Clin Infect Dis 2000). Smoking is also the most prevalent risk factor in HIV-infected adults in the Swiss HIV Cohort Study (57%), followed by low HDL cholesterol (37%), high triglycerides (36%) and hypertension (26%) (**TR Glass et al**, HIV Med 2006). It is vital that behaviorally modifiable risk factors such as smoking also be targeted for intervention.

Some recent studies suggested that HIV-infected patients may be receptive to smoking cessation treatment. A recent trial including 95 participants who received a cellular telephone intervention showed that patients receiving an intervention via cellular telephone in addition to the usual care components were 3.6 times more likely to quit smoking compared with participants who received usual care (**DJ Vidrine et al**, AIDS 2006). A prospective, pilot study in Switzerland of counseling and nicotine replacement therapy yielded similar encouraging results (**L Elzi et al**, Antivir Ther 2006). Smoking cessation programs should therefore be encouraged – and smoking cessation interventions should take into account and treat symptoms of depression and co-dependency in addition to nicotine dependence and motivation, in order to maximize their chances of success (**A Bénard et al**, AIDS Patient Care STD 2007).

2.2.4 The Metabolic Syndrome

In 2001, the US National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) guidelines introduced the Metabolic Syndrome as a risk factor additional to elevated

low-density lipoprotein (LDL) cholesterol. The Metabolic Syndrome is a constellation of risk factors of metabolic origin that are accompanied by increased risk for cardiovascular disease and type 2 diabetes. The two major underlying components of the Metabolic Syndrome are obesity and insulin resistance; risk associated with obesity is best identified by increased waist circumference (abdominal obesity). Many patients will develop type 2 diabetes, which further increases risk for cardiovascular disease. The role of antiretroviral drugs in the pathogenesis of Metabolic Syndrome is unclear; in a cohort of 1218 patients followed for a median of 27 months, Metabolic Syndrome was found in 20% (a rate of 7.5 cases per 100 patient years). Patients exposed to stavudine were more likely to develop Metabolic Syndrome, whereas atazanavir was found to be protective (**J Young et al**, J Clin Epidemiol 2009).

Metabolic syndrome has been associated with the use of antiretroviral therapy. cART-related lipodystrophy is particularly associated with Metabolic Syndrome (K Samaras et al, Diabetes Care 2007. The clustering of the lipodystrophy syndrome-associated metabolic abnormalities have striking similarities with the MS. We demonstrated in our paper ([appendix, publication # 5, H Wand, A Calmy et al; Three year incidence of Metabolic Syndrome and type 2 diabetes mellitus after initiation of antiretroviral therapy in HIV-infected individuals, AIDS 2007; 21:2445-53](#)) that the presence of Metabolic Syndrome increases the potential risk of developing both cardiovascular disease and diabetes in a cohort of previously untreated patients initiating antiretroviral therapy.

2.2.5 HIV RNA replication: a new cardiovascular risk factor?

The link between cART and an increased risk of cardiovascular events demonstrated in most epidemiologic studies has generally been attributed to adverse effects of therapy on lipid and glucose metabolism. Other studies, however, suggest that cART actually decreases cardiovascular events. A large, randomized clinical trial including 5,472 HIV-infected patients, known as Strategies for Management of Anti-Retroviral Therapy, or SMART,

demonstrated that people receiving intermittent cART had a 50% increased risk of cardiovascular disease compared to those receiving continuous cART, with this increased risk diminishing after continuous cART was resumed (**W El Sadr et al**, N Engl J Med 2006; **W El Sadr et al**, Ann Intern Med 2008). These results were surprising as it was expected that the intermittent arm of SMART trial would, by virtue of less drug exposure, have less cardiovascular disease than patients exposed to continuous cART. It has been suggested a direct, pro-inflammatory effect of HIV independent of CD4+ lymphocyte count could explain this paradoxical finding. In the SMART trial, a pre-planned substudy regularly measured four inflammatory biomarkers: high sensitivity C-reactive protein (hsCRP), interleukin (IL)-6, amyloid A, and amyloid P, and two coagulation markers: D-dimer and prothrombin fragment 1+2. In an analysis of a random 250 SMART participants from each arm after one month on study, the researchers found that individuals who interrupted cART showed significant increases in IL-6 and D-Dimer levels than controls on continuous cART, and that the magnitude of these increases correlated with the degree of viral load increase after cART interruption.

These data from a randomized trial are supported through the large database of the Boston Hospital information system. Triant et al (**VA Triant et al**, J Acquir Immune Defic Syndr. 2009) tested the association of elevated CRP (defined by the upper range of the available assay) and HIV with myocardial infarction. After adjustment for demographic data, hypertension, diabetes, and dyslipidaemia, the HIV-infected patients with elevated CRP had a markedly increased relative risk of myocardial infarction.

We have attached in the appendix 1 our own analysis of key inflammatory markers in a large cART interruption trial (**Appendix, publication # 6. A Calmy, A Gayet-Ageron et al. HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial, AIDS 2009;23:929-39**). We designed a post-hoc analysis of a subgroup of participants in the STACCATO trial who had no prior cART exposure at entry, and measured plasma levels of soluble mediators associated with cardiovascular risk before cART initiation, on cART when plasma HIV RNA was undetectable, and then in the randomized phase of the

trial when patients either continued cART or suspended cART, in order to test if and how the values of the different cardiovascular markers correlated with HIV replication.

The realization that uncontrolled viraemia contributes to cardiovascular disease has major implications in the management of chronic HIV infection. Most importantly, it suggests that, despite the evidence that cART may be responsible for dyslipidaemia and glucose intolerance, stopping or interrupting cART may even be more deleterious for cardiovascular disease.

2.2.6 Integrating risk factors in cardiovascular risk estimates:

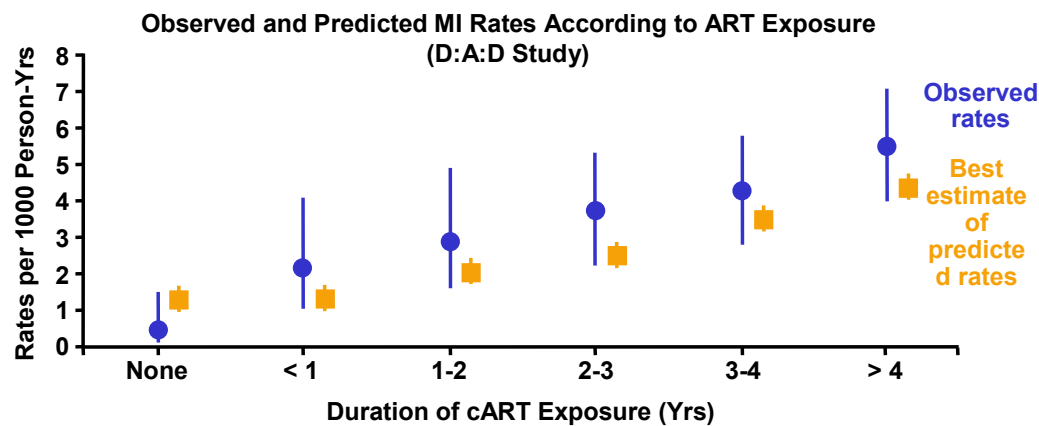
The Framingham score

In the general population, the Framingham equation is largely used in clinical practice to estimate the risk for developing a cardiovascular event over a given period. In Switzerland, data suggest that around 2% of HIV-individuals included in the Swiss HIV cohort study have a high risk (more than 20% risk of a myocardial infarction at 10 years) and 13% a moderate risk (between 10 and 20%) (**TR Glass et al**, HIV Med 2006).

Is cardiovascular risk in HIV disease reasonably estimated using the Framingham equation?

Analysis of the D:A:D study data suggest that the Framingham equation is a reasonable tool, but probably underestimates somewhat the risk (**M Law et al**, HIV Med 2006). This finding suggests that this equation can also be used to estimate change in risk with various interventions. Figure 7 represents the observed rates (the top bar) of myocardial infarction in D:A:D study, compared with the estimates of predicted rates (the below bar) using the Framingham equation.

Figure 7 Observed and predicted myocardial infarction rates according to duration of antiretroviral exposure



Reproduced from HIV Med. 2006 (reproduced with permission, M Law et al, HIV Med. 2006)

Asymptomatic myocardial ischemia was detected in about 10% of a large cohort of adults with no history of cardiovascular disease (**A Carr et al**, AIDS 2008), emphasizing the need for universal cardiovascular risk assessment of HIV-infected adults prior to the initiation of cART and then perhaps every year or so thereafter.

2.2.7 Prevention and management: how to reduce cardiovascular risk patients with HIV infection?

The above data collectively suggest that cardiovascular risk will best be addressed by suppressing HIV RNA with antiretroviral drugs that cause the least amount of metabolic disturbance.

As abacavir and didanosine have been associated, in a lipid-independent manner, with an increased risk of myocardial infarction (**CA Sabin et al**, Lancet 2008); it may be prudent to avoid both drugs in patients with high underlying cardiovascular disease risk if suitable alternative regimens are available. If not, patients' absolute cardiovascular disease risk in the presence of abacavir may require more aggressive management of traditional cardiovascular risk factors, with a particular emphasis on smoking cessation. Of note,

recently, most cohorts have shown a better management of cardiovascular risks factors in HIV-infected patients than in the general population and, perhaps as a result, the incidence of cardiovascular disease has been stable or even decreasing over the recent years (**TR Glass et al**, HIV Med 2006).

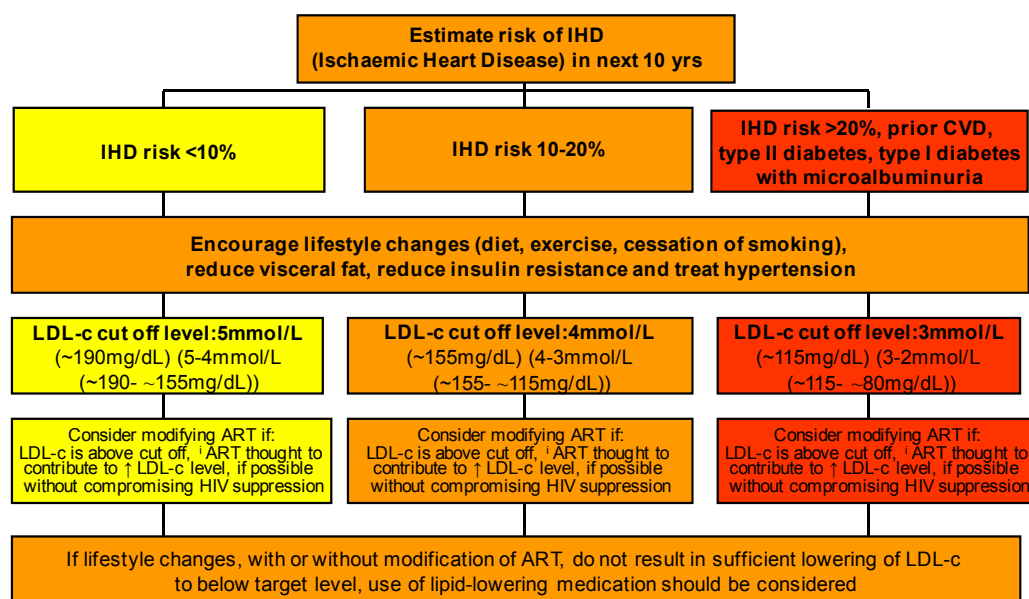
Lifestyle factors to address in those with significant total risk include smoking cessation, the control of blood pressure, diabetes, and appropriate exercise and diet. In general, elevated total cholesterol is addressed first by changes to cART or by the initiation of a statin that unlikely to interact with cART; pravastatin is most used as its metabolism is unaffected by cART, although its effects may be less than in HIV-uninfected adults (**E Martinez et al**, Curr Opin HIV AIDS 2008). Plasma levels of more potent statins such as rosuvastatin and atorvastatin can be increased by protease inhibitors, increasing risk of statin toxicity so these statins should be initiated at lower-than-normal doses. Ezetimibe also is effective at lowering LDL cholesterol but data on its use for HIV-infected individuals are limited (**J Stebbing et al**, J Antimicrob Chemother. 2009, **E Martinez et al**, Curr Opin HIV AIDS 2008). Fibrates are probably more appropriate for those with elevated triglycerides or low levels of HDL cholesterol. The best approach to a low HDL cholesterol level would either be control of HIV-replication, initiation of a fibrate, or drugs that allowed for improvement of HIV lipodystrophy, which is strongly linked to low levels of HDL cholesterol. Of note, all these intervention are not equivalent in terms of preventing cardiovascular disease. A systematic review of randomized, controlled trials published up to June 2003, comparing any lipid-lowering intervention with placebo or usual diet with respect to mortality. In this review, statin therapy was a more potent cholesterol-lowering intervention than fibrate therapy (**M Studer et al**, Arch Intern Med 2005).

The other efficient option to treat dyslipidaemia is switching boosted protease inhibitors, efavirenz and/or some nucleoside analogues to other virologically-active, more “lipid-neutral” cART. As cART is generally permanent, switching is an attractive option as it avoids permanently treating drug toxicity with another drug.

Metformin improves insulin sensitivity and systolic blood pressure but is associated with adverse reactions that may limit its use in patients on cART.

Several algorithms have been suggested to improve the management of cardiovascular risk factors in HIV-infected individuals. The following figure illustrates the central role of a comprehensive assessment of global cardiovascular risk for a given individual. Lipid-lowering interventions are only provided once lifestyle intervention, and cART switch, if applicable, have been tried.

Figure 8 EACS (European) guidelines for the management of cardiovascular risk



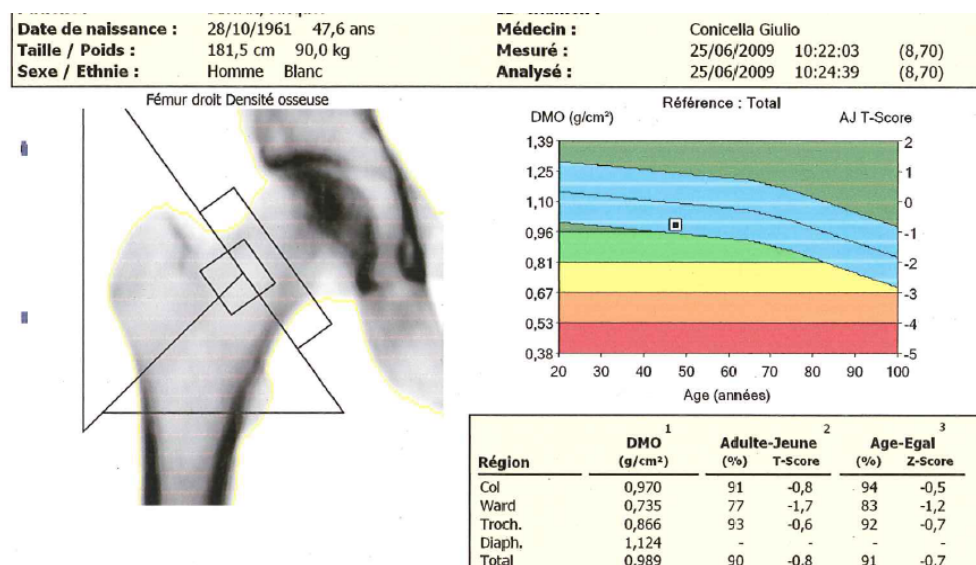
Accessed from <http://www.eacs.eu/guide/index.htm>

2.3 Low bone mineral density

Osteopenia, osteoporosis and osteonecrosis have been reported in patients infected with HIV; their etiologies remain unclear. Alterations in bone mineral density (BMD) result from the balance between osteolytic activities of osteoclasts and regenerative activities of osteoblasts. Reductions in BMD directly correlate with the risk of bone fractures. For every standard deviation reduction in vertebral BMD, for example, there is a 2-fold increased risk of vertebral fracture (**PJ Meunier**, Clin Ther 1999).

DXA is the reference method to measure BMD, which is reported as a T-score, which is the difference in standard deviations between the measured BMD and the mean values of young adults of the same sex and ethnicity. The World Health Organization defines osteoporosis on the basis on the T-score (T score below -2.5 defines osteoporosis, T score below -1 defines osteopenia) (**Consensus development conference**, Am J Med 1993). Figure 10 shows the DXA scan report of a patient with a request for bone DXA, Geneva, Switzerland (Lunar Prodigy).

Figure 9 A bone DXA report – Bone mineral density of the right trochanter



2.3.1 Description and prevalence

Symptomatic osteonecrosis affected 0.1 to 1.3% of HIV-infected patients, with asymptomatic osteonecrosis (detected with magnetic resonance imaging) in 4% (**CG Morse et al**, Clin Infect Dis 2007). Eighty-five percent of cases involved one or both femoral heads. Controlled epidemiological studies do not support a direct link with cART, and around 33% of HIV-infected individuals with osteonecrosis have traditional risk factors such as use of corticosteroids, alcohol abuse or use of megestrol acetate.

A systematic review of 12 cross-sectional studies found that HIV-infected adults had a 6.4-fold increased odds ratio of reduction in BMD and a 3.7-fold increased odds ratio of osteoporosis compared with HIV-uninfected controls (**TT Brown et al**, AIDS 2006). This suggests that low BMD is one of the most frequent metabolic complications associated with HIV and cART. “Classic” risk factors such as low body mass index, history of weight loss, corticosteroid use, and smoking, together with the duration of HIV infection, were also identified in HIV-infected adults as risk factors for low BMD (**K Mondy et al**, Clin Infect Dis 2003). Most studies, however, do not include sufficient women to draw sex-specific conclusions.

Prospective studies are less numerous but suggest that BMD declines over the first one to two years after cART initiation and then may remain relatively stable. One randomized trial compared tenofovir with stavudine (given together with lamivudine and efavirenz). At week 144, there was a greater decrease from baseline of BMD in the lumbar spine and hip in patients treated with tenofovir as compared to those with treated with stavudine (**JE Gallant et al**, JAMA 2004).

The role of cART or of particular drug classes are not firmly established, but use of boosted PIs has been associated with a greater frequency of osteopenia in a systematic review including 12 cross-sectional studies, with an odds ratio of 1.57 (1.05-2.34) for protease inhibitor recipients (**TT Brown et al**, AIDS 2006).

Table 8 reports three randomized clinical trials reporting PI use and BMD outcome in HIV-infected individuals (**C Duvivier et al**, AIDS 2009, **P Tebas et al**, 2007, and **TT Brown et al**, J Acquir Immune Defic Syndr. 2009).

Table 6 PI use and BMD outcome in randomized trials

Author	N	duration (wks)	PI	Non-PI	Criteria of judgment	PI effect on BMD (vs non PI arm)
Tebas, 2007	157	96	NFV	EFV	Total BMC	No
Brown, 2009	106	96	LPV/r	EFV	Total BMD	No
Duvivier, 2009	71	48	LPV/r, IDV/r	EFV, NVP	Hip, Spine	Spine: -4.9% v -1.5% Hip: -2.8% v -2.7%

Recent data however suggest a role for the NRTI backbone in the BMD loss experienced by HIV-infected individuals on cART. In a randomized trial comparing lopinavir/ritonavir (LPV/r) + ZDV/3TC with LPV/r + nevirapine (NVP) in 50 cART-naïve men, there was more BMD loss at 24 months in the NRTI-containing group, both at the femoral neck (-6.3% +/- 1.0% compared to -2.3% +/- 0.9%, between-group p=0.0006) and at the lumbar spine (-5.1% +/- 0.8% compared to -2.6% +/- 0.7%, between-group p=0.07) (**MG Van Vonderen et al**, AIDS 2009).

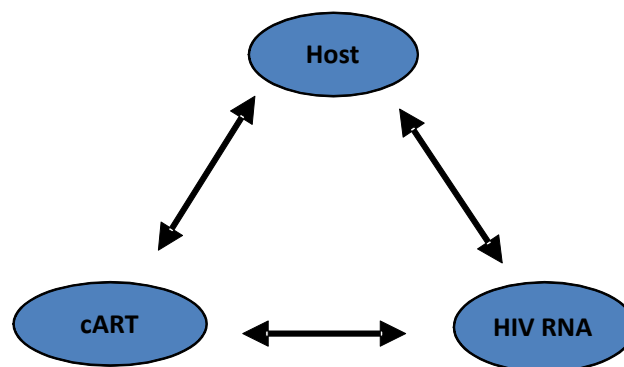
The relative importance of classic risks factors in comparison with antiretroviral drugs exposure is unclear. We aimed at determining the prevalence of low BMD, its relationship with numerous potential risk factors, including proximal renal tubular dysfunction in a cohort of HIV-infected adults receiving combined antiretroviral treatment (ART) (**A Calmy, CA Fux, et al; Low bone mineral density, renal dysfunction and fracture risk in HIV Infection: a cross-sectional study. Journal of Inf Dis. 2009: 200:1764-54, appendic, publication # 7).**

2.3.2 Pathogenesis

The relative role of cART in low BMD is unclear as reduced BMD is common in both cART-naïve and cART-treated patients. Several hypotheses have been raised to explain the possible role of HIV-related reductions in BMD: osteoclast stimulation through cytokines (TNF- α , IL-6) and decreased muscle and fat mass; PI-associated losses in BMD due to PI inhibition of 1,25-dihydroxy vitamin D₃ production (**M Cozzolino**, AIDS 2003); increased osteoclast or reduced osteoblast activity (**RG Jain**, J Biol Chem 2002, **AP Malizia**, AIDS Res Hum Retrovirus 2007); and disequilibrium in the nuclear factor kappa B ligand (RANKL)/osteoprotegerin system (**MW Wang**, J Clin Invest 2004). RANKL and osteoprotegerin are potent agonists and antagonists, respectively, of osteoclast formation and activity. Furthermore, efavirenz has been shown to alter vitamin D metabolism by inducing CYP450 (**K Gyllensten**, AIDS 2006), while tenofovir has been associated with BMD reductions, possibly through hypophosphataemic osteomalacia as a consequence of drug-related proximal renal tubulopathy (**JE Gallant**, JAMA 2004, **MJ Parsonage**, HIV Med 2005, **RI Gafni**, Pediatrics 2006, **M Essig**, J Acquir Immune Defic Syndr 2007).

In summary, BMD loss in HIV-infected individuals is likely to be a complex interplay of different factors.

Figure 10 Multifactorial origin of BMD loss in HIV-infected individuals



2.3.3 Clinical relevance and possible prevention of bone disease

Until recently, the clinical relevance with regards to an increased risk of fragility fracture of low BMD with cART, was uncertain. A large cohort study reported that HIV-infected adults had significantly higher prevalence of vertebral, hip, wrist, and combined fractures compared to non HIV-infected adults (**VA Triant et al**, J Clin Endocrinol Metab 2008). Overall, 2.87 fractures per 100 persons were noted in the HIV group compared with 1.77 in controls ($p < 0.0001$), a fracture prevalence increase of more than 60% that was independent of race and sex. Co-medications, HIV surrogate markers for disease severity as well as other potential risk factors such as trauma were, however, not available in this study based on a regional database.

A substudy from the SMART trial compared 98 participants randomized to the drug conservation arm (intermittent therapy) to 116 patients on the viral suppression arm (continuous therapy) followed up for a mean of 2.4 years; they observed that continuous ART was associated with progressive decline in BMD (differences in mean BMD change were 1.4% for hip (hip DXA; $P=0.002$) and 1.3% for spine (spine DXA; $P = 0.03$) in favor of the intermittent arm. In the parent study, 10 of 2753 participants in the continuous group and two of 2720 in the intermittent group reported serious fractures (hazard ratio 4.9; 95% CI 1.1-22.5; $P = 0.04$). In conclusion, this suggests that not only does BMD decrease when cART is continued, but that this BMD loss may be clinically significant (**B Grund et al**, AIDS 2009).

2.3.4 Management and treatment

Although the National US Osteoporosis Foundation does not recommend BMD screening for all patients with HIV, it explicitly states that post-menopausal women and men over 50 should be considered for BMD testing if the risk factor profile (hypogonadism, alcohol abuse, steroid exposure, low body weight or height loss) suggests cause for concern (**National Osteoporosis Foundation**, www.nof.org).

The World Health Organization recently developed a fracture risk assessment tool to estimate 10-year fracture risk (WHO fracture risk assessment tool, www.shef.ac.uk/FRAX/reference.htm). This algorithm has not been validated yet in HIV-positive populations, but may be a useful tool to assess the need for treatment in an individual patient.

Figure 11 Front page of the FRAX tool™ website for the calculation of the 10 years risk.

The screenshot shows the FRAX tool website interface. At the top, the URL is <http://www.shef.ac.uk/FRAX/tool.jsp?locationValue=12>. The main heading is "Calculation Tool". On the left, there are sections for "Weight Conversion" (pound to kg) and "Height Conversion" (inch to cm), each with a "convert" button. The main form area is titled "Questionnaire:" and includes a "Country" dropdown set to "France", a "Name / ID" field, and a link "About the risk factors". The questionnaire consists of 12 items:

1. Age (between 40-90 years) or Date of birth: Age: [] Date of birth: Y: [] M: [] D: []
2. Sex: ☐ Male ☐ Female
3. Weight (kg): []
4. Height (cm): []
5. Previous fracture: ☒ No ☐ Yes
6. Parent fractured hip: ☒ No ☐ Yes
7. Current smoking: ☒ No ☐ Yes
8. Glucocorticoids: ☒ No ☐ Yes
9. Rheumatoid arthritis: ☒ No ☐ Yes
10. Secondary osteoporosis: ☒ No ☐ Yes
11. Alcohol 3 more units per day: ☒ No ☐ Yes
12. Femoral neck BMD: [] [Select] []

At the bottom right of the questionnaire, there are "Clear" and "Calculate" buttons.

Preventative measures to minimize BMD loss or promote BMD increase include increase in physical exercise, sufficient ingestion of calcium and vitamin D, and elimination of risk factors such as alcohol abuse, and smoking are warranted.

The optimal treatment for low BMD in HIV-infected adults is unknown. Bisphosphonates are pyrophosphate analogues that inhibit bone resorption by binding to hydroxaapatite crystals. Several bisphosphonate have been tested in osteopenic HIV-infected adults on cART to improve BMD (**K Mondy et al**, J Acquir Immune Defic Syndr 2005). Alendronate for 48 weeks increased BMD in the lumbar spine, hip and trochanter, but not at the femoral neck

(GA McComsey et al, AIDS 2007). The third generation, long-acting bisphosphonate zoledronate also significantly increases BMD, and reduces fracture rates in postmenopausal women with osteoporosis in the general population (DM Black et al, New Engl J Med 2007). Bolland et al (MJ Bolland et al, J Clin Endocrinol Metab 2007) and Huang et al (J Huang et al, AIDS 2009) conducted randomized, placebo-controlled trials using zoledronate given once yearly (4 and 5 mg) to treat osteopenia/osteoporosis in HIV-infected patients on cART. In these studies, BMD significantly improved in zoledronate recipients as compared with minimal changes in those receiving placebo.

Results of these four trials are summarized in table 9.

Table 7 Effect of antiresorptive therapy in HIV-infected individuals

First author	Bisphosphonate	n	Duration (months)	T score baseline	Effect (primary endpoint, in % change)
Huang	zoledronate*	30	12	-1.5 (lumbar) -1.3 (hip)	• lumbar (T-score): mean +3.7% increase (controls: 0.7%) • hip (T score): mean +3.2 % increase (controls: -1.8%) • lumbar (BMD): +8.9% (versus +2.6% in placebo)
Bolland	zoledronate*	43	24	-0.5	• hip (BMD): 3.8% (versus -0.8 in placebo) • lumbar (BMD): +3.4% (versus 1.1% in placebo arm)
McComsey	alendronate**	80	48	-2.1	• hip (BMD): +4% (versus 1.3% in the placebo arm)
Mondy	Alendronate**	31	12	-1.5 (lumbar) -1.02 (hip)	• lumbar: +5.2% (BMD) versus 1.3%

* zoledronate is used 5 mg once a year, ** alendronate is used 70 mg each week

Patients included in these four randomized studies were mostly male with longstanding and well-treated HIV infection; all had undetectable plasma HIV RNA, and CD4+ lymphocyte count median above 400 cells/mm³. However, no study reported the results of overall risk fracture evaluation; inclusion criteria were T-score value that would not have warranted antiresorptive therapy in most current guidelines, unless other major risk factors for bone disease would be present or a patient had experienced a fragility fracture.

Besides these limitations, we can have confidence that antiresorptive therapy is effective. However, the question as to when this therapy is indicated in HIV-infected individuals still needs careful evaluation and validation.

This introduction chapter was largely inspired by our two published reviews (attached in Appendix, publications # 1 and 2 respectively):

A Calmy, B Hirschel, DA Cooper, A Carr. **A new era of antiretroviral drug toxicity.** *Antiviral Therapy* 2009, 14:165-79

A Calmy, B Hirschel, DA Cooper, A Carr. **Clinical update: adverse effects of antiretroviral therapy.** *Lancet* 2007; 370:12-4

3. THESIS SUMMARY

The analyses presented in this thesis confirm that the detection and management of, and the therapeutic approaches to cART-related adverse events are challenging.

The first part of this research focused on mechanisms and risk factors for developing lipodystrophy syndrome and metabolic abnormalities with the aim of identifying preventative strategies as these side-effects are known to limit cART adherence and to reduce quality of life in HIV-infected patients on an otherwise efficient, stable antiretroviral regimen.

3.1 Thesis main findings

3.1.1 Drug-sparing or class-sparing strategies in lipodystrophy

In the absence of validated treatment to cure established lipodystrophy, the most effective approach is to avoid it by limiting the use of those antiretroviral drugs that give rise to its occurrence.

Stavudine and zidovudine can induce lipoatrophy in treatment-naïve patients initiating cART (**MG Van Vonderen et al**, PLoS One, 2009; **SH Lowe et al**, HIV Clin Trial 2007). The use of NRTI-sparing regimens reduce the incidence of lipodystrophy (**RH Haubrich et al**, AIDS 2009, **MG Van Vonderen et al**, PLoS one 2009). Our multicohort study (**A Calmy, K Petoumenos; Combination antiretroviral therapy without a nucleoside analogue: Experience from 334 patients in three cohorts. HIV Med. 2007; 3:171-80, appendix, publication # 3**) demonstrated that the strategy of avoiding NRTIs, albeit virologically safe and effective, can commonly lead to sometimes severe dyslipidaemia (hypertriglyceridaemia in 63% and hypercholesterolaemia in 44% of patients over 2 years). Metabolic data of the largest (n=753) randomized, clinical trial directly comparing an NRTI-sparing regimen (lopinavir/r and efavirenz) with two NRTIs and either lopinavir/r or efavirenz were recently published (**RH Haubrich et al**, AIDS 2009) and confirmed that the use of lipid-lowering agents was

significantly greater in the NRTI-sparing arm (25% vs 11-13%) with a significantly increase in total cholesterol plasma levels. However, this study also showed that lipoatrophy (defined as a 20% or greater loss in peripheral fat mass by week 96) was less frequent in patients initiating cART with NRTI-sparing cART (9% versus 32% and 17% in the NRTI-efavirenz and NRTI-lopinavir/r arms, respectively). A NRTI-sparing strategy combining lopinavir/r with nevirapine may limit cholesterol increase, but this combined regimen has only been assessed in smaller studies. One such study (**C Duvivier et al**, J Antimicrob Chemother 2008) was stopped early because of a higher rate of virological failure in the NRTI-sparing arm. Recently data investigated 50 treatment-naïve HIV-infected men who were randomized to zidovudine/lamivudine+lopinavir/r or to NRTI-sparing therapy (nevirapine+lopinavir/r). An increase in visceral fat in the NRTI-sparing group was found with no evidence of limb fat loss; however, total and LDL cholesterol levels increased more in the nevirapine group (**MG Van Vonderen et al**, PLoS One 2009). In our collaborative cohort analysis, the relatively limited sample size did not allow us to assess differences between different types of NRTI-sparing regimens.

Boosted PI monotherapy were tested both in naïve (**JF Delfraissy et al**, AIDS 2008) and in treatment-experienced patients (**J Arribas et al**, J Acquir Immune Defic Syndr. 2009, **C Katlama et al**, IAS 2009, **J Arribas et al**, IAS 2009). Most earlier monotherapy studies involved lopinavir/ritonavir and found a higher risk of failure with monotherapy than with a standard regimen except in patients with a prolonged virological suppression prior to the start of monotherapy (**A Hill et al**, AIDS 2008); recent data on darunavir/ritonavir monotherapy have demonstrated that darunavir/ritonavir monotherapy was not inferior to darunavir/ritonavir plus two nucleosides in people who switched to one of these regimens with a viral load under 50 copies (**C Katlama et al**, IAS 2009, **J Arribas et al**, IAS 2009). Although no new or unexpected treatment-related problems arose during the trial, the trial duration was short and these data do not support the use of darunavir/r monotherapy without further validation.

In conclusion, accumulating data suggest that avoiding NRTIs is a useful strategy to prevent

lipoatrophy, but the price to pay in terms of incident dyslipidaemia may be too high for this strategy to be broadly recommended, particularly for those at significant cardiovascular risk. However, with the increasing availability of two new drug classes (integrase inhibitors and entry inhibitors), as well as of new drugs in existing classes (e.g. etravirine and darunavir), new NRTI-sparing strategies can now be assessed.

3.1.2 Switching drugs: the role of newer antiretroviral drugs

Switching strategies – for instance, from a tNRTI to abacavir or tenofovir – have already been shown to be effective in reducing the occurrence of lipoatrophy and, to a lesser extent, dyslipidaemia and should remain a key consideration for these complications (**A Martin et al**, AIDS 2004; **GJ Moyle et al**, AIDS 2006; **E Ribeira et al**, HIV Clin Trial 2008, **M Fischer et al**, J Acquir Immun Defic Syndr 2009). Furthermore, patients who switched cART when virologically suppressed were found to be more likely to achieve recommended cholesterol targets compared to those not switching cART (**TR Glass et al**, HIV Clin Trial 2007). For this strategy to be broadly applicable, however, the toxicity of the new antiretroviral drugs has to be considered: the question is not only which drug to avoid, but also what substitute should be used.

Results from clinical trials in the late 1990s suggested that lipoatrophy was most likely linked to thymidine analogues such as stavudine or zidovudine because of their known mitochondrial toxicity. One interpretation of the ACTG5142 study discussed above is that efavirenz may promote lipoatrophy (**RH Haubrich et al**, AIDS 2009). The alternative hypothesis is that lopinavir/r or ritonavir alone may increase fat mass. Recent studies exploring the use of atazanavir with or without ritonavir boosting support the latter hypothesis (**GA Mc Comsey et al**, Clin Infect Dis 2009). Nevertheless, despite some evidence of a protective effect of ritonavir in preventing the development of subcutaneous fat atrophy, the other adverse events related to the use of boosted PIs, as described in our cohort analysis, makes it difficult to suggest switching to protease inhibitor-based ART as a treatment for

lipoatrophy. Indeed, NRTI-sparing regimens were explored to address the issue of fat atrophy, but ritonavir-sparing regimens are also one way of avoiding pharmacokinetic interactions, lipid elevations and gastrointestinal intolerance.

Ritonavir-sparing regimens also are a new road for optimizing antiretroviral drug therapy. One such combination is raltegravir and atazanavir. The metabolism of raltegravir is inhibited by atazanavir through the inhibition of UDP-glucuronosyl transferase 1A1 (UGT1A1). While the licensed raltegravir dose is administered twice daily, the interaction with atazanavir makes a once daily combination of the two drugs theoretically possible (**M Neely et al**, IAS Conference 2009). Also, non-PI boosters, such as GS9350 (Gilead Sciences) or SPI 452 (Sequoia Pharmaceuticals), are being developed that may allow for potent once-daily protease inhibitor therapy with a potentially lower toxicity profile. Preliminary data from two Phase I studies involving HIV-negative volunteers reported no lipid elevations when using these alternative boosters, offering hope for ritonavir-sparing new PI-containing regimens (**S Gulnik et al**, **A Mathias et al**, CROI 2009).

New antiretroviral drugs may allow for simpler regimens and better tolerability. In our analysis ([Appendix, Nguyen A, Calmy A, et al, for the Swiss HIV Cohort Study. Lipodystrophy is not what it used to be. Data from the Swiss HIV Cohort Study, HIV Med 2008; 17:142-150, publication # 4](#)) of more than 5500 patients within a prospective cohort, we found that lipodystrophy became less prevalent when comparing patients who started treatment between 2000 and 2002 to patients who started different cART regimens after 2002. Supporting this result, the number of drug substitutions due to lipodystrophy within the cohort steadily decreased from 2003 to 2006. This suggests that the prevalence of lipodystrophy serious enough to warrant a change of treatment is decreasing. What will be the impact of newer antiretroviral drugs such as raltegravir or maraviroc? Their mechanism of action and *in vitro* data suggest they are unlikely to cause lipodystrophy. However, their use is mostly restricted to experienced patients, so it is difficult to assess their potential impact on lipodystrophy incidence.

3.1.3 Treating lipoatrophy: new approaches?

We conducted a randomized trial to assess new approaches for alleviating lipoatrophy in patients who had been on long-term, tNRTI-based cART and who had consequently developed severe lipoatrophy ([Appendix, A Calmy, M Bloch, R Norris et al. No significant effect of uridine or pravastatin for HIV lipoatrophy in men who have ceased thymidine nucleoside analogue therapy: a randomized trial. HIV Med 2009, in press, publication # 8](#)).

This trial looked at two compounds that had been found to be effective in small, pilot trials, namely uridine and pravastatin. Our negative results (lack of significant efficacy on limb fat with pravastatin or uridine) were disappointing and emphasize the need to carefully select cART regimens and screen and monitor patients for lipodystrophy. Additional data on these compounds are expected to be released soon; a Phase II/III, randomized, double-blind, placebo-controlled trial of uridine supplementation in HIV Lipoatrophy (ACTG A5229, clinical trial.gov NCT00307164) is about to be completed and will compare the use of daily uridine versus placebo in patients with lipoatrophy, but with concomitant use of zidovudine or stavudine. Another phase II study (clinical trial.gov, NCT00471614) is ongoing and is testing the effect of uridine supplementation on insulin sensitivity as measured by hyperinsulinemic, euglycemic clamp in patients with impaired insulin sensitivity on zidovudine or stavudine. The results of these trials are unlikely to change the management of lipoatrophy for the majority of patients whose treatment has already been changed to non-tNRTI-containing regimens. Also, the cost of uridine is around \$US 300 per month, a price that will prevent most patients in need from accessing this drug.

New clinical trials on lipoatrophy are now focusing on the correction of facial lipoatrophy through dermal fillers (clinical trial.gov, NCT00360932, NCT00333684, NCT00931268). Research on new molecules to combat lipoatrophy is needed and both basic and clinical research should be encouraged. With the rapid roll-out of stavudine in resource limited

settings, lipodystrophy is likely to be a “disease of the poor” with the consequence of not attracting sufficient funding to generate new research.

3.1.4 Metabolic Syndrome

Lipodystrophy is not only a potentially stigmatising cosmetic side-effect, but also includes a cluster of alterations such as low HDL cholesterol, large waist circumference and insulin resistance that are all elements of Metabolic Syndrome. Each of the individual component of Metabolic Syndrome, such as low HDL cholesterol, high triglycerides, high waist circumference, hypertension, and impaired glucose metabolism, are known risk factors for cardiovascular disease, although the extent to which the sum of these components provides additional information with regards to cardiovascular risk in the general population is under debate (R Kahn et al, Diabetes Care 2005).

In our randomized trial of treatment-naïve, HIV-infected adults initiating cART (INITIO), MS was found in a surprisingly low prevalence (8%) at baseline when compared to north American cohorts (16 to 25%) (Appendix, H Wand, A Calmy et al; Three year incidence of Metabolic Syndrome and type 2 diabetes mellitus after initiation of antiretroviral therapy in HIV-infected individuals, AIDS 2007; 21:2445-53, publication # 5).

However, Metabolic Syndrome incidence accumulated over the first three years of cART, and was found to be a risk factor for both cardiovascular disease and diabetes. The presence of Metabolic Syndrome at baseline was associated with a 2.5-fold increased incidence of cardiovascular disease of borderline statistical significance and a 3.3-fold, statistically significant increase in the risk of diabetes, while incident Metabolic Syndrome was significantly associated with both incident cardiovascular disease and diabetes. We were not able to distinguish whether certain drugs or drug classes were more prone to cause Metabolic Syndrome.

Our data differed in certain respects from those generated from the observational D:A:D cohort, in which Metabolic Syndrome at study enrolment was associated with a 2.9-fold higher risk of cardiovascular disease (95% CI 2.34-3.59). However, baseline Metabolic Syndrome no longer predicted the risk of cardiovascular disease in the multivariate analysis (adjusted relative risk 0.85; 95% CI 0.61-1.17) (**SW Worm et al**, Diabetes Care 2009).

It is unclear whether Metabolic Syndrome is a better predictor of cardiovascular disease than its individual components; it is, however, the only known predictor so far for type 2 diabetes in this high risk population. Lifestyle modification (including exercise and dietary counselling) and metformin are two strategies that are likely to improve the health status of individuals with HIV-associated Metabolic Syndrome and should be considered for HIV-infected adults with Metabolic Syndrome and possibly its components.

3.1.5 HIV and cardiovascular risk

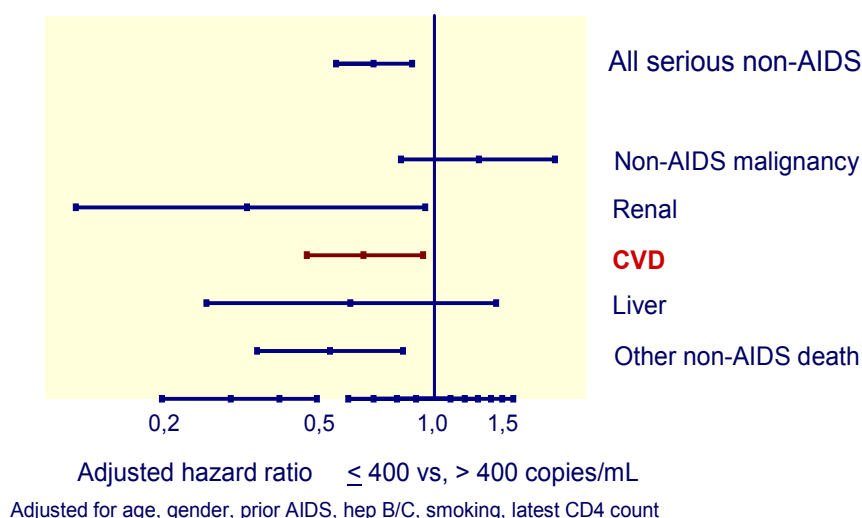
We carried out a substudy within a cART interruption trial (STACCATO trial) to explore the hypothesis that HIV RNA promoted inflammation ([Appendix, A Calmy A, A Gayet-Ageron et al. HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial. AIDS. 2009;23:929-39, publication # 6](#)). We reported various biomarker changes in 145 Thai participants (97 in the interruption arm, 48 in the continuous therapy arm) and observed a significant increase in pro-inflammatory cytokines such as CCL2 and s-VCAM-1 during cART interruption, together with decreases in the anti-inflammatory proteins interleukin-10 or adiponectin. These changes did not completely reverse when cART was restarted.

Taken together, our STACCATO findings, as well as those from the SMART study, showing that inflammatory proteins such as interleukin-6 [IL-6] and D-dimer increase in patients interrupting cART, suggest that cART interruption is associated with physiological changes -- including increased inflammation and blood coagulation -- that may increase the risk of cardiovascular events. Moreover, in the SMART study, IL-6 and D-dimer were strongly

related to all-cause mortality (**LH Kuller et al**, PLoS Med 2008). Similarly, in a large US cohort, patients with HIV and elevated C-reactive protein [CRP] had a 4-fold greater risk of death as compared with non-HIV-infected individuals with normal CRP (**VA Triant et al**, J Acquir Immune Defic Syndr 2009). Marin et al also have shown in a small French cohort that HIV RNA replication was associated with an increased risk of death from cardiovascular disease (adjusted hazard ratio: 3.86, 95% CI 1.57-9.51 for a latest HIV RNA less than 5.0 log₁₀/mL compared with a value above 5.0 log₁₀/mL) (**S Marin et al**, AIDS 2009).

The deleterious role of HIV RNA replication goes beyond the increased risk of cardiovascular disease, as viral replication influences a range of AIDS and non AIDS events: a SMART substudy found that the adjusted (including the most recent CD4+ lymphocyte count) hazard ratio for serious non-AIDS events according to the most recent HIV RNA level was 0.70 (95% CI 0.58-0.88); this association was strongest for renal, cardiovascular disease, and other non-AIDS deaths (**AN Phillips et al**, AIDS 2008) (Figure 13).

Figure 12 HIV RNA and associated risk of serious non-AIDS events: evidence from the SMART study



Provided by A Phillips (reproduced with permission)

Inflammatory changes appear to be mediated by alterations in HIV RNA levels rather than a direct or indirect effect of the drugs themselves. The switch from another PI to atazanavir in treatment-experienced patients did not result in improvement of endothelial function despite significantly improved serum lipids (**AJ Flammer et al**, Heart 2009).

Based on these consistent results, it may be prudent to monitor cardiovascular and selected metabolic biomarkers in HIV-infected patients, although the most important approach is to achieve sustained virological suppression. However, routine measurement of these markers would require prospective demonstration that particular levels of one or more markers were sufficiently sensitive and specific for predicting cardiovascular events, particularly in patients at clinically substantial risk of cardiovascular disease, and that such prediction was independent of existing cardiovascular risk prediction tools such as the Framingham equation.

3.1.6 Bone disease

The use of certain types of cART has been found to be deleterious to bone mineral density (BMD). HIV-infected adults have a 6.7-fold greater prevalence of low BMD compared to non HIV-infected adults and a 3.7-fold greater prevalence of osteoporosis (**TT Brown et al**, AIDS 2006); cART-treated patients also carry a higher prevalence of low bone mineral density compared with untreated patients. However, few studies have addressed the relative impacts of HIV, host characteristics, or cART regimens. In a cross-sectional study, we assessed biological and clinical risk factors of individuals followed-up in the ambulatory clinic of St Vincent's hospital (appendix 6). Most were patients successfully treated with cART for more than 10 years. After controlling for confounding, the use of a boosted protease inhibitor remained the variable most strongly associated with a low BMD. Vitamin D deficiency was found in about 12% of our participants, but no other classical or modifiable risk factor (except body mass index) was associated with low BMD in this survey.

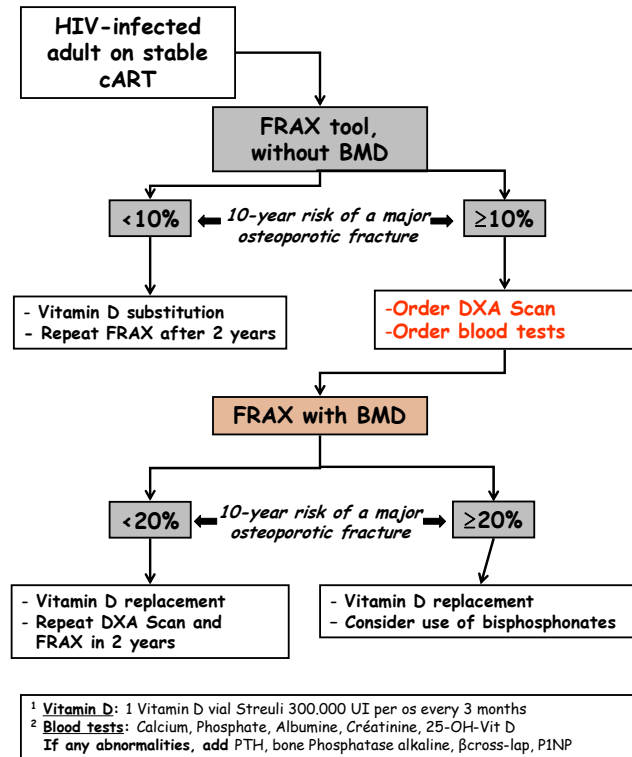
The FRAX[®] tool has been recently developed by WHO to evaluate fracture risk of patients. It is based on models that incorporate risks associated with clinical risk factors such as age, previous fracture, rheumatoid arthritis or glucocorticoid use as well as BMD at the femoral neck and is based on the same model that was used to develop the Framingham score to predict cardiovascular risk. We found that the use of FRAX tool was highly dependant on the threshold chosen to inform on antiresorptive treatment, but that even in the absence of BMD data the FRAX[®] tool provided some useful information on fracture risk.

The question of the threshold at which antiresorptive treatment should be given is still debated. American guidelines from the National Osteoporosis Foundation (www.nof.org, accessed 3rd June 2009) suggest prescribing antiresorptive therapy if the 10-year risk for hip fracture is above 3% or above 20% for any major osteoporotic fracture. This threshold should be validated in HIV-infected patients. Other guidelines recommend antiresorptive therapy taking age into account (**JA Kanis et al**, Osteoporosis Int, 2008) leading to substantial variations in the number of patients theoretically needing antiresorptive therapy

How can we translate these findings into clinical practice? An evaluation of bone fragility with the FRAX tool used without BMD should be considered in all stable HIV-infected patients on efficient cART. If this screening shows a moderate or a high probability of fracture within a 10-year period, then DXA is recommended and specific treatment might be indicated. The care provider should also be aware of risk factors for low BMD, such as premature menopause and vitamin D deficiency. One limitation, however, of a broad bone fragility screening policy is that the bone-specific treatment in young adults, especially in younger men, is not yet standardised and the long term safety and efficacy of bisphosphonates are not understood.

Figure 14 shows an algorithm to help deciding when a DXA Scan might be appropriate, and when antiresorptive treatment could be appropriate.

Figure 13 Screening algorithm for bone disease in HIV patients



In summary, this research has several clinical implications:

- **NRTI-class sparing strategies are effective at suppressing viraemia but increase the risk of ART-related dyslipidaemia.**
- **Risk factors for lipoatrophy and lipohypertrophy are different, supporting the hypothesis that peripheral fat loss and central visceral fat gain may constitute different processes, warranting different therapeutic approaches.**
- **Switching drugs away from thymidine analogues remains the most useful strategy to combat lipoatrophy as therapeutic agents such as uridine or pravastatin have so far failed to reproducibly show any efficacy.**
- **Physicians should consider screening for Metabolic Syndrome so as to predict both cardiovascular disease and type 2 diabetes in HIV-infected individuals.**
- **HIV RNA promotes inflammation and so may be a risk factor for non-AIDS disease, adding further importance to the goal of sustained viral suppression.**
- **Finally, bone mineral density merits more attention. Our studies show that vitamin D depletion is a common feature in HIV-infected adults, and validation of a prediction score such as the WHO FRAX® tool is warranted.**

4. THESIS LIMITATIONS

4.1 Choice of adverse events

This thesis could not cover the entire spectrum of anti-HIV drugs and HIV-related adverse events. The focus was on selected long-term metabolic, cardiovascular, and adipose complications associated with the use of cART. It did not examine earlier, life-threatening toxicities (e.g. abacavir hypersensitivity, lactic acidosis or Stevens-Johnson syndrome), and common, but rarely severe adverse events (e.g. gastro-intestinal intolerance, anaemia, sleep disturbance, hyperbilirubinaemia). Other end organ diseases such as renal or bone diseases were not extensively discussed in this thesis.

Finally, cART can generally lead to adverse events that are not considered as drug toxicities such as Immune Reconstitution Inflammatory Syndrome (IRIS; also known as Immune Reconstitution Disease) a pathological inflammatory response to either previously treated infection or untreated subclinical infection. IRIS is common among HIV-infected patients co-infected with *M. tuberculosis*, *M. avium* complex or *C. neoformans*. At-risk patients include those with advanced HIV disease, starting cART in close proximity to the diagnosis of an opportunistic infection, and with a very rapid decline in HIV RNA levels after the initiation of cART. The aetiology of IRIS is unknown but is presumed to occur at least in part as a consequence of cART-related immune restoration leading to a powerful immune response to microbial antigens. IRIS is relatively common, and is seen in 7-36% of patients starting cART in a context of diagnosed tuberculosis (**SD Lawn et al**, Am J Respir Crit Care Med 2008)

4.2 Methodology

Antiretroviral toxicity is not usually a primary endpoint in randomized, clinical trials and the methodology used in most of our analyses reflects this limitation. We performed cohort analyses to assess the efficacy and safety of NRTI-sparing cART. While cohort analyses have the disadvantage of not controlling for treatment selection bias (no blinding) or a priori

control of confounding (randomisation), they allows for longer follow-up periods than are general achievable (affordable) via randomised trials. We were able to analyse three cohorts in France and Australia for up to 24 months with routine clinical and biological assessments. Our data were consistent with findings from two subsequently published, randomized trials (**RH Haubrich et al**, AIDS 2009, **MG von Vonderen**, PLoS One 2009).

For one of the presented publication, we performed prospective laboratory assessments and data analyses from a randomized trial for which the main endpoint was virological efficacy of treatment strategies in cART-treated adults. The use of stored samples has been argued as a potential source of measurement bias (samples degrading over time). In our analysis however, samples had been specifically collected and stored as part of these trials and we analysed all samples in a reference, centralized laboratory with strict quality control.

To evaluate cART-related low BMD, we implemented a cross-sectional study in the ambulatory setting of St Vincent's Hospital, Sydney. Although an imperfect design (use of non-standardized questionnaire) we were able to more fully characterize the relative impact of "classic" risk factors by a detailed questionnaire complemented by a large range of biological tests as well as anthropometric and radiological analyses.

To rigorously assess the efficacy of two potential investigational drugs for HIV lipodystrophy we performed a randomized, controlled trial. We were, unfortunately, unable to access pravastatin or uridine as placebo and so used an open-label design. Cost was the main reason preventing use of pravastatin placebo (stability requirement, external quality control cost made the placebo manufacture out of our budget); for uridine the manufacturer of uridine would not provide or agree to sell us placebo. To minimise the sample size to investigate both agents, we used a factorial design. To have a better understanding of clinical value of each agent, we followed participants for 24 weeks, not 12 as in previous controlled studies. Despite a longer trial duration and the use of a factorial design allowing for a smaller sample size, we can not rule out the possibility that a larger, longer, placebo-controlled randomized trial may have yielded a positive result.

4.3 Study populations

Toxicity is a major reason for discontinuation of first-line cART in most studies (TT Vo et al, Journal of Infect Dis 2008). The durability of initial regimens is stable and data from the Swiss HIV cohort study report that intolerance accounted for 51% of treatment changes, with patient wishes and physician decisions each accounting for a further 15% of changes (virological failure only explained 7% of switches). However, most data regarding discontinuation of first-line cART regimens are derived from Caucasian men living in Europe, the United States or Australia.

The studies conducted in Geneva and Sydney also mainly reported adverse events in Caucasian men. Several reports now confirm that toxicity is also a major reason for drug substitution in resource limited settings – with even more important consequences as the drug formulary is limited.

We did not address adverse events in children for several reasons: specific outcomes of such as psychomotor development, growth, and adherence require different study designs. Furthermore, and most importantly, our study research sites were located in Australia where HIV-infection in children is (fortunately) very rare. That said, it is of key importance not to exclude children from HIV/AIDS research and long term adverse events are even more critical in the setting of life-long cART, as paediatric HIV care, while rare in the developed world, is an important part of HIV care in the developing world.

4.3.1 Adverse events in women

Several aspects of cART may differ between men and women. Few clinical trials performed in Western countries had sufficient enrolment to address gender-distinct cART responses. So far few definite differences have been reported for virological, immunological or tolerability outcomes between men and women. There is relatively little knowledge as to what extent pharmacokinetics, pharmacodynamics, tolerability, hormones and pregnancy may affect these differences.

Some data suggest there is an increase of pancreatitis, hepatic steatosis, lactic acidosis, lipodystrophy, and less lipid abnormalities (particularly triglycerides) in women. Certain acute toxicities, such as liver function abnormalities and rash, as in nevirapine toxicity, are more common in women (**S Bersoff-Matcha et al**, Clin Infect Dis 2001, **A Calmy et al**, Antivir Ther 2009).

As an illustration on how these differences may have impacted on our research, we will take the example of Metabolic Syndrome. We have shown that the prevalence of Metabolic Syndrome at baseline was 11% and 9% (ATP-III and IDF criteria, respectively). During follow-up, progression to Metabolic Syndrome among participants without Metabolic Syndrome at baseline was 32% (ATP-III) and 22% (IDF). In contrast, among participants of the Women Health Initiative study, Metabolic Syndrome was identified in 33% of HIV-infected women. Metabolic Syndrome in these women was primarily associated with low HDL cholesterol levels and high triglycerides, as opposed to hypertension, glucose, or waist circumference as found in the mostly male participants of the INITIO study.

Pregnancy is also a major challenge; at least 10 percent of HIV-infected women of child-bearing age are pregnant, yet little is known about the efficacy, pharmacology, and safety of antiretroviral drugs in pregnant women. Recent data suggest that antiretroviral drugs are well tolerated during pregnancy, with the most frequent adverse event being mild gastro-intestinal disorders (**DM Zuk et al**, Ann Pharmacother 2009). Including women in clinical trials of cART should be a priority. The solution may come from the Southern hemisphere; in sub-Saharan Africa, most patients receiving antiretrovirals are female (**EJ Mills et al**, Lancet 2009) and this is reflected now in an increase in women in clinical trial recruitment (CARINEMO trial, ANRS 1246, M Bonnet, personal communication).

4.3.2 Adverse events in resource-limited settings

Due to availability, cost and convenience, most patients in resource-limited settings are put on a first-line fixed dose cART comprising nevirapine, lamivudine and either zidovudine or stavudine (**F Renaud-Théry et al**, AIDS 2007). Due to limited drug options, the duration of a first-line regimen is generally greater than in Western countries: in TAHOD, the TREAT ASIA cohort, 50% of the patients are still on the first prescribed regimen after 4 years (**J Zhou et al**, HIV Med 2007).

The demographics of the AIDS epidemic is very different in resource poor settings: the majority of patients accessing care have late stage disease, are women of childbearing age, and often present with co-infections such as tuberculosis, cryptococcosis, malaria and bacterial pneumonia (**R Subbaraman et al**, Clin Infect Dis 2007). However, toxicity is also a leading cause for treatment change: in Khayelitsha, South Africa, analysis of 1700 adults on cART showed that approximately 10% of patients switched one agent due to toxicity over 36 months (**A Boulle et al**, Antivir Ther 2007). The rates were similar for stavudine (8.5%), zidovudine (8.7%), and nevirapine (8.9%), although changes related to stavudine occurred later in the course of the treatment. Of note, occurrence of tuberculosis is also a leading cause of cART change, as most guidelines recommend switching nevirapine to efavirenz in those initiating rifampicin (**A Calmy et al**, AIDS 2006).

While the initial focus of attention has been on starting as many patients as possible on potent cART, limited attention has been given to side effects in resource-limited settings. Moreover, in many settings no or very little laboratory monitoring is available. Thus, most data collected has relied on clinical data and subjective reports to assess adverse event.

The rates of specific drug-related side-effects seem to be different in resource-limited settings compared to those reported in the Europe, US and Australia (**R Subbaraman et al**, Clin Infect Dis 2007). Cohorts from South Africa have demonstrated an exceptionally high rate of lactic acidosis (up to 12.3 cases per 1,000 patient-years), especially among women with a high body mass index (**A Boulle et al**, Antivir Ther 2007, **D Stead et al**, Antivir Ther

2008). The high rate of hyperlactataemia in South Africa together with numerous reports of high rates of neuropathy and lipoatrophy, led WHO to changes guidelines in 2006 to recommend that first-line cART regimens move away from a stavudine-based regimen to include tenofovir, abacavir or zidovudine (**Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach**, WHO guidelines, 2006 revision). However, Stevens-Johnson syndrome and severe hepatic reactions are very rarely reported, although it is difficult to rule out the possibility that side-effects that require lab monitoring are missed.

As most treatment cohorts in resource limited settings are less than five years old, almost no data are available on long-term side-effects, and very few studies have looked systematically at metabolic, lipodystrophy and cardiovascular events in these populations.

Overall, the spectrum of adverse events in general, and direct drug toxicity in particular, may be different in resource-limited settings compared to Western settings, and may lead to different recommendations with regards to clinical or laboratory monitoring.

5. CHALLENGES

There are several issues to address in order to improve research into cART-associated complications. Firstly, standardized data collection should be encouraged. Secondly, an international perspective on cART tolerability needs to be developed to include patients from diverse origins. And finally, researchers have to keep in mind that patients will be exposed to cART for a long time: not only will patients live longer, but it is likely that cART will be initiated earlier in course of HIV disease in the coming years – tolerability of cART regimens will be a key factor for determining when to start treatment.

5.1 How to best encourage data collection for adverse events?

Long-term side effects deserve special attention as most of the new drugs are marketed on the basis of clinical trial data. Most of these studies are too short and report insufficient adverse event data. Although the most common cause for treatment cessation in randomized trials (9.0%) or in cohorts (51%), adverse events were only reported in about half of all clinical trials evaluating initial cART (**A Carr et al**, AIDS 2009, **TT Vo et al**, J of Infect Dis 2008). Moreover, adverse events are more likely to be reported in studies that were in phase 2 or 3 (i.e. prior to commercialisation), when the studies were academia-sponsored, and when overall study duration was less than 36 months. Randomized clinical trials also have also inherent limitations in generating safety signals (**G Bisson et al**, AIDS 2003). Sample size of registration trials is generally powered to detect differences in medium-term drug efficacy and so are underpowered to detect rare or late-onset adverse events (such as lactic acidosis or cardiovascular disease), and there are wide exclusion criteria to ensure participant safety and a homogeneous study population make it difficult to generalize to different groups of patients.

What is therefore the role of cohort studies in detecting rates of adverse events? The longitudinal nature of the cohort makes it particularly suitable for evaluating the effect of duration of treatment exposure on the development of adverse events. The example of abacavir stresses the difficulty of attributing causality to a statistically significant association: abacavir as a risk factor for myocardial infarction was detected in a large, well characterized cohort study (**CA Sabin et al**, Lancet 2008), a risk that was not reported in any randomized trial.

Table 8 Summary of studies on the association between exposure to abacavir and the risk of myocardial infarction (MI)

Study	Design	CV Events	Effect of ABC?
D:A:D ^[1] (N of MI = 580)	Observational cohort	Prospective, predefined	Yes
FHDH ^[2] (N of MI = 289)	Case control study	Prospective, MI retrospectively validated	Yes 1 st yr of exposure
SMART ^[3] (N of MI = 19)	RCT, observational analyses	Prospective, predefined	Yes
STEAL ^[4] (N of MI = 3)	RCT	Prospective	Yes
GSK analysis ^[5] (N of MI = 11)	12 RCTs	Retrospective database search	No
ALLRT ACTG A5001 ^[6] (N of MI = 27)	5 RCTs	Retrospective by 2 independent reviewers	No
HEAT ^[7] (N of MI = 0)	RCT	Prospective	No

References: 1. Lundgren JD, et al. CROI 2009. Abstract 44LB.2. Lang S, et al. CROI 2009. Abstract 43LB.3. SMART. AIDS. 2008;22:F17-F24.4. Cooper DA, et al. Clin infect Dis 2009 in press.5. Cutrell A, et al. IAC 2008. Abstract WEAB0106.6. Benson C, et al. CROI 2009. Abstract 721.7. Smith KY, et al. AIDS 2009

Abbreviations: RCT stands for randomized clinical trial. MI stands for myocardial infarction.

*slide provided by Dominique Costagliola and presented at IAS conference 2009 (reproduced with permission)

However, cohort studies have inherent limitations as biases and confounding can never be completely ruled out. The relationship between abacavir and heart disease disappeared after adjustment for renal disease or cocaine use in recent data from the US Veteran Affairs cohort (**R Bedimo et al**, IAS Conference 2009) and from the French Hospital Database

cohorts (**D Costagliola**, IAS conference 2009). These findings highlight the need for careful controlling of confounders in observational studies.

After a drug has been marketed, the major (and sometimes only) mechanism of post-marketing surveillance is spontaneous reporting. Obvious limitations of this approach exist, not least underreporting, which is a major issue in countries without a standardized pharmacovigilance database. In the US, it is estimated that the Federal Drug Administration (FDA) receives reports of less than 1% of suspected serious adverse events (**HD Scott et al**, Am J Med, 1986). The development of automated and standardised databases may be able to overcome these limitations and provide signals that could be then be followed up by different analyses.

From a clinical point of view, most trials or cohort do use surrogate markers such as lipids, glucoses or transaminasis to assess long term toxicities such as lipodystrophy. With the new concept of non-infectious co-morbidities occurring in HIV-infected patients even with moderate immunosuppression, we should question whether these are the most relevant surrogate endpoints. Identifying patients at risks for end-organ diseases with more accurate tools over long term period is warranted. For example, measuring carotid intima thickness may better predict the risk for cardiac event than the unsensitive Framingham equation in such population.

In summary, randomized clinical trials are rarely designed to signal new or rare adverse event and observational cohort with well defined endpoints, such as used in the D:A:D cohort, are key element for drug surveillance. However results from observational data have to be analysed with caution; there is a need for effective and reactive pharmacovigilance strategies, including strategies that can be used in resource limited settings, as the current pre-licensing trials are not well suited to detecting rare or late-onset adverse reactions.

5.2 International perspectives of clinical research

From 2002 onwards, the international drive to scale up antiretroviral therapy in Africa gained considerable momentum. With almost four millions individuals receiving antiretroviral therapy

in resource-limited settings, it is no longer acceptable to restrict large clinical trials or cohorts solely to Western Europe, Australia and United States. Because trial participants are the first people exposed to new pharmacological products, and because this data is normally used to obtain drug approval, it is important that the information generated can be generalized to different patient populations.

Most of the knowledge on adverse events was based on data generated in wealthy countries. It is critical that the roll out of cART programs in resource limited settings be accompanied by operational research including surveillance and standardized data collection for adverse events.

5.3 Longer exposure to ARV drugs

Patients will likely be exposed to antiretroviral therapy for longer periods of time as mortality decreases and evidence accumulates on the benefit of earlier treatment initiation.

5.3.1 Population age

Since the 1980s a growing proportion of HIV-infected patients are over the age of 50 (**B Lederberger et al**, 15th Conference on Retroviruses and Opportunistic Infections 2008).

Drug-related toxicity and tolerability are influenced by age, in part because albumin levels decrease and because of changes in the cytochrome p450 enzyme system leading to slower drug clearance.

Parallel to age-related changes in drug metabolism explaining some changes in drug tolerability in ageing patients, cART has been associated with early onset of age-related comorbidities such as low BMD, cognitive impairment or visceral fat accumulation. Because mitochondrial dysfunction and oxidative stress are involved in the ageing process, and because mitochondrial toxicity is attributed to certain anti-HIV drugs, some researchers have suggested that both were related. Indeed, Caron and al exposed human fibroblasts to NRTI and a range of oxidative agents and observed proliferation, cell-cycle arrest, senescence-

associated beta-galactosidase activity, and changes in morphology and concluded that fibroblasts exposed to stavudine and zidovudine, but not to didanosine, lamivudine, abacavir or tenofovir were prematurely senescent. The authors hypothesised that some NRTIs may have a direct toxic role in the premature senescence seen in HIV-infected individuals (**M Caron et al**, Antivir Ther 2008).

Other factors such as lifestyle risk factors probably account for premature ageing in this population (**SG Deeks et al**, BMJ 2009); these includes physical inactivity, decrease vitamin D or calcium intake, and higher rate of cigarette smoking and depression, all of which are known cardiovascular or osteopenia risk factors. Persistent immune dysfunction and continuous inflammation also play a role in premature senescence – accounting in the process of premature senescence observed in several manifestations of HIV infection.

In summary, regardless of whether HIV or antiretroviral drugs affect the development of co-morbidities, the importance of cART management in an ageing population deserves to be emphasized. The prevalence of many comorbid conditions, such as dyslipidaemia, diabetes, cardiovascular disease, osteoporosis, and cognitive impairment all increase with age (**CA Sabin et al**, HIV Med 2008). Therefore, it is likely that using cART in older HIV-infected patients with prevalent co-morbidities will make the comprehensive care of these older patients a challenging clinical endeavour.

5.3.2 Earlier treatment initiation

The optimal time to initiate cART in asymptomatic patients has been controversial for many years. Since the tolerability of newer drugs appears to be better, the question of starting antiretroviral regimen earlier in the course of the HIV disease has become a burning issue. Yet the supporting evidence for the benefit of earlier therapy is increasing. Among more than 8000 patients with CD4⁺ lymphocyte counts between 351 to 500 cells/mm³ included in a US cohort collaboration, deferral of cART until the CD4⁺ lymphocyte count had fallen below 350

was associated with an increase of 69% in the risk of death – the majority of deaths being related to non AIDS-defining illnesses (**M Kitahata et al**, N Engl J Med 2009). This adds to the body of evidence initiated with the unexpected results from the SMART study showing that non-AIDS complications occurred more commonly in the intermittently treated group (drug conservation [DC] group) – and suggests that cART cannot be as deleterious as untreated HIV infection.

Randomised trials are underway to demonstrate in the setting of a prospective clinical trial, less prone to bias and confounding than cohort analysis, that all HIV patients should be treated regardless of CD4+ lymphocyte count (TasP trial, ANRS [French agency for AIDS research], **B Bazin**, personal communication; POPART trial, MRC, **J Weber**, personal communication) or with high CD4+ lymphocyte cell count (START trial).

A randomised trial recently provided strong evidence that early cART initiation, at least from the perspective of resource limited settings, significantly improves survival. The CIPRA HT 001 study enrolled 816 HIV-infected adults aged 18 and older with early HIV disease and CD4+ T cell counts between 200 and 350 cells/mm³ and assigned the participants to immediate cART or to defer cART until their CD4+ lymphocyte counts dropped below 200 cells/mm³ or they were diagnosed with AIDS. An interim data analysis revealed a 4-fold reduced risk of death and a 2-fold reduction in incident tuberculosis among patients who started the treatment earlier (CIPRA HT001 trial).

If the ongoing and planned clinical trials confirm these finding for higher CD4+ lymphocyte count at treatment initiation, it will of course be even more critical to individualize cART to minimize adverse events and allow more prolonged cART to be safely prescribed.

In such a context, addressing the safe durability of a first prescribed cART regimen is key. An ideal regimen will be safe across all patient groups. The current regimen used in more than 90% of national guidelines in sub-Saharan Africa, however, includes nevirapine. Nevirapine however is unsuitable for use in patients above 400 CD4 cells/μl for men, and

250 CD4 cells/ μ l for women (Package insert, available at: www.boehringer-ingenelheim.com/corporate/news/information_packs/documents/VIRAMUNE_Backgrounder_website.pdf). Efavirenz, from the same drug class and with recognized efficacy, is unsuitable for universal treatment, as it is currently classified FDA class D for pregnancy. Other options need to be developed.

It is not yet clear whether universal access can be achieved using the current formulary; the tolerability of current drugs in various populations including pregnant women will be essential. The role of more recent drugs, such as raltegravir, darunavir or rilpivirine may be of interest due to their promising safety profile; however experience in large and diverse population is lacking.

6. NEW ROADS FOR CLINICAL RESEARCH

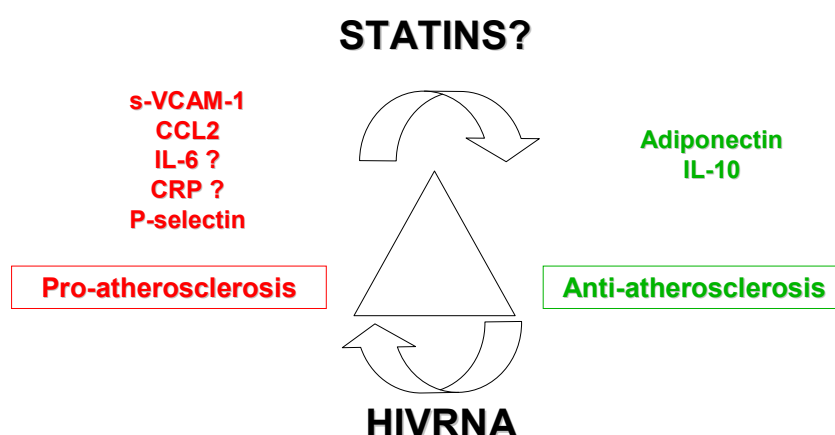
The monitoring and the management of long-term toxicities should be viewed with an international perspective, in the light of new data informing the recommendations regarding the “when to start” issue and within the context of the availability of newer antiretroviral agents. Three key points are likely to shape future research directions, and these are summarized in the next three chapters.

6.1 HIV is an inflammatory disease

Cardiovascular disease among HIV-positive individuals was first thought to be a complication related at least partly to change in cardiovascular risk factors (dyslipidaemia, insulin resistance) observed in patients on PI-based cART. However, after the results of the SMART trial, it was clear that cardiovascular disease was not only a cART-related complication (**W El Sadr et al**, N Engl J Med 2006). One hypothesis to explain the SMART unexpected findings of increased risks of non-HIV morbidity and mortality after HAART cessation was that HIV RNA triggers an inflammatory process. As a consequence, several organ diseases such as ischaemic heart disease, that were thought to be related to anti-HIV drugs adverse events, are now understood to be at least partly linked to uncontrolled HIV RNA replication.

If HIV RNA replication promotes inflammation, which in turn is responsible for a wide range of AIDS and non AIDS-related events, then the options are 1) to suppress HIV RNA replication, or 2) to suppress inflammation. This concept is further illustrated in the following figure.

Figure 14 Relationship between inflammation and HIV RNA



Two studies are planned or ongoing to assess the above relationship. These are:

1. Anti-inflammatory effect of statin in a large, unselected population of HIV-infected patients (clinical trial.gov NCT00673582).
3. Anti-inflammatory effect of HDL-increasing agents (ER niacin-lapopiprant) in HIV-infected patients on boosted PI (proof-of-concept trial)

6.2 Genetic prediction of drug toxicity

The sequencing of the human genome and the large-scale identification of genome polymorphisms have provided opportunities for understanding the genetic basis for individual differences in drug responses, a field known as toxicogenetics. Toxicogenetics establishes links between the variability in the response to ART, especially the susceptibility to adverse reactions, to an individuals' genetic background. Host variability in antiretroviral efficacy and tolerability includes both immunogenetic factors (HLA immune response genes) and pharmacogenetic factors (drug metabolism, transporter or receptor genes). For example, HLA screening has demonstrated the involvement of genetic factors in abacavir (**S Mallal et al**, Lancet 2002) and nevirapine hypersensitivity (**AM Martin et al**, AIDS 2005; **DW Haas et al**, Clin Infect Dis 2006). Efavirenz neurotoxicity is increased in patients harbouring certain CYP2B6 polymorphism (**DW Haas et al**, AIDS 2004).

Recent studies suggest that single-nucleotide polymorphisms (SNPs) in several genes involved in lipid metabolism and lipid transport in the general population (ABCA1, APOA5, APOC3, APOE, CETP) might modulate plasma triglyceride and HDL cholesterol levels in HIV-infected patients (**JM Bard et al**, Antivir Ther 2006, **PE Tarr et al**, Antivir Ther 2007). Also, lipodystrophy has been linked to an accumulation of mtDNA mutations (this is also found in animal models of ageing). While genetic prediction for dyslipidaemia or lipodystrophy is not yet possible, with greater availability of genetic testing, the inclusion of an algorithm in clinical practice may not be far off.

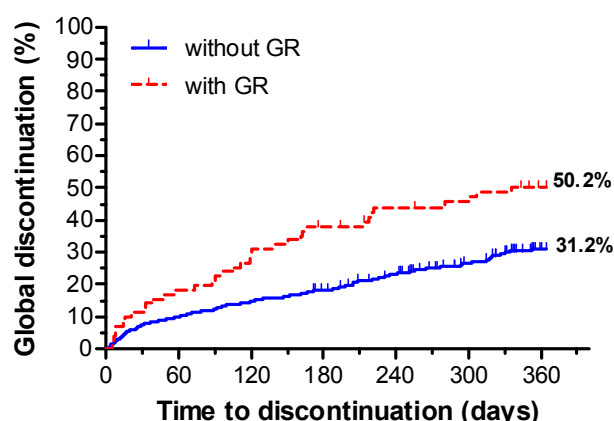
How might host genetic information improve HIV-treatment tolerance?

The tolerability of certain drugs is related to well-documented biological disturbances, leading to clinical symptoms. A patient might cease a drug because of the occurrence of such symptoms.

The proof of concept of how such information could be used was recently provided by Swiss researchers. Investigators from the Swiss HIV Cohort Study searched for 13 pharmacogenetic markers thought to signal antiretroviral toxicity on 9 genes; they focused on efavirenz, atazanavir, lopinavir, tenofovir and abacavir, as summarized in Table 11 (**S Columbo et al**, International Workshop on Clinical Pharmacology of HIV Therapy 2009).

The study involved 577 treatment-naïve people starting their first antiretroviral regimen. Within the first year of treatment, 190 people (33%) stopped one or more antiretrovirals because of toxicity. The aim of the study was to analyse the influence of pharmacogenetic markers to predict the discontinuation of atazanavir, efavirenz, and tenofovir.

Figure 15 Time to drug discontinuation in individuals with and without risk genetic markers



Courtesy A Telenti, reproduced with permission. GR: genetic risk

Figure 16 shows that pharmacogenetic markers predicted discontinuation of atazanavir, efavirenz, and tenofovir, which was consistent with the hypothesis that individuals carrying risk genetic markers will discontinue initial cART more frequently than individuals without such markers.

How might host genetic information optimizing drug dosage?

Another interesting avenue for pharmacogenetics in a public health approach would be the prediction of some metabolic pathways allowing for drug dose-reduction in certain populations. Host sources of variability in antiretroviral efficacy and tolerability includes both immunogenetic factors (HLA immune response genes) and pharmacogenetic factors (drug metabolism enzyme, drug transporter or drug receptor genes). For example, HLA screening has demonstrated the involvement of genetic factors in the abacavir (**S Mallal et al**, Lancet 2002) and nevirapine HSR (**AM Martin et al**, AIDS 2005, **DW Haas et al**, Clin Infect Dis 2006). Efavirenz neurotoxicity is increased in patients harbouring certain CYP2B6 polymorphism (**DW Haas et al**, AIDS 2004).

Polymorphism of the cytochrome P450 2B6 gene at position 516 slows efavirenz clearance and increases median efavirenz concentrations 2-3 fold, thus increasing efavirenz exposure (**M Rotger et al**, Eur J Clin Pharmacol 2008). Central nervous system efavirenz toxicity is more frequent in African-Americans, who harbour this particular genotype more frequently.

Individualized drug prescriptions based on genetic patterns might therefore increase the safety and certainly the durability of drugs. How this will be integrated in current practice, and for whom? How might we individualise drug prescription in countries where the public health approach only recommends using two regimens and genetic tests are unavailable? Will population genetics help us to design cART regimens safe for specific populations? We will need to address all these issues to shape a safer future for HIV therapy around the world as well as ensure equitable access to these new techniques.

Ongoing study in Geneva within the Swiss HIV Cohort Study:

Evaluation of a Bayesian approach for dose reduction in patients treated by a efavirenz based regimen, with documented virological efficacy and high plasma levels and its association with genetic polymorphism of cytochrome P450 2B6.

6.3 The potential of newer antiretroviral agents

If more patients start cART even earlier on in the course of their HIV disease, drugs will have to be potent, safe, well tolerated and easy to take (once daily, use of fixed dose combination, no refrigeration, no food intake requirement).

It is already clear that toxicity issues influence drug production, and for the first time in the history of HIV drugs, some antiretroviral drugs have been withdrawn from the market. The soft-gel version of saquinavir has been discontinued, and the same company also announced that it will discontinue zalcitabine (ddC), mainly because of its poor tolerance. Similarly, toxicity concerns have caused the termination of several clinical development programs: Bristol-Myers Squibb announced earlier in 2009 that it will not be moving forward with the extended-release version of d4T although the compound was already FDA approved. Increased levels of amylase were found in patients on the extended release D4T and might in part have explained this decision. In 2005, GlaxoSmithKline (GSK) terminated clinical trials of aplaviroc, an experimental CCR5 antagonist, because of an unacceptable risk of severe hepatotoxicity. In phase 2b/3 trials of maraviroc, another CCR5 antagonist, a high incidence of non Hodgkin lymphomas and Kaposi's sarcoma was seen. An independent review concluded that this high rate was consistent with the specific population studied. Long-term data however are needed to firmly rule out any liver disease or cancer associated with CCR5 blockade.

The following table summarized recently approved drugs and newer drugs still in development.

Table 9 New drugs

Drug formulation	Date of FDA approval if marketed	Principal characteristics
Darunavir	2008	PI Indicated for treatment naïve and treatment experience patients with a booster. Encouraging results in monotherapy.
Raltegravir	2007	First Integrase inhibitor indicated for treatment naïve and treatment experienced multi class resistant patients.
Maraviroc	2008	First CCR5 Receptor Inhibitor indicated for treatment experienced, multiresistant patients with only CCR5 tropism.
Etravirine	2007	New generation of NNRTI. Active against mutant NNRTI resistant HIV strains.
Vicriviroc	NA	CCR5 Receptor inhibitor in phase III trials.
Rilpivirine	NA	New generation of NNRTI. Once a day dosing. Undergoing Phase III study.
Elvitegravir	NA	Integrase inhibitor undergoing Phase II-III with a booster GS-9350
S/GSK1349572	NA	Integrase inhibitor. Phase II trials ongoing.
GS-9350	NA	CYP 3A inhibitor, non ARV booster. Entering phase II-III studies. Developed for combination with elvitegravir.
SPI-452	NA	CYP 3A inhibitor, non ARV booster. Only preclinical studies done.

NA *Not applicable*

The safety profile of drugs marketed since 2007 seems favourable, with the caveat already discussed on the known limitations of randomized clinical trials to detect rare or long-term adverse events.

As an example, the first integrase inhibitor licensed for clinical use (FDA, in 2007) is raltegravir. In two randomized, double-blind, placebo-controlled trials, serious drug-related adverse events occurred in 2.2% of raltegravir recipients versus 0% of placebo recipients and adverse events leading to drug discontinuation were reported for 1.7% of the patients. Nausea, headache, fever and creatine phosphokinase elevations were among the few

adverse events reported (**RT Steigbigel et al**, N Engl J Med 2008). Cancers have been reported in early studies on pre-treated patients receiving raltegravir. Many of those cancers were of the type expected in very immunodeficient individuals and additional risk factors such as smoking, chronic viral hepatitis and papillomavirus were often present and, with longer follow-up, the cancer rate was similar to that in controls. No evidence of mutagenicity or genotoxicity was observed *in vitro* during tests of mutagenesis, DNA breakage, or *in vitro* and *in vivo* during chromosomal aberration studies. Overall, raltegravir is well tolerated at its licensed dose and toxicity seems to be minimal with the available follow-up time. Post-marketing studies will need to include more women, who comprised only 13% of participants included in clinical trials.

In summary, newer drugs seem to have a better safety profile than older ones. If their tolerability holds up in post-marketing surveillance some currently prevalent side-effects such as lipodystrophy may disappear within the next decade as these newer drugs become more widely used. Severe forms of mitochondrial toxicity such as lactic acidosis have already completely disappeared in wealthy countries where stavudine (marketed in 1994), and to a lesser extent, zidovudine (marketed in 1987), were replaced by tenofovir (FDA approved 2001) and abacavir (FDA approved 1998). However, the fact that stavudine and zidovudine are still in widespread use in developing countries provides a warning that efforts must be made to ensure the newer drugs are affordable and available in developing countries if the majority are to benefit from their superior tolerability.

Table 10

Future research directions in cART-related complications

Encourage standardized and international data collection	• Extend randomized clinical trial duration • Broaden populations and adverse events studied in observational cohorts • Broaden pharmacovigilance reporting
Cardiovascular research	• Biomarkers study to assess each phase of atherosclerosis processess before and after abacavir initiation in patients with virological suppression. • Assess the management algorithm for cardiovascular risk prevention in HIV infected patients by comparing cART change with other standard interventions. • Establish the biological plausability for the abacavir association with the increased risk of cardiovascular disease both in animal models and in humans. • Optimize the existing cardio-vascular prevention measures.
Inflammatory component of HIV disease	• Evaluate the use of statin in a large, non selected HIV-infected population on ART (NCT00673582). • evaluate the anti-inflammatory effect of HDL increase in a non-selected HIV-infected population (proof-of-concept trial)
Basic sciences	• Investigate the pathogenesis of visceral fat hypertrophy
Pharmacogenetics	• Drug dose optimization • Population based polymorphism characterisations
New drugs	• Determine the role of new drugs in long term end organ toxicity • Determine the role of new drugs in the management of lipo hyper and atrophy

Take home messages

The arrival of cART in 1996 revolutionized HIV disease to the same extent as penicillin did for pneumococcal disease in 1945. In 2009, HIV has become a chronic, manageable disease with most challenges related to long-term antiretroviral safety and tolerability. The average lifetime duration of drug exposure is likely to increase as the CD4+ lymphocyte count threshold at which cART is initiated continues to increase and because the improved potency of cART results in better control of HIV replication.

As life-long treatment is likely to be the rule for the next few years, and as adverse events does not resolve by interrupting treatment, drug toxicity is a burning issue for the management of HIV-infected patients.

This thesis focused on recent clinical development in the toxicity issues and several conclusions and recommendations can be drawn. Treatment strategies for peripheral subcutaneous lipoatrophy are disappointing: we investigated two new promising agents such as uridine, a nucleoside that counteract mitochondrial toxicity, or pravastatin, and were not able to demonstrate any efficacy of these two agents. The only validated therapeutic option to reverse lipoatrophy remains the switch from a thymidine nucleoside analogue to a non-thymidine. Moreover, the lipid abnormalities and glucose disturbances associated with the lipodystrophy syndrome resemble the metabolic syndrome and may increase the cardiovascular risk of these patients. Therefore, physicians should consider screening for MS in the routine clinical care. It is however likely that the incidence of lipodystrophy will decrease as the safety profile of the new drugs is better regarding lipid levels, glucose intolerance and fat distribution.

We demonstrated that the class sparing strategy may be effective at suppressing viraemia, but increase the risk of ART-related dyslipidemia. Again, new drug strategies are promising and should help us building better tolerated NRTIs-sparing regimens. HIV RNA replication and modifications of specific cardiovascular risk biomarkers (s-VCAM-1, CCL2, adiponectin

and IL-10) are associated independently of known confounders. We therefore suggest that HIV RNA be considered as an additional risk factors for HIV-infected patients and should prompt for virological suppression whenever possible. Intervention strategies should be studied for HIV-infected patients with high plasma levels of inflammatory markers and with ongoing HIV RNA replication

As new drugs are marketed with data on a limited number of patients and a limited follow-up (48 weeks), pharmacovigilance is warranted. Newer drugs seem to have a better safety profile than older ones; as a result, lipodystrophy and mitochondrial toxicity may disappear within the next decade. However, the majority of people on cART live in the developing world, where old, toxic drugs no longer used in the West are the backbone of treatment. As much as the newer medications with better side-effect profiles are to be welcomed, they will only benefit the minority unless their widespread access is assured.

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[Appendix]

Publications liées à la présente thèse

1. A Calmy, B Hirschel, DA Cooper, A Carr. A new era of antiretroviral drug toxicity. *Antiviral Therapy* 2009, 14:165-79
2. A Calmy, B Hirschel, DA Cooper, A Carr. Clinical update: adverse effects of antiretroviral therapy. *Lancet* 2007; 370:12-4
3. A Calmy, K Petoumenos, et al for the Aquitaine Cohort, the Australian HIV observational Database and the St Vincent's Hospital Cohort. Combination antiretroviral therapy without a nucleoside analogue: Experience from 334 patients in three cohorts. *HIV Med.* 2007; 3:171-80
4. Nguyen A, Calmy A, et al, for the Swiss HIV Cohort Study. Lipodystrophy is not what it used to be. Data from the Swiss HIV Cohort Study, *HIV Med* 2008; 17:142-150
5. H Wand, A Calmy et al; Three year incidence of Metabolic Syndrome and type 2 diabetes mellitus after initiation of

antiretroviral therapy in HIV-infected individuals, AIDS 2007; 21:2445-53

6. A Calmy, A Gayet-Ageron et al. HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial. AIDS 2009;23:929-39
7. A Calmy, CA Fux, et al; Low bone mineral density, renal dysfunction and fracture risk in HIV Infection: a cross-sectional study. J Infect Dis. 2009; 200: 1764-54
8. A Calmy, M Bloch, et al. No significant effect of uridine or pravastatin for HIV lipoatrophy in men who have ceased thymidine nucleoside analogue therapy: a randomized trial. HIV Med 2009, in press.

Review

A new era of antiretroviral drug toxicity

Alexandra Calmy^{1*}, Bernard Hirschel¹, David A Cooper^{2,3} and Andrew Carr³

¹Geneva University Hospital, HIV Unit, Geneva, Switzerland

²National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia

³HIV, Immunology and Infectious Diseases Unit, St Vincent's Hospital, Sydney, Australia

*Corresponding author: E-mail: alexandra.calmy@hcuge.ch

The spectrum of drugs used in HIV-infected patients has dramatically changed since triple antiretroviral combinations were introduced, albeit at the expense of some severe adverse events, in 1996. Abandonment of stavudine in countries that can afford it, new drugs from new classes with a wide therapeutic window and the impressive scale-up of drug access in resource-limited settings

are several of the key new events. Drug safety is likely to be the most important factor to distinguish one antiretroviral regimen from another. We review life-threatening adverse events, adverse events of new investigational or recently marketed drugs, adverse events with a genetic component and tissue-specific adverse events of fat, heart, bone, kidney and liver.

Introduction

A total of 24 antiretroviral drugs have been approved since 1986. The efficacy of combination antiretroviral therapy (ART) for compliant ART-naïve patients is now >90% and this percentage might be difficult to improve. The main factors that distinguish one ART regimen from another are simplicity, toxicity and cost in some countries [1]. Zalcitabine has been withdrawn for toxicity concern and stavudine extended-release, although approved by the US Food and Drug Administration (FDA) in 2002, is not marketed.

Episodic ART causes more AIDS, deaths, myocardial infarctions (MI), end-stage liver disease and end-stage renal failure than intermittent ART, suggesting that, once initiated, ART should be permanent [2].

We review life-threatening adverse events, adverse events of new investigational drugs or recently marketed drugs, adverse events with a genetic component and tissue-specific adverse events of fat, heart, bone, kidney and liver. We will not address specific populations, such as children, individuals receiving post-exposure prophylaxis and issues related to mother-to-child transmission.

A summary of major and minor adverse events for the most popular anti-HIV drugs, classified by site of action, is provided in Table 1.

Immediate life-threatening adverse events

Background

A drug is approved when its benefits are thought to outweigh its risks, not because there is no risk. There are

several, life-threatening adverse events associated with antiretroviral drugs and some of these effects have been seen only after drug approval. If a drug incurs a significant risk of serious or life-threatening adverse event, the FDA will request a black box warning to be added to the drug label. Updated information on such adverse events can be found in the US and European guidelines [3,4].

Lactic acidosis

Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) can inhibit mitochondrial DNA polymerase γ , decrease mitochondrial DNA and RNA levels and decrease mitochondrial function [5,6]. These effects could result in hyperlactataemia and a large spectrum of clinical and biological abnormalities that include peripheral and autonomic neuropathy, skeletal and cardiomyopathy, steatohepatitis, pancreatitis and lipoatrophy.

Severe steatohepatitis and/or pancreatitis can lead to lactic acidosis, a life-threatening condition [7], which clinically manifests as subacute onset of nausea, fatigue, weight loss, abdominal pain, dyspnoea and eventual circulatory collapse. Lactic acidosis is rare (0.85–2.7 per 1,000 person years), although higher rates have been described in southern Africa [8–10]. Factors associated with lactic acidosis are higher age, female sex, high body mass index, lipoatrophy, low CD4⁺ T-cell count, hypertriglyceridaemia and use of stavudine and didanosine [11–17], although it has been described with all NRTIs except abacavir.

Table 1. Severe and non-severe side effects of common anti-HIV drugs classified by action site

Registered anti-HIV drug by action site	Abbreviation	Severe adverse event	Common adverse event (>5%)	Management (apart from drug cessation)
NRTIs				
Abacavir	ABC	HSR ^a , myocardial infarction	–	Prevent HSR with <i>HLA-B*5701</i> -testing, stopping abacavir treatment immediately and permanently if HSR is suspected. Consider an alternative in patients at high cardiovascular risk.
Didanosine	ddl	Lactic acidosis ^b , steatohepatitis, pancreatitis (1–7%), peripheral neuropathy	Nausea	Didanosine and stavudine should not be used concomitantly during pregnancy.
Lamivudine or emtricitabine	3TC or FTC	Rare, severe exacerbation of hepatitis B infection reported in patients who discontinued lamivudine or emtricitabine	–	–
Stavudine	d4T	Lactic acidosis ^b , pancreatitis, steatohepatitis, rapidly ascending neuromuscular weakness (rare)	Peripheral neuropathy, lipoatrophy	Didanosine and stavudine should not be used concomitantly during pregnancy.
Tenofovir disoproxil fumarate	TDF	GFR decrease, tubular disease, osteopaenia, exacerbation of hepatitis B infection after withdrawal	–	Avoid concomitant nephrotoxic drugs.
Zidovudine	AZT	Lactic acidosis ^b , lipoatrophy, anaemia (Hb<7 g/dl; 1–4%), neutropaenia (1–2%)	Headache, asthaenia, nausea, nail pigmentation	–
NNRTIs				
Efavirenz	EFV	Rash, hepatotoxicity, Stevens–Johnson syndrome (0.1%), fetal CNS malformations	CNS symptoms such as insomnia, dizziness, vivid dreams (>50%); ^c headaches, increased transaminase levels	–
Etravirine	ETV	Rash	Nausea	–
Nevirapine	NVP	Rash, HSR ^a , hepatotoxicity, Stevens–Johnson syndrome (0.3%)	Increase in transaminase levels	Dose increase (14 days lead-in period), avoid nevirapine treatment in female patients with CD4 ⁺ T-cell count >250 cells/μl and in male patients with CD4 ⁺ T-cell count >400 cells/μl. Do not rechallenge.
PIs^d				
Ritonavir	RTV or r	Coadministration of ritonavir even at a low dose with certain drugs (antihistamines, hypnotics, antiarrhythmics or ergot alkaloids) might lead to severe adverse events as a result of drug interactions	–	–
Atazanavir	ATV	First degree atrioventricular block, nephrolithiasis	Indirect hyperbilirubinaemia	–
Darunavir/ritonavir	DRV/r	Stevens–Johnson syndrome, erythema multiforme, hepatotoxicity	Skin rash (7%), nausea, diarrhoea	Use with caution in patients with sulphonamide allergy (sulphonamide moiety).

^aAbacavir and nevirapine hypersensitivity reaction (HSR) can be life-threatening. ^bRandom lactate level is not useful for screening. ^cIncreased risk when combined with methadone. ^dMost protease inhibitors (PIs) can cause gastrointestinal disorders. All ritonavir-boosted PIs can cause dyslipidaemia (where a statin with minimal or no P450 interaction might be preferable and simvastatin and lovastatin should not be used) and so are associated with cardiovascular disease and possibly lipodystrophy. CNS, central nervous system; CPK, creatine phosphokinase; GFR, glomerular filtration rate; Hg, haemoglobin; NNRTI, non-nucleoside/nucleotide reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor.

Table 1. Continued

Registered anti-HIV drug by action site	Abbreviation	Severe adverse event	Common adverse event (>5%)	Management (apart from drug cessation)
Fosamprenavir	FPV	–	Skin rash (19%), nausea, diarrhoea	–
Indinavir	IDV	Nephrolithiasis	Dry skin, ingrown nails	–
Lopinavir/ritonavir	LPV/r	–	Hypertriglyceridaemia, asthenia, nausea, diarrhoea	–
Saquinavir	SQV	–	Headache, diarrhoea	–
Tipranavir	TPV	Cerebral haemorrhage (platelet inhibition), hepatotoxicity	Nausea, diarrhoea	Avoid platelet inhibitors.
Integrase inhibitors				
Raltegravir	RAL	No attributable severe adverse events described	Headache, diarrhoea, fever, CPK increase	–
Fusion and entry inhibitors				
Enfuvirtide	T-20	Hypersensitivity (<1%)	Local infection site reaction (90%), increased risk of bacterial pneumonia	Do not rechallenge.
Maraviroc	MVC	Possible lymphoma, hepatotoxicity	Dizziness, rash, orthostatic hypotension, upper respiratory tract infections	–

Hepatotoxicity

Hepatotoxicity is common and associated with most antiretroviral drugs. Mechanisms of antiretroviral hepatotoxicity include direct antiretroviral toxicity, hypersensitivity, immune reconstitution in those with chronic viral hepatitis and steatohepatitis secondary to NRTI mitochondrial toxicity (Table 2). Liver toxicity is more frequent among patients with chronic viral hepatitis or increased baseline hepatic transaminases and in alcohol abusers [18].

Nevirapine produces a symptomatic drug-induced hepatitis in 2.5–11% of patients, mainly during the first 12 weeks [19]. Approximately 50% of these reactions are associated with fever, rash or arthralgias, suggesting that hypersensitivity underlies many such cases. Severe, life-threatening and fatal cases of hepatotoxicity have also occurred. Because immunocompetence is a significant risk factor, guidelines recommend that nevirapine be initiated only in men and women with CD4⁺ T-cell counts <400 and <250 cells/ μ l, respectively, with a particular caution for those with chronic viral hepatitis. Patients already receiving fully suppressive ART who switch to nevirapine above these thresholds might not have this greater risk of hepatitis [20].

Drug-induced hepatitis has also been reported with darunavir. From June 2006 to December 2007, 13 post-marketing cases of suspected drug-induced hepatitis were reported, 2 of which were fatal. During the same period, other hepatic adverse events such as neoplasm or cirrhosis in patients with comorbidities and advanced HIV type-1 disease were also reported, 14 of which were fatal [21].

Hypersensitivity

Stevens–Johnson syndrome and toxic epidermal necrolysis are severe forms of cutaneous hypersensitivity that are mainly associated with non-NRTI (NNRTI; 0.3–0.5% for nevirapine, 0.1% for efavirenz and 0.1% for etravirine), as well as amprenavir, darunavir and abacavir [4].

The abacavir hypersensitivity reaction (HSR) occurs in 5–8% of unselected Caucasians after a median 10 days of exposure; its frequency is much less in African and Asian populations. Symptoms include combinations of fever, constitutional symptoms, rash and gastrointestinal and respiratory signs. Clinical symptoms for the diagnosis of HSR are non-specific. A positive epicutaneous patch test 6–10 weeks after clinically suspected abacavir HSR confirms true immunologically-mediated hypersensitivity [22]. Permanent discontinuation of abacavir is mandated after HSR, as death can occur with abacavir rechallenge [23]. This HSR is mediated by CD8⁺ T-lymphocytes expressing the human leukocyte antigen (HLA)-B*5701 [24].

Management

Drug cessation is essential for any potentially life-threatening adverse event. When possible, drugs with different half-lives should be ceased progressively to allow for concurrent disappearance from plasma, in order to reduce the likelihood of monotherapy and secondary drug resistance. For example, if nevirapine has to be stopped, coadministered drugs with a shorter half-life should be continued for ≥ 7 days, unless these drugs are contributing to severe toxicity.

Table 2. Types of liver disease relating to antiretroviral therapy

Cause	Hyperlactataemia	Hypersensitivity	Isolated hepatitis	Immune restoration	Hepatitis B flare
Antiretroviral agent	Didanosine, stavudine, zidovudine	Nevirapine	Nevirapine	Any	Stopping lamivudine, emtricitabine and/or tenofovir
Risk factors	Duration of exposure, female sex, obesity, ribavirin, pregnancy	Higher CD4 ⁺ T-cell count	HCV RNA and ALT increase	CD4 ⁺ T-cell <100 cells/ μ l, chronic viral hepatitis, <i>Mycobacterium avium</i> complex bacteraemia, cytomegalovirus viraemia, recent tuberculosis	HBV DNA plus pre-pregnancy
Clinical features					
Onset	Late	Early ^a	Early/late	Early	Early
Fever	No	Common	Occasional	Yes	Yes
Rash	No	Common	No	No	No
Jaundice	No	Occasional	Occasional	Common	Common
Dyspnoea	Yes	No	No	No	No
Liver failure	Occasional	Uncommon	Uncommon	More common	Common
Laboratory features					
ALT>10 $\times$ ULN	No	Common	Common	Common	Common
Lactate >2 mmol/l	Yes	No	No	No	No
Therapy	Cease nucleoside analogue treatment	Cease antiretroviral therapy	Cease nevirapine and treat HCV	Possible steroid treatment, cease antiretroviral therapy if severe and treat OI	Restart HBV therapy

^aMean of 21 days after initiation. ALT, alanine aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; OI, opportunistic infection; ULN, upper limit of normal.

Genetics and drug toxicity

Background

Host sources of variability in antiretroviral efficacy and tolerability include both immunogenetic factors (HLA immune response genes) and pharmacogenetic factors (drug metabolism enzyme, drug transporter or drug receptor genes). For example, HLA screening has demonstrated the involvement of genetic factors in the abacavir [22] and nevirapine HSR [25,26]. Efavirenz neurotoxicity is increased in patients harbouring certain CYP2B6 polymorphisms [27].

Description and prevalence

An association between abacavir HSR and carriage of the class I allele *HLA-B*5701* was reported in 2002 [23,24]. A randomized trial showed that immunologically-confirmed HSR is extremely unlikely to occur in *HLA-B*5701*-negative Caucasians [22]. Nevertheless, 33–50% of *HLA-B*5701*-positive patients do not develop HSR. The additional factors required for HSR are unknown.

Polymorphism of the cytochrome P450 2B6 gene at position 516 slows efavirenz clearance and increases median efavirenz concentrations two- to threefold, thus increasing efavirenz exposure [28]. Central nervous system efavirenz toxicity is more frequent in African-Americans, who harbour this particular genotype more frequently; however, this polymorphism is not limited to

African-Americans. Determination of P450 2B6 genotype and plasma efavirenz concentrations might permit dose reductions in patients who are CYP2B6 *6/*6 and *6/*26 carriers [29].

Nevirapine hypersensitivity has been associated with an interaction between *HLA-DRB1*0101* and the CD4⁺ T-cell percentage, consistent with a CD4⁺ T-lymphocyte-dependent immune response to nevirapine-derived antigens [25]. A case-control study explored associations between genetic variants in *MDR1*, *CYP2B6*, *CYP3A4* and *CYP3A5* and nevirapine-associated hepatotoxicity in South Africa and found that the *MDR1* position 3435 T allele was associated with decreased risk of hepatotoxicity [26].

Management

*HLA-B*5701* testing to prevent abacavir HSR is the best studied example of the incorporation of a pharmacogenetic test into routine care. The applicability of this test to diverse ethnic groups is unclear. As *HLA-B*5701* is less prevalent in sub-Saharan Africa and in Southeast Asia, *HLA-B*5701* testing might not be cost-effective in these regions. Most HIV-infected patients live in resource-limited settings and it is unlikely that such an expensive and technically challenging test will be available or affordable for such patients. Drugs that need less intense clinical monitoring will be favoured in resource-limited settings.

Cardiovascular disease

Background

Cardiovascular complications of HIV, such as cardiomyopathy or pericarditis, have been reduced by ART, as both are related to advanced HIV disease. However, the exact magnitude of the ART effect is unknown [30]. Premature cardiovascular disease (CVD) is more common. Soon after the introduction of potent ART, concern was raised about a possible increased risk of coronary heart disease [31]. Both ART and HIV infection itself appear to contribute to CVD by inducing dyslipidaemia, insulin resistance and possibly vascular dysfunction.

Description and prevalence

The Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study, a global prospective study of >30,000 HIV-infected adults, found that the incidence of MI increased with longer exposure to ART (adjusted relative rate 1.16 per year of ART exposure) [32,33]. This increased risk was primarily driven by duration of protease inhibitor (PI) therapy [33]. Half the PI risk for MI was explained by lipids levels, particularly with regard to levels of total and high-density lipoprotein (HDL) cholesterol [34]. The remaining half remains unexplained.

The DAD study also found that neither zidovudine nor stavudine therapy, which can cause dyslipidaemia and insulin resistance, were associated with CVD. However, recent and ongoing abacavir or didanosine therapy was each independently associated with an increased risk of MI, by 90% and 46%, respectively. The 90% excess risk associated with abacavir appears high compared with the 16% annual excess risk associated with PI therapy. Abacavir is not known to have any other long-term toxicity, so the mechanism by which abacavir might cause CVD is unknown. It has been suggested that abacavir be avoided in those with high MI risk (>20% over 10 years by the Framingham equation) [35,36].

The SMART trial demonstrated that people receiving intermittent ART had an increased risk of CVD compared with those receiving continuous ART, with this increased risk diminishing after continuous ART was resumed [37]. Two mechanisms have been proposed to explain these findings. Firstly, HIV infection is associated with reductions in the level of HDL cholesterol and a lower total to HDL cholesterol ratio [38]. HIV can inhibit macrophage export of HDL cholesterol [39]. In turn, most types of effective ART increase HDL cholesterol levels [40]. Another possible cause is endothelial inflammation from active HIV replication. Some biomarkers associated with inflammation, blood coagulation or endothelial dysfunction change with ART interruption [41,42]. These markers include D-dimer (a marker of thrombosis), interleukin-6 (a proinflammatory cytokine) and vascular cell adhesion molecule-1.

Management

The Framingham equation reasonably estimates the 10-year risk of MI in adults receiving ART, although it might slightly underestimate the true risk [43]. This suggests that conventional risk factors explain much of the cardiovascular risk in this population.

Careful management of classical risk factors is essential. Smoking cessation, early diagnosis of hypertension, glucose intolerance and hyperlipidaemia should be included in routine care of adults. Because dyslipidaemia is often multifactorial, its treatment might require multiple approaches, including antiretroviral changes. The effect of any specific antiretroviral agent on lipids can be difficult to determine as most drugs are used in combination. Moreover, interpretation might be complicated by lipid changes that occur as HIV wasting improves in response to combination ART. Low-density lipoprotein cholesterol tends to increase with most antiretrovirals and boosted PIs tend to increase triglycerides. HDL cholesterol is increased by all regimens that effectively suppress HIV replication, but perhaps somewhat more with the NNRTI drug class. New antiretroviral agents, such as raltegravir and CCR5 blockers, don't appear to adversely affect lipids, although they might not increase HDL cholesterol levels to the same extent as efavirenz [44]. Effects of switching therapy have been extensively studied in randomized trials and have shown positive results. Switching zidovudine or stavudine to tenofovir or abacavir, and a PI to nevirapine, abacavir or unboosted atazanavir has improved dyslipidaemia [45–49]. However, the risk of failing after antiretroviral change has to be carefully balanced against what could be a small lipid improvement. If additional medication is needed, statins are the most efficient lipid-lowering medication; however, cytochrome interaction might limit the choice of statins. Table 3 provides information regarding the management of cardiovascular risk factors in an antiretroviral-treated study population.

Whether measurement of plasma levels of D-dimer and interleukin-6 can be used as predictive tools to assess cardiovascular risk is unclear and merits further studies.

Lipodystrophy

Background

HIV lipodystrophy is characterized by peripheral, subcutaneous lipoatrophy in the face, arms, legs and buttocks and central fat accumulation in the neck, breasts and abdomen (referred to as lipohypertrophy). Lipodystrophy is associated with low levels of HDL cholesterol, insulin resistance, and, less commonly, hyperglycaemia [50,51].

NRTI-associated mitochondrial toxicity is increasingly implicated in lipoatrophy [52]. Mitochondrial DNA polymerase γ is inhibited by some NRTIs (mainly

stavudine) and thus causes depletion of mitochondrial DNA resulting in mitochondrial dysfunction. Adverse effects of PIs on adipocyte differentiation has been described in cultured cells, probably acting at the step of sterol regulatory element-binding protein maturation and nucleus localization [53]. These PI effects have not been reported *in vivo*.

It is unclear whether fat accumulation and lipodystrophy are part of the same phenomenon or not. If central fat accumulation is a redirection of fat normally stored in peripheral adipocytes, then the best treatment of fat accumulation would be treatment of lipodystrophy. If, however, fat accumulation is an effect of aging, of recovery from HIV wasting or a separate direct effect

Table 3. Management of metabolic abnormalities and cardiovascular risks factors

Metabolic abnormality	Screening	Intervention	Comments for all metabolic abnormalities considered, low-lipid diet, low-calorie diet and increased aerobic exercise
Predominantly increased TG	Before starting cART and annually thereafter	Antiretroviral switch, fibrates, fish oil, niacin	Contribution of increased TG to cardiovascular risk unclear. TG decreased by 20–25%, possibly a greater effect with fenofibrate than with gemfibrozil. Fibrates have minimal effect on insulin resistance and TC and LDL cholesterol might be less effective than in HIV-negative adults. Fish oil and extended-released formulations of niacin can also be used. Lopinavir/ritonavir is the drug that is the most frequently associated with TG increase.
Increased TC and LDL cholesterol	Before starting cART and annually thereafter	Antiretroviral switch, HMG-CoA reductase inhibitor (statin)	Switching from stavudine to abacavir or tenofovir does not carry excess risk of virological failure in a virologically suppressed patient. Switch from boosted protease inhibitor to nevirapine or unboosted atazanavir. Cardiovascular benefit of lowering TC not proven to be similar in HIV-positive and HIV-negative individuals. Switching strategies has not been compared in a randomized manner with aggressive management of cardiovascular risk factors. With statin therapy, TC and LDL cholesterol decreased by approximately 25%, but there was no change in insulin resistance or TG. Pravastatin is preferred as the initial cholesterol-lowering agent because it has no significant cytochrome P450-mediated interaction with ART. Rosuvastatin and atorvastatin should be used with caution for patients on boosted protease inhibitors. Efavirenz decreases statin exposure (careful increase of statin dose should be considered). Statins might improve endothelial function. There is insufficient data on when or how to use ezetimide in HIV-infected individuals.
Low levels of HDL cholesterol	Before starting cART and annually thereafter	HIV replication, antiretroviral switch, fibrate	Effect of HIV RNA replication greater than ART effect: reducing viral replication is the most effective way to increase HDL cholesterol. Antiretroviral switch (nevirapine might increase HDL cholesterol levels). Lifestyle changes warranted (for example, increased exercise).
Type 2 diabetes mellitus	Fasting blood glucose at treatment initiation and every 6 months thereafter	Metformin thiazolidinedione	Medication only indicated in case of established diabetes mellitus (fasting blood glucose >7 mmol/l). Dietary guidelines can be applied to HIV-infected patients. Metformin improves insulin resistance, blood pressure and possibly hypertriglyceridaemia. It also decreases visceral fat accumulation. Thiazolidinedione improves insulin resistance. Rosiglitazone increases TC and LDL cholesterol. Pioglitazone might improve fat atrophy. Treatment should target the normalization of haemoglobin A _{1c} .

Cardiovascular risk factors should be evaluated using the Framingham equation. If >15% at 10 years then the following should be considered: stop smoking, control blood pressure, disorders of glucose metabolism and atherogenic lipid abnormalities. National Cholesterol Education Project Treatment guidelines should be used for HIV-infected individuals. Furthermore, cessation of abacavir should also be considered if a substitution is possible when cardiovascular risk is >15% at 10 years as calculated using the Framingham equation. ART, antiretroviral therapy; cART, combined antiretroviral therapy; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylgluaryl-coenzyme A; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides.

of ART, then the two conditions need to be managed separately. Risk factors for fat atrophy and accumulation are not identical [54], some cross-sectional studies have found no association between lipoatrophy and central lipohypertrophy [55] and improvement in fat atrophy after NRTI cessation has not been associated with visceral fat loss [56], suggesting that the second hypothesis might be more likely.

Description and prevalence

The prevalence of lipodystrophy ranges from 20% to 70% of patients receiving ART, depending on the type of ART, with much lower prevalence in more recent studies of newer agents, probably because of the avoidance of thymidine analogue nucleoside reverse transcriptase inhibitor (tNRTI) therapy [57]. Indeed the use of abacavir or tenofovir-based backbone regimens together with efavirenz is associated with a very low rate of lipoatrophy after 3 years of therapy [58,59], implying a dominant effect of tNRTI treatment in lipoatrophy.

Efavirenz has not traditionally been linked with the development of lipodystrophy [60]. One randomized, prospective study [61] revealed greater limb fat loss with nelfinavir treatment compared with efavirenz treatment over 144 weeks. A substudy of another trial of patients randomized to received either efavirenz or unboosted atazanavir with a zidovudine and lamivudine backbone showed similar effects of efavirenz and atazanavir on total and regional fat [62]. With this in mind, results from the ACTG 5142 study are more surprising: 32% of patients receiving two NRTIs plus efavirenz developed DEXA-defined lipoatrophy compared with 18% of patients receiving two NRTIs plus lopinavir/ritonavir and only 8% of patients receiving efavirenz and lopinavir/ritonavir [63]. The higher incidence of fat loss in those receiving efavirenz as compared with those receiving lopinavir/ritonavir was not expected. It is unknown whether this finding represents lipoatrophy from efavirenz or adipocyte growth from lopinavir/ritonavir. The 8% rate for tenofovir plus lamivudine was similar to that of the lopinavir/ritonavir-efavirenz group, suggesting that tenofovir and lamivudine cause little if any lipoatrophy. Increasing limb fat mass (without an increase in visceral fat or insulin resistance) has also been observed in other recent studies that evaluated boosted fosamprenavir or tipranavir with tenofovir and lamivudine [64,65].

Lipoatrophy and fat accumulation are not trivial: they can stigmatize patients and might reduce treatment adherence. Moreover, the obesity and associated lipid and glucose disturbances resemble the metabolic syndrome (MS). Although there are still controversies regarding the clinical significance of MS in HIV-infected patients receiving ART, HIV-infected adults with MS have more subclinical atherosclerosis [66] and have greater 3-year risks of incident diabetes and possibly of MI [67].

Management of lipoatrophy

Treatment strategies are disappointing: switching from tNRTI therapy has shown only modest improvements (around 0.4 kg per year) in limb fat mass [68,69], which suggests that lipoatrophy might take well over 5 years to resolve for many patients without additional intervention.

Reconstructive surgery (face-filling) is at least partially effective, but costly. Polyactic-L-acid (PLA) has been approved by the FDA. A randomized trial of PLA every 2 weeks on four occasions that included volumetric computerized tomography found PLA was safe, but increased facial soft tissue volume by only 3% [70]. Several medical interventions have been evaluated for lipoatrophy. Thiazolidinediones are agonists of the peroxisome proliferator-activated receptor- γ and are insulin-sensitizing drugs used for the treatment of type 2 diabetes mellitus. In young adults with congenital lipodystrophy, treatment with a thiazolidinedione (troglitazone, now withdrawn because of hepatotoxicity) resulted in improvement of peripheral fat mass [71]. Thiazolidinedione therapy had little if any benefit in six randomized trials evaluating rosiglitazone or pioglitazone [72,73]. Uridine is a pyrimidine precursor and so might replenish intracellular pyrimidine pools. *In vitro*, uridine abrogates the mitochondrial toxicity to adipocytes and hepatocytes of stavudine and zidovudine, but not of didanosine [74]. NucleomaxX[®], a nutritional supplement that increases plasma uridine levels, increased limb fat by 0.9 kg over 3 months in lipoatrophic adults receiving a tNRTI (zidovudine or stavudine) relative to placebo, an effect seemingly far greater than with tNRTI switching [75]. One question remaining is whether uridine might have any benefit in patients no longer receiving a tNRTI.

Limb fat increased significantly with 12 weeks of pravastatin 40 mg [76], an effect not related to its cholesterol-lowering effect, suggesting a different mechanism. This effect was not observed, however, in another small randomized study [77].

Management of lipohypertrophy

Metformin, growth hormones and growth hormone analogues might reduce visceral fat accumulation. Metformin and growth hormone both improve visceral adiposity in HIV-infected individuals, but effects were overall modest and adverse effects of growth hormone were frequent [78,79]. Growth hormones were denied approval for the treatment of HIV-related fat accumulation.

Visceral fat was reduced by 15.2% in lipodystrophic adults randomized to receive the growth hormone-releasing hormone analogue, tesamorelin (2 mg daily for 26 weeks) [80]. Tesamorelin, however, is not yet approved for this indication and is not available commercially.

Prevention

The optimal management of lipodystrophy includes prevention through the use of drugs, such as abacavir, lamivudine, tenofovir, emtricitabine, nevirapine, lopinavir/ritonavir and atazanavir/ritonavir. The effect of new drug classes, such as integrase or CCR5 inhibitors, is unknown. Lifestyle interventions, including a caloric-restrictive diet and aerobic exercise might reduce central fat accumulation, but might aggravate lipoatrophy [81]. The management of related lipid and glucose disturbance do not differ in general from HIV-uninfected patients, but drug interactions might limit therapeutic options (Table 3). It remains unknown whether changing ART or lipid-lowering therapy is the safest and more effective strategy.

Osteoporosis and osteonecrosis

Background

Osteopaenia, osteoporosis and osteonecrosis have been reported in patients infected with HIV and their aetiologies remain unclear. In particular, the role of ART is unclear as reduced bone mineral density (BMD) is common in both ART-naïve and ART-treated patients.

Description and prevalence

Symptomatic osteonecrosis affects 0.1–1.3% of HIV-infected patients and asymptomatic osteonecrosis (detected with magnetic resonance imaging) affects 4% of patients [82]. More than 85% of cases involved one or both femoral heads. Controlled epidemiological studies do not support a direct link with ART and approximately one-third of HIV-infected individuals with osteonecrosis have traditional risk factors, such as use of corticosteroids, alcohol abuse or use of megestrol acetate.

A systematic review of cross-sectional studies found that HIV-infected adults had a 6.4-fold increased odds ratio of reduction in BMD and a 3.7-fold increased odds ratio of osteoporosis compared with uninfected controls [83]. Classic risk factors, such as low body mass index, history of weight loss, corticosteroid use and smoking, together with the duration of HIV infection were identified [84]. Most studies do not include sufficient women to draw sex-specific conclusions. The role of ART as such is not established, but use of boosted PIs has been associated with a greater frequency of osteopaenia [83].

Prospective studies are less numerous, but suggest that BMD decreases over the first 1–2 years after ART initiation and then might remain relatively stable. One randomized trial compared tenofovir with stavudine (administered together with lamivudine and efavirenz). At week 144, there was a greater decrease from baseline of BMD in the lumbar spine and hip in patients treated with tenofovir as compared with those on stavudine [59]. A large cohort study reported that HIV-infected

adults had significantly higher prevalence of vertebral, hip, wrist and combined fractures compared with non-HIV-infected patients [85]. If confirmed, this would suggest that more aggressive diagnosis and management of low BMD is warranted.

Management

Preventive measures (such as physical exercise, sufficient ingestion of calcium and vitamin D) and elimination of risk factors (such as alcohol, tobacco and poor diet) are warranted. Alendronate treatment for 48 weeks increased BMD in HIV-infected adults with established osteopaenia [86]. It is reasonable to screen patients with numerous risk factors for osteopaenia with DEXA.

The World Health Organization (WHO) recently developed a fracture risk assessment tool to estimate 10-year fracture risk [87]. This algorithm has not yet been validated in HIV-positive populations, but might be a useful tool to assess the need for treatment in an individual patient.

Renal disease

Background

Renal toxicity has been mainly associated with two drugs: indinavir and tenofovir. Severe toxicity is rare; history of renal disease, as well as concomitant use of nephrotoxic drugs are recognized risk factors [88]. More rarely, atazanavir has been associated with nephrolithiasis.

Description and prevalence

Indinavir nephrotoxicity generally manifests with renal colic, but can also present as gradual onset tubulointerstitial nephritis or, rarely, acute renal failure. Reduced doses of indinavir cause less nephrotoxicity [89].

Tenofovir is associated with a direct tubular and a glomerular toxicity and its use has been linked to a 10% reduction in estimated glomerular filtration rate (eGFR) over the first year, after which time renal function appeared to stabilize [59,90]. In an analysis of 1,111 patients in two randomized trials, small differences in eGFR over time were noted, but no clinically relevant renal disease or adverse events were demonstrated in antiretroviral-naïve patients treated with tenofovir through 144 weeks [91]. Renal events were the most commonly reported serious adverse drug reactions in post-marketing safety data including 455,392 person-years of exposure to tenofovir [92]. A renal serious adverse event of any type was observed in 0.5% of patients and graded increases in serum creatinine occurred in 2.2%. In cohort studies, approximately 2–3% of adults receiving tenofovir for an average of about 12 months had moderate or severe renal dysfunction (eGFR < 60 ml/min) [93]. Risk factors in these cohorts were advanced HIV disease, anaemia, diabetes,

Box 1. Specific issues related to antiretroviral drugs in resource-limited settings

Issues related to antiretroviral drugs

- Use of fixed-dose combination (stavudine plus lamivudine plus nevirapine)
- Efavirenz teratogenicity
- Abacavir hypersensitivity
- Renal toxicity of tenofovir
- Long-term use of stavudine
- Lactic acidosis

Management

- Provide enough drug formulary to be able to switch a single drug in case of toxicity
- Pregnant women in the first trimester should avoid efavirenz, but efavirenz is safe in the second and third trimesters
- Risk of hypersensitivity reactions on abacavir are less frequent in sub-Saharan Africa than in western Europe and in the US
- Clinical guidelines to manage hypersensitivity in resource-limited settings should be provided
- Tenofovir use might be restricted to patients without risks factors for renal diseases (for example, older age, diabetes and hypertension) unless creatinine monitoring is available twice yearly

hypertension, use of tenofovir and use of low-dose ritonavir, which increased plasma tenofovir levels by approximately 30%.

Clinicians should be alert to the manifestations of tubular dysfunction, such as hypophosphataemia, metabolic acidosis, glycosuria and aminoaciduria, which can develop at any time in patients receiving tenofovir, particularly in those with other renal risk factors [94].

Management and perspective

Renal function (eGFR) should be assessed in all patients receiving tenofovir or indinavir approximately every 3 months by use of the Modification of Diet in Renal Disease equation, which is a more sensitive and accurate measure of renal dysfunction than serum creatinine level [95].

Toxicity in resource-limited settings

First-line ART is now reaching >3 million people in low and middle income countries [96]. Several dilemmas, including the balance between cost and toxicity, is a major ongoing challenge in resource-limited settings (Box 1).

Because of the limited availability, cost and convenience of fixed-dose combinations, most patients receive a first-line regimen, including nevirapine, lamivudine, zidovudine or stavudine. In the rush to put as many patients as possible on a potent ART, limited attention has been given to side effects. Very little or no laboratory monitoring is available. Thus, most available data relied on clinical observation and subjective report to assess adverse events.

Stavudine toxicity is of similar or greater extent in resource-limited setting to that observed in Caucasian populations of wealthy countries; 34% of patients presented with lipodystrophy in Rwanda and 46% in western India [97,98]. In South Africa, the main causes of stavudine cessation were peripheral neuropathy, sensory neuropathy, lactic acidosis and lipodystrophy [99]. Although well tolerated over the first 6 months, nearly a third of patients discontinued stavudine as a result of toxicity within 3 years, a higher rate than the 8% discontinuation rates for zidovudine and nevirapine over the same period [99].

The use of stavudine has decreased greatly in countries that can afford second-generation alternative NRTIs. The WHO now recommends that stavudine be used at the dose of 30 mg twice daily for all adults regardless of weight [100], but also recommends that first-line regimens move away from a stavudine-based regimen to tenofovir, abacavir or even zidovudine [101].

Two years after these recommendations, only three countries have adopted tenofovir as a first-line regimen in national guidelines. Among other factors, such as cost or formulation, concern regarding renal toxicity is an obstacle to wider use of tenofovir in countries without access to routine laboratory monitoring. An analysis of creatinine clearance among all 3,316 participants in the Development of Antiretroviral Therapy in Africa (DART) study showed that 45% of participants had mild renal insufficiency as estimated by eGFR at baseline, but only 7% was moderate and 0.2% was grade 3 or 4 [102]. Over 96 weeks, serum creatinine increased over the first 60 weeks. Of the patients who subsequently developed grade 3 or 4 renal impairment, tenofovir use was not a significant risk factor. Several questions, such as the relevance of a weight-based creatinine clearance calculation in severely wasted patients, are still debated. Using baseline body weight to calculate creatinine clearance might exclude treatment from those who could benefit from tenofovir.

The spectrum of adverse events in resource-limited settings can differ from those observed in Europe, North America and Australia [103], perhaps because of different demographics (Box 2). Most patients that access care at a late stage of disease are women. Moreover, exposure to malnutrition, sepsis and various diseases, either opportunistic infections (such as tuberculosis and cryptococcus) or not (such as malaria), is highly prevalent. Stevens–Johnson syndrome and severe hepatitis are very rarely reported, but it is difficult to rule out the possibility that side effects requiring laboratory monitoring are missed by the current, non-standardized reporting system. In India, 1,443 ART-naïve patients received stavudine- or zidovudine-containing regimens and rash (66%), hepatotoxicity (27%) and anaemia (23%) were the most common adverse events reported

[104]. The incidence of nevirapine-related hepatotoxicity and rash in a Thai adult population was similar to that reported in other cohorts. However, pregnant women with high CD4⁺ T-cell counts were at increased risk of severe hepatotoxicity [105]. In a report from the DART trial conducted in Uganda and Zimbabwe, 219 (6.6%) of 3,314 participants developed grade 4 anaemia by week 48. Abacavir HSR was reported in 2% of participants, a lower rate than reported in Caucasian populations (8%) [106]. Zidovudine-related anaemia was less prevalent in patients on prior stavudine-containing ART [107].

Female-specific incidence rates have rarely been calculated as treatment was previously only available in wealthy countries where HIV-infected patients were mostly male; it is now important to take the opportunity to analyse data from countries in which more than half of treated patients are women. A review of 1,735 patients initiating ART in Soweto, South Africa showed an exceptionally high rate of lactic acidosis in women (16.1 cases per 1,000 patient years) relative to men (1.2 cases per 1,000 patient years) [108]. Nevirapine, the most common drug used in first-line regimen beside stavudine, is also associated with a sex difference in toxicity. The fact that this was only found in post-marketing studies in early 2004 is attributable to the fact that the drug was not initially studied in large populations of women [109].

No data are available on long-term side effects and very few studies have looked systematically at metabolic abnormalities, body composition and CVD in these populations. A systematic and standardized data collection should be encouraged.

Recommendations to reduce adverse events in resource-limited settings include the replacement of stavudine, advocacy for the availability of a larger drug formulary to allow drug substitution, the use of point-of-care laboratory monitoring and initiation of ART before the onset of advanced deficiency.

New and investigational drugs

Raltegravir

In two randomized double-blind placebo-controlled trials, serious drug-related adverse events occurred in 2.2% of raltegravir recipients versus 0% of placebo recipients and adverse events leading to drug discontinuation were reported for 1.7% of the patients. Nausea, headache, fever and creatine phosphokinase increases were among the few adverse events reported [110,111]. Cancers have been reported in early studies on pretreated patients receiving raltegravir. Many of those cancers were of the type expected in very immunodeficient individuals and additional risk factors, such as smoking, chronic viral hepatitis and papillomavirus infection, were often present and

Box 2. Specific issues related to the characteristics of the patient population in resource-limited settings

Issues related to the characteristics of the patient population

- Gender
- HIV late stage disease
- Concomitant infection
 - Tuberculosis
 - Malaria
 - Malnutrition
- Lack of data on new drugs for specific populations
- Immune reconstitution syndrome

Comments

- Clinical trials for registration and approval of new compounds must be conducted in resource-limited settings in addition to developed countries

the cancer rate was similar to that in controls with a longer follow-up. No evidence of mutagenicity or genotoxicity was observed *in vitro* during microbial mutagenesis tests, assays for DNA breakage or during *in vitro* and *in vivo* chromosomal aberration studies.

Overall, raltegravir is well tolerated at the effective dose and toxicity seems to be minimal with the available follow-up time. Post-marketing studies will need to include more women, who comprised only 13% of participants included in clinical trials.

CCR5 inhibitors

The development of CCR5 inhibitors drug class was marked by the discontinuation of the development of aplaviroc because of hepatotoxicity [112]. Also, the occurrence of increased rates of lymphoma in vicriviroc (another CCR5 inhibitor) recipients in one study raised the fear that this class might promote haematological malignancy by inhibition of CCR5 [113,114].

Maraviroc, another CCR5 antagonist, was approved in 2007 on the basis of the MOTIVATE trials conducted in ART-experienced adults [115]. Although discontinuation rates as a result of side effects were slightly higher in the maraviroc groups compared with the placebo groups, the differences were not statistically significant. Hepatotoxicity has been rarely reported and can be preceded by symptoms of hypersensitivity. Maraviroc can bind α receptors and can cause hypotension at doses higher than used in clinical practice as well as sinus congestion in a small proportion of patients. The drug was associated with an increased risk of malignancy in one study, but this has not been found in other studies. Although this class of drug raises the theoretical risk of interference with cardiac potassium conduction, no evidence of abnormal cardiac conduction (prolonged QT interval) or arrhythmias has been evident in extensive prelicensing investigation.

Table 4. Recent advances in the understanding of antiretroviral toxicity

Adverse event	New learning points	Most promising therapies
Lipoatrophy	Largely preventable by avoidance of stavudine and zidovudine. Contribution of current generation protease inhibitors are less certain (lopinavir/ritonavir, atazanavir/ritonavir, fosamprenavir/ritonavir and darunavir/ritonavir). Annual DEXA should be considered in those receiving stavudine, zidovudine or a protease inhibitor.	Stavudine and zidovudine substitution is helpful, but improvement is very gradual. Pioglitazone (in those not receiving stavudine). Drugs under investigation include uridine and pravastatin.
Central fat accumulation	Treatment directions limited by uncertainty as to whether central fat accumulation is a direct drug effect or is secondary to lipoatrophy.	Growth hormone treatment. Tesamorelin treatment (growth hormone-releasing hormone analogue). Metformin treatment.
Dyslipidaemia	Diet and exercise supervised by a dietitian reduces total cholesterol and triglycerides.	Protease inhibitor and/or thymidine analogue nucleoside/nucleotide reverse transcriptase inhibitor cessation. Pravastatin, low-dose atorvastatin or rosuvastatin for hypercholesterolaemia. Fibrate for hypertriglyceridaemia. Target value similar to HIV-negative individuals.
Insulin resistance or diabetes	Fasting glucose is a poor tool for diagnosis of diabetes. Oral glucose tolerance testing should be considered in higher risk patients.	Standard diabetic treatment guidelines should be followed.
Cardiovascular disease	Withdrawal of ART increases risk, perhaps because of reduced HDL cholesterol levels or increased inflammation. Traditional risk factors affect risk more than ART. Abacavir might increase cardiovascular risk, particularly in those already at high risk (Framingham score >20% at 10 years).	The Framingham score should be used to assess cardiovascular risk in all HIV-positive individuals and to monitor responses to intervention. Similar targets for dyslipidaemia correction should be used. All modifiable risk factors, such as smoking, hypertension and diabetes, along with increased total cholesterol should be addressed. Suppressing HIV replication reduces cardiovascular risk.
Hepatotoxicity	Nevirapine should be initiated only in ART-naïve men and women with CD4 ⁺ T-cell counts <400 and <250 cells/μl, respectively. Didanosine is associated (rarely) with hepatic fibrosis, nodular regenerative hyperplasia and portal hypertension.	Hepatotoxicity less prevalent in patients starting nevirapine when virologically suppressed.
Hypersensitivity	Abacavir hypersensitivity strongly linked to <i>HLA-B*5701</i> ancestral haplotype.	Molecular testing for <i>HLA-B*5701</i> prevents almost all, if not all, immunologically-mediated abacavir hypersensitivity.
Osteoporosis	Tenofovir is associated with slightly increased risk of osteopaenia over 3 years (lumbar spine), but not with increased fracture rate. Role of routine screening (bone mineral densitometry) is unknown.	Alendronate treatment can be considered.
Nephrotoxicity	Tenofovir is associated with slightly increased risk of grade 3–4 proximal tubular nephropathy.	Creatinine and measurement of creatinine clearance at baseline and follow-up at least twice a year.
Enfuvirtide injection site reactions	Occurs in 98% of patients and does not abate over time. Has substantially affected enfuvirtide use.	Might be less severe with use of a needle-free injection device.

ART, antiretroviral therapy; HDL, high-density lipoprotein.

Recommendations and conclusions

The arrival of triple antiretroviral combinations in 1996 revolutionized HIV disease to the same extent as did penicillin for pneumococcal disease in 1945. In 2008, HIV/AIDS has become a chronic disease with most challenges related to the long-term safety and tolerability of drugs. A summary of recent advances in the understanding of antiretroviral toxicity can be found in Table 4.

As new drugs are generally marketed with data on a limited number of patients and a limited follow-up (48 weeks), pharmacovigilance is warranted. Accessing updated information is crucial with regards to new drugs safety data and use of validated references, such as treatment guidelines or regulatory authorities website, is a useful tool. The newer drugs seem to have a better safety profile than older ones; if their tolerability holds up, lipodystrophy and mitochondrial toxicity might disappear within the next decade. Interruption trials have

raised the role of HIV RNA replication in several organ diseases that were thought to be a result of drug-related adverse events.

Disclosure statement

The authors declare no competing interests.

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Clinical update: adverse effects of antiretroviral therapy

See [New Drug Class](#) page 81

See [Online](#) for webtable

The adverse effects of antiretroviral therapy (ART) cause substantial morbidity and compromise adherence, which can lead to drug resistance. Treatment guidelines recommend against universal immediate ART, partly because of toxicities (table). The webtable lists licensed ARTs.

HIV lipodystrophy is characterised by peripheral subcutaneous lipoatrophy, relative central fat accumulation, lipomatosis, dyslipidaemia, insulin resistance, and hyperlactataemia. Over the first 6 months of ART, limb and visceral fat increase, often followed by a progressive and selective loss of limb fat.¹ Zidovudine and stavudine are the drugs most associated with lipoatrophy. About 30% of patients receiving stavudine for 2 years developed lipoatrophy compared with 6% of tenofovir recipients.² Abacavir, emtricitabine, lamivudine, and tenofovir do not seem to induce much lipoatrophy; the protease inhibitor nelfinavir accelerated lipoatrophy, but another such drug, lopinavir, might prevent it.³ Lipoatrophy can be largely prevented by avoidance of stavudine and zidovudine. Switching stavudine or zidovudine to tenofovir or abacavir improves lipoatrophy, but normalisation can take years.⁴ Cessation of protease inhibitor therapy may improve visceral adiposity.

Thiazolidinediones have a small or no effect on HIV lipoatrophy.⁵ Uridine and pravastatin may improve lipoatrophy more rapidly and substantially than zidovudine or stavudine switching.^{6,7} Cosmetic interventions, such as poly-L-lactic acid injections, modestly improve facial lipoatrophy. Growth hormone and a growth-hormone releasing-hormone analogue improve visceral abdominal fat accumulation, but aggravate lipoatrophy and, at least for growth hormone, insulin resistance and dyslipidaemia.¹ Metformin also improves visceral adiposity, but the effect is modest.

Most protease inhibitors (except unboosted atazanavir, but including low-dose ritonavir), efavirenz, stavudine, and zidovudine increase total cholesterol, LDL-cholesterol, and triglyceride concentrations.¹ The change in risk of cardiovascular disease with ART (estimated by the Framingham equation) is generally not substantial unless other cardiovascular risk factors are present, perhaps partly because most potent ART also increases HDL-cholesterol concentrations.

Diabetes mellitus occurs in 6–10% of those receiving ART. Risk factors include increasing age, obesity, family

history, lipoatrophy and fat accumulation (and the ARTs that cause them), metabolic syndrome, and hepatitis C infection.⁸ Stavudine, indinavir, ritonavir (even at the low boosting dose) and lopinavir, but not atazanavir or amprenavir, induce insulin resistance acutely, but long-term effects are unknown. Fasting blood glucose is a poor measure of insulin resistance and diagnoses less than half of all diabetes in HIV-infected adults. When other cardiovascular risks factors are present, an oral glucose tolerance test should be considered. Metformin improves insulin sensitivity and systolic blood pressure, and may reduce visceral fat. Thiazolidinediones also improve insulin sensitivity; rosiglitazone, but not pioglitazone, aggravates dyslipidaemia.

A large prospective cohort study reported a 16% relative increase in the incidence of myocardial infarction per year of ART exposure, an effect mainly associated with the duration of protease inhibitor therapy.⁹ About half of this association was explained by hypercholesterolaemia, hypertriglyceridaemia, and low HDL-cholesterol. Nevertheless, traditional risk factors (older age, being male, smoking, hypertension, diabetes, pre-ART dyslipidaemia) are collectively more important than ART. The overall cardiovascular risk associated with ART is declining, possibly because of the increasing use of lipid-lowering drugs, less smoking, and use of more lipid-neutral ART.⁹ Surprisingly, however, intermittent ART modestly increased rather than decreased the cardiovascular event rate compared with continuous ART. This increase may relate to the observed slight increase in the ratio of total cholesterol to HDL-cholesterol with intermittent ART.¹⁰

All cardiovascular risk factors should be assessed before starting ART and about annually thereafter. Cessation of smoking is likely to be the single most effective intervention. Intensive diet and exercise over 6 months improved central obesity and systolic blood pressure in HIV-infected adults with metabolic syndrome, but lipid abnormalities did not improve.¹¹

For hypercholesterolaemia, pravastatin is most used because its metabolism is unaffected by ART, although its effects may be less than in HIV-uninfected adults. Plasma concentrations of more potent statins, such as rosuvastatin and atorvastatin, can be increased by protease inhibitors, which increases the risk of statin

toxicity. If used, these statins should be started at lower than normal doses. Ezetimide is also effective at lowering LDL-cholesterol. The other option for dyslipidaemia is to switch boosted protease inhibitors, efavirenz, and/or some nucleoside analogues to other virologically active lipid-neutral ART. Because ART is generally given long-term, switching is an attractive option because it avoids permanently treating drug toxicity with another drug.

Mechanisms of hepatotoxicity include direct anti-retroviral toxicity, hypersensitivity, immune reconstitution in those with chronic viral hepatitis, and steatohepatitis secondary to mitochondrial toxicity caused by nucleoside reverse-transcriptase inhibitors. Liver toxicity is generally more frequent in patients with chronic viral hepatitis or increased baseline hepatic aminotransferases and in people who abuse alcohol. 5% of nevirapine-exposed patients develop hepatitis in the first 3 months of therapy, and in half of these a rash also occurs. Because immunocompetence is a significant risk factor, guidelines recommend that nevirapine be started only in ART-naïve men and women with CD4+ lymphocyte counts less than 400 and 250 cells per μL , respectively.¹² Patients already receiving ART who switch to nevirapine above these CD4 thresholds may not have this greater risk of hepatitis.¹³ Idiopathic hepatic fibrosis was found in 2% of HIV-infected adults and was related to cumulative didanosine exposure. Moreover, long-term didanosine therapy is rarely linked to nodular regenerative hyperplasia of the liver.¹⁴

Tenofovir can induce nephrotoxicity. In two phase III trials, no tenofovir-induced grade 3 or 4 nephrotoxicity (estimated glomerular filtration rate [eGFR] less than 50 mL per min per 1.73 m^2) was observed, although eGFR fell by a mean 10%.¹⁵ In cohorts, however, about 4% of tenofovir recipients had decreased creatinine clearance of more than 50% after a median 12 months, a rate about 60% greater than that without tenofovir.¹⁶ Risk factors for this significant GFR decline were a low CD4+ count, hypertension, anaemia, impaired renal function, injection drug use, diabetes, and the use of a boosted protease inhibitor, which increases tenofovir levels by about 30%. Consensus guidelines recommend that eGFR be estimated before ART and at least every 6 months in those receiving tenofovir.

Abacavir causes a hypersensitivity reaction, including fever, rash, fatigue, and gastrointestinal symptoms after a mean 10 days in 8% of unselected white adults, but less

Side-effect	New learning points	Most promising therapies*
Lipoatrophy	Largely preventable by avoidance of stavudine and zidovudine Contribution of protease inhibitors less certain Consider annual DEXA in those receiving stavudine, zidovudine, or protease inhibitor	Stavudine and zidovudine cessation helpful but improvement very gradual Pioglitazone (in those not receiving stavudine) Uridine Pravastatin
Central fat accumulation	Treatment directions limited by uncertainty about whether central fat accumulation is direct drug effect or secondary to lipoatrophy	Growth hormone Growth-hormone releasing-hormone analogue Metformin
Dyslipidaemia	No proven benefit for diet or exercise	Protease inhibitor and/or tNRTI cessation Pravastatin, low-dose atorvastatin, or rosuvastatin
Insulin resistance/diabetes	Fasting glucose is poor tool for diagnosis of diabetes Consider oral glucose tolerance testing in higher-risk patients	Follow standard diabetic treatment guidelines
Cardiovascular disease	Withdrawal of ART increases risk, perhaps because of declines in HDL-cholesterol Traditional risk factors affect risk more than ART	Address all risk factors, such as smoking, hypertension and diabetes, not just raised total cholesterol
Hepatotoxicity	Nevirapine be initiated only in ART-naïve men and women with CD4+ lymphocyte counts <400 and 250 cells per μL , respectively Didanosine associated rarely with hepatic fibrosis, nodular regenerative hyperplasia, and portal hypertension	Nil
Hypersensitivity	Abacavir hypersensitivity strongly linked to HLAB*5701 ancestral haplotype	Molecular testing for HLAB*5701 may prevent most abacavir hypersensitivity
Osteoporosis	Tenofovir associated with small increased risk of osteopenia over 3 years, but not with increased fracture rate Role of routine screening (bone mineral densitometry) unknown	Alendronate
Nephrotoxicity	Tenofovir associated with small increased risk of grade 3–4 nephrotoxicity	Nil
Enfuvirtide injection-site reactions	Occurs in 98% of patients and does not abate over time Has substantially affected use of enfuvirtide	Maybe less severe with use of needle-free injection device

DEXA=dual-energy X-ray absorptiometry. tNRTI=thymidine nucleoside reverse-transcriptase inhibitor. *In addition to drug withdrawal and avoidance in higher-risk patients.

Table: Recent advances in understanding of antiretroviral toxicity

in black Americans and Africans. Deaths have occurred after unsupervised rechallenge, which is contraindicated. Adults with the HLA-B*5701 haplotype are at very high risk of abacavir hypersensitivity. Molecular screening for HLA-B*5701 has positive and negative predictive values of about 80% and 96%, respectively. Screening decreased hypersensitivity from 9% to 2%, and lowered the cessation rate in those with uncertain symptoms.¹⁷

HIV-infected adults receiving ART have more osteopenia than uninfected adults. The relative contributions of ART, HIV, and patients' characteristics are unknown. Tenofovir is associated with significantly more osteopenia than stavudine. No study has been large enough to assess whether the low bone-mineral density (BMD) will

translate into an increased fracture rate. Measurement of BMD should be considered in high-risk patients. Alendronate for 48 weeks significantly increased BMD in osteopenic adults on ART.

There are some important differences in the pattern of toxicity in resource-poor settings, in which most patients start ART with nevirapine, lamivudine, and either stavudine or zidovudine. Because of the few alternatives, patients often endure chronic toxicity to receive the benefits of ART. A South African study showed a 1.5% annual risk of symptomatic hyperlactataemia with stavudine (although rechallenge with zidovudine in some patients was reported as safe).¹⁸ Risk of nevirapine rash was 2.8 times higher in Thai adults than white adults.¹⁹ In Africa, grade 2–4 anaemia occurred in 12% of adults, compared with 2% in Europe and the USA.²⁰ Low CD4+ count, female sex, anaemia, low body-mass index, and being ART-naïve were the main risk factors. Issues of toxicity led WHO to change its ART guidelines in 2006 and exclude stavudine from first-line ART. If used, the recommended dose of stavudine is now 30 mg twice daily, regardless of weight.

10–25% of patients starting ART develop an immune reconstitution inflammatory syndrome: fever, a constitutional illness, and inflammation at the site of previously unrecognised replication of an opportunistic pathogen, such as *Mycobacterium tuberculosis*. This illness is more common in patients with advanced immunosuppression. The syndrome is not a drug-specific side-effect, rather a consequence of rapidly improving immune function.

More potential toxicities are now studied preclinically, in particular mitochondrial toxicity. Several drugs have been or will soon be withdrawn from the market, in part for toxicity: soft-gel saquinavir (diarrhoea, nausea), zalcitabine (peripheral neuropathy), and extended-release stavudine (pancreatitis, lipodystrophy, and peripheral sensory neuropathy). A continuing issue for a disease requiring life-long therapy is the short duration of registrational randomised trials, usually no more than 2 years. There is a need for systematic pharmacovigilance.

*Alexandra Calmy, Bernard Hirschel, David A Cooper, Andrew Carr

St Vincent's Hospital, Sydney 2010, NSW, Australia (A Calmy, DAC, A Carr); Service des Maladies Infectieuses, Hôpital Cantonal Universitaire de Genève, Geneva, Switzerland (A Calmy, BH); and National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, NSW, Australia (DAC) acalmy@gmail.com

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ORIGINAL RESEARCH

Combination antiretroviral therapy without a nucleoside reverse transcriptase inhibitor: experience from 334 patients in three cohorts

A Calmy,¹ K Petoumenos,² C Lewden,^{3,4} M Law,² F Bocquentin,⁴ K Hesse,¹ D Cooper,^{1,2} A Carr¹ and F Bonnet^{2,3,4} for the Aquitaine Cohort, Australian HIV Observational Database and St Vincent's Hospital Cohort study groups*

¹St Vincent's Hospital, Sydney, Australia, ²National Centre in HIV Epidemiology and Clinical Research, Sydney, Australia, ³INSERM U593, Université Victor Segalen Bordeaux 2, France, and ⁴Centre d'Information et de Soins de l'Immunodéficience Acquise (CISIH), CHU de Bordeaux, Bordeaux, France

Background

Toxicity and resistance may limit the use of HIV nucleoside reverse transcriptase inhibitors (NRTIs). We assessed the safety and activity of regimens that did not include an NRTI.

Method and patients

We analysed NRTI-sparing regimens using pooled data from three cohorts in Australia and France where HIV RNA viral load, CD4 lymphocyte count and metabolic parameters are assessed prospectively. The inclusion criterion was the commencement of any antiretroviral combination excluding NRTIs.

Results

A total of 334 (3.9%) of 8477 patients were included in the present study for a median follow-up time of 105 weeks. Therapeutic combinations were one nonnucleoside reverse transcriptase inhibitor (NNRTI) plus one protease inhibitor (PI) (58%), two PIs (26%), one PI (16%), and one NNRTI plus two PIs (8%). At baseline, the median CD4 lymphocyte count was 264 cells/ μ L (interquartile range 164–446 cells/ μ L) and 25% of patients had plasma HIV RNA below 500 HIV-1 RNA copies/mL. In intent-to-treat analysis, 64% of patients had HIV RNA < 500 copies/mL at 6 months and 68% at 24 months. The mean CD4 lymphocyte count increase was 60 cells/ μ L (95% confidence interval 41–76 cells/ μ L) at 6 months and 111 cells/ μ L (95% confidence interval 82–140 cells/ μ L) at 24 months. Prognostic factors for having HIV RNA < 500 copies/mL at 6 months included independently having undetectable HIV RNA at baseline and being naïve for NNRTIs. The proportion of patients with triglycerides > 2.3 mmol/L increased from 32% to 63% at 6 months and to 62% at 24 months (P -trend = 0.002), and those with total cholesterol > 6.2 mmol/L increased from 18% to 38% at 6 months and to 44% at 24 months (P -trend < 0.001), with an increased risk for patients treated with NNRTI + PIs. Forty-one per cent of patients discontinued their NRTI-sparing regimen.

Conclusion

In these antiretroviral-experienced patients, NRTI-sparing therapy appeared to have satisfactory virological and immunological efficacy. However, hyperlipidaemia was frequent and requires monitoring of cardiovascular risk factors.

Keywords: antiretroviral regimen, cohort, nucleoside reverse transcriptase inhibitor

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Correspondence: Dr Alexandra Calmy, HIV Immunology and Infectious Diseases Clinical Services Unit, St Vincent's Hospital, Victoria Street, Darlinghurst, 2010 NSW, Australia. Tel: 0061 (2) 8382 3872; fax: 0061 (2) 8382 4749; e-mail: acalmy@stvincents.com.au

*All investigators are listed in the Appendix.

Introduction

International guidelines recommend starting antiretroviral therapy with a combination of three drugs including two nucleoside or nucleotide reverse transcriptase inhibitors

(NRTIs) with either a protease inhibitor (PI) or a nonnucleoside reverse transcriptase inhibitor (NNRTI) [1]. The 2006 recommendations of the International AIDS Society-USA Panel suggest that changing a first regimen in experienced patients should be individualized from case to case according to the results of drug resistance testing and treatment history [1]. Many resistance mutations to NRTIs confer cross resistance. The tolerability and toxicity of NRTIs are other key issues when choosing a potentially life-long antiretroviral regimen, as these can compromise adherence. NRTIs can inhibit DNA γ -polymerase, and induce mitochondrial dysfunction, resulting in plasma hyperlactataemia and a large spectrum of illnesses: peripheral neuropathy, myopathies, steatohepatitis, pancreatitis, lipoatrophy, renal tubular acidosis, postnatal encephalopathy and lactic acidosis [2–5]. NRTI cessation is often required for these toxicities to improve or resolve. In these cases other antiretroviral combinations without NRTIs may have to be administered.

NRTI-sparing regimens have also been considered as an option for a first treatment [6] but, to date, very few data are available on their efficacy and their short- and long-term tolerance in clinical practice [7–9]. We hypothesized that, despite the lack of knowledge, NRTI-sparing regimens have been prescribed in clinical practice. Thus, we conducted an intercohort study to assess the use of these combinations.

Patients and method

We included patients recruited in the ANRS C03 Aquitaine Cohort, France, the Australian HIV Observational Database, and the Cohort of St Vincent's Hospital, Sydney, Australia.

The Aquitaine Cohort database is the hospital-based information system of the Groupe d'Epidémiologie Clinique du SIDA en Aquitaine (GECSA). Anonymous data on a predefined set of demographic, laboratory and clinical variables are collected at each patient's visit [10]. The Australian HIV Observational Database (AHOD) has prospectively collected data for HIV-infected adults at 27 sites throughout Australia since 1999. Anonymous data on a predefined set of demographic, laboratory (excluding metabolic data) and clinical variables are collected every 6 months [11]. The hospital-based cohort of St Vincent's Hospital, Sydney, Australia collects epidemiological, clinical and biological data and therapeutic histories of HIV-infected adults followed in the clinic. Data are electronically recorded on the day of the visit by a research nurse and clinicians.

Data were pooled based on the most recent data merge (at the time of analysis) for each of the three cohorts (31 December 2004 for the Aquitaine Cohort, 1 March 2004 for AHOD, and 30 March 2005 for St Vincent's Hospital).

The inclusion criterion in the present study was the commencement of any antiretroviral combination excluding NRTIs and tenofovir since 1 January 1997. NRTI-sparing regimens were categorized as protease inhibitor (PI) regimens (one or more PIs), or nonnucleoside reverse transcriptase inhibitor (NNRTI) + PI regimens (one NNRTI + one or more PIs). Ritonavir used as a booster was not counted as a PI. Data recorded up to 3 months prior to the start of an NRTI-sparing regimen were considered baseline data for the present analysis. Patients were categorized as lost to follow-up if no information was recorded for more than 12 months prior to the cut-off date. Reasons for starting or stopping NRTI-sparing regimens determined by the physician were: virological failure, toxicity (including lipodystrophy), poor adherence, patient choice, other and unknown. Mean change in biological variables [including HIV-1 RNA viral load, CD4 T-lymphocyte count, total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides] from baseline was determined for every 3-month period, up to 24 months. If not otherwise stated, all the analyses were conducted using the intent-to-treat approach (ITT): all analyses, including descriptive and endpoint statistics, included all patient follow-up data up to the time of censoring, regardless of their combination therapy at each time-point.

Factors associated with virological response (defined as a viral load <500 HIV-1 RNA copies/mL plasma at 6 months) and with immunological response (defined as an increase of at least 20% in the CD4 T-lymphocyte count at 6 months compared with the baseline value) were examined using multivariate logistic regression models. Covariates examined at baseline included: age, gender, calendar period of initiation of NRTI-sparing regimen by quartile (1999 and before, 2000–2001, 2002, and 2003 and after), known duration of HIV infection (<10 years vs ≥ 10 years), AIDS stage, duration of antiretroviral therapy (<5 years vs ≥ 5 years), number of previous antiretroviral regimens (0–1, 2–3, 4–6 and >6), number of NRTIs previously received (0, 1–3 and >3 NRTIs), number of NNRTIs previously received (0 vs ≥ 1), number of PIs previously received (0, 1–2 and >2 PIs), type of NRTI-sparing regimen (PI only vs PI + NNRTI), HIV-1 RNA at the start of the NRTI-sparing regimen (≤ 500 vs >500 copies/mL plasma) and CD4 T-lymphocyte counts stratified at baseline (<50 , 50–200, 201–350 and >350 cells/ μ L). The multivariate model was determined using the forward stepwise approach, considering only covariates that were significant at the 0.25 level in univariate analysis. A Cox proportional hazard model was used to assess the factors associated with NRTI-sparing regimen interruption.

General estimating equation (GEE) models accounting for repeated measures within individuals were used to

assess trends in mean change in CD4 T-lymphocyte count from baseline to months 3, 6, 9, 12, 15, 18 and 24. GEE methods were also used to assess trends in the proportion of patients with HIV-1 RNA below 500 copies/mL plasma from baseline to 24 months and trends in the proportion of patients with total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol above the cut-off for primary prevention [12]. All these analyses were adjusted by cohort. Analyses were performed using STATA software version 8.0 [13].

Results

Patient characteristics

A total of 334 (3.9%) of 8477 patients in the three cohorts (82 from the St Vincent's Hospital Cohort, 156 from the Aquitaine Cohort and 96 from AHOD) received an NRTI-sparing regimen during their follow-up and were included in the present analysis. Patient characteristics are shown in Table 1. NRTI-sparing regimens included the following combinations: one NNRTI plus one PI (50%), two PIs (26%), one PI (16%), and one NNRTI plus two PIs (8%). In NNRTI plus PI combinations ($n = 193$) the most frequent regimen used was efavirenz plus lopinavir/r ($n = 38$, 20%) followed by nevirapine plus indinavir ($n = 19$, 10%), nevirapine plus lopinavir/r ($n = 18$, 9%), and nevirapine plus saquinavir ($n = 16$, 8%). The most common PI used as a single agent ($n = 55$) was lopinavir/r ($n = 17$, 31%), followed by indinavir ($n = 10$, 18%) and saquinavir ($n = 7$, 13%). In the double PI regimen ($n = 86$), the most frequent combinations were saquinavir plus atazanavir ($n = 31$, 36%), saquinavir plus lopinavir ($n = 20$, 23%) and amprenavir plus lopinavir ($n = 19$, 22%).

The median follow-up time until censoring date was 105 weeks [interquartile range (IQR) 39–196]. Fifty-nine per cent of patients were still on an NRTI-sparing regimen at the censoring date. Baseline characteristics did not differ between patients lost to follow up ($n = 34$, 10%) and other patients (data not shown). Thirty-one patients (10%) experienced an AIDS-related illness ($n = 26$: Kaposi sarcoma, $n = 5$; disseminated *Mycobacterium avium intracellulare* infections, $n = 5$; cytomegalovirus infection, $n = 4$; non-Hodgkin lymphoma, $n = 2$; recurrent pneumopathy, $n = 2$; oesophageal candidiasis, $n = 2$; toxoplasmosis, $n = 2$; others, $n = 4$) and/or death ($n = 14$) during follow up.

Virological and immunological response

The proportion of patients with HIV RNA <500 copies/mL plasma rose from 25% at baseline to 64% at 6 months, 64%

Table 1 Epidemiological, biological and therapeutic characteristics of 334 patients starting an antiretroviral combination excluding a nucleoside reverse transcriptase inhibitor (NRTI)

Characteristics	
Gender (% male)	87
Age (years) [median (IQR)]	43.2 (38.1–50.2)
Transmission group (%)	
Men who have sex with men	60
Heterosexual contact	16
Injecting drug users	7
Blood recipients	2
Undetermined	15
CDC stage C (%)	33
Period of inception of NRTI-sparing regimen (%)	
1999 and before	21
2000–2001	15
2002	25
2003 and after	39
Known duration of HIV infection (years) [median (IQR)]	9.6 (6.7–13.4)
Duration of antiretroviral therapy before NRTI-sparing regimen (years) [median (IQR)]	5.2 (2.1–8.1)
Number of prior antiretroviral regimens [median (IQR)]	4 (2–7)
Number of NNRTIs received [median (IQR)]	1 (0–1)
Number of PIs received [median (IQR)]	2 (1–3)
Reasons for stopping NRTI (%)	
Virological failure	41
Toxicity	22
Poor adherence	10
Patient's choice	2
Others (protocol, pregnancy)	12
Unknown	12
NRTI-sparing combination therapy ^a (%)	
One NNRTI + one PI	50
Two PIs	26
One PI	16
One NNRTI + two PIs	8
Positive HCV antibodies ($n = 288$) (%)	15
Positive HBs antigen ($n = 273$) (%)	5
CD4 count (cells/ μ L) [median (IQR)]	264 (164–446)
HIV-1 RNA (copies/mL) [median (IQR)]	19550 (500–118650)
Total cholesterol (mmol/L) ($n = 143$) [median (IQR)]	5.0 (4.0–6.0)
Total cholesterol >6.2 mmol/L (%)	18
HDL-cholesterol (mmol/L) ($n = 90$) [median (IQR)]	1.0 (0.8–1.2)
HDL-cholesterol <0.9 mmol/L (%)	31
Triglycerides (mmol/L) ($n = 145$) [median (IQR)]	1.7 (1.2–3.1)
Triglycerides >2.3 mmol/L (%)	32

^aRitonavir used as a booster is not counted as one PI in these combinations. CDC, Centers for Disease Control and Prevention; Hepatitis B HBs Ag CHBs, HCV, hepatitis C virus; HDL, high-density lipoprotein; IQR, interquartile range; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor.

at 1 year and 68% at 2 years (Fig. 1). If patients were censored when NRTI-sparing therapy was stopped (as treated analysis), then the proportion of patients achieving plasma HIV RNA <500 copies/mL plasma on an NRTI-sparing therapy rose from 25% at baseline to 65% at 6 months, 66% at M12 and 79% at 24 months. Mean [95% confidence interval (CI)] CD4 T-lymphocyte count change

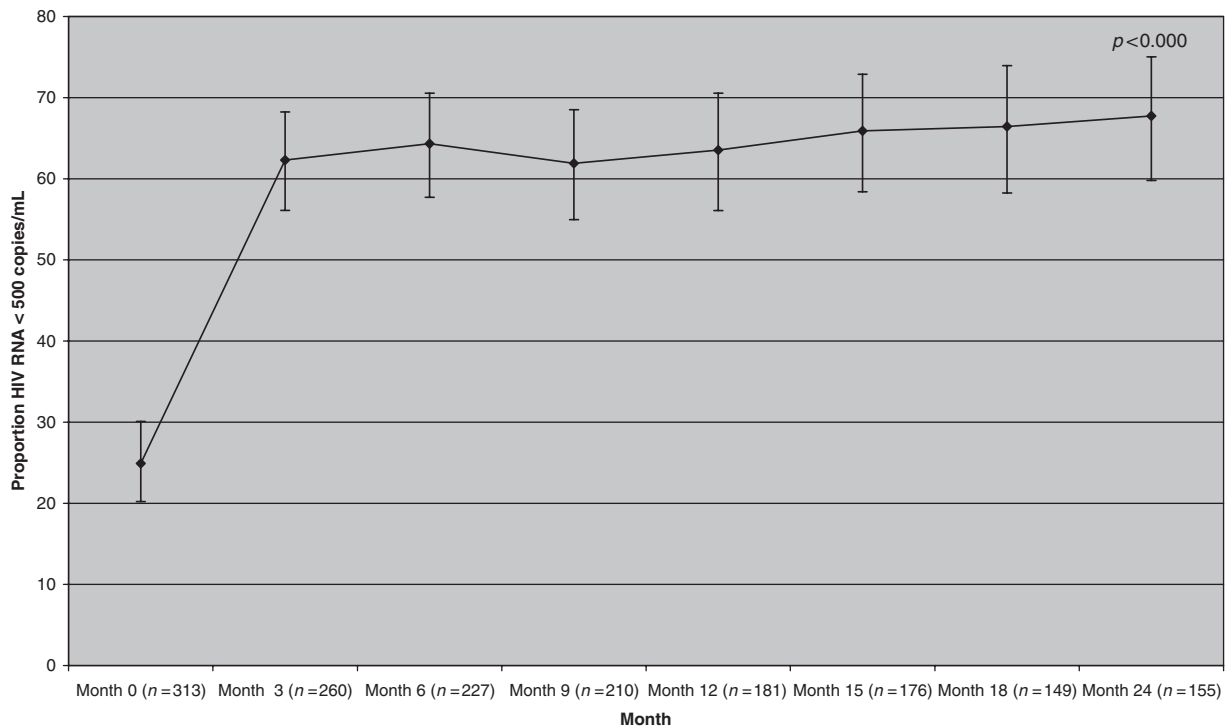


Fig. 1 Proportion of patients with HIV RNA < 500 copies/mL after starting a nucleoside reverse transcriptase inhibitor (NRTI)-sparing regimen.

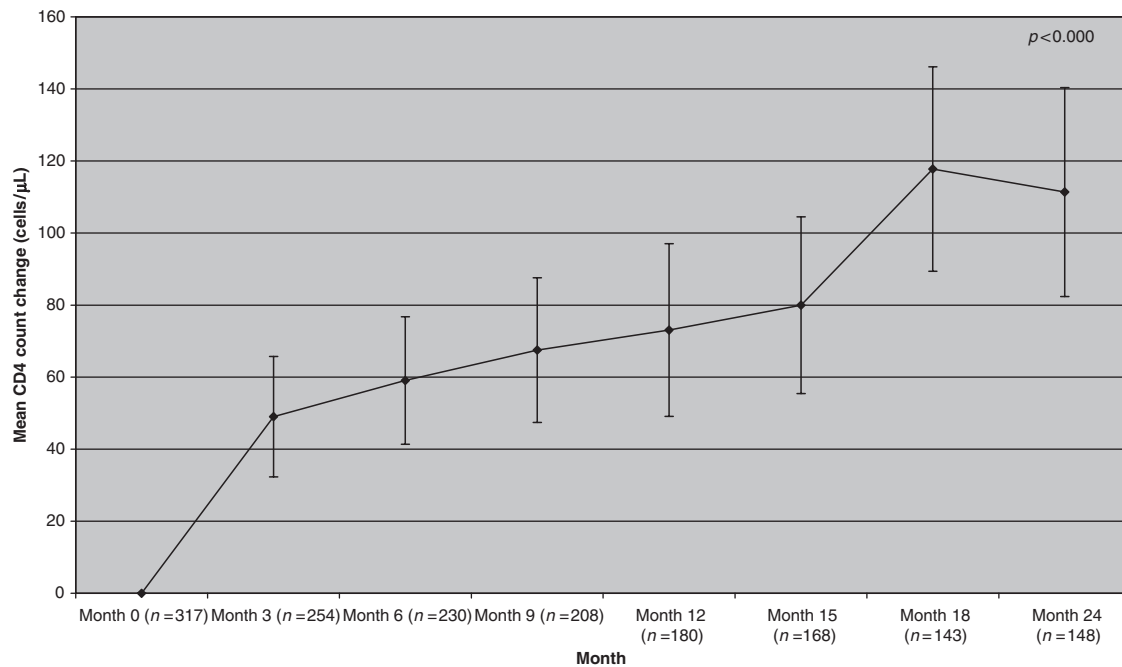


Fig. 2 Mean change in CD4 lymphocyte count from baseline in patients starting a nucleoside reverse transcriptase inhibitor (NRTI)-sparing regimen.

was + 60 cells/μL (41–77, $n = 230$) at 6 months, 73 cells/μL cells at M12 (49–97, $n = 180$) and 111 cells/μL cells at 24 months (82–140, $n = 148$) (Fig. 2).

In patients starting NRTI-sparing therapy with HIV RNA < 500 copies/mL, 89%, 93% and 87% had undetectable HIV RNA at 6, 12 and 24 months of follow-up, respectively.

Table 2 Factors associated with virological response (HIV RNA <500 copies/mL at 6 months) in patients starting a nucleoside reverse transcriptase inhibitor (NRTI)-sparing regimen (logistic regression)

Characteristic	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-value (P-trend)	OR (95% CI)	P-value (P-trend)
Age >42 years	0.95 (0.55–1.65)	0.85		
Female gender	1.05 (0.47–2.31)	0.91		
Duration of antiretroviral therapy >5 years	1.81 (1.01–3.25)	0.05	1.70 (0.83–3.48)	0.15
Duration of HIV seropositivity >10 years	1.03 (0.60–1.78)	0.91		
Non-AIDS stage	0.52 (0.29–0.91)	0.02	0.57 (0.31–1.07)	0.08
Number of previous antiretroviral regimens				
0–1	1.00		1.00	
2–3	0.53 (0.20–1.41)	0.20 (0.07)	0.60 (0.19–1.84)	0.37 (0.48)
4–6	0.56 (0.22–1.46)	0.24	0.86 (0.28–2.65)	0.79
>6	0.40 (0.16–1.00)	0.05	0.59 (0.20–1.78)	0.35
Number of previous NRTIs				
0	1.00			
1–3	0.54 (0.14–2.13)	0.38 (0.04)		
>3	0.50 (0.13–1.86)	0.30		
Number of previous PIs				
0	1.00		1.00	
1–2	0.97 (0.43–2.20)	0.94 (0.01)	1.26 (0.51–3.16)	0.61 (0.10)
>2	0.40 (0.18–0.90)	0.03	0.57 (0.22–1.48)	0.25
Previous NNRTIs				
0	1.00			
1–2	0.51 (0.29–0.90)	0.02	0.31 (0.16–0.61)	0.001
NRTI-sparing regimen				
PI only	1.00		1.00	
NNRTI + PI	1.60 (0.91–2.81)	0.10	1.60 (0.80–3.23)	0.19
CD4 count (cells/ μ L)				
<50	1.00		1.00	
50–199	1.82 (0.85–3.90)	0.12 (0.03)	2.00 (0.86–4.64)	0.11 (0.31)
200–349	1.42 (0.68–2.95)	0.35	1.74 (0.77–3.96)	0.19
≥ 350	0.17 (0.06–0.48)	0.001	0.27 (0.08–0.88)	0.03
Missing	3.40 (0.38–30.66)	0.28	1.55 (0.01–169.43)	0.86
HIV RNA (copies/mL)				
<500	1.00		1.00	
>500	0.17 (0.06–0.45)	0.001 ($<10^{-3}$)	0.14 (0.05–0.40)	0.001 ($<10^{-4}$)
Missing	0.39 (0.06–2.51)	0.32	0.53 (0.07–4.28)	0.55
Calendar period				
1997–1999	1.00		1.00	
2000–2001	3.51 (1.63–7.53)	0.001 ($<10^{-2}$)	6.09 (2.54–14.64)	0.001 ($<10^{-3}$)
2002	4.65 (2.08–10.37)	0.001	7.41 (2.94–18.66)	0.001
2003–2004	2.94 (1.06–8.18)	0.038	4.12 (1.28–13.29)	0.002

CI, confidence interval; OR, odds ratio; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

In patients starting NRTI-sparing therapy with HIV RNA >500 copies/mL who were NNRTI naïve, 68%, 57% and 69% had undetectable HIV RNA at 6, 12 and 24 months, respectively. Among patients who had already received NNRTI in a previous regimen, 50%, 59% and 58% had undetectable HIV RNA at 6, 12 and 24 months, respectively. In patients starting NRTI-sparing therapy with HIV RNA >500 copies/mL and with PI Nonotherapy on bithrapy 44%, 57% and 46% had undetectable HIV RNA at 6, 12 and 24 months, respectively.

Factors associated with HIV RNA <500 copies/mL at 6 months are shown in Table 2. Starting NRTI-sparing regimen after 2000, ($P=0.001$), being NNRTI naïve at baseline ($P=0.001$) and having undetectable viraemia at

baseline ($P<0.001$) were independently associated with a HIV RNA <500 copies/mL at 6 months.

Factors associated with an immunological response, defined as an increase of at least 20% in the CD4 T-lymphocyte count at 6 months, were assessed. Patients starting an NRTI-sparing regimen with a baseline CD4 count between 50 and 200 cells/ μ L [odds ratio (OR) 5.33; 95% CI 2.40–11.85; $P<0.001$], between 201 and 350 cells/ μ L (OR 8.44; 95% CI 3.75–19.00; $P<0.001$), and above 350 cells/ μ L (OR 5.65; 95% CI 2.07–15.39; $P<0.001$) had better immune reconstitution than patients starting an NRTI-sparing regimen at <50 cells/ μ L. HIV RNA >500 copies/mL at baseline was also associated with a CD4 increase (OR 4.38; 95% CI 1.95–9.86; $P<0.001$). When immune reconstitution

was defined as a 40% increase in CD4 count, the same associations were found (data not shown).

Safety assessment

One hundred and thirty-seven patients (41%) stopped the NRTI-sparing regimen during their follow-up: 30% for toxicity, 16% for virological failure, 14% because of poor adherence, 8% because it was the patient's choice, 6% for inclusion in therapeutic protocol, and 27% for unknown reasons. In patients starting an NRTI-sparing regimen with undetectable viral load, the main reason for stopping NRTIs was toxicity (36%).

The median duration of NRTI-sparing regimen was 52 (IQR 23–118) weeks before stopping or censoring. Analysis of prognostic factors for ceasing an NRTI-sparing regimen showed that a patient starting an NRTI-sparing regimen because of lipodystrophy or NRTI-related toxicity was less likely to stop this regimen (OR 0.62; 95% CI 0.39–1.00; $P = 0.05$). No other predictors for cessation of this regimen were found either in univariate or in multivariate analysis (data not shown). There was no difference in the time to stopping the NRTI-sparing regimen between patients on NNRTI plus PI regimens and those on PI-only regimens (data not shown).

Lipids, hepatic transaminases and glucose levels were assessed every 3 months up to 24 months in the St Vincent's and Aquitaine Cohorts. Glucose levels and liver enzymes did not significantly change over time (Table 3).

However, we observed a significant increase in the proportion of patients with lipid levels above the (National Cholesterol Education Program NCEP) cut-offs for primary prevention: the percentage of patients with total cholesterol above the threshold of 6.2 mmol/L was 18% at baseline ($n = 143$), rising to 37% at 3 months ($n = 140$) and to 44% at 24 months ($n = 78$) (P -trend < 0.001). The proportion of patients with HDL-cholesterol below 0.9 mmol/L increased slightly from 31% at baseline to 44% at 6 months, with a decrease to 21% at 24 months (P -trend = 0.009). Hypertriglyceridaemia, defined as levels of triglycerides above 2.3 mmol/L, increased from 32% ($n = 145$) at baseline to 62% at 3 months ($n = 140$) and to 62% at 24 months ($n = 76$) (P -trend = 0.002).

The proportions of patients with a rise in total cholesterol and/or triglycerides above threshold values were significantly higher in the group of patients treated with NNRTI plus PI than in patients treated with PI alone: the proportion of patients with total cholesterol > 6.2 mmol/L increased from 14% at baseline to 21% at 6 months and 20% at 24 months in patients treated with PI alone, and from 21% at baseline to 47% at 6 months and 49% at 24 months in patients treated with NNRTI plus PI ($P = 0.005$); the proportion of patients with triglycerides > 2.3 mmol/L increased from 32% at baseline to 47% at 6 months and 40% at 24 months in patients treated with PI alone and from 33% at baseline to 72% at 6 months and 67% at 24 months in patients treated with NNRTI plus PI ($P = 0.046$).

Table 3 Evolution of percentage of patients with metabolic abnormalities after starting a nucleoside reverse transcriptase inhibitor (NRTI)-sparing regimen (Aquitaine and St Vincent's Cohorts) ($n = 238$)

	Month 0	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 24
Cholesterol								
Available data	143	140	132	111	92	89	76	78
≤ 6.2 mmol/L (%)	82.5	62.9	62.1	70.3	62.0	57.3	59.2	56.4
> 6.2 mmol/L (%)	17.5	37.1	37.9	29.7	38.0	42.7	40.8	43.6
HDL-cholesterol								
Available data	90	72	69	53	54	53	52	52
< 0.9 mmol/L (%)	31.1	44.4	43.5	28.3	29.6	37.7	38.5	21.2
≥ 0.9 mmol/L (%)	68.9	55.6	56.5	71.7	70.4	62.3	61.5	78.9
Triglycerides								
Available data	145	140	132	110	92	89	78	76
≤ 2.3 mmol/L (%)	67.6	37.9	37.1	47.3	37.0	42.7	32.0	38.2
> 2.3 mmol/L (%)	32.4	62.1	62.9	52.7	63.0	57.3	68.0	61.8
Glucose								
Available data	155	138	120	106	95	37	75	77
≤ 11 mmol/L (%)	97.4	97.8	97.5	98.1	96.8	100	98.7	98.7
> 11 mmol/L (%)	2.6	2.2	2.5	1.9	3.2	0	1.3	1.3
AST								
Available data	180	157	136	116	104	102	84	81
≤ 100 IU/L (%)	91.1	95.5	92.7	94.8	95.2	96.1	95.2	93.8
> 100 IU/L (%)	8.9	4.5	7.5	5.2	4.8	3.9	4.8	6.2

The P -trend values are: $P < 0.001$ for cholesterol, $P = 0.009$ for HDL-cholesterol, $P = 0.001$ for triglycerides, $P = 0.711$ for glucose, and $P = 0.123$ for SGOT. HDL, high-density lipoprotein; AST, (aspartate aminotransferase).

Discussion

This study assessed the clinical, virological and immunological outcomes of NRTI-sparing regimens in clinical practice. Overall, our data suggest that the use of NRTI-sparing regimens may be virologically effective in pretreated patients, as 64% of patients with available data were under the threshold of 500 copies/mL at 6 months of follow up and 68% at 24 months. Ninety-one per cent of patients starting an NRTI-sparing regimen with HIV RNA <500 copies/mL still had undetectable HIV RNA at 6 months. Similarly, an NRTI-sparing regimen led to immune restoration even in these heavily experienced patients. However, a significant increase in the proportion of patients with lipid levels above the NCEP threshold, particularly in patients treated with a combination therapy including NNRTI and PI, was observed.

The use of NRTI-sparing regimens increased after 2000, but the number of patients is still small. Only 334 patients on an NRTI-sparing regimen were identified out of over 8000 included in three large cohorts since 1997 (3.9%). The typical patient was treatment experienced with moderately advanced and long-standing HIV disease, 33% of them having a prior AIDS-defining illness.

There are several limitations to this study. First, all analyses were based on data from three different cohorts, with variations in the amount of patient follow-up and disease stage. However, wherever possible, all analyses were stratified by cohort in an attempt to minimize any bias. Secondly, as this cohort analysis was entirely observational, patients on an NRTI-sparing regimen would have self-selected this regimen, with varying reasons for commencing an NRTI-sparing regimen and with numerous NRTI-sparing regimen combinations. Thirdly, there were no standardized guidelines for reporting reasons for stopping or commencing treatment, across or even within cohorts.

Our results are consistent with the limited data already published. The most frequent regimen in our patients was a PI in combination with an NNRTI. This regimen has been studied previously in naïve patients in a large randomized trial comparing three arms (unboosted indinavir plus efavirenz, efavirenz plus zidovudine-lamivudine, and unboosted indinavir plus zidovudine-lamivudine). The percentage of patients with plasma viral load <400 copies/mL at 48 weeks was 53% in the group assigned to indinavir plus efavirenz, as opposed to 70% in the group assigned to efavirenz plus two NRTIs [14]. Indinavir, however, is now rarely used as a single PI in naïve or experienced patients. Our study shows that most patients with an NNRTI-containing regimen used lopinavir/ritonavir (LPV/r). A pilot study recently demonstrated a satisfactory outcome in 21 experienced (but NNRTI naïve) and 65 naïve

patients on LPV/r plus efavirenz: 73% of patients achieved a plasma viral load <400 copies/mL [9]. A trial of efavirenz plus LPV/r and efavirenz plus two NRTIs following a first suppressive three- or four-drug regimen with a median follow-up time of 110 weeks suggested a trend, although not statistically significant, towards a higher rate of virological failure in the LPV/r plus efavirenz arm ($P = +0.088$) [15]. The combination of LPV/r and nevirapine was studied in naïve patients and results at 48 weeks showed that 11 of the 14 naïve patients had undetectable viraemia at week 48 [16]. Overall, NNRTI plus PI combinations have been found to be effective in some small-scale comparative trials in naïve or pretreated patients. Very recently, the results of a large randomized, open-label, prospective trial comparing three class-sparing regimens (two NRTIs plus LPV/r vs two NRTIs plus efavirenz vs LPV/r plus efavirenz) for naïve subjects have been made available: compared with a standard regimen of efavirenz plus two NRTIs, the NRTI-sparing regimen of LPV/r plus efavirenz had a similar virological outcome and safety [17].

Different combinations of double-PI boosted regimens have been studied, mostly in pretreated subjects. In the LOPSAQ study, a combination of LPV/r with saquinavir without an NRTI backbone was given to 121 patients with advanced HIV disease and multiple regimen failure [18]. Preliminary 24-week results were encouraging, with immune reconstitution and the median viral load decreasing from 5.2 to 2.1 log₁₀ copies/mL. Smaller studies performed on heavily treated patients using LPV/r plus indinavir [19] or a LPV/r plus amprenavir [20] regimens have shown similar results.

Our results for a wide range of NRTI-sparing regimens in 334 mostly heavily pretreated patients (of whom 25% only had undetectable viraemia at baseline) compare well with those obtained even in naïve patients with NNRTI plus PI or double boosted PIs and suggest a use for these regimens in specific situations. However, the main predictive factor of virological response at 6 months was being naïve to NNRTIs, suggesting the importance of this class in such a combination.

The indication to start an NRTI-sparing regimen was reported to be virological failure in 40% of patients. Twenty-five per cent of patients starting an NRTI-sparing regimen were virologically suppressed at the start of the NRTI-sparing regimen, suggesting that, for these patients at least, toxicity was a leading cause of stopping NRTIs.

Among patients identified as having started an NRTI-sparing regimen, the rate of discontinuation was 41%. Reasons for stopping are sometimes difficult to assess: clinicians may choose between competing reasons, and the assessment is subjective. However, reasons for stopping the NRTI-sparing regimens were reported to be toxicity for 30% of patients and virological failure for 16%. This high

rate of discontinuation is consistent with previously reported data. Allavena *et al.* reported a 24% rate of discontinuation in patients in their study, and a third of the patients on a LPV/r-containing regimen were reported to stop for toxicity reasons [9]. Staszewski *et al.* found an even higher discontinuation rate of 50% in patients on an efavirenz/LPV/r regimen [14]. However, we have shown that patients with previous NRTI toxicity were less likely to discontinue an NRTI-sparing regimen and thus could represent a target population for these combinations.

An increase of lipid values was observed in patients treated with an NRTI-sparing regimen. The percentage of patients with total cholesterol >6.2 mmol/L increased from 18% at baseline to 44% at 24 months, and the percentage of patients with triglycerides >2.3 mmol/L increased from 32% at baseline to 62% at 24 months. Furthermore, patients on a combination of PI plus NNRTI were more likely to have an atherogenic lipid profile than patients on PI only. Such findings have been reported by others studying NRTI-sparing regimens, including the previously mentioned open-label study of 86 patients on LPV/r and efavirenz [9]. In that study, the authors observed a rapid rise in lipid levels during the first 8 weeks of treatment; later, lipids levels remained stable up to 48 weeks. In another open-label study, patients failing NRTIs were switched to indinavir/r 800/100 mg twice a day (bid) plus efavirenz 600 mg once a day [8]. This regimen gave a durable virological response, but a pro-atherogenic metabolic profile developed and nephrotoxicity occurred, requiring indinavir dose reductions. In lipoatrophic patients, small pilot nonpublished studies have evaluated the benefit of a switch to a PI-containing/NRTI-sparing regimen compared with a maintenance NRTI-containing regimen and showed that the combination lopinavir/efavirenz was associated with a significant improvement in body fat [21], but also with a greater increase in triglycerides and total cholesterol, compared with the NRTI arm [22].

Overall, NRTI-sparing regimens show encouraging long-term results in terms of virological and immunological safety. However, long-term toxicity, particularly regarding the metabolic profile and cardiovascular outcome, remains a concern and may depend on the type of regimen used.

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- Clinical coordination:* D. Lacoste, D. Malvy, I. Pellegrin, J. F. Moreau, M. Dupon, P. Morlat, J. L. Pellegrin and J. M. Ragnaud.
- Participating hospital departments (participating physicians):* Bordeaux University Hospital: J. Beylot (P. Morlat, N. Bernard, D. Lacoste and F. Bonnet), C. Beylot (M. S. Doutre), C. Conri (J. Constans), P. Couzigou and H. Fleury (B. Masquelier and I. Pellegrin), M. Dupon (I. Chossat), J. L. Pellegrin (P. Mercie), M. Le Bras (D. Malvy, F. Djossou and J. P. Pivetaud), J. F. Moreau (J. L. Taupin), J. M. Ragnaud (C. De La Taille, H. Dutronc and D. Neau); Dax Hospital: M. Loste (I. Blanchard, L. Caunègre and A. Pons); Bayonne Hospital: F. Bonnal (Y. Blanchard, S. Farbos and M. C. Gemain); Libourne Hospital: J. Ceccaldi, B. Darpeix and P. Legendre; Villeneuve sur Lot Hospital: E. Buy.
- Data management and analysis:* S. Lawson-Ayayi, E. Balestre, G. Palmer and D. Touchard.
- Data collection:* M. J. Blaizeau, M. Decoin, S. Delveaux, A. M. Formaggio, M. Pontgahet and B. Uwamaliya.

The AHOD study group

New South Wales: M. Gotowski, S. Taylor and L. Stuart-Hill, Bligh Street Clinic, Tamworth. E. Jackson, D. Hunter and L. Lewis, Blue Mountains Sexual Health and HIV Clinic, Katoomba. M. Block, D. Austen, D. Quan, A. Gowers and C. Anderson, Holdsworth House General Practice, Darlinghurst. K. Brown and N. Skobalj, Illawarra Sexual Health, Warrawong. C. O'Connor and B. Allam, Livingstone Road Sexual Health Centre, Marrickville. D. Templeton, Macquarie Sexual Health Centre, Dubbo. M. T. Liang, Nepean Sexual Health and HIV Clinic, Penrith. D. Allen and B. Strazdinis, Holden Clinic, Gosford. D. Smith and J. Armishaw, SHAIDS, Lismore. D. Cooper, A. Carr and M. Lacey, St Vincents Hospital, Sydney. B. Donovan, C. Pell and B. Judd, Sydney Sexual Health Centre, Sydney. R. Finlayson and R. Richardson, Taylor Square Private Clinic, Darlinghurst. D. Ellis, The Medical and Vein Centre, Coffs Harbour. D. Baker*, J. Kidd, R. McFarlane and R. Vale, 407 Bourke Street, Surry Hills. P. Canavan,* National Association of People Living with HIV/AIDS (NAPWA). P. Rawstorne,* National Centre in HIV Social Research, University of New South Wales, Sydney. B. Mulhall,* University of Sydney, Sydney. M. Law,* K. Petoumenos* and D. Smith,* NCHECR, Darlinghurst.

Northern Territory: B. Hughes, H. Lyttle and P. Knibbs, Communicable Disease Centre, Royal Darwin Hospital, Darwin.

South Australia: G. Rogers, S. Markinson, C. Sullivan, F. Downey, M. Curry, J. Oddy and J. Thompson, The Care and Prevention Programme, Adelaide University, Adelaide.

Queensland: M. Kelly and H. Magon, AIDS Medical Unit, D. Brisbane. Sowden and A. Walker, Blackall Terrace Specialist

Appendix: composition of the three cohort study groups

The Aquitaine Cohort study group

Organization and methodology: G. Chêne, F. Dabis, R. Thiebaut and R. Salamon.

Centre, Blackall Terrace. D. Orth, G. Lister and D. Youds, Gladstone Road Medical Centre, Highgate Hill. J. Chuah,* N. Wendt, W. Fankhauser and B. Dickson, Gold Coast Sexual Health Clinic, Miami. D. Russell, J. Leamy and C. D'arcy Evans, Sexual Health Program, Cairns Base Hospital, Cairns. *Victoria*: T. Reid and J. Laing, Melbourne Sexual Health Centre, Carlton. I. Woolley, T. Korman, A. Padiglione and K. Visvanathan, Monash Medical Centre, Clayton. N. Roth,* B. Eu, S. Strecker, D. Russell and H. Wood, Prahran Market Clinic, South Yarra. A. Mijch,* J. Hoy, A. Pierce, M. Bryant, C. McCormack and K. Watson, The Alfred Hospital, Prahran. J. Anderson,* R. Moore, D. Russell, G. McGovern,

R. McNair and K. Lowe, The Carlton Clinic, Carlton. N. Medland, The Centre Clinic, St Kilda.

Western Australia: S. Mallal,* M. French, A. Cain, J. Skett and C. Moore, Department of Clinical Immunology, Royal Perth Hospital, Perth.

*Steering committee member.

The St Vincent's Hospital Cohort study group

Participating physicians: D. Cooper, A. Carr, S. Pett, P. Mallon, A. Winston, C. Wheatherall, S. Millikan, B. Brew and A. Calmy.
Database manager: K. Hesse.

ORIGINAL RESEARCH

Lipodystrophy and weight changes: data from the Swiss HIV Cohort Study, 2000–2006

A Nguyen,¹ A Calmy,¹ V Schiffer,¹ E Bernasconi,² M Battegay,³ M Opravil,⁴ J-M Evison,⁵ PE Tarr,⁶ P Schmid,⁷ T Perneger,⁸ B Hirschel¹ and the Swiss HIV Cohort Study*

¹Infectious Disease/HIV Unit, University Hospital Geneva, Geneva, Switzerland, ²Infectious Disease Service, Hospital Lugano, Lugano, Switzerland, ³Division of Infectious Diseases, University Hospital Basel, Basel, Switzerland, ⁴Division of Infectious Diseases, University Hospital Zurich, Zurich, Switzerland, ⁵Infectious Disease Service, University Hospital Bern, Bern, Switzerland, ⁶Infectious Disease Service, University Hospital Lausanne, Lausanne, Switzerland, ⁷Infectious Disease Unit, Hospital St-Gall, St-Gall, Switzerland and ⁸Quality of Care Service, University Hospital Geneva, Geneva, Switzerland

Background and Objectives

Combination antiretroviral therapy (cART) is changing, and this may affect the type and occurrence of side effects. We examined the frequency of lipodystrophy (LD) and weight changes in relation to the use of specific drugs in the Swiss HIV Cohort Study (SHCS).

Methods

In the SHCS, patients are followed twice a year and scored by the treating physician as having 'fat accumulation', 'fat loss', or neither. Treatments, and reasons for change thereof, are recorded. Our study sample included all patients treated with cART between 2003 and 2006 and, in addition, all patients who started cART between 2000 and 2003.

Results

From 2003 to 2006, the percentage of patients taking stavudine, didanosine and nelfinavir decreased, the percentage taking lopinavir, nevirapine and efavirenz remained stable, and the percentage taking atazanavir and tenofovir increased by 18.7 and 22.2%, respectively.

In life-table Kaplan–Meier analysis, patients starting cART in 2003–2006 were less likely to develop LD than those starting cART from 2000 to 2002 ($P < 0.02$). LD was quoted as the reason for treatment change or discontinuation for 4% of patients on cART in 2003, and for 1% of patients treated in 2006 (P for trend < 0.001). In univariate and multivariate regression analysis, patients with a weight gain of ≥ 5 kg were more likely to take lopinavir or atazanavir than patients without such a weight gain [odds ratio (OR) 2, 95% confidence interval (CI) 1.3–2.9, and OR 1.7, 95% CI 1.3–2.1, respectively].

Conclusions

LD has become less frequent in the SHCS from 2000 to 2006. A weight gain of more than 5 kg was associated with the use of atazanavir and lopinavir.

Keywords: antiretroviral therapy, body weight, cohort studies, fat accumulation, fat loss, lipodystrophy, nonnucleoside reverse transcriptase inhibitors, nucleoside reverse transcriptase inhibitors, protease inhibitors, Swiss HIV Cohort Study

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Introduction

Combination antiretroviral therapy (cART) against HIV infection has diminished AIDS-related morbidity and

mortality [1]. Suppression of viral replication to below the detection level has become a treatment goal that can also be reached for patients with triple class treatment failure.

However, patients on cART often develop long-term side effects, particularly lipodystrophy (LD) syndrome, a combination of fat atrophy (in the limbs, buttocks and face) and visceral fat accumulation, with or without lipid and glucose metabolism disturbances [2–4]. The definition of 'LD' remains a challenge [39]. Proposals for a case definition

*All investigators are listed in the Appendix.

Correspondence: Dr Alexandra Calmy, Division des Maladies infectieuses, Geneva University Hospital, rue Micheli-du-Crest 24, 1211 Genève 14, Switzerland. Tel: + 41 22 3729808; fax: + 41 22 3729599; e-mail: aacalmy@gmail.com

have been put forward, but necessitate dual energy x-ray absorptiometry (DEXA) and computed tomography (CT) scans [3–7]; while invaluable for small-scale studies, they are not practical for routine use in patients. Instead, larger cohorts and epidemiological studies most often rely only on the subjective impressions of patients and/or physicians [8].

LD may decrease compliance with therapy for aesthetic reasons [9], but also may be associated with an increased risk of cardiovascular diseases [5,9], insulin resistance [10,11], and diabetes [6,12,13].

The aetiology of LD in HIV-infected patients is still unclear [3–7]. LD was initially attributed to prolonged therapy with protease inhibitors (PIs) [14–16], but all current drug classes have been involved in LD [17–19].

Lipoatrophy is related to the mitochondrial toxicity of nucleoside reverse transcriptase inhibitors (NRTIs), whose action is not limited to inhibition of reverse transcription, but extends to mitochondrial DNA polymerase γ , the main enzyme involved in replication of mitochondrial DNA. Inhibition of this enzyme is associated with abnormal mitochondrial morphology and may induce apoptosis of adipocytes and lipoatrophy [20,21]. In contrast, the pathogenesis of fat accumulation is not clear. Proposed mechanisms abound: highly active antiretroviral therapy (HAART) may have an impact on cytokines and cytokine receptors [22] and appetite regulators [23], as well as on the differentiation and functions of the adipocytes [20,21,24] and growth hormone metabolism [25,26]. Another theory holds that abdominal and visceral fat accumulation is a consequence of lipoatrophy, because the atrophic fatty tissues are unable to take up fat from the plasma [4]. Of note, a pure phenotype of fat atrophy or fat accumulation is only seen in 10% of patients. The paradox that central fat increases while subcutaneous fat decreases can be explained by the difference between visceral fat (mainly comprising brown adipocytes) and subcutaneous fat (comprising mainly white adipocytes): thus exposure to antiretrovirals may affect subcutaneous adipose tissue differently from visceral adipose tissue [27]. Because HAART entails the combined use of several drugs, it has been difficult to link the various manifestations of LD to a particular molecule. Cross-sectional and prospective studies demonstrated that stavudine (d4T) was the drug most frequently associated with fat atrophy [28,29]. Other risk factors include race, age and HIV disease severity for fat atrophy, and race, sex and sedentary lifestyle for fat accumulation [4].

The present paper attempts to analyse these data in relation to the type of cART administered. It focuses on the period from 2003 to 2006, coincident with the introduction of atazanavir (ATV) and the introduction of new NRTIs.

Patients and methods

The Swiss HIV Cohort Study (SHCS) is a nationwide prospective study based on voluntary participation of persons infected with HIV-1. The rationale, organization and baseline characteristics of the study have been described elsewhere in detail [30], and a continuously updated description can be found at the Swiss HIV Cohort website [31].

LD is coded based on the subjective impression of the treating physician as 'fat accumulation' or 'fat loss', and is routinely recorded 6-monthly in the SHCS database. More objective measurements, such as DEXA scans or computed tomography (CT) scans, were not available for this study.

Incidence of LD in patients starting cART

All study participants who had previously been antiretroviral-naïve, but who started cART between 1 January 2000 and 31 December 2006, were eligible for inclusion. The time to the occurrence of 'fat loss' or 'fat accumulation', or a treatment change because of LD noted at one of the 6-monthly cohort visits, was measured according to Kaplan–Meier. Two groups were compared with the log-rank test: patients starting cART between 2000 and 2002 and patients starting cART between 2003 and 2006.

Prevalence of weight changes, fat loss and fat accumulation in patients on cART

To establish how frequently antiretroviral treatment was changed because of 'fat loss' or 'fat accumulation', we included all patients on cART with at least one follow-up visit between 2003 and 2006. The number of patients on cART with a discontinuation or change of treatment because of 'fat loss' or 'fat accumulation' was determined and related to the total number of patients on cART during the same year, considering only the first change or discontinuation in that year. A *P*-trend analysis for the prevalence of discontinuation or change in general, and the prevalence of discontinuation or change in particular because of 'fat loss or fat accumulation', was performed.

Analysis of factors associated with weight gain, weight loss, fat loss and fat accumulation

In order to analyse factors associated with gain of weight, patients who gained ≥ 5 kg between two consecutive visits were compared with patients who did not have such gain of weight. In order to exclude from consideration the weight gain corresponding to correction of AIDS-related cachexia,

we included in this analysis only patients with a baseline body mass index (BMI) above 20 for men and above 18 for women.

Factors associated with a weight gain ≥ 5 kg between the first two follow-up cohort visits between 2003 and 2006 were examined using univariate analysis followed by multivariate logistic regression. Gender, ethnic origin, diabetes status based on the guidelines of the American Diabetes Association (ADA) [32], smoking status and cART (current exposure, i.e. cART received during the period when weight changed or fat loss/accumulation occurred) were entered as categorical variables; for age, BMI, CD4 cell count, RNA viral load, plasma cholesterol (mmol/L), and triglyceride (mmol/L), quartiles were compared (see Table 1). The baseline was defined as the first visit of the first two follow-up cohort visits between 2003 and 2006.

The multivariate model was constructed using the forward stepwise approach, including covariates that remained significant at the 0.1 level in univariate analysis. We repeated the analysis for factors associated with weight loss, fat accumulation and fat loss.

Analyses were performed using the SPSS version 11.0 for Windows statistical software package (SPSS Inc., Chicago, IL, USA).

Results

Use of antiretroviral drugs in SHCS, 2003–2006

In each year from 2000 to 2006, between 5203 and 6016 patients had a follow-up visit in the SHCS. Figure 1 shows the proportion of patients treated with the 10 most frequently used drugs, plus d4T. Between 2003 and 2006, use of zidovudine (ZDV), lamivudine (3TC), didanosine (ddI) and d4T declined, use of lopinavir (LPV), abacavir (ABC), efavirenz (EFV) and nevirapine (NVP) remained stable, and use of ATV, emtricitabine (ETC) and tenofovir (TDF) increased by 18.7, 8.2 and 22.2%, respectively.

Incidence of LD in treatment-naïve patients

Figure 2 shows the likelihood of occurrence of LD in patients who were cART-naïve, and started treatment between 2000 and 2006. Patients starting treatment in 2003–2006 were significantly less likely to experience LD than those starting between 2000 and 2002 (log rank = 0.02). From 2000 to 2002, most patients were started on 3TC (96.9%), ZDV (88.5%), d4T (4.2%), TDF (0.8%), EFV (38.1%), nelfinavir (NFV) (28.8%) and LPV (13.8). From 2003 to 2006, the corresponding percentages were 83.7% for 3TC, 10.8% for ETC, 64.2% for ZDV, 0.7% for d4T, 30.5% for TDF, 41.8% for EFV, 3.8% for NFV,

Table 1 Baseline characteristics of patients

Population characteristics: demographic	Baseline value for physician visit in 2003–2006 (<i>n</i> = 5427 patients) (%)
Female gender	32.2
Age (years)	
17–37	31.5
38–42	26.3
43–49	22.1
50–88	20.1
Ethnic origin [†]	
White	82.6
Black	12.0
Hispano-American	2.0
Asian	3.0
Body mass index*	
<25	70
26–30	23
>30	5
cART use	
Zidovudine	54.2
Abacavir	24.3
Stavudine	14.3
Didanosine	15.8
Atazanavir	5
Lopinavir	22.4
Indinavir	3.7
Nelfinavir	15.9
Efavirenz	30.2
Nevirapine	10

Population characteristics: laboratory variables	Baseline value for physician visit in 2003–2006 (<i>n</i> = 5427 patients) (%)
CD4 (cells/ μ L)	
2–301	32.7
302–446	22.9
447–628	21.9
629–3010	21.7
RNA viral load (log copies/mL)	
<1.6	55.0
≥ 1.6 and <2.7	11.8
≥ 2.7 and <4.3	12.9
≥ 4.3	17.4
Cholesterol (mmol/L)	
≥ 1.2 and <4.3	26.8
≥ 4.3 and <5	19.9
≥ 5 and <5.9	24.0
≥ 5.9	23.9
Triglycerides (mmol/L)	
≥ 0.2 and <1.13	24.6
≥ 1.13 and <1.75	23.6
≥ 1.75 and <2.8	23.3
≥ 2.8	22.9
Current smoker	46.7
Diabetes mellitus	0.5
Fat loss without fat accumulation	10.4
Fat accumulation without fat loss	12.3
Fat loss and fat accumulation combined	10.3

cART, combination antiretroviral therapy; *2% of population had unknown heights, thus BMI was not available; [†]0.4% of population had unknown ethnic origins.

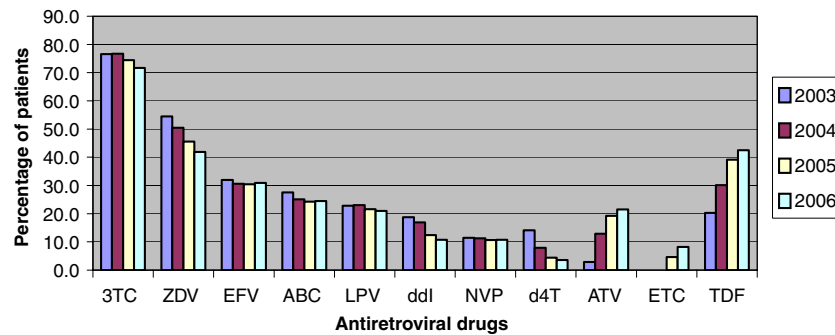


Fig. 1 Proportion of patients treated with frequently used antiretroviral drugs in 2003–2006. 3TC, lamivudine; ABC, abacavir; ATV, atazanavir; d4T, stavudine; ddI, didanosine; EFV, efavirenz; ETC, emtricitabine; LPV, lopinavir; NVP, nevirapine; TDF, tenofovir; ZDV, zidovudine.

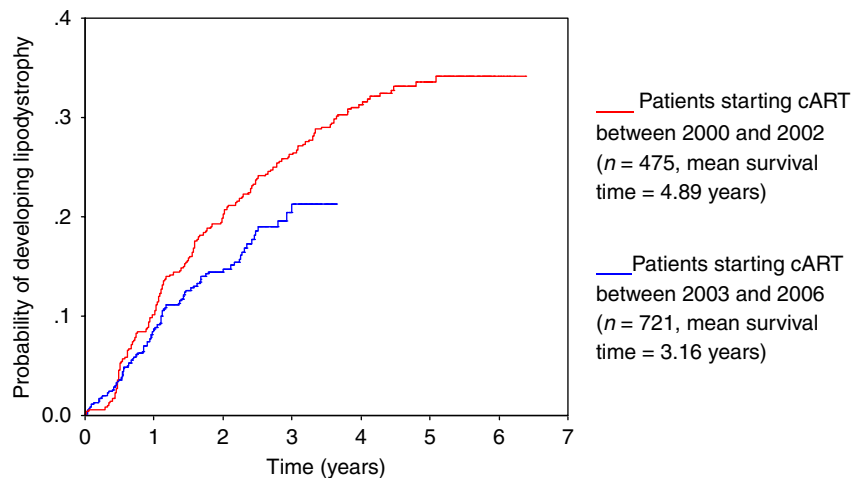


Fig. 2 Probability of developing lipodystrophy since 2000 for the groups of naïve patients starting combination antiretroviral therapy (cART) in 2000–2002 and 2003–2006, respectively.

38.5% for LPV and 7.5% for ATV. Overall, the proportion of patients initiating cART with ATV, ritonavir-boosted LPV (LPV/r) or TDF was higher in the 2003–2006 period than in the 2000–2002 period.

Treatment changes because of LD

A total of 5777 patients were followed in the SHCS in 2003, and 6016 during 2006. Baseline characteristics are summarized in Table 1: 32% of the patients were female, and they had a median age of 42.5 years [interquartile range (IQR) 37.5–48.5 years]. The number of patients treated with cARTs was 5269 (91.2%) in 2003 and 5530 (91.9%) in 2006. In 2003, 44% underwent a change or discontinuation of treatment, while in 2006 the proportion decreased to 17% ($P < 0.001$). LD was quoted as the reason for treatment change or discontinuation for 4% of patients on cARTs in 2003, but for only 1% of patients treated in 2006 (P for trend < 0.001).

10.4% of patients were diagnosed with only fat loss and 12.3% with only fat accumulation. Overall, 33.9% had a diagnosis of either fat loss and fat accumulation or fat loss or fat accumulation.

We determined the proportion of patients who had a 'fat loss' or a 'fat accumulation' diagnosis before our baseline. 90.4% of ABC-treated patients diagnosed with fat accumulation and 93.4% of those diagnosed with fat loss had had this diagnosis for more than 2 years. For ZDV-treated patients, we observed similar results (83.1% for fat accumulation and 86.4% for fat loss).

Factors involved in weight changes and fat redistribution (see Tables 2 and 3)

Weight changes

Weight gain of ≥ 5 kg

The mean BMI of our population was 23.7 kg/m². In 2003, of the patients with eligible BMIs (> 18 kg/m²), 494 (7.03%)

Table 2 Univariate analysis of factors associated with weight gain, weight loss and lipodystrophy*

Characteristic	Weight gain ≥ 5 kg		Weight loss ≥ 5 kg		Fat accumulation		Fat loss	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Female gender	1.1	0.50	1.3	0.17	1.3	0.001	0.71	0.001
Age (years)								
17–37	1.0							
38–42	0.7	0.002	0.7	0.03	1.4	0.001	2.4	0.001
43–49	0.8	0.002	0.9	0.44	1.9	0.001	3.3	0.001
50–88	0.8	0.10	0.8	0.09	3.0	0.001	4.2	0.001
Body mass index								
< 25	1.0							
26–30	1.5	0.001	0.8	0.80	1.0	0.50	0.8	0.002
> 30	1.0	0.80	0.2	0.70	1.0	0.70	0.9	0.50
Ethnic origin								
White	1.0							
Black	1.1	0.48	1.1	0.37	1.3	0.01	0.3	0.001
Hispano-American	1.0	0.99	0.8	0.63	1.0	0.90	0.7	0.16
Asian	0.7	0.26	0.8	0.57	0.9	0.64	0.5	0.01
Smoking	1.0	0.96	1.5	0.001	0.7	0.001	1.1	0.33
Diabetes	3.4	0.01	2.2	0.15	3.2	0.01	1.2	0.68
CD4 (cells/ μ L)								
2–301	1.0							
302–446	0.5	0.001	0.7	0.04	1.4	0.001	1.4	0.001
447–628	0.5	0.001	0.6	0.001	1.6	0.001	1.6	0.001
629–3010	0.4	0.001	0.6	0.002	1.6	0.001	1.9	0.001
Plasma HIV RNA (log copies/mL)								
< 1.6	1.0							
≥ 1.6 and < 2.7	1.2	0.19	1.1	0.67	0.9	0.26	0.8	0.12
≥ 2.7 and < 4.3	1.2	0.17	1.2	0.17	0.7	0.01	0.7	0.001
≥ 4.3	2.1	0.001	1.4	0.02	0.5	0.001	0.4	0.001
Total cholesterol (mmol/L)								
≥ 1.2 and < 4.3	1.0							
≥ 4.3 and < 5	0.9	0.33	0.8	0.21	1.2	0.17	1.3	0.02
≥ 5 and < 5.9	0.7	0.02	0.8	0.04	1.3	0.01	1.4	0.001
≥ 5.9	0.5	0.001	0.8	0.07	1.9	0.001	2.1	0.001
Triglycerides (mmol/L)								
≥ 0.2 and < 1.13	1.0							
≥ 1.13 and < 1.75	1.3	0.03	1.3	0.09	1.3	0.01	1.592	0.001
≥ 1.75 and < 2.8	1.0	0.78	1.3	0.09	1.7	0.001	2.109	0.001
≥ 2.8	0.9	0.52	1.0	0.89	2.4	0.001	3.842	0.001
Drugs								
Zidovudine	1.0	0.93	0.8	0.21	0.8	0.02	0.5	0.001
Abacavir	0.7	0.005	0.8	0.19	1.5	0.001	1.8	0.001
Stavudine	0.8	0.33	0.8	0.16	1.0	0.70	1.0	0.76
Didanosine	0.9	0.47	1.1	0.34	1.0	0.75	1.0	0.56
Atazanavir	1.7	0.001	0.9	0.58	1.0	0.64	1.2	0.03
Lopinavir	2.4	0.001	1.2	0.56	1.0	0.85	0.7	0.04
Indinavir	0.7	0.29	1.0	0.89	1.9	0.001	1.2	0.45
Nelfinavir	1.0	0.77	0.9	0.55	1.7	0.001	1.4	0.01
Efavirenz	1.0	0.94	0.7	0.02	1.4	0.001	1.3	0.01
Nevirapine	0.9	0.42	0.7	0.04	1.6	0.001	1.3	0.02

CI, confidence interval; OR, odds ratio; *values in bold indicate P -value ≤ 0.05 .

experienced a rapid gain of weight of ≥ 5 kg within a 6-month period, as compared with 491 (6.49%) in 2004, 517 (6.57%) in 2005, and 222 (5.51%) in 2006 (difference not significant).

Diabetes [odds ratio (OR) 3.4, $P < 0.01$], viral load ≥ 4.3 log HIV-1 RNA copies/mL (OR 2.1, $P < 0.001$), ATV (OR 1.7, $P < 0.001$) and LPV/r (OR 2.4, $P < 0.001$) use, and BMI < 25 and > 30 (OR 1.5, $P < 0.001$) were associated with a ≥ 5 kg

weight gain (Table 2). Age above 37 years, a CD4 count > 300 cells/ μ L, a plasma cholesterol value ≥ 5 mmol/L and the use of ABC (OR 0.7, $P < 0.01$) were associated with a lower probability of weight gain. In multivariate analysis, ATV (OR 2, $P = 0.001$) and LPV/r (OR 1.7, $P < 0.001$) were associated with weight gain. A high CD4 cell count was a protective factor for weight gain (OR 0.6, $P < 0.001$), being associated with a lower probability of weight gain (see

Table 3 Multivariate analysis of factors associated with weight gain, weight loss and lipodystrophy [lipoatrophy (fat loss) and lipohypertrophy (fat gain)]

Characteristic	Weight gain ≥ 5 kg		Weight loss ≥ 5 kg		Fat accumulation		Fat loss	
	OR	P-value	OR	P-value	OR	P-value	OR	P-value
Drugs								
Zidovudine					0.78	0.001	0.42	<0.001
Abacavir							1.35	<0.001
Stavudine								
Didanosine								
Atazanavir	1.96	0.001						
Lopinavir	1.65	<0.001						
Indinavir								
Nelfinavir					1.35	0.001		
Efavirenz								
Nevirapine								
Female gender			1.25	0.045			1.26	0.009
Age (years)								
17–37								
38–42					1.57	<0.001	1.90	<0.001
43–49					2.24	<0.001	2.49	<0.001
50–88					3.19	<0.001	3.12	<0.001
Ethnic origin								
White								
Black					1.74	<0.001	0.46	<0.001
Hispano-American								
Asian							0.64	0.081
Current smoker			1.46	0.001	0.79	0.001		
Diabetes mellitus								
CD4 (cells/ μ L)								
2–301								
302–446	0.58	<0.001	0.74	0.029	1.27	0.016	1.34	0.006
447–628	0.61	0.001	0.64	0.003	1.36	0.02	1.53	<0.001
629–3010	0.51	<0.001	0.65	0.003	1.45	<0.001	1.76	<0.001
RNA viral load (log copies/mL)								
< 1.6								
≥ 1.6 and < 2.7								
≥ 2.7 and < 4.3					0.79	0.037	0.82	0.099
≥ 4.3					0.63	<0.001	0.58	<0.001

OR, odds ratio.

Table 3). Patients who gained >5 kg had a mean BMI of 25.4 (after weight gain), compared with 23.6 in patients who gained <5 kg (Table 3).

Weight loss of >5 kg

A CD4 count >300 cells/ μ L (OR 0.7, $P<0.03$), a cholesterol value ≥ 5 mmol/L (OR 0.7, $P<0.1$) and EFV use (OR 0.7, $P<0.03$) were associated with a lower probability of weight loss ≥ 5 kg, while smoking (OR 1.5, $P<0.001$), plasma HIV RNA ≥ 4.3 log copies/mL (OR 1.4, $P<0.02$) and triglyceride values <1.75 mmol/L (OR 1.3, $P<0.1$) were associated with loss of weight (Table 2). In multivariate analysis, female gender (OR 1.3, $P=0.04$) and smoking (OR 1.5, $P<0.001$) were associated with increased risk of weight loss, while a high CD4 count >300 cells/ μ L (OR 0.7, $P=0.003$) was protective for weight loss (Table 3).

Fat distribution

Fat accumulation (lipohypertrophy)

Older age (OR 3, $P<0.001$), white ethnic origin (OR 1.3, $P<0.01$), diabetes (OR 3.2, $P<0.01$), CD4 count >300 cells/ μ L (OR 1.5, $P<0.001$), cholesterol values ≥ 5 mmol/L (OR 1.3, $P<0.01$), triglyceride values ≥ 1.75 mmol/L (OR 1.7, $P<0.001$), and ABC (OR 1.5, $P<0.001$), IDV (OR 1.9, $P<0.001$), NFV (OR 1.7, $P<0.001$) and EFV use (OR 1.4, $P=0.001$) were associated with the presence of fat accumulation. Smoking (OR 0.7, $P<0.001$), RNA viral load ≥ 4.3 log copies/mL (OR 0.5, $P<0.001$) and ZDV use (OR 0.8, $P<0.02$) were associated with less reported fat accumulation (Table 2). In multivariate analysis, older age (OR 3.2, $P<0.001$), black ethnic origin (OR 1.7, $P<0.001$), high CD4 cell count (OR 1.3, $P<0.02$) and NFV use (OR 1.4, $P=0.001$) predicted the presence of lipohypertrophy. Being a smoker (OR 0.8,

$P = 0.02$), having a high RNA viral load (> 4.3 log copies/ml, $P < 0.001$) or being on ZDV (OR 0.8, $P = 0.001$) was associated with less fat accumulation (Table 3).

Fat loss (lipoatrophy)

Older age (OR 4.2, $P < 0.001$), a higher CD4 cell count (OR 1.9, $P < 0.001$), a higher cholesterol value (OR 2.1, $P < 0.001$), a higher triglyceride value (OR 3.9, $P < 0.001$), and ABC (OR 1.8, $P < 0.001$), ATV (OR 1.2, $P < 0.03$), NFV (OR 1.4, $P < 0.01$) and EFV (OR 1.3, $P < 0.01$) use were associated with greater lipoatrophy. BMI > 25 and < 30 (OR 0.8, $P < 0.002$), black ethnicity (OR 0.3, $P < 0.001$) and Asian ethnicity (OR 0.5, $P < 0.01$), viral load ≥ 4.3 log copies/mL (OR 0.4, $P < 0.001$), and ZDV (OR 0.5, $P < 0.001$) and LPV (OR 0.7, $P < 0.04$) use were associated with less atrophy (Table 2). Finally, multivariate analysis showed that lipoatrophy was associated with female gender (OR 1.3, $P = 0.009$), older age (OR 2.5, $P < 0.001$) high CD4 cell count (OR 1.8, $P < 0.001$) and ABC use (OR 1.35, $P < 0.001$). An absence of fat loss was associated with black ethnic origin (OR 0.5, $P > 0.001$), high RNA viral load (OR 0.6, $P < 0.001$) and use of ZDV (OR 0.4, $P < 0.001$) (Table 3).

Discussion

In this study, in which more than 5500 patients were investigated within a large prospective cohort, we found that LD has become less frequent since 2003.

LD was discovered in the late 1990s, when the most frequently used drugs were d4T and indinavir. By 2006, neither was among the 10 most used antiretrovirals in Switzerland. While the use of thymidine analogues was almost universal in initial treatment in 2000, d4T is no longer recommended in the guidelines for treatment initiation [33] and had nearly disappeared by 2006, whereas ZDV tends to be replaced by TDF. Randomized prospective studies show that lipoatrophy develops less frequently on TDF than on d4T [34]. Randomized studies represent a particular situation and their results are not always reproduced in clinical practice. However, in this instance, when patients who started treatment in the 2000–2002 period were compared with those who started treatment later, we found that the probability of developing LD had decreased, while the pattern of drug prescription had significantly changed. In addition, in an analysis of all patients (not just the newly treated), the number of visits resulting in a change of treatment because of LD was found to have steadily decreased from 2003 to 2006. This indicates that, in the SHCS, the prevalence of LD serious enough to warrant a change of treatment is decreasing.

DEXA scans of patients taking standard NRTI-based cART show an early increase in limb fat before a steady and

progressive limb fat loss from 3 months onwards [35]. What will happen when thymidine analogues are no longer used? One may speculate that the atrophic effect on fat tissue will disappear, but that the influence of the other constituents of cART on fat will persist. For instance, if PIs contribute to fat accumulation, their unopposed actions might lead to gain of weight.

Our results lend some support to this hypothesis, because substantial weight gain (≥ 5 kg within 6 months) was indeed associated with the use of the PIs LPV and ATV. By restricting our analysis to patients with a BMI > 20 in men, and > 18 in women, we attempted to exclude patients where AIDS-related cachexia was corrected through HAART. Nonetheless, it is still unclear whether the weight gain represents an undesirable side effect, or rather the return to desirable normalcy. As expected, the average BMI in weight gainers (25.4) was higher than the average BMI of other patients, but was still lower than the average BMI of American patients [36].

Cohort studies have inherent advantages (for instance, long follow-up and large size) but also disadvantages. Lack of randomization and masking favour bias and confounding.

Among other well-known risk factors for lipoatrophy (older age, low CD4 and high HIV RNA), we showed that ABC was associated in both univariate and multivariate analyses with a diagnosis of lipoatrophy. This was puzzling, because randomized trials demonstrated that patients randomized to ABC had greater recovery of limb fat mass compared with those remaining on thymidine analogues [37]. Examination of individual case records in the SHCS revealed that many ABC-treated patients had previously been diagnosed with lipoatrophy, leading to replacement of ZDV or d4T in patients with atrophy [37,38] by ABC; as atrophy persists for years, so does the association with ABC. In the context of this observational cohort, association does not equal causation.

Other study limitations include the lack of objective measures for fat loss and fat redistribution. However, SHCS patients are treated by specialized physicians skilled in the detection and the management of LD. Diet or lifestyle variables are not routinely recorded in the database and may limit the ability to interpret weight changes. Moreover, the database does not allow assessment of the use of ritonavir as a booster in ATV-treated patients.

In our analysis we made no distinction between current and cumulative exposure to individual ART agents. However, we have demonstrated that LD was generally diagnosed long before inclusion in our analysis in 2003.

We have scanned the medical literature and conference abstracts to search for other evidence of changing patterns

and incidence of LD, but without success. The preliminary data we report here should stimulate additional research on the prevalence of LD and weight changes in large cohorts. It will be of particular interest to examine the impact of new drug classes, such as integrase or entry inhibitors, on the long-term pattern of fat redistribution and weight changes.

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Appendix: composition of the SHCS cohort

The members of the Swiss HIV Cohort Study are M. Battegay, E. Bernasconi, J. Böni, H. Bucher, Ph. Bürgisser, A. Calmy, S. Cattacin, M. Cavassini, R. Dubs, M. Egger, L. Elzi, P. Erb, M. Fischer, M. Flepp, A. Fontana, P. Francioli, H. Furrer, M. Gorgievski, H. Günthard, H. Hirsch, B. Hirschel, I. Hösli, Ch. Kahlert, L. Kaiser, U. Karrer, C. Kind, Th. Klimkait, B. Ledergerber, G. Martinetti, B. Martinez, N. Müller, D. Nadal, M. Opravil, F. Paccaud, G. Pantaleo, M. Rickenbach, C. Rudin, P. Schmid, D. Schultze, J. Schüpbach, R. Speck, P. Taffé, P. Tarr, A. Telenti, A. Trkola, P. Vernazza, R. Weber and S. Yerly.

Composition of the University Hospital Geneva Cohort

Participating physicians

B. Hirschel, V. Schiffer, E. Boffi, S. Inoubli, A. Gayet, C. Morel-Boyce, S. Emonet, A. Calmy and A. Nguyen

Database manager

A. Nguyen

Metabolic syndrome, cardiovascular disease and type 2 diabetes mellitus after initiation of antiretroviral therapy in HIV infection

Handan Wand^a, Alexandra Calmy^b, Dianne L. Carey^a,
Katherine Samaras^c, Andrew Carr^b, Matthew G. Law^a,
David A. Cooper^{a,b}, and Sean Emery^a, on behalf of the INITIO
Trial International Coordinating Committee

Background: Metabolic syndrome (MS) identifies individuals at risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). Little is known about MS and its consequences following initiation of antiretroviral therapy (ART).

Methods: HIV-infected adults (881) initiating ART were evaluated for prevalence and incidence of MS and subsequent diagnosis of CVD and T2DM over a 3-year period. MS was defined by criteria of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (*Third Report*; ATP-III) or of the International Diabetes Federation (IDF).

Results: The prevalence of baseline MS was 8.5% and 7.8% (ATP-III and IDF, respectively). During follow-up, 234 (12/100 patient-years) (ATP-III) and 178 (8/100 patient-years) (IDF) progressed to MS. MS at baseline had a borderline association with increased risk of CVD [ATP-III: hazard ratio (HR), 2.56; 95% confidence interval (CI), 0.86–7.60; $P=0.095$; IDF: HR, 2.89; 95% CI, 0.98–8.63; $P=0.058$] and was significantly associated with an increased risk of T2DM (ATP-III: HR, 4.34; 95% CI, 1.83–10.25; $P=0.001$; IDF: HR, 3.33; 95% CI, 1.35–8.17; $P=0.009$). Incident MS was significantly associated with an increased risk of both CVD (ATP-III: HR, 2.73; 95% CI, 1.07–6.96; $P=0.036$; IDF: HR, 3.05; 95% CI, 1.20–7.75; $P=0.019$) and T2DM (ATP-III: HR, 4.89; 95% CI, 2.22–10.78; $P<0.0001$; IDF: HR, 4.84; 95% CI, 2.20–10.64; $P<0.0001$).

Conclusions: Substantial progression to MS occurs within 3 years following initiation of ART. Since baseline and incident MS identifies individuals at risk for subsequent CVD and T2DM, it warrants evaluation in patients commencing ART.

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Introduction

Metabolic syndrome (MS) is an aggregation of central obesity and metabolic abnormalities that confer an increased risk of cardiovascular disease (CVD) and type 2 diabetes (T2DM) [1]. Approximately 25% of the US general population has MS [2]. The clinical utility of MS

for predicting cardiovascular risk beyond individual risk factors is subject to intense international discussion [3]. Findings from a recent British male cohort put the prevalence of MS at 26% and established the validity of MS as a simple clinical tool for identifying subjects predisposed to T2DM [4]. In most general populations studied to-date, the prevalence of MS increases with age

From the ^aNational Centre in HIV Epidemiology and Clinical Research, University of New South Wales, the ^bSt Vincent's Hospital, and the ^cGarvan Institute for Medical Research, Sydney, Australia.

Correspondence to Associate Professor S. Emery, National Centre for HIV Epidemiology and Clinical Research, University of New South Wales, 376 Victoria St, Sydney, NSW 2010, Australia.

E-mail: semery@nchechr.unsw.edu.au

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and varies with ethnicity. There are several definitions of MS, the most widely promulgated being the definitions in the *Third Report* of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III; ATP-III) and the International Diabetes Federation (IDF) [5,6]. The ATP-III definition requires three of five simple clinical measures [waist circumference, triglycerides, high density lipoprotein (HDL) cholesterol, blood pressure and glucose]. The IDF definition uses a gender- and race-specific waist circumference threshold and places a greater emphasis on abdominal obesity, making it an essential requirement for diagnosis.

Combination antiretroviral therapy (ART) for HIV-1 infection is frequently complicated by lipodystrophy (peripheral fat loss and relative visceral obesity), dyslipidaemia and insulin resistance [7,8]. The clustering of these metabolic and morphological abnormalities has striking similarities with MS. HIV-infected adults receiving ART have an increased incidence of elevated blood pressure and cardiovascular morbidity [9–13] and might be at increased risk of developing MS and its complications. A better understanding of links between MS and subsequent morbidities in this patient population might allow for more effective clinical management of patients.

Data on the prevalence of MS in HIV-infected populations are limited and were acquired using different methodologies and applied to different populations. A Spanish study demonstrated MS prevalence at 17%, while a US study described a prevalence of 26% [14–16]. An international cohort, examined cross-sectionally, determined a prevalence of 14% and 18% by IDF and ATP-III criteria, respectively [17]. A recent report indicated 24% prevalence of MS in a US cohort. In this setting, the incidence of MS was determined as 1.2/100 patient-months [18].

No study has examined the predictive value of MS for CVD or T2DM in any population commencing ART. INITIO, an international, multicentre, randomized clinical trial, compared three treatment strategies for initial ART [19] and this allowed the examination of (a) the prevalence of MS, CVD and T2DM in treatment-naïve HIV-infected participants, (b) the 3-year incidence of MS, CVD and T2DM; and (c) the predictive factors for the development of MS, CVD and T2DM.

Methods

Study aims and participants

Individuals (881) commencing randomly assigned regimens of initial ART in INITIO were evaluated for the

prevalence, incidence of MS and subsequent CVD and T2DM. Demographic and clinical features were examined at baseline and during follow-up for associations with MS and subsequent diagnoses of CVD or T2DM. All analyses were posthoc.

INITIO participants were randomized in a 1 to 1 to 1 ratio to receive the nucleoside reverse transcriptase inhibitors didanosine and stavudine together with efavirenz (288), nelfinavir (305), or efavirenz plus nelfinavir (288). Episodes of study drug intolerance were managed by switches within drug class. This preserved the randomized treatment strategy and was well adhered to during study conduct [19]. Median follow-up was 192 weeks [interquartile range (IQR), 165–215]. All participants provided written, informed consent that was approved by participant institution review committees.

Assessments

All participants were assessed for the components of MS at baseline and every 12 weeks thereafter. At each visit, blood pressure was measured and blood collected for HDL cholesterol, triglyceride and glucose determination. In addition, body habitus parameters (weight, umbilical waist and maximum hip circumference) were measured and ART changes recorded. Height was recorded at baseline. Blood pressure and anthropometric assessments were not subject to external quality control. Procedures were standardized by protocol. Blood pressure was assessed in a seated subject who had rested for at least 5 min prior to recording. Waist and hip measurements were performed using a flexible tape applied loosely to defined landmarks in subjects without outer clothing. Weight was measured without shoes or other heavy garments.

Definitions

MS was defined using ATP-III and IDF criteria [5,6]. ATP-III MS comprises three or more of the following: (a) fasting glucose ≥ 6.1 mmol/l; (b) fasting triglycerides ≥ 1.7 mmol/l; (c) HDL cholesterol < 1.04 mmol/l; (d) systolic blood pressure > 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg; and (e) waist circumference > 102 cm for men and > 88 cm for women. IDF MS requires central obesity as defined by a waist circumference > 94 cm for men and > 80 cm for women. In addition, two of the following four factors are required: (a) fasting glucose ≥ 5.6 mmol/l; (b) fasting triglycerides ≥ 1.7 mmol/l; (c) HDL cholesterol < 1.29 mmol/l; and (d) systolic blood pressure > 130 mmHg or diastolic blood pressure ≥ 85 mmHg. When nonfasting blood was collected, triglyceride and glucose thresholds were 2.26 mmol/l (200 mg/dl) and 8 mmol/l, respectively [20,21].

All spontaneously reported cardiovascular events were reviewed, and the following events included in these analyses: arrhythmia, shock or heart failure,

congestive cardiac failure, pulmonary oedema, angina, ischemic heart disease, myocardial infarction, other/unspecified CVD. Symptomatic and asymptomatic cardiomyopathy and hypertension were not included. Patients with T2DM at baseline or a clinical history of CVD at baseline were not included in the analyses of cardiovascular events. Diagnosis of T2DM during INITIO was defined by a fasting glucose >7 mmol/l or a nonfasting glucose >11.1 mmol/l in the absence of symptoms of diabetes [21]. CVD and T2DM diagnoses included for analysis were not subject to independent verification.

The formulation of the Framingham risk score used was that of Anderson [22]. The Framingham equation was used to estimate the risk of CVD (defined as myocardial infarction, fatal coronary heart disease plus angina and coronary insufficiency) over 10 years for each participant at baseline, which was then compared with the MS estimates of risk for CVD and T2DM.

Statistical analysis

Participants were assessed for MS according to both ATP-III and IDF criteria at each nominal study week using a time-window approach with available data. Time to incident MS was defined as the time from randomization to the study week of first MS. The last visit was defined as the week 156 data. Any patient with data missing at intermittent study weeks was recorded as not having MS at that study visit. Factors associated with MS at baseline were assessed using χ^2 tests and two-sample *t*-tests. Factors considered were sex, age, body mass index (BMI), smoking status, hip circumference, waist/hip ratio, CD4 cell count, $\log_{10}$ HIV RNA, HIV disease stage at baseline, and the components of MS (waist circumference, systolic and diastolic blood pressure, triglycerides, glucose and HDL) and total cholesterol.

Time to MS in participants without MS at baseline was summarized using Kaplan–Meier survival plots. Survival plots were compared using the log-rank test. Risk factors for incident MS were assessed using Cox regression adjusted for age, sex and smoking status. Risk factors considered were as for baseline MS plus randomized ART arm. To assess the role of ART exposure, participants randomized to receive nelfinavir or nelfinavir plus efavirenz (on a common backbone of didanosine plus stavudine) were treated as one group and compared with the group randomized to receive efavirenz (plus backbone didanosine plus stavudine). Multivariate models considered all variables statistically significant ($P < 0.05$) in initial analyses and used forward stepwise methods.

The effects of MS and the other covariates on the risk of CVD events and T2DM during the study follow-up were evaluated by Cox regression analyses. In these analyses, participants with established CVD or T2DM at baseline were excluded, and MS was fitted as a time-dependent

covariate. There was no adjustment of *P* values for multiple comparisons. All analyses were performed using Stata Statistical Software 8.2 (Stata Corporation, College Station, Texas, USA).

Results

A total of 881 randomized subjects commenced allocated regimens of ART (288 to efavirenz, 305 to nelfinavir and 288 to efavirenz plus nelfinavir) and formed the intention-to-treat population. After 3 years, a total of 741 (84%) subjects remained in follow-up (246 efavirenz, 258 nelfinavir and 237 efavirenz plus nelfinavir). At every scheduled assessment, 75–89% of patients provided required datasets for the stated analyses. The mean age of the 881 INITIO participants included in these analyses was 38.7 years (SD, 10). The mean BMI was 23.0 kg/m² (SD, 3.6) and 21% were female.

During follow-up, approximately 25% of patients at each visit provided fasted blood samples. As such, approximately 75% of the cut-offs for serum triglycerides and glucose were at the higher level described in the Methods.

Prevalence of metabolic syndrome at baseline

Prior to initiation of ART, 75 (8.5%) and 69 (7.8%) participants had MS using the ATP-III criteria and IDF definitions, respectively. Table 1 presents the baseline demographic and clinical characteristics of participants according to the two MS definitions. Participants with MS (ATP-III or IDF) at baseline were significantly older and had a higher BMI, hip circumference and waist/hip ratio. Each MS component was significantly associated with the presence of MS at baseline.

Incidence of metabolic syndrome

During follow-up, 234 (12/100 patient-years) and 178 (8/100 patient-years) developed MS according to ATP-III and IDF definitions, respectively. Among the 234 participants who developed ATP-III-defined MS, the most prevalent MS-defining presentation was high triglycerides in association with high blood pressure and elevated glucose (in 50%; data not shown). A further 30% had presentations comprising high blood pressure with high triglycerides and high waist circumference; high blood pressure with high triglycerides and high waist circumference; high triglycerides with high glucose and low HDL cholesterol; and high triglycerides with high glucose and low HDL cholesterol. The Kaplan–Meier survival curves of MS for both definitions are shown in Fig. 1. The overall incidence was slightly higher for ATP-III-defined MS than for that defined by IDF. There were no significant differences in MS incidence between randomly allocated regimens of ART based on the comparison of efavirenz recipients with the combined

Table 1. Baseline characteristics of 881 INITIO participants by metabolic syndrome criteria.

Baseline characteristic	ATP-III definition			IDF definition		
	Not present (n = 806)	Present (n = 75)	P value	Not present (n = 812)	Present (n = 69)	P value
Male [No. (%)]	634 (79)	60 (80)	0.82	640 (79)	54 (78)	0.88
Mean age [years (SD)]	38 (10)	44 (10)	< 0.0001	38 (10)	47 (10)	< 0.0001
Mean BMI [kg/m ² (SD)]	23 (3)	26 (4)	< 0.0001	22 (3)	28 (3)	< 0.0001
Smoking status [No. (%)]						
Never	338 (42)	31 (42)	0.58	339 (42)	28 (41)	0.045
Past	133 (17)	16 (21)		130 (16)	19 (27)	
Current	326 (41)	28 (38)		332 (41)	22 (32)	
Mean hip circumference [cm (SD)]	92 (9)	100 (10)	< 0.0001	92 (9)	104 (9)	< 0.0001
Mean waist/hip ratio (SD)	0.89 (0.08)	0.93 (0.08)	< 0.0001	0.89 (0.08)	0.96 (0.08)	< 0.0001
Mean CD4 cell count [cells/μl (SD)]	220 (170)	227 (179)	0.72	217 (171)	258 (162)	0.058
Mean plasma HIV RNA [log ₁₀ copies/ml (SD)]	4.94 (0.72)	5.00 (0.77)	0.47	4.95 (0.72)	4.87 (0.69)	0.40
Total cholesterol [mmol/l (SD)]	4.27 (1.01)	4.52 (1.20)	0.05	4.27 (1.00)	4.49 (1.10)	0.12
HIV stage [No. (%)]						
Asymptomatic	473 (59)	77 (63)	0.72	471 (58)	59 (72)	0.10
Symptomatic non-AIDS	161 (20)	15 (20)		165 (20)	11 (16)	
AIDS	170 (21)	13 (17)		174 (22)	9 (13)	
Mean metabolic syndrome components (SD)						
Waist circumference (cm)	82 (10)	93 (11)	< 0.0001	82 (9)	99 (8)	< 0.0001
Systolic blood pressure (mmHg)	118 (14)	133 (13)	< 0.0001	118 (14)	132 (14)	< 0.0001
Diastolic blood pressure (mmHg)	75 (10)	83 (10)	< 0.0001	75 (10)	83 (10)	< 0.0001
Triglycerides (mmol/l)	1.48 (1.00)	2.72 (2.00)	< 0.0001	1.55 (1.00)	2.03 (1.00)	0.0009
Glucose (mmol/l)	4.84 (1.00)	5.72 (2.00)	< 0.0001	4.87 (1.00)	5.52 (1.00)	< 0.0001
HDL-cholesterol (mmol/l)	1.05 (0.40)	0.80 (0.20)	< 0.0001	1.05 (0.40)	0.83 (0.20)	< 0.0001

ATP-III, criteria of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF, International Diabetes Federation definition; BMI, body mass index; HDL, high density lipoprotein.

results for recipients of nelfinavir and nelfinavir plus efavirenz (*P* = 0.46 and 0.39; respectively; Fig. 2).

Multivariate analyses of baseline covariates associated with the development of MS are summarized in Table 2. High baseline BMI, waist/hip ratio, diastolic blood pressure, or triglyceride and low baseline HDL cholesterol were all significantly associated with an increased

risk of ATP-III-defined MS. In contrast, only high BMI, hip circumference, waist/hip ratio and triglyceride levels were significantly associated with IDF-defined MS.

Metabolic syndrome as a predictor of cardiovascular events

During follow-up, 21 CVD events were reported among 19 (2.2%) individuals. These events included 11 episodes

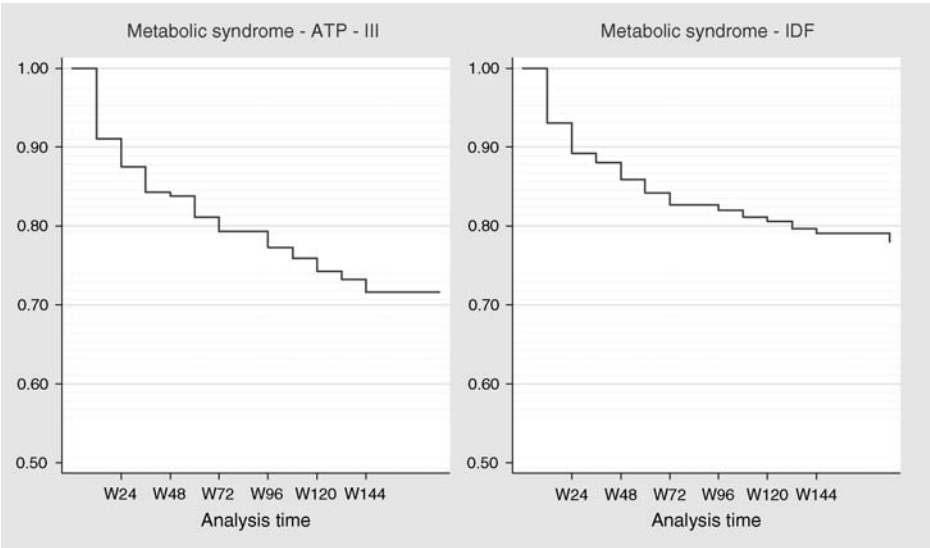


Fig. 1. Survival curves for progression to metabolic syndrome during study follow up as defined by the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP-III) or the International Diabetes Federation (IDF).

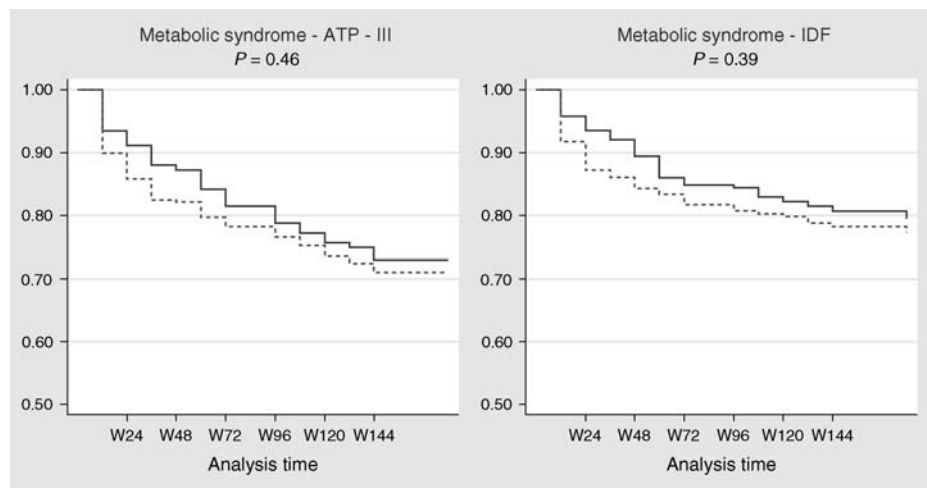


Fig. 2. Survival curves for progression to metabolic syndrome as defined by the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP-III) or the International Diabetes Federation (IDF) during study follow up, by baseline treatment groups: didanosine/stavudine/efavirenz (—); didanosine/stavudine/nelfinavir or didanosine/stavudine/nelfinavir/efavirenz (---). *P* values obtained by log rank test.

of shock/heart failure, 6 episodes of angina/ischaemic heart disease, 2 episodes of arrhythmia and 1 episode for each of myocardial infarction and unspecified CVD. Factors associated with developing a CVD event are summarized in Table 3. IDF-defined MS at baseline showed a borderline, nonsignificant association with an increased risk of CVD [hazard ratio (HR), 2.89; 95% confidence interval (CI), 0.98–8.63; *P* = 0.058], whereas ATP-III-defined MS at baseline did not show a significant association (HR, 2.56; 95% CI, 0.86–7.60; *P* = 0.095). Progression to MS during follow-up by ATP-III and IDF definitions was significantly associated with an increased risk of developing cardiovascular events during follow-up (ATP-III: HR, 2.73; 95% CI, 1.07–6.96; *P* = 0.036; IDF: HR, 3.05; 95% CI, 1.20–7.275; *P* = 0.019). Of MS components, only waist circumference and systolic blood pressure were significantly associated with cardiovascular events during follow-up. Higher baseline Framingham

score was not significantly associated with an increased risk of CVD (HR, 1.29 95% CI, 0.35–4.82; *P* = 0.7).

Metabolic syndrome as a predictor of type 2 diabetes

The incidence of T2DM during 3 years of follow-up was 5% (per 100 patient-years) derived from a total of 41 diagnoses. In total, 13 of these patients had more than one episode of casual plasma glucose > 11.1 mmol/l. Participants with ATP-III- or IDF-defined MS at baseline showed a significantly increased risk of developing T2DM during follow-up (ATP-III: HR, 4.34; 95% CI, 1.83–10.25; *P* = 0.001; IDF: HR, 3.33; 95% CI, 1.35–8.17; *P* = 0.009). Several individual components of MS (waist circumference, systolic blood pressure, triglycerides and glucose) were significant predictors of incident T2DM (Table 3). Progression to MS during follow-up according to both ATP-III and IDF definitions

Table 2. Multivariate risk factors for progression to metabolic syndrome.

Baseline characteristic ^a	ATP-III definition		IDF definition	
	HR ^b (95% CI)	<i>P</i> value	HR ^b (95% CI)	<i>P</i> value
BMI (kg/m ²)	1.14 (1.09–1.20)	< 0.0001	1.10 (1.02–1.19)	0.012
Hip circumference (cm)	—	—	1.03 (1.00–1.06)	< 0.0001
Waist/hip ratio (> 0.90)	1.45 (1.04–2.02)	0.028	2.55 (1.59–4.09)	< 0.0001
Metabolic syndrome components				
Diastolic blood pressure (10 mmHg)	1.03 (1.01–1.04)	< 0.0001	—	—
Triglycerides (mmol/l)	1.32 (1.18–1.49)	< 0.0001	1.29 (1.16–1.43)	< 0.0001
HDL cholesterol (mmol/l)	0.32 (0.17–0.60)	< 0.0001	—	—

ATP-III, criteria of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF, International Diabetes Federation definition; HR, hazard ratio; CI, confidence interval; BMI, body mass index; HDL, high density lipoprotein.

^aAll factors are adjusted for age, gender and smoking status.

^bHazard ratios are per unit change for each covariate as indicated; a dash indicates a nonsignificant HR and omission of the characteristic from the multivariate model.

Table 3. Risk factors for cardiovascular disease and type 2 diabetes mellitus.

Baseline characteristic ^a	CVD ^b		T2DM ^c	
	HR ^d (95% CI)	<i>P</i> value	HR ^d (95% CI)	<i>P</i> value
Baseline metabolic syndrome				
ATP-III definition	2.56 (0.86–7.60)	0.095	4.34 (1.83–10.25)	0.001
IDF definition	2.89 (0.98–8.63)	0.058	3.33 (1.35–8.17)	0.009
On-study metabolic syndrome				
ATP-III definition	2.73 (1.07–6.96)	0.036	4.89 (2.22–10.78)	<0.0001
IDF definition	3.05 (1.20–7.75)	0.019	4.84 (2.20–10.64)	<0.0001
ART class				
NNRTI	1.00		1.00	
PI	0.83 (0.30–2.31)	0.73	0.84 (0.33–2.13)	0.72
NNRTI + PI	0.49 (0.15–1.64)	0.25	1.00 (0.41–2.47)	0.99
BMI (kg/m ²)	1.03 (0.92–1.15)	0.59	1.13 (1.04–1.22)	0.003
Hip circumference (cm)	1.01 (0.97–1.06)	0.64	1.05 (1.02–1.09)	0.003
Waist/hip ratio ^e (>0.90 male, >0.80 female)	4.92 (1.48–16.40)	0.009	1.54 (0.61–3.90)	0.36
CD4 cell count (per 100 cells/ml higher)	1.00 (0.99–1.003)	0.28	1.00 (0.99–1.002)	0.82
Plasma HIV RNA (per log ₁₀ copies/μl higher)	1.25 (0.64–2.45)	0.52	0.97 (0.61–1.54)	0.90
Total cholesterol (mmol/l)	1.28 (0.84–1.93)	0.25	1.37 (1.01–1.84)	0.04
HIV disease stage				
Asymptomatic	1.00		1.00	
Symptomatic, non-AIDS	1.57 (0.58–4.26)	0.38	0.33 (0.10–1.10)	0.071
AIDS	0.99 (0.31–3.10)	0.98	0.97 (0.45–2.07)	0.93
Metabolic syndrome components				
Waist circumference (cm)	1.03 (1.00–1.07)	0.072	1.05 (1.02–1.08)	<0.0001
Systolic blood pressure (10 mmHg)	1.02 (0.99–1.04)	0.13	1.03 (1.01–1.05)	0.001
Diastolic blood pressure (10 mmHg)	1.02 (0.99–1.06)	0.17	0.99 (0.96–1.02)	0.71
Triglycerides (mmol/l)	1.04 (0.71–1.52)	0.84	1.30 (1.04–1.61)	0.019
Glucose (mmol/l)	1.21 (0.87–1.69)	0.26	1.84 (1.49–2.28)	<0.0001
HDL-cholesterol (mmol/l)	2.58 (0.87–7.56)	0.10	0.44 (0.13–1.48)	0.19
Framingham risk score ^f (>3%)	1.29 (0.35–4.82)	0.70	1.10 (0.42–2.67)	0.90

CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus; ATP-III, criteria of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF, International Diabetes Federation definition; HR, hazard ratio; CI, confidence interval; BMI, body mass index; HDL, high density lipoprotein; ART, antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

^aAll characteristics are per unit change for each covariate as indicated.

^bCVD as described in Methods.

^cT2DM as described in Methods.

^dAll Hazard ratios are adjusted for age, gender and smoking status.

^eIncluded in the model as a time-varying covariate.

^fCalculated using available data from approximately 500 patients.

was associated with a significantly increased risk of developing T2DM (ATP-III: HR, 4.89; 95% CI, 2.22–10.78; $P < 0.0001$; IDF: HR, 4.84; 95% CI, 2.20–10.64; $P < 0.0001$). These relative risk values were higher than those for the individual risk factors. The baseline median glucose levels for patients who later developed T2DM were 5.4 mmol/l (IQR, 5.0–6.2) and 5.2 mmol/l (IQR, 5.0–6.1) for those with metabolic syndrome defined by ATP-III and IDF criteria, respectively. Higher baseline Framingham score did not predict risk of T2DM (HR, 1.10 95% CI, 0.42–2.67; $P = 0.9$).

Discussion

In this study of 881 HIV-infected adults commencing combination ART and followed for 3 years, the baseline prevalence of MS was 8.5% assessed by ATP-III criteria and 7.8% assessed by IDF criteria. The presence of MS at baseline was associated with at least a 2.5-fold increase in

risk for development of CVD ($P = 0.095$) and at least a 3.3-fold increase in risk of T2DM ($P = 0.009$). Incident MS was significantly associated with the development of CVD and T2DM. This findings, based on relatively few incident cases of CVD (21) or T2DM (41), is striking.

Our analyses showed that baseline and incident MS had greater predictive value than a baseline dichotomized Framingham Risk Score for subsequent CVD and T2DM. These results provide an evidential basis for the use of MS in risk determination. Previous investigations have indicated that the Framingham Risk Score underestimates myocardial infarction in HIV-infected patients treated with ART but overestimates myocardial infarction in HIV-infected patients who are not treated with ART [23]. In both patient groups, the Framingham Risk Score was sensitive. In the present study, we employed a more extensive list of CVD events than myocardial infarction alone. These differences in endpoint definition might have contributed to the lack of prediction for CVD events provided by the Framingham

Risk Score at baseline. It is also possible that the Framingham Risk Score lacks precision when assessed in a treatment-naïve, HIV-infected subject (there are physiological features not included in the Framingham equation that are important and independent determinants of CVD risk). This would argue for assessment of CVD risk to include MS as well as the Framingham Risk Score. An additional benefit from this approach would be the assessment of risk for T2DM.

The prevalence of MS in the US general population exceeds 23% and is approximately 26% in British men [2,4]. The prevalence of MS in this HIV-infected cohort was substantially less than these HIV-negative populations. It was also lower than that reported elsewhere for HIV-positive populations, where prevalence ranged between 14 and 24% [14–18]. INITIO participants were young (mean age 38.7 years), recruited in diverse clinical settings (119 sites in 21 countries), and 21% were female. The relatively low baseline prevalence of MS may reflect the differing demographic characteristics of study participants. The influence of age and race on MS has been well described; the potential contribution of underlying HIV wasting in the absence at baseline of ART should also be considered. Uncontrolled HIV replication also affects serum lipid concentrations (reducing HDL and low density lipoprotein cholesterol but increasing triglycerides) [24–26]. These derangements in lipid metabolism would be expected to increase the prevalence of MS in the INITIO population at baseline, and particularly for the ATP-III definition. Although higher MS prevalence has been described in HIV-positive cohorts, these studies were cross-sectional and predominantly included individuals already taking ART [14–17,27,28]. The INITIO trial provided a unique opportunity to examine MS progression in HIV-infected, treatment-naïve individuals. Importantly, the lower rates of MS in this population suggests that HIV infection *per se* probably does not contribute to MS or may perhaps confer protection from components of the syndrome. Whether ART-naïve patients are at reduced risk of subsequent CVD or T2DM is unclear, particularly in light of emerging data from randomized trials and cohorts indicating rates of CVD are higher in people with HIV who are not treated with ART [29,30].

Despite low baseline prevalence, we observed substantial rates of progression to MS (12/100 patient-years by ATP-III and 8/100 patient-years by IDF) over the 3 years following initiation of ART. These estimates are substantially higher than those recently reported in the prospective follow up of a US cohort [18]. It seems probable the difference arises because we assessed incidence in a truly treatment-naïve cohort while only 25% (135) of the recently reported population were ART naïve at baseline and it is not clear how many of these actually commenced ART during follow-up.

The most prevalent progression to MS was elevated triglycerides in association with increased blood pressure and glucose. In HIV-negative populations, the most common features associated with MS are obesity and hypertension. Elevated blood pressure and triglycerides were also reported as common components in the prospective follow up of a US HIV-positive cohort and the most frequent abnormalities that led to MS diagnosis in a cross-sectional HIV-infected cohort [18,31]. Prevention and management of MS in HIV-positive patients might need to be tailored accordingly.

This study found the risk of T2DM increased at least 3.3-fold in those with MS at baseline (ATP-III: HR, 4.34; 95% CI, 1.83–10.25; IDF: HR, 3.33; 95% CI, 1.35–8.17). In addition, incident MS was significantly associated both with a diagnosis of T2DM and with the development of CVD. These findings are consistent with observations made in the general populations studied to date [30]. The adjusted HR values for MS were higher than those for the individual MS components. This finding is important, indicating a higher predictive value from MS than the predictive value of its individual components. The validity of MS has been under substantial international debate and this study is one of the first to use prospective data to support the multiplicative effects of the combined risk factors over the assessment of individual risk factors [3].

We did not observe any significant differences in rates of progression to MS between the randomized treatment groups in INITIO. This is despite the well-described impact of ART class or drugs on the components of MS [7,9–14,18,31–33]. There was departure from the protocol-defined initial regimens of therapy in INITIO [19]. It is possible this dilution tended to mask any real differences arising from the different regimens in this study. Similarly, all patients received a common nucleoside reverse transcriptase inhibitor component in their ART regimen, didanosine and stavudine. This too, may have masked any contributions of either nelfinavir or efavirenz on progression to MS in this cohort.

There are some important caveats to this work. First, all of the analyses were post hoc and there was no adjustment for multiple analyses. There is a possibility of type I errors. Second, our CVD endpoints were assessed from spontaneous study site reports that were not subjected to any type of validation. It is also true that our analyses included only a limited number of defined clinical events (21 CVD and 41 T2DM), resulting in very limited overall power. Third, it should be noted that the INITIO regimens would no longer be recommended for initial therapy [34]. Currently recommended initial ART would be expected to induce less dyslipidaemia and lipodystrophy. Fourth, we do not have reliable data on race or ethnicity and cannot comment on any racial differences within our cohort. Finally, we were not able to

confirm that assessment of serum triglycerides or glucose was performed on blood drawn from fasted subjects for all but a minority of scheduled visits. We selected an applicable definition of T2DM and selected a CVD diagnosis that we felt to be clinically related to atherosclerosis and vascular disease. Collectively, we urge caution against overinterpretation of these findings.

Debate continues on the validity of MS for clinical management. We did not observe substantial differences between the ATP-III and IDF assessment of MS in this cohort. Because the IDF system now adjusts for race, we would propose that it is likely to be more applicable. Our data indicate that MS is less prevalent in HIV-infected individuals who are ART naive compared with that in the general population. We observed an incident rate of progression to MS in HIV-infected patients treated with combination ART somewhat higher than previously reported. The finding that MS possibly identifies those at increased risk of CVD and T2DM may be of value for selecting patients for risk assessment and possibly preventive strategies. This is particularly true given the need for lifelong ART in people with HIV-1 infection. The impact of preventive strategies would need evaluation.

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INITIO Trial International Coordinating Committee. D.A. Cooper (joint Chair NCHECR), P. Yeni (joint Chair Groupe Hospitalier Bichat), J.-P. Aboulker (INSERM SC10), F. Antunes (National Principal Investigator, Portugal), A. Babiker (MRC CTU), M. Becker (Roche), N. Boukli (INSERM SC10), F. Brun-Vezinet (joint chair Virology Group), D. Carey (NCHECR), D. Churchill (joint National Principal Investigator, UK), B. Conway (National Principal Investigator, Canada), C. Chazallon (INSERM SC10), J. Darbyshire (MRC CTU), S. De Wit (National Principal Investigator, Belgium), B. Dusak (Dupont), S. Emery (NCHECR), M. Flepp (National Principal Investigator, Switzerland), M. Floridia (ISS), J. Gatell (National Principal Investigator, Spain), P.-M. Girard (National Principal Investigator, France), R.L.

Goodall (MRC CTU), R. Hemmer (National Principal Investigator, Luxembourg), M. Hooker (MRC CTU), M. Law (NCHECR), C. Loveday (joint chair Virology Group), J. Lundgren (National Principal Investigator, Denmark), D. Manion (Dupont), V. Meiffredy (INSERM SC10), A. Mijch (National Principal Investigator, Australia and New Zealand), F. Mulcahy (National Principal Investigator, Ireland), A. Orani (National Principal Investigator, Italy), C. Pharo (GSK), M. Ristola (National Principal Investigator, Finland), B. Salzberger (joint National Principal Investigator, Germany), E. Sandstrom (National Principal Investigator, Sweden), M. Schechter (National Principal Investigator, Brazil), S. Schnittman (Bristol Myers Squibb), M. Seligmann (Chair Immunology Group), S. Staszewski (joint National Principal Investigator, Germany), M. Stek (Merck), W. Verbiest (VIRCO), J. Weber (joint National Principal Investigator, UK).

Coordinating Trial Centres. Australia/New Zealand/Brazil: National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney (D. Carey, S. Emery, W. Lee, S. Phipps, T. Sharkey); Canada: Department of Pharmacology and Therapeutics, University of British Columbia, Vancouver (B. Conway, R. Dimayuga, M. Jones, S. Jutha, D. Kraus, B. Zastre); Denmark/Sweden/Finland: Copenhagen HIV Programme, Hvidovre (U. Dragsted, A. Grønholdt, K. Jensen, J. Lundgren, D. Møllerup, L. Skinnes); France/Belgium/Luxembourg/Spain/Portugal: INSERM SC10, Paris (J.-P. Aboulker, N. Boukli, C. Chazallon, B. Guillon, S. Kahi, L. Léger, V. Meiffredy, A.-S. Rodier, Y. Saidi); UASP: Hospital Clinic, Barcelona (A. Cruceta); Germany: Coordinating Centre for Clinical Studies (KKS), Philipps University, Marburg (B. Lenz, J. Rochon, C. Schade-Brittinger, M. Wittenberg, H. Wolf); Italy: Laboratory of Virology and Department of Clinical Research and Evaluation, Istituto Superiore di Sanità, Rome (R. Bucciardini, M. Floridia, V. Fragola, C.M. Galluzzo, E. Germinario, M. Guidi, F. Innocenti, M. Massella, A. Mattei, M. Mirra, M.I. Paoloni, C. Polizzi, M. Pirillo, S. Vella); Switzerland: Clinic for Infectious Diseases and Hospital Epidemiology, University Hospital, Zurich (M. Flepp, E. Gremlich, A. Mosimann); UK and Ireland: MRC Clinical Trials Unit, London (A. Babiker, J. Darbyshire, R. Goodall, M. Hooker, F. Hudson, D. Johnson, P. Kelleher, A. Poland, K. Taylor, J. Wait, R. Withnall); Overall coordination: MRC CTU (London); databases held and maintained: National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia; INSERM SC10, Paris, France; Coordinating Centre for Clinical Studies, Philipps University, Marburg, Germany; Laboratory of Virology and Department of Clinical Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy; MRC Clinical Trials Unit, London, UK.

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HIV increases markers of cardiovascular risk: results from a randomized, treatment interruption trial

Alexandra Calmy^a, Angèle Gayet-Ageron^a, Fabrizio Montecucco^b,
Alain Nguyen^a, Francois Mach^b, Fabienne Burger^b,
Sasiwimol Ubolyam^c, Andrew Carr^d, Kiat Ruxungtham^{c,e},
Bernard Hirschel^a, Jintanat Ananworanich^{c,f},
on behalf of the STACCATO Study Group*

Objectives: Plasma soluble inflammatory molecules are associated with the risk of ischaemic cardiovascular events. We investigated whether HIV replication modified the levels of these proteins in a combination antiretroviral therapy (cART) interruption trial.

Method and results: In 145 HIV-infected Thai patients (62% women, median CD4 cell count 271 cells/ μ l, median plasma HIV-RNA 4.66 log₁₀ copies/ml) included in the Swiss–Thai–Australia Treatment Interruption Trial (STACCATO) trial, leptin, adiponectin, C-reactive protein, soluble vascular cell adhesion molecule-1 (s-VCAM-1), P-selectin, chemokine ligand 2, chemokine ligand 3, interleukin (IL)-6, IL-10, granulocyte macrophage colony-stimulating factor and D-dimer were measured before cART was initiated, after cART had suppressed HIV replication to less than 50 copies/ml plasma (median 8 months) and again 12 weeks after randomization to continued cART ($n = 48$) or interrupted cART ($n = 97$). Multiple linear regression and logistic regression were used to investigate the association between each cardiovascular marker and plasma HIV-RNA. Initiation of cART resulted in significant declines in s-VCAM-1, P-selectin, leptin and D-dimer, whereas mediators with anti-inflammatory properties, such as adiponectin and IL-10, increased. At 12 weeks after randomization, we found positive associations between levels of s-VCAM-1 and chemokine ligand 2 with an increase in plasma HIV-RNA ($r = 0.271$, $P = 0.001$ and $r = 0.24$, $P = 0.005$, respectively), whereas levels of adiponectin decreased for each 1 log increase in plasma HIV-RNA ($r = -0.24$, $P = 0.002$). Detectable IL-10 was less likely (odds ratio = 0.64, 95% confidence interval = 0.43–0.96) for each 1 log increase in plasma HIV-RNA.

Conclusion: Plasma levels of several inflammatory, anti-inflammatory and endothelial activation markers of cardiovascular disease are associated with HIV-RNA replication.

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Keywords: cardiovascular risk, HIV, HIV-RNA replication, inflammation, marker

^aHIV Unit, ^bFoundation for Medical Research, Cardiology Division, Geneva University Hospital, Geneva, Switzerland, ^cHIV Netherlands Australia Thailand Research Collaboration, Bangkok, Thailand, ^dSt. Vincent's Hospital, Sydney, New South Wales, Australia, ^eDepartment of Medicine, Faculty of Medicine, Chulalongkorn University, and ^fSouth East Asia Research Collaboration with Hawaii, Bangkok, Thailand.

Correspondence to Alexandra Calmy, MD, HIV Unit, Geneva University Hospital, 15 rue Micheli-Du-Crest, 1205 Geneva, Switzerland.

Tel: +41 22 3729808; fax: +41 22 3729599; e-mail: acalmy@gmail.com

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Introduction

Combination antiretroviral therapy (cART) has dramatically reduced both mortality and morbidity for infections associated with HIV infection [1]. Non-AIDS events, including acute cardiovascular disease, account for a substantial proportion of all deaths in patients starting cART with CD4⁺ lymphocyte counts above 200 cells/ μ l [2,3]. The metabolic effects of cART can be expected to increase the risk of cardiovascular events. The risk of myocardial infarction (MI) increases with longer exposure to cART [4], although traditional risk factors still remain the main determinants of cardiovascular risk in HIV-infected adults [5].

Paradoxically, intermittent, CD4 lymphocyte count-guided cART was associated with a 50% greater risk of MI relative to continuous cART [6]. One possible cause is HIV-induced endothelial activation [7].

Although established cardiovascular risk factors are widely used to assess cardiovascular risk in the general population, novel serum markers of endothelial activation and inflammation may also be involved in the pathogenesis of atherosclerosis [8]. Subclinical arterial inflammation is a prognostic factor in atherosclerosis [8]. Twenty-four weeks of cART improved endothelial dysfunction as measured by brachial artery flow-mediated dilation (FMD) [9]. The only variable associated with an improvement in FMD was the reduction in plasma HIV viral load.

We hypothesized that HIV replication could modify the serum levels of cardiovascular endothelial and inflammatory markers. We selected 12 biomarkers that have been associated with proatherosclerotic processes, including endothelial activation [soluble vascular cell adhesion molecule-1 (s-VCAM-1) and P-selectin], systemic inflammation [chemokine ligand 3 (CCL3), CCL2, granulocyte macrophage colony-stimulating factor (GM-CSF), C-reactive protein (CRP), interleukin-6 (IL-6) and IL-10], platelet (P-selectin) and coagulation cascade (D-dimer) activation (Table 1) [10–26]. Adipose tissue-derived hormones (such as leptin or adiponectin), both of which are correlated with cardiovascular diseases in epidemiological studies, have also been analysed [24–26].

We designed a post-hoc analysis comprising a subgroup of participants in the Swiss–Thai–Australia Treatment Interruption Trial (STACCATO) trial who had no prior ART exposure at entry and measured plasma levels of soluble mediators associated with cardiovascular risk before cART initiation, on cART when plasma HIV-RNA was undetectable and then in the randomized phase of the trial when patients either continued cART or suspended cART in order to test whether and how the values of the different cardiovascular markers correlated with HIV replication [27].

Participants and methods

Participants

The STACCATO trial [27] included 490 patients from Thailand, Australia and Switzerland. Patients were treated with cART for at least 6 months until plasma HIV-RNA was below 50 copies/ml and CD4⁺ lymphocyte count was more than 350 cells/ μ l when they were randomized 1:2 to continue cART (continued cART arm) or interrupted cART [structured treatment interruption (STI) arm]. Participant cART comprised ritonavir-boosted saquinavir and stavudine/didanosine until March 2003, when stavudine/didanosine was replaced by tenofovir/lamivudine or tenofovir/emtricitabine. To be eligible for the present analysis, participants had to be ART-naïve and have frozen serum samples available from before initiation of cART, at randomization when all patients were on cART and 12 weeks after randomization to continued or interrupted cART. One hundred and forty-five patients, all from seven STACCATO sites in Thailand, were included in the present analysis (48 patients in the continued cART arm and 97 in the STI arm).

Assessments

All participants were assessed on three occasions: before initiation of cART, when virologically suppressed at randomization and 12 weeks after randomization. Participants randomized in the STI arm also had samples analysed at the final visit in which they were re-treated with cART for 12 up to 24 weeks.

Real-time whole blood and ethylenediaminetetraacetic acid (EDTA) plasma were collected for measuring fasting total cholesterol (TC), triglycerides, glucose, CD4⁺ lymphocyte count and HIV-RNA. Plasma for storage was centrifuged at 800g for 10 min within 6 h of collection and then stored at -70°C for measurement of leptin, adiponectin, CRP, s-VCAM-1, P-selectin, CCL2, CCL3, IL-6, IL-10, GM-CSF, D-dimer and high-density lipoprotein (HDL) cholesterol.

Coronary risk profile was calculated using Framingham equation before the first antiretroviral treatment [28]. Using the Framingham equation, patients were categorized at high (>20% risk or patients with a history of cardiovascular disease), moderate (10–20% risk), low (<10% risk) 10-year risk of coronary heart disease or an unknown 10-year risk when the predicted risk of coronary heart disease could not be calculated because of missing values.

Cardiovascular risk marker level detection

Plasma leptin, adiponectin, s-VCAM-1, P-selectin, CCL2, GM-CSF, IL-6, IL-10 and CCL3 levels were measured by colorimetric enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota, USA). EDTA plasma D-dimer levels were measured by

Table 1. Name, role and abbreviations of soluble mediators associated with cardiovascular risk tested in the study.

Marker	Functions	HIV infection	Cardiovascular risk with higher levels
Soluble vascular cell adhesion molecule-1	Adhesion molecule released in endothelial dysfunction and activation	Increased serum levels [10]	Increased [11]
P-selectin	Adhesion molecule with a key role in promoting adherence of leukocyte to endothelium and marker of platelet activation	Not known	Increased [12]
Macrophage inflammatory protein-1 alpha or CCL3	CC chemokine involved in monocyte recruitment into atherosclerotic plaques and specific ligand for CCR5	Decreased serum levels [13]	Increased [14]
Monocyte chemoattractant protein-1 or CCL2	Proinflammatory CC chemokine attracting monocytes in inflammatory sites	Increased serum levels [15]	Increased [16]
Granulocyte macrophage colony stimulating factor	Growth factor with proinflammatory activities on leukocytes	Decreased in certain HIV cohorts [17]	Controversial [18]
C-reactive protein	Acute-phase reactant, mainly secreted by the liver with proinflammatory activities	Controversial [19]	Increased [20]
Interleukin-6	Proinflammatory cytokine	Controversial [19]	Increased [21]
Interleukin-10	Anti-inflammatory cytokine and antiatherosclerotic cytokine which is also associated with the development of lymphoproliferative disorders in HIV patients	Controversial [22]	Decreased [23]
D-dimer	Fibrin degradation products	Not known	Increased [19]
Leptin	Adipocytokine with immunomodulatory activities on leukocytes	Decreased serum levels in lipodystrophy [24]	Increased [25]
Adiponectin	Adipocytokine with anti-inflammatory properties on leukocytes and vascular cells	Controversial [24]	Decreased [26]

CCL, chemokine ligand; CCR5, chemokine (C-C motif) receptor 5.

enzyme-linked fluorescent assay (Roche Diagnostics Systems, Basel, Switzerland). EDTA plasma D-dimer values were validated by statistical correlation with sodium citrate plasma D-dimer values in normal individuals (data not shown). CRP was measured by high-sensitivity nephelometric latex immunoassay (Roche Diagnostic systems, Basel, Switzerland). The limits of detection for leptin was 31.25 pg/ml, 62.50 pg/ml for adiponectin, 6.25 ng/ml for s-VCAM-1, 125 pg/ml for P-selectin, 15.6 pg/ml for CCL2, 31.25 pg/ml for GM-CSF, 0.156 pg/ml for IL-6, 0.78 pg/ml for IL-10, 7.8 pg/ml for CCL3, 45 ng/ml for D-dimer and 0.11 µg/ml for CRP. Glucose, triglycerides, TC, low-density lipoprotein (LDL) cholesterol and HDL cholesterol were measured at fasting state and expressed in milligram per decalitre.

Statistical analysis

Patient characteristics were described before treatment. Participants included in the present analysis were compared for demographical and clinical variables with the entire naive Thai population of STACCATO using χ^2 test or Fisher's exact test for categorical variables and Student's *t*-test for means of continuous variables. When we compared cardiovascular markers between two time periods, we used paired Student's *t*-test or the Wilcoxon signed-rank test for continuous variables.

For most cardiovascular markers, results were expressed as medians [interquartile range (IQR)], except for GM-CSF, IL-10 and IL-6, each of which were presented as the proportion of patients with values above the limit of detection as normal distribution was not assessed for those three markers. We used a Wilcoxon signed-rank test or a McNemar's test to compare the values of each cardiovascular marker before and after cART and at randomization versus after final cART re-treatment.

Pearson's correlation coefficient was used to assess correlations between cardiovascular markers (s-VCAM-1, adiponectin, CCL2, CCL3, P-selectin, leptin, CRP and D-dimer) and log₁₀ plasma HIV-RNA before cART and at week 12, as a linear relationship between those markers and log₁₀ HIV-RNA before cART exists and normal distribution was also assessed.

The estimation of factors associated with changes in the levels of s-VCAM-1, adiponectin, CCL2, CCL3, P-selectin, leptin, CRP and D-dimer at week 12 was calculated using a multiple linear model adjusted on the main independent covariable, HIV-RNA, and on other well known demographical and clinical factors of HIV infection. We provide results of the unadjusted and adjusted β slope for a 10-fold (1 log) difference in HIV-RNA and the associated *P*-value. Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, multicollinearity and homoscedasticity. We used a backward, stepwise procedure that examined all variables selected in univariable analysis with

$P < 0.25$ and all clinically relevant interaction terms (between treatment use and log₁₀ HIV-RNA and all interaction terms between log₁₀ HIV-RNA and variables in the final model). First, all interaction terms with $P > 0.05$ were removed from the model. Then we eliminated the main covariates that were nonsignificant ($P > 0.05$) but kept in the model all potentially confounding variables. Residual analysis was used to assess the adequacy of the fitted, multiple, linear model.

We present the mean value of s-VCAM-1, adiponectin, CCL2 and IL-10 assorted with their 95% confidence interval (CI) by three HIV-RNA strata. We divided HIV-RNA into quartiles and defined three strata: undetectable HIV-RNA (<50 copies/ml or 25th percentile), detectable below the 75th percentile (50–10 000 copies/ml) or detectable above the 75th percentile (>10 000 copies/ml). A *P*-test for linearity of each mean marker across the three HIV-RNA strata was assessed using analysis of variance.

For GM-CSF, IL-10 and IL6, we used a logistic regression model to assess the risk of a value above the limit of detection for each 1 log difference of HIV-RNA at week 12. We controlled for demographical and clinical factors that are potentially associated with the assessed risk. We provided unadjusted and adjusted odds ratios with their 95% CIs. The Hosmer and Lemeshow goodness-of-fit test was interpreted as acceptable if the corresponding *P*-value of the Pearson's χ^2 statistic with 8 DF was more than 0.05.

Regression models were adjusted on the following factors: as continuous variable, age, Centers for Disease Control and Prevention (CDC) stage at baseline (A stage as the reference), risk factors for HIV infection (heterosexual sex as the reference), sex (male as the reference), weight, white blood cell count, platelet count, CD4⁺ lymphocyte count, TC, glucose, LDL cholesterol, triglycerides and TC/HDL cholesterol ratio. All analyses were done with Statistical Package for the Social Sciences, version 11.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Patients' characteristics

Of the 216 Thai patients (67.1%) enrolled into STACCATO, we included all 145 patients with available samples. We did not find any difference regarding demographic and clinical variables between the 145 Thai patients with available samples and the 216 Thai naive patients (data not shown). Baseline (pre-cART) demographic and biological characteristics of the study population are shown in Table 2. Ninety patients were women (62%). Mean age was 33.9 years ($\pm$ SD 8.1), and only four (3%) patients were classified as CDC stage C. Median baseline CD4⁺ lymphocyte count was 271 cells/µl (IQR = 231–328), and median

Table 2. Patients' characteristics at baseline before starting combination antiretroviral therapy.

Demographic data and biological parameters	
Women, <i>n</i> (%)	90 (62.1)
Age, years, mean ($\pm$ SD)	33.9 ($\pm$ 8.1)
CDC stage, <i>n</i> (%)	
A	97 (66.9)
B	44 (30.3)
HIV route of infection, <i>n</i> (%)	
Heterosexual	128 (88.3)
Men who have sex with men	13 (9.0)
Intravenous drug use	1 (0.7)
Weight, kg, mean ($\pm$ SD)	57.5 ($\pm$ 10.7)
Systolic blood pressure, mmHg, mean ($\pm$ SD), <i>n</i>	115 ($\pm$ 12.4), 131
Current smoking status, <i>n</i> (%), <i>N</i>	18 (12.4), 93
CD4 cell count, cells/ μ l, median (IQR; minimum–maximum)	271 (234–328; 167–515)
Log ₁₀ HIV-RNA, copies/ml, median (IQR; minimum–maximum)	4.66 (4.17–5.12; 2.23–5.88)
Glucose, mg/dl, median (IQR; minimum–maximum)	88 (82–94; 69–374)
Total cholesterol, mg/dl, median (IQR; minimum–maximum)	172 (144–198; 76–268)
LDL cholesterol, mg/dl, median (IQR; minimum–maximum)	99 (75–118; 23–194)
HDL cholesterol, mg/dl, median (IQR; minimum–maximum)	49 (41–58; 15–100)
Triglycerides, mg/dl, median (IQR; minimum–maximum)	106 (76–162; 38–426)
Predictive risk of coronary heart disease, <i>n</i> (%)	
High risk	0
Moderate risk	9 (6.2)
Low risk	71 (49.0)
Unknown risk	65 (44.8)

n = 145 unless specified otherwise. CDC, Centers for Disease Control and Prevention; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein.

log₁₀ HIV-RNA was 4.66 (IQR = 4.17–5.12). At randomization to cART continuation or interruption, patients had been treated for a median of 8.0 months (IQR = 6.4–12.4), and the median CD4⁺ lymphocyte count was 442 cells/ μ l (IQR = 392–531).

Changes in cardiovascular markers after combination antiretroviral therapy and their correlation with HIV-RNA

The values of each cardiovascular marker before cART initiation and on cART (i.e. at randomization) are presented in Table 3. We showed a significant decrease in the values of endothelial activation markers (s-VCAM-1 and P-selectin), inflammatory hormone (leptin) and fibrin degradation product (D-dimer) after a median 8 months of cART ($P \leq 0.001$, $P < 0.001$, $P = 0.03$ and $P = 0.005$, respectively). CCL2 decrease was of borderline statistical significance ($P = 0.06$).

Adiponectin, an adipose tissue-derived hormone with anti-inflammatory properties, showed a significant increase after cART initiation ($P = 0.05$). Additionally, the proportion of anti-inflammatory cytokine, IL-10, values above the limit of detection was significantly lower on cART than before treatment ($P = 0.001$).

Pearson's correlation coefficient between each cardiovascular marker before cART initiation and log₁₀ HIV-RNA was not significant, except for D-dimer ($P = 0.03$) and CRP ($P = 0.04$) for which there was a slightly significant positive correlation with log₁₀ HIV-RNA before treatment was initiated. Between randomization

and week 12, we observed a significant increase in s-VCAM-1, CCL-2, P-selectin and D-Dimer ($P < 0.001$, $P = 0.007$, $P = 0.01$, $P = 0.01$, respectively) and a significant increase in adiponectin ($P = 0.009$) (data not shown).

Association of HIV-RNA with cardiovascular markers 12 weeks after randomization

Of the 145 patients included, 48 (33.3%) were randomized to the continued cART arm and 97 (66.7%) to the STI arm (cART interruption). Table 4 presents the median values and IQR for each cardiovascular marker (s-VCAM-1, adiponectin, CCL2, CCL3, P-selectin, leptin, CRP and D-dimer) 12 weeks after randomization and the proportion of patients with GM-CSF, IL-6 and IL-10 above the limit of detection 12 weeks after randomization for the STI and continued cART groups.

s-VCAM-1 and CCL2 were positively associated with each 1 log increase in HIV-RNA ($r = 0.271$, $P = 0.001$ and $r = 0.238$, $P = 0.005$, respectively); this association remains after adjustment for age, platelet, LDL cholesterol and white blood count for s-VCAM-1 and after adjustment for HIV route of infection and TC for CCL2. Adiponectin was negatively associated with each 1 log increase in HIV-RNA ($r = -0.248$, $P = 0.002$) after adjustment for sex and triglycerides. Detectable IL-10 was significantly less likely with each 1 log increase in HIV-RNA after adjustment for sex and route of HIV infection. We assessed the colinearity between cART and HIV-RNA and verified that cART was not an effect modifier in the relation between each cardiovascular marker and HIV-RNA.

Table 3. Cardiovascular and metabolic markers before and after a median of 8 months of combination antiretroviral therapy and their correlation with HIV-RNA before combination antiretroviral therapy.

Biological markers	Before treatment				On treatment			<i>P</i>
	25th	50th	75th	<i>r</i> ^a (<i>P</i>)	25th	50th	75th	
s-VCAM-1 (ng/ml)	1818.7	2266.5	2672.9	0.078 (<i>P</i> = 0.36)	1069.5	1533.5	2183.3	<0.001
Adiponectin (mg/ml)	0.03	4.5	7.1	−0.059 (0.48)	2.9	4.6	6.5	0.05
CCL2 (pg/ml)	15.6	15.6	77.7	0.116 (0.18)	15.6	15.6	33.8	0.06
CCL3 (pg/ml)	8.0	13.3	26.1	0.093 (0.26)	8.0	9.8	27.6	0.49
P-selectin (ng/ml)	39.1	90.3	148.6	−0.004 (0.97)	22.8	54.9	106.4	<0.001
Leptin (ng/ml)	2.6	7.6	20.2	−0.109 (0.19)	2.7	6.5	12.4	0.03
CRP (mg/l)	0.3	0.6	1.6	0.173 (0.04)	0.3	0.6	1.7	0.56
D-dimer (ng/ml)	192.9	275.8	411.7	0.256 (0.03)	156.2	223.1	312.6	0.005
Under the limit of detection (%)					Under the limit of detection (%)			<i>P</i>
GM-CSF (≤31.25 pg/ml)			98.6			97.9		0.99
IL-6 (≤0.156 pg/ml)			95.2			93.8		0.77
IL-10 (≤0.78 pg/ml)			90.2			73.8		0.001

CCL, chemokine ligand; CRP, C-reactive protein; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; s-VCAM, soluble vascular cell adhesion molecule.

^a*r*, Pearson's correlation coefficient between HIV-RNA and each cardiovascular marker.

Figure 1 shows mean values and their 95% CI by HIV-RNA strata for s-VCAM-1, CCL2, adiponectin and IL-10 across three strata of HIV-RNA (≤50, 50–10 000 and >10 000 copies/ml); s-VCAM-1 and CCL2 increased as HIV-RNA increased, and adiponectin and IL-10 plasma concentrations decreased as HIV-RNA increased. All these changes are highly statistically significant (*P* trend = 0.001, 0.003, 0.004 and 0.001, respectively).

Markers plasma concentration at study termination (interrupted combination antiretroviral therapy arm)

Patients randomized to the STI arm had a median of two cycles of treatment interruption during STACCATO trial. We compared the values of each cardiovascular

biomarker between randomization (when patients had experienced a median 8 months of cART) and study termination, after a mean (SD) time of 20.4 (15.8) weeks of cART re-treatment. We found that s-VCAM-1, CCL2 and P-selectin were significantly higher at study termination (*P* < 0.001, *P* < 0.02, *P* < 0.04, respectively) than after randomization. Similarly, the protective factor, adiponectin, was significantly lower at study termination than after randomization (*P* < 0.001), and the proportion of IL-10 under the limit of detection was significantly higher at study termination than after randomization (*P* = 0.04).

Cardiovascular risk in the study population

Regarding paired values of lipids and glucose, TC (*P* < 0.001), triglycerides (*P* = 0.004), HDL cholesterol

Table 4. Univariate and multivariate linear regression and logistic regression models assessing the association between cardiovascular markers and log₁₀ HIV-RNA at week 12.

Cardiovascular markers ^a	Week 12			
	STI (off cART) (<i>n</i> = 97)	CT (on cART) (<i>n</i> = 48)	<i>r</i> ^b (<i>P</i>)	Adjusted <i>r</i> (<i>P</i>)
s-VCAM-1 (ng/ml)	2322.1	2054.9*	0.270 (0.001)	0.271 (0.001)
Adiponectin (mg/ml)	3.7	4.7*	−0.199 (0.02)	−0.248 (0.002)
CCL2 (pg/ml)	15.6	15.6*	0.239 (0.005)	0.238 (0.005)
CCL3 (pg/ml)	8.0	14.7	−0.144 (0.08)	−0.153 (0.07)
P-selectin (ng/ml)	87.1	65.1*	0.065 (0.44)	0.080 (0.36)
Leptin (ng/ml)	7.2	7.6	−0.007 (0.93)	−0.008 (0.90)
CRP (mg/l)	0.6	0.8	−0.128 (0.13)	−0.126 (0.14)
D-dimer (ng/ml)	270.9	247.9	−0.016 (0.87)	0.018 (0.85)
Below the limit of detection (%)			Unadjusted OR (95% CI)	Adjusted OR (95% CI, <i>P</i>)
GM-CSF (≤31.25 pg/ml)	99.0	95.7	0.79 (0.29–2.16)	0.97 (0.34–2.81, 0.96)
IL-6 (≤0.156 pg/ml)	92.7	100	1.08 (0.60–1.93)	1.27 (0.65–2.47, 0.49)
IL-10 (≤0.78 pg/ml)	89.4	63.0	0.67 (0.46–0.99)	0.64 (0.43–0.96, 0.03)

cART, combination antiretroviral therapy; CCL, chemokine ligand; CI, confidence interval; CRP, C-reactive protein; CT, continued cART; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; OR, odds ratio; STI, s-VCAM, soluble vascular cell adhesion molecule.

^aAll results are presented in median.

^bPearson correlation coefficient.

**P* < 0.05 using Mann–Whitney test.

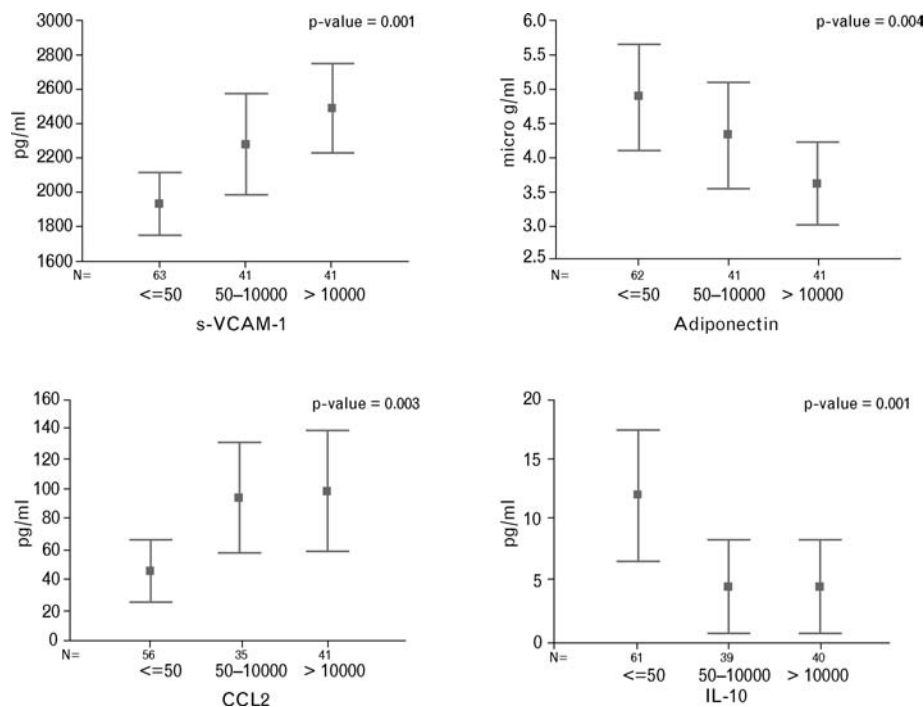


Fig. 1. Cardiovascular markers distribution across the three strata of HIV-RNA at week 12. CCL, chemokine ligand; IL, interleukin; s-VCAM, soluble vascular cell adhesion molecule.

($P < 0.001$) and glucose ($P = 0.02$) were significantly increased after cART initiation. TC, HDL cholesterol and LDL cholesterol had a significant negative correlation with HIV-RNA before treatment ($r = -0.19$, $P = 0.02$; $r = -0.29$, $P < 0.001$; $r = -0.17$, $P = 0.04$, respectively). None of these metabolic markers was significantly correlated with HIV-RNA at week 12 (data not shown).

The Framingham equation was calculated before cART was initiated. We showed that 49% of patients had a low risk, 6.2% had a moderate risk and no patient had a high predictive risk of coronary heart disease (Table 2).

Discussion

Long-term cART increases the risk of acute cardiovascular events in HIV-infected adults [29]. Continuous therapy (for years) with a protease inhibitor, but not with a nonnucleoside reverse transcriptase inhibitor, has been associated with an increased risk of MI. This is probably due in part to the dyslipidaemic effects of protease inhibitor therapy [4]. On the contrary, a similar increased incidence of acute cardiovascular events occurs during interruption of antiretroviral treatment [6]. This suggests that HIV itself increases the cardiovascular risk. The present study investigates the possible correlation between HIV replication and quantitative values of cardiovascular risk markers after interruption of cART.

In the present study, we show that HIV-RNA replication is associated with increased levels of s-VCAM-1 and CCL2 and decreased levels of adiponectin and IL-10. These strong associations persisted after adjustment for known cardiovascular risk factors. No significant correlation was observed between patients treated and those who stopped cART at week 12 of the trial for the other cardiovascular markers such as CRP, leptin, CCL3, IL-6, D-dimer and GM-CSF. All values reported in the present study were found within the normal ranges previously published among HIV-infected [30] and HIV-uninfected patients [31–37,23,38], except for s-VCAM-1, which was found to be as high among our HIV-infected study population as in patients with type II diabetes or end-stage renal failure [39,40]. Our work points to possible molecular mechanisms of the proatherosclerotic effect of HIV-RNA in the cascade leading to a cardiovascular event.

The biomarkers investigated in the present study are involved in endothelial activation (s-VCAM-1 and P-selectin), systemic inflammation (CCL2, CCL3, GM-CSF, CRP, IL-6 and IL-10), platelet (P-selectin), adipocyte (adiponectin and leptin) and the coagulation cascade (D-dimer).

Several studies have suggested a direct action of different HIV components in the modulation of inflammatory processes independent of CD4 cell counts. Virus proteins, such as Tat and Nef, influence monocyte functions (i.e.

cytokine and chemokine production) and survival [41,42]. On the contrary, HIV induces endothelial activation [43,44]. Furthermore, HIV also interferes with adipose tissue homeostasis independent of antiretroviral drug-induced lipodystrophy. Hypertriglyceridaemia, associated with the wasting syndrome, occurs in advanced HIV disease [45].

Less is known about a possible direct effect of HIV in the coagulation cascade. Recent investigations in the Strategies for Management of AntiRetroviral Therapy (SMART) Trial [46] suggest that the coagulation product, D-dimer, is strongly related to all-cause mortality in HIV patients. HIV-induced dysregulation of the complex cross-talk between leukocytes, endothelial cells and adipose tissue promotes atherosclerosis up to and including acute ischaemic complications. The modifications of the soluble markers under investigation in our study (s-VCAM-1, CCL2, adiponectin and IL-10) may be key active players in the HIV-induced increase in cardiovascular risk.

The significant correlation between HIV-RNA and s-VCAM-1, CCL2, adiponectin or IL-10 persisted after adjustment for known confounders (lipids, glucose, age, etc.). We did not adjust our multiple regression models on treatment use because the correlation between HIV-RNA and treatment use was above 0.70, indicating multicollinearity. We also verified that treatment use was not an effect modifier in the association between the cardiovascular biomarker studied and HIV-RNA.

Inflammatory cells and soluble mediators, which play a central role in all phases of atherosclerotic inflammation, have been investigated to assess their role as independent cardiovascular risk factors [11,12,14,16,18–21,23,25,26]. Although their clinical use is still controversial and not recommended because of the low specificity and high cost [8], previous studies clearly show a strong correlation between these markers and the incidence of acute cardiovascular events [32,35,38]. On the basis of these premises, together with the absence of acute cardiovascular events reported at the end of STACCATO trial, the present study only focused on the association between cardiovascular biomarkers and HIV-RNA replication but not on the risk of cardiovascular events in HIV patients.

Among the biomarkers, CRP is also implicated in several conditions not related to atherosclerosis [47]. We observed normal levels (about 1 mg/l) independent of cART use. The absence of CRP modification suggests that our STACCATO study population was not affected by clinical infections or other systemic inflammatory states, which might modify the other tested cardiovascular biomarkers [27].

CCL3 is a selective ligand for CCR5, the HIV coreceptor, and therefore, a potential HIV antagonist,

but we did not observe any correlation between CCL3 and HIV-RNA [36]. These data might be explained by a direct virus interference with CCL3 plasma levels, which could thus limit the use of CCL3 as a possible cardiovascular risk marker in HIV-positive patients.

D-dimers decreased after a median 8 months of cART and correlated with HIV-RNA, but we did not show any significant association with HIV-RNA replication in the adjusted analysis at week 12 for this marker. Our results can be compared to those of the SMART trial [46]. In the SMART analysis [46], six biomarkers were assessed at study entry and 1 month following randomization in the interruption and continuous treatment arm, respectively. After 1 month of cART interruption in SMART, both IL-6 and D-dimer levels rose significantly with a median D-dimer increase of 0.05 µg/ml after 1 month off ART in the interruption group. In STACCATO, we also showed a significant increase in D-dimer off cART of a level similar to what was observed in SMART (+0.07 µg/ml, $P=0.01$).

We did not report a similar finding for IL-6. However, we used a method with a detection limit of 0.0156 pg/ml, and we documented more than 75% of the patients with a value below our detection limit. With a more sensitive method, we might have found results similar to those reported in SMART.

After 1 month of cART interruption in SMART, both IL-6 and D-dimer levels rose significantly but were stable in those who continued cART. Moreover, higher levels of IL-6 and D-dimer were associated with a large increase in the likelihood of death. As noted, only two deaths and no MI occurred in STACCATO, precluding evaluation of cardiovascular events or deaths. We also had a majority of women, which may make comparison with the mostly male population in SMART difficult.

HIV could directly induce monocyte/macrophage, endothelial and adipocyte activation. These cells can increase atherosclerotic inflammation by upregulating the secretion of inflammatory molecules (CCL2) and down-regulating anti-inflammatory factors (adiponectin and IL-10). The altered profile of these soluble mediators further contributes to endothelial activation and atherosclerosis development. Injured endothelial cells release the adhesion molecule, s-VCAM-1, in the vessel lumen, which should be considered as a marker of endothelial activation. Therefore, the present study shows a possible direct effect of HIV replication on inflammatory processes underlying atherosclerosis.

Our relatively small and young population limited the power of our study to correlate the biomarker changes with clinical events. Therefore, our data should be considered as an assessment of cardiovascular risk without clinical endpoints but validated by previously published

studies [32,35,38]. These limitations, together with the selection of an exclusively Thai population, reduce the generalizability of our findings.

In conclusion, our study associates HIV-RNA replication and modifications of specific cardiovascular risk biomarkers (s-VCAM-1, CCL2, adiponectin and IL-10) independent of known confounders. In the general population, the use of rosuvastatin in apparently healthy individuals with elevated high-sensitivity CRP levels reduced the incidence of major cardiovascular events. Intervention strategies should be studied for HIV-infected patients with high plasma levels of inflammatory markers and with ongoing HIV-RNA replication [48].

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The STACCATO Study Group (Thailand): Protocol advisors: Praphan Phanuphak (The HIV Netherlands Australia Thailand Research Collaboration, Bangkok, Thailand), David A. Cooper (The National Centre in HIV Epidemiology and Clinical Research, Sydney, Australia).

Investigators: Kiat Ruxrungham, Jintanat Ananworanich and Reto Nuesch (HIV-NAT, Bangkok), Ploench Chetchotisakd (Khon Kaen University, Khon Kaen), Wisit Prasithsirikul (Bamrasnaradura Institute, Nonthaburi), Sasisopin Kiertiburanakul (Ramathibodi Hospital, Mahidol University, Bangkok), Warangkana Munsakul (Bangkok Metropolitan Administration Medical College and Vajira Hospital, Bangkok), Phitsanu Raksakulkarn and Wirat Klinbuayam (Sanpatong Hospital, Chiang Mai, Thailand) and Somboon Tansuphasawadikul (Buddhachinnaraj Hospital, Phitsanulok, Thailand).

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Low Bone Mineral Density, Renal Dysfunction, and Fracture Risk in HIV Infection: A Cross-Sectional Study

Alexandra Calmy,^{1,2,5} Christoph A. Fux,⁶ Richard Norris,^{1,2} Nathalie Vallier,⁵ Cécile Delhumeau,⁵ Katherine Samaras,³ Karl Hesse,^{1,2} Bernard Hirschel,⁵ David A. Cooper,^{1,2,4} and Andrew Carr^{1,2}

¹HIV, Immunology and Infectious Diseases Unit and ²Centre for Applied Medical Research, St Vincent's Hospital, ³Garvan Institute for Medical Research, and ⁴National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia; ⁵HIV Unit, Geneva University Hospital, Geneva, and ⁶University Clinic for Infectious Diseases and University of Berne, Switzerland

Background. Reduced bone mineral density (BMD) is common in adults infected with human immunodeficiency virus (HIV). The role of proximal renal tubular dysfunction (PRTD) and alterations in bone metabolism in HIV-related low BMD are incompletely understood.

Methods. We quantified BMD (dual-energy x-ray absorptiometry), blood and urinary markers of bone metabolism and renal function, and risk factors for low BMD (hip or spine T score, -1 or less) in an ambulatory care setting. We determined factors associated with low BMD and calculated 10-year fracture risks using the World Health Organization FRAX equation.

Results. We studied 153 adults (98% men; median age, 48 years; median body mass index, 24.5; 67 [44%] were receiving tenofovir, 81 [53%] were receiving a boosted protease inhibitor [PI]). Sixty-five participants (42%) had low BMD, and 11 (7%) had PRTD. PI therapy was associated with low BMD in multivariable analysis (odds ratio, 2.69; 95% confidence interval, 1.09–6.63). Tenofovir use was associated with increased osteoblast and osteoclast activity ($P \leq .002$). The mean estimated 10-year risks were 1.2% for hip fracture and 5.4% for any major osteoporotic fracture.

Conclusions. In this mostly male population, low BMD was significantly associated with PI therapy. Tenofovir recipients showed evidence of increased bone turnover. Measurement of BMD and estimation of fracture risk may be warranted in treated HIV-infected adults.

Low bone mineral density (BMD), including premature osteopenia and osteoporosis, is common in persons infected with human immunodeficiency virus (HIV) [1–5]. A review of cross-sectional studies found that HIV-infected adults had a 6.4-fold increased odds ratio (OR) of osteopenia and a 3.7-fold increased OR of osteoporosis compared with uninfected controls [1]. “Classic” risk factors identified were low body mass index, weight loss, corticosteroid use, and smoking, together with the duration of HIV infection [2, 6].

Dynamics in BMD depend on the balance between osteolytic activities of osteoclasts and regenerative activities of osteoblasts. Reductions in BMD directly correlate with the risk of bone fractures. Every reduction of 1 standard deviation (SD) in vertebral BMD, for example, resulted in a 2-fold increased risk

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Reprints or correspondence: Dr Calmy, HIV Unit, Geneva University Hospital, 4 rue Gabrielle Perret-Gentil, 1205 Geneva, Switzerland (acalmy@gmail.com).

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of vertebral fracture [7]. The mechanism of low BMD in HIV-infected adults is uncertain, as are the relative effects of classic risk factors, HIV itself, and specific antiretroviral therapies on BMD and fracture risk in treated HIV-infected patients.

Particular drugs and drug classes have been associated with low BMD. Tenofovir (TDF) is a nucleotide analogue reverse-transcriptase inhibitor shown to reduce BMD [8, 9]. Low BMD has been attributed to the use of HIV protease inhibitors (PIs), but other antiretrovirals have also been implicated [10, 11]. Studies published to date have not evaluated the roles of specific antiretrovirals in the context of a broader examination of classic risk factors.

Tenofovir can induce proximal renal tubular dysfunction (PRTD). This specific reabsorption defect of glomerular filtration products can result in excessive renal phosphate, uric acid, and bicarbonate losses, as well as proteinuria and glucosuria, particularly in patients with preexisting nephropathy [12]. PRTD might promote loss of BMD through renal phosphate wasting [13].

The risk of a fracture in an individual patient not only depends on BMD but is also associated with numerous other factors, including age, sex, alcohol use, and smoking. The World Health Organization (WHO) recently issued the FRAX equation to calculate the 10-year risk of fracture based on key risk factors [14]. We hypothesized that the comparison between FRAX- and BMD-derived fracture risks may provide better insight into the significance of low BMD seen in the context of long-term antiretroviral treatment (ART) and may help identify HIV-infected patients at greater risk of fracture at any given BMD value.

In the current study, we determined the prevalence of low BMD and its relationship with numerous potential risk factors, including PRTD, tenofovir, and PI therapy, in a cohort of HIV-infected adults receiving combined antiretroviral treatment. We also estimated the 10-year fracture risk.

MATERIALS AND METHODS

Study design and participants. We performed a cross-sectional analysis in a hospital outpatient-based cohort. All patients who were receiving antiretroviral treatment and attending the HIV outpatient clinic at St Vincent's Hospital (Sydney, Australia) for routine appointments between January and April 2007 were invited to participate, except for those with an active opportunistic condition. The protocol was approved by the St Vincent's Hospital Human Research Ethics Committee. All patients provided written informed consent.

Assessments. The following were evaluated by means of a questionnaire administered by a study nurse or physician: patient characteristics (age, sex, and duration of HIV infection), body composition (height, weight, body mass index, fat mass percentage, and lipodystrophy), risk factors for low BMD (pre-

vious fracture, prior fracture in a first-degree relative, smoking status, corticosteroid use, alcohol consumption, and concomitant medications), and type and duration of antiretroviral treatment.

Blood samples were collected after a minimum 10-h overnight fast for determination of serum creatinine levels, liver transaminase levels, metabolic parameters (total alkaline phosphatase [ALP], lactate, glucose, lipids [total, high-density lipoprotein and low-density lipoprotein cholesterol, and triglycerides]), HIV-related parameters (CD4⁺ lymphocyte count and HIV load), and risk factors for bone disease (calcium, phosphate, bone-specific ALP [bALP], 25-hydroxyvitamin D, total testosterone, parathyroid hormone [PTH], and osteocalcin). Creatinine clearance was calculated using the Cockcroft Gault formula (calculated glomerular filtration rate [GFR]) and analyzed as a continuous variable. From a spot urine sample we measured albumin, creatinine, glucose, phosphate, and hydroxyproline.

25-Hydroxyvitamin D was quantified using a competitive protein-binding assay (DiaSorin), total testosterone by radioimmunoassay (RIA) (ImmunoChem double-antibody testosterone iodine 125 RIA kit), PTH by RIA (Siemens Medical Solutions Diagnostics), osteocalcin by an in-house RIA, and bALP by Tandem Ostase immunoassay (Beckman Coulter). The respective lower limits of detection for these 5 assays were 15 nmol/L, 0.1 nmol/L, 1 pmol/L, 3 µg/L, and 0.1 µg/L.

WHO criteria were used to classify patients as having osteoporosis (hip or spine T score, -2.5 or less; ie, 2.5 SDs below the mean BMD value for young adults of the same sex and race) or osteopenia (hip or spine T score, -1 or less). Patients classified as osteopenic or osteoporotic were compared with the "normal BMD group" (hip and spine T score above -1 SD). The Z score compares the BMD with the mean BMD for individuals of the same age and sex; any Z score greater than -2 was considered to be within the normal range.

PRTD was defined by the presence of ≥ 2 of the following 4 pathologies [13]: (1) renal tubular phosphate loss, defined as a ratio of maximal reabsorption capacity (tubular phosphate) to GFR of <0.8 , as determined with the normogram of Walton and Bijvoet [15], which corrects the fractional excretion of phosphate ($[\text{phosphate}_{\text{urine}}/\text{phosphate}_{\text{serum}}]/[\text{creatinine}_{\text{urine}}/\text{creatinine}_{\text{serum}}]$) for the respective serum phosphate level; (2) a ratio of urine albumin to urine creatinine of >2.5 mg/mmol; (3) a urine glucose level of >1 mmol/L with a fasting plasma glucose level of ≤ 7.1 mmol/L; and (4) a plasma bicarbonate level of <20 mmol/L.

When bALP, the hydroxyproline-creatinine ratio, PTH, osteocalcin, and 25-hydroxyvitamin D were used as categorical variables, the upper limits of normal were set at 20.9 µg/L, 15 µmol/mmol, 7 pmol/L, 18.0 µg/L, and 35 nmol/L, respectively.

We defined a boosted PI (bPI) as any HIV PI given with a ritonavir dose of 100 or 200 mg daily.

Dual-energy x-ray absorptiometry (DXA) was performed on a GE Lunar Prodigy DXA machine (GE Healthcare; software version 7.51). The in vivo precision for the bone measurement using the DXA technique is 0.5%–1.5% at the lumbar spine.

The FRAX tool integrates clinical risk factors (age, sex, weight, height, previous fracture, parent hip fracture, current smoking, current glucocorticoid use, rheumatoid arthritis, secondary osteoporosis, and alcohol use (≥ 3 units/day) to produce a score computed with or without BMD (T score) at the femoral neck. The FRAX algorithm outputs are the 10-year probabilities of hip fracture and of a major osteoporotic fracture.

Statistical analysis. All statistical tests were 2-sided, with a threshold of 5%. Continuous variables are reported using medians and interquartile ranges, except when stated otherwise. Logistic regression was used to determine factors associated with low BMD. Demographic (age, duration of HIV infection in years, prior fractures, family history of fracture, smoking status, and alcohol consumption) anthropometric (weight, height, and body composition parameters), treatment-related (current use of lipid-lowering drugs, steroids, antihypertensive therapy, proton pump inhibitors, hormonal substitution or nonsteroidal anti-inflammatory drugs; current or past use of tenofovir, zidovudine, abacavir, bPI, or nonnucleoside reverse-transcriptase inhibitors), HIV-related (CD4⁺ lymphocyte count and HIV load) and pathophysiologically plausible biologic variables (cGFR, testosterone, PTH, and 25-hydroxyvitamin D levels) were included in a univariable analysis. All variables with $P < .2$ in the univariable analysis were entered in a multivariable logistic model. The model was adjusted for patient age. FRAX scores were compared between groups using a 2-sided, non-parametric Mann-Whitney *U* test. Statistical analysis was performed using SPSS software, version 15 (SPSS).

RESULTS

Patients. The 153 participants were mostly men with long-standing HIV infection and a high rate of lipodystrophy (Table 1). Viral replication was undetectable in 127 patients (83%). Sixty-seven participants (44%) were currently receiving tenofovir, 81 (53%) were receiving a bPI, and 40 (26%) were receiving both tenofovir and a bPI. Eighteen patients (12%) had low plasma 25-hydroxyvitamin D levels (<35 nmol/L).

Prevalence of low BMD. Sixty-five (42%) patients had low BMD (osteopenia or osteoporosis), 6 (4%) had osteoporosis, and 9 (6%) had a Z score less than -2 .

Prevalence of PRTD. Eleven patients (7.2%) had PRTD, all with impaired fractional tubular resorption of phosphorus and albuminuria; 1 patient also had glucosuria. No patient with PRTD had a low plasma bicarbonate level. When we alternatively defined PRTD solely on the basis of impaired fractional

Table 1. Baseline Characteristics of Study Patients

Patient characteristics	All patients (n = 153)
Demographic data	
Male sex	150 (98.0)
Age, years	48 (42.5–55.0)
Duration of HIV infection, years	13 (7–19)
Body mass index ^a	24.5 (22.5–27.0)
Undetectable HIV RNA level	127 (83.0)
CD4 ⁺ cell count, cells/mm ³	513 (360–735)
Lipodystrophy at ≥ 1 site	92 (60.1)
Creatinine level, μ mol/L	83.0 (74.5–93.0)
cGFR, mL/min	103.7 (83.9–122.9)
Risk factors	
Previous fracture	52 (34)
Lipid-lowering treatment	40 (26.1)
Antihypertensive treatment	33 (21.6)
Alcohol consumption >3 units/week	74 (48.4)
Smoking, cigarettes per day	15 (5–25)
Coffee consumption, drinks per day	1 (0–2)
Systolic blood pressure, mm Hg	126 (115.5–126.0)
Biological data	
Bone ALP level, μ g/L	15.2 (11.9–20.3)
Osteocalcin level, μ g/L	14.0 (10.0–17.5)
25-hydroxyvitamin D level, nmol/L	66.0 (46.0–87.5)
Testosterone level, nmol/L	16.4 (13.1–21.0)
PTH level, pmol/L	4.4 (3.0–6.7)
Hydroxyproline-creatinine ratio, μ mol/mmol	13.2 (10.4–16.1)
Treatment	
Tenofovir	
History of any use	87 (56.9)
Duration of use, months	28 (16–51)
Currently receiving	67 (43.8)
Duration for current recipients, months	33 (16–53)
Boosted PI	
History of any use	103 (67.3)
Duration of use, months	51 (27–77)
Currently receiving	81 (52.9)
Duration for current recipients, months	56 (36.5–80.5)
Zidovudine	
History of any use	85 (55.6)
Duration of use, months	35 (10.5–71.0)
Currently receiving	4 (2.6)
Duration for current recipients, months	113.5 (62.3–132.5)
Abacavir	
History of any use	88 (57.5)
Duration of use, months	51 (18.0–82.3)
Currently receiving	64 (41.8)
Duration for current recipients, months	54 (18.0–84.8)
NNRTI	
History of any use	111 (72.5)
Duration of use, months	58 (28–85)
Currently receiving	74 (48.4)
Duration for current recipients, months	77 (47.8–92.0)
History of any PI use	
Lopinavir-ritonavir	58 (37.9)
Indinavir	58 (37.9)
Saquinavir	53 (34.6)
Atazanavir	41 (26.8)

NOTE. Data are no. (%) of patients or median (interquartile range). ALP, alkaline phosphatase; cGFR, calculated glomerular filtration rate; HIV, human immunodeficiency virus; IQR, interquartile range; NNRTI, nonnucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; PTH, parathyroid hormone.

^a Calculated as weight in kilograms divided by the square of height in meters

tubular resorption of phosphorus in a post hoc analysis, we identified 27 patients (17.6%).

PRTD was associated with a longer duration of HIV infection and longer exposure to tenofovir (with 8 of the 11 patients with PRTD exposed to tenofovir for ≥ 2 years) or bPIs (Table 2). BMD was slightly lower and cGFR significantly decreased in patients with PRTD ($P = .002$). Seven of the 11 patients (64%) had evidence of altered bone metabolism with either increased osteoclast activity (hydroxyproline-creatinine ratio) (6 patients [55%]), increased osteoblast activity (osteocalcin and/or bALP) (5 patients [46%]), or both (4 patients [37%]). Of the 27 patients with increased fractional excretion of phosphorus, 5 (19%) had elevated plasma PTH, 16 (59%) had evidence of altered bone metabolism, and 14 (52%) had low BMD; 18 (67%) had been exposed to tenofovir for a median of 33.5 months.

Risk factors for low BMD. Univariable analysis revealed that patients with higher body mass index (OR, 0.87; 95%

confidence interval [CI], 0.77–0.98), higher testosterone levels (OR, 0.94; 95% CI, 0.89–0.99), or higher creatinine clearance (OR, 0.99; 95% CI, 0.97–1.00) were less likely to have low BMD; any use of bPI, however, was significantly associated with low BMD (OR, 2.83; 95% CI, 1.36–5.92) (Table 3). Current tenofovir, bPI, thymidine analogue, abacavir, or nonnucleoside reverse-transcriptase inhibitor therapy; current lipodystrophy; and use of concomitant medications were not significant. In multivariable analysis, the history of any use of bPI remained significant (OR, 3.10; 95% CI, 1.30–7.21). Higher testosterone levels were also a significant protective factor (OR, 0.93; 95% CI, 0.88–0.99). Of note, “classic” risk factors, such as prior fracture, use of steroids, and alcohol consumption, were not risk factors for low BMD after adjustment for combination antiretroviral therapy exposure.

BMD and bone metabolism by antiretroviral treatment exposure. Low BMD was consistently more frequent in patients treated with tenofovir or bPI, but differences only reached

Table 2. Patient Characteristics According to the Presence or Absence of Proximal Tubular Renal Dysfunction (PRTD)

Patient characteristics ^a	PRTD present (n = 11)	PRTD absent (n = 142)	P
Demographic and HIV disease characteristics			
Male sex	10 (90.9)	117 (82.4)	.695
Age, years	55 (45–58)	48 (42–54)	.169
HIV duration, years	21.0 (15.0–23.0)	12.0 (7.0–18.0)	.006
Undetectable plasma HIV RNA level	10 (90.9)	117 (82.4)	>.99
CD4 ⁺ cell count, cells/mm ³	464 (420–620)	517 (360–756)	.601
Tenofovir exposure, months	35 (0–48)	0 (0–23)	.019
bPI exposure, months	56 (42–92)	23 (0–57)	.009
Body composition and bone mineral density			
Body mass index ^a	24.1 (21.6–24.9)	24.6 (22.9–27.2)	.046
Total fat, %	19.0 (17.4–23.6)	21.7 (16.6–26.9)	.557
Spine T score	−0.80 (−1.50 to 0.40)	−0.30 (−1.30 to 0.70)	.454
Hip T score	−0.60 (−1.30 to −0.20)	−0.55 (−1.10 to 0.20)	.406
Osteopenia (T score less than −1)	6 (66.7) ^b	59 (45.4) ^b	.304
Bone metabolism, vitamin D, and bone-related hormones			
Parathyroid hormone level, pmol/L	3.1 (2.6–6.8)	4.4 (3.1–6.5)	.402
Elevated PTH level	2 (18.2)	30 (22.6) ^b	>.99
bALP level, μ g/L	18 (14–23)	15 (12–20)	.328
Elevated bALP level	4 (40.0) ^b	26 (20.3) ^b	.224
Osteocalcin, μ g/L	12.3 (10.5–23.3)	14.0 (10.0–17.4)	.811
Elevated osteocalcin level	3 (27.3)	28 (21.2) ^b	.704
Hydroxyproline-creatinine ratio	15.2 (11.4–19.3)	13.01 (10.41–16.0)	.154
Elevated hydroxyproline-creatinine ratio	6 (54.5)	46 (34.6) ^b	.205
Plasma 25-hydroxyvitamin D level, nmol/L	88 (48–101)	66 (45–81)	.388
Low plasma 25-hydroxyvitamin D level	1 (9.1)	17 (12.7) ^b	>.99
cGFR	68.1 (54.4–82.7)	104.9 (87.6–123.7)	.002

NOTE. Data are no. (%) of patients or median (interquartile ranges). Statistically significant *P* values are shown in boldface font. bALP, bone alkaline phosphatase; bPI, boosted protease inhibitor; cGFR, calculated glomerular filtration rate; HIV, human immunodeficiency virus; NNRTI, non-nucleoside reverse-transcriptase inhibitor; PTH, PI, protease inhibitor; PTH, parathyroid hormone.

^a Calculated as weight in kilograms divided by the square of height in meters

^b Percentages were calculated using the number of patients with available data as the denominator.

Table 3. Parameters Associated with Low Bone Mineral Density (T score less than –1)

Characteristic	Univariable analysis		Multivariable analysis ^a	
	OR (95% CI)	P	OR (95% CI)	P
Age ^a	1.01 (0.98–1.05)	.415	1.04 (0.99–1.10)	.113
Body mass index	0.87 (0.77–0.98)	.018	0.87 (0.74–1.02)	.094
Current antihypertensive therapy	0.55 (0.24–1.26)	.156	0.46 (0.17–1.27)	.133
cGFR	0.99 (0.97–1.00)	.043	0.99 (0.97–1.01)	.565
Testosterone level	0.94 (0.89–0.99)	.022	0.93 (0.88–0.99)	.027
PRTD ^a	2.40 (0.57–10.04)	.331	1.54 (0.29–8.29)	.613
Tenofovir (history of any use)	1.58 (0.81–3.11)	.181	1.32 (0.60–2.92)	.488
bPI (history of any use)	2.83 (1.36–5.92)	.006	3.10 (1.30–7.21)	.011
NNRTI (history of any use)	0.54 (0.25–1.15)	.109	0.49 (0.20–1.16)	.106

NOTE. Statistically significant *P* values are shown in boldface font. bPI, boosted PI; cGFR, calculated glomerular filtration rate; CI, confidence interval; NNRTI, nonnucleoside reverse-transcriptase inhibitor; OR, odds ratio; PRTD, proximal renal tubular dysfunction.

^a Analyses have been adjusted for age and PRTD.

statistical significance for the Z score in bPI recipients, the spine T and Z scores in bPI recipients, and the hip Z and T scores in tenofovir recipients (Table 4). Levels of ALP (data not shown), its bone isoenzyme (bALP), osteocalcin, and urinary hydroxyproline excretion were significantly higher in individuals receiving tenofovir ($P \leq .002$), suggesting increases in both osteoblast and osteoclast activity. Interestingly, among 48 (35%) of 138 patients with elevated bALP or osteocalcin levels, 32 (68%) of 47 also had a high urinary hydroxyproline levels. Among the 52 (36%) of 144 patients with elevated urine hydroxyproline levels, 32 (63%) of 51 had elevated levels of bALP or osteocalcin. There was also a trend toward higher PTH levels in tenofovir-treated patients ($P = .07$). Patients currently receiving bPI were more likely to have elevated plasma osteocalcin levels ($P = .004$), but ALP and bALP and the hydroxyproline-creatinine ratio did not change significantly. There was no significant relationship between bPI duration ($P = .194$, by test for trend) or tenofovir duration ($P = .731$, by test for trend) and the prevalence of low BMD. Remarkably, significantly fewer patients treated with TDF showed a pathological fractional excretion of phosphate.

Ten-year estimation of fracture risk. The FRAX score computed without BMD provided similar fracture risks for patients with normal BMD and those with low BMD (Table 5). The inclusion of BMD data in the equation significantly increased the calculated risk of fractures in patients with osteopenia, whereas it significantly reduced fracture risks of patients with normal BMD. The mean 10-year risk of fracture of the whole study population estimated by the FRAX equation (computed with the BMD) was 1.2% for hip fracture and 5.4% for major osteoporotic fracture.

Twenty-two (15.8%) of 139 patients had a 10-year probability of a major osteoporotic fracture of $>7.5\%$ (the threshold at which bisphosphonate therapy is considered to be cost-effective

[16]), and only 3 (2.2%) had a 10-year probability of major osteoporotic fracture of $>20\%$. We compared the characteristics of patients with a FRAX score above the 7.5% threshold with those of the rest of the study population. We did not find any significant difference between these 2 groups with regard to risk factors, demographic characteristics, renal function, or duration of antiretroviral treatment by class (data not shown).

DISCUSSION

In agreement with other studies, we found low BMD to be common in HIV-infected men, with 47% of patients having WHO-defined osteopenia or osteoporosis [1]. Use of a bPI was independently associated with low BMD. Few studies have looked at BMD after adjustment for HIV-independent risk factors, HIV-related parameters, and antiretroviral treatment characteristics. A meta-analysis of 12 cross-sectional studies calculated a pooled OR for low BMD of 1.57 for PI-treated versus PI-untreated patients [17]. However, concomitant disease and treatment variables were not evaluated. Recently, osteopenia was found to be more common in premenopausal HIV-infected women receiving PI-based therapy (17%) than in premenopausal, uninfected women (7%) [18]. In the Aquitaine cohort an association was reported between BMD and nadir CD4⁺ cell count in women, but, again, no adjustment for known risk factors was performed [19].

In contrast, we assessed the risk for low BMD adjusted for a large range of classic, HIV-related, and antiretroviral treatment variables, including PRTD. Only bPIs and low testosterone remained significantly associated with low BMD in multivariable analysis, suggesting a causative role for PIs in the pathogenesis of osteopenia in patients with stable, mostly virologically suppressed HIV disease. With $<10\%$ of our study population having severe immunosuppression (CD4⁺ cell

Table 4. Patient Characteristics, According to Current Tenofovir (TDF) or Boosted Protease Inhibitor (bPI) Exposure

Patient characteristic ^a	Current TDF exposure			Current bPI exposure		
	Yes (<i>n</i> = 67)	No (<i>n</i> = 86)	<i>P</i>	Yes (<i>n</i> = 81)	No (<i>n</i> = 72)	<i>P</i>
Demographic and HIV disease						
Male sex	66 (98.5)	84 (97.7)	>.99	78 (96.3)	72 (100)	.248
Age, years	47 (42–55)	49 (43–55)	.354	47 (43–54)	49.5 (42–57)	.538
HIV infection duration, years	14 (7–20)	12 (6.8–17.3)	.433	15 (9–20)	10 (5.3–16.0)	.003
Undetectable HIV RNA level	56 (83.6)	71 (82.6)	.867	68 (84)	59 (81.9)	.742
CD4 ⁺ cell count, cells/mm ³	480 (330–696)	537 (430–780)	.044	504 (350–708)	519 (410–766)	.465
Body composition and BMD						
Body mass index ^a	24.8 (22.9–27.2)	24.3 (22.5–26.6)	.325	24.8 (22.7–27.4)	24.3 (22.5–26.5)	.445
Total fat, %	21.4 (18.0–27.4)	21.3 (16.2–26.8)	.315	21.0 (17.1–28.5)	21.8 (16.4–25.3)	.561
Leg fat, %	15.8 (11.0–21.3)	15.5 (9.1–21.0)	.694	15.6 (8.4–23.6)	15.6 (12.2–19.5)	.504
Trunk fat, %	26.5 (22.1–33.1)	26.4 (20.4–33.0)	.459	25.8 (22.0–34.5)	27.1 (19.5–31.5)	.633
Spine T score	−0.6 (−1.6 to 0.5)	−0.1 (−1.1 to 0.8)	.103	−0.6 (−1.6 to 0.4)	−0.1 (−0.9 to 0.9)	.032
Spine Z score	−0.2 (−1.3 to 0.6)	0.2 (−0.6 to 1.1)	.050	−0.1 (−1.4 to 0.6)	0.2 (−0.6 to 1.1)	.021
Hip T score	−0.8 (−1.4 to 0.2)	−0.4 (−1.0 to 0.4)	.010	−0.7 (−1.4 to 0.0)	−0.5 (−1.0 to 0.3)	.208
Hip Z score	−0.3 (−1.0 to 0.3)	0.3 (−0.6 to 0.7)	.003	−0.2 (−0.9 to 0.5)	0.0 (−0.6 to 0.7)	.137
Osteopenia according to T score, no. (%)	30 (52.6) ^b	35 (42.7) ^b	.248	39 (53.4) ^b	26 (39.4) ^b	.098
Z score less than −2 SDs	6 (10.5) ^b	3 (3.7) ^b	.160	8 (11.0) ^b	1 (1.5) ^b	.035
BMD	1.18 (1.11–1.23)	1.19 (1.12–1.23)	.532	1.18 (1.11–1.22)	1.20 (1.12–1.26)	.041
Bone metabolism, vitamin D, and bone-related hormones						
Bone ALP level, μg/L	17.1 (13.6–22.8)	13.8 (11.1–17.6)	.002	15.6 (12.0–21.1)	15.0 (11.7–17.4)	.218
Osteocalcin level, μg/L	14.6 (11.3–20.2)	12.8 (9.0–15.8)	.004	14.2 (11.0–20.4)	12.5 (12.5–18.3)	.004
Hydroxyproline-creatinine ratio	15.4 (12.9–18.6)	11.3 (9.5–14.4)	<.001	13.7 (11.0–17.5)	11.8 (9.6–15.7)	.057
Plasma 25-hydroxyvitamin D level, nmol/L	68.0 (49–95)	62.5 (42.8–79.0)	.183	69 (51.5–89.0)	60 (41–81)	.102
Testosterone level, nmol/L	17.1 (13.4–21.7)	16.0 (12.9–19.0)	.202	16.4 (12.5–21.7)	16.5 (14.1–20.8)	.647
PTH level, pmol/L	4.9 (3.3–7.3)	4.1 (2.8–6.1)	.065	4.7 (3.1–7.0)	4.2 (2.8–6.3)	.336
Renal function						
cGFR	101.3 (82.6–130.2)	105.3 (84.5–119.7)	.966	105.4 (82.8–121.5)	101.1 (83.0–127.5)	.766
PRTD	7 (10.5) ^b	4 (4.7)	.213	8 (9.9)	3 (4.2)	.219
Pathologic fractional excretion of phosphate	4 (6.8)	16 (19.8)	.030	11 (14.9)	9 (13.6)	.836

NOTE. Data are no. (%) of patients or median (interquartile ranges). Statistically significant *P* values are shown in boldface font. ALP, alkaline phosphatase; BMD, bone mineral density; cGFR, calculated glomerular filtration rate; HIV, human immunodeficiency virus; PRTD, proximal renal tubular disease; PTH, parathyroid hormone; SD, standard deviation.

^a Calculated as weight in kilograms divided by the square of height in meters

^b Percentages were calculated using the number of patients with available data as the denominator.

count, <200 cells/μL), and 83% having suppressed viremia, it is difficult to conclude that HIV replication and disease severity are important contributors to the observed low BMD in our population. PI-associated loss of BMD may be due to altered 1,25-dihydroxyvitamin D₃ production [20] or stimulated osteoclast or inhibited osteoblast activity [21, 22]. Although low BMD is also found in untreated HIV-infected individuals, a randomized prospective study demonstrated greater BMD loss with continuous antiretroviral treatment than with intermittent antiretroviral treatment [23]. A recent study showed a median decrease of 4.1% in lumbar spine BMD and 2.7% in hip BMD

48 weeks after antiretroviral treatment initiation, with greater decrements in the PI-treated patients [24]. Another study examined the change in total BMD in HIV-infected persons randomized to efavirenz or lopinavir-ritonavir. There was an average 2.4% loss in BMD during a period of 96 weeks, regardless of treatment. Interestingly, the switch to bPI monotherapy after 24 weeks was not associated with improvement in BMD [25].

The effect of PIs on BMD has been controversial. Earlier cross-sectional studies suggesting a negative PI effect were not adjusted for HIV-independent and HIV-related confounding factors. Moreover, individual PIs may have differential effects

Table 5. Ten-Year Fracture Risks for Patients with or without Low Bone Mineral Density (BMD), According to FRAX Scores Computed with or without BMD

	Patients with normal BMD (n = 74)			Patients with low BMD (n = 65)			Overall population (n = 139)		
	FRAX score computed with- out BMD	FRAX score computed with BMD	P	FRAX score computed without BMD	FRAX score computed with BMD	P	FRAX score computed with- out BMD	FRAX score computed with BMD	P
Fracture risk									
Hip fracture risk	0.40 (0.20–1.00)	0.20 (0.10–0.50)	<.001	0.40 (0.20–1.20)	0.90 (0.50–2.40)	<.001	0.40 (0.20–1.00)	0.50 (0.10–1.00)	.575
Major osteoporotic fracture risk	4.10 (2.90–6.00)	3.55 (2.70–5.20)	<.001	3.80 (2.60–6.90)	4.80 (3.80–7.90)	<.001	4.40 (2.90–6.40)	4.10 (2.90–6.20)	.467

NOTE. Data are median (interquartile range), unless otherwise indicated. Low BMD was defined as a T score lower than 1 SD below the mean. P values refer to the difference between the fracture risks computed with and without BMD.

on BMD. Our results reinforce the possible role of bPI in reductions in BMD [26].

Although T and Z scores for both hip and spine were consistently lower and osteopenia more prevalent in patients receiving tenofovir-based regimens, we could not demonstrate a statistically significant association between current or cumulative tenofovir use and osteopenia or osteoporosis. The lack of significance despite this consistent pattern may be due to the relative short median tenofovir exposure time (28 months), which is only approximately one-half the median PI exposure time. Thus, exposure time may have been too short to result in significant quantitative differences in BMD. This concern is nourished by the significant higher osteoblast and osteoclast activity in tenofovir recipients, which, together with a trend for increased PTH levels, might indicate developing osteomalacia. To clarify these concerns, long-term follow-up data are needed.

Tenofovir has been associated with renal tubular toxicity and subsequent renal phosphate wasting [27]. Renal phosphate wasting may lead to increased bone turnover and hence elevated serum ALP. Significant tenofovir-related increases in ALP were identified after the initiation of tenofovir-based antiretroviral treatment but not tenofovir-sparing regimens in both treatment-naïve and treatment-experienced patients [28].

Eleven patients (7.2%) had PRTD, which did not correlate with low BMD in multivariable analysis. The discriminatory power of the study for PRTD was limited by the lack of information on proteinuria (rather than albuminuria) or specific markers for tubular proteinuria. However, excessive phosphaturia in the fasting state and in the absence of vitamin D or PTH disturbances—as documented in our patients—is considered highly specific for proximal renal tubulopathy. Furthermore, HIV-associated nephropathy and diabetes mellitus, 2 main causes of nontubular proteinuria, were not evident in our patients. We therefore believe that PRTD truly represents tubulopathy. When we alternatively defined PRTD solely on the basis of impaired fractional tubular resorption of phosphorus in a post hoc analysis, we identified 27 patients (17.6%); the fact that most of them had no hyperparathyroidism suggests

that a mild form of tubulopathy was present in these patients. Again, there was no association between osteopenia and alterations in bone metabolism (data not shown).

In the current era, with a growing proportion of HIV-infected persons aged >50 years, bone health is becoming a more important comorbid factor. It is unclear whether the high rates of osteopenia in men <50 years old will translate into increased fracture rates after an additional 10–20 years of antiretroviral treatment. A very large cohort study recently reported fracture prevalence to be >60% greater in HIV-infected adults than in HIV-uninfected adults [29]. BMD screening may be even more relevant as effective therapies become available. For example, the use of intravenous zoledronate appears to be a well tolerated and effective therapy for HIV-associated bone loss [30, 31]. Although the National US Osteoporosis Foundation does not recommend BMD screening for all patients with HIV, it explicitly states that postmenopausal women and men >50 years of age should be considered for BMD testing if the risk factor profile suggests cause for concern [32].

To improve the ability to predict subsequent fragility fracture in our patients, we used the WHO FRAX equation [14]. There are different recommendations for defining the threshold at which antiresorptive treatment is recommended. British guidelines recommend using a threshold based on age—that is, 7.5% for a man 45 years of age; this recommendation could translate into treatment for up to 16% of our HIV-infected population, significantly more than the 4.3% identified by documented osteoporosis only [16]. The National Osteoporosis Foundation recommendations suggest treating only patients with a risk for major osteoporotic fracture above a threshold of 20% at 10 years, meaning that only one-half of the patients (2.2% vs 4.3%) with osteoporosis would be identified [32]. In a relatively young population such as ours, with well-identified nonclassic risk factors (chronic disease, antiretroviral therapy), use of an age-dependent threshold as in the British recommendations may be more appropriate.

The FRAX score computed without BMD seems unable to discriminate adequately between patients with and those with-

out osteopenia, and its guidance on when to initiate antiretroviral therapy is highly dependent on the chosen threshold, with such therapy recommended for 2.2% of our population at a 20% ten-year risk of major osteoporotic fracture, and 16% at a 7.5% ten-year risk.

Considering that the FRAX score includes only classic, HIV-independent risk factors and that HIV positivity and treatment have been associated with lower BMD, the score provides a very conservative fracture risk estimate for HIV-positive populations. Similar to rheumatoid arthritis, HIV infection promotes a chronic inflammatory state that may turn out to be an independent risk factor for bone fracture to be included in a FRAX-like score.

Taken together, these observations argue for using a FRAX score computed without BMD only as a screening tool in all HIV-positive patients with no indications for DXA scanning. Given our findings and published data, BMD measurement may be appropriate for HIV-positive postmenopausal women and men >50 years of age, all HIV-positive patients with documented hypogonadism, and bPI and/or tenofovir recipients.

This study has several limitations resulting from its cross-sectional nature. In particular, antiretroviral treatment regimens had not been chosen randomly, and drug-independent effects on BMD or bone metabolism may therefore have been falsely attributed to bPI or tenofovir treatment. The effect of insufficient vitamin D or testosterone levels as well as low body mass index may be obscured by the low rate of pathologic values found in the study population. Moreover, the use of the FRAX tool has not been validated for young HIV-positive individuals or for Australians. Given the epidemiology in Australia, our study results apply only to HIV-positive male patients and therefore cannot be generalized to women.

In conclusion, we found a high prevalence of low BMD in HIV-infected adults receiving combination antiretroviral therapy, particularly in those receiving a bPI. The use of a tool such as the WHO FRAX tool warrants further validation studies in HIV-infected patients.

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Paper # 8

No significant effect of uridine or pravastatin for HIV lipotrophy in men who have ceased thymidine nucleoside analogue therapy: a randomized trial

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Alexandra Calmy, MD^{1,2}, Mark Bloch, MD³, Handan Wand, PhD⁴, Cécile Delhumeau, PhD², Robert Finlayson, MD⁵, Martina Rafferty¹, Richard Norris¹, Bernard Hirschel, MD², David A Cooper, MD^{1,4}, Andrew Carr, MD for the URISTAT study group*

¹HIV, Immunology and Infectious Diseases Unit, and Clinical Research Program, Centre for Applied Medical Research, St Vincent's Hospital, Sydney, Australia, ²Geneva University Hospital, Geneva, Switzerland, ³Holdsworth House Medical Practice, Sydney, Australia, ⁴National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia, ⁵Taylor Square Private Clinic, Sydney, Australia.

**Composition of the URISTAT study group in the Acknowledgment section*

Corresponding author: Alexandra Calmy, MD.

University Hospital Geneva

25 rue Micheli-du-Crest

1205 Geneva, Switzerland

Ph: +41 22 3729 812

Fax: +41 22 3729 599

Email address: acalmy@gmail.com

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Keywords: HIV lipoatrophy; limb fat; pravastatin; uridine

Short title: Uridine or pravastatin for HIV lipoatrophy

Abstract

Background Lipoatrophy can complicate thymidine nucleoside analogue (tNRTI)-based antiretroviral therapy (ART). Lipoatrophy may be less likely with ART including ritonavir-boosted lopinavir (lopinavir/r). Small, placebo-controlled studies found that uridine (in tNRTI recipients) and pravastatin improved HIV lipoatrophy over 12 weeks. Today, most patients with lipoatrophy receive non-tNRTI-based ART; uridine's effect in such patients is unknown.

Methods We performed a prospective, randomised trial in lipoatrophic adults with plasma HIV RNA <50 copies/ml on tNRTI-sparing ART including lopinavir/r. Patients received uridine (36g tid on 10 consecutive days/month; n=10), pravastatin 40mg nocte (n=12), uridine plus pravastatin (n=11) or neither (n=12) for 24 weeks. The primary endpoint was mean change in limb fat mass by dual-energy x-ray absorptiometry (DXA). With 20 patients per intervention, the study had 80% power to detect a mean difference between each treatment and controls of 0.5 kg, assuming a standard deviation of 0.9 and 2-alpha=5%.

Results Of 45 participants (all men, median 49.5 years, limb fat 2.6 kg), two discontinued pravastatin and one participant stopped both pravastatin and uridine. The difference between the mean changes in limb fat mass for uridine versus no uridine was 0.03kg (95%CI -0.35,+0.28; p=0.79). The respective difference for pravastatin was -0.03kg (95%CI -0.29,+0.34; p=0.84). Pravastatin slightly decreased total cholesterol (0.44 mmol/l; p=0.099). Visceral adipose tissue measured by computed tomography did not change significantly.

Conclusion In this population and at the doses used, neither uridine nor pravastatin for 24 weeks significantly increased limb fat mass.

Introduction

HIV lipodystrophy is characterized by subcutaneous lipoatrophy in the face, arms, legs, and buttocks and relative central fat accumulation (lipohypertrophy) in the neck, breasts, and abdomen [1]. Thymidine-based nucleoside analogue reverse transcriptase inhibitor (tNRTI)-associated mitochondrial toxicity is implicated in lipoatrophy [2-4]. Mitochondrial DNA polymerase- γ is inhibited by some NRTIs (mainly tNRTIs) and thus causes depletion of mtDNA-encoded enzymes resulting in mitochondrial dysfunction. tNRTIs can also deplete adipose mitochondrial RNA [5]. Lipoatrophy can be largely prevented through the use of drugs such as abacavir, lamivudine, tenofovir, emtricitabine and ritonavir-boosted lopinavir (lopinavir/r) [6-7], but treatment strategies are disappointing: switching from a tNRTI to a non-tNRTI produced only modest improvements in limb fat mass over 2 years [8-9]. Reconstructive surgery with poly-L-lactic acid is transiently effective but costly [10]. Thiazolidinedione therapy failed to show efficacy in published, randomized trials [11-12].

Uridine is a pyrimidine precursor and so might replenish intracellular pyrimidine pools. *In vitro*, uridine abrogates the mitochondrial toxicity to adipocytes and hepatocytes of the tNRTIs stavudine (d4T) and zidovudine (AZT), but not of didanosine [13]. Uridine supplementation increased limb fat by 0.9 kg relative to placebo over 12 weeks in lipoatrophic adults receiving a tNRTI, an increase far greater and more rapid than observed after replacement of a tNRTI with other drug [14]. A small, non-randomized study found that uridine supplementation for 32 weeks was well tolerated, did not affect HIV viral load, and was associated with a subjective improvement in lipoatrophy [15]. However, whether uridine increases limb fat mass in patients no longer receiving a tNRTI remains unanswered.

Another promising agent is pravastatin. Limb fat and subcutaneous, abdominal fat increased significantly with 12 weeks of pravastatin 40 mg nocte in HIV-infected men with hypercholesterolaemia [16]; the magnitude of increase was not related to its cholesterol-lowering effect, suggesting a mechanism independent of HMGCoA reductase. This unexpected effect was not observed, however, in another randomized study [17].

We assessed the safety and efficacy of uridine and pravastatin in HIV-infected adults receiving a lopinavir/r-containing antiretroviral regimen with moderate to severe subcutaneous lipoatrophy despite cessation of tNRTI therapy.

Materials and Methods

Participants

Subjects were recruited at two University Hospitals (St Vincent's Hospital, the HIV, Immunology and Infectious Diseases Unit, Sydney, Australia, and Geneva University Hospital, HIV Unit, Switzerland) and in two primary care clinics in Sydney (Holdsworth House Medical Practice, Taylor Square Private Clinic) from November 2006 up to March 2008.

Eligibility criteria were: subcutaneous lipoatrophy in at least two body sites (of moderate or greater severity in at least one site) according to both the patient and their enrolling physician; stable antiretroviral therapy and plasma HIV viral load less than 50 copies/ml for at least the preceding three months; no grade 3 or 4 laboratory value (except triglycerides for Australian sites) and the provision of written, informed consent. Exclusion criteria were: tNRTI therapy in the preceding year; prior virological failure on lopinavir/r; requirement for statin therapy because of known ischaemic, cardiovascular disease or clinically significant hyperlipidaemia; statin therapy within the preceding 3 months; current anabolic steroid, growth hormone or supra-physiological corticosteroid therapy; intolerance to any component of the randomised drugs (including sweeteners or milk protein); and prior use of uridine.

The protocol was approved by the Human Research Ethics Committees of St Vincent's and Geneva University Hospitals. The study was conducted in accordance with the ethical principles laid out in the Declaration of Helsinki (1996) and Good Clinical Practice guidelines (Consolidated guidelines [E6] issued by the International Conference on Harmonization [ICH] in May 1996) and was registered in the Australian and New Zealand Clinical Trials Registry (ANZCTR number:12608000307303).

Study design and procedures

Lopinavir/r was chosen as the background 'third drug' for all participants to reduce treatment heterogeneity and because use of lopinavir/r has been associated with stable or increasing limb fat mass [7,18]. Participants who were receiving another protease inhibitor or a non-nucleoside RTI as the 'third drug' switched this drug to lopinavir/r at screening. After 4 weeks (baseline), participants who continued to tolerate their lopinavir/r-based antiretroviral therapy were randomized in a 1:1:1:1 ratio using a block-randomization technique to receive uridine (36g three times daily for 10 consecutive days per month), pravastatin 40mg nocte, uridine plus pravastatin, or neither drug for 24 weeks. At week 24, all participants were offered 6 months of uridine and pravastatin.

Oral uridine supplementation was provided as NucleomaxX® (purchased from Pharma Trade Healthcare, Spanga, Sweden), a dietary supplement with a high content (17%, 36 g per sachet) and availability of uridine. The uridine dose was based on previous studies showing efficacy of uridine supplementation for lipoatrophy at this dose [13] and rapid entry of exogenous uridine from plasma into cells where uridine pools turn over with a half-life of 13-18 h [19]. Participants could adapt their uridine dose to 1 sachet daily (for 30 days/months) for significant gastro-intestinal intolerance to tds uridine, as diarrhea is a known side-effect of uridine [20].

Assessments

Clinical and biological assessments were performed at randomisation, week 4, week 12 and week 24. Anthropometric parameters (weight, umbilical waist circumference and maximum hip circumference) were measured at each visit. Height was recorded at baseline. Blood was collected for fasting total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose and insulin, as well as safety measures (hepatic transaminases, creatinine, electrolytes and full blood count).

Patients randomized to receive uridine had a uridine plasma concentration performed at baseline, week 1 and week 24. All uridine plasma levels were quantified using high performance liquid chromatography (HPLC) with ultraviolet detection at a wavelength of 262 nm; the range of detection was 0.25 to 10 µg/mL and the coefficient of variation less than 10%. Plasma was extracted using acetonitrile to precipitate plasma proteins. The extract was centrifuged at 13000 rpm for 5 min to separate the supernatant from the precipitant. The supernatant was evaporated to dryness at 50°C and the residue suspended in mobile phase. An aliquot of the resuspended fluid was injected onto the HPLC. Separation was performed on a Phenomenex Aqua column (250 X 4.6 mm) with a mobile phase of water containing phosphate buffer.

Quantitative HIV-1 RNA (viral load) was measured on a Roche COBAS TaqMan HIV-1 test (COBAS AmpliPrep, Roche Diagnostic, Basel, Switzerland) at baseline and at weeks 4, 12, and 24.

Adherence was assessed by pill count and empty pack return at all Australian sites by a study pharmacist.

Body composition

Body composition was quantified at baseline, week 12 and week 24 by dual-energy X-ray absorptiometry (DXA). DXA scans were performed on a GE Lunar Prodigy machine (Madison, WI, USA) using a software version 7.51. Cross-validation between sites was done using a body composition phantom.

Visceral and subcutaneous abdominal fat areas at the L2, L3 and L4 levels [21] were measured at baseline and at week 24 for patients in Sydney (n=39) by computed tomography (CT) on a single Philips Brilliance CT (Philips® Medical System, USA).

Statistical analysis

The primary endpoint was the change in limb fat from baseline at week 24 as assessed by DXA. The factorial design and a sample size of 40 patients (10 per group, and so 20 patients receiving uridine compared with 20 controls, and 20 patients receiving pravastatin compared with 20 controls), and assuming no interaction between uridine and pravastatin, 10% loss to follow-up, a standard deviation (SD) of 0.9 and 2- α =5%, the study had 80% power to detect a mean difference between treatment of 0.50 kg by intention to treat analysis.

Baseline characteristics were summarized using median (interquartile range). Analysis of variance (ANOVA) was used to confirm the lack of significant two-way interaction between uridine and pravastatin.

Change from randomization to week 24 in limb fat and other body composition, chemistry and haematology parameters were compared using a Student t-test with a threshold of 5% for each treatment (uridine versus non-uridine groups and pravastatin versus non-pravastatin groups). For qualitative variables, we used a chi-squared test or Fisher's exact test with a threshold of 5%.

All efficacy analyses compared the randomised treatment groups on an intention to treat basis regardless of treatments received during the study, including all patients with data at randomisation and at least one follow-up visit. Primary efficacy analyses used a last value-carried forward approach for any patients permanently lost to follow-up. Secondary analyses only included available data.

Statistical analysis was performed using STATA Release 10.0 (Stata Statistical Software: Release 10.0, Stata Corporation, College Station, USA).

Results

Participants

Of 47 patients screened, 16 patients (36%) switched to lopinavir/r from another protease inhibitor (n=13), didanosine (n=1) or a NNRTI (n=2) at study commencement. One

patient was not randomized because of intolerance to lopinavir/r and one patient withdrew consent before randomisation for personal reasons (figure 1).

Forty-five men (median 49.5 years, limb fat 2.6kg) were randomized to uridine (n=10), pravastatin (n=12), uridine plus pravastatin (n=11) or neither drug (n=12). Median CD4+ lymphocyte count was 588 (410, 618) cells/mm³. There was no significant difference at baseline between the four groups for clinical, metabolic and body composition characteristics (table 1). The median duration of prior stavudine exposure was 41 months [IQR 12-60] and 10 months for zidovudine [IQR 0-47]. Zidovudine users stopped this drug a median 128 months [111-132] prior to study commencement, whereas stavudine users stopped it a median 67 months [46-89] prior to study initiation.

Treatment

After randomization, two participants discontinued pravastatin and one additional subject stopped both uridine and pravastatin (figure 1). One patient was lost to follow-up from week 8. Five participants modified their uridine dose to 1 sachet daily on 30 days/month because of diarrhoea. Overall adherence (including for patients who discontinued therapy or were lost to follow-up), as measured by pills and sachet count, was 91% for uridine recipients and 88% for pravastatin recipients.

Antiretroviral therapy remained unchanged during the study follow-up for 40 patients (89%); 5 patients (11%) changed lopinavir/r between week 4 and week 12, mainly for gastrointestinal disturbances (four to atazanavir/r, and one to fosamprenavir/r).

Body composition

Limb fat increased modestly in all groups (table 2). At week 24, the difference between the mean changes in limb fat mass between those who received uridine and those who did not receive uridine was 0.03kg (95%CI -0.35,+0.28; p=0.79) (figure 2; table 2). The difference between those who received pravastatin and those who did not receive pravastatin was -0.03kg (95%CI -0.29,+0.34; p=0.84). Uridine increased lean mass (without

weight change) by 1.0kg ($p=0.03$) and there was a non-significant increase in lean mass in patients assigned to pravastatin (0.9kg, $p=0.07$). Total visceral adipose tissue, subcutaneous adipose tissue and truncal fat did not change significantly with either intervention.

In a post-hoc analysis, the non-significant change in limb fat mass with uridine was also observed when the analysis was confined to the 16 participants who only received uridine at the planned dose of 36g three times a day for 10 consecutive days each month (limb fat difference for the 16 uridine recipients versus uridine controls, -0.08kg (95%CI -0.44,+0.29; $p=0.68$). The relationship between changes in cholesterol and changes in limb fat in pravastatin recipients is not significant (ρ 0.17, $p=0.50$).

In a post-hoc analysis, we stratified patients by baseline limb fat mass, BMI (<25% percentile, between 25 and 75%, and >75%), age and tNRTI duration (using median values to define categories). We found no effect of any of these variables on the magnitude of limb fat change with either intervention (data not shown).

Safety

Pravastatin decreased total cholesterol (mean change 0.4 mmol/l for pravastatin recipients versus pravastatin controls; $p=0.099$) and serum bicarbonate (mean relative decline 2 mmol/l, $p=0.005$), but in no other metabolic parameter including HDL and estimated LDL (table 3). Uridine caused a significant but modest decrease in serum potassium (mean change -0.2 mmol/l, $p=0.05$). There was no loss of virological control with either intervention.

Five (11%) participants developed sustained grade 3 or 4 hypertriglyceridaemia (4 of who had initiated lopinavir/r at screening), three patients developed grade 3 or 4 elevation in creatine kinase and one patient developed grade 3 thrombocytopenia. Two serious adverse events were reported; one participant with known cardiomyopathy was hospitalized for third-degree heart block at week 1 (uridine and pravastatin arm) and another participant was hospitalized for gastroenteritis at baseline (uridine arm).

Uridine plasma concentration

Plasma uridine concentrations increased significantly after one week of uridine supplementation from a median 1.4 µg/mL (1.2, 1.6) to 23.2 µg/mL (20.2, 26.0) ($p=0.012$) for participants in the uridine only group, and from a median 1.4 µg/mL (1.2, 1.7) to 21.5 µg/mL (18.5, 26.0) ($p=0.001$) for participants in the combined uridine-pravastatin group. In contrast, plasma uridine was stable in participants not receiving uridine (data not shown).

At week 24, 20 days after the last dose of uridine in those receiving uridine at the planned dose, the median plasma uridine concentration was 0.8 µg/mL [0.3, 4.7] in the uridine group and 0.7 µg/mL [0.1, 3.3] in the combined uridine-pravastatin group.

Discussion

In this population of lipoatrophic men receiving stable tNRTI-sparing ART including lopinavir/r, neither uridine nor pravastatin significantly increased limb fat mass over 24 weeks. Our data are not in keeping with encouraging results from small, randomized, placebo-controlled trials in which limb fat increased by 0.89kg after 12 weeks of uridine supplementation (with the same dose and formulation as tested in the present trial [14]) and by 0.72kg with the same dose of pravastatin [16].

There are several possible reasons that might explain why we found no significant effect with either intervention. Firstly, as we powered our study to detect a change of at least 0.5kg, which is the smallest increase that is likely to be observed clinically, smaller changes with uridine or pravastatin may have been missed with our sample size.

Secondly, all our patients had moderate to severe lipoatrophy, as confirmed by a standardized questionnaire (2) and by baseline limb fat (median 2.5 kg), which is lower than in the previous trials of uridine (3.1 kg) and pravastatin (5.0 kg). Patients with more severe lipoatrophy may respond less well to these interventions. We were not powered to detect whether larger changes may be observed across the range of baseline limb fat at study entry. A longer period of observation, as well as stratification based on baseline limb fat at

study entry would be needed in order to assess whether our intervention would yield different results for different lipoatrophy severity at entry. Of note, our patient baseline characteristics were similar to those included in the ROSEY trial, which failed to show efficacy of rosiglitazone in a randomized, blinded manner [21].

Thirdly, patients who previously responded to uridine supplementation were all receiving a tNRTI. In contrast, we only included patients who had not received a tNRTI for at least 12 months, mostly because of lipoatrophy. Supplementation with the pyrimidine precursor uridine has been proposed to abrogate mitochondrial adverse effects of tNRTIs and to reverse mitochondrial DNA depletion. *In vitro* data support the use of uridine in patients exposed to stavudine or zidovudine [22], although no changes in fat or blood mtDNA was observed in a pilot trial on safety and effect of uridine on mitochondrial indices [15]. The *in vitro* effects of uridine on tNRTI-affected adipocytes exposed to drugs such as abacavir and tenofovir are unknown.

Further, uridine absorption may have been suboptimal even though uridine plasma levels increased 17-fold one week after commencing uridine. Previous studies have shown that NucleomaxX increased serum uridine concentrations in humans from about 5 μM to more than 150 μM [23]. Poor adherence to a 3-time per day sachet is possible, but mean adherence was over 90%. Lastly, although we used the same dose that was effective in adults receiving a tNRTI, the optimal dose of uridine is not known and it is conceivable that a higher dose might be effective in this population.

We also observed no increase in limb fat mass with pravastatin. HMG-Co-A reductase inhibitors (statins) are predominantly used to manage hypercholesterolemia but have a range of additional effects (e.g. anti-inflammatory effects) beyond cholesterol reduction [24]. Participants in the study by Mallon et al were similar to ours (mostly men taking a protease inhibitor but no longer a tNRTI) with the notable exceptions that they all had hypercholesterolaemia (greater than 6.5 mmol/l) and were not selected for lipoatrophy. Our study was powered to detect clinically detectable increases in limb fat mass, and could

not reliably determine whether change in limb fat was greater in those with higher total cholesterol levels ($\rho=0.17$, $p=0.51$).

Lean mass increases in uridine recipients, although creatine kinase plasma levels did not change. We did not assess dietary intake, but the absence of changes in weight, albumin level and cholesterol level suggest that there was no major change in nutritional status with uridine.

We did not observe any severe or unexpected safety signal with uridine or pravastatin; in particular, there was no loss of virological control. Also, the sugar-cane derived dietary supplement did not appear to have had a deleterious effect on glucose homeostasis. Only four patients interrupted their assigned treatment allocation, and five patients switched to one uridine sachet daily, mainly because of diarrhoea. Diarrhoea might also explain the slight decrease observed in plasma potassium levels.

Eleven percent of our patients developed grade 3 and 4 hypertriglyceridaemia after study commencement; these changes were asymptomatic and not require any change in therapy. This increase was mainly associated with the recent initiation of lopinavir/r; such an increase has been observed in previous studies.

In conclusion, none of the two trial regimens proposed in our study were proven efficient for these patient population. Switching from a tNRTI such as stavudine or zidovudine to tenofovir or abacavir remains the most proven option for treating lipoatrophy. But with a fat increase of 0.40 kg per year after tNRTI cessation, lipoatrophy may take over 5 years to resolve for many patients without additional intervention, at least with those with severe lipoatrophy [26]. Innovative antiretroviral regimens using either new drugs (raltegravir, etravirine), or new treatment strategies (NRTI-sparing regimens) may warrant further evaluation in patients suffering severe lipoatrophy.

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Author Contributions

Study concept and design: Calmy, Carr

Analysis and interpretation of data: Calmy, Carr, Delhumeau, Wand, Bloch, Hirschel, Finlayson

Data extraction: Wand, Delhumeau

Drafting of the manuscript: Calmy

Critical revision of the manuscript for important intellectual content: all authors

Statistical analysis: Wand, Delhumeau

Generated allocation sequence and assigned patients to their randomisation group: Wand

(The randomization form had to be faxed to Handan Wand, at the National Center for HIV

Epidemiology and Research, and receipt of the randomization was provided within one working day)

Study supervision: Carr

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Table 1 Patient characteristics

Parameter	Total	Uridine only	Uridine plus pravastatin	Pravastatin only	Delayed arm
Number of subjects, n (%)	45 (100)	10 (22)	11 (24)	12 (27)	12 (27)
Age (years)	49 (45, 54)	53 (49, 57)	49 (43, 54)	46 (42,57)	47 (44, 52)
Prior AIDS diagnosis, n (%)	15 (34)	3 (30)	2 (20)	4 (33)	6 (50)
CD4 cell count (cells/mm ³)	588 (410, 618)	519 (400, 698)	630 (418, 703)	672 (461, 778)	522 (400, 618)
NRTI backbone					
tenofovir (current use)	20 (44)	5 (50)	4 (36)	7 (58)	4 (33)
abacavir (current use)	14 (31)	4 (40)	3 (27)	4 (33)	3 (25)
prior stavudine (months duration)	48 (31.5, 66.5)	53 (38, 74.5)	70.5 (40.5, 82.5)	51 (14, 62)	41 (38, 48)
prior zidovudine (months duration)	37 (10, 60.5)	59 (8,104)	40 (15, 46)	48 (30, 108)	12 (9, 54)
Switched to LPV/r at screening, n (%)	16 (36)	2 (20)	4 (36)	6 (50)	4 (33)
Body composition					
body mass index, kg/m ²	24 (22;25)	23 (21;24)	23.5 (22;25)	24 (24;25)	24 (21;26)

limb fat, kg	2.58 (1.93;4.88)	2.13 (1.57;2.81)	2.73 (1.52;5.26)	3.03 (2.21;5.31)	2.53 (1.83.5;4.94)
limb fat, %	19.2 (13.9;27.9)	16.6 (11.3;19.2)	19.7 (13.7;27.9)	25.4 (15.9;35.2)	19.6 (11.2;30.6)
trunk fat, kg	7.83 (5.18;10.08)	7.39 (4.92;9.13)	6.33 (4.86;9.96)	9.21 (7.39;13.70)	7.87 (3.74;8.91)
trunk fat, %	21.3 (16.2;25.3)	20.55 (16.2;24)	19.2 (14;23.2)	24.6 (19.4;29.7)	21.9 (11.1;24.75)
total fat, kg	10.95 (7.58;15.17)	10.06 (6.66;11.57)	8.95 (7.30;15.17)	12.40 (9.97;19.00)	11.03 (5.93;14.78)
total fat, %	16 (11.9;18.4)	14.80 (11.2;17.1)	13.50 (11.9;17.5)	18.30 (13.7;23.1)	17.00 (8.45;18.9)
lean body mass, kg	61.3 (54.2;65.1)	59.93 (51.22;66.60)	62.04 (54.15;66.25)	62.54 (55.09;64.43)	59.78 (55.41;64.71)
lean mass, %	0.84 (0.82;0.88)	0.85 (0.83;0.89)	0.86 (0.82;0.88)	0.83 (0.77;0.86)	0.83 (0.81;0.92)
visceral adipose tissue, cm ²	38.31 (20.2;57.54)	21.54 (11.67;47.36)	43.19 (20.2;55.81)	31.89 (22.77;50.45)	46.87 (30.42;59.93)
subcutaneous abdominal fat, cm ²	12.69 (6.83;29.25)	9.51 (6.34;13.62)	11.63 (7.57;17.96)	16.30 (12.69;41.88)	17.78 (4.86;29.25)
Lipid and glycaemic parameters					
Total cholesterol, mmol/L	5.5 (4.7;6.15)	5.3 (4.8;7.7)	5.3 (5.1;5.6)	5.6 (4.5;6.4)	5.6 (4.6;6.2)
estimated LDL cholesterol, mmol/L	3.2 (2.6;3.7)	3.2 (2.9;4.15)	2.9 (2.5;3.4)	2.8 (2.4;3.3)	3.5 (2.5;4.1)
HDL cholesterol, mmol/L	1.1 (0.8;1.1)	1.1 (0.9;1.3)	0.9 (0.8;1.1)	1 (0.9;1.3)	1.1 (0.81;1.2)
Triglycerides, mmol/L	2.7 (1.7;4.2)	2.7 (2;3.7)	2.1 (1.7;4.1)	3.9 (2.0;6.2)	2.3 (1.5;3.5)

Insulin, mU/L	9.9 (7;18.2)	9.3 (7.5;23.8)	9.5 (6.7;17.3)	10.6 (6.8;18.2)	11 (7.9;16.4)
Glucose, mmol/L	5.1 (4.7;5.6)	5.1 (4.8;5.3)	4.8 (4.6;5.6)	5.2 (4.7;5.7)	5.1 (4.8;5.6)
HOMA, mUmmol/L ²	2.3 (1.5;4.3)	2.2 (1.9;5.1)	2.0 (1.3;6.0)	2.7 (1.5;4.1)	2.6 (1.6;4.0)

Liver function

Alanine aminotransferase, U/L	29 (22;45)	35.5 (24;43)	27 (21;32)	30.5 (24;42)	28 (21;49)
Alkaline phosphatase, U/L	84 (64.5;98)	83.5 (74;96)	87 (58;93)	84 (64.5;112.5)	77.5 (60;106.5)

Biochemistry

Creatine kinase, U/L	145 (83;222)	149 (122;211)	104 (78;156)	143 (96.5;232)	152 (120.5;239.5)
Anion gap, mmol/L	10 (8;11)	10 (10;11)	9.5 (8;11)	9 (8;11)	10 (8.5;12)
Lactate, mmol/L	1.5 (1;2)	1.6 (1.2;2.7)	1.35 (1;2)	1.5 (1.15;1.7)	1.7 (1.1;2.6)
Uridine, µg/mL	1.4 (1.2;1.6)	1.4 (1.2;1.6)	1.5 (1.2;1.9)	1.4 (1.3;1.5)	1.4 (1.2;1.5)

If not indicated differently, data are median [IQR].

Table 2 **Body composition parameters changes**

	Uridine groups	No uridine		Pravastatin groups	No pravastatin	
	Mean change (95% CI)	Mean change (95% CI)	P-value*	Mean change (95% CI)	Mean change (95% CI)	P-value*
Limb fat, kg	0.15 (-0.04;0.34)	0.12 (-0.14;0.37)	0.790	0.12 (-0.13;0.38)	0.15 (-0.06;0.35)	0.839
Limb fat, %	0 (0;0)	0 (0;0)	0.856	0 (0;0)	0 (0;0)	1.000
Trunk fat, kg	0.25 (-0.24;0.75)	0.86 (-1.36;3.08)	0.598	0.79 (-1.68;3.26)	0.37 (-0.04;0.78)	0.731
Total fat, kg	0.41 (-0.25;1.07)	0.57 (-0.24;1.38)	0.748	0.46 (-0.45;1.37)	0.52 (-0.06;1.10)	0.917
Total fat, %	0.4 (-0.4;1.2)	0.9 (0.0;1.7)	0.379	0.8 (-0.2;1.7)	0.5 (-0.2;1.2)	0.700
Visceral adipose tissue, cm ²	-3 (-6;1)	0 (-5;4)	0.425	-3 (-9;3)	0.9 (-3.5;3.7)	0.370
Subcutaneous adipose tissue, cm ²	1 (0;3)	0 (-5;4)	0.626	0 (-5;6)	0 (-3;4)	0.955
Lean mass, kg	0.52 (-0.30;1.35)	-0.51 (-1.01;-0.01)	0.029	-0.48 (-1.20;0.25)	0.40 (-0.25;1.06)	0.066
Lean mass, %	0 (-0.01;0.01)	0 (-0.02;-0.00)	0.165	0 (-0.02;-0.00)	0 (-0.01;0.00)	0.361
Weight, kg	0.4 (-0.7;1.5)	-0.10 (-2.41;2.21)	0.657	0.55 (-1.91;3.01)	-0.05 (-1.44;1.35)	0.615
Body mass index, kg/m ²	0.1 (-0.3;0.4)	-0.02 (-0.45;0.40)	0.781	-0.08 (-0.51;0.36)	0.09 (-0.24;0.42)	0.529

*P values are for comparisons of intervention and control groups

Table 3 Safety and metabolic parameters

	Uridine groups Mean change (95% CI)	No uridine Mean change (95% CI)	p-value*	Pravastatin groups Mean change (95% CI)	No pravastatin Mean change (95% CI)	p-value*
Lipid and glycaemic parameters						
Total cholesterol, mmol/L	-0.2 (-0.5;0.1)	-0.4 (-0.9;-0.0)	0.397	-0.5 (-0.9;-0.2)	-0.1 (-0.5;0.2)	0.099
LDL* cholesterol, mmol/L	-0.2 (-0.5;0.2)	-0.3 (-0.7;0.0)	0.433	-0.4 (-0.9;-0.0)	-0.1 (-0.4;0.1)	0.176
Triglycerides, mmol/L	-0.4 (-1.1;0.2)	0.5 (-0.6;1.6)	0.185	0.3 (-1.1;1.7)	-0.1 (-0.5;0.2)	0.518
HDL** cholesterol, mmol/L	0.1 (-0.0;0.2)	0.0 (-0.0;0.1)	0.659	0.1 (-0.0;0.2)	0.0 (-0.1;0.1)	0.304
Glucose, mmol/L	-0.1 (-0.6;0.4)	-0.3 (-0.6;0.0)	0.137	-0.3 (-0.8;0.2)	-0.1 (-0.3;0.1)	0.474
Insulin, mU/L	12.3 (-11.3;35.9)	-3.4 (-11.9;5.0)	0.196	-3.9 (-13.8;6.0)	10.1 (-9.4;29.6)	0.203
Liver function						
Alanine aminotransferase, U/L	4 (-1.7;10.5)	-2 (-7.8;2.9)	0.087	0.8 (-4.5;6.2)	0.3 (-6.2;6.9)	0.880
Asparate aminotransferase, U/L	2 (-1.9;6.6)	2 (-2.5;6.9)	0.956	1.5 (-2.4;5.6)	3 (-2.1;8.1)	0.644
Albumin, g/L	0 (-0.9;1.4)	0 (-0.4;1.3)	0.775	0.8 (-0.1;1.7)	-0.1 (-1.2;0.9)	0.163
Alkaline phosphatase, U/L	-2 (-8.7;5.2)	-5 (-12.9;2.0)	0.452	-6.5 (-15.7;2.7)	-1 (-5.7;3.5)	0.281
Biochemistry						
Sodium, mmol/L	2 (1;3)	0.5 (-0.5;1.6)	0.064	1.1 (-0.1;2.3)	1.3 (0.0;2.5)	0.790
Potassium, mmol/L	-0.1 (-0.3;0)	0.1 (-0.1;0.2)	0.045	0.0 (-0.1;0.1)	0 (-0.1;0.0)	0.502
Chloride, mmol/L	2 (1;3)	0 (-1.3;1.2)	0.048	0.3 (-1.0;1.7)	0.9 (-0.2;2.1)	0.522
Bicarbonate, mmol/L	-0.3 (-1;1)	0 (-1.2;1.2)	0.720	-1.2 (-2.1;-0.3)	0.9 (-0.2;2.1)	0.005
Lactate, mmol/L	0.1 (-0.3;0.5)	0.2 (-0.3;0.8)	0.496	0.5 (-0.1;1.1)	-0.1 (-0.5;0.2)	0.065
Urea, mmol/L	0 (-1;1)	0.2 (-0.5;0.9)	0.451	0.3 (-0.4;1.1)	-0.2 (-1.0;0.5)	0.249
Creatinine, umol/L	-1 (-5;3)	0.3 (-3.5;4.2)	0.553	-0.6 (-4.3;3.0)	-0.1 (-4.3;4.2)	0.820
Amylase, mmol/L	9 (-5;23)	4 (-7;16)	0.614	3 (-9;15)	9 (-4;23)	0.456
Creatine kinase, U/L	117 (-48;282)	111 (-105;327)	0.962	89 (-78;255)	138 (-100;376)	0.772
HIV viral load, copies/mL	-1 (-17;16)	14 (-25;53)	0.491	4 (-21;29)	10 (-27;47)	0.787

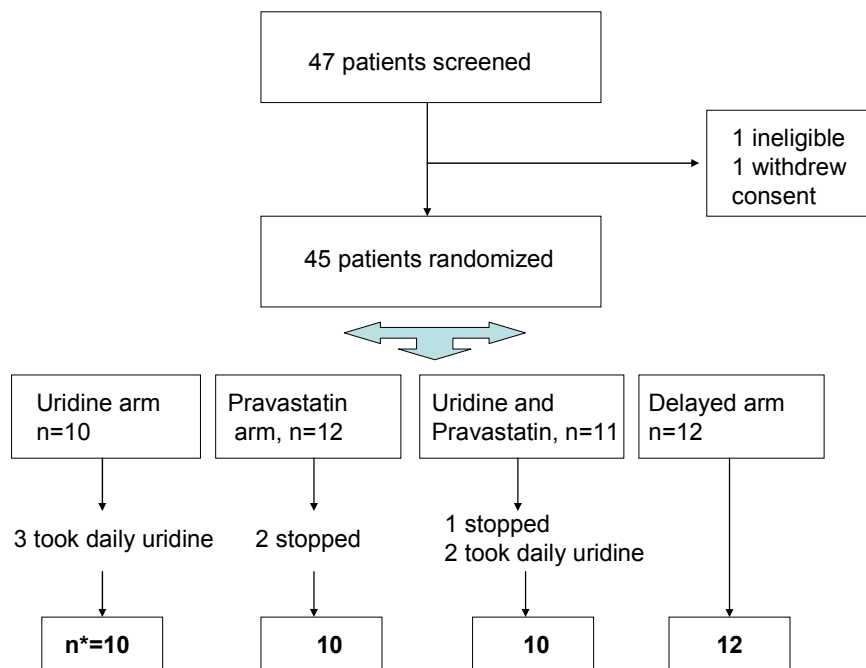
Values are mean (standard deviations). *LDL Cholesterol stands for Low-density lipoprotein cholesterol
**HDL stands for High-density lipoprotein.

Figure legends

Figure 1 Participant disposition

Figure 2 Mean (SD) change in limb fat mass

Figure 1



n : number of patients included in the ITT analysis of the primary endpoint*

Figure 2

