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Mitochondrial toxicity in HIV-infected patients both off and on antiretroviral treatment: a continuum or distinct underlying mechanisms?

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Mitochondrial toxicity contributes to serious adverse effects observed in HIV-infected individuals treated with nucleoside reverse transcriptase inhibitors (NRTIs). However, similar mitochondrial abnormalities have recently been found even in treatment-naive patients, suggesting that chronic HIV *per se* could contribute to the toxicity observed in NRTI-exposed individuals. This review gives a current status of the field, with particular focus on recent observations suggesting that distinct mechanisms might cause such toxicity in both NRTI-exposed individuals and those naive to antiretroviral treatment.

Keywords: HIV-1, mitochondria, DNA polymerase gamma, nucleoside reverse transcriptase inhibitors, persistent immune activation

Introduction

Combination antiretroviral therapy (ART) has over the last decade proven its excellent clinical efficacy.[1] Increasing attention has therefore been focused on long-term adverse effects of ART, including the devastating fat distribution changes observed in the lipodystrophy syndrome and the increased risk for cardiovascular disease.[2–5] However, although much effort has been made in understanding the possible underlying mechanisms for such adverse effects, our insight is still incomplete and reliable surrogate markers for ART-related toxicity are not accessible. It should be noted, though, that some nucleoside reverse transcriptase inhibitors (NRTIs; Table 1) have been noticeably associated with many of these long-term adverse effects.[6] The triphosphate forms of NRTIs compete with endogenous nucleotides for HIV DNA synthesis by the HIV enzyme reverse transcriptase (RT), thus acting as chain terminators. Unfortunately, NRTIs can to a variable extent also be substrates for mitochondrial DNA (mtDNA) polymerase γ and, as a result, interrupt replication even of mtDNA. It was hypothesized early on that mitochondrial toxicity caused by inhibition of DNA polymerase γ (DNA pol γ) was responsible for NRTI-associated adverse effects.[6] First, zidovudine-associated skeletal myopathy was observed early after the introduction of the drug in 1987, and subsequently ragged-red fibres and reduction of mtDNA content as well as morphological changes in the mitochondria were demonstrated in skeletal muscle.[7,8] Second, mitochondrial changes were also observed in HIV-infected patients with peripheral neuropathy, insulin resistance, hyperlactataemia and lactic acidosis, the latter being a life-threatening condition often associated with hepatosteatosis and/or pancreatitis.[9–13] In 1999, it was postulated that NRTI-associated mitochondrial toxicity might play an essential role also in the lipodystrophy syndrome[14,15] and was particularly associated with exposure to the thymidine analogue stavudine.[16] Against this background, assessments of mitochondrial toxicity in patients on ART therefore became a relevant area for clinical research using peripheral blood mononuclear cells (PBMC) as a readily available cellular material. Later, it was learned that mtDNA content may be quite unaffected in PBMC, while at the same time it is decreased in specific tissues.[7,10,17] Another complicating factor was the observation that mtDNA was reduced in PBMC not only in patients treated with certain NRTIs, but also in ART-naive patients.[18–22] Thus, HIV infection *per se* appears to be associated with mitochondrial toxicity in HIV-infected individuals, at least in PBMC, and this phenomenon complicated the data interpretation of NRTI toxicity in ART-exposed patients.

The objective of this review is to discuss the possible mechanisms underlying mitochondrial toxicity in relation to treatment with NRTIs as well as to untreated HIV infection. Enhanced understanding of the mechanisms behind these effects is relevant for future management of long-term ART and adverse effects in HIV-infected patients.

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Table 1. Nucleoside and nucleotide reverse transcriptase inhibitors and their relation to mitochondrial toxicity and clinically adverse effects as well as evidence of reversibility of mitochondrial toxicity

Nucleoside[a] or nucleotide[b] analogues	Mitochondrial toxicity	Clinically adverse effects related to mitochondrial toxicity	Evidence of reversibility
Thymidine analogues[a]			
zidovudine (AZT/ZDV)	yes	myopathy,[7,8] lipoatrophy[13,16,17]	
stavudine (d4T)	yes	lactic acidosis,[89,124] lipoatrophy,[125] insulin resistance[11]	dose reduction[126,127]
Inosine analogue[a]			
didanosine (ddI)	yes	peripheral neuropathy,[128] lactic acidosis,[9] nephropathy[129]	
Cytidine analogues[a]			
zalcitabine (ddC)	yes	peripheral neuropathy[10]	
lamivudine (3TC)	no		
emtricitabine (FTC)	no		
Guanosine analogue[a]			
abacavir (ABC)	no		switch-studies[52,59,130]
Adenosine analogue[b]			
tenofovir disoproxil fumarate (TDF)	no?	nephropathy?[131]	switch-studies[60]

Mitochondria: structure and function in human cells

Mitochondria are small intracellular organelles (diameter of 0.5–1 μm) located in the cellular cytoplasm and were first discovered by Altman in 1890.[23] In the early 1960s it was found that mitochondria contain their own, almost exclusively maternally inherited, DNA[24] and in 1981, the mitochondrial genome, consisting of 16569 bp, was sequenced.[25] All cells contain mitochondria, with the exception of erythrocytes. A single cell may contain hundreds to thousands of mitochondria, particularly cells with a high energy demand, and each mitochondrion contains 2–10 copies of mtDNA.[26–28] Mitochondria are highly motile and were recently shown to undergo both fusion and fission through complex mechanisms.[29,30] Because mitochondria lack several enzymes for DNA repair, they are particularly susceptible to mutation.[24] In fact, the mutation rate of mtDNA is >10 times higher than that of nuclear DNA.[31] However, mitochondrial dysfunction probably requires a minimum threshold level of either mutant mtDNA or mtDNA mass to become clinically relevant.[32]

Mitochondria have a double lipid membrane, with an inner membrane that is folded into numerous cristae surrounding the matrix space. This matrix contains copies of the mtDNA genome, which encodes subunits of four of the five complexes of the oxidative phosphorylation (OXPHOS) system located in the inner membrane of the mitochondrion.[33] The OXPHOS system is responsible for providing most of the energy to cells; however, mitochondria also play a key role in apoptosis,[34] β-oxidation of free fatty acids and calcium homeostasis.

NRTI-related effects on mitochondria: differences between drugs and tissue-specific susceptibilities

Effects on DNA pol γ

The nuclear-encoded mitochondrial DNA pol γ is the only DNA polymerase found in mitochondria and is therefore crucial for mtDNA replication as well as for mtDNA repair capacity.[24,35] DNA pol γ consists of a catalytic subunit of both polymerase and 3′–5′ exonuclease activity, which is associated with the mitochondrial DNA pol γ accessory subunit that stimulates the catalytic and exonuclease activity of the larger catalytic subunit.[32] Inhibition of mtDNA replication subsequently leads to a reduction of mtDNA content, and thereby decreased synthesis of the mtDNA-encoded protein subunits of the OXPHOS system and reduced ATP production, as well as enhanced generation of reactive oxygen species.[6,36] Quantification of the mtDNA content has therefore frequently been used as a tool for determining mitochondrial toxicity in clinical research. However, reduced mtDNA content along with normal mtRNA levels encoded by mitochondrial genes has also been found, suggesting possible up-regulating mechanisms for mitochondrial transcription.[37]

Nevertheless, many NRTIs do hamper mtDNA synthesis *in vitro*, by acting as competitive substrates to endogenous nucleotides and chain terminators at the nucleotide-binding site of DNA pol γ, because NRTIs lack the 3′-hydroxyl group necessary for further elongation of the mtDNA strand. NRTIs might also persist in the mtDNA strand because of inefficient DNA pol γ exonuclease activity.[33,38] Generally, both

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endogenous nucleosides and NRTIs must become phosphorylated to triphosphate forms to be substrates for DNA polymerases in DNA synthesis. The triphosphate forms of zalcitabine, didanosine, stavudine and lamivudine were all well incorporated into the elongating mtDNA strand *in vitro*, but less so for tenofovir, zidovudine and abacavir.[39–41] One factor that may be important for a lower clinical toxicity profile of lamivudine is that this NRTI appears to be more efficiently removed by DNA pol γ exonuclease activity than other NRTIs.[42]

The less effective incorporation of zidovudine triphosphate into mtDNA may be outweighed by a number of other mechanisms that might explain the toxicities of zidovudine. First, even zidovudine monophosphate might act as a substrate for mtDNA synthesis and may also inhibit DNA pol γ exonuclease activity.[42] Second, zidovudine has also been related to decreased levels of complex IV of the OXPHOS system, even in absence of mtDNA depletion, impairment of the ADP/ATP translocase as well as inhibition of adenylate kinase, an enzyme involved in ATP formation.[39,43–45] In a recent review, Lund and Wallace proposed that zidovudine might also compete with NADH directly at complex I.[46] Decreased OXPHOS activity may also lead to increased electron leakage into the matrix and generation of reactive oxygen species, which in turn initiate a cascade of further oxidative damage.[47] In fact, zidovudine exposure was also associated with oxidative stress and reduced ATP production *in vitro*.[48]

Effects on thymidine kinases (TKs)

Endogenous thymidine and cytidine, as well as the thymidine analogues zidovudine and stavudine, are phosphorylated to monophosphates by the nucleotide kinases TK1, which is localized in the cellular cytoplasm, or TK2 in the mitochondria. Zidovudine may even inhibit TK2, and cause reduced levels of endogenous nucleotides and thereby decreased synthesis of mtDNA.[49] Reduction of endogenous nucleotides could also be caused by favouring the transport of phosphorylated NRTIs into the mitochondria compared with endogenous nucleotides.[50]

TK1 is expressed only during the S phase in mitotic dividing cells, while TK2 is expressed in post-mitotic tissue like skeletal muscle, adipocytes and neurons.[33] Whereas mtDNA changes in these tissues have particularly been associated with exposure to didanosine and zalcitabine, treatment with stavudine has been especially associated with mtDNA changes in peripheral fat.[8,51–58] In addition, zidovudine may induce mtDNA depletion in skeletal muscle[7] and, although to a lesser extent, has also been associated with lipoatrophy.[59,60] It might therefore be speculated that the susceptibility to mitochondrial changes mainly depends on TK2 in resting tissues like fat, muscle and neurons with low TK1, despite the greater affinity for TK1 than TK2 of thymidine analogues. Because PBMC probably lack TK1,[33] this could possibly explain the observation that exposure to didanosine, which is not a substrate for thymidine kinases, seems to be more likely associated with mtDNA depletion in PBMC compared with thymidine analogues.[20,61–63] In keeping with this notion, exposure to didanosine and tenofovir was recently associated with a depleted CD4+ T cell count, despite virological suppression.[64] This could possibly be linked to apoptosis caused by mitochondrial toxicity.

On the other hand, increased total TK activity has recently been associated with cellular activation in HIV infection[65] and it might be speculated that even TK1 activity could be increased in PBMC in HIV-infected individuals.[65,66] In keeping with this, increased intracellular concentrations of NRTIs were found in PBMC from patients with low CD4+ T cell counts, and the concentrations in fact decreased when the CD4+ T cell count and immune activation improved.[67,68] As reviewed by Anderson *et al.*,[68] intracellular NRTI triphosphate concentration is probably a key factor for mitochondrial toxicity. Increased intracellular concentrations of zidovudine and lamivudine were also found in women relative to men, and it should be noted that both women and patients with low nadir CD4+ T cell counts seem particularly susceptible to mitochondrial toxicity.[68–70]

To summarize, substantial differences in both phosphorylation rates and cellular uptake of NRTIs might explain the variability of mtDNA-related toxicity between cell types. This could explain the observed reduction of mtDNA in susceptible adipocytes from patients with lipoatrophy, who apparently had no corresponding depletion of mtDNA in PBMC.[52,71–73]

mtDNA mutations and reduction in gene expression

NRTI may clearly cause mitochondrial dysfunction by mechanisms other than inhibition of DNA pol γ, for example by predisposing, pre-existing mutations or accumulation of mtDNA mutations as a result of NRTIs. Although the spontaneous mutation rate is generally higher in mtDNA compared with nuclear DNA, data on mtDNA mutations in HIV-infected patients have been inconsistent in relation to ART. First, in PBMC, a longitudinal study of HIV-infected patients found such mutations in PBMC soon after initiating combined ART,[74] while another study found neither mtDNA mutations nor reduced mtDNA content in PBMC from patients who had or had not developed lipoatrophy.[75] Second, in muscle tissue, we found mtDNA deletions in NRTI-exposed patients,[76] whereas reduced mtDNA in adipose tissue from NRTI-exposed patients with lipoatrophy but no concomitant mtDNA mutations was found in another study.[77]

Another factor for reduced mitochondrial function is a decreased expression of mitochondrial genes, again detected in adipocytes[78–80] and even in the absence of reduced mtDNA. In the study by Mallon *et al.*,[81] decreased mRNA levels corresponding to the mtDNA-encoded subunits of the OXPHOS system were found, in addition to down-regulation of mitochondrial and nuclear genes involved in lipid metabolism.[81] Another explanation for the differences between solid tissue and PBMC is that mitochondrial gene expression in PBMC was normal, despite concurrent depletion of mtDNA found in PBMC from patients exposed to stavudine and didanosine, suggesting a compensatory up-regulation of mitochondrial transcription or translation.[37] Taken together, more in-depth studies are required to understand mitochondrial toxicity and the impact of deletions, mutations and regulation of mtDNA gene expression for clinical practice.

Host factors

Because not all individuals treated with specific NRTIs develop adverse effects related to mitochondrial toxicity, a number of host factors have been suggested, such as female sex, white race,

older age, hepatitis B and C co-infection, nadir CD4+ T cell counts, and decreased creatinine clearance.[82–87]

Gender

Lactic acidosis in stavudine-exposed patients has been reported more frequently in women,[69,88,89] with a particular increased risk in obese women.[83] Women might also be more susceptible to fat distribution changes.[85,86] These phenomena could possibly be related to the previously discussed pharmacological differences between men and women.[68] In fact, normal plasma concentrations but higher intracellular triphosphate concentrations of zidovudine and lamivudine were found in women compared with men.[67] Recently, focus has also been placed on the stage of HIV infection, because nadir CD4+ T cell counts of <100 cells/mm^3 appear to be associated with lipoatrophy and polyneuropathy.[70,87] It might be speculated that this could be attributed to the enhanced susceptibility due to dysregulated lipid metabolism in advanced HIV with high levels of tumour necrosis factor α (TNF-α).[68]

Inborn mitochondrial variants

Pre-existing mutations in both mtDNA and nuclear DNA as subclinical inborn variants will affect all mitochondria and, therefore, possibly predispose to overt mitochondrial toxicity in chronic HIV.[75] Recently, a mutation in *POLG*, the nuclear gene encoding DNA pol γ, was found in a woman with stavudine-associated lactic acidosis, suggesting an increased risk because of the pre-existing mutation in this nuclear gene.[90] Mitochondrial haplogroup T was associated with increased risk for peripheral neuropathy in individuals treated with stavudine and didanosine,[55] whereas haplogroup J possibly protects from lipoatrophy.[91]

Age

Older age, which is associated with accumulated mtDNA mutations in non-HIV,[92] was also an independent predictor for peripheral neuropathy.[55] Age-related mtDNA mutations might be even more challenged in the near future, because individuals treated with combination ART not only become older, but also have a long cumulative exposure period to thymidine analogues and didanosine, with some patients even having been exposed to zalcitabine, the first drug taken off the market due to profound mitochondrial toxicity.

Other factors

Co-morbidities and other concomitant medication may also contribute to an increased risk for mitochondrial toxicity in HIV-infected patients, such as individuals with HIV and hepatitis C co-infection treated with ribavirin in combination with didanosine, who have a substantial risk for lactic acidosis.[93–95]

HIV and its effect on mitochondria

HIV infection *per se* appears to affect mitochondria and may therefore contribute to the mitochondrial toxicity observed in NRTI-exposed individuals. In fact, reduced mtDNA content, decreased OXPHOS activity and reduced mitochondrial membrane potential ($\Delta\Psi_m$) have been found in ART-naive patients, mainly in PBMC.[18,19,21,22,96] In fact, $\Delta\Psi_m$ even correlated with the CD4+ T cell count.[96] Further, mitochondrial changes have been observed also in tissue from treatment-naive patients. First, decreased mtDNA content was found in skeletal muscle in a few treatment-naive individuals.[54] Second, reduced levels of both mitochondrial- and nuclear-encoded proteins necessary for mitochondrial function were found in adipocytes from both treatment-naive and NRTI-exposed patients.[78]

The underlying mechanisms for the mitochondrial changes observed in *untreated* HIV infection have been less clear; although, recently, we[62] and others[97] found that reduced mtDNA content was related to markers of persistent immune activation in ART-naive individuals, at least in PBMC. Although the hallmark of chronic HIV infection is substantial CD4+ T cell loss,[98] it should be noted that lymphocyte proliferation and turnover rates become increased not only among CD4+ T lymphocytes (2-fold), but particularly among hyperactivated CD8+ T lymphocytes (6-fold)[99] and B lymphocytes.[100] In fact, the enhanced proliferation and turnover of CD4+ T cells probably overcompensates the actual HIV-related CD4+ T cell death rate. Moreover, the increased proliferation of both CD8+ T cells and B cells is probably the result of chronic immune stimulation, partly from HIV but also from other sources, such as antigen leaking across mucosal membranes.[101,102] Such phenomena might play a role also in CD4+ T cells.[98,103] Persistent immune activation in itself might therefore hamper mtDNA synthesis in untreated HIV-infected individuals, as supported by the observation by Casula *et al.*,[18] who found mtDNA loss in PBMC after seroconversion in a retrospective longitudinal study. One explanation could be increased rates of mitochondrial apoptosis induced by proinflammatory cytokines as well as HIV proteins, as recently reviewed by Shedlock *et al.*[104] Some HIV gene products may in fact aggravate mitochondrial dysfunction (Figure 1).[34,105–109] Proinflammatory cytokines, such as TNF-α, interferon α and γ, and interleukin 2 and 6, may also in turn induce apoptosis.[109,110] This notion is in agreement with a recent study in which mitochondrial dysfunction was associated with increased PBMC apoptosis in HIV progressors but not in long-term non-progressors.[111] Nevertheless, the complexity is illustrated by data suggesting that HIV proteins also may be antiapoptotic, since Tat might protect from apoptosis by down-regulation of caspase-10 activity.[110]

The expression of the activation marker CD38 on CD8+ T cells has long been the best predictor of disease progression in chronic HIV infection.[112] Recently, programmed cell death-1 (PD-1) was suggested to play an important immunopathophysiological role through its involvement in reversible T cell exhaustion[113] and we subsequently found that expression of PD-1 had even better prognostic significance.[103] In our studies, mtDNA was substantially decreased in CD8+ T cells and B cells of ART-naive patients, both PBMC subsets known to be particularly hyperactivated in untreated chronic HIV infection. Even though mtDNA was less decreased in CD4+ T cells than in CD8+ T cells, mtDNA was significantly lower in untreated rapid progressors compared with slow progressors. In fact, mtDNA depletion in all these lymphocyte subsets correlated strongly with markers of persistent immune activation (CD38 and PD-1) on CD8+ T cells.[62] Because HIV infection also induces B cell dysfunction,[114,115] this might explain the highly significant depletion of mtDNA also in B cells from ART-naive

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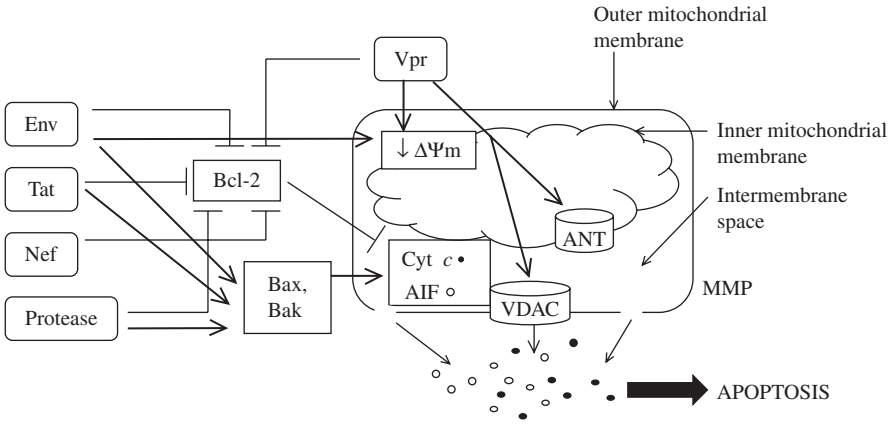

Figure 1. Mitochondria are key organelles in apoptosis, and the HIV peptides Tat, Env, Vpr and Nef, as well as protease, might trigger apoptosis either by inducing apoptotic signals or by blocking anti-apoptotic signals. Thus, HIV might provoke the mitochondrial membrane potential (MMP), the point of no return in apoptosis. MMP, in turn, causes release of pro-apoptotic components, such as apoptosis-inducing factor (AIF) and cytochrome *c* (Cyt *c*), from the intermembrane space in the mitochondria to the cellular cytoplasm. Proteins of the Bcl-2 family play a significant role in this apoptotic process, and might be both pro- and antiapoptotic. HIV peptides might not only activate the pro-apoptotic Bcl-2 family members Bax and Bak, but also inhibit antiapoptotic Bcl-2 family members, thus inducing MMP by both mechanisms. HIV peptides like Env might also directly disrupt the MMP ΔΨm, and Vpr might act on the transition pores adenine nucleotide translocator (ANT) and voltage-dependent anion carrier (VDAC), subsequently leading to apoptosis.

patients.[62] We therefore suggested that persistent immune activation particularly hampers mtDNA in lymphocytes from untreated HIV-infected patients, whereas we previously did not find mtDNA depletion in muscle tissue from such patients.[76]

Non-NRTIs and mitochondrial toxicity

Although non-NRTIs do not inhibit DNA pol γ, a recent study found that both efavirenz and nevirapine might reduce mitochondrial $\Delta\Psi_m$ in PBMC, and current treatment with nevirapine was also in fact associated with apoptosis of lymphocytes.[116] In keeping with this notion, efavirenz was associated with apoptosis *in vitro*, induced by the intrinsic mitochondrial apoptotic pathway.[117] On the other hand, protease inhibitors probably protect $\Delta\Psi_m$, thereby preventing apoptosis induced by the mitochondria.[118] This might be in agreement with clinical experience, because treatment with efavirenz rather than boosted lopinavir was more likely associated with lipoatrophy at 96 weeks of combination therapy with a backbone regimen of stavudine or zidovudine regimens,[119] in contrast to a minimal risk for lipoatrophy when efavirenz was combined with either abacavir or tenofovir.[119,120]

Conclusions and future perspectives

Although the pathogenesis of NRTI-related mitochondrial toxicity was first explained by the inhibition of DNA pol γ,[121] a number of other factors might also contribute.[46,81] For example, decreased mtRNAs but no concomitant depletion of mtDNA have been found,[81] and also reduced mtDNA content but normal levels of mtDNA-encoded proteins have been observed in both PBMC and tissue.[37,122] Until recently, quantification of mtDNA content was frequently used in studies of mitochondrial toxicity in HIV-infected individuals. However, the functional and thereby clinical significance of mtDNA changes, such as numerical loss, in terms of mitochondrial dysfunction are not always clear. MtDNA content might even be unaffected in PBMC while decreased in specific tissues and this in fact highlights the limitation of quantifying mtDNA content in PBMC in relation to clinically adverse effects. Finally, HIV-associated reduction of mtDNA in the ART-naive phase could possibly also contribute to mitochondrial toxicity even in NRTI-treated patients. However, this issue is not at all elucidated, suggesting the relevance for longitudinal assessments of mtDNA content in treatment-naive patients after initiation of ART. Such data could provide better understanding of the impact of reduced immune activation after starting effective ART, which might explain improved mtDNA content in peripheral blood in some patients,[22,123] and the accumulating negative effects of certain NRTIs. Future studies should relate different mitochondrial assays to the quantification assay of mtDNA content. Also, more in-depth studies should elucidate the possible role of mtDNA loss in specific lymphocyte subsets in relation to drug-associated toxicity and possibly also disease progression in the ART-naive phase of HIV infection.

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References

1. Palella FJ Jr, Delaney KM, Moorman AC *et al.* Declining morbidity and mortality among patients with advanced human

immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998; **338**: 853–860.

2. Carr A, Samaras K, Burton S *et al*. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS* 1998; **12**: F51–8.

3. Carr A, Miller J, Law M *et al*. A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome. *AIDS* 2000; **14**: F25–32.

4. Friis-Møller N, Sabin CA, Weber R *et al*. Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med* 2003; **349**: 1993–2003.

5. Friis-Møller N, Reiss P, Sabin CA *et al*. Class of antiretroviral drugs and the risk of myocardial infarction. *N Engl J Med* 2007; **356**: 1723–35.

6. Lewis W, Dalakas MC. Mitochondrial toxicity of antiviral drugs. *Nat Med* 1995; **1**: 417–22.

7. Arnaudo E, Dalakas M, Shanske S *et al*. Depletion of muscle mitochondrial DNA in AIDS patients with zidovudine-induced myopathy. *Lancet* 1991; **337**: 508–10.

8. Dalakas MC, Illa I, Pezeshkpour GH *et al*. Mitochondrial myopathy caused by long-term zidovudine therapy. *N Engl J Med* 1990; **322**: 1098–105.

9. Bissuel F, Bruneel F, Habersetzer F *et al*. Fulminant hepatitis with severe lactate acidosis in HIV-infected patients on didanosine therapy. *J Intern Med* 1994; **235**: 367–71.

10. Dalakas MC, Semino-Mora C, Leon-Monzon M. Mitochondrial alterations with mitochondrial DNA depletion in the nerves of AIDS patients with peripheral neuropathy induced by 2′3′-dideoxycytidine (ddC). *Lab Invest* 2001; **81**: 1537–44.

11. Fleischman A, Johnsen S, Systrom DM *et al*. Effects of a nucleoside reverse transcriptase inhibitor, stavudine, on glucose disposal and mitochondrial function in muscle of healthy adults. *Am J Physiol Endocrinol Metab* 2007; **292**: E1666–73.

12. Lo JC, Kazemi MR, Hsue PY *et al*. The relationship between nucleoside analogue treatment duration, insulin resistance, and fasting arterialized lactate level in patients with HIV infection. *Clin Infect Dis* 2005; **41**: 1335–40.

13. Olano JP, Borucki MJ, Wen JW *et al*. Massive hepatic steatosis and lactic acidosis in a patient with AIDS who was receiving zidovudine. *Clin Infect Dis* 1995; **21**: 973–6.

14. Brinkman K, Smeitink JA, Romijn JA *et al*. Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. *Lancet* 1999; **354**: 1112–5.

15. Kakuda TN, Brundage RC, Anderson PL *et al*. Nucleoside reverse transcriptase inhibitor-induced mitochondrial toxicity as an etiology for lipodystrophy. *AIDS* 1999; **13**: 2311.

16. Mallal SA, John M, Moore CB *et al*. Contribution of nucleoside analogue reverse transcriptase inhibitors to subcutaneous fat wasting in patients with HIV infection. *AIDS* 2000; **14**: 1309–16.

17. Shikuma CM, Hu N, Milne C *et al*. Mitochondrial DNA decrease in subcutaneous adipose tissue of HIV-infected individuals with peripheral lipoatrophy. *AIDS* 2001; **15**: 1801–9.

18. Casula M, Bosboom-Dobbelaer I, Smolder K *et al*. Infection with HIV-1 induces a decrease in mtDNA. *J Infect Dis* 2005; **191**: 1468–71.

19. Chiappini F, Teicher E, Saffroy R *et al*. Prospective evaluation of blood concentration of mitochondrial DNA as a marker of toxicity in 157 consecutively recruited untreated or HAART-treated HIV-positive patients. *Lab Invest* 2004; **84**: 908–14.

20. Maagaard A, Holberg-Petersen M, Kvittingen EA *et al*. Depletion of mitochondrial DNA copies/cell in peripheral blood mononuclear cells (PBMC) in HIV-1 infected treatment-naive patients. *HIV Medicine* 2006; **7**: 53–8.

21. Miró Ò, López S, Martinez A *et al*. Mitochondrial effects of HIV infection on the peripheral blood mononuclear cells of HIV-infected patients who were never treated with antiretrovirals. *Clin Infect Dis* 2004; **39**: 710–6.

22. Miura T, Goto M, Hosoya N *et al*. Depletion of mitochondrial DNA in HIV-1-infected patients and its amelioration by antiretroviral therapy. *J Med Virol* 2003; **70**: 497–505.

23. Altmann R. Die Elementarorganismen und ihre Beziehungen zu den Zellen. Leipzig: Veit, 1890.

24. Graziewicz MA, Longley MJ, Copeland WC. DNA polymerase gamma in mitochondrial DNA replication and repair. *Chem Rev* 2006; **106**: 383–405.

25. Anderson S, Bankier AT, Barrell BG *et al*. Sequence and organization of the human mitochondrial genome. *Nature* 1981; **290**: 457–65.

26. Shuster RC, Rubenstein AJ, Wallace DC. Mitochondrial DNA in anucleate human blood cells. *Biochem Biophys Res Commun* 1988; **155**: 1360–5.

27. Wiesner RJ, Ruegg JC, Morano I. Counting target molecules by exponential polymerase chain reaction: copy number of mitochondrial DNA in rat tissues. *Biochem Biophys Res Commun* 1992; **183**: 553–9.

28. Smeitink J, van den Heuvel L, DiMauro S. The genetics and pathology of oxidative phosphorylation. *Nat Rev Genet* 2001; **2**: 342–52.

29. Hoppins S, Lackner L, Nunnari J. The machines that divide and fuse mitochondria. *Annu Rev Biochem* 2007; **76**: 751–80.

30. Detmer SA, Chan DC. Functions and dysfunctions of mitochondrial dynamics. *Nat Rev Mol Cell Biol* 2007; **8**: 870–9.

31. Wallace DC. Mitochondrial genetics: a paradigm for aging and degenerative diseases? *Science* 1992; **256**: 628–32.

32. Falkenberg M, Larsson NG, Gustafsson CM. DNA replication and transcription in mammalian mitochondria. *Annu Rev Biochem* 2007; **76**: 679–99.

33. Kakuda TN. Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. *Clin Ther* 2000; **22**: 685–708.

34. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 2007; **87**: 99–163.

35. Shapiro TA, Englund PT. The structure and replication of kinetoplast DNA. *Annu Rev Microbiol* 1995; **49**: 117–43.

36. Brinkman K, ter Hofstede HJM, Burger DM *et al*. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. *AIDS* 1998; **12**: 1735–44.

37. Miró Ò, López SP, Rodriguez de la Concepción MP *et al*. Upregulatory mechanisms compensate for mitochondrial DNA depletion in asymptomatic individuals receiving stavudine plus didanosine. *J Acq Immune Defic Syndr* 2004; **37**: 1550–5.

38. Lewis W, Day BJ, Copeland WC. Mitochondrial toxicity of NRTI antiviral drugs: an integrated cellular perspective. *Nat Rev Drug Disc* 2003; **2**: 812–22.

39. Birkus G, Hitchcock MJ, Cihlar T. Assessment of mitochondrial toxicity in human cells treated with tenofovir: comparison with other nucleoside reverse transcriptase inhibitors. *Antimicrob Agents Chemother* 2002; **46**: 716–23.

40. Johnson AA, Ray AS, Hanes J *et al*. Toxicity of antiviral nucleoside analogs and the human mitochondrial DNA polymerase. *J Biol Chem* 2001; **276**: 40847–57.

41. Martin JL, Brown CE, Matthews-Davis N *et al*. Effects of antiviral nucleoside analogs on human DNA polymerases and mitochondrial DNA synthesis. *Antimicrob Agents Chemother* 1994; **38**: 2743–9.

42. Lim SE, Copeland WC. Differential incorporation and removal of antiviral deoxynucleotides by human DNA polymerase gamma. *J Biol Chem* 2001; **276**: 23616–23.

43. Pan-Zhou XR, Cui L, Zhou XJ *et al*. Differential effects of antiretroviral nucleoside analogs on mitochondrial function in HepG2 cells. *Antimicrob Agents Chemother* 2000; **44**: 496–503.

44. Barile M, Valenti D, Passarella S *et al*. 3′-Azido-3′-deoxythmidine uptake into isolated rat liver mitochondria and impairment of ADP/ATP translocator. *Biochem Pharmacol* 1997; **53**: 913–20.

45. Barile M, Valenti D, Quagliariello E *et al*. Mitochondria as cell targets of AZT (zidovudine). *Gen Pharmacol* 1998; **31**: 531–8.

46. Lund KC, Wallace KB. Direct, DNA pol-gamma-independent effects of nucleoside reverse transcriptase inhibitors on mitochondrial bioenergetics. *Cardiovasc Toxicol* 2004; **4**: 217–28.

47. Wallace DC. Mitochondrial diseases in man and mouse. *Science* 1999; **283**: 1482–8.

48. Yamaguchi T, Katoh I, Kurata S. Azidothymidine causes functional and structural destruction of mitochondria, glutathione deficiency and HIV-1 promoter sensitization. *Eur J Biochem.* 2002; **269**: 2782–8.

49. Lynx MD, McKee EE. 3′-Azido-3′-deoxythymidine (AZT) is a competitive inhibitor of thymidine phosphorylation in isolated rat heart and liver mitochondria. *Biochem Pharmacol* 2006; **72**: 239–43.

50. Côté HC. Possible ways nucleoside analogues can affect mitochondrial DNA content and gene expression during HIV therapy. *Antivir Ther* 2005; **10** Suppl 2: M3–11.

51. Buffet M, Schwazinger MAB, Gourlain K *et al*. Mitochondrial DNA depletion in adipose tissue of HIV-infected patients with peripheral lipoatrophy. *J Clin Virol* 2005; **33**: 60–4.

52. Cherry CL, Gahan ME, McArthur JC *et al*. Exposure to dideoxynucleosides is reflected in lowered mitochondrial DNA in subcutaneous fat. *J Acquir Immune Defic Syndr* 2002; **30**: 271–7.

53. Cui L, Locatelli L, Xie MY *et al*. Effect of nucleoside analogs on neurite regeneration and mitochondrial DNA synthesis in PC-12 cells. *J Pharmacol Exp Ther* 1997; **280**: 1228–34.

54. Haugaard SB, Andersen O, Pedersen SB *et al*. Depleted skeletal muscle mitochondrial DNA, hyperlactatemia, and decreased oxidative capacity in HIV-infected patients on highly active antiretroviral therapy. *J Med Virol* 2005; **77**: 29–38.

55. Hulgan T, Haas D, Haines JL *et al*. Mitochondrial haplogroups and peripheral neuropathy during antiretroviral therapy: an adult AIDS clinical trials group study. *AIDS* 2005; **19**: 1341–9.

56. Jones SP, Qazi N, Morelese J *et al*. Assessment of adipokine expression and mitochondrial toxicity in HIV patients with lipoatrophy on stavudine- and zidovudine-containing regimens. *J Acquir Immune Defic Syndr* 2005; **40**: 565–72.

57. Nolan D, Hammond E, Martin A *et al*. Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. *AIDS* 2003; **17**: 1329–38.

58. Pace CS, Martin AM, Hammond EL *et al*. Mitochondrial proliferation, DNA depletion and adipocyte differentiation in subcutaneous adipose tissue of HIV-positive HAART recipients. *Antivir Ther* 2003; **8**: 323–31.

59. Martin A, Smith DE, Carr A *et al*. Reversibility of lipoatrophy in HIV-infected patients 2 years after switching from a thymidine analogue to abacavir: the MITOX Extension Study. *AIDS* 2004; **18**: 1029–36.

60. Moyle GJ, Sabin CA, Cartledge J *et al*. A randomized comparative trial of tenofovir DF or abacavir as replacement for a thymidine analogue in persons with lipoatrophy. *AIDS* 2006; **20**: 2043–50.

61. Côté HC, Yip B, Asselin JJ *et al*. Mitochondrial:nuclear DNA ratios in peripheral blood cells from human immunodeficiency virus (HIV)-infected patients who received selected HIV antiretroviral drug regimens. *J Infect Dis* 2003; **187**: 1972–6.

62. Maagaard A, Holberg-Petersen M, Løvgården G *et al*. Distinct mechanisms for mitochondrial DNA loss in T and B lymphocytes from HIV-infected patients exposed to nucleoside reverse-transcriptase inhibitors and those naive to antiretroviral treatment. *J Infect Dis* 2008; **198**: 1474–81.

63. Reiss P, Casula M, de Ronde A *et al*. Greater and more rapid depletion of mitochondrial DNA in blood of patients treated with dual (zidovudine + didanosine or zidovudine + zalcitabine) vs. single (zidovudine) nucleoside reverse transcriptase inhibitors. *HIV Med* 2004; **5**: 11–4.

64. Nies-Kraske E, Schacker TW, Condoluci D *et al*. Evaluation of the pathogenesis of decreasing CD4(+) T cell counts in human immunodeficiency virus type 1-infected patients receiving successfully suppressive antiretroviral therapy. *J Infect Dis* 2009; **199**: 1648–56.

65. Turriziani O, Butera O, Gianotti N *et al*. Thymidine kinase and deoxycytidine kinase activity in mononuclear cells from antiretroviral-naive HIV-infected patients. *AIDS* 2005; **19**: 473–9.

66. Anderson PL, Zheng JH, King T *et al*. Concentrations of zidovudine- and lamivudine-triphosphate according to cell type in HIV-seronegative adults. *AIDS* 2007; **21**: 1849–54.

67. Anderson PL, Kakuda TN, Kawle S *et al*. Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in HIV-infected individuals. *AIDS* 2003; **17**: 2159–68.

68. Anderson PL, Kakuda TN, Lichtenstein KA. The cellular pharmacology of nucleoside- and nucleotide-analogue reverse-transcriptase inhibitors and its relationship to clinical toxicities. *Clin Infect Dis* 2004; **38**: 743–53.

69. Moyle GJ, Datta D, Mandalia S *et al*. Hyperlactataemia and lactic acidosis during antiretroviral therapy: relevance, reproducibility and possible risk factors. *AIDS* 2002; **16**: 1341–9.

70. Moyle G. Clinical manifestations and management of antiretroviral nucleoside analog-related mitochondrial toxicity. *Clin Ther* 2000; **22**: 911–36.

71. Gerschenson M, Shiramizu B, LiButti DA *et al*. Mitochondrial DNA levels of peripheral blood mononuclear cells and subcutaneous blood mononuclear cells and subcutaneous tissue from thigh, fat and abdomen of HIV-1 seropositive and negative individuals. *Antivir Ther* 2005; **10**: M83–9.

72. Cherry CL, Nolan D, James IR *et al*. Tissue-specific associations between mitochondrial DNA levels and current treatment status in HIV-infected individuals. *J Acquir Immune Defic Syndr* 2006; **42**: 435–40.

73. Shikuma CM, Gerschenson M, Chow D *et al*. Mitochondrial oxidative phosphorylation protein levels in peripheral blood mononuclear cells correlate with levels in subcutaneous adipose tissue within samples differing by HIV and lipoatrophy status. *AIDS Res Hum Retrovir* 2008; **24**: 1255–62.

74. Martin AM, Hammond E, Nolan D *et al*. Accumulation of mitochondrial DNA mutations in human immunodeficiency virus-infected patients treated with nucleoside-analogue reverse-transcriptase inhibitors. *Am J Hum Gen* 2003; **72**: 549–60.

75. McComsey G, Tan DJ, Lederman M *et al*. Analysis of the mitochondrial DNA genome in the peripheral blood leukocytes of HIV-infected patients with or without lipoatrophy. *AIDS* 2002; **16**: 513–8.

76. Maagaard A, Holberg-Petersen M, Kollberg G *et al*. Mitochondrial (mt)DNA changes in tissue may not be reflected by depletion of mtDNA in peripheral blood mononuclear cells in HIV-infected patients. *Antivir Ther* 2006; **11**: 601–8.

77. Walker UA, Bickel M, Lutke Volksbeck SI *et al*. Evidence of nucleoside analogue reverse transcriptase inhibitor—associated genetic and structural defects of mitochondria in adipose tissue of HIV-infected patients. *J Acquir Immune Defic Syndr* 2002; **29**: 117–21.

78. Giralt M, Domingo P, Guallar JP *et al*. HIV-1 infection alters gene expression in adipose tissue, which contributes to HIV-1/HAART-associated lipodystrophy. *Antivir Ther* 2006; **11**: 729–40.

79. Galluzzi L, Pinti M, Guaraldi G *et al*. Altered mitochondrial RNA production in adipocytes from HIV-infected individuals with lipodystrophy. *Antivir Ther* 2005; **10** Suppl 2: M91–9.

80. McComsey GA, Libutti DE, O'Riordan M *et al*. Mitochondrial RNA and DNA alterations in HIV lipoatrophy are linked to antiretroviral therapy and not to HIV infection. *Antivir Ther* 2008; **13**: 715–22.

81. Mallon PW, Unemori P, Sedwell R *et al*. In vivo, nucleoside reverse-transcriptase inhibitors alter expression of both mitochondrial and lipid metabolism genes in the absence of depletion of mitochondrial DNA. *J Infect Dis* 2005; **191**: 1686–96.

82. Bonnet F, Bonarek M, Morlat P *et al*. Risk factors for lactic acidosis in HIV-infected patients treated with nucleoside reverse-transcriptase inhibitors: a case-control study. *Clin Infect Dis* 2003; **36**: 1324–8.

83. Currier JS. Sex differences in antiretroviral therapy toxicity: lactic acidosis, stavudine, and women. *Clin Infect Dis* 2007; **45**: 261–2.

84. Fleischer R, Boxwell D, Sherman KE. Nucleoside analogues and mitochondrial toxicity. *Clin Infect Dis* 2004; **38**: e79–80.

85. Galli M, Veglia F, Angarano G *et al*. Gender differences in antiretroviral drug-related adipose tissue alterations. Women are at higher risk than men and develop particular lipodystrophy patterns. *J Acquir Immune Defic Syndr* 2003; **34**: 58–61.

86. Gervasoni C, Ridolfo AL, Trifiro G *et al*. Redistribution of body fat in HIV-infected women undergoing combined antiretroviral therapy. *AIDS* 1999; **13**: 465–71.

87. Lichtenstein KA, Delaney KM, Armon C *et al*. Incidence of and risk factors for lipoatrophy (abnormal fat loss) in ambulatory HIV-1-infected patients. *J Acquir Immune Defic Syndr* 2003; **32**: 48–56.

88. Bolhaar MG, Karstaedt AS. A high incidence of lactic acidosis and symptomatic hyperlactatemia in women receiving highly active antiretroviral therapy in Soweto, South Africa. *Clin Infect Dis* 2007; **45**: 254–60.

89. John M, Mallal S. Hyperlactatemia syndromes in people with HIV infection. *Curr Opin Infect Dis* 2002; **15**: 23–9.

90. Yamanaka H, Gatanaga H, Kosalaraksa P *et al*. Novel mutation of human DNA polymerase gamma associated with mitochondrial toxicity induced by anti-HIV treatment. *J Infect Dis* 2007; **195**: 1419–25.

91. Hulgan T, Tebas P, Canter JA *et al*. Hemochromatosis gene polymorphisms, mitochondrial haplogroups, and peripheral lipoatrophy during antiretroviral therapy. *J Infect Dis* 2008; **197**: 858–66.

92. Chan DC. Mitochondria: dynamic organelles in disease, aging, and development. *Cell* 2006; **125**: 1241–52.

93. Bani-Sadr F, Carrat F, Pol S *et al*. Risk factors for symptomatic mitochondrial toxicity in HIV/hepatitis C virus-coinfected patients during interferon plus ribavirin-based therapy. *J Acquir Immune Defic Syndr* 2005; **40**: 47–52.

94. Butt AA. Fatal lactic acidosis and pancreatitis associated with ribavirin and didanosine therapy. *AIDS Read* 2003; **13**: 344–8.

95. Moreno A, Quereda C, Moreno L *et al*. High rate of didanosine-related mitochondrial toxicity in HIV/HCV-coinfected patients receiving ribavirin. *Antivir Ther* 2004; **9**: 133–8.

96. Sternfel T, Schmid M, Tischleder A *et al*. The influence of HIV infection and antiretroviral therapy on the mitochondrial membrane potential of peripheral mononuclear cells. *Antivir Ther* 2007; **12**: 769–78.

97. Casula M, Vrisekoop N, Wit FW *et al*. Mitochondrial DNA decline in T cells of HIV-1 seroconverters may be dependent on immune activation. *J Infect Dis* 2007; **196**: 371–6.

98. Douek DC, Picker LJ, Koup RA. T cell dynamics in HIV-1 infection. *Annu Rev Immunol* 2003; **21**: 265–304.

99. Sachsenberg N, Perelson AS, Yerly S *et al*. Turnover of CD4+ and CD8+ T lymphocytes in HIV-1 infection as measured by Ki-67 antigen. *J Exp Med* 1998; **187**: 1295–303.

100. Rieckmann P, Poli G, Kehrl JH *et al*. Activated B lymphocytes from human immunodeficiency virus-infected individuals induce virus expression in infected T cells and a promonocytic cell line, U1. *J Exp Med* 1991; **173**: 1–5.

101. Sodora DL, Silvestri G. Immune activation and AIDS pathogenesis. *AIDS* 2008; **22**: 439–46.

102. Brenchley JM, Price DA, Schacker TW *et al*. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006; **12**: 1365–71.

103. Holm M, Pettersen FO, Kvale D. PD-1 predicts CD4 loss rate on chronic HIV-1 infection better than HIV RNA and CD38 but not in cryopreserved samples. *Curr HIV Res* 2008; **6**: 49–58.

104. Shedlock DJ, Hwang D, Choo AY *et al*. HIV-1 viral genes and mitochondrial apoptosis. *Apoptosis* 2008; **13**: 1088–99.

105. Raidel SM, Haase C, Jansen NR *et al*. Targeted myocardial transgenic expression of HIV Tat causes cardiomyopathy and mitochondrial damage. *Am J Physiol Heart Circ Physiol* 2002; **282**: H1672–8.

106. Jacotot E, Ravagnan L, Loeffler M *et al*. The HIV-1 viral protein R induces apoptosis via a direct effect on the mitochondrial permeability transition pore. *J Exp Med* 2000; **191**: 33–46.

107. Deniaud A, Brenner C, Kroemer G. Mitochondrial membrane permeabilization by HIV-1 Vpr. *Mitochondrion* 2004; **4**: 223–33.

108. Riedl SJ, Salvesen GS. The apoptosome: signalling platform of cell death. *Nat Rev Mol Cell Biol* 2007; **8**: 405–13.

109. Buenz EJ, Badley AD. Impact of mitochondrial regulation of apoptosis on the pathogenesis and treatment of HIV-1-induced immunodeficiency. *Mitochondrion* 2004; **4**: 235–54.

110. Cossarizza A. Apoptosis and HIV infection: about molecules and genes. *Curr Pharm Des* 2008; **14**: 237–44.

111. Peraire J, Miró Ò, Saumoy M *et al*. HIV-1-infected long-term non-progressors have milder mitochondrial impairment and lower mitochondrially-driven apoptosis in peripheral blood mononuclear cells than typical progressors. *Curr HIV Res* 2007; **5**: 467–73.

112. Giorgi JV, Lyles RH, Matud JL *et al*. Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. *J Acquir Immune Defic Syndr* 2002; **29**: 346–55.

113. Barber DL, Wherry EJ, Masopust D *et al*. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2006; **439**: 682–7.

114. Ho J, Moir S, Malaspina A *et al*. Two overrepresented B cell populations in HIV-infected individuals undergo apoptosis by different mechanisms. *Proc Natl Acad Sci USA* 2006; **103**: 19436–41.

115. Moir S, Malaspina A, Ogwaro KM *et al*. HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals. *Proc Natl Acad Sci USA* 2001; **98**: 10362–7.

116. Karamchand L, Dawood H, Chuturgoon AA. Lymphocyte mitochondrial depolarization and apoptosis in HIV-1-infected HAART patients. *J Acquir Immune Defic Syndr* 2008; **48**: 381–8.

117. Pilon AA, Lum JJ, Sanchez-Dardon J *et al*. Induction of apoptosis by a nonnucleoside human immunodeficiency virus type 1 reverse transcriptase inhibitor. *Antimicrob Agents Chemother* 2002; **46**: 2687–91.

118. Vlahakis SR, Bennett SA, Whitehead SN *et al*. HIV protease inhibitors modulate apoptosis signaling in vitro and in vivo. *Apoptosis* 2007; **12**: 969–77.

119. Haubrich RH, Riddler SA, DiRienzo AG *et al*. Metabolic outcomes in a randomized trial of nucleoside, nonnucleoside and protease inhibitor-sparing regimens for initial HIV treatment. *AIDS* 2009; **23**: 1109–18.

120. Podzamczer D, Ferrer E, Martinez E *et al*. How much fat loss is needed for lipoatrophy to become clinically evident? *AIDS Res Hum Retrovir* 2009; **25**: 563–7.

121. Lewis W, Kohler JJ, Hosseini SH *et al*. Antiretroviral nucleosides, deoxynucleotide carrier and mitochondrial DNA: evidence supporting the DNA pol gamma hypothesis. *AIDS* 2006; **20**: 675–84.

122. Kim MJ, Jardel C, Barthelemy C *et al*. Mitochondrial DNA content: an inaccurate biomarker of mitochondrial alteration in HIV-related lipodystrophy. *Antimicrob Agents Chemother* 2008; **52**: 1670–6.

123. Casula M, Weverling GJ, Wit FW *et al*. Mitochondrial DNA and RNA increase in peripheral blood mononuclear cells from HIV-1-infected patients randomized to receive stavudine-containing or stavudine-sparing combination therapy. *J Infect Dis* 2005; **192**: 1794–800.

124. Boubaker K, Flepp M, Sudre P *et al*. Hyperlactatemia and antiretroviral therapy: the Swiss HIV Cohort Study. *Clin Infect Dis* 2001; **33**: 1931–7.

125. Mallon P Sr, Duarte N, Kelleher A *et al*. Changes in adipose tissue mitochondrial DNA quality and quantity—relation to HIV disease, lipoatrophy and exposure to stavudine. *XVI International AIDS Conference*, *Toronto*, *2006*. Abstract THAB0106.

126. McComsey GA, Lo RV III, O'Riordan M *et al*. Effect of reducing the dose of stavudine on body composition, bone density, and markers of mitochondrial toxicity in HIV-infected subjects: a randomized, controlled study. *Clin Infect Dis* 2008; **46**: 1290–6.

127. Sánchez-Conde M, Mendoza C, Jiménez-Nacher I *et al*. Reductions in stavudine dose might ameliorate mitochondrial-associated complications without compromising antiviral activity. *HIV Clin Trials* 2005; **6**: 197–202.

128. Moore RD, Wong WM, Keruly JC *et al*. Incidence of neuropathy in HIV-infected patients on monotherapy versus those on combination therapy with didanosine, stavudine and hydroxyurea. *AIDS* 2000; **14**: 273–8.

129. Côté HC, Magil AB, Harris M *et al*. Exploring mitochondrial nephrotoxicity as a potential mechanism of kidney dysfunction among HIV-infected patients on highly active antiretroviral therapy. *Antivir Ther* 2006; **11**: 79–86.

130. McComsey GA, Paulsen DM, Lonergan JT *et al*. Improvements in lipoatrophy, mitochondrial DNA levels and fat apoptosis after replacing stavudine with abacavir or zidovudine. *AIDS* 2005; **19**: 15–23.

131. Saumoy M, Vidal F, Peraire J *et al*. Proximal tubular kidney damage and tenofovir: a role for mitochondrial toxicity? *AIDS* 2004; **18**: 1741–2.