AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

Check for updates

Upregulation of Apoptosis Pathway Genes in Peripheral Blood Mononuclear Cells of HIV-Infected Individuals with Antiretroviral Therapy-Associated Mitochondrial Toxicity

Yram Foli,[a] Musie Ghebremichael,[b] Min Li,[a] Elijah Paintsil[a,b,c,d]

Department of Pediatrics, Yale School of Medicine, New Haven, Connecticut, USA[a]; Ragon Institute and Harvard Medical School, Cambridge, Massachusetts, USA[b]; Department of Pharmacology, Yale School of Medicine, New Haven, Connecticut, USA[c]; Department of Epidemiology & Public Health, Yale School of Medicine, New Haven, Connecticut, USA[d]

ABSTRACT A case-control study of the effect of antiretroviral therapy (ART) on apoptosis pathway genes comprising 16 cases (HIV infected with mitochondrial toxicity) and 16 controls (HIV uninfected) was conducted. A total of 26 of 84 genes of the apoptosis pathway were differentially expressed. Two of the upregulated genes, DFFA and TNFRSF1A, classified 75% of study participants correctly as either a case or control. Thus, apoptosis may be in the causal pathway of ART-associated mitochondrial toxicity. These two genes could be markers for detecting and monitoring ART-induced mitochondrial toxicity.

KEYWORDS antiretroviral therapy, mitochondrial toxicity, apoptosis, biomarker

Antiretroviral therapy (ART) has markedly improved outcomes of HIV infection (1–4). However, the therapeutic benefit of ART is sometimes limited by long-term toxicities (5, 6). Although newer and next-generation ART may have less toxicity, ART-induced toxicity remains a substantial burden globally (7, 8). There are reports that contemporary ART, such as some integrase-based regimens, may be associated with neuropsychiatric toxicity (9, 10). The underlying mechanisms of ART-induced toxicity are still debatable. Nucleoside reverse transcriptase inhibitors (NRTIs) have been associated with toxicities such as skeletal muscle myopathy, lactic acidosis, lipodystrophy, peripheral neuropathies, cardiomyopathies, and pancytopenia (11–14). These toxicities have been implicated in the inhibition of mitochondrial DNA (mtDNA) polymerase gamma (Pol-γ) by NRTIs (15). A recent review of emerging studies concluded that mitochondrial dysfunction cannot be explained solely by Pol-γ inhibition (16). Furthermore, protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) do not inhibit Pol-γ, and yet they also cause toxicities similar to those associated with mitochondrial dysfunction. Moreover, HIV-infected patients are at increased risk for chronic conditions, such as cardiovascular disease, renal disease, bone disease, and diabetes mellitus, compared to non-HIV patients (17). These toxicities have been associated with ART (18–20).

We recently found elevated plasma concentrations of cytochrome *c* (CYCS) protein in HIV treatment-experienced patients with toxicity compared with HIV treatment-experienced patients without toxicity (21). CYCS is a mitochondrial protein and pro-apoptotic signal that, when released from the mitochondria, activates effector caspases 3 and 7, resulting in apoptosis (programmed cell death) (22). Other investigators have found that several components of ART can cause apoptosis, which is a function of the mitochondrion (23, 24). Thus, biomarkers of apoptosis could be used to detect and

Received 10 March 2017 **Returned for modification** 5 April 2017 **Accepted** 30 May 2017

Accepted manuscript posted online 5 June 2017

Citation Foli Y, Ghebremichael M, Li M, Paintsil E. 2017. Upregulation of apoptosis pathway genes in peripheral blood mononuclear cells of HIV-infected individuals with antiretroviral therapy-associated mitochondrial toxicity. Antimicrob Agents Chemother 61:e00522-17. https://doi.org/10.1128/AAC.00522-17.

Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to Elijah Paintsil, elijah.paintsil@yale.edu.

Y.F. and M.G. contributed equally to this work.

Downloaded from http://aac.asm.org/ on October 15, 2019 by guest

TABLE 1 Demographic and clinical characteristics of study participants

Variable	HIV-uninfected participants[a]	HIV-infected participants with toxicity[a]
Gender (*n*)		
Female	5	5
Male	11	11
Age (range) (yrs)	33–66	33–66
Race (%)		
White non-Hispanic	25	25
White Hispanic	6	6
African American	69	69
Mean CD4 count (range) (count/μl)	NA[b]	523 (86–1336)
Mean viral load (range) (copies/ml)	NA	2,087 (20–29297)
Mean duration of exposure to nucleoside analogs (range) (yrs)	NA	8.18 (1–17)
Toxicities (%)		
Single	NA	63
Multiple	NA	38
Basis of treatment regimen (%)		
NNRTI	NA	25
PI	NA	44
Integrase	NA	6
PI/NNRTI	NA	13
PI/integrase	NA	6
Integrase/NNRTI	NA	6

[a]Sample size (*n*) = 16.
[b]NA, not applicable.

monitor ART-induced toxicity (25, 26). In the current study, we sought to investigate the differential expression profile of genes of the apoptosis pathway in HIV-infected patients with ART-associated mitochondrial toxicity (cases) versus HIV-uninfected individuals (controls).

We included 32 participants in this case-control study of the effect of ART on apoptosis pathway genes. A case comprised an HIV-infected individual ($n = 16$) diagnosed with ART-associated mitochondrial toxicity by his or her provider based on clinical and/or laboratory evidence of one or more of the following ART toxicities: hyperlipidemia, anemia, elevated liver function tests, thrombocytopenia, lactic acidosis, elevated blood urea nitrogen or creatinine, peripheral neuropathy, lipodystrophy, and/or pancytopenia (27). Cases were matched to HIV-uninfected controls ($n = 16$) by age, sex, and race/ethnicity. The study protocol was approved by the institutional review board of the Yale School of Medicine. All participants gave their written informed consent before participation in the study. The demographic and clinical characteristics of cases are listed in Table 1. Out of the 16 cases, only 3 had detectable viremia: C02 (29,297 copies/ml), C019 (1,508 copies/ml), and C023 (215 copies/ml). To investigate the impact of ART on apoptosis pathway-specific genes, quantitative PCRs were done using the Human Apoptosis RT^2 Profiler PCR Array kit (SuperArray Biosciences) as previously published (28). We identified differentially expressed genes on the basis of false discovery rate (FDR) adjusted *P* value using empirical Bayes moderated tests. The FDR was controlled using Benjamini and Hochberg algorithm. We identified 26 out of the 84 genes that were differentially expressed between the cases and controls (Fig. 1A). Of note, the gene profiles of one case (C007) and 2 controls (002HC and 012HC) segregated with controls and cases, respectively. There was no significant association between gene expression and disease characteristics (e.g., viral and CD4$^+$ T-cell count) among cases. Of the 26 genes, 18 were proapoptotic (TNFRSF1A, CYCS, DFFA, ABL1, LTBR, CASP7, FASLG, BAD, TRAF2, BAK1, CIDEA, TNFRSF11B, CASP14, BIK,

Downloaded from http://aac.asm.org/ on October 15, 2019 by guest

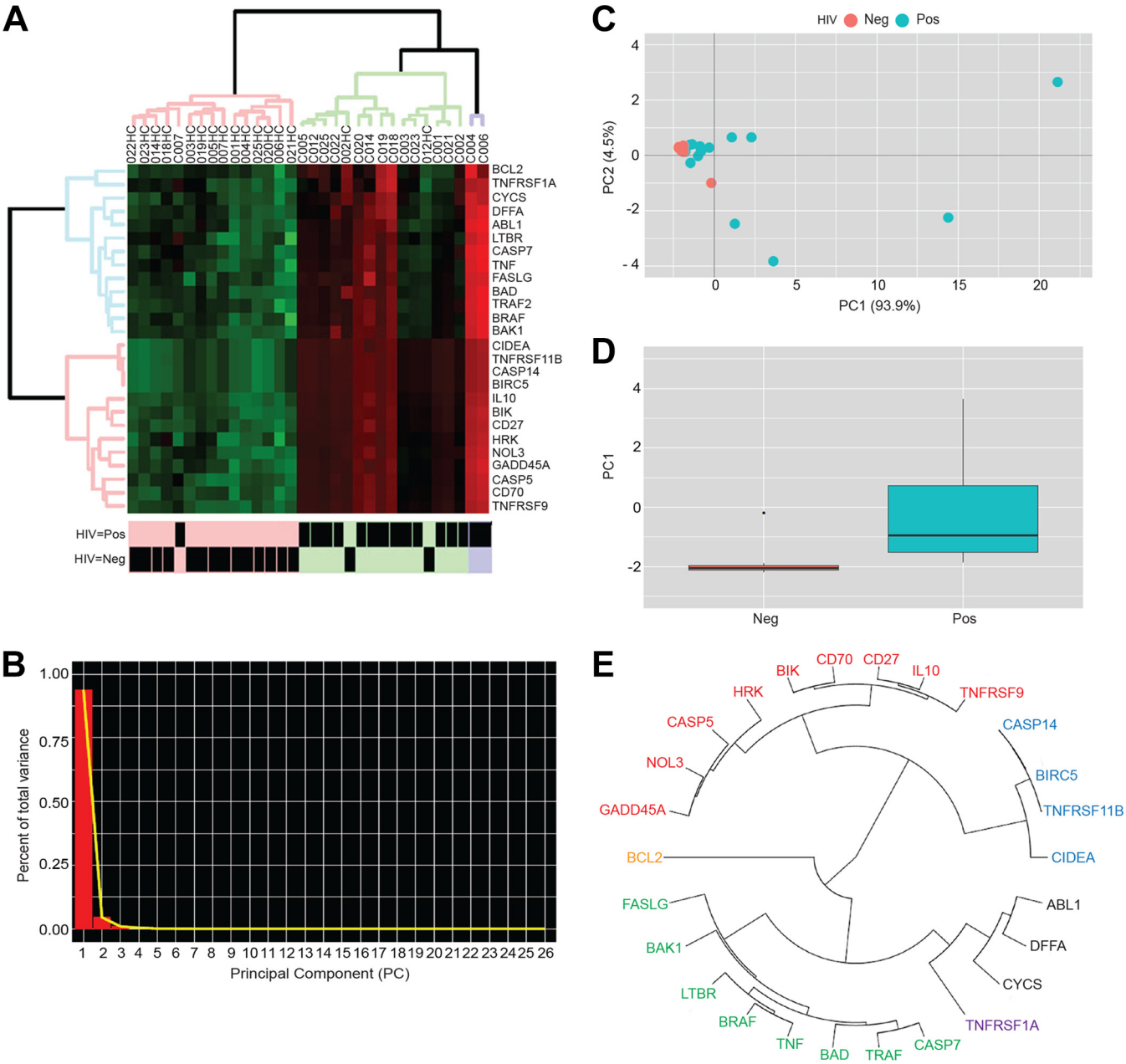

FIG 1 Differentially expressed apoptosis pathway genes. (A) Heat map of significantly differentially expressed genes between HIV-infected patients with mitochondrial toxicity (HIV = Pos) and HIV-uninfected patients (HIV = Neg). Columns represent the subjects (identification numbers with "HC" at the end represent HIV-uninfected patients, and identification numbers with "C" in front represent HIV-infected patients), and rows represent genes. Gene expression levels are color-coded; red, black, and green represent high, medium, and low expression levels, respectively. (B) Screen plot depicting the proportion of the total variance accounted for by each of the principal components. (C) Score plot of the first principle component (PC1) versus the second principle component (PC2). (D) Box plots comparing scores of the first principle component (PC1) between the two groups. (E) Clusters formed by performing unsupervised analyses on the transcriptional profile data of the 26 genes using hierarchical clustering analysis.

GADD45A, CASP5, CD70, and TNFRSF9), 5 were antiapoptotic (BCL2, BRAF, BIRC5, IL-10, and NOL3), and 3 had overlapping functions (CD27, HRK, and TNF).

Principal-component analysis (PCA) was used to group the 26 differentially expressed correlated genes to uncorrelated summary variables called principal components (PC) (Fig. 1B). The PCA revealed that cases and controls formed two distinct groups, which were primarily separated on the first principal component (PC1) (Fig. 1C). PC1 accounted for 94% of the total variance of the 26 genes, with high positive correlation with all 26 genes (Fig. 1C). As illustrated in Fig. 1D, the overall gene expression score (PC1) was significantly higher in cases than in controls ($P < 0.05$).

Downloaded from http://aac.asm.org/ on October 15, 2019 by guest

Furthermore, unsupervised hierarchical clustering analysis divided the 26 differentially expressed genes into four major groups (Fig. 1E). To further identify the key genes which contributed to the differences in profile, penalized regression was used to select the best subset of genes from the 26 genes which were differentially expressed between the two groups. This analysis selected two genes: DFFA and TNFRSF1A. DFFA is a proapoptotic gene in the executioner pathway, and TNFRSF1A is a proapoptotic gene in the extrinsic pathway. To assess the discriminatory power of DFFA and TNFRSF1A, we then developed a classifier model to classify study participants into groups based on these two selected genes. The classifier model correctly classified 75% of the participants into their respective groups.

In this exploratory study, we found 26 out of the 84 genes that were differentially expressed between the two groups. To the best of our knowledge, this is the first report of the association of these 26 genes with ART-associated mitochondrial toxicity. Although these genes belong to the three main apoptosis pathways (i.e., intrinsic, extrinsic and executioner), we observed a preponderance of proapoptotic genes ($n = 18$) to antiapoptotic genes ($n = 5$). The relative ratio of expression of proapoptotic to antiapoptotic genes determines the fate of a cell (29). Our findings suggest that apoptosis may be part of the causal pathway of ART-associated mitochondrial toxicity.

In many cell types, the extrinsic and intrinsic pathways converge to induce apoptosis, which requires the involvement of the mitochondria (30). The extrinsic signaling pathway of apoptosis is initiated by transmembrane death receptors, which are members of the tumor necrosis factor (TNF) receptor gene superfamily (TNFRSF) (31). Members of the TNF receptor family bind to extrinsic ligands, leading to the activation of the initiator caspase-8, which eventually results in the death of the cell (31). In our study, "case patients" had upregulation of TNFRSF9, LTBR, TNF, TNFRSF11B, and TNFRSF1A; proteins encoded by these genes are members of the TNF receptor superfamily. We also observed an upregulation of TNF receptor-associated factor (TRAF) gene. The intrinsic apoptotic pathway is initiated by a diverse array of non-receptor-mediated stimuli such as DNA damage, growth factor withdrawal, radiation, toxins, hypoxia, hyperthermia, viral infections, and free radicals (32). This pathway is closely regulated by the BCL-2 family, a group of related proteins which regulate the integrity of the mitochondrial membrane (32). Damaged mitochondrial outer membranes cannot produce energy by oxidative phosphorylation and leak apoptogenic factors, such as CYCS and Smac/DIABLO into the cytosol (29). CYCS, among other factors, is thought to induce caspase activation, which leads to apoptosis (33). We observed upregulation of proapoptotic genes, such as BAD, BAK1, and BIK. We also observed an upregulation of the CYCS gene.

Studies of apoptotic gene expression in HIV infection using both *in vitro* and *in vivo* models in the absence of treatment have demonstrated increased expression of proapoptotic genes of intrinsic, extrinsic, and executioner pathways (34, 35). However, on initiation of ART, studies have reported downregulation of proapoptotic genes (36) and upregulation of antiapoptotic genes (37). The aforementioned studies of HIV treatment-experienced patients focused on either a single pathway of apoptosis or a restricted number of apoptosis-related genes. Balestrieri et al. studied the expression profile of 19 apoptosis-related genes in 12 treatment-naive patients initiating ART. They found that after 12 months of ART, the expressions of proapoptotic genes (FAS, FAS-L, FAF-1, FADD, CASPASE-8, DR3, TRAIL, TRADD, and BAX) were significantly downregulated compared with those at time zero, while the expressions of antiapoptotic genes (BCL-2, BCL-XL, and MCL-1) were significantly upregulated (38). Furthermore, Pitrak et al. recently observed increased activation of caspase 8 (extrinsic pathway), caspase 9 (intrinsic pathway), and caspases 3 and 7 (executioner pathway) at baseline compared to 6 months after initiating ART in 10 HIV treatment-naive patients (39). Altogether, considering these data, we surmise that HIV infection increases apoptosis, while ART reduces apoptosis. The effect of ART on apoptosis in these studies is contrary to our finding of increased expression of proapoptotic genes. This may be explained by the difference in the study populations. Our study compared HIV treatment-experienced

Downloaded from http://aac.asm.org/ on October 15, 2019 by guest

patients who were diagnosed with ART-associated mitochondrial toxicity with HIV-uninfected patients. However, our finding is consistent with findings from other studies, both *in vitro* and *in vivo*, that reported increased apoptosis in association with ART-induced clinical toxicities (40–42). The findings of these studies suggest that markers of apoptosis could be more sensitive and specific biomarkers of ART toxicity (25). In our exploratory study, upregulation of 2 (DFFA and TNFRSF1A) out of the 84 genes successfully classified 75% of study participants correctly as either a case or control. Thus, if these genes are validated in further studies, quantitative PCR assays of these genes could serve as biomarkers for ART-induced toxicity.

Our study has several limitations. First, like all cross-sectional studies, we have not provided incontrovertible proof that the upregulated genes were caused by ART-induced toxicity in the HIV-infected individuals, as we did not have their gene profile prior to starting ART. Second, the diagnosis of mitochondrial toxicity was not confirmed with tissue biopsy. Third, the small sample size limits the generalization of our findings. Fourth, we did not study HIV-infected patients on ART without toxicity as controls. However, our findings are consistent with those of other investigators, suggesting that apoptosis may be part of the causal pathway of ART-associated mitochondrial toxicity. Therefore, further well-designed prospective studies are needed.

ACKNOWLEDGMENTS

We thank the patients and staff of the Nathan Smith Clinic Yale–New Haven Hospital for their participation and support during the study. In addition, we acknowledge Warren Andiman for his critical review of the manuscript.

This study was supported by grants from the National Institutes of Health to E.P. (KO8AI074404 and R01 HD074252). M.G. was supported by a grant from the NIH (P30AI060354).

REFERENCES

1. Palella FJ, Jr, Baker RK, Moorman AC, Chmiel JS, Wood KC, Brooks JT, Holmberg SD, Investigators HIVOS. 2006. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr 43:27–34. https://doi.org/10.1097/01.qai.0000233310.90484.16.
2. Palella FJ, Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD, HIV Outpatient Study Investigators. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 338:853–860. https://doi.org/10.1056/NEJM199803263381301.
3. Fang CT, Chang YY, Hsu HM, Twu SJ, Chen KT, Lin CC, Huang LY, Chen MY, Hwang JS, Wang JD, Chuang CY. 2007. Life expectancy of patients with newly-diagnosed HIV infection in the era of highly active antiretroviral therapy. QJM 100:97–105. https://doi.org/10.1093/qjmed/hcl141.
4. Egger M, May M, Chene G, Phillips AN, Ledergerber B, Dabis F, Costagliola D, D'Arminio Monforte A, de Wolf F, Reiss P, Lundgren JD, Justice AC, Staszewski S, Leport C, Hogg RS, Sabin CA, Gill MJ, Salzberger B, Sterne JA. 2002. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. Lancet 360:119–129. https://doi.org/10.1016/S0140-6736(02)09411-4.
5. Lenert LA, Feddersen M, Sturley A, Lee D. 2002. Adverse effects of medications and trade-offs between length of life and quality of life in human immunodeficiency virus infection. Am J Med 113:229–232. https://doi.org/10.1016/S0002-9343(02)01156-7.
6. Powderly WG. 2002. Long-term exposure to lifelong therapies. J Acquir Immune Defic Syndr 29(Suppl 1):S28–40. https://doi.org/10.1097/00126334-200202011-00005.
7. Smyth K, Affandi JS, McArthur JC, Bowtell-Harris C, Mijch AM, Watson K, Costello K, Woolley IJ, Price P, Wesselingh SL, Cherry CL. 2007. Prevalence of and risk factors for HIV-associated neuropathy in Melbourne, Australia 1993–2006. HIV Med 8:367–373. https://doi.org/10.1111/j.1468-1293.2007.00478.x.
8. Haubrich RH, Riddler SA, DiRienzo AG, Komarow L, Powderly WG, Klingman K, Garren KW, Butcher DL, Rooney JF, Haas DW, Mellors JW, Havlir DV, Team ACTGAS. 2009. Metabolic outcomes in a randomized trial of nucleoside, nonnucleoside and protease inhibitor-sparing regimens for initial HIV treatment. AIDS 23:1109–1118. https://doi.org/10.1097/QAD.0b013e32832b4377.
9. Troya J, Bascunana J. 2016. Safety and tolerability: current challenges to antiretroviral therapy for the long-term management of HIV infection. AIDS Rev 18:127–137.
10. Hoffmann C, Welz T, Sabranski M, Kolb M, Wolf E, Stellbrink HJ, Wyen C. 2017. Higher rates of neuropsychiatric adverse events leading to dolutegravir discontinuation in women and older patients. HIV Med 18:56–63. https://doi.org/10.1111/hiv.12468.
11. Gertner E, Thurn JR, Williams DN, Simpson M, Balfour HH, Jr, Rhame F, Henry K. 1989. Zidovudine-associated myopathy. Am J Med 86:814–818. https://doi.org/10.1016/0002-9343(89)90478-6.
12. Brinkman K, ter Hofstede HJ, Burger DM, Smeitink JA, Koopmans PP. 1998. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. AIDS 12:1735–1744. https://doi.org/10.1097/00002030-199814000-00004.
13. Montaner JS, Cote HC, Harris M, Hogg RS, Yip B, Harrigan PR, O'Shaughnessy MV. 2004. Nucleoside-related mitochondrial toxicity among HIV-infected patients receiving antiretroviral therapy: insights from the evaluation of venous lactic acid and peripheral blood mitochondrial DNA. Clin Infect Dis 38(Suppl 2):S73–S79. https://doi.org/10.1086/381449.
14. Moyle G. 2000. Clinical manifestations and management of antiretroviral nucleoside analog-related mitochondrial toxicity. Clin Ther 22:911–936; discussion 898. https://doi.org/10.1016/S0149-2918(00)80064-8.
15. Lewis W, Dalakas MC. 1995. Mitochondrial toxicity of antiviral drugs. Nat Med 1:417–422. https://doi.org/10.1038/nm0595-417.
16. Apostolova N, Blas-Garcia A, Esplugues JV. 2011. Mitochondrial interference by anti-HIV drugs: mechanisms beyond Pol-γ inhibition. Trends Pharmacol Sci 32:715–725. https://doi.org/10.1016/j.tips.2011.07.007.
17. Effros RB, Fletcher CV, Gebo K, Halter JB, Hazzard WR, Horne FM, Huebner RE, Janoff EN, Justice AC, Kuritzkes D, Nayfield SG, Plaeger SF, Schmader KE, Ashworth JR, Campanelli C, Clayton CP, Rada B, Woolard NF, High KP. 2008. Aging and infectious diseases: workshop on HIV

Downloaded from http://aac.asm.org/ on October 15, 2019 by guest

infection and aging: what is known and future research directions. Clin Infect Dis 47:542–553. https://doi.org/10.1086/590150.
18. Hoy J, Grund B, Roediger M, Ensrud KE, Brar I, Colebunders R, Castro ND, Johnson M, Sharma A, Carr A, INSIGHT SMART Body Composition Substudy Group. 2013. Interruption or deferral of antiretroviral therapy reduces markers of bone turnover compared with continuous therapy: the SMART body composition substudy. J Bone Miner Res 28:1264–1274. https://doi.org/10.1002/jbmr.1861.
19. El-Sadr WM, Lundgren J, Neaton JD, Gordin F, Abrams D, Arduino RC, Babiker A, Burman W, Clumeck N, Cohen CJ, Cohn D, Cooper D, Darbyshire J, Emery S, Fatkenheuer G, Gazzard B, Grund B, Hoy J, Klingman K, Losso M, Markowitz N, Neuhaus J, Phillips A, Rappoport C, Strategies for Management of Antiretroviral Therapy Study Group. 2006. CD4$^+$ count-guided interruption of antiretroviral treatment. N Engl J Med 355:2283–2296. https://doi.org/10.1056/NEJMoa062360.
20. Ryom L, Mocroft A, Kirk O, Worm SW, Kamara DA, Reiss P, Ross M, Fux CA, Morlat P, Moranne O, Smith C, Lundgren JD, D:A:D Study Group. 2013. Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. J Infect Dis 207:1359–1369. https://doi.org/10.1093/infdis/jit043.
21. Langs-Barlow A, Selvaraj S, Ogbuagu O, Shabanova V, Shapiro ED, Paintsil E. 2015. Association of circulating cytochrome *c* with clinical manifestations of antiretroviral-induced toxicity. Mitochondrion 20:71–74. https://doi.org/10.1016/j.mito.2014.11.004.
22. Renz A, Berdel WE, Kreuter M, Belka C, Schulze-Osthoff K, Los M. 2001. Rapid extracellular release of cytochrome c is specific for apoptosis and marks cell death *in vivo*. Blood 98:1542–1548. https://doi.org/10.1182/blood.V98.5.1542.
23. Vlahakis SR, Bennett SA, Whitehead SN, Badley AD. 2007. HIV protease inhibitors modulate apoptosis signaling in vitro and in vivo. Apoptosis 12:969–977. https://doi.org/10.1007/s10495-007-0755-3.
24. Karamchand L, Dawood H, Chuturgoon AA. 2008. Lymphocyte mitochondrial depolarization and apoptosis in HIV-1-infected HAART patients. J Acquir Immune Defic Syndr 48:381–388. https://doi.org/10.1097/QAI.0b013e3181799662.
25. Hooker DJ, Cherry CL. 2009. Apoptosis: a clinically useful measure of antiretroviral drug toxicity? Expert Opin Drug Metab Toxicol 5:1543–1553. https://doi.org/10.1517/17425250903282781.
26. Hooker DJ, Gorry PR, Ellett AM, Wesselingh SL, Cherry CL. 2009. Measuring and monitoring apoptosis and drug toxicity in HIV patients by ligation-mediated polymerase chain reaction. J Cell Mol Med 13:948–958. https://doi.org/10.1111/j.1582-4934.2008.00612.x.
27. Kakuda TN. 2000. Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. Clin Ther 22:685–708. https://doi.org/10.1016/S0149-2918(00)90004-3.
28. Silva JC, Ferreira-Strixino J, Fontana LC, Paula LM, Raniero L, Martin AA, Canevari RA. 2014. Apoptosis-associated genes related to photodynamic therapy in breast carcinomas. Lasers Med Sci 29:1429–1436. https://doi.org/10.1007/s10103-014-1547-y.
29. Packham G, Stevenson FK. 2005. Bodyguards and assassins: Bcl-2 family proteins and apoptosis control in chronic lymphocytic leukaemia. Immunology 114:441–449. https://doi.org/10.1111/j.1365-2567.2005.02117.x.
30. Letai AG. 2008. Diagnosing and exploiting cancer's addiction to blocks in apoptosis. Nat Rev Cancer 8:121–132. https://doi.org/10.1038/nrc2297.
31. Locksley RM, Killeen N, Lenardo MJ. 2001. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 104:487–501. https://doi.org/10.1016/S0092-8674(01)00237-9.
32. Elmore S. 2007. Apoptosis: a review of programmed cell death. Toxicol Pathol 35:495–516. https://doi.org/10.1080/01926230701320337.
33. Jin Z, El-Deiry WS. 2005. Overview of cell death signaling pathways. Cancer Biol Ther 4:139–163. https://doi.org/10.4161/cbt.4.2.1508.
34. Corbeil J, Sheeter D, Genini D, Rought S, Leoni L, Du P, Ferguson M, Masys DR, Welsh JB, Fink JL, Sasik R, Huang D, Drenkow J, Richman DD, Gingeras T. 2001. Temporal gene regulation during HIV-1 infection of human CD4$^+$ T cells. Genome Res 11:1198–1204. https://doi.org/10.1101/gr.GR-1802R.
35. Genini D, Sheeter D, Rought S, Zaunders JJ, Susin SA, Kroemer G, Richman DD, Carson DA, Corbeil J, Leoni LM. 2001. HIV induces lymphocyte apoptosis by a p53-initiated, mitochondrial-mediated mechanism. FASEB J 15:5–6. https://doi.org/10.1096/fj.00-0336fje.
36. Grelli S, Campagna S, Lichtner M, Ricci G, Vella S, Vullo V, Montella F, Di Fabio S, Favalli C, Mastino A, Macchi B. 2000. Spontaneous and anti-Fas-induced apoptosis in lymphocytes from HIV-infected patients undergoing highly active anti-retroviral therapy. AIDS 14:939–949. https://doi.org/10.1097/00002030-200005260-00005.
37. Airo P, Torti C, Uccelli MC, Malacarne F, Palvarini L, Carosi G, Castelli F. 2000. Naive CD4$^+$ T lymphocytes express high levels of Bcl-2 after highly active antiretroviral therapy for HIV infection. AIDS Res Hum Retroviruses 16:1805–1807. https://doi.org/10.1089/08892220050195766.
38. Balestrieri E, Grelli S, Matteucci C, Minutolo A, d'Ettorre G, Di Sora F, Montella F, Vullo V, Vella S, Favalli C, Macchi B, Mastino A. 2007. Apoptosis-associated gene expression in HIV-infected patients in response to successful antiretroviral therapy. J Med Virol 79:111–117. https://doi.org/10.1002/jmv.20768.
39. Pitrak DL, Novak RM, Estes R, Tschampa J, Abaya CD, Martinson J, Bradley K, Tenorio AR, Landay AL. 2015. Short communication: apoptosis pathways in HIV-1-infected patients before and after highly active antiretroviral therapy: relevance to immune recovery. AIDS Res Hum Retroviruses 31:208–216. https://doi.org/10.1089/aid.2014.0038.
40. Domingo P, Matias-Guiu X, Pujol RM, Francia E, Lagarda E, Sambeat MA, Vazquez G. 1999. Subcutaneous adipocyte apoptosis in HIV-1 protease inhibitor-associated lipodystrophy. AIDS 13:2261–2267. https://doi.org/10.1097/00002030-199911120-00008.
41. Cherry CL, Lal L, Thompson KA, McLean CA, Ross LL, Hernandez J, Wesselingh SL, McComsey G. 2005. Increased adipocyte apoptosis in lipoatrophy improves within 48 weeks of switching patient therapy from stavudine to abacavir or zidovudine. J Acquir Immune Defic Syndr 38:263–267.
42. Pettersen JA, Jones G, Worthington C, Krentz HB, Keppler OT, Hoke A, Gill MJ, Power C. 2006. Sensory neuropathy in human immunodeficiency virus/acquired immunodeficiency syndrome patients: protease inhibitor-mediated neurotoxicity. Ann Neurol 59:816–824. https://doi.org/10.1002/ana.20816.

Downloaded from http://aac.asm.org/ on October 15, 2019 by guest