Workshop report

Key data from the 11th International Workshop on Adverse Drug Reactions and Co-Morbidities in HIV

*Richard E Boehme[1] and Stephen Cameron[2]**

[1]International Medical Press, Hamilton, NJ, USA
[2]International Medical Press, London, UK

*Corresponding author: stephen.cameron@intmedpress.com

The effectiveness of current antiretroviral therapies has led to more patients receiving long-term therapy, and increasing numbers of older patients. The complex interactions between HIV infection, ageing, comorbid conditions and drug-related toxicities, and their combined effects on patient health and quality of life, were the subject of the *11th International Workshop on Adverse Drug Reactions and Co-Morbidities in HIV*. Key topics included the metabolic effects of antiretroviral agents, cardiovascular disease, bone metabolism and age-related changes in disease and response to drug therapy. Data presented at the meeting reflect the growing recognition of common pathways that contribute to progression of mutiple types of disease and adverse drug reactions.

Introduction

The relative contributions and interactions of highly active antiretroviral therapy (HAART), HIV infection, comorbidities, genetics and lifestyle risk factors with respect to the development of medical complications are complex, and pose significant challenges for healthcare providers [1]. Both HIV infection and HAART have been associated with multiple serious complications, including lipodystrophies, dyslipidaemia, cardiovascular disease, insulin resistance and other metabolic disorders, bone disease, kidney disease, sarcopaenia, cognitive impairment and liver disease [2]. Although links between some of these conditions are well established, such as between dyslipidaemia and cardiovascular disease, there remain many unanswered questions concerning the relative effects of different HAART regimens, the potential involvement of common biologies in the aetiology of complications, and the interaction between HIV-related effects with risk factors seen in the general population. Ultimately, the aim is to gain a practical understanding of these issues in order to tailor antiretroviral treatment so that the risks of complications are minimized, while maintaining fully effective suppression of viral replication.

The *11th International Workshop on Adverse Drug Reactions and Co-Morbidities in HIV*, held in Philadelphia, PA, USA, provided a forum for presentation and discussion of new research findings pertaining to complications of antiretroviral therapy (ART) of HIV infection. The workshop was accredited for continuing medical education by the Accreditation Council for Continuing Medical Education, and was attended by physicians and researchers from the Americas, Europe and the Asia-Pacific region. This report summarizes key reports from the workshop pertaining to adipose tissue metabolism and lipodystrophy, metabolic disorders, cardiovascular disease, bone disorders, mitochondrial metabolism, and other toxicities and complications.

Adipose tissue metabolism and lipodystrophy

Changes in adipose tissue distribution have been associated with HIV infection and with ART; however, relationships between HIV infection and changes in the different types of adipose tissue are uncertain. Carl Grunfeld [3] reported results from the FRAM 2 study that compared levels of intermuscular adipose tissue (IMAT), measured by MRI, in 425 HIV-infected patients and 211 uninfected control subjects. The FRAM 1 study previously showed that subcutaneous adipose tissue (SAT) is reduced in HIV infection, but visceral adipose tissue (VAT) is preserved or increased [4,5]. Previous studies suggested that IMAT is similar to VAT with respect to associations with insulin resistance, diabetes and age-related increases. However, contrary to expectations, the present analysis showed a significant

reduction of IMAT in HIV-infected subjects, similar to reductions of SAT and fat in striated muscle (SM). Leg SAT had the strongest association with IMAT in HIV-infected subjects; VAT had the strongest association in controls. Serum levels of C-reactive protein (CRP) were associated with total IMAT, more so in controls than in HIV-infected subjects, and a multivariate analysis of possible HIV-related contributing factors showed that use of stavudine (d4T), didanosine (ddI) and nelfinavir was independently associated with IMAT and SAT reductions in HIV-infected subjects, suggesting that IMAT and SAT share some cellular origins.

It is well recognized that lipodystrophy can occur in HAART recipients and these effects vary with different antiretroviral agents or combinations of agents. Several reports at the conference addressed the incidence and clinical characteristics of lipodystrophy in relation to treatment received, and the molecular mechanisms associated with these events. David A Cooper [6], on behalf of the Altair study, reported that ART type is an independent predictor of lipoatrophy in patients initiating HAART. This study compared virological and metabolic outcomes for three different ART regimens in 322 treatment-naive patients, randomly assigned 1:1:1 to receive tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) plus either efavirenz (EFV), ritonavir-boosted atazanavir (ATV/r) or zidovudine (AZT) plus abacavir (ABC). At week 48, the ATV/r and AZT/ABC groups were non-inferior to the EFV group with respect to HIV RNA response (the primary study end point); however, the AZT/ABC group was significantly inferior to the EFV group by multiple secondary measures, including safety and tolerability. EFV recipients showed increased plasma low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and glucose compared with the other groups at 48 weeks; the AZT/ABC group was essentially neutral with respect to lipids. In an anthropometric and metabolic substudy of the 227 study subjects with complete data at week 48, VAT was highest in the EFV group at week 48; however, differences with the other groups were not significant. SAT increased in the EFV and ATV/r groups, but did not change in the AZT/ABC group. Soft tissue dual-energy X-ray absorptiometry (DEXA) scans showed increased total body fat and peripheral fat in the EFV and ATV/r groups, and decreases in the AZT/ABC group. Significantly higher proportions of AZT/ABC recipients had peripheral lipoatrophy (defined as ≥10% or ≥20% loss of limb fat by DEXA) compared with the EFV group; a significantly lower proportion of ATV/r recipients had ≥20% lipoatrophy compared with the EFV group. Multivariate analysis identified use of AZT/ABC, low HIV RNA at baseline, high triglycerides at 24 weeks, glucose at baseline and Asian ethnicity as independent predictors of peripheral lipoatrophy. Overall, the quadruple nucleoside/nucleotide-containing arm exhibited consistently lower metabolic effects compared with the EFV arm, whereas ATV/r was less likely than EFV to cause significant peripheral lipoatrophy when combined with TDF/FTC over 48 weeks.

Ionescu *et al.* [7] reported that switching from AZT to TDF in ART regimens improved adipose tissue distribution and metabolic parameters. In this study, patients with undetectable HIV RNA who had received AZT-containing regimens for an average of 5 years were randomly assigned to switch from AZT to TDF (n=9) and compared with patients who continued AZT (n=7). The patients were generally overweight, sedentary and in their fifties, with some insulin resistance. Despite 5 years of AZT use, there was little evidence of mitochondrial dysfunction at baseline. Patients who had switched to TDF and assessed on a stationary bicycle showed a trend towards improved peak power and heart rate. Body fat generally increased in the TDF group and decreased in the AZT group, with no changes in lean tissue. Paradoxically, lactate area under the curve measured during exercise increased in the TDF group and decreased in the AZT group; this was attributed to a TDF-associated increase in exercise capacity and power output. TDF was associated with an increase in anaerobic myocardial work.

Vrouenraets *et al.* [8] reported that in the BASIC study, TDF/FTC combined with either ritonavir-boosted saquinavir or ATV/r had similar virological efficacy and similar potentially favourable effects on lipids after 48 weeks of therapy in 123 treatment-naive patients. No significant changes in insulin sensitivity were observed; both treatment arms had no effects on glucose metabolism, conserved adipose tissue and similarly reduced glomerular filtration rate. ATV/r recipients gained significantly more SAT.

Podzamczer *et al.* [9] reported metabolic findings from a 48-week randomized comparison of TDF/FTC plus nevirapine (NVP) or ATV/r in 569 treatment-naive patients. At week 48, NVP recipients showed increases in total cholesterol, HDL-C and LDL-C, but a reduction in the total cholesterol:HDL-C ratio and no change in triglycerides. By contrast, ATV/r recipients showed smaller increases in total cholesterol, HDL-C and LDL-C, but increases in triglycerides and the total cholesterol:HDL-C ratio. Discontinuations were more frequent with NVP (21.8% and 28.2% for NVP once daily and twice daily, respectively) than with ATV/r (9.3%).

Relatively less information is available concerning lipodystrophy in HIV-infected children and adolescents. Alam *et al.* [10] described risk factors in 473 HIV-infected youths with a median age of 13.5 years and a median ART duration of 8.8 years. Approximately two-thirds were clinically asymptomatic. Clinical evidence of altered fat distribution were found in >40%

of the cohort, of which approximately one-third were lipoatrophic, one-third lipohypertrophic and one-third both. In a univariate analysis, the presence of any evidence of altered fat distribution was significantly associated with White race, severe HIV disease, maternal lipodystrophy, Tanner puberty stage V and the use of d4T or EFV. The association of body fat changes with non-HIV-related parameters suggests that factors other than ART may play a role in their pathogenesis.

The mechanisms of ART-related lipodystrophy remain uncertain. Several reports concerned the varying effects of different antiretroviral treatment regimens on adipocyte metabolism and gene expression. Shahmanesh [11,12] reported long-term follow-up results in patients receiving regimens containing EFV plus either TDF/FTC or AZT plus lamivudine (3TC) with respect to lipid metabolism, body fat and adipocyte gene expression. After 6 months, genes involved with fatty acid uptake, lipogenesis, lipolysis, cortisol and glucose were up-regulated in both treatment groups relative to seronegative controls, whereas expression of genes involved with β-oxidation decreased. At 18 months, both treatment groups showed increases in VAT, trunk fat, peripheral fat and SAT, although not all changes were significant. Genes involved with lipogenesis, lipolysis and β-oxidation were down-regulated, generally to a greater extent in the AZT/3TC group versus TDF/FTC, suggesting a switch from aerobic to anaerobic metabolism.

As reported by Patrick W Mallon, Distler *et al.* [13] compared the effects of AZT/3TC, ritonavir-boosted lopinavir (LPV/r) or AZT/3TC plus LPV/r in healthy HIV-negative subjects after 2 and 6 weeks with respect to body fat, serum lipids and adipocyte gene expression. At 6 weeks, the AZT/3TC group showed a decrease from baseline in adipocyte mitochondrial copy number, whereas the LPV/r group showed an increase. Correlation analyses showed different patterns of gene expression according to treatment group. At baseline, expression of the mitochondrial gene CytB correlated with the nuclear gene activator peroxisome proliferator-activated receptor γ coactivator 1 (PGC-1α) and with peroxisome proliferator-activated receptor γ (PPARγ). Upon exposure to LPV/r but not to AZT/3TC, these correlations were no longer present, suggesting a pathway whereby HIV protease inhibitors (PIs) might blunt the effects of nucleoside reverse transcriptase inhibitors (NRTIs) on adipose gene expression.

Martone *et al.* [14] compared HIV-infected lipodystrophic HAART recipients with uninfected controls with respect to the expression of 48 different genes in biopsy-derived SAT. Expression of CCAAT/enhancer binding protein-α (C/EBPα) was greater in uninfected subjects than in the HIV-infected patients; the reverse was true for adiponectin Q, suggesting a role for these proteins in lipoatrophy. The authors suggested that C/EBPα deficiency may lead to development of defective adipose tissue, whereas adiponectin Q overexpression may increase fatty acid catabolism.

George MN Behrens [15] reported data suggesting that physiological concentrations of CRP in patients with HIV infection may promote the antiadipogenic potential of d4T, possibly contributing to interpatient variability in d4T-associated lipoatrophy. CRP has previously been shown to inhibit adiponectin gene expression and secretion in cultured 3T3-L1 adipocytes [16]. In this study of 3T3-L1 cells exposed to AZT, d4T and/or CRP during differentiation, AZT alone or d4T combined with CRP reduced lipogenesis, indicated by reduced triacylglyceride accumulation and reduced expression of the adipocyte markers PPARγ and C/EBPα. Coadministration of the PPARγ activator rosiglitazone had no effect on reduced adipogenesis, but partially reversed the reduction of adiponectin production by AZT and d4T. CRP also amplified d4T-induced reduction of adipogenic markers involved in lipid accumulation.

Shikuma *et al.* [17] described a biopsy study in which the effects of different antiretroviral treatment regimens on apoptosis in SAT were assessed. There were no significant differences in apoptotic parameters according to treatment group. The analysis showed a higher apoptosis index in pre-adipocytes than in adipocytes in both HIV-infected and -uninfected subjects. Apoptosis indices were not significantly altered in lipodystrophic patients, suggesting that another form(s) of cell death, or decreased production of adipocyte precursors, is involved. However, Veloso *et al.* [18] reported different results from a cross-sectional case control study that compared apoptosis markers in biopsy-derived SAT. This study concerned 38 HIV-infected patients receiving HAART, including 25 lipodystrophic, 13 non-lipodystrophic patients and 21 uninfected controls. In this study, SAT adipocyte apoptosis and expression of Fas ligand, a pro-apoptotic marker of the death receptor pathway, were significantly higher in HIV-infected lipodystrophic patients than in the other two groups. Expression of the anti-apoptotic protein Bcl-2 was significantly higher in both HIV-infected groups compared with the uninfected controls. Together, these data suggest that SAT adipocyte apoptosis is increased in HIV-infected lipodystrophic patients and driven primarily through the death receptor pathway.

Growth hormone levels have been reported to be reduced in some HIV-infected patients with increased VAT [19]; two reports concerned with the potential benefits of restoring growth hormone were presented. The effects of physiological doses of growth hormone on body composition and metabolic parameters were evaluated by Lo *et al.* [20]. Data were reported from an 18-month crossover extension of a prospective placebo-controlled trial in 48 HIV-infected patients on stable antiretroviral regimens

who had increased VAT and low growth hormone. In the primary analysis prior to the cross-over, growth hormone treatment increased insulin-like growth factor-1 (IGF-1) levels and reduced VAT compared with placebo, but had no effect on SAT. Growth hormone treatment improved blood pressure and plasma lipids, but impaired glucose homeostasis (fasting and 2 h glucose levels) despite the increases in IGF-1. After switching from growth hormone to placebo in the extension study, VAT and IGF-1 levels returned to baseline rapidly. Similar to these findings, two 6-month studies had previously shown that tesamorelin, a synthetic analogue of growth hormone releasing factor, improved VAT and plasma lipids without affecting SAT [21]. In an analysis of health-related quality-of-life assessments completed in conjunction with those studies, Turner *et al.* [22] reported that patient-reported belly, body and weight images improved in tesamorelin recipients and were correlated with reduction of VAT.

Metabolic disorders

Metabolic disorders have been described in HIV-infected patients receiving HAART; however, little information has been reported concerning the long-term incidence and risk factors for new-onset diabetes in European HAART recipients. Jacqueline Capeau [23] presented results from the APROCO-COPILOTE cohort on the long-term incidence of new-onset diabetes in 643 French HIV-infected patients, initially enrolled in 1997–1998 at the time that first-generation PIs were introduced, and followed for 9 years. The population was primarily Caucasian and 46% were treatment-naive at enrolment. The incidence of new onset diabetes during follow-up was high and similar for men and women (10.8 and 11.4 cases/1,000 patient-years, respectively). Older age at PI initiation was significantly associated with diabetes for both genders, increasing from 4.8 cases/1,000 patient-years for patients <30 years of age to 31.7 cases/1,000 patient-years for patients >50 years. In all age groups, the incidence of diabetes was approximately fourfold higher than that observed in the Augsburg cohort study of diabetes in non-HIV-infected individuals. Risk factors were those typically associated with diabetes in the general population, including familial history, higher body mass index (BMI) and waist-to-hip ratio and older age. In addition, incident diabetes was significantly associated with use of d4T or indinavir (IDV), suggesting drug-induced insulin resistance. Diabetes was not associated with any markers of HIV infection. Cardiovascular risk was increased in patients with diabetes, indicating the importance of targeted intervention.

Brown *et al.* [24] reported that systemic inflammation, even after ART-induced viral suppression, might contribute to diabetes pathogenesis among HIV-infected patients. Abnormalities of glucose metabolism are common in this group. In addition to the typical risk factors for diabetes that are seen in the general population, some antiretroviral drugs and HIV-related factors might contribute to diabetes risk in HIV-infected patients through multiple mechanisms. The potential role of chronic inflammation was assessed in this case control study, in which four inflammatory markers were assessed at baseline and 48 weeks after initiation of ART: CRP, interleukin (IL)-6 and soluble tissue necrosis factor receptors (sTNFR) -1 and -2. The 55 cases in the study had normal glucose at baseline and developed diabetes at least 48 weeks after ART initiation; matched controls were HIV-infected patients who remained normoglycaemic throughout follow-up. Results indicated no significant differences between cases and controls in the use of particular antiretroviral agents. In the overall study population, median CRP levels increased over the 48 weeks; the other inflammatory markers decreased. Odds ratio analysis indicated that higher levels of markers of tumour necrosis factor-α (TNF-α) activity (sTNFR1 and 2) and CRP, but not IL-6, were associated with the risk of incident diabetes.

Kim *et al.* [25] reported a high incidence of microalbuminuria in HIV-infected type-2 diabetics. Microalbuminuria is an early marker of kidney damage and is associated with increased cardiovascular and renal disease. In the pre-HAART era, microalbuminuria was prevalent in HIV-infected patients, ranging from 19% to 30%. Today, the incidence is reported as 8.7–11%. Among non-HIV-infected type-2 diabetics, the incidence of microalbuminuria has been reported as 25%. Kim *et al.* [25] assessed the prevalence of microalbuminuria among 73 HIV-infected type-2 diabetics in comparison to 61 type-2 diabetics without HIV infection and 82 HIV-infected patients without diabetes. Demographically, both diabetic groups were older than the HIV-infected/no diabetes group, with higher proportions of African-Americans and higher mean BMIs. Microalbuminuria was significantly more common among patients with HIV infection and diabetes (34%) than in patients with only HIV infection (13%) or diabetes (16%). In multivariate analysis, both HIV infection and diabetes were independent predictors of microalbuminuria. HIV-infected subjects with microalbuminuria had significantly greater cumulative exposure to ABC, possibly reflecting early endothelial injury and less exposure to IDV compared with HIV-infected subjects without microalbuminuria. There were no significant relationships between microalbuminuria and exposure to any other antiretroviral agents.

Ritonavir and other PIs have been reported to contribute to insulin resistance and other metabolic changes that can increase the risk of cardiovascular disease. It is recognized that HAART might have a direct adverse effect on myocardium: left ventricular

diastolic dysfunction has been seen in >60% of HAART recipients and is associated with reduced exercise tolerance [26]. Diabetes is a significant risk factor for diastolic dysfunction. Both ritonavir and LPV acutely reduce systemic and myocardial glucose uptake and, in TG9 mice with advanced dilated cardiomyopathy, ritonavir acutely reduces left ventricular end diastolic diameter (LVEDD) [27]. Paul W Hruz [28] reported results of a study designed to determine whether improving myocardial glucose transport might mitigate these effects, using the TG9 mouse model of dilated cardiomyopathy (reduced contractile function). In these mice, glucose transport blockade by ritonavir precipitates acute decompensated heart failure and accelerated death. To assess the potential benefits of increasing systemic and myocardial glucose uptake, the investigators administered the glucagon-like peptide 1 (GLP-1) agonist exenatide or the angiotensin-converting enzyme (ACE) inhibitor captopril to TG9 mice beginning at 56 days of age, and ritonavir beginning at 75 days. Although captopril did not alter glucose tolerance or reverse the adverse effects of ritonavir, exenatide markedly improved glucose tolerance and myocardial glucose uptake. Exenatide both extended survival of control mice and reversed the accelerated death seen in ritonavir-treated mice. These results provide a rationale for studying the effect of impaired glucose homeostasis on cardiac function in HIV-infected patients receiving PIs, and suggest that improving glucose disposal may help preserve cardiac function.

The use of d4T has been associated with insulin resistance and diabetes [29]. In HIV-uninfected populations, these conditions have been associated with altered expression of adipocyte-derived cytokines. Saeedi *et al.* [30] found that d4T reduced adiponectin expression and secretion and increased retinol binding protein-4 (RBP-4) secretion from cultured 3T3-L1 pre-adipocytes. Expression of monocyte chemotactic protein-1 (MCP-1), RBP-4 and plasminogen activator inhibitor-1 (PAI-1) was increased. These results provide a possible cellular mechanism for d4T-induced insulin resistance.

Cardiovascular disease

HIV infection and ART have been associated with increased risks of cardiovascular disease. These are associated with the physical changes of atherosclerosis, as well as functional changes in the endothelium. Ganz [31] described the relationship of physical and functional arterial changes, and non-invasive methods that can be used to assess endothelial function and atherosclerosis. Acetylcholine, serotonin, thrombin and bradykinin activate the nitric oxide synthetase pathway that mediates endothelial relaxation in healthy arteries. This pathway is regulated by shear stress, insulin, exercise and Rho kinase; however, in atherosclerotic arteries, acetylcholine, sympathetic activation and exercise paradoxically cause constriction and flow-mediated dilation (FMD) is impaired [32]. Nitric oxide has emerged as a key antiatherogenic molecule; studies have shown that nitric oxide deficiency augments atherosclerosis in hypercholesterolaemic mice.

Endothelial function is a sensitive indicator of arterial health. Multiple risk factors can impair endothelial function before atherosclerosis is apparent, including well-known factors such as dyslipidaemia, diabetes and smoking, and novel factors such as inflammation, obesity and physical inactivity [31]. Correspondingly, multiple studies have shown that abnormal endothelial function and FMD predict subsequent arteriosclerosis and cardiovascular events. Conversely, many interventions that improve cardiovascular outcomes have been shown to improve endothelial function, including lipid-lowering drugs, antihypertensives, ACE inhibitors, calcium channel blockers, smoking cessation, weight reduction and exercise.

Hypertension is associated with impaired endothelium-mediated relaxation even in arteries free of atherosclerosis, contributing to ischaemia [31]. This latter effect also suggests that non-invasive endothelial function tests can be accomplished in readily accessible arteries. For example, brachial artery dilation assessed by ultrasound imaging has been correlated with endothelial function in coronary arteries. Several other methods have also been used to assess endothelial function, including venous occlusion plethysmography, peripheral arterial tonometry, coronary angiography, coronary Doppler and pulse wave velocity and arterial stiffness.

Endothelial function, assessed by FMD, is impaired in HIV-infected patients and the use of PIs is also associated with endothelial dysfunction [31]. Treatment with pravastatin improves FMD. In a study of the effect of different ART regimens on endothelial function in treatment-naive HIV-infected patients, PI-sparing regimens, non-nucleoside reverse transcriptase inhibitor (NNRTI)-sparing regimens and NRTI-sparing regimens all improved endothelial function as early as 4 weeks after the initiation of therapy. This finding suggests that ART *per se* or the combination of different drug classes is important in determining the effect on endothelial function

Measurement of coronary artery calcium (CAC) is an investigational non-invasive method for early detection of atherosclerosis. Guaraldi *et al.* [33] reported a high prevalence of subclinical atherosclerosis in HIV-infected patients, assessed by multidetector electron beam computed tomography (EBCT) of CAC. CAC scores obtained with this technique have been associated with coronary atherosclerotic disease burden. In

this study, sequential EBCT scans were obtained over a median 333 days from 132 HIV-infected men receiving ART and without prior cardiovascular disease. CAC did not change in 77 patients, regressed in 11 (including 8 of the 10 who had high CAC at baseline) and progressed in 44. Of the latter, 75% had ≥15% increase in CAC score, which is considered clinically meaningful. Independent predictors of CAC progression were older age, high LDL-C, high VAT and higher $CD4^+$ T-cell count. The association of CAC progression with VAT is consistent with previous reports and might be a particular problem for HIV-infected patients with lipodystrophy, where VAT may be disproportionately high in relation to BMI. The positive association of $CD4^+$ T-cell count with CAC progression is striking. Although the mechanism is uncertain, it might be related to the promotion of chronic inflammatory processes related to immune activation.

Measurement of arterial stiffness using pulse wave analysis is another non-invasive method for assessing atherosclerosis. Richardson *et al.* [34] reported that arterial stiffness was higher in HIV-infected patients with typical cardiac risk factors and in those with lower viral load. Increased cardiovascular risk has been associated with untreated HIV replication, derived from increases in the total cholesterol to HDL-C ratio, increases in conventional cardiovascular disease markers and increased CRP levels. Cardiovascular disease in HIV-infected patients has also been associated with longer exposure to PIs (related to dyslipidaemia) and, in some studies, to ABC exposure. Aortic stiffness is associated with reduced left ventricular function and coronary artery disease, and is an independent predictor of cardiovascular mortality. This study assessed the relationship of arterial stiffness with HIV viral load and cardiac biomarkers in 201 HIV-infected patients, 176 of whom were receiving ART. Arterial stiffness was assessed either directly, following arterial catheterization, or indirectly by applanation tonometry to generate an augmentation index (AIx). Results showed an association between higher aortic stiffness and the presence of established cardiac risk factors, and also with lower HIV viral load. The latter finding suggests that the types of ART used by study subjects may have had a more detrimental effect on arterial stiffness than did HIV replication.

ABC use has controversially been associated with an increased risk of acute myocardial infarction (AMI). To further explore this potential relationship, Young *et al.* [35] reported the results of a 24-week interim analysis of an ongoing open-label pilot evaluation of ABC/3TC plus raltegravir in 35 treatment-naive HIV-infected patients. At baseline, 37% of subjects had a BMI≥25 kg/m^2, 43% were smokers and 20% had a family history of cardiovascular disease. Most subjects had low Framingham risk scores and no evidence of metabolic syndrome. At 24 weeks, although most subjects maintained their baseline cardiovascular risk profiles, three subjects shifted to high-risk profiles due to increased LDL-C. Two of these three also developed metabolic syndrome. These data support a generally favourable lipid profile for ABC/3TC plus raltegravir, but suggest that adverse changes in lipids may occur in some patients.

Hileman *et al.* [36] reported negative results from a clinical evaluation of salsalate for reducing endothelial activation (and potentially cardiovascular risk) in 40 ART recipients with stable viral load ≤400 copies/ml. Salsalate, a non-steroidal anti-inflammatory agent, is a potent inhibitor of the IKK-β/nuclear factor-κB (NF-κB) pathway that is a central integrator of proinflammatory signals. Salsalate has been shown to improve endothelial function in untreated HIV-infected patients. In this open-label study, with subjects randomly assigned to receive salsalate or no salsalate, endothelial activation was assessed by FMD of the brachial artery. After 13 weeks, there were no significant differences in FMD between the salsalate and control groups. Plasma levels of IL-6 and D-dimer increased in the salsalate group, but there were no significant changes in any other biomarker associated with inflammation or glucose metabolism. However, many patients required salsalate dose reduction due to poor tolerability; therefore, insufficient salsalate doses may have contributed to its lack of effect.

Although vertical transmission of HIV infection is decreasing globally, left ventricular dysfunction (LVD) has been observed at birth, and persisting into childhood, in HIV-uninfected children born to HIV-infected mothers [37]. Cade *et al.* [38] described the results of efforts to find more effective non-invasive methods of identifying and monitoring cardiac function in these children. Two-dimensional speckle tracking echocardiography (2DSE) may have advantages over conventional echocardiography for diagnosing and monitoring myocardial strain and subclinical LVD. Decreased myocardial strain is associated with lower myocardial contractility, regional O_2 consumption and perfusion; thus, 2DSE may be useful for assessing left ventricular function. In this study, myocardial strain was evaluated in eight HIV-negative neonates born to HIV-infected mothers and exposed to HIV and HAART *in utero*, and compared with eight HIV-negative neonates born to HIV-negative mothers. Results showed that infants born to HIV-infected mothers had significantly lower global longitudinal strain and higher heart rates, compared with the control group, indicating subclinical LVD. The mechanisms underlying this difference are unknown, but may include NRTI-mediated myocardial mitochondrial dysfunction, maternal–fetal nutrient abnormality *in utero*, placental abnormality or maternal lifestyle. Continuing follow-up

may determine whether cardiac abnormalities in these neonates persist into adulthood and increase risks of cardiovascular morbidity.

Rader [39] reviewed studies of genetic factors that contribute to lipid disorders and cardiovascular risk. HIV infection and ART are both risk factors for dyslipidaemia and atherosclerosis, which in turn increase the risk of potentially catastrophic cardiovascular disease. Both LDL-C and HDL-C are independent predictors of coronary heart disease (CHD), and a number of studies have focused on identifying genes that affect plasma levels of LDL-C and HDL-C as a strategy for discovering new chemotherapeutic targets. Observations that individuals with a genetic deficiency of microsomal triglyceride transfer protein (MTP) have no serum LDL-C prompted research that led to the discovery of the MTP inhibitor, lomitapride. This agent has shown marked suppression of LDL-C in patients with familial hypercholesterolaemia and may also be effective in less severely affected individuals. Mutations of proprotein convertase subtilisin/kexin type-9 (PCSK9), a liver-secreted protein that targets LDL-C receptors for degradation, have been associated with reduced serum LDL-C and protection against CHD. More recent genome-wide association studies have identified additional genetic loci associated with LDL-C plasma levels and biology (such as the 1p13 locus, which contains four genes plus non-coding regions). Further assessments are underway to determine the feasibility of therapeutic interventions directed at these targets.

Although there is a well-established causal link between CHD risk and LDL-C, individuals with genetically low HDL-C do not consistently exhibit increased CHD risk, and low HDL-C is often accompanied by other cardiovascular risk factors [39]. Although it is uncertain whether drugs that specifically increase plasma HDL-C will have a beneficial effect on CHD risk, genes that encode potential targets for HDL-C-related drugs are being evaluated with respect to their role in lipid metabolism and potential for therapeutic intervention. Apolipoprotein A-1 (ApoA-1) is the major protein component of HDL-C; overexpression in mice increases plasma HDL-C through induction of macrophage-to-feces reverse cholesterol transport, reducing CHD risk. In human ApoA-1 transgenic mice, both EFV and NVP increase HDL-C levels and reverse cholesterol transport. Similarly in humans, NVP treatment has been linked with increased levels of ApoA-1 and HDL-C. Another potential chemotherapeutic target is cholesteryl ester transfer protein (CETP). CETP deficiency is associated with increased HDL-C levels. Torcetrapib, a CETP inhibitor, markedly increased HDL-C in clinical trials; however, clinical development was stopped due to off-target toxicity before a possible link between CETP inhibition and CHD could be established. Endothelial lipase and chemokine C–X–C motif ligand 12 (CXCL12) are additional proteins influencing serum HDL-C that may provide future drug targets. Increasing the repertoire of available agents for treating dyslipidaemia may allow more individualized therapy to address issues of tolerability and non-response.

Bone metabolism

Both HIV infection and ART regimens, especially those containing PIs, have been associated with reduced bone mineral density (BMD). Cotter *et al.* [40] described the results of *in vitro* studies that suggest a direct interaction between HIV and mesenchymal stem cells (MSC), resulting in increased differentiation into adipocytes and reduced differentiation into osteoblasts. MSC were exposed under differentiating conditions to sera from either uninfected control subjects or treatment-naive HIV-infected subjects. Sera from the HIV-infected subjects induced a pro-adipogenic phenotype, including increased adipocyte formation and expression of PPARγ and lipoprotein lipase, and also reduced expression of chicken ovalbumin upstream promoter transcript factor-1 (COUP TF-1). This transcription factor interacts with HIV TAT and is expressed at higher levels during MSC differentiation into osteoblasts versus adipocytes, suggesting a possible mechanism for the effect of HIV on MSC differentiation.

The presence and/or degree of osteopaenia caused by ART can vary with the type of treatment. Moyle *et al.* [41] reported that after 48 weeks, treatment with TDF/FTC plus EFV (n=193), compared with ABC/3TC plus EFV (n=192), was associated with a greater loss of BMD (measured by DEXA) in the hip and lumber spine, and greater associated changes in serum markers of osteopaenia. Consistent with previous studies [42], BMD (assessed by DEXA) decreased in both study groups, but to a significantly greater extent in the TDF/FTC group. Similarly, the proportion of patients meeting the World Health Organization osteopaenia criteria increased to a greater extent between baseline and week 48 with TDF/FTC than with ABC/3TC. BMD changes correlated with bone biomarkers. Plasma levels of C-terminal telopeptide of type-1 collagen (CTx), osteocalcin, bone-specific alkaline phosphatase and procollagen type-1 amino-terminal propeptide (P1NP) were all significantly higher in the TDF/FTC group than in the ABC/3TC group at week 24, and all but CTx were significantly higher at week 48. However, the mechanisms underlying the apparent treatment-related differences in osteopaenia have not been determined.

Both ART and vitamin D deficiency have been shown to contribute to reduced BMD in HIV-infected

individuals. In particular, an increased prevalence of vitamin D deficiency among EFV recipients has been reported [43]. Brown *et al.* [44] reported that EFV-containing ART regimens can adversely affect vitamin D metabolism. Subjects were enrolled from a cohort identified previously for a study of bone turnover associated with ART initiation. A total of 51 patients received regimens that included EFV; 36 received regimens without EFV. Serum levels of 25-hydroxyvitamin D were assessed at baseline and 6–12 months after ART initiation. Vitamin D deficiency was common in this population at baseline, with levels <32 ng/ml in 84% of subjects and ≤15 ng/ml (defined as hypovitaminosis D) in 33% of subjects. Low vitamin D levels were associated with non-White race, winter season and longer duration of HIV infection. At 6–12 months after ART initiation, patients receiving EFV-containing regimens showed significant reductions from baseline in vitamin D levels, compared with a small increase in levels for patients receiving regimens without EFV. Comparing the groups after ART, those receiving EFV had significantly lower levels of vitamin D and a significantly higher incidence of hypovitaminosis D. No associations were seen between vitamin D level and the use of any particular NRTI. Although the mechanism underlying the effect of EFV on vitamin D levels is uncertain, it may be associated with the known induction of CYP24A (vitamin D 24-hydroxylase) by EFV, causing increased catabolism of vitamin D.

Effros *et al.* [45] reviewed the results of several studies that suggested that bone homeostasis is subject to immune regulation, with T-cells playing an important role. In diseases such as rheumatoid arthritis, inflammatory bowel disease and periodontal disease, chronic immune activation is associated with loss of bone mass. Ageing, HIV disease and atherosclerosis are associated with low bone mass and dyslipidaemia; dyslipidaemia associated with ART may exacerbate T-cell-mediated bone loss in HIV-infected patients. Persons with lower bone density (for example post-menopausal women) tend to have higher serum lipid levels and, correspondingly, lipid-lowering drugs retard bone loss.

Soluble receptor activator of NF-κB ligand (RANKL) is a key regulator of bone remodelling, interacting with RANK that is expressed on multiple cell types, including osteoclasts [45]. Osteoprotegrin (OPG) is a decoy receptor for RANKL that is expressed on and secreted by activated T-cells and is involved in T-cell and dendritic cell communication and survival. *In vitro*, oxidized lipids induce production of RANKL and lectin-like oxidized LDL-C receptor-1 (LOX-1) by unstimulated T-cells and reduce OPG expression. LOX-1 plays a crucial pathogenic role in atherosclerosis. Chronic activation of T-cells *in vitro* leads to replicative senescence, which is accelerated by oxidized lipids. In dyslipidaemic mice with bone loss, an increased RANKL:OPG ratio is seen and there is increased T-lymphocyte osteoclastogenic cytokine messenger RNA. In the bone marrow, there is a higher proportion of activated memory T-cells, supporting osteoclast formation. These results suggest that ageing and HIV may have additive effects on bone loss.

Although changes in BMD are often observed in HIV-infected individuals, the risk of bone fractures associated with these changes are uncertain. Falutz *et al.* [46] reported application of FRAX scoring to an HIV-infected cohort to assess the risk of fractures in this population. The FRAX score uses femoral neck BMD measurements and multiple other measures related to disease, personal characteristics and lifestyle to predict the 10-year risk of hip or major osteoporotic fractures. In this study of 130 HIV-infected males with a median age of 48 years, only 9% had normal BMD measurements, whereas 64% were osteopaenic and 27% were osteoporotic. FRAX scoring indicated that this relatively young population had fracture risks normally associated with a significantly older population, and these risks were likely to increase as the patients aged.

A roundtable discussion involving the speakers from the bone metabolism session focused on the unexplained reduction of BMD that typically accompanies the initiation of ART. This reduction is typically of a similar magnitude to that seen following menopause, and is accompanied by some up-regulation of osteoclasts and down-regulation of osteoblasts. The reduction in inflammation that is associated with ART should be good for bone markers; however, it is not. Additional data concerning the trajectory of BMD during the year preceding ART may help clarify the ART-associated changes.

HIV-infected patients often have many risk factors for bone loss, such as lipodystrophy, drug use and smoking. Paradoxically, among HIV-infected individuals, men may be at greater risk for low BMD than women [47]. In addition, men who have sex with men without HIV infection were reported to have a higher than expected rate of low BMD [48]. Age-related bone loss is an increasing issue for HIV-infected patients. Another complicating factor to reduction of BMD is the unknown contribution of the weight gain that frequently accompanies the initiation of ART. Further work is needed to understand the contributions of HIV infection, ART, lifestyle factors and other disease-related factors to loss of BMD in HIV-infected patients.

The potential value of adjunctive therapies to improve BMD was also addressed by the roundtable. Options include vitamin D and calcium supplements, which have positive but generally modest effects on BMD; statins, which have a favourable impact on BMD in women (no data in HIV infection); and bisphosphonates. Overall, these interventions have

little demonstrated benefit for reducing fracture risk for younger osteopaenic individuals. Indeed, the clinical impact of reduced BMD in non-elderly HIV-infected individuals is uncertain and possibly minimal. There are few data suggesting an increased risk of fractures in this group. It was suggested that HIV-infected individuals >50 years of age should receive BMD screening (DEXA) routinely as a strategy for identifying those individuals with severe bone loss who require treatment. However, screening of younger patients was not advocated, as the costs associated with routine screening were seen to be prohibitive, and non-significant reductions of BMD may trigger unnecessary and potentially toxic treatment.

Mitochondrial dysfunction

Mitochondrial dysfunction has been associated with the use of some antiretroviral agents, particularly nucleoside analogues. Several reports addressed the clinical effects and molecular mechanisms associated with ART-associated mitochondrial toxicities. Glover *et al.* [49] assessed the effects of different ARTs on exercise capacity and mitochondrial function in 30 HIV-infected subjects. Results showed that the use of AZT, ddI and d4T were associated with diminished aerobic exercise capacity, compared with 14 matched uninfected controls, and lower mitochondrial DNA (mtDNA) and mitochondrial oxidative enzyme capacity in muscle. These adverse effects were present in previous as well as current users of those agents, suggesting that the deleterious effects in muscle may persist after the drugs are discontinued.

Hulgan *et al.* [50] reported that metabolic complications associated with ART can differ according to mtDNA haplogroup. For many metabolic complications, associations with NRTIs suggest a mitochondrial aetiology. The circular DNA of mitochondria encodes 13 oxidative phosphorylation proteins; mtDNA haplogroups are defined by patterns of single nucleotide polymorphisms (SNPs) and may affect disease susceptibilities. Haplogroup prevalence varies globally; previous work suggested some associations between ART-associated lipodystrophy and mtDNA haplogroup. The report by Hulgan *et al.* [50] was derived from analysis of samples from a class-sparing study in which 757 treatment-naive subjects were randomly assigned to one of three groups: EFV plus LPV/r, EFV plus 3TC plus a second NRTI (d4T, AZT or TDF) or LPV/r plus 3TC plus a second NRTI. Metabolic outcomes included increased total cholesterol in the NRTI-sparing group and increased lipoatrophy with d4T or AZT (relative to TDF) and with EFV (relative to LPV/r). The haplogroup analysis included 245 non-Hispanic White subjects from this study, who were assigned to 1 of the 9 European haplogroups by mtDNA SNP genotyping. Virological response did not differ across haplogroups. At baseline, haplogroup I, which is found most frequently in Spain, showed higher body fat and cholesterol compared with the other haplogroups. During 96 weeks of subsequent treatment, haplogroup I showed decreases in extremity fat and non-HDL-C in contrast to the other haplogroups, which showed increases in these parameters. A significantly higher proportion of patients in haplogroup I, compared with the non-I haplogroups, showed lipoatrophy at week 96 (≥20% decrease in extremity fat). These results suggest that mtDNA variation may influence metabolic changes during ART.

Another haplogroup analysis by Arenas-Pinto *et al.* [51] concerned 12 European HIV-infected patients with severe hyperlactataemia or lactic acidosis, the *sine qua non* of mitochondrial dysfunction. Of the 12 patients, 11 were receiving at least 1 dideoxynucleoside and 7 were receiving ddI and d4T simultaneously. However, bearing in mind the very small sample size, there was no evident association between mitochondrial dysfunction and haplotype.

AZT induces myopathy in HIV-infected patients by interfering with mtDNA replication. Lebrecht *et al.* [52] reported that in Wistar rats, AZT leads to mtDNA depletion in primarily slow muscle fibres. This effect was reversed, in part, by feeding the rats a dietary supplement containing high uridine bioavailability.

EFV has been reported to induce bioenergetic stress in hepatic cells by inhibiting mitochondrial O_2 respiration and decreasing ATP production. Blas-García *et al.* [53] reported that in cultured hepatic Hep3B cells, EFV but not NVP significantly inhibited complex-I-mediated mitochondrial respiration, reducing O_2 consumption and inducing accumulation of reactive oxygen species (ROS). The results suggest the effect is specific to EFV and not the NNRTI class as a whole; however, the concentration of EFV used may have been supratherapeutic and so of uncertain clinical significance.

Other toxicities and complications

Ageing

The effect of ageing on HIV-infected individuals was a recurring issue at the conference, where associations between ageing and multiple drug- and disease-related parameters have emerged. Campisi [54] reviewed cellular mechanisms of ageing and their relationships to HIV infection and ART. Ageing can be defined as a process that turns a more fit organism into a less fit organism, with associated increases in disease susceptibility. Age is the most significant risk factor for many of the most prevalent medical conditions, for example, neurodegeneration, osteoporosis, cancer, cardiovascular disease, diabetes, sarcopaenia, and impaired liver and

lung function. A key issue is whether there is a common biology that links ageing with these diverse conditions.

Cellular damage is an important cause of ageing, caused by epigenetic drift, evolutionary antagonistic pleiotropy, germline mutations, somatic mutations or mitochondrial dysfunction [54]. The greatest sources of somatic damage are ROS that are normal by-products of mitochondrial metabolism. Many antiretroviral NRTIs inhibit mitochondrial polymerase; this may increase ROS leading to damage of mtDNA, RNA and protein, causing cell death or triggering cellular senescence – described as compromised cellular and tissue renewal – and leading to inflammation. In this context, inflammation was described as a primary driving force for all age-related diseases, including cancer.

Apoptosis and cellular senescence are potent tumour suppressor mechanisms [54]. These have caretaker functions that protect the genome and promote longevity, as well as gatekeeper functions that eliminate damaged cells. Senescent cells arrest proliferation irreversibly and can accumulate in many tissues. There are marked changes in gene expression, leading to growth arrest, resistance to apoptosis and altered function. The senescence-associated secretory phenotype (SASP) results in altered secretion of cytokines by senescent cells, affecting the cellular microenvironment in multiple ways. Normal tissue and cellular functions are disrupted, and pro-malignant phenotypes can be conferred on neighbouring cells, inhibiting tissue regeneration and/or promoting tumourigenesis.

SASP components are involved in tissue repair, suggesting that the senescence response has evolved to enhance tissue regeneration [54]. Some SASP cytokines activate natural killer (NK) cells to cause destruction of cancer cells, but this also leads to chronic inflammation and accelerates ageing through the associated release of oxidants. Although the proportion of senescent cells in a tissue is small (approximately 1% even in most aged tissues), senescence can still drive inflammation through the effects of SASP on neighbouring cells.

Renal toxicity

TDF has been associated with renal tubular dysfunction and, in more extreme cases, with Fanconi's syndrome with tubular damage or acute tubular necrosis. Evidence suggests mitochondrial toxicity as a possible mechanism. Cotter *et al.* [55] reported the results of *in vitro* studies showing that TDF may induce fibrogenic changes and reduce cell proliferation in the renal proximal tubular epithelium, potentially contributing to TDF-associated renal toxicity. After 1 week of TDF treatment of HK-2 cells, an immortalized line of renal proximal tubule epithelial cells, cell numbers decreased significantly, with associated increases in the apoptosis marker caspase and the kidney injury markers neutrophil gelatinase-associated lipocalin (N-GAL) and fatty acid binding protein-1 (FABP1). TDF also triggered morphological changes and marked increases in transforming growth factor-β (TGF-β), connective tissue growth factor (CTGF) and collagen, which are responses associated with epithelial mesenchymal transition (EMT). EMT is a process whereby epithelial characteristics are lost, with cells becoming more fibroblast-like. *In vitro*, this can lead to fibrosis, and EMT is known to play a role is disease processes affecting the kidney. Although there is currently little clinical evidence of TDF-associated renal fibrosis, disruption of cell function and/or morphology may contribute to the lesions observed in biopsies. TGF-β is known to drive reduced renal epithelial proliferation, increased apoptosis and EMT; thus, increased TGF-β production may contribute to TDF-associated tubular damage.

Sleep apnea

Brown *et al.* [56] reported that obstructive sleep apnea (OSA) is highly prevalent in HIV-infected men, even those with normal BMI. This study investigated possible associations between OSA and systemic inflammation in 58 HIV-infected men receiving HAART, 41 HIV-infected men not receiving HAART and 60 uninfected men. Subjects were evaluated in a nocturnal sleep study with measurement of plasma markers of systemic inflammation. Moderate-to-severe OSA was found in 57% of the uninfected group, and in 41% and 44% of the HIV-infected treated and untreated groups, respectively. TNF-α, sTNFR-1, sTNFR-2 and IL-6 were all increased more in the HIV-infected groups than in the uninfected group, independent of age, race and BMI, and generally more in the untreated than in the HAART-treated group. In the untreated HIV-infected group, TNF-α and IL-6 were increased more in those with severe OSA than in those without, after adjusting for age, race and BMI. However, no relationship was observed between inflammatory markers and moderate-to-severe OSA in the other two groups; thus, OSA was associated with systemic inflammation in HIV-infected men not receiving HAART, but this association was not present in HIV-infected HAART recipients or uninfected men. These discrepant findings suggest that the relationship between OSA and HIV infection may not be causal.

Cognitive impairment

Cognitive impairment appears to be present in >30% of HIV-infected adults and is associated with increasing age [57]. Although generally mild, it can adversely affect driving ability and adherence to medication. Possible aetiologies of cognitive impairment despite effective ART include inadequate control of HIV in monocytes and brain, pre-HAART brain injury, neurological

toxicity of ART, coexisting morbidity and conventional influences such as cerebrovascular disease and Alzheimer's disease. Vascular risk is increased by ART, and risk factors such as diabetes, dyslipidaemia and smoking are frequent in HIV-infected adults. HIV infection is itself an independent risk factor for cardiovascular disease. HIV and cerebrovascular disease are both subcortical processes that involve cerebral white matter and the basal ganglia, and may have similar clinical presentations of cognitive dysfunction.

Jahanshad *et al.* [57] investigated whether the cognitive impact of cerebrovascular disease can be measured in HIV-infected individuals. Subjects were stratified according to the presence or absence of HIV infection and the presence or absence of cerebrovascular risk factors (hypertension, smoking, high cholesterol and diabetes), and assessed with respect to memory, psychomotor, motor and working memory parameters. Results showed that overall, HIV-infected patients scored lower in cognitive assessments. The presence of cerebrovascular risk factors further impaired neuropsychological performance in the HIV-infected group. Unsurprisingly, multiple risk factors had additive effects on cognitive impairment and a greater impact on HIV-infected subjects than on uninfected subjects, especially with respect to motor skills.

In a related study, diffusion tensor imaging (DTI), a magnetic resonance imaging technique, was used to measure the local rate and direction of water diffusion in different brain areas of 30 HIV-infected subjects [57]. DTI results were compared on the basis of the presence or absence of vascular risk factors. In the whole brain analysis, there were no significant differences for any risk factor; however, some trends and significant differences were seen in regional analyses. The results suggest that DTI may help define the impact of cerebrovascular disease in high-risk populations.

Malignancies

Multiple types of malignancies have been associated with HIV infection and related immunosuppression. Biggar [58] reviewed studies that addressed the extent to which immunity controls the expression of different types of cancer. Kaposi's sarcoma (KS), some types of non-Hodgkin's lymphoma (NHL), cervical cancer and a few other malignancies have been linked to HIV infection by time, place and affected population. The incidences of KS and NHL increased markedly, particularly in New York City and San Francisco, in 1981 coincident with the start of the AIDS epidemic, and subsequently decreased sharply in the mid to late 1990s with the advent of HAART. Other malignancies that are more common in HIV-infected patients include anal cancer, Hodgkin's lymphoma, liver cancer, leukaemia, lung cancer, oropharangeal cancer, brain cancer and myeloma. Some of these cancers are linked to viral infections, but not all (for example, lung and lip cancers); other contributing factors remain uncertain but may include lifestyle, diagnostic bias or misclassification. The contribution of immunosuppression is uncertain and probably variable, but many cancers that are more common in HIV-infected patients are also more common in organ transplant recipients receiving immunosuppressive therapy.

The relationship of cancer incidence with $CD4^+$ T-cell counts varies by type of cancer [58]. A significant relationship has been demonstrated for KS, NHL and hepatocellular carcinoma (linked to hepatitis B virus or hepatitis C virus [HCV] infection), but the incidences of human papillomavirus-related anal and cervical cancers in HIV-infected individuals is only variably associated with $CD4^+$ T-cell counts. A link has been demonstrated between $CD4^+$ T cell-count and cervical intraepithelial neoplasia, but not invasive cervical cancer. Interestingly, the incidence of Hodgkin's lymphoma in HIV-infected patients is directly related to $CD4^+$ T-cell count and increases in patients receiving HAART. However, it is uncertain if HIV-related immunosuppression reduces the incidence of Hodgkin's lymphoma or reduces the ability to see it: 97% of the mass of a Hodgkin's tumour is composed of cells attracted by cytokines, which are not recruited with severe immunosuppression.

It is also uncertain whether $CD4^+$ T-cell counts are always the right measure of immune function with respect to cancers; $CD8^+$ T-cells may be more important in some cases [58]. It remains unclear why KS and NHL are so closely associated with $CD4^+$ T-cell counts, whereas other cancer types, even those with viral aetiologies, show a much weaker relationship.

Hepatitis C virus coinfection

Sarrazin [59] reviewed new antiviral drugs in development for treatment of HCV infection, focusing on adverse event profiles and pharmacokinetic interactions. Coinfection with HIV and HCV is common. Current therapy for HCV – pegylated interferon (PEG-IFN) and ribavirin (RBV) – achieves a sustained virological response (SVR) in approximately half of treated patients. There are no other options at present for the patients who fail to achieve an SVR, and many patients are not candidates for PEG-IFN/RBV therapy due to contraindications or other issues. Improved therapies for HCV are therefore needed.

HCV offers multiple molecular targets for intervention and many compounds are in preclinical or clinical development [59]. Most of these specifically targeted antiviral therapy for HCV (STAT-C) compounds are inhibitors of HCV protease or RNA polymerase; however, multiple drugs with other mechanisms are also in development. Clinical data to date suggest that monotherapy with any of these agents will be inadequate due to the emergence

of resistance, lack of sufficient antiviral effect and/or low SVR rates. Combining HCV protease inhibitors with PEG-IFN/RBV has promise from an efficacy perspective, with less resistance than STAT-C monotherapy and higher SVR rates than those achieved with PEG-IFN/RBV alone. However, the significant tolerability issues associated with PEG-IFN/RBV remain, and are potentially amplified by coadministration of a STAT-C compound.

Development of a number of STAT-C agents has been stopped due to toxicity [59]. These toxicities have been of multiple types, including cardiac, renal and gastrointestinal. Tolerability is also an ongoing issue for compounds that remain in development. The HCV protease inhibitor telapravir, combined with PEG-IFN/RBV, has been associated with a 25% adverse-event-related discontinuation rate in a Phase II trial, with a hypersensitivity rash and gastrointestinal disorders being the most common adverse events. Combining the HCV protease inhibitor bocepravir with PEG-IFN/RBV elicited a high rate of anaemia (partially reversible with erythropoetin) and was associated with marked increases in the rates of dysgeusia, neutropaenia and vomiting, compared with rates seen with PEG-IFN/RBV alone. Other STAT-C drugs in development are variously associated with gastrointestinal abnormalities (common with HCV protease inhibitors and some HCV polymerase inhibitors); headache (reported with several HCV polymerase inhibitors); and, less frequently, hepatic insufficiency, renal toxicity, neutropaenia, rash and prolongation of the cardiac QT interval.

Drug–drug interactions are also being studied for STAT-C compounds [59]. Telapravir and bocepravir require three times daily dosing; ritonavir boosting of the HCV PI narlepravir allows twice daily dosing, providing similar virological responses to telapravir three times daily. Combinations of STAT-C compounds are also being evaluated, for example, the HCV polymerase inhibitor R7128 with the HCV protease inhibitor R7227. With this combination, no adverse drug–drug interactions and no serious adverse events were reported over 14 days; however, a diverse array of generally low-grade adverse events and laboratory abnormalities were seen.

Current issues for development of STAT-C compounds include resistance, cross-genotype activity, optimal dosing schedules, adverse events, evaluation of drug combinations without PEG-IFN/RBV, drug interactions and ritonavir boosting [59]. There are no data for these compounds in HIV-coinfected patients or in patients with advanced liver disease, although a study of telapravir in HIV coinfection is planned.

Conclusions

The success of ART has led to a growing number of HIV-infected patients remaining on therapy for ever-increasing durations and a progressive increase in the number of older patients. Beyond the primary need of maintaining suppression of viral replication, there has been increasing recognition of the need to understand the contributions of drug toxicities and comorbid conditions to patient health, quality of life and ultimately survival. HIV-infected individuals, whether receiving ART or not, have a higher risk of acquiring many serious diseases that are not directly related to HIV infection, such as cardiovascular disease, diabetes, osteopaenia, kidney disease, cognitive disorders and some types of cancer. It is evident that many of these conditions and toxicities are influenced by multiple factors, including both HIV-related factors and other risk factors commonly reported for the general population. Ageing and physical senescence add yet another dimension to the understanding of disease in HIV infection.

Substantial effort has been directed towards understanding how HIV-specific and ART-specific risk factors interact with other influences with respect to the many morbidities found in HIV-infected individuals. The proceedings of the workshop demonstrate continuing progress in understanding these relationships, but also serve to illustrate the many questions that remain.

Disclosure statement

The authors declare no competing interests.

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