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# NEW FRONTIERS FOR PEOPLE LIVING WITH HIV: WHAT ARE THE CHALLENGES WE NOW FACE?



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# MANAGING HIV-ASSOCIATED INFLAMMATION AND AGEING IN THE ERA OF MODERN ART

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**ABSTRACT**

Objectives: This paper aims to address the concerns around ongoing immune activation, inflammation, and resistance in those ageing with HIV that represent current challenges for clinicians.

Methods: Presentations at a symposium addressing issues of ageing with HIV infection were reviewed and synthesised.

Results: The changing natural history and demographics of human immunodeficiency virus (HIV)-infected individuals means new challenges in contemporary management. In the early years of the epidemic, management was focussed on acute, potentially life-threatening AIDS-related complications. From initial monotherapy with first-generation antiretroviral therapy (ART), the development of combination highly active ART (HAART) allowed HIV control but ART toxicities, treatment adherence and drug resistance emerged as major issues. Today, the availability of potent and tolerable ART has made viral suppression achievable in most people living with HIV (PLHIV), and clinicians are confronted with managing a chronic condition among an ageing population. The combination of diseases of ageing and the co-morbidities associated with HIV-infection, even when well controlled, results in a complex set of challenges for many older PLHIV. There is a growing

appreciation that many non-AIDS-related co-morbidities are caused, at least in part, by persistent, low-grade immune activation, inflammation, and hypercoagulability, despite suppressive ART.

Conclusions: In order to further improve HIV management, it is important to understand the enduring effects of chronically suppressed HIV infection, the potential contribution of these factors to the ageing process, the possibility of drug resistance, and the impact of different treatment strategies, including early ART initiation.

**Key words:** HIV infection; antiretroviral therapy; inflammation; immune activation; co-morbidities; ageing; resistance

## INTRODUCTION

It has been almost 40 years since the first report of acquired immunodeficiency syndrome (AIDS) (1) followed by the identification of human immunodeficiency virus (HIV) as the causative agent (2, 3). In that time, the clinical management of HIV has continued to evolve. In the early days of the epidemic, HIV was a progressive, almost universally fatal infection (4). Hospital admissions were common for AIDS-related conditions and with limited treatment options, no viral load (VL) testing, no resistance testing, and relatively short-term benefits from therapy, clinical management was acute and often palliative in nature.

From around 1996 the complexity of clinical management began to increase in response to advances in HIV treatments and scientific knowledge. The benefits of combination antiretroviral therapy (ART) were demonstrated, VL testing became available and mortality/morbidity rates dropped sharply (5). As hospital admissions for HIV/AIDS declined, clinical care arrangements evolved to encompass a mix of specialist and generalist services. However, by the late 1990s, clinicians were faced with the emerging, unpredicted effects of early ART regimens, including lipodystrophy, cardiovascular disease (CVD), diabetes risks and changes in bone mineral density (6,

7). Issues of drug resistance and cross-resistance became an increasing problem as failure to successive ART regimens accumulated. Resistance testing and therapeutic drug monitoring began to be used, and the terms 'treatment failure' and 'salvage therapy' became common place as many people living with HIV (PLHIV) were unable to be stabilised on ART due to toxicities and side effects, sub-standard ART regimens, and problems with treatment adherence (6).

Each successive evolution of ART has been associated with greater simplicity and less toxicity (8), and the combinations recommended by today's HIV guidelines make viral suppression an achievable and sustainable goal in most PLHIV who are identified early and who adhere to their regimens (9, 10) ,as well as the majority of patients with longterm HIV who may have experienced sub-optimal regimens and previous treatment failure.

In most developed countries, the median age of PLHIV has passed 50 years,

a figure which has doubled since the introduction of ART and is continuing to increase globally (11).

However, durable viral suppression and the prevention of AIDS-related illnesses have not presented the challenges they may have in the past, PLHIV are living longer (12), yet they continue to experience an elevated risk of chronic health complications, particularly CVD (13-15), cancer (16, 17), cognitive impairment (18) and frailty (19). This elevated risk is often independent of traditional risk factors that are more prevalent in PLHIV, such as smoking, alcohol and dyslipidaemia (20). Rather than dealing with acute, potentially life-threatening complications, clinicians are now confronted with managing a chronic condition with an increasing prevalence of non-AIDS-related co-morbidities (21). Recent literature has reshaped our understanding of morbidity and mortality among PLHIV in the current era, and there is growing appreciation that many non-AIDS-related co-morbidities may be driven least in part, by persistent, low-grade immune activation inflammation and hypercoagulability despite suppressive ART (13, 22-26).

The changing natural history and demographics of HIV means that contemporary management must evolve and respond to these new challenges. An understanding of the often-complex interplay between chronic low-level inflammation and non-AIDS-related morbidities will be necessary in

order to prioritise and optimise health outcomes in an aging population of PLHIV. This paper discusses concerns around ongoing immune activation,, ageing, and the possibility of drug resistance that represent current challenges for clinicians. Significant progress has been made in understanding the mechanisms associated with these issues, and solutions are needed to transform HIV into a condition whereby healthy ageing without chronic illness is possible.

## DRIVERS OF IMMUNE DYSFUNCTION AND PERSISTENT INFLAMMATION

There is now consistent evidence indicating that despite successful viral suppression, ART reduces but does not normalise the inflammatory response to HIV. Indeed, markers of both innate and adaptive immune activation persist in ART-treated PLHIV with undetectable HIV RNA (24, 27-33). The causes of persistent inflammation in virologically suppressed PLHIV are multifactorial and complex. First, translocation of microbial products from the intestinal lumen to the systemic circulation is a central factor that determines the severity of HIV-associated chronic immune activaton and subsequently, non-AIDS comorbidities (34, 35). Damage to the gut-associated lymphoid tissue (GALT) occurs early in HIV infection leading to increased microbial translocation across the gut wall and into the systemic circulation. The release of bacterial products provokes a persistent, systemic activation of the innate immune system that triggers and maintains inflammation (34-37). ART does not completely reverse the damage to the gut mucosal epithelia during early HIV infection nor does it completely restore HIV-associated microbial dysbiosis (38-41).

Second, HIV persistence in certain anatomical compartments, such as the lymph nodes, central nervous system (CNS) and gastrointestinal tract is recognised as potential driver of IA (42-46).

HIV can persist in PLHIV on ART in multiple forms: in both a latent and transcriptionally active state, in quiescent or proliferating cells, in multiple T-cell subsets, monocytes and macrophages; as well as tissue sites and as both defective and intact virus (47). Several studies have demonstrated a strong association between markers of inflammation and viral persistence in PLHIV on ART (48). Although both pathways are likely to be interrelated, whether HIV persistence drives immune activation or vice versa, remains the subject of further research.

Third, clinical or subclinical co-infections are a potential source of chronic immune activation in ART-treated HIV infection (49). Co-infection with hepatitis B virus (HBV), hepatitis C virus (HCV), or cytomegalovirus (CMV) are common with HIV infection and has been linked to heightened levels

of plasma lipopolysaccharide (LPS), a marker of microbial translocation that activates the coagulation process, and CD8+ T-cell activation, respectively (50-52).

Finally, inflammatory lipids may contribute to immune activation in ART-treated HIV infection. Levels of oxidized low-density lipoprotein (oxLDL) are higher in PLHIV compared to HIV-uninfected individuals, and oxLDL levels correlate with markers of monocyte activation (53).

## CHRONIC INFLAMMATION AND NON-AIDS-RELATED MORBIDITY AND MORTALITY

The first strong evidence for an association between biomarkers of innate immune activation, hypercoagulability and increased risk of non–AIDS-defining morbidity and mortality was reported in the pivotal SMART trial (54). The trial showed that plasma levels of interleukin-6 (IL-6) and D-dimer (a marker of coagulation) were significantly associated with all-cause mortality among ART-treated individuals. Multiple studies have subsequently confirmed these findings across a broad range of CD4+ T-cell counts and for both ART-treated and -naïve individuals (13, 55-58). Since then,

elevated levels of high-sensitivity C-reactive protein (hs-CRP) (57, 59, 60), fibrinogen (60), soluble tumour necrosis factor receptor 1 and 2 (sTNFR1, sTNFR2) (26), bacterial translocation (e.g. LPS) (61), and monocyte/macrophage activation (soluble CD14 [sCD14]) (62, 63), among others, have also been found to predict non-AIDS-defining morbid events in PLHIV on suppressive ART.

Several markers related to the adaptive immune system also continue to demonstrate prognostic significance in ART-treated individuals. The rate at which CD4+ T-cells increase after ART initiation is highly variable; however, CD4+ T-cell recovery is associated with reduced morbidity and mortality in HIV-infected individuals (8, 64, 65). Even in PLHIV with baseline CD4+ T-cell counts >500 cells/μL, further increases are still associated with a slight benefit in terms of mortality

(65). A persistently low CD4+/CD8+ T-cell ratio despite virological control reflects a higher risk of morbidity in ART-treated PLHIV (67-71). Evidence from longitudinal studies suggests that early ART initiation (e.g. within 6 months of infection) might contribute to more rapid and robust CD4+/CD8+ T-cell ratio normalisation than deferred initiation (e.g. >2 years) (71), a finding that adds additional support to the need for early HIV diagnosis and early initiation of ART (9, 10).

Mechanistic evidence to support this observation suggests that HIV decreases the proportion of CD28−CD8+ T-cells expressing CD57, a marker of proliferative history and poor proliferative capacity (71). This CD8+ T-cell abnormality is evident within the first few months of HIV infection and can be largely reversed by early ART initiation, but not when ART is delayed by even a few years. Research suggests that the expansion of CD-57 expressing CD8+ T-cells, reflected as a low CD4+/CD8+ T-cell ratio, identifies a subgroup of individuals with a number of immunological abnormalities and a poor prognosis (73). These findings explain, in part, why during ART-mediated viral suppression, some individuals achieving CD4+ T-cell counts >500 copies/μL experience a simultaneous decline in CD8+ T-cell counts, leading to normalisation of the CD4+/CD8+ T-cell ratio, while others maintain high levels of circulating CD8+ T-cells and hence a persistently low

CD4+/CD8+ T-cell ratio.

In PLHIV on suppressive ART, markers of persistent immune activation and inflammation have been linked to a host of morbidities, including type 2 diabetes (74), neurocognitive dysfunction (75), renal disease (76), chronic obstructive pulmonary disease (77), and depression (78). However, most research to date has focussed on the role of chronic inflammation in the pathogenesis of CVD, including myocardial infarction (MI), stroke, heart failure and sudden cardiac death (79-81). This is understandable given that CVD accounts for 11-30% of mortality in PLHIV (82, 83), and while there is a relatively high prevalence of traditional CVD risk factors in populations of PLHIV, this does not adequately account for the observed higher prevalence (20, 84).

Immune activation and inflammation play important roles in the pathogenesis of atherosclerosis in both HIV-infected and non-infected persons (81, 85). However, vascular inflammation occurs earlier in PLHIV despite effective ART (25), and PLHIV are more likely to develop atherosclerotic plaques compared to HIV-negative controls matched for age and traditional risk factors (86). There is now a consistent body of evidence from prospective and cross-sectional studies to suggest that monocytes play a central role in the development of atherosclerosis. Plasma levels of the monocyte activation marker sCD14 are associated with coronary artery calcification (87) and more rapid progression of carotid intima media thickness (88), while sCD163 (another marker of monocyte activation) is associated with arterial wall inflammation (24).

Several inflammatory mechanisms, particularly related to monocyte activation, provide important mechanistic insight into the role of monocytes as a possible driver of CVD in ART-treated PLHIV (89). It is hypothesised that HIV may promote an atherogenic phenotype that functions independent of traditional risk factors and that despite viral suppression, chronic inflammation results in the activation of pro-inflammatory monocytes and secretion of chemokines from the endothelium.

Activation of the endothelium and of monocytes results in greater monocyte adherence with subsequent transendothelial migration to form foam cells or lipid-laden macrophages (which are associated with plaque progression) (89-91). Furthermore, the characteristics of such plaques (e.g. high lipid content, increased numbers of inflammatory cells) are features common to unstable, vulnerable plaques with a high risk of rupture (92).

While there is consistent evidence for a relationship between markers of inflammation and several non-AIDS-related comorbidities, it is important to note that the majority of biomarkers presently used to evaluate systemic immune activation and inflammation have yet to be validated as surrogate markers for individual end-organ diseases in PLHIV and often do not clearly and fully reveal the underlying

mechanisms and causal pathways involved (93). Prentice et al. developed criteria that are sufficient to validate surrogate end points in phase 3 trials (94). These criteria essentially require that the surrogate must be a correlate of the true clinical outcome and fully capture the net effect of treatment on the clinical outcome. Although the first criterion is usually easy to verify, the second is not (95). Randomised controlled trials that evaluate the effects on both biomarkers and the event rates for end-organ disease are the strongest approach to help clarify the central causal relationships and are required to help validate some hypothesised associations and eliminate others. For this reason, the current guidelines issued by the U.S. Department of Health and Human Services provide no instructions for the changes in immune markers in ART-treated PLHIV, emphasis instead is placed on monitoring modifiable risk factors for comorbid conditions (9).

## INFLAMMATION AND AGEING

The cause of age-related comorbidities in PLHIV is likely to be multifactorial given the complex mix of HIV infection, ART regimens, chronic viral co-infections and lifestyle/behavioural factors. Nonetheless, HIV and ageing share several immune-related changes: pro-inflammatory biomarkers, including IL-6, TNF, and D-dimer are commonly found in PLHIV on suppressive ART as well as in

elderly HIV-negative individuals (34). The general process of ageing is accompanied by a low-level chronic systemic inflammatory state that contributes strongly to frailty, a clinical syndrome of increased vulnerability due to an age-related deterioration in reserve across multiple physiological systems (96). Frailty is defined as the presence of at least three of five of the following: physical slowness, fatigue, low physical activity, weakness, and weight loss (96), is now recognised as an important HIV-associated condition (95). As expected, the prevalence of frailty is much higher in PLHIV >50 years compared to the general population (97). Frailty in PLHIV has also been shown to be predictive of all-cause morbidity, mortality and progression to AIDS (97, 98), and to be associated with measures of innate and adaptive immune activation despite suppressive ART (97-100).

In a cross-sectional study from Australia, investigators compared the prevalence of frailty and its relationship with quality of life (QoL) in 93 HIV-positive men >50 years of age on suppressive ART (19). Frailty was assessed using three measures: the Frailty Phenotype (101), Frailty Index (102) and Edmonton Frail Scale (103). They found the prevalence of frailty to be 10.8%: approximately double that observed in the age-matched general population (104). Frailty was significantly associated with poorer QoL regardless of which frailty instrument was used.

Although adults ageing with HIV are subject to the same risk factors for age-related diseases and conditions as uninfected adults, they differ in prevalence of harmful health behaviours. Smoking, alcohol, and substance abuse are modifiable risk factors for comorbidities, yet their prevalence is higher among PLHIV than the general population (105). However, PLHIV also experience ongoing HIV-associated immune activation and inflammation (106) and often the legacy of chronic exposure to more toxic ART, likely leading to organ system injury (107). Against this background, the question as to whether HIV accentuates or accelerates ageing has been a topic of considerable debate. Accentuated aging occurs when there is an increased burden of age-related-damage, but the year-on-year change in damage

remains constant over time, In contrast, accelerated aging occurs when the decline arises earlier than expected and implies a progressive increase in the rate of decline (108).

To address this question, the COBRA study compared established biomarkers of ageing between PLHIV aged ≥45 years (n=134), life-style-comparable HIV-negative controls (n=79), and aged-matched blood donors (n=35) (109). Biological age was estimated using a validated algorithm based on 10 biomarkers, and linear regression analysis was used to determine relationships between 'age advancement' and HIV status/parameters, lifestyle, CMV, HBV and HCV infections. Overall, age advancement was greatest in PLHIV and was related to prior immunodeficiency and cumulative saquinavir exposure. Furthermore, chronic HBV, higher anti-CMV IgG titre, higher CD8+ T-cell

count, and nadir CD4+ T-cell count were independently associated with age advancement. These results suggest that PLHIV on suppressive ART experience accentuated ageing compared with HIV-negative individuals with similar lifestyles. The results also emphasise the importance of controlling coinfections in PLHIV. Of note, the same cohort over a period of 2 years found no evidence for accelerated brain ageing comparing HIV-positives and negatives (110)

In Australia, 46% of all PLHIV are >50 years of age (107). Similar figures are observed in the US and Europe (111), and modelling predicts that the prevalence of PLHIV >50 years will exceed 70% by 2035 (108). The changing natural history of HIV and the ageing profile of PLHIV has considerable implications for treatment as well as policy and planning of health services. Furthermore, understanding the relative contribution of HIV infection, ART and lifestyle factors to the development of these comorbidities in PLHIV is imperative to developing screening, prevention and advocacy programs for the rapidly expanding older population of PLHIV (105).

In the context of improving care for PLHIV, there are many unique issues associated with ageing and HIV. For example, understanding the age distribution of PLHIV is important in terms of expected comorbidities and mapping appropriate services. Even among PLHIV receiving treatment

in the current era, potential differences in the disease trajectory between those starting ART early compared to those who are treated late require consideration. Multimorbidity is also much higher in PLHIV compared to age-matched controls (105), as is polypharmacy (111). Indeed, a recent survey of Australian HIV clinicians found that management of comorbidities and access to mental health services were among their key concerns (21).

To date, the optimal model of care for managing ageing PLHIV has not been established. Today's HIV clinicians require not only knowledge of ART but also expertise in preventing and managing the comorbidities associated with chronic inflammation, including many of the complications typically associated with ageing. It stands to reason that traditional HIV clinics may not have the

resources or appropriate expertise to deal with these often-complex cases and there is a strong argument for the development of additional models of care. Upskilling HIV healthcare providers in the care needs of elderly PLHIV, involving geriatricians in HIV consultations, or the provision of dedicated clinics for elderly PLHIV, are just some of the potential models for consideration.

## INTERVENTIONS TO TARGET IMMUNE DYSFUNCTION AND INFLAMMATION

To the extent that immune activation and chronic inflammation persist in HIV, contemporary PLHIV may benefit from anti-inflammatory treatment given in addition to ART. A growing number of strategies and novel therapies designed to targeted inflammatory pathways implicated in disease progression have been assessed in PLHIV, albeit with mixed results.

### *Early ART*

Regardless of regimen, ART initiation consistently leads to decreases in most systemic inflammatory markers, indices of T-cell, and monocyte activation, although rarely to the levels seen in HIV-uninfected individuals (112). However, until recently most studies studied PLHIV who

initiated ART at relatively advanced stages of disease (28, 32, 106, 113, 114), and little was known about the effects of earlier initiation. The findings from two relatively new trials: RV254/SEARCH010 (115) and a sub-analysis of ART initiation within one year of HIV diagnosis in adults from the START study (116) provide additional information in this area and suggest that early ART initiation attenuates inflammation more than initiating ART later in the course of chronic HIV infection.

In the RV254/SEARCH010 study ART was initiated in 78 acutely infected participants presenting to voluntary testing centres in Bangkok, Thailand (115). Early ART initiation (within 2 days) resulted in the normalisation of several inflammatory markers, namely, IL-6, IL-6R, TNF and D-

dimer. Levels of hs-CRP and sCD14 also decreased with ART; however, they remained elevated compared with HIV-uninfected participants. These findings are consistent with those from a sub-analysis of the START study, where HIV-positive adults with CD4+ T-cell counts >500 cells/μL were randomised to immediate versus deferred ART, and biomarkers were measured from stored plasma prior to randomisation and at month 8 (116). In participants randomised to immediate ART (median time from HIV diagnosis was 1.0 year), modest declines (e.g. 12-21%) were observed between baseline and month 8 for the inflammatory markers IL-6, D-dimer, serum amyloid A (SAA), interleukin-27 (IL-27), soluble intercellular adhesion molecule-1 (sICAM), and soluble vascular cell adhesion molecule-1 (sVCAM). Furthermore, participants with the highest VLs at baseline had the greatest declines in biomarker levels. In contrast, levels of D-dimer were elevated at month 8 in the deferred ART group, while hs-CRP increased in both the immediate and deferred groups.

It is important to note that the RV254/SEARCH010 and START studies (immediate treatment arm) differed substantially in terms of how soon after infection treatment was started. In RV254/SEARCH010, ART was initiated during acute infection, while in START, ART was

initiated at CD4+ T-cell counts >500 copies/mL, potentially well into the course of chronic infection. However, both results show that earlier ART initiation reduces some biomarker levels of systemic inflammation, coagulation, and vascular injury compared with ART deferral. For example, the normalisation of D-dimer levels with early ART, in association with decreased HIV burden, suggests a reduced risk of prothrombotic events. Other biomarker levels, namely sCD14 and hs-CRP remained elevated, suggesting that early ART initiation is insufficient to completely resolve the chronic inflammation associated with all-cause morbidity and mortality risk. The results also suggest that additional immunomodulatory therapies during early infection may be necessary to avert potential long-term consequences.

***Canakinumab and methotrexate***

The durable association between inflammation and atherosclerosis has prompted significant interest in strategies to inhibit inflammatory proteins upstream of hs-CRP, particularly IL-6 and interleukin-1β (IL-1β). The strongest evidence to date for targeting inflammatory pathways for the prevention of CVD comes from the CANTOS study, albeit in HIV-uninfected participants (117). CANTOS randomised patients with a prior history of MI and a hs-CRP level >2 mg/L to canakinumab, a selective IL-1β monoclonal antibody, or placebo. At a median follow-up of 3.7 years, canakinumab (150 mg every 3 months) significantly reduced the rate of recurrent CV events compared to placebo. The risk reduction was greatest among patients with the largest reductions in IL-6 and hs-CRP levels (117, 118), suggesting that the benefit was related to the targeting of the IL-1β–IL-6–CRP pathway. However, canakinumab was associated with a higher incidence of fatal infection or sepsis, as well as neutropenia and thrombocytopenia, than was placebo.

In addition to the CANTOS results, Hsue et al. recently demonstrated that IL-1β inhibition with canakinumab reduces atherosclerotic inflammation in HIV infection (119). Ten ART-treated

PLHIV ≥40 years of age with established CVD or one CVD risk factor and CD4+ T-cell counts ≥400 cells/μL, received a single subcutaneous dose of 150 mg canakinumab. Participants were followed for 12 weeks with fluorodeoxyglucose–positron emission tomography/computed tomography (FDG-PET/CT) performed at weeks 0 and 8 to measure arterial inflammation along with assessments of systemic inflammation and immune activation. At week 8, systemic markers of inflammation (hs-CRP, IL-6 and sCD163) decreased significantly compared to baseline, while a 10% reduction in arterial inflammation was observed on FDG-PET/CT. The authors note that the 10% decrease in

arterial inflammation is intermediate compared to reductions observed with low-dose and high-dose statins in HIV-uninfected individuals (120).

Considering both the potential for infection with immune deficiency as well as the unique features of HIV-associated inflammation, ongoing and future research will need to consider balancing risks and benefits associated with canakinumab and other anti-inflammatory strategies in the context of HIV infection. Indeed, any adjunctive intervention will need to establish safety (or entail minimal risk) and not compromise ART effectiveness, given the excellent outcomes now experienced among PLHIV treated early with ART.

It is also noteworthy that alternative approaches to inhibiting inflammatory pathways have produced contrasting results to those observed for canakinumab. The CIRT trial assessed low-dose methotrexate (15 to 20 mg weekly) or matching placebo in 4,786 patients with previous MI or multivessel coronary disease who were HIV-negative and additionally had either type 2 diabetes or metabolic syndrome (121). The trial was designed in parallel with CANTOS and was based on findings from observational studies, in which patients with rheumatoid arthritis and psoriatic arthritis who

received low-dose methotrexate had fewer CV events than patients who received other therapies or placebo (122, 123). However, CIRT was stopped early after low-dose methotrexate failed to lower IL-1β, IL-6, or hs-CRP levels and was not associated with fewer CV events compared to placebo. Methotrexate was also associated with elevations in liver enzymes, reductions in leukocyte counts and haematocrit, and a higher incidence of non–basal-cell skin cancers than placebo. Similar results were reported by Hsue et al. in a phase 2 trial of PLHIV at increased risk of atherosclerotic events treated with low-dose methotrexate (5–15 mg/week) or placebo for 24 weeks (124). In that trial low-dose methotrexate had no effect on the primary endpoint of brachial artery flow-mediated dilation, nor did it have any positive effect on a panel of inflammatory biomarkers (hs-CRP, IL-6, IP-10, sCD163, sCD14, D-dimer, fibrinogen, VCAM).

An important observation to come from CANTOS and CIRT is that despite abundant data to indicate that inflammation contributes to atherothrombosis, there is mechanistic diversity between the inflammatory pathways involved. Understanding these differences will be crucial for future studies targeting inflammation as a means of reducing CV events.

***Ruxolitinib and edoxaban***

Several potent immune-based therapeutics and anticoagulants are also being pursued in ongoing trials of ART-treated PLHIV. Once such therapy is ruxolitinib, a Janus kinase (JAK) inhibitor with demonstrated reduction in markers of systemic inflammation in patients with myelofibrosis, psoriasis, polycythaemia and rheumatoid arthritis (125). In an open-label trial, HIV-positive participants on stable ART for >2 years, with CD4+ T-cells >350 cells/μL, were randomised to receive ruxolitinib (10 mg BID) for 5 weeks as part of their ongoing treatment (n=40) or to continue taking ART alone (n=20) (126). At week 5 participants in the ruxolitinib-treatment arm demonstrated significant decreases in sCD14, relative to the ART-alone arm. However, a non-significant decrease in IL-6 was observed in the ruxolitinib-treated arm versus the ART-alone arm.

With regards to anticoagulants, oral edoxaban (30 mg/day), a direct factor Xa inhibitor, was recently investigated in the placebo-controlled TACTICAL-HIV trial (127). Forty-four PLHIV receiving ART with VL <200 copies/mL and D-dimer levels ≥100 ng/mL were enrolled. Following 4 months of treatment, edoxaban did not lower biomarkers associated with inflammation or monocyte activation; however, relative to placebo, edoxaban was associated with a 40% reduction in D-dimer levels, a 36% reduction in transactivator of transcription (TAT) levels, and a 7% reduction in the International Normalised Ratio.

***Statin therapy***

The anti-inflammatory activity of HMG co-enzyme A reductase inhibitors or 'statins' is well documented (128), and several clinical trials have assessed the benefits of statins on surrogate markers of CVD in ART-suppressed PLHIV (129-131). In the SATURN-HIV trial, rosuvastatin (10 mg/day) significantly reduced levels of sCD14 (an independent predictor of morbidity in HIV disease) (62), by approximately 10% at 48 weeks in PLHIV on stable ART (129). Significant reductions were also observed in several markers of vascular inflammation, including lipoprotein-associated phospholipase A2 (Lp-PLA2) and T-cell activation (both CD4+ and CD8+). Similar results were observed for pitavastatin but not for pravastatin in the INTREPID trial (131). Two hundred and fifty-two PLHIV on stable ART were randomised (1:1) to receive pitavastatin (4 mg/day) or pravastatin (40 mg/day), with markers of immune activation and arterial inflammation assessed at baseline and following 52 weeks of therapy. Relative to pravastatin, pitavastatin was associated with significantly greater reductions in sCD14, oxLDL and Lp-PLA2. In another randomised, placebo-controlled trial involving 40 PLHIV with subclinical coronary atherosclerosis, atorvastatin significantly reduced non-calcified plaque volume and high-risk plaque features [e.g. LDL-cholesterol and Lp-PLA2 relative to placebo (132).

Although these results are limited by the fact that some statins interact with concurrent ART (133) while others interfere with metabolic control (134), collectively the data imply that by reducing both the procoagulant phenotype of monocytes and inflammation associated with the vascular endothelium (which may act as a substrate for clot formation), statins may reduce coagulation potential and hence the risk of CVD. To accurately answer that question, studies are now needed to evaluate whether the statin-induced reduction of cardiovascular biomarkers will translate into a reduction in morbidity and mortality observed in PLHIV. The ongoing REPRIEVE study has been designed to address this issue (128). REPRIEVE involves approximately 7,500 PLHIV on stable ART randomised to receive 4 mg/day of pitavastatin or placebo for the duration of the study. The

primary endpoint is time to the first event, which is a composite of major CV events (e.g. atherosclerotic or other CVD death, nonfatal MI, unstable angina hospitalisation, coronary or peripheral arterial revascularisation, nonfatal stroke or transient ischaemic attack, or urgent peripheral arterial disease ischaemic event [acute or chronic limb ischaemia, amputation, etc.]). Results from the REPRIEVE study are expected by 2021.

## RESISTANCE TO ART

Incomplete suppression of HIV infection by ART can result in drug resistance. Historically, suboptimal activity of ART was a potential cause of resistance (135); however, most modern ART regimens used for first-line therapy are sufficiently potent to completely suppress HIV replication and have a genetic barrier to resistance high enough to maintain long-term virological suppression (9, 10). As a result, most cases of virological failure and drug resistance arise from incomplete adherence, which exposes the virus to incompletely suppressive antiviral levels.

Patterns of drug resistance at virological failure vary according to regimen, baseline resistance and patient adherence levels. Because all recommended first-line ART regimens contain a cytidine

analogue and because of the relatively high frequency at which this mutation arises, M184V is the most commonly acquired resistance mutation (136). The emergence of other nucleoside reverse transcriptase inhibitor (NRTI) mutations depends on the second NRTI in the regimen. Furthermore, virological failure on the 'first generation' integrase strand transfer inhibitors (INSTIs): raltegravir (RAL)- and elvitegravir (EVG), is not frequently found (137).

Resistance has rarely been observed in patients failing first-line, 3-drug dolutegravir (DTG)-based regimens (138, 139). The SINGLE and SPRING-2 studies investigated the efficacy and safety of DTG + abacavir/lamivudine (ABC/3TC) versus EFV/TDF/FTC and DTG + 2 NRTIs versus raltegravir (RAL) + 2 NRTIs, respectively, in ART-naïve PLHIV and VL >1,000 copies/mL (139-141). No resistance mutations were detected to DTG-containing regimens through week 96; however, 7/419 (1.6%) and 5/419 (1.1%) cases of resistance were identified in the EFV- and RAL-containing regimens, respectively.

It is important to consider that EVG and RAL have overlapping resistance profiles, while DTG and bictegravir (BIC) retain antiviral efficacy against many HIV isolates with primary resistance substitutions (142). Sax et al. recently performed a post-hoc analysis of Gilead study 380-4030 to examine the impact of pre-treatment resistance mutations on treatment outcomes (143). Genotype

data were available for 470/565 (83%) participants, and on retrospective screening the prevalence on NRTI resistance mutations was 17% and 12% in the BIC/FTC/TAF arm and DTG + FTC/TAF arm, respectively. At week 48, no participant with pre-existing NRTI resistance had HIV-1 RNA ≥50 c/mL in either group. These results support the potential utility of BIC or DTG in PLHIV with pre-existing NRTI resistance mutations. Moreover, BIC resistance is yet to be observed in phase III trials (134-137).

After M184V, K65R is the most common NRTI resistance mutation in PLHIV with virological failure on a TDF-containing regimen (144). K65R reduces TDF susceptibility by approximately two-fold and historically, but this mutation usually occurs with 184V which partially restores TDF susceptibility. However, it was not possible to administer the additional tenofovir (TFV) necessary

to overcome the mutation. TAF; however, is more stable in plasma and is predominantly hydrolysed to TFV intracellularly by cathepsin A (146). This results in intracellular levels of TFV that are approximately seven-times higher relative to 300 mg TDF (145) and therefore sufficient to suppress the K65R mutant.

In other situations, virologically suppressed PLHIV need a treatment switch for reasons not related to virological failure but to improve convenience, address drug-intolerance, prevent long-term drug toxicity, or eliminate drug-drug interactions (147). In these cases, there are changes in drug pressure that can affect the emergence of resistance. Furthermore, archived resistance mutations persist among virologically suppressed PLHIV (142). Therefore, guidelines suggest that switching be performed after careful review of previous history of regimens, failures and resistance (9, 10).

Preventing resistance remains a priority in HIV care. Drugs with high barriers to resistance assist with preventing resistance, but even drugs with lower barriers to resistance that are found in simple, and well tolerated

ART regimens can provide effective longterm therapy Continuous patient education on the risk of non-adherence is another important factor in preventing resistance. Once established, resistance evolves, diversifies, and may become more difficult to

manage. Fortunately, there are now several drugs available that retain antiviral activity against HIV with mutations that confer resistance to multiple drugs.

***Is there a link between low-level viraemia and resistance to ART?***

In clinical practice, an important problem is how to advise individuals with low-level viraemia (LLV; e.g. 20-1,000 copies/mL) despite an initial virological response to ART and good adherence. In these situations, it can be difficult to determine whether persistent LLV is due to resistance or related to viral release from reservoirs. Several research groups have attempted to answer this question using different avenues of inquiry.

In the first, the Antiretroviral Therapy Cohort Collaboration analysed data from 17,902 participants from high income countries in order to assess the association between LLV (≥2 VLs above threshold) and virological failure, defined as two consecutive VLs >500 copies/mL or one VL >500 copies/mL followed by ART modification or AIDS event/death (148). Participants identified with LLV were divided into two groups: a) 50-199 copies/mL (n=624), and b) 200-499 copies/mL (n=482). After a median follow-up of 2.3 and 3.1 years for virological and clinical outcomes, respectively, the authors found that LLV within the range of 200-499 copies/mL was strongly associated with virological failure (adjusted hazard ratio 3.97, 95% confidence interval 3.05-5.17) but not with AIDS event/death. In contrast, LLV in the range of 50-199 copies/mL was not associated with virological failure. It is postulated that viral loads below 200 copies/mL reflect release of archived virus from the latent reservoir whereas levels above 200 indicate new viral replication. However, in large cohort studies like this, non-adherence may be an important driver for both LLV and resistance and may therefore not specifically address whether LLV on ART is linked to resistance.

In another study from Belgium, Vancoillie et al. identified 18 subjects with LLV (20-250 copies/mL) from a longitudinal cohort of 621 PLHIV on suppressive ART (149). To determine

whether LLV was linked to ongoing viral replication, next-generation sequencing of env and pol genes was performed before and during LLV. Over a median period of 4.8 years, there was no evidence of genetic evolution and no evidence that LLV was due to ongoing replication in any subject.

Finally, in 10 PLHIV with demonstrated LLV for >6 months (with or without ART switch or intensification) and no suspicion of non-adherence, Halvas et al. assessed whether the source of LLV was due to ongoing viral replication or to an expanded clone of cells that carry intact virus (150). The median time on ART, median time with non-suppressible viraemia, and VL at referral

were 10 years, 3.2 years and 98 copies/mL, respectively. Peripheral blood mononuclear cells (PBMCs) and plasma samples were collected at two or more time points, and single-genome sequencing was performed on plasma HIV RNA, cell-associated HIV DNA, and P24+ culture supernatants from quantitative viral outgrowth assays. The clonal cellular origin was assessed by phylogenetics and integration site analysis and confirmed by sequencing the integrated provirus and the flanking host sequences.

The percentage of identical plasma sequences in participants ranged from 28-86%. Furthermore, in 7/9 participants, the HIV DNA sequences inside PBMCs matched those observed in plasma, and in 5/9 participants, the ex-vivo viral sequences were identical to those observed in plasma. The results demonstrate that cell clones carrying intact proviruses can produce clinically relevant levels of viremia and should be considered within the context of clinical management.

Collectively, the studies described above suggest that, in the absence of adherence issues, LLV may not be due to drug resistance but rather the result of viral release from clonal expansion of infected cells, referred to as replicones. The identification of mechanisms to eliminate or suppress replicones to achieve HIV-remission off ART is the focus of ongoing research.

## CONCLUSIONS

Although HIV suppression has become more achievable with simplified and more effective ART, paradoxically HIV management has become more complex in PLHIV as they survive longer and experience more HIV-related co-morbidities as the diseases associated with ageing. We know now that low-grade immune activation, inflammation and hypercoagulability, persist in PLHIV despite suppressive ART. This phenomenon, together with co-infections, co-morbidities, and adverse lifestyle factors, is an important contributor to several non-AIDS-related comorbidities, including the ageing process

itself. The harmful effects of immune activation and inflammation must always be considered when managing

PLHIV, and clinicians must be watchful for signs and symptoms of potential non-AIDS-related comorbidities, particularly CVD and malignancies, as reflected in management guidelines from the U.S. DHHS and European AIDS Clinical Society (9, 10).

Knowledge about this subject is increasing; however, important questions remain regarding the role of chronic inflammation in the pathogenesis of different comorbidities. In addition, many of the strategies investigated to date to manage HIV-related inflammation have limited applicability to current practice. Despite these issues, the field is currently at a turning point where the potential to mitigate the excess risk of inflammatory-related comorbidities in PLHIV is within reach. In doing so, we will need to consider how to appropriately prioritise prevention, screening and treatment, and research will need to embrace the complex interaction between HIV, inflammation and ageing in order to maintain a high QoL for PLHIV while continuing to extend survival.

At this point, the following practical steps to controlling inflammation in PLHIV should be considered: (151) a) ART should be initiated as soon as possible, b) prevention and treatment of

coinfection should be a priority, c) clinicians should aggressively screen for and treat any comorbid condition, d) all PLHIV should be advised to stop smoking, and e) where appropriate, consideration should be given to switching ART to the least toxic regimen.

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**Authors' contributions**

All authors contributed to design and helped to draft the manuscript. All authors read and approved the final manuscript.

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