Open Forum Infectious Diseases

MAJOR ARTICLE

Very-Low-Level Viremia, Inflammatory Biomarkers, and Associated Baseline Variables: Three-Year Results of the Randomized TANGO Study

Ruolan Wang,[1] Mark Underwood,[1] Josep M. Llibre,[2] Enrique Bernal Morell,[3] Cynthia Brinson,[4] José Sanz Moreno,[5] Stefan Scholten,[6] Richard Moore,[7] Parminder Saggu,[8] James Oyee,[8] Riya Moodley,[9] Brian Wynne,[1] Michelle Kisare,[9] Bryn Jones,[9] and Mounir Ait-Khaled[9]

[1]ViiV Healthcare, Durham, North Carolina, USA, [2]Infectious Diseases Division and Fight Infections Foundation, Hospital Universitari Germans Trias i Pujol, Barcelona, Spain, [3]Department of Infectious Diseases, Hospital General Universitario Reina Sofía, Murcia, Spain, [4]Central Texas Clinical Research, Austin, Texas, USA, [5]Departamento de Medicina Interna, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Spain, [6]Praxis Hohenstaufenring, Cologne, Germany, [7]Northside Clinic, Fitzroy North, Australia, [8]GSK, Brentford, United Kingdom, and [9]ViiV Healthcare, Brentford, United Kingdom

Background. We compared proportions of participants with target detected, target not detected (TND), and elevated viral load (VL) and assessed baseline variables associated with week 144 inflammatory biomarker levels between dolutegravir-lamivudine (DTG/3TC) and tenofovir alafenamide–based regimens (TBRs) in the TANGO study (post hoc).

Methods. TANGO is an open-label, multicenter, phase 3 study that randomized adults with VL <50 copies/mL to switch to once-daily fixed-dose DTG/3TC or continue TBR. At baseline and each study visit, the VL was measured. Elevated VL event frequencies were assessed, including "blips." Interleukin 6, D-dimer, high-sensitivity C-reactive protein, soluble CD14, and soluble CD163 were measured at baseline and at week 144. Log_e-transformed week 144 biomarker levels were compared between treatment groups using an analysis of covariance model adjusting for baseline variables.

Results. High, comparable proportions of participants had VL <40 copies/mL and TND at week 144 (DTG/3TC, 279 of 369 [76%]; TBR, 267 of 372 [72%], intention-to-treat exposed Snapshot analysis; adjusted difference, 3.9% [95% confidence interval, −2.5% to 10.2%]), with similar TND proportions at all postbaseline visits (123 of 369 [33%] vs 101 of 372 [27%], respectively). Similar proportions of DTG/3TC participants had ≥1 postbaseline VL ≥50 copies/mL (28 of 369 [8%] vs 42 of 372 [11%] for TBR), primarily blips (18 of 369 [5%] and 26 of 372 [7%], respectively). Week 144 inflammatory biomarker levels were low and comparable between groups and associated with multiple demographic and baseline characteristics, including baseline biomarker levels, indicating a multifactorial inflammatory response.

Conclusions. Week 144 biomarker levels were low and generally comparable between treatment groups, reflecting similar, robust, and durable viral suppression observed using the stringent TND end point.

Trial registration: ClinicalTrials.gov, NCT03446573.

Keywords. 2-drug regimen; dolutegravir-lamivudine; inflammation; integrase strand transfer inhibitor; virologic suppression.

Chronic inflammation is associated with an increased risk of comorbid conditions and may result in elevated levels of immune response biomarkers [1]. People with human immunodeficiency virus (HIV) (PWH) have multiple possible causes for acute and chronic inflammation, including viral replication [1]. Various studies have reported high levels of inflammation and immune activation in treatment-naive PWH before initiating antiretroviral therapy (ART) [2–4]. While using highly effective ART significantly decreases HIV-related inflammation and immune activation [5], chronic inflammation persists and increased inflammatory biomarker levels are still observed in PWH compared with healthy individuals without HIV [6–10]. Some studies have found that low-level viremia is associated with elevated levels of circulating markers of inflammation [6–9], which have been linked to development of comorbid conditions and increased morbidity and mortality rates in virologically suppressed PWH [1, 9]. The inflammatory response is complex and multifactorial, and in PWH on suppressive ART, it may be driven by many other factors aside from residual viremia or persistent low-level viral replication.

Elevated levels of inflammatory biomarkers may result from unique or overlapping root causes, representing multiple inflammatory pathways or mechanisms, including systemic inflammation (interleukin 6 [IL-6] and high-sensitivity C-reactive protein [hs-CRP]), coagulation dysregulation

Received 06 July 2023; published online 9 December 2023

Correspondence: Ruolan Wang, MSc, ViiV Healthcare, Clinical Virology and Translational medical Research, 406 Blackwell St, Ste 300, Durham, NC 27701 (ruolan.h.wang@viivhealthcare.com).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofad626

(D-dimer), and monocyte and macrophage activation (soluble CD14 and CD163 [sCD14 and sCD163) 11, 12]. Many emotional stressors and lifestyle factors have been linked to chronic inflammation in the general population [1, 13]. Other factors that can contribute to inflammation include bacterial translocation, comorbid conditions, and coinfections [14]. To provide the most effective recommendations for improved outcomes in virologically suppressed PWH, it is important to consider these and other factors that affect the inflammatory landscape independently of aging or controllable lifestyle factors.

In the phase 3 TANGO study, switching to dolutegravir-lamivudine (DTG/3TC) demonstrated durable and noninferior efficacy and good safety versus continuing tenofovir alafenamide–based regimens (TBRs) in virologically suppressed adults [15, 16]. Both treatment groups demonstrated durable antiviral efficacy and a low incidence of incomplete virologic suppression at week 144, with high and comparable proportions of participants having HIV-1 RNA levels <40 copies/mL and target not detected (TND; a qualitative measure for a viral load [VL] below the 40 copies/mL threshold of the HIV-1 RNA assay) [15]. In TANGO, changes from baseline to week 144 in $CD4^+/CD8^+$ ratio and adjusted inflammatory biomarker levels were small (with no consistent pattern of change) and similar between treatment groups, supporting the observed virologic outcomes [15]. Here we report results from post hoc analyses of the TANGO study assessing proportions of participants with HIV-1 RNA levels <40 copies/mL, target detected (TD) or TND, and the frequency of elevated VL events through week 144. To better understand which baseline factors were associated with week 144 inflammatory biomarker levels in TANGO participants, inflammatory biomarker levels at week 144 were compared between treatment groups using an analysis of covariance (ANCOVA) model adjusting for baseline variables for each biomarker.

METHODS

Participants and Study Design

The TANGO study (ClinicalTrials.gov, NCT03446573) is a multicenter, phase 3, open-label, randomized, noninferiority study to assess the efficacy and safety of switching to DTG/3TC versus continuing TBR in virologically suppressed adults with HIV-1. Detailed methods have been published [15, 16]. Briefly, eligible adults on uninterrupted ART for >6 months and currently on a stable TBR were randomized 1:1 (stratified by baseline third agent class) to either switch to once-daily DTG/3TC (50 mg/300 mg fixed-dose combination) or continue their current TBR for 144 weeks.

Procedures

Plasma samples were collected at baseline, every 4 weeks through week 12 and every 12 weeks thereafter, and at study withdrawal. HIV-1 RNA assays were conducted at Q^2 Solutions using the Abbott RealTime HIV-1 assay (Abbott Molecular). The assay measures VL from 40 to 10 000 000 copies/mL and provides qualitative TD or TND outcomes for VLs <40 copies/mL. The primary end point in TANGO was the proportion of participants with HIV-1 RNA levels ≥50 copies/mL at week 48, assessed using the US Food and Drug Administration Snapshot algorithm.

This post hoc analysis summarizes the proportion of participants with Snapshot analysis VL <40 copies/mL and TND at week 144; assesses the proportion of participants with very-low-level viremia (VL <40 copies/mL and TD or TND) and low-level viremia (VL ≥40 to <50 copies/mL or ≥50 copies/mL) at baseline and at each visit; and identifies the frequency of elevated VL events, including virologic "blips," which were defined as VLs between 50 and 200 copies/mL with adjacent VL values <50 copies/mL, by week 144.

Blood samples were collected at baseline and at weeks 48, 96, and 144 for inflammatory biomarker assessment. Levels of IL-6, hs-CRP, sCD14, and sCD163 (from serum) and D-dimer (from plasma) were measured at Q^2 Solutions Laboratory. Quantifications of inflammatory biomarker levels were derived using a high-sensitivity sandwich enzyme-linked immunosorbent assay (ELISA) and a Synergy 2 microplate reader (BioTek) for IL-6; a Roche cobas 8000/c502 analyzer (Roche Diagnostics) for hs-CRP; a sandwich ELISA and a Tecan GENios Pro microplate reader (Tecan Systems) for sCD14 and sCD163; and a BCS coagulation analyzer (Siemens) for D-dimer (by means of immunoturbidity).

Ethical Approval

This study was performed in accordance with the ethical principles of the Declaration of Helsinki using a protocol reviewed and approved by national, regional, or investigational center ethics committees or institutional review boards. TANGO was conducted in accordance with International Conference on Harmonization Good Clinical Practice and the Declaration of Helsinki, with protocol approvals obtained before participant screening.

Patient Consent Statement

Written informed consent was obtained from all participants before study initiation.

Statistical Analysis

Analyses of quantitative VLs and qualitative TD/TND measures were performed in the intention-to-treat exposed (ITT-E) population, which included all participants who received ≥1 dose of study treatment and was based on randomized treatment assignment. Proportions of participants with VL <40 copies/mL and TND at week 144 were analyzed using a

Table 1. Changes in Quantifiable and Nonquantifiable Viral Load by Baseline Viral Load Category Through Week 144: Intention-to-Treat Exposed Population

	DTG/3TC (n = 369)			TBR (n = 372)		
VL Subcategory	TND	TD	≥40 Copies/mL	TND	TD	≥40 Copies/mL
Participants with baseline VL data[a]	302 (82)	51 (14)	11 (3)	303 (81)	59 (16)	9 (2)
Participants with postbaseline VL data[b]						
≥1 VL ≥50 copies/mL[c]	19 (6)	7 (14)	2 (18)	32 (11)	9 (15)	1 (11)
≥1 VL ≥40 and <50 copies/mL	5 (2)	5 (10)	2 (18)	11 (4)	5 (8)	1 (11)
≥1 VL <40 copies/mL and TD	161 (53)	33 (65)	7 (64)	166 (55)	39 (66)	6 (67)
All VLs <40 copies/mL and TND	117 (39)	6 (12)	0 (0)	94 (31)	6 (10)	1 (11)

Postbaseline categories are mutually exclusive and determined by the highest VL observed. Five participants on DTG/3TC with baseline VL <40 copies/mL and 1 on TBR with baseline VL ≥50 copies/mL were not presented owing to the absence of postbaseline VL data.

Abbreviations: DTG/3TC, dolutegravir-lamivudine; TBR, tenofovir alafenamide–based regimen; TD, target detected; TND, target not detected; VL, viral load.

[a]Number of participants with postbaseline VL data (percentage based on total number in treatment group).

[b]Percentages based on number of participants with postbaseline data.

[c]"Blips," defined as VLs between 50 and 200 copies/mL with adjacent VL <50 copies/mL, are included in this category.

Cochran-Mantel-Haenszel test adjusting for baseline third agent class.

Biomarker analyses were performed in the safety population, which included all participants who received ≥1 dose of study treatment and was based on actual treatment received. Baseline geometric means are reported as actual values. Reported week 144 adjusted geometric means and 95% confidence intervals (CIs) were calculated using an ANCOVA model with $\log_e$-transformed biomarker data, adjusting for treatment, sex, race, age, and baseline characteristics (body mass index [BMI], smoking status, TD/TND status, biomarker levels, Centers for Disease Control and Prevention [CDC] HIV disease category, and $CD4^+/CD8^+$ ratio). IL-6 data were also adjusted for baseline hs-CRP levels. The hs-CRP data were also adjusted for baseline lipid-modifying agent use and levels of triglycerides, total cholesterol, and low-density and high-density lipoprotein cholesterol. Adjusted week 144 geometric mean biomarker levels were compared between treatment groups.

RESULTS

Study Participants

A total of 743 participants were enrolled and randomized to switch to DTG/3TC (n = 371) or continue TBR (n = 372). Demographic and baseline characteristics in the ITT-E population were balanced between treatment groups (Supplementary Table 1) [16]. The median age (range) was 40 (20–74) and 39 (18–73) years in the DTG/3TC and TBR groups, respectively. Most participants in each group were male (93% for DTG/3TC and 91% for TBR), white (80% and 78%, respectively), and not Hispanic or Latin American (81% and 82%).

VL Assessments

Per visit, the proportions of participants with VL <40 copies/mL and TND were high and comparable in both treatment groups through week 144. The proportions with VL <40 copies/mL and TD as well as VL ≥40 copies/mL per visit were also comparable between groups (Supplementary Figure 1). Regardless of baseline VL category, 33% (123 of 369) and 27% (101 of 372) of participants in the DTG/3TC and TBR groups, respectively, had VL <40 copies/mL and TND at every visit through week 144 (Table 1). More participants with TND at baseline versus other baseline VL categories had postbaseline TND at every visit, and 92% (278 of 302) of participants on DTG/3TC versus 86% (260 of 303) on TBR with TND at baseline never had a postbaseline VL ≥40 copies/mL.

The occurrence of elevated VL events was infrequent in both groups through week 144. However, numerically fewer participants had elevated VL in the DTG/3TC group (28 of 369 [8%]) than in the TBR group (42 of 372 [11%]; Supplementary Table 2). In both treatment groups, observed elevated VL events were primarily blips (DTG/3TC, 18 of 369 [5%]; TBR, 26 of 372 [7%]) as opposed to multiple consecutive elevated VL events. Of note, none of the participants with archived M184V/I mutation (all detected as mixture with wild type: DTG/3TC, n = 4; TBR, n = 3) had elevated VL events through week 144.

As reported elsewhere [15], similar proportions of participants in each treatment group had VL <40 copies/mL and TND at week 144 (Snapshot analysis, ITT-E; DTG/3TC, 279 of 369 [76%]; TBR, 267 of 372 [72%]; adjusted treatment difference, 3.9% [95% CI, −2.5% to 10.2%]; Table 2). The proportion of participants categorized as having virologic nonresponse using the more stringent HIV-1 RNA measurement of ≥40 copies/mL or TD criterion at week 144 was low and similar in both treatment groups (DTG/3TC, 41 of 369 [11%]; TBR, 53 of 372 [14%]). Most participants with virologic nonresponse or who were not TND had VL <40 copies/mL and were TD (DTG/3TC, 37 of 41 [90%]; TBR, 35 of 53 [66%]).

Table 2. Summary of Study Outcomes at Week 144: Snapshot Analysis for Intention-to-Treat Exposed Population

Outcome	Study Participants, No. (%)	
	DTG/3TC (n = 369)	TBR (n = 372)
Virologic response (VL <40 copies/mL and TND)[a]	279 (76)	267 (72)
Virologic nonresponse or not TND	41 (11)	53 (14)
Data in window and VL <40 copies/mL and TD[b]	37 (10)	35 (9)
Data in window and VL ≥40 copies/mL	1 (<1)	2 (<1)
Discontinued for lack of efficacy	0	5 (1)
Discontinued for other reason while VL ≥40 copies/mL or VL <40 copies/mL and TD	3 (<1)	10 (3)
Change in ART	0	1 (<1)
No virologic data	49 (13)	52 (14)
Discontinued study due to adverse event or death	23 (6)	6 (2)
Discontinued study for other reason with VL <40 copies/mL and TND or no on-treatment VL	22 (6)	46 (12)
On study but missing data in window	4 (1)[c]	0

Abbreviations: ART, antiretroviral therapy; DTG/3TC, dolutegravir-lamivudine; TBR, tenofovir alafenamide–based regimen; TD, target detected; TND, target not detected; VL, viral load.

[a]The adjusted treatment difference between the DTG/3TC and TBR groups was 3.9% (95% confidence interval, −2.5% to 10.2%).

[b]Participants with human immunodeficiency virus 1 RNA levels <40 copies/mL with missing interpretation for TD/TND are assumed to be TD.

[c]Four participants had missing data in the window owing to the impact of coronavirus disease 2019.

Inflammatory Biomarkers

At baseline and at week 144, the geometric mean IL-6 levels (95% CIs) were 1.64 (1.52–1.78) and 1.73 (1.58–1.89) ng/L, respectively, for the DTG/3TC group and 1.67 (1.54–1.80) and 1.58 (1.46–1.72) ng/L for the TBR group. From the ANCOVA analysis, the adjusted geometric means (95% CIs) at week 144 were 1.91 (1.57–2.33) ng/L for the DTG/3TC group and 1.72 (1.41–2.09) ng/L for the TBR group. There was no difference in adjusted week 144 IL-6 levels between treatment groups (ratio, 1.11 [95% CI, 1.00–1.24]; $P = .053$). Higher IL-6 levels at week 144 were associated with higher baseline BMI, baseline smoking status, increasing age, and higher baseline IL-6 level (Figure 1*A* and Supplementary Table 3).

At baseline and at week 144, D-dimer geometric means (95% CIs) were 1.69 (1.59–1.79) and 1.59 (1.50–1.69) nmol/L fibrinogen-equivalent units, respectively, for the DTG/3TC group and 1.66 (1.58–1.76) and 1.57 (1.48–1.66) nmol/L fibrinogen-equivalent units for the TBR group. From the ANCOVA analysis, the adjusted geometric means (95% CIs) at week 144 for the DTG/3TC and TBR groups were 1.89 (1.65–2.16) and 1.82 (1.59–2.07) nmol/L fibrinogen-equivalent units, respectively. Week 144 D-dimer levels were similar between treatment groups (ratio, 1.04 [95% CI, .96–1.12]; $P = .33$). Higher D-dimer levels at week 144 were associated with baseline obesity, baseline VL <40 copies/mL and TD compared with baseline VL <40 copies/mL and TND, increasing age, and higher baseline D-dimer level (Figure 1*B* and Supplementary Table 3).

At baseline and at week 144, geometric mean hs-CRP levels (95% CIs) were 1.37 (1.23–1.53) and 1.11 (0.98–1.26) mg/L, respectively, in the DTG/3TC group and 1.30 (1.16–1.46) and 1.13 (1.00–1.28) mg/L in the TBR group. From the ANCOVA analysis, the adjusted geometric means (95% CIs) at week 144 for the DTG/3TC and TBR groups were 1.45 (1.07–1.95) and 1.47 (1.10–1.98) mg/L, respectively. Week 144 hs-CRP levels were similar between treatment groups (ratio, 0.98 [95% CI, .84–1.15]; $P = .81$). Higher hs-CRP levels at week 144 were associated with female sex, higher baseline BMI, higher baseline triglyceride levels, and higher baseline hs-CRP levels (Figure 2 and Supplementary Table 3).

At baseline and at week 144, the geometric mean sCD14 levels (95% CIs) were 1.61 (1.57–1.64) and 1.18 (1.11–1.25) $\times 10^6$ ng/L, respectively, for the DTG/3TC group, and 1.58 (1.55–1.61) and 1.28 (1.21–1.35) $\times 10^6$ ng/L for the TBR group. From the ANCOVA analysis, the adjusted geometric means (95% CIs) at week 144 were 0.99 (0.86–1.15) and 1.08 (0.94–1.25) $\times 10^6$ ng/L, respectively, for the DTG/3TC and TBR groups. The sCD14 levels at week 144 were slightly lower in the DTG/3TC group than in the TBR group (ratio, 0.92 [95% CI, .85–1.00]; $P = .04$). Higher sCD14 levels at week 144 were associated with more advanced baseline CDC HIV disease category and higher baseline sCD14 level. Lower sCD14 levels at week 144 were associated with use of DTG/3TC, female sex, black or African American race compared with white race, and baseline VL ≥40 copies/mL compared with baseline VL <40 copies/mL and TND (Figure 3*A* and Supplementary Table 3).

At baseline and at week 144, the geometric mean sCD163 levels (95% CIs) were 660.89 (630.54–692.70) and 559.11 (534.93–584.38) µg/L, respectively, for the DTG/3TC group and 642.03 (615.26–669.97) and 533.64 (510.80–557.50) µg/L for the TBR group. From the ANCOVA analysis, the adjusted geometric means (95% CIs) at week 144 for the DTG/3TC and TBR groups were 574.04 (521.42–631.97) and 550.78 (500.79–605.75) µg/L, respectively. The week 144 sCD163 levels were similar between treatment groups (ratio, 1.04 [95% CI, .99–1.10]; $P = .14$). Higher sCD163 levels at week 144 were associated with increasing age and higher baseline sCD163 levels (Figure 3*B* and Supplementary Table 3).

DISCUSSION

In alignment with the previously demonstrated durable and noninferior efficacy of switching to DTG/3TC compared with continuing TBR through 3 years [15], there was no difference between treatment groups in the proportion of participants maintaining virologic suppression when using the more stringent VL measurement of <40 copies/mL and TND threshold through 3 years. Similar proportions of participants in both treatment groups maintained postbaseline TND at all available

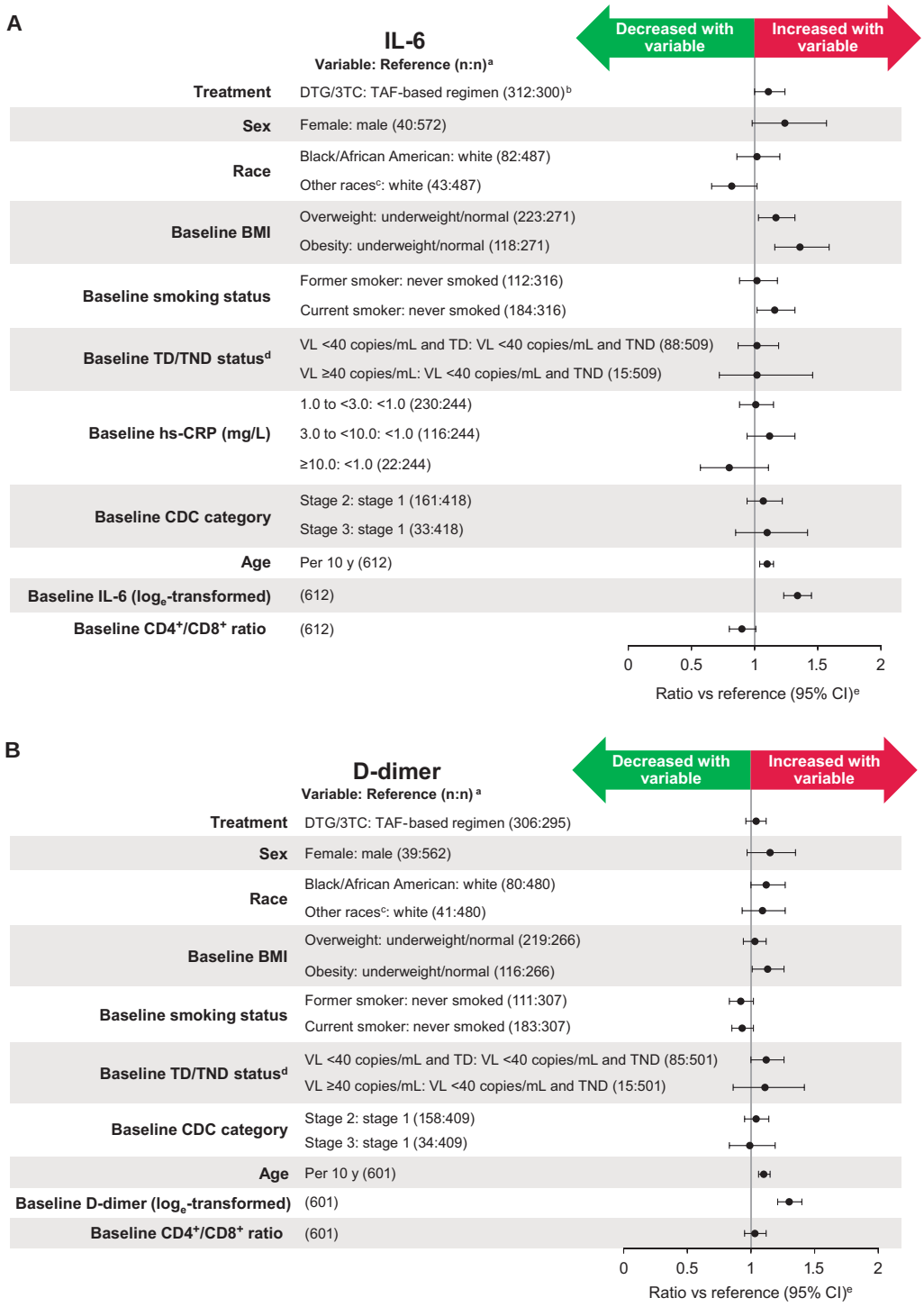

Figure 1. Variables associated with interleukin 6 (IL-6) (*A*) and D-dimer (*B*) at week 144. [a]Number of participants with nonmissing biomarker data at week 144. [b]Lower limit for the estimated treatment ratio is 0.9985. [c]Including American Indian or Alaska Native, Asian, Native Hawaiian or other Pacific Islander, mixed white race, and multiple races. [d]Quantified viral load (VL) from 40 to 10 000 000 copies/mL and qualitative target detected (TD) or target not detected (TND) outcomes for VL <40 copies/mL, provided by Abbott RealTime human immunodeficiency virus (HIV) 1 assay. [e]Ratio calculated using an analysis of covariance model on log$_e$-transformed biomarker data adjusting for treatment, sex, race, age, and baseline characteristics (body mass index [BMI], smoking status, TD/TND status, biomarker value, Centers for Disease Control and Prevention [CDC] HIV disease category, and CD4$^+$/CD8$^+$ ratio). IL-6 data were also adjusted for baseline high-sensitivity C-reactive protein (hs-CRP). Abbreviations: CI, confidence interval; DTG/3TC, dolutegravir-lamivudine; TAF, tenofovir alafenamide.

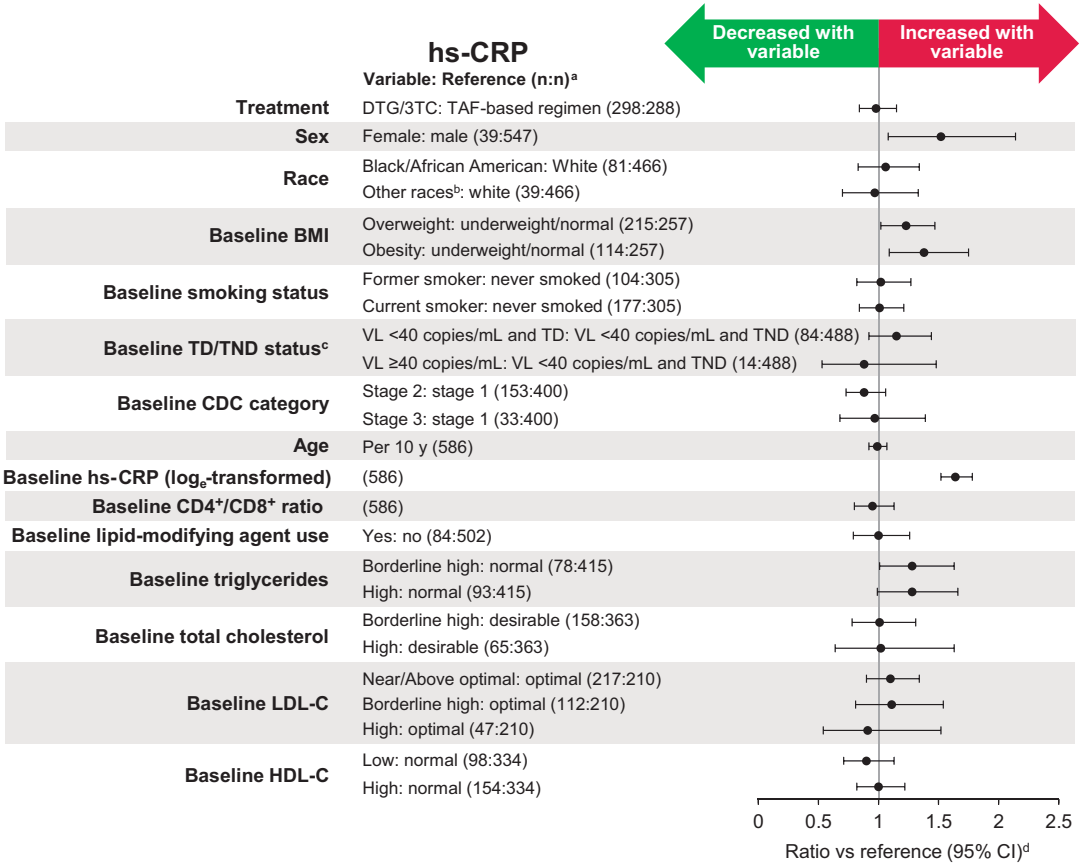

Figure 2. Variables associated with high-sensitivity C-reactive protein (hs-CRP) at week 144. [a]Number of participants with nonmissing biomarker data at week 144. [b]Including American Indian or Alaska Native, Asian, Native Hawaiian or other Pacific Islander, mixed white race, and multiple races. [c]Quantified viral load (VL) from 40 to 10 000 000 copies/mL and qualitative target detected (TD) or target not detected (TND) outcomes for VL <40 copies/mL, provided by Abbott RealTime human immunodeficiency virus (HIV) 1 assay. [d]Ratio calculated using an analysis of covariance model on log$_e$-transformed biomarker data adjusting for treatment, sex, race, age, and baseline characteristics (body mass index [BMI], smoking status, TD/TND status, biomarker value, Centers for Disease Control and Prevention [CDC] HIV disease category, and CD4$^+$/CD8$^+$ ratio). hs-CRP data were also adjusted for baseline lipid-modifying agent use, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). Abbreviations: CI, confidence interval; DTG/3TC, dolutegravir-lamivudine; TAF, tenofovir alafenamide.

visits through week 144. Ninety-two percent of participants (278 of 302) on DTG/3TC versus 86% (260 of 303) on TBR with TND at baseline never had a postbaseline VL ≥40 copies/mL. "Blips" were the most common elevated VL event observed throughout the study and occurred at low and similar frequencies in both treatment groups.

Adjusted inflammatory biomarker levels were low and comparable in the DTG/3TC and TBR groups at week 144, indicating similar robust and durable virologic suppression. As previously reported, minimal and similar changes in biomarker levels from baseline to week 144 were observed between treatment groups after adjustment for factors related to HIV status, lifestyle, coinfection status, and demographic characteristics [15]. Similar biomarker outcomes were also observed at week 48 in the SALSA trial in virologically suppressed PWH who switched to DTG/3TC or continued current 3- or 4-drug antiretroviral regimens [17].

The high and equivalent proportions of participants with VL <40 copies/mL and TND status and low frequency of blips in both treatment groups through week 144 in this post hoc analysis indicate that there was no suboptimal viral suppression after switching to DTG/3TC versus continuing TBR and therefore no replication-associated reason to expect increases in inflammatory biomarker levels. ART–mediated viral suppression is the most strongly recommended strategy to reduce ongoing inflammation in PWH, and it is unlikely that an effective ART regimen would increase inflammation or immune activation under similar conditions of virologic suppression unless it contained toxic components [18]. While further research is warranted to understand persistent inflammation in PWH with virologic suppression <50 copies/mL, these data do not indicate increased inflammation after switching to the 2-drug regimen DTG/3TC versus continuing a 3- or 4-drug TBR.

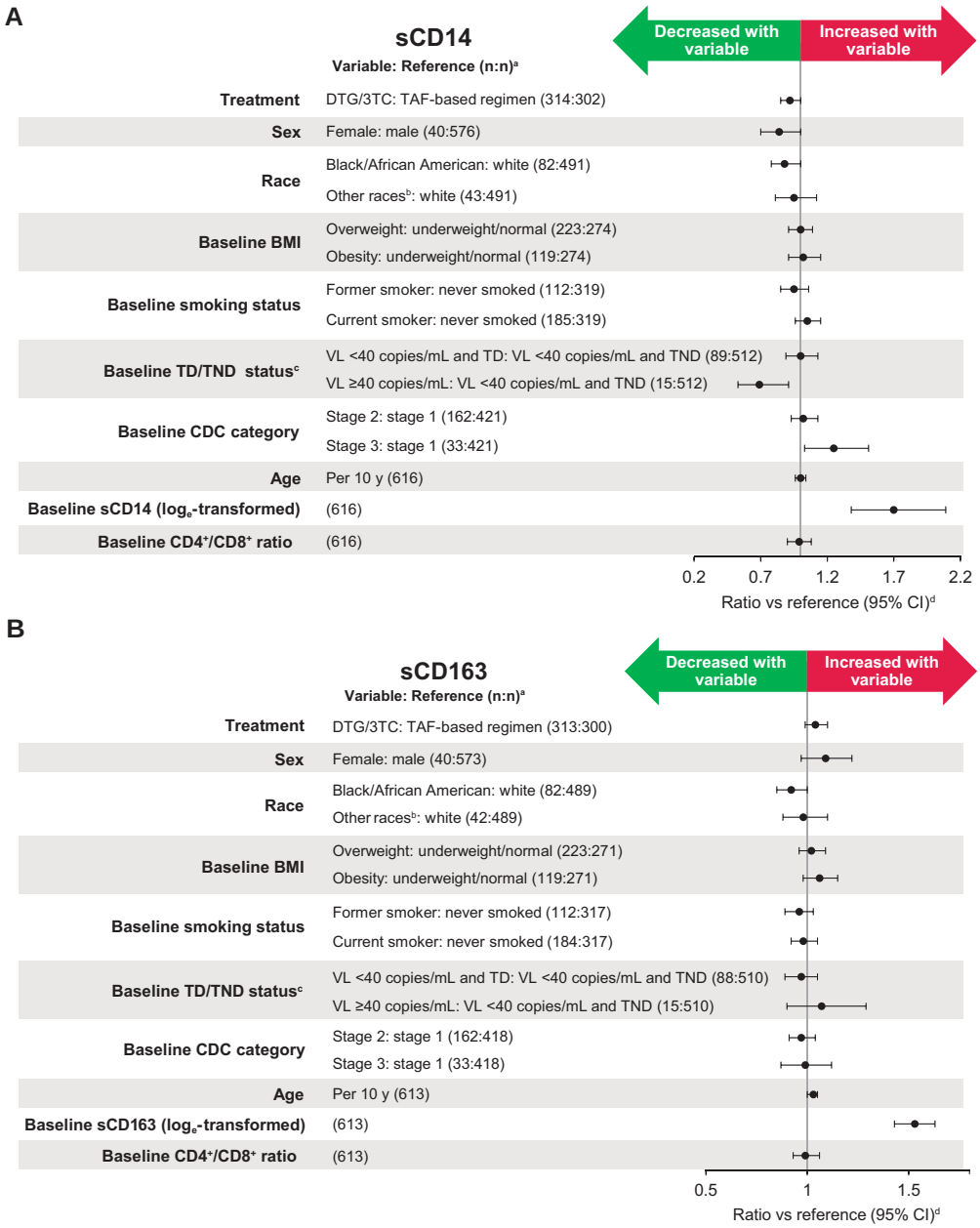

Figure 3. Variables associated with soluble CD14 (sCD14) (*A*) and soluble CD 163 (sCD163) (*B*) at week 144. [a]Number of participants with nonmissing biomarker data at week 144. [b]Including American Indian or Alaska Native, Asian, Native Hawaiian or other Pacific Islander, mixed white race, and multiple races. [c]Quantified viral load (VL) from 40 to 10 000 000 copies/mL and qualitative target detected (TD) or target not detected (TND) outcomes for VL <40 copies/mL provided by Abbott RealTime human immunodeficiency virus (HIV) 1 assay. [d]Ratio calculated using an analysis of covariance model on log$_e$-transformed biomarker data adjusting for treatment, sex, race, age, and baseline characteristics (body mass index [BMI], smoking status, TD/TND status, biomarker value, Centers for Disease Control and Prevention [CDC] HIV disease category, and CD4$^+$/CD8$^+$ ratio). Abbreviations: CI, confidence interval; DTG/3TC, dolutegravir-lamivudine; TAF, tenofovir alafenamide.

Even in PWH with sustained plasma VL <50 copies/mL, viruses can continue to persist and replicate in ways that evade ART or the immune response [19]. The incomplete suppression of viral reservoirs leads to residual viremia and a persistent inflammatory state [14]. Current guidelines recommend against modifying or intensifying ART in PWH who are virologically suppressed as a method of further reducing inflammation, and research has supported that 2-drug versus 3- or 4-drug regimens have similar inflammatory effects in virologically suppressed PWH. The ASPIRE [20], GEMINI [21], LAMIDOL [22], and TANGO [15] studies found that participants receiving a 2-drug regimen of DTG and 3TC versus 3- or 4-drug regimens had similar levels of residual viremia for up to 3 years. The LAMIDOL study also observed no change

in viral reservoir during the first year after switching from a 3-drug regimen to DTG and 3TC in virologically suppressed participants. Furthermore, a pilot study in suppressed PWH found a low frequency of genital shedding with DTG and 3TC that was within the range observed with 3-drug regimens [23], and the DOLAM study found no evidence of central nervous system HIV replication or changes in cerebrospinal fluid inflammatory markers 48 weeks after switching from a 3-drug regimen to DTG and 3TC.

The landscape of inflammatory biomarker analyses has expanded since their initial consideration as indicators of increased mortality risk based on results from the Strategies for Management of Anti-Retroviral Therapy (SMART) trial [24]. One nested study within an observational cohort predicted a higher risk of D-dimer and hs-CRP quartile increase over time in PWH who switched to a 2-drug regimen versus continuing a 3-drug regimen [25]. However, these analyses were performed using a small population (2-drug regimen, n = 58 [DTG/3TC, n = 7]; 3-drug regimen, n = 90), and models did not adjust for certain known significant confounders, such as coinfections and lifestyle factors [9].

Using mixed-models repeated-measures analyses adjusting for additional covariates (eg, race, BMI, smoking status, and hepatitis C virus coinfection), similar, minimal changes from baseline in inflammatory biomarkers were generally observed at week 144. Small fluctuations from baseline occurred for sCD14, with a greater decrease from baseline in the DTG/3TC group observed at weeks 48 and 144 (but not week 96); conversely, for IL-6, a greater decrease was observed in the TBR group at weeks 48 and 144 (but not week 96). No differences were observed for D-dimer, hs-CRP, or sCD163 [15]. With respect to IL-6, differences in adjusted IL-6 levels were not seen between treatment groups in the SALSA study at week 48 [17] or in the randomized DEBATE trial at 12 months, in which virologically suppressed PWH switched to either DTG/3TC (n = 33) or bictegravir-emtricitabine–tenofovir alafenamide (n = 33), with an analysis adjusting for fewer baseline factors. While the clinical significance of these small fluctuations in inflammatory biomarkers is currently unclear, these data do not suggest a consistent directionality on the impact of inflammation with DTG/3TC or 3- or 4-drug TBRs [16, 26].

In this current ANCOVA analysis, there was no statistically significant difference in adjusted week 144 IL-6 levels between DTG/3TC and TBR groups. Here, our randomized comparisons from a controlled trial demonstrate that inflammatory biomarkers in PWH are associated with confounding variables regardless of ART regimen, including lifestyle variables such as smoking and an individual's preexisting inflammatory state.

Multiple demographic, lifestyle, and baseline HIV disease factors were independently associated with inflammatory biomarker levels at week 144, indicating the multifactorial aspect of the inflammatory response in PWH. Increasing age was associated with higher week 144 IL-6, D-dimer, and sCD163 levels. Higher baseline BMI was associated with higher week 144 IL-6, D-dimer, and hs-CRP levels, consistent with prior analyses [9, 24]. Female sex was associated with higher hs-CRP and lower sCD14 levels; however, this population was small (n = 58 total, with 39–40 participants having available biomarker data at baseline and week 144) [16]. In addition, lower week 144 sCD14 levels were associated with black or African American race and baseline VL ≥40 copies/mL, albeit the latter category was small (n = 20 total, with 15 participants having available biomarker data at baseline and at week 144). Higher sCD14 levels were associated with advanced CDC HIV disease category at baseline. Higher IL-6 levels were associated with baseline smoking status, and higher hs-CRP levels were associated with baseline triglycerides.

Importantly, all week 144 biomarker levels were strongly associated with their respective baseline levels across all biomarkers. Because TANGO participants were virologically suppressed by prior ART at the switch, these baseline biomarker levels indicate the relative association of persistent inflammation among an individual's conditions, comorbid conditions, or other factors—including preexisting virologic, inflammatory, and immunologic set points established before ART initiation—that may determine inflammation levels during treatment [5]. These findings suggest a strong association between inflammation and individual characteristics in virologically suppressed PWH.

Although PWH with persistent low-level or undetectable viremia have still shown increased levels of inflammatory biomarkers compared with individuals without HIV [6, 10], current HIV treatment guidelines do not recommend clinical monitoring of inflammatory biomarkers for several reasons. First, no standardized methods for measuring biomarker levels exist [14]. In addition, biomarker levels can fluctuate for a multitude of non–HIV-related reasons (eg, exercise, stress, hormones, and infection) [13, 27], and there are no commonly accepted thresholds for any biomarkers to define what constitutes a clinically meaningful change [14]. Some biomarkers, such as hs-CRP and D-dimer, are nonspecific indicators of the inflammatory response [14], and elevated levels could result from multiple causes. Finally, although the inflammatory biomarkers evaluated in this study have been investigated in the context of HIV, most biomarkers have not yet been validated as predictors of precise clinical events or HIV disease outcomes [14].

The strength of these post hoc analyses is the randomized comparison, though there are some limitations. Some covariate groups were small (eg, VL ≥40 copies/mL, female sex, individuals in the "other races" subgroup, and CDC HIV disease category stage 3). Biomarkers were quantified and assessed from a single discrete week 144 sample; however, inflammatory biomarker levels in a single individual can fluctuate daily for various reasons (eg, acute infections or intense exercise) [13, 27, 28]. Collecting and assessing multiple successive

biomarker samples may provide a more consistent representation of an individual's biomarker levels as they naturally vary across time. The ANCOVA models adjusted for many baseline variables but could not adjust for certain pre-ART disease characteristics known to affect chronic inflammation in PWH, such as the $CD4^+$ nadir [9] and the time between HIV diagnosis and ART initiation [4], because these data were not collected during enrollment. It is expected that uncontrolled variables will exist in all analyses owing to the number of possible conditions and lifestyle factors that could contribute to the inflammatory landscape.

These results support that switching to the 2-drug DTG/3TC regimen has durable noninferior efficacy with similar effects on inflammation compared with continuing a 3- or 4-drug TBR over 3 years. High and comparable proportions of participants maintained viral suppression in the DTG/3TC and TBR groups, including with the more stringent measure of VL <40 copies/mL and TND at week 144. Long-term inflammatory biomarker levels were low and comparable between treatment groups and were independently associated with multiple demographic, lifestyle, and baseline HIV disease characteristics.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Acknowledgments. The authors thank the study participants; their families and caregivers; the investigators and site staff who participated in the study; and the ViiV Healthcare, GSK, Pharmaceutical Product Development, and Phastar study team members. They also acknowledge Nisha George for her contributions to data analysis. Editorial assistance was provided under the direction of the authors by Lindsay Walton, PhD, and Jennifer Rossi, MA, ELS, MedThink SciCom, with funding from ViiV Healthcare.

Author contributions. Study conception: B. W., B. J., and M. A.-K. Study design: M. U., J. O., B. W., and M. A.-K. Data acquisition: R. W., M. U., E. B. M., C. B., J. S. M., S. S., R. Moore, P. S., J. O., R. Moodley, B. W., and M. A.-K. Data analysis: R. W., M. U., P. S., J. O., and M. A.-K. Data interpretation: R. W., P. S., J. O., B. W., M. K., B. J., and M. A.-K. Drafting of the manuscript: R. W., M. U., P. S., M. K., and M. A.-K. Critical revision of the manuscript for important intellectual content: R. W., M. U., J. M. L., P. S., J. O., R. Moodley, B. W., M. K., B. J., and M. A.-K. All authors approved the manuscript for publication.

Data availability. Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

Financial support. This work was supported by ViiV Healthcare.

Potential conflicts of interest. R. W., M. U., J. O., R. Moodley, B. W., M. K., B. J., and M. A.-K. are employees of ViiV Healthcare or GSK and own stock in GSK. J. M. L. has received consulting fees and honoraria from Gilead, Janssen-Cilag, and ViiV Healthcare and travel support from Gilead. E. B. M. has received honoraria and travel support from Gilead, Janssen, MSD, and ViiV Healthcare. C. B. has received grants, paid to her institution, from Gilead; consulting fees and honoraria from Gilead and ViiV Healthcare; and travel support from Gilead, Merck, and ViiV Healthcare. J. S. M. has received honoraria from Gilead, Janssen, MSD, and ViiV Healthcare and travel support from Gilead and Janssen. S. S. has received grants from Gilead, GSK, Heidelberg ImmunoTherapeutics, INSTO, Kassenärztliche Vereinigung Nordrhein, RKI, and ViiV Healthcare; honoraria from Cepheid, Gilead, Janssen, and ViiV Healthcare; payment for expert testimony from Gilead; and travel support from Gilead, Janssen, and ViiV Healthcare. S. S. has also participated in advisory boards for Gilead and ViiV Healthcare and is a member or controller of Landeskommission AIDS North-Rhine Westphalia (NRW), AIDS Hilfe NRW, NÄAGNO, and Dagnä. P. S. is a complimentary worker on behalf of GSK. R. Moore certifies no potential conflicts of interest.

References

1. Gabuzda D, Jamieson BD, Collman RG, et al. Pathogenesis of aging and age-related comorbidities in people with HIV: highlights from the HIV ACTION workshop. Pathog Immun **2020**; 5:143–74.
2. Kelesidis T, Tran TTT, Stein JH, et al. Changes in inflammation and immune activation with atazanavir-, raltegravir-, darunavir-based initial antiviral therapy: ACTG 5260s. Clin Infect Dis **2015**; 61:651–60.
3. Baker JV, Neuhaus J, Duprez D, et al. Changes in inflammatory and coagulation biomarkers: a randomized comparison of immediate versus deferred antiretroviral therapy in patients with HIV infection. J Acquir Immune Defic Syndr **2011**; 56:36–43.
4. Baker JV, Sharma S, Grund B, et al. Systemic inflammation, coagulation, and clinical risk in the START trial. Open Forum Infect Dis **2017**; 4:ofx262.
5. Gandhi RT, McMahon DK, Bosch RJ, et al. Levels of HIV-1 persistence on antiretroviral therapy are not associated with markers of inflammation or activation. PLoS Pathog **2017**; 13:e1006285.
6. Hattab S, Guiguet M, Carcelain G, et al. Soluble biomarkers of immune activation and inflammation in HIV infection: impact of 2 years of effective first-line combination antiretroviral therapy. HIV Med **2015**; 16:553–62.
7. Bastard JP, Soulié C, Fellahi S, et al. Circulating interleukin-6 levels correlate with residual HIV viraemia and markers of immune dysfunction in treatment-controlled HIV-infected patients. Antivir Ther **2012**; 17:915–9.
8. Wada NI, Jacobson LP, Margolick JB, et al. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. AIDS **2015**; 29:463–71.
9. Borges ÁH, O'Connor JL, Phillips AN, et al. Factors associated with plasma IL-6 levels during HIV infection. J Infect Dis **2015**; 212:585–95.
10. Schouten J, Wit FW, Stolte IG, et al. Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the $AGE_{h}IV$ Cohort Study. Clin Infect Dis **2014**; 59:1787–97.
11. Deeks SG, Tracy R, Douek DC. Systemic effects of inflammation on health during chronic HIV infection. Immunity **2013**; 39:633–45.
12. Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med **2019**; 25:1822–32.
13. Del Giudice M, Gangestad SW. Rethinking IL-6 and CRP: why they are more than inflammatory biomarkers, and why it matters. Brain Behav Immun **2018**; 70:61–75.
14. Llibre JM, Cahn PE, Lo J, et al. Changes in inflammatory and atherogenesis biomarkers with the 2-drug regimen dolutegravir plus lamivudine in antiretroviral therapy–experienced, virologically suppressed people with HIV-1: a systematic literature review. Open Forum Infect Dis **2022**; 9:ofac068.
15. Osiyemi O, De Wit S, Ajana F, et al. Efficacy and safety of switching to dolutegravir/lamivudine versus continuing a tenofovir alafenamide-based 3- or 4-drug regimen for maintenance of virologic suppression in adults living with human immunodeficiency virus type 1: results through week 144 from the phase 3, noninferiority TANGO randomized trial. Clin Infect Dis **2022**; 75:975–86.
16. van Wyk J, Ajana F, Bisshop F, et al. Efficacy and safety of switching to dolutegravir/lamivudine fixed-dose 2-drug regimen vs continuing a tenofovir alafenamide-based 3- or 4-drug regimen for maintenance of virologic suppression in adults living with human immunodeficiency virus type 1: phase 3, randomized, noninferiority TANGO study. Clin Infect Dis **2020**; 71:1920–9.
17. Llibre JM, Brites C, Cheng CY, et al. Efficacy and safety of switching to the 2-drug regimen dolutegravir/lamivudine versus continuing a 3- or 4-drug regimen for maintaining virologic suppression in adults living with human immunodeficiency virus 1 (HIV-1): week 48 results from the phase 3, noninferiority SALSA randomized trial. Clin Infect Dis **2023**; 76:720–9.
18. Llibre JM, Cahn PE, Barber TJ. Commentary: long-term changes of inflammatory biomarkers in individuals on suppressive three-drug or two-drug antiretroviral regimens. Front Immunol **2022**; 13:904689.
19. Halvas EK, Joseph KW, Brandt LD, et al. HIV-1 viremia not suppressible by antiretroviral therapy can originate from large T cell clones producing infectious virus. J Clin Invest **2020**; 130:5847–57.

20. Li J, Sax P, Marconi V, et al. No significant changes to residual viremia after switch to dolutegravir and lamivudine in a randomized trial. J Int AIDS Soc **2018**; 21(suppl 8):e25187.
21. Underwood M, Urbaityte R, Wang R, et al. DTG + 3TC in GEMINI-1 & -2: HIV-1 replication at <50 c/mL and VL 'blips' through 144 weeks [poster PEB163]. Presented at: 11th IAS Conference on HIV Science; July 18-21, **2021**; virtual.
22. Charpentier C, Peytavin G, Burdet C, et al. Residual HIV-1 RNA, HIV-1 DNA, and drug plasma Cmin in dual DTG + 3TC, ANRS 167 LAMIDOL [poster 491]. Presented at: Conference on Retroviruses and Opportunistic Infections; March 4-7, **2019**; Seattle, WA.
23. Gianella S, Marconi VC, Berzins B, et al. Genital HIV-1 shedding with dolutegravir (DTG) plus lamivudine (3TC) dual therapy. J Acquir Immune Defic Syndr **2018**; 79:e112–4.
24. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med **2008**; 5:e203.
25. Serrano-Villar S, López-Huertas MR, Jiménez D, et al. Long-term changes of inflammatory biomarkers in individuals on suppressive three-drug or two-drug antiretroviral regimens. Front Immunol **2022**; 13:848630.
26. Osiyemi O, Ajana F, Bisshop F, et al. Switching to DTG/3TC fixed-dose combination (FDC) is non-inferior to continuing a TAF-based regimen in maintaining virologic suppression through 144 weeks (TANGO study) [poster 900]. Presented at: IDWeek 2021; September 29-October 3, **2021**; virtual.
27. Singh T, Newman AB. Inflammatory markers in population studies of aging. Ageing Res Rev **2011**; 10:319–29.
28. Autio TJ, Koskenkorva T, Leino TK, Koivunen P, Alho OP. Longitudinal analysis of inflammatory biomarkers during acute rhinosinusitis. Laryngoscope **2017**; 127: E55–61.