A Phase 1b/2a study of the safety, pharmacokinetics and antiviral activity of BIT225 in patients with HIV-1 infection

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Objectives: BIT225 (N-carbamimidoyl-5-(1-methyl-1H-pyrazol-4-yl)-2-naphthamide), a novel acyl-guanidine, is a novel antiviral drug that blocks Vpu ion channel activity and has anti-HIV-1 activity in vitro. The antiviral effect of BIT225 is most pronounced in cells of the myeloid lineage. With infected circulating monocytes and tissue-resident macrophages representing a key cellular reservoir of HIV-1, BIT225 has a potential role in the eradication of the virus from the host.

Patients and methods: BIT225-004 is a Phase 1b/2a, placebo-controlled, randomized study of the safety, pharmacokinetics and antiviral activity of BIT225 in 21 HIV-1-infected, ART-naive subjects. Twenty-one subjects were enrolled and received BIT225 (400 mg twice daily) or placebo treatment for 10 days (randomized 2:1). The anti-HIV-1 effect of BIT225 in the monocyte reservoir was measured in CD14+ monocytes isolated from the peripheral blood on days 1 (pre-dose), 5, 10 and 20; isolated monocytes were co-cultured ex vivo with MT4 T cells. De novo HIV-1 replication was measured by p24 activity of released virus into the culture supernatant to day 25 of co-culture. In addition, monocyte samples were collected for analysis by RT–PCR total HIV-1 DNA single-copy assay.

Results: Measurement of HIV-1 directly within the patient's monocyte population indicated that BIT225 treatment significantly reduced the viral burden in myeloid lineage cells, which was more evident in those individuals with the highest viral loads. In addition, BIT225-treated subjects demonstrated a significantly reduced level of monocyte activation (sCD163) compared with the placebo controls.

Conclusions: This study's unique design demonstrates that BIT225 can significantly reduce the dissemination of HIV-1 from infected monocytes. This has important ramifications for diminishing the seeding/re-seeding of the viral reservoir.

Introduction

BIT225 (N-carbamimidoyl-5-(1-methyl-1H-pyrazol-4-yl)-2-naphthamide) is a first-in-class antiviral drug that blocks Vpu ion channel activity, resulting in disrupted HIV-1 assembly within the host cell and a substantial loss of infectivity of the progeny virus.1-6 BIT225 demonstrates encouraging anti-HIV-1 activity in primary human CD14+ monocyte-derived macrophages (MDMs) and significantly reduces virus release from MDMs with a mean (±SE) effective concentration (EC50) of 2.25 ± 0.23 μM and a mean (±SE) toxic concentration (TC50) of 284 ± 9 μM.6 While BIT225 demonstrates anti-HIV-1 activity in T cells,7 the effects are greater in cells of the myeloid lineage as a result of differences in viral assembly and release. In CD4+ T cells HIV-1 assembles at the plasma membrane,8 whereas in macrophages assembly is within internal multi-vesicular bodies (MVBs), where progeny viruses accumulate in high numbers and possess markers of these compartments.5-8

Viral reservoirs are a significant obstacle to the eradication of HIV-1 infection. Despite the availability of combination ART to HIV-1 infected individuals, HIV-1 avoids eradication through the establishment and survival of latently infected cellular reservoirs.9-12 While the majority of latently infected cells are found within the resting CD4+ T cells, predominantly central and transitional memory subsets,11 monocytes and macrophages of the myeloid lineage also contribute to the overall reservoir.12-16 While there is evidence that circulating monocytes are infected,12,15,17 they generally only reside in the periphery for around a day before entering the tissues and differentiating into macrophages, although recently it has been reported that macrophages within the periphery in addition to those in the various organs are capable of self-renewal...
Block viral replication within cells of the myeloid lineage often requires higher concentrations of antiretroviral drugs to penetrate these cell types compared with CD4+ T cells, making these cells an enduring reservoir during ART. Anatomical sanctuary sites exist, such as brain-resident macrophages, where drug penetration is poor and evaluating drug efficacy is challenging. Infected cells within these reservoir sites may be responsible for the persistent viremia observed in some individuals on long-term ART. HIV-1 has been shown to replicate in CD14+ monocytes in vivo, even in those individuals receiving ART, and when therapy is discontinued in virally suppressed patients infectious virus originating from monocytes can be recovered. Further supporting the continued low-level viral replication in some of the treated individuals is the ongoing presence of two long terminal repeat (LTR) circles in CD14+ monocytes, although this finding remains contentious. Despite long-term ART, increased immune activation persists and results in increased mortality. The permanently elevated levels of immune activation are further indication of ongoing viral replication even in the presence of ART. Here, we report on the antiviral effects of BIT225 in the setting of a Phase 1b/2a clinical trial conducted at Siriraj Hospital, Bangkok, Thailand, in HIV-1-positive individuals. Using a novel co-culture assay that measures infectious virus from patient CD14+ monocytes, and directly quantifying total HIV-1 DNA with RT–PCR, we demonstrated that treatment with BIT225 significantly reduced the level of HIV-1 within these cells. Our findings demonstrated that even a short, i.e. 10 day, treatment with BIT225 resulted in a decrease in monocyte immune activation, as measured by sCD163. These results provide evidence that BIT225 can target and reduce the viral burden in cells of the myeloid lineage in a clinical setting.

Methods

Trial design

Participants were recruited at a single site, Siriraj Hospital, in Bangkok, Thailand. The primary objective was the evaluation of the safety and tolerability of 400 mg of BIT225 twice daily compared with placebo in male and female individuals aged 18–65 years with HIV-1 infection (viral load >5000 copies/mL; CD4+ count >350 cells/μL) that were ART-naïve or treatment-experienced patients that had received no antiretroviral treatment within 90 days of study screening. Secondary objectives included the pharmacokinetic evaluation of 400 mg of BIT225 administered daily on day 1 and 10 and twice daily on days 2–9; the assessment of the antiviral activity of BIT225 as measured in real time in isolated HIV-1-infected CD14+ monocytes and cultured ex vivo; and assessment of the ability of BIT225 to cross the blood–brain barrier by sampling the CSF of a subset of patients at a single timepoint (day 9 or 10). Twenty-one subjects were recruited and randomly assigned to receive either BIT225 or placebo (lactose powder) at a 2:1 ratio. BIT225 or placebo was administered as a powder reconstituted with 25 mL of Ora-Sweet SFTM taste-masking agent.

Ethics statement

The study was approved by the ethics review board at Siriraj Hospital, Bangkok, Thailand. All patients provided written informed consent. The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki (October 1996). The trial was registered on the Australian New Zealand Clinical Trials Registry (ANZCTR; Trial ID ACTRN12612000696897).

Pharmacokinetic assessments

Blood samples were collected for pharmacokinetic analysis on day 1 at 0 h (pre-dose), 30, 60, 90 and 120 min post-dose, then at 3, 4, and 24 h post-dose. Blood samples were collected for pharmacokinetic analysis on day 10 at 0 h (pre-dose) and 30, 60, 90 and 120 min after the last dose, then at 3, 4, 6, 8, 10 and 12 h and on day 12 at 48 h after the last dose. Blood samples were also collected pre-dose on day 5.

Plasma samples were analysed for BIT225 concentrations using a validated method (CR Pharma Services Pty Ltd, Thebarton, Australia). Briefly, BIT225 was extracted from human plasma using liquid–liquid extraction. The analytes were separated by HPLC on a C18 reverse-phase column and the eluate was monitored by an API4000 MS/MS detector. Samples were spiked with an internal standard and assayed against a calibration curve.

HIV-1 RNA plasma viral load assay

Blood samples for HIV-1 plasma viral load were collected at the pre-screen and on days 1 (pre-dose) 2, 5, 10, 11 and 20. HIV-1 RNA was quantified using the COBAS TaqMan HIV-1 assay (Roche Diagnostics). Absolute CD4+ T cell counts were determined by flow cytometry on days 0, 5, 10 and 20.

CD14+ monocyte isolation and co-culture assay

For all patients, blood was collected on days 1 (pre-dose), 5 and 10 of dosing and at a follow-up visit 10 days after treatment (day 20). Plasma was stored and CD14+ monocytes were isolated from the 21 study participants by magnetic bead sorting (Miltenyi Biotech, Gladbach, Germany) at each of these four timepoints. In real time, freshly isolated CD14+ monocytes were combined with uninfected MT4 T cells and co-cultured ex vivo for 25 days to amplify virus originating from the isolated monocyte cells. HIV-1 replication in the co-culture supernatant was determined by p24 ELISA (Perkin Elmer, MA, USA) after 5, 10, 15, 20 and 25 days of co-culture. Thus, for each individual, four co-cultures were set up and sampled over a 25 day period for HIV-1 that originated from the infected individuals’ circulating monocytes.

Total HIV-1 DNA quantification in monocytes

Total DNA was extracted from isolated CD14+ monocytes collected at days 1 (pre-dose) 5, 10 and 20 using the Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany) according to the manufacturer’s instructions. Total HIV-1 DNA was quantified by a real-time PCR assay specific for clade E HIV-gag using the clade E SK1-15 (5′-AGTGGGAGGACCACGAGCAATGCAAAT-3′) and clade E SKC1B (5′-TATCGTAGTCCCTGCTATG-3′) primers and incorporated a fluorescent locked nucleic acid (LNA) probe, SKLNA2-3′ (5′-6-FAM AT(C|A|T)GAGGAA|G|C-T|G|C-BHQ-1′-3′) with LNAs shown in brackets). A TOPO cloned patient insert plasmid was used as a 10-fold serially diluted standard curve from 102 to 10 copies.

Total HIV-1 DNA copies were normalized using the Applied Biosystems (CA, USA) TaqMan β-actin detection reagents with an FAM-labelled probe. The standard curve was constructed from purified human buffy coat DNA. The assay was performed on a Bio-Rad iQ5 multicolour real-time PCR detection system (CA, USA), and consisted of 1 cycle of 95 °C for 3 min followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. All standard
dilutions and patient samples were run in triplicate and quantified total HIV-1 DNA was expressed as copies/500 ng DNA.

**Monocyte activation markers**

Levels of plasma sCD163 were quantified by ELISA according to the manufacturer’s recommendations (Trillium Diagnostics, Bangor, ME, USA). Plasma samples at days 1 (pre-dose), 5, 10 and 20 were batch tested in addition to 11 healthy HIV-1 seronegative individuals, to determine a normal range for this biomarker.

**Statistical analysis**

Pharmacokinetic parameters were derived from plasma sample data using non-compartmental methods employing WinNonlin® Phoenix version 6.3 (Pharsight, St Louis, MO, USA). Computer software (Statview 4.5; Abacus, Berkeley, CA, USA) was used for all statistical calculations. Comparisons between BIT225 and placebo controls were carried out by use of the Mann–Whitney U-test, with a significance cut-off of \( P < 0.05 \), unless otherwise stated.

**Results**

**Study participants**

Twenty-one individuals were enrolled in the study and the two treatment arms were comparable for baseline characteristics (Table 1 and Figure S1, available as Supplementary data at JAC Online).

**Safety**

Adverse events (AEs) were reported from day 1 (time of administration of BIT225) to day 20. The majority of AEs were mild (Grade 1) in severity (Table S1), with the most frequently occurring AEs being headache (85.7% with BIT225 versus 28.6% with placebo), nausea (57.1% with BIT225 versus 28.6% with placebo) and vomiting (35.7% with BIT225 versus 14.3% with placebo). A total of four BIT225-treated subjects experienced a Grade 2 AE; no subject experienced a Grade 3 AE.

Although in most subjects the reported AEs were transient and self-limiting, two individuals prematurely discontinued BIT225, both due to headache, nausea and vomiting after the first dose (Grades 1 and 2).

**Pharmacokinetic analysis**

**Plasma**

The pharmacokinetics of BIT225 400 mg were assessed following single and repeated doses. Significant plasma concentrations were attained on day 1 after a single dose. AUC(0–12) values indicated an \(~3\)-fold accumulation from day 1 to 10 and moderate inter-subject variability was observed in \( C_{\text{min}} \) and \( C_{\text{max}} \). \( C_{\text{max}} \) averaged \((±SE) 2110 ± 239\ ng/mL\) on day 1 and increased to an average of \( 4770 ± 569\ ng/mL\) on day 10. The median \( T_{\text{max}} \) for day 1 was 4.0 h (IQR 3–4 h) and on day 10 it was 3.5 h (IQR 0.5–4.5 h).

As calculated from the day 10 data, the mean (± SE) steady-state \( C_{\text{max}} \) was \( 4770 ± 569\ ng/mL\) and mean \( C_{\text{min}} \) was \( 3390 ± 488\ ng/mL\) (Figure S2). These are equivalent to \(~16\ \mu M\) \( (C_{\text{max}})\) and \(~11\ \mu M\) \( (C_{\text{min}})\) (the mol. wt of BIT225 is 293.32). The steady-state plasma concentration of BIT225 in the treated individuals was therefore at least five times the EC50 value for BIT225, \( 2.25 ± 0.23\ \mu M\), as determined for BIT225 in vitro, using in HIV-1BaL-infected MDMs.

The terminal half-life was calculated from the observed terminal phase rate constant estimated by linear regression through at least three data points in the terminal phase of the log concentration–time profile using data for the last dose on day 10. The pharmacokinetic sampling on day 1 was of insufficient duration and frequency to enable accurate determination of terminal half-life, hence it was calculated from the day 10 data. The half-life had a mean of \( 16.7 ± 3.0\ h\) and ranged from 6.76 to 40.1 h.

| Table 1. Baseline characteristics of the study participants |
|-----------------------------|-----------------------------|-----------------------------|
| Treatment group (no. of subjects) | BIT225 (n=14) | placebo (n=7) | Total |
| Gender, n (%) |  |  |  |
| female | 7 (50.0) | 3 (42.9) | 10 (47.6) |
| male | 7 (50.0) | 4 (57.1) | 11 (52.4) |
| Withdraw | 2 | 0 | 2 |
| Age (years), mean (SD) | 32.1 (11.5) | 25.6 (5.0) | 29.9 (10.2) |
| Race, n (%) |  |  |  |
| Asian | 12 (85.7) | 7 (100) | 19 (90.5) |
| white | 2 (14.3) | 0 | 2 (9.5) |
| Weight (kg), mean (SD) | 59.4 (7.9) | 60.7 (9.4) | 59.8 (8.2) |
| BMI (kg/m²), mean (SD) | 22.3 (2.4) | 22.4 (3.1) | 22.3 (2.5) |
| Plasma viral load (copies/mL), median (IQR) | 29049 (11846–53553) | 27897 (18580–57698) | 27897 (12007–53644) |
| Log10 viral load (copies/mL), median (IQR) | 4.46 (4.07–4.73) | 4.45 (4.24–4.75) | 4.45 (4.08–4.73) |
| CD4 count (cells/mm³), median (IQR) | 428 (335–562) | 482 (463–563) | 451 (337–584) |

The treated and placebo groups were comparable for baseline characteristics.
BIT225 was measured in CSF in two patients at ~4–5 h after the day 10 dose. CSF concentrations were 87.8 and 24.4 ng/mL in the two patients and the respective CSF/plasma ratios were 2.5 and 1.5%, indicating that BIT225 penetrates the blood–brain barrier.

**Plasma HIV-1 viral load and CD4+ T cell count**

BIT225 had no significant effect upon patient HIV-1 viral load or CD4+ T cell count. The mean plasma HIV-1 level in the placebo arm was relatively stable: it was 38,341 copies/mL pre-dose, peaked at 50,913 copies/mL on day 5 and returned to 32,778 copies by day 20, 10 days post-therapy. Similarly, in the BIT225-treated cohort, excluding the two individuals that withdrew prior to day 5, mean copy number was 51,494 copies/mL pre-dose and peaked on day 20 with 69,677 copies/mL. Mean CD4+ T cell counts remained relatively constant throughout the study period in both cohorts, with the placebo arm fluctuating from +5 to −67 cells/mm³ and from +32 to −97 cells/mm³ with respect to baseline.

**Antiviral activity in myeloid cells**

**Total HIV-1 DNA as determined by RT–PCR**

Total HIV-1 DNA could only be detected at all four timepoints in the isolated monocytes from 6 of the 12 individuals receiving BIT225 (2 additional subjects withdrew from the study and were excluded from these analyses due to incomplete sample sets). In individuals where a signal was absent at one or more of the timepoints, the level of virus detected was generally low (<200 copies/500 ng) in these cells.

The placebo cohort demonstrated a significantly higher pre-dose total HIV-1 DNA copy number in the monocyte cells. HIV-1 DNA copy number in the placebo cohort was 7851 ± 2981 compared with 1493 ± 593 copies/500 ng in the BIT225-treated patients (P = 0.02). In the control group, the HIV-1 DNA copy number in the isolated monocytes remained stable throughout the study, with 7851 ± 2981, 6000 ± 4731, 6974 ± 3836 and 7098 ± 3066 copies/500 ng at pre-dose and days 5, 10 and 20, respectively (n = 7). In contrast, there was a reduction in total HIV-1 DNA copy number in the monocytes from the drug cohort during the course of treatment with BIT225 at day 10 (P = 0.09). The mean (±SE) total HIV-1 copy number in the BIT225-treated group was 1493 ± 593, 1414 ± 655, 547 ± 157 and 1419 ± 1183 copies/500 ng DNA at pre-dose and days 5, 10 and 20, respectively (Figure 1).

Analysis of individual responses from each subject demonstrated that three of the six BIT225-treated individuals demonstrated significant reductions in total HIV-1 DNA levels within their monocytes; the other three subjects’ responses remained unchanged. Of note, these three individuals with the greatest observable BIT225-induced effects also demonstrated the highest levels of HIV-1 within the circulating monocytes pre-dose. This suggests that a drug response can be detected provided there is a high detectable level of virus at the initiation of therapy for BIT225 to act upon (Figure 2).

Ten days of BIT225 treatment resulted in a 2.7-fold decrease or 63% reduction in the mean total HIV-1 DNA copy number in the monocytes of the BIT225-treated individuals (n = 6) compared with baseline (P = 0.09; Figure 3). The three treated individuals

**HIV-1 co-culture transfer assay**

The amount of virus originating from the CD14+ monocytes in the placebo group (n = 7) remained constant throughout the study, with no differences observed in the HIV-1 replication rate in the co-cultures at the four sampled timepoints: pre-dose and days 5, 10 and 20 (Figure 3). In the BIT225-treated arm (n = 12), a reduced amount of virus was detected in the isolated patient monocytes that were collected after 5 and 10 days of drug treatment, compared with pre-dose levels. This lower level of virus in the co-cultured cells was evident at days 5 and 10 of BIT225 therapy and persisted to day 20, 10 days after the drug was stopped. This is indicative of less HIV-1 present within the myeloid

**Figure 1.** Mean total HIV-1 DNA in monocytes of HIV-1-seropositive individuals following 10 days of BIT225 treatment. The bars show the standard error.

**Figure 2.** Reduction in mean HIV-1 copy number in monocytes of HIV-1-seropositive individuals following 10 days of BIT225 treatment. The individual responses of the six patients from whom detectable total HIV-1 DNA was isolated are shown as broken lines; the mean response at baseline versus the end of BIT225 treatment on day 10 is shown as a solid line with SE bars (n = 6; P = 0.09).
compartment in the drug-treated patients and a post-drug treatment effect after cessation of BIT225 treatment on day 10.

To further analyse the BIT225 antiviral effect, the 12 BIT225-treated patients were split into two groups on the basis of their pre-dose plasma HIV-1 RNA viral loads relative to the median cohort HIV-1 RNA viral load at that timepoint. Group A included subjects with higher viral loads (\(>\log_{10} 4.43\)) and Group B those with lower viral loads (\(<\log_{10} 4.3\)) at study entry. Subjects with the highest viral loads had a significant reduction in virus in their CD14\(^+\) monocytes following treatment with BIT225. In this group, monocytes isolated at day 5 demonstrated significantly less virus within the co-cultures at days 15 and 20 of co-culture (\(P=0.05\) and 0.04, respectively) and there was a general trend of less HIV-1 originating from monocytes during BIT225 therapy, even 10 days following the cessation of BIT225 (Figure 4a). In contrast, analysis of virus levels within the monocytes of treated individuals with lower plasma viral loads (Group B; \(n=6\)) remained constant throughout the study (Figure 4b). These data are similar to previous findings with the HIV-1 total DNA assay on HIV-1 infected monocytes, where a high level of virus before treatment allowed a measurable drug response after treatment (Figure 3).

Figure 3. Reduction in virus burden within CD14\(^+\) monocytes following treatment. Although the levels of HIV-1 within the monocyte population remained steady throughout the trial in the placebo arm (left panel), a reduction in the amount of HIV-1 within the monocytes of individuals receiving BIT225 treatment was observed (right panel). The mean p24 titres in co-culture supernatants are shown (with SE bars) for monocytes isolated pre-dose, on day 5 of treatment and on day 10 and 10 days following treatment for the placebo- and BIT225-treated individuals.

Figure 4. BIT225 therapy results in a significant reduction in HIV-1 within the CD14\(^+\) monocytes of (a) patients with higher viral loads (Group A; \(n=6\)) compared with (b) those with lower viral loads (Group B; \(n=6\)). Individuals with higher levels of plasma HIV-1 at baseline demonstrated a greater responses following 10 days of BIT225.
reasonably high plasma viral loads (days 5, 10 and 20, respectively. Despite enrolled individuals having plasma sCD163 levels were comparable to those in uninfected 1263 relatively constant across the four timepoints, with 1275 HIV-1 replication within this myeloid population. 1286 baseline, (2187 sCD163 levels were significantly (736 Pearson’s test, sCD163 levels were significantly (<0.04) decrease in sCD163 levels, from 1992 ± 187 ng/mL pre-dose to 1802 ± 184 ng/mL at day 5, the level normalizing at day 10 to 2087 ± 163 ng/mL. At day 20, 10 days after BIT225 cessation, sCD163 levels were significantly (<0.04) elevated from baseline, (2187 ± 205 ng/mL), suggesting the resumption of HIV-1 replication within this myeloid population.

In the placebo cohort (n = 7), sCD163 plasma levels remained relatively constant across the four timepoints, with 1275 ± 141, 1263 ± 176, 1449 ± 238 and 1334 ± 196 ng/mL at pre-dose and days 5, 10 and 20, respectively. Despite enrolled individuals having reasonably high plasma viral loads (> log 3.5 copies/mL at entry), plasma sCD163 levels were comparable to those in uninfected healthy controls throughout the course of the study (Figure 5).

Discussion

BIT225 is a first-in-class antiviral compound that is capable of inhibiting HIV-1 production in the myeloid cells of infected individuals. In this Phase 1b/2a double-blind, placebo-controlled, safety, pharmacokinetic and antiviral study of BIT225, 10 days of BIT225 treatment was well tolerated in subjects infected with HIV-1. AEs were generally mild in severity, Grade 1 and self-limited. Four BIT225-treated subjects experienced Grade 2 AEs; two of them prematurely discontinued BIT225, both due to headache, nausea and vomiting after the first dose. BIT225 has since been re-formulated as a capsule, which may assist in reducing these incidences. In addition, dose levels in future trials are anticipated to be reduced, and there is potential for once-a-day dosing. Plasma BIT225 concentration–time data indicated that significant concentrations were attained in all dosed subjects on day 1, with concentrations rising to steady state by day 10, with adequate plasma levels achieved. Analysis of CSF demonstrated that the drug was able to cross the blood–brain barrier. Even during a short duration of treatment, BIT225 reduced the viral burden in circulating peripheral monocytes, measured directly by quantifying total HIV-1 DNA and additionally in an infectious virus co-culture assay. The magnitude of response correlated with the individuals’ viral loads pre-dosing, such that greater drug effects were observed in those individuals with the highest plasma HIV-1 loads on study entry. Additionally, the antiviral effects of BIT225 were associated with a transient reduction in the immune activation of these myeloid cells, as measured by sCD163 plasma levels. No such changes were observed within the matched placebo controls.

The finding that BIT225 can cross the blood–brain barrier has implications for HIV-associated neurocognitive disorders (HANDs). Infiltration of the CNS by infected macrophages and the propagation of virus by astrocytes and microglial cells create long-lived reservoirs with quantifiable viraemia even 2 years after the initiation of ART, 33 which are likely to originate from these myeloid cells. The efficacy of ART against monocytes/macrophages has been demonstrated to correlate with cognitive function and a drug targeting cells of the myeloid lineage may improve HIV-1 neurocognitive impairment.26

Determining the role and importance of the myeloid cells in primary HIV-1 infection and viral persistence is difficult due to the logistical issues of accessing these cells directly from the tissues. However, macrophages are widely acknowledged to be a major target for infection by HIV-1, with vaginal macrophages reported to be highly permissive and dendritic cells having been implicated in early infection within the mucosa and assisting in viral dissemination, rapidly transporting HIV-1 from the periphery to the lymph nodes, resulting in high levels of HIV-1 replication.34,35 By directly measuring total HIV-1 DNA within the circulating monocyte cells, the most accessible myeloid population, this study demonstrates that BIT225 reduces the viral burden in these cells following 10 days of treatment. The finding of a reduction in integrated virus within the CD14+ monocytes was supported using a co-culture assay to measure the release of infectious viruses that originate from the CD14+ monocytes isolated from BIT225-treated individuals. Unsurprisingly, the activity of BIT225 was greatest in those individuals with a high viral burden at study entry, where an antiviral response could be clearly delineated from the limits of detection, i.e. the background of the assay. Further, the reduction in immune activation, as measured by the myeloid-specific marker sCD163, suggests a lower level of viral replication within these cells. The fact that virus can be detected within these circulating cells and that BIT225 has an effect is promising. Monocytes circulating in the periphery account for a small percentage of all myeloid cells and remain in circulation only briefly before extravasation and differentiation in the peripheral tissues, with macrophages found in every organ system.35,36 A drug targeting HIV-1 replication within the myeloid cells and limiting virus dissemination has a clear clinical benefit.

Despite combination ART reducing plasma HIV-1 RNA levels to below detection, cell-associated HIV-1 DNA can be detected in

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**Figure 5.** Mean plasma levels of the monocyte activation marker sCD163 in the BIT225-treated group (n = 12) and placebo controls (n = 7). Individuals were treated with BIT225 for 10 days and were then followed up for 10 days. The mean (SE bars) for the placebo cohort (open circles) and the BIT225-treated group (closed circles) are shown, with the mean (SE bar) for HIV-1-uninfected controls (normal range: n = 11), represented by the cross.

**Quantification of monocyte activation**

As a prelude to this study, a normal range of the activation marker sCD163 was determined in a cohort of 11 HIV-1-uninfected Caucasian individuals. The mean (±SE) sCD163 level was 1378 ± 151 ng/mL (range 680–2427 ng/mL) in this healthy cohort.

The plasma level of the activation marker sCD163 was quantified in the individuals enrolled in the trial, at the four timepoints (pre-dose and days 5, 10 and 20) by ELISA. High plasma levels of sCD163 significantly correlated with higher HIV-1 viral loads pre-dose and throughout BIT225 therapy (P = 0.0001, r = 0.53).

Treatment with BIT225 (n = 12) resulted in a significant (P < 0.04) decrease in sCD163 levels, from 1992 ± 187 ng/mL pre-dose to 1802 ± 184 ng/mL at day 5, the level normalizing at day 10 to 2087 ± 163 ng/mL. At day 20, 10 days after BIT225 cessation, sCD163 levels were significantly (P = 0.04) elevated from baseline, (2187 ± 205 ng/mL), suggesting the resumption of HIV-1 replication within this myeloid population.

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Despite combination ART reducing plasma HIV-1 RNA levels to below detection, cell-associated HIV-1 DNA can be detected in
most patients receiving ART. Interruption of treatment results in the rapid rebound of virus from latent reservoirs and from cells and/or sanctuary sites where drug penetration is poor. Current approaches employing latency-reversing or reactivation agents result in productive infection within these cells, with the potential to both reduce and re-seed the reservoir. The generation of large numbers of de novo virions, using agents such as vorinostat and romidepsin, requires effective ART that targets both T cell and myeloid infected cells to be fully effective and prevent a net increase in the reservoir size. By targeting the cells of the myeloid lineage and preventing seeding/re-seeding of the reservoirs, BIT225 has a potential role in the eradication strategy of HIV-1.

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Transparency declarations
J. W., C. L., G. E., K. McB. and M. M. were all employed by Biotron Limited at the time of the study. R. L. M. is a Medical Consultant for Biotron Limited. There are no conflicts of interest for W. R.

Author contributions
W. R. was the principal investigator of the study and responsible for recruitment of patients. J. W., C. L., G. E., M. M. and R. L. M. were involved with the design and implementation of the study. J. W. performed the co-culture and scCD163 assays and K. McB. performed the single copy RT–PCR assay.

Supplementary data
Figures S1 and S2 and Table S1 are available at JAC Online (http://jac.oupjournals.org/).

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