A Phase 2a Study of the Safety, Pharmacokinetics and Antiviral Activity of BIT225 in Patients with HIV-1 Infection

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Introduction

Viral reservoirs are a significant obstacle to eradication of HIV-1 infection. Macrophages are an early target for HIV-1 infection and serve as long term reservoirs of the virus. Therapeutic strategies aimed at fully eradicating HIV-1 from the host must also target these infected cells to be fully effective.

The discovery that specific viral proteins have ion channel activity (viroporins) led Biotron to design a library of >250 compounds with >70% active against the Vpu target. BIT225 was selected as the lead compound from this library. It demonstrates encouraging anti-HIV-1 activity in primary human CD14$^+$ monocyte-derived macrophages (MDMs). BIT225 significantly reduces virus release from MDMs with an EC_{50} of 1.1 ± 0.4 μM and a TC_{50} of 212 μM.

Here we report on the antiviral effects of BIT225 in the setting of a recent Phase 1b/2a clinical trial conducted at the Siriraj Hospital, Bangkok, Thailand in HIV-1$^+$ individuals. Using a novel co-culture assay measuring infectious virus from patient CD14$^+$ monocytes, we have demonstrated that treatment with BIT225 significantly reduced the level of HIV-1 within these cells. The results provide evidence that BIT225 can target and reduce the viral burden in cells of the myeloid lineage in a clinical setting.

Aim

The aim of this study was to examine the effect of 10 days of BIT225 treatment on the level of active HIV-1 infection in the myeloid cellular reservoirs, which persist irrespective of active virus replication in T cells. This used a novel endpoint-analysis method for e*x vivo* measurement of HIV-1 output from cells of the CD14$^+$ monocyte lineage after isolation from BIT225-treated individuals.

In contrast to HIV-1 clinical trials using T cell-targeting drugs, this trial did not aim to measure a direct decrease in HIV-1 viral load, nor increase in CD4$^+$ T-cell count: Such effects would be unlikely for a short monotherapy trial of BIT225, a compound which was designed to target HIV-1 replication in macrophages and myeloid cellular reservoirs.

Study Design

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

Primary objective

The safety and tolerability of 400 mg of BIT225 BID compared with placebo in patients with HIV-1 infection that were antiretroviral therapy naïve.

Secondary objectives

- The pharmacokinetics of 400 mg of BIT225 administered daily on day 1 & 10 and twice daily on days 2 – 9.
- The antiviral activity of BIT225.

Study design

- A randomised, placebo controlled, parallel, double-blind study of BIT225 in patients with HIV-1 infection who are antiretroviral therapy naïve.
- Open to males and females, aged 18 to 65 years, with HIV-1 infection (viral load >5,000 copies/mL; CD4$^+$ count >350 cells/mm^3) and that are antiretroviral therapy naïve.
- 14 patients received 400 mg BIT225 and 7 received placebo.

Samples, CD14$^+$ monocyte isolation and co-culture assay

For all patients, blood was collected on days 0, 5, and 10 of dosing and a follow up visit at day 20. Plasma was stored and CD14$^+$ monocytes isolated from the 21 study participants by magnetic bead sorting at each of these 4 time points.

At each of the 4 time points, total DNA was extracted from the isolated CD14$^+$ monocytes and total HIV-1 DNA copies quantitated using RT-PCR. Total HIV-1 DNA copy number was not detected in all samples at all time points (placebo n=7; BIT225 n=6).

In real time, isolated CD14$^+$ monocytes were combined with MT4 T cells and co-cultured *ex vivo* for 25 days. HIV-1 replication in the co-culture was determined by p24 ELISA of the co-culture supernatant after 5, 10, 15, 20 and 25 days of co-culture.

Results – Antiviral Efficacy

Table 1. Baseline characteristics of the study participants. The treated and placebo groups were well matched at baseline, with no significant differences between the two groups in any of the parameters measured.

	Total	Placebo	BIT225
n	21	7	14
Female	10	3	7
Male	11	4	7
Withdrew	2	0	2*
Mean Age	29.2	27	30.4
***HIV-1 VL** (copies/mL)*			
Median	27,199	20,521	27,997
Range	3,560 – 276,930	6,109 – 81,829	3,560 – 276,930
Log	4.43	4.29	4.45
Range	3.55 – 5.44	3.79 – 4.91	3.55 – 5.44
***CD4 Count** (cells/mm3)*			
Median	475	482	441
Range	261 – 835	261 – 617	299 – 835

** Discontinued due to headache, nausea and vomiting (grade 1 & 2)*

Ten days of BIT225 was generally well tolerated. The pharmacokinetic data suggests that adequate BIT225 levels in the plasma were achieved *in vivo.* As expected, this short duration of BIT225 had no effect upon patient HIV-1 viral load or CD4$^+$ T cell count.

Figure 1. BIT225 results in a reduction in total HIV-1 DNA in the monocytes of HIV-1$^+$ individuals with 10 days of treatment. Total HIV-1 DNA in individuals receiving placebo remained stable throughout the study.

Mean ±SE copy number was 1493 ±593, 1414 ±655, 547 ±157 and 1419 ±1183 copies/500 ng DNA at days 0, 5, 10 & 20 respectively.

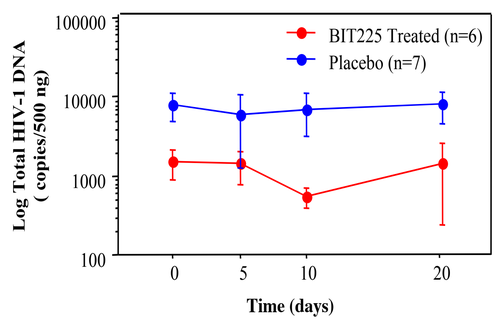


Figure 2. BIT225 results in a mean reduction of 63% in HIV-1 copy number in the monocytes of the HIV-1 seropositive individuals following 10 days of treatment (p=0.09).

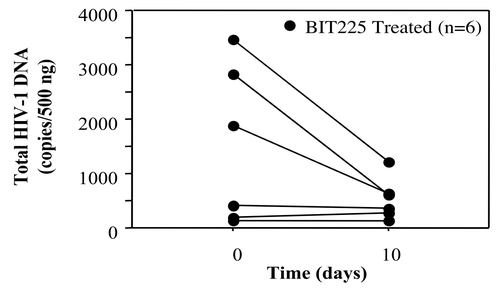
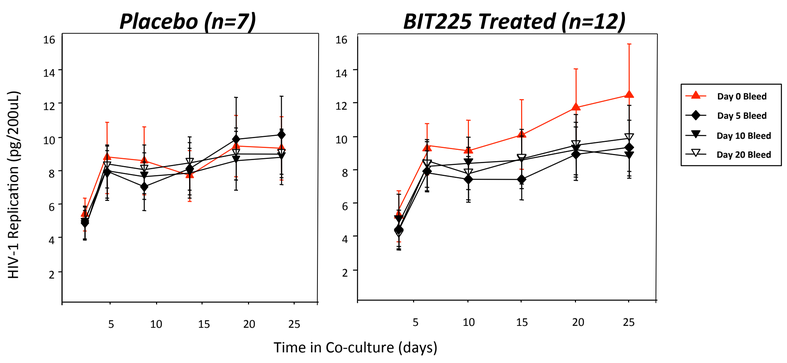


Figure 3. BIT225 therapy results in a reduction in virus burden within the CD14$^+$ monocytes.

The amount of virus within the CD14$^+$ monocytes in the placebo group (n=7) remained constant throughout the study, no differences were observed in the HIV-1 replication rate in the co-cultures of cells collected from the 4 time points in the trial.

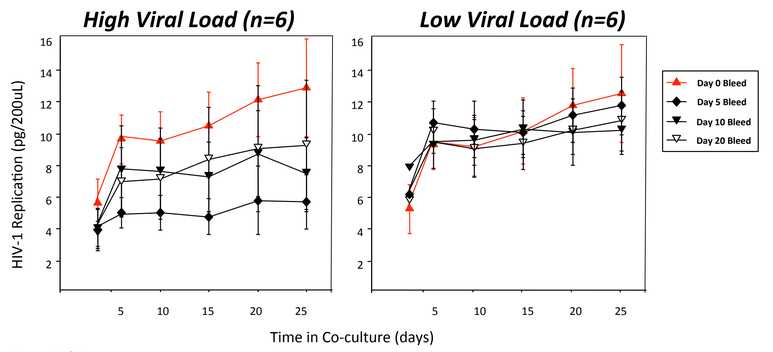
In the BIT225 treated arm (n=12), a reduced amount of virus was detected in the co-cultured cells from blood collected after 5 days of drug treatment when compared to the day 0 bleed. This lower level of virus persisted out to the day 20 bleed, indicative of less HIV-1 present within the myeloid compartment in the drug-treated patients.



Results

Figure 4. BIT225 therapy results in a significant reduction in HIV-1 within the CD14$^+$ monocytes of patients with high viral loads.

When the 12 treated patients were split in to 2 groups, determined by the median viral load, those patients with high viral loads (>4.43) demonstrated significantly less virus within the co-cultures of cells collected during BIT225 therapy.



Mann-Whitney p=

0 v 5	0.25	0.09	0.1	**0.05**	**0.04**	0.2
0 v 10	0.25	0.31	0.31	0.42	0.44	0.28
0 v 20	0.25	0.28	0.31	0.37	0.35	0.54

Conclusion

1. By directly measuring total HIV-1 DNA within the patients' monocyte cells, representing their myeloid population, we have shown that BIT225 reduces the viral burden in these cells following 10 days of treatment ***Figure 1.***
2. This response was greatest in those patients with higher total HIV-1 DNA at baseline ***Figure 2***.
3. This observed reduction of virus within the CD14$^+$ monocyte compartment by BIT225, was also supported using a co-culture assay. The assay measures replication competent virus originating from these cells. A reduced *infectious* viral burden within the monocyte population was observed following 10 days of BIT225 ***Figure 3***.
4. In the co-culture assay, the drug effect was more evident in those individuals with higher viral loads ***Figure 4.***
5. No such changes in virus burden within the CD14$^+$ monocytes were observed in the placebo cohort.
6. As expected with a short duration single drug regimen targeting myeloid lineage cells, no changes in viral load or the CD4$^+$ T cell count were observed during the treatment period.
7. BIT225 was well tolerated with adequate plasma levels achieved. Analysis of CSF demonstrated that the drug is able to cross the blood brain barrier.
8. Since completion of this trial, an optimised capsule formulation of BIT225 has been developed and tested in healthy volunteers.

Treatment with BIT225 reduced the virus burden in monocyte reservoirs, particularly for those individuals with high viral loads. By targeting these cells and preventing (re)seeding of the myeloid reservoirs, BIT225 has a potential role in the eradication strategy of HIV-1.

Further information

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