BIT225 Therapy Reduces HIV-1 Burden in Monocyte Cells And Decreases Immune Activation

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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.

BIT225 is a first in class antiviral drug that blocks Vpu ion channel activity resulting in disrupted HIV-1 assembly within the host cell. It demonstrates encouraging anti-HIV-1 activity in primary human CD14$^+$ monocyte-derived macrophages (MDM). BIT225 significantly reduces virus release from MDM with an EC_{50} of 1.1 ± 0.4 μM and a TC_{50} of 212 μM.

Here we report on the effects of treating HIV-1$^+$ individuals with BIT225 in the setting of a recent Phase 1b/2a clinical trial conducted at the Siriraj Hospital, Bangkok, Thailand. In these treated individuals we have demonstrated that BIT225 significantly reduces both monocyte activation, as measured by sCD163, and the level of infectious HIV-1 within the circulating CD14$^+$ monocyte cells.

The results provide evidence that BIT225 can target and reduce the viral burden in cells of the myeloid lineage and dampen the activation of the immune system 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:

1. HIV-1 viral burden in the circulating myeloid cellular reservoirs.
This was quantitated using both RT-PCR on total DNA and a novel endpoint-analysis method for the *ex vivo* measurement of infectious HIV-1 output from the CD14$^+$ monocyte cells after isolation from BIT225-treated individuals.

2. Immune activation, as measured using the monocyte markers sCD163 and neopterin

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

- 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 sCD163 and neopterin was evaluated by ELISA at the end of study. CD14$^+$ monocytes were 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.

Ten days of BIT225 was generally well tolerated. The pharmacokinetic data suggests that adequate BIT225 levels in the plasma were achieved *in vivo.*

	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)*

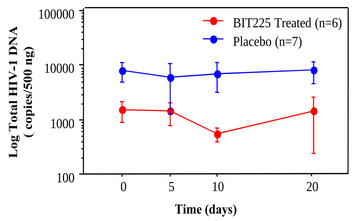


Figure 1. BIT225 results in a reduction in total HIV-1 DNA in the monocytes of HIV-1$^+$ individuals with 10 days of treatment. Total HIV-1 DNA in individuals receiving placebo remained stable throughout the study. Mean ±SE copy number in the treated was 1493 ±593, 1414 ±655, 547 ±157 and 1419 ±1183 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). The response is greatest in those individuals with higher viral loads at baseline.

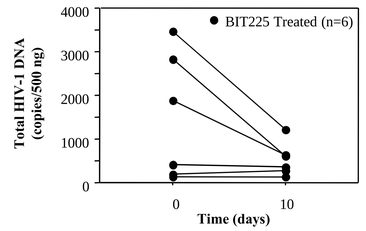
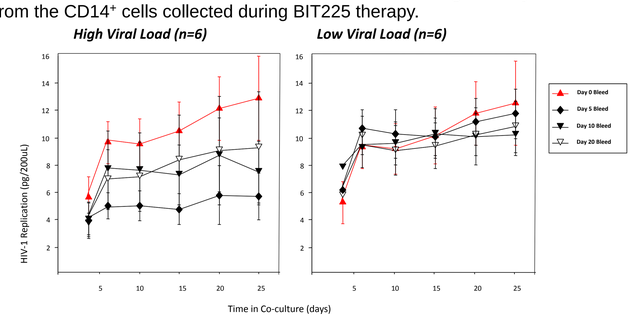


Figure 3. BIT225 therapy results in a significant reduction in HIV-1 within the CD14$^+$ monocytes of patients with high viral loads. The amount of virus within the CD14$^+$ monocytes in the placebo group (n=7) remained constant throughout the study, no differences were observed in the HIV-1 replication 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, indicative of less HIV-1 present within the myeloid compartment in the drug-treated patients.

When the 12 treated patients were split in to 2 groups, determined by the median viral load at baseline, those patients with high viral loads (>4.43) demonstrated significantly less virus within the co-cultures from the CD14$^+$ cells collected during BIT225 therapy.

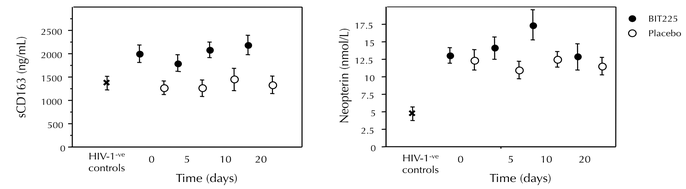


Mann-Whitney p=

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

Results – Immune Activation

Figure 4. Plasma levels of the monocyte activation markers (a) sCD163 and (b) neopterin for the BIT225 treated (n=12) and placebo controls (n=7). Individuals were treated with BIT225 for 10 days and were followed up 10 days after. The normal range for HIV-1 uninfected controls (n=11) is also represented.
High plasma levels of sCD163 significantly correlated with higher HIV-1 viral loads at baseline and throughout BIT225 therapy (p=0.0001, r=0.53). Significantly higher levels of plasma sCD163 were observed in the BIT225 treated arm versus the placebo, most likely a result of the higher viral loads within the treated cohort throughout the study.
Treatment with BIT225 (n=12) resulted in a significant (p=0.04) decrease in sCD163 levels at day 5, that normalised at day 10. At day 20, 10 days after BIT225 cessation, sCD163 levels were significantly (p=0.04) elevated from baseline, suggesting the resumption of HIV-1 replication within this myeloid population.



Conclusion

BIT225 is a first in class antiviral that is capable of inhibiting viral production in the myeloid cells of HIV-1 infected individuals.

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.*** This response was greatest in those patients with higher total HIV-1 DNA at baseline ***Figure 2***.
2. This observed reduction of virus within the CD14$^+$ monocyte compartment by BIT225, was supported using a co-culture assay that measures replication competent virus originating from these cells. A reduced *infectious* viral burden within the monocyte population was observed following 10 days of BIT225, with the drug effect more evident in those individuals with higher viral loads ***Figure 3***.
3. Even during a short duration of treatment, BIT225 transiently reduced monocyte immune activation in HIV-1 infected individuals, as measured by sCD163 levels. Neopterin levels remained elevated, most likely driven by IFN-γ from activated T cells ***Figure 4***.
4. BIT225 was well tolerated with adequate plasma levels achieved. Analysis of CSF demonstrated that the drug is able to cross the blood brain barrier where it has the potential to reduce both viral burden within this sanctuary site and HIV-1 associated neurocognitive disorders (HAND).

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

Further information

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