BIT225, a Novel Inhibitor of HIV-1 Release from HIV-1 Reservoirs of the Myeloid Lineage

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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. Biotron has previously completed preclinical safety studies and a Phase 1 dose escalation study in healthy volunteers with BIT225 under its HIV program.

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 utilise a novel endpoint-analysis method with a drug designed to target the myeloid cellular reservoirs of HIV-1, that exist in the presence (or absence) of active virus replication in T-cells. The new method examines the level of active HIV-1 infection in cells of the CD14$^+$ monocyte lineage during 10 days of BIT225 treatment, by examining HIV-1 output from these cells e*x vivo* after isolation from BIT225-treated individuals.

In contrast to HIV-1 clinical trials using T cell-targeting drugs, the aim of this trial was not to measure a direct decrease in HIV-1 viral load, nor increase in CD4$^+$ T-cell count; such an effect would be unlikely for a short monotherapy trial of BIT225, which targets HIV-1 replication in the macrophages.

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.
- Evaluate BIT225 levels in cerebrospinal fluid (CSF) at day 10.

Study design

- A randomised, parallel, double-blind study of BIT225 in patients with HIV-1 infection that 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, 10 and 20 of dosing. Plasma was stored and CD14$^+$ monocytes isolated from the 21 study participants by magnetic bead sorting at each of these 4 time points. In addition, CD14$^+$ and CD14$^-$ (T cell) samples were stored for single copy HIV-1 RNA & DNA assays.

At each of the 4 time points, the 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 of culture.

Results – Antiviral Efficacy & CSF Analysis

Figure 1. 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, with no differences 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.

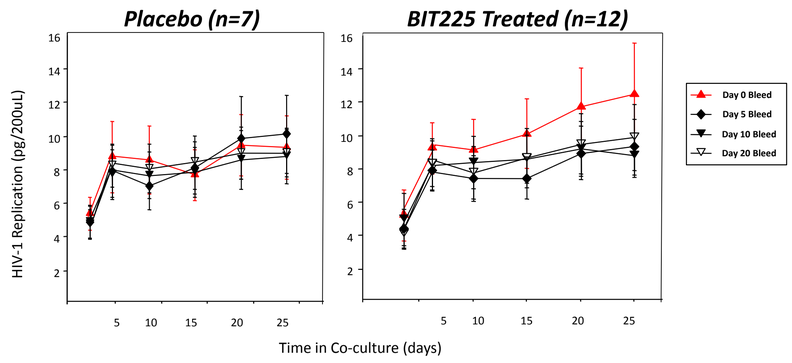
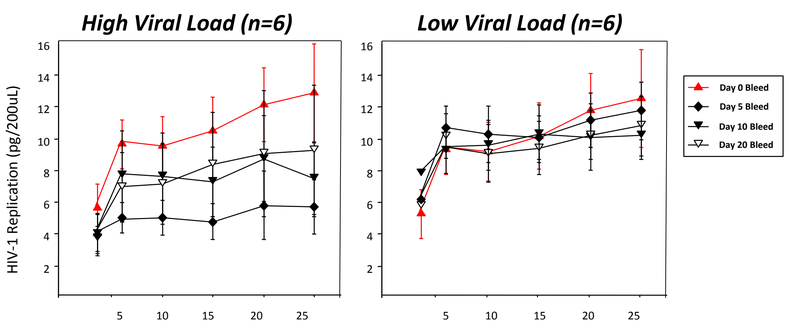


Figure 2. BIT225 therapy results in a significant reduction in HIV-1 within the CD14$^+$ monocytes of patients with high viral loads.

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.



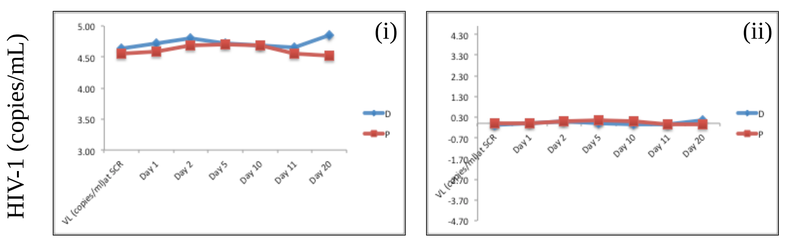
Mann-Whitney p=

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

Figure 3. As expected, 10 days of BIT225 treatment had no effect upon patient (a) HIV-1 viral load and (b) CD4$^+$ T cell count.

Plotted are (i) absolute numbers and (ii) changes from baseline (day 0). There were no differences between the 14 treated (blue) versus 7 placebo (red) for either parameter measured.

(a) HIV-1 viral load (copies/mL)



(b) CD4$^+$ T cell counts (cells/mm^3)

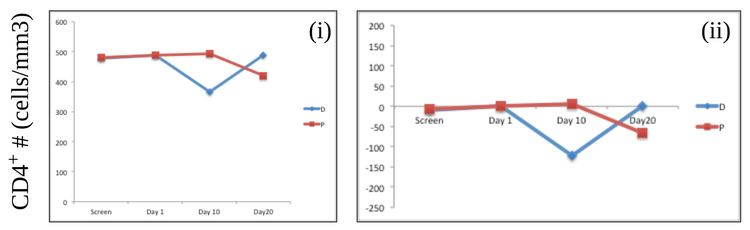


Table 1. Concentration of BIT225 in the CSF.

Samples of CSF were collected from two subjects at 4 hours post-dosing on day 10. Drug was detectable in the CSF, albeit at lower levels than those seen in plasma.

Subject	CSF (ng/ml)	Plasma (ng/ml)
402	87.8	3550
418	24.4	1620

(Lower limit of quantitation of HPLC assay for BIT225 in CSF is 0.200 ng/ml)

Results – Baseline Characteristics & Safety

Table 2. 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)*

Table 3. A summary of the adverse events of the study participants.

Ten days of BIT225 treatment was well tolerated by the participating HIV-1$^+$ individuals. Headaches and nausea were the most common adverse events and were the reason for withdrawal or temporary discontinuation.

Adverse Event Number of Events (%)	BIT225 (n=14)	Placebo (n=7)
Headache	12 (86)	2 (28)
Nausea	9 (64)	2 (28)
Vomiting	5 (36)	1 (14)
Fever	5 (36)	0
Numbness/Parasthesia	5 (36)	0
Dizziness	4 (29)	0
Rash	3 (21)	1 (14)
Itchiness	3 (21)	0

Two patients interrupted BIT225 for 2-4 days due to headache, dizziness, nausea and vomiting One patient interrupted for 2 days due to palpitations

Since completion of this trial, an optimised capsule formulation of BIT225 has been developed and tested in healthy volunteers.

Conclusion

1. By measuring HIV-1 within the patients' monocyte cells, representing their myeloid population, we have shown that BIT225 treatment significantly reduces the viral burden in these cells ***Figures 1 and 2.*** The reduction was more evident in those individuals with higher viral loads ***Figure 2.***
2. 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 ***Figure 3.***
3. Analysis of CSF demonstrates that the drug is able to cross the blood brain barrier ***Table 1.***
4. BIT225 has acceptable safety and tolerability in HIV-1$^+$ individuals ***Table 3.***

Treatment with BIT225 demonstrated a significant effect upon the virus burden in the monocytes of 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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- John Wilkinson -

Biotron Limited, Australia

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BIT225
(N-[5-(1-Methyl-1H-pyrazol-4-yl)-napthalene-2- carbonyl]-guanidine)

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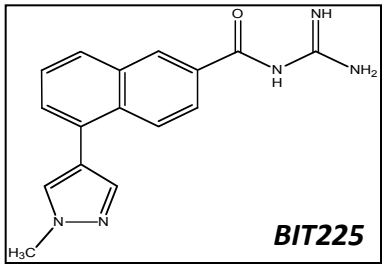
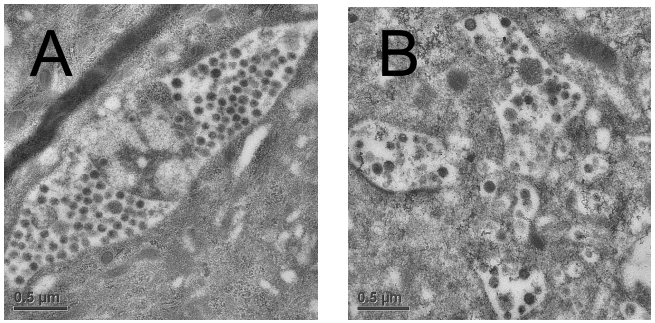
- First-in-class drug targeting HIV-1 within cells of the myeloid lineage (selected as lead from ~250 compound library designed to target Vpu)

- Anti-HIV-1 activity in primary human $CD14^+$ MDM assay:
  - *>90% inhibition of HIV-1 release (RT & p24)*
  - *IC_{50} of ~1.1 ± 0.4 uM TC_{50} of 212 uM*



- Also active in DCs
- Targets Vpu ion channels, with no effect on HIV-2
- No effect on reverse transcription or on the RTase or protease enzymes
- Acts post-integration
- EM suggests defects in virion packaging/budding

- Good safety and PK profiles in preclinical toxicology studies and Phase 1 human trials



Visualisation by EM of (A) DMSO (B) BIT225 treated cells

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Significant HIV-1 Reduction in Human Macrophages *in vitro* with BIT225

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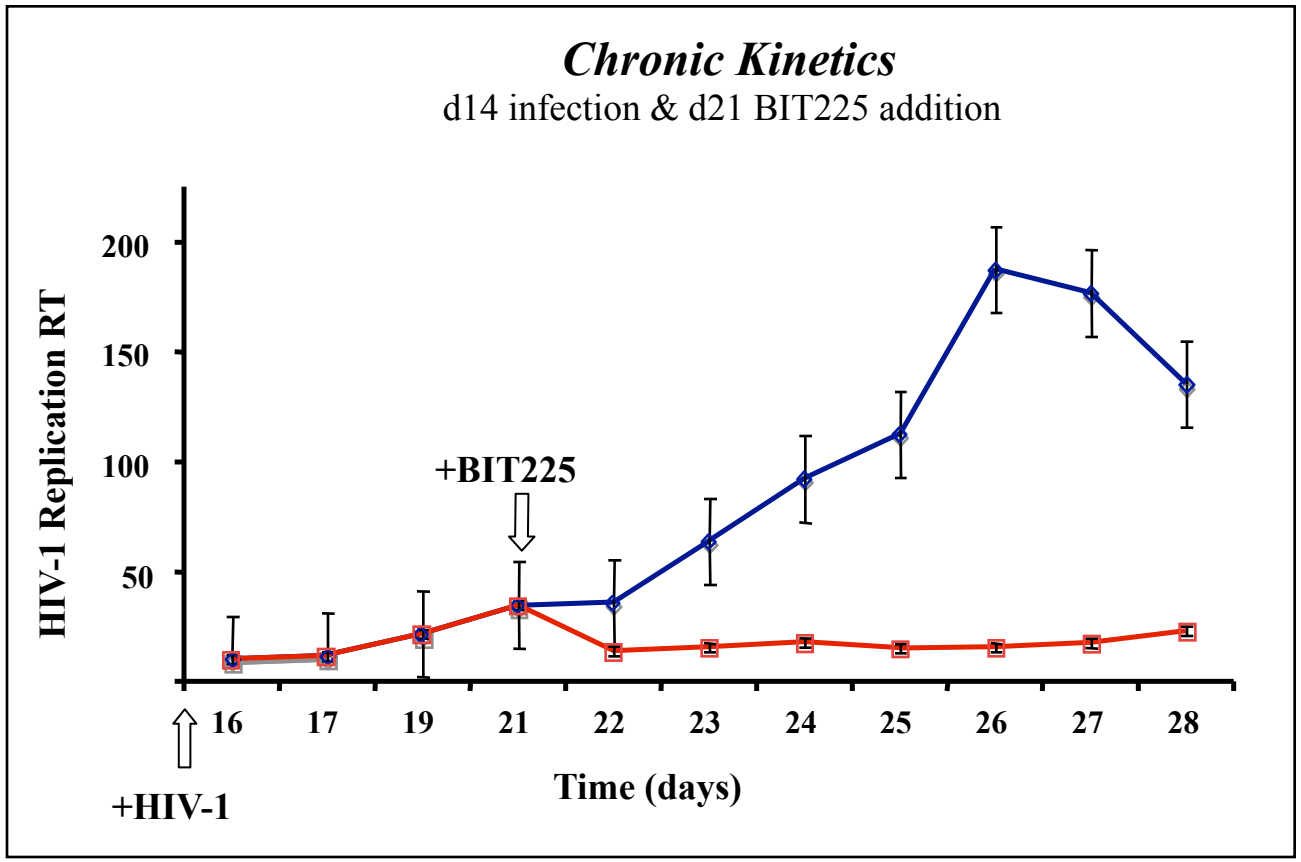

Khoury et al., Antimicrobial Agents and Chemotherapy. 2009

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Monocytes and HIV-1 Infection

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- $CD14^+$ monocytes (~30%) are long lived cells with reports that once infected they can disseminate virus for 6 weeks *in vitro* (Sharova *et al* EMBO J 2005)
- The minor $CD16^+$ subset (5-10% of monocytes) are preferentially infected; higher CCR5 levels (Ellery *et al* JI 2007)
- Circulate in the blood for ~1 day before entering the tissue -> MØ
  - Important wrt transmission and seeding the tissues (brain)
- HIV-1 can be isolated from monocytes (Wang *et al* Plos One 2013), their HPC precursors (Carter *et al* Nat Med 2010) and thought to contribute to viral persistence (Le Douce *et al* Retrovirology 2010)
- Treatment regimens fail to inhibit HIV-1 DNA persistence in monocytes (Sonza *et al* AIDS 2001; Zhu *et al* JV 2002; Llewellyn *et al* JLB 2006) but they are not a major reservoir in elite suppressors (Spivak *et al* JV 2011)

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A Phase 1b/2a Trial with BIT225

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BIT225-004, a Phase 1b/2a, Placebo-Controlled, Randomised Study of the Safety, Pharmacokinetics and Antiviral Activity of BIT225 in Patients with Human Immunodeficiency Virus-1 Infection

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BIT225-004: Synopsis

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Primary objective

The safety and tolerability of 400 mg of BIT225 BID compared with placebo in patients with HIV-1 infection that are 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
- Evaluate BIT225 levels in cerebrospinal fluid at day 10 (optional day 9)

Study design

- A randomized, parallel, double-blind study of BIT225 in patients with HIV-1 infection that are antiretroviral therapy naïve
- 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 receiving 400 mg BIT225 and 7 receiving placebo

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BIT225 Antiviral Activity in a Clinical Setting

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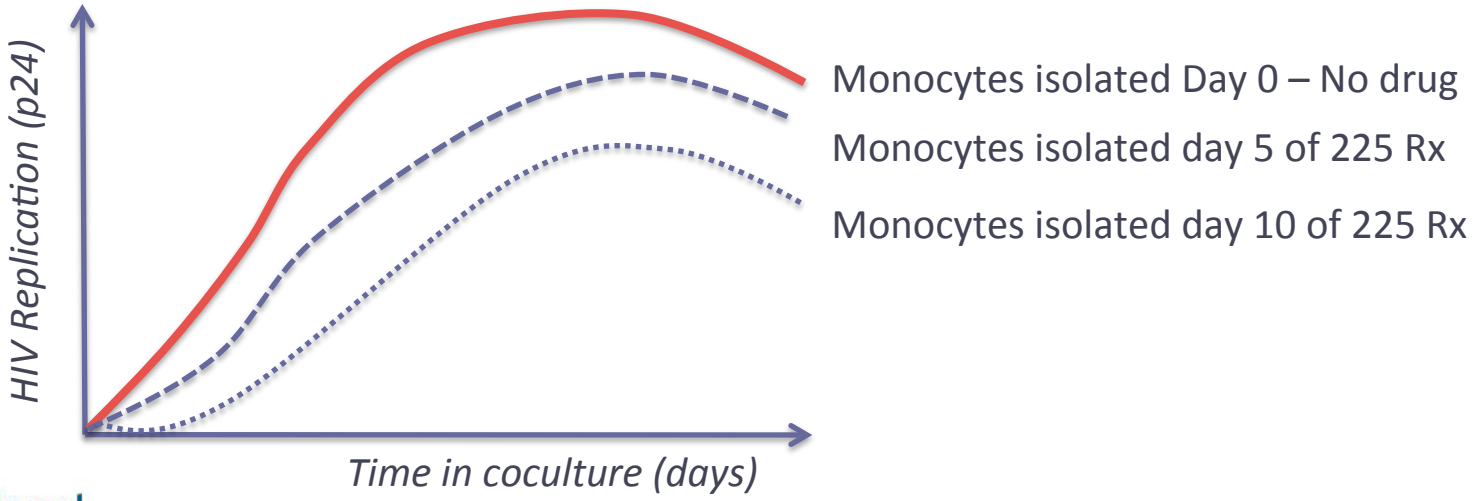
In a study of only 10 days with a drug targeting cells of the myeloid lineage, dramatic decreases in HIV-1 viral load and concomitant increases in $CD4^+$ T cell number are unlikely to be observed. Issues with access to macrophages

Aim: To determine the effect of BIT225 on the viral burden in circulating $CD14^+$ monocytes in HIV-1^+ individuals

Method: $CD14^+$ monocytes were isolated with magnetic beads on days 0, 5, 10 and 20 and co-cultured with MT4 HIV-1^- T cells for 25 days



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Monocyte Co-Culture Assay

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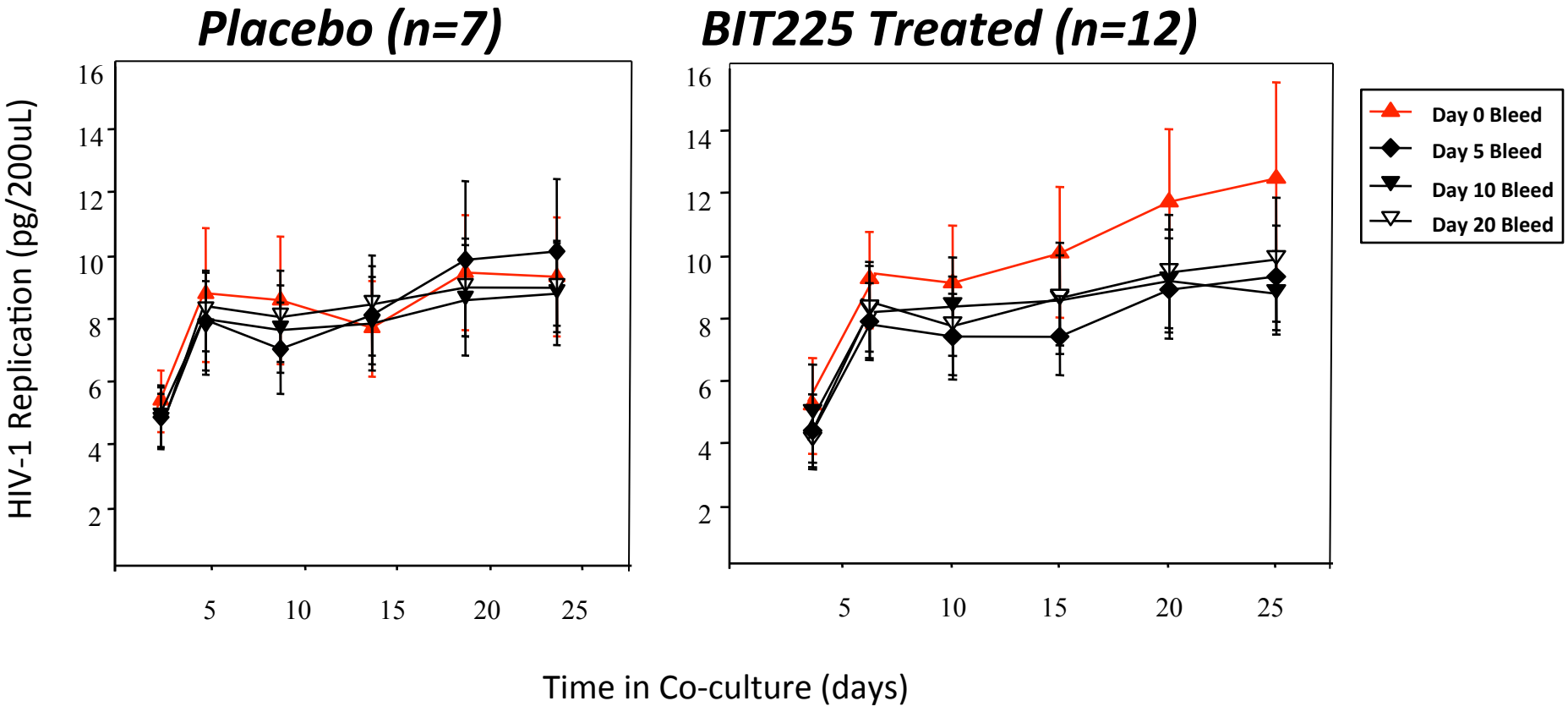

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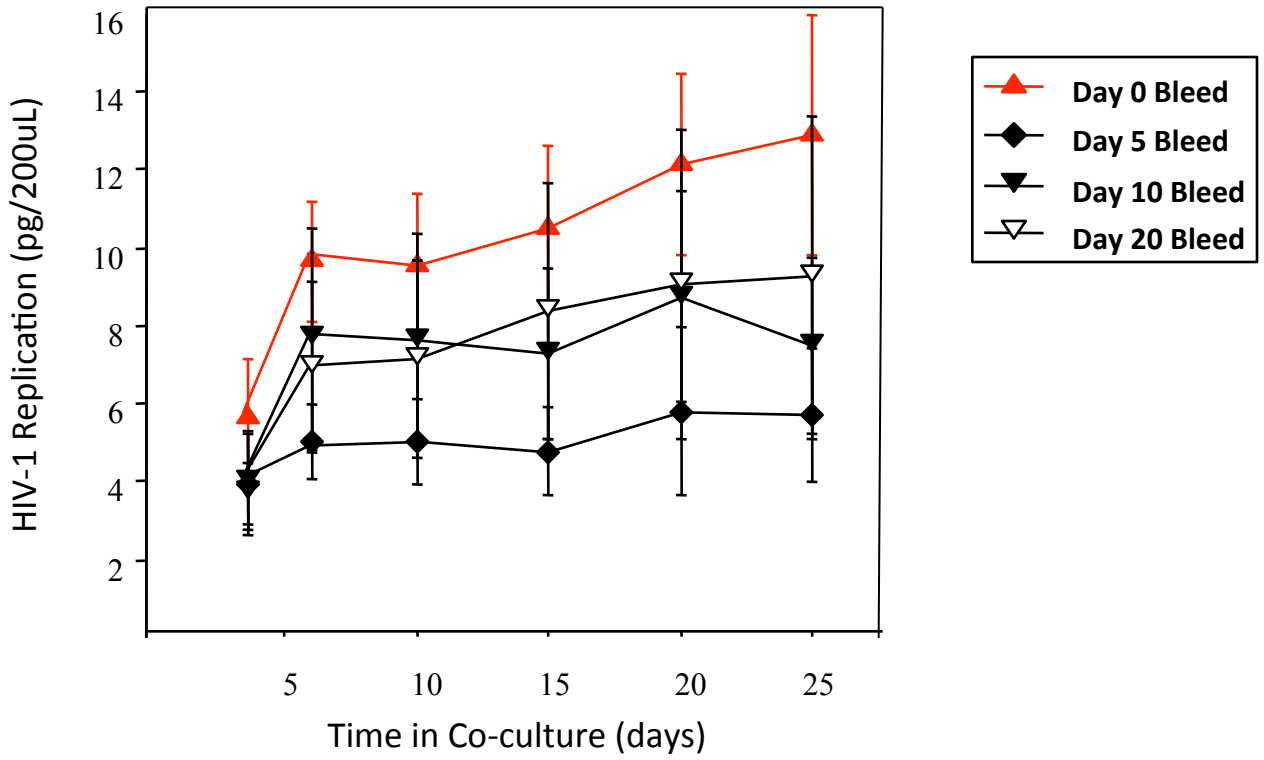
Monocyte Co-Culture Assay
BIT225 Treated: High Viral Load n=6



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

Monocyte Co-Culture Assay
BIT225 Treated: Low Viral Load n=6

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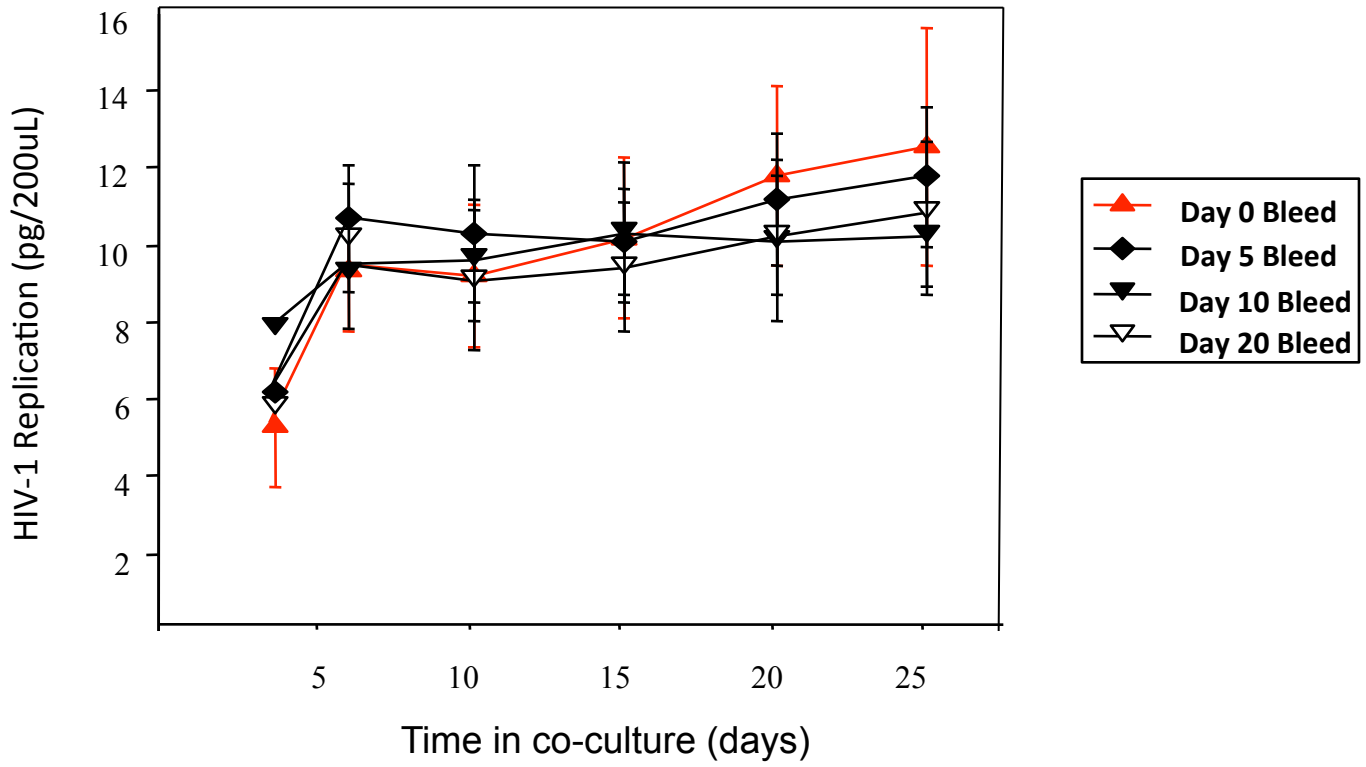

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In Summary

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- This study strengthens our previous findings *in vitro* and *ex vivo*, supporting the role for BIT225 as a novel drug targeting HIV-1 within the myeloid compartment
- In those patients with high HIV-1^{+} viral loads, treatment with BIT225 for 10 days significantly reduced the amount of infectious HIV-1 within the circulating CD14^{+} monocyte population
  - Single Copy HIV-1 RT-PCR Analysis: For 21 patients at the 4 bleeds, RNA and DNA (in triplicate) has been isolated and stored for HIV RNA and HIV DNA analysis
- By targeting these cells and preventing the (re)seeding of the reservoirs, is there a potential role for BIT225 in the eradication strategy?

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