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

- John Wilkinson -

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

• 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 $\approx 1.1 \pm 0.4 \text{ uM}$ $TC_{50}$ 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]
Significant HIV-1 Reduction in Human Macrophages *in vitro* with BIT225

*Chronic Kinetics*

d14 infection & d21 BIT225 addition

Khoury et al., *Antimicrobial Agents and Chemotherapy*. 2009
CD14+ monocytes (~30%) are long lived cells with reports that once infected they can disseminate virus for 6 weeks \textit{in vitro} (Sharova \textit{et al} EMBO J 2005)

The minor CD16+ subset (5-10% of monocytes) are preferentially infected; higher CCR5 levels (Ellery \textit{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 \textit{et al} Plos One 2013), their HPC precursors (Carter \textit{et al} Nat Med 2010) and thought to contribute to viral persistence (Le Douce \textit{et al} Retrovirology 2010)

Treatment regimens fail to inhibit HIV-1 DNA persistence in monocytes (Sonza \textit{et al} AIDS 2001; Zhu \textit{et al} JV 2002; Llewellyn \textit{et al} JLB 2006) but they are not a major reservoir in elite suppressors (Spivak \textit{et al} JV 2011)
A Phase 1b/2a Trial with BIT225

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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**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/mm³) and that are antiretroviral therapy naïve
- 14 patients receiving 400 mg BIT225 and 7 receiving placebo
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
Monocyte Co-Culture Assay

**Placebo (n=7)**

**BIT225 Treated (n=12)**

HIV-1 Replication (pg/200μL) vs. Time in Co-culture (days)

- Placebo (n=7)
- BIT225 Treated (n=12)

Legend:
- Day 0 Bleed
- Day 5 Bleed
- Day 10 Bleed
- Day 20 Bleed
Monocyte Co-Culture Assay
BIT225 Treated: High Viral Load n=6

Mann-Whitney p=

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Day 0 Bleed
Day 5 Bleed
Day 10 Bleed
Day 20 Bleed
Monocyte Co-Culture Assay
BIT225 Treated: Low Viral Load n=6

HIV-1 Replication (pg/200μL)

Time in co-culture (days)

Day 0 Bleed
Day 5 Bleed
Day 10 Bleed
Day 20 Bleed
In Summary

- 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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Poster: MOLBPE11