BIT225-009: Significant Immunological Outcomes after 12 weeks of BIT225 and Antiretroviral Therapy in an HIV-1 Phase 2 Clinical Trial

Luscombe CA[1], Ewart GD[1], Thomson AS[1], Avihingsanon A[2], Supparatpinyo K[3], Sivaporn G[2], Win M[2], Murphy RL[4], and Miller M[1].
[1]*Biotron Limited, North Ryde, Australia;* [2]*HIVNAT, Bangkok, Thailand;* [3]*Chiang Mai University, Chiang Mai, Thailand;* [4]*Northwestern University, Chicago, USA*

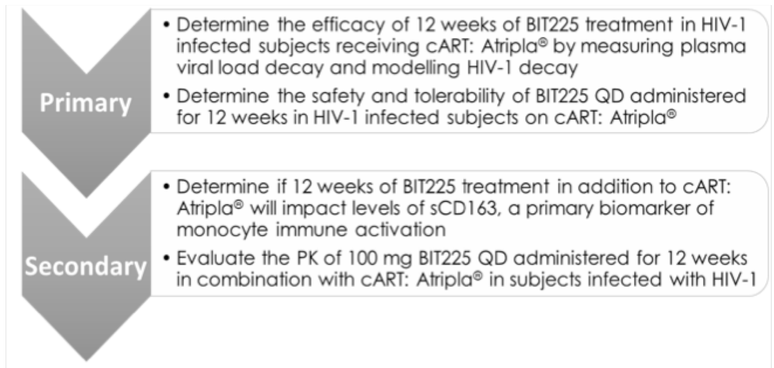
Background

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; eradication strategies must also target these infected cells to be fully effective.

BIT225 (N-carbamimidoyl-5-(1-methyl-1H-pyrazol-4-yl)-2-naphthamide) is an HIV-1 Vpu inhibitor that targets virus assembly and is most effective in cells of monocyte lineage. This Phase 2 trial was designed to investigate whether BIT225 provides additional virological or immunological benefits on top of that seen with antiretroviral therapy (ART) Atripla in subjects commencing ART.

Study Design

- A randomized, placebo controlled, double-blind study of BIT225 in patients with HIV-1 commencing ART (males and females, aged 18 to 65 years, viral load >5,000 copies/mL; $CD4^+$ count >100 cells/mm^3, ART naïve).
- BIT225 or placebo added to ART (Atripla) for first 12 weeks of treatment
- 36 HIV-1^{+ve}, treatment-naïve subjects, randomized 2:1 (drug:placebo)
  - N=9 (100 mg BIT225 or placebo QD) for PK (*not reported on here)*
  - N=27 (200 mg BIT225 or placebo QD) for safety, impact on viral load and kinetics, and impact on immunological markers



Results – Demographics and Adverse Events

Table 1. Baseline demographics of the study participants.

Parameter	BIT225 200mg n=18	Placebo n=9
Gender (n (%)) Male	17 (94.4%)	7 (77.8%)
Female	1 (5.6%)	2 (22.2%)
Race (n (%)) - Asian	18 (100.0%)	9 (100.0%)
Age (yr) - Mean	26.1	24.4
BMI (kg/m^2) - Mean	21.44	19.8
Height (cm) - Mean	170.9	165.7
Weight (kg) - Mean	63.15	54.67
HIV-1 Viral Load (copies/mL) - Mean	92790	48689
CD4+ (cells/mm^3) - Mean	455	541

The treated and placebo groups were well matched at baseline, with no significant differences between the two groups in any of the parameters measured (Table 1). Twelve weeks of 200mg QD BIT225 was generally well tolerated, with no SAEs reported (Table 2).

Table 2. Most Common Treatment-Emergent AEs (Safety Population)

AE Reported Term	BIT225 200mg n=18 (n%)	Placebo n=9 (n%)	Total n=27 (n%)
Dizziness	13 (72.2)	8 (88.9)	21 (77.8)
Nausea	6 (33.3)	6 (66.7)	12 (44.4)
Headache	9 (50.0)	2 (22.2)	11 (40.7)
Vomiting	4 (22.2)	3 (33.3)	7 (25.9)
Pyrexia	6 (33.3)	0 (0.0)	6 (22.2)
Rash	5 (27.8)	1 (11.1)	6 (22.2)
Upper Respiratory Tract Infection	4 (22.2)	2 (22.2)	6 (22.2)
Rash maculo-papular	4 (22.2)	1 (11.1)	5 (18.5)
Rhinorrhoea	4 (22.2)	0 (0.0)	4 (14.8)

Further Information and Acknowledgements

cluscombe@biotron.com.au, mmiller@biotron.com.au

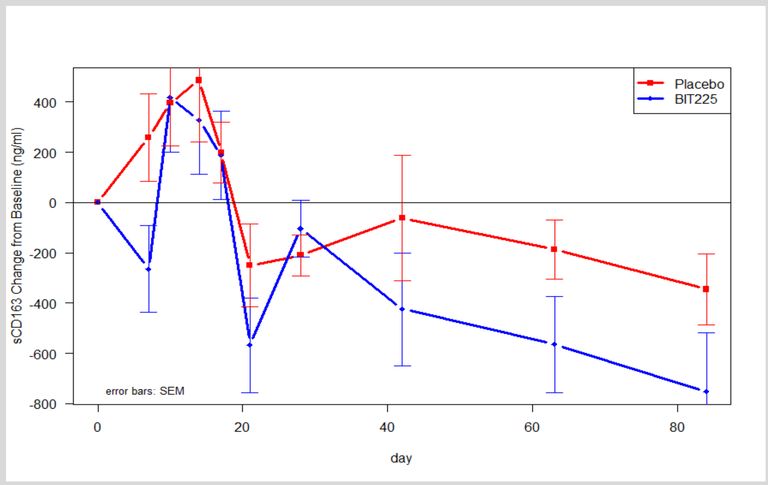
We thank the trial participants for their involvement in this study, staff at ACLIRES Bangkok, HIV-NAT Bangkok, and Chiang Mai University Hospital, Chiang Mai, Thailand.

Results – Immunological Outcomes

1. sCD163

Soluble CD163 (sCD163) is a primary biomarker specific for monocyte and macrophage immune activation. This marker is strongly correlated with macrophage-mediated pathogenesis and is also a better predictor than T-cell activation markers of all-cause morbidity and mortality in HIV-1 patients who are on successful suppressive anti-retroviral therapy.

Figure 1. Soluble CD163 change from baseline by Treatment Group Mean during 12 Weeks of BIT225 QD or placebo treatment with ART.



Levels of the sCD163 showed significantly greater reduction from baseline (P<0.05, general linear model) in the BIT225 treated cohort over the treatment period (Figure 1). Analysis was performed by two-way ANOVA for BIT225 versus Placebo, controlling for day of treatment, using R statistical software by fitting a generalised linear model: $\beta 0+\beta 1.day+ \beta 1.IRx.$, where *IRx* takes the values 0 (for BIT225 treatment), or 1 (for Placebo). The estimate $\beta 1 = 208 \pm 99$ (SE) ng/ml indicates a statistically larger overall decrease in sCD163 for the BIT225 treated group (P = 0.036).

2. $CD8^+$ Cells

Figure 2. Activated $CD8^+$ cell numbers proportional to baseline during 12 Weeks of BIT225 QD or placebo treatment with ART.

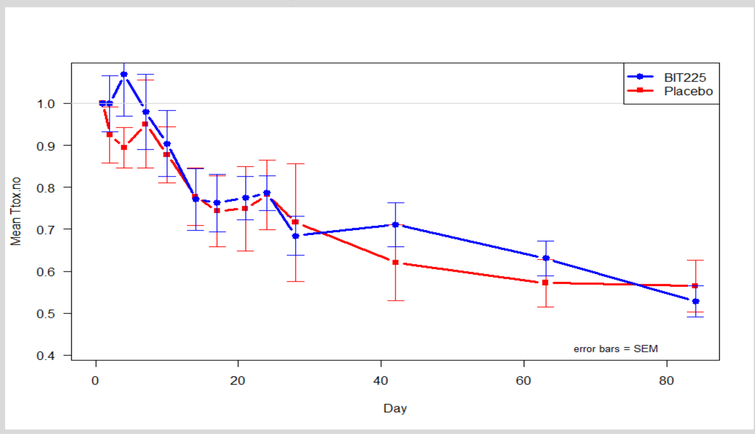


Figure 2 shows significant difference in CD8 cell response. The data showed a statistically significant (P<0.05, Welch's T-test) rise in the mean $CD8^+$ cell numbers as proportion of baseline in the initial days of BIT225 treatment compared to placebo.

3. Activated $CD4^+$ Cells

Figure 3. Activated $CD4^+$ cell numbers proportional to baseline during 12 Weeks of BIT225 QD or placebo treatment with ART.

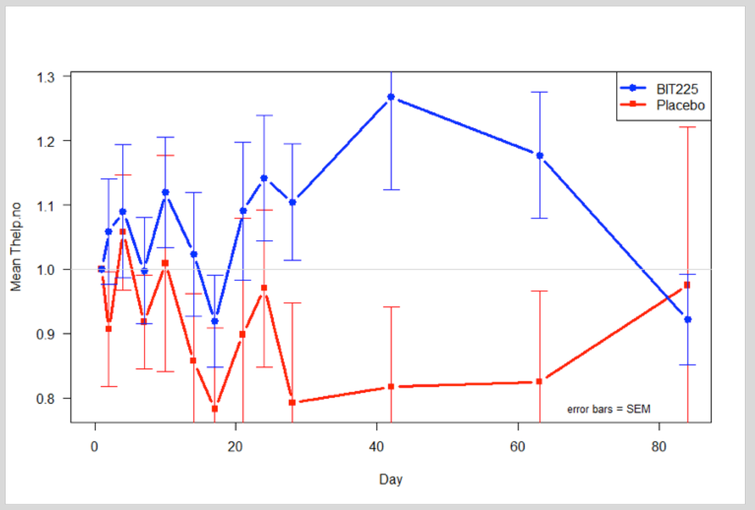
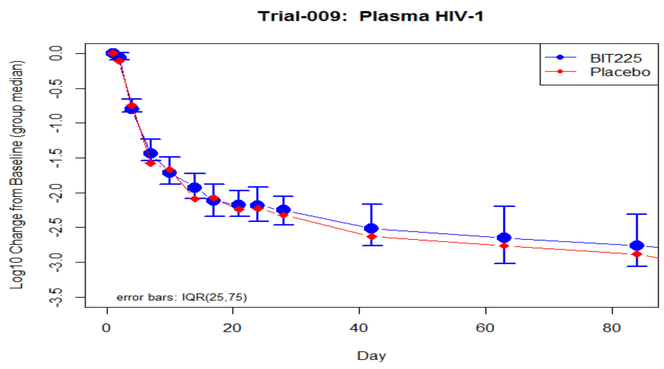


Figure 3 shows significant differences in activated CD4 cell responses. There was a statistically significant (P<0.01, general linear model) sustained delay in decline of $CD4^+$ activated T-cell numbers during the BIT225 treatment period.

Results – Virological Outcomes

Figure 4. Plasma HIV-1 RNA change from baseline (median) during 12 Weeks of treatment with BIT225 or placebo and ART.



The design of this study was to intentionally drive plasma HIV-1 RNA levels towards undetectable limits. The addition of BIT225 to ART did not have a significant impact on the plasma HIV-1 RNA decline kinetics, as expected due to the effectiveness of ART (Figure 4).

A second assessment of virological outcomes, an assessment of cell-associated virus in specific cell populations, is ongoing. This may provide additional virologic information on relative differences in HIV-1 decay in different blood cell populations.

References

Kumar A, Abbas W, Herbein G. HIV-1 latency in monocytes/macrophages. 2014. Viruses 6 (4), 1837-1860; doi: 10.3390/v6041837

Khoury G, Ewart G, Luscombe C, Miller M, Wilkinson J. Antiviral efficacy of the novel compound BIT225 against HIV-1 release from human macrophages. 2010. Antimicrob Agents Chemother Feb; 54(2) 835-845. doi: 10.1128/AAC.01308-09

Khoury G, Ewart G, Luscombe C, Miller M, Wilkinson J. The antiviral compound BIT225 inhibits HiV-1 replication in myeloid dendritic cells. 2016 AIDS Res Ther Feb 8: 13: 7 doi: 10.1186/s 12981-016

Conclusion

The results indicate that BIT225 is having a unique effect, over and above viral suppression seen with ART:

- The BIT225 cohort had a significantly greater reduction of the macrophage activation marker sCD163, a good indicator of all cause morbidity and mortality in HIV-1 patients. Additional reduction of this marker in patients taking BIT225 demonstrates significant immunological benefit. **The sCD163 result suggests a potential role of BIT225 for treatment of high risk patients.**
- The BIT225 cohort had a significant initial increase in $CD8^+$ T cells despite viral load rapidly declining.
- The BIT225 cohort had a significant sustained delayed decrease in activated $CD4^+$ cells.

The initial increase in $CD8^+$ cells indicates that in the BIT225 cohort the cells are exposed to a unique antigen source. Sustained levels of activated $CD4^+$ cells in this cohort indicates that the exposure continues over several weeks. The source of this viral antigen is potentially replication incompetent virus released from macrophages. BIT225 has been reported to cause defective HIV-1 assembly resulting in production of non-infectious virus in macrophages (Khoury *et al.*, 2010) and dendritic cells *in vitro* (Khoury *et al.*, 2016*).* The commencement of a decline of activated CD4+ cell levels at week 6 suggests that virus from these reservoir cells is being eradicated, and cleared by week 12 when levels return to those seen in the ART + placebo cohort.

The sustained activation of $CD4^+$ T cells potentially produces interferon-gamma, a non cytolytic virus-replication inhibiting cytokine. The observed reduction in macrophage immune activation and unique immune system stimulation following BIT225 treatment suggest an increase in potential protection from new HIV cell infections, which may impact the HIV reservoir size and persistence.

Additional investigations are ongoing to determine what additional immunological triggers BIT225 treatment may have induced such as the production of broad neutralizing antibodies.

The stimulation of the immune system in the BIT225 cohort makes this treatment approach a unique and potentially clinical beneficial step towards HIV-1 eradication. By targeting and preventing (re)seeding of the myeloid reservoirs, BIT225 has a potential role in the eradication strategy of HIV-1.