

12 March 2020

The Manager Companies  
ASX Limited  
20 Bridge Street  
SYDNEY NSW 2000

(3 pages by email)

Dear Madam,

### **BIT225 RESTORES IMMUNE FUNCTION IN HIV-1 INFECTIONS**

The Directors of Biotron Limited ('Biotron') are pleased to advise that the attached poster presentation entitled "Phase 2 Trial OF VPU Inhibitor BIT225 in Combination with Antiretroviral Therapy" is being presented at the annual Conference on Retroviral and Opportunistic Infections (CROI) this week.

The Company has previously reported that its BIT225-009 Phase 2 HIV-1 clinical trial showed that BIT225 induced statistically significant changes to key immune cell populations. These changes were not seen in this or other trials with current approved anti-HIV-1 drugs.

Since completing the clinical trial and releasing positive results in late 2018, Biotron has continued to characterise the unique mechanism of action of its lead antiviral drug, BIT225. The latest data, presented at CROI, further characterises the previously reported immune modulating effects of BIT225.

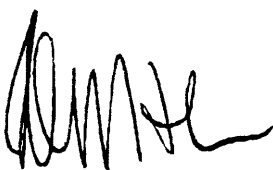
The new data shows that BIT225 "unmasks" HIV-infected cells that remain in the body despite treatment with approved anti-HIV-1 drugs. These infected reservoir cells are usually hidden from the immune system and are the reason why life-long drugs are necessary to keep the HIV-1 infection under control.

The data indicate that the addition of BIT225 to anti-HIV-1 drugs stimulates the innate immune system so that the body's cells can "see" the HIV-infected reservoir cells and take the necessary steps to eliminate any residual virus.

The results are encouraging and may have profound implications for the future treatment and cure of HIV-1 infection.

CROI is the pre-eminent international HIV-1 conference and brings together top basic, translational, and clinical researchers from around the world to share the latest studies, important developments, and best research methods in the ongoing battle against HIV/AIDS and related infectious diseases. Due to concerns relating to the COVID-19 outbreak the 2020 CROI meeting is virtual.

Yours sincerely



Peter J. Nightingale  
Company Secretary

## **About Biotron**

Biotron Limited is engaged in the research, development, and commercialisation of drugs targeting significant viral diseases with unmet medical need. The Company has BIT225 in clinical development for HIV-1 and a promising preclinical program for HBV. In addition, Biotron has several earlier stage programs designing drugs that target a class of virus protein known as viroporins which have a key role in the virus life cycle of a very broad range of viruses, many of which have caused worldwide health issues such as Coronavirus, Dengue, Ebola, Middle East Respiratory virus, Influenza and Zika viruses.

## **Enquiries**

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# PHASE 2 TRIAL OF VPU INHIBITOR BIT225 IN COMBINATION WITH ANTIRETROVIRAL THERAPY

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## BACKGROUND

Vpu is a HIV-1 encoded membrane protein with multiple regulatory functions that enhance HIV-1 replication fitness and promote innate immune evasion in multiple cell types including monocytes. BIT225 inhibits HIV-1 replication in myeloid cells *in vitro*. BIT225 has been studied in patients with chronic HIV-1 infection receiving antiretroviral therapy (ART).

## METHODS

- A randomized, placebo controlled, double-blind, Phase 2 study of 100mg and 200mg BIT225 in individuals infected with HIV-1 commencing ART (males and females, aged 18-65yrs, viral load >5,000 copies/mL; CD4<sup>+</sup>count >100 cells/mm<sup>3</sup>, ART naïve).
- Participants were recruited from two sites in Thailand, treated with BIT225 or placebo in addition to ART (Atripla<sup>®</sup>) for 12 weeks.
- Individuals were randomized 2:1 (BIT225:placebo). Markers of viral replication and immune functions were investigated.

## The Vpu inhibitor BIT225, in combination with Atripla<sup>®</sup> promotes innate immune restoration in ART naïve participants infected with HIV-1

## RESULTS

Thirty-six patients were enrolled. Plasma HIV-1 RNA levels declined with similar viral decay kinetics in all cohorts over the 12 week study period. In contrast, significant changes were observed for multiple immune markers between the placebo and BIT225 cohorts (200mg cohort only shown). Levels of the monocyte activation marker sCD163 showed significantly greater reduction from baseline ( $P < 0.05$ , general linear model, two-way ANOVA) in the 200mg BIT225 treated cohort compared to ART alone over the 12 week treatment period. There was a statistically significant increase in activated CD8<sup>+</sup>, CD4<sup>+</sup> cells, and NK cells in the BIT225 cohort. There was a transient statistically significant increase in plasma IL-21 production in the first 3 weeks of BIT225 therapy. There were no significant changes to plasma TNF- $\alpha$ , IL-6, and interferon- $\gamma$  in either cohort over the treatment period.

The elevated NK cell numbers over the treatment period observed in the BIT225 cohort compared to placebo cohort, suggests enhanced NK cell recruitment and activation to eliminate HIV infected cells. This may be mediated via Vpu-related cell-signaling mechanisms. It is possible that there has been restoration of non-neutralizing antibody immune function.

The transient but statistically significant increase in plasma IL-21 is a unique finding in the BIT225 treatment cohort. ART and BIT225 treatment increase IL-17 production (results not shown). This data together supports restoration of Th17 cell number and perhaps function to restore the gut related immune barrier. The IL-21 data may also indicate that Tfh cell functions are being restored. Tfh cells produce IL-21 and are involved in neutralizing and non-neutralizing antibody mediated immune functions.

## CONCLUSIONS

The addition of BIT225 to ART resulted in unique stimulation of multiple arms of the innate immune system. The increased numbers of CD8<sup>+</sup>, CD4<sup>+</sup> and NK cells are consistent with enhanced recognition of HIV-1 infected cells. Vpu has been associated with reducing cell surface expression/function of numerous cellular proteins/receptors involved in viral antigen presentation to CD4<sup>+</sup>, CD8<sup>+</sup> T cells and NK cells. The production of IL-21 by Tfh, Th17, and/or NK cells is a unique immunological consequence of addition of BIT225 to ART and offers the potential for treatment targeting different HIV-1 compartments during standard therapy.

The T cell, NK cell, sCD163 and IL-21 data together suggest that the addition of BIT225 to ART stimulates antigen presentation as well as T cell and NK cell priming, and may result in changes to the immune system similar to that of long term non-progressors.

BIT225's immune modulating effects may have utility in improving HIV-1 induced chronic immune activation outcomes and aid in future eradication strategies.

## ADDITIONAL KEY INFORMATION

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- Clinical trial participants
- ACLIRES staff and management
- Staff associated with the studies at HIV-NAT, Thai Red Cross AIDS Research Centre and Chiang Mai University Hospital.

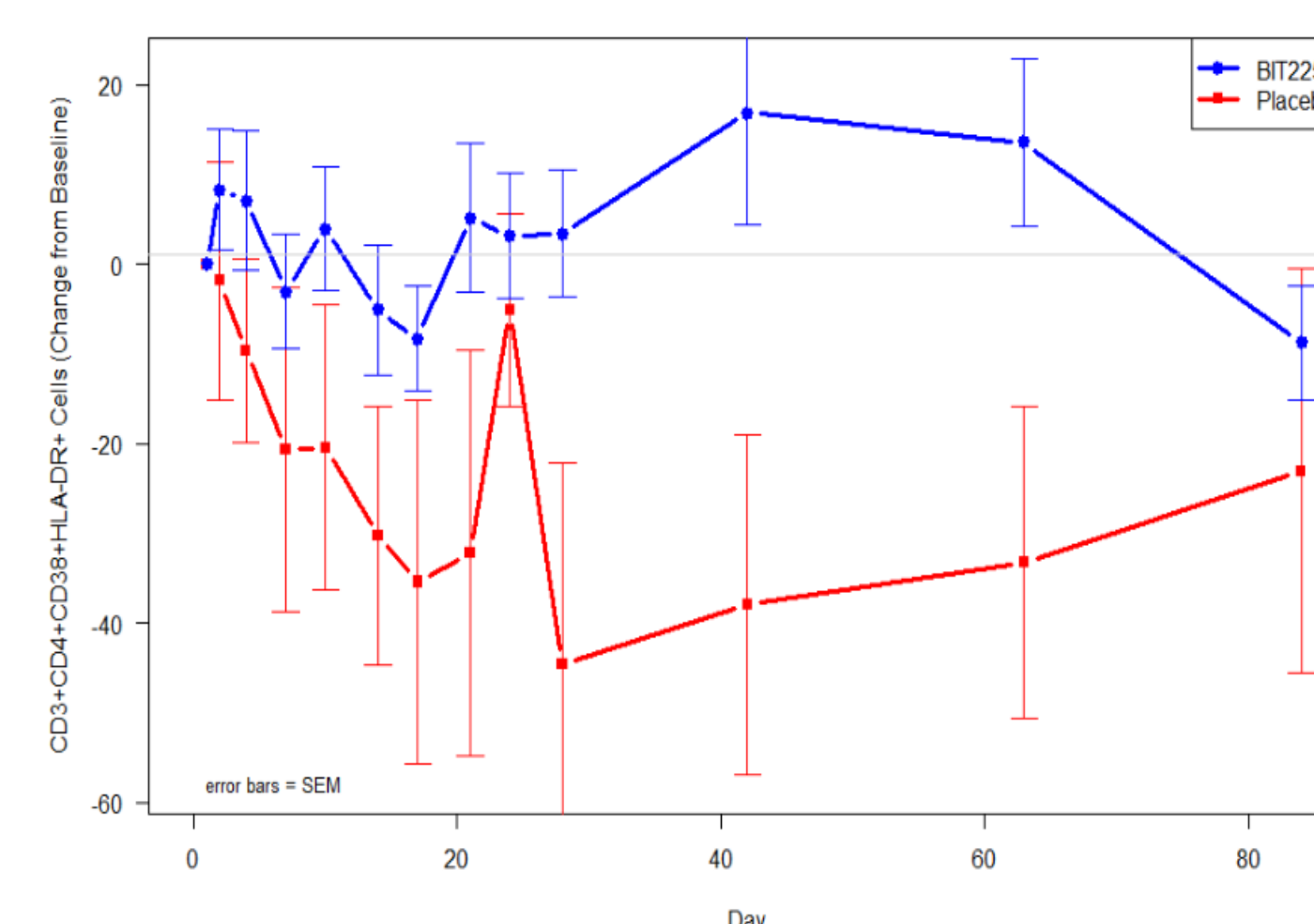
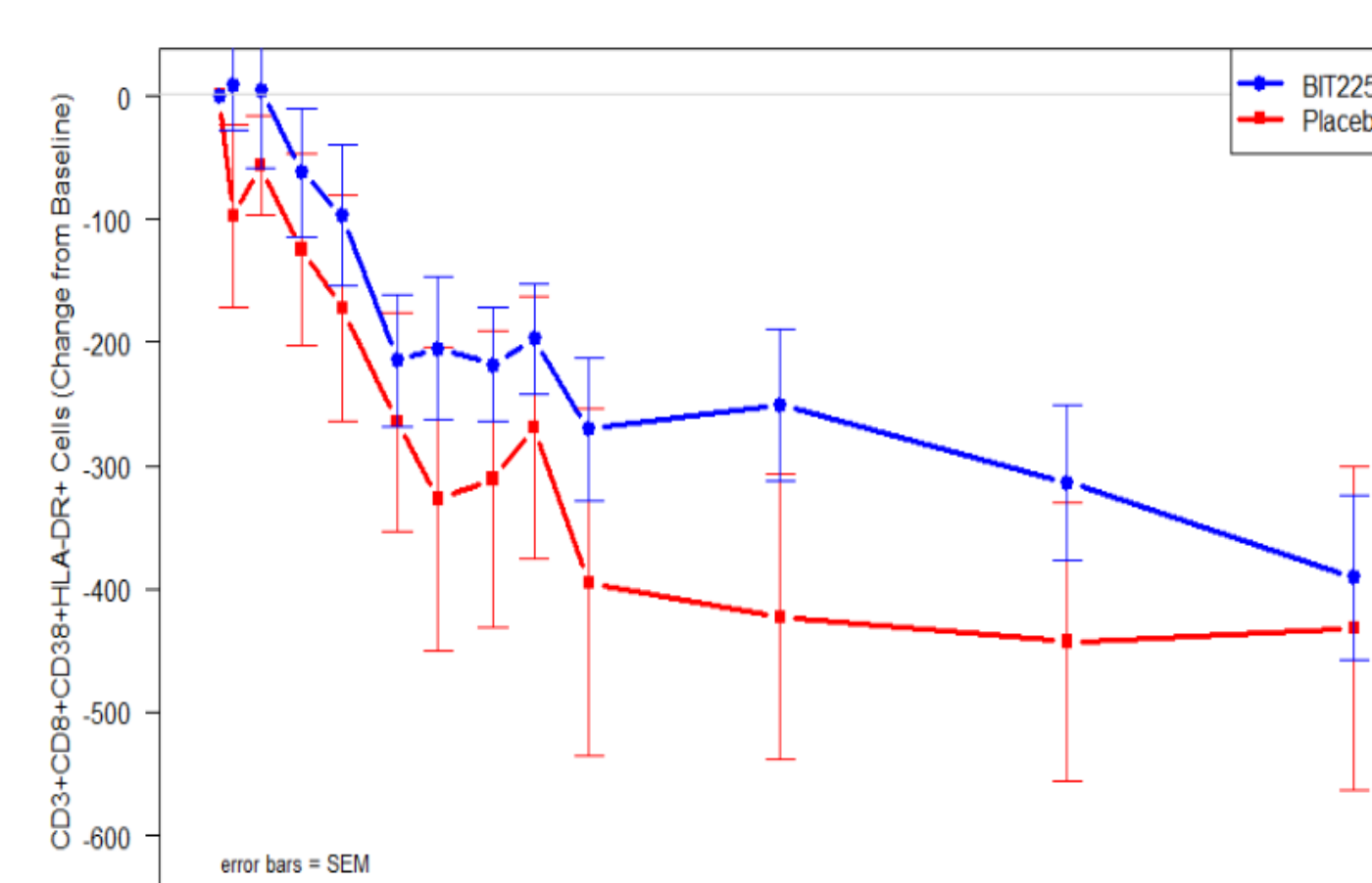


Figure 1

A. Group mean change from baseline of activated CD4<sup>+</sup> T cells (CD4<sup>+</sup>/HLA-DR<sup>+</sup>/CD38<sup>+</sup>) over 12 week treatment period with 200 mg BIT225 QD (blue circles) or placebo (red squares) and Atripla<sup>®</sup>. There was a statistically significant ( $P < 0.01$ , linear model) sustained delay in decline of CD4<sup>+</sup> activated T-cell numbers during the BIT225 treatment period, compared to placebo.



B. Group mean change from baseline of activated CD8<sup>+</sup> cell (CD8<sup>+</sup>/HLA-DR<sup>+</sup>/CD38<sup>+</sup>) numbers during 12 weeks of 200 mg BIT225 QD (blue circles) or placebo (red squares) treatment with Atripla<sup>®</sup>. The linear model showed that the BIT225 cohort had a smaller decrease in activated CD8<sup>+</sup> T-cells during the BIT225 treatment period: Estimated average difference 85 cells/ml ( $\pm 29$ , SEM), which was statistically significant ( $P < 0.01$ ).

Figure 2. Time course of mean soluble CD163 (sCD163) ng/mL change from baseline during 12 weeks of treatment with Atripla<sup>®</sup> plus 200 mg BIT225 QD (blue circles) or placebo (red diamonds). Two-way ANOVA for BIT225 versus Placebo, controlling for day of treatment, was done using R statistical software by fitting the linear model:  $\beta_0 + \beta_1 \cdot \text{day} + \beta_2 \cdot I_{\text{BIT225}}$ , where  $I_{\text{BIT225}}$  takes the values 0 (BIT225 treatment), or 1 (Placebo). The estimate:  $\beta_2 = 208 \pm 99$  (SE) ng/ml indicates a statistically larger overall decrease in sCD163 for the BIT225 treated group ( $P = 0.036$ ). The difference between placebo and control at Day 7 is statistically significant by Welch's T-test ( $P = 0.045$ ).

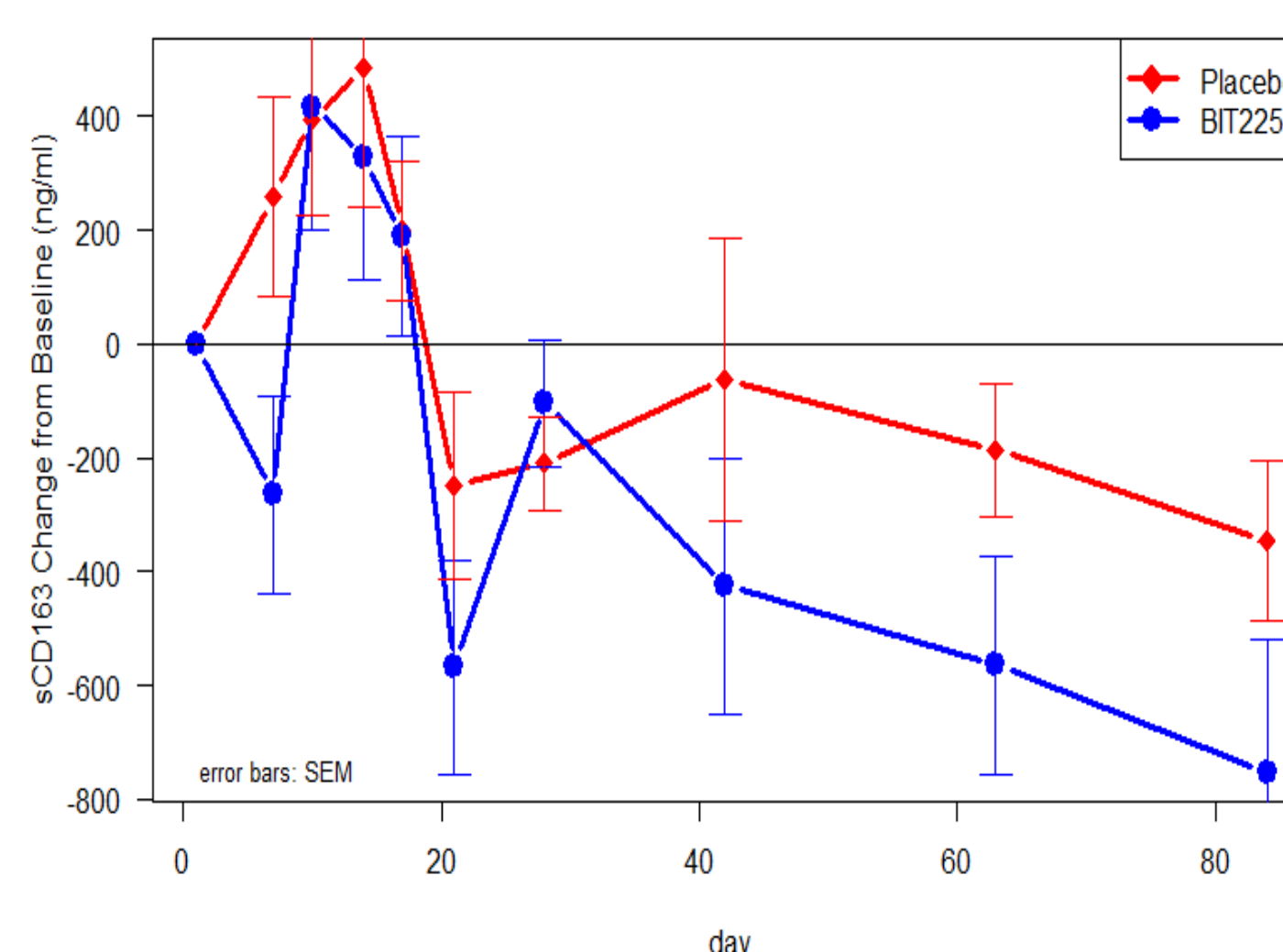


Figure 3. Group mean change from baseline of NK cell (CD8<sup>+</sup>/HLA-DR<sup>+</sup>/CD38<sup>+</sup>) numbers during 12 weeks of 200 mg BIT225 QD (blue circles) or placebo (red squares) treatment with Atripla<sup>®</sup>. The BIT225 cohort had a smaller decrease in NK cells during the BIT225 treatment period: Estimated average difference 71 cells/ml ( $\pm 23$ , SEM), which was statistically significant ( $P < 0.01$ ).

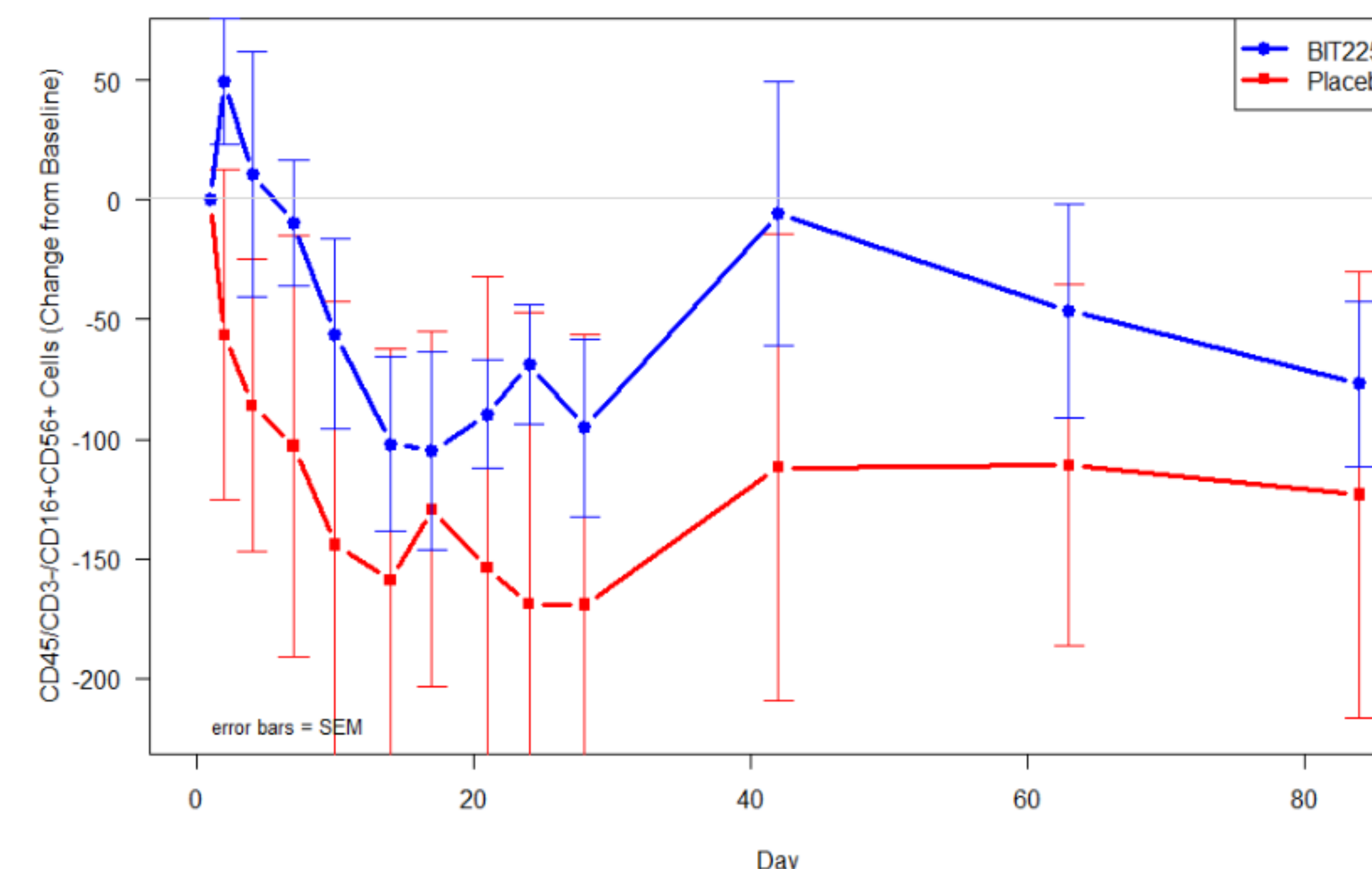


Figure 4. Time course of plasma IL-21 (ng/mL) change from baseline during 12 Weeks of treatment with 200 mg BIT225 QD (blue triangles) or placebo (red circles) and Atripla<sup>®</sup>. ANOVA analysis found that the difference between BIT225 and placebo cohorts over the first 3 weeks was statistically significant ( $P = 0.02$ ).

