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The Manager Companies
ASX Limited
20 Bridge Street
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(3 pages by email)

Dear Madam,

BIT225 REVERSES HIV-INDUCED IMMUNE EVASION

The Directors of Biotron Limited ('Biotron') are pleased to advise that the Company is presenting new data on its lead HIV-1 drug, BIT225, this week at the 23rd International AIDS Conference (AIDS 2020). The data indicate that via its inhibition of the HIV-1 viral protein Vpu, BIT225 enhances the immune response to HIV.

BIT225 is unique in its ability to both directly inhibit the virus and augment the immune responses against it.

The Company has previously reported that its BIT225-009 Phase 2 HIV-1 clinical trial demonstrated safety and tolerability, as well as the expected viral suppression in those treated with standard anti-HIV drugs (ART) alone or with ART plus BIT225. The BIT225 group also showed statistically significant changes in key immune cell populations. The new data being presented this week at the conference explain the mechanisms by which BIT225 induces these changes.

"The latest results provide key information on how BIT225 directly modifies immune responses to HIV-1 infection. It helps explain the immune changes that we saw in the Phase 2 clinical trial and gives us even more confidence in our product", said Dr Michelle Miller, Biotron's Managing Director.

During HIV-1 infection, in order to attenuate host immune responses against the virus, specific cell surface markers that normally signal the immune system to attack the virus are downregulated. This downregulation enables the virus to evade the immune response and persist. These cells constitute a reservoir for the virus that enables it to persist despite ART.

The new data show that BIT225 restores these cell surface markers to normal. In addition, the new data also indicate that BIT225 increases a key marker linked to functionality, meaning that T cells are able to move around the body and restore immune function.

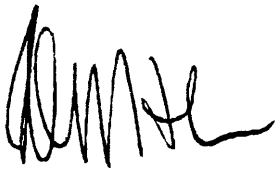
The Vpu protein of HIV-1 is largely responsible for downregulation of cell surface markers that attenuate immune responses. This new study used wild-type virus as well as virus that had Vpu removed to show that BIT225's effects on the immune system are mediated directly through its inhibition of Vpu. These effects are additional to the known antiviral effect of BIT225.

The new data support and further extend Biotron's previous report that BIT225 "unmasks" HIV-infected cells and promotes immune recognition of the virus. Immune system recognition initiates the host defence processes including the clearance of virus.

In combination with results from the Phase 2 clinical trial, the results reported here support further clinical study of the potential anti-viral and immunological benefits of BIT225 therapy in combination with ART.

A copy of the poster presentation, entitled “Vpu inhibitor BIT225 alters T cell activation and homing plasma membrane receptor expression on CD4+ T cells (CD28 and CCR7) and monocyte-derived macrophages (CD80 and CD86)”, is attached.

Yours sincerely



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Vpu inhibitor BIT225 alters T cell activation and homing plasma membrane receptor expression on CD4⁺T cells (CD28 and CCR7) and monocyte-derived macrophages (CD80 and CD86)

LBPEA03

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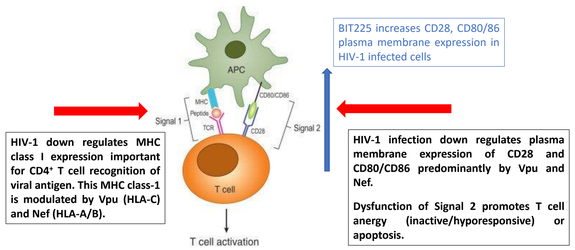
BIT225, a Vpu inhibitor, is able to counteract the HIV-1 induced downregulation of key cellular receptors essential for T cell activation and response

BACKGROUND

Chronic HIV-1 infection occurs in-part because the virus encodes accessory proteins to misdirect the host immune system so that HIV-1-infected cells can survive and propagate virus. In a recent Phase 2 clinical trial (BIT225-009) the addition of BIT225 to Atripla ® therapy resulted in a delayed decline in CD4⁺ T cell activation. This *in vitro* study investigated if there was a Vpu-dependent mechanism that could result in better activation and function of CD4⁺ T cells during ART. The costimulatory receptor of T cell activation is CD28 and its counterparts are CD80 and CD86 on macrophages, dendritic cells and B cells. Experiments were designed to identify the role of accessory proteins Vpu and Nef expression on CD4⁺ T cell activation and homing plasma membrane (PM) receptor expression on CD4⁺ T cells (CD28, CCR7 respectively) and monocyte-derived macrophages (MDM)(CD80, CD86).

Modified Schematic of T cell receptor activation adapted from Sharma et al., 2018.



1. CD4⁺ T CELL RECEPTOR FUNCTION

Two steps are required for T cell activation:

A. The binding of the PM TCR(CD3) on the T cell with its partner MHC/antigen complex on PM of APC. Binding of the CD4⁺ coreceptor.
B. The costimulatory binding of T cell PM CD28 with its partner receptor, either CD80 or CD86, on PM of APC.

This study investigates the potential modulation of B7 family molecules (CD28, CD80 and CD86) that are essential for the co-stimulatory signaling of the TCR. Approximately 95% of plasma CD4⁺ T cells express CD28 and they are abundant on both resting and activated T cells. The two step TCR signaling combination has 3 outcomes. If the TCR and CD28 are engaged, it results in IL-2 production and IL-2 receptor expression which drives T cell proliferation and differentiation to become a limited number of subtypes. If the TCR is ligated alone then the T cell enters a state of anergy or apoptosis. If only the CD28 is ligated, there is no effect.

HIV-1 utilizes 2 accessory proteins, Vpu and Nef , to reduce the efficacy of the costimulatory TCR recognition step by decreasing the PM expression the key receptors on both CD4⁺ T cells and MDM (Ramirez et al., 2019). This redundancy implies it is an important mechanism for HIV-1 to hide from the immune system. This is the first report of therapeutic intervention (BIT225) of this process that may have important implications for future treatment strategies. The data is consistent with the sustained increased plasma CD4⁺ T cell numbers observed in vivo during 12 weeks of Atripla® and 200 mg QD of BIT225 (Avihingsanon et al., 2018, Avihingsanon et al., 2020).

METHODS

CD4⁺ T cells and monocytes were extracted from the blood of three donors. MDM were grown in culture from monocytes isolated from PBMCs. The MDM or CD4⁺ T cells were infected with VSVG-pseudotyped wildtype, Vpu-, Nef- and Vpu-/Nef- HIV-1_{NL4-3}. The expression of PM receptors CD28, CCR7, CD80 and CD86 was measured by flow cytometry. Statistical analysis of the data was performed by ANOVA.

RESULTS

HIV-1_{NL4-3} infection of CD4⁺ T cells results in downmodulation of PM expression of CD28 and CCR7. Downregulation of CD28 and CCR7 is induced by Vpu expression. CD28 and CCR7 are expression also impacted by Nef. BIT225 treatment increases the expression of CD28 and CCR7 in a Vpu-dependent manner. Infection of MDM with VSVG-pseudotyped HIV-1_{NL4-3} resulted in decreased expression of CD80 and CD86. BIT225 treatment of MDM infected with VSVG-pseudotyped HIV-1_{NL4-3} resulted in a partial reversal of downmodulation of CD86 which was partially Vpu-independent. BIT225 treatment partially restores CD80 expression. The effect on CD80 expression is Vpu-dependent. BIT225 had no observable effect on Vpu-/Nef- HIV-1_{NL4-3} mutants, primarily because double deletion of Vpu and Nef returns receptors to uninfected levels.

CD4⁺/CD28⁺ T cells

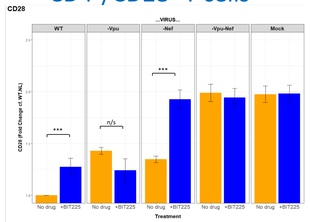


Figure 1. Plasma Membrane expression of CD28 on CD4⁺ T cells treated with 3 μM of BIT225 at 72 hours post-infection with HIV-1_{NL4-3} or mutants deficient in Vpu, Nef or both Vpu and Nef.

Plasma membrane expression of CD28 on CD4⁺ T cells was decreased by infection with wildtype (WT) HIV-1_{NL4-3} over the 72 hour experiment. The expression of CD28 was down-modulated by the expression of Vpu and Nef. BIT225 treatment of WT infected cells resulted in a partial restoration of CD28 expression that was dependent on Vpu expression only. The double deletion of Vpu and Nef resulted in restoration of CD28 expression to levels comparable to unifected cells. BIT225 reduces Vpu-related PM CD28 downregulation as a result of HIV-1 infection.

MDM /CD80

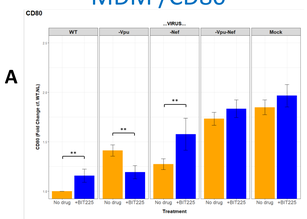
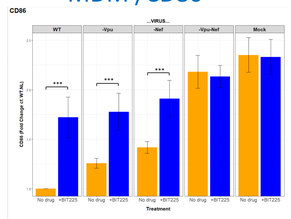


Figure 2. Plasma Membrane expression of CD80 or CD86 on MDM treated with 3 μM of BIT225 at 72 hours post-infection with HIV-1_{NL4-3} or mutants deficient in Vpu, Nef or both Vpu and Nef.

A. Plasma membrane expression of CD80 on MDM was decreased by infection with WT HIV-1_{NL4-3} over the 72 hour experiment. The PM expression of CD80 was modulated by the expression of Vpu and Nef. BIT225 treatment of WT infected cells resulted in a partial restoration of PM CD80 expression. The double deletion of Vpu and Nef resulted in restoration of CD80 expression to levels comparable to uninfected cells. The BIT225 treatment response was dependent on Vpu expression.

MDM /CD86



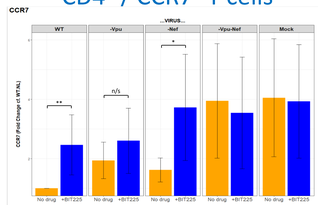
B. Plasma membrane expression of CD86 on MDM was decreased by infection with WT HIV-1_{NL4-3} over the 72 hour experiment. The PM expression of CD86 was modulated by the expression of Vpu and Nef. BIT225 treatment of WT infected cells resulted in a partial restoration of PM CD86 expression. The double deletion of Vpu and Nef resulted in restoration of CD86 expression to levels comparable to uninfected cells. Unlike for the other receptors, the CD86 BIT225 treatment response was similar in the viruses with Vpu or Nef single deletions, implying a novel mechanism that is largely independent of Vpu expression.

2. T CELL HOMING and DIFFERENTIATION FUNCTION: CD4⁺/ CCR7

T cell function/differentiation is affected by the environment and education the cell is exposed to during its circulation through the blood and immune tissues/organs. A critical homing signal is the expression of PM Chemokine receptor 7 (CCR7). This study determined that PM CCR7 expression on CD4⁺ T cells was decreased by HIV-1 infection. The PM expression of CCR7 was reduced when Vpu was expressed. This Vpu dependence on CCR7 expression was eliminated by treatment with BIT225. This data suggest that CD4⁺ T cell function may be improved when BIT225 is added to ART .

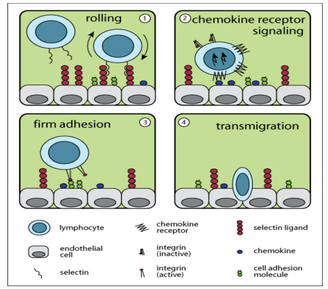
Figure 3. Plasma Membrane expression of CCR7 on CD4⁺ T cells treated with 3 μM of BIT225 at 72 hours post-infection with HIV-1_{NL4-3} or mutants deficient in Vpu, Nef or both Vpu and Nef.

CD4⁺ / CCR7⁺ T cells



Statistical methods: Data were converted to "fold-change" by division with Donor- and Time- matched values from the untreated WT.NL4.3 dataset. Two-way ANOVA was used to statistically assess the within-virus difference between BIT225 treated and untreated means. The linear model: ***Fold-Change = β_0 + β_1*Time+ β_2*I_{BIT225}*** was fitted to the dataset (N=18) for each virus which included measurements from three donors at three time-points (24h, 48h & 72h); either treated with BIT225 (3 mM) or not (DMSO control). The P-values for whether the estimate of β_2 was significantly non-zero are summarised by the asterisks in Figures 1 to 3:

*** P<0.001; ** P<0.01; * P<0.05; n/s P>0.05



HIV-1 infection downregulates PM expression of CCR7 on CD4⁺ T cells.

BIT225 treatment increases PM expression of CCR7. This may in part reestablish the appropriate migration and homing to anatomical sites in the body of the CCR7⁺/CD4⁺T cells. This may result in more efficient recognition and elimination of HIV-1 infected cells.

Modified schematic from British Society of Immunology: T lymphocyte migration occurs via a multistep pathway.
https://www.immunology.org/public-information/bitesized-immunology/systems-and-processes/t-lymphocyte-transmigration

CONCLUSIONS

BIT225 is a clinical stage Vpu inhibitor, the first in class. A phase II study demonstrated safety and tolerability, as well as the expected viral suppression in those treated with ART alone or ART plus BIT225. Those in the BIT225 arm had a persistent elevation of activated CD4 cells, which has prompted further study, including the data presented here. These data suggest that BIT225 treatment can counteract Vpu-mediated downregulation of membrane expressed CD28 & CCR7 on CD4+ T cells, as well as CD80 & CD86 on monocyte derived macrophages (MDM) infected with HIV-1. The mechanism of action of BIT225 requires the presence of Vpu for modulation, and restoration of CD28 & CCR7 on CD4⁺ T cells, and CD80 on MDM. BIT225 treatment-related enhancement of CD86 expression on MDMs infected with the Vpu- mutant virus indicates that an additional mechanism of BIT225 action operates in MDM. BIT225 thus appears to enhance the costimulatory, signal 2, of TCR activation by increasing expression of both the receptor, and ligand on CD4+ T cells and MDM, respectively. These findings, and those of enhanced homing indicate novel attributes of BIT225 Vpu inhibition that suggest enhanced host immune surveillance of HIV. In the clinical trial enhanced CD4 activation, seen only in the BIT225 treated group, was persistent, even in the setting of rapidly falling HIV RNA. Further studies will explore the hypothesis that Vpu inhibition permits the detection of virus-infected cells that were neither eradicated by suppressive ART, nor readily identified by the host immune system. BIT225 treatment may be a valuable addition to future antiretroviral treatment, and in consideration of eradication strategies.

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Ramirez et al., 2019 Cells 8:1020 doi: 10.3390/cells8091020
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