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**Investor Zoom Webinar 11:00am AEDT today (25 November 2021)**
You are invited to register using this link:
https://zoom.us/webinar/register/WN_RvXVWdr2SveoVoRtnBM3SQ

*Questions may be tabled as you register or during the webinar*

25 November 2021

The Manager Companies
ASX Limited
20 Bridge Street
SYDNEY NSW 2000

(9 pages by email)

Dear Madam

**BIOTRON DRUG EFFECTIVE AGAINST COVID-19 IN ANIMALS**

- **BIT225 administered orally significantly reduced viral load in the lungs and blood of animals challenged with SARS-CoV-2.**
- **BIT225 protected against severe disease, indicated by the significant prevention of body weight loss in animals treated with BIT225 compared to non-treated controls.**
- **Pro-inflammatory cytokines were also significantly reduced in the lungs and blood of BIT225 treated animals compared with controls. Raised pro-inflammatory cytokines are associated with severe illness in people with COVID-19. Eliminating the "cytokine storm" is essential for successful treatment.**
- **The results show statistically and clinically significant efficacy of BIT225 in this model of COVID-19.**
- **In a cell culture *in vitro* study BIT225 was active against the highly infectious delta variant of SARS-CoV-2, reducing the level of virus by more than 99.99% compared to controls.**
- **BIT225 is a clinical stage drug in development for treatment of HIV-1, with over 200 people dosed in trials to date. It is an oral drug, suitable for once-a-day dosing and has a well characterised safety profile. Biotron Limited is now seeking to accelerate BIT225 into clinical trials in SARS-CoV-2-infected patients.**

The Directors of Biotron Limited (ASX: BIT) are pleased to announce that the Company's lead clinical asset, BIT225, has demonstrated substantial and clinically meaningful efficacy against SARS-CoV-2 in a series of animal and cell-based studies performed at The SCRIPPS Research Institute, La Jolla, CA, USA.

BIT225 was tested in a COVID-19 mouse model (K18-hACE2). These mice have been engineered to be infectable by SARS-CoV-2 which then produces a range of pathologies including pulmonary disease. This model is routinely used to assess the ability of drugs to target SARS-CoV-2 and treat COVID-19 disease.

The study in the COVID mice showed that BIT225 given orally (by mouth) significantly reduced virus load in the lungs of treated mice when compared with control mice that were given drug-free control material (known as vehicle control). There was also a reduction in virus in the blood. The reduction in virus was dose-dependent - i.e. reduction in viral load was greater at the higher dose.

Increased levels of pro-inflammatory cytokines ('cytokine storm') are linked to severe illness and death in people infected with SARS-CoV-2 virus. Controlling this cytokine storm is essential for successful treatment of COVID-19.

BIT225 significantly reduced all assayed pro-inflammatory cytokines and chemokines, including IL-6, IL-1α, IL-1β, TNF-α, TGF-β and MCP-1, in the lungs and blood of BIT225-treated mice compared to control mice.

During the course of infection with SARS-CoV-2, K18 mice generally develop severe disease that is reflected in the loss of body weight. The animals treated with BIT225 did not lose weight throughout the study and, in fact, significantly increased their weight in line with growth expectations of the age of the animals.

The impact of BIT225 on the proinflammatory cytokines and on overall health, indicated by preventing loss of body weight, indicates clinically significant benefit of BIT225.

In addition to the *in vivo* animal study, BIT225 was tested in an *in vitro* study in cell cultures to assess the ability of the drug to inhibit the highly infectious delta strain. The data showed that BIT225 reduced the delta virus in the cell cultures by more than 99.99% (over 4 logs reduction).

**The *in vivo* study demonstrates that BIT225 is highly effective antiviral agent and protects the animals from severe disease. The *in vitro* study demonstrates that BIT225 is also active against the highly infectious delta strain of SARS-CoV-2.**

BIT225 belongs to a new class of antiviral drugs known as viroporin inhibitors. It targets key viral-encoded proteins known as viroporins that are central to establishing and maintaining infections through modulation of the body's immune system.

BIT225 is Biotron's lead antiviral clinical-stage, investigational, small molecule antiviral drug. It is an oral drug, suitable for once-a-day dosing and has a well characterised safety profile. The drug has been evaluated in nine clinical trials involving healthy volunteers, patients with HIV-1 infection, patients co-infected with Hepatitis C virus (HCV) and HIV-1 and patients with HCV (as monotherapy and in combination with pegylated interferon-alfa and ribavirin). Formal pre-clinical studies have assessed safety over 24 weeks of dosing.

Recently the Company commenced two Phase 2 HIV-1 trials in Australia and Thailand to assess the impact of BIT225 on the HIV-1 reservoir and key markers of improved health outcomes.

Biotron is now in discussions with its USA advisors and consultants to expedite progression of BIT225 into human trials for treatment of SARS-CoV-2 infection. The Company has sufficient drug product on hand after recently completing the manufacture of several kilograms of additional clinical grade (cGMP) drug.

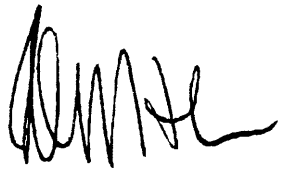
The Chair of Biotron's Scientific Advisory Board, Professor Rob Murphy, Professor of Medicine and Biomedical Engineering, John P. Phair Professor of Infectious Diseases at Northwestern University, Chicago, said, "These very encouraging results in a SARS-CoV-2 animal model demonstrate a robust antiviral response that justifies further study in humans. BIT225 is a novel antiviral drug that has been safely used in over 200 patients with other RNA viral diseases including HIV and hepatitis C. This is drug that should be studied as a COVID-19 treatment in the very near future."

Biotron's Managing Director, Michelle Miller, said "These results suggest that BIT225 may have benefit over other known antiviral agents. We will actively pursue all avenues to progress this Biotron drug into human trials as quickly as possible."

Experimental details and results are set out in an Addendum below.

This announcement has been approved for release by the Company's Managing Director.

Yours sincerely



Peter J. Nightingale
Company Secretary

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**ADDENDUM**

**EXPERIMENT DETAILS**

***In vivo* study in K18-hACE2 mice**

Transgenic mice expressing human ACE2 under the control of the cytokeratin 18 promoter (K18-hACE2 mice) were inoculated intranasally with $10^4$ PFU of SARS-CoV-2 (2019n-CoV/US-WA1/2020). The mice were dosed 12 hourly for 14 doses by oral gavage (Day 7 = last day):

- Group 1: 5 mice dosed with 100 mg/kg BIT225
- Group 2: 5 mice dosed with 300 mg/kg BIT225
- Group 3: 5 mice dosed with vehicle control (methylcellulose, benzyl alcohol, polysorbate 80)

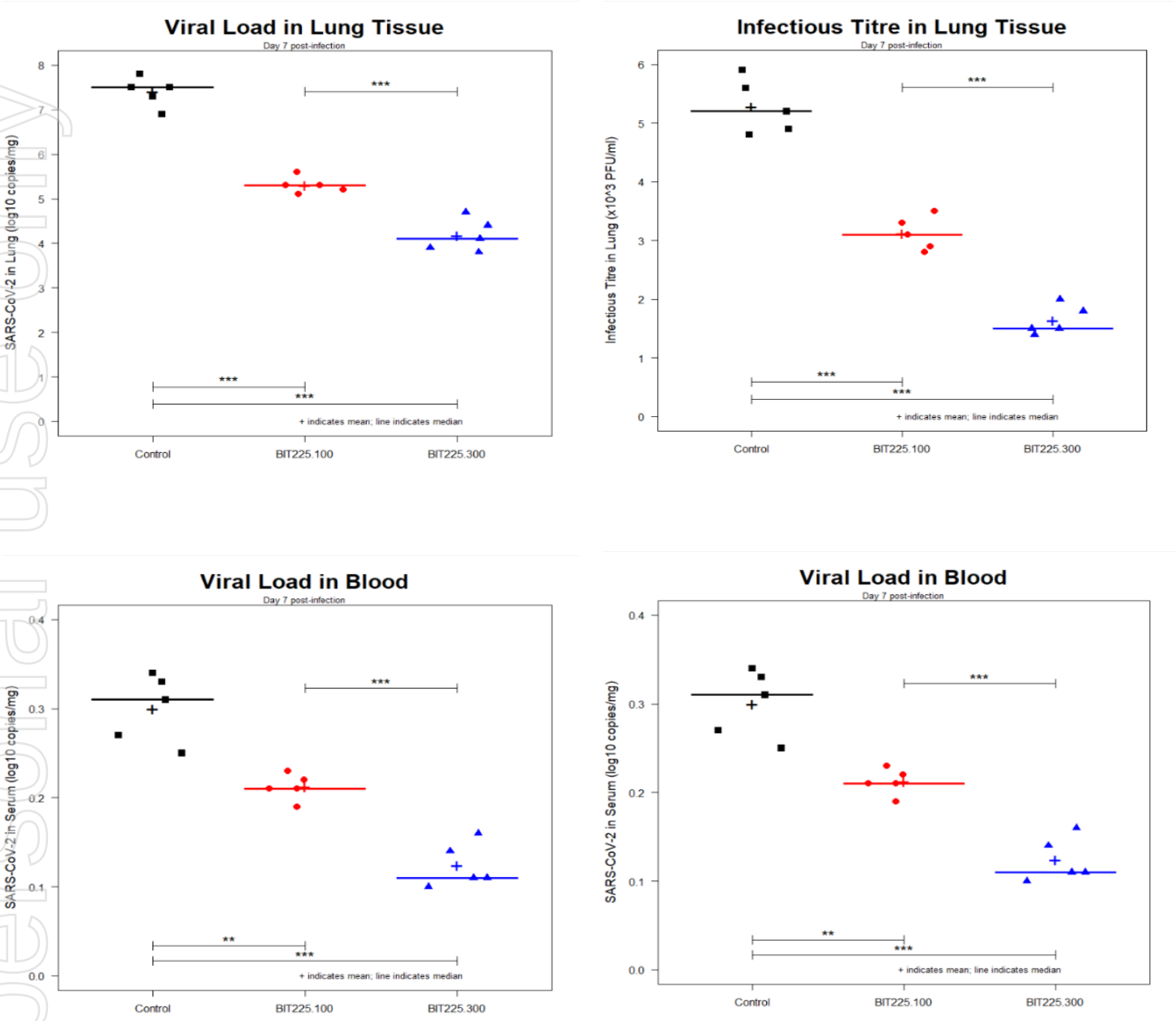
Body weight and general health was monitored daily. The mice were sacrificed on Day 7 and blood and lung samples taken.

The impact of BIT225 on viral loads was determine by two methods. In the first method the number of viral copies in blood and lung tissue was determined by qRT-PCR using standard kits and methods. In the second method the amount of infectious virus in blood and lung tissue was determined by plaque assay using standard methods.

Quantitation of virus copies and amount of infectious virus in lung homogenate and blood showed that both doses of BIT225 significantly reduced viral load compared to vehicle control.

**Figure 1** plots show data values for each mouse, with median (line) and mean ("+") indicated by treatment group. Compound treatments versus vehicle and BIT225/AD178 (300 mg/kg) versus BIT225/AD178 (100 mg/kg) statistical comparisons were by T-test (n = 5 per group). Asterisks indicate strength of statistical significance based on P-values (*** $P<0.001$, ** $P<0.01$; * $P<0.05$; ns $P>0.05$). In all cases, the 300 mg/kg dose of BIT225 significantly reduced viral load compared to the 100 mg/kg dose.

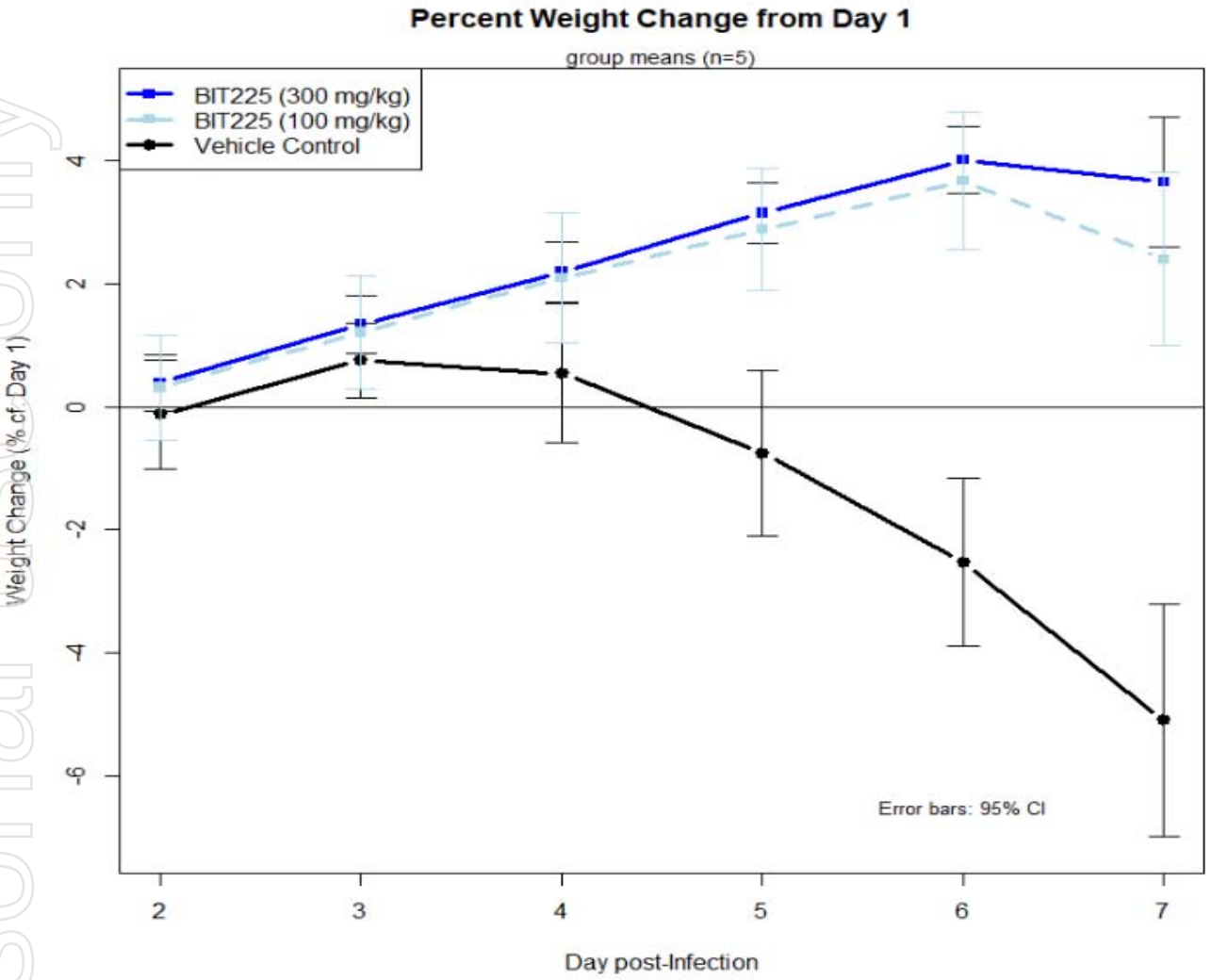
**FIGURE 1:**




Assessment of the body weights of the mice throughout the study show a pronounced downward trend in the mice treated with vehicle control from about Day 4, which is not observed in mice treated with 100 or 300 mg/kg BIT225.

**Figure 2** shows mean % weight change for mice treated with 300 mg/kg of BIT225 and mice treated with vehicle control. Between Day 0 and Day 7, mice treated with vehicle control showed a mean weight reduction of 5.1 % ($P = 0.005$), while mice treated with 300 mg/kg of BIT225 showed a mean weight increase of 3.7% % ($P < 0.01$).

**FIGURE 2:**



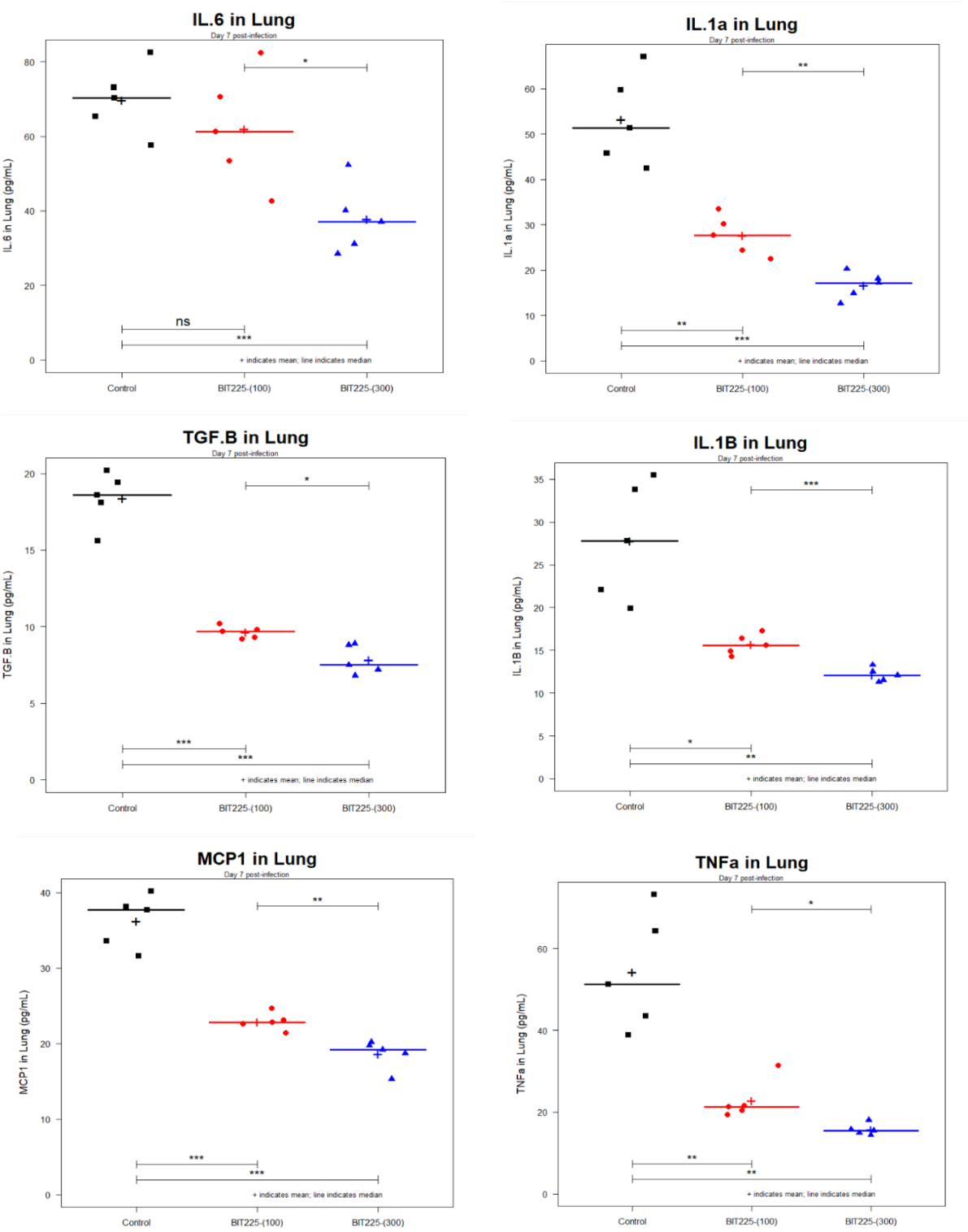

The increase in weight of mice treated with BIT225, compared to the decrease in weight of mice treated with vehicle control, indicates a reduction in the severity of disease and complications associated with SARS-CoV-2 infection.

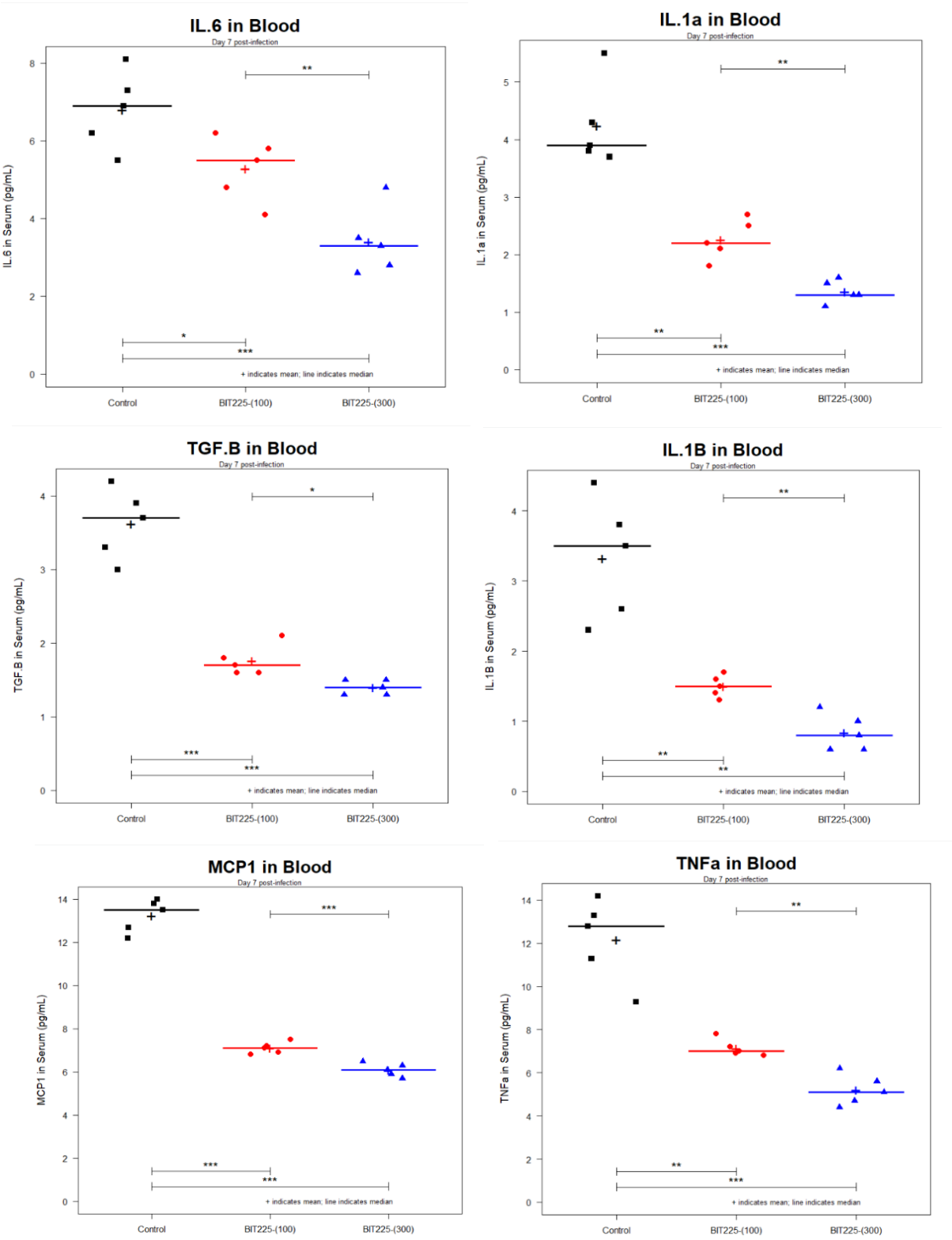
Inflammation was measured by determining amounts of the proinflammatory cytokines interleukin-6 (IL-6), interleukin-1alpha (IL-1α), interleukin-1beta (IL-1 β), tumour necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β) and the proinflammatory chemokine monocyte chemoattractant protein-1 (MCP-1) using commercially available ELISA kits.

The 12 plots following (**Figure 3**) show quantitation data for six cytokines: IL-6; IL-α; TNF-α;TGF-β; MCP-1 and IL-1β, measured in both lung homogenates and blood serum. BIT225 treatment versus vehicle and BIT22 5(300 mg/kg) versus BIT225 (100 mg/kg) statistical comparisons were by T-test (n = 5 per group). Asterisks indicate strength of statistical significance based on P-values (*** P<0.001, ** P<0.01; * P<0.05; ns P>0.05).

For all six cytokines, the high dose of BIT225 is associated with statistically significant reduction in both lung tissue and serum, compared to mice treated with vehicle control. In addition, the high dose of BIT225 has greater effect than the low dose.

**FIGURE 3:**






**In summary, the *in vivo* results demonstrate that BIT225 inhibits SARS-CoV-2 replication, reduces infectious viral load, reduces the production of pro-inflammatory cytokines and chemokines, and reduces the severity of complications associated with SARS-CoV-2 infection in this animal model of COVID-19.**

***In vitro* study of BIT225 against Delta Strain of SARS-CoV-2**

To assess the ability of BIT225 to inhibit replication of SARS-CoV-2 delta strain Vero and Calu 3 cells were infected with either SARS-CoV-2 US WA1/2020 (WA1) or US PHC658/2021 (Delta) and treated with BIT225 at a range of concentrations - 0, 0.3, 0.6, 1.25, 2.5, 5 & 10 µM (in triplicate). Virus levels were assessed after four days by measuring the number of virus copies released by the cells into the culture medium using qRT-PCR (copies/me ($log_{10}$)) using standard methods.

The results show that BIT225 has efficacy against both the delta and WA1 strains of SARS-CoV-2. There were similar levels of activity against both strains as shown in the table below:

| Assay | Cell | Virus | $EC_{50}$ (µM) | Lower 95%CI | Upper 95%CI |
|---|---|---|---|---|---|
| PCR | Calu 3 | Delta | 4.24 | 3.77 | 4.71 |
| | | WA1 | 4.28 | 3.68 | 4.88 |
| | Vero | Delta | 4.08 | 3.5 | 4.67 |
| | | WA1 | 3.67 | 3.21 | 4.12 |

**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 promising preclinical programs for SARS-CoV-2 and 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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