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17 March 2022

The Manager Companies
ASX Limited
20 Bridge Street
SYDNEY NSW 2000 (4 pages by email)

Dear Madam

CONFIRMATION OF BIT225 EFFECTIVENESS AGAINST COVID-19 IN ANIMALS

Further to the Company's announcement on 25 November 2021, the Directors are pleased to advise that the Company's lead clinical asset, BIT225, has again demonstrated substantial and clinically meaningful efficacy against SARS-CoV-2 in a series of animal studies performed at The SCRIPPS Research Institute, La Jolla, CA, USA.

- **BIT225 protected against severe disease, indicated by the significant prevention of body weight loss and absence of death in SARS-CoV-2-infected animals treated with BIT225 compared to non-treated controls.**
- **BIT225 administered orally significantly reduced virus levels in the lungs of animals challenged with SARS-CoV-2.**
- **The results show statistically and clinically significant efficacy of BIT225 in this model of COVID-19.**

Experimental details of this repeat study ('Study 2'), set out in the Addendum below, are the same as for the first 300mg dosage study ('Study 1') announced on 25 November 2021 with the exception that, due to the good health of the mice receiving BIT225 at the end of Study 1, the treatment period was extended from 7 days for Study 1 to 12 days for Study 2.

In both studies, BIT225 was tested in a COVID-19 mouse model (K18-hACE2) that is routinely used to assess the ability of drugs to target SARS-CoV-2 and treat COVID-19 disease.

In Study 2, shown below in the Addendum, body weights of the mice throughout the studies show a pronounced downward trend in SARS-CoV-2-infected mice treated with vehicle control (i.e. drug-free control) from about Day 4, which is not observed in SARS-CoV-2-infected mice treated with BIT225.

No mice receiving daily BIT225 by oral gavage developed any signs of disease, and all continued to gain weight as per age expectations through to Day 12 when the study was terminated (See Figure 1, below).

In contrast, all mice in the control, drug-free group died by Day 8 post-infection with SARS-CoV-2 from severe COVID.

As in the previous study announced in November 2021, BIT225-treated mice in Study 2 had significantly lower viral loads of SARS-CoV-2 in lung compared to vehicle (drug-free) controls.

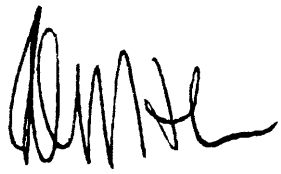
Biotron's Managing Director, Michelle Miller, said;

"The results from the extended, repeat Study 2 confirm the results from Study 1, showing a clear clinical benefit from the treatment with BIT225.

Supported by these results, and in consultation with USA based advisors and consultants, Biotron has submitted a proposal to the USA Food and Drug Administration (FDA) to conduct a human clinical trial to assess the efficacy of BIT225 for the treatment of COVID-19 under the Coronavirus Treatment Acceleration Program, a special emergency program for potential coronavirus therapies.

Funding the clinical development of BIT225 for this indication will be sought from potential partners and non-equity funding sources."

Yours sincerely



Peter J. Nightingale
Company Secretary

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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.

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

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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 12 days by oral gavage (Day 12 = last day of the study):

- Group 1: 7 mice dosed with 300 mg/kg BIT225
- Group 2: 7 mice dosed with vehicle control

Body weight and general health was monitored daily. The primary outcomes and endpoints measured were survival time and body weight. Mice losing >30% body weight compared to their baseline pre-infection weight were euthanised and counted as a death due to virus event. All surviving mice were sacrificed on Day 12 and lung samples taken.

Figure 1 (below) shows data values of weights for each mouse, expressed as percentage change in weight from baseline (pre-infection) weight. Statistical comparisons were by T-test (n = 7 per group). Asterisks indicate strength of statistical significance based on P-values (*** P<0.001; ** P<0.01).

All mice in the vehicle group (black squares) died or had reached the humane endpoint of >30% body weight loss from baseline at days 7 or 8 post-infection. In contrast, all mice treated with BIT225 (blue triangles) for 12 days had increased body weight relative to baseline (in line with age-related growth expectations) and survived.

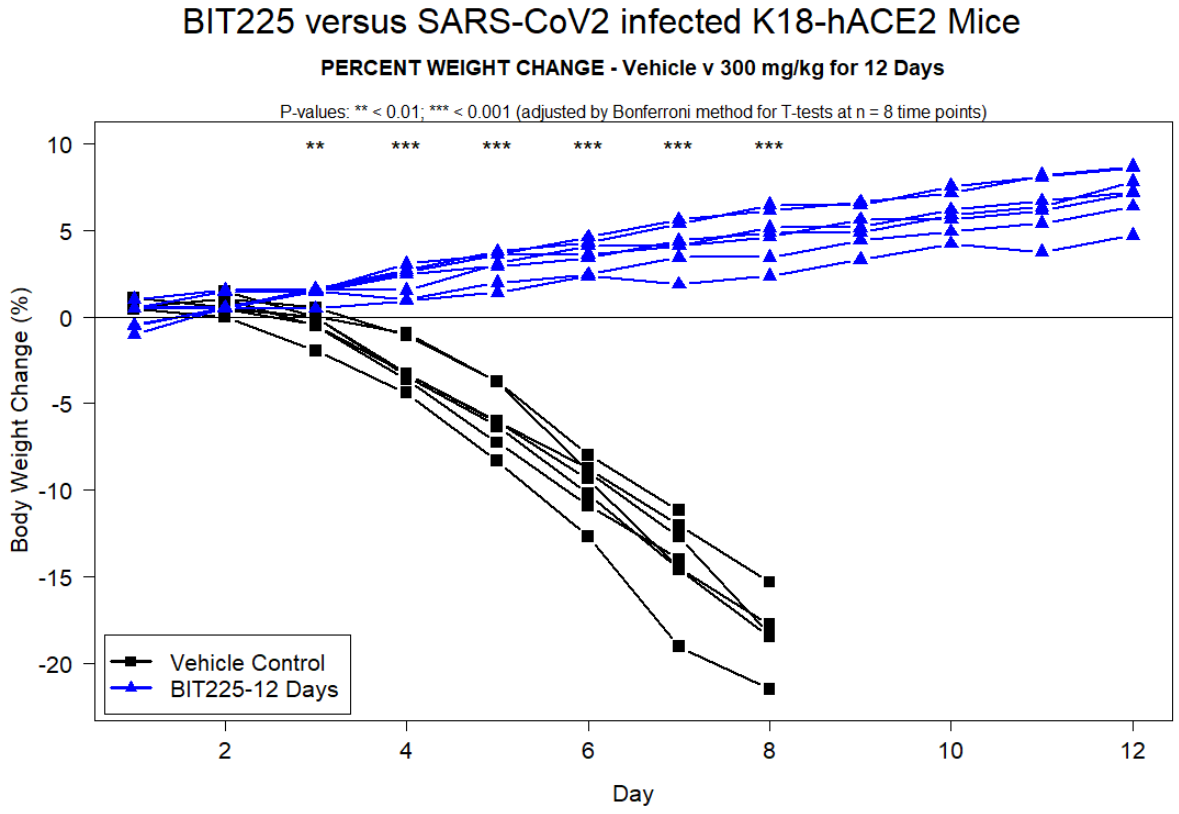


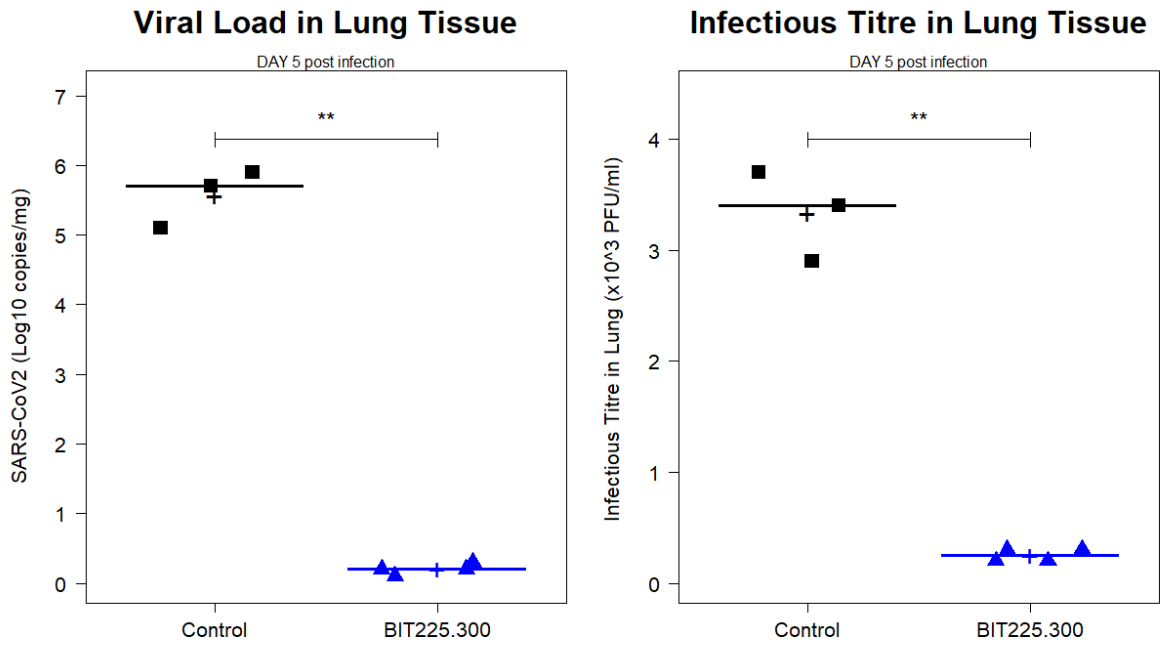
Figure 1

A satellite sub-study was run concurrently to compare viral load endpoints at Day 5 for BIT225 (300 mg/kg) vs vehicle control (n=4 mice per group). This satellite sub-study was necessary as viral loads could not be measured at later time points due to death of control mice from COVID.

Two methods were employed to assess virus levels in lungs harvested and homogenised at Day 5. In the first method the number of viral copies was determined by qRT-PCR using standard kits and methods. In the second method the amount of infectious virus was determined by plaque assay using standard methods.

As shown in **Figure 2** (below) the quantitation of virus copies and amount of infectious virus in lung homogenate showed that BIT225 (blue triangles) significantly reduced viral load compared to vehicle control (black squares). T-tests were used to compare the intra-day group means (P-values indicated as: ** $P < 0.01$).

Both the viral loads and infectious titres in the BIT225-treated mice were at or near the lower limit of detection of virus which indicate very little, if any, virus remained. In contrast, viral loads in the vehicle control mice were approximately 6 $\log_{10}$ higher and infectious titres were approximately 3500 times higher that in the BIT225 treated mice.



In summary, the *in vivo* results confirm previous findings that BIT225 inhibits SARS-CoV-2 replication, reduces viral loads in the lungs and prevents development of severe COVID-19 disease and death associated with SARS-CoV-2 infection in this animal model of COVID-19.