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The Manager Companies
ASX Limited
20 Bridge Street
SYDNEY NSW 2000 (2 pages by email)

Dear Madam,

LEAD SERIES FOR SARS-CoV-2 PROGRAM

The Directors are pleased to provide an update on its ongoing program to develop compounds with activity against SARS-CoV-2, the coronavirus that causes COVID-19.

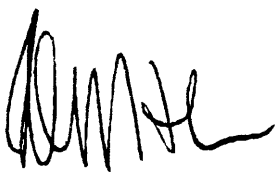
The Company previously advised on 7 September 2020 that several of its proprietary compounds had demonstrated antiviral activity against SARS-CoV-2 in laboratory studies. At that time, Biotron outlined its plan to extend its testing program to a series of newly designed and synthesised compounds. Since then, the Company has been screening this series of new, proprietary compounds in a range of different cell-culture assays designed to test the antiviral activity of the compounds against SARS-CoV-2.

The Directors are now pleased to report that as a result of this extended testing program, the Company has identified its top three anti-SARS-CoV-2 compounds. These three compounds have inhibited SARS-CoV-2 replication in three different cell culture models of SARS-CoV-2 virus infection. The assays were performed by collaborators at the Scripps Research Institute, La Jolla, CA, USA. Details of the methods used are set out below in an Addendum to this announcement.

Biotron's Managing Director, Michelle Miller, said; "The antiviral activity shown across a range of different assays by these three novel compounds against SARS-CoV-2 is encouraging. Identification of this potential lead series of compounds is a positive step for Biotron as we work towards new ways to treat this disease. The results underscore the versatility of Biotron's approach to designing and developing drugs to target serious virus infections."

These compounds are now progressing through preliminary safety studies as well as bioavailability studies in mice. When complete, the compounds are expected to be tested in animal model(s) of COVID-19 infection reflecting the diverse presentation of this disease. It is expected that these planned studies will be completed in mid-2021.

Yours sincerely



Peter J. Nightingale
Company Secretary

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ADDENDUM

In vitro susceptibility of viruses to an antiviral agent is usually assessed using a quantitative assay to measure virus replication in the presence of increasing concentrations of the product compared to replication in the absence of the product. The effective concentration is the concentration of product at which virus replication is inhibited by 50 percent (EC_{50} for cell-based assays).

Biotron has selected its lead series for its SARS-CoV-2 program. The three compounds have shown good activity with low micromolar EC_{50}s against SARS-CoV-2 in a series of three different *in vitro* cell culture models of infection.

The first assay measured the ability of the compounds to inhibit replication of SARS-CoV-2 in cells infected with the virus. The compounds were tested in triplicate across seven concentrations of each compound. The readout from the assay was the inhibition of release of SARS-CoV-2 nucleocapsid protein. Nucleocapsid protein is a well conserved highly immunogenic phosphoprotein and the level of this protein in the cell cultures is a direct measurement of the amount of virus present. A commercially available ELISA Kit was used to quantitate the nucleocapsid protein. All three compounds showed dose-response inhibition of nucleocapsid release, demonstrating their ability to inhibit replication of the virus. All three compounds demonstrated low micromolar EC_{50}s in this assay.

The second assay was a plaque assay, which is another way to measure the ability of compounds to inhibit replication of viruses. This type of assay is a very accurate method for the direct quantification of infectious virus and antiviral compounds through the counting of discrete plaques (infectious units and cellular dead zones) in cell culture. Seven concentrations of each compound were tested in triplicate in Vero cells were infected with SARS-CoV-2. After 4 days of incubation the wells were fixed, dyed with a coloured stain which is taken up by the plaques, and the number of plaques in each well was counted on a light box to determine the number of plaque forming units per ml. The approved antiviral drug Remdesivir was included as a positive control. The three compounds were all able to inhibit the formation of plaques to the same extent as Remdesivir, demonstrating low micromolar EC_{50}s. The assay was repeated using a second cell line (Calu3 cells), with similar results.

The third assay measured the ability of the compounds to inhibit replication of SARS-CoV-2 in cells infected with the virus. As in the first assay, compounds were tested in triplicate across seven concentrations of each compound. The readout was the direct measurement of the amount of virus present using a quantitative reverse-transcritpase polymerase chain reaction assay (qRT-PCR) which provides a very sensitive measurement of the level of virus present. The approved antiviral drug Remdesivir was included as a positive control. The three compounds were all able to inhibit replication of the virus to the same level or better than Remdesivir, demonstrating low micromolar EC_{50}s.

All three assays generated a dose-response curves showing inhibition of the virus by each of the three compounds. The three compounds performed as well as Remdesivir in inhibiting the virus in two assays in which the approved drug was included as a positive control.

These three compounds are now being assessed for their suitability for progression to the next stage of development through a series of *in vitro* safety studies including bioavailability studies in mice. These studies will provide key information to support dosing in animal model(s) of SARS-CoV-2 infection.

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.

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

Enquiries

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