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14 November 2025

The Manager Companies
ASX Limited
20 Bridge Street
SYDNEY NSW 2000 (3 pages by email)

Dear Madam,

HEPATITIS B VIRUS PROGRAM UPDATE

The Directors of Biotron Limited ('Biotron' or 'the Company') (ASX: BIT) are pleased to provide an update on the Biotron's Hepatitis B Virus (HBV) program, as summarised below:

- BIT-HBV001, Biotron's lead HBV drug, shows anti-HBV activity in two different mouse models of HBV infection,
- BIT-HBV001 shows superior anti-HBV activity to Tenofovir, the first-line treatment for HBV, against specific replication markers in i*n vitro*, cell-based assays of HBV infection,
- BIT-HBV001 improves the anti-HBV activity of Tenofovir when used in combination in an *in vitro*, cell-based combination (synergism) study with Tenofovir,
- Filing of a patent application relating to a series of novel, undisclosed small molecule compounds, including BIT-HBV001, for treatment of serious viral infections including HBV.

Biotron has previously reported that it has novel compounds with strong antiviral activities *in vitro* against markers of HBV replication in a variety of experimental cell lines as well as primary human hepatocytes.

In June 2025 the Company reported the completion of the first stage of a series of studies in two mouse models that are commonly used to study liver diseases and infection, with its lead anti-HBV drug (BIT-HBV001). The results, reported at that time, demonstrated the safety of the drug in mice and allowed for the calculation of the correct dosage of drug in further mouse studies.

In the second stage, which is now complete, the antiviral activity of BIT-HBV001 was assessed in the two mouse models. The first model (Tg05-C57Bl/6) utilises mice that have

been genetically engineered to express HBV in the liver; the second model (MUP-uPA-SCID/Beige-Balb/c) utilises mice that have been engrafted with human hepatocytes so can be infected with HBV and produce infectious virus.

The latest results, generated at the SCRIPPS Research Institute, La Jolla, CA, USA, confirm the antiviral activity of BIT-HBV001 *in vivo*.

In both models, mice (in triplicate) were dosed with drug every 10 hours for 16 days, after which liver and blood samples were collected and analysed for levels of specific HBV markers.

The data from the Tg05 model demonstrated that Biotron's HBV drug significantly reduced HBV DNA levels (~65% decrease) by day 16. However, levels of HBsAg - another HBV marker – remained unchanged.

The data from the MUP-uPA model demonstrated that Biotron's HBV drug once again significantly reduced HBV DNA levels (`62% decrease). In this model levels of HBsAg were also significantly reduced (~ 48% decrease).

The reduction of both HBV DNA and HBsAg in the MUP-uPA mice, in contrast to only reducing HBV DNA in the Tg05 mice, reflects the mode of action of Biotron's unique HBV compounds.

A follow-up *in vitro* antiviral activity cell-based study was then performed to compare BIT-HBV001 with Tenofovir (TDF), the current first-line treatment for HBV, against five (5) HBV replication end points. Cells (NTCP+HepG2) were pre-treated for one hour with a series of 2-fold serial dilutions of either BIT-HBV001 or TDF in triplicate, infected with HBV, incubated for 13 days and then analysed for HBV replication end points.

The results of this *in vitro* study demonstrated that BIT-HBV001 was able to strongly inhibit all five HBV replication end points (HBV DNA 99.97% inhibition, HBsAg 97% inhibition, HBeAg 97.6% inhibition, cccDNA 77.3% inhibition, pgRNA 99.4% inhibition).

In contrast, TDF was only able to inhibit HBV DNA (99.9% inhibition); it was inactive or very mildly active (less than 7%) against all other HBV replication end points.

Tenofovir, while the current standard of care treatment for chronic HBV, has limitations in that it does not cure or eradicate HBV. Rather, it suppresses the virus and requires ongoing treatment to prevent virus break through. **The inhibition of cccDNA by BIT-HBV001 suggests a role in eradication of the virus and is a strong differentiating factor for this new class of HBV drug.**

An *in vitro* cell-based combination study of BIT-HBV001 with TDF was then performed to determine whether there was any additional benefit in combining the two drugs. NTCP+HepG2 cells were pre-treated for one hour with a checkboard of 2-fold serial dilutions of BIT-HBV-001 and TDF, infected with HBV, incubated for 13 days, and then analysed for HBV replication end points.

The results demonstrated a high degree of synergy for the HBV DNA end point when the

two drugs were combined. **This indicates that lower amounts of each drug can be used in combination achieve the same level of HBV inhibition.**

As expected there was no synergism with regard to the other end points, as TDF is inactive against them. However, TDF did not antagonise (negatively impact) on BIT-HBV001's strong activity against those other end points.

Michelle Miller, Biotron's Managing Director, said: "These latest animal and cell-based study results significantly advance Biotron's very promising HBV program. This was an extensive series of experiments with our best HBV drug against a complex challenging virus. We now have a clearer understanding of the mechanism of action of this new class of drug, which has demonstrated advantages over the current standard of care first-line treatment. The inhibition of cccDNA, a marker of HBV persistence by BIT-HBV001, is particularly encouraging."

A patent has been filed for BIT-HBV001 and associated compounds, for their composition (structures), method of manufacture, and use.

Chronic infection with HBV can lead to complications such as cirrhosis and liver cancer, which cause close to one million deaths worldwide each year. Over 2 billion people worldwide have been infected with HBV. The World Health Organisation estimates that over 250 million are chronically infected. There remains an unmet need for HBV cure, which will require new drugs that stop persistent viral infection.

Yours sincerely

Marcelo Mora
Company Secretary

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This announcement has been approved by the Company's Board of Directors.

Enquiries

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