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ASX:NSB

ASX ANNOUNCEMENT
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EMTINB™ DEMONSTRATES A POSITIVE TREATMENT EFFECT IN MULTIPLE SCLEROSIS ANIMAL STUDY

HIGHLIGHTS

- **NeuroScientific's lead peptide candidate EmtinB™ has demonstrated a statistically significant positive treatment effect in a Gold Standard MOG-EAE induced model of Multiple Sclerosis**
- **All EmtinB™ dose levels showed an initial delay in the progression of EAE clinical symptoms displaying a likely neuroprotective effect**
- **EmtinB™ administered at 10mg/kg resulted in a statistically significant positive treatment effect in EAE Clinical Scores**
- **EmtinB™ administered at 10mg/kg and 20mg/kg resulted in a statistically significant treatment effect in the maintenance of body weight**

NeuroScientific Biopharmaceuticals Ltd (ASX: **NSB**) (**"NeuroScientific"** or **"the company"**), a clinical-stage drug development company, is pleased to announce positive results of lead drug candidate EmtinB™ in a gold standard animal model of Multiple sclerosis (MS). The study was undertaken by leading contract research partner Biospective, Canada.

Study Design

The study was conducted in the myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (MOG-EAE) mouse model, the gold-standard animal model for replicating the inflammatory mechanisms of human MS. The study evaluated EmtinB™ across three dose groups, including 10mg/kg (Group B) & 20mg/kg (Group C) dosed daily, and an exploratory group of 40mg/kg dosed once every 3 days (Group D) with the drug administered for a period of 21-days following the onset of initial clinical symptoms in the mice.

Treatment effect was determined through comparison to the control group (Group A) which were animals that were disease induced (MOG-EAE), but were administered vehicle only (placebo) on a daily basis for the 21 day treatment period duration, acting as a negative control.

The study was appropriately powered to provide confidence in results with N=16 animals in each group for Groups A-C, with the exploratory Group D inclusive of N=10 animals only.

Key Clinical Assessments performed

(a) The daily assessment of clinical symptoms for the duration of the study (21 days) was performed using a standardised scoring system (Table 1). Higher scores mean more severe symptoms. Day-on-day EAE Clinical Score and Cumulative EAE Clinical Scores were analysed. With the induction of the model there is an aggressive and progressive deterioration in the EAE Clinical Score.

Table 1: EAE Clinical Score Standardised Definitions

Clinical Score	Description
0	No clinical symptoms
0.5	Loss of tone in the tip of tail
1	Complete loss of tail tone (floppy tail)
1.5	Floppy tail and weak hind limbs
2	Floppy tail, clasping of hind limbs when lifted, and weakness of hind limbs when walking
2.5	Floppy tail, clasping of hind limbs when lifted, and partial paralysis of hind limbs when walking
3	Floppy tail and complete hind limb paralysis
3.5	Floppy tail, complete hind limb paralysis, and unable to right when overturned
4	Limp tail, complete hind limbs paralysis, unable to right, and partial front leg weakness/paralysis
4.5	Complete hind and partial front leg paralysis, no movement around the cage. >20% weight loss
5	Moribund (euthanatized)

(b) Body Weight was measured on a daily basis, with body weight variation from baseline being analysed. On induction of the model, body weight typically declines, with the worsening of EAE Clinical Scores.

Mechanism of Action (Biomarker) Assessments

(a) Analysis of concentrations of neurofilament light polypeptide (NfL) in cerebrospinal fluid (CSF) and plasma at the terminal time point following the 21-day treatment period.

(b) Quantitative assessment of the expression of myelin basic protein (detected using the antibody MBP), activated T cells (detected using the antibody CD3), microglia and macrophages (detected using the antibody Iba-1) and astrocytes (detected using the antibody GFAP) in tissue sections from spinal cords at the terminal time point following the 21-day treatment period.

EmtinB™ treated mice display a likely neuroprotective effect through the delay in the initial progression of EAE Clinical Scores

Mice treated at all dose levels displayed an initial delay in the progression of worsening EAE Clinical Scores. As visually clear in **Figure 1**, note A, we see EmtinB™ treated Groups B to D show a rightward shift in the early days of dosing, indicating a delay in progression of induced symptoms when compared against Group A (negative control). This delay is statistically significant in the day-to-day analysis at Day 1 for Groups B ($p= 0.0008$) and C ($p= 0.0004$) and in Group D at Day 1 ($P=0.0023$) and Day 2 ($p<0.0001$).

This delay was further confirmed in the Cumulative EAE Clinical Score values, see in **Figure 2** note A, where significant difference from Group A is seen at Day 1 and 2 in Group B ($p<0.0092$) and Group C ($p<0.03$). In Group D we see a longer delay period in symptom progression when compared to negative control with significant effect seen from Day 1 through Day 7 in cumulative data (daily p range from 0.0001 to 0.0402).

This delay in the worsening of EAE clinical scores in the early stage of treatments is most likely indicative of a neuroprotective effect caused by EmtinB™.

10mg/kg EmtinB™ daily dosing displays a treatment effect through an overall improvement in EAE Clinical Scores for latter period of treatment

Mice treated with 10mg/kg EmtinB™ daily (Group B) had a day-on-day statistically significant difference noted in EAE Clinical Scores from Day 10 through Day 21 (final day) of dosing when compared to the negative control, Group A. Period of statistical significance can be seen in **Figure 1** note B, with significance p value range of <0.0003 and 0.013. This substantial time period of significance is noted as a positive treatment effect.

This positive treatment effect in Group B (10mg/kg) is also shown in **Figure 2** note B, where cumulative EAE Clinical Scores are improved on the control Group A, with significance seen from Day 14 through Day 21 (completion of treatment period) with significance p value range of 0.0088 and 0.0497.

A trend for a similar treatment effect was noted in Group C (20mg/kg) but no days in the latter treatment period met statistical significance. The 40mg/kg dosed every 3 days (Group D) did not show any treatment effect, which is consistent with previous *in vivo* investigations, where there is a reduction in efficacy at higher dose levels.

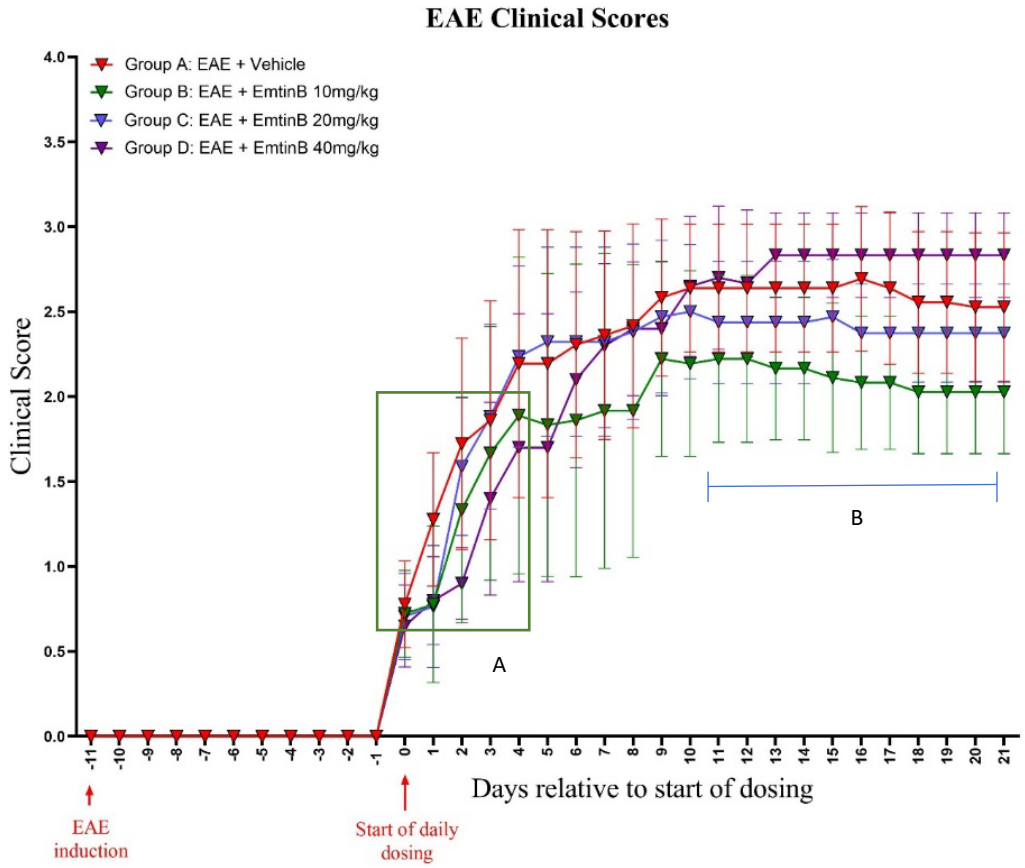

Figure 1 - Daily EAE Clinical Scores.

A: range of protective effect; B: Treatment effect from Day 14 through Day 21 study completion in Group B

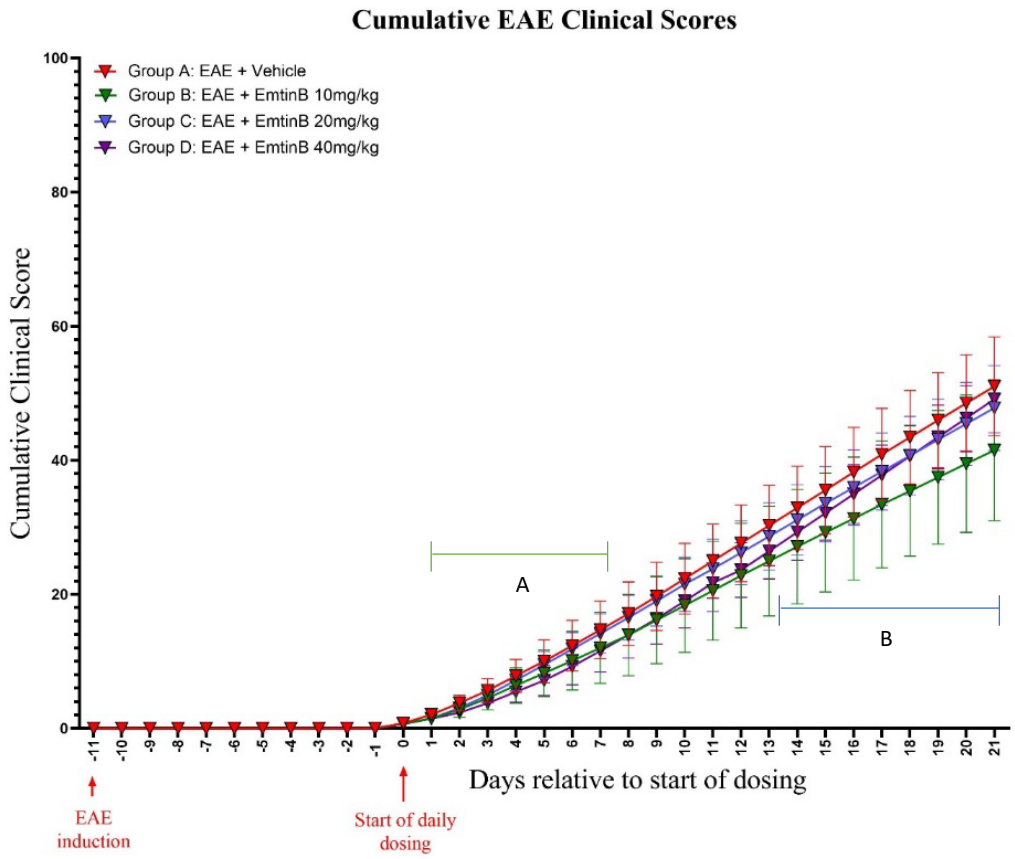

Figure 2- Cumulative EAE Clinical Scores

A: range of protective effect; B: Treatment effect from Day 14 through Day 21 study completion in Group B

EmtinB™ administered at 10mg/kg and 20mg/kg resulted in a statistically significant positive effect in the maintenance of body weight

Body weight change is a key feature of this animal model, with a decline in body weight typical with decline in clinical symptoms of the animals.

As visually depicted in **Figure 3,** there is a clear decline in body weight in the negative control group A (red), with no clear difference with Group D which was dosed 40mg/kg every 3 days. However we see a prolonged maintenance of body weight in the 10mg/kg (Group B, green) for the duration of the treatment period, with a statistically significant difference compared to the negative control, seen from Day 4 through the completion of treatment (Day 21) with a p value range from <0.0001 to 0.0138.

A statistically significant difference in body weight variation between the 20mg/kg (Group C) and the negative control (Group A) was seen on Day 1 and from Day 7 to Day 18 with a p value range of 0.03 and 0.004, and when in the absence of significance, there is a trend of improvement on the other treatment days.

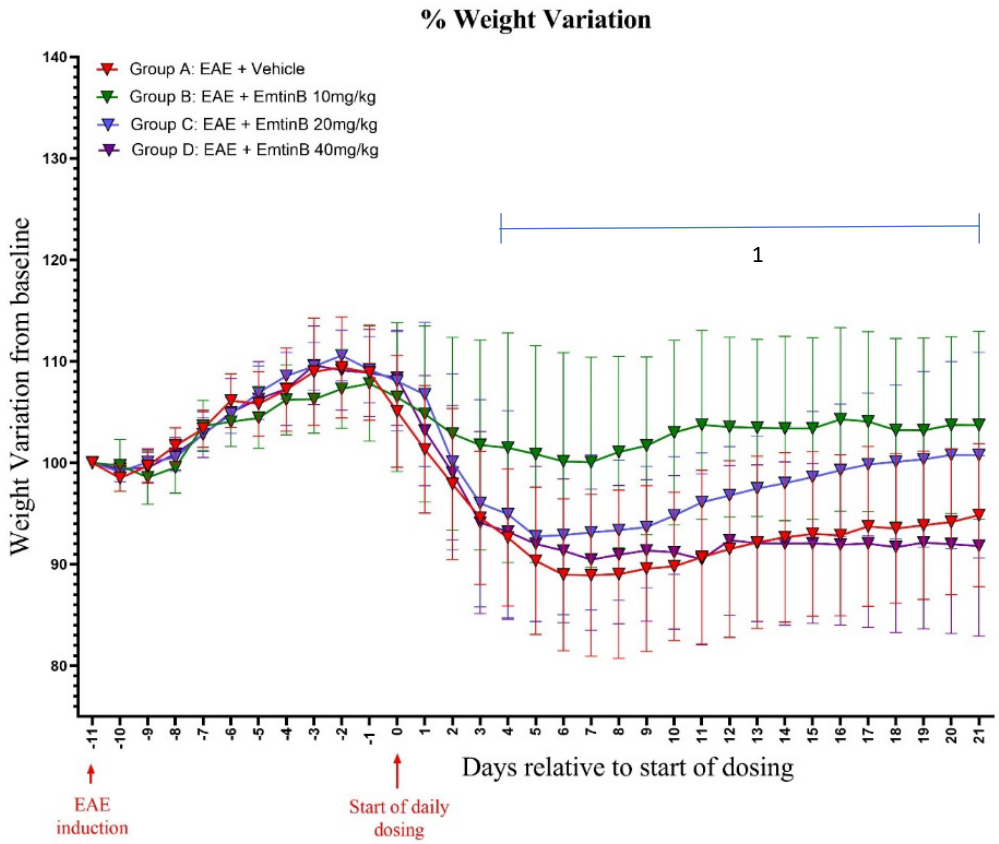

Figure 3 -Percentage Weight Variation from Baseline

1. Period of statistically significant results at the 10mg/kg Group B

Mechanism of Action Assessments

All assessments performed for mechanism of action (biomarkers) were only able to be collected on termination of the animal, thus all assessments for this study were performed following the completion of the 21-day treatment period.

Unfortunately, there was not a clear correlation of the biomarkers evaluated to the significant treatment effect seen clinically, as noted above.

The biomarkers that are included in this assessment are very dependent on the mechanism of action of the drug under investigation and the state of disease at the time of assessment. Following discussion internally and with the contract research provider it was determined that the lack of correlation is most likely due to a late collection of samples, and that a correlation may have been seen if samples were collected at a midpoint in the treatment period. This midpoint collection was not possible in this initial study (as NSB wanted to ensure power of the study through to 21 days), but NSB will determine if a future study may provide a more complete understanding of the mechanism of action of EmtinB™. NSB has previously disclosed information relating to *in vitro* biomarker investigations confirming the likelihood of neuro-inflammation modulation as well as *in vitro* work confirming axon regeneration and protection and other data that speaks to the mechanism which may explain the clinical benefits seen within this study.

NeuroScientific's Interim CEO and Chairman, Paul Rennie commented: *"NSB is very pleased to report this data demonstrating that EmtinB™ had a statistically significant positive clinical effect in this industry standard preclinical model of MS. This preclinical data will be used to complete the efficacy section for NSB's planned submission for its first in human Phase I clinical trial. Further progress has also been made in the safety and purity sections for the HREC submission. NSB management is focused on preparing its HREC submission by late Q2 or Q3 CY 2023."*

Conclusion

Following review of the final report from this study in the Gold Standard animal model of Multiple Sclerosis, NSB is very pleased with the significant treatment effect seen in the EAE Clinical Scores and the maintenance of body weight, particularly in the 10mg/kg group, providing confirmation of a likely target therapeutic dose range of 10-20mg/kg in a mouse (equivalent to an adult human dose range of 50mg to 115mg). NSB believes that this study further supports EmtinB™ acting as a neuroprotective agent and treatment for neurodegenerative disease, in particular Multiple Sclerosis.

This announcement is authorised by the Board of NeuroScientific Biopharmaceuticals Ltd.

-ENDS-

For more information please contact:

Paul Rennie
Interim CEO and Chairman
prennie@neuroscientific.com
+ 61 8 6382 1805

Abby Macnish Niven
CFO & Company Secretary
ir@neuroscientific.com
+ 61 8 6382 1805

About NeuroScientific Biopharmaceuticals Ltd

NeuroScientific Biopharmaceuticals Limited (ASX: NSB) is a company developing peptide-based pharmaceutical drugs that target a number of neurodegenerative conditions with high unmet medical demand. The company's product portfolio includes EmtinB™, a therapeutic peptide initially targeting Alzheimer's disease and glaucoma, as well as other Emtin peptides (EmtinAc, EmtinAn, and EmtinBn) which have demonstrated similar therapeutic potential as EmtinB™. For more information, please visit www.neuroscientific.com

About EmtinB™

EmtinB™ is a peptide-based compound that binds to surface-based cell receptors from the LDLR family, activating intracellular signalling pathways that stimulate neuroprotection, neuroregeneration and modulate neuroinflammation. EmtinB™ is modelled on a specific active domain of the complex human protein called Metallothionein-IIA, which is produced as part of the human body's innate immune response to cell injury.

Our preclinical research has established that EmtinB™ is highly specific and selective for its target receptor, safe and well tolerated at high concentrations, and is able to penetrate the blood brain barrier. A series of Phase I clinical studies will be conducted to establish the safety profile of EmtinB™ in humans.