POSITIVE PRECLINCAL DATA ON EFFICACY OF ARG-007 IN NEW INDICATION – GLOBAL CEREBRAL ISCHAEMIA

Highlights:

- *ARG-007 reduces brain cell death in CA1 hippocampal neurons compared with saline injected controls in a four-vessel occlusion (4-VO) animal model of global cerebral ischaemia.*

- *The data provides additional evidence of the neuroprotective potential of ARG-007 in new indications of cerebral ischaemia following cardiac arrest and cardiac surgery.*

- *Results from this study will further support the efficacy data package for the Phase 1 clinical trial.*

Perth, Australia; 30 MARCH 2022 - Argenica Therapeutics Limited (ASX: AGN) ("Argenica" or the "Company"), is pleased to announce positive results from a preclinical study assessing the efficacy of ARG-007 in protecting brain cells following blood flow disruption to the brain (cerebral ischaemia), as seen following cardiac arrest and certain cardiac surgeries.

The preclinical study found that ARG-007, administered after disruption of blood flow to the brain utilising a four-vessel occlusion (4-VO) rat model, reduced cell death in hippocampal neurons (CA1 neurons) in the brain. The data was compared to un-injured (sham) animals and injured (4-VO) animals who received saline as the treatment (vehicle). These results provide further evidence of ARG-007's neuroprotective capabilities in a new therapeutic area of global cerebral ischaemia, which can occur following cardiac arrest or from certain cardiac surgeries where cardiac output is decreased.

The 4-VO rat model is used by researchers to mimic the pathological conditions of blood flow disruption to the brain, and subsequent brain injury, that can follow a cardiac arrest or certain cardiac surgeries. The 4-VO model occludes the key arteries leading to the brain, thereby disrupting blood flow to the brain, resulting in global cerebral ischaemia. This model is used to replicate brain injury caused by blood flow disruption to the brain, and the subsequent and sudden restoration of blood flow to the brain once cardiac rhythm is restored. This cerebral ischaemia, and subsequent restoration of blood flow, can cause significant brain injury and cognitive impairment in patients.

Despite the risks and impact of cerebral ischaemia following cardiac arrest and in certain cardiac surgeries, there is no established neuroprotective standard of care for the treatment and management of global cerebral ischaemia[1].

Chief Executive Officer, Dr Liz Dallimore said: "We are very encouraged by these results on the efficacy of ARG-007 in the 4-VO animal model. Brain injury resulting from a reduction in blood flow to the brain following cardiac arrest and cardiac surgery can result in long-term neurological deficits in these patients. This data provides further confirmation that our lead candidate, ARG-007, can provide neuroprotection in applications beyond focal stroke, HIE and TBI. We will now consider ways to progress the results of this study into clinical studies."

FURTHER INFORMATION ABOUT THE STUDY

Argenica commissioned MD Biosciences Neuroscience Discovery Services, a preclinical contract research organisation that specialises in central nervous system (CNS) drug development, to undertake an assessment of ARG-007's efficacy in a four-vessel occlusion (4-VO) animal model, which mimics cerebral ischaemia, or disruption of blood flow to the brain, that can occur following cardiac arrest and certain cardiac surgeries.

This study aimed to determine whether a dose of 300 nmol/kg of ARG-007, as previously shown to be efficacious in preclinical animal stroke models[2,3], would also prove efficacious at reducing neuronal cell death, and therefore a potential neuroprotective therapeutic, in an animal model of global ischaemia that mimics brain injury following cardiac arrest or cardiac surgery.

4 VESSEL OCCLUSION (4-VO) – GLOBAL CEREBRAL ISCHAEMIA – MODEL

The 4-VO rat model has been widely employed by researchers to investigate the mechanisms which cause brain damage after a reduction in blood flow to the brain. This 4-VO model in the rat induces transient forebrain ischaemia, and subsequent neuronal cell death in the CA1 hippocampus region of the brain. The 4-VO rat model has been widely used to test the effects of neuroprotective drugs on neuronal cell death.

Specifically, the 4-VO rat model involves permanent occlusion of the two vertebral arteries and transient occlusion of the two carotid arteries. This vessel occlusion mimics the reduction in blood flow, or cerebral ischaemia, following cardiac arrest, coronary bypass surgery, aorta repair, and other cardiac surgeries. The reduction in cerebral blood flow results in brain injury in the forebrain, specifically hippocampal damage and presents as a loss of CA1 neurons in the hippocampus region of the brain -which plays a major role in learning and memory.

[1] Katz, A., Brosnahan, S. B., Papadopoulos, J., Parnia, S., & Lam, J. Q. (2022). Pharmacologic neuroprotection in ischemic brain injury after cardiac arrest. *Annals of the New York Academy of Sciences*, *1507*(1), 49–59.

[2] Milani, D., Clark, V. W., Feindel, K. W., Blacker, D. J., Bynevelt, M., Edwards, A. B., Anderton, R. S., Knuckey, N. W., & Meloni, B. P. (2021). Comparative Assessment of the Proteolytic Stability and Impact of Poly-Arginine Peptides R18 and R18D on Infarct Growth and Penumbral Tissue Preservation Following Middle Cerebral Artery Occlusion in the Sprague Dawley Rat. *Neurochemical research*, *46*(5), 1166–1176.

[3] Meloni BP, South SM, Gill DA, Marriott AL, Déziel RA, Jacques A et al (2019) Poly-arginine peptides R18 and R18D improve functional outcomes after endothelin-1-induced stroke in the Sprague Dawley rat. J Neuropathol Exp Neurol 78(5):426–435

The study demonstrated the extent of hippocampal neuronal cell death (CA1 neurons), using hematoxylin and eosin (H&E) staining, following cerebral ischaemia and subsequent treatment with either saline (vehicle) or a single intravenous dose of ARG-007 (treatment).

STUDY DESIGN

Animals were assigned to either:

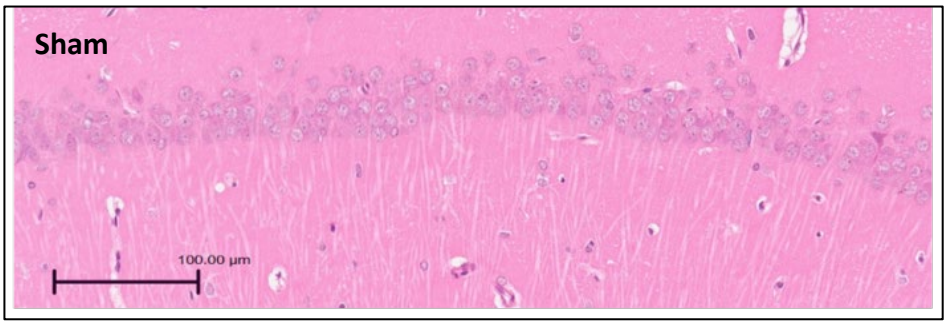
- the sham (un-injured non-ischaemic) group, whereby they underwent surgery but did not have their vertebral and carotid arteries occluded and were not given saline or ARG-007;
- the vehicle group (4-VO plus saline), whereby they underwent surgery, had their vertebral and carotid arteries occluded and were given a placebo (saline); or
- the treatment group (4-VO plus 300 nmol/kg ARG-007), whereby they underwent surgery, had their vertebral and carotid arteries occluded and were given 300 nmol/kg of ARG-007.

For the vehicle and treatment groups, following the vessel occlusion surgery and subsequent reperfusion of the carotid arteries, either ARG-007 or saline was administered intravenously over 10 minutes in a volume of 1 ml/kg, and the rats left to recover. This was deemed study day 1. On study day 8 the brain tissue was processed for histological staining with H&E, which is used for visualising neurons. Normal neurons at CA1 region of hippocampus were counted using AI techniques (see below).

RESULTS

Viability of CA1 Neurons

Hippocampal cell (CA1 neurons) death was assessed using H&E staining histology as shown in histological sections (slides) of the hippocampus below.



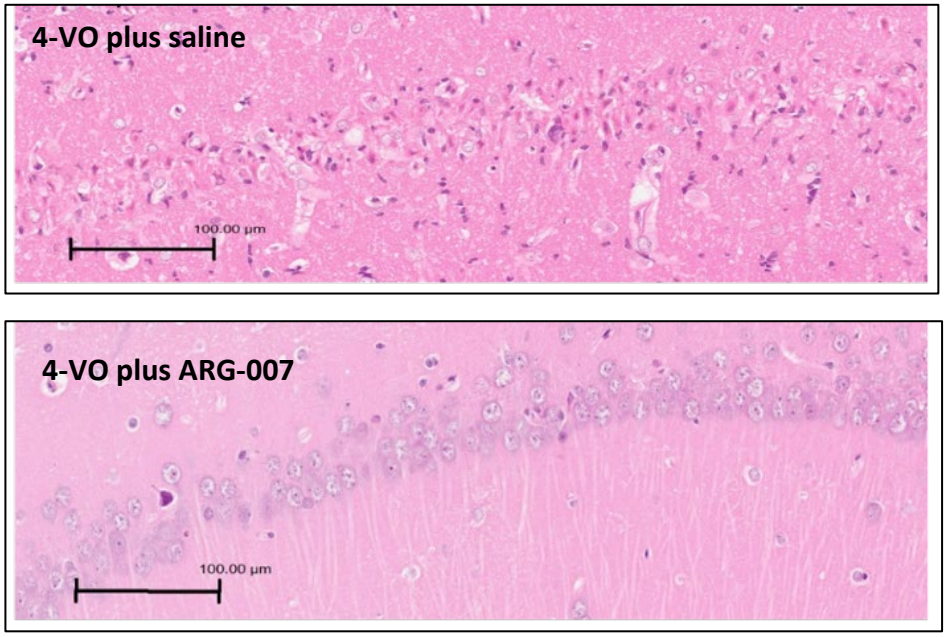


Figure 1. H&E histology of CA1 neurons in the hippocampus.

Following H&E staining, the slides were scanned using a high-resolution digital scanner (DP-200 by Roche). The digital files were analysed using an artificial intelligence (AI) software (STUDIO™ by Deepathology) and the viable neurons in the CA1 region of the hippocampus were counted and their number was normalized according to the area.

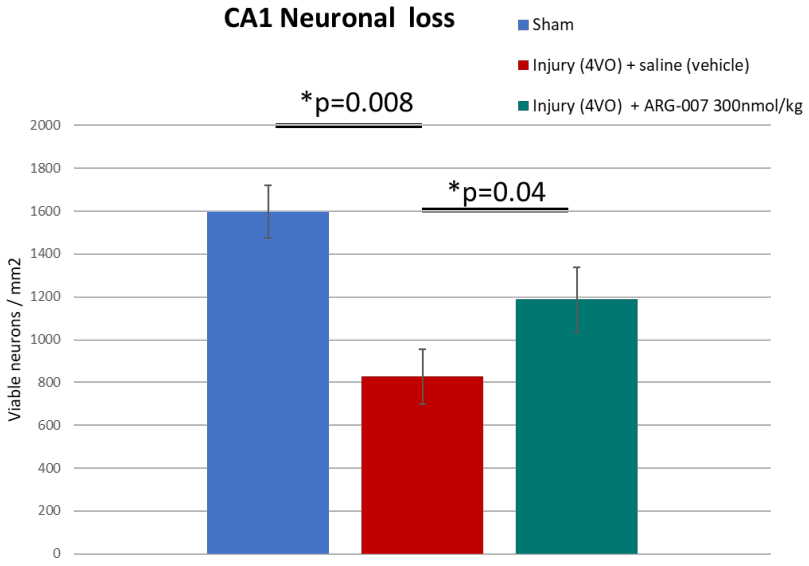


Figure 2. CA1 neuronal viability in sham (n=5), injury + saline (vehicle control) (n=10) and injury + 300 nmol/kg ARG-007 (n=11). Students t-test (two-sample assuming equal variances; one-tailed at α=0.05)

The mean number of viable CA1 neurons (number / mm^2) was statistically significantly reduced ($p<0.05$ t-test) in the vehicle group (injury plus saline) compared with the sham group (see Figure 2 above, p=0.008). Hence, the 4-VO surgery caused a demonstrable loss of CA1 neurons. Treatment with ARG-007 at a dose of 300 nmol/kg showed an increase in the mean number of viable CA1 neurons (number / mm^2) compared with vehicle group (see Figure 2 above, p=0.04). This indicates that following cerebral ischaemia a single dose of ARG-007, can protect CA1 neurons in the hippocampus from dying.

CONCLUSION

The current study shows that treatment with a single dose of ARG-007 following global cerebral ischaemia, using the 4-VO animal model, protects CA1 hippocampal neurons from cell death when compared to saline controls. This preclinical study provides evidence of the neuroprotective properties of ARG-007 in a global cerebral ischaemia model, indicating the potential use of ARG-007 as a neuroprotective therapeutic following cardiac arrest or to protect against neuronal cell death as a result of various cardiac surgeries. The data will also be used to provide further supporting evidence of the neuroprotective efficacy of ARG-007 as the Company begins its Phase 1 clinical trial to assess the safety of ARG-007 in healthy human volunteers. The Company will now explore the potential application of ARG-007 as a neuroprotective therapeutic for global cerebral ischaemia following cerebral blood flow disruption from cardiac arrest and certain cardiac surgeries and consider ways to progress this research into clinical trials.

This announcement has been approved for release by the Board of Argenica

For more information please contact: info@argenica.com.au

ABOUT ARGENICA

Argenica (ASX: AGN) is developing novel therapeutics to reduce brain tissue death after stroke and improve patient outcomes. Our lead neuroprotective peptide candidate, ARG-007 has been successfully demonstrated to improve outcomes in pre-clinical stroke models and is in the process of being verified for its safety and toxicity before commencing Phase 1 clinical trials in humans. The aim is for our therapeutic to be administered by first responders to protect brain tissue against damage during a stroke with further potential to enhance recovery once a stroke has taken place.