# ARG-007 PROVIDES PROLONGED REDUCTION OF BRAIN INJURY IN LATEST PRECLINICAL HYPOXIC ISCHAEMIC ENCEPHALOPATHY STUDY

**Highlights:**

- *ARG-007 shown to significantly reduce brain injury caused by both ischemia (reduced blood flow) and vasogenic oedema (brain swelling) in a full-term-equivalent animal model of hypoxic-ischaemic encephalopathy (HIE).*

- *Data shows a 300nmol/kg dose of ARG-007 maintained over a **70% reduction** in total brain injury **from one week out to four weeks post injury**.*

- *Doses of 100 and 300 nmol/kg of ARG-007 also **reduced vasogenic oedema** (brain swelling) at 48 hours post injury by 35.4% and 32.9% respectively.*

- *Argenica is currently completing preclinical studies required to initiate an Investigational New Drug Application with the US Food and Drug Administration (FDA). The efficacy studies are funded by a recent Stan Perron Charitable Foundation Grant.*

**Perth, Australia; 20 April 2023 -** Argenica Therapeutics Limited (ASX: AGN) ("Argenica" or the "Company"), a biotechnology company developing novel therapeutics to reduce brain tissue death after brain injury, is pleased to announce the latest positive preclinical data in hypoxic ischaemic encephalopathy (HIE); also referred to as perinatal asphyxia or perinatal hypoxia-ischaemia, showing the effect of a single dose of ARG-007 lasting out to four weeks.

**TOTAL BRAIN INJURY EFFICACY**

Argenica has previously demonstrated ARG-007 reduces neuronal cell death caused by ischaemia out to 48 hours post the reduction in blood flow to the brain in a term animal model of HIE (see announcement dated 29 September 2022). The present study examined the efficacy of a single dose of ARG-007 in the same model of HIE when brain injury was assessed at timepoints beyond 48h, namely 1 week, 2 weeks, 3 weeks, and 4 weeks, to determine whether the effect of ARG-007 was temporary or prolonged, the importance of which is discussed later in this announcement.

The study utilised clinically relevant magnetic resonance imaging (MRI) to quantify the extent of total brain injury in both ARG-007 treated HIE animals, and saline treated HIE control

animals. At time points beyond 48 hours, MRI detects total brain injury caused by combined ischaemia induced neuronal cell death and vasogenic oedema (accumulation of fluid in the extracellular space in the brain) induced neuronal cell death.

This preclinical study was conducted in a rat model of HIE equivalent to a term infant (human gestational age ≥37 weeks). The study showed ARG-007 **significantly reduced total brain injury** caused by combined ischaemia and vasogenic oedema **at all time points assessed**.

The study, conducted by Dr Adam Edwards at the Perron Institute for Neurological and Translational Science (the Perron Institute), assessed the efficacy of ARG-007 at 3 different doses (100, 300 and 600 nmol/kg) compared with saline placebo groups. The 300nmol/kg dose was the most efficacious, resulting in a **73.96% reduction in total brain injury at 1 week** which was maintained out to 4 weeks after HIE (**70.3% reduction at 4 weeks**, see figure 1 below).

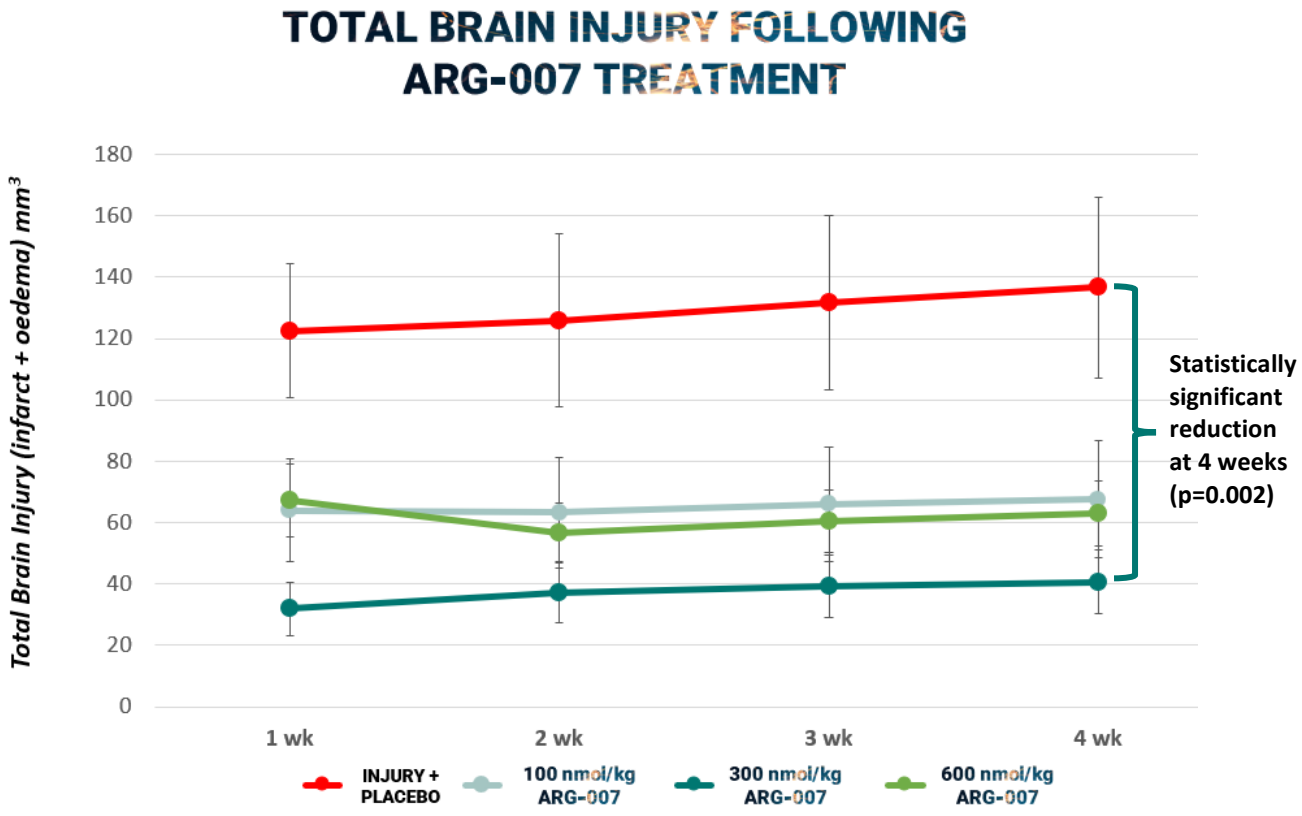


**Figure 1.** Total Brain Injury (infarct volume from ischaemia and vasogenic oedema, $mm^3$) in control (injury + saline) animals and treatment (ARG-007) animals. Following injury (ischaemia-hypoxia), treatment animals received ARG-007 at varying doses (nmol/kg). Following treatment, the 300 nmol/kg ARG-007 treated groups showed a significantly greater reduction in total brain injury compared with the saline control group (p=0.002).

## EFFICACY ON VASOGENIC OEDEMA

To assess whether ARG-007 had a specific effect on vasogenic oedema, the volume of vasogenic oedema was assessed in a term rat model of HIE using MRI at 48 hours post HIE insult. Vasogenic oedema is a key symptom of HIE insult in the early stages and can lead to later stage neuronal cell death. Vasogenic oedema occurs when the blood brain barrier is compromised, resulting in protein-rich fluid accumulating in the extracellular space around brain cell, causing brain swelling. This can then convert to neuronal cell death in areas of the brain outside the area of the initial infarct, leading to further brain injury. Therefore, any reduction in vasogenic oedema in the first 48 hours will result in more salvageable brain tissue and better functional outcomes.

A single dose of ARG-007 (either 100, 300 and 600 nmol/kg) was administered post HIE injury. The data shows ARG-007 administered at doses of 100 and 300 nmol/kg resulted in statistically **significant reductions in vasogenic oedema volume of 35.4% and 32.9%** respectively (see figure 2 below) at 48 hours post HIE.

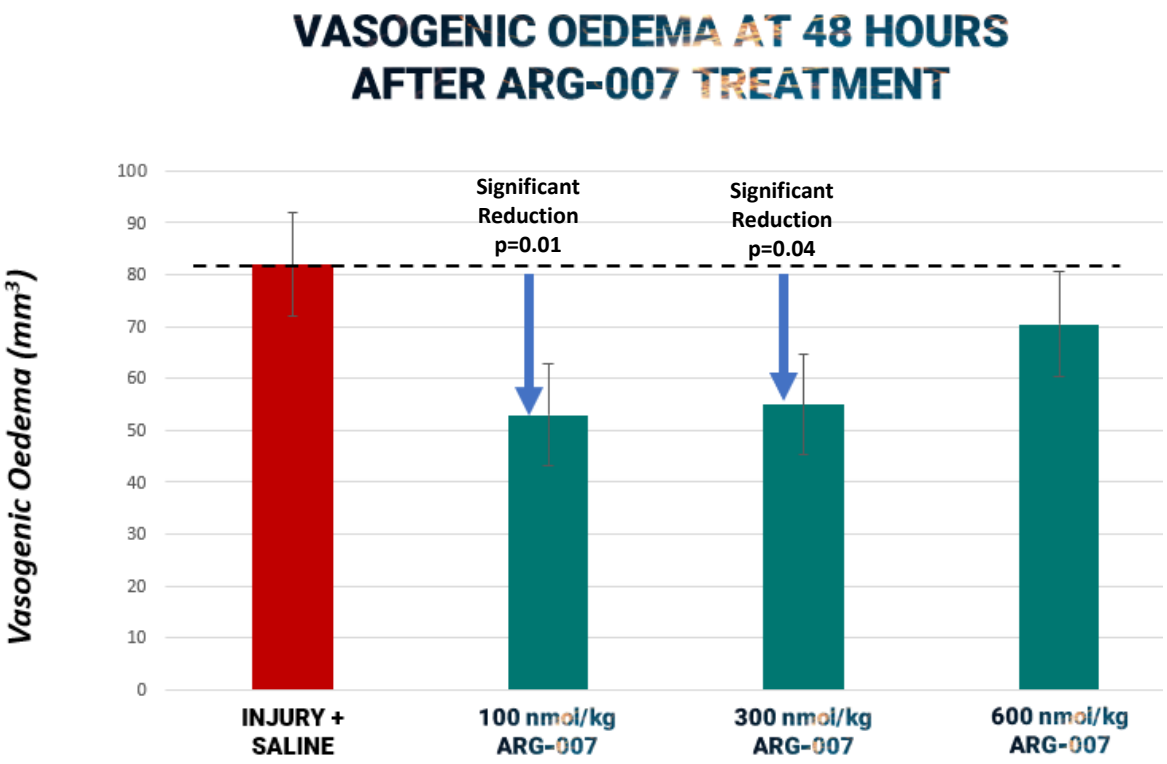


**Figure 2.** Vasogenic oedema ($mm^3$) in control (injury + saline) animals and treatment (ARG-007) animals. Following injury (ischaemia-hypoxia), treatment animals received ARG-007 at varying doses (nmol/kg). Following treatment, the 100 nmol/kg and the 300 nmol/kg ARG-007 treated groups showed a significantly greater reduction in total vasogenic oedema compared with the saline control group (p=0.01 and 0.04, respectively).

The data showing the reduction in vasogenic oedema at 48 hours post HIE (Figure 2), combined with the 48-hour infarct volume data showing a 62% and 86% reduction in infarct volume in the 100 and 300 nmol/kg doses respectively (announced on 29 September 2022), confirms the broader therapeutic effect of both the 100 and 300 nmol/kg doses of ARG-007 in a term model of HIE at 48 hours post the HIE event.

**IMPORTANCE OF PROLONGED PROTECTION AGAINST NEURONAL CELL DEATH IN HIE**

It is important to note that HIE is **not a single event** related to the hypoxic-ischaemia (HI), or a reduction in oxygen and/or blood flow, but rather an ongoing process causing neuronal cell death over hours to days after the initial HI event. The neuronal cell death can continue to occur even after blood flow is restored to the brain. There are three distinct phases of neuronal cell death following HI that can last out to days post the initial HI insult. The first phase is caused by the reduction in blood flow to the brain, resulting in the accumulation of toxins, metabolic failure of neurons, and oedema (brain swelling) which leads to neuronal cell death. This sets off the primary neuronal cell death, or initial infarct.

Once blood flow is restored in these babies, there is then a latent phase whereby neuronal cell death stabilises – this phase lasts approximately six hours. The third phase of cell death then occurs, starting between six and 15 hours post the HI event and can last days. This phase is characterised by overactivation of glutamate receptors which starts a cascade of neurotoxicity, leading to significant neuronal cell death[1]. This third phase is associated with marked encephalopathy (brain damage). Neuroprotective strategies are aimed at protecting brain cells from this third and prolonged wave of neuronal cell death. Therefore, a neuroprotective drug must have a prolonged effect to give the brain the best chance of protection against this third wave of cell death.

Argenica's Managing Director, **Dr Liz Dallimore said**: "The preclinical data we are generating on the efficacy of ARG-007 in HIE is extremely encouraging. Whilst HIE is a rare paediatric condition, it has devastating outcomes for these babies, and a treatment is desperately needed. The fact that ARG-007's effect in this preclinical model lasts out to 4 weeks indicates the treatment effect is sustained, protecting against the devastating third wave of brain cell death seen in HIE. This data, plus the additional data we will gather in larger animal studies, will provide a solid base to move into clinical trials for HIE."

**NEXT STEPS**

Argenica has engaged global contract research organisation Labcorp Drug Development's (Labcorp) paediatric regulatory team to develop a regulatory and clinical trial strategy for ARG-007 in HIE in newborns. Labcorp has extensive experience in planning and running global paediatric clinical trials, as well as obtaining regulatory investigational new drug applications, and orphan drug designation from the Food and Drug Administration (FDA) for therapies

[1] Favié LMA, Cox AR, van den Hoogen A, Nijboer CHA, Peeters-Scholte CMPCD, van Bel F, Egberts TCG, Rademaker CMA, Groenendaal F. Nitric Oxide Synthase Inhibition as a Neuroprotective Strategy Following Hypoxic-Ischemic Encephalopathy: Evidence From Animal Studies. Front Neurol. 2018 Apr 19;9:258.

targeting rare paediatric therapies. To meet requirements to undertake clinical trials in HIE in the US, Argenica has initiated a preclinical juvenile toxicology study and preclinical efficacy studies in a large animal term model of HIE. If these studies illicit positive results, then the Company's aim is to commence a Phase 1/2 trial in HIE in the US. The preclinical efficacy studies are generously funded by a grant from the Stan Perron Charitable Foundation (see announcement dated 30 March 2022). Results of these studies, as well as the Company's engagement with the FDA, will be announced as they come to hand.

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

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**ABOUT ARGENICA**

Argenica (ASX: AGN) is developing novel therapeutics to reduce brain tissue death after stroke and other types of brain injury and neurodegenerative diseases to improve patient outcomes. Our lead neuroprotective peptide candidate, ARG-007, has been successfully demonstrated to improve outcomes in pre-clinical stroke models, traumatic brain injury (TBI) and hypoxic ischaemic encephalopathy (HIE). The Company has recently completed a Phase 1 clinical trial in healthy human volunteers to assess the safety and tolerability of a single dose of ARG-007. Argenica is now progressing towards a Phase 2 clinical trial in ischaemic stroke patients, as well as continuing to generate preclinical data in other neurological conditions, including in TBI, HIE and Alzheimer's Disease.

**ABOUT HIE**

HIE, **a rare paediatric condition,** is a type of brain injury sustained by newborns whereby the brain doesn't receive enough oxygen or blood flow for a period. There are several causes of HIE, including placental rupture, umbilical cord problems, or other factors. The condition may develop during pregnancy, labour, and delivery, or during the postnatal period. Whilst some babies will only sustain mild effects, others will have severe and permanent disability including cerebral palsy, developmental delays, or severe disability. Currently the only treatment for HIE in newborn term infants is brain cooling, or hypothermia, however studies show that whilst this treatment may be well tolerated and safe for term babies, in 31-55% of babies the treatment has been shown to be ineffective at providing improved neurological outcomes[2]. This treatment is not appropriate for preterm babies, and there are currently no therapies available for preterm babies who suffer HIE.

[2] Shankaran S. Therapeutic hypothermia for neonatal encephalopathy. Curr Treat Options Neurol. 2012;14(6):608–19

**APPENDIX A**

**Study Details**

This study was undertaken by Dr Adam Edwards (Argenica's Neonatal Scientific Advisor and Senior Post Doctoral Research Fellow at the Perron Institute) to determine the efficacy of ARG-007 in reducing total brain injury following HIE in an established term animal model, equivalent to >37 weeks gestation in humans. The study compared the efficacy of ARG-007 against saline administered injury controls.

In newborn infants, HIE is one of the most serious complications in term (greater than 37 weeks gestation) infants, affecting around 2.5 per 1000 live births in developed countries[3].

Given the demonstrated efficacy of ARG-007 in attenuating the development of an infarct in a term model of HIE at 48 hours post injury (see announcement dated 29 September 2022), the present study evaluated the efficacy of varying doses of ARG-007 on the vasogenic oedema as measured by MRI 48 hours post injury and examined the efficacy of the same single dose of ARG-007 in a term-equivalent animal model of HIE compared to a saline control injured postnatal day (PND) 10 pups at timepoints beyond 48h, namely 1 week, 2 weeks, 3 weeks and 4 weeks to determine whether the effect of this single ARG-007 administration would endure. This study also builds on the previous preclinical studies supporting the use of ARG-007 as a treatment for preterm HIE[4,5] and provides further supporting efficacy data to progress this treatment towards clinical trials.

***Methods***

A model of HIE in perinatal rats (10-day-old, equivalent to human term infants with respect to brain development) was used in this study in which blood flow to the brain was blocked by occluding the right common and internal carotid arteries and subjecting animals to a period of hypoxia (detailed methodology previously reported[6]). Immediately following hypoxia animals received either a dose of ARG-007 (100, 300 or 600 nmol/kg) or a dose of saline as controls. A total of 38 animals across the saline and ARG-007 dosed groups were assessed.

[3] Graham EM, Ruis KA, Hartman AL, Northington FJ, Fox HE. A systematic review of the role of intrapartum hypoxia ischemia in the causation of neonatal encephalopathy. Am J Obstet Gynecol. 2008; 199(6):587-95.
[4] Edwards, A. B., Cross, J. L., Anderton, R. S., Knuckey, N. W., & Meloni, B. P. (2018). Poly-arginine R18 and R18D (D-enantiomer) peptides reduce infarct volume and improves behavioural outcomes following perinatal hypoxic-ischaemic encephalopathy in the P7 rat. *Molecular brain*, *11*(1), 8.)
[5] Edwards, A. B., Anderton, R. S., Knuckey, N. W., & Meloni, B. P. (2018). Assessment of therapeutic window for poly-arginine-18D (R18D) in a P7 rat model of perinatal hypoxic-ischaemic encephalopathy. *Journal of neuroscience research*, *96*(11), 1816–1826.
[6] Edwards, A.B., Feindel, K.W., Cross, J.L., Anderton, R.S., Clark, V.W., Knuckey, N.W., Meloni, B.P. (2017). Modification to the Rice-Vannucci perinatal hypoxic-ischaemic encephalopathy model in the P7 rat improves the reliability of cerebral infarct development after 48 hours. *Journal of neuroscience methods*, 288, 62-71.

Animals were assessed for either vasogenic oedema at 48 hours post HIE and treatment, or total brain injury (total area of brain injury associated with both neuronal cell death and vasogenic oedema) at 4 time points following injury and treatment – 1 week, 2 weeks, 3 weeks and 4 weeks. The data is expressed as either vasogenic oedema volume in $mm^3$ or total brain injury in $mm^3$ (Figure 1). All injury was measured on a combination of apparent diffusion coefficient (ADC) and T2-weighted scans captured via magnetic resonance imaging (MRI).

Statistics for the data were performed in Prism with an ANOVA + Fisher's post hoc analysis. $P<0.05$ is considered a statistically significant difference.

***Results***

A dose-dependent response following administration of ARG-007 from 100– 600 nmol/kg was observed, with a statistically significant reduction in vasogenic oedema at 48 hours post injury seen in the 100 and 300 nmol/kg treatment groups (p = 0.01 and 0.04, respectively).

A dose-dependent response following administration of ARG-007 from 100 – 600 nmol/kg was observed, with reduction in total brain injury volume most significant at the 300 nmol/kg doses (Figure 1). Results demonstrated that 100, 300 and 600 nmol/kg doses of ARG-007 significantly reduces total brain injury up to 4 weeks (last measurement) after injury and treatment when compared to saline (injured) controls ($p$ = 0.02, 0.002, and 0.01, respectively).

***Conclusion***

Doses of 100 and 300 nmol/kg of ARG-007 significantly reduced total brain injury volume following HIE in a preclinical term animal model, equivalent to ≥37 weeks gestation in humans. The efficacy of ARG-007 was sustained up to 4 weeks post injury, indicating that a single dose of ARG-007 following HIE is sufficient to provide protection against brain injury that will have an impact of the third phase of neuronal cell death seen in HIE, and therefore having a lasting impact on functional outcomes.

ARG-007 at a dose of 300 nmol/kg demonstrated a greater mean reduction in total brain injury when compared to doses of 100 and 600 nmol/kg. This dose also showed a reduction in vasogenic oedema at 48 hours post injury, suggesting a 300 nmol/kg dose is the most efficacious in a term model of HIE. This is further supported by a previous study (announced 29 September 2022) showing a dose of 300 nmol/kg significantly (p=0.01) reduced infarct volume at 48 hours post HIE in a term animal model.