ARG-007 IS AN EFFECTIVE STAND-ALONE THERAPY IN PRECLINICAL STUDY OF TERM HYPOXIC ISCHAEMIC ENCEPHALOPATHY

Highlights:

- *The preclinical study determined the reduction in total brain injury following hypoxic ischaemic encephalopathy (HIE) following standard of care therapeutic hypothermia and ARG-007, and the combination of the two, to determine if there was any benefit to combining ARG-007 with standard of care hypothermia.*

- *The study showed that **48 hours post-HIE**, ARG-007 demonstrated a **46% reduction in total brain injury** compared to saline placebo and a **42% reduction in total brain injury** compared to therapeutic hypothermia. The study further demonstrated that ARG-007 sustained a reduction in total brain injury when examined **4 weeks post-HIE**, with a single dose of ARG-007 showing a **52% reduction in total brain injury** compared to saline placebo **and 57% reduction in total brain injury** compared to therapeutic hypothermia.*

- *These findings indicate ARG-007 could be suitable as a combined neuroprotection therapy for HIE with hypothermia or as a stand-alone therapy when hypothermia cannot be used.*

- *Given the consistency in brain injury reduction seen with ARG-007 treatment in small animal studies, these latest results now draw these studies to completion. Argenica will now complete larger animal studies to confirm these positive results, as well as complete juvenile toxicology studies required to advance ARG-007 into clinical trials for infants suffering HIE.*

- *Argenica's preclinical HIE studies are being funded by a non-dilutive grant from the Stan Perron Charitable Foundation (SPCF), which is sufficient to complete all pre-clinical efficacy studies required to initiate a clinical trial in newborn infants.*

Perth, Australia; 18 October, 2023 - Argenica Therapeutics Limited (ASX: AGN) ("Argenica" or the "Company"), a company developing novel neuroprotective therapeutics to reduce brain tissue death in neurological conditions, is pleased to announce its lead drug candidate ARG-007 has shown to be significantly effective at reducing total brain injury in a preclinical model of hypoxic-ischaemic encephalopathy (HIE).

HIE is a type of newborn brain damage caused by oxygen deprivation and limited blood flow. This results in damage to brain cells which begins almost immediately when blood flow and

oxygen delivery to the brain is impaired. There is currently no therapeutic drug on the market to protect brain cells following HIE, instead therapeutic hypothermia (cooling the body to maintain a targeted temperature) is used which can prevent or minimize permanent brain damage in some babies, however, can result in a number of complications, including cardiovascular and respiratory complications, and is not recommended in patients in low to middle income countries[1]. With and without therapeutic hypothermia, many infants with HIE go on to develop permanent health conditions. These include cerebral palsy, cognitive disabilities, epilepsy, hearing and vision impairments, and many more.

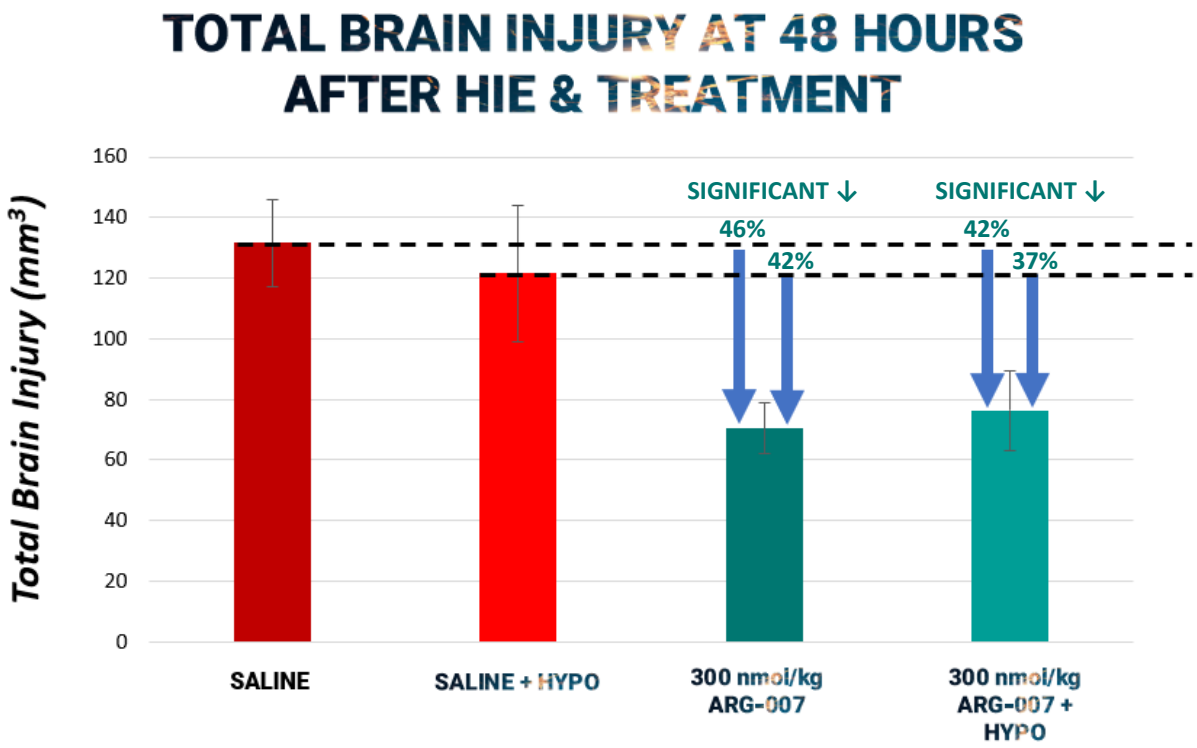

Figure 1. Total brain injury (mm^3) in HIE control (saline and saline + therapeutic hypothermia "hypo") animals and treatment (ARG-007 and ARG-007 + hypo) animals, 48 hours post injury & treatment. Following injury (ischaemia-hypoxia), control animals received either saline (placebo control) or saline with hypothermia (treatment control); treatment animals received either ARG-007 (300 nmol/kg) or ARG-007 (300 nmol/kg) with hypothermia. Following injury there was no difference in total brain injury between the control saline animals and the control saline + hypo animals, indicating that hypothermia alone does not reduce brain injury following HIE in this animal model. Whereas the ARG-007 treated groups showed a statistically significant reduction in total brain injury from saline (46.47% reduction with ARG-007 alone; p=0.0009 and 42.01% reduction with ARG-007 +hypo; p=0.0028); and from saline + hypo (42.07% reduction with ARG-007 alone; p=0.0098 and 37.25% reduction with ARG-007 + hypo; p=0.0229). The addition of ARG-007 to hypothermia treatment did not result in an additional statistically significant reduction in total brain injury volume.

These results confirmed that ARG-007 alone significantly reduced the volume of total brain injury 48 hours following HIE, compared to the positive control group which received a saline injection instead of ARG-007 (46% reduction; Figure 1). The study also assessed, for the first time, the comparative neuroprotection of ARG-007 versus therapeutic hypothermia, which is

[1] Thayyil S,et al Hypothermia for moderate or severe neonatal encephalopathy in low-income and middle-income countries (HELIX): a randomised controlled trial in India, Sri Lanka, and Bangladesh. Lancet Glob Health. 2021 Sep;9(9):e1273-e1285

currently the only treatment available which improves neurological outcomes for term infants with HIE. Compared with a preclinical model of therapeutic hypothermia, ARG-007 showed a significant reduction in the total brain injury (42% reduction; Figure 1), whereas therapeutic hypothermia alone did not significantly reduce total brain injury.

The study also assessed total brain injury at 4 weeks post injury (and treatment) to determine the length of time the neuroprotection lasted from this single administration. At 4 weeks, the results confirmed that ARG-007 alone maintained a significant reduction in total brain injury compared to saline controls (52% reduction; Figure 2). Similarly, compared with hypothermia alone, ARG-007 maintained a significant reduction in the total brain injury (57% reduction; Figure 2).

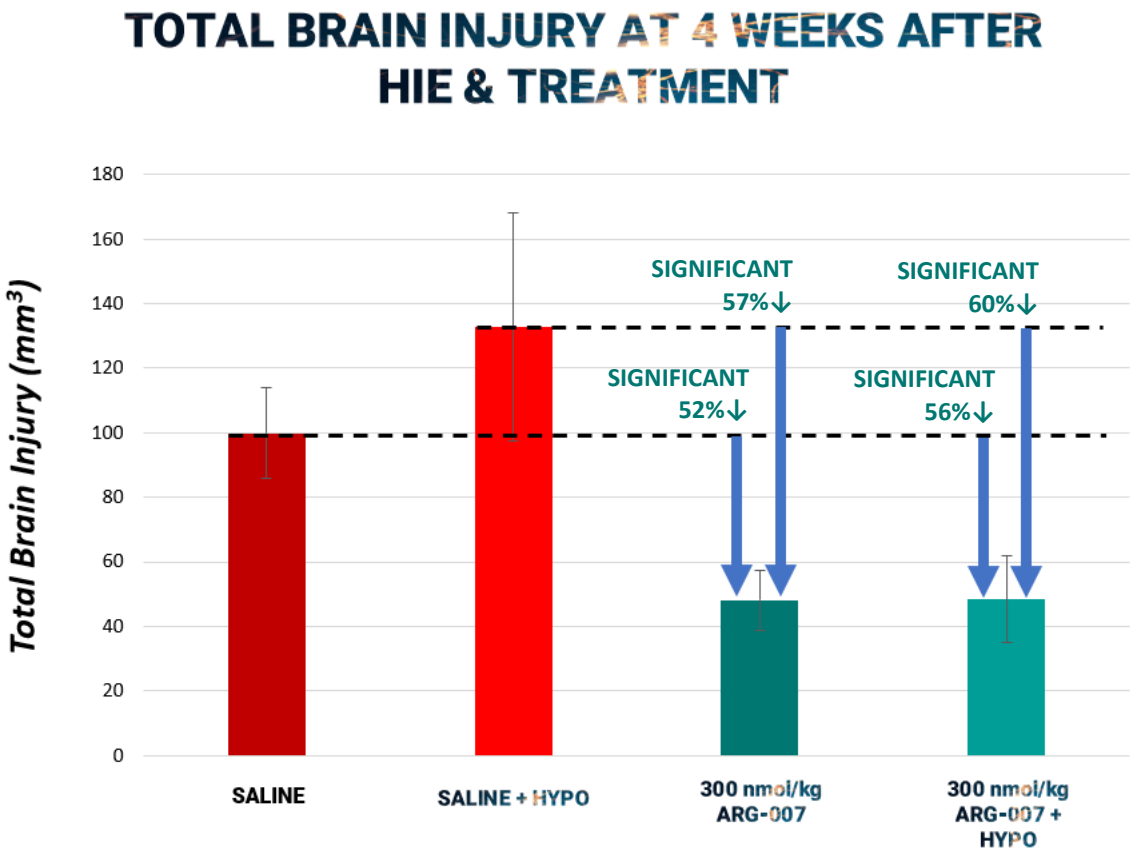

Figure 2. Total brain injury (mm^3) in HIE control (saline and saline + hypothermia "hypo") animals and treatment (ARG-007 and ARG-007 + hypo) animals, 4 weeks post injury & treatment. Animals were assessed again at the 4-week time point following injury (ischaemia-hypoxia). At this time point, whilst there appeared to be an increase in total brain injury between the control saline animals and the control saline + hypo animals, this increase was not statistically significant (p=0.6268), indicating that hypothermia alone does not reduce or increase total brain injury following HIE in this animal model. As seen at 48 hours, at the 4-week time point, the ARG-007 treated groups showed a significantly greater reduction in total brain from saline (51.62% reduction with ARG-007 alone; p=0.0129 and 55.66% reduction with ARG-007 +hypo; p=0.0093); and from saline + hypo (56.52% reduction with ARG-007 alone; p=0.0071 and 60.15% reduction with ARG-007 + hypo; p=0.0052). This indicates that the effect of ARG-007 treatment lasts out to at least 4 weeks.

Taken together, these results show that a single dose of ARG-007 administered after HIE injury significantly reduces total brain injury, when compared to both the injury alone (saline placebo) and therapeutic hypothermia treatment (saline plus hypothermia), to cause long-lasting neuroprotection. As ARG-007 appears effective regardless of whether hypothermia is used, ARG-007 could be delivered either as an additional therapy with hypothermia or as a standalone therapy.

NEXT STEPS

This study now draws to completion the rat efficacy studies of ARG-007 in HIE. The Company will now work with Dr Adam Edwards, Senior Postdoctoral Research Fellow at the Perron Institute and Argenica's Neonatal Scientific and Regulatory Advisor, to complete larger animal studies in both term and pre-term models of HIE, to be conducted at the University of Aarhus, Denmark and the University of Western Australia, respectively. These studies will examine ARG-007 efficacy in models that more closely resemble the newborn human condition. In addition, the larger animal model of term HIE has been selected due to its use in extensively validating the current standard of care, therapeutic hypothermia. Pilot studies for both these preclinical studies are currently in the planning stages, with preliminary data expected in calendar year 2024. Further, the juvenile toxicology studies required to commence a clinical trial in infants have also been initiated. Together, these studies will allow Argenica to progress ARG-007 into a clinical trial in HIE in the future.

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.

APPENDIX A
Further Study & HIE Information

This study was undertaken by Dr Adam Edwards and Prof Bruno Meloni (Argenica's CSO) at the Perron Institute to determine the efficacy of ARG-007 in reducing total brain injury in the brain following hypoxic-ischaemic encephalopathy (HIE) in an established term animal model, equivalent to 37 weeks gestation in humans. The study compared the efficacy of ARG-007 against a preclinical model of therapeutic hypothermia treatment. The induction of therapeutic hypothermia using targeted temperature management in newborn term infants may be used as standard of care to help protect brain cells.

In newborn infants, HIE is one of the most serious complications affecting preterm (less than 37 weeks gestation) and term (greater than 37 weeks gestation) infants, affecting around 2.5 per 1000 live births in developed countries[2].

HIE occurs when the brain does not receive enough oxygen or blood flow for a period of time. It may occur at any time prior to labour, during labour and delivery, or immediately following delivery. The initial injury that is caused by a loss or reduction of oxygen supply is followed by progressive brain cell death due to excitotoxicity, oxidative stress, and inflammation[3,4]. The physiological effects resulting from the interruption to blood flow and/or oxygen in the brain can vary greatly depending on the length of time the disruption occurs as well as the location of the disruption. Some children may only display mild effects whilst others will have severe permanent disability including cerebral palsy, cognitive impairment, or developmental delay.

Clinically, treatment to reduce brain injury for HIE is very limited. For term babies in developed countries with access to specialised equipment, treatment predominately consists of exposing babies to moderate hypothermia (33.5°C for 72h) as a way of providing neuroprotection. 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[5]. Further, its use in earlier preterm infants is associated with increased mortality and adverse effects and therefore it is not used for these babies.

Given the demonstrated efficacy of ARG-007 in several preclinical stroke models as well as a late-preterm animal and term animal model of HIE, the present study examined the efficacy of ARG-007 in a term neonatal animal model of HIE compared to exposure to therapeutic

[2] 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.
[3] Leonardo CC, Pennypacker KR. Neuroinflammation and MMPs: potential therapeutic targets in neonatal hypoxic-ischemic injury. *J Neuroinflammation* (2009) 6:13
[4] Thornton C, Hagberg H. Role of mitochondria in apoptotic and necroptotic cell death in the developing brain. *Clin Chim Acta* (2015) 451:35–8
[5] Shankaran S. Therapeutic hypothermia for neonatal encephalopathy. Curr Treat Options Neurol. 2012;14(6):608–19

hypothermia. This study builds on the previous preclinical studies supporting the use of ARG-007 as a treatment for perinatal HIE[6,7,8].

Methods

A model of HIE in perinatal rats (PND10, equivalent to term infants) was used in this study in which blood flow to the brain from the right common and internal carotid arteries were occluded, and animals were subsequently subjected to hypoxia (detailed methodology previously reported[9]). Immediately following hypoxia animals received either a dose of ARG-007 (300 nmol/kg) or a dose of saline to act as a placebo control to ARG-007 treatment. Some groups were then cooled to maintain a standard preclinical hypothermia therapy core temperature (33.5°C for 4 hours). Those animals not subject to hypothermia were maintained at normal core temperature (37°C).

The experimental groups are outlined below:

- Injury + Saline placebo (negative control to determine injury features)
- Injury + Saline placebo + hypothermia (standard of care treatment control)
- Injury + ARG-007
- Injury + ARG-007 + hypothermia.

Animals were assessed for total brain injury (area of infarct and oedema in mm^3) utilising magnetic resonance imaging, with results shown for the 48 hours and 4 weeks time points post HIE and treatment –. Statistical significance was determined using Analysis of Variance (ANOVA), with each experimental group assessed against each other.

Results

The analysis aimed to determine whether there was any statistically significant difference between each group.
At the 48-hour time point there was no difference in volume of total brain injury between the control saline animals and the hypothermia treated HIE animals (control saline plus hypothermia), indicating that hypothermia alone does not reduce total brain injury following HIE in this animal model at 48 hours. Comparing the saline control animals to the ARG-007 treated groups, there was a statistically significantly reduction in total brain injury from both the ARG-007 alone (46.47% reduction; p=0.0009) group and the ARG-007 plus hypothermia

[6] 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.)
[7] 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.
[8] ASX Announcements on 3 November 2021, 29 September 2022, and 20 April 2023.
[9] 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.

group (42.01% reduction; p=0.0028). Further, when comparing the saline plus hypothermia group to the ARG-007 treatment groups, there was a statistically significant reduction in total brain injury from the ARG-007 alone group (42.07% reduction; p=0.0098) and the ARG-007 plus hypothermia group (37.25% reduction; p=0.0229). At the 48-hour time point, the co-administration of ARG-007 to hypothermia treatment caused no additional significant reduction in total brain injury.

When analysing total brain injury data from the animals at the 4-week time, whilst there appeared to be an increase in total brain injury volume between the control saline animals and the hypothermia treated animals, this increase was not statistically significant (p=0.6268), indicating that hypothermia alone does not reduce or increase total brain injury following HIE in this animal model even 4 weeks later. Comparing the saline control animals to the ARG-007 treated groups, there was a statistically significantly reduction in total brain injury from both the ARG-007 alone (51.62% reduction; p=0.0129) group and the ARG-007 plus hypothermia group (55.66% reduction; p=0.0093). Further, when comparing the saline plus hypothermia group to the ARG-007 treatment groups, there was a statistically significant reduction in total brain injury from the ARG-007 alone group (56.52% reduction; p=0.0071) and the ARG-007 plus hypothermia group (60.15% reduction; p=0.0052). This indicates that the effect of ARG-007 treatment on HIE lasts out to at least 4 weeks.

Conclusion

A single dose of 300 nmol/kg ARG-007 significantly reduces total brain injury following HIE in a term animal model, which appears to last for at least 4 weeks. The significant reduction in brain cell death following a single dose of ARG-007 provides Argenica with additional data to continue to progress the development of ARG-007 as a therapeutic for HIE in term babies.

Co-administration of ARG-007 with hypothermia in this preclinical model appears to have no additional beneficial effect on reducing total brain injury. As ARG-007 appears effective regardless of whether hypothermia is used, ARG-007 could be delivered either as an additional therapy with hypothermia or as a standalone therapy where standard of care therapeutic hypothermia is not suitable.