ARG-007 SIGNIFICANTLY REDUCES EFFECTS OF TRAUMATIC BRAIN INJURY IN PRECLINICAL STUDY

Highlights:

- ***ARG-007 significantly reduced damage to brain cells*** *in a ferret animal model of mild to moderate traumatic brain injury (modTBI), a model that closely resembles the gross anatomy of the human brain.*

- *The observed therapeutic effects of ARG-007 in the modTBI model included:*

  - *A **significant reduction in the accumulation of key proteins**, amyloid precursor protein and neurofilament M-14.9, which are known markers of brain cell injury following modTBI. The protein levels following ARG-007 treatment in brain regions associated with memory and mental functioning were reduced back to the equivalent to non-injured animals**.***

  - *A **significant reduction in the level of inflammation markers** GFAP and Iba1 back to levels seen in non-injured animals, in the brain region associated with memory. This is important because inflammation in the brain following TBI is a cause of secondary brain injury which usually lasts far beyond the initial injury.*

- *This study expands on previous published and announced data in rodent models of mod-TBI[1]. It provides Argenica with **further robust evidence** regarding the potential of ARG-007 as a treatment following TBI.*

- *Argenica will continue to advance the preclinical efficacy studies in TBI including validating this study in a larger ferret study.*

Perth, Australia; 15 May 2024 - Argenica Therapeutics Limited (ASX: AGN) ("Argenica" or the "Company"), a biotechnology company developing novel therapeutics to reduce brain tissue death after stroke and other neurological conditions, is pleased to announce its results from a pilot study assessing the efficacy of ARG-007 in a ferret preclinical model of mild to moderate traumatic brain injury (modTBI).

[1] ASX Announcement dated 22 June 2023 – ARG-007 Protects Brain Cells in Moderate Traumatic Brain Injury Model; and Chiu et al, 2020, *Effect of Polyarginine Peptide R18D Following a Traumatic Brain Injury in Sprague-Dawley Rats.*

The study showed that ARG-007 was able to:

- **significantly reduce damage to the axons of brain cells** (neurons) as measured by amyloid precursor protein (APP) and neurofilament M-14.9 (RMO-14) accumulation, both validated biomarkers of axonal injury.
- significantly **reduce the expression of key inflammatory markers** in microglia/macrophages (as assessed via IBA1) and astrocytes (as assessed via GFAP) post-injury in areas of the brain responsible for **memory and mental functioning**.
- treatment with ARG-007 improved **motor function in a balance and coordination test** post injury.

The study, undertaken by the University of Adelaide and partially funded by Argenica's CRC-P grant[2], was a pilot study to assess the therapeutic potential of ARG-007 in protecting brain cell (neuron) integrity following modTBI. The study utilised an established ferret preclinical model of mild to moderate TBI. Ferrets were chosen because their brains more closely resemble the gross anatomy of the human brain with respect to features such as sulci and gyri (the raised and folded structures) in the cerebral cortex and white matter content compared with the rodent brain, and therefore is more likely to better replicate the pathophysiological outcomes of TBI in humans.

Dr Liz Dallimore, **Managing Director of Argenica**, commented "*The data generated from this new model of TBI is greatly encouraging because it supports and affirms the positive findings in our previous studies in rodents, showing ARG-007 has significant potential as a therapeutic drug following TBI. We look forward to continuing to work with the University of Adelaide to expand this program of work to ensure we have robust preclinical data to build a strong scientific and clinical rationale to progress ARG-007 into a Phase 2 clinical trial in TBI patients.*"

Based on the positive results shown in this study, the Company will investigate the performance of ARG-007 in a larger scale ferret preclinical study to further validate this data. Taken together with previous rodent studies, this will provide Argenica with a robust preclinical data set on the efficacy of ARG-007 in TBI.

[2] Announcement dated 20 January, 2023 – Argenica Awarded $1.2M Grant for Traumatic Brain Injury Project Under the CRC-P Program.

STUDY OVERVIEW

Background

Traumatic Brain Injury (TBI) occurs when a mechanical force is applied to the head and is a major health concern worldwide, representing the greatest contribution to death and disability more than any other traumatic insult (Dewan *et al.*, 2018). The initial impact or blow to the head sets in motion a secondary injury that causes ongoing neuronal injury and dysfunction (McKee and Daneshvar, 2015). Patients who suffer a TBI can present with cognitive, motor and behavioural symptoms, with patients also reporting difficulties with memory, attention, balance and co-ordination (van Donkelaar *et al.*, 2006; Barman *et al.*, 2016).

Many of these symptoms of TBI are the result of diffuse injury to the axons of neurons caused by the the movement of brain within the skull (van Donkelaar *et al.*, 2006; Lima Santos *et al.*, 2021). Axons are the long thin projections of neurons (brain cells), responsible for communicating information across long distances, with bundles of axons known as white matter tracts. Damage to axons disrupts information being transferred from brain cell to brain cell leading to functional deficits, with the type of deficit dependant on the white matter tract involved. Importantly, the majority of axonal injury in TBI is not due to physical tearing of axons at the time of the initial injury (Buki and Povlishock, 2006) but is caused by the initiation of a number of secondary injury processes (Johnson *et al.*, 2013), which are therefore amenable to treatment. This secondary injury can be caused by cellular damage from the initial impact causing an inflammatory response, which is triggered by the activation of resident glial cells including microglia/macrophages and astrocytes (Corrigan *et al.*, 2016). This inflammatory response can also cause further cellular injury by triggering pathways that culminate in oxidative damage and activation of cell death (Donat *et al.*, 2017; Mira *et al.*, 2021). Accordingly, the application of a therapy such as ARG-007, that may reduce this secondary inflammatory response as well as damage to axons of brain cells, could offer a strong therapeutic rationale to treat TBI.

Aims & Methods

The aim of this pilot study was to investigate whether ARG-007, delivered intravenously at either a 100 nmol/kg or 300 nmol/kg dose, can improve motor and cognitive outcome with an associated reduction in axonal injury and neuroinflammation at 3 days following a mild-moderate diffuse head injury in ferrets. The animals were randomly allocated via a random number generator to sham (uninjured) (n = 9) or injury (n = 25), with injured ferrets administered either vehicle (saline; n = 10), 100nmol/kg (0.3 mg/kg) R18D (n =8) or 300nmol/kg (0.9 mg/kg) R18D (n = 7).

The animals were subject to a range of validated motor and cognitive tests, following which key white matter tract brain regions associated with damage following TBI were analyzed for TBI biomarkers, specifically the brain regions of the corpus callosum (mental functioning), the

fornix (memory), external medullary laminae and internal capsule (motor and sensory control).

STUDY RESULTS HIGHLIGHTS

ARG-007 Significantly Reduces Accumulation of axonal injury protein markers APP and RMO-14

Damage to neurons following TBI can be assessed via the accumulation of proteins that are transported along the neuronal shaft, or axon. The current gold standard used for this assessment is the amyloid precursor protein (APP), with damaged axons showing accumulation of APP within 30 minutes of injury and peaking within the first 24-72 hours. Further, neurofilaments within brain cells that aid maintenance of brain cell structure can also be damaged following a TBI. Assessing the amount of neurofilament M-14.9 (RMO-14) is a key marker for axonal injury. RMO-14 increases in injured axons within 30 minutes following a TBI and also peaks within the first 24-72 hours when axonal injury is maximal.

Results from this study show a single administered dose of either 100nmol/kg or 300nmol/kg of ARG-007 at 30 minutes post-injury prevented the accumulation of both APP and RMO-14 in neurons, as assessed 3 days after injury, suggesting neuroprotection of axons from ARG-007 following modTBI (Figures 1 & 2).

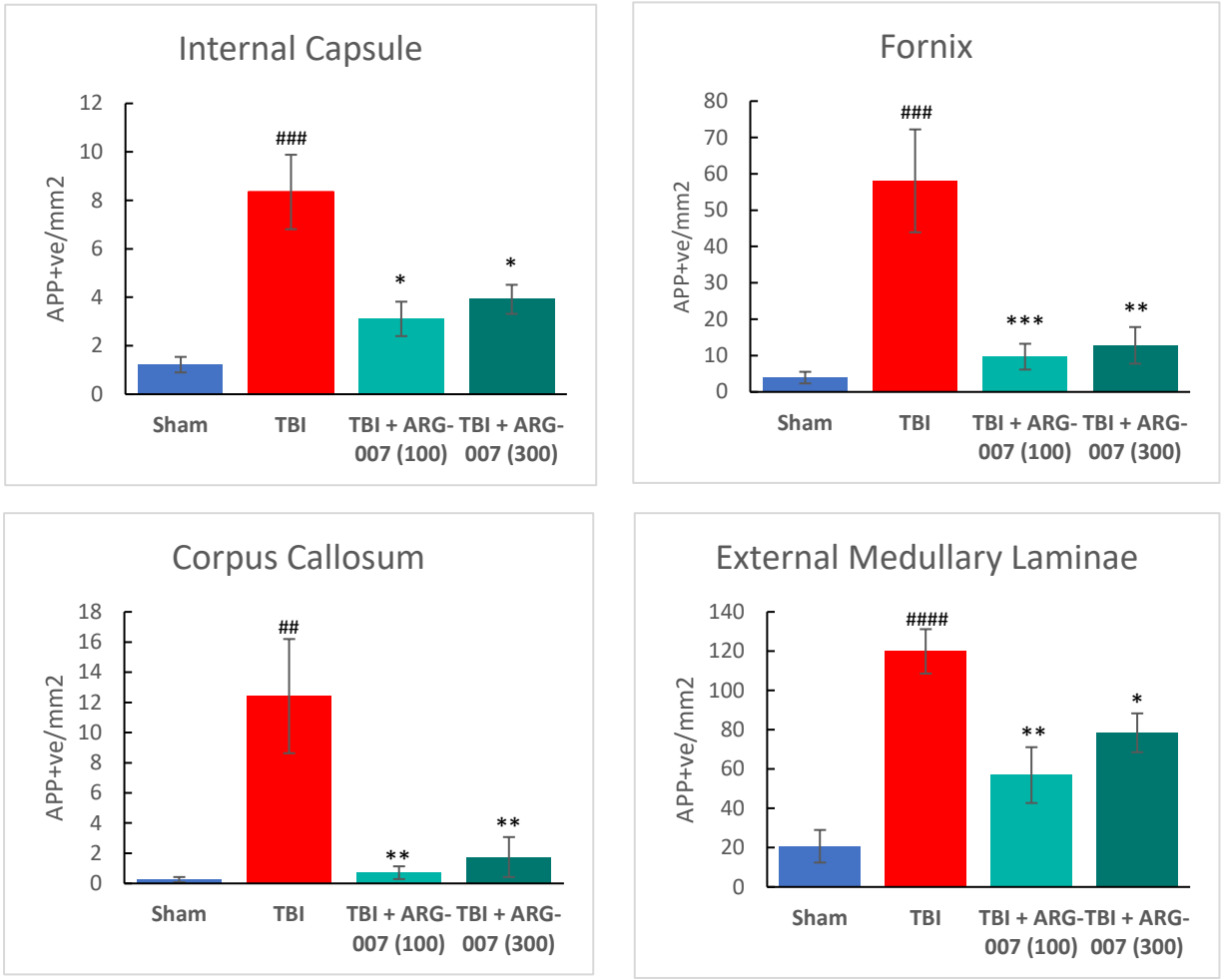

FIGURE 1: ARG-007 protected axons from injury as assessed by APP accumulation in all white matter tract brain regions analysed.

Both 100nmol/kg and 300nmol/kg doses of ARG-007 showed reductions in APP accumulation in key white matter tract regions of the brain being the internal capsule, fornix, corpus callosum and external medullary laminae. Evaluation of axonal injury via accumulation of the amyloid precursor protein (APP) within key white matter tracts. ##p<0.01, ###p<0.001, ####p< .0001 compared to sham animals to confirm injury impairment, and ***p<0.001, **p<0.01 *p<0.05 compared to TBI:Vehicle treated animals to confirm therapeutic response of ARG-007.

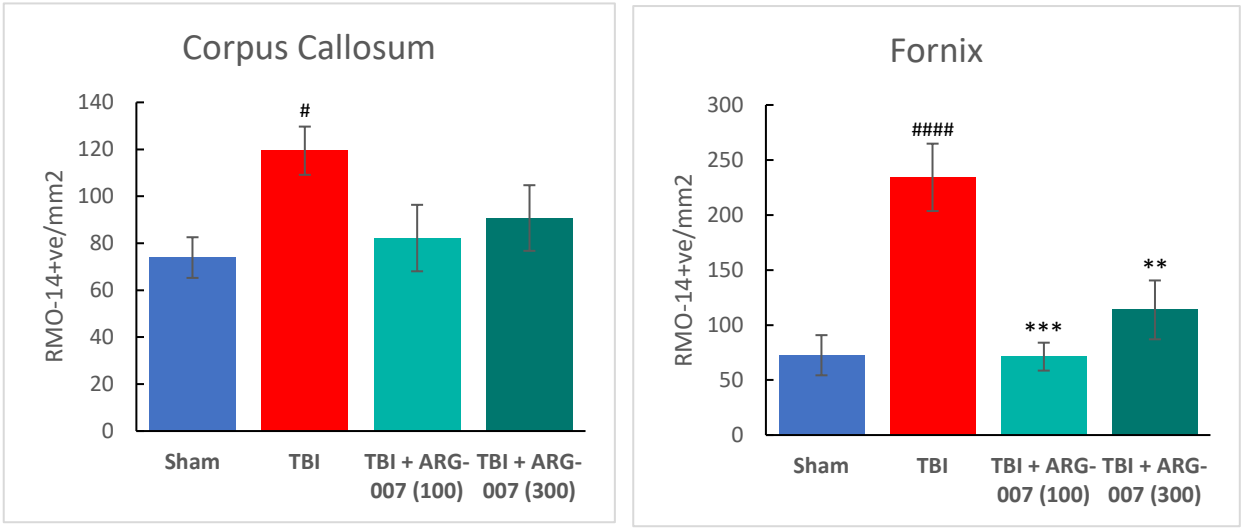

FIGURE 2: ARG-007 protected axons from injury as assessed by RMO-14 accumulation in white matter tract brain regions associated with mental functioning and memory.
Calculation of the number of RMO-14+ axons within the corpus callosum and fornix, with a significant difference seen in the fornix in both the 100 and 300 nmol/kg doses. ####p<0.0001, #p<0.05 compared to sham animals to confirm injury impairment, ***p<0.001, **p<0.01 compared to TBI:Vehicle treated animals to confirm therapeutic response of ARG-007.

ARG-007 Reduces Neuroinflammation Response Following TBI

To investigate the effect of modTBI and ARG-007 treatment on neuroinflammation, the level of GFAP and Iba1 expression, protein markers of astrocyte and microglia/macrophage activation, respectively, was quantified. Astrocytes and microglia/macrophage are activated during the inflammatory response to enhance release of pro-inflammatory molecules. In the fornix, the region of the brain associated with memory, ARG-007 reduced GFAP and Iba1 density to levels significantly lower than that of the vehicle-treated modTBI group, and on par with the level in the sham injured controls (Figure 3).

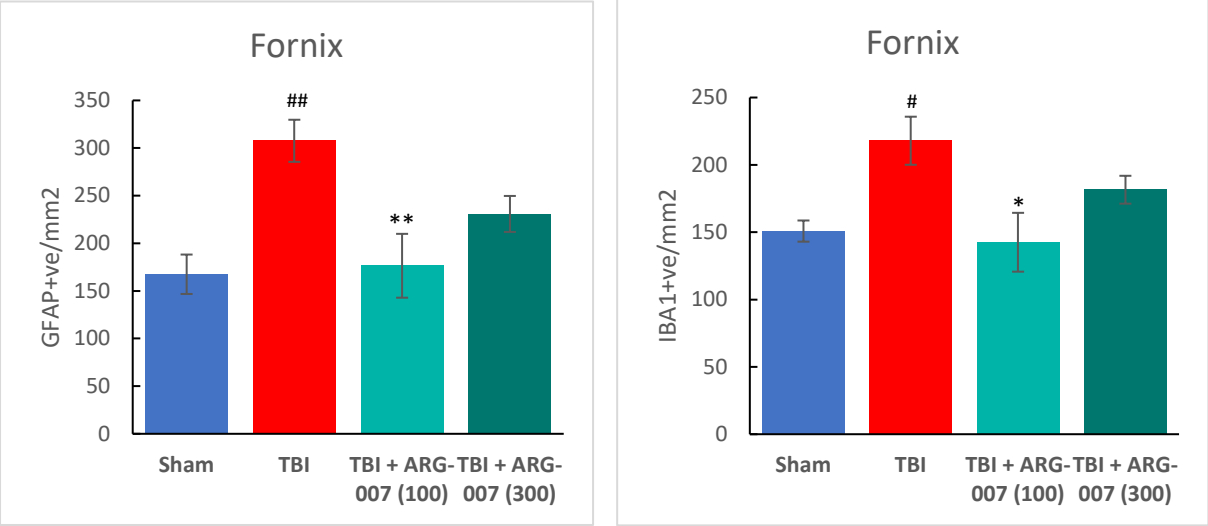

FIGURE 3: ARG-007 reduced inflammation as assessed by the number of GFAP and IBA1 positive cells in white matter tract brain region associated with memory.
Calculation of the number of GFAP+ and IBA1+ cells within key regions of interest showing significant increases in the fornix following TBI injury, and which was attenuated in ARG-007 100 nmol/kg treated animals. ##, p<0.01, #p<0.05 compared to sham ferrets to confirm injury impairment, **p<0.01, *p<0.05 compared to TBI:Vehicle ferrets to confirm therapeutic response of ARG-007.

ARG-007 Improved Balance and Coordination Following ModTBI

Following injury, preliminary investigations of functional motor and cognition were also undertaken using standardised assessment techniques. Whilst the study wasn't powered to test the treatment effect on such functional outcomes following ARG-007 administration, it was encouraging to see statistical significance of ARG-007 in improving balance and coordination in the ladder walk task of motor performance. A further large-scale study is warranted to fully establish the concordance of the observed impact of ARG-007 on injury response proteins and functional improvement in motor and cognition.

CONCLUSION

This pilot study in a model of TBI that mimics key clinical features, including the presence of cortical folds in the brain (gyrification), indicates that a single IV dose of ARG-007 at both 100nmol/kg or 300nmol/kg may prevent functional deficits following injury, with an associated significant reduction in axonal injury biomarkers. This is the first study to demonstrate benefits of ARG-007 treatment in a gyrencephalic model (convoluted brain similar to humans) of diffuse modTBI.

Based on the positive results shown in this study, the Company will investigate the performance of ARG-007 in a larger scale ferret preclinical study to further validate this data and explore its correlation to potential functional (motor and cognition) improvement. Taken together with previous rodent studies, this will provide Argenica with a robust preclinical data set on the efficacy of ARG-007 in TBI.

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 completed a Phase 1 clinical trial in healthy human volunteers to assess the safety and tolerability of a single dose of ARG-007. Argenica has now initiated 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.