# ARGENICA TO PROGRESS CLINICAL DEVELOPMENT OF ARG-007 IN TRAUMATIC BRAIN INJURY FOLLOWING POSITIVE PRECLINICAL DATA

**Highlights:**

- *A second preclinical study of ARG-007 in a large cohort ferret model of moderate **traumatic brain injury** (modTBI) showed **significant long-lasting reduction in brain cell damage and inflammation** following injury, consistent with the findings from the initial 3-day pilot study.*

- *The findings from this latest preclinical study extended on those data from the initial 3-day pilot study[1]. In this second study, **ARG-007 was able to significantly reduce both axonal injury and inflammation out to 14 days post-injury**. Further, ARG-007 successfully reduced motor and cognitive deficits observed two weeks following the modTBI. Collectively, the data over the two studies demonstrates that ARG-007 can supress injury over both the acute phase and well as late chronic stage of injury, an important outcome for the drug's prospective clinical utility.*

- *This ferret model closely resembles the gross anatomy of the human brain and, taken together with previously generated data in modTBI[2] from a number of rat studies and the pilot ferret study, provides **a robust preclinical data package**, thereby providing a strong scientific rationale for ARG-007 as a therapy for moderate TBI – a huge unmet global medical need.*

- *Argenica is now establishing a globally renowned Clinical Advisory Committee to be chaired by leading Australian neurologist Clinical Professor Terry O'Brien and supported by world leading TBI neurology clinicians from across Australia and the US, to provide advice and input into a clinical development plan for ARG-007 in TBI.*

**Perth, Australia; 18 June 2025 -** Argenica Therapeutics Limited (ASX: AGN) ("Argenica" or the "Company"), a biotechnology company developing novel therapeutics to reduce brain tissue

[1] ASX Announcement dated 15 May 2024

[2] ASX Announcements dated 22 June 2023, 15 May 2024 and 4 February 2025, as well as published data including Chiu et al, 2020, *Effect of Polyarginine Peptide R18D Following a Traumatic Brain Injury in Sprague-Dawley Rats*

death after stroke and other neurological conditions, is pleased to announce positive preclinical data in a large cohort ferret study assessing ARG-007 efficacy out to a 14-day duration post moderate traumatic brain injury (modTBI). Results from this recently completed preclinical study, together with previously completed studies[2], provides Argenica with a robust preclinical package which consistently exhibits significant protection conferred by ARG-007 in the treatment of modTBI.

TBI affects 69 million people globally annually, with no approved therapies available that can protect against the devastating brain injury sustained. The global TBI treatment market represents a significant commercial opportunity for Argenica, with ARG-007 poised to address a critical gap.

The latest ferret preclinical study showed ARG-007 was able to:

- **significantly reduce damage to the axons of brain cells** (neurons) as measured by amyloid precursor protein (APP) and neurofilament light (NFL) 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** and **improved memory** on a novel object recognition test, post injury.

Importantly, this study expanded on the previous ferret pilot study by assessing the extended duration effect of ARG-007 out to 14 days (compared to 3 days in the previous study), **confirming brain cell (neuron) protection was long lasting**, rather than transient. These biomarkers provide a translationally robust set of outcome measures for TBI research and drug development, paving the way for assessment of efficacy of ARG-007 in future clinical development in TBI patients. The fact that ARG-007 is effective at reducing both axonal injury and inflammation biomarkers up to 14 days post injury confirms the drug tackles the key secondary-injury cascades driving disability after TBI, in turn leading to a higher likelihood of functional benefits.

The study, undertaken by the University of Adelaide and partially funded by Argenica's CRC-P grant[3], was an extended duration study in a large cohort of ferrets to assess the therapeutic potential of ARG-007 in protecting brain cell (neuron) integrity for an extended period over both the acute and chronic phase of injury following modTBI. The study utilised an established ferret preclinical model of modTBI. 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

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

pathophysiological outcomes of TBI in humans. Animals were randomly allocated via a random number generator to sham (uninjured) (n = 11) or injury (n = 45). Injured ferrets were administered either vehicle (saline), a single dose of intravenous 100nmol/kg (0.3 mg/kg) ARG-007 (delivered 30 minutes post injury) or a double dose of ARG-007 with the second dose delivered subcutaneously 24 hours post injury at either 100nmol/kg or 300nmol/kg. There was no statistically significant difference between the results of the single dose of ARG-007 and the double dose groups of ARG-007 in any of the biomarker analysis. As only the single dose of ARG-007 produced statistical differences on behaviour assessments, only the single dose ARG-007 treatment animal data has been presented across the biomarker and behavioural analysis below. Further details of this study are provided in Appendix 1.

Dr Liz Dallimore, **Managing Director of Argenica**, commented "*This latest preclinical study in TBI provides exciting new data indicating the multifaceted protection provided by ARG-007 following modTBI is persistent and long lasting, rather than transient. This is critical for a TBI therapeutic, as we know damage can continue to occur weeks after the initial injury due to protein aggregation and inflammation. This data completes a comprehensive preclinical package on the efficacy of ARG-007 in modTBI and provides sound rationale to progress ARG-007 into clinical trials in TBI patients. We look forward to working with the newly established Clinical Advisory Committee to help us design a Phase 2 trial over the coming months.*"

*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 recently completed dosing in 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.

**APPENIDX 1 – 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 large cohort ferret study was to investigate whether ARG-007, delivered intravenously initially 30 minutes post injury, as well as the impact of a second dose, can improve motor and cognitive outcome with an associated reduction in axonal injury and neuroinflammation at 14 days following a mild-moderate diffuse head injury in ferrets. The animals were randomly allocated via a random number generator to sham (uninjured) (n = 11) or injury (n = 45), with ferrets administered either vehicle (saline; n = 12), a single dose of intravenous 100nmol/kg (0.3 mg/kg) ARG-007 (n =11) 30 minutes after injury or a double dose of ARG-007 with the second dose delivered subcutaneously 24 hours after injury at either 100nmol/kg (n=11) or 300nmol/kg (n-11).

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).

Statistical analysis: For IBA1 and GFAP a one-way ANOVA was performed followed by individual pair-wise comparisons. For NFL and APP protein biomarkers a multivariate analysis of variance (MANOVA) was performed, with pair-wise comparisons via LSD post-hoc testing. All graphs are presented as mean±SEM (standard error of the mean).

**STUDY RESULTS HIGHLIGHTS**

**There was no statistically significant difference between the results of the single dose of ARG-007 and the double dose groups of ARG-007 in any of the biomarker analysis. As only the single dose of ARG-007 produced statistical differences on behaviour assessments, only the single dose ARG-007 treatment animal data has been presented across the biomarker and behavioural analysis below.**

***ARG-007 Significantly Reduces Accumulation of axonal injury protein markers APP and NFL***

Damage to neurons following TBI 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 light (NFL) is a key biomarker for structural alterations within neuronal axons. NFL increases in injured axons within minutes following a TBI and continues to increase in the days to weeks post TBI. Residual lesions of NFL can persist for months after a severe TBI.

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

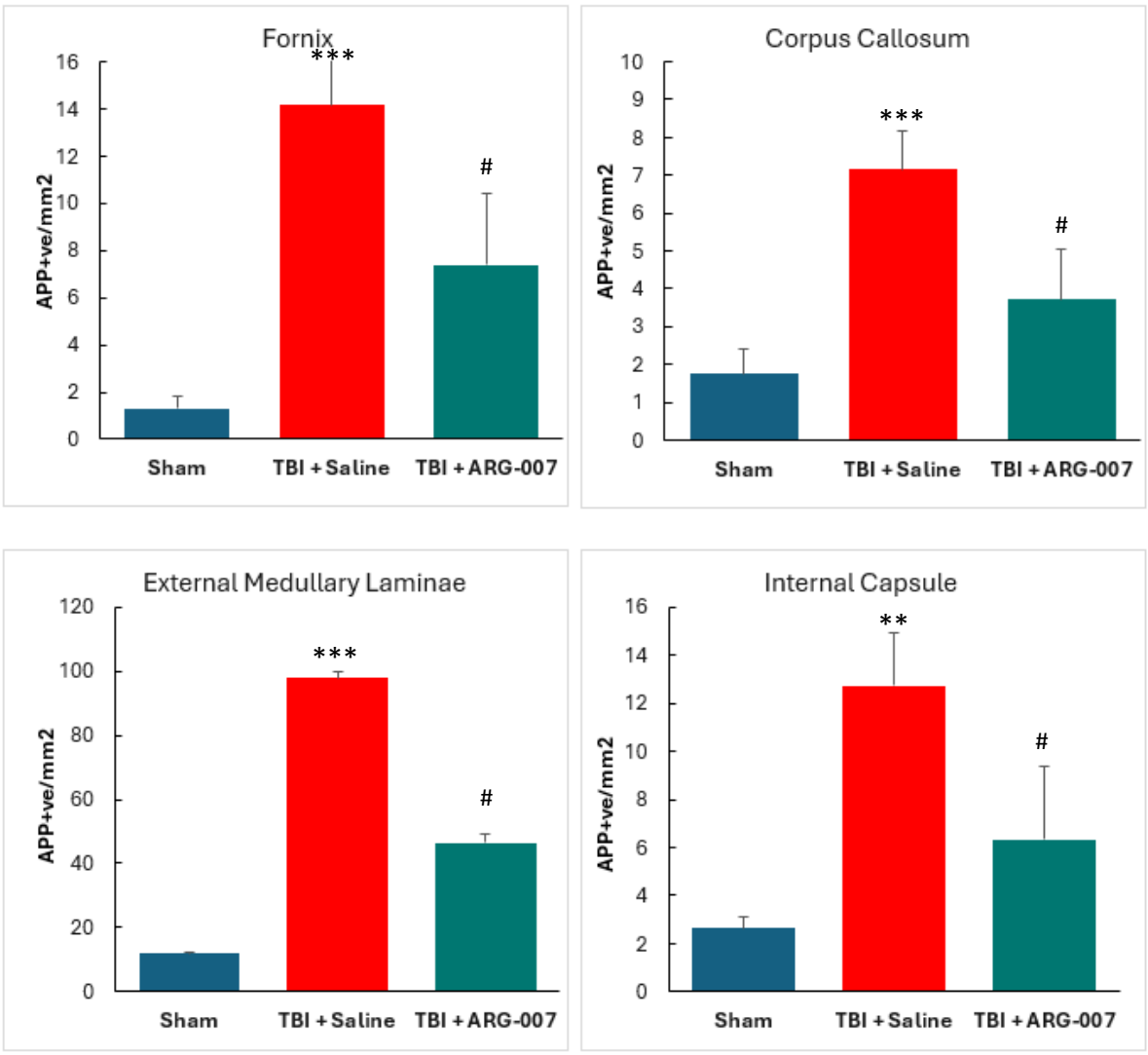


**FIGURE 1: ARG-007 protected axons from injury as assessed by APP accumulation in white matter tract brain regions.** *Injury significantly increased APP+ axons in all regions (***p<0.001, **p=0.01, TBI Injury + saline compared to shams) with ARG-007 treatment significantly reducing axonal injury in all four brain regions (#-p<0.05 TBI + ARG-007 compared to TBI Injury + Saline ferrets); Sham n-11, TBI+Saline n=9, TBI+ARG-007 n=10.*

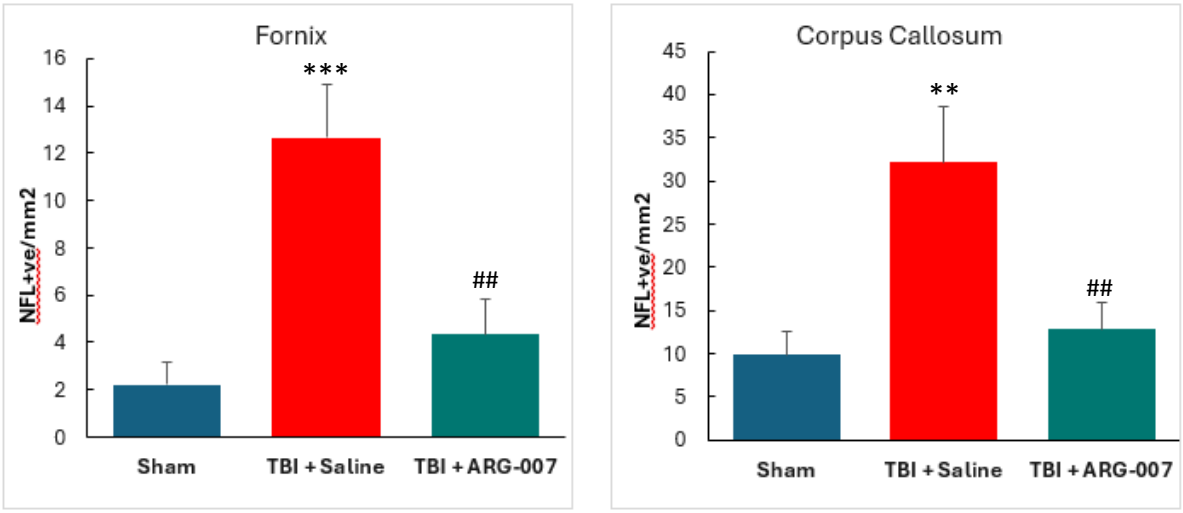


**FIGURE 2: Examination of the number of NFL+ lengths and bulbs in the corpus callosum and fornix.** *Injury significantly increased NFL+ axons in both the corpus callosum and fornix (**p=0.001; ***p<0.001, TBI Injury + saline compared to shams), with ARG-007 significantly reducing axonal injury in the corpus callosum and fornix ( ##p<0.01 TBI + ARG-007 compared to TBI Injury + Saline ferrets); Sham n-11, TBI+Saline n=10, TBI+ARG-007 n=10*

### *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 biomarkers 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). The level of Iba1 was also significantly reduced in the corpus callosum and the level of GFAP was also significantly reduced in the internal capsule (data not shown).

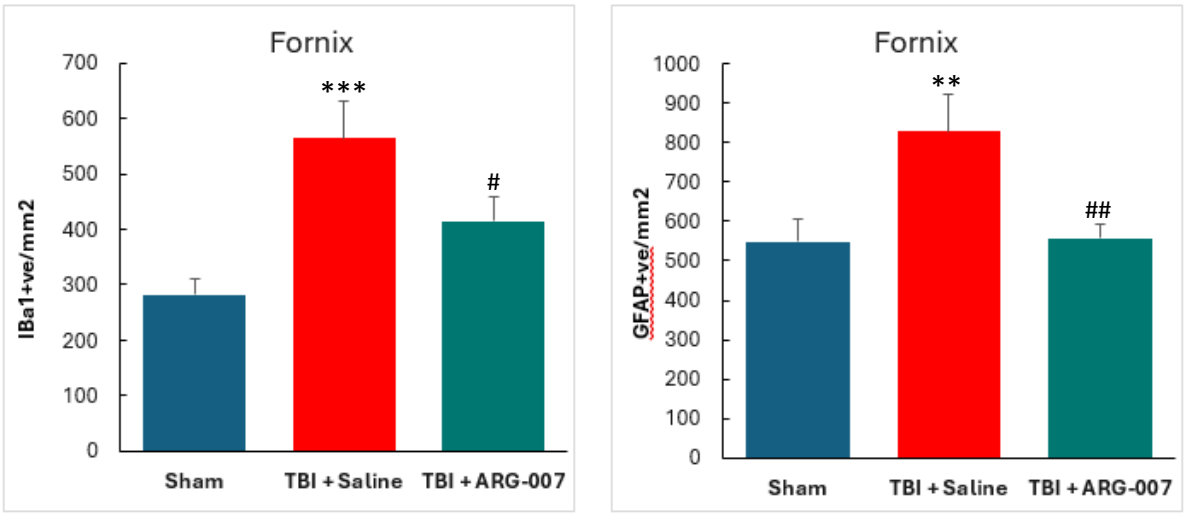


**FIGURE 3: ARG-007 reduced inflammation as assessed by the number of IBA1 and GFAP positive cells in white matter tract brain region associated with memory.** *Injury significantly increased inflammatory markers Iba1+ and GFAP in fornix (**p<0.01; ***p<0.001, TBI Injury + saline compared to shams), with ARG-007 significantly reducing axonal injury in the fornix (#p<0.05, ##p<0.01 TBI + ARG-007 compared to TBI Injury + Saline ferrets); Sham n=9, TBI+Saline n=10, TBI+ARG-007 n=9*

### *ARG-007 Improved Balance and Coordination, and memory following ModTBI*

Following injury, functional motor and cognition assessments were also undertaken using standardised techniques. For all motor and cognitive tests performed where TBI injury alone resulted in a deficit, ARG-007 administration resulted in a statistically significant improvement in balance and coordination in the ladder walk task of motor performance and the novel object recognition assessment of memory recognition. Specifically, balance and coordination were assessed on the challenging ladder walk task, to examine more subtle effects of injury on motor performance, with ferrets tested on day 1, 8 and 14 post-injury. A single dose of ARG-007 was sufficient to significantly improve outcomes ($p<0.05$) at all time points tested (day 14 shown in Figure 4), Recognition memory was tested in the novel object recognition (NOR) task where animals were first exposed to two identical objects and then provided with different novel objects one and twenty-four hours later to test short and long-term recognition memory. Ferret receiving single dose ARG-007 showed a significant difference to TBI + saline animals at both one and 24-hours later (24-hour data shown in Figure 4).

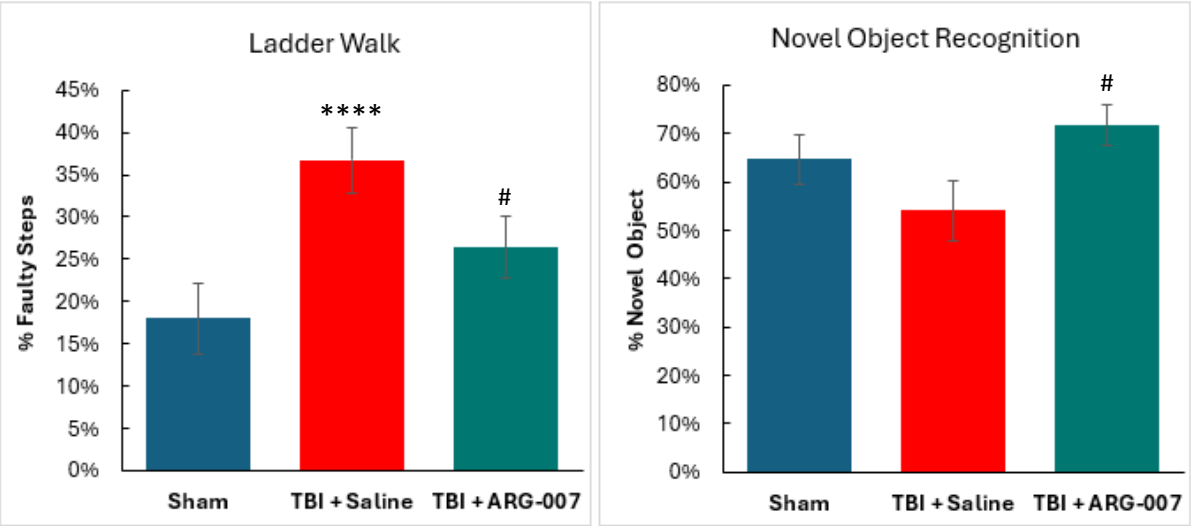


**FIGURE 4: ARG-007 improved both motor coordination as assessed by the percentage of faulty steps on a ladder, and memory as assessed by a novel object recognition test.** *TBI+Saline ferrets had significantly more faulty steps than sham ferrets at day 14, which was significantly improved with one dose of ARG-007 post-injury. In the novel object recognition test TBI+ARG-007 ferrets spent significantly more time with the novel object 24 hours after injury than TBI+saline ferrets (****p<0.0001; #p<0.05).*

## CONCLUSION

This larger extended 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 100nmol/kg may prevent functional deficits following injury, with an associated significant reduction in axonal injury and inflammation biomarkers. The results from this main study not only confirm the findings from the previous pilot ferret study but also confirm that a single dose of ARG-007 soon after injury results in a sustained prolonged benefit out to 14 days post injury.

Taken together with previous rodent studies, this will provide Argenica with a robust preclinical data set on the efficacy of ARG-007 in TBI.