ARG-007 PREVENTS UPTAKE AND AGGREGATION OF KEY NEURODEGENERATIVE PROTEIN LINKED TO PARKINSON'S AND ALZHEIMER'S DISEASES

Highlights:

- *Preclinical data published this week in the scientific journal* Biomedicines *shows ARG-007 (R18D) significantly reduces cellular uptake of Alpha-Synuclein (α-syn) protein aggregates* ***by 84%.***

- *Further, preclinical data confirms an inhibitory effect of ARG-007 on α-syn aggregation in a cell free assay, with the inhibitory effect increasing with increasing doses of ARG-007, resulting in reduction of α-syn aggregation* ***by 90%.***

- *Intracellular uptake and accumulation of insoluble protein deposits, such as α-syn, has emerged as a common element of major age-related neurodegenerative disorders, including Parkinson's Disease and Alzheimer's Disease.*

- *This data, taken together with data previously announced on the ability of ARG-007 to reduce Amyloid-Beta (Abeta) aggregation by greater than 50%, strengthens ARG-007's potential position as a broad neuroprotective therapeutic for a range of neurodegenerative diseases, including Alzheimer's Disease and Parkinson's Disease.*

Perth, Australia; 1 August 2023 - Argenica Therapeutics Limited (ASX: AGN) ("Argenica" or the "Company"), a biotechnology company developing novel therapeutics to reduce brain tissue death after stroke and other brain injuries, is pleased to announce preclinical data on the efficacy of ARG-007 in reducing the cellular uptake and aggregation of Alpha-Synuclein (α-syn) – a key hallmark of a number of neurodegenerative diseases including Parkinson's disease[1] and Alzheimer's Disease[2].

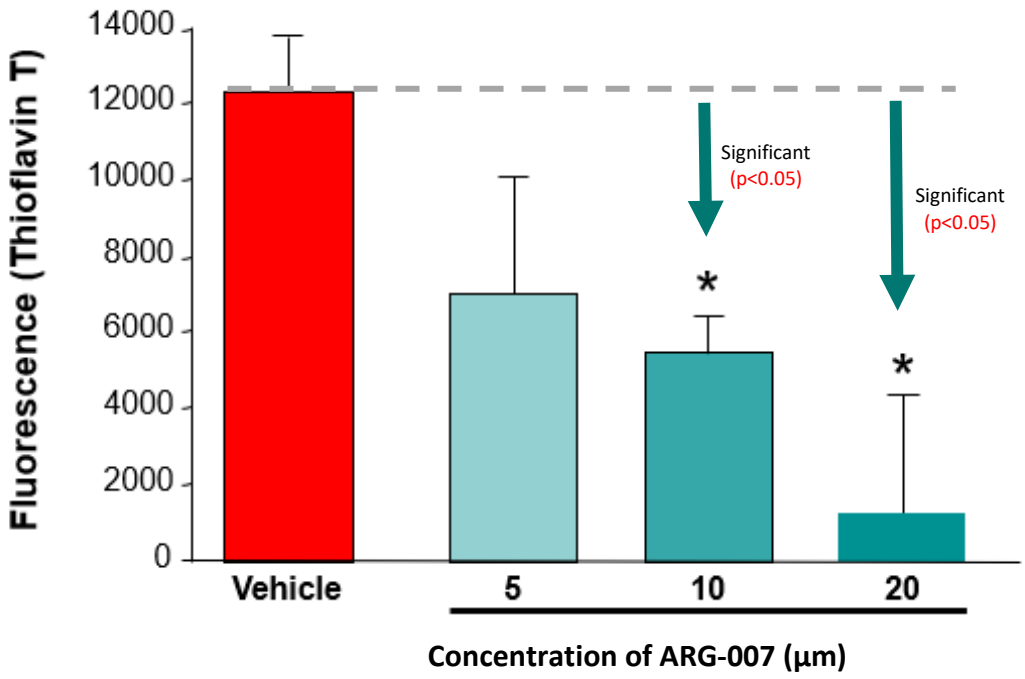
Intracellular accumulation of insoluble protein deposits such as α-syn has emerged as a common element of major age-related neurodegenerative disorders. Thus, targeting α-syn aggregation and cellular uptake could be an effective therapeutic strategy for neurodegenerative conditions including Parkinson's Disease and Alzheimer's Disease.

[1] Calabresi, P., Mechelli, A., Natale, G. *et al.* Alpha-synuclein in Parkinson's disease and other synucleinopathies: from overt neurodegeneration back to early synaptic dysfunction. *Cell Death Dis* **14**, 176 (2023).
[2] Twohig, D., Nielsen, H.M. α-synuclein in the pathophysiology of Alzheimer's disease. *Mol Neurodegeneration* **14**, 23 (2019).

Data published this week in the scientific journal *Biomedicines,* from the laboratory of Argenica's Chief Scientific Officer, Prof Bruno Meloni, sought to determine whether ARG-007 (R18D), with its confirmed neuroprotective actions, had the capacity to prevent cellular uptake of α-syn aggregates.

Data presented in the published study confirms ARG-007 significantly reduces the cellular uptake of α-syn protein aggregates into enteroendocrine cells, which are a key site of α-syn misfolding and aggregation. Further, without ARG-007 treatment, the paper confirms that the α-syn protein aggregates are readily taken up into cells[3].

Importantly, additional data recently generated in Prof Meloni's laboratory also confirms a dose-dependent inhibitory effect of ARG-007 on α-syn aggregation in a cell free assay. The study tested the efficacy of three different concentrations of ARG-007 (5, 10 or 20 µM) to reduce human α-syn aggregation in an *in vitro* model. The study demonstrated that as the dose level of ARG-007 increased, so too did the inhibitory effect on α-syn increase, with the highest level (20 µM concentration) of ARG-007 tested showing a reduction of α-syn protein aggregation by 90% compared to untreated controls (see below and Figure 2 of Appendix 1).



ARG-007 inhibits α-synuclein protein aggregation in cell free assay. ARG-007 (5, 10 or 20 µM) incubated with α-synuclein monomer (20 µM), α-synuclein seed aggregates (10 nM) and the fluorescent marker Thioflavin T (25 µM) at 37°C for 24 hours. Thioflavin T fluorescence measured at 450 nm excitation/485 nm emission. * = $p < 0.05$; N = 3.

[3] Gorecki, A.M.; Spencer, H.; Meloni, B.P.; Anderton, R.S. The Poly-Arginine Peptide R18D Interferes with the Internalisation of α-Synuclein Pre-Formed Fibrils in STC-1 Enteroendocrine Cells. *Biomedicines* 2023, *11*, 2089. https://doi.org/10.3390/biomedicines11082089

One of the key hallmarks of Parkinson's Disease is the accumulation of aggregates of α-syn (known as Lewy bodies) in neurons, and its immunoreactivity is a highly specific biomarker for Parkinson's Disease. This data confirms ARG-007 reduces both cellular uptake and aggregation of α-syn, two critical components of the progression of neurodegenerative diseases such as Parkinson's Disease. Further, the results from the data may also be beneficial for Alzheimer's Disease as clinical data is beginning to emerge regarding the presence of α-syn pathology in patients with that disease[4].

This data, taken together with data presented on the ability of ARG-007 to reduce Amyloid-Beta (Abeta) aggregation (announced 9 February 2023) and the broader neuroprotective potential of ARG-007, *strengthens the underlying scientific hypothesis that ARG-007's may have therapeutic potential as a broad neuroprotective with possible application in* a range of neurodegenerative diseases, including Alzheimer's Disease and Parkinson's Disease.

Dr Liz Dallimore, Argenica's Managing Director, said "The data released today is extremely encouraging. If you also consider it with our Abeta data previously announced, it is even more exciting and encouraging. The scientific community now understands that neurodegenerative diseases are extremely complex, however the aggregation and accumulation of several proteins in the brain appear to be an important contributor. The ability of a therapy such as ARG-007 to work on a number of these protein aggregates is very important from a scientific perspective, and we look forward to progressing preclinical studies in this area further."

NEXT STEPS

Argenica will continue to progress preclinical studies looking at the efficacy of ARG-007 in Alzheimer's Disease, with data from a mouse model of Alzheimer's Disease expected early calendar year 2024. Further consideration will now also be given to potential Parkinson's Disease animal studies Argenica's Chief Scientific Officer, Prof Meloni, can initiate.

[4] Twohig, D., Nielsen, H.M. α-synuclein in the pathophysiology of Alzheimer's disease. *Mol Neurodegeneration* **14**, 23 (2019).

APPENDIX 1 – STUDY DATA

Details of the α-syn cellular uptake study can be found in the recently published paper: Gorecki, A.M.; Spencer, H.; Meloni, B.P.; Anderton, R.S. The Poly-Arginine Peptide R18D[5] Interferes with the Internalisation of α-Synuclein Pre-Formed Fibrils in STC-1 Enteroendocrine Cells. *Biomedicines* 2023, *11*, 2089. https://doi.org/10.3390/biomedicines11082089

The study used an *in vitro* model of enteroendocrine cells (STC-1 cells) to study the uptake of α-syn into cells, utilising fluorescent tags and immunohistochemistry to quantify the amount of α-syn, as well as ARG-007 uptake. STC-1 cells were pre-treated with R18D for 10 minutes prior to and during incubation with pre-formed α-syn fibrils (PFF-488) for two hours. Media was aspirated after two hours, and cells were fixed and immunostained for human α-syn for confocal microscopy.

The data shows that treatment with R18D clearly prevents cellular uptake compared to the PFF-488 group (PFF; no treatment). The mean fluorescence intensity of human α-syn was significantly higher in the PFF group compared to untreated controls and R18D-PFF group ($p < 0.05$). See Figure 1 below.

To examine the capacity of R18D/ARG-007 to inhibit α-syn aggregation, different concentrations of R18D (5, 10 or 20 µM) were incubated with recombinant human α-syn protein monomer (20 µM: Abcam; ab218818) and recombinant human α-syn aggregates or seeds (10 nM: Abcam; ab218819). The mixture also contained the fluorescent dye Thioflavin T (25 µM) to detect and quantify the formation of α-syn protein aggregates.

Thioflavin T fluorescent emission is directly proportional to the level of α-syn aggregates. The α-syn aggregation assay was performed at 37°C, over 24 hours before fluorescent intensity (450 nm excitation/485 nm emission) measured using the Cytation 5 microplate reader. After the 24-hour incubation, R18D reduced α-syn aggregation in a concentration dependent manner, with the 10 µM and 20 µM concentrations reducing aggregation by 55% and 90%, respectively ($p = 0.048$ and $p = 0.004$; $N = 3$). See Figure 2 below.

[5] Note ARG-007 is termed R18D in the scientific literature.

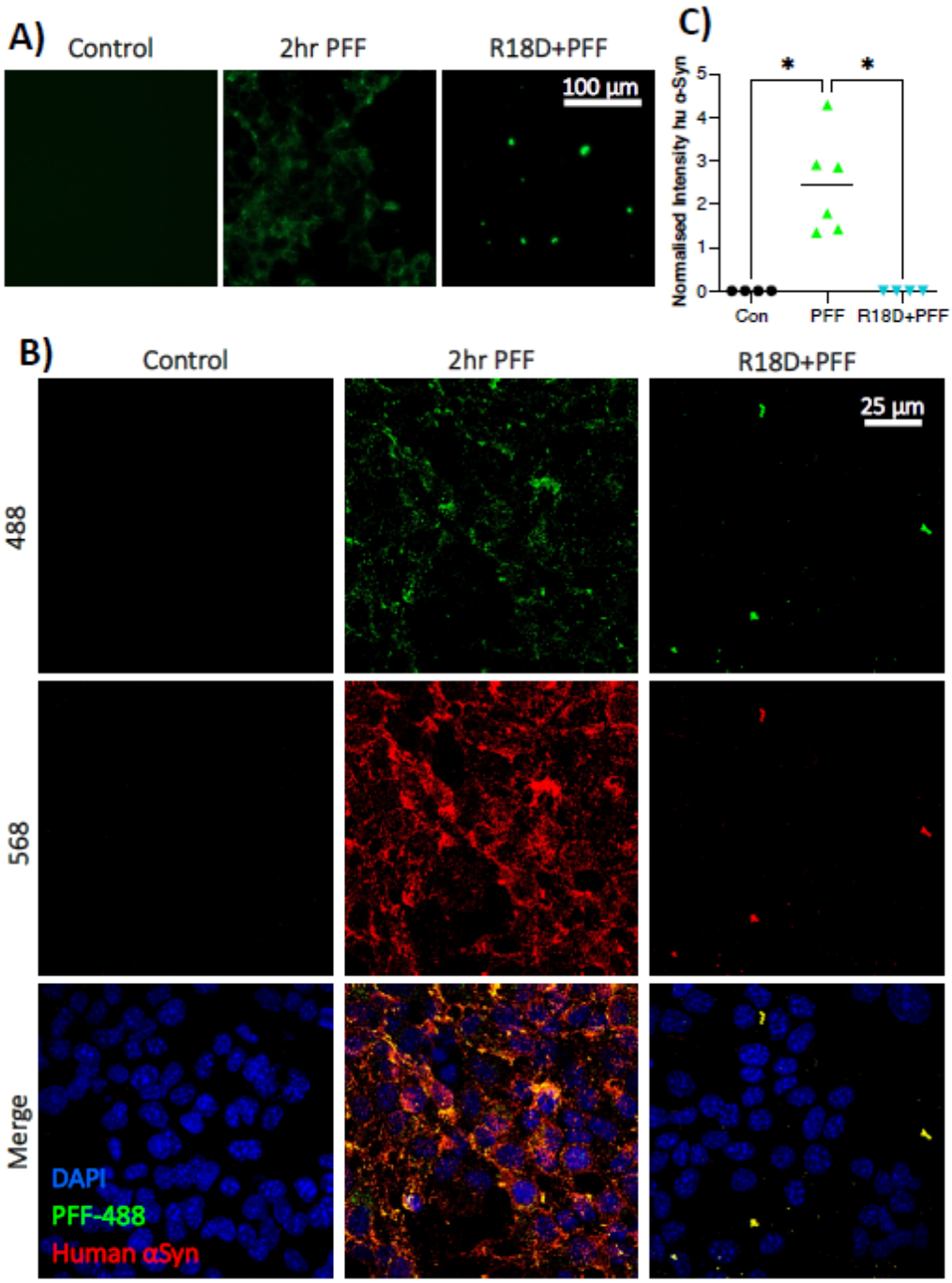


Figure 1. Cationic arginine-rich peptide R18D prevents PFF-488 uptake by STC-1 cells *in vitro*. STC-1 cells were pre-treated with R18D for 10 minutes prior to and during incubation with PFF-488 for two hours. (A) Live cell imaging (20x magnification) demonstrates that R18D prevents PFF uptake (scale bar represents 100 µm). (B) Media was aspirated after two hours, and cells were fixed and immunostained for human α-synuclein and DAPI for confocal microscopy. Treatment with R18D clearly prevents cellular uptake compared to the PFF-488 group. Images are maximum intensity projections of confocal z-stacks acquired at 100x magnification (scale bar represents 25 µm). (C) Mean fluorescence intensity of human α-synuclein was significantly higher in the PFF-488 group compared to untreated controls and PFF-R18D group, * = $p < 0.05$.

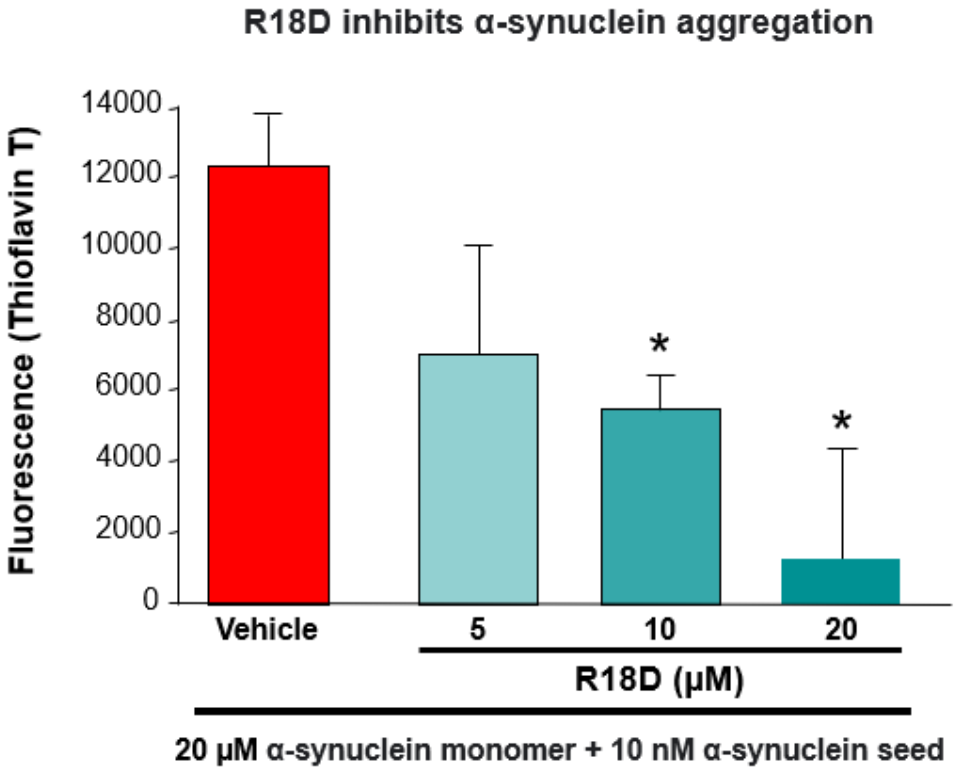


Figure 2: R18D inhibits α-synuclein protein aggregation in cell free assay. R18D (5, 10 or 20 μM) incubated with α-synuclein monomer (20 μM), α-synuclein seed aggregates (10 nM) and Thioflavin T (25 μM) at 37°C for 24 hours. Thioflavin T fluorescence measured at 450 nm excitation/485 nm emission. * = $p < 0.05$; N = 3.

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.