PRECLINICAL DATA SHOWS ARG-007 INHIBITS ONE OF THE MAIN CAUSES OF ALZHEIMER'S DISEASE

Highlights:

- *Preclinical data has shown ARG-007 significantly inhibited the aggregation of human recombinant Amyloid-Beta (Abeta) in a cell-free Abeta aggregation assay model.*
- *Abeta aggregation is thought to be one of the main causes of Alzheimer's Disease, with the Abeta accumulation in senile plaques causing memory loss and confusion.*
- *At 16 hours following ARG-007 administration, a 25 µM dose of ARG-007 reduced Abeta aggregation by more than 50% compared to vehicle controls.*
- *Argenica will now progress to animal studies to further confirm the efficacy of ARG-007 in Alzheimer's Disease and will update the market as milestones are meet.*
- *The global Alzheimer's therapeutics market size was valued at USD4.04 billion in 2021 and is expected to expand at a compound annual growth rate (CAGR) of 16.2% from 2022 to 2030.*

Perth, Australia; 9 February 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 positive initial preclinical data on ARG-007's ability to inhibit human recombinant Amyloid-Beta (Abeta) aggregation in a preclinical (*in vitro*) model of Alzheimer's Disease. Abeta aggregation is thought to be one of the main causes of Alzheimer's Disease, with the Abeta accumulation in senile plaques causing memory loss and confusion.[1]

Argenica engaged leading preclinical Contract Research Organisation QPS, based in Austria, to undertake the study. The aim of the study was to determine the effects of ARG-007 in comparison to controls in inhibiting human recombinant Abeta aggregation using the cell-free Abeta aggregation assay.

In this study, three concentrations of ARG-007 were used to determine the drug's efficacy in inhibiting human recombinant Abeta aggregation – 2.5 µM, 7.5 µM and 25 µM. Abeta

[1] Li et al, 2021, **Amyloid-Beta Influences Memory via Functional Connectivity During Memory Retrieval in Alzheimer's Disease**, *Front. Aging Neurosci*, Volume 13

aggregation was assessed at 4 hours, 10 hours and 16 hours post administration of ARG-007. The results of this study show ARG-007 has a positive effect in inhibiting Abeta aggregation at the 10 hour and 16 hour post administration time points, compared to the vehicle controls. At the 16-hour time point, when Abeta had reached maximum aggregation, all three concentrations of ARG-007 showed a large significant reduction in Abeta aggregation compared to the control, with the 25 µM showing a greater than 50% reduction in Abeta aggregation, as shown in the graph below:

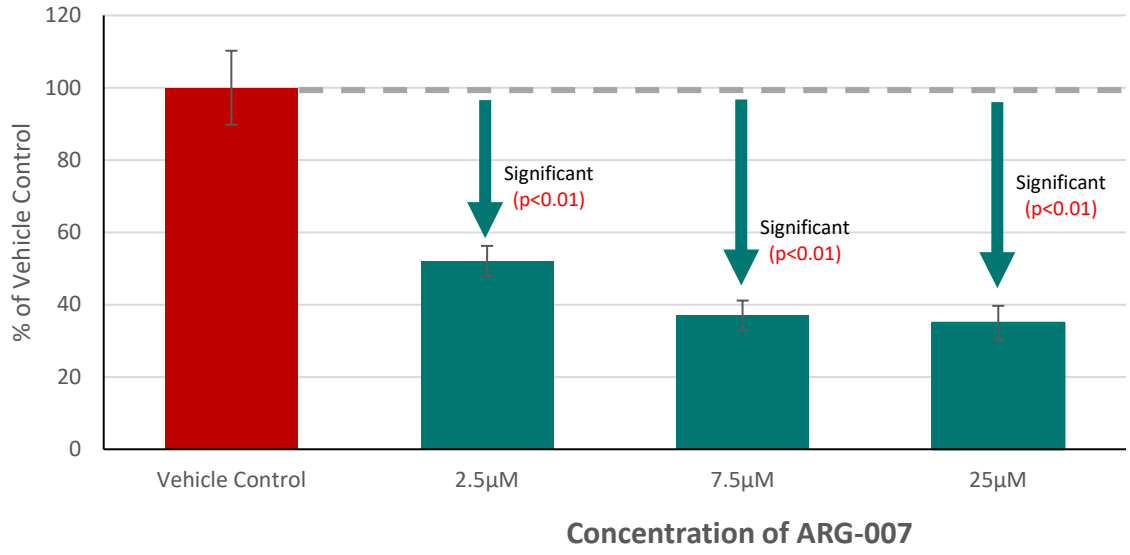

Figure 1. An assessment of the extent of Abeta aggregation inhibition at Abeta plateau, 16 hours after the administration of ARG-007, at three different concentrations of the drug, 2.5 µM, 7.5 µM and 25 µM. Data are shown as % of vehicle control (VC) and displayed as bar graphs with group means +SEM (n=4 per group). Statistical analysis involved a two-tailed ANOVA followed by Bonferroni's Multiple comparison Test (*post hoc* test) compared to VC *p<0.05; **p<0.01; ***p<0.001.

This *in vitro* (in petri dish) cell-free Abeta aggregation assay model provides important insights into the pathogenesis of Alzheimer's Disease by simulating the disease in a less complex environment compared to *in vivo* (in living organism) systems. It provides preliminary information on mechanisms and possible protective roles of ARG-007 in Alzheimer's Disease.

Dr Liz Dallimore, Argenica's Managing Director, said "This is extremely encouraging data showing a potential new indication for ARG-007. It is well recognised that Abeta aggregation in the brain plays a key role in initiating Alzheimer's Disease, and therefore a safe therapeutic drug that can reduce Abeta aggregation is a huge opportunity. We look forward to continuing to progress this exciting opportunity into further animal studies."

The global Alzheimer's Disease therapeutics market size was valued at USD4.04 billion in 2021 and is expected to expand at a compound annual growth rate (CAGR) of 16.2% from 2022 to

2030[2]. According to the World Health Organization, the economic cost burden will range from USD 1.3 trillion to 2.8 trillion by 2030[2]. Alzheimer's Disease accounts for 60-70% of all cases of dementia, with government and non-government organizations investing extensively in the development of diagnostics and therapies for the disease as a result of the rising prevalence of the disease worldwide[3]. The only approved drug to treat Alzheimer's Disease, Aducanumab, targets the Abeta protein, however concerns have been raised regarding its safety[4].

Further details of this *in vitro* preclinical study are provided in Appendix 1.

NEXT STEPS

Argenica has now further engaged QPS to undertake an *in vivo* study in 5xFAD mice, a model of familial Alzheimer's Disease. The aged mice will receive multiple doses of ARG-007 over a an extended prior of time, with results to assess the effect on Abeta levels and plaques, Tau protein levels, neuroinflammation, and neurodegeneration. Results of this study will be announced to the market as they come to hand.

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

For more information please contact: info@argenica.com.au

ABOUT ARGENICA

Argenica (ASX: AGN) is developing novel therapeutics to reduce brain tissue death after stroke and improve patient outcomes. Our lead neuroprotective peptide candidate, ARG-007 has been successfully demonstrated to improve outcomes in pre-clinical stroke models and is in the process of being verified for its safety and toxicity before commencing Phase 1 clinical trials in humans. The aim is for our therapeutic to be administered by first responders to protect brain tissue against damage during a stroke with further potential to enhance recovery once a stroke has taken place.

[2] **https://www.grandviewresearch.com/industry-analysis/alzheimers-therapeutics-market**

[3] **Alzheimer's Therapeutics Market Share, Size, Trends By Drug Class, By Distribution Channel, By Region, Segment Forecast, 2022 - 2030**

[4] Gleason A, Ayton S and Bush AI, 2022, **Does the FDA-approved Alzheimer drug aducanumab have a place in the Australian pharmacopoeia?**, *The Medical Journal of Australia*, Volume 216, Issue 4, pgs 172-174.

APPENDIX 1 – STUDY OUTLINE

Study Aim and Design:

The aim of the study was to examine the effects of ARG-007 in comparison to controls to counteract Abeta aggregation in the cell-free $Abeta_{1-42}$ aggregation assay. The test substance (ARG-007) was transferred to QPS' lab by Argenica. The effects of ARG-007 at three different concentrations were then tested in the cell-free $Abeta_{1-42}$ aggregation assay using human recombinant monomeric $Abeta_{1-42}$ in comparison to the reference item Tannic acid (positive control) and the vehicle control.

Overview of Abeta Aggregation Assay:

The Abeta aggregation assay involves incubating the Abeta peptide in a buffer solution over time and monitoring the aggregation of the peptide. To measure the Abeta aggregation, ThioflavinT (THT) is added to the Abeta and buffer solution. THT binds to amyloid fibrils (i.e. aggregated Abeta) and gives a strong fluorescence signal at a wavelength of approximately 482 nm when excited at 450 nm. The amount of fluorescence can then be quantified on a fluorescence reader. The fluorescence reading determines the kinetics, or rate, at which the Abeta aggregates. Typically, there is a lag in Abeta aggregation followed by a rapid growth phase of aggregation, which then plateaus. The initial "lag phase" of this curve represents the formation of oligomeric nuclei. This is followed by a rapid "elongation" or growth phase, in which the nuclei subsequently grow into larger, fibrils due to the incorporation of additional monomers, until a plateau is reached that is considered the endpoint of amyloid formation.

In the current experiments the rapid period of Abeta aggregation is happening at approximately the 8-12 hour time points from initiation of the experiment, and from 12 to 16 hours Abeta aggregation plateaus (see Figure 1).

Materials and Methods:

The cell-free $Abeta_{1-42}$ aggregation assay was performed according to QPS's standard operating procedure (SOP) NMET152. A 222 µM (=1 mg/mL) Aβ1-42 (rPeptide CatNo. A-1167-2) stock was prepared in 5 mM Tris. Thereafter, Aβ1-42 was immediately diluted within TBS (50 mM Tris, 150 mM NaCl, pH 7.4) to yield the 2x Aβ working solution containing 50 µM Aβ1-42. 20 µL of this 2x Aβ working solution was combined with 10 µL 160 µM THT solution and 10 µL 4x concentrated Test (i.e., 10, 30 and 100 µM) or Reference item (R.I. i.e., 400 µM) solution in black half area plates, to have final concentrations of 25 µM Aβ, 40 µM THT and the respective concentrations of ARG-007. or R.I. in the well. Appropriate Vehicle and buffer controls were included in the analysis.

The assay was carried out with four technical replicates. In this study, the 16 h time point is when the $Abeta_{1-42}$ aggregation plateaued. Immediately after preparation a baseline measurement was carried out and kinetics of $Abeta_{1-42}$ aggregation was monitored at every

10 min for 16 h while incubating at 37°C, by using 450 nm excitation and 485 nm emission fluorescence mode on a Multimode Reader Cytation 5 (BioTek).

Statistics:
Basic statistical analysis was performed. Data are shown as % of vehicle control (VC) and displayed as bar graphs with group means +SEM (n=4 per group). Two-way ANOVA followed by Bonferroni's Multiple comparison Tet (*post hoc* test) compared to VC *p<0.05; **p<0.01; ***p<0.001.

Results:

The study was performed to examine the effects of ARG-007, in comparison to controls, to counteract Abeta$_{1-42}$ aggregation in the cell-free Abeta$_{1-42}$ aggregation assay using human recombinant monomeric Abeta$_{1-42}$.

The aggregation of Abeta$_{1-42}$ was monitored over time. For analysis of group differences, the change in relative fluorescence units (ΔRFU) from 4 h, 10 h or 16 h and 10 min measurement was calculated, representing the increase in THT signal over the time of aggregation. In this experiment, at 4 h and 10 h the assessments were taken during the lag phase of Abeta$_{1-42}$ aggregation and while aggregation was increasing, respectively, and the 16 h time point is when the plateau in Abeta$_{1-42}$ aggregation was reached in the controls. A reduction in ΔRFU values by adding different concentrations of ARG-007 or the reference item can be interpreted as a positive effect in inhibition of Abeta$_{1-42}$ aggregation.

A strong increase in fluorescence was observed over time, indicating formation of amyloid fibrils, which could be counteracted with the reference item Tannic Acid (TA) (Fig. 1A). The reference item led to a significant decrease of all calculated ΔRFUs compared to vehicle control (Fig. 1B). This confirmed the validity of the assay.

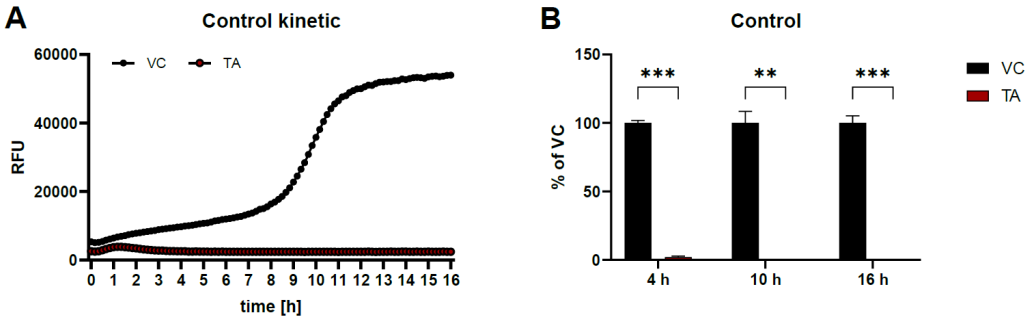

Figure 1: Effect of the reference item Tannic Acid on Abeta1-42 aggregation
(A) Kinetics of Abeta$_{1-42}$ aggregation with vehicle (black) or reference item Tannic Acid (TA) (red) incubation monitored over 16 h. (B) Kinetics (ΔRFU) using subtraction of RFU from 10 min measurement from the RFU from the 4 h, 10 h or 16 h measurement. Data are shown as % of vehicle control (VC) and displayed as bar graphs with group means +SEM (n=4 per group). Two-tailed ANOVA followed by Bonferroni's Multiple comparison Test (*post hoc* test) compared to VC **p<0.01; ***p<0.001.

Evaluation of Abeta$_{1-42}$ aggregation after 4 h revealed a significant increase with all tested concentrations of **ARG-007** compared to vehicle control. However, and importantly, ARG-007 significantly inhibited Abeta aggregation in the growth phase of aggregation (between 8 to 10 hours later). Further, calculating ΔRFU at the 16 h time point, when Abeta amyloid fibril formation and aggregation had plateaued, showed a significant reduction in Abeta$_{1-42}$ aggregation with all tested concentrations of ARG-007 (Fig. 2 A,B). Importantly, the data at the 16 h time point, when the plateau in Abeta$_{1-42}$ aggregation was reached, demonstrated a dose dependent reduction in ΔRFU values for ARG007, i.e. a lower formation of amyloid fibrils indicating ARG-007 may have the potential to inhibit Abeta$_{1-42}$ aggregation *in vivo*.

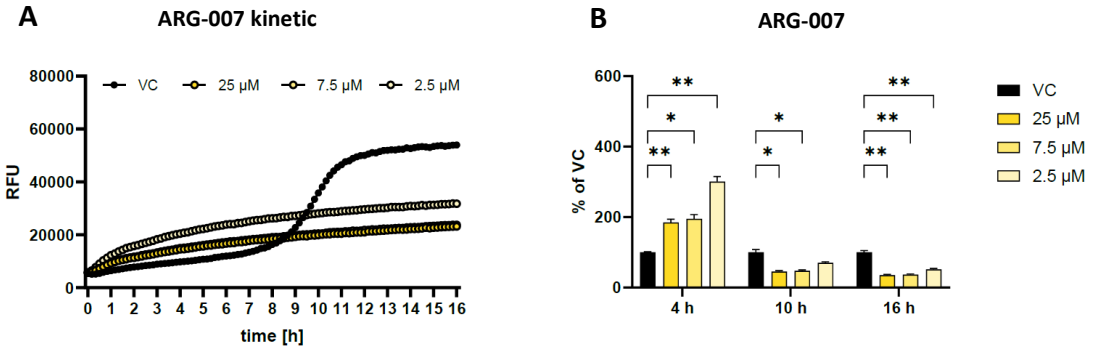

Figure 2: Effect of ARG-007 on Abeta1-42 aggregation
(A) Kinetics of Abeta1-42 aggregation with vehicle (black) or ARG-007 (yellow) incubation monitored over 16 h. (B) Kinetics (ΔRFU) using subtraction of RFU from 10 min measurement from the RFU from the 4 h, 10 h or 16 h measurement. Data are shown as % of vehicle control (VC) and displayed as bar graphs with group means +SEM (n=4 per group). Two-tailed ANOVA followed by Bonferroni's Multiple comparison Test (*post hoc* test)
compared to VC *p<0.05; **p<0.01; ***p<0.001.

The data in figure 1 shows that at the point when Abeta formation is rapidly expanding (10 hour time point) and then the aggregation plateauing (16 hour time point), ARG-007 is having an inhibitory effect, preventing Abeta aggregation from moving into the rapid expansion phase. This provides preliminary confirmation that ARG-007 could be a useful therapeutic in preventing rapid Abeta aggregation in Alzheimer's Disease.