PRECLINICAL DATA SHOWS ARG-007 INHIBITS A SECOND MAIN CAUSE OF ALZHEIMER'S DISEASE

Highlights:

- *Preclinical data from two separate studies shows **ARG-007 significantly inhibits** the cellular uptake and aggregation of **tau protein** in two different* in vitro *Alzheimer's disease models.*

- *The aggregation of tau protein into tangles inside brain cells, and the spread of abnormal tau within the brain through cellular uptake, is thought to be a significant cause of Alzheimer's disease, with the spread and cellular uptake of abnormal tau a key hallmark of Alzheimer's disease progression[1].*

- *These two studies confirm ARG-007 has the capacity to **significantly reduce the uptake of abnormal tau into brain cells**, with a **68% and 49% reduction** seen in the two different studies, thereby potentially limiting the spread of abnormal tau between brain cells.*

- *The studies also confirm ARG-007 **inhibits intracellular tau aggregation by up to 89% in the first study and 35% in the second study**.*

- *This data, together with previously reported data[2], now confirms ARG-007 has a significant impact on a number of key proteins implicated in neurodegenerative diseases, namely **beta-amyloid, alpha-synuclein, and now tau**, making ARG-007 an exciting multi-modal protein targeting therapeutic.*

- *Argenica is continuing to progress* in vivo *animal studies to further confirm the efficacy of ARG-007 in Alzheimer's disease and will update the market as milestones are met.*

Perth, Australia; 3 November 2023 - Argenica Therapeutics Limited (ASX: AGN) ("Argenica" or the "Company"), a biotechnology company developing novel therapeutics to reduce brain tissue death after brain injury and neurodegeneration, is pleased to announce positive initial preclinical data on ARG-007's ability to inhibit the cellular uptake of abnormal tau and aggregation of tau protein in brain cells (neurons) in two different preclinical (*in vitro*) models of Alzheimer's disease. The aggregation of tau protein within neurons (intracellular tau

[1] Naseri NN, Wang H, Guo J, Sharma M, Luo W. The complexity of tau in Alzheimer's disease. Neurosci Lett. 2019 Jul 13;705:183-194. doi: 10.1016/j.neulet.2019.04.022. Epub 2019 Apr 25. PMID: 31028844; PMCID: PMC7060758.
[2] ASX Announcements dated 9 February 2023 and 1 August 2023.

tangles) and the transfer of abnormal aggregated tau between neurons (tau cellular uptake) are thought to be significant contributors to Alzheimer's disease.[3]

ARG-007 IMPACT ON TAU UPTAKE IN NEURONS

Two different *in vitro* studies, undertaken by two different laboratories, were used to determine ARG-007's efficacy in inhibiting tau protein seed uptake into neurons and neuron-like cells. Varying concentrations of ARG-007 were tested, and each study identified a concentration which showed a statistically significant reduction in tau cellular uptake from control cells that had received no ARG-007 treatment.

The first study, undertaken by Creative Biolabs in New York, used tau seeds isolated from human Alzheimer's disease patients. The tau seeds were incubated with mouse cortical neurons (brain cells) to determine the extent to which the aggregated tau protein (seeds) was taken up into neurons. The results showed a **68.2% reduction in cellular uptake** at the 0.0375 µM concentration (Figure 1) compared to neurons exposed to tau seeds but with no treatment. The data was normalised to the no tau no treatment control as the baseline.

The second study was undertaken by Argenica's Chief Scientific Officer, Professor Bruno Meloni, at the Perron Neuroscience Research Institute. Professor Meloni utilised a human neuroblastoma neuronal-like cell line (SH-SY5Y) and incubated them with recombinant tau seeds. Professor Meloni tested five different concentrations of ARG-007 and compared the inhibition of tau uptake in SH-SY5Y cells that were not treated with ARG-007. This data was expressed as a percentage of tau uptake inhibition, with the no treatment control taken as no inhibition. The data showed an **48.7% inhibition in cellular uptake** of tau at the 0.8 µM concentration of ARG-007 (Figure 2). The data was normalised to the no tau no treatment control as the baseline.

The findings from these studies indicate that ARG-007 has the capacity to inhibit the uptake of tau into neurons, indicating a potential mechanism to limit the spread of tau aggregates between neurons. The spread of abnormal tau protein from neuron to neuron through cellular uptake is thought to be a leading cause of Alzheimer's disease progression[4]**..**

[3] Wegmann S, Biernat J, Mandelkow E. A current view on Tau protein phosphorylation in Alzheimer's disease. **Curr Opin Neurobiol.** 2021 Aug;69:131-138.
[4] Naseri NN, Wang H, Guo J, Sharma M, Luo W. The complexity of tau in Alzheimer's disease. Neurosci Lett. 2019 Jul 13;705:183-194. doi: 10.1016/j.neulet.2019.04.022. Epub 2019 Apr 25. PMID: 31028844; PMCID: PMC7060758.

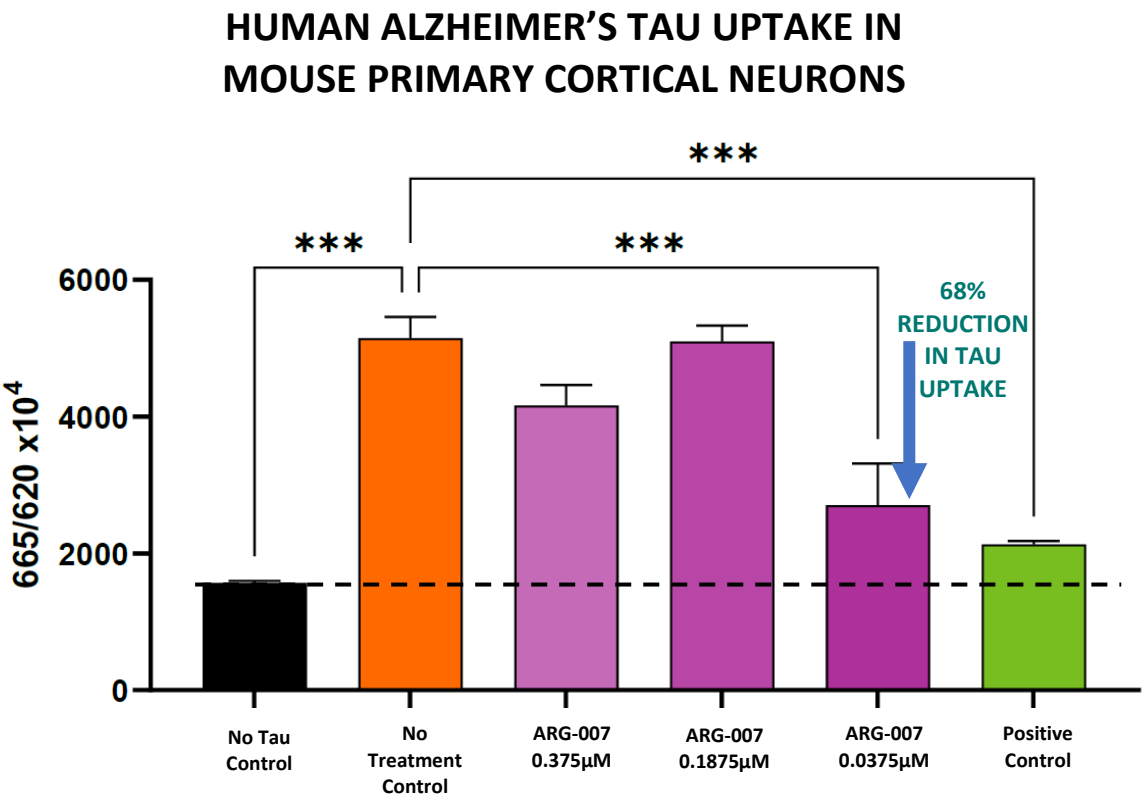

Figure 1. An assessment of the extent of cellular uptake of tau by cortical neurons from huma n tau seeds taken from human Alzheimer's disease patients. The administration of ARG-007, at three different concentrations of the drug, 0.375 µM, 0.1875 µM and 0.0375 µM, shows a statistically significant reduction in tau uptake in the 0.0375 µM treatment group compared to the no treatment control. Tau uptake was analysed with a tau aggregation assay (Cisbio) as signal ratio (665/620 nm x10^4). Data are displayed as bar graphs with group means +SEM (n=5-6 per groups, no treatment control n=12). One-way ANOVA followed by Dunnett's Multiple Comparison Test (*post hoc* test) compared to no treatment control (AD seed control). Outlier according to Grubbs outlier test as well as values above a threshold of 9,500 x 10^4 excluded. *P<0.05, **p<0.01, ***p<0.001.

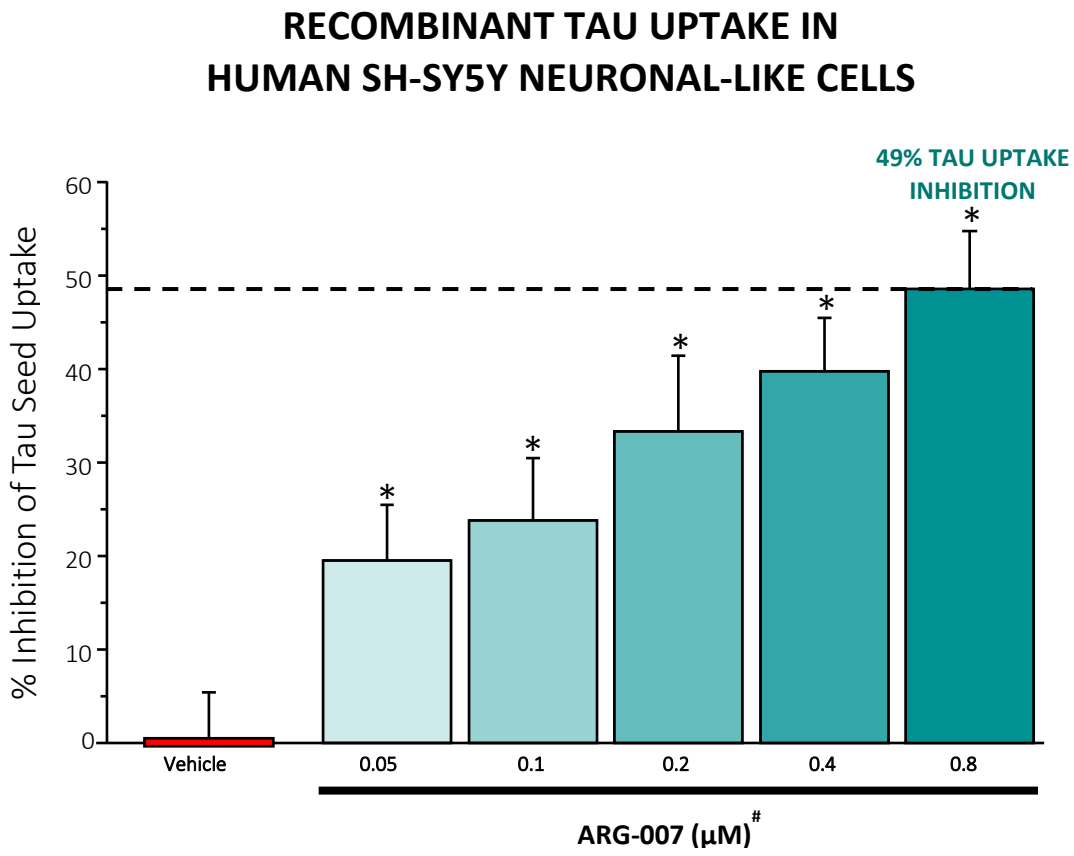

Figure 2: ARG-007 inhibits tau seed uptake in human SH-SY5Y cells. Tau seed (0.08 µM) was pre-incubated with R18D (0.05, 0.1, 0.2, 0.4 or 0.8 µM) for 15 minutes at room temperature prior to the addition of 50 µL of the mixture to SH-SY5Y cells cultured in 50 µL of medium (final volume 100 µL). Following a 48-hour exposure to SH-SY5Y cells, tau seed uptake was assessed by measuring intracellular tau aggregation levels using a homogenous time-resolved fluorescence (HTRF) tau aggregate assay (Cisbio). Fluorescence data have been transformed, with control (no tau seeds and no ARG-007 treatment; data not shown) taken as background fluorescence and subtracted from all experimental groups, and vehicle (tau seeds and no ARG-007 treatment) taken as 0% inhibition of tau seed uptake. Data are means ± SE; N = 6, *P < 0.05 compared with vehicle. # Concentration during 15-minute pre-incubation.

ARG-007 IMPACT ON TAU AGGREGATION INSIDE NEURONS

The aggregation of tau into neurofibrillary tangles is a major hallmark of Alzheimer's disease. Both the Creative Biolabs study and the study undertaken by Professor Meloni examined the impact ARG-007 has on tau aggregation inside cells, the results are presented in Appendix 1. Both studies showed ARG-007 significantly reduced intracellular tau aggregation compared to no treatment controls.

The Creative Biolabs study found statistically significant reductions across all concentrations of ARG-007 tested (Appendix 1, Figure 3), with the 0.0375 µM concentration resulting in an **89% reduction in intracellular tau aggregation,** when data is normalised to the no tau no treatment control**.**

Professor Meloni's study found statistically significant reductions with the 0.1, 0.2 and 0.4 µM concentrations of ARG-007, with the 0.4 µM concentration resulting in a **35% inhibition of intracellular tau aggregation** (Appendix 1, Figure 4).

These studies in combination with previously reported data on the ability of ARG-007 to reduce beta-amyloid aggregation[5] suggest ARG-007 could have a significant impact on reducing the effects of key proteins implicated in Alzheimer's disease.

Dr Liz Dallimore, **Argenica's Managing Director**, said "This ability of ARG-007 to reduce tau cellular uptake and abnormal aggregation of tau within neurons in these two preclinical studies is extremely encouraging. It provides further confirmation that ARG-007 can act on multiple targets and in multiple ways, making it an ideal drug candidate for neurodegenerative conditions such as Alzheimer's disease. 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 USD 4.04 billion in 2021 and is expected to expand at a compound annual growth rate (CAGR) of 16.2% from 2022 to 2030[6]. 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 organisations investing extensively in the development of diagnostics and therapies for the disease as a result of the rising prevalence of the disease worldwide[7]. There are currently no approved drugs targeting tau protein aggregation and cellular uptake.

Further details of these *in vitro* preclinical studies are provided in Appendix 1.

NEXT STEPS

Argenica is continuing to work with QPS to undertake an *in vivo* study in 5xFAD mice, a model of familial Alzheimer's disease. The mice will receive multiple doses of ARG-007 over a an extended period of time, with results to assess the effect on beta-amyloid 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

[5] ASX Announcement dated 9th February 2023 – Preclinical Data shows ARG-007 inhibits one of the main causes of Alzheimer's Disease.
[6] https://www.grandviewresearch.com/industry-analysis/alzheimers-therapeutics-market
[7] Alzheimer's Therapeutics Market Share, Size, Trends By Drug Class, By Distribution Channel, By Region, Segment Forecast, 2022 - 2030

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 1

Creative Biolabs Study Outline

Study Aim and Design:

The aim of the study was to examine the effect of ARG-007 at three concentrations on cellular tau aggregation and uptake in mouse primary cortical neurons.

Brain tissue from Alzheimer's disease patients (AD, Braak V/VI) was purchased from Netherland Brain Bank by Creative Biolabs and sarcosyl-insoluble tau seeds were extracted according to the manuscript from Julian et al., (2021).

Primary cortical neurons were isolated from E18 C57BL/6 mouse embryos, cultivated and on day *in vitro* (DIV) 9 used for experimentation. Cells were then treated with human AD tau-seeds and ARG-007 at 3 concentrations for 48 hours with Lipofectamine 2000 (LP2000) to assess intracellular tau seeding and without LP2000 to assess tau cellular uptake. Controls included a no seed control (no seed), and AD tau- seeded cells treated with either vehicle (VC) or reference item (RI; HT7 anti-tau monoclonal antibody). The experiments were performed with n = 5-6 treatment replicates and 12 vehicle control replicates per study.

Tau aggregation was evaluated with a Cisbio Homogeneous Time Resolved Fluorescence (HTRF) aggregation detection kit (Cisbio; 6FTAUPEG) and all results were compared with the VC.

Materials and Methods:
Primary cortical neurons were prepared from timed pregnant wild-type C57BL/6JRccHsd mice at E18.

Tau uptake assay
20 µg total protein/well of brain extracts (AD-tau seeds) were mixed with 7.5 pmol/well (5.625 µg/mL final concentration) of RI, vehicle (PBS) or ARG-007 at 3 different concentrations and incubated overnight at 4°C. On DIV 9, neurons were exposed to the AD-seed mixture (20 µL of 10x stocks per well) for 48 hours at 37°C. Cells were harvested on DIV 11 and tau aggregates measured using the HTRF detection kit.

Tau intracellular seeding assay
2.5 µg total protein/well of brain extracts (AD-tau seeds; ~60 ng tau per µg protein) were mixed with RI positive control or vehicle (PBS) or ARG-007 at 3 different concentrations.

After an overnight incubation at 4°C, the AD-seeds (2.5 µg total protein/well) as well as the AD-seed mixtures were incubated with LP2000 (1 µL/2.5 µg protein) in Opti-MEM for 10 minutes at room temperature before exposure to DIV 9 neurons for 48 hours at 37°C. Cells were harvested on DIV 11 and tau aggregates measured using the HTRF detection kit.

HTRF detection kit assay

Tau aggregation kits were purchased from Cisbio (kit#6FTAUPEG) and used according to manufacturer's protocol.

Aggregated tau protein is detected using one specific monoclonal antibody, labelled either with Tb-Cryptate (donor) or with d2 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a laser light source triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm).

The antibody labelled with d2 or Tb binds to tau protein, when tau protein aggregates the antibody labelled with d2 or Tb come then to a close proximity generating FRET. Signal intensities are proportional to the number of aggregates formed.

Briefly, cells were lysed with 50 µL 1x lysis buffer supplemented with blocking reagent. Samples were used undiluted or 1:2 diluted for the tau aggregation measurement. The anti-human tau-d2 conjugate as well as the anti-human tau-Tb^{3+}-cryptate conjugate were diluted 1:50 in diluent solution and premixed. Thereafter, 10 µL of the lysates and 2.5 µL premixed conjugates were applied to a white 396 well plate and incubated approximately 20 hours at room temperature on a shaker. Fluorescence emission at two different wavelengths (665 nm and 620 nm) was performed on a multilabel plate counter (Victor 3V, PerkinElmer, SOP NEQU190). The signal ratio was calculated using the following formula: (Signal 665 nm / Signal 620 nm) x 10^4.

Statistics:

Basic statistical analysis was performed in Graph Pad Prism9. Data are presented as bar graphs with group means + Standard Error of Mean (SEM). Outliers were analysed using Grubbs (Alpha = 0.05) outlier test and are indicated in the data tables in the appendix. Group differences were evaluated by One-way ANOVA followed by Dunnett's multiple comparisons test compared to VC (vehicle control including AD-tau seeds) *p<0.05 **p<0.01 ***p<0.001. A separate evaluation was done for data obtained from the uptake assay, excluding all values above 9,500 x 10^4 665/620 ratio.

Results:

This study was performed to test the efficacy of ARG-007 at 3 concentrations on cellular tau seeding and uptake in primary cortical neurons.

As expected, tau uptake and intracellular tau aggregation (seeding) was detectable as increased HTRF signal after treatment of cells with human AD-tau seeds (VC; no treatment control) compared to no tau control. The HT7 antibody, used as a RI, significantly reduced the AD-tau associated tau aggregation in the tau uptake and tau seeding assay (Figures 1 & 3).

All concentrations of ARG-007 significantly reduced intracellular tau aggregation compared to vehicle treated AD-tau seed control (VC) (Figure 3), with the most efficacy concentration reducing aggregation by 82%.

When evaluating the effect of ARG-007 on tau uptake, upon exclusion of values above 9,500 x 10^4 665/620 ratio from uptake data, reflecting wells with extremely high HTRF signals, a significant 49% reduction of tau uptake at the lowest tested ARG-007 concentration was detectable (Figure 1).

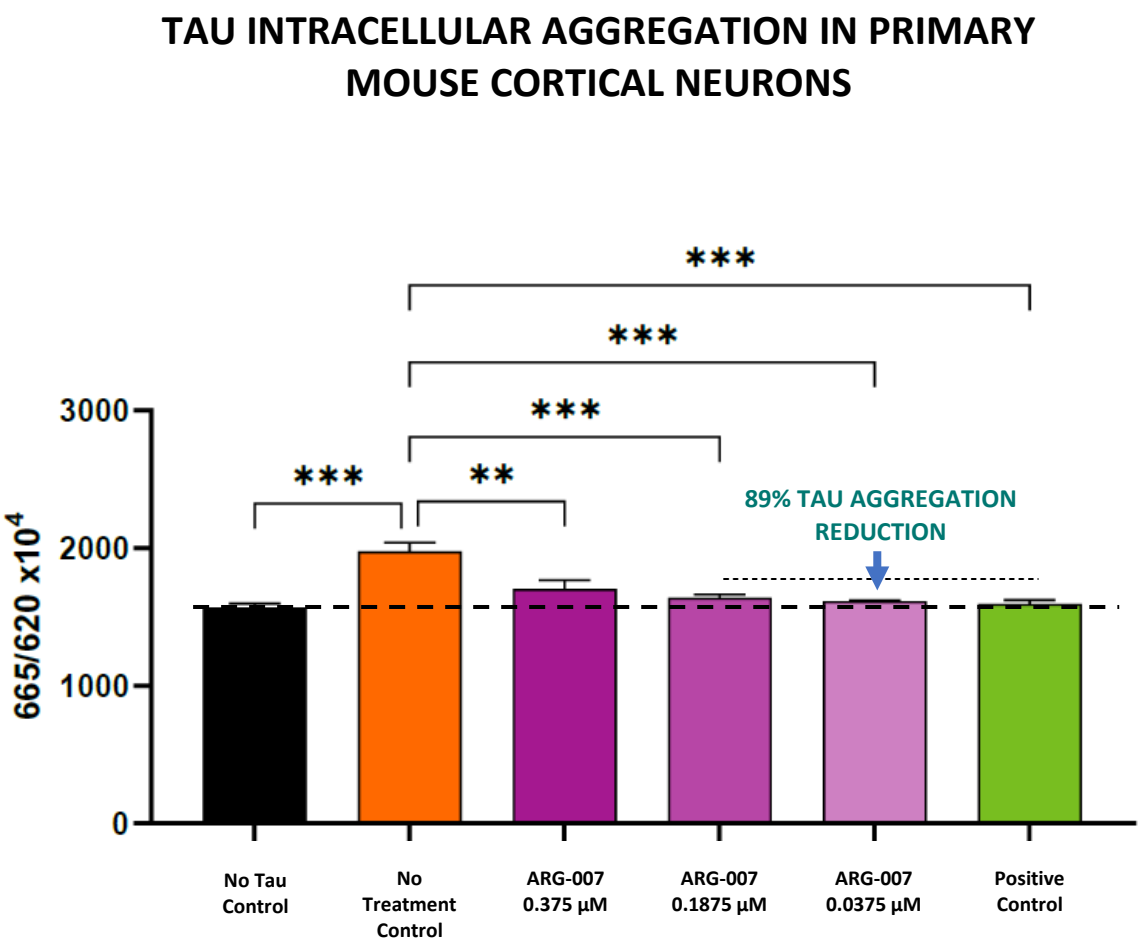

Figure 3. An assessment of the extent of tau aggregation within neurons from human tau seeds taken from human Alzheimer's disease patients. The administration of ARG-007, at three different concentrations of the drug, 1.061 µg/ml, 0.53 µg/ml and 0.1061 µg/ml, shows a statistically significant reduction in tau aggregation compared to the no treatment control. Tau seeding was analysed with a tau aggregation assay (Cisbio) as signal ratio (665/620 nm x10^4). Data are displayed as bar graphs with group means +SEM (n=5-6 per groups, no treatment control n=12). One-way ANOVA followed by Dunnett's Multiple Comparison Test (*post hoc* test) compared to no treatment control (AD seed control). Outlier according to Grubbs outlier test excluded. **p<0.01, ***p<0.001. Data provided by Creative Biolabs.

Conclusion:
The findings from this study indicate that ARG-007 has the capacity to inhibit intracellular tau aggregation (seeding) and tau cellular uptake in primary neuronal cultures.

Professor Meloni Study Outline

Study Aim and Design:
The aim of the study was to examine the effect of ARG-007 at five concentrations on cellular tau aggregation and uptake in human SH-SY5Y cells. Recombinant human tau protein aggregates [mutated P301S] purchased from Abcam (cat # ab246003) were used for the study. The experiments were performed with n = 6 replicates per condition.

SH-SY5Y human neuroblastoma cells
SH-SY5Y cells were cultured in Dulbecco's modified eagle medium (DMEM), supplemented with 2% foetal bovine serum (FBS).

Tau uptake assay
ARG-007 at concentration of 0.05, 0.1, 0.2, 0.4 or 0.8 µM or vehicle (DMEM/2% FBS) were incubated with tau seeds (Abcam ab246003) at 80 nM in DMEM/2% FBS for 15 minutes at room temperature. After the incubation period, SH-SY5Y cells were exposed to the tau seed mixtures for 48 hours at 37°C. A control included no tau seeds/no ARG-007. After the 48 hour incubation, cells were harvested and tau aggregates measured using the HTRF kit (Cisbio; 6FTAUPEG).

Tau intracellular seeding assay
SH-SY5Y cells were treated with tau seeds (Abcam ab246003) at a concentration of 80 nM for 2 hours at 37°C before the addition of ARG-007 at 5 different concentrations (0.025, 0.05, 0.1, 0.2 or 0.4 µM) or vehicle (DMEM/2% FBS). A control included no tau seeds/no ARG-007. Cells were incubated with the tau seeds for a further 46 hours at 37°C. After the 48-hour incubation with tau seeds, cells were harvested and tau aggregates measured using the HTRF kit.

Statistics:
Statistical differences between groups were evaluated by one-way ANOVA followed by post-hoc Fisher's LSD test. A P-value < 0.05 when compared with the vehicle control was considered statistically significant.

Results:
This study was performed to test the efficacy of ARG-007 at 5 concentrations on cellular tau seeding and uptake in SH-SY5Y neuronal-like cells.

As expected, tau uptake and tau intracellular aggregation (seeding) was detectable as increased HTRF signal after treatment of cells with recombinant tau seeds (data not shown).

All concentrations of ARG-007 significantly inhibited tau seed uptake compared with the vehicle treated cells (Figure 3). The highest ARG-007 concentration examined reduced tau seed uptake by 49%.

Similarly, ARG-007 at the 3 highest concentartions significantly reduced intracellular tau aggregation compared with the vehicle treated cells (Figure 4). The highest ARG-007 concentration examined reduced intracellular tau aggregation uptake by 35%.

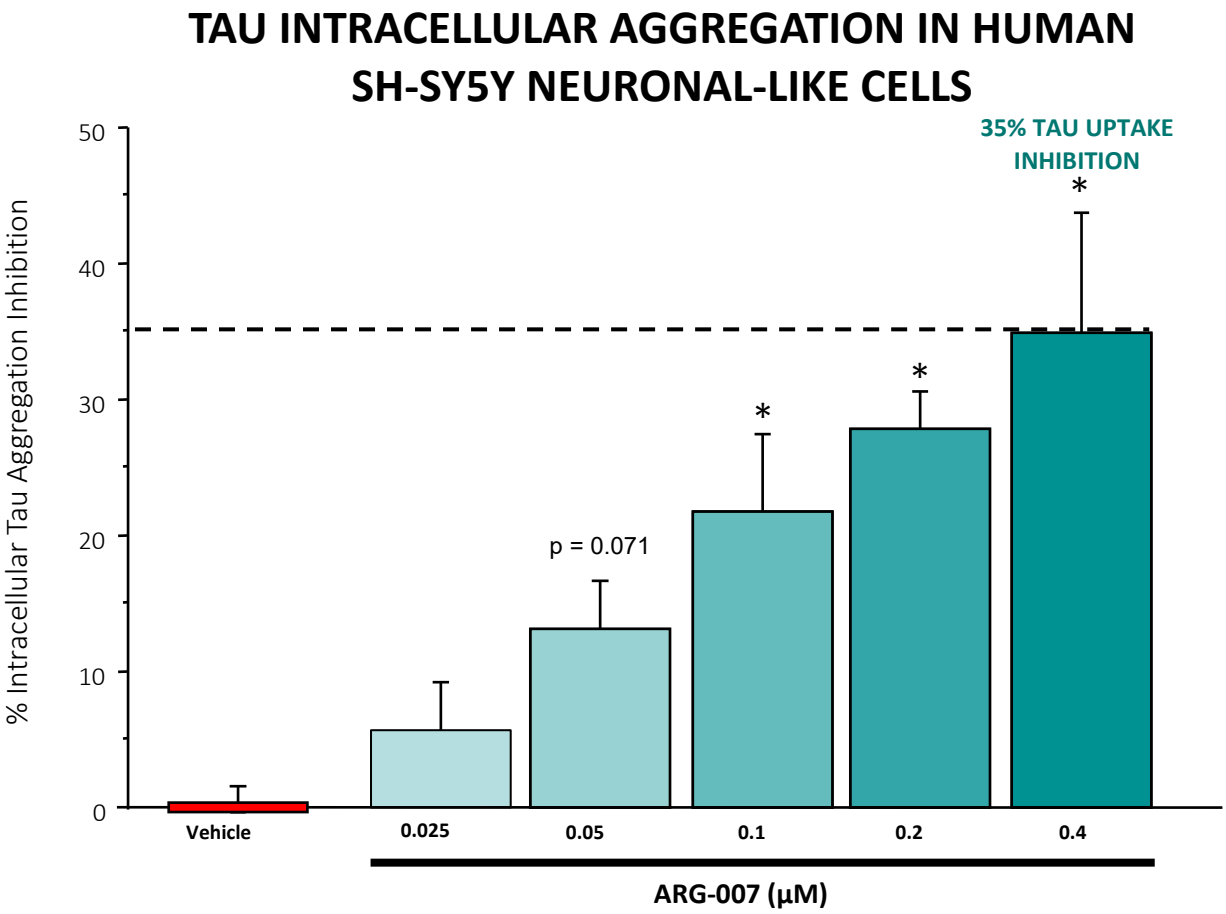

Figure 4: ARG-007 inhibits intracellular tau aggregation in human SH-SY5Y cells. SH-SY5Y cells were exposed to tau seeds (0.08 µM) for 2-hours prior to treatment with R18D (0.025, 0.05, 0.1, 0.2 or 0.4 µM) for 46-hours. After the 48-hour tau seed exposure, intracellular tau aggregation levels were measured using a homogenous time-resolved fluorescence (HTRF) tau aggregate assay (Cisbio). Fluorescence data has been transformed, with control (no tau aggregates and no ARG-007; data not shown) taken as background and subtracted from all experimental groups, and vehicle (tau seeds and no ARG-007) taken as 0% inhibition of intracellular tau aggregation. Data are means ± SE; N = 6, *P < 0.05 compared with vehicle.

Conclusion:

The findings from this study indicate that ARG-007 has the capacity to inhibit intracellular tau aggregation (seeding) and tau cellular uptake in neuronal-like SH-SY5Y cells.