Drugging Stress Granules and RNA Metabolism to Treat Tauopathies

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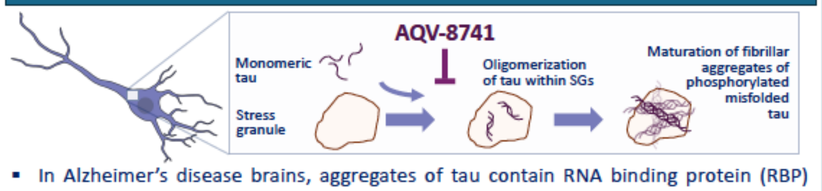
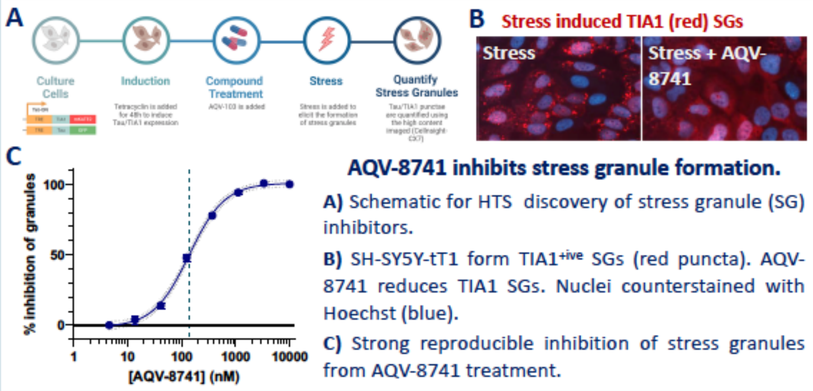
1. Introduction



- In Alzheimer's disease brains, aggregates of tau contain RNA binding protein (RBP) markers characteristic of stress granules.
- tau co-condensates with RBPs in stress granules to regulate the translational stress-response. This facilitates the pathological conversion of tau into oligomers (o.tau), which exhibit pronounced pathogenicity (PMID: 33619090).
- Inhibiting this event (by knock out of Tia1) decreases o.tau formation and delays disease progression in PS19 tau transgenic mice (PMID: 29273772, 34453888).
- Aquinnah Pharmaceuticals identified small molecules that can inhibit stress granules containing tau by high throughput screen.

2. High Throughput Screen



AQV-8741 inhibits stress granule formation.

A) Schematic for HTS discovery of stress granule (SG) inhibitors.

B) SH-SY5Y-tT1 form TIA1^{+ive} SGs (red puncta). AQV-8741 reduces TIA1 SGs. Nuclei counterstained with Hoechst (blue).

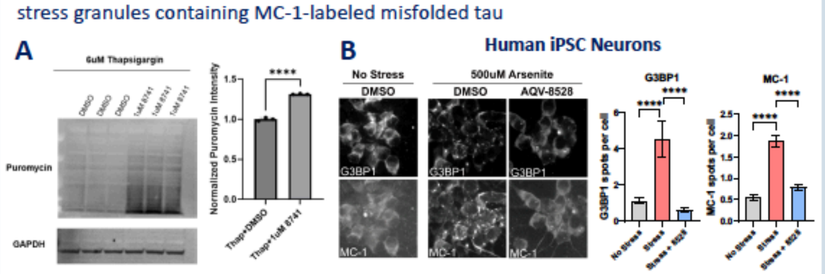
C) Strong reproducible inhibition of stress granules from AQV-8741 treatment.

3. *In Vitro* Efficacy

AQV-8741 and AQV-8528 relieve stress induced translational inhibition and reduce tau pathology.

A) AQV-8741 restores protein synthesis following inhibition by thapsigargin-induced stress. SUnSET assay of puromycin incorporation into nascent proteins.

B) In human iPSC-derived cortical neurons, AQV-8528 inhibits formation of G3BP1-stress granules containing MC-1-labeled misfolded tau



4. IND Enabling Studies: Well Tolerated in Rat and Dog

Mouse Tau PS19 (Efficacy Study)

Compound	AQV-103
Following 28 day QD PO dosing in PS19 mice	30 and100 mg/kg
gross pathology	No adverse effects
blood chemistries	No adverse effects
tissue pathology	No adverse effects

Rat Safety

Compound	AQV-103R
SAD & 28 days (QD PO)	**30, 100 & 300 mg/kg**
gross pathology	No adverse effects
clinical chemistry	No adverse effects
tissue pathology	No adverse effects
hematology	No adverse effects

Dog Safety

Compound	AQV-103R
SAD & 28 days (QD PO)	**30, 100 & 300 mg/kg**
gross pathology	No adverse effects
clinical chemistry	No adverse effects
hematology	No adverse effects

Rat: AQV-103R Oral treatment vehicle, 30, 100, 300 mg/kg orally for 28 days GLP study

- No drug related deaths
- No drug related clinical observations were noted across dose groups
- No drug associated macro-observations at necropsy
- No differences in organ weights
- No clinically significant changes in hematology parameters, hemostasis
- No clinically significant changes in liver, kidney function, Irwin test, CNS, respiratory
- No histopathology changes observed

No Observed Adverse Effect Level (NOAEL) = 300 mg/kg

Dog: AQV-103R Oral treatment vehicle, up to 300 mg/kg: 28 days (daily QD), GLP study

- Doses: 30, 100, 300 mg/kg
- Body weights and body condition scores for both drug doses were not different from vehicle
- No significant difference in CV, pulmonary, hematology or liver and renal function tests
- Histopathology pending

28 Day Study completed

No drug related deaths

No Observed Adverse Effect Level (NOAL) = 300 mg/kg

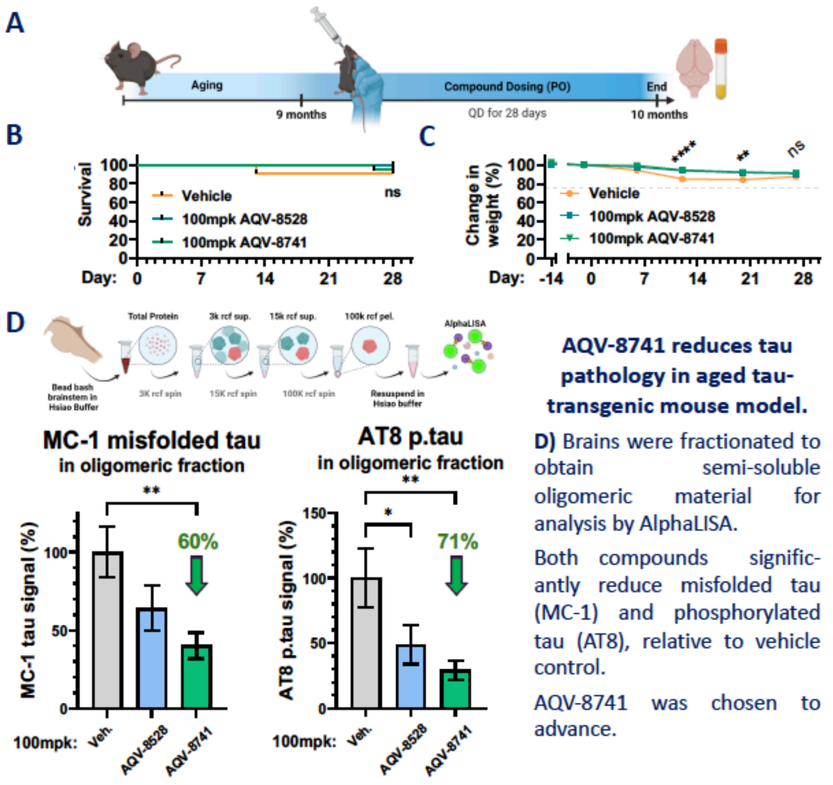
Ames Negative

5. *In Vivo* Efficacy

AQV-8741 reduces tau pathology in aged tau-transgenic mouse model.

A) Schematic for daily oral dosing of PS19 mice for 28-days, from 9-mo to 10-mo old.

B, C) AQV-8741 and AQV-8528 are well tolerated, with no deleterious effect on survival or body weight.



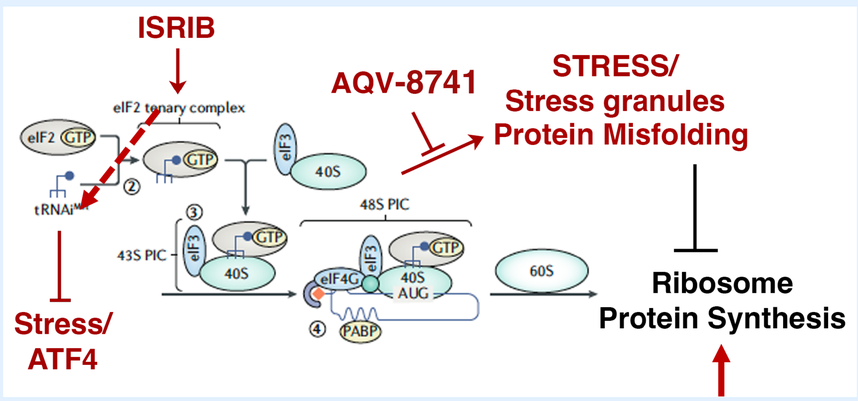
AQV-8741 reduces tau pathology in aged tau-transgenic mouse model.

D) Brains were fractionated to obtain semi-soluble oligomeric material for analysis by AlphaLISA.

Both compounds significantly reduce misfolded tau (MC-1) and phosphorylated tau (AT8), relative to vehicle control.

AQV-8741 was chosen to advance.

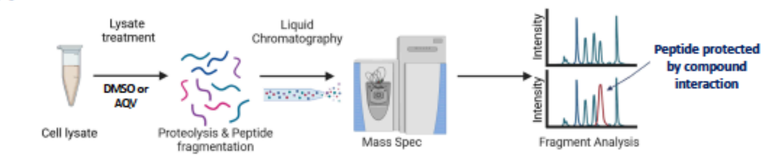
7. Proposed Pathway Distinct From ISRIB: AQV-8741 Increases Protein Synthesis AND Removes Protein Aggregates (unlike ISRIB)



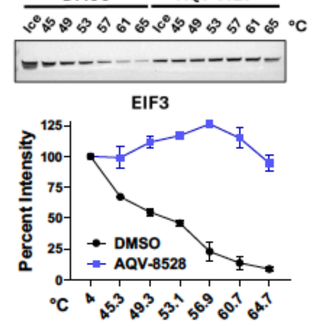
6. Target ID and PD Biomarkers

AQV-8741 targets a member of EIF3 complex, regulating its stress-interactome and leading to recovery of protein synthesis.

A **LiPs discovery of EIF3 target of AQV-8741**



B **TSA of EIF3 in lysates**



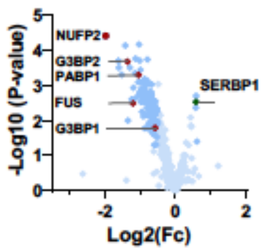
A) Target identification using Limited-proteolysis mass spectroscopy.

B) Target validation included siRNA screen (not shown) and thermal shift assay (TSA), in which the thermal stability of EIF3 is increased by addition of AQV-8528 in SH-SY5Y cell lysates.

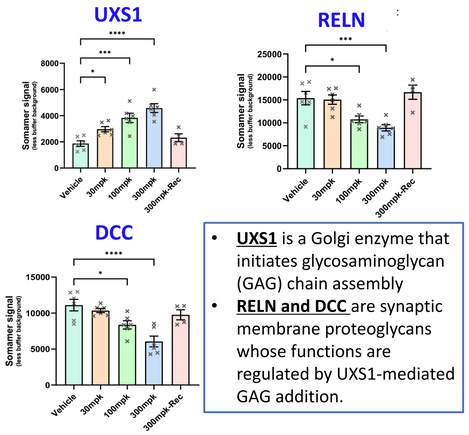
C) EIF3 increases interaction with SG components (including G3BP1 and 2) during stress (not shown), but is reversed with AQV-8528 treatment.

Critically, EIF3 interacts with SERBP1, which is important for clearance of SGs. AQV-8528 increases the interaction of EIF3 with SERBP1 (in stressed cells). PPIs determined by EIF3-IP/MS.

C **AQV-8528 alters stress-interactome of EIF3**



D **Plasma (Dog) PD Biomarkers after 28 day treatment: Somalogic Aptamers**



E

PD biomarkers in development:

- Plasma: DCC, UXS1, RELN & others
- PBMCs: ↑ Protein synthesis
- CSF: Additional PD biomarkers (e.g., PCDH8) also present

- **UXS1** is a Golgi enzyme that initiates glycosaminoglycan (GAG) chain assembly
- **RELN and DCC** are synaptic membrane proteoglycans whose functions are regulated by UXS1-mediated GAG addition.

8. AQV-8741: Proposed Phase 1 Clinical Trial Design

Phase 1a: Normal healthy volunteers; male and female 18-65 years old; QD oral drug dosing
Objectives: SAD → MAD; Assessing safety, PK, & PD drug biomarker

Double Blind SAD: Primary outcome: safety/tolerability and PK; and **Exploratory**: pharmacodynamic drug activity
48 subjects: 6 groups n=8/group (6 active, 2 placebo) up to 3 additional groups may be added based on results
Double Blind MAD: Primary outcome safety/tolerability and PK; and **Exploratory**: pharmacodynamic drug activity
Total 24 subjects: 3 dose groups n=8/group (6 active, 2 placebo). 3 additional groups may be added based on results
Each cohort: QD PO day 1-5

Phase 1b:
Cognitively impaired subjects: MMSE 15-22, pTau217+ & MTBR Tau-243+, male & female, aged 60-80 years old
Primary: safety/tolerability and PK; and **Exploratory:** AD and PD biomarkers @ baseline, 14 days & 28 days (end of study), to assess potential drug induced AD biomarker changes, to support Phase 2 dose selection & design.
Highest safe drug dose from Phase 1a (N=9) and placebo (N=3) for QD oral daily dosing for 28 days

Enrollment criteria:1) MMSE score of 15-22 on intake followed by being positive for 2) pTau217 & 3) MTBR Tau-243

- 28 day study biomarker collection times: Bloods taken at 0, 2 and 4 weeks; CSF taken at 0 and 4 weeks
- Estimated subjects screened: to identify 12 cognitively impaired subjects for Phase 1b enrollment: 60 -140

9. Conclusions

AQV-8741 a novel therapeutic approach that:

- **inhibits stress granules, relieves chronic stress-induced translational inhibition, and reduces tau pathologies,**
- **is well tolerated in IND-enabling preclinical safety studies,**
- **is an oral, brain penetrant drug candidate.**

Aquinnah Pharmaceuticals identified small molecules, AQV-8741 (the R-enantiomer of AQV-8528), that modulate the eIF3 complex which dampens the chronic stress response, and dramatically reduces the production of tau pathologies in human cortical neurons and tau transgenic mice.

AQV-8741 resulted in no drug related adverse events when orally administered to rats and dogs in GLP 28-day toxicology studies (up to 300mg/kg/day).

Target engagement PD biomarkers, directly related to the target and to the translational stress-response, are detectable in either human PBMCs or dog plasma.

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