A Single- and Multiple-Ascending Dose Study to Evaluate the Safety and Pharmacokinetics of Oral PU-AD, Epichaperome Inhibitor to Treat Alzheimer's Disease

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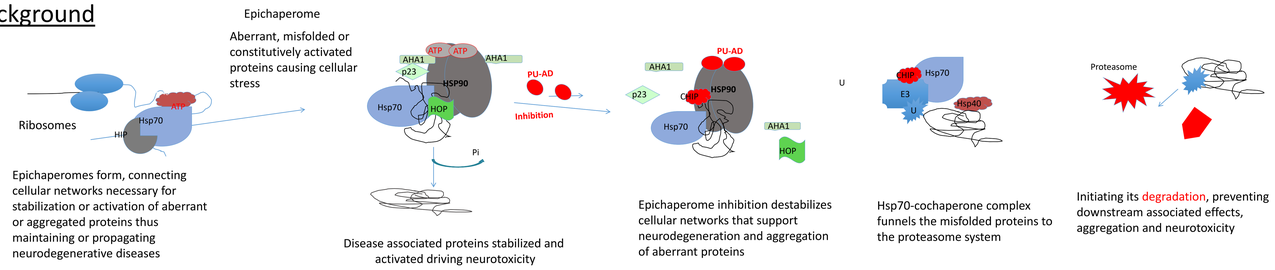
Background

- Neurodegenerative diseases, such as Alzheimer's Disease (AD), are in part caused by the breakdown of regulatory pathways designed to prevent aggregation and accumulation of disease-associated proteins
- Cellular proteostasis is tightly controlled by the protein quality control system of interacting networks regulating synthesis, folding or refolding, activation and degradation of proteins
- Disturbances of cellular proteostasis (such as molecular crowding, aggregation of proteins, decrease of proteolytic activity due to aging or oxidative stress) induce a stress response resulting in the formation of epichaperomes
- Epichaperome complexes, which form only in diseased cells, are absent in normal cells and become central nodes of cellular networks in diseased cells
- Epichaperomes maintain and drive the pathologic cellular phenotype by stabilizing aberrant proteins, preventing their degradation and, in some cases, aiding in their aggregation
- Inhibition of epichaperomes by PU-AD restores proteostasis by initiating protein degradation and rescues neuronal functions
- The novel epichaperome inhibitor PU-AD was well-tolerated and had favorable plasma PK and CSF exposure in the Phase 1 study in healthy volunteers and has entered a Phase 2 trial in AD patients



PU-AD specifically targets epichaperomes in AD brain

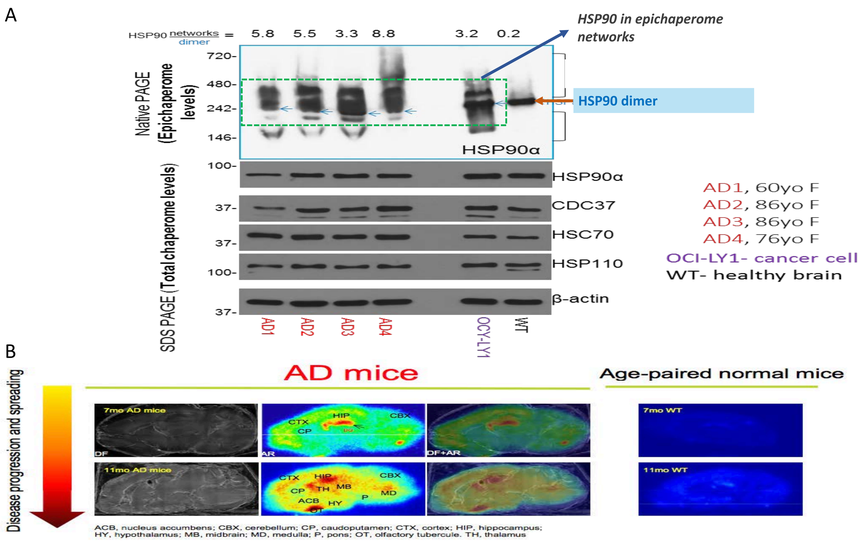


Figure 1. (A) Postmortem human brain samples were collected from patients with Alzheimer's disease (AD) or healthy patient controls (WT). Samples were homogenized and separated on native gels. Gels were immunoblotted with anti-HSP90α. Extracts were run on 7% SDS-PAGE. Membranes were blotted with anit-HSP110, anti-HSC70, anit-HSP90α, and anti-CDC37. In healthy brains, HSP90 forms a dimer (see the orange arrow). During AD, however, HSP90 will be incorporated in epichaperomes (see green box). PU-AD specifically binds to the HSP90 when part of an epichaperome and not to HSP90 in normal cells. (B) Sectioning and staining to map epichaperome localization via autoradiography (AR) using ^{131}I-PU-AD, is shown for a representative mouse brain. Dark field (DF) microscopy images were taken to visualize brain anatomy. Brain sections show epichaperome localization and its distribution in PS19 (n = 5) mouse brains and its absence in WT (n = 5) mouse brains. As tauopathy progresses (from age 7 to 11 months), brain epichaperome levels increase.

PU-AD treatment results in reduced levels of ptau and restores spatial memory/learning in AD mice

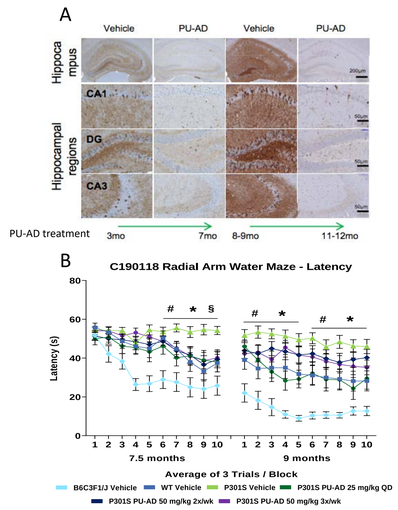


Figure 2. (A) Representative immunostaining of ptau (AT8) in indicated brain areas of PS19 mice treated with vehicle or PU-AD at 7 months or 11-12 months of age. Epichaperome inhibition in PS19 mice not only blocked the hyperphosphorylation of human tau species (see PU-AD treatment of mice from 3 mo to 7 mo of age), but also reduced the already formed hyperphosphorylated and aggregated tau (see PU-AD treatment of mice from 8-9 mo to 11-12 mo of age). Slides were counterstained with hematoxylin (B) P301S mice were treated orally with PU-AD starting at 5 months of age with either 25mg/kg daily or 50 mg/kg 2x or 3x a week. At 7.5 and 9 months of age, spatial memory and learning was evaluated using the Radial Arm Water Maze (Latency). PU-AD treatment improved spatial memory and learning compared to untreated P301S mice (green line) and at 9 months of age mice treated daily were indistinguishable from wild type mice.

Clinical Trial Design

Part 1: Single Ascending Dose in 3 cohorts receiving a single oral dose of 10, 20, or 30 mg of PU-AD or placebo

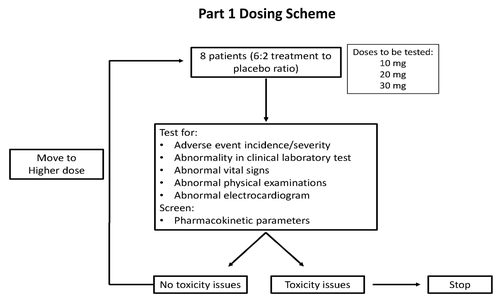
Objective: To evaluate the safety and tolerability of single doses of PU-AD in healthy subjects

Part 2: Multiple Ascending Dose in 2 cohorts receiving QD oral dose of 20 or 30 mg of PU-AD or placebo for 7 days

Objective: To evaluate the safety and tolerability of multiple doses of PU-AD in healthy subjects over 60 years of age

Part 3: Bioavailability of 30 mg of PU-AD oral solution and tablet formulations

Objective: To determine the relative BA of a single 30 mg dose of PU-AD administered as an oral tablet compared to an oral solution of PU-AD in healthy subjects



Adverse Events during Trial

	Cohort	Adverse Event	Relationship	Severity
Part 1: Single Dose	1	NA	NA	NA
	2	HEADACHE	RELATED	GRADE 1
	2	DROWSINESS	RELATED	GRADE 1
	2	HEADACHE	RELATED	GRADE 1
	3	FLU LIKE SYMPTOMS	RELATED	GRADE 1
	3	DRY MOUTH	RELATED	GRADE 1
	4	LEFT HIP DISCOMFORT	NOT RELATED	GRADE 1
Part 2: 7 days QD	4	ASYMPTOMATIC DECREASED SYSTOLIC BLOOD PRESSURE	NOT RELATED	GRADE 1
	4	CONSTIPATION	NOT RELATED	GRADE 1
	4	HEADACHE	NOT RELATED	GRADE 2
	4	LEFT FOREARM PHLEBITIS	NOT RELATED	GRADE 1
	4	HEADACHE	RELATED	GRADE 2
	4	HEADACHE	RELATED	GRADE 1
	5	LUMBAR PAIN	NOT RELATED	GRADE 1
	5	DIARRHEA	NOT RELATED	GRADE 1
	5	HEADACHE	NOT RELATED	GRADE 1
	5	HEADACHE	NOT RELATED	GRADE 2

Table 1. Relatedness and severity of all adverse events. There were no serious adverse events (SAEs) or deaths reported in Study PU-AD-01-001, and no subject experienced an AE leading to discontinuation from the study. The majority of AEs were reported as Grade 1 and self limited. All doses were well-tolerated in clinical subjects with no dose limiting AEs observed. Overall, the safety findings in Study PU-AD-01-001 indicate a highly favorable safety profile of PU-AD.

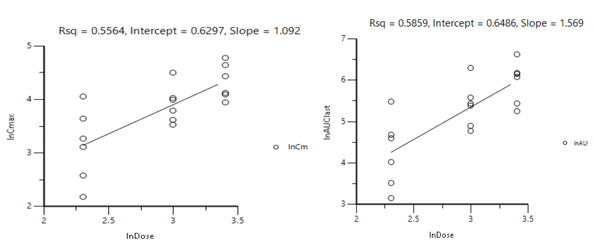
Human Pharmacokinetics

A

PK Parameters of Single Oral Administration of 10, 20, or 30 mg PU-AD

Dose (mg)	10	20	30
T_{max} (h)	2 (1:3)	2 (1:3)	2 (1:6)
C_{max} (ng/mL)	23 (77)	50 (36)	77 (34)
$AUC_{0-\infty}$ (h*ng/mL)	192 (57)	240 (56)	398 (61)
$t_{1/2}$ (h)	2.2 (17.2)	1.7 (13.0)	2.2 (34.4)

B



C

PK Parameters of Oral Administration of 20 mg or 30 mg PU-AD QD for 7 Days

	Day 1		Day 7	
Dose (mg)	20	30	20	30
T_{max} (h)	1 (1:2)	2 (1:4)	1 (1:2)	1 (0.5:3)
C_{max} (ng/mL)	106 (49)	162 (58)	115 (45)	216 (46)
$t_{1/2}$ (h)	2.6 (30.2)	3.2 (67.6)	3.23 (20.7)	3.5 (19.3)
$AUC_{0-\infty}$ (h*ng/mL)	415 (63)	927 (68)	492 (40)	952 (46)
Rac ((h*ng/mL)/(h*ng/mL))	NA	NA	1.205 (22.0)	1.170 (29.6)
Mean CSF concentration (ng/mL)	NA	NA	0.92 (0.48)	1.50 (0.68)

D

PK Parameters of 30 mg PU-AD Oral Solution and Tablet

	Solution	Tablet
T_{max} (h)	2 (1:4)	2 (1:3)
C_{max} (ng/mL)	80 (57)	105 (61)
$t_{1/2}$ (h)	2.2 (36.2)	2.0 (39.5)
AUC_{last} (h*ng/mL)	329 (53)	325 (70)
$AUC_{0-\infty}$ (h*ng/mL)	357 (50)	393 (59)

E

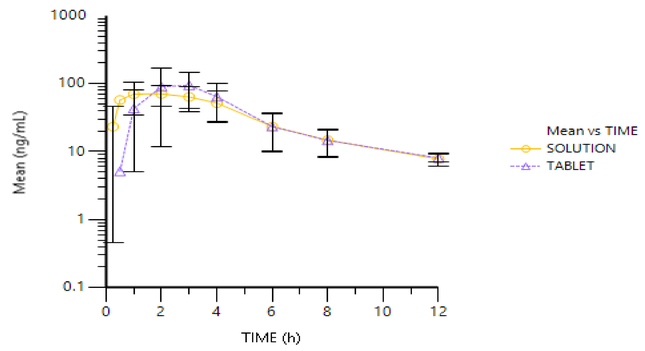


Figure 3. (A) Summary of the PK parameters of a single oral administration of 10, 20, or 30 mg of PU-AD. (B) Dose proportionality assessment of PU-AD. PK was dose-proportional between 10 mg to 30 mg. (C) Summary of PK Parameters of Oral Administration of 20 or 30 mg PU-AD QD for 7 days. PU-AD showed minimal accumulation with repeated dosing. (D) Summary of PK Parameters of 30 mg of PU-AD oral solution and tablet. (E) Plasma concentration-time curves for 30 mg of PU-AD oral solution and tablet. The bioavailability of a single 30 mg dose of PU-AD administered as an oral tablet and an oral solution of PU-AD is comparable in healthy subjects. $AUC_{0-\infty}$ = AUC from time 0 to infinity; C_{max} = maximum observed plasma concentration; $t_{1/2}$ = terminal elimination half-life; T_{max} = time to maximum concentration. Geometric means (CV%) are presented for C_{max} and AUCs; median (minimum:maximum) for t_{max} values presented were rounded); arithmetic mean (CV%) for all other parameters. Mean CSF concentrations (N=6 for both solution and tablet) are presented as arithmetic mean and standard deviation (SD).

Conclusions and Future Directions

- Epichaperomes play a critical role in the pathogenesis of neurodegenerative diseases
- Epichaperomes present a potential therapeutic target, offering high specificity and potentially greater efficacy than targeting a single protein
- PU-AD, a novel and selective epichaperome inhibitor, shows efficacy in animal AD models
- In humans, PU-AD was well-tolerated after single oral doses of 10, 20, and 30 mg
- PU-AD was well-tolerated after multiple doses of 20 and 30 mg in healthy volunteers and in elderly subjects >60 years of age
- PU-AD systemic exposures were comparable between liquid and solid dose formulations
- Pharmacokinetic profile of PU-AD was successfully defined in humans
- PU-AD detected in CSF samples provides evidence of brain penetration
- PU-AD is rapidly absorbed and shows PK linearity between 10 to 30 mg single dose
- PU-AD will be evaluated for safety and efficacy in human patients with Alzheimer's Disease

Acknowledgments

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