AZP2006 a new clinical candidate for the treatment of PSP

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AlzProtect
Therapeutic Solutions for Neurodegeneration

INTRODUCTION

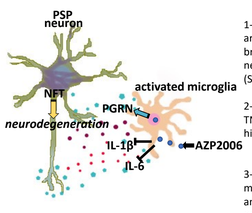
AZP2006 is a disease-modifying small molecule that readily crosses the blood-brain barrier. It increases the neurotrophic factor Progranulin (PGRN) levels and displays neuroprotective properties promoting neuron survival, neurite outgrowth and synaptogenesis.

PGRN is a secreted glycoprotein primarily expressed in mature neurons and microglia known to promote neuronal survival and to enhance neurite outgrowth in cultured neurons. PGRN protein reduction has been associated with increased Tau pathology in human and rodents, supporting the notion that its reduction might contribute to phosphorylation and intraneuronal accumulation of Tau protein. Inflammatory stimuli in the brain are also relevant to exacerbate Tau phosphorylation. Since PGRN could act as an anti-inflammatory factor in neuroinflammation, it is hypothesized that Tau phosphorylation would be accelerated in the inflammatory status resulting from reduced PGRN levels.

AZP2006 is currently developed for the treatment of Progressive Supranuclear Palsy (PSP). Orphan drug designation has been granted to AZP2006 by the EMA and the US FDA. AZP2006 increasing the levels of PGRN, targets the pathophysiology of PSP : 1) decreasing phosphorylated Tau and 2) decreasing associated neuroinflammation.

RESULTS

Hypothesis of AZP2006 MOA

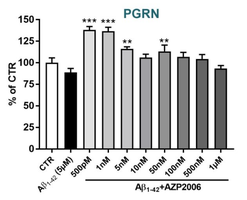


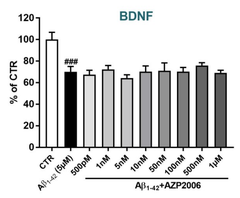
PSP	AZP2006	*Proposed effects of AZP2006*
1- Activated microglia are present in those brain regions with neurodegeneration (SN and STR).	*1° Neuroprotection* Is triggered by its effect on recruited and activated microglia that lead to a substantial release of PGRN.	**Effect on Motor Impairment**
2- IL-1β, IL-6 and TNFα are significantly higher in SN.	*2° Neuroinflammation* Reduced, its effect on microglia could decrease IL-1β and IL-6 levels.	**Effect on Neuroinflamation**
3- NFT, NT and TA are mainly in brainstem and basal ganglia. The presence of NFT in cortical areas is correlated to dementia.	*3° Tau phosphorylation* Is reduced, which may limit tau aggregation and NTF formation, preventing neurodegeneration and memory deficits.	**Effect on Cognitive Deficits**

STR: Striatum (Caudate, Putamen and Pallidum), Pallidum (GPi, GPe-Globus Pallidum internus and externus), SN: Substantia Nigra, NFT: neurofibrillary tangles, NT: neuropil threads, TA: tufted astrocytes.

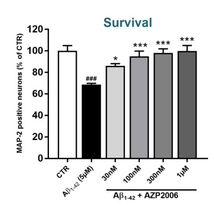
AZP2006 increases PGRN and promotes neuronal survival via PGRN

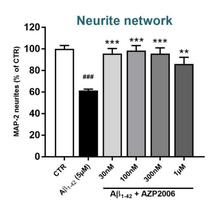
1) In primary rat cortical neurons cultured with microglia and injured for 72h with $A\beta_{1-42}$ oligomers, AZP2006 increased the level of secreted PGRN, but not of the brain-derived neurotrophic factor (BDNF).



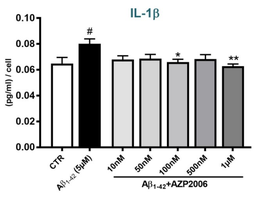


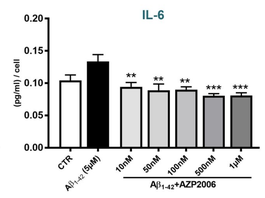
2) Increased neuron survival and neurite network protection were associated to the increase of released PGRN but not BDNF evidencing the specificity of AZP2006 effect.

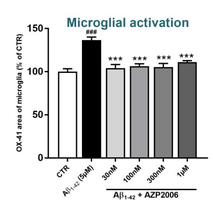


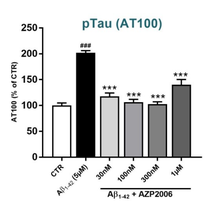


3) AZP2006 abolished the release of IL-1β, IL-6, microglia activation and and Tau phosphorylation

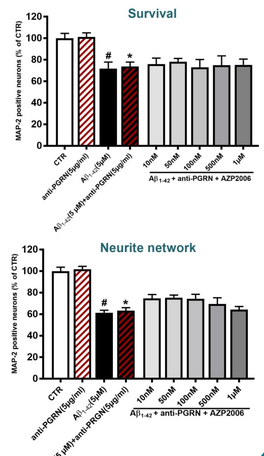








4) Anti-PRGN antibody abolished the AZP2006 effect on cell survival and neurite network.

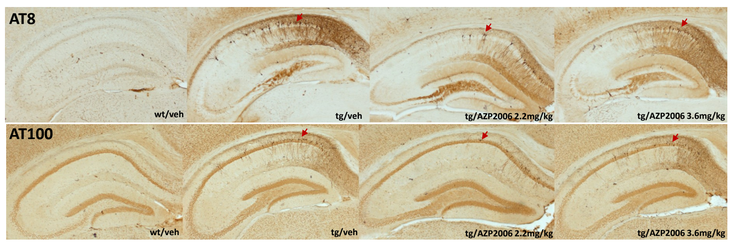


*Data expressed as percentage of control show the mean ± SEM (100% = no Aß1-42, no compound). One-way ANOVA followed by Dunnett's test, n=6. *, ** and ### or *** indicate p<0.05, p<0.01 and p<0.001 respectively vs Aß1-42 condition (*) or vs CTR condition (#).*

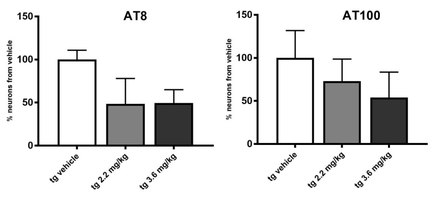
AZP2006 decreases Tau phosphorylation and improves memory in mice

*THY-Tau22 mouse model**

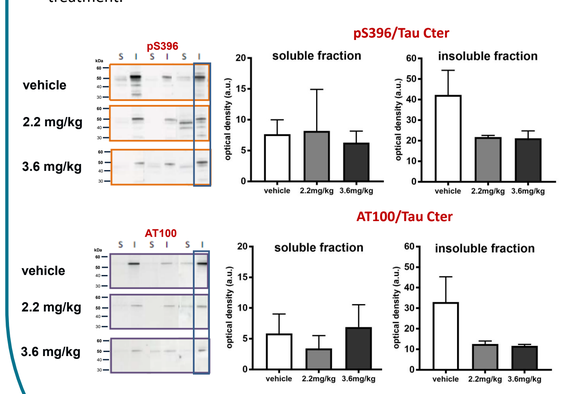
The transgenic THY-Tau22 mice model expresses human 4R tau isoform containing exon 10 with the double mutations G272V and P301S. The aim was to investigate the protective effect of AZP2006 on pathogenic Tau phosphorylation and cognitive deficits.
Three month-old females C57BL/6 wt and THY-Tau22 (n=10/group) were treated p.o. (drinking water) with vehicle, 2.2 or 3.6 mg/kg/day of AZP2006 for 3 or 5 months.



Tau phosphorylation evaluated by immunohistochemistry (AT8 and AT100) was performed after 3 months. Accumulation of phosphorylated tau (pS396 and AT100) was evaluated by fractioning (Triton soluble and insoluble) followed by WB after 5-month treatment. Cognition (learning and memory by Morris Water Maze test –MWM) was evaluated after 3 and 5-months of treatment.

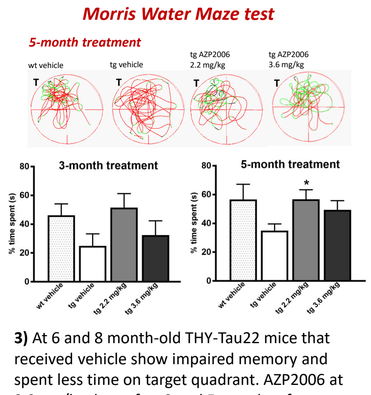


1) AZP2006 prevented the increase of Tau phosphorylation in the hippocampus after 3 months of treatment (6-month-old).



2) The 5-month treatment with AZP2006 reduced Tau hyperphosphorylation (pS396) and abnormal phosphorylation (AT100) in hippocampal insoluble fraction.

Morris Water Maze test



3) At 6 and 8 month-old THY-Tau22 mice that received vehicle show impaired memory and spent less time on target quadrant. AZP2006 at 2.2 mg/kg dose after 3 and 5 months of treatment prevented the decrease *(*p<0.05 Student t test, n=4-5).*

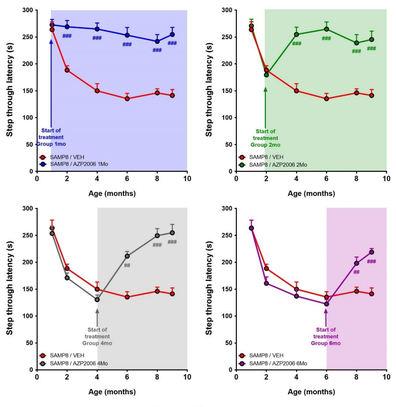
** Study performed in collaboration with Dr Melnyk, Dr Sergeant and Dr Buée from the UMR-S 1172, France*

SAMP-8 mouse model

The potential protective effect of the long-term administration of AZP2006 was tested in the mouse model of accelerated ageing: the Senescence-Accelerated Mouse-Prone 8 (SAMP-8). This model displays hyperphosphorylation of Tau, abnormal Aβ accumulation, increased oxidative stress and gliosis. SAMP-8 mice also show impaired immune response and deficits of learning and memory.
SAMP-8 mice were treated p.o. (drinking water) with vehicle or 3mg/kg/day AZP2006 from the age of 1, 2, 4 or 6 months and evaluated each 2 months until the age of 10 months.

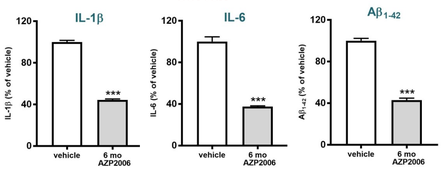
Memory deficits determined by the step-through and escape latencies in the Step-Through Passive Avoidance (STPA) and by the spontaneous alternation in a Y-Maze test (not shown) (n=10-12).

1) A significant prevention (animals treated from 1 month) or recovered the memory lost (animals treated from 2, 4 or 6 months of age) was observed after the treatment with AZP2006.



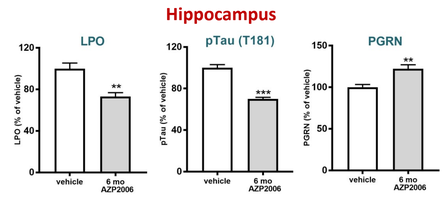
Data show mean ± SEM. One-way ANOVA, significant differences when compared with vehicle treated animals. #, ## and ### indicate p<0.05, p<0.01 and p<0.001 respectively. (n=7-12).

Cortex



2) After 4 months of treatment, the levels of IL-1β, IL-6 and $A\beta_{1-42}$ were reduced in the cortex (n=4-7).

Hippocampus



3) In the hippocampus, AZP2006 treatment reduced the lipid peroxidation (LPO) and pTau (T181), while increased the levels of secreted PGRN (n=4-7).

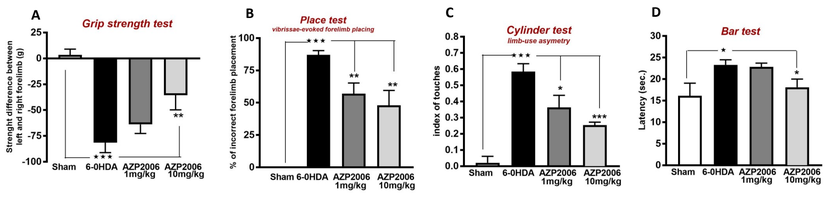
*Data show the mean ± SEM, One -way ANOVA followed by Dunnett's test. Significant differences when compared with vehicle treated animals *, ** and *** indicate p<0.05, p<0.01 and p<0.001 respectively.*

AZP2006 restores motor functions

Nigro-striatal lesion induced by 6-hydroxydopamine #

The experimental model of nigro-striatal lesion induced by 6-hydroxydopamine (6-OHDA) was used to assess the ability of AZP2006 to restore motor and sensorimotor functions in the rat.
Two-month Wistar male rats (n=10 /group) were unilaterally lesioned (20µg/rat into the right Substantia Nigra pars compacta (SNpc). AZP2006 (1 or 10 mg/kg) was administered p.o. (gavage) 30 min after the 6-OHDA infusion. On day 21, apomorphine (50µg/kg s.c.) induced rotation test was performed to verify the efficacy of the lesion. Clear spontaneous limb asymmetries, due to motor impairment of limbs contralateral to the injected hemisphere were evaluated by motor evaluation tests.

1) AZP2006 prevented the motor dysfunction due to dopamine depletion in SNpc and the striatum



A) Grip strength test: data show the difference in the strength force between the left forelimb (contralateral) – right forelimb
B) Percentage of unsuccessful forelimb placing responses in the Place test
C) Index of touches (number of ipsi - contra touches) relative to the total number of touches (ipsi+contra+both) in the limb-use asymmetry cylinder test
D) Latency to remove both paws in the Bar test

*One-way ANOVA followed by Newman-Keuls Multiple Comparison Test, **p<0.01 and ***p<0.001, n=6-10.*

** Study performed by Dr Salomé in collaboration with Dr Bordet from the U 1171, France*

Summary of the effects of AZP2006 on cellular and pathological characteristics of PSP

Cellular characteristics	Effect of AZP2006	Test system *in vitro*
Neuronal loss and loss of synapses (basal ganglia, pallidus, subthalamic nucleus and substantia nigra, and subcortical nucleus: brainstem, including the dentate nucleus, pontine nucleus)	Protection of synapses (PSD-95), induction of synaptogenesis (SYP/PSD-95 coloc.) Increase neuron survival (MAP-2) Increase neurite outgrowth (MAP-2)	Hippocampus of lesioned mice ($A\beta_{25-35}$). Primary hippocampal, cortical and dopaminergic neurons (with or w/o microglia) injured with $A\beta_{1-42}$ peptide, Glu, 6-OHDA, MMP^+ or α-syn.
Neurofibrillary tangles Abnormal accumulation of tau protein	Protection and reduction of Tau hyperphosphorylation (AT100, AT8, pT181, pS396)	Primary cortical neurons (with or w/o microglia) injured with $A\beta_{1-42}$ peptide or okadaic acid. SY5Y hTau cells (Tau 441, 2N4R). Hippocampus of lesioned mice ($A\beta_{25-35}$) and Tg mice (THY-Tau22)
Inflammation and gliosis	Reduces microglia activation (OX-41) Reduces cytokine release (IL-6, IL-1β, TNFα)	Primary cortical and dopaminergic neurons with microglia injured with $A\beta_{1-42}$ peptide. Hippocampus of SAMP8 mice
Pathological characteristics	**Effect of AZP2006**	**Test system *in vivo***
Subcortical dementia	Prevents and reverses memory deficits (working and spatial memory)	THY-Tau22 transgenic mice, $A\beta_{25-35}$ lesioned mice, SAMP8 mice
Motor deficits	Prevent motor impairment	6-OHDA lesioned rat

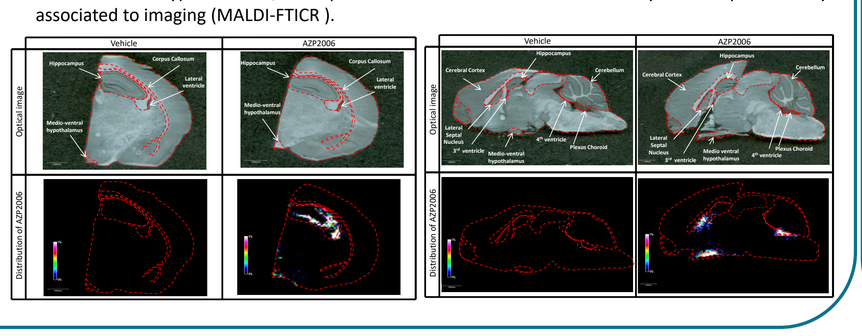
AZP2006 readily crosses the blood brain barrier

In vitro system	Endothelial cell permeability coefficient (related to tested concentrations)	Classification
Bovine brain capillary endothelial cells	Pe values : 1,05 (0,18) to 1,55 (0,12) depending on the tested concentrations (from 1 to 25µM)	Moderate permeability
Human brain like endothelial cells	Pe values : 1,54 (0,33) to 1,83 (0,57) depending on the tested concentrations (from 1 to 25µM)	Moderate permeability

In vivo study	Calculated Log BB (brain to plasma)	Results/findings
Daily oral administration of AZP2006 2mg/kg by drinking water to female mice	From 1,37 to 1,53 depending on the administration period (from 4 to 9 months)	AZP 2006 readily crosses the BBB logBB>>0,3 In addition AZP2006 was also quantified in CSF (data not shown)
Daily oral administration of AZP2006 1;2 or 4mg/kg by gavage to female mice	From 1,08 to 1,35 depending on the administration period (from 8 to 14 days)	AZP 2006 readily crosses the BBB logBB>>0,3

AZP2006 distribution in brain

AZP2006 was detected by on tissue after a 3-month daily oral administration with AZP2006 at 2.2 mg/kg (drinking water). AZP2006 was mainly identified in the hippocampus (CA2/CA3 and DG), the medio-ventral hypothalamus, the plexus choroid and the ventricles by mass spectrometry associated to imaging (MALDI-FTICR).



AZP2006 batches overview and related studies

AZP2006 batch denomination	Synthetis scale (expressed in salt form eq.)	Main uses
AZP2006 « pharmacological » batch	0,5kg	-All *in vitro* and *in vivo* pharmacological studies -PK and ADME studies including DDI assessment
AZP2006 GMP 1st clinical batch	>2kg	-Clinical Phase 1 in male HV (SAD+MAD) -PK and food effect clinical trial in male HV
AZP2006 2nd « tox » batch (GLP compliance)	2,3 kg scale	-Long term toxicology studies (6 months in rats and 9 months in dogs -Reprotoxicity studies
^{14}C AZP2006	52,2mCi	QWBA in toxicological species (rat and dogs)
AZP2006 GMP 2nd clinical batch	>2kg targeted (*on going*)	Phase 2 clinical trial

AZP2006 Drug Substance and Drug Product

AZP2006 denomination	Main features	Main characteristics	Stability
AZP2006 Drug Substance	Produced in 5 chemicals steps under salt form	Highly soluble	Up to 5 years
AZP2006 Drug Product (liquid form)	Water based solution(DS in water) without any preservative or excipients	Strength from 2 to 20mg/mL	Shelf life Up to 18 months
AZP2006 Drug Product (solid form)	Currently under investigation		

Preclinical and clinical key features of AZP006

- Safety pharmacology studies : AZP2006 does not show any significant effect
- Distribution: AZP2006 is rapidly absorbed following oral administration and crosses the BBB
- Metabolism : one main metabolite (M2) in dog and human
- Toxicity studies : AZP2006 orally administered once daily for up to 20 weeks (ongoing) in rats and 32 weeks dogs (ongoing) do not show any relevant finding
- Clinical studies : oral administration of AZP2006 (liquid formulations) to healthy human adults for up to 10 days was well tolerated, had a good safety profile.

AZP2006 was well tolerated in Healthy Subjects

AZP2006C01: A safety, tolerability and pharmacokinetics of single doses of AZP2006 orally administered to healthy volunteers in a randomized, double-blind, placebo controlled First-In-Man phase I study

- The administrated doses were 3, 5, 10, 30, 90, 180 and 360 mg free base eq.. Eight healthy male subjects (6 verums and 2 placebos) were included in each dose level.
- Safety and tolerability : No effect on Physical examination and Vital signs, ECG, Psychometrics tests of awareness, and no serious Adverse Events

AZP2006C02: A randomized double blind, placebo controlled multiple dose escalation study in healthy male volunteers to study the safety, tolerability and pharmacokinetics of AZP2006

- The administrated doses were 30, 60 and 120 mg daily for 10 days in 10 healthy male subjects (8+2).
- Safety and tolerability : No effect on Physical examination and Vital signs, ECG, Psychometrics tests of awareness, and no serious Adverse Events

Ongoing Phase 1b and Future Phase 2 clinical trials

AZP2006C03: A randomized, open label, single dose, cross over study to investigate the potential food effect on pharmacokinetic parameters of 60 mg of AZP2006 administered orally to healthy male subjects

- Primary Objectives: To determine the impact of concomitant food intake on the PK parameters of AZP2006 and its metabolite (M2) after a single oral administration of 60 mg of AZP2006 in healthy male subjects.

AZP2006C04: A multi-center, randomized, double-blind, placebo controlled, parallel group study to assess, tolerability, safety, pharmacokinetics and effect of AZP2006 on cerebrospinal fluid biomarkers in 30 patients with Progressive Supranuclear Palsy

- Primary Objectives: to determine the tolerability, the safety and the pharmacokinetics of AZP2006 (12 week-treatment)
- Secondary Objectives: Effect of AZP2006 on cerebrospinal fluid biomarkers (12 week-treatment)

Biomarkers

- Level of Tau: D1, D29 and D90
- Phospho-Tau: D1, D29 and D90
- β-amyloid: D1, D29 and D90
- Progranulin levels will be assessed in the CSF (on D1, D29, D90) (TBD)

CONCLUSIONS

AZP2006 is an investigational product that is being developed for the treatment of PSP. We demonstrated that AZP2006 is orally active and crosses the BBB. The combined MOAs described above and supported by data provide a strong rationale to envisage AZP2006 as PSP disease-modifying drug, targeting PSP's pathophysiological roots and positively impacting disease progression.
In addition to the nonclinical data, safety, toxicology studies (up to 6 months in rats and 9 months in dogs) and tolerability data from first in man study (SAD and MAD up to 10 days) in healthy volunteers make AZP2006 a suitable candidate for clinical evaluation in PSP patients.