HER-096 Is a Novel Brain-Penetrating Peptidomimetic That Promotes Proteostasis and Reduces Neuroinflammation in an Aged Mouse Model of Synucleinopathy

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SUMMARY

Intraputamenal CDNF infusion has previously been tested in a Phase 1 study in moderately advanced Parkinson's disease (PD). A more patient-friendly route of administration will support further development of a disease-modifying therapy for PD and allow access to earlier stage patients.

HER-096 is a synthetic peptidomimetic compound developed based on the UPR-modulating site of human CDNF protein. HER-096 tolerates proteolysis and can penetrate the blood-brain barrier (BBB) allowing systemic administration for treatment of neurodegenerative diseases.

METHODS

A peptide library was created based on the GRP78-binding side of CDNF. A set of in vitro activity assays were used for screening for optimal activcity.

Lead optimization process consisted of modifications to the peptide backbone to improve metabolic stability but also to preserve BBB penetration and target-modulation properties. HER-096 is a ~1000 Da peptidomimetic derived from this lead optimization process (Figure 1).

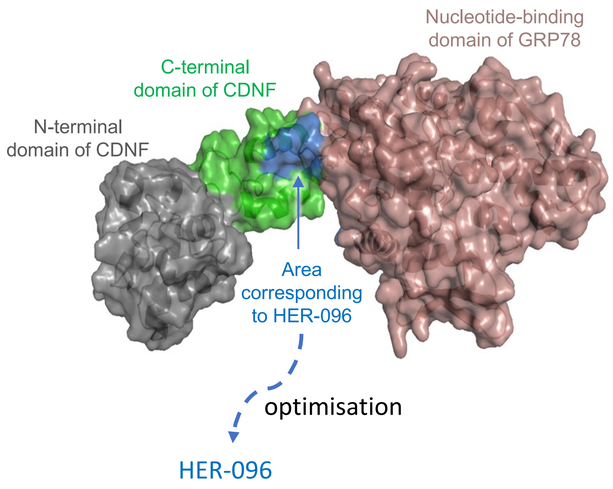

Figure 1. HER-096 was developed based on the GRP78-binding site located in the C-terminal domain of the human CDNF protein.

In vitro models of BBB penetration, neuroprotection and metabolism.

In vivo pharmacology studies in healthy mice, rats and dogs addressing pharmacokinetics, brain distribution and excretion.

In vivo pharmacodynamic studies in an aged mouse alpha-synuclein injection (bilateral substantia nigra) model with chronic glucocerebrosidase (GBA) inhibition by conduritol B-epoxide (CBE) (Figure 2). HER-096 was administered subcutaneously (s.c.) three times of week for 4 or 5 weeks starting from day 0 or day 7 after model induction.

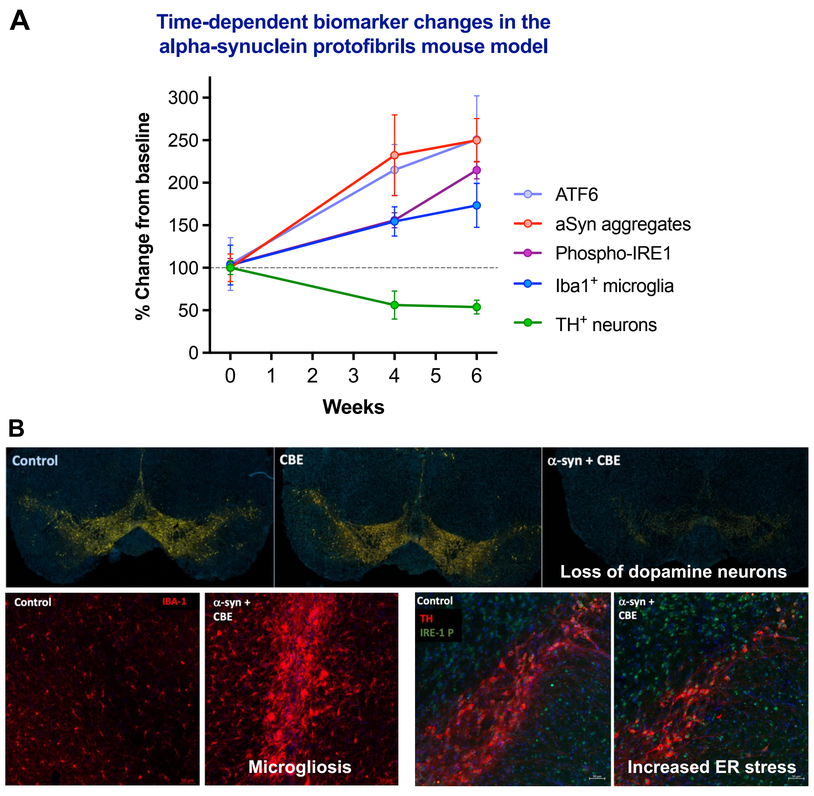

Figure 2. Aged mouse model of synucleinopathy. *(**A**) Development of the different pathology markers in substantia nigra of mouse α-synuclein protofibril model is shown over time as compared to baseline. ER stress and the UPR were activated in dopamine neurons as shown by an increase in phosphorylation of IRE1 and increased nuclear localization of ATF6. This leads to an increase in α-synuclein aggregation (a-syn) and induction of neuroinflammation as seen by increased microglial activation visualized by Iba-1 staining. Finally, the model shows progressive loss of dopamine (TH+) neurons. (**B**) Representative images of immunohistochemical staining demonstrate the loss of dopamine neurons, increased microgliosis and ER stress in mouse substantia nigra 4 weeks after intrastriatal α-synuclein protofibril injection and GBA inhibition.*

RESULTS

HER-096 shows neuroprotective activity *in vitro* that depends on modulation of Unfolded Protein Response (UPR) pathway activity

HER-096 shows a similar potency and mechanism of action as CDNF promoting dopamine cell survival (Figure 3A), reducing α-synuclein aggregation (Figure 3B) and modulating UPR (Figure 3C) in an in vitro midbrain culture treated with the dopamine toxin 1-methyl-4-phenylpyridinium. Neuroprotective activity of HER-096 is abolished upone application of UPR pathways inhibitors (Figure 3D-E).

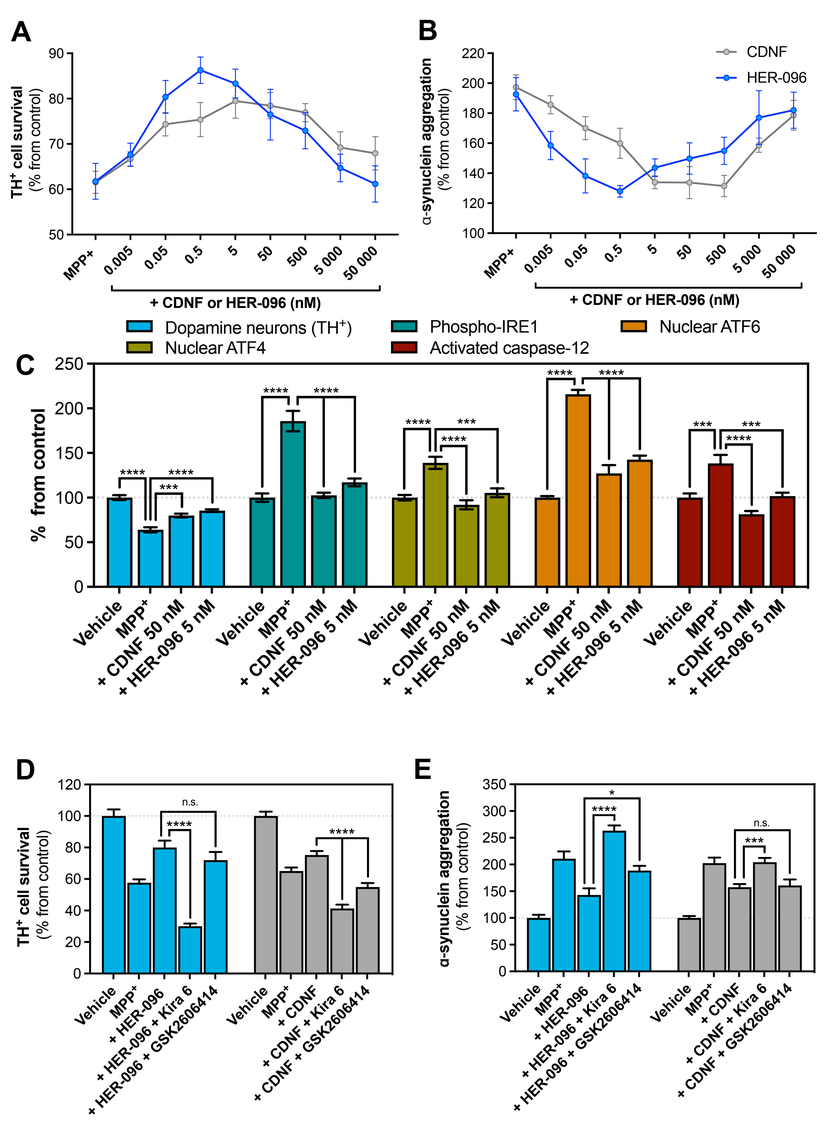

Figure 3. HER-096 protects dopamine neurons via modulation of UPR pathway activity. *Comparison on effect of CDNF and HER-096 on dopamine neurons survival (**A**) and a-synuclein aggregation in dopamine neurons (**B**) after 48 h of MPP+ exposure. (**C**) Effect of CDNF and HER-096 on dopamine neuron survival and activation UPR-associated pathway markers in dopamine neurons. (**D, E**) Change of HER-096 and CDNF neuroprotective activity on MPP+-injured dopamine neurons upon treatment on inhibitors of PERK (GSK2606414) and IRE1 (Kira6) signaling. N=4-6. *p<0.05, ***p<0.001, *****p<0.0001, One-way ANOVA with uncorrected Fisher's LSD test for pairwise comparison versus α-synuclein group. Results are presented as a mean percentage from the control level +/- SEM.*

HER-096 is metabolically stable and able to penetrate to CSF and ISF after subcutaneous administration in healthy animals

S.c. administered HER-096 was found at therapeutic levels in the rat cerebrospinal fluid (CSF) and interstitial fluid (ISF)(Figure 4) and in dog CSF, with extended brain half-life compared to plasma.

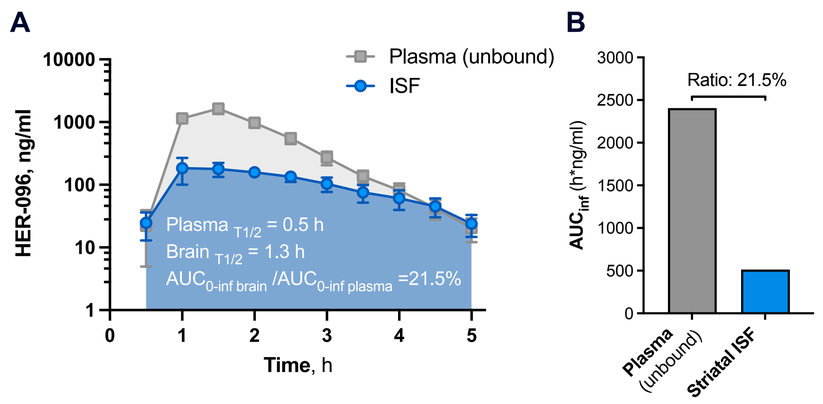

Figure 4. Brain penetration of HER-096 in rats and dogs. *(**A**) Unbound plasma and ISF concentration measured from microdialysate samples collected simultaneously from probes installed to rat striatum and jugular vein for 5 h after subcutaneous bolus administration of HER-096 at 15 mg/kg. N=4. Results are presented as a mean value +/- SEM. (**B**) Unbound plasma and CSF AUC_{inf} were used for estimation of ISF-to-plasma ratio.*

HER-096 demonstrates neuroprotective activity and modulates UPR pathway in an *in vivo* model of Parkinson's disease in aged mice

HER-096 delivered s.c. 3 times per week for 4 weeks modulated UPR activity, protected dopamine neurons, and significantly reduced α-synuclein aggregation and microgliosis in a mouse model relevant to human synucleinopathy (Figure 5).

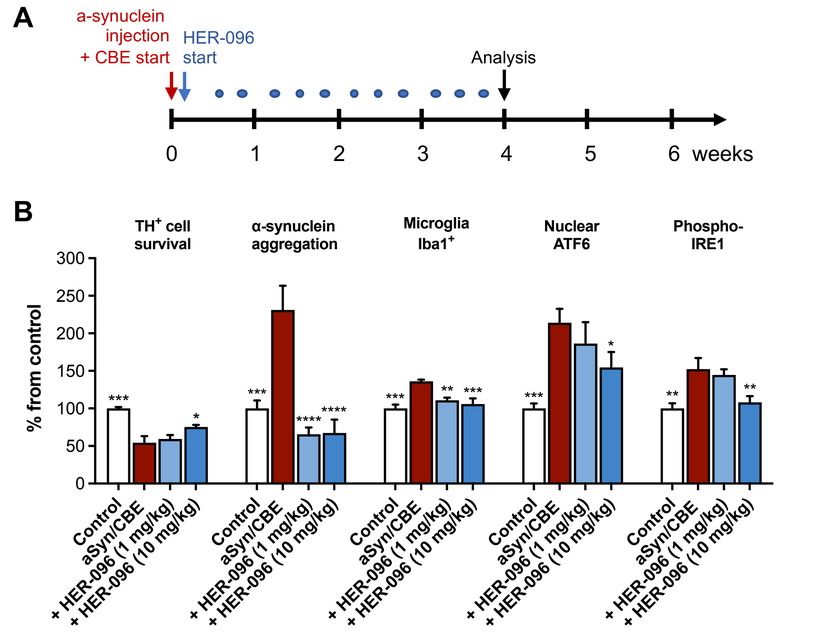

Figure 5. Target pathway modulation and microgliosis-reducing effects of HER-096 in the substantia nigra following subcutaneous administration. *(**A**) HER-096 was administered s.c. in dose 1 or 10 mg/kg three times per week for four weeks starting from the day of model induction. (**B**) HER-096 significantly promoted the dopamine neurons (TH+) survival, reduced the a-synuclein accumulation and microglial (Iba1+) activation. HER-096 reduced the nuclear translocation of ATF6 and IRE1 phosphorylation in TH+ cells. N=4-5. *p<0.05, **p<0.01, ***p<0.001, *****p<0.0001, One-way ANOVA with uncorrected Fisher's LSD test for pairwise comparison versus vehicle group (a-synuclein/CBE). Results are presented as a mean percentage from the control level +/- SEM.*

Also, HER-096 was effective if the treatment started 1 week after induction of the pathology in mice. Efficacy dose range studies suggested that an effective therapeutic range is 2.5-60 mg/kg in the mouse model, based on dopamine cell survival and α-synuclein aggregation (Figure 6). HER-096 was also able to improve proteinopathy-induced impairment of motor function as measured by bar test and grid walking test (after s.c. 3 times/week for 5 weeks).

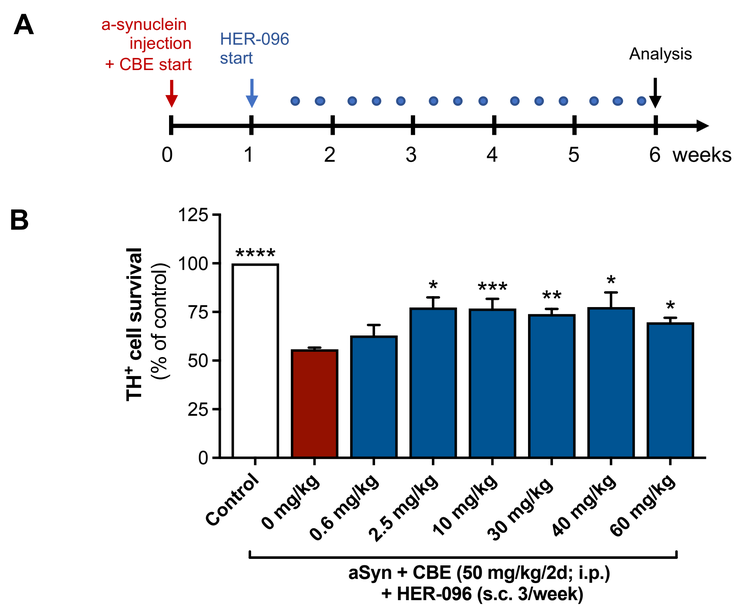

Figure 6. Effective dose range of s.c. HER-096 in the mouse model of synucleinopathy. *(**A**) HER-096 was administered s.c. three times per week for five weeks starting one week after model induction. (**B**) HER-096 significantly promoted dopamine neuron survival at dose levels ranging from 2.5 mg/kg to 60 mg/kg N=5-12. *p<0.05, **p<0.01, ***p<0.001, two-way ANOVA with study and dose as factors followed by uncorrected Fisher's LSD test for pairwise comparison of mean dose effect versus vehicle group (0 mg/kg). Results are presented as a mean percentage from the control level +/- SEM.*

CONCLUSIONS

HER-096 is a novel synthetic peptidomimetic compound developed based on the UPR-modulating site of the human CDNF protein. HER-096 is metabolically stable and can penetrate the blood-brain barrier allowing systemic administration for treatment of neurodegenerative diseases. HER-096 with a more patient-friendly route of administration is a novel candidate for further development of a disease-modifying therapy for Parkinson's disease. A Phase 1a single-ascending dose study in healthy volunteers will begin in April 2023.

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