THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

Therapeutics Development Initiative 2008 – Academic Track
Application Coversheet - Checklist

Principal Investigator Name:	**Jack**	**Rogers**	**Ph.D.**
	First Name	Last Name	Suffix

Institution: **Massachusetts General Hospital**

Project Title: **RNA-based therapeutics for PD directed to the alpha-synuclein 5'untranslated Region.**

Required: (applicable for non-profit & for-profit entities unless specified)

Scientific Information

- ☐ **Narrative – 6 page maximum**
- ☐ **Literature Citations – 3 page maximum**
- ☐ **Budget:**
  - **Budget Details – listed in United States Dollars (using MJFF template)**
  - **Budget Justification**
  - **Other Funding Sources**
- ☐ **Personnel Information**
  - **Biographical Sketches – 2 page maximum per investigator**
  - **Letters of Collaboration**
  - **Intellectual Property Letters**
- ☐ **Approval Documents**
  - **Relevant IRB, IACUC, AAALAC, etc. approval letters**
  - **Letter of Institutional Approval**

Support Documents

Appendix 1: Dose-responsive suppression (IC-50) of α-synuclein 5'UTR directed luciferase reporter gene translation by *Strophanthidine*, *Sarmentogenin, Gitoxigenin-diacetate, Mycophenolic acid* (Natural Product leads found to be potent translation inhibitors of the α-syn 5'UTR conferred luciferase reporter gene expression. Dose-response assays of translation inhibition and cytotoxicity).

Appendix 2: Friedlich, A., Tanzi, R., and Rogers, J., Mol Psychiatry. 2007;12 (3): 222-223

NARRATIVE

Therapeutic Description: Our goal is to prevent α-synucleinopathy, by targeting the 5' untranslated region (5'UTR) of α-synuclein (α-syn) mRNA to develop potent α-syn translation inhibitory drugs that selectively maintain compensatory β- and γ-syn expression.

α-Syn is the ~15 kd protein implicated in the pathogenesis of neurodegenerative α-synucleinopathies (1), including Parkinson's disease (PD), the most prevalent movement disorder in humans. Others include Dementia with Lewy bodies (LBs), LB variant of Alzheimer's disease (AD), and multiple system atrophy. In these disorders, α-syn undergoes a conformational change and oligomerization, causing a toxic gain of function, subsequent neurodegeneration and deposition of α-syn aggregates, most commonly in LBs, but also in dystrophic neuritis, axonal spheroids and glial cytoplasmic inclusions (2). Mutations to the signaling kinase, LRRK-2, activates inflammatory causes of PD (3) (in which our laboratory has experience (4)). However, increased iron in the Substantia nigra appears close to the pathogenesis of PD (5).

Recently, Lewy body dementia (LBD) brains were shown to exhibit lowered α-syn mRNA but higher insoluble protein, suggesting mis-regulation of α-syn mRNA translation in addition to protein clearance by chaperones (6). α-Syn and amyloid-β peptide (Aß) pathomechanisms converge via interaction of the two amyloidogenic proteins; co-precipitating into ß-pleated oligomers and insoluble fibrils (7-9), although Aß and α-syn rarely, if ever, localize to the same brain amyloid deposits (10).

Thus, our discovery of an iron responsive element (IRE-Type II) in the 5'untranslated region (5'UTR) functioning to suppress amyloid precursor protein (APP) translation when intracellular iron is chelated (11)*, encouraged us to then identify a related, but distinct, motif in α-syn mRNA.* IREs are RNA stem loops in the UTRs of ferritin and transferrin-receptor mRNAs that are critical to iron homeostasis (12,13) (Fig 1). The pattern of RNA binding protein interaction with the α-syn IRE, however, was different form the canonical IREs of iron homeostasis (see Fig. 2).

Thus use of the α-syn 5'UTR RNA target is unique enough to identify neural α-syn translation blockers that are sufficiently selective not to change neural β- and γ-syn expression (unrelated 5'UTRs) or cause side-effects towards iron metabolism and homeostasis (a complete knock out of α-syn expression in mice has no influence on viability, which supports our strategy to limit α-syn expression and maintain compensatory β-syn expression (14).

Prior to initiating a high throughput screen (HTS) of 250,000 small molecules for potent α-syn 5'UTR directed inhibitors at the Yale University Genome Center (Letter, Dr. Lars Branden) (Fig. 1), we already have identified and ranked 17 translation inhibitors of α-syn from 720 natural products (NPs). Here we found that the cardiac glycosides *strophanthidine, sarmentogenein, gitoxigenin* selectively and potently inhibited α-syn 5'UTR driven translation of a luciferase reporter gene while maintaining 100% cell viability (Table 1). The half-lives and pharmacodynamics of these cardinolides are well described. This project will also identify top novel α-syn RNA targeted translation inhibitors and ultimately provide a new oral therapeutic approach for PD additional to current anti-fibrilization agents (or SiRNAs, that have to be administered by catheter to the brain).

We have previously demonstrated that the AD experimental drug, (-)-phenserine (PS), a physostigmine analogue and an acetylcholinesterase inhibitor (AChEi) that reached clinical assessment inhibited APP translation though its 5'UTR (15,16). PS proved well tolerated in phase III clinical trials for AD (17). By contrast, posiphen, the (+)-enantiomer of PS, lacks AChEi activity and thus can be administered at 8-fold higher doses (18). It recently underwent dose

escalating phase 1 clinical assessment in humans and was well tolerated (reaching doses of 80 and 120 mg vs. 20 for PS). PS approved to be a well-tolerated compound that consistently reduced α-syn expression in SH-SY5Y cells over a similar dose range as its action on APP (and Aβ) (Fig. 3). Posiphen, spiked into our 384 well transfection-based NP screen, was found to selectively inhibit α-syn 5'UTR conferred translation to a greater extent than PS. We will hence pursue posiphen for its clear capacity to lower α-syn expression in addition to the cardiac glycosides in both tissue culture and animal models (Fig. 3). We plan to use a well known transgenic mouse model for PD to test the anti α-synefficacy of (i) posiphen (ii) the cardiac glycosides (cardinolides:strophantidine/ sarmentogenin) each as α-syn 5'UTR blockers, additional to (iii) novel α-syn 5'UTR translation inhibitors (deriving from our ongoing HTS screen).

Therapeutic Rationale and Current Stage of Development:- The 5'UTR of the α-synuclein transcript folds into a unique RNA stem loop encoding a functional iron responsive element secondary structure that is related to (but totally distinct from) the ferritin and APP 5'UTR specific IREs. This RNA sequence provides an excellent drug target to screen / identify suppressors of α-syn translation since this 5'UTR encodes a highly specific but functional version of an IRE stem loop that is not encoded by the β- and γ-syn transcripts.

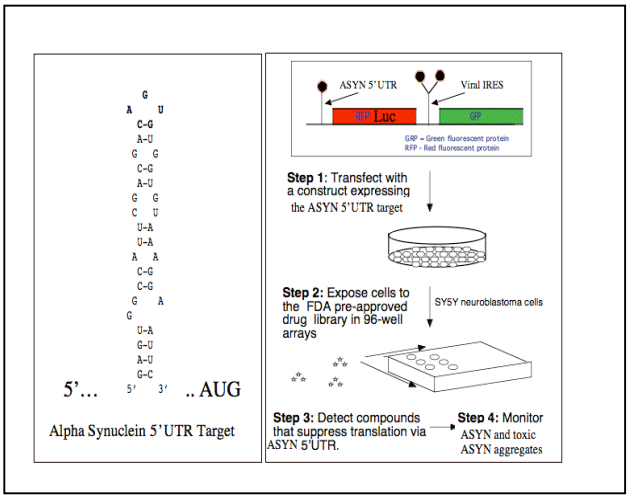

Figure 1: Dicistronic construct designed to screen small molecule inhibitors of α-syn translation: Left Panel: the unique RNA stem-loop target in the 5'UTR of the transcript for α-syn (RNA computer predicted by the multi-fold program of Zuker et al., 2003). **Right Panel:** Transfection based screen for α-syn 5'UTR directed compounds that limit luciferase/RFP reporter translation pIRES (α-syn), that maintain GFP expression from an IRES for use of this RNA targeting technology for identifying translation inhibitors of APP mRNA (precedent for AD therapeutics: see our Refs (11) (19)). Potent AD specific inhibitors of the APP 5'UTR inhibitors were already screened and can be downloaded from PUBCHEM at the NCBI websites as AID: 1285 describing our primary screen for inhibitors of Alzheimer's amyloid precursor protein (APP) translation (translation blockers).

As for APP mRNA and ferritin mRNA, intracellular Fe chelation with desferrioxamine repressed neural α-synu translation acting through the IRE in the α-syn 5'UTR(11,19). The Iron-regulatory Proteins, IRP1and IRP2, control iron homeostasis by binding to IRE stem loops in the 5' UTR of ferritin mRNAs (translational repression) and the 3'UTR of transferrin-receptor (TfR) mRNA during iron deficit. Since the IRE-like stem loop structure predicted in the 5' UTR of α-syn, was ~50% sequence homologous to the ferritin-H IRE (Fig 2), we sought to determine whether this α-syn 5'UTR sequence exhibited the same profile of RNA binding as the ferritin-H IRE.

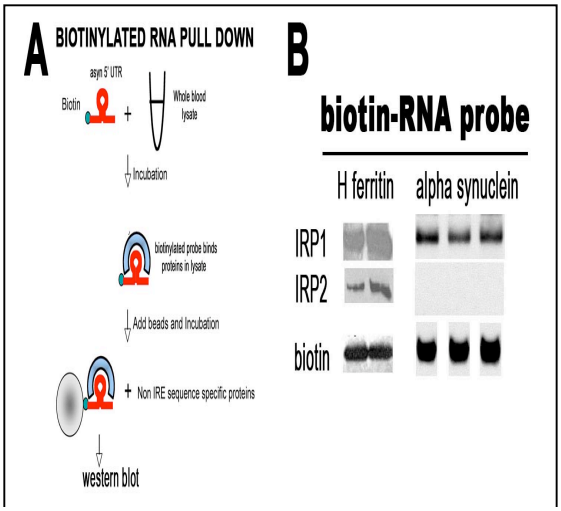

Figure 2: The α-syn 5'UTR uniquely bound to IRP1 whereas the canonical ferritin IRE interacted with IRP-1 and IRP2, and thus is a selective drug target for PD therapeutics: A: Schematic of the pull down assay with biotinylated RNA probes encoding α-syn & H-ferritin IREs employed in a protein capture assay to identify the IRP binding specificity of their RNA stem loops (Western blot). **Panel B**: Biotinylated RNA probes for α-syn IRE co-precipitated with

IRP1, but not IRP2 whereas the H-ferritin 5' UTR IRE probe co-precipitated both IRP-1 and IRP-2 (Westerns).

The biotin pull down assays, shown in Figure 2, demonstrated that the α-syn 5'UTR selectively interacted with IRP1, and not IRP2 in human blood and (not shown) human brain lysates. By contrast, the H-ferritin IRE interacted with both IRP-1 & IRP-2 consistent with IRP2 being the more important controller for iron homeostasis (controlling ferritin translation and TfR mRNA stability by iron). These findings add weight to the use of this 5'UTR as a desirable and selective drug target to identify selective suppressors of α-syn expression *in vitro* and *in vivo*.

With strong preliminary data that α-syn mRNA 5' UTR contains a cis-acting translational regulatory element that selectively binds IRP1, we evaluated the α-syn mRNA 5' UTR as a target for pharmacologic suppression of α-syn protein production. As precedent, APP 5'UTR directed inhibitors, some of which are in clinical trials, can be effectively identified and subsequently found not to change APLP-1 and APLP-2 expression in SH-SY5Y cells (20,21), and we reported that APP 5'UTR inhibitors successfully reduced amyloid burden in a transgenic mouse model for AD (22)).

Because of the 50% sequence similarities between the APP and α-syn 5' UTRs (Fig 3A), we predicted overlap in the spectrum of drugs that suppress APP translation through the APP mRNA 5' UTR with those that suppress α-synu through its 5' UTR. Posiphen, as a RNA directed drug that passed phase 1 safety trials in human subjects, selectively repressed neural α-syn translation via its 5'UTR (Fig.1), with clear evidence for PS enhancing translational repression (by IRP1). Transfection experiments showed posiphen was 10 times more effective as a blocker of α-syn 5'UTR driven translation relative to the related but distinct APP 5'UTR element.

Thus we assessed whether PS and posiphen would similarly suppress α-syn expression in SH-SY5Y dopaminergic neurons. Indeed PS inhibited of APP expression by half at 5 μM (Fig 3B), whereas it inhibited α-sy expression at an IC-50 of 7μM. By comparison, posiphen suppressed expression of α-syn and APP, each with an IC-50 of 5 μM.

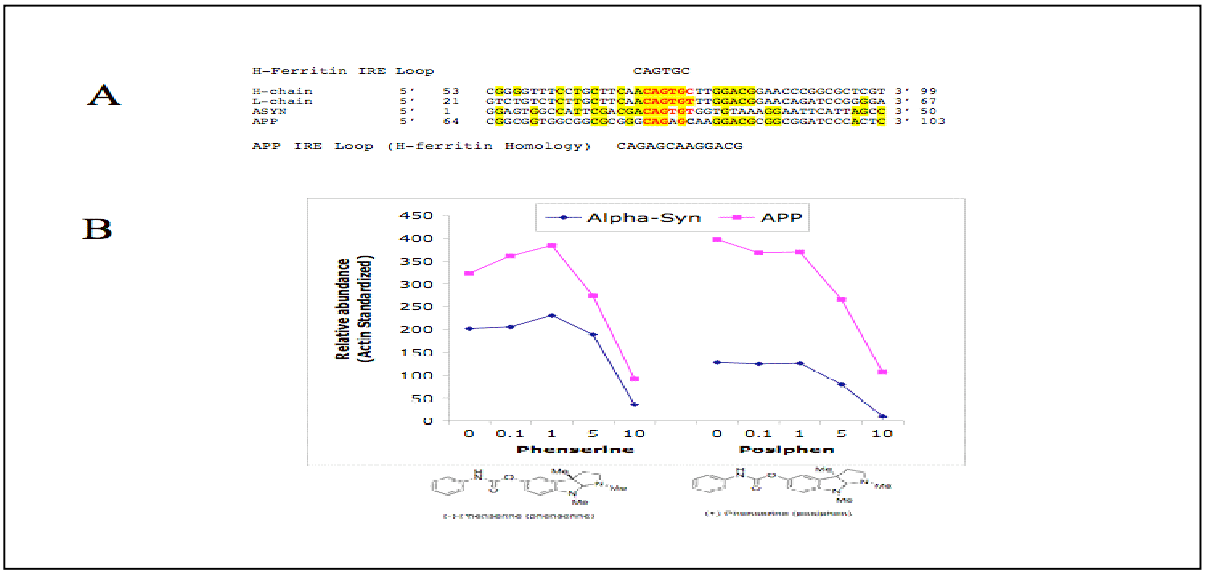

Figure 3: Phenserine and posiphen each decrease levels of α-syn in a dose-dependent manner in cultured cells as previously reported for APP**. Panel A:** The 5'UTRs of both APP and α-syn show homology to each other and also 50% homology to the Iron-responsive Element (IRE) in H-ferritin mRNA (23). **Panel B:** SH-SY5Y cells were treated with PS and posiphen for 48 hours with concentrations ranging from 0 to 10 μM and inhibited α-syn expression (IC-50 =5 μM) without cell based toxicity.

Using stable α-syn 5'UTR–luciferase H4 cell lines, transfected with the dicistronic screening construct shown in Figure 1, we set up a high throughput screening campaign of 720 NPs (see Table 1). Shown are four (of 17 available) α-syn 5'UTR directed inhibitors assayed in neural cells (a HTS of the complete library is planned with Dr. Lars Branden (letter attached)).

Lead Compound	*APP Specific Inhibition (Max 10 μM)*	*ASYN Specific Inhibition (Max 10 μM)*	*Toxicity Alamar Blue at IC-50*	*IC-50 when drug inhibits a-syn 5'UTR driven lucif. activity by half.*
Harmine	50% Inhibition	65%	Trophic	Anti-Parkinsonian Stimulant.
Posiphen	85% Inhbition	75%	Non toxic	Phase I Tolerant. (IC-50 = 50 nM.
Sarmentogenin	5% Inhibition	100%	Non toxic	Glycoside/ IC-50 = 500 nM
Strophanthidine	5% Inhibition	100%	Non Toxic	Glycoside/ IC-50 = 400 nM
Gitoxigneinin	5% Inhibition	100%	Non Toxic	Glucoside/ IC-50 – 80 nM
Mycophenolic acid	5% Inhibition	50%	Non toxic	Immunosuppressant IC-50 = 100nM

Table 1: Selectivity of top alpha-syn 5'UTR directed inhibitors screened from 720 MLSCN NPs. Posiphen was incorporated into this screen as a positive control. The other 5 natural products shown are from the top 17 α-syn 5'UTR inhibitors thus far identified. Percent inhibition in the Alamar Blue assay was calculated and compared with percent inhibition of luciferase activity. Compounds for which the difference was greater than 40, and which scored as a hit in the luciferase assay were then being evaluated in a dose-response assay (appendix 1). Leads were pursued for further testing in this proposal if they passed the criterion of maintaining APP 5'UTR activity (specificity control).

Our current leads include the plant cardenolides, *sarmentogenin* and *strophanthidine*, *ditoxigenin-diacetate* and *mycophenolic acid (immunosuppressant used in kidney transplants).* α-Syn 5'UTR inhibitors were checked to ensure target selectivity - where all top cardiac glycosides caused no change to APP 5'UTR target expression. *Harmine* inhibited α-syn expression and is already used as a therapeutic agent for PD (to be used as +ve control for our purposes), although *harmine* did reduced APP 5'UTR mediated expression in our assays.

Development Plan and Timeline:

We will characterize and establish the optimal doses and formulations of top α-syn 5'UTR inhibitors, including that of posiphen, relative to our NPs, the cardenolides as listed in Table I, each to be tested in the in-house α-syn transgenic mouse model available to our laboratory (24). PS/posiphen and its optimized derivatives from Dr. Greig's medicinal chemistry program within the Intramural Research Program of NIA, NIH, are available to test for α-syn efficacy using the α-syn transgenic mouse model (ASYN) (24), with induced α-syn expression (and dopamaminergic fibrilized alpha-syn).

These in-house ASYN transgenic animals (24), will be tested for posiphen efficacy as we have already achieved for the anti-amyloid efficacy in AD mouse models (25). A concern for dual action drugs that possess AChE activity is that PS, similar to most other AChEiss, is dose-limiting in animal models and humans by cholinergically mediated central actions (tremor in animals and nausea in humans, respectively) (26). As posiphen lacks AChE action (18,26), this concern is circumvented. Our prior studies demonstrate that PS can be maximally administered at 7.5 mg/kg whereas posiphen can be escalated to 75 mg/kg (18,26). Furthermore, we predict that as with APP, concentrations in brain below amounts found in cell culture will effectively lower α-syn levels.

Specific Aim 1: Characterize the top α-syn translation inhibitors in cultured neural cells :

By use of quantitative Western blot analysis, we will use both H4 and SH-SY5Y neural cells lines to establish the effective concentrations of each of our leads to inhibit α-syn expression (IC-50). In the first instant, the cardiac glycosides *sarmentogenin* and *strophanthidine*, are currently being

assessed for their capacity to inhibit α-syn correlated to their cytotoxicity (Alamar Blue assay, MTS assay) in these neural cells lines. Similar experiments were already reported to establish the cytotoxicity of digitoxin analogs and other related cardiac glycosides in human tumor cells (27). Western blot assays will establish the lowest doses of each agent required to inhibit neuronal α-syn and not β- and γ- syn expression. Prior to beginning animal tests, we will test minimum formulated doses of each of the compounds (in Table I) required to reduce α-syn expression in primary cortical dopaminergic neurons. For example, individually tested and ranked *sarmentogenin*, *strophanthidine* and Gitoxigenin Diacetate will be dose respsonse optimized in combinations for anti α-syn efficacy in cell lines and monitored closely for toxicity since cardiac glycosides have a narrow therapeutic window. To ensure selectivity, treatment with each drug should be compensated by β-syn and γ-syn expression. In support of this rationale, RNA targeting with macrolide antibiotics inhibits bacterial ribosomal RNA function (28) and β-syn and γ-syn knockout mice are viable (29).

Timeline: Progressing from Year-1 into Year-2 we anticipate conducting a continuation of this project in a milestone driven basis in dialogue with the scientific staff at the Michael J Fox Foundation. We anticipate establishing an optimal formulation of our leads and have in hand the best strategy for administering our leads to the transgenic mice for evaluating their anti α-syn efficacy *in vivo*.

Specific Aim 2: Test and validate a-synuclein inhibitors using an animal model of PD.

At MGH we have been provided transgenic mice that express wild-type α-syn on a C57BL/6 x DBA/2 mixed genetic background (30). These mice were well characterized and develop α-syn neuropathology by 2 months of age, therefore providing an excellent *in vivo* model for preclinical drug suppression of human α-syn production where the α-syn 5'UTR drives the expression of wild-type and mutant α-syn. Handling and breeding of these mice will be undertaken with Drs. Castelvetri (MGH) and Friedlich (MGH))(Letters Provided).

The results of our screen for α-syn directed translation inhibitors from 720 NPs (in 384 well format) are shown in Table 1. These Nps maintain excellent cell viability by the Alamar Blue test in H4 cells, and one drug, harmine, already has an indication for PD. Since their pharmacology and *in vivo* dosing profiles are published these are excellent lead candidates to validate the α-syn 5'UTR as a genuine anti α-syn drug target. We will test these leads (together with posiphen compared to placebo) in the α-syn transgenic mouse model for PD available at MGH (24).

In vivo drug efficacy against a-synulein induced PD pathology will be tested in a 3 month study of 6 cohorts of α-syn transgenic mice treated with (i) *posiphen*, (ii) *phenserine* (iii) *dose optimized Strophanthidine*/ (iv) dose optimized *sarmentogenine, (v) harmine* (+ve control), (vi) placebo (-ve control) (n=14) (age and sex matched). We will orally provide these natural product drugs at lowest dose to the mice in a protocol similar to tests that we have already published to assess the anti amyloid efficacy of FDA drugs in AD transgenic mice (22,25). Drug doses will be estabshed from the published ADME literature. In the case of posiphen we already established that 75 mg/kg/day (i.p.) was a well tolerated dose for posiphen in mice, 3 week trial.

In the case of the cadenolides, we have access to published work that reports the cytotoxicity of several cardenolides, including ouabain (a close analog of strophanitidne), and digitoxigemnis (a close anlog of digitoxenin) with primary tumor cell lines, as others have been developing the use of these glycosidse for the alternative indication of anti-cancer therapy (see ref (27)). Indeed glycosides are now being tested as novel anticaner agents with results demonstrating a wide range of cytotoxicity depending on the drug ((31)). Of interest to our work, there is literature available that describes the formulation and administrative path of caredeloisdes, to mice, for example Chen et al., 1952 (32).

Outcomes measurement and techniques: After 3 month treatment (2 mo - 5 mo interval) mice will be assessed to measure potential improvement of movement deterioration by use of the rotor-rod grasping test. These PD mice exhibit movement disorder starting at 3 months of age progressing to 12 months. Following rotor-rod movement testing, mice will be sacrificed and the pathological markers of PD associated with α-syn accumulation will be measured by standard protocols (i.e., immunohistochemistry of α-syn and tyrosine hydoxylase (TH) and biochemical measurement of dopamine levels in collected striatal samples (by ELISA). We will test the relative capacity of each drug to offset the accumulation of α-syn into brain LBs, their relative capacity to lessen α-syn induced neuronal inclusion formation, their capacity to improve levels of striatal neuron TH and dopamine. Disease progression in these PD specific lesions has previously been established in our α-syn transgenic mice and our purpose is to determine whether α-syn 5'UTR inhibitor leads, indeed, exhibit disease associated efficacy *in vivo* (see letters of Collaboration Drs. Castelvetri and Fridlich.

Time line: Progression to the experiments outlined in Specific Aim 2 (Year-2) are to proceed in a "milestone" driven protocol based on evaluations (at the end of Year-1) as to the capacity of our most attractive leads to limit α-syn (but maintain β-syn and γ-syn) in neural cell cultures. We already have established convincing Western blot data showing posiphen reduced α-syn levels in SH-SY5Y cells (Fig 2) and also in H4 cells (not shown). These decisions will be made in consultation with the scientific staff of M. J. FOX Foundation depending on progress made in Year-1 with our current best lead α-syn translation inhibitors including *sarmentogenin* and *strophanthidine and in a low dose cocktail*.

During the course of Year-1 even more potent selective drugs will become available from our cell culture drug optimization and characterizations (IC-50 and LD-50 of current leads). At the end of Year-1 proceeding to Year-2, we will have assessed optimal lead dosing of our NP leads best suited for their testing parallel with posiphen and PS delivered to the α-syn transgenic mice. These new potentially more potent α-syn hit inhibitors are in the screening pipeline.

Future Development Requirements: (other future collaboration).

The immediate advantage of this project is that we are guaranteed results with posiphen and a lead cardiac glycoside, maybe even more potent compound processed by medicinal chemistry optimization (Dr. Nigel Greig heads drug design within NIA and has other leads for this drug class, and we have the additional capacity to further work closely with Dr. Xudong Huang, Greg Cuny, each well respected medicinal chemists at BWH). With this team, we will chemically optimize the HTS leads from the screening pipeline for the Columbia/Yale Genome Center (appendix 3)). This effort will improve our chances for animal testing for the pharmacokinetic potential (by linear metabolism array analysis prior to animal testing).

The transgenic mice expressing wild-type and mutant human α-syn (Science 287:1265; 2000) on a C57BL/6 x DBA/2 mixed genetic background. These mice are well characterized and develop α-syn neuropathology by 3-4 months of age, Thereby providing an excellent in vivo model for preclinical drug development to suppress production of human α-syn protein production and interdict pathogenesis.

References

1. Ueda, K., Fukushima, H., Masliah, E., Xia, Y., Iwai, A., Yoshimoto, M., Otero, D. A., Kondo, J., Ihara, Y. & Saitoh, T. (1993) Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proc Natl Acad Sci U S A 90: 11282-11286.

2. Lee, V. M., Giasson, B. I. & Trojanowski, J. Q. (2004) More than just two peas in a pod: common amyloidogenic properties of tau and alpha-synuclein in neurodegenerative diseases. Trends Neurosci 27: 129-134.

3. Taylor, J. P., Mata, I. F. & Farrer, M. J. (2006) LRRK2: a common pathway for parkinsonism, pathogenesis and prevention? Trends Mol Med 12: 76-82.

4. Cahill, C. M. & Rogers, J. T. (2008) Interleukin-1beta induction of IL-6 is mediated by a novel phosphatidylinositol 3-kinase dependent AKT/Ikappa B kinase alpha pathway targeting activator protein-1. J Biol Chem.

5. Oakley, A. E., Collingwood, J. F., Dobson, J., Love, G., Perrott, H. R., Edwardson, J. A., Elstner, M. & Morris, C. M. (2007) Individual dopaminergic neurons show raised iron levels in Parkinson disease. Neurology 68: 1820-1825.

6. Cantuti-Castelvetri, I., Klucken, J., Ingelsson, M., Ramasamy, K., McLean, P. J., Frosch, M. P., Hyman, B. T. & Standaert, D. G. (2005) Alpha-synuclein and chaperones in dementia with Lewy bodies. J Neuropathol Exp Neurol 64: 1058-1066.

7. Jensen, P. H., Hojrup, P., Hager, H., Nielsen, M. S., Jacobsen, L., Olesen, O. F., Gliemann, J. & Jakes, R. (1997) Binding of Abeta to alpha- and beta-synucleins: identification of segments in alpha-synuclein/NAC precursor that bind Abeta and NAC. Biochem J 323 (Pt 2): 539-546.

8. Conway, K. A., Harper, J. D. & Lansbury, P. T. (1998) Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. Nat Med 4: 1318-1320.

9. Han, H., Weinreb, P. H. & Lansbury, P. T., Jr. (1995) The core Alzheimer's peptide NAC forms amyloid fibrils which seed and are seeded by beta-amyloid: is NAC a common trigger or target in neurodegenerative disease? Chem Biol 2: 163-169.

10. Trojanowski, J. Q. & Lee, V. M. (2001) Parkinson's disease and related neurodegenerative synucleinopathies linked to progressive accumulations of synuclein aggregates in brain. Parkinsonism Relat Disord 7: 247-251.

11. Rogers, J. T., Randall, J. D., Cahill, C. M., Eder, P. S., Huang, X., Gunshin, H., Leiter, L., McPhee, J., Sarang, S. S. et al. (2002) An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. J Biol Chem 277: 45518-45528.

12. Thomson, A. M., Rogers, J. T. & Leedman, P. J. (1999) Iron-regulatory proteins, iron-responsive elements and ferritin mRNA translation. Int J Biochem Cell Biol 31: 1139-1152.

13. Thomson, A. M., Cahill, C. M., Cho, H. H., Kassachau, K. D., Epis, M. R., Bridges, K. R., Leedman, P. J. & Rogers, J. T. (2005) The Acute Box cis-Element in Human Heavy Ferritin mRNA 5'-Untranslated Region Is a Unique Translation Enhancer That Binds Poly(C)-binding Proteins. J Biol Chem 280: 30032-30045.

14. Chandra, S., Fornai, F., Kwon, H. B., Yazdani, U., Atasoy, D., Liu, X., Hammer, R. E., Battaglia, G., German, D. C. et al. (2004) Double-knockout mice for alpha- and beta-synucleins: effect on synaptic functions. Proc Natl Acad Sci U S A 101: 14966-14971.

15. Shaw, K. T., Utsuki, T., Rogers, J., Yu, Q. S., Sambamurti, K., Brossi, A., Ge, Y. W., Lahiri, D. K. & Greig, N. H. (2001) Phenserine regulates translation of beta -amyloid precursor protein mRNA by a putative interleukin-1 responsive element, a target for drug development. Proc Natl Acad Sci U S A 98: 7605-7610.

16. Venti, A., Giordano, T., Eder, P., Lahiri, D. K., Greig, N. H. & Rogers, J. T. (2004) The Integrated Role of Desferrioxamine and Phenserine Targeted to an Iron-Responsive Element in the APP-mRNA 5'-Untranslated Region. Ann N Y Acad Sci 1035: 34-48.

17. Kadir A, Andreasen N, Almkvist O, Wall A, Forsberg A, Engler H, Hagman G, Lärksäter M, Winblad B, Zetterberg H, Blennow K, Långström B, Nordberg A. (2008) Effect of phenserine treatment on brain functional activity and amyloid in Alzheimer's disease. Ann Neurol. 63:621-31.

18. Lahiri DK, Chen D, Maloney B, Holloway HW, Yu QS, Utsuki T, Giordano T, Sambamurti K, Greig NH. (2007) The experimental Alzheimer's disease drug posiphen [(+)-phenserine] lowers amyloid-beta peptide levels in cell culture and mice. J Pharmacol Exp Ther. 320:386-96.

19. Bandyopadhyay, S., Huang, X., Cho, H., Greig, N. H., Youdim, M. B. & Rogers, J. T. (2006) Metal specificity of an iron-responsive element in Alzheimer's APP mRNA 5'untranslated region, tolerance of SH-SY5Y and H4 neural cells to desferrioxamine, clioquinol, VK-28, and a piperazine chelator. J Neural Transm.: 237-247.

20. Morse, L. J., Payton, S. M., Cuny, G. D. & Rogers, J. T. (2004) FDA-Preapproved Drugs Targeted to the Translational Regulation and Processing of the Amyloid Precursor Protein. J Mol Neurosci 24: 129-136.

21. Payton, S., Cahill, C. M., Randall, J. D., Gullans, S. R. & Rogers, J. T. (2003) Drug discovery targeted to the Alzheimer's APP mRNA 5'-untranslated region: the action of paroxetine and dimercaptopropanol. J Mol Neurosci 20: 267-275.

22. Tucker, S., Ahl, M., Cho, H. H., Bandyopadhyay, S., Cuny, G. D., Bush, A. I., Goldstein, L. E., Westaway, D., Huang, X. & Rogers, J. T. (2006) RNA Therapeutics Directed to the Non Coding Regions of APP mRNA, In Vivo Anti-Amyloid Efficacy of Paroxetine, Erythromycin, and N-acetyl cysteine. Curr Alzheimer Res 3: 221-227.

23. Friedlich, A. L., Tanzi, R. E. & Rogers, J. T. (2007) The 5'-untranslated region of Parkinson's disease alpha-synuclein messengerRNA contains a predicted iron responsive element. Mol Psychiatry 12: 222-223.

24. Masliah, E., Rockenstein, E., Veinbergs, I., Mallory, M., Hashimoto, M., Takeda, A., Sagara, Y., Sisk, A. & Mucke, L. (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. Science 287: 1265-1269.

25. Tucker, S., Ahl, M., Bush, A., Westaway, D., Huang, X. & Rogers, J. T. (2005) Pilot study of the reducing effect on amyloidosis in vivo by three FDA pre-approved drugs via the Alzheimer's APP 5' untranslated region. Curr Alzheimer Res 2: 249-254.

26. Greig NH, S. K., Yu QS, Brossi A, Bruinsma GB, Lahiri DK. (2005.) An overview of phenserine tartrate, a novel acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. . Curr Alzheimer Res. 2: 281-290.

27. Johansson, S., Lindholm, P., Gullbo, J., Larsson, R., Bohlin, L. & Claeson, P. (2001) Cytotoxicity of digitoxin and related cardiac glycosides in human tumor cells. Anticancer Drugs 12: 475-483.

28. Porse, B. T., Kirillov, S. V., Awayez, M. J. & Garrett, R. A. (1999) UV-induced modifications in the peptidyl transferase loop of 23S rRNA dependent on binding of the streptogramin B antibiotic, pristinamycin IA. RNA 5: 585-595.

29. Dauer, W., Kholodilov, N., Vila, M., Trillat, A. C., Goodchild, R., Larsen, K. E., Staal, R., Tieu, K., Schmitz, Y. et al. (2002) Resistance of alpha -synuclein null mice to the parkinsonian neurotoxin MPTP. Proc Natl Acad Sci U S A 99: 14524-14529.

30. Mucke, L., Yu, G. Q., McConlogue, L., Rockenstein, E. M., Abraham, C. R. & Masliah, E. (2000) Astroglial expression of human alpha(1)-antichymotrypsin enhances alzheimer-like pathology in amyloid protein precursor transgenic mice. Am J Pathol 157: 2003-2010.

31. Newman, R., P, Y., Pawlus AD & KI., B. (2008) Cardiac glycosides as novel cancer therapeutic agents. Mol Intervention 8: 36-49.

32. Chen, K. K., Anderson, R. & Rose, C. (1952) Comparison of 11-hydroxy cardiac steriods with compound F adn cortisone. J Pharmacol Exp Ther 106: 314-318.