Proposal for “Grow Iowa Values Fund” Grant Program

Title: Testing of lead PK compounds in preclinical animal models of Parkinson’s disease.

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Collaborator: George A Kraus, Ph.D. Director, IPRT Administration, ISU, Ames, IA 50011. 515-294-8902; gakraus@iastate.edu

Company partners: PK Biosciences Corporation (since 2006; 4 employees) Vellareddy Anantharam, Ph.D., Vice President 2501 N. Loop Drive, Suite 1600, Ames, IA 50010, 515 450-4836; info@pkbio.com

EXECUTIVE SUMMARY

Parkinson’s disease (PD) is a major debilitating neurodegenerative disorder characterized by a selective degeneration of nigral dopaminergic neurons in the brain. PD affects more than 1.5 million people in the U.S. with over $2.5 billion spent annually for pharmaceuticals. The existing treatment approach for PD is symptomatic and fails to prevent the progression of the disease. Development of neuroprotective agents to delay progression of the disease is the subject of active research, patent applications and clinical trials. Lack of understanding of the molecular mechanisms underlying the disease severely hinders the development of neuroprotective drugs for PD. Fortunately, we have identified a key apoptotic cell death pathway in PD cell culture and animal models that involves aberrant activation of a novel protein kinase-C, namely PKCδ, and inhibition of the kinase protects the dopaminergic neurons, suggesting that PKCδ is a promising therapeutic target for PD. This work led to the filing of two U.S. patent applications in 2005 (patent pending: SN: 11/262,677 and 11/479,173) with ISURF. Additionally, we established the biopharmaceutical company PK Biosciences Corp., in Ames to further develop novel neuroprotective strategies for treatment of PD. In 2008, PK Biosciences, in collaboration with Dr. Kraus’ organic synthesis group, secured SBIR phase-1 funding from NIH to synthesize and characterize a library of PKCδ small molecule inhibitors (PK analogs). This led to the identification of three lead PK compounds with excellent neuroprotective activity in PD cell culture models. The next logical step of this very promising project is to test the lead PK analogs in well-established preclinical animal models of PD to determine in vivo neuroprotective efficacy. Funding from GIVF will be used to demonstrate the feasibility of the study in PD animal models, and will provide preliminary data for a SBIR-Phase-II application. Thus, the requested GIVF funding will support this promising drug discovery project at its critical juncture to transition from SBIR-phase-I to SBIR-phase-II in developing an effective neuroprotective drug for treatment of PD. The ultimate success of the proposed neuroprotective technology can be expected to create many new opportunities in Iowa, including economic and job growth and a viable biotechnology industry.
Background and Rationale: Parkinson’s disease (PD) is a major progressive neurodegenerative disorder characterized by the cardinal motor symptoms of rigidity, bradykinesia, tremors, and postural instability. A pathological hallmark of PD includes a significant loss of dopaminergic neurons in the substantia nigra leading to a dramatic depletion of dopamine in the striatum. The etiology of PD cases has been estimated to be >95% sporadic and <5% familial. PD incidence increases with age, with the mean age of onset around 55 years. Early onset PD cases have been linked significantly to genetic factors. The degenerative process of PD has been proposed to be influenced by genetic susceptibility, environmental neurotoxin exposure, mitochondrial respiratory failure, excessive free radical injury, excitotoxicity, and aging [1-9].

Neuroprotection and PD: The existing treatment approach (primarily levodopa and dopamine receptor agonists) for PD aims to control symptoms but fails to prevent the progression of the neurodegenerative process and produces severe side effects including dyskinesia (the inability to control muscles). The discovery of levodopa for the treatment of PD represents one of the most remarkable success stories in the history of medicine. However, the drug confers only symptomatic relief of what remains an inexorably progressive neurodegenerative disorder. Hence, novel neuroprotective agents designed to interfere with the basic pathogenic mechanism of cell death in PD are clearly needed. Several lead compounds representing different classes of pharmacological agents have been explored for neuroprotective potential in PD but the clinical trials have not been encouraging. We have in the past five years performed translational research based on solid mechanistic studies aimed at identifying a small molecule kinase inhibitor of PKCδ with greater neuroprotective efficacy and a high therapeutic index for PD. Scheme-1 summarizes the overall progress to date and the long term goal of our economic development in the neuropharmacology area.

Research Design: Hypothesis: Novel PK analogs will be effective in preventing neurodegenerative processes in a preclinical model of Parkinson’s disease

Specific Aim: To determine the potential neuroprotective effects of the lead PK analogs in the MPTP-induced preclinical animal models of Parkinson’s disease Using SBIR phase-I funding, we screened our PK chemical library consisting of first, second and third generation PK analogs in the well-characterized cell culture model of PD known as mesencephalic dopaminergic neuronal cells (N27 cells) [10-17]. Among the first generation analogs tested, analog PK08102 most effectively protects against MPP⁺-induced apoptotic cell death. Results obtained with subsequent screening of the second and third generation analogs
suggested that PK08202 and PK09301 most effectively prevent MPP⁺-induced neurotoxicity. Verification of the neuroprotective effect of selective and potent PK compounds in a whole animal model of PD is the next logical step. From the results obtained from cell culture studies, we propose to test the potential neuroprotective effect of PK compounds PK08102 and PK09301 in the well-characterized C57 black mouse model of MPTP dopaminergic neurotoxicity. The overall plan is outlined in the Scheme 2. The following section describes the experimental design and methods for the proposed studies.

**Characterization of the neuroprotective efficacy of the lead novel compounds in the MPTP treated murine model of PD.**

C57 black mice, at postnatal week 12-14 (approx. 25 g), will be arranged by weight and randomized into control, MPTP and MPTP plus PK analog treated groups. Each group will have 20 animals (10 animals for neurochemical/biochemical and 10 animals for immunohistochemical studies). We will test two doses of each analogs and use total of 200 animals for the studies. Animals will be injected with MPTP at a dose of 25 mg/kg (i.p.) once a day for five days to induce Parkinsonism. The drug treated groups will receive once daily 1, 3 or 10 mg/kg (p.o.) of each PK compound (PK08102 and PK09301). The PK compound treatment will begin along with the MPTP treatment and will continue for six days post MPTP-treatment. Seven days post-MPTP treatment, animals will be subjected to behavioral, neurochemical and biochemical studies.

**Behavioral testing of animals:** Since chemical-induced neurological deficits have traditionally been diagnosed by clinical behavioral evaluation, we propose to evaluate motor signs in MPTP-treated mice that reflect difficulty in initiation of ambulatory activity and changes in ambulatory activity patterns by measuring the locomotor activity with a Versamax computerized activity monitoring system (Accuscan, Columbus, OH). Following the behavior measurements, animals will be sacrificed and neurochemical/biochemical and histopathological changes in the brain will be assessed.

**Neurochemical/biochemical measurements:** Brains from animals designated for neurochemical/biochemical measurements will be removed and then striatum and nigral tissues will be separated. We will measure dopamine and its metabolites (DOPAC, HVA) from one half of the tissue using an HPLC-electrochemical procedure, and the other half will be used for biochemical analyses. These include immunoprecipitation PKCδ kinase assays and Western blot analyses of cleaved caspase-3.

**Immunohistological analysis of nigral brain tissue:** The second set of animals will be used for immunohistological studies as described previously in our publication [19]. After the treatments, animals will be perfused transcardially with 4% paraformaldehyde and 40 micron brain sections will be processed for immunohistochemical studies and evaluated for TH, GAD, microglia and astroglial activation, and caspase-3 cleavage. All methods will be performed as described previously in our lab [11, 14, 16-21].
**Time line and extramural funding plan:** We request 18 months of funding for the proposed studies. The proposed animal studies are time consuming and labor intensive. The 18 month time window will allow generation of sufficient preliminary data for submission of a strong SBIR phase-II application in order to secure the funding. In phase-II, the focus will be on optimization of a lead PK drug candidate, efficacy studies in a primate model and preclinical toxicological evaluation.

**Commercialization Plan and Potential Economic Impact of the Proposal:** Parkinson's disease (PD), a devastating neurodegenerative disorder affecting several million people worldwide, inflicts a tremendous social and economic burden on modern society. In 2004, the global neurological degenerative disease (NDD) pharmaceutical market was valued at $5.7 billion, of which PD treatments accounted for $2.5 billion. The total NDD market achieved a compound annual growth rate of 23% in 2000-2004.\(^1\) Recent studies estimated that Parkinson's disease costs approximately $5 billion in the U.S. alone, and over $25 billion per year globally [22-25]. The existing treatment approach (levadopa and dopamine receptor agonists) for PD is symptomatic not only fails to prevent the progression of the neurodegenerative process but it also produces severe side effects including dyskinesia. Quite simply, no drugs are currently available to effectively treat progressive neuronal cell death in PD. A neuroprotective drug for PD would have a huge market value. The average medication cost per patient is estimated at $2,500 a year, and surgical procedures typically cost up to $100,000 per patient. The current worldwide market for the treatment of Parkinson's disease is estimated to be approximately $1.5 billion. This proposal represents translational research based on solid mechanistic studies aimed at identifying a small molecule kinase inhibitor of PKC\(\delta\) with greater neuroprotective efficacy and high therapeutic index in animal models of PD. At the end of the project, we will have generated animal data required for a collaborative partnership between Iowa State and PK Biosciences Corp.. The company aims to translate mechanistic studies of key protein kinases (PK) into therapeutic strategies for treatment of neurodegenerative disorders. The company was awarded the SBIR phase-I award to test novel PK analogs in cell culture models of PD. The results from the SBIR-I study warrant extension of study to preclinical animal models. The company is currently negotiating a licensing option with the PKC\(\delta\)-based neuroprotection technology (U.S. patent pending: serial numbers 11/262,677 and 11/479,173) from OIPTT at Iowa State. The GIVF will also enhance the value of IP. PK Biosciences received GIVF two years ago to develop a PKC\(\delta\) peptide based gene therapy technology for PD. We reported encouraging results from the study and will pursue this technology further once PK Bioscience completes the licensing agreement with OIPTT. The ultimate success of the proposed neuroprotective strategy can be expected to create many new opportunities in Iowa, including economic and job growth and a viable biotechnology industry.

Our PK neuroprotective technology was ranked as one of the top neuroprotective approaches by a Foresight Science & Technology\(^{TM}\) Company's review, which was initiated by NIH. According to the review, there is only one competitor Teva Pharmaceutical Industries, which recently markets Azilect (rasagiline) for PD and generates annual sales of $1 billion. Azilect is not a mechanism-based drug and it shows only moderate activity in slowing the disease's progression. The long term effect of the drug is not known. We expect to capture similar sales of our drug when finally approved by FDA.
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**Budget Justification:** Please detail the planned expenditures and indicate the nature of sources of cost-shared funds (i.e. cash or in-kind).

**Cost-shared Funds:** The equal matching fund will be used from

AGK: Prof Anumantha G Kanthasamy salary savings (34% $56,100)

GAK: Prof George A Kraus’s salary savings (15%, $27,000).

VA: Dr. Vellareddy Anantharam’s salary savings (10% $9,000)

PKB: PK Biosciences facilities usage ($10,000)

Drklab: Dr. Kanthasamy’s lab facilities usage ($26,000)

**Equipment:** A refrigerated centrifuge and incubator is requested for brain tissue samples.

**Chemicals, Biochemicals and Radioligands:** A number of biological reagents and other normal lab reagents are required for the project.

**Histology and immunohistochemistry supplies:** Slides, coverslips, Permount, slide boxes, Tissue-tek, Histo Free, paraformaldehyde, 1° antibodies, 2° antibodies, ABC complex, etc..

**Animal studies** A significant amount of the budget requested is for animal purchase, maintenance and animal studies.
Bibliography


PI Name: Anumantha Kanthasamy

Project Title: Testing of lead PK compounds in preclinical animal models of Parkinson’s disease

College Ranking 1 of 8

Recommendation: Fund X Fund if Possible ___ Do Not Fund ___

Could be high impact if drug testing proves to be protective; impact of this testing technology may be very specific for PD only

SECTION I: TECHNICAL MERIT (60% of recommendation)

Low 1 2 3 4 5 High

Considerations:
• What is the scientific merit of the proposed project?
• Is the project technically feasible to accomplish in the listed time frame?
• Does the budget seem reasonable?
• Does the PI/team of researchers have the qualifications necessary to carry out the work?

Justification:

REVIEWER 1.
• Funds requested to test in animal models
• PI and Co-PIs well qualified; science would be well-founded
• Salary cost share for PI seems high for this project; 34%?
• Otherwise budget seems justified

REVIEWER 2. This project is addressing PK compounds for application in preclinical animal models of Parkinson’s Disease (PD). PD affects about 1.5 million people in the US and treatment costs are in excess of $2.5 billion. This project is a continuation of previous work to identify novel proteins associated with PD. It has led to the filing of two patents in the area and has established a biotechnology company with a view to commercial application. The work has already secured phase I NIH SBIR; and this project is seeking funds to support data collection and feasibility studies for a phase II NIH SBIR. Three lead PK analogs have been identified and this proposal seeks to assess these analogs in PD animal models to identify if they have protective effects in controlling the disease.

The project would appear to be feasible in the timeframe proposed.

The budget seems reasonable for the work proposed.

The PI and team appear to be well qualified for the work proposed – they already have partial proof of concept and need to confirm this using animal models.

SECTION II: BROADER IMPACTS (30% of recommendation)

Low 1 2 3 4 5 High

Considerations:
• What is the probability that this project could be used to leverage future funding from non-ISU sources?
• What is the potential to increase ISU’s research capabilities or capacity?
• What is the potential to enhance learning opportunities for students?

Justification:

REVIEWER 1.
• PI is owner in company
• If the PI is involved with the company that is partnering, ISU makes sure they have a COI management plan in place before they receive the funding and will identify an alternate ISU PI to provide oversight for the project.
• PI very successful in NIH research funding; testing of compounds can leverage federal funding
• Testing system scope may be very narrow/specific
• Student impact- minimal unless used for summer research scholar program

REVIEWER 2. This project will easily secure non ISU funds – the group have already secured NIH SBIR phase 1 and look well poised to secure phase 2.

The project will increase ISU capabilities in PD research – this is evident from the record of the researchers involved and their work to date.

The project will enhance learning in human health disease using animal models and probably has broad interest t students in vet med as well as biology, biochemistry and human health fields.

SECTION III: COMMERCIAL POTENTIAL (10% of recommendation)

Low  1  2  3  4  5  High

Considerations:
• What is the likelihood that new intellectual property will be generated?
• What is the likelihood of eventual commercial success?

Justification:

REVIEWER 1
• Information on PK industries website does not state annual sales or number of employees
• Success depends on how versatile the testing model, broad scope versus narrow and if it is unique to the PK company. Pharma has a lot of resources for animal model testing/drug discovery
• High likelihood of new IP; seems to be going forward presently
• No doubt PD is significant; unsure of impact of this testing system for other diseases
• Largest impact is proof of drug protection for PD and if this is next step; could be very high impact for compound/treatment success

REVIEWER 2. The likelihood for commercial application is relatively high given the impact of FD annually in the US with no cure currently in sight – however there is treatment for symptoms but this does not address neuroprotection which this proposal is seeking to address. The investigators in this project have identified three potential candidates as neuroprotectors with patents already in place for other aspects of the research highlighting the investigators potential to pursue IP. Commercial success would appear to be self evident based on the current annual costs for treatment of PD in the US ($1.5 billion). Commercial feasibility is already evident in the NIH Foresight Science and Technology Review, which has also identified/ recognized the potential of the work.
Early stage drug development - Parkinson’s Disease

Project would follow positive tissue tests with animal models

Commercial Potential:

1) Will this project lead to a new Iowa business/company, or increase the sales/profitability of a recently started Iowa company?

   Yes

   Please comment on the above: PK Biosciences Corporation (2006) is in the process of licensing technology from ISURF and is well positioned for the technology development phase of the project. Strategy to partner with an established pharmaceutical partner during regulatory trials abates significant concern for the ability to commercialize.

2) Will this project increase the sales or profitability of an existing Iowa business/company?

   Possible

   What is the probability of commercial success: Much too early to determine probability for commercial success. Preliminary data is promising, but successfully developing drug candidates into commercial products is a long and extremely expensive process.

   This project is part of the process

3) Are competitors identified, is the advantage of the proposed technology clear.

   Yes

   Please comment on the above: Current drugs treat symptoms, this technology has potential to be neuroprotective and reverse/halt progression of the disease. Current therapies slow Parkinson’s but don’t halt progression. This drug candidate may halt progression.

4) Is there a clear strategy for entry to the market, start up or existing Iowa business/company.

   Yes

   Please comment on the above: Company has strategy to partner with established pharmaceutical company. Industry information supports the willingness of big pharma to in-license technologies.

5) Please make any other comments related to the commercial potential of this proposal: This project, if funded, would demonstrate feasibility of study in Parkinson’s disease animal models, which would be critical for securing SBIR phase 2 funding.
**Intellectual Property Evaluation**

1. Have any current ISURF invention disclosures been identified as background IP?   **Yes**
   
   If Yes:
   
   a) What is/are the ISURF number(s)?

   03172
   03411
   03655

   b) What is the IP/patent status?

   Patent pending

   c) What is the licensing status?

   PK Biosciences is negotiating a licensing with ISURF

2. Does this project have the potential to generate new intellectual property?  **Yes**
   
   (please explain)

   New compounds

3. Based on your current knowledge, without having the opportunity to receive input from the principle investigator or to conduct a market or technology assessment, what will be the barriers to commercializing this technology?

   - Proof of concept for drug development
   - Drug development costs