Therapeutic Pipeline Program - Spring 2015 - Pre-Proposal

Principal Investigator

Applicant Name

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Project Details

Project Information

Please confirm the program you wish to apply for
Therapeutic Pipeline Program Spring 2015

Project Title
Exploratory study of Safety and Efficacy of Ambroxol in Patients with Parkinson's Disease

Project Duration
In whole months (two-year maximum pre-clinical stage/three-year maximum clinical stage
36

INSTITUTIONAL APPROVAL: Appropriate institutional approval must be obtained for any proposed work before funding can begin (e.g. IACUC, IRB, AAALAC, Biohazard Safety Committee, etc.)

I have applied for the necessary institutional approval
<None>

Budget Information

Direct Costs Amount (In United States Dollars)

$499,660.00

Indirect Costs Amount (In United States Dollars)
Indirect Costs cannot exceed 25% for non-profits and 10% for industy of direct costs

$49,966.00

Total Costs (In United States Dollars)
Please click the calculator icon for your total.

Is this your first application to The Michael J. Fox Foundation for Parkinson's Research?
No

Is this your first application focusing on Parkinson's disease research?
No

"Where did you hear about this RFA?"
Michael J. Fox Foundation Email Announcement

If Other Please Specify Source
Colleague;
Michael J. Fox Foundation Website

Key Personnel Information

The following pages are for Key Personnel information. Please enter the contact information and role (CoPI, Paid or Unpaid Collaborator, Consultant, etc) for any key personnel.

1

Key Personnel 1 - Name

First Name	Middle Name	Last Name	Suffix (Ex: MD, PhD)
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E-mail

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Key Personnel 5 - Name

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Ex: MD, PhD

PositionTitle
(Organization Position)

Role
(CoPI, Paid or Unpaid Collaborator, Consultant, etc.)

Key Personnel 5 - Contact Information

Mailing Address

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Phone Extension

Fax

E-mail

Additional Personnel

Please list any additional Key Personnel that were not included on the previous pages.
For each person, list 1- NAME, 2 - LABEL (CoPI, Paid or Unpaid Collaborator, Consultant, etc.), 3 - INSTITUTION, 4 - MAILING ADDRESS, 5 - EMAIL, 6 - PHONE

Abstract

Project Abstract (Brief summary of the goals of your project)
(300 word limit)

Substantial evidence exists that recessive alleles of Gaucher Disease (GD)-associated glucocerebrosidase (GCase) mutations strongly predispose carriers to Parkinson's disease (PD). GCase misfolding and mis-trafficking are involved in the etiology of both GD and PD. Ambroxol, a drug that has been used for nearly four decades in various pulmonary disorders, has been found to be one of the best pharmacological chaperones for promoting proper GCase folding. Ambroxol significantly increases GCase activity and reduces oxidative stress in fibroblasts isolated from both GD and PD patients harboring GCase gene mutations. Ambroxol also readily crosses the blood-brain barrier in mice upon oral administration, thus indicating good CNS penetrability.

In a pilot trial, one-year treatment with Ambroxol effectively improved symptoms of two Type 1 GD patients, resulting in improvements in hemoglobin and platelet counts, and reductions in spleen volume and chitotriosidase levels. In another pilot study, the addition of Ambroxol to Cerezyme® (miglucerase, GCase) therapy improved seizures and myoclonus symptoms more than Cerezyme® alone in two patients with Type 3 GD. Since Type 3 GD is a form of GD having CNS involvement, it is expected that

Ambroxol should also exhibit effective target engagement in the brains of PD patients. Both sets of results suggest that Ambroxol can provide clinical benefit to patients with GD genetic lesions.

Zywie LLC (formerly ExSAR Corporation) proposes a 2-step pilot clinical trial. Step 1 is to assay GCase and alpha-synuclein (a protein implicated in PD) in monocytes isolated from PD patients before and after ex vivo treatment with Ambroxol. Step 2 is to treat, with Ambroxol or placebo, those PD patients whose monocyte GCase activities are increased by Ambroxol observed in Step 1. The proposed pilot trial is a dose-escalation cross-over study design. Safety and efficacy (based on motor function and biomarker assessment) will be assessed during the study.

Confirmation

Confirmation

Please type initials here

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may be grounds for denying the grant request. I agree to accept responsibility for the scientific conduct of the project, provide the required progress reports, and meet other requirements specified if a grant is awarded as a result of this application.

KL

Attachments

Title	File Name
Project Summary Template	Project Summary Template - Robert Johnston.pdf

Files attached to this form may be deleted 120 days after submission.

THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

2015 PIPELINE PROGRAMS
THERAPEUTIC PIPELINE: AN EDMOND J. SAFRA CORE PROGRAM

PROJECT SUMMARY TEMPLATE

Please limit this to 3 pages maximum

Principal Investigator: Robert Johnston
Institution/Company: Zywie LCC (formerly ExSAR Corporation)
Project Title: Exploratory study of Safety and Efficacy of Ambroxol in Patients with Parkinson's Disease

THERAPEUTIC	The therapeutic, Ambroxol, is a small molecule drug currently used in clinics worldwide, except in the US, for various pulmonary disorders. The proposed mode of action against Parkinson Disease (PD) is that Ambroxol acts as a pharmacological chaperone which stabilizes the mutant enzyme, the Gaucher Disease (GD)-related glucocerebrosidase (GCase), thus allowing the mutant GCase to escape its degradation in the endoplasmic reticulum and resulting in an increase in GCase activities. Reduced GCase activity is the cause of GD and has recently been shown to be associated with the development of PD due to pathogenesis of sporadic synucleinopathies (Campbell et al. 2012; Cullen et al. 2011; Gegg et al. 2012; Jeyakumar et al. 2005; Mazzulli et al. 2011; McNeill et al. 2014; Neumann et al. 2009; Shachar et al. 2011). By increasing GCase activities in GD GCase-related PD patients, the progression of PD can be halted. Ambroxol is a potential treatment for PD based on the following "key" studies: (1) Ambroxol is a "repositioned" drug which has been used in the clinic for approx. 4 decades for various pulmonary disorders worldwide, except in the US. It has a good safety profile. (2) Ambroxol was found to be one of the best pharmacological chaperones for GD gene-related GCase, based on high output screen of 1,040 small molecules from the National Institute of Neurological Disorders and Stroke (NINDS) library which are FDA-approved or currently in clinical use (Maegawa et al. 2009). (3) Ambroxol significantly increased GCase activities and reduced oxidative stress in fibroblasts isolated from both GD and PD patients with GCase gene mutations (McNeill et al. 2014). Also, Ambroxol, as in the case with recombinant GCase, eliminated the phenotype (such as elevated TNF-alpha, IL-6, and IL-1b) of GD exhibited by macrophages generated by directed differentiation of human induced pluripotent stem cells (iPSC) derived from Type 1, 2, and 3 GD patients (Panicker et al. 2014). These studies indicated the potential of using Ambroxol to treat GD as well as GCase-gene related to PD. (4) Ambroxol significantly penetrates into the brain in Thy1-alphaSyn PD mice (funded by MJFF, TDI Fall 2011Program). Thus, oral administration of Ambroxol should access the brain of PD patients to exert its therapeutic effects. (5) A pilot clinical trial sponsored by ExSAR (current name Zywie, LLC) showed that Ambroxol is effective against Type 1 GD in 2 out of 12 Type 1 GD patients treated with Ambroxol for 1 year, resulting in improvements in hemoglobin and platelet counts, and reductions in spleen volume and chitotriosidase levels (Zimran et al. 2013). Since testing of sensitivity to Ambroxol was not an eligibility requirement for this trial, the low treatment response rate in this trial may be due to the inappropriate GCase gene allele (i.e., non-chaperone-responsive mutations) among the study subjects. The low treatment response rate may also be due to the dose being too low, as the two responders have the lowest body weight (and therefore the highest drug exposure) among the 12 subjects. Regardless of the reasons for the low response rate, the results from this pilot trial indicate that Ambroxol can provide clinical benefit to patients with GD gene-related GCase. (6) Ambroxol is safe and effective in Type 3 GD patients, according to preliminary data from an ongoing pilot clinical trial conducted by a Japanese investigator, Dr. Kousaku Ohno (San-in Rosai Hospital) (personal communication). The preliminary data showed that in two patients (Patients 1 and 2), the addition of Ambroxol to Cerezyme® (miglucerase, GCase) therapy improved seizures and myoclonus symptoms more than Cerezyme® alone. There were no adverse events associated with one year of treatment with Ambroxol at doses as high as 15 mg/kg in these patients. Ambroxol was detected in the central spinal fluid (CSF) of ~20% of blood concentration in Patient 1, indicating that Ambroxol can penetrate the CNS to a significant degree, consistent with an animal study using the Thy1-alphaSyn mouse model of PD. Since Type 3 GD is a CNS form of GD, Ambroxol should also be safe and effective against GD gene-related PD. Due to the progress described above, it is reasonable to start testing the safety and efficacy of Ambroxol in PD patients in a pilot clinical study, as suggested in the current pre-proposal.

	The strengths and weaknesses in developing Ambroxol as a treatment for PD are summarized below: *Strengths:* - Ambroxol is safe and well tolerated, according to numerous acute, subacute and chronic animal toxicology studies, animal safety studies, more than 100 clinical trials, and approx. 4 decades of use in the clinic. It has a clear safety record based on nearly 5 million patient-years of use. The development of Ambroxol for PD is unlikely to be derailed by any toxicity issues. - Ambroxol was identified as one of the best pharmacological chaperones for GD gene-related GCase. - Ambroxol is effective against fibroblasts from GD and PD patients with GCase gene mutations, and macrophages from iPSC of Type 1, 2 and 3 GD patients, *in vitro*. - Ambroxol penetrates into the brain to a significant degree according to a study using the Thy1-alphaSyn mouse model of PD and according to assessment of concentration is CSF in a Type 3 GD patient. Thus, Ambroxol should have access to the brain of PD patients with oral administration. - Ambroxol improves symptoms of Type 1 GD in 2 patients treated with 1 year of Ambroxol, thus supporting Ambroxol being effective against GD gene-related GCase. - Ambroxol is safe and effective in improving the CNS symptoms in 2 Type 3 GD patients. Thus, Ambroxol may also be safe and effective in patients with GD gene-related PD. - Zywie, LCC (former name ExSAR Corporation) has had an active IND for conducting clinical trials to investigate the safety and efficacy of Ambroxol in Type 3 GD patients in the US. This IND can be modified for conducting the proposed pilot trial in PD patients. *Both Strength and Weakness:* - Isofagomine (AT2101, afegostat tartarate, and Plicera™, by Amicus Pharmaceuticals) and Ambroxol have a similar mode of action. They both bind near the active site of GCase and stabilize the folded form, allowing delivery of increased quantities of enzyme to the lysosomal compartment. It is a strength because Phase 1 and 2 results show that Isofagomine is safe and increases GCase activities in healthy volunteers and Type 1 GD patients, thus validating pharmacological chaperones being a viable therapeutic approach for the GD form of GCase. It is a weakness because only 1 of 18 study subjects treated with Isofagomine exhibited clinically benefit, minimizing the response rate of pharmacological chaperones. One reason for the low response rate may be because testing for sensitivity to Isofagomine was not an eligibility requirement, and therefore patients in this trial may have inappropriate GCase gene allele (i.e., non-chaperone-responsive mutations). Another reason for the low response rate may be that Ambroxol binds to GCase with a Kd of 5.2 uM at pH 7.0 whereas Isofagomine binds more tightly to GCase but also dissociates more slowly in the acid pH found in lysosome. The excessive un-dissociated Isofagomine in lysosomes may reduce the effectiveness in enhancing GCase levels. This is consistent with *in vitro* studies on glucosylceramide substrate reduction in mutant (N370S/N370S) fibroblasts treated with comparable concentrations (20 uM of Isofagomine and Ambroxol), that Ambroxol significantly reduced the cellular glucosylceramide burden whereas Isofagomine was no more than placebo treatment (Maegawa et al, 2009). Accordingly, better response rate with Ambroxol than Isofagomine is expected, especially when the proposed pilot clinical trial involves testing of sensitivity to Ambroxol as an eligibility requirement. *Weakness:* - Only 7-15% of PD patients in some populations carry a mutant GCase gene (Sidransky et al, 2009; Velayati et al, 2010), and the frequency of reduced GCase activities in the brain in PD patients may be as low as 4.18% according to a study using British PD patients (Neumann et al. 2009). Therefore, Ambroxol may potentially be effective in as few as 4.18% of PD patients. A potential therapy for 4% out of approx. 30 million PD patients in the US (Willis et al. 2010), or for approx. 1.6 million PD patients, is a great achievement in the fight against PD. On the other hand, Ambroxol may actually be effective in PD patients that do not carry a mutant GCase gene, as Ambroxol may increase GCase activities in subjects with non-Gaucher gene defect due to stabilization of GCase from degradation in the endoplasmic reticulum. This is reflected by an increase in GCase activities in white blood cells isolated from healthy volunteers treated with Isofagomine, a pharmacological chaperone being developed by Amicus Pharmaceuticals. For this reason, the proposed clinical trial to be submitted to MJFF clinical trial involves testing of sensitivity to Ambroxol as an eligibility requirement, prior to treating PD patients with Ambroxol.
INDICATION	The intended indication for Ambroxol is treatment of GD-gene related PD. By functioning as a pharmacological chaperone for GD-related GCase, Ambroxol is expected to increase GCase activities in the brain of patients with GD-gene related PD.
TARGET & PATHWAY	The target is the GD form of GCase in PD patients. GCase is involved in the catabolic breakdown of glycosphingolipids in the cells, and some naturally occurring mutations of human GCase gene causes

	GCase to misfold. People who have the defective GCase gene allele from both parents can develop GD. GCase defects have also been linked to PD, although the exact mechanism in causing PD is not fully understood. It could be due to disruption of cellular homeostasis caused by lowered lysosomal GCase activity levels, leading to increases in cellular glucosylceramide concentrations, perturbation of intracellular Ca levels, and concomitant increases in α-synuclein aggregation. Modest doses of Ambroxol can "chaperone" the folding of some mutants of GCase (including the common N370S allele) and boost levels of native-like activity in cells expressing the defective enzyme (Maegawa et al, 2009). The GD form of GCase mutations has been genetically linked to PD. Mutations of the human GCase gene that causes misfolding, and inactivation of GCase strongly predisposes carriers of these alleles to PD (Sidransky et al, 2009). GCase mutations are actually the most common genetic risk factor associated with PD, and a single mutant GCase allele can increase the risk of PD and Lewy body disorders fivefold (Velayati et al, 2010). Although GD is a relatively rare disease, many more individuals are carriers of the defective GCase alleles (as many as 7-15% of PD patients in some populations carrying a mutant GCase gene (Sidransky et al, 2009; Velayati et al, 2010).
STAGE OF DEVELOPMENT	Ambroxol is a repositioned drug that has approx. 4 decades of clinical use for various pulmonary disorders. For the development of Ambroxol for GD and PD, it is currently in the clinical phase of drug development. The proposed study will be the first clinical trial in patients with PD.
DEVELOPMENT PLAN	The proposed study is a pilot proof-of-concept study of Ambroxol in PD patients. It has 2 steps. Step 1 involves identifying potential positive responders to Ambroxol, by screening of PD patients whose GCase activities in monocytes would be increased by Ambroxol ex vivo. GCase gene of the positive responders identified in this step will be sequenced to correlate results from Step 2. Step 2 is a dose-escalation trial of Ambroxol with cross-over double blinded study design, involving treatment with Ambroxol of the responders identified in Step 1. Monocyte GCase activites (biomarker), symptoms related to PD, and safety will be monitored during the course of the study. If the results from the proposed pilot trial are promising, a Phase 2 and then Phase 3 trial will be conducted in the future, involving a proportionally large number of PD patients. The results from the future Phase 2 and 3 trials will form the basis for a New Drug Application (NDA) and appropriate dossier for US and worldwide approval in the use of Ambroxol to treat GD gene-related PD.
IP/PATENT LANDSCAPE	Ambroxol has been around since the 1970's and is no longer covered by third party patents. Zywie has exclusively licensed from the Hospital for Sick Children and McMaster University (Canada), and the patent portfolio covers the method of using Ambroxol for the treatment of certain disorders. Issued claims have already been granted directed to the use of Ambroxol for treating patients who have both GD and PD, and continuing applications have been filed directed to claims for the use of Ambroxol for the treatment of all PD patients regardless of whether the patient also has GD. In preliminary searches, no other third party patents have been identified as problematic.
FUTURE OBJECTIVES	Our ultimate goals are to demonstrate in well-design clinical trials the safety and efficacy of Ambroxol in the treatment of GD gene-related PD and obtain regulatory approval of Ambroxol to treat PD patients. The expected steps and timeline leading to achieving these goals are: - To conduct the proposed pilot clinical trial upon availability of funding (3 years) - To conduct the future Phase 2 trial (4 years) - To have a Pre-Phase 3 meeting with FDA and then conduct the future Phase 3 trial (6 years) - To compose and generate NDA for FDA submission (0.45 year) - FDA review, comment, Q&A, and approval of Ambroxol (0.75 year)

References

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