Title: Safety and Tolerability Study of R(+)Pramipexole in Alzheimer's Disease

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BACKGROUND AND SIGNIFICANCE:

The looming problem

Dementia due to Alzheimer's disease (AD) type pathology currently afflicts 3.5-5 million Americans, with resulting annual costs for medical and hospice care and lost wages of caregivers estimated to be 150-200 billion dollars. Because the major risk for developing AD is aging, and given the projected 2 to 2.5-fold increase in the age-vulnerable population as the "baby boomer" generation ages, projected expenditures for AD care in 15-20 years could equal the baseline Department of Defense budget. In addition to the immense personal tragedies inflicted by AD, the looming medical and socioeconomic disaster posed by this one condition of brain aging should stimulate massive efforts to both understand its pathophysiology and develop disease trajectory-altering therapies.

What are the roadblocks limiting disease-altering AD therapy development?

The vast majority of AD (~95%) occurs sporadically without clear autosomal genetic cause. While certain genes such as APOE4 increase risk of acquiring AD, they certainly are not causal. Genes felt to be causal for genetic (familial) AD that represents ~5% of the AD population involve either one of multiple known mutations of amyloid precursor protein (APP) or mutations in one of the two known presenilin genes (PS1 or PS2). Because cells expressing APP only or with PS mutations (but not PS mutations alone) overproduce 40-42 amino acid long peptide fragments of APP, known as beta amyloid 1-40 or 1-42, because AD brains characteristically accumulate beta amyloid as visible plaques, and in light of the *in vitro* neurotoxicity of beta amyloids (esp the 1-42 peptide), a causal theory known as the "beta amyloid cascade hypothesis" was advanced ~15 years ago and has dominated AD investigations and experimental therapeutics. Arguments for and against this hypothesis have been both many and at times stridently debated. Opponents argue that this hypothesis has now been tested in humans with several different types of beta amyloid lowering therapies and found to be deficient. Proponents argue that the theory either has not yet had the proper testing in humans, and/or that the toxic species are low molecular weight soluble oligomeric forms of beta amyloid (as opposed to insoluble plaques). Therapies that either reduce beta amyloid production (beta- or gamma-secretase inhibitors) or remove accumulated beta amyloid (immunotherapies) continue to be developed and enter testing in humans with AD. As a result, the amyloid cascade hypothesis will "get its day in court", but there is clearly a substantial risk of failure in pursuing solely this single therapeutic avenue.

In addition to whatever conceptual barriers that have arisen as a result of the dominance of the amyloid cascade hypothesis (some have compared its vocal proponents to a "scientific cartel"), a more vexing problem relates to defining other "targets". Traditional pharma drug development has relied upon defining specific molecular targets, developing and screening multiple candidates against that target, and selecting the most efficacious/least toxic for potential development to use in humans. For a complex and likely heterogeneous disease such as AD, the identities of the targets causal for disease progression are not clear. A major premise of this application is that the molecular target approach for altering neurodegenerative diseases is too limiting and likely to fail. That has been the case so far in Parkinson's disease [3], the second most prevalent neurodegenerative disease of adults. Rather, the approach taken in this application is to try and define consistent abnormalities in systems biologic processes that are defective in AD, then apply therapies that correct these abnormal biological processes. This proposal advances a process-altering, not a molecular target-driven approach.

Elevated oxidative stress appears early in AD and can drive increased beta amyloid accumulation

Most eukaryotic life on earth lives in atmospheric oxygen and is dependent on oxidative decarboxylation of fuels and reduction of oxygen to water by mitochondrial respiration to produce storage energy (ATP). Highly differentiated, non-mitotic cells such as neurons are energy intensive, very dependent on mitochondrial respiration, and as a consequence must inactivate relatively large amounts of reactive oxygen species (ROS) such as superoxide anion and its diffusible dismutation product hydrogen peroxide. When ROS production rates exceed inactivation rates, a state of "oxidative stress" exists, wherein proteins, lipids and nucleic acids are oxidatively damaged. Because most ROS normally arise from the small inefficiency of mitochondrial respiration, mitochondrial components

particularly experience oxidative stress damage. Paradoxically, oxidative damage to mitochondrial DNA, RNA and respiratory proteins appears to increase further the inefficiency of respiration, driving up ROS production rates. This leads to a downward spiral over time in mitochondrial respiration due to oxidative damage, formulated as the "mitochondrial hypothesis of aging", at least as applied to energy-intensive, non-mitotic tissues such as brain, heart, retina and muscle.

Relative oxidative stress in brain tissue can be monitored in living subjects by assay of certain oxidized small lipid molecules known as F2-isoprostanes/neuroprostanes [4,5,6,7,8]. In deceased subjects, brain tissues can be assayed for the same molecules, as well as oxidized proteins, other lipids and nucleic acids. For subjects with mild cognitive impairment (MCI), a pre-AD state for a certain fraction, elevated oxidative stress markers are present in spinal fluid and brain tissues [4,5,9,10,11,12,13,14,15,16,17,18,19]. For mice expressing familial AD genes, brain oxidative stress is elevated long before beta amyloid accumulation begins [6]. For AD brains, elevated oxidative stress could be responsible for appearance of elevated beta amyloid peptides, as expression of beta secretase enzyme (necessary for cleaving the N-terminal part of beta amyloid from APP) is increased by oxidative stress, and activity of insulin degrading enzyme (neprilysin), which degrades beta amyloid peptide, is decreased by oxidative stress [20,21,22,23,24].

mtDNA in sporadic AD can contribute to oxidative stress and increased beta amyloid production

Human mitochondria have multiple copies of their own small (~16.6 kbase) circular, maternally inherited DNA that codes for 13 essential respiratory proteins. Human brain mtDNA accumulates point mutations and oxidative damage with aging. Individual brain neurons also experience clonal expansion of mtDNA's containing sizeable (5-7 kbase) deletions that cannot produce essential respiratory proteins. As a result, age-related damage to brain neuronal mtDNA, leading to increased oxidative stress, is an attractive hypothesis for risk of developing neurodegenerative conditions such as AD.

In our previous studies we have tested the hypothesis that AD patients harbor pathogenic mtDNA's by creating cybrid cells that selectively express mtDNA against constant nuclear genetic and neuronal cell culture backgrounds [25]. We found that compared to cybrid cells made from age-matched non-AD controls, cybrids from AD subjects had increased oxidative stress, impaired mitochondrial functions and secreted increased beta amyloid peptides [15,25,26]. While not providing insight into the specifics of mtDNA abnormalities, and not proving causality of mtDNA to initiate AD pathogenesis, our cybrid studies implicated mtDNA-driven oxidative stress as a likely contributor to beta amyloid overproduction in AD.

If abnormal mtDNA's produce impaired respiration and increased oxidative stress in AD brain neurons as our cybrid studies suggest is possible, and if such increased oxidative stress further damages mitochondrial components and increases beta amyloid production, then reduction of mitochondrial oxidative stress should exert a disease-slowing effect in AD. This benefit, if present, would neither support nor refute the amyloid cascade hypothesis, since oxidative stress by itself is damaging to neuronal integrity. However, there is also no *a priori* reason to reject the possibility of multiple interacting drivers of neurodegeneration. To return to the initial argument advanced, what this application proposes is development of approaches to reduce mitochondrially generated oxidative stress as a systems biological problem in AD, rather than target a specific macromolecule.

What about regional vulnerability?

A consistent, defining characteristic of adult neurodegenerative diseases is regional vulnerability, meaning that selected neuronal populations degenerate at increased rates relative to other neurons. For AD, loss of hippocampal and nucleus basalis neurons is greatest, followed by certain cortical neuronal populations. For PD, nigral dopamine and other brainstem aminergic neurons are most vulnerable. How can this be?

The honest answer is that causes of regional vulnerability are both intriguing and remain elusive. Altering their mechanisms may offer great therapeutic benefits. Yet, rational experiments to alter pathogenic mechanisms can proceed in the absence of knowing how regional vulnerability occurs. In the case of AD and PD, overlapping of clinical phenotypes and pathologies with disease progression implicates similar disease mechanisms, at least in some subjects.

SPECIFIC AIMS

The primary aim of this protocol is to test the safety and tolerability of R-Pramipexole in humans with probable AD. We will also explore changes in oxidative stress and brain metabolic consequences as a "proof of concept" exploratory aim.

Primary Aim: Assess the safety and tolerability of R-Pramipexole in probable AD. Twenty subjects with probable AD will initiate treatment with 100 mg/day of R(+) pramipexole for one month. If tolerated, the subjects will increase to 200 mg/day for one month and then 300 mg/day for the next 4 months. Participants will be contacted by phone every two weeks to determine any adverse events. They will also be examined by a physician, have their vital signs measured and have routine safety blood labs performed every two months.

Exploratory Aim: Assess the effect of R-Pramipexole on oxidative stress and brain metabolic consequences. Prior to initiation of R(+)PPX dosing, we will obtain plasma and spinal fluid for oxidative stress markers and obtain brain glucose metabolism PET scans (2FDG PET). During the last month of 300 mg/day dosing, obtain plasma and spinal fluid samples and repeat 2FDG PET scan.

PRELIMINARY DATA

R(+)pramipexole is a mitochondrially concentrated antioxidant

Pramipexole (PPX) is a benzothiazole synthesized in the 1980's as an apomorphine analogue [27]. The stereospecific synthesis yielded S(-)PPX (Figure 1), which possessed potent and full agonist actions at the D2 family of brain dopamine receptors. S(-)PPX was developed for clinical use in humans and was approved in 1997 by the FDA as Mirapex®, for treatment of Parkinson's disease symptoms due to nigrostriatal dopamine deficiency.

PPX synthesis also yielded the R(+) PPX enantiomer (Figure 1), which was subsequently shown to possess very weak potency at D2 receptors. However, both R(+) and S(-) PPX are present at physiological pH as lipophilic, doubly charged cations, a property that predicted to the sponsor that they would be concentrated into mitochondria. Because PPX also possessed a favorable reduction potential that predicted it would inactivate all common ROS and reactive nitrogen species (RNS), R(+)PPX had potential as a mitochondrially concentrated ROS/RNS scavenger that should be tolerated in very high doses because of its weak potency at D2 receptors.

R(+)PPX is concentrated into mitochondria (Figure 2) and scavenges mitochondrial superoxide (Figure 3).

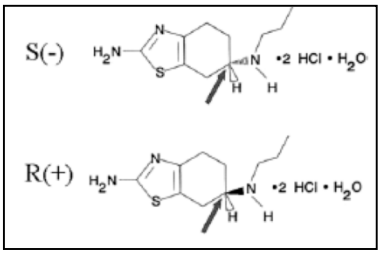

Figure1. Structures of S(-) and R(+) pramipexole. Arrow points to asymmetric carbon.

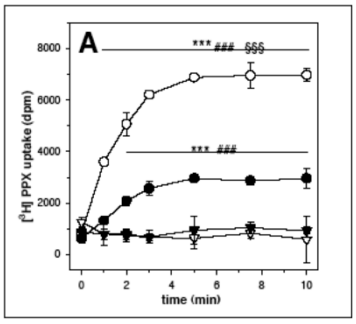

Figure 2. Uptake of [3H] PPX into mitochondria. Mitochondria (control; open circles, bars), were lysed (Triton-X 100;1% (v/v); open triangles), sonicated vigorously (SMP; filled triangles) or uncoupled with FCCP (1 µM; filled circles) and preincubated for 2.5 min in malate/pyruvate–containing (2.5/5 mM) incubation buffer (all 0.2 mg/ml). Uptake was started by addition of [^{3}H] PPX (3 µCi/ml) and terminated at the indicated time. From Fig 2 of [1]

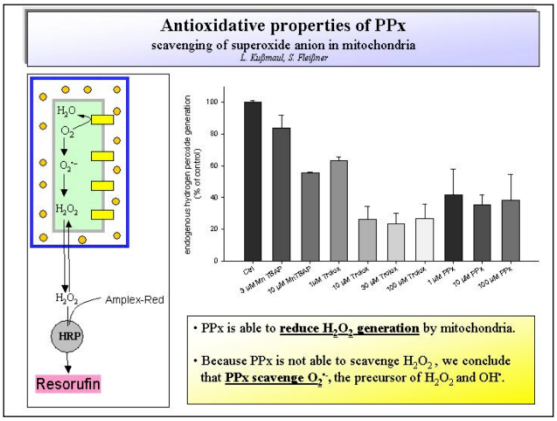

Figure 3. PPX directly scavenges superoxide anion (O2*-) thus reducing mitochondrial hydrogen peroxide production in isolated mitochondria. TBAP= MnSOD (SOD2) mimetic; TROLOX= water soluble vitamin E. Note that PPX at 1 uM is maximally potent at scavenging superoxide , is more potent than TBAP, and is slightly less potent than high levels of TROLOX (unpublished data from Boerrhinger-Ingelheim shared with JPB in videoconference)

R(+)PPX is also concentrated into brain >6-fold from plasma (Figure 4) and achieves steady state plasma levels of ≥ 1 μM in humans dosed with 300 mg/day. (Figure 5)

	plasma [μM]	Brain [μM]	accumulation factor brain/plasma
PPX	1.0 ± 0.2	5.4 ± 1.0	6.7 ± 1.3
SND	0.43 ± 0.09	2.5 ± 1.0	6.4 ± 1.2

Figure 4. Brain accumulation of S(-) PPX ("PPX") and R(+) PPX ("SND") after 4 days of 200 mg/kg/day intake in mice. From [1]

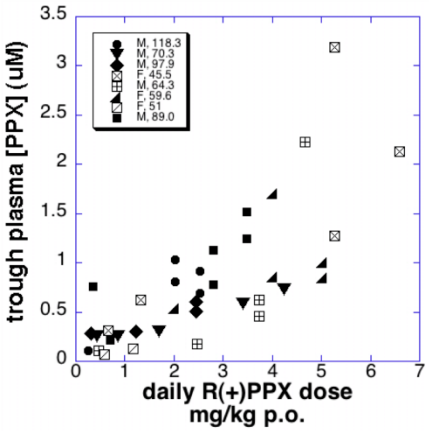

Figure 5. Steady state trough plasma levels in A subjects dosed with vary R(+)PPX daily mg. Gende and wt (kg) shown in legend. From [2]

R(+)PPX can safely be administered to humans up to 300 mg/day and slows progression of ALS

Based on the preclinical pharmacology of R(+)PPX and his prediction that it would be tolerated in much higher doses than S(-)PPX (limited to 4.5-6 mg/day), the sponsor(Bennett) obtained a physician-sponsor IND from the FDA to administer R(+)PPX to humans with ALS. Dosing began in spring, 2004. With private funding, the sponsor carried out a series of small Phase 1-2 studies in ALS subjects, culminating in a dose escalation study to 300 mg/day carried out in 2006-07. In each study, the sponsor obtained data showing a slowing of disease progression in ALS, but the numbers were too small and the studies were all comparative dose, open label that did not allow definitive statements about efficacy [2]

In 2006 R(+)PPX was licensed to Knopp Neurosciences that began its own commercial development of R(+)PPX. In Dec, 2009 Knopp announced its very positive Phase 2b results from a placebo-controlled trial in early ALS subjects (http://www.knoppneurosciences.com/index.php?section=news&subsection=news&id=60)

In the case of ALSFRS-R, the number of treatment failures, defined as the loss of 6 points or greater in ALSFRS-R scores from baseline, totaled 9 subjects (or 33%), in the placebo group; 8 subjects (35%), in the 50 mg/day group, 4 subjects (15%) in the 150 mg/day group, and 2 subjects (8%) in the 300 mg/day group (p=0.014).

In the case of pulmonary function, the number of treatment failures, defined as a reduction in forced vital capacity of 20% or greater from baseline, totaled 8 subjects (30%) in the placebo group, 3 subjects (13%) in the 50 mg group, 3 subjects (12%) in the 150 mg group, and 1 subject (4%) in the 300 mg group (p=0.028).

Both the PI and Knopp have observed excellent safety records of R(+)PPX. The sponsor has searched for evidence of suppression of serum prolactin, as a consequence of *in vivo* D2 dopamine receptor activity, in ALS patients undergoing dose escalation with R(+)PPX. He found no evidence of suppression of prolactin levels (Figure 6). This suggests that at a daily dose of 300 mg/day, his preparation of R(+)PPX has no detectable activity at central D2 dopamine receptors and should be well tolerated by subjects with mild dementia.

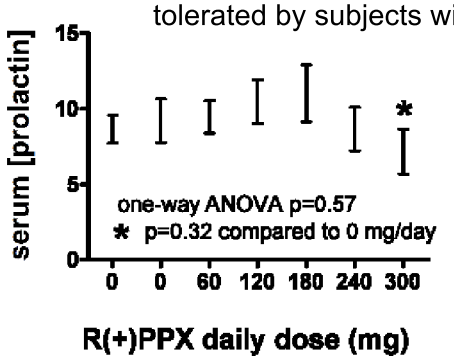

Figure 6. Lack of effect of R(+)PPX oral dosing on serum prolactin levels. Shown are daily R(+)PPX doses during the dose escalation study of 8 ALS subjects and the mean +/- SEM of their serum prolactin levels. ANOVA did not reveal any effect of PPX dose on prolactin. There was no significant difference between serum prolactin levels at 0 and 300 mg/day PPX. (Wang, Keller and Bennett, unpublished)

Thus, at this point in time R(+)PPX has been administered to ~170 subjects, ~50 of whom have taken 300 mg/day for 6-9 months. R(+)PPX has an excellent safety record with no drug-related serious adverse events reported. It has excellent preclinical data supporting brain and mitochondrial concentration and ROS scavenging. Its clinical use in humans with the devastating disease ALS demonstrated efficacy in slowing disease progression in a placebo-controlled trial. It is entering definitive Phase 3 efficacy testing in ALS and is ready to be tested in probable AD in this trial.

While the sponsor (Bennett) and Knopp Neurosciences maintain a collegial relationship, each has his/its own IND, R(+)PPX supply, and independently pursues clinical testing of R(+)PPX. Knopp is presently completely focused on pursuing Phase 3 testing of R(+)PPX in ALS. The sponsor of this proposal has amended his IND to pursue testing of R(+)PPX in AD and PD subjects. The sponsor declares a financial interest in the uses of R(+)PPX in degenerative illnesses.

AD subjects show early increases in brain oxidative stress and reductions in cortical energy metabolism

In recent years several groups have argued persuasively that mitochondrial dysfunction and increased oxidative stress are early deficits in humans with AD and transgenic mice expressing familial AD genes. It is not necessary to repeat the details of these arguments here; the reader is referred to specific reviews for details [9,10,11,12,13,14,28,29,30].

One of the very recent reviews of this subject [10] is particularly passionate in its arguments and summarizes most forcefully the basic argument. Bonda, et al [10] argue that increased beta amyloid production occurs as part of an antioxidant response to early oxidative stress. This beta amyloid response paradoxically turns into its own neurodegenerative stimulus, stimulating increased oxidative stress in its own right. This positive feed-forward "vicious" neurodegenerative cycle is well established by the time clinical symptoms appear and may become increasingly difficult to overcome as the disease progresses. Bonda, et al, and others, argue for early initiation of effective antioxidant therapy, at the earliest sign of cognitive impairment.

Under normal dietary (non-fasting) circumstances, most brain energy is formed from glucose metabolism. Rates of accumulation of deoxyglucose provide measure of regional brain glucose metabolic rates, which reflect cytosolic glycolysis and ultimately pyruvate metabolism in mitochondria. Robust cerebral cortical glucose metabolism is reduced in MCI subjects in a pattern that recapitulates more severe metabolic reductions once AD is clinically established.

Recent advances in understanding the utility of cerebral glucose metabolism, studied with FDG-PET, to discriminate MCI from AD and follow progression of AD have begun to appear as a result of the Alzheimer's Disease Neuroimaging Initiative (ADNI). The results of a very recently reported study using statistical brain mapping of FDG-PET scans in MCI and early/probable AD subjects [31] are shown in Figure 7. The authors found multiple cortical areas of decline in both MCI and early AD subjects. Notably, they calculated that *66 AD subjects will be required in each treatment arm of a randomized clinical trial to detect with 80% power at p=0.05 a 25% change in decline of FDG accumulation over a 12 month period.*

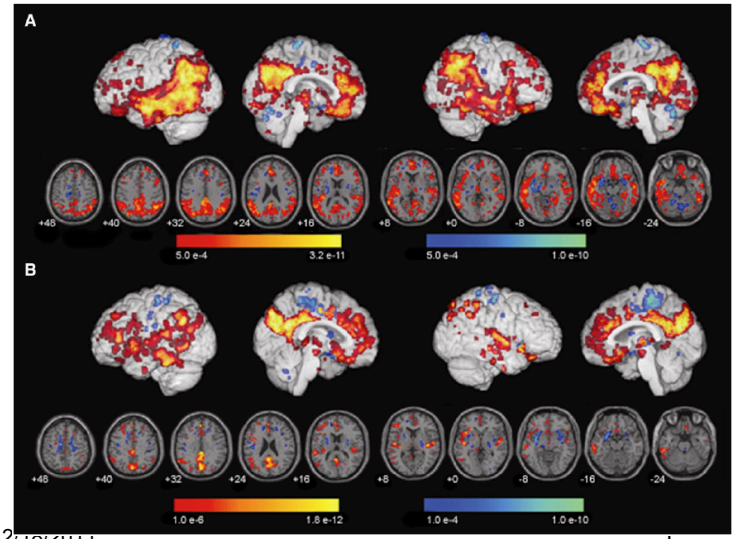

Figure 7. Statistical brain maps of the spared (non-declining) regions of interest (ROI's) (in the blue color scale) and the ROI's where over twelve-months there wa a decline in brain glucose metabolism (in the red-to-yell color scale). Shown in **A** are probable AD patients and **B** MCI patient groups. The brain maps were generated usir baseline and follow-up images acquired in the each patie group's training set. Note anatomic similarity of involve brain regions between the AD and MCI groups and the overall more extensive involvement of regions that declined over 12 months in the AD compared to MCI group.

Available antioxidants have not been helpful

As recently reviewed by Dumont, et al, [12] several trials of antioxidants have been carried out in AD subjects. Compounds showing some positive activity in transgenic mouse models but generally failing in human subjects included vitamin E, idebenone (CoenzymeQ10 analogue) cloquinol, curcumin and dimebon. One possibility for failure of these compounds in humans, in addition to kinetic issues, is that none are predicted to accumulate into brain mitochondria. If one is testing the hypothesis that mitochondrially generated ROS are driving elevated oxidative stress damage in AD, then brain and mitochondrial concentration of the ROS scavenger are critical. R(+)PPX satisfies these criteria (see Figures 2 and 4 above).

EXPERIMENTAL DESIGN AND METHODS

General

This is a safety and tolerability study of R(+)PPX in subjects with probable AD. R(+)PPX is an orally active, brain and mitochondrially concentrated free radical scavenger that has shown excellent safety and tolerability in Phase 1 and 2 studies in >100 ALS subjects. Its lack of *in vivo* D2 dopamine receptor activity (based on not lowering serum prolactin) at daily doses up to 300 mg/day contrasts with the potent dopamine receptor-limiting dosing of S(-)PPX (Mirapex) at 4.5-6.0 mg/day. Pharmacokinetic studies carried out by the PI show that daily R(+)PPX doses of 300 mg/day frequently yield steady-state trough plasma PPX levels >1 μM, which predict highly neuroprotective brain PPX levels of >6 μM [2].

R(+)PPX slows the progression of disability and decline of respiratory capacity in early ALS subjects (Knopp Phase 2b, Phase 3 pending). The scientific rationale for the current proposed study in probable AD is that oxidative stress is an early abnormality in human AD and transgenic animal models of familial AD. Increased brain oxidative stress is likely causal for AD pathogenesis and can stimulate net production of beta amyloid by increasing beta amyloid synthesis (beta secretase expression) and inhibiting beta amyloid degradation (neprilysin activity). The sponsor's group has shown that mtDNA from AD subjects expressed in a cybrid model can drive increased oxidative stress and beta amyloid production, which is reduced by PPX. The current study will not define how oxidative stress is increased in AD brain, rather it will correct that problem by treatment with a well tolerated and novel mitochondrial antioxidant.

The sponsor has extensive experience testing R(+)PPX in ALS and has an active physician-sponsor IND that has been amended to allow testing of R(+)PPX in AD subjects (see DSMB charter in appendix for more information). Because of conflicts of interest, the sponsor will not be involved at all in recruitment, treatment or evaluation of AD subjects; that will occur at Kansas University Medical Center (KUMC), with Dr. Jeff Burns as the Principal Investigator. James Bennett, Jr.,MD, PhD will function as Sponsor in this study but will have no direct involvement in clinical care.

The primary goal of the study is to establish the safety and tolerability of the study medication in individuals with AD. Exploratory variables involve changes in brain and peripheral oxidative stress assayed by isoprostane levels in spinal fluid and serum, respectively, change in cognitive function and improvement in brain glucose metabolism assayed by FDG-PET scan. The goal of the present study is to establish that up to 300 mg/day of R(+)PPX is tolerated by subjects with probable AD, lowers oxidative stress and may show a trend of improving brain glucose metabolism.

Subject selection and recruitment

Subjects with probable AD will be identified and recruited through the Memory Disorders Clinic at the University of Kansas Medical Center (KUMC). This clinic is under the direction of Dr. Jeff Burns, an accomplished AD clinical investigator.

KU Alzheimer and Memory Program

The KU AMP, directed by Dr. Burns, has been an active and rapidly expanding research group at KUMC since 2004. In this short time, the AMP has developed the infrastructure and expertise for the identification, recruitment, and characterization of nondemented and probable AD research participants, established a history of successful collaborations, attracted both new and experienced investigators

into the field of AD research, and has established an early track record of scientific productivity primarily along a programmatic theme of the role of metabolic dysfunction in AD and aging. These efforts have led to recent funding for two R01s assessing the neurobiological effects of exercise in older adults with and without AD.

Additionally, the AMP also supports clinical trial activities through the AD Clinical Trial Unit, initiated and directed by Dr. Burns. The Clinical Trial Unit staff includes three clinicians (Burns, Anderson, and Anne Arthur, ARNP), a director of research (Anita Macan), and a research nurse (Cherie Parker). Pat Laubinger provides oversight and planning for the Clinical Trial Unit through her role as the administrative director of the AMP.

Alzheimer and Memory Clinic

Dr. Burns directs the Alzheimer and Memory Clinic, the major referral clinic for the area and the state of Kansas and the clinical study site for this application. The clinic evaluates more than 600 patients annually, 300 of which are new evaluations, and is staffed by four clinicians (Burns, Anderson, Swerdlow, and Arthur) in addition to a multidisciplinary team (social worker, pharmacist, psychometrician, and a nurse). AMP research evaluations are not conducted in the clinic. Rather, patients interested in research who appear to meet criteria for entry are referred to the AMP for enrollment into specific studies.

Clinic Database: Patients evaluated in the clinic are asked to provide consent to be contacted in the future for possible consideration of research studies. Additionally, consent is provided to store health information in a database. The database contains information regarding patient diagnosis, medications, medical history, and basic cognitive performance data (MMSE and memory testing [logical memory]). The information collected was chosen to provide basic information regarding common inclusion and exclusion criteria to best target potential recruitment into our ongoing investigational studies and clinical trials.

Since initiating the database in September 2004, information on a total of 945 unique patients has been entered into our database. Of these, 486 were diagnosed with AD (mean age 74.7 years, MMSE 19.8, and logical memory II 1.5) and 112 were diagnosed with MCI (mean age 72.5, MMSE = 26.8, logical memory II = 5.1). Forty patients included in the database have a diagnosis of dementia with Lewy bodies and 25 with frontotemporal dementia. The use of the database has been helpful in identifying participants for recruitment into research studies. Patients identified as potentially meeting enrollment into the UDS Registry or clinical trials are flagged in the database and referred to our recruitment coordinator for contact and further screening. The database has been an effective and efficient way to recruit participants from our active clinic population.

Alzheimer's Disease: Individuals with AD meet NINDS-ADRDA criteria for possible of probable AD and have a score of 14 - 26 inclusive on the MMSE. NINDS-ADRDA criteria are operationalized as 1) memory impairment, 2) the gradual onset and progression of impairment in memory and in at least one other cognitive domain (as demonstrated by clinical history and neuropsychological testing) and 3) the absence of clinical or laboratory evidence of other disorders that could account for memory or cognitive decline or if a second disorder is present it is not considered the primary cause of dementia.

VCU laboratory site

The physician-sponsor is a faculty member at Virginia Commonwealth University and has no relationship with Dr. Burns or KUMC. Because the /sponsor has a financial interest in the commercial development of R(+)PPX, it is critical that he have no relationship with Dr. Burns/KUMC and have no influence on subject recruitment or study execution. However, the sponsor will provide analytical services for this application. Serum and spinal fluid specimens will be analyzed blinded as to subject identity and time in study. The sponsor has available facilities for LC-MS analysis of PPX and isoprostane levels.

Inclusion and Exclusion Criteria at Entry	
Inclusion Criteria	**Exclusion criteria**
• Informed consent provided by the participant or the participant's legally acceptable representative • Age 55 years or older • Diagnosis of Probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) • Community dwelling with a caregiver able and willing to accompany the participant on all visits, if necessary. Caregiver must visit with the subject>5 times a week and accompany participant to study visits. • Rosen Modified Hachinski score of 4 or less • MMSE score of 14-26 inclusive • Stable doses of medications for at least 30 days prior to screening. • Reliable person to administer study drug to the participant twice a day for the duration of the study • Imaging study (CT or MRI) of the brain compatible with AD or age-related changes after onset of memory problems (absence of significant abnormalities that may explain cognitive decline (such as, multiple lacunar infarcts or a single prior infarct > 1 cm^3, micro-hemorrhages or evidence of a prior hemorrhage > 1 cm^3, evidence of cerebral contusion encephalomalacia, aneurysm, vascular malformation, or space-occupying lesion such as an arachnoid cyst or brain tumor) • Adequate visual and auditory abilities to perform all aspects of the cognitive and functional assessments	• Significant neurological disease, other than AD, that may affect cognition • Current clinically-significant systemic illness that is likely to result in deterioration of the patient's condition or affect the patient's safety during the study • History of clinically-evident stroke • Clinically-significant infection within the last 30 days • Myocardial infarction or symptoms of active coronary artery disease (e.g., angina) in the last two-years. • Uncontrolled hypertension within the last 6 months • History of cancer within the last 5 years (except non-metastatic basal or squamous cell carcinoma) • History of drug or alcohol abuse as defined by DSM-IV criteria within the last 2 years • Insulin-dependent diabetes mellitus • Clinically significant depression (Geriatric Depression Scale score >5). • History of kidney disease or renal insufficiency (serum creatinine level >1.5)

Treatment protocol

Overview (see calendar of events below): After providing signed informed consent, participants will undergo a screening visit to assess study participant eligibility. Neuropsychological testing (ADAS-Cog) will be performed at baseline and every two months (visits 2, 3, 4, and End of Study (EOS)). A lumbar puncture and blood draw (for isoprostane analysis) and fluoro-deoxy-glucose positron emission tomography (FDG-PET) will be performed at baseline and after 6 months of treatment to serve as exploratory "proof of concept" measures to explore the effect of R-Pramipexole on key biological targets. Safety visits with laboratory testing and physical assessments will occur every two months. Follow up phone calls to the participants and their study partner will be conducted every two weeks during treatment to assess for possible adverse events.

Screening Visit: A screening visit will occur in the GCRC and last approximately 2 hours. Participants will be accompanied by a study partner and provide informed consent. Demographics, medical history, and medications will be collected by a study coordinator. The study coordinator will administer the mini-mental status exam, Geriatric Depression Scale and Hachinski Ischemic Scale. Vital signs will be assessed (height, weight, blood pressure, respiratory rate, temperature) and non-fasting blood and urine will be collected for routine safety labs including a chemistry panel (sodium, potassium, chloride, CO2, BUN, glucose, calcium, total protein, total bilirubin, albumin, alkaline phosphatase, AST and ALT), hematology panel (complete blood count including differential), and a microscopic urinalysis. A study clinician will perform a physical and neurological exam and review the inclusion / exclusion criteria and AD diagnostic criteria to determine study participant eligibility.

Baseline Assessments: Baseline assessments will occur within four weeks of the screening visit and will consist of two or three visits to KUMC: 1) Cognitive testing at the KU Alzheimer and Memory Program (<1 hour), 2) FDG PET scanning in the radiology department (1.5 hours), and 3) GCRC visit for a blood draw and lumbar puncture (2 hours).

Cognitive Testing: A study psychometrician will perform a standard battery of cognitive tests on all participants utilizing the Alzheimer's Disease Assessment Scale – Cognitive subscale (ADAS-Cog). The ADAS-Cog is composed of 11 subtests of memory and language and is frequently used for clinical trials.

Positron Emission Tomography (PET) Scans: Fluoro-deoxy-glucose (FDG) PET imaging will be performed on all participants at baseline and after treatment for 6 months. Participants will be asked to fast for at least four hours prior to the scanning session. The participant's blood glucose will be checked prior to scanning and must be < 180 mg/dL. After the injection of 5 mCi of tracer, subjects will sit in a quiet, dimly lit room for 30 minutes after which they are placed in the scanner. Procedures will be identical to those used extensively by this group in the Alzheimer's Disease Neuroimaging Initiative.

Lumbar Puncture: Participants will arrive in the GCRC in the early morning after an overnight fast for blood and CSF collection. Fasting blood (14 cc) will be drawn for isoprostane analysis. Dr. Burns will perform all lumbar punctures. Lidocaine will be used for topical anesthesia and an atraumatic 24 gauge Sprotte needle will be inserted for CSF collection at the L3/4 or L4/5 interspace. 2 cc of fluid will be discarded and 2 cc sent to the local laboratory for routine cell counts, protein, and glucose. Ten milliliters of blood will be collected and spun. The serum sample will be sent to the protocol investigator for isoprostane analyses. After completion of the procedure, participants will be remain in the GCRC; have breakfast; and rest for about 30-45 minutes before being discharged home with post lumbar puncture instructions. A phone call to the participant 24 hours after the lumbar puncture will be performed to assess for any problems following this procedure. The FDG Pet Scan and the lumbar puncture must be completed on two different days at least 24 hours apart.

Safety Visits: Participants will have in-person safety assessments (1.5 hours) in the GCRC every 8 weeks. Vital signs and weight will be measured; concurrent medications and adverse events will be assessed. A physical and neurological examination will be performed by a study clinician. A targeted symptom checklist will be reviewed with the participant and study partner at each visit to assess occurrence of adverse events including those most associated with the use of pramipexole (Mirapex, see table 1 of appendix). Blood and urine will be collected for safety labs. Study drug will be dispensed at safety visits i.e. visits 1, 2, and 3. Cognitive testing (ADAS-Cog) will be administered at each safety visit.

Study Drug Administration: R(+)PPX is provided by the sponsor to the KUMC investigational pharmacy as the water soluble dihydrochloride salt, in the form of powder manufactured under GMP conditions by Quality Chemical Laboratories, the supplier of drug for all of Dr. Bennett's studies. The investigational pharmacy prepares a water solution at 10 mg/ml. This solution has been tested by the sponsor and is stable at 4 degrees for at least two months.

Treatment is initiated after the Baseline visit at 100mg a day (50 mg bid in morning and early evening) for 1 month. If tolerated, dosing will increase to 200mg a day (100 mg bid) for a second one-month interval. If at the end of four months of being on study drug, the participant is not experiencing side effects the study drug will be increased to 300mg a day (150 mg bid) where it will remain for the next 4 months. Subjects will be seen every two months for safety assessments prior to any dose escalation and will also be contacted every two weeks by phone (see below). If a participant experiences mild, non-serious adverse events possibly due to the study drug, the study clinician will determine the causality and make a decision whether to continue the study medication and at what dosage. If study drug is discontinued temporarily (>10 days), it will be titrated to the target dose with dose increases no more frequent than once a month.

Drug Accountability: Participants in the study will return any unused drug and study drug containers at each of the safety visits. Study partners will be asked to account for any missed dosages of the study drug and the reason for missing the dosing. Participants are encouraged to maintain 100 percent compliance with dosing. The study partner and the participant will be advised of their

compliance at each safety visit before the study drug is dispensed and means and measures to increase compliance will be initiated. All unused study drug and containers will be returned to the investigational pharmacy for disposal.

Phone Calls: Participants and their study partner will be contacted by the study coordinator every two weeks by phone after starting to take the study medication to review concurrent medications and adverse events.

End-of-Treatment Assessments (Visit 5): Final outcome assessments will be completed during week 24 visit/ Visit 4 and consist of identical procedures to the baseline assessments (see above): 1) Cognitive testing at the KU Alzheimer and Memory Program (1 hour), 2) FDG PET scanning in the radiology department (1.5 hours), and 3) GCRC visit for a blood draw and lumbar puncture (2 hours). In addition the participant will have a physical/ neurological examination, safety laboratory sampling, vital signs, concurrent medications and adverse events. The participant will return all remaining study drug and containers at this visit. No additional medication will be dispensed.

End-of-Study Visit: Participants will return two weeks after completing the R(+)PPX treatment for a final physical and neurological exam and review of adverse events.

Data analysis

Primary Aim: Safety

Subject safety is the primary variable and item of greatest concern in this study. The extensive dosing experience with R(+)PPX in ALS subjects, many of whom have mild dementia, combined with its lack of detectable D2 dopamine receptor activity *in vivo* at 300 mg/day dosing, support its safety in probable AD subjects. Safety and tolerability will be evaluated by frequent contact with subjects and caregivers and lab safety studies every two months. The frequency and severity of adverse events will be examined and their potential causal relationship to the study drug (as determined by the study physician) will be assessed (the adverse events related to Mirapex [pramipexole] are listed in table 1 of appendix). This information will be used to guide dose selection and inform a more definitive safety and efficacy study. Additionally, cognitive performance data will be acquired every two months to assess the cognitive trajectory to explore possible dose-related changes in cognitive decline over the 6 months of intervention.

Exploratory Aims

Oxidative stress

Isoprostanes are stable oxidative products of polyunsaturated fatty acids. Oxidative stress will be evaluated by following serum isoprostane levels over the course of dose escalation and by assaying csf isoprostane before and after R(+)PPX. A very sensitive LC-MS assay will be used. Serum and CSF PPX levels will also be assayed by LC-MS. To assess change in isoprostane levels with treatment, we will compare baseline (pre-treatment) and 6-month (post-treatment) isoprostane levels using a paired t-test. We hypothesize that isoprostane levels will be reduced after 6 months of R(+)PPX treatment.

2-FDG PET

Changes in cerebral glucose metabolism as a proxy for mitochondrial respiration will be assayed at baseline and after a period of several months dosing with R(+)PPX at 300 mg/day. Multiple regions will be compared, with particular attention paid to hippocampus. The statistical mapping approach described by Chen, et al [31] will be used. Regional FDG uptake will be compared pre- and post-PPX dosing. Correlations will also be sought with assays of oxidative stress reduction, to see if greater reductions in brain oxidative stress (reflected by reductions in isoprostane levels) are reflected in elevations in cortical 2-FDG.

Conclusions

An abundance of compelling evidence supports the early appearance of oxidative stress as a pathogenic event in AD. Oxidative stress is elevated in brains of persons with pre-AD condition of MCI and in transgenic mice expressing familial AD genes prior to the amyloidosis seen in these models. Our

studies in the mitochondrial transgenic cybrid model of AD supports mtDNA as a contributor to AD oxidative stress and beta amyloid production. *In toto*, reduction of oxidative stress early in the course of AD should reduce the trajectory of neurodegeneration.

The sponsor studied S(-) and R(+) PPX in his laboratory beginning in 1997 and has clinically tested R(+) pramipexole in ALS subjects under a physician-sponsor IND since 2004. R(+)PPX is now commercially licensed and under separate development for treating ALS by Knopp Neurosciences. R(+)PPX is a brain and mitochondrially concentrated antioxidant that can be safely administered to humans at daily doses up to 300 mg/day and has no detectable DA receptor activity *in vivo*. At this dose many subjects achieved trough steady-state PPX levels of >1 μM, predicting brain levels of >6 μM that are clearly in the ROS scavenging and neuroprotective levels. R(+)PPX at 300 mg/day substantially improves the course of ALS in short-term studies and will enter Phase 3 testing later this year.

The present application seeks to introduce R(+)PPX treatment to subjects with early AD. In this protocol the subjects will undergo careful dose escalation from 100 mg/day to 300 mg/day R(+)PPX over 4 months. Subjects will have regular serum, combined with baseline and late treatment spinal fluid samples, assayed for isoprostanes as measures of oxidative stress. Serum and spinal fluid will also be assayed for [PPX]. As a measure of cerebral metabolism, brain 2-FDG PET scans will be obtained at baseline and after several months of dosing with 300 mg/day R(+)PPX.

The goal of the present study is to determine if R(+)PPX can be administered in high doses to probable AD subjects, and if so, whether oxidative stress is reduced and cerebral metabolism is increased. Hopefully the results from this study will justify the cost of a larger early efficacy study in probable AD.

Data Management

The KU Alzheimer and Memory Program currently uses an electronic database system, called REDCap (Research Electronic Data Capture), for much of its data management needs. REDCap was developed by Vanderbilt University, with collaboration from a consortium of institutional partners, for electronic collection and management of research and clinical trial data. This secure system is hosted by the Center for Research Methods and Data Analysis (CRMDA) at the University of Kansas Lawrence (KU-L) campus. The CRMDA hosts a KU-L Information Technology-controlled, HIPAA-certified REDCap database server on which project data will be stored. The REDCap system provides secure, web-based applications that have an intuitive interface for users to enter data and real time validation rules (with automated data type and range checks) at the time of entry. These systems offer easy data manipulation with audit trails and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). REDCap was developed specifically around HIPAA-Security guidelines and all web-based information transmission is encrypted. REDCap is used at many institutions and currently supports > 170 academic/non-profit consortium partners on six continents and 13,000 research end-users (www.project-redcap.org). Brad Amstein, a member of the Alzheimer and Memory Program at KUMC, is responsible for working with the CRMDA and the REDCap system to design the database, its forms, the data entry process, and reports related to adverse events and pertinent safety data for this research study.

Data Safety and Monitoring Plan

All adverse events (AE's) will be reported to the PI and sponsor in a timely fashion. The study coordinator will enter the AE data from the source documentation into REDCap within 5 business days from receipt of the initial call to report the AE. Any serious adverse event will be reported to the PI and sponsor immediately and then to the General Clinical Research Center and the Human Subject's Committee according to reporting guidelines.

A Data Safety and Monitoring Board (DSMB, see DSMB charter attached below) will meet quarterly to review AE's and the overall safety of the ongoing trial. The DSMB is composed of three experienced clinicians: Maral Mouradian, MD from the Robert Wood Johnson Medical School, Un Kang, MD from

the University of Chicago, and Fred Wooten, MD from the University of Virginia. Dr. Mouradian will be the acting Chairman.

The DSMB will have access to de-identified participant medical records and other study-related records. The investigator agrees to cooperate with the monitor (s) to ensure that any problems detected in the course of these monitoring visits are resolved. Personal contact between the DSMB and the investigator will be maintained throughout the clinical trial to assure that the investigator is fulfilling his obligations and the facilities used in the clinical trial remain acceptable.

The DSMB will perform a quarterly review of lab safety data, results of physical/ neurological assessments and subject adverse event/SAE reports. The DSMB can advise and recommend temporary or permanent withdrawal of a subject or that the entire study be placed on hold or terminated.

All grade 2 and above AE's according to CTCAE v3.0 will be reviewed by the DSMB members quarterly. To facilitate this review, a list of each AE grade 2 or higher (by CTCAE v3.0) will be generated from REDCap. Each AE listed will include the grade, the type of event, and the potential relationship of the AE to the study intervention. The PI will be notified within 24 hours by the study coordinator of all AE's grade 3 or higher. Should a study participant experience an AE in any category rated 3 or greater according to CTCAE v3.0, that individual will be removed from the study immediately. Should three or more subjects (15% of target enrollment) have AE's in any category that is grade 3 or higher according to CTCAE v3.0 that are possibly or probably related to study intervention, the study will be terminated.

Schedule of Study Procedures and Assessments

Week #	-4	-2	0	2	4	6	8	10	12	14	16	18	20	22	24	26
Visit Name	**Scr**	**B**	**1**	P/C	P/C	P/C	**2**	P/C	P/C	P/C	**3**	P/C	P/C	P/C	**4**	EOS
Informed Consent	X															
NINDS-ADRDA	X															
Hachinski Ischemic Scale	X															
Geriatric Depression Scale	X															
MMSE	X															
Demographics	X															
Medical History	X	X														
Medication History	X	X														
Body height/ weight *	X		X				X				X				X	X
Vital Signs	X		X				X				X				X	X
Baseline Sx Checklist	X		X				X				X				X	X
Physical/Neuro Exam	X						X				X				X	X
Safety labs	X						X				X				X	X
Inclusion/ Exclusion	X															
ADAS-Cog		X					X				X				X	
FDG PET scan-brain		X													X	
Lumbar puncture		X													X	
Isoprostane analysis **		X													X	
Dispense Study Drug			X				X				X					
Drug Accountability					X		X				X				X	
Concurrent Medications			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events			X	X	X	X	X	X	X	X	X	X	X	X	X	X

Scr: Screen; B: Baseline; P/C: Phone Call follow-up; EOS: End of Study; MMSE: Mini Mental Status Exam; NINDS-ADRDA: National Institute of Neurological Disorders and Stroke - Alzheimer's Disease and Related Disorders Association criteria for AD;
ADAS-Cog: Alzheimer's Disease Assessment Scale - Cognitive subscale;
FDG-PET: Fluoro-deoxyglucose Positron Emission Tomography;

***** Height done only at screening
*** Isoprostane analysis- CSF and plasma*

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Appendix:

1. **Table of Treament-Emergent Adverse Events from Mirapex**
2. **Data Safety and Monitoring Board (DSMB) Charter**

Table 1: Treatment-Emergent Adverse-Event* Incidence in Double-Blind, Placebo-Controlled Trials in Early Parkinson's Disease (Events ≥ 1% of Patients Treated with MIRAPEX tablets and Numerically More Frequent than in the Placebo Group)

Body System/ Adverse Event	MIRAPEX N=388	Placebo N=235
Body as a Whole		
Asthenia	14	12
General edema	5	3
Malaise	2	1
Reaction unevaluable	2	1
Fever	1	0
Digestive System		
Nausea	28	18
Constipation	14	6
Anorexia	4	2
Dysphagia	2	0
Metabolic & Nutritional System		
Peripheral edema	5	4
Decreased weight	2	0
Nervous System		
Dizziness	25	24
Somnolence	22	9
Insomnia	17	12
Hallucinations	9	3
Confusion	4	1
Amnesia	4	2
Hypesthesia	3	1
Dystonia	2	1
Akathisia	2	0
Thinking abnormalities	2	0
Decreased libido	1	0
Myoclonus	1	0
Special Senses		
Vision abnormalities	3	0
Urogenital System		
Impotence	2	1

*Patients may have reported multiple adverse experiences during the study or at discontinuation; thus, patients may be included in more than one category.

Source: Mirapex (pramipexole) package insert

IND 60,948.
R(+) Pramipexole Treatment of Alzheimer's or Parkinson's Disease.
Data Safety and Monitoring Board (DSMB) Charter

Purpose: The Sponsor (James P. Bennett, Jr. M.D., Ph.D.) obtained IND 60,948 for use of the experimental neuroprotective drug R(+) pramipexole dihydrochloride (PPX) to treat patients with amyotrophic lateral sclerosis (ALS). The IND was removed from clinical hold at the end of 2002, and Phase I studies were initiated by the Sponsor in March, 2004, followed by Phase II studies in 2005. In 2006 the license for testing of R(+)PPX was acquired by Knopp Neurosciences (now Knopp Biosciences). Knopp obtained its own commercial IND and carried out Phase I/II studies. Following the completion of a successful Phase IIB study in 2009, the license for Phase III clinical testing in ALS was acquired from Knopp by Biogen-Idec in August, 2010.

The Sponsor of IND 60,948 has retained an active physician-sponsor IND and in 2006-2007 amended his IND to carry out studies of R(+)PPX in patients with Alzheimer's disease (AD) and Parkinson's disease (PD). This DSMB is chartered to monitor safety and tolerability of R(+)PPX in AD or PD patients. No protocols involving efficacy of slowing disease progression are currently being considered. Should such efficacy studies be proposed, this Charter will be amended.

Composition: This DSMB will consist of at least three neurologist-physicians who have experience with experimental therapeutics in humans. One member will function as Chair, based on mutual agreement of the members. Participation on this DSMB is voluntary, follows solicitation by the Sponsor and is compensated at an annual rate of $2,000 per annum.

DSMB review of clinical studies: Members of the DSMB will receive by electronic means all of the safety laboratory and clinical data on each participant. The DSMB members will also receive all Adverse Event (AE) and Serious Adverse Event (SAE) reports generated during the study. It is anticipated that such data submissions will occur on a monthly basis. The DSMB will meet quarterly. These meeting can occur electronically or by telephone conference, depending on the wishes of the DSMB members. SAE reports will be provided by the Sponsor to the DSMB based on FDA regulations. All SAE's are reported within 72 hours, and all deaths, expected or unexpected, are reported within 24 hours.

The DSMB will provide to the Sponsor its advice regarding any safety/tolerability issues that arise. This advice can include recommendation that a particular subject be temporarily or permanently withdrawn from the study or that the entire study be placed on hold or terminated.

DSMB Reports: The advice provided to the Sponsor by the DSMB, and Sponsor's responses, will become part of the IND record and will be filed by the Sponsor with the Sponsor's IND annual reports.