FINAL REPORT

FOR CRADA NO. C-07-04

BETWEEN

BROOKHAVEN SCIENCE ASSOCIATES

AND

CENERX BIOPHARMA, INC.

Project Entitled: PET Studies of CX-157

Brookhaven PI: Joanna Fowler

Submitted by: Michael J. Furey
Manager, Research Partnerships
Brookhaven National Laboratory

DISCLAIMER

FINAL REPORT

FOR CRADA NO. C-07-04

BETWEEN

BROOKHAVEN SCIENCE ASSOCIATES

AND

CENERX BIOPHARMA, INC.

Project Entitled: PET Studies of CX-157

Brookhaven PI: Joanna Fowler

Submitted by: Michael J. Furey
Manager, Research Partnerships
Brookhaven National Laboratory

DISCLAIMER

Brookhaven National Laboratory
Memorandum

Date: December 26, 2008
To: Michael Furey
From: Joanna S. Fowler
Subject: CRADA with CeNeRx Biopharma, Inc- (No. BNL-C-07-04)

This project has been completed except for the preparation of a manuscript for publication (which is in progress). We attach an abstract of this work which was just presented as a poster at the annual American College of Neuropsychopharmacology meeting in Phoenix, AZ on December 8, 2008. A full report prepared by BNL and CeNeRx is also available if it is needed.

- *Significant Accomplishments*: We completed measuring Brain MAO A activity in 15 subjects at baseline and after different doses of CX157 and also at different times after each dose. Fifty five scans were completed. We determined that plasma levels of the drug are a surrogate marker for the degree of MAO A inhibition in the brain.
- *Significant problems*: none
- *Industry Benefits realized*: Based on the results of this study, CeNeRx chose the dose and timing for CX157 for a multi-center clinical trial for depression which is now underway. They used the results of this study to raise funds for the multi-center clinical trial.
- *Laboratory benefits realized*: We established the ability to do a complex supervised dosing protocol and also the ability to house subjects overnight at the laboratory with no significant problems. We will also have a publication in a prominent journal which will provide an example of our ability to do this type of study in a timely fashion and to provide results which are needed in order for the company to proceed to phase 2 studies.
- *Recommended follow-on work:* This specific project is complete. However, we have established a good relationship with CeNeRx and expect that they would call on us in the future for any imaging studies.
- *Potential benefits from follow-on work*: The benefits from follow on work would be to provide more support for the clinical infrastructure of PET imaging group. This is also an example of how PET technology can facilitate the development of new drugs.

ACNP Abstract:

Evidence for Robust and Reversible Brain MAO-A Inhibition by CX157 in Healthy Normal Volunteers

Joanna S. Fowler, Jean Logan, Albert J. Azzaro, Robert M. Fielding, Daniel Burch, Gene-Jack Wang, Frank Telang, Barbara Hubbard, Millard Jayne, Pauline Carter, Scott Carter, Youwen Xu, Colleen Shea, Lisa Muench, David Alexoff and Karen Apelskog

Background: According to the World Health Organization, Major Depressive Disorder (MDD) is predicted to become the second leading cause of disease burden worldwide (after HIV) by 2030 (Mathers CD, Loncar D, 2006, *PLoS Med.* **3** (11): e442). This creates a sense of urgency to develop more effective antidepressant medications. CX157 (3-fluoro-7-(2,2,2-trifluoroethoxy)phenoxathiin-10,10-dioxide) is an investigational compound currently being developed for the treatment of MDD. Pre-clinical experiments have demonstrated specific, competitive, and reversible inhibition of brain MAO-A activity associated with elevated levels of brain monoamine neurotransmitters in animal models (Data on file at CeNeRx BioPharma, Inc., Cary, North Carolina) suggesting that CX157 could effectively treat MDD with minimal hypertensive effects associated with dietary tyramine (the "cheese effect"). Three phase I safety trials, with single doses up to 120 mg, or repeated doses up to 60 mg TID for 14 days have shown that the drug was well-tolerated. The objectives of this study were to assess the *in vivo* potency and duration of action of CX157 as a reversible inhibitor of brain MAO-A in humans using PET and [^{11}C]clorgyline, a MAO-A-specific radiotracer (Fowler et al., 1996, PNAS 93:14065-14069), and to examine the relationship between brain MAO-A inhibition and plasma CX157 concentration.

Methods: This study was carried out at Brookhaven National Laboratory and approved by the local Institutional Review Board. Fifteen normal healthy male non-smokers (33.4 ± 9.0 yrs of age), were studied. Twelve of the subjects each received a baseline and 2 hour scan after a single oral dose of CX157 (20, 40, 60 or 80 mg), followed by 2 or 3 more scans over the next 24 hours. To capture all of the time points, some of the subjects were dosed and scanned on 2 different days, one week apart. Three other subjects were scanned at baseline and then received oral doses of 40 mg of CX157 BID for seven days (10:30 AM and 10:30 PM) followed by PET scans at 2, 5, 8 and 12 hrs post last dose. All CX157 dosing was supervised. Plasma samples obtained from each subject prior to dosing and at various time points, post-dosing, including the time of the PET scans, were analyzed for CX157 using a validated LC/MS assay. Time-activity curves from each brain region and arterial plasma values were used to estimate brain MAO-A using a 3-compartment model following the injection of 7.1±0.6 mCi of [^{11}C]clorgyline. A composite MAO-A [^{11}C]clorgyline binding value was obtained from each brain region and compared to the baseline value to obtain the fraction of MAO-A inhibition following each dose and at each time-point. The relationship between brain MAO-A inhibition and plasma CX157 concentration was assessed by plotting % MAO-A inhibition vs plasma CX157 level.

Results: Brain MAO-A inhibition by CX157 was robust and rapid following oral dosing with a peak effect observed at the 2 hr time-point. Inhibition of MAO-A at 2 hr averaged 54.6±8.9% and 72.4±8.7% for the 40 and 80 mg single doses, respectively (n=3/dose). Brain MAO-A inhibition rapidly declined to 10.7±9.3% of baseline at 12 hours and recovered completely by 24 hr following the single 40 mg dose. MAO-A inhibition by CX157 was more sustained with BID dosing, with 12-hr inhibition increasing to 23.1±3.7% vs 10.7±9.3% for the single dose. MAO-

A inhibition was proportional to CX157 plasma exposure, despite signifiscant intersubject variability in pharmacokinetics.

Discussion: CX157 entered the brain rapidly after oral dosing producing a dose- and time-related inhibition of brain MAO-A in human subjects. MAO-A inhibition peaked early then declined over the 24 hours after a single-dose. Multiple twice-daily dosing with CX157 produced a more sustained inhibition of MAO-A over a 12 hr period, suggesting that repeated dosing could achieve continuous therapeutic levels of MAO-A inhibition. Brain MAO-A inhibition was directly correlated with plasma levels indicating the usefulness of CX157 plasma levels as a biomarker for brain MAO-A inhibition for future studies.

Acknowledgments: This research was carried out at Brookhaven National Laboratory under contract DE-AC02-98CH10886 and was supported by CeNeRx BioPharma, Inc and by GCRC grant #MO1RR10710.