



CounterACT Neurotherapeutics Screening (CNS) Program

Executive Summary

The NIH NIAID Chemical Countermeasures Research Program (CCRP) includes established animal screening models to identify and accelerate the research and development of novel medical countermeasures (MCMs) for organophosphorus (OP) chemical threats that target the central nervous system. These models are available through the CNS Program to support translational studies evaluating the potential *in vivo* efficacy of test compounds against the lethal and/or non-lethal effects of OP chemical threat agents. Studies are conducted through the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD; <http://chemdef.apgea.army.mil>). If accepted into the CNS program, studies are performed at **no cost** to the investigator/supplier.

The main purpose of the CNS program is to provide applicants with important pre-application proof-of-principle efficacy data in support of a research application to the NIH-wide CounterACT peer-reviewed grant program. The CNS program does **not** replace the need to establish direct collaborations with laboratories that are certified to work with restricted chemical agents within those applications submitted in response to NIH research solicitations.

The proposed studies must not overlap, but may be conducted in concert with, studies performed by other CCRP efficacy and/or preclinical research support services. Participants will retain custody of and have primary rights to the data developed, subject to Government rights of access consistent with current U.S. Government (USG) policies.

Before, during, and subsequent to entry into the program, the USG is not required to obtain for the participants any proprietary rights, including intellectual property rights, or any materials needed by the applicant to perform the project. Participants are advised to establish a separate Material Tech-Transfer or Lab Service Agreement between themselves and the NIH-supported laboratories before commencing any studies.

Program Description and Goal

OP-induced seizures result from overstimulation of susceptible brain circuits by abnormally high levels of the excitatory neurotransmitter acetylcholine, which rapidly builds up after inhibition of the enzyme acetylcholinesterase by nerve agent¹. These seizures rapidly progress to a condition known as *status epilepticus* (SE), a medical emergency that responds to only a subset of known anticonvulsant drugs. Moreover, the longer these seizures persist the more difficult they are to stop pharmacologically², and in the case of chemical warfare nerve agents (NAs), as little as 20 min of continuous seizure activity is sufficient to produce neuropathology, the severity and extent of neuropathology is then proportional to the duration of the seizures^{3,4}.

¹ McDonough JH. *Neurosci. Biobehav. Rev.* 1997; 21(5):559-579.

² Shih TM, et al. *Toxicol. Appl. Pharmacol.* 2003; 188(2):69-80.

³ Lallement G, et al. *Neuroreport* 1994; 5(17):2265-2268.

⁴ McDonough JH, et al. *Neurotoxicology* 1995; 16(1):123-132.



The current MCM approach to treat OP-induced seizures includes the administration of atropine, pralidoxime chloride (2-PAM Cl), and a benzodiazepine-based anticonvulsant (diazepam/midazolam). Though efficacious to an extent, overall improvement in both mortality and morbidity outcomes is still highly desired. The goal of the CNS program is to identify novel neurotherapeutics that may be administered with the approved treatments, in a civilian first-responder setting, to more effectively suppress SE activity and/or mitigate neuropathology after OP exposure.

Screening Models

The CNS program employs the following *in vivo* screening models:

- 1) OP Diisopropylfluorophosphate-(DFP) induced electrographic SE model in male, Sprague-Dawley rats (125-175 g) surgically prepared with surface cortical electrodes one week before actual exposure to record brain electroencephalographic (EEG) activity.
 - a. Implanted animals will be pre-treated with pyridostigmine (PB, 0.026 mg/kg, i.m.) at 30 min before injection of DFP (4.0-6.0 mg/kg, s.c.), followed by co-administration of atropine methyl nitrate (AMN, 2.0 mg/kg, i.m.) and 2-PAM (25 mg/kg, i.m.) within 1 min later. The test neurotherapeutic compound +/- midazolam (1.78 mg/kg, i.m.) will be administered at 60 min after the occurrence of the first electrographic seizure, based on the appearance of repetitive spikes and sharp waves in the EEG.
 - b. The model utilizes 24-hr EEG recordings with objective, semi-automated, and quantitative methods of data analysis^{5,6} to determine the efficacy of different investigational compounds at suppressing DFP-induced electrographic SE.
 - c. Additional studies will quantify neuronal death under these conditions to assess neuroprotection via Fluoro-Jade B staining in at least four perfused brain areas that are highly susceptible to DFP-induced brain damage.

- 2) NA Soman-(GD) induced electrographic SE model in male, Sprague-Dawley rats (250-300 g at time of surgery) also surgically prepared with surface cortical electrodes to record brain EEG activity.
 - a. Animals are pretreated with the oxime HI-6 (125 mg/kg, i.p.); 30 min later the animals are challenged with 1.6xLD₅₀ GD (180 µg/kg, s.c.), at a dose that elicits EEG seizure activity in 100% of the animals. The animals will receive AMN (2.0 mg/kg, i.m.) within 1 min after GD challenge.
 - b. At 20 min following the onset of electrographic seizure activity, animals will receive standard medical countermeasures for NA intoxication via intramuscular injection (0.45 mg/kg atropine sulfate, 25.0 mg/kg 2-PAM-Cl, 1.78 mg/kg midazolam) along with a dose of the test neurotherapeutic compound previously identified from the DFP model. EEG is also continuously recorded and 24 hr later at the end of the recording period, the animals are perfused, the brain stained with Fluoro-Jade B and/or hematoxylin and eosin (H&E), then evaluated to rate the degree of pathology by examining up to five brain areas highly susceptible to NA-induced damage^{7,8}.

⁵ Lehmkuhle MJ, et al. J Neurophysiol 2009; 101:1660-70.

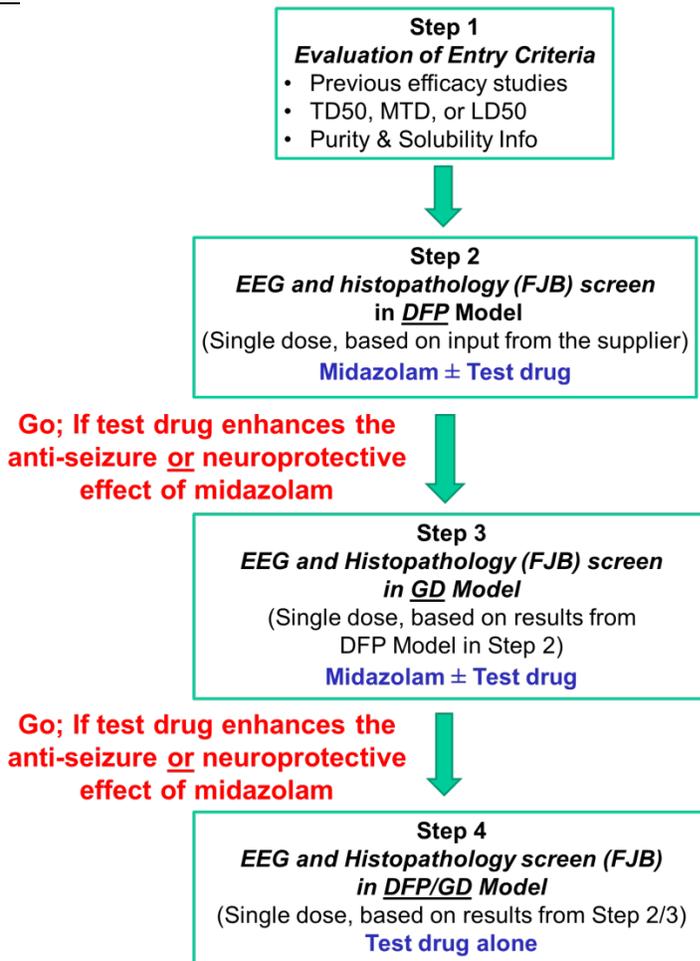
⁶ Pouliot W, et al. Neuroscience 2013; 231:145-56.

⁷ McDonough JH, et al. Neurotoxicology 1995; 16(1):123-132.

⁸ McDonough JH, et al. Neurotoxicology 1998; 19(3):381-391.



Screening Approach



The test neurotherapeutic compound may be coded throughout the study, so that experiments are performed under blind procedures.

A final Study Report (SR), including task background, methodology, assumptions, specific data collected, analyses conducted, conclusions and recommendations, will be delivered to the investigator / supplier at the conclusion of the study.

Eligibility Criteria

NIH will accept applications from individual Principle Investigators (PIs) from academic institutions, government laboratories, and/or companies. PIs may consult with NIH to determine eligibility.

- a) Non-domestic (non-U.S.) Entities (Foreign Institutions) are not eligible to apply.
- b) Non-domestic (non-U.S.) components of U.S. Organizations are not eligible to apply.



The CNS program is available to all investigators within and outside of the CounterACT grant program with promising medical countermeasure(s) that would be responsive to the mission of the CCRP.

A minimum requirement for the test neurotherapeutic supplier is to provide documentation regarding compound toxicity, solubility, purity, and previous *in vivo* efficacy studies related to this effort. Supplier must be able to provide sufficient quantity of the compound with purity $\geq 95\%$ by NMR or HPLC analysis for evaluation in up to 60 animals based on the highest ED50 value of the previous efficacy studies.

Solubility information would facilitate determination of the best vehicle and route of administration (IM or IP). The optimal solvent/vehicle is saline. Toxicity data, such as the median toxic dose 50 (TD50), maximum tolerated dose (MTD), or median lethal dose 50 (LD50), and previous efficacy studies would assist in identifying the range of doses to be considered for evaluation and aid in prioritizing the compounds to be tested. These information are required before testing of analogs and congeners as well.

For additional information or an application to enroll in the CNS program, please contact (preferably by email):

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