**Dennis. E Kyle, PhD**
Examples of Current Projects

## Project Title

"Discovery of host genetic factors associated with susceptibility to infections with brain-eating amoebae"

*Naegleria fowleri* is a small, free-living amoebae (FLA) that is found ubiquitously in warm freshwater and soil. Most FLA in the environment feed on bacteria and are of no medical importance, yet *N. fowleri* causes a serious, usually fatal disease known as primary amoebic meningoencephalitis (PAM). Infection occurs following instillation of water containing amoebae into the nasal passages. *N. fowleri* then crosses the olfactory epithelium and migrates along the olfactory nerves to the frontal lobes of the brain where it causes extensive inflammation and pathology. Unfortunately, PAM results in fatality in >97% of cases, hence the opportunistic pathogen has garnered the name "brain-eating amoebae." The infection progresses rapidly with onset of symptoms within 5-10 days and death by 14 -17 days post-infection. Infections have been associated with any source of freshwater that is not properly chlorinated; these include recreational activities in lakes or ponds, splash pads, white water rafting, slip-n-slides, wave pools, thermal springs, Neti pots, and ritual ablutions. Although PAM is a rare disease, there is significant evidence it is underdiagnosed globally due to similar symptomology with viral or bacterial encephalitides and lack of widely available diagnostics.

In this proposal we will test the hypothesis that host genetic factors contribute significantly to susceptibility to *N. fowleri*. We propose to use the well-defined, homozygous progeny of the NIH Collaborative Cross (CC) mouse collection to identify genetic determinants of susceptibility to *N. fowleri* infection. The CC mice originate from five common inbred mouse strains and three wild-derived strains. The CC lines comprise a large panel of recombinant inbred strains developed to enhance standard genetic mapping capabilities. The choice of founder strains and breeding design produced high level recombination and uniform distribution of genetic variation with lower long range linkage disequilibrium, thus providing higher statistical power and locus resolution quantitative trait loci (QTL) mapping. The CC mouse collection is >45 million segregating polymorphisms that encompasses ~90% of the genetic diversity of *Mus musculus*, thus capturing vastly more naturally evolved genetic diversity than a typical inbred cross.

Genetic regions that influence infection and PAM severity will be identified by QTL mapping of phenotypic variation in 30 CC lines infected with *N. fowleri*. The QTL mapping is greatly strengthened by the availability of extensive genome sequences and annotations for all CC lines. Consequently, discrete infection/virulence phenotypes measured in these lines can be readily mapped to their underlying host genetic loci and candidate genes. In this study we propose to use two phenotypes of PAM disease severity in each of the CC lines. First, we will determine the mean survival times (MSTs) in the CC lines after *N. fowleri* infection. Each mouse will be infected by instillation of 1000 amoebae (V067 strain) into a single nare of an anesthetized mouse. We chose *N. fowleri* V067 because we found it to be the least virulent, thus offering the potential to identify host genetic factors that drive the phenotype toward either enhanced susceptibility to infection (i.e., lower MSTs) or toward factors that inhibit or limit the infection. Our extensive prior data demonstrate reproducible MSTs for multiple *N. fowleri* strains in outbred mice; however, for these studies we will include a founder inbred line for comparisons between experiments. In addition, mice of both sexes will be included to assess sex-linked differences in the measured phenotypes. The second phenotype for QTL mapping will quantification of a small-RNA of *N. fowleri* that is secreted in extracellular vesicles. In previous studies we validated that copy numbers of an amoeba specific small-RNA (Nf smRNA-1) in urine can be used to estimate biomass of the amoebae and follow progression of disease.

## Project Title

"Optimizing 2-Amido-pyrazines as Fast-Acting Antimalarials"

Malaria is endemic in 85 countries and 50% of the world population is at risk for contracting the disease[1]. Successful malaria control efforts have led to reductions in malaria morbidity and mortality from 2000 to 2015. However, in part due to the Covid-19 pandemic, cases increased by 24 million and deaths increased by 69,000 from 2019 to 2020[2] (WHO). This sudden turnaround in malaria control highlights the importance of treating patients with effective blood schizonticides[3]. Currently, the frontline treatment for uncomplicated falciparum malaria are artemisinin combination therapies (ACTs). Alarmingly, artemisinin resistance is established in the Greater Mekong Subregion and has been recently reported in Sub-Saharan Africa, where over 90% of malaria cases occur[4-7]. Therefore, the most significant need for treatment, control, and elimination is discovery and validation of new drug targets and development of new, rapidly acting blood schizonticides against those targets[8].

To address this unmet medical need, we discovered the 2-amido-pyrazine (APZ) scaffold that kills asexual *P. falciparum* blood stages at single-digit nanomolar concentrations. The straightforward synthesis from readily available and affordable starting materials, favorable physicochemical properties, promising pharmacokinetics, and oral bioavailability with frontrunner **APZ-2214** renders the APZ class attractive for lead optimization and development. The scientific premise of this proposal is grounded on our recent discovery with **APZ-2214** demonstrating that our orally bioavailable APZ chemotype exhibits fast and effective killing (6 h lag time, $PCT_{99.9}$ of 67 h) similar to chloroquine, thus making it appealing for further development. Our overarching hypothesis is that optimized APZs have potential to back-up, or possibly succeed, the fast-acting artemisinin component of treatment regimens that are threatened by artemisinin drug resistance. There are no previously published medicinal chemistry efforts for the APZs, and our preliminary data suggest a new target, therefore, the proposed work is both novel and innovative. Our multi-PI proposal combines the expertise of parasitologists (Maher/Kyle), a medicinal chemist (Manetsch), a computer-aided drug designer (Cui), a pharmacologist (Lombardo), and a computational biologist (Ruberto). Herein, we propose the medicinal chemistry optimization of APZs, evaluation of their *in vitro* and *in vivo* efficacy in established malaria models, and studies into the mechanism of action of APZs.

**Impact:** The APZ series has potential to treat blood stages of multidrug resistant *P. falciparum* malaria. The series has a quick mechanism of action and parasite reduction ratio, similar to 4-aminoquinolines, which could be used in combination with artemisinin derivatives, legacy antimalarials, or new schizonticides as part of a future combination therapy. Development of a drug with these properties would have a tremendous global public health impact and could significantly enhance current malaria elimination efforts.