SUMMARY OF PROPOSED RESEARCH

Please provide a one-page summary of the research plan, rationale, objectives, and specific goals of the proposed research.

Infection with the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* occurs in 60-70% of adult CF patients in Canada and is associated with increased morbidity and mortality, irrespective of lung function. Most infections result from colonization by environmental strains that, once established, remain persistently associated with the host for decades and are recalcitrant to further therapy. The factors driving the transition to chronic infection, and possible therapeutic interventions to prevent or delay this transition, remain unclear.

We suggest that a major selective force in the transition from a free-living environmental strain to chronic infection of the CF lung is oxidative stress. Bacteria growing in the CF lung experience three independent sources of oxidative stress: anaerobic respiration and/or fermentation in the mucous layer that generates reactive nitrogen intermediates such as NO, oxidative burst by the host immune system and, somewhat controversially, the use of antibiotics whose main killing mechanism may involve oxidative damage. The combined effect of these three factors, we hypothesize, causes strong selection to mitigate oxidative stress and maintain an appropriate redox balance. Consistent with this hypothesis, we have found that isolates of *P. aeruginosa* from CF patients across Ontario show signatures of strong selection on genes and genetic pathways associated with maintaining redox balance and mitigating various sources of oxidative stress. Furthermore, an expanded analysis of many more strains from diverse sources revealed that genes with redox function experience stronger positive selection in isolates from CF patients compared to those from the environment. These results suggest that restoring redox balance in the lungs of children, for example through prophylactic treatment with an antioxidant like *N*-acetylcysteine, might prevent adaptation to oxidative stress and so delay or prevent the onset of chronic infection.

The research outlined in this proposal will evaluate the idea that establishing a chronic infection requires adaptations to mitigate oxidative stress and proposes a means to prevent those adaptations from evolving in the first place. We leverage the results of a large (~500 genome) cross-sectional study using comparative genomics to identify the unique signatures of selection on CF isolates (Aim 1) with the power of experimental evolution to test these ideas directly (Aim 2). Together with molecular biology and genetic techniques that allow us to verify the effects of candidate mutations (Aim 3) and an analysis of parallel evolution of the same mutations or genes between experimental and natural isolates (Aim 4), we will be in a position to provide a comprehensive view of the genetic pathways to chronic infection and the role of oxidative stress in this process. We will contribute to improving the health of CF patients by providing a proof-of-principle experiment to evaluate whether antioxidant therapy is effective at delaying the evolution of OSR, and so the development of chronic infection, and by expanding the fundamental knowledge base about the causes of chronic infection in the CF lung.