1. Project Title

CB-839 Targets Cardiac Mitochondrial Glutaminolysis to Mitigate Acute Myocardial Injury

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2. Abstract of Project

The objective of this research project is to investigate how mitochondrial health influences cardiac adaptation to acute myocardial injury, with a long-term goal of reducing the progression to heart failure. Using a murine model of ischemia–reperfusion (I/R) injury, we will examine the role of mitochondrial glutaminase (GLS1), a key enzyme mediating glutamine oxidation (glutaminolysis), which may compromise myocardial resilience under pathological stress.

Glutamine-dependent anaplerosis is activated during oxidative stress in acute myocardial injury. Although this response may provide short-term metabolic support, increased GLS1 activity is also associated with maladaptive remodeling and fibrosis. We hypothesize that in vivo GLS1 inhibition will reduce I/R-induced damage and attenuate adverse cardiac remodeling. Despite the clinical importance of this pathway, the mechanistic contributions of glutaminolysis to myocardial infarction remain insufficiently defined.

To test this hypothesis, we will use CB-839 (telaglenastat), a highly selective, orally available GLS1 inhibitor that targets both GAC and KGA isoforms. CB-839 suppresses mitochondrial glutamine metabolism and has demonstrated excellent tolerability in early oncology trials, with up to 96% inhibition of tumor glutaminase activity. Its predecessor, BPTES, has been shown to attenuate pressure overload–induced cardiac hypertrophy, supporting GLS1 inhibition as a cardioprotective strategy.

FBV mice (8–9 weeks old) will receive CB-839 (200 mg/kg, oral gavage) or vehicle control for 6–18 days. I/R injury will be induced by transient occlusion of the left anterior descending (LAD) coronary artery for 30 minutes, followed by 24 hours of reperfusion.

We will evaluate whether CB-839 enhances myocardial adaptation to I/R by improving mitochondrial respiration, preserving mitochondrial integrity, and suppressing pathological glutaminolysis. Mitochondrial respiration will be measured using oxygen polarography, and cardiac function will be assessed by echocardiography.

The findings are expected to offer mechanistic insights into how glutaminolysis regulates cardiac adaptation to injury and may identify GLS1 inhibition as a therapeutic strategy for myocardial infarction and heart failure. This work has the potential to contribute toward improving human healthspan through novel approaches to cardioprotection.

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3. Significance of the Research

Myocardial infarction affects more than 800,000 Americans annually and carries a 20% mortality rate. Aging amplifies susceptibility to ischemia–reperfusion injury, a major driver of heart failure. Mitochondrial dysfunction—manifested by impaired energy production and redox imbalance—is central to heart failure pathogenesis.

Glutaminolysis increases markedly in post-ischemic myocardium and is implicated in:

- redox dysregulation
- inefficient energy coupling
- impaired myocardial recovery
- progressive cardiac fibrosis and dysfunction

This project tests the hypothesis that pharmacological GLS1 inhibition with CB-839 can improve myocardial resilience to acute myocardial injury and reduce the risk of subsequent heart failure. Using CB-839 as the therapeutic model, this study will help establish whether targeting dysregulated glutaminolysis provides a novel cardioprotective strategy.

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4. Goals and Objectives

This project aims to train medical students in biomedical research while exploring how GLS1-mediated glutaminolysis and mitochondrial health influence the heart's response to acute injury. The objectives are:

- To evaluate how in vivo GLS1 inhibition affects myocardial adaptation to acute I/R injury
- To assess changes in mitochondrial bioenergetics and cardiac function
- To provide hands-on training in echocardiography, mitochondrial isolation, spectroscopic assays, and data analysis

To ensure feasibility within an eight-week summer fellowship, the study will focus on characterizing cardiac and mitochondrial phenotypes in a pharmacological acute I/R model.

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5. Research Methods

A. In Vivo Mouse Model of Myocardial I/R

- The myocardial I/R protocol will follow previously published methods.
- FBV mice treated with CB-839 or vehicle will undergo 30 minutes of LAD ligation followed by 24 hours of reperfusion.
- Cardiac function will be assessed under anesthesia using echocardiography.
- Hearts will then be excised for TTC staining to delineate infarct size and risk region.
- Tissue from the risk region will be used for mitochondrial isolation.

B. Mitochondrial Isolation and Respiration Analysis

- Mitochondria will be isolated from non-ischemic and post-ischemic tissue via differential centrifugation.
- Respiration will be assessed at 30°C using a Clark-type oxygen electrode.
- NADH-linked (malate/glutamate) and succinate-linked respiration will be measured.
- Enzymatic activities of ETC complexes will be quantified as previously described.
- GLS1 activity will be measured by glutamine-to-glutamate conversion coupled to glutamate dehydrogenase.

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6. Data Analysis

1. **Echocardiography:**
    - Ejection fraction, fractional shortening
    - LV internal diameters (systole/diastole)
    - LV volumes
    - Relative wall thickness

- Heart weight/body weight ratio
- Mitral valve E/A ratio

2. **Mitochondrial assays:**
   - OCR first-derivative kinetics
   - Complex I activity via NADH oxidation. Complex II activity via DCPIP reduction
   - Complex III and Complex IV activities via ferricytochrome c reduction and ferrocytochrome c oxidation
3. **Statistics:**
   - Data reported as mean ± SEM
   - Between-group comparisons using one-way ANOVA

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7. Contribution to the Overall Research Program

By integrating echocardiographic analysis, mitochondrial bioenergetics, and pharmacological GLS1 modulation, this pilot project will expand our understanding of how mitochondrial glutaminolysis influences cardiac recovery after I/R injury. These studies are expected to:

- Reveal new insights into mitochondrial determinants of cardiac resilience
- Identify GLS1 as a potential therapeutic target
- Provide foundational data for future grant applications
- Support long-term investigations into cardioprotection and heart failure prevention

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Summer Research Fellow Training and Monitoring Plan

1. **Student Requirements and Procedures**

a. Students will begin by reviewing key publications from Dr. Chen's group and the 2025 summer research poster. They will receive training on acute myocardial injury models, echocardiography, mitochondrial isolation, spectroscopic assays, and data interpretation.

b. Echocardiography training will be supervised by Dr. Vahagn Ohanyan.

c. Students will participate in regular 1:1 meetings with Dr. Chen and joint meetings with Dr. Chen and Dr. Ohanyan.

d. Training schedule:

- Weeks 1–3: animal model and pharmacological treatment
- Weeks 4–6: mitochondrial biology and polarographic analysis
- Weeks 7–8: data analysis and interpretation

e. Students will attend weekly Cardiovascular Interest Group meetings and present research updates.

f. Students will participate in the summer research journal club.

g. Students will present a research poster at Summer Research Day.

2. **Protocol Availability**
   Protocols for CB-839 and BPTES treatment are established in the PI's laboratory. All required equipment and personnel are available.
3. **Research Location**
   Research activities will be conducted in RGE laboratories and the echocardiography facility in the C-building on the NEOMED campus.