Cardiovascular disease (CVD) is the leading cause of death in the United States and a growing concern worldwide. Patients who are diagnosed with cardiac disease, or suffer a myocardial infarction, commonly face protracted regimens of pharmaceuticals. Unfortunately, these treatments provide diminishing returns as they are symptomatic therapies that do not correct the underlying cause of the condition. Gene and cell replacement strategies offer long term corrective alternatives. Recent studies in the University of Washington's Heart and Muscle Mechanics (HAMM) lab show encouraging results that engineered mutations in myofilament proteins can affect Ca^{2+} responsiveness, help restore cardiac function, and may provide viable therapies for certain cardiomyopathies.[1,2] My goal is to advance the translation of this research from the lab to the clinic through *in vivo* studies that assess these variants in the whole organ.

As a researcher in the HAMM lab I pursue new treatments that will improve and save lives. We investigate the mechanisms of cardiac function and dysfunction in order to develop therapies that address underlying changes in the myocardium that result from damage or disease. This work is fundamentally concerned with the contraction of cardiac muscle, which is regulated by intracellular calcium ion concentration ($[Ca^{2+}]$). Specifically, contraction is initiated by the binding of Ca^{2+} ions to the TnC subunit of the troponin complex (Tn) on the thin filament. The interplay between Ca^{2+} sensitivity and cooperative cross-bridge binding between myosin heads and the actin filament is very important in the beat-to-beat regulation of cardiac function. Disruption of this mechanism may underlie the etiology of many conditions, as mutations associated with hypertrophic, dilated and restrictive cardiomyopathies (HCM, DCM, and RCM) cause changes in the $[Ca^{2+}]$-sensitivity of force production in cardiac muscle.[3]

Current therapeutic options for patients with HCM, DCM and RCM are limited. Pharmaceutical treatments for these forms of CVD are 'upstream' of the myofilaments, targeting intracellular $[Ca^{2+}]$ release. Significant side effects may arise from these treatments, such as incomplete muscle relaxation that leads to continued heart failure.[3] Targeting the $[Ca^{2+}]$ 'sensor', cTnC, instead of $[Ca^{2+}]$ release itself, may be more effective at treating isolated dysfunctions. In collaboration with researchers in the HAMM lab, specifically Drs. Regnier, Razumova and Korte, I study the effects of engineered cTnC variants on force generation in multicellular rat cardiac muscle preparations. The lab has developed and characterized a series of cTnC variants with single amino acid mutations that alter the Ca^{2+} responsiveness of cardiac muscle. One variant, L48Q, causes enhanced contractility at physiological $[Ca^{2+}]$ in demembranated rat cardiac muscle and shows promise as a treatment for cardiopathologies with decreased cardiac pumping capacity.[2] Additionally, L48Q cTnC improves cardiomyocyte performance following infarction.[1]

Involvement with the mechanistic testing of L48Q provides valuable experience for my examination of a new cTnC variant, L57Q. In order to characterize the steady state and kinetic parameters of the force-Ca^{2+} relationship at different sarcomere lengths, I employed passive exchange protocols to substitute native Tn with whole Tn complexes containing L57Q cTnC. Initial results from these experiments demonstrate decreased $[Ca^{2+}]$-sensitivity of cardiac tissue and suggest L57Q may be a viable option for treating HCM (which often involves increased cardiomyocyte Ca^{2+} sensitivity of contraction). Unfortunately, L57Q also lowered the maximal

[1] Korte, F.S., D. Wang, J. Dai, K. Buckley, C.E. Murry, V. Daggett, and M. Regnier. Mutations that alter cTnC Ca2+ binding affect interactions with cTnI and cardiomyocyte Ca2+ handling. *J. Muscle Research and Motility, 2010*.

[2] Kreutziger, K.L., N. Piroddi, C. Tesi, C.Poggesi, and M. Regnier. Cooperative activation and tension kinetics in cardiac muscle are strongly modulated by calcium binding kinetics of troponin C. (Accepted with revisions)

[3] Willott, R. et al, Mutations in Troponin that cause HCM, DCM, AND RCM: What can we learn about thin filament function? *J Mol Cell Cardiol* (2009).

force production of our samples.[4] I am currently conducting further mechanistic studies to elucidate how this variant affects strong myosin cross-bridge binding in cardiac activation, whether strong cross bridges can sufficiently maintain the 'open' conformation of Tm to allow full force production even with weak cTnC regulation, and if lower levels of L57Q may reduce Ca^{2+}-sensitivity without decreasing maximal force production. These experiments will provide valuable assessment of this variant and aid the future design of novel [Ca^{2+}] -desensitizing therapeutics in the HAMM lab. Experience with these increasingly independent projects motivates me to further expand my abilities through the design and completion of *in vivo* studies. Examining L57Q cTnC in a mouse model of HCM will provide clinically valuable information; however, challenges related to gene delivery must be addressed.

Genetically engineered therapeutics are currently limited by delivery mechanisms. In particular, viral vectors used to transmit genetic material are known to cause a potentially dangerous immune response in humans. Failures with viral delivery methods have previously cast doubt on the overall viability of gene therapy. In an address to Amgen scholars this summer, Dr. Robert Langer of MIT asserted that genetic therapies pose "the greatest current challenge in drug delivery". Fortunately, current advances in both viral and non-viral methods have returned gene therapy to the forefront of medical research. A recently successful mechanism, zinc finger nucleases, provides an interesting option to explore the *in vivo* therapeutic effect of the HAMM lab's cTnC variants.

Zinc finger nucleases have shown the ability to efficiently modify the genomes of a variety of model organisms through sequence-specific targeting, cleavage, and modification in locations of the genome not associated with oncogene activation.[5] I am eager to employ this technique in the HAMM lab to replace native cTnC genes with genes coding for the potential therapeutic cTnC variants L48Q and L57Q. Of particular interest is the ability of L48Q to restore function in a model of DCM and the ability of L57Q to restore function in a model of HCM. Functional changes *in vivo* will be investigated using echocardiography, which can provide information about alterations in ventricular diameter during systole and diastole, ejection fraction, and fractional shortening of the myocardium. These measurements represent important functional parameters that are commonly utilized in a clinical setting to evaluate global cardiac function. Examining the effect of engineered cTnC variants in animal models will integrate my understanding of cardiac contraction and gene modification while contributing significantly towards the clinical application of important research results generated in the HAMM lab. This project will also provide essential knowledge as I continue to explore the vast potential of gene therapy throughout my graduate education and career.

Studies at the HAMM lab and other collaborating labs increase our understanding of the mechanisms of cardiac function and show the potential for gene therapy to treat CVD. This novel research will provide cardiac patients valuable alternatives to symptomatic pharmaceuticals, surgeries, and transplant lists. Gene therapy has the potential to reduce suffering, save lives, and replace outdated, ineffective treatments. I look forward to accelerating this transition and am excited to continue work on engineered variants of cTnC which may restore proper heart function after damage or disease.

Signed:

Date:

[4] Turtle, C.W. The effect of L57Q cTnC on the Ca^{2+} and sarcomere length sensitivity of force in rat demembranted trabeculae. *University of Washington Amgen Scholars Program 2010*.

[5] Urnov, F.D. et al, Genome editing with engineered zinc finger nucleases. Nature Reviews Genetics 11, 636-646.