Regenerative Medicine Minnesota
Final Report
Due: 2/28/2018

Grant Title: Novel Cell-Free Peptide Therapeutics for Cardiac Repair and Regeneration

Grant Number: RMM 11215 TR001

Principal Investigator: John Burnett, Jr, MD

Project Timeline: 2/1/2016 - 1/31/2018

Progress to Date:

The objective of our highly translational application was to advance an innovative **cell-free peptide therapeutic strategy** with a designer peptide that was engineered by the applicant for optimizing the heart's intrinsic reparative and regenerative systems following myocardial infarction. Our long-term goal is to prevent and/or reverse post-MI HF.

Specifically, our application advanced the preclinical development of the novel designer peptide NPA7 invented by the applicant. This innovative peptide was designed to co-activate Mas and GC-A receptors representing a first-in-class multivalent peptide therapeutic agent.

Our two specific aims and progress are summarized below:

Aim 1: Establish *in vitro* the protective properties of NPA7 in suppressing apoptosis in human cardiomyocytes, promoting angiogenesis in human endothelial cells and reducing proliferation of human cardiac fibroblasts.

We have been highly successful in addressing Aim 1 with progress in all three-cell types. *In vitro* studies have validated dual pGC-A/MasR activation by NPA7 and of respective second messengers. Specifically, *in vitro* in human cardiomyocytes, NPA7 was a potent inhibitor of apoptosis. In contrast, NPA7 did not induce angiogenesis *in vitro.* In ongoing studies in human cardiac fibroblasts we validated co-presence of both the MasR and GC-A. Proliferation studies are currently underway.

Aim 2: Establish *in vivo* in a model of MI-induced permanent myocardial injury the protective actions of NPA7 in enhancing myocardial function with suppression of apoptosis, increasing angiogenesis, and inhibiting fibrosis.

We have been highly successful in accelerating our *in vitro* studies into a large animal model. The scope of Aim 2 also broadened to support our strategy of securing a preIND meeting with the FDA in 2018. Here our goal is to begin to lay the foundation for a first in human study in normal human volunteers to support a human trial of NPA7 in the post acute MI setting. Thus, to support preclinical pharmacology for a potential IND, we recently completed studies on subcutaneous delivery of NPA7, cardiorenal responses, pharmacokinetics and pharmacodynamics, peptide stability and NPA7 metabolites. Thus, plasma and urinary cGMP, cardiorenal and plasma renin responses to SQ injection 2.89 nmol/kg (10µg/kg) or to IV bolus injection 667pmol/kg (2.31µg/kg) were determined over 3 hours in normal canines (n=5 of each group) *in vivo*. *Ex vivo* we established NPA7 stability and investigated key NPA7 metabolites in canine serum using liquid chromatography-mass spectrometry (LC-MS).

In vivo, increases in plasma and urinary cGMP with SQ NPA7 were sustained up to 2 to 3 hours respectively ($p<0.05$ to baseline) while IV NPA7 was sustained only to 1 hr (Plasma cGMP T1/2 min SQ: 258±91 vs. IV: 81±7 min $p<0.05$). Deceasing of BP was mild in SQ NPA7 from 60 min to 120 min ($p<0.05$ to baseline), while IV NPA7 deceased BP within 5 min. Reductions in PCWP were more sustained with SQ NPA7 (3 hrs. $p<0.05$) vs. IV NPA7 (2 hrs. $p<0.05$) as was natriuresis (SQ NPA7: 2 hrs. $p<0.05$; IV NPA7: 30 min, p<, 0.05). SQ NPA7 suppressed plasma renin activity that was greater than IV NPA7 at 2 hrs. ($p<0.05$). NPA7 was highly stable in canine plasma (disappearance time 120 min). We also identified 2 major NPA7 metabolites NPA71-27 and NPA71-28.

These IND enabling studies establish that SQ NPA7 is rapidly absorbed and has prolonged cGMP activating, cardiorenal enhancing and renin suppressing actions in normal canines. Further NPA7 when delivered *in vivo* is safe and well tolerated. It also is stable in serum. These studies support NPA7 as a novel therapeutic strategy, which enhances cardiorenal function acutely, suppresses the renin angiotensin system, and can be safely delivered SQ. Thus, NPA7 represents a first-in-class innovative multivalent designer peptide being developed for the prevention and treatment of HF as a cell-free peptide therapeutic strategy. These studies will accelerate our strategy to move NPA7 from the laboratory into human clinical trials.

Please list any of the following that have resulted from the Minnesota Regenerative Medicine grant funding:

Publications and/or manuscripts submitted for publication: A publication is currently in review at *Circulation Research.*
Disclosures/patents: A US patent has previously been awarded #9102707 B2 on 8/21/2012.
Grant applications and/or awards: In 2017 we were awarded a second new NIH RO1 to support research related to NPA7 (RO1 HL134668: Novel Peptide Therapeutics for Cardiorenal Protection in HF; period of funding: 8/1/2017 to 7/31/2021; total award $1.3M).

Budget Update:
All funding has been spent to support the research application.

Reporting to all Minnesotans: While more people are surviving a heart attack, the heart muscle remains injured and can lead to congestive heart failure with retention of salt and water by the kidney, shortness of breath and weakness. Our drug being developed at Mayo Clinic could protect the heart as well as the kidney promoting healing of the heart and protecting the kidney after heart attack thus reducing the risk for heart failure.