AWARD NUMBER:  W81XWH-16-1-0180

TITLE:  Targeting Extracellular Histones with Novel RNA Biodrugs for the Treatment of Acute Lung Injury

PRINCIPAL INVESTIGATOR:  Paloma H Giangrande

RECIPIENT:  The University of Iowa
            Iowa City, IA 52242

REPORT DATE:  October 2017

TYPE OF REPORT:  Annual

PREPARED FOR:  U.S. Army Medical Research and Materiel Command
                Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:  Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
Targeting Extracellular Histones with Novel RNA Biodrugs for the Treatment of Acute Lung Injury

Extracellular (or circulating) histones have been proposed as the causative agent of acute lung injury (ALI). The goal of this proposal is to develop a therapeutic to neutralize (inactivate) circulating histones and prevent the morbidity and mortality associated with multiple organ dysfunction/acute respiratory distress syndrome (MODS/ARDS) and ALI that can be easily delivered in combat and field situations. To accomplish this goal, we developed novel bio-reagents (RNA aptamers) that bind to those histones known to cause MODS/ARDS and ALI but do not bind to other proteins or cells in blood. The RNA aptamers were evaluated for their ability to inhibit histone-mediate 1. cytotoxicity, 2. platelet aggregation, 3. TLR activation and 4. calcium influx. In this report, we provide evidence for the in vitro efficacy of three individual RNA aptamers (KU5, KU7 and KU9). Future efforts will focus on evaluating safety and in vivo efficacy of the aptamers in murine models of ALI. Finally, the levels of circulating histones will also be quantitated in samples from ALI patients.

Acute lung injury (ALI), acute respiratory distress syndrome (ARDS), multiple organ dysfunction syndrome, extracellular histones, circulating histones,
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>4</td>
</tr>
<tr>
<td>2. Keywords</td>
<td>4</td>
</tr>
<tr>
<td>3. Accomplishments</td>
<td>5</td>
</tr>
<tr>
<td>4. Impact</td>
<td>14</td>
</tr>
<tr>
<td>5. Changes/Problems</td>
<td>15</td>
</tr>
<tr>
<td>6. Products</td>
<td>16</td>
</tr>
<tr>
<td>7. Participants &amp; Other Collaborating Organizations</td>
<td>19</td>
</tr>
<tr>
<td>8. Special Reporting Requirements</td>
<td>22</td>
</tr>
<tr>
<td>9. Appendices</td>
<td>23</td>
</tr>
</tbody>
</table>
1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

A challenging medical problem often observed in critically ill patients is that following a severe injury or illness, even those organs not directly affected by the original problem subsequently become dysfunctional. This condition, known as multiple organ dysfunction syndrome (MODS) may be reversible, but there is no treatment to prevent it from happening and of those that develop MODS, the risk of death is 40%. The most common organ involved in MODS is the lungs (referred to as acute respiratory distress syndrome or ARDS). Trauma (blast and explosive) has obvious relevance to the military; however, other equally relevant causes of MODS/ARDS are acute lung injury (ALI) from smoke/chlorine gas inhalation, burns, radiation, influenza and severe infection. Only recently have investigators recognized that each of these various conditions are caused by damaged tissues releasing histones into the circulation. Histones normally reside in the nucleus and partner with the DNA, but when extracellular histones have toxic effects to the lungs and other organs. The goal of this proposal is to develop a therapeutic to neutralize (inactivate) circulating histones and prevent the morbidity and mortality associated with MODS/ARDS and ALI that can be easily delivered in combat and field situations. To accomplish this goal, novel bio-reagents (RNA aptamers) that will bind to histones but not to other circulating proteins or cells will be tested in human cultured cells and in mice for their ability to prevent histone-mediated toxicity and ALI. During the first year of the award, studies have successfully evaluated the effect of extracellular histones on endothelial cell calcium influx, TLR activation, cytotoxicity, and on platelet activation. Furthermore, additional characterization of RNA aptamers have been completed and have been found to attenuate cell death and platelet aggregation in vitro. In addition, histones have been measured in serum obtained from patients with lung injury. Since histones are highly conserved across species from yeast to humans, the bio-reagents developed and validated in this proposal can be immediately tested in preclinical animal models and human clinical trials. Furthermore, as a drug to prevent the development of MODS/ARDS and ALI in high risk patients, these bio-reagents have significant advantages as compared to other possible therapeutics because they are very stable and not as susceptible to fluctuations in temperature, do not require special handling conditions, do not cause allergic responses, and will be easy to deliver. In addition to having relevance to military situations, the therapeutics derived from this application would have wide benefit to the general population in reducing morbidity and mortality associated with MODS/ARDS and ALI.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Acute lung injury (ALI), acute respiratory distress syndrome (ARDS), multiple organ dysfunction syndrome, extracellular histones, circulating histones, histones
3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

**What were the major goals of the project?**

<table>
<thead>
<tr>
<th>Goals of project as stated in the approved SOW:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific Aim 1: In vitro characterization and optimization of RNA aptamers that selectively bind to human histone.</strong></td>
</tr>
<tr>
<td><strong>Major Task 1: Local IRB/IACUC Approval</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Local IRB approval</td>
</tr>
<tr>
<td>Local IACUC approval</td>
</tr>
<tr>
<td><strong>Milestone achieved: regulatory approvals obtained</strong></td>
</tr>
<tr>
<td><strong>Major Task 2: In vitro - functional efficacy of aptamers</strong></td>
</tr>
<tr>
<td>Measurement of calcium influx by fura-2</td>
</tr>
<tr>
<td>Cell line used: human pulmonary microvascular endothelial cells; Clonetics</td>
</tr>
<tr>
<td>Measurement of TLR activation by cytokine assay kit</td>
</tr>
<tr>
<td>Cell lines used: human pulmonary microvascular endothelial cells; Clonetics</td>
</tr>
<tr>
<td>Detection of cell toxicity</td>
</tr>
<tr>
<td>Cell lines used: human pulmonary microvascular endothelial cells; Clonetics</td>
</tr>
<tr>
<td>Measure platelet thrombi formation Human platelets (healthy donors)</td>
</tr>
<tr>
<td>Measure platelet surface marker expression Human platelets (healthy donors)</td>
</tr>
<tr>
<td>Ex vivo experiment using blood from patients with ALI</td>
</tr>
<tr>
<td><strong>Milestones Achieved: Determination of efficacy of aptamers on histone-mediated toxicity in cultured cells and human platelets.</strong></td>
</tr>
</tbody>
</table>
Specific Aim 2: Evaluate efficacy and safety of histone-specific RNA aptamers in vivo

<table>
<thead>
<tr>
<th>Major Task 3: Evaluation of efficacy in inhalation injury model (chlorine inhalation and smoke inhalation models)</th>
<th>Timeline</th>
<th>Site 1 (Initiating PI)</th>
<th>Site 2 (Partnering PI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessment of minimal effective dose (MED) C57/BL6 mice (~10 wks of age): 10 mice per dose (up to 5 doses) per treatment (3 total) per mouse model = up to 300 mice.</td>
<td>Months</td>
<td>Team</td>
<td>Team</td>
</tr>
<tr>
<td></td>
<td>18-26</td>
<td>Dr. Miller Dr. Tighe Dr. Gunn</td>
<td>Dr. Giangrande Dr. Smith</td>
</tr>
<tr>
<td>Assessment of alveolar permeability and inflammation by bronchoalveolar lavage</td>
<td>18-26</td>
<td>Dr. Miller Dr. Tighe Dr. Gunn</td>
<td></td>
</tr>
<tr>
<td>Lung histology by histopathologic staining and analysis</td>
<td>26-30</td>
<td>Dr. Miller Dr. Tighe Dr. Gunn</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Major Task 4: Evaluation of efficacy in influenza lung injury model</th>
<th>Timeline</th>
<th>Team</th>
<th>Team</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessment of minimal effective dose (MED) C57/BL6 mice (~10 wks of age): 10 mice per dose (up to 5 doses) per treatment (3 total) per mouse model = up to 150 mice.</td>
<td>Months</td>
<td>Team</td>
<td>Team</td>
</tr>
<tr>
<td></td>
<td>18-26</td>
<td>Dr. Miller Dr. Tighe</td>
<td>Dr. Giangrande Dr. Smith</td>
</tr>
<tr>
<td>Assessment of alveolar permeability and inflammation by bronchoalveolar lavage</td>
<td>18-26</td>
<td>Dr. Miller Dr. Tighe</td>
<td>Dr. Giangrande Dr. Comellas</td>
</tr>
<tr>
<td>Lung histology by histopathologic staining and analysis</td>
<td>26-30</td>
<td>Dr. Miller Dr. Tighe</td>
<td>Dr. Giangrande Dr. Comellas</td>
</tr>
</tbody>
</table>

Milestone(s) Achieved: Completion of assessment of efficacy of aptamers on ALI in mice

<table>
<thead>
<tr>
<th>Major Task 5: Evaluation of safety</th>
<th>Timeline</th>
<th>Team</th>
<th>Team</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rising dose and repeated dose toxicology studies to establish a no observed adverse event level (NOAEL) NOAEL will be determined for lead aptamer from in vitro and in vivo studies. Immune-competent BALB/c mice (~10 wks of age): An NOAEL will be declared at the level at which 0 out of 6 mice experience an adverse effect. Up to 60 mice are anticipated.</td>
<td>Months</td>
<td>Team</td>
<td>Team</td>
</tr>
<tr>
<td></td>
<td>30-36</td>
<td>Dr. Miller</td>
<td>Dr. Giangrande Dr. Smith</td>
</tr>
<tr>
<td>Assessment of potential immunostimulation in humanized mice</td>
<td>30-36</td>
<td>Dr. Miller</td>
<td>Dr. Giangrande</td>
</tr>
</tbody>
</table>

Milestone Achieved: Completion of assessment of safety of aptamers on histone-mediated toxicity in mice
What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

The following major activities under specific aim 1 were accomplished during the first reporting period (months 1-12):

### Specific Aim 1: In vitro characterization and optimization of RNA aptamers that selectively bind to human histone.

<table>
<thead>
<tr>
<th>Major Task 1: Local IRB/IACUC Approval</th>
<th>Timeline</th>
<th>Site 1 (Initiating PI)</th>
<th>Site 2 (Partnering PI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local IRB approval</td>
<td>Months</td>
<td>Team</td>
<td>Team</td>
</tr>
<tr>
<td>Local IRB approval</td>
<td>1</td>
<td>Dr. Miller</td>
<td>Dr. Giangrande</td>
</tr>
<tr>
<td>Local IACUC approval</td>
<td>1</td>
<td>Dr. Miller</td>
<td>Dr. Giangrande</td>
</tr>
</tbody>
</table>

**Milestone achieved:** regulatory approvals obtained

<table>
<thead>
<tr>
<th>Major Task 2: In vitro - functional efficacy of aptamers</th>
<th>Timeline</th>
<th>Team</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement of calcium influx by fura-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell line used: human pulmonary microvascular endothelial cells; Clonetics</td>
<td>1-12</td>
<td>Dr. Miller Dr. Giangrande</td>
</tr>
<tr>
<td>Measurement of TLR activation by cytokine assay kit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell lines used: human pulmonary microvascular endothelial cells; Clonetics</td>
<td>1-12</td>
<td>Dr. Giangrande</td>
</tr>
<tr>
<td>Detection of cell toxicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell lines used: human pulmonary microvascular endothelial cells; Clonetics</td>
<td>1-12</td>
<td>Dr. Miller Dr. Giangrande</td>
</tr>
<tr>
<td>Measure platelet thrombi formation Human platelets (healthy donors)</td>
<td>6-18</td>
<td>Dr. Miller Dr. Giangrande Dr. Smith Dr. Dayal</td>
</tr>
</tbody>
</table>

**Major Task 1 – 100% completed**
Local IRB and IACUC regulatory approvals were obtained from both sites.

**Major Task 2 – 75% completed**
Measurement of calcium influx (Fig 1). These studies were performed at Duke University (Site 1) by Dr. Miller and his group. The goal of these studies is to examine the effect of histones on endothelial calcium levels. Using fura 2-AM as an indicator of intracellular calcium, we found that low concentrations of histones cause the release of intracellular calcium stores (Fig 1A); whereas at high concentrations of histones, an early influx of calcium is from extracellular source and a later increase in calcium from intracellular stores (Fig 1B-D, n=9-10 cells per bar). Over the next couple of months, we will further explore the mechanisms of calcium influx and will test the ability of RNA aptamers to neutralize histones and protect from histone-mediated calcium influx. These experiments are waiting for the chemical synthesis of the histone aptamers from TriLink Biotechnologies with anticipated completion within the next month.
Figure 1. Histone-mediated calcium influx. (A) Cultured human umbilical vein endothelial cell line (EA.hy926) were incubated in fura 2-AM to establish a baseline intracellular calcium signal (relative ratio of fluorescence at 340/380) and 12.5 μM histones added at time “0”. Blue line is in the presence and red line in the absence of extracellular calcium. (B) Cultured human pulmonary microvascular endothelial cells were incubated in fura 2-AM to establish a baseline intracellular calcium signal (relative ratio of fluorescence at 340/380) and 200 μM histones added at time “0”. Blue line is in the presence and red line in the absence of extracellular calcium. Summary data of the maximum change in intracellular calcium is shown at the early peak (C) and the late peak (D).
Measurement of TLR activation by cytokine assay kit (Fig. 2). These studies were performed at The University of Iowa (Site 2) by Dr. Giangrande and her group. The objective of these studies was to confirm that histones result in TLR activation and to determine whether the RNA aptamers reverse histone-mediated TLR activation. We have shown that calf thymus histone treatment of cells results in activation of IL-6, cytokine whose expression is upregulated upon TLR activation. We are in the process of performing these experiments with the histone aptamers. These experiments have been on hold due to a delay in the chemical synthesis of the histone aptamers from TriLink Biotechnologies. We anticipate receipt of the order during the next 2 weeks and will be able to perform these studies.

Fig 2. Effect of histone aptamers on histone-mediated TLR activation. (A) Interleukin-6 (IL-6) levels were used as a measure of TLR activation. EaHy926 cells were seeded at 80% confluence in a 96 well plate. The cells were treated for 16h with varying concentrations of calf thymus histones (ranging from 0 10 100 μg/mL). After 16 hours cell supernatants were collected and processed using the IL-6 ELISA kit from Abcam according to manufacturer’s recommendations. (B) Ea.Hy926 cells were seeded as in part A. The cells were treated for 16 hours with either vehicle, aptamer alone (50 μg/mL), histone alone (50 μg/mL) or histone plus aptamer (at varying histone: aptamer molar ratios - 1:1, 1:0.5, 1:0.25, 1:0.125, 1:0.0625). After 16 hours, the supernatants were collected and processed as in part A above.
Detection of cell toxicity (Fig. 3). These studies were performed at The University of Iowa (Site 2) by Dr. Giangrande and her group. The objective of these studies was to determine the effect of histones on viability of human pulmonary microvascular endothelial cells and to assess the effect of the RNA aptamers on histone-mediated cytotoxicity. Human endothelial cells were incubated with calf thymus histones alone (no aptamer) or in the presence of the therapeutic RNA aptamers KU5, KU7 or KU9. We observed that when administered to a human endothelial cell line (EA.hy926), calf thymus histones cause a dose-dependent cell death (Fig. 3A; no aptamer, inverted grey triangle). Aptamers (KU5, KU7 and KU9) that specifically bind histones, have a dose-dependent protective effect in neutralizing histone-induced cytotoxicity (Fig. 3A and B).

**Fig. 3.** Aptamer reversal of histone-mediated cytotoxicity of endothelial cells. A) Dose response of histones at constant aptamers concentrations: Cells were treated with 1.2uM of each aptamer and a decreasing amount of calf thymus histones for 24h. Cell viability was determined using the MTS assay. All samples had 4 biological replicates except the 0 ug/ml of untreated cells which had 32 samples. B) Dose response of aptamer at fixed histone concentration. Cells were treated with 180ug/mL of calf thymus histone and a decreasing amount of each aptamer for 24h. Cell viability was assessed as in part A.

Measurement of platelet thrombin formation using platelets derived from healthy donors (Fig. 4). These studies were performed at The University of Iowa (Site 2) by Dr. Giangrande and her group. The objective of these studies was to show that the RNA aptamers can inhibit histone-mediated platelet aggregation. The release of histones from dying cells is associated with microvascular thrombosis and tissue ischemia. Histone H4 and, to a lesser extent H3, are responsible for directly inducing aggregation of human platelets. In preliminary data, we show that histone H4 and H3 induce pronounced platelet aggregation, which can be inhibited by the addition of aptamers (from a non-selected aptamer library) (Fig. 4A). Importantly, a more pronounced inhibition of platelet aggregation is observed with selected aptamer pools (round 3 pool for H4 and round 5 pool for H3). In contrast, the selected aptamer pools had no effect on collagen-mediated platelet aggregation (negative control), and heparin reverses histone mediated platelet aggregation (positive control) (Fig. 4B). Aptamer inhibition of histone-mediated platelet aggregation was achieved with a 1:4 aptamer to histone molar ratio (Fig. 4C – data shown for aptamer KU7). Together, these data confirm that the aptamers can prevent the functional effect of histones in vitro and provide the rationale for proposing that these aptamers have the potential to attenuate histone-mediated injury in vivo.
Additional Achievements ahead of schedule:

Major Task 2 – 50% completed
Ex vivo experiment using blood from intensive care unit (ICU) patients with ALI (Fig. 5).
These studies were completed with collaboration between the two sites, the assay performed at The University of Iowa (Site 2) by Dr. Giangrande and her group. The objective of these studies was to show that histone levels are elevated in serum from patients with ALI. Serum from patients with sepsis with and without evidence of lung involvement was evaluated for circulating histones using immunostaining for histone H3 (histone implicated in ALI). Further analysis will evaluate the relationship of serum histone levels with severity of illness.
Major Task 3 – 10% completed.

1. Evaluation of efficacy in smoke inhalation injury model. These studies were completed at Duke University (Site 1) by Dr. Miller and his colleagues. Experiments were begun to examine the role of extracellular histones in acute lung injury (ALI) by smoke inhalation. The particulate matter from smoldering or flammable wood smoke was delivered intra-tracheal in mice and 24 hours later tissue and blood harvested. As shown in the table below, the flammable wood smoke induced a more robust inflammatory response in the bronchoaveolar lavage (BAL) fluid as compared to smoldering smoke. The BAL will be analyzed for cytokines and histone levels. The lung was saline perfused in vivo, inflated prior to fixation, and will be examined for pathologic changes and immunostained for histones. Future studies will evaluate the ability of RNA aptamers to attenuated lung inflammation and injury.

<table>
<thead>
<tr>
<th></th>
<th>Cells/ml</th>
<th>Macs/ml</th>
<th>% Macs</th>
<th>Neut/ml</th>
<th>% Neut</th>
<th>Eos/ml</th>
<th>% Eos</th>
<th>Lymph/ml</th>
<th>% Lymph</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>15640</td>
<td>15249</td>
<td>97.5%</td>
<td>146</td>
<td>0.9%</td>
<td>0</td>
<td>0.0%</td>
<td>245</td>
<td>1.6%</td>
</tr>
<tr>
<td>WS smold</td>
<td>14105</td>
<td>12973</td>
<td>92.0%</td>
<td>1006</td>
<td>7.1%</td>
<td>0</td>
<td>0.0%</td>
<td>126</td>
<td>0.9%</td>
</tr>
<tr>
<td>WS flam</td>
<td>30019</td>
<td>22337</td>
<td>74.4%</td>
<td>5674</td>
<td>18.9%</td>
<td>272</td>
<td>0.9%</td>
<td>1737</td>
<td>5.8%</td>
</tr>
</tbody>
</table>

Table. Cellular content of BAL after smoke inhalation injury in mice. Mice were administered intra-tracheal PBS (control), smoldering wood smoke (WS smold), or flammable wood smoke (WS flam) particulate matter and BAL fluid evaluated 24 hours later. N=6 mice per group.
What opportunities for training and professional development has the project provided?

Kevin Urak (graduate student) – Kevin meets with Dr. Giangrande on a daily to weekly basis to design and troubleshoot experiments and interpret data. Dr. Giangrande and Kevin also have regular (monthly) skype calls with Dr. Miller and his group to troubleshoot and discuss progress made. In addition, Kevin has had the opportunity to present the work accomplished under this project at weekly lab meetings/data clubs and symposiums at the University of Iowa (ex. 4th Annual Abboud Cardiovascular Research Center (ACRC) Symposium) and at several scientific conferences outside of Iowa including: the American Society for Gene and Cell Therapy (ASGCT – oral presentation), Oligonucleotide Therapeutics Society (OTS – oral presentation) and RNA Consortium (poster). Abstracts and poster submitted to the scientific conferences have been included under Appendix.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

The goals for reporting period 2 include:

1. Complete platelet aggregation and activation studies. These studies will be performed by Dr. Giangrande (Site 2 PI) and her group. These studies are being performed in collaboration with Dr. Dayal at the University of Iowa. Platelets from healthy donors will be obtained. Platelets will be treated with or without histones and RNA aptamers. We will determine platelet aggregation times and platelet surface marker expression as a measure of platelet activation.

2. Complete studies evaluating the efficacy of RNA aptamers to protect pulmonary endothelial and epithelial cells from histone-mediated injury (calcium influx, TLR activation, apoptosis).

3. Perform quantification of histone levels in blood from patients with ALI. These studies will be performed by Dr. Giangrande (Site 2 PI) and her group at the University of Iowa. Dr. Giangrande’s group is currently developing several methods (ex. ELISA, immune blot and aptamer filter binding assay) to enable the robust detection of histones in human plasma/serum. Patient samples will be provided by Dr. Comellas (University of Iowa).

4. Begin evaluation of efficacy of RNA aptamers in murine models of inhalation (chlorine inhalation and smoke inhalation). These studies will be performed by Dr. Miller (Site 1 PI) at Duke University. Dr. Miller is working in collaboration with Dr. Tighe and Dr. Gunn to set up the animal models and for sample and data collection. Mouse organs, serum and bronchoalveolar lavage fluid (BAL) will be analyzed at Duke and some sent to Dr. Giangrande (Site 2 PI) for further analysis.

5. Begin evaluation of efficacy of RNA aptamers in murine influenza lung injury model. These studies will be performed by Dr. Giangrande (Site 2 PI) and her group at the University of Iowa. Dr. Giangrande is working with Dr. Comellas (University of Iowa) and Dr. Kevin Legge (collaborator - University of Iowa) to set up the animal model of influenza. Mouse organs, serum and bronchoalveolar lavage fluid (BALF) will be collected and processed for histone levels and organ pathology.
4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report at this time. However, if this study is successful then the RNA aptamers can be immediately tested in preclinical animal models and human clinical trials as a drug to prevent the development of MODS/ARDS and ALI in high risk patients. These bio-reagents have significant advantages as compared to other possible therapeutics because they are stable and not as susceptible to fluctuations in temperature, do not require special handling conditions, do not cause allergic responses, and will be easy to deliver.

What was the impact on other disciplines?

Nothing to report. However, since histones are responsible for multiple diverse causes of MODS/ARDS, including trauma, burns, major surgery, pancreatitis, sepsis, ischemia/reperfusion, etc., if this study is successful than the findings will have broad application to many other disciplines.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.
5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

**Changes in approach and reasons for change**
*Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

| Nothing to report |

**Actual or anticipated problems or delays and actions or plans to resolve them**
*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

| Nothing to report |

**Changes that had a significant impact on expenditures**
*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

| A revised budget for Dr. Giangrande was approved by Jennifer E. Hayden (Contract Specialist) on April 26, 2017 to purchase a new equipment (PCR machine) to replace her old equipment that had stopped working. The new PCR machine was needed to perform the TLR activation studies described above. This change did not result in a significant impact on expenditures and all objectives were met. |

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

| Nothing to report |

**Significant changes in use or care of human subjects**

| Nothing to report |
Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

• Publications, conference papers, and presentations
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

  Nothing to report

  Books or other non-periodical, one-time publications. Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

  Nothing to report

  Other publications, conference papers, and presentations. Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year...
Conference presentations (oral)

1. Oligonucleotide Therapeutics Society (OTS), September 25-28, 2016, Montreal, Quebec, Canada. Neutralization of Extracellular Histones with Nucleic Acid Aptamers for the Treatment of Critical Illness. **Kevin Urak**, MS, University of Iowa, Iowa City, IA


- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the
understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- data or databases;
- biospecimen collections;
- audio or video products;
- software;
- models;
- educational aids or curricula;
- instruments or equipment;
- research material (e.g., Germplasm; cell lines, DNA probes, animal models);
- clinical interventions;
- new business creation; and
- other.
7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change.”

<table>
<thead>
<tr>
<th>Name:</th>
<th>Paloma H Giangrande, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role:</td>
<td>PI Site 2</td>
</tr>
<tr>
<td>Nearest person month worked:</td>
<td>4.8</td>
</tr>
<tr>
<td>Contribution to Project:</td>
<td>Dr. Giangrande was responsible for overseeing the aptamer in vitro studies performed in specific aim 1.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name:</th>
<th>Brian J Smith, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role:</td>
<td>Biostatistician Site 2</td>
</tr>
<tr>
<td>Nearest person month worked:</td>
<td>0.3</td>
</tr>
<tr>
<td>Contribution to Project:</td>
<td>Dr. Smith was responsible for developing the statistical analysis for this proposal. He has performed the data management and statistical programming tasks of the analysis for all in vitro experiments performed under period 1.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name:</th>
<th>Li-Hsien Lin, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role:</td>
<td>Associate Research Scientist Site 2</td>
</tr>
<tr>
<td>Nearest person month worked:</td>
<td>12</td>
</tr>
<tr>
<td>Contribution to Project:</td>
<td>Dr. Lin has assisted Kevin on the in vitro studies performed under this project period. She is also working towards generating data for the proposed PK/PD and safety studies (project periods 2 and 3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name:</th>
<th>Kevin Urak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role:</td>
<td>Graduate student Site 2</td>
</tr>
<tr>
<td>Nearest person month worked:</td>
<td>4 (effort only)</td>
</tr>
<tr>
<td>Contribution to Project:</td>
<td>Kevin was responsible for performing the aptamer selections and generating all in vitro data under project period 1.</td>
</tr>
<tr>
<td>Funding Support:</td>
<td>Kevin Urak has received an AHA fellowship which provides full salary support.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name:</th>
<th>Giselle Blanco</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role:</td>
<td>research Assistant Site 2</td>
</tr>
<tr>
<td>Nearest person month worked:</td>
<td>12</td>
</tr>
<tr>
<td>Contribution to Project:</td>
<td>Giselle was responsible for assisting Kevin with the in vitro studies performed under specific aim 1. Specifically, she generated the data pertaining to the TLR studies.</td>
</tr>
</tbody>
</table>
Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed. Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner’s contribution to the project (identify one or more)
• Financial support;
• In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
• Facilities (e.g., project staff use the partner’s facilities for project activities);
• Collaboration (e.g., partner’s staff work with project staff on the project);
• Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and
• Other.

Nothing to report
8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

See attached files: 3 abstracts and 1 poster

Abstracts submitted to scientific conferences/symposiums
1. Oligonucleotide Therapeutics Society (OTS), September 25-28, 2016, Montreal, Quebec, Canada. Neutralization of Extracellular Histones with Nucleic Acid Aptamers for the Treatment of Critical Illness. Kevin Urak, MS, University of Iowa, Iowa City, IA (oral)


3. 4th Annual Abboud Cardiovascular Research Center (ACRC) Symposium, March 30, 2017, University of Iowa. Treatment of myocardial depression in sepsis by neutralization of extracellular histones with nucleic acid aptamers. Kevin Urak, MS, University of Iowa, Iowa City IA. USA (poster)

Posters presented at scientific conferences/symposiums
1. 4th Annual Abboud Cardiovascular Research Center (ACRC) Symposium, March 30, 2017, University of Iowa. Treatment of myocardial depression in sepsis by neutralization of extracellular histones with nucleic acid aptamers. Kevin Urak, MS, University of Iowa, Iowa City IA. USA (poster)

2. 11th Annual RNA Consortium, RNA Consortium, Duarte CA, May 5-6, 2017. Treatment of myocardial depression in sepsis by neutralization of extracellular histones with nucleic acid aptamers. Kevin Urak, MS, University of Iowa, Iowa City IA. USA (poster)
Treatment of myocardial depression in sepsis by neutralization of extracellular histones with nucleic acid aptamers

Kevin Urak1,2, Ofonime Udofot1, Giselle Blanco1, Li-Hsien Lin1, Francis Miller Jr.3,4, Paloma Giangrande1,2,5,6,7

1Department of Internal Medicine, University of Iowa, Iowa City, IA, 52242, USA
2Molecular & Cellular Biology Program, University of Iowa, Iowa City, IA, 52242, USA
3Department of Medicine, Duke University, Durham, NC, 27708, USA
4Department of Internal Medicine, Veterans Affairs Medical Center, Durham, NC, 27708, USA
5Department of Radiation Oncology University of Iowa, Iowa City, IA, 52242, USA
6Medical Scientist Training Program, University of Iowa, Iowa City, IA 52242, USA
7Abboud Cardiovascular Research Center, University of Iowa, Iowa City, IA 52242, USA

Sepsis is the leading cause of morbidity and mortality in noncoronary intensive care units in the western world. Septic patients often develop myocardial dysfunction, leading to a phenomenon known as myocardial depression in sepsis (MDIS). This is mediated by the release of histones into the extracellular space by apoptotic and necrotic cells, and/or neutrophil extracellular traps (NETs). In this study, we have identified RNA aptamers that bind with high affinity and specificity to those histones implicated in MDIS. We employed Systemic Evolution of Ligands by Exponential Enrichment (SELEX) technology to identify RNA aptamers that bind with high affinity and specificity to those histones (H3/H4) implicated in MDIS. Aptamer toxicity was assessed both in vitro (lung-derived endothelial and epithelial cells) and in vivo (mouse model of multiple organ dysfunction), and its reversal effects on cytotoxicity and platelet aggregation mediated by histones was evaluated. We identified RNA aptamers that were able to bind with high affinity (low nM- pM range) and specificity to those histones (H3/H4) implicated in MDIS, but not to other proteins present in blood or on cells. We confirmed that aptamers reversed the platelet aggregation and cytotoxicity induced by the H3/H4 histones. Currently, we are evaluating the efficacy/safety of these RNA bio-drugs in cardiomyocytes in culture and in established murine models of sepsis in protecting it from myocardial dysfunction. In conclusion, we present a robust preclinical data on a novel class of therapeutics against histones that may be potentially effective in the treatment of septic patients with MDIS.

4th Annual Abboud Cardiovascular Research Center (ACRC) Symposium Poster Session 2017, Iowa City IA. USA

Location:
University of Iowa
Medical Education Research Facility
Prem Sahai Auditorium
375 Newton Road, Iowa City, Iowa

Date & Time:
8:00 am to 5:00 pm
Sepsis is the leading cause of morbidity and mortality in noncoronary intensive care units in the western world. Sepsis is a systemic inflammatory response syndrome (SIRS) that is triggered by a wide range of microorganisms, leading to a phenomenon known as multiple organ dysfunction syndrome (MODS). The increasing incidence of sepsis is associated with an increased incidence of MODS. In the United States, more than 1.7 million cases of sepsis occur annually, with more than 250,000 deaths. Mortality rates remain high at 25-35%, despite advances in medical care.

There is no single drug to treat MODS, and most treatments are directed at treating the underlying pathologies. One approach is to target the initial events of MODS, such as inflammation, oxidative stress, and endothelial dysfunction. The focus of this study was to develop a new strategy to target MODS by developing RNA aptamers that bind with high affinity and specificity to histones implicated in MODS.

Aptamers are short, single-stranded DNA or RNA molecules that can be selected in vitro for the ability to bind to a target molecule. They are rapidly evolving as a new class of therapeutic agents, with applications ranging from drug delivery to drug discovery. In this study, we used SELEX (Systematic Evolution of Ligands by EXponential Enrichment) to select RNA aptamers that bind with high affinity and specificity to histones implicated in MODS.

We employed SELEX to identify RNA aptamers that bind with high affinity and specificity to histones (H3/H4) implicated in MODS. We used Systemic Evolution of Ligands by Exponential Enrichment (SELEX) technology to identify RNA aptamers that bind with high affinity and specificity to those histones (H3/H4) implicated in MODS. Aptamer toxicity was assessed both in vitro (lung-derived endothelial and epithelial cells) and in vivo (mouse model of pulmonary organ dysfunction), and its reversal effects on cytokinetics and platelet aggregation mediated by histones were evaluated. We identified RNA aptamers that were able to bind with high affinity (low nM-pM range) and specificity to those histones (H3/H4) implicated in MODS but not to other proteins present in blood or on cells. We confirmed that aptamers reversed the platelet aggregation and cytokinetics induced by the H3/H4 histones. Currently, we are evaluating the efficacy/safety of these RNA bio-drugs in cardiomyocytes in culture and in established murine models of sepsis in protecting it from myocardial dysfunction. In conclusion, we present a robust preclinical data on a novel class of therapeutics against histones that may be potentially effective in the treatment of septic patients with MODS.

**Figure 1. Aptamer Inhibitors of Extracellular Histones for the Treatment of Critical Illness**

**Figure 2. Protein-Based SELEX**

**Figure 3. Selected RNA Aptamers Specificity, Sensitivity, and Stability**

**Figure 4. RNA Aptamers Inhibit Histone-Mediated Cytotoxicity**

**Figure 5. RNA Aptamers Inhibit Histone-Mediated Platelet Aggregation**

**Future Directions**

1. Determine ability of selected H3 and H4 aptamers to inhibit histone-mediated platelet activation and translocation.
2. Determine efficacy of selected aptamers to inhibit extracellular histone cytotoxicity in human and murine cardiomyocytes.
3. Establish clinically relevant in vivo murine models of histone-mediated illness or trauma such as ischemia and reperfusion to determine efficacy of selected aptamers in vivo.
4. Evaluating the efficacy and safety of these RNA bio-drugs in cardiomyocytes in culture and in established murine models of sepsis in protecting it from myocardial dysfunction.

**Acknowledgements**

Funding

- Carver Collaborative Pilot Grant 2015
- OVPRED Internal Funding Initiative 2015
- DOD

Collaborators

- Steve Lentz
- Sanjana Dayal
- Mary Wilson
- Yani Chen

**References**

1. University of Iowa, Iowa City IA, 52246, USA
2. Duke University School of Medicine and Durham Veterans Affairs Medical Center, Durham, NC 27710, USA
73 - Treatment of Sepsis by Neutralization of Extracellular Histones with Nucleic Acid Aptamers

May 10, 2017, 4:30 - 4:45 PM

Maryland ABC

Keywords
Aptamers, Oligonucleotide-Based Therapies, Infectious Diseases, Sepsis

Author/Speaker
Kevin T. Urak¹, Francis J. Miller², Paloma Giangrande¹

¹Internal Medicine, University of Iowa, Iowa City, IA, ²Department of Medicine, Duke University, Durham, NC

Abstract/Presentation Description
Sepsis is the leading cause of morbidity and mortality in noncoronary healthcare units in the Western world. Septic patients often develop myocardial dysfunction, coagulation abnormalities, and increased endothelial permselectivity leading to multiple organ dysfunction syndrome (MODS) and acute respiratory distress syndrome (ARDS). Recent evidence suggests that the molecular mechanism responsible for MODS/ARDS associated with sepsis involves extracellular histones. Histones are normally present in the nucleus of eukaryotic cells. However, apoptotic and necrotic cells, and/or neutrophil extracellular traps (NETs), release histones into the extracellular space. Once in the extracellular fluid, histones activate toll-like receptor (TLR) pathways and increase cell influx, resulting in platelet aggregation, endothelial cell activation, and cytokine release. This self-propagating tissue injury is a significant contributor to development of MODS/ARDS, for which there is currently no treatment other than supportive care and a mortality rate approaching 40%. We hypothesized that neutralization of extracellular histones with nucleic acid aptamers (RNA molecules) can prevent the morbidity and mortality associated with sepsis. We employed Systemic Evolution of Ligands by Exponential Enrichment technology to identify RNA aptamers that bind with high affinity (low nM range) and specificity to those histones (H3 and H4) known to cause MTE. We confirmed that H3/H4 induce pronounced platelet aggregation, which can be inhibited by the selected RNA aptamers. Furthermore, we demonstrated that histone-induced cytotoxicity can be reversed by treatment with the RNA aptamers both in vitro (lung-derived endothelial and epithelial cells) and in a mouse model of MODS/ARDS. Current efforts are focused on evaluating the efficacy and safety of these RNA bio-drugs in other established murine models of sepsis (e.g., cecal ligation and puncture). In conclusion, we present robust preclinical data on a novel class of therapeutics against circulating histones that may be potentially effective in a common clinical condition with high morbidity, mortality, and expense.
Treatment of Sepsis by Neutralization of Extracellular Histones with Nuc... septic patients.
Neutralization of extracellular histones with nucleic acid aptamers for the treatment of critical illness

Kevin Urak\textsuperscript{1,3}, Francis Miller Jr.\textsuperscript{2}, Paloma Giangrande\textsuperscript{1,3,4}

Affiliation(s)
\textsuperscript{1}Department of Internal Medicine, \textsuperscript{3}Molecular & Cellular Biology Program, \textsuperscript{4}Department of Radiation Oncology University of Iowa, Iowa City, IA, USA 52242, USA

Multiple organ dysfunction syndrome (MODS) is an insidious and life threatening sequelae in patients suffering major trauma or illness. With prompt care patients with major trauma can survive the initial injury, but soon after organs not directly affected by the original injury or illness may become dysfunctional. Breathing problems will develop that require placement on a ventilator, the kidneys will stop working requiring dialysis, the liver will not function normally, and the patient will bleed from every orifice. Coordinated efforts in the intensive care unit may reverse MODS at great cost, but there is currently no treatment to prevent MODS. Of those that develop MODS (200,000 case/year in the US alone), the risk of death is 40%. The most common organ involved in MODS is the lung (referred to as acute respiratory distress syndrome or ARDS). Trauma, smoke inhalation, burns, radiation, severe infection and blood transfusions can each cause ARDS and lead to acute lung injury. Only recently have investigators recognized that there is a common element to these conditions: damaged tissues releasing histones into the circulation. Histones are basic proteins found in chromatin. They normally reside in the nucleus of the cell and partner with DNA. However, when released from dead cells, histones have toxic effects on the lungs and other organs. We hypothesized that neutralization of extracellular histones with nucleic acid aptamers can prevent the morbidity and mortality associated with MODS/ARDS. We have employed a state-of-the art technology available in our laboratory to identify RNA aptamer bio-reagents that bind with high affinity (low nM - pM range) and specificity to those histones (H3 and H4) known to cause MODS/ARDS but not to other circulating proteins or cells. In preliminary data, we show that histones H3/H4 induce pronounced platelet aggregation, which can be inhibited with the addition of the selected RNA aptamers. Ongoing experiments are evaluating (1) the ability of the histone RNA aptamers to prevent toxicity of lung-derived endothelial and epithelial cells and (2) the efficacy and safety of these bio-reagents in established murine models of MODS/ARDS (e.g. inhalation lung injury, transfusion-related acute lung injury). In conclusion, this work will establish a paradigm change in the treatment of critically ill patients by identifying novel therapeutic bio-reagents potentially effective in a wide-variety of common clinical conditions with high degree of morbidity, mortality and expense and for which there is currently no effective treatment.

Kevin Thomas Urak (MS)
Graduate Research Assistant
University of Iowa
375 Newton rd.
Iowa City, Iowa
United States
Sepsis is the leading cause of morbidity and mortality in noncoronary intensive care units in the western world. Sepsic patients often develop myocardial dysfunction, leading to a phenomenon known as myocardial depression in sepsis (MDIS). This is mediated by the release of histones into the extracellular space by apoptotic and necrotic cells, and/or neutrophil extracellular traps (NETs). In this study, we have identified RNA aptamers that bind with high affinity and specificity to those histones implicated in MDIS. Apteran toxicity was assessed both in vitro (lung-derived endothelial and epithelial cells) and in vivo (mouse model of multiple organ dysfunction), and its reversal effects on cytokotoxicity and platelet aggregation mediated by histones were evaluated. We identified RNA aptamers that were able to bind with high affinity (low nM-PM range) and specificity to those histones (H3/H4) implicated in MDIS but not to other proteins present in blood or on cells. We confirmed that aptamers reversed the platelet aggregation and cytokotoxicity induced by the H3/H4 histones. Currently, we are evaluating the efficacy/safety of these RNA bio-drugs in cardiomyocytes in culture and in established murine models of sepsis in protecting it from myocardial dysfunction. In conclusion, we present a robust preclinical data on a novel class of therapeutics against histones that may be potentially effective in the treatment of septic patients with MDIS.

Future Directions
1. Determine ability of selected H4 and H3 aptamers to inhibit histone-mediated platelet activation and translocation.
2. Determine efficacy of selected aptamers to inhibit extracellular histone cytotoxicity in human and murine cardiac cells.
3. Establish clinically relevant in vivo murine models of histone-mediated illness or trauma such as ischemia and reperfusion to determine efficacy of selected aptamers.
4. Evaluating the efficacy and safety of these RNA bio-drugs in cardiomyocytes in culture and in established murine models of sepsis in protecting it from myocardial dysfunction.

Acknowledgements
Funding
- Carver Collaborative Pilot Grant 2015
- OVPRED Internal Funding Initiative 2015
- DOD

Collaborators
- Steve Lentz
- Sanjana Dayal
- Mary Wilson
- Yani Chen