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TITLE: Targeting Extracellular Histones with Novel RNA Bio-drugs for the Treatment of Acute Lung Injury

PRINCIPAL INVESTIGATOR: Francis Miller

CONTRACTING ORGANIZATION: Duke University
DURHAM, NC 27705

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6. AUTHOR(S)
Francis J Miller, MD

E-Mail: francis.miller@duke.edu;

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Duke University
200 West Main St
Suite 820 Erwin Square Plaza
Durham, NC 27705-0000

Subcontract to:
UNIVERSITY OF IOWA, THE
105 JESSUP HALL
IOWA CITY IA 52242-1316

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

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.

15. SUBJECT TERMS

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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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TABLE OF CONTENTS

1. INTRODUCTION:

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:

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Goals of project as stated in the approved SOW:

Specific Aim 1: In vitro characterization and optimization of RNA aptamers that selectively bind to human histone.	**Timeline**	**Site 1 (Initiating PI)**	**Site 2 (Partnering PI)**
Major Task 1: Local IRB/IACUC Approval	*Months*	*Team*	*Team*
Local IRB approval	1	Dr. Miller	Dr. Giangrande
Local IACUC approval	1	Dr. Miller	Dr. Giangrande
Milestone achieved: regulatory approvals obtained	*1*		
Major Task 2: In vitro - functional efficacy of aptamers	*Months*	*Team*	*Team*
Measurement of calcium influx by fura-2 Cell line used: human pulmonary microvascular endothelial cells; Clonetics	1-12	Dr. Miller	Dr. Giangrande
Measurement of TLR activation by cytokine assay kit Cell lines used: human pulmonary microvascular endothelial cells; Clonetics	1-12		Dr. Giangrande
Detection of cell toxicity Cell lines used: human pulmonary microvascular endothelial cells; Clonetics	1-12	Dr. Miller	Dr. Giangrande
Measure platelet thrombi formation Human platelets (healthy donors)	6-18	Dr. Miller	Dr. Giangrande Dr. Smith Dr. Dayal
Measure platelet surface marker expression Human platelets (healthy donors)	6-18	Dr. Miller	Dr. Giangrande Dr. Smith Dr. Dayal
Ex vivo experiment using blood from patients with ALI	6-24	Dr. Miller	Dr. Giangrande Dr. Smith Dr. Comellas
Milestones Achieved: Determination of efficacy of aptamers on histone-mediated toxicity in cultured cells and human platelets.	*24*		

Specific Aim 2: Evaluate efficacy and safety of histone-specific RNA aptamers in vivo	**Timeline**	**Site 1 (Initiating PI)**	**Site 2 (Partnering PI)**
Major Task 3: Evaluation of efficacy in inhalation injury model (chlorine inhalation and smoke inhalation models)	*Months*	*Team*	*Team*
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.	18-26	Dr. Miller Dr. Tighe Dr. Gunn	Dr. Giangrande Dr. Smith
Assessment of alveoloar permeability and inflammation by bronchoalveolar lavage	18-26	Dr. Miller Dr. Tighe Dr. Gunn	
Lung histology by histopathologic staining and analysis	26-30	Dr. Miller Dr. Tighe Dr. Gunn	
Major Task 4: Evaluation of efficacy in influenza lung injury model	*Months*	*Team*	Team
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.	18-26	Dr. Miller Dr. Tighe	Dr. Giangrande Dr. Smith
Assessment of alveoloar permeability and inflammation by bronchoalveolar lavage	18-26	Dr. Miller Dr. Tighe	Dr. Giangrande Dr. Comellas
Lung histology by histopathologic staining and analysis	26-30	Dr. Miller Dr. Tighe	Dr. Giangrande Dr. Comellas
Milestone(s) Achieved: Completion of assessment of efficacy of aptamers on ALI in mice	30		
Major Task 5: Evaluation of safety	*Months*	*Team*	*Team*
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.	30-36	Dr. Miller	Dr. Giangrande Dr. Smith
Assessment of potential immunostimulation in humanized mice	30-36	Dr. Miller	Dr. Giangrande
Milestone Achieved: Completion of assessment of safety of aptamers on histone-mediated toxicity in mice	*36*		

What was accomplished under these goals?

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.	**Timeline**	**Site 1 (Initiating PI)**	**Site 2 (Partnering PI)**
Major Task 1: Local IRB/IACUC Approval	*Months*	*Team*	*Team*
Local IRB approval	1	Dr. Miller	Dr. Giangrande
Local IACUC approval	1	Dr. Miller	Dr. Giangrande
Milestone achieved: regulatory approvals obtained	*1*		
Major Task 2: In vitro - functional efficacy of aptamers	*Months*	*Team*	*Team*
Measurement of calcium influx by fura-2 Cell line used: human pulmonary microvascular endothelial cells; Clonetics	1-12	Dr. Miller	Dr. Giangrande
Measurement of TLR activation by cytokine assay kit Cell lines used: human pulmonary microvascular endothelial cells; Clonetics	1-12		Dr. Giangrande
Detection of cell toxicity Cell lines used: human pulmonary microvascular endothelial cells; Clonetics	1-12	Dr. Miller	Dr. Giangrande
Measure platelet thrombi formation Human platelets (healthy donors)	6-18	Dr. Miller	Dr. Giangrande Dr. Smith Dr. Dayal

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.

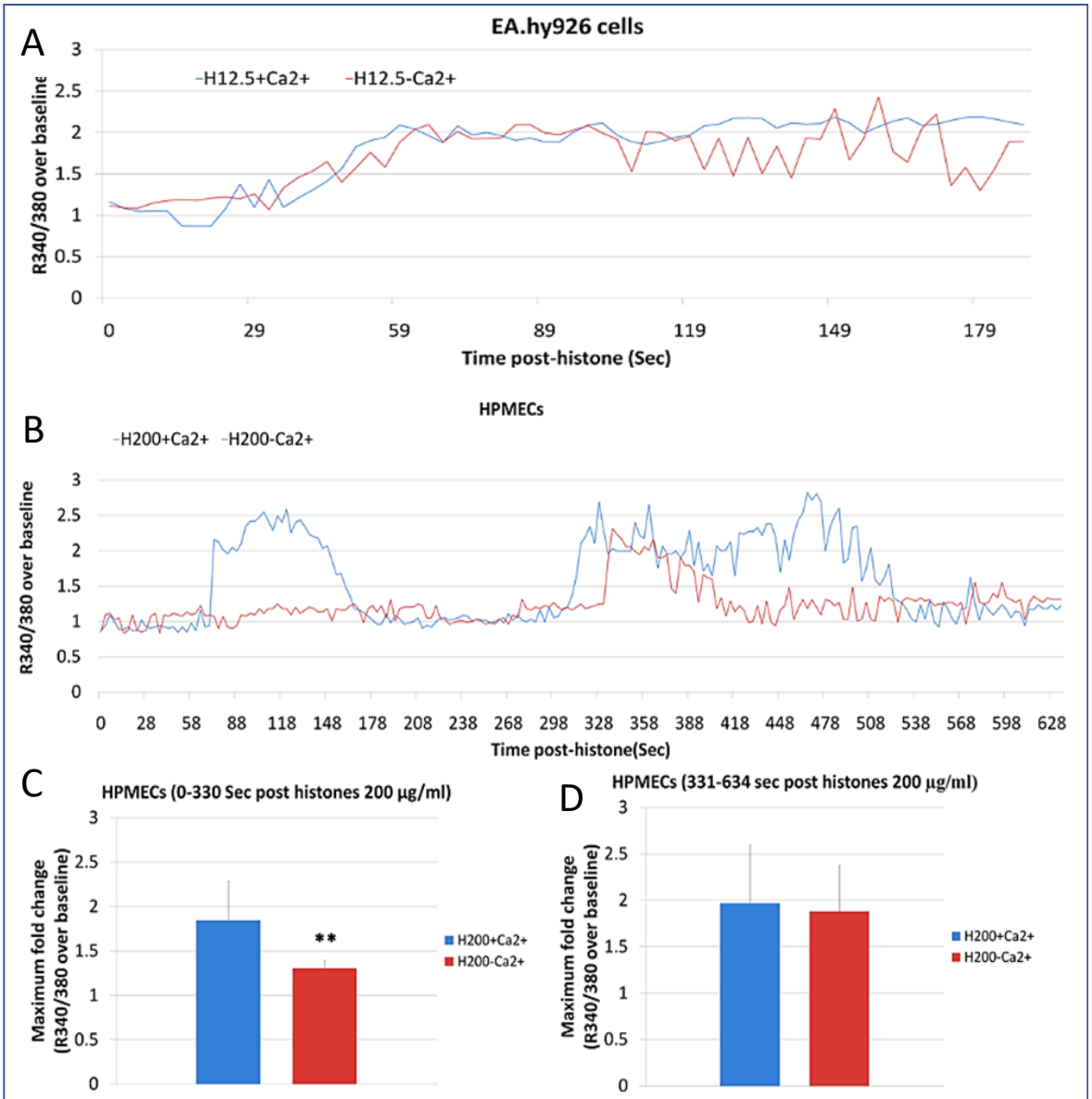

Figure 1. Histone-mediated calcium influx. (A) Cultured human umbilical vein endothelial cell line (EA.hy926) were incubated in fura 2-AM to establish a baseline intracellular calcium signal (relative ratio of fluorescence at 340/380) and 12.5 µM histones added at time "0". Blue line is in the presence 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.

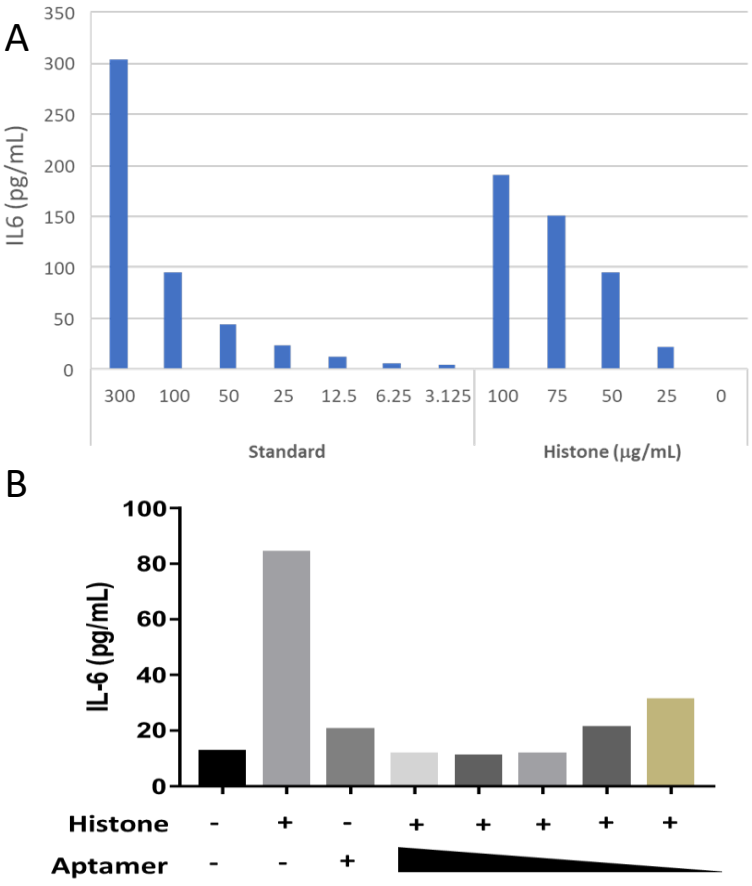

Fig 2. Effect of histone aptamers on histone-mediated TLR activation. (A) Interleukin-6 (IL-6) levels were used as a measure of TLR activation. EaHy926 cells were seeded at 80% confluency in a 96 well plate. The cells were treated for 16h with varying concentrations of calf thymus histones (ranging from 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:.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***).

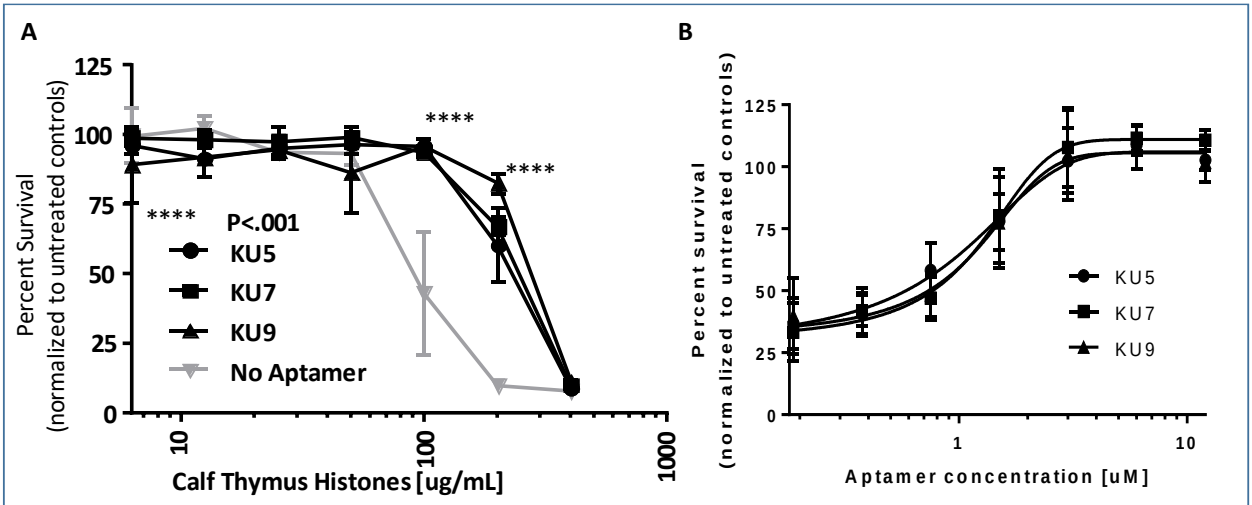

Fig. 3. Aptamer reversal of histone-mediated cytotoxicity of endothelial cells. A) Dose response of histones at constant aptamers concentrations: Cells were treated with 1.2uM of each aptamer and a decreasing amount of calf thymus histones for 24h. Cell viability was determined using the MTS assay. All 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.*

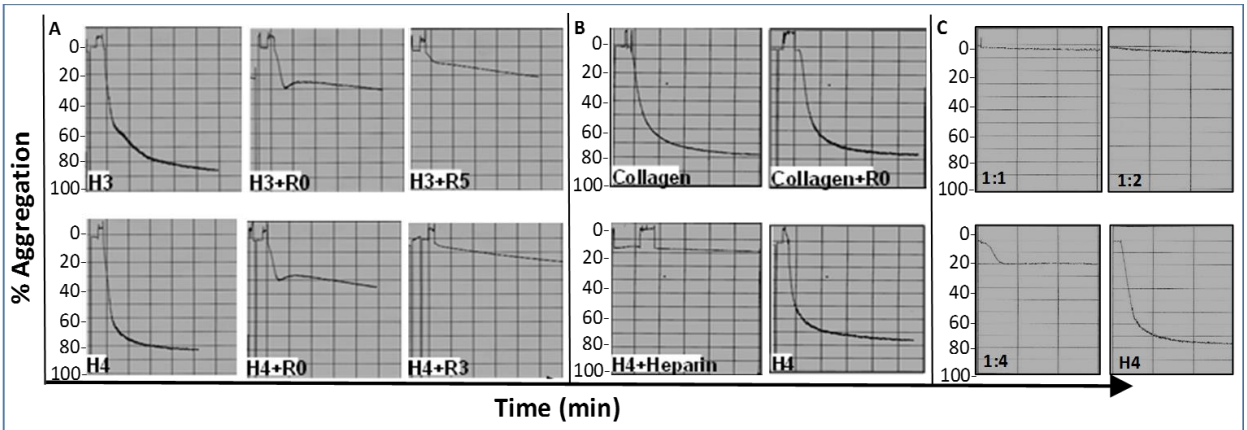

Fig. 4. Histone aptamers inhibit histone-mediated platelet aggregation. Platelet aggregation was performed with washed human platelets and quantitated using an aggregometer at 2 min intervals. **(A)** Histones H3 and H4 induce platelet aggregation. Addition of the unselected round 0 aptamer pool (R0) reduces platelet aggregation. A more pronounced inhibition of platelet aggregation is observed with selected RNA pools (R5 for H3 and R3 for H4). **(B)** RNA aptamers have no effect on collagen-mediated platelet aggregation. Heparin (1U/mL) reverses histone-mediated platelet aggregation. **(C)** Selected aptamer KU5 showed pronounced inhibition of histone H4 at an aptamer:histone molar ration of 1:4.

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.

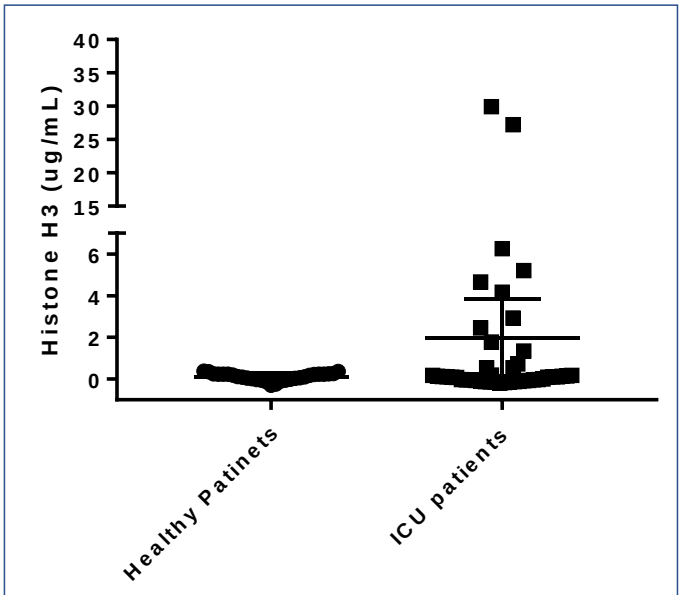

Fig. 5. Levels of serum histone H3 in healthy and ICU patients. Serum samples were collected from healthy donors or patients admitted to the ICU. Samples were processed then quantified by immuno-staining with a human histone H3 antibody.

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.

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

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?

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:

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:

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

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:

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

  Journal publications.

Nothing to report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers, and presentations.

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
2. American Society for Gene and Cell Therapy (ASGCT), May 10-13, 2017, Washington DC. Treatment of Sepsis by Neutralization of Extracellular Histones with Nucleic Acid Aptamers. Kevin Urak, University of Iowa, Iowa City, IA

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**
  N/A

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	***Francis Miller, MD***
Project Role:	*PI Site 1*
Nearest person month worked:	*3.0*
Contribution to Project:	*Dr. Miller assisted in the design and interpretation of all studies performed at site 1 and site 2. He was responsible for overseeing the completion of the in vitro calcium studies performed in Major Task 1 and the in vivo studies of Major Task 2 that are described above.*
Name:	***Kamie Snow***
Project Role:	*Research Scientist*
Nearest person month worked:	*6.0*
Contribution to Project:	*Ms. Smith was responsible for submitting all necessary regulatory documents (IACUC, IRB, safety, etc). She was responsible for completion of the animal experiments described in Major Task 2.*
Name:	***Beilei Lei, PhD***
Project Role:	*Associate Research Scientist Site 2*
Nearest person month worked:	*1.5*
Contribution to Project:	*Dr. Lei was responsible for collection of the calcium influx experiments described in Major Task 1.*
Name:	***Michael Gunn, MD***
Project Role:	*Co-Investigator*
Nearest person month worked:	*0.6*
Contribution to Project:	*Dr. Gunn assisted in the development of animal studies described in Major Task 3 regarding models of acute lung injury. .*
Name:	***Alejandro Comellas, MD***
Project Role:	*Co-Investigator, Site 2*
Nearest person month worked:	*0.6*
Contribution to Project:	*Dr Comellas assisted in the interpretation of lung injury data described in Major Task 2 and in the identification of patients at site 2 (Univ of Iowa) with acute lung injury for studies outlined in Major Task 3. .*

Name:	***Sanjana Dayal, PhD***
Project Role:	*Co-Investigator, Site 2*
Nearest person month worked:	*0.6*
Contribution to Project:	*Dr. Dayal was responsible for assisting with the data on platelet reactivity described in Major Task 1.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

See attached files: 3 abstracts and 2 posters

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)

2. American Society for Gene and Cell Therapy (ASGCT), May 10-13, 2017, Washington DC. Treatment of Sepsis by Neutralization of Extracellular Histones with Nucleic Acid Aptamers. 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 Urak[1,2], Ofonime Udofot[1], Giselle Blanco[1], Li-Hsien Lin[1], Francis Miller Jr.[3,4], Paloma Giangrande[1,2,5,6,7]
[1]*Department of Internal Medicine, University of Iowa, Iowa City, IA, 52242, USA*
[2]*Molecular & Cellular Biology Program, University of Iowa, Iowa City, IA, 52242, USA*
[3]*Department of Medicine, Duke University, Durham, NC, 27708, USA*
[4]*Departmetn of Internal Medicine, Veterans Affairs Medical Center, Durham, NC, 27708, USA*
[5]*Department of Radiation Oncology University of Iowa, Iowa City, IA, 52242, USA*
[6]*Medical Scientist Training Program, University of Iowa, Iowa City, IA 52242, USA*
[7]*Abboud Cardiovascular Research Center, University of Iowa, Iowa City, IA 52242, USA*

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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:
Thursday March 30, 2017.
8:00 am to 5:00 pm

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[2]Duke University School of Medicine and Durham Veterans Affairs Medical Center, Durham, NC 27710, USA

Collaborators

University of Iowa Carver College of Medicine

University of Iowa Health Care

ABSTRACT

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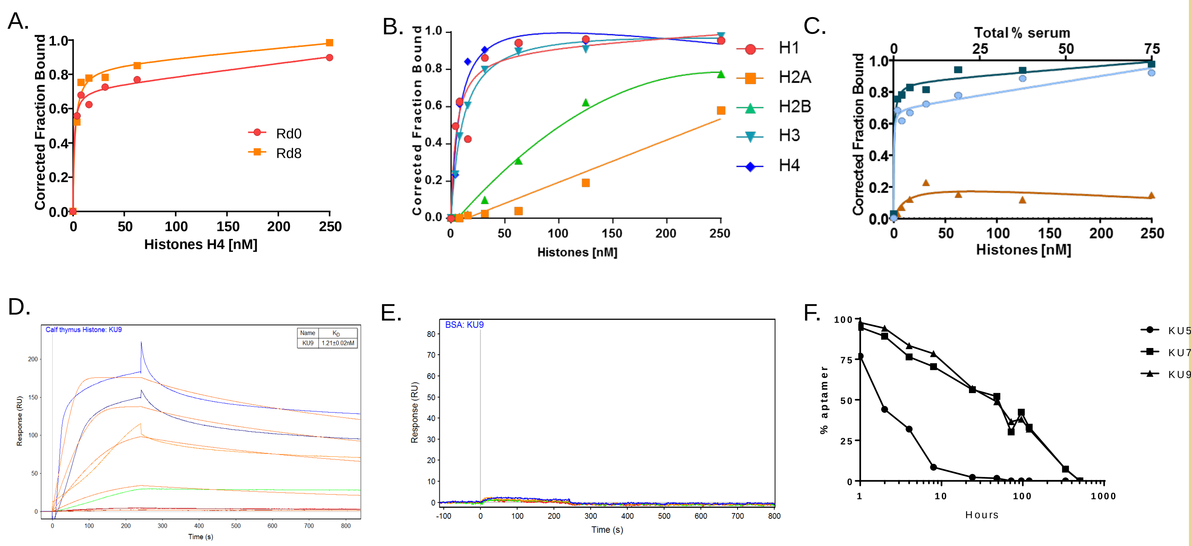

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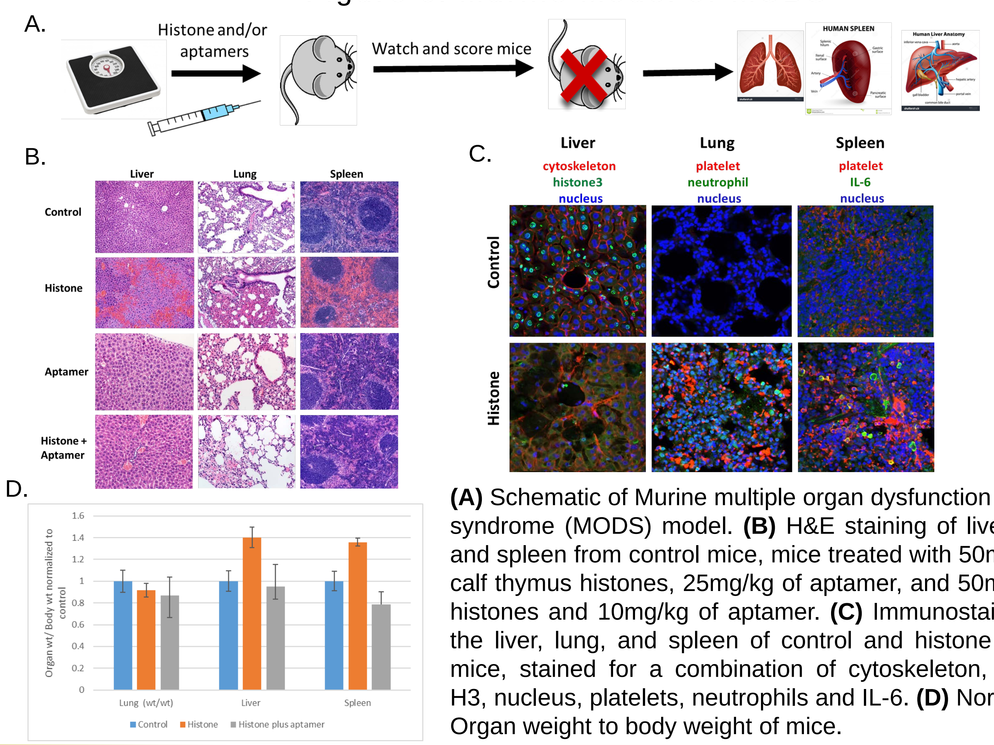

(A) Schematic of Murine multiple organ dysfunction syndrome (MODS) model. **(B)** H&E staining of liver, lung, and spleen from control mice, mice treated with 50mg.kg of calf thymus histones, 25mg/kg of aptamer, and 50mg/kg of histones and 10mg/kg of aptamer. **(C)** Immunostaining on the liver, lung, and spleen of control and histone treated mice, stained for a combination of cytoskeleton, histone H3, nucleus, platelets, neutrophils and IL-6. **(D)** Normalized Organ weight to body weight of mice.

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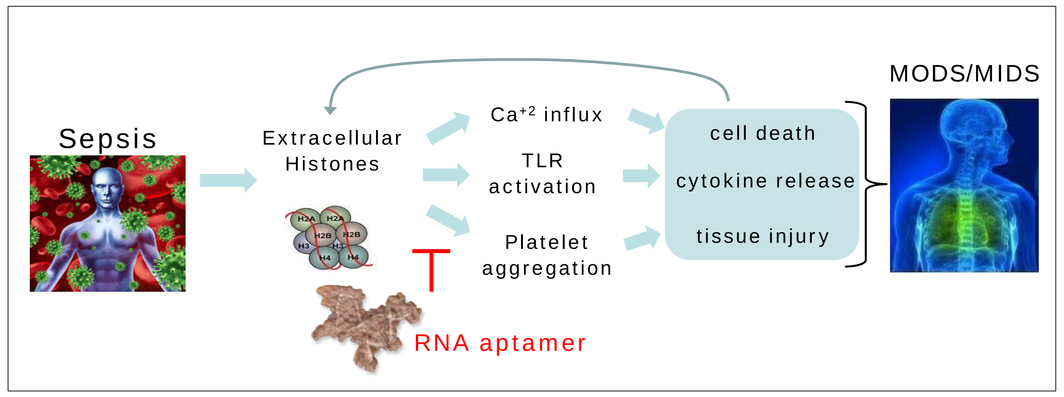

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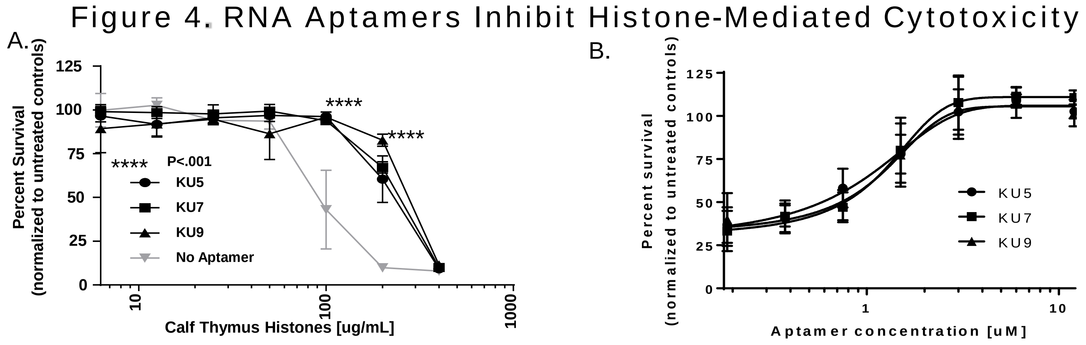

(A) EA.Hy926 cells were treated with 1.2uM of the respected aptamer and a decreasing amount of calf thymus histones. Cell viability was determined by MTS assay. A 2way ANOVA test determined that there was a significant difference between all aptamers and the no aptamer treatment at the 100 and 200 ug/ml concentration of calf thymus histones. **(B)** A549 cells were treated with 180ug/mL of Calf Thymus Histone and a decreasing amount of Aptamer.

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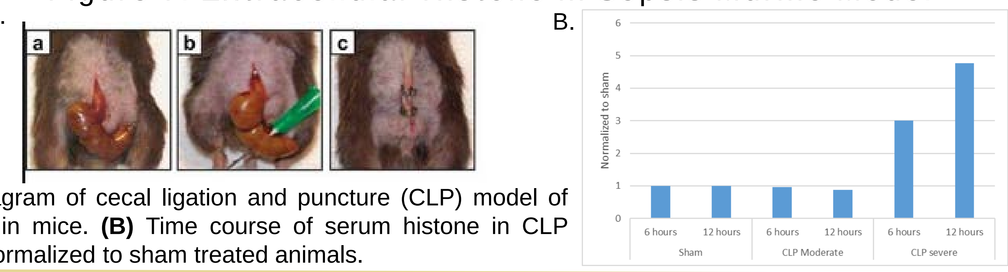

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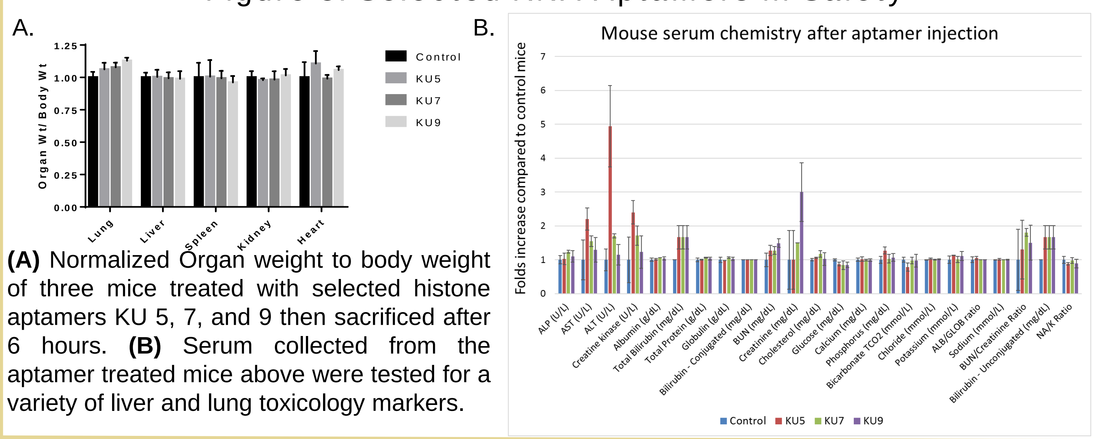

(A) Normalized Organ weight to body weight of three mice treated with selected histone aptamers KU 5, 7, and 9 then sacrificed after 6 hours. **(B)** Serum collected from the aptamer treated mice above were tested for a variety of liver and lung toxicology markers.

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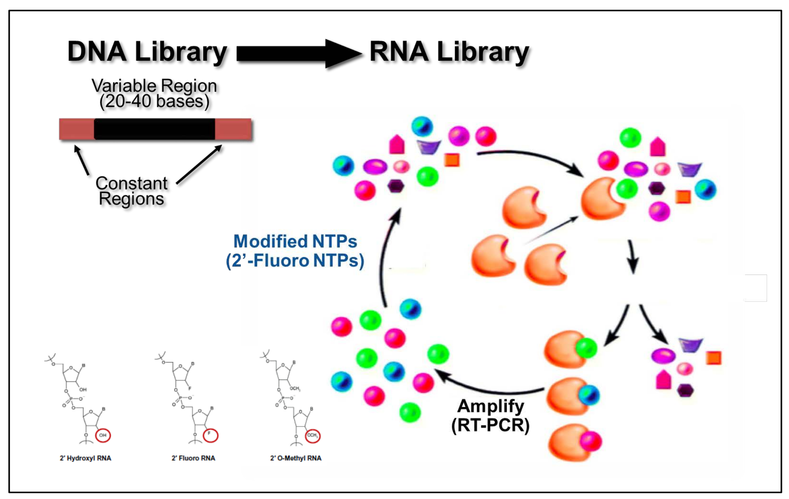

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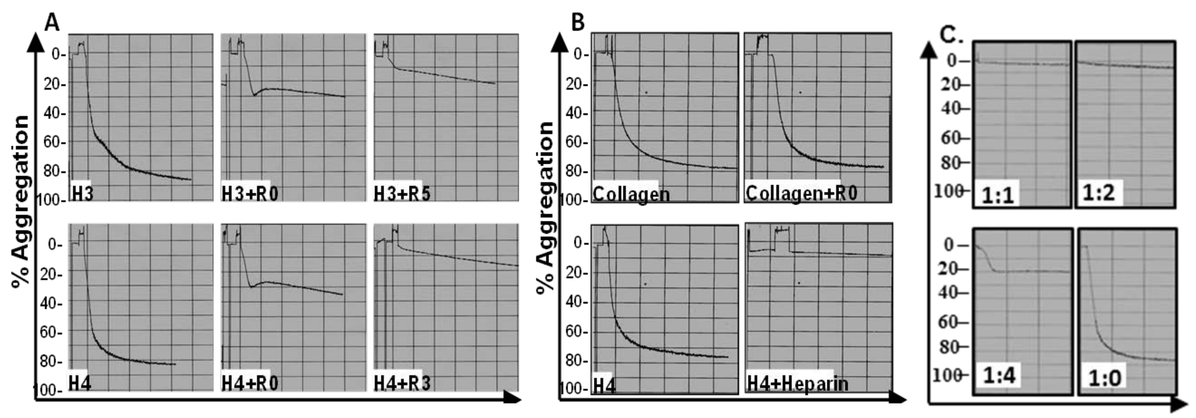

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Future Directions

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Acknowledgements

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Neutralization of extracellular histones with nucleic acid aptamers for the treatment of critical illness

Kevin Urak[1,3], Francis Miller Jr.[2], Paloma Giangrande[1,3,4]
Affiliation(s)
[1]Department of Internal Medicine, , [3]Molecular & Cellular Biology Program, [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

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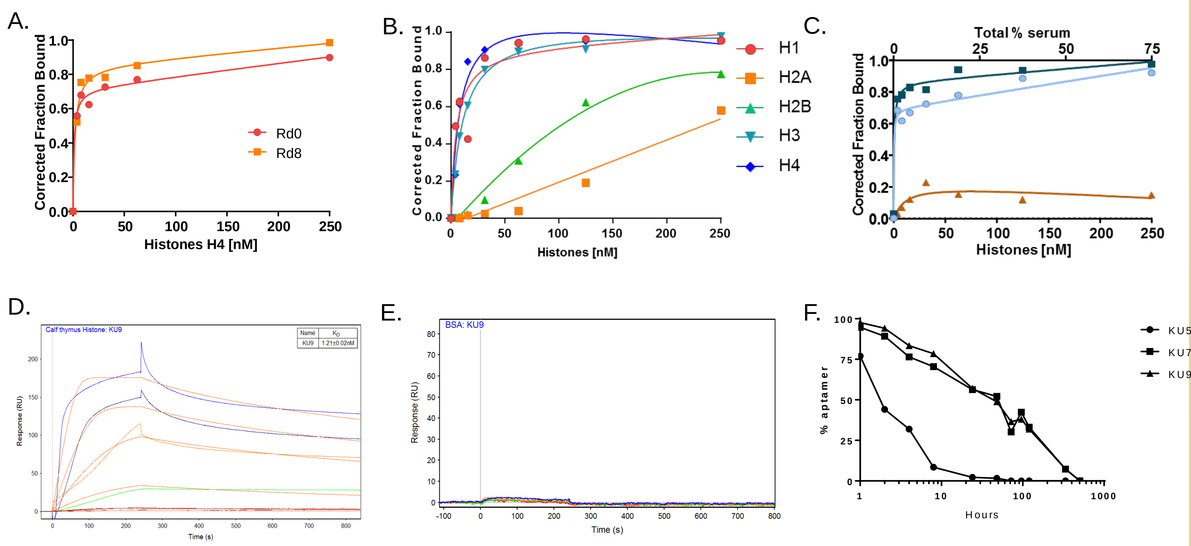

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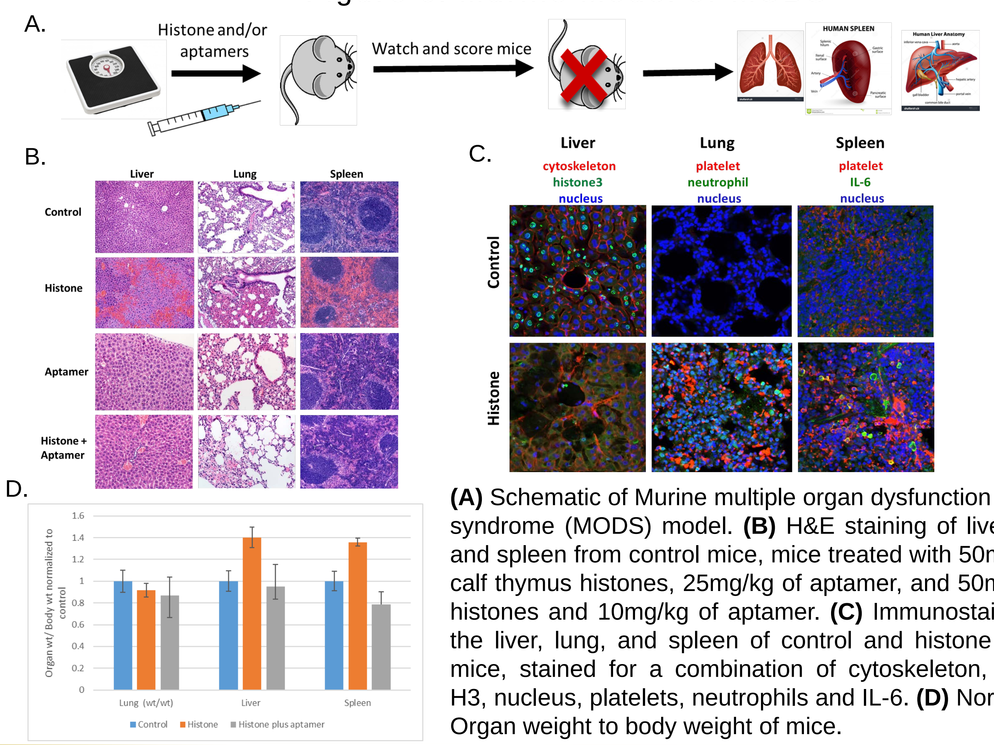

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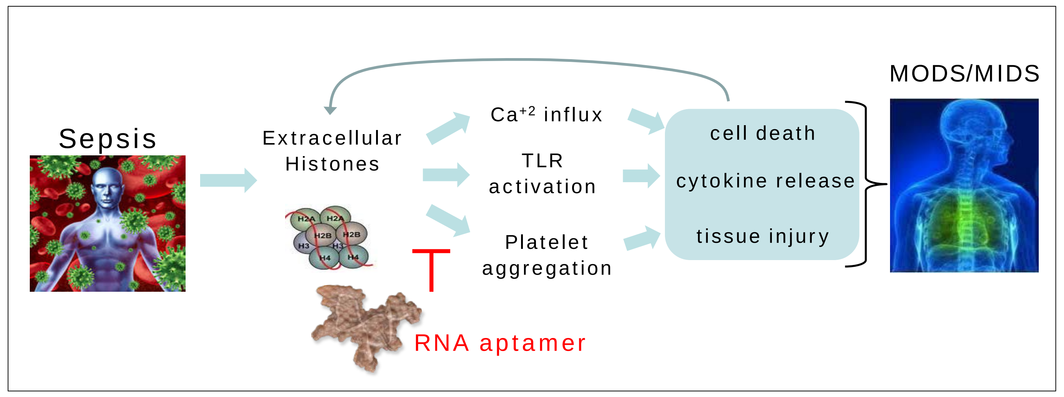

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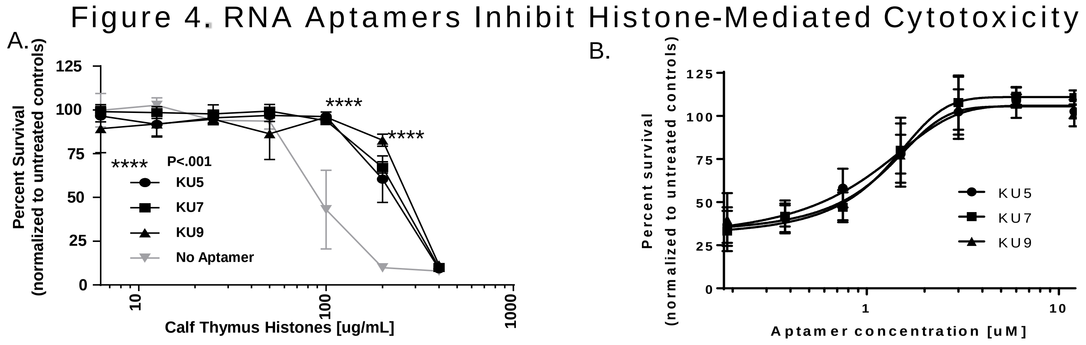

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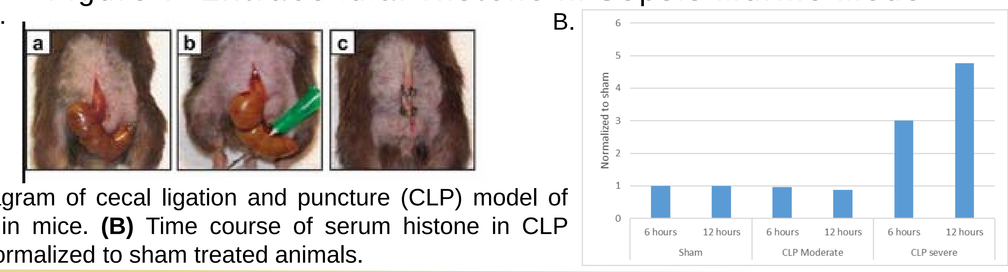

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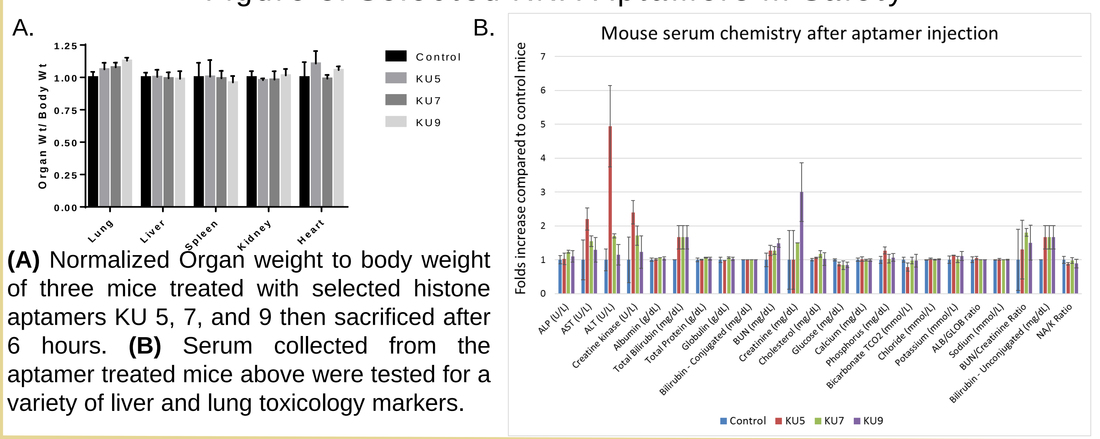

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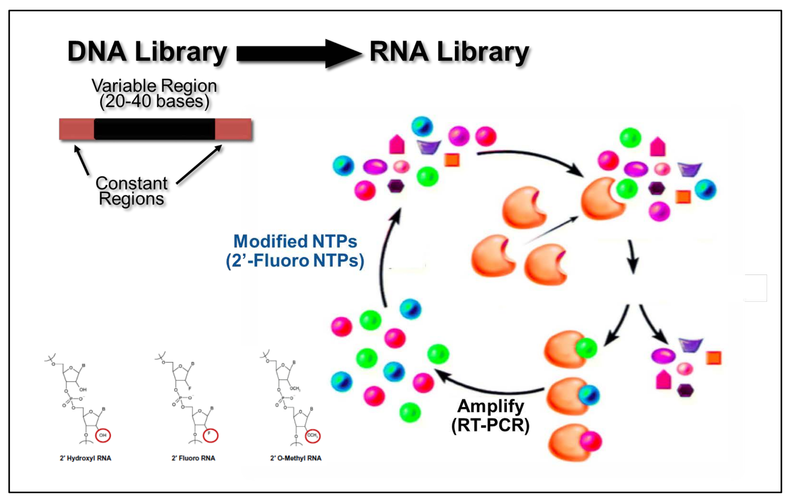

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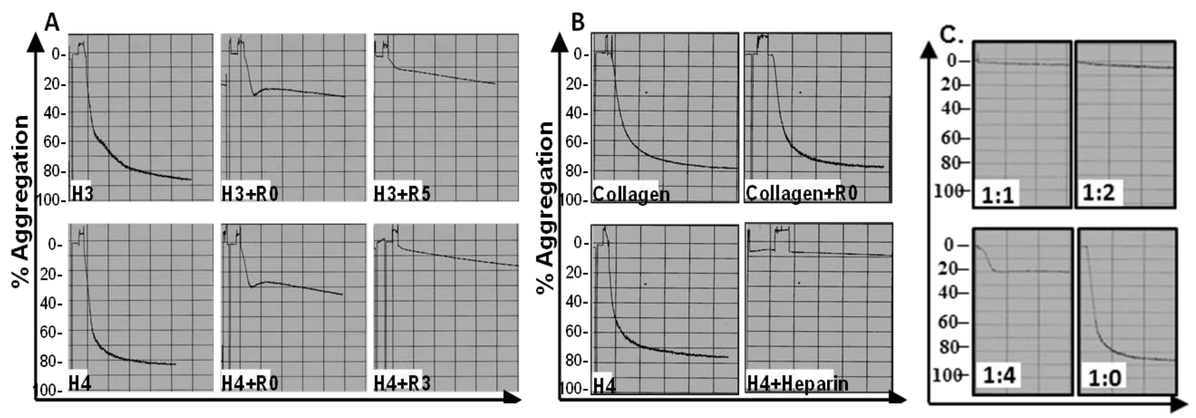

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← Search Results

Session 143 - Oligonucleotide Therapeutics

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. Urak[1], Francis J. Miller[2], Paloma Giangrande[1]

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

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