A. COVER PAGE

Project Title: Validation of non-electrophile Nrf2 activators for WTC relevant pulmonary indications	
Grant Number: 1 U01OH012054-01	**Project/Grant Period**: **06/01/2020 - 03/31/2022**
Reporting Period: **06/01/2020 - 03/31/2022**	**Date Submitted**: 9/2/2022
Program Director/ Principal Investigator Michael Darin Cameron Senior Scientific Director Scripps Florida Jupiter, FL michaelcameron@ufl.edu	**Administrative Official Information**
Change of Contact PD/PI: **No**	
Human Subjects: **No**	**Vertebrate Animals:** **Yes**
hESC: **No**	**Inventions/Patents:** **No**

B. ACCOMPLISHMENTS

B.1. What are the major goals of the project?

There were two goals in this one year project. A novel non-electrophilic Nrf2 activator was to be evaluated in pulmonary fibrosis models and analogs were to be synthesized containing a photo-activated cross linker to define the active site.

B.2. What did you accomplish under these goals?

Project initiation was slowed due to Covid19 initiated capacity restrictions. The planned in vivo experiments require multiple researchers to be present. Because of this we initially focused on Aim2. The lead Nrf2 activator was modified to incorporate an amide. The amide was used to couple photo-activated diazirine probes using a short and a long linker. These were evaluated and did not activate Nrf2. We used a backup strategy and developed a fluorescence polarization assay and examined the binding of the test compounds at the interface between Nrf2 and its binding partner Keap1. The compounds were found to bind to a unique binding site which opens up new areas to explore for the modulation of Nrf2 activity. As Covid19 restrictions were lifted, we initiated in vivo studies using a bleomycin model. The test compounds were shown to increase the mRNA levels of Nrf2 target genes in the lungs of mice and elevated the concentration of the key cellular antioxidant, glutathione. Tolerability and toxicity were not observed after dosing the test compounds. We proceeded to a bleomycin-induced lung injury model. Bleomycin intercalates into DNA and causes DNA double-strand breaks, culminating in high systemic toxicity three to seven days post-dose and severe pulmonary fibrotic injury within four weeks. The test compound has poor solubility and requires the use of micelle-based excipients to solubilize and dose the compound. These excipients proved to be incompatible with the bleomycin model. The use of alternative formulations and oral dosing were unsuccessful and resulted in minimal plasma levels of the test compound. With the limited remaining time on the one-year award, the compounds were tested in an asbestos model of lung injury where the formulation did not cause a problem. Pulmonary injury was evaluated four weeks after asbestos insult. We were surprised to see that when compared to control mice dosed with saline, asbestos upregulated the expression of Nrf2 target genes. The test compounds further increased the level of Nrf2 target genes in the asbestos treated group. Objective markers of pulmonary function, cytokine levels, and fibrosis were measured. Pulmonary function was not observed to decline over the first 28 days. Mice treated with the non-electrophile Nrf2 activators had decreased levels of both pro-inflamatory cytokines and mRNA for colegen synthesis enzymes, but the change did not reach statistical significance. Due to the progressive nature of asbestos injury, the four week study may have been too short and evaluating the impact over three to six months may give a better indication of the potential of the Nrf2 activators to modulate pulmonary injury.

B.3. Competitive Revisions/Administrative Supplements

n/a

B.4. What opportunities for training and professional development did the project provide?
The project included a post-doctoral researcher that was able to express and purify protein, establish a fluorescence polarization assay, and further evaluate compounds in bleomycin and asbestos models.

B.5. How did you disseminate the results to communities of interest?
In Progress

B.6 - What do you plan to do during the next reporting period to accomplish the goals?
This was a one year project.

C. PRODUCTS

C.1. Publications, conference papers, and presentations
None

C.2. Website(s) or other Internet site(s) – include URL(s)
None

C.3. Technologies or techniques

n/a

C.4. Inventions, patent applications, and/or licenses
n/a

C.5. Other products and resource sharing
n/a

D. PARTICIPANTS

D.1. What individuals have worked on the project? Please include calendar, academic, and summer months.

Commons ID	S/K	Name	Degrees(s)	Role	Cal	Aca	Sum	Foreign	Country	SS
cameronmichael		Michael Cameron	Ph.D.	PI	3			No	n/a	
		Susan Khan	AAS	Res. Asst	2.45			no	n/a	
		Katalin Toth	Ph.D.	Post Doc	9.81			no	n/a	
kameneck		Ted Kamenecka	Ph.D.	Co-I	0.91			no	n/a	

ZHUD180		Di Zhu	Ph.D.	Post Doc	3.79			no	n/a	
CGPARKER		Christopher Parker	Ph.D.	Co-I	1.16			no	n/a	
WAWEIGBY		W. Wang	Ph.D.	Post Doc	5.64			no	n/a	

D.2 Personnel updates

a. Level of Effort:
b. New Senior/Key Personnel:
c. Changes in Other Support:
d. New Other Significant Contributors:

E. IMPACT

E.1 - What is the impact on the development of human resources, if applicable?
n/a

E.2 - What is the impact the Public Health Relevance and Impact? The investigator should address how the findings of the project relate beyond the immediate study to improved practices, prevention or intervention techniques, legislation, policy, or use of technology in public health.

This project tested a novel mechanism to address pulmonary fibrotic conditions important to WTC responders. The experiments provided encouraging results on the activation of the protective pathways regulated by Nrf2 using non-electrophiles. This has the potential to activate this pathway without causing harm to the host. Cellular studies showed target modulation, including increased expression level of Nrf2 targets and an increase in cellular levels of antioxidants such as glutathione. These were confirmed with intraperitoneal dosing of the test compounds in mice.

F. CHANGES

F.1 – Changes in approach and reasons for change, including changes that have a significant impact on expenditures

The planned use of photo-activated probes to examine the binding site did not work because none of the prepared photo probes activated Nrf2. We believe they do not bind to the appropriate region. An alternative strategy was utilized where a fluorescence polarization assay was established to examine the direct inhibition of Nrf2-Keap1 binding. We synthesized a compound reported by the pharmaceutical company Glaxo-Smith-Kline to interrupt this interaction as a positive control. The assay worked very well and we were able to show the compounds we were exploring have a unique mechanism that does not involve binding at the Nrf2-Keap1 interface. This is not entirely surprising because none of the redox-active cysteines that exhibit biological control of the Nrf2 system in vivo are located near the Nrf2-Keap1 interface.

F.2 - Actual or anticipated challenges or delays and actions or plans to resolve them

This was a one-year project. We extended the project timelines to run the compound through an asbestos model.

F.3 - Significant changes to human subjects, vertebrate animals, biohazards, and/or select agents

none

G. Special Reporting Requirements

G.1 Special Notice of Award Terms and Funding Opportunities Announcement Reporting Requirements
n/a

G.2 Responsible Conduct of Research

n/a

G.3 Mentor's Research Report or Sponsor Comments

n/a

G.4 Human Subjects

G.4.a Does the project involve human subjects?
No

G.4.b Inclusion Enrollment Data
n/a

G.4.c ClinicalTrials.gov
n/a

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?
No

G.5 Human Subject Education Requirement

Are there personnel on this project who are newly involved in the design or conduct of human subject's research?
No

G.6 Human Embryonic Stem Cells (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?
No

G.7 Vertebrate Animals

Does this project involve vertebrate animals?
Yes

G.8 Project/Performance Sites
Scripps Florida

G.9 Foreign Component
None

G.10 Estimated Unobligated Balance

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

no

G.11 Program Income

Is program income anticipated during the next budget period?
No

G.12 F&A Costs

Is there a change in performance sites that will affect F&A costs?
No

I. OUTCOMES

I. Provide a concise summary of the outcomes or findings of the award, written for the general public in clear and comprehensible language, without including any proprietary, confidential information or trade secrets

Note: project outcome information will be made public in NIH RePORTER

Project initiation was slowed due to Covid19 initiated capacity restrictions. The planned in vivo experiments require multiple researchers to be present. Because of this we initially focused on Aim2. The lead Nrf2 activator was modified to incorporate an amide. The amide was used to couple photo-activated diazirine probes using a short and a long linker. These were evaluated and did not activate Nrf2. We used a backup strategy and developed a fluorescence polarization assay and examined the binding of the test compounds at the interface between Nrf2 and its binding partner Keap1. The compounds were found to bind to a unique binding site which opens up new areas to explore for the modulation of Nrf2 activity. As Covid19 restrictions were lifted, we initiated in vivo studies. The test compounds were shown to increase the mRNA levels of Nrf2 target genes in the lungs of mice and elevated the concentration of the key cellular antioxidant, glutathione. Tolerability and toxicity were not observed after dosing the test compounds. We proceeded to a bleomycin-induced lung injury model. Bleomycin intercalates into DNA and causes DNA double-strand breaks, culminating in high systemic toxicity three to seven days post-dose and severe pulmonary fibrotic injury within four weeks. The test compound has poor solubility and requires the use of micelle-based excipients to solubilize and dose the compound. These excipients proved to be incompatible with the bleomycin model. The use of alternative formulations and oral dosing were unsuccessful and resulted in minimal plasma levels of the test compound. With the limited remaining time on the one-year award, the compounds were tested in an asbestos model of lung injury where the formulation did not cause a problem. Pulmonary injury was evaluated four weeks after asbestos insult. We were surprised to see that when compared to control mice dosed with saline, asbestos upregulated the expression of Nrf2 target genes in lung tissue. The test compounds further increased the level of Nrf2 target genes in the asbestos-treated group. Objective markers of pulmonary function, cytokine levels, and fibrosis were measured. Pulmonary function was not observed to decline over the first 28 days. Mice treated with the non-electrophile Nrf2 activators had decreased levels of both pro-inflammatory cytokines and mRNA for collagen synthesis enzymes, but the change did not reach statistical significance. Due to the progressive nature of asbestos injury, the four-week study may have been too short, and evaluating the impact over three to six months may give a better indication of the potential of the Nrf2 activators to modulate pulmonary injury.