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TITLE: Innovative Therapy and Underlying Mechanism of Neuraminidase-1-Driven Pulmonary Fibrosis

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CONTRACTING ORGANIZATION: Baltimore Research & Education Foundation

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14. ABSTRACT
Tissue fibrosis contributes to multiple diseases within every organ and tissue. Better therapies need to be developed. We recently identified an enzyme, neuraminidase (NEU) 1, as a key player in fibrosis. Specific Aim 1 to preclinically develop a novel, NEU1-targeting, antifibrotic therapy. In the first year of funding, we performed experiments to comparatively assess various routes of drug delivery based on evaluation of sustained inhibition of NEU1. The results indicate that parenteral (injected) route provides the most efficient as well as prolonged inhibition of NEU1 compared with the oral route. This is an important finding that will support further development of the drug towards transition to human trials beyond the scope of this project. In addition to this work, we identified a protein called MUC1 as a NEU1 substrate and demonstrated that desialylation enhances its downstream signaling. Specific Aim 2 to explore whether MUC1 mediates the profibrotic effect of NEU1 will be pursued in the second year of this project.

15. SUBJECT TERMS
fibrosis, therapy, preclinical development, neuraminidase, mucin, profibrotic mechanism

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose, and scope of the research.*

This project addresses an important unmet clinical need in Military Service Members and Veterans: pulmonary fibrosis. The purpose is to explore the suitability of a disease mediator, known as NEU1, as a potential therapeutic target for treating pulmonary fibrosis in these patients. In the translational arm of this work (Aim 1), NEU1 inhibitors are being preclinically developed as future drugs. In the mechanistic arm (Aim 2), the disease-driving NEU1 – MUC1 axis is explored.

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

Lung, fibrosis, therapy, neuraminidase-1, mucin-1, bleomycin, preclinical

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1. Identify an optimal route of C9-BA-DANA and III-32B5 in vivo delivery that allows for best magnitude and duration of sialidase inhibition in the lung. Month 1–8. 100% completed.
Major Task 2. For the identified route of delivery, establish the minimal effective dose of C9-BA-DANA and III-32B5 in chronic bleomycin model in the therapeutic regimen. Months 6–15. 70% completed.
Major Task 3. Correlate the levels of desialylated and total MUC1- ED with the NEU1-selective inhibitor-induced decline in sialidase activity and collagen accumulation. Months 12–15. 0% completed.
Major Task 4. Compare the profibrotic effect of sialylated, desialylated, and non-glycosylated MUC1-ED glycoforms in primary cell culture. Months 12 – 15. 10% completed.
Major Task 5. In vivo effects of MUC1-ED glycoforms acting alone or in combination with bleomycin on lung inflammation and fibrosis. Months 16 – 18. 0% completed.

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.

Major Task 1 was fully completed, as initially planned. We have identified the route for the NEU1 inhibitor delivery, and based on this information, started and advanced the studies in Major Task 2. In Task 2, the dosing was titrated down to 1 - 5 mg doses, with 5 mg ensuring reliable effect, whereas with 1 mg, the effect is less pronounced and more variable. We are completing the relevant studies in the chronic model; these will be completed by month 15 as planned and approved. Major Tasks 3 and 5 were planned and approved for the second year of the project, and are being initiated currently. In Major Task 4, only a regulatory sub-task to obtain a relevant approval has been completed, as planned. The experimental work under Task 4 was planned and approved for the second year of the project, and it has just been initiated.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Lab personnel were trained in a one-on-one setting on the basis of drug administration routes as well as drug pharmacodynamics, especially as it relates to inhibition of neuraminidase activities. The PI, Dr. Luzina, routinely participates in journal clubs and seminars, where she shares her knowledge on the molecular and cellular mechanisms of pulmonary fibrosis, the roles of neuraminidases and mucins in this pathology, and emerging pharmacological approaches to their therapeutic targeting. This knowledge is shared with other investigators, as well as postdoctoral fellows and graduate students on campus.

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

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

We are in the end of the first year of the study, meaning that data collection is continuing, data are being analyzed and integrated, and a research manuscript is being planned. In the second year of the study, the manuscript will be prepared. It will then be submitted for consideration for publication in a peer-reviewed scientific periodical. The results will be shared with other scientists as well as patients and care givers through this publication.

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The work will progress exactly as initially planned and approved. Specifically, Major Tasks 3 and 5 will be pursued, and the efforts in Specific Task 4 will continue.

4. **IMPACT:** D*escribe 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We are continuing to analyze the rapidly accumulating data, but several important trends have already emerged. First, we observed that the oral route of administration of NEU1 inhibitors is inferior to systemically injected routes. In future development of these prospective drugs, either an inhaled formulation will need to be considered, or a parenteral route will have to be used. We also discovered that inhibition of neuraminidase activity lasts much longer—up to 48 hours and likely beyond—than expected after a single dose. The mechanism is yet unclear, but the importance is that the dosing in humans will likely be only once or twice weekly, which is beneficial in terms of patients' compliance with the therapy.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Neuraminidases also play important roles in atherosclerosis, diabetes, and cancer, and their inhibition in those pathologies is also likely to be therapeutically beneficial. In the end of this study, we will disseminate our finding to the broad scientific community, which will benefit drug development for those major diseases with unmet clinical needs, including in Military Service Members and especially Veterans.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

In the past, we filed a relevant patent application through the Baltimore VA Medical Center and University of Maryland. This Department of Defense-funded project goes beyond the scope of that patent application, but there is a possibility—yet to be confirmed—that future intellectual property may, or may not, be generated in this project. This will be clear in the end of the second year of the study, and relevant determinations will be performed at that time.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:*

There have been no changes in the approach. The project has been and continues to be performed exactly as initially planned and approved.

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.

No major problems or delays are expected. There have been several minor delays associated with delivery of reagents and supplies, due to the COVID-19 pandemic. We have negotiated these delays by working with different providers and adjusting scheduling of specific experiments, so that the overall plans have not been affected.

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.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

This study has been determined to be "Non-human subject research" by the Institutional Review Board as was planned and completed under Major Task 4 subtask 1.

No changes have taken place or are expected.

Significant changes in use of biohazards and/or select agents

No changes

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).*

We are still accumulating data. A publication will require the data that are going to be accumulated in the second year of the project.

  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 (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **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. Describe the technologies or techniques were shared.

The scope of this project is based on utilizing existing technologies and does not call for the development of new technologies or techniques.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. 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;*
  - *physical 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.*

Nothing to report

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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Irina G. Luzina, MD, PhD
Project Role: Principal Investigator
Nearest person month worked: 6
Contribution to Project: Dr. Luzina, Principal Investigator, is responsible for the design and conduct of the experiments, interpretation and reporting of the results, and regulatory compliance.

Name: Virginia Lockatell, BSc
Project Role: Research Specialist
Nearest person month worked: 3.25
Contribution to Project: Ms. Lockatell performed experiments outlined in the Research Plan, specifically focusing on the experiments in animals described in Aim 1. Her work included bleomycin model, animal treatment with NEU1 inhibitors, tissue processing, experiments in cell culture, collagen assays and ELISA assays.

Name: Erik Lillehoj, PhD
Project Role: Co-Investigator
Nearest person month worked: 1.8
Contribution to Project: Dr. Lillehoj was involved in preparation of sialylated, desialylated, and nonglycosylated MUC1-ED to be used in Aim 2. Dr. Lillehoj contributed to experimental planning and data analysis and interpretations.

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:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (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

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

Innovative Therapy and Underlying Mechanism of Neuraminidase-1-Driven Pulmonary Fibrosis

PR202031/FY20 PRMRP-Discovery Award
W81XWH2110041

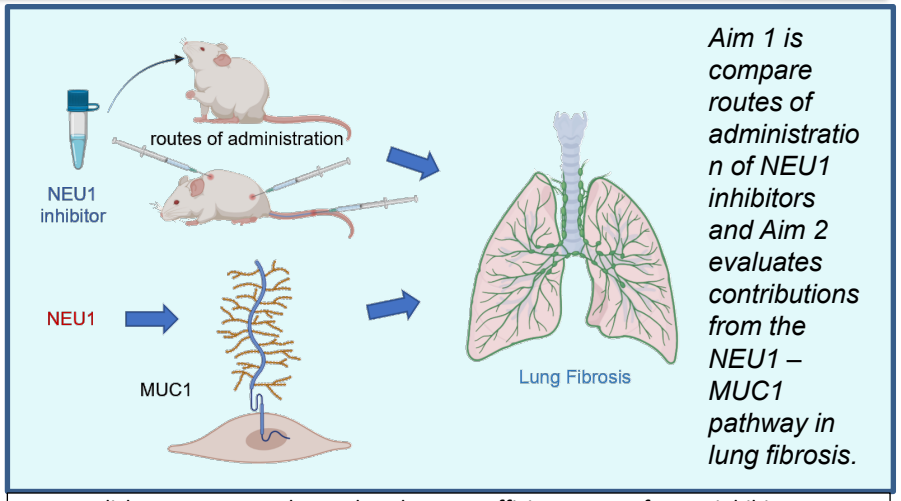
PI: Luzina, Irina **Org:** VA Maryland Health Care System **Award Amount:** $220,000

Study/Product Aim(s)

Pharmacological inhibition of an enzyme, NEU1, is explored as a method to treat fibrosis. The role of a mucin, MUC1, is expored in the mechanism of fibrosis and in responsiveness to therapy. Specific Aims:
1. Preclinically develop novel NEU1-selective small molecule inhibitors as a therapeutic intervention for tissue fibrosis.
2. Establish whether the sialylation state of MUC1 ectodomain defines the degree of its shedding into a soluble form, which in turn provokes tissue fibrosis.

Approach

We performed experiments to comparatively assess various routes of drug delivery based on evaluation of sustained inhibition of NEU1. The results indicate that parenteral (injected) route provides the most efficient as well as prolonged inhibition of NEU1 compared with the oral route. In the second year, we will explore whether MUC1 mediates the profibrotic effect of NEU1. Work progresses as initially approved.



Aim 1 is compare routes of administratio n of NEU1 inhibitors and Aim 2 evaluates contributions from the NEU1 – MUC1 pathway in lung fibrosis.

Accomplishment: We now know that the most efficient route of NEU1 inhibitor delivery is parenteral (injected), whereas the oral route is less preferred. This knowledge will guide future transition to early clinical trials in humans.

Timeline and Cost

Activities CY	2021	2022
Major Task 1		
Major Task 2		
Major Task 3		
Major Task 4		
Major Task 5		
Estimated Budget ($K)	**$110,000**	**$110,000**

Updated: (place date of last update)

Goals/Milestones (Example)

☑ **Major Task 1.** Identify an optimal route of C9-BA-DANA and III-32B5 in vivo delivery for best therapeutic effect.
☑ **Major Task 2.** For the identified route of delivery, establish the minimal effective dose of C9-BA-DANA and III-32B5 in chronic bleomycin model in the therapeutic regimen.
☐ **Major Task 3.** Correlate the levels of desialylated and total MUC1- ED with the NEU1-selective inhibitor-induced decline in sialidase activity and collagen accumulation.
☐ **Major Task 4.** Compare the profibrotic effect of sialylated, desialylated, and non-glycosylated MUC1-ED glycoforms in primary cell culture.
☐ **Major Task 5.** In vivo effects of MUC1-ED glycoforms acting alone or in combination with bleomycin on lung inflammation and fibrosis.

Budget Expenditure to Date
Projected Expenditure: $220,000
Actual Expenditure: $87,255

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.*