

**AWARD NUMBER:** W81XWH-22-1-0027

**TITLE:** NRF2 Pathway Activation in the Lung Tumor Microenvironment Macrophages

**PRINCIPAL INVESTIGATOR:** Ana Sofia Mendes Leal

**CONTRACTING ORGANIZATION:** Michigan State University

**REPORT DATE:** JANUARY 2023

**TYPE OF REPORT:** Annual report

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

**DISTRIBUTION STATEMENT:** Approved for public release; distribution is 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.

# REPORT DOCUMENTATION PAGE

*Form Approved*  
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

<b>1. REPORT DATE</b> JANUARY 2023		<b>2. REPORT TYPE</b> ANNUAL		<b>3. DATES COVERED</b> 1JAN2022 - 31DEC2022	
<b>4. TITLE AND SUBTITLE</b>  NRF2 Pathway Activation in the Lung Tumor Microenvironment Macrophages				<b>5a. CONTRACT NUMBER</b> W81XWH-22-1-0027	
				<b>5b. GRANT NUMBER</b>	
				<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b>  Ana Sofia Mendes Leal  E-Mail:				<b>5d. PROJECT NUMBER</b>	
				<b>5e. TASK NUMBER</b>	
				<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  Michigan State University 426 Auditorium Rd. East Lansing, MI 48824-1046				<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012				<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
				<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for Public Release; Distribution Unlimited					
<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT:</b> Lung is physiologically populated by numerous immune cells, that can be skewed to support the growth of tumor cells. My main goal is to understand how cancer cells interact with the surrounding environment, creating an immunosuppressive tumor microenvironment, to promote growth. This knowledge can ultimately render new vulnerabilities that can be addressed by the development of new drugs or re-purposing of known drugs, eventually leading to an increase in survival of lung cancer patients. Bone marrow derived macrophages (BMDM) from A/J mice show an increased expression of NRF2 and HO-1 upon stimulation with conditioned media from lung cancer cells, this increase is accompanied with increased expression of CD206, arginase, IL10 and others, showing the ability of cancer cells to skew macrophages towards a tumor promoting phenotype. Moreover, CD11 and CD3 cells were isolated from A/J mice injected with vinyl carbamate (or saline for control group). Both CD11 and CD3 cells, showed an increased expression of HO-1 2 weeks after vinyl carbamate injection. These data suggest that NRF2->HO-1 pathway is activated in macrophages early in the progression of lung cancer.					
<b>15. SUBJECT TERMS</b>  Lung cancer, NRF2→HO-1 pathway, macrophages, clodronate liposomes					
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b>
<b>a. REPORT</b>	<b>b. ABSTRACT</b>	<b>c. THIS PAGE</b>			<b>19b. TELEPHONE NUMBER</b> (include area code)
U	U	U	UU	20	USAMRDC

## TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	6
2. Keywords	6
3. Accomplishments	6
4. Impact	11
5. Changes/Problems	12
6. Products	14
7. Participants & Other Collaborating Organizations	17
8. Special Reporting Requirements	19
9. Appendices	19

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

Historically, activation of the Nrf2 pathway was considered beneficial for cancer prevention. Nrf2 deficiency enhances susceptibility to carcinogens, and Nrf2 activators prevent or delay tumor development in many preclinical models, regardless of cancer type or the carcinogen used. Cells in the lung, which are continuously exposed to abundant reactive oxygen and nitrogen species as well as carcinogens, especially rely on this important defense pathway to maintain cellular homeostasis. Upon oxidative/electrophilic stress or heme accumulation, the negative regulator Keap1 is inactivated and targeted for proteasomal degradation while Nrf2 translocates to the nucleus. In the nucleus, NRF2 dimerizes with Mafs promoting the transcription of antioxidant target genes, including HO-1. Both levels of NRF2 and HO-1 (HMOX1) predict survival in lung adenocarcinoma, when KRAS is overexpressed. However, the role of NRF2 in the functionality of the immune cells within the TME in lung cancer and the role of this pathway in carcinogenesis are poorly understood. This work aims to fully understand the role of NRF2→HO-1 expressing macrophages in lung cancer progression in the presence on KRAS mutations.

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

Lung cancer, NRF2→HO-1 pathway, macrophages, clodronate liposomes

**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.*

**Specific Aim 1:** Immune suppressive HO-1 positive macrophages promote tumor growth in KRAS mutated lung cancer

Major Task 1B: Determine the effects of lung cancer conditioned media on the upregulation of NRF2 and HO-1 in RAW264.7 cells and BMDM from A/J and NRF2 KO mice

Major Task 1C: Assess the effects of macrophage depletion in lung cancer progression in A/J mice

Major Task 1E: Evaluate the effects of NRF2 KO macrophages chimeras on lung cancer progression

**Specific Aim 2:** NRF2 activated macrophages in KRAS transformed tumors decrease the activity of anti-tumor T cells, by increasing exhaustion phenotypes in cytotoxic T cells.

Major Task 2A: Evaluation of in vitro T cell activation by NRF2 wild type and KO macrophages

Major Task 2B: In vivo evaluation of T cell activation/exhaustion in NRF2 wild type mice and macrophage KO chimeras. N=48 (24 WT, 24 NRF2 chimeras), 6/group/time point

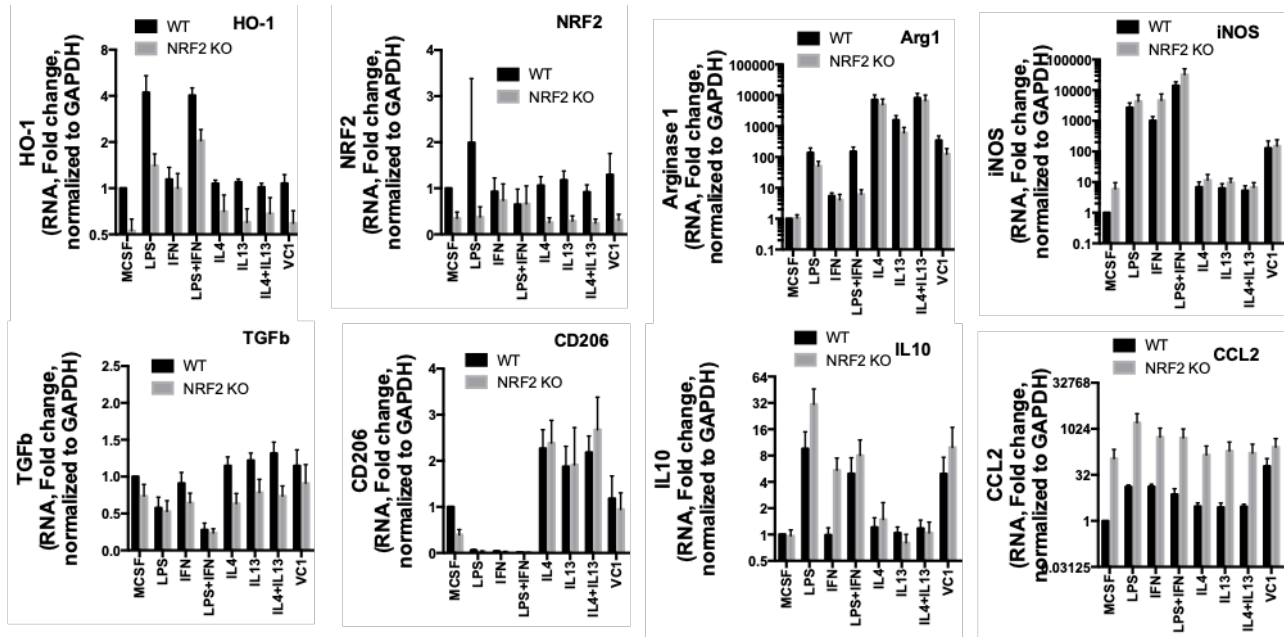
## What was accomplished under these goals?

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.

During the period here reported the major goal was to establish a robust colonies of A/J mice of both genotypes, wild type, and KO. While WT mice reproduce well with litters on average 1 month apart, and with expected numbers of pups (5-6), KO A/J mice are less productive. Typically, the first litter of pups died with the first days of birth, or if the pups survive, they are smaller in size and the number of pups/litter is on average much smaller, with only 2-4 pups per litter. Due to this unexpected trait, accruing sufficient numbers of mice for the in vivo studies is taking longer than expected.

## **Specific Aim 1: Immune suppressive HO-1 positive macrophages promote tumor growth in KRAS mutated lung cancer**

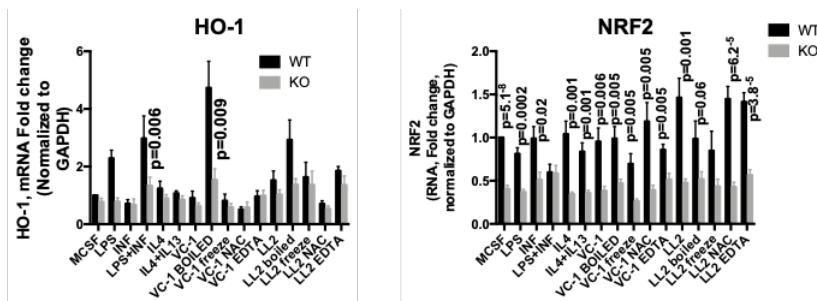
**Major Task 1B:** Determine the effects of lung cancer conditioned media on the upregulation of NRF2 and HO-1 in RAW264.7 cells and BMDM from A/J and NRF2 KO mice – subtasks 2-5



**Figure 1: Conditioned media from murine lung cancer cells increases HO-1 expression in wild type (WT) bone marrow derived macrophages (BMDM).** BMDM were collected from both wild type (WT) and NRF2 KO (KO) mice and cultured for 5 days with M-CSF (10 ng/ml). On day 5 media was replaced for media containing 1%FBS and LPS (1 ng/ml), interferon gamma (IFN) (10 ng/ml), IL4 (10 ng/ml), IL13 (10ng/ml) or conditioned media from murine lung cancer cells (VC1), and BMDM cultured for additional 24 hours. Conditioned media was generated by culturing VC1 cells at 70-80% confluency for 24 hours in 1%FBS media. Levels of the indicated markers were determined by RT-PCR.

To determine the effects of KRAS mutation on macrophage polarization and the role of NRF2→HO1 pathway, bone marrow derived macrophages (BMDM) from both A/J wild type and

full body NRF2 KO were utilized. Monocytes are collected from the marrow of the femur and tibia bones and differentiated into macrophages using M-CSF (10 mg/ml) for 5 days. On day 5 media is replaced with reduced serum (1% FBS) and BMDM are further stimulated with LPS (1ng/ml), interferon (IFN) (10 ng/ml), IL4 (10ng/ml), IL13 (10 ng/ml) or conditioned media from lung cancer cells (VC1). VC1 cells were generated from A/J mice bearing lung tumors. Conditioned media is generated by culturing VC1 cells at 70-80 confluence for 24 hours in reduced serum (1% FBS). As expected, levels of HO-1 and NRF2 are higher in BMDM of WT mice compared with NRF2 KO mice, in all conditions, with exception of interferon stimulation. Likely HO-1 levels in interferon stimulation are not dependent on NRF2 activation/presence (Figure 1). To further characterize the BMDM generated, mRNA levels of macrophage polarization markers (iNOS, Arginase, CCL2, CD206, IL10 and TGFb) were analyzed. No differences were observed in the levels of iNOS,



**Figure 2: Boiled conditioned media from lung cancer cells increases expression of HO-1 in BMDM.** Levels of HO-1 and NRF2 in wild type (WT) and NRF2 KO (KO) bone marrow derived macrophages stimulated with inflammatory cytokines or conditioned media from two murine lung cancer cells, for 24 hours. Conditioned media was boiled (15 minutes) or frozen (3 cycles of freeze/thaw) or treated with antioxidant NAC (1M) or chelator EDTA (1M).

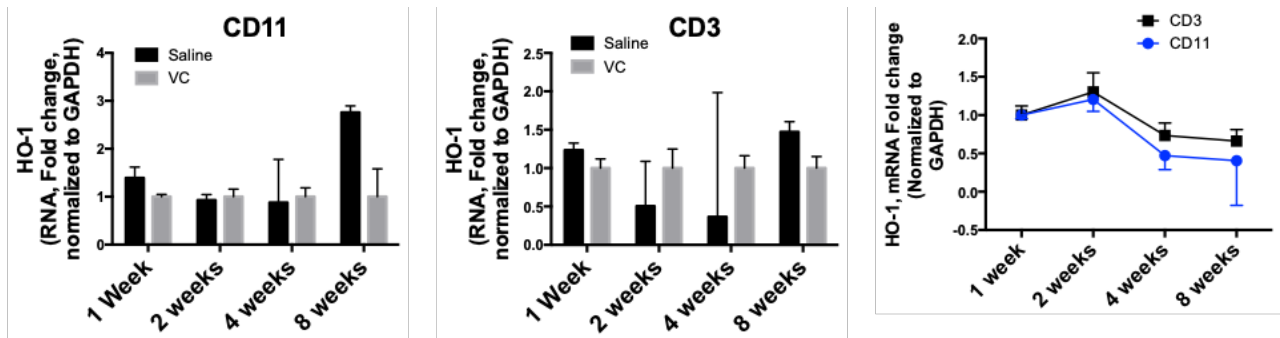
Arginase, CCL2, CD206, IL10 and TGFb between WT and KO BMDM. Levels of other markers, such as IL1b, IL33 and PDL1 are underway. Additionally, protein levels of HO-1 and NRF2 will be tested. RAW cells (macrophage-like cells) were already treated with the same conditions as in Figure 1 and western blots will be run in the future. Flow cytometry will be used to confirm the data generated by RT-PCR for cell

surface markers and cytokines.

To determine the factors that are secreted from cancer cells that lead to the increase in HO-1, BMDM were stimulated with conditioned media from two murine cell lines, VC1 and commercially available LL2 cells. Interestingly, in both cell lines the levels of HO-1 are elevated after the media is boiled for 15 minutes. Small decreases are observed when conditioned media is treated with NAC (N-acetyl cysteine), but no changes are observed with EDTA (Figure 2). NAC can act both as an antioxidant and a chelator. Both data combined suggest that cancer cells are secreting an oxidative specie that is likely conjugated with a protein waste, I do hypothesize that cancer cells are secreting heme, and that upon boiling releases iron, that can strongly activate HO-1 in macrophages. To further elucidate this, boiled conditioned media will be treated with EDTA, NAC and Iron Chelator IV, 21H7. Levels of HO-1 will be determined. Additionally, RT PCR for macrophage polarization markers (iNOS, Arginase, CCL2, CD206, IL10 and TGFb) will be analyzed.

*Subtask 6:* Isolate CD11b positive cells from lungs of A/J WT and NRF2 mice after stimulation with vinyl carbamate. Lungs will be collected every week for 8 weeks. CD11b cells will be characterized by RT-PCR and/or western blot.

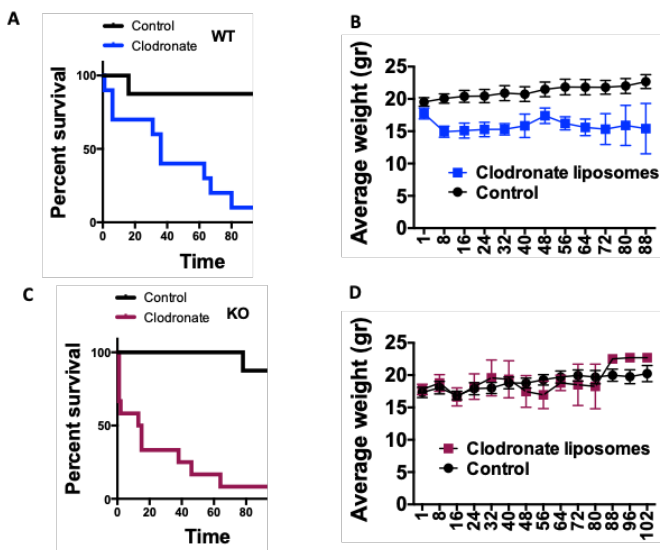
To determine the evolution of HO-1 expression in macrophages in the lung after tumorigenesis initiation with vinyl carbamate, lung of A/J mice were collected at 1, 2, 4, and 8 weeks after the last vinyl carbamate injection. Vinyl carbamate is a carcinogenic compound that will lead to the formation of lung adenocarcinomas in A/J mice approximately 12 weeks after administration. Typically, 2 injections are given to 7–8-week-old A/J mice with 1 week of interval. Saline injections



**Figure 3: Vinyl carbamate stimulation increases the expression of HO-1 in CD11 and CD3 cells.** HO-1 levels on CD11 and CD3 cells isolated from lungs of A/J mice after vinyl carbamate stimulation. 7-8 weeks old A/J mice were injected with vinyl carbamate or saline on days 1 and 7. Lungs were collected and digested to obtain a single cell suspension. CD3 and CD11 cells were isolated by magnetic cell sorting. HO-1 levels in CD11 positive (A) or CD3 positive (B) cells from lungs stimulated with saline or vinyl carbamate (VC) after 1, 2, 4 and 8 weeks after the last vinyl carbamate injection. All values are normalized to the respective saline. (C) HO-1 mRNA levels over time in CD11 and CD3 positive cells, week 1 was assumed as 1. (n=4, females)

were done as controls. Mice were sacrificed and lungs were collected 1, 2, 4, and 8 weeks after the last vinyl carbamate injection. The right lobe was store in buffed formalin for further analysis by IHC. The remain lobes were lysed using a mixture of collagenase, dispase and DNase, to obtain a single cell suspension. Cells were counted to normalize to the recommended concentrations before incubating with the respective reagents for magnetic isolation of CD11 and CD3 cells. CD11 and CD3 cells isolated from each mouse (4 females per group) were tested for HO-1 levels by RT-PCR. Levels of HO-1 were similar in vinyl carbamate and saline stimulated mice in CD11 cells. In CD3 cells HO-1 levels were elevated compared to saline on weeks 2 and 4 after stimulation. To compare the evolution of the HO-1 levels over time, all CD11 and CD3 samples from mice stimulated with vinyl carbamate, levels were compared with cells isolated at week 1. Both CD11 and CD3 cells show an increase in the levels at week 2 and decreases at weeks 4 and 8 after vinyl carbamate stimulation (Figure 3). Male mice are currently being enrolled.

**Major Task 1C: Assess the effects of macrophage depletion in lung cancer progression in A/J mice**



**Figure 4: Survival of A/J wild type (WT) (A) and NRF2 KO (KO) (C) mice when injected with clodronate liposomes.** 7-8 weeks old A/J mice were injected with clodronate liposomes or control liposomes on day 1 followed by vinyl carbamate on day 2. Vinyl carbamate was again injected on day 7 and clodronate liposomes or control liposomes were injected twice weekly until the end of the study (12 weeks). Average weight over time for WT (B) and KO mice (D). N=8-12.

To determine if macrophages with HO-1 upregulation are important players in lung carcinogenesis and lung cancer progression, clodronate liposomes were used to deplete macrophage population. A single clodronate liposome ip injection was administered before stimulation with vinyl carbamate. Clodronate liposome administration is continued until the end point (12 weeks), with 2 doses a week. Vinyl carbamate is administered twice with 1 week interval. Control/empty liposomes are used as control. Unexpectedly, the combination of vinyl carbamate and clodronate liposomes was lethal in both wild type and NRF2 KO mice. On average, wild type mice receiving

both clodronate and vinyl carbamate survive for 36 days and KO NRF2 mice survive 14 days after the first injection of clodronate liposomes (Figure 4). Only one mouse on each cohort survives until the end point of 12 weeks after the first clodronate liposome injection. Macrophages were successfully depleted in the two surviving mice. Wild type mice receiving clodronate liposomes show a minor weight loss in the first week that is not recovered over time. NRF2 KO mice do not show a difference between the mice receiving clodronate liposomes and mice receiving control liposomes.

To overcome the lethality observed mice will be given one initial dose of clodronate liposomes on day one and a dose of vinyl carbamate on day 2. Mice will be allowed to recover for 1 week and the clodronate liposome administration will be reestablished. A single dose of vinyl carbamate will be used, since is sufficient for tumor induction. Mice will be given additional nutritional support in the form of gel food, to increase food and water intake.

**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.*

Nothing to Report

**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.*

Nothing to Report

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

*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.*

Increase breeding of A/J mice and more closely monitoring for more viable pregnancies and healthy pups. Most mice should be available for enrollment as late as April. Most experiments involving mice should be completed by September.

- 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?**

*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).*

Nothing to Report

**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.*

Nothing to report

**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.*

Nothing to report
-------------------

**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:*

**Changes in approach and reasons for change**

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.*

Toxicity in the combination of vinyl carbamate and clodronate liposomes: reduce the 2 doses of vinyl carbamate to 1 dose; and allow mice time to recover before administering clodronate liposomes after vinyl carbamate. Gel food is now provided to increase mice nutritional uptake.

Reduced breeding ability in A/J NRF2 KO mice: increase breeding

**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.*

Difficulties and maintaining student worker in the summer: Most of mouse colony work is done by students, however, students are rarely available in the summer months (May to September). This reduces the ability to process samples and enroll mice necessary for the objectives.

**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**

Nothing to report

**Significant changes in use or care of vertebrate animals**

Due to mortality of animals receiving both clodronate liposomes and vinyl carbamate, nutritional support is now provided in the form of gel food. Gel food allows the animal to have access to enriched nutrients and water.

**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 (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.*

Nothing to report

- **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: *Ashley Rowell*  
Project Role: *Student Worker*  
Researcher Identifier (e.g. ORCID ID): *n/a*  
Nearest person month worked: *3*

Name: *Lizbeth Lockwood*  
Project Role: *Technician*  
Researcher Identifier (e.g. ORCID ID): *n/a*  
Nearest person month worked: *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?**

*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 Principal Investigator (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://ebrap.org/eBRAP/public/index.htm> for each unique award.*

**QUAD CHARTS:** *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil/Pages/Resources.aspx>) 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.*