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TITLE: Regulation of Lung Cancer Dormancy and Recurrence by Stress-Activated Neutrophils

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CONTRACTING ORGANIZATION: The Wistar Institute of Anatomy & Biology

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Fort Detrick, Maryland 21702-5012

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1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
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Regulation of Lung Cancer Dormancy and Recurrence by Stress-Activated Neutrophils

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Philadelphia, PA 19104-4265

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Lung cancer is the second most common cancer in veterans. It represents critical medical challenge for veterans in their families. This proposal seeks to address the major problem – lung cancer recurrence after successful initial treatment and its therapeutic correction. We, for the first time, will establish the role of stress and PMN in reactivation of tumor dormant cells and lung cancer recurrence and identify the mechanism of this process. We will determine the link between the presence of norepinephrine and S100A9 and lung cancer recurrence in cancer patients. We will test, for the first time, the possibility of reducing lung cancer recurrence by blocking S100A9/A proteins and β2 adrenergic receptor. The main objective of this study is to identify the mechanism of recurrence of lung cancer and to determine therapeutic targeting strategy to control this process. We propose that reactivation of dormant tumor cells in lung cancer could be caused by prolonged stress with release of neuroendocrine adrenergic hormones. These hormones induce activation of PMN resulting in their sequestration in lungs with release of S100A9/A8 proteins. We propose that binding of S100A9/A8 to receptors on PMN promotes lipid peroxidation via induction of reactive oxygen species and activation of myeloperoxidase (MPO). Local release of oxidized lipids directly drives re-activation of dormant tumor cells. Targeting of S100A9/A8 with Tasquinimod and use of β2-adrenergic receptor blockade can substantially reduce tumor recurrence. We expect to offer novel mechanism of therapeutic regulation of lung cancer recurrence. If successful, it may have major impact for patients' survival.

15. SUBJECT TERMS
mechanism of recurrence of lung cancer, S100A9/A8, Tasquinimod

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

Lung cancer is the second most common cancer in veterans. It represents critical medical challenge for veterans in their families. This proposal seeks to address the major problem – lung cancer recurrence after successful initial treatment and its therapeutic correction. We, for the first time, will establish the role of stress and PMN in reactivation of tumor dormant cells and lung cancer recurrence and identify the mechanism of this process. We will determine the link between the presence of norepinephrine and S100A9 and lung cancer recurrence in cancer patients. We will test, for the first time, the possibility of reducing lung cancer recurrence by blocking S100A9/A proteins and β2 adrenergic receptor. The main objective of this study is to identify the mechanism of recurrence of lung cancer and to determine therapeutic targeting strategy to control this process. We propose that reactivation of dormant tumor cells in lung cancer could be caused by prolonged stress with release of neuroendocrine adrenergic hormones. These hormones induce activation of PMN resulting in their sequestration in lungs with release of S100A9/A8 proteins. We propose that binding of S100A9/A8 to receptors on PMN promotes lipid peroxidation via induction of reactive oxygen species and activation of myeloperoxidase (MPO). Local release of oxidized lipids directly drives re-activation of dormant tumor cells. Targeting of S100A9/A8 with Tasquinimod and use of β2-adrenergic receptor blockade can substantially reduce tumor recurrence. We expect to offer novel mechanism of therapeutic regulation of lung cancer recurrence. If successful, it may have major impact for patients' survival.

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

mechanism of recurrence of lung cancer, S100A9/A8, Tasquinimod

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: To identify the mechanism of PMN-mediated reactivation of dormant tumor cells in the models of genetic and chemotherapy-induced dormancy

Major Task 1: Identify the mechanism of PMN effect on reactivation of tumor dormancy

Subtask 1. Establish mouse tumor models 30 mice using mouse cell lines KPR, LL2 and human commercial (ATCC) cell lines A549, H1299 cell lines.

Subtask 2. Mechanism of the effect of S100A9/A8 proteins on PMN ability to reactivate dormant cells. Assayed via cell counts, Western blots, activity assays, and antibody inhibition experiments.

Subtask 3. The role of oxidized lipids in reactivation of dormant tumor cells. Assessed with LC-MS, MS, and MS/MS, and by tumor cell count after exposure to norepinephrine (NE).

Milestone(s) Achieved: **(1)** Mouse tumor models are established**, (2)** Specific mechanism of S100A9/A8 effect on PMN is identified, **(3)** Profile of oxidized lipids generated in PMN in response to S100A9/A8 is determined.

ACURO Approval will be obtained before start of the project

Major Task 2 Determine the effect of stress on PMN ability to reactivate dormant cells.

Subtask 1. Effect of stress (NE exposure) on reactivation of dormant tumor cells by PMN in vitro

Subtask 2. Validation of mouse model of stress by measuring NE levels.30 mice (KPRA, LL2cisA, A459cisA)

Subtask 3. Effect of stress on reactivation of dormant tumor cells by PMN - 60 mice

Subtask 4. The role of FGF signaling in regulation of PMN mediated reactivation of tumor dormancy: determine whether the effect on FGF pathway can be reproduced by treatment of cells with NE, and whether it would be cancelled in the presence of PMN that are deficient for MPO.

Milestone(s) Achieved: **(1)** Establishing mouse model of stress and activation of dormancy; **(2)** Identification of the effect of PMN on reactivation of tumor dormancy in stress in vitro and in vivo**; (3)** Identification of the mechanism of FGF involvement in reactivation of tumor dormancy

Specific Aim 2: To determine therapeutic effect of targeting S100A9/A8 and β2-adrenergic receptors on re-activation of dormant tumor cells.

Major Task 3. Therapeutic regulation of tumor dormancy in vivo.

Subtask 1. Regulation of tumor recurrence in the model of chemotherapy-induced senescence – 120 mice

Assess the ability of Tasquinimod to abrogate reactivation effect of PMN in stress induced models (KPRA, LL2cisA, A459cisA). Similar experiments will be performed with β2-adrenergic receptor blocker ICI-118,551. C57BL/6 mice with orthotopic LL2 tumor will be treated with chemotherapy until complete tumor regression, then exposed to stress. Mice will be treated with Tasquinimod and ICI-118,551 as above, and reactivation of tumor growth will be measured (40 mice)

Major Task 4. Evaluation of clinical correlation between the presence of S1009/A8 and NE and tumor recurrence – 120 patients

HRPO Approval

Subtask 1. Collect blood samples from patients with stage I-II NSCLC who undergo complete tumor resection (n=120)

Subtask 2. Determine whether the amount of S100A9/A9 and NE in plasma of cancer patients correlates with the time of recurrence.

Milestone(s) Achieved: **(1)** Identification of the effect Tasquinimod and ICI-118,551 on reactivation of tumor dormancy, **(2)** Identification of the effect Tasquinimod and ICI-118,551 on reactivation of tumor dormancy caused by chemotherapy, **(3)** Establishing link between S100A9, NE and early recurrence in patients with NSCLC.

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.

Tumor recurrence years after seemingly successful treatment of primary tumors is one of the major causes of mortality in cancer patients. Reactivation of dormant tumor cells is largely responsible for this phenomenon. Using models of lung and ovarian cancer, we found a specific mechanism that may govern this process mediated by stress and neutrophils. Stress hormones cause rapid release of S100A8/A9 proteins by neutrophils. S100A8/A9 induce activation of myeloperoxidase resulting in accumulation of oxidized lipids. These lipids up-regulate fibroblast growth factor pathway in tumor cells causing tumor cell exit from the dormancy and formation of tumor lesions. Higher serum levels of S100A8/A9 were associated with shorter time to recurrence in patients with lung cancer after complete tumor resection. Targeting of S100A8/A9 or β2 adrenergic receptors abrogated stress induced reactivation of dormant tumor cells. These observations demonstrate a mechanism linking stress, and specific neutrophils activation with early recurrence in cancer.

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

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

N/A

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).

This work open an opportunity to reduce tumor recurrence in cancer patients by interfering with multiple mechanisms discovered in this study.

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

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

No changes in approach or objectives.

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.

There was no problems and delays. Action plan is mot applicable since project is terminated.

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.

No changes

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

Human subjects accrual for this study did not take place. Protocol was approved by IRB and submitted to ACRO for its approval in September of 2019. However, the review was delayed until February 2020 apparently due to changes in personnel. Covid19 pandemic prevented from patients accrual.

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).*

One manuscript is currently submitted after revision. Information will be provided after it is accepted for publication.

  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).*

None

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.*

None

- **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.

None

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

None

- **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.

None

- **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.*

None

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".

The following personnel devoted effort to this project in the time period specified:
D. Gabrilovich 1.3 cm
A. Hashimoto 7 cm.

Name:	*Dmitry Gabrilovich*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*PI and oversight for the project*
Funding Support:	*This grant.*
Name:	*Ayumi Hashimoto*
Project Role:	*Postdoctoral Associate*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*7*
Contribution to Project:	*Performed vertebrate animal experiments*
Funding Support:	*This grant.*

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.

All active support ended when Dr. Gabrilovich left Wistar.

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*

University of Pittsburgh, Pittsburgh, PA
Collaboration – Dr. Valerian Kagan

8. SPECIAL REPORTING REQUIREMENTS

Here per contract:

Award Expiration Transition Plan: The Award Expiration Transition Plan (available on https://ebrap.org/eBRAP/public/Program.htm) must be submitted as an appendix to the final report.

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.*