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TITLE: Targeting a stress-derived immunosuppressive adenosine pathway in tumors resistant to checkpoint inhibitors

PRINCIPAL INVESTIGATOR: Yong Qin

CONTRACTING ORGANIZATION: The University of Texas MD Anderson Cancer Center Houston, TX

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14. ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) and uveal melanoma (UM), a subtype of melanoma that begins in the eye, are not responsive to immune checkpoint inhibitors (CPIs), such as ipilimumab or pembrolizumab. The accumulation of adenosine in the tumor microenvironment acts as a negative regulator for both the activation and effector phases of the anti-tumor T cell response. It is known that CD73 and A2AR are crucial factors in the immunosuppressive adenosine pathway. Up till now, a major gap lies in our knowledge of the role of adenosine pathway driving immune s uppression in UM and PDAC. In this study, we will investigate a new mechanism for immune resistance driven by aberrant adenosine signaling by analyzing the immune profiles of UM and PDAC tumors relevant to CD73 and A2AR. We will also attempt to demonstrate how blockade of CD73 and A2AR to reverse the immunosuppressive tumor microenvironment. Furthermore, we will explore the strategy of combining CD73/A2AR inhibitors with immune checkpoint blockade (anti-PD-1 therapy) to overcome the immune resistance of UM and PDAC.

15. SUBJECT TERMS
NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	14	19b. TELEPHONE NUMBER *(include area code)*

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

Pancreatic ductal adenocarcinoma (PDAC) and uveal melanoma (UM), a subtype of melanoma that begins in the eye, are not responsive to immune checkpoint inhibitors (CPIs), such as ipilimumab or pembrolizumab. The accumulation of adenosine in the tumor microenvironment acts as a negative regulator for both the activation and effector phases of the anti-tumor T cell response. It is known that CD73 and A2AR are crucial factors in the immunosuppressive adenosine pathway. Up till now, a major gap lies in our knowledge of the role of adenosine pathway driving immune suppression in UM and PDAC. In this study, we will investigate a new mechanism for immune resistance driven by aberrant adenosine signaling by analyzing the immune profiles of UM and PDAC tumors relevant to CD73 and A2AR. We will also attempt to demonstrate how blockade of CD73 and A2AR to reverse the immunosuppressive tumor microenvironment. Furthermore, we will explore the strategy of combining CD73/A2AR inhibitors with immune checkpoint blockade (anti-PD-1 therapy) to overcome the immune resistance of UM and PDAC.

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

Pancreatic Ductal Adenocarcinoma, Uveal Melanoma, Adenosine signaling Pathway, CD73, A2AR, Immune Checkpoint Inhibitors, Tumor Microenvironment, Combinational Therapy, Immune Resistance.

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.

PROPOSED START DATE August 01, 2019

IRB/HRPO Approval
To establish institution-approved IRB protocols for this project (Proposed 05/01/2019-07/30/2019) IACUC/ACURO approval (Proposed: 02/01/2020 – 05/01/2020)

The research protocol, "Characterization of Immune Signature Related to Adenosine Pathway in Tumors Resistant to Immunotherapy" (PA19-0801), was submitted by Dr. Yong Qin and approved by the University of Texas MD Anderson Cancer Center (MDACC) Institutional Review Board 2 (IRB2) on 09 October 2019. On 11 June 2020, The U.S. Army Medical Research and Development Command (USAMRDC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) reviewed the protocol and found that it complies with applicable DOD, U.S. Army, and USAMRDC human subjects protection requirements. The protocol PA19-0801 was activated at MDACC on 6/18/2020.

The IACUC protocol, "Evaluation of the antitumor immune response of combining checkpoint inhibitors with blockade of adenosine signaling (co-inhibition of CD73 and A2AR) in humanized models of uveal melanoma and pancreatic ductal adenocarcinoma" (00001979-RN00), was submitted by Dr. Yong Qin to the Institutional Animal Care and Use Committee at MDACC on 03/20/202. The protocol is currently under review by the IACUC at MDACC.

Due to the approval date of the IRB protocol and pending IACUC protocol, the research works of the following major tasks were not conducted during 08/01/2019 – 7/30/2020.

Specific Aim 1: To Quantify and evaluate CD73 and A2AR expressions and their clinical relevance in uveal melanoma (UM) and pancreatic ductal adenocarcinoma (PDAC) tumors.

Major Task 1: To characterize profiles of CD73 and A2AR in UM and PDAC tumors. (Proposed: Months 1-5)

Major Task 2: To characterize the immune infiltrates and their correlations with CD73 and A2AR in UM and PDAC tumors. (Proposed: Months 5-16)

Specific Aim 2: To enhance immune response in UM and PDAC tumors by small inhibitors targeting CD73 and A2AR, and further examine their anti-tumor effects in combination with CPIs.

Major Task 1: To examine the effects of CD73 and A2AR inhibitors on the growths of UM and PDAC cells in vitro. (Proposed: Months 1-5)

Major Task 2: To examine the efficacy of CPIs (anti-PD-1 or anti-CTLA4 drug) with CD73/A2AR inhibitors on antitumor immune cell response, especially for cytotoxic T lymphocytes, in vitro. (Proposed: Months 5-15)

Major Task 3: To test the efficacy of combining CPIs (anti- PD-1 or anti-CTLA4 drug) with blockade of adenosine signaling (co-inhibition of CD73 and A2AR) on antitumor immune response in humanized mouse models of UM and PDAC. (Proposed: Months 10-24)

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.

1) Major activities;

During 08/01/2019 – 7/30/2020, the IRB and IACUC protocols to supports this research project were developed. The IRB protocol was approved by MDACC IRB and The U.S. Army Medical Research and Development Command (USAMRDC), Office of Research Protections (ORP),

Human Research Protection Office (HRPO). The IACUC protocol is currently under review by the Institutional Animal Care and Use Committee at MDACC.

No research work has been conducted for this project from 08/01/2019 – 7/30/2020, due to the delay of IRB and IACUC protocol approval and the pandemic. There was no result or key outcome to report.

4) Other achievements.
In collaboration with Dr. Sapna Patel at MDACC, 20 metastatic UM tumors were identified from the R21 project led by Dr. Patel (NCT01585194). The pathologist further evaluated these tumor tissues for the study of Specific Aim 1 of this project.

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.

As a junior faculty (Instructor) at MDACC, this project provided training for Dr. Yong Qin to independently develop IRB and IACUC protocols and manage a grant as a principal investigator. Dr. Sapna Patel and Faculty Committee at the Department of Melanoma Research Oncology at MDACC provide mentorship and support for Dr. Qin's professional development.

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.

Due to the new appointment of Dr. Yong Qin as the tenure-track assistant professor at the University of Texas at El Paso (UTEP), Dr. Qin will lead and establish collaboration between UIEP and MDACC for this project. Dr. Qin and Dr. Sapna Patel will collaborate to conduct the proposed study.

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).

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
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.

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.

1. As a junior faculty and fist-time PI, Dr. Qin had to get all certifications for establishing research protocols at MDACC. The prolonged time for developing the IRB and IACUC protocols had delayed the research works of this project. With the help from the Department of Melanoma Research Oncology at MDACC, the IRB protocol (PA19-0801) was activated on 6/18/2020. The IACUC protocol is currently under review by the committee at MDACC. 2. Dr. Qin was delayed to come back to the States during the oversea travel in January 2020, due to the restriction of international travel caused by the COVID-19 pandemic. Although Dr. Qin was back to work on 3/16, all research works and laboratories at MDACC were shut down on 3/23/2020 in response to the pandemic. On 6/17/2020, Limited research (Phase II opening) was permitted by MDACC for the research group of Dr. Qin and others. The protocol review and research works related to this project were significantly postponed. After re-opening research at MDACC, we expected the review process for the IACUC protocol would be expedited.

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.

The shutdown of the research laboratories at MDACC had significantly impeded the research works related to this project. The lack of on-site study and remote work delayed the protocol review and postponed the conduction of relevant experiments.

Dr. Yong Qin will start a new job as an Assistant Professor at the University of Texas at El Paso on 7/1/2020. Dr. Qin's job transition of will affect the grant and institutional transitions.

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

Nothing to Report.

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:	Yong Qin
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-4743-938X
Nearest person month worked:	3
Contribution to Project:	Dr. Qin had established IRB and IACUC protocols to support research works of this project.
Funding Support:	The fund from the Department of Melanoma Medical Oncology at MDACC.

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://ers.amedd.army.mil*** *for each unique award.*

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