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TITLE:
Enhancing Immunotherapy with Novel Combinations to Improve the Treatment of Primary and Metastatic Colon Cancer"

PRINCIPAL INVESTIGATOR:
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CONTRACTING ORGANIZATION:
University of Illinois at Chicago

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14. ABSTRACT

Our goal is to improve the control of advanced colon cancer by enhancing anti-tumor immune responses by stimulating the immune system to increase the infiltration and activation of anti-tumor lymphocytes in colon cancer tumors. We believe that the tumor microenvironment can be manipulated to enhance cytotoxic lymphocyte infiltration and activation, and that immune-checkpoint blockade with anti-CTLA4 therapy can be simultaneously utilized to augment a clinically relevant immune response. Once it is known how to enhance immunostimulatory signals in the tumor microenvironment and simultaneously suppress immunosuppressive influences, a new strategy for the management of colon cancer with the potential for durable anti-tumor responses and improved survival is possible. In this time period we have prepared data to investigate immunostimulation of colon cancer cells, preparing them for enhanced lymphocyte proliferation and susceptibility to anti-CTLA4 checkpoint blockade.

15. SUBJECT TERMS
Immunotherapy, colon cancer, LIGHT, CTLA4, checkpoint blockade, TIL

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

Our goal is to improve the control of advanced colon cancer by enhancing anti-tumor immune responses by stimulating the immune system to increase the infiltration and activation of anti-tumor lymphocytes in colon cancer tumors. We believe that the tumor microenvironment can be manipulated to enhance cytotoxic lymphocyte infiltration and activation, and that immune-checkpoint blockade with anti-CTLA4 therapy can be simultaneously utilized to augment a clinically relevant immune response. Once it is known how to enhance immunostimulatory signals in the tumor microenvironment and simultaneously suppress immunosuppressive influences, a new strategy for the management of colon cancer with the potential for durable anti-tumor responses and improved survival is possible.

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

Immunotherapy, colon cancer, LIGHT, CTLA4, checkpoint blockade, TIL

2. **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.

The goals of the project were to develop and test strategies to deliver immunostimulatory signals into the microenvironment, and to then perform pre-clinical studies combining selective delivery of immunostimulatory cytokines with systemic checkpoint blockade. We endeavored to increase anti-tumor immune responses by impacting regulatory inhibition in the tumor microenvironment. Our major goals for year 1 were to elucidate the mechanistic impact of CTLA4 blockade on LIGHT-induced TIL trafficking, proliferation, activation, and exhaustion; and to quantify the impact of combined treatment on T-regulatory cells. This was projected in the SOW to be done over the first 12 months.

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.) The major activity was to determine the effect of combination immunotherapy on T-cell proliferation, activation, and exhaustion. Over the next 2-3 quarters, it was also planned for tumors to be harvested for immunohistochemistry.
2.) The specific objectives were to utilize colorectal cancer flank tumors of CT26LIGHT inducible cells that had received LIGHT + anti-CTLA4 or isotype control. Cells from these spleens and tumors were previously harvested and in vitro analysis was performed herein in preparation for the larger proposed experiments. V
3.) We were excited to demonstrate that splenocytes in combination immunotherapy treated cells expressed significantly higher amounts of ICOS in CD4+ and CD8+ T-cells. Furthermore, CD8 cells expressed more Ki67 and granzyme B. Combined, these exciting data support the contention that combination immunotherapy stimulated CD8 T-cell proliferation, activation, and increased CTLA-4 responsiveness (due to increased ICOS), which supports our overall hypothesis and goals of the study. There was not a difference in activation markers CD44, 62L, or total CD25 or in Treg in the spleen. In the last few months we also were able to build on these findings and evaluate a pilot of saved tumor infiltrating lymphocytes from combination treated cells. In this analysis, Ki67 trended to increase on CD4 and CD8 lymphocytes with combination immunotherapy, while there was no increase in granzyme B expression on CD8 cells, or differences in exhaustion markers. There was a trend of decreased Treg infiltrating the tumor with LIGHT+anti-CTLA4, and tetramer staining confirmed tumor specificity of T-cells in both groups. These findings required further evaluation as planned in the larger in vivo experiments of specific aim 1 that were to follow.
4.) In preparation for quantification of tumor infiltrating lymphocytes of various lineages proposed to be tested in large scale immunohistochemistry, we optimized our imaging platform using fluorescent immunohistochemistry.

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."

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.

We presented our data at department research meetings and with the UIC Cancer Center.

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.

Nothing to report. Final report

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).

Our preliminary data in preparation for more intensive in vivo studies, has implied that our mechanism to increase the immunogenicity of colon cancer cells may also sensitize the cells to CTLA4 checkpoint blockade, which when administered, may increase cytotoxic T-cell proliferation.

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.

Mechanisms to increase sensitivity to checkpoint blockade will be applicable to multiple tumor types.

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.*

As we improve the efficacy of immunotherapy, the public knowledge of tumor immunology will be improved, and a distinction between chemotherapy and immunotherapy will be easier to comprehend.

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:*

Nothing to report – all has been previously reported in writing.

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.

ACURO response to the submission given at the time of the award was not received for many months. It was recently received and clarifications were made with our office of animal care.

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

none

Significant changes in use or care of vertebrate animals

none

Significant changes in use of biohazards and/or select agents

none

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. Limited time period

- **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: *Ajay Maker*
Project Role: *PI*
Research Identifier (ORCID): *0000-0002-8234-2762*
Nearest person month worked: *2CM*
Contribution to Project: directly supervised Dr. Qiao and directed all aspects of the proposed studies, overall experimental activity, and coordinated and supervised the efforts of other participants

Name: *Guilin Qiao*
Project Role: *Research Assistant*
Nearest person month worked: *5CM*
Contribution to Project: responsible for maintaining cell lines, performing in vitro assays and in vivo studies, flow cytometry

Name: *Bellur Prabhakar*
Project Role: *Co-I*
Researcher Identifier (e.g. ORCID ID): *0001-9815-9850*
Nearest person month worked: *1CM*
Contribution to Project: assisted Dr. Maker in the design of T-cell assays and the design of in vivo models proposed in the grant.

*funding supports from this award

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

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.*