

AWARD NUMBER: W81XWH-19-1-0603

TITLE: Targeting the Microbiome to Enable Immunotherapeutic Efficacy in Pancreatic Carcinoma

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CONTRACTING ORGANIZATION: New York University

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# REPORT DOCUMENTATION PAGE

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<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> Our goal is to perform clinical trials in PDA patients testing strategies to modulate the microbiome to enhance the efficacy for immunotherapy. However, the optimal regimen has not been defined. We will perform experiments in mouse PDA models and in human organotypic systems which are designed to define the most efficacious microbiome modulatory regimens - either antibiotics or probiotics - to combine with immunotherapy. In Aim 1 we will test the immune-activating and tumor-protective effects of specific antibiotic and probiotic regimens in mouse models of PDA and an innovative microfluidic-based organotypic model derived from freshly resected human PDA. In Aim 2 we will determine the regimen that most effectively synergizes with immunotherapy. Aim 3 will encompass the first clinical trial in PDA targeting the microbiome as a strategy to enable immunotherapeutic efficacy. Collectively, these Aims will lead to a new treatment paradigm for PDA patients that targets the microbiome.					
<b>15. SUBJECT TERMS</b> Pancreatic Cancer, Microbiome, immunotherapy, immune suppression, bacteria, probiotics, cancer.					
<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>
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**1. INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The PRCRP Topic Area to be addressed is “Pancreatic cancer”. The PRCRP Military Relevance Focus Area is “Gaps in cancer treatment”. Pancreatic ductal adenocarcinoma (PDA) is characterized by immune-tolerance and resistance to immunotherapies. The microbiome has emerged as an important factor regulating health and disease. More specifically, we and other groups have shown that the microbiome has a pathogenic role in promoting the development of PDA and in mitigating response to therapy. Our recent published work indicates that the PDA-associated microbiome is markedly expanded by more than 1000-fold compared with the normal pancreas (Pushalkar et al, *Cancer Discovery* 2018). Further, we found that mouse and human PDA-bearing hosts exhibit bacterial dysbiosis in the gut. Moreover, we found that the microbiome corrupts tumor immunity in PDA. Ablation of the microbiome in PDA was tumor-protective, upregulated expression of checkpoint receptors on T cells, and enabled efficacy for immunotherapy in mouse models of PDA. Based on these data, the microbiome is an attractive target in the treatment of PDA.

**Hypothesis/Objective:** Our overarching hypothesis is that targeting pathogenic bacteria will augment innate and adaptive immunity in human PDA and enable successful immunotherapy of this disease. Our objective is to identify specific bacterial species and cocktails associated with immunogenic activation of the PDA tumor microenvironment. We will then translate the knowledge gained from our experiments in mouse models and human pre-clinical models to a Phase I clinical trial testing the safety and efficacy of bacterial ablation in combination with  $\alpha$ PD-1 treatment in PDA patients. We expect that this approach will broach an era of successful immunotherapy of PDA.

**2. KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).* **Pancreatic Cancer, Microbiome, immunotherapy, immune suppression, bacteria, probiotics, cancer.**

**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.*

Aim 1. To determine effect of modulation of the microbiome on innate and adaptive immunity in PDA

- a. To determine the optimal antibiotic and probiotic regimens to slow disease progression and enhance tumor immunity in mouse models of PDA
- b. To determine the influence of select probiotic bacterial taxa on tumor immune response and tumor viability in a microfluidic-based organotypic tumor model derived from freshly resected human PDA

Aim 2. To determine whether targeting the microbiome enables efficacy for immunotherapy in PDA

- a. To determine the optimal antibiotic and probiotic regimens to enable efficacy for checkpoint or costimulatory receptor-based immunotherapy in PDA in mouse models
- b. To determine whether select bacterial taxa enable efficacy for combination immunotherapy in a microfluidic-based organotypic tumor model derived from freshly resected human PDA

Aim 3. To conduct a Phase I ‘window of opportunity’ clinical trial in resectable PDA patients treated with antibiotics plus checkpoint-receptor based

- a. To determine the safety and efficacy of treatment with antibiotics plus checkpoint-receptor based immunotherapy in PDA patients
- b. To determine the effect of antibiotics plus checkpoint-receptor based immunotherapy on systemic and intra-tumoral immunity in human PDA.

### **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.*

**Please note: Covid-19 has impacted the work of this year. The grant was awarded in September 2019 and we started initial planning and recruitment of laboratory research staff. The clinical trial was activated in March 2019 (NCT03891979). In early March 2020 there was a major disruption in work due to COVID19. New York City was the most affected city in the country at that time. New York University paused all the research activities, both laboratory and clinical, in the middle of March 2020. Research activities were allowed to reopen in late June with limited capacity.**

During this shutdown period all research staff were advised to work from home on existing data analysis on microbiome and PDA. **No patient recruitment was done.** Given the difficulty accruing to the study even prior to the pandemic as well as the changing landscape in the treatment of resectable pancreatic cancer, discussion was begun regarding amending the clinical protocol study design. We try to address Aim 1 whether there is any functional changes in the microbiome which can help in developing optimal probiotics which can be used in combination with immunotherapy in PDA.

Major findings:

1. To investigate the association between the dysbiotic gut microbiome and the altered host metabolic or signaling pathways, we performed PICRUST analysis. The electron transfer carriers ( $p=0.0001$ ) and secretion system ( $p=0.003$ ) were differentially enriched in the gut of PDA patients than of non-PDA controls. The pathways of amino acid, ascorbate, and aldarate ( $p=0.01$ ), nucleotide ( $p=0.0004$ ), glycan biosynthesis and metabolism ( $p=0.05$ ), cofactors and vitamins ( $p=0.03$ ), signal transduction mechanisms ( $p=0.03$ ) and bladder cancer ( $p=0.002$ ) were significantly expanded in PDA gut. In addition, bacterial motility proteins ( $p=0.04$ ), replication, recombination and repair proteins ( $p=0.02$ ) and pathways involving bacterial invasion of epithelial cells ( $p=0.001$ ) were differentially downregulated in non-PDA control guts. On contrary, pathways involved in oxidative phosphorylation ( $p=0.004$ ), peroxisome proliferator-activated receptors (PPAR) signaling ( $p=0.005$ ) and adipocytokine signaling ( $p=0.02$ ) were upregulated in the non-PDA gut. Moreover, alanine, aspartate and glutamate ( $p=0.01$ ) and histidine metabolism ( $p=0.04$ ) as well as fatty acid biosynthesis were significantly decreased in the PDA gut.

2. We assessed the host metabolic changes in response to the dysbiotic pancreatic microbiota in PDA and healthy (non-PDA) patient population. We found that the pathways of PPAR signaling ( $p=0.05$ ) and ether lipid metabolism ( $p=0.02$ ) were significantly overrepresented while, protein machinery for replication, recombination and repair ( $p=0.05$ ) were significantly underrepresented in cancer cohorts. The metabolic pathways for fatty acid, tryptophan, lipid metabolism and biosynthesis, valine, leucine and isoleucine biodegradation, oxidative phosphorylation and bacterial toxins were enriched in PDA pancreata. In contrast, flavone and flavonol biosynthesis, alanine, aspartate and glutamate metabolism, amino acid metabolism, glycan biosynthesis and metabolism, signal transduction mechanisms and electron transfer carriers were upregulated in non-PDA pancreata.

To conclude, these host-microbiota interactions are chiefly driven by accessible metabolites produced by bacteria and those that they utilize as substrates. Thus, amalgamating metabolome and microbiome as a unique approach to functionally distinguish the microbiota in terms of their metabolic activity in relation to cancer will increase our understanding of this complex interactions. Next funding cycle we will expand our finding and larger sample size. We will also include longitudinal investigation of the microbiome and the metabolites production in the gut and the pancreata at different stages of pancreatic cancer to explicate the functions of metabolically upregulated pathways in oncogenic progression.

3. Clinical trial was closed to accrual March 2020 given difficulty enrolling resectable pancreatic cancer patients to a study without pre-operative cytotoxic chemotherapy, need for pause due to the pandemic, and transition of PI from one institution to another. Since activation, the treatment landscape has changed for early stage pancreatic cancer and currently most academic centers are employing pre-operative chemotherapy. As a result, the decision was made to redesign study to align with standard of care and better allow for more expeditious enrollment.

Next funding cycle we will open the redesigned clinical trial (see attached schema, Appendix 1) which will allow for pre-operative multi-agent chemotherapy as well as a window of opportunity to test the combination of microbiome depletion with antibiotics in combination with immune checkpoint inhibition. We will plan to report interim accrual numbers and possibly early results from the paired biopsies.

**What opportunities for training and professional development has the project provided?**

Nothing to Report

**How were the results disseminated to communities of interest?**

My co-PI presented at invited seminar at AACR meeting: The Microbiome, Viruses, and Cancer. February 2020

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

- Develop optimal antibiotic and probiotic regimens to slow disease progression and enhance tumor immunity in mouse models of PDA
- To determine the optimal antibiotic and probiotic regimens to enable efficacy for checkpoint or costimulatory receptor-based immunotherapy in PDA in mouse models
- Obtain human subject approval for new recruitment
- Submit manuscript on functional analysis of microbiome in PDA.

**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?**

Nothing to Report

**What was the impact on other disciplines?**

Nothing to Report

**What was the impact on technology transfer?**

Nothing to Report

**What was the impact on society beyond science and technology?**

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**

Nothing to Report

**Changes that had a significant impact on expenditures**

Nothing to Report

**Significant changes in use or care of human subjects, vertebrate animals, 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**

**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).*

NO

**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).*

NO

**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.*

## **AACR meeting: The Microbiome, Viruses, and Cancer**

### **Plenary Session 4: Tumor-Associated Microbiota**

Regulation of pancreatic oncogenesis by pathogens  
New York University College of Dentistry, New York, New York  
<https://www.aacr.org/meeting/microbiome-2020/program/>  
2020  
.... Orlando, Florida

Deepak Saxena,  
February 21-24,

Hyatt Regency Orlando

#### **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. In addition to a description of the technologies or techniques, describe how they will be 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. State whether an application is provisional or non-provisional and indicate the application number. 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;*
- *biospecimen 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 AND OTHER COLLABORATING ORGANIZATIONS

### Individuals who have worked on 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."

Name:	George Miller
Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Dr. Cohen was the Principal Investigator overseeing the project.</i>
Funding Support:	

Name:	<i>Wei Wang</i>
Project Role:	<i>Post Doc</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	<i>4.8</i>
Contribution to Project:	<i>Laboratory experimental design and analysis</i>

Funding Support:	
------------------	--

Name:	<i>Sorin Arman Shadaloey</i>
Project Role:	<i>Technician</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	<i>7.2</i>
Contribution to Project:	<i>Bench research</i>
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

## 8. SPECIAL REPORTING REQUIREMENTS

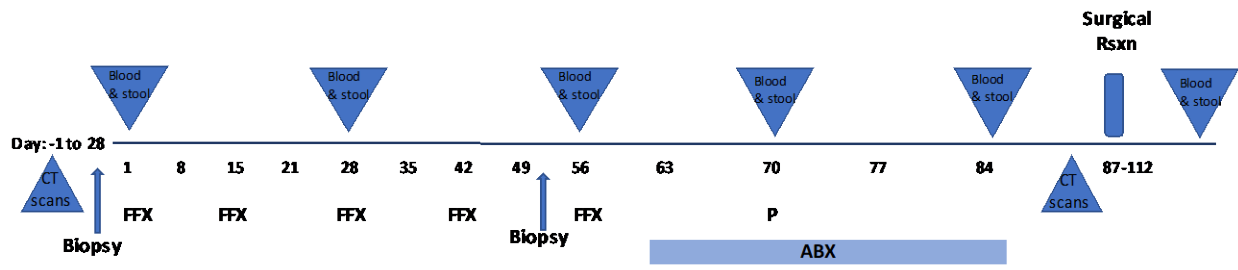
**COLLABORATIVE AWARDS:** For collaborative awards, independent reports are required from **BOTH** the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ebrap.org> 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. Reminder: Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.**

### Appendix 1: Clinical protocol schema



**Intervention:**  
 FFX: 5FU 2400mg/m<sup>2</sup>, oxaliplatin 85mg/m<sup>2</sup>, Leucovorin 400mg/m<sup>2</sup>, irinotecan 150m/m<sup>2</sup>  
 P: Pembrolizumab 200mg IV  
 Abx:  
 Ciprofloxacin 500mg PO BID & Metronidazole 500mg PO TID