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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

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.

15. SUBJECT TERMS

Pancreatic Cancer, Microbiome, immunotherapy, immune suppression, bacteria, probiotics, cancer.

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1. INTRODUCTION:

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 αPD-1 treatment in PDA patients. We expect that this approach will broach an era of successful immunotherapy of PDA.

2. KEYWORDS: Pancreatic Cancer, Microbiome, immunotherapy, immune suppression, bacteria, probiotics, cancer.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

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 immunotherapy 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?

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:

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:

Changes in approach and reasons for change

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:

Publications, conference papers, and presentations

Journal publications.

NO

Books or other non-periodical, one-time publications.

NO

Other publications, conference papers, and presentations.

AACR meeting: The Microbiome, Viruses, and Cancer

Plenary Session 4: Tumor-Associated Microbiota

Regulation of pancreatic oncogenesis by pathogens Deepak Saxena, New York University College of Dentistry, New York, New York https://www.aacr.org/meeting/microbiome-2020/program/ . February 21-24, 2020 Hyatt Regency Orlando .... Orlando, Florida

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

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:	*Deirdre Cohen*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-6178-9266
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Cohen has redesigned and re-written clinical protocol.*
Funding Support:	

Name:	*Fangxi Xu*
Project Role:	*Jr Research Scientist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*11*
Contribution to Project:	*Bench research*
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

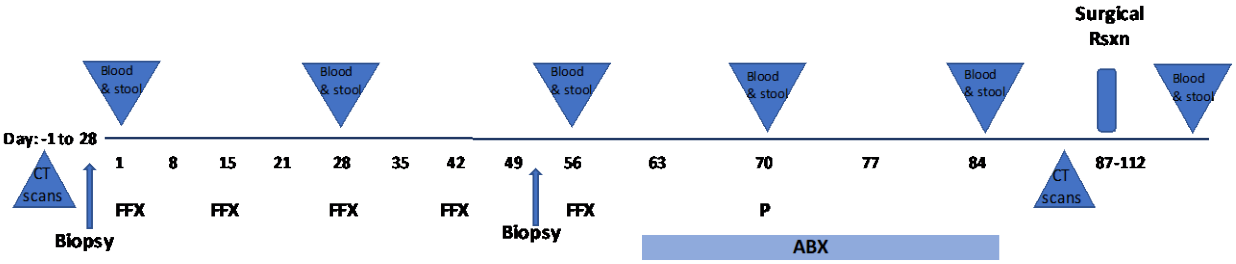
8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES.

Appendix 1: Clinical protocol schema



Intervention:
FFX: 5FU 2400mg/m², oxaliplatin 85mg/m², Leucovorin 400mg/², irinotecan 150m/m²
P: Pembrolizumab 200mg IV
Abx:
Ciprofloxacin 500mg PO BID & Metronidazole 500mg PO TID