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

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Icahn School of Medicine at Mount Sinai
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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.

16. SECURITY CLASSIFICATION OF:	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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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 αPD-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 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?
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 research staff. However, in early March 2020 there was major disruption in work due to COVID19. New York City was the most affected city in the country. New York University closed down all the research activities in the middle of March 2020. The labs were allowed to reopen in late June with limited capacity. Currently, we are working with restricted capacity in our institution.

Project 1 PI Dr. Xin Li:

Subtask 1 - Regulatory review and approval by the USAMRMC Animal Care and Use Review Office (ACURO)
Completed and approved by ACURO

Subtask 3 – Determine the optimal antibiotic and probiotic regimens to slow disease progression in mouse models of PDA. An orthotopic PdxCre; LSL-KrasG12D; p53R172H (KPC) model will be used as tumor bearing mice to receive antibiotics or bacteria by oral gavage.

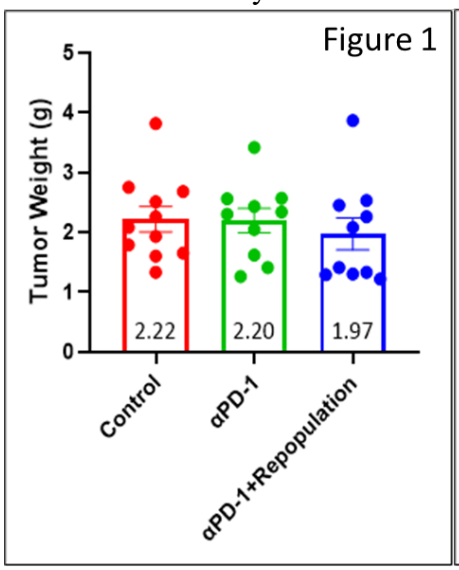
Experimental strategy. We postulate that immunogenic reprogramming of the microbiome will enhance tumor immunity in PDA. Our preliminary work that suggested an important role for the microbiome in corrupting anti-tumor immunity in PDA was performed in germ-free mice and in mice treated with broad spectrum antibiotics. However, while the use of germ-free mice and broad spectrum antibiotics are valid as proof of principal, there are critical questions that must be answered in more discriminating preclinical models to facilitate clinical translation. Specifically, we will need to determine whether a more selective antibiotic regimen which targets specific bacterial populations, rather than the entire microbiome, is protective. Further, we will test our hypothesis that re-population with probiotic immune-activating bacteria will enhance anti-tumor immunity in PDA.

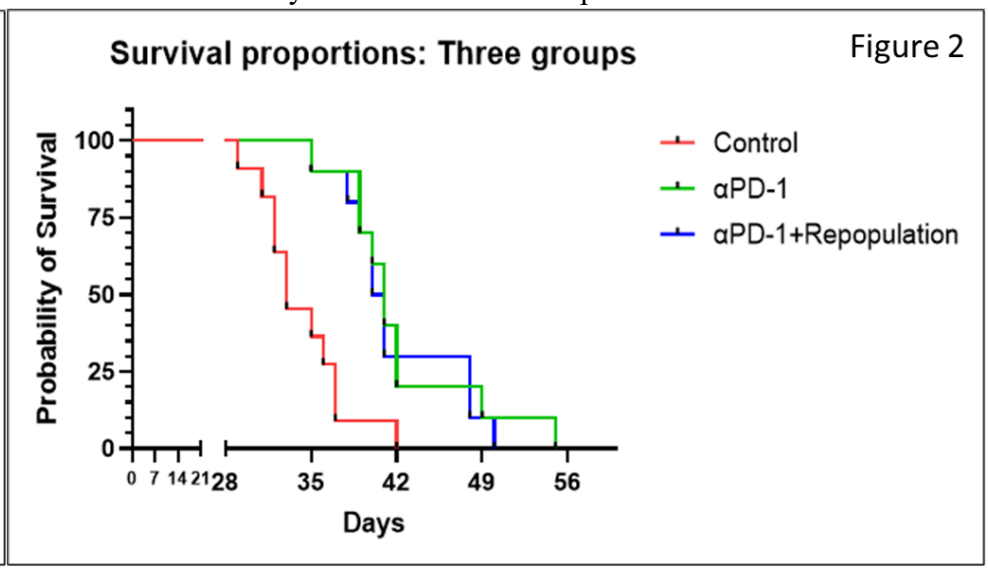
We selected Treatments regimens which included:
(i) broad spectrum antibiotics (vancomycin, neomycin, flagyl, ampicillin)
(ii) vancomycin alone
(iii) neomycin alone
(iv) flagyl alone
(v) ampicillin alone
(vi) ampicillin + flagyl

Mouse Model: We postulate that modulation of the microbiome with the selective antibiotics and/or probiotic repopulation will enhance anti-tumor immunity. Because of many experimental conditions are proposed to test, we require a high throughput in vivo system. Therefore, we initially employed an orthotopic PdxCre;LSL-KrasG12D;p53R172H (KPC) model where KPC cells are implanted in the pancreas of WT hosts. This high throughput system will allow for testing of all our antibiotic and probiotic conditions. In our initial experiment we tested one antibiotic combination and one formulation. Mice were sacrificed at experimental endpoints to measure tumor progression and intra-tumoral myeloid cell and T cell programming. We also tested the survival rate in another cohort.

(i) Tumor size and survival rate:
We used 10 -11 mice/group and implanted 40,000 cells in all of them. After depletion with a combined antibiotic, PD-1 antibody (αPD-1) was injected to two groups with one of them received probiotic formulation by oral gavage using published protocol. We used broad spectrum antibiotic in this experiment. Survival in the control and treated groups was measured by Kaplan-Meier analyses.

Results: In our initial experiment we established the feasibility of experiments and dosage. The analysis indicated there were no major differences in the tumor weight (Figure 1) however the animals with αPD1 and αPD1-repopulated with probiotic survived much longer as compared to the control group (Figure 2). We noticed the cocktail of probiotics has low solubility in the vehicle solution which could compromise the efficacy of the probiotics. We will have to modify our protocol for formulation. We have tried a few combinations and noticed that some probiotics have better solubility and we will consider to use them only in our next in vivo experiment.





Project 2 PI Dr. Deepak Saxena:

Subtask 2 – Culture probiotics and generate cocktails to share with Project 1.

In addition, based on our 16S bacterial DNA sequencing experiments we have identified a number of bacterial strains that may be protective against PDA. We have chosen these bacterial taxa as putative 'probiotics' based on their greater abundance in healthy control compared to PDA patients in our 16S analysis and an established literature designating these strains as either having immune activating effects or protecting against pathogenic bacterial colonization. Therefore, we will also treat mice with either single strains or select combinations of:

(i) Lactobacillus - Enhances innate immunity.
(ii) Akkermansia muciniphila – Improves gut barrier integrity limiting translocation.
(iii) Bifidobacterium bifidum – Helps colonization of other probiotics, enhances immunity.
(iv) Escherichia coli Nissle 1917 or Streptococcus thermophilus – Improves survival capacities of 'immunogenic' bacteria.
(v) Bifidobacterium lactis - Increases the intestinal barrier against pathogens.

As negative controls, we will also repopulate with tumor-promoting strains B. pseudolongum and F. nucleatum which we have shown promote accelerated oncogenesis.

We initiated developing probiotics using various combination of Bacteria. As proposed in Aim1 we selected 8 bacterial strains. The bacterial cultures were obtained from ATCC.

Bacterial Growth & Preparation

Bacterial strain	Liquid Growth Medium	Aerobic/Anaerobic	Growth time	Cfu/mL @ **<time>**
Lactobacillus acidophilus ATCC#4356	MRS Broth (BD 288130)	Anaerobic	14h	~3.00×10^8 cfu/mL
Lactobacillus casei ATCC#393	MRS Broth (BD 288130)	Anaerobic	14h	~2.0×10^9 cfu/mL

Lactobacillus paracasei ATCC#25302	MRS Broth (BD 288130)	Anaerobic	14h	~2.8×10^9 cfu/mL
Lactobacillus reuteri ATCC#23272	MRS Broth (BD 288130)	Aerobic	TBD	~1.9×10^9 cfu/mL
Akkermansia muciniphila ATCC#BAA-835	BHI Broth (BD 237500)	Anaerobic	TBD	TBD
Streptococcus thermophilus ATCC#19258	BHI Broth (BD 237500)	Aerobic	TBD	TBD
Bifidobacterium breve ATCC #15700	Modified Reinforced Clostridial (custom)	Anaerobic	TBD	TBD
Ruminococcus bromii ATCC#27255	PYG Broth (AS-822)	Anaerobic	TBD	TBD

Bacterial cells were grown to log-phase in 100mL of the appropriate medium (see table above).
- Cells btwn 10^{8-10} cfu/mL, depending on species.
- 100mL culture volume was produce approx. 10^{10-12} cfu total

Cells were harvested by centrifugation at 4000×g/5min/4°C, and washed 3x with sterile distilled water[1].
Resuspend cells in 5mL cryoprotectant solution (see below).

Cryoprotectant Solution

Gelatin-glycerol medium:
1.5% (w/v) gelatin
1.0% (v/v) glycerol
Mix in MilliQ water, sterilize by autoclaving on liquid cycle at 121°C/30min.
Allow to cool before using.

Microencapsulation Procedure
Polymer mix:
1.0% (w/v) xanthan gum
0.75% (w/v) gellan gum
Mix in MilliQ water, sterilize by autoclaving on liquid cycle at 121°C/15min.
Allow to cool before using.

Hardening solution:
0.2M sterile $CaCl_2$

Mix cryoprotectant-cell suspension with polymer solution (1:1) (5mL)
- 10mL should give ~10^{9-11} cfu/mL
- Collect 100µL for cfu-determination (pre-freeze drying)

Load cell suspension into syringe fitted with 21G (or similar) needle.[2]
Drop-wise, add cell suspension to $CaCl_2$ solution.

[1] To completely remove any media residue

- Allow beads to harden for 30min by sitting in $CaCl_2$ solution.

Collect beads on sterile gauze, rinse with MilliQ water.
Aliquot beads to 5g/50mL conical tube, resuspend in 5mL cryoprotectant media.
Freeze overnight at -80°C.
Lyophilize next day: may have to work out conditions, safe point to start seems -40°C/48h.

Viability Testing

Initial bacterial titer

Perform several serial-fold dilutions on 100µL aliquot from above. (to at least 10^{-8})
Plate duplicate plates from 10^{-6} and upwards.

- 100µL per plate, per dilution
- $total\ cfu = (plate\ count) \times 10 \times 10^{dilution\ factor}$

Viability of cells after lyophilization

Weigh empty 50mL conical tubes beforehand.
Weigh after lyophilization: $dry\ pellet\ weight = (pre)weight - (post)weight$

Resuspend lyophilized pellet in equal volume appropriate medium for bacterial strain.
Perform several serial-fold dilutions. (to at least 10^{-8})
Plate duplicate plates from 10^{-6} and upwards.

- 100µL per plate, per dilution
- $total\ cfu = (plate\ count) \times 10 \times 10^{dilution\ factor}$
- $\frac{cfu}{g} = total\ \frac{total\ cfu}{dry\ pellet\ weight}$

Perform viability test immediately after lyophilization, and at (time period[s]) afterwards.

Results:

We have developed 3 different formulation:

1. POC18 and 19: Using 6 strains combination.
2. POC20 (LL): Using two lactobacillus strains which has better solubility.

These formulations are being test in Project 1 (Dr Li) using KPC orthotopic models.

Project 3 PI Dr. Deirdre Cohen:

Specific Aim 3: To conduct window of opportunity clinical trial in resectable PDA patients with antibiotics plus checkpoint receptor based immunotherapy.

Subtask 1 To determine the microbiome composition in tumor tissue prior to and following treatment with antibiotics, chemotherapy and PD1 inhibition.

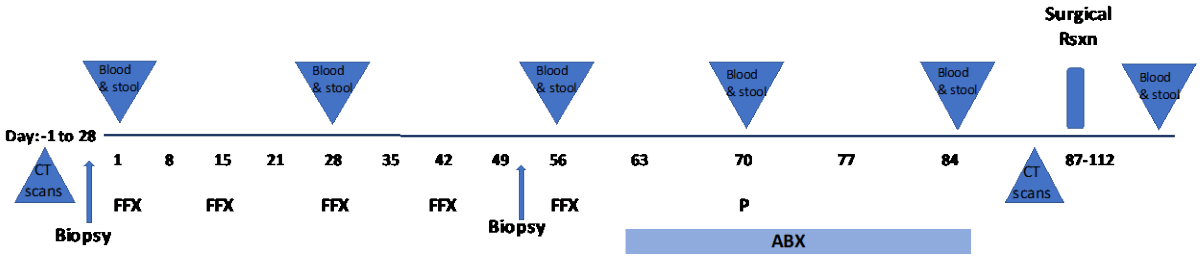
Subtask 2 To determine the microbial signature in the intestines of enrolled patients with PDAC using 16S sequencing

The clinical trial was redesigned and the protocol rewritten to include pre-operative multi-agent chemotherapy followed by a window of opportunity to test the combination of microbiome depletion with antibiotics in combination with immune checkpoint inhibition. Following protocol revision, it was reviewed and approved by the Mount Sinai Biostatistics Design Workshop. Subsequently, the study was presented at the GI Disease Focus Group and approved to move forward for review by the Mount Sinai scientific review committee (PRMC). Most recently, the study was deferred by the PRMC in order to clarify and address various concerns regarding

feasibility and design. The protocol has been resubmitted and we are now awaiting approval. In parallel, ongoing negotiation between clinical study sponsor and Mount Sinai is underway. Following PRMC approval, we will submit an IND application to the FDA. In parallel, the protocol will be submitted to the Mount Sinai IRB for review and approval.

Next funding cycle we will open the redesigned clinical trial (see attached schema, Appendix 1) which will enable us to collect the biological samples (tumor tissue and stool) in order to perform subtasks 1 and 2 of Aim 3. We will plan to report interim accrual numbers and possibly early results from the paired biopsies.

Clinical protocol schema



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

Project 1, 2 and 3 (PI Li, Saxena, Cohen)

We tried addressing 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. The manuscript on this finding is under revision. The 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.

Training and Professional Development

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

Dissemination of Results

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.

Present invited seminar at AACR meeting: The Microbiome, Viruses, and Cancer. February 2020

Plans for Next Reporting Period

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.

1. Continue working on developing optimal antibiotic and probiotic regimens to slow disease progression and enhance tumor immunity in mouse models of PDA
2. Continue working on optimal antibiotic and probiotic regimens to enable efficacy for checkpoint or costimulatory receptor-based immunotherapy in PDA in mouse models.
3. Obtain human subject approval for new recruitment.
4. Submit manuscript on functional analysis of microbiome in PDA.

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:*

Impact on principal discipline.

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

Impact on other disciplines.

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

Impact on technology transfer.

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

Impact on society.

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.

We proposed to use either Patient-derived oganotypic tumor spheroids (PDOTS) system or human 3D organotypic cancer model which are recently validated as a platform for evaluating immunotherapies. If it is possible we will plan to use commercial available animal or human 3D organotypic pancreatic cancer models.

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.

Nothing to Report

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
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).*

  Smruti Pushalkar, Fangxi Xu, Scott Thomas, Constantinos P. Zambirinis, Donnele Daley, Qianhao Li, Mautin Hundeyin, Mykhaylo Usyk, Deirdre Cohen, George Miller, Xin Li and Deepak Saxena. 2021. Microbial Dysbiosis and Altered Functional Metabolic Pathways in Pancreatic Ductal Adenocarcinoma. Cancers (under review)

  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.*

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 AND OTHER COLLABORATING ORGANIZATIONS

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."

Individuals who have worked on project

Name:	Deepak Saxena
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-5506-5827
Nearest person month worked:	1
Contribution to Project:	Design experiment and analysis

Funding Support:	

Name:	Xin Li
Project Role:	PI Project 2
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Design experiment and analysis
Funding Support:	
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:	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 (Project 2)
Funding Support:	

Name:	Yuqi Guo
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5
Bench research	Bench research (Project 1)
Funding Support:	

Name:	
Project Role:	
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Change in support since last reporting period

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.

The PI of Project 1 has been changed to Dr Xin Li, Professor, NYU College of Dentistry. It has been approved by Science Officer (SO)/Grants Officer Representative (GOR).

Other organizations.

- *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 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.***