AWARD NUMBER: W81XWH-21-1-0163

TITLE: Selective Metabolic Dependencies in Kidney Cancer with Mitochondrial Mutations

PRINCIPAL INVESTIGATOR: Dr. Pere Puigserver, PhD

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute

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Fort Detrick, Maryland 21702-5012

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1. REPORT DATE AUGUST 2023	**2. REPORT TYPE** Final Technical Report	**3. DATES COVERED** 15APR2021 - 14APR2023

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6. AUTHOR(S) Dr. Pere Puigserver, PhD, E-Mail: pere_puigserver@dfci.harvard.edu	**5d. PROJECT NUMBER**
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) *Dana-Farber Cancer Institute* *450 Brookline Avenue* *Boston, MA. 02215-5450*	**8. PERFORMING ORGANIZATION REPORT NUMBER**
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12. DISTRIBUTION / AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Chromophobe renal cell carcinoma (CRCC) accounts for 5% of RCC and the only treatment for patients is surgery. Mitochondrial deletions are common in these tumors and based on our previous work they might constitute a vulnerability in CRCC. We focus on whether inhibition of NADPH producing enzymes is a vulnerability in two CRCC cell lines using pharmacological and genetic tools in vitro and xenograft models. We have discovered that CRCC cells are more sensitive to the inhibition of NADPH production enzymes. We have used genetic and pharmacological inhibition of PPP pathway enzyme and ME1 finding that blocking these enzymes results in important cell death in CRCC cells. Importantly, the inhibition of PPP pathway enzyme causes sensitivity to ferroptosis inhibitors. Thus, we conclude that PPP pathway enzymes, alone or in combination with ferroptotic activators, are potential targets in CRCC.

15. SUBJECT TERMS

kidney cancer, mitochondrial mutations, NADPH, pentose phosphate pathway, malic enzyme, oxidative stress, chromophobe renal cell carcinoma

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

This award entitled "Selective Metabolic Dependencies in Kidney Cancer with Mitochondrial Mutations" was aimed to investigate whether genetic and pharmacological inhibition of PPP (G6PDH) and ME1 enzymes in human chromophobe kidney cancer cell lines, UOK276 and Chromo-A, tumors confer specific dependencies that could be used as drug-targeted therapies for these tumors. These vulnerabilities were proposed to be
tested in chromophobe cell lines and xenografts in nude mice. The scope of this research was that chromophobe tumors carrying mitochondrial DNA mutations, similar to mitochondrial disease mutant cells,will be sensitive to PPP and ME1 inhibitors providing novel potential therapies to treat these kidney cancer types.

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

kidney cancer, mitochondrial mutations, NADPH, pentose phosphate pathway, G6PDH, malic enzyme,

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.

This award entitled "Selective Metabolic Dependencies in Kidney Cancer with Mitochondrial Mutations" was aimed to investigate whether genetic and pharmacological inhibition of PPP (G6PDH) and ME1 enzymes in human chromophobe kidney cancer cell lines, UOK276 and Chromo-A, tumors confer specific dependencies that could be used as drug-targeted therapies for these tumors. These vulnerabilities were proposed to be tested in chromophobe cell lines and xenografts in nude mice. The scope of this research was that chromophobe tumors carrying mitochondrial DNA mutations, similar to mitochondrial disease mutant cells, will be sensitive to PPP and ME1 inhibitors providing novel potential therapies to treat these kidney cancer types.

The objective of this application was to investigate whether genetic and pharmacological inhibition of PPP and ME1 enzymes in human chromophobe cell lines and tumors with specific mitochondrial, confer specific dependencies and vulnerabilities that could be used as drug-targeted therapies for this subset of kidney tumors. The major goals were: 1) To determine the vulnerabilities of human derived chromophobe renal cell carcinoma cells (UOK276 and Chromo-A) with mitochondrial DNA deletions. 2) Determine the vulnerabilities of human derived chromophobe renal cell carcinoma cells with mitochondrial DNA deletions in xenograft mouse models. In goal 1, milestone # 1 establish the sensitivity of human chromophobe renal carcinoma cells (UOK276 and Chromo-A cell lines) to glucose 6 phosphate dehydrogenase and malic enzyme 1 inhibitors; milestone # 2 establish human chromophobe renal carcinoma cell lines with specific ablations of glucose 6 phosphate dehydrogenase and malic enzyme and establish the sensitivity to cell death. In goal 2, milestone #1, establish the growth effects of these two small molecule inhibitors on human derived chromophobe renal cancer xenografts;

milestone #2 establish the growth effects of CRISPR guides against 6-phosphoguconate dehydrogenase and malic enzyme on human derived papillary renal xenografts. See below for accomplishment of these milestones.

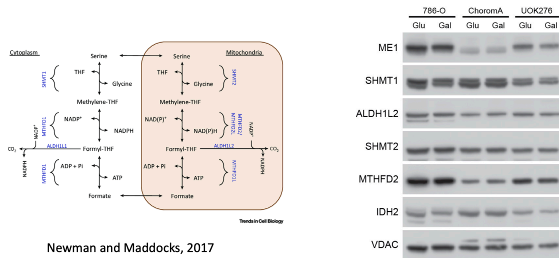

Fig. 1. Chromo-A carcinoma cells have decreased levels of ME1. Left panel, metabolic cellular pathways that produce NADPH. Right panel, western blot showing the amounts of the indicated proteins.

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.

In **major goal 1** that determines the vulnerabilities of human chromophobe renal carcinoma cells, we have accomplished **milestone 1**. We have established the sensitivity of these two cell lines to glucose 6 phosphate dehydrogenase and ME1 enzyme inhibitors, we have found that only Chromo-A but not UOK276 are sensitive to ME1 enzyme inhibition, but not the glucose 6 phosphate dehydrogenase inhibition. We have predicted that this might be explained by the lack of mitochondrial mutations in UOK276 cells; however, deletion of mitochondrial genes NDUFA9 or NDUF11 deletion in these cells does not sensitize to ME1 inhibition. **Milestone #2** we have deleted glucose 6 phosphate dehydrogenase (G6PDH) and ME1 in these chromophobe renal cell carcinoma cells showing that deletion of G6PDH, but not ME1 (as predicted) sensitizes these cells to ME1 inhibitors, as well as RSL3 (GPX4 inhibitor that triggers ferroptosis. In **major goal 2**, that determines the vulnerabilities of human derived chromophobe renal cell carcinoma cells with mitochondrial DNA deletions in xenograft mouse models, the milestones have only been accomplished due to significant failures to generate reliable xenograft models. We proposed to establish the growth effects of these two small molecule inhibitors on human derived chromophobe renal xenografts. We have had the technical problem that UOK276 were not sensitive to these inhibitors, and even when mitochondrial deletions were made were not growing in xenografts. As it relates to the Chromo-A, the main technical problem is the slow growth of these cells in xenograft models and has been impossible to establish these models. As reported last year, we generated PTEN deletion in Chromo-A cells, and in some cases formed tumor, but because of Chromo-A have already PTEN mutations, it was difficult to rationalize the experiments, and additional repeats the growth in xenograft was not consistent. Here are the major activities, specific objectives and significant results and outcomes that were accomplished:

Aim 1- To determine the vulnerabilities of human derived chromophobe renal cell carcinoma cells (UOK276 and Chromo-A) with mitochondrial DNA deletions. (Accomplished). **Major Task 1**- Use of glucose 6 phosphate dehydrogenase (6-AN) and malic enzyme 1 (compound 1) inhibitors to analyze cell death in human chromophobe renal cell carcinoma cells. **Subtask 1**: Regulatory review and approval by the ACURO (Accomplished). **Subtask 2**: Determine specific doses of these small molecule inhibitors of the sensitivity to cell death (Accomplished). **Milestone # 1** Establish the sensitivity of human chromophobe renal carcinoma cells (UOK276 and Chromo-A cell lines) to glucose 6 phosphate dehydrogenase and malic enzyme 1 inhibitors. (Accomplished). Specific Experiments performed to support the Major Task 1. **Fig. 1** shows that Chromo-A cells have decreased of ME1 and mitochondrial one-carbon metabolism, however, exhibit elevated expression of IDH2, as a potential mechanism to generate NADPH. We hypothesized that it is the decreased expression of ME1 that makes sensitive to ME1 inhibitors, in contrast to UOK276. To further support the sensitivity of this carcinoma cell line to NADPH, we performed experiments in glucose restriction conditions (**Fig. 2**) demonstrating that under these conditions Chromo-A cells are more sensitive because of the inability to compensate NADPH deficit caused by inhibition of the PPP (glucose restriction limits the metabolic flux through this pathway resulting in decreases in cellular NADP production. One of the consequences of deficits in NADPH production is also the vulnerability to ferroptosis, which can be caused through cysteine depletion or GPX4 (enzyme that protects against ROS) inhibition (using RSL3 chemical inhibitor). Thus, we have cultured carcinoma cells under cysteine depletion or treated with RSL3. **Fig. 3** shows that Chromo-A or UOK276 carcinoma cells are more sensitive to ferroptotic activators (cysteine depletion or RSL3 treatment) compared to 786-O renal carcinoma cells. Of note, these cells are also considered ferroptotic vulnerable, denoting the high susceptibility to ferroptosis of chromophobe renal carcinoma cells, this vulnerability could be potentially translated to the clinics. These studies have been included in a publication in Proc Natl Acad Sci USA 2022, 119(28), in collaboration with Dr. Henske at Brigham and Women's Hospital) showing the hypersensitivity of chromophobe carcinoma cells to ferroptotic activators. Consistent with this sensitivity to NADPH, it is interesting that Chromo-A were more sensitive to ME1 inhibition, but not the G6PDH inhibition (6AN) (**Fig. 4**). However, and importantly, UOK276 were strongly sensitive to the dual pharmacological inhibition of G6PDH and ME1 (**Fig. 5**). Taken together, the results of these experiments indicate that targeting NADPH production enzymes (particularly ME1)

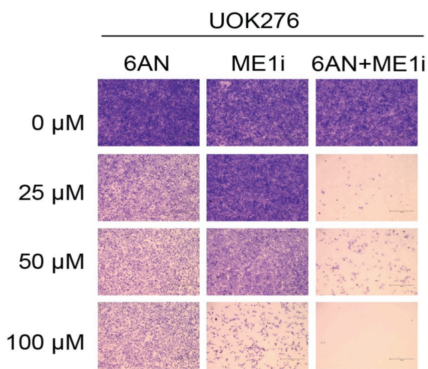

Fig. 6. Inhibition of G6PDH strongly synergize with ME inhibition to promote cell death in UOK276A renal carcinoma cells. Renal carcinoma cells were treated with the different concentrations of ME1 inhibitor (ME1i) and G6PDH inhibitor (6AN).

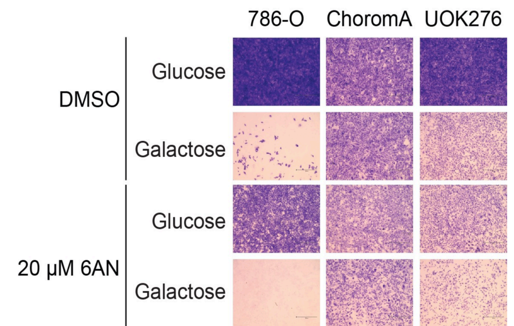

Fig. 2. Chromo-A renal carcinoma cells are resistant to glucose restriction (galactose). The indicated renal carcinoma cells were treated with the G6PDH inhibitor (6AN) with different culture glucose medium conditions.

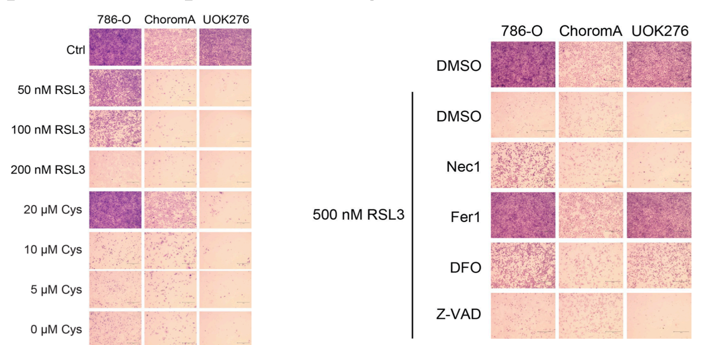

Fig. 3. Chromo-A and UOK276 renal carcinoma cells are sensitive to ferroptotic activators. The indicated renal carcinoma cells were treated with the different ferroptotic activators and cysteine depletion.

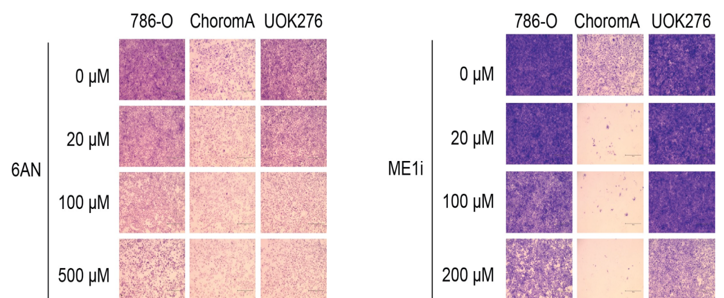

Fig. 4. Inhibition of ME1 strongly promotes cell death in Chromo-A renal carcinoma cells. The indicated renal carcinoma cells were treated with the different concentrations of ME1 inhibitors (ME1i) and G6PDH inhibitors (6AN).

alone, or in combination with ferroptotic activators is a potential new treatment that needs to be considered for chromophobe renal cancer patients.

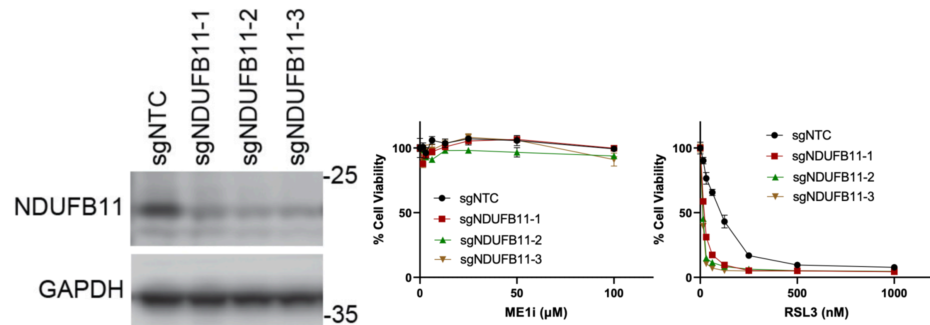

Fig. 9. NDUFB11 KO UOK276A renal carcinoma cells are not sensitive to ME1 inhibition, but sensitive to ferroptosis activation. Indicated renal carcinoma cells were treated with ME1 inhibitor (ME1i) and GPX4 inhibitor (RSL3).

Major Task 2. Generation of CRISPR edited human chromophobe renal carcinoma cells deleting 6-Glucose 6 phosphate dehydrogenase (NADPH) and malic enzyme 1 (ME1). (Accomplished). **Subtask 1:** Determine the sensitivity of human chromophobe renal carcinoma cells (UOK276 and Chromo-A) to CRISPR edited 6-phosphoguconate dehydrogenase and malic enzyme 1. Two different guides will be used for each gene, including control guides. (Accomplished). **Milestone # 1** Establish human chromophobe renal carcinoma cell lines with specific ablations of glucose 6 phosphate dehydrogenase and malic enzyme and establish the sensitivity to cell death (Accomplished). Specific Experiments performed to support the Major Task 2. We have generated CRISPR-mediated deletion in UOK276, as due to the low growth rate it was technically difficult to select in Chromo-A renal carcinoma cells. We have used two different sgCRISPR guides to target ME1 (substantial decreases in ME1 protein levels), and as predicted, no effects on sensitivity to ME1 inhibition, however it moderately promoted the sensitivity to the GPX4 ferroptotic activator RSL3 (**Fig. 6**). Similar experiments were performed using two different sgCRISPR guides to target G6PDH (substantial decreases were reached with these guides to reduce G6PDH protein levels) in UOK276 (**Fig. 7**). Very importantly, and consistent with the experiments performed in the Major Task 1, genetic deletion of G6PDH in chromophobe renal carcinoma cells significantly sensitizes to ME1 inhibition and RSL3, during 72 hours and 48 hours treatment. These experiments are very significant because they show that combinatorial targeting of these two enzymes cause a synthetic lethality in chromophobe cancer cells. Future experiments will determine this potential combination to support treatment of these renal carcinomas. In addition, because it was difficult to assess mitochondrial deletions or strong mitochondrial failures in the two chromophobe renal carcinoma cells, we generated specific deletions of two different proteins that are part of complex I, NDUFA9 and NDUFB11. **Figs. 8 and 9** show that deletion of these two proteins did not significantly affect cell death when cells are treated with ME1 inhibition,

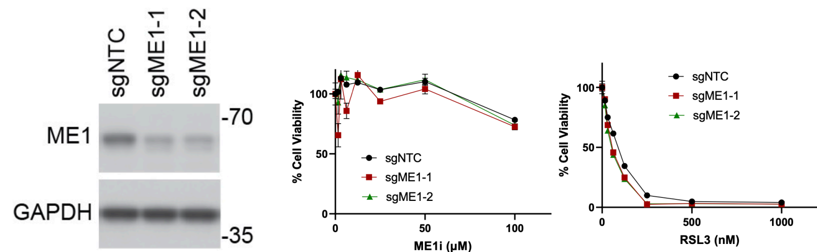

Fig. 7. ME1 KO UOK276A renal carcinoma cells are not sensitive to ME1 inhibition, but moderately sensitive to ferroptosis. Indicated renal carcinoma cells were treated with ME1 inhibitor (ME1i) and GPX4 inhibitor (RSL3).

Fig. 7. G6PDH KO UOK276A renal carcinoma cells are strongly sensitive to ME1 inhibition or ferroptosis activation. Indicated renal carcinoma cells were treated with ME1 inhibitor (ME1i) and GPX4 inhibitor (RSL3).

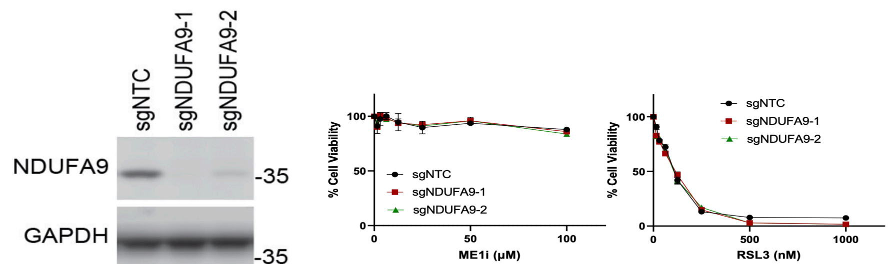

Fig. 8. NDUFA9 KO UOK276A renal carcinoma cells are not sensitive to ME1 inhibition or ferroptosis activation. Indicated renal carcinoma cells were treated with ME1 inhibitor (ME1i) and GPX4 inhibitor (RSL3).

however in the case of NDUFB11 significant sensitivity to RSL3 or ferroptosis is observed. These experiments indicate that potential mitochondrial respiratory inhibition synergizes with ferroptotic activators. Future studies will determine whether pharmacological inhibition of complex I would also provide sensitivity to NADPH production enzyme inhibitors or ferroptosis.

Aim 2- Determine the vulnerabilities of human derived chromophobe renal cell carcinoma cells with mitochondrial DNA deletions in xenograft mouse models. (Partially accomplished). **Major Task 1**. Determine the effects of 6AN (6-phosphoguconate dehydrogenase inhibitor) and Compound-1 (malic enzyme 1 inhibitor) on xenograft models of human derived chromophobe renal carcinoma cell lines. (Partially accomplished). We have established two new edited UOK276 and Chromo-A cell lines, however were not able to consistently grow in xenograft models. In the case of Chromo-A, as reported in the previous technical report, we generated a PTEN deletion, that in some cases, formed tumors in xenografts after 2 months, however because of the lack of consistency, and the fact that Chromo-A have already PTEN mutations, it was difficult to rationalize the cause of tumor formation, and the experiments were not performed. **Subtask 1**: Determine tumor growth in the mice described in major task 1 (Not accomplished). **Milestone # 1** Establish the growth effects of these two small molecule inhibitors on human derived chromophobe renal xenografts. (Not accomplished). **Major Task 2.** Determine the effects of CRISPR guides against 6-phosphoguconate dehydrogenase and malic enzyme 1 on xenograft models of human derived chromophobe renal carcinoma cell lines. (Not accomplished). **Subtask 1**: Determine tumor growth in the mice described in major task 2 (Not accomplished). **Milestone # 1** Establish the growth effects of CRISPR guides against 6-phosphoguconate dehydrogenase and malic enzyme on human derived papillary renal xenografts (Not accomplished). Future studies would need to address and develop consistent xenograft models for chromophobe renal carcinomas to test the outcomes of aim 1, targeting G6PDH and ME1, alone or in combination with ferroptotic activators.

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."

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."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

"Nothing to 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).

"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.

"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).*

Zhang L, Hobeika CS, Khabibullin D, Yu D, Filippakis H, Alchoueiry M, Tang Y, Lam HC, Tsvetkov P, Georgiou G, Lamb C, Stone E, Puigserver P, Priolo C, Henske EP. Hypersensitivity to ferroptosis in chromophobe RCC is mediated by a glutathione metabolic dependency and cystine import via solute carrier family 7 member 11. Proc Natl Acad Sci U S A. 2022 Jul 12;119(28):e2122840119. doi: 10.1073/pnas.2122840119. Epub 2022 Jul 8. PMID: 35867762; PMCID: PMC9651629.

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:	Pere Puigserver
Project Role:	PD/PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-0884-2718
Nearest person month worked:	1
Contribution to Project	Supervision, direction, guidance, and reporting of all aspects of the project.
Funding support	N/A

Name:	Deyang Yu
Project Role:	Research Fellow
Researcher Identifier (e.g. ORCID ID):	0000-0003-4321-1142
Nearest person month worked:	1
Contribution to Project	Generation of groups of mice, and injections, and experimental design.
Funding support	N/A

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 following grants of Dr. Puigserver have ended:

5 R01 DK081418-12 (PI: Puigserver) 07/25/2018 – 03/31/2023 (NCE) 1.92 calendar months/year
NIH/NIDDK annual direct costs
total costs
Thermogenic chromatin remodeling control and fat tissue communication

Goals: The outcomes from this application will identify the transcriptional and epigenetic mechanisms that control adjustable thermogenesis in response to cold and overnutrition driven through the YY1/INO80 chromatin remodeling complex.

Specific Aims: 1) Transcriptional and epigenetic regulatory analysis of how the YY1/INO80 chromatin remodeling complex controls mitochondrial/thermogenic and secreted proteins gene expression programs in brown adipose cells; 2) Metabolic and bioenergetics analysis mediated by the YY1/INO80-dependent thermogenic and secreted gene expression programs in brown and beige adipose cells; 3) Energy and metabolic analysis in response to cold- and diet-induced thermogenesis mediated through the YY1/INO80 transcriptional complex and GDF15 secreted protein.
Funding Agency Contact: Corinne M. Silva, Program Official, NIDDK
Overlap: No

5 R01 DK089883-09 (PI: Puigserver) 04/01/2017 – 03/31/2023 (NCE) 0.48 calendar montl
NIH/NIDDK annual direct costs
Total costs

Energy expenditure and metabolic effects through brown/beige adipose Clk2 kinase

Goals: The major goals of this grant are to identify the molecular mechanisms by which insulin controls Clk2 kinase activity, the molecular mechanisms by which CLK2 suppress hepatic gluconeogenesis, and to test Clk2 metabolic functionality *in-vitro* and in *in-vivo* mouse models.

Specific Aims: 1) Molecular and functional analysis of how insulin controls Clk2 kinase activity; 2) Molecular and functional analysis of Clk2 suppression on hepatic gluconeogenesis; 3) Effects of Clk2 on hepatic glucose and lipid metabolism in mice.
Funding Agency Contact: Corinne M. Silva, Program Official, NIDDK
Overlap: No

W81XWH-21-1-0163
(PI: Puigserver) 04/15/2021 – 04/14/2023 (NCE) 1.08 calendar months/year
DOD CDMRP - KCRP annual direct costs
total costs
Selective metabolic dependencies in kidney cancer with mitochondrial mutations

Goals: The objective of this application is to investigate whether genetic and pharmacological inhibition of PPP and ME1 enzymes in human PRCC cell lines and tumors confer specific dependencies that could be used as drug-targeted therapies for this subset of kidney tumors.

Specific Aims: 1) To determine the vulnerabilities of human derived papillary renal cell carcinoma cells with mitochondrial DNA deletions. 2) Determine the vulnerabilities.

The following grants for Dr. Puigserver are now active:

(PI: Puigserver) 02/01/2022 – 01/31/2025 1.2 calendar months
The Mathers Charitable Foundation total costs
Mechanisms of cellular pathogenic mitochondrial heteroplasmy

Goals: This research will significantly advance our understanding of basic human biology and medicine focusing on the causes of genetic variation of mitochondrial organelles that leads to severe tissue damage and ultimately death.

Specific Aims: Aim 1. Identification of genes that suppress somatic mitochondrial heteroplasmy in MELAS heteroplasmic cell lines. Aim 2. Validation and characterization of these genes. Aim 3. Establishing the mechanisms of action and cellular processes that control mitochondrial heteroplasmy load.

Funding Agency Contact: Zach Handelman, Director of Operations

Overlap: No

ME210011 (Puigserver) 06/01/2022 – 05/31/2025 2.04 calendar months
DOD CDMRP – MRP Idea Award annual direct costs
total costs
Defective tumor mitochondria drives intrinsic immunogenicity through MHC class 1 antigen presentation and T cell engagement

Goals: To use specific drugs that selectively and partially inhibit mitochondrial bioenergetics in tumor cells that will initiate an immune attack to kill melanoma cells.

Specific Aims: Specific Aim 1. Proteomic identification of immune-linked proteins that change in response to selective mitochondrial complex I subunit inhibition in melanomas formed in immunocompetent mice. Specific Aim 2. Identification of melanoma complex I inhibition-dependent peptide antigens that cause T cell engagement. Specific Aim 3. Analysis of specific proteins that are required or sufficient for MHC class 1 peptide antigen presentation and T cell engagement.

Funding Agency Contact: Mark Wilkison, Grants Management Specialist

Overlap: No.

(Puigserver) 12/01/2022 – 11/30/2025 1.56 calendar months
Dana-Farber Cancer Institute Innovation Research Fund annual direct costs
Total costs

R01 DK136640-01 (Puigserver) 08/01/2023 – 07/31/2027 1.56 calendar months
NIH annual direct costs
total costs
Regulatory mechanisms of mitochondrial cristae biogenesis and thermogenic function

Goals: The premise of this application is that the ER signals to the mitochondrial protein import to control cristae biogenesis and form competent thermogenic adipocytes protecting against lower temperatures and obesity/T2D.

Specific Aims: 1) Determine the regulatory mechanisms of cristae biogenesis and thermogenic function through PERK activation; 2) Determine the mechanisms of cold-dependent mitochondrial protein import coupled to thermogenic function; 3) Analysis of mitochondrial cristae formation and metabolic/energetic function during cold- and diet-induced thermogenesis.

Funding Agency Contact: Maren Laughlin

Overlap: None

Mitochondrial and lipid tumor NKT immunogenicity

Goals: The objective is to identify the mechanisms and endogenous lipids that load into CD1d to recruit NKT cells and cause tumor rejection.

Specific Aims: The research plan is focused on three specific objectives: 1) Identification and analysis of endogenous lipid antigens that are loaded into CD1d and cause tumor recruitment of NKT cells. 2) Analysis of the different choline/betaine pathway enzymes that cause tumor NKT cell infiltration and rejection. 3) Generation of specific lipid nanoparticles containing lipid antigens that cause tumor NKT cells infiltration and rejection. Although we will use mainly melanoma as a tumor model in this research plan, in the different aims we will also employ other cancer models, including breast.

Funding Agency Contact: Maren Laughlin

Overlap: None

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.*

None.

8. SPECIAL REPORTING REQUIREMENTS

Not applicable.

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.*

   None.