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TITLE: Coaxing Senescence in Retroperitoneal Liposarcomas

PRINCIPAL INVESTIGATOR: Jonathan R. Pollack, MD, PhD

CONTRACTING ORGANIZATION: Stanford University

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6. AUTHOR(S)

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5e. TASK NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Retroperitoneal liposarcomas are rare, but can progress with high mortality, where current medical therapies are ineffective. All carry amplification of the CDK4 cell-cycle promoter, and CDK4 inhibitors can induce cell senescence, but not all liposarcomas respond. Our broad objective is to discover drugs or druggable targets that in combination with CDK4 inhibition might drive liposarcoma cell senescence or death. Specific aims include a small-molecule screen of bioactive compounds, and a CRISPR genetic screen of druggable protein targets. During the reporting period, we have encountered some challenges, including a sarcoma research community reluctant to share cell lines, and an inability to reproduce certain previously published findings in the field. Nevertheless, we have made substantial progress, including validating 4 liposarcoma lines with CDK4 amplification, characterizing CDK4 inhibitor responses (senescence phenotype assays), piloting drug combination assays with candidate synergizing compounds, and generating stable Cas9-expressing cell subclones. We are on target to complete our studies during the no-cost extension period, where completion will identify new treatment avenues for this rare but deadly cancer.

15. SUBJECT TERMS

Liposarcoma, CDK4 inhibitor, senescence, novel therapeutics

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.*

   Retroperitoneal liposarcomas are rare cancers, but can dedifferentiate and progress with high mortality. Current medical therapies are largely ineffective. Retroperitoneal liposarcomas carry amplification of the CDK4 gene, which promotes cell-cycle progression. CDK4 inhibitors have been reported to induce liposarcoma cell senescence, but not all liposarcomas respond by senescence. Our broad objective has been to discover drugs or druggable targets that in combination with CDK4 inhibition might drive all liposarcoma cells to senescence or death. Our specific aims include (1) Completing a small-molecule screen of bioactive compounds that in combination with CDK4 inhibition drive liposarcoma cell senescence; and (2) Completing a CRISPR genetic screen of druggable protein whose knockout in combination with CDK4 inhibition drives liposarcoma cell senescence.

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

   Sarcoma, Retroperitoneal liposarcoma, CDK4 amplification, CDK4 inhibitor, senescence, drug combinations, CRISPR screen, novel therapeutics, repurposed therapeutics

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.

   Specific Aim 1: Complete a small-molecule screen of bioactive and FDA-approved drugs that in combination with CDK4 inhibition drive liposarcoma cell senescence and validate top hits.

   Major Task 1: Identify compounds/drugs that drive senescence in CDK4 inhibitor-resistant liposarcoma lines. (by Month 6) [approx. 40% completed]

   Major Task 2: Validate top hits from compound/drug screen (by Month 12) [0% completed]

   Specific Aim 2: Complete a CRISPR genetic screen of druggable proteins whose knockout in combination with CDK4 inhibition drive liposarcoma cell senescence and validate top hits.

   Major Task 1: Identify druggable proteins whose gene knockout drives senescence in CDK4 inhibitor-resistant liposarcoma lines. (by Month 7) [approx. 40% completed]

   Major Task 2: Validate top hits from CRISPR genetic screen (by Month 12) [0% completed]

   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.

For Specific Aim 1 (small-molecule screen), our major activities/accomplishments include:

(i) Obtaining and characterizing four human liposarcoma cell lines from cell repositories (ATCC) and academic colleagues.

(ii) Validating CDK4 amplification in liposarcoma cell lines by Q-PCR (Figure 1).

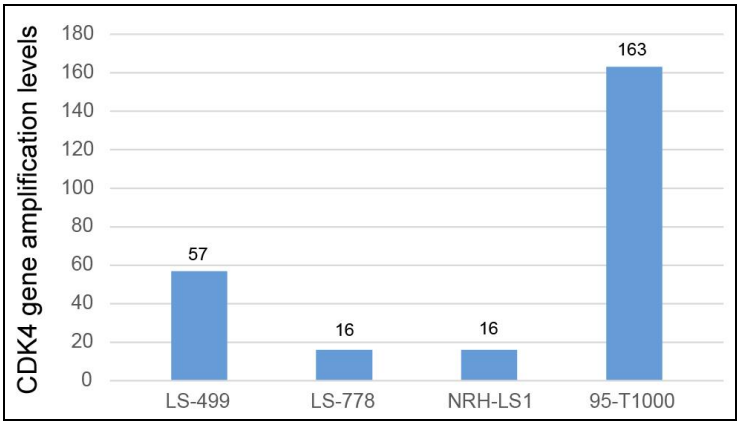

Fig 1. Validating CDK4 amplification in liposarcoma cell lines. CDK4 amplification quantified by TaqMan qPCR, normalized to control gene RNaseP, and reported relative to normal human DNA. Gene amplification levels are reported relative to the normal diploid copy number.

(iii) Characterizing cell senescence responses in liposarcoma cell lines, and control human cancer cell lines, following challenge with CDK4/6 inhibitor (1μM palbociclib) for 7 days. Senescence assayed by β-galactosidase assay (Figure 2A, B).

(iv) Characterizing cell senescence responses in control cancer cell lines reported in the literature to be sensitive (H1975) or resistant (H358 and H3122) to palbociclib-induced senescence (Figure 2B).

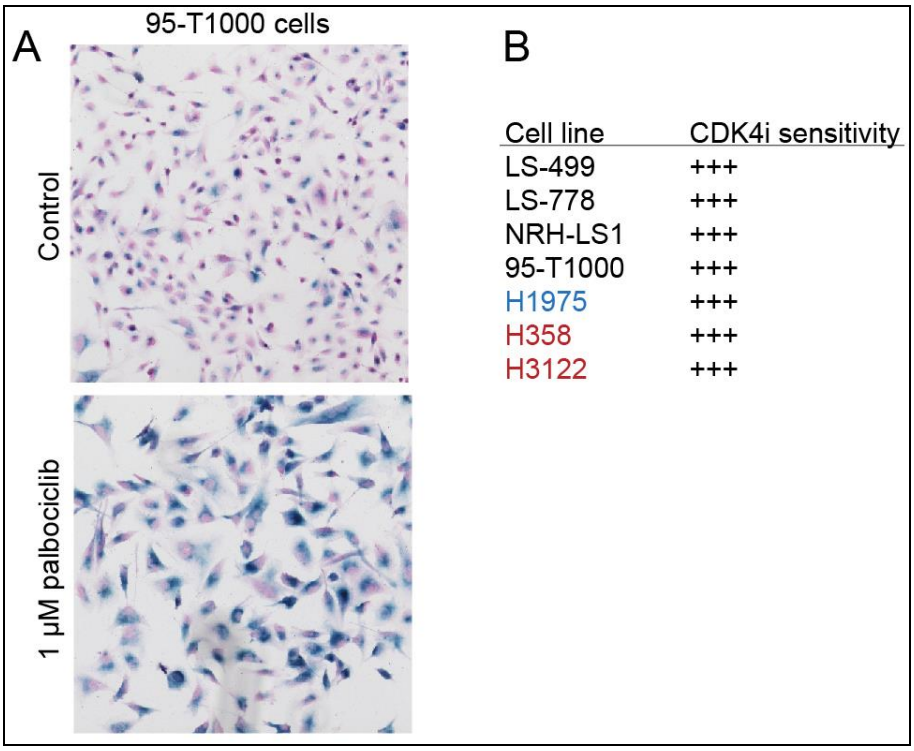

Fig 2. Characterizing cell senescence responses in liposarcoma and control cancer cell lines. (A) Liposarcoma cell line 95-T1000 undergoes senescence in response to CDK4 inhibition by 1 μM palbociclib treatment for 7 days. Senescence assayed by β-galactosidase staining. Note the large, flattened cells positive for β -galactosidase (blue staining) in the treated versus control cells. **(B)** Assaying liposarcoma cells lines reveals palbociclib sensitivity, including in control cancer lines (H358 and H3122) reported in the literature to be resistant.

(v) Miniaturizing cell senescence and cell viability assays, in advance of small molecule screen.

(vi) Piloting experiments with candidate positive-control compounds – those that in combination with palbociclib induce cell senescence or cell death – including navitoclax, fisetin, and dasatinb-quercetin.

For Specific Aim 2 (CRISPR/Cas9 genetic screen), major activities/accomplishments include:

(i) Obtaining, characterizing and validating human liposarcoma cell lines with CDK4 amplification, and characterizing their senescence responses to CDK4 inhibition (as detailed above, and in Figures 1 and 2).

(ii) Piloting creation of stable liposarcoma cells lines expressing Cas9 (for CRISPR genetic screens) by stable transfection.

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

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.

During the next reporting period (no-cost extension), we will finish the small-molecule and CRISPR screens. We will then evaluate top screen hits. Finally, we will draft a manuscript with findings.

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.

During the reporting period, we have characterized the senescence responses of liposarcoma and other cancer cell lines. Of note, we have been unable to reproduce the published finding that some cancer cell lines are resistant to palbociclib-driven senescence (Figure 2B). Therefore, for our small-molecule and CRISPR screens, we are now using cell death in place of senescence as an assay endpoint to identify compounds/targets that synergize with palbociclib against liposarcoma cells. Cell death is a stronger endpoint than cell senescence, and should provide increased benefit to patients. We therefore consider this change in the choice of endpoint screening assay to be of insufficient significance as to require prior approval of the agency.

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.

During the reporting period, our progress was significantly impacted by delays in obtaining the needed liposarcoma cell lines under executed MTAs. In particular, fewer than half of the investigators we had contacted either responded (over multiple attempts), or were willing to share their published cell lines. As a newcomer to the field of liposarcoma research, I found this quite disappointing. Ultimately, by contacting additional investigators, we were able to obtain sufficient liposarcoma cell lines to complete our studies. However, what should have taken 1 month (to obtain needed cell lines) ended up taking over 4 months.

During the reporting period, our progress was also impacted by COVID laboratory personnel restrictions and supply chain delays. In addition, we encountered problems receiving international shipments of liposarcoma cell lines, where COVID restrictions caused delays and failures of transit through customs. Those problems were temporary and are now resolved.

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.

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **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".

Name:	Jonathan Pollack, MD, PhD
Project Role:	PD/PI
Researcher Identifier:	ORCHID ID: 0000-0002-0212-7721
Nearest person months worked:	1
Contribution to Project:	Dr. Pollack has served as Project Director, where he has managed project personnel and overseen all aspects of experiment planning, analysis and interpretation.
Funding Support:	N/A

Name:	Anna Sikes, MD
Project Role:	Research Scientist
Researcher Identifier:	NA
Nearest person months worked:	2
Contribution to Project:	Dr. Sikes has carried out all wet-bench experiments, and has contributed to data analysis and interpretation.
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.

Previously active grants that have closed:

Title:	Stanford Molecular and Cellular Characterization Laboratory (PI: James Brooks)
Time Commitment:	1.2 calendar months
Supporting Agency:	NIH/NCI
Project Number:	U01CA196387
Performance Period:	9/16/2015-8/31/2021
Level of Funding:	
Project Goals:	The goals of this proposal are to comprehensively characterize the molecular genetic alterations in early prostate cancer lesions.
Specific Aims:	The specific aims are (1) To investigate the early genomic evolution of good and bad outcome prostate cancer; and (2) Define the genomic heterogeneity of good and bad outcome prostate cancer.
Role:	Co-Investigator
Overlap:	None.

Previous pending grants that are now active:

Title:	Stanford O'Brien Urology Research Center (PI: James Brooks)
Time Commitment:	3.6 calendar months
Supporting Agency:	NIH (SPO 208184)
Project Number:	U54DK130065
Performance Period:	7/1/2021 – 6/30/2026
Level of Funding:	
Project Goals:	The goal of this pending application is to create a Benign Urology research center at Stanford, with a research focus on benign prostatic hyperplasia (BPH), plus an educational focus to train future researchers in benign urology.
Specific Aims:	The proposed Center has three Research Projects, aimed to (1) Investigate the role of prostate stroma in BPH pathogenesis; (2) Define the role of the immune microenvironment in BPH pathogenesis; and (3) Build an atlas of BPH spanning MRI to histology, cells and molecules.
Role:	Co-Investigator
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.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

None

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