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TITLE: DNA Palindromes as a Novel Tumor Marker in Extracellular Vesicles from Liquid Biopsy

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of our project is to develop a novel, highly sensitive Next Generation Sequencing (NGS)-based assay to identify tumor-specific chromosome aberrations in plasma from liquid biopsies in women with very early stages of breast cancer. To accomplish the goal, we have developed a method to enrich DNA palindromes, a common form of structurally aberrant DNA in tumor cells. We have shown previously that we can identify cancer-specific DNA palindromes in breast tumors by sequencing the enriched DNA (Genome-wide Analysis of Palindrome Formation, GAPF). In the first year of the grant period, we have optimized the procedure and established a pipeline for the sequencing data analysis. We also obtained 38 tumor/buffy coat (normal)/plasma pairs from biobank and completed DNA extraction from tumors and buffy coat samples. We enriched DNA palindromes for the three sets of tumor/normal pairs and constructed DNA sequencing libraries. We have submitted the libraries and are waiting for the data.

We also rigorously investigated DNA contents that are circulating in plasma. We showed that most of the DNA plasma are in the large extracellular vesicles, which support the feasibility of our approach. The results were published (PMID 30108686).

15. SUBJECT TERMS

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 goal of our project is to develop a novel, highly sensitive Next Generation Sequencing (NGS)-based assay to identify tumor-specific chromosome aberrations in plasma from liquid biopsies in women with very early stages of invasive breast cancer. Tumor DNA in circulation is a convincing sign of invasive tumors and allows us to distinguish invasive tumors from non-invasive carcinoma in situ. To accomplish the goal, we will apply our method to enrich a form of structurally aberrant DNA (DNA palindromes). Using the genomic approach (Genome-wide Analysis of Palindrome Formation, GAPF), we have shown that DNA palindromes (inverted repeats) are widespread in cancer genomes and provide a platform for subsequent gene amplification. DNA palindromes arise when DNA double-strand breaks (DSBs) are repaired illegitimately and initiate genomic amplification through a series of DSBs and chromosome fusions (Breakage-Fusion-Bridge cycles, BFB cycles). Because BFB cycles are shown to promote chromosomal rearrangements at the early stage of tumorigenesis, DNA palindromes in plasma DNA could be a useful marker for early detection of invasive breast cancer.
A crucial aspect of GAPF is that it amplifies DNA palindromes and eliminates non-palindromic DNA that derives from normal cell DNA contaminated in the sample. There are other platforms for the detection of tumor DNA in plasma, such as tumor-specific mutation detection and DNA methylation profiles; however, none of the methods can physically eliminate signals from contaminated non-tumor DNA. Therefore, the sensitivity of the tests suffers a lot when tumor DNA fraction is very small, for example, in the plasma samples of patients with the early stage of breast tumors. In this regard, we have seen the excellent signal (DNA palindromes) to noise (non-palindromic DNA) ratio in our GAPF assay, which is consistently more than several hundred-fold. We have improved the assay for the past year so that we were able to perform the assay with as little as 20 ng of DNA. Thus, GAPF can overcome the limitations associated with liquid biopsy in which the excess of normal cell DNA obscures the detection of tumor DNA.

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

DNA palindrome, Inverted repeat, Breakage-Fusion-Bridge cycles, Gene amplification, circulating tumor DNA, DNA biomarker.

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.

1. We will generate GAPF-seq data by (1) creating DNA enriched with palindromes, (2) creating sequencing libraries and (3) sequencing libraries at Genomics Core.
2. We will analyze GAPF-seq data and identify tumor-specific palindromes in tumor tissue DNA.

3. We will isolate large extracellular vesicles from plasma.
4. We will investigate and optimize DNA isolation methods from vesicles.
5. We will generate GAPF-seq data from plasma DNA by (1) creating DNA enriched with palindromes, (2) creating sequencing libraries and (3) sequencing libraries at Genomics Core.
6. We will enroll women with suspicious mass in mammograms and collection of plasma and buccal mucosa to the study.

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.

1. Major activities

a. Technological improvement

We have made improvements for our enrichment strategy of DNA palindromes so that we can now perform the assay with a very small amount of DNA (20ng). The improvements were prompted because we encountered inconsistent results of GAPF from breast tumor/normal leukocyte pairs. Inconsistency includes (1) the poor enrichment of DNA palindromes, measured by quantitative PCR for the enrichment of a pre-existing DNA palindrome in normal genomes, and (2) the poor recovery of palindromic DNA, measured by the Qubit.

Poor enrichment of DNA palindromes was suspected by the qPCR assay for the pre-existing palindrome at 19q13.2. We routinely monitor the enrichment of palindromes and the depletion of background DNA by qPCR using the library DNA that is constructed by the DNA undergone GAPF processes. While the depletion of background DNA has always been excellent, the enrichment of the palindrome was not as efficient as we expected. To address the problem, we investigated the effect of formamide that is added into the DNA during the heat-denaturation and rapid renaturation steps. Formamide was added because it promotes the denaturation of GC-rich DNA. However, formamide is known to stabilize the single-stranded DNA and potentially prevents the intra-strand self-annealing of palindromic DNA, resulting in the reduced enrichment of palindromes. We tested GAPF with or without formamide and evaluated the enrichment of the pre-existing IR. The enrichment is consistently better without formamide than with formamide (**Fig. 1**). Accordingly, we decided to conduct GAPF without formamide.

The unique aspect of GAPF-seq is that GAPF physically eliminates background normal DNA and enrich (cancer-) specific features. Because it eliminates the vast majority of DNA, the **efficient recovery of DNA** is crucial. We have been using a magnetic-beads based recovery of DNA; however, the amount of recovered DNA is, in most of the time, less than 2 ng of DNA out of 100 ng. Such a low amount of recovery may reflect an efficient elimination of background DNA. However, we noticed the inconsistency of recovery, as the decrease of starting material does not always result in the decrease of recovery. Further, the procedure of the magnetic-beads based recovery is somewhat cumbersome. Therefore, we tested a new silica membrane-based

procedure. We found that the new procedure is easier, and the yield is proportional to the starting material of DNA.
These improvements are now implemented to the procedure and should facilitate the identification of DNA palindromes with a small amount of input DNA.

b. GAPF-seq data analysis pipeline
We designed an algorithm to search for cancer-specific (de novo) palindromes, and the results were presented at the 2019 San Antonio Breast Cancer Symposium. *De novo* palindromes can arise anywhere in the genome. We focused on the genomic regions with non-duplicated, single-copy regions for *de novo* palindrome detection for two reasons. First, regions containing duplicated sequences can harbor germline palindromes pre-existing in the genome. Whether palindromes detected in such regions are true *de novo* is questionable. Second, confident mapping of Illumina reads to duplicated regions is challenging because highly similar sequences offer multiple potential alignments, thereby lowering mapping quality scores.

We applied a mapping quality score 40 to filter for the reads that align uniquely to the reference genome. **The reads that remain after the filtering likely originate from palindromes that arise *de novo* by genome rearrangements in tumors**. We then developed an algorithm for identifying bins with a high number of reads throughout the entire genome and generating a palindrome profile for the sample. The number of reads within each bin was divided by a per-million scaling factor (for example, the scaling factor is 100 for a run with 100 million reads) in order to adjust for the total sequencing depth of a particular sequencing run (**adjusted read coverage, ARC**). We expect that bins containing high ARC will demarcate DNA palindromes.

De novo palindromes formed by this mechanism can span several million base pairs. Prior to denaturation/renaturation, we digested tumor DNA containing such palindromes by rare-cutting restriction enzymes, and dsDNA after renaturation only forms from the DNA that contains the centers of palindromes. Since the DNA fragments are ranging from a few kb to 20 kb, we expect dsDNA after denaturation and quick renaturation to be less than 10 kb. Therefore, our algorithm considers five consecutive 1 kb bins with an ARC > 1.5.

- Normalizing to sequencing depth uses a “per million” scaling factor where the number of reads in each bin is divided by the total number of millions of reads (e.g., a scaling factor of 20 for 20 million reads).
- Adjusted read coverage (ARC) > 1.5
- For palindromes, five (5) consecutive bins need to be enriched above this threshold (i.e., the palindrome must span 5 kb)

Using these criteria, we identified 170 palindromes in the tumor DNA (ID: 14-709T), whereas we only identified two palindromes in the normal DNA (14-709N) (**Fig. 2**). The palindromes in the normal DNA were also seen in tumor DNA, suggesting that they are germline palindromes and not represented in the reference genome.

c. Optimal DNA isolation methods from vesicles.
With the co-investigator Dolores Di Vizio, we tested two methods (a silica membrane-based approach and a classical phenol/chloroform-based method) for isolating DNA from large extracellular vesicles. We consistently saw two-to-three times more yields using a phenol/chloroform-based method than a silica membrane-based method). We decided to employ a phenol/chloroform-based method for DNA extraction.

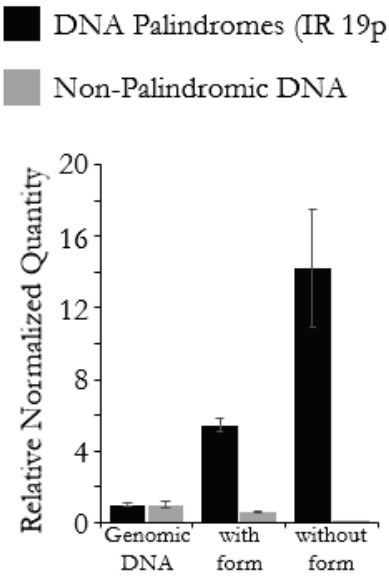

Figure 1 The enrichment of palindromic DNA (black bars) and non-palindromic DNA (gray bars) with or without formamide, measured by quantitative PCR.

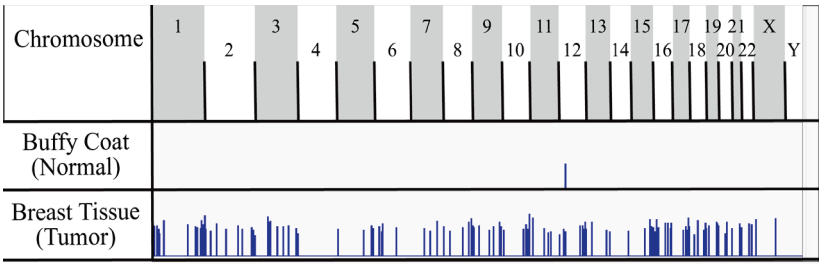

Figure 2 Genome-wide palindrome profiles (in single-copy regions) for matched breast tumor (14-709T) and buffy coat samples (14-709N).

2. Specific objectives, including goals not met

Because of the need for developing the improved and consistent approach for GAPF, we have not generated the palindrome profiles of breast tumor/matched normal DNA pairs (major goals 1 and 2). With the improved method and a new bioinformatic algorithm, we are working hard to finish the analysis.

Isolating DNA from large EVs is another goal of the second year. Dr. Di Vizio recently published a new protocol for isolating large EVs (Mariscal et al. *J. Extracellular Vesicles* 9, 1764192, 2020). Accordingly, we employ the new approach for isolating large EVs from plasma and extract DNA (major goals 3 and 5).

Due to the renovation and the temporal relocation of the Cedars-Sinai breast center, we were unable to enroll and collect the plasma samples from women with abnormal signs in mammograms (major goal 6). Now that the renovation is finished and the new breast center is operating, we will start the enrollment and collection.

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.

The funding supports Michael Murata, Ph.D. Dr. Murata joined our lab in January 2019 and has received a training opportunity for next-generation sequencing data analysis. Working with the staff at the High-Performance Computing Resources in our institute, Dr. Murata has developed a pipeline for the analysis of GAPF-seq data.

The funding also provides a training opportunity for Fumie Igari M.D. Ph.D., who joined our lab in February 2020 as a visiting scientist. She is a breast surgeon at the Juntendo University Hospital in Tokyo, Japan. She has received opportunities for training in molecular biology and bioinformatics. The hands-on experience in the updated technologies of liquid biopsy will benefit her professional development as a breast surgeon.

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.

Although we experience delays in our project, we have established systems that will overcome the delays. The systems include (1) the improved method for palindrome enrichment, (2) a robust bioinformatics pipeline, which we will further implement statistical robustness by working with the biostatistical core at Cedars-Sinai, and (3) a newly developed, efficient method of large EV isolation. These methods should facilitate our procedures for the analysis of (1) the palindrome profiles of 33 tumor/normal/plasma DNA from cancer patients and (2) the analysis of 30 large EV DNA in plasma from 30 women with abnormal signs of mammograms.

Also, our visiting scientist Dr. Igari is helping us with the wet bench work, providing more staff members for completing the project.

The new breast center is now up and running. The problem was that, because the breast center was temporally out of campus, we do not have the way to transport samples to the Biobank. Now that the center is back on campus, we will be able to transport the samples without any issues. Dr. Armando Giuliano sees 10-20 new patients every week, so we should be able to recruit 30 patients within half a year.

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.

We took the delays in our project very seriously and added a staff member to help us completing the project. Our established systems, including (1) the improved method for palindrome enrichment, (2) a robust bioinformatic pipeline and (3) a new method of large EV isolation should facilitate the completion.

The new breast center is now up and running. The problem was that, because the breast center was temporally out of campus, we do not have the way to transport samples to the Biobank. Now the center is back on campus, we will be able to transport the samples without any issues. Dr. Armando Giuliano sees 10-20 new patients every week, so recruiting 30 patients can be done in 6 months.

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.

As described in the delay section of this report, due to the technological improvement and sample transport issues this caused a slight decrease of expenditure for completing the constructions of the proposed DNA sequencing libraries during year 2. We originally budgeted $26,400 and spent $5,383.93 on sequencing costs. With the addition of staff and facilities now back on our campus we fully anticipate being able to complete the proposed DNA sequence libraries needed to complete the aims proposed and utilize the funds budgeted for those costs.

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

Not applicable

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).*

1. **Tanaka, H.*** and Watanabe, T. (2020). Mechanisms underlying recurrent genomic amplification in human cancers. *Trends in Cancer* 6: 462-477. PMID 32383436, https://doi.org/10.1016/j.trecan.2020.02.019. (*corresponding author) (Acknowledgement of federal support, YES)

2. Lahiguera Á., Hyrossová P., Figueras A., Garzón D., Moreno R., Soto-CerratoV., McNeish I., Serra V., Lazaro C., Barretina P., Brunet J., Menéndez J., Matias-Guiu X., Vidal A, Villanueva A., Taylor-Harding B., **Tanaka H**., Orsulic S., Junza A., Yanes O., Muñoz-Pinedo C., Palomero L., Pujana M.À., Perales J. C. and Viñals F. (2020) Tumors defective in homologous recombination rely on oxidative metabolism: relevance to treatments with PARP inhibitors1. *EMBO Molecular Medicine*, PMID 32400970 (Acknowledgement of federal support, no)

3. Huang, F., **Tanaka, H.**, Rutgars, J. and Knudsen B. (2020) Mutant POLQ and POLZ/REV3L DNA polymerases may contribute to the favourable survival of patients with tumours with POLE mutations outside the exonuclease domain. *BMC Medical Genetics* (accepted, *in press*) (Acknowledgement of federal support, no)

4. Suzuki R., Murata M.M., Manguso N., Watanabe T., Mouakkad-Montoya L., Igari F., Rahman M M., Qu Y., Cui X., Giuliano A.E., Takeda S. and **Tanaka H**. The fragility of structurally diverse duplication block triggers recurrent genomic amplification. (*Nucleic Acids Research* under review) (Acknowledgement of federal support, YES)

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.*

Poster presentation at the 2019 San Antonio Breast Cancer Meeting
Title: DNA palindromes as novel genomic targets for unbiased, genome-wide, and rapid pan-cancer screening
Murata M.M., Mouakkad-Montoya L., Giuliano A.E., and **Tanaka H**

- **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:	*Hisashi Tanaka*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-9223-4186*
Nearest person month worked:	*1.2*
Contribution to the Project	*Dr. Tanaka is a PI and overseeing the project.*
Funding Support:	*NIH NCI R01 CA149385 and the Margie Petersen Breast Cancer Research Laboratory*
Name:	*Dolores Di Vizio*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to the Project	*Dr. Di Vizio is co-investigator assisting the PI with .*
Funding Support:	*NIH NCI R01 CA2188526, NIH NCI R01CA234557, NIH NCI P01 CA098912, Luke Wu-Jeu Change Discovery Fund, NIH NCI P01 CA233452*
Name:	*Michael Murata*
Project Role:	*Post Doctoral Scientist*
Fellow Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project	*Dr. Murata has tested the methods to for enriching DNA palindromes from cancer and normal DNA. Dr. Murata has developed an algorithm for the analysis.*
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.

Nothing to report

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.*

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.