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TITLE: Liquid Biopsy Detection of Structural Variant Breakpoints to Monitor Ovarian Cancer Clonal Evolution: A Pilot Study

PRINCIPAL INVESTIGATOR: R. Tyler Hillman, M.D., Ph.D.

CONTRACTING ORGANIZATION: The University of Texas MD Anderson Cancer Center
Houston, TX

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R. Tyler Hillman, MD, PhD, Department of Gynecologic Oncology & Reproductive Medicine
E-Mail: RTHillman@mdanderson.org

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UT MD Anderson Cancer Center
1515 Holcombe Blvd
Houston, TX 77030

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project aims to develop a platform to map and monitor HGSOC sub-clonal evolution by integrating whole genome sequencing(WGS)of tumor biopsies and the detection of structural variants(SVs) by cell-free DNA(cfDNA)-based approach. The major achievements regarding tumor biopsies: multi-site whole genome sequencing on pre-treatment HGSOC biopsy specimens from two cases has been successfully performed. Confident calls of SVs have been determined by multiple methods. Reconstruction of sub-clonal by SVs and identification and validation of SVs are the priorities. Modification of current protocol to enroll prospective cases has been approved. The WGS of multi-site biopsies from the first case met the criteria is ongoing. The significant progress has been made to streamline workflows of digital droplet PCR assays for detection of SVs from synthetic cfDNA and determine the analytical parameters. Assays to consistent isolation of cfDNA from plasma is established.

15. SUBJECT TERMS

Liquid biopsy, Structural variant, High grade serous ovarian cancer

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1. Introduction

The overall hypothesis of this research is that detection of structural variants (SVs) from circulating free DNA (cfDNA) can be used to map ovarian cancer subclone evolutionary trajectories during primary treatment and beyond, with implications for prognostic stratification and the development of novel maintenance strategies. The primary objective of this protocol is to determine the test performance characteristics of a novel cfDNA-based approach for detecting clonal and sub-clonal SVs among patients with epithelial ovarian cancer undergoing primary treatment.

2. Keywords

Liquid biopsy, Structural variants, High grade serous ovarian cancer

3. Accomplishments

- **What were the major goals of the project?**

Liquid Biopsy Detection of Structural Variant Breakpoints to Monitor Ovarian Cancer Clonal Evolution: A Pilot Study	**Timeline**	**Site 1**	**Progress**
Major Task 1 Determine the subclonal composition of pre-treatment high grade serous ovarian cancer among excellent responders to neo-adjuvant chemotherapy.*	Months		
Subtask 1 – Obtain institutional review board approval for use of patient data and biospecimens.	1-3	X	Completed
Subtask 2 – Obtain Human Research Protection Office (HRPO) approval for use of patient data and biospecimens.	1-3	X	Completed
Subtask 3 – Perform multi-site whole genome sequencing on pre-treatment biopsy specimens from excellent responders to neo-adjuvant chemotherapy.	3-6	X	Completed
Subtask 4 – Determine the subclonal architecture of pre-treatment high grade serous ovarian cancer using orthogonal mutation classes.	3-6	X	Completed
Milestone(s) Achieved: Identification and classification of clonal and subclonal mutations, including structural variants, from pilot cohort [see below for biospecimen sources and utilization].			Milestone Achieved
Major Task 2 Develop and validate a novel assay to detect structural variant breakpoints from liquid biopsies.			
Subtask 1 – Design digital droplet PCR assays for detection of structural variant breakpoints from circulating free DNA.	6-12	X	Completed
Subtask 2 – Determine the analytical parameters of pre-treatment clonal structural variant breakpoint detection using digital droplet PCR.	10-16	X	In progress
Subtask 3 – Determine the analytical parameters of pre-treatment subclonal structural variant breakpoint detection using digital droplet PCR.	10-16	X	In progress
Subtask 4 – Perform structural variant-based reconstruction of tumor subclonal architecture from circulating free DNA obtained at interval cytoreductive surgery.	16-24	X	In progress
Milestone(s) Achieved: Development and validation of novel circulating free DNA assay for the detection of clonal and subclonal structural variants [see below for biospecimen sources and utilization].			Milestone Not Yet Achieved

- **What was accomplished under these goals?**

Major Task 1: Determine the subclonal composition of pre-treatment high grade serous ovarian cancer among excellent responders to neo- adjuvant chemotherapy.

1) ***Completed*** Subtask 1: Obtain institutional review board approval for use of patient data and biospecimens.

   a. Human subjects approval was obtained by the MD Anderson Cancer Center Institutional Review Board, protocol #2022-0078.

2) ***Completed*** Subtask 2 – Obtain Human Research Protection Office (HRPO) approval for use of patient data and biospecimens.

   a. The U.S. Army Medical Research and Development Command (USAMRDC),Office of Research Protections (ORP), Human Research Protection Office (HRPO) reviewed the protocol and found that it complies with applicable DOD, U.S. Army, and USAMRDC human subjects protection requirements. HRPO approval memorandum was received May 6, 2022.

3) ***Completed*** Subtask 3 – Perform multi-site whole genome sequencing on pre- treatment biopsy specimens from excellent responders to neo-adjuvant chemotherapy.

   a. We performed whole genome sequencing on multisite cryopreserved tumor biopsy samples and white blood cells obtained from 4 cases contained within the MD Anderson Cancer Center Multidisciplinary Gynecologic Oncology Tumor Bank. Sequencing data was quality controlled and aligned to the reference genome using standard procedures. Structural variants were called using a consensus method.

4) ***Completed*** Subtask 4 – Determine the subclonal architecture of pre-treatment high grade serous ovarian cancer using orthogonal mutation classes.

   a. High confidence structural variant calls were identified in Subtask 3 using multiple independent calling algorithms and applying a consensus method. We next performed subclonal reconstruction to identify structural variants common to most biopsy sites (Figure 1A) or unique to a single biopsy site (Figure 1B).

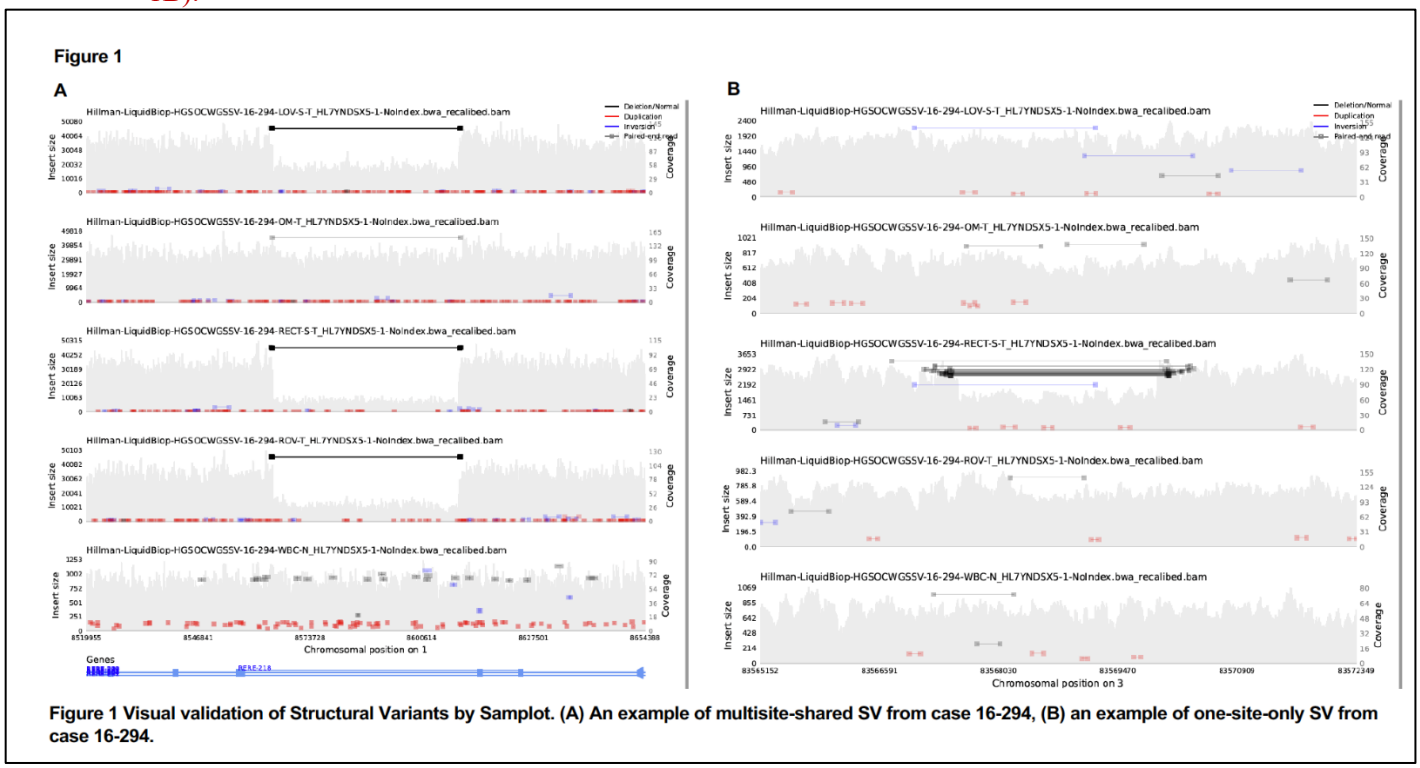

Figure 1 Visual validation of Structural Variants by Samplot. (A) An example of multisite-shared SV from case 16-294, (B) an example of one-site-only SV from case 16-294.

Major Task 2: Develop and validate a novel assay to detect structural variant breakpoints from liquid biopsies.

1) ***Completed*** Subtask 1 – Design digital droplet PCR assays for detection of structural variant breakpoints from circulating free DNA.

    a. We have developed and optimized an algorithmic workflow to design PCR primers for specific amplification of structural variants identified from multisite tumor biopsies in Major Task 1.

2) ***In Progress*** Subtask 2 – Determine the analytical parameters of pre-treatment clonal structural variant breakpoint detection using digital droplet PCR.

    a. We generated and validated synthetic cell free DNA from ovarian cancer cell lines and used this approach to validates analytical parameters of this approach. Consistent isolation and characterization of cfDNA from plasma have been established by using commercial kit (*QIAamp Circulating Nucleic Acid Kit, QIAGEN, cat# 55114*). Using quantitative Taqman PCR and digital droplet PCR, we determined the analytical limit of blank (LoB) and limit of detection (LoD) for detection of clonal structural variant breakpoints using this method.

3) ***In Progress*** Subtask 3 – Determine the analytical parameters of pre-treatment subclonal structural variant breakpoint detection using digital droplet PCR.

    a. Experiments are ongoing to determine the analytical parameters of pre-treatment subclonal structural variant breakpoint detection using digital droplet PCR.

4) ***In Progress*** Subtask 4 – Perform structural variant-based reconstruction of tumor subclonal architecture from circulating free DNA obtained at interval cytoreductive surgery.

    a. Work on Subtask 4 will await completion of Major Task 2, Subtasks 1-3 in order to have in place a fully optimized and characterized workflow for performing structural variant-based reconstruction of tumor subclonal architecture from circulating free DNA obtained at interval cytoreductive surgery.

- **What opportunities for training and professional development has the project provided?**

Jian Li, PhD, is a post-doctoral fellow who is partially supported by the project. Her training activities have given her additional expertise and skills in the research applications of high throughput sequencing technologies including nucleic acid extraction and library preparation. In addition she is developing skills in using the R statistical computing environment for the analysis of structural variants identified from whole genome sequencing. Dr Li meets with the principal investigator on a weekly basis to discuss progress on the project and her other career development goals.

- **How were the results disseminated to communities of interest?**

Nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

Determine the analytical parameters of pre-treatment subclonal structural variant breakpoint detection using digital droplet PCR and perform structural variant-based reconstruction of tumor subclonal architecture from circulating free DNA obtained at interval cytoreductive surgery. Work towards achieving these goals is ongoing.

4. Impact

- **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to report.

- **What was the impact on other disciplines?**

Nothing to report.

- **What was the impact on technology transfer?**

Nothing to report.

- **What was the impact on society beyond science and technology?**

Nothing to report.

5. Changes/Problems

Nothing to report.

6. Products

- **Publications, conference papers, and presentations**
  - **Journal publications.** *Nothing to report.*
  - **Books or other non-periodical, one-time publications.** *Nothing to report.*
  - **Other publications, conference papers, and presentations.** *Nothing to report.*
- **Website(s) or other Internet site(s).** Nothing to report.
- **Technologies or techniques.** Nothing to report.
- **Inventions, patent applications, and/or licenses**. *Nothing to report.*
- **Other Products.** Nothing to report.

7. Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

Name:	Robert Tyler Hillman
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID)	0000-0002-9832-9925
Nearest person month worked:	1
Contribution to the Project:	Supervised experiments and supported project member's progress and career development.
Funding Support	*N/A*

Name:	Jian Li
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID)	0009-0000-4412-7968
Nearest person month worked:	6

Contribution to the Project:	Sample process, experimental design, troubleshooting
Funding Support	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report.

- **What other organizations were involved as partners?**

Nothing to report.

8. Special Reporting Requirements

N/A

COLLABORATIVE AWARDS:

N/A

QUAD CHARTS:

N/A

9. Appendices