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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Genetic screening is now recommended for all women diagnosed with ovarian cancer as a basis for guiding cancer prevention (such as oophorectomy) and treatment (such as PARP inhibitors). These procedures and treatments can potentially save lives. However, many of the genetic alterations found in screens of ovarian cancer genes like BRCA2 are variants of uncertain significance (VUS) which do not generally inform clinical care. As a basis for better interpreting the significance of missense BRCA2 VUS, we have developed an expression system to characterize their effects on homologous recombination and cellular resistance to PARP inhibitors. In the past year, we have generated expression constructs containing 8 benign and 8 pathogenic missense BRCA2 variants for validation of our system. We have also developed expression constructs and cells for 29 missense BRCA2 VUS for which we also have clinical/genetic data. Results of HR assays and clinical/genetic data for these VUS will be used for multifactorial statistical models of pathogenicity.

15. SUBJECT TERMS

BRCA2, BRCA1, PALB2, RAD51C, Tumor Suppressors, Homologous Recombination, PARP Inhibitor, Variants of Uncertain Significance, Functional Stratification, Multifactorial Tests

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1. Introduction

The purpose of this project is to empower genetic screens for ovarian cancer by developing functional assays to classify variants of uncertain significance (VUS) in genes that function as tumor suppressors by mediating homologous recombination (HR). Beginning with *BRCA2*, we will validate and perform functional assays to predict the effects of missense VUS on cancer risk using HR assays and on treatment response to PARP inhibitors. These results will also be combined with clinical and genetic data in a multifactorial statistical analysis. Additionally, we will develop expression systems for functional tests of variants of other ovarian cancer genes related to HR, including *PALB2*, *RAD51C*, *RAD51D*, *BRCA1* and *BRIP1*.

2. Keywords

BRCA2, Tumor Suppressors, Homologous Recombination, PARP Inhibitors, Variants of Uncertain Significance, Functional Stratification, Multifactorial Tests

3. Accomplishments

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 is a progress report for the second year (6/15/2019–6/14/2020) of a three year project.

***Aim 1*. We will establish rapid functional assays of *BRCA2* VUS, and initiate characterization to predict pathogenicity as well as increased sensitivity to a PARP inhibitor utilized to treat ovarian cancer.**

Major Task 1: Validate HR assays in non-transformed cells, to predict cancer risk, using benign & pathogenic *BRCA2* standards (Months 1-6). 85% completed at present.

Major Task 2: Predict cancer risk for 125 selected *BRCA2* VUS based on HR assays in non-transformed *BRCA2*-deficient cells, and perform multifactorial analyses that integrate results from these functional assays with genetic/pathologic/biophysical data on VUS (Months 3-33). 30% completed at present (work on Major Task 2 is outlined to continue into year 3).

Major Task 3: Validate PARP inhibitor sensitivity assays in PE01 *BRCA2*-deficient ovarian cancer cells for predictions of the effect of specific *BRCA2* variants on the response to treatment (Months 1-6). 60% completed at present.

Major Task 4: Predict the effect of 125 selected *BRCA2* VUS on cellular sensitivity to the PARP inhibitor olaparib in reconstituted PE01 *BRCA2*-deficient ovarian cancer cells (Months 3-33). 30% completed at present (work for Major Task 2 is outlined to continue into year 3).

***Aim 2*. We will establish functional assays with a high volume capacity for *BRIP1*, *RAD51C*, *RAD51D*, *PALB2*, and *BRCA1* VUS to predict pathogenicity and increased cellular sensitivity to a PARP inhibitor.**

Major Task 1: Design and order codon-optimized cDNAs in a lentiviral vector for *BRIP1*, *RAD51C* & *RAD51D* (Months 12-13), and *PALB2* and *BRCA1* (Months 24-25) from GenScript. 50% completed at present (work for Major Task 1 is outlined to continue into year 3).

Major Task 2: Validate homologous recombination (HR) assays to predict cancer risk using non-transformed cells with genetic deficiencies for *BRIP1*, *RAD51C*, *RAD51D*, *PALB2*, and *BRCA1* (Months 14-36). 15% completed at present (work for Major Task 2 is mostly outlined to be completed in year 3).

Major Task 3: Validate PARP sensitivity assays to predict the effect of variants of *BRIP1*, *RAD51C*, *RAD51D*, *PALB2* and *BRCA1* on therapeutic response (Months 14-36). 15% completed at present (work for Major Task 3 is mostly outlined to be completed in year 3).

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.

Aim 1.

Major Task 1: Validate HR assays in non-transformed cells, to predict cancer risk, using benign & pathogenic *BRCA2* standards (Months 1-6). Eight benign (K2411T, A2717S, K2472T, K2729N, E2856A, K2950N, V3079I & V3098H), and eight pathogenic (W2626C, I2627F, L2647P, L2653P, D2723H, G2748D, R3052W & D3095E) variants have been selected to serve as standards for validation of HR reporter assays, as planned. These were selected based upon information in the ClinVar and ENIGMA databases, including segregation, domain of the protein, and evolutionary conservation and frequency in the general population. Further, as planned, these variants (standards) were ordered from GenScript to generate lentiviral expression constructs for full-length BRCA2 with and without variants.

HR assays in U2OS-DR cells with transient transfection of a siRNA depleting endogenous BRCA2 have been performed following reconstitution with the V2969M and Y3098H benign variants, and the G2748D and R3052W pathogenic variants. The benign variants had full activity similar to WT BRCA2co and the pathogenic variants were deficient for HR with activities similar to the empty vector (full loss-of-function). While tests for other benign and pathogenic standards were due to be completed within the first year of the project, they have not yet been completed due to a delay discussed below resulting from difficulties generating *BRCA2*-deficient cells containing the HR reporter (*Section 5. Changes/Problems*).

Major Task 2: Predict cancer risk for 125 selected *BRCA2* VUS based on HR assays in non-transformed *BRCA2*-deficient cells, and perform multifactorial analyses that integrate results from these functional assays with genetic/pathologic/biophysical data on VUS (Months 3-33). In year 1, we identified ~30 missense BRCA2 VUS in the C-terminal DBD where the benign/pathogenic standards are also localized. There are individuals/families in the Ohio State University Cancer Registry that have each of these variants, so upon completion of our functional tests there will be clinical data available for Amanda Toland (co-I) to perform multifactorial statistical analyses of the pathogenicity for each variant. Following completion of year 2, we have already ordered each of these missense BRCA2 VUS from GenScript and inserted them into the lentiviral expression construct: R2418G, K2434T, S2483N, L2587F, P2589S, V2610M, W2619S, H2623R, I2628T, R2651T, T2662K, I2664M, T2681R, S2709G, V2739I, I2752F, A2770D, R2784W, R2784Q, S2810G, G2812E, R2842C, Q2925H, A2942T, L2972W, K3059N, D3064Y, C3069F, and Y3092C. *BRCA2*-deficient U2OS-DR cells have been reconstituted with 8 of these VUS (see also Table 1): V2739I, I2752F, A2770D, R2784W, R2784Q, S2810G, G2812E and R2842C. In our initial results, 5 (63% of those assayed) had compromised HR activities indicative of a potentially increased risk of developing cancer but none displayed full loss-of function (LOF). The five VUS with intermediate activity were: A2770D, R2784W, R2784Q, G2812E and R2824C. While the initial plan was for HR assays of a majority of the 125 missense BRCA2 VUS to be completed by the end of year 2, this has been delayed by difficulties generating suitable *BRCA2*-deficient cells which contain an HR reporter (see *Section 5. Changes/Problems* for more detail).

Major Task 3: Validate PARP inhibitor sensitivity assays in PE01 *BRCA2*-deficient ovarian cancer cells for predictions of the effect of specific *BRCA2* variants on the response to treatment (Months 1-6). As described for Major Task 1, 8 benign and 8 pathogenic variants were selected to serve as standards for validation of PARP inhibitor sensitivity assays, and lentiviral expression constructs for expression of full-length BRCA2 with and without these variants have now been generated.

PARP inhibitor assays with cells containing benign (neutral) and pathogenic (deleterious) variants were to be completed within the first year of the project, but have not yet been completed due to results

demonstrating the need to change the cell type these assays are being conducted in. These delays are discussed below in *Section 5. Changes/ Problems*.

Major Task 4: Predict the effect of 125 selected *BRCA2* VUS on cellular sensitivity to the PARP inhibitor olaparib in reconstituted PE01 *BRCA2*-deficient ovarian cancer cells (Months 3-33). We have identified ~30 missense BRCA2 VUS in the C-terminal DBD where the benign/pathogenic standards are also localized, and generated lentiviral-BRCA2co expression constructs containing these VUS, as described above for Major Task 2. At the end of year 2 of this project, 25 of these missense BRCA2 VUS have been successfully expressed in *BRCA2*-deficient cells and tested for cellular resistance to the PARP inhibitor olaparib (see Table 1). To date, we have identified 5 (T2662K, S2709G, R2784W, R2784Q and R2842C) which conferred increased sensitivity to olaparib. That represents 25% of the VUS assayed to this point. In each, case there was partial resistance to olaparib, as compared to the activity of the empty vector. Importantly, 2 (A2770D and G2812E, underlined in Table 1) conferred compromised HR but did not alter resistance to olaparib. While completion of assays of cellular resistance to olaparib for the majority of 125 BRCA2 VUS was initially outlined by the end of year 2, this has been delayed by the need to switch recipient cells for this assay (see *Section 5. Changes/Problems* for more detail).

Table 1. List of BRCA2 VUSs generated in the lentiviral-Flag-HA-BRCA2 codon-optimized construct and tests performed on them to date (through June 14, 2020)

VUS	Olaparib sensitivity	HR
R2418G	No	
K2434T	No	
L2584F	No	
S2483N	No	
L2584F	No	
P2589S	No	
V2610M	No	
H2623R	No	
I2628T	No	
R2651T	No	
T2662K	Intermediate	
I2664M	No	
S2709G	Intermediate	
V2739I	No	Normal
I2752F	No	Normal
A2770D	No	Intermediate
R2784W	Intermediate	Intermediate
R2784Q	Intermediate	Intermediate
S2810G	No	Normal
G2812E	No	Intermediate
R2842C	Intermediate	Intermediate (severe)
Q2925H	No	
A2942T	No	
K3059N	No	
D3064Y	No	
C3069F	No	

Olaparib: No – normal reconstitution of resistance when expressed in BRCA2$^{-/-}$ cells. Intermediate indicates decreased resistance but greater than cells containing the empty vector.

HR: Normal indicates similar to activity of WT BRCA2co. Intermediate indicates reduced HR compared to WT BRCA2 but greater than the activity of the empty vector. Not tested yet if left blank.

***Aim 2*.**

Major Task 1: Design and order codon-optimized cDNAs in a lentiviral vector for *BRIP1*, *RAD51C* & *RAD51D* (Months 12-13), and *PALB2* and *BRCA1* (Months 24-25) from GenScript. We have initiated the design of codon-optimized cDNAs in a lentiviral vector for *BRIP1*, *RAD51C* and *RAD51D*, which was outlined to be completed by the end of year 2. Much of the work, specifically the design and generation of codon-optimized cDNAs for *PALB2* and *BRCA1*, will be undertaken in year 3 of this project as planned.

Major Task 2: Validate homologous recombination (HR) assays to predict cancer risk using non-transformed cells with genetic deficiencies for *BRIP1*, *RAD51C*, *RAD51D*, *PALB2*, and *BRCA1* (Months 14-36). We have reconstituted *PALB2*-deficient cells with 3 benign variants (D134N, E672Q and G998E) and 3 pathogenic variants (L35P, R753X and R1086X) of PALB2 ahead of schedule, since this was planned for year 3. The benign variants display HR activities similar to WT PALB2 and the pathogenic variants have HR function similar to the empty vector (full LOF). Work validating assays to test the effects of BRIP1, RAD51C and RAD51D VUS, while planned for year 2 of this project, have been delayed by an institutionally-directed slowdown resulting from the COVID-19 outbreak. This will be completed in year 3, along with validation of assays for BRCA1 variants which was already outlined for completion in year 3.

Major Task 3: Validate PARP sensitivity assays to predict the effect of variants of *BRIP1*, *RAD51C*, *RAD51D*, *PALB2* and *BRCA1* on therapeutic response (Months 14-36).
While generation of expression constructs for 3 benign variants and 3 pathogenic variants of PALB2 and reconstitution of cells has been completed, generation of lentiviral expression constructs for codon-optimized *BRIP1*, *RAD51C* and *RAD51D* containing benign and pathogenic standards is ongoing. Although the latter was planned for year 2, it has been delayed because of an institutionally-directed slowdown due to the COVID-19 outbreak. Reconstitution of cells for these genes with or without variants, and completion of PARP sensitivity assays will be completed in year 3, along with validation of assays for BRCA1 variants which was already outlined for completion in year 3.

4. Impact

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

Until recently, there was no system for testing the impact of BRCA2 variants based upon expression of the full-length human BRCA2 protein in human cells. Instead, previous systems relied upon expression of human BRCA2 in rodent cells. Because of the limited homology between human and rodent BRCA2, this has restricted assays to regions that are more highly conserved. While M. Ikegami et al. [2020 Nature Communications 11(1):2573] reported a transposase-based expression system for human BRCA2 in human cells, their assays focused on drug sensitivity which is not deemed by the field to be as reliable as HR assays for predicting pathogenicity. Further, there are notable concerns about the ability of their system to fully reconstitute drug resistance to the levels seen in *BRCA2*-proficient cells and to reliably classify known benign/likely benign/likely pathogenic/pathogenic missense variants. To date, our system has not had these issues. We will test the expected full or no loss-of function variants of BRCA2 that Ikegami et al. could not adequately distinguish in our system, both for HR and olaparib sensitivity. We expect that this will establish our system as uniquely capable of evaluating the pathogenicity of missense BRCA2 variants throughout the protein, including regions of lower conservation.

Further, of 8 missense BRCA2 VUS tested, we have already found 4 that compromise HR. This supports the importance of functionally classifying the impact of missense BRCA2 VUS to better guide clinical care of patients harboring these variants. Notably, 2 of these (A2770D and G2812E) appear to have diminished HR capacity but do not affect cellular resistance to the PARP inhibitor olaparib. This uncoupling of HR and resistance to olaparib will be important to the cancer genetics field because it underscores the need to separately conduct HR and olaparib sensitivity assays for specific variants to predict effects on cancer risk and therapeutic response, respectively.

What was the impact on other disciplines?
The finding of novel variants that compromise BRCA2 function in HR and cellular resistance to olaparib will be useful to molecular biologists and biochemists, as well as biologists interested in DNA repair, the

maintenance of genome stability and cancer therapeutics. Such findings will increase understanding of BRCA2 function and may identify domain-specific activities. Further, the finding that A2770D and G2812E diminish BRCA2 function in HR but not its ability to mediate cellular resistance to olaparib will similarly yield unique insight into BRCA2 function and should be of interest to those working in the DNA repair, cell biology and cancer therapeutics fields.

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

Changes in approach and reasons for change
For Aim 1, originally, we expected to receive hTERT immortalized FA-D1 fibroblasts with a genetic deficiency for *BRCA2* from our collaborator, Helmut Hanenberg, which contained a GFP reporter for HR as a non-transformed model to predict the effects of BRCA2 variants on cancer risk. However, Dr. Hanenberg did not even have hTERT-immortalized FA-D1 fibroblasts, so we began by generating several such distinct cell lines. At that point, we decided that by generating pairs of hTERT-immortalized and Lg T-transformed FA-D1 fibroblasts, with subsequent introduction of the HR reporter and benign/pathogenic variants, we could compare their ability to predict the pathogenicity of BRCA2 variants. Subsequently, we discovered that introduction of an HR reporter into cells with a genetic deficiency for *BRCA2*, utilizing CRISPR-Cas9 or viral transduction approaches, dramatically reduces cell growth. For this reason, the resulting cells could not be used for robust and reliable HR assays. Thus, in year 2, as an alternative, we tested U2OS-DR cells already containing the HR reporter, along with depletion of endogenous BRCA2 utilizing a siRNA, with 10 missense BRCA2 variants (*Section 3. Accomplishments*).

Also, while tests of cellular resistance to olaparib were originally outlined in Aim 1 for PE01 ovarian cancer cells, following reconstitution with BRCA2 using our expression system, cell growth was not sufficient to support reliable assays. As an alternative, we are now utilizing Lg T antigen transformed FA-D1 fibroblasts, genetically-deficient for *BRCA2*, to distinguish the effects of neutral and deleterious BRCA2 variants on drug sensitivity.

Actual or anticipated problems or delays and actions or plans to resolve them
We first attempted to introduce the HR reporter at the AAVS1 locus using CRISPR-Cas9 mediated DNA double-strand breaks (DSBs). While we were successful in introducing the HR reporter and in generating clonal cell populations, the cells displayed progressively poorer growth presumably due to high levels of off-target cutting of DNA in cells already compromised for HR (due to deficiency for *BRCA2*). This has delayed *Major Tasks 1 & 2* of Aim 1 because we need to have cells with the HR reporter prior to introducing benign/pathogenic variants or BRCA2 VUS. To circumvent this problem, we also attempted to introduce the GFP-DR HR reporter by the approach typically used in the literature: transfection with the linearized reporter plasmid followed by drug (puromycin in this case) and clonal selection. Utilizing this approach, *BRCA2*-deficient cells containing the HR reporter again grew poorly. Finally, we attempted to utilize U2OS-DR cells with the HR reporter already integrated and in which we depleted endogenous BRCA2 utilizing a siRNA. While the assays were successful (see Table 1), we noticed significant variability between technical replicates which we attribute to the need to transiently transfect the cells with a plasmid encoding the I-SCEI endonuclease that induces DSBs. As an alternative to be utilized for ongoing studies, we have developed U2OS-DR cells with a stably expressing shRNA that strongly depletes endogenous BRCA2. Reconstitution of these cells with different BRCA2 VUS is ongoing. For *Major Tasks 3 & 4* of Aim 1, we found that PE01 *BRCA2*-deficient cells from an ovarian cancer patient grew increasingly slower following exogenous expression of full-length BRCA2 with or variants. We determined that due to this very slow rate of growth, assays in PE01 cells would not yield robust and reliable results. For this reason, we are now instead successfully employing *BRCA2*-deficient fibroblasts from a Fanconi anemia patient (D1 complementation group).

For Aim 2, reconstitution of deficient cells with codon-optimized cDNAs for *BRIP1*, *RAD51C* and *RAD51D* (*Major Tasks 2 & 3*) outlined for year 2 of this project was delayed because of decreased lab activity due to the COVID-19 outbreak, but work is ongoing.

Changes that had a significant impact on expenditures
Nothing to report for year 2 of the project

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report for year 2 of the project

Significant changes in use or care of human subjects
Nothing to report for year 2 of the project

Significant changes in use or care of vertebrate animals
Nothing to report for year 2 of the project

Significant changes in use of biohazards and/or select agents
Nothing to report for year 2 of the project

6. Products

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

Journal publications.

Nothing to report for year 2 of the project

Books or other non-periodical, one-time publications.

Nothing to report for year 2 of the project

Other publications, conference papers and presentations.

H. Abe, K.G. Alavattam, Y.C. Hu, Q. Pang, P.R. Andreassen, R.S. Hegde and S.H. Namekawa. 2020. The initiation of meiotic sex chromosome inactivation sequesters DNA damage signaling from autosomes in mouse spermatogenesis. Current Biology 30, 408-420. *Published.*

Y. Dong, X. Zhao, X. Feng, Y. Zhou, X. Yan, Y. Zhang, J. Bu, D. Zhan, Y. Hayashi, Y. Zhang, Z. Xu, R. Huang, J. Wang, T. Zhao, Z. Xiao, Z. Ju, P.R. Andreassen, Q.F. Wang, W. Chen and G. Huang. 2019. SETD2 mutations confer chemoresistance in acute myeloid leukemia partly through altered cell cycle checkpoints. Leukemia 33, 2585-2598. *Published.*

R. Jayavaradhan, D.M. Pillis, M. Goodman, F. Zhang, Y. Zhang, P.R. Andreassen* and P. Malik. 2019*. CRISPR-Cas9 fusion to dominant-negative 53BP1 enhances HDR and inhibits NHEJ specifically at Cas9 target sites. Nature Communications 10(1), 2866. * denotes co-corresponding authorship. *Published.*

Y. Yu, K. Choi, J. Wu, P.R. Andreassen, P.J. Dexheimer, M. Keddache, H. Brems, R.J. Spinner, J.A. Cancelas, L.J. Martin, M.R. Wallace E. Leguis, K.S. Vogel and N. Ratner. 2020. NF1 patient missense variants predict a role for ATM in modifying neurofibroma initiation. Acta Neuropathologica 139, 157-174. *Published.*

- **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.

  Modular Expression Systems for Gene Expression and Methods of Using Same, International Patent US2019/55808 filed Oct 2019. This follows up Provisional Application 62/744,831 in Oct. 2018.

- **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:	*Paul R. Andreassen, PhD* Unchanged
Name:	*Yue Zhang, MD* Unchanged
Name: *Nearest person months worked:*	*Fan Zhang, MS* 6
Name:	*Amanda E. Toland, PhD* Unchanged
Name:	*Helmut Hanenberg, MD*

Unchanged

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

Two grants of the PI, Dr. Paul R. Andreassen, which were pending at the end of the last reporting period, have been subsequently awarded:

NIH R01 award GM134731
Study entitled, “Functional characterization of the role of distinct domains of ATM and the impact of sequence variants on the DNA damage response”.
20% Effort.
PI.
08/20/19 – 05/31/23

Doris Duke Charitable Foundation: Sickle Cell Disese/Advancing Cures Program 2019
Study entitled, “Reducing error-prone repair for therapeutic correction of sickle cell anemia
10% Effort
Co-Investigator (PI: Punam Malik)
09/01/19 – 08/31/22

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Nothing to report

8. Special Reporting Requirements

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