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TITLE: Testing Chemoprevention Strategies in a Genetically Engineered Mouse Model of High-Grade Serous Carcinoma

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14. ABSTRACT

Given the challenges associated with effecting cures for women with tubo-ovarian high-grade serous carcinoma (HGSC), an enhanced focus on preventing these tumors is warranted. Genetically engineered mouse models (GEMMs) of cancer provide tractable and relatively rapid systems with which to test cancer prevention strategies. We recently developed *Ovgp1-iCreERT2* mice that also carry floxed *Brca1, Trp53, Rb1,* and *Nf1* TSG alleles (hereafter called *BPRN* mice). Transient tamoxifen (TAM) treatment of these mice consistently results in oviductal HGSCs after several months. The long latency and time for disease progression render our *BPRN* model ideally suited to test potential chemopreventive agents for their ability to prevent or delay HGSC development. This project aims to test the hypotheses that PARP inhibition (Aim 1) and/or treatment with lipophilic statins (Aim 2) will delay or prevent HGSC development in our GEMMs. We have largely completed Major Task 1 for each Aim, as proposed. For Aim 1, cohorts of *BPRN* mice (n=21 each) have been generated for each of 5 groups (control and four different olaparib treatment regimens). For Aim 2, cohorts of *BPRN* (n=20 each) and *ApcPten* (*AP*) mice (n=17 each) have been generated for each of 3 groups (control and two different simvastatin treatment regimens). Most of the mice have been TAM-injected to induce tumors, have begun the proposed drug treatments, and are in the monitoring phase of the work.

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1. **Introduction:** Given the many challenges associated with effecting cures for women with tubo-ovarian high-grade serous carcinoma (HGSC), an enhanced focus on preventing these tumors is warranted. For women with hereditary predisposition to ovarian cancer, choosing if, and when, to undergo risk-reducing bilateral salpingo-oophorectomy (RRSO) is a challenge. Identification of effective and well-tolerated chemopreventive strategies could provide at-risk women with an opportunity to delay, or even forego, RRSO and avoid the negative consequences of premature menopause. Genetically engineered mouse models (GEMMs) of cancer provide tractable and relatively rapid systems with which to test cancer prevention strategies. We recently developed *Ovgp1-iCreERT2* mice that also carry floxed *Brca1, Trp53, Rb1,* and *Nf1* TSG alleles (hereafter called *BPRN* mice). Transient tamoxifen (TAM) treatment of these mice consistently results in oviductal HGSCs after several months. The long latency and time for disease progression render our *BPRN* model ideally suited to test potential chemopreventive agents for their ability to prevent or delay HGSC development. We have also used *Ovgp1-iCreERT2* mice to conditionally inactivate *Apc* and *Pten* (*AP*) in the oviductal epithelium. The *AP* mice develop a different type of cancer known as endometrioid carcinoma. This project aims to test the hypotheses that PARP inhibition (PARPi) and/or treatment with lipophilic statins will delay or prevent HGSC (or endometrioid carcinoma) development in our GEMM(s). Specifically, Aim 1 will test effects of continuous and intermittent PARPi (olaparib) on HGSC pathogenesis in *BPRN* mice. Aim 2 will test whether a lipophilic statin (simvastatin) prevents or delays tumor development and/or progression in our *BPRN* model of HGSC and/or *AP* model of endometrioid carcinoma.

2. **Keywords:** cancer prevention, high-grade serous carcinoma, endometrioid carcinoma, olaparib, simvastatin, genetically engineered mouse model, PARP inhibition

3. **Accomplishments:** This project has two specific aims:

Specific Aim 1: To test preventive effects of continuous and intermittent PARP inhibition on HGSC pathogenesis in post-TAM *BPRN* mice.

Major Task 1: Generate sufficient numbers of female *BPRN* mice for Aim 1 (0-12 months).
What was accomplished: We have achieved the 12 month milestones associated with this goal as indicated in our Statement of Work (SOW). Specifically, our local IACUC protocol (PRO00008343) was successfully amended to allow treatment of mice with olaparib-compounded chow and ACURO approval was also obtained. We have generated five cohorts of female *BPRN* mice (n=21 each). The five cohorts are as follows: 1) control; 2) constant oral high dose (200 mg/kg) olaparib; 3) constant oral low dose (50 mg/kg) olaparib; 4) intermittent oral high dose (200 mg/kg) olaparib; and 5) intermittent oral low dose (50 mg/kg) olaparib. All 105 mice were treated with TAM at 6-8 weeks of age to induce HGSC formation. TAM-treated mice are in the process of being fed olaparib compounded chow (ad lib) on a continuous or intermittent basis. Our pharmacokinetics core has confirmed that drug levels in the chow are correct, and that plasma levels of drug reflect dosing (Table 1). All mice are currently in the monitoring phase of the project, ranging from 4 mo to 9 mo post-TAM.

Table 1. Olaparib concentration in mouse plasma and compounded chow.

Plasma samples were obtained from 3 mice, and 3 aliquots of compounded chow were tested.

BLQ: below limit of quantification. NA: not applicable

Olaparib Dose (mg/kg)	Olaparib Concentration (ng/mL) in Plasma			
	Replicate 1	Replicate 2	Replicate 3	Average
Control	BLQ	BLQ	BLQ	NA
50	4.87	4.82	9.13	6.27
200	85.5	89.3	41.9	72.23

Olaparib Dose (mg/kg)	Olaparib Concentration (mg/kg) in Food			
	Replicate 1	Replicate 2	Replicate 3	Average
50	47.32	48.16	49.84	48.44
200	185.08	188.16	173.88	182.37

Specific Aim 2: To test whether lipophilic statins prevent or delay tumor development and/or progression in our *BPRN* model of HGSC and/or *AP* model of endometrioid carcinoma

Major Task 1: Generate sufficient numbers of female *BPRN* and *AP* mice for Aim 2.
What was accomplished: We largely achieved the 12 month milestones associated with this goal as indicated in our Statement of Work (SOW). Specifically, our IACUC protocol (PRO00008343) was successfully amended to allow treatment of mice with simvastatin-compounded chow and ACURO approval was also obtained. We have generated three cohorts of female *BPRN* mice (n=20 each) and have nearly finished generating three cohorts of *AP* mice (n=17, with goal of n=20). For each genotype, the three cohorts are as follows: 1) control; 2) oral high dose (120 mg/kg) simvastatin; and 3) oral low dose (12 mg/kg) simvastatin. We have been unable to breed new animals since mid-March, when our laboratory was shut down in response to the COVID-19 pandemic. Laboratory work is just re-starting and we anticipate being able to fill the *AP* cohorts in the next few months. Until our Unit for Laboratory Medicine is fully functional, we are not permitted to breed new experimental animals. Existing *BPRN* and *AP* mice bred for this project were treated with TAM at 6-8 weeks of age to induce HGSC or endometrioid carcinoma formation, respectively. The TAM-treated mice have been receiving simvastatin-compounded or control chow (ad lib) on a continuous basis. Our pharmacokinetics core is in the process of confirming that drug levels in the chow are correct, and that plasma levels of drug reflect dosing. This has been delayed due to the shut-down of our core facilities in response to the COVID-19 pandemic.

What opportunities for training and professional development has the project provided: Nothing to report.

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. Once laboratory operations fully resume, we will complete the *AP* mouse cohorts for Specific Aim 2, treat the newly bred mice with TAM, and initiate drug treatment for animals not already being dosed. We are ahead of schedule with studies proposed in Aim 1, and a bit behind for studies proposed in Aim 2 (due to the COVID-19 pandemic). We do not anticipate being unable to complete the proposed work on schedule, assuming lab work can fully resume this summer. For Aim 1, we will continue to provide cohorts of post-TAM *BPRN* mice with standard chow or chow containing olaparib during the entire 12-month monitoring period (Major Task 2, Subtask 1). We will euthanize and necropsy olaparib or control-treated mice following 12 months (or earlier if humane endpoints are reached) (Major Task 2, Subtask 2) and begin analyzing tissues for presence and extent of oviductal tumors in these cohorts (Major Task 3). For Aim 2, once we have completed generating the desired cohorts, remaining mice will be treated with TAM and all mice will be treated with control or simvastatin chow as indicated above (Major Task 2, Subtask 1). We will euthanize and necropsy simvastatin or control-treated mice following 12 months (or earlier if humane endpoints are reached) (Major Task 2, Subtask 2) and begin analyzing tissues for presence and extent of oviductal tumors in these cohorts (Major Task 3).

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

Changes in approach and reasons for change: We anticipate no changes in the approach described in our original application.

Actual or anticipated problems or delays and actions or plans to resolve them. As indicated above, breeding of animals to fill *AP* mouse cohorts for the Aim 2 studies was halted during our lab shut-down in response to the COVID-19 pandemic. This led to an unanticipated delay in completing Major Task 1 of Aim 2. Assuming laboratory work can fully resume by mid-summer, we believe we can complete this project on schedule. If our Unit for Laboratory Animal Medicine does not fully activate and we cannot fill the Aim 2 cohorts, we will complete the work with mice already available. As indicated above, each cohort already includes at least 17 of the desired 20 mice.

Changes that had a significant impact on expenditures: We have had reduced supply expenditures since mid-March while our lab was shut down, but do not expect this to have a major impact on the overall budget now that lab activities are resuming.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents. No major changes to report. Two ACURO protocol amendments were approved on 07/15/19 (OC180232.e001) and 03/16/20 (OC180232.e002). Both amendments were necessitated by changes to our institutional protocol (IACUC PRO00008343) for studies unrelated to this award.

6. Products

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	Kathleen Cho
Project Role	Principal Investigator
Researcher Identifier (ORCID)	ORCID 0000-0003-0500-9998
Nearest person month worked	1
Contribution to project	General project oversight, experimental design, data interpretation
Funding support	In addition to funding from this grant, Dr. Cho receives funding support from the National Institutes of Health

Name	Yali Zhai
Project Role	Co-Investigator
Researcher Identifier (ORCID)	N/A
Nearest person month worked	2
Contribution to project	Breeding colony management, genotyping, animal husbandry, experimental design and execution, data interpretation
Funding support	In addition to funding from this grant, Dr. Zhai receives funding support from the National Institutes of Health

Name	Rong Wu
Project Role	Co-Investigator
Researcher Identifier (ORCID)	N/A
Nearest person month worked	5
Contribution to project	Breeding colony management, genotyping, animal husbandry, experimental design and execution, data interpretation
Funding support	In addition to funding from this grant, Dr. Wu receives funding support from the National Institutes of Health

Name	Xiaoman Hou
Project Role	Graduate student
Researcher Identifier (ORCID)	N/A
Nearest person month worked	3
Contribution to project	Breeding colony management, genotyping, animal husbandry, experimental design and execution, data interpretation
Funding support	Ms. Hou is a PhD candidate fully funded by her home institution (Fudan University, Shanghai, China)

Name	Lixing Chen
Project Role	Graduate student
Researcher Identifier (ORCID)	N/A
Nearest person month worked	3
Contribution to project	Breeding colony management, genotyping, animal husbandry, experimental design and execution, data interpretation
Funding support	Ms. Chen is a PhD candidate fully funded by her home institution (Central South University, Third Xiangya Hospital, Changsha, Hunan, China)

Has there been a change in the active other support of the PI or key personnel since the last reporting period? Nothing to report.

What organizations were involved as partners? Nothing to report.

8. **Special Reporting Requirements** N/A

9. **Appendices** None