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CONTRACTING ORGANIZATION: UNIVERSITY OF NORTH DAKOTA
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

KEY RESEARCH ACCOMPLISHMENTS:

- Successful synthesis and purification of 5F02 analogs with different chemical properties.
- The effect of histone-dependent PARP-1 inhibitors on androgen-dependent and -independent activation of AR signaling was evaluated.
- The functional antitumor activity of 5F02 and its analogs was examined in androgen-dependent and castration-resistant PC cells. 5F02 and its analogs demonstrated superior antitumor activity compared with NAD-like clinically relevant PARP-1 inhibitors olaparib, veliparib, and rucaparib.
- Physicochemical and ADME properties of 5F02 and its analogs were examined.

15. SUBJECT TERMS
PARP-1, PARG, PARP-1 inhibitors, histone-dependent PARP-1 regulation, poly(ADP-ribose), prostate cancer cells

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Unclassified	Unclassified	Unclassified	Unclassified	15	19b. TELEPHONE NUMBER *(include area code)*

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The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Androgen ablation has been the mainstay treatment for advanced prostate cancer (PC). Importantly, androgen receptor (AR) signaling is vital not only for the initiation of PC, which is initially androgen-dependent, but also for castration-resistant disease. However, AR-mediated functions are not completely abrogated by existing androgen deprivation therapies (ADT) and their therapeutic failure is often accompanied by various molecular alterations, such as androgen-independent AR activation and AR structural alterations, including expression of constitutively active AR variants that lack the ligand-binding domain (AR-Vs). PARP-1 serves as a functional modulator of AR transcriptional activity. Our proprietary histone-dependent PARP-1 inhibitors suppress AR transcriptional function and are therefore effective against both androgen-dependent and -independent routes of AR activation. Our studies show that our lead histone-dependent PARP-1 inhibitor 5F02 demonstrates superior antitumor activity compared with clinically relevant NAD-like PARP-1 inhibitors and antiandrogen agents in both androgen-dependent and castration-resistant cell models of human PC. The overall objective of our proposal is to examine the therapeutic potential of histone-dependent PARP-1 inhibitors and to investigate the molecular mechanisms underlying their antitumor activity. The proposed studies will provide valuable insight into new avenues for potential treatment of advanced prostate cancer.

KEYWORDS: *Provide a brief list of keywords (limit to 20 words).* **PARP-1, PARG, PARP-1 inhibitors, histone-dependent PARP-1 regulation, poly(ADP-ribose),** prostate **cancer cells**

ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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.

To Specific Aim 1: To identify histone-dependent PARP-1 inhibitors with the superior antitumor activity: Task 6: To examine pharmacokinetic (PK) profile of histone-dependent PARP-1 inhibitors. The following parameters will be calculated: Cmax, Tmax, half-life, and area under the curve (AUC). These studies will be performed in collaboration with the Moulder Center for Drug Discovery Research. Task 7: Evaluate acute and chronic toxicity of histone-dependent PARP-1 inhibitors. Evaluate acute and chronic toxicity of histone-dependent PARP-1 inhibitors. A single escalating dose study will be used to examine acute toxicity in C.B17/Icr-scid mice. Chronic toxicity will be examined using a 3-week course of treatment.

To Specific Aim 2: To investigate the molecular mechanism underlying PARP-1-dependent control of PC malignant growth. Task 2: To determine the effects of PARP-1 and PARG dysregulation during the onset of PC. We will knock down PARP-1 by expressing anti-PARP-1 shRNA in PC cells. Upon treatment with shRNA, we

will monitor the expression of NF-kappa B-dependent genes. Task 3: To determine genomic sites of PARP-1 and pADPr occupancy in PC chromatin. We will determine genome-wide binding sites that PARP-1 occupies in chromatin and compare the distribution of these sites in normal prostate cells and PC-derived cells.

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.

INTRODUCTION:

Androgen ablation has been the mainstay treatment for advanced prostate cancer (PC). Importantly, androgen receptor (AR) signaling is vital not only for the initiation of PC, which is initially androgen-dependent, but also for castration-resistant disease. However, AR-mediated functions are not completely abrogated by existing androgen deprivation therapies (ADT) and their therapeutic failure is often accompanied by various molecular alterations, such as androgen-independent AR activation and AR structural alterations, including expression of constitutively active AR variants that lack the ligand-binding domain (AR-Vs). PARP-1 serves as a functional modulator of AR transcriptional activity. Our proprietary histone-dependent PARP-1 inhibitors suppress AR transcriptional function and are therefore effective against both androgen-dependent and -independent routes of AR activation. Our studies show that our lead histone-dependent PARP-1 inhibitor 5F02 demonstrates superior antitumor activity compared with clinically relevant NAD-like PARP-1 inhibitors and antiandrogen agents in both androgen-dependent and castration-resistant cell models of human PC. The overall objective of our proposal is to examine the therapeutic potential of histone-dependent PARP-1 inhibitors and to investigate the molecular mechanisms underlying their antitumor activity. The proposed studies will provide valuable insight into new avenues for potential treatment of advanced prostate cancer.

BODY:

We are investigating the antitumor activity of histone-dependent PARP-1 inhibitors against PC cells, as proposed in the statement of work. The results we obtained in the past year are consistent with our hypothesis and reinforce our experimental rationale. Specific progress is reported below.

As it was indicated in our previous report, we performed analysis of several newly synthesized analogs of our lead histone-dependent PARP-1 inhibitor 5F02. Some of the novel agents were more potent than 5F02 in suppressing viability of androgen-dependent LNCaP cells. However, these agents were less effective than 5F02 in suppressing viability of castration-resistant AR-negative PC-3 and DU-145 cells suggesting that antitumor activity of these compounds is mediated primarily via inhibition of AR signaling. The development of therapeutics that overcome aberrant AR signaling in castration-resistant PC is an unmet medical need for treating lethal prostate cancer. Therefore, in the past year, we have synthetized and tested several novel 5F02

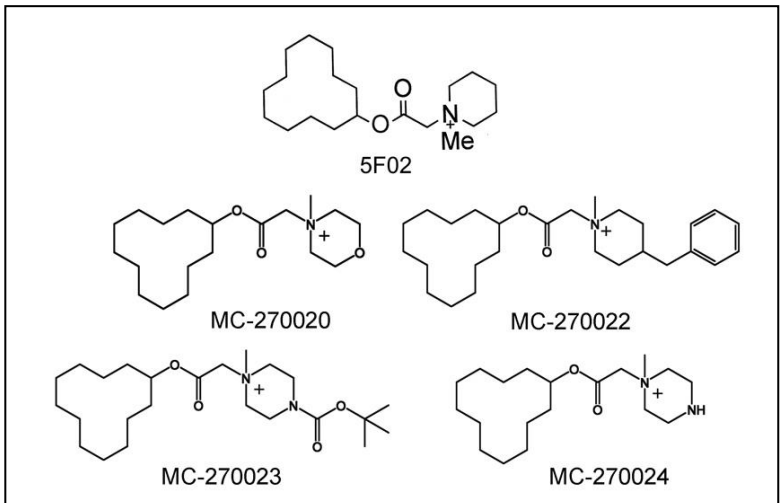


Figure 1. 5F02 and its novel analogs.

derivatives in the search of compounds able to inhibit growth of castration-resistant PC cells **(Fig. 1)**. We compared the effect of treatment with 5F02 and its novel analogs on the viability of androgen-dependent LNCaP and castration-resistant PC-3 and DU-145 PC cells. Treatment with MC-270020, MC-270023, and MC-270024 had no significant effect on the viability of all three tested PC cell lines, whereas the inhibitory effect of MC-270022 was more pronounced than that of 5F02 and NAD-like PARP-1 inhibitor olaparib **(Fig. 2)**.

We also determined the role of mutations in DNA damage-repair genes for sensitivity of PC cells to histone-dependent PARP-1 inhibitors. We focused our studies on the role of deficient expression of BRCA1, ATM, and CHEK2 in mediating synthetic lethality upon treatment with PARP-1 inhibitors. Initially, we planned to perform these experiments using PTEN-negative parental PC-3 PC cells and PC-3 cells with stable expression of PTEN generated in our laboratory and described previously (Makhov et al. Mol Cancer Ther. 2012 Jul;11(7):1510-7). However, several recent reports identified a synthetic lethal genetic interaction between PTEN and ATM (Kawahara et al., Biomed Rep. 2017 Nov; 7(5): 391–399; Li et al., Experimental Cell Research 2018 May 1, 366 (1): 24-33). Furthermore, several studies indicated that co-concurrent loss of PTEN and BRCA1 could potentially restore the HR repair efficiency in cells with defection of either BRCA1 or PTEN alone (Peng et al., Nat Commun. 2014; 5: 3361; O'Kane et al. Trends Mol Med. 2017 Dec;23(12):1121-1137). Therefore, for these studies we generated CRISPR/Cas9 knockouts of BRCA1, ATM, and CHEK2 using PTEN-positive DU-145 PC cells. No significant difference in sensitivity to 5F02 and olaparib was noted between wild-type and transformed DU-145 cells (**Fig. 3**).

It is critical to the success of this proposal that we have a firm understanding of the *in vivo* pharmacokinetics (PK) of our lead compounds. This will ensure that sufficient systemic exposure is achieved to support efficacy in an *in vivo* setting. Therefore, we investigated the PK profiles of 5F02 and MC-270022. The results of these studies are presented in **Figures 4** and **5** and **Tables 1** and **2**. These experiments were performed by our co-Investigator John Gordon, Ph.D. at the Moulder Center for Drug Discovery Research at Temple University School of Pharmacy. 5F02 displayed more favorable PK characteristics than that of MC-270022. MC-270022 presented slow distribution and metabolism after IV injection. It is possible that MC-270022 could be accumulated in tissues/organs through the distribution, and then remain prolonged exposure above 24 hours. Also, MC-27002 exhibited poor bioavailability after oral administration. The related factors of the poor bioavailability could include, but not limited to, compound's intestinal stability, lipophilicity, and strong polarity.

In parallel, we analyzed the plasma stability of 5F02 and MC-270022 to provide basic information about the properties of new probe and tool compounds. As demonstrated in **Figure 6**, 5F02 displayed better plasma stability than MC-270022 ($t_{1/2}$ 107.8 min vs. 31.9 min respectively). Procaine was used as a highly unstable reference compound. As indicated in **Figure 2**, the *in vitro* antitumor activity of MC-270022 was more potent than that of 5F02. However, 5F02 demonstrated more favorable PK profile and plasma stability compared with MC-270022. The greater complexity of the *in vivo* environment may have been responsible for these differences.

C_{max}, μg/ml	T_{max}, h	AUC_{0-24}, ug/ml*h	$T_{1/2}$, h	CL, L/h	Vss, L
0.6	2.0	1.87	4.56	0.065	0.3

Table 1. Summary of calculated PK parameters for the results of the study presented in Figure 4.

	C_{max}, μg/ml	T_{max}, h	AUC_{0-24}, ug/ml*h	$T_{1/2}$, h	CL, L/h	Vss, L
IV	0.28	1.0	0.79	23.9	0.065	101.67
PO	0.029	1.0	0.067	5		352.33

Table 2. Summary of calculated PK parameters for the results of the study presented in Figure 5.

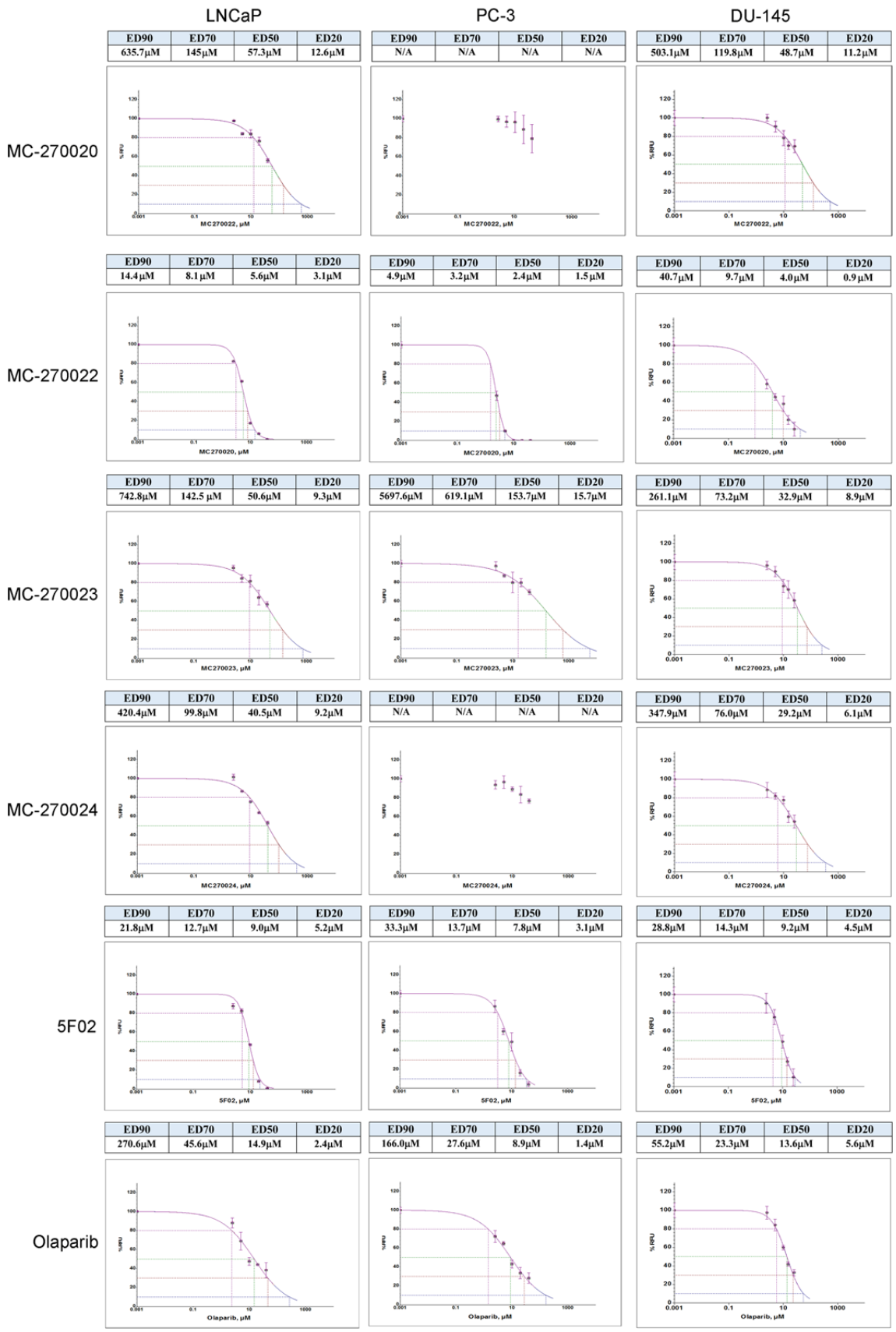


Figure 2. The effect of histone-dependent and NAD-like PARP-1 inhibitors on viability of androgen-dependent LNCaP and castration-resistant PC-3 and DU-145 human PC cells. Cells were treated with escalating concentrations of various PARP-1 inhibitors for 96 hours. Cellular viability was assessed using the CellTiter Blue assay. The effective doses (ED) were calculated using XLfit software.

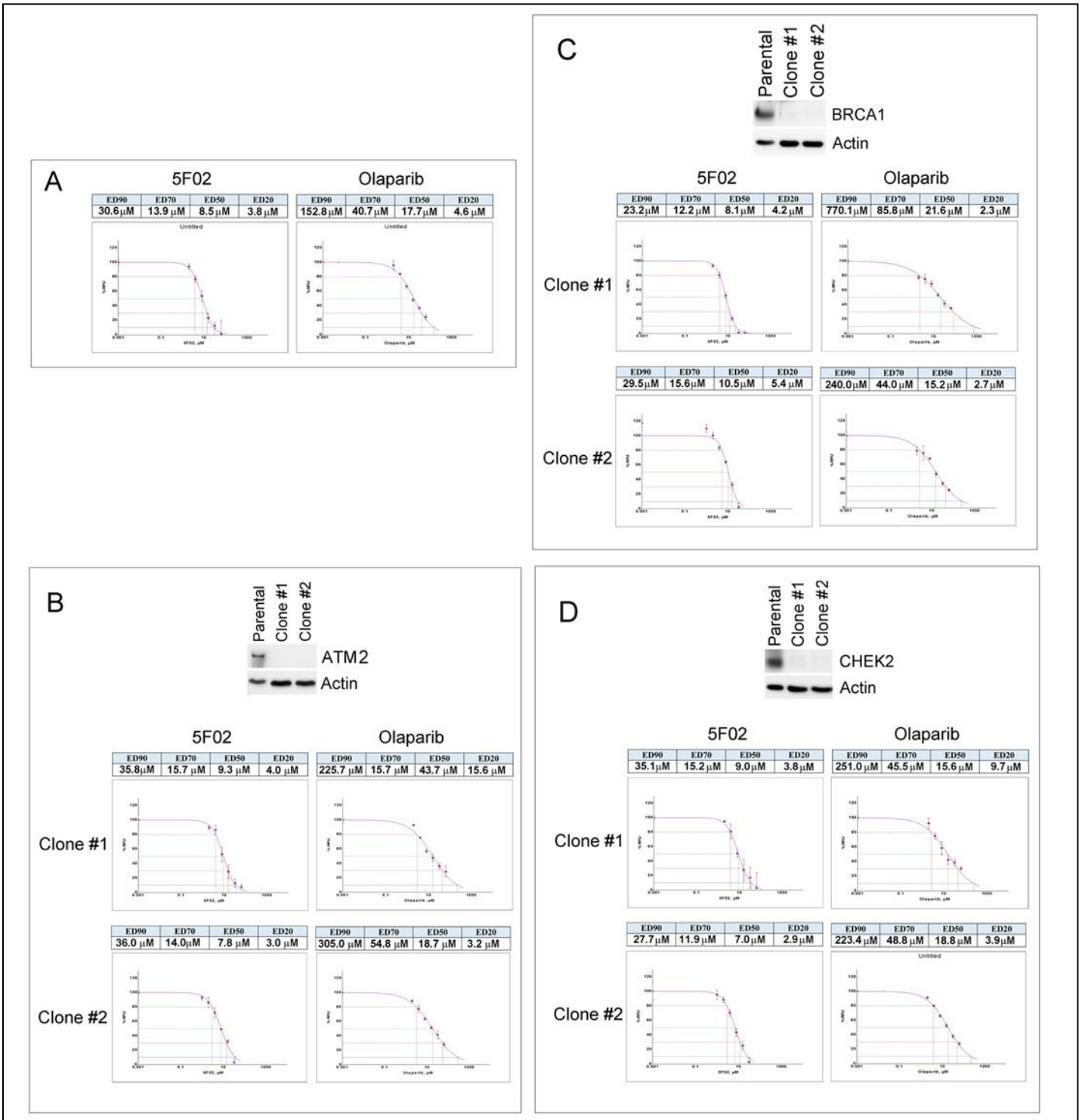


Figure 3. The effect of 5F02 and olaparib on viability of parental DU-145 PC cells and DU-145 cells with CRISPR/Cas9 knockouts of ATM, BRCA1 or CHEK2. Parental DU-145 cells and DU-145 cells with knockout of ATM, BRCA1 or CHEK2 were treated with either 5F02 or olaparib for 72 hrs. Samples were assayed for firefly luciferase activity. Cellular viability was assessed using the CellTiter Blue assay. The effective doses (ED) were calculated using XLfit software.

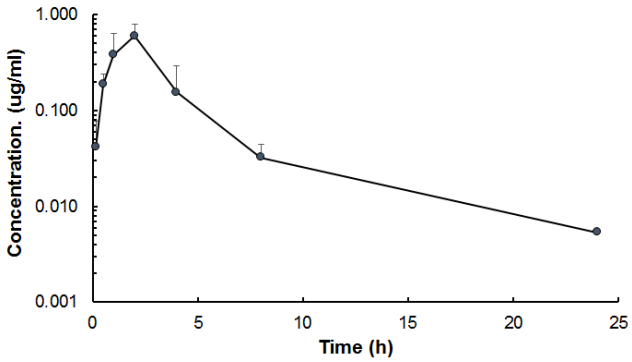


Figure 4. 5F02 levels in mice plasma samples following administration of 5F02 at 5 mg/kg.

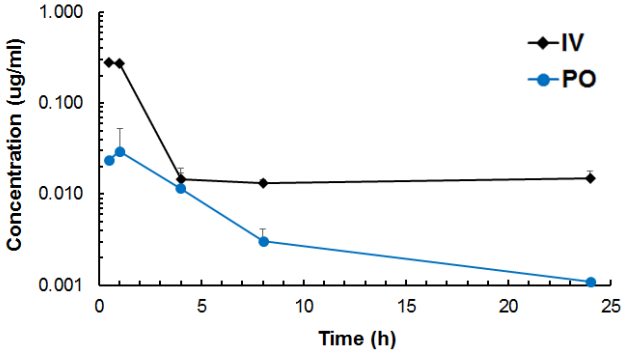
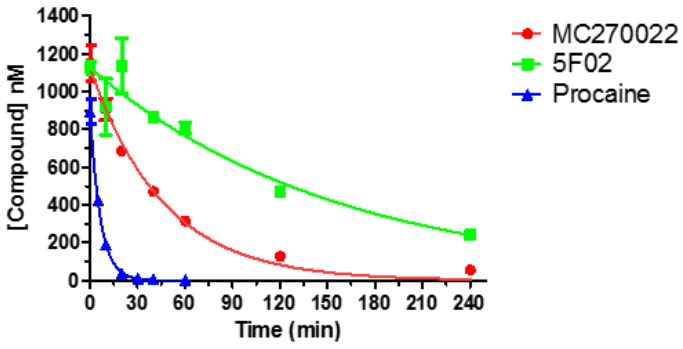


Figure 5. MC-270022 levels in mice plasma samples following IV and PO administration of MC-270022 at 5 mg/kg.



Compound	Media	t1/2 (min)
MC270022	Plasma	31.9
5F02	Plasma	107.8
Procaine	Plasma	4.6

Figure 6. Mouse plasma stability of 5F02 and MC-270022.

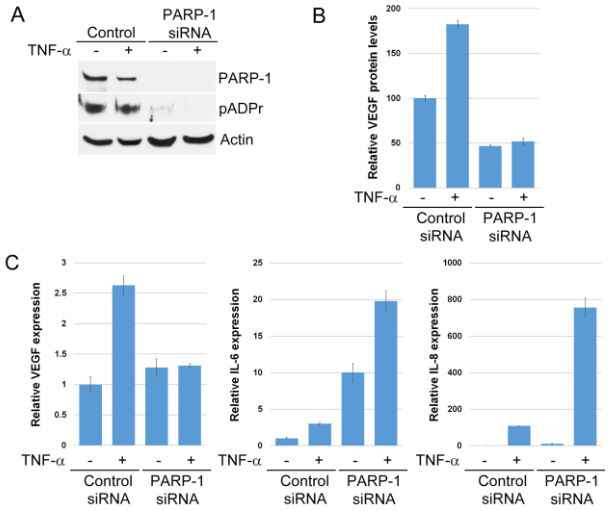


Figure 7. PC-3 PC and 769-P RCC cells after PARP-1 knockdown with siRNA. 48 hours after siRNA transfection cells were stimulated o/n with TNF-a.

Determine the in vitro binding affinity of novel compounds. The C-terminal catalytic domain of PARP-1 binds to histone H4, resulting in prolonged activation of this enzyme and sustained production of pADPr. The classical PARP-1 inhibitors stabilize binding of PARP-1 to the activator histone H4, while histone-dependent PARP-1 inhibitors disrupt PARP-1 binding to histone H4. We examined whether the ability of histone-dependent PARP-1 inhibitors to disrupt PARP-1 binding to histone H4 correlates with their anti-tumor efficacy. As we demonstrated in Figure 8, MC-270022, the compound demonstrating the most potent anti-cancer activity, also demonstrated the superior histone H4 binding ability compared with all other tested histone-dependent PARP-1 inhibitors.

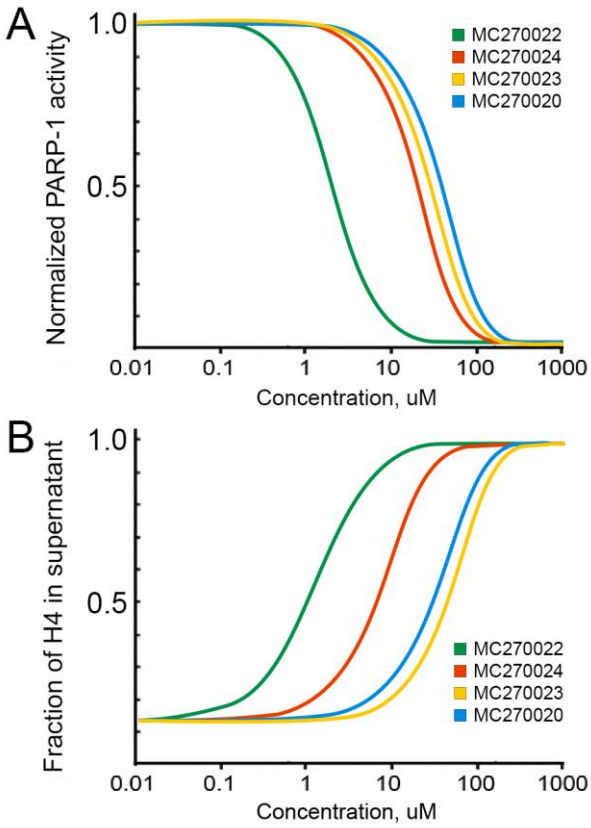


Figure 8. Activities of non-NAD-like PARP-1 inhibitors. A. Non-NAD-like inhibitors disrupt PARP-1 activation by histone H4. PARP-1 inhibition assay in cell free system using non-NAD-like PARP-1 inhibitor MC270020, MC270022, MC270023, MC270024. Reduction of pADPr accumulation (inhibition of PARP-1 activity) was detected by the ELISA assay for inhibitor-treated reaction mixtures relative to DMSO-treated mixtures. **B.** Non-NAD-like inhibitors disrupt PARP-1 binding to histone H4. *in vitro* binding assay: PARP-1 protein was covalently coupled to CnBr beads and preincubated with solution containing different concentration of non-NAD-PARP-1 inhibitors and then histone H4 was added. The reactions were incubated for 30 minutes and then beads were precipitated. The amounts of unbound histone H4 in supernatant were examined by the ELISA assay.

KEY RESEARCH ACCOMPLISHMENTS:

- Successful synthesis and purification of 5F02 analogs with different chemical properties.
- The effect of histone-dependent PARP-1 inhibitors on androgen-dependent and -independent activation of AR signaling was evaluated.
- The functional antitumor activity of 5F02 and its analogs was examined in androgen-dependent and castration-resistant PC cells. 5F02 and its analogs demonstrated superior antitumor activity compared with NAD-like clinically relevant PARP-1 inhibitors olaparib, veliparib, and rucaparib.
- Physicochemical and ADME properties of 5F02 and its analogs were examined.

CONCLUSION: Contemporary therapeutic agents, such as abiraterone and enzalutamide, have shown impressive results in pre- and post-chemotherapy settings, prolonging the survival of patients with CRPC. However, nearly all patients ultimately develop resistance to anti-androgen therapeutics. Therapeutic failure of anti-androgen therapeutics is often accompanied by various molecular alterations resulting in androgen-independent activation of AR signaling pathway. PARP-1 supports AR transcriptional function. Therefore, PARP-1 inhibitors can be effective against both androgen-dependent and -independent activation of AR signaling. Our group was the first to identify agents that specifically target the histone-dependent route of PARP-1 activation, a mechanism that is unique to PARP-1. The proposal described herein strives to harness the therapeutic potential of our proprietary histone-dependent PARP-1inhibitors and apply our findings to the development of therapeutics for patients with castration-resistant prostate cancer. The proposed strategy not only carries promise as a stand-alone approach, but likely can also dovetail with existing pharmaceutical tactics for prostate cancer. If the idea presented here is proven viable, significant clinical rewards are expected.

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.

Part of experiments described in the report were carried out by undergraduate student Keely Walker with an assistance from a very talented graduate school student, Gbolahan Bamgbose. Gbolahan joined my lab during the fall of 2019. Our current findings were presented as two posters at the UND annual research conference. Three manuscripts are now in preparation. All students contributed to writing and data analysis for these manuscripts and are listed as co-authors.

Gbolahan Bamgbose presented the results of his project at the UND summer retreat.

Keely Walker, undergraduate student in the lab, have devoted 100% of their research time to this project. Participating in this project, she has now mastered most of the techniques used in the lab and are now training two incoming high school and undergraduate students. Her efforts have been recognized by his admission to the MD program at the UND, Grand Forks, starting in the Fall of 2019.

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.

Students working in my lab have reported their findings to their home group seminar series. Our results will be reported as posters during one international meeting this summer and at the annual UND Epigenetics Symposium.

All recombinant plasmids, transgenic constructs, and recombinant cell lines obtained in specific ams 1 - 2 with have been shared with other research teams: Don Sinclair (Simon Fraser University, Canada); Alexey Veraksa (University of Massachusetts Boston, MA); Kolja Becker (Institute of Molecular Biology gGmbH (IMB)); Krzysztof Jagla, Ph.D (University of Clermont-Ferrand, France).

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.

To Specific Aim1: 1) To determine the role of mutations in DNA damage-repair genes for sensitivity of PC cells to histone-dependent PARP-1 inhibitors. The proposed studies will address the causal role of the mutations in DNA-repair genes for sensitivity of PC cells to histone-dependent PARP-1 inhibitors. These studies will be performed using parental (PTEN-negative) and PTEN-expressing PC-3 cells. 2) To examine pharmacokinetic (PK) profile of histone-dependent PARP-1 inhibitors. The following parameters will be calculated: Cmax, Tmax, half-life, and area under the curve (AUC). These studies will be performed in collaboration with the Moulder Center for Drug Discovery Research. 120 male C.B17/Icr-scid mice will be used for these experiments. 3) Evaluate acute and chronic toxicity of histone-dependent PARP-1 inhibitors. Evaluate acute and chronic toxicity of histone-dependent PARP-1 inhibitors. A single escalating dose study will be used to examine acute toxicity in C.B17/Icr-scid mice. Chronic toxicity will be examined using a 3-week course of treatment. 160 male C.B17/Icr-scid mice will be used for these experiments. *To Specific Aim2:* 1) To determine the effects of PARP-1 and PARG dysregulation during the onset of PC. We will knock down PARP-1 by expressing anti-PARP-1 shRNA in PC cells. Upon treatment with shRNA, we will monitor the expression of NF-kappa B-dependent genes. 2) To determine genomic sites of PARP-1 and pADPr occupancy in PC chromatin. We will determine genome-wide binding sites that PARP-1 occupies in chromatin and compare the distribution of these sites in normal prostate cells and PC-derived cells.

IMPACT: *Describe 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).

Traditionally, research on cancer epigenetics has been focused on investigating either histone or DNA modifications. In contrast, this proposal targets PARP-1, a protein that simultaneously functions as an effector and as an epigenetic mark. We identify cancer-related genes targeted by PARP-1 through a genome-wide approach. Products of these genes can serve as biomarkers for PARP-1 inhibitor sensitivity in future clinical trials involving our new histone-dependent inhibitors. In addition, the antitumor activity of a novel class of histone-dependent PARP-1 inhibitors is tested in primary PC cells, as well as a xenograft animal model of human PC. Technologically, we have developed a method of identifying histone-dependent PARP-1 inhibitors using the histone-dependent route of PARP-1 activation. Thus, we bypass off-target effects of classical NAD-dependent PARP-1 inhibitors. Also, since the histone-dependent activation route is unique to PARP-1, our novel histone-dependent PARP-1 inhibitors afford greater specificity.

We explore the efficacy of novel PARP-1 inhibitors against castration-resistant PC cells, which are notoriously difficult to treat, as they are resistant to most conventional therapeutic regimens. Notably, conventional PARP-1 inhibitors act via an AR-dependent route of transcription activation (3); thus, they are only effective against AR-positive PC cells. As shown in our studies, our histone-dependent PARP-1 inhibitors, on the other hand, have a completely different molecular mechanism of action (see below) and effectively suppress growth of both AR-positive and AR-negative PC cells, both *in vitro* and *in vivo*.

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.

My laboratory is a part of the UND medical school. The university provides outstanding education opportunities for diverse students to engage in hands-on research in STEM disciplines. Research completed for this grant in my laboratory has been an integral part of undergraduate and graduate student training. All experimental systems developed for this project were used to train undergraduate students (100% effort on the project) and several high school students. Two of our students, who made major contributions to the project, will be continuing their education as a Ph.D. student at UND.
Improvement of K-12 education: Collaborating with Ms. Sarina Bauer, an advanced biology teacher at Sacred Heart High School (East Grand Forks, MN), members of my research team and I engage high school students

in research on pADPr metabolism at the lab. Atreyi Ghatak (researcher in the lab) and I gave a number of presentations at the Sacred Heart School, explaining the significance of our research and introducing research opportunities in my laboratory. All interested students have visited my laboratory for a tour and a brief interview about their interests.

CHANGES/PROBLEMS: *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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.

Nothing to Report

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.

Nothing to Report

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.

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

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).*

Thomas C, Ji Y, Wu C, Datz H, Boyle C, MacLeod B, Patel S, Ampofo M, Currie M, Harbin J, Pechenkina K, Lodhi N, Johnson SJ, Tulin AV. Hit and run versus long-term activation of PARP-1 by its different domains fine-tunes nuclear processes. Proc Natl Acad Sci U S A. 2019 May 14;116(20):9941-9946.

Karpova Y, Wu C, Divan A, McDonnell ME, Hewlett E, Makhov P, Gordon J, Ye M, Reitz AB, Childers WE, Skorski T, Kolenko V, Tulin AV. Non-NAD-like PARP-1 inhibitors in prostate cancer treatment. Biochem Pharmacol. 2019 Sep;167:149-162.

> **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. In addition to a description of the technologies or techniques, describe how they will be 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. State whether an application is provisional or non-provisional and indicate the application number. 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;

biospecimen 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

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project? OSR will send a table for you to complete

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."

Alexei Tulin (PI)
Vladimir Kolenko (PI)
Danping Gou (Scientific Associate)
Atreyi Ghartak (Scientific Associate)
Iaroslava Karpova (postdoctoral associate)
Haily Datz (undergraduate student) (UND, ND, USA)
Victor Gromoff (undergraduate student) (UND, ND, USA)
Breanna McLain (undergraduate student) (UND, ND, USA)
Cody Boyle (undergraduate student) (UND, ND, USA)
Brett MacLeod (undergraduate student) (UND, ND, USA)
Sayem Bhuiyan (graduate student) (UND, ND, USA)

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? OSR will compare previous Other Support to Current Other Support

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.

Fox Chase Cancer Center, Philadelphia USA
Temple University, Philadelphia USA

•Name
Dr. Vladimir Kolenko, Ph.D.
Dr. John Gordon, Ph.D.

•Location
Fox Chase Cancer Center, Philadelphia USA
Temple University, Philadelphia USA

•Partner's contribution to the project
Collaborators

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating 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.*

Nothing to Report

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

Nothing to Report

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.*

Nothing to Report