AWARD NUMBER: W81XWH-19-1-0850

TITLE: Topical Nitric Oxide Therapy to Treat Cervical Neoplasias and Prevent HPV-Associated Cancers

PRINCIPAL INVESTIGATOR: Carri Geer, Ph.D.

CONTRACTING ORGANIZATION: Novan, Inc., Durham, NC

REPORT DATE: October 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2022	Annual	30Sep2021-29Sep2022

4. TITLE AND SUBTITLE
Topical Nitric Oxide Therapy to Treat Cervical Neoplasias and Prevent HPV-Associated Cancers

5a. CONTRACT NUMBER
W81XWH-19-1-0850

5b. GRANT NUMBER
CA180741

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The goal of this research is to develop a nitric oxide-releasing vaginal suppository that can be self-administered by female patients as a treatment for cervical neoplasias to eradicate latent HPV-18 infection and inhibit disease progression to cancer. The suppository will contain our proprietary NO-releasing drug, NVN1000, that has been shown to have antiviral efficacy against HPV-18. We have developed five prototype formulations using excipients determined to be compatible with NVN1000 and appropriate analytical methods for determining formulation stability. From the 12 week stability data, we selected a lead prototype candidate, although it appears refrigeration will be required to maintain stability. No further analysis of this formulation is planned. Through our collaborator at the University of Alabama Birmingham, we have also established a NVN1000 dose and application frequency that successfully inhibits HPV-18 replication in infected human raft cultures while minimizing cytotoxity and are in the process of evaluating the MOA of NO against E6 and E7 oncoprotiens.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	17	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

The goal of this research is to develop a nitric oxide-releasing vaginal suppository that can be self-administered by female patients as a treatment for cervical neoplasias to eradicate latent HPV infection and inhibit disease progression to cancer. This goal will be reached by formulating a stable vaginal suppository with well-characterized physical chemical properties suitable for intravaginal administration and evaluating the effect of varying concentrations and treatment durations of NVN1000 against HPV-18 in human raft cell culture in vitro studies.

2. KEYWORDS:

Nitric oxide, Human Papillomavirus, HPV-18, Vaginal Suppository, Antiviral, Cervical Intraepithelial Neoplasias

3. ACCOMPLISHMENTS:

What were the major goals of the project?

	Timeline (months)	**% Complete**
Specific Aim 1: Formulate a stable vaginal suppository with well-characterized physical chemical properties suitable for intravaginal administration.		
1.1 Formulation development of a nitric oxide releasing vaginal suppository.	12-14	75%
Deliverable 1: Selection of excipients compatible with NVN1000 following conduct of pre-formulation excipient compatibility studies.	1.5-2.0	100%
Deliverable 2: Creation of 5 prototype vaginal suppository formulations for evaluation of performance and stability.	2.0-2.5	100%
Deliverable 3: Development of a stability indicating chromatography analytical method development for the routine characterization of vaginal suppository at release and over time on stability. – Corresponding method development report.	2.0-3.0	100%
~~*Deliverable 4: Execution of stability testing program at 3 recommended ICH Climate conditions for up to 6 months in duration targeting at least one formulation having the minimum acceptable stability for clinical use. Corresponding stability report.*~~	7.0-8.0	0% (Aim no longer being pursued)
2.1 In vitro dissolution testing of vaginal suppository.	5.0-6.0	0% (Aim no longer being pursued)
~~*Deliverable 1: Development of an in vitro dissolution test method in simulated vaginal fluid utilizing the*~~	1.5-2.0	

~~*chemiluminescent nitric oxide analyzer employed in the characterization of other nitric oxide releasing drug products. – Corresponding method development report.*~~		
~~*Deliverable 2: Screening of the 5 prototype vaginal suppositories following dissolution in simulated vaginal fluid to determine acceptable loadings of NVN1000 for continued development based on appropriate pH thresholds.*~~	0.5-1.0	
~~*Deliverable 3: Generation of real time nitric oxide release kinetics of all of the lead prototype vaginal suppositories that have acceptable pH values upon dissolution.*~~	2.0-3.0	
3.1 Additional Performance Testing	4.0-5.0	0% (Aim no longer being pursued)
~~*Deliverable 1: Execution of condom compatibility testing and identification of a lead prototype suppository that does not impact condom integrity.*~~	1.0-1.5	
~~*Deliverable 2: Execution of mucoadhesion testing in an in vitro perfusion model and identification of a lead prototype suppository that has greatest mucoadhesive performance.*~~	1.0-1.5	
~~*Deliverable 3: Publish a Pharmaceutical Development Report integrating pre- formulation, formulation, analytical characterization, and performance testing results to assist with the preparation of future regulatory submissions to the FDA.*~~	1.0-2.0	
Specific Aim 2: Evaluate the effect of varying concentrations and treatment durations of NVN1000 in HPV-18 infected human raft cell cultures.		
2.1 **Evaluation of NVN1000's antiviral activity across a higher range of concentrations in vitro**	10.0	100%
Deliverable 1: Regulatory review and approval by the USAMRMC Human Research Protection Office (HRPO)	2.0-4.0	100%
Deliverable 2: Established minimally effective dose response of NVN1000 required to inhibit viral replication >90% qPCR. Data at 3, 4, 5 and 6 mg/ml administered to uninfected PHKs and HPV-18 infected PHK raft cultures.	4.0-6.0	100%
2.2 **Optimization of NVN1000 frequency of administration to HPV-18 infected cultures**	6.0	60%
Deliverable 1: Data for application once every other day	2.0-3.0	100%
Deliverable 2: Data for application once every three days	2.0-3.0	100%
Deliverable 3: Final report on minimally effective dose and frequency of application to inhibit >90% viral replication.	2.0-3.0	100%
Deliverable 4: Establish recovery and rebound of HPV DNA replication once NVN1000 challenge is	2.0-3.0	25%

removed. Data on drug-free chase experiments.		
2.3 **Further the understanding of nitric oxide's mechanism of action against E6 and E7 oncoproteins.**	6.0	10% (Waiting on completion of 2.2)
Deliverable 1: Data from in situ assays	1.0-2.0	
Deliverable 2: Data from biochemical assays	1.0-2.0	
Deliverable 3: Data from RNA-sequencing assays	2.0-2.5	
Deliverable 4: Final report and Manuscript on the impact of higher concentrations and longer exposure duration of NVN1000 on E6 and E7 oncoprotein activity, DNA damage, and markers of apoptosis.	1.0-2.0	

What was accomplished under these goals?

Specific Aim 1:
In July 2022, Novan met with SO/GOR Emilee Senkevitch and received permission to stop any further work on Specific Aim 1 (as indicated in Accomplishments section table above). Based on the data from the prototype formulations (as detailed in the Year 2 annual report) and the funds remaining, we determined that we were unlikely to meet the Go/No-go goal of 12 weeks of room temperature stability for the prototype formulation. These funds are being rebudgeted to Specific Aim 2.

Specific Aim 2.2, Deliverables 3 and 4, Specific Aim 2.3:
Every 2nd day of 4 mg/ml NVN1000 was established as the minimally effective dose required to inhibit viral replication while also minimizing toxicity on uninfected PHK raft cultures.

UAB has harveseted raft cultures, has completed the treated and untreated exposure periods under the optimized exposure regimen, and has prepared paraffin blocks of the formalin fixed tissue. Samples for RNA and DNA isolation and protein analysis have also been taken. They are currently in the process of completing histology of the raft cultures, and will be sending samples for RNA analysis. HPV-18 DNA amplification, expression of E6 and E7 proteins, and other biomarkers will be determined by immunoblot analysis. As a part of this experiment, recovery and rebound of the HPV DNA replication after removal of treatment will be established.

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?

Q4-2022 to Q1 2023: Complete Aims 2.3, Deliverable 4 and Aim 2.3, Deliverables 1-3

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:

In July 2022, Novan met with SO/GOR Emilee Senkevitch and received permission to stop any further work on Specific Aim 1 (as indicated in Accomplishments section table above). Based on the data from the prototype formulations and the funds remaining, we determined that we were unlikely to meet the Go/No-go goal of 12 weeks of room temperature stability for the prototype formulation. We were allowed to rebudget remaining funds to Aim 2, which is still in progress. Additionally, we requested and were granted a 1 year no-cost extension.

Actual or anticipated problems or delays and actions or plans to resolve them

Although Aim 2 has been significantly delayed by lab moves, raft culture contamination, and material availability delays, the remaining experiments are now underway.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

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.

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?

- From project initiation through the date of this report, none of our personnel have worked more than one-person month on the project. These lower personnel hours were expected during this stage of the project because our personnel have been operating in supporting and oversight roles while our collaborator, MedPharm, have been performing against the key deliverables under Aim 1.1 of the project.

While none of our personnel have worked more than one-person month to date, we deemed it appropriate to list those key personnel who we expect to work more than one-person month on the project during the total project duration.

Name	**Carri Geer, Ph.D.**
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	Year 1 – 1.8 calendar months, Year 2 – 0.9 calendar months, Year 3 – 0.1 calendar months; Year 4 – 0.1 calendar months
Contribution to Project:	Leading the work performed by Novan personnel, consultants and subcontractors and working closely with Novan's Pharmaceutical Development team and MedPharm to formulate a nitric oxide-releasing vaginal suppository and with collaborators at the University of Alabama – Birmingham.
Name	**Hussaini Qhattal, Ph.D.**
Project Role:	(former) Associate Director of Product Development
Researcher Identifier (e.g. ORCID ID):	N/A

Nearest person month worked:	Year 1 – 2.8 calendar months, Year 2 – 1.8 calendar months, Year 3 – 0.1 calendar months
Contribution to Project:	Leading the formulation development with MedPharm

Name	**Shashank Jain, Ph.D. (Replaced Hussaini Qhattal in Q2 2021)**
Project Role:	Director of Product Development
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	Year 3 – 0.0 calendar months
Contribution to Project:	Leading the formulation development with MedPharm
Nearest person month worked:	Year 4 – 0.1 calendar months
Contribution to Project:	Leading the analytical development with MedPharm.

Name	**Benjamin "BJ" Privett, Ph.D.**
Project Role:	Principal Scientist
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	Year 1 – 4.5 calendar months, Year 2 – 1.8 calendar months, Year 3 – 0.1 calendar months, Year 4 – 0.1 calendar months
Contribution to Project:	Will be characterizing the nitric oxide release profile of the prototype when in contact with SVF. Working closely with MedPharm to guide experiments, analyze data, and summarize major conclusions. Serves as the grant manager and assisting in vendor oversight.

Name	**Shaylyn Walter**
Project Role:	Scientist
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	Year 1 – 2.5 calendar months, Year 2 – 0.6 calendar months
Contribution to Project:	Will be providing primary support during conduct of the in vitro dissolution testing of the vaginal suppository formulation, including support during performance of other activities.

Name	**Dan Riccio**
Project Role:	Senior Director, Drug Substance Development
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	Not in original personnel list, will replace some hours planned for Carri Geer and Tammy Payne.
Contribution to Project:	Supporting and offering guidance for formulation and analytical development activities and vendor oversight.

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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

QUAD CHARTS:

CA180741: Topical Nitric Oxide Therapy to Treat Cervical Neoplasias and Prevent HPV-Associated Cancers

PI: Carri Geer, Ph.D., Novan, Inc., NC **Budget:** $1,112,679.00

Topic Area: CDMRP **Mechanism:** W81XWH-18-PRCRP-IPA

Research Area(s): 0104, 0800, 0801, 0803 **Award Status:** 30-SEP-2019 TO 29-SEP2023

Study Goals:

The goal of this study is to develop a nitric oxide-releasing vaginal suppository that can be self-administered by female patients as a treatment for cervical neoplasias to eradicate latent HPV infection and inhibit disease progression to cancer.

Specific Aims:

The aims are to 1) formulate a stable vaginal suppository with well-characterized physical chemical properties suitable for intravaginal administration and 2) evaluate the effect of varying concentrations and treatment durations of NVN1000 against HPV-18 in human raft cell culture in vitro studies.

Key Accomplishments and Outcomes:

1. Established a range of potential suppository excipients that are compatible with NVN1000
2. Developed prototype formulations and methods of manufacture that result in initial NVN1000 stability
3. Evaluated stability of lead prototypes over 8 weeks and established that refrigeration may be required or additional development may be needed to achieve room temperature stability
4. Identified lead prototype with sufficient compatibility with NVN1000
5. Confirmed the antiviral efficacy of NVN1000 against HPV-18 in a human raft cell culture model.
6. Established a minimally effective dose of 4 mg/mL NVN1000 every 2nd day.

Publications: none to date

Patents: none to date

Funding Obtained: none to date

9. APPENDICES: *No appendices.*