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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 have selected a lead prototype candidate, although it appears refrigeration will be required to maintain stability. 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.

15. SUBJECT TERMS
None listed.

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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	95% (method development in progress)
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% (waiting on completion of 1.1, Deliverable 2)
2.1 In vitro dissolution testing of vaginal suppository.	5.0-6.0	0% (waiting on selection of prototype
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.		formulations in 1.1)
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% (waiting on selection of prototype formulations in 1.1)
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	50%
Deliverable 4: Establish recovery and rebound of HPV DNA replication once NVN1000 challenge is	2.0-3.0	0%

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	0% (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?

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.

Specific Aim 1.1, Deliverable 2:

1. NVN1000 Content and Purity

Refrigerated (2-8 °C) and room temperature (25 °C) stability at 4, 8, and 12 weeks has been completed for the previously selected 5 formulation prototypes. Although week 0-8 timepoints were reported in previous progress reports, the week 12 timepoint was completed during this report period and is highlighted below in blue. The percentage recovery (%) and percentage peak purity (% area) of NVN1000 in formulations was assessed at t=0 and following t=4, 8 and 12 weeks at 2-8 and 25 °C, and the results have been presented in Table 1 and Table 2, respectively.

The mean recoveries for all formulations at t=0 was within 90 – 110% NVN1000, indicating all were successfully extracted, and that no substantial drop in the content of NVN1000 in the formulations was observed. The results for the percentage peak purity of NVN1000 in the formulations was in alignment with this, where no notable drop in NVN1000 purity was observed compared to a QC2 standard of ca. 400 µg/mL NVN1000. At this stage of the project MedPharm included the relative response factors (RRFs) provided by the Sponsor for the known impurities of NVN1000 (N-nitroso-MAP3, N-nitro-MAP3, nitrite and nitrate) in the calculations for NVN1000 percentage peak purity. This resulted in higher purities (ca. 95% area compared to ca. 92% area), although they trended consistently across formulations.

Following t=4 weeks of storage at 2-8 °C, minimal NVN1000 degradation occurred in S06, with ca. 5% area drop, and then little change was observed at the subsequent timepoints (ca. 91% area). Additionally, only a slight decrease in the percentage peak purity of NVN1000 was noted in S17 (95.97% area at t=0, compared to 93.59% area following t=4 weeks at 2-8 °C). Following t=8 weeks of storage, a substantial drop (ca. 25% area) in the percentage peak purity of NVN1000 in S17 was observed, with this continuing at the t=12 week timepoint (40% area) and the variability between replicates also increased.

For the remaining formulations, degradation of >30% area was observed following t=12 weeks at 2-8 °C, and at 25 °C degradation of >75% area was noted in all formulations following t=12 weeks. It should be noted that there was a high level of variability between replicates for the percentage peak purity and the percentage recovery of NVN1000. Due to the consistency of the data at t=0, and during formulation development, it is hypothesized that the variability is not due to inefficient extractions. However, it is possible that the variability between replicates was a result of heterogeneously distributed NVN1000 within each sample due to the fact NVN1000 is present as a

suspension and the generic manufacturing method employed. This may have caused some samples to have a larger surface area of NVN1000 particulates in contact with the formulation vehicle, potentially causing an increased rate of degradation compared to others.
Currently, S06 is the most promising formulation when stored at 2-8 °C indicating that NVN1000 may be more stable in PEG 1000 than the hard-fat excipients. Additionally, it is likely that refrigeration of the final product will be required due to the sensitivity of NVN1000 to heat.

Table 1. Percentage recovery (%) of NVN1000 in the developed formulations at t=0 and following up to t=12 weeks at 2-8 and 25 °C.

Formulation	Percentage recovery (%) of NVN1000						
	T=0	T=4 weeks		T=8 weeks		T=12 weeks	
		2-8 °C	25 °C	2-8 °C	25 °C	2-8 °C	25 °C
S06	96.10 (91.19 - 99.29)	88.47 (79.76 - 94.92)	57.11 (53.17 - 60.07)	89.85 (83.59 - 98.35)	25.70 (13.79 - 34.35)	97.90 (91.81 - 101.78)	13.58 (0.00 - 21.92)
S07	100.08 (96.45 - 102.97)	75.35 (67.72 - 79.59)	11.99 (6.05 - 16.14)	55.72 (52.80 - 60.73)	10.95 (0.65 - 24.26)	33.94 (28.93 - 40.77)	0.44 (0.00 - 1.32)
S08	98.58 (96.77 - 99.71)	76.98 (74.66 - 80.44)	26.52 (9.40 - 37.69)	64.61 (39.26 - 96.02)	1.09 (0.49 - 1.59)	54.09 (36.82 - 70.85)	0.30 (0.00 - 0.90)
S12	93.27 (91.25 - 94.84)	78.77 (45.42 - 96.26)	59.12 (30.40 - 76.28)	41.90 (33.04 - 49.50)	35.77 (9.06 - 50.71)	70.34 (13.66 - 99.09)	14.43 (1.30 - 29.04)
S17	92.51 (86.69 - 97.32)	92.97 (90.12 - 94.74)	1.26 (0.68 - 1.72)	66.62 (30.47 - 94.37)	1.39 (0.04 - 3.60)	40.19 (19.89 - 64.09)	0.00 (0.00 - 0.00)

Table 2. Percentage peak purity (% area) of NVN1000 in the developed formulations at t=0 calculated with and without RRFs and following up to t=12 weeks at 2-8 and 25 °C calculated with the RRFs, n=3.

Formulation	Percentage peak purity (% area) of NVN1000							
	T=0		T=4 weeks		T=8 weeks		T=12 weeks	
	Without RRFs	With RRFs	2-8 °C	25 °C	2-8 °C	25 °C	2-8 °C	25 °C
QC2 (ca. 400 µg/mL NVN1000)	92.29*	96.00*	N/A					
S06	91.55 (91.13 - 91.92)	95.45 (95.18 - 95.67)	90.92 (87.97 – 93.24)	66.94 (63.33 - 69.43)	90.70 (87.38 - 93.77)	37.63 (26.25 - 47.28)	91.01 (90.56 - 91.28)	19.32 (0.00 - 30.67)
S07	92.08 (91.96 - 92.20)	95.74 (95.66 - 95.82)	83.24 (78.17 - 85.99)	23.78 (13.84 - 30.21)	71.32 (68.80 - 75.69)	16.23 (1.59 - 29.73)	46.61 (42.01 - 54.73)	0.95 (0.00 - 2.86)
S08	92.84 (92.84 - 92.85)	96.27 (96.27 - 96.28)	86.23 (83.35 - 91.79)	37.40 (21.35 - 47.20)	74.99 (59.39 - 91.19)	2.81 (1.26 - 4.02)	59.77 (51.54 - 67.49)	0.71 (0.00 - 2.12)
S12	91.83 (91.71 - 92.02)	95.58 (95.47 - 95.79)	83.47 (63.42 - 93.80)	69.00 (50.66 - 79.73)	59.08 (51.75 - 64.71)	51.32 (20.31 - 72.22)	68.39 (24.22 - 90.57)	22.93 (3.05 - 41.61)
S17	92.35 (92.31 - 92.40)	95.97 (95.90 - 96.03)	93.59 (92.38 - 94.20)	1.84 (1.04 - 2.52)	70.00 (37.61 - 94.14)	1.86 (0.12 - 4.67)	40.00 (22.42 - 63.34)	0.00 (0.00 - 0.00)

RRFs – Relative Response Factors
(*) – n=1

2. Melting Profile

The melting profile of the vehicle formulations at 37 °C was assessed at t=0 and following up to t=12 weeks at 2-8 and 25 °C. The time until full melt was observed and is detailed in Table 3. The 12 week timepoint was completed during this reporting period and is highlighted in blue below. Only vehicle formulations were assessed, as previous experiments showed that there was no material difference between the melt profile of active and vehicle formulations.
At t=0, the melt profile of the formulations was comparable to those determined during formulation development, with slight variations to be expected from a subjective observation. The exception to this was S07, where full melt was not observed following t=60 mins, whilst a melt time of 30 - 35 mins was observed during formulation

development. During assessment it was noted that the outer layer of the suppository did melt following t=30 mins, with no further change after this point. It is possible that this suppository was non-homogenous.
No material changes in the melting profile of any of the formulations was noted following t=12 weeks at 2-8 or 25 °C with the exception of S07. As S07 did not melt following t=60 mins at t=0, this formulation was further assessed for up to 2 hours at the subsequent timepoints. Following t=60 mins, the vials were periodically agitated to aid melting, and high levels of variability between timepoints and temperatures were observed. These results are not in alignment with formulation development, and it is unlikely to be due to heterogeneity due to the consistently long melt times for this formulation. Further investigations are required to inform a hypothesis, and these could comprise multiple set ups with some containing different batches of each excipient to determine if the variability in the manufacturing method has caused the change in melting profile. However, based on the NVN1000 content and purity data which shows S07 is unlikely to be the lead formulation due to substantial NVN1000 degradation no investigations are currently planned or recommended.

Table 3. Melting time of the developed vehicle formulations at t=0 and following up to t=12 weeks at 2-8 and 25 °C.

Formulation	Melting time (min)						
	T=0	T=4 weeks		T=8 weeks		T=12 weeks	
		2-8 °C	25 °C	2-8 °C	25 °C	2-8 °C	25 °C
S06	35	20	25	20	25	20	25
S07	>60	80*	110*	60	105*	50	70*
S08	20	20	25	20	25	20	20
S12	10	10	10	10	10	10	10
S17	20	25	20	25	25	20	25

(*) Following t=60 mins the vial was periodically agitated to aid melting.

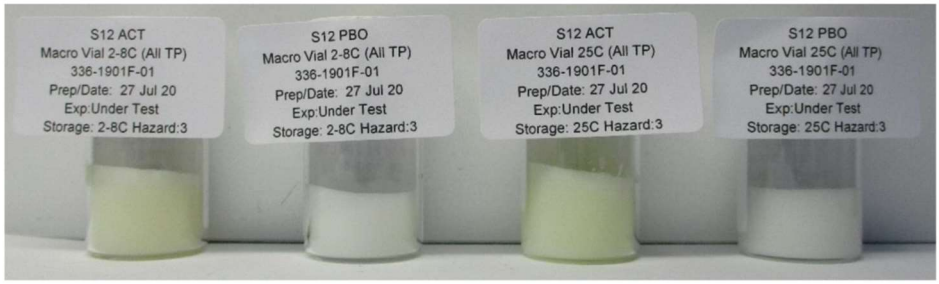
3. Macroscopic Appearance

The macroscopic appearance of the formulations was assessed at t=0 and following up to t=12 weeks at 2-8 and 25 °C, and the results have been presented in Table 4, with representative images for week 12 in Table 5. The 12 week timepoint was completed during this reporting period and is highlighted in blue below. All formulations were described as opaque solids, with all actives being described as faint yellow, except S06 and S08 ACT which were white. This faint yellow coloration for active formulations was also observed during formulation development. S06 was also the exception to the trend for the placebos, where all other placebos were described as white, whilst the placebo for S06 was faint grey. It should be noted, that whilst a color chart is used to improve the accuracy of color observations, these are still a subjective measure.
Following up to t=12 weeks of storage at 2-8 and 25 °C, there was no material change in the macroscopic appearance of the majority of the formulations. The exceptions to this were the active samples of S06 which had turned faint yellow following t=4 weeks at 25 °C, and at both temperatures following t=8 and 12 weeks. This color change also occurred in S08 following t=4 and 8 weeks at 25 °C, and at both temperatures following t=12 weeks. S06 placebo had also turned white at 2-8 and 25 °C. A yellow coloration can be indicative of API degradation, and therefore this will be monitored throughout stability. Additionally, two layers where observed in S06 active at every timepoint at 25 °C, where the top appeared more yellow than the bottom. It is possible that this is due to separation of the excipients during initial formulation processing, however further investigation would be required to confirm if this is the case, and alternative manufacturing methods may resolve this issue. Additionally, this more dominant yellow discoloration at the top of the sample indicates that it is possible NVN1000 was not homogenously distributed throughout the sample, and degradation of the API has caused the yellow discoloration.

Table 4. Macroscopic appearance of the developed formulations at t=0 and following up to t=12 weeks at 2-8 and 25 °C.

Formulation	Macroscopic appearance						
	T=0	T=4 weeks		T=8 weeks		T=12 weeks	
		2-8 °C	25 °C	2-8 °C	25 °C	2-8 °C	25 °C
S06 ACT	White, opaque, solid	No obvious change from t=0	Faint yellow, opaque, solid (two layers observed)	Faint yellow, opaque, solid	Faint yellow, opaque, solid (two layers observed)	Faint yellow, opaque, solid	Faint yellow, opaque, solid (two layers observed)
S06 PBO	Faint grey, opaque, solid	White, opaque, solid					
S07 ACT	Faint yellow, opaque, solid	No obvious change from t=0					
S07 PBO	White, opaque, solid						
S08 ACT	White, opaque, solid	No obvious change from t=0	Faint yellow, opaque, solid	No obvious change from t=0	Faint yellow, opaque, solid	Faint yellow, opaque, solid	
S08 PBO		No obvious change from t=0					
S12 ACT	Faint yellow, opaque, solid	No obvious change from t=0					
S12 PBO	White, opaque, solid						
S17 ACT	Faint yellow, opaque, solid	No obvious change from t=0					
S17 PBO	White, opaque, solid						

Table 5. Macroscopic images of the developed formulations following t=12 weeks.

System	Macroscopic images
S06	
S07	
S08	
S12	
S17	

4. Microscopic Appearance

The microscopic appearance of the formulations was assessed at t=0 and following t=12 weeks at 2-8 and 25 °C, and the results have been presented in Table 6 and Table 7. As with the observations during formulation development, it was challenging to identify drug particulates in the microscopy due to the large number of excipient particulates present in the formulations.

Table 6. Microscopic appearance of the developed formulations at t=0.

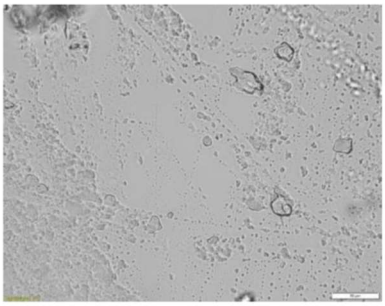
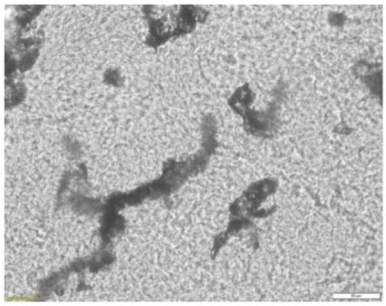
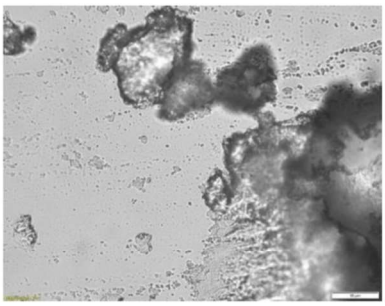
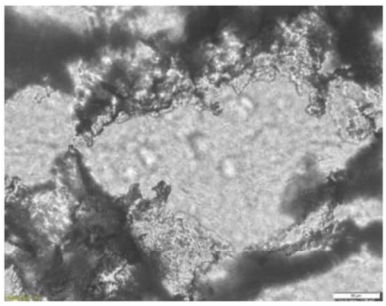
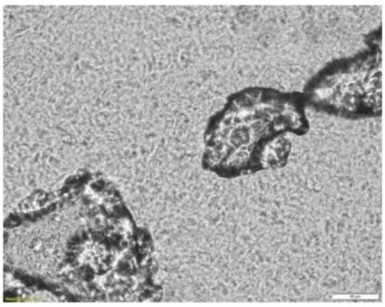
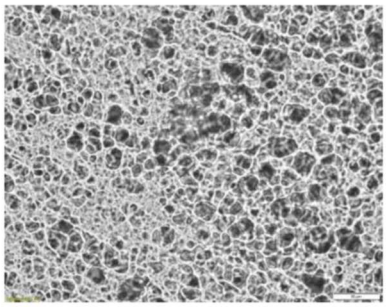
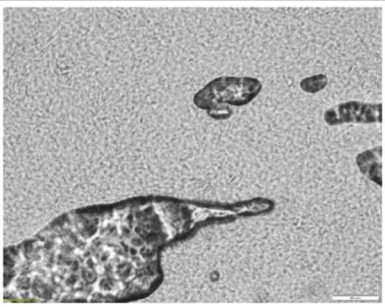
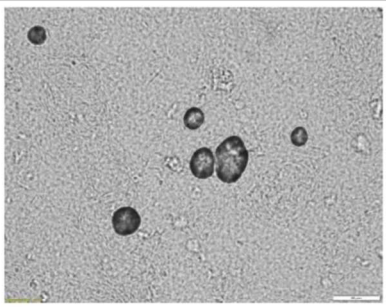
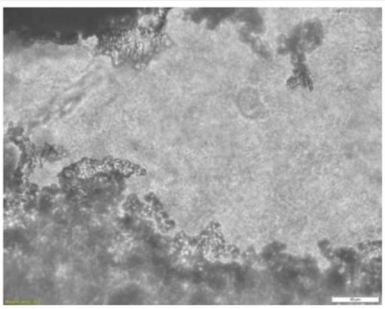
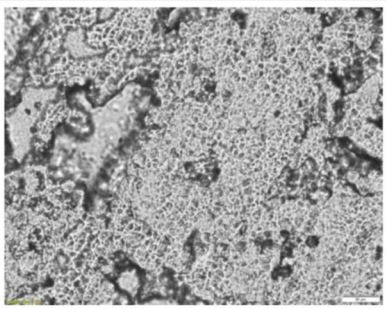
System	Microscopic appearance at t=0	
	Vehicle	Active
S06		
S07		
S08		
S12		
S17		

Table 7. Microscopic appearance of the developed formulations following t=12 weeks at 2-8 and 25 °C.

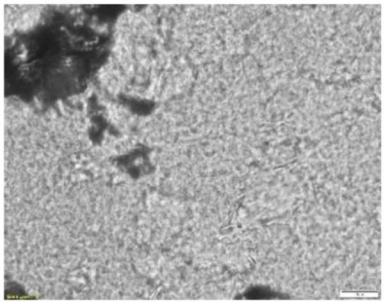
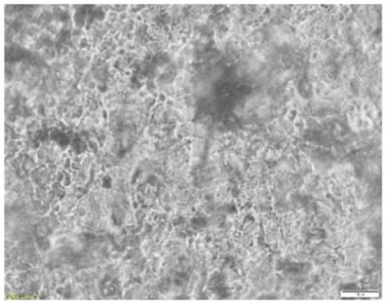
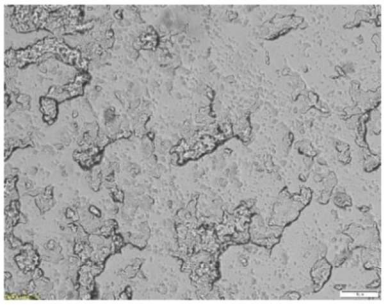
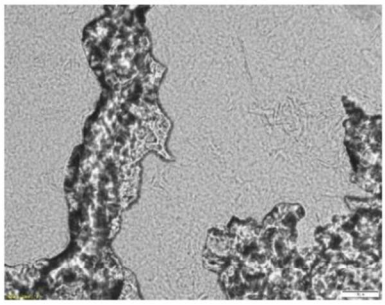
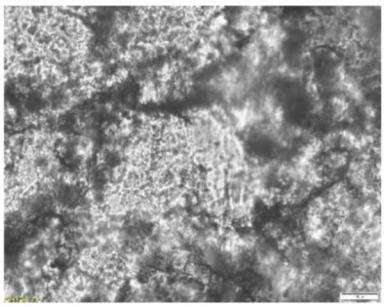
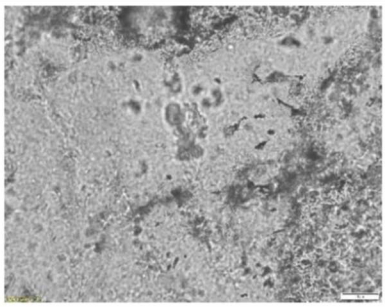
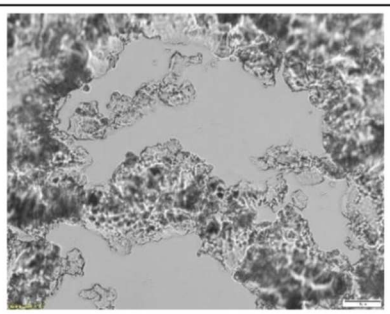
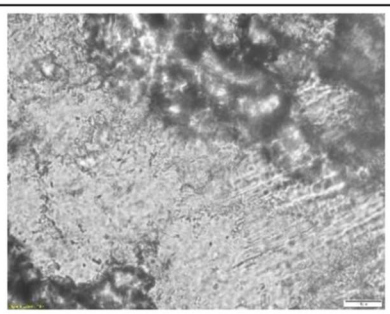
System		Microscopic appearance at t=12 weeks	
		2-8 °C	25 °C
S06	Active		
	Vehicle		
S07	Active		
	Vehicle		

Table 7 (continued). Microscopic appearance of the developed formulations following t=12 weeks at 2-8 and 25 °C.

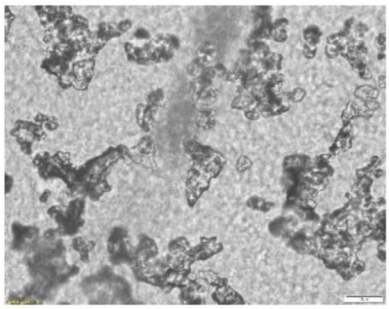
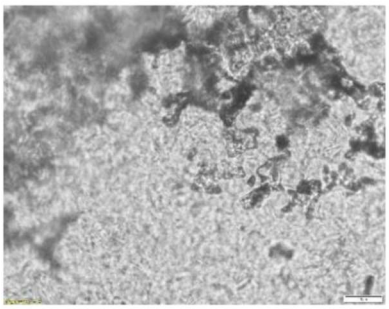
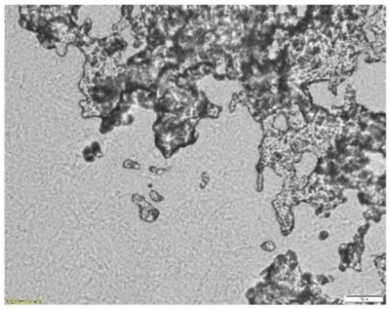
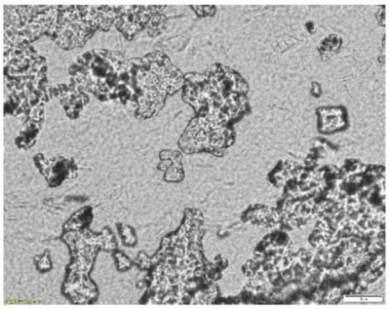
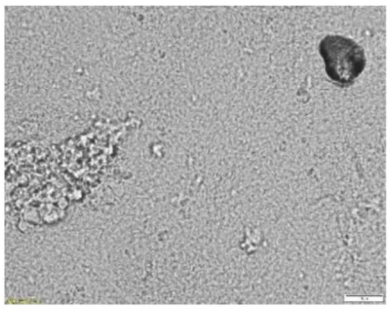
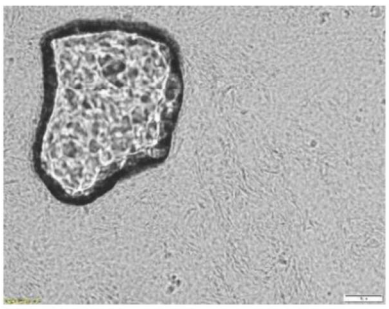
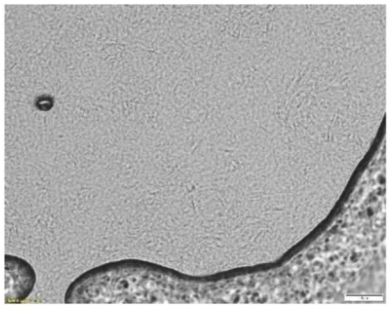
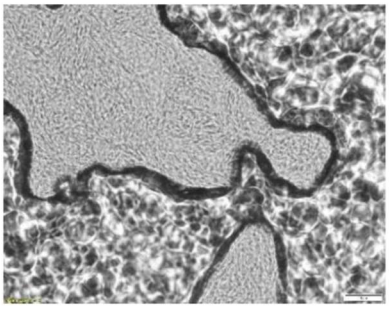
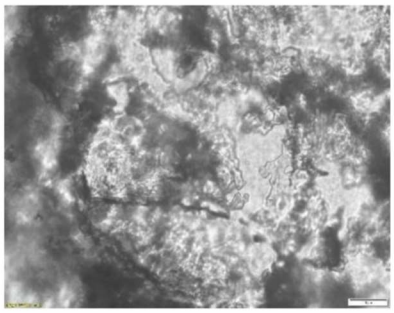
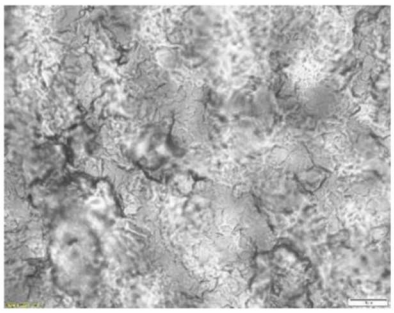
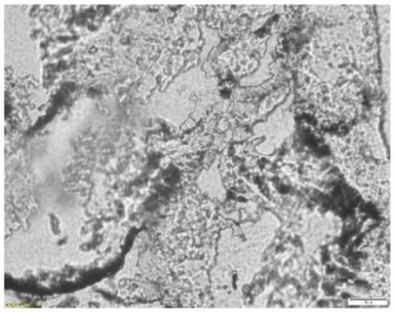
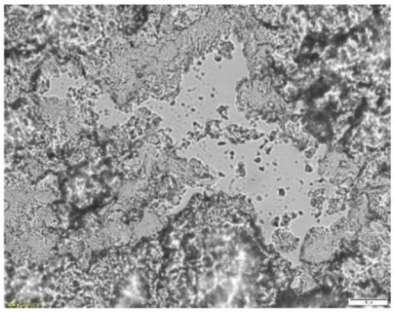
System		Microscopic appearance at t=12 weeks	
		2-8 °C	25 °C
S08	Active		
	Vehicle		
S12	Active		
	Vehicle		

Table 7 (continued). Microscopic appearance of the developed formulations following t=12 weeks at 2-8 and 25 °C.

System		Microscopic appearance at t=12 weeks	
		2-8 °C	25 °C
S17	Active		
	Vehicle		

5. Selection of Formulations for Manufacturing Assessment

Based on the results of the stability testing, a decision matrix has been prepared to aid the selection of formulation(s) for manufacturing assessment (Table 9). The parameters and criteria assessed are presented in Table 8.
MedPharm recommends the selection of S06 (coloured in green) due to its superior chemical stability at 2-8 °C.

Table 8. Parameters and criteria assessed in the decision matrix.

Parameter	Criteria	
Purity (% peak area) of NVN1000 following t=12 weeks at 2-8 °C	1	>90%
	2	80 – 90%
	3	<80%
Melting time following t=12 weeks at 2-8 °C	1	<20 mins
	2	20 – 30 mins
	3	>30 mins
Macroscopic appearance (actives)	1	No change from t=0
	2	Colour change from t=0
	3	Formulation separation observed at 25 °C

Table 9. Decision matrix to aid in the selection of formulations for the manufacturing assessment.

Parameter	S06	S07	S08	S12	S17
Purity (% peak area) of NVN1000 following t=12 weeks at 2-8 °C	1	3	3	3	3
Melting time following t=12 weeks at 2-8 °C	2	3	2	1	2
Macroscopic appearance (actives)	3	1	2	1	1
Recommended for progression	Y	N	N	N	N

Y: Yes; N: No

Following t=12 weeks, S06 appears to be the most promising formulation candidate based on NVN1000 purity at 2-8 °C, indicating that NVN1000 may be more stable in PEG 1000 than the hard-fat excipients. All formulations were unstable at 25 °C and as such it is likely that refrigeration of the final product will be required. No material difference in the melt profile of any formulation was observed up to t=12 weeks at 2-8 or 25 °C, except for S07, where highly variable melt times were observed. In terms of visual appearance, no changes from t=0 were observed, with the exception of the active samples for S06 and S08 which had become faint yellow at 25 °C (and t=8 weeks 2-8 °C for S06), with a stronger yellow discoloration in the top of the S06 samples potentially indicating separation of the excipients or heterogeneously distributed NVN1000. It may be possible to resolve these issues with S06 by improving the manufacturing process for the formulation.

Novan is currently working with MedPharm to develop a manufacturing procedure for S06 that improves the homogeneity and stability of the formulation. This work will include the establishment of a suitable manufacturing method at the ~150g scale to enable further DOE assessment using stir temperature and stir time on stability and homogeneity. Some additional work will be performed on optimizing the extraction method specifically for prototype S06. **Due to the additional work needed to formulate a stable and homogenous vaginal suppository prototype, sufficient funding may not be available to complete condom compatibility and mucoadhesion experiments.**

Starting in 1Q2021 Novan moved out of its previous facility and began the process of renovating a new facility. Although Novan's new office space opened in 2Q2021, our analytical lab is under construction and due to Covid-related construction and supply-chain delays, will not be ready for occupancy until Q42021. While MedPharm could have continued formulation work during Q1-Q3 2021, Aim 1.2 necessitates coordination with Novan's analytical lab for timely in vitro dissolution testing of formulations by Novan. Accordingly, this work was intentionally delayed and may not restart until Q42021. We don't expect any additional data to be generated towards this aim until at least Q4 2021 or early 2022.

Specific Aim 2.2, Deliverables 1 and 2:
Based on the data generated in Q3 2020 and reported in the last Quarterly Progress Report, the following frequency and concentration combinations were found to be suitable to prevent viral DNA replication, abrogation of HPV oncogene activity with least cytotoxic effects: daily or every 2nd day of 4 mg/ml, every 2nd day of 5 mg/ml or every 3rd day of 6 mg/ml NVN1000. UAB is in the process of examining which of these is least toxic on uninfected PHK raft cultures in parallel experiments, while exerting long lasting therapeutic effects against HPV-18 RCs. Starting in Q4 2020, these experiments went on hold due to a lab move and laboratory renovations at UAB. Construction and continued Covid-related delays have prevented work on this aim from progressing. We now expect that experiments towards this aim will resume sometime in 4Q 2021.

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?

Q4-2021:

- Examine cytoxicity of optimized NVN1000 dosing regimes to uninfected raft cultures
- Evaluate durability of antiviral efficacy using drug-free chase experiments.

Q4-2021 or Q1-2022:

- Commence a manufacturing assessment, DOE, and stability studies aimed at scaling up prototype S06, improving stability, and improving NVN1000 homogeneity.
- Initiate development and performance of in vitro dissolution testing, NO release kinetics testing, and pH performance testing for vaginal suppository candidates.

4. **IMPACT:**

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

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

As noted above, work on Specific Aim 1 is delayed until late Q4 or early 2022 due to a planned shutdown and move of Novan's analytical lab. Some lab renovation-related delays to Specific Aim 2 experiments at UAB were also noted above.
Due to the extra work and expense required for formulating a stable and homogenous prototype formulation, we anticipate that sufficient funding may not be available for executing condom compatibility and mucoadhesion testing of the final formulation.

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

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 1 – 0.6 calendar months, Year 2 – 0.3 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
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.

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 30-SEP2022

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 and established a dosing protocol that minimizes cytotoxicity

Publications: none to date
Patents: none to date
Funding Obtained: none to date

9. APPENDICES: *No appendices.*