CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

217424Orig1s000

CLINICAL MICROBIOLOGY/VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS, CDER/OND/OAP
CLINICAL VIROLOGY CONSULT REVIEW
NDA 217424 (eCTD 0001) DATE REVIEWED: 04/24/2023

CDER/OND/OAP/DAV Reviewer: Michael Thomson, Ph.D.
Date of Consult Request: 03/01/2023

FDA Center requesting consult: Center for Drug Evaluation and Research (CDER)
Office of Immunology and Inflammation (OII)
Division of Dermatology and Dentistry (DDD)
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Initial Submission Dates:
Correspondence Date: 01/05/2023
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Review Complete Date: 04/24/2023
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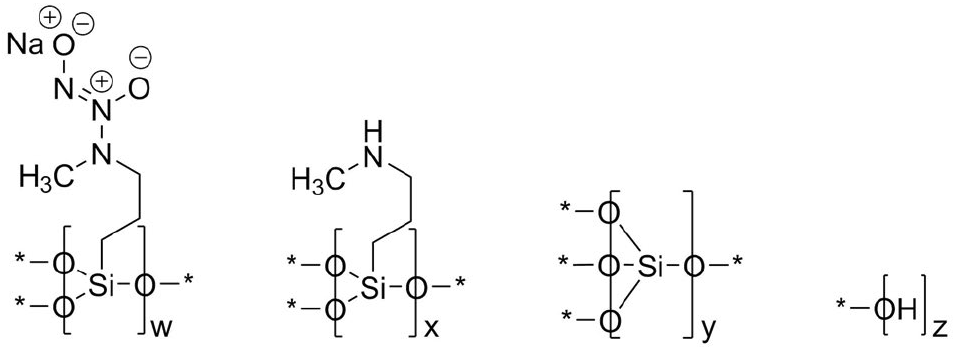
Regulatory History (Virology-Related Submissions Reviewed):

Submission	Received	Assigned	Content
SDN 001 (eCTD 0001)	01/05/2023	03/02/2023	Original NDA submission
SDN 008 (eCTD 0008)	03/20/2023	07/07/2023	Draft labeling

Product Names: SB206 Gel, (b) (4) Zelsuvmi™, NVN1000 Gel, berdazimer sodium
Structure:



* Denotes shared oxygen atom between bonded constituents; resulting bonds are Si-O-Si or Si-OH

SB206

Empirical Formula: $[(C_4H_9N_3NaO_{3.5}Si)_3(C_4H_{10}NO_{1.5}Si)_1(SiO_2)_6(HO_{0.5})_5]_{0.1n}$
Molecular weight: Due to the insoluble nature of berdazimer sodium the molecular formula, molecular mass and average molecular weight range cannot be determined
Drug category: Cytotoxic agent
Indication: Topical treatment of molluscum contagiosum virus infection in adult and pediatric patients (b) (4) (b) (4) of age and older
Dosage Form/Route of administration: 10.3% (103 mg berdazimer per gram) / topical

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Abbreviations: CC_{50}, 50% cytotoxic concentration; CRPV, cottontail rabbit papillomavirus; EC_{50}, 50% effective concentration; HPV, human papillomavirus; iNOS, inducible nitric oxide synthase; MCV, molluscum contagiosum virus; NO, nitric oxide; PFU, plaque-forming units; PHK, primary human keratinocytes; PV, papillomavirus; PCR, polymerase chain reaction; TLR, toll-like receptor

Purpose of Consult

This review is to address a consult request from CDER/OII/DDD to perform a virology review of NDA 217424, berdazimer sodium, for the topical treatment of molluscum contagiosum (MC) infection. The consult is to evaluate (b) (4) non-clinical studies of berdazimer sodium; no virological assessments were conducted in the pivotal clinical trials.

BACKGROUND

SB206 gel combines berdazimer sodium, a nitric oxide (NO) releasing macromolecule, with a buffered hydrogel for topical administration. Different formulations of berdazimer sodium have been assessed in prior pre-IND and IND submissions for a variety of indications. SB206 gel for the treatment of MC was originally assessed under IND 137015, which was not reviewed by the Division of AntiVirals. (b) (4)

According to the Applicant, SB206 gel releases NO, which has bactericidal and virucidal activity, presumably through direct damage to various biomolecules by oxidation and nitration reactions (Wink et al., 1991; Nguyen et al., 1992; Fang, 1997). NO is a short-lived, lipophilic gas, that is endogenously produced and important for defending against infection (reviewed in Schairer et al., 2012). Inducible nitric oxide synthase (iNOS) is the main enzyme for the synthesis of NO. In general, endogenous NO is considered protective against viruses, being produced by immune effector cells and critical to the innate immune response (Wink et al., 2011; Uehara et al., 2015; Akaike and Maeda, 2000). NO may have direct antiviral effects, including inhibition of viral enzymes through nitrosylation, damage to viral DNA through oxidative and nitrosative stress, and suppression of viral transcription factors (Garren et al., 2021). NO is also important to the modulation of vascular physiology, including vasodilation, leading to improved oxygenation. However, NO can have different physiological effects depending on the tissue and concentration, with high concentrations leading to apoptosis and cell cycle arrest (Garren et al., 2021). NO is also one of the key components in the formation of the tumor microenvironment caused by chronic infection, and plays a role in cancer initiation and progression (Vahora et al., 2016).

The cytotoxic effect of exogenously administered NO has been investigated in a number of published studies, using a variety of delivery systems. In a study of gaseous NO to manage wound infection, no cytotoxic effects were observed at levels of up to 200 ppm (6.7 mM, potentially much lower than the concentration of NO released from berdazimer sodium in the clinic) to human dermal fibroblasts, keratinocytes and endothelial cells (Ghaffari et al., 2006; Ghaffari et al., 2007). In a study using NO-releasing nanoparticles, human lung fibroblasts were treated for 24 hours and only minimal cytotoxicity was observed (Friedman et al., 2008). NO releasing patches have been tested for wound healing, and were shown to safely deliver up to 400 ppm (13 mM) NO (Jones et al., 2012). Earlier studies tested NO-releasing cream containing nitrate and ascorbic acid on the skin of volunteers, and showed that the NO was pro-inflammatory and toxic to DNA, leading to the accumulation of p53 and subsequent apoptosis (Ormerod et al., 1999). A study using NO-loaded zeolite ointment showed impairment of 3T3 fibroblast viability in a 24 hour cell culture assay, and that the cytotoxicity

resulted from the NO release rather than the ointment itself (Neidrauer et al., 2014). However, this cytotoxic effect was not recapitulated in the context of wound healing in obese rats.

The Applicant stated that based on pharmacology studies performed to date, the virucidal activity of SB206 gel derives from a direct chemical effect on viral proteins, and with respect to the proposed MC indication considers berdazimer sodium to be an inhibitor of the MC160 protein, derived from early gene expression. Currently there is no cell culture system or animal model available for propagating MCV. Study reports for inhibition of MCV particles, vaccinia virus replication, and HPV using raft cultures, were included as supportive evidence for antiviral activity. (b) (4) In general, based on the relative size and number of possible host targets with respect to any potential viral target, the Applicant's hypothesis of a direct antiviral mechanism appears unlikely. They conducted a cytotoxicity assessment only for the vaccinia virus model, and did not demonstrate a substantially different cytotoxicity concentration (CC_{50} value) from the antiviral activity (EC_{50} value) claimed for the MCV cell culture model.

NONCLINICAL STUDIES

Activity of berdazimer sodium against vaccinia virus (study report phar-22-002)

The Applicant conducted an experiment to evaluate the antiviral activity of berdazimer sodium against vaccinia virus (Western Reserve [WR] strain), an orthopoxvirus which is distantly related to MCV, a molluscipoxvirus. Hence, the relevance of these studies is not clear, although MCV is predicted to encode several vaccinia virus homologs of extracellular virion-specific proteins (Senkevich et al., 1997; Monticelli et al., 2020). In addition, whether vaccinia virus could become resistant to berdazimer sodium was evaluated in a second experiment.

Methods

A recombinant vaccinia virus (VVEGIR) was used for these experiments, which expresses green fluorescent protein (mNeonGreen) under an early promoter, and a red fluorescent protein (mKate2) under an intermediate promoter. The cell line BSC40 (African green monkey kidney) was used for infection studies. To prepare berdazimer sodium, it was first resuspended with DMSO, then diluted in cell culture medium (DMEM) at pH 4.0, followed by a 1:1 dilution with DMEM pH 6.5.

To evaluate toxicity, 1 x 10^4 cells/well BSC40 were seeded into a 96-well plate and incubated overnight. Berdazimer sodium in cell culture medium was serially diluted from 1,400 µg/mL (highest starting concentration in the wells; equates to approximately 1.2 mM based on the empirical formula, although the Applicant notes that the molecular formula, mass and MWt are indeterminate because of the insoluble nature of berdazimer sodium) and added to the cells, with negative control cells with only DMSO, and positive toxicity cells with mitomycin C at 125 µg/mL. After overnight incubation at 37°C, toxicity was assessed using CellTiterGlo® to measure ATP levels, and read with a luminometer.

> **Note**: A concentration of 1,400 µg/mL is high for an antiviral drug; small molecules which progress to clinical development are typically in the nanomolar range (e.g., 10 nM or 5 ng/mL for a 500 MWt drug), which allows room for separation from cytotoxic effects.
>
> **Note**: Cytotoxicity may not be apparent after an overnight incubation, and should be assessed for longer, and for the same period of time as the antiviral activity (3 days; see below). The Applicant should also have assessed in both stationary and proliferating cells over multiple cell divisions.

To determine the antiviral activity of berdazimer sodium, $5x10^5$ cells/well BSC40 cells were seeded into a 12-well plate and incubated overnight. Berdazimer sodium was serially diluted in cell culture medium and added to the cells the following day, with DMSO as a negative control. After 30 minutes at 37°C, 1 x 10^3 PFU/well VVEGIR was added, and plates incubated overnight at 37°C. Wells were dosed again with the same amounts of berdazimer sodium. At 3 days post-infection, cells were harvested, virus released with 3 freeze-thaw cycles, and plaque assay conducted to determine infectivity.

For direct virus particle treatment, 5 x 10^4 plaque-forming units (PFU) of VVEGIR were mixed with different dilutions of berdazimer sodium in DMEM (pH 6.5). The mixture was incubated overnight at room temperature, then treated virions serially 10-fold diluted in DMEM (pH 7.4) and the number of infectious particles in each dilution determined using plaque assay with standard methodology.

For resistance assessment, 1 x 10^6 per well BSC40 cells were seeded into a 6 well plate and incubated overnight. Berdazimer sodium was diluted to 50 µg/mL (equates to 44 µM based on empirical formula) in DMEM (pH 6.5) and added to the cells for 30 minutes at 37°C. Vaccinia virus (VVEGIR) was added at 1 x 10^3 PFU/well and cells incubated overnight at 37°C. The next day, cells were treated again with the same concentration of berdazimer sodium. Three days post-infection, cells were harvested, and virus released from cells using 3 freeze-thaw cycles. Cell lysate was then inoculated onto fresh BSC40 cells which had been treated with 100 µg/mL (equates to 87 µM based on empirical formula) berdazimer sodium and the incubation/harvesting procedures repeated as above. The cell culture passage was repeated two more times, and after the final treatment, virus was plaque purified using BSC40 cells in the presence of berdazimer sodium at 80 µg/mL. For comparison, VVEGIR which had not been passaged was assessed on a separate plate in parallel.

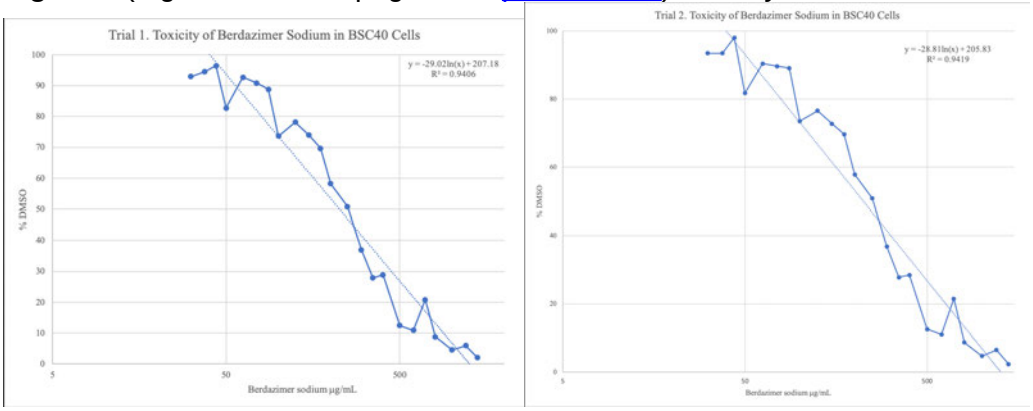
Results

Cytotoxicity

Dose-response curves for berdazimer sodium diluted onto BSC40 cells are shown in **Figure 1**. The CC_{50} values for the two experiments were 225.1 µg/mL and 216.2 µg/mL, respectively. Mitomycin C, used as a positive control for toxicity, showed >65% toxicity in both experiments.

> **Virology Note**: The close similarity of the two dose-response curves is unusual for a cell-based assay, and may indicate that two reads from the same plate were taken rather than two independent experiments being conducted.

Figure 1 (Figures 1 and 2, pages 8-9, phar-22-002) Toxicity of berdazimer sodium in BSC40 cells*



* Y-axes show the viability of BSC40 cells as a percentage of the DMSO controls, using CellTiterGlo® as a readout

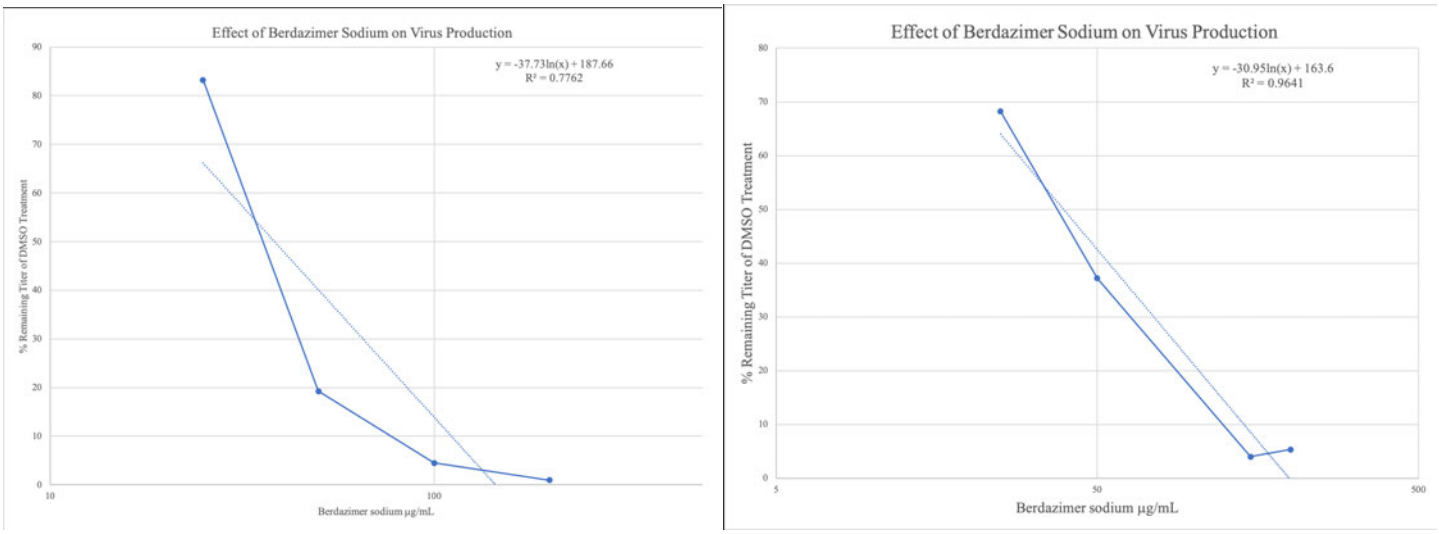
Virology Note: The proposed clinical product contains 10.3% berdazimer sodium, or 103 mg of drug per gram of gel. The highest concentration of berdazimer sodium used in the cytotoxicity studies was 1.4 mg/mL, so it would appear that at the concentration to be used in the clinic is significantly higher than tested in these experiments. However, whether berdazimer sodium would have a similar CC_{50} value in epidermal cells, or epidermal cells presumably weakened by infection with MCV, was not determined, so the relevance of this experiment to the clinical application is not clear.

Antiviral activity

The activity of berdazimer sodium against vaccinia virus (VVEGIR) was assessed in BSC40 cells. **Figure 2** shows the dose-response curves for two experiments. The EC_{50} values for berdazimer sodium were determined as 41.6 µg/mL and 39.2 µg/mL for the two experiments, respectively.

Virology Note: While it would have been preferable to conduct a parallel toxicity assessment, it is likely that the vaccinia virus itself would cause cytotoxicity in the time frame assessed, confounding the results. Based on the CC_{50} results shown above of approximately 200 µg/mL (measured after overnight incubation) and the EC_{50} values of approximately 40 µg/mL (measured after 3 days of exposure), it appears there is only a 5-fold therapeutic index. Given such a small difference, and the different exposure times for cytotoxicity and antiviral activity assessments, it is questionable whether berdazimer has any direct antiviral activity in this setting, and seems likely that any effects were from cytotoxicity (Sidwell and Huffman, 1971). Also, as noted above, the concentrations of drug used in these experiments appears to be significantly below the concentration to be used in the clinic, so unless the cytotoxicity is significantly different in epidermal cells, it seems likely that any effect clinically would not be separable from cytotoxicity.

Figure 2 (Figures 3 and 4, pages 11-12, phar-22-002) Inhibition of vaccinia virus production after exposure to berdazimer sodium*



* Y-axes show the PFU of virus as a percentage of the infectivity remaining in the DMSO controls

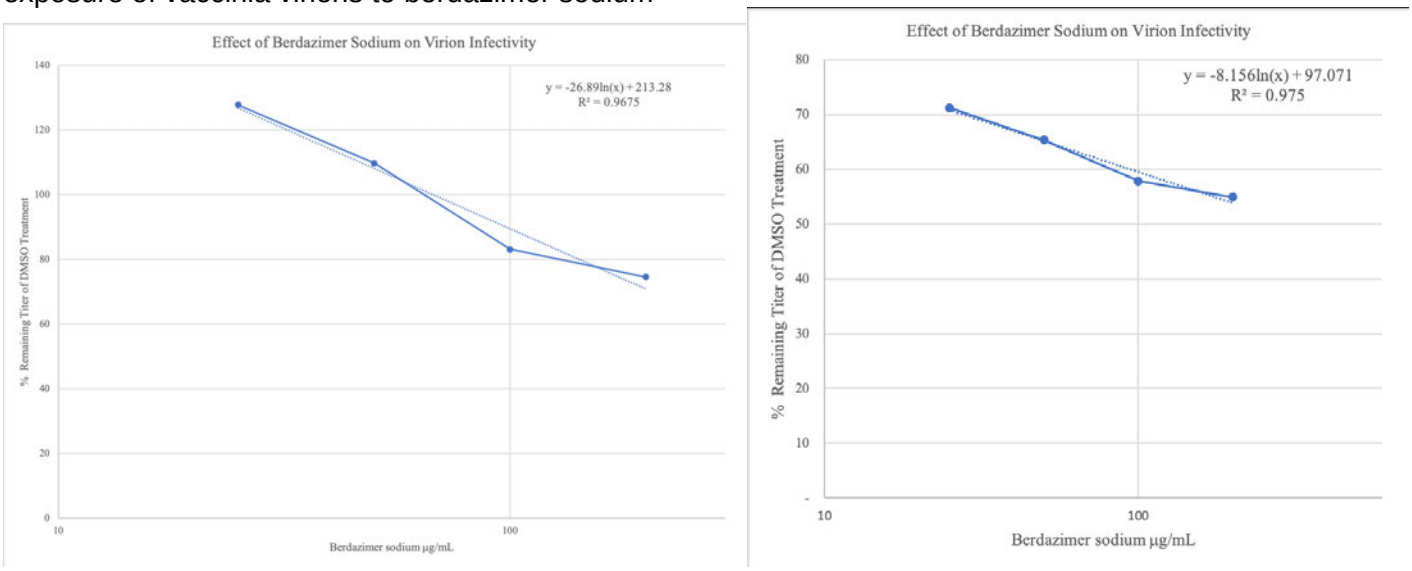
Direct vaccinia virus particle treatment

Vaccinia virus particles were treated directly with different concentrations of berdazimer sodium, added to BSC40 cells, and after 3 days infectivity from cell lysates assessed by plaque assay (**Figure 3**). For the two

experiments conducted, the EC_{50} values were determined as 433.6 µg/mL and 321.0 µg/mL, respectively (equate to 378 µM and 280 µM, respectively, based on empirical formula).

> **Virology Note**: Given that in both experiments the infectivity of treated particles did not reach below 50% of those in the DMSO control, it is not possible to determine EC_{50} values. Even assuming the EC_{50} values to be accurate, they are approximately 10-fold higher than those determined for infectious cell culture, and above the CC_{50} value. The highest concentration tested, 200 µg/mL, is approximately the CC_{50} value for berdazimer sodium in BSC40 cells, so the reduction in infectivity is probably a result of drug carry-over into the cell culture causing cytotoxicity.

Figure 3 (Figures 5 and 6, pages 14-15, phar-22-002) Inhibition of vaccinia virus particle infectivity after exposure of vaccinia virions to berdazimer sodium*



* Y-axes show the PFU of virus as a percentage of the infectivity remaining in the DMSO controls

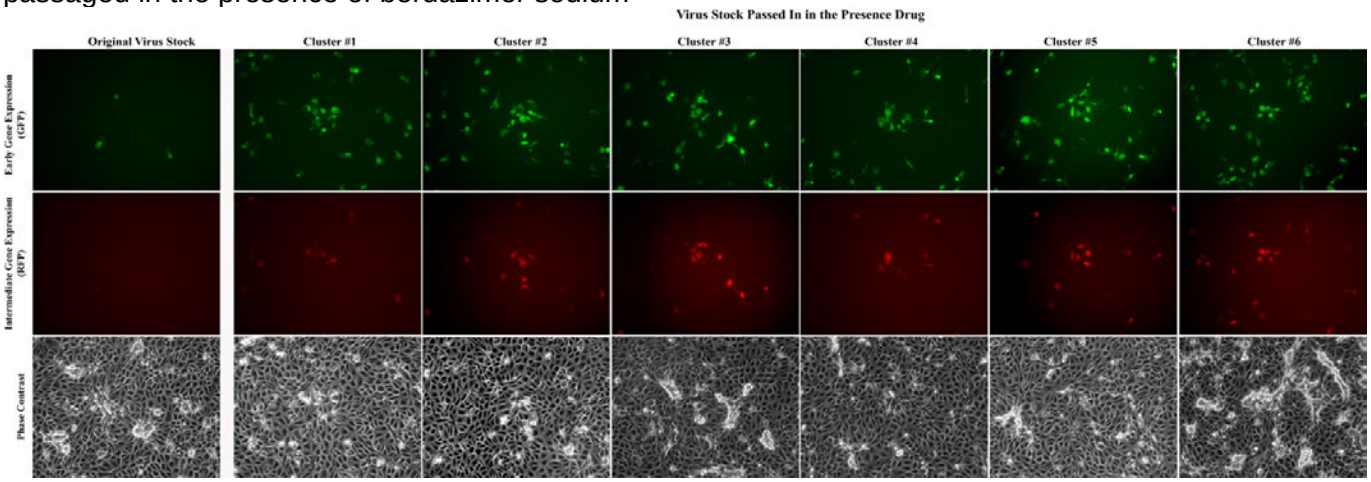
Resistance selection in cell culture

Recombinant vaccinia virus (VVEGIR) was serially passaged in BSC40 cells in the presence of berdazimer sodium (2 passages at 50 µg/mL, and 3 passages at 100 µg/mL) to select for resistant virus, using reporters in the virus expressed from early (mNeonGreen) or intermediate (mKate2) promoters to evaluate replication. Following passage, plaque-purified virus was added to cell monolayers and fluorescence recorded (**Figure 4**). According to the Applicant, cells fluorescing only green indicated that the replication did not go beyond early gene expression, whereas cells fluorescing both green and red results from both early and intermediate gene expression, and fluorescence in adjacent cells indicates cell to cell spread of virus. Because the post-passaged virus fluoresced both green and red and adjacent cells fluoresced in the presence of 80 µg/mL berdazimer sodium, as compared with the parental virus, the Applicant believes virus resistance had developed. No further characterization of the putative resistant virus was conducted.

> **Virology Note**: Berdazimer sodium was used below the reported CC_{50} value of approximately 200 µg/mL in BSC40 cells, but above the EC_{50} value of approximately 40 µg/mL. However, fluorescence was measured after one day of infection, whereas CC_{50} and EC_{50} values were determined over 3 days, so it is not clear that the concentration of berdazimer sodium used for the final evaluation was sufficient to cause either cytotoxicity or have antiviral activity in the time frame used.

Based on the images in **Figure 4**, there is low-level red fluorescence across all "clusters", which are presumably different plaque-purified viruses, and a near absence of green and red fluorescence from cells infected with parental virus. However, based on the methodology provided, the amount of virus was not standardized between parental and plaque picked viruses, so the difference could simply be the result of low infectivity of the parental virus compared with passaged virus.

Figure 4 (Figure 7, page 16, phar-22-002) Fluorescent evaluation of vaccinia virus VVEGIR* which had been passaged in the presence of berdazimer sodium



* Virus expresses green fluorescent protein (mNeonGreen) under an early promoter, and red fluorescent protein (mKate2) under an intermediate promoter.

Conclusions

Berdazimer sodium was evaluated for cytotoxicity and antiviral activity against recombinant vaccinia virus in BSC40 cells, and had a 5-fold therapeutic index based on CC_{50} and EC_{50} values of approximately 200 and 40 µg/mL, respectively. It is possible that the antiviral activity is simply a result of cytotoxicity/cytostasis given the small therapeutic index and the lack of direct inactivation for this oxidizing agent. Also, it is likely that the concentration of drug being used clinically is much higher, and it is not known whether BSC40 cells, an African green monkey kidney cell line, is a relevant model for epidermal cell infection. Hence, overall, no conclusions can be drawn with respect to antiviral activity of berdazimer sodium as tested in these experiments, or at the concentrations to be used clinically. The cell culture resistance passage experiments are also inconclusive because it is not clear that the Applicant used the same amount of input virus when comparing parental and passaged viruses, and the evaluation of phenotype was conducted after one day of infection rather than 3 days as for the antiviral activity studies.

Activity of berdazimer sodium against molluscum contagiosum virus (study report phar-22-003)

MCV does not replicate in cell culture, but can enter cells and express early genes. Berdazimer sodium was evaluated for its direct impact on MCV particles and subsequent expression of the early gene MC160, involved in immune response evasion (Nichols and Shisler, 2006).

Methods

MCV was extracted from dermal lesions and purified over a sucrose cushion. Virus was quantitated by PCR targeting the viral polymerase gene. Purified MCV was suspended in cell culture medium (DMEM, pH 6.5). Berdazimer sodium was mixed with DMSO then diluted in DMEM (pH 4.0). A 2-fold dilution series was made in DMEM pH 6.5 and added to MCV (1×10^5 particles in each dilution) to give final concentrations from 400

µg/mL to 50 µg/mL. The mixtures were incubated overnight at room temperature, then DMEM pH 7.4 added, diluting the mixture 5-fold (top concentration of berdazimer sodium of 80 µg/mL) and the treated virions inoculated onto BSC40 cells (1 x 10^5 cells per test). Cells were incubated overnight at 37°C, then harvested and lysed using radioimmunoprecipitation (RIPA) buffer. Lysed cells were clarified by centrifugation and analyzed by western blot using anti-MC160 polyclonal antisera followed by horseradish peroxidase (HRP) conjugated donkey anti-rabbit antibody and mouse anti-actin followed by Alexa-647 conjugated donkey anti mouse antibody. HRP and fluorescence signals were detected using a Kodak Image Station 40000MM Pro (Carestream Health Inc.).

Results

Western blotting showed that expression of MC160 was decreased in cell lysates inoculated with MCV treated with berdazimer sodium (**Figure 5**). The response appeared to increase with dose, based on the signal intensity normalized to actin, and an EC_{50} value of 192.9 µg/mL was determined from the dose-response curve (**Figure 6**).

Figure 5 (Figure 1, page 5, phar-22-003) Western Blot of MC160 gene expression after exposure of molluscum contagiosum virions to berdazimer sodium

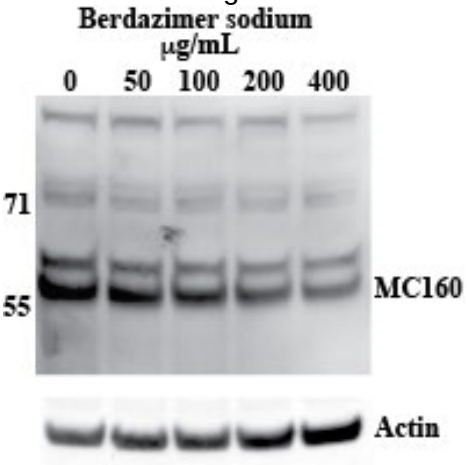
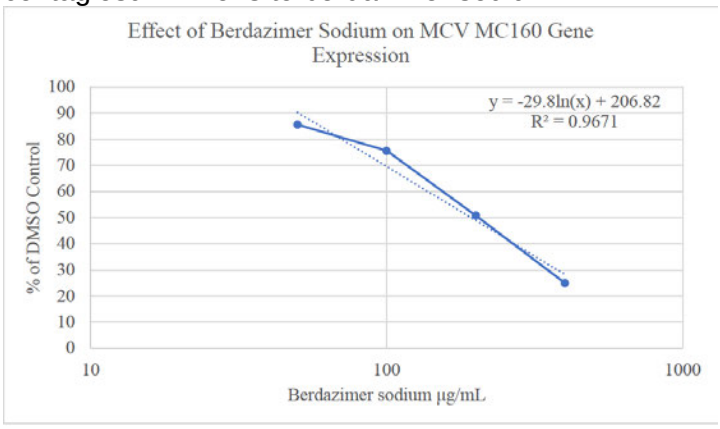


Figure 6 (Figure 2, page 6, phar-22-003) Inhibition of MC160 gene expression after exposure of molluscum contagiosum virions to berdazimer sodium



Virology Note: The Applicant stated that the diluted concentration of berdazimer sodium during incubation with BSC40 cells was not higher than 80 µg/mL, and therefore not caused by cytotoxicity.

However, the CC_{50} value determined for BSC40 cells was approximately 200 µg, the same concentration needed to reduce the infectivity of MCV by 50%, so for all practical purposes it is not possible to separate activity from toxicity. In any case, an EC_{50} value of approximately 40 µg/mL (the concentration of berdazimer sodium used on cells at the EC_{50} value after diluting 1/5), would only give a therapeutic index of 5, which is indicative of cytotoxic effects. Typically, a 10-fold window would be needed to rule out a cytotoxic mechanism (Sidwell and Huffman, 1971).

Conclusions

The Applicant conducted an experiment to assess the effect of berdazimer sodium on MCV particles before infecting cells, given that the virus does not replicate in cell culture. They determined an EC_{50} value of approximately 200 µg/mL, the same as the CC_{50} value in BSC40 cells, which indicates that to impact virus infectivity/replication a cytotoxic concentration would have to be used. Clinically, it is likely that the concentration of berdazimer sodium applied to skin is significantly higher, and therefore likely cytotoxic.

Activity of berdazimer sodium against human papilloma virus (study phar-15-006, which includes a publication by Banerjee et al., 2019, and no separate study report)

The Applicant used a human epithelium raft culture model of HPV infection to demonstrate virucidal activity. The model recapitulates HPV-16 or -18 infected squamous epithelium in culture to study the virus replication cycle (Chow, 2015).

Methods

Primary human keratinocytes (PHK) recovered from neonatal circumcisions are seeded on dermal equivalent consisting of collagen and fibroblasts. The assembly is then raised onto a stainless-steel grid (raft) and allowed to grow at the air-media interface for 10 days or longer during which the cells stratify and differentiate into a squamous epithelium. Utilizing Cre-LoxP recombination, extrachromosomal HPV-18 genomic plasmid is generated in PHKs transfected with a recombinant parental plasmid containing the neomycin resistance marker gene. After a 2-3 day G418 selection, resistant PHKs are used within a week of transfection to develop raft cultures over a period of up to 3 weeks.

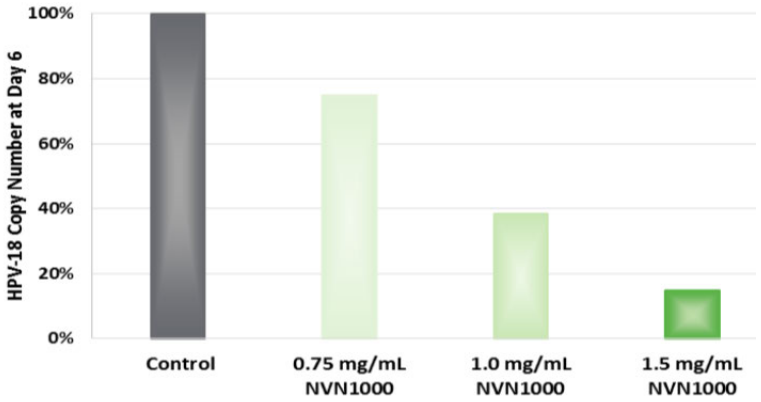
To model topical application, vehicle or berdazimer sodium (0.75, 1.0, or 1.5 mg/mL) was added on top of the growing epithelium daily from Day 6. After 1 hr exposure, the compound was removed by gentle aspiration to maintain the air-liquid interface. Raft cultures were harvested on Day 13, 14 or 16, and 12 hours before each harvest one culture of each treatment set exposed to 5-bromo-2'-deoxyuridine (BrdU). Harvested raft cultures were used for histology, BrdU staining to measure DNA synthesis, and other immunofluorescence and immunoblot assays. In-situ hybridization and real-time PCR were used to detect and quantify HPV-18 DNA.

> **Virology Note:** The proposed clinical product contains 10.3% berdazimer sodium, and the highest concentration used in the raft culture studies was 0.15% (1.5 mg/mL), which is approximately 70-fold lower than the concentration that is intended to be used in the clinic. Hence the relevance of the raft culture findings to any clinical mechanism of action is not clear.

Results

Following daily exposure from Day 7 through Day 12, berdazimer sodium demonstrated a dose-dependent reduction in HPV DNA copy, with an ~85% reduction in cells incubated with 1.5 mg/mL of drug relative to control-treated cultures (**Figure 7**).

Figure 7 (Figure 10, page 21, pharmacology) Effect of berdazimer sodium on relative copy number of HPV-18 determined by real-time PCR of raft culture-derived DNA



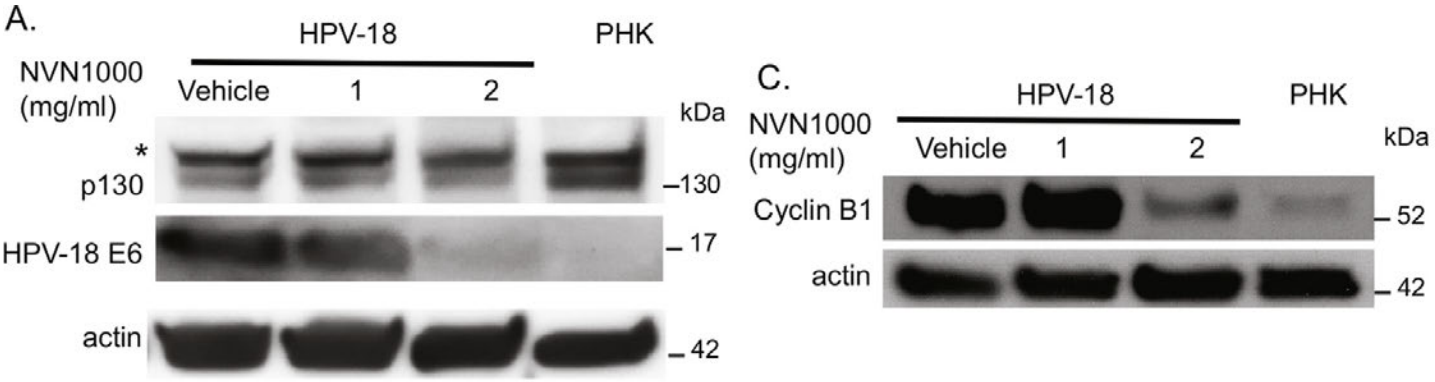
Relative viral DNA copy number per cell as determined by qPCR from parallel cultures from the same experiment.

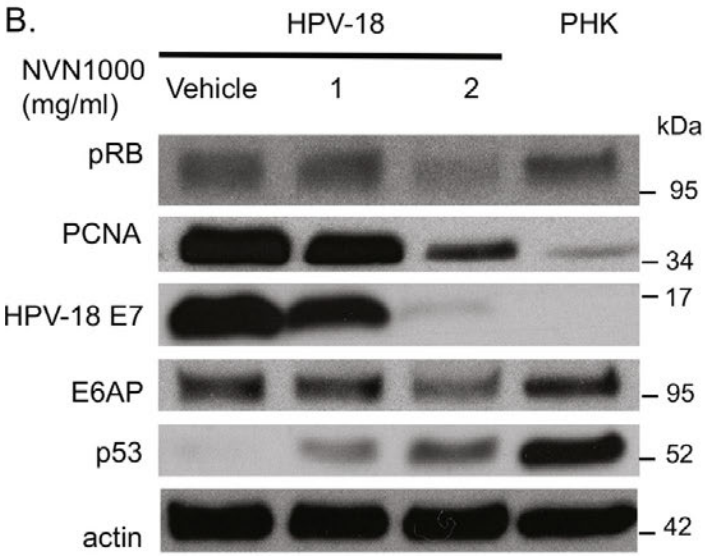
> **Virology Note**: Application of the drug was associated with a reduction in HPV DNA and mitotic activity; however, these effects might also be seen with non-specific cytotoxicity. The Applicant also stained for HPV L1 capsid protein, and saw a dose-dependent reduction in staining from Day 7 to Day 12 in cultures treated with berdazimer sodium. Again, the decrease in detectable L1 capsid protein and presumably progeny virus reduction could have resulted from a virucidal effect or a non-specific cytotoxic effect.

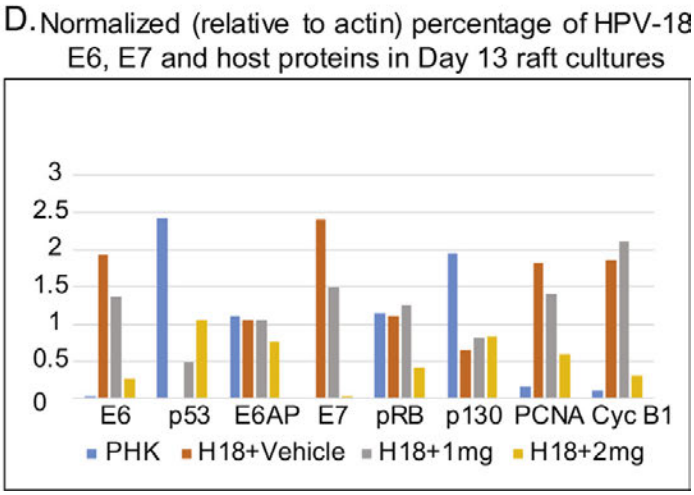
The impact of NVN1000 on E6 and E7 protein levels in raft culture was assessed (**Figure 8**). Amounts of both proteins were reduced relative to the actin control. In addition, an increase in p53 and a reduction in the E7-induced proteins PCNA (proliferating cell nuclear antigen, a DNA polymerase processivity factor) and cyclin B1 was observed.

> **Virology Note**: The authors of the Banerjee et al., 2019 publication suggested that NO may stabilize p53 protein, resulting in an increase in P53 which may contribute to the reduction of S phase progression and viral DNA amplification. However, it's not clear that any changes observed were HPV-specific, or simply from cytotoxic/cytostatic effects of berdazimer sodium.

Figure 3. (Figure 4, Banerjee et al., 2019) Immunoblot analyses of raft cultures with HPV-18 E6 and E7 proteins and increasing concentrations of berdazimer sodium







Raft cultures were treated for 1 h daily with topical application of the agent from day 7 to day 12 and harvested on day 13. The lysates were derived from parallel cultures from one of the three independent experiments described above. Panels A, B and C each represent a separate blot, with actin serving as a loading control. Panel D is densitometric quantification of viral and host protein bands. In panel A, * indicates a nonspecific cross-reactive protein. NVN1000 = berdazimer sodium.

Conclusions

A published study describing the effects of berdazimer sodium on HPV raft cultures was included as supportive evidence, presumably of general antiviral activity. Like the studies with vaccinia virus and MCV, there was no clear evidence that the observed reductions in HPV DNA and HPV proteins, effects on p53, cell cycle and associated host cell proteins, were mediated via antiviral activity rather than non-specific cytotoxicity. Furthermore, the relevance of HPV raft cultures to MCV infection of epidermal cells is not clear.

Antiviral activity of berdazimer sodium in the cottontail rabbit papillomavirus model (study reports phar-14-006, phar-14-008)

The Cottontail Rabbit Papillomavirus (CRPV) model has been used extensively to conduct studies investigating immunological features of HPV infection, virus-induced carcinogenesis, and host immune-mediated regression (Christensen, 2005; Cladel et al., 2008). In this model, the skin on the backs of rabbits is inoculated with wild-type CRPV DNA or E8 knock-out CRPV DNA (mE8-CRPV); the sites inoculated with mE8-CRPV develop papillomas that are slower growing, and thought to more closely model the course of human papilloma growth.

Methods

CRPV studies were conducted at (b) (4). For these studies, different formulations of berdazimer sodium were tested (**Table 1**).

Table 1 (Table 1, page 8, pharmacology) Active gel comparisons

Ingredient	NVN1000 Suspension (mg/kg/day)	SB205 20% Active Gel [a] (% w/w)	SB207 20% Active Gel [s] (% w/w)
Berdazimer sodium	(b) (4)		
Isopropyl alcohol			

Hexylene glycol	[redacted] (b) (4)		
Cyclomethicone			
Hydroxypropylcelluose, (b) (4)			
[redacted] (b) (4)			
Total	n/a	100	100

Note: The strengths listed in column headers reflect the berdazimer sodium concentration before mixing with the hydrogel
[a] When combined with proton donating hydrogel, SB205 and SB207 gel nominal strength is 10%.
[redacted] (b) (4)

In the first experiment (study report phar-14-006), A total of 32 New Zealand White rabbits were divided into 8 treatment groups (4 animals/group), including two control groups, two groups treated with fast-releasing SB205 gel, two groups with slow-releasing SB2015 gel, one group with single phase NVN1000 ointment, and a positive control group (0.3% cidofovir in cremophor). Admixture formulations were made by with the relevant gel phase mixed in a 1:1 fashion with the corresponding hydrogel phase prior to topical administration.

Rabbits were infected with wild-type CRPV (wtCRPV) and E8 knock-out CRPV (mE8-CRPV) on Day 1. From two weeks post viral DNA inoculation, animals were treated topically with the test article formulation once daily, 5 days a week (Monday through Friday) for 5 weeks. The applied dose formulations were spread out onto the skin surface in a uniform layer. Papillomas were measured weekly in three axes (length x width x height) and recorded as a geometric mean diameter (GMD) in millimeters. Body weights were determined weekly.

In the second experiment (phar-14-008), different doses of SB207 gel were evaluated. A total of 30 rabbits were divided into 6 treatment groups (n=5/group), including placebo, 2% SB207 gel, 4% SB207 gel, 8% SB207 gel, 10% SB207 gel and positive control 5% imiquimod cream (TLR-7 agonist used for the treatment of genital warts). Rabbits were infected with wild-type CRPV (wtCRPV) and E8 knock-out CRPV (mE8-CRPV) on Day 1. All animals received topical treatment once a day, five times a week (M-F) of the appropriate test article formulation for a total duration of eight weeks. Papillomas and body weights were evaluated as for the first experiment. In addition, two tissue samples were taken from the papilloma, or area where the papilloma should have developed, and one was used for cytokine analysis and the other for histological analysis.

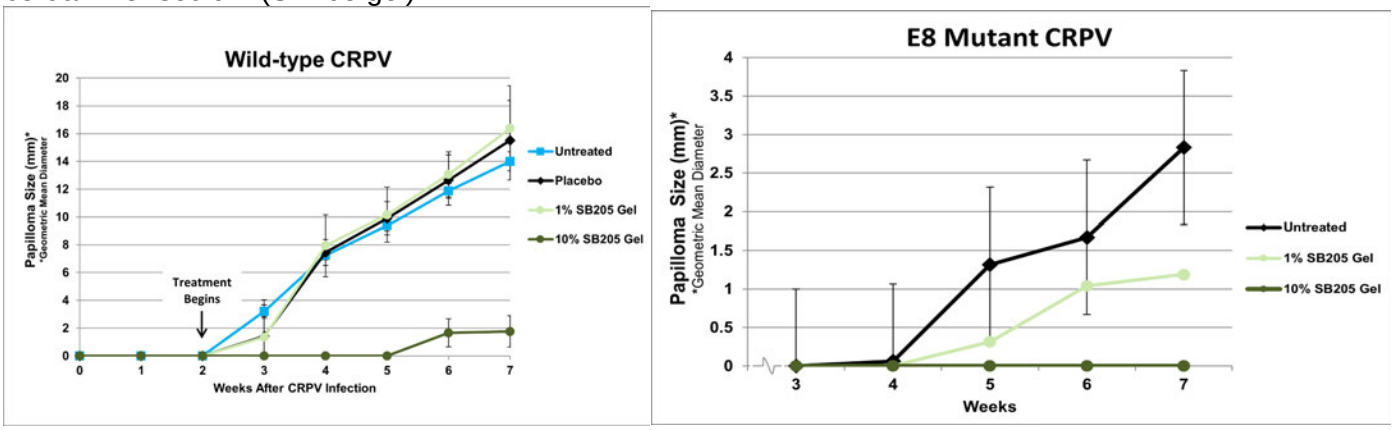
Results

Only the results of SB205 gel, the formulation relevant to the NDA application, are described here. Scarified sites inoculated with the wild-type CRPV develop papillomas that are measurable three weeks following inoculation of viral DNA, whereas sites inoculated with the E8 mutant CRPV develop slower growing papillomas, thought to model the progression of human papillomas more closely. **Figure 4** shows the impact of SB205 gel on wtCRPV and mE8-CRPV papilloma growth.

For wild-type CRPV, treatment with placebo or 1% SB205 gel did not impact papilloma size, but 10% SB205 gel caused a significant decrease (88%) in the mean diameter compared with untreated controls. This was comparable to the effect observed using 0.3% cidofovir, which is cytotoxic (Mertens et al., 2016). For five weeks after inoculation of wild-type CRPV DNA, all sites treated with 10% SB205 gel lacked measurable papillomas, whereas all other treatment groups had measurable papillomas as early as three weeks.

For mE8-CRPV papillomas, at the end of topical treatment (Week 7), 1% and 10% SB205 gel reduced the mean diameter by 58% and 100%, respectively, compared with untreated controls. Treatment of E8 mutant CRPV-inoculated sites with 0.3% cidofovir resulted in complete inhibition of mutant papilloma growth following topical therapy (Week 7).

Figure 4. (Figures 11 and 12, pages 23-24, pharmacology) Inhibition of wtCRPV papilloma growth with berdazimer sodium (SB205 gel)

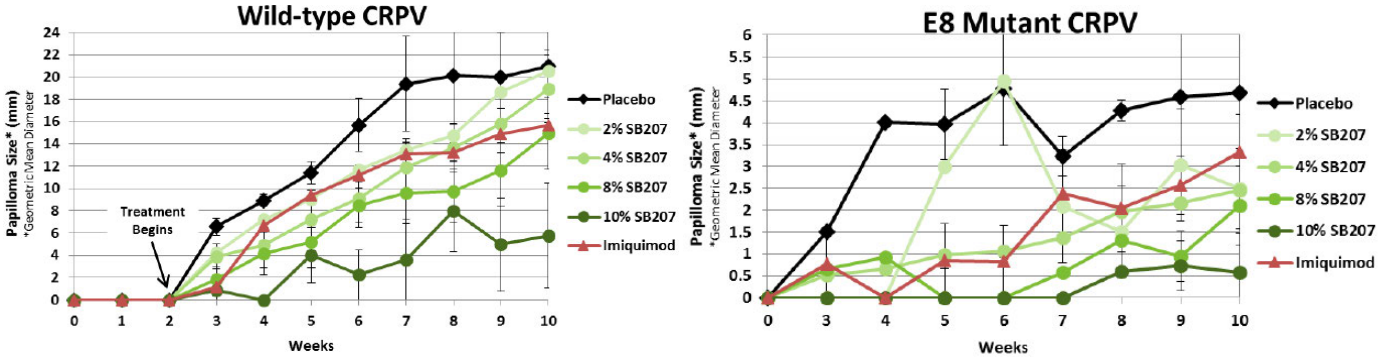


Papillomas were allowed to grow for one week following the cessation of topical treatment and the frequency of animals without any papilloma growth was determined (data not copied into review). For the wild-type CRPV, 1/4 animals treated with 10% SB205 or 0.3% cidofovir which had shown complete inhibition of papilloma growth showed papilloma growth one week after treatment cessation. For the mE8 CRPV-infected animals, all those treated with 10% SB205 or 0.3% cidofovir which had achieved complete inhibition of papilloma growth at Week 7 did not show papilloma growth one-week post-treatment.

> **Virology Note:** SB205 gel appeared to be administered prior to formation of papillomas. Besides being a different virus from the proposed indication, the study does not model the effect of drug as it is intended to be used in the clinic (i.e., after virus-induced lesions are visible).

Figure 5 shows the impact on papilloma growth for different concentrations of SB207 Gel administered for 8 weeks in the wtCRPV and mE8-CRPV models, using imiquimod as a positive control. At the end of this study (Week 10), against wtCRPV, only 8% and 10% SB207 gel appeared to impact the size of papillomas, with the 10% dose causing an 82% reduction compared with placebo control. In the mE8-CRPV study, for doses of 2%, 4%, 8% and 10% SB207 gel, at Week 10 a decrease in papilloma size compared with placebo control of 47%, 47%, 55% and 88%, respectively, was observed. A total of 4 of 5 rabbits in the 10% SB207 gel treatment group did not develop palpable papillomas while the papilloma of the 5th rabbit had a GMD of 0.58 mm at study end.

Figure 5 (Figures 2 and 3, pages 7-8, phar-14-008) Dose responsive inhibition of wtCRPV and E8 mutant CRPV papilloma growth with berdazimer sodium (SB207 gel)



> **Virology Note:** Like the study shown in **Figure 4** above, in these dose/response studies, treatment appears to have been initiated prior to the formation of papillomas. In the mE8-CRPV experiment, they did not show data from Weeks 1 and 2, giving the appearance of linear papilloma growth from the inoculation time point to Week 3 (**Figure 5**). Presumably there was no measurable papilloma growth from Weeks 0 to 2, as seen in the other studies.

For the histological examination, a qualitative assessment of inflammatory cells showed a low level of inflammation for all treatment groups. In addition, 4 of 5 rabbits treated with 10% SB207 gel and 2 of 5 treated with 8% SB206 gel did not have intra-nuclear basophilic inclusions, which the Applicant stated was indicative of a complete lack of viral infection.

> **Virology Note:** A lack of inclusion bodies does not necessarily indicate that the cells were not infected. HPV infection is associated with inclusion bodies, and these bodies tend to vary in appearance and size depending on the HPV subtype (Roberts et al., 2007). However, the detection method of these bodies (i.e., microscopy) is likely to have poor sensitivity, and only sample a small fraction of the cell and potentially infected tissue. Absence of viral infection should be confirmed using a sensitive method to detect HPV DNA, for example real-time PCR.

For the cytokine analyses, there was significant variability among the gene expression observed from tissue biopsies of a single treatment group, as well as fluctuation in the expression observed from the triplicate samples from a single tissue biopsy. This variability prevented normalization of data and evaluation of relative levels of gene expression between treatment groups. However, overall, there appeared to be a heterogenous response and upregulation of certain inflammatory markers, which were not observed in untreated animals or those receiving low doses of SB207 gel.

Conclusions

A cottontail rabbit papillomavirus model was used to study the effect of berdazimer sodium on papilloma growth. The relevance of this model to MCV is not known, and the experimental design in which the berdazimer sodium gel was administered prior to the formation of papillomas does not reflect the intended use of the product in the clinic where virus-induced lesions are present prior to administration. Irrespective of the model's relevance, it's not clear that any effect on papilloma growth, including intracellular, cytokine profile and histological changes, was indistinguishable from a cytotoxic effect.

CONCLUSIONS

A consult from the Division of Dermatology and Dentistry was requested to evaluate (b) (4) NDA application for berdazimer sodium treatment of MCV lesions. Because MCV does not propagate in cell culture and no animal model exists, the Applicant relied on models of a distantly related poxvirus and other unrelated DNA viruses, and assessment of the impact of berdazimer sodium on extracellular MCV particles. However, the relevance of these surrogate models to MCV is not known. There are several issues with the interpretation of data that berdazimer sodium has antiviral activity in the assays conducted.

The main concern is that activity was essentially indistinguishable from cytotoxicity in the cell line in which MCV and vaccinia virus were tested (BSC40). In addition, it was not clear that BSC40 was a relevant model for the epidermal cells in which MCV replicates, and the concentration of drug tested appeared significantly lower than would be used clinically. For MCV, the relevance of treating free particles prior to cellular uptake is not clear, and the impact on gene expression observed could have been caused by cytotoxicity. In addition, direct treatment of vaccinia virus particles with berdazimer sodium at concentrations around the CC_{50} value did not inactivate the virus, consistent with a cytotoxic effect. Finally, the relevance of the papilloma virus raft cultures

and animal models to MCV infection is not clear, and in any case the effects on HPV and cellular markers could have been caused by cytotoxicity. Overall, there was no clear evidence of an antiviral effect of berdazimer sodium in the models assessed, (b) (4)

RECOMMENDATIONS

On review of the non-clinical data provided by the Applicant, we find no clear evidence of an antiviral effect of berdazimer sodium on MCV, or other viruses assessed. Our main concerns are as follows:

- The cytotoxicity of berdazimer sodium in the BSC40 cell line used for vaccinia virus and MCV experiments was approximately 200 µg/mL, only 5-fold above the EC_{50} value in both cases, and determined from overnight exposure compared with 3 days for the EC_{50} evaluation in the vaccinia study. Regardless of the exposure time, a therapeutic index below 10 is typically indicative of a cytotoxic mechanism. For the MCV experiment in which extracellular particles were tested, an EC_{50} value of 200 µg/mL was determined. Even though there was a 5-fold dilution prior to adding to BSC40 cells, for all practical purposes, the concentration of berdazimer sodium needed initially was the same as the CC_{50} value.
- The relevance to the proposed indication of experiments in which extracellular virus particles were treated with berdazimer sodium prior to adding to cells is not clear. For the vaccinia virus experiment, the infectivity of treated particles did not reach below 50% of those in the DMSO control at the highest concentration tested, so it is not possible to determine EC_{50} values in this case.
- The concentration of berdazimer sodium tested non-clinically appeared to be significantly lower than proposed for clinical use. Hence, at clinical concentrations it seems likely that the product would be cytotoxic. No data were provided for cytotoxicity in a cell line which may be more relevant to the epidermal cells which MCV infects.
- For the vaccinia virus resistance experiment, the assay readout was conducted one day following infection, whereas the EC_{50} value was determined over 3 days, so it is not clear that the concentration of berdazimer sodium used was sufficient to have antiviral activity in the time frame used. In addition, the amount of virus was not standardized between parental and passaged viruses, so the difference seen in the assay readouts could simply be the result of low infectivity of the parental virus compared with passaged virus.
- The relevance of the papilloma virus raft cultures and animal models to MCV infection is not clear. For the cottontail rabbit model, the product was added prior to the formation of papillomas, which is not consistent with the intended clinical use (i.e., once viral lesions have formed). In any case, the effects observed of berdazimer sodium on HPV and cellular markers could equally have been caused by cytotoxicity.

(b) (4)

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CONCURRENCES

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