Novel Nanoviricides® Highly Effective Against Varicella Zoster Virus in Cell Culture

Stock Symbol (NYSE MKT) : NNVC

www.nanoviricides.com

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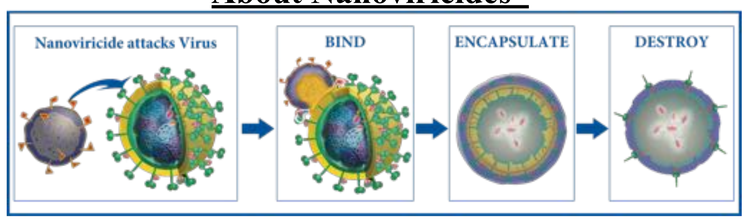
Abstract

Varicella Zoster virus (**VZV**) primary infection causes chickenpox, followed by latency in ganglia and neurons, and can reactivate decades later causing herpes zoster (shingles), usually upon immunosuppression resulting from age, stress, or other factors. Classical shingles presents as a painful unilateral dermatomal vesicular rash as virus spreads to the skin through peripheral nerves. In severe cases, VZV can reactivate in or around the eye which can cause facial disfiguration or blindness. There are about 1 million cases annually and the lifetime risk of developing shingles is at least 30%. While there is a shingles vaccine, it is not effective post-breakout, is only ~50% effective in preventing disease, and cannot be given to immunosuppressed people. **Topical treatment of shingles remains an unmet medical need, and would enable high concentration of active drug locally for rapid treatment with minimal systemic effects. NanoViricides, Inc. is developing broad-spectrum drugs against herpesviruses for both topical and systemic use**. Our novel nanoviricide® class of drug candidates are designed to specifically attack enveloped virus particles by specially designed small chemical ligands and dismantle them with the polymeric micelle which is covalently attached. Our approach of designing ligands to mimic virus binding sites on cellular receptors promises that a virus cannot escape the nanoviricide drug due to mutation(s). We present two nanoviricide drug candidates that are highly effective against VZV *in vitro*, and one that was not. These candidates differ only in the chemical structure of the ligands, demonstrating ligand-defined virus specificity. Candidates were non-cytotoxic in all cell lines tested. The active candidates inhibited VZV up to 5x better than acyclovir-sodium (the current standard of care), and completely inhibited VZV protein production/infection *in vitro*. NanoViricides, Inc. is advancing these candidates further into *ex vivo* dermal studies towards IND filing.

About Nanoviricides®



NanoViricides, Inc. is a global leader in the development of nanomedicine drugs against viruses. The Company has eight anti-viral treatments in development in various pre-clinical stages. Of these, certain topical drugs in the HerpeCide™ program are in advanced preclinical stage and are expected to enter human clinical studies in the very near future. We are a development-stage company creating special-purpose nanomaterials for anti-viral drugs based on a novel, first-in-class mechanism. The Company's novel nanoviricide® class of drug candidates are designed to specifically attack enveloped virus particles, on the same sites that they use to bind to cells, and dismantle them. Our approach promises that a virus cannot escape our nanoviricide® due to mutations.

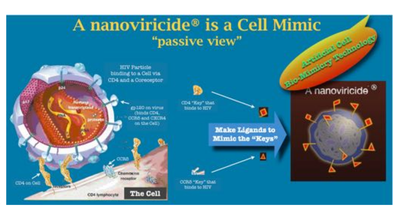
Beyond Antibodies and Vaccines: A Nanoviricide® is A Nanomachine that Completes the Task of Destroying Virus without Help from the Patient's Immune System

Antibodies have been developed as drugs against viruses. However, an antibody only binds by two points to the virus. Moreover, the patient's immune system needs to be healthy for the clearance of the complex by the bodily systems of complement fixation and immune response. Vaccines only train the body into producing antibodies against the virus in the vaccine. Because antibodies are highly specific, antibodies and vaccines are easily overcome by viruses by virtue of rapid mutations in the field. Limitations of antibody cocktail approaches and of vaccines are now well known. For example, clinical studies found that the zMapp antibody cocktail was ineffective against the Ebola epidemic in 2015, and the 2015 influenza seasonal vaccine was only 23% effective.

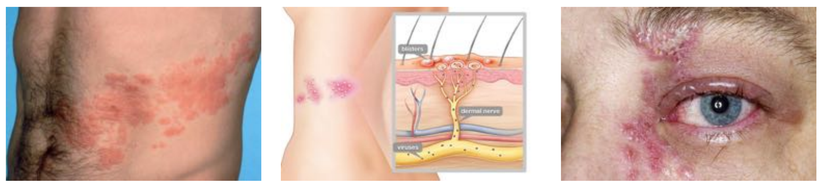
In contrast, a nanoviricide® should work against all strains of the virus because they still bind the same way to the same host cell receptor. A nanoviricide® attacks the virus with hundreds of virus-binding sites on its surface. The nanoviricide® is capable of dismantling the virus in the same way as strong soap solutions do, and the resulting complexes are fully biodegradable in the body.

Key Issue of Drug Resistance from Viral Mutations is Unlikely with Nanoviricides® due to Unique Biomimetic Technology

The nanoviricide® technology platform mimics the host cell. We design and develop a virus-binding ligand that mimics the site on the host cell receptor to which the virus binds. This ligand is then chemically attached to a special polymer to make a nanoviricide®. The virus is expected to be fooled into binding to the nanoviricide®, like a venus-fly-trap. The nanoviricide® is then expected to engulf the virus and possibly destroy it. To the extent that the ligand accurately maps to the receptor binding site, the virus would not be able to escape the nanoviricide® no matter how much it mutates, because such mutants would not be able to bind efficiently to the natural receptor, and would not be very pathogenic.



Introduction



Images from WebMD.com

There are eight herpesviruses known to infect humans. Varicella Zoster virus (**VZV**) is a member of *Herpesviridae* and is also known as human herpesvirus 3 (HHV-3). Primary infection with VZV causes chickenpox in children, followed by latency in nerves, including the cranial nerve ganglia, dorsal root ganglia, and autonomic ganglia, and can reactivate decades later to cause herpes zoster (shingles) in adults usually upon immune compromise resulting from age, stress, or other factors. **Classical shingles presents as the virus exits peripheral nerve endings and infects proximal skin cells, further spreading to the skin causing a unilateral vesicular rash in dermatomal distribution with stinging pain. In severe cases, disfiguring facial presentation can occur, and the virus can also reactivate around the eye or even in the retina which may lead to blindness.** There are about 1 million cases annually and the lifetime risk of developing shingles is at least 30%. While there is a shingles vaccine, it is not effective after the beginning of a breakout, is only about 50% effective in preventing disease, and cannot be given to immunosuppressed people. New vaccines are being sought after.

Topical treatment of shingles remains an unmet medical need, in spite of its dermal presentation. A topical treatment would enable high concentration of active drug locally for rapid resolution of pathology with minimal systemic effects. **NanoViricides, Inc. is developing different broad-spectrum drugs against the herpesvirus family for topical use and for systemic use.** Our novel nanoviricide® class of drug candidates are designed to specifically attack enveloped virus particles by virtue of specially designed small chemical ligands, and to dismantle them by virtue of the polymeric micelle to which the ligands or covalently attached. Our approach of designing the ligands to mimic virus binding site on cellular receptor promises that a virus cannot escape the nanoviricide® drug due to mutation(s).

Methods

CELL-BASED ELISAs: We have successfully developed a cell-based ELISA in 96-well black, clear-bottom plates which allows us a higher-throughput screening of compounds compared to other methods. With appropriate antibody selection, we have successfully applied this ELISA technique to quantitation of different viruses. In the VZV assay presented here, VZV is pre-incubated with compounds and then added to ARPE-19 cells and incubated for 6 days (see **Figure 1** and **Figure 2**). This gives the virus time to replicate and express viral proteins. Cells are then fixed in formalin, so viral protein expression can be relatively measured compared to uninfected controls. We can compare uninfected cells, infected cells with no treatment, and cells treated with our compounds or positive controls such as Acyclovir Sodium (ACV Na+). We can then compare relative infection levels between all groups in terms of quantification of reduction in protein expression. A reduction in protein expression compared to untreated, infected controls suggests a decrease in virus quantity, and serves as a quantitatively comparable estimate of virus production/growth/spread.

Figure 1: VZV ELISA Optimization of Infection. In order to determine the best concentration of virus to use for our cell-based ELISAs, ARPE-19 cells were seeded in 96-well plates 24 hours prior to infection. Serial dilutions of our VZV stock virus preps were tested and plates were fixed in formalin at various time points. Absorbance was measured to determine optimum concentration of virus to use in our studies

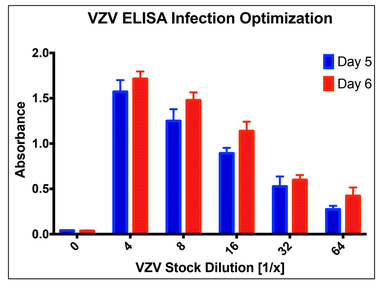
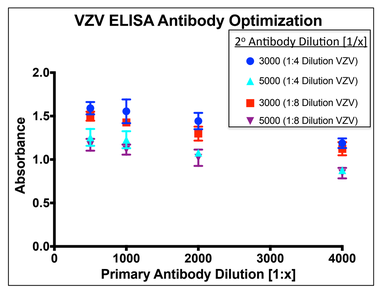


Figure 2: VZV ELISA Optimization of Antibody Dilutions. In order to determine the best primary and secondary concentration of antibody (Ab) to use in our cell-based ELISAs, ARPE-19 cells were seeded in 96-well plates 24 hours prior to infection. Based on Figure 1, plates were infected with either 1:4 or 1:8 dilution of virus stock, and fixed in formalin after 6 days. 4 different primary Ab dilutions and 2 different secondary Ab concentrations were used to optimized our assay.



CYTOTOXICITY/CELL-VIABILITY ASSAY: We used the Promega CellTiter-Glo Luminescent Cell Viability Assay (Cat# G7571) to determine if any of our compounds were toxic and caused cell death on their own. This assay allows us to differentiate effects caused by toxicity and actual virus suppression

Results

Figure 3: Initial screening of multiple compounds. In order to narrow down our list of in-house compounds to determine which are effective against VZV, we used our cell-based ELISA to screen many compounds. ARPE-19 cells were seeded in 96-well plates 24 hours prior to infection. VZV was incubated with 4 different concentrations of each compound for 1 hour prior to infection of the cells. After 6 days, plates were fixed in formalin. Cell-based ELISAs were performed and percent infection relative to untreated infected controls was determined by measuring absorbance.

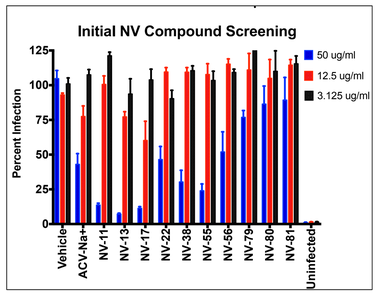


Figure 4: Compounds NV-118 and NV-121 are non-toxic to cells *in vitro*. In order to determine if our compounds, controls, or vehicles were toxic to the cells themselves, we performed a cell-viability assay. ARPE-19 cells were seeded in 96-well plates 24 hours prior to addition of 4 different concentrations of compounds. After 6 days (same day as the plates are fixed for ELISA measurement), cell viability was determined by Promega's CellTiter-Glo Luminescent Cell Viability Assay. These are the combined results of **2 separate** experiments (n=6). Selected compounds are shown.

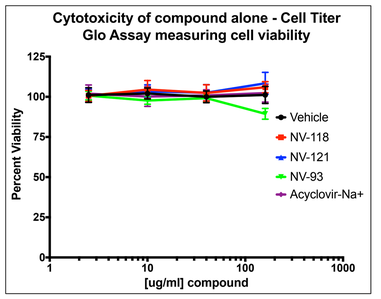
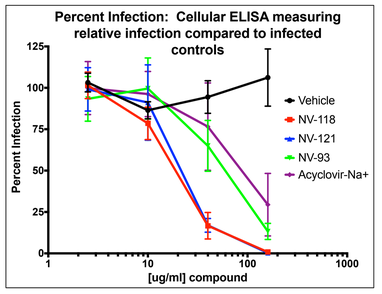


Figure 5: Compounds NV-118 and NV-121 are extremely effective inhibiting VZV *in vitro*. In contrast, NV-93 was less effective. ARPE-19 cells were seeded in 96-well plates 24 hours prior to infection. VZV was incubated with 4 different concentrations of compounds, inhibitors, and vehicles for 1 hour prior to infection of the cells. After 6 days, plates were fixed in formalin. Cell-based ELISAs were performed and percent infection relative to untreated infected controls was determined by measuring absorbance. Combined results of **2 separate** experiments (n=6) shown.



Conclusions

- **We present here two nanoviricide® active drug candidates, NV-118 and NV-121, that are highly effective against VZV *in vitro*, and one, NV-93, that was comparatively ineffective. The three candidates differ only in the chemical structures of their ligands, demonstrating ligand-directed virus specificity.**
- **All three compounds were found to be non-cytotoxic in ARPE-19 cells as well as every other cell line tested.**
- **These two active candidates were found to inhibit VZV up to 5 times better than acyclovir-sodium treatment (the current standard of care) *in vitro*, and completely inhibited VZV protein production/infection at the highest dose.**

Future Directions

- NanoViricides, Inc. is now advancing these two drug candidates further into *ex vivo* dermal and toxicology studies towards IND filing for clinical trials as expeditiously as possible.
- NanoViricides, Inc. is also planning to test these active drug candidates against other human herpesviruses for efficacy and potency and to determine if there is broad-spectrum activity.
- NanoViricides, Inc. is unique in the field in that we now have our own multi-kg-scale, cGMP-capable clinical drug manufacturing capability. This facility is anticipated to enable rapid translation to the clinic (as opposed to using an external manufacturer) of our drug candidates.

Acknowledgements

I would like to specifically thank our CEO Dr. Eugene Seymore, President and Chairman Dr. Anil R. Diwan, CSO Dr. Randall W. Barton, CFO Meeta R. Vyas, Vice President of R&D Dr. Jayant Tatake, and our Board of Directors for all their help and support.

I would also like to thank everyone at NanoViricides, Inc. and AllExcel, Inc. for their hard work and dedication to this project.