

## Nitric Oxide-Releasing Cyclodextrins for Treating COVID-19 Infections

COVID-19 Proposal (\$900k)

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Nitric oxide has proven efficacy against SARS-CoV (*J. Virol.* 2005; 79: 1966-1999). The National Institute of Allergy and Infectious Diseases is currently evaluating antiviral properties of the compound in preclinical animal models. For patients with SARS-CoV, it was shown that nitric oxide can (1) reduce the time to hospital discharge, (2) reduce the need for ventilatory support, and (3) improve appearance of infection on lungs via chest radiograph (*Clin. Infect. Diseases.* 2004; 39:1531–1535).

My lab has been developing novel water-soluble, macromolecular nitric oxide (NO) donors for the treatment of pulmonary infections that are amenable for nebulization. Our most promising compounds are based on highly loaded nitric oxide donor-modified cyclodextrin derivatives that release nitric oxide in a controlled manner with variable release kinetics (*J. Am. Chem. Soc.* 2018; 140: 14178-14184). We have extensive preclinical data that demonstrates that these compounds are efficacious against a variety of pulmonary pathogens. It is expectedly broad spectrum based on its mechanisms of action (both oxidative and nitrosative stress.) Others have previously published that delivery of NO via a low molecular weight NO donor molecule inhibited replication of the SARS-CoV in a concentration-dependent manner:

1. Akerstrom et al., *J. Virol.* 2005; 79:1966-1999
2. Xu et al., *Free Rad. Biol. & Med.* 2006; 41:19-28
3. Akerstrom et al., *Virology* 2009; 395:1-9.

It is well known that individuals with impaired endogenous NO production (e.g., those with diabetes, high blood pressure, cancers) are at greatest risk for serious complications related to COVID-19 infections. In virally infected cells, nitric oxide *inhibits viral replication* by binding directly to key enzymes or by inducing apoptosis, or programmed cell death, in these cells where tumor suppressors have been degraded or disabled. Nitric oxide and its reactive by-products also cause a reduction in the palmitoylation of nascently expressed spike (S) protein which affects the fusion between the S protein and its cognate receptor, Angiotensin Converting Enzyme 2. In turn, this reduces viral uptake by mammalian cells.

While the powerful benefits of nitric oxide have long been recognized, there is a scarcity of nitric oxide-based therapeutic products due to the challenges associated with controlling, or tuning, the delivery of nitric oxide because it is a gas. In addition, the poor stability and low storage capacity of low molecular weight (i.e., small molecule) nitric oxide-donor molecules, the inability to target specific tissues, and the toxicity of most small molecule NO donor precursors have all prevented the effective development of a nitric oxide-based anti-infective therapeutic. We believe the following key components of NO donor-modified cyclodextrins will fuel the creation of a respiratory therapeutic for treating COVID-19 infections that overcomes these limitations.

- High water-solubility that facilitates delivery of NO directly to the lungs via solution avoiding systemic adverse side-effects
- Tunable release of NO based on NO donor chemistry/modification employed
- Favorable toxicity at high doses
- Localized release and local acting (for the relevant anatomical location of the disease)
- Established broad-spectrum antibacterial and antiviral activity with the ability to prevent blood-clots—all of which are necessary for patient survival

With established broad-spectrum antibacterial and ability to prevent clot formation, support of our proposal could at a minimal provide treatments for the symptoms of COVID-19 that lead to morbidity. Our therapeutic platform also has the potential to reduce viral replication without engendering antimicrobial resistance nor destabilizing the gut microbiome while promoting blood flow to the lungs. As such, this therapeutic represents a major step forward in the treatment of COVID-19 infections is not a repurposed drug.

Vast Therapeutics, a North Carolina based company that Dr. Schoenfisch co-founded, has in-licensed a portfolio of drug candidates from the University of North Carolina at Chapel Hill, including the NO-releasing cyclodextrins. Over the last year, Vast Therapeutics spent >\$1.5M constructing GMP production capability for high pressure nitric oxide loading reactions. This capability was designed for Phase 1 GMP manufacturing but will also be ideal for preparing GLP materials at scale for evaluating the performance of the proposed drug candidates in animal models and toxicology studies. Vast Therapeutics, through an already established partnership with the Schoenfisch lab and UNC-Chapel Hill, has also scaled the modification of cyclodextrin with NO donor precursors and developed appropriate analytical methods for assessing purity, impurities, and drug stability. In this proposed extended partnership with UNC and the Schoenfisch Laboratory, Vast Therapeutics will support the IND-enabling toxicology program (cost share of \$750k) for the lead compound with greatest therapeutic efficacy as determined by in vitro antibacterial/antiviral testing and most favorable cell/tissue cytotoxicity.

During the first phase of this project, the Schoenfisch lab developed and selected multiple NO-release drug candidates at laboratory scale (10-gram scale) which was evaluated through in vitro assays (assessing antibacterial, antiviral, toxicological characteristics). The best compounds were selected through physiochemical considerations common to respiratory drugs delivered by nebulization, with a particular focus on reducing adverse effects. Of importance, one of our drug candidates, BIOC76, has shown preliminary anti-viral activity against SARS-CoV2. In the second phase of this project, 8 drug candidates were transferred to Vast Therapeutics for deeper in vitro screenings against multiple (>100) bacterial agents, scale-up (100-gram scale), stability testing, and formulation development for delivery by nebulization. Based on the literature and our preliminary results, we believe that this technology will have efficacy against SARS-CoV2. By augmenting our research and development efforts, the timeline for first-in antiviral human clinical trials will be substantially accelerated and could begin as soon as Q4 2020.

At this time, we are prepared for the third phase of this project which includes the conclusion of drug down-selection of a lead candidate for pre-clinical antiviral testing, further scale-up (1-kilogram scale), bioanalytical method development, and IND-enabling toxicology program in two species (standard for respiratory products) for determination of maximum tolerated doses and safety studies of repeat dosing. Stage appropriate toxicology evaluations will be completed according International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Vast Therapeutics has already developed a full toxicology program using consultants specializing in inhalation toxicology. Our inhalation toxicology contract research partner is Covance in Somerset, NJ. Inhalation toxicology studies of our lead candidate will be conducted with daily dosing for up to 28 days in duration to support up to 28-day clinical trials and will include toxicokinetic (TK) assessment of systemic exposure and histopathology of the respiratory tract and a full list of systemic tissues in addition to other standard evaluations (ICH M3[R2] and ICH [S3A]).

Vast Therapeutics, and its parent company KnowBIO, have extensive experience in designing and running clinical trials in large patient populations (>3,000 patients) with attention to the safety and efficacy of nitric oxide-based therapeutics. Relationships with multiple clinical research organizations (CROs), including IQVIA, Rho, and Cato Research are in place to enable oversight of clinical trials of this scale and significance. Vast Therapeutics has been working since 2016 to develop nitric oxide-releasing therapeutics for treating respiratory infections with a focus on chronic bacterial infections. In this time, Vast Therapeutics has developed strong relationships with many of the world's leading clinical pulmonologists, several of which

serve on the Vast's Scientific Advisory Board. With deep knowledge of the safety and efficacy of nitric oxide, our capability to design and execute an appropriate clinical trial combined with ownership of patent rights related to the proposed treatment, uniquely positions us to mitigate the severity of this pandemic in the United States and abroad.

As a sign of KnowBIO's desire for continued partnership for years after this specific project, KnowBIO will also increase its sponsorship to the Schoenfisch lab at UNC-Chapel Hill to \$250k per year and extend the current agreement.

#### Budget and Milestones

**An overall budget of \$900k is requested for the proposed work.** The work will be carried out in two phases with *Phase 1* being supported by an initial tranche of \$500k. Successful completion of *Phase 1* will trigger the remaining funds (\$400k) to help offset the costs of the IND-enabling toxicology program (*Phase 2*).

#### Phase 1 (\$500k)

The following activities will be completed by September 30, 2020.

1. Selection and scale-up of a lead drug candidate with acceptable purity for IND-enabling toxicology program
2. Development of an appropriate formulation for delivering drug
3. In vitro antibacterial and antiviral testing (both because our drug has multiple mechanisms of action that may benefit COVID-19 infections beyond antiviral activity alone).
4. Bioanalytical method development (to enable tracking in plasma of drug, the NO that is released, and elements of the formulation)
5. Characterization and stability testing of both drug solution and product

#### Phase 2 (\$400k)

The following IND-enabling toxicology studies in rodent and dog species will be completed by December 30, 2020. The IND-enabling toxicology studies represent the gateway for first-in human clinical trials. The as-designed cost of this IND-enabling work exceeds \$1.5m. As such, Vast Therapeutics will contribute more than \$750k towards this expense.

1. 7-day maximum tolerated dose study to support 28-day repeat dosing study
2. 28-day repeat dosing study for safety assessment and determination of acceptable dose for human clinical trials.

EHRA Salary	\$29k
SHRA Salary	
Grad Student	
Temps	
Fringe Pool	\$4k
Non-Personnel Expenses	\$867k
<b>Total</b>	<b>\$900k</b>