PROJECT 2:
THERAPEUTICS II: PRECLINICAL STUDIES OF NOVEL THERAPEUTIC AGENTS IN MOUSE MODELS FOR TARGET/DRUG VALIDATION, PHARMACOKINETIC (PK) STUDIES, AND EFFICACY USING MOUSE-ADAPTED SARS-COV-2 VIRUS

Team

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This project is part of a two-pronged effort to accelerate the prevention and treatment of COVID-19 in NC and the rest of world. There are > 750 proposed therapies to prevent/treat COVID-19. Consequently, there is a need to prioritize these therapies based on estimated time to clinic and therapeutic index (TI, efficacy/safety).

We propose to create with the Baric lab a world-class in vivo mouse facility to test therapies to treat both components of COVID-19 disease: 1) the virus-dominated early damage phase; and 2) the late inflammation/repair phase. As relevant, these strategies will be tested for prophylaxis as well. The Baric lab will focus on anti-viral approaches. This component of a mouse therapeutics core focuses on the inflammation/repair components of COVID-19. A combination of paired genetically modified mice and pharmacologic agents assembled from national, UNC, and NC biotech sources will be studied, including: 1) antioxidant anti- inflammatories, including Nerf 2 KO mice and inhaled SOD (from A. Kabanov, SOP);
anti-ER stress, including XBP1s and ERN2 KO mice and Kira-6 (IREX Pharmaceuticals); 3) inflammasome- targeted anti-inflammatories, including IL1R and NLRP3 KO mice and commercially available therapeutics, e.g., anakinra; and 4) mucolytic/pro-repair molecules, including Muc5b cys-ala mice and the thiol-based P2119 agent (Parion Sciences) and anti-sense oligonucleotide/ endosomal disrupters (Initos Sciences).

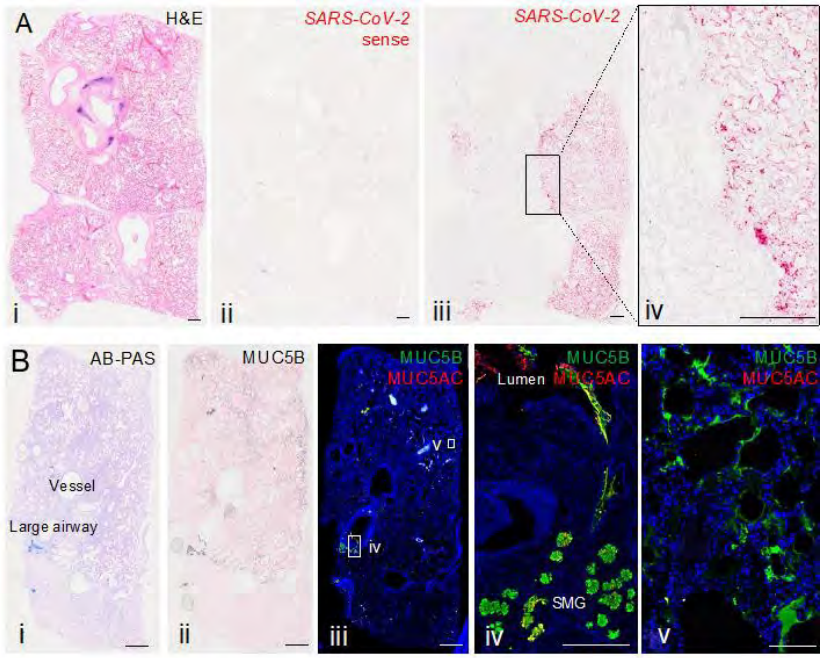

Fig. 1. COVID-19 autopsy lung. (**A**) H&E staining of lung parenchyma with SARS-CoV-2 *in situ* hybridization (RNAscope) localization of virus to focal lung segments. (**B**) AB-PAS carbohydrate stain, and MUC5B IHC (**Bii**) and combined MUC5AC/MUC5B fluorescence stains (**Biii-v**) denoting accumulated mucins in airways and lung parenchyma (Hou, J., et al, *Cell*, 2020, in press).

For viral exposure, a BSL2 platform of influenza (PR8) will be complemented for targets of interest by BSL3 studies of mouse-adapted SARS-CoV-2. World-class near GLP aerosol exposure, mouse imaging (CT, ^{19}F-MRI, ^{129}Xe- MRI), and sophisticated cellular, proteomic, and viral readouts will describe

results. Promising target compounds from the studies, and compounds emerging from Baric screens, will be studied in aerosol/systemic *in vivo* PK studies featuring measurements of drug concentrations in airway surface, epithelial cell, and systemic compartments to accelerate translation to human clinical studies.

Several key novel synergistic features not cited above could be realized upon creation of a pandemic respiratory viral mouse models facility with a focus on SARS-CoV-2. Such synergies include:

- Access to > 20 human COVID-19 autopsy lungs from New Mexico, Oklahoma, and NYC (see **Fig. 1**). The ability to compare and contrast *in vitro* cell culture data, mouse data, and human data is obviously invaluable to vet the preclinical systems.
- Expansion of interests to non-epithelial targets: Clearly the syndrome of COVID-19 is inextricably linked to:
  1) local and systemic immunologic responses; and 2) endothelial/vascular responses. Indeed, virtually every organ of the body is primarily or secondarily affected by the COVID-19 syndrome. The mouse models facility will offer a model system to organ-level investigators, systems biologists, and mathematical modelers.
- Expansion of research activities to better utilize mouse models: Major efforts are underway to generate the pulmonary region-specific cultures from mice that have been so powerful in human studies. In parallel, novel mouse bronchoscopy and optical coherence tomography studies are feasible to better characterize the upper airway mucus plugging in mice that is a feature of human COVID-19 disease.

Note, the budget justifications that accompany the scientific/synergy justifications for the fusion of the Baric/O'Neal/Boucher laboratories in this facility are noted in the proposal budget sections.

IMPACT TO THE STATE (300 Word Limit)

- *Description of the problem or challenge being addressed and how the problem impacts those in the state of North Carolina*
- *Describe how the proposed research will provide impactful solutions to the described problem to help the state of North Carolina*

This project is part of a broad UNC-based effort to accelerate the prevention and treatment of COVID-19 in NC and the rest of world. There are > 750 proposed therapies to prevent/treat COVID-19. Consequently, there is a need to prioritize these therapies based on estimated time to clinic and therapeutic index (TI, efficacy/safety).

We propose to create with the Baric lab a world-class *in vivo* mouse facility to test therapies to treat both components of COVID-19 disease: 1) the virus-dominated early damage phase; and 2) the late inflammation/repair phase. As relevant, these strategies will be tested for prophylaxis as well. The Baric lab will focus on anti-viral approaches. This component of a mouse therapeutics core focuses on the inflammation/repair components of COVID-19. A combination of paired genetically modified mice and pharmacologic agents assembled from national, UNC, and NC biotech sources will be studied, including: 1) antioxidant anti-inflammatories, including Nerf 2 KO mice and inhaled SOD (from A. Kabanov, SOP); 2) anti-ER stress, including XBP1s and ERN2 KO mice and Kira-6 (IREX Pharmaceuticals); 3) inflammasome-targeted anti-inflammatories, including IL1R and NLRP3 KO mice and commercially available therapeutics, *e.g.*, anakinra; and 4) mucolytic/pro-repair molecules, including Muc5b cys-ala mice and the thiol-based P2119 agent (Parion Sciences) and anti-sense oligonucleotide/endosomal disrupters (Initos Sciences). For viral exposure, a BSL2 platform of influenza (PR8) will be complemented for targets of interest by BSL3 studies of mouse-adapted SARS-CoV-2. World-class near GLP aerosol exposure, mouse imaging (CT, ^{19}F-MRI, ^{129}Xe-MRI), and sophisticated cellular, proteomic, and viral readouts will describe results. Promising target compounds from these studies, and compounds emerging from Baric screens, will move into aerosol/systemic *in vivo* PK studies featuring measurements of drug concentrations in airway surface, epithelial cell, and systemic compartments to accelerate translation to human clinical studies. Note, see budget justification for Baric/O'Neal/Boucher laboratory budget synergies.

MILESTONES (300 word limit)
Description of what will be accomplished and what can be delivered by August 31, 2020, and by Dec. 31, 2020. The start date will be June 1, 2020.

August 31, 2020 Milestones

1) Create an Advisory Board to prioritize selection of targets/therapeutic agents: The board will include experts in antiviral therapies, *e.g*., David Margolis, aerosol delivery, *e.g*., Tony Hickey, drug delivery, *e.g*., Rudy Juliano, and clinical investigators, *e.g*., Shannon Carson. The board will interface with leaders in COVID-19 therapeutics nationally/internationally, with UNC researchers, including in Pharmacy (Tropsha, Kabanov) and the Baric lab, and NC biotech companies.

2) Targets/compounds selected for study: 6 combined gene-targeted mouse models/therapeutic agents will be completed.

December 31, 2020 Milestones

1) Create a world-class mouse testing facility, integrated with the Baric lab, to screen/vet candidate therapies for COVID-19 and future pandemic respiratory viral infections, including influenza. The facility will include aerosol exposure capabilities, drug deposition and measurement capabilities utilizing imaging, HPLC, and mass spec approaches, coupled to broad-based mouse lung phenotyping capabilities.

2) Create a testing facility responsive to the NC academic and biotech communities for the rapid development of NC-based therapeutics.

3) Characterize another 6 targets in gene-targeted mice/with therapeutic agents.

4) Advancement of at least one candidate to IND/clinical trial status.

BUDGET JUSTIFICATION (200 word limit)
Funds are limited. We encourage all teams to revisit their budget and determine if it can be reduced.

Note: The O'Neal/Boucher and Baric lab budgets are complementary to synergize/accomplish the program interactive goals.

Total Budget= $781,077

Personnel
$183,920

Dr. Wanda O'Neal (Assoc. Prof. of Medicine, 5% effort) and Dr. Boucher (Prof. of Medicine, 0% effort) will oversee the facility. All co-investigators will assist Core activities at 0% effort. Two research technicians are budgeted for exposure, PK measurements, and mouse phenotyping.

Dr. Baric will oversee two graduate students, research staff and project manager for viral studies.

Supplies
$247,157

To supply required inbred, genotyped mice and supplies for extensive mouse phenotyping ($172,845) and for viral studies ($74,312).

Equipment
$345,000

1) Buxco aerosol twin tower device: The Buxco device is the industry standard for aerosol delivery of test compounds to mice, rats, and hamsters. We have operational aerosol expertise with Gregory Smith and oversight with Tony Hickey. We have optimized measurements of presented doses, *i.e*., to filters, and deposited doses, *i.e*., drug in lungs (microdialysis approaches) and systemic dose. Note, GLP conditions could be achieved with adequate future manpower for FDA documentation.

2) HPLC: A Waters HPLC will measure drugs/agents delivered in high concentrations and/or that fluoresce in the three relevant compartments, *i.e*., airway surface, intracellular, and plasma.

3) Plate reader: The plate reader will measure safety aspects of drug exposure, including toxicity readouts, *i.e*., LDH, HMGB1, and readouts of lung health/function, *e.g*., CCSP, PRR4, SPB.

Tuition
$5,000