PROJECT 1:
THERAPEUTICS I: TRANSITIONING/OPTIMIZING NEW DRUGS FOR PULMONARY DELIVERY

Team
PI: Richard Boucher, MD (SOM)
Co-investigators: Charles Esther, PhD (SOM), Ray Pickles, PhD (SOM), Brian Button, PhD (SOM), Alessandra Livraghi-Butrico, PhD (SOM), Claire Doerschuk, MD (SOM), Matt Wolfgang, PhD (SOM), Alexander Kabanov, PhD (SOP), Gauri Rao, PhD (SOP)

This project is an effort to accelerate drug development for the prevention and treatment of COVID-19 infections. There are > 750 proposed therapies to prevent/treat COVID-19, which emphasizes the need to prioritize therapies based on estimated time to clinic and therapeutic index (TI, efficacy/safety). A unique opportunity exists in pulmonary antiviral therapeutics because of the opportunity to deliver compounds via the aerosol route to improve their TI and overcome systemic delivery issues. As noted in Figures 1 and 2, the aerosol route may be particularly favorable because viral entry/infection is an airway surface event.

Accordingly, this application seeks to generate a world-class human respiratory epithelial testing facility to characterize the pharmacokinetic (PK) and pharmacodynamic (PD) properties of antiviral drugs in respiratory epithelia in vitro delivered by aerosol vs systemic routes. The facility will concentrate initially on two broad classes of compounds that have promise for the near-term treatment of COVID-19 disease. First, it will examine drugs whose activities depend on concentrations at the airway surface, e.g., the protease inhibitor camostat and monoclonal/peptide SARS-CoV-2 binders. Second, it will characterize drugs that act intracellularly, e.g., the nucleoside inhibitors remdesivir and NHC and the anti-malarial hydroxychloroquine. Note, the antiviral activity of intracellular drugs depends on their metabolism and accumulation in intracellular compartments, which are currently poorly characterized in human respiratory epithelia nor is the preferred route known. The PK system employs novel aerosolized delivery of test agents to human pulmonary epithelial cells under direct confocal visualization, combined with imaging/mass spectrometric-based analyses of test agent distribution between airway surface and intracellular compartments. All confocal-based PK studies are coupled to pharmacodynamic (PD) model coronavirus test systems (GFP – NL63, MHV) to provide a drug-screening platform that will identify: 1) optimal compounds for development; and 2) preferred route, i.e., system vs aerosol. These in vitro
studies in Therapeutics I will inform in vivo mouse studies in Therapeutics II.

Although we have emphasized antiviral therapies in our Impact Statement, the proposed facility will be suitable for testing a wide variety of therapeutic agents targeted at airway epithelia. The goals of the facility are to provide data relevant to dosing route (aerosol vs systemic), dose, and dosing frequency of these agents. Classes of therapeutic agents, in addition to the small molecule antiviral agents cited in the Impact Statement, will include:

- Anti-inflammatory agents, e.g., inflammasome-targeted therapies.
- Antioxidant agents – targeted at Nerf2 pathways and/or directly at oxidants, e.g., H₂O₂. Agents could include small molecules (e.g., P2119) or inhaled proteins (e.g., SOD).
- Mucolytics – targeted at mucus that obstructs endotracheal tubes and is the nidus for bacterial infection.
- Anti-biofilm agents: Preliminary data from UNC suggest ~60% of SARS-CoV-2-infected ICU patients culture Pseudomonas aeruginosa from their lower airways. Because P. aeruginosa typically grows as biofilms in static airway mucus, novel biofilm agents require testing.
- Anti-fibrotic agents – ~25% of SARS1-infected subjects exhibited pulmonary fibrosis one year post infection. Hence, novel or clinically approved anti-fibrotic agents, e.g., pirfenadone, require optimal formulation.

Hence, we envision this facility as a broad-based, therapeutics agonist facility to spur drug development for the COVID-19 syndrome.
This project is designed to provide critical human in vitro data to synergize with in vivo (including mouse model) data to accelerate drug development for the prevention and treatment of COVID-19 infections. There are > 750 proposed therapies to prevent/treat COVID-19, which emphasizes the need to prioritize therapies based on estimated time to clinic and therapeutic index (TI, efficacy/safety). A unique opportunity exists in pulmonary antiviral therapeutics because of the opportunity to deliver compounds via the aerosol route to improve their TI and overcome systemic delivery issues.

Accordingly, this application seeks to generate a world-class human respiratory epithelial testing facility to characterize the pharmacokinetic (PK) and pharmacodynamic (PD) properties of antiviral drugs in respiratory epithelia in vitro delivered by aerosol vs systemic approaches. The facility will concentrate initially on two broad classes of compounds that have promise for the near-term treatment of COVID-19 disease. First, it will examine drugs whose activities depend on concentrations at the airway surface, e.g., the protease inhibitor camostat and monoclonal/peptide SARS-CoV-2 binders. Second, it will characterize drugs that act intracellularly, e.g., the nucleoside inhibitors remdesivir and NHC and the anti-malarial hydroxychloroquine. Note, the antiviral activity of intracellular drugs depends on their metabolism and accumulation in intracellular compartments, which are currently poorly characterized in human respiratory epithelia. The PK system employs novel aerosolized delivery of test agents to pulmonary epithelial cells representing all major pulmonary regions under direct confocal visualization, combined with imaging/mass spectrometric-based analyses of test agent distribution between airway surface and intracellular compartments. All confocal-based PK studies are coupled to pharmacodynamic (PD) model coronavirus test systems (GFP – NL63, MHV) to provide a drug-screening platform that will identify: 1) prioritized compounds for development; and 2) preferred route, i.e., system vs aerosol. These in vitro studies will inform in vivo mouse studies in Therapeutics II.
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, in aerosol delivery, e.g., Tony Hickey, in drug delivery, e.g., Rudy Juliano, and clinical "users", e.g., Shannon Carson. The board will interface with leaders in COVID-19 therapeutics nationally/internationally, UNC researchers, including in Pharmacy (Tropsha, Kabanov) and the Baric lab, and NC biotech companies.

2) Targets/compounds selected for study: 10 combined assessments of pulmonary epithelial PK and antiviral activity (PD) will be completed.

**December 31, 2020 Milestones**

1) Create a world-class *in vitro* human pulmonary epithelial PK/PD testing facility to screen/vet candidate therapies for COVID-19 and future pandemic respiratory viral infections, including influenza. The facility will provide epithelial cultures from all pulmonary regions (nasal, bronchial, bronchiolar, and alveolar) coupled to imaging, HPLC, and mass spec approaches for measurement of drug PK coupled to antiviral pharmacodynamic (PD) measurements. As indicated, assays and components can be transferred to READi at the end of the funding period.

2) Create a database that catalogs the therapeutic index (TI) of test compounds and data describing parameters that may guide dosing route (aerosol vs systemic) and dosing intervals.

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

4) Characterize another 10 targets/compounds for PK/PD relationships and calculation of TI.

5) Data from at least 1-2 compounds will be utilized for IND/first-in-man human studies.
BUDGET JUSTIFICATION (200 word limit)

- Funds are limited. We encourage all teams to revisit their budget and determine if it can be reduced.

Total Budget= $607,190

**Personnel**
$83,276

Charles Esther, MD/PhD, Prof. Dept. of Pediatrics (5% effort), and Richard Boucher, MD, Marsico Lung Institute (0% effort), will be Co-Principal Investigators of the Project. All other co-investigators will be at 0% effort.

Three full-time technicians are requested for the project: 1) 2.0 techs will focus on pulmonary epithelial PK studies, including exposure, PK analyte, and imaging analyses; and 2) 1.0 techs will perform viral exposure/test article experiments.

**Supplies**
$113,914

Costs are dominated by cell culture expenses (cell purchases, media) and viral production.

**Equipment**
$410,000

Confocal microscope:
Confocal microscopy is a novel/key component in our *in vitro* human pulmonary epithelial testing system. This system delivers aerosols to culture surfaces at rates that mimic *in vivo* rates to visual deposition online. Confocal visualization is key as many agents have off-target effects on cellular function, *i.e.*, fluid secretion, cilia beat, that can be detected *in vitro* and, hence, avoid the need for live animal toxicology studies. Importantly, a number of the test compounds fluoresce, particularly in the UV wave lengths, so that PK studies can be performed on line, *i.e.*, measures of initial deposition/concentration, cellular uptake, and duration of compound retention in apical vs intracellular compartments.