Abstract:
The pandemic COVID19 caused by the novel Coronavirus (SARS-CoV-2) has created unprecedented global challenges for health care delivery. Resources of health care systems are overwhelmed by the escalating needs for invasive mechanical ventilatory support and critical care provision. In critically ill patients, mortality rates range from 20-60%, with substantial heterogeneity related to age, sex, and comorbid conditions. Our understanding of the biological underpinnings of the clinical heterogeneity of patients with COVID19 is extremely limited, and therefore clinical care for the associated organ failures in the ICU remains largely supportive. Furthermore, nearly all patients with COVID19 in the Intensive Care Unit (ICU) receive empiric broad-spectrum antibiotics for fear of a bacterial respiratory co-infection, which may lead to an antimicrobial resistance crisis from such indiscriminate use of antibiotics. We propose the COronavirus and LUng Microbiome INteractions in Acute Respirator'y illness (CO-LUMINARY) study to address the biological heterogeneity and the need for better diagnostics of co-infection in COVID19. Through integrative studies of lung microbiota and innate immunity with advanced metagenomic assays of the SARS-CoV-2 genome, the lung microbiome and the host-transcriptome, we will be able to define significant predictors of outcome and COVID19 biological endotypes, as well as to uncover the rates of bacterial or fungal co-infection in patients with COVID19. Our research has the potential to allow for targeted therapeutic interventions (e.g. anti-inflammatory drugs tailored to hyperinflammatory COVID19 patients) and for critically needed antimicrobial stewardship during the pandemic.

Progress Report:
1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

Since the receipt of this award in May 2020, I have been actively involved in clinical and translational research in hospitalized patients with COVID-19. I have been leading the recruitment efforts for critically and moderately-ill inpatients with COVID-19 from several units within the UPMC system with collection of clinical data and biospecimens in two prospective cohort studies. To date, we have enrolled 120 critically ill patients from medical and surgical intensive care units, and in 160 moderately ill inpatients from hospital wards. I have been coordinating the efforts around clinical data acquisition, database management, data analysis, as well as biospecimen repository management, and sample sharing with multiple investigators within the University of Pittsburgh. I have conducted a series of translational studies as proposed in the COLUMINARY study design, with extensive analyses of plasma samples with both microbial and host-response molecular assays. This work has led to several publications as outlined below, as well as important preliminary data for upcoming proposals.

There have been pandemic-related delays in the execution of my proposed work, as they relate to the supply chain of important analytes and laboratory supplies for the conduct of experiments. A central piece of the experiments involved the conduct of metagenomic sequencing in respiratory samples; such experiments have been delayed due to a several months-long delay in delivery of the necessary device and supplies for sequencing from Oxford Nanopore Technologies.
2. **If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?**

The remaining funds of my pilot CTSI award will be utilized to complete the Nanopore metagenomics sequencing of nasal/oral swabs as well as lower respiratory tract samples, to systematically profile the respiratory tract microbiome in patients with COVID-19. I will then merge the respiratory microbiome data with my rich data set of blood-based microbiome and host immunology signatures to then systematically analyze and understand the role of the microbiome and secondary infections in COVID-19.

3. **How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?**

My translational work within the scope of the COLUMINARY grant has led to the following findings:

a. Mechanically-ventilated patients with COVID-19 have similar physiologic and radiographic findings compared to patients with non-covid severe pneumonias (prior to 2020), yet COVID-19 patients require longer courses of mechanical ventilation support for reasons that appear to be unrelated to the severity of the illness (perhaps due to different clinical practices during the pandemic).

b. Critically ill patients with COVID-19 have lower levels of systemic inflammatory biomarkers compared to patients with bacterial pneumonia, but similar levels to other types of viral pneumonia observed prior to 2020.

c. Higher amounts of circulating microbial cell-free DNA in the bloodstream of patients with COVID-19 are predictive of mortality and may reflect the harmful impact of secondary infections in patients with covid 19. Importantly, our retrospective analysis indicated that some of these secondary infections were unrecognized at the time of clinical care.

d. Plasma metagenomic sequencing can reveal in non-invasive ways both secondary pneumonias as well as other deep-seated infections in any patient with COVID-19.

e. Critically ill patients with COVID-19 have much higher levels of inflammatory biomarkers compared to moderately ill inpatients, making the former group most likely to benefit from immunomodulatory therapies. The work conducted thus far has offered us important lessons with implications beyond COVID-19. Utilizing the framework applied in these studies, we will further investigate the ability to improve our understanding of different subphenotypes of critical illness syndromes as well as to develop faster and sensitive diagnostic tools for secondary infections in the ICU.

4. **Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.**

Work conducted in the context of this award just lead to the following publications (1 first author, 1 co-first author, 1 senior author, 2 co-author):


I also have multiple manuscripts in different stages of development, including two in revision stage, one under submission, and several in development with regards to the translational work conducted as part of my funded work in COVID-19.

I am also the senior author of two abstracts presented at the American thoracic society 2021 meeting on May 16, 2021. Of note, the abstract to be presented by Dr. Drohan has received an ATS scholarship award.


I am also going to utilize the preliminary data generated from the COLUMINARY study for an upcoming R03 NIH proposal, which will be followed by an independent investigator R01-level proposal in the next six-nine months.

I am deeply thankful to the Pitt CTSI and the David Scaife Family (DSF) Charitable Foundation for their generous support of my research in COVID-19.
Abstract:

The novel coronavirus, previously dubbed 2019-nCoV, and now officially named SARS-CoV-2 which caused the coronavirus disease (COVID-19) pandemic outbreak and was first detected in Wuhan, China in December 2019. Safe vaccines that rapidly induce long-lasting virus-specific immune responses are urgently needed. As an initial attempt to develop a SARS-CoV-2 vaccine, we constructed two subunit vaccines, a monomeric S1 (rSARS-CoV-2-S1) and a trimeric S1 (rSARSCoV-2-S1fRS09) and recombinant adenoviral vectors encoding the S1 (Ad.SARS-CoV-2-S1). The safety profile and growth characteristics of these vaccine platforms make them suitable rSARS-CoV-2 vaccine candidates for preclinical testing. One key aspect of validating candidate vaccines in vivo is the establishment of an appropriate challenge animal model. However, this is not a straightforward task for Sars-CoV-2 coronavirus, since it uses the human ACE2 receptor to infect the host which is not conserved in mice. While a mouse exists that expresses hACE2, its expression levels and locations are not physiologic, making it a poor model for vaccine and therapeutic drug testing. Here we propose to generate a mouse that expresses human ACE2 in proper locations and amounts. We will use CRISPR/Cas9 technology to knock-in a cassette just at the mouse Ace2 start codon, using a plasmid targeting vector as template DNA for homologous recombination. These transgenic mice will be immunized with the different vaccines and challenged with wt SARS-CoV-2 virus and pathogenicity and clinical signs observed. In future work they can be used for in vivo drug testing; they could be licensed out to many companies for this purpose.

Progress Report:

1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

During the first round of funding, we generated human Ace2 knock-in mice by replacing the endogenous gene. We had 6 independent founders and bred the mice to a homozygous/ hemizygous state. We established a stable breeding colony without the need for genotyping litters. We verified these mice by sequencing and performed immunofluorescent microscopy to test for human Ace2 protein expression. We undertook initial experiments in the regional biocontainment facility under biosafety level (BSL) 3 conditions in which Ace2 heterozygous and homozygous mice were infected with SARS-CoV-2 intratracheally. We are still in the process of analyzing the whole dataset (sera, tissue preservation, flow cytometry, swaps for viral qRT-PCR) but could confirm SARS-CoV-2 Spike 1 protein-specific germinal center reaction in cervical lymph nodes by multicolor flow cytometry. To this end, we generated SARS-CoV-2 Spike 1 protein and SARS-CoV-2 Spike 1 receptor-binding domain (RBD) fluorescent tetramers to identify the specific germinal center and memory B cells in these mice. We recently published our adenovirus-based SARS-CoV-2 vaccine and established all experimental techniques which we are proposing to use in our human Ace2 knock-in mice. We bred up 2 cohorts of human Ace2 knock-in mice to be transferred to the BSL3 facility to start testing our SARS-CoV-2 vaccine in this animal in a real infection setup. In
summary, during the initial funding period we generated, confirmed, and bred a stable colony of human Ace2 knock-in mice, established all required experimental techniques, performed initial experiments, and set the stage to successfully carry out all proposed experimental procedures.

2. *If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?*

The funding will be used to maintain the breading of the TG Ace2 knock-in mice colony and perform immunization/challenge studies in the BSL3 facility.

3. *How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?*

A key aspect of validating candidate vaccines in vivo is the establishment of an appropriate challenge animal model. Sars-CoV-2 coronavirus uses the human ACE2 receptor to infect the host which is not conserved in mice. Although a mouse existed that expresses hACE2, its expression levels and locations are not physiologic, making it a poor model for vaccine and therapeutic drug testing. What we have generated here is a mouse that expresses human ACE2 in proper locations and amounts. We believe that this animal model will have an impact to test novel vaccines and therapeutics against SARS-CoV2 and more broadly to all the viruses that use hACE2 as an entry receptor.

4. *Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.*

Some of the work related to this project led to a publication co-authored by Drs. Gambotto, Falo, and Shlomchik in which CTSI and DSF Charitable Foundation funding source was acknowledged.

Abstract:
There are rapidly emerging reports of neurological symptoms associated with COVID 19 infection ranging from loss of smell/taste to acute encephalopathy, stroke-like symptoms, coma, seizures, brainstem dysfunction, and necrotizing encephalopathy. SARS-CoV 2 shares significant homology with SARS-CoV, which is neuroinvasive especially to the brainstem. If indeed some COVID 19 patients develop serious neurologic manifestations, it is of utmost importance that we capture and characterize this phenomenon, determine its prevalence, risk factors, and impact on COVID 19 patient outcomes. We rapidly assembled a multi-center, international research consortium to study this emerging phenomenon by leveraging an existing large research network through the Neurocritical Care Society. We have designed a PRAGMATIC study that is feasible to conduct in the current extremely restricted circumstances in the inpatient and ICU areas. We developed COMMON DATA ELEMENTS (CDEs) to capture the most important neurological features of COVID 19 with PRAGMATIC DESIGN such that all data elements can be captured remotely through electronic health record +/- telephone call to the clinical team in COVID 19 clinical areas. Given some sites may be overwhelmed due to COVID 19 crisis, we further divided the CDEs into core (basic, extremely pragmatic, all sites collect this data) and supplemental (optional additional data-capable sites collect this). From these CDEs, we developed simple data collection tools and IRB templates to share with sites to facilitate site initiation ASAP. We have over 50 sites registered from 14 countries and all continents except Australia.

Progress Report:
1. **What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?**

We have accomplished a lot with the award. This award supports our work as the central coordinating center for the GCS-NeuroCOVID consortium, for which I serve as the lead PI. **To date, our consortium has over 133 registered adult study sites, over 109 registered pediatrics study sites, spanning all populated continents across the globe (except Antarctica).** We have launched 2 studies in the adult population (MANIFESTATIONS and OUTCOMES) one study in the pediatrics population (please see links below).

   - [https://www.neurocriticalcare.org/research/covid-19-research-opportunities](https://www.neurocriticalcare.org/research/covid-19-research-opportunities)
   - [https://clinicaltrials.gov/ct2/show/NCT04496128](https://clinicaltrials.gov/ct2/show/NCT04496128)
   - [https://clinicaltrials.gov/ct2/show/NCT04418609](https://clinicaltrials.gov/ct2/show/NCT04418609)

We have formed formal collaborative alliances with the European Academy of Neurology for a joint consortium study with their ENERGY cohort, as well as formal collaborations with research consortia in South America through the Latin American Brain Injury Consortium (LABIC) and the POSSIBLE Network. We have published several papers, including the large paper from our MANIFESTATIONS study (see below).

We currently have over 12,000 patients' data submitted for the MANIFESTATIONS study from over 30 sites, and over 3,000 patients' data submitted for the OUTCOMES study from over 16 sites. We are actively working on data verification and will soon proceed to data analysis, with multiple future manuscripts planned. We are also actively preparing to launch the next phase of the GCS-NeuroCOVID consortium study to focus on the subacute and long-term outcomes of this study population.
We have submitted 2 funding applications - one as an investigator-initiated multicenter R01 and one as a consortium study in response to an NIH ROA.

2. **If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?**

We are carefully using the remaining funds to support data analysis from two major studies of the GCS-NeuroCOVID Consortium as discussed under question # 1. Funds support our ongoing effort on data verification in over 15,000 patients data from over 30 global sites, and will then support data analysis efforts and subsequent manuscripts and publications. Funds also support our effort as the central coordinating center for the GCS-NeuroCOVID Consortium.

3. **How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?**

We are currently the largest global consortium studying the neurological impacts of COVID-19, a collaboration that now includes more than 200 medical centers in more than 30 countries. Our newly released manuscript provides the largest cohort study to date on neurological manifestations in acutely hospitalized COVID-19 patients. Several important contributions from our work include: 1) design and implement one of the largest global cohort studies on neurological impacts of COVID-19, 2) develop and harmonize common data elements in neurological impacts of COVID-19 across several large collaborative networks and consortium, including translation of CRFs and case definitions into multiple languages, and 3) contribute to the generation of global guidelines including the WHO COVID-19 guidance.

4. **Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.**


*More than 70 outlets picked up the story, including locally in print, radio, and TV (Pittsburgh Trib, WESA, and WPXI), nationally (US News and World Report, Forbes, and others), and internationally (El Financiero in Mexico, Il Giornale in Italy, India Times and MSN India, and more).*


Abstract:

The scale and transmissibility characteristics of the COVID-19 pandemic present a number of important challenges to the healthcare provider, including personal risk of infection and both material and human resource austerity. Direct physical assessment of a suspected COVID-19 case places the healthcare provider in immediate proximity to the patient, requiring personal protective equipment that may be unavailable in peak pandemic conditions and may delay patient vital sign assessment and downstream treatment, placing the patient at risk for cardiorespiratory deterioration. Our group seeks to address the unique synergistic dilemma of these collective concerns through the development of a smartphone-deployed physiologic monitoring tool (COVID-Insight) tailored to COVID-19 that is based on an existing video image analysis system, Mobile CV Insight™ (mCVI™). mCVI™ uses continuous signal analysis of multi-channel video image data, available ubiquitously in modern digital cameras, including those in smartphones, to derive the pulse rate (PR), respiratory rate (RR), and oxygen saturation (SpO2) with minimal lag from 1.5 to 2 meters away. We propose a rapid observational clinical study to compare mCVI™ PR, RR and SpO2 data with simultaneously recorded pulse oximeter-derived values (Masimo radical 7) in 60 symptomatic hypoxemic patients being screened for COVID-19 in our UPMC Presbyterian Emergency Department. Data recorded from both mCVI™ and Masimo™ oximeters will be extracted, cleaned and analyzed to calibrate, validate and refine the mCVI algorithms tailored to COVID-19 for the COVID-Insight tool. We will then use COVID-Insight readouts to develop therapeutic and triage protocols.

Progress Report:

1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

The main thrust of our study is to refine existing video-based vital sign measurement technology to accommodate hypoxemia associated with COVID-19 and other conditions using data from patients recruited from UPMC Presbyterian Emergency Department. During the period of the award, our team began by successfully setting up the computational research infrastructure for the study. This included building custom software for preprocessing, analysis and de-identification of the video and standard clinical monitor data streams obtained from each patient. After IRB approval of our clinical research plan, we then worked with the MACRO service at Pitt to train and deploy our study data collectors, who then collected data from 60 patients in the period from August to December 2020. In total, more than 3 hours of accumulated video data and concurrent physiologic monitoring data were collected, using two different high fidelity cameras, with most patients tolerating 2 to 5 minutes of video monitoring. Data were extracted from native formats and processed by our study team, including Dr. Salcido and student researcher Connor Willson, deriving plethysmographic waveforms from the primary video streams. Simultaneously, data bundles were prepared, including isolation of regions of interest and patient de-identification, for sharing with our industry partner for algorithm refinement. The pandemic did create supply chain, organizational/logistical, and personal delays, and created difficulties in data collection refinement during the early stages of the clinical phase.
2. *If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?*

Recently, we received IRB approval to recruit an additional 40 patients, who will be recruited with additional controls for heterogeneity of lighting, motion, and camera orientation. These additional patients will help create an even more robust signal analysis algorithm in our final deliverable. An additional student will also be trained to work with this data, netting a positive impact for trainees in our department.

3. *How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?*

Our project has moved forward the understanding of real-world conditions in the clinical environment where video-based vital signs assessment will be deployed for the current or future pandemics, or analogous circumstances of resource austerity. Future assumptions about stable and consistent environmental conditions and patient motion under observation with this technology will be greatly informed by our findings. We have also expanded the applications of this technology with concurrent projects for the Department of Defense, where the technology may be useful for battlefield injury monitoring. Additional utility for patients progressing to cardiac arrest during opioid overdose is being explored.

4. *Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.*

The study team is currently preparing a manuscript describing the levels and impacts of environmental conditions on the performance of the technology. Also, an invention disclosure is in progress for an application of the technology for performing vital signs assessment in children with neurodevelopmental disorders, including autism.
Abstract:

COVID-19 infection results in a spectrum of clinical presentations ranging from asymptomatic individuals to respiratory failure and death. The more severe forms of the disease have been associated with age and the presence of other diseases. However, the mechanisms of protection and immune pathogenesis are not understood. Cross-reactive antibodies against endemic coronavirus (229E, HKU1, NL63, and OC43) may have an effect on infection and presentation of the disease. Planning and evaluation of mitigation strategies have been very difficult due to limited information necessary for the computation models. Our main hypothesis is that host genetic background and environmental exposures to endemic coronaviruses are important factors determining virus transmission rates and clinical outcomes. The overarching goal of this pilot project is to develop critical tools to study the correlations among the host genetics background, immune responses, and clinical outcomes. The specific tasks involved in this project are 1) To develop a cohort of adult COVID19 cases based on hospitalized UPMC patients and electronic medical records; 2) To develop serological panels for COVID19 assay development, evaluation, and validation; 3) To develop and evaluate quantitative IgA, IgG3 and total IgG assays to detect exposure to COVID19 and endemic coronaviruses for use in clinical diagnostics, epidemiological studies and vaccine efficacy evaluations; 4) To determine the plasma levels of cytokines and soluble complement factors; 5) To genotype COVID-19 patients for 4,627 variants in 1,191 genes; 6) To investigate correlations between humoral immune responses (antibodies, cytokines, and complement factors) with host genetic background and clinical outcomes.

Progress Report:

1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

We have developed serological assays for S, N, S1 and RBD antigens for SARS-CoV-2 and assays for S, and N antigens from 229E, OC43, HUK1, NL63. The assays for IgG, IgA are working superbly.

We tested 54 samples from patients with severe and moderate disease for 12 complement factors and adipokines. We also tested 124 samples from a pediatrics cohort from Sara Weller for all 4 C.C.C. Data analysis is ongoing.

We found that neutralization titers and anti-S antibody titers do not correlate with reduction of viral RNA in the blood. Rapid antibody rising and higher titers are associated with severe cases. BMI is correlated with C3a levels and severe disease indicating that there is higher complement activity in obese population. Resistin and Leptin levels correlated with BMI. High IgA titers and (IgG/IgA ratios) are correlated with lower C3a levels and less severe disease.

We could also successfully infect primary human adipocytes with SARS-CoV-2 in vitro.
The goals of our project and accomplishments are as follows:

- We developed a cohort of adult COVID19 cases based on hospitalized UPMC patients and electronic medical records. We received access to 24 ICU patient samples that included clinical covariates, treatment information, and COVID-19 outcomes.

- We developed serological panels for COVID19 assay development, evaluation and validation.

- We developed and evaluated quantitative IgA, IgG3 and total IgG assays to detect exposure to COVID19 and endemic coronaviruses for use in clinical diagnostics, epidemiological studies and vaccine efficacy evaluations.

- We determined the plasma levels of cytokines and soluble complement factors. Some cytokine analysis remains to be measured but complement analyses were performed. Our research discovered that proinflammatory cytokines secreted by fat cells or adipocytes were elevated in COVID-19 patients.

- We investigated correlations between humoral immune responses (antibodies, cytokines, and complement factors) with host genetic background and clinical outcomes.

This is a work in progress, but initial analysis found that many of the Pittsburgh patient cohorts were obese and had elevated levels of proinflammatory cytokines secreted by adipocytes. Based on our findings, we began investigating the role of adipocytes on COVID-19. Here, we have a major additional discovery. We are the first group to demonstrate that human adipose tissue can be infected by the SARS-CoV-2 virus. This led us to submit an R01 application further investigating our discovery (see below for more details).

The pandemic did indeed affect productivity. Our research staff was unable to perform any on-site research from the date we first received funding to about the first week in July 2020. Even after restrictions were eased, our staff had limited access to the lab and the scheduling of experiments was at times delayed consequently.

2. If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?

The goals we would like to achieve with our remaining funds are to complete all analyte measurements that were proposed by the study and complete the computational analyses described in our application, as mentioned above. Our future aims are to publish our discoveries and continue to pursue our interest in investigating the role of obesity on COVID-19 severity.

3. How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?

Our research has impacted the COVID-19 pandemic by beginning to elucidate the interaction between obesity and COVID-19 severity. On a broader scale, we aim to elucidate how adipocytes or fat cells contribute to SARS-CoV-2 infection severity and other viral infections.
4. *Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.*

We are currently finalizing our research and writing two manuscripts, one focused on antibody responses and another focused on the role of adipokines.

We have submitted an R01 application titled “Pathogenic role of adipocytes during SARS-CoV-2 infections“ (1R01AI165623-01) that was made possible through the generous funding and resources we received through this award.
Abstract:

The impact of maternal SARS-CoV2 infection on the developing fetus/newborn is largely unknown. This pilot prospective cohort will investigate vertical transmission of SARS-CoV2 to infants born to infected mothers and its impact on infant growth and neurodevelopment during the first six months of life. Specifically, Aim 1 will assess occurrence of vertical SARS-CoV2 transmission by examining infant specimens for virus and virus-specific antibodies (Ab). Aim 2 will focus exclusively on maternal breastmilk, examining breastmilk for virus and virus-specific Ab. Aim 3 will characterize longitudinal growth and neurodevelopment of exposed infants using validated assessments and compare to population means. In Aim 4, we will preserve collected specimens from this unique cohort in a biorepository for future use. To achieve these aims, we will recruit 20 pregnant women within UPMC who test positive for SARS-CoV2 at some point during pregnancy and collect new or residual specimens including blood, nasal swabs, placenta/placenta swabs, and breastmilk at enrollment, delivery, two and six months postpartum. Medical history, cord blood/blood, stool/anal swabs, and nasal swabs will be collected on infants at birth, two and six months. We will assess infant growth using Fenton Growth Curves and neurodevelopment using Bayley Scales of Infant Development and the Ages and Stages Questionnaire at two and six months. Our team of assembled experts in immunology, virology, and clinical and translational research ensures the rigor of this research, for which, there is a critical and immediate need to inform the clinical care of pregnant women and their newborns during this pandemic.

Progress Report:

1. **What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?**

Since receiving the award, we have completed recruitment for this study screening a total of 67 pregnant women with diagnosed SARS-CoV2 infection during pregnancy and enrolling 26. We have also completed all 26 telephone interviews, 24 delivery visits, 16 two-month and four 6-month follow-up visits. We have collected 90 blood, 70 nasal, 22 placenta, 30 breastmilk and 35 stool specimens from consented mothers and infants. We have also started specimen analysis including evaluation of SARS-CoV2 antibodies in mother and infant blood, and SARS-CoV2 PCR analysis of mother and infant nasal swabs and blood.

2. **If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?**

With our remaining funds, we will continue the longitudinal follow-up of these mothers and infants over the next several months completing their 2 and 6 month follow-up appointments, where we collect specimens from mother and infant as well as obtain anthropometric data and complete two neurodevelopmental evaluations of the child. We will also complete specimen analysis, which includes finishing SARS-CoV2 antibody analysis of
blood and breastfeeding specimens, SARS-CoV2 PCR of remaining nasal swabs, blood, breastfeeding, stool and placenta and complete immunofluorescence staining and analysis of placenta specimens.

3. **How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?**

The results of our work, though preliminary at this time, provide reassurance against the risks of vertical transmission from SARS-CoV2 to the infant with data thus far showing no evidence of viral transmission (negative infant nasal swabs at birth and absent IgM response in blood) and reassuring neurodevelopmental outcomes of children exposed in utero. This is in-line with similar cohort studies showing an overall low risk of vertical transmission of SARS-CoV2. There have been no other studies reporting on longer-term neurodevelopmental outcomes of infants exposed in utero, which will be a novel finding our study will contribute. Importantly, we have seen several adverse birth events among our cohort including evidence of microcephaly and preterm birth resultant from placenta abruption during maternal SARS-CoV2 infection. While this is a small cohort and these findings will need further investigation, this could suggest that exposure in utero may affect the fetus indirectly. Potential mechanism including placenta inflammation or insufficiency or perhaps vertical transmission that has not yet been identified (additional PCR testing of other samples has not yet been completed). These data have significant implications for the COVID-19 pandemic and the delivery of SARS-CoV2 vaccines to pregnant women. While risk of vertical transmission is low, the potential increased risk for maternal and perinatal complications resultant from a SARS-CoV2 infection during pregnancy reinforces the importance of pregnant women being prioritized and targeted for vaccination through national vaccine campaign efforts and for future research.

4. **Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.**

**Presentations**

Rick AM. “Vertical Transmission, Passive Immunity, and Neurodevelopmental Outcomes for Infants Exposed to SARS-CoV2 in utero: Preliminary Data from a Prospective Cohort Study”. Pediatric Discovery Day, Children’s Hospital of Pittsburgh, April 2021.

**Pending Funding**

CTSI COVID-19 Disparities Pilot Program (Pitt) Rick (PI) 05/2021-04/2022

SARS-CoV2 Vaccination in Pregnant and Lactating Women: Characterizing Immunogenicity, Passive Protective Immunity and Exploring Vaccine Attitudes

Role: Principal Investigator

Infectious Diseases Clinical Research Consortium (DMID) Munoz/Beigi (Co-PIs) 07/2021-10/2023

Observational, Prospective Cohort Study of the Immunogenicity and Safety of SARS-CoV-2 Vaccines Administered during Pregnancy or Postpartum and Evaluation of Antibody Transfer and Durability in Infants

Role: Co-Investigator
Abstract:
Currently, the world is facing a burgeoning pandemic due to Coronavirus disease-19 (COVID-19) caused by SARS-CoV-2. There are no effective treatments or prophylactics against COVID-19. SARS-CoV-2 transmission occurs predominantly through oral and nasal routes leading to high viral replication in the oropharynx and nasopharynx. Therefore, we hypothesize that prophylactic use of antivirals against SARS-CoV-2 via a nasal spray can lead to virus inactivation at the sites of viral entry and replication. This proposal aims to deploy Q-griffithsin (Q-GRFT), a lectin obtained from Nicotiana benthamiana plants that targets mannose residues on the surface of viral glycoproteins with nanomolar affinity and neutralizes a broad spectrum of coronaviruses. Studies in human bronchial airway epithelium cultures (EpiAirwayTM – AIR-100) confirm that QGRFT inhibits SARS-CoV-2 infection. Furthermore, intranasal GRFT has shown protection against SARS and MERS in animal models. This work will focus on developing a nasal spray for Q-GRFT delivery to the nasopharynx and oropharynx regions. Studies including drug-excipient compatibility, stability, and toxicity, and permeability in epithelial cultures will be used to optimize the nasal product. Ergonomic studies with potential users will be used to obtain actuation parameters impacting spray characteristics during use. This critical and timely work is highly translational and positioned for success due to already available IND-enabling studies for Q-GRFT. Given Q-GRFTs’ broad-spectrum activity against coronaviruses, it has potential to thwart future infections. The ultimate goal is to advance the product to the clinic within this year. This product can be highly applicable to frontline healthcare workers and at-risk populations.

Progress Report:
1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

Since receiving the award, our team has made considerable progress. The intranasal spray formulation has been characterized for various attributes including pH, Osmolality, drug content, spray characteristics, and toxicity. The nasal dosage form has met the specifications and found to be non-toxic in an EpiAirway™ tissue model. Spray characteristics of a nasal spray are important attributes for consistent and uniform delivery of the nasal solution to the targeted areas to achieve high protection against SAR-CoV-2 infection. Methods for spray characteristics, namely, droplet size distribution, plume geometry, and spray pattern are being developed. We have confirmed that the nasal spray distributes product to the targeted nasal regions (e.g. nasopharynx) using a nasal cast model. The initiation of spray characterization has been delayed due to contractual delays, however, we have been on track to complete this work within the requested extension period.

2. If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?

Our request for extension has been granted to complete the spray characterization work which was delayed due to an administrative delay in contract establishment with our identified vendor. We will be completing the spray
characterization methods work within the requested extension. The next steps associated with advancing this product is filing of the IND and advancement of the product to the clinic. This will be accomplished through support from NCATS, PA DCED, & R.K. Mellon Foundation for IND enabling studies and funding from the NCI for the Phase 1 clinical trial.

3. **How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?**

The Q-GRFT nasal spray is being developed as a preventative against COVID-19. This work is an important component of our goal to advance the Q-GRFT nasal spray product through IND-enabling studies and clinical studies funded by other grant mechanisms. Despite vaccine rollout, we believe that this product will play an important role in protection of those who cannot mount an adequate response to a vaccine or those who are not able to be vaccinated for any reason. Further, the impact of this work has potential beyond COVID-19. Given the subnanomolar activity of Q-GRFT against SARS-CoV, MERS-CoV, and possibly future strains of coronaviruses or resistant strains of SARS-CoV-2, the Q-GRFT nasal spray could protect against future pandemics or endemic respiratory viruses. Additionally, this work will support us in future applications focused on developing nasal dosage forms against other local, regional, and systemic diseases.

4. **Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.**

We plan to present the data generated from this work at scientific conferences and publish in peer-reviewed journals.
Abstract:
The COVID-19 pandemic has stretched healthcare resources in many parts of the world to the breaking point, and we are observing capacity constraints in the US. Uncertainty remains regarding the time course of the epidemic, the risk factors that predict poor outcomes, and the optimal combination of strategies for mitigation. International experiences vary tremendously, from the near-disaster in northern Italy to less aggressive outbreaks in Singapore and South Korea. We have incorporated a representation of COVID-19 into the Framework for Reconstructing Epidemic Dynamics (FRED) an agent-based modeling tool that represents the entire US population and their daily activities. We have been testing possible mitigation strategies such as closing schools and increased social distancing, but in order to allow us to evaluate more specific containment strategies we need to add several components to FRED that are not currently part of the tool. The purpose of this COVID-19 pilot grant is to extend the capabilities of FRED to develop better tools for policy makers to use to inform COVID-19 decision making. We will accomplish this in the following specific aims: Aim 1. Incorporate individual and neighborhood perception of risks and impact on individual behavior. Health behaviors such as self-isolation can depend on self-perceptions of susceptibility, severity, potential benefits, barriers, and other factors. These can be modified based on trust of governmental authority and on peer network attitudes which differ by race, culture, and socioeconomics. These can be explicitly represented in behavior change probability modeling within each agent in FRED. Aim 2. Incorporate data-driven seasonal forcing into the infectivity of the virus. The seasonal variation in infectivity observed in influenza and the endemic coronaviruses, which may substantially change the epidemic curve in the summer months Aim 3. Incorporate and evaluate a series of potential mitigation, social distancing relaxation, and optimal resource allocation strategies. The addition of increased model detail will allow a series of mitigation and social distancing relation strategies to be evaluated.

Progress Report:
1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

The Pandemic did not produce delays in the work, but the constantly changing landscape of the pandemic altered the importance of some of the aims of the project. As vaccination became more widespread, the issues related to vaccine priority receded in importance, and issues of vaccine hesitancy and the ability to relax mitigation strategies became more important.

Aim 1. Incorporate individual and neighborhood perception of risks and impact on individual behavior. Health behaviors such as self-isolation can depend on self-perceptions of susceptibility, severity, potential benefits, barriers, and other factors. These can be modified based on trust of governmental authority and on peer network attitudes which differ by race, culture, and socioeconomics. These can be explicitly represented in behavior change probability modeling within each agent in FRED.

Outcomes for Aim 1: Dr. Burke and his epidemiology graduate student advisee Kavya Hiryur worked on the development of an Agent-Based Computational Model of COVID-19 Vaccine Hesitancy. A number of factors,
including demographic and geographic characteristics, affect an individual’s likelihood to accept a vaccine. We built an agent-based model that can be applied to simulate interactions and behavior change over time.

**Aim 2. Incorporate data-driven seasonal forcing into the infectivity of the virus.** The seasonal variation in infectivity observed in influenza and the endemic coronaviruses, which may substantially change the epidemic curve in the summer months.

Outcomes for Aim 2: We successfully incorporated seasonal forcing into the model. In figure 1, the panel on the right illustrates the difference in the basic COVID model with and without seasonal forcing, and the panel on the right demonstrates that the model (with seasonal forcing) is able to replicate the actual death rates in Allegheny county.

**Aim 3. Incorporate and evaluate a series of potential mitigation, social distancing relaxation, and optimal resource allocation strategies.** The addition of increased model detail will allow a series of mitigation and social distancing relation strategies to be evaluated.

Outcomes for Aim 3: We have compared multiple strategies to relaxation of mitigation strategies. For example, in a proposal to the Governor’s office (funding pending) we have demonstrated the potential impact of reducing the intensity of social distancing in PA.
2. If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?

We have used the CTSI funding to enhance the FRED COVID-19 modeling tool and have used that to secure CDC funding and work with the State and the University to evaluate various mitigation strategies. We will continue ongoing COVID modeling work as part of the CDC Influenza Modeling Groups, which have been provided COVID-19 supplements. Next steps include:

- Incorporating multiple strains of COVID-19 with different infectiousness
- Enhancing the immunology model to use accumulating data on the waning of immunity from both COVID-19 infection and the various vaccination types.
- Enhance the understanding of spread in children to better model the reopening of schools in the fall

3. How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?

We built a conceptual model representing COVID-19 vaccine hesitancy that allows stakeholders and policymakers to understand and evaluate the best methods to combat vaccine hesitancy in the population.

We enhanced our modeling tool to project potential COVID-19 deaths and demonstrate the potential impact of reducing the intensity of social distancing in PA.

4. Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.

The most important outcome that we can report is that the CTSI funding allowed us to apply for and receive a supplement ($375,000) to our CDC-funded Pitt Influenza Modeling Center to expand the modeling center's work into COVID-19. That supplement (a second $375,000) has been renewed for the Influenza parent grant.
Abstract:
Mortality associated with the Covid-19 outbreak is driven by pneumonia and ARDS for which no specific treatment is available. The host response against SARS-CoV-2 infection is characterized by hyperproduction of cytokines. This cytokine storm, promotes lung inflammation and correlates with mortality. In the absence of preventive vaccines, anti-inflammatory strategies are required to treat Covid-19-ARDS. MSCs exhibit potent anti-inflammatory effects that limit acute lung injury. In multicenter clinical trials (START), we demonstrated the safety of MSCs in severe ARDS. MSC showed promise in ARDS treatment during the Wuhan pandemic. FDA granted fast track designation to MSCs for the treatment of ARDS. However, the need for specialized infrastructure, length of time, and high cost required for MSC procurement prevent their rapid deployment during pandemic illnesses. MSCs ameliorate lung injury via secretion of paracrine factors: proteins, and extracellular vesicles. MSCs use extracellular vesicles to inhibit macrophage activation and limit the release of cytokines. In contrast to cells, MSC-derived extracellular vesicles can be manufactured as a shelf-stable product ready for deployment for the treatment of Covid-19-ARDS. In response to Pitt CTSI COVID-19 Grant Program, we propose the following Aims: 1) To determine, in vitro, the efficacy of MSC-derived extracellular vesicles to suppress the secretion of INFγ, TNF, and IL6 by mononuclear cells from subjects afflicted by Covid-19 ARDS; 2) To procure IND enabling animal data to conduct a phase I clinical trial of MSC-derived extracellular vesicles in Covid-19 ARDS; 2) To scale production and determine the global distribution of MSC-derived extracellular vesicles.

Progress Report:
1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

The idea behind the grant was to level further support from federal agencies as we were keenly aware that the cost for such our project exceeded the available funds. To that effect, we wrote several administrative supplements to NIH institutes. However, these initiatives were not successful and we proceeded to explore a more translational avenue to assess the value of MSCs in subjects affected by COVID19 associated ARDS. Here progress was steady and we secured, in collaboration with the University of Minnesota, an FDA IND to treat these patients in a randomized (2:1) placebo control trial. Subsequently, in January 2021 we requested the CTSI authorization to change the use of the funds to support the clinical enterprise. A positive response to the
request was granted by CTSI and we requested formal IRB permission to treat patients here at the University of Pittsburgh.

2. If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?

Subsequently, we initiated recruitment of subjects into the study: 9 subjects were consented and 8 fully treated at the University of MN. Of these patients, 2 withdrew shortly after initiation of the protocol, 2 died during the study, and the rest completed the protocol. After separation from mechanical ventilation and discharge from the hospital, 2 additional patients declined the outpatient clinical follow-up. Although the DSMB did not identify any infusion-related adverse events, we have not been able to secure solid funding (DOD gave our grant a score of 2 but provided no funding) to cover the cost associated with this study. Consequently, patient recruitment has been placed on permanent hold and we have requested the DSMB permission to finish the study and unblind patients so that we can understand the impact of treatment on these subjects.

3. How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?

At this junction, we learned that the acute events associated with the COVID-19 respiratory distress have been modified by some of the therapeutic interventions such as high dose of dexamethasone, the introduction of IL 6 receptor antagonists, remdesivir, and use of monoclonal antibodies is helpful. With regards to the use of mesenchymal stem cells, we had a press release announcement by Novartis indicating that their study on over 600 subjects will not meet the primary endpoint. However, this endpoint was not realistic as it was expected to reduce ARDS mortality by 40% Our current data, based on the experience accumulated on these 9 subjects is that all of them experience serious chronic sequelae as they sustain long-term abnormalities in their pulmonary function testing, renal, and neurocognitive function.

4. Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.

Not at this time.
Abstract:

The emergence of SARS-CoV-2 infection from Wuhan China at the end of 2019 and its subsequent global spread has led to an unprecedented collaborative effort among scientists to develop effective treatment and prevention. Among the strategies under investigation are both active (vaccines) and passive immunization (convalescent serum and/or neutralizing monoclonal antibodies). Both strategies rely, at least partly, on the ability of anti-SARS-CoV-2 antibodies to bind surface-exposed viral proteins and block viral entry into cells. A critical unknown is the capacity of SARS-CoV-2 to escape immune recognition through genetic variation. Immune escape variants could thwart efforts to develop effective immune-based interventions for treatment and prevention. In this project, we will focus on whether SARS-CoV-2 variants can escape antibody recognition. Since the key viral target for antibody neutralization is the receptor-binding domain (RBD) of the exterior viral spike protein (S) that binds to the receptor ACE2 on the host cell surface prior to viral entry, the most likely mechanism of antibody escape is genetic variation in the RBD. Genetic variation in RBDs of SARS, MERS, and SARS-CoV-2 prevents neutralization of SARS-CoV-2 by some SARS- or MERS-specific neutralizing antibodies. We will investigate whether genetic variation in RBD of SARS-CoV-2 can lead to viral escape from neutralizing antibodies induced by vaccines or given as treatment including convalescent plasma therapy, which is being implemented nationwide through an FDA emergency IND and neutralizing monoclonal antibodies, discovered at the University of Pittsburgh Center for Antibody Therapeutics.

Progress Report:

1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

We have performed analyses on N=189,429 amino acid (AA) sequences of SARS-CoV-2 S available in the GISAID database to search for AA variants of S that appear in >0.3% of sequences (<0.1% for RBD). Using these sequence alignments, we have identified 19 mutations that have a prevalence >0.3% outside of the RBD and 5 mutations in the RBD >0.1%. We have also established an infectivity and neutralization assay based on lentivirus pseudotyped with full-length human codon-optimized SARS-CoV-2 S. Full-length S is used as wild-type (WT) and desired mutations are introduced into this WT-S sequence using PCR-based site-directed mutagenesis. The first panel of mutants was tested for infectivity and neutralization with a handful of SARS-CoV-2-specific monoclonal antibodies discovered at Pitt’s Center for Antibody Therapeutics (CAT), one of which (Ab1) is in pre-clinical development. The wild-type PSV assay has also been used to contribute to several other projects interrogating SARS-CoV-2-S-specific neutralizing antibody responses.

The pandemic initially caused some difficulties in obtaining reagents and materials. But it also allowed us to focus on COVID work and allowed me, as a junior investigator, to quickly begin to build my area of research.

2. If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?

Funds have been spent, but we plan to continue testing newly emerged mutants and newly discovered mAbs from CAT. We also plan to write up our protocol to publish in Cell Star Protocols. We also plan to continue to use
WT PSV assay for various other projects interrogating SARS-CoV-2-S-specific neutralizing antibody responses, including vaccine breakthroughs.

3. **How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?**

Our project has contributed to the ongoing pre-clinical development of SARS-CoV-2-S-specific monoclonal antibody Ab1, and to the discovery of a monoclonal antibody more effective in neutralizing currently circulating variants (Ab6). The WT PSV assay was also used to contribute to a project on an immunocompromised patient with an intractable infection that led to intra-host viral evolution (Hensley et al, CID, 2021. doi: 10.1093/cid/ciab072). Beyond the COVID-19 pandemic, our PSV assay and mutagenesis system can be applied to many viral surface proteins to probe viral entry and neutralization, allowing their study in a BSL2 setting quickly and easily. This could include any newly emerging viruses.

4. **Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.**

- We were granted funding for assessing activity of Ab1 against circulating variants from the company Novimab, and UPMC leadership funding for assessing activity of various mAbs, convalescent sera and post-vaccine sera against circulating variants
- We published a perspective in Science entitled “The Emerging Plasticity of SARS-CoV-2” (doi: 10.1126/science.abg4493)
- We submitted an R21 in response to RFA/PA: PAR20-177 in August 2020 (was not funded)
- We are submitting an R21 focused on the limits of SARS-CoV-2 evolution in response to immunity

The WT PSV assay contributed to/is contributing to other projects:
- The Hensley et al manuscript mentioned in #3 above
- The manuscript “Antibody Responses after mRNA-based COVID-19 Vaccination in Residential Older Adults: Implications for Reopening”, submitted to the Journal of the American Medical Directors Association
- The assay is being with NIH support for AIDS Clinical Trials Groups (ACTG) study A5401: ACTIV-2 Outpatient Monoclonal Antibodies and Other Therapies)
Abstract:

Recent influenza and SARS-CoV2 global pandemics underscore the urgent need for improved diagnostics in viral pneumonia. Currently, there is no reliable method to determine which patients will have mild/moderate disease versus those that will progress to severe illness. A diagnostic blood test would be extremely useful in guiding patient care decisions with regard to hospital admission and supportive care. Our group has recently begun to evaluate immune protein biomarkers and cells in blood from children and adults with viral pneumonia. We currently have access to human blood samples collected at the time of presentation from outpatients and inpatients through CDC-funded FLU VE and HAIVEN networks. These samples are taken as early as possible in illness and will be used in combination with medical record review to evaluate outcomes. Currently, these networks are focused on influenza patients, however it is anticipated that we will soon collect SARS-CoV2 samples. We propose to study blood biomarkers of severe viral pneumonia in influenza-infected adults as a model for pandemic pneumonia viruses. In the event that SARS-CoV2 samples are available we will compare and contrast immune responses with influenza. These data sets would be analyzed using machine learners such as support vector machines to identify predictors of viral pneumonia severity. Our goal is to identify a panel of markers, perhaps 3-5 proteins or cells, which robustly predict the severity of illness in adults. This cross-discipline approach has the greatest possibility of defining severity biomarkers with high utility in influenza and SARS-CoV2 treatment.

Progress Report:

1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

During the funding period, we focused on analyzing banked samples from inpatients and outpatients infected with influenza during the 2018-19 and 2019-20 seasons. The goal of the project was to immunophenotype influenza severity using blood samples and eventually compare with COVID-19. Analyses were delayed by the initial laboratory shut down at Pitt but were able to resume in full during the Fall of 2020. We measured the concentration of 65 cytokines in blood plasma from approximately 80 patients. We then obtained patient demographic and clinical course information from the electronic medical record. These data are required for stratification of patients by severity and biological variables. The statistical team has developed a database for cytokine and de-identified patient data for analyses. Machine learning analyses have begun using this data set to identify peripheral biomarkers of severe viral disease. Pitt policies regarding the collection of COVID-19 samples precluded us from obtaining acute infection blood samples until the Winter/Spring of 2021. We have now banked nearly 100 samples from inpatients and outpatients with COVID-19. The laboratory is preparing for analyses of these samples.

2. If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?

We have applied for an NCE for the project to complete the study as planned. We will analyze cytokines in the COVID-19 samples mentioned above and enhance our data set by conducting flow cytometry work on peripheral blood mononuclear cells. These two approaches will require additional cytokine kits and flow cytometry
antibodies to conclude the project data collection. Once completed, we will provide these data and clinical information to the statistical group to analyze. This will allow for completion of the study Aims to compare influenza and COVID-19 disease.

3. How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?

The project is not yet completed and has not resulted in an impactful publication or presentation yet. However, the funding provided has focused the group on COVID-19 research and has led to several efforts to collect COVID-19 related patient samples. We have been able to bank blood samples from convalescent patients to study immune memory to COVID-19. We have also collected blood samples from patients before and after COVID-19 vaccination. These samples have greatly increased the scope of our COVID-19 research and likely would not have occurred without funding to direct our efforts. Data from convalescent patients are nearing publication and directly inform the need for vaccination in young adults who were previously infected with SARS-CoV-2.

4. Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.

We applied for an NIH NIAID R21 in 2020 in response to an urgent COVID-19 RFA. This was not funded. We have recently leveraged the COVID-19 samples collected in the last year to apply for PA Department of Health funding. We expect to obtain funds for studies of convalescent patients and vaccine recipients focused on T cell memory. At this time, we have not published our results as the project is not completed.
Abstract:
COVID-19, caused by SARS-CoV-2, is a pandemic with over 1.8 million cases that has a range of presentations from asymptomatic disease to respiratory failure requiring life support measures. Asymptomatic shed and less severe pediatric disease make current symptom- and level of acuity-based testing, limitations driven by supply shortages, wholly inadequate to estimate disease prevalence in children, an important population for community spread. To improve estimates, we aim to measure disease prevalence and geographic spread in children using SARS-CoV-2 antibody testing and electronic medical record data. We hypothesize viral testing limitations have led to under-reporting of the true extent of infection in the pediatric population, therefore also underestimating the pediatric role in community viral transmission. Residual pediatric blood samples from the clinical laboratory will be collected and tested using a clinical assay for measuring SARS-CoV-2 antibodies developed in the Clinical Immunopathology Laboratory at UPMC allowing for determination of past infection of both symptomatic and asymptomatic patients. Leveraging these results with Electronic Medical Record (EMR) data will provide a powerful tool for assessing success of containment measures, the role of children in community spread, immunity, and informing public health actions. We expect that the prevalence of COVID-19 is significantly higher than currently reported as it is based on limited viral PCR testing. Prevalence data are vital and can be obtained quickly and applied immediately to inform decision-making about containment measures, population immunity, and provide antibody levels from patients to be used as surrogates for vaccine efficacy.

Progress Report:
1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

Since receiving this award we were able to provide the ACHD with red phase and yellow phase seroprevalence of children in SWPA and specifically Allegheny county. We characterized seroprevalence and outcomes in immune-suppressed children and found that, unlike their adult counterparts, their outcomes were not significantly worse than immune-competent children. We also characterized the need for orthogonal serologic testing in children, a population that may be subject to unusually high false-positivity rates in low prevalence areas.

2. If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?

Our funding is mostly completed, and we are investigating some pediatric-specific SARS-COV-2 mechanisms.

3. How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?

Our work was the first SARS-CoV-2 seroprevalence study specifically in the pediatric population with important implications for back-to-school decisions, pediatric testing for prior asymptomatic exposure, and outcomes for immune-compromised children.
4. Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.


Under Review: Pediatric SARS-CoV-2 Seroprevalence During Mitigation Procedures in Southwestern Pennsylvania
Abstract:
COVID-19 requires no introduction since the entire globe is dealing with a worldwide pandemic due to SARS-CoV-2. Most individuals infected with the virus will have mild self-limiting symptoms, and some may even be asymptomatic. This has made control of virus spread very difficult and has necessitated the drastic measures of social distancing and governmental shelter in place orders. With testing currently restricted to acute cases and even that being limited due to resource shortages, it is impossible to know how widespread the disease is within the community or the hospital setting. These data are desperately needed to augment public health efforts to control the spread of the virus and furthermore would hasten our return to normal societal behaviors. In this proposal, we seek to develop critical serologic assays that can quantitate human humoral immune responses to SARS-CoV-2. These data will inform how widespread the disease is in the Pittsburgh community since we will identify previously infected individuals whose symptoms were not severe enough to necessitate hospitalization or acute phase testing. Importantly, these assays will speed up return to normalcy since individuals who have seroconverted are expected to be immune to re-infection or at least protected from severe disease, and therefore safe to return to work and school settings that would put them in contact with others who are potentially infected. By using our extensive virological capabilities, validated patient samples and state-of-the-art biocontainment laboratories our goal is to provide in-depth immunological granularity to support clinical research, diagnostics and ultimately clinical trials.

Progress Report:
1. **What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?**
   - Two SARS-CoV-2 ELISA assays were developed- one using the nucleocapsid antigen and another using a portion of the spike antigen known as RBD. These assays are semi-quantitative and measure the amount of virus-specific antibodies in a patient blood sample.
   - ELISA assays were validated using over 200 control samples- this included samples obtained from patients pre-COVID who had a number of different infectious/inflammatory conditions as well as samples from over 100 patients with known COVID at various times post-infection. This was enabled via our close collaborations with UPMC clinical labs.
   - ELISA assays demonstrated excellent correlation with WHO International Standards
   - A focus reduction neutralization assay (FRNT) was developed using live SARS-CoV-2 virus- which measures the amount of virus-inactivation activity there is in a patient blood sample.
   - A PGH community serosurvey was conducted at two-time points in the pandemic- in the Fall of 2020 and again in Feb of 2021, just after the winter wave of cases.
   - The only pandemic-related delays have been delays in supplies due to supply chain issues.

2. **If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?**
   - There is no funding remaining, although there are several outstanding invoices due to COVID-related manufacturing delays.
• The next step would be responding to any reviewer requests for additional studies.

3. **How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?**
   - This work demonstrates the importance of using serologic assays that can distinguish infected from vaccinated individuals for studies of the epidemiology of COVID-19.
   - This work demonstrates how a moderate cohort of samples can be used to predict community COVID seroprevalence.
   - This work revealed that the RBD titer that correlated with virus neutralization activity was lower in those who were infected than in those who were vaccinated, suggesting a qualitative difference in RBD-directed antibodies following infection vs vaccination.
   - This work has enabled numerous other COVID studies by UPMC clinical labs and other Pitt investigators (Pediatrics, Family Medicine, Biology, and MMG) by providing FRNT assay support.
   - This work has also enabled numerous other COVID studies at the CVR - animal model development, pre-clinical vaccine studies in both mice and non-human primates.
   - This work emphasizes the capabilities of the CVR to rapidly respond to an emerging pandemic. This is even more significant when you consider the fact that none of the CVR virologists were experts in Coronaviruses prior to the pandemic, demonstrating our ability to rapidly pivot and meet the needs of our community.

4. **Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.**
   - We have a PA DOH grant pending.
   - A manuscript describing this work was prepared and submitted - happy to send along a copy once it is accepted for publication.
Abstract:
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has driven a pandemic of 2019 coronavirus disease (COVID-19). Although COVID-19 risk factors include male sex, hypertension, and chronic lung disease, asthma may be a specific risk in younger patients. Yet, underlying mechanisms are unclear. The enzyme, ST6β-galactoside α-2,6-sialyltransferase 1 (ST6GAL1) catalyzes the transfer of sialic acid to galactose-containing substrates. Blocking ST6GAL1 prevents epithelial cell adsorption and replication of influenza through a reduction in these α-2,6 sialylated residues. While the target proteins for this effect are unknown, ST6GAL1 was shown by us to be critical for α-2,6 sialylation of the tethered and secreted mucin, MUC4β. Tethered mucins play critical roles in host defense and sialylation of MUC4β drives goblet cell differentiation and reduce primary human airway epithelial cell (AEC) proliferation in response to Type-2 immune stimulus. Airway MUC4β and ST6GAL1 are both elevated in Type-2 asthmatic patients. In mice, AEC Muc4 protected against related SARS-CoV infection, which uses the same cellular receptor as SARS-CoV-2, and was more protective in female compared to male mice, consistent with male predisposition for severe COVID-19. The mechanisms for this protective role remain unclear as neither viral titers nor lung inflammation were impacted. MUC4β sialylation-status was not reported, but could also be critical. These observations suggest that targeting sialylated mucin pathways could influence COVID-19 outcomes. Therefore, we hypothesize that the sialylation state of MUC4β is critical for determining its anti-SARS-CoV-2 effects in primary human AECs and that this sialylation links to underlying asthma, race, male sex and resultant COVID-19 infections.

Progress Report:
1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?
We have exposed multiple primary polarized human airway epithelial cells to SARS-COV2 under “healthy” and “asthmatic”/IL13 conditions. We have measured viral uptake and proliferation, as well as cell death. We have knocked down the mucin MUC4 and a sialylating enzyme, ST6GA1, and shown that ST6GAL1 appears to be protective against viral uptake and proliferation.

In addition, we sent out 2 surveys on COVID and its impact on ~600 asthma patients and 600 healthy controls on 2 occasions, following an initial baseline survey in March-April 2020. The last one was sent out in February, just following the winter peak, and captured approximately 60 cases of COVID, many of which are still having symptoms months later. We are in the process of analyzing risk factors for “long haul” COVID, as well as the economic toll of COVID.

We were limited in two ways. Supplies for these experiments were not available for many months. These epithelial cells require culturing on specialized plates which were not available for 3-4 months. Although we tried substitutes they did not work well. Secondly, since we were unable to perform bronchoscopies, our
supply of “fresh” human cells was limited. Although we grew cells that had been cryopreserved (frozen), these cells did not grow as well, and in fact, transfection of these cells was nearly impossible. Finally, the IL-13 which we purchased was shown to have a yet unknown contaminant which made many of our IL-13 stimulation experiments suspect.

2. If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?

We will need to repeat many of our IL-13 experiments and effectively knockdown MUC4 and ST6GAL1 under these conditions. In addition, we are still analyzing the COVID surveys, as there is a tremendous amount of data there. We are hoping to address personal and community preparedness from our first very early COVID survey with outcomes 1 year later.

3. How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?

We are beginning to understand the factors that predict better or worse outcomes from COVID based on individual disease and community preparedness. We are evaluating the impact of underlying asthma on prolonged shortness of breath post COVID. Although we do not have any certain results yet, our early data suggest that mucins and their sialylation will play a role in epithelial responses to SARS-CoV2.

4. Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.

We submitted two NIH applications that were not funded. We expect to have several publications on the COVID-19 surveys soon.
Abstract:
Investigation of the novel and highly transmissible Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of Coronavirus Disease 2019 (COVID-19), generally requires the use of expensive and scarce high biosafety level containment (BSL-3) facilities, limiting the number of researchers who can contribute to these initiatives. A single-cycle SARS-CoV-2 virus, which infects cells and progresses through the virus life cycle without producing new infectious virus particles (i.e. replication-defective), would permit more widespread research studies into multiple aspects of SARS-CoV-2 virology at a reduced biosafety level (BSL-2). Replacing an accessory protein gene in the viral genome with a fluorescent (e.g. mNeonGreen) or bioluminescent (e.g. nanoLuciferase) protein gene and production of virus from multiple vectors would generate a reporter virus that would facilitate research activities including super-resolution and live-cell imaging to study SARS-CoV-2 biology and high-throughput screening of virus-interacting host cell factors and drug candidates. Herein, we propose to leverage our knowledge and experience with single-cycle HIV-1 reporter viruses to develop a single-cycle SARS-CoV-2 reporter virus system, expressing fluorescent/bioluminescent proteins, for use by our lab and other laboratories in a BSL-2 setting for imaging and high-throughput drug screening studies. We further propose to utilize this virus for imaging studies performed in the Center for Biologic Imaging (CBI) to understand post-entry virus life cycle events, particularly hijacking of host cell proteins and pathways. This single cycle SARS-CoV-2 reporter virus system would facilitate the study of SARS-CoV-2 and the identification of unique virus-host interactions that can be targeted by novel therapeutics.

Progress Report:

1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

Last May, we received the SARS-CoV-2 genome in the form of 7 cDNAs encoded in individual plasmids and bacterial artificial chromosomes (BACs). Using molecular biology techniques, we successfully engineered 3 versions of the replication-competent SARS-CoV-2 genome in BACs: full-length SARS-CoV-2, SARS-CoV-2 encoding a green fluorescent protein, and SARS-CoV-2 encoding a bioluminescent protein (luciferase). We confirmed that transfection of full-length SARS-CoV-2 RNA transcribed from the BAC constructs produced viruses that replicated in cell culture. SARS-CoV-2 encoding luciferase was shown to be transmitted intranasally in hACE-2 mice and detectable by whole-body imaging by the Klimstra laboratory. To be able to study SARS-CoV-2 infection of cells outside of the RBL, we have produced 2 molecular clones in which one or two viral genes have been eliminated. We have shown that these constructs did not produce infectious virus. Currently, we are producing the replication-defective viruses with the missing viral proteins added separately into cells to demonstrate that infectious virus can be produced to infect cells but that it will not produce new viruses. This may allow us to perform a single cycle of replication studies at a lower biosafety level. In addition, we rebuilt the live-cell microscope in the RBL to visualize fluorescently labeled SARS-CoV-2 infection of cells. The pandemic delayed progress both by limiting the time that personnel could work in the laboratory, due to working in shifts and the requirement for RBL access and delays in shipments of several critical reagents.
2. **If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?**

Our funding is completed, but we are currently performing the following three experiments. First, production of replication-defective, infectious viruses to request downgrade for use outside of the BSL-3/RBL laboratory. Second, production of a BAC encoding the SARS-CoV-2 nsp3 protein fused to a far-red fluorescent protein and nsp4 to allow expression in cells, which will allow visualization of nsp3 induction of cellular SARS-CoV-2 replication structures by fluorescence microscopy. Third, we are evaluating transfection of full-length SARS-CoV-2 DNA (instead of transcribed RNA) for more efficient production of virus. In the future, we plan to introduce sequences from SARS-CoV-2 variants of concern (e.g. B.1.1.7) into our constructs to understand how specific mutations may influence SARS-CoV-2 replication in human cells.

3. **How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?**

We hope to receive Environmental Health and Safety and Institutional Biosafety Committee approval to study SARS-CoV-2 replication at a more convenient biosafety level. This will allow us and others to study a single cycle of SARS-CoV-2 replication more easily for screening drug candidates and understanding the biology of how the virus infects human cells. In addition, the use of reporter viruses allows the visualization of specific SARS-CoV-2 replication steps in cells as well as visualization of infection in tissues of animal models that will allow the design of better therapies to inhibit replication and prevent COVID-19.

4. **Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.**

We anticipate preparation of a manuscript for publication on the replication-defective SARS-CoV-2 constructs in the next 1-2 months. In early February, we submitted a R21 to NIAID/NIH, which will be reviewed in June.
Abstract:

SARS-CoV-2 is associated with the outbreak of COVID-19 pandemic. SARS-CoV-2 virus utilizes its trimeric surface spike (S) protein to bind a human ACE2 receptor via a receptor-binding domain (RBD). Structures of S/RBD-hACE2 complex have been determined, which serve as the structural basis for an understanding of viral entry and future therapeutic development. Here, we are compelled to propose to leverage a revolutionary nanobody (Nb) platform that we have recently developed and patented to generate and characterize novel anti-SARS-CoV-2 Nbs. Our technologies enable identification and structural characterizations of thousands of highly divergent, ultrahigh-affinity (sub-nM KD) Nbs comparable to the most successful therapeutic IgG antibodies. Camelid nanobodies (Nbs) are characterized by their small size, excellent solubility, and can be easily bioengineered and manufactured. Because of their low immunogenicity and high stability, they can be a rapid point-of-care diagnostic and can potentially be used for inhaled delivery to the lungs. The superiority of Nbs in recognizing cryptic epitopes for viral neutralization has also been demonstrated. Our overarching goal is to identify and characterize a large repertoire of drug-quality NbSARS-2 to block SARS-CoV-2 RBD binding to its cognate receptor hACE2. We expect a combination of multiple conformational Nbs, similar to HIV broadly neutralizing antibodies (cocktails), will efficiently block viral entry and infection. By fusing the inhibitory NbSARS-2 with our NbHSA, we will drastically improve the pharmacokinetics and drug efficacy of the fusion Nbs towards a novel, highly effective Nb-based therapy. Moreover, successful completion of this project will discover critical viral neutralization epitopes to facilitate rational vaccine development.

Progress Report:

1. What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?

We have recently identified > 8,000 high-affinity, multi-epitope nanobodies (Nbs) that target the receptor-binding domain (RBD) of SARS-CoV-2 glycoprotein (Xiang et al. 2020. Science). This large repertoire of neutralizing Nbs includes sub-pM affinity binders that inhibit viral infection at sub-ng/ml concentration, which are unprecedented for antibody fragments. Bioengineering of multi-epitope/ multivalent Nbs further improves the potency to < 0.1 ng/ml and may prevent mutational escape. These thermostable Nbs can be rapidly produced from microbes and resist lyophilization, and aerosolization. This work has been well received worldwide. In the follow-up structural studies, we have unraveled a plethora of mechanisms of virus neutralization. Systematic structure-function studies reveal that potent neutralizing Nbs are highly resistant to the circulating variants of concern. High-resolution cryoEM analysis of 9 structures reveals novel and conserved epitopes inaccessible to large human antibodies (Sun et al. 2021. Under review). This new study has provided critical insights into how Nbs uniquely target the virus and will inform the design of novel sarbecovirus/ pan-coronavirus therapeutic and vaccine development. Critically, we have demonstrated the high preclinical efficacy of a lead Nb (or any Nb) for inhalation treatment of SARS-CoV-2 infection (Nambuli et al. 2021. Science Advances in press). Aerosol delivery of our lead Nb facilitates deposition throughout the respiratory tract and dose minimization to 0.2 mg/kg. Inhalation treatment decreases lung viral titers by 6 logs leading to drastically mitigated lung pathology and prevents pneumonia.
2. **If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?**

We will apply NIH funding for the possibility to extend this highly promising study into NHP study and possible clinical trials.

3. **How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?**

Combined with the marked stability and low production cost, this novel therapy may provide a convenient and cost-effective option to the pandemic.

4. **Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.**


Inhalable Nanobody (PiN-21) prevents and treats SARS-CoV-2 infections in Syrian hamsters at ultra-low doses. Sham Nambulli, Yufei Xiang, Natasha L. Tilston-Lunel, Linda J. Rennick, Zhe Sang, William B. Klimstra, Douglas S. Reed, Nicholas A. Crossland, **Yi Shi** and **W. Paul Duprex.** Science Advances (in press)

Dapeng Sun, Zhe Sang, Yong Joon Kim, Yufei Xiang, Tomer Cohen, Anna K. Belford, Alexis Huet, James F. Conway, Ji Sun, Derek J. Taylor, Dina Schneidman-Duhovny, Cheng Zhang, Wei Huang, and Yi **Shi** (revision) preprint available at bioRxiv

Patent:
Pitt Ref.: 05391
Disclosure Title: Therapeutic Nanobodies for SARS-CoV-2 Viral Neutralization
Innovators: Yi Shi (University of Pittsburgh); Yufei Xiang (University of Pittsburgh)
Abstract:

Cell-penetrating peptides, also known as protein transduction domains, are 6-30 amino acid long peptides, able to cross cell membrane barriers while carrying cargoes, up to several times their size, in an intact functional form. Our prior work using phage display identified a 12-amino acid long Cardiac Targeting Peptide, so named due to its ability to transduce heart tissue in 15mins after peripheral intravenous injection. Studies into its mechanism of transduction using an alanine scan identified two alanine “mutant” versions of it, S7A and R11A that targeted lung tissue after intravenous or intra-tracheal administration, as well as transduced human bronchial epithelial cells robustly in vitro. SARS-CoV-2 virus, the agent causing a worldwide COVID-19 pandemic, gains access through the inhalational route using the ACE2 receptors to gain entry into lung epithelial cells and multiply. The current grant aims to develop an anti-viral agent specific to the SARS-CoV-2 by designing siRNAs targeting key structural envelope and nuclear viral proteins, and deliver them specifically to the lungs using the two, novel lung targeting peptides (LTPs). The siRNAs will be selected such that there is no interference with normal human mRNA encoding proteins. The siRNA and LTPs will have a disulfide bond between them that will be broken on exposure to the intracellular reducing environment, releasing the cargo siRNA into lung epithelial cells. This will allow the siRNA to interact with the viral mRNA and interrupt the protein synthesis process, thus aborting the cycle of replication and further viral propagation.

Progress Report:

1. **What have you accomplished since receiving this award? Did the pandemic cause any delays in your work?**

   We were able to refine the chemical conjugation of siRNA to N-terminal of lung targeting peptides using a conjugate disulfide bond between the two. This allows for the bond to break inside the reducing environment of the cell and release of the siRNA. The pandemic did not slow us down.

2. **If you have funding remaining, what work remains to be done? If your funding is completed, what are your next steps?**

   We have some funding remaining. Even though the lung targeting peptides were able to internalize siRNA, in our COVID studies, and studies relating to cystic fibrosis, the siRNAs were not active and did not give us the expected knockdown. We would like to use the remaining funding to figure out the reasons behind this failure to produce the expected biological effects that the siRNAs do in concert with Lipofectamine.

3. **How has your project had an impact on the COVID-19 pandemic? What are the broader impacts of your work beyond the COVID-19 pandemic?**

   Unfortunately, we did not produce an effective anti-viral that we had hoped to against COVID-19. However, the knowledge gleaned from trying to place siRNAs on peptides, has led to enhanced services now available through peptide synthesis facility of University of Pittsburgh. Furthermore, we have utilized this knowledge to conjugate
siRNA against BAG3 to lung targeting peptides, and miRNAs to cardiac targeting peptides. The miRNA linked to cardiac targeting peptide is being tested by Dr. Ian Gallicano, a collaborator at Georgetown University, with very promising results.

4. Has your project had any outcomes in the form of publications, presentations, intellectual property, or further funding? Please include any applications that are pending or were not accepted.

Since this was a negative study, we will not be attempting to publish these results unless we can figure out the reason behind siRNAs being delivered and not being active intracellularly. We had applied for a provisional patent on the use of lung targeting peptides to deliver siRNAs as anti-viral therapies (for COVID-19, influenza, etc.). However, that provisional patent was not converted given our lack of efficacy with the siRNAs.