AWARD NUMBER: W81XWH-21-1-0070

TITLE: Development of TMPRSS2 Antibody as an Antiviral Treatment for SARS-CoV-2 (COVID-19)

PRINCIPAL INVESTIGATOR: Ya-Wen Chen

CONTRACTING ORGANIZATION: University of Southern California, Los Angeles, CA

REPORT DATE: February 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
February 2022	Annual	15Jan2021-14Jan2022

4. TITLE AND SUBTITLE

Development of TMPRSS2 Antibody as an Antiviral Treatment for SARS-CoV-2 (COVID-19)

5a. CONTRACT NUMBER
W81XWH-21-1-0070

5b. GRANT NUMBER
W81XWH-21-1-0070

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Ya-Wen Chen

E-Mail: yawen.chen@mssm.edu

5d. PROJECT NUMBER
0011560695-0001

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Southern California
3720 S Flower St., FL 3,
Los Angeles, CA 90007-4318

8. PERFORMING ORGANIZATION REPORT NUMBER

1

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

To enter a host cell, SARS-CoV-2 uses its spike protein to bind to the ACE2 cellular receptor and then is primed by the type II transmembrane serine protease TMPRSS2. We aim to develop an efficient antiviral via a monoclonal antibody of TMPRSS2, AL20, to impede the entrance of the virus into cells, specifically into lung epithelial cells. This past year is the first year of this funding award and the COVID-19 pandemic and the delayed approval from the HRPO significantly impacted our research activities in a negative way. Despite the challenges and unusual circumstances, we still made progress and determined that there is no cytotoxicity when treated cells with AL20. In addition, we showed that alveolar type II (AT2) cells generated from human pluripotent stem cells (hPSCs) in the 2D and 3D lung models were susceptible to both SARS-CoV-2 pseudovirus and live virus. Furthermore, we showed treating with AL20 inhibits the SARS-CoV-2 pseudovirus transduction in Calu-3 cell line and the 2D hPSC-AT2 cells. During the next reporting period, we hope to make up the lost time and effort toward completion of the proposed research.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	17	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. Introduction

In December of 2019, a novel coronavirus, now referred to as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), struck Wuhan, China and unleashed the current Coronavirus disease (COVID-19) pandemic. This proposal aims to develop an efficient antiviral to impede the entrance of the virus into cells, specifically into lung epithelial cells. Thanks to recent studies, we know which "door" (a receptor called ACE2) and "key" (a protease called TMPRSS2) the virus uses to enter cells. Our goal is to remove the "key" so the virus cannot open the "door" and enter host cells. In Aim 1, we will determine if TMPRSS2 antibodies block SARS-CoV-2 infection *in vitro*. In Aim 2, we will investigate mechanisms of AL20, a lead TMPRSS2 monoclonal antibody, in inhibiting SARS-CoV-2 cellular entry. In Aim 3, we will humanize the lead TMPRSS2 antibody and determine the minimum effective dose against SARS-CoV-2 infection *in vivo*.

2. Keywords

SARS-CoV-2, COVID-19, hPSC-derived lung organoid, TMPRSS2, ACE2, respiratory infectious disease, protease, monoclonal antibody.

3. Accomplishments

WHAT WERE THE MAJOR GOALS OF THE PROJECT?

The major goals of the projects are:

1. To determine if TMPRSS2 antibodies block SARS-CoV-2 infection *in vitro.*

2. To understand the mechanisms of AL20, a TMPRSS2 monoclonal antibody, in inhibiting SARS-CoV-2 entry.

3. To humanize the lead TMPRSS2 antibody and determine the minimum effective dose against SARS-CoV-2 infection *in vivo*.

WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

1) Major activities

This past year is the first year of this funding award and the COVID-19 pandemic significantly impacted our research activities in a negative way. Most of the research activities were suspended or only partially allowed due to university-wide and state-wide policies. All administrative and university research committees, such as institutional animal care and use committee (IACUC) and institutional review boards (IRB), worked remotely increased the difficulties to obtain approvals from the HRPO and ACURO in time. In addition, there was a misunderstanding from the HRPO on the commercially available cell lines we proposed to use in the projects. The HRPO requested us to submit an IRB approval and the consent form of the NIH-approved, commercially available human embryonic stem cell line, RUES2. It took about a year

to get this misunderstanding resolved. The delayed approval of the HRPO further negatively impacted the progress of the project.

Despite the challenges and unusual circumstances, we were able to complete the major activities listed in the follows:

1. Determine the cytotoxicity of the AL20 in Calu-3 cells.

2. Determine cytotoxicity of the AL20 in 2D hPSC-AT2 cells.

3. Confirm SARS-CoV-2 pseudovirus infection in 2D hPSC-AT2 cell.

4. Confirm AL20's efficacy against SARS-CoV-2 pseudovirus infection in 2D hPSC-AT2 cells.

5. Confirm SARS-CoV-2 pseudovirus infection in 3D hPSC-AT2 cells.

6. Confirm SARS-CoV-2 live virus infection in 2D hPSC-AT2 cells.

7. Confirm SARS-CoV-2 live virus infection in 3D hPSC-AT2 cells.

8. Label AL20 using classic Alex Fluor dyes and determine if fluorescent labeled AL20 internalized into hPSC-AT2 cells.

2) Specific objectives

1. To determine if AL20 has cytotoxicity when treated with cells.

2. To determine if 2D hPSC-AT2 and 3D hPSC-AT2 can be transduced by SARS-CoV-2 pseudovirus.

3. To determine if 2D hPSC-AT2 and 3D hPSC-AT2 can be infected by SARS-CoV-2 live virus.

4. To determine if AL20 blocks SARS-CoV-2 pseudovirus transduction *in vitro*.

3) Significant results or key outcomes

1. We found no cytotoxicity when treating cells with the lead TMPRSS2 antibody, AL20, at a dose as high as 500 ug/ml in the Calu-3, Huh7.5, Vero, Vero-E6, HeLa, HeLa-ACE2, and human pluripotent stem cell (hPSC)-derived 2D lung cultures **(Fig 1a** and not shown**)**. We used one embryonic stem cell line, RUES2, and two iPS cell lines, mRNA and SV, to generate 2D lung cultures for the cytotoxicity tests. With the lead antibody showed no signs of cytotoxicity in 9 cell lines we tested; we concluded there is no cytotoxicity when using monoclonal TMPRSS2 antibody, AL20, to treat cells.

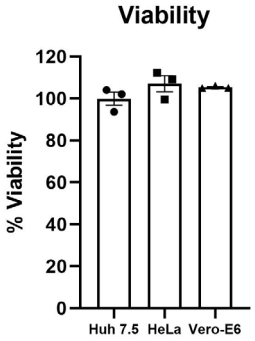

Figure 1. AL20 has no cytotoxicity when treated with cells. Viability of Huh7.5, HeLa and Vero-E6 cell lines treated with 500 μg/ml AL20 for 96 hours.

2. We found the transduction rate of Calu-3 cells is not high (~5-7%) and not showing consistency of infection between experiment to experiment **(Fig. 2a)**. Though we did observe a reduction of pseudovirus cellular entry to Calu-3 cells after antibody treatments, the experimental results varied **(Fig. 2b)**. We concluded that Calu-3 might not be a good cell line to obtain meaningful data in terms of the efficacy of the AL20.

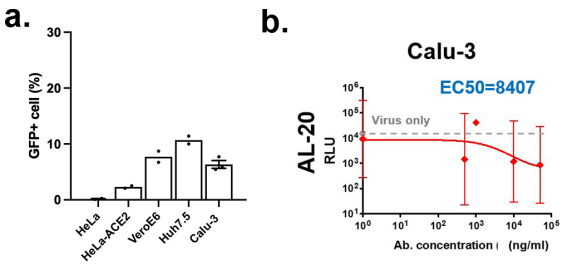

Figure 2. Transduction of SARS-CoV-2-GFP pseudovirus in cell lines. (a) Quantification of GFP+ cells in SARS-CoV-2-GFP transduced cell lines. **(b)** Calu-3 cell were pre-treated with different concentrations of AL20 for one hour followed by SARS-CoV-2 pseudovirus with luciferase reporter. Luciferase activity was measured 24 hours post infection.

3. When we transduced hPSC-derived 2D AT2 cells with SARS-CoV-2-GFP pseudotyped virus, we found a dramatically increased in pseudotyped virus transduction rate **(Fig. 3)**. This indicates that the 2D hPSC-AT2 cells might serve as a better model for drug screening compared to conventional cell lines. Indeed, we observed a better inhibitory effect of SARS-CoV-2-GFP viral entry when treated 2D-hPSC AT2 cells with the AL20 **(Fig. 4)**.

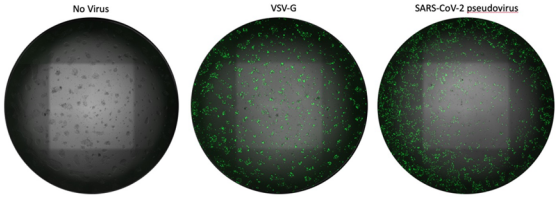

Figure 3. Transduction of SARS-CoV-2-GFP pseudovirus in 2D hPSC-AT2 cells. 2D hPSC-derived AT2 cells were transduced with SARS-CoV-2-GFP pseudovirus. GFP expression was visualized and 24 hours post transduction (n=3 biological independent experiments).

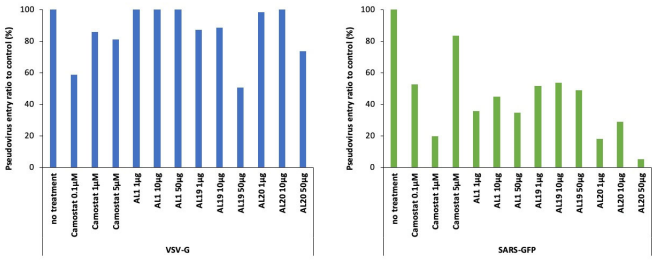

Figure 4. Inhibition of SARS-CoV-2-GFP pseudovirus cellular entry in 2D hPSC-derived AT2 cells. 2D hPSC-derived AT2 cells were pre-treated with different concentrations of TMPRSS2 monoclonal antibodies and Camostat (control) for one hour followed by either VSV-GFP (control virus) or SARS-CoV-2-GFP pseudovirus. GFP positive cells was quantified 24 hours post infection.

4. We have previously developed a lung organoid model, primarily containing AT2 cells, from hPSCs. We found expression of ACE2 and TMPRSS2 increased as the organoids matured in culture both at mRNA **(Fig. 5a)** and at protein levels **(Fig. 5b)**. Furthermore, the amounts of ACE2 and TMPRSS2 are higher in lung organoids compared to conventional cell lines used for SARS-CoV-2 studies **(Fig. 5)**. Comparable to the cell lines, lung organoids were readily susceptible to SARS-CoV-2 Spike pseudotyped vectors with efficient vector entry (about 25%) observed **(Fig. 6)**. We further tested SARS-CoV-2 live virus infection in lung organoids. Unlike other infection methods where organoid cultures were either disrupted mechanically or enzymatically digested into

single cells, we exposed the cultures to virus by simply replacing the culture media with virus-containing media at corresponding multiplicity of infections (MOIs). This allowed us to preserve the structure and physiological location of cells in the organoids. SARS-CoV-2 nucleocapsid proteins (NPs) were readily detected one day post infection (dpi) **(Fig. 7a)**. The majority of cells remained viable at 4 dpi, but sustained viral damage **(Fig. 7b)**. Both quantitative reverse transcription polymerase chain reaction (qRT-PCR, **Fig. 7c**) and plaque assay **(Fig. 7d)** indicated that live SARS-CoV-2 virus can infect and replicate in lung organoids. These results demonstrate that lung organoids provide an authentic model for respiratory viral pathogenesis, recapitulating respiratory viral infection in the host and serving as valuable tools to study COVID-19.

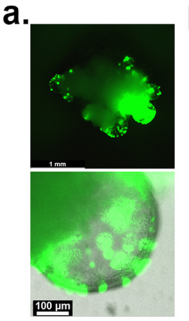

Figure 6. Transduction of SARS-CoV-2-GFP pseudovirus in lung organoids. hPSC-derived lung organoids were transduced with SARS-CoV-2-GFP pseudovirus. GFP expression was **(a)** visualized and **(b)** quantified by flow cytometry 24 hours post transduction (n=2 to 3 biological independent experiments).

5. To investigate the potential mechanisms of AL20 in inhibiting SARS-CoV-2 cellular entry, we first labeled AL20 with classic Alexa Fluor dye (AL20-Alexa-555). When treated hPSC-AT2 cells with AL20 for 24 hours, we observed the internalization of the AL20-Alexa-555 **(Fig. 8)**.

4) Other achievements

Nothing to report.

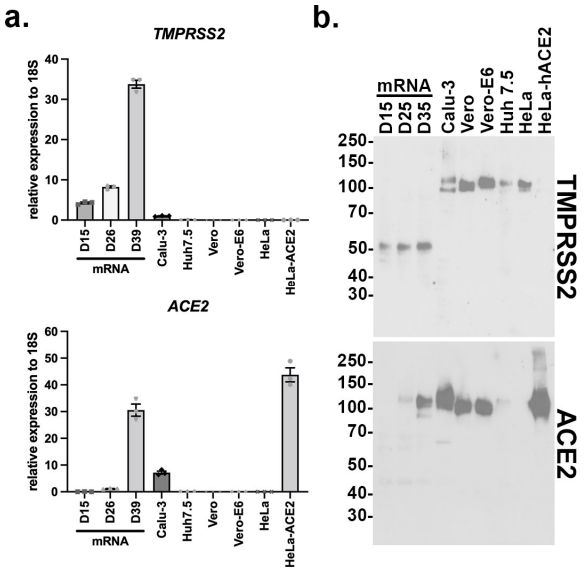

Figure 5. Expression of TMPRSS2 and ACE2 in lung organoids and cell lines. Total RNA **(a)** and protein expression **(b)** of ACE2 and TMPRSS2 in hPSC-derived lung organoids of different differentiation stages and in indicated cell lines. D= day. (n=3 biological independent experiments).

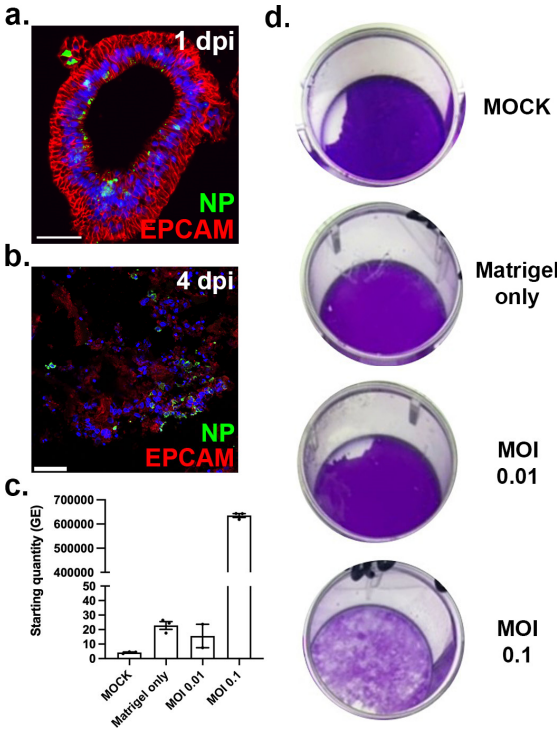

Figure 7. Infection of live SARS-CoV-2 in lung organoids and cell lines. d50 lung organoid derived from RUES2 hPSCs infected with live SARS-CoV-2. **(a)** 24 hours post infection. Scale bar = 50 µm . **(b)** 96 hours post infection. Scale bar = 50 µm. qRT-PCR **(c)** and plaque assay **(d)** using media collected from 4 dpi infected organoids.

WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

The PI, Ya-Wen Chen, has attended virtual meetings of American Thoracic Society and International Society for Stem Cell Research. These conferences broadened her knowledge in pathogenesis of SARS-CoV-2, as well as the use of human pluripotent stem cell-derived models on SARS-CoV-2.

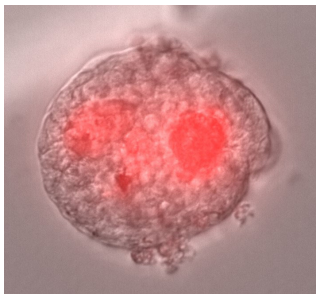

Figure 8. Internalization of AL20. Cells were incubated with AL20-Alexa-555 for 24 hours. Image taken 24 hours post incubation.

HOW WERE THE RESULTS DISSEMINATED TO COMMUNITIES OF INTEREST?

The PI was invited to give seminars at several organizations regarding the findings of the project.

WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

During the next reporting period, we plan to focus on completing the works on live SARS-CoV-2. We will test the inhibitory ability of AL20 at a viral MOI of 0.1 using 6 doses of AL20 in our 2D and 3D hPSC-derived lung cultures. We will quantify the virus infection rate on AT2 cells via immunofluorescent staining and RT-qPCR. After we determine an optimal dose of AL20 in inhibiting SARS-CoV-2 live virus entry, we will determine the minimum effective dose of AL20 against SARS-CoV-2 infection *in* vivo. In addition, we will focus on determining if AL20 blocks SARS-CoV-2 infection via reducing the TMPRSS2 protein level on cell surface using the AL20-Alexa-555. We will also determine if TMPRSS2 antibodies block SARS-CoV-2 infection via reducing its proteolytic activity on cell surface.

4. Impact

WHAT WAS THE IMPACT ON THE DEVELOPMENT OF THE PRINCIPAL DISCIPLIN(S) OF THE PROJECT?

Nothing to report.

WHAT WAS THE IMPACT ON OTHER DISCIPLINES?

Nothing to report.

WHAT WAS THE IMPACT ON TECHNOLOGY TRANSFER?

Nothing to report.

WHAT WAS THE IMPACT ON SOCIETY BEYONG SCIENCE AND TECHNOLOGY?

Nothing to report.

5. Changes/Problems

CHANGES IN APPROACH AND REASONS FOR CHANGE

Nothing to report.

ACTUAL OR ANTICIPATED PROBLEMS OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

This past year is the first year of this funding award and the COVID-19 pandemic significantly impacted our research activities in a negative way. Most of the research activities were suspended or only partially allowed due to university-wide and state-wide policies. All administrative and university research committees, such as institutional animal care and use committee (IACUC) and institutional review boards (IRB), worked remotely increased the difficulties to obtain approvals from the HRPO and ACURO in time. In addition, there was a misunderstanding from the HRPO on the commercially available cell lines we proposed to use in the projects. The HRPO requested us to submit an IRB approval and the consent form of the NIH-approved, commercially available human embryonic stem cell line, RUES2. It took about a year to get this misunderstanding resolved. The delayed approval of the HRPO further negatively impacted the progress of the project. During the next reporting period, we hope to make up the lost time and effort toward completion of the proposed research.

CHANGES THAT HAD SIGNIFICANT IMPACT ON EXPENDITURES

Nothing to report.

SIGNIFICANT CHANGES IN USE OR CARE OF HUMAN SUBJECTS, VETEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. Products

PUBLICATIONS, CONFERENCE PAPERS, AND PRESENTATIONS

Journal publications

Nothing to report.

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers and presentations

National

Mar. 2021 – Hastings Symposium, Los Angeles, CA

Local societies

Jan. 2021 – Keeping Up With COVID-19, University of Southern California, Los Angeles, CA

Feb. 2021 – Research seminar, Black Family Stem Cell Institute/Cell, Developmental, and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY

Oct. 2021 – Research seminar, Department of Pharmacology and Regenerative Medicine, The University of Illinois College of Medicine, Chicago, IL

WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report.

TECHNOLOGIES OR TECHNIQUES

Nothing to report.

INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Nothing to report.

OTHER PRODUCTS

Nothing to report.

7. Participants & Other Collaborating Organizations

WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Name:	Ya-Wen Chen, PhD
Project Role:	PI
Researcher Identifier:	ORCID ID:
Nearest person month worked:	1.2
Contribution to Project:	Dr. Chen oversaw and directed all aspects of the project and supervised personnel.

Name:	Lu Tian, PhD
Project Role:	Postdoctoral Scientist
Researcher Identifier:	N/A
Nearest person month worked:	3.6
Contribution to Project:	Dr. Tian is the main person who perform most of the experiments.

Name:	Irving Garcia
Project Role:	Technician
Researcher Identifier:	N/A
Nearest person month worked:	1.8
Contribution to Project:	Mr. Garcia generated the SARS-CoV-2 pseudovirus used in the study.

Name:	Carrie Zhang
Project Role:	Graduate Student
Researcher Identifier:	N/A
Nearest person month worked:	1.2
Contribution to Project:	Ms. Zhang performed the Western Blot verifying the expression of ACE2 and TMPRSS2 in both hPSC-derived lung cells and other cell lines.

HAS THERE BEEN A CHANGE IN THE ACTIVE OTHER SUPPORT OF THE PD/PI(S) OR SENIOR/KEY PERSONNEL SINCE THE LAST REPORTING PERIOD?

The following previously active grants have closed since the last reporting period.

Title of the project	Identification of Progenitors in Lung Organoid from Human Pluripotent Stem Cells
Project number	N/A
Level (%) of effort	30%
Performance period	07/01/2018-06/30/2021
Supporting agency	Francis Family Foundation (PI: Chen)
Supporting agency POC	Shari Hockenbery shari@francisfoundation.org 800 West 47th St. Ste. 717 Kansas City, MO 64112
Specific aims/tasks	Aim 1: Characterization of the P63+Krt5+CD104+ cells in d170 MLBOs. Aim 2: Propagation of the P63+Krt5+CD104+ cells in d170 MLBOs.
Goals of the project	To study if the cells identified in the human lung organoids are the human equivalent of the distal stem cells observed in others in injured mouse lungs.

Title of the project	Roles of hypoxia and Wnt signaling in progenitor cell fate decision during lung injury
Project number	N/A
Level (%) of effort	20%
Performance period	07/01/2020-06/30/2021
Supporting agency	Baxter Foundation (PI: Chen)
Supporting agency POC	
Specific aims/tasks	Aim 1: To investigate differentiation potentials of human SAEPs from lung organoids and in vitro expansion.

	Aim 2: To identify cell surface marker(s) for human SAEPs and investigate mechanisms that promote AT2 differentiation
Goals of the project	The goal of the proposed study is to determine the differentiation potential of human small airway epithelial progenitors (SAEPs) towards AT2 and Krt5+ cells.
Title of the project	Development of a host PIKFYVE kinase inhibitor for the treatment of COVID-19
Project number	DISC2COVID19-11901
Level (%) of effort	10%
Performance period	06/15/2020-06/14/2021
Supporting agency	National California Institute for Regenerative Medicine (CIRM) (PI: Ichida)
Supporting agency POC	
Specific aims/tasks	Aim 1: Confirm ASR-149's efficacy against SARS-CoV-2 pseudovirus and live replication competent SARS-CoV-2 virus infection in human iPSC-lung type II cells.
	Aim 2: Use antisense oligonucleotides to verify that PIKFYVE inhibition blocks entry of SARS-CoV-2 pseudovirus and live replication competent SARS-CoV-2 virus in human iPSC-lung type II cells.
Goals of the project	The goal of the proposed study is to confirm that our novel lead PIKFYVE inhibitor, ASR-149, blocks the infection of SARS-CoV-2 into human alveolar type II cells.
Title of the project	Development of TMPRSS2 antibody as an antiviral treatment for SARS-CoV-2
Project number	N/A
Level (%) of effort	10%
Performance period	08/01/2020-07/31/2021
Supporting agency	Keck School of Medicine Internal Award

Supporting agency POC	Dawn Muench 1975 Zonal Ave. KAM B41 Los Angeles, CA 90033
Specific aims/tasks	1) Determine cytotoxicity of TMPRSS2 antibodies 2) Screening of TMPRSS2 antibodies against SARS-CoV-2 cellular entry 3) Investigate potential mechanisms of TMPRSS2 antibodies in inhibiting SARS-CoV-2 cellular entry
Goals of the project	The goal of the proposed study is to screen the TMPRSS2 antibodies against SARS-CoV-2 cellular entry to generate preliminary data for extramural funding applications.

The following previously pending grants or grants submitted after last reporting period are now active.

Title of the project	Airway reconstruction via stem cell-based therapy
Project number	PR201225
Level (%) of effort	10%
Performance period	02/01/2021-01/31/2023
Supporting agency	Department of Defense Discovery Award (PI: Chen)
Supporting agency POC	Darrell L. Ellsworth, PhD Science Officer GoldBelt Frontier Supporting the Congressionally Directed Medical Research Programs (CDMRP) United States Army Medical Research Development Command (USAMRDC) Fort Detrick, MD 21702 Email: darrell.l.ellsworth2.ctr@mail.mil
Specific aims/tasks	Aim 1: Determine conditions for de-epithelialization and recellularization of mouse tracheas. Aim 2: Characterize repopulated ivBCs on de-epithelialized tracheas ex vivo. Aim 3: in vivo potential of ivBCs-bioengineered mouse trachea.

Goals of the project	The goal of this proposal focus on the potential of these cells to drive regeneration of injured airways.
Potential overlap with this DoD proposal	None.

Title of the project	Generation of multilineage adrenal gland organoids using human pluripotent stem cells
Project number	1R21HD106118-01
Level (%) of effort	5%
Performance period	07/01/2021-06/30/2023
Supporting agency	The National Institutes of Health R21 (PI: Zeltner)
Supporting agency POC	Glen Nuckolls nuckollg@mail.nih.gov
Specific aims/tasks	Aim 1: Characterize adrenal glands (AG) progenitor cell types from human pluripotent stem cells (hPSCs) in 2D. Aim 2: Optimize the culture conditions for the AG organoid. Aim 3: Investigate human AG specific developmental events. Aim 4: Characterization of AG organoids functionality in vitro, in xenotransplants and at the single cell level.
Goals of the project	The goal of this proposal is to develop complex, multilineage AG organoids using hPSCs
Potential overlap with this DoD proposal	None.

Title of the project	TMPRSS2 as a potential target for treatments of COVID-19 and respiratory infectious viruses in lung
Project number	1R56HL159712-01
Level (%) of effort	25%
Performance period	09/20/2021-08/31/2022
Supporting agency	The National Institutes of Health/NHLBI R56 (PI: Chen)

Supporting agency POC	Sara Lin sara.lin@nih.gov
Specific aims/tasks	Aim 1. Test efficacy of AL20 for blocking entry of SARS-CoV-2 in lung cells.
Goals of the project	The goal of this project is to establish a model to study live SARS-CoV-2 using the lung organoid model and determine if TMPRSS2 is an effective target to block SARS-CoV-2 infection.
Potential overlap with this DoD proposal	None.

Title of the project	Development of Monothiol Human Thioredoxin-1 (ORP100S) as an Inhaled Treatment for Acute Viral Lung Injury
Project number	PR210859
Level (%) of effort	10%
Performance period	02/01/2022-01/31/2023
Supporting agency	Department of Defense (PI: Heifetz)
Supporting agency POC	Robin Walker, PhD Science Officer, Peer Review Medical Research Program (PRMRP) robin.k.walker5.ctr@mail.mil
Specific aims/tasks	Specific Aim 1: In vitro pharmacodynamics of ORP100S in SARS-Cov-2, influenza and bleomycin acute lung injury models. Specific Aim 2: In vivo pharmacodynamics of ORP100S in a Syrian golden hamster SARS-CoV-2 postexposure prophylaxis infection model. Specific Aim 3: In vivo pharmacodynamics of inhaled ORP100S in an oropharyngeal mouse influenza model. Specific Aim 4: Development and validation of a hybrid immunocapture-LC-MS/MS bioanalytical method for quantitation of ORP100S in BALF and serum/plasma matrices from rat and NHP.

Goals of the project	Our overall DoD study objective is to establish proof of concept for subsequent translational evaluation of non-systemically acting, topically-delivered monothiol human thioredoxin variant ORP100S as a potential treatment for ARDS and other manifestations of severe COVID-19 disease. In particular, we will be in charge to investigate in Aim 1 the ability of ORP100S to protect human AT2 cells, the stem cell of alveoli, following infection with SARS-CoV-2 and influenza, including reduction of cytokine storm and inflammation vs. controls in stem cell-derived three-dimensional lung bud organoids. To better understand effects of ORP100S we will compare and contrast the ability of ORP100S to protect against SARS-CoV-2- and influenza with bleomycin-mediated acute lung injury
Potential overlap with this DoD proposal	None.

WHAT OTHER ORGANIZATIONS WERE INVOVLED AS PARTNERS?

Nothing to report.

8. Special Reporting Requirements

COLLABORATIVE AWARDS:

Nothing to report.

QUAD CHARTS:

Nothing to report.

9. Appendices