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PRINCIPAL INVESTIGATOR: Sumit K Chanda, Ph.D.

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14. ABSTRACT The overall goal of DABRA is to disrupt the paradigm for the treatment of respiratory viral infection through the development of broad-spectrum inhibitors. These antivirals will effectively treat infections caused by multiple respiratory viruses that impact military readiness, including influenza (A and B), coronaviruses, human rhinoviruses, respiratory syncytial virus, and Adenoviruses. In the longer term, we expect these efforts to provide critical proof-of-concept studies that will pave the way for development broad-spectrum therapies for other classes of (non-respiratory) viruses.					
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1. INTRODUCTION:

To overcome the current challenge of treating respiratory viral infections that negatively impact military readiness, we propose to develop first-in-class broad-spectrum respiratory antiviral therapies. Towards this end, we hypothesize that host-directed compounds that either inhibit dependency factors required for viral replication or activate cellular innate immune programs will provide safe and effective therapeutic activity to achieve this goal.

2. KEYWORDS:

DABRA (Development and Advancement of Broad-spectrum Respiratory Antivirals), IND (investigational new drug), IAV (influenza A virus), SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), FDA (Federal Drug Administration), IC50 (50 % inhibitory concentration)

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The goal of Dr. Chanda's laboratory in Year 1 is to investigate antiviral breadth and characterize antiviral response of four candidate compounds M4, M85, KIN269, and KIN1502 plus one additional compound KIN 1400. Antiviral activities of the compounds against SARS-CoV-2 was investigated according to the expansion of scope.

Project 1 Specific Aim 2 Major Task 1 : Investigate antiviral breadth (40% completion)

What was accomplished under these goals?

1) Major activities;

Antiviral activity of M4, M85, M85 derivatives, KIN269, KIN1502, and KIN1400 against BSL2 influenza A (IAV) virus was tested using A549 cells. Antiviral activity of the candidate compounds against SARS-CoV-2 was also tested using Vero E6, 293T-ACE-2, and Hela-ACE2 cells.

According to the expansion of scope, clofazimine was evaluated in vitro and in vivo as a repurposing drug candidate for the treatment of SARS-CoV-2 infection.

2) Specific objectives;

Evaluate the antiviral activity of candidate compounds against IAV and SARS-CoV-2.

3) Significant results;

Antiviral activities of M4, M85, and M85 derivatives were tested against Influenza A WSN. Oseltamivir (Tamiflu) was used as a positive control. M85 and M4 inhibited the infection of A549 cells with the IC₅₀ values of Oseltamivir (Tamiflu), M85, and M4 in A549 cells were 0.6 ~ 19 nM, 12 ~ 39 nM, and 181 nM, respectively (Figure 1). The IC₅₀ values of M85 derivatives and M85 structure-activity relationship is reported in Olson Lab's part.

Antiviral activities of M4, M85, and M85 derivatives, against SARS-CoV-2 were tested. M4 and M85 did not inhibit the infection of Vero E6 cells with SARS-CoV-2. These 2 compounds inhibited the infection of 293T-ACE2 cells. The IC₅₀ values of M4 and M85 in 293T-ACE2 cells were 2.0 and ~1.3 μ M, respectively. M4 and M85 hardly inhibited the infection of Hela-ACE2 cells. The IC₅₀ values of M4 and M85 in Hela-ACE2 cells were 5.8 and ~0.42 μ M, respectively (Figure 2).

The Chanda Lab also continued efforts related to the expansion of scope to evaluate clofazimine as a part of ReFRAME repurposing screen against SARS-CoV-2. Clofazimine was one of the hit compounds from ReFRAME screen and selected as a repurposed drug candidate for potential treatment of COVID-19. Clofazimine has been found to be well tolerated in humans, showing a desirable safety profile at doses of 200 mg/day in human, a C_{max} of > 861nM, and a selectivity index (CC₅₀/EC₅₀) around 30~50 against SARS-CoV-2 infection.

Clofazimine reduced MERS-CoV replication in VeroE6 cells with an EC₅₀ of 1.48 $\pm$ 0.17 μ M (Figure 3a). Immunofluorescence staining for MERS-CoV NP illustrated effective suppression of virus infection in cells upon clofazimine treatment (upper panel, Figure 3b), and demonstrated by the flow cytometry analysis that the percentage of MERS-CoV-infected cells after clofazimine treatment decreased from 44.6% (DMSO) to 23.0% (clofazimine) at 24 hours post-infection (hpi) in human hepatocellular Huh7 cells (lower panel, Figure 3b). Using SARS-CoV-2 or MERS-CoV infection as models, we characterized the antiviral activity of clofazimine in a more physiologically human embryonic stem cell-derived cardiomyocytes (CM) and human primary small airway epithelial cells (HSAEpC) that robustly support SARS-CoV-219 and MERS-CoV20 replication, respectively. In a dose-dependent manner, 10 μ M clofazimine treatment reduced SARS-CoV-2 titer by >3-log₁₀ and that of MERS-CoV by 2-log₁₀ when compared with the DMSO control (Figure 3c). Next, we assessed the antiviral activity of clofazimine in an ex vivo lung culture system. Donor lung tissues were infected with SARS-CoV-2 or MERS-CoV for 24 h with drug treatment starting at 2 hpi. The results revealed that clofazimine potently antagonized viral replication in human lung tissues that reflect the primary site of SARS-CoV-2 and MERS-CoV replication (Figure 3d). To explore whether clofazimine confers cross-protection against other epidemic and seasonal CoVs, we performed viral load reduction assays for SARS-CoV-1, hCoV-229E and hCoV-OC43 in corresponding cell lines that support virus replication. Under the non-toxic concentration, viral yields in the cell culture supernatant was decreased by ~2-log₁₀ in SARS-CoV-1-infected VeroE6 cells, by ~4-log₁₀ in hCoV-229E infected-human embryonic lung fibroblasts (HELFI), and by ~3-log₁₀ in hCoV-OC43-infected monkey kidney cells BSC1 (Figure 3e). Importantly, clofazimine showed negligible cytotoxicity in the matching cell line and concentrations as described above. Overall, clofazimine exhibited broad-spectrum anti-CoV efficacy, and antagonized both SARS-CoV-2 and MERS-CoV replication in human primary cell and ex vivo lung models.

To understand the impact of clofazimine on the viral life cycle, antiviral activity was first evaluated by a time of addition assay in a single infectious cycle. Treatment with clofazimine during inoculation strongly inhibited SARS-CoV-2 infection, indicating that clofazimine exerts inhibitory effect on viral entry. Intriguingly, clofazimine also blocked SARS-CoV-2 infection at a post-entry step as evidenced by the observed reduction of viral infection when clofazimine was added at 5 hours post-inoculation (Figure 4a). To further evaluate the impact of clofazimine on viral entry, we employed vesicular stomatitis virus (VSV) based SARS-CoV-2 S pseudotyped virions. Clofazimine treatment dramatically reduced the infectivity of both SARS-CoV-1 S and SARS-CoV-2 S pseudotyped virions in VeroE6 cells. Interestingly, clofazimine did not impact MERS-CoV S pseudotyped virus particles (Figure 4b), and this lack of entry inhibition may contribute to a lower potency observed for MERS-CoV. To confirm clofazimine also inhibits post-entry steps of viral replication, we evaluated the impact of clofazimine on viral RNA production by electroporating in vitro transcribed viral RNA into VeroE6 cells, which enables to bypass clofazimine-mediated inhibition on the entry process, and directly measure RNA synthesis (Figure 4c). As expected, remdesivir could effectively reduce the negative-stranded RNA in a dose-dependent manner (Figure 4d). Intriguingly, viral RNA levels were also reduced by 1~1.5 logs in the cells treated with clofazimine at high concentration (above 5 μ M) (Figure 4e). However, no significant effect was observed on electroporated GFP mRNA translation (Figure 4f). Collectively, these results demonstrated that clofazimine blocked SARS-CoV-2 infection by interfering with spike-mediated entry, as well as viral RNA replication, likely through inhibition of multiple targets.

Clofazimine is useful for the treatment of disease due to multidrug resistant *M. tuberculosis*, as well as leprosy and certain chronic skin diseases. Previous pharmacokinetics studies revealed that clofazimine absorption varies from 45 to 62% following oral administration in leprosy patients. Co-administration of a 200 mg dose of clofazimine with food resulted in a C_{max} of 0.41 mg/L (equivalent to 0.861 μ M) with a T_{max} of 8h. Administered in a fasting state, however, the corresponding C_{max} of clofazimine was 30% lower while the time to C_{max} was 12h. Intriguingly, clofazimine exerts anti-inflammatory properties due to the suppression of macrophage activity, which may further mitigate the high levels of inflammatory cytokines and chemokines reported in severe COVID-19 in addition to its direct antiviral effects. To determine the in vivo antiviral efficacy of clofazimine, we employed a golden Syrian hamster model that serves as a suitable tool to study antiviral effects and disease pathogenesis. Since administration of clofazimine with a high fat meal provides better bioavailability, we delivered the drug through oral route utilizing corn oil as vehicle. To faithfully simulate the prescribed human dosage, an equivalent hamster dose of 25 mg/kg/day was converted based on body surface area. Clofazimine given on 3 consecutive days exhibited no significant observable toxicity to the animals. Remdesivir was included as a positive control drug and dosed at 15mg/kg/day via intraperitoneal route based on its effective dosage in SARS-CoV-infected mice.

Clofazimine has a relatively long duration of action with the mean elimination half-life approximately 25 days, thus we performed both prophylactic and therapeutic regimens with hamsters intranasally challenged with 105 PFU of SARS-CoV-2 (Figure 5a). Expectedly, the vehicle-treated control hamsters developed the clinical signs of lethargy, hunched back posture, and rapid breathing starting from 2 dpi, whereas the hamsters treated with clofazimine did not develop clinical signs. After clofazimine treatment, less body weight loss was observed from 1 dpi to 11 dpi in prophylaxis group and 5 dpi to 9 dpi in therapeutic group, respectively ($p < 0.05$). Oral administration of clofazimine post-virus-challenge shortened the time of body weight rebound from 6 dpi to 3 dpi (Figure 5b).

At 4 dpi when viral loads peaked with significant histopathological changes, clofazimine decreased virus plaque forming units in lung tissues by ~ 1 to $2 \cdot \log_{10}$ (Figure 5c). Consistently, suppression of SARS-CoV-2 viral gene copies in hamster lungs were confirmed in the clofazimine-treated hamsters (Figure 5d). Besides, the capacity of clofazimine to diminish SARS-CoV-2 shedding were evaluated by nasal wash (upper respiratory tract) and fecal (gastrointestinal tract) samples. At 4dpi after remdesivir or clofazimine therapy, no significant reduction of infectious virus titer in the hamster nasal wash was detected; whereas an average of 2-fold reduction was achieved with prophylactic clofazimine administration ($p < 0.05$, Figure 5e). Irrespective of prophylactic or therapeutic clofazimine administration, significantly lower viral loads were found in the animal feces at 4 dpi when compared with the vehicle group, probably due to the high local concentration of clofazimine in the hamster gastrointestinal tract after oral administration (prophylaxis $p < 0.05$ and therapeutic $p < 0.001$, Figure 5f). Remdesivir, however, did not alleviate the virus shedding from the feces. Overall, we demonstrate in vivo that clofazimine antagonizes SARS-CoV-2 replication in the lung and provides partial reduction of virus shedding in feces.

To ascertain if clofazimine improves the disease pathogenesis, we determined the serum IL-6 level that has been correlated with respiratory failure and adverse clinical outcome. Substantially decreased serum IL-6 level was generally detected in both clofazimine and remdesivir groups when compared with the vehicle control (Figure 5g). Previous reports have shown that clofazimine can inhibit lymphocyte function in cell culture. To explore if this is the case in the hamster model, animal sera were collected at 14 dpi for measuring anti-NP antibody by ELISA-based enzyme immunoassay. Apparently, similarly high levels of antibody responses were triggered in vehicle and clofazimine groups, indicating insignificant suppression of humoral immune response of B lymphocyte by clofazimine while the viral replication was reduced (Figure 5h).

Furthermore, RNA-seq of hamster lung tissues revealed that 73 genes were significantly up-regulated (fold change > 1.5 , $p < 0.05$ in prophylactic clofazimine administration group comparing with vehicle controls at 4 dpi. Out of the 73 up-regulated genes, 34 (46.6%) were mapped to immune response related biological processes by Gene Ontology analysis, indicating that a stronger host immune response against SARS-CoV-2 was induced when clofazimine was used as a prophylactic agent. Within the 34 genes, 13 genes were mapped to “Leukocyte differentiation” GO category. Interestingly, two major histocompatibility complex (MHC) class II molecules, i.e. H2-Aa and H2-Ab1 were up-regulated by clofazimine, implying that clofazimine might potentiate the antigen presenting capacity during infection. In addition, transcription factors Fos, Junb and Egr1 were also on the list of upregulated genes, which is consistent with the transcriptomic analysis on Caco-2 cells treated by clofazimine. When clofazimine was applied after infection, most of these genes also showed a trend of up-regulation compared with vehicle controls, though the change was less significant than that of prophylactic clofazimine. However, most of the immune response-related genes were not changed after administering clofazimine to uninfected. These results indicate that clofazimine may prime the animal host for better control of SARS-CoV-2 infection without undue activation of host immune system.

To determine the severity of lung damage at 4dpi, histological examination of hematoxylin and eosin (H&E) stained lung tissues was performed. Control hamster lungs after prophylactic or therapeutic vehicle showed severe pathological changes, which were evidenced by large areas of consolidation and massive alveolar space infiltration. Apparently, cell infiltrations were also observed in endothelium of blood vessel as well as perivascular and peribronchiolar regions (black arrows, Figure 5i).

In contrast, clofazimine or remdesivir-treated lung exhibited improved morphology and milder infiltrations. Generally, prophylactic administration conferred more dramatic improvements of lung pathology when compared with therapeutic administration, which might be attributed to the relatively long T_{max} of clofazimine. Nevertheless, both prophylactic and therapeutic treatment with clofazimine reduced SARS-CoV-2 disease in the lung. Taken together, clofazimine conferred protection against SARS-CoV-2 challenge by reducing the virus replication and the associated inflammatory dysregulation.

Remdesivir is considered the standard of care for the treatment of COVID-19. To understand the impact of combinatorial treatment of remdesivir and clofazimine on SARS-CoV-2 replication, we conducted a matrixed dose response analysis. We found that co-application of clofazimine and remdesivir impacts SARS-CoV-2 replication in a manner that extends beyond the additive combinatorial activity predicted by the Bliss independence model (maximal Bliss Synergy Score of 44.28, Figure 6a), which indicates that these two drugs harbor a synergistic antiviral relationship. Clofazimine can be safely dosed at 200 mg/day for the treatment of leprosy, which results in average serum concentrations of 1.79 μ M, although the bioavailable fraction of the molecule will be a function of plasma protein binding. The addition of 1.25 μ M clofazimine in an in vitro cell assay with a 10% concentration of FBS resulted in a nearly 20-fold decrease in concentrations of remdesivir required to inhibit viral replication by 90% (Figure 6b). Importantly, the combination of drugs did not elicit additional cellular cytotoxicity (Figure 6c).

To explore their antiviral synergy in vivo and to recapitulate the scenario that most COVID-19 patients will be treated after disease onset, SARS-CoV-2-infected hamsters were given oral clofazimine and intraperitoneal remdesivir together, with the first doses given 24 h after virus challenge. As a control, 15 mg/kg/day remdesivir was given as standard dosing. In the combinatorial group, 10-fold lower dosing, i.e. 1.5 mg/kg/day remdesivir and reduced clofazimine dosing, i.e. 15mg/kg/day (corresponding to 100 mg/human/day) were given (Figure 6d). Generally, co-administration of the two drugs ameliorated the SARS-CoV-2 induced weight loss at 3 dpi ($p < 0.05$). Similar to the vehicle control, none of the monotherapy groups including the standard dosing remdesivir group achieved any clinical benefit. At 4 dpi, significant improvement of weight loss was observed in remdesivir + clofazimine group, when compared with the vehicle control ($p < 0.01$) or low dose remdesivir group ($p < 0.05$, Figure 6e). Monotherapy of low dosing remdesivir caused marginal reduction of lung virus titer; additional application of clofazimine, however, not only exhibited potent synergy and inhibited virus replication by $\sim 2\text{-log}_{10}$ ($p < 0.01$, Figure 6f), but also restricted virus replication in the focal bronchiolar epithelial cells from spreading to alveolar areas. Importantly, the synergistic effect between remdesivir and clofazimine suppressed virus shedding in the nasal wash (Figure 6g), which was not achievable with mono- and therapeutic-remdesivir or clofazimine treatment (Figure 6e). The result is also evidenced by the immunofluorescence staining of hamster nasal turbinate with significantly diminished NP antigen expression ($p < 0.01$) as observed in the epithelium after the combinatorial treatment (Figure 6h and 6i). Taken together, the antiviral synergy between remdesivir and clofazimine significantly improve the effect of monotherapy, with reduced body weight loss, suppressed pulmonary virus titer, and nasal virus shedding, as well as decreased drug dosages.

Given the suboptimal clinical efficacies for Remdesivir, potential issues of shortages due to the complex synthesis steps required to manufacture the compound (Jarvis C&EN 2020) possible adverse events or toxicity issues (Singh Diabetes Metab Syndr 2020; Wang Lancet 2020), and likely emergence of resistance to a monotherapy (Ledford Nature 2020), our primary objective is to have an effective, safe, inexpensive, easily manufactured, FDA approved therapeutic that can be repurposed for the treatment of SARS-CoV-2 infections. In view of the potentially long pandemic dynamics and pressures on healthcare capacity, our in vivo proof of concept data within animal models for SARS-CoV-2, as well as the cost, safety, tolerability data for clofazimine, it should be considered as one of the countermeasures for global control of the COVID-19 pandemic (Kissler Science 2020). Remdesivir at US \$3,120 per treatment course, may not be affordable in developing countries and is given by IV infusion and is therefore only for inpatient treatment. Routine administration of clofazimine (US \$1.43 per 100 mg/day) can be administered orally and thus as an outpatient COVID-19 prophylactic option would greatly relieve the critical care burden worldwide. It is currently manufactured by Novartis and distributed by governments and NGOs around the world. We have submitted applications to Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV), BARDA, and the COVID-19 Early Treatment Fund to obtain funding to initiate clinical trials with Clofazimine for COVID-19. Establishing clinical efficacy of Clofazimine within Phase II trials could represent a game changing option for managing this pandemic globally.

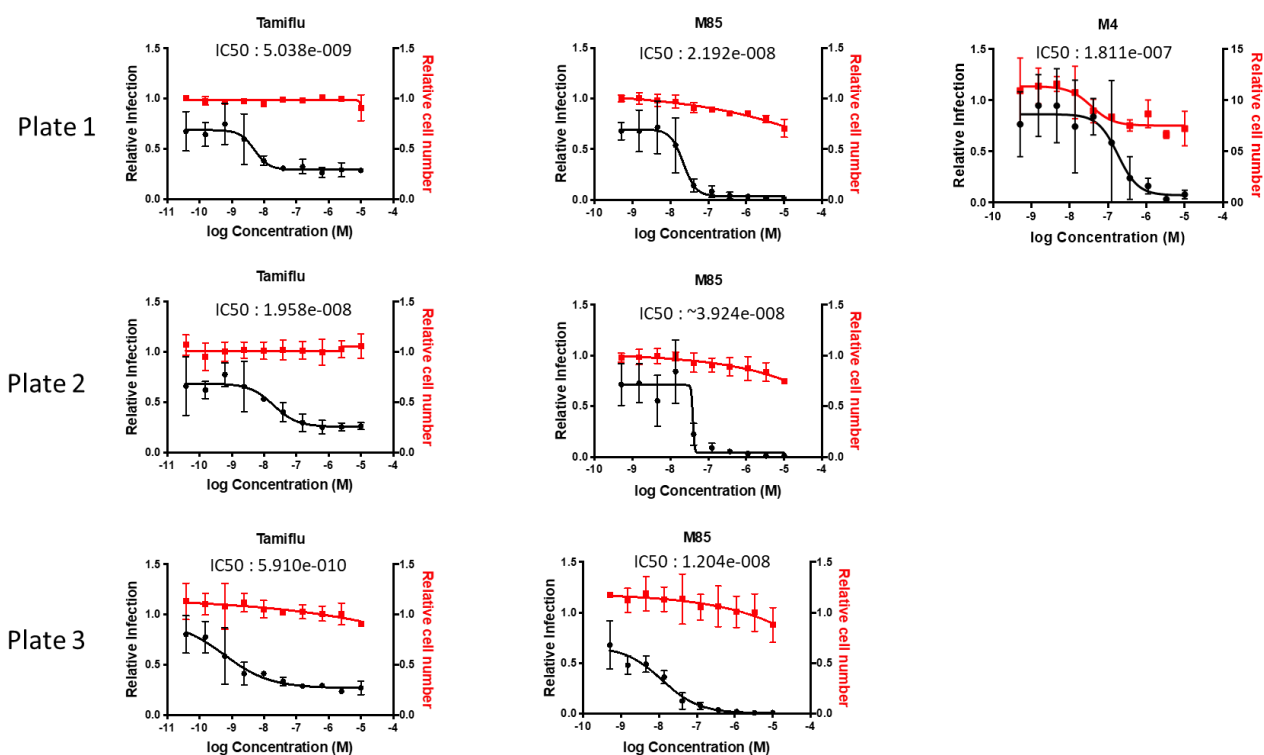
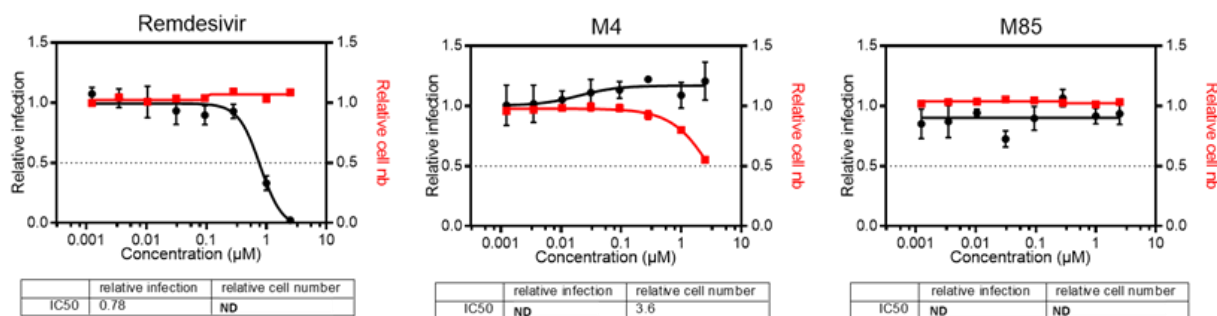
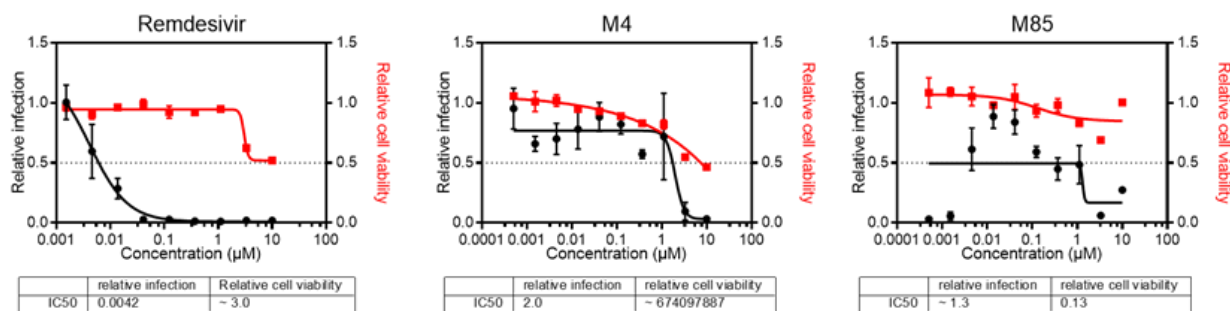


Figure 1. Dose-response relationships of M4, M85, and Osetamivir (Tamiflu). A549 cells were pre-treated for 16 h with increasing concentrations of the indicated compounds and then infected with influenza A WSN in the presence of the compound. Thirty six hours of post-infection, cells were fixed, and immunofluorescence imaging was performed. For each condition, the percentage of infection was calculated as the ratio between the number of infected cells stained for NP and the total amount of cells stained with DAPI.

SARS-COV2: VERO E6 CELLS



SARS-COV2: 293T-ACE2 CELLS



SARS-COV2: HELA-ACE2 CELLS

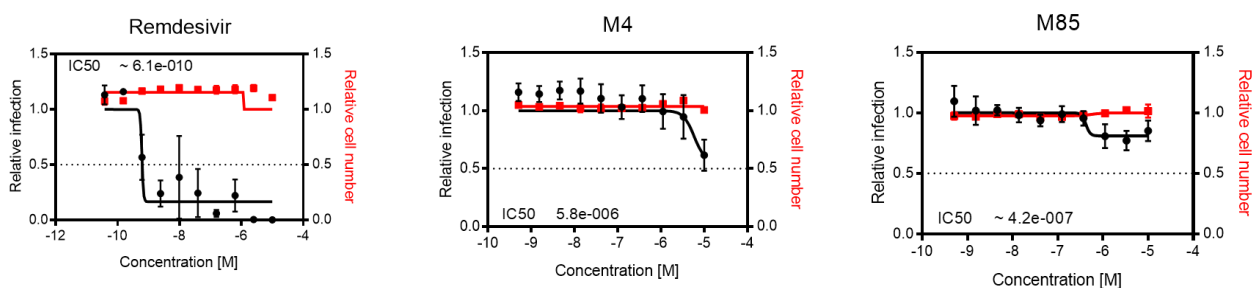


Figure 2. Dose-response relationships of M4, M85, and Remdesivir. Vero E6 cells, 293T-ACE2 and HeLa-ACE2 cells were pre-treated for 16 h with increasing concentrations of the indicated compounds and then infected with SARS-CoV-2 in the presence of the compound. Twenty four hours of post-infection, cells were fixed, and immunofluorescence imaging was performed. For each condition, the percentage of infection was calculated as the ratio between the number of infected cells stained for CoV NP and the total amount of cells stained with DAPI.

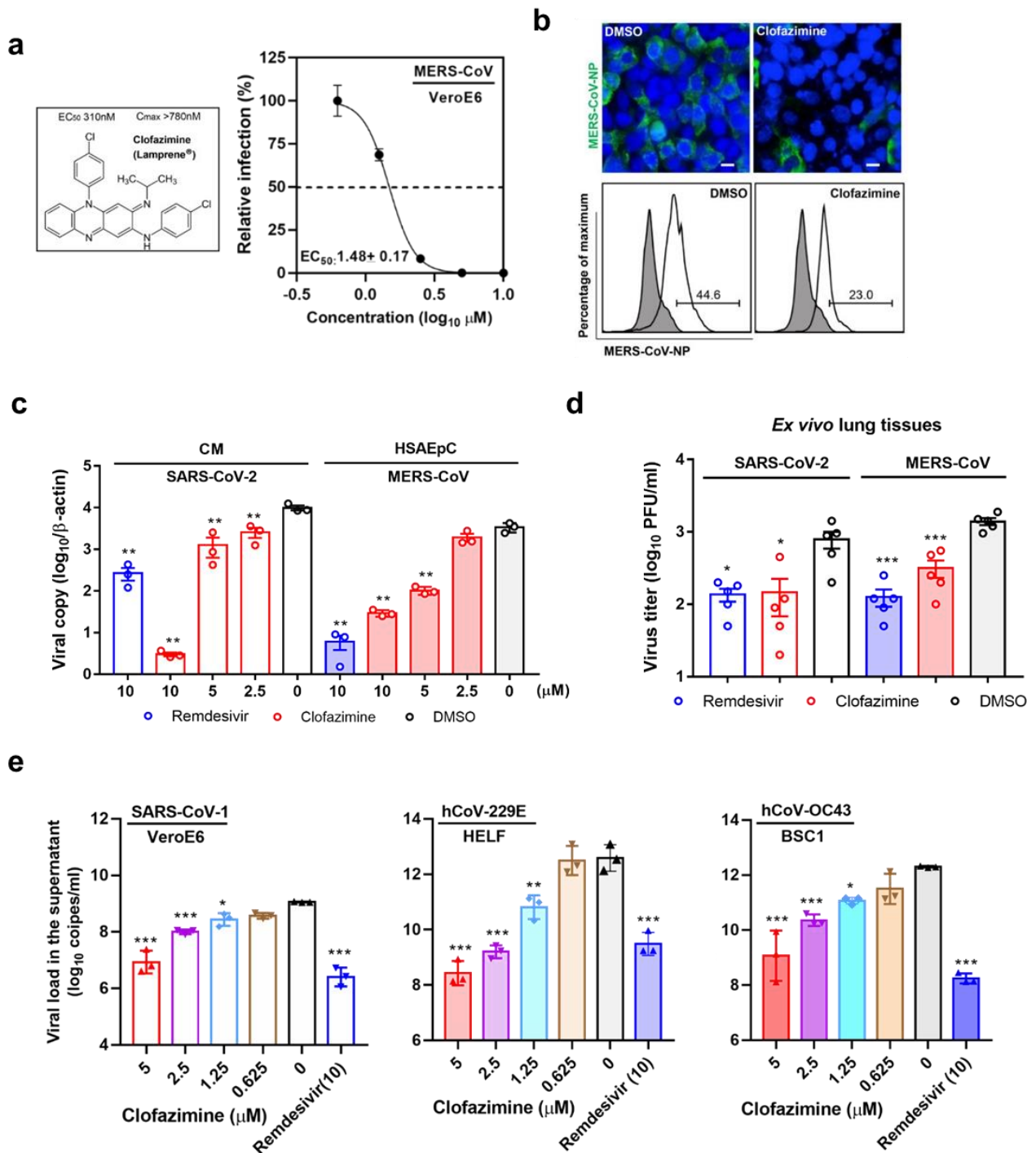


Figure 3. Clofazimine inhibited a broad-spectrum of human-pathogenic CoVs replication in human cellular models. (a) Chemical structure of clofazimine (brand name Lamprene) has an EC₅₀ of 310 nM against SARS-CoV-2 and peak serum concentration C_{max} >780 nM through oral administration in patients¹⁶. Besides, clofazimine inhibited MERS-CoV replication in a dose-dependent manner. EC₅₀ was achieved by plaque reduction assay on VeroE6 cells and plotted using logistic non-linear regression model (GraphPad). (b) MERS-CoV-infected Huh7 cells (0.01 MOI) were treated with or without clofazimine (5 μM) for 24 h. Upper panel: immunofluorescence staining of MERS-CoV-NP antigen (green), and Huh 7 cell nucleus (blue). Scale bar: 20μm. Lower panel: MERS-CoV-NP positive cells quantitated by flow cytometry. The experiments were performed twice with representative images and quantifications shown.

(c) Clofazimine inhibited SARS-CoV-2 (0.1 MOI) replication in human primary CMs and MERS-CoV (1MOI) replication in human primary HSAEpCs. Cell lysates were collected at the 24hpi with viral load determined by RT-qPCR assays. Data represent mean $\pm$ SD for n=3 biological replicates. One-way ANOVA when compared with DMSO group (0 μ M). **p<0.01. (d) Ex vivo human lung tissues were infected with SARS-CoV-2 or MERS-CoV-2 with an inoculum of 5 $\times$ 10⁵ PFU. After two hours, the inoculum was removed before clofazimine (10 μ M) or remdesivir (10 μ M) or DMSO (0.1%) was added and incubated for 24 h. Supernatants were collected for quantification of viral titer by plaque assay. Error bars represent SD for n=5 biological replicates. Student's t-test. *p<0.05 and ***p<0.001. (e) Antiviral activity of clofazimine against SARS-CoV (0.01MOI, 48hpi), HCoV-229E (0.001MOI, 72hpi), and HCoV-OC43 (0.001MOI, 72hpi) in cell lines as indicated. Viral load in the cell culture supernatant was quantified by RT-qPCR assays. Data represent mean $\pm$ SD for n=3 biological replicates. One-way ANOVA when compared with 0 μ M group, ***p<0.001, **p<0.01 and *p<0.05.

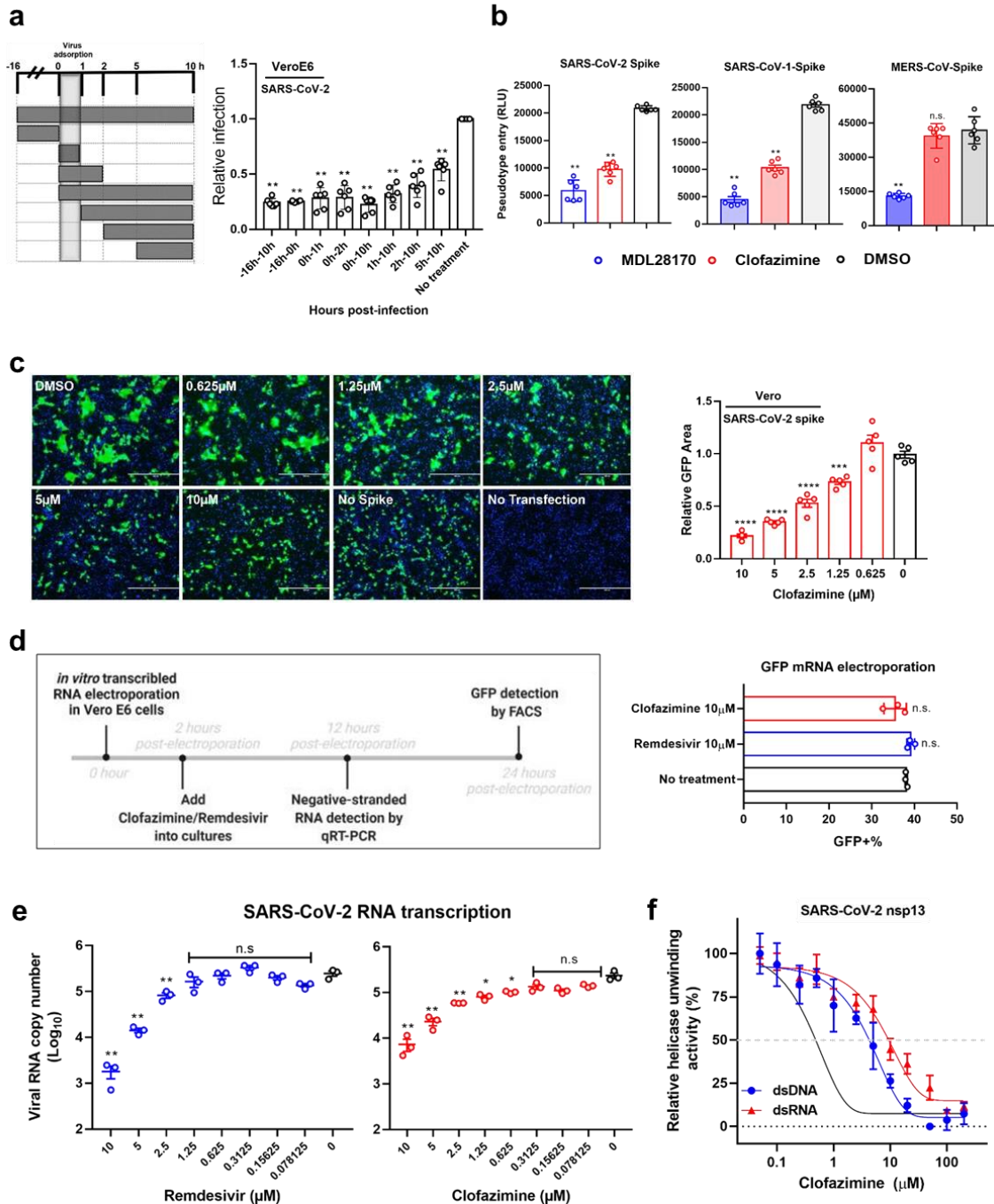


Figure 4. Clofazimine interferes with multiple steps of virus life cycle. (a) Time-of-addition assay. VeroE6 were inoculated with SARS-CoV-2 for 1 h, and the inoculum was then removed. Cells were also incubated with clofazimine at a concentration of 5 μ M and at time-points indicated. Infection at 10 hpi was quantified by immunostaining for CoV NP. Data are normalized to the average of DMSO-treated wells for each corresponding time-point and are presented as mean $\pm$ SD for n=6 independent experiments. Two-way ANOVA followed by Tukey post-hoc test was performed as statistical analysis. (b) VSV-based pseudotyped viral particles assay. VeroE6 cells were pre-treated with the indicated compounds (2.5 μ M) for 2 h and then infected with SARS-CoV-1 S or SARS-CoV-2 S or MERS-CoV S pseudotyped particles harboring firefly luciferase for additional 2 h. Luciferase signals were quantified at 24 h post-inoculation. Error bars represent SEM for n=6 independent experiments. One-way ANOVA followed by Dunnett post-test was performed for statistical analysis. * p < 0.05, ** P < 0.01. MDL28170 was utilized as a positive control entry inhibitor. (c) Clofazimine inhibited SARS-CoV-2 spike-mediated cell-to-cell fusion. Vero cells were co-transfected with 1 μ g SARS-CoV-2 spike plasmid and 0.4 μ g EGFP plasmid for 8 h before treated with clofazimine or DMSO (0.1%) as indicated concentration. Confocal images were acquired at 48 h post-transfection. Scale bar: 400 μ m. Quantification of the inhibitory effect is based on the GFP positive area using ImageJ software. Error bars represent SEM for n=5 randomly selected images. **** p < 0.0001 and *** p < 0.001 when compared with the 0 μ M (DMSO) group by One-way ANOVA. (d-e) In vitro transcribed viral RNA based on viral replication assay. VeroE6 cells were electroporated with in vitro transcribed viral RNA. At 2 h after seeding, the cells were treated with (e) remdesivir (blue circles) or clofazimine (red circles) at the indicated dose. Negative-stranded RNA was then quantified at 12 h post-electroporation. Error bars represent SEM for n=3 independent experiments. GFP mRNA was utilized as a negative control and its translation was not affected by remdesivir or clofazimine. Error bars represent SEM for n=3 independent experiments. * P < 0.05, ** P < 0.01, n.s. > 0.05 when compared with the 0 μ M group by One-way ANOVA. (f) Titration of the DNA-unwinding activity and RNA-unwinding activity of the SARS-CoV-2 helicase by clofazimine at varying concentrations as indicated by a FRET-based assay. The black curve represents a positive control inhibitor (ranitidine bismuth citrate) using DNA-based substrate. Data represent mean $\pm$ SD for n=3 biological replicates. For clarify, mean and SD values of the control inhibitor are not shown.

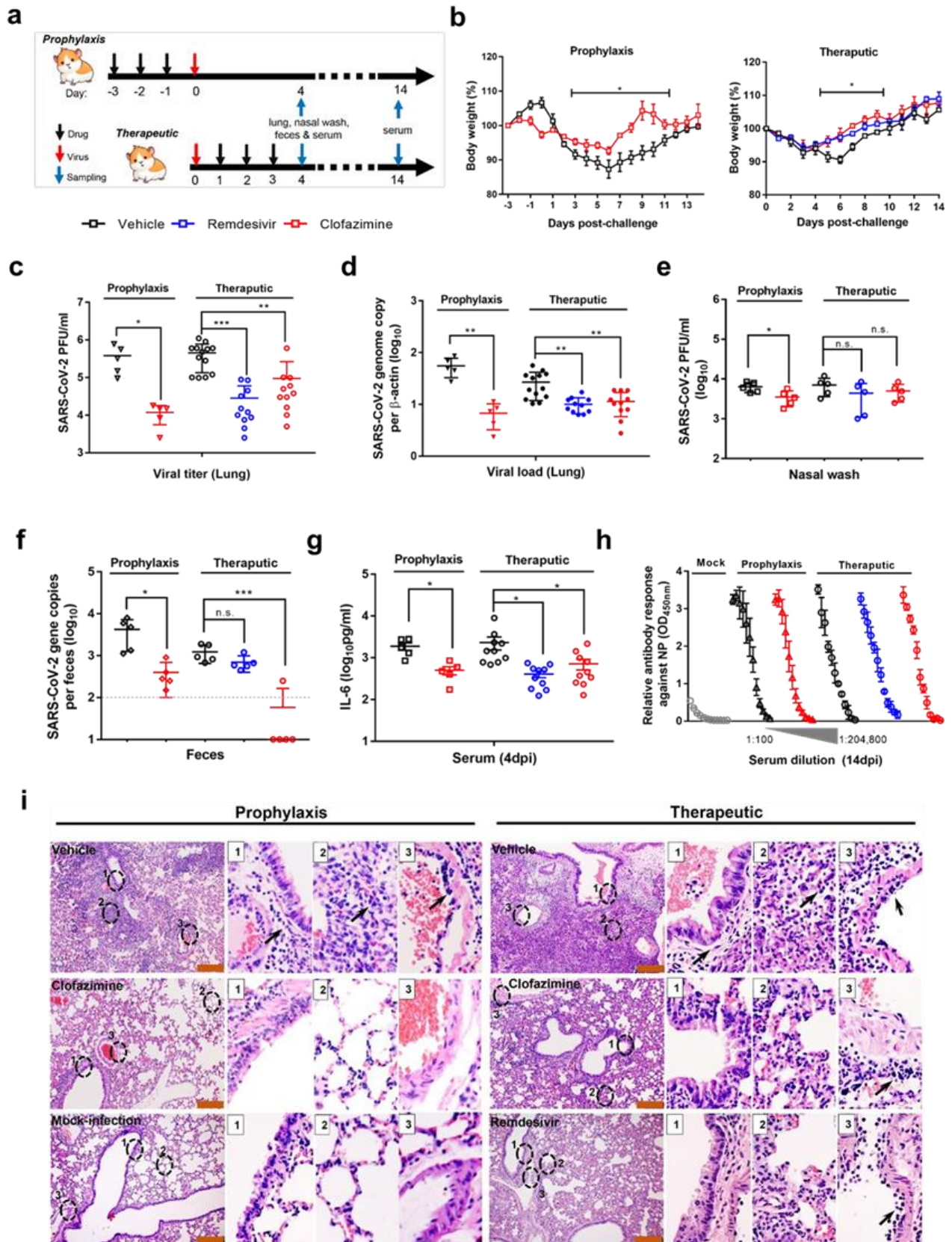


Figure 5. Prophylactic and therapeutic treatment with clofazimine reduces SARS-CoV-2 disease. (a) Experimental design: prophylactic treatment used oral administration of clofazimine given at -3, -2 and -1 dpi (25 mg/kg/day), followed by virus challenge at 0dpi through intranasal route (105 PFU/hamster); therapeutic post-exposure administration of clofazimine was performed at 1, 2, and 3 dpi applying the same drug dosage and virus inoculum. Lung tissue, nasal wash, fecal and serum samples were collected at indicated days post-infection. Remdesivir (15mg/kg, blue symbols) was included as a control in the therapeutic regimen through intraperitoneal injection. (b) Daily body weights of the hamster (n=3). Asterisks indicate statistically significant differences ($p<0.05$) as determined by two-way ANOVA and Tukey's multiple comparison test. (c and d) Viral yield in the hamster lung tissue, after prophylactic (n=5) or therapeutic treatment (n=11 or 13), were harvested at 4 dpi and titrated by plaque assay (c) and RT-qPCR assay (d), respectively. Student's t-test for prophylaxis groups and one-way ANOVA for therapeutic groups comparing with the vehicle group (black symbols). (e) Hamster nasal washes, collected on 4 dpi and under hamster anesthesia, were subjected to live virus titration by plaque assays (n=5). (f) Hamster feces, freshly collected at 4 dpi, were subjected to SARS-CoV-2 viral copy detection by RT-qPCR assays (n=5). For statistical analysis purpose, a value of 10~100 was assigned for any data point below the detection limit (the dotted line). (g) The disease severity marker IL-6 level in hamster serum was quantified by ELISA at 4 dpi. (h) Hamster exhibited normal humoral immune response after SARS-CoV-2 infection and clofazimine treatment. Enzyme immunoassay (EIA) for immunoglobulin G against SARS-CoV-2 nucleoprotein was performed in hamster sera of the indicated groups collected at 14 dpi. The sera were serially-diluted (from 1:100 to 1: 204800) before adding to the NP-coated ELISA plate (n=3). (i) Representative images of H&E-stained lung tissue section from hamsters treated with different groups indicated. Numbered circled areas are shown in magnified images to the right, illustrating the severity of (1) bronchiolar and/or peribronchiolar cell death; (2) alveoli destruction and/or alveolar infiltration; (3) blood vessel and perivascular infiltration. Black arrows indicate sites of infiltrations. These representative images were selected from a pool of over 15 images captured in three randomly selected hamsters per group. Scale bar, 200 μm . For all statistical analysis, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

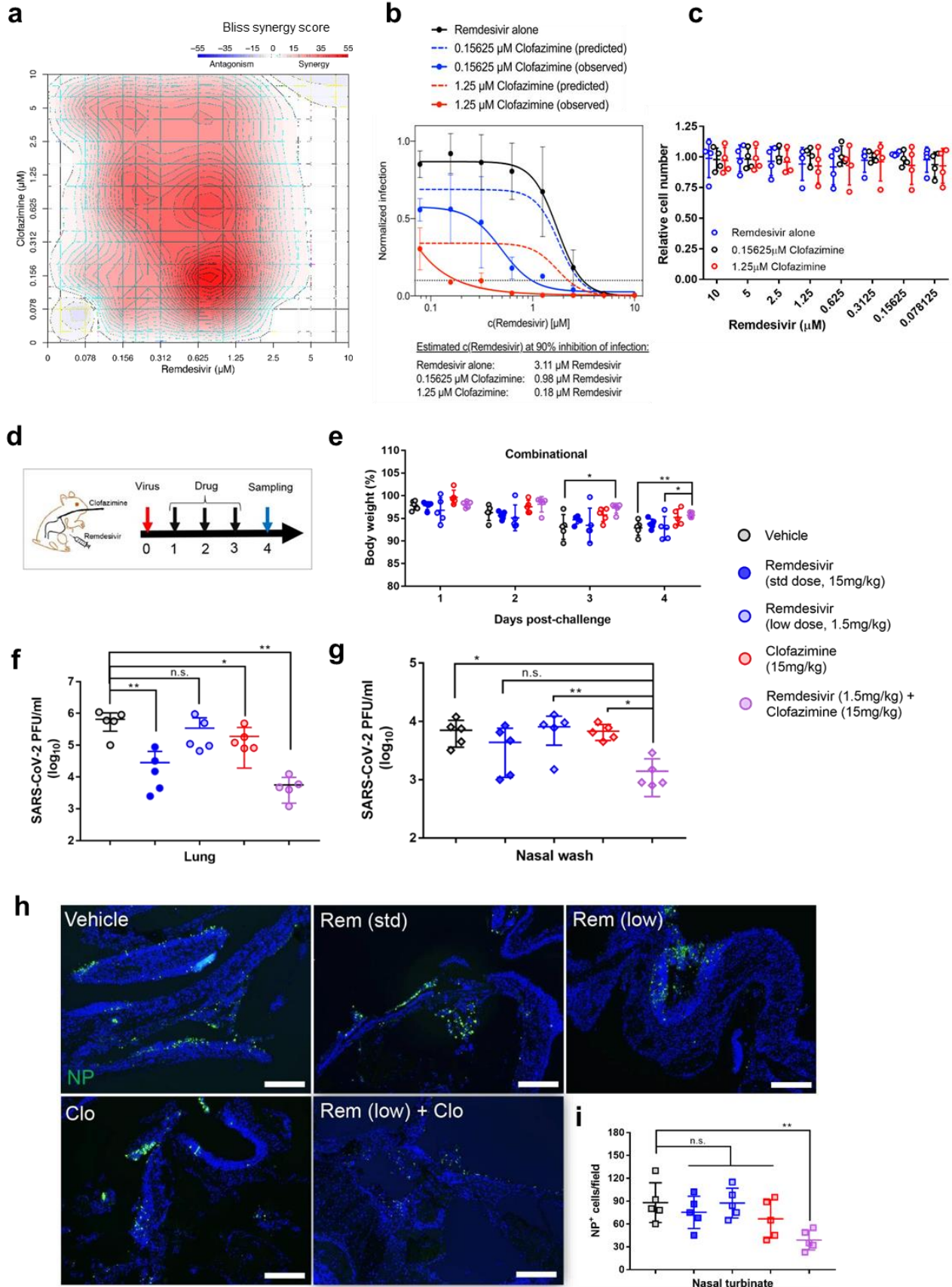


Figure 6. Clofazimine exhibits antiviral synergy with remdesivir in vitro and in vivo. VeroE6 cells were pre-treated for 16 h with increasing concentrations of the indicated compound and then infected with SARS-CoV-2 at a MOI of 0.01. Thirty hours after infection, cells were fixed and analyzed by immunofluorescence imaging. For each condition, the percentage of infection was calculated as the ratio of the number of infected cells stained for SARS-CoV-2 NP to the number of cells stained with DAPI. (a) Topographic two-dimensional map of synergy scores determined in synergy finder. Color gradient indicates synergy score (red–highest score). X-axis: remdesivir up to 10 μ M, y-axis: clofazimine up to 10 μ M. (b) Dose response analysis of remdesivir alone (black) and in combination with 0.15625 μ M (blue) or 0.625 μ M (red) clofazimine. The observed compound activities are represented by solid lines, while the predicted additive combinatorial activities are indicated by dashed lines. The dotted black line denotes 90% inhibition of infection. Data are normalized to mean values for DMSO-treated wells and represent mean $\pm$ SEM of 2 independent experiments. (c) Counting of cell numbers in each drug combination as indicated. Shown is mean $\pm$ SD. (d) Experimental design of in vivo combination therapy: after virus challenge of 105 PFU/hamster at day 0, oral administration of clofazimine (15 mg/kg) and/or intraperitoneal injection of remdesivir (1.5 mg/kg) was performed at 1, 2, and 3 dpi. Lung tissue, nasal turbinate and nasal wash samples were collected at 4 dpi. Remdesivir alone (15 mg/kg) was included as a control through intraperitoneal injection. Vehicle control hamsters received oral administration of corn oil and intraperitoneal injection of 2% DMSO in 12% SBE- β -CD. (e) Daily body weights of the hamsters (n=5). Asterisks indicate statistically significant differences as determined by two-way ANOVA and Tukey's multiple comparison test. (f) Virus in the hamster lung tissue harvested at 4 dpi was titrated by plaque assay. One-way ANOVA when compared with the vehicle control group. (g) Virus titer in the nasal wash was determined by plaque assays. One-way ANOVA when compared with the combinational group that receiving 1.5 mg/kg of remdesivir and 15 mg/kg of clofazimine. * p < 0.05, ** p <0.01 and n.s. indicates non-significant for (e), (f) and (g). (h) Representative image of infected cells by immunofluorescence staining in nasal turbinate at 4 dpi. SARS-CoV-2 N protein (NP) was detected by specific antibody (green) and cell nuclei were stained by DAPI (blue). Scale bar 200 μ m. (i) NP positive cells per 50 $\times$ field per hamster's nasal turbinate section (n=5). ** p <0.01 when compared with the vehicle-treated group by one-way ANOVA.

What opportunities for training and professional development has the project provided?

A part of the accomplishments (Figure 1 – 4) is achieved by postdoctoral associates and National Research Service Award (NRSA) postdoctoral fellow at Chanda Lab.

How were the results disseminated to communities of interest?

A part of the accomplishments (Figure 3 – 6) were published in a scientific journal Nature and disseminated to communities of interest.

What do you plan to do during the next reporting period to accomplish the goals?

We will continue testing of antiviral efficacy of M compounds, KIN compounds, and their derivatives using SARS-CoV-2 and BSL2 influenza A virus strains along with cytotoxicity testing to continue SAR study and promote affinity tagging and chemoproteomics analysis.

Project 1 Specific Aim 1 Major Task 3

Genetic and biochemical validation for M85 will be completed by Y2Q3 (Dec 2021),

Project 1 Specific Aim 2 Major Task 1

Investigate antiviral breadth will be completed by Y2Q4 (Apr 2022).

Project 1 Specific Aim 2 Major Task 4

Comparison of Phosphoproteome upon IAV infection +/- M85 in WT, EGFR KO, PI3K KO, and EGFR/PI3K KO Calu3 cells will be completed by Y2 Q3 (Jan 2022).

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

The overall goal of DABRA is to achieve a significant impact on the landscape of available broad-spectrum inhibitors for treatment of multiple respiratory viruses that impact military readiness, including influenza (A and B), coronaviruses, human rhinoviruses, respiratory syncytial virus, and Adenoviruses. Specifically, we expect to complete preclinical developmental studies to advance up to two lead compound series to Development Candidate stage, facilitating further IND-enabling activities (i.e., GLP/GMP scale-up, comprehensive toxicology and pharmacokinetic studies, chemistry).

What was the impact on other disciplines?

In the longer term, we expect to establish novel paradigms for the therapeutic development of host-directed and broad-spectrum therapies as safe and effective prophylactic and therapeutic approach. According to the expansion of scope, anti-SARS-CoV-2 drug repurposing research was conducted. The research results impacted preclinical and also clinical study for the treatment of COVID-19.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

The research publications described in the PRODUCTS section improved public knowledge and understandings as to treatment for SARS-CoV-2 infection.

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Sanford Burnham Prebys Medical Discovery Institute has been operating at reduced capacity due to the COVID-19 pandemic. Chanda laboratory has delays in initiating Project 1 Specific Aim 1 Major Task 3 Target selection and validation for M85, and Project 1 Specific Aim 2 Major Task 1 Investigate antiviral breadth.

Actual or anticipated problems or delays and actions or plans to resolve them

The COVID-19 pandemic affected operation rate of our laboratory. It is uncertain how long the situation lasts. The continuing pandemic makes it unpredictable when we will be able to resume normal full activities. However, we will accelerate the task in the next year by increasing personnel.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

- **Publications, conference papers, and presentations**
Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing.
Riva L, Yuan S, Yin X, Martin-Sancho L, Matsunaga N, Pache L, Burgstaller-Muehlbacher S, De Jesus PD, Teriete P, Hull MV, Chang MW, Chan JF, Cao J, Poon VK, Herbert KM, Cheng K, Nguyen TH, Rubanov A, Pu Y, Nguyen C, Choi A, Rathnasinghe R, Schotsaert M, Miorin L, Dejoze M, Zwaka TP, Sit KY, Martinez-Sobrido L, Liu WC, White KM, Chapman ME, Lendy EK, Glynne RJ, Albrecht R, Ruppin E, Mesecar AD, Johnson JR, Benner C, Sun R, Schultz PG, Su AI, García-Sastre A, Chatterjee AK, Yuen KY, Chanda SK. *Nature*. 2020 Oct;586(7827):113-119. doi: 10.1038/s41586-020-2577-1. Epub 2020 Jul 24. PMID: 32707573

Clofazimine broadly inhibits coronaviruses including SARS-CoV-2.
Yuan S, Yin X, Meng X, Chan JF, Ye ZW, Riva L, Pache L, Chan CC, Lai PM, Chan CC, Poon VK, Lee AC, Matsunaga N, Pu Y, Yuen CK, Cao J, Liang R, Tang K, Sheng L, Du Y, Xu W, Lau CY, Sit KY, Au WK, Wang R, Zhang YY, Tang YD, Clausen TM, Pihl J, Oh J, Sze KH, Zhang AJ, Chu H, Kok KH, Wang D, Cai XH, Esko JD, Hung IF, Li RA, Chen H, Sun H, Jin DY, Sun R, Chanda SK, Yuen KY. *Nature*. 2021 Mar 16. doi: 10.1038/s41586-021-03431-4. Online ahead of print. PMID: 33727703

MDA5 Governs the Innate Immune Response to SARS-CoV-2 in Lung Epithelial Cells.
Yin X, Riva L, Pu Y, Martin-Sancho L, Kanamune J, Yamamoto Y, Sakai K, Gotoh S, Miorin L, De Jesus PD, Yang CC, Herbert KM, Yoh S, Hultquist JF, García-Sastre A, Chanda SK. *Cell Rep*. 2021 Jan 12;34(2):108628. doi: 10.1016/j.celrep.2020.108628. PMID: 33440148

SARS-CoV-2 Infection Depends on Cellular Heparan Sulfate and ACE2.
Clausen TM, Sandoval DR, Spliid CB, Pihl J, Perrett HR, Painter CD, Narayanan A, Majowicz SA, Kwong EM, McVicar RN, Thacker BE, Glass CA, Yang Z, Torres JL, Golden GJ, Bartels PL, Porell RN, Garretson AF, Laubach L, Feldman J, Yin X, Pu Y, Hauser BM, Caradonna TM, Kellman BP, Martino C, Gordts PLSM, Chanda SK, Schmidt AG, Godula K, Leibel SL, Jose J, Corbett KD, Ward AB, Carlin AF, Esko JD. Cell. 2020 Nov 12;183(4):1043-1057.e15. doi: 10.1016/j.cell.2020.09.033. Epub 2020 Sep 14. PMID: 32970989

Topoisomerase 1 inhibition therapy protects against SARS-CoV-2-induced inflammation and death in animal models.

Yuin Ho JS, Wing-Yee Mok B, Campisi L, Jordan T, Yildiz S, Parameswaran S, Wayman JA, Gaudreault NN, Meekins DA, Indran SV, Morozov I, Trujillo JD, Fstchyan YS, Rathnasinghe R, Zhu Z, Zheng S, Zhao N, White K, Ray-Jones H, Malysheva V, Thiecke MJ, Lau SY, Liu H, Junxia Zhang A, Chak-Yiu Lee A, Liu WC, Aydiillo T, Salom Melo B, Guccione E, Sebra R, Shum E, Bakker J, Kaufman DA, Moreira AL, Carossino M, Balasuriya UBR, Byun M, Miraldi ER, Albrecht RA, Schotsaert M, Garcia-Sastre A, Chanda SK, Jeyasekharan AD, TenOever BR, Spivakov M, Weirauch MT, Heinz S, Chen H, Benner C, Richt JA, Marazzi I. bioRxiv. 2020 Dec 1:2020.12.01.404483. doi: 10.1101/2020.12.01.404483. Preprint. PMID: 33299999

Functional Landscape of SARS-CoV-2 Cellular Restriction.

Martin-Sancho L, Lewinski MK, Pache L, Stoneham CA, Yin X, Pratt D, Churas C, Rosenthal SB, Liu S, De Jesus PD, O'Neill AM, Gounder AP, Nguyen C, Pu Y, Oom AL, Miorin L, Rodriguez-Frandsen A, Urbanowski M, Shaw ML, Chang MW, Benner C, Frieman MB, García-Sastre A, Ideker T, Hultquist JF, Guatelli J, Chanda SK. bioRxiv. 2020 Sep 30:2020.09.29.319566. doi: 10.1101/2020.09.29.319566. Preprint. PMID: 33024967

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

- **Press release** - [COVID-19: Scientists identify human genes that fight infection | SBP \(sbpdiscovery.org\)](https://sbpdiscovery.org) (4/16/2021)
- **Press release** - [Leprosy drug holds promise as at-home treatment for COVID-19](#) (3/16/21)
- **Press release** - [Scientists identify “immune cop” that detects SARS-CoV-2](#)(1/12/21)
- **Press release** - [Researchers use live virus to identify 30 existing drugs that could treat COVID-19](#) (4/18/2020)
- **Press release** - [Nature study identifies 21 existing drugs that could treat COVID-19](#) (7/14/2020)
- **Press release** - [New Coronascope tool will help unlock big-data insights for COVID-19](#) (9/17/2020)

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**

A US Provisional Application No. 63/010,630 was filed prior to the April 15, 2021 deadline. Approval is pending.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Sumit Chanda
Project Role: PD/PI
Nearest person month worked: 2.39
Contribution to Project: Dr. Chanda directed and oversaw the whole project.

Name: Laura Martin-Sancho
Project Role: Senior Postdoctoral Associate
Nearest person month worked: 3.54
Contribution to Project: Dr. Martin-Sancho conducted antiviral testing.

Name: Anne Beall
Project Role: Postdoctoral Associate
Nearest person month worked: 3.79
Contribution to Project: Dr. Beall conducted antiviral testing.

Name: Kristina Herbert
Project Role: Research Assistant Professor
Nearest person month worked: 1.22 (Termed)
Contribution to Project: Administrative management

Name: Naoko Matsunaga
Project Role: Senior Staff Scientist
Nearest person month worked: 1.07
Contribution to Project: Project management

Name: Andrey Rubanov
Project Role: Research Assistant II
Nearest person month worked: 0.86 (Termed)
Contribution to Project: Compound management

Name: Peter Teriete
Project Role: Research Assistant Professor
Nearest person month worked: 0.75 (Termed)
Contribution to Project: Compound management

Name: Xin Yin
Project Role: Postdoctoral Associate
Nearest person month worked: 1.63 (Termed)
Contribution to Project: Dr. Yin conducted antiviral testing

Name: Raul Baluyut
Project Role: Lab Assistant I
Nearest person month worked: 0.10
Contribution to Project: Technical assistance and lab maintenance

Name: Hyunwoo Shin
Project Role: Lab Assistant I
Nearest person month worked: 1.0
Contribution to Project: Technical assistance and lab maintenance

Name: Grifin Berge
Project Role: Research Associate I
Nearest person month worked: 0.22
Contribution to Project: Compound management

Name: Supriya Sadhukhan
Project Role: Research Associate I
Nearest person month worked: 0.62
Contribution to Project: Technical assistance

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

See below.

PREVIOUS, CURRENT, AND PENDING SUPPORT

CHANDA, SUMIT

ACTIVE

4 U19 AI118610-05 (IOF) (PI: Fernandez-Sesma) 06/01/2019-05/31/2020 0.68 calendar
Icahn School of Medicine at Mount Sinai/NIH

High-throughput Analysis of Pathogen-driven Host Factor Relocalization

Project goals: to develop and evaluate a high-throughput screening platform to identify alternations in host protein subcellular localization after dengue virus (DENV) infection and evaluate the impact of these factors in DENV replication.

Specific Aims: To develop and execute a high-throughput screening platform to identify alterations in host protein subcellular localization after DENV infection 2) To evaluate the impact of these factors in DENV replication.

Grants Management Officer: Roberta D. Wolcott, wolcottr@niaid.nih.gov, 240-669-2964

Overlap: None

4 U19 AI118610-06 (PI: Fernandez-Sesma) 06/01/2020-05/31/2020 0.36 calendar
Icahn School of Medicine at Mount Sinai/NIH

Dengue Human Immunology Project Consortium (DHIPC)

Project goals: to apply genome-wide siRNA screens using human monocyte-derived dendritic cells (MDDCs) to characterize DENV-induced innate immune activation and dengue virus-host interactions networks to functionally address the virus-host interactions that are critical in the activation of the innate immune system and in restricting viral replication.

Specific Aim: to apply genome-wide siRNA screens using human monocyte-derived dendritic cells (MDDCs) to characterize DENV-induced innate immune activation and dengue virus-host interaction networks to functionally address the virus-host interactions that are critical in the activation of the innate immune system and in restricting viral replication

Grants Management Officer: Roberta D. Wolcott, wolcottr@niaid.nih.gov, 240-669-2964

Role: Project lead

Overlap: None

5 R01 AI124843-05 NCE (PI: Chanda) 06/17/2016-05/31/2022 0.36 calendar
NIH/NIAID

Development of SMAC mimetics as Latency-Reversing Agents

Project goals: the combinatorial activities of HDACi and Smac mimetics will enable clinically potent and efficacious reactivation of HIV-1 in aviremic patients, while limiting toxicity risks associated with systemic activation of canonical NF- κ B signaling.

Specific Aims: 1) determine if clinical-stage Smac mimetics can promote HIV-1 latency reversal at reported maximum tolerated doses (MTD) 2) development of next-generation Smac mimetic optimized for HIV-1 LRA activity 3) characterize the molecular impact of Smac mimetics on HIV latency.

Grants Management Officer: Jason A. Lundgren, lundgrenj@mail.nih.gov

Role: PD/PI

Overlap: None

5 R01 AI125453-04 (PI: Basler) 02/15/2017-01/31/2022 0.46 calendar
Georgia State University/NIH

Small Molecule Inhibitors of Ebola Virus Polymerase Function

Project goals: to identify small molecules that target different components of the EBOV RDRP complex and to use this as a guide to identify synergistic compound combinations for novel therapeutics against EBOV and other filoviruses.

Specific Aims: 1) Perform a high throughput screen of the 385,000 compound NIH Molecular Libraries Small Molecule Repository (MLSMR) collection and validate the hits 2) Test hits for pan-filovirus activity and perform SAR studies to identify leads for further development 3) Evaluate antiviral activity of hits against EBOV and define mechanism of action

Grants Management Officer: Roberta D. Wolcott, wolcott@niaid.nih.gov, 240-669-2964

Role: Co-investigator

Overlap: None

5 R01 AI127302-04 (PI: Chanda) 02/28/2017-01/31/2022 0.91 calendar
NIH/NIAID (PI portion)

Innate Immune Surveillance of HIV-1 During Transmission and Systemic Infection

The goals of this grant are to determine the PAMP sensed by a novel innate immune receptor to HIV-1, PQBP1, interrogate potential immune evasion mechanisms of HIV-1 to PQBP1/cGAS, and investigate the functional consequences of this pathway.

Specific Aims: 1) Investigate the induction of the PQBP1/cGAS antiviral pathway by HIV-1. 2) Investigate role of polyglutamylation in regulation of PQBP1-dependent response to HIV-1. 3) Elucidate and characterize signaling molecules that govern the PQBP1/cGAS innate response to HIV-1.

Grants Management Officer: Yesenia Isabela Mendoza, yesenia.mendoza@nih.gov, 240-627-3671

Role: PD/PI

Overlap: None

5 U19 AI135972-03 (PI: Garcia-Sastre) 01/20/2018-12/31/2022 1.20 calendar
Icahn School of Medicine at Mount Sinai/NIH

Fluomics: The Next Generation – Project 2

Project goals: In project 2, we propose to utilize leading edge OMICS approaches including systems biology approaches and various genetic tools to identify ex vivo molecular signatures that correlate with clinical disease outcomes. The elucidation and characterization of factors that govern outcome-related biomarker signatures offer the potential for the development of novel antiviral therapies.

Specific Aims: 1) identification of human ex vivo molecular signatures that correlate with influenza disease severity 2) elucidation of factors that govern viral replication and host immune responses 3) validation and mechanistic evaluation of key drivers that impact influenza severity.

Grants Management Officer: Jenny L. Greer, 984-287-332, jenny.greer@nih.gov

Role: Project lead

Overlap: None

5 U19 AI135972-03 (PI: Garcia-Sastre) 01/01/2019-12/31/2022 0.46 calendar
Icahn School of Medicine at Mount Sinai/NIH

Fluomics: The Next Generation – Core A

Project goals: to create oversight for the projects and cores of this grant.

Specific Aims: 1) To establish an organizational structure centered on an Executive Committee responsible for monitoring overall program progress, implementing a Pilot Research Program, and making decisions on staffing plans, allocation of resources, core usage and other policies, 2) To coordinate conference calls and organize annual meetings between the PIs, selected key personnel, the Steering Committee and NIAID, 3) To assist the investigators of this consortium in reagents and data sharing, manuscript preparations, public release of data and submission of annual progress reports to the NIH, 4) To Provide Training Opportunities in Systems Biology for the Infection Disease Scientist.

Grants Management Officer: Jenny L. Greer, 984-287-332, jenny.greer@nih.gov

Role: Core lead

Overlap: None

5 R01 CA229818-03 (PI: Chanda) 07/11/2018-06/30/2022 0.91 calendar
NIH/NCI (PI portion)

Early Development of Small Molecule Dendritic Cell Immunopotentiators for the Treatment of Solid Tumors

Project goals: the proposed study has a strong potential of generating T cell responses against tumor-specific antigens expressed by a patient's individual cancer potentially offering a novel immunotherapeutic approach to treat cancer.

Specific Aims: 1) Cross validation of hit compounds that activate MDDCs 2) Establish anti-tumor efficacy of immunomodulatory compounds in murine melanoma model 3) delineate *in vivo* and *ex vivo* biomarkers that act as surrogates of *in vivo* efficacy.

Grants Management Officer: Candace M Cofie, candace.cofie@nih.gov, 240-276-5721

Role: PD/PI

Overlap: None

3 U19 AI135972 S1 (PI: Garcia Sastre)

03/30/2020-12/31/2021

0.27 calendar

NIH/ISMMS

Fluomics: The Next Generation (Coronavirus Administrative Supplement)

Project goals: we propose to utilize leading edge OMICS approaches including systems biology approaches and various genetic tools to identify *ex vivo* molecular signatures that correlate with clinical disease outcomes. The elucidation and characterization of factors that govern outcome-related biomarker signatures offer the potential for the development of novel antiviral therapies for Corona Virus.

Specific Aims: 1) identification of human *ex vivo* molecular signatures that correlate with Corona Virus disease severity 2) elucidation of factors that govern viral replication and host immune responses 3) validation and mechanistic evaluation of key drivers that impact Corona Virus severity.

Grants Management Officer: Tseday G Girma, tseday.girma@nih.gov

Role: Co-Investigator

Overlap: None

W81XWH-20-1-0270 (PI: Chanda/Garcia Sastre)

04/15/2020-04/14/2024

2.40 calendar

Department of the Army

(PI portion)

Development and advancement of broad-spectrum respiratory antivirals.

Project goals: The goal of DABRA is to *exploit, harness, and integrate* the advances from our respective labs to respond to this critical unmet medical need. This will be achieved through pre-investigational new drug (pre-IND) development of a unique portfolio of *host-directed* broad-spectrum antivirals. The proposal will focus on four assets that have been heavily vetted and all have clinical potential.

Specific Aims: 1) Host target identification and validation 2) Characterization of broad-spectrum antiviral activities 3) determining cellular response to antiviral treatment.

Grants Management Officer: Joshua L. Disbennett, 301-619-7349, joshua.l.disbennett.civ@mail.mil

Role: MPI PD/PI

Overlap: None

75N93019C00046 (PI: Garcia Sastre)

09/30/2019 – 09/29/2024

0.91 calendar

NIH Contract

C-GAS/STING Inducing Compounds as Novel Adjuvants

Project goals: are to identify and develop 1 to 2 promising novel adjuvants that activate the endogenous and powerful cGAS/STING pathway to facilitate innate and adaptive immune protection, and the induction of broad protective B and T cell responses when used as adjuvants of an inactivated influenza virus vaccine.

Specific Aims: 1) to design and execution all medicinal chemistry and ADME/PK work related to the contract, including data analysis and reporting, and guiding/interfaces the project personnel to ensure successful milestone delivery. The medicinal chemistry will also perform hit assessment, compound synthesis and optimization, overseeing SAR, interpreting the biological data and selecting compounds for *in vitro* ADME studies to be in the Exploratory Pharmacology Core.

Grants Management Officer: Wolfgang W. Leitner, 240-627-3575, wleitner@mail.nih.gov

Role: MPI PD/PI and Project lead

Overlap: None

2 P30 CA030199-39S1 NCE (PI: Ronai) NIH/NCI <i>Cancer Center Support Grant (CCSG)</i> In response to the immediate public health emergency caused by the novel coronavirus, SARSCoV-2 which causes the pneumonia like disease named COVID-19, we intend to screen the ReFRAME drug repurposing library to identify compound combinations that can be used to inhibit SARS-CoV-2 infection. Grants Management Officer: Sarah Lee, 240-276-6280, sarah.lee@nih.gov Role: Project Lead Overlap: None	05/01/2020-04/30/2022	0.46 calendar
3 R01 CA202021-05S1 (PI: Ronai) NIH/NCI <i>Control of Protein Synthesis by the UPS Under Stress</i> The underlying hypothesis of this proposal is that targeting components of the eIF4F complex is expected to provide novel therapeutic modality for inhibition of SARS-CoV-2 replication, while exerting anti-neoplastic effects across broad spectrum of cancers. Grants Management Officer: Candace M Cofie, candace.cofie@nih.gov, 240-276-5721 Role: Co-Investigator Overlap: None	07/01/2020-06/30/2021	0.12 calendar
SA 20-023 (PI: Chanda) Eli Lilly & Company <i>Research Service Agreement</i> Pilot study with up to 12 samples comparing results in the pseudotyped virus and authentic virus Immunofluorescence assay; both using Vero E6 cells and run in triplicate. Grants Management Officer: Daniel Skovronsky, skovronsky_daniel@lilly.com Role: PD/PI Overlap: None	04/04/2020-04/28/2021	0.18 calendar
2176 NCE (PI: Chanda) Fast Grants <i>A Drug Repurposing Strategy for SARS-CoV-2 Vaccine Adjuvants</i> The major goal of the project is to apply an unbiased approach to screen a comprehensive drug repurposing library of ~12,000 clinically tested small molecules with the aim of identifying safe drugs that stimulate innate immune pathway(s). By activating innate immune responses, which play a key role in promoting maturation of dendritic cells (DC), which are major antigen presenting cells capable of priming naïve CD4+ T cells, these adjuvants should be capable of potentiating the immune response to all vaccines, including the SARS-CoV-2 vaccine. Grants Management Officer: Tyler Cowen, fastgrantscovid19@gmail.com Role: PI/PD Overlap: None	09/01/2020-08/31/2021	0.09 calendar
CSRA 20-006 (PI: Chanda) Boehringer Ingelheim <i>Genomewide siRNA screening to identify targets driving pro-fibrotic epithelia-fibroblast crosstalk and CRISPR screening to identify pro-fibrotic/pro-inflammatory pathways in senescent fibroblasts</i> Project goals: Identify and characterize novel target to treat autoimmune diseases. Grants Management Officer: Gaylene Anderson, 203-482-8520, gaylene.anderson@boehringer-ingelheim.com Role: Project lead Overlap: None	06/30/2020-12/31/2023	0.48 calendar
RCA 20-001 (PI: Chanda)	01/04/2021-01/03/2022	0.09 calendar

Scripps Research Institute

An Integrated Drug Discovery Consortium for NTDs (IDDC-NTD)

Project goals: Prof Sumit Chanda, Immunity and Pathogenesis Program, and his team at the Infectious and Inflammatory Diseases Center within SBP will screen the ReFRAME collection against DENV-2 strain 16881. They will reconfirm hits in dose-response and run counterscreens for hit triage. This will include the assessment of cytotoxicity in uninfected cells as well as profiling for activity against DENV-1, -3 and -4.

Specific Aims: N/A

Grants Management Officer: Karen Wolff, kwolff@scripps.edu

Role: Project lead

Overlap: None

1 R01 AI158552-01 (PI: Ware)

08/12/2020-07/31/2025

0.09 calendar

NIH/NIAID

Overriding the Immune Evasion Tactics of Coronavirus

The goal is to search for antiviral drugs that will help restore the immune response to virus infections. Our experiments seek to identify new information on how coronavirus alters the immune system to help in the design of new therapies.

Grants Management Officer: Tamia Powell, powellty@niaid.nih.gov, 240-669-2982

Role: Co-Investigator

Overlap: None

PREVIOUS

1 R01 AI104972-05 (PI: Diamond)

01/01/2013-12/31/2017

0.91 calendar

NIH/NIAID

ISG Control of Flavivirus Infection

Project goals: to identify and characterize interferon-stimulated genes that act in concert to form a first line of innate defense during flaviviral infection.

Specific Aims: 1) to define inhibitory ISGs that restrict flavivirus infection 2) to characterize the mechanism of action of ISGs 3) to determine the contribution of specific ISGs to anti-WNV immunity *in vivo*.

Grants Management Officer: Mable Nee, neema@mail.nih.gov, 301-496-4221

Role: PI

1 R01 AI105184-03S1 (PI: Chanda)

08/17/2016-07/31/2017

0.36 calendar

NIH

Molecular Determinants of TLR Trafficking (Supplement)

Project goals: to elucidate our understanding of PQBP1's role in the cGAS innate immune pathway with focused efforts on HIV-1 PAMP characterization.

Specific Aims: 1) Elucidate features of the DNA pathogen associated molecular pattern (PAMP) sensed by host pathogen recognition receptor (PRR) 2) Understand how the DNA PAMP and host PRR interaction promotes innate induction

Grants Management Officer: Tamia Y. Powell, powellty@niaid.nih.gov, 240-669-2982

Role: PI

5 R01 DA033773-05 (PI: Wolinsky)

04/15/2012-03/31/2017

0.46 calendar

NIH/NIDA

A systems-level approach to studying HIV/AIDS susceptibility and substance abuse

Project goals: to characterize the role of addiction-related cellular proteins in the control of HIV replication.

Using both gain-of-function and loss-of-function approaches. In addition, Dr. Chanda will also lead the biocomputational efforts to integrate various system-level datasets to globally understand the impact of addiction-related genes on networks of HIV-host interactions. Functional and computational characterization of these viral-host interactions will provide a molecular insight towards the impact of drug addiction upon rates of HIV replication.

Specific Aims: 1) Functionally validate the role of identified factors whose expression is highly regulated upon HIV-1 infection in both innate immune response and viral replication 2) Determine the collaborative effects of multiple genes within complex pathways that regulate innate response and viral replication with respect to HIV susceptibility and drug abuse

Grants Management Officer: Pamela G. Fleming, pfleming@mail.nih.gov, 301-253-8729

Role: Co-Investigator

2 P50 GM085764-07 (PI: Ideker)

09/01/2015-05/31/2017

1.2 calendar

NIH/NIGMS

San Diego Center for Systems Biology: From Maps to Models

Project 3: Chanda (Subcontract PI) \$6,188

Project goals: to employ systems-based approaches to comprehensively characterize the regulation of the response

to extracellular pro-inflammatory stimuli. Additionally, we propose to elucidate and characterize genetic interactions between regulatory factors that coordinately regulate these events.

Specific Aims: 1) Acquisition of data on spatiotemporal cellular states within tissue to construct maps of the dynamic tissue organization 2) Data-driven model parameterization 3) Specific forward predictions

Grants Management Officer: Robert Altieri, Robert.altieri@nih.gov

Role: Co-Investigator

5 U19 AI106754-05 (PI: Garcia-Sastre)

06/26/2013-05/31/2018

1.20 calendar

NIH

Multiscale analysis of influenza host-pathogen interactions: Fluomics – Project 1

Ex vivo interrogation of the genetic and biochemical networks controlling influenza virus infection

Project goals: To use a systematic approach to generate predictive models of influenza virus pathogenesis which will 1) allow us to identify biomarkers for predicting pathogenic potential of new influenza viruses, b) give us the ability to predict which populations may be more susceptible to disease based on genetic variability, and c) provide avenues to explore for new, host-directed, therapeutic interventions

Specific Aims: 1) characterization of the impact of influenza virus infection on the cellular transcriptome, epigenome, proteome and metabolome, 2) Analyses of genetic and biochemical influenza virus-host interaction networks, 3) Network identification and perturbations for refining the model, 4) Characterization of candidate molecules implicated by the model in influenza virus infection.

Grants Management Officer: Cheryl Y. Wall, cheryl.wall@nih.gov, 240-669-2956

Role: Co-Investigator

5 U19 AI106754-05 (PI: Garcia-Sastre)

06/26/2013-05/31/2018

0.24 calendar

NIH

Multiscale analysis of influenza host-pathogen interactions: Fluomics – Core A

Project goals: To provide the administrative infrastructure to facilitate coordination of 3 scientific projects, 3 cores, the exchange of information, viruses, samples and reagents between different laboratories, monitoring of scientific progress and performance, resolution of technical problems and prioritization of experiments, organization of travel and temporal accommodation of personnel during the U19 annual meetings and the program meeting with NIAID

Specific Aims: 1) To establish an organizational structure centered on an Executive Committee responsible for monitoring overall program progress, implementing a Pilot Research Program, and making decisions on staffing plans, allocation of resources, core usage and other policies, 2) To coordinate conference calls and organize annual meetings between the PIs, selected key personnel, the Steering Committee and NIAID, 3) To assist the investigators of this consortium in reagents and data sharing, manuscript preparations, public release of data and submission of annual progress reports to the NIH, 4) To Provide Training Opportunities in Systems Biology for the Infection Disease Scientist.

Grants Management Officer: Cheryl Y. Wall, cheryl.wall@nih.gov, 240-669-2956

5 U19 AI106754-05 (PI: Garcia-Sastre)	06/26/2013-05/31/2018	0.96 calendar
NIH		
<i>Multiscale analysis of influenza host-pathogen interactions: Fluomics – Core F</i>		
Project goals: To develop and maintain a central repository for all data and resources generated by the Center, and ensure that these materials are readily accessible to internal project investigators and external scientific community		
Specific Aims: 1) To Consolidate and Assimilate Systems-Driven Data Generated in the Program, 2) To Integrate Publicly Available Datasets for Host-Pathogen Interactions and Innate Signaling and Responses, 3) To Ensure Timely Dissemination of Resources and Data to the Scientific Community.		
Grants Management Officer: Cheryl Y. Wall, cheryl.wall@nih.gov, 240-669-2956		
Role: Co-Investigator		
5 R01 AI105184-04 (PI: Chanda)	08/01/2014-07/31/2018	0.48 calendar
NIH/NIAID		
<i>Molecular Determinants of TLR Trafficking</i>		
Project Goals: To characterize factors and processes that promote faithful endosomal localization of TLR7 and TLR9		
Specific Aims: 1) To identify and characterize the E3 ligase that is required for TLR9 ubiquitination, 2) To investigate the non-degradative role of ESCRT (Endosomal Sorting Complex Required for Transport) in mediating endosomal TLR activation, 3) To investigate the role of additional cofactors that modulates endosomal targeting of TLR9, 4) To investigate the role of TLR9 ubiquitination and ESCRT function in TLR9-expressing cell subsets.		
Grants Management Officer: Cheryl Y. Wall, cheryl.wall@nih.gov, 240-669-2956		
5 P50 GM085764-09 (PI: Ideker)	09/01/2015-05/31/2019	0.46 calendar
UCSD/NIH		
<i>Center for Systems Biology of Cellular Stress Responses – Project 4</i>		
Project goals: to systematically study the interactions between the organism and the environment, with the aim of developing large multi-species maps of gene-environment interactions and analyzing these maps to exploit fundamental rules and principles of interaction that can be used predictively.		
Specific Aims: 1) using evolved resistance to map gene-environment interactions at single base resolution in two species 2) using genome-wide libraries to map gene-environment interactions systematically in two species 3) a general cross-species model of drug-gene interaction.		
Grants Management Officer: Robert Altieri, Robert.altieri@nih.gov, 631-849-1872		
Role: Co-Investigator		
5 P01 CA177322-05 (PI: Sun)	09/19/2014-08/31/2019	0.91 calendar
UCLA/NIH		
<i>Innate Immune Responses and Vaccines Against Tumor-Associated Herpesviruses</i>		
Project goals: is the identification of innate response pathways targeted by KSHV for immune evasion and to find out host factors that restrict and assist viral replication.		
Specific Aims: 1) To Elucidate γ -herpesvirus-Host Immune Interactions that Impact Viral Replication, 2) To Characterize interactions between γ -herpesvirus factors and Host Regulators of Innate Immune Responses, 3) To Identify and Validate Highly Immunogenic Viral Mutants for Vaccine Development.		
Grants Management Officer: Tracie McGraw, mcgrawth@nih.gov, 301-631-3020		
Role: Co-Investigator		
CSRA 16-08 (PI: Chanda/Ware)	04/01/2016-09/30/2019	0.12 calendar
Boehringer Ingelheim		
<i>Identification and Characterization of Novel Targets in Immunology and Respiratory Diseases</i>		
Project goals: to investigate the molecular basis of inflammatory and autoimmune diseases such as SLE and IBD, and identify novel targets and candidate lead molecules for therapeutic development. Studies will include		

ex vivo genetic studies, proteomics, and in vivo CRISPR/knockout mice at SBP combined with BI's exceptional drug development capabilities.

Specific Aims: 1) Candidate gene and pathway validation 2) Novel targets for inflammatory bowel diseases and lung fibrosis 3) The DEAD-box helicase autoimmune target platform

Grants Management Officer: Mark Ralph, mark.ralph@boehringer-ingelheim.com, 203-798-5235

Role: PD/PI

5 R21 AI134581-02 (PI: Chanda)

08/08/2018-07/31/2020

0.12 calendar

NIH

A Novel Conformation of the ZIKV Protease Provides a Unique 3D Scaffold for Discovering Allosteric Protease Inhibitors as Direct Anti-Virals, via HT and Virtual Screening, and Protein Engineering

Project goals: to validate allosteric binding using biophysical techniques, co-crystallization, and enzymology; and direct inhibitor optimization by co-crystallization to obtain ~5 inhibitor suitable for future therapeutic development

Specific Aims: 1) screen compound libraries for allosteric inhibitors 2) validate high affinity allosteric binding and to direct inhibitor optimization.

Grants Management Officer: Trevor Alford, trevor.alford@nih.gov, 240-669-2916

Role: PD/PI

5 P30 CA030199-38 (PI: Powis)

07/29/2015-04/30/2020

0.46 calendar

NIH/NCI

(PI portion)

Cancer Center Support Grant (CCSG)

The Sanford Burnham Medical Research Institute's Cancer Center is dedicated to revealing the fundamental molecular causes of cancer and to applying this knowledge to the health of humans. Through continued commitment to collaborative and multidisciplinary research, our plans seek to propel our scientific activities onward to deliver results that will have a transformational impact in cancer research and medicine. No support is provided for research use.

Grants Management Officer: Candace M Cofie, candace.cofie@nih.gov, 240-276-5721

Role: Project Lead

PENDING

1 UM1 AI164561-01 (MPI: Chanda)

07/01/2021-06/30/2026

0.22 calendar

NIH/NIAID

(PI Portion)

Reversing Immune Dysfunction for HIV-1 Eradication

The major goal of this project is to understand the molecular and cellular basis for the loss of immune homeostasis in HIV infected patients. This knowledge will be harnessed to create a therapeutic paradigm encompassing immune restorative treatments, utilized in concert with enhanced viral reactivation and elimination strategies, to establish a HIV-1 cure.

Specific Aims 1 Understanding Metabolic Control of Innate Immune Dysregulation and HIV-1 Persistence. 2) Restoring Adaptive Immune Control of HIV-1 infection. 3) Reactivation, Clearance, and Elimination of the HIV-1 Reservoir for a Sterilizing Cure.

Grants Management Officer: Pending

Role: Contact PI.

OVERLAP

There is no scientific or budgetary overlap.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

University of Hong Kong
Georgia State University
Northwestern University
Scripps Research Institute / CALIBR
Kyoto University / HiLung, Inc.
WuXi Apptec

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ebrap.org/eBRAP/public/index.htm> for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil/Pages/Resources.aspx>) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

AWARD NUMBER: W81XWH-20-1-0270

TITLE: Development and Advancement of Respiratory Antivirals

PRINCIPAL INVESTIGATOR: Michael Gale, Jr. / Adolfo Garcia-Sastre

CONTRACTING ORGANIZATION: Sumit K Chanda, Ph.D.

REPORT DATE: April 30, 2021

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: Choose Distribution Statement A or B. (Reference https://mrdc.amedd.army.mil/index.cfm/resources/researcher_resources/reporting/technical for additional information.)

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

- 1. INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The overall goal of this Program is to bring up to two lead candidate antiviral drugs to the selection of a Developmental Candidate (DC), and one back up, to enable GMP/GLP studies and support an investigational new drug (IND) filing in the United States. Our research program focuses on developing drugs that target the host innate immune response. The innate immune response is critical for antiviral immunity and it serves to control virus replication and spread, and to direct the adaptive immune response. Our studies show that IRF3 is a central and essential component of the innate immune response that controls virus infection, including respiratory and many other viral infections. For these innate immune activating compounds, we aim to define the cellular mechanism of action, elucidating specifically why these compounds function to block replication of viruses without activating IFN responses that might lead to cytokine storm. Our research program is working toward the following aims in understanding the mechanism of action of RIG-I and IRF-3 direct innate immune antivirals: Aim 1: Characterize the cellular response and defining IRF3 target genes, Aim 2: Define Viral response and Aim 3: Determining the IRF3 target genes with anti-viral activity. During this funding period, we also received a supplement to select candidate antiviral drugs against SARS-CoV-2. We proposed to execute at SBP a high throughput screen (HTS) of a best-in-class repurposing library: ReFRAME and to validate the best hits in ex vivo and in vivo animal models at ISMMS.

- 2. KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Antiviral, therapeutics, drug, IRF-3, RIG-I, innate immunity, SARS-CoV2, virus

- 3. ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Project 3: Defining MOA for Broad-spectrum Respiratory Antivirals that Activate Innate Immunity

Specific Aim 1: <u>Identify and validate the host targets of KIN269 and KIN1502.</u>	Proposed Timeline	% completion	Site Gale
Major Task 1: KIN269 target identification.	Months		
Subtask 1: Identify KIN269 targets within several cell types.	1-3	0% (COVID-delay)	Gale
Subtask 2: Validate targets.	3-5	0% (COVID-delay)	Gale
<i>Milestone #1: Identification of KIN269 binding partners.</i>	4-5	0%	Gale
Major Task 2: Assessing KIN269-target protein binding.	Months		
Subtask 1: Assess direct KIN269-target protein binding:	6-7	5%	Gale
Subtask 2: Biochemical validation of target-KIN269 binding and determine minimal motif for compound engagement (structure-function binding analysis):	7-12	0% (COVID-delay)	Gale
<i>Milestone #2: Confirm biochemical interaction between target and KIN269 and map minimal motif of target and KIN269 that is required for interaction.</i>	6-12	0% (COVID-delay)	Gale
Major Task 3: Requirement for target protein for innate immune actions of KIN269.	Months		
Subtask 1: Establish target knockdown using CRISPR/Cas9 Calu3 and THP-1 cell lines	1-4	0% (COVID-delay)	Gale
Subtask 2: Assess IRF3 phosphorylation and distribution in presence of KIN269.	3-12	50% (COVID-delay)	Gale
<i>Milestone #3: Confirmed requirement for target binding protein in innate immune activity of KIN269.</i>	12	5% (COVID-delay)	Gale
Specific Aim 2: <u>Define the host cellular and virus responses to RIG-I- and IRF-3-targeted small molecule therapeutics.</u>	Timeline		Site Gale & García-Sastre
Major Task 1: Mechanisms of virus control.	Months		
Subtask 1: Evaluate antiviral action of KIN269 and KIN1502 over the virus replication cycle:	6-12	0% (COVID-delay and technical delay)	García-Sastre
Subtask 2: Define the stage of the viral life cycle impacted	12-16	0%	García-

by compound treatment: Use Calu3 cells with [IC ₉₀] of compound.			Sastre
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Major Task 2: Antiviral breadth.	Months	% Completion	Site
Subtask 1: Compound ability to promote nuclear translocation of IRF3:	16-18	50%	García-Sastre
Subtask 2: Testing of antiviral efficacy	18-22	0%	García-Sastre
Subtask 3: Subtask 3: Testing of broad-spectrum antiviral efficacy (John Morrey – Utah State see LOS):	22-24	0%	García-Sastre
Subtask 4: Efficacy testing in murine & human primary cells:	24-26	0%	García-Sastre
<i>Milestone #4: Defined breadth of anti-respiratory virus effect induced by KIN269 and KIN1502 treatment.</i>	26	0%	García-Sastre
Major Task 3: Host transcriptional response.	Months		
Subtask 1: transcriptional profiling of primary HBEC and Calu-3 cells:	10-36	0%	Gale
Subtask 2: Validation of differentially expressed genes from treatment with KIN269 and KIN1502	36-40	0%	Gale
<i>Milestone #5: Determine breadth and duration of the pharmacologic response to KIN269 and KIN1502</i>	40	0%	Gale
<u>Specific Aim 3: Define the mechanisms of action and the IRF3 target antiviral genes that direct the viral suppression by RIDE compounds.</u>	Timeline	% completion	Site
Major Task 1: Defining the mechanism of action and IRF3 target genes of RIDE compound antiviral actions.	Months		
Subtask 1: Assess antiviral activity if IRF3-dependent, IFN-independent cDNAs:	28-30	0%	García-Sastre
Subtask 2: Validate IRF3 gene antiviral activity:	30-34	0%	García-Sastre
Subtask 3: Assess gene induction by compounds:	35-38	0%	García-Sastre

Subtask 4: Validate compound antiviral activity is dependent on gene induction:	38-40	0%	García-Sastre
Milestone #6: Determine mechanism of action of RIDE compound antiviral actions.	40	0%	García-Sastre
Major Task 2: Modeling KIN269 and KIN1502 antiviral actions	40-44		
Milestone #7: Identify predicted sites of viral control over IAV infection and other respiratory viruses	44	0%	Gale
Major Task 3: In vivo studies to validate host target - Evaluate antiviral activity of KO mouse strains (KO of target(s) of interest) after respiratory virus infection	36-48		Gale /García-Sastre
Milestone #8: Validate in vivo antiviral viral control over IAV infection and other respiratory viruses	48	0%	Gale /García-Sastre

Expansion of Scope 1: Testing and Development of Lead Assets To Limit SARS-CoV-2 Replication

Specific Aim 1: Optimization of potency and <i>in vitro</i> ADME properties of leads.	Timeline	% Completion	Site
Major Task 1: Optimization of M4 (back-up), M85 (lead), KIN 269 (back-up), and KIN1502 (lead).	Months		
Subtask 1: Synthesis of ~10-25 additional analogs of each asset (Olson) and evaluation in SARS-CoV-2 antiviral efficacy assays of the entire 85-225 analogs per asset as well as parent molecules (Chanda/Gale) and Tier 1 in vitro ADME for the ~3 additional analogs per asset.	1-12 13-24	25%	Chanda/Gale (efficacy testing) Olson analog synthesis and in vitro ADME
Milestone #1: Optimized leads (~20 analogs for each asset over all projects) with an antiviral $IC_{50} < 50$ nM and $SI > 25$.	12 24	5%	Chanda/Gale, Olson
Subtask 2: Testing for SARS-CoV-2 Antiviral Efficacy within human primary cells and primary animal cells.	4-14 16-26	0%	Chanda/Gale

Subtask 3: Testing of pan-CoV efficacy (testing an additional 3 analogs per asset in vitro against SARS and MERS - outsourced: John Morrey)	6-15 18-27	0%	(outsourced)
Milestone #2: Optimized leads (~6 additional analogs of each asset) with an antiviral $IC_{50} < 50$ nM and SI > 25 against SARS-CoV-2 and potentially additional CoVs	15 27	0%	Chanda/Gale
Specific Aim 2: Optimization of in vivo PD/PK/tox properties.	Timeline	% Completion	Site
Major Task 1: In vivo PD/PK/Tox	Months		
Subtask 1: In vivo pharmacokinetics on an additional 3-6 analogs per asset:	7-21 19-30	0%	Olson/ García-Sastre
Subtask 2: In vivo pharmacodynamics on an additional 3-6 analogs per asset:	7-21 19-30	0%	Olson/Chanda/ García-Sastre/ Gale
Subtask 3: In vivo toxicology on an additional 3-6 analogs per asset.	8-22 20-31	0%	Olson/ García-Sastre
Milestone #3: Compounds, ~3 total per KIN or M series, that meet the PK criteria outlined in Table 2-2 of the Narrative.	22 31	0%	Olson/Chanda/ García-Sastre/ Gale

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Progress report from the Gale laboratory

For Quarter 1, Year 1:

- 1) Major activities. We have initiated activities related to the expansion of scope 1 to develop lead assets to limit SARS-CoV2 replication, Major Task 1: Optimization of KIN269 and KIN1502. These activities have included the synthesis of KIN269 and KIN1502 and the generation of a virus stock of SARS CoV2 that will be used in our planned experiments. We have added KIN1400 to our study design, a novel RIG-I agonist compound.
- 2) Specific Objectives. Our overall objective for these efforts were to support specific aim 1: optimization of potency and in vitro ADME properties of leads.

- 3) **Significant Results/Key Outcomes.** We have successfully synthesized large batches of KIN269 and KIN1400, which will be used for future experiments. Both compounds were evaluated for purity by HPLC, NMR and TLC and we achieved >95% purity for KIN269 and >97% purity for KIN1400 (Figure 1). We also had KIN1502 synthesized with greater than 95% purity as determined by NMR and HPLC (Figure 2). We also have generated virus stocks of a primary isolate of SARS-CoV2/Seattle/2020 that will be used in the planned SARS-CoV2 analyses. We have determine viral titers of our stock via plaque assays and verified viral replication of the stock virus via infection of Vero cells (Figure 3).
- 4) **Other Achievements.** We developed, submitted, and received UW IACUC and ACURO approval for animal protocols. See regulatory section below for details.

For Quarter 2, Year 1:

- 1) **Major activities.**
 - A) We have initiated activities related to Project 3: Defining MOA for Broad-spectrum Respiratory Antivirals that Activate Innate Immunity, Major Task 3: Requirement for target protein for innate immune actions of KIN269. These activities have included evaluating the ability of KIN269, KIN1400 and KIN1502 to induce phosphorylation of IRF3 in the human lung-derived Calu3 cell line after compound treatment. We also evaluated the ability of KIN1502 to induce protein expression of the innate immune ISGs RIG-I and IFITM1 in Calu3 cells after treatment.
 - B) We have also continued efforts related to the expansion of scope 1 to develop lead assets to limit SARS-CoV2 replication, Major Task 1: Optimization of KIN269 and KIN1502. These activities have included evaluating potential cytotoxicity of KIN269, KIN1400, and KIN1502 in Calu3 and HEK293-ACE2 cells, which are the two cell lines used by the lab as in vitro models of SARS-CoV2 infection. HEK293-ACE2 cells are a human embryonic kidney cell line that expresses a high level of human ACE2 protein, which is required for SARS-CoV2 viral entry into the cell during infection. We have also completed the initial antiviral testing of KIN269, KIN1400, and KIN1502 activity against SARS-CoV2 infection in Calu3 cells.
- 2) **Specific Objectives.** Our overall objective for these efforts were to support (A) specific aim 1 of project 3 to identify and validate the host targets of KIN269 and KIN1502 and (B) specific aim 1 of the expansion of scope 1 to optimize the potency and in vitro ADME properties of leads.
- 3) **Significant Results/Key Outcomes.** We completed a timecourse dose-titration study to define the toxicity induced by KIN269, KIN1400, and KIN1502 in Calu3 and HEK293 ACE2 cells and found that with a cut-off of 80% viability, KIN1502 was not toxic to Calu3 and HEK293 ACE2 cells after treatment with compound for 72 hours at any dose up to 10uM. In contrast, KIN269 and KIN1400 induced cellular toxicity in Calu3 and HEK293 ACE2 cells with both compounds reducing cell viability to lower than 80% after 0.1uM treatment in Calu3 cells and 1uM treatment in HEK293 ACE2 cells (Figure 4 & 5). We also found that KIN1502 induced IRF3 phosphorylation of serine residue 386 after 24 hours of compound treatment in Calu3 cells. In additional KIN1502 induced protein expression of interferon stimulated genes RIG-I and IFITM1 (Figure 6). At 24 hours post-treatment, KIN269 and KIN1400 did not induce IRF3

phosphorylation of S386 or IFITM-1 expression in Calu3 cells. These results confirm that KIN1502 treatment in Calu3 cells is triggering innate immune response pathways. Finally, we have found that KIN1502 had dose-dependent activity against SARS-CoV2 infection in Calu3 cells upon 72 hours post-compound treatment (Figure 7). Based on these results, we are moving forward with designing medicinal chemistry analogs to improve potency of KIN1502 and developing intranasal formulations to evaluate KIN1502 efficacy as a therapeutic against SARS-CoV2 infection in vivo.

- 4) Other Achievements. We have been working with the formulations group to develop intranasal formulations for KIN1502 that would be tested by the program as a potential therapeutic to treat SARS-CoV2 infection to prevent COVID19.

For Quarter 3, Year 1

- 1) Major activities

We have continued activities related to Project 3: Defining MOA for Broad-spectrum Respiratory Antivirals that Activate Innate Immunity. These activities included evaluating the ability of KIN1502 to induce phosphorylation of IRF3 in human liver-derived HepG2-hNTCP cells. We also used immunofluorescence analysis (IFA) to evaluate the ability of KIN1502 to induce IRF3 translocation to the nucleus. In addition, we produced HepG2-hNTCP cells expressing non-targeting guide RNA (HepG2-hNTCP-NT), or guide RNA for knockout (KO) of RIG-I expression (HepG2-hNTCP-RKO) or MDA5 (HepG2-hNTCP-MKO) using CRISPR-Cas9 genome editing technology and used these cell lines to determine if the RLRs RIG-I or MDA5 are required in KIN1502-induced IRF3 activation. Finally, we evaluated the innate immune gene expression pathways that are induced in HepG2-hNTCP cells 24 and 72 hours after KIN1502 treatment compared to the known poly U/UC RNA PAMP RIG-I agonist.

- 2) Specific objectives

Our overall objective for these efforts were to support (A) specific aim 1 of project 3, major task 3, subtask 2 to assess IRF3 phosphorylation and distribution in the presence of KIN1502 and (B) specific aim 2 of project 3, major task 2, subtask 1 to evaluate the compound ability of KIN1502 to promote nuclear translocation of IRF-3. Two additional objectives were to (C) evaluate the requirement of RLR receptors RIG-I and MDA5 in KIN1502 IRF3 pathway activation and (D) to define the innate immune activation pathways induced by KIN1502 using transcriptional profiling.

Significant results

In Quarter 2, we reported that KIN1502 induced IRF3 phosphorylation and induced protein expression of interferon stimulated genes (ISGs) after treatment in the human-derived lung calu3 cell line. In Quarter 3, we confirmed similar results using the human-derived liver HepG2-hNTCP cell line that was originally designed for in vitro modeling of HBV infection. We found that KIN1502 induced dose-dependent phosphorylation of IRF3 and induction of the ISG IFIT1 in HepG2-hNTCP cells after 24 and 48 hrs of treatment (Figure 8). Our findings reveal that KIN1502 can induce IRF3 phosphorylation in two different human cell lines derived from two different tissue types.

We also confirmed KIN1502 induced IRF3 activation by stimulating IRF3 nuclear translocation (Figure 8). For this work, we treated HepG2-hNTCP cells with 10 μ M KIN1502 or 200 ng/mL poly-U/UC PAMP formulated in liposome for 24 h. Control cells were treated with DMSO or infected with Sendai virus (SenV; a potent activator of RIG-

I-dependent signaling), or transfected with 200 ng/mL X-RNA negative control in liposome, a non-PAMP/non-signaling 5'ppp-containing 100 nt RNA motif from the HCV genome with similar mass to the poly-U/UC PAMP⁽¹⁾. Similar to SenV control, both KIN1502 and poly-U/UC PAMP but neither DMSO nor X-RNA treatment specifically induced innate immune activation as marked by IRF3 translocation into the nucleus.

To ascertain whether the activation of IRF3 in cells treated with KIN1502 was dependent on RIG-I rather than occurring as an off-target effect of IRF3 agonist treatment, we produced HepG2-hNTCP cells expressing non-targeting guide RNA (HepG2-hNTCP-NT), or guide RNA for knockout (KO) of RIG-I expression (HepG2-hNTCP-RKO) or MDA5 (HepG2-hNTCP-MKO) using CRISPR-Cas9 genome editing technology. Immunoblot analysis of the different cell populations shows that HepG2-hNTCP-RKO or HepG2-hNTCP-MKO cells do not express detectable levels of RIG-I or MDA5, respectively (data not shown). Treatment of cells with KIN1502 (Figure 9) shows that the HepG2-hNTCP-RKO cells do not respond to treatment, whereas HepG2-hNTCP-NT control cells and HepG2-hNTCP-MKO cells fully respond to KIN1502 to accumulate phosphorylated/activated IRF3 concomitant with expression of IFIT1. HepG2-hNTCP-MKO cells serve as an RLR KO control to reveal the specificity of KIN1502 and poly-U/UC PAMP for triggering RIG-I-dependent IRF3 activation⁽¹⁾. These results demonstrate that the IRF3 activation induced by KIN1502 specifically signals through RIG-I, defining KIN1502 as a RIG-I agonist that activates IRF3 to impart innate immune activation and antiviral activity.

Finally, we assessed mRNA expression across a panel of innate immune response genes, including IFNs, ISGs, and direct IRF3-target genes, for response to KIN1502 or poly-U/UC PAMP (Figure 9). This assessment revealed that while Poly-U/UC PAMP treatment induced type I and type III IFN and ISG expression, KIN1502 treatment induced only IRF3 direct target gene expression including IFIT1, CXCL10, RSAD2, and IFITM1. This difference is consistent with the signaling properties of each molecule and the nature of IFN expression, as IFN expression relies on activation of both IRF3 and NF- κ B. KIN1502 specifically activates IRF3 but not NF- κ B⁽²⁾. In contrast, the poly-U/UC PAMP triggers RIG-I signaling of both transcription factors to induce IRF3-target genes, IFNs, and hence ISGs^(1,3). Taken together, these results show that KIN1502 induces innate immune activation in treated cells that initiates with IRF3 activation and the induction of IRF3-target genes.

All of the above significant results were featured in our recent iScience publication⁽⁴⁾.

For Quarter 4, Year 1

1) Major activities.

In the Gale laboratory, the major activities for Quarter 4, Year 1 focused on troubleshooting the loss of activity for the KIN1502 compound and preparation of the KIN1502 library for SAR analysis. A description of these activities are featured below.

KIN1502 activity loss. Our scientists observed loss of activity with KIN1502 when evaluated in antiviral assays and in innate immune activation assays. To troubleshoot this activity loss, we worked with chemists to evaluate the possibility of KIN1502 stock degradation. KIN1502 integrity was evaluated by proton NMR analysis (Figure 10) and HPLC analysis. Several KIN1502 lots were also evaluated in innate immune assays

and by NMR and HPLC to determine if the activity loss was associated with specific lots.

KIN1502 library preparation. In quarter 4, we facilitated the transfer of over 1,000 KIN compounds from Kineta, Inc. to the Gale laboratory. Once transferred, the Gale laboratory, generated a digital log inventory of this library. We identified and located 84 KIN1502 derivatives within the larger KIN library, and these 84 compound derivatives will be used in an SAR to determine how chemical structure relates to antiviral activity. The Gale laboratory aliquoted 10-20mg of each KIN1502 derivative and shipped the 84 KIN1502 derivative library along with structural information to SBP so that SBP can initiate the SAR analysis.

2) Specific objectives.

For the Gale lab, our overall objective for these efforts were to support (A) specific aim 1, 2, and 3 for the troubleshooting of KIN1502 activity loss, and (B) specific aim 1 of the expansion of scope 1 to optimize the potency and in vitro ADME properties of leads.

3) Significant Results/Key Outcomes.

For the Gale lab, the proton NMR and HPLC results were consistent and revealed that KIN1502 lots were not degraded. Thus KIN1502 activity loss cannot be contributed to compound chemical degradation.

4) Other Achievements. None.

For Year 1:

Goals not met. Due to COVID-19 related delays in our research efforts and loss of KIN1502 activity, we have not been able to initiate research efforts for Major Tasks 1 and 3 for Project 3. Details on these delays and timeline impact are discussed below.

Progress Report for the García-Sastre Laboratory

The García-Sastre lab has established several rodent models of SARS-CoV-2 infection and tested the antiviral and therapeutic activities of several compounds in these models:

1. The hamster model of SARS-CoV-2 infection.

The García-Sastre group has infected intranasally Golden Syrian hamsters (6-weeks of age) with SARS-CoV-2 and evaluated body weight loss, viral titers in nasal washes, lungs, intestine and other organs at different days postinfection. These experiments were performed under an approved IACUC protocol and at a BSL3 facility at ISMMS. The virus caused around 15% body weight drop at 6 days postinfection and the animals recovered. Viral titers were observed in the nose and lungs of the animals as well as in the intestine, kidney, olfactory bulb and heart. This animal model is being used available for testing the antiviral activity of selected compounds.

2. The transgenic K18-hACE2 and the adenovirus transduced hACE2 mouse models of SARS-COV-2 infection.

The García-Sastre group has compared the K18-hACE2 transgenic model to adenovirus-mediated delivery of hACE2 to the mouse lung. He has shown that K18-hACE2 mice replicate virus to high titers in the nasal turbinates, lung and brain, with high lethality, and cytokine/chemokine production. In contrast, adenovirus-mediated delivery results in viral replication to lower titers limited to the nasal turbinates and lung, and no clinical signs of infection with a challenge dose of 10⁴ plaque forming units. The K18-hACE2 model provides a stringent model for testing the ability of vaccines and antivirals to protect against disease, whereas the adenovirus delivery system has the flexibility to be used across multiple genetic backgrounds and modified mouse strains. These results were published: R. Rathnasinghe, S. Strohmeier, F. Amanat, V. L. Gillespie, F. Krammer, A. García-Sastre, L. Coughlan, M. Schotsaert & Melissa Uccellini. Comparison of transgenic and adenovirus hACE2 mouse models for SARS-CoV-2 infection. *Emerging Microbes & Infection*, 9:2433-2445 (2020).

3. A mouse-adapted SARS-CoV-2 animal model.

This mouse model is based on mouse adaptation of the SARS-CoV-2 strain USA-WA1/2020 (BEI resources; NR-52281), and allowed his lab to test the importance of the N501Y mutations present on SARS-CoV-2 variants in evading or not humoral immunity conferred by natural infection or by vaccination. Specifically, the García-Sastre laboratory has “mouse-adapted” SARS-CoV-2 by serial passaging a clinical virus isolate in the lungs of mice. They then used low doses of this virus in mouse models for advanced age, diabetes and obesity. Similar to SARS-CoV-2 infection in humans, the outcome of infection with mouse-adapted SARS-CoV-2 resulted in enhanced morbidity in aged and diabetic obese mice. Mutations associated with mouse adaptation occurred in the S, M, N and ORF8 genes. Interestingly, one mutation in the receptor binding domain of the S protein results in the change of an asparagine to tyrosine residue at position 501 (N501Y). This mutation is also present in the newly emerging SARS-CoV-2 variant viruses reported in the U.K. (20B/501Y.V1, B.1.1.7 lineage) that is epidemiologically associated with high human to human transmission. This mutation is also present in the B.1.351 and P.1 variants of concern. We found that human convalescent and post vaccination sera can neutralize the newly emerging N501Y virus variant with similar efficiency as that of the reference USA-WA1/2020 virus, suggesting that current SARS-CoV-2 vaccines will protect against the 20B/501Y.V1 strain

In addition, the García-Sastre laboratory has established *ex vivo* protocols for the determination of the inhibitory impact of compounds in human cells. Several of the inhibitors identified in our publication with the Chanda lab (Riva et al, *Nature*, 2020, 586(7827):113-119) were validated in human cells as inhibiting SARS-CoV-2 replication. Additional inhibitors tested in cells by the García-Sastre lab were also recently published in Bouhaddou et al, *Cell*, 2020, 182(3):685-712. Our previously published SARS-CoV-2 highlighted 332 host proteins that are likely to play a role in the viral life cycle of SARS-CoV-2. In that work we tested 47 existing drugs that were known to modulate these identified host proteins, with many of these drugs showing significant antiviral activity against SARS-CoV-2 in cell culture(6). Of the inhibitors tested, those that targeted the eukaryotic translation machinery (eIF4H interacts with SARS-CoV-2 Nsp9) demonstrated particularly potent antiviral activities. Zotatafin, an inhibitor of eIF4A (a partner of eIF4H), had a 90% inhibitory concentration (IC₉₀) of 154 nM and ternatin-4, an inhibitor of eEF1A which has potential interactions with multiple coronavirus proteins, had an IC₉₀ of 15 nM against SARS-CoV-2 in Vero E6 cells. In an effort to further explore the therapeutic potential of host eEF1A as a target for the treatment of COVID-19, we evaluated the eEF1A inhibitor plitidepsin (aplidin), which has limited clinical approval for the treatment of multiple myeloma. We found that the drug plitidepsin (aplidin), which has limited clinical approval, possesses antiviral activity (IC₉₀ = 0.88 nM) 27.5-fold more potent than remdesivir against SARS-CoV-2 *in vitro*, with limited toxicity in cell culture. Through the use of a drug resistant mutant, we found that the antiviral activity of plitidepsin against SARS-CoV-2 is indeed mediated through inhibition of the known target eEF1A. We have demonstrated the *in vivo* efficacy of plitidepsin treatment in two mouse models of SARS-CoV-2

infection with a reduction of viral replication in the lungs by two orders of magnitude using prophylactic treatment. Our results indicate that plitidepsin is a promising therapeutic candidate for COVID-19.

In more recent publications, we found that the proposed antiviral drug famotidine does not inhibit SARS-CoV-2 replication (Malone, P. et al. *Frontiers in Pharmacology*, doi.org/10.3389/fphar.2021.633680 (2021)). That TOP1 inhibition therapy protects against SARS-CoV-2-induced lethal inflammation in both hamster models and mouse models of SARS-CoV-2 infection (Ho et al., *Cell*, in press). We continue to use hamsters and mouse models to evaluate the therapeutic impact of drugs found by our collaborators in disease induced by influenza and/or SARS-CoV-2 infection.

References

1. Saito T., Owen D.M., Jiang F., Marcotrigiano J., Gale M., Jr. Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. *Nature*. 2008;454:523–527. PMID: PMC2856441
2. Bedard K.M., Wang M.L., Proll S.C., Loo Y.M., Katze M.G., Gale M., Jr., Iadonato S.P. Isoflavone agonists of IRF-3 dependent signaling have antiviral activity against RNA viruses. *J. Virol.* 2012;86:7334–7344. PMID: PMC3416323
3. Schnell G., Loo Y.M., Marcotrigiano J., Gale M., Jr. Uridine composition of the poly-U/UC tract of HCV RNA defines non-self recognition by RIG-I. *PLoS Pathog.* 2012;8:e1002839. PMID: PMC3410852
4. Lee, S., Goyal, A., Perelson, A.S., Ishida, Y., Saito, T., Gale, M. Jr. Suppression of hepatitis B virus through therapeutic activation of RIG-I and IRF3 signaling in hepatocytes. *iScience*. 2021. 24(1):101969. PMID: PMC7797372.

Figure 1, Panels A, B, C. Quality control analysis of KIN269 by (A) TLC, (B) NMR, and (C) HPLC.

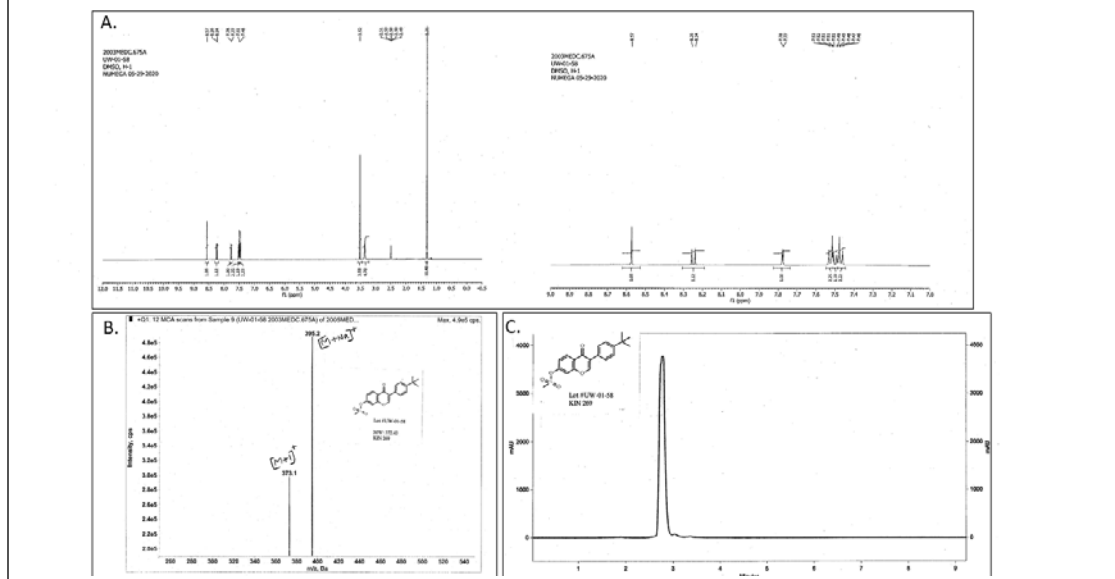


Figure 1, Panels D, E, F. Quality control analysis of KIN1400 by (D) TLC, (E) NMR, and (F) HPLC.

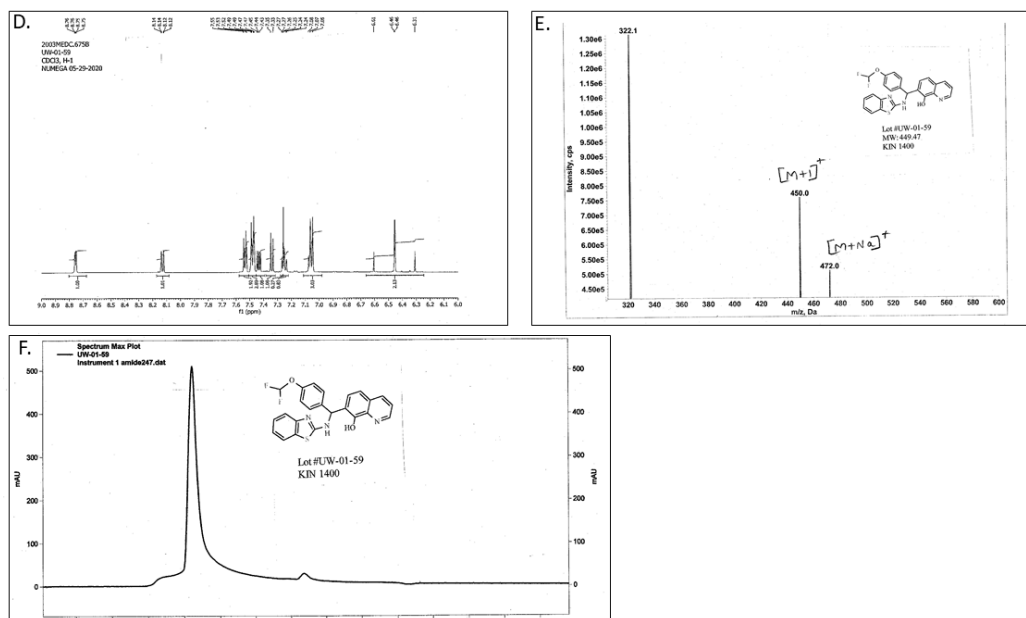
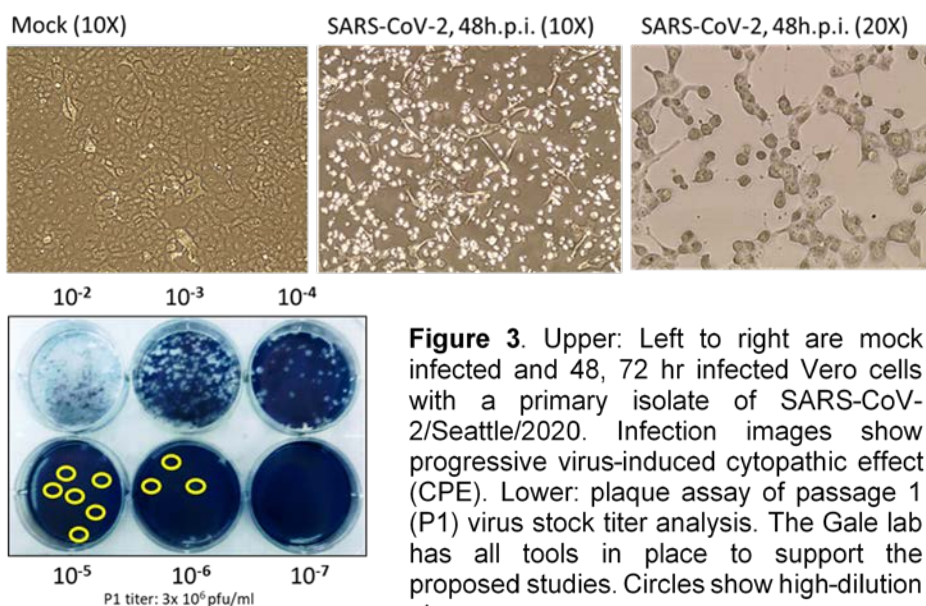
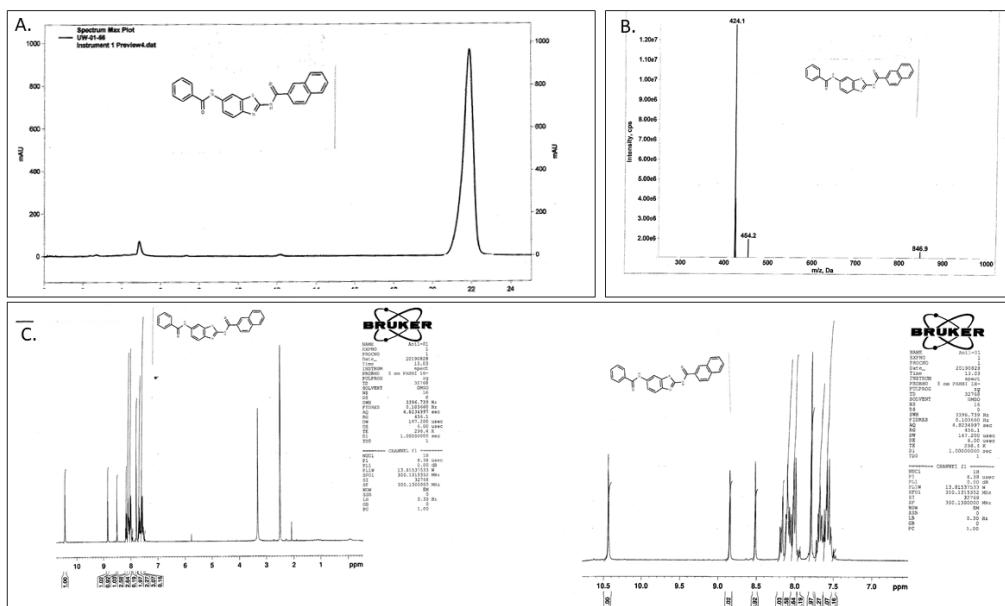


Figure 2, Panels A, B, C. Quality control analysis of KIN1502 by HPLC (A) and NMR (B & C.)



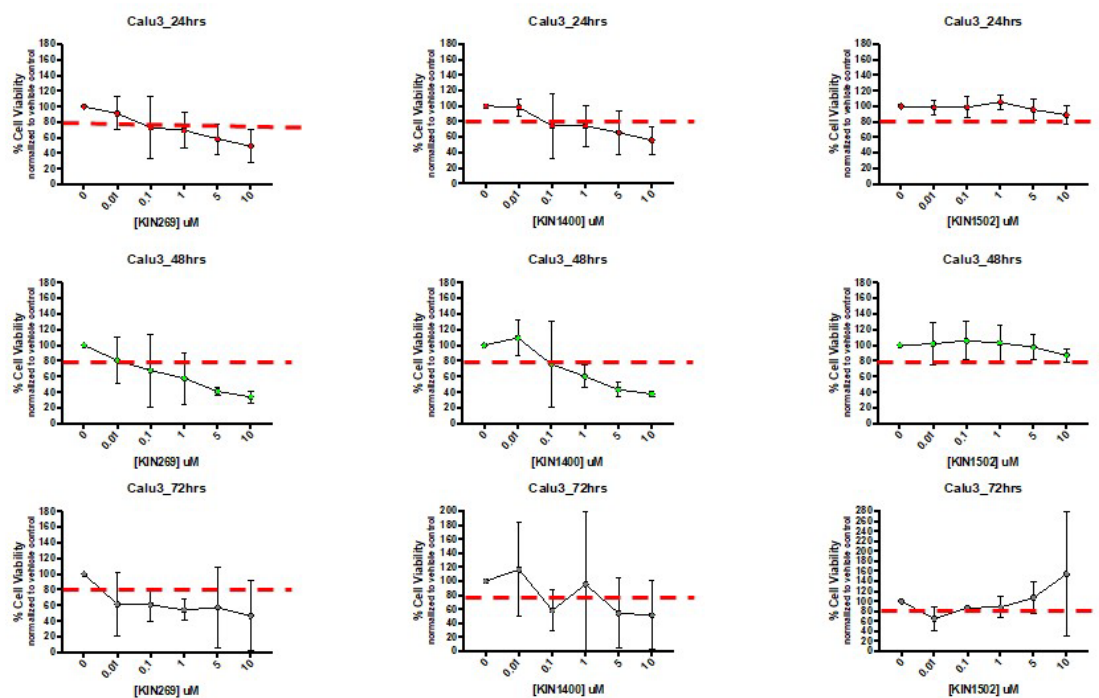


Figure 4. Cell viability of Calu3 cells at 24, 48, and 72 hours after treatment with KIN269, KIN1400, and KIN1502. Cells were treated with indicated doses of compound in 5% DMSO.

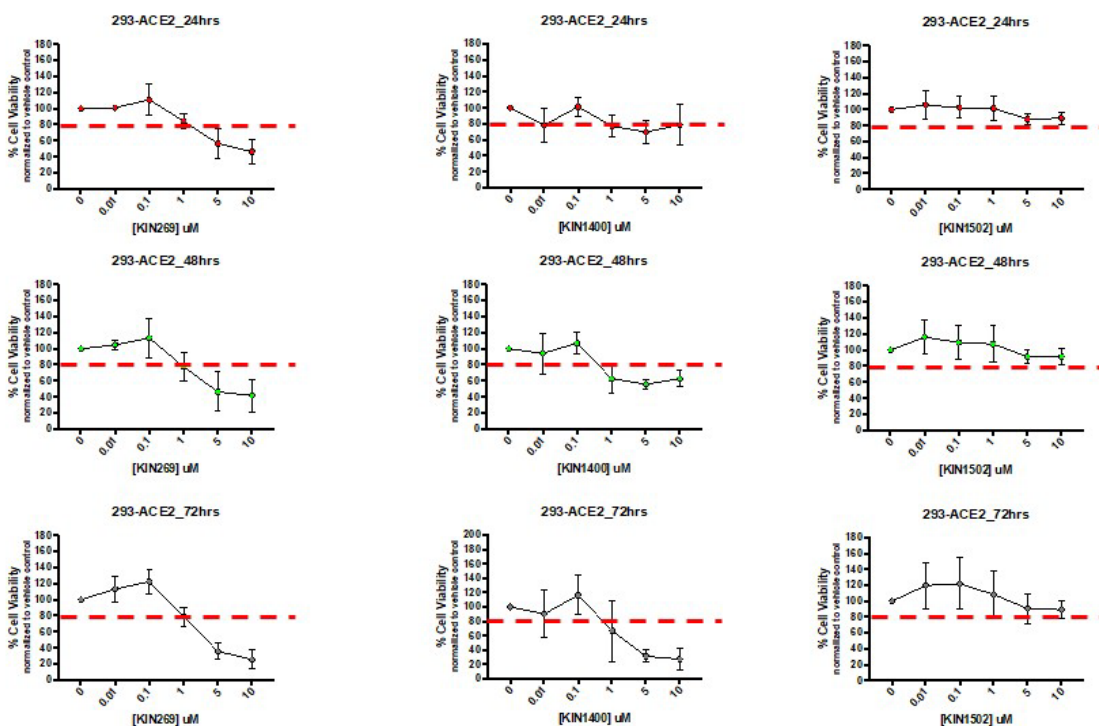


Figure 5. Cell viability of HEK293 ACE2 cells at 24, 48, and 72 hours after treatment with KIN269, KIN1400, and KIN1502. Cells were treated with indicated doses of compound in 5% DMSO.

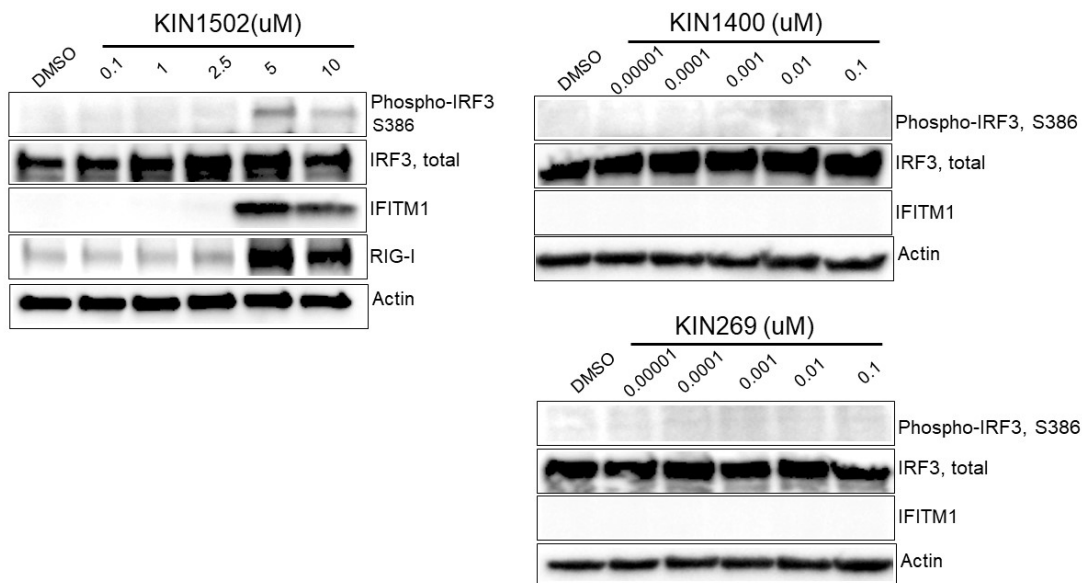


Figure 6. KIN1502 induces phosphorylation of S386 on IRF3 and IFITM1 and RIG-I in Calu3 cells. Cells were treated with compound for 24 hours. Cell lysates were evaluated by immunoblotting with antibodies that specifically detect phosphorylated IRF3 S386, IFITM1, and RIG-I. Total IRF3 and actin immunoblots are included as a loading control. Compounds were suspended in 5% DMSO solution at the indicated doses.

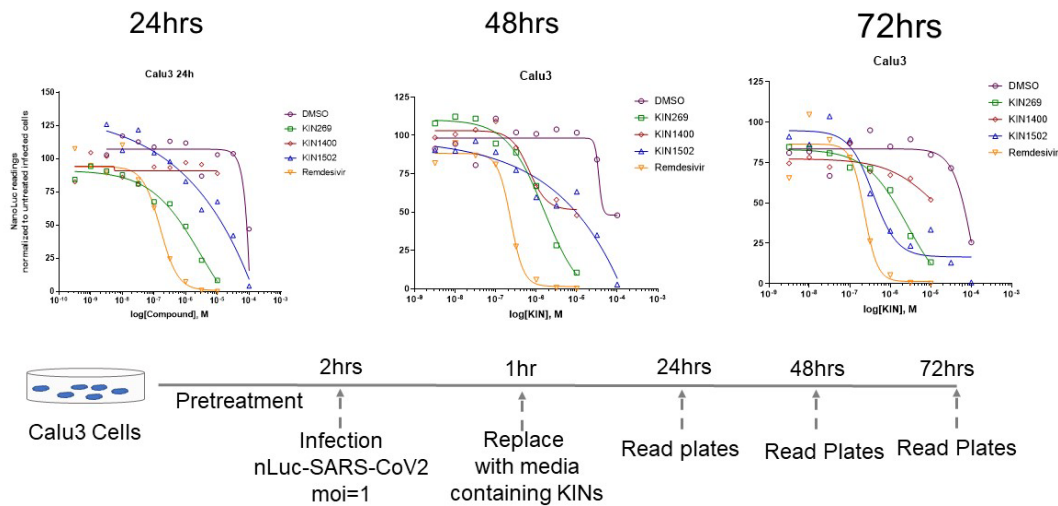
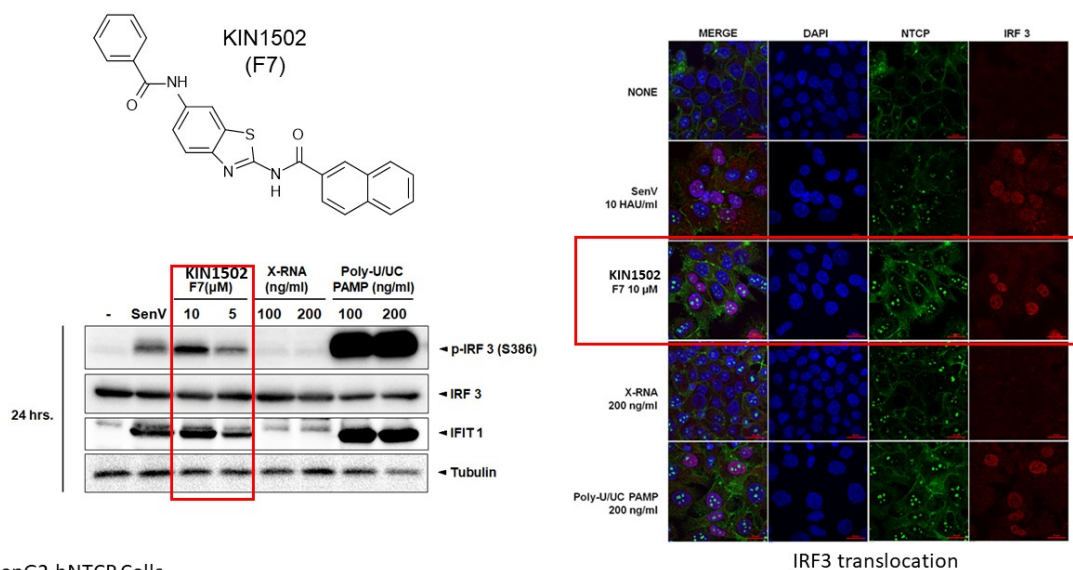


Figure 7. KIN1502 has dose-dependent antiviral activity against SARS-CoV2 in Calu3 cells. Cells were pre-treated with compounds for 2 hours prior to infection with SARS-CoV2 containing a luciferase gene (nLuc-SARS-CoV2). Virus was removed by washing cells with fresh media containing KIN compounds 1 hour after the infection was initiated. SARS-CoV2 levels in each culture was determined by luminescence and readings were normalized to the luminescence measured in untreated infected cells. Compounds were suspended in 5% DMSO solution. Remdesivir is included as a positive control.



HepG2-hNTCP Cells

Figure 8. KIN1502 induces IRF-3 activation and nuclear translocation. (A) Structure of KIN1502 (F7). (B) KIN1502 and poly-U/UC induce IRF3 activation and innate immune gene expression. Sendai virus, KIN1502, X-RNA, and poly-U/UC PAMP were administered to HepG2-hNTCP for 24h. The cell lysates were resolved by SDS-PAGE and then subjected to immunoblotting. The levels of protein expression by the p-IRF3 (S386 phosphorylation active form of IRF3), IRF3 (total IRF3), and IFIT1 relative to the expression level of tubulin were determined using respective antibodies. (C) Immunofluorescence analysis of IRF3 translocation. HepG2-hNTCP cells were cultured in medium containing 2.5% DMSO, infected with Sendai virus (SenV; 10 HAU/ml), or treated with KIN1502 (10 μM), X-RNA (200 ng/mL in liposome), or poly-U/UC PAMP (200 ng/mL in liposome) for 24 h. Cells were fixed with 3% paraformaldehyde, stained by using mouse anti-hNTCP (green) and rabbit anti-IRF3 (red) antibodies, and counterstained with DAPI (blue). Scale bars, 20 μm.

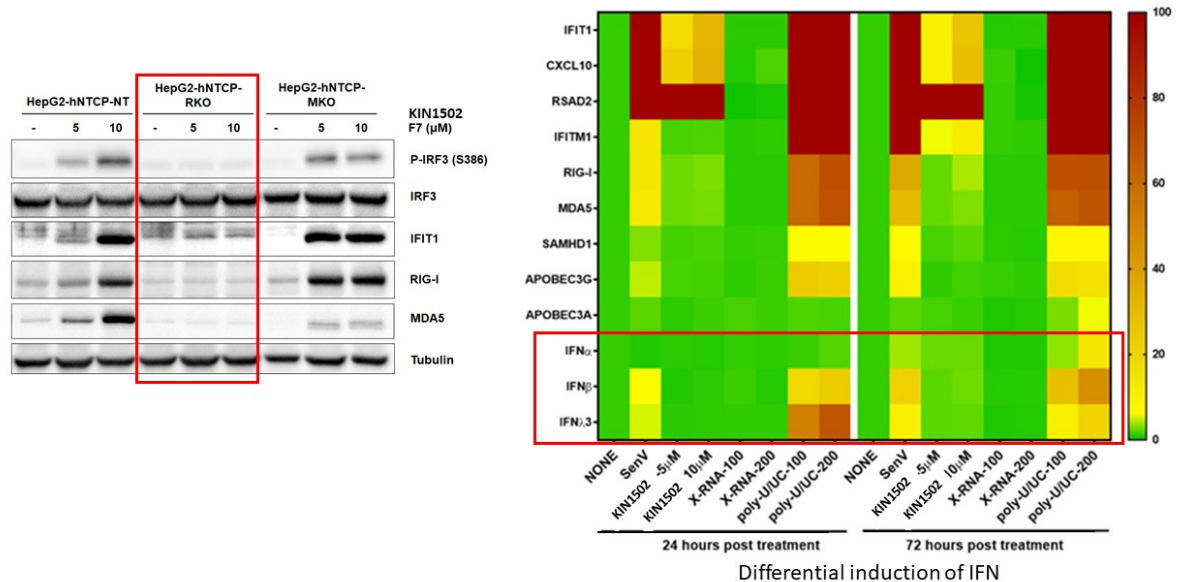


Figure 9. KIN1502-induced innate activation requires RIG-I and produces a differential induction of IFN when compared to other known RLR agonists. (A) KIN1502 was administered for 24 h to HepG2-hNTCP-NT, RKO, and MKO cells. Cells were harvested and analyzed by immunoblot. The levels of protein expression by p-IRF3 (S386 phosphorylated), IRF3 (total IRF), IFIT1, RIG-I, and MDA5 relative to the expression level of tubulin were determined using respective antibodies. (B) Gene expression analysis. HepG2-hNTCP cells were treated with SenV, KIN1502, X-RNA, and poly-U/UC-RNA as described above for 24 and 72 h; then total cellular RNA was purified from harvested cells. The levels of expression of innate immune genes IFIT1, CXCL10, IFITM1, RSAD2, RIG-I, MDA5, SAMHD1, APOBEC3A, APOBEC3G, IFN- α , IFN- β , and IFN- λ 3 were measured by qRT-PCR and normalized to the level of GAPDH expression. Each are shown as the mean fold induction over that achieved with 2.5% DMSO treatment from three independent experiments.

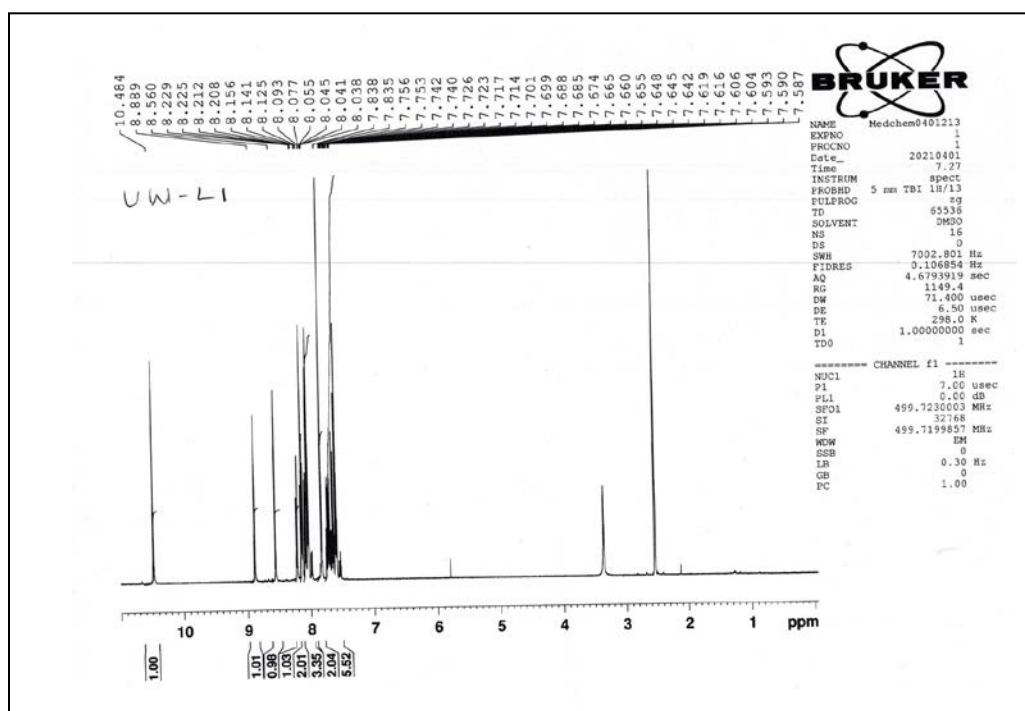


Figure 10. Proton NMR analysis of KIN1502. Analysis of 3 separate lots of KIN1502 were identical and revealed no evidence of chemical degradation with >95% purity in all lots.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or

one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the Gale laboratory, we plan on continuing to troubleshoot the KIN1502 loss of activity. We will evaluate various mechanisms of increasing KIN1502 solubility with sonication, heat, and changing how the compound is handled when diluting into culture media. Once potential biotin labeling sites have been identified with the SAR, we will initiate pull down assays. We will also initiate antiviral SARS CoV-2 and influenza in vitro assays once active KIN1502 derivatives have been identified with the SAR.

In the García-Sastre laboratory, we will continue testing selected compounds with antiviral activity against influenza and SARS-CoV-2 viruses and with PK data in appropriate mice and hamster models.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The major impact to the principal discipline is that KIN1502 induces innate immune activation in treated cells that initiates with IRF3 activation and the induction of IRF3-target genes.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

The results on plitidepsin antiviral activity generated in the García-Sastre lab have supported the initiation of a phase 3 clinical trial to evaluate the efficacy and safety of plitidepsin in hospitalized patients with moderate COVID-19 (NEPTUNO; NCT04784559).

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

In the last quarter, we have experienced a loss of KIN1502 activity as measured by innate immune activation assays and antiviral assays. Chemical analysis (proton NMR and HPLC) revealed that the loss of activity was not due to degradation of compound stocks. We now believe that loss of activity is due to inherent insolubility of the compound when diluted in tissue culture media. We are trying a variety of methods (see above section “plans for next reporting period” for plans on increasing compound solubility, which we hope will restore compound activity. In addition, our institution implemented personnel restrictions in the laboratories in response to the COVID-19 pandemic, which in turn restricted our capacity to complete mechanism of action studies in Aim 1 of Project 3. We are now back at full capacity for personnel, and plan on working to complete the originally proposed studies.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

We have had delay on expenditures due to COVID-19 pandemic-related limitations on personnel as described above.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable.

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:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

Nothing to report.

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Lee, S., Goyal, A., Perelson, A.S., Ishida, Y., Saito, T., Gale, M. Jr. Suppression of hepatitis B virus through therapeutic activation of RIG-I and IRF3 signaling in hepatocytes. *iScience*. 2021. 24(1):101969. PMID:PMC7797372. (yes)

R. Rathnasinghe, S. Strohmeier, F. Amanat, V. L. Gillespie, F. Krammer, A. García-Sastre, L. Coughlan, M. Schotsaert & Melissa Uccellini. Comparison of transgenic and adenovirus hACE2 mouse models for SARS-CoV-2 infection. *Emerging Microbes & Infection*, 9:2433-2445 (2020). (yes)

M. Bouhaddou, D. Memon, B. Meyer, K. M. White, V. V. Rezell, M. C. Marrero, B. J. Polacco, J. E. Melnyk, S. Ulferts, R. M. Kaake, J. Batra, A. L. Richards, E. Stevenson, D. E. Gordon, A. Rojc, K. Obernier, J. M. Fabius, M. Soucheray, L. Miorin, E. Moreno, C. Koh, Q. D. Tran, A. Hardy, R. Robinot, T. Vallet, B. E. Nilsson-Payant, C. Hernandez-Armenta, A. Dunham, S. Weigang, J. Knerr, M. Modak, D. Quintero, Y. Zhou, A. Dugourd, A. Valdeolivas, T. Patil, Q. Li, R. Hüttenhain, M. Cakir, M. Muralidharan, M. Kim, G. Jang, B. Tutuncuoglu, J. Hiatt, J. Z. Guo, J. Xu, S. Bouhaddou, C. J. P. Mathy, A. Gaulton, E. J. Manners, E. Félix, Y. Shi, M. Goff, J. K. Lim, T. McBride, M. C. O'Neal, Y. Cai, J. C. J. Chang, D. J. Broadhurst, S. Klippsten, E. De Wit, A. R. Leach, T. Kortemme, B. Shoichet, M. Ott, J. Saez-Rodriguez, B. R. tenOever, D. Mullins, E. R. Fischer, G. Kochs, R. Grosse*, A. García-Sastre*, M. Vignuzzi*, J. R. Johnson*, K. M. Shokat*, D. L. Swaney*, P. Beltrao*, N. J. Krogan*. The global phosphorylation landscape of SARS-CoV-2 infection. *Cell*, 182:685-712 (2020). PMID: PMC7321036. *Equal contribution. (yes)

L. Riva, S. Yuan, X. Yin, L. Martin-Sancho, N. Matsunaga, L. Pache, S. Burgstaller-Muehlbacher, P. D. De Jesus, P. Teriete, M. V. Hull, Max W. Chang, J. F.-W. Chan, J. Cao, V. K.-M. Poon, K. M. Herbert, K. Cheng, T.-T. H. Nguyen, A. Rubanov, Y. Pu, C. Nguyen, A. Choi, R. Rathnasinghe, M. Schotsaert, L. Miorin, M. Dejez, T. P. Zwaka, K.-Y. Sit, L. Martinez-Sobrido, W.-C. Liu, K. M. White, M. E. Chapman, E. K. Lendy, R. J. Glynne, R. Albrecht, E. Rupp, A. D. Mesecar, J. R. Johnson, C. Benner, R. Sun, P. G. Schultz, A. I. Su, A. García-Sastre, A. K. Chatterjee, K.-Y. Yuen & S. K. Chanda. Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing. *Nature*, 586:113-119 (2020). PMID: PMC7603405. (yes)

D. E. Gordon, J. Hiatt¹, M. Bouhaddou, V. V. Rezeli, S. Ulferts, H. Braberg, A. S. Jureka, K. Obernier, J. Z. Guo, J. Batra, R. M. Kaake, A. R. Weckstein, T. W. Owens, M. Gupta, S. Pourmal, E. W. Titus, M. Cakir, M. Soucheray, M. McGregor, Z. Cakir, G. Jang, M. J. O'Meara, T. A. Tummino, Z. Zhang, H. Foussard, A. Rojc, Y. Zhou, D. Kuchenov, R. Hüttenhain, J. Xu, M. Eckhardt, D. L. Swaney, J. M. Fabius, M. Ummadi, B. Tutuncuoglu, U. Rathore, M. Modak, P. Haas, K. M. Haas, Z. Z. C. Naing, E. H. Pulido, Y. Shi, I. Barrio-Hernandez, D. Memon, E. Petsalaki, A. Dunham, M. C. Marrero, D. Burke, C. Koh, T. Vallet, J. A. Silvas, C. M. Azumaya, C. Billesbølle, A. F. Brilot, M. G. Campbell, A. Diallo, M. S. Dickinson, D. Diwanji, N. Herrera, N. Hoppe, H. T. Kratochvil, Y. Liu, G. E. Merz, M. Moritz, H. C. Nguyen, C. Nowotny, C. Puchades, A. N. Rizo, U. Schulze-Gahmen, A. M. Smith, M. Sun, I. D. Young, J. Zhao, D. Asarnow, J. Biel, A. Bowen, J. R. Braxton, J. Chen, C. M. Chio, U. S. Chio, I. Deshpande, L. Doan, B. Faust, S. Flores, M. Jin, K. Kim, V. L. Lam, F. Li, J. Li, Y.-L. Li, Y. Li, X. Liu, M. Lo, K. E. Lopez, A. A. Melo, F. R. Moss III, P. Nguyen, J. Paulino, K. I. Pawar, J. K. Peters, T. H. Pospiech Jr., M. Safari, S. Sangwan, K. Schaefer, P. V. Thomas, A. C. Thwin, R. Trenker, E. Tse, T. K. M. Tsui, F. Wang, N. Whitis, Z. Yu, K. Zhang, Y. Zhang, F. Zhou, D. Saltzberg, QCRG Structural Biology Consortium, A. J. Hodder, A. S. Shun-Shion, D. M. Williams, K. M. White, R. Rosales, T. Kehrer, L. Miorin, E. Moreno, A. H. Patel, S. Rihn, M. M. Khalid, A. Vallejo-Gracia, P. Fozouni, C. R. Simoneau, T. L. Roth, D. Wu, M. A. Karim, M. Ghousaini, I. Dunham, F. Berardi, S. Weigang, M. Chazal, J. Park, J. Logue, M. McGrath, S. Weston, R. Haupt, C. J. Hastie, M. Elliott, F. Brown, K. A. Burness, E. Reid, M. Dorward, C. Johnson, S. G. Wilkinson, A. Geyer, D. M. Giesel, C. Baillie, S. Raggett, H. Leech, R. Toth, N. Goodman, K. C. Keough, A. L. Lind, Zoonomia Consortium, R. J. Klesh, K. R. Hemphill, J. Carlson-Stevermer, J. Oki, K. Holden, T. Maures, K. S. Pollard, A. Sali, D. A. Agard, Y. Cheng, J. S. Fraser, A. Frost, N. Jura, T. Kortemme, A. Manglik, D. R. Southworth, R. M. Stroud, D. R. Alessi, P. Davies, M. B. Frieman, T. Ideker, C. Abate, N. Jouvenet, G. Kochs, B. Shoichet, M. Ott, M. Palmarini, K. M. Shokat, A. García-Sastre*, J. A. Rassen*, R. Grosse*, O. S. Rosenberg, K. A. Verba*, C. F. Basler*, M. Vignuzzi*, A. A. Peden*, P. Beltrao* & N. J. Krogan*. Comparative host-coronavirus protein interaction networks reveal pan-viral disease mechanisms. *Science*, 370(6521):eabe9403. doi: 10.1126/science.abe9403 (2020). PMID: PMC7808408. *Equal contribution (yes)

K. M. White, R. Rosales, S. Yildiz, T. Kehrer, L. Miorin, E. Moreno, S. Jangra, M. B. Uccellini, R. Rathnasinghe, L. Coughlan, C. Martinez-Romero, J. Batra, A. Rojc, M. Bouhaddou, J. M. Fabius, K. Obernier, M. DeJozes, M. J. Guillén, A. Losada, P. Avilés, M. Schotsaert, T. Zwaka, M. Vignuzzi, K. Shokat, N. J. Krogan & A. García-Sastre. Plitidepsin has potent preclinical efficacy against SARS-CoV-2 by targeting the host protein eEF1A. *Science*, 371:926-931. doi: 10.1126/science.abf4058 (2021). PMID: PMC7963220. (yes)

Malone, P. Tisdall, P. Fremont-Smith, Y. Liu, X.-P. Huang, K. M. White, L. Miorin, E. Moreno, A. Alon, E. Delaforge, C. D. Hennecker, G. Wang, J. Pottel, R. V. Blair, C. J. Roy, N. Smith, J. M. Hall, K. M. Tomera, G. Shapiro, A. K. Mittermaier, A. C. Kruse, A. García-Sastre, B. Roth, J. Glasspool-Malone & D. O. Ricke. COVID-19: Famotidine, histamine, mast cells, and mechanisms. *Frontiers in Pharmacology*, doi.org/10.3389/fphar.2021.633680 (2021). (yes)

S. Y. Ho, B. W.-Y. Mok, L. Campisi, T. Jordan, S. Yildiz, S. Parameswaran, J. A. Wayman, N. N. Gaudreault, D. A. Meekins, S. V. Indran, I. Morozov, J. D. Trujillo, Y. S. Fstkhyan, R. Rathnasinghe, Z. Zhu, S. Zheng, N. Zhao, K. White, H. Ray-Jones, V. Malysheva, M. J. Thiecke, S.-Y. Lau, H. Liu, A. J. Zhang, A. C.-Y. Lee, W.-C. Liu, S. Jangra, A. Escalera, T. Aydiello, B. S. Melo, E. Guccione, R. Sebra, E. Shum, J. Bakker, D. A. Kaufman, A. L. Moreira, M. Carossino, U. B. R. Balasuriya, M. Byun, R. A. Albrecht, M. Schotsaert, A. García-Sastre, S. K. Chanda, E. R. Miraldi, A. D. Jeyasekharan, B. R. TenOever, M. Spivakov, M. T. Weirauch, S. Heinz, H. Chen, C. Benner, J. A. Richt & I. Marazzi. TOP1 inhibition therapy protects against SARS-CoV-2-induced lethal inflammation. *Cell*, in press.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities.

Nothing to report.

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- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

University of Washington

Name	Michael Gale
Project Role	PI
ID	870002824
Nearest Person Month worked.	0.86
Contribution to project	Overall project oversight and direction.
Name	Jackie Berhorst
Project Role	Research Coordinator
ID	867009172
Nearest Person Month worked.	0.84
Contribution to project	Ordering supplies and fiscal management.
Name	Tien-Ying Hsiang
Project Role	Research Scientist
ID	85002763
Nearest Person Month worked.	2.29
Contribution to project	SARS CoV2 antiviral testing.
Name	Amina Negash
Project Role	Research Scientist
ID	859005436
Nearest Person Month worked.	9.86
Contribution to project	KIN compound toxicity studies and innate immune activation assays.
Name	Bryan Turnbull
Project Role	Research scientist
ID	84905402
Nearest Person Month worked.	3.53
Contribution to project	Assist with antiviral assays.
Name	Kathleen Voss
Project Role	Research scientist
ID	849008699
Nearest Person Month worked.	0.76
Contribution to project	Assist with antiviral assays.
Name	Megan Knoll
Project Role	Research scientist
ID	864007187
Nearest Person Month worked.	1.23
Contribution to project	Assist with compound innate immune activation assays.

Name	Elyse Verstelle
Project Role	Laboratory Manager
ID	848002117
Nearest Person Month worked.	0.31
Contribution to project	Compound inventory, shipment, storage, and equipment maintenance.

Icahn School of Medicine at Mount Sinai

Name	Dr. Adolfo Garcia-Sastre
Project Role	PI
Nearest Person Month worked.	1.20
Contribution to project	Project administration and oversight
Name	Dr. Ignacio Mena
Project Role	Research Faculty
Nearest Person Month worked.	2.40
Contribution to project	Animal work under BSL3 conditions
Name	Dr. Carles Martinez
Project Role	Research Faculty
Nearest Person Month worked.	2.40
Contribution to project	Animal work under BSL3 conditions
Name	Dr. Naoko Imai
Project Role	Research Faculty
Nearest Person Month worked.	1.00
Contribution to project	Animal work under BSL3 conditions
Name	Dr. Arturo Marin
Project Role	Postdoctoral Fellow
Nearest Person Month worked.	9.00
Contribution to project	Data analysis
Name	Arantza Cobela
Project Role	Technician
Nearest Person Month worked.	4.00
Contribution to project	Assist lab staff with animal work
Name	Anastasija Cupic
Project Role	Technician
Nearest Person Month worked.	1.80
Contribution to project	Assist lab staff with animal work
Name	Richard Cadagan
Project Role	Technician
Nearest Person Month worked.	0.60
Contribution to project	Assist lab staff with animal work

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Yes, please see attached other support document.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

CHANGES IN ACTIVE SUPPORT

GALE, MICHAEL

CURRENT

4U19 AI100625-09 (PI: M. Heise, R. Baric) 8/5/12-8/31/22 0.45 CM (3.75%)
\$745,000 total direct costs

Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross

The goal of this project is: The major goals of this project are to identify conserved genetic networks that regulate immune responses across biodefense pathogens, organ compartments, and species to provide valuable therapeutic targets for broad-based intervention strategies.

Specific Aims: The Gale laboratory project specifically focuses on the identification of genetic components that contribute to the innate immune and inflammatory responses and determine West Nile Virus (WNV) infection and immunity.

Grants Management Specialist: Tina M. Carlisle, (240) 669-2947, carlisl@niaid.nih.gov

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

R01 AI 118916-05 (PI: M. Gale) 3/1/16-2/28/22 0.9 CM (7.5%)
\$1,619,445 total direct costs

The host response to hepatitis C virus

The goal of this project focus on defining the molecular mechanisms of hepatic innate immunity that impart acute to chronic HCV infection outcome. We have shown that retinoic acid inducible gene-I (RIG-I) initiates the hepatic innate immune response upon recognition and binding of the pathogen associated molecular pattern (PAMP) consisting of 5' triphosphate and the poly-uridine/cytosine (pU/UC) motif within the HCV RNA

Specific Aims: 1) Define the RIG-I/PAMP interactions of pathogen recognition and differential innate immune signaling from T/F genomes during acute HCV infection; 2) Determine the role of RIG-I acetylation in translocon assembly and function; 3) Identify the NS3/4A/MAVS interface that mediates interaction to control the RIG-I pathway.

Grants Management Specialist: Laura A. Pone, (240) 669-2951, amidonl@niaid.nih.gov

Role: PI

Overlap: No scientific or budgetary overlap.

P51 OD 010425-59 (PI: D. Anderson) 7/21/20-4/30/22 0.18 CM (1.5%)
\$82,860 total direct costs

Nonhuman Primate Systems Biology

"Division of Nonhuman Primate Systems Biology" (M. Gale)

This is the core grant of the Washington National Primate Research Center. The Division of Nonhuman Primate Systems Biology functions as a national resource for the development of nonhuman primate genomic and proteomic resources and for the incorporation of genomics and proteomics into nonhuman primate research.

The Division includes a High-Throughput Molecular Profiling Core and a Statistical Analysis and Computational Modeling Core.

Specific Aims: Provide the IT infrastructure and laboratory information management system for high-throughput data management and analysis.

Grants Management Specialist: Jean Richelsen, (301) 594-9446, richelsj@mail.nih.gov

Role: Associate Director, Core Staff Scientist, and Head of Division of Nonhuman Primate Systems Biology.

Overlap: No scientific or budgetary overlap.

HHSN272201800008C (PI: M. Gale) 7/1/19-6/30/25 0.58 CM (4.84%)

\$12,162,731 total direct costs

Nonhuman Primate Core Functional Genomics Laboratory for AIDS Vaccine Research and Development

The goal of this project provides high-throughput transcriptomic measurements for nonhuman primate samples from animals enrolled in priority protocols conducted by the Simian Vaccine Evaluation Unit (SVEU) and for samples from other investigator-initiated studies as selected by the DAIDS Contract Officer Representative.

Specific Aims: The core uses a LabKey laboratory information management system as a key component for tracking samples, organizing and reporting study results, and managerial project monitoring.

Grants Management Specialist: Robert J. Corno, (240)627-3032, cornorj@niaid.nih.gov

Role: PI

Overlap: No scientific or budgetary overlap.

5 R01AI127463-05 (PI: M. Gale)

12/15/16-11/30/21

0.9 CM (7.5%)

\$1,754,791 total direct costs

Mechanisms of hepatic innate immune activation by HCV and HBV

The major goal of this project is to define the virus and host processes that trigger and control liver inflammation during chronic hepatitis C and B virus infection.

Specific Aims: Our study design comprises two Aims to investigate the hypothesis that HCV activation of the NLRP3 inflammasome in liver macrophages drives hepatic inflammation, innate immune activation, and chronic hepatitis through a virion-induced inflammasome-cytokine loop that underlies immune activation and liver disease.

Grants Management Specialist: Trevor T. Alford, (240) 747-7398, trevor.alford@nih.gov

Role: PI

Overlap: No scientific or budgetary overlap.

R56 AI 141494-01A1 (PI: D. Fuller)

6/18/19-5/31/21

0.084 CM (.7%)

\$127,496 total direct costs

CD180 targeted immunotherapeutic for chronic HBV in HIV infected patients

The major goal of this project is to develop a novel vaccine platform that retains its potency even in an immune compromised individual. If successful, this strategy will be developed for immunotherapy of chronic HBV in HIV infected patients.

Specific Aims: Our study design comprises two Aims to investigate the hypothesis that HCV activation of the NLRP3 inflammasome in liver macrophages drives hepatic inflammation, innate immune activation, and chronic hepatitis through a virion-induced inflammasome-cytokine loop that underlies immune activation and liver disease.

Grants Management Specialist: Laura A Pone, (240) 669-2951, ponel@niaid.nih.gov

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

1 R01 AI 140888-02 (PI: Sacha/Hansen)

6/01/18-5/31/23

0.06 CM (0.5%)

\$218,995 total direct costs

Understanding unconventional CD8+ T cell responses in protection from HIV

The major goal of this project is understanding unconventional CD8+ T cell Responses in Protection from HIV to determine if RhCMV protection against SIV can be recapitulated in a second species.

Specific Aims: UW will perform statistical assessment of differential expression, network modeling, and functional analysis. Both the generated raw RNA-seq sequencing data and the processed data will be stored at UW until results are published at which time the UW will disseminated these data sets to the scientific community through public repositories like NCBI's GEO/SRA.

Grants Management Specialist: Nicole Guidetti, (301) 761-6934, nicole.guidetti@nih.gov

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

OPP1193500 (PI: M. Gale)

8/01/18-12/31/21

0.6 CM (5%)

\$872,987 total direct costs

Transcriptomic analysis of the protective host response to CMV-TB and CMV-SIV vaccination

The major goal of this project is to determine the host gene signature associated with protection and vaccine efficacy and to define outcome predictor gene signatures of 1) CMV-Tb vaccine protection against Mtb challenge, and 2) for CMV-SIV challenge against SIV infection.

Specific Aims: To perform mRNA-seq transcriptomics and bioinformatics analyses on samples from nonhuman primate SIV/Tb preclinical vaccination studies to understand the mechanisms of vaccine efficacy and programming of immunity toward vaccine application for human use.

Grants Management Specialist: Karen Makar, Karen.Makar@gatesfoundation.org

Role: PI

Overlap: No scientific or budgetary overlap.

14011.00.007.0000 (PI: M. Gale)

9/22/17-3/17/22

0.06 CM (0.5%)

SN272201700010I/HHSN27200007

\$556,181 total direct costs

Transcriptomics of RhCMV/SIV Vector-Vaccinated Macaques

The major goal of this project is to elucidate the immunologic mechanisms of leading HIV-1 prophylactic vaccines and eradication strategies. Our overall hypothesis is that understanding the mechanism of action of these immunologic interventions will facilitate the clinical development of these concepts and will lead to the next generation of vaccines and eradication strategies against HIV infection.

Specific Aims: 1) using tropism-modified 68-1 RhCMV/SIV vectors that either elicit only MHC-E-restricted or only MHC-II T cells to define the relative contribution of MHC-E-vs. MHC-I-restricted responses to efficacy, and 2) to confirm efficacy of attenuated (spread-deficient) 68-1 RhCMV/SIV vectors (pp71-deleted and MiR-124-restricted) at both high dose and low dose, respectively.

Grants Management Specialist: Karen Makar, Karen.Makar@gatesfoundation.org

Role: PI

Overlap: No scientific or budgetary overlap.

R01 AI052056-17A1 (PI: Watkin)

12/01/18-11/30/21

0.18 CM (1.5%)

\$236,518 total direct costs

The functional significance of CTL escape

The major goal of this project is to provide RNA isolation and QC, mRNA-seq library construction and QC, and sequencing for approximately 65 samples/year for five years. Using the mRNA-seq data sets generated.

Specific Aims: Perform statistical assessment of differential expression, network modeling, and functional analysis.

Grants Management Specialist: Annie Noel Grimes, (301) 761-7315, annie.grimes@nih.gov

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

R01 AI145296-03 (PI: M. Gale)

3/01/19-2/29/24

0.9 CM (7.5%)

\$3,237,089 total direct costs

Innate immune regulation of Zika virus infection

The major goal of this project is to investigate the hypothesis that (i) contemporary-emerging Asian and American ZIKV strains replicate more efficiently in specific cell types because of their inherent ability to antagonize JAK-STAT signaling pathways, and that ii) reduced STAT signaling results in injury to the developing placenta and fetus in vivo, resulting in fetal demise.

Specific Aims: We will conduct three Aims 1) Identify the molecular mechanisms of Jak-STAT regulation by pathogenic ZIKV strains; 2) Determine the outcome of Jak-STAT signaling control and innate immune defenses on maternal/fetal cell tropism and function in ZIKV infection, and 3) Define the role of IFN defenses in protection against maternal/fetal/newborn disease in vivo in a mouse model of ZIKV infection.

Grants Management Specialist: Laura A Pone, (240) 669-2951, ponel@niaid.nih.gov

Role: PI

Overlap: No scientific or budgetary overlap.

R21 AI145359-02 (PI: M. Gale)

6/17/19-5/31/21

0.27 CM (2.25%)

\$275,000 total direct costs

Using the Collaborative Cross to discover immunoregulatory mechanisms that control WNV infection

The major goal of this project is focuses on evaluating WNV disease susceptibility and host innate immune responses to WNV infection in the Collaborative Cross (CC) model. We have identified unique quantitative trait loci (QTL) encoding host genes that are linked to immune-regulatory phenotypes of WNV control.

Specific Aims: 1) Apply the CC to identify gene(s) regulating peripheral viral control in the spleen, and 2) define novel virus stimulated genes (VSG) in the CC that control neuroinvasion and neuropathogenesis of WNV infection.

Grants Management Specialist: David Madoo, (301) 761-6446, david.madoo@nih.gov

Role: PI

Overlap: No scientific or budgetary overlap.

75N93019C00070 (PI: K. Urdahl)

9/30/20-9/29/26

0.84 CM (7%)

\$3,765,453 total direct costs

Immune Mechanisms of Protection Against Mycobacterium Tuberculosis Center (Cascade IMPAc-TB)

The major goal of this project is to inform the rational design of an effective TB vaccine by identification of immune responses that are capable of controlling and potentially even eradicating Mtb. We will achieve this goal by identifying and dissecting protective pathways in natural and vaccine-induced immunity through integrated analyses of tissue-specific and systemic immunity in mice, non-human primates (NHP), and humans.

Specific Aims: 1) determine early immune events (key genes and pathways) that lead to Mtb eradication or containment prior to overt disease, 2) generate a gene signature that predicates protection by the RhCVM/TB vaccine platform and 3) correlate transcriptional host response with protection outcome and other virologic and immunologic phenotypes.

Grants Management Specialist: Wolfgang Leitner, (240) 627-3575, wleitner@niaid.nih.gov

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

R01 AI143265-01A1 (PI: K. Adams Waldorf/M. Gale)

7/01/19-6/30/24

0.45 CM (3.75%)

\$927,508 total direct costs

JAK-STAT Control of Zika Virus-Induced Fetal Injury

The major goal of this project is to define the viral-host interactions that support ZIKV infection, replication, and maternal-fetal transmission, and to develop a clinically relevant animal model for studies of pathogenesis. Relevant animal models are essential to define the outcome of viral-host interaction within the progression of ZIKV infection in order to fully understand ZIKV disease, and importantly to evaluate the efficacy of vaccines and therapeutics to control ZIKV infection and emergence.

Specific Aims: In Aim 1, we will determine how ZIKV mediates a broad JAK-STAT signaling suppression (STAT 1-6) in vitro and ex vivo to control innate immune defenses, viral trafficking and injury. In Aim 2, we will determine how viral control of JAK-STAT impacts vertical transmission and pathogenesis of fetal brain injury in vivo in a nonhuman primate model of the congenital ZIKV syndrome.

Grants Management Specialist: Racine Ann-marie Allen, (301) 761-7004, racine.allen@nih.gov

Role: Multi PI

Overlap: No scientific or budgetary overlap.

75N93019C00043 (PI: J. Nelson)

9/30/19-9/29/24

0.45 CM (3.75%)

\$1,541,038 total direct costs

Development of Novel STING Pathway Agonists

The major goal of this project is to complete a high-throughput adjuvant screening program that will identify small molecule compounds that enhance activity of STING to enhance protective immunity for protein-based

vaccines to Zika virus (ZIKV).

Specific Aims: to identify and comprehensively analyze the molecular and cellular events, and cellular response networks of candidate adjuvant compounds and innate immune signaling factors. Another focus of the project is to determine mechanism of action and pathway mapping using a comprehensive approach that includes functional genomics and gene knockouts in human and mouse cells. We will also perform preclinical evaluation of lead adjuvant compounds for adjuvant activity with ZIKV virus-like particles (VLPs).

Grants Management Specialist: Wolfgang Leitner, (240) 627-3575, wleitner@niaid.nih.gov

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

1UM1AI148684 (PI: D. Stephens)

12/5/19-11/30/26

1.32 CM (11%)

\$1,532,965 total direct costs

Leadership Group of Infectious Diseases Clinical Research Consortium (IDCRC)

The major goal of this project is to develop, implement, and evaluate laboratory research supporting the agenda of the leadership group (LG) and NIH Program for the IDCRC.

Specific Aims: Aim 1. Maintain organizational infrastructure of the LOU and VETU linkage. Aim 2. Identify, design and implement laboratory research plan in conjunction with the LG, and develop and implement ancillary studies to support VETU research and clinical activities. I) List off and describe core group services; II) Summarize and show table of specialty services; III) describe how ancillary. Aim 3. Implement the sharing and distribution of samples and study-related data across the IDCRC and approved outside partners, and provide quality control and quality assurance of the services provided to the IC-CRC by the LOU. Aim 4. Train the next generation of researchers in laboratory assay development and application for basic and clinical studies.

Grants Management Specialist: Michael W. Fato, (240) 627-3304, mfato@niaid.nih.gov

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

W81XWH-20-1-0270 (PI: S. Chanda)

4/15/20-4/14/24

1.2 CM (10%)

\$1,412, 018 total direct costs

Development and Advancement of Broad-spectrum Respiratory Antivirals

The major goal of this project is to to advance the development of broad-spectrum antivirals targeting host cellular factors involved in virus replication, virulence, or innate restriction against respiratory viruses.

Respiratory viruses, such as influenza A viruses (IAV), which have the potential to trigger global pandemics, rhinoviruses, and respiratory syncytial viruses (RSV), which typically cause the common cold and bronchiolitis, respectively, as well as adenoviruses, and parainfluenza viruses, show many commonalities in disease presentation despite being genetically distinct. This makes broad-spectrum antivirals for treatment without pathogen identification a high priority goal.

Specific Aims: Characterize the cellular response and defining IRF3 target genes. We will perform global analysis of the host transcriptome and proteome in compound-treated, mock, and virus-infected cells.

Grants Management Specialist: Joshua L. Disbennett, (301) 619-7349, joshua.l.disbennett.civ@mail.mil

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

U01AI151698 (PI: W. Van Voorhis)

5/22/20-4/30/25

0.6 CM (5%)

\$501,240 total direct costs

University of Washington Arboviral Research Network (UWARN)

The major goal of this project is for The University of Washington Arboviral Research Network (UWARN) will address emerging and re-emerging infectious diseases by carrying out research on arboviruses, viruses transmitted by insects to humans that include current high burden viruses like dengue, chikungunya, and Zika viruses. Arboviruses also include emerging and re-emerging viruses such as Mayaro, Una, Usutu, Japanese encephalitis, yellow fever, Crimean-Congo hemorrhagic fever, and Oropouche viruses. UWARN will leverage the UW Metacenter for Pandemic Preparedness predictive, evaluative, and training components, and the UW

Department of Global Health greater world-wide connections to proactively respond to arboviral emergence.
Specific Aims: Characterize the cellular response and defining IRF3 target genes. We will perform global analysis of the host transcriptome and proteome in compound-treated, mock, and virus-infected cells.
Grants Management Specialist: Jenna L. Briggs, (301) 761-5137, jenna.briggs@nih.gov
Role: Co-Investigator
Overlap: No scientific or budgetary overlap.

U01AI151698 (PI: D. Barouch) **5/22/20-4/30/25** **0.18 CM (1.5%)**
\$929,081 total direct costs

Viral Reservoir Biomarkers in SIV-Infected Macaques

The major goal of this project is To discover circulating markers to determine the size of the HIV reservoir in the body, to clinically test interventions that might reduce the size, and to discover novel interventions to induce durable, antiretroviral-free remission of HIV

Specific Aims: To discover circulating markers to determine the size of the HIV reservoir in the body, to clinically test interventions that might reduce the size, and to discover novel interventions to induce durable, antiretroviral-free remission of HIV.

Grants Management Specialist: Colleen Silcox, colleen.silcox@gatesfoundation.org

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

U01AI151698 (PI: J. Estes) **5/22/20-4/30/25** **0.12 CM (1%)**
\$636,356 total direct costs

Viral Reservoir & Cellular Immune Neighborhood Guided Biomarker Discovery

The major goal of this project is to design, develop and maintain a LabKey-based Laboratory Information Management System (LIMS) for data capture, storage and dissemination of the multiply types of data that will be generated by this program. Additionally, using the LIMS comprehensive database, the Gale laboratory will perform computational data analysis with the overarching goal of integrating these data types to identify key biological pathways, genes, gene networks, and virus-host interactions within the molecular, cellular, and systems processes that control SIV rebound following ART discontinuation. This work will define biomarkers of virus and host control of SIV infection to inform strategies for implementing a cure to HIV infection.

Specific Aims: To discover circulating markers to determine the size of the HIV reservoir in the body, to clinically test interventions that might reduce the size, and to discover novel interventions to induce durable, antiretroviral-free remission of HIV.

Grants Management Specialist: Colleen Silcox, colleen.silcox@gatesfoundation.org

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

A167212 (PI: M. Gale) **4/01/21-3/31/22** **0.36 CM (3%)**
\$159,903 total direct costs

Antiviral activity of light activating dyes against coronavirus infection

The major goal of this project is 1) Assessment of various permeable materials/meshes treated with Light Activated Dyes (LADs) for viral inactivation efficacy. 2) Assessment of various LADS in combination with each other to determine optimal viral inactivation.

Specific Aims: Determine the antiviral efficacy of light activated dyes (LADs) against coronavirus infection of cultured cells. Evaluate the antiviral efficacy of LAD coating on PPE materials for preventing SARS CoV-2 transmission to cells after virus exposure.

Grants Management Specialist: Paul Rolley, 765.491.3240, prolley@singletto.com

Role: PI

Overlap: No scientific or budgetary overlap.

INV-017205 AM01 (PI: P. Drain)

5/22/20-4/30/25

0.36 CM (3%)

\$379,171 total direct costs

Developing and Evaluating Point-of-Care Antigen and Immunoassays for COVID-19 and Cytokine Release Syndrome among people being screened for SARS-CoV-2 infection in Seattle

The major goal of this project is to evaluate the longitudinal dynamics of SARS-CoV-2 infection and testing kinetics via viability (culture), viral load (PCR), S & N antigens, viral sequence diversity, host transcriptional response and antibody detection using laboratory and point-of-care (POC) tests.

Specific Aims: To conduct viral isolation, sequencing, replication, phenotype assessment and S & antigen detection potential across acute time points; to conduct whole blood transcriptional profiling across acute and post-infection time points; to correlate SARS-CoV-2 viral load (PCR) with viability (culture), S & N antigen detection, and IgG/IgM/broadly neutralizing antibody titers during the acute infection (first 14 days); to correlate SARS-CoV-2 S & N antigen detection with viral load (PCR); viability (culture), and IgG/IgM antibody titers, and to link viral genotype and phenotype features with blood transcriptome and acute infection outcomes.

Grants Management Specialist: N/A

Role: Co-Investigator

Overlap: No scientific or budgetary overlap.

PENDING

None

OTHER SUPPORT

GARCÍA-SASTRE, A.

ACTIVE

HHSN272201400008C (García-Sastre PI) 04/01/2014-08/01/2022 0.06 calendar
NIH/NIAID \$77,928,947

Center for Research on Influenza Pathogenesis (CRIP)

This NIH contract is dedicated to determining factors affecting the pathogenicity, host tropism and innate immune responses of animal influenza viruses.

Point of Contact at Funding Agency: FELENT, ERIN, erin.felent@nih.gov

U19AI118610 (Fernandez-Sesma PI, García-Sastre Core Lead) 06/24/2015-05/31/2022 0.12 calendar
NIH/NIAID \$40,072,431

Dengue Human Immunology Project Consortium (DHIPC)

The objective of this Core is to manage the administrative and fiscal responsibility of the Infrastructure and Opportunity Fund (IOF) for the Human Immunology Project Consortium Network supported by the NIAID under the U19 mechanism.

Point of Contact at Funding Agency: KELLY, HALONNA R, kellyhr@mail.nih.gov

R01AI125524 (Lynch PI, García-Sastre MPI) 05/25/2016-04/30/2022 0.12 calendar
NIH/NIAID \$2,634,829

University of Pennsylvania

Splicing and nuclear transport of influenza virus mRNA

These studies aim to uncover novel mechanisms of, and connections between, alternative splicing and nuclear transport and how these processes are subverted by the influenza virus. The studies also aim to reveal host vulnerabilities targeted by influenza virus that can potentially be used to devise new therapeutic options.

Point of Contact at Funding Agency: BOZICK, BROOKE ALLISON, brooke.bozick@nih.gov

R01AI127302 (Chanda PI, García-Sastre MPI) 02/28/2017-01/31/2022 0.46 calendar
NIH/NIAID \$4,354,702

Sanford Burnham Prebys Medical Discovery Institute

Innate Immune Surveillance of HIV-1 During Transmission and Systemic Infection

We plan to study the factors mediating innate recognition and host responses to HIV-1 infection and their impact on transmission.

Point of Contact at Funding Agency: SHANKAR, UDAY K, uday.shankar@nih.gov

R01AI127658 (Blander PI, García-Sastre Co-I) 04/01/2017-08/31/2021 0.06 calendar
NIH/NIAID \$2,096,335

Weill Cornell Medicine

Novel vita-vaccine formula combines safety of dead and efficacy of live vaccines

The goal of this study is the use of bacterial mRNAs as superior vita-adjuvants that restore the signatures of microbial viability to dead vaccines and improve existing inactivated and subunit vaccines for protection against either bacterial or viral diseases.

Point of Contact at Funding Agency: LAPHAM, CHERYL K, clapham@mail.nih.gov

R33AI119304 (García-Sastre PI) 07/13/2017-06/30/2021 0.06 calendar
NIH/NIAID \$1,500,584

Chemical intervention of influenza virus RNA nuclear export

Influenza virus infection is a significant health and economic burden with limited therapeutic options. The goal of this grant is to develop a new strategy for the identification of a new class of antivirals.

Point of Contact at Funding Agency: KRAFFT, AMY, krafft@niaid.nih.gov

R01AI127775 (Manicassamy PI, García-Sastre Co-I) 12/08/2017-11/30/2022 0.06 calendar
NIH/NIAID \$2,216,793

University of Iowa

Endothelial cell tropism in the pathogenesis and host response against influenza viruses

This project will investigate how viral cell tropism contributes to the pathogenesis of highly pathogenic influenza strains.
Point of Contact at Funding Agency: BOZICK, BROOKE ALLISON, brooke.bozick@nih.gov

U19AI135972 (García-Sastre PI)	01/20/2018-12/31/2022	1.30 calendar
NIH/NIAID	\$10,744,782	

Fluomics: The Next Generation

The overall goal of this application is to use an OMICS integrated systems biology approach to identify and validate novel host targets for therapeutic intervention during influenza A virus infection.

Point of Contact at Funding Agency: SHABMAN, REED SOLOMON, reed.shabman@nih.gov

R01CA229818 (Chanda PI, García-Sastre MPI)	07/11/2018-06/30/2022	0.91 calendar
NIH/NCI	\$2,144,721	

Sanford Burnham Prebys Medical Discovery Institute

Early development of small molecule dendritic cell immunopotentiators for the treatment of solid tumors

We will test the synergistic effect of hit compounds that activate MDDCs in NDV oncolytic activities.

Point of Contact at Funding Agency: SOMMERS, CONNIE L, sommersc@mail.nih.gov

P01AI097092 (Palese PI, García-Sastre Proj. Lead)	08/01/2018-07/31/2023	0.12 calendar
NIH/NIAID	\$5,396,683	

Toward a Universal Influenza Virus Vaccine

We are attempting to design novel universal influenza virus vaccines which would be cross-protective against different strains and thus last longer, avoiding the necessity of annual revaccinations.

Point of Contact at Funding Agency: GORDON, JENNIFER L, jennifer.gordon2@nih.gov

W81XWH-18-1-0488 (Krammer PI, García-Sastre Co-I)	09/01/2018-08/31/2021	0.12 calendar
DoD/Department of the Army	\$5,085,000	

GMP Production of Candidate Pan-Group 2 Influenza A Virus Vaccines

In this application we propose to develop a vaccine that provides broad protection from influenza infections caused by group 2 viruses like H3N2 and H7N9 based on the conserved stalk domain of the hemagglutinin.

Point of Contact at Funding Agency: SANCHEZ, CATHERINE, catherine.n.sanchez.civ@mail.mil

R01AI141226 (García-Sastre PI)	01/07/2019-12/31/2023	0.60 calendar
NIH/NIAID	\$3,866,211	

Toward a universal influenza virus vaccine based on live attenuated NS1-deleted influenza viruses

We plan to manufacture two live attenuated NS1-deleted influenza virus vaccines expressing chimeric group 1 HA proteins.

Point of Contact at Funding Agency: GORDON, JENNIFER L, jennifer.gordon2@nih.gov

HHSN272201800010I (Sayeed PI, García-Sastre Co-I)	01/23/2019-12/31/2025	0.06 calendar
NIH/NIAID	\$381,122	

IAVI

Manufacture of influenza for human challenge clinical trials

Dr. García-Sastre will provide input on the rescue of the GMP seed viruses for the GMP human challenge virus stocks for this project.

Point of Contact at Funding Agency: SAYEED, EDDY, esayeed@iavi.org

R01AI142086 (Banchereau PI, García-Sastre Co-I)	02/14/2019-01/31/2024	0.06 calendar
NIH/NIAID	\$1,745,936	

The Jackson Laboratory

High-resolution single cell profiling of vaccine responsiveness in the elderly

Antibodies against influenza virus vaccine induced in our proposed studies will be characterized by the hemagglutination inhibition (HI) assay.

Point of Contact at Funding Agency: PRABHUDAS, MERCY R, mprabhudas@niaid.nih.gov

U19AI142733 (Palucka PI, García-Sastre Co-I)	03/05/2019-02/29/2024	0.12 calendar
NIH/NIAID	\$6,600,000	

The Jackson Laboratory

Modulation of Lung Immune Responses to Viral Infection

The García-Sastre lab will prepare all influenza viruses used in this proposal including recombination viruses with differential cell type tropism and reporter viruses.

Point of Contact at Funding Agency: JIANG, CHAO, jiangc3@mail.nih.gov

R21AI147201 (Shapira PI, García-Sastre Co-I) 09/01/2019-08/31/2021 0.06 calendar
NIH/NIAID \$466,250

Columbia University

Leveraging experimental and computational tools to define molecular functions of non-coding RNAs in innate immune responses to viral infection

We propose to take a data-driven approach to identify and functionally characterize the role of a novel lncRNA that we have identified to function as an immunological rheostat in epithelial cells.

Point of Contact at Funding Agency: LANE, MARY CHELSEA, lanemc@niaid.nih.gov

75N93019C00051 (Krammer PI, García-Sastre Co-I) 09/10/2019-09/09/2026 1.08 calendar
NIH/NIAID \$32,524,307

Sinai-Emory CIVIC

The SEM CIVIC aims to develop improved seasonal and universal influenza virus vaccines that induce long lasting protection against drifted seasonal, zoonotic and future pandemic influenza viruses.

Point of Contact at Funding Agency: FELENT, ERIN, erin.felent@nih.gov

U01EB029085 (Benam PI, García-Sastre Co-I) 09/17/2019-06/30/2024 0.06 calendar
NIH/NIBIB \$829,208

University of Pittsburgh

A Microphysiological Mimicry of Human Lung-Bone Marrow Organ-Organ Crosstalk On-a-Chip

The García-Sastre lab will provide and titrate influenza A viruses (IAVs), and compare results generated using Organ-on-a-Chip in Dr. Benam's laboratory against other ex vivo and in vivo data generated in his laboratory.

Point of Contact at Funding Agency: RAMPULLA, DAVID, david.rampulla@nih.gov

75N93019C00046 (García-Sastre PI) 09/30/2019-09/29/2024 1.08 calendar
NIH/NIAID \$3,424,685

C-GAS/STING Inducing Compounds as Novel Adjuvants

This proposal focuses on the discovery of small molecules that induce innate stimulation of primary human monocyte derived dendritic cells (MDDCs) and have potent and immunestimulatory (adjuvant) properties through activation of the cGAS/STING pathway.

Point of Contact at Funding Agency: RALIS, GEORGE, ralisg@niaid.nih.gov

U19AI135972 (García-Sastre PI) 03/30/2020-12/31/2022 0.18 calendar
NIH/NIAID \$1,339,416

Fluomics: The Next Generation (Coronavirus NOSI Supplement)

This supplement will identify compounds with known human dosing and safety profiles that inhibit SARS-CoV-2 in vitro and in a mouse model, leading to their rapid advancement into human clinical trials for the prophylaxis and/or treatment of SARS-CoV-2 infections.

Point of Contact at Funding Agency: SHABMAN, REED SOLOMON, reed.shabman@nih.gov

HR0011-19-2-0020 (Shoichet PI, García-Sastre Co-I) 04/01/2020-08/31/2021 0.27 calendar
DARPA \$1,305,000

University of California, San Francisco

COVID-19 Supplement - Targeted Polypharmacology for Novel Analgesics (Panacea)

The goal of this project is to discover drugs and leads to treat Covid-19. A combination of chemoinformatics approaches—for rapid drug repurposing—and molecular docking approaches—for new lead discovery against viral targets—are used.

Point of Contact at Funding Agency: DONAGHUE, PETER, desmond.donaghue@darpa.mil

U19AI142733 (Palucka PI, García-Sastre Co-I) 04/08/2020-02/28/2023 0.06 calendar
NIH/NIAID \$518,190

The Jackson Laboratory

Modulation of Lung Immune Responses to Viral Infection (Coronavirus Administrative Supplement)

The García-Sastre lab will carry out the work related to viral exposure and identification of lung epithelial cells targeted by SARS-CoV-2 using single cell RNA-seq technology as well as generating antibodies for tissue analysis.
Point of Contact at Funding Agency: JIANG, CHAO, jiangc3@mail.nih.gov

2020-215611 (5384) (García-Sastre PI) 04/15/2020-04/14/2021 0.06 calendar
Open Philanthropy Project/SVCF \$627,700
Studies to ascertain the prevalence of SARS-CoV-2 infections and to mitigate the medical and economic impact of the COVID-19 pandemic

Here we propose to leverage an existing longitudinal research cohort to determine seroprevalence of SARS-CoV-2 infections and disease severity in a well-defined group of individuals.

Point of Contact at Funding Agency: YOUNGS, HEATHER, heather@openphilanthropy.org

W81XWH-20-1-0270 (Chanda PI, García-Sastre MPI) 04/15/2020-04/14/2024 1.20 calendar
DoD/Department of the Army \$677,243
Sanford Burnham Prebys Medical Discovery Institute
Development and Advancement of Broad-spectrum Respiratory Antivirals (DABRA)
DABRA will conduct pre-investigational new drug (pre-IND) development of a unique portfolio of host-directed and innate immune-activating broad-spectrum antivirals.
Point of Contact at Funding Agency: GERSCH, ANNMARIE, annmarie.gersch.ctr@mail.mil

75N93019C00045 (Evans PI, García-Sastre Co-I) 04/23/2020-09/29/2022 0.06 calendar
NIH/NIAID \$846,730
University of Montana
Rapid advancement of a safe and effective vaccine targeting SARS-CoV-2 (COVID-19) (Administrative Supplement)

The team at Mount Sinai will develop mouse and ferret COVID-19 challenge models for testing vaccinated animals.

Point of Contact at Funding Agency: RALIS, GEORGE, ralisg@niaid.nih.gov

AGR-17705 (García-Sastre PI) 05/08/2020-05/07/2021 0.06 calendar
7 Hills Pharma \$15,000
Testing of anti-SARS-CoV-2 titers in mouse sera from vaccinated mice by microneutralization assays
Mouse sera from vaccinated mice provided by 7 Hills Pharma will be tested for anti-SARS-CoV-2 titers by microneutralization assays in the BSL3 facility at ISMMS.
Point of Contact at Funding Agency: MARATHI, UPENDRA, upendra@7hills.co

N/A (Gallego PI, García-Sastre Co-I) 06/01/2020-05/31/2022 0.06 calendar
CaixaImpulse \$104,514
Fundación Universidad Católica de Valencia
RNA-targeted small-molecule agents against SARS-CoV-2
We propose to evaluate the effect of compounds with potent antiretroviral activity on SARS-CoV-2 replication, subsequently explore structure-activity relationships by testing chemical analogues and determine the antiviral mechanism of the most potent compounds.
Point of Contact at Funding Agency: CARMEN TUDELA MIÑANA, ADRIANA, ac.tudela@ucv.es

N/A (García-Sastre PI) 06/22/2020-06/21/2021 0.06 calendar
The JPB Foundation \$750,000
COVID-19 related research to develop antiviral therapies and determine SARS-CoV-2 seroprevalence, antibody levels, and immunity
We propose to repurpose FDA approved compounds into primary human systems and animal models to test efficacy and support ongoing clinical trials to expedite the timeline for human use. Additionally, we propose to ascertain if “seroconverters,” who have developed antibodies after exposure to SARS-CoV-2, are immune against reinfection and what level of antibodies is protective.
Point of Contact at Funding Agency: BAUTISTA, JULIO, jbautista@jpbfoundation.org

AGR-21604 (García-Sastre PI) 07/02/2020-07/01/2021 0.06 calendar
Pfizer Inc. \$47,460

Assess protection provided through passive transfer of human sera/plasma to Golden Syrian Hamsters challenged with SARS-CoV-2

We will assess protection provided through passive transfer of human sera/plasma to Golden Syrian Hamsters challenged with SARS-CoV-2

Point of Contact at Funding Agency: KANEVSKY, ISIS, isis.kanevsky@pfizer.com

R21AI149013 (Forst PI, García-Sastre Co-I) 07/10/2020-06/30/2022 0.06 calendar
NIH/NIAID \$254,250

Multi-scale analysis of single cell sequencing data to dissect the complexity of influenza infections

This application aims to investigate the genetic predisposition, key pathways and processes against influenza infections using existing public data sets and immune system specific information from the public Influenza Research Database.

Point of Contact at Funding Agency: BOZICK, BROOKE ALLISON, brooke.bozick@nih.gov

R35HL135834 (Cardoso PI, García-Sastre Co-I) 08/15/2020-07/31/2021 0.06 calendar
NIH/NHLBI \$946,449

Columbia University

Mechanisms Controlling Expansion and Lineage Specification of Airway Progenitors in Development and Disease (Administrative Supplement)

The goal of this Administrative Supplement project is to elucidate the cellular and molecular changes elicited by the SARS-CoV-2 in the airway epithelium by identifying and pharmacologically targeting master regulator (MR) proteins that mechanistically regulate SARS-CoV-2 interactions in airway epithelial cells.

Point of Contact at Funding Agency: LIN, SARA, sara.lin@nih.gov

N/A (Chanda PI, García-Sastre Co-I) 09/01/2020-08/31/2021 0.06 calendar
Fast Grants/Mercatus Center \$27,500

Sanford Burnham Prebys Medical Discovery Institute

A Drug Repurposing Strategy for SARS-CoV-2 Vaccine Adjuvants

The goal of this grant is to identify dendritic cell immunopotentiators amongst existing drugs, which by stimulating innate immune pathway(s) can serve to adjuvant SARS-CoV-2 vaccine activity.

Point of Contact at Funding Agency: O'ROURKE, CAMEY, emergentventures@mercatus.gmu.edu

N/A (García-Sastre PI) 09/10/2020-09/09/2021 0.06 calendar
Kenall Manufacturing \$33,900

The use of 405nm visible light to inactivate SARS-CoV-2

We will use 405nm visible light to inactivate SARS-CoV-2.

Point of Contact at Funding Agency: YAHNKE, CLIFFORD J, cliff.yahnke@kenall.com

R01AI150837 (Lim PI, García-Sastre Co-I) 09/17/2020-08/31/2025 0.12 calendar
NIH/NIAID \$421,878

Enhanced susceptibility to Zika virus during pregnancy: a role for maternal dengue antibodies

In this application, we will investigate how antibodies against dengue virus enhance infection and pathogenesis of Zika virus in the context of pregnancy.

Point of Contact at Funding Agency: MORABITO, KAITLYN MELISSA, dambachkm@mail.nih.gov

U54CA260560 (Hirsch PI, García-Sastre MPI) 09/30/2020-08/31/2025 0.73 calendar
NIH/NCI \$3,968,419

Vulnerability of SARS- CoV-2 Infection in Lung Cancer Based on Serological Antibody Analyses

This U54 grant aims to provide new information on the determinants of the susceptibility of lung cancer patients to SARS-CoV-2 infection and uncover potential therapeutic strategies.

Point of Contact at Funding Agency: KRUEGER, KARL E, kk317h@nih.gov

AGR-22018 (García-Sastre PI) 10/08/2020-10/07/2021 0.06 calendar
Pfizer Inc. \$42,375

Evaluation of compounds in Ad-ACE2 model of CoV-2 infection

The García-Sastre lab will test up to six Pfizer compounds in the Ad-hACE2 mouse model and provide whole blood to Pfizer for evaluation of compound levels in the blood.

Point of Contact at Funding Agency: COOPER, EVAN, evan.cooper@pfizer.com

N/A (García-Sastre PI)	10/30/2020-10/29/2021	0.06 calendar
ImmunityBio, Inc.	\$200,000	

Testing COVID-19 vaccine candidates in hamsters

The García-Sastre lab will test COVID-19 vaccine candidates in hamsters.

Point of Contact at Funding Agency: SAFRIT, JEFFREY, jeffrey.safrit@nantkwest.com

AGR-22361 (García-Sastre PI)	11/30/2020-11/29/2021	0.06 calendar
N-fold LLC	\$47,459	

Examining the antiviral activity of cyclodextrin in the hamster model of COVID-19

The García-Sastre lab will examine the antiviral activity of cyclodextrin formulated at 8% concentration in synthetic mucous in the hamster model of COVID-19.

Point of Contact at Funding Agency: SOSIN, HOWARD, howard@sosin.net

AGR-22479 (Krammer PI, García-Sastre Co-I)	01/01/2021-12/31/2021	0.06 calendar
Toscana Life Sciences	\$189,840	

Explore mAb protective level in the hamster animal model in a dose titration study and transmission study

In this study, we will explore mAb protective level of SARS-CoV-2 in the animal model.

Point of Contact at Funding Agency: GIORDANO, CINZIA, c.giordano@toscanalifesciences.org

R01AI148180 (Mebratu PI, García-Sastre Co-I)	01/01/2021-12/31/2025	0.12 calendar
NIH/NIAID	\$462,696	

Brigham and Women's Hospital

The role of Bik in the replication and severity of influenza A virus

The goal of this application is to dissect the role of Bik in promoting viral replication, and to identify host cellular factors that contribute to influenza susceptibility and severity.

Point of Contact at Funding Agency: BOZICK, BROOKE ALLISON, brooke.bozick@nih.gov

75N93019R00028 (García-Sastre PI)	04/01/2021-03/31/2028	1.80 calendar
NIH/NIAID	\$6,001,311	

NIAID Centers of Excellence for Influenza Research and Response (CEIRR)

This NIH contract is dedicated to investigating the ecology, epidemiology, diversity, evolution, pathogenesis, host tropism, transmission and immunogenicity of influenza A viruses.

Point of Contact at Funding Agency: SMITH, MARY, mary.smith@nih.gov

PENDING

AGR-16652A1 (García-Sastre PI)	03/04/2021-03/03/2023	0.06 calendar
Avimex	\$50,000	

Generation of NDV vaccine vectors for avian influenza

We plan to generate new NDV-HA and control vaccine viruses to test against novel strains of avian influenza.

Point of Contact at Funding Agency: ROSAS, GUILLERMO, guillermo.rosas@avimex.com.mx

R01AI142086 (Banchereau PI, García-Sastre Co-I)	06/01/2021-05/31/2023	0.12 calendar
NIH/NIAID	\$185,772	

The Jackson Laboratory

High-resolution single cell profiling of vaccine responsiveness in the elderly (Supplement)

The García-Sastre laboratory at ISMMS will determine the antibody titers in sera against S, N and RBD, and of SARs-CoV-2 and human coronavirus, as well as the corresponding virus neutralizing titers.

Point of Contact at Funding Agency: PRABHUDAS, MERCY R, mprabhudas@niaid.nih.gov

R01AI164485 (Goldfeld PI, García-Sastre Co-I)	07/01/2021-06/30/2026	0.12 calendar
NIH/NIAID	\$423,750	

Boston Children's Hospital

Host immunity and interrupting lethal virus infection

The García-Sastre laboratory will conduct experiments to generate and validate interferon competent cell lines and then work in BSL3 conditions to assess the antiviral activity of NTZ on SARS-2 CoV.
Point of Contact at Funding Agency: Pending Assignment

R21AI163920 (Alarcon PI, García-Sastre Co-I) 07/01/2021-06/30/2023 0.12 calendar
NIH/NIAID \$233,063
Yale University

Targeting the methyltransferase activities of SARS-CoV-2

The García-Sastre laboratory will screen and characterize the methyl-transferase inhibitors for anti-SARS-CoV-2 activity in cell culture.

Point of Contact at Funding Agency: Pending Assignment

R01AI000000 (Fontoura PI, García-Sastre MPI) 07/01/2021-06/30/2026 0.60 calendar
NIH/NIAID \$915,300
University of Texas, Southwestern

The Nsp1 Protein of SARS-CoV-2 Targets the Host mRNA Nuclear Export Machinery

The Garcia-Sastre laboratory at Icahn School of Medicine at Mount Sinai will conduct experiments on NSP-1 mutant SARS-CoV-2.

Point of Contact at Funding Agency: Pending Assignment

R21OH000000 (Mulholland PI, García-Sastre Co-I) 07/01/2021-06/30/2023 0.24 calendar
NIH/NIOSH \$147,493

Using Androgen Receptor Targeted Therapy to Overcome the Adverse Effects of COVID19 Exposure in WTC First Responders

This study will test whether androgen receptor targeted therapies can block the function of Tmprss2, a hormone regulated enzyme, in efforts to reduce SARS-CoV-2 infection.

Point of Contact at Funding Agency: Pending Assignment

R01AI000000 (Gusella PI, García-Sastre Co-I) 07/01/2021-06/30/2024 0.12 calendar
NIH/NIDDK \$1,271,250

Novel models for studying SARS-CoV2-associated renal injury

This proposal aims to establish both in vivo and in vitro systems to study the mechanisms of renal damage in COVID patients.

Point of Contact at Funding Agency: Pending Assignment

STTR (Bogunovic PI, García-Sastre Co-I) 07/01/2021-06/30/2022 0.12 calendar
NIH/NIAID \$90,000
Lab11 Therapeutics LLC

Transient Gene Therapy as a Broad Spectrum Antiviral

The García-Sastre laboratory will be responsible for testing modRNA cocktail delivered IV and IN in mice against Influenza A virus challenge and in hamsters against SARS-CoV-2.

Point of Contact at Funding Agency: Pending Assignment

P01AI000000 (Banchereau PI, García-Sastre Co-I) 08/01/2021-07/31/2026 0.24 calendar
NIH/NIAID \$641,098

The Jackson Laboratory

Dynamic Assessment of the Immune System post COVID19 (DAISCO19)

The García-Sastre laboratory at ISMMS will determine the magnitude and functionality of the antibody response against the vaccines used in the study.

Point of Contact at Funding Agency: Pending Assignment

R01000000 (Iyengar PI, García-Sastre Co-I) 09/01/2021-08/31/2026 0.60 calendar
NIH \$4,843,496

COVID-19 dependent cardiac disease

We will integrate a combination of clinical and experimental data and use machine learning algorithms to develop and implement predictive models that can based on standard clinical examination to predict if COVID-19 patients can develop heart disease 1-2 years after viral infection.

Point of Contact at Funding Agency: Pending Assignment

R01AI000000 (Evans PI, García-Sastre MPI) 09/01/2021-08/31/2026 1.00 calendar
NIH/NIAID \$4,132,422

Zika virus nonstructural protein 5 inhibition of interferon signaling

This project seeks to elucidate how the flavivirus nonstructural 5 (NS5) protein impacts host innate immune responses by using innovative molecular virology approaches.

Point of Contact at Funding Agency: Pending Assignment

UG3TR003870 (Nicola PI, García-Sastre Co-I) 01/01/2022-12/31/2022 0.12 calendar
NIH/NCATS \$169,500

Repurpose.AI, Inc.

Drug repurposing through chemical feature-based deep learning for COVID-19 therapeutics

The Garcia-Sastre laboratory at ISMMS will test the in vivo impact of compounds on SARS-CoV-2 infection.

Point of Contact at Funding Agency: Pending Assignment

U01AI000000 (Banchereau PI, García-Sastre Co-I) 03/01/2022-02/28/2027 1.20 calendar
NIH/NIAID \$692,640

The Jackson Laboratory

A deep longitudinal analysis of next generation influenza vaccines in older adults

The García-Sastre laboratory at ISMMS will determine the magnitude and functionality of the antibody response against the vaccines used in the study.

Point of Contact at Funding Agency: Pending Assignment

OVERLAP

No scientific overlap.

TERMINATED

R01AI088770 (Durbin PI, García-Sastre Co-I) 04/15/2010-03/31/2017 0.80 calendar
NIH/NIAID \$3,662,327

New York University

A novel approach to RSV vaccination

This grant explores the use of Newcastle disease virus (NDV) vectors expressing RSV antigens as RSV vaccines. As part of this project, the Garcia-Sastre lab will generate the recombinant NDV vectors.

Point of Contact at Funding Agency: KIM, SONNIE, sonnie.kim@nih.gov

P01AI090935 (Young PI, García-Sastre Co-I of subproject) 08/15/2010-07/31/2015 1.00 calendar
NIH/NIAID \$20,346,044

Salk Institute for Biological Sciences

Global innate immune responses to HIV-1 infection

This subproject in part of a multi-investigator program project entitled “Global innate immune responses to HIV-1 infection” to study the interactions between HIV and innate immunity. The subproject studies the functional relationships between HIV restriction factors and innate immune pathways.

Point of Contact at Funding Agency: CHURCH, ELIZABETH S, elizabeth.church@nih.gov

R01AI089539 (Roth PI, García-Sastre Co-I) 07/01/2010-06/30/2015 0.60 calendar
NIH/NIAID \$1,999,249

University of Texas Southwestern

Novel inhibitors of viral replication

In this project, novel inhibitors of influenza virus replication will be identified by our collaborators and we will investigate their antiviral activities in a mouse model.

Point of Contact at Funding Agency: KRAFFT, AMY, krafft@niaid.nih.gov

2010-ST-061-AG0001-02 (Richt PI, García-Sastre Co-I of subproject) 07/01/2010-06/30/2016 0.60 calendar
DHS \$2,124,119

Kansas State University

Center of Excellence for Emerging & Zoonotic Animal Disease (CEEZAD)

Under this grant, the García-Sastre lab will generate NDV vectors expressing HA antigens as possible vaccines against avian and swine influenza for birds and pigs.

Point of Contact at Funding Agency: HOBER, STEPHANIE, shober@vet.k-state.edu

U19AI089987 (Banchereau PI, García-Sastre Co-I) 07/12/2010-06/30/2015 0.60 calendar
NIH/NIAID \$17,002,769

Baylor University

Systems analysis vaccine responses in healthy and hyporesponsive humans

This Center is based at the Baylor Institute for Immunology Research at Dallas, Texas and is composed of five academic research centers and one Biotech Company that are located in five states. The Center focuses on analyzing the immune responses to vaccination of several cohorts of healthy individuals of different age groups from 5 to 65 years and several cohorts of hyporesponsive individuals. The García-Sastre lab provides with influenza virus expertise and reagents to analyze immune responses in individuals vaccinated with influenza virus vaccine.

Point of Contact at Funding Agency: JOHNSON, DAVID R, drjohnson@niaid.nih.gov

HHSN272201000054C (Sealfon PI, Garcia-Sastre Co-I) 09/30/2010-09/29/2015 0.72 calendar
NIH/NIAID \$17,219,467

Modeling Viral Immunity and Antagonism

This major goal of this five-institution research program is to develop an integrated experimental data-based model of the molecular responses of human dendritic cells to infection by pathogenic virus.

Point of Contact at Funding Agency: KITSOULIS, REGINA E, kitsoulisre@niaid.nih.gov

R01DA033773 (Wolinsky PI, Garcia-Sastre Co-I) 04/15/2012-03/31/2017 0.48 calendar
NIH/NIDA \$4,610,911

Northwestern University

A system-level approach in HIV/AIDS susceptibility and substance abuse

The goal of this project is to assess the role in innate immune responses of the identified variants that affect HIV infection in the context of substance abuse.

Point of Contact at Funding Agency: SATTERLEE, JOHN S, satterleej@mail.nih.gov

R21AI112717 (Dewhurst PI, García-Sastre Co-I) 07/01/2015-06/30/2016 0.24 calendar
NIH/NIAID \$440,300

University of Rochester

Enhanced live attenuated influenza virus with improved safety and immunogenicity

The overall goal of this grant is to determine the molecular basis for efficient replication of quail H9N2 influenza virus in human lung epithelial cells at 37°C even though it lacks the mutations in the polymerase genes that allow mammalian adaptation of other avian influenza viruses.

Point of Contact at Funding Agency: SALOMON, RACHELLE, salomonra@mail.nih.gov

426 (Richt PI, García-Sastre Co-I) 01/01/2016-12/31/2016 0.24 calendar
Kansas Bioscience Authority \$25,425

Kansas State University

Development of recombinant Newcastle Disease Viruses expressing immunogenically relevant proteins from important pathogens of mammalian livestock species

The goal of this project is to develop recombinant Newcastle Disease Viruses expressing immunogenically relevant proteins from important pathogens of mammalian livestock species and testing immunogenicity of recombinant viruses in pigs.

Point of Contact at Funding Agency: HOBER, STEPHANIE, shober@vet.k-state.edu

AGR-9532A1 (Palese PI, García-Sastre Co-I) 01/01/2015-12/31/2017 0.12 calendar
Merck Sharp & Dohme Corp. \$2,158,500

Preclinical R&D for evaluation of the armed New Castle Disease Viruses (NDV) for anti-tumor efficacy in combination with immunomodulatory antibodies

The goal of this project is the identification of innate immune mechanisms playing a role in NDV-mediated immune activation.

Point of Contact at Funding Agency: NAJI, ZUBIA, zubia_naji@merck.com

426 (Richt, PI, García-Sastre Co-I)	01/01/2017-03/31/2018	0.12 calendar
Kansas Bioscience Authority	\$8,475	
Kansas State University		

Efficacy of NDV-vectored HPAI H5 vaccine in chickens via water/air mass application immunization

The goal of this project is to develop recombinant Newcastle Disease Viruses expressing immunogenically relevant proteins from important pathogens of mammalian livestock species and testing immunogenicity of recombinant viruses in chickens.

Point of Contact at Funding Agency: HOBBER, STEPHANIE, shober@vet.k-state.edu

U19AI106754 (García-Sastre)	06/26/2013-05/31/2018	2.13 calendar
NIH/NIAID	\$25,992,160	

Multiscale analysis of influenza host-pathogen interactions: Fluomics

The overall goal of this application is to use an OMICS integrated systems biology approach to identify and validate novel host targets for therapeutic intervention during influenza A virus infection.

Point of Contact at Funding Agency: CHANDRAMOULISWARAN, ISHWAR, ishwar.chandramouliswaran@nih.gov

HHSN272201300023C (Gale PI, García-Sastre Co-I)	09/30/2013-09/29/2018	0.36 calendar
NIH/NIAID	\$12,975,048	

University of Washington

Development of KIN-1148 as a novel innate immune adjuvant for emerging RNA virus vaccines

This program aims to develop and optimize KIN1148, a novel small-molecule adjuvant that acts through the Retinoic acid inducible gene-I (RIG-I) pathway and the interferon regulatory factor (IRF)-3 transcription factor to stimulate innate immunity and protection following viral infection.

Point of Contact at Funding Agency: IRETON, RENEE, rireton@uw.edu

AGR-7811 (Palese PI, García-Sastre Co-I)	01/01/2014-12/31/2018	0.12 calendar
GlaxoSmithKline	\$4,200,000	

Development of a supra seasonal influenza virus vaccine

The long term objective of this proposal is to develop a universal influenza virus vaccine that provides long-lasting protection against seasonal and pandemic influenza virus infections without the need of annual re-vaccination for infants, children and adults.

Point of Contact at Funding Agency: VAN DER WIELEN, MARIE, marie.x.van-der-wielen@gsk.com

R21AI129486 (García-Sastre PI)	08/01/2017-07/31/2019	0.91 calendar
NIH/NIAID	\$466,125	

Interferon signaling inhibition by the Zika virus NS5 protein

The proposed experiments in this R21 will advance our knowledge on a novel mechanism by how ZIKV antagonize IFN-I antiviral responses, setting the basis for future potential studies on ZIKV vaccines, animal models and antivirals.

Point of Contact at Funding Agency: WOODSON, SARA ELAINE, sara.woodson@nih.gov

AGR-17705 (García-Sastre PI)	03/01/2019-02/29/2020	0.06 calendar
7 Hills Pharma	\$30,000	

Adjuvant properties of 7HP349

The ability of compound 7HP349 to increase the induction of HAI antibodies by the inactivated influenza virus vaccine and to increase protection from morbidity and mortality from master homologous influenza virus challenge will be tested in mice.

Point of Contact at Funding Agency: MARATHI, UPENDRA, upendra@7hills.co

OPP1084518 (Krammer PI, García-Sastre Co-I)	11/05/2014-03/31/2020	0.06 calendar
Bill & Melinda Gates Foundation	\$1,181,082	

Evaluation of safety and immunogenicity of chimeric HA expressing live-attenuated and inactivated universal influenza virus vaccines in phase I clinical trials

The long term objective of this proposal is to develop a universal influenza virus vaccine that provides long-lasting protection against seasonal and pandemic influenza virus infections without the need of annual re-vaccination for infants, children and adults.

Point of Contact at Funding Agency: SACKETT, KATE, kate.sackett@gatesfoundation.org

U01HL146240 (Wolinsky PI, García-Sastre Co-I)	09/18/2019-03/31/2020	0.06 calendar
NIH/NIAID	\$60,000	

Northwestern University

Northwestern CORE Clinical Research Site: Trans-omics for HIV/AIDS Research

The García-Sastre lab will determine whether a particular host factor identified as a potential candidate of increasing or decreasing HIV acquisition and/or induced disease is a regulator of antiviral responses.

Point of Contact at Funding Agency: ALTEKRUSE, SEAN FITZGERALD, sean.altekruse@nih.gov

R21AI142337 (Aggarwal PI, García-Sastre Co-I)	11/12/2018-10/31/2020	0.12 calendar
NIH/NIAID	\$466,125	

Structure guided discovery of chemical probes for targeting Zika methyltransferase

This application aims to develop antivirals targeting the methyltransferase (MTase) domain at the N-terminus of the nonstructural protein NS5 of Zika virus that is key to the replication of the virus, using crystallography coupled with chemical, biophysical and cellular characterizations.

Point of Contact at Funding Agency: DAVIS, MINDY I, davismi2@mail.nih.gov

AWARD NUMBER: W81XWH-20-1-0270

TITLE: Development and Advancement of Respiratory Antivirals

PRINCIPAL INVESTIGATOR: Steven Olson, Ph.D. (Subaward PI)

CONTRACTING ORGANIZATION: Sumit K Chanda, Ph.D.

REPORT DATE: 04/30/2021

TYPE OF REPORT: Annual Report

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1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The Program leverages two complementary strategies for the development of broad-spectrum host directed antivirals, with MOA to medicinal chemistry, ADME/T, and in vivo efficacy studies conducted in parallel. Taken together, the project leverages a significant body of preliminary studies that have identified four novel and potent early stage host-directed pan-respiratory antiviral molecules, brings together a unique and complementary set of highly experienced investigators, and incorporates a dynamic and integrated programmatic structure. These features will enable the preclinical development and clinical transition of paradigm-shifting therapies for the treatment of respiratory viral infections that impact troop readiness.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Medicinal chemistry, structure-activity relationship, SAR, potencies, antiviral activity, metabolism, pharmacokinetics, DMPK

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The overall goal of this Program is to identify lead candidate antiviral drugs leading to the selection of a Developmental Candidate (DC), and one back up. We plan to advance the DS into GMP/GLP preclinical studies to support an investigational new drug (IND) filing in the United States.

Dr. Olson serves as a co-investigator and his laboratory is in charge of optimization of potency and in vitro ADME properties of the Hit-to-lead series. M85 and M4 exhibit relatively good potency, $IC_{50} < 500$ nM against IAV. The primary objective is to improve the drug like properties of the molecule using the Tier 1 in vitro ADME assays described herein, while maintaining or increasing potency.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Dr. Olson's laboratory:

Major activities:

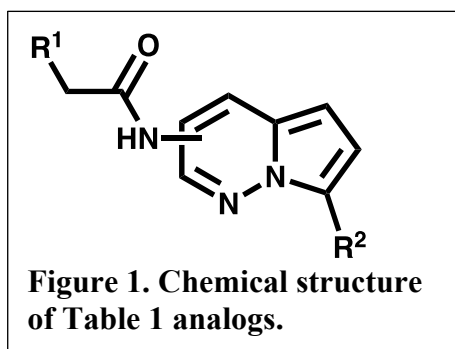
1. A broad set of M85 analogs were tested against both Influenza A and SARS CoV-2 in the cellular infection assay. Structure-activity relationships (SAR) from the data set were analyzed and led to the design of novel M85 analogs.
2. M85 analogs are synthesized by the medicinal chemists and tested in the Influenza A and SARS CoV-2 infection assays. Feedback from the assay data creates additional SAR, leading to the design of the next generation of analogs.

Specific Objectives:

1. The medicinal chemistry effort is focused on advancing the M85 series of compounds and developing structure-activity relationships that provide a rational approach for the discovery of improved analogs. Our continuing goal is to discover broad spectrum antivirals and understand the SAR responsible for differential antiviral activity.
2. The program is currently working to advance M85 analogs for both Influenza A and SARS CoV-2. Primarily, we needed to establish whether the SAR for M85 analogs against Influenza A is identical or consistent with the SAR for M85 analogs against M85. Although M85 itself is active in both assays, determining whether the relative potencies are consistent for all of our analogs is important for planning the direction of the medicinal chemistry research.

Significant results and outcomes:

Selectivity between Influenza A and SARS CoV-2. M85 was potent against both Influenza A and SARS CoV-2, providing additional evidence that M85 exhibits broad antiviral activity. Close analogs of M85 (**Table 1**) were also active, but the relative potencies were not consistent for the two viruses, indicating there are additional mechanistic variables that must be considered when designing broad spectrum inhibitors. Possible explanations include differential expression of kinase proteins responsible for viral entry or varied expression of small molecule efflux pumps between Influenza A and SARS CoV-2. Additional SAR will help elucidate the mechanism and overcome the differential activity. Specifically, substitution of the M85 thiophene ring (R¹) was allowed such that substitution with *m*-methoxyphenyl retained SARS CoV-2 activity and lost Influenza A activity, whereas indole substitution retained Influenza A activity and lost SARS CoV-2 activity. Additional analogs are planned to further elucidate



Compound	R ¹	Substitution	R ²	Influenza A IC ₅₀ (μM)	SARS CoV-2 IC ₅₀ (μM)	Cell Prolif IC ₅₀ (μM)
M-85		meta		0.12	~1.3	~ 10
F-0478		meta		>10	1.3	~ 10
F-1033		meta		>10	0.22	> 10
F-1914		meta		> 10	2.7	> 10
F-1932		meta		2.6	> 10	~ 10
F-0811		para		> 10	> 10	> 10

Table 1. Structure activity relationships for M85 series analogs against Influenza A and SARS CoV-2.

these differences. Substitution of the 3-pyridyl ring (R2) with 4-pyridyl or thiophene resulted in compounds that retained SARS CoV-2 potency, but lost potency against Influenza A.

Expanding the structure-activity relationships. The initial M85 drug discovery effort for Influenza A indicated that improvements in activity may be found by varying the arylacetamide group (R, **Table 1**). We used this data as a starting point for our medicinal chemistry strategy to improve the potency of analogs and further understand the relationships between Influenza A activity and SARS CoV-2 activity (**Table 2**).

SBI#	Shaw ID	R-Group	IC ₅₀ (μM)	CC ₅₀ (μM)	SI
1153218	11	H ₂ N-	>50	>50	na
0231370	1 (M85)		1.9	>50	26
	3		4.8	>50	10
1153449	17		5.7	15.9	3
1153448	10		>50	>50	na
0231372	8		28	>50	2
1153447	9		22	>50	2
1153451	12		20	>50	2
	24		>50	>50	na

SBI#	Shaw ID	R-Group	IC ₅₀ (μM)	CC ₅₀ (μM)	SI
	2		>50	>50	na
	5		39	>50	1
	6		>50	>50	na
	4		>50	>50	na
1153450	7		>50	>50	na
1153285			>50	>50	na
1153219			>50	>50	na
1153220			>50	>50	na

Table 2. Early SAR for substitution at the meta position of the phenyl ring of M85.

During the initial SAR campaign, a novel biphenyl aminopyridazine was also identified as potent against influenza A (**Table 3**, Shaw ID 14). To follow up on this discovery, we proposed that additional aminopyridazines may also show activity. We are encouraged that two of the analogs showed good activity against influenza A, SBI-0236159 and SBI-1153412 at 1.1 μM and 0.60 μM (**Table 4**). This is an additional lead series that we should be able to advance in the next year.

Shaw ID	Structure	IC ₅₀ (μM)	CC ₅₀ (μM)	SI
13		>50	>50	na
14		33	>50	2

Table 3. Discovery of aminopyridazines active against Influenza A.

SBI#	Structure	IC ₅₀ (μM)	CC ₅₀ (μM)	SBI#	Structure	IC ₅₀ (μM)	CC ₅₀ (μM)
0236159		1.1	>9	1153409		>9	>9
0236162		>9	>9	1153410		>9	>9
0236211		>9	>9	1153411		>9	>9
0236213		>9	>9	1153412		0.60	>9
0236214		>9	>9	1153413		>9	>9

Table 4. Aminopyridazine SAR.

We also expanded our efforts to synthesize novel analogs in the triazolopyridazine series, as shown in **Table 5**. Since substitution of the thiophene of M85 (**Table 2**, Shaw ID: 1) resulted in activity against both viruses, we believe this is the ideal position for in-depth medicinal chemistry exploration. As a result, we synthesized a set of 40 new analogs at the 3-amino position of the phenyl ring (**Table 5**). In addition to SBI-0231370 which had an IC₅₀ of 0.022 μM against influenza A, both the thiazole analog SBI-1153572 and imidazole SBI-1153572 showed good activity at 0.23 μM and 0.40 μM, respectively.

SBI#	R-Group	IC50 (uM)	SBI#	R-Group	IC50 (uM)	SBI#	R-Group	IC50 (uM)	SBI#	R-Group	IC50 (uM)
1153218		>9	1153449		1.6	1153255		>9	1153219		0.42 *
0231370		0.022	1153201		>9	1153198		>9	1153285		>9
1153180		>9	1153200		>9	1153284		>9	1153448		>9
1153183		>9	1153220		>9	1153315		>9	1153175		>9
1153199		>9	1153420		>9	1153316		>9	1153178		>9
1153187		3.6	1153572		0.23	1153414		>9	1153179		>9
1153181		>9	1153282		0.40	1153415		>9	1153567		>9
1153186		>9	1153283		1.0	1153416		>9	1153570		>9
1153182		>9	1153184		2.7	1153451		>9	1153423		>9
1153185		>9	0231372		>9	1153450		>9	1153447		>9

Table 5: The activity of amide-linked triazolopyridazine analogs against Influenza A.

Pharmacokinetics and drug metabolism major activity: In vivo assessment of KIN1502 drug metabolism and pharmacokinetics properties.

Pharmacokinetics and drug metabolism specific objectives: The goal of these studies is to determine the optimal formulation, dose and route of administration of KIN1502. This work will ensure that KIN1502 reaches therapeutically effective levels in the lung target tissue to enable a positive outcome from our planned in vivo efficacy studies.

This work has not yet commenced. Pharmacokinetic studies using KIN1502 formulated in cyclodextrins as described above were expected in this quarter. However, the limited solubility and need to develop intranasal dosing techniques required a delay in these studies. As such, efforts in this quarter were focused on securing IACUC approval (see below) for intranasal dosing, validation of this technique and discussion of the technical hurdles in assessing drug exposure in the lung epithelium versus total drug exposure in the lungs.

Pharmacokinetics and Drug Metabolism Summary of Accomplishments:

Much of year 1 was focused on establishing protocols and experimental procedures, and obtaining baseline ADME and DMPK properties for our lead series represented by KIN1502. As discussed below, and in previous progress reports, initial assessment of ADME properties revealed a series of limitations to the use of KIN1502 in vivo. Most notably, the compound is poorly soluble, limiting the overall dose that can be administered. Formulation studies revealed that using cyclodextrin excipients increased solubility by ~10x. In addition to solubility, overall systemic drug exposure was low when administered orally. To address this, the project team requested that the DMPK group develop an intranasal dosing approach to maximize drug exposure to the lung epithelium. The DMPK team evaluated options and developed a plan to enable this study with the addition of intranasal dosing. IACUC approval for this method and route of administration was secured, and follow-on DMPK studies of KIN1502 administered intranasally are planned for the coming months.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

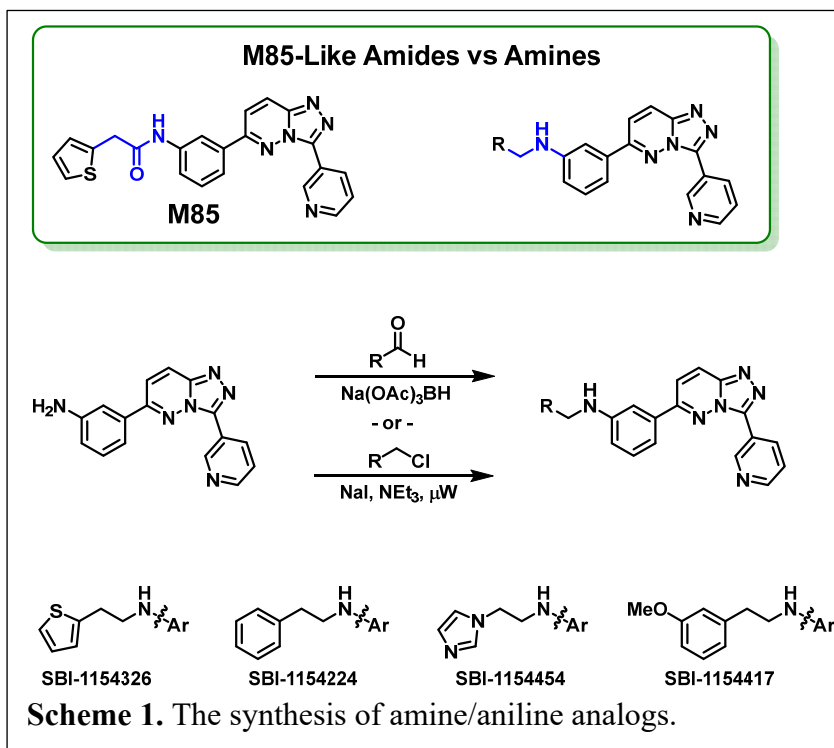
What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state "Nothing to Report."

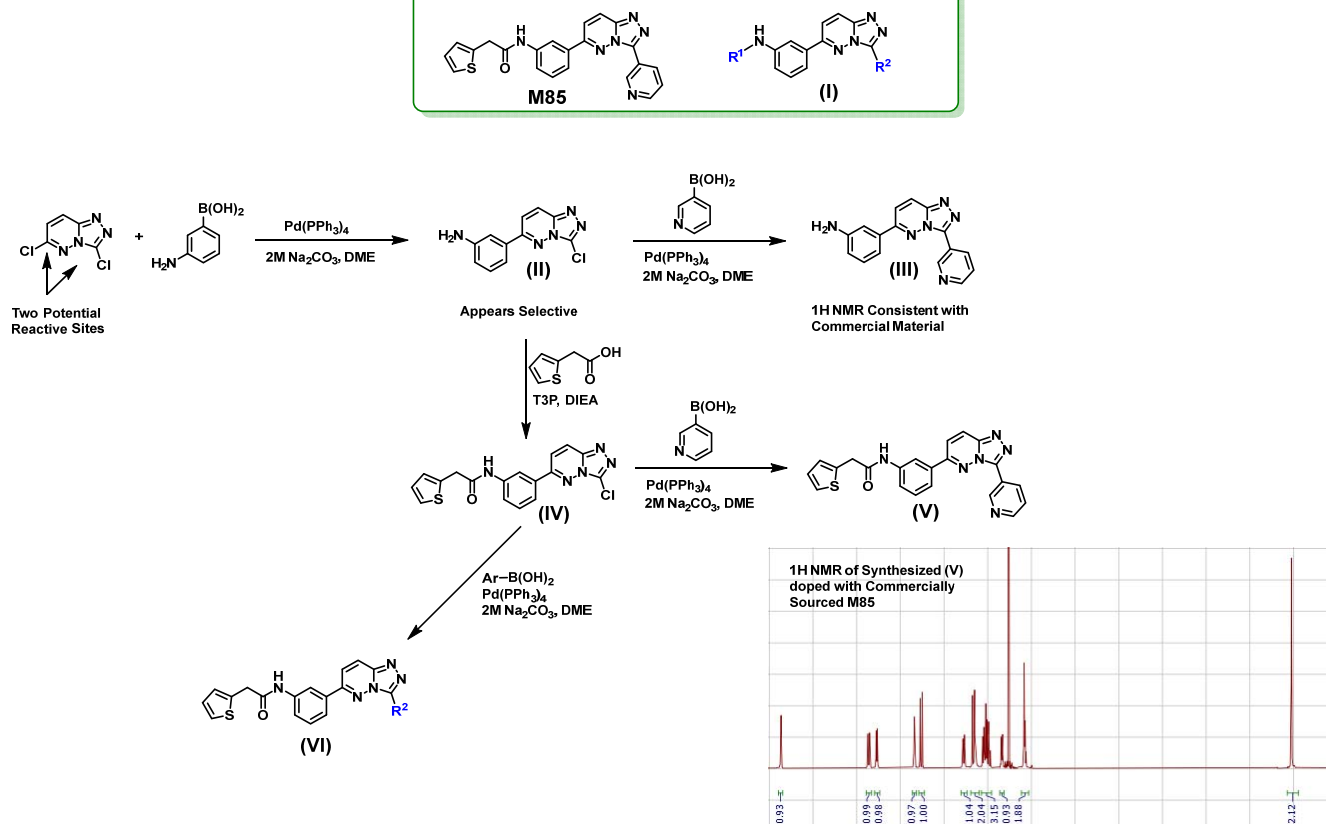
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Recently, we prepared *amine* analogs based on the M85 scaffold that contain substituents that parallel the most potent *amides* found in **Table 5**. These compounds were prepared as shown in **Scheme 1** using either reductive amination or halide displacement under microwave heating conditions. Over the next month, we will expand this chemistry to additional analogs, as we would expect the basic amines to improve solubility.

We also propose to investigate various R²-substituents while maintaining the remainder of the M85 scaffold (**Scheme 2**). To accomplish this this substitution, we explored a regioselective Suzuki reaction on substrate (I). Initial analysis suggested the reaction was highly selective for the desired product. Since the reaction at either chloro group would give a similar proton NMR spectrum, the resultant test substrate was elaborated to material which could be purchased commercially, and then ultimately to the synthesis of M85. Doping the resultant compound with commercially purchased M85 gave a proton NMR spectrum for only M85. These structure proofs give further confidence to the connectivity/selectivity from this route. The remaining aryl-chloride is sufficiently reactive to allow for investigation of analogs corresponding to (VI). Indeed, we have begun to make analogs in this position as shown in **Figure 6**.



Selective Suzuki Route to R² Analogues



Scheme 2. The synthesis of R² analogs using the Suzuki cross-coupling reaction.

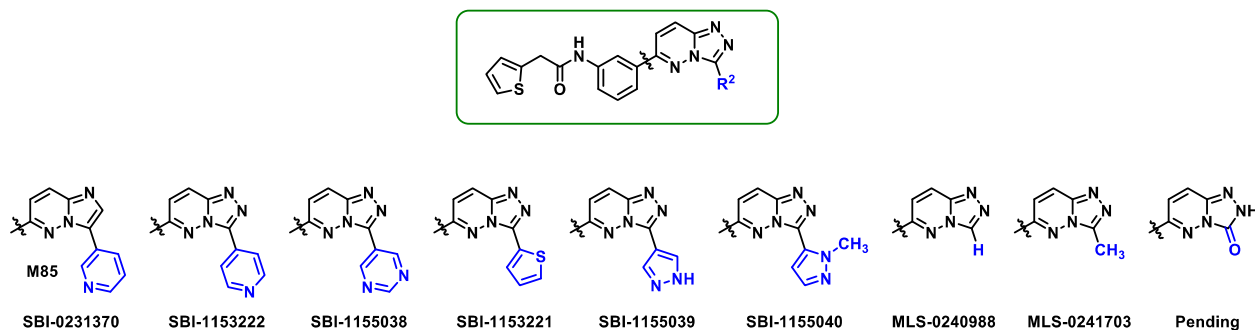
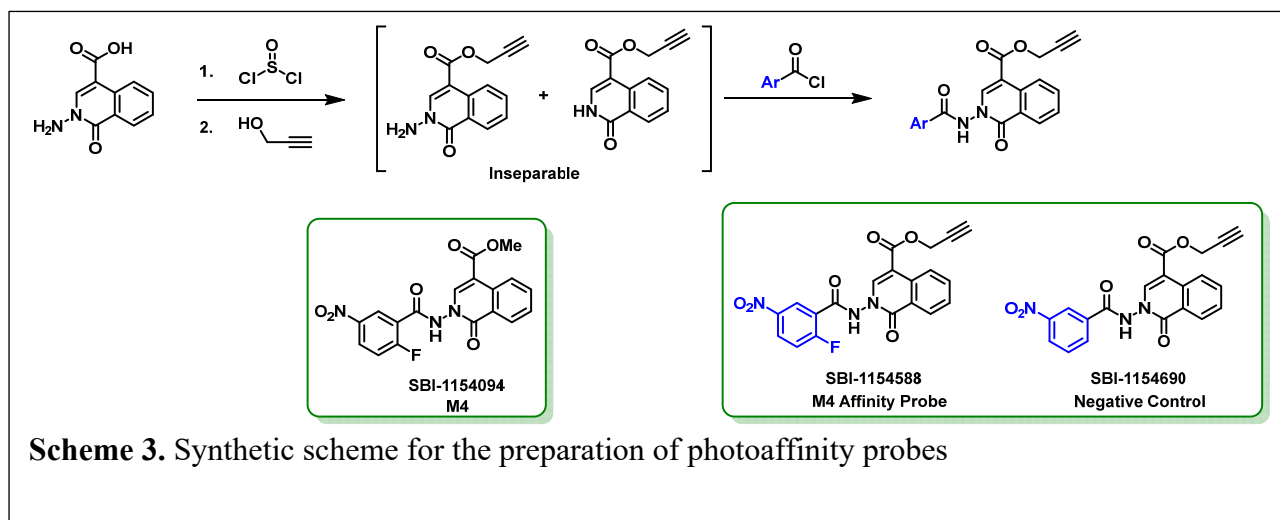


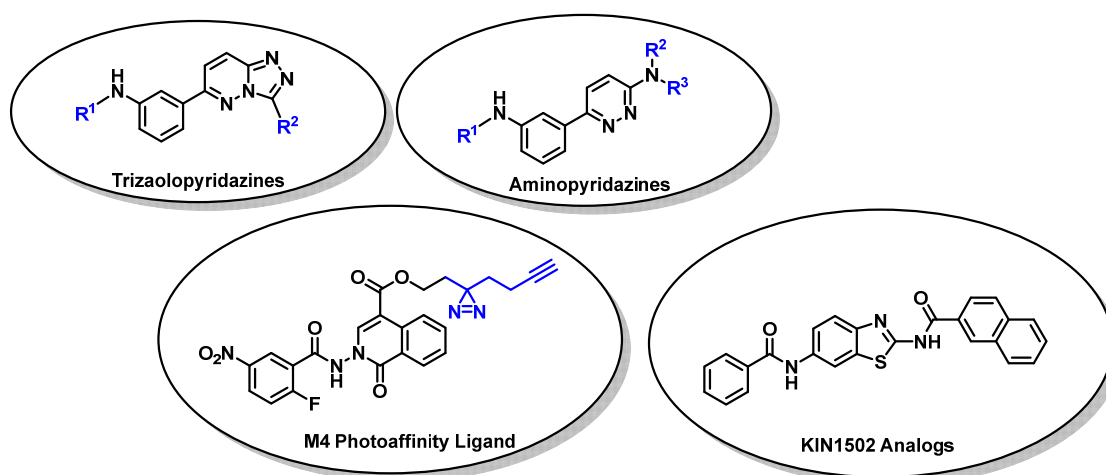
Figure 6. Proposed analogs to prepare analogs with diverse heterocycles at the R² position

As part of our research strategy, we proposed chemoproteomic studies of antiviral compound M4. Our approach requires analogs with an affinity for the target protein(s) that also contain a non-interfering alkyne handle to “pull-down” the protein targets for analysis using azide “click” chemistry and Biotin-Streptavidin purification. We know from experience that the *para*-nitro-fluorophenyl group is quite reactive toward nucleophiles and anticipate that this may be a significant and irreversible interaction within the binding site of the protein. To this end we have prepared the affinity probe and the

corresponding negative control compound as shown in **Scheme 3**. Pending verification of their activity in our cellular assays these probes will be utilized in the Bollong proteomics lab at Scripps Research Institute.



Forthcoming medicinal chemistry efforts will focus on expanding on active series within the M85 portfolio, preparing a photoaffinity probe based on the M4 scaffold, and further investigating antiviral SAR of a sourced library of KIN1502 analogs. Additionally, activity of the simplified aminopyridazine series will be explored through the synthesis of a focused set of analogs designed to mimic key elements of the triazolopyridazine (M85) series.



Pharmacokinetics and Drug Metabolism Plans: In the coming months, we will refine our intranasal dosing techniques and pharmacokinetics study design. We anticipate assessing the tissue and systemic exposure of KIN1502 after intranasal dosing at a series of escalating dose levels to determine the targeted compounds exposure in the lung. We will also determine the extent of systemic exposure on KIN1502 after intranasal dosing. It is anticipated that this approach will be used for new analogs of KIN1502 as well as other scaffolds that are developed by the medicinal chemistry team.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

PROTOCOL (3 of 3 total):

Protocol [ACURO Assigned Number]: PR190558.e001

Title: “In Vivo Assessment of Plasma Levels in Mice”

Target required for statistical significance: 372

Target approved for statistical significance: 372

SUBMITTED TO AND APPROVED BY:

Approved by SBP IACUC on February 6, 2020

Protocol was approved by ACURO on April 10, 2020

Amendment of IACUC Protocol to include intranasal dosing was approved March 10, 2021

STATUS:

- Protocol has been approved.
- IACUC protocol was amended to include intranasal dosing of compounds
- Animal studies have not been initiated.
- Currently no administrative, technical, or logistical issues are impacting progress of the study.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

- **Publications, conference papers, and presentations**
Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status*

of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk () if presentation produced a manuscript.*

Nothing to report.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*

- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

Name:	Mary Smith
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	1234567
Nearest person month worked:	5
Contribution to Project:	Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support:	The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name: Steve Olson
 Project Role: Subaward PI
 Researcher Identifier (e.g. ORCID ID): eRA Commons: STEVENOLSON
 Nearest person month worked: 1 (1.10 calendar months)
 Contribution to Project: PI and drug discovery lead for the project.

Name: Jon Covell
 Project Role: Medicinal Chemist
 Researcher Identifier (e.g. ORCID ID): Not Available
 Nearest person month worked: 4 (4.28 calendar months)
 Contribution to Project: Dr. Covell participated in the planning and design of new, improved analogs.

Name: Jing Zhu
 Project Role: Scientist
 Researcher Identifier (e.g. ORCID ID): Not Available
 Nearest person month worked: 2 (1.69 calendar months)
 Contribution to Project: Dr. Zhu worked on DMPK for this project.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Yes, updated other support attached.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ebrap.org/eBRAP/public/index.htm> for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil/Pages/Resources.aspx>) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

CURRENT/COMPLETED SUPPORT

Steve Olson, Ph.D., Sanford Burnham Prebys Medical Discovery Institute

CURRENT

[NEW]

Title: Small molecule inhibitors of LMPTP: an obesity drug target

Grant #: R01 DK106233 (Bottini, Olson*)

Time Commitment: 0.60 calendar months

Supporting Agency: NIH/NIDDK

Funding Agency Contact: Christina Coriz, Grants Management Specialist, Phone: 301-594-8848, Email: corizc@niddk.nih.gov

Performance Period: 07/01/2015 – 05/31/2023

Funding Level: \$100,000

Goals: The goal of this project is to continue investigation on a promising candidate molecule for the treatment of diabetes. This work seeks to improve potency of the small molecular inhibitor, as well as test in vitro efficacy, in vitro ADME, in vivo PK, in vivo efficacy in mouse models, and preliminary clinical evaluations.

Specific Aims:

Aim 1. Preclinical validation of LMPTP inhibitor efficacy in human hepatocytes and in mouse models of obesity-induced diabetes.

Aim 2. Preclinical validation of LMPTP inhibitor safety in mice.

Aim 3. Generation of an optimized LMPTP inhibitor candidate.

Role: PD/PI

Overlap: None

**Effective 1/1/2021*

Title: Sanford Burnham Prebys Medical Discovery Institute Dedicated CBC Center

Grant #: 16X113 MSA Management (Jackson, Vuori)

Time Commitment: 0.90 calendar months

Supporting Agency: The Chemical Biology Consortium - National Cancer Institute via Master Service Agreement w/ Leidos Biomedical Research, Inc.

Funding Agency Contact: Bonnie Beard, Email: beardb@mail.nih.gov

Performance Period: 04/01/2016 – 08/30/2021

Funding Level: \$90,062 annual direct costs

Goals: The objective of this project is to perform mutually agreed upon services in support of the Chemical Biology Consortium awarded projects.

Specific Aims: N/A

Role: Executive Director, Medicinal Chemistry

Overlap: None

Title: Inhibitors of the Artemis endonuclease for Cancer Chemotherapy

Grant #: 16X113-Artemis (Olson)

Time Commitment: 0.05 calendar months

Supporting Agency: The Chemical Biology Consortium - National Cancer Institute via Master Service Agreement w/ Leidos Biomedical Research, Inc.

Funding Agency Contact: Bonnie Beard, Email: beardb@mail.nih.gov

Performance Period: Completed: 08/01/2016 – 08/30/2021

Funding Level: \$7,883

Goals: The objective is to perform mutually agreed upon services in support of the Artemis project.

Specific Aims: N/A

Role: Subaward PI

Title: Small Molecule Inhibitors of Ebola Virus Polymerase Function

Grant: R01 AI125453 (Basler)

Time Commitment: 0.24 calendar months

Supporting Agency: Georgia State University/NIH

Funding Agency Contact: Roberta D. Wolcott, NIAID Grants Management Specialist. Email: wolcott@niaid.nih.gov, Phone: 240-669-2964

Performance Period: 02/15/2017 – 01/31/2022

Funding Level: \$100,000 annual direct costs (\$19,835 Prebys Center Directs)

Goals: The goal of this project is to perform a high throughput screen, using previously validated assays, optimize the antiviral activity against EBOV of validated hits, and determine the mechanisms of action.

Specific Aims:

Aim 1. Perform a high throughput screen of the 350,000 compound NIH Library and validate the hits.

Aim 2. Optimize hits through SAR studies and identify leads for further development.

Aim 3. Evaluate antiviral activity of hits against EBOV and define mechanism of action.

Role: Collaborator

Overlap: None

Title: Validating and Characterizing a New Melanoma Therapeutic Target

Grant #: R01 CA217625 (Courtneidge)

Time Commitment: 0.60 calendar months

Supporting Agency: Oregon Health and Science University/NIH

Funding Agency Contact: Viviana Knowles, Grants Management Specialist, Email: Viviana.knowles@nih.gov, Phone: 240-276-5157

Performance Period: 08/01/2017 – 07/31/2021

Funding Level: \$170,771 annual direct costs

Goals: The goal of the project is to define atomic resolution structures of TAO3 kinase, focusing on the catalytic domain in complex with small-molecule activity modulators and in vivo ligands, and to develop a potent and selective TAO3 inhibitor with defined ADME properties, which will also serve as a tool compound to validate TAO3 as a target and as a lead for further optimization to an in vivo active compound.

Specific Aims:

Aim 1. To determine the mechanism by which TAO3 facilitates invadopodia formation and tumor growth.

Aim 2. To determine whether the background of driver mutations affects sensitivity to loss of TAO3.

Aim 3. To develop small molecule TAO3 inhibitors and test them in in vivo tumor growth assays

Role: Subaward PI

Overlap: None

Title: Discovery of SHP2 Allosteric Inhibitors

Grant #: 16X113-SHP2 (Tautz, Cosford, Olson)

Time Commitment: 0.30 calendar months

Supporting Agency: The Chemical Biology Consortium - National Cancer Institute via Master Service Agreement w/ Leidos Biomedical Research, Inc.

Funding Agency Contact: Bonnie Beard, Email: beardb@mail.nih.gov

Performance Period: Completed: 10/19/2017 – 5/15/2021

Funding Level: \$343,121 annual direct costs (\$218,606 Prebys Center directs)

Goals: The protein tyrosine phosphatase (PTP) SHP2 is an oncoprotein and new candidate for targeted cancer therapy, including in leukemias and HER2-positive and triple-negative breast cancers (TNBC). This project seeks to identify allosteric SHP2 inhibitors via high-throughput screening (HTS) of small molecule libraries using a protein thermal shift (PTS) assay.

Specific Aims: N/A

Role: Principal Investigator

Overlap: None

Title: Early Development of Small Molecule Dendritic Cell Immunopotentiators for the Treatment of Solid Tumors

Grant #: R01 CA229818 (Chanda, Garcia-Sastre)

Time Commitment: 0.24 calendar months

Supporting Agency: Icahn School of Medicine at Mount Sinai/NIH

Funding Agency Contact: Candace M Cofie, Grants Management Specialist. Email: candace.cofie@nih.gov
Phone: (240) 276-5721

Performance Period: 07/11/2018 – 06/30/2022

Funding Level: \$517,007 annual direct costs (\$24,900 Prebys Center directs)

Goals: This proposal aims to develop small molecules identified in a high throughput screening (HTS) assay to significantly improve the therapeutic efficacy of the oncolytic Newcastle Disease Virus (NDV) through the activation of antigen presentation cells (APCs), resulting in an enhanced anticancer immune response in the tumor microenvironment.

Specific Aims:

Aim 1. Cross validation of hit compounds that activate MDSCs.

Aim 2. Establish anti-tumor efficacy of immunomodulatory compounds in murine melanoma model.

Aim 3. Delineate in vivo and ex vivo biomarkers that act as surrogates of in vivo efficacy.

Role: Co-Investigator

Overlap: None

Title: Large-Scale Screen with a Novel Assay against RNA Editing to Identify Anti-Trypanosomal Agents

Grant #: R01 AI143593 (Salavati)

Time Commitment: 0.24 calendar months

Supporting Agency: McGill University/NIH

Funding Agency Contact: Gregory P. Smith, Grants Management Specialist, Email: gsmith@niaid.nih.gov,
Phone: 240-669-2993

Performance Period: 03/06/2019 – 02/28/2022

Funding Level: \$123,170 annual direct costs (year 2 direct costs, year 3 NOA pending)

Goals: The goal of this project is to identify inhibitors of this essential process, utilizing the capacity of a FRET-based assay for a sensitive “mix and measure” in vitro monitoring of RNA editing activity in *T. brucei*, which we developed in a previous R21 assay development grant.

Specific Aims:

Aim 1. Editosomes and RNAs will be prepared, while we further miniaturize to 1536-well the 384-well RNA editing assay, and implement it in an HTS campaign.

Aim 2. Confirm RNA editing inhibitors and determine their potency (IC₅₀), and will assess growth inhibition of the bloodstream forms of *T. brucei*.

Aim 3. Elucidate the nascent structure-activity relationships (SAR) of chemical scaffolds and optimize the selected hits to improve target specificity and potency of inhibitors. It will then investigate the MOA and cross-species effect of the best inhibitory compound(s), followed by scale-up to benchmark their ADME/T & PK properties.

Role: Executive Director, Medicinal Chemistry

Overlap: None

Title: Brain pathology and function in a chronic mouse model of ZIKV transmission

Grant #: R01 NS105969 (Tersikh, Olson, Muotri)

Time Commitment: 0.96 calendar months

Supporting Agency: UCSD/NIH

Funding Agency Contact: NIH Grants Management Specialist: Yvonne C. Talley, Email: talleyy@mail.nih.gov,
Phone: 301-496-7432

Performance Period: 04/01/2019 – 03/31/2024

Funding Level: \$427,499 (\$37,460 Prebys Center directs)

Goals: The goal of this project is to determine, both the dynamics of ZIKV infection in the developing brain, and the detrimental effect of the pathogen on brain development, after either maternal infection (mimicking a mosquito bite) and sexual transmission.

Specific Aims:

Aim 1. Determine the dynamics of fetal brain infection and investigate perinatal consequences following ZIKV vertical transmission in SJL mice

Aim 2. Determine structural and functional consequences to the adult brain following congenital ZIKV infection in SJL mice

Aim 3. Determine the best treatment regimens for ZIKV infection using inhibitors of different stages of the viral life cycle and their combinations to prevent vertical transmission of ZIKV

Role: Principal Investigator

Overlap: None

Title: Targeting Neuroinflammation in AD with Novel CX3CR1 Agonists

Grant #: R01 AG062557 (Smith, Nash)

Time Commitment: 1.20 calendar months

Supporting Agency: University of South Florida/NIH

Funding Agency Contact: Manda C. Richards, Grants Management Specialist, Email: manda.richards@nih.gov, Phone: 301-827-7977

Performance Period: 05/01/2019 – 02/29/2024

Funding Level: \$475,953 annual direct costs

Goals: The goal of this project is to develop orally bioavailable potent CX3CR1 agonist compounds suitable for use as chemical probes in the development of a novel drug target for the treatment of tau pathology, neuron atrophy, and neurodegeneration in Alzheimer's disease.

Specific Aims:

Aim 1. Establish and validate outcome measures for the lead-optimization campaign.

Aim 2. Determine the potency, efficacy, and selectivity of novel CX3CR1 agonist compounds in a battery of existing bioassays in vitro.

Aim 3. Measure the physicochemical and pharmacological properties of improved CX3CR1 agonists.

Aim 4. Execute efficacy studies in vivo using transgenic mouse models of AD, including rTg4510 and APP-PS1 mice.

Role: Co-Investigator

Overlap: None

Title: High-Throughput Screen for Discovery of Ciliary Neurotrophic Factor (CNTF) Receptor Agonists for the Treatment of Amyotrophic Lateral Sclerosis (ALS)

Grant #: R33 NS110960 (Rossoll)

Time Commitment: 0.24 calendar months

Supporting Agency: Mayo Clinic/NIH

Funding Agency Contact: Jeannette Gordon, Grants Management Specialist, Email: jg82s@nih.gov, Phone: 301-496-3813

Performance Period: 05/01/2019 – 04/30/2022

Funding Level: \$137,500 annual direct costs (year 2 direct costs, year 3 NOA pending)

Goals: The goal of this project is to develop a cell-based high throughput screen, and follow-on biological assays to discover and validate small molecules that protect motor neuron function and survival by specifically activating CNTFR but not IL-6R complexes.

Specific Aims:

R61 Phase Specific Aims:

Aim 1. Develop an HTS assay for selective CNTFR agonists and conduct a pilot screen.

Aim 2. Optimize secondary assays in neuronal cell lines and primary neurons.

R33 Phase Specific Aims:

Aim 1. Conduct high-throughput screening of a large and diverse library for selective CNTFR agonists.

Aim 2. Characterize promising therapeutic agents from the HTS screen.

Aim 3. Perform focused medicinal chemistry approach to develop early lead compounds.

Role: Executive Director, Medicinal Chemistry

Overlap: None

[NEW]

Title: The Development of TGR5 Antagonists for the Treatment of Cholangiopathies

Grant #: R01 DK117133 (Olson*, LaRusso)

Time Commitment: 1.80 calendar months

Supporting Agency: NIH/NIDDK

Funding Agency Contact: Kevin Reeves, Grants Management Specialist. Email: reeveskm@mail.nih.gov

Phone: 301-594-6285 Fax: 301-480-3777

Performance Period: 09/15/2019 – 6/30/2023

Funding Level: \$356,331 annual direct costs (\$147,642 Prebys Center directs)

Goals: The objective is to develop antagonists to inhibit TGR5 activity, thus reducing hepatic cystogenesis in PLD.

Specific Aims:

Aim 1. To design and synthesize TGR5 antagonists orally active in vivo. Through iterative cycles of chemistry, we will identify compounds suitable for in vivo efficacy studies.

Aim 2. To clarify the mechanisms of action of SBI-319 analogs in PLD. We will 1) assess SBI-319 analog effects on: (i) cAMP production in cystic cholangiocytes; (ii) cholangiocyte proliferation; and (iii) growth of hepatic cystic structures in 3D cultures; 2) examine the expression of TGR5 and Gas proteins, and their coupling upon treatment with SBI-319. Aim 3. To evaluate the efficacy of TGR5 antagonists as a treatment for PLD. We will assess effects of SBI-319 analogs on hepatic and renal cystogenesis and clarify the role of TGR5 inhibition in disease progression in vivo.

Role: Contact Principal Investigator

Overlap: None

**Effective 1/1/2021*

Title: C-GAS/STING Inducing Compounds as Novel Adjuvants

Grant #: 75N93019C00046 (Chanda, Garcia-Sastre, Sekaly)

Time Commitment: 0.36 calendar months

Supporting Agency: NIH Contract

Funding Agency Contact: Wolfgang W. Leitner, MSc PhD, Contracting Officer Representative. Email: wleitner@mail.nih.gov Phone: 240-627-3575

Performance Period: 09/30/2019 – 09/29/2024

Funding Level: \$399,960 annual directs (\$74,508 Prebys Center annual directs)

Goals: The goal of this project is to identify and develop 1 to 2 promising novel adjuvants that activate the endogenous and powerful cGAS/STING pathway to facilitate innate and adaptive immune protection, and the induction of broad protective B and T cell responses when used as adjuvants of an inactivated influenza virus vaccine.

Specific Aims:

To design and execution all medicinal chemistry and ADME/PK work related to the contract, including data analysis and reporting, and guiding/interfaces the project personnel to ensure successful milestone delivery. The medicinal chemistry will also perform hit assessment, compound synthesis and optimization, overseeing SAR, interpreting the biological data and selecting compounds for in vitro ADME studies to be in the Exploratory Pharmacology Core.

Role: Co-Investigator

Overlap: None

[NEW]

Title: IGNITE: Development of Novel Cyclophilin B/PIKfyve Inhibitors for Treatment of Malignant Peripheral Nerve Sheath Tumors

Grant #: R61 NS111085 (Olson*, Bram)

Time Commitment: 0.96 calendar months

Supporting Agency: NIH/NINDS

Funding Agency Contact: NIH Grants Management Specialist: Yvonne C. Talley, Email: talleyy@mail.nih.gov, Phone: 301-496-7432

Performance Period: 12/15/2019 – 11/30/2021

Funding Level: \$373,751 annual direct costs (\$175,000 Prebys Center directs) (year 1 direct costs, year 2 NOA pending)

Goals: The goal of this R61/R33 proposal is to conduct preclinical in vitro and in vivo testing of SBI298 and a focused set of related analogs, to demonstrate that it has sufficient biological activity to warrant further development for treatment of MPNST.

Specific Aims:

R61 Phase Specific Aims

1. To scale up the synthesis and confirm the purity, stability, and solubility of SBI298.
2. To perform a limited structure activity relationship (SAR) study to ensure that SBI298 is the optimal compound for studies in the R33 phase.
3. To conduct PK studies in mice to determine tissue levels of SBI298 in mice with MPNST tumors and to verify target engagement.
4. To assess variability of the mouse tumor models to estimate needed sample sizes for R33 studies.

R33 Phase Specific Aims

1. To determine pharmacodynamic metabolism, conversion and excretion studies of SBI298 or an improved analog in mice.
2. To determine the activity of SBI298 or an improved analog in xenograft models of MPNST and relate tumor responses to tissue concentrations and AUC measurements.
3. To determine the overall response and survival outcomes of mice bearing MPNST xenografts in response to treatment with SBI298 or an improved analog.

Role: Contact Principal Investigator

Overlap: None

**Effective 1/1/2021*

[THIS GRANT]

Title: Development and Advancement of Broad-spectrum Respiratory Antivirals

Grant #: W81XWH-20-1-0270/ PR190558 (Chanda, Garcia-Sastre)

Time Commitment: 1.20 calendar months

Supporting Agency: US Department of Defense

Funding Agency Contact: Abigail Strock, Grants Officer. Email: Abigail.l.strock.civ@mail.mil. Phone: 301-619-2342

Performance Period: 04/15/2020 – 04/14/2024

Funding Level: \$2,276,645 annual direct costs (\$255,839 Prebys Center directs)

Goals: The primary goal of DABRA, is to develop 1-2 antivirals that can be transitioned to preclinical scale up and GLP/GMP studies, and secondarily, to advance a follow-on antiviral portfolio based on targeting host factors through a preclinical development pipeline.

Specific Aims:

Aim 1. Host Target Identification and Validation. This aim focuses on the identification of broad-spectrum antiviral compound targets through chemoproteomic, genetic screening, and biochemical techniques.

Aim 2. Characterization of Broad-spectrum Antiviral Activities. We will characterize the phylogenetic breadth and potency of anti-respiratory viral activity in cell lines and primary cells.

Aim 3. Determining Cellular Response to Antiviral Treatment. Elucidating the impact of the antiviral compounds on cell physiology will allow us to further understand mechanism of action and identify critical PD markers to assess compound target engagement.

Role: Executive Director, Medicinal Chemistry

Overlap: None

[RENEWAL]

Title: Cancer Center Support Grant

Grant #: P30 CA030199 (Powis)

Time Commitment: 1.20 calendar months

Supporting Agency: NIH/NCI

Funding Agency Contact: Candace M Cofie, Grants Management Specialist. Email: candace.cofie@nih.gov;

Phone: (240) 276-5721

Performance Period: 05/29/2020 – 04/30/2025

Funding Level: \$2,192,919 annual direct costs (Core 7: \$107,152)

Goals: The Sanford Burnham Medical Research Institute's Cancer Center is dedicated to revealing the fundamental molecular causes of cancer and to applying this knowledge to the health of humans. Through continued commitment to collaborative and multidisciplinary research, our plans seek to propel our scientific activities onward to deliver results that will have a transformational impact in cancer research and medicine.

Specific Aims: N/A

Role: Executive Director, Medicinal Chemistry

Overlap: None

[NEW]

Title: Farnesyl Transferase Inhibitors to Treat Tauopathies

Grant #: T-PEP-20-672021 (Kosik, Olson)

Time Commitment: 1.20 calendar months

Supporting Agency: Alzheimer's Association/Tau Consortium

Funding Agency Contact: Daniela Gallardo, Subaward Officer, Office of Research. Email:

gallardo@research.ucsb.edu. Phone: 805.896.7027

Performance Period: 07/01/2020 – 06/30/2022

Funding Level: \$170,455 annual direct costs

Goals: The goal of this project is to perform lead optimization medicinal chemistry to discover farnesyltransferase inhibitors that reach pharmacologically-relevant brain concentrations and maintain high in vitro potency.

Specific Aims:

Specific Aims not available. Scope of work as follows:

The Conrad Prebys Center for Chemical Genomics (Prebys Center) will perform medicinal chemistry, a primary farnesyltransferase inhibition assay, and ADME/T studies.

1. Medicinal Chemistry

- a. Conception: The Prebys Center will conceive novel farnesyltransferase inhibitors through the use of computer-aided drug discovery and known co-crystal structures of inhibitors bound to farnesyltransferase, physical property-based predictions and structure-activity relationships. Initial targets are outlined in Aim 1, and feedback loops will be employed to identify additional generations of compounds
- b. Synthesis, purification and characterization: The Prebys Center chemists will synthesize farnesyltransferase inhibitors in state-of-the-art chemical laboratories. The molecules are purified by standard or reverse-phase chromatographic techniques and characterized by HPLC and NMR spectroscopy.

2. Farnesyltransferase Inhibition: The Prebys Center will develop a high-throughput, fluorescence-based farnesyltransferase inhibition assay to determine the potency of each analog synthesized by the group.

3. ADME/T studies (in vitro assays for compound absorption, distribution, metabolism, excretion and toxicology): The Prebys Center drug metabolism group has the standard ADME/T assays validated and available for use or ongoing drug discovery programs. Critical first tier assays for this program are permeability, P-gp efflux, and stability in mouse and human liver microsomes. Second tier assays will determine free fraction in plasma and brain.

Role: Principal Investigator

Overlap: None

[NEW]

Title: Therapeutic Targeting of a Lipid Kinase Regulator of Mutant p53 in Breast Cancer

Grant #: W81XWH-20-BCRP-BTA12 / BC200704P1 (Cryns, Chung)

Time Commitment: 0.00 calendar months; No effort until year 2: 0.60 calendar months

Supporting Agency: University of Wisconsin/United States Department of Defense, CDMRP

Funding Agency Contact: Darrell Beaver, Grants Specialist, Phone: 301-619-4019, Email:

Darrell.L.Beaver4.mil@mail.mil

Performance Period: 02/01/2021 – 01/31/2024

Funding Level: \$238,918 annual direct costs, year 1 award

Goals: The objective of this project is to identify potent and selective inhibitors of PIPKIalpha and test their activity in halting breast tumor growth.

Specific Aims:

Aim 1. Determine the effects of inducibly silencing PIPKIalpha on breast tumors that express mutant p53

Aim 2. Perform a large scale (up to 350,000 compounds) high-throughput screen (HTS) and follow-up to identify novel *potent* and *selective* PIPKIa inhibitors.

Aim 3. Determine the mechanism of inhibition (MOI) of promising PIPKIalpha inhibitors and available analogs and assess their activity in cellular and murine models of TNBC.

Role: Executive Director, Medicinal Chemistry

Overlap: None

COMPLETED (since last reporting period)

Nothing to report.

CURRENT/COMPLETED SUPPORT

Bradley Savall, Ph.D., Sanford Burnham Prebys Medical Discovery Institute

CURRENT

Title: Reversing Pulmonary Fibrosis through Dopamine D1 Receptor Agonism

Grant #: W81XWH-19-1-0030 / PR181132P1 (Savall/Tschumperlin)

Time Commitment: 1.20 calendar months

Supporting Agency: United States Department of Defense, CDMRP

Funding Agency Contact: Jennifer E. Hayden, Grants Specialist, Phone: 301-619-1363, Email: jennifer.e.hayden.civ@mail.mil

Performance Period: 09/01/2019 – 08/31/2022

Funding Level: \$373,455 annual direct costs (year 1 direct costs; year 2 NOA pending)

Goals: The goal of this project is to develop novel D1 receptor agonists that are not BBB penetrant, thereby eliminating their potential neurological side effects, and establish their efficacy in preclinical models of pulmonary fibrosis.

Specific Aims:

Aim 1. Design, synthesize and test novel and selective D1R agonists and demonstrate their efficacy in cultured human lung fibroblasts including those from subjects with IPF.

Aim 2. Identify the dose, route and dosing interval effects of our novel D1R agonist(s) on biodistribution, pharmacokinetics, and on-target efficacy in mice.

Aim 3. Evaluate the efficacy of D1R agonists in reversing fibrosis in both young and aged mice in two experimental pulmonary fibrosis models, bleomycin injury and adenoviral-TGF- β .

Role: Principal Investigator

Overlap: None

Title: IGNITE: Development of novel cyclophilin B/PIKfyve inhibitors for treatment of Malignant Peripheral Nerve Sheath Tumors

Grant #: R61 NS111085 (Olson, Bram)

Time Commitment: 0.48 calendar months

Supporting Agency: NIH/NINDS

Funding Agency Contact: Yvonne C. Talley, Grants Management Specialist, Phone: 301-496-7432, Email: talleyy@mail.nih.gov

Performance Period: 12/15/2019 – 11/30/2021

Funding Level: \$323,167 annual direct costs (\$124,999 to Prebys Center)

Goals: The goal of this proposal is to conduct preclinical in vitro and in vivo testing of SBI298 and a focused set of related analogs to demonstrate that it has sufficient biological activity to warrant further development for treatment of MPNST.

Specific Aims:

R61 Phase Specific Aims:

Aim 1. To scale up the synthesis and confirm the purity, stability, and solubility of SBI298.

Aim 2. To perform a limited structure activity relationship (SAR) study to ensure that SBI298 is the optimal compound for studies in the R33 phase.

Aim 3. To conduct PK studies in mice to determine tissue levels of SBI298 in mice with MPNST tumors and to verify target engagement.

Aim 4. To assess variability of the mouse tumor models to estimate needed sample sizes for R33 studies.

R33 Phase Specific Aims:

Aim 1. To determine pharmacodynamic metabolism, conversion and excretion studies of SBI298 or an improved analog in mice.

Aim 2. To determine the activity of SBI298 or an improved analog in xenograft models of MPNST and relate tumor responses to tissue concentrations and AUC measurements.

Aim 3. To determine the overall response and survival outcomes of mice bearing MPNST xenografts in response to treatment with SBI298 or an improved analog.

Role: Associate Director, Medicinal Chemistry

Overlap: None

[NEW]

Title: HTS Targeting HIV-1 Protease Autoprocessing for First in Class Drug Discovery

Grant #: R01 AI150223 (Chen, Chung, Sherman)

Time Commitment: 0.24 calendar months

Supporting Agency: NIH/NIAID

Funding Agency Contact: Gerard Lacourciere, Program Official, Email: gerard.lacourciere@nih.gov

Performance Period: 01/01/2020 – 12/31/2023

Funding Level: \$150,369 annual direct costs

Goals: The objective of the project is to carry out high-throughput screens with an established primary assay and characterize the hit compounds with follow-up assays to identify small molecules selectively targeting HIV-1 protease precursor autoprocessing, an essential viral-specific process that has not been previously exploited for anti-HIV drug development.

Specific Aims:

Aim 1: Identify and confirm positive hits from natural product extracts (NPEs)

Aim 2: Identify, confirm and validate hits from small molecule libraries (~350K compounds)

Aim 3: Examine biological efficacy and MOA of validated hits towards lead identification

Role: Co-Investigator

Overlap: None

[NEW]

Title: Novel Antagonists of the N-terminal Domain of the CRF Receptor Type 1 for Alzheimer's Disease

Grant #: R01 AG065385 (Rissman)

Time Commitment: 0.60 calendar months

Supporting Agency: Veterans Medical Research Foundation/NIH

Funding Agency Contact: Manda C. Richards, Grants Management Specialist, Phone: 301-827-7977, Email: manda.richards@nih.gov

Performance Period: 01/15/2020 – 12/31/2023

Funding Level: \$149,164 annual direct costs

Goals: The objective of this project is discovery of new CRFR1 antagonists that represent new chemical scaffolds by specifically targeting the *N*-terminal extracellular domain.

Specific Aims:

Aim 1. Complete an HTS using our novel TR-FRET assay to identify and confirm novel antagonist hits of CRF binding to the *N*-terminal extracellular domain of CRFR1 (ECD) on a large chemical library.

Aim 2. Validate the potency, specificity, functional antagonism and emergent SAR of confirmed hits.

Aim 3. Perform hit-to-lead and lead optimization through 2° critical path assays, characterized in 3° biologically relevant brain slice assays followed by scale up and characterization, benchmarking of their ADME/T, PK and brain penetrance properties.

Role: Co-investigator

Overlap: None

[THIS GRANT]

Title: Development and Advancement of Broad-spectrum Respiratory Antivirals

Grant #: PR190558 / W81XWH-20-1-0270 (Chanda/Garcia-Sastre)

Time Commitment: 0.96 calendar months

Supporting Agency: US Department of Defense

Funding Agency Contact: To be determined.

Performance Period: 04/15/2020 – 04/14/2024

Funding Level: \$2,276,645 annual direct costs (\$255,839 Prebys Center directs)

Goals: The primary goal of DABRA, is to develop 1-2 antivirals that can be transitioned to preclinical scale up and GLP/GMP studies, and secondarily, to advance a follow-on antiviral portfolio based on targeting host factors through a preclinical development pipeline.

Specific Aims:

Aim 1. Host Target Identification and Validation. This aim focuses on the identification of broad-spectrum antiviral compound targets through chemoproteomic, genetic screening, and biochemical techniques.

Aim 2. Characterization of Broad-spectrum Antiviral Activities. We will characterize the phylogenetic breadth and potency of anti-respiratory viral activity in cell lines and primary cells.

Aim 3. Determining Cellular Response to Antiviral Treatment. Elucidating the impact of the antiviral compounds on cell physiology will allow us to further understand mechanism of action and identify critical PD markers to assess compound target engagement.

Role: Associate Director, Medicinal Chemistry

Overlap: None

[NEW]

Title: Optimization and Characterization of "MYC Degradors" for Pediatric Medulloblastoma

Grant #: R61 NS116191 (Savall/Wechsler-Reya)

Time Commitment: 0.96 calendar months

Supporting Agency: NIH/NINDS

Funding Agency Contact: Yvonne C. Talley, Grants Management Specialist, Phone: 301-496-7432, Email: talleyy@mail.nih.gov

Performance Period: 05/15/2020 – 04/30/2022

Funding Level: \$250,000 annual direct costs (\$150,000 Prebys Center directs)

Goals: The objective of this project is to validate our approach of identifying and optimizing a “MYC degrader” using a disease-relevant signature biomarker assay to inhibit progression of MYC-driven MB, with a long-term goal to develop novel therapies for treatment of G3 MB and other MYC-driven cancers.

Specific Aims:

R61 Phase Specific Aims.

Aim 1. Scale up synthesis and confirm purity, metabolic stability and solubility of current lead SBI1242.

Aim 2. Perform SAR studies around SBI1242 to optimize ADME/DMPK properties and BBB penetration.

Aim 3. Conduct PK studies on SBI1242 to determine best route of administration and in vivo BBB penetration.

Aim 4. Conduct PK/PD studies using SBI1242 (or an improved analog) in our orthotopic PDX mouse model in NSG mice, monitoring cellular MYC levels as a biomarker of activity.

R33 Phase Specific Aims.

Aim 1. Scale up synthesis and confirm purity, metabolic stability and solubility of optimized lead.

Aim 2. Perform in-vivo experiments evaluating toxicity & frequency of administration.

Aim 3. Perform in-vivo experiments evaluating effects on tumor growth and survival of G3 MB PDX mice.

Role: Principal Investigator

Overlap: None

[NEW]

Title: A Pharmacochaperone-based strategy for identifying chemical probes of brain-derived orphan GPCRs

Grant #: R01 MH123826 (Smith/Jackson)

Time Commitment: 0.60 calendar months

Supporting Agency: NIH/NIMH

Funding Agency Contact: Maggie C. Paolini, Grants Management Specialist, Phone: 301-443-2746, Email: maggie.paolini@nih.gov

Performance Period: 06/05/2020 – 03/31/2025

Funding Level: \$480,441 annual direct costs

Goals: The primary goal of this project is the identification and validation of small molecule probes that will enable the interrogation of orphan receptors and their role in brain function.

Specific Aims:

Aim 1. Develop a panel of assays of brain derived oGPCRs suitable for HTS screening.

Aim 2. Execute screens of the SBP compound collection to identify chemical probes of oGPCRs.

Aim 3. Verify structure, establish structure-activity-relationships, and determine physicochemical properties of probes.

Aim 4. Validate the utility of these novel tools in human iPSC-derived neurons.

Role: Co-Investigator

Overlap: None

[NEW]

Title: Mechanism of Action of a Novel Golgi-Targeted Anti-Cancer Agent

Grant #: R01 CA254397 (Field, Jackson)

Time Commitment: 1.44 calendar months

Supporting Agency: Case Western Reserve University/NCI

Funding Agency Contact: Joy Kearse, Grants Management Specialist, Phone: 301-631-2002, Email:

kearsej@mail.nih.gov

Performance Period: 09/01/2020 – 08/31/2025

Funding Level: \$234,596 annual direct costs

Goals: Our overall goal is to determine the mechanism of action of ML1377, including its direct target, and its potential to be developed into a cancer therapeutic. Our overall goal is to determine the mechanism of action of ML1377, including its direct target, and its potential to be developed into a cancer therapeutic.

Specific Aims:

AIM 1: Determine how ML1377 controls the MYO18A/F-actin interaction

AIM 2: Identify direct mechanistic target(s) of ML1377

AIM 3: Validate mechanistic targets of ML1377 as targets for cancer therapeutics

Role: Co-Investigator

Overlap: None

COMPLETED (since last reporting period)

Title: PHGDH Inhibitors

Grant #: 16X113-PHGDH (Moscat)

Time Commitment: 2.40 calendar months

Supporting Agency: The Chemical Biology Consortium - National Cancer Institute via Master Service Agreement w/ Leidos Biomedical Research, Inc.

Funding Agency Contact: Bonnie Beard, Senior Subcontracts Administrator, Email:

Bonnie.Beard@mail.nih.gov, Phone: 301-846-5169

Performance Period: 10/27/2017 – 08/30/2021

Funding Level: \$315,694 (\$203,833 Prebys Center)

Goals: The goal of this project is to identify potent inhibitors of PHGDH that possess diverse mechanisms of action with the ultimate goal of deriving a highly potent drug with differential activity against cancer cells.

Specific Aims: N/A

Role: Chemistry Lead

Overlap: None

PREVIOUS/CURRENT/PENDING SUPPORT

Layton H. Smith, Ph.D., Sanford Burnham Prebys Medical Discovery Institute

CURRENT

Title: Small molecule inhibitors of LMPTP: an obesity drug target

Grant #: R01 DK106233 (Bottini, Olson)

Time Commitment: 0.36 calendar months:

Supporting Agency: NIH/NIDDK

Funding Agency Contact: Christina Coriz, Grants Management Specialist, Phone: 301-594-8848, Email: corizc@niddk.nih.gov

Performance Period: 07/01/2015 – 05/31/2023

Funding Level: \$100,000

Goals: The goal of this project is to continue investigation on a promising candidate molecule for the treatment of diabetes. This work seeks to improve potency of the small molecular inhibitor, as well as test in vitro efficacy, in vitro ADME, in vivo PK, in vivo efficacy in mouse models, and preliminary clinical evaluations.

Specific Aims:

Aim 1. Preclinical validation of LMPTP inhibitor efficacy in human hepatocytes and in mouse models of obesity-induced diabetes.

Aim 2. Preclinical validation of LMPTP inhibitor safety in mice.

Aim 3. Generation of an optimized LMPTP inhibitor candidate.

Role: PD/PI

Overlap: None

Title: Probe Discovery for tRNA Methylation

Grant #: R01 GM126210 (Hou, Smith)

Time Commitment: 1.68 calendar months

Supporting Agency: Thomas Jefferson University/NIH

Funding Agency Contact: Miles Fabian, Program Official, email: fabianm@nigms.nih.gov

Performance Period: 06/01/2018 – 05/31/2021

Funding Level: \$86,587 annual direct costs

Goals: The objective of this project is to conduct HTS of the NIH compound collection with the goal of identifying compounds that inhibit tRNA methylation.

Specific Aims:

Aim 1. Use an enzyme-based fluorescence assay to isolate active inhibitors of EcTrmD.

Aim 2. Use cheminformatics to prioritize hits, and assess hits in a multitude of secondary assays to determine their inhibition potency and modality.

Aim 3. Screen hits with our whole-cell assays to isolate compounds that inhibit cell growth and display phenotypes specific to TrmD deficiency, including reduced drug efflux.

Role: PD/PI

Overlap: None

Title: Molecular Mechanism of Castration Resistant Prostate Cancer Recurrence and Therapeutic Strategies

Grant #: R01 CA227025 (Mahajan/Feng)

Time Commitment: 0.60 calendar months

Supporting Agency: Washington University School of Medicine/NIH

Funding Agency Contact: Lisa Gibbs, PTE Financial Contact, Washington University, Email:

WUAPScanner@wumail.wustl.edu; Phone: 314-935-5710

Performance Period: 12/14/2018 – 11/30/2023

Funding Level: \$13,120 annual direct costs

Goal: The overall objective of this proposal is to examine the role of ACK1/acK609-AR/ACK1 signaling loop in CRPC recurrence and to perform drug development, biomarker, and preclinical therapeutic studies necessary to credential (R)-9bMS and its potent derivative SG4-176 as a treatment approach, with the long-term goal of ultimately advancing this therapy to the clinic.

Specific Aims:

Not available. Per the statement of work: Dr. Smith will provide subject matter expertise in drug discovery and preclinical development. He will advise on the overall strategy for advancing R9B towards clinical development. Specifically, Dr. Smith will develop a critical path for advancing R9B to include Non-GLP and GLP studies with a long-term goal of enabling IND. To achieve this goal, he will recommend in vitro and in vivo studies to assess the pharmacological properties of R9B, interpret results, and advise on follow-up experiments. He will assist Dr. Mahajan in the design of toxicology studies and the interpretation of the results thereof. In addition, he will help draft manuscripts, reports, and new proposals that meet the overall objective of bringing R9B to clinical development.

Role: Subcontract PI

Overlap: None

Title: Targeting Neuroinflammation in AD with Novel CX3CR1 Agonists

Grant #: R01 AG062557 (Smith, Nash)

Time Commitment: 2.16 calendar months

Supporting Agency: NIH/NIA

Funding Agency Contact: Manda C. Richards, Grants Management Specialist, Email: manda.richards@nih.gov, Phone: 301-827-7977

Performance Period: 05/01/2019 – 02/29/2024

Funding Level: \$475,953 annual direct costs

Goals: The goal of this project is to develop orally bioavailable potent CX3CR1 agonist compounds suitable for use as chemical probes in the development of a novel drug target for the treatment of tau pathology, neuron atrophy, and neurodegeneration in Alzheimer's disease.

Specific Aims:

Aim 1. Establish and validate outcome measures for the lead-optimization campaign.

Aim 2. Determine the potency, efficacy, and selectivity of novel CX3CR1 agonist compounds in a battery of existing bioassays in vitro.

Aim 3. Measure the physicochemical and pharmacological properties of improved CX3CR1 agonists.

Aim 4. Execute efficacy studies in vivo using transgenic mouse models of AD, including rTg4510 and APP-PS1 mice.

Role: PD/PI

Overlap: None

Title: Development of SBI-553, an Allosteric Modulator of NTR1, for the Treatment of Substance Use Disorders

Grant #: UG3 DA050316 (Pinkerton, Caron, Barak, Guptill)

Time Commitment: 1.20 calendar months

Supporting Agency: NIH/NIDA

Funding Agency Contact: Amy Connolly, Grants Management Specialist, Email: connolla@mail.nih.gov, Phone: (301) 827-4457

Performance Period: 09/30/2019 – 08/31/2021

Funding Level: \$1,101,222 annual direct costs (\$615,493 Prebys Center annual directs)

Goals: The goal of this project is to develop SBI-553, a novel brain penetrant allosteric NTR1 modulator discovered through our previous NIH-sponsored grants as a treatment for Opioid Use Disorders.

Specific Aims:

Aim 1. Complete PK and preclinical safety profile of SBI-553 in a relevant non-rodent species

Aim 2. Identify backup compound to SBI-553

Aim 3. Conduct definitive cellular and in vivo pharmacology studies with SBI-553 (and/or potential backup) in models of addiction to inform best clinical indication and clinical endpoints (PK/PD).

Role: Co-Investigator

Overlap: None

[THIS GRANT]

Title: Development and Advancement of Broad-spectrum Respiratory Antivirals

Grant #: W81XWH-20-1-0270/ PR190558 (Chanda, Garcia-Sastre)

Time Commitment: 1.20 calendar months

Supporting Agency: US Department of Defense (w/Icahn School of Medicine at Mount Sinai)

Funding Agency Contact: Abigail Strock, Grants Officer. Email: Abigail.l.strock.civ@mail.mil. Phone: 301-619-2342

Performance Period: 04/15/2020 – 04/14/2024

Funding Level: \$2,276,645 annual direct costs (\$255,839 Prebys Center directs)

Goals: The primary goal of DABRA, is to develop 1-2 antivirals that can be transitioned to preclinical scale up and GLP/GMP studies, and secondarily, to advance a follow-on antiviral portfolio based on targeting host factors through a preclinical development pipeline.

Specific Aims:

Aim 1. Host Target Identification and Validation. This aim focuses on the identification of broad-spectrum antiviral compound targets through chemoproteomic, genetic screening, and biochemical techniques.

Aim 2. Characterization of Broad-spectrum Antiviral Activities. We will characterize the phylogenetic breadth and potency of anti-respiratory viral activity in cell lines and primary cells.

Aim 3. Determining Cellular Response to Antiviral Treatment. Elucidating the impact of the antiviral compounds on cell physiology will allow us to further understand mechanism of action and identify critical PD markers to assess compound target engagement.

Role: Executive Director, Preclinical Research

Overlap: None

Title: A Pharmacochaperone-based strategy for identifying chemical probes of brain-derived orphan GPCRs

Grant #: R01 MH123826 (Smith, Jackson)

Time Commitment: 1.80 calendar months

Supporting Agency: NIH/NIMH

Funding Agency Contact: Maggie C. Paolini, Grants Management Specialist. Email: maggie.paolini@nih.gov
Phone: 301-443-2746 Fax: 301-480-1956.

Performance Period: 06/05/2020 – 03/31/2025

Funding Level: \$480,441 annual direct costs (year 1 direct costs, year 2 NOA pending)

Goals: The primary goal of this project is the identification and validation of small molecule probes that will enable the interrogation of orphan receptors and their role in brain function.

Specific Aims:

Aim 1. Develop a panel of assays of brain derived oGPCRs suitable for HTS screening.

Aim 2. Execute screens of the SBP compound collection to identify chemical probes of oGPCRs.

Aim 3. Verify structure, establish structure-activity-relationships, and determine physicochemical properties of probes.

Aim 4. Validate the utility of these novel tools in human iPSC-derived neurons.

Role: Contact PD/PI

Overlap: None

Title: Mechanism of Action of a Novel Golgi-Targeted Anti-Cancer Agent

Grant #: R01 CA254397 (Field/Jackson)

Time Commitment: No effort until year 3

Supporting Agency: Case Western Reserve University/NIH

Funding Agency Contact: Joy Kears, Grants Management Specialist, Phone: 301-631-3002, Email: kearsej@mail.nih.gov

Performance Period: 09/01/2020 – 05/31/2025

Funding Level: \$226,197 annual direct costs, year 1

Goals: Through a phenotypic screen, we have identified a small molecule inhibitor of the GOLPH3 pathway that preferentially kills cancerous cells compared to untransformed cells. The goal of this project is to determine the mechanism of action of this compound to provide new insight into mechanisms of oncogenic transformation and enable hit-to-lead optimization for new cancer therapeutic agents.

Specific Aims:

AIM 1: Determine how ML1377 controls the MYO18A/F-actin interaction

AIM 2: Identify direct mechanistic target(s) of ML1377

AIM 3: Validate mechanistic targets of ML1377 as targets for cancer therapeutics

Role: Co-Investigator

Overlap: None

Title: Defining chromostasis - a candidate regulator of healthy aging and longevity

Grant #: R01 AG071464 (Adams)

Time Commitment: No effort requested until year 4

Supporting Agency: NIH/NIA

Funding Agency Contact: Terry Pham, Grants Management Specialist, Phone: 301-496-9666, Email: terry.pham@nih.gov

Performance Period: 09/11/2020 – 05/31/2025

Funding Level: \$458,256 annual direct costs (\$0 Prebys Center annual direct costs)

Goals: The objective of this project is to test the proposed hypothesis that cells possess mechanisms of chromatin homeostasis, or chromostasis, that preserve chromatin integrity, suppress phenotypic instability and so slow the pace of aging.

Specific Aims:

Aim 1. Manipulate chromostasis and assess the impact on phenotypic plasticity

Aim 2. Identify the genes involved in chromostasis

Aim 3. Test whether chromostasis promotes healthy aging and longevity

Role: Co-Investigator

Overlap: None

PENDING

Title: Clinically Viable Inhibitors of SARS-CoV-2 Cysteine Proteases

Grant #: R01 AI161359 (Smith, J., Jackson)

Time Commitment: 0.24 calendar months

Supporting Agency: NIH/NIAID

Funding Agency Contact: To be determined.

Performance Period: 09/01/2020 – 08/31/2024

Funding Level: \$2,821,036 annual direct costs (\$1,552,019 Prebys Center annual direct costs)

Goals: The objective of this project is to bring forward orally bioavailable inhibitors of the two CoV-processing proteases. We have assembled a team of experts in protease function, computer aided drug design, medicinal chemistry structural biology, and virology.

Specific Aims:

Aim 1. Develop potent and metabolically stable inhibitors of PLpro that can be advanced into preclinical development.

Aim 2. Develop potent and metabolically stable inhibitors of 3CLpro that can be advanced into preclinical development.

Aim 3. Test the efficacy of optimized inhibitors in the Syrian Hamster model of SARS-CoV-2 infection.

Role: Co-Investigator

Overlap: None

Title: High Specificity Inhibitors of ACK1/TNK2 as Novel therapeutics for Castration Resistant Prostate Cancer

Grant #: R01 CA267808 (Olson/ Mahajan)

Time Commitment: 0.60 calendar months

Supporting Agency: NIH

Funding Agency Contact: To be determined.

Performance Period: 09/01/2021 – 08/31/2026

Funding Level: \$576,650 (\$365,098 Prebys Center direct costs)

Goals: We previously identified a mechanism of therapeutic resistance in prostate cancer and developed a small molecule, (R)-9b, to suppress drug-resistant prostate tumor growth. The goal of this proposal is to optimize (R)-9b, while also identifying additional novel chemical matter as a backup to (R)-9b, and test the most promising drug candidates in cellular and animal models of resistant prostate cancer.

Specific Aims:

Aim 1. Design, synthesize and test novel ACK1 inhibitors.

Aim 2. Identify back-up compound by HTS.

Aim 3. Determine the physicochemical and pharmacological properties of optimized Ack1 inhibitors.

Aim 4. Apply selective and potent inhibitors of ACK1 to in vivo mouse models.

Role: PD/PI

Overlap: None

Title: Preclinical Discovery of Novel Farnesyltransferase Inhibitors for the Treatment of Alzheimer's Disease and Related Tauopathies

Grant #: R01 AG071694-01A1 (Olson)

Time Commitment: 0.24 calendar months

Supporting Agency: NIH

Funding Agency Contact: To be determined.

Performance Period: 09/01/2021 – 08/31/2025

Funding Level: \$617,284 (\$325,350 Preby Center direct costs)

Goals: The goal of this project is to develop a novel class of disease-modifying drugs for the treatment of AD that will ultimately increase tau degradation to prevent tangle formation.

Specific Aims:

Aim 1. Redesign known FTIs to identify BBB penetrant compounds.

Aim 2. Identify novel inhibitors of farnesyltransferase.

Aim 3. Lead optimization of farnesyltransferase inhibitors.

Role: PD/PI

Overlap: None

Title: Mechanisms of Protection from Noise-Induced Hearing Loss

Grant #: W81XWH-20-HRRP-FRA (Friedman/Hoelz)

Time Commitment: 0.12 calendar months

Supporting Agency: UCSD/Department of Defense

Funding Agency Contact: To be determined.

Performance Period: 10/01/2021 – 09/30/2023

Funding Level: \$31,103 Prebys Center direct costs

Goals: The goal of this proposal is to determine the molecular mechanistic basis for AMPK-dependent protection from noise induced hearing loss (NIHL) and establish the groundwork for targeted prevention and/or treatment of NIHL.

Specific Aims:

Aim 1. The identification of cellular factors associated with susceptibility to NIHL.

Aim 2. Structural and functional analysis of AMPK nucleoplasmic shuttling.

Aim 3. Identification of additional factors in the AMPK pathway leading to susceptibility to NIHL.

Role: Subcontract PD/PI

Overlap: None

PREVIOUS (last 5 years)

Title: Identification of Allosteric Ligands for Hepatic Nuclear Factor 4-alpha

Grant #: R01 DK097475 (Rastinejad)

Time Commitment: 0.60 calendar months

Supporting Agency: National Institutes of Health / National Institute of Diabetes and Digestive and Kidney Disease

Funding Agency Contact: Ronald N. Margolis, Ph.D., Bg 2DEM Rm 693A, 6707 Democracy Blvd, Bethesda, MD 20817; MargolisR@extra.niddk.nih.gov

Performance Period: 12/05/2012 – 05/31/2016

Funding Level: \$257,468 annual direct costs

Goals: The goal of this project was to identify potentiators of DNA binding that benefit certain MODY1 and HH disease populations where receptor-DNA binding is compromised by point mutations in HNF4.

Specific Aims:

Aim 1. We will conduct a MLPCN library screen that identifies molecules enhancing the DNA-binding affinity of the full-length HNF4 protein.

Aim 2. We will implement dose response studies, validate the “hits” with a counter-screen and a separate cell-based assay format, and optimize molecules using SAR by catalog and medicinal chemistry.

Aim 3. We will study the mechanism of action of our “hits” through structural studies, and test our probes for their ability to rescue the DNA binding loss associated with HNF4 disease-linked mutations.

Overlap: None

Title: Cellular Sentinels Toxicity Platform: Image-based toxicity screens of human stem cell-derived neurons, cardiomyocytes, and hepatocytes

Grant #: FA8650-13-2-6453 (Smith, Jackson)

Time Commitment: 1.20 calendar months

Supporting Agency: Department of the Air Force, 711th Human Performance

Funding Agency Contact: Mr. Charlie Dean, 711th HPW/XPH, (937)938-3174, charles.dean@wpafb.af.mil

Performance Period: 08/21/2013 – 11/20/2016

Funding Level: \$667,429 annual direct costs

Goals: The objective of this project was the development and validation of a suite of high-throughput, multiplexed assays of cellular phenotype and physiology for the purpose of assessing the potential toxicity of large collections of chemicals with unknown toxicological risk.

Specific Aims:

Step 1 Assay Development: Optimize cell production scale-up, plating parameters, assay performance, reliability and robustness statistics. Adapt to automation.

Step 2 Assay Validation: Validate assays using control compounds with known toxicity for each cell type. Generate “signatures” for compound class toxicity.

Step 3 Pilot Phase: Evaluate 50-500 compounds with unknown toxicity for each cell module, and compare ability to identify potential toxins based on established set of signatures of toxicity. Advance compounds identified as potentially toxic to Tier 2 cellular physiology assays.

Step 4 Translation Phase: Evaluate 500-5,000 compounds/yr with further development phase involving miniaturization to 1536 well format. Advance compounds identified as potentially toxic to Tier 2 cellular physiology assays.

Role: Co-PD/PI

Title: Identification of Rev-Erb alpha/beta nuclear receptor modulators

Grant #: R01 DK101520 (Rastinejad)

Time Commitment: 0.62 calendar months

Supporting Agency: NIH/DIDDK

Funding Agency Contact: Diana O'Donovan, Grants Management Specialist, Email: odonovand@extra.niddk.gov, Phone: 301-594-8868

Performance Period: 04/01/2014 – 03/31/2017

Funding Level: \$288,484 annual direct costs

Goals: The goal of this project was to conduct high throughput screening programs, secondary/confirmatory assays and gene expression studies to identify the most promising Ver-Erb alpha/beta modulators.

Specific Aims:

Aim 1. We will perform two MLPCN Library Screens that identify molecules that can alter the DNA-binding activities of full-length Rev-Erb alpha and Rev-Erb beta proteins.

Aim 2. We will implement dose response studies, validate all the “hits” using a counter-screen, employ a secondary cell-based transcription assay, confirm direct receptor binding, and optimize our most promising molecules using SAR by catalog and medicinal chemistry approaches.

Aim 3. We will Examine the effects of our molecules on Rev-Erb target genes, to identify those modulators with potentially therapeutic profiles for treating obesity, type II diabetes and hyperlipidemia.

Overlap: None

Title: A chemical biology strategy to validate the apelin receptor as a therapeutic target for diabetic retinopathy

Grant #: 2-SRA-2014-146-Q-R (Smith)

Time Commitment: 1.20 calendar months

Supporting Agency: Juvenile Diabetes Research Foundation

Funding Agency Contact: Tamara Siskind, JDRF Administrator; Email: tsiskind@jdrf.org

Performance Period: 05/01/2014 – 10/31/2016

Funding Level: \$238,636 annual direct costs (\$113,637 Prebys Center annual directs)

Goals: The objective of this program is to validate the apelin receptor (APJ) as a therapeutic target for the treatment of diabetic retinopathy (DR).

Specific Aims:

(1) Design and synthesize novel APJ antagonists;

(2) Test the potency, selectivity and efficacy of novel APJ antagonists in vitro;

(3) Execute proof-of-concept studies of novel APJ antagonists in the oxygen induced retinopathy (OIR) and branch vein occlusion (BVO) models of DR in mice.

Overlap: None

Title: Developing Disease-Modifying Chemical Probes to Inhibit Alpha Synuclein Aggregation

Grant #: MJFF 2014, 9224 (McLean, Smith)

Time Commitment: 1.20 calendar months / no minimum required effort

Supporting Agency: Mayo Clinic/Michael J Fox Foundation

Funding Agency Contact: Research Finance Chair, Susan Uhlenkamp, Mayo Clinic; Phone: 507-284-5773; email: uhlenkamp.susan@Mayo.edu

Performance Period: 05/01/2014 – 10/31/2016

Funding Level: \$143,370 annual direct costs

Goals: The objective of the program is to screen the NIH collection for drugs that inhibit alpha-synuclein aggregation and toxicity using a sensitive, cell-based assay that can monitor alpha-synuclein aggregation.

Specific Aims: N/A

Overlap: None

Title: Selective Inhibitors of Plasmodium falciparum G6PD as Novel Antimalarials

Grant #: R01 AI104916 (Pinkerton)

Time Commitment: 0.0 calendar, no minimum required effort

Supporting Agency: NIH/NIAID

Funding Agency Contact: Julie M. Bergerud, Grants Management Specialist, Email: waughj@mail.nih.gov,

Phone: (240)669-2967

Performance Period: 08/15/2014 – 07/31/2017

Funding Level: \$72,783 annual direct costs

Goals: The goal of the project was to develop PfGluPho inhibitors to kill the Plasmodium parasite and treat malaria.

Specific Aims:

Aim 1. Design and synthesize novel analogs of ML276 and ML304.

Aim 2. Test in vitro potency and selectivity, in cellulo activity and toxicity of ML276 and ML304 analogs and evaluate their physicochemical properties.

Aim 3. Determine pharmacokinetics (PK) and potential off-target effects.

Aim 4. Assess potential hemolytic risk in human RBCs and execute proof-of-concept studies of novel PfGluPho inhibitors in a malaria mouse model.

Overlap: None

Title: Lead optimization of brain penetrant Neurotensin Receptor 1 agonists

Grant #: R33 DA038019 (Pinkerton)

Time Commitment: 0.24 calendar months

Supporting Agency: NIH/NIDA

Funding Agency Contact: Amy Connolly; Email: connolla@mail.nih.gov; Phone: (301) 827-4457

Performance Period: 04/01/2015 – 06/30/2020

Funding Level: \$250,000 annual direct costs

Goals: The goal of this project is to provide an orally active, brain-penetrant preclinical NTR1 agonist aimed at Meth addiction treatment based on a lead series, developed through the NIH's MLPCN program, by performing preliminary SAR and a full lead optimization campaign.

Specific Aims:

R21 Phase Specific Aims:

Aim 1: Design and synthesize optimized NTR1 agonists that are orally bioavailable.

Aim 2: Assess the potency and selectivity of NTR1 agonists.

Aim 3: Evaluate novel NTR1 agonists using in vitro ADME/T and in vivo pharmacokinetic (PK) assays, including brain penetration.

R33 Phase Specific Aims:

Aim 1: Design and synthesize optimized NTR1 agonists that are orally active in vivo.

Aim 2: Assess the potency and selectivity of NTR1 agonists in relevant in vitro assays.

Aim 3: Evaluate novel NTR1 agonists using in vitro ADME/T and in vivo pharmacokinetic (PK) assays, including brain penetration.

Aim 4: Characterize lead NTR1 agonists in rodent models of drug addiction.

Overlap: None

Title: Small molecule discovery for GC-A activators

Grant #: R01 DK103850 (Burnett)

Time Commitment: 0.36 calendar months

Supporting Agency: Mayo Clinic/NIH

Funding Agency Contact: Christina Coriz, Grants Management Specialist, Email: corizc@niddk.nih.gov,
Phone: 301-594-8848

Performance Period: 07/01/2015 – 06/30/2018

Funding Level: \$92,767 annual direct costs

Goals: To identify small molecule GC-A agonists.

Specific Aims:

- (1) Perform the MLSMR Library Screen to identify molecules that can activate the GC-A receptor-signaling pathway.
- (2) Implement dose-response studies, validate all “Hits” using a counter-screen, employ secondary cell-based endogenously-expressing assays, confirm receptor signaling, and optimize our most promising molecules using SAR analog-by-catalogue, and medicinal chemistry approaches.
- (3) Establish the cGMP activating actions of our most promising GC-A activating candidates in cultured human vascular endothelial cells, vascular smooth muscle cells, glomerular mesangial cells, cardiac fibroblasts and adipocytes, all of which abundantly express the GC-A receptor.

Overlap: None

Title: Identifying CXCR4 Receptor Agonists to Improve Diabetic Healing

Grant #: R01 DK105010 (Liechty)

Time Commitment: 0.38 calendar months

Supporting Agency: NIH/NIDDK

Funding Agency Contact:

Performance Period: 06/15/2016 – 05/31/2019

Funding Level: \$158,046 annual direct costs

Goals: The goal is to expand the testing funnel and test a small library as proof of concept studies for a larger high throughput screen to identify CXCR4 agonists for diabetic wound healing.

Specific Aims:

- 1.0 Develop and optimize CXCR4 cell-based assay for HTS
- 2.0 Conduct a High Throughput Screen of the NCATS SMR Library
- 3.0 Validate hits from the NCATS SMR library in Concentration-response
- 4.0 Conduct Analog-by-catalog and minimal synthetic chemistry around scaffolds

Overlap: None

Title: Florida Translational Research Program at Sanford Burnham Prebys Medical Discovery Institute at Lake Nona

Grant #: COHK8 (Smith)

Time Commitment: No minimum required effort

Supporting Agency: State of Florida, Department of Health

Funding Agency Contact: Jennifer Drake, Government Analyst II, Florida Department of Health, Public Health Research Unit; Phone: 850-245-4444 x3927. Address: 4052 Bald Cypress Way, Tallahassee, Florida, 32399

Performance Period: 07/01/2016 – 06/30/2017

Funding Level: \$2,871,795 annual direct costs

Goals: The goal of the contract is to successfully complete the scope of work and related tasks.

Specific Aims: N/A.

Overlap: None

Title: Developing Disease-Modifying Chemical Probes to Inhibit Alpha Synuclein Aggregation

Grant #: 9224 (Smith, McLean)

Time Commitment: 0.36 calendar months

Supporting Agency: The Michael J. Fox Foundation (with Mayo Clinic)

Funding Agency Contact: Randall S. Jones, Operations Manager, Email: Jones.randall@mayo.edu, Phone: 507-284-4391

Performance Period: 04/01/2017 – 03/31/2018

Funding Level: \$90,000 annual direct costs

Goals: The goal of the project was to validate and execute a high-throughput screen of the SBP collection of small molecules to identify small molecules that inhibit the oligomerization of α -synuclein.

Specific Aims: Not applicable.

Overlap: None

Title: Targeting Transcription-Coupled DNA Supercoiling for Discovering Antibiotics against Bacterial DNA Gyrase

Grant #: R21 AI125973 (Leng)

Time Commitment: 0.6 calendar months calendar months

Supporting Agency: Florida International University/NIAID

Funding Agency Contact: Regnier A. Jurado, Director, Research Management Solutions, Florida International University, Email: juradora@fiu.edu; Phone: not available.

Performance Period: 07/21/2017 – 01/08/2020

Funding Level: \$75,662 annual direct costs

Goals: The objective of this project was to identify small molecule probes of prokaryotic DNA gyrase.

Specific Aims:

Aim 1. Develop a novel in vitro biochemical assay to screen inhibitors targeting bacterial DNA gyrase.

Aim 2. Screen the NCATS compound collection to identify bacterial DNA gyrase inhibitors.

Aim 3. Validate hits and identify DNA gyrase poisons using a newly developed cell-based method targeting TCDS.

Overlap: None

Title: Therapeutic Agonist of CX3CR1 for Alzheimer's Disease

Grant #: R21 AG055080 (Nash)

Time Commitment: 0.60 calendar months calendar months

Supporting Agency: University of South Florida/NIH

Funding Agency Contact: Jessica Perez, Grants Management Specialist, Email: perezj@nia.nih.gov, Phone: 301-496-1472

Performance Period: 03/01/2018 – 02/29/2020

Funding Level: \$74,691 annual direct costs

annual direct costs

Goals: The goal of this project was to identify small molecule agonists of the human Cx3CR1 receptor, which could then be used to investigate the hypothesis that a brain penetrant CX3CR1 agonist can reduce the disease progression in a mouse model of Alzheimer's disease.

Specific Aims:

(1) Conduct a high throughput screen of the NCATS compound collection

(2) Validate hits from the primary screen in concentration response

(3) Conduct analog-by-catalogue and synthetic chemistry around chemical scaffolds

Overlap: None

Title: Identification of Hypoxia-Inducible Factor-2alpha Activators for Chronic Kidney Disease Anemia

Grant #: R01 DK118297 (Rastinejad)

Time Commitment: 0.60 calendar months

Supporting Agency: NIH

Funding Agency Contact: Carolyn Kofa, Grants Management Specialist, Phone: (301) 594-7687, Email: kofac@extra.niddk.nih.gov

Performance Period: 06/01/2018 – 04/30/2022 (Award closed out 08/09/2018 due to the departure of the PD/PI)

Funding Level: \$355,792 original annual direct costs; \$57,746 revised annual direct costs

Goals: The objective of this program is to find first-in-class HIF-2alpha/ARNT activators that can potentially benefit CKD anemia patients.

Specific Aims:

- (1) Using the NIH MLPCN chemical library, employ a high-throughput biochemical screen to find small molecules that stabilize the HIF-2alpha/ARNT protein heterodimer, a requirement for HIF-2alpha function activity as a transcription factor
- (2) Conduct comprehensive biochemical and cell-based assays to validate and prioritize the most promising activators
- (3) Carry out hit-to-lead optimization, together with pharmacological and mechanistic studies to accelerate the path toward preclinical development

Overlap: None

AWARD NUMBER: W81XWH-20-1-0270

TITLE: Development and Advancement of Respiratory Antivirals

PRINCIPAL INVESTIGATOR: Hong M. Moulton

CONTRACTING ORGANIZATION: Sumit K Chanda, Ph.D.

REPORT DATE: April 27, 2021

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: Choose Distribution Statement A or B. (Reference https://mrdc.amedd.army.mil/index.cfm/resources/researcher_resources/reporting/technical for additional information.)

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Dr. Moulton's laboratory at Oregon State university will be design and synthesize all peptide-conjugated morpholino oligomers (PPMOs) for the proposal. PPMOs will be used to modulate expression of selected genes in vitro and in vivo.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

PPMO, Antisense oligonucleotides, Morpholino oligomers

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

For each gene target, one to two PPMO sequences will be designed, synthesized, purified, analyzed in Dr. Moulton's lab. In addition, the lab is responsible for QC & QA of all the PPMOs to be used for the project. Furthermore, Moulton Lab will provide the expertise for the use of PPMOs in proposed experiments carried out by the consortium. Moulton lab will work with the consortium in designing cell culture and animal experiments, in analyzing the results, troubleshooting problems and writing reports for NIH and manuscripts for publications.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

We have our PPMO design strategies in place and have assembled and tested all the reagents and equipment necessary to synthesize and purify PPMO. As part of our Quality Control measures for post-synthesis PPMO, we have made arrangements for use of the Mass Spec facility at Oregon State University and put several PPMO through the process there. As a reagent for the DoD program, we have prepared a negative control PPMO, which could be shipped at any time.

We have optimized a few key parameters of a chemical reaction that links the starting materials PMO and peptide. We have found that an undesired side-product can be minimized by reducing reaction time from 90 to 30 minutes and temperature from 45 to 25 °C. We now can produce higher quality PPMO with an improved protocol.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

We mentored and trained an undergraduate Honors student majored in Chemical Engineering. A part of the accomplishments listed above is a part of his Honors research thesis.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We are prepared to design, synthesize, purify and quality-control custom-PPMOs upon request for this project.

- 4. IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The improved method is expected to generate higher quality of PPMO products for the project.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The improved method has been already used for producing higher quality of PPMOs for other research communities in the fields of genetic and infectious diseases.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Research conditions resulting from COVID-19 have delayed the requests for PPMO production from other centers.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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

No changes.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a*

periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

With the improvement we have made in our synthesis method, we now can produce higher quality PPMOs.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

Name: Mary Smith

Project Role: Graduate Student

Researcher Identifier (e.g. ORCID ID): 1234567

Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.

Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name: Hong Moulton

Project Role: PD

Nearest person month worked: 1

Contribution to Project: Dr. Moulton has coordinated with Dr. Sumit's team and OSU to set up the administrative aspects of the grant, participated in the monthly meeting, secured supplies and personnel required, supervising personnel for PPMO method development, synthesis and analysis.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

See attached below.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other. S*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ebrap.org/eBRAP/public/index.htm> for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil/Pages/Resources.aspx>) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

PREVIOUS

1. U19AI106754 (PI: Adolfo Garcia-Sastre) 06/26/2013 - 05/31/2018 4.0 CM
(OSU total cost)

Title: Multiscale analysis of influenza host-pathogen interactions: Fluomics

Project goal: To generate predictive models of influenza virus pathogenesis which will a) allow us to identify biomarkers for predicting pathogenic potential of new influenza viruses, b) give us the ability to predict which populations may be more susceptible to disease based on genetic variability, and c) provide avenues to explore for new, host-directed, therapeutic interventions.

Specific Aims: 1) analyze and integrate global responses during infection with three pathogenically diverse influenza viruses, 2) analyze the impact of selected perturbations in global response outcomes using a targeted – OMICS approach, and 3) validate the impact of key host factors in virus replication, host responses and disease.

Grants Officer: Cheryl Wall, Phone: 240-669-2956, Email: wallcy@niaid.nih.gov

Overlap: No scientific or budgetary overlap.

2. 1R41AI122462-01 (PI: Moulton and Amanna) 08/01/2017 – 07/31/2018 3.8 CM
NIH/NIAID Total Cost:

Title: Development of a novel platform to enhance intracellular bioavailability of antisense morpholino oligomers

Project goal: To test an advanced PPMO approach using our established *in vitro* dengue virus model system, with the goal of validating our novel approach through the creation of an anti-dengue virus PPMO with a significantly improved selective index profile compared to current PPMO

Specific Aims: Synthesize novel PPMO containing endosomolytic viral fusion peptides and test in a dengue virus model system

Grants Management Specialist: Tseday G Girma, Phone: 240-669-5896, Email: tseday.girma@nih.gov

Overlap: No scientific or budgetary overlap.

CURRENT

- 1.U19AI135972 (PI: Garcia-Sastre,Adolfo) 02/01/2018 – 12/31/2022 1.4 CM
(OSU total cost)

Title: Fluomics: The Next Generation

The goal of this project is: To obtain multiple OMICS-based systems level measurements and integrate them using modeling approaches and machine learning algorithms to identify and validate 1) host-virus networks that modulate influenza A virus disease severity, 2) biomarkers in blood that reflect the activation states of these networks and 3) novel host targets for therapeutic interventions.

Specific Aims: To analyze and integrate global responses during infection with influenza viruses resulting in different disease outcomes, and identify networks associated with resilient and pathogenic outcomes. 2) To identify blood biomarkers associated with resilient and pathogenic outcomes. 3) To identify and validate key drivers as therapeutic targets of disease.

Grants Management Specialist: Caleb D. Waller, (301)761-6643, caleb.waller@nih.gov

Role: Co-I

Overlap: No scientific or budgetary overlap.

2. W81XWH-20-1-0270 (PI: Chanda, S) 04/15/2020-04/14/2024 1.4 CM
(OSU total cost)

Title: Fluomics: The Next Generation

The goal of this project is: To obtain multiple OMICS-based systems level measurements and integrate them using modeling approaches and machine learning algorithms to identify and validate 1) host-virus networks that modulate influenza A virus disease severity, 2) biomarkers in blood that reflect the activation states of these networks and 3) novel host targets for therapeutic interventions.

Specific Aims: To analyze and integrate global responses during infection with influenza viruses resulting in different disease outcomes, and identify networks associated with resilient and pathogenic outcomes. 2) To identify blood biomarkers associated with resilient and pathogenic outcomes. 3) To identify and validate key drivers as therapeutic targets of disease.

GMS: Joshua L. Disbennett, (301)-619-7349, Joshua.l.disbennett.civ@mail.mil

Role: Project PI

Overlap: No scientific or budgetary overlap.

3. 2 R44 TR 002692 – 02 (PI: Juliano, R)

07/01/20-06/30/22

2.4 CM

(OSU total cost)

Title: Therapy of Pulmonary Diseases with Peptide-Oligonucleotide Conjugates and Small Molecule Enhancing Compounds

Project goal: This proposal describes a novel technology to facilitate the therapeutic use of oligonucleotides by improving their intracellular delivery. We will use a special type of oligonucleotide called a P-PMO in combination with a small molecule termed an OEC. We will use the P-PMO+OEC combination to correct an RNA splicing defect in certain patients with cystic fibrosis. We will also use this approach to reduce over-production of mucus that is a problem in many pulmonary diseases.

Specific Aims: 1) Optimization of the P-PMO/SSO+OEC Combination. 2) Pharmacodynamics of the P-PMO/SSO+OEC Combination. 3) Pre-clinical Safety Evaluation. 4) Development of P-PMO/SSO+OEC Combinations for Correction of CFTR Splicing Mutations. 5) Development of P-PMO/SSO+OEC Combinations for Modulation of Mucin Hypersecretion.

Grants Management Specialist: Artisha Wright, (301)435-0845, artisha.wright@nih.gov

Role: Project Principal Investigator

Overlap: No scientific or budgetary overlap.

PENDING

1U3RAI156433-01 (PI: Moulton, H)

05/2021 – 04/2023

1.2 CM

Total cost:

Title: Evaluation of a PPMO Inhibitor of Lassa Virus Infection and Disease in a Guinea Pig Model

Project goal: To evaluate if PPMO targeting highly conserved sequences in Lassa virus (LASV) will inhibit the LASV replication and pathogenesis in a guinea pig model.

Specific Aims: 1) Identification of an effective PPMO for targeting LASV virus, 2) Evaluation of the toxicity and antiviral efficacy of a candidate PPMO in a guinea pig model of LASV disease

Specific Aims:

Grants Management Specialist: Roberta Wolcott, (240)669-2964, Wolcottr@niaid.nih.gov

Role: PI

Overlap: No scientific or budgetary overlap.

AWARD NUMBER: W81XWH-20-1-0270

TITLE: Development and Advancement of Respiratory Antivirals

PRINCIPAL INVESTIGATOR: Michael Bollong

CONTRACTING ORGANIZATION: Sumit K Chanda, Ph.D.

REPORT DATE:

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: Choose Distribution Statement A or B. (Reference https://mrdc.amedd.army.mil/index.cfm/resources/researcher_resources/reporting/technical for additional information.)

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE	<i>Form Approved</i> <i>OMB No. 0704-0188</i>
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

In the target identification group led by Michael Bollong, the identity of host proteins targeted by novel antiviral lead molecules will be identified and biophysical and genetic methods will be used to characterize their antiviral mechanism.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Pharmacological pan-antiviral; Target identification; chemical proteomics

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

- Probe molecules will be synthesized and evaluated for activity
- Host protein targets will be identified using an established MS/MS-enabled chemo-proteomics pipeline.
- Putative protein targets will be validated using classical genetics methods (e.g., overexpression, shRNA-mediated knockdown),
- biophysical characterization of target proteins (e.g., CETSA, SPR, *in vitro* crosslinking)

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

- 1) *Major activities:* Dr. Bollong evaluated existing structure activity relationship of the M4 antiviral scaffold and has evaluated the literature from similar to chemical matter, generating facile synthetic routes to the core scaffold. Using recently established data from Chanda and team, he has designed a series of readily achievable photo-activatable affinity probes. He and his team have strategically mapped a number of synthetic routes for the chemical synthesis of photo-activatable probes and prioritized their efforts based on perceived likelihood of probe activity and synthetic complexity. He is working with the SBP chemistry team to design and synthesize these molecules.
- 2) *Specific objectives:* Aid in the design and synthesis of photo-activatable affinity probe small molecules.
- 3) *Significant results:* To date, Bollong team have generated the above synthetic routes. Have generated a protocol for evaluating the covalent interaction of the M4 scaffold with glutathione.
- 4) *Other achievements:* There are no other achievements to report.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In the next reporting period, Bollong and SBP chemistry team will synthesize 2-4 photo-activatable affinity probe molecules. The team will also synthesize the M4 parent lead compound to ensure reproducibility of synthesis and ensure purity during preparative purification steps. The initial in situ fluorescent labeling of cells with target identification probes will be performed to assess their selectivity and determine which method of target identification will be employed or if additional probe molecules will need to be synthesized. M4 and related analogs will be evaluated for reactivity with GSH to understand potential covalent reactivity with putative targets.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

that

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Books or other non-periodical, one-time publications. Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report

Other publications, conference papers and presentations. Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Nothing to report

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.

Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name: Michael J. Bollong
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): orcid.org/0000-0001-9439-1476
Nearest person month worked: 1
Contribution to Project: Dr. Bollong designed photo-activatable affinity probes to be used in this project and has procured reagents to begin synthetic and biological work.

Name: Edyta M. Grzelak
Project Role: Postdoctoral associate
Researcher Identifier (e.g. ORCID ID): orcid.org/0000-0002-3033-2917
Nearest person month worked: 1.5
Contribution to Project: Dr. Grzelak has helped design analogs and generate methodology to evaluate GSH reactivity with M4 series.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

See other support below.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ebrap.org/eBRAP/public/index.htm> for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil/Pages/Resources.aspx>) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

OTHER SUPPORT

SCRIPPS RESEARCH

Michael Bollong

CURRENT:

PR190558 (Chanda) 12/01/2020-11/30/2024 0.60 calendar
DoD

Development and Advancement of Broad-Spectrum Respiratory Antivirals

In the target identification group led by Michael Bollong, we will determine the identity of host proteins targeted by novel antiviral lead molecules and use biophysical and genetic methods to characterize their antiviral mechanism. Probe molecules will be synthesized by the Chatterjee group and protein targets identified using an established MS/MS-enabled chemo-proteomics pipeline. Putative protein targets will be validated using classical genetics methods (e.g., overexpression, shRNA-mediated knockdown), biophysical characterization of target proteins (e.g., CETSA, SPR, *in vitro* crosslinking), and biochemical/functional characterization (e.g., enzymatic assays) where possible. Our goal will be to demonstrate a correlation between cellular antiviral activity and target engagement across analogs in a chemical series. Successful completion of the proposed work will yield both biophysical- and genetics-based evidence of the proteins responsible for host-mediated antiviral activity of lead molecules.

PENDING:

R21ES033772 09/01/2021 – 08/31/2023 2.00 calendar
NIH

"Pharmacological Activation of Metal-responsive Transcription Factor 1 (MTF-1)"

Small molecule drugs that activate metal-responsive transcription factor-1 (MTF-1) have the potential to transform the treatment of individuals exposed to heavy metals as well as provide new treatment options for various diseases in which oxidative stress is a damaging component. At present, no chemical tools exist to modulate the transcriptional activity of MTF-1 and therapeutic options for diseases with metal-associated pathologies are limited. The objective of this project is to map the druggable space of the MTF-1 pathway. Using unbiased, high throughput, cell-based chemical screening we will identify additional, non-toxic chemical matter for activating MTF-1 and use road tested target identification experiments to elucidate their associated mechanism(s) of action, thereby providing new chemical matter and druggable targets to activate this underexplored transcriptional response.

No overlap.

PREVIOUS:

None

AWARD NUMBER: W81XWH-20-1-0270

TITLE: Development and Advancement of Respiratory Antivirals

PRINCIPAL INVESTIGATOR: Megan Shaw, Ph.D.

CONTRACTING ORGANIZATION: Sumit K Chanda, Ph.D.

REPORT DATE: 30 April 2021

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: Choose Distribution Statement A or B. (Reference https://mrdc.amedd.army.mil/index.cfm/resources/researcher_resources/reporting/technical for additional information.)

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

DABRA aims to develop broad-spectrum respiratory antivirals that act by targeting cellular factors. Dr Shaw participates in Project 1 which focuses on the M4 and M85 compounds series, and aims to characterize their antiviral properties and cellular targets.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Antiviral drug, host target, broad-spectrum, respiratory virus

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Dr. Shaw serves as a co-investigator on this proposal and participates in Project 1. Specifically, she is involved in the following aspects:

- 1) Genetic and biochemical target validation of host-directed compounds
- 2) Characterizing antiviral mechanisms for host-directed compounds
- 3) Characterizing the cellular actions of host-directed antiviral compounds
- 4) Determining the resistance profiles of host-directed antiviral compounds
- 5) Providing overall guidance to all aspects of Project 1 based on her prior experience.

Milestones:

Life-cycle mapping of M85: Complete (100%)

Life-cycle mapping of M4: Delayed

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities

- Mechanism of action studies for compounds in Project 1 (M85 and M4)
- Development of reagents and assays to allow for antiviral assays with SARS-CoV-2

2) Specific objectives

- Life cycle mapping and mechanism of action studies on M85
- Isolation and growth of SARS-CoV-2 wild type and B1.351 (South African variant) viruses
- Development of SARS-CoV-2 entry assay for use in mechanism of action studies

3) Key outcomes

- Life-cycle mapping of M85 has been accomplished. In summary the data show that:
 - 1) M85 inhibits the entry stage of the influenza virus life-cycle
 - 2) M85 specifically targets the endocytosis of virus particles
 - 3) M85 does not block endosomal acidification
 - 4) Immunofluorescence shows that M85 traps virus close to the cell surface, potentially indicating a block in formation of endocytotic pits, endocytotic vesicles, or early endosome trafficking.
- Both WT and B1.351 SARS-CoV-2 have been isolated, cultured, and titered.
- The spike proteins of WT and B1.351 SARS-Co-2 have been cloned for the purposes of developing an entry assay

4) other achievements

- Dr. Shaw has provided expert advice on interpretation of antiviral assays
- Equipment for expanding cell culture abilities on the project has been purchased
- A BSc(Hons) student working on the project graduated in December 2020.
- A PhD student has been recruited for the project and began in March 2021

Life-cycle mapping of M4 has been delayed due to the impact of COVID on the ability to recruit students in 2020, and the closure of university campus for 4 months. The recruitment of a PhD student in March 2021 will help to address this.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Training in molecular virology and cell culture was provided to an undergraduate student.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

- 1) Begin characterization of M4 mechanism of action by performing life cycle mapping (revised deadline is Oct 2021)
- 2) Generate SARS-CoV-2 pseudoparticles for use in entry assays.
- 3) Participate in discussions with the Project team to evaluate the latest data

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Due to the emergence of SARS-CoV-2, this virus has been added to the respiratory viruses of interest. For this reason we are establishing live SARS-CoV-2 replication assays and developing specific SARS-CoV-2 entry assays for examining the activity of antiviral compounds

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The COVID pandemic has had a significant impact on the progress of this project. When the contract was awarded, South Africa was in the highest level of lockdown and there was no access to University campus until the end of July 2020. Since then, necessary permits have been obtained and researchers have been allowed on campus according to capacity regulations. The most significant impact on our work is the procurement of supplies and equipment which are all sourced from international suppliers. Recruitment of personnel was particularly difficult during this time and this caused a delay in the initiation of work on the M4 small molecule. This issue has been resolved with the recruitment of a graduate student in March 2021

Uncertainty still remains as to the situation with the COVID-19 pandemic and the impact this may have on our ability to operate at 100%. Vaccine rollout has yet to begin in South Africa which means that we are anticipating a 3rd wave of infections in the coming months.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

The following impacted expenditures:

- 1) Closure of University campus for 4 months during the COVID pandemic
- 2) Delay in recruitment of graduate student due to COVID pandemic

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

All work with live SARS-CoV-2 is performed in a BSL3 laboratory by trained personnel

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

O'Hanlon R, Leyva-Grado VH, Sourisseau M, Evans MJ, Shaw ML. An Influenza Virus Entry Inhibitor Targets Class II PI3 Kinase and Synergizes with Oseltamivir. ACS Infect Dis. 2019 Oct 11;5(10):1779-1793.

Published

Acknowledgement of federal support: No (prior to initiation of award)

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*

- *new business creation; and*
- *other.*

Titered, and sequenced, stocks of WT and B1.351 SARS-CoV-2.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

*Name: Mary Smith
 Project Role: Graduate Student
 Researcher Identifier (e.g. ORCID ID): 1234567
 Nearest person month worked: 5
 Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
 Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Megan Shaw
 Project Role: PI
 Researcher Identifier: 0000-0002-1267-0891 (ORCID)
 Nearest person month worked: 1.2
 Contribution to Project: Consultant; supervisor; antiviral mechanism of action studies

Name: Tasnim Suliman
 Project Role: Postdoctoral Fellow
 Nearest person month worked: 4.0
 Contribution to Project: SARS-CoV-2 isolation; antiviral assays, supervision of undergrad student

Name: Ame Daniels
 Project Role: Undergraduate student
 Nearest person month worked: 3.0
 Contribution to Project: cloning of SARS-CoV-2 spike

Name: Jihaan Adonis
 Project Role: Graduate student
 Nearest person month worked: 1.0
 Contribution to Project: joined in March 2021. Will focus on M4 antiviral studies

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

New active grant:

CURRENT

SAMRC (co-PIs: PREISER/SHAW) 03/23/2021 – 05/31/2022 1.2
CM

200,000 ZAR to SHAW

“Replication kinetics of SARS-CoV-2 variant 501Y.V2 and quantification of viral loads”

The overall goal is to determine whether SARS-CoV-2 variants (including 501Y.V2 or other variants yet to emerge) display differences in replication kinetics in vitro that may drive altered viral loads and clinical characteristics in vivo.

Aims:

- 1) To compare the replication kinetics of SARS-CoV-2 501Y.V2 with earlier variants in South Africa (and any other variants).
- 2) To quantify RNA viral loads over the course of SARS-CoV-2 infection and correlate with infectious virus titers.

Grants Management Specialist: Rizwana Mia, email: Rizwana.Mia@mrc.ac.za

Role: co-PI

Overlap: No scientific or budgetary overlap.

Closure of previously active grant:

HHSN272201400008C GARCIA-SASTRE (PI) 04/01/2019 – 03/31/2021
NIH/NIAID

“NIAID Centers of Excellence for Influenza Research and Surveillance (CEIRS)”

The goal of this study is to perform surveillance of influenza viruses in South African children with pulmonary tuberculosis and investigate influenza virus sequence diversity in this population.

My role: PI of subcontract

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

University of Stellenbosch, Tygerberg medical campus

Cape Town, South Africa

Access to BSL3 facility that allows for project staff to work with live SARS-CoV-2

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ebrap.org/eBRAP/public/index.htm> for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil/Pages/Resources.aspx>) should be updated and submitted with attachments.*

- 9. APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*