AWARD NUMBER: W81XWH-19-1-0100

TITLE: Broad-Spectrum Inhibitors of Future Emerging Viruses

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Covington, LA

REPORT DATE: March 2021

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
March 2021	Annual	01Mar2020-28Feb2021

4. TITLE AND SUBTITLE
Broad-Spectrum Inhibitors of Future Emerging Viruses

5a. CONTRACT NUMBER
W81XWH-19-1-0100

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
0011421640

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Tulane National Primate Research Center
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8. PERFORMING ORGANIZATION REPORT NUMBER

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

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

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The goal of our project is to implement a strategy for proactive acquisition of broad-spectrum antivirals against emerging viral pathogens prior to their entry into human populations during outbreaks. If successful, our longer-term goal is to develop broad-spectrum antiviral drugs that can be made available to military and medical personnel at risk of infection during deployment to affected geographical locales. The project addresses three of the FY18 PRMRP Topic Areas. "Emerging infectious diseases" is addressed directly, "vaccine development for infectious diseases" is addressed in spirit though our technology is not a vaccine and, as this project starts by consideration of the flavivirus family, "Guillain-Barre' Syndrome" is relevant since neurotropic species of the virus family are causally associated with GBS.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	8	19b. TELEPHONE NUMBER *(include area code)*

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REPORT OUTLINE

1. **INTRODUCTION:**

Emerging zoonotic viruses will continue to pose a risk to human populations for the foreseeable future. Among the RNA viruses causing human outbreaks/epidemics/pandemics over the past fifty years have come from 20 RNA virus families. Examples of specific virus species and their families include SARS *coronavirus*, Ebola *filovirus*, Chikungunya *togavirus*, Lassa fever *arenavirus*, Zika *flavivirus*, Sin nombre *bunyavirus*, and Nipah *paramyxovirus*. The *flavivirus* family contains more than 50 arthropod-borne virus (arbovirus) species including Dengue (DENV), Zika (ZIKV), West Nile (WNV), Japanese encephalitis (JEV), Yellow fever (YFV), St. Louis encephalitis (SLEV), Powassan (POWV), and Omsk hemorrhagic fever viruses (OHFV). **The goal of our Discovery project was to target the flaviviruses for identifying broad-spectrum antiviral compounds for use against constellations of known, as yet unknown viruses, as well as viruses that will arise in the future through evolution**. Our initial approach has been to use innovative artificial intelligence (AI)-based molecule screening (AtomNet) against an aligned 3D protein set from multiple related flaviviruses. We hypothesized that this would facilitate the identification of small molecule hits that would be effective in blocking the replication of a spectrum of related viruses within the family. While outside the scope of the Discovery project, we proposed that one or more follow up projects would enable development, procurement, and availability broad-spectrum antivirals to military and medical personnel at risk of infection before future viral outbreaks occur.

2. **KEYWORDS:**

Emerging virus, RNA virus, flavivirus, broad-spectrum small molecule inhibitor

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

Major goals of the project were to carry out parallel small molecule screening against the conserved flavivirus NS3 protease (Major Task 1), to empirically screen these AI-generated hits using an enzymatic NS3 protease assay (Major Task 2), and to identify a set of potential small molecule inhibitors. If successful, we would then use AI to define effective chemical scaffolds, carry out a second round of screening, and then evaluate efficacy against a set of diverse species in the flavivirus family (Major Tasks 3 and 4).

- **What was accomplished under these goals?**

This past year has been challenging due to the COVID-19 pandemic. The Tulane National Primate Research Center (TNPRC), where the work of the current project is being carried out, was charged with developing tractable NHP models of SARS-CoV-2 induced pathogenesis to be used for evaluation of COVID-19-related antiviral therapeutics and vaccine assessment. The faculty expert in virology, including me, were expected to contribute substantively in time and effort to this goal. Moreover, BSL-2 laboratory access was constrained due to mandated social distancing protocols in BSL-2 containment. A synopsis of the project and progress is provided below.

Comparison of 3D structures of the active site of NS3 from multiple flavivirus species revealed regions of higher order variation and commonality around the active site of the enzyme.

For example, figure 1 compares the structure of the NS3 active site for two of the flaviviruses, and parallel analysis was carried out for additional active sites from multiple flaviviruses.

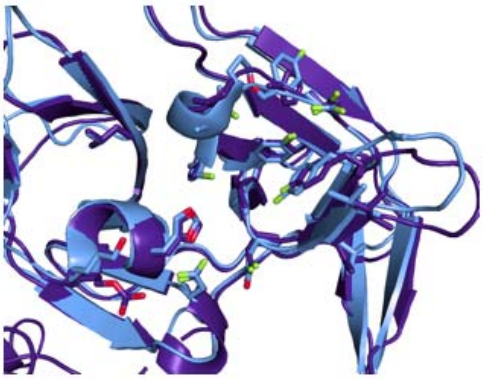

Fig. 1. Similarity in the NS3 active sites of two flaviviruses.

We used AI to screen the potential interaction between a small molecule library composed of about 8 million molecules with the NS3 active site of several flaviviruses and then empirically determined efficacy in an enzymatic assay for NS3 protease activity. The reaction contained recombinant WNV NS3 peptide, and a fluorescence resonance energy transfer (FRET) peptide cleavable by serine proteases including NS3. Thus, NS3 activity was monitored by cleavage, dequenching, and fluorescence detection (Fig. 2). Approximately 200 compounds were screened in the assay. Two of the molecules initially appeared to inhibit NS3 PR activity, with further investigation indicating that 2 compounds had IC50s of about 50 uM and 2 uM. Unfortunately, subsequent data indicated that both compounds likely have an intrinsic fluorescence quenching effect, which caused them to score as false positives. Consequently, we are expanding the library further and will use our highly tractable cleavage assay to evaluate additional molecules. It should be noted that the AI approach that we are using (AtomNet) has been used in more than 600 independent projects with a success rate of about 75%. Thus, we think it worthwhile to carry out additional screening.

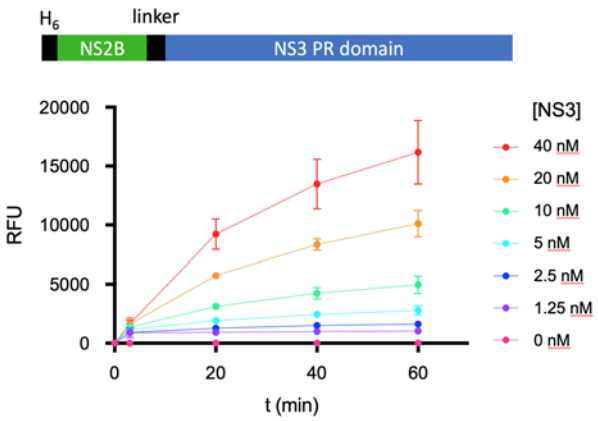

Fig. 2. NS3 protease assay NS3 dependence. WNV NS3 PR was expressed with an N-terminal his6 tag, a 47 a.a. peptide from NS2B, and a 10 a.a. linker. The standard NS3 assay contained 20 uM FRET substrate, L-Pyro-GRTKR-AMC (pERTKR-AMC) and increasing amounts of recombinant WNV NS3 as indicated. Substrate cleavage was monitored by fluorescence (excitation=380 nm, emission=460 nm) over 60 min. The standard reaction for screening the compound library contained 20 nM NS3. The Z' value of the standard reaction = 0.72.

Several of the ER membrane complex (EMC) proteins have recently been identified as host factors required for the replication of multiple flaviviruses. The EMC is required for correct insertion of membrane-associated proteins with intracellular membranes. It is reasonable to posit that the EMC is required for correct association of the flavivirus polyprotein. Flavivirus replication takes place in association with intracellular membranes (Fig. 3) and this association is important for the

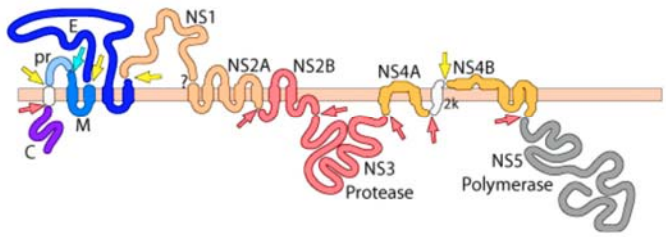

Fig. 3. Membrane-associated precursor protein. Pink arrows indicate sites cleaved by NS2B/NS3 protease. We posit that the EMC is required for correct membrane insertion.

correct function of multiple viral proteins including NS3. We would also like to carry out a parallel complementary AI-based approach to identify an inhibitor of one of the EMC components, EMC8, as this may yield another type of broad-spectrum inhibitor of the flaviviruses.

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

Nothing to report

- **How were the results disseminated to communities of interest?**

Nothing to report

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

As described above, we will screen additional small molecules in the NS3 PR assay. In addition, we would like to carry out a complementary AI screen against a potential broad-spectrum target for the flavivirus family.

4. **IMPACT:**
   - **impact on the development of the principal discipline(s) of the project?**

Nothing to report

- **What was the impact on other disciplines?**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Nothing to report

5. **CHANGES/PROBLEMS:**

Our main goal, to use AI to identify broad-spectrum inhibitors that are effective in blocking the replication of a constellation of species in the flavivirus family, remain unchanged. As described previously, we will use our initial strategy to attempt identification of a small molecule inhibitor of NS3 protease.

- **Changes in approach and reasons for change**

In addition to our original approach, we seek to try a complementary approach that targets a protein required for the replication of many flaviviruses. Since our original approach has so far proved to be unsuccessful we would like to try using AI to target a protein required for correct NS3 function in the natural context of membrane association. We will seek discussion and agency approval for this part of the project.

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

We do not anticipate further delays in the project due to COVID-19 or other factors.

- **Changes that had a significant impact on expenditures**

Nothing to report

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

Not applicable

- **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**

Not applicable

6. **PRODUCTS:**
   - **Publications, conference papers, and presentations**

Nothing to report

     - **Journal publications.**

Nothing to report

     - **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)**

Nothing to report

   - **Technologies or techniques**

Nothing to report

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

Nothing to report

   - **Other Products**

Nothing to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   - **What individuals have worked on the project?**

Antonito Panganiban, no change

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

Nothing to report

- **What other organizations were involved as partners?**

Nothing to report

8. **SPECIAL REPORTING REQUIREMENTS**

Not applicable

9. **APPENDICES:**

none