AWARD NUMBER: W81XWH-19-1-0027

TITLE: “DEVELOPMENT OF A RECOMBINANT VSV-BASED VACCINE FOR LASSA FEVER”

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14. ABSTRACT

Lassa virus (LASV) can cause severe and sometimes fatal hemorrhagic fever in humans. It has been estimated that LASV infects over 300,000 individuals per year across West Africa, causing over 3,000 deaths. The case fatality rate for Lassa fever (LF) has historically ranged from 15% to 50% in hospitalized patients with a recent multi-year study in Sierra Leone reporting a 69% rate. Previous and recent importation of LF into Europe and the US by travelers on commercial airlines illustrates the potential for spread outside the endemic area. In addition to causing morbidity and mortality as a naturally acquired infection, LASV is also categorized as a Category A Priority Pathogen by several US Government agencies because of the concern for deliberate misuse. In addition, LASV was recently listed on the World Health Organization's (WHO) 2018 List of Priority Pathogens. Geographically, LASV is restricted to West Africa and has two endemic regions: the Mano River Region (Sierra Leone, Guinea, Liberia) and Nigeria, where LASV infections are documented annually. Sequence analysis of LASV isolates from these regions show a remarkably high level of genetic diversity, with at least four lineages of LASV described that correlate with geographical regions. LASV lineages I, II, and III are localized in Nigeria and appear to be ancestral to lineage IV viruses that are found in and around Sierra Leona, Liberia, and Guinea. Two additional lineages have been proposed for isolates from Mali and Ivory Coast, representing a fifth potential lineage; isolates from Togo represent a sixth potential lineage. The genetic heterogeneity within LASV raises questions about the efficacy of a potential universal LASV vaccine that could elicit a protective immune response capable of preventing infection from all strains of LASV.

Currently, there are no licensed vaccines for the prevention of LF. Several experimental platforms have been evaluated as potential LF vaccines, with most candidates utilizing the LASV glycoprotein as an immunogen. While the majority of these platforms demonstrated a robust immune response in laboratory animals, many have failed to fully protect against lethal disease in relevant disease models and only a few have been assessed against a non-homologous LASV challenge. Previously, we reported that a single injection of a recombinant vesicular stomatitis virus (rVSV)-based LF vaccine expressing the glycoprotein precursor of LASV strain Josiah (rVSV-LASVGPC) completely protected nonhuman primates (NHPs) against a lethal challenge with the homologous lineage IV LASV Josiah strain. Recently, we developed a new uniformly lethal NHP model of LF using a contemporary and genetically diverse LASV lineage II isolate from a recent outbreak in Nigeria. Directly relevant to this proposal, we just completed the in-life portion of a NHP study that addresses the issue of whether or not the LASV lineage IV Josiah GPC expressed by our prototype rVSV vector can protect NHPs against a genetically diverse LASV from LASV lineage II. In brief, the goal of the study was to assess the protective efficacy of a quadra-valent filovirus plus LASV vaccine. Importantly, all NHPs vaccinated with the quadra-valent vaccine containing rVSV-LASVGPC based on lineage IV Josiah strain GPC were completely protected against lethal heterologous challenge with the new contemporary lineage II LASV isolate.

The main objective of this proposal is to develop a rVSV-based vaccine against LASV that can provide both rapid protection and long term immunity against the Nigeria strain of LASV. Data obtained from this work will be invaluable in moving this vaccine to advanced development. During the current reporting period, we achieved a major success with the initial study to begin to define the minimum interval between vaccination and exposure to LASV-Nigeria required for protection. Importantly, we demonstrated that 6/6 NHPs immunized with a single injection of our rVSV-LASVGPC Josiah strain vaccine 7 days before high dose exposure to heterologous LASV-Nigeria were completely protected from disease and death, while 3/3 control animals that received a nonspecific vaccine succumbed to Lassa fever. This data shows that the rVSV-LASVGPC Josiah vaccine can provide rapid protection against heterologous LASV-Nigeria. Further studies during the next reporting period will determine whether the interval required for protection can be reduced even further. These data have particular importance regarding impact on outbreak response and ring vaccination approaches.

15. SUBJECT TERMS
Lassa virus, Lassa fever, vaccine

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2020 ANNUAL REPORT for W81XWH1910027: "Development of a Recombinant VSV-Based Vaccine for Lassa Fever"

1. **INTRODUCTION:**
   The goal of the study is advance the development of a recombinant vesicular stomatitis virus (rVSV)-based vaccine that expresses glycoprotein C (GPC) of Lassa virus (LASV). This will be accomplished by determining the ability of the rVSV-LASVGPC vaccine to serve as a rapid acting vaccine in nonhuman primates (NHPs), determining the durability of the rVSV-LASVGPC vaccine in NHPs, and determining the correlates of protection of the rVSV-based rVSV-LASVGPC vaccine in NHPs.

2. **KEYWORDS:**
   Lassa virus, Lassa fever, vaccine, virology

3. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project?**

Note that the original Aim 1, to determine the neurovirulence of the rVSV-LASVGPC vaccine in mice, was not funded by the Sponsor. The other Aims and progress for this reporting period are as follows:

1. **Specific Aim 2: Determine the ability of the rVSV-LASVGPC vaccine to serve as a rapid acting vaccine in cynomolgous macaques.** *Original target date: Months 8 to 20.*
   a. Major Task 2: Determine the ability of the rVSV-LASVGPC vaccine to serve as a rapid acting vaccine in nonhuman primates (NHPs) at with a 7-day interval between vaccination and virus challenge.
      i. Subtask 1. In-life portion of Aim 2-1. [6 NHPs + 3 NHP controls = 9 NHPs total]; **completed on 30 March 2020.**
      ii. Subtask 2: Assess humoral and cellular immune responses, clinical pathology assays, viremia by PCR and plaque assay; perform necropsies on NHPs from Subtask 1 [Cell line: Vero E6 to assess viral load by plaque assay]; **25% complete; clinical pathology assays completed by 30 March 2020; necropsies completed by 30 March 2020**.
   b. Major Task 3: Determine the ability of the rVSV-LASVGPC vaccine to serve as a rapid acting vaccine in NHPs at with a 3 or 14 day interval between vaccination and virus challenge.
      i. Subtask 1. In-life portion of Aim 2-2 [6 NHPs + 3 NHP controls = 9 NHPs total]; **0% complete. Space reserved and study scheduled to start on 18 October 2020.**
      ii. Subtask 2: Assess humoral and cellular immune responses, clinical pathology assays, viremia by PCR and plaque assay; perform necropsies on NHPs from Subtask 1 [Cell line: Vero E6 to assess viral load by plaque assay]; **0% complete (see above).**

2. **Specific Aim 3: Determine the durability of the rVSV-LASVGPC vaccine in NHPs.** *Original target date: Months 7 to 30.*
   a. Major Task 4: Determine protection using prime only or prime-boost regimens.
      i. Subtask 1. In-life portion of Aim 3. [9 vaccinated NHPs + 9 unvaccinated controls = 18 NHPs total]; **less than 5% complete; the vaccine phase of this study lasts for ~ 12 months; animals were vaccinated on 14 July 2020 and monthly bleeds initiated.**
      ii. Subtask 2: Assess humoral and cellular immune responses, clinical pathology assays, viremia by PCR and plaque assay; perform necropsies on NHPs from Subtask 1. [Cell line: Vero E6 to assess viral load by plaque assay]; **No work scheduled for the current reporting period.**

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

Major Task 2: Subtask 1. The In-life portion of Aim 2.1. [6 NHPs + 3 NHP controls = 9 NHPs total] was completed on 30 March 2020. Importantly, all 6 NHPs that received the specific rVSV-LASVGPC Josiah strain and were challenged with heterologous LASV-Nigeria showed no evidence of clinical disease and survived to the study endpoint (day 35), while all three

control animals that received an irrelevant vaccine (rVSV-EBOV 76) succumbed to Lassa fever on postexposure day 12 (**Figure 1**).

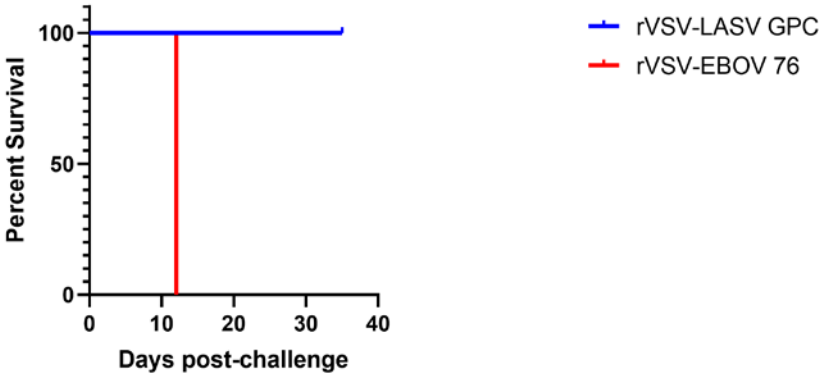

Figure 1. Survival outcome of cynomolgus monkeys specifically vaccinated with rVSV-LASVGPC Josiah strain (n = 6) or a nonspecific rVSV-EBOV 76 vaccine and challenged 7 days later with heterologous LASV-Nigeria.

Major Task 2: Subtask 2: Clinical pathology assays, necropsies, and gross pathology analysis were performed, and formalin-fixed tissues were embedded in paraffin.

Major Task 4: Subtask 1. The in-life portion of Aim 3 [9 vaccinated NHPs + 9 unvaccinated controls = 18 NHPs total] was initiated on 14 July 2020 with the 18 animals being vaccinated, which begins the approximately 12-month vaccine phase of the durability study.

- **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?**
  Major Task 2: Subtask 2: In the next reporting period, the remaining work from the 7-day interval NHP study will be performed and data analyzed. Specifically, this will include performing the PCR and plaque assays, humoral and cellular assays, and histopathology as well as and all of the data.

  Major Task 3 Subtask 1 and 2: Based on the success of the 7-day interval vaccine study, a 3-day interval vaccine study will be performed with the vaccination scheduled for 18 October 2020 and LASV-Nigeria challenge scheduled for 21 October 2020. It is anticipated that Subtask 3.1 will be completed during the next study period and that most (>75%) of Subtask 3.2 will be completed during the next reporting period.

  Major Task 4: Subtask 1. The vaccination portion of this study will continue, with monthly sample collection occurring throughout the next reporting period as originally scheduled with animals being challenged with heterologous LASV-Nigeria in July 2021.

4. **IMPACT:**
   - **What was the 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:**
   - **Changes in approach and reasons for change**
     Nothing to report.

   - **Actual or anticipated problems or delays and actions or plans to resolve them**
     All non-COVID-19 research at UTMB was shut down from 24 March 2020 until 18 May 2020 due to the COVID-19 pandemic (the in-life portion only of challenge studies that had already been initiated were allowed to be completed). This mandated shut down resulted in a substantial backlog in BSL-4 activities as many studies had to be delayed and rescheduled. Our main focus for this project during this reporting period, and as a result of the SARS-CoV-2 pandemic, has been to stay on schedule with the in-life portion of the originally proposed NHP work. We have stayed on track so far with the in the in-life portion of the project, and should stay on track with the in-life portion of the NHPs for this reporting period. Fortunately, decisions regarding work during the current reporting period and the next reporting period are determined by survival rather than by analysis of the host response. Likewise, the durability study has not yet been impacted because the first 12 months of that task is the vaccination phase. The main work impacted by the pandemic has been the performance of assays and analysis of data collected (e.g., PCR assays, host response assays, pathology). It is possible that small delays in the performance of assays and analysis of data may occur during the next reporting period as a result of the delays caused by the pandemic. In the event that some assays or data analysis initially planned for the next reporting period are delayed, we will conduct any such efforts during the Year 3 reporting period.

   - **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**
     Nothing to report.

   - **Significant changes in use or care of human subjects.**
     Nothing to report.

   - **Significant changes in use or care of vertebrate animals.**
     Nothing to report.

   - **Significant changes in use of biohazards and/or select agents.**
     Nothing to report.

6. **PRODUCTS:**
   - **Publications, conference papers, and presentations**
     - **Journal publications.**
       Nothing to report.

     - **Books or other non-periodical, one-time publications.**
       Nothing to report.

     - **Other publications, conference papers, and presentations.**
       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?**

Name:	Project Role:	Researcher Identifier (ORCID ID):	Nearest person month worked:	Contribution to Project:	Funding Support:
Thomas W. Geisbert	Principal Investigator	0000-0003-0858-1877	1	Provided general oversight, directing research, participating in NHP experiments.	NA
Krystle W. Agans	BSL-4 Research Technician	0000-0002-7319-6935	1	Performing various procedures with NHP clinical samples including hematology, clinical chemistry, virology, and RT-PCR assays.	
Robert Cross	BSL-4 Research Scientist	0000-0001-7718-1522	1	Assisting Dr. Geisbert with hands-on procedures with the NHPs, including phlebotomy, vaccination, clinical observations, euthanasia, and necropsy. Also performing virology assays on clinical samples.	
Daniel Deer	BSL-4 Research Technician	none	1	Hands-on procedures with the NHPs, including phlebotomy, clinical observations, euthanasia, and necropsy.	
Karla Fenton	BSL-4 Veterinary Pathologist	0000-0002-5530-6969	1	Hands-on procedures with the NHPs, including, clinical observations, euthanasia, and necropsy, and histology.	
Joan Geisbert	BSL-4 Technical Director	none	1	Laboratory set-up, supervision of the BSL-4 research associates and postdoctoral fellows, and hands-on participation in these experiments including vaccination, challenge, and phlebotomy of the NHPs.	

Name:	Project Role:	Researcher Identifier (ORCID ID):	Nearest person month worked:	Contribution to Project:	Funding Support:
Kevin Melody	BSL-4 Postdoctoral Fellow	0000-0003-2713-5338	1	Hands-on procedures with the NHPs, including phlebotomy, vaccination, clinical observations, euthanasia, and necropsy. Also performing clinical pathology, immunology, and virology assays on clinical samples.	NA
Kimberly Schuenke	Program Administrator	0000-0002-6661-7631	1	Assisted with establishment of grant and prepared reports.	
Courtney Williams	BSL-4 Postdoctoral Fellow	0000-0003-3389-0137	1	Performing various procedures with NHP clinical samples including hematology, clinical chemistry, virology plaque, and immunology assays.	

- **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**
   - **COLLABORATIVE AWARDS:**
     Not applicable.
   - **QUAD CHARTS:**
     See next page for updated Award Chart.

9. **APPENDICES:**
   Not applicable.

Award #W81XWH1910027: "Development of a Recombinant VSV-Based Vaccine for Lassa Fever"

PI: Thomas W. Geisbert, PhD, University of Texas Medical Branch at Galveston, Texas

Budget: $1,864,897.00

Topic Area: Vaccine Development for Infectious Diseases

Mechanism: Peer Reviewed Medical Research Program (PRMRP) Investigator-Initiated Research Award

Research Area(s): 0608 - Biological Prevention / Prophylactic Vaccines; 0499 – Pathobiology, Not Otherwise Specified

Award Status: 01SEP2019 to 31AUG2022

Study Goals:

The goal of the study is advance the development of a recombinant vesicular stomatitis virus (rVSV)-based vaccine that expresses glycoprotein C (GPC) of Lassa virus (LASV).

Specific Aims:

1. To determine the ability of the rVSV-LASVGPC vaccine to serve as a rapid acting vaccine in nonhuman primates (NHPs).
2. To determine the durability of the rVSV-LASVGPC vaccine in NHPs.
3. To determine correlates of protection of the rVSV-based rVSV-LASVGPC vaccine in NHPs.

Note that the original Aim 1 (to determine the neurovirulence of the rVSV-LASVGPC vaccine in mice) was deleted at the request of DoD.

Key Accomplishments and Outcomes:

Publications: none to date

Patents: none to date

Funding Obtained: none to date