AWARD NUMBER: W81XWH-18-1-0071

PROPOSAL TITLE: Development of Live-Attenuated Old World Arenavirus Vaccines Based on Temperature-Sensitive Viruses

PRINCIPAL INVESTIGATOR: David Topham, PhD

CONTRACTING ORGANIZATION: University of Rochester

REPORT DATE: FEBRUARY 2022

TYPE OF REPORT: Final Technical Progress Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
FEBRUARY 2022	Final Technical Progress Report	05/01/2018-10/31/2021

4. TITLE AND SUBTITLE

Development of Live-Attenuated Old World Arenavirus Vaccines Based on Temperature-Sensitive Viruses

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0071

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

David Topham

E-Mail: David_Topham@urmc.rochester.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University Rochester
518 Hylan Building
Rochester, NY 14627-0001

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 In this application we will test the novel hypothesis that a temperature sensitive (ts)-based strategy can be used to develop safe, stable, immunogenic and protective live-attenuated vaccines (LAV) for the treatment of Old World Arenavirus (OWA) disease in humans. Although a few ts mutants have been previously used to generate attenuated viruses for their implementation as LAV (e.g. influenza), this will be the first demonstration that this ts-based approach could be used to develop LAV for the treatment of OWA. Importantly, our studies will also provide essential information on the biology of OWA and how mutations in their genome affect viral fitness at different temperatures. Moreover, results from this proposal will allow us to demonstrate that our ts-based approach also represents an excellent strategy to generate valid OWA surrogates that could be used safely in BSL2 containment to facilitate the study of these important human pathogens without the use of restrictive BSL4 laboratories. Because of the safety concerns and costs associated with hemorrhagic fever (HF)-causing OWA work under BSL4 facilities, we will use, as a proof of concept, the prototype OWA lymphocytic choriomeningitis virus (LCMV). To that end, we will combine the identification of mutations found in our ts r3LCMV individual clones with the power of reverse genetic approaches to generate unique rLCMV containing mutation(s) responsible of the ts phenotype (rLCMV/ts). The generated rLCMV/ts will be evaluated for their potential as safe, stable, immunogenic and effective LAV, using the extensively validated and well characterized animal model of LCMV infection and associated disease. Our long-term goal is to implement the same ts-based approach to develop LAV to combat disease caused by HF-causing OWA (e.g. LASV), currently outside the scope of this proof-of-concept exploratory proposal.

15. SUBJECT TERMS
Old World Arenavirus, biosafety level, hemorrhagic fever virus, live-attenuated vaccines, lymphocytic choriomeningitis virus, Lassa virus,reverse genetics, temperature sensitive mutants.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	42	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

Page

1. INTRODUCTION

Arenaviruses comprise important human pathogens and some of them, chiefly the old world arenavirus (OWA) Lassa virus (LASV) in West Africa, cause hemorrhagic fever (HF) disease and represent a serious public health concern within their endemic regions. Notably, increased traveling into and from endemic areas has led to the importation of Lassa fever (LF) cases into non-endemic metropolitan areas around the globe, including the United States (US). Moreover, novel HF-causing OWA are likely to emerge, as illustrated by Lujo virus (LUJV) that caused an outbreak of viral HF in Southern Africa in 2008. Evidence also indicates that the prototype member in the family lymphocytic choriomeningitis virus (LCMV) is a neglected human pathogen of clinical significance. Besides the impact on public health, several arenaviruses, including LASV, pose a credible bioweapons threat and are classified as NIAID Category A Priority Pathogens. No FDA-licensed arenavirus vaccines are available and current anti-arenavirus therapy is limited to the use of ribavirin, which is only partially effective and associated with side effects. The significance of HF-causing OWA in human health and Biodefense readiness, together with the limited existing armamentarium to combat them, demonstrate the urgent need of developing effective countermeasures to combat HF-causing OWA infection in humans.

In this application we will test the novel hypothesis that a temperature sensitive (ts)-based strategy can be used to develop safe, stable, immunogenic and protective live-attenuated vaccines (LAV) for the treatment of OWA HF disease in humans. Our studies will provide a comprehensive assessment of the feasibility of using, for the first time, a ts-based approach to develop LAV to combat OWA infections. Although a few ts mutants have been previously used to generate attenuated viruses for their implementation as LAV (e.g. influenza), this will be the first demonstration that this ts-based approach could be used to develop LAV for the treatment of OWA in humans. Importantly, our studies will also provide essential information on the biology of OWA and how mutations in their genome affect viral fitness at different temperatures. Moreover, results from this proposal will allow us to demonstrate that our ts-based approach also represents an excellent strategy to generate valid OWA surrogates that could be used safely in BSL2 containment to facilitate the study of these important human pathogens without the use of restrictive BSL4 laboratories. These ts OWA will provide investigators with the capability to work with these viruses outside BSL4 containment to facilitate, for instance, the study of OWA-host cell interactions and to identify and characterize therapeutics for the treatment of these important human pathogens. Because of the safety concerns and costs associated with HF-causing OWA work under BSL4 facilities, we will use, as a proof of concept, the LCMV system. The prototype OWA LCMV provides us with a BSL2 agent to demonstrate that ts mutations can convert a virulent LCMV into a suitable attenuated viral form with features of LAV. To that end, we will combine the identification of mutations found in our ts r3LCMV individual clones with the power of reverse genetic approaches to generate unique rLCMV containing mutation(s) responsible of the ts phenotype (rLCMV/ts). The generated rLCMV/ts will be evaluated for their potential as safe, stable, immunogenic and effective LAV, using the extensively validated and well characterized animal model of LCMV infection and associated disease.

2. KEYWORDS

Biosafety level, hemorrhagic fever virus, lymphocytic choriomeningitis virus, Lassa virus, live-attenuated vaccine, reverse genetics, Old World arenavirus, temperature sensitive mutants.

3. ACCOMPLISHMENTS

3A. Major project goals

Specific Aims (SA):
SA 1. Identify the mutations responsible for the temperature sensitive phenotype of LCMV.
Timeline (Months): 1-6
Status: Complete

Major tasks:
Major Task 1. High-throughput next generation sequencing (NGS) to identify the mutations responsible for the ts phenotype of r3LCMV/ts individual clones.
Timeline (Months): 1-3
Status: Complete

Major Task 2. Generation and characterization of rLCMV/ts mutants
Timeline (Months): 4-6
Status: Complete

Milestone(s):
Milestone 1. Identification of the mutations responsible of the ts phenotype of r3LCMV/ts individual clones.
Status: Complete

Milestone 2. Generation of ts rLCMV/ts.
Status: Complete

Local IRB Approval: Submitted and approved at Texas Biomedical Research Institute.
Local IACUC Approval: Not applicable.

SA 2. Determine the genetic and phenotypic stability of rLCMV/ts
Timeline (Months): 7-12
Status: Not completed

Major tasks:
Major Task 3. Assess the genetic and phenotypic stability or rLCMV/ts in cultured cells.
Timeline (Months): 7-9
Status: Not completed

Major Task 4. Determine the genetic and phenotypic stability of rLCMV/ts in vivo.
Timeline (Months): 10-12
Status: Not completed.

Milestone(s):
Milestone 1. Stability of rLCMV/ts in cultured cells.
Status: Not completed.

Milestone 2. Stability of rLCMV/ts in vivo.
Status: Not completed.

Local IRB Approval: Submitted and approved at Texas Biomedical Research Institute.
Local IACUC Approval: Submitted and approved at Texas Biomedical Research Institute.

ACURO Approval: Submitted and approved.

SA 3. Characterize the safety, immunogenicity and protective efficacy of selected rLCMV/ts
Timeline (Months): 13-18
Status: Partially completed

Major tasks:
Major Task 5. Characterize the ability of rLCMV/ts to induce fatal LCM.
Timeline (Months): 13-14
Status: Completed.

Major Task 6. Assess the immunogenicity of rLCMV/ts.
Timeline (Months): 15-16
Status: Not completed.

Major Task 7. Evaluate the protection efficacy of rLCMV/ts against a lethal challenge with rLCMV/WT.
Timeline (Months): 17-18
Status: Completed.

Milestone(s):
Milestone 1. Safety of rLCMV/ts.
Status: Completed.

Milestone 2. Immunogenicity of rLCMV/ts.
Status: Not completed.

Milestone 3. Protection efficacy of rLCMV/ts.
Status: Completed.
Local IRB Approval: Submitted and approved at Texas Biomedical Research Institute.
Local IACUC Approval: Submitted and approved at Texas Biomedical Research Institute.
ACURO Approval: Submitted and approved.

3A. What was accomplished under these goals?

r3LCMV recapitulates LCMV infections: A r3LCMV platform in which each of the two viral S segments is altered to replace one of the viral genes with a reporter gene has been recently developed (**Fig. 1A**). The physical separation of the viral glycoprotein (GP) and NP into two different S segments imposes a strong selective pressure to package and maintain all viral segments necessary to produce a replication-competent r3LCMV/WT (**Fig. 1A**). Notably, r3LCMV/WT exhibit rLCMV/WT growth properties in cultured cells (**Fig. 1B**) and are both genetically and phenotypically stable both in vitro and in vivo. Markedly, levels of GFP and Gluc expression in r3LCMV/WT are similar to those of NP and GP, respectively, in rLCMV/WT (**Fig. 1C**), demonstrating that r3LCMV/WT recapitulates rLCMV infections. *These results demonstrate that r3LCMV/WT can be used as a valid surrogate to evaluate rLCMV/WT infection in cultured cells.*

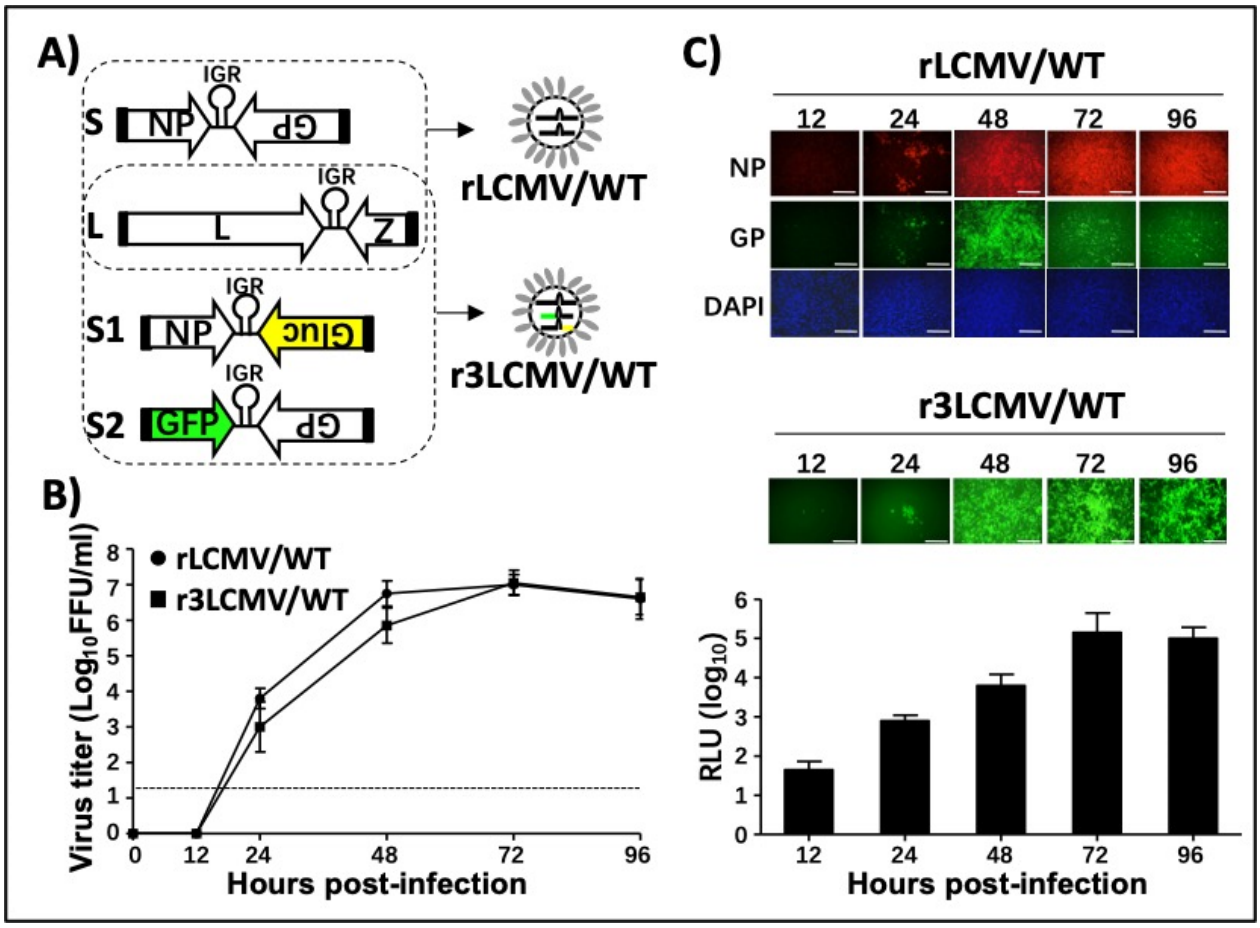

Figure 1. r3LCMV GFP/Gluc recapitulates rLCMV/WT infections. A) Schematic representation of rLCMV/WT and r3LCMV/WT genome segments: Black boxes: non-coding regions. IGR: intergenic regions. L: polymerase. Z: matrix-like protein. NP: nucleoprotein. GP: glycoprotein. GFP: green fluorescent protein. Gluc: Gaussia luciferase.**B) Growth properties of rLCMV/WT and r3LCMV/WT:** Vero cells (6 well-plate format, triplicates) were infected (multiplicity of infection 0.01) with rLCMV/WT (circles) or r3LCMV/WT (squares). Viral titers in tissue culture supernatants (TCS) at the indicated hours post-infection were determined by immunofocus assay (FFU/ml) for rLCMV/WT and by fluorescence microscopy for r3LCMV/WT. Dotted line indicates the limit of detection (20 FFU/ml)**. C) r3LCMV/WT reporter gene expression recapitulate viral protein expression:** Replicates of Vero cells infected as before were evaluated for NP and GP expression levels (rLCMV/WT, top) by immunofluorescence assay using LCMV GP and NP monoclonal antibodies 83.6 and 1.1.3, respectively. DAPI was used for nuclear staining. Reporter gene expression in r3LCMV/WT infected cells (bottom) was evaluated by fluorescence microscopy (GFP) and luminiscence (Gluc). Representative images are illustrated. Scale bar = 100 μm. Average and standard deviation are shown.

r3LCMV/WT grows efficiently at different temperatures: Before passaging the r3LCMV/WT at low temperatures for the selection of ts sensitive viruses (r3LCMV/ts), we first evaluated the viral growth kinetics of r3LCMV/WT in Vero cells at different temperatures and times post-infection (**Fig. 2A**). As determined by GFP (**Fig. 2B**) and Gluc (**Fig. 2C**) expression, r3LCMV/WT is able to replicate at all tested (25-39oC) temperatures, although, as expected, more efficiently and faster at higher (37-39oC) than lower (25-33oC) temperatures. Importantly, reporter gene expression levels, both GFP and Gluc, correlate with viral titers. *These results demonstrate that r3LCMV/WT can efficiently replicate at all tested temperatures (25-39oC) and that reporter gene expression (GFP and Gluc) can be used as a valid surrogate of viral replication.*

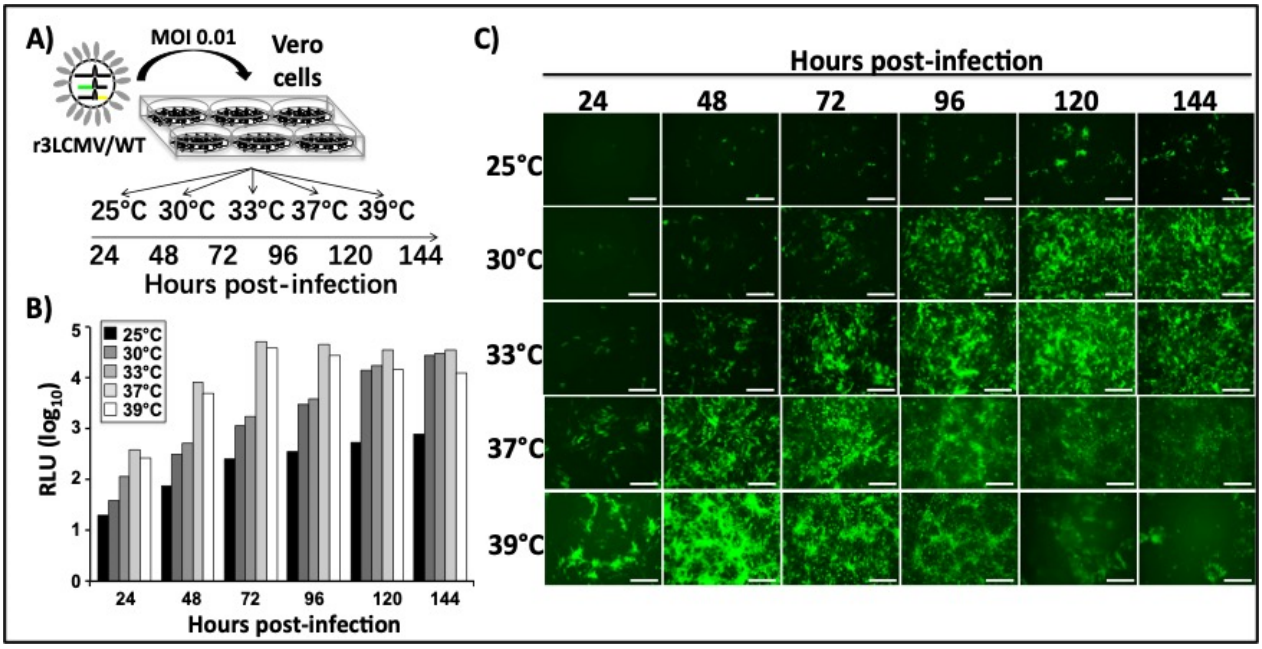

Figure 2. r3LCMV/WT grows efficiently at different temperatures. A) Schematic representation of the assay: Vero cells (6-well plates, triplicates) were infected (moi of 0.01) with r3LCMV/WT and incubated at the indicated temperatures. At different hours post-infection (24, 48, 72, 96, 120 and 144) reporter gene expression was evaluated by GFP expression using fluorescence microscopy (**B**) and Gluc using luminiscence (**C**). Representative images are shown. Scale bar 100 µm.

Generation and characterization of a r3LCMV/ts: We next examined whether 10 serial passages (P10) of r3LCMV/WT in Vero cells at low (25oC) temperature (**Fig. 3A**) resulted in the introduction of mutations that affected viral replication at high (37 and 39oC) temperatures. As determined by GFP (**Fig. 3B**) and Gluc (Fig. 3C), r3LCMV P10 exhibited restricted growth kinetics at low (25-33oC) temperatures in contrast to the replication of r3LCMV/WT that replicated at all tested temperatures. *These results demonstrate that after 10 passages at 25oC we have been able to generate a r3LCMV/ts (P10) that only replicates at low (25-33oC) temperatures.*

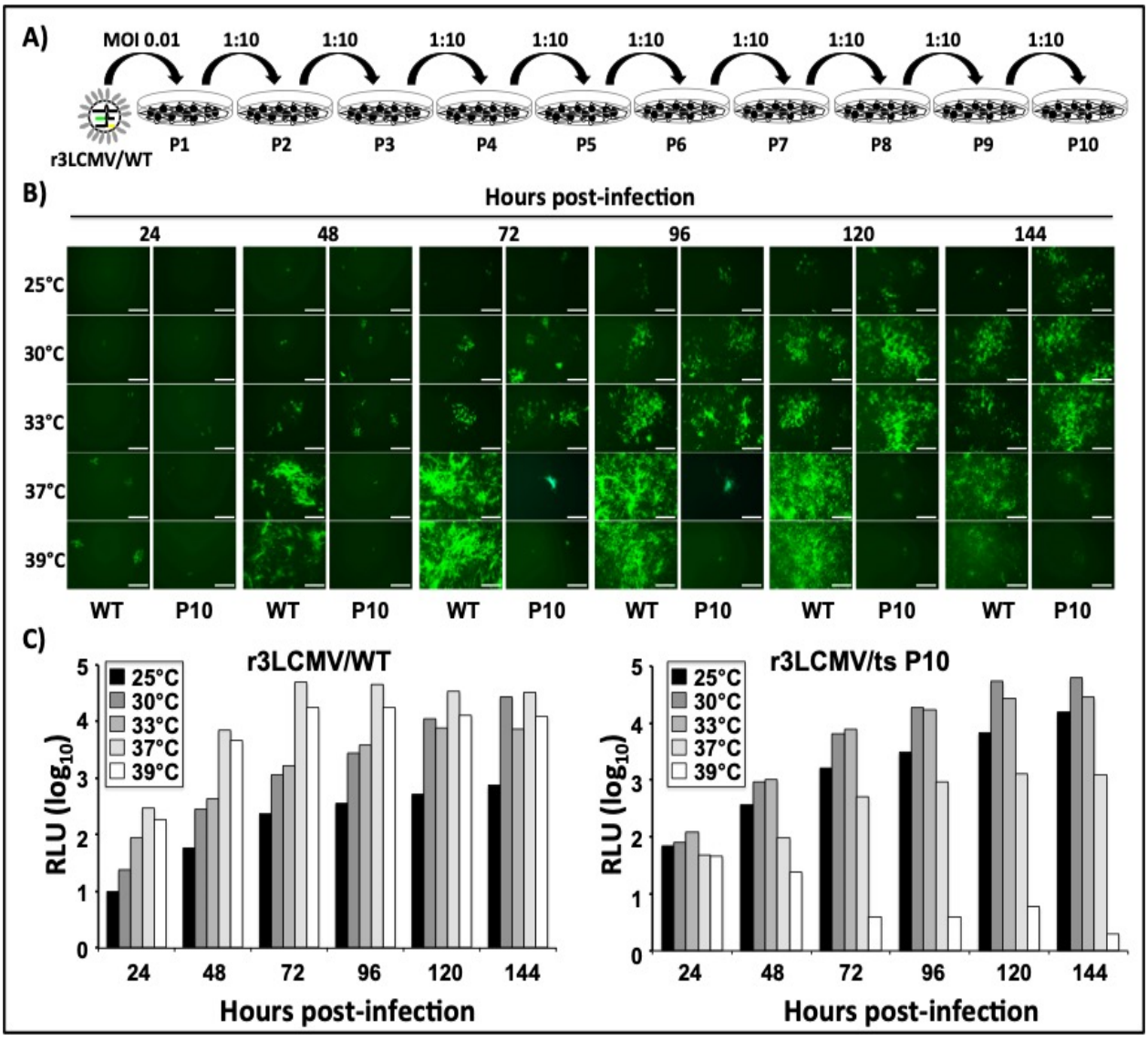

Figure 3. Generation and characterization of a ts r3LCMV/WT. A) Schematic representation of the assay: Vero cells (6-well plate format) were infected (moi 0.01) with r3LCMV/WT and incubated at 25oC for 10 days. At 10 days post-infection (~ 50% virus infection as determined by GFP expression under a fluorescence microscope) TCS were collected. A 1:10 dilution of the TCS was used to infect fresh monolayers of Vero cells (6-well plate format) and incubated for 10 days for the rest of the serial passages (total 10 passages). **B-C) r3LCMV/WT P0 and ts P10 viral replication:** Vero cells (6-well plate format) were infected (moi 0.01) with r3LCMV/WT (P0) or r3LCMV/WT ts (P10) and incubated at the indicated temperatures. At different times post-infection, viral replication was evaluated by GFP expression under a fluorescence microscope (**B**). TCS from infected cells at different temperatures and times post-infection were also evaluated for Gluc expression (**C**) using a luciferase assay. Representative images are illustrated. Scale bar = 100 µm.

Identification of individual r3LCMV/ts clones: We conducted serial 10-fold dilutions of our r3LCMV/ts (**Fig. 4A**) to isolate individual clones that were evaluated for their ability to replicate at different temperatures (25-39oC). As determined by GFP (**Fig. 4B**) and Gluc (**Fig. 4C**) expression, we were able to isolate individual r3LCMV/ts clones that could replicate only at 25-33oC. Notably, r3LCMV/ts clones grew in Vero cells to high titers (~106-107 PFU/ml) at 33oC. *These results demonstrate the ability to generate r3LCMV/ts that grow efficiently at low (25-33oC) but not at high (37-39oC) temperatures.*

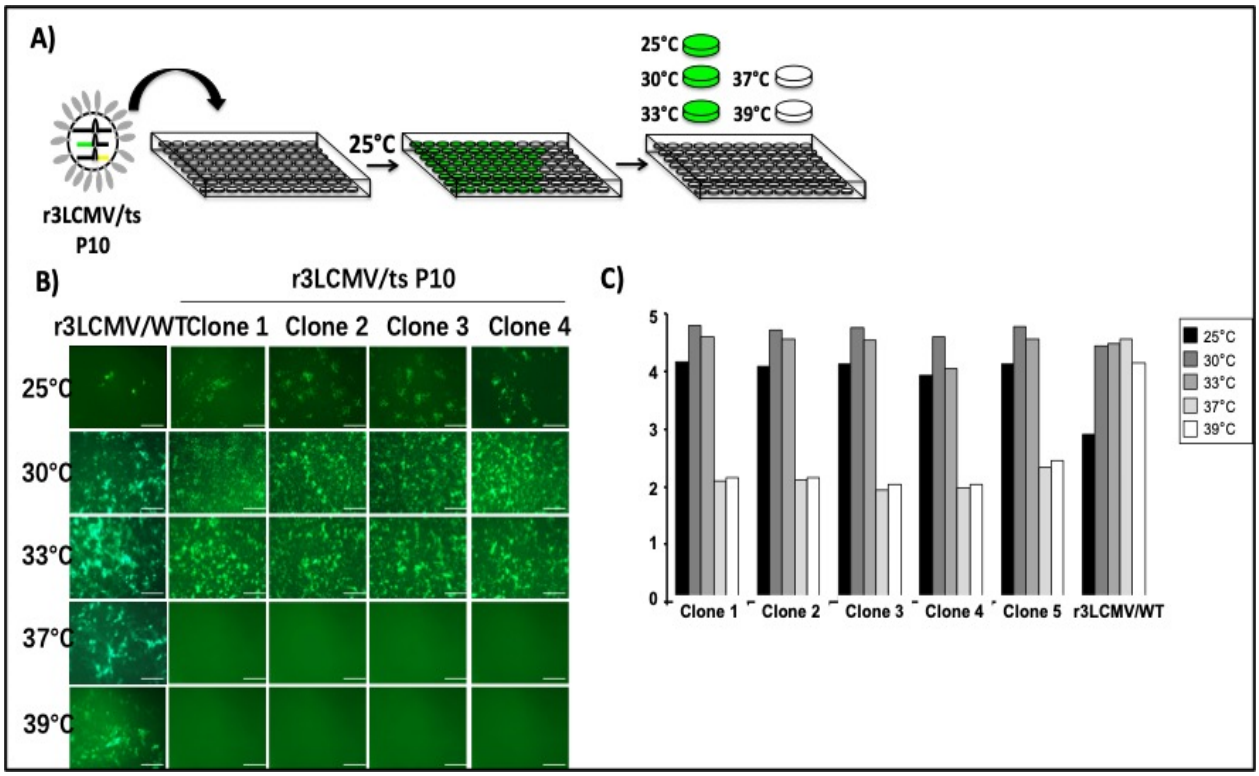

Figure 4. Isolation of r3LCMV/ts clones. A) Schematic representation of the assay: Vero cells (96-well plates) infected (MOI 0.01) with 10-fold serial dilutions of r3LCMV/ts P10 (Fig. 3) were incubated at 25oC. Ten days p.i., individual clones that replicate at low (25-33oC) but not high (37-39oC) temperatures were collected and amplified in fresh Vero cells (33oC). **B-C) Viral replication:** Vero cells (12-well plates, triplicates) were infected (MOI 0.01) with individual r3LCMV/ts clones at the indicated temperatures. Virus replication was assessed by GFP (**B**) and Gluc (**C**) at the indicated h p.i. Representative images (only 120 h p.i.) are shown. Scale bars 100 µm.

Viral growth kinetics of individual r3LCMV/ts clones: We next evaluated the ability of the selected individual r3LCMV/ts clones to replicate at different temperatures (25-39oC) by looking at Gluc (**Fig. 5A**) and GFP (**Fig. 5B**) expression at different times post-infection. Similar to our preliminary studies, all the individual r3LCMV/ts clones replicated efficiently at low temperatures (25-33oC) but were affected to replicate at 39oC. Importantly, clones 1, 3 and 4 showed some viral replication (although minimal when compared to r3LCMV/WT) at 37oC (**Figs. 5A and 5B**). Notably, clone 2 was able to efficiently replicate at 37oC (**Figs. 5A and 5B**). *These results demonstrate that all the selected individual r3LCMV/ts clones were affected to replicate at 39oC, contrary to the situation with r3LCMV/WT, and that clones 1, 3 and 4 (but not clone 2) were also affected in viral replication at 37oC.*

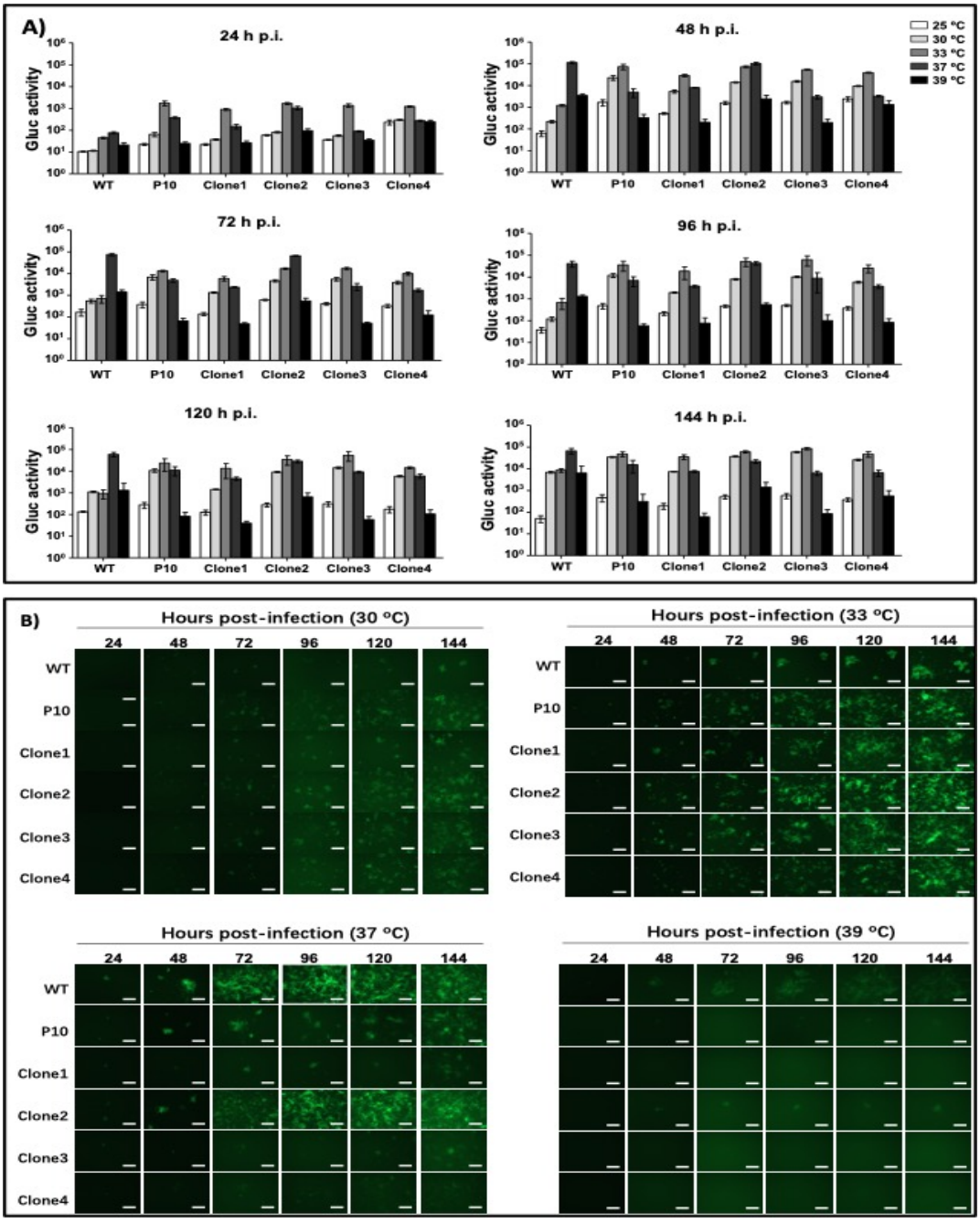

Figure 5. Growth kinetics of selected individual ts r3LCMV clones: A total of four individual ts r3LCMV clones (1-4) were selected and expanded at 30 ºC. Stocks for the ts r3LCMV clones were used to infect Vero cells (MOI 0.01). Gluc activity in the TCS from infected cells was determined at 24, 48, 72, 96, 120 and 144 h post infection **(A).** Representative GFP representative images were captured at the same times post-infection **(B).**

LCMV NP S25P and S25T are partially responsible for the ts phenotype of r3LCMV/ts clones: We next, infected (MOI 0.01) 10-cm dishes of Vero cells (33oC) with r3LCMV/ts clones 1, 3 and 4 (able to replicate at 25-33oC temperatures, **Fig. 5**) as well as clone 2 (able to replicate at 25-37oC, **Fig. 5**). At 6 days p.i., r3LCMV/ts were purified from the TCS by ultracentrifugation. RNA from purified viruses was extracted with Trizol and used for the amplification of the viral GP, NP and Z genes by RT-PCR and sequencing. No mutations in the reporter genes (GFP or Gluc) were identified in the r3LCMV/ts clones. Likewise, no mutations in the Z protein were identified in any of the r3LCMV/ts clones. Single amino acid substitutions in GP were identified in all the clones (**Table 2**). Notably, all r3LCMV/ts clones, except clone 2, contained a single amino acid substitution at position 25 (S25P) in NP (**Table 1**). Clone 2 contained a S25L change. We next introduce the identified S25P and S25L mutations in LCMV NP and used a minigenome (MG) assay to evaluate if these amino acid changes confer NP the ability to replicate at low but not at high temperatures (**Fig. 6**). Mutant NPs containing a S25T and a S25D changes were also included in these assays (**Fig. 6**). LCMV NP S25P and S25L were not severely affected in viral replication at 30oC as compared to LCMV NP WT. Notably, S25P substitution had a more drastic effect, but still some activity, on the MG at 33oC. No MG activity was observed with NP S25P at 37-39oC. This effect was less dramatic with S25L since it was able to efficiently replicate, although to less extend as compared to NP WT at all tested temperatures. *These results demonstrate that S25P/L are, at least in part, responsible for the ts phenotype of the identified r3LCMV/ts individual clones. Moreover, these results, together with those in* ***Figs. 4-5*** *suggest the presence of additional mutations in r3LCMV/ts clones responsible for the lack of viral replication at high (37-39oC) temperatures. It is worth noting that S25 is highly conserved in all OWA except LUJV and Mobala, which have a T25 (****Fig. 7****).*

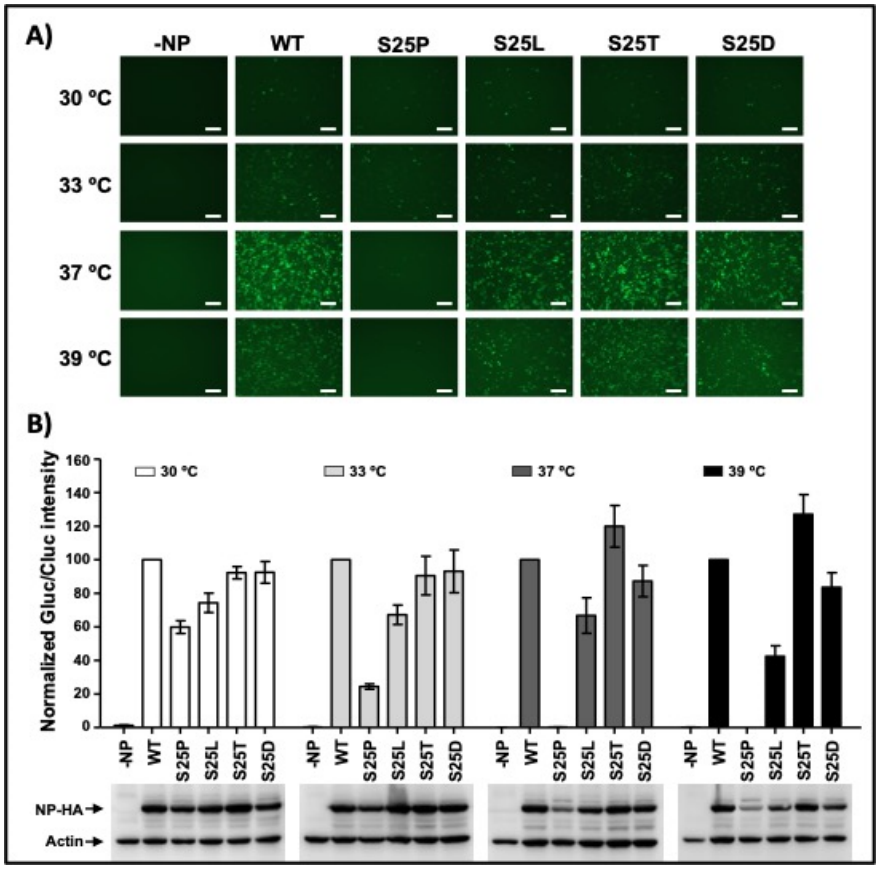

Figure 6. Effect of LCMV NP mutation at position S25 in minigenome (MG) activity at different temperatures: Human 293T cells (12-well plates, triplicates) were transfected (triplicates) with the indicated NP expression plasmids (WT, S25P, S25L, S25T or S25D) together with pCAGGS L, a LCMV MG plasmid, and a SV40 Cypridina luciferase to normalize transfection efficiencies. At 48 h, MG activity was evaluated by GFP (**A**) and Gluc (**B**) expression. NP expression levels were determined by Western blot (**C**). Representative images are illustrated. Scale bar 100 mm.

S25 is highly conserved among OWA: Based on our previous results demonstrating that LCMV NP S25 plays an important role in viral replication and transcription as determined in our MG experiments (**Fig. 6**), we next assess the conservation of this amino acid residue in other OWA NPs. As shown in **Fig. 7**, all OWA NPs, expect LUJV and MOBV has a S at position 25. Notably, LUJV and MOBV contain a T at position 25 that has a similar activity in viral replication and transcription **(Fig. 6).**

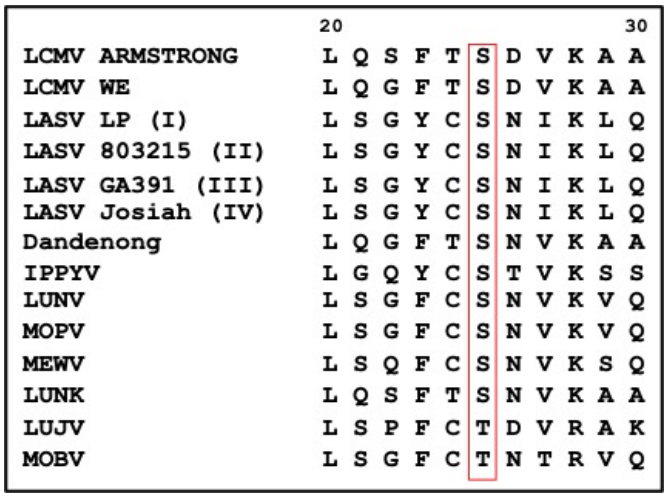

Figure 7. Conservation of S25 in different OWA: Sequence comparison of different LCMV strains and LASV lineages (I-IV) as well as representative virus in the OWA. IPPYV: Ippy virus; LUNV: Luna virus; MOPV: Mopeia virus; MEWV: Merino Walk virus; LUNK: Lunk virus; LUJV: Lujo virus; MOBV: Mobala virus.

Effect of mutations in LASV NP viral replication and transcription: Based on our results with LCMV NP that demonstrate the important role of S25 in viral replication and transcription (**Fig. 6**), we next evaluated the role of this amino acid in LASV NP (**Fig. 8**). Similar to our assays with LCMV NP, we introduced the identified S25P and S25L mutations, as well as S25T and S25D, in LASV NP and used a MG assay to evaluate if these amino acid changes altered the ability of LASV NP to replicate and transcribe at different temperatures (**Fig. 8**). Similar to our results with LCMV, LASV NP S25P and S25L were not affected in viral replication at 30oC as compared to LASV NP WT. Notably, both S25P and S25L substitutions had a more drastic effect, but still some activity, on the MG at 33oC. No MG activity was observed with LASV NP S25P or S25L at 37-39oC. *These results demonstrate that S25P/L substitutions in LASV NP also affect its ability to replicate and transcribe in a MG assay at high temperatures (37-39oC) but not at permissive (30-33oC) temperatures.*

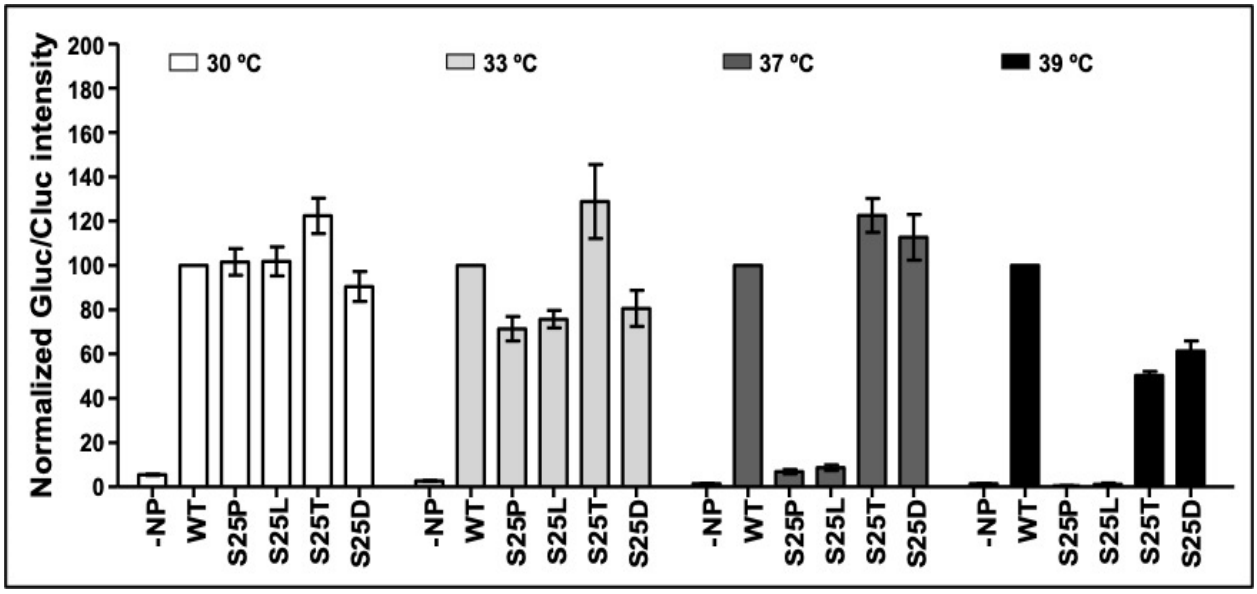

Figure 8. Effect of LASV NP mutation at position S25 in MG activity at different temperatures: Human 293T cells (12-well plates, triplicates) were transiently transfected (triplicates) with the indicated LASV NP expression plasmids (WT, S25P, S25L, S25T or S25D) together with pCAGGS L, a LASV MG plasmid, and a SV40 Cypridina luciferase to normalize transfection efficiencies. At 48 h post-transfection, MG activity was evaluated by Gluc expression.

Identification of other mutations responsible for the ts phenotype of LCMV: We have identified and isolated individual r3LCMV/ts clones able to replicate at low (25-33oC) but not high (37-39oC) temperatures (**Figs. 4 and 5**). Furthermore, we have identified a single amino acid substitution (S25) in LCMV NP responsible, at least in part, for the ts phenotype of the r3LCMV/ts clones (**Fig. 6**). However, we have not evaluated the presence of additional mutations in the viral polymerase protein (L) or in the small (S) and large (L) viral RNA segment non-coding (NCR) and intergenic (IGR) regions that could be responsible for the ts phenotype observed in our r3LCMV/ts clones. To evaluate if other mutations in the viral genome are responsible of the ts phonotype, we sequenced the entire viral genome of our r3LCMV/ts clones using NGS and compared to our original r3LCMV/WT. In addition to the mutations observed in NP (**Table 1**), we have also identified mutations in the viral GP (**Table 2**) and L (**Table 3**). No mutations were found in the viral Z or NCRs (data not shown).

Table 1. Mutations in the NP of r3LCMV/ts by NGS

		NP									
Position	WT	P10		Clone 1		Clone 2		Clone 3		Clone 4	
		nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid
133	T	C/1977(89%) T/243(11%)	NP(S25P) NP(S25S)	C/842(76%) T/260(24%)	NP(S25P) NP(S25S)	C/2397(97%) T/71(3%)	NP(S25P) NP(S25S)	C/1624(100%)	NP(S25P)	C/2980(99%)	NP(S25P)
134	C							T/1612(100%)	NP(S25L)		
170	A			A/347(71%) G/296(29%)	NP (N37N) NP (N37S)						
183	G					A/520(23%) G/1784(77%)	NP(G41G) NP(G41G)				
488	T					C/928(82%) T/192(17%)	NP(I143T) NP(I143I)				
1122	T	C/33(5%) T/641(95%)	NP(P354P) NP(P354P)			C/742(100%)	NP(P354P)				
1707	T							C/341(28%) T/876(82%)	NP(I549I) NP(I549I)		

Table 2. Mutations in the GP of r3LCMV/ts by NGS

		GP									
Position	WT	P10		Clone 1		Clone 2		Clone 3		Clone 4	
		nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid
1577	C	T/3165(99%)	GP(K256K)	T/3165(99%)	GP (K256K)	T/2555(100%)	GP (K256K)	T/3259(100%)	GP(K256K)		
1868	T					C/4407(80%) T/1131(20%)	GP (V158V) GP (V158V)				
1919	T					C/4464(80%) T/1102(20%)	GP (I141V) GP (I141I)				
1921	T					C/4433(80%) T/1097(20%)	GP (I141V) GP (I141I)				

Table 3. Mutations in the L of r3LCMV/ts by NGS

		L									
Position	WT	P10		Clone 1		Clone 2		Clone 3		Clone 4	
		nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid	nt/Reads(%)	Amino acid	nt/Reads(%)	Amino ac
930	T	C/572(66%) T/299(34%)	L(I300T) L(I300I)	C/139(100%)	L(I300T)	C/249(100%)	L(I300T)	C/695(100%)	L(I300T)	A/373(100%)	L(I300K)
976	T	C/670(65%) T/362(35%)	L(T315T) L(T315T)	C/159(100%)	L(T315T)	C/295(100%)	L(T315T)	C/844(100%)	L(T315T)	C/432(99%)	L(T315T
1246	T	C/644(61%) T/406(39%)	L(S405S) L(S405S)	C/172(99%)	L(S405S)	C/250(100%)	L(S405S)	C/825(100%)	L(S405S)	C/481(100%)	L(S405S
1329	A									G/482(100%)	L(E433G
1550	G							A/474(100%)	L(A507T)		
2254	A					C/160(88%) A/21(12%)	L(E741D) L(E741E)				
2654	G	A/243(23%) G/799(77%)	L(E875K) L(E875E)	A/159(99%)	L(E875K)	A/187(94%) G/13(7%)	L(E875K) L(E875E)	A/606(100%)	L(E875K)		
2763	G							A/267(24%) G/864(76%)	L(R911H) L(R911R)		
2954	T							C/166(26%) T/477(74%)	L(F975L) L(F975F)		
4893	A	C/241(26%) A/681(74%)	L(N1621T) L(N1621N)								
4959	C	G/614(60%) C/408(40%)	L(T1643R) L(T1643T)	G/169(100%)	L(T1643R)	G/281(100%)	L(T1643R)	G/815(100%)	L(T1643R)	G/423(100%)	L(T1643F
5940	G			A/143 (100%)	L(R1970K)						
6377	A	G/318(23%) A/1049(77%)	L(K2116E) L(K2116K)								

***In vitro* characterization of the identified LCMV L mutations in viral replication and transcription**: Our NGS sequencing analysis identified 3 amino acid changes (I300T, E875K and T1643R) in the LCMV L (**Table 3**) that could be responsible for the differences in viral replication and transcription observed in our individual r3LCMV/ts clones. To assess the contribution of these amino acid changes, alone or in combination, in LCMV replication and transcription, we used our previously described MG assay. We introduced the identified I300T, E875K and T1643R (individually or in combination) in our pCAGGS L expression plasmid and evaluated if these amino acid changes affected the ability of LCMV L to replicate at low but not at high temperatures (**Fig. 9**). All LCMV L mutants were able to replicate to levels compared to LCMV L WT at 33oC. Notably, LCMV mutant E875K was affected in viral replication and transcription at high (37-39oC) temperatures (**Fig. 9**). However, LCMV L mutants I300T and T1643R replicate similarly to LCMV L WT at 37-39oC (**Fig. 9**). *These results suggest that LCMV L E875K might be responsible, alone or in combination with LCMV NP S25, in the ts phenotype observed with our ts r3LCMV clones.*

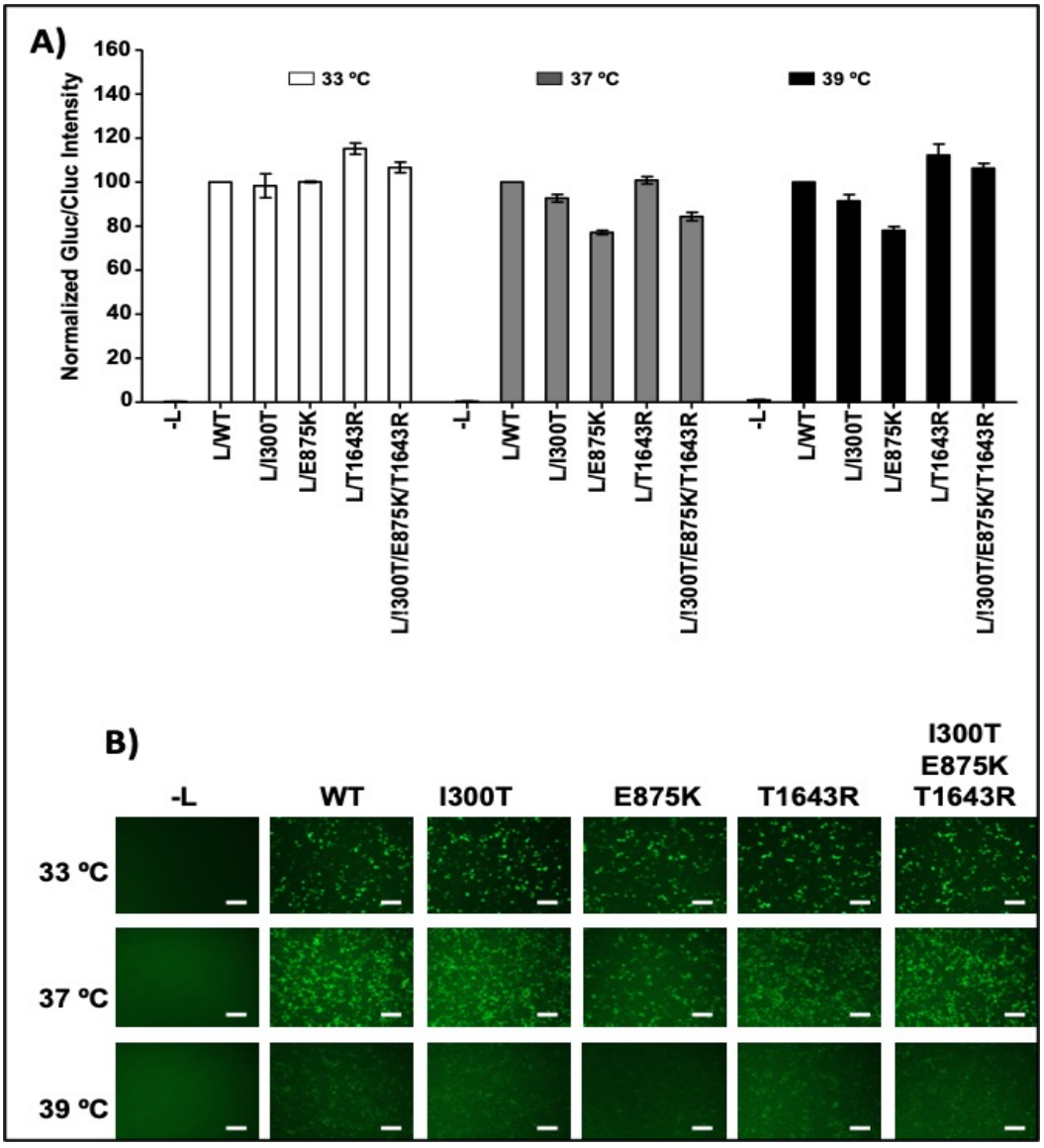

Figure 9. Effect of LCMV L mutations at position I300, E875 and T1643 in MG activity at different temperatures (33, 37 and 39C): Human 293T cells (12-well plates, triplicates) were transfected (triplicates) with the indicated LCMV L expression plasmids (WT, I300T, E875K, T1643R or I300T/E875K/T1643R) together with pCAGGS NP, a LCMV MG plasmid, and a SV40 Cypridina luciferase to normalize transfection efficiencies. At 48 h post-transfection, MG activity was evaluated by Gluc (**A**) and GFP (**B**) Representative images are illustrated. Scale bar 100 mm.

Contribution of LCMV NP and L mutations in viral replication and transcription: Based on our previous results using individual NP or L mutations, we next used our MG assay to assess the contribution of LCMV NP (S25P) and L (I300T, E875K and T1643R alone or in combination) mutations, together, in viral replication and transcription (**Fig. 10**). Our preliminary data demonstrate that the combination of both LCMV NP and L mutations clearly affect viral replication and transcription even at the lowest temperature (33oC). Notably, the combination of LCMV NP and L mutations were completely affected in viral replication and transcription at non permissive temperatures (37-39oC). The conservation of I300, E875 and T1643 in different OWA L proteins is shown in **Fig. 11.**

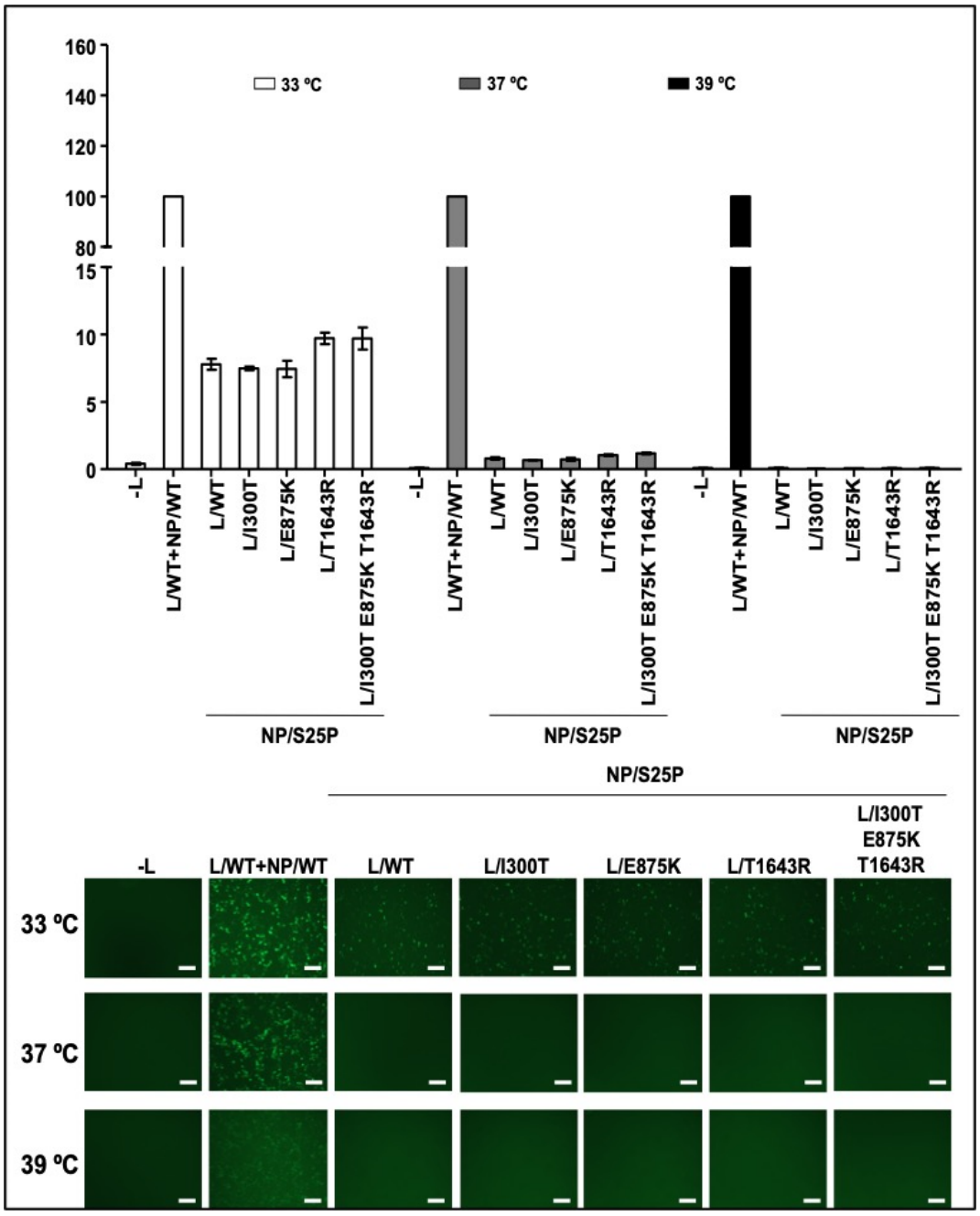

Figure 10. Effect of LCMV NP and L mutations in MG activity at different temperatures: Human 293T cells (12-well plates, triplicates) were transfected (triplicates) with the indicated LCMV NP and L expression plasmids together with a LCMV MG plasmid, and a SV40 Cypridina luciferase to normalize transfection efficiencies. At 48 h post-transfection, MG activity was evaluated by Gluc (**Top**) and GFP (**Bottom**) expression. Representative images are illustrated. Scale bar 100 mm.

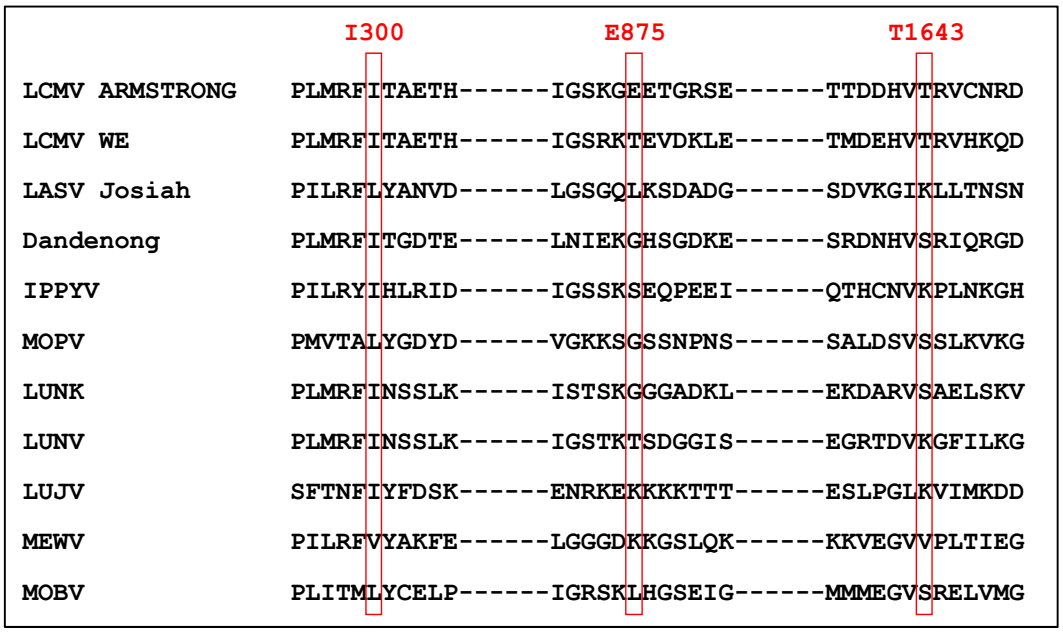

Figure 11. Conservation of I300, E875, and T1643 in different OWA L proteins: Sequence comparison of different LCMV strains and representative virus in the OWA. IPPYV: Ippy virus; LUNV: Luna virus; MOPV: Mopeia virus; MEWV: Merino Walk virus; LUNK: Lunk virus; LUJV: Lujo virus; MOBV: Mobala virus.

Safety and protection efficacy of rLCMV/ts: To demonstrate the safety and protection efficacy of rLCMV/ts mutants for their implementation as live-attenuated vaccines, we assessed the virulence and the ability of r3LCMV/ts clone 3 to induce a protective immune response *in vivo* using the fatal lymphocytic choriomeningitis (LCM) disease mouse model induced by intracranial (i.c.) inoculation with 10^3 plaque forming units (PFU) of LCMV WT in WT B6 mice. We selected r3LCMV/ts clone 3 based on its ability to replicate only at low (25-33ºC) but not high (37-39ºC) temperatures. Mice infected with rLCMV/WT developed the expected fatal LCM within 8 days. In contrast, all mice infected with r3LCMV/ts clone 3 survived and remained free of clinical symptoms throughout the 12-day duration of the experiment (**Fig. 12**). We next assessed whether a single intraperitoneal (i.p.) immunization with 10^5 PFU of r3LCMV/ts clone 3 was capable of inducing protective immunity against a lethal challenge with LCMV WT (**Fig. 13**). Mice vaccinated with r3LCMV/ts clone 3 survived and remained free of clinical symptoms throughout the duration (12 days) of the experiment. All mock (PBS)-immunized mice developed severe LCM symptoms and died within the 8 days after rLCMV/WT challenge (**Fig. 13**).

% Survival (N = 8)				
Days p.i. (i.c., 10^3 PFU)	6	7	8	12
PBS	100	100	100	100
rLCMV/WT	100	37.5	0	-
rLCMV/ts 3	100	100	100	100

Figure 12. Safety of r3LCMV/ts clone 3: Six-week-old B6 mice (N=8) were immunized (i.c.) with 10^3 PFU of r3LCMV/WT, r3LCMV/ts clone 3, or mock (PBS)-immunized. Animals were monitored daily for morbidity and mortality for 12 days.

Days post-challenge (i.c. 10^3 PFU; LCMV WT)		% Survival (n=8)			
		6	7	8	12
Primary infection	PBS	100	25	0	-
	r3LCMV/WT	100	100	100	100
	r3LCMV/ts 3	100	100	100	100

Figure 13. Protective efficacy of r3LCMV/ts clone 3: Six-week-old B6 mice (N=8) were immunized (i.p.) with 10^5 PFU of r3LCMV/ts clone 3, r3LCMV/WT, or mock (PBS)-immunized. Four weeks later, mice were lethally challenged, i.c., with 10^3 PFU of LCMV WT and monitored daily for morbidity and mortality for 12 days.

3B. What opportunities for training and professional development has the project provided?

The project has provided training and professional development opportunities to Dr. Chengjin Ye. Dr. Ye is a post-doctoral fellow in Dr. Martinez-Sobrido's laboratory. He has been mentored by Dr. Martinez-Sobrido and his research during his post-doctoral training has mainly be focused in this project.

3C. Dissemination of results to communities of interest.

Our results have not yet been presented in any scientific venues because of the still ongoing COVID-19 pandemic that have prevented us to attend conferences.

3D. What do you plan to do during the next reporting period to accomplish the goals?
Nothing to Report.

4. IMPACT

4A. What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report.

4B. What was the impact on other disciplines?
Nothing to Report.

4C. What was the impact on technology transfer?
Nothing to Report

4D. What was the impact on society beyond science and technology?
Nothing to Report.

5. CHALLENGES/PROBLEMS

5A. Changes in approach and reasons for change
Progress on this project has been severely delayed and affected as result of several extenuating circumstances. Our laboratory relocated from University of Rochester to Texas Biomedical Research Institute in February 2020. Shortly after the relocation of our laboratory to Texas biomedical Research Institute, the SARS-CoV-2 pandemic was declared a national threat that limited operations and research capacity at Texas Biomedical Research Institute, including research on this project. In response to the start of the COVID-19 pandemic, San Antonio City and Bexar County officials implemented efforts to prevent the spread of the COVID-19 disease and maintain the health of our communities, including Texas Biomedical Research Institute. Texas Biomedical Research Institute Leadership acted quickly, in accordance with San Antonio City and Bexar County, to comply with efforts to ensure the safety and health of employees and their families. As within the rest of the country, several operations were impacted and limited, including starting our scientific work in the Institute. Moreover, and because of the pandemic, COVID-19 studies were prioritized for the severity of the pandemic, severely limiting research resources in other areas during this time. These limited operations created a backlog of IACUC approvals, BSC approvals, research studies, access to supplies, and laboratory operations. Because of these two unexpected reasons (relocation of the laboratory from University of Rochester to Texas Biomedical Research Institute and the COVID-19 pandemic), we have not been able to accomplish several of the goals initially outlined in the

proposal. With resources at Texas Biomedical Research Institute slowly increasing to reach closer to normal operations capacity recently, our laboratory is slowly establishing and gearing up to execute on contracts and grants. However, the emergency of several SARS-CoV-2 variants of concern (VoC) during the last two years since the emergence of the COVID-19 pandemic, Texas Biomedical Research Institute, San Antonio City, and Bexar County, have been impacted. For this specific project, remaining tasks were planned to be executed as soon as possible and although Texas Biomedical Research Institute leadership was committed to ensuring that we have resources available to successfully complete the goals of this research project the still ongoing pandemic impact, including the emerge of SARS-CoV-2 VoC during the last two years, have severely impacted the project. In addition, during this time, personnel at Texas Biomedical Research Institute and in the laboratory, have encounter cases of COVID-19 that also significantly affected progress in the project, especially during the emerge of SARS-CoV-2 delta and, more recently, omicron VoC. The pandemic's impact and prevalence during the last two years have been unpredictable, including resources available to successfully complete the work outlined in the proposal.

5B. Actual or anticipated problems or delays and actions or plans to resolve them

Two of the major problems with this project have been: 1) the relocation or our laboratory from University of Rochester to Texas Biomedical Research Institute in San Antonio, Texas, in February 2020; and, 2) the SARS-CoV-2 pandemic early 2020 (and still ongoing). After joining Texas Biomedical Research Institute, and because the SARS-CoV-2 pandemic, research at Texas Biomedical Research Institute, in response to the COVID-19 pandemic, was significantly affected.

5C. Changes that had a significant impact on expenditures

None

5D. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

None.

6. PRODUCTS

6A. Publications, conference papers, and presentations

Nothing to report.

6B. Website(s) or other Internet site(s)

Nothing to report.

6C. Technologies or techniques

Nothing to report.

6D. Inventions, patent applications, and/or licenses

Nothing to report.

6E. Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7A. What individuals have worked on the project?

Name: David J. Topham
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000000294358673
Nearest person month worked: 0 months
Contribution to Project: Dr. David Topham recently took over as contact PI at the University of Rochester upon Dr. Luis Martinez-Sobrido leaving the University of Rochester. Dr. Topham provides contract award administrative oversight. All work is being performed at Texas Biomedical Research Institute, under the direction of Dr. Martinez-Sobrido.
Funding Support: This award

Name: Luis Martinez-Sobrido
Project Role: Subaward PI
Researcher Identifier (e.g. ORCID ID): 0000-0001-7084-0804
Nearest person month worked: 0.96 months
Contribution to Project: Dr. Martinez-Sobrido directly oversaw or supervised all project studies. Dr. Martinez-Sobrido is well experienced in the molecular biology and virological aspects of arenavirus. He has worked on arenavirus for more than 10 years and is expert in the use of plasmid-based reverse genetics techniques to rescue recombinant arenavirus, the identification and characterization of arenavirus interferon antagonist proteins, the development of trisegmented and single-cycle infectious arenavirus, and in the implementation of the codon optimization approach for the generation of safe vaccines
for the treatment of arenavirus infections.
Funding Support: This award

Name: Chengjin Ye
Project Role: Post-doctoral fellow
Researcher Identifier (e.g. ORCID ID): Not available
Nearest person month worked: 7 months
Contribution to Project: Dr. Ye has been responsible of conducting the experiment described in this proposal. Dr. Ye has more than 5 years of experience working with negative- and positive-stranded RNA viruses, last of them in Dr. Martinez-Sobrido's laboratory. Dr. Ye is an expert in reverse genetic techniques to generate recombinant wild-type and mutated negative- and positive-stranded RNA viruses, and work with animal model of virus infection.
Funding Support: This award

7B. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes. In our initial proposal we included Snezhana Dimitrova as Technical Associate. Ms. Dimitrova has not relocated to Texas Biomed. There was a change in PI from Dr. Martinez Sobrido-Martinez to Dr. David Topham. Dr. Topham at the University of Rochester is the prime award/contact PI and Dr. Martinez-Sobrido is the Subaward PI at Texas Biomedical Research Institute.

7C. What other organizations were involved as partners?

None.

8. SPECIAL REPORTING REQUIREMENTS

None.

9. APPENDICES

OTHER SUPPORT

TOPHAM, D.J.

ACTIVE

HHSN272201400005C (Topham) 04/01/21-03/31/22 .6 Calendar
NIH/NIAID TPC
"NIAID Centers of Excellence in Influenza Research and Surveillance - Administrative support for the following non-severable options:

- Nayak Option 16D/16D EA - Effect of frequency and type of influenza vaccination on the development of the anti-influenza CD4 T cell and B cell response
- Topham Option 16J - Increasing the vaccine cross-protection against IAVs by improving the NA antigen response
- Sant Option 16I - Unique features of CD4 T cell epitope distribution, localization and effector functions elicited by intranasal administration of HA-ferritin vaccines
- Sant Option 17A EA - Potentiating broadly protective local immunity to influenza virus through a novel vaccine platform
- Subbararo Option 12B/12B EA – The effect of prior natural infection or vaccination ('imprinting') on subsequent response to influenza vaccine in children

Role: PD/PI

HHSN272201400005C (Topham) 08/30/18-03/31/22 0 Calendar
NIH/NIAID TPC
"NIAID Centers of Excellence in Influenza Research and Surveillance - 16J - Increasing the vaccine cross-protection against IAVs by improving the NA antigen response"
The deliverables of this study are (1) Development of CVVs that encode for currently recommended NA antigens with variations in stability AND (2) Evaluations of different strategies to extrinsically stabilize NA during the CVV isolation process and to increase NA yields from CVVs
Role: Project PI

HHSN272201400005C (Topham) 08/30/18-09/30/21 0 Calendar
NIH/NIAID TPC
"NIAID Centers of Excellence in Influenza Research and Surveillance – 16GEA - Memory B cell and serum antibody responses compared in healthy adults receiving licensed seasonal influenza vaccines…"
This project addresses whether adaptations in the influenza hemagglutinin during culture in eggs affects the antigenicity of the vaccine, diminishing induction of protective immunity. Further, it addresses the goals of understanding vaccine failures, which is the major overall theme of the NYICE and CEIRS
Role: Project PI

HHSN272201400005C (Topham) 04/08/20-05/20/22 .6 Calendar
NIH/NIAID TPC
"NIAID Centers of Excellence in Influenza Research and Surveillance – 12CEA - Natural history of SARS-CoV-2 in comparison to influenza A virus: a multi-site study focused in the Southern Hemisphere and equatorial regions"

The COVID-19 pandemic demands a strong and sustained research response aimed at informing policy decisions and the design of effective countermeasures. To help meet this challenge, the CEIRS Network proposes a unified human surveillance effort designed to gather critical information on the spectrum of disease, risk factors, duration of viral shedding, viral genomics, viral dynamics within and between populations an innate and memory immune responses to infection.
Role: Project PI

5 P01 AI102851-07 (Fowell) 9/1/19-8/31/24 2.4 Calendar
NIH/NIAID TPC
"Tissue Regulation of T Cell Function: Project 3 - Formation, Positioning, Motility, and Function of Tissue Resident Memory CD8+ T cells After Influenza Infection" (TPC - Topham, Project 3)
To determine the mechanisms that determine differentiation, establishment, and maintenance of TRM subsets after influenza infection; Investigate mechanisms of T cell-epithelial cell-matrix interactions required for motility and positioning in the airways; and determine the functions of CD49a and CD103 in optimizing immune protection.
Role: Project 3 PI

5 R01 AI129988-04 (Takimoto) 09/25/17-08/31/22 1.2 Calendar
NIH/NIAID TPC
"Influenza virus host shutoff mechanism"
The proposed research will characterize the novel influenza virus protein PA-X for its activity to shutoff host gene expression.
Role: Co-Inv

000792 - UNIV/Cornell Subaward (Topham) 08/08/17-07/31/22 .24 Calendar
NIH/NIAID - Prime 5 U01 AI131348-04 (Rudd) TPC (Rochester Subaward)
"Roles for Developmentally Regulated microRNAs in Neonatal Immunity"
The major goal of this grant is to identify the key gene regulatory networks that underlie cell-intrinsic differences between neonatal and adult CD8+ T cells.
Role: Subaward PI

5 UM1 AI148450-02 (Falsey, Branche) 12/11/19-11/30/26 1.2 Calendar
NIH/NIAID TPC
"University of Rochester Vaccine and Treatment Evaluation Unit (VTEU)"
The University of Rochester VTEU site can offer the necessary scientific, clinical, administrative, and organizational structure to support these endeavors: evaluation of vaccines, preventive biologics, therapeutics, diagnostics, predictive markers and devices for the treatment and prevention of infectious diseases.
Role: Co-Investigator

W81XWH1810071 (Topham) 05/01/18-10/31/21 0 Calendar
DOD/ARMY TPC
"Development of Live-Attenuated Old World Arenavirus Vaccines Based on Temperature-Sensitive Viruses"
The major goals of this project are to characterize the safety, immunogenicity and protective efficacy of selected rLCMV/ts in mice.
Role: PI

PENDING

R21 (Dumont) 09/01/21-08/31/23 .36 Calendar
NIH PC
"Development of the S2 subunit of the SARS-CoV-2 Spike as an Immunogen"
The overall goal of this project is to develop new classes of immunogens that can elicit immune responses directed at the spike S2 subunit as a step towards development of escape-resistant pancoronavirus vaccines.
Role: Co-Investigator

R21 AI159415 Resubmission (Thakar) 09/01/21-08/31/23 .6 Calendar
NIH TPC
"Mechanistic modeling to discover control in unterferon and cytokine/chemokine signaling in influenza virus and coronavirus infections"
We propose to re-use valuable open- access data collected by BRCs to address the significant research gap in understanding the balance of early innate anti-viral response to influenza and SARS-CoV2 across viral strains and cell-types.
Role: Co-Investigator

U01 AI165330 (Topham, Evans, Falsey) 01/01/22-12/31/23 1.2 Calendar
NIH/NIAID TPC
"A Phase 1, Open-Label Study to Evaluate the Effect of Varied Boosting Regimens on the Immune Response to ChAdOx1-MERS"
We intend to conduct a large, first-of-its-kind 10-arm study to examine the relation between pre-immune responses and post-boost measurements, and to begin to build models that may be used by other investigators to further this area of investigation.
Role: MPI

DP2 (Anderson) 07/01/21-06/30/26 .24 Calendar
NIH TPC
"The airway microbiota as a driver of clinical severity following influenza infection"
The goal of this proposal is to test how host responses to influenza virus infection are modulated by the airway microbiota
Role: Co-Investigator

R21 (McGrath) 07/01/21-06/30/23 .24 Calendar
NIH TPC
"Small extracellular vesicle-based detection of anti-viral B cell subsets"
We propose to develop a novel method to detect these antigen specific B cell subtypes, including those resident in lymphoid organs and peripheral tissues. If successful, this could be adapted into a high throughput method for rapid screening of populations to assess their B cell subset responses to specific antigens.
Role: Co-Investigator

OVERLAP
There is no scientific overlap in the above applications.

MARTINEZ-SOBRIDO, LUIS

ACTIVE

R01AI141607 PI: de Figueiredo 2/1/2020 – 5/31/2024 0.60 calendar
NIH/NHLBI
Development of a High-Throughput Microfluidics-Enabled Functional Assay for Rapidly Identifying Neutralizing Antibodies
Goal: The major goal of this project is to test the working hypothesis that PRESCIENT can identify in a mixed population of individual human hybridoma cells neutralizing antibodies against pH1N1 virus.
Specific Aims: We will generate stocks of influenza pandemic A/California/04/09 H1N1 (pH1N1) and A/Wyoming/3/03 H3N2 influenza A viruses as well as influenza B/Brisbane/60/08 mCherry-expressing viruses that will be used to optimize the performance of PRESCIENT (Aim 1), to test the working hypothesis that PRESCIENT can identify in a mixed population of individual human hybridoma cells neutralizing antibodies against pH1N1 virus (Aim 2) and to identify influenza A H1N1, H3N2; and B virus neutralizing antibodies from EBV-immortalized patient-derived B cells (Aim 3).
Role: Co-Investigator
Program Officer: Mary Chelsea Lane, NIH/NIAID, BG 5601FL RM 8A19, 5601 Fishers Lane, Rockville, MD 20852
No overlap

R01 AI142985 PI: de la Torre 2/1/2020 – 3/31/2023 0.90 calendar
National Institutes of Health
Roles of the Nucleoprotein 3'-5' Exonuclease Domain in Arenavirus Biology
Goal: The major goals of this project are to provide a better understanding of arenavirus-host innate defense interactions, which can facilitate the development of novel strategies to combat human pathogenic mammarenaviruses.
Specific Aims: Morbidity and mortality associated with human mammarenavirus infections involve a failure of the host's innate immune response to restrict virus multiplication at early stages of infection, thus compromising the initiation of an effective innate and adaptive immune response to control and eliminate the virus. Studies in this application will provide a better understanding of arenavirus-host innate defense interactions, which can facilitate the development of novel strategies to combat human pathogenic mammarenaviruses.
Role: Co-Investigator
Program Officer: Lesley Conrad Dupuy, NIH/NIAID, BG 5601FL RM 8E66, 5601 Fishers Lane, Rockville, MD 20852
No overlap

R01 AI145332 PI: Kobie 2/1/2020 – 8/31/2023 0.96 calendar
National Institutes of Health
Dynamics of the protective vaccine-induced human influenza neuraminidase B cell response
Goal: The major goals of this project are to define how various influenza vaccine types impact the characteristics of neuraminidase specific antibodies in humans and what properties of neuraminidase specific antibodies are most effective at preventing influenza infection against diverse strains.
Specific Aims: Dr. Martinez Sobrido will coordinate all the studies related to assess the ability of IIV-induced human NA B cell lineages to inhibit infection and transmission (Aim 2). He will coordinate with Dr. Kobie all the studies related to evaluate the Fc -dependence of NA-specific mAb protection

mechanism (Aim 3). He will also be responsible for obtaining the required IACUC approval for the **experiments proposed in this application that would require the use of animals (Aims 2 and 3).**
Role: Co-Investigator
Program Officer: Sonnie Kim, NIH/NIAID, BG 5601FL RM 8E12, 5601 Fishers Lane, Rockville, MD 20852
No overlap

R01 HL091968 PI: O'Reilly 2/1/2020 – 8/31/2022 0.42 calendar
National Institutes of Health
Effects of Neonatal Hyperoxia on Alveolar Development and Infection
Goal: The major goal of this project is to test the hypothesis that neonatal hyperoxia enhances sensitivity to IAV infection by inducing epigenetic changes in proliferating AEC2s that are maintained even when they become AEC1s and these changes are mediated by the persistent expression of Ki67.
Specific Aims: Preterm infants are often exposed to an inappropriate oxygen environment at birth that causes persistent lung disease later in life through poorly understood mechanisms. This application uses a unique mouse model to understand how excess oxygen at birth alters lung development and the host response to influenza A virus infection. The research is important because the scientific discoveries could stimulate development of new therapies designed to improve health of people born preterm.
Role: Co-Investigator
Program Officer: Aruna R Natarajan, NHLBI, BG RKL1 RM 403-A1, 6705 Rockledge Drive, Bethesda, MD 20817
No overlap

N/A PI: Ivanov 7/1/2020 – 6/30/2022 (NCE) 0.24 calendar
San Antonio Partnership for Precision Therapeutics
Blocking SARS-CoV-2 evasion from innate antiviral defenses
Goal: The major goal of this project is to determine the effectivity of AT-100 post-infection in blocking SARS-CoV-2 infectivity.
Specific Aims: Aim 1: Development of a robust HTS assay of Orf10-CUL2 binding. (2) Aims 1 & 2: Identification of small-molecule inhibitors of Orf10-CUL2 binding. (3) Aim 3: Development of the Orf10 SARS-CoV-2 virus and its pilot evaluation using cell-based assays and the mouse model. (5) Pilot synthesis of a targeted compound library to evaluate structure-activity relationships for one most promising molecular scaffold.
Role: Co-Investigator
Grants Officer: Liz Tullis, San Antonio Partnership for Precision Therapeutics
No overlap

N/A PI: Gupta 7/1/2020 – 6/30/2022 (NCE) 0.24 calendar
San Antonio Partnership for Precision Therapeutics
Mechanism-based Targeting of an RNA Processing Pathway of SARS-CoV-2
Goal: We aim to determine the structural basis of RNA processing in host immune and inflammatory response critical for viral survival and growth and will conduct HTS screening, medicinal chemistry, and in vitro and in vivo studies towards rapid clinical translation.
Specific Aims: 1) Identification of novel antiviral ligands; 2) On-target inhibitors of SARS-CoV-2 nsp10, 13, 14, 16; and, 3) A targeted drug discovery platform for SARS-CoV-2 and emerging coronaviruses.
Role: Co-Investigator
Grants Officer: Liz Tullis, San Antonio Partnership for Precision Therapeutics
No overlap

W81XWH1910496 PI: Martinez-Sobrido 5/1/2020 – 10/31/2023 0.90 calendar
Department of Defense
Development of Live-Attenuated Vaccine Platform Against Hemorrhagic fever causing Arenaviruses
Goal: The major goal of this project is to demonstrate that recombinant forms of the prototypic mammarenavirus lymphocytic choriomeningitis virus (LCMV) containing a codon deoptimized (CD) nucleoprotein (NP) expressing glycoproteins (GP) of HF-causing LASV and JUNV (rLCMV/NPCD/GPHF) can be used for the development of a safe and protective individual or blended live-attenuated vaccine (LAV) to combat HF disease caused by LASV and JUNV infections.
Specific Aims: Aim 1. Generate and characterize rLCMV/NPCD expressing GPs from LASV and JUNV (rLCMV/NPCD/GPHF). Aim 2. Determine the stability of rLCMV/NPCD/GPHF in cultured cells and in vivo. Aim 3. Assess the safety and immunogenicity in mice of individual and blended rLCMV/NPCD/GPHF. Aim 4. Potential of a rLCMV/NPCD/GPHF individual and blended approach as a safe and cross-protective LAV against HF-causing arenaviruses in guinea pigs.
Role: Award PI
Grants Management Specialist: Stephanie Davis, 820 Chandler Street, Fort Detrick, MD 21705-5104
No overlap

INV-019155 PI: Torrelles 9/16/2020 – 12/31/2022 1.80 calendar
Bill & Melinda Gates Foundation
COVID-19 CTA: Preclinical models for SARS-CoV-2 infection
Goal: The major goal of this project is to determine if human monoclonal antibodies (hmAbs) shown in vitro to block SARS-CoV-2 infection are also effective, alone or in combination, in vivo using two rodent animal models of SARS-CoV-2 infection and associated COVID-19 disease.
Specific Aims: Evaluate a dose titration of the positive control reference standard mAb in both the mouse and hamster models in prophylactic mode (mAb administered 24hr prior to virus challenge) and therapeutic mode (mAb administered 24hr after virus challenge). Evaluate efficacy of mAbs from CoVIC or other grantees/partners of the foundation as requested, singly or in combination, using a single dose level in prophylactic mode in one of the two models (TBD) following the experimental time-line.
Role: Co-Investigator
Grants Officer: Jacqueline Kirchner, P.O. Box 23350, Seattle, WA 98102
No overlap

W81XWH2110095 PI: Shetty 2/1/2021 – 1/31/2023 0.6 calendar
Department of Defense
Development of a Novel Drug Candidate, CSP7, for the Treatment of COVID-19
Goal: The main goal of this project is to determine the efficacy of the novel drug candidate, CSP7, for the treatment of COVID-19.
Specific Aims: 1: To determine whether systemically delivered CSP7 improves overall survival by inhibiting ALI and accelerated remodeling associated with CoV2 infection using hACE2 mice. 2: To determine whether better efficacy can be achieved by airway inhalation of CSP7 DP using hACE2 mice infected with CoV2.
Role: Co-Investigator
Grants Officer: Abigail Strock, Fort Detrick CDMRP 1120 Fort Detrick, Federick, MD 21702
No overlap

W81XWH2110103 PI: Pertsemlidis 2/01/2021 – 1/31/2023 0.60 calendar
Department of Defense
SARS-CoV-2 Viral RNAs as Unique Biomarkers and Therapeutic Targets

Goal: The purpose of this study is to identify and characterize novel small RNAs produced by SARS-CoV-2 and exploit them as biomarkers and therapeutic targets. We propose a combination of computational biology, synthetic biology, genome editing, and chemistry approaches. The successful delivery of a v-miRNA inhibitor in cell culture will establish a foundation for preclinical and clinical characterization.
Specific Aims: First, we will combine miRNA prediction, miRNA target prediction, and small RNA expression profiling of infected cells, to identify the unique small RNAs produced by the SARS- CoV-2 virus. Second, we will develop novel v-miRNA sensors based on converting an analog (miRNA) input into a digital output and we will tune the sensitivity and specificity of the sensor and evaluate v-miRNAs as biomarkers of the presence of SARS-CoV-2 infection through quantifying v-miRNAs levels in patient biomaterials. Third, we will optimize the chemistry and delivery of an inhibitor of v-miRNAs in vitro, through incorporation of modified bases and backbone chemistries and conjugation with a ligand for the receptor exploited by SARS-CoV-2 during infection. Fourth, we will evaluate inhibition of v-miRNAs as a therapeutic strategy for the treatment of COVID-19.
Role: Co-Investigator
Grants Officer: Abigail Strock, Fort Detrick CDMRP 1120 Fort Detrick, Federick, MD 21702
No overlap

Subcontract No. ID07200010-501-1PI: Mallory 7/01/2021 – 9/25/2022 0.38 calendar Department of Defense
Viral and Microbial Decontamination Strategies for Medical Treatment Facilities, Close Quarters, and Austere Settings (Zeolites task order)
Goal: The major goals of this project are to test the hypothesis that surfaces treated with zeolite will become inhospitable to bacterial and destroy the virulence of viruses coming in contact with treated surfaces for extended periods of time without reapplications.
Specific Aims: Aim 1: Synthesis of zeolites, transition-metal exchanged zeolites, characterization and formulation of coatings. Aim 2: Assess the acute antimicrobial efficacy of zeolites on textiles, hard surfaces, and other synthetic materials in a controlled laboratory setting. Aim 3: Determine potential synergistic and temporal effects of zeolites in pro-oxidant environments against coronaviruses including SARS-CoV-2 and bacteria.
Role: Co-Investigator
Contracting Officer: David J. Kilbourne, GSA
No overlap

Subcontract No. ID07200010-501-2 PI: Mallory 7/01/2021 – 9/25/2022 0.25 calendar Department of Defense
Viral and Microbial Decontamination Strategies for Medical Treatment Facilities, Close Quarters, and Austere Settings (CIMR Efficacy task order)
Goal: The major goals of this project are testing the efficiency of a H2O2 generating Continuous Infection Microbial Reduction device (CIMR) against SARS-CoV-2 and other respiratory pathogens, such as viruses: Influenza (seasonal H1N1/H3N2, and influenza B) and adenovirus; bacteria [Streptococcus pneumonia (Gram positive), and Legionella pneumophila (gram negative)]; and mold (Aspergillus fumigatus).
Specific Aims: Aim 1: Synthesis of zeolites, transition-metal exchanged zeolites, characterization and formulation of coatings. Aim 2: Assess the acute antimicrobial efficacy of zeolites on textiles, hard surfaces, and other synthetic materials in a controlled laboratory setting. Aim 3: Determine potential synergistic and temporal effects of zeolites in pro-oxidant environments against coronaviruses including SARS-CoV-2 and bacteria.
Role: Co-Investigator
Contracting Officer: David J. Kilbourne, GSA

No overlap

1R43AI165089-01 PI: Huang 7/9/2021 – 6/30/2022 0.6 calendar
National Institutes of Health
Developing a Thermostable SARS-CoV-2 RBD-particle Vaccine
Goal: Our overall goal is to demonstrate that RBD-particles can elicit robust and protective antibody responses against SARS-CoV-2 with thermostability after lyophilization.
Specific Aims: To assess the impact of lyophilization on the conformational and thermal stability of the resulting lyophilized vaccine, evaluated by biochemical and biophysical assays, and its efficacy will be assessed by functional immunogenicity in mice. This project will assess the feasibility of breaking the cold-chain requirements for a next-generation particle vaccine system, which could be critical for resource-limited settings. A transgenic mouse model for SARS-CoV- 2 infection will be used to study the thermostability on protection induced by the lyophilized, RBD particle vaccine.
Role: Co-Investigator
Grants Management Specialist: Ashley Colette Ranellone No overlap

1R01AI161363-01 PI: Gupta 8/01/2021 – 7/31/2026 0.6 calendar
National Institutes of Health
Mechanism-based Targeting of the RNA Processing Machinery of SARS-CoV-2
Goal: The major goal of this project is to determine the prophylactic and therapeutic activities of the identified compounds in vitro in cultured cells and in vivo in K18 human angiotensin converting enzyme 2 (hACE2) transgenic (wild-type SARS-CoV-2) or wild-type (mouse adapted SARS-CoV-2) mice and/or golden Syrian hamsters to determine their antiviral activity against SARS-CoV-2.
Specific Aims: In Aim 1, we will resolve a series of new structures of nsp16/nsp10 proteins captured in every step of the methyl transfer by X-ray crystallography. The structural data will be validated by detailed biochemical and biophysical studies. We will resolve the biochemical and structural determinants of the assembly of viral RNA capping machinery, and identify factors underlying integrity of RNA genome. In Aim 2, we will develop a novel molecular tool to study temporal distribution of the RNA methylation during viral infection. We will examine new models for combinatorial inhibition of viral proteins by drug repurposing or novel small molecules. Finally, we will use our recently established reverse genetics approaches based on the use of a bacterial artificial chromosome (BAC) to generate recombinant (r)SARS-CoV2 containing mutations in nsp16 to determine their contribution in viral replication in cultured cells and pathogenesis in vivo using our recently described K18 human angiotensin converting enzyme 2 (hACE2) mouse model of SARS-CoV-2 infection and associated coronavirus disease 2019 (COVID-19).
Role: Co-Investigator
Grants Management Specialist: Nicole A. Guidetti
No overlap

Subaward from UTHSCSA PI: Gupta 9/1/2021 – 8/31/2022 0.01 calendar
San Antonio Medical Foundation
Repurposing Drugs for COVID-19 Therapy
Goal: The major goal of this project is to evaluate the toxicity and anti-SARS-CoV-2 activity of the selected compounds with in vitro using cell cultures and in vivo using mice/hamsters.
Specific Aims: Aim 1. Repurposing of Drugs for COVID-19 therapy. Aim 2: In vitro and in vivo testing of antiviral compounds.
Role: Co-Investigator
Program Officer: Pam Leissner, San Antonio Medical Foundation, PO Box 29736, SATX 78229
No overlap

75N93021C00014 PI: Garcia-Sastre 09/01/2021 – 9/01/2023 0.6 calendar
Centers of Excellence for Influenza Research and Response
A multi-fluorescent microneutralization assay (MFMA) to rapidly evaluate SARS-CoV-2 neutralizing antibody (NAb) responses (NIAID Centers of Excellence for Influenza Research and Response)
Goal: The major goal of this project is to develop a novel multi-fluorescent microneutralization assay (MFMA) using different fluorescent-expressing recombinant (r)SARS-CoV-2 for the rapid, sensitive, and simultaneous identification of neutralizing antibody (NAb) responses against SARS-CoV-2, including those with broadly neutralizing activity against variants of concern (VoC), within the same well.
Specific Aims: Aim 1: We will generate both wild-type (WT) and fluorescent rSARS-CoV-2, including SARS-CoV-2 USA-WA1/2020 (WA-1), and B.1.351 (South Africa), P.1. or 20J/501Y.V3 (Japan), B.1427 (California), and B.1.617 (India) VoC. Aim 2: We will demonstrate that our approach provides a robust and reproducible signal of sufficient intensity that can be easily measured above background levels during individual infection with different fluorescent rSARS-CoV-2 to identify NAbs using a collection of well-characterized hMAbs available in our laboratory.
COR and Program Officer: Marciela M. DeGrace, NIAID/NIH/DHHS
Role: Pilot grant PI
No overlap

1R01AI161175-01A1 PI: Kobie 9/01/2021 – 8/31/2024 1.2 calendar
National Institutes of Health
The origin and future protective activity of SARS-CoV-2 RBD specific neutralizing antibodies
Goal: The major goal of this project is to perform the experiments related to the use of SARS-CoV-2 in vitro and in vivo on the mouse model.
Specific Aims: Perform the experiments related to the use of SARS-CoV-2 in vitro and in vivo on the mouse model.
Program Officer: Erik J. Stemmy, National Institute of Allergy and Infectious Diseases
Role: Co-Investigator
No overlap

75N95C21P00127 PI: Martinez-Sobrido 9/03/2021 – 8/25/2022 0.01 calendar
National Institutes of Health
Evaluating the efficacy of ROC-35 in a SARS-CoV-2 humanized ACE2-transgenic mouse model
Goal: The major goal of this project is to evaluate the efficacy of ROC-35 in a SARS-CoV-2 humanized ACE2-transgenic mouse model.
Contracting Officer: Rhanda Lopez, National Center for Advancing Translational Sciences
Role: Award PI
No overlap

Research Collaboration Agreement PI: Martinez-Sobrido 5/15/2022 – 5/14/2025 0.01 calendar
IrsiCaixa (Spain)
Novel monoclonal antibodies to halt the entry of enveloped viruses into immune cells
Goal: The main goal of this project will be testing the ability of α-Siglec-1 mAbs to block enveloped virus in animal models.
Role: PI
No overlap

CDMRP Stanford Subaward PI: Einav 9/30/2022 – 9/30/2026 0.60 calendar
Congressionally Directed Medical Research Program
Repurposing of pan-ErbB inhibitors to protect from coronaviral infection, inflammation and lung injury

Goal: The overall goal of this proposal is to test the antiviral activity of pan-ErbB inhibitors against coronavirus infection, inflammation and lung injury.
Role: Co-Investigator
No overlap

PENDING

INV-033376 PI: Truong 6/1/2022 – 12/31/2023 1.20 calendar
Bill & Melinda Gates Foundation
Prevention of Respiratory Viruses Aerosols Transmission Using Inhaled VHH mAbs
Goal: The major goal of this project is to evaluate the ability of human monoclonal antibodies to prevent infection and transmission of SARS-CoV-2 and influenza virus in hamsters.
Specific Aims: Determine 1) whether antiviral mAbs manufactured in spirulina are well tolerated when inhaled; and 2) whether inhaled mAbs that are directed in the upper airways (e.g. nasopharynx) are effective in reducing aerosol transmission from infected hosts and preventing aerosol infection to naïve individuals. Role: Co-Investigator
No overlap

P01 Subaward (21-109) PI: Modjarrad 6/1/2022 – 5/31/2024 0.60 calendar
National Institutes of Health
Pan-coronavirus vaccine utilizing adjuvanted Spike- and RBD-ferritin nanoparticles
Goal: The overall goal of this P01 proposal is to develop a universal vaccine for the treatment of coronavirus infections.
Specific Aims: Project 1**-Specific Aim 1. Determine the antibody specificity, and function of antibodies elicited in non-human primates by SARS-CoV-2 and MERS-CoV immunogens. Specific Aim 2. Determine the antibody repertoire, specificity, and function of antibodies elicited in SARS-CoV-2 SpFN-vaccinated individuals. Specific Aim 3. Determine how prior MERS-CoV exposure, whether by vaccination or previous infection, shapes B cell responses following COVID-19 infection or vaccination.** Project 2**-Specific Aim 1. Design and assess next-generation pan-sarbecovirus nanoparticle immunogenicity. Specific Aim 2. Design and assess pan-merbecovirus nanoparticle immunogenicity. Specific Aim 3. Down-select a pan-sarbecovirus and pan-merbecovirus vaccine in murine and hamster challenge studies. Specific Aim 4. Test the protective effect of a pan-sarbecovirus vaccine and a pan-merbecovirus vaccine in sarbecovirus and merbecovirus NHP challenge studies.**
Role: Co-Investigator
No overlap

TSRI LASV U19 subaward PI: Chanda 6/1/2022 – 5/31/2027 0.60 calendar
National Institutes of Health
Center for Antiviral Medicines & Pandemic Preparedness (CAMPP) – Project 6
Goal: Our contribution to project 6 in this U19 is to assess the antiviral activity of compounds against LASV in vitro.
Role: Project Lead
No overlap

TSRI AViDD U19 TSRI Subaward PI: Chanda 6/1/2022 – 5/31/2027 0.24 calendar
National Institutes of Health
Center for Antiviral Medicines & Pandemic Preparedness (CAMPP) – Project 4

Goal: Our contribution to this project will be to test the antiviral activity of compounds against LASV.
Role: Co-Investigator
No overlap

Emory AViDD U19 Subaward PI: Painter 6/1/2022 – 5/31/2027 1.20 calendar
National Institutes of Health
Antiviral Countermeasure Development Center (AC DC)
Goal: The goal of this application is to participate in a research project to identify antivirals for the treatment of SARS-CoV-2 infection in vitro and in vivo (NHP) as part of the Emory Antiviral Countermeasure Development Center (AC DC) U19.
Role: Co-Investigator
No overlap

UCSF U19 subaward PI: Krogan 6/1/2022 – 5/31/2027 0.60 calendar
National Institutes of Health
QCRG (QBI Coronavirus Research Group) Pandemic Response Program
Goal: The overall goal of this U19 is to find antivirals for the treatment of SARS-CoV-2 and other viral infections. Research at Texas Biomed will focus on developing recombinant SARS-CoV-2.
Role: Project Lead
No overlap

UTHSCSA R01 Subaward PI: Muniswamy 7/1/2022-6/30/2027 0.24 calendar
National Institutes of Health
SARS-CoV-2 Infection Enhances Mitochondrial PTP Complex Activity to Perturb Cardiac Energetics in Humans
Goal: The major goal of this project is to assess changes in mitochondrial complexes during SARS-CoV-2 infection.
Role: Co-Investigator
No overlap

U. of Chicago R01 Subaward PI: Chen 7/1/2022-6/30/2024 0.30 calendar National Institutes of Health **SARS-CoV-2-Induced Host Organelle Remodeling: Impacts on Pathogenesis**
Goal: The overall goal of this proposal is to assess how SARS-CoV-2 infection affects intracellular remodeling of organelles and the impact on SARS-CoV-2 infection and associated COVID-19 disease.
Role: Co-Investigator
No overlap

Mt. Sinai R01 Subaward PI: Johnson 7/1/2022-6/30/2024 0.24 calendar National Institutes of Health
Functional interactions between mitogen-activated protein kinases (MAPKs) and SARS-CoV-2
Goal: The overall goal of this collaborative proposal will assess the interaction between MAPKs (mitogen-activated protein kinases) and SARS-CoV-2.
Role: Co-Investigator
No overlap

UAB R01 Subaward PI: Kobie 7/1/2022-6/30/2024 0.60 calendar
National Institutes of Health

Induction of universal coronavirus S2-based humoral immunity
Goal: In this R01 proposal we will identify and characterize, in vitro and in vivo, human monoclonal antibodies targeting the S2 domain of the SARS-CoV-2 spike protein.
Role: Co-Investigator
No overlap

UAB R21 Subaward PI: Petit 7/1/2022-6/30/2024 0.60 calendar
National Institutes of Health
Host-pathogen interactions of SARS-CoV-2 nonstructural protein 1
Goal: Our contribution to this project will be to generate recombinant SARS-CoV-2 containing mutations in the nsp1.
Role: Co-Investigator
No overlap

R01 Subaward PI: Sahay 7/1/2022-6/30/2023 0.60 calendar
National Institutes of Health
Rapid generation of decoy ACE2 using mRNA based nano-therapeutics for the treatment of COVID-19
Goal: We will evaluate the ability of nanotherapeutic-delivered mRNA to inhibit SARS-CoV-2 infection in vivo. We will test whether LNP delivered hsACE2 can inhibit live SARS-CoV-2 virus infection in K18-hACE2 mice model.
Role: Co-Investigator
No overlap

R01 Subaward PI: de la Torre 7/1/2022-6/30/2027 0.60 calendar
National Institutes of Health
Novel chemical proteomic approaches to discover druggable SARS-CoV-2-host cell protein interactions
Goal: The overall goal of this proposal is to determine the effects of primary hits on seasonal CoV OC43, SARS-CoV, MERS-CoV, and influenza A virus (IAV) in SA 1; characterize the mechanisms of action of validated hits on CoV OC43, SARS-CoV, MERS-CoV, and IAV in SA2; and, assess the effect on SCoV2 infection in vivo of existing drugs for identified FFF host target proteins in SA 3.
Role: Co-Investigator
No overlap

R01 Subaward PI: Veiseh 9/1/2022-8/31/2027 0.30 calendar
National Institutes of Health
Cell Based Immunomodulation of SARS-CoV2 Induced Lung Inflammation and Damage
Goal: Our contribution to this project will be the delivery of optimal capsule candidates(s) in the hamster and NHP models to the respiratory track and perform the studies/measures proposed.
Role: Co-Investigator
No overlap

R01 Subaward PI: de la Torre 12/1/2022-11/30/2027 0.72 calendar
National Institutes of Health
Creating a Lassa Fever Live-Attenuated Vaccine with Unbreachable Attenuation
Goal: Our contribution to this project will be the delivery of optimal capsule candidates(s) in the hamster and NHP models to the respiratory track and perform the studies/measures proposed.
Role: Co-Investigator
No overlap

SBIR Subaward PI: Tung 12/1/2022-11/30/2024 0.30 calendar
National Institutes of Health
Lentiviral vector presenting SARS-CoV-2 protein
Goal: The overall goal of this project is to analyze several vaccine candidates providing protection against SARS-CoV-2 infection, evaluating infection dynamics, lung immunopathology, neutralizing antibody titers, etc. in vaccinated and unvaccinated mice.
Role: Co-Investigator
No overlap

SBIR Subaward PI: Branco 12/1/2022-11/30/2025 0.36 calendar
National Institutes of Health
Evaluation of Highly Protective Pan-Lassa Virus Human Monoclonal Antibodies as Candidate Therapeutics for LCMV Infection
Goal: The objective of this project is to evaluate the dose-dependent protective efficacy and pharmacokinetics (PK) of individual GP-specific BNhMAbs against LCMV.
Role: Co-Investigator
No overlap

R21 Subaward PI: Tumanov 12/1/2022-11/30/2024 0.30 calendar
National Institutes of Health
Novel animal models to study organ-specific SARS-CoV-2-induced pathology
Goal: The main goals of this award are to test if hACE2 expression restricted to type II alveolar epithelial cells and club cells is necessary and sufficient for SARS-CoV-2-induced lung immunopathology and also to test if SARS-CoV-2 infection exacerbates intestinal inflammation using mice with specific expression of hACE2 in intestinal epithelial cells.
Role: Co-Investigator
No overlap

U19 Subaward PI: Garcia-Sastre 01/01/2023-12/31/2027 0.60 calendar
National Institutes of Health
SARS-CoV-2 adaptations through a Systems Biology Lens (SYVIL) – Technology Core B
Goal: The overall goal of this U19 application is to characterize SARS-CoV-2 host interactions to understand the biology of viral infections.
Role: Co-Investigator
No overlap

CDMRP PI: Martinez-Sobrido 10/01/2022-09/30/2024 0.24 calendar
Congressionally Directed Medical Research Programs
A live-attenuated vaccine for the treatment of SARS-CoV-2 infection
Goal: The central goal of this application is to test the novel hypothesis that a codon deoptimized (CD)-based approach can be used for the development of a safe, immunogenic, and protective live-attenuated vaccine (LAV) to combat COVID-19 disease caused by SARS-CoV-2 infection.
Role: PI
No overlap

CDMRP PI: Torrelles 10/01/2022-09/30/2024 0.24 calendar
Congressionally Directed Medical Research Programs

Novel animal models to study organ-specific SARS-CoV-2-induced pathology
Goal: The goal of this proposal is to characterize these novel mouse models and define the impact of SARS-CoV-2 specifically on lung immunopathology and intestinal disease. Our central hypothesis is that mice with conditional expression of hACE2 in Rosa26 locus represent a robust platform to study tissue-specific SARS-CoV-2-induced pathology.
Role: Co-PI
No overlap

PREVIOUS

7R21AI135284-03 PI: Martinez-Sobrido 2/1/2020 – 12/31/2021 0.40 calendar
National Institutes of Health
Attenuation of Lassa virus via codon deoptimization
Goal: This project will characterize the safety, immunogenicity and protective efficacy of selected rLCMV/ts in mice. This contract involves virulence, immunogenecity and protective efficacy studies.
Specific Aims: 1. Generate and characterize LCMV/LASVGPCD. 2. A Lassa Fever Live-Attenuated Vaccine Based on Codon Deoptimization of the Viral Glycoprotein Gene. 3. Assess the safety, immunogenicity and protective efficacy of rLCMV/LASVGPCD in mice. 4. Assess the safety and potential of rLASV/GPCD as a LAV against LASV.
Role: Award PI
Program Officer: Patricia Repik, NIH/NIAID, Bldg. 5601FL RM8E59, 5601 Fishers Lane, Rockville, MD 20852
No overlap

W81XWH1810071 PI: Topham 2/1/2020 – 10/31/2021 0.60 calendar
Department of Defense
Development of Live-Attenuated Old World Arenavirus Vaccines Based on Temperature-Sensitive Viruses
Goal: This project will characterize the safety, immunogenicity and protective efficacy of selected rLCMV/ts in mice.This contract involves virulence, immunogenecity and protective efficacy studies.
Specific Aims: 1. Identify the mutations responsible for the temperature sensitive phenotype of LCMV. 2. Determine the genetic and phenotypic stability of rLCMV/ts. Characterize the safety, immunogenicity and protective efficacy of selected rLCMV/ts.
Role: Co-Investigator
Grants Specialist: Lisa Sawyer, lisa.m.sawyer22.civ@mail.mil, 810 Schreider Street, Fort Detrick, MD 21702-5000
No overlap

HHSN272201400005C PI: Topham 04/01/18-03/31/21 0.6 calendar
NIH/NIAID
Centers for Excellence on Influenza Research and Surveillance - Pandemic response to influenza virus infections
Major goal: To conduct a pandemic research plan that includes pre-pandemic risk assessment and emergency pandemic response plan.
Specific Aims: To characterize influenza strains and establish a pandemic research plan including pre-pandemic risk assessment and emergency pandemic strains.
Role: Project PI

Program Officer: Diane J. Post, NIH/NIAID/DMID, Room 3212, 6610 Rockledge Drive, Bethesda, MD 20817
No overlap

HHSN272201400005C PI: Topham 02/01/20-03/15/21 0.6 calendar
NIH/NIAID
Centers for Excellence on Influenza Research and Surveillance – Option 15B - Natural history and mechanism of protection of human vaccine induced influenza B virus (IBV) neuraminidase (NA) broadly neutralizing monoclonal antibodies (BNhmAbs)
Goal: The overall goal of this application is to develop and validate, for the first time, a novel MFMA using different fluorescent-expressing influenza viruses for the rapid, sensitive and simultaneous identification of NAbs, including broadly NAbs, against currently circulating IAV and IBV within the same well.
Specific Aims: to determine the natural history of how influenza B virus (IBV) broadly neutralizing human monoclonal antibodies (BNhmAbs) are induced in response to seasonal vaccination and their ability to prevent IBV infection and transmission, and their mechanism of action. This knowledge will provide the basis for the development of immunotherapeutics to fill the existing gap for treatments against IBV. Moreover, a detailed characterization of the conserved epitopes recognized by these IBV NA BNmAbs would facilitate the generation of immunogens able to induce similar broadly neutralizing Ab responses in humans and the development of a universal vaccine to combat IBV.
Role: Project PI
Program Officer: Diane J. Post, NIH/NIAID/DMID, Room 3212, 6610 Rockledge Drive, Bethesda, MD 20817
No overlap

DOD PR171042 PI: Martinez-Sobrido 05/01/18-10/31/20 1.2 calendar
W81XWH-1810071-PRMRP-DA
Identification and characterization of temperature sensitive mutations in Old World Arenavirus
Major Goal: The central goal of this application is to test, for the first time, the novel hypothesis that a temperature sensitive (ts)-based approach can be used to develop safe, immunogenic, stable and protective live-attenuated vaccines (LAV) to combat OWA HF disease in humans.
Role: Subaward PI
Program Officer: Abigail Strock/DoD Grants Officer, Fort Detrick CDMRP 1120 Fort Detrick, Federick, MD 21702.
No overlap

R21/R33 AI119321 PI: Arnold 07/01/15-06/30/17 0.6 cal. months
NIH/NIAID
Drug development targeting influenza A cap-snatching endonuclease
Major goal: Antiviral drugs are essential for treating people infected with influenza viruses and represent the best option for controlling outbreaks of flu pandemics. Tamiflu's limited efficacy in humans and existence of Tamiflu resistant strains of influenza highlights the need for new anti-influenza drugs. We are using a coordinated multidisciplinary approach to develop novel anti-influenza drugs that will retain efficacy against seasonal, drug- resistant, and potentially pandemic viral strains.
Role: PI of subcontract
Program Officer: Amy Krafft, NIH/NIAID, Bldg. 5601FL RM8E15, 5601 Fishers Lane, Rockville, MD 20852
No overlap

R21 AI119775 (Paessler) 07/01/15-06/30/17 1.2 cal. months

NIH/NIAID
Development of a safe and valid surrogate to study Lassa virus
Major goal: Because of the high (BSL4) level of biocontainment required to work with LASV, we propose to generate a valid virus surrogate to study LASV using widely available BSL2 facilities with the ultimate goal of accelerating research for this important human biodefense pathogen.
Role: PI of subcontract
Program Officer: Patricia Repik, NIH/NIAID, Bldg. 5601FL RM8E59, 5601 Fishers Lane, Rockville, MD 20852
No overlap

R21 AI128097 PI: Martinez-Sobrido 12/19/16-11/30/18 1.2 calendar
NIH/NIAID
Development of Human Broadly Neutralizing Monoclonal Antibodies Against New World Arenaviruses
Major goal: The main purpose of this project is to generate cross-reactive NWA GP-specific human V region monoclonal antibodies (hVMAbs) using sequential plasmid DNA immunization of the VelocImmune mouse with selected NWA GPs that will be characterized for their cross-reactivity and broadly neutralizing (BN) activity against HF NWA both in cell-based assays and in vivo.
Specific Aims: In this exploratory R21 application we propose first to generate cross-reactive variable (V) region humanized monoclonal antibodies (hVMAbs) against NWA surface GPs using sequential plasmid DNA immunization of VelocImmune mice with GPs from the genetically distantly related NWA (Aim 1). We will characterize these hVMAbs regarding their breadth of reactivity against GPs of representative members from all three NWA clades (Aim 2), including all known HF-causing NWA. We will select hVMAbs exhibiting broad NWA cross-reactivity to identify those with broadly neutralizing activity (BNhVMAbs) against NWA in cell-based assays (Aim 3) and to initially assess their in vivo therapeutic activity using infection of the mouse with recombinant LCM viruses expressing NWA HF-causing GPs of interest (Aim 4).
Role: PI
Program Officer: Patricia Repik, NIH/NIAID, Bldg. 5601FL RM8E59, 5601 Fishers Lane, Rockville, MD 20852
No overlap

R21 AI121550 PI: Martinez-Sobrido 02/15/16-01/31/19 (NCE) 1.2 calendar
NIH/NIAID
Broadly Neutralizing Antibodies Against Human Pathogenic Old-World Arenaviruses
Major goal: The main goals of this project are to evaluate a panel of 103 Lassa virus (LASV) glycoprotein (GP)-specific human monoclonal antibodies (hMAbs) isolated from Lassa fever (LF) survivors provided by Dr. Robinson (Tulane University) to identify those exhibiting cross-reactive and broadly neutralizing (BN) activity against several Old World arenaviruses (OWA), including strains from all LASV lineages (I-IV). We will also identify and initially characterized the epitopes recognized by the identified OWA BNhMAbs.
Specific Aims: In this application we propose first to evaluate a panel of 103 LASV glycoprotein (GP)-specific human monoclonal antibodies (hMAbs) derived from 14 different Lassa fever (LF) survivors (provided by Dr. Robinson at Tulane University) for their cross-reactivity (Aim 1) and range of neutralizing activities (Aim 2) against a collection of different Old World Arenavirus (OWA) and genetically distantly related strains of LASV and LCMV lineages. We will then identify the subunits, regions and specific amino acid residues of LASV GP recognized by selected OWA BNhMAbs via mutation-function studies and characterization of selected viral variants resistant to neutralization by the selected BNhMAbs (Aim 3). In addition, we will use antibody binding competition assays to identify OWA BNhMAbs that recognize the same or overlapping epitopes.

Role: PI
Program Officer: Patricia Repik, NIH/NIAID, Bldg. 5601FL RM8E59, 5601 Fishers Lane, Rockville, MD 20852
No overlap

P01 AI102851 PI: Fowell 06/01/14-05/31/19 0.6 cal. months NIH/ NIAID
Tissue Regulation of T Cell Function – Project 3 – CD8 T cell transepothelial migration in the influenza-infected trachea
Major goal: Pathogen control ultimately requires the recruitment and activation of immune effectors to specific infected tissue micro-environments. The central hypothesis is that the specific tissue and the local inflammatory milieu will shape T cell recruitment and effector function, thus ultimately controlling the magnitude and functional diversity of the immune response. The objective of this Program Project is to bring together scientific expertise in leukocyte migration and effector function to address fundamental effector T cell processes in infected tissues. We focus on four key events/checkpoints in the emergent immune response in infected tissue: effector T cell exit from the bloodstream.
Specific Aims: Aim 1: Determine the mechanisms that determine differentiation, establishment, and maintenance of TRM subsets after influenza infection. Aim 2: Investigate mechanisms of T cell-epithelial cell-matrix interactions required for motility and positioning in the airways. Aim 3: Determine the functions of CD49a and CD103 in optimizing immune protection.
Role: Co-Investigator
Program Officer: N/A
No overlap

***Should any of the pending grants be awarded, Dr. Martinez-Sobrido will adjust his effort as not to exceed 12 calendar months.**