AWARD NUMBER: W81XWH-17-1-0168

TITLE: Development of a New, More Effective Live-Attenuated Influenza Vaccine: An Essential Platform for Future Pandemic Protection

PRINCIPAL INVESTIGATOR: Stephen Dewhurst, PhD

CONTRACTING ORGANIZATION: University of Rochester
601 Elmwood Avenue
Rochester, NY 14642-0001

REPORT DATE: SEPTEMBER 2019

TYPE OF REPORT: Annual Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
SEPTEMBER 2019	ANNUAL	1 Sep 2018 – 31 Aug 2019

4. TITLE AND SUBTITLE
Development of a New, More Effective Live-Attenuated Influenza Vaccine: An Essential Platform for Future Pandemic Protection

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
W81XWH-17-1-0168

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

6. AUTHOR(S)
Stephen Dewhurst, PhD

E-Mail: Stephen Dewhurst@urmc.rochester.edu

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Rochester
500 Joseph C. Wilson Blvd., Rochester NY 14627-0001

Emory University
1648 Pierce Dr NE, Atlanta, GA 30307

Icahn School of Medicine at Mt. Sinai
1 Gustave L. Levy Pl, New York, NY 10029

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel 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
Vaccination is the most cost-effective approach by which the spread of a pandemic influenza virus could be prevented, and severe disease reduced. However, current influenza vaccines have had poor efficacy. Thus, it is very important to develop a new and more effective live-attenuated influenza vaccine (LAIV). We hypothesize that by understanding the molecular basis for the temperature sensitive (ts) and attnenuated (att) phenotypes of LAIV, it will be possible to develop a new, more effective live-attenuated influenza vaccine that leverages LAIV's superior ability to protect against infection by diverse influenza viruses. Our goal is to develop a new and improved LAIV that has enhanced safety and efficacy, due to (1) a greater temperature sensitivity than current LAIVs, resulting in viral replication only in the lower temperatures of the nasal cavity and extreme upper airway and (2) high levels of virus gene expression but poor replication – resulting in abundant protein expression (and immunogenicity) but minimal production of infectious progeny virus.

15. SUBJECT TERMS
Influenza, vaccine, pandemic, LAIV, live-attenuated, live-attenuated influenza vaccine

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	21	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

PROGRESS REPORT

PROPOSAL TITLE: Development of a New, More Effective Live-Attenuated Influenza Vaccine: An Essential Platform for Future Pandemic Protection

AWARD NUMBER: W81XWH-17-1-0168

PI: Stephen Dewhurst, Ph.D.

1. INTRODUCTION

Vaccination is the most cost-effective approach by which the spread of a pandemic influenza virus could be prevented, and severe disease reduced. However, current influenza vaccines have had poor efficacy. Thus, it is very important to develop a new and more effective live-attenuated influenza vaccine (LAIV). We hypothesize that by understanding the molecular basis for the temperature sensitive (ts) and attnenuated (att) phenotypes of LAIV, it will be possible to develop a new, more effective live-attenuated influenza vaccine that leverages LAIV's superior ability to protect against infection by diverse influenza viruses. Our goal is to develop a new and improved LAIV that has enhanced safety and efficacy, due to (1) a greater temperature sensitivity than current LAIVs, resulting in viral replication only in the lower temperatures of the nasal cavity and extreme upper airway and (2) high levels of virus gene expression but poor replication – resulting in abundant protein expression (and immunogenicity) but minimal production of infectious progeny virus.

2. KEY WORDS

Influenza, vaccine, pandemic, LAIV, live-attenuated, live-attenuated influenza vaccine

3. ACCOMPLISHMENTS

3A: Major project goals
NYI: Not yet initiated/achieved

AIM/TASK	Timeline	Responsible Site	Status
SPECIFIC AIM 1 To identify the mechanisms by which the current LAIV mutations affect viral polymerase function			
Major Task 1 To study the effects of the LAIV mutations on the biochemical activity of the purified viral polymerase	Months		
Subtasks: Purification of polymerase proteins; Functional analysis of pol proteins	1-12	Emory	In Progress. Purification of selected proteins has been achieved. Functional studies are still ongoing because the purification of biochemically active polymerase has been challenging.

Milestone(s) Achieved: Identification of biochemical correlates of LAIV polymerase temperature sensitivity	12	Emory	In Progress
Major Task 2 To study the effects of the LAIV mutations, both alone and in combination, on genomic replication versus transcription by the viral polymerase			
Subtasks: Construction of minigenome plasmids; Performance of cell-based minigenome assays	1-12	UR	Completed. See Year 1 report.
Milestone(s) Achieved: Identification of LAIV mutations associated with increased temperature sensitivity in minigenome assays	12	UR	Completed. See Year 1 report.
Major Task 3 To study the effects of the LAIV mutations, both alone and in combination, on intracellular trafficking of the viral polymerase			
Subtasks: Construction of viral mutants; Analysis of viral mutants	9-20	UR	In Progress. See below.
Milestone(s) Achieved: Identification of LAIV mutations associated with temperature sensitive changes in nuclear/cytoplasmic trafficking of the viral polymerase	20	UR	In Progress. Note that we have deferred analyses of nuclear/cytoplasmic trafficking of the polymerase in favor of studies on the temperature sensitivity of viral replication (Task 4). Our rationale is that a *ts* viral replicative phenotype is more predictive of attenuation and more directly on the critical path for vaccine discovery/development.
Major Task 4 To study the effects of the LAIV mutations, both alone and in combination, on the temperature sensitivity of viral replication			
Subtasks: Construction of viral mutants; Analysis of viral mutants	5-12	UR	In Progress. Many mutants have been constructed and tested. However, we have found that the viral genetic background can exert an unexpectedly strong effect on the extent of temperature sensitivity conferred by LAIV mutations. As a result, these experiments are still ongoing.

Milestone(s) Achieved: Identification of LAIV mutations associated with temperature sensitive changes in virus replication	12	UR	In Progress. We have identified many LAIV mutations associated with *ts* changes in viral replication. However, testing of other mutations is still ongoing (see above).
SPECIFIC AIM 2 To identify the mechanisms by which other polymerase mutations associated with *ts* and *att* phenotypes affect viral polymerase function			
Major Task 5 To study the effects of the other polymerase mutations on the biochemical activity of the purified viral polymerase	Months		
Subtasks: Purification of polymerase proteins; Functional analysis of pol proteins	13-24	Emory	In Progress. Purification of selected proteins has been achieved; functional studies are still ongoing because the purification of biochemically active polymerase has been challenging.
Milestone(s) Achieved: Identification of biochemical correlates of polymerase temperature sensitivity	24	Emory	In Progress
Major Task 6 To study the effects of the other polymerase mutations, both alone and in combination, on genomic replication versus transcription by the viral polymerase			
Subtasks: Construction of minigenome plasmids; Performance of cell-based minigenome assays	13-24	UR	NYI. Note that we have deferred minigenome analyses in favor of studies on the temperature sensitivity of viral replication (Task 8). Our rationale is that a *ts* viral replicative phenotype is more predictive of attenuation and more directly on the critical path for vaccine discovery/development.
Milestone(s) Achieved: Identification of pol mutations associated with increased temperature sensitivity in minigenome assays	24	UR	NYI. See above.
Major Task 7 To study the effects of the other polymerase mutations, both alone and in combination, on intracellular trafficking of the viral polymerase			

Subtasks: Construction of viral mutants; Analysis of viral mutants	24-36	UR	NYI
Milestone(s) Achieved: Identification of pol mutations associated with temperature sensitive changes in nuclear/cytoplasmic trafficking of the viral polymerase	36	UR	NYI
Major Task 8 To study the effects of the other polymerase mutations, both alone and in combination, on the temperature sensitivity of viral replication			
Subtasks: Construction of viral mutants; Analysis of viral mutants	13-20	UR	In Progress. We have found that the viral genetic background can exert an unexpectedly strong effect on the impact of some pol mutations of the viral ts phenotype. As a result, these experiments are still ongoing.
Milestone(s) Achieved: Identification of pol mutations associated with temperature sensitive changes in virus replication	20	UR	In Progress. See above. Some novel, enhanced LAIVs have already been identified.
SPECIFIC AIM 3 To evaluate the in vivo replicative capacity, safety and protective efficacy of novel LAIVs containing rationally designed combinations of mutations			
Major Task 9 To identify the relative contribution of LAIV mutations to viral attenuation in mice			
Local IACUC Approval	13	UR	Approval obtained
Subtasks: Assess viral virulence and replication in mice; Assess lung damage/pathology in infected mice	14-18	UR	Completed
Milestone(s) Achieved: Identification of novel LAIVs with greater *in vivo* attenuation than the unmodified U.S. and Russian LAIVs	18	UR	Completed
Major Task 10 To test whether enhanced LAIVs retain immunogenicity and protective efficacy in mice			
Local IACUC Approval	13	UR	Approval obtained
Subtask: Assess immunogenicity and protective efficacy of novel LAIVs in mice	19-24	UR	In Progress. Studies of immunogenicity and protective efficacy in mice are in progress (some have already been completed)

Milestone(s) Achieved: Identification of novel, enhanced LAIVs that retain immunogenicity and protective efficacy in mice	24	UR	In Progress. Some novel, enhanced LAIVs have already been identified, while others are still being evaluated.
Major Task 11 To confirm that mutations that enhance the safety of LAIV in mice also do so in a ferret model			
Local IACUC Approval	13	Mt Sinai	Approval obtained
Subtasks: Analysis of virus replication and histopathology; Analysis of pulmonary safety; immunization/challenge studies	15-36	Mt Sinai	In Progress.
Milestone(s) Achieved: Identification of new and more effective LAIVs for future development as candidate universal influenza vaccines.	36	Mt Sinai	NYI

3A. What was accomplished under these goals?

3A1. A single amino acid substitution in PB1 is sufficient to fully attenuate a pandemic H1N1 influenza A virus, when combined with the *ts, ca* and *att* mutations from the A/Ann Arbor/6/60 H2N2 master donor virus

The five mutations responsible for the ts, ca and att phenotype of the U.S. LAIV (derived from A/Ann Arbor/6/60 H2N2) are located in three viral segments: the viral polymerase subunits PB2 (N265S), and PB1 (K391E, D581G, and A661T); and the viral NP (D34G). Interestingly, when this genetic signature was introduced into the backbone of H1N1 Iinfluenza A viruses (IAVs), different levels of attenuation were observed. While the mutations were able to confer a ts, ca and att phenotype to A/Puerto Rico/8/34, the same amino acid changes did not significantly change the virulence of the 2009 pandemic A/California/04/09 (pH1N1). In order to attenuate the pH1N1, we replaced the natural and conserved leucine at position 319 with glutamine (L319Q) in PB1 and analyzed the in vitro and in vivo properties of pH1N1 viruses containing either PB1 L319Q alone or in combination with the 5 mutations of the A/Ann Arbor/6/60 H2N2 MDV using two relevant animal models of influenza viral infection and transmission, ferrets and guinea pigs. Our data demonstrate that the amino acid substitution L319Q in the pH1N1 PB1, alone or in combination with the 5 ts, ca and att mutations of the A/Ann Arbor/6/60 H2N2 MDV results in reduced pathogenicity (ferrets) and transmission (guinea pigs), and an increased ts phenotype. These results demonstrate the feasibility of generating a fully attenuated MDV based on the contemporary pH1N1 backbone.

Table 1. MLD_{50} of WT and LAIV pH1N1 and PR8 viruses: Data previously published by our groups (PMID: 28637750, 24965472, 27122587, 25552727).

Virus	MLD_{50} (PFU) [a]
pH1N1 (Cal/09) WT	$2.5x10^2$
pH1N1 (Cal/09) LAIV	$4.34x10^3$
PR8 WT	$2.5x10^1$
PR8 LAIV	$3.16x10^4$

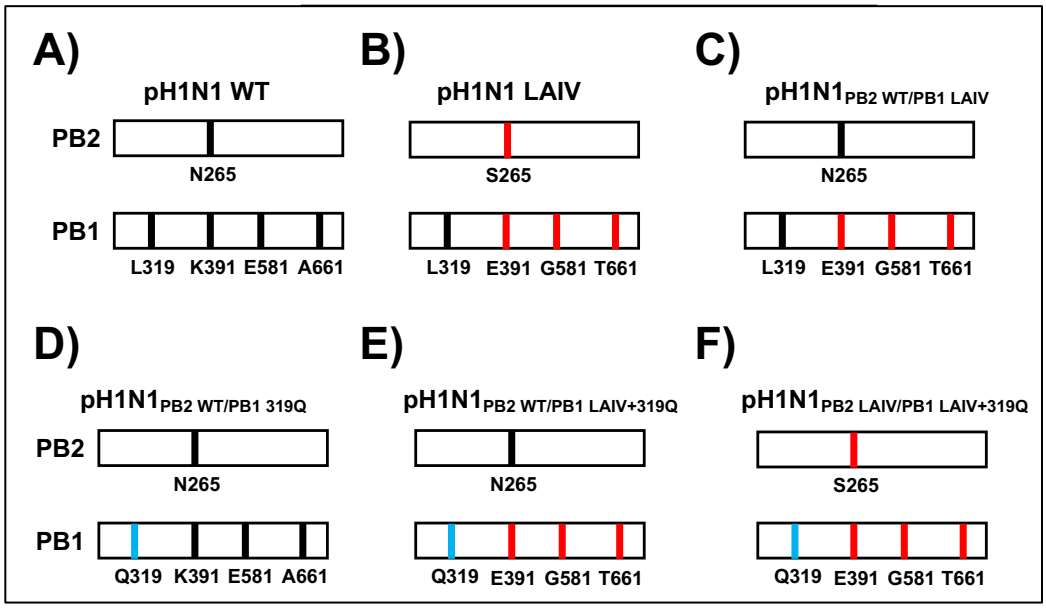

Figure 1. Schematic representation of the PB2 and PB1 viral segments in the different pH1N1 viruses. The PB2 and PB1 viral segments of pH1N1 WT (**A**), pH1N1 LAIV (**B**), $pH1N1_{PB2\ WT/PB1\ LAIV}$ (**C**), $pH1N1_{PB2\ WT/PB1\ 319Q}$ (**D**), $pH1N1_{PB2\ WT/PB1\ LAIV+319Q}$ (**E**), and $pH1N1_{PB2\ LAIV/PB1\ LAIV+319Q}$ (**F**) viruses are shown. WT PB2 (N265) and PB1 (L319, K391, E581, and A661); LAIV PB2 (S265) and PB1 (E391, G581, and T661); and Q319 amino acids are indicated by black, red or blue lines, respectively.

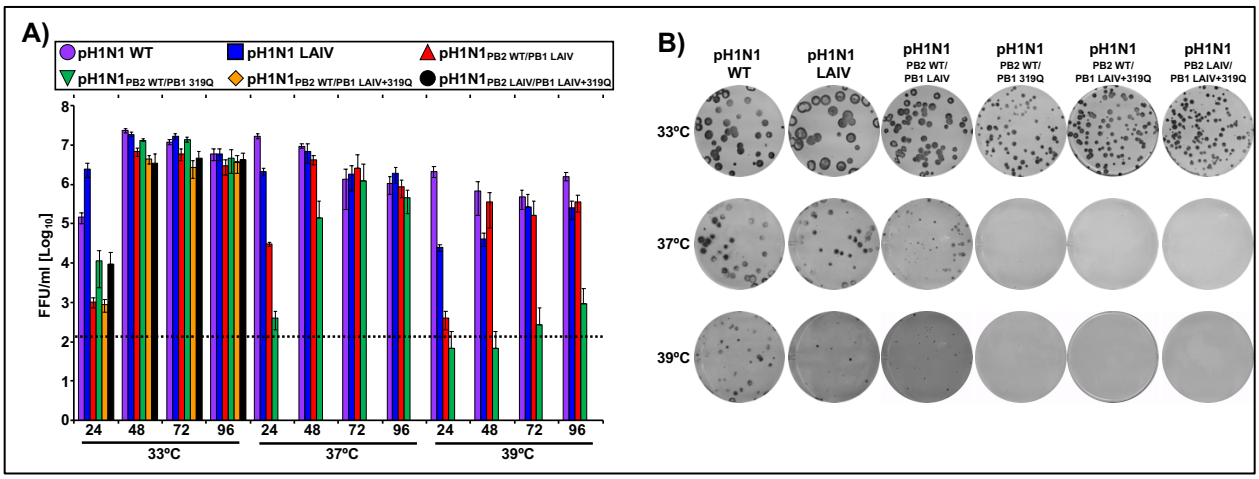

Figure 2. Characterization of the pH1N1 viruses. A) **Viral growth kinetics:** Tissue culture supernatants of MDCK cells infected at a low MOI (0.001) with the indicated pH1N1 WT and mutant viruses at 33ºC, 37ºC, and 39ºC were analyzed at the indicated times post-infection (24, 48, 72 and 96 h) by immunofocus assay using an anti-NP monoclonal antibody (HB-65). Data represent the means and SDs of the results determined from triplicate wells. The dashed line indicates the limit of detection (200 FFU/ml). **B**) **Plaque assays:** Confluent monolayers of MDCK cells were infected with the indicated pH1N1 viruses and incubated at 33ºC, 37ºC, and 39ºC for 3 days. The plaque phenotype was assessed by immunostaining with the anti-NP monoclonal antibody HB-65.

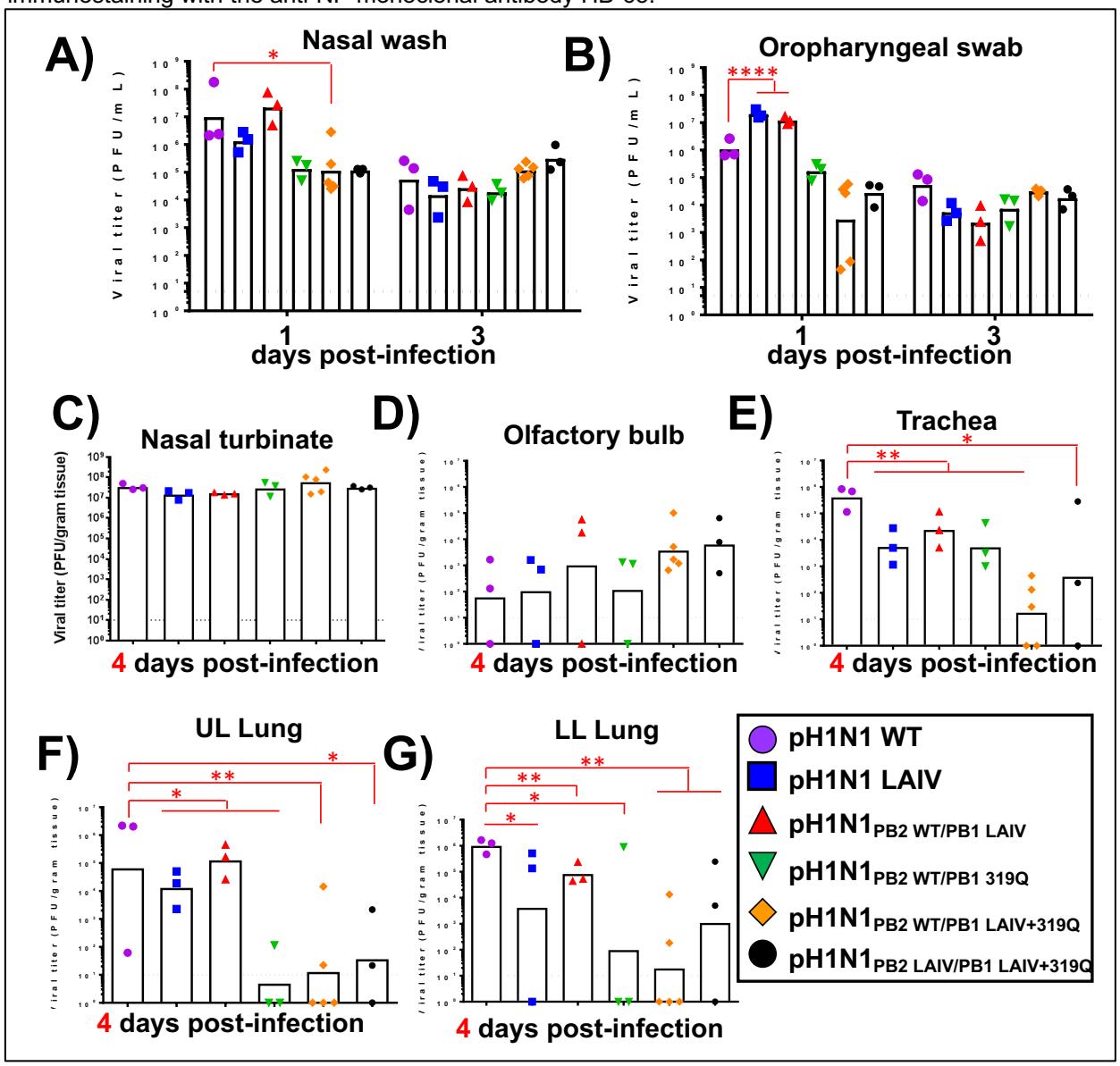

Figure 3. Characterization of the pH1N1 WT and mutant viruses in ferrets. Three to five 4 to 5-month-old castrated **male Fitch** ferrets were inoculated with $1x10^7$ PFU of the indicated WT and mutant pH1N1 viruses and viral replication was measured on days 1 and 3 post-infection in the nasal washes (**A**) or oropharyngeal swab (**B**) swabs; and at day **4** post-infection in the nasal turbinate (**C**), olfactory bulb (**D**), trachea (**E**), and the upper left (UL, **F**) or lower left (LL, **G**) lungs by plaque assay (PFU/ml). Dashed lines indicate the limit of detection (10 PFU/ml). Data in (A-B) were compared to pH1N1 WT virus infected animals and analyzed by two-way ANOVA followed by a Sidak's multiple comparison test (multiple time points). Data in (C-G) were compared to pH1N1 WT virus infected animals with one-way ANOVA followed by a Dunnett's multiple comparison test (single time point). The asterisks refer to the level of significance. *: $p<0.05$; ****: $p<0.0001$.

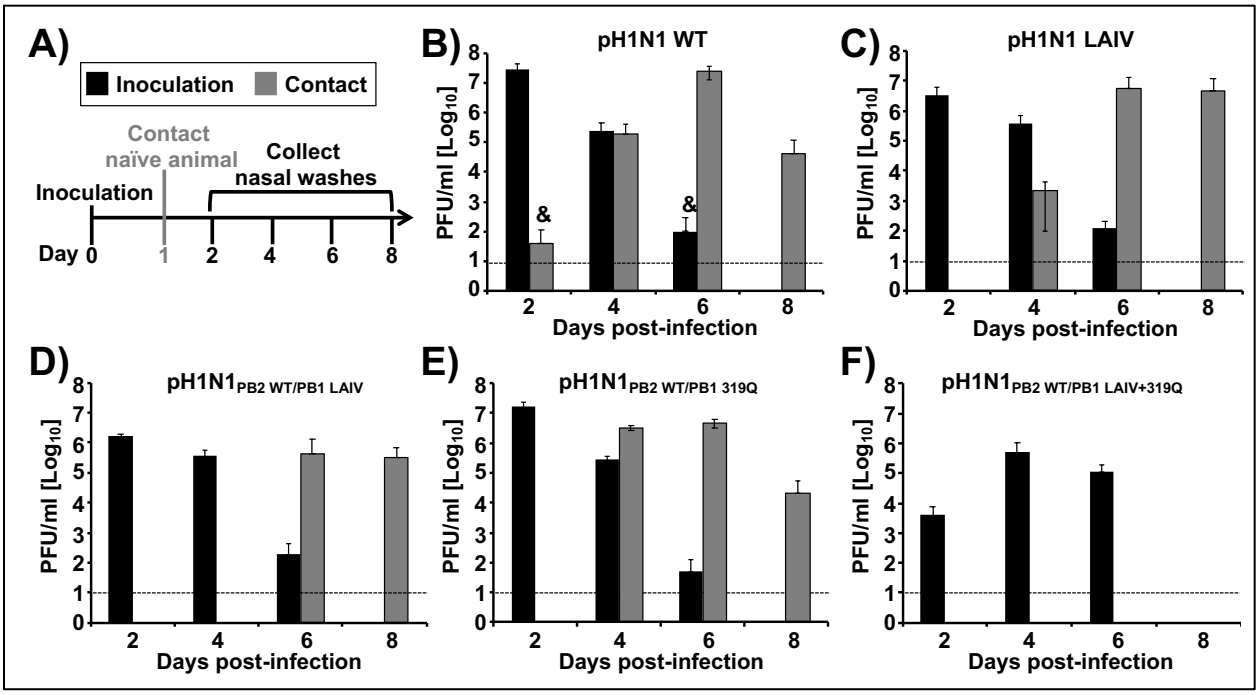

Figure 4. Transmission of WT and mutant pH1N1 viruses in guinea pigs. A) **Schematic representation of the experimental design:** Four guinea pigs were inoculated with $1x10^4$ PFU of pH1N1 WT (**B**), pH1N1 LAIV (**C**), $pH1N1_{PB2\ WT/PB1\ LAIV}$ (**D**), $pH1N1_{PB2\ WT/PB1\ 319Q}$ (**E**), or $pH1N1_{PB2\ WT/PB1\ LAIV+319Q}$ (**F**) virus (black). Then, at 24 hours post-infection, infected guinea pigs (black) were placed in a cage with one uninfected guinea pig (gray). Nasal washes were collected for 8 days at 48 hours intervals, starting at day 2 post-infection for the inoculated animals (24 hours post-contact). Presence of the viruses in the nasal wash samples were determined by plaque assay (PFU/ml) using the NP monoclonal antibody HB-65. Dashed lines indicate the limit of detection (10 PFU/ml). &, Virus detected in one out of 4 guinea pigs.

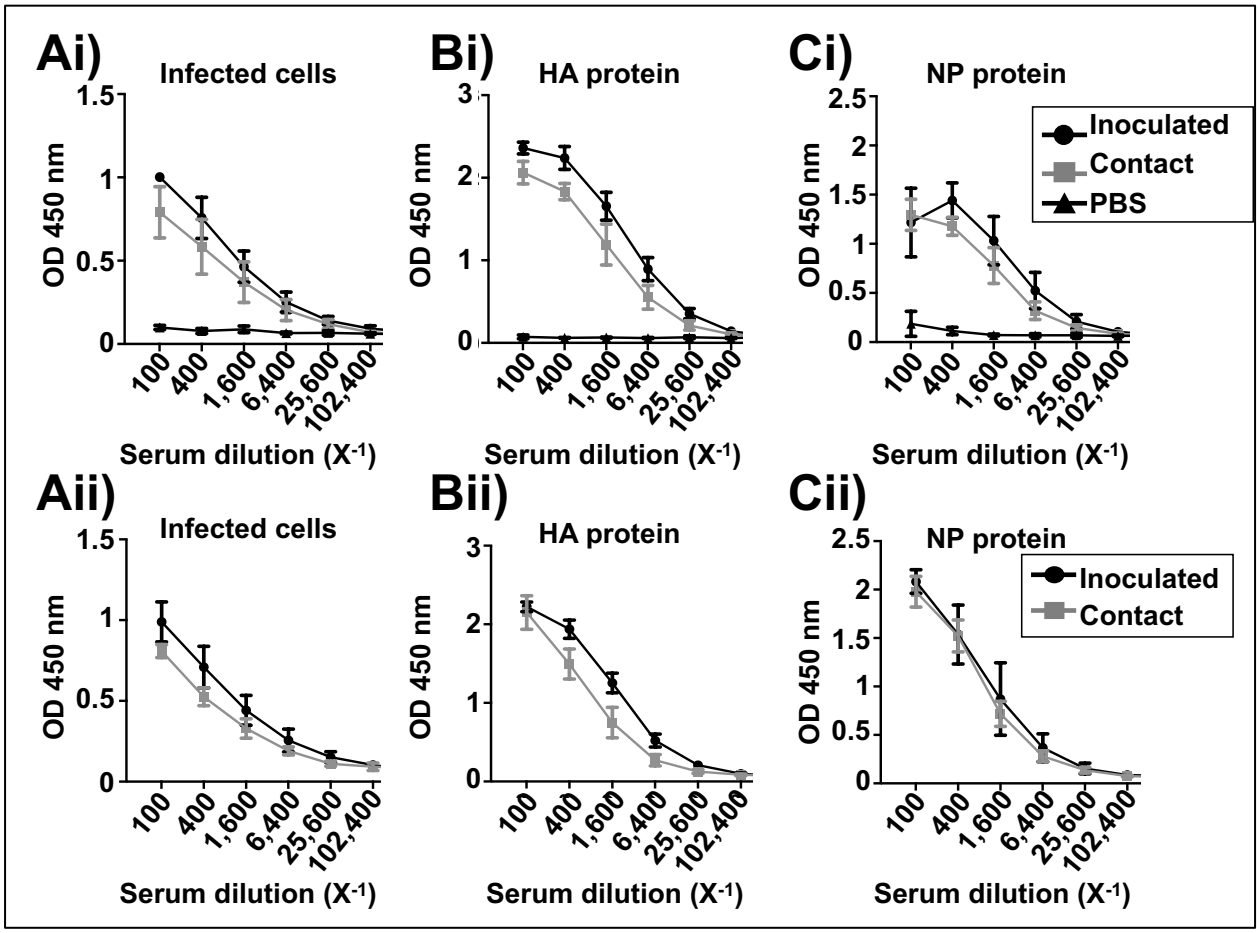

Figure 5. Immunogenicity of pH1N1 LAIV in infected guinea pigs. Guinea pigs from the transmission experiment (**Figure 5**), infected with pH1N1 WT (**Ai, Bi and Ci**) or pH1N1 LAIV (**Aii, Bii and Cii**) viruses were bled at 21 days post-infection and sera were collected and evaluated by ELISA using 1:4 dilutions of serum (starting dilution, 1:100) for IgG antibodies against total virus proteins using cell extracts of pH1N1 WT virus-infected MDCK cells **(A)**, against recombinant HA **(B)** or NP **(C)** viral proteins. OD, optical density. Data represent the means and SDs of each group (N=4).

Table 2. Immunogenicity of pH1N1 viruses. Hemagglutination inhibition (HAI) assays were used to evaluate the presence of neutralizing antibodies. [a]Data were calculated from 4 immunized or mock-immunized (PBS) animals. ND, not determined.

Immunization[a]	Mean HAI titer
PBS	< 20 (ND)
pH1N1 WT-Inoculation	640
pH1N1 WT-Contact	640
pH1N1 LAIV-Inoculation	320
pH1N1 LAIV-Contact	320

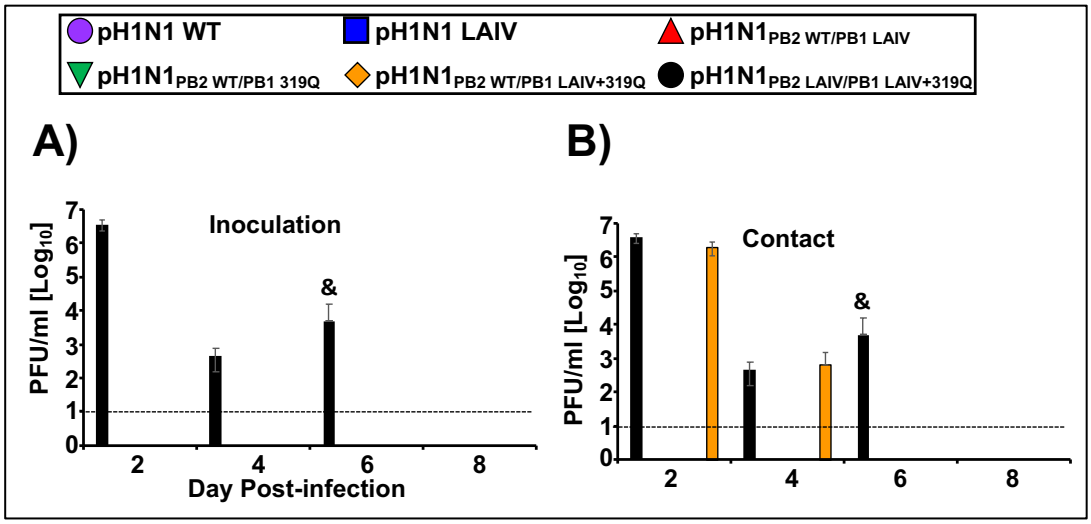

Figure 6. Vaccinated guinea pigs are protected against a homologous challenge. Guinea pigs from the transmission experiment (**Figure. 5**; inoculation (**A**) or contact (**B**)), were challenged 21 days later with $1x10^4$ PFU of pH1N1 WT. A group (n = 4) of mock-vaccinated animals was included as a control. To evaluate viral replication, nasal washes were collected for 8 days at 48 hour intervals, starting from 2 day post-infection. Presence of virus in the nasal wash samples was determined by plaque assay (PFU/ml) using the NP monoclonal antibody HB-65. Dashed lines indicate the limit of detection (10 PFU/ml). &, Virus detected in one out of the 4 guinea pigs. The same mock group has been included in panels A and B.

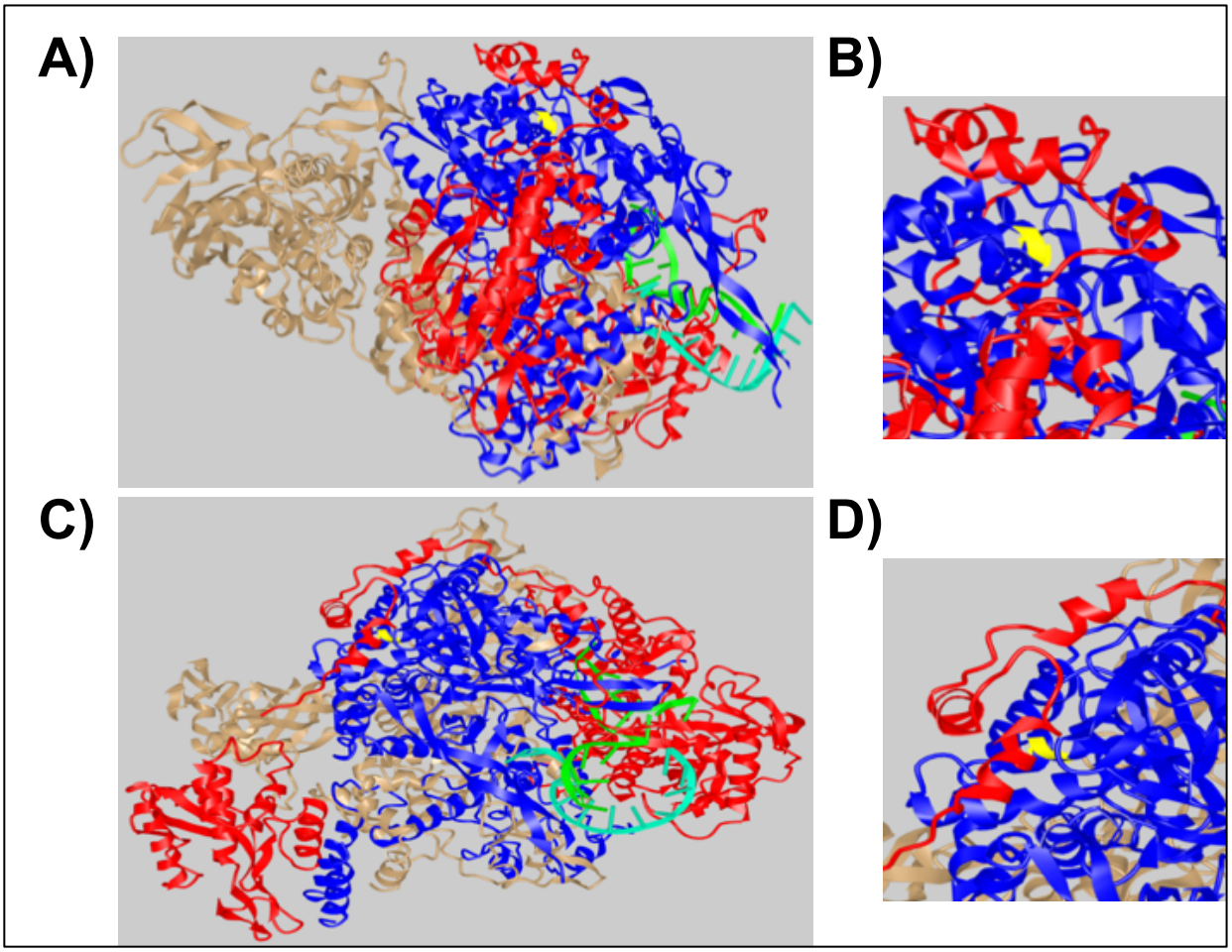

Figure 7. Structure of IAV polymerase complex indicating the position of residue 319 in PB1. The IAV polymerase complex structure (PDB code 4WSB) is shown in two different views with a zoom of the amino acid 319 (A and B or C and D), and the same coloring is retained in all images. PB2, PB1 and PA subunits are shown in brown, blue and red, respectively. Amino acid 319 in PB1 is indicated in yellow. The 5′ and 3′ vRNAs are shown in green and cyan, respectively

Development of reverse genetics for the US MDV A/Ann Arbor/6/60 H2N2 LAIV

In order to evaluate how the mutations in the polymerase complex of the United States (US) Master Donor Virus (MDV) A/Ann Arbor/6/60 H2N2 LAIV affect viral genome replication and gene transcription, we first built a plasmid-based reverse genetic system that we could manipulate to generate recombinant Viruses (Figure 8). To this end we started with the plasmids encoding the six internal proteins from the MDV A/Ann Arbor/6/60 H2N2 LAIV, which contain the 5 mutations (PB2 N265S; PB1 K391E, E581G, A661T; and NP D34G) that confer the *ca* and *ts* phenotype to the MDV A/AA/6/60 LAIV and the HA and NA of influenza A/California/04/09 H1N1. We also generated a wild-type (WT) A/Ann Arbor/6/60 H2N2 to demonstrate that the introduced mutations are responsible for the ts phenotype of the MDV A/Ann Arbor/6/60 H2N2 LAIV (Figure 8). Similar to the US MDV A/Ann Arbor/6/60 H2N2 LAIV, we are currently developing similar plasmid-based reverse genetics techniques to rescue the Russian MDV A/Leningrad/17/57 LAIV.

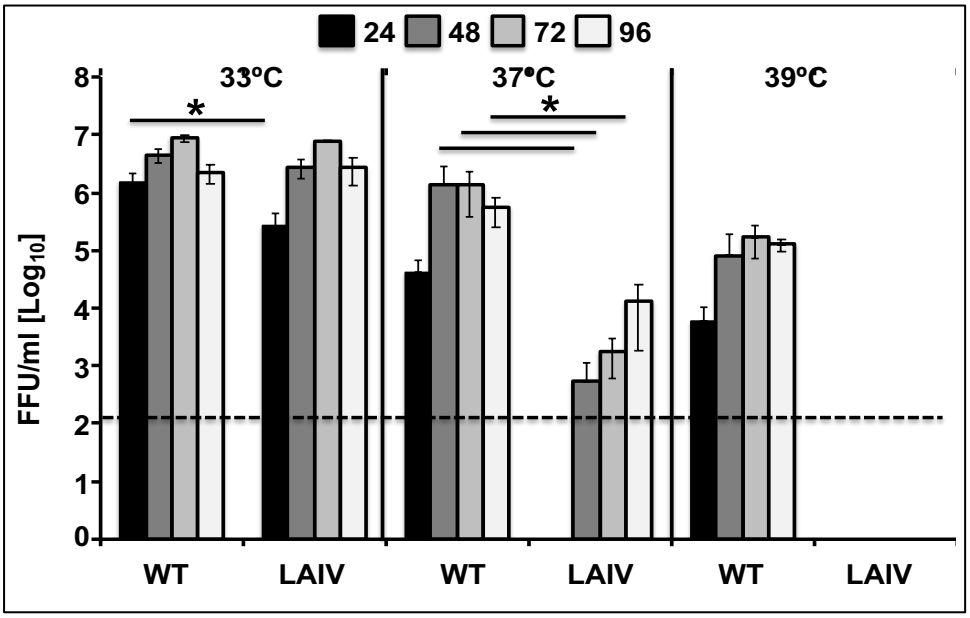

Figure 8. Multicycle growth kinetics of WT and mutant MDV A/AA/6/60 LAIV at different temperatures: TCS from MDCK cells (5×10^5, 12-well plates, triplicates) infected at low multiplicity of infection (MOI, 0.001) with WT A/AA/6/60 or MDV A/AA/6/60 LAIV at 33ºC, 37ºC, and 39ºC were analyzed at the indicated h p.i. (24, 48, 72 and 96) by immunofocus assay using an anti-NP mAb (HB-65). Data represent the means and SDs of the results determined from triplicate wells. The dashed line indicates the limit of detection (200 fluorescent forming units, FFU/ml).

3A2. Studies on the biochemical activity of the purified viral polymerase.

We have initiated studies to examine the temperature sensitivity of the purified viral polymerase. A very preliminary first experiment is shown in Fig. 9. We are still in the process of optimizing this assay.

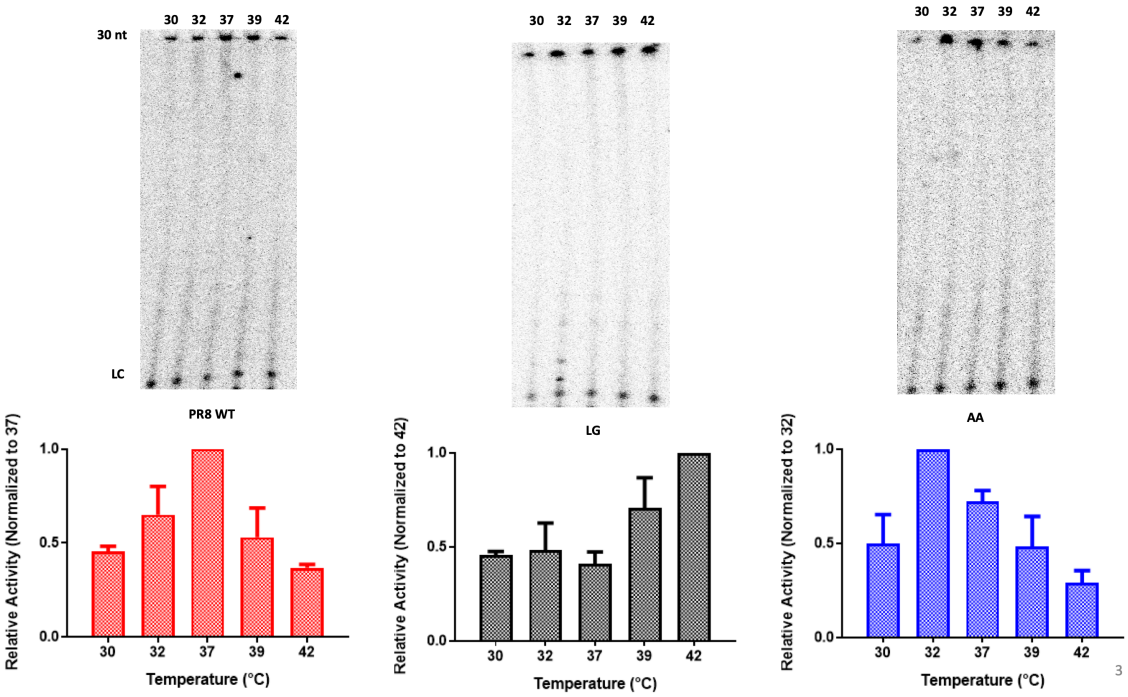

Figure 9. Preliminary analysis of the biochemical activity of the purified viral polymerase, in a primer extension assay. A simple primer extension assay was performed on an ApG-primed influenza virus RNA template, using 3 different, biochemically purified viral polymerases. These polymerases correspond to: (1) wild-type A/PR/8/34 (PR8 WT), (2) PR8 bearing the L319Q mutation in PB1 (LG), (3) PR8 containing the 4 polymerase mutations from the U.S. LAIV (PB2 N265S; PB1 K391E, E581G, A661T) (AA). The upper panel shows a representative primer extension assay. The lower panel shows data from triplicate assays (mean, SD). Data were normalized to 100% polymerase activity for each enzyme preparation. The data show that the polymerase from WT PR8 virus functions best at 37oC (as expected), while the polymerase from the AA virus functions best at 32oC (suggestive of a cold-adapted phenotype, which would be consistent with the derivation of this virus). This analysis is being repeated under a range of different experimental conditions, in order to better understand the data.

3B. What opportunities for training and professional development has the project provided?
The project has provided training and professional development opportunities to the following individuals:

1. **Andrew Smith.** MD/PhD trainee in Dr. Dewhurst's lab. Training activities: Mr. Smith successfully defended his PhD thesis in 2019, with the major focus of his thesis being on this project. Professional development: Mr. Smith is completing medical school, as part of his M.D./Ph.D. program.
2. **Justin Leach.** PhD trainee in Dr. Dewhurst's lab. Training activities: Mr. Leach is being mentored by Dr. Dewhurst. Professional development: Mr. Leach successfully entered his 2nd year of graduate training, and plans to complete his thesis with Dr. Dewhurst.
3. **Sarah Belanger.** Undergraduate student conducting independent research in Dr. Dewhurst's lab. Training activities: Ms. Belanger was initially mentored in her research by Mr. Chamberlain, and more recently by Mr. Leach. Professional development: Ms. Belanger learned a broad range of new technical skills under Mr. Chamberlain and Mr. Leach's guidance.
4. **Kara Farquharson.** M.S student conducting independent research in Dr. Dewhurst's lab. She is a participant in the NIH-funded Rochester Bridges to the Doctorate Program (RB2D; R25 GM107739), which seeks to increase the number of deaf and hard-of-hearing students (DHH) who enter biomedical Ph.D. programs. Training activities: Ms. Farquharson was mentored by Dr. Dewhurst,. Professional development: Ms. Farquharson learned a broad range of new technical skills, and is presently applying to Ph.D. programs.

3C. Dissemination of results to communities of interest.
Our results were presented in the following venues:

1. Invited research seminars by Dr. Dewhurst, at the University of Rochester (2018, 2019), as well as Michigan State University (2019).
2. Summer poster presentations by Ms. Farquharson at the University of Rochester (2019), and the Rochester Institute of Technology (RIT).

3D. Plans for next reporting period.
Our plans for the next reporting period remain unchanged from the original SOW

4. IMPACT

4A. What was the impact on the development of the principal discipline(s) of the project?
The five mutations responsible for the ts, ca and att phenotype of the U.S. LAIV (derived from A/Ann Arbor/6/60 H2N2) are located in three viral segments: the viral polymerase subunits PB2 (N265S), and PB1 (K391E, D581G, and A661T); and the viral NP (D34G). Interestingly, when this genetic signature was introduced into the backbone of H1N1 Iinfluenza A viruses (IAVs), different levels of attenuation were observed. While the mutations were able to confer a ts, ca and att phenotype to A/Puerto Rico/8/34, the same amino acid changes did not significantly change the virulence of the 2009 pandemic A/California/04/09 (pH1N1). In order to attenuate the pH1N1, we replaced the natural and conserved leucine at position 319 with glutamine (L319Q) in PB1 and analyzed the in vitro and in vivo properties of pH1N1 viruses containing either PB1 L319Q alone or in combination with the 5 mutations of the A/Ann Arbor/6/60 H2N2 MDV using two relevant animal models of influenza viral infection and transmission, ferrets and guinea pigs. Our data demonstrate that the amino acid substitution L319Q in the pH1N1 PB1, alone or in combination with the 5 ts, ca and att mutations of the A/Ann Arbor/6/60 H2N2 MDV results in reduced pathogenicity (ferrets) and transmission (guinea pigs), and an increased ts phenotype. These results demonstrate the feasibility of generating a fully attenuated MDV based on the contemporary pH1N1 backbone.

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. CHANGES/PROBLEMS

5A. Changes in approach and reasons for change
No changes in approach

5B. Actual or anticipated problems or delays and actions or plans to resolve them
Biochemical studies on the purified viral polymerase (Major Task 1) have been delayed due to the unanticipated complexity of producing biochemically active polymerase. The Emory site has agreed to continue these experiments at no-cost during the coming project year, so no significant problems are expected. Other aspects of the project remain on schedule.

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
No journal publications or books to date. An article is presently under review.

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:	Stephen Dewhurst
Project Role:	PI
Research Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001--7729-7920
Nearest person month worked:	2
Contribution to Project:	Dr. Dewhurst directly oversaw or supervised all project studies
Funding Support:	This award

Name:	Baek Kim
Project Role:	PI (Emory Subcontract)
Research Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-7986-4335
Nearest person month worked:	1
Contribution to Project:	Dr. Kim directed all studies at the Emory site (biochemical studies on the viral polymerase)
Funding Support:	This award

Name:	Randy Albrecht
Project Role:	PI (Icahn School of Medicine at Mt. Sinai Subcontract)
Research Identifier (e.g. ORCID ID):	https://orcid.org/0000-0003-4008-503X
Nearest person month worked:	3
Contribution to Project:	Dr. Albrecht organized, directed, and conducted the ferret vaccination studies.

Funding Support:	This award

Name:	Luis Martinez-Sobrido
Project Role:	Coinvestigator (UR)
Research Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-7084-0804
Nearest person month worked:	1
Contribution to Project:	Dr. Martinez-Sobrido assisted Dr. Dewhurst with all minigenome, virus replication and viral immunogenicity/challenge studies at the Rochester site.
Funding Support:	This award

Name:	Changyong Feng
Project Role:	Coinvestigator (UR)
Research Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Feng has performed all statistical analyses for this project.
Funding Support:	This award

Name:	Jeffrey Chamberlain
Project Role:	Technician (UR)
Research Identifier (e.g. ORCID ID):	N/A (No ORCID ID or equivalent)
Nearest person month worked:	4
Contribution to Project:	Mr. Chamberlain assisted with plasmid production, minigenome assays and assays of virus replication.
Funding Support:	This award

Name:	Andrew Smith
Project Role:	Graduate Student (UR)
Research Identifier (e.g. ORCID ID):	N/A (No ORCID ID or equivalent)
Nearest person month worked:	10
Contribution to Project:	Mr. Smith assisted with plasmid production, minigenome assays and assays of virus replication.
Funding Support:	This award

Name:	Gines Avila Perez
Project Role:	Postdoctoral Trainee in Martinez-Sobrido lab (UR)
Research Identifier (e.g. ORCID ID):	N/A (No ORCID ID or equivalent)

Nearest person month worked:	1
Contribution to Project:	Dr. Avila Perez assisted with plasmid production, minigenome assays and assays of virus replication.
Funding Support:	This award

Name:	Aitor Nogales
Project Role:	Research Assistant Professor in Martinez-Sobrido lab (UR)
Research Identifier (e.g. ORCID ID):	N/A (No ORCID ID or equivalent)
Nearest person month worked:	1
Contribution to Project:	Dr. Nogales assisted with plasmid production, minigenome assays and assays of virus replication.
Funding Support:	This award

Name:	Sarah Belanger
Project Role:	Undergraduate Student (UR)
Research Identifier (e.g. ORCID ID):	N/A (No ORCID ID or equivalent)
Nearest person month worked:	12
Contribution to Project:	Ms. Belanger assisted Mr. Chamberlain with plasmid production and minigenome assays.
Funding Support:	This award

Name:	Kara Farquharson
Project Role:	M.S. Student (RIT)
Research Identifier (e.g. ORCID ID):	N/A (No ORCID ID or equivalent)
Nearest person month worked:	3
Contribution to Project:	Ms. Farquharson assisted Mr. Leach with viral mutagenesis and viral replication assays in vitro.
Funding Support:	NIH R25 GM107739

Name:	Justin Leach
Project Role:	PhD Graduate Student
Research Identifier (e.g. ORCID ID):	N/A (No ORCID ID or equivalent)
Nearest person month worked:	12
Contribution to Project:	Mr. Leach has assisted with viral mutagenesis, viral replication assays in vitro, and studies of viral pathogenicity and immunogenicity in mice.
Funding Support:	University of Rochester internal funds

Name:	Wen-Chun Liu
Project Role:	Postdoc (Icahn School of Medicine at Mt. Sinai Subcontract)
Research Identifier (e.g. ORCID ID):	https://orcid.org/0000-0003-4008-503X
Nearest person month worked:	3
Contribution to Project:	Dr. Liu organized and conducted the ferret vaccination studies. Dr. Liu also analyzed the virus titers from the tissue specimens and prepared tissue specimens for histo-pathological assessment.
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?
Changes in the active support for the PD/PI(s) and senior/key personnel have occurred, and are included in this report.

7C. What other organizations were involved as partners?
As stated in the original grant application, and in the corresponding Notice of Grant Award from NIH, the University of Rochester (Rochester, NY; primary institution), Emory University (Atlanta, GA; subcontracting institution), and the Icahn School of Medicine at Mt. Sinai (New York, NY; subcontracting institution) were involved in the second year of this project.

8. SPECIAL REPORTING REQUIREMENTS

None

9. APPENDICES

None

OTHER SUPPORT

DEWHURST, Stephen

ACTIVE AWARDS

W81XWH-17-1-0168 (Dewhurst, PI) 10/01/17-09/30/20 2.4 calendar
DOD $391,901 ADC
Title: Development of a New, More Effective Live-Attenuated Influenza Vaccine: An Essential Platform for Future Pandemic Protection
Major goal: To develop a new LAIV that has greater temperature sensitivity than current LAIVs and selectively favors virus gene expression over viral replication. The ultimate goal is to identify new and more effective LAIVs for development as candidate universal influenza vaccines.
Role: PI
Program Official: Abigail Strock/DoD Grants Officer, Fort Detrick CDMRP 1120 Fort Detrick, Frederick, MD 21702

P30 AI078498 (Dewhurst, PI) 05/01/13-04/30/20 NCE 1.8 calendar
NIH/NIAID $1,006,421 ADC
Title: University of Rochester Center for AIDS Research
Major goal: To implement and administer a Center for AIDS Research at the U of Rochester.
Role: Overall PI, Director for Core A, Assoc Director for Core B
Program Official: Gregory Smith, 240-669-2993, RM 4G70, 5601 Fishers Lane, Rockville, MD 20852

BAA-NIAID-DMID AI2012154 (Topham, PI) 04/01/15-03/31/20 0.12 calendar
NIH/NIAID $132,038 ADC
Title: Centers for Excellence on Influenza Research and Surveillance
Major goal: Drs. Martínez-Sobrido and Stephen Dewhurst will be responsible for the pandemic research plan that includes pre-pandemic risk assessment and emergency pandemic response plan.
Role: Co-Investigator
Program Official: Diane J. Post, NIH/NIAID/DMID, Room 3212, 6610 Rockledge Drive, Bethesda, MD 20817

R01 AI150463 (Wedekind, PI) 09/30/17-07/31/21 0.12 calendar
NIH $250,793 ADC
Title: Cyclic Peptide Inhibitors of HIV-1 Proliferation
Major goal: To develop novel inhibitors of HIV-1 replication, by targeting the HIV-1 TAR RNA structure, using cyclic peptides.
Role: Co-Investigator
Program Official: Gerard Lacourciere, 301-761-7477, RM 9G55, 5601 Fishers Lane, Rockville, MD 20852

R01 GM117005 (Kielkopf, PI) 03/1/16-02/29/20 0.12 calendar
NIH $192,500 ADC
Title: Structural Control of Human CoFactors for Retroviral Gene Expression
Major goal: To understand the molecular mechanisms for a human protein to coordinate production of HIV-1 RNAs, and ultimately to exploit this, for the development of novel anti-HIV therapeutics.
Role: Co-Investigator
Program Official: Michael Sakalian, 301-402-4374, RM 2AS13M, 45 Center Dr, Bethesda, MD 20814

R21 AI143494 (Miller, B., PI) 01/03/19-12/31/20 0.60 calendar
NIH $125,000 ADC
Title: Targeting CCR5 FrameShifting with Synthetic Molecules
Major goal: To develop small molecules which interact with the structured CCR5 RNA element, the frameshift-stimulatory sequence (FSS), to block CCR5 expression - thus inhibiting cellular susceptibility to HIV infection.
Role: Co-Investigator
Program Official: Roger Miller, RM 9G50, 5601 Fishers Lane, Rockville, MD 20852

1014095 BWF (Dewhurst, Bennett, MPI) 02/02/15-02/01/20 1.44 calendar
Burroughs Wellcome Fund Institutional Program $500,000 ADC
Unifying Population and Laboratory Based Sciences
Title: Infection and Immunity: From Molecules to Populations
Major goal: To train predoctoral students at the intersection of basic/translational research in infectious diseases and immunity, and population health
Role: Co-PI
Program Official: Victoria McGovern, 919-991-5112, 21 T.W. Alexander Drive, Research Triangle Park, NC 27709

T32 AI118689 (Dewhurst, PI) 07/01/15-06/30/20 no salary support
NIH/NIAID $228,966 ADC
Title: Infection and Immunity: The Pathogenesis of Host-Microbe Interactions
Major goal: To train predoctoral students in infection and immunity research.
Role: PI
Program Official: Stephanie Coomes, 301-761-6855, RM 7G67, 5601 Fishers Lane, Rockville, MD 20852

DP7 DE024857 (Dewhurst, Peyre, Baas, MPI) 09/01/14-08/31/19 0.12 calendar
NIH/OD $231,701 ADC
Title: The University of Rochester BEST Training Program
Major goal: To develop and implement a novel training program for biomedical researchers, intended to prepare them for a greater breadth career opportunities.
Role: MPI
Program Official: Patricia Labosky, 301-594-4863, RM 8180A, 6001 Executive Blvd, Rockville, MD 20852

K12 GM106997 (Dewhurst, Doolittle, MPI) 07/01/15-03/30/21 0.60 calendar
NIH/NIGMS $888,200 ADC
Title: Rochester Partnership to Advance Research and Academic Careers in Deaf Scholars
Major goal: To develop and implement a unique biomedical research training program for deaf scholars.
Role: MPI
Program Official: Mercedes Rubio, 301-451-3137, RM 2AS49A, 45 Center Dr, Bethesda, MD 20814

Grant Dated 01/01/19 (Dewhurst, PI) 01/01/19-12/31/19 0.60 calendar
Gilead Sciences, Inc. $50,000 ADC
Title: Curiosity-Driven, Humanizing Portraits of HIV: A Narrative and Art Based Communication Model to Improve Patient Care
Major Goal: To improve the lives of persons living with HIV and with mental health diagnoses (which present a major risk factor for acquisition of HIV and for poor health outcomes in persons living with HIV). The project will do this by bringing a powerful series of portraits of persons affected by HIV and by mental health challenges, along with other related media, to a national audience – thereby educating, raising awareness,

provoking dialogue and increasing empathy. Our aim is to destigmatize HIV, through humanizing, on a nationwide basis.
Role: PI
Program Official: Gilead Sciences, Inc. Grants Coordinator, 650-522-1696, 333 Lakeside Dr, Foster City, CA 94404

NO OVERLAP

KIM, Baek

ACTIVE AWARDS

8R01AI150451-09 (Kim) 07/01/12-07/31/20 2.4 calendar
NIH/NIAID $199,500 ADC
Title: SAMHD1 controls dNTP pool and HIV sensitivity to NRTIs
Major Goals: 1) To determine the effect of SAMHD1/Vpx on HIV-1 sensitivity to NRTIs in human primary macrophages and activated $CD4^+$ T cells.
Role: PI
Program Official: Michael Sakalian

5 R01 AI136581-02 (Kim) 01/19/18-12/31/22 4.0 calendar
NIH/NIAID $250,000 ADC
Title: Lentivirus Replication Strategy and Pathogenesis
Major goal: 1)To investigate distinct enzyme kinetic adaptation strategies between RTs of SAMHD1 non-counteracting HIV-1 and counteracting SIV/HIV-2 by genetic and biochemical approaches. 2)To investigate the anti-SAMHD1 strategies of non-primate lentiviruses (FIV, EIAV and BIV) in nondividing cell types. 3)To investigate the comparative rNTP incorporation by HIV-1, HIV-2 and SIV in macrophages, rNTP incorporation hot spots, and effects of rNTP incorporation on viral mutagenesis
Role: PI
Program Official: Paula Acevedo (GMS), 301-435-2860

5 R01 MH116695-02 (Schinazi /Tyor) 03/10/2018 – 12/31/2022 1.2 calendar
NIH/NIMH $441,584
Title: Towards Suppression and Elimination of HIV in the CNS
Major goal: The focus is to investigate new adjunctive therapeutic strategies from three distinct classes of compounds to target improved therapy targeting myeloid cells as viral reservoirs in the CNS (i.e., microglia, MP) and eliminate these reservoirs and/or reduce risk of developing HAND.
Role: Co-investigator
Program Official: John E Fonda (GMS), 301-402-8158

ST Pharm, Ltd EPEX 50411 (Kim) 02/1/2019 – 01/31/2020 0.12 calendar
Effect of STP0404 on HIV Rebound
Role: PI
Program Official: Seohyun Ahn, Ph.D., Head of New Drug Development, seohyun.ahn@stpharm.co.kr

NO OVERLAP

ALBRECHT, Randy

ACTIVE AWARDS

HHSN272201400008C (PD/PI García-Sastre) 04/01/14-03/31/21
NIH/NIAID $1,594 0.12 calendar
Title: Center for Research on Influenza Pathogenesis
Major Goal: This project is a research component of the NIAID Center of Excellence for Influenza Research and Surveillance, and is focused on examining the replication, pathogenesis, and transmission of influenza viruses.
Role: Co-Investigator
Program Official: Dr. Marciela M. Degrace, marciela.degrace@nih.gov NIH/NIAID/DMID, 5601 Fishers Lane, Rockville, MD 20852

U19AI117873 (PD/PI Sealfon) 05/08/15-04/30/20
NIH/NIAID $57,062 0.72 calendar
Title: **Modeling Early Immunity to Human Influenza Infection**
Major Goal: This subproject, part of a multi-investigator U19, entitled, "Immunity to Influenza in Primary Lung EpithelialCells," will quantify the responses of fully differentiated primary HTBE to IAV infection.
Role: Core Lead
Program Official: Dr. Timothy A. Gondre-Lewis, tglewis@niaid.nih.gov, NIH/NIAID/DMID, 5601 Fishers Lane Rockville, MD 20852

P01AI097092 (PD/PI Palese) 08/01/18-06/30/23
NIH/NIAID $15,938 1.20 calendar
Title: **Toward a Universal Influenza Virus Vaccine**
Major Goal: The overall focus of this program project grant is to advance our understanding of immunity and antibody responses to generate a broadly-protective influenza virus vaccine. The goal of Project 3 is to evaluate the immune responses to influenza virus vaccines and efficacy of immunotherapeutics in the ferret model.
Role: Co-Investigator
Program Official: Dr.Jennifer L.Gordon, jennifer.gordon2@nih.gov, NIH/NIAID/DMID, 5601 Fishers Lane Rockville, MD 20852

W81XWH1810488 (PR171792) (PD/PI Krammer) 09/01/18-08/31/21
Department of the Army $15,938 1.20 calendar
Title: **GMP production of candidate pan-group 2 influenza A virus vaccines**
Major Goal: The focus of this study is to develop a universal flu vaccine that provides protection against all influenza A viruses (group 1 and group 2) and will abolish the need for annual reformulation and re-administration of the vaccine each year.
Role: Co-Investigator
Program Official: Catherine Sanchez, catherine.n.sanchez.civ@mail.mil, Department of Defense, Congressionally Directed Medical Research Programs (CDMRP),

R03AI142046 (PD/PI Albrecht) 09/01/18-08/31/20
NIH/NIAID $50,000 0.24 calendar
Title: **Developing immune reagents to increase the preclinical value of the ferret model**
Major Goal: The focus of this grant is to develop immunological reagents for the ferret model of influenza which will then be used in a pilot study that will examine whether autophagy pathways can be stimulated to enhance the ferret immune response to influenza virus vaccine.
Role: PI
Program Official: Dr.Teresa Hauguel, hauguelt@niaid.nih.gov, NIH/NIAID/DMID, 5601 Fishers Lane Rockville, MD 20852

R01AI141226 (PD/PI García-Sastre) 01/07/19-12/31/23
NIH/NIAID $15,938 1.20 calendar
Title: **Toward a universal influenza virus vaccine based on live attenuated NS1-deleted influenza viruses**
Major Goal: We plan to manufacture two live attenuated NS1-deleted influenza virus vaccines expressing chimeric group 1 HA proteins.
Role: Co-Investigator
Program Official: Dr.Jennifer L.Gordon, jennifer.gordon2@nih.gov, NIH/NIAID/DMID, 5601 Fishers Lane Rockville, MD 20852

R21AI144525 (PD/PI Evans) 01/21/19-12/20/20
NIH/NIAID $7,969 0.60 calendar
Title: **Adapting hepatitis C virus to infect ferrets**
Major Goal: Our focus is to study how host and viral factors influence HCV species-tropism, and examine the capacity for HCV to adapt to overcome these limitations in a ferret model system.
Role: Co-Investigator
Program Official: Dr. Rajen Koshy, rkoshy@niaid.nih.gov, NIH/NIAID/DMID, 5601 Fishers Lane Rockville, MD 20852

N/A (PD/PI tenOever) 03/08/19-03/07/20
Institut Pasteur, DARPA $54,400 0.24 calendar
Title: **Defective interference of viral INF**
Major Goal: This research program is a component of DARPA's INTERfering and Co-Evolving Prevention and Therapy (INTERCEPT) program, which aims to harness therapeutic interfering particles as a novel, adaptive medical countermeasure to infection by influenza viruses. The therapeutic efficacy of novel interfering particles will be tested in the ferret model of influenza.
Role: Co-Investigator
Program Official: Dr. Bradley Ringeisen, DARPA Program Officer, 675 North Randolph Street, Arlington, VA 22203-2114

NO OVERLAP